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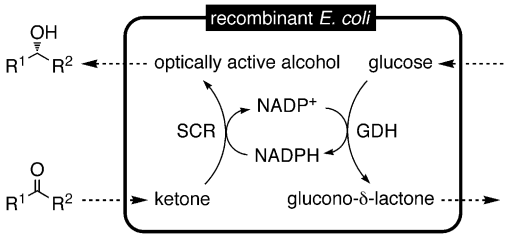
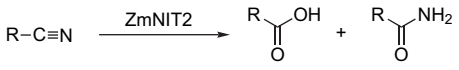
Tetrahedron

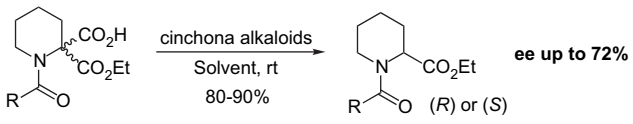
Tetrahedron Vol. 62, No. 26, 2006

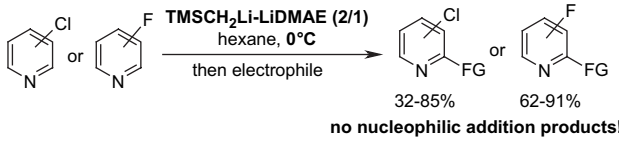
Contents

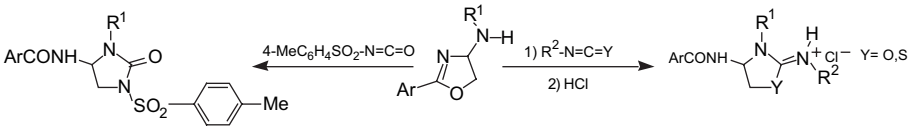
ARTICLES

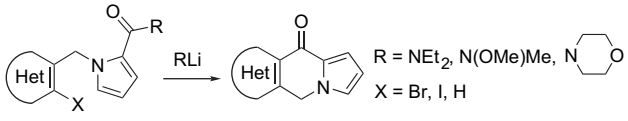


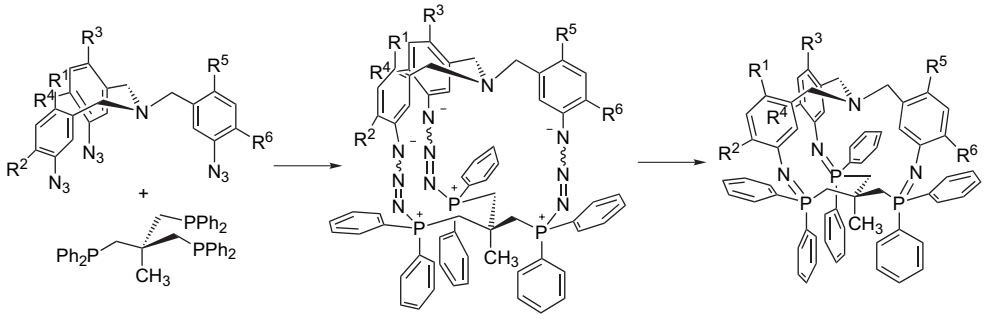


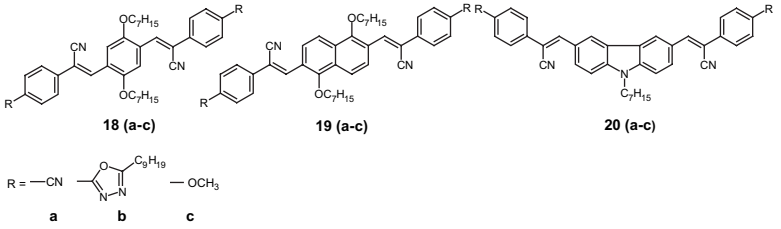




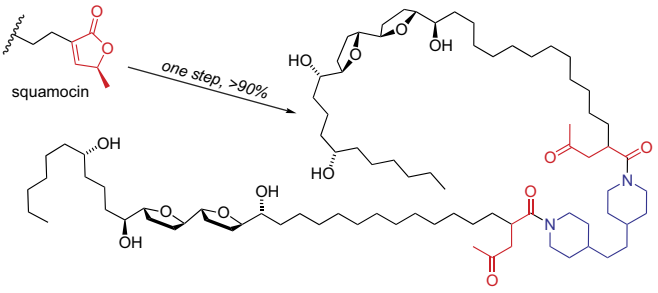




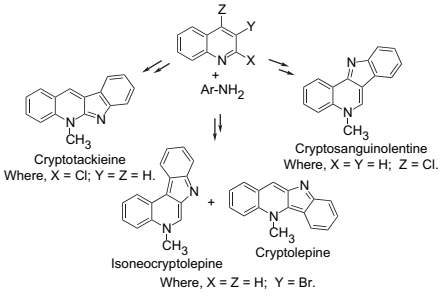


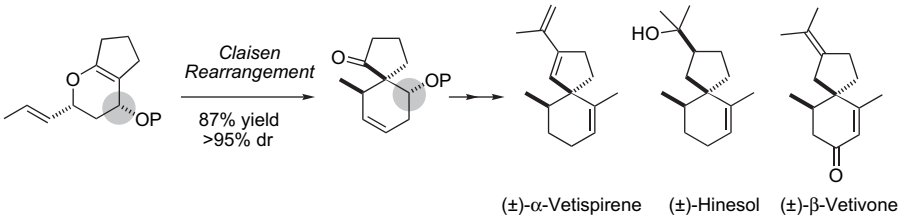


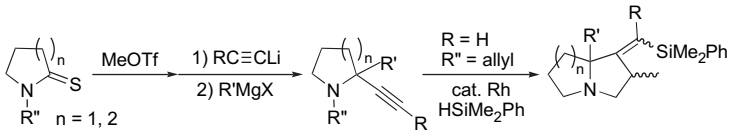
A series of cyano-substituted model compounds (**18**–**20**) for OLEDs were prepared and the influence of electron releasing and electron-withdrawing substituents on α-cyanostyryl moieties was investigated as far as their emissive and absorptive properties were concerned.

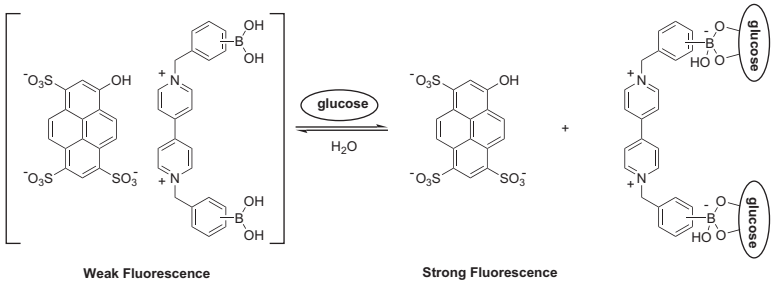


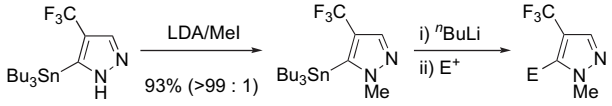
A three-step synthesis of the indoloquinoline alkaloids and their new methyl derivatives has been described, which may be useful as new antiplasmodial drugs and DNA intercalating agents.

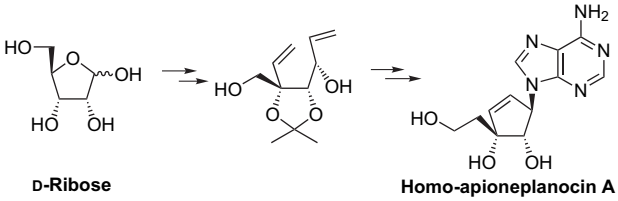




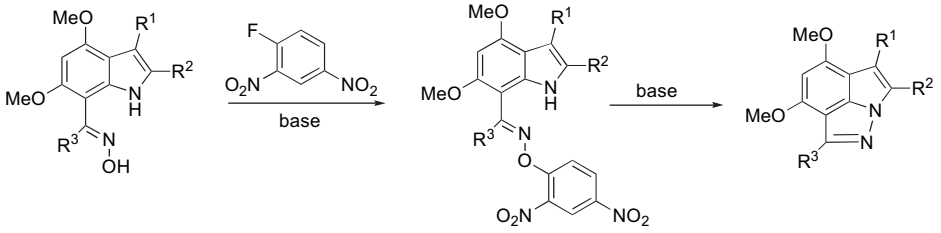


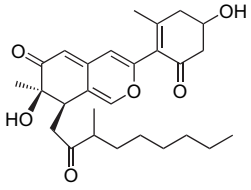




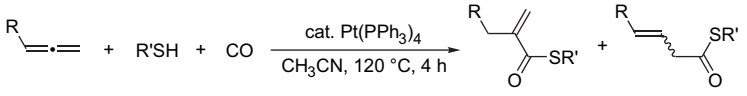


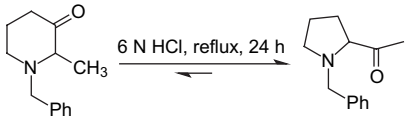
Homo-apioneplanocin A was efficiently synthesized via stereoselective hydroxymethylation, regio- and chemoselective hydroboration, and chemoselective oxidation as key steps from D-ribose.

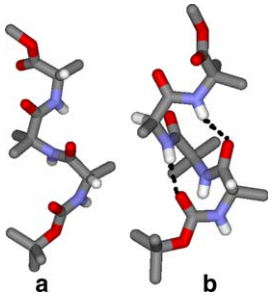


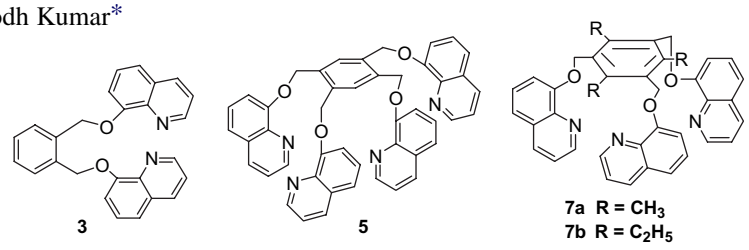


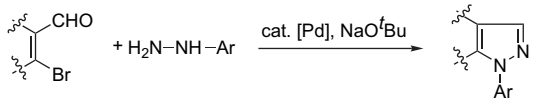
Four new antibiotic azaphilones named cohaerins C–F along with binapthyl were isolated and characterized from the xylariaceous fungus *Annulohypoxylon cohaerens*.

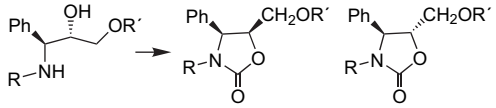


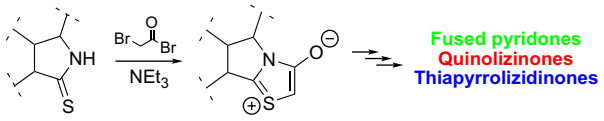




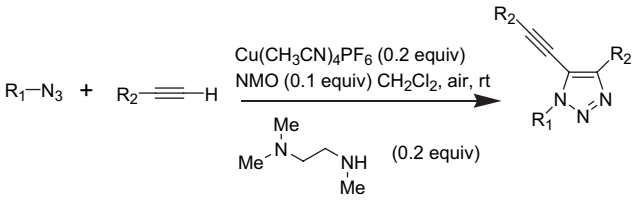


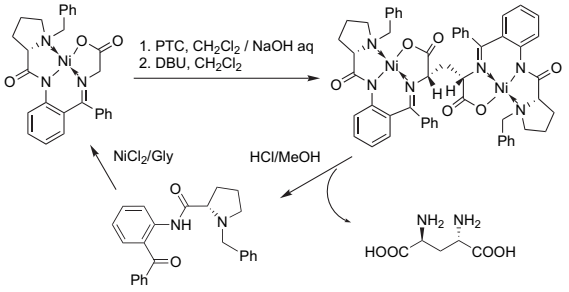


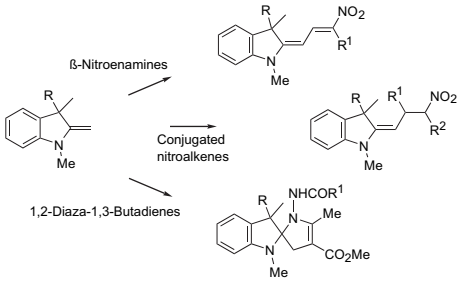


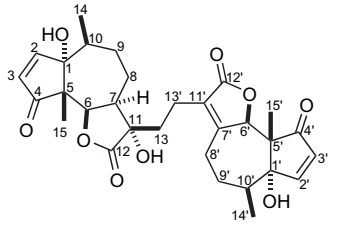


Substituted thiapyrrolizidinones and fused pyridones, and quinolizinones were synthesized efficiently by thioisomünchnone/1,3-dipolar cycloaddition/desulfurization cyclization cascade in a one-pot procedure from thioamides.









dichrocepholide D

*Corresponding author
ⓘ+ Supplementary data available via ScienceDirect

COVER

The self-assembly of tris(3-azidobenzyl)amines with 1,1,1-tris[(diphenylphospino)methyl]ethane (*triphos*) *via* tripod–tripod coupling proceeds successfully to give chiral macrobicyclic triphosphazides. The heating of these macrobicyclic cages induces a remarkable stepwise triple extrusion of molecular nitrogen to afford tri-λ^5-phosphazenes which preserve the chiral, propeller-like topology of their precursors. The molecular structure of one of the tri-λ^5-phosphazenes ($R^1{=}R^3{=}R^5{=}$Br, $R^2{=}R^4{=}R^6{=}$H) is shown projected along the threefold axis [from the tribenzylamine fragment (right) and also from the *triphos* fragment (left)]. Six phenyl groups have been replaced by hydrogen atoms for clarity. *Tetrahedron* **2006**, *62*, 6190–6202.

Indexed/Abstracted in: AGRICOLA, Beilstein, BIOSIS Previews, CAB Abstracts, Chemical Abstracts. Current Contents: Life Sciences, Current Contents: Physical, Chemical and Earth Sciences, Current Contents Search, Derwent Drug File, Ei compendex, EMBASE/Excerpta Medica, Medline, PASCAL, Research Alert, Science Citation Index, SciSearch

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ISSN 0040-4020

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SCIENCE DIRECT
Tetrahedron
Tetrahedron 62 (2006) 6143–6149

Asymmetric reduction of ketones using recombinant *E. coli* cells that produce a versatile carbonyl reductase with high enantioselectivity and broad substrate specificity

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Received 3 April 2006; revised 19 April 2006; accepted 21 April 2006
Available online 6 May 2006

Abstract—The gene encoding a versatile biocatalyst that shows high enantioselectivity for a variety of ketones, SCR (*Saccharomyces cerevisiae* carbonyl reductase), has been identified, cloned, and expressed in *Escherichia coli*. Two types of expression systems with high NADPH-regenerating capacities have been constructed. One is the tandem system, where the genes encoding SCR and GDH (glucose dehydrogenase) are located in the same plasmid, and the other is the two-plasmid system, where each of the SCR and GDH genes is located in separate plasmids that can coexist in one *E. coli* cell. Asymmetric reduction of ketones with the recombinant *E. coli* cells gave synthetically useful 20 alcohols, 11 of which were enantiomerically pure. The productivity of one of these products was as high as 41 g/L.

1. Introduction

Asymmetric synthesis with biocatalysts has attracted much attention from the viewpoint of green chemistry.[1–2] Among them, versatile biocatalysts capable of showing high enantioselectivity and broad substrate specificity simultaneously have found broad utility because this characteristic feature is useful for asymmetric synthesis of various compounds needed in a laboratory as well as a quite important compound.[3] The discovery and development of a new versatile biocatalyst with high efficiency is therefore an important subject, contributing to the significant enlargement of the library of synthetically useful biocatalysts.

Carbonyl reductases from various microorganisms have been used to prepare optically active alcohols from carbonyl compounds.[2] A *Saccharomyces cerevisiae* (bakers' yeast) carbonyl reductase (SCR) previously reported by us showed catalytic activity for various ketones, such as α-chloro ketones, α-acetoxy ketones, α-keto esters, β-keto esters, γ-keto esters, and β-diketones, and 13 out of 20 alcohols obtained had enantiomeric purities of >98% ee.[4a,b] Despite the potential of this enzyme as a versatile biocatalyst, its utility was restricted by its low expression level in *S. cerevisiae* and the laborious purification procedure. These drawbacks can be overcome by constructing an SCR gene expression system. The synthetic power of recombinant carbonyl reductases from various origins has recently been demonstrated.[5–13] In particular, a number of recombinant whole-cell reductions with high efficiency have been reported.[6–8] Recently, we have identified and cloned the gene coding for SCR, and have investigated the capabilities of the recombinant SCR by conducting both enzymatic and whole-cell reductions of various ketones.[4c] Here we report asymmetric reduction of various ketones with recombinant *Escherichia coli* expressing the gene encoding SCR, demonstrating the utility and power of the versatile biocatalyst.

2. Results

2.1. Identification, cloning, and expression of the gene

The amino-acid sequence analysis of purified SCR suggested strongly that the gene encoding SCR is *Gre2* (*YOL151w*). Stewart and co-workers have reported a library of *S. cerevisiae* reductases, which includes Gre2.[5] In this study, the SCR gene was PCR-cloned from the genomic DNA of an *S. cerevisiae* strain that we have used previously, and an expression plasmid, pESCR, was constructed (Fig. 1). As shown in Table 1, enzymatic activity (90 U per 1 g of wet cells) of SCR heterologously expressed in *E. coli* BL21(DE3) harboring pESCR was 800-fold higher than that (0.11 U per 1 g of wet cells) of SCR in *S. cerevisiae*.

Keywords: Alcohol; Asymmetric synthesis; Biotransformation; Reduction.
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doi:10.1016/j.tet.2006.04.061

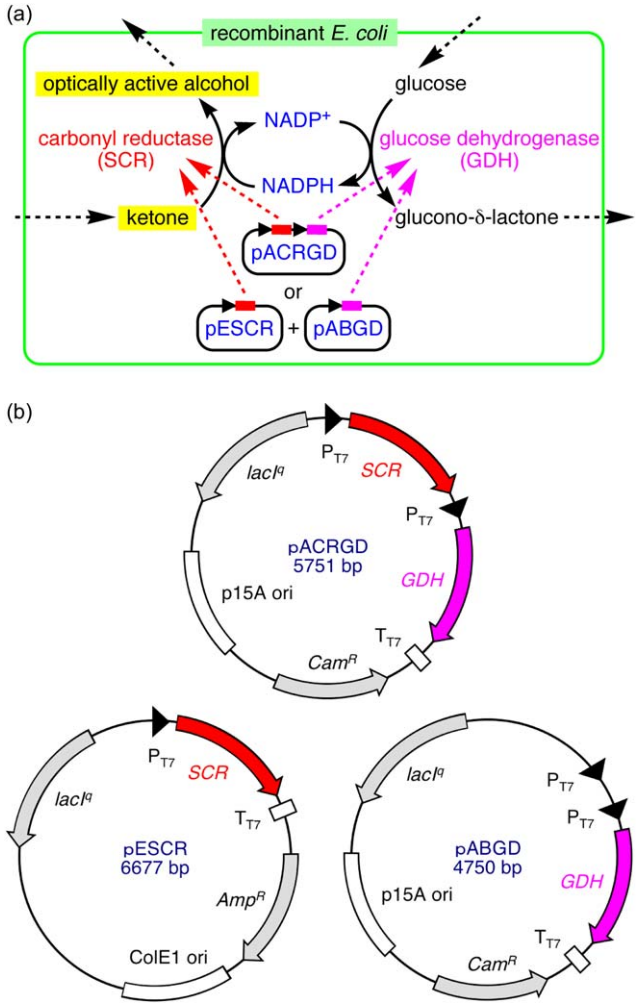

Figure 1. (A) Recombinant *E. coli* cell coexpressing SCR and GDH genes to produce optically active alcohols. In this paper, *E. coli* harboring pACRGD is called the tandem system, while that harboring pESCR and pABGD is called the two-plasmid system. (B) Structures of expression plasmids used in this study. *SCR*: SCR gene, *GDH*: GDH gene, Amp^R: ampicillin-resistance gene, Cam^R: chloramphenicol-resistance gene, P_{T7}: T7 promoter, and T_{T7}: T7 terminator.

Cofactor regeneration is essential for high productivity of alcohols. Kataoka and co-workers have successfully employed a glucose dehydrogenase (GDH) from *Bacillus megaterium* to regenerate NADPH in vivo.[6] We constructed an expression plasmid having the SCR and GDH genes (tandem system), pACRGD, as shown in Figure 1. pACYCDuet-1 (Novagen) was selected as an expression vector because it has two multiple cloning sites, each preceded by a T7 promoter, which is suitable for coexpression of two genes. The GDH gene was subcloned into pACYCDuet-1 to give pABGD, into which the SCR gene was subcloned to give pACRGD. Using *E. coli* BL21(DE3) harboring pACRGD, a high level of production of SCR and GDH was confirmed by SDS-PAGE (Fig. 2), and the SCR and GDH activities were 60 and 15,000 U per 1 g of wet cells, respectively (Table 1).

Table 1. Enzymatic activity per 1 g of wet cells

Host	Plasmid	SCR activity (U)	GDH activity (U)
E. coli BL21(DE3)	pESCR	90	—
E. coli BL21(DE3)	pACRGD	60	15,000
E. coli BL21(DE3)	pESCR+pABGD	100	5800
Bakers' yeast	—	0.11	—

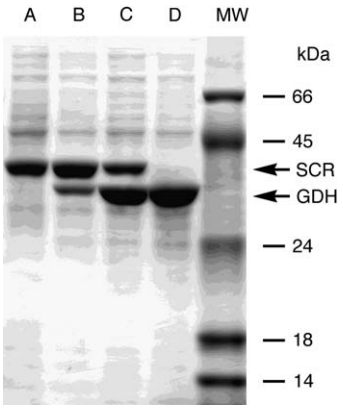

Figure 2. SDS-PAGE of the cell lysates of *E. coli* BL21(DE3) transformed with (A) pESCR, (B) pESCR+pABGD, (C) pACRGD, and (D) pABGD after cultivation and induction.

2.2. Asymmetric reduction of ketones with the tandem system

The whole-cell reductions of various ketones were conducted with the recombinant *E. coli* (tandem system). The experimental procedure is described in Section 5.7, and the results are summarized in Figure 3. Although 3 mmol of ketone was typically used, the amount of a very reactive one, ethyl pyruvate (**7a**), was increased to 6 mmol, and that of some ketones with modest reactivity was decreased to 1 mmol. Although the reaction did proceed without addition of $NADP^+$, the addition of a catalytic amount (0.4 mol %) of $NADP^+$ increased the conversion (not optimized). Figure 3 clearly shows that the inherent characteristic features of SCR are retained in the recombinant SCR. The absolute configurations of all the 16 alcohols **1b**–**16b** obtained with the recombinant *E. coli* were the same as those obtained previously with SCR purified from *S. cerevisiae*. The enantiomeric purities in the former are as high as those in the latter in most cases. Both aliphatic and aromatic ketones were successfully reduced. Not only enantioselectivity but also regioselectivity for 2,4-hexanedione (**16a**) was complete, with the less hindered carbonyl group being reduced exclusively, as observed previously.[4a,b]

As a control, *E. coli* BL21(DE3) harboring pACYCDuet-1 was used, and very low conversions (0% conversion in many cases) were confirmed by ^1H NMR in all cases. This suggests that although *E. coli* produces its own enzymes, its ketone-reducing and/or cofactor-regenerating capacity for 1–6 mmol of ketone is very low. Furthermore, *E. coli* BL21(DE3) strains harboring either pESCR or pABGD, which lack either GDH or SCR gene, were also examined for α-acetoxyacetophenone (**5a**) under the same reaction conditions, which resulted in very low conversions (4% and 0%). All these results indicate that SCR is responsible for the reduction of all the ketones and that both SCR and GDH are essential for the high-turnover catalysis.

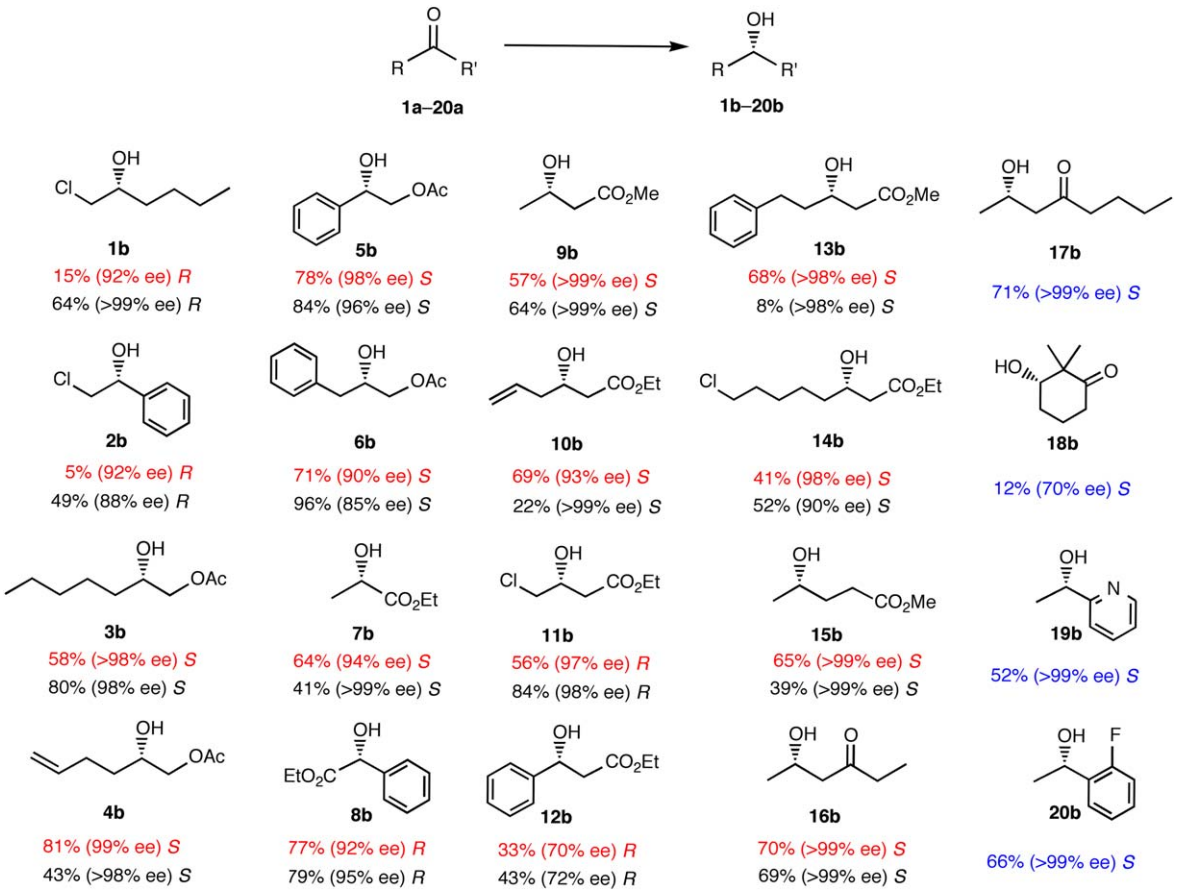

Figure 3. Asymmetric reduction of ketones with SCR. The data are shown in the following order: isolated yield, enantiomeric purity, and absolute configuration. The results obtained with recombinant *E. coli* (tandem system) and that (two-plasmid system) are shown in red and blue, respectively. The results obtained with SCR purified from bakers' yeast are taken from the lit.[4b] and are shown in black. Conditions for the tandem system: substrate (3.0 mmol for **1a**–**6a**, **8a**–**9a**, **11a**, and **15a**–**16a**, 6.0 mmol for **7a**, 1.0 mmol for **10a** and **12a**–**14a**), wet cells of *E. coli* BL21(DE3) harboring pACRGD (2 g), glucose (6.0 mmol), $NADP^+$ (12 μmol), 0.1 M phosphate buffer (pH 7.0, 50 mL), 30 °C. Conditions for the two-plasmid system: substrate (4.0 mmol for **17a**, 1.0 mmol for **18a**–**20a**), wet cells of *E. coli* BL21(DE3) harboring pESCR and pABGD (2 g), glucose (2 equiv), $NADP^+$ (12 μmol), 0.1 M phosphate buffer (pH 7.0, 10 mL), 30 °C. The pH of the reaction mixture was kept constant by adding 2 N NaOH. The experimental procedure is described in Section 5.7.

Mori and co-workers have demonstrated that (*S*)-3-hydroxy-2,2-dimethylcyclohexanone ((*S*)-**18b**) can be used in the total synthesis of more than 10 natural products.[14] Based on the stereochemical trend shown in Figure 3, we expected that (*S*)-**18b** might be obtained by the asymmetric reduction of 2,2-dimethylcyclohexane-1,3-dione (**18a**) using SCR. As a result of the reduction with the recombinant *E. coli* (tandem system), unfortunately, we detected only a trace amount of product by ^{1}H NMR.

2.3. Asymmetric reduction of ketones with the two-plasmid system

In view of the very low conversion of **18a**, we tried to improve the reducing power by increasing the expression level of SCR. We had two plasmids that can coexist in one *E. coli* cell, pESCR and pABGD (Fig. 1). Because the copy number of the former is higher than that of the latter, *E. coli* cells harboring the two plasmids (two-plasmid system) may produce SCR more efficiently than that harboring pACRGD (tandem system). As expected, enzymatic activity (Table 1) and SDS-PAGE (Fig. 2) clearly indicate that SCR is produced more efficiently in the two-plasmid system than in the tandem system. Because the whole-cell reduction was found to be faster in higher concentrations, the amount of buffer solution was decreased from 50 mL to 10 mL (data not shown). When the time courses of the reduction of a substrate with moderate reactivity, 2,4-octanedione (**17a**), were compared (Fig. 4), the two-plasmid system showed the best performance, giving (*S*)-2-hydroxy-4-octanone ((*S*)-**17b**) with complete enantioselectivity and regioselectivity in 71% isolated yield, which corresponds to the relatively high productivity of 41 g/L. In contrast, *E. coli* transformants harboring either pESCR or pABGD resulted in very low conversions (Fig. 4), which indicates again that both SCR and GDH are essential for the high-turnover catalysis.

Using the two-plasmid system, we conducted the reduction of cyclic β-diketone **18a** again, and found that the reaction proceeded slowly. The product (*S*)-**18b** was isolated in 12% yield with 70% ee (Fig. 3). Despite the long reaction time (24 h), the substrate **18a** remained in the reaction mixture (18% conversion). Using the two-plasmid system, we further examined other substrates **19a** and **20a**, which contain nitrogen and fluorine atoms, respectively, and obtained optically pure alcohols (*S*)-**19b** and (*S*)-**20b** successfully (Fig. 3).

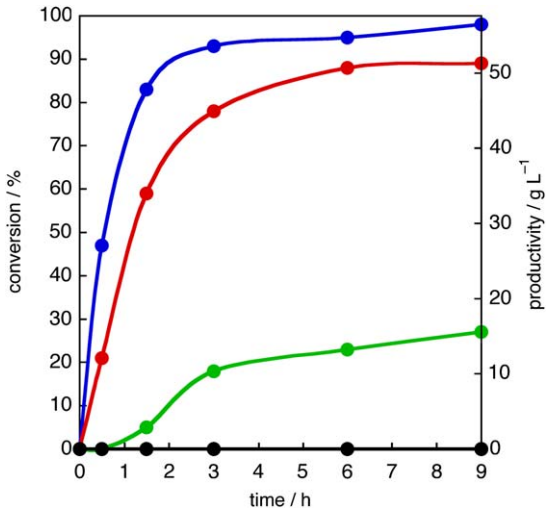

Figure 4. Time courses for reductions of **17a** with *E. coli* BL21(DE3) transformants harboring pACRGD (red), both pESCR and pABGD (blue), pESCR (green), or pABGD (black). Conditions: **17a** (4.0 mmol), wet cells of *E. coli* BL21(DE3) transformant (2 g), glucose (8.0 mmol), $NADP^+$ (12 μmol), 0.1 M phosphate buffer (pH 7.0, 10 mL), 30 °C. The pH of the reaction mixture was kept constant by adding 2 N NaOH. The reactions were monitored by ^{1}H NMR.

3. Discussion

Versatile biocatalysts capable of showing high enantioselectivity and broad substrate specificity simultaneously are very useful for asymmetric syntheses of various chiral compounds. In this study, the gene encoding a versatile biocatalyst, SCR, has been identified, cloned, and expressed in *E. coli*. Two types of systems coproducing SCR and GDH (tandem and two-plasmid systems) have been constructed (Fig. 1), and these recombinant *E. coli* cells afforded 20 optically active alcohols, 11 of which had enantiomeric purities of >98% ee (Fig. 3). Some of the obtained alcohols have been used in the total synthesis of natural products and biologically active compounds: e.g., (*R*)-**11b** for carnitine,[15] (*R*)-**12b** for fluoxetine,[16] (*S*)-**13b** for dihydrokawain,[17] (*S*)-**14b** for xestospongin A,[18] and (*S*)-**15b** for pyrenophorin.[19] (*S*)-**18b** is an extremely useful intermediate for the total synthesis of various natural products such as polygodial, dihydroactinidiolide, *O*-methyl pisiferic acid, juvenile hormone III, and (−)-K-76.[14] SCR, which can produce these practical alcohols, is a synthetically useful, versatile biocatalyst.

Coenzyme regeneration is essential for an efficient catalytic reduction of ketones.[6] NADPH is oxidized to $NADP^+$ by the SCR-catalyzed reduction of ketone, while $NADP^+$ is reduced back to NADPH by the GDH-catalyzed oxidation of glucose (Fig. 1). This catalytic cycle is realized only when both the two enzymes are produced in vivo. Indeed, only the tandem and two-plasmid systems, coexpressing the two genes, gave the product in good yields (Fig. 4). In the latter case, the relatively high productivity (41 g/L based on the isolated yield) of (*S*)-**17b** was achieved; the productivity based on the conversion was 56 g/L. In contrast, *E. coli* strain harboring pESCR showed much lower conversions, which suggests that $NADP^+$ formed by the SCR-catalyzed reaction was slowly reduced to NADPH by small amount of enzymes originating from *E. coli* itself. *E. coli* strain harboring pABGD exhibited 0% conversion because of the absence of SCR.

The two-plasmid system showed a higher reducing power than the tandem system did. Figure 4 shows that the initial rate of the former is about 2-fold faster than that of the latter. This is a result of the modulation of the production level of SCR and GDH. Because glucose is the natural substrate for GDH, GDH activity in the tandem system is sufficiently high (Table 1). In contrast, because the ketones used in this study are unnatural substrates for SCR, SCR activity is low in the tandem system. Obviously, a lower amount of GDH and a higher amount of SCR would be ideal. In the two-plasmid system, the copy number of pESCR in the cell is higher than that of pABGD. Indeed, SDS-PAGE (Fig. 2) indicated that SCR was produced more efficiently in the two-plasmid system than in the tandem system, and in the former case, improved SCR activity (100 U per 1 g of wet cells) and reduced GDH activity (5800 U per 1 g of wet cells) were observed (Table 1). This modulation of the production level of SCR and GDH led to the higher reducing power of the two-plasmid system.

Some products were isolated in low yields with incomplete enantiomeric purities (Fig. 3). For example, the useful synthetic intermediate (*S*)-**18b** was isolated in 12% yield with 70% ee despite the use of the two-plasmid system. The structural alteration of SCR itself may be effective for further improvement. For example, SCR can be used as a parent enzyme for directed evolution or rational design approaches.

4. Conclusion

The gene encoding a versatile biocatalyst that shows high enantioselectivity for a variety of ketones, SCR, has been identified, cloned, and expressed in *E. coli*. Two types of expression systems with high NADPH-regenerating capacities have been constructed. One is the tandem system, where the genes encoding SCR and GDH are located in the same plasmid, and the other is the two-plasmid system, where each of the SCR and GDH genes is located in separate plasmids that can coexist in one *E. coli* cell. These recombinant *E. coli* cells coproducing SCR and GDH are easy-to-use, synthetically useful biocatalysts to give a variety of optically active alcohols, and 11 out of 20 alcohols obtained had enantiomeric purities of >98% ee. When the two coexpression systems were compared in terms of the conversion of 2,4-octanedione, the two-plasmid system showed better performance, giving (*S*)-2-hydroxy-4-octanone with complete enantioselectivity and regioselectivity in 71% isolated yield, which corresponds to the productivity of 41 g/L.

5. Experimental

The pressed bakers' yeast was purchased from Oriental Yeast, and SCR from bakers' yeast was purified to homogeneity as described previously.[4a] Two internal amino-acid sequences of SCR were determined at APRO Life Science Institute, and the homology search was performed by BLAST analysis. All the DNA manipulations and bacterial transformations were carried out according to the standard

protocols or manufacturers' instructions, unless otherwise stated.[20] *E. coli* JM109 (Toyobo) and pGEM-T (Promega) were routinely used as a host and a cloning vector, respectively, and *E. coli* BL21(DE3) (Stratagene) was used as a host for pET-11a (Stratagene), pACYCDuet-1 (Novagen), and their derivatives. *TaKaRa Ex Taq* DNA Polymerase was purchased from Takara Bio. Restriction enzymes were purchased from Toyobo, Stratagene, or Roche, and T4 DNA ligase was purchased from Toyobo or New England Biolabs. Agarose for electrophoresis was purchased from Nacalai Tesque. QIAprep Spin Miniprep Kit, QIAquick PCR Purification Kit, QIAquick Nucleotide Removal Kit, and QIAquick Gel Extraction Kit (Qiagen) were routinely used for DNA isolation and purification. Synthetic oligonucleotides were obtained from Sigma Genosys Japan. DNA sequencing was performed on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) at Advanced Science Research Center of Okayama University, and then analyzed with MacVector 7.0 (Oxford Molecular). All the genes cloned into the plasmids were fully sequenced in both directions. NADPH and $NADP^+$ were purchased from Oriental Yeast. The amount of proteins was determined by the method of Bradford using BSA as the standard.[21] TLC was performed on Merck silica gel 60 F_{254}, and silica gel column chromatography was performed using Fuji Silysia BW-127 ZH (100–270 mesh). ^{1}H NMR spectra were measured in $CDCl_3$ at 600, 500, 300, or 200 MHz.

5.1. Cloning of the SCR gene and construction of an expression plasmid, pESCR

Two internal peptides of the purified SCR were sequenced: AAWEFLEENR and DLLIPAVDGVK, both of which hit Gre2 (YOL151w) of *S. cerevisiae* by BLAST analysis. The SCR gene (*GRE2*, *YOL151w*) was amplified by PCR from the genomic DNA of bakers' yeast purchased from Oriental Yeast. The primers used for PCR are as follows: SC-CR-3F (5′-CGATTTTCAAACAAACAGATAGCAG-3′) and SC-CR-4R (5′-AAAATGCGCAGAGATGTACT AGATG-3′), which correspond to 5′- or 3′-noncoding regions encompassing the gene. The conditions for the 100 μL PCR mixture were as follows: 0.5 μM each primer, 0.2 mM each dNTP, genomic DNA (400 ng), 5 U of *TaKaRa Ex Taq* DNA Polymerase, 1.5 mM $MgCl_2$, and 10 μL of PCR buffer. PCR was done for 30 cycles of 98 °C for 10 s and 55 °C for 30 s followed by a final extension at 72 °C for 1 min. The amplified DNA fragment was directly cloned into pGEM-T to yield pGSCR from transformed *E. coli* JM109 cells. The SCR gene was subcloned into an expression vector, pET-11a. The primers used for PCR are as follows: SC-CR-5F (5′-ATCACACGCCCTTA *CATATG*TCAG-3′) and SC-CR-6R (5′-CG*GGATCC*TTAA AGTTTATATTCTGCCCTC-3′), where the restriction sites for *Nde*I and *Bam*HI are italicized. The conditions for the 100 μL PCR mixture were as follows: 0.5 μM each primer, 0.2 mM each dNTP, pGSCR (100 pg), 5 U of *TaKaRa Ex Taq* DNA Polymerase, 1.5 mM $MgCl_2$, and 10 μL of PCR buffer. PCR was done for 30 cycles of 98 °C for 10 s and 55 °C for 30 s followed by a final extension at 72 °C for 1 min. The amplified DNA fragment was digested with *Nde*I and *Bam*HI, and then ligated into pET-11a that had been treated with the same restriction enzymes. *E. coli* BL21(DE3) harboring pESCR was obtained by transformation of the competent cells.

5.2. Construction of a coexpression plasmid having SCR and GDH genes, pACRGD

Genomic DNA of *B. megaterium* NBRC (formerly IFO) 15308 was isolated according to the lit.[22] The glucose dehydrogenase (GDH) gene was amplified by PCR with the primers BM-GD-1F (5′-ATGTATACAGATTTAAAAGAT AAAGTAGTT-3′) and BM-GD-2R (5′-TTAGCCTCTTC CTGCTTGG-3′).[23] The conditions for the 100 μL PCR mixture were as follows: 0.5 μM each primer, 0.2 mM each dNTP, genomic DNA (80 ng), 5 U of *TaKaRa Ex Taq* DNA Polymerase, 1.5 mM $MgCl_2$, and 10 μL of PCR buffer. PCR was done for 30 cycles of 98 °C for 10 s and 50 °C for 30 s followed by a final extension at 72 °C for 5 min. The amplified DNA fragment was directly cloned into pGEM-T to yield pGBGD from transformed *E. coli* JM109 cells. The GDH gene was subcloned into an expression vector, pACYCDuet-1. The primers used for PCR are as follows: BM-GD-10F (5′-CCATGGCCGCGGGCATA-3′) and BM-GD-11R (5′-CGC*GGTACC*GATTTTAGCCTCTTC-3′), where the restriction site for *Kpn*I is italicized. The conditions for the 100 μL PCR mixture were as follows: 0.5 μM each primer, 0.2 mM each dNTP, pGBGD (3.7 ng), 2.5 U of *TaKaRa Ex Taq* DNA Polymerase, 1.5 mM $MgCl_2$, and 10 μL of PCR buffer. PCR was done for 30 cycles of 98 °C for 10 s and 48 °C for 30 s followed by a final extension at 72 °C for 5 min. The amplified DNA fragment was digested with *Nde*I and *Kpn*I, and then ligated into pACYCDuet-1 that had been treated with the same restriction enzymes. *E. coli* BL21(DE3) harboring pABGD was obtained by transformation of the competent cells. The SCR gene was then subcloned into pABGD. The primers used for PCR are as follows: SC-CR-10F (5′-CACGCCC TTAA*TCATGA*GTGTTTTTGTTTC-3′) and SC-CR-6R (5′-CG*GGATCC*TTAAAGTTTATATTCTGCCCTC-3′), where the restriction sites for *Rca*I and *Bam*HI are italicized. The conditions for the 100 μL PCR mixture were as follows: 0.5 μM each primer, 0.2 mM each dNTP, pGSCR (370 ng), 2.5 U of *TaKaRa Ex Taq* DNA Polymerase, 1.5 mM $MgCl_2$, and 10 μL of PCR buffer. PCR was done for 30 cycles of 98 °C for 10 s and 55 °C for 30 s followed by a final extension at 72 °C for 5 min. The amplified DNA fragment was digested with *Rca*I and *Bam*HI, and then ligated into pABGD that had been treated with *Nco*I and *Bam*HI. *E. coli* BL21(DE3) harboring pACRGD was obtained by transformation of the competent cells.

5.3. Preparation of *E. coli* harboring pESCR and pABGD

E. coli BL21(DE3) harboring pABGD was transformed with pESCR and grown on an LB plate containing ampicillin (100 μg/mL) and chloramphenicol (34 μg/mL). The transformation was confirmed by agarose gel electrophoresis and enzymatic activity.

5.4. Sole expression of SCR gene

After *E. coli* BL21(DE3) cells harboring pESCR were grown in LB medium (3 mL) containing ampicillin (100 μg/mL) at

37 °C for 16 h with shaking at 230 rpm, 2 mL of the culture was transferred to the same medium (200 mL) in a 1-L Erlenmeyer flask. The culture was shaken at 200 rpm at 37 °C, and IPTG (0.1 mM) was added when OD_{600} reached 0.6–0.8. The cells were further incubated at 30 °C for 16 h with shaking at 200 rpm, harvested by centrifugation (9000 rpm, 4 °C, 5 min), and washed with 0.1 M phosphate buffer (pH 7.0, 50 mL). The cell pellet was stored at −20 °C until it was used.

5.5. Coexpression of SCR and GDH genes

E. coli BL21(DE3) cells harboring pACRGD (tandem system) were grown in LB medium (3 mL) containing chloramphenicol (34 μg/mL) at 37 °C for 15 h with shaking at 230 rpm, and those harboring pESCR and pABGD (two-plasmid system) were grown in LB medium (3 mL) containing ampicillin (100 μg/mL) and chloramphenicol (34 μg/mL) in the same way. The culture (3 mL) was transferred to the same medium (300 mL) in a 1-L Erlenmeyer flask, and shaken at 200 rpm at 37 °C. IPTG (0.1 mM) was added when OD_{600} reached 0.6–0.8. The cells were further incubated at 28 °C for 18 h with shaking at 200 rpm, harvested by centrifugation (7000 rpm, 4 °C, 10 min), and washed with 0.1 M phosphate buffer (pH 7.0, 50 mL). The cell pellet was stored at −20 °C until it was used for asymmetric reduction.

5.6. Enzyme activity

SCR activity was determined as described previously,[4a] after cell disruption by sonication followed by centrifugation. SCR activity of 1.0 U is defined as the amount of enzyme that oxidizes 1.0 μmol of NADPH per minute at 25 °C.[4a] GDH activity was determined as follows. *E. coli* cells having GDH activity were lysed by sonication for 1 min in an ice bath (×10). Centrifugation (20,000 rpm, 4 °C, 30 min) gave a cell-free extract (CFE) as the supernatant. After appropriately diluted, CFE (300 μL) was added to a solution (2.5 mL) of glucose (100 mM) and $NADP^+$ (2.0 mM) in 60 mM Tris buffer (pH 8.0) in a UV-cuvette thermostated at 25 °C. After the solution was quickly shaken, the reaction rate was measured by following the increase in the absorbance of NADPH at 340 nm as a function of time. In this paper, 1.0 U of GDH activity is defined as the amount of enzyme that reduces 1.0 μmol of $NADP^+$ per minute at 25 °C under the above reaction conditions.

5.7. General procedure for whole-cell asymmetric reduction

In the case of the tandem system, to a mixture of glucose (1.08 g, 6.0 mmol), $NADP^+$ (10 mg, 12 μmol), and *E. coli* BL21(DE3) cells harboring pACRGD (2.0 g) in 0.1 M phosphate buffer (pH 7.0, 50 mL) was added the substrate (typically, 3.0 mmol). In the case of the two-plasmid system, to a mixture of glucose (0.36 g, 2.0 mmol), $NADP^+$ (10 mg, 12 μmol), and *E. coli* BL21(DE3) cells harboring pESCR and pABGD (2.0 g) in 0.1 M phosphate buffer (pH 7.0, 10 mL) was added the substrate (typically, 1.0 mmol). The mixture was stirred with a magnetic stirrer in a water bath at 30 °C. The progress of the reaction was monitored by TLC and/or ^{1}H NMR. In the two-plasmid system, the pH of the reaction mixture was kept constant by adding 2 N NaOH. After an appropriate reaction time, the cells were precipitated by centrifugation (10,000 rpm, 4 °C, 10 min), and solid NaCl was added. The products were extracted with EtOAc or Et_2O several times. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The product was purified by column chromatography and/or distillation. Although the control reaction with *E. coli* BL21(DE3) harboring pACYCDuet-1 was performed in a similar way, the isolation of the product was not attempted because of a very low conversion as determined by ^{1}H NMR.

5.8. Identification of products

Alcohols **1b**–**16b** were characterized as described previously.[4a,b] **17b**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.90 (t, J=7.8 Hz, 3H), 1.18 (d, J=6.0 Hz, 3H), 1.31 (sext, J=7.8 Hz, 2H), 1.56 (quint, J=7.8 Hz, 2H), 2.42 (t, J=7.8 Hz, 2H), 2.50 (dd, J=9.0, 17.4 Hz, 1H), 2.60 (dd, J=3.0, 17.4 Hz, 1H), 3.14 (br s, 1H), 4.20–4.23 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 13.8, 22.26, 22.27, 25.7, 43.3, 50.3, 63.8, 212.6; $[\alpha]_D^{27}$ +64.3 (*c* 1.84, $CHCl_3$), >99% ee (*S*), lit.[24] $[\alpha]_D$ +50 (*c* 1.8) for (*S*)-**17b** with >99% ee. **18b**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.13 (s, 3H), 1.18 (s, 3H), 1.61 (br d, J=4.2 Hz, 1H), 1.64–1.70 (m, 1H), 1.81–1.86 (m, 1H), 2.00–2.07 (m, 2H), 2.37–2.45 (m, 2H), 3.70–3.73 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 19.5, 20.5, 22.7, 28.8, 37.1, 51.1, 77.7, 214.5; $[\alpha]_D^{27}$ +15.0 (*c* 0.80, $CHCl_3$), 70% ee (*S*), lit.[14] $[\alpha]_D^{21}$ +23.0 (*c* 2.0, $CHCl_3$) for (*S*)-**18b** with 96.0–98.8% ee. **19b**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.50 (d, J=6.6 Hz, 3H), 4.30 (br s, 1H), 4.89 (q, J=6.6 Hz, 1H), 7.18–7.21 (m, 1H), 7.27–7.28 (m, 1H), 7.67–7.70 (m, 1H), 8.53 (d, J=4.2 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 24.2, 68.8, 119.8, 122.2, 136.8, 148.1, 163.0; $[\alpha]_D^{25}$ −27.4 (*c* 1.03, $CHCl_3$), >99% ee (*S*), lit.[25] $[\alpha]_D$ −24.5 (*c* 0.50, $CHCl_3$) for (*S*)-**19b** with 98% ee. **20b**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.52 (d, J=6.6 Hz, 3H), 1.95 (br s, 1H), 5.20 (q, J=6.6 Hz, 1H), 7.00–7.03 (m, 1H), 7.14–7.16 (m, 1H), 7.22–7.26 (m, 1H), 7.47–7.50 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 24.0, 64.6, 115.3 (d, J=21.9 Hz), 124.3 (d, J=3.5 Hz), 126.6 (d, J=4.7 Hz), 128.8 (d, J=8.0 Hz), 132.6 (d, J=13.3 Hz), 159.7 (d, J=245.7 Hz); ^{19}F NMR (282 MHz, $CDCl_3$) δ −121.1 (m); $[\alpha]_D^{25}$ −47.8 (*c* 1.46, $CHCl_3$), >99% ee (*S*), lit.[26] $[\alpha]_D^{21}$ +47.2 (*c* 1.35, $CHCl_3$) for (*R*)-**20b** with 97% ee.

5.9. Determination of enantiomeric purities

The enantiomeric purities of the obtained alcohols **2b**, **3b**, **5b**–**9b**, **12b**, **13b**, **15b**, and **16b** were determined as reported previously.[4a,b] The enantiomeric purities of **1b** (acetate form), **17b** (acetate form), **18b** (acetate form), **19b**, and **20b** were determined by capillary GC with a CP-cyclodextrin-β-2,3,6-M-19 column (Chrompack, ϕ 0.25 mm×25 m). GC for **1b** (acetate form): Inj. 250 °C, Col. 80 °C, Det. 220 °C, (*R*) 32.7 min, (*S*) 37.8 min. GC for **17b** (acetate form): Inj. 250 °C, Col. 110 °C, Det. 220 °C, (*S*) 30.2 min, (*R*) 31.6 min. GC for **18b** (acetate form): Inj. 250 °C, Col. 110 °C, Det. 220 °C, (*S*) 45.0 min, (*R*) 47.5 min. GC for **19b**: Inj. 300 °C, Col. 70 °C, Det. 250 °C, (*R*) 82.6 min, (*S*) 85.4 min. GC for **20b**: Inj. 300 °C, Col. 99 °C, Det. 250 °C, (*R*) 24.2 min, (*S*) 27.4 min. Alcohol **4b** was

converted to the corresponding tosylate, which was analyzed by HPLC with a chiral column (Daicel Chemical Industries): Chiralpak AD-H, hexane/*i*-PrOH=9/1, 0.5 mL/min, 254 nm, (*S*) 21.2 min, (*R*) 22.1 min. The enantiomeric purities of **10b**, **11b**, and **14b** were determined by 600 MHz ^{1}H NMR after conversion to the corresponding MTPA esters.

Acknowledgements

We thank Dr. Hiroaki Yamamoto (Daicel Chemical Industries) and Professor Tetsuo Toraya (Okayama University) for the determination of the internal amino-acid sequences of SCR and useful advice on gene expression, respectively. This work was supported by a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science (JSPS) and by a grant from Venture Business Laboratory of Okayama University. We are grateful to the SC-NMR Laboratory of Okayama University for the measurement of NMR spectra.

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Tetrahedron 62 (2006) 6150–6154

Enzymatic nitrile hydrolysis catalyzed by nitrilase ZmNIT2 from maize. An unprecedented β-hydroxy functionality enhanced amide formation

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Received 15 February 2006; revised 13 April 2006; accepted 21 April 2006
Available online 12 May 2006

Abstract—To explore the synthetic potential of nitrilase ZmNIT2 from maize, the substrate specificity of this nitrilase was studied with a diverse collection of nitriles. The nitrilase ZmNIT2 showed high activity for all the tested nitriles except benzonitrile, producing both acids and amides. For the hydrolysis of aliphatic, aromatic nitriles, phenylacetonitrile derivatives and dinitriles, carboxylic acids were the major products. Unexpectedly, amides were found to be the major products in nitrilase ZmNIT2-catalyzed hydrolysis of β-hydroxy nitriles. The hydrogen bonding between the hydroxyl group and nitrogen in the enzyme–substrate complex intermediates that disfavors the loss of ammonia and formation of acyl–enzyme intermediate, which was further hydrolyzed to acid, was proposed to be responsible for the unprecedented β-hydroxy functionality assisted high yield of amide formation.

1. Introduction

Biocatalysts have attracted much attention in environmental science, synthetic organic chemistry, microbiology, and biotechnology. Hydrolytic biocatalysts are becoming optimal tools for the synthesis of pharmaceutically and biologically important molecules.[1] In particular, among hydrolytic biocatalysts, the nitrilase superfamily enzymes have evoked substantial commercial interest because of their very high synthetic potential.[2–4] Two important enzymes of this superfamily are nitrilase (EC 3.5.5.1) and nitrile hydratase (EC 4.2.1.84). Nitrilases catalyze hydrolysis of nitriles to their corresponding carboxylic acids and ammonia, while nitrile hydratases hydrolyze nitriles to amides.[5] These enzymes are highly demanding biocatalysts in drug synthesis, biodegradation of nitrile wastes, and agricultural development.[6,7] The chemical industry has considerable interest in the utilization of nitrilases for the chemo-, regio-, or enantioselective production of carboxylic acids from nitriles under mild conditions,[8–13] because chemical hydrolysis of nitriles usually requires strong basic or acidic conditions and elevated reaction temperature, that often results in the undesirable side reactions. For example, elimination of the OH group occurs in the chemical hydrolysis of β-hydroxy nitriles to yield unsaturated by-products.[14] However, the total synthetic applicability of these biocatalysts has not been fully achieved because of the paucity of available enzymes.[7,15]

Several nitrilases have been characterized from plants and microorganisms.[16–22] Recently, Glawischnig et al. cloned and characterized a nitrilase ZmNIT2 from maize (*Zea mays*), and demonstrated that it was involved in the biosynthesis of a plant hormone auxin.[23] This nitrilase was reported to convert indole-3-acetonitrile to indole-3-acetic acid at least 7–20 times more efficiently than AtNIT1/2/3 from *Arabidopsis thaliana*. The nitrilase ZmNIT2 also showed no substrate inhibition for indole-3-acetonitrile that was observed for nitrilases from *A. thaliana*.[19] This characteristic property is particularly important for enzymes to be used as an efficient catalyst in organic synthesis, because useful processes usually require high concentration of reactants to achieve optimal space productivity. These unique features stimulated us to explore the synthetic application potential of the nitrilase ZmNIT2 from maize. Therefore, we studied the substrate specificity of this nitrilase with a collection of nitriles with structural diversity. The nitrilase ZmNIT2 was found to be active toward aliphatic nitriles, phenylacetonitrile derivatives, and aromatic nitriles. In these cases, acids were obtained as the major products with concomitant formation of amides. On the contrary, nitrilase ZmNIT2 catalyzed the hydrolysis of β-hydroxy nitriles, producing amides as the major products instead of acids.

2. Results and discussion

Nitrilase ZmNIT2 gene from maize was over-expressed in *Escherichia coli* and the encoded protein was purified

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doi:10.1016/j.tet.2006.04.069

from cell-free extract by following the literature method.[23] This His-tagged enzyme was stable during storage under different conditions. For example, the lyophilized enzyme was stable for months at 4 °C. Ninety percent of the initial activity was recovered after being stored in potassium phosphate buffer (pH 7.2, 10 mM) with 50% glycerol at −20 °C for a month.

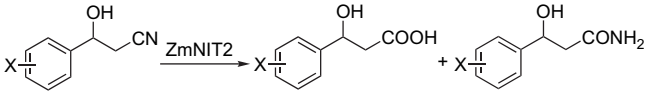
The substrate specificity of this nitrilase was studied by treating nitrile substrates with the purified enzyme in potassium phosphate buffer as described in Section 4. The products were analyzed and characterized by GC, HPLC, and NMR spectroscopy. The results are summarized in Tables 1 and 2. It can be seen that nitrilase ZmNIT2-catalyzed hydrolysis of various nitriles with diverse structures to afford the corresponding acids with concomitant amide formation. It was surprising that nitrilase ZmNIT2 efficiently catalyzed the hydrolysis of substituted benzonitrile derivatives and crotonitrile to give acids as the major products, although it showed low activity for benzonitrile (entries 1–4 in Table 1). Benzyl cyanide and its substituted counterparts were also efficiently hydrolyzed to acids with concomitant formation of amides. The amount of amide formed in the reaction was dependent on the substituent on the benzene ring. Racemic mandelonitrile was hydrolyzed by nitrilase ZmNIT2 to enantiopure (*R*)-mandelic acid.

Table 1. Hydrolysis of various nitriles catalyzed by nitrilase ZmNIT2

Entry	Nitrile	Conversion (%)[a]	
		Acid	Amide
1	Benzonitrile	Overall yield <5	
2	4-Hydroxy-3-methoxybenzonitrile	83	17
3	4-Acetylbenzonitrile	68	31
4	Crotonitrile	79	20
5	Benzyl cyanide	90	10
6	3,4-(Methylenedioxy)-phenylacetonitrile	74	26
7	2,4-Dimethoxyphenylacetonitrile	67	33
8	Mandelonitrile	45[b]	<2
9	Allyl cyanide	80	20
10	*n*-Butyronitrile	80	19
11	Valeronitrile	73	26
12	Hexanenitrile	64	36
13	Heptanenitrile	60	40
14	3-Phenylpropionitrile	84	16
15	4-Phenylbutyronitrile	83	17
16	2-Methylthioacetonitrile	76	24
17	1,4-Dicyanobutane	89	10
18	2-Methylglutaronitrile	83	17

[a] The conversion was determined by GC, HPLC, and NMR analysis.
[b] The product was (*R*)-mandelic acid with 100% ee.

Table 2. Hydrolysis of β-hydroxy nitriles catalyzed by nitrilase ZmNIT2



Entry	Substrate	Conversion (%)[a]	
		Acid	Amide
1	4-CH_3	14	85
2	4-F	17	73
3	4-Cl	13	85
4	4-Br	12	88
5	4-OCH_3	37	63
6	4-CH_3CO	27	68

[a] The conversion was determined by HPLC and NMR analysis.

As shown in Table 1, nitrilase ZmNIT2 also efficiently catalyzed the hydrolysis of aliphatic nitriles to the corresponding carboxylic acids as major products. The acid and amide product distribution was affected by the structure of aliphatic nitriles. It appeared that the amount of amide increased as the chain length became longer. Dinitriles were converted to di-acids and di-amides, and no mixed amide–acid was detected. Selective hydrolysis to mono-acid or amide was not achieved. Methylthioacetonitrile was hydrolyzed to methylthioacetic acid and methylthioacetamide.

Nitrilase-catalyzed hydrolysis of β-hydroxy nitriles results in β-hydroxy carboxylic acids, which are important precursors of β-blockers and β-amino alcohols.[14] Although biocatalytic transformation of β-hydroxy nitriles to the corresponding β-hydroxy carboxylic acids and/or amides has been achieved with nitrile hydratase/amidase-based microbial biocatalysts,[24–27] to our best knowledge, the hydrolysis of β-hydroxy nitriles catalyzed by isolated nitrilases has not been reported. Therefore, the activity of nitrilase ZmNIT2 toward a series of β-hydroxy nitriles was examined. In contrast to the nitriles in Table 1, when β-hydroxy nitriles were treated with nitrilase ZmNIT2 under the same condition the major products were amides with the conversion of 63 to 88%, along with acids as minor products (Table 2). Although the hydrolysis of mandelonitrile was highly enantioselective, nitrilase ZmNIT2 showed little enantioselectivity for the hydrolysis of β-hydroxy nitriles (less than 40% ee, data were not shown and the absolute configurations were not determined). This is consistent with the observation in most cases that a chiral carbon atom at the β-position to the reaction center would be recognized with much more difficulty than the one at α-position.[25]

There are several reports on the formation of amides as minor products in nitrilase-catalyzed hydrolysis of nitrile substrates.[15,16,28–31] The unexpected large amount of amides from β-hydroxy nitriles catalyzed by nitrilase ZmNIT2 was worthy to be further investigated. 3-(4-Fluorophenyl)-3-hydroxypropionitrile and 3-(4-methylphenyl)-3-hydroxypropionitrile were used for detailed study. To gain insight on the mechanism of amide formation, reaction mixtures were analyzed at different time intervals and the conversions of acid and amide were determined. The ratios of amide/acid in the hydrolysis of 3-(4-fluorophenyl)-3-hydroxypropionitrile after 2 and 6 h were 4.7 and 4.5, respectively, while those for hydrolysis of 3-(4-methylphenyl)-3-hydroxypropionitrile were 7.1 and 6.5. The results indicated that the ratio of product amide to acid kept fairly constant as the reaction proceeded. The same time independence of amide/acid ratio was observed for the hydrolysis of 3-phenylpropionitrile. The product mixture, which was obtained from the hydrolysis of 3-(4-fluorophenyl)-3-hydroxypropionitrile after 6 h, was incubated with a fresh enzyme overnight. The amide/acid ratio did not show meaningful change. This ruled out the possibility that enzyme deactivation was responsible for the nearly constant amide/acid ratio. The concurrent formation of amide and acid clearly suggested that the acid was not produced from amide as in the

sequential hydrolysis of nitrile catalyzed by nitrile hydratase and amidase. This was further supported by the observation that hexanoamide was not a substrate of nitrilase ZmNIT2.

We also investigated effect of temperature, pH, and solvent on the product distribution of amide and acid. The results are presented in Figures 1–3. From the results it can be seen that temperature, pH, and solvent did not exert significant impact on the product distribution of amide and acid. This is consistent with the reported independence of the amide to acid ratio on reaction conditions.[28]

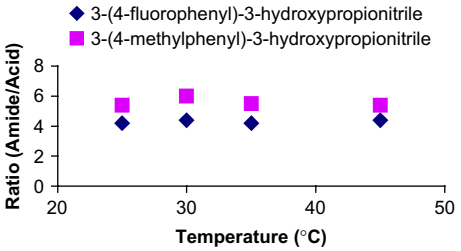


Figure 1. Temperature effect on the product distribution in hydrolysis of β-hydroxy nitriles.

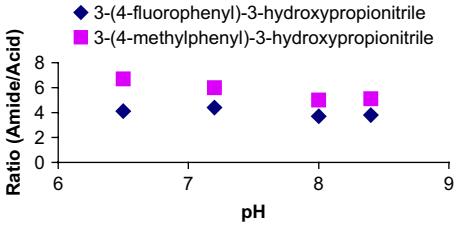


Figure 2. pH effect on the product distribution in hydrolysis of β-hydroxy nitriles.

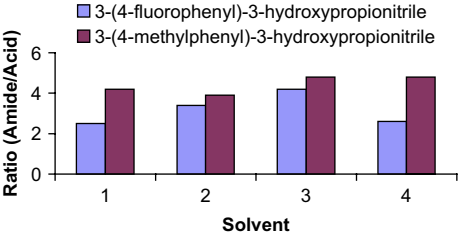
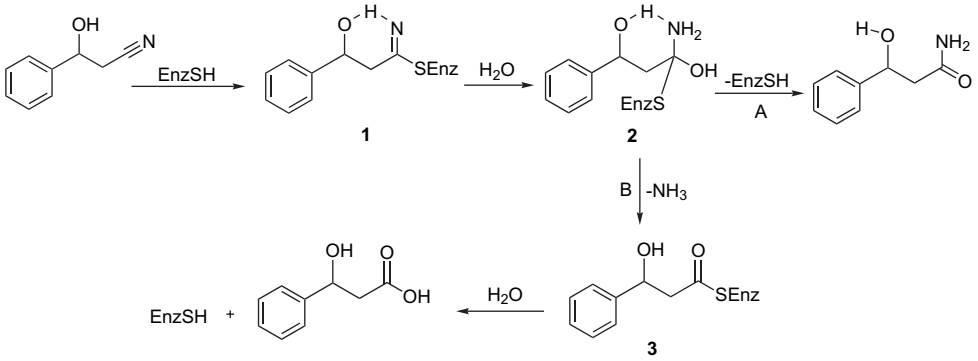


Figure 3. Solvent effect on the product distribution in hydrolysis of β-hydroxy nitriles (1. *t*-BuOH; 2. MeOH; 3. acetonitrile; 4. acetone).

Nitrilase possesses a cysteine residue in its catalytic site and the proposed reaction mechanism for the hydrolysis of nitriles involves the formation of an enzyme–substrate complex where the thiol residue of the enzyme forms a covalent bond with nitrile carbon.[29,32,33] Addition of a H_2O molecule to the complex generates a tetrahedral intermediate. Loss of ammonia from this tetrahedral intermediate gives an acyl–enzyme intermediate, which is further hydrolyzed to produce carboxylic acid and release the enzyme for next catalytic cycle. In a few cases, small amount of amide was also obtained, and it was due to cleavage of the C–S bond in the tetrahedral intermediate, that furnished amide and released enzyme.[1]

Recently, Effenberger et al. have found that amides were major products in the hydrolysis of α-fluoroarylacetonitriles and π-conjugated nitriles with acceptor groups (CN, NO_2) catalyzed by AtNIT1 from *A. thaliana*. The stabilization of the tetrahedral intermediate in the enzyme–substrate complex by these electron-withdrawing groups was proposed to be responsible for the preference of amide formation.[15] Hydrolysis of nitriles catalyzed by nitrilase ZmNIT2 should also involve participation of cysteine thiolate as a nucleophilic entity to form an enzyme–substrate complex.[29,32,33] Hydrogen bond formation is a very common feature in enzyme catalysis, when polar amine group either from substrate or from enzyme residue is in close proximity to hydroxy group from similar environment. It has also been observed that hydrogen bonding could greatly enhance the reaction rate in enzyme catalysis.[34,35] Therefore, when β-hydroxy nitriles were substrates, it was possible that the hydrogen bond between the lone pair of electrons on the nitrogen atom and the β-OH group was formed in the enzyme–substrate complex (**1**) because they were in close proximity, as shown in Scheme 1. Addition of H_2O generated the tetrahedral intermediate (**2**), which had two fates. One was loss of ammonia to form intermediate (**3**), which was further hydrolyzed to carboxylic acid (pathway B). The other one was cleavage of the C–S bond to yield amide (pathway A). These two pathways were competitive and the existence of hydrogen bond in intermediate (**2**) made pathway A favorable over pathway B, because loss of ammonia required extra energy to break down the six-membered cyclic hydrogen bond system. Therefore, amides were the major products in the hydrolysis of β-hydroxy nitriles catalyzed by nitrilase ZmNIT2. To assess the importance of intra-molecular bonding in promoting amide formation, the hydrolysis of



Scheme 1. Proposed hydrogen bonding facilitated amide formation in ZmNIT2-catalyzed hydrolysis of β-hydroxy nitriles.

4-phenylbutyronitrile and 4-hydroxy-4-phenylbutyronitrile was tested. As predicted, the carboxylic acids were the major products with amide/acid ratios being 0.2 for both substrates. Because formation of seven-membered ring was disfavored in this case, γ-hydroxyl groups did not promote the amide formation. This indicated that β-hydroxyl group played a critical role in the formation of large amount of β-hydroxy amides. Since the tetrahedral intermediate (**2**) was stabilized by intra-molecular hydrogen bonding, which was minimally affected by media, the reaction conditions did not have great effect on the product distribution as shown in Figures 1–3.

One more concern was that the tetrahedral intermediate (**2**) in Scheme 1 might be bound in a metal coordinative structure replacing the proton by a metal ion. This structure could be destructed by a strong chelating ligand such as EDTA. Thus addition of EDTA to the reaction mixture should reduce the amide formation. However, the amount of amide was not reduced when the reaction was carried out in the presence of EDTA, indicating metal ion was not involved in ZmNIT2-catalyzed nitrile hydrolysis.

3. Conclusions

Nitrilase ZmNIT2 from maize efficiently catalyzed the hydrolysis of a broad range of nitriles with diverse structures to afford the corresponding carboxylic acids and amides. For most nitriles such as aliphatic, aromatic nitriles, and phenylacetonitrile derivatives, carboxylic acids were produced as the major products. In contrast, nitrilase ZmNIT2 catalyzed the hydrolysis of *β*-hydroxy nitriles to afford amides as the major products. The unprecedented β-hydroxy functionality enhanced amide formation might be due to the hydrogen bonding between the hydroxyl group and nitrogen in the reaction intermediates, that prevented the loss of ammonia and formation of acyl–enzyme intermediate, thus led to the formation of amide as major product. Because the crystal structure of nitrilase ZmNIT2 is not known, how the intramolecular hydrogen bonding in the tetrahedral intermediate (**2**) facilitates the elimination of cysteine to give amide needs to wait for further studies.

4. Experimental

The GC analysis was performed on a Hewlett-Packard 5890 series II plus gas chromatograph. The HPLC analysis was performed on an Agilent 1100 series high-performance liquid chromatography system. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz Bruker AVANCE DRX-400 Multinuclear NMR spectrometer. All the nitriles, amide, and acid standards were purchased from Aldrich or prepared by following the literature procedures.[14,25]

4.1. Expression of nitrilase ZmNIT2 gene in *E. coli*

Plasmid DNA containing nitrilase ZmNIT2 gene (gift from Professor Erich Glawischnig at Technische Universität München, also available from our laboratory)[23] was transformed into BL21(DE3)pLysS *E. coli* strain (Novagen). Overnight pre-cultures were diluted into LB containing 100 μg/ml of ampicillin and 34 μg/ml of chloramphenicol, the cells were induced with 0.1 mM of IPTG when optical density at 595 nm was 0.6. The bacterial cultures were incubated at 30 °C on an orbital shaker at 180 rpm for another 4 h. The cells were harvested.

4.2. Preparation of cell-free extract and purification of nitrilase ZmNIT2 enzyme

The cultures of *E. coli* BL21(DE3)pLysS were harvested by centrifugation. The cell pellet was resuspended in potassium phosphate lysis buffer (10 mM, pH 7.2, 1 mM DTT), and the cell was lysed by homogenizer. The cell-free extract was mixed with equal volume of PEI solution (0.25% polyethyleneimine MW 40–60K, 6% NaCl, 100 mM Borax, pH 7.4) to remove lipids.[36] After centrifugation, the supernatant was precipitated with 30% ammonium sulfate. The resulting precipitate was collected after centrifugation and dissolved in potassium phosphate buffer (10 mM, pH 7.2, 1 mM DTT). The lysate was desalted by gel filtration into potassium phosphate buffer (10 mM, pH 7.2, 1 mM DTT), and lyophilized.

4.3. Hydrolysis of nitriles catalyzed by nitrilase ZmNIT2

A typical experimental procedure was as follows: the nitrile (45 mM) in 1 ml of potassium phosphate buffer (100 mM, pH 7.15) was treated with 1 mg of lyophilized enzyme ZmNIT2. The reaction mixture was shaken for 14 h at 30 °C, and then acidified with a few drops of 1 N HCl solution to adjust pH to 3–4. The products and/or unreacted nitriles were extracted into 1 ml of ethyl acetate. The extract was dried over sodium sulfate and subjected to HPLC or GC analysis to determine the conversion. For GC analysis, the acids were converted to methyl ester derivatives using freshly prepared ethereal solution of diazomethane at 0 °C. For NMR analysis, the solvent was removed from the extract and the residue was dissolved in chloroform-*d* or acetone-d_6. The products were identified by comparing the retention time with authentic sample, or ^{1}H and ^{13}C NMR data with those in the literature.[14,25,37]

Acknowledgements

We thank Professor Erich Glawischnig at Technische Universität München for providing us the plasmid of nitrilase ZmNIT2 gene from maize (*Zea mays*), and Southern Methodist University for start-up support.

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Tetrahedron 62 (2006) 6155–6165

Organocatalyzed route to enantioenriched pipecolic esters: decarboxylation of an aminomalonate hemiester

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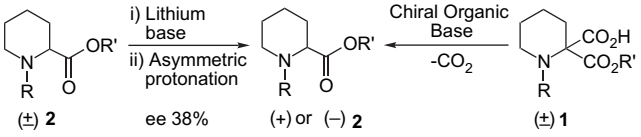
Received 3 April 2006; revised 14 April 2006; accepted 20 April 2006
Available online 8 May 2006

Abstract—Enantioenriched pipecolic esters were prepared in good yields in the decarboxylation, at room temperature, of *N*-protected piperidinohemimalonates catalyzed by cinchona alkaloids. Enantiomeric excesses as high as 72% were obtained when using 9-*epi*-cinchonine and the *N*-benzoyl substituted piperidinohemimalonate. A detailed study of the different reaction parameters revealed that the selectivity of this noncovalent organocatalyzed reaction is strongly dependent on the solvent, toluene or carbon tetrachloride being the best ones. The whole process based on the malonic acid synthesis was successfully tested on a 10 mmolar scale and established a practical alternative to the asymmetric protonation of lithium enolates.

1. Introduction

Organocatalysis,[1] which is the acceleration of chemical reactions with a substoichiometric amount of an organic compound is a well-known concept in living systems since many organic reactions are catalyzed by metal-free enzymes, eventually inducing a high level of asymmetry. In recent years it has been established that much smaller chiral organic molecules are able to achieve comparable performances.[2] Although remarkable results in asymmetric organocatalytic reactions were reported in the sixties and seventies,[3] these works were overlooked until recently, by the prominence of transition-metal-catalyzed asymmetric transformations. Today, due to environmental concerns, there is a need for metal-free chemistry. As a consequence, organocatalysis has emerged as a new tool to efficiently perform organic reactions with high enantioselectivity under very mild and simple conditions.

As part of a program on the synthesis of a selective M_2 muscarinic receptor antagonist,[4] we developed an efficient enantioselective route to pipecolamides (ee's: 95–99%) in order to have access to both enantiomers.[5] It was based on the asymmetric protonation of a lithium enolate. However, attempts to use this methodology to prepare the corresponding esters afforded ee's lower than 40%.[6] Moreover, the deracemization used capricious *sec*-butyl lithium, required low and difficult-to-adjust temperatures, and stoichiometric amounts of the chiral reagent. Therefore, we considered the asymmetric decarboxylation of a malonyl analogue of pipecolate as an organocatalyzed alternative to the chiral protonation of pipecolyl enolates (Scheme 1).



Scheme 1. Asymmetric protonation versus asymmetric decarboxylation.

The first example of an enantioselective decarboxylation was reported by Marckwald in 1904.[7] For more than 70 years this reaction received little attention.[8] In the seventies and eighties, metal-mediated asymmetric decarboxylations were developed. First, stoichiometric amounts of chiral cobalt–amine complexes were shown to induce high level of enantioselectivity in the decarboxylation of α-alkyl-α-aminomalonic acids.[9] In 1987, Maumy used a catalytic combination of copper(I) and cinchonidine to promote the asymmetric decarboxylation of a monoalkyl phenylmalonate hemiester with an ee of 31%.[10] Later, based on Darensbourg's work,[11] Brunner's group[12] and then Hénin–Muzart[13] proved that copper was not necessary, thus developing the first organocatalytic asymmetric decarboxylations.

Keywords: Organocatalysis; Enantioselective decarboxylation; Pipecolate; Cinchona alkaloids.
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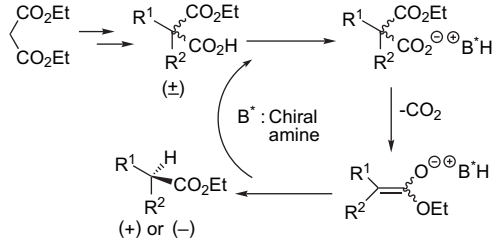
0040–4020/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2006.04.062

Then, Brunner studied the decarboxylation of an α-cyanocarboxylic acid and of 2-aminomalonic acid derivatives with chiral bases. Enantiomeric excesses (ee's) up to 72% were reached.[14] More than 25–30 chiral bases derived mainly from the 9-*epi* configuration of the parent alkaloid were tested. The role of the C9 configuration of the alkaloid was not investigated. Related to this organocatalyzed reaction are the palladium-induced enantioselective debenzylation–decarboxylation.[15] Finally, a decarboxylase, isolated from a bacterium strain, was shown to catalyze the enantioselective decarboxylation of arylalkylmalonic acids with ee's up to 97%.[16] The main drawback of these reagents remains their high degree of specificity for the substrate.[17]

In this paper we described our extensive work on the asymmetric decarboxylation of malonyl pipecolates **1** catalyzed by cinchona alkaloids to synthesize enantiomerically enriched pipecolate ester **2** (Scheme 1). The selectivity of the reaction was studied as a function of different reaction parameters (solvent, concentration, temperature), of the substrate (nitrogen substituent) and of the organic base. We examined also the effect of inversion of configuration at C9 of cinchona derivatives on the catalyst performance.

2. Results and discussion

The malonic acid synthesis is a classical but yet a useful method for the synthesis of α,α′-disubstituted carboxylic acids. The principle of the organocatalyzed asymmetric malonic acid synthesis is depicted in Scheme 2. Sequential alkylations followed by monosaponification of a malonate ester generate a racemic acid–ester, which is subjected to the decarboxylation in the presence of a chiral base.



Scheme 2. Organocatalyzed asymmetric decarboxylation.

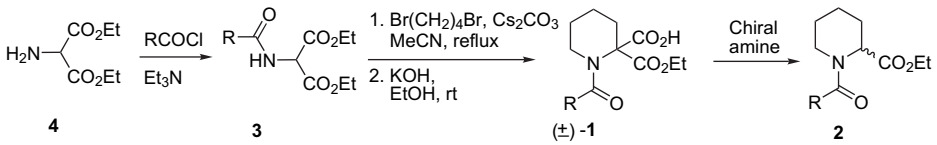
When using an optically pure amine, we expect a rapid deprotonation of the carboxylic acid by the amine resulting in the formation of a diastereoisomeric mixture of salts. Then, in appropriate conditions (solvent, temperature) the unstable carboxylate looses carbon dioxide affording an intermediate enolate, which should be rapidly protonated to generate an enantiomerically enriched α-substituted ester. On the asymmetric point of view, the chirality of the product is introduced at the final step and the whole process resembles the enantioselective protonation of enolates.[18] According to the pK_a of the species involved in this route, a catalytic amount of an enantiomerically pure amine as chiral organic catalyst can be used.[14] Moreover, the protonation step should be an irreversible process under the reaction conditions and the optical activity of the product should not be altered by any of the other species.

2.1. Evaluation of the reaction parameters

Compound **1a** was easily synthesized from commercially available acetamido malonate **3a**. Preliminary investigations showed that triethylamine was able to promote the decarboxylation under simple and mild conditions to generate **2a**. Several chiral amines were screened and cinchona alkaloids were the most efficient bases to induce asymmetry.[19] The study of the different reaction parameters on the enantioselectivity was undertaken with **1b**, easily detected by HPLC, and prepared in a three-step sequence starting from aminomalonate **4** (Scheme 3).

The optically pure pipecolates **2** were prepared independently and fully characterized in order to determine the enantiomeric excess and absolute configuration of the enantiomers formed in asymmetric decarboxylations. In our first experiments, the reactions were conducted in THF, the solvent of choice for the decarboxylations.[14] A quick survey of the four commercially available cinchona alkaloids revealed that cinchonine (**CN**), used in a 1/1 ratio with **1b**, induced the best ee of 33%. Therefore, **CN** was selected as the base for the study of the different reaction parameters on the efficiency and enantioselectivity of the reaction (Table 1).

This first set of data showed that the temperature, the concentration and the amount of base used have no significant influence on the enantioselectivity of the decarboxylation, at least in the ranges studied. The conversion of the reaction was decreased at 0 °C or when a substoichiometric amount of **CN** was used (entries 4 and 7). The effect of the temperature deserves some comments: on one hand, there was no gain of selectivity while cooling down the reaction to 0 °C. Below this temperature, one would expect such a slow rate for the decarboxylation that the reaction would loose its original benefit. On the other hand, heating the mixture to 60 °C did not affect the selectivity while increasing the



4

3

R	Yield (%)
a Me-	-
b Ph-	88
c *p*-MeOC$_6$H$_4$-	81
d *p*-NO$_2$C$_6$H$_4$-	62
e α-Naphyl	91

(±) **-1**

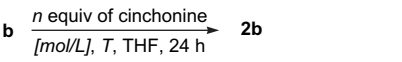
R	Yield (%)
a Me-	65
b Ph-	38
c *p*-MeOC$_6$H$_4$-	45
d *p*-NO$_2$C$_6$H$_4$-	39
e α-Naphyl	23

2

R
a Me-
b Ph-
c *p*-MeOC$_6$H$_4$-
d *p*-NO$_2$C$_6$H$_4$-
e α-Naphyl

Scheme 3. Synthesis of substrates.

Table 1. Decarboxylation of **1b**: ee's as a function of concentration, temperature and the amount of base



Entry	[**1b**] (mol/L)	T (°C)	n	Yield (%)[a]	ee (%)[b]
1	0.01	20	1	88	34
2	0.025	20	1	91	33
3	0.05	20	1	96	34
4	0.05	0	1	70	34
5	0.05	60[c]	1	89	37
6	0.05	20	2	83	36
7	0.05	20	0.5	65	36

[a] Isolated yields.
[b] Determined by chiral HPLC. The (*R*) product was obtained as the major enantiomer.
[c] Complete conversion after 12 h.

reaction rate as already observed by Brunner (entry 5).[14] Therefore the effect of a substoichiometric amount of the alkaloid (entry 7) could be compensated by a slight increase of the temperature to keep the reaction time in a reasonable scale.

The choice of the solvent is an important issue in organocatalysis. Acetonitrile was the solvent used for metal-mediated asymmetric decarboxylations[10,12] and THF was preferred in the few examples of organocatalytic decarboxylations.[14] From Table 1, we selected our conditions for studying the effect of the solvent on the enantioselectivity of the reaction. In order to have a complete reaction in 24 h at room temperature, we chose to use **1b** and **CN** (1 equiv) at a concentration of 0.05 mol/L (Table 2).

A strong influence of the solvent on the enantioselectivity of the decarboxylation was observed. The ee's were moderate in THF, poor in the more polar CH_3CN and very low in ionic liquid ([bmim]PF_6), whereas they reached 61–63% in nonpolar solvents like toluene or CCl_4.[20] Unlike in Brunner's work,[14] even though the reaction partners were only slightly soluble in those solvents, a very good conversion and a good level of selectivity were observed. In the nonpolar solvents cyclohexane and perfluorocyclohexane, both the enantioselectivity and the conversion decreased dramatically (entries 10 and 11). This could be due to the insolubility of the reaction components in the used solvents. However, to confirm the tendency observed in Table 2 for other substrates and amines we decided to pursue the study by carrying out each decarboxylation separately in two different solvents. CCl_4 was the solvent giving here the best selectivity and THF was the solvent, which gave the best results in Brunner's work. At this point, we checked for the possibility of having a deracemization[21] of the *N*-benzoylpipecolate **2b**, after decarboxylation–protonation of compound **1b**. Therefore we mixed racemic product **2b** with a stoichiometric amount of **CN** in CCl_4 and heated the solution at 70 °C for three days. We applied the same conditions to enantiopure product **2b** synthesized independently. In both the cases no change of the ee's occurred.

Table 2. Decarboxylation of **1b**: solvent effect on the ee of **2b**

Entry	Solvent	υ[a]	ε[b]	Yield (%)[c]	ee (%)[d]
1	[bmim]PF_6	>5	30–40	88	17
2	CH_3CN	3.9	37.5	91	21
3	THF	1.7	7.5	96	33
4	CH_2Cl_2	1.6	9.0	70	46
5	$CHCl_3$	1.2	4.8	89	45
6	Et_2O	1.1	4.3	85	52
7	*Toluene*	*0.4*	*2.4*	*91*	*61*
8	1,4-Dioxane	0	2.2	87	43
9	*CCl_4*	*0*	*2.2*	*92*	*63*
10	Cyclohexane	0	2.0	62	45
11	C_6F_{12}	0	—	61	19

[a] υ: Dipolar moment.
[b] ε: Dielectric constant.
[c] Isolated yield.
[d] Determined by chiral HPLC. The (*R*) product was obtained as the major enantiomer.

2.2. Screening cinchona alkaloid analogues on the decarboxylation of malonate hemiesters 1

In a first step towards the optimization of the selectivity, we synthesized various substrates and bases. Concerning the starting hemiesters **1**, we focused on the piperidine protected by an aroyl group starting from the initial observation that a benzoyl group clearly improved the selectivity compared to an acetyl group.[22] Precursors **1c–e** (Scheme 3) bearing *para*-methoxy, *para*-nitrobenzoyl or α-naphtoyl substituent on the piperidine nitrogen atom were thus prepared. The cinchona alkaloid structure was kept as a common scaffold for the bases, changing only the nature and the configuration of the functional group at C9. All used chiral bases are presented in Figure 1 and were either synthesized according to literature procedures[14,23] or by using classical reactions. All compounds derived from quinidine (**QD**) and cinchonine (**CN**), which have the same configurations at C8 and C9 (8*R*, 9*S*) and for some of these, we also prepared the C9 epimer [along this article '*epi*' refers to the configuration C9 (*R*)]. These bases bear at C9 different functional groups (alcohol: '**QD**' and '**CN**', ether: '**OMe**', benzamide: '**Amide**', phenylcarbamate: '**PhCarb**' or *tert*-butylcarbamate: '***t*-BuCarb**'). We also tested bis-alkaloids **(DHQD)$_2$PYR**, **(DHQD)$_2$AQN**, **(CN)$_2$PYR** and cyclic ethers ('**Cyclic**').

Because of the crucial role of the solvent, the decarboxylation of the *N*-acetyl derivative **1a** with the whole set of bases was reinvestigated in several solvents. The two most significant results were obtained in Et_2O by using bases **QD** and **QD–Amide** with ee's, respectively, of 36% and 33%. With the other bases or solvents, the enantioselectivity of the reaction was consistently lower than 30%, not sufficient to draw any conclusions regarding the relation structure–enantioselectivity of the base.[24]

The main results of the decarboxylation, in carbon tetrachloride or in THF, of *N*-benzoylated substrate **1b** in the presence of some of the bases shown in Figure 1, are summarized in Table 3. They show the dramatic influence of the methoxy group on the quinoline ring (i.e. quinidine or cinchonine as the parent alkaloids). The ee's in Table 3, which were obtained with six different bases derived from quinidine **QD** (entries 1–3, 5 and 6), are low with the exception of the one obtained with the bis-alkaloid **(DHQD)$_2$PYR** (entry 4). The decrease of the selectivity is intriguing considering the remote distance on the base of the methoxy group from the nitrogen of the quinuclidine ring. The ee's obtained with bases derived from cinchonine are much higher, reaching 72%

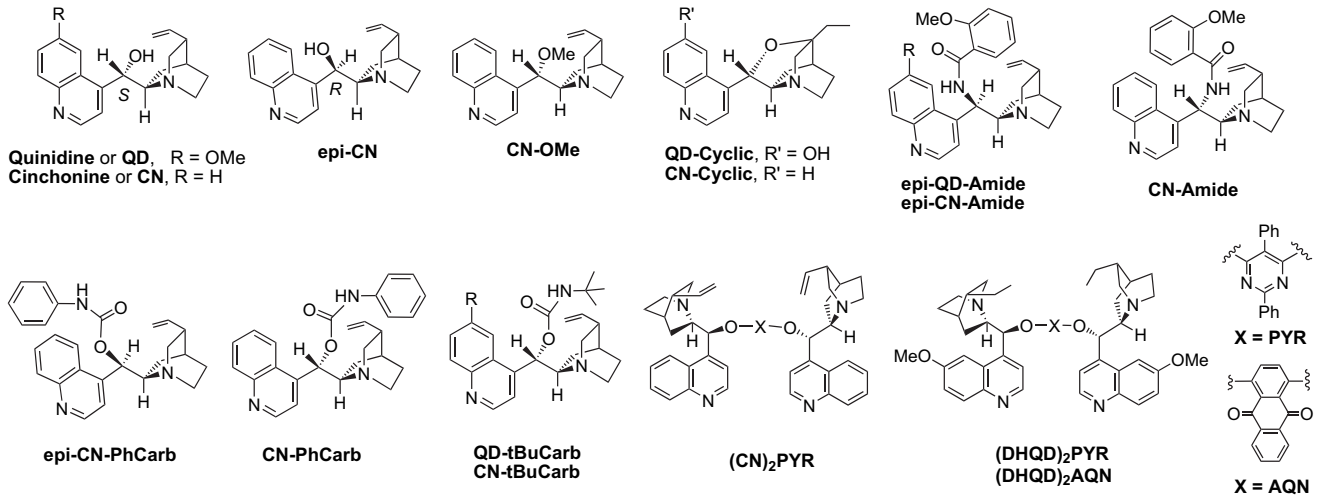


Figure 1. Cinchona derivatives used as bases.

Table 3. Decarboxylation of **1b** with cinchonine and quinidine type alkaloids[a]

Entry	Base	C_9	ee % (configuration)[c]	
			CCl_4	THF
1	**QD**	*S*	18 (*S*)	18 (*R*)
2	*epi*-**QD–Amide**[b]	*R*	9 (*S*)	0
3	**QD**–*t*-**BuCarb**	*S*	7 (*S*)	—
4	**(DHQD)$_2$PYR**	*S*	*64* (*S*)	50 (*S*)
5	**(DHQD)$_2$AQN**	*S*	9 (*S*)	—
6	**QD–Cyclic**	*S*	—	2 (*R*)
7	**CN**	*S*	*64* (*R*)	33 (*R*)
8	*epi*-**CN**	*R*	*72* (*R*)	*66* (*R*)
9	**CN**–*t*-**BuCarb**	*S*	50 (*S*)	39 (*S*)
10	**CN–PhCarb**	*S*	17 (*R*)	6 (*R*)
11	*epi*-**CN–Amide**[b]	*R*	42 (*S*)	42 (*S*)
12	**CN–Amide**[b]	*S*	9 (*S*)	—
13	**(CN)$_2$PYR**	*S*	*63* (*S*)	—
14	**CN–OMe**	*S*	10 (*R*)	—
15	**CN–Cyclic**	*S*	11 (*S*)	19 (*S*)

[a] Reaction conditions: 0.1 mmol of **1b** and 0.1 mmol of the base were left at room temperature for 24 h.
[b] Reactions were carried out for three days.
[c] Absolute configuration of the major enantiomer. ee's were measured by chiral HPLC.

with *epi*-**CN** in CCl_4 (entry 8). However, the correlation between the selectivity, the absolute configuration of the major enantiomer and the configuration of the base at C9 deserves some preliminary comments.

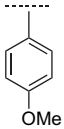
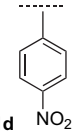
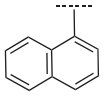
For example, **CN** and *epi*-**CN** induced in CCl_4 similar ee's (entries 7 and 8) and the same enantioselectivity, in favour of the *R* product. However, *epi*-**CN–Amide** gave a reasonable enantioselectivity in favour of the *S* product but opposite to that of *epi*-**CN** (entries 7 and 11). Another surprising result was the comparison between **CN**–*t*-**BuCarb** favouring the *S* product whereas **CN**, having the same absolute configuration, generated mainly the *R* product (entries 9 and 7). More generally, a simple relation between the absolute configuration of the base at C9 and the major enantiomer obtained cannot be established, even when considering only significant results (above 40–50% ee).

Noteworthy is the comparison between the base developed by Brunner, namely (9*R*)-*epi*-**CN–Amide**, and its epimeric analogue (9*S*)-**CN–Amide**: when having an amide group on the C9 of the base, only the 9*R* configuration (*epi*-**CN–Amide**) is able to induce a good level of selectivity, a point not previously addressed. Interestingly, bis-alkaloids derived from **QD** or **CN** (same configuration at C9) with a diphenylpyrimidine linker, **(DHQD)$_2$PYR** or **(CN)$_2$PYR**, were able to induce the same and good enantioselectivity but opposite to that of **CN** (entries 4 and 13). We did not observe the same deleterious effect on the selectivity from the methoxy group on the quinoline moiety as mentioned previously with monoalkaloids since the value of the ee obtained for pipecolate **2b** with **(DHQD)$_2$PYR** or **(CN)$_2$PYR** is identical to the one obtained with **CN** in CCl_4. Finally, the decarboxylations catalyzed by ether derivatives of **CN**, **CN–Cyclic** or **CN–OMe**, gave nearly racemic mixtures of **2b**. These results point out the role of the hydroxyl group of **CN** in the overall chiral induction process. From all the data presented in Table 3, one can conclude that the highest enantioselectivity can be reached when the piperidine was protected with a benzoyl group (compared to an acetyl) and when there is no methoxy group on the quinoline ring of the base.

We pursued the study by varying the electronic nature and the size of the aromatic ring on the piperidine substituent to strengthen the π-stacking interactions. Compounds **1c**–**1e** were tested with the **CN** derivatives (Table 4).

The ee's obtained with the *N*-(4-methoxy)-benzoyl derivative **1c** were slightly lower than that of **1b** (Table 4, entries 1–6). The same tendency was also observed with **1e** having a naphtoyl substituent (Table 4, entries 13–15), and even lower selectivity were obtained in these reactions particularly with the bis-alkaloid **(DHQD)$_2$PYR**. Surprising results came from substrate **1d** bearing a nitro substituent on the benzoyl moiety. ee's higher than 60% were achieved for pipecolate **2d** by using *epi*-bases (Table 4, entries 8, 10 and 11) in CCl_4, whereas **CN** and **(DHQD)$_2$PYR** were not able to induce noticeable ee's. The major problem associated with **1d** was its low stability since it underwent partial spontaneous decarboxylation even when stored below 10 °C. This made its preparation difficult and the results were on the selectivities not easy to explain as compared with the other *N*-aroylated substrates.[25] It should be emphasized that the

Table 4. Decarboxylation of **1c–1e** with cinchonine type alkaloids[a]

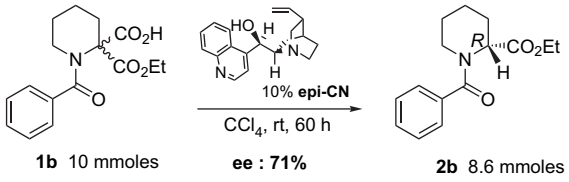
Entry	Starting material R	Base	C_9	ee % (configuration)[c]	
				CCl_4	THF
1		**CN**	*S*	51 (*R*)	48 (*R*)
2		*epi*-**CN**	*R*	*67* (*R*)	*69* (*R*)
3		**CN–*t*-BuCarb**	*S*	41 (*S*)	43 (*S*)
4		**CN–PhCarb**	*S*	9 (*S*)	7 (*S*)
5		*epi*-**CN–Amide**[b]	*R*	36 (*S*)	33 (*S*)
6	**1c**	**(DHQD)$_2$PYR**	*S*	55 (*S*)	35 (*S*)
7		**CN**	*S*	13 (*R*)	11 (*R*)
8		*epi*-**CN**	*R*	*63* (*R*)	20 (*R*)
9		**CN–*t*-BuCarb**	*S*	50 (*S*)	3 (*S*)
10		*epi*-**CN–PhCarb**	*R*	*65* (*S*)	—
11		*epi*-**CN–Amide**[b]	*R*	*67* (*S*)	30 (*S*)
12	**1d**	**(DHQD)$_2$PYR**	*S*	9 (*S*)	—
13		**CN**	*S*	59 (*R*)	45 (*R*)
14		*epi*-**CN**	*R*	47 (*R*)	51 (*R*)
15		**(DHQD)$_2$PYR**	*S*	37 (*S*)	11 (*S*)
	1e				

[a] Reaction conditions: 0.1 mmol of **1b** and 0.1 mmol of the base were left at room temperature for 24 h.
[b] Reactions were carried out for three days.
[c] Absolute configuration of the major enantiomer. ee's were measured by chiral HPLC.

'*epi*' configuration gave better enantioselectivities with equal functional group (**CN** vs *epi*-**CN**, **CN–Amide** vs *epi*-**CN–Amide** and **CN–PhCarb** vs *epi*-**CN–PhCarb**). Improvement of the enantioselectivity was clearly observed for **1d** when replacing THF by CCl_4 (Table 4, entries 8, 9 and 11), whereas no sharp differences were obtained between these solvents with other substrates, for example, the reactions of **1b** with *epi*-**CN** and *epi*-**CN–Amide** (Table 3, entries 8 and 11). However, the best results were generally obtained in carbon tetrachloride.

2.3. Organocatalysis

At this point of the study, our main initial goal was to develop catalytic conditions that could be used on larger amounts of starting material. Preliminary results showed that a substoichiometric amount (50%) of the base was able to promote the asymmetric decarboxylation of our substrate without altering the selectivity (Table 1, entry 7). Hemimalonate **1b** gave consistently the highest ee's with several bases. *epi*-**CN** was also the most efficient base to promote the asymmetric decarboxylation. Having in hands the conditions (base, solvent, substrate) to reach a reasonable control of the enantioselectivity, we demonstrated the possibility of carrying out the reaction under a catalytic amount of chiral base (10%) and on a 10 mmol scale (Scheme 4).



Scheme 4. Preparative organocatalyzed decarboxylation.

The conditions giving the best and most reproducible results (ee: 70–72%) were thus used for the organocatalytic experiment. A mixture of 10 mmol of piperidine **1b** and 10% mol of *epi*-**CN** in CCl_4 stirred at room temperature for 60 h afforded pipecolate **2b** in 86% isolated yield and 71% ee. This result demonstrated that organocatalysis was a well suited methodology for asymmetric decarboxylations and illustrated the potential of this reaction on a more practical scale. This reaction was carried out under very simple and mild conditions. Moreover, the possibility of recovering the catalyst by an acid–base treatment during the work-up is a real plus.

3. Conclusions

The malonic acid synthesis has been largely exploited for the preparation of carboxylic acid derivatives before the advent of selective and powerful methodologies using metal enolates. In its asymmetric organocatalytic version, it was used recently as an alternative route to α-cyano substituted derivatives and linear α-amino acids. The results presented in this work widen the scope of this reaction for the preparation of enantioenriched pipecolic esters derivatives with ee's higher than 70%, which stand as some of the best enantioselectivities obtained for enantioselective decarboxylation. Moreover, the reaction was tested with the same efficiency on a multigram scale. The importance of key parameters on the selectivity, the low polarity of the solvent and the aromatic nature of the nitrogen substituent of the substrate were demonstrated. The temperature was shown to influence only the rate of the decarboxylation and not the enantioselection. A full set of quinidine–cinchonine type of base was prepared. A clear trend showed that the cinchonine analogues (vs quinidine derivatives) were the best catalysts. A steric repulsion between the methoxy group of the quinoline and our substrate was probably occurring with the quinidine family. This study highlighted the little studied effect (compared to other reactions using cinchona alkaloid catalysts) of the configuration at C9 of the base. In general for a given chemical group, the '*epi*' configuration afforded better enantioselectivities and the alcohol group, i.e. *epi*-**CN** is, so far, the best functionality tested. On the asymmetric perspective, this reaction should be viewed as an organocatalyzed enantioselective protonation performed under very simple metal-free conditions, at room temperature and using a catalytic amount of the chiral reagent. Our current efforts are focusing on preparing novel cinchonine derivatives that will provide improved enantioselectivities for the asymmetric decarboxylation of malonyl compounds. Furthermore, this study performed on a model compound, will be generalized to other malonyl derivatives to extend the scope of the methodology.

4. Experimental

4.1. General

Solvents (THF, CH_2Cl_2, MeCN, Et_2O) were dried and purified from Pure-Solv™ 400 Solvent Purification System. CCl_4 was distilled on P_2O_5 and stored over $CaCl_2$. Triethylamine (NEt_3), toluene, $CHCl_3$ and 1,4-dioxane were distilled from CaH_2. All commercially available compounds were used as received. Thin layer chromatography was performed on silica gel 60 F-254 plates (0.1 mm,

Merck) with iodine and/or UV detection. Chromatographic separations were achieved on silica gel columns (Kieselgel 60, 40–63 μm, Merck). Analytical high performance liquid chromatography (HPLC) was carried out with a Waters instrument [detector M996 (200–400 nm) and pump 600]. All NMR spectra were recorded on a Bruker Avance DPX 250 instrument (250 MHz ^{1}H, 62 MHz ^{13}C) using $CDCl_3$ and TMS as solvent and reference, respectively. Chemical shifts (δ) are given in parts per million and coupling constants (J) in hertz. Mass and high resolution mass spectra (HRMS) were obtained on a Waters-Micromass Q-Tof micro instrument. IR spectra were recorded on a Perkin–Elmer 16 PC FTIR spectrometer. Optical rotations were measured on a Perkin–Elmer 241 LC polarimeter in a 10 cm cell. $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2/g. Analytical data were performed with a Thermoquest NA 2500 instrument. Melting points were determined on a Gallenkamp apparatus and are uncorrected.

4.2. General procedure for the acylation of diethyl aminomalonate (4)

To a stirred solution of diethyl aminomalonate **4** (2.2 g, 10 mmol, 1 equiv) and NEt_3 (4.2 mL, 30 mmol, 3 equiv) in CH_2Cl_2 (150 ml) was added at 0 °C acid chloride (10 mmol, 1 equiv). After stirring 15 h at room temperature, the reaction mixture was diluted with CH_2Cl_2, washed with HCl (1 N) and extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure.

4.2.1. 2-(Benzoylamino)propanedioic acid diethyl ester (3b). Purification of the crude product by recrystallization from AcOEt/heptane (1/10) afforded **3b** as a white solid in 88% yield (2.3 g from 2 g aminomalonate **4**): mp 63 °C; ^{1}H NMR δ 1.32 (t, J=7.1 Hz, 6H), 4.2–4.4 (m, 4H), 5.35 (d, J=7.0 Hz, 1H), 7.10 (br d, J=7.0 Hz, 1H), 7.4–7.5 (m, 3H), 7.8–7.9 (m, 2H); ^{13}C NMR δ 14.1 (CH_3), 56.9 (CH), 62.8 (CH_2), 127.4 (CH), 128.7 (CH), 132.2 (CH), 133.1 (C), 166.5 (C), 166.9 (C); IR (KBr) 3430, 2990, 1740, 1666, 1513, 1022 cm^{-1}; MS (EI) m/z (%) 280 (M$^+$, 3), 262 (2), 234 (8), 207 (12), 189 (9), 161 (14), 105 (100), 77 (25); Anal. Calcd for $C_{14}H_{17}NO_5$: C, 60.21; H, 6.14; N, 5.02. Found: C, 60.09; H, 6.28; N, 5.12.

4.2.2. 2-(4-Methoxybenzoylamino)propanedioic acid diethyl ester (3c). Purification of the crude product by flash chromatography on silica gel (CH_2Cl_2/AcOEt, 90/10) afforded amide **3c** as a white solid in 81% yield (7.5 g from 5.4 g aminomalonate **4**): mp 106 °C; ^{1}H NMR δ 1.32 (t, J=7.5 Hz, 6H), 3.85 (s, 3H), 4.2–4.4 (m, 4H), 5.35 (d, J=7.0 Hz, 1H), 6.94 (d, J=8.5 Hz, 2H), 7.05 (br d, J=7.0 Hz, 1H), 7.82 (d, J=8.5 Hz, 2H); ^{13}C NMR δ 14.1 (CH_3), 55.5 (CH_3), 56.9 (CH), 62.7 (CH_2), 113.9 (CH), 125.4 (C), 129.3 (CH), 162.8 (C), 166.3 (C), 166.6 (C); IR (KBr) 3432, 1780, 1662, 1492, 1264 cm^{-1}; MS (EI) m/z (%) 309 (M$^+$, 14), 264 (6), 237 (2), 219 (2), 191 (5), 135 (100), 107 (6), 77 (10); Anal. Calcd for $C_{15}H_{19}NO_6$: C, 58.25; H, 6.19; N, 4.53. Found: C, 58.11; H, 6.28; N, 4.59.

4.2.3. 2-(4-Nitrobenzoylamino)propanedioic acid diethyl ester (3d). Purification of the crude product by recrystallization from AcOEt/heptane (1/10) afforded **3d** as a white solid in 62% yield (4.0 g from 5.3 g of aminomalonate **4**): mp 138 °C; ^{1}H NMR δ 1.35 (t, J=7.2 Hz, 6H), 4.2–4.4 (m, 4H), 5.34 (d, J=6.7 Hz, 1H), 7.26 (br d, J=7.2 Hz, 1H), 8.04 (d, J=8.5 Hz, 2H), 8.34 (d, J=8.5 Hz, 2H); ^{13}C NMR δ 14.4 (CH_3), 57.3 (CH), 63.3 (CH_2), 124.2 (CH), 128.9 (CH), 138.9 (C), 150.4 (C), 165.2 (C), 166.4 (C); IR (KBr) 3053, 2985, 1755, 1740, 1677, 1529, 1264 cm^{-1}; MS (EI) m/z (%) 325 (M$^+$, 6), 279 (8), 251 (26), 206 (32), 150 (100), 134 (5), 120 (9), 104 (15), 92 (13); HRMS (EI) calcd for $C_{14}H_{16}N_2O_7$ 325.1036, found 325.1027.

4.2.4. 2-(α-Naphtoylamino)propanedioic acid diethyl ester (3e). Purification of the crude product by recrystallization (CH_2Cl_2/AcOEt: 10/1) afforded **3e** as a white solid in 91% yield (3.0 g from 2.65 g aminomalonate **4**): mp 72 °C; ^{1}H NMR δ 1.35 (t, J=7.0 Hz, 6H), 4.2–4.4 (m, 4H), 5.47 (d, J=7.0 Hz, 1H), 7.04 (br d, J=7.0 Hz, 1H), 7.4–7.6 (m, 3H), 7.73 (d, J=7.2 Hz, 1H), 7.8–8.0 (m, 2H), 8.4 (d, J=8.0 Hz, 1H); ^{13}C NMR δ 14.4 (CH_3), 57.3 (CH), 63.2 (CH_2), 125.0 (CH), 125.7 (CH), 126.1 (CH), 126.9 (CH), 127.7 (CH), 128.7 (CH), 130.6 (C), 131.6 (CH), 133.2 (C), 134.1 (C), 166.9 (C), 169.4 (C); IR (neat) 3266, 2982, 1753, 1737, 1638, 1524, 1349, 1282, 1236, 1157, 781 cm^{-1}; MS (EI) m/z (%) 329 (M$^+$, 27), 284 (7), 237 (7), 211 (3), 155 (100), 127 (29); HRMS (ESI) calcd for $C_{18}H_{20}NO_5$ [M+H]$^+$ 330.1341, found 330.1339.

4.3. Typical procedure for the piperidine ring construction

To a stirred solution of 1,4-dibromobutane (2.5 mL, 21 mmol, 1.05 equiv) and Cs_2CO_3 (15.0 g, 46 mmol, 2.3 equiv) in MeCN (425 mL) was added acylaminomalonic acid diethyl ester (**3**) (20 mmol, 1.0 equiv) in MeCN (20 mL) over 15 h at 70 °C. After 24 h stirring at the same temperature, the reaction mixture was cooled to room temperature and concentrated under reduced pressure. Water (200 mL) was added and the mixture was extracted with CH_2Cl_2 (5×100 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure.

4.3.1. 1-Acetyl-piperidine-2,2-dicarboxylic acid diethyl ester. Purification of the crude product by bulb-to-bulb distillation (200 °C, 0.02 mbar) afforded the title compound (2.25 g from 2.15 g of commercially available starting material **3a**; 84%) as a clear oil; ^{1}H NMR δ 1.29 (t, J=7.0 Hz, 3H), 1.4–1.5 (m, 2H), 1.6–1.7 (m, 2H), 2.03 (s, 3H), 2.2–2.3 (m, 2H), 3.3–3.4 (m, 2H), 4.2–4.3 (m, 4H); ^{13}C NMR δ 14.3 (CH_3), 20.7 (CH_2), 22.6 (CH_3), 24.4 (CH_2), 32.7 (CH_2), 45.4 (CH_2), 62.1 (CH_2), 69.0 (C), 168.6 (C), 173.2 (C); IR (NaCl) 2980, 2944, 2866, 1734, 1668, 1446, 1272 cm^{-1}; MS (EI) m/z (%) 272 (M$^+$+1, 30), 230 (10), 226 (100); HRMS (ESI) calcd for $C_{13}H_{21}NO_5Na$ [M+Na]$^+$ 294.1317, found 294.1320.

4.3.2. 1-Benzoylpiperidine-2,2-dicarboxylic acid diethyl ester. Purification of the crude product by flash chromatography on silica gel (CH_2Cl_2/AcOEt: 97/3) afforded the title compound as a clear oil in 63% yield (6.0 g from 8.0 g of starting material **3b**). ^{1}H NMR δ 1.32 (t, J=7.0 Hz, 6H), 1.60 (br s, 4H), 2.3–2.4 (m, 2H), 3.3–3.4 (m, 2H), 4.2–4.4 (m, 4H), 7.3–7.5 (m, 5H); ^{13}C NMR δ 14.0 (CH_3), 21.0 (CH_2), 24.1 (CH_2), 32.3 (CH_2), 47.0 (CH_2), 61.9 (CH_2), 69.0 (C), 127.0 (CH), 128.4 (CH), 129.9 (CH), 135.7 (C),

168.1 (C), 173.2 (C); IR (NaCl) 3408, 2981, 1733, 1651, 1390, 1230 cm^{-1}; MS (EI) *m/z* (%): 333 (M^+, 6), 260 (71), 228 (5), 105 (100), 77 (24); HRMS (ESI) calcd for $C_{18}H_{24}NO_5$ $[M+H]^+$ 334.1654, found 334.1654.

4.3.3. 1-(4-Methoxybenzoyl)piperidine-2,2-dicarboxylic acid diethyl ester. Purification of the crude product by flash chromatography on silica gel (CH_2Cl_2/AcOEt: 50/50) afforded the title compound as a clear oil in 63% yield (1.5 g from 2 g of starting material **3c**). ^1H NMR δ 1.30 (t, J=7.2 Hz, 6H), 1.61 (br s, 4H), 2.3–2.4 (m, 2H), 3.3–3.4 (m, 2H), 3.83 (s, 3H), 4.2–4.4 (m, 4H), 6.91 (d, J=8.6 Hz, 2H), 7.48 (d, J=8.6 Hz, 2H); ^{13}C NMR δ 14.4 (CH_3), 21.6 (CH_2), 24.7 (CH_2), 32.8 (CH_2), 47.6 (CH_2), 55.8 (CH_3), 62.2 (CH_2), 69.5 (C), 114.2 (CH), 128.2 (C), 129.5 (CH), 161.4 (C), 168.6 (C), 173.5 (C); IR (NaCl) 3447, 2940, 1731, 1576, 1512, 1420, 1003, 840 cm^{-1}; MS (EI) *m/z* (%) 363 (M^+, 4), 318 (5), 290 (35), 228 (4), 135 (100), 107 (6), 92 (4), 77 (10); HRMS (ESI) calcd for $C_{19}H_{26}NO_6$ $[M+H]^+$ 364.1760, found 364.1760.

4.3.4. 1-(4-Nitrobenzoyl)piperidine-2,2-dicarboxylic acid diethyl ester. Purification of the crude product by flash chromatography on silica gel (CH_2Cl_2/AcOEt: 10/0.4) afforded the title compound as a white solid in 60% yield (1.4 g from 2 g of starting material **3d**); mp 128 °C; ^1H NMR δ 1.34 (t, J=7.2 Hz, 6H), 1.5–1.7 (m, 4H), 2.3–2.4 (m, 2H), 3.2–3.3 (m, 2H), 4.2–4.4 (m, 4H), 7.68 (d, J=8.7 Hz, 2H), 8.29 (d, J=8.7 Hz, 2H); ^{13}C NMR δ 14.4 (CH_3), 21.1 (CH_2), 24.3 (CH_2), 32.6 (CH_2), 47.3 (CH_2), 62.5 (CH_2), 69.5 (C), 124.2 (CH), 128.3 (CH), 142.4 (C), 148.8 (C), 168.1 (C), 171.5 (C); IR (KBr) 2950, 2253, 1731, 1654, 1525, 1348, 1230, 912 cm^{-1}; MS (EI) *m/z* (%) 378 (M^+, 4), 333 (4), 305 (100), 259 (5), 150 (52), 120 (6), 104 (9); HRMS (ESI) calcd for $C_{18}H_{23}N_2O_7$ $[M+H]^+$ 379.1505, found 379.1519.

4.3.5. 1-(1-Naphtoyl)piperidine-2,2-dicarboxylic acid diethyl ester. Purification of the crude product by flash chromatography on silica gel (CH_2Cl_2 100% then CH_2Cl_2/AcOEt: 97/3) afforded the title compound as a white solid in 31% yield (0.5 g from 1.4 g of starting material **3e**): mp 105 °C; ^1H NMR δ 1.38 (q, J=7 Hz, 6H), 1.4–1.6 (m, 4H), 2.3–2.5 (m, 2H), 3.1–3.2 (m, 2H), 4.3–4.4 (m, 4H), 7.4–7.6 (m, 4H), 7.8–7.9 (m, 2H), 8.1–8.2 (m, 1H); ^{13}C NMR δ 14.6 (CH_3), 21.2 (CH_2), 24.6 (CH_2), 32.9 (CH_2), 46.7 (CH_2), 62.6 (CH_2), 69.2 (C), 124.4 (CH), 125.5 (CH), 125.6 (CH), 126.9 (CH), 127.5 (CH), 128.6 (CH), 129.7 (CH), 130.1(C), 133.7 (C), 134.5 (C), 168.5 (C), 168.9 (C), 172.9 (C); IR (NaCl) 2982, 2949, 2252, 1732, 1650, 1380, 1237, 910, 740 cm^{-1}; MS (EI) *m/z* (%) 383 (M^+, 11), 337 (10), 310 (39), 236 (6), 155 (100), 127 (24); HRMS (ESI) calcd for $C_{22}H_{25}NO_5$ $[M+Na]^+$ 406.1630, found 406.1637.

4.4. General procedure for preparation of acid

KOH (5–15 equiv) was added to the *N*-protected piperidine-2,2-dicarboxylic acid diester (12.4 mmol) in EtOH (10 mL). The reaction mixture was stirred at room temperature for a time given in each case. Around 70–80% of EtOH were removed under vacuum at a temperature below 20 °C (*caution: in order to avoid degradation, EtOH should not be completely evaporated*). $NaHCO_3$ (aqueous solution, 10%, 50 mL) was added and the aqueous layer was washed with Et_2O (50 mL) then acidified to pH 1 and extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and then concentrated at a temperature below 20 °C to give the crude product.

4.4.1. 1-Acetylpiperidine-2,2-dicarboxylic acid monoethyl ester (1a). Saponification: 12 h using 5–7 equiv of KOH. Purification of the crude product by recrystallization from EtOAc/pentane (1/1) afforded **1a** as a white solid in 82% yield (2.03 g from 2.76 g of diester): mp 101–102 °C; ^1H NMR δ 1.29 (t, J=7.0 Hz, 3H), 1.6–1.9 (m, 4H), 2.15 (s, 3H), 2.2–2.3 (m, 1H), 3.6–3.7 (m, 2H), 4.2–4.3 (m, 2H); ^{13}C NMR δ 14.1 (CH_3), 19.3 (CH_2), 22.1 (CH_3), 23.7 (CH_2), 32.2 (CH_2), 44.9 (CH_2), 62.7 (CH_2), 67.3 (C), 170.7 (C), 171.2 (C), 174.3 (C); IR (KBr) 3500, 3052, 2983, 1734, 1652, 1419, 1264 cm^{-1}; MS (EI) *m/z* (%) 199 ($M^+ - CO_2$, 21), 156 (15), 126 (67), 85 (100); Anal. Calcd for $C_{11}H_{17}NO_5$: C, 54.31; H, 7.04; N, 5.76. Found: C, 54.32; H, 7.19; N, 5.67.

4.4.2. 1-Benzoylpiperidine-2,2-dicarboxylic acid monoethyl ester (1b). Saponification: 24 h using 12–15 equiv of KOH. Purification of the crude product by recristallization from EtOAc/pentane (1/1) afforded **1b** as a white solid in 60% yield (1.10 g from 2.00 g of diester): mp 106 °C; ^1H NMR δ 1.29 (t, J=7.0 Hz, 3H), 1.8–1.9 (m, 4H), 2.0–2.1 (m, 1H), 2.4–2.5 (m, 1H), 3.4–3.5 (m, 1H), 3.7–3.8 (m, 1H), 4.28 (q, J=7.0 Hz, 2H), 7.4–7.5 (m, 5H); ^{13}C NMR δ 14.0 (CH_3), 19.0 (CH_2), 23.3 (CH_2), 31.46 (CH_2), 46.5 (CH_2), 63.0 (CH_2), 67.6 (C), 127.6 (CH), 128.7 (CH), 131.0 (CH), 134.5 (C), 170.0 (C), 171.2 (C), 175.2 (C); IR (KBr) 3420, 2949, 1734, 1636, 1405, 909, 700 cm^{-1}; MS (ESI) *m/z* (%) 306 (M+1, 50), 288 (26), 260 (100), 232 (7), 216 (9), 156 (69), 105 (26); HRMS (ESI) calcd for $C_{16}H_{20}NO_5$ $[M+H]^+$ 306.1341, found 306.1336.

4.4.3. 1-(4-Methoxybenzoyl)piperidine-2,2-dicarboxylic acid monoethyl ester (1c). Saponification: 24 h using 10 equiv of KOH. Purification of the crude product by recrystallization from EtOAc/pentane (4/6) afforded **1c** as a white solid in 72% yield (1.00 g from 1.50 g starting material): mp 138 °C; ^1H NMR δ 1.27 (t, J=7.5 Hz, 3H), 1.7–1.8 (m, 4H), 2.0–2.1 (m, 1H), 2.4–2.5 (m, 1H), 3.3–3.4 (m, 1H), 3.8–3.9 (m, 1H), 3.85 (s, 3H), 4.2–4.3 (m, 2H), 6.95 (d, J=8.6 Hz, 2H), 7.56 (d, J=8.6 Hz, 2H); ^{13}C NMR δ 14.0 (CH_3), 19.0 (CH_2), 23.4 (CH_2), 31.2 (CH_2), 47.0 (CH_2), 55.4 (CH_3), 62.9 (CH_2), 68.0 (C), 113.8 (CH), 125.8 (C), 130.3 (CH), 162.3 (C), 169.5 (C), 171.0 (C), 176.1 (C); IR (KBr) 3415, 3052, 1606, 1420, 1264, 741 cm^{-1}.

4.4.4. 1-(4-Nitrobenzoyl)piperidine-2,2-dicarboxylic acid monoethyl ester (1d). Saponification: 48 h using 15 equiv of KOH. Progress of the saponification was monitored by ^1H-NMR. Purification of the crude product by recrystallization from EtOAc/pentane (4/6) afforded **1d** as a white solid in 66% yield (800 g from 1.3 g of diester): mp 120 °C; ^1H NMR δ 1.33 (t, J=7.0 Hz, 3H), 1.6–1.8 (m, 4H), 2.2–2.3 (m, 1H), 2.3–2.4 (m, 1H), 3.4–3.5 (m, 2H), 4.35 (m, 2H), 7.6–7.7 (m, 2H), 8.2–8.3 (m, 2H); ^{13}C NMR δ 14.1 (CH_3), 18.9 (CH_2), 23.2 (CH_2), 31.7 (CH_2), 46.2 (CH_2), 63.4 (CH_2), 67.0 (C), 124.2 (CH), 128.3 (CH), 141.1 (C), 149.0 (C), 170.3 (C), 171.6 (C), 172.1 (C); IR (KBr) 3400, 3052, 2883, 1733, 1652, 1525, 1420, 1350, 1264, 750 cm^{-1}; MS

(ESI) *m/z* (%) 351 ([M+H]$^+$, 24), 333 (20), 305 (100) 277 (13.6); HRMS (ESI) calcd for $C_{16}H_{18}N_2O_7$ [M+H]$^+$ 351.1192, found 351.1160.

4.4.5. 1-(1-Naphtoyl)piperidine-2,2-dicarboxylic acid monoethyl ester (1e). Saponification: 24 h using 10 equiv of KOH. Purification of the crude product by recrystallization from EtOAc/pentane (4/6) afforded **1e** as a white solid in 75% yield (400 mg from 580 mg of diester): mp 70 °C; ^{1}H NMR δ 1.3–1.4 (m, 3H), 1.5–1.7 (m, 4H), 2.1–2.2 (m, 1H), 2.3–2.4 (m, 1H), 3.2–3.4 (m, 2H), 4.2–4.5 (m, 2H), 7.3–7.6 (m, 4H), 7.8–7.9 (m, 2H), 8.0–8.2 (m, 1H), 8.2–8.4 (br s, H acid); ^{13}C NMR δ (2 rotamers) 14.4 (CH_3), 18.6 (CH_2), 19.0 (CH_2), 23.2 (CH_2), 23.5 (CH_2), 31.7 (CH_2), 32.0 (CH_2), 45.3 (CH_2), 46.0 (CH_2), 63.7 (CH_2), 66.6 (C), 66.8 (C), 123.6 (CH), 124.3 (CH), 124.9 (CH), 125.2 (CH), 125.4 (CH), 126.5 (CH), 126.7 (CH), 127.1 (CH), 127.5 (CH), 128.1 (CH), 128.5 (CH), 129.4 (C), 129.9 (CH), 132.9 (C), 133.2 (C), 170.0 (C), 170.2 (C), 172.8 (C), 173.0 (C), 173.7 (C); IR (neat) 2944, 1733, 1646, 1375, 1229, 1020, 780; MS (ESI) *m/z* (%) 356 ([M+H]$^+$, 30.1), 338 (8), 310 (28), 155 (100); HRMS (ESI) calcd for $C_{22}H_{21}NO_5$ [M+H]$^+$ 356.1498, found 356.1500.

4.5. Typical decarboxylation procedure

The organic base (0.25 mmol, chiral amine or Et_3N) was added under nitrogen to *N*-protected-piperidine-2,2-dicarboxylic acid monoethyl ester (**1**) (0.25 mmol) in distilled aprotic solvent (10 mL). The mixture was stirred for 24 h at room temperature. The solvent was removed under reduced pressure (temperature not exceeding 20 °C). After acidification (HCl 1 N), the mixture was extracted with CH_2Cl_2, and the combined organic layers were dried ($MgSO_4$), and then concentrated (temperature not exceeding 20 °C) to give the crude product. Purification was carried out by flash chromatography, and then the enantiomeric excess was determined by HPLC analysis using a Chiralpak AD-H column (250×4.6 mm i.d., 5 μm); mobile phase: 95% of *n*-heptane and 5% of a mixture of MeOH/EtOH: 70/30; flow rate: 1 mL/min; variable temperature and wavelength detection.

4.6. Organocatalyzed decarboxylation of piperidinomalonate hemiester (1b)

epi-Cinchonine (0.3 g, 1.0 mmol) was added under nitrogen to piperidine-2,2-dicarboxylic acid monoethyl ester (**1b**) (3.0 g, 10 mmol) in CCl_4 (10 mL). The mixture was stirred for 48 h at room temperature. The solvent was removed under reduced pressure (temperature not exceeding 20 °C). After acidification (HCl 1 N), the mixture was extracted with CH_2Cl_2, and the combined organic layers were dried ($MgSO_4$), and then concentrated (temperature not exceeding 20 °C) to give the crude product. Purification was carried out by flash chromatography on silica gel to afford **2b** as a colourless oil (2.25 g, 86% yield). The enantiomeric excess was determined by HPLC (ee: 71%).

4.7. General procedure for the synthesis of optically pure pipecolic acid derivatives (HPLC references)

(*S*)-(−)-1-(*tert*-Butoxycarbonyl)-2-piperidinecarboxylic acid (229 mg, 1 mmol) was dissolved in a solution of HCl in EtOH (20 mL, 10% v/v) and stirred for 15 h at room temperature. The solvent was evaporated. To the crude product dissolved in a mixture of CH_2Cl_2 (20 mL) and pyridine (2 mL) was added the acid chloride (3 equiv). After 5 h stirring at room temperature, the reaction mixture was diluted with CH_2Cl_2, washed with HCl (1 N) and extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. After purification on silication gel, the product was subjected to HPLC analysis. All retention times are given for both enantiomers by comparison for the (*R*) enantiomer with the racemic mixtures synthesized by decarboxylation of malonyl derivatives **1** in the presence of Et_3N.

4.7.1. (*S*)-1-Acetylpiperidine-2-carboxylic acid ethyl ester (2a). Purification of the crude product by flash chromatography on silica gel (AcOEt/CH_2Cl_2: 6/4) afforded **2a** as a colourless oil (179 mg, 90% yield): $[\alpha]_D^{20}$ −60 (*c* 5.0, $CHCl_3$); HPLC retention times: (*S*) 11.0 min; (*R*) 8.5 min (*T*: 35 °C, λ: 203 nm); ^{1}H NMR δ (two rotamers a/b: 75/25) 1.27 (t, *J*=6.8 Hz, 3H, a+b), 1.2–1.7 (m, 5H, a+b), 2.07 (s, 3H, b), 2.14 (s, 3H, a), 2.2–2.3 (m, 1H, a+b), 2.6–2.7 (m, 1H, b), 3.30 (dt, *J*=12.4, 2.3 Hz, a), 3.6–3.7 (m, 1H, a), 4.1–4.2 (m, 2H, a+b), 4.4–4.6 (m, 2H, b), 5.3–5.4 (m, 1H, a); ^{13}C NMR δ (two rotamers) 14.5 (a+b, CH_3), 21.0 (b, CH_2), 21.1 (a, CH_2), 21.7 (b, CH_3), 21.9 (a, CH_3), 24.7 (b, CH_2), 25.6 (a, CH_2), 26.9 (a, CH_2), 27.5 (b, CH_2), 39.5 (b, CH_2), 44.4 (a, CH_2), 52.0 (a, CH), 57.2 (b, CH), 61.3 (a, CH_2), 61.8 (b, CH_2), 170.9 (a, C), 171.1 (b, C), 171.6 (a+b, C); IR (NaCl) 2940, 2862, 1735, 1648, 1424, 1200 cm^{-1}; MS (EI) *m/z* (%) 199 (M$^+$, 10), 154 (4), 126 (57), 84 (100); HRMS (EI) calcd. for $C_{10}H_{17}NO_3$ 199.1208, found 199.1202.

4.7.2. (*S*)-1-Benzoylpiperidine-2-carboxylic acid ethyl ester (2b). Purification of the crude product by flash chromatography on silica gel (AcOEt/CH_2Cl_2: 6/4) afforded **2b** as a colourless oil (222 mg, 85% yield). $[\alpha]_D^{20}$ −75 (*c* 0.80, $CHCl_3$); HPLC retention times: (*S*) 14.5 min.; (*R*) 15.4 min; (*T*: 20 °C, λ: 205 nm); ^{1}H NMR δ (two rotamers a/b: 70/30) 1.27 (t, *J*=6.8 Hz, 3H, a+b), 1.3–1.8 (m, 5H, a+b), 2.20 (d, *J*=12.5 Hz, 1H, b), 2.35 (d, *J*=12.5 Hz, 1H, a), 2.83 (t, *J*=12.5 Hz, 1H, b), 3.23 (t, *J*=12.5 Hz, 1H, a), 3.63 (d, *J*=13.5 Hz, 1H, a), 4.2–4.3 (m, 2H, a+b), 4.42 (s, 1H, b), 4.64 (d, *J*=13.5 Hz, 1H, b), 5.49 (d, *J*=4.0 Hz, 1H, a), 7.3–7.5 (m, 5H, a+b); ^{13}C NMR δ (two rotamers) 14.6 (a+b, CH_3), 21.5 (a+b, CH_2), 25.0 (b, CH_2), 25.8 (a, CH_2), 27.0 (a, CH_2), 27.7 (b, CH_2), 40.3 (b, CH_2), 46.2 (a, CH_2), 52.6 (a, CH), 58.8 (b, CH), 61.6 (a, CH_2), 61.9 (b, CH_2), 126.7 (b, CH), 127.2 (a, CH), 128.8 (a, CH), 129.0 (b, CH), 129.9 (a+b, CH), 136.4 (a+b, C), 171.2 (b, C), 171.4 (a, C), 171.9 (a, C), 172.2 (b, C); IR (NaCl) 2939, 2861, 1732, 1633, 1417, 1200, 1139, 1005, 699 cm^{-1}; MS (EI) *m/z* (%) 261 (M$^+$, 11), 216 (2), 188 (100), 156 (5), 105 (100), 77 (27); HRMS (ESI) calcd for $C_{15}H_{20}NO_3$ [M+H]$^+$ 262.1443, found 262.1441.

4.7.3. (*S*)-1-(4-Methoxybenzoyl)piperidine-2-carboxylic acid ethyl ester (2c). Purification of the crude product by flash chromatography on silica gel (AcOEt/CH_2Cl_2: 6/4) afforded **2c** as a colourless oil (200 mg, 69% yield). $[\alpha]_D^{20}$ −65 (*c* 1.0, $CHCl_3$); HPLC retention times: (*S*) 34.0 min; (*R*) 26.0 min (*T*: 35 °C, λ: 240 nm); ^{1}H NMR δ (two rotamers

a/b: 60/40) 1.30 (t, J=7.1 Hz, 3H, a+b), 1.4–1.8 (m, 5H, a+b), 2.1–2.4 (br m, 1H, a+b), 3.7–3.9 (br m, 1H, b), 3.2–3.4 (br m, 1H, a), 3.7–3.8 (br m, 1H, a), 3.83 (s, 3H, a+b), 4.23 (q, J=7.1 Hz, 2H, a+b), 4.5–4.7 (br m, 2H, b), 5.45 (br s, 1H, a), 6. 91 (d, J=8.6 Hz, 2H, a+b), 7.40 (d, J=8.6 Hz, 2H, a+b); ^{13}C NMR δ (2 rotamers) 14.3 (a+b, CH_3), 21.3 (a+b, CH_2), 24.7 (b, CH_2), 25.6 (a, CH_2), 26.8 (a, CH_2), 27.3 (b, CH_2), 40.2 (b, CH_2), 46.1 (a, CH_2), 52.6 (a, CH), 55.4 (a+b, CH_3), 58.8 (b, CH), 61.3 (a+b, CH_2), 113.7 (a+b, C), 127.5 (a+b, CH), 128.0 (a+b, CH), 129.05 (a+b, C), 160.8 (a+b, C), 171.2 (a+b, C), 171.6 (a+b, C); IR (NaCl) 3455, 2940, 2243, 1731, 1632, 1512, 1422, 1003, 912, 840, 728 cm^{-1}; MS (EI) m/z (%) 291 (M^+, 9), 218 (34), 135 (100), 107 (5); HRMS (ESI) calcd for $C_{16}H_{21}NO_4$ $[M+H]^+$ 292.1549, found 292.1540.

4.7.4. (*S*)-1-(4-Nitrobenzoyl)piperidine-2-carboxylic acid ethyl ester (2d). Purification of the crude product by flash chromatography on silica gel (AcOEt/CH_2Cl_2: 6/4) afforded **2d** as a colourless oil (260 mg, 85% yield). $[\alpha]_D^{20}$ −60 (c 0.45, $CHCl_3$); HPLC retention times: (*S*) 23.5 min; (*R*) 31.0 min; (*T*: 35 °C, λ: 262 nm); ^{1}H NMR δ (2 rotamers a/b: 75/25) 1.33 (t, J=7.1 Hz, 3H, a+b), 1.3–1.8 (m, 5H, a+b), 2.23 (d, J=13.2 Hz, 1H, b), 2.39 (d, J=13.2 Hz, 1H, a), 2.86 (t, J=12.9 Hz, 1H, b), 3.29 (t, J=12.9 Hz, 1H, a), 3.47 (d, J=12.9 Hz, 1H, a), 4.2–4.3 (m, 2H, a+b, and 1H, b), 4.65 (br d, J=13.9 Hz, 1H, b), 5.47 (d, J=4.8 Hz, 1H, a), 7.5–7.6 (m, 2H, a+b), 8.2–8.3 (m, 2H, a+b); ^{13}C NMR δ (two rotamers) 14.7 (a+b, CH_3), 21.4 (a+b, CH_2), 24.9 (b, CH_2), 25.7 (a, CH_2), 26.9 (a, CH_2), 27.6 (b, CH_2), 40.5 (b, CH_2), 46.2 (a, CH_2), 52.8 (a, CH), 58.7 (b, CH), 61.9 (a, CH_2), 62.3 (b, CH_2), 124.3 (a, CH), 124.4 (b, CH), 127.9 (b, CH), 128.3 (a, CH), 142.6 (a+b, C), 148.7 (a+b, C), 169.7 (a+b, C), 171.0 (a+b, C); IR (NaCl) 3445, 2933, 1734, 1640, 1522, 1430, 1347, 1173, 1021, 852 cm^{-1}; MS (EI) m/z (%) 306 (6), 233 (100), 150 (42), 120 (5), 104 (9); HRMS (ESI) calcd for $C_{15}H_{19}N_2O_5$ $[M+H]^+$ 307.1294, found 307.1278.

4.7.5. 1-(1-Naphtoyl)piperidine-2-carboxylic acid ethyl ester (2e). Purification of the crude product by flash chromatography on silica gel (AcOEt/CH_2Cl_2: 6/4) afforded **2e** as a colourless oil (245 mg, 79% yield). $[\alpha]_D^{20}$ −94 (c 1.3, $CHCl_3$); HPLC retention times: (*S*) 9.0 min; (*R*) 13.7 min; (*T*: 20 °C, λ: in nm); ^{1}H NMR δ (four rotamers a/b/c/d in approximate ratio: 51/24/15/10, respectively) 1.16, 1.28, 1.38, 1.43 (d, c, b, a, respectively) (t, J=7.1 Hz, 3H), 1.3–1.8 (m, 5H, a+b+c+d), 2.0–2.1 (m, 1H, c+d), 2.4–2.5 (m, 1H, a+b), 2.97 (td, J=13.4 and 3.2 Hz, 1H, c), 3.0–3.2 (m, 1H, a+b+d), 3.3–3.4 (m, 1H, a+b), 4.0–4.1 (m, 2H, d), 4.12 (br d, J=5.0 Hz, 1H, d), 4.2–4.4 (m, 2H, a+b+d and 1H, c), 4.8–4.9 (m, 1H, c+d), 5.73 (d, J=5.0 Hz, 1H, a+b), 7.4–7.6 (m, 4H, a+b+c+d), 7.8–7.9 (m, 2.5H), 8.13 (d, J=8.1 Hz, 0.5H); ^{13}C NMR δ (four rotamers a, b, c, d) 14.4 (d, CH_3), 14.6 (c, CH_3), 14.7 (b, CH_3), 14.8 (a, CH_3), 21.5–21.6 (a+b+c+d, CH_2), 25.3 (d, CH_2), 25.4 (c, CH_2), 25.8 (a, CH_2), 26.2 (b, CH_2), 27.1 (a, CH_2), 27.2 (b, CH_2), 27.8 (d, CH_2), 28.2 (c, CH_2), 39.3 (d, CH_2), 40.0 (c, CH_2), 45.6 (a, CH_2), 46.0 (b, CH_2), 52.4 (a+b, CH), 58.4 (c, CH), 58.5 (d, CH), 61.8–61.9 (a+b+c+d, CH_2), 123.5, 123.8, 124.1, 124.3, 124.6, 124.8, 125.0, 125.3, 125.6, 125.7, 125.8, 126.1, 126.3, 126.8, 126.9, 127.3, 127.4, 127.5, 128.5, 128.6, 128.8, 128.9, 129.2, 129.4, 129.5, 129.7, 129.8, 129.9, 130.1, 130.2, 133.7, 133.8, 134.1, 134.46, 134.4, 134.5, 134.7, 134.8 (aromatic carbons of rotamers a, b, c, d), 170.8–171.0–171.1 (a+b+c+d, C), 171.2 (b, C), 171.3 (d, C), 171.4 (c, C), 171.5 (a, C); IR (NaCl) 3442, 3016, 1731, 1628, 1437, 1215, 756; MS (EI) m/z (%) 311 (20), 265 (5), 238 (50), 155 (100), 127 (28); HRMS (ESI) calcd for $C_{19}H_{21}NO_3$ $[M+H]^+$ 312.1600, found 312.1597.

4.8. Synthesis of cinchona alkaloid derivatives

4.8.1. *N*-(9-Deoxycinchonin-9-yl)-2-methoxybenzamide—CN–Amide. To (8*R*, 9*S*)-9-amino-(9-deoxy)-cinchonine (0.530 g, 1.83 mmol) in CH_2Cl_2 (5 mL) were added Et_3N (1.62 mL, 11.52 mmol) and DMAP (0.032 g, 0.26 mmol). The mixture was cooled to 0 °C and *o*-anisoyl chloride (0.641 g, 3.76 mmol) in CH_2Cl_2 (10 mL) was added. Stirring was continued at 0 °C for 30 min then at room temperature for three days. After addition of NaOH (2 N, 10 mL), the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. Purification by flash chromatography over silica gel (CH_2Cl_2/MeOH: 95/5) afforded **CN–Amide** (0.703 g, 90%) as a white solid: mp 88–89 °C; $[\alpha]_D^{20}$ +13.2 (c 0.26, EtOH); ^{1}H NMR δ 1.5–1.8 (m, 4H), 1.8–2.0 (m, 1H), 2.1–2.3 (m, 1H), 2.6–2.9 (m, 4H), 3.4–3.5 (m, 1H), 3.86 (s, 3H), 4.9–5.1 (m, 2H), 5.9–6.1 (m, 1H), 6.2–6.3 (m, 1H), 6.93 (d, J=8.4 Hz, 1H), 7.0–7.1 (m, 1H), 7.3–7.5 (m, 2H), 7.5–7.6 (m, 1H), 7.6–7.7 (m, 1H), 8.0–8.2 (m, 2H), 8.30 (d, J=8.5 Hz, 1H), 8.38 (d, J=8.5 Hz, 1H), 8.90 (d, J=4.6 Hz, 1H); ^{13}C NMR δ 25.4 (CH_2), 26.6 (CH_2), 28.2 (CH), 40.3 (CH), 48.4 (CH_2), 49.4 (CH_2), 50.2 (CH), 56.1 (CH_3), 59.4 (CH) 111.4 (CH), 114.9 (CH_2), 118.7 (CH), 121.2 (C), 121.5 (CH), 123.8 (CH), 126.9 (CH), 127.4 (C), 129.2 (CH), 130.3 (CH), 132.5 (CH), 133.1 (CH), 140.4 (CH), 148.0 (C), 148.9 (C), 150.1 (CH), 157.4 (C), 165.0 (C); IR (KBr) 3482, 3357, 2938, 1600, 1541, 1243, 1023, 756 cm^{-1}; MS (EI) m/z (%) 427 (M^+, 48), 386 (10), 332 (16), 292 (88), 135 (100), 77 (27); HRMS (ESI) calcd for $C_{27}H_{30}N_3O_2$ $[M+H]^+$ 428.2338, found 428.2334.

4.8.2. Cinchonin-9-yl phenylcarbamate—CN–PhCarb. Phenyl isocyanate (1.44 g, 12.10 mmol) was added to a solution of cinchonine (2.73 g, 9.26 mmol) in toluene (30 mL). The reaction mixture was heated at 110 °C for 16 h. Toluene was removed under reduced pressure. The crude product was purified by flash chromatography over silica gel (CH_2Cl_2/MeOH: 97.5/2.5) to afford **CN–PhCarb** (2.88 g, 75%) as a white solid: mp 201–202 °C [lit.[13] 190–191 °C]; $[\alpha]_D^{20}$ +51 (c 0.8, $CHCl_3$) [lit.[13] +53 (c 0.54, $CHCl_3$)]; ^{1}H NMR δ 1.4–1.6 (m, 2H), 1.8–2.0 (m, 2H), 2.25 (q, J=8.2 Hz, 1H), 2.6–2.8 (m, 2H), 2.90 (d, J=8.9 Hz, 2H), 3.34 (q, J=8.5 Hz, 1H), 5.0–5.2 (m, 2H), 5.9–6.1 (m, 1H), 6.57 (d, J=7.9 Hz, 1H), 6.79 (br s, 1H), 7.06 (t, J=7.1 Hz, 1H), 7.2–7.4 (m, 5H), 7.44 (d, J=4.5 Hz, 1H), 7.5–7.6 (m, 1H), 7.6–7.7 (m, 1H), 8.13 (d, J=8.3 Hz, 1H), 8.25 (d, J=8.4 Hz, 1H), 8.90 (d, J=4.5 Hz, 1H); ^{13}C NMR δ 24.4 (CH_2), 26.4 (CH_2), 27.8 (CH), 39.8 (CH), 49.0 (CH_2), 49.7 (CH_2), 59.9 (CH), 74.0 (CH), 115.1 (CH_2), 119.1 (CH), 119.2 (CH) 123.8(CH), 123.9 (CH), 126.6 (C), 127.1 (CH), 129.2 (CH), 129.4 (CH), 130.4 (CH), 138.0 (C), 140.6 (CH), 146.3 (C), 148.6 (C), 150.0 (CH), 153.0 (C); IR (KBr) 3240, 3064, 2938, 1730, 1600, 1545, 1445, 1317, 1219, 1053, 758 cm^{-1}; MS (ESI) m/z (%) 414 (M+1, 17), 295 (3), 277 (100), 246 (2), 234 (4);

HRMS (ESI) calcd for $C_{26}H_{28}N_3O_2$ $[M+H]^+$ 414.2182, found 414.2162.

4.8.3. Cinchonin-9-yl *tert*-butylcarbamate—CN–*t*-BuCarb. Tertiobutyl isocyanate (1.20 g, 12.10 mmol) and one drop of dibutyltin laurate were added to cinchonine (2.73 g, 9.26 mmol) in toluene (30 mL). The reaction mixture was heated at 110 °C for 48 h. Toluene was removed under reduced pressure. The crude product was purified by flash chromatography over silica gel (acetone) to afford **CN–*t*-BuCarb** (3.60 g, 99%) as a white solid: mp 115–116 °C; $[\alpha]_D^{20}$ +71 (*c* 1.0, $CHCl_3$); ^{1}H NMR δ 1.28 (s, 9H), 1.4–1.6 (m, 2H), 1.8–2.0 (m, 2H), 2.1–2.3 (m, 1H), 2.6–2.9 (m, 5H), 3.2–3.3 (m, 1H), 4.76 (br s, 1H), 5.0–5.2 (m, 2H), 5.9–6.1 (m, 1H), 6.48 (d, *J*=7.1 Hz, 1H), 7.39 (d, *J*=4.5 Hz, 1H), 7.57 (td, *J*=7.4, 1.1 Hz, 1H), 7.70 (td, *J*=7.4, 1.2 Hz, 1H), 8.11 (d, *J*=8.3 Hz, 1H), 8.22 (d, *J*=8.2 Hz, 1H), 8.88 (d, *J*=4.5 Hz, 1H); ^{13}C NMR δ 24.1 (CH_2), 26.4 (CH_2), 27.8 (CH), 28.8 (CH_3), 39.8 (CH), 48.9 (CH_2), 49.7 (C), 50.6 (CH_2), 59.8 (CH), 72.9 (CH), 114.6 (CH_2), 118.4 (CH), 123.6 (CH), 126.2 (C), 126.7 (CH), 129.0 (CH), 130.2 (CH), 140.6 (CH), 146.3 (C), 148.4 (C), 149.9 (CH), 153.7 (C); IR (neat) 3278, 2952, 1723, 1506, 1262, 1202 1098, 1049, 770 cm^{-1}; MS (ESI) *m/z* (%) 394 (M^++1, 45), 295 (14), 277 (100), 234 (3); HRMS (ESI) calcd for $C_{24}H_{32}N_3O_2$ $[M+H]^+$ 394.2495, found 394.2482.

4.8.4. *epi*-Cinchonin-9-yl phenylcarbamate—*epi*-CN–PhCarb. Phenyl isocyanate (1.052 g, 8.83 mmol) was added to *epi*-cinchonine (2 g, 6.79 mmol) in toluene (22 mL). The reaction mixture was heated at 110 °C for 5 h. Toluene was removed under reduced pressure. The crude product was purified by flash chromatography over silica gel (CH_2Cl_2/MeOH: 97.5/2.5) to afford *epi*-**CN–PhCarb** (2.53 g, 90%) as a white solid: mp 108 °C; $[\alpha]_D^{20}$ +115.4 (*c* 0.58, $CHCl_3$); ^{1}H NMR δ 0.9–1.0 (m, 1H), 1.1–1.3 (m, 1H), 1.7–1.4 (m, 3H), 2.1–2.3 (m, 1H), 2.7–3.0 (m, 3H), 3.1–3.3 (m, 1H), 3.50–3.67 (m, 1H), 5.0–5.2 (m, 2H), 5.75–5.95 (m, 1H), 6.69 (d, *J*=10.21 Hz, 1H), 6.93 (t, *J*=7.0 Hz, 1H), 7.05–7.27 (m, 4H), 7.58 (d, *J*=4.4 Hz, 1H), 7.66 (t, *J*=7.3 Hz, 1H), 7.78 (t, *J*=7.3 Hz, 1H), 8.19 (d, *J*=8.2 Hz, 1H), 8.52 (d, *J*=8.3 Hz, 1H), 8.59 (br s, 1H), 8.99 (d, *J*=4.4 Hz, 1H); ^{13}C NMR δ 24.2 (CH_2), 26.1 (CH_2), 27.2 (CH), 39.0 (CH), 47.4 (CH_2), 48.6 (CH_2), 59.6 (CH), 71.6 (CH), 115.0 (CH_2), 119.3 (CH), 120.0 (CH), 123.3 (CH), 123.7 (CH), 126.8 (C), 127.1 (CH), 128.6 (CH), 129.6 (CH), 130.4 (CH), 138.0 (C), 139.8 (CH), 144.1 (C), 148.7 (C), 149.9 (CH), 153.5 (C); IR (neat) 3241, 3062, 2938, 1725, 1601, 1544, 1314, 1222, 1054, 758 cm^{-1}; MS (ESI) *m/z* (%) 414 (M+1, 12), 295 (3), 277 (100), 234 (4); Anal. Calcd for $C_{26}H_{27}N_3O_2$: C, 75.52; H, 6.58; N, 10.16. Found: C, 75.39; H, 6.94; N, 10.16.

4.8.5. 4,6-bis(Cinchonin)-2,5-diphenylpyrimidine—$(CN)_2$PYR. A mixture of 4,6-dichloro-2,5-diphenylpyrimidine (301 mg, 1 mmol), cinchonine (589 mg, 2 mmol) and KOH (560 mg, 10 mmol) in toluene (30 mL) was stirred at room temperature for 10 min then at 115 °C for 15 h with azeotropic removal of water. After cooling to room temperature, water was added and the aqueous layer was extracted ($CHCl_3$). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was purified by flash column chromatography over silica gel (EtOAc/MeOH: 98.5/1.5) to afford **$(CN)_2$PYR** (534 mg, 65%) as a white solid: mp 115–116 °C; $[\alpha]_D^{20}$ –127.5 (*c* 0.45, $CHCl_3$); ^{1}H NMR δ 1.1–1.7 (m, 8H), 1.7–1.9 (t, *J*=11.0 Hz, 2H), 2.0–2.2 (m, 2H), 2.5–2.9 (m, 8H), 3.0–3.2 (m, 2H), 4.7–5.0 (m, 4H), 5.2–5.4 (m, 2H), 6.7–7.9 (m, 18H), 8.14 (d, *J*=8.3 Hz, 2H), 8.29 (d, *J*=8.3 Hz, 2H), 8.87 (d, *J*=4.3 Hz, 2H); ^{13}C NMR δ 22.7 (CH_2), 26.3 (CH_2), 28.4 (CH), 40.5 (CH), 49.8 (CH_2), 49.9 (CH_2), 60.0 (CH), 76.8 (CH), 104.7 (C), 114.7 (CH_2), 118.2 (CH), 123.6 (CH), 126.0 (C), 126.6 (CH), 127.7 (CH), 128.6 (CH), 129.1 (CH), 130.4 (CH), 130.5 (CH), 131.2 (C), 136.3 (C), 140.4 (CH), 146.9 (C), 148.5 (C), 150.0 (CH), 161.0 (C), 166.3 (C); IR (KBr) 3064, 2936, 2869, 1578, 1539, 1414, 1361, 1110 cm^{-1}; HRMS (ESI) calcd for $C_{54}H_{53}N_6O_2$ $(M+H)^+$ 817.4230, found 817.4223.

Acknowledgements

We gratefully acknowledge the 'Ministère de la Recherche et des Nouvelles Technologies' for fellowships T.S., 'PunchOrga' Network (Pôle Universitaire Normand de Chimie Organique), CNRS (Centre National de la Recherche Scientifique), the 'Région Basse-Normandie', and the European Union (FEDER funding) for financial support.

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24. We noticed a lack of reproducibility of certain results: as an example we could not reproduce the enantiomeric excess of 52% obtained with the base **QD–Amide** in THF, see Ref. 19.
25. For compound **2d**, we checked for the asymmetric deprotonation–reprotonation according to the conditions described for **2b** at the end of Section 2.1. Again, no such effect was observed for this product.

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Tetrahedron 62 (2006) 6166–6171
Tetrahedron

$TMSCH_2Li$–LiDMAE: a new nonnucleophilic reagent for C-2 lithiation of halopyridines

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Received 21 March 2006; revised 18 April 2006; accepted 20 April 2006
Available online 19 May 2006

Abstract—A new superbasic reagent has been discovered by combining $TMSCH_2Li$ and LiDMAE in hexane. This reagent was found highly efficient for the C-2 lithiation of sensitive chloro- and fluoropyridines. The metallation occurred chemo- and regioselectively at 0 °C avoiding the nucleophilic addition or substrate degradation commonly obtained with other alkyllithiums even at lower temperatures.

1. Introduction

The C-2 lithiation of the pyridine ring has proved to be a synthetically important process to introduce opportune functionalities subsequently giving access to numerous pharmacophores, polydentate ligands (e.g., bipyridines) and chiral catalysts. Several methodologies have been developed to lithiate selectively the C-2 position. The bromine–lithium exchange was probably the most useful since adequately placed bromine ensured the selectivity.[1] However, the reactions were performed at low temperatures to avoid substrate degradation and the availability of C-2 brominated pyridines often restricted the reaction to derivatives with a low degree of functionality. Another way was to increase the alpha proton's acidity by reacting the substrate with a Lewis acid.[2] The intermediate pyridinium salt was then efficiently lithiated with LiTMP but mixtures of mono- and bifunctional derivatives are always obtained. The hydrogen–lithium exchange appeared as the more elegant process since brominated precursors and a tedious activation step were avoided. However, this was probably the more complicated approach since the base used had to be sufficiently basic to abstract the alpha protons while not nucleophilic to prevent side addition at the electrophilic C-2 position. The success was met by associating *n*-BuLi with $Me_2N(CH_2)_2OLi$ in nonpolar solvents such as hexane or toluene.[3] The obtained reagent named BuLi–LiDMAE effected clean alpha metallations of numerous pyridine derivatives with tolerance of sensitive functionalities such as chlorine,[4] acidic methyles,[5] anisyles[6] and pyrrolyl groups.[7] BuLi–LiDMAE is now a well-recognized reagent included in the portfolio of metallating agents.[8] However, it still suffers from drawbacks, which need to be overcome. All lithiations require an excess of base (typically 3–4 equiv) implying the use of the corresponding excess of electrophilic reagent to avoid its consumption by the base before trapping the lithiopyridine. This was problematic when an expensive electrophile was used or could complicate the purification step, the side addition product being present in the medium. Furthermore the newly introduced substituent could be attacked by the excess of basic reagent if sensitive such as an aldehyde. Since BuLi–LiDMAE is an *n*-BuLi-containing reagent, nucleophilicity towards the most electrophilic heterocycles remains problematic and low temperatures (typically −80 to −100 °C) are required to metallate substrates such as halopyridines.

Since we are continuously looking for the most efficient and easily handled reagent we have launched a research program aiming at replacing *n*-BuLi by other alkyllithiums. It is well known that the degrees of aggregation and solvation have a significant impact on the reactivity and selectivity of organolithium complexes. Generally the smaller the aggregate size the more reactive they are. In contrast to *n*-BuLi, branched *i*-PrLi, *s*-BuLi and *t*-BuLi are not hexameric, but rather tetrameric, in hexanes. So the association of such bases with LiDMAE should provide more reactive lithiating agents usable in lower amounts than *n*-BuLi–LiDMAE. The steric effects are also expected to induce lower nucleophilicity towards sensitive heterocycles potentially allowing the metallation to be performed at more practical higher temperatures (0 °C or rt). Unfortunately, our first attempts to associate LiDMAE with classical branched alkyllithiums (*s*-BuLi or *t*-BuLi) only led to insoluble mixtures unreactive

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0040–4020/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2006.04.059

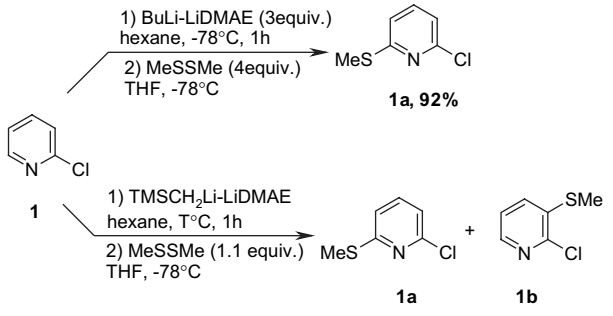
or slightly nucleophilic towards pyridines,[9] and we decided to examine other lithiating agents.

A literature survey reported the beneficial effects of silicon-containing reagents and substrates on several transformations. Accelerating effects and enantiomeric excess enhancement have been observed in asymmetric additions in the presence of silylated catalysts.[10] Moreover, silyl moieties are also known to bring some thermal and air stability to lithiating agents such as in lithium hexamethyldisilazide (LiHMDS).[11] Unfortunately, the consequence is also a decrease of the basicity (p*K*a 29.5 vs 35.7 for LDA in THF) impeding, for example, abstraction of acidic pyrimidinone protons.[12] Recently, the bulky Me_3SiCH_2Li ($TMSCH_2Li$) has been reported to be a promising base for ortholithiation of some pyridine derivatives when reacted in the presence of small amounts of diisopropylamine (DIA) in THF.[13] Thus, we felt that such an apparently nonnucleophilic reagent could be an excellent candidate for promoting alpha lithiation of pyridine derivatives when associated with LiDMAE in nonpolar solvents. Therefore, we investigated the reactivity of various $TMSCH_2Li$–LiDMAE combinations towards electrophilic chloro- and fluoropyridines.

2. Results and discussion

2.1. Lithiation of chloropyridines

The study was initiated with the metallation of 2-chloropyridine for which the behaviour towards *n*-BuLi–LiDMAE is well known (Scheme 1). As shown 3 equiv of this reagent at −78 °C in hexane were needed to metallate selectively **1** in 92% yield.[4a] Higher temperatures (e.g., 0 °C) gave nucleophilic addition and substrate degradation.[4a] **1** was then treated with $TMSCH_2Li$–LiDMAE combinations in hexane under various temperatures and stoichiometries (Table 1). $TMSCH_2Li$–LiDMAE mixtures were prepared according to procedures previously reported for BuLi–LiDMAE.[4–7]



Scheme 1. Lithiation of **1** with BuLi–LiDMAE and $TMSCH_2Li$–LiDMAE.

The experiments in Table 1 clearly underline the differences between *n*-BuLi and $TMSCH_2Li$. At first, the latter did not react with **1** even at 0 °C (entries 1–3) while *n*-BuLi gave addition even at −70 °C leading to a dihydropyridone.[14] Another interesting feature was the effect of the $TMSCH_2Li$–LiDMAE ratio on the selectivity. Indeed while BuLi and LiDMAE had to be used in a 1/1 ratio to ensure selective C-6 lithiation, such a stoichiometry gave mixtures of **1a** and **1b** with the new reagent (entries 4–6). In contrast the 2/1 ratio gave the best selectivities leading exclusively to **1a** whatever the temperature (entries 7–10). The best result was obtained when the metallation was performed at 0 °C yielding **1b** cleanly in an excellent yield (96%, entry 10) without any nucleophilic addition product. Entries 11 and 13 demonstrated that the 2/1 ratio must be adopted since a decrease of the amount of $TMSCH_2Li$ or the use of a 2/2 ratio induced a loss of the regioselectivity. Attempts to metallate at rt did not give any reaction, maybe due to the reagent degradation at such a temperature (entry 12). Finally LiDMAE used in substoichiometric amount also effected a clean metallation despite a slight decrease in the conversion (entry 14). Lower amounts led to much less efficient processes. The low nucleophilicity of $TMSCH_2Li$ is remarkable since all experiments were realized using only 1–1.1 equiv of electrophile. This means that $TMSCH_2Li$ was less nucleophilic than the formed pyridyllithium allowing efficient introduction of the functionality on the ring. Such a reagent stoichiometry was prohibited with BuLi–LiDMAE and excess of electrophile was necessary (Scheme 1) except on rare occasions when base compatible electrophiles such as TMSCl or $ClSnBu_3$ were used.[15]

Table 1. Conditions screening for lithiation of **1** with $TMSCH_2Li$–LiDMAE[a]

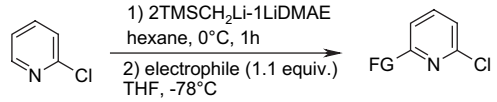
Entry	$TMSCH_2Li$ (equiv)	LiDMAE (equiv)	*T* (°C)	S.M. (%)[b]	**1a** (%)[b]	**1b** (%)[b]
1	1	0	−78	>99[c]	—	—
2	2	0	−78	>99[c]	—	—
3	2	0	0	99	tr	—
4	1	1	−78	72	22	6
5	1.5	1.5	−78	43	47	10
6	2	2	−78	64	17	19
7	2	1	−78	54	46	tr
8	2	1	−40	20	80	tr
9	2	1	−20	7	93	tr
10	2	1	0	3	96 (92)[d]	—
11	2	2	0	30	38	30
12	2	1	20	>99[c]	—	—
13	1.5	1	0	25	42	32
14	2	0.5	0	20	80	—

[a] All reactions performed on 1.8 mmol of **1**.
[b] GC yields, S.M.=starting material.
[c] No reaction occurred.
[d] Isolated yield in parentheses.

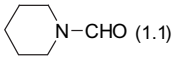
To gain additional information about the reactivity of the new superbase, we examined various conditions for the quenching step in order to examine the pyridyllithium versus $TMSCH_2Li$ nucleophilicity. The temperature was first maintained at 0 °C (conditions: entry 10, Table 1) after the metallation step, the electrophile was then introduced at the same temperature. Under these conditions, **1a** was obtained in very good yield (85%). Moreover, when THF usually added to release the pyridyllithium from aggregates to enhance its reactivity was omitted, **1a** was still obtained in excellent yield (89%). These experiments additionally stress the low nucleophilicity of $TMSCH_2Li$ allowing one to perform the metallation in more practical conditions.

The scope of the reaction was then investigated by reaction with a range of electrophiles such as halides, aldehydes or amides, which were introduced in stoichiometric amounts (Table 2). For reasons of solubility and to minimize degradation of these sensitive reagents, it was preferred to perform the trapping step at −78 °C using THF as a co-solvent.

Table 2. Metallation of **1** and reaction with sensitive electrophiles[a]

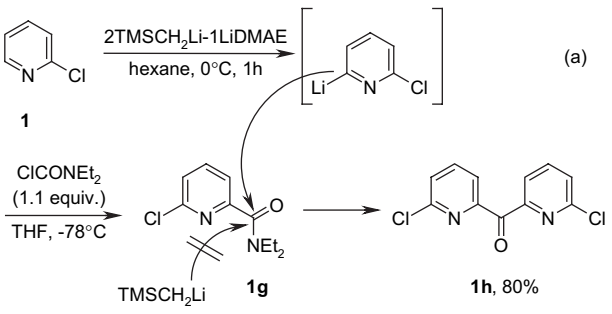


1 **1c-g**

Entry	Electrophile (equiv)	FG	Product, yield (%)[b]
1	CBr_4 (1.1)	Br	**1c**, 58
2	DMF (1.1)	CHO	**1d**, 62
3	 (1.1)	CHO	**1d**, 81
4	$PhCONMe_2$ (1.1)	COPh	**1e**, 46
5	$PhCONMe_2$ (1.1)	COPh	**1e**, 95[c]
6	PhCHO (1.1)	PhCH(OH)	**1f**, 60[d]
7	PhCHO (2)	PhCH(OH)	**1f**, 80
8	$ClCONEt_2$ (1.1)	$CONEt_2$	**1g**, 7[e]
9	$ClCONEt_2$ (3)	$CONEt_2$	**1g**, 20[e]
10	$ClCONEt_2$ (3)	$CONEt_2$	**1g**, 32[e,f]

[a] All reactions performed on 1.8 mmol of **1**.
[b] Isolated yields.
[c] The electrophile was added at 0 °C.
[d] The remaining part was unreacted **1**.
[e] Bis-(6-chloro-pyridin-2-yl)-methanone **1h** was the main product.
[f] Reverse addition.

As shown, all the expected products were synthetized in good yields using only 1.1 equiv of electrophile. Impressive was the stability of aldehyde **1d** that is usually highly electrophilic towards *n*-BuLi. A notable improvement was obtained using piperidine carboxaldehyde instead of DMF. Expectedly, PhCHO was found to be more sensitive to nucleophilic attack by the excess of $TMSCH_2Li$ nevertheless providing the corresponding alcohol **1f** in 60% yield, the remaining product being only unreacted **1**. The yield could be raised to 80% by employing an additional equivalent of benzaldehyde. Finally, the reaction of the diethylcarbamide (entries 8–10) was handicapped by fast subsequent reaction of the lithiopyridine with the target amide **1g** leading to the corresponding bis-(6-chloro-pyridin-2-yl)-methanone **1h** in good yields (60–83%) (see Scheme 2). A 32% yield of **1g** could, however, be obtained using an excess of carbamide and a reversed addition order. Interestingly, the amide **1g** was not attacked by the excess of $TMSCH_2Li$ but only by the pyridyllithium. This contrasted with the reactivity of BuLi–LiDMAE which always led to the corresponding pyridylbutylketone.[16]

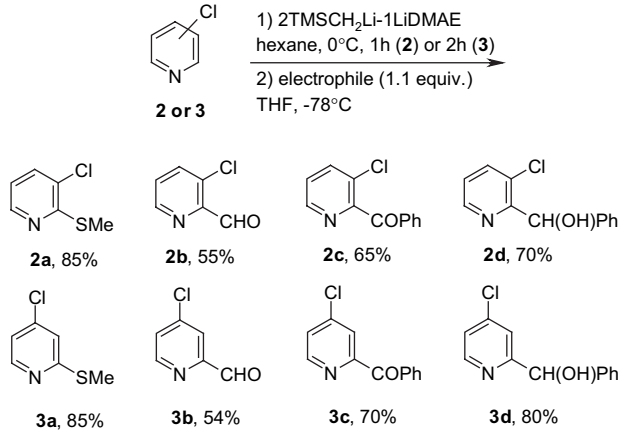


Scheme 2. Formation of dipyridylketone **1h** (a) and classical reaction with *n*-BuLi (b).

Thus the new $TMSCH_2Li$–LiDMAE reagent effected the first clean alpha lithiation of **1** at 0 °C while temperatures under −78 °C were needed with other lithiating agents. Thanks to its low nucleophilicity, the reagent also generally allowed the use of electrophiles in stoichiometric amounts.

Then we turned to the extension of this promising selective reagent to the metallation of 3- and 4-chloropyridines **2** and **3**, the latter being the most electrophilic in this series. First conditions screening showed that $TMSCH_2Li$ behaved in the same way with these isomers. No reaction occurred in the absence of LiDMAE and the conditions used for 2-chloropyridine could be applied successfully for the C-2 lithiation of **2** and **3** except that the metallation time had to be extended to 2 h for the complete conversion of **3**. The reaction performed at 0 °C did not give any nucleophilic addition product even with **3** nor did it result in isomerisation leading to C-4 lithiation with **2**. The reactivity was different from those of *n*-BuLi–LiDMAE which had to be used in excess (3–4 equiv) at −60 and −78 °C for the metallation of **2** and **3**, respectively, to avoid nucleophilic addition and C-4 lithiation of **2**.[4b]

A range of sensitive electrophiles was then reacted with lithiated **2** and **3**. All reactions were clean and gave the expected products in good to high yields (Scheme 3).



Scheme 3. Preparation of C-2 substituted chloropyridines under mild conditions.

From these excellent results obtained under mild conditions and using low amounts of reagents we decided to examine further the scope of the reaction by investigating the metallation in the fluoropyridine series.

2.2. Lithiation of fluoropyridines

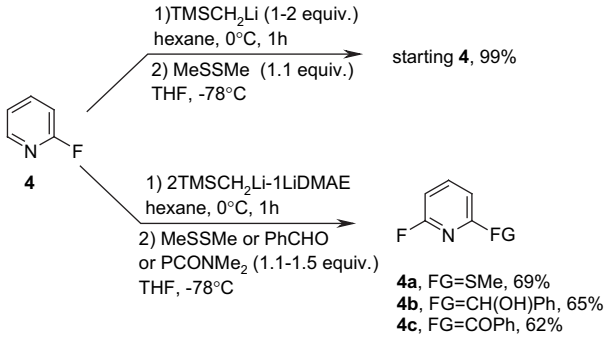
Fluoropyridines are important substrates for many reasons. At first fluorine is far more electronegative than chlorine inducing dramatic changes in the reactivity of the pyridine ring. The consequences are a strong increase of the electrophilicity[17] and proton acidities especially for those *ortho* to fluorine, so major changes could be expected on chemo- and regioselectivity.

These electronic properties have also made the introduction of fluorine in heterocyclic structures of high interest for the pharmaceutical purpose. Indeed, fluorine and hydrogen are isosteric atoms; consequently, steric or chelating effects

able to interfere with the substrate–biological site interactions never accompany the electronic effect.

Recently, 2-fluoropyridine (**4**) has been lithiated successfully at C-6 using 3 equiv of BuLi–LiDMAE. The temperature had to be maintained at −100 °C to avoid substrate degradation.[18] 3-Fluoropyridine (**5**) has been lithiated with various basic systems and low temperatures were always employed (−40 to −78 °C). The C-2 lithiation of this substrate was less easily directed since protons at C-4 were also highly activated by the fluorine group. This implied a thorough control of temperature and the appropriate lithiating agent.[8c,d,19] A few examples of 4-fluoropyridine lithiation have been reported also at low temperatures. The formed C-3 lithio intermediate was often instable leading to pyridine formation lowering the synthetic interest of this substrate.[19] Moreover, 4-fluoropyridine is not easily available from standard commercial sources and its preparation is quite tedious.

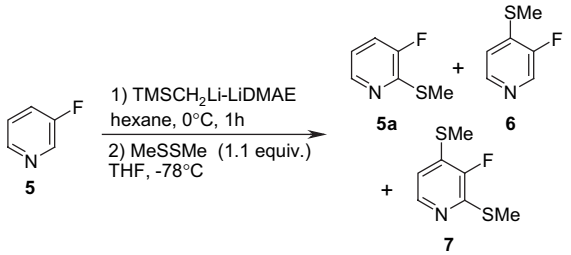
So we rather focused on the lithiation of 2-fluoropyridine **4** and 3-fluoropyridine **5**. Our first observation was surprising since **4** did not react at all with $TMSCH_2Li$ (1–2 equiv) at 0 °C in hexane indicating the low nucleophilicity of the reagent towards the substrate. This first positive outcome encouraged us to attempt the lithiation with $TMSCH_2Li$–LiDMAE. We were pleased to obtain a quantitative C-6 lithiation at 0 °C! The reaction medium did neither reveal nucleophilic addition product nor material loss due to degradation. The 2/1 $TMSCH_2Li$–LiDMAE ratio was found to be the best to ensure efficient reaction. Sulfenyl, alcohol and ketone functionalities were introduced in good yields. It must be pointed out here that the isolated yields were lower than the GC yields due to the volatility of the products (Scheme 4).



Scheme 4. Clean lithiation of **4** with $TMSCH_2Li$–LiDMAE and preparation of derivatives.

The lithiation of 3-fluoropyridine **5** was then investigated (Table 3). A first blank test was realized with $TMSCH_2Li$ at 0 °C (entry 1). The absence of nucleophilicity was clearly established since the metallation was complete and gave a mixture of C-2 and C-4 functionalized derivatives **5a** and **6**. At this stage a decrease of the metallation temperature could have been envisioned but we first decided to maintain the temperature at 0 °C and to attempt to control the selectivity only by adding LiDMAE to the basic system. Under these conditions, the incorporation of LiDMAE in the 2/1 ratio significantly favoured the C-2 versus C-4 lithiation

Table 3. Effect of the $TMSCH_2Li$–LiDMAE ratio on the lithiation selectivity of **5**[a]

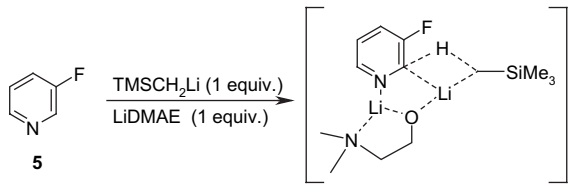


Entry	$TMSCH_2Li$ (equiv)	LiDMAE (equiv)	S.M. (%)[b]	**5a** (%)[b]	**6** (%)[b]	**7** (%)
1	2	0	—	53	45	—
2	2	1	—	61	38	—
3	1	1	—	87	12	tr
4	2	2	—	90	—	9

[a] All reactions performed on 1.84 mmol of **1**.
[b] GC yields, S.M.=starting material.

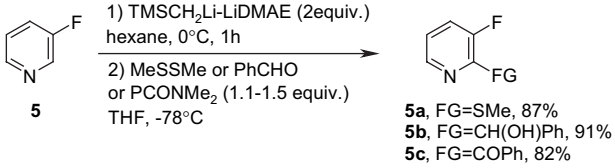
(entry 2). A stoichiometric amount of LiDMAE led to a far more selective metallation giving mainly **5a** in 87% besides **6** in 12% yield. An increase of the global base amount to 2 equiv gave the best selectivity affording **5a** in 90% yield with the formation of the bismetallation product **7**.

As shown in Table 3, the **5a**–**6** ratio was strongly dependent on the amount of LiDMAE. This underlined the severe competition between the acidities of H-4 and H-2. The $TMSCH_2Li$–LiDMAE (1/1) reagent probably ensured the formation of an aggregate with the pyridine nitrogen which was sufficiently robust to maintain the basic reagent near the H-2 proton and promote the selective lithiation (Scheme 5). This was different with the 2/1 ratio where $TMSCH_2Li$ could be released from the aggregates to deprotonate the other position.



Scheme 5. Proposed aggregate for selective C-2 lithiation.

The reaction was finally examined with a range of electrophiles leading selectively to the corresponding derivatives in excellent yields, which were found better than with 2-fluoropyridine (Scheme 6). The bismetallation product was never obtained after reaction with aldehydes of amides supporting a subsequent lithiation of **5a** during the quenching step with MeSSMe.[7]



Scheme 6. C-2 lithiation of 3-fluoropyridine.

3. Conclusion

A new superbasic reagent has been discovered by combining $TMSCH_2Li$ and LiDMAE in hexane. This reagent, when used in a 2/1 ratio was found highly efficient for the C-2 lithiation of sensitive chloro- and fluoropyridines. The metallation occurred chemoselectively at 0 °C without any trace of the nucleophilic addition commonly observed with other alkyllithiums even at lower temperatures. The usually tedious regioselective control of 3-fluoropyridine lithiation was achieved by simply adjusting the $TMSCH_2Li$–LiDMAE ratio to 1/1. Moreover, the low nucleophilicity of the base allowed using only stoichiometric amounts of electrophilic reagents. Although using the more expensive $TMSCH_2Li$, the newly reported C-2 lithiating reagent is a profound improvement of the known BuLi–LiDMAE superbase for lithiation of halopyridines. Work is now progressing to further investigate the scope of the $TMSCH_2Li$–LiDMAE superbase especially with more electrophilic heterocycles.

4. Experimental

4.1. General

All solvents were distilled and stored over sodium wire before use. 2-Dimethylaminoethanol was distilled under nitrogen and stored on molecular sieves. $TMSCH_2Li$ (FMC Lithium) was used as a 0.92 M solution in hexanes. All reagents were commercially available and used as such except for 4-chloropyridine, which was released from its hydrochloride salt by basic treatment (aq K_2CO_3). ^1H and ^{13}C NMR spectra were obtained in $CDCl_3$ (unless otherwise stated) on a Bruker AC400 instrument at 200 and 50 MHz, respectively. GC experiments were performed on a Shimadzu chromatograph (FID detection) through a 15 m capillary HP1 column. Products **1a**, **1c–e**,[4a] **1f**,[18] **2a–c**,[4b] **3a–c**[4b] and **4b**[18] were found spectroscopically identical to the previously reported compounds.

4.2. General procedure for C-2 lithiation of chloropyridines 1–3 and 2-fluoropyridine 4

$TMSCH_2Li$ (6 mL, 5.52 mmol) was added dropwise to a solution of 2-dimethylaminoethanol (164 mg, 1.84 mmol) in hexane (6 mL) at 0 °C. After 30 min of stirring, a solution of the appropriate halopyridine (1.84 mmol) in hexane (6 mL) was then added dropwise. The solution was then stirred for 1 h (2 h for **3**) at the same temperature and then treated at −78 °C with a solution of the appropriate electrophile (2.02 mmol), except for benzaldehyde (3.68 mmol), in THF (18 mL). The temperature was maintained at −78 °C for 1 h and at 0 °C for 30 min. Hydrolysis was then performed at this temperature with water (10 mL). The reaction medium was then extracted twice with ether (25 mL), the organic layer dried over $MgSO_4$ and evaporated under vacuum. The crude product was first subjected to GC analysis and purified if needed by chromatography on silica gel eluting with hexane–AcOEt mixtures using a gradient from 95/5 to 90/10.

4.2.1. 6-Chloro-pyridine-2-carboxylic acid diethylamide (1g).[20] Yield, 32%. ^1H NMR ($CDCl_3$): δ=1.35 (t, J=7.3 Hz, 6H), 3.49 (q, J=7.3 Hz, 4H), 7.38 (d, J=7.6 Hz, 1H), 7.54 (d, J=7.6 Hz, 1H), 7.75 (t, J=7.6 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ=15.4, 43.5, 121.9, 125.1, 139.7, 150.1, 155.3, 203.1.

4.2.2. Bis-(6-chloro-pyridin-2-yl)-methanone (1h). Yield, 80%. ^1H NMR ($CDCl_3$): δ=7.55 (d, J=8.0 Hz, 2H), 7.87 (t, J=7.5 Hz, 2H), 8.04 (d, J=7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ=124.1, 127.8, 139.5, 151.1, 153.7, 189.6. Anal. Calcd for $C_{11}H_6Cl_2N_2O$: C, 52.20; H, 2.39; N, 11.07%. Found: C, 51.97; H, 2.23; N, 11.16%.

4.2.3. (3-Chloro-pyridin-2-yl)-phenyl-methanol (2d).[21] Yield, 70%. ^1H NMR ($CDCl_3$): δ=5.31 (br s, 1H), 6.03 (s, 1H), 7.24–7.39 (m, 6H), 6.69 (dd, J=7.9 and 1.3 Hz, 1H), 8.57 (dd, J=4.8 and 1.3 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ= 72.1, 123.8, 126.9, 128.1, 128.4, 128.5, 130.1, 137.9, 141.7, 146.2, 157.5.

4.2.4. (4-Chloro-pyridin-2-yl)-phenyl-methanol (3d).[22] Yield, 80%. ^1H NMR ($CDCl_3$): δ=4.93 (br s, 1H), 5.72 (s, 1H), 7.17–7.41 (m, 7H), 8.44 (d, J=5.8 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ=75.2, 121.9, 124.2, 127.1, 128.7, 128.8, 142.5, 149.1, 163.2.

4.2.5. 2-Fluoro-6-methylsulfanyl-pyridine (4a).[23] Yield, 69%. ^1H NMR ($CDCl_3$): δ=2.55 (s, 3H), 6.59 (dd, J=6.9 and 0.9 Hz, 1H), 7.07 (dd, J=7.9 and 1.9 Hz, 1H), 7.59 (d, J=7.9 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ=12.5, 104.1, 118.5, 140.65, 157.5, 163.4.

4.2.6. (6-Fluoro-pyridin-2-yl)-phenyl-methanone (4c). Yield, 62%. ^1H NMR ($CDCl_3$): δ=7.15 (dd, J=8.1 and 1.2 Hz, 1H), 7.46–7.60 (m, 2H), 7.62 (q, J=6.9 Hz, 1H), 7.94–8.10 (m, 4H). ^{13}C NMR ($CDCl_3$): δ=112.8, 113.4, 122.3, 128.1, 131.2, 133.5, 142.2, 142.4, 160.2, 164.1, 192.1 ppm. Anal. Calcd for $C_{12}H_8FNO$: C, 71.64; H, 4.01; N, 6.96%. Found: C, 71.35; H, 4.23; N, 6.69%.

4.3. General procedure for C-2 lithiation of 3-fluoropyridine 5

$TMSCH_2Li$ (8 mL, 7.36 mmol) was added dropwise to a solution of 2-dimethylaminoethanol (328 mg, 3.68 mmol) in hexane (8 mL) at 0 °C. After 30 min of stirring, a solution of **5** (178 mg, 1.84 mmol) in hexane (8 mL) was then added dropwise. The solution was then stirred for 1 h at the same temperature and then treated at −78 °C with a solution of the appropriate electrophile (1.1 or 1.5 mmol for benzaldehyde) in THF (24 mL). The temperature was maintained at −78 °C for 1 h and at 0 °C for 30 min. Hydrolysis was then performed at this temperature with water (3 mL). The reaction medium was then extracted with ether (10 mL), the organic layer dried over $MgSO_4$ and evaporated under vacuum. The crude product was first subjected to GC analysis and purified if needed by eluting with hexane–AcOEt mixtures using a gradient from 95/5 to 90/10.

4.3.1. 3-Fluoro-2-methylsulfanyl-pyridine (5a). Yield, 87%. ^1H NMR ($CDCl_3$): δ=2.58 (s, 3H), 6.99 (m, 1H), 7.26 (dt, J=8.5 and 0.9 Hz, 1H), 8.28 (dd, J=5.9 and 1.5 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ=12.1, 119.7, 120.6, 120.9, 145.1, 148.3, 158.6 ppm. Anal. Calcd for C_6H_6FNS: C, 50.33; H, 4.22; N, 9.78%. Found: C, 50.47; H, 4.18; N, 9.52%.

4.3.2. (3-Fluoro-pyridin-2-yl)-phenyl-methanol (5b). Yield, 91%. ^{1}H NMR ($CDCl_3$): δ=5.35 (s, 1H), 5.98 (s, 1H), 7.14–7.41 (m, 7H), 8.36 (dd, J=4.6 and 1.5 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ=70.3, 123.5, 123.8, 124.2, 126.9, 127.9, 128.6, 142.3, 144.0, 149.1, 154.3, 158.4 ppm. Anal. Calcd for $C_{12}H_{10}FNO$: C, 70.93; H, 4.96; N, 6.89%. Found: C, 70.68; H, 4.83; N, 6.93%.

4.3.3. (3-Fluoro-pyridin-2-yl)-phenyl-methanone (5c). Yield, 87%. ^{1}H NMR ($CDCl_3$): δ=7.44–7.63 (m, 5H), 7.92 (d, J=4.9 Hz, 6H), 8.52 (dd, J=5.7 and 1.4 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ=124.7, 125.1, 127.0, 128.5, 130.5, 133.9, 135.9, 144.9, 156.1, 160.2, 191.1 ppm. Anal. Calcd for $C_{12}H_8FNO$: C, 71.64; H, 4.01; N, 6.96%. Found: C, 71.57; H, 4.10; N, 7.04%.

Acknowledgements

The authors would like to thank FMC Corporation, Lithium Division for financial support.

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The reaction of 4-amino-2-oxazolines with isocyanates and isothiocyanates. Synthesis and X-ray structures of polysubstituted 2-imidazolidinones, 1,3-oxazolidines and 1,3-thiazolidines

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Received 14 March 2006; revised 10 April 2006; accepted 20 April 2006
Available online 11 May 2006

Abstract—Reactions of 4-alkylamino-2-phenyl-2-oxazolines **1** with isocyanates and isothiocyanates provide unprecedented efficient and regioselective heterocycle–heterocycle transformations. Compounds **1** reacted rapidly with tosyl isocyanate yielding directly 3-alkyl-4-benzamido-1-tosyl-2-imidazolidinones **4** in almost quantitative yields. The corresponding ureido intermediates **2** were not isolable species. However, the reactions with non-sulfonylated isocyanates or isothiocyanates were slower, leading to the expected ureido and thioureido derivatives **5**, which were easily and efficiently transformed to either polysubstituted 2-imino-1,3-oxazolidine or 2-imino-1,3-thiazolidine hydrochlorides **7**, respectively, by treatment with hydrochloric acid. The possible reasons for this disparity in chemical behaviour are discussed. X-ray crystallographic structures for 4-benzamido-3-methyl-1-tosyl-2-imidazolidinone **4b**, 4-[1-isopropyl-3-(4-nitrophenyl)ureido]-2-phenyl-2-oxazoline **5e**, (*Z*)-3-benzyl-4-benzamido-2-phenylimino-1,3-oxazolidine hydrochloride **7a** and (*Z*)-3-benzyl-4-benzamido-2-phenylimino-1,3-thiazolidine hydrochloride **7b** have been determined.

1. Introduction

2-Oxazolines are remarkably versatile synthetic intermediates.[1–4] The vast number of transformations[1] reported over the last few years have led to a renewed interest in the chemistry of these compounds. However, only a few examples of transformations of 2-oxazolines into other heterocycles have been described; most work on this subject has been focused on either hydrogenation or dehydrogenation processes to give products retaining the original ring system.[1]

As a result of our research project on new methods for the synthesis of heterocyclic compounds, based on using chloral as a key starting material, we developed an efficient and general preparative procedure that provided novel 4-amino-2-aryl-2-oxazolines[5,6] **1** from chloralamides. Continuing this project, we focused the work on the reactions of these compounds with isocyanates and isothiocyanates. Given the peculiar structural arrangement of the expected ureido and thioureido derivatives, it was considered that these reactions could be the starting point for attractive approaches to novel heterocyclic compounds. In preliminary communication[7,8] we reported the successful application of this synthetic methodology for preparing novel 2-imidazolidinones, 1,3-oxazolidines and 1,3-thiazolidines. In this paper we describe full details of this work and new results of the preparative procedures, together with spectral and X-ray crystallographic data for the hitherto unknown classes of compounds newly accessed. Differences in chemical behaviour observed for ureido and thioureido intermediates may be attributed to crucial electronic effects associated with the presence or absence of the sulfonyl group.

2. Results and discussion

4-Alkylamino-2-aryl-2-oxazolines **1** were treated with *p*-toluenesulfonyl isocyanate in ether solution at room temperature (Scheme 1). The reactions occurred quickly under such mild experimental conditions. The instantaneous formation of a white solid precipitate was observed in all cases. The resulting voluminous crude solid products were isolated by filtration and identified by the usual analytical methods

Keywords: Oxazolines; Ureas; Thioureas; Imidazolidinones; Oxazolidines; Thiazolidines.
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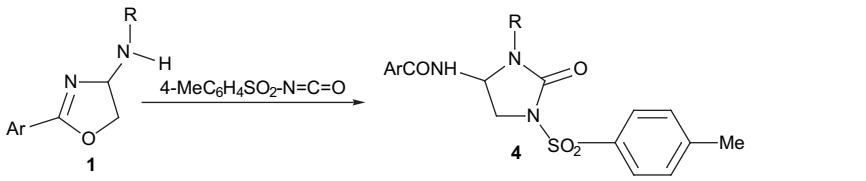
doi:10.1016/j.tet.2006.04.058



	Ar	R
a	C_6H_5	$C_6H_5CH_2$
b	C_6H_5	CH_3
c	C_6H_5	$(CH_3)_2CH$
d	3-ClC_6H_4	$C_6H_5CH_2$
e	3,4,5-$(CH_3O)_3C_6H_2$	$C_6H_5CH_2$

Scheme 1.

as highly pure 3-alkyl-4-benzamido-1-tosyl-2-imidazolidinones **4**. Yields were nearly quantitative. IR and NMR spectra for crude and recrystallized products showed negligible differences.

The structural assignment of these compounds was corroborated by X-ray crystallographic analysis of 4-benzamido-3-methyl-1-tosyl-2-imidazolidinone **4b**. The molecular structure is illustrated in Figure 1. Selected intramolecular distances (crystallographic numbering of atoms) and selected bond angles are given in Table 1. Suitable single crystals for this analysis were obtained from hexane/chloroform and contained one molecule of chloroform per molecule of **4b**. A thermogravimetric analysis of this compound showed a sharp peak at 106.2 °C, corresponding to quantitative loss of chloroform. The crystallographic analysis showed the crystal packing to be determined by N–H···O and Cl···O interactions.

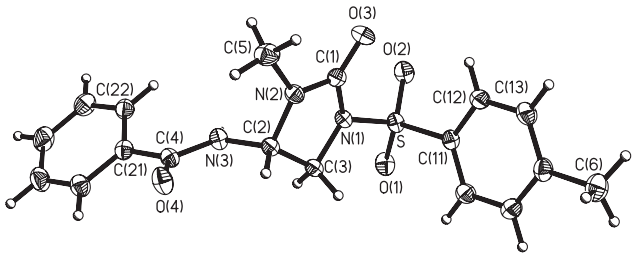


Figure 1. Molecular structure of product **4b**, showing the crystallographic numbering system used.

A particularly facile rearrangement of the ureido intermediates **2** to imidazolidinones **4** appears as a key step in this transformation (Scheme 1). However, ureido derivatives are, in general, stable crystalline compounds that can be used for the separation and characterization of amines. In contrast, 2-oxazolines are characterized by a high reluctance to undergo alteration of the ring system by nucleophilic attacks, and are indeed commonly used as protecting or activating groups in strongly nucleophilic media.[9] Therefore, the pronounced lability evidenced by intermediates **2** indicates the existence of some special factors that promote a remarkable enhancement of electrophilic activity at C-5. A reasonable explanation for this might be a protonic autoactivation induced by the sulfonyl group, which would generate an internal acidic centre. In conjunction with the cogeneration of a benzamido group, this would strongly facilitate an

Table 1. Selected bond lengths and bond angles in crystal structure of **4b**

Lengths (Å)

O(3)–C(1)	1.213(2)	O(4)–C(4)	1.229(2)
N(1)–C(1)	1.418(2)	N(1)–C(3)	1.474(2)
N(2)–C(1)	1.349(2)	N(2)–C(5)	1.453(2)
N(2)–C(2)	1.458(2)	N(3)–C(4)	1.351(2)
N(3)–C(2)	1.449(2)	C(2)–C(3)	1.537(2)

Angles (°)

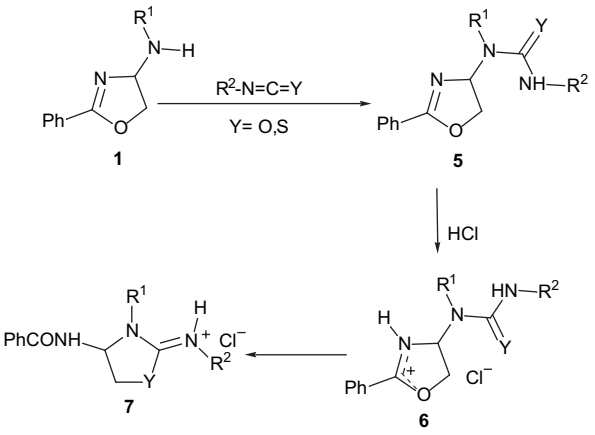
C(1)–N(1)–C(3) 110.l9(13)	C(3)–N(1)–S 120.49(11)	C(1)–N(2)–C(2) 113.88(13)
C(4)–N(3)–C(2) 122.79(14)	O(3)–C(1)–N(2) 127.28(16)	N(3)–C(2)–N(2) 112.19(13)
N(2)–C(2)–C(3) 102.29(12)	C(1)–N(1)–S 122.86(11)	C(1)–N(2)–C(5) 121.19(14)
C(5)–N(2)–C(2) 123.46(14)	O(3)–C(1)–N(2) 127.3(2)	N(2)–C(1)–N(1) 106.57(13)
N(3)–C(2)–C(3) 112.11(13)	N(1)–C(3)–C(2) 102.25(13)	

intramolecular attack at that site. Therefore, a rearrangement process involving a ring opening of the oxazolinic intermediates **3** with simultaneous ring closure to form the corresponding imidazolidinones **4** could take place easily in this way. This type of rearrangement is as yet unknown in the literature. A paper containing a remotely related process reported the conversion of 5-aryl(or benzyl)-3-(2-bromoethyl)1,3,4-oxadiazol-2(3*H*)ones to 1-acylamino-3-alkylimidazolin-2-ones by treatment with primary alkylamines.[10c]

The isolation of the presumably labile sulfonylureido intermediates **2** was attempted by carrying out the reactions at subambient temperature. Thus, oxazolines **1d**,**e** reacted with tosyl isocyanate at 0 °C yielding the targeted ureido derivatives **2d**,**e** instead of imidazolines **4d**,**e**. These products demonstrated a great tendency towards the postulated rearrangement to give the corresponding imidazolidinones **4d**,**e**. In agreement with this discussion, compounds **2d**,**e** showed mass spectra and melting points fully coincident with those of **4d**,**e**. However, they were clearly distinguishable by analyzing the samples with non-aggressive low-temperature analytical techniques such as IR and NMR spectroscopy.

2-Imidazolidinones[11] are compounds of great interest. As far as we know, direct conversion of 2-oxazolines into 2-imidazolidinones has not yet been reported. The wide variety of 2-oxazolines **1** available[5,6] confers a high versatility on this new approach to specifically polysubstituted 2-imidazolidinones. The most extended methodology for preparing 2-imidazolidinones involves carbonylation of diamines with various reagents[12] and reactions of diols,[12a] aminols[12a] and 1,2-dicarbonyl compounds[13] with urea. In general, the limitations found for these procedures are caused by lack of mildness in the required experimental conditions or a deficient regiocontrol in the formation of more complex products. It should be noted that the synthesis of specifically substituted 2-imidazolidinones has received significant attention.[10] It is also worth considering that certain polysubstituted 2-imidazolidinones exhibit a wide range of therapeutic[14] and other important properties and applications.[15,16] Of special importance is the anticancer activity[14c,17] found in several *N*-arylsulfonyl-4-phenyl-2-imidazolidinones whose structure is closely related to that of the products **4** described here.

The above results strongly favoured the study of the behaviour of the oxazolines **1** in reactions with simple aryl isocyanates instead of tosyl isocyanate, since the role as activator agent presumed for the sulfonyl group of ureides **2** would be evidenced by these experiments. Thus, aminooxazolines **1** were treated with aryl isocyanates in dry ether at room temperature (Scheme 2). In contrast to the reactions with *p*-toluenesulfonyl isocyanate, which occurred almost instantaneously, a longer reaction time was necessary, although solid precipitates were also formed. These were easily isolated by filtration, and were identified as highly pure 4-[1-alkyl-3-arylureido]-2-phenyl-2-oxazolines **5** rather than products analogous to compounds **4**. IR and NMR spectra for crude and crystallized products showed negligible differences. Yields were almost quantitative. The molecular structure of one of these novel compounds, 4-[1-isopropyl-3-(4-nitrophenyl)ureido]-2-phenyl-2-oxazoline **5e**, was determined by X-ray crystallography. The molecular structure is illustrated in Figure 2. Selected intramolecular distances (crystallographic numbering of atoms) and selected bond angles are given in Table 2.



entry	Y	R^2	R^1
a	O	C_6H_5	$C_6H_5CH_2$
b	S	C_6H_5	$C_6H_5CH_2$
c	S	$4\text{-}CH_3OC_6H_5$	$C_6H_5CH_2$
d	O	$4\text{-}ClC_6H_4$	$C_6H_5CH_2$
e	O	$4\text{-}NO_2C_6H_4$	$(CH_3)_2CH$
f	O	$C_6H_5CH_2$	$C_6H_5CH_2$
g	O	$(CH_3)_2CH$	$C_6H_5CH_2$
h	O	$C_6H_5CH_2$	$(CH_3)_2CH$
i	O	$CH_3CH_2CH_2$	$C_6H_5CH_2$

Scheme 2.

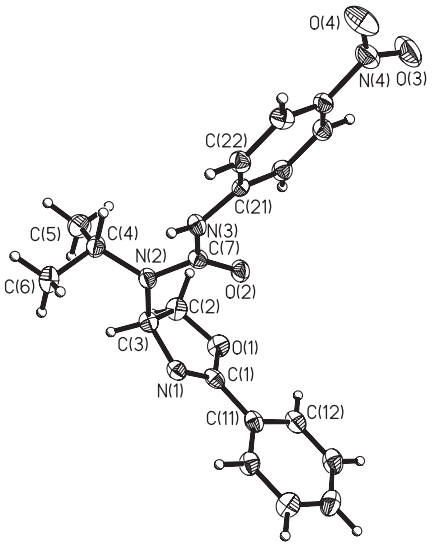


Figure 2. Molecular structure of product **5e**, showing the crystallographic numbering system used.

Compounds **5** were much more stable than the tosylated intermediates **2**. In contrast to the lability exhibited by compounds **2d**,**e**, the non-sulfonylated ureido derivatives **5** were stable enough to be handled in solution and to permit prolonged storage without need of any special care. Given the absence of the sulfonyl group, this relative stability appears to be attributable to the lack of the protonic autoactivation postulated above. In order to seek a firm support to this

Table 2. Selected bond lengths and bond angles in crystal structure of **5e**

Lengths (Å)			
O(1)–C(1)	1.3515(18)	N(1)–C(3)	1.4697(19)
N(2)–C(3)	1.4608(18)	C(2)–C(3)	1.541(2)
O(1)–C(2)	1.4485(19)	N(1)–C(1)	1.2796(19)

Angles (°)		
C(1)–O(1)–C(2) 106.00(11)	N(1)–C(1)–C(11) 125.60(14)	O(1)–C(2)–C(3) 104.31(12)
C(1)–N(1)–C(3) 106.96(13)	N(1)–C(1)–O(1) 118.39(14)	O(1)–C(1)–C(11) 116.01(13)
N(2)–C(3)–N(1) 111.33(12)	N(1)–C(3)–C(2) 104.10(12)	

hypothesis, it was considered that the treatment of ureides **5** with a mineral acid would circumvent the non-presence of the sulfonyl group. Consequently, solutions of compounds **5** in ether were treated with concentrated hydrochloric acid resulting in almost instantaneous reactions with formation of solid precipitates. The products were crystallized and characterized as the corresponding 2-arylimino-1,3-oxazolidine or 2-arylimino-1,3-thiazolidine hydrochlorides **7**. Crude and crystallized products showed negligible spectroscopic differences. Yields were almost quantitative. The molecular structure of one of these compounds, (*Z*)-3-benzyl-4-benzamido-2-phenylimino-1,3-oxazolidine hydrochloride **7a**, was corroborated by X-ray crystallography. The structure is illustrated in Figure 3. Selected intramolecular distances (crystallographic numbering of atoms) and selected bond angles are given in Table 3. It is of significant interest that similar treatment of ureides **2d**,**e** with hydrochloric acid provided the corresponding imidazolidinones **4d**,**e** instead of oxazolidine hydrochloride products.

Analogous reactions of aminooxazolines **1** with isothiocyanates instead of isocyanates were carried out, providing parallel results. Highly pure 4-[1-alkyl-3-arylthioureido]-2-phenyl-2-oxazolines **5** were isolated in high yields. Such compounds are also indefinitely stable, but were quickly and quantitatively convertible to (*Z*)-3-alkyl-4-benzamido-2-phenylimino-1,3-thiazolidine hydrochlorides **7** by treatment with hydrochloric acid. The molecular structure of one of these compounds, (*Z*)-3-benzyl-4-benzamido-2-phenylimino-1,3-thiazolidine hydrochloride **7b**, was determined by X-ray crystallography. The structure is illustrated in Figure 4. Selected intramolecular distances (crystallographic numbering of atoms) and selected bond angles are given in Table 4.

There are no precedents for compounds of type **7**. As far as we know, this is the first time that direct conversions of 2-oxazoline rings to either 1,3-oxazolidine or 1,3-thiazolidine ring systems have been reported. The high versatility of the reactions[5,6] involved in preparing the starting materials **1** implies a concomitantly wide versatility for these synthetic approaches. The described preparative procedures in separate steps could also be carried out in a one-pot process, which allows quick and highly efficient preparations of the same compounds. It should be noted that the synthesis of either 2-iminooxazolidines or 2-iminothiazolidines has

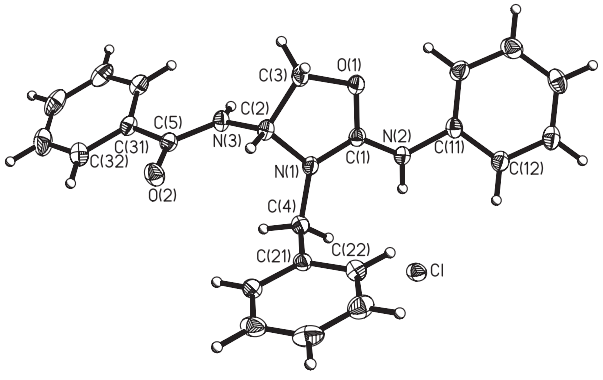


Figure 3. Molecular structure of product **7a**, showing the crystallographic numbering system used.

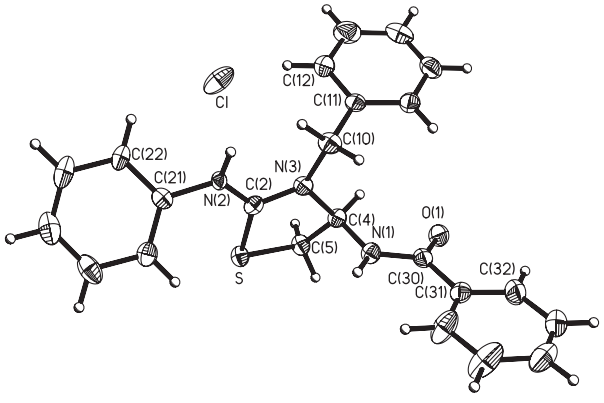


Figure 4. Molecular structure of product **7b**, showing the crystallographic numbering system used.

Table 3. Selected bond lengths and bond angles in crystal structure of **7a**

Lengths (Å)			
C(1)–O(1)	1.3182(14)	C(1)–N(1)	1.3204(14)
C(1)–N(2)	1.3241(15)	C(2)–N(3)	1.4350(14)
C(2)–N(1)	1.4776(14)	C(2)–C(3)	1.5331(17)
C(3)–O(1)	1.4632(14)		

Angles (°)		
N(1)–C(2)–C(3) 100.70(9)	O(1)–C(3)–C(2) 105.66(9)	C(1)–N(1)–C(4) 128.23(10)
C(1)–N(1)–C(2) 110.87(9)	C(4)–N(1)–C(2) 120.73(9)	C(1)–N(2)–C(11) 126.77(10)
C(1)–O(1)–C(3) 108.24(9)	C(5)–N(3)–C(2) 122.28(9)	O(1)–C(1)–N(1) 113.88(10)
O(1)–C(1)–N(2) 120.03(10)	N(1)–C(1)–N(2) 126.08(10)	N(3)–C(2)–N(1) 111.29(9)
N(3)–C(2)–C(3) 113.76(9)		

Table 4. Selected bond lengths and bond angles in crystal structure of **7b**

Lengths (Å)

S–C(2)	1.7448(13)	S–C(5)	1.8135(12)
C(2)–N(2')	1.323(7)	C(2)–N(3)	1.3338(16)
N(3)–C(4)	1.4635(15)	N(3)–C(10)	1.4802(16)
C(4)–N(1)	1.4518(16)	C(4)–C(5)	1.5226(17)
C(10)–C(11)	1.5047(19)	N(2')–C(21')	1.427(7)
C(30)–O(1)	1.2331(16)	C(30)–N(1)	1.3557(16)
C(30)–C(31)	1.4988(19)		

Angles (°)

C(2)–S–C(5)	90.45(6)	N(2')–C(2)–N(3)	129.5(3)	N(2')–C(2)–S	116.7(3)
N(3)–C(2)–S	113.75(9)	C(2)–N(3)–C(4)	114.62(10)	C(2)–N(3)–C(10)	125.97(10)
C(4)–N(3)–C(10)	117.41(10)	N(1)–C(4)–N(3)	109.57(9)	N(1)–C(4)–C(5)	115.03(11)
N(3)–C(4)–C(5)	105.64(10)	C(4)–C(5)–S	106.30(8)	N(3)–C(10)–C(11)	110.64(10)
C(2)–N(2')–C(21')	127.7(6)	O(1)–C(30)–N(1)	120.70(13)	O(1)–C(30)–C(31)	120.88(12)
N(1)–C(30)–C(31)	118.42(12)				

special interest because certain members of these types of compound exhibit important therapeutic and biological activities.[18]

2-Imino-1,3-oxazolidines have usually been prepared by either dehydration of *N*-(β-hydroxyethyl)-*N*,*N*′-diarylureas[19] or reaction of oxiranes with carbodiimides,[20] whereas syntheses of 2-iminothiazolidines are based on reactions of 1,2-dihaloalkanes with thioureas,[21] treatment of 2-haloamines or γ-halocrotonic acid derivatives with isothiocyanates,[22] cycloaddition between aziridines and isothiocyanates[23] and treatment of α-haloketone imines with potassium thiocyanate.[24]

With regard to the relatively high electrophilic activity of the oxazoline moieties (Schemes 1 and 2), the evidence described above clearly shows that both protonation and generation of a benzamido group would be the main factors operating to facilitate the unusual nucleophilic attacks occurring at C-5. Consequently, in each type of rearrangement, analogous activated intermediates (**3** or **6**) would be involved. However, this primary assumption needs to be considered in conjunction with the participation of either nitrogen or oxygen and sulfur as nucleophilic active centres. From this perspective it can be concluded that nucleophilicities of the ureido moieties are crucially influenced by electronic differences substantial enough to cause a total disparity in the results. The different reaction modalities may thus be explained taking into consideration that ureas and thioureas are attacked[25] by electrophilic agents, preferentially at the oxygen or sulfur atoms, respectively; this seems mainly attributable to a relatively high electron density at such heteroatoms. This is the case for the formation of products **7** via intermediates **6**. The formation of products **4** via intermediates **3**, however, would be a consequence of the strong electron withdrawing effect of the sulfonyl group, which would substantially decrease the nucleophilicity at the oxygen.

In conclusion, the first direct conversions of 2-oxazolines into either 2-imidazolidinones or 1,3-oxazolidines **7** (Y=O) or 1,3-thiazolidines **7** (Y=S) derivatives are reported. The formation of these products involves hitherto unknown rearrangement processes. The preparative methods described here are of significant synthetic utility, providing directly polysubstituted heterocyclic products of potential biological interest with a full control of the substitution pattern. Versatility, good yields, easy availability of starting materials, mildness and simple experimental procedure are noteworthy advantages of these approaches, which provide potential access to previously unattainable compounds. It seems feasible to extend the described synthetic methodology to prepare a wide variety of heterocyclic compounds.

3. Experimental

3.1. General

NMR spectra were determined on Bruker AC-200 or Varian Unity 300 Unity instruments with tetramethylsilane as internal reference. Electron-impact mass spectra were obtained on Hewlett–Packard 5995 and Autospect 5000 VG spectrometers under an ionizing voltage of 70 eV. IR spectra (Nujol emulsions) were recorded on a Nicolet Impact 400 spectrophotometer. Microanalyses were performed on a Carlo Erba EA-1108 analyzer. Melting points were determined on a Kofler hot-plate melting point apparatus, and are uncorrected.

X-ray crystallographic data were collected using Mo Kα radiation (λ=0.71073 Å). For compounds **4b** and **5e** a Siemens P4 diffractometer was used (ω-scans, $2\theta_{max}$ 55° and 50°, respectively); for **7a** and **7b** a Bruker SMART 1000 CCD (ω- and ø-scans, $2\theta_{max}$ 60°). Structures were refined anisotropically on F^2 using the program SHELXL-97 (G.M. Sheldrick, University of Göttingen). Hydrogen atoms were included using rigid methyl groups or a riding model, except for compound **4b** C-5 methyl group which is disordered over two sites. Crystal data are given below for each compound individually.

3.2. Preparation of 4-benzamido-3-alkyl-1-tosyl-2-imidazolidinones (4) and 4-(1-alkyl-3-tosylureido)-2-aryl-2-oxazolines (2)

A solution of *p*-toluenesulfonyl isocyanate (1 mmol) in dry ether (3 mL) was added dropwise at room temperature to a stirred solution of the appropriate aminooxazoline **1** (1 mmol) in dry ether (5 mL). A white precipitate formed almost instantaneously. The solid products **4** were filtered off, washed with cold ether and crystallized from a mixture of hexane/chloroform or hexane/dichloromethane. The reactions carried out at 0 °C yielded the ureido intermediates **2** instead of the final products **4**.

3.2.1. 4-(1-Benzyl-3-tosylureido)-2-(3-chlorophenyl)-2-oxazoline (2d). Yield 88%; crystallization from petroleum ether/dichloromethane gave white powder; mp 222–223 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 2.43 (s, 3H), 4.20 (dd, 1H, J=10.0 Hz, J=6.3 Hz), 4.28 (d, 1H, J=17.1 Hz), 4.39 (d, 1H, J=17.1 Hz), 4.49 (t, 1H, J=10.0 Hz), 6.35–6.37 (m, 1H), 7.26–7.49 (m, 11H), 7.75–7.87 (m, 3H); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ (ppm): 21.73 (CH_3), 46.49 (CH_2), 71.66 (CH_2), 77.83 (CH), 126.87 (CH), 126.99 (CH), 128.22 (C), 128.34 (CH), 128.44 (CH), 128.84 (CH), 129.41 (CH), 129.50 (CH), 129.93 (CH), 132.50 (CH), 134.71 (C), 136.14 (C), 136.28 (C), 144.63 (C), 151.99 (CO), 166.30 (C=N); MS (EI, 70 eV) m/z (rel intensity, %): 483 [M$^+$] (1), 392 (23), 328 (94), 173 (66), 155 (15), 139 (86), 131 (43), 111 (44), 106 (48), 91 (100), 77 (9), 65 (31); IR (Nujol) ν_{max}/cm^{-1} 3321, 1678, 1647, 1462, 1349, 1246, 1165, 1088, 1069, 967, 713, 664; Anal. Calcd for $C_{24}H_{22}ClN_3O_4S$ (483.97): C, 59.56; H, 4.58; N, 8.68; S, 6.63. Found: C, 59.43; H, 4.61; N, 8.72; S, 6.51.

3.2.2. 4-(1-Benzyl-3-tosylureido)-2-(3,4,5-trimethoxyphenyl)-2-oxazoline (2e). Yield 96%; crystallization from petroleum ether/dichloromethane gave white powder; mp 247–250 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 2.43 (s, 3H), 3.85 (s, 6H), 3.88 (s, 3H), 4.20 (dd, 1H, J=10.0 Hz, J=6.0 Hz), 4.29 (d, 1H, J=15.9 Hz), 4.39 (d, 1H, J=16.0 Hz), 4.45 (t, 1H, J=10.0 Hz), 6.29–6.32 (m, 1H), 7.15 (s, 2H), 7.24–7.36 (m, 7H), 7.78 (d, 2H, J=8.0 Hz); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ (ppm): 21.69 (CH_3), 46.67 (CH_2), 56.32 (CH_3O), 60.99 (CH_3O), 71.66 (CH_2), 77.88 (CH), 106.08 (CH), 121.33 (CH), 127.02 (CH), 128.17 (CH), 128.40 (CH), 129.24 (CH), 129.44 (CH), 136.30 (C), 136.45 (C), 141.92 (C), 144.51 (C), 152.07 (C), 153.18 (C), 167.34 (C=N); MS (EI, 70 eV) m/z (rel intensity, %): 539 [M$^+$] (7), 384 (2), 328 (51), 211 (26), 195 (69), 173 (38), 106 (5), 91 (100), 77 (4), 65 (9); IR (Nujol) ν_{max}/cm^{-1} 1692, 1630, 1586, 1467, 1382, 1345, 1228, 1165, 1129, 1084, 1013, 819, 724, 681; Anal. Calcd for $C_{27}H_{29}N_3O_7S$ (539.60): C, 60.10; H, 5.42; N, 7.79; S, 5.94. Found: C, 59.98; H, 5.46; N, 9.84; S, 5.89.

3.2.3. 3-Benzyl-4-benzamido-1-tosyl-2-imidazolidinone (4a). Yield 95%; crystallization from petroleum ether/chloroform gave white needles; mp 215–216 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 2.47 (s, 3H), 3.90–3.98 (m, 2H), 4.23 (d, 1H, J=15.0 Hz), 4.53 (d, 1H, J=15.3 Hz), 5.80 (m, 1H), 7.18–7.53 (m, 11H), 7.77 (d, 2H, J=7.8 Hz), 7.88 (d, 2H, J=8.1 Hz); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ (ppm): 21.80 (CH_3), 45.48 (CH_2), 49.53 (CH_2), 58.69 (CH), 127.49 (CH), 127.95 (CH), 128.15 (CH), 128.48 (CH), 128.59 (CH), 128.82 (CH), 129.89 (CH), 132.22 (CH), 133.15 (C), 134.55 (C), 136.24 (C), 145.23 (C), 153.49 (CO), 167.59 (CO); MS (EI, 70 eV) m/z (rel intensity, %): 449 [M$^+$] (2), 358 (6), 328 (55), 294 (11), 173 (37), 155 (5), 131 (27), 105 (95), 91 (100), 77 (40); IR (Nujol) ν_{max}/cm^{-1} 3311, 1743, 1640, 1523, 1464, 1377, 1166, 1123, 1098 cm^{-1}; Anal. Calcd for $C_{24}H_{23}N_3O_4S$ (449.52): C, 64.13; H, 5.16; N, 9.35; S, 7.13. Found: C, 64.23; H, 5.20; N, 9.39; S, 7.09.

3.2.4. 4-Benzamido-3-methyl-1-tosyl-2-imidazolidinone (4b). Yield 97%; crystallization from hexane/chloroform gave colourless prisms; mp 216–218 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 2.45 (s, 3H), 2.79 (s, 3H), 3.90 (dd, 1H, J=10.2 Hz, J=8.4 Hz), 4.05 (dd, 1H, J=10.3 Hz, J=2.4 Hz), 5.87 (td, 1H, J=8.4 Hz, J=2.4 Hz), 7.29 (d, 2H, J=8.1 Hz), 7.42 (t, 2H, J=7.8 Hz), 7.55 (t, 1H, J=5.1 Hz), 7.83 (d, 2H, J=8.4 Hz), 7.97–8.03 (m, 3H); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ (ppm): 21.68 (CH_3), 28.00 (CH_3), 48.72 (CH_2), 59.69 (CH), 127.68 (CH), 127.92 (CH), 128.50 (CH), 129.82 (CH), 132.05 (CH), 133.31 (C), 134.50 (C), 145.04 (C), 153.41 (CO), 168.00 (CO); MS (EI, 70 eV) m/z (rel intensity, %): 373 [M$^+$] (1), 252 (19), 218 (20), 155 (13), 105 (100), 91 (47), 77 (41); IR (Nujol) ν_{max}/cm^{-1} 3310, 1732, 1667, 1537, 1464, 1366, 1265, 1171, 1134, 857, 814, 760, 666; Anal. Calcd for $C_{19}H_{20}Cl_3N_3O_4S \equiv C_{18}H_{19}N_3O_4S(CHCl_3)$ (492.80): C, 46.31; H, 4.09; N, 8.53; S, 6.51. Found: C, 46.24; H, 4.11; N, 8.49; S, 6.58.

Crystallographic data for **4b**. $CHCl_3$: $C_{19}H_{20}Cl_3N_3O_4S$, triclinic, space group $P(-1)$, a=5.9314(6), b=13.013(2), c=15.650(2) Å, α=109.983(8), β=99.405(10), γ=96.709(10)°, V=1100.4 Å^3, Z=2. A colourless prism 0.60×0.38×0.28 mm was used to measure 5540 reflections at T=173 K, of which 4945 were unique (R_{int}=0.010). Refinement proceeded to wR_2=0.0948 (all data), R_1=0.0351 and GOF=1.07 [$I>2\sigma(I)$]. Maximum residual electron density was 0.82 eÅ^{-3}.

3.2.5. 4-Benzamido-3-isopropyl-1-tosyl-2-imidazolidinone (4c). Yield 96%; crystallization from petroleum ether/chloroform gave white micro needles; mp 222–224 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.22 (m, 6H), 2.45 (s, 3H), 3.84–4.03 (m, 3H), 5.98 (t, 1H, J=8.4 Hz), 7.26–7.56 (m, 6H), 7.84–7.90 (m, 4H); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ (ppm): 19.23 (CH_3), 21.34 (CH_3), 21.78 (CH_3), 45.47 (CH), 50.40 (CH_2), 57.05 (CH), 127.39 (CH), 128.24 (CH), 128.80 (CH), 129.84 (CH), 132.31 (CH), 133.33 (C), 134.60 (C), 145.09 (C), 152.78 (CO), 166.93 (CO); MS (EI, 70 eV) m/z (rel intensity, %): 401 [M$^+$] (1), 358 (31), 280 (75), 265 (24), 216 (8), 155 (6), 125 (87), 105 (100), 91 (90), 83 (70), 77 (61); IR (Nujol) ν_{max}/cm^{-1} 3405, 1723, 1667, 1526, 1464, 1351, 1240, 1225, 1171, 1138, 1108, 716, 669; Anal. Calcd for $C_{20}H_{23}N_3O_4S$ (401.48): C, 59.83; H, 5.77; N, 10.47; S, 7.99. Found: C, 59.90; H, 5.81; N, 10.41; S, 8.05.

3.2.6. 3-Benzyl-4-(3-chlorobenzamido)-1-tosyl-2-imidazolidinone (4d). Yield 98%; crystallization from petroleum ether/dichloromethane gave white needles; mp 220–221 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 2.47 (s, 3H), 3.85 (dd, 1H, J=10.5 Hz, J=8.1 Hz), 3.99 (dd, 1H, J=10.0 Hz, J=2.1 Hz), 4.23 (d, 1H, J=15.0 Hz), 4.56 (d, 1H, J=15.0 Hz), 5.78 (td, 1H, J=8.7 Hz, J=2.1 Hz), 7.16–7.36 (m, 8H), 7.50 (d, 1H, J=8.1 Hz), 7.70–7.88 (m, 5H); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ (ppm): 21.79 (CH_3), 45.44 (CH_2), 49.37 (CH_2), 58.56 (CH), 125.92 (CH), 127.80 (CH), 128.09 (CH), 128.49 (CH), 128.85 (CH), 129.88 (CH), 132.17 (CH), 134.29 (C), 134.75 (C), 135.09 (C), 136.13 (C), 145.34 (C), 153.68 (CO), 166.39 (CO); MS (EI, 70 eV) m/z (rel intensity, %): 483 [M$^+$] (1), 328 (34), 173 (30), 155 (6), 139 (68), 106 (20), 91 (100), 77 (5); IR (Nujol) ν_{max}/cm^{-1} 3297, 1745, 1639, 1529, 1466, 1365, 1254, 1165, 1106, 810, 747, 703; Anal. Calcd for $C_{24}H_{22}ClN_3O_4S$ (483.97): C, 59.56; H, 4.58; N, 8.68; S, 6.63. Found: C, 59.38; H, 4.63; N, 8.61; S, 6.61.

3.2.7. 3-Benzyl-4-(3,4,5-trimethoxybenzamido)-1-tosyl-2-imidazolidinone (4e). Yield 94%; crystallization from hexane/dichloromethane gave white needles; mp 250 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.44 (s, 3H), 3.71 (s, 3H), 3.82 (m, 7H), 4.17 (d, 1H, J=15.3 Hz), 4.22 (t, 1H, J=9.3 Hz), 4.38 (d, 1H, J=15.0 Hz), 5.69 (td, 1H, J=8.5 Hz, J=3.3 Hz), 7.09–7.12 (m, 4H), 7.22–7.24 (m, 3H), 7.48 (d, 2H, J=8.0 Hz), 7.90 (d, 2H, J=8.0 Hz), 9.10 (d, 1H, J=8.5 Hz); ^{13}C NMR (DMSO-d_6, 75.4 MHz) δ (ppm): 21.11 (CH_3), 44.33 (CH_2), 48.10 (CH_2), 56.15 (CH_3O), 58.49 (CH), 60.13 (CH_3O), 105.28 (CH), 127.31 (CH), 127.54 (CH), 127.78 (CH), 128.34 (CH), 128.40 (C), 129.74 (CH), 134.90 (C), 136.56 (C), 140.64 (C), 144.76 (C), 152.57 (C), 152.72 (C), 166.24 (CO); MS (EI, 70 eV) m/z (rel intensity, %): 539 [M$^+$] (8), 384 (3), 328 (66), 211 (34), 195 (84), 173 (53), 155 (7), 131 (25), 106 (8), 91 (100), 77 (6), 65 (13); IR (Nujol) ν_{max}/cm^{-1} 3304, 1744, 1637, 1584, 1525, 1501, 1465, 1378, 1331, 1239, 1174, 1130, 1002, 844, 813, 740, 670; Anal. Calcd for $C_{27}H_{29}N_3O_7S$ (539.60): C, 60.10; H, 5.42; N, 7.79; S, 5.94. Found: C, 60.02; H, 5.37; N, 8.85; S, 6.01.

3.3. Preparation of 4-[1-alkyl-3-arylureido]-2-phenyl-2-oxazolines and 4-[1-alkyl-3-arylthioureido]-2-phenyl-2-oxazolines (5)

A solution of the appropriate isocyanate or isothiocyanate (1.8 mmol) in dry ether (9 mL) was added dropwise at room temperature to a stirred solution of the corresponding aminooxazoline **1** (1.2 mmol) in dry ether (9 mL). After 1 h the solid precipitate was filtered off and crystallized from a suitable solvent.

3.3.1. 4-(1-Benzyl-3-phenylureido)-2-phenyl-2-oxazoline (5a). Yield 81%; crystallization from petroleum ether/dichloromethane gave white needles; mp 122–125 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 4.30 (dd, 1H, J=10.1 Hz, J=6.3 Hz), 4.40 (d, 1H, J=17.4 Hz), 4.52 (d, 1H, J=17.4 Hz), 4.63 (t, 1H, J=9.8 Hz), 6.61 (br s, 1H), 6.67 (dd, 1H, J=9.3 Hz, J=6.3 Hz), 6.95–7.53 (m, 13H), 7.99 (d, 2H, J=7.5 Hz); ^{13}C NMR ($CDCl_3$, 50.4 MHz) δ (ppm): 46.86 (CH_2), 71.72 (CH_2), 77.96 (CH), 119.84 (CH), 123.30 (CH), 126.94 (C), 127.07 (CH), 128.14 (CH), 128.57 (CH), 128.77 (CH), 128.90 (CH), 129.35 (CH), 132.24 (CH), 137.67 (C), 138.68 (C), 155.71 (CO), 166.87 (C=N); MS (EI, 70 eV) m/z (rel intensity, %): 371 [M$^+$] (1), 250 (5), 146 (15), 131 (19), 119 (19), 105 (25), 91 (100), 77 (28); IR (Nujol) ν_{max}/cm^{-1} 3295, 1632, 1532, 1446, 1377, 1315, 1173, 965, 756, 700; Anal. Calcd for $C_{23}H_{21}N_3O_2$ (371.43): C, 74.37; H, 5.70; N, 11.31. Found: C, 74.28; H, 5.71; N, 11.21.

3.3.2. 4-(1-Benzyl-3-phenylthioureido)-2-phenyl-2-oxazoline (5b). Yield 92%; crystallization from hexane/dichloromethane gave white needles; mp 99–102 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 4.38 (dd, 1H, J=10.3 Hz, J=6.3 Hz), 4.62 (d, 1H, J=17.0 Hz), 4.79 (d, 1H, J=17.0 Hz), 4.85 (t, 1H, J=10.0 Hz), 7.13–7.52 (m, 15H), 7.98 (d, 2H, J=7.0 Hz); ^{13}C NMR ($CDCl_3$, 50.4 MHz) δ (ppm): 49.10 (CH_2), 72.98 (CH_2), 82.16 (CH), 125.28 (CH), 126.01 (CH), 126.90 (CH), 128.34 (CH), 128.54 (CH), 128.72 (CH), 128.80 (CH), 129.45 (CH), 132.29 (CH), 136.25 (C), 139.23 (C), 167.40 (C=N), 182.94 (C=S); MS (EI, 70 eV) m/z (rel intensity, %): 387 [M$^+$] (16), 266 (43), 240 (58), 207 (8), 182 (26), 175 (32), 167 (70), 148 (32), 137 (23), 121 (24), 105 (69), 91 (100), 77 (70), 65 (22); IR (Nujol) ν_{max}/cm^{-1} 3313, 1637, 1524, 1463, 1378, 1347, 1321, 1144, 1094, 1051, 967, 697; Anal. Calcd for $C_{23}H_{21}N_3OS$ (387.50): C, 71.29; H, 5.46; N, 10.84; S, 8.28. Found: C, 71.33; H, 5.49; N, 10.95; S, 8.25.

3.3.3. 4-[1-Benzyl-3-(4-methoxyphenyl)thioureido]-2-phenyl-2-oxazoline (5c). Yield 86%; crystallization from hexane/dichloromethane gave white needles; mp 118–120 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 3.76 (s, 3H), 4.37 (dd, 1H, J=10.2 Hz, J=6.3 Hz), 4.63 (d, 1H, J=17.1 Hz), 4.77 (d, 1H, J=17.1 Hz), 4.84 (t, 1H, J=9.9 Hz), 6.81 (d, 2H, J=8.7 Hz), 7.03 (d, 2H, J=9.0 Hz), 7.25–7.53 (m, 10H), 7.97 (d, 2H, J=7.2 Hz); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ (ppm): 48.87 (CH_2), 55.48 (CH_3O), 72.94 (CH_2), 82.28 (CH), 114.01 (CH), 126.80 (CH), 127.09 (C), 127.46 (CH), 128.25 (CH), 128.52 (CH), 128.80 (CH), 129.39 (CH), 132.24 (CH), 136.30 (C), 157.96 (C), 167.32 (C=N), 183.49 (C=S); MS (EI, 70 eV) m/z (rel intensity, %): 417 [M$^+$] (30), 296 (85), 270 (230), 237 (36), 212 (58), 197 (69), 165 (75), 148 (27), 121 (28), 105 (73), 91 (100), 77 (68), 65 (21); IR (Nujol) ν_{max}/cm^{-1} 3314, 1639, 1519, 1467, 1346, 1280, 1093, 1052, 967, 826, 727, 703, 670; Anal. Calcd for $C_{24}H_{23}N_3O_2S$ (417.52): C, 69.04; H, 5.55; N, 10.06; S, 7.68. Found: C, 68.97; H, 5.47; N, 9.98; S, 7.74.

3.3.4. 4-[1-Benzyl-3-(4-chlorophenyl)ureido]-2-phenyl-2-oxazoline (5d). Yield 90%; crystallization from THF/hexane gave white powder; mp 169–172 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm): 4.29 (dd, 1H, J=10.0 Hz, J=6.3 Hz), 4.39 (d, 1H, J=16.7 Hz), 4.51 (d, 1H, J=16.7 Hz), 4.61 (t, 1H, J=10.0 Hz), 6.62 (dd, 1H, J=9.2 Hz, J=6.4 Hz), 6.68 (s, 1H), 6.58–7.53 (m, 12H), 7.98 (d, 2H, J=6.8 Hz); ^{13}C NMR δ ($CDCl_3$, 50.4 MHz) δ (ppm): 46.93 (CH_2), 71.61 (CH_2), 78.07 (CH), 121.03 (CH), 126.87 (C), 127.05 (CH), 128.22 (CH), 128.59 (CH), 128.78 (CH), 128.87 (CH), 129.39 (CH), 132.32 (CH), 137.36 (C), 137.53 (C), 155.58 (CO), 166.97 (C=N); MS (EI, 70 eV) m/z (rel intensity, %): 405 [M$^+$] (6), 284 (53), 258 (11), 251 (16), 161 (8), 153 (46), 146 (37), 131 (53), 118 (39), 105 (48), 91 (100), 77 (38); IR (Nujol) ν_{max}/cm^{-1} 3378, 1676, 1633, 1593, 1526, 1494, 1455, 1374, 1337, 1226, 1106, 1011, 828, 706; Anal. Calcd for $C_{23}H_{20}ClN_3O_2$ (405.88): C, 68.06; H, 4.97; N, 10.35. Found: C, 67.94; H, 4.99; N, 10.42.

3.3.5. 4-[1-Isopropyl-3-(4-nitrophenyl)ureido]-2-phenyl-2-oxazoline (5e). Yield 89%; crystallization from petroleum ether/chloroform gave yellow prisms; mp 160–164 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.28 (d, 3H, J=6.6 Hz), 1.36 (d, 3H, J=6.6 Hz), 4.29 (t, 1H, J=9.6 Hz), 4.47 (sept., 1H, J=6.6 Hz), 4.64 (t, 1H, J=9.6 Hz), 5.82 (t, 1H, J=9.6 Hz), 7.28 (d, 2H, J=9.3 Hz), 7.51 (t, 2H, J=7.8 Hz), 7.62 (t, 1H, J=7.8 Hz), 7.68 (br s, 1H), 8.03–8.07 (m, 4H); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ (ppm): 21.09 (CH_3), 21.89 (CH_3), 46.58 (CH), 71.81 (CH_2), 76.57 (CH), 118.14 (CH), 125.11 (CH), 126.24 (C), 128.70 (CH), 128.90 (CH), 132.97 (CH), 142.38 (C), 145.15 (C), 154.73 (CO), 167.51 (C=N); MS (EI, 70 eV) m/z (rel

intensity, %): 368 [M$^+$] (5), 247 (25), 217 (14), 203 (51), 189 (18), 175 (11), 164 (45), 146 (100), 134 (51), 118 (43), 105 (94), 90 (60), 83 (53), 77 (57); IR (Nujol) ν_{max}/cm^{-1} 3235, 3281, 3131, 1680, 1635, 1598, 1545, 1505, 1463, 1371, 1307, 1113, 1079, 1028, 848, 704; Anal. Calcd for $C_{19}H_{20}N_4O_4$ (368.39): C, 61.95; H, 5.47; N, 15.21. Found: C, 62.02; H, 5.56; N, 15.15.

Crystallographic data for **5e**: $C_{19}H_{20}N_4O_4$, monoclinic, space group $P2_1/n$, a=12.3880(12), b=11.5752(12) Å, c=13.6715(12) Å, β=110.444(4)°, V=1836.9(3) Å^3, Z=4. A colourless prism 0.42×0.22×0.20 mm was used to measure 3736 reflections at T=173 K, of which 3234 were unique (R_{int}=0.013). Refinement proceeded to wR_2=0.0803 (all data), R_1=0.0344 and GOF=0.901 [$I>2\sigma(I)$]. Maximum residual electron density was 0.12 eÅ^{-3}.

3.4. One-pot synthesis of (*Z*)-3-alkyl-4-benzamido-2-arylimino-1,3-oxazolidine and (*Z*)-3-alkyl-4-benzamido-2-arylimino-1,3-thiazolidine hydrochlorides (7)

To a well-stirred solution of aminooxazoline **1** (1 mmol) in dry ether (10 mL) a solution of the corresponding isocyanate or isothiocyanate (1 mmol) in dry ether (10 mL) was added dropwise, and the reaction mixture was stirred at room temperature for 1 h. Then, hydrochloric acid (0.15 mL; 35%) was added and the solid product was filtered off and crystallized from the appropriate solvent.

3.4.1. (*Z*)-3-Benzyl-4-benzamido-2-phenylimino-1,3-oxazolidine hydrochloride (7a). Yield 92%; crystallization from ethanol gave colourless prisms; mp 164–165 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ (ppm): 4.63 (d, 1H, J=16.1 Hz), 4.84 (dd, 1H, J=9.6 Hz, J=3.6 Hz), 5.10 (t, 1H, J=9.3 Hz), 5.52 (d, 1H, J=16.0 Hz), 6.00 (td, 1H, J=9.4 Hz, J=3.3 Hz), 7.23–7.60 (m, 13H), 7.90 (d, 2H, J=7.0 Hz), 9.82 (d, 1H, J=8.1 Hz); ^{13}C NMR (DMSO-d_6, 50.4 MHz) δ (ppm): 45.46 (CH_2), 64.87 (CH), 74.02 (CH_2), 119.58 (CH), 124.07 (CH), 126.51 (C), 126.95 (CH), 127.68 (CH), 128.10 (CH), 128.47 (CH), 128.68 (CH), 129.23 (CH), 132.26 (CH), 132.87 (C), 134.07 (C), 158.71 (C=N), 166.90 (CO); MS (EI, 70 eV) *m*/*z* (rel intensity, %): 371 [M$^+$−36] (1), 250 (1), 226 (2), 181 (2), 145 (77), 117 (63), 105 (43), 90 (100), 77 (61); IR (Nujol) ν_{max}/cm^{-1} 3212, 3185, 1682, 1665, 1596, 1531, 1462, 1378, 1278, 1153, 1070, 997, 944, 839, 767; Anal. Calcd for $C_{23}H_{22}ClN_3O_2$ (407.89): C, 67.73; H, 5.44; N, 10.30. Found: C, 67.46; H, 5.31; N, 10.24.

Crystallographic data for **7a**: $C_{23}H_{22}ClN_3O_2$, triclinic, space group $P(-1)$, a=9.7195(8), b=9.9757(8), c=11.2373(10) Å, α=90.287(3), β=112.816(3), γ=98.369(3)°, V=991.35 Å^3, Z=2. A colourless prism 0.35×0.18×0.15 mm was used to measure 15 487 reflections at T=143 K, of which 5751 were unique (R_{int}=0.047). Refinement proceeded to wR_2=0.0992 (all data), R_1=0.0373 and GOF=1.05 [$I>2\sigma(I)$]. Maximum residual electron density was 0.39 eÅ^{-3}.

3.4.2. (*Z*)-3-Benzyl-4-benzamido-2-phenylimino-1,3-thiazolidine hydrochloride (7b). Yield 88%; crystallization from acetonitrile gave colourless prisms; mp 172–174 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ (ppm): 3.46 (dd, 1H, J=11.9 Hz, J=2.8 Hz), 3.91 (dd, 1H, J=11.9 Hz, J=7.8 Hz), 4.71 (d, 1H, J=15.9 Hz), 5.49 (d, 1H, J=15.9 Hz), 6.17 (td, 1H, J=8.0 Hz, J=2.8 Hz), 7.33–7.63 (m, 14H), 7.91 (d, 2H, J=7.1 Hz), 9.75 (d, 1H, J=8.0 Hz); ^{13}C NMR (DMSO-d_6 50.4 MHz) δ (ppm): 33.85 (CH_2), 48.81 (CH_2), 70.35 (CH), 125.33 (CH), 127.86 (CH), 127.99 (CH), 128.37 (CH), 128.78 (CH), 129.64 (CH), 132.10 (CH), 133.09 (C), 134.64 (C), 166.74 (CO); MS (EI, 70 eV) *m*/*z* (rel intensity, %): 387 [M$^+$−36] (11), 266 (44), 240 (45), 182 (20), 167 (76), 148 (24), 121 (27), 105 (63), 91 (100), 77 (68), 65 (22); IR (Nujol) ν_{max}/cm^{-1} 3146, 2692, 1650, 1626, 1587, 1519, 1487, 1463, 1378, 1181, 766, 700; Anal. Calcd for $C_{23}H_{22}ClN_3OS$ (423.96): C, 65.16; H, 5.23; N, 9.91; S, 7.56. Found: C, 65.23; H, 5.19; N, 9.87; S, 7.60.

Crystallographic data for **7b**: $C_{23}H_{22}ClN_3OS$, orthorhombic, space group *Pbca*, a=16.8839(16), b=10.7096(10), c=23.290(2) Å, V=4211.2 Å^3, Z=8. A colourless prism 0.38×0.21×0.21 mm was used to measure 33 360 reflections at T=143 K, of which 6161 were unique (R_{int}=0.058). The phenyl group at N2 is disordered over two positions. Refinement proceeded to wR_2=0.0928 (all data), R_1=0.0357 and GOF=1.03 [$I>2\sigma(I)$]. Maximum residual electron density was 0.32 eÅ^{-3}.

3.4.3. (*Z*)-3-Benzyl-4-benzamido-2-(4-methoxyphenyl)-imino-1,3-thiazolidine hydrochloride (7c). Yield 90%; crystallization from ethanol gave white powder; mp 173–182 °C dec; ^{1}H NMR (DMSO-d_6, 200 MHz) δ (ppm): 3.48 (dd, 1H, J=8.0 Hz, J=2.7 Hz), 3.80 (s, 3H), 3.92 (dd, 1H, J=7.9 Hz, J=7.8 Hz), 4.68 (d, 1H, J=16.0 Hz), 5.50 (d, 1H, J=16.0 Hz), 6.19 (td, 1H, J=8.0 Hz, J=2.7 Hz), 7.05 (d, 2H, J=8.7 Hz), 7.36–7.59 (m, 11H), 7.92 (d, 2H, J=7.9 Hz), 9.82 (d, 1H, J=8.0 Hz); ^{13}C NMR (DMSO-d_6, 50.4 MHz) δ (ppm): 33.88 (CH_2), 48.88 (CH_2), 55.56 (CH_3O), 70.84 (CH), 114.76 (CH), 127.20 (CH), 127.87 (CH), 127.99 (CH), 128.04 (CH), 128.35 (CH), 128.78 (CH), 131.43 (C), 132.10 (CH), 133.03 (C), 134.32 (C), 159.10 (C), 166.72 (CO); FAB$^+$, 418 (100); IR (Nujol) ν_{max}/cm^{-1} 3198, 2732, 2650, 1626, 1519, 1459, 1376, 1247, 1177, 1032, 828, 710; Anal. Calcd for $C_{24}H_{24}ClN_3O_2S$ (453.99): C, 63.49; H, 5.33; N, 9.26; S, 7.06. Found: C, 63.73; H, 5.47; N, 9.16; S, 7.03.

3.4.4. (*Z*)-3-Benzyl-4-benzamido-2-(4-chlorophenyl)-imino-1,3-oxazolidine hydrochloride (7d). Yield 94%; crystallization from methanol gave white powder; mp 167 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ (ppm): 4.60 (d, 1H, J=16.1 Hz), 4.81 (dd, 1H, J=8.0 Hz, J=3.4 Hz), 5.07 (t, 1H, J=8.1 Hz), 5.44 (d, 1H, J=16.1 Hz), 5.98 (td, 1H, J=8.2 Hz, J=3.3 Hz), 7.12–7.65 (m, 12 H), 7.87 (d, 2H, J=6.9 Hz), 9.70 (d, 1H, J=8.1 Hz); ^{13}C NMR δ (DMSO-d_6, 50.4 MHz) δ (ppm): 45.53 (CH_2), 64.64 (CH), 73.68 (CH_2), 121.13 (CH), 125.59 (CH), 127.61 (CH), 128.03 (CH), 128.48 (CH), 128.67 (CH), 129.17 (CH), 132.23 (CH), 132.97 (C), 134.35 (C), 139.13 (C), 139.26 (C), 154.88 (C=N), 166.95 (CO); FAB$^+$, 406 (100); IR (Nujol) ν_{max}/cm^{-1} 3166, 2670, 1684, 1660, 1531, 1490, 1464, 1377, 1284, 1152, 1095, 1004, 959, 837, 809, 705; Anal. Calcd for $C_{23}H_{21}Cl_2N_3O_2$ (442.34): C, 62.45; H, 4.79; N, 9.50. Found: C, 62.13; H, 4.88; N, 9.43.

3.4.5. (*Z*)-4-Benzamido-3-isopropyl-2-(4-nitrophenyl)-imino-1,3-oxazolidine hydrochloride (7e). Yield 97%;

crystallization from ethanol/pentane gave white needles; mp 119–121 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ (ppm): 1.23 (d, 3H, J=6.2 Hz), 1.43 (d, 3H, J=6.2 Hz), 4.36–4.39 (m, 1H), 4.97–5.07 (m, 2H), 6.17–6.19 (m, 1H), 7.62–7.82 (m, 5H), 8.11 (d, 2H, J=9.1 Hz), 8.28 (d, 2H, J=7.4 Hz), 9.69 (s, 1H); ^{13}C NMR δ (DMSO-d_6, 50.4 MHz) δ (ppm): 21.57 (CH_3), 47.98 (CH), 69.49 (CH), 74.14 (CH_2), 119.35 (CH), 124.52 (CH), 129.47 (CH), 129.92 (CH), 135.71 (CH), 141.34 (C), 146.77 (C), 153.78 (C=N), 170.02 (CO); MS (EI, 70 eV) *m*/*z* (rel intensity, %): 247 (2), 223 (4), 203 (5), 164 (18), 145 (80), 138 (69), 117 (76), 108 (62), 90 (100), 77 (26); IR (Nujol) ν_{max}/cm^{-1} 3258, 2576, 1677, 1613, 1502, 1464, 1376, 1312, 1244, 1077, 855, 703; Anal. Calcd for $C_{19}H_{21}ClN_4O_4$ (404.85): C, 56.37; H, 5.23; N, 13.84. Found: C, 56.14; H, 5.57; N, 13.86.

3.4.6. (*Z*)-3-Benzyl-2-benzylimino-4-benzamido-1,3-oxazolidine hydrochloride (7f). Yield 90%; crystallization from ethanol/hexane gave colourless prisms; mp 164 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ (ppm): 4.53 (d, 1H, J=11.5 Hz), 4.58 (s, 2H), 4.83 (dd, 1H, J=9.3 Hz, J=3.7 Hz), 5.06 (t, 1H, J=8.8 Hz), 5.14 (d, 1H, J=11.5 Hz), 5.97 (td, 1H, J=8.0 Hz, J=3.6 Hz), 7.28–7.63 (m, 13H), 7.88 (d, 2H, J=7.1 Hz), 9.82 (d, 1H, J=7.9 Hz), 11.34 (t, 1H, J=5.9 Hz); ^{13}C NMR (DMSO-d_6, 50.4 MHz) δ (ppm): 45.06 (CH_2), 45.43 (CH_2), 65.10 (CH), 73.66 (CH_2), 127.71 (CH), 127.72 (CH), 127.83 (CH), 128.07 (CH), 128.16 (CH), 128.26 (CH), 128.45 (CH), 128.65 (CH), 132.24 (CH), 132.91 (C), 134.18 (C), 136.71 (C), 159.79 (C=N), 167.01 (CO); MS (EI, 70 eV) *m*/*z* (rel intensity, %): 264 (17), 240 (75), 145 (43), 117 (22), 106 (100), 91 (90), 77 (47); IR (Nujol) ν_{max}/cm^{-1} 3168, 1689, 1658, 1536, 1466, 1378, 1276, 1125, 1020, 952, 714; Anal. Calcd for $C_{24}H_{24}ClN_3O_2$ (421.92): C, 68.32; H, 5.73; N, 9.96. Found: C, 67.03; H, 5.88; N, 9.84.

3.4.7. (*Z*)-3-Benzyl-4-benzamido-2-isopropylimino-1,3-oxazolidine hydrochloride (7g). Yield 89%; crystallization from THF gave colourless prisms; mp 171 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ (ppm): 1.30 (t, 6H, J=6.7 Hz), 3.99 (sept., 1H, J=6.6 Hz), 4.46 (d, 1H, J=16.3 Hz), 4.79 (dd, 1H, J=8.0 Hz, J=3.6 Hz), 5.04 (t, 1H, J=8.8 Hz), 5.09 (d, 1H, J=16.3 Hz), 5.89 (td, 1H, J=8.0 Hz, J=3.5 Hz), 7.29–7.58 (m, 8H), 7.83 (d, 2H, J=7.0 Hz), 9.62 (d, 1H, J=7.9 Hz), 10.10 (d, 1H, J=7.4 Hz); ^{13}C NMR δ (DMSO-d_6, 50.4 MHz) δ (ppm): 19.31 (CH_3), 19.62 (CH_3), 42.16 (CH_2), 43.75 (CH), 62.15 (CH), 70.82 (CH_2), 124.89 (CH), 125.11 (CH), 125.29 (CH), 125.73 (CH), 125.98 (CH), 129.50 (CH), 130.23 (C), 131.55 (C), 156.26 (C=N), 164.26 (CO); MS (EI, 70 eV) *m*/*z* (rel intensity, %): 337 (6), 322 (12), 252 (61), 216 (16), 201 (24), 191 (51), 146 (55), 131 (29), 106 (85), 91 (100), 77 (45); IR (Nujol) ν_{max}/cm^{-1} 3130, 1695, 1657, 1531, 1460, 1379, 1287, 1157, 1101, 1081, 716; Anal. Calcd for $C_{20}H_{24}ClN_3O_2$ (373.88): C, 64.25; H, 6.47; N, 11.24. Found: C, 63.79; H, 6.51; N, 11.05.

3.4.8. (*Z*)-2-Benzylimino-4-benzamido-3-isopropyl-1,3-oxazolidine hydrochloride (7h). Yield 95%; crystallization from THF/pentane gave white needles; mp 171 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ (ppm): 1.15 (d, 3H, J=6.6 Hz), 1.39 (d, 3H, J=6.6 Hz), 4.22 (sept., 1H, J=6.6 Hz), 4.54 (s, 2H), 4.65 (dd, 1H, J=8.5 Hz, J=3.0 Hz), 4.89 (t, 1H, J=8.5 Hz), 6.06 (td, 1H, J=8.0 Hz, J=3.1 Hz), 7.30–7.65 (m, 8H), 7.91 (d, 2H, J=6.8 Hz), 9.76 (d, 1H, J=7.8 Hz), 10.71 (s, 1H); ^{13}C NMR (DMSO-d_6, 50.4 MHz) δ (ppm): 18.83 (CH_3), 20.90 (CH_3), 45.71 (CH_2), 46.51 (CH), 63.04 (CH), 73.80 (CH_2), 127.51 (CH), 127.59 (CH), 127.71 (CH), 128.66 (CH), 132.27 (CH), 133.27 (C), 137.09 (C), 158.09 (C=N), 166.48 (CO); MS (EI, 70 eV) *m*/*z* (rel intensity, %): 337 (1), 252 (7), 216 (39), 192 (45), 191 (43), 174 (19), 146 (37), 145 (38), 121 (19), 105 (79), 91 (100), 77 (61); IR (Nujol) ν_{max}/cm^{-1} 3175, 1683, 1539, 1456, 1364, 1298, 1281, 1215, 1081, 1038, 710; Anal. Calcd for $C_{20}H_{24}ClN_3O_2$ (373.88): C, 64.25; H, 6.47; N, 11.24. Found: C, 64.13; H, 6.49; N, 11.06.

3.4.9. (*Z*)-3-Benzyl-4-benzamido-2-propylimino-1,3-oxazolidine hydrochloride (7i). Yield 92%; crystallization from ethanol/pentane gave colourless prisms; mp 170 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 0.93 (t, 3H, J=7.5 Hz), 1.58–1.70 (m, 2H), 3.32 (m, 2H), 4.48 (d, 1H, J=16.2 Hz), 4.80 (dd, 1H, J=8.7 Hz, J=3.9 Hz), 5.05 (t, 1H, J=8.7 Hz), 5.13 (d, 1H, J=16.2 Hz), 5.91 (td, 1H, J=8.1 Hz, J=3.3 Hz), 7.30–7.61 (m, 8H), 7.86 (d, 2H, J=8.1 Hz), 9.70 (d, 1H, J=8.0 Hz), 10.59 (s, 1H); ^{13}C NMR (DMSO-d_6, 75.4 MHz) δ (ppm): 11.00 (CH_3), 22.08 (CH_2), 44.01 (CH_2), 44.87 (CH_2), 64.90 (CH), 73.42 (CH_2), 127.58 (CH), 127.95 (CH), 128.37 (CH), 128.59 (CH), 132.13 (CH), 132.89 (C), 134.19 (C), 159.62 (C=N), 166.94 (CO); MS (EI, 70 eV) *m*/*z* (rel intensity, %): 337 (3), 252 (64), 192 (36), 146 (49), 106 (53), 90 (100), 77 (68); IR (Nujol) ν_{max}/cm^{-1} 3217, 3185, 1695, 1666, 1537, 1465, 1275, 1113, 996, 807, 712; Anal. Calcd for $C_{20}H_{24}ClN_3O_2$ (373.88): C, 64.25; H, 6.47; N, 11.24. Found: C, 63.86; H, 7.12; N, 11.37.

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC-299402 (**4b**), CCDC-298631 (**5e**), CCDC-243016 (**7a**) and CCDC-243018 (**7b**). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

Acknowledgements

We gratefully acknowledge the financial support of the Ministerio de Educación y Ciencia (project CTQ2004-06427) and the Consejería de Economía, Industria e Innovación of the Comunidad Autónoma de la Región de Murcia (project 2I04SU005). B.M. thanks the Fundación Cajamurcia for a grant.

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Tetrahedron 62 (2006) 6182–6189
Tetrahedron

Intramolecular cyclisation of functionalised heteroaryllithiums. Synthesis of novel indolizinone-based compounds

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Received 10 March 2006; revised 7 April 2006; accepted 20 April 2006
Available online 11 May 2006

Dedicated to the memory of Professor Marcial Moreno Mañas

Abstract—The intramolecular cyclisation of heteroaryllithiums derived from *N*-heteroarylmethylpyrrole-2-carboxamides takes place smoothly at low temperature when *N*-methoxy-*N*-methyl and morpholine amides are used as internal electrophiles. Halogen–lithium exchange using *n*-BuLi is the method of choice to achieve metalation on the quinoline and pyridine derivatives, while directed lithiation (LDA) works better for furan. In the case of thiophene both methodologies can be applied. These metalation–cyclisation sequences provide a useful entry to several types of indolizidine based compounds (pyrrolo[1,2-*b*]acridinones, pyrrolo[1,2-*g*]quinolones, thieno and furo[3,2-*f*]indolizinones).

1. Introduction

The synthetic utility of lithium–halogen exchange reaction for the metalation of aromatic substrates, though mechanistically controversial,[1] is well established and has been subjected to several comprehensive reviews.[2] A particularly useful application[3,4] of this reaction has been the facile construction of benzo-fused carbocyclic[5] and heterocyclic[6] ring systems via intramolecular reaction of the so-generated aryllithium compounds with internal electrophiles, a metalation–cyclisation process pioneered by Parham.[7]

On the other hand, the ease with which halogenated heterocycles may be prepared regioselectively make the use of these compounds as substrates for permutational halogen–metal interconversions extremely attractive. Organolithium derivatives of all simple heterocycles at all possible positions have been made by this method.[2,8] Thus, heteroaryllithiums may be employed in Parham cyclisation for the synthesis of several types of heterocyclic systems.[9] For instance, Avendaño[10] used a tandem directed *ortho*-metalation/metal–halogen exchange reaction for the synthesis of 1,8-diazanthracene-9,10-diones. It was necessary to prepare the second heteroaryllithium, a 2-lithiopyridine intermediate, by halogen–metal exchange, since directed metalation was unsuccessful due to the presence of competitive *ortho*-metalation sites. Other 3-lithio- and 2-lithiopyridine derivatives have been used as intermediates in the Parham cyclisation for the synthesis of azatetralones[11] and dipyridocycloheptenones,[9] respectively. Maddaluno[12] has also used 2-lithiopyridine intermediates in the intramolecular carbolithiation of propargyl acetals that provided furo[3,2-*b*]pyridines. Pearson[13] has demonstrated that 3-lithiopyridines and 3-lithiofurans, generated by metal–halogen exchange with *t*-butyllithium, may be used for the synthesis of indanones derived from monic acid. Mesityllithium was found to be an excellent selective lithiating agent to prepare heteroaryllithium compounds having alkoxycarbonyl groups, whose intramolecular cyclisation led to an important precursor for the synthesis of camptothecin.[14] Selnick[15] developed a new route to thieno[2,3-*b*]thiophenes by a metal–halogen exchange initiated intramolecular acylation of 3-bromothiophenes with Weinreb amides as internal electrophiles. A recent enantioselective synthesis of polyhydroxylated piperidines used as a key step for the formation of heteroaryllithiums, derived from 5-bromo-oxazoles or thiazoles, which cyclised to give the corresponding oxazolo- and thiazolo[4,5-*c*]pyridones. [16]

In connection with our interest in Parham cyclistions, we have developed an anionic cyclisation approach towards the construction of the pyrrolo[1,2-*b*]isoquinolone core present in some natural products such as the lycorine class of *Amaryllidaceae* alkaloids[17] and the phenanthroindolizidine alkaloids.[18] Thus, we have shown[19] that *N*-(*o*-iodobenzyl)-pyrrole-2-carboxamides tolerate lithium–iodine exchange reaction conditions,[20] allowing the efficient synthesis of

Keywords: Lithiation; Lithium–halogen exchange; Heteroaryllithium compounds; Parham cyclisation; Heterocycles; *ortho*-Lithiation.
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0040–4020/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2006.04.057

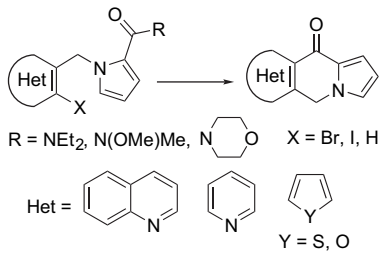


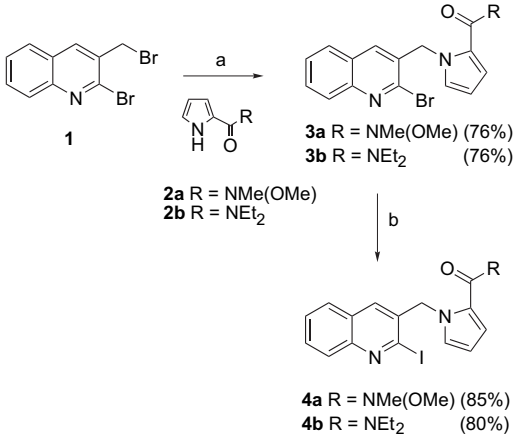
Figure 1.

the pyrrolo[1,2-*b*]isoquinoline nucleus. The *N*-methoxy-*N*-methyl and morpholine amides behave as excellent internal electrophiles, improving the results obtained with *N*,*N*-diethyl amides. This procedure has also been applied to the construction of fused seven and eight-membered rings, opening also new routes to other heterocyclic systems (pyrrolo[1,2-*a*]benzazepines and pyrrolo[1,2-*a*]benzazocines).

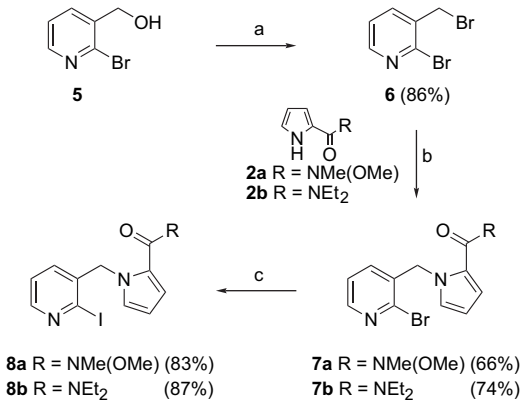
Therefore, we decided to expand this procedure by using heteroaryllithium compounds as intermediates in this aromatic metalation–cyclisation sequence and thus afford convenient access to fused indolizidinone systems.[21] For this purpose, we employed a series of *N*-(2-haloheteroarylmethyl)pyrrole-2-carboxamides as substrates for the Parham cyclisation. We chose both electron-deficient (pyridine, quinoline) and electron-rich (thiophene, furan) heteroaromatic ring systems that incorporated *N*,*N*-diethyl- and *N*-methoxy-*N*-methyl or morpholine amides as internal electrophiles (Fig. 1).

2. Results

Our first task was to extend the scope of our carbanionic heterocyclisation method to the synthesis of pyrrolo[1,2-*b*]acridones and pyrrolo[1,2-*g*]quinolones. Thus, we first prepared *N*-(2-bromoquinolylmethyl)pyrrole-2-carboxamides **3a**,**b** by N-alkylation of the corresponding pyrrole-2-carboxamide **2** with 2-bromo-3-bromomethylquinoline **1**[22] under standard conditions. These carboxamides were converted into the corresponding iodinated derivatives **4** by a bromine–iodine exchange reaction following Buchwald procedure (NaI, CuI, *N*,*N*′-dimethylethylene diamine)[23] (Scheme 1). The same sequence of reactions was applied to obtain the *N*-(2-halopyridylmethyl)pyrrole-2-carboxamides **7** and **8**, prepared from 2-bromo-3-hydroxymethylpyridine (**5**)[24] as described in Scheme 2.

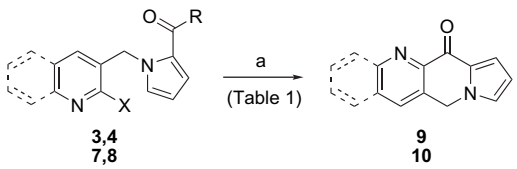


Scheme 1. Reagents: (a) KOH, DMSO, rt; (b) NaI, CuI (5% mol) $MeHN(CH_2)_2NHMe$, dioxane, reflux.



Scheme 2. Reagents: (a) PBr_3, CH_2Cl_2, rt; (b) KOH, DMSO, rt; (c) NaI, CuI (5% mol) $MeHN(CH_2)_2NHMe$, dioxane, reflux.

Next, we applied the metalation–cyclisation sequence to these amides. However, when the metalation of **3b** was performed with *t*-BuLi (2 equiv) under usual conditions (−78 °C, 3 h), a complex mixture of compounds was obtained. Only the corresponding dehalogenated amides were isolated in low yields (23–25%), both quenching at low temperature or allowing the mixture to warm up to room temperature before quenching. Although *t*-BuLi usually tends to undergo addition reaction with electron-deficient heterocycles, no addition product was detected. Therefore, *n*-BuLi was tested as metalating agent. Several experimental conditions were tried and we found that cyclisation of amides **3** and **4** took place smoothly when the heteroaryllithium was generated with *n*-BuLi (2.2 equiv) at low temperature for a shorter period of time (−90 °C, 5 min) and the reaction was quenched at low temperature (Scheme 3, Table 1, entries 1–4). No cyclisation products were isolated when heteroaryllithiums derived from **3** and **4** were allowed to reach room temperature. The effect of the halogen atom on the halogen–lithium exchange reaction was as expected, and better yields of the pyrrolo[1,2-*b*]acridone **9** were obtained with iodides **4** than with bromides **3**, particularly when *N*,*N*-diethylcarboxamides were used as internal electrophiles. This result suggested that in this case the extra stabilisation of the intermediate formed after cyclisation by formation of an internal chelate with Weinreb amides is needed to obtain good yields. In a similar fashion, the halogen–metal exchange reaction on amides **7** and **8** gave access to pyrrolo[1,2-*a*]quinolone **9** in moderate to good yields, obtaining the best results when X=I, and Weinreb amide was used as internal electrophile (Scheme 3, Table 1, entries 5–8).

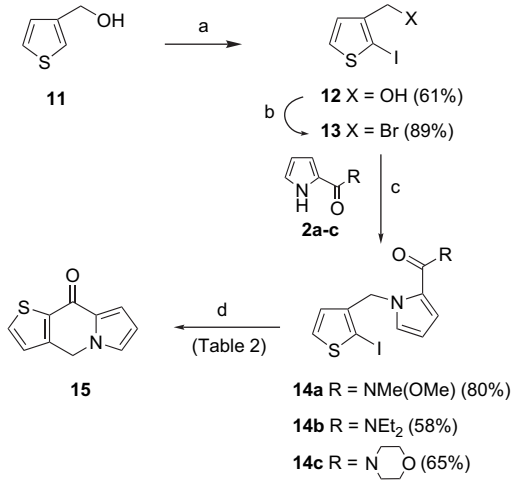


Scheme 3. Reagents: (a) *n*-BuLi (2 equiv), −90 °C, 5 min.

Table 1. Parham cyclisation of amides **3**–**4**, **7**–**8**

Entry	Substrates	X	R	Product	Yield (%)
1	**3a**	Br	NMe(OMe)	**9**	61
2	**3b**	Br	NEt_2	**9**	28
3	**4a**	I	NMe(OMe)	**9**	85
4	**4b**	I	NEt_2	**9**	83
5	**7a**	Br	NMe(OMe)	**10**	70
6	**7b**	Br	NEt_2	**10**	60
7	**8a**	I	NMe(OMe)	**10**	85
8	**8b**	I	NEt_2	**10**	80

We then began to test the feasibility of this Parham type cyclisation with electron-rich heterocycles. In view of the results obtained with quinoline and pyridine systems, and our previous results,[19] no attempts to perform bromine–lithium exchange were undertaken, and we decided to test this metalation–cyclisation sequence using also a morpholine amide as internal electrophile. Thus, the preparation of the *N*-thiophenylmethylpyrroles **14a**–**c** was achieved in three steps from 3-hydroxymethylthiophene (**11**), as depicted in Scheme 4.



Scheme 4. Reagents: (a) I_2, CF_3CO_2Ag, $CHCl_3$, 0 °C; (b) PBr_3, rt; (c) KOH, DMSO, rt; (d) *t*-BuLi (2.2 equiv), −78 °C (see Table 2).

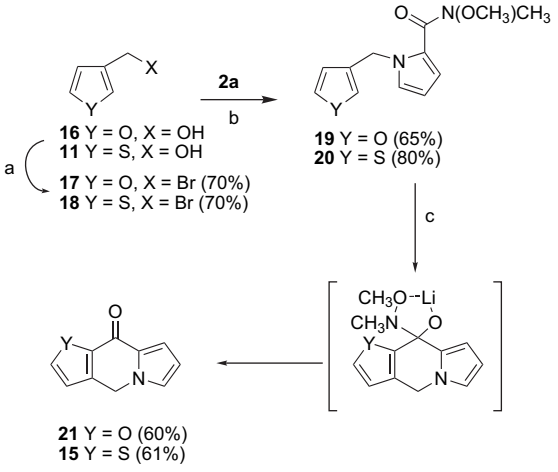
It was necessary to optimise the cyclisation conditions for this electron-rich heterocycle, and we finally chose two methods (A and B), using *t*-BuLi as metalating agent and quenching the reaction at low temperature, or allowing the reaction mixture to reach room temperature before quenching (Table 2). In this case, iodine–lithium exchange worked properly at −78 °C when Weinreb amide was used as internal electrophile (**14a**) and the reactions were quenched at low temperature. However, the use of *N*,*N*-diethyl amide **14b** only allowed the isolation of the thienoindolizidine **15** in modest yields (42%) under the same reaction conditions.[25] Besides, under method B conditions only dehalogenated amide was isolated (77% yield). This result is in agreement with our previously reported results[19] on the Parham cyclisation of *N*-arylmethylpyrrole-2-carboxamides with the *N*,*N*-diethylcarbamoyl group as internal electrophile. The iodine–lithium exchange on morpholine amide **14b** also took place efficiently, though it was necessary to use 3.5 equiv of the organolithium. The so-generated 2-lithiothiophene added smoothly to the amide. The intermediate was probably stabilised by chelate formation and only evolves to the ketone in aqueous media. Thus, the use of this type of amide also prevents elimination of the *N*,*N*-dialkyl group under basic conditions.

Table 2. Parham cyclisation of amides **14a**–**c**

Entry	Substrates	R	**15**, Yield (%)	
			Method A[a]	Method B[b]
1	**14a**	NMe(OMe)	71	49
2	**14b**	NEt_2	42	[c]
3	**14c**	Morpholine[d]	67	63

[a] *t*-BuLi (2.2 equiv), −78 °C, 3 h.
[b] *t*-BuLi (2.2 equiv), −78 °C, 3 h; →rt, 4 h.
[c] Dehalogenated amide was isolated (77%).
[d] *t*-BuLi (3.5 equiv) was used.

We then try to apply an analogous sequence to the construction of the furoindolizidine skeleton. However, since all attempts to prepare the 2-iodo-3-hydroxymethylfuran failed, we decided to generate the heteroaryllithium intermediates by hydrogen–lithium exchange. Therefore, we prepared the *N*-furylmethylpyrrole-2-carboxamide **19** from 3-furanmethanol **16** (Scheme 5). In this case, the intermediate 3-bromomethylfuran **17** was very unstable, so it was prepared and used in the alkylation step without prior purification. Although it is known that electron-rich five-membered aromatic heterocycles can be lithiated using alkyllithiums,[2b] in our case the use of *n*-BuLi or *t*-BuLi always resulted in addition to the amide carbonyl, so the corresponding ketones were the only reaction products.



Scheme 5. Reagents: (a) PBr_3, rt; (b) KOH, DMSO, rt; (c) LDA (2 equiv), −78 °C, 3 h.

However, LDA was capable of deprotonating **19** and the resulting 2-lithiofuran derivative gave the furo[3,2-*f*]indolizidone **21** in moderate yield (60%). In view of these results, the *ortho*-lithiation–cyclisation sequence was also applied to the non-halogenated *N*-thienylmethylpyrrole **20**, under the same reaction conditions, which provided the expected thienoindolizidone **15** in moderate yield (61%).

3. Conclusion

We have shown that the intramolecular cyclisation of heteroaryllithiums provides a useful entry to several types of

indolizidine based compounds starting from *N*-heteroarylmethylpyrrole-2-carboxamides. Halogen–lithium exchange reaction using *n*-BuLi (−90 °C, 5 min) is the method of choice to achieve metalation on the quinoline and pyridine derivatives, while directed lithiation (LDA, −78 °C) works better for furan. In the case of the thiophene both methodologies can be applied, but *t*-BuLi is needed to perform the iodine–lithium exchange. In all cases, cyclisation takes place smoothly when the reactions are quenched at low temperature. As expected, *N*-methoxy-*N*-methyl and morpholine amides behave as excellent internal electrophiles, improving the results obtained with *N*,*N*-diethyl amides. Therefore, these anionic heterocyclic annulation reactions represent a convenient approach to the preparation of pyrrolo[1,2-*b*]acridinones, pyrrolo[1,2-*g*]quinolones, as well as thieno and furo[3,2-*f*]indolizinones.

4. Experimental

4.1. General

Melting points were determined in unsealed capillary tubes and are uncorrected. IR spectra were obtained on KBr pellets (solids) or neat (oils). NMR spectra were recorded at 20–25 °C, running at 250 MHz for ^{1}H and 62.8 MHz for ^{13}C in $CDCl_3$ solutions. Assignment of individual ^{13}C resonances is supported by DEPT experiments. ^{1}H–{^{1}H} NOE experiments were carried out in the difference mode by irradiation of all the lines of a multiplet.[26] Mass spectra were recorded under electron impact at 70 eV. GC–MS analyses were performed using a TRB-1 column (methyl polysiloxane, 30 m×0.25 mm×0.25 μm). TLC was carried out with 0.2 mm thick silica gel plates. Visualisation was accomplished by UV light. Flash column chromatography[27] on silica gel was performed with Kieselgel 60 (230–400 mesh). All solvents used in reactions were anhydrous and purified according to standard procedures.[28] Organolithium reagents were titrated with diphenylacetic acid periodically prior to use. All air- or moisture-sensitive reactions were performed under argon; the glassware was dried (130 °C) and purged with argon.

4.2. Iodination of 11. Synthesis of 3-hydroxymethyl-2-iodothiophene (12)

A solution of I_2 (3.28 g, 12.9 mmol) in dry $CHCl_3$ (20 mL) was added over a suspension of CF_3COOAg (2.85 g, 12.9 mmol) and 3-hydroxymethylthiophene **11** (1.47 g, 12.9 mmol) in $CHCl_3$ (20 mL). The reaction mixture was stirred at 0 °C during 30 min, the resulting AgI precipitate was filtered through a Celite pad, and the resulting solution was washed with satd $Na_2S_2O_3$. The organic phase was dried (Na_2SO_4) and the solvent was evaporated. Flash column chromatography (silica gel, 30% hexane/AcOEt) afforded **12** as a colourless oil (1.88 g, 61%): IR (neat) 3333 cm^{-1}; ^{1}H NMR ($CDCl_3$) 3.66 (s, 1H), 4.48 (s, 2H), 6.92 (d, *J*=5.5 Hz, 1H), 7.40 (d, *J*=5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) 61.6, 74.6, 127.7, 131.0, 145.1; MS (EI) *m*/*z* (rel intensity) 242 (M^++2, 4), 241 (M^++1, 6), 240 (M^+, 72), 223 (12), 113 (44), 112 (11), 111 (20), 96 (11), 85 (100), 84 (39), 83 (10), 82 (13), 81 (10), 69 (14), 58 (18), 57 (11). Anal. Calcd for C_5H_5IOS: C, 25.02; H, 2.10. Found: C, 25.22; H, 2.15.

4.3. Synthesis of bromides. General procedure

PBr_3 (0.19 mL, 2 mmol) was added over a solution of alcohols **5**, **12**, **16** or **11** (1 mmol) in dry CH_2Cl_2 (10 mL), and the reaction mixture was stirred at rt for 16 h. Solvent was evaporated and the resulting oil was treated with satd $NaHCO_3$. The resulting aqueous phase was extracted with CH_2Cl_2 (3×15 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo, yielding bromides **6**, **13**, **17** or **18**.

4.3.1. 2-Bromo-3-bromomethylpyridine (6).[29] According to the general procedure, **5** (2 g, 10 mmol) was treated with PBr_3 (1.9 mL, 20 mmol). After work-up, flash column chromatography (silica gel, 20% hexane/AcOEt) afforded **6** as a colourless oil (2.3 g, 86%): ^{1}H NMR ($CDCl_3$) 4.41 (s, 2H), 7.12 (dd, *J*=7.5, 4.8 Hz), 7.62 (dd, *J*=7.5, 1.8 Hz, 1H), 8.13 (dd, *J*=4.8, 1.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) 31.2, 122.4, 134.3, 138.9, 144.1, 149.8.

4.3.2. 3-Bromomethyl-2-iodothiophene (13). According to the general procedure, **12** (457 mg, 1.9 mmol) was treated with PBr_3 (0.3 mL, 3.8 mmol). After work-up, flash column chromatography (silica gel, 20% hexane/AcOEt) afforded **13** as a colourless oil (507 mg, 89%): IR (neat) 2923, 1720 cm^{-1}; ^{1}H NMR ($CDCl_3$) 4.45 (s, 2H), 6.99 (d, *J*=5.5 Hz, 1H), 7.44 (d, *J*=5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) 28.9, 78.3, 128.3, 131.6, 142.0; MS (EI) *m*/*z* (rel intensity) 305 (M^++2, 1), 304 (M^++1, 8), 303 (M^+, 1), 223 (100), 96 (38), 70 (22), 69 (19). Anal. Calcd for C_5H_4BrIS: C, 19.82; H, 1.33. Found: C, 19.14; H, 1.53.

4.3.3. 3-Bromomethylfurane (17).[30] According to the general procedure, **16** (0.2 mL, 2.3 mmol) was treated with PBr_3 (0.4 mL, 4.6 mmol). After work-up, flash column chromatography (silica gel, 20% hexane/AcOEt) afforded **17** as a colourless oil (261 mg, 70%): ^{1}H NMR ($CDCl_3$) 4.38 (s, 2H), 6.45 (s, 1H), 7.40 (s, 1H), 7.48 (s, 1H); ^{13}C NMR ($CDCl_3$) 23.5, 110.7, 118.6, 140.7, 143.6.

4.3.4. 3-Bromomethylthiophene (18).[31] According to the general procedure, **11** (0.2 mL, 2.3 mmol) was treated with PBr_3 (0.4 mL, 4.6 mmol). After work-up, flash column chromatography (silica gel, 20% hexane/AcOEt) afforded **18** as a colourless oil (283 mg, 70%): ^{1}H NMR ($CDCl_3$) 4.52 (s, 2H), 6.95 (s, 2H), 7.22 (s, 1H); ^{13}C NMR ($CDCl_3$) 33.9, 119.8, 125.7, 129.1, 135.6.

4.4. Alkylation reactions. General procedure

Pyrrole-2-carboxamide **2a**, **2b** or **2c**[19] (1 mmol) was added over a suspension of powdered KOH (224 mg, 4 mmol) in DMSO (5 mL). The mixture was stirred at rt for 2 h, bromide **1**, **6**, **13**, **17** or **18** (2 mmol) was added, and the reaction mixture was stirred for 3 h. H_2O (10 mL) was added and the resulting aqueous phase was extracted with CH_2Cl_2 (3×10 mL). The combined organic extracts were washed with brine (3×10 mL), dried (Na_2SO_4) and concentrated in vacuo. Flash column chromatography (silica gel) afforded the corresponding carboxamides **3a**,**b**, **7a**,**b**, **14a**–**c**, **19** or **20**.

4.4.1. 1-(2-Bromoquinolin-3-ylmethyl)pyrrole-2-carboxylic acid methoxy methyl amide (3a). According to the

general procedure, *N*-methoxy-*N*-methylpyrrole-2-carboxamide **2a** (308 mg, 2 mmol) was treated with KOH (448 mg, 8 mmol) in DMSO (10 mL), and bromide **1** (1.2 g, 4 mmol). After work-up, flash column chromatography (silica gel, 30% hexane/AcOEt) afforded **3a** as a white solid, that was crystallised from Et_2O (585 mg, 76%): mp (Et_2O) 136–138 °C; IR (KBr) 1613 cm^{-1}; ^1H NMR ($CDCl_3$) 3.24 (s, 3H), 3.66 (s, 3H), 5.72 (s, 2H), 6.30 (dd, *J*=3.7, 2.6 Hz, 1H), 6.91 (dd, *J*=2.6, 1.8 Hz, 1H), 7.10 (dd, *J*=3.7, 1.8 Hz, 1H), 7.14 (s, 1H), 7.44–7.51 (m, 1H), 7.59–7.69 (m, 2H), 7.99 (d, *J*=8.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) 33.4, 52.1, 61.0, 108.8, 117.6, 123.0, 127.1, 127.4, 127.5, 127.8, 128.2, 130.0, 133.4, 134.9, 141.3, 147.4, 161.7; MS (EI) *m/z* (rel intensity) 375 (M^++2, 3), 373 (M^+, 6), 316 (20), 315 (97), 314 (21), 313 (100), 264 (28), 223 (16), 222 (82), 221 (14), 220 (88), 209 (12), 208 (13), 207 (63), 206 (17), 205 (17), 178 (11), 176 (30), 141 (20), 140 (33), 103 (21). Anal. Calcd for $C_{17}H_{16}BrN_3O_2$: C, 54.56; H, 4.31; N, 11.23. Found: C, 54.88; H, 4.42; N, 11.19.

4.4.2. 1-(2-Bromoquinolin-3-ylmethyl)pyrrole-2-carboxylic acid diethyl amide (3b). According to the general procedure, *N*,*N*-diethylpyrrole-2-carboxamide **2b** (336 mg, 2 mmol) was treated with KOH (448 mg, 8 mmol) in DMSO (10 mL), and bromide **1** (1.2 g, 4 mmol). After work-up, flash column chromatography (silica gel, 30% hexane/AcOEt) afforded **3b** as a white solid, that was crystallised from Et_2O (585 mg, 76%): mp (Et_2O) 136–138 °C; IR (KBr) 1615 cm^{-1}; ^1H NMR ($CDCl_3$) 1.02 (t, *J*=6.7 Hz, 6H), 3.38 (q, *J*=6.7 Hz, 4H), 5.48 (s, 2H, CH_2), 6.16 (dd, *J*=3.8, 2.8 Hz, 1H), 6.42 (d, *J*=2.8, 1.6 Hz, 1H), 6.82 (br s, 1H), 7.43–7.48 (m, 2H), 7.60–7.65 (m, 2H), 7.95 (d, *J*=8.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) 13.4, 40.9, 51.0, 107.6, 111.6, 125.4, 125.8, 127.1, 127.2, 127.5, 128.1, 130.2, 132.5, 136.4, 141.8, 147.4, 163.1; MS (EI) *m/z* (rel intensity) 387 (M^++2, 47), 385 (M^+, 46), 316 (8), 315 (42), 314 (17), 313 (42), 312 (10), 307 (18), 306 (79), 305 (7), 286 (17), 285 (17), 235 (10), 234 (22), 233 (72), 223 (14), 222 (97), 221 (16), 220 (100), 207 (75), 206 (30), 205 (46), 141 (40), 140 (66), 115 (7), 114 (15), 113 (10), 103 (14), 100 (16), 72 (20). Anal. Calcd for $C_{19}H_{20}BrN_3O$: C, 59.08; H, 5.22; N, 10.88. Found: C, 59.18; H, 5.53; N, 10.56.

4.4.3. 1-(2-Bromopyridin-3-ylmethyl)pyrrole-2-carboxylic acid methoxy methyl amide (7a). According to the general procedure, *N*-methoxy-*N*-methylpyrrole-2-carboxamide **2a** (481 mg, 3.12 mmol) was treated with KOH (701 mg, 12.5 mmol) in DMSO (10 mL), and bromide **6** (1.6 g, 6.25 mmol). After work-up, flash column chromatography (silica gel, 30% hexane/AcOEt) afforded **7a** as an oil (668 mg, 66%): IR (neat) 1624 cm^{-1}; ^1H NMR ($CDCl_3$) 3.25 (s, 3H), 3.66 (s, 3H), 5.56 (s, 2H), 6.25 (dd, *J*=4.0, 2.6 Hz, 1H), 6.78 (dd, *J*=7.5, 1.6 Hz, 1H), 6.84 (dd, *J*=2.6, 1.8 Hz, 1H), 7.04 (dd, *J*=4.0, 2.0 Hz, 1H), 7.13 (dd, *J*=7.5, 4.8 Hz, 1H), 8.22 (dd, *J*=4.8, 1.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) 33.0, 51.4, 60.6, 108.4, 116.7, 122.5, 122.7, 127.5, 135.4, 135.9, 140.6, 147.9, 161.2; MS (EI) *m/z* (rel intensity) 325 (M^++2, 3), 323 (M^+, 4), 266 (13), 265 (100), 264 (13), 263 (100), 184 (15), 170 (50), 156 (19), 155 (21). Anal. Calcd for $C_{13}H_{14}BrN_3O_2$: C, 48.16; H, 4.35; N, 12.96. Found: C, 48.24; H, 4.53; N, 12.85.

4.4.4. 1-(2-Bromopyridin-3-ylmethyl)pyrrole-2-carboxylic acid diethyl amide (7b). According to the general procedure, *N*,*N*-diethylpyrrole-2-carboxamide **2b** (762 mg, 4.49 mmol) was treated with KOH (1.03 g, 18.3 mmol) in DMSO (10 mL), and bromide **6** (2.3 g, 9.2 mmol). After work-up, flash column chromatography (silica gel, 30% hexane/AcOEt) afforded **7b** as an oil (2.28 mg, 74%): IR (neat) 1616 cm^{-1}; ^1H NMR ($CDCl_3$) 1.10 (t, *J*=6.7 Hz, 6H), 3.23 (q, *J*=6.7 Hz, 4H), 5.33 (s, 2H), 6.14 (dd, *J*=4.0, 2.6 Hz, 1H), 6.38 (dd, *J*=4.0, 2.0 Hz, 1H), 6.84 (dd, *J*=2.6, 1.8 Hz, 1H), 7.04 (dd, *J*=7.5, 1.6 Hz, 1H), 7.13 (dd, *J*=7.5, 4.8 Hz, 1H), 8.19 (dd, *J*=4.8, 1.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) 13.4, 41.3, 50.6, 107.7, 111.4, 122.9, 125.1, 126.0, 135.7, 137.0, 141.5, 148.5, 163.0; MS (EI) *m/z* (rel intensity) 337 (M^++2, 43), 335 (M^+, 44), 266 (14), 265 (99), 264 (18), 263 (100). Anal. Calcd for $C_{15}H_{18}BrN_3O$: C, 53.58; H, 5.39; N, 12.49. Found: C, 53.46; H, 5.30; N, 12.53.

4.4.5. 1-(2-Iodothiophen-3-ylmethyl)pyrrole-2-carboxylic acid methoxy methyl amide (14a). According to the general procedure, *N*-methoxy-*N*-methylpyrrole-2-carboxamide **2a** (154 mg, 1 mmol) was treated with KOH (224 mg, 4 mmol) in DMSO (5 mL), and bromide **13** (610 mg, 2 mmol). After work-up, flash column chromatography (silica gel, 20% hexane/AcOEt) afforded **14a** as a white solid, that was crystallised from Et_2O (301 mg, 80%): mp (Et_2O) 91–92 °C; IR (KBr) 1620 cm^{-1}; ^1H NMR ($CDCl_3$) 3.32 (s, 3H), 3.63 (s, 3H), 5.43 (s, 2H), 6.15 (dd, *J*=4.0, 2.6 Hz, 1H), 6.60 (d, *J*=5.5 Hz, 1H), 6.85 (dd, *J*=2.6, 1.8 Hz, 1H), 6.92 (dd, *J*=4.0, 1.6 Hz, 1H), 7.34 (d, *J*=5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) 33.6, 49.0, 60.8, 74.6, 107.9, 116.4, 122.8, 127.0, 127.8, 130.9, 143.1, 162.1; MS (EI) *m/z* (rel intensity) 376 (M^+, 1), 316 (23), 249 (21), 223 (31), 191 (6), 190 (14), 189 (100), 188 (6), 161 (6), 160 (9), 96 (14), 70 (6). Anal. Calcd for $C_{12}H_{13}IN_2O_2S$: C, 38.31; H, 3.48; N, 7.45. Found: C, 38.26; H, 3.45; N, 7.32.

4.4.6. 1-(2-Iodothiophen-3-ylmethyl)pyrrole-2-carboxylic acid diethyl amide (14b). According to the general procedure, *N*,*N*-diethylpyrrole-2-carboxamide **2b** (225 mg, 1.3 mmol) was treated with KOH (304 mg, 5.4 mmol) in DMSO (5 mL), and bromide **13** (821 mg, 2.7 mmol). After work-up, flash column chromatography (silica gel, 30% hexane/AcOEt) afforded **14b** as an oil (305 mg, 58%): IR (KBr) 1609 cm^{-1}; ^1H NMR ($CDCl_3$) 1.14 (t, *J*=7.1 Hz, 6H), 3.45 (q, *J*=7.1 Hz, 4H), 5.22 (s, 2H), 6.09 (d, *J*=2.8 Hz, 1H), 6.34 (dd, *J*=2.8, 1.6 Hz, 1H), 6.63 (d, *J*=5.5 Hz, 1H), 6.81 (d, *J*=1.6 Hz, 1H), 7.33 (d, *J*=5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) 13.5, 40.9, 48.3, 75.0, 107.0, 111.1, 124.8, 125.8, 128.0, 130.9, 143.0, 163.5; MS (EI) *m/z* (rel intensity) 388 (M^+, 5), 262 (19), 261 (100), 223 (41), 190 (16), 189 (52), 188 (18), 168 (92), 162 (13), 160 (14), 100 (11), 97 (16), 96 (24), 94 (10), 72 (33), 70 (15), 56 (11). Anal. Calcd for $C_{14}H_{17}IN_2OS$: C, 43.31; H, 4.41; N, 7.21. Found: C, 43.22; H, 4.31; N, 7.35.

4.4.7. 1-(2-Iodothiophen-3-ylmethyl)pyrrole-2-carboxylic acid morpholino amide (14c). According to the general procedure, 2-morpholinocarbonylpyrrole **2c** (163 mg, 0.91 mmol) was treated with KOH (203 mg, 3.62 mmol) in DMSO (5 mL), and bromide **13** (548 mg, 1.81 mmol). After work-up, flash column chromatography (silica gel, 20% hexane/AcOEt) afforded **14c** as an oil (237 mg,

65%): IR (KBr) 1609 cm^{-1}; ^1H NMR ($CDCl_3$) 3.51–3.56 (m, 4H), 3.63–3.67 (m, 4H), 5.21 (s, 2H), 6.08 (dd, *J*=4.0, 2.8 Hz, 1H), 6.27 (dd, *J*=4.0, 1.6 Hz, 1H), 6.60 (d, *J*=5.6 Hz, 1H), 6.83 (dd, *J*=2.8, 1.6 Hz, 1H), 7.32 (d, *J*=5.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) 45.3, 48.2, 66.7, 75.1, 107.2, 112.8, 124.3, 125.5, 128.0, 131.0, 142.8, 162.7; MS (EI) *m/z* (rel intensity) 486 (M^+, 3), 360 (23), 359 (100), 307 (17), 273 (6), 267 (9), 266 (55), 114 (7), 70 (8). Anal. Calcd for $C_{14}H_{15}IN_2O_2S$: C, 41.80; H, 3.76; N, 6.96. Found: C, 41.84; H, 3.63; N, 6.61.

4.4.8. 1-(Furan-3-ylmethyl)pyrrole-2-carboxylic acid methoxy methyl amide (19). According to the general procedure, *N*-methoxy-*N*-methylpyrrole-2-carboxamide **2a** (382 mg, 2.3 mmol) was treated with KOH (520 mg, 9.3 mmol) in DMSO (5 mL), and bromide **17** (747 mg, 4.6 mmol). After work-up, flash column chromatography (silica gel, 20% hexane/AcOEt) afforded **19** as an oil (369 mg, 65%): IR (KBr) 1624 cm^{-1}; ^1H NMR ($CDCl_3$) 3.30 (s, 3H), 3.59 (s, 3H), 5.35 (s, 2H), 6.13 (dd, *J*=4.0, 2.6 Hz, 1H), 6.29 (s, 1H), 6.32 (dd, *J*=2.6, 1.8 Hz, 1H), 6.89 (dd, *J*=4.0, 1.8 Hz, 1H), 7.32–7.34 (m, 2H); ^{13}C NMR ($CDCl_3$) 33.8, 43.5, 60.9, 107.7, 110.1, 116.6, 122.7, 122.8, 126.9, 140.2, 143.2, 162.5; MS (EI) *m/z* (rel intensity) 235 (M^++1, 1), 234 (M^+, 8), 175 (11), 174 (94), 146 (22), 81 (100). Anal. Calcd for $C_{12}H_{14}N_2O_3$: C, 61.52; H, 6.02; N, 11.96. Found: C, 61.84; H, 6.13; N, 12.04.

4.4.9. 1-(Thiophen-3-ylmethyl)pyrrole-2-carboxylic acid methoxy methyl amide (20). According to the general procedure, *N*-methoxy-*N*-methylpyrrole-2-carboxamide **2a** (154 mg, 1 mmol) was treated with KOH (224 mg, 4 mmol) in DMSO (5 mL), and bromide **18** (354 mg, 2 mmol). After work-up, flash column chromatography (silica gel, 20% hexane/AcOEt) afforded **20** as an oil (200 mg, 80%): IR (KBr) 1620 cm^{-1}; ^1H NMR ($CDCl_3$) 3.27 (s, 3H), 3.55 (s, 3H), 5.51 (s, 2H), 6.15 (dd, *J*=4.0, 2.6 Hz, 1H), 6.85–6.93 (m, 3H), 7.01 (dd, *J*=2.6, 1.8 Hz, 1H), 7.21 (dd, *J*=4.0, 1.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) 33.6, 47.2, 60.5, 107.9, 116.2, 121.7, 122.6, 125.6, 126.6, 126.8, 139.4, 162.1; MS (EI) *m/z* (rel intensity) 250 (M^+, 3), 191 (9), 190 (64), 97 (100). Anal. Calcd for $C_{12}H_{14}N_2O_2S$: C, 57.58; H, 5.64; N, 11.19. Found: C, 57.33; H, 5.55; N, 11.09.

4.5. Iodination of 3a,b and 7a,b. General procedure

A solution of **3a**,**b** or **7a**,**b** (1 mmol) in dry dioxane (5 mL) was added via cannula over a suspension of NaI (300 mg, 2 mmol), CuI (5% mol) and *N*,*N*′-dimethylethylene diamine (10% mol) in dioxane (10 mL). The mixture was refluxed for 16 h. H_2O (10 mL) was added and the resulting aqueous phase was extracted with CH_2Cl_2 (3×10 mL). The combined organic extracts were washed with brine (3×10 mL), dried (Na_2SO_4) and concentrated in vacuo. Flash column chromatography (silica gel) afforded the corresponding iodides **4a**,**b** and **8a**,**b**.

4.5.1. 1-(2-Iodoquinolin-3-ylmethyl)pyrrole-2-carboxylic acid methoxy methyl amide (4a). According to the general procedure, **3a** (374 mg, 1 mmol) was treated with NaI (300 mg, 2 mmol), CuI (5% mol) and *N*,*N*′-dimethylethylene diamine (10% mol). After work-up, flash column chromatography (silica gel, 30% hexane/AcOEt) afforded **4a** as an oil (358 mg, 85%): IR (KBr) 1617 cm^{-1}; ^1H NMR ($CDCl_3$) 3.19 (s, 3H), 3.62 (s, 3H), 5.67 (s, 2H), 6.27 (dd, *J*=3.7, 2.6 Hz, 1H), 6.88 (dd, *J*=2.6, 1.8 Hz, 1H), 7.07 (dd, *J*=3.7, 1.8 Hz, 1H)*, 7.08 (s, 1H)* 7.42–7.44 (m, 1H), 7.53–7.60 (m, 2H), 7.95 (d, *J*=8.5 Hz, 1H) (*Partially overlapped signals); ^{13}C NMR ($CDCl_3$) 33.5, 56.1, 61.0, 108.9, 117.2, 127.2, 127.4, 127.4, 127.5, 127.8, 128.5, 129.9.0, 130.1, 133.3, 135.7, 148.6, 168.7; MS (EI) *m/z* (rel intensity) 421 (M^+, 7), 361 (100), 315 (6), 268 (48), 234 (61), 205 (27), 141 (44). Anal. Calcd for $C_{17}H_{16}IN_3O_2$: C, 48.47; H, 3.82; N, 9.97. Found: C, 48.23; H, 3.59; N, 9.92.

4.5.2. 1-(2-Iodoquinolin-3-ylmethyl)pyrrole-2-carboxylic acid diethyl amide (4b). According to the general procedure, **3b** (386 mg, 1 mmol) was treated with NaI (300 mg, 2 mmol), CuI (5% mol) and *N*,*N*′-dimethylethylene diamine (10% mol). After work-up, flash column chromatography (silica gel, 30% hexane/AcOEt) afforded **4b** as an oil (347 mg, 80%): IR (KBr) 1619 cm^{-1}; ^1H NMR ($CDCl_3$) 1.08 (t, *J*=6.7 Hz, 6H), 3.44 (q, *J*=6.7 Hz, 4H), 5.46 (s, 2H), 6.21 (dd, *J*=3.6, 2.8 Hz, 1H), 6.42 (dd, *J*=3.6, 1.8 Hz, 1H), 6.82 (br s, 1H), 7.33 (s, 1H), 7.50–7.53 (m, 1H), 7.63–7.69 (m, 2H), 8.02 (d, *J*=8.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) 13.3, 41.2, 54.8, 107.6, 111.5, 122.4, 125.8, 127.0, 127.1, 127.4, 128.1, 129.9, 132.5, 134.5, 134.9, 148.4, 163.0; MS (EI) *m/z* (rel intensity) 434 (M^++1, 13), 433 (M^+, 41), 306 (100), 268 (33), 234 (45), 207 (44), 205 (42), 141 (44), 140 (44), 100 (49). Anal. Calcd for $C_{19}H_{20}IN_3O$: C, 52.67; H, 4.65; N, 9.69. Found: C, 52.48; H, 4.57; N, 9.72.

4.5.3. 1-(2-Iodopyridin-3-ylmethyl)pyrrole-2-carboxylic acid methoxy methyl amide (8a). According to the general procedure, **7a** (324 mg, 1 mmol) was treated with NaI (300 mg, 2 mmol), CuI (5% mol) and *N*,*N*′-dimethylethylene diamine (10% mol). After work-up, flash column chromatography (silica gel, 30% hexane/AcOEt) afforded **8a** as an oil (310 mg, 83%): IR (KBr) 1622 cm^{-1}; ^1H NMR ($CDCl_3$) 3.23 (s, 3H), 3.66 (s, 3H), 5.44 (s, 2H), 6.23 (dd, *J*=4.0, 2.6 Hz, 1H), 6.60 (dd, *J*=7.5, 1.6 Hz, 1H), 6.80 (dd, *J*=2.6, 1.8 Hz, 1H), 7.02 (dd, *J*=4.0, 2.0 Hz, 1H), 7.08 (dd, *J*=7.5, 4.8 Hz, 1H), 8.17 (dd, *J*=4.8, 1.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) 33.4, 51.8, 61.0, 108.8, 117.0, 123.1, 127.7, 127.8, 135.9, 136.3, 141.0, 148.4, 161.7. Anal. Calcd for $C_{13}H_{14}IN_3O_2$: C, 42.06; H, 3.80; N, 11.32. Found: C, 42.35; H, 3.88; N, 11.47.

4.5.4. 1-(2-Iodopyridin-3-ylmethyl)pyrrole-2-carboxylic acid diethyl amide (8b). According to the general procedure, **7b** (335 mg, 1 mmol) was treated with NaI (300 mg, 2 mmol), CuI (5% mol) and *N*,*N*′-dimethylethylene diamine (10% mol). After work-up, flash column chromatography (silica gel, 30% hexane/AcOEt) afforded **8b** as an oil (346 mg, 87%): IR (KBr) 1621 cm^{-1}; ^1H NMR ($CDCl_3$) 1.05 (t, *J*=6.7 Hz, 6H), 3.37 (q, *J*=6.7 Hz, 4H), 5.20 (s, 2H), 6.10 (dd, *J*=4.0, 2.6 Hz, 1H), 6.34 (dd, *J*=4.0, 2.0 Hz, 1H), 6.69 (dd, *J*=2.6, 1.8 Hz, 1H), 6.80 (dd, *J*=7.5, 1.6 Hz, 1H), 7.06 (dd, *J*=7.5, 4.8 Hz, 1H), 8.11 (dd, *J*=4.8, 1.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) 13.4, 41.3, 50.6, 107.7, 111.4, 122.9, 125.1, 126.0, 135.7, 137.0, 141.5, 148.5, 163.0 (CO). Anal. Calcd for $C_{15}H_{18}IN_3O$: C, 47.01; H, 4.73; N, 10.96. Found: C, 47.11; H, 4.66; N, 10.89.

4.6. Metalation–cyclisation reactions

Only experimental procedures of the best yielding method for the synthesis of **9**, **10**, **15** and **21** are given.

4.6.1. Synthesis of 11*H*-pyrrolo[1,2-*b*]acridin-4-one (9). To a solution of iodinated pyrrole-2-carboxamide **4a** (421 mg, 1 mmol) in dry THF (15 mL), *n*-BuLi (1.4 mL of a 1.6 M solution in hexanes, 2.2 mmol) was added at −90 °C, and the resulting mixture was stirred at this temperature for 5 min. The reaction was quenched by the addition of satd NH_4Cl (10 mL). Et_2O (15 mL) was added, the organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2 (3×15 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo. Flash column chromatography (silica gel, 50% hexane/AcOEt) afforded pyrrolo[1,2-*b*]acridinone **9** (199 mg, 85%): IR (KBr) 1650 cm^{-1}; ^1H NMR ($CDCl_3$) 5.62 (s, 2H), 6.50 (dd, *J*=4.0, 2.4 Hz, 1H), 7.15 (br s, 1H), 7.38 (dd, *J*=4.0, 1.2 Hz, 1H), 7.62–7.68 (m, 1H), 7.76–7.82 (m, 1H), 7.85 (d, *J*=8.3 Hz, 1H), 8.20 (s, 1H), 8.43 (d, *J*=8.3 Hz, 1H); ^{13}C NMR ($CDCl_3$) 46.7, 112.6, 115.9, 126.3, 126.9, 127.3, 128.3, 129.7, 130.5, 131.3, 131.8, 133.9, 146.4, 148.8, 173.1. MS (EI) *m*/*z* (rel intensity) 235 (M^++1, 21), 433 (M^+, 100), 233 (29), 206 (44), 205 (81), 166 (10), 151 (9), 140 (18), 114 (10), 103 (13). Anal. Calcd for $C_{15}H_{10}N_2O$: C, 76.91; H, 4.30; N, 11.96. Found: C, 76.49; H, 4.27; N, 11.94.

4.6.2. Synthesis of 5*H*-pyrrolo[1,2-*g*]quinolin-10-one (10). According to the procedure described for the synthesis of **9**, pyrrole-2-carboxamide **8a** (371 mg, 1 mmol) was treated with *n*-BuLi (1.4 mL of a 1.6 M solution in hexanes, 2.2 mmol) at −90 °C for 5 min. After work-up, flash column chromatography (silica gel, 50% hexane/AcOEt) afforded pyrrolo[1,2-*g*]quinolone **10** (156 mg, 85%): IR (KBr) 1640 cm^{-1}; ^1H NMR ($CDCl_3$) 5.39 (s, 2H), 6.37 (dd, *J*=4.0, 2.4 Hz, 1H), 7.05 (br s, 1H), 7.18 (dd, *J*=4.0, 1.2 Hz, 1H), 7.40 (dd, 7.0, 4.0 Hz, 1H), 7.70 (dd, *J*=7.0, 1.6 Hz, 1H), 8.72 (dd, *J*=4.0, 1.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) 46.9, 112.6, 115.7, 127.3, 128.1, 130.3, 131.7, 133.6, 146.2, 148.0, 173.1. Anal. Calcd for $C_{11}H_8N_2O$: C, 71.73; H, 4.37; N, 15.21. Found: C, 71.56; H, 4.25; N, 15.04.

4.6.3. Synthesis of 5*H*-thieno[3,2-*f*]indolizin-9-one (15).[25] To a solution of iodinated pyrrole-2-carboxamide **14a** (199 mg, 0.53 mmol) in dry THF (15 mL), *t*-BuLi (0.73 mL of a 1.6 M solution in pentane, 1.17 mmol) was added at −78 °C, and the resulting mixture was stirred at this temperature for 3 h. The reaction was quenched by the addition of satd NH_4Cl (10 mL). After standard work-up, flash column chromatography (silica gel, 30% hexane/AcOEt) afforded thieno[3,2-*f*]indolizinone **15**, that was crystallised from ethanol (71 mg, 71%): mp (EtOH) 160–163 °C (lit.[6] 168–170 °C); IR (KBr) 1632 cm^{-1}; ^1H NMR ($CDCl_3$) 5.33 (s, 2H), 6.39–6.40 (m, 1H), 7.05−7.11 (m, 3H), 7.67 (d, *J*=5.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) 46.3, 111.5, 112.5, 125.2, 125.8, 129.6, 133.4, 136.1, 141.8, 170.4. MS (EI) *m*/*z* (rel intensity) 191 (M^++2, 5), 190 (M^++1, 13), 189 (M^+, 100), 160 (33), 134 (5), 83 (5).

4.6.4. Synthesis of 5*H*-furo[3,2-*f*]indolizin-9-one (21). To a solution of pyrrole-2-carboxamide **19** (199 mg, 0.53 mmol) in dry THF (15 mL), LDA (10 mL of a 0.28 M solution in THF, 2.8 mmol) was added at −78 °C, and the resulting mixture was stirred at this temperature for 3 h. The reaction was quenched by the addition of satd NH_4Cl (10 mL). After standard work-up, flash column chromatography (silica gel, 50% hexane/AcOEt) afforded furo[3,2-*f*]indolizinone **21** (146 mg, 60%): IR (KBr) 1654 cm^{-1}; ^1H NMR ($CDCl_3$) 5.27 (s, 2H), 6.37 (dd, *J*=4.3, 2.6 Hz, 1H), 6.56 (d, *J*=1.8 Hz, 1H), 7.07 (dd, *J*=2.6, 1.6 Hz, 1H), 7.11 (dd, *J*=4.2, 1.6 Hz, 1H), 7.68 (d, *J*=1.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) 44.3, 109.5, 111.4, 112.8, 126.0, 129.4, 131.0, 146.2, 147.5, 165.6. MS (EI) *m*/*z* (rel intensity) 174 (M^++1, 14), 173 (M^+, 100), 172 (21), 145 (16), 144 (10), 117 (46), 116 (15), 90 (31). Anal. Calcd for $C_{10}H_7N_2O$: C, 69.36; H, 4.07; N, 8.09. Found: C, 69.28; H, 4.10; N, 8.10.

Acknowledgements

Financial support from MCYT (BQU2003-03239), and Universidad del País Vasco/Euskal Herriko Unibertsitatea is gratefully acknowledged. We also thank MEC for a grant (J.R.).

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Tetrahedron 62 (2006) 6190–6202
Tetrahedron

New macrobicyclic triphosphazides and triphosphazenes formed by self-assembly of tripodal triazides with triphosphanes

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Received 9 March 2006; revised 19 April 2006; accepted 20 April 2006
Available online 11 May 2006

Abstract—The self-assembly of tris(3-azidobenzyl)amines with 1,1,1-tris[(diphenylphosphino)methyl]ethane via tripod–tripod coupling proceeds successfully to give chiral macrobicyclic triphosphazides. The heating of these macrobicyclic cages induces a remarkable stepwise triple extrusion of molecular nitrogen to afford tri-λ^5-phosphazenes, which preserved the chiral, propeller-like topology of their precursors. The replacement of one benzylic arm by an *ortho*-phenethylic or propylenic one still allows the success of the self-assembly. However, the quaternization of the pivotal nitrogen atom of the triamine in the form of *N*-oxide prevented its coupling with the triphosphane.

1. Introduction

The synthesis, structure, and properties of numerous macrobicyclic cages have been intensively studied during the past few decades. In fact, several synthetic strategies may be devised for the construction of such molecular architectures. The more direct one is the tripod–tripod coupling, a molecular self-assembly process that implies the formation of three bonds in a single step.[1] A reaction of this type requires first complementary components containing two or more interaction sites capable of establishing multiple connections, and second the reversibility of the connecting events in order to allow the full exploration of the energy hypersurface of the system.[2] A major drawback of tripod–tripod coupling is the occurrence of extensive side reactions, which minimize the yield of the expected bicyclic product. Only in limited cases,[3] such processes have been carried out in synthetically useful yields, provided that fine tuning of reagents, reactions, and conditions could be achieved.

The imination reaction of tervalent phosphorus compounds with organic azides is known as Staudinger reaction,[4] a two-step process involving the initial nucleophilic attack of a P^{III} center, usually a tertiary phosphane $(R^1)_3P$, to the terminal nitrogen atom of an azide R^2N_3 followed by dinitrogen extrusion from the intermediate phosphazide $(R^1)_3PN_3R^2$ giving the λ^5-phosphazene $(R^1)_3P{=}NR^2$ (also known as phosphine imine or iminophosphorane). Only in a few instances, the primary imination products, phosphazides, have been isolated.[5]

In previous communications,[6] we reported the preparation of the first examples of chiral C_3-symmetric, macrobicyclic triphosphazides formed by two tripodal subunits by means of triple P–N bond formation in Staudinger reaction, and without recourse to high-dilution conditions.

Here we present the results of a detailed study on the preparation, characterization, and properties of a variety of macrobicyclic triphosphazides and tri-λ^5-phosphazenes formed by self-assembly of pivotal triazides bearing a tribenzylamine skeleton with 1,1,1-tris[(diphenylphosphino)methyl]ethane. The validity of this method has been extended to other triazides where one benzylic arm is replaced by an *ortho*-phenethylic or propylenic one.

2. Results and discussion

2.1. Preparation of tris(3-azidobenzyl)amines (1)

A wide range of new tris(3-azidobenzyl)amines **1** of C_3, C_s, and C_1 local symmetries (Fig. 1 and Table 1) were prepared by standard procedures.

As a representative example, the synthesis of the asymmetric triazides **1c** and **1d** is outlined in Scheme 1, which is based on the sequential reduction of 2-chloro-5-nitrobenzaldehyde to 5-amino-2-chlorobenzyl alcohol, which is further converted into the corresponding azidobenzyl alcohol in the usual way (diazotization/azidation). This alcohol reacted

Keywords: Azides; Phosphazides; λ^5-Phosphazenes; Macrobicycles; Cage compounds; Propeller; Tripod–tripod coupling.
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doi:10.1016/j.tet.2006.04.056

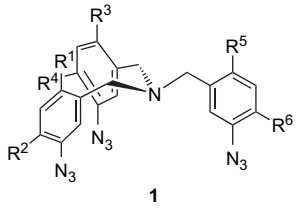


Figure 1. Tripodal triazides **1**.

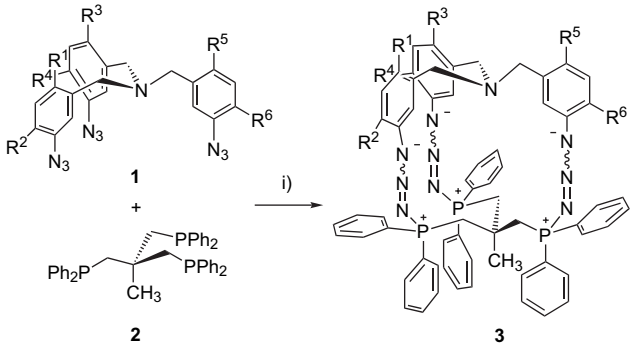
Table 1. Tris(3-azidobenzyl)amines **1**

Compound	R^1	R^2	R^3	R^4	R^5	R^6
1a	H	H	H	H	H	H
1b	Br	H	Br	H	Br	H
1c	Br	H	Br	H	Cl	H
1d	Br	H	Cl	H	H	H
1e	H	CH_3	H	CH_3	H	CH_3
1f	H	CH_3	H	CH_3	H	H
1g	H	CH_3	H	H	H	H

with thionyl chloride to yield 5-azido-2-chlorobenzyl chloride that was converted into the corresponding primary benzylamine by Gabriel reaction. Further alkylation with 5-azido-2-bromobenzyl bromide afforded **1c** and the expected secondary amine, which is alkylated by 3-azidobenzyl iodide finally yielding **1d**. A detailed description for the synthesis of the rest of compounds **1** prepared, as well as their spectroscopic data, can be found in Supplementary data.

2.2. Tripod–tripod coupling of triazides 1 with 1,1,1-tris[(diphenylphosphino)methyl]ethane (2)

The tripod–tripod coupling of triazides **1** with 1,1,1-tris[(diphenylphosphino)methyl]ethane (**2**) was carried out in diethyl ether solution at room temperature. The resulting macrobicyclic triphosphazides **3** (Scheme 2) precipitated from the reaction medium as yellow solids and were obtained in good yields, with the only exception of **3e** that could not be prepared (Table 2). In this last case (Table 2, entry 5), the material isolated from the reaction was a complex mixture, which seems to contain compounds combining both phosphazide and phosphazene units, as indicated by its ^{1}H,

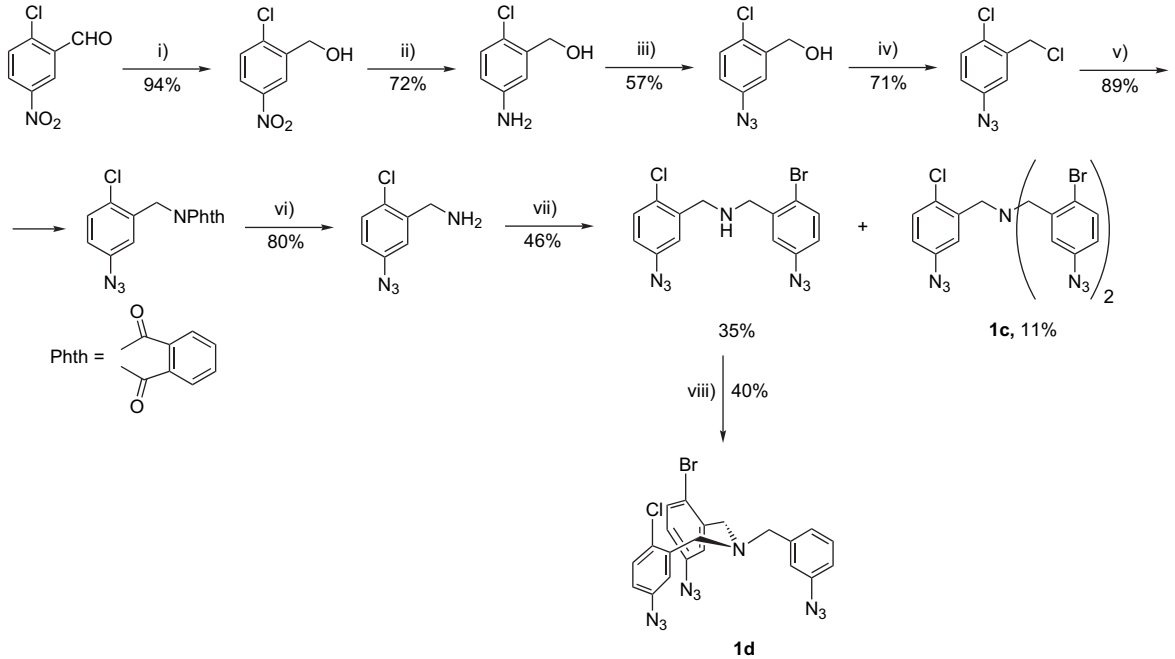


Scheme 2. Synthesis of triphosphazides **3**. Conditions: (i) Et_2O, 25 °C, 3 h.

Table 2. Triphosphazides **3**

Entry	Compound	R^1	R^2	R^3	R^4	R^5	R^6	Yield (%)
1	**3a**	H	H	H	H	H	H	79
2	**3b**	Br	H	Br	H	Br	H	63
3	**3c**	Br	H	Br	H	Cl	H	80
4	**3d+3d′**	Br	H	Cl	H	H	H	66
5	**3e**	H	CH_3	H	CH_3	H	CH_3	—[a]
6	**3f**	H	CH_3	H	CH_3	H	H	77
7	**3g**	H	CH_3	H	H	H	H	79

[a] Complex mixture.



Scheme 1. Synthesis of triazides **1c** and **1d**. Reagents and conditions: (i) $NaBH_4$, MeOH, 0→25 °C, 24 h; (ii) Fe–AcOH, EtOH, reflux, 4 h; (iii) $NaNO_2$, dil H_2SO_4, 0 °C, 30 min, then NaN_3, 25 °C, 16 h; (iv) $SOCl_2$, CH_2Cl_2, 0 °C, 3 h; (v) potassium phthalimide (KNPhth), DMF, 80 °C, 12 h; (vi) $N_2H_4\cdot H_2O$, EtOH, reflux, 3 h; (vii) 5-azido-2-bromobenzyl bromide, 1,4-dioxane, reflux, 3 h, then Et_3N, 25 °C, 2 h, (viii) 3-azidobenzyl iodide, 1,4-dioxane, reflux, 8 h, then Et_3N, 25 °C, 3 h.

$^{13}C\{^1H\}$, and $^{31}P\{^1H\}$ NMR spectra. The structural determination of compounds **3** was accomplished by means of their analytical and spectral data. Full characterization of **3a** was discussed in a previous communication.[6c]

Selected physical data of new triphosphazides **3b–g** were essentially coincident with those of the previously reported **3a**, indicative of their tridimensional arrangements where the lone pair and CH_3 group on the bridgehead atoms are directed in and out, respectively, from the bicyclic cavity and possessing propeller-like topology in solution and in the solid state. The intrinsic chirality of these species was shown by the anisochronous C*H*$_2$N and C*H*$_2$P methylene protons in their ^1H NMR spectra, as well as by the appearance of the *ipso*, *ortho*, *meta*, and *para* carbons of the *Ph*P rings as two sets of signals. The observed magnetic equivalence of the three arms of macrobicycles **3a** and **3b** in their solution ^1H and $^{13}C\{^1H\}$ NMR spectra is in contrast with their inequivalence in the solid state. The X-ray crystal structure of **3a**, shows two zwitterionic PN_3 fragments ($^+P–N{=}N–N^-$) of *Z* configuration with respect to the central N=N bond whereas the third one is *E*. This dissimilitude of NMR data can only be understood if, in $CDCl_3$ solution at 298 K, the fluxionality of **3a** and **3b** involves *E*/*Z* equilibration of the phosphazide fragments to yield a bicyclic skeleton of C_3-symmetric mean conformation. Indicative of such equilibration is the observed broad signals in their ^1H and $^{13}C\{^1H\}$ NMR spectra, and more notoriously in their $^{31}P\{^1H\}$ NMR spectra in $CDCl_3$ at 298 K, where a very broad singlet around δ 3.0 ($\Delta\nu_{½}$=972 Hz for **3a** and 608 Hz for **3b**) is observed. This signal is somewhat thinner when the spectra were run in CD_2Cl_2 solution, appearing at δ 8.93 ($\Delta\nu_{½}$=425 Hz) and 5.36 ($\Delta\nu_{½}$=484 Hz) for **3a** and **3b**, respectively. The assumption of this equilibration is also in accord with the results of low temperature NMR experiments. Thus, on cooling at 253 K in $CDCl_3$ solution, the broad singlet in the $^{31}P\{^1H\}$ NMR spectrum of **3a** separated out into two broad singlets, which at 213 K resolved into two well-separated multiplets of complex fine structure, centered at δ 2 and 24, which probably are associated with the two types of phosphorus atoms: those included into PN_3 units of *Z* and *E* configuration, respectively (Fig. 2). These values are similar to those observed in the solid state by the CPMAS-NMR spectrum of **3a**, which shows one signal at δ 1.31 and other at 24.13, the first one of double intensity.

To our knowledge, the characterization of phosphazides as *Z* or *E* geometrical isomers (in their zwitterionic $^+P–N{=}N–N^-$ forms, equivalent to *s-cis* or *s-trans* in the neutral P=N–N=N form, respectively) by spectroscopic means has not been generalized up to now. From the $^{31}P\{^1H\}$ NMR data of compounds **3** in solution, we conclude that the chemical shifts for the phosphorus atoms belonging to intracyclic phosphazide units are quite different depending on their geometry. Thus, we propose the approximate shift values of δ 23 and δ 0 for phosphazides with *E* and *Z* geometry, respectively.

The molecular fluxionality of **3f** and **3g** is not so accused as that of their counterparts commented above. Their $^{31}P\{^1H\}$ NMR spectra show two well-defined singlets in the ranges established above for *E* and *Z* phosphazide units. Thus, compound **3f** shows two signals at δ 2.14 and 21.58, this last one

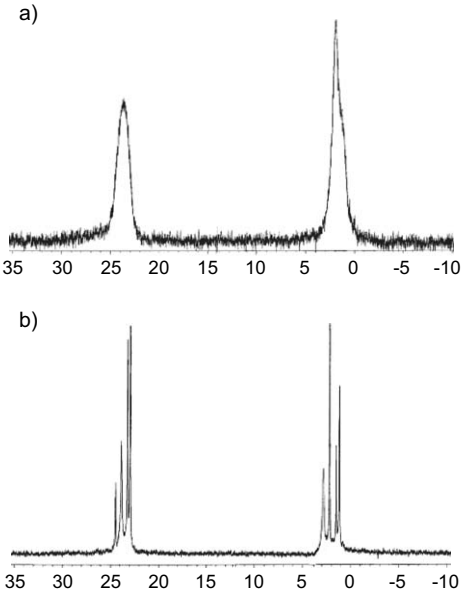


Figure 2. $^{31}P\{^1H\}$ NMR spectra of triphosphazide **3a** recorded at (a) 253 K and (b) 213 K.

of double intensity, assignable to the phosphorus atoms of the units with *Z* (arm without CH_3) and *E* configuration (arms with CH_3), respectively. By contrary, the triphosphazide **3g** shows a slightly broad singlet at δ 5.12 of double intensity than that appearing at δ 21.50. These data can be interpreted by assuming that the CH_3 group in *ortho*-position to the N terminus of a PN_3 function exerts some steric influence on the geometry of the nearby phosphazide fragment. This may result in preference for the *E* configuration of that phosphazide arm in order to minimize repulsive interactions with the *ortho*-methyl group (Fig. 3).

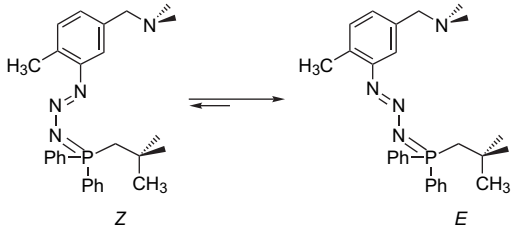
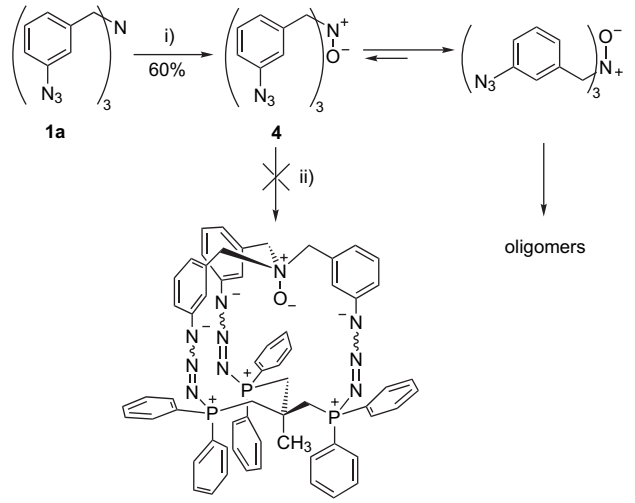


Figure 3. *E*/*Z* equilibrium of the phosphazide fragments, displaced toward *E* as a result of the presence of the *ortho*-CH_3 group.

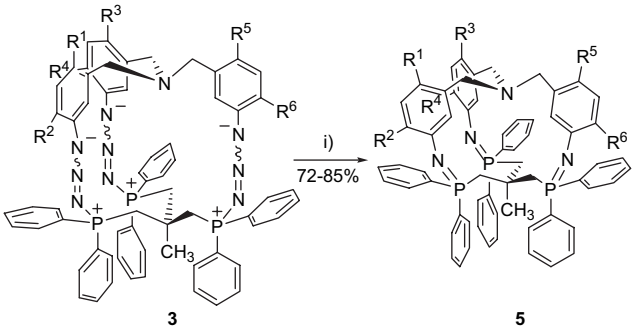
The coupling of triphos with tris(3-azidobenzyl)amine *N*-oxide **4**, prepared by reaction of **1a** with *m*CPBA in 60% yield, did not yield the expected triphosphazide, instead giving rise to oligomeric products (Scheme 3). We reasoned that this result can be a consequence of a low population, in the conformational equilibrium of **4**, of the reactive conformer with the optimal tridimensional structure for the success of the tripod–tripod coupling, other more populated conformations leading to oligomers. Alternatively, the putative macrobicycle could be highly unstable as a result of electronic repulsions between the negatively charged O and N atoms.



Scheme 3. Reaction of tris(3-azidobenzyl)amine *N*-oxide (**4**) with triphosphane **2**. Reagents and conditions: (i) *m*CPBA, $CHCl_3$, 0 °C, 4 h; (ii) 2, Et_2O, 25 °C, 3 h.

2.3. Macrobicyclic tri-λ^5-phosphazenes from triphosphazides

When compounds **3** were heated at 333 K in $CDCl_3$ solution for 24 h they were cleanly converted into tri-λ^5-phosphazenes **5** in 72–85% yields, by triple extrusion of molecular N_2 (Scheme 4). The dinitrogen expulsion from each PN_3 unit can be understood as the second mechanistic step of Staudinger imination reaction.[4,7]



	R^1	R^2	R^3	R^4	R^5	R^6
5a	H	H	H	H	H	H
5b	Br	H	Br	H	Br	H
5c	Br	H	Br	H	Cl	H
5d + 5d'	Br	H	Cl	H	H	H
5e	H	CH_3	H	CH_3	H	CH_3
5f	H	CH_3	H	CH_3	H	H
5g	H	CH_3	H	H	H	H

Scheme 4. Synthesis of tri-λ^5-phosphazenes **5**. Conditions: (i) $CDCl_3$, 60 °C, 24 h.

The structural determination of the cage compounds **5** was accomplished by its analytical and spectroscopic data. Full characterization of **5a** and **5b** was discussed in our previous communication.[6d] The X-ray analysis of **5b** was described there, and is shown here for convenience (Fig. 4). The molecule is located on a noncrystallographic threefold axis passing through the two bridgehead atoms of the bicyclic cage, and its propeller-like shape is clearly apparent when the molecule is viewed along this axis. Both propeller units, the upper tribenzylamine core and the lower *tert*-pentane fragment, present the same helical twist sense.[8]

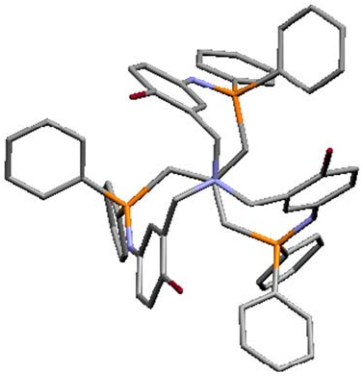


Figure 4. A perspective view of compound **5b** as projected along the threefold axis.

Since the physical data of the new tri-λ^5-phosphazenes **5c–g** were essentially coincident with those of the previously reported **5a** and **5b**, we assumed the same tridimensional arrangements for the new members of the series prepared here.

In the ^{1}H NMR spectra of compounds **5**, the protons of the pivotal CH_3 group appear as a singlet between −0.78 and −0.69 ppm, notably upfield relative to those in the phosphane (δ 0.95).[9] These data are consistent with a conformation in solution in which the three pseudoaxial phenyl groups are flanking the pivotal methyl group, situation that is favored by stabilizing [CH···π] interactions.[10]

The simplicity of the NMR of C_3-symmetric **5a**, **5b**, and **5e** indicates high symmetry. The ^{31}P{^{1}H} NMR spectra of these compounds show only one sharp singlet between −1.43 and 0.49 ppm, shifted 25.87–27.79 ppm downfield relative to that of the parent triphosphane δ −27.3,[11] in accordance with previous reports on acyclic λ^5-phosphazenes.[4b–d] In their ^{1}H and ^{13}C{^{1}H} NMR spectra, only one set of signals is observed for the three equivalent arms of the bicyclic structure. The methylene protons of the CH_2N and CH_2P groups are magnetically inequivalent as a consequence of their diastereotopic nature, accounting for the chirality of these propeller-shaped compounds.

The different coupling patterns shown by the two CH_2P protons (an apparent triplet and an apparent quintuplet) in the ^{1}H NMR spectra are particularly significant. The first signal is due to what may be called the *pseudoaxial* (nearly parallel to the local C_3-axis) methylene proton, ^3J(H,H)≈ ^2J(H,P)=14.4–14.5 Hz, whereas the second one, with a separation between lines of 6.8–8.4 Hz, corresponds to the *pseudoequatorial* proton, which is further split by a long-distance coupling with the phosphorus atom in one of the other arms in relative *M* planar disposition to that proton, as revealed by the crystal structure of **5b**. The observation of these coupling patterns in the solution spectrum of **5b** clearly indicates that the solid state and the room temperature solution conformation of compounds **5** could be nearly identical.

In the ^{13}C{^{1}H} NMR spectra of compounds **5a**, **5b**, and **5e**, the two phenyl rings linked to each phosphorus atom are

magnetically inequivalent, and show notable differences in the ^1J(C,P) coupling constants of their two *ipso* carbons (see Section 4). This is a consequence of the environment of the phosphorus atoms,[6a] which causes the neat differentiation of the two *Ph*P rings: one *pseudoaxial* and the other *pseudoequatorial*.

Compounds **5c**, **5f**, and **5g** with only two chemically identical arms, exhibit two singlets in a 2:1 intensity ratio in their $^{31}P\{^1H\}$ NMR. Their ^1H and $^{13}C\{^1H\}$ MNR spectra are very complex as a result of the diastereotopicity of the two chemically equivalent arms (see Section 4).

The tri-λ^5-phosphazene derived from the constitutionally chiral, benzyl 4-bromobenzyl 4-chlorobenzyl amine **1d** was obtained as an approximately equimolecular mixture of two diastereoisomers, **5d** and **5d′**. These two diastereoisomers were not separated, the composition of the mixture (1:1) being deduced by integration in its ^1H NMR spectrum. The $^{31}P\{^1H\}$ NMR spectrum of this mixture shows six singlets of equal intensity, which correspond to the two sets of three inequivalent phosphorus atoms, each one associated to one diastereoisomer. The equimolecular diastereomeric composition of the mixture is also evidenced in some regions of its $^{13}C\{^1H\}$ NMR spectrum, such as the CH_2N zone in the range δ 55.13–58.10.

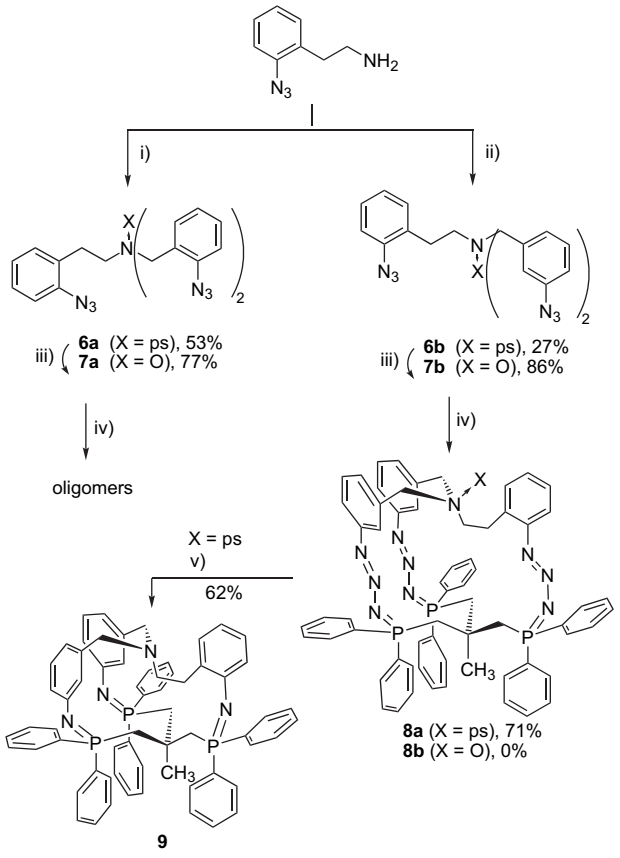
We wondered if the triple extrusion of dinitrogen in **3** leading to **5** takes place either simultaneously in the three arms or stepwise, in one arm after the other. The mass spectrometric data, MS-FAB+, of the reaction mixture at different times, from 10 min up to 6 h, revealed that the dinitrogen extrusion occurs stepwise, since we could detect in those spectra molecular ions corresponding to macrobicyclic species in which phosphazide arms coexisted with λ^5-phosphazene arms.

2.4. Structural variations in the triazide component

Having established the efficiency of the self-assembly between triphosphane **2** and triazides derived from tribenzylamine with its three azido groups situated either in *ortho*[6a,b] or *meta* positions, we next examined if this coupling strategy is still valid for the assembly of **2** with other tripodal triazides of slightly different structure, as for instance those bearing one arm of different length and/or nature than the other benzylic ones. With this aim, we prepared the triazides **6a** and **6b** by alkylation of 2-(2-azidophenyl)ethylamine[12] with 2 equiv of the appropriate azidobenzyl iodide. The quaternization of the pivotal nitrogen atom of **6** with *m*CPBA gave rise to the corresponding *N*-oxides **7** (Scheme 5).

The tripodal coupling of these new azides with **2** was successful only in the case of triazide **6b** giving rise to triphosphazide **8a**, whereas **6a**, **7a**, and **7b** afforded oligomeric products (Scheme 5).

The structure determination of **8a** was accomplished by comparison of its spectroscopic data with those previously obtained for other triphosphazides. Its ^{31}P NMR spectrum shows three signals, two (at δ 19.93 and 21.28) attributed to the benzylic diastereotopic *E* arms and the third one (at δ −0.55) corresponding to the phenethylic *Z* arm. In the solid state, its CPMAS-NMR shows similar shift values, δ −3.12,



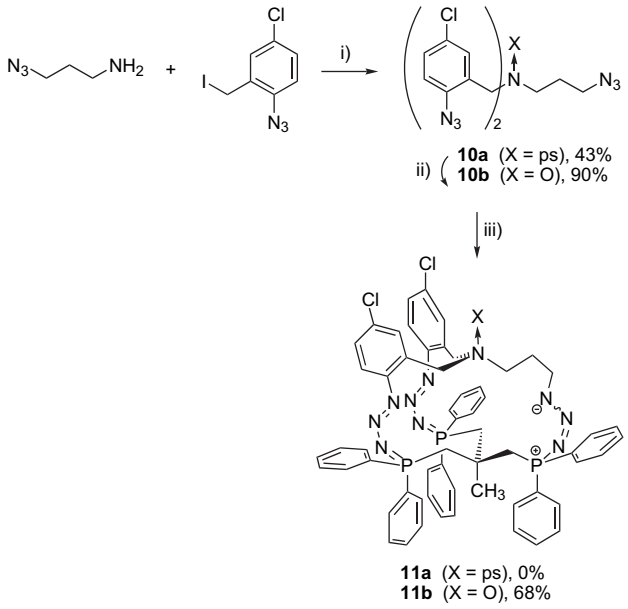
Scheme 5. Synthesis of tri-λ^5-phosphazene **9**. Reagents and conditions: (i) (a) 2-azidobenzyl iodide, 1,4-dioxane, reflux, 4 h; (b) excess Et_3N, rt, 2 h; (ii) (a) 3-azidobenzyl iodide, 1,4-dioxane, reflux, 4 h; (b) excess Et_3N, rt, 2 h; (iii) *m*CPBA, $CHCl_3$, 0 °C, 4 h; (iv) triphos (**2**), Et_2O, 25 °C, N_2 atm, 3 h; (v) $CDCl_3$, 60 °C, 24 h.

21.02, and 25.24. Molecular models (CPK) accommodate best an out arrangement for the lone pair on the bridgehead nitrogen atom.

The thermal treatment of **8a** yielded tri-λ^5-phosphazene **9** in 62%, which shows spectroscopic data in accordance with its structure (Scheme 5).

Finally, we tested if the change of one benzylic arm by other aliphatic one of similar length (three carbon atoms) allowed the tripod–tripod coupling. Thus, we prepared triazide **10a** by alkylation of 3-azidopropylamine[13] with 2-azido-5-chlorobenzyl iodide (Scheme 6). The quaternization of its pivotal nitrogen with *m*CPBA gave rise to the corresponding *N*-oxide **10b**.

The reaction of bis(2-azido-5-chlorobenzyl)(3-azidopropyl)amine (**10a**) with triphosphane **2** did not provide the expected macrobicycle, instead oligomeric products were obtained. However, the reaction of the *N*-oxide **10b** afforded a yellow microcrystalline solid, its spectroscopic data and elemental analysis were in agreement with the structure of triphosphazide **11b** (Scheme 6). Probably, macrobicycle **11a** could not be obtained because of its instability in solution caused by the pyramidal inversion of its pivotal nitrogen atom, which favors the dissociation of the phosphazide arms, as we have demonstrated in other similar cases.[6b] In contrast, the stability of triphosphazide **11b** is notably greater as a



Scheme 6. Synthesis of triphosphazide **11b**. Reagents and conditions: (i) (a) 1,4-dioxane, reflux, 4 h; (b) excess Et_3N, rt, 2 h; (ii) *m*CPBA, $CHCl_3$, 0 °C, 4 h; (iii) 2, Et_2O, 25 °C, N_2 atm, 3 h.

result of the higher configurational stability imposed by the *N*-oxide function, which obviously blocks such inversion. Moreover, the pivotal oxygen atom of **11b** avoids electronic repulsive interferences with the phosphazide nitrogen atoms by orienting itself out from the internal cavity.

3. Conclusions

A wide range of examples of chiral macrobicyclic triphosphazides have been prepared by tripod–tripod coupling of several triazides derived from the tribenzylamine skeleton with 1,1,1-tris[diphenylphosphino]methyl]ethane. These cage compounds show molecular fluxionality, associated with the *E*/*Z* isomerization of its PN_3 units, and could be converted into tri-λ^5-phosphazenes by nonsimultaneous dinitrogen extrusion in its three phosphazide fragments. The reported method was also suitable for the synthesis of macrobicyclic triphosphazides where one benzylic arm has been substituted by another *ortho*-phenethylic or propylenic fragment.

4. Experimental

4.1. General

Coupling reactions were carried out under nitrogen and using solvents that were dried by routine procedures. Column chromatography was performed with the use of silica gel (70–200 μm) as a stationary phase. All melting points were determined on a Kofler hot-plate melting point apparatus and are uncorrected. IR spectra were determined as Nujol emulsions or films on a Nicolet Impact 400 spectrophotometer. NMR spectra were recorded at 25 °C on a Brucker AC200 (200 MHz) or a Varian Unity 300 (300 MHz). ^1H and ^{13}C chemical shifts were reported in parts per million downfield of internal tetramethylsilane (TMS) and ^{31}P chemical shifts were externally referenced to 85% aqueous phosphoric acid or ammonium hydrogen phosphate. Abbreviations of coupling patterns are as follows: s, singlet; d, doublet; t, triplet; q, quadruplet. The mass spectra were recorded on a Hewlett-Packard 5993C spectrometer (EI) or on a VG-Autospec spectrometer (FAB^+). Microanalyses were performed on an EA 1108 Carlo Erba instrument.

4.2. Materials

Compounds 2-chloro-5-nitrobenzyl alcohol,[14] 5-amino-2-chlorobenzyl alcohol,[15] 3-azidobenzyl alcohol,[16] 3-azidobenzyl bromide,[17] and 2-azidobenzyl iodide[6b] were prepared following previously reported procedures. The syntheses and data of 3-azido-4-methylbenzyl alcohol, 3-azidobenzyl chloride, 3-azido-4-methylbenzyl chloride, 3-azido-4-methylbenzyl iodide, 3-azidobenzylamine, 3-azido-4-methylbenzylamine, bis(3-azidobenzyl)amine, bis(3-azido-4-methylbenzyl)-amine, bis(5-azido-2-bromobenzyl)amine, tris(3-azidobenzyl)amine (**1a**), tris(5-azido-2-bromobenzyl)-amine (**1b**), tris(3-azido-4-methylbenzyl)amine (**1e**), bis(3-azido-4-methylbenzyl)(3-azidobenzyl)amine (**1f**), bis(3-azidobenzyl)(3-azido-4-methylbenzyl)amine (**1g**), tris(3-azidobenzyl)amine *N*-oxide (**4**), bis(2-azidobenzyl)[(2-azidophenyl)ethyl]amine (**6a**), bis(3-azidobenzyl)[(2-azidophenyl)ethyl]amine (**6b**), bis(2-azidobenzyl)[(2-azidophenyl)ethyl]amine *N*-oxide (**7a**), bis(3-azidobenzyl)[(2-azidophenyl)ethyl]amine *N*-oxide (**7b**), bis(2-azido-5-chlorobenzyl)(3-azidopropyl)amine (**10a**), and bis(2-azido-5-chlorobenzyl)(3-azidopropyl)amine *N*-oxide (**10b**) are given in Supplementary data.

4.3. Preparation of 5-azido-2-chlorobenzyl alcohol

A solution of sodium nitrite (3.30 g, 48 mmol) in H_2O (30 mL) was added dropwise to an ice-cooled solution of 5-amino-2-chlorobenzyl alcohol (5.20 g, 33 mmol) in a mixture of H_2O (40 mL) and concentrated sulfuric acid (7.3 mL). The resulting mixture was stirred at that temperature for 30 min. A solution of sodium azide (4.42 g, 68 mmol) in H_2O (25 mL) was then added dropwise. After stirring for 16 h at room temperature, the precipitated solid was isolated by filtration, washed with H_2O (100 mL), air-dried, and recrystallized from abs EtOH. Yield: 57%; mp 86–87 °C (colorless prisms); ^1H NMR (200 MHz, $CDCl_3$): δ=2.55 (br s, 1H, OH), 4.72 (s, 2H, CH_2), 6.86 (dd, J(H,H)=8.5, 2.5 Hz, 1H, H4), 7.17 (d, J(H,H)=2.5 Hz, 1H, H6), 7.29 (d, J(H,H)=8.5 Hz, 1H, H3); $^{13}C\{^1H\}$ NMR (50.3 MHz, $CDCl_3$): δ=62.27 (CH_2), 118.67, 119.05, 128.06 (*q*), 130.41, 139.12 (*q*), 139.94 (*q*); IR (Nujol): ν=3251 (OH), 2122 (N_3) cm^{-1}; MS (70 eV, EI): *m*/*z* (%)=185 (8) [M^++2], 183 (15) [M^+], 157 (100); $C_7H_6ClN_3O$ (183.59): Calcd C 45.79, H 3.29, N 22.89; found C 45.64, H 3.17, N 22.96.

4.4. Preparation of 5-azido-2-chlorobenzyl chloride

Thionyl chloride (4.16 g, 35 mmol) was added dropwise to an ice-cooled solution of 5-azido-2-chlorobenzyl alcohol (5.51 g, 30 mmol) in dry CH_2Cl_2 (40 mL) and the reaction mixture was stirred at 0 °C for 3 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel; diethyl ether/*n*-hexane 1:1). Yield: 71%; yellow oil; ^1H NMR (200 MHz,

$CDCl_3$): δ=4.64 (s, 2H, CH_2), 6.92 (dd, J(H,H)=8.6, 2.6 Hz, 1H, H4), 7.12 (d, J(H,H)=2.6 Hz, 1H, H6), 7.35 (d, J(H,H)=8.6 Hz, 1H, H3); $^{13}C\{^1H\}$ NMR (50.3 MHz, $CDCl_3$): δ=43.09 (CH_2), 120.31 (q), 121.09, 129.75, 131.00, 136.59 (q), 139.33 (q); IR (film): ν=2117 (N_3) cm^{-1}; MS (70 eV, EI): m/z (%)=205 (8) [M^++4], 203 (38) [M^++2], 201 (42) [M^+], 175 (61), 77 (100); $C_7H_5Cl_2N_3$ (202.04): Calcd C 41.61, H 2.49, N 20.80; found C 41.43, H 2.53, N 20.71.

4.5. Preparation of 5-azido-2-bromobenzyl bromide

A solution of bromine (3.5 g, 22 mmol) in C_6H_6 (35 mL) was added dropwise to a cooled solution of triphenylphosphane (5.8 g, 22 mmol) in C_6H_6 (100 mL). The mixture was stirred at that temperature for 45 min, when a solution of 5-azido-2-bromobenzyl alcohol (5 g, 22 mmol) in C_6H_6 (20 mL) and triethylamine (2.2 g, 22 mmol) was added. The reaction mixture was stirred at room temperature for 2 h. The triethylammonium bromide was separated by filtration, and the solvent removed under reduced pressure. The resulting material was chromatographed (silica gel; ethyl acetate/*n*-hexane 1:4). Yield: 81%; mp 50–51 °C (yellow prisms from ethyl acetate/*n*-hexane); ^1H NMR (300 MHz, $CDCl_3$): δ=4.54 (s, 2H, CH_2), 6.84 (dd, J(H,H)=8.6, 2.6 Hz, 1H, H4), 7.11 (d, J(H,H)=2.6 Hz, 1H, H6), 7.53 (d, J(H,H)=8.6 Hz, 1H, H3); $^{13}C\{^1H\}$ NMR (75.4 MHz, $CDCl_3$): δ=32.54 (CH_2), 119.70 (q), 120.70, 121.62, 134.56, 138.70 (q), 140.20 (q); IR (Nujol): ν=2113 (N_3) cm^{-1}; MS (70 eV, EI): m/z (%)=293 (6) [M^++4], 291 (4) [M^++2], 289 (4) [M^+], 103 (100); $C_7H_5Br_2N_3$ (290.94): Calcd C 28.90, H 1.73, N 14.44; found C 28.83, H 1.89, N 14.31.

4.6. Preparation of 3-azidobenzyl iodide

Sodium iodide (2.85 g, 19 mmol) was added to a solution of 3-azidobenzyl chloride (2.2 g, 13 mmol) in dry acetone (25 mL), and this reaction mixture was stirred at room temperature for 12 h. The precipitated sodium chloride was separated by filtration. From the filtrate, the solvent was removed under reduced pressure and the resulting material was chromatographed (silica gel; diethyl ether/*n*-hexane 1:1). Yield: 91%; yellow oil; ^1H NMR (200 MHz, $CDCl_3$): δ=4.37 (s, 2H, CH_2), 6.88 (d, J(H,H)=7.7 Hz, 1H, H4), 7.00 (s, 1H, H2), 7.12 (d, J(H,H)=7.7 Hz, 1H, H6), 7.25 (t, J(H,H)=7.7 Hz, 1H, H5); $^{13}C\{^1H\}$ NMR (50.3 MHz, $CDCl_3$): δ=4.34 (CH_2), 118.46, 119.15, 125.22, 130.12, 140.31 (q), 141.15 (q); IR (film): ν=2114 (N_3) cm^{-1}; MS (70 eV, EI): m/z (%)=259 (6) [M^+], 207 (100); $C_7H_6IN_3$ (259.05): Calcd C 32.46, H 2.33, N 16.22; found C 32.39, H 2.28, N 16.20.

4.7. Preparation of *N*-(5-azido-2-chlorobenzyl)-phthalimide

A mixture of 5-azido-2-chlorobenzyl chloride (4.54 g, 25 mmol) and potassium phthalimide (5.56 g, 30 mmol) in dry DMF (30 mL) was stirred at 80 °C for 12 h. After cooling to room temperature, the mixture was poured on ice/H_2O (500 mL) and the precipitated solid was isolated by filtration, washed with H_2O (2×100 mL), and air-dried under vacuum. The solid was then recrystallized from chloroform/diethyl ether. Yield: 89%; mp 225–227 °C (white needles); ^1H NMR (300 MHz, $CDCl_3$): δ=4.94 (s, 2H, CH_2), 6.82 (d, J(H,H)=2.5 Hz, 1H, H6), 6.90 (dd, J(H,H)=8.4, 2.5 Hz, 1H, H4), 7.35 (d, J(H,H)=8.4 Hz, 1H, H3), 7.73–7.82 (m, 2H, H_{arom}), 7.86–7.90 (m, 2H, H_{arom}); $^{13}C\{^1H\}$ NMR (75.4 MHz, $CDCl_3$): δ=39.28 (CH_2), 119.25, 119.49, 123.69, 129.04 (q), 130.90, 131.96 (q), 134.31, 135.27 (q), 139.14 (q), 167.69 (q, CO); IR (Nujol): ν=2116 (N_3), 1725 (CO) cm^{-1}; MS (70 eV, EI): m/z (%)=314 (4) [M^++2], 312 (8) [M^+], 249 (82), 130 (100); $C_{15}H_9ClN_4O_2$ (312.71): Calcd C 57.61, H 2.90, N 17.92; found C 57.73, H 2.84, N 17.89.

4.8. Preparation of 5-azido-2-chlorobenzylamine

$N_2H_4\cdot H_2O$ (5 mL) was added to a solution of *N*-(5-azido-2-chlorobenzyl)phthalimide (5.26 g, 18 mmol) in EtOH (75 mL), and the mixture was stirred at reflux temperature for 3 h. After cooling to room temperature, NaOH 10% (50 mL) was added and the resulting solution was extracted with CH_2Cl_2 (3×50 mL). The combined organic extracts were washed with brine (100 mL) and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the resulting oil was chromatographed (silica gel; ethyl acetate/methanol 1:1). Yield: 80%; yellow oil; ^1H NMR (200 MHz, $CDCl_3$): δ=1.58 (br s, 2H, NH_2), 3.90 (s, 2H, CH_2), 6.84 (dd, J(H,H)=8.6, 2.6 Hz, 1H, H4), 7.08 (d, J(H,H)=2.6 Hz, 1H, H6), 7.30 (d, J(H,H)=8.6 Hz, 1H, H5); $^{13}C\{^1H\}$ NMR (50.3 MHz, $CDCl_3$): δ=44.22 (CH_2), 118.49, 118.24, 129.08 (q), 130.56, 139.03 (q), 142.24 (q); IR (film): ν=3397, 3326 (NH_2), 2115 (N_3) cm^{-1}; MS (70 eV, EI): m/z (%)=184 (26) [M^++2], 182 (39) [M^+], 154 (100); $C_7H_7ClN_4$ (259.05): Calcd C 46.04, H 3.86, N 30.68; found C 45.89, H 3.80, N 30.83.

4.9. Preparation of (5-azido-2-bromobenzyl)(5-azido-2-chlorobenzyl)amine and bis(5-azido-2-bromobenzyl)(5-azido-2-chlorobenzyl)amine (1c)

5-Azido-2-bromobenzyl bromide (4.4 g, 15 mmol) was added to a solution of 5-azido-2-chlorobenzylamine (3.65 g, 20 mmol) in dry dioxane (100 mL). The mixture was heated at reflux temperature for 3 h. After cooling to room temperature, triethylamine (2.0 g, 20 mmol) was added, and the mixture then stirred for 2 h. The triethylammonium bromide was separated by filtration, and the solvent was removed under reduced pressure. From the resulting residue, a mixture of secondary and tertiary amines was separated by chromatography (silica gel; ethyl acetate/*n*-hexane 1:9). (5-Azido-2-bromobenzyl)(5-azido-2-chlorobenzyl)-amine: Yield: 35%; mp 111–113 °C (yellow prisms from chloroform); ^1H NMR (200 MHz, $CDCl_3$): δ=1.87 (br s, 1H, NH), 3.85 (s, 2H, CH_2), 3.87 (s, 2H, CH_2), 6.79 (dd, J(H,H)=8.5, 2.8 Hz, 1H, H4/H4′), 6.86 (dd, J(H,H)=8.5, 2.8 Hz, 1H, H4/H4′), 7.14 (d, J(H,H)=2.8 Hz, 2H, H6+H6′), 7.31 (d, J(H,H)=8.5 Hz, 1H, H3/H3′), 7.48 (d, J(H,H)=8.5 Hz, 1H, H3/H3′); $^{13}C\{^1H\}$ NMR (50.3 MHz, $CDCl_3$): δ=50.43 (CH_2), 52.89 (CH_2), 118.95, 119.13 (q), 119.29, 120.40, 120.55, 129.62 (q), 130.69, 133.92, 139.01 (q), 139.13 (q), 139.71 (q), 140.73 (q); IR (Nujol): ν=3166 (NH), 2116 (N_3) cm^{-1}; MS (70 eV, EI): m/z (%)=396 (12) [M^++5], 394 (36) [M^++3], 392 (36) [M^++1], 363 (39), 103 (100); $C_{14}H_{11}BrClN_7$ (392.64): Calcd C 42.83, H 2.82, N 24.97; found C 42.91, H 2.77, N 24.82. Bis(5-azido-2-bromobenzyl)(5-azido-2-chlorobenzyl)amine (**1c**): Yield: 11%; mp 125–127 °C (brown prisms from ethyl

acetate/*n*-hexane); ^{1}H NMR (200 MHz, $CDCl_3$): δ=3.80 (s, 4H, CH_2), 3.82 (s, 2H, CH_2), 6.72 (dd, *J*(H,H)=8.5, 2.8 Hz, 2H, H4), 6.79 (dd, *J*(H,H)=8.5, 2.8 Hz, 1H, H4′), 7.25–7.30 (m, 4H, H3′+H6+H6′), 7.45 (d, *J*(H,H)=8.5 Hz, 2H, H3); ^{13}C{^{1}H} NMR (50.3 MHz, $CDCl_3$): δ=56.27 (CH_2), 58.78 (CH_2), 119.21, 119.28 (*q*), 119.54, 120.25, 120.34, 129.80 (*q*), 130.77, 133.99, 137.91 (*q*), 139.08 (*q*), 139.58 (*q*), 139.79 (*q*); IR (Nujol): ν=2113 (N_3) cm^{-1}; MS (70 eV, EI): *m*/*z* (%)=604 (27) [M$^+$+4], 602 (36) [M$^+$+2], 600 (22) [M$^+$], 102 (100); $C_{21}H_{15}Br_2ClN_{10}$ (602.67): Calcd C 41.85, H 2.51, N 23.24; found C 41.70, H 2.47, N 23.09.

4.10. Preparation of (3-azidobenzyl)(5-azido-2-bromobenzyl)(5-azido-2-chlorobenzyl)amine (1d)

3-Azidobenzyl iodide (2.6 g, 10 mmol) was added to a solution of (5-azido-2-bromobenzyl)(5-azido-2-chlorobenzyl)-amine (3.9 g, 10 mmol) in dry dioxane (100 mL), and the mixture was stirred at reflux temperature for 8 h. After cooling to room temperature, triethylamine (1.5 g, 13 mmol) was added, and the mixture was stirred for 3 h. The triethylammonium iodide was separated by filtration. From the filtrate, the dioxane was evaporated to dryness and the residue was purified by column chromatography (silica gel; ethyl acetate/*n*-hexane 1:9); Yield: 40%; brown oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.68 (s, 2H, CH_2), 3.71 (s, 2H, CH_2), 3.73 (s, 2H, CH_2), 6.72 (dd, *J*(H,H)=8.4, 2.9 Hz, 1H, H_{arom}), 6.79 (dd, *J*(H,H)=8.5, 2.8 Hz, 1H, H_{arom}), 6.91 (br d, *J*(H,H)=7.9 Hz, 1H, H_{arom}), 7.06 (br s, 1H, H_{arom}), 7.16 (br d, *J*(H,H)=7.6 Hz, 1H, H_{arom}), 7.25–7.32 (m, 4H, H_{arom}), 7.44 (d, *J*(H,H)=8.4 Hz, 1H, H_{arom}); ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$): δ=55.50 (CH_2), 58.08 (CH_2), 58.88 (CH_2), 118.10, 119.00, 119.11, 119.35, 120.29, 120.41, 121.05 (*q*), 125.15, 129.78 (*q*), 129.89, 130.69, 133.90, 138.21 (*q*), 139.01 (*q*), 139.72 (*q*), 139.87 (*q*), 140.31 (*q*), 140.60 (*q*); IR (film): ν=2112 (N_3) cm^{-1}; MS (70 eV, EI): *m*/*z* (%)=524 (26) [M$^+$+2], 522 (25) [M$^+$], 103 (100); $C_{21}H_{16}BrClN_{10}$ (523.78): Calcd C 48.16, H 3.08, N 26.74; found C 48.00, H 3.21, N 26.67.

4.11. General procedure for the preparation of the triphosphazides 3

Two solutions, one of the corresponding tris(3-azidobenzyl)-amine **1** (1.5 mmol) in diethyl ether or dichloromethane (10 mL) and the other of 1,1,1-tris[(diphenylphosphino)-methyl]ethane (**2**) (1.3 g, 1.5 mmol) in diethyl ether (10 mL), were simultaneously added to a round-bottom flask containing diethyl ether (15 mL) under nitrogen atmosphere at room temperature over a period of 30 min with stirring. The resulting mixture was then stirred for 3 h. The precipitated pale yellow solid was filtered, washed with diethyl ether (3×10 mL), and dried under vacuum.

4.11.1. Triphosphazide 3a. Yield: 79%; mp>350 °C (yellow prisms from dichloromethane/diethyl ether); ^{1}H NMR (300 MHz, $CDCl_3$): δ=−0.26 (br s, 3H, CH_3), 3.52–3.62 (m, 3H, CH_AH$_B$P), 3.63 (d, *J*(H,H)=14.3 Hz, 3H, CH_AH$_B$N), 3.81–3.93 (m, 6H, CH$_A$$H_B$N+CH$_A$$H_B$P), 6.80–7.10 (m, 9H, H_{arom}), 7.18 (t, *J*(H,H)=7.5 Hz, 3H, H_{arom}), 7.25–7.50 (m, 18H, H_{arom}), 7.59 (d, *J*(H,H)=7.8 Hz, 3H, H_{arom}), 7.90–8.10 (m, 9H, H_{arom}); ^{1}H NMR (200 MHz, CD_2Cl_2): δ=−0.23 (s, 3H, CH_3), 3.59–3.73 (m, 3H, CH_AH$_B$P), 3.78 (d, *J*(H,H)=14.7 Hz, 3H, CH_AH$_B$N), 3.85 (d, *J*(H,H)=14.7 Hz, 3H, CH$_A$$H_B$N), 3.94 (pseudot, *J*(H,H)(H,P)=14.5 Hz, 3H, CH$_A$$H_B$P), 7.07–7.16 (m, 9H, H_{arom}), 7.31–7.52 (m, 21H, H_{arom}), 7.63 (br d, *J*(H,H)=8.0 Hz, 3H, H_{arom}), 7.95 (br s, 3H, H_{arom}), 8.03–8.13 (m, 6H, H_{arom}); ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$): δ=26.58 (br s, CH_3), 40.05 (m, CH_2P), 40.68 (q, ^{2}*J*(C,P)=3.5 Hz, CH_3*C*), 57.35 (CH_2N), 119.00 (br s, two signals), 125.78 (*q*), 128.65, 128.71 (d, ^{3}*J*(C,P)=10.1 Hz, *m*C-PhP), 128.73 (d, ^{3}*J*(C,P)=11.6 Hz, *m*C-PhP), 129.85 (d, ^{1}*J*(C,P)=83.6 Hz, *i*C-PhP), 131.09 (d, ^{2}*J*(C,P)=9.1 Hz, *o*C-PhP), 131.48 (d, ^{4}*J*(C,P)=2.0 Hz, *p*C-PhP), 132.16 (d, ^{1}*J*(C,P)=105.8 Hz, *i*C-PhP), 132.26 (d, ^{4}*J*(C,P)=1.0 Hz, *p*C-PhP), 132.92 (d, ^{2}*J*(C,P)=7.5 Hz, *o*C-PhP), 141.55 (*q*), 150.39 (*q*); ^{13}C{^{1}H} NMR (50.3 MHz, CD_2Cl_2): δ=26.49 (CH_3), 39.81 (m, CH_2P), 40.62 (q, ^{2}*J*(C,P)=3.5 Hz, CH_3*C*), 57.17 (CH_2N), 117.21 (br s), 121.96 (br s), 125.86 (*q*), 127.40 (d, ^{1}*J*(C,P)=108.8 Hz, *i*C-PhP), 128.75, 128.78 (d, ^{3}*J*(C,P)=11.6 Hz, *m*C-PhP), 128.85 (d, ^{3}*J*(C,P)=11.2 Hz, *m*C-PhP), 130.14 (d, ^{1}*J*(C,P)=82.4 Hz, *i*C-PhP), 130.96 (d, ^{2}*J*(C,P)=9.1 Hz, *o*C-PhP), 131.54 (d, ^{4}*J*(C,P)=2.8 Hz, *p*C-PhP), 132.30 (d, ^{4}*J*(C,P)=2.2 Hz, *p*C-PhP), 132.90 (d, ^{2}*J*(C,P)=7.5 Hz, *o*C-PhP), 142.01 (*q*), 150.25 (*q*); ^{31}P{^{1}H} NMR (121.4 MHz, $CDCl_3$): δ=3.76 (br s, $\Delta\nu_{½}$=972 Hz); ^{31}P{^{1}H} NMR (81.01 MHz, $CDCl_3$): δ=8.93 (br s, $\Delta\nu_{½}$=425 Hz); ^{31}P{^{1}H} NMR (CPMAS, 121.4 MHz, H_3PO_4 85%): δ=1.31 (s, 2P, *Z*-PN_3), 24.13 (s, 1P, *E*-PN_3); ^{31}P{^{1}H} NMR (CPMAS, 121.4 MHz, $(NH_4)_2HPO_4$): δ=2.72 (s, 2P, *Z*-PN_3), 25.44 (s, 1P, *E*-PN_3); IR (Nujol): ν=1440 (CP), 1122 (NP) cm^{-1}; MS (FAB+): *m*/*z* (%)=1036 (9) [M$^+$+1], 307 (100); $C_{62}H_{57}N_{10}P_3$ (1035.10): Calcd C 71.94, H 5.55, N 13.53; found C 71.79, H 5.68, N 13.39.

4.11.2. Triphosphazide 3b. Yield: 63%; mp (decomp.) 291–293 °C (yellow prisms from dichloromethane/diethyl ether); ^{1}H NMR (300 MHz, $CDCl_3$): δ=−0.23 (br s, 3H, CH_3), 3.46–3.58 (m, 3H, CH_AH$_B$P), 3.83 (d, *J*(H,H)=16.6 Hz, 3H, CH_AH$_B$N), 3.86 (pseudot, *J*(H,H)(H,P)=14.2 Hz, 3H, CH$_A$$H_B$P), 4.05 (d, *J*(H,H)=16.6 Hz, 3H, CH$_A$$H_B$N), 6.92–6.94 (m, 6H, H_{arom}), 7.14–7.26 (m, 9H, H_{arom}), 7.34–7.41 (m, 12H, H_{arom}), 7.49 (dd, *J*(H,H)=8.4, 1.8 Hz, 3H, H_{arom}), 7.99–8.04 (m, 6H, H_{arom}), 8.22 (br s, 3H, H_{arom}); ^{1}H NMR (200 MHz, CD_2Cl_2): δ=−0.29 (br s, 3H, CH_3), 3.48–3.54 (m, 3H, CH_AH$_B$P), 3.84 (d, *J*(H,H)=16.7 Hz, 3H, CH_AH$_B$N), 3.86 (pseudot, *J*(H,H)(H,P)=12.9 Hz, 3H, CH$_A$$H_B$P), 4.11 (d, *J*(H,H)=16.7 Hz, 3H, CH$_A$$H_B$N), 6.95–6.98 (m, 6H, *m*H-PhP), 7.15 (dd, *J*(H,H)(H,P)=8.5, 8.1 Hz, 6H, *o*H-PhP), 7.26 (t, *J*=7.4 Hz, 3H, *p*H-PhP), 7.40–7.44 (m, 9H, H_{arom}), 7.47 (br d, *J*(H,H)=8.7 Hz, 3H, H_{arom}), 7.58 (d, *J*(H,H)=8.7 Hz, 3H, H_{arom}), 7.96–8.02 (m, 6H, *o*H-PhP), 8.20 (br s, 3H, H_{arom}); ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$): δ=26.41 (br s, CH_3), 40.54 (m, CH_2P), 40.87 (q, ^{2}*J*(C,P)=3.0 Hz, CH_3*C*), 58.45 (CH_2N), 118.10 (br s), 119.89 (*q*), 121.20 (br s), 127.20 (d, ^{1}*J*(C,P)=110.0 Hz, *i*C-PhP), 128.73 (d, ^{3}*J*(C,P)=11.5 Hz, *m*C-PhP), 128.80 (d, ^{3}*J*(C,P)=11.3 Hz, *m*C-PhP), 129.04 (d, ^{1}*J*(C,P)=81.8 Hz, *i*C-PhP), 130.96 (d, ^{2}*J*(C,P)=9.3 Hz, *o*C-PhP), 131.70 (br s, *p*C-PhP), 132.31 (br s, *p*C-PhP), 132.84 (d, ^{2}*J*(C,P)=7.0 Hz, *o*C-PhP), 133.47, 139.52 (*q*), 149.53 (*q*); ^{13}C{^{1}H} NMR (50.3 MHz, CD_2Cl_2): δ=26.35 (CH_3), 39.97 (m, CH_2P), 40.82 (q, ^{2}*J*(C,P)=3.6 Hz, CH_3*C*), 58.43 (CH_2N), 117.56 (br s), 119.62 (*q*), 121.96 (br s), 127.38 (d, ^{1}*J*(C,P)=107.2 Hz, *i*C-PhP), 128.77 (d,

^3J(C,P)=11.7 Hz, *m*C-PhP), 128.91 (d, ^3J(C,P)=11.3 Hz, *m*C-PhP), 129.39 (d, ^1J(C,P)=82.0 Hz, *i*C-PhP), 130.90 (d, ^2J(C,P)=9.2 Hz, *o*C-PhP), 131.69 (d, ^4J(C,P)=2.9 Hz, *p*C-PhP), 132.32 (d, ^4J(C,P)=2.2 Hz, *p*C-PhP), 132.81 (d, ^2J(C,P)=7.6 Hz, *o*C-PhP), 133.54, 139.89 (*q*), 149.66 (*q*); $^{31}P\{^1H\}$ NMR (121.4 MHz, $CDCl_3$): δ=2.46 (br s, $\Delta\nu_{1/2}$= 608 Hz); $^{31}P\{^1H\}$ NMR (81.01 MHz, $CDCl_3$): δ=5.36 (br s, $\Delta\nu_{1/2}$=484 Hz); $^{31}P\{^1H\}$ NMR (CPMAS, 121.4 MHz, $(NH_4)_2HPO_4$): δ=−0.91 (s, *Z*-PN_3); IR (Nujol): ν=1438 (CP), 1109 (NP) cm^{-1}; MS (FAB+): *m*/*z* (%)=1275 (10) [M^++7], 1273 (7) [M^++5], 1271 (4) [M^++3], 1269 (6) [M^++1], 638 (100); $C_{62}H_{54}Br_3N_{10}P_3$ (1271.79): Calcd C 58.55, H 4.28, N 11.01; found C 58.42, H 4.43, N 10.88.

4.11.3. Triphosphazide 3c. Yield: 80%; >350 °C (yellow prisms from chloroform-*d*/diethyl ether); ^1H NMR (300 MHz, $CDCl_3$): δ=−0.25 (br s, 3H, CH_3), 3.46–3.59 (m, 3H, CH_AH_BP), 3.78–3.90 (m, 6H, CH_AH_BN+ CH_AH_BP), 4.01–4.15 (m, 3H, CH_AH_BN), 6.91–6.93 (m, 3H, H_{arom}), 7.13–7.26 (m, 9H, H_{arom}), 7.35–7.58 (m, 18H, H_{arom}), 7.98–8.06 (m, 6H, *o*H-PhP), 8.23 (br s, H_{arom}); $^{13}C\{^1H\}$ NMR (75.4 MHz, $CDCl_3$): δ=26.40 (br s, CH_3), 39.97 (m, CH_2P), 40.82 (q, ^2J(C,P)=3.1 Hz, CH_3C), 55.87 (CH_2N), 58.44 (CH_2N), 118.26 (br s, two signals), 119.86 (*q*), 121.72 (br s, two signals), 127.19 (d, ^1J(C,P)= 106.8 Hz, *i*C-PhP), 128.72 (d, ^3J(C,P)=11.5 Hz, *m*C-PhP), 128.78 (d, ^3J(C,P)=11.0 Hz, *m*C-PhP), 129.04 (d, ^1J(C,P)= 82.0 Hz, *i*C-PhP), 129.59 (*q*), 130.20, 130.97 (d, ^2J(C,P)= 9.3 Hz, *o*C-PhP), 131.67 (br s, *p*C-PhP), 132.29 (br s, *p*C-PhP), 132.82 (d, ^2J(C,P)=6.9 Hz, *o*C-PhP), 133.47, 138.00 (*q*), 139.54 (*q*), 149.02 (*q*), 149.54 (*q*); $^{31}P\{^1H\}$ NMR (121.4 MHz, $CDCl_3$): δ=2.90 (br s, $\Delta\nu_{1/2}$=545 Hz); IR (Nujol): ν=1459 (CP), 1111 (NP) cm^{-1}; MS (FAB+): *m*/*z* (%)=1252 (10) [M^++5+Na], 1229 (6) [M^++5], 1227 (4) [M^++3], 638 (100); $C_{62}H_{54}Br_2ClN_{10}P_3$ (1227.34): Calcd C 60.67, H 4.44, N 11.41; found C 60.54, H 4.57, N 11.34.

4.11.4. Triphosphazide 3d/3d′. Yield: 66%; ^1H NMR (300 MHz, $CDCl_3$): δ=−0.25 [s, 6H, CH_3 (d+d′)], 3.44–3.62 [m, 6H, CH_AH_BP (d+d′)], 3.78–4.07 [m, 18H, CH_2N (d+d′)+CH_AH_BP (d+d′)], 6.87–6.97 [m, 12H, H_{arom} (d+d′)], 7.04 [d, J(H,H)=7.5 Hz, 4H, H_{arom} (d+d′)], 7.16–7.22 [m, 18H, H_{arom} (d+d′)], 7.35–7.38 [m, 20H, H_{arom} (d+d′)], 7.47–7.59 [m, 8H, H_{arom} (d+d′)], 7.90–8.06 [m, 12H, H_{arom} (d+d′)], 8.20 [br s, 4H, H_{arom} (d+d′)], 8.25 [br s, 2H, H_{arom} (d+d′)]; $^{13}C\{^1H\}$ NMR (75.4 MHz, $CDCl_3$): δ=26.44 [br s, CH_3 (d+d′)], 38.80–40.50 [m, 3 CH_2P (d+d′)], 40.75 [q, ^2J(C,P)=3.5 Hz, CH_3C (d+d′)], 55.13 [CH_2N (d/d′)], 55.54 [CH_2N (d/d′)], 57.75 [CH_2N (d/d′)], 57.87 [2 CH_2N (d/d′)], 58.10 [CH_2N (d/d′)], 118.26 [br s, d/d′)], 119.90 [*q*, (d/d′)], 120.04 [*q*, (d/d′)], 121.72 [br s, (d+d′)], 125.44 [*q*, (d+d′)], 126.97 [br d, ^1J(C,P)=107.3 Hz, *i*C-PhP (d+d′)], 128.30–130.26 (a+b), 130.97 [br d, ^2J(C,P)=9.1 Hz, *o*C-PhP (d/d′)], 131.02 [br d, ^2J(C,P)=8.7 Hz, 2 *o*C-PhP (d/d′)], 131.49 [br s, *p*C-PhP (d/d′)], 131.64 [br s, 2 *p*C-PhP (d/d′)], 132.21 [br s, *p*C-PhP (d/d′)], 132.22 [br s, 2 *p*C-PhP (d/d′)], 132.83 [m, 2 *o*C-PhP (d/d′)], 133.22 [d, ^2J(C,P)=6.4 Hz, *o*C-PhP (d/d′)], 138.15 [*q*, (d/d′)], 138.31 [*q*, (d/d′)], 139.71 [*q*, (d/d′)], 139.85 [*q*, (d/d′)], 140.92 [2 *q*, (d/d′)], 148.86 [*q*, (d/d′)], 148.91 [*q*, (d/d′)], 149.36 [*q*, (d/d′)], 149.41 [*q*, (d/d′)], 150.76 [2 *q*, (d/d′)]; $^{31}P\{^1H\}$ NMR (121.4 MHz, $CDCl_3$): δ=2.40 (br s, $\Delta\nu_{1/2}$=726 Hz); IR (Nujol): ν=1455 (CP), 1108 (NP) cm^{-1}; MS (FAB+): *m*/*z* (%)=1171 (6) [M^++2+Na], 1148 (4) [M^++2], 638 (100); $C_{62}H_{55}BrClN_{10}P_3$ (1148.45): Calcd C 64.84, H 4.83, N 12.20; found C 64.71, H 4.92, N 12.17.

4.11.5. Triphosphazide 3e. Yield: 30%; mp (decomp.) >350 °C (microcrystalline solid from diethyl ether); IR (Nujol): ν=1445 (CP), 1091 (NP) cm^{-1}; MS (FAB+): *m*/*z* (%)=1100 (8) [M^++Na], 1077 (6) [M^+], 641 (100); $C_{65}H_{63}N_{10}P_3$ (1077.18): Calcd C 72.48, H 5.90, N 13.00; found C 72.35, H 5.76, N 12.86.

4.11.6. Triphosphazide 3f. Yield: 77%; mp (decomp.) >350 °C (microcrystalline solid from diethyl ether); ^1H NMR (300 MHz, $CDCl_3$): δ=−0.28 (br s, 3H, CH_3), 2.50 (s, 3H, CH_3-Ar), 2.53 (s, 3H, CH_3-Ar), 3.34 (d, J(H,H)= 15.0 Hz, 2H, CH_AH_BN), 3.39 (d, J(H,H)=14.8 Hz, 1H, CH_AH_BN), 3.44–3.82 (m, 6H, CH_AH_BN+CH_AH_BP), 4.08 (pseudot, J(H,H)=15.2 Hz, 3H, CH_AH_BP), 6.88–6.96 (m, 9H, H_{arom}), 7.12–7.28 (m, 4H, H_{arom}), 7.38–7.49 (m, 18H, H_{arom}), 7.99–8.05 (m, 6H, H_{arom}), 8.08 (br s, 1H, H_{arom}), 8.17 (br s, 1H, H_{arom}), 8.19 (br s, 1H, H_{arom}); $^{31}P\{^1H\}$ NMR (121.4 MHz, $CDCl_3$): δ=2.14 (br s, 1P, *Z*-PN_3), 21.58 (br s, 2P, *E*-PN_3); IR (Nujol): ν=1450 (CP), 1108 (NP) cm^{-1}; MS (FAB+): *m*/*z* (%)=1086 (13) [M^++Na], 1064 (7) [M^++1], 638 (100); $C_{64}H_{61}N_{10}P_3$ (1063.16): Calcd C 72.30, H 5.78, N 13.17; found C 72.17, H 5.63, N 13.04.

4.11.7. Triphosphazide 3g. Yield: 79%; mp (decomp.) 316–318 °C (microcrystalline solid from diethyl ether); ^1H NMR (300 MHz, $CDCl_3$): δ=−0.27 (br s, 3H, CH_3), 2.55 (s, 3H, CH_3-Ar), 3.39–3.90 (m, 9H, CH_AH_BN+CH_AH_BP), 3.95 (pseudot, J(H,H)(H,P)=14.4 Hz, 3H, CH_AH_BP), 6.92–7.04 (m, 10H, H_{arom}), 7.14–7.57 (m, 22H, H_{arom}), 7.80–8.05 (m, 8H, H_{arom}), 8.20 (br s, 1H, H_{arom}); $^{13}C\{^1H\}$ NMR (75.4 MHz, $CDCl_3$): δ=19.97 (CH_3-Ar), 26.68 (CH_3C), 39.50–40.20 (m, 3 CH_2P), 40.82 (q, ^2J(C,P)=3.3 Hz, CH_3C), 57.31 (CH_2N), 57.35 (CH_2N), 58.24 (CH_2N), 115.57, 119.00 (br s), 125.77, 126.26, 128.70–133.24, 138.21 (2 *q*), 141.60 (*q*), 149.64 (br s, 3 *q*); $^{31}P\{^1H\}$ NMR (121.4 MHz, $CDCl_3$): δ=5.12 (br s, 2P, *Z*-PN_3), 21.50 (br s, 1P, *E*-PN_3); IR (Nujol): ν=1440 (CP), 1114 (NP) cm^{-1}; MS (FAB+): *m*/*z* (%) 1049 (12) [M^+], 640 (100); $C_{63}H_{59}N_{10}P_3$ (1049.13): Calcd C 72.12, H 5.67, N 13.35; found C 71.99, H 5.56, N 13.27.

4.12. General procedure for the preparation of tri-λ^5-phosphazenes 5

A solution of the corresponding triphosphazide (1 mmol) in $CDCl_3$ (10 mL) was heated at 60 °C in a flask immersed in an oil bath for 24 h. After cooling, the solvent was removed under reduced pressure and the crude product was crystallized.

4.12.1. Tri-λ^5-phosphazene 5a. Yield: 75%; mp 236–238 °C (colorless prisms from chloroform/*n*-hexane); ^1H NMR (300 MHz, $CDCl_3$): δ=−0.78 (s, 3H, CH_3), 2.94 (d, J(H,H)=12.3 Hz, 3H, CH_AH_BN), 3.20 (pseudoquint, J(H,H) (H,P)=7.5 Hz, 3H, CH_AH_BP), 3.57 (d, J(H,H)=12.3 Hz, 3H, CH_AH_BN), 3.97 (pseudot, J(H,H)(H,P)=14.4 Hz, 3H, CH_AH_BP), 6.37 (s, 3H, H_{arom}), 6.49–6.52 (m, 3H, H_{arom}), 7.09–7.11 (m, 6H, H_{arom}), 7.20–7.50 (m, 24H, H_{arom}), 7.85

(dd, J(H,H)=7.5, 2.1 Hz, 6H, *o*H-PhP); $^{13}C\{^1H\}$ NMR (75.4 MHz, $CDCl_3$): δ=27.42 (CH_3), 37.48 (m, CH_2P), 39.05 (q, ^2J(C,P)=3.4 Hz, CH_3C), 57.68 (CH_2N), 118.56, 122.52 (d, ^3J(C,P)=12.6 Hz, *s-cis-C*H=C–N=P), 125.29 (d, ^3J(C,P)=28.7 Hz, *s-trans-C*H=C–N=P), 128.20, 128.37 (d, ^3J(C,P)=12.6 Hz, *m*C-PhP), 128.71 (d, ^3J(C,P)=11.5 Hz, *m*C-PhP), 130.66 (d, ^1J(C,P)=85.1 Hz, *i*C-PhP), 131.21 (d, ^4J(C,P)=1.8 Hz, *p*C-PhP), 131.49 (d, ^2J(C,P)=8.6 Hz, *o*C-PhP), 131.70 (d, ^4J(C,P)=1.1 Hz, *p*C-PhP), 132.12 (d, ^2J(C,P)=9.2 Hz, *o*C-PhP), 132.41 (d, ^1J(C,P)=102.1 Hz, *i*C-PhP), 141.99 (*q*), 151.33 (*q*); $^{31}P\{^1H\}$ NMR (121.4 MHz, $CDCl_3$): δ=0.22; IR (Nujol): ν=1459 (CP), 1114 (NP) cm^{-1}; MS (FAB+): *m/z* (%)=950 (100) [M^+ −1]; $C_{62}H_{57}N_4P_3$ (951.06): Calcd C 78.30, H 6.04, N 5.89; found C 78.15, H 5.89, N 5.75.

4.12.2. Tri-λ^5-phosphazene 5b. Yield: 74%; mp (decomp.) 316–318 °C (colorless prisms from chloroform); ^1H NMR (300 MHz, $CDCl_3$): δ=−0.69 (s, 3H, CH_3), 3.23 (pseudoquint, J(H,H)(H,P)=8.4 Hz, 3H, CH_AH$_B$P), 3.45 (d, J(H,H)=13.3 Hz, 3H, CH_AH$_B$N), 3.65 (d, J(H,H)=13.3 Hz, 3H, CH$_A$$H_B$N), 3.95 (pseudot, J(H,H)(H,P)=14.5 Hz, 3H, CH$_A$$H_B$P), 6.55–6.57 (m, 3H, H_{arom}), 6.96 (dd, J(H,H)=8.6, 2.5 Hz, 6H, H_{arom}), 7.25–7.42 (m, 24H, H_{arom}), 7.81–7.87 (m, 6H, H_{arom}); $^{13}C\{^1H\}$ NMR (75.4 MHz, $CDCl_3$): δ=27.59 (CH_3), 38.09 (m, CH_2P), 39.10 (q, ^2J(C,P)=3.2 Hz, CH_3C), 56.46 (CH_2N), 111.71 (*q*), 123.69 (d, ^3J(C,P)=12.2 Hz, *s-cis-C*H=C–N=P), 127.07 (d, ^3J(C,P)=27.8 Hz, *s-trans-C*H=C–N=P), 128.50 (d, ^3J(C,P)=12.8 Hz, *m*C-PhP), 128.87 (d, ^3J(C,P)=11.0 Hz, *m*C-PhP), 130.23 (d, ^1J(C,P)=90.5 Hz, *i*C-PhP), 131.13 (d, ^2J(C,P)=9.3 Hz, *o*C-PhP), 131.60 (br s, *p*C-PhP), 131.81 (br s, *p*C-PhP), 131.91 (d, ^1J(C,P)=105.2 Hz, *i*C-PhP), 131.99 (d, ^2J(C,P)=9.3 Hz, *o*C-PhP), 132.60, 139.65 (*q*), 150.77 (*q*); $^{31}P\{^1H\}$ NMR (121.4 MHz, $CDCl_3$): δ=0.49; IR (Nujol): ν=1457 (CP), 1119 (NP) cm^{-1}; MS (FAB+): *m/z* (%)=1190 (48) [M^++6], 1188 (84) [M^++4], 1186 (60) [M^++2], 1184 (15) [M^+], 154 (100); $C_{62}H_{54}Br_3N_4P_3$ (1187.75): Calcd C 62.70, H 4.58, N 4.72; found C 62.58, H 4.44, N 4.57.

4.12.3. Tri-λ^5-phosphazene 5c. Yield: 79%; mp (decomp.) 325–326 °C (colorless prisms from chloroform); ^1H NMR (300 MHz, $CDCl_3$): δ=−0.73 (s, 3H, CH_3), 3.23 (pseudoquint, J(H,H)(H,P)=6.8 Hz, 3H, CH_AH$_B$P), 3.34 (d, J(H,H)=13.3 Hz, 2H, CH_AH$_B$N), 3.38 (d, J(H,H)=13.1 Hz, 1H, CH_AH$_B$N), 3.61 (d, J(H,H)=13.3 Hz, 2H, CH$_A$$H_B$N), 3.65 (d, J(H,H)=13.1 Hz, 1H, CH$_A$$H_B$N), 3.89 (pseudot, J(H,H)(H,P)=14.7 Hz, 3H, CH$_A$$H_B$P), 6.48–6.50 (m, 3H, H_{arom}), 6.96 (dd, J(H,H)(H,P)=8.6, 2.7 Hz, 2H, H_{arom}), 7.01 (dd, J(H,H)(H,P)=8.6, 2.7 Hz, 1H, H_{arom}), 7.13 (d, J(H,H)=8.7 Hz, 2H, H_{arom}), 7.15 (d, J(H,H)=8.7 Hz, 1H, H_{arom}), 7.27–7.49 (m, 24H, H_{arom}), 7.78–7.89 (m, 6H, H_{arom}); $^{13}C\{^1H\}$ NMR (75.4 MHz, $CDCl_3$): δ=27.50 (CH_3), 37.91 (ddd, ^1J(C,P)=46.5 Hz, ^3J(C,P)=11.9, 3.9 Hz, 3 CH_2P), 38.96 (q, ^2J(C,P)=3.9 Hz, CH_3C), 53.59 (2 CH_2N), 56.19 (CH_2N), 111.52 (2 *q*), 121.99 (*q*), 123.40 (d, ^3J(C,P)=12.3 Hz, *s-cis-C*H=C–N=P), 123.72 (d, ^3J(C,P)=12.3 Hz, 2 *s-cis-C*H=C–N=P), 126.62 (d, ^3J(C,P)=26.6 Hz, *s-trans-C*H=C–N=P), 127.16 (d, ^3J(C,P)=28.2 Hz, 2 *s-trans-C*H=C–N=P), 128.61 (d, ^3J(C,P)=13.4 Hz, *m*C-PhP), 128.86 (d, ^3J(C,P)=11.9 Hz, *m*C-PhP), 129.43 (d, ^4J(C,P)=4.5 Hz), 131.08 (d, ^2J(C,P)=8.8 Hz, *o*C-PhP), 131.46 (d, ^1J(C,P)=81.2 Hz, *i*C-PhP), 131.66 (d, ^4J(C,P)=2.7 Hz, *p*C-PhP), 131.91 (d, ^4J(C,P)=2.1 Hz, *p*C-PhP), 131.96 (d, ^2J(C,P)=9.3 Hz, *o*C-PhP), 132.54 (d, ^4J(C,P)=4.1 Hz), 137.99 (*q*), 139.67 (*q*), 150.33 (d, ^2J(C,P)=1.4 Hz, *q*), 150.95 (d, ^2J(C,P)=1.6 Hz, *q*), the resonance of one *i*C-PhP was not observed; $^{31}P\{^1H\}$ NMR (121.4 MHz, $CDCl_3$): δ=0.29 (s, 2P), 0.39 (s, 1P); IR (Nujol): ν=1437 (CP), 1110 (NP) cm^{-1}; MS (FAB+): *m/z* (%)=1146 (10) [M^++6], 1144 (29) [M^++4], 1142 (38) [M^++2], 1140 (21) [M^+], 154 (100); $C_{62}H_{54}ClBr_2N_4P_3$ (1143.30): Calcd C 65.13, H 4.76, N 4.90; found C 65.01, H 4.65, N 4.77.

4.12.4. Tri-λ^5-phosphazenes 5d+5d′. Yield: 83%; ^1H NMR (300 MHz, $CDCl_3$): δ=−0.74 [s, 6H, CH_3 (d+d′)], 3.05–3.70 [m, 36H, CH_AH$_B$P (d+d′)+CH_2N (d+d′)], 3.80–4.04 [m, 12H, CH$_A$$H_B$P (d+d′)], 6.39–6.52 [m, 5H, H_{arom} (d+d′)], 6.93–7.04 [m, 5H, H_{arom} (a+b)], 7.10–7.13 [m, 4H, H_{arom} (d+d′)], 7.26–7.48 [m, 34H, H_{arom} (a+b)], 7.80–7.88 [m, 8H, H_{arom} (d+d′)]; $^{13}C\{^1H\}$ NMR (75.4 MHz, $CDCl_3$): δ=27.48 [CH_3 (d+d′)], 37.34–38.41 [m, CH_2P (d+d′)], 39.02 [q, ^2J(C,P)=3.5 Hz, CH_3C (d+d′)], 53.26 [CH_2N (d/d′)], 53.54 [CH_2N (d/d′)], 56.81 [CH_2N (d/d′)], 56.13 [CH_2N (d/d′)], 57.88 [CH_2N (d/d′)], 57.91 [CH_2N (d/d′)], 111.81 [*q* (d/d′)], 111.94 [*q* (d/d′)], 118.59 (d+d′), 122.21 [*q* (d/d′)], 122.25 [d, ^3J(C,P)=11.6 Hz, *s-cis-C*H=C–N=P (d+d′)], 122.30 [*q* (d/d′)], 123.97 [d, ^3J(C,P)=12.7 Hz, *s-cis-C*H=C–N=P (d+d′)], 124.20 [d, ^3J(C,P)=12.2 Hz, *s-cis-C*H=C–N=P (d/d′)], 124.27 [d, ^3J(C,P)=12.2 Hz, *s-cis-C*H=C–N=P (d/d′)], 125.41 [d, ^3J(C,P)=28.4 Hz, *s-trans-C*H=C–N=P (d+d′)], 126.57 [d, ^3J(C,P)=29.0 Hz, *s-trans-C*H=C–N=P (d+d′)], 127.11 [d, ^3J(C,P)=28.4 Hz, *s-trans-C*H=C–N=P (d+d′)], 128.35–132.60 (d+d′), 138.25 [*q* (d/d′)], 138.31 [*q* (d/d′)], 139.95 [*q* (d/d′)], 139.99 [*q* (d/d′)], 141.57 [*q* (d+d′)], 149.99 [*q* (d/d′)], 150.19 [*q* (d/d′)], 150.65 [*q* (d/d′)], 150.83 [*q* (d/d′)], 151.35 [*q* (d+d′)]; $^{31}P\{^1H\}$ NMR (121.4 MHz, $CDCl_3$): δ=−0.11 (s, 1P), −0.05 (s, 1P), 0.84 (s, 1P), 0.88 (s, 1P), 1.04 (s, 1P), 1.16 (s, 1P); IR (Nujol): ν=1442 (CP), 1123 (NP) cm^{-1}; MS (FAB+): *m/z* (%)=1066 (31) [M^++4], 1064 (89) [M^++2], 1062 (74) [M^+], 154 (100); $C_{62}H_{55}ClBrN_4P_3$ (1064.41): Calcd C 69.96, H 5.21, N 5.26; found C 69.82, H 5.08, N 5.12.

4.12.5. Tri-λ^5-phosphazene 5e. Yield: 85%; mp 301–303 °C (colorless prisms from chloroform/*n*-hexane); ^1H NMR (300 MHz, $CDCl_3$): δ=−0.69 (s, 3H, CH_3), 2.50 (s, 9H, CH_3-Ar), 2.87 (d, J(H,H)=12.3 Hz, 3H, CH_AH$_B$N), 3.14 (pseudoquint, J(H,H)(H,P)=6.8 Hz, 3H, CH_AH$_B$P), 3.58 (d, J(H,H)=12.3 Hz, 3H, CH$_A$$H_B$N), 4.04 (pseudot, J(H,H)(H,P)=14.4 Hz, 3H, CH$_A$$H_B$P), 6.43 (d, J(H,H)=7.2 Hz, 3H, H_{arom}), 6.48 (s, 3H, H_{arom}), 7.05 (dd, J(H,H)=7.1, 2.7 Hz, 3H, H_{arom}), 7.20–7.36 (m, 27H, H_{arom}), 7.84–7.89 (m, 3H, H_{arom}); $^{13}C\{^1H\}$ NMR (75.4 MHz, $CDCl_3$): δ=19.56 (CH_3-Ar), 28.26 (CH_3C), 37.46 (m, CH_2P), 38.86 (q, ^2J(C,P)=3.5 Hz, CH_3C), 57.52 (CH_2N), 118.16, 122.09 (d, ^3J(C,P)=12.2 Hz, *s-cis-C*H=C–N=P), 128.18, 128.40 (d, ^3J(C,P)=11.6 Hz, *m*C-PhP), 128.62 (br s, *p*C-PhP), 131.05 (d, ^3J(C,P)=8.7 Hz, *m*C-PhP), 131.33 (br s, *p*C-PhP), 132.19 (d, ^1J(C,P)=81.8 Hz, *i*C-PhP), 132.21 (d, ^2J(C,P)=9.3 Hz, *o*C-PhP), 133.19 (d_{left} *i*C-PhP), 139.88 (*q*), 149.59 (*q*), the resonance of one *o*C-PhP and the quaternary atom *s-trans-C*=C–N=P was not observed; $^{31}P\{^1H\}$ NMR (121.4 MHz, $CDCl_3$): δ=−1.43; IR (Nujol): ν=1437

(CP), 1116 (NP) cm^{-1}; MS (FAB+): *m/z* (%)=994 (55) [M^++1], 993 (100) [M^+], 992 (78) [M^+−1]; $C_{65}H_{63}N_4P_3$ (993.14): Calcd C 78.61, H 6.39, N 5.64; found C 78.46, H 6.25, N 5.49.

4.12.6. Tri-λ^5-phosphazene 5f. Yield: 82%; mp 288–290 °C (colorless prisms from chloroform/*n*-hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ=−0.73 (s, 3H, CH_3), 2.51 (s, 3H, CH_3-Ar), 2.52 (s, 3H, CH_3-Ar), 2.88 (d, *J*(H,H)=12.3 Hz, 1H, CH_AH$_B$N), 2.89 (d, *J*(H,H)=12.3 Hz, 1H, CH_AH$_B$N), 2.91 (d, *J*(H,H)=12.1 Hz, 1H, CH_AH$_B$N), 3.12–3.23 (m, 3H, CH_AH$_B$P), 3.56 (d, *J*(H,H)=12.1 Hz, 1H, CH_AH$_B$N), 3.58 (d, *J*(H,H)=12.2 Hz, 2H, CH$_A$$H_B$N), 3.94 (pseudot, *J*(H,H)(H,P)=14.6 Hz, 1H, CH$_A$$H_B$P), 4.02 (pseudot, *J*(H,H)(H,P)=14.4 Hz, 1H, CH$_A$$H_B$P), 4.10 (pseudot, *J*(H,H)(H,P)=14.4 Hz, 1H, CH$_A$$H_B$P), 6.40–6.50 (m, 4H, H_{arom}), 7.06–7.10 (m, 3H, H_{arom}), 7.15–7.43 (m, 30H, H_{arom}), 7.87 (m, 3H, H_{arom}); ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$): δ=19.58 (CH_3-Ar), 19.61 (CH_3-Ar), 27.89 (*C*H$_3$C), 36.20–38.80 (m, CH_2P), 38.86 (q, ^{2}*J*(C,P)=3.5 Hz, CH_3*C*), 57.35 (CH_2N), 57.58 (CH_2N), 57.65 (CH_2N), 118.10, 118.14, 118.74, 122.04 (d, ^{3}*J*(C,P)=12.0 Hz, *s-cis-C*H=C–N=P), 122.08 (d, ^{3}*J*(C,P)=12.8 Hz, *s-cis-C*H=C–N=P), 122.39 (d, ^{3}*J*(C,P)=12.8 Hz, *s-cis-C*H=C–N=P), 125.01 (d, ^{3}*J*(C,P)=28.4 Hz, *s-trans-C*H=C–N=P), 128.10–133.10, 139.74 (*q*), 139.78 (*q*), 142.11 (*q*), 149.59 (2 *q*), 150.99 (*q*); ^{31}P{^{1}H} NMR (121.4 MHz, $CDCl_3$): δ=−1.90 (s, 1P, CH_3–Ar–N=P), −1.23 (s, 1P, CH_3–Ar–N=P), 1.98 (s, 1P, H–Ar–N=P); IR (Nujol): ν=1439 (CP), 1117 (NP) cm^{-1}; MS (FAB+): *m/z* (%)=1022 (3) [M^++Na], 980 (56) [M^++1], 979 (100) [M^+]; $C_{64}H_{61}N_4P_3$ (979.12): Calcd C 78.51, H 6.28, N 5.72; found C 78.38, H 6.16, N 5.58.

4.12.7. Tri-λ^5-phosphazene 5g. Yield: 78%; mp 269–270 °C (colorless prisms from chloroform/*n*-hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ=−0.75 (s, 3H, CH_3), 2.52 (s, 3H, CH_3-Ar), 2.90 (d, *J*(H,H)=12.3 Hz, 1H, CH_AH$_B$N), 2.91 (d, *J*(H,H)=12.2 Hz, 1H, CH_AH$_B$N), 2.92 (d, *J*(H,H)=12.2 Hz, 1H, CH_AH$_B$N), 3.13–3.26 (m, 3H, CH_AH$_B$P), 3.56 (d, *J*(H,H)=12.1 Hz, 2H, CH$_A$$H_B$N), 3.58 (d, *J*(H,H)=12.2 Hz, 1H, CH$_A$$H_B$N), 3.92 (pseudot, *J*(H,H)(H,P)=14.6 Hz, 1H, CH$_A$$H_B$P), 4.00 (pseudot, *J*(H,H)(H,P)=14.4 Hz, 1H, CH$_A$$H_B$P), 4.07 (pseudot, *J*(H,H)(H,P)=14.4 Hz, 1H, CH$_A$$H_B$P), 6.39 (s, 1H, H_{arom}), 6.44–6.50 (m, 4H, H_{arom}), 7.07–7.10 (m, 3H, H_{arom}), 7.18–7.48 (m, 30H, H_{arom}), 7.80–7.91 (m, 3H, H_{arom}); ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$): δ=19.58 (CH_3-Ar), 27.58 (*C*H$_3$C), 36.83–38.15 (m, 3 CH_2P), 38.91 (q, ^{2}*J*(C,P)=4.1 Hz, CH_3*C*), 57.44 (CH_2N), 57.50 (CH_2N), 57.73 (CH_2N), 118.08, 118.61, 118.65, 122.06 (d, ^{3}*J*(C,P)=12.3 Hz, *s-cis-C*H=C–N=P), 122.40 (d, ^{3}*J*(C,P)=12.0 Hz, *s-cis-C*H=C–N=P), 122.44 (d, ^{3}*J*(C,P)=13.4 Hz, *s-cis-C*H=C–N=P), 125.06 (d, ^{3}*J*(C,P)=28.4 Hz, *s-trans-C*H=C–N=P), 125.15 (d, ^{3}*J*(C,P)=28.4 Hz, *s-trans-C*H=C–N=P), 128.20–132.90, 139.66 (*q*), 141.99 (*q*), 142.02 (*q*), 149.61 (*q*), 151.11 (2 *q*); ^{31}P{^{1}H} NMR (121.4 MHz, $CDCl_3$): δ=−1.76 (s, 1P, CH_3–Ar–N=P), 1.35 (s, 1P, H–Ar–N=P), 1.95 (s, 1P, H–Ar–N=P); IR (Nujol): ν=1438 (CP), 1118 (NP) cm^{-1}; MS (FAB+): *m/z* (%)=965 (55) [M^+], 964 (100) [M^+−1]; $C_{63}H_{59}N_4P_3$ (965.09): Calcd C 78.40, H 6.16, N 5.81; found C 78.45, H 6.02, N 5.68.

4.13. Procedure for the preparation of triphosphazides 8a and 11b

Two solutions, one of bis(3-azidobenzyl)[2-(2-azidophenyl)-ethyl]amine (**6b**) (0.64 g, 1.5 mmol) or bis(2-azido-5-chlorobenzyl)(3-azidopropyl)amine *N*-oxide (**10b**) (0.67 g, 1.5 mmol) in diethyl ether or dichloromethane (10 mL) and the other of 1,1,1-tris[(diphenylphosphino)methyl]-ethane (**2**) (1.3 g, 1.5 mmol) in diethyl ether (10 mL) were simultaneously added to a round-bottom flask containing diethyl ether (15 mL) under nitrogen atmosphere at room temperature over a period of 30 min with stirring. The resulting mixture was then stirred for 3 h. The precipitated pale yellow solid was filtered, washed with diethyl ether (3×10 mL), and dried under vacuum.

4.13.1. Triphosphazide 8a. Yield: 71%; mp 298–300 °C (yellow prisms from dichloromethane/diethyl ether); ^{1}H NMR (300 MHz, $CDCl_3$): δ=−0.06 (br s, 3H, CH_3), 2.70–2.79 (m, 2H, ArCH_2CH$_2$N), 3.16 (td, *J*(H,H)=12.4, 5.2 Hz, 1H, CH$_2$CH_AH$_B$N), 3.32–3.85 (m, 8H, CH$_2$CH$_A$$H_B$N+2 CH$_2$N+3 C$H_AH_B$P), 3.91 (pseudot, *J*(H,H)(H,P)=16.0 Hz, 1H, CH$_A$$H_B$P), 4.03 (pseudot, *J*(H,H)(H,P)=16.0 Hz, 1H, CH$_A$$H_B$P), 4.07 (pseudot, *J*(H,H)(H,P)=10.1 Hz, 1H, CH$_A$$H_B$P), 6.61 (td, *J*=7.9, 2.9 Hz, 1H, H_{arom}), 6.86 (td, 1H, *J*=7.8, 3.1 Hz, H_{arom}), 6.93–7.57 (m, 34H, H_{arom}), 7.81–8.25 (m, 6H, H_{arom}); ^{13}C{^{1}H} NMR (75.4 MHz, $CDCl_3$): δ=26.06 (*C*H$_3$C), 31.11 (Ar*C*H$_2$CH$_2$N), 36.90 (m, CH_2P), 39.40 (m, CH_2P), 40.09 (q, ^{2}*J*(C,P)=3.2 Hz, CH_3*C*), 40.50 (m, CH_2P), 51.37 (ArCH$_2$*C*H$_2$N), 59.06 (CH_2N), 59.25 (CH_2N), 114.61, 116.08, 117.61, 122.91, 125.05, 125.08 (d, ^{1}*J*(C,P)=109.6 Hz, *i*C-PhP), 125.51, 126.05, 126.32, 127.27 (d, ^{1}*J*(C,P)=82.9 Hz, *i*C-PhP), 127.56 (d_{right}, *i*C-PhP), 128.48–128.14, 129.50 (d_{left}, *i*C-PhP), 129.77 (d, ^{1}*J*(C,P)=91.6 Hz, *i*C-PhP), 129.90 (d, ^{2}*J*(C,P)=9.3 Hz, *o*C-PhP), 130.88 (d, ^{2}*J*(C,P)=8.7 Hz, *o*C-PhP), 130.95, 131.22 (d, $^2J_{CP}$=8.7 Hz, *o*C-PhP), 131.65, 131.93 (d, ^{2}*J*(C,P)=7.5 Hz, *o*C-PhP), 132.47, 132.72 (d, ^{2}*J*(C,P)=8.7 Hz, *o*C-PhP), 132.93, 133.29 (d, ^{2}*J*(C,P)=8.1 Hz, *o*C-PhP), 133.74 (*q*), 141.36 (*q*), 141.83 (*q*), 147.38 (*q*), 151.34 (*q*), 152.04 (*q*); ^{31}P{^{1}H} NMR (121.4 MHz, $CDCl_3$): δ=−0.55 (s, 1P, *Z*-PN$_3$), 19.93 (s, 1P, *E*-PN$_3$), 21.28 (s, 1P, *E*-PN$_3$); ^{31}P{^{1}H} NMR (CPMAS, 121.4 MHz, $(NH_4)_2HPO_4$): δ=−3.12 (*Z*-PN$_3$), 21.02 (*E*-PN$_3$), 25.24 (*E*-PN$_3$); IR (Nujol): ν=1440 (CP), 1106 (NP) cm^{-1}; MS (FAB+): *m/z* (%)=1072 (M^++Na, 2), 1050 (M^++1, 2), 154 (100); $C_{63}H_{59}N_{10}P_3$ (1049.13): Calcd C 72.12, H 5.67, N 13.35; found C 71.85, H 5.58, N 13.08.

4.13.2. Triphosphazide 11b. Yield: 68%; mp 206–208 °C (yellow prisms from dichloromethane/diethyl ether); ^{1}H NMR (300 MHz, $CDCl_3$): δ=−0.20 (br s, 3H, CH_3), 1.96–1.99 (m, 1H, CH$_2$CH_AH$_B$CH$_2$N), 2.47–2.49 (m, 1H, CH$_2$CH$_A$$H_BCH_2$N), 2.81 (br s, 2H, C$H_2CH_2CH_2$N), 2.97–3.17 (m, 2H, CH$_2CH_2CH_AH_B$N+C$H_AH_B$P), 3.47 (pseudot, *J*(H,H)(H,P)=17.4 Hz, 1H, CH_AH$_B$P), 3.51 (pseudot, *J*(H,H)(H,P)=17.4 Hz, 1H, CH_AH$_B$P), 3.57–3.74 (m, 2H, CH$_A$$H_B$P), 3.90 (d, *J*(H,H)=12.4 Hz, 2H, CH_AH$_B$N), 3.99–4.07 (m, 1H, CH$_2$CH$_2$CH$_A$$H_B$N), 4.31 (d, *J*(H,H)=16.0 Hz, 1H, CH$_A$$H_B$N), 4.66–4.73 (m, 1H, CH$_A$$H_B$P), 5.00 (d, *J*(H,H)=16.0 Hz, 1H, CH$_A$$H_B$N), 6.98–7.05 (m, 3H, H_{arom}), 7.25–7.57 (m, 23H, H_{arom}), 7.91–8.02 (m, 6H, H_{arom}), 8.16–8.20 (m, 2H, H_{arom}), 8.33 (d, *J*(H,H)=2.0 Hz, 1H, H_{arom}),

8.45 (d, J(H,H)=1.8 Hz, 1H, H_{arom}); $^{13}C\{^1H\}$ NMR (75.4 MHz, $CDCl_3$): δ=23.31 ($PN_3CH_2\mathit{C}H_2CH_2N$), 26.14 ($\mathit{C}H_3C$), 36.20 (m, CH_2P), 38.41 (dd, ^1J(C,P)=42.8 Hz, ^3J(C,P)=13.9 Hz, CH_2P), 40.02 (q, ^2J(C,P)=3.2 Hz, $CH_3\mathit{C}$), 40.09 (dd, ^1J(C,P)=40.5 Hz, ^3J(C,P)=13.2 Hz, CH_2P), 57.39 ($PN_3CH_2CH_2\mathit{C}H_2N$), 65.44 ($CH_2N$), 67.08 ($CH_2N$), 68.17 ($CH_2N$), 116.99, 117.54, 125.43 (d, ^1J(C,P)=101.7 Hz, *i*C-PhP), 128.77 (d, ^3J(C,P)=11.6 Hz, *m*C-PhP), 128.93 (d, ^3J(C,P)=12.2 Hz, *m*C-PhP), 129.01, 129.08 (d, ^3J(C,P)=10.4 Hz, *m*C-PhP), 129.49 (d, ^3J(C,P)=11.6 Hz, *m*C-PhP), 129.88, 130.57 (d, ^2J(C,P)=9.5 Hz, *o*C-PhP), 131.17 (br s, *p*C-PhP), 131.74–132.85, 145.97 (*q*); $^{31}P\{^1H\}$ NMR (121.4 MHz, $CDCl_3$): δ=−0.40 (s, 1P, *Z*-PN_3), 0.40 (s ancho, 1P), 5.95 (s, 1P, *Z*-PN_3); IR (Nujol): ν=1406 (CP), 1106 (NP) cm^{-1}; MS (FAB+): *m*/*z* (%)=1094 (M^++1+Na, 8), 1074 (M^++4, 4), 1073 (M^++3, 3), 1072 (M^++2, 6), 183 (100); $C_{58}H_{55}Cl_2N_{10}OP_3$ (1071.95): Calcd C 64.99, H 5.17, N 13.07; found C 65.13, H 5.29, N 13.11.

4.14. Preparation of tri-λ-phosphazene 9

This compound was prepared following the procedure described above for the preparation of tri-λ-phosphazenes **4**.

4.14.1. Tri-λ-phosphazene 9. Yield: 62%; mp 228–230; ^1H NMR (300 MHz, $CDCl_3$): δ=−0.44 (s, 3H, CH_3), 2.40–2.78 (m, 5H, 3 $C\mathit{H}_A\mathit{H}_BP$+Ar$C\mathit{H}_2C\mathit{H}_2N$), 3.02 (d, J(H,H)=13.5 Hz, 1H, $C\mathit{H}_A\mathit{H}_BN$), 3.10 (d, J(H,H)=12.4 Hz, 1H, $C\mathit{H}_A\mathit{H}_BN$), 3.27–3.42 (m, 1H, $C\mathit{H}_A\mathit{H}_BN$), 3.53–3.58 (m, 1H, $CH_A\mathit{H}_BN$), 3.64 (d, J(H,H)=12.4 Hz, 1H, $CH_A\mathit{H}_BN$), 3.76–3.81 (m, 1H, $CH_A\mathit{H}_BP$), 3.91 (d, J(H,H)=13.4 Hz, 1H, $CH_A\mathit{H}_BN$), 4.21 (pseudot, J(H,H)(H,P)=13.9 Hz, 1H, $CH_A\mathit{H}_BP$), 5.49 (pseudot, J(H,H)(H,P)=13.9 Hz, 1H, $CH_A\mathit{H}_BP$), 6.04 (d, J(H,H)=7.6 Hz, 2H, H_{arom}), 6.50–6.65 (m, 2H, H_{arom}), 6.88–7.56 (m, 32H, H_{arom}), 7.67–7.93 (m, 6H, H_{arom}); $^{13}C\{^1H\}$ NMR (75.4 MHz, $CDCl_3$): δ=29.07 ($\mathit{C}H_3C$), 34.01 (Ar$\mathit{C}H_2CH_2N$), 37.12 (dd, ^1J(C,P)=47.6 Hz, ^3J(C,P)=7.1 Hz, CH_2P), 38.59 (q, ^2J(C,P)=3.6 Hz, $CH_3\mathit{C}$), 39.92 (dd, ^1J(C,P)=47.6 Hz, ^3J(C,P)=7.1 Hz, CH_2P), 44.82 (dd, ^1J(C,P)=95.1 Hz, ^3J(C,P)=7.1 Hz, CH_2P), 53.73 (CH_2N), 56.01 (CH_2N), 61.04 (CH_2N), 116.42, 117.48, 118.15, 120.29 (d, ^3J(C,P)=11.6 Hz), 122.33 (d, ^3J(C,P)=13.9 Hz), 123.23 (d, ^3J(C,P)=12.8 Hz), 124.76 (d, ^3J(C,P)=28.4 Hz), 125.02 (d, ^3J(C,P)=29.0 Hz), 125.98, 128.24–133.15, 133.48 (d, ^2J(C,P)=9.3 Hz, *o*C-PhP), 134.73 (d, ^3J(C,P)=23.8 Hz, *q*), 141.66 (*q*), 143.90 (*q*), 149.90 (*q*), 151.50 (*q*), 151.53 (*q*); $^{31}P\{^1H\}$ NMR (121.4 MHz, $CDCl_3$): δ=0.76 (s, 1P), 1.44 (s, 1P), 8.16 (s, 1P); IR (Nujol): ν=1436 (CP), 1122 (NP) cm^{-1}; MS (FAB+): *m*/*z* (%)=966 (M^++1, 57), 965 (M^+, 100); $C_{63}H_{59}N_4P_3$ (965.09): Calcd C 78.40, H 6.16, N 5.81; found C 8.23, H 5.88, N 5.58.

Acknowledgements

This work was supported by the MEC and FEDER (Project CTQ2005-02323/BQU) and Fundación Séneca-CARM (Project 00458/PI/04). J.B. also thanks the MEC for a fellowship.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.04.056.

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Synthesis of protected derivatives and short peptides of antAib, a novel C^{α}-tetrasubstituted α-amino acid of the Ac_5c type possessing a fused anthracene fluorophore

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Received 28 February 2006; revised 18 April 2006; accepted 20 April 2006
Available online 19 May 2006

Abstract—The N^{α}-Boc and N^{α}-Fmoc protected derivatives of 2-amino-2,3-dihydro-1*H*-cyclopenta[*b*]anthracene-2-carboxylic acid (antAib), a novel fluorescent, achiral, α-amino acid, rigid analogue of the known 9-antAla and 2-antAla residues, and belonging to the class of $C_i^{\alpha} \rightarrow C_i^{\alpha}$ cyclized, $C^{\alpha,\alpha}$-disubstituted glycines (strong β-turn and helix inducers in peptides), were synthesized in seven steps from 1,2,4-trimethylbenzene. The UV absorption and fluorescence properties of Boc–antAib–OEt and Boc–antAib–OH are also described. Solution syntheses of the short peptides Boc–antAib–L-Ala–OMe, Fmoc–L-Ala–antAib–L-Ala–OMe, as well as Boc–Aib–antAib–L-Ala–OMe and the side product 2,5-dioxopiperazine *cyclo*-[antAib–L-Ala], are presented as examples of the coupling ability at both C- and N-termini of the antAib residue.

1. Introduction

Fluorescence spectroscopy has become a highly valuable technique for conformational studies of biopolymers, the development of peptide-based chemosensors, and biochemical research in general.[1] Incorporation of a fluorescent probe into a peptide chain may be achieved by reaction with side-chain functional groups or the direct use of fluorophore-bearing amino acids. In this connection, *synthetic* fluorescent amino acids may exhibit significant advantages over the related *protein* (Trp, Tyr) residues in terms of potentially different and improved properties. In previous studies, we took advantage of the fluorescence, the increased rigidity, and the axial chirality of 2′,1′:1,2;1″,2″:3,4-dinaphthcyclohepta-1,3-diene-6-amino-6-carboxylic acid (Bin)[2] (Fig. 1), a $C^{\alpha,\alpha}$-disubstituted glycine derived from 1,1′-binaphthyl, to carry out photophysical studies involving intramolecular energy transfer (fluorescence quenching) and intramolecular spin polarization (CIDEP) effects in conformationally constrained peptide-based systems.[3] However, interpretation of the data was complicated by the nonplanar structure of the Bin 1,1′-binaphthyl core. To circumvent this problem, we have now designed 2-amino-2,3-dihydro-1*H*-cyclopenta-[*b*]anthracene-2-carboxylic acid (antAib), a new fluorescent α-amino acid residue which is based on a planar anthracene core and, like Bin, belongs to the class of $C_i^{\alpha} \rightarrow C_i^{\alpha}$ cyclized, $C^{\alpha,\alpha}$-disubstituted glycines (effective β-turn and helix inducers in peptides[4]). The achiral antAib residue may be regarded either as a rigid analogue of the known 9-antAla[5] (or its cyano derivative Flu[1a]) and 2-antAla[5b] residues,

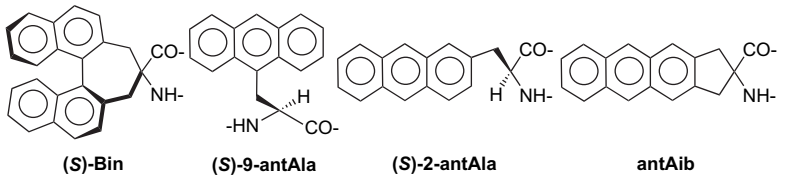

Figure 1. Chemical structure of the antAib residue compared with Bin,[2] 9-antAla,[5] and 2-antAla.[5b]

Keywords: Anthracene; Aromatic amino acid; $C^{\alpha,\alpha}$-Disubstituted glycine; Fluorescent amino acid.
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doi:10.1016/j.tet.2006.04.055

with the spatial disposition of the anthracene side-chain fluorophore relative to the α-carbon atom being completely defined, or as an anthracene-fused 1-aminocyclopentanecarboxylic acid (Ac_5c). Only quinonic derivatives of antAib have been synthesized previously by different methods.[6]

2. Results and discussion

For the synthesis of antAib, the known dimethyl anthracene-2,3-dicarboxylate **5** was used as a key intermediate. This compound was readily prepared by using an easily reproducible, published, procedure (Fig. 2),[7] in which Friedel–Crafts acylation of 1,2,4-trimethylbenzene **1** with benzoyl chloride gave the resulting benzophenone derivative **2** in 67% yield. In the second step, the two-stage oxidation of all three methyl groups present in **2** afforded the tricarboxylic acid **3** (61%), which upon cyclization in concentrated sulfuric acid formed the anthraquinone dicarboxylic acid **4** (88%). Reduction of the quinone part of **4** with activated zinc dust and ammonium hydroxide gave 2,3-anthracene dicarboxylic acid as a soft solid precipitate which could not be collected easily by filtration (see Section 3). Therefore, it was not purified, but collected by centrifugation and then directly esterified in refluxing methanol (MeOH) containing 98% H_2SO_4 to afford the dimethyl ester **5** in 71% overall yield after extraction followed by crystallization from acetone/methanol.

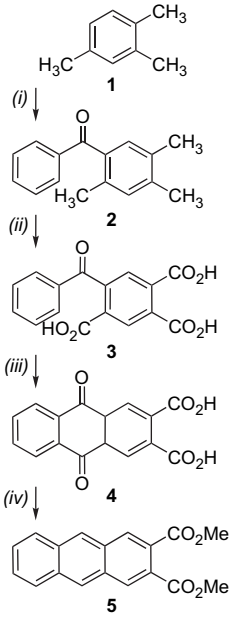

Figure 2. Synthesis of dimethyl anthracene-2,3-dicarboxylate **5**. (i) C_6H_5COCl; $AlCl_3$; 0 °C to rt; 67%. (ii) (1) 20% aq HNO_3; reflux; (2) 10% aq NaOH; $KMnO_4$; reflux; (3) H^+; 62%. (iii) 98% H_2SO_4; 120–130 °C; 87%. (iv) (1) Activated Zn powder; 20% aq NH_4OH; reflux; (2) 98% H_2SO_4; MeOH; reflux (71%).

Reduction of the diester **5** was accomplished under various experimental conditions. The expected diol was not isolated, but treated directly with acetic anhydride (Ac_2O) in pyridine[8,9] to afford the corresponding 2,3-(bis)-acetoxymethyl-anthracene **6** (Fig. 3). The use of lithium aluminum hydride in THF (tetrahydrofuran) as the reducing agent always resulted in partial over-reduction of the central ring with formation of 2,3-(bis)-acetoxymethyl-9,10-dihydroanthracene **7** along with **6**, in a ratio depending on reaction time and temperature (Table 1). Large-scale separation of the desired compound **6** from the side product **7** by chromatography was not possible because of their close R_f values (as observed by analytical TLC). However, their separation by fractional crystallization from acetone allowed the recovery of pure **6** in 46% yield from the first crop.

To transform **5** into the corresponding diol without over-reduction of the central ring, the best method we found was the use of sodium bis(2-methoxyethoxy)aluminum hydride (RedAl®) at low temperature, as proposed by Sun and Desper in a similar case.[8] Here, another side product was formed, the mono-reduced 2-acetoxymethyl-3-methyl-anthracene **8**, but in a very minor proportion relative to **6** (3% vs 86% isolated yields) when the reaction was conducted at low temperature (−15 °C to +3 °C) for a short period of time (1 h 15 min) (Table 1). Furthermore, the separation of **8** from **6** by chromatography was easy. Subsequent crystallization from boiling MeOH/CH_2Cl_2 afforded analytically pure diacetate **6** in 81% yield.

Bromuration of the diacetate **6** with HBr in acetic acid[9] readily furnished 2,3-(bis)-bromomethyl-anthracene **9** (Fig. 4) in 98% yield after crystallization from boiling MeOH/CH_2Cl_2. The dibromide **9** was then used as an electrophile for the bis-alkylation of ethyl isocyanoacetate under the phase transfer conditions developed by Kotha and Brahmachary,[6b] with potassium carbonate as a base and tetra-*n*-butylammonium ($^nBu_4N^+$) hydrogen sulfate as the catalyst, in acetonitrile at 80 °C. The resulting 2-isocyano-2,3-dihydro-1*H*-cyclopenta[*b*]anthracene-2-carboxylic acid ethyl ester **10a** was not isolated, but rather the crude reaction mixture was submitted to acid hydrolysis to afford directly the desired α-amino

Table 1. Product distribution (%) after reduction of the diester **5** followed by acetylation

Reduction conditions		6	7	8
$LiAlH_4$ (THF)	0 °C to rt (2 h)	*30*[a]	*70*[a]	
$LiAlH_4$ (THF)	−14 °C to −2 °C (1 h)	*82*[a] 36[b]	*18*[a]	
$LiAlH_4$ (THF)	−60 °C to −15 °C (2 h)	*85*[a] 46[b]	*15*[a]	
RedAl® (THF)	0 °C (2 h 30 min)	54[b]		10[b]
RedAl® (THF)	0 °C (1 h 15 min)	73[b]		5[b]
RedAl® (THF)	−15 °C to +3 °C (1 h 15 min)	86[b]		3[b]

[a] Ratio determined by ^1H NMR.
[b] Isolated yield.

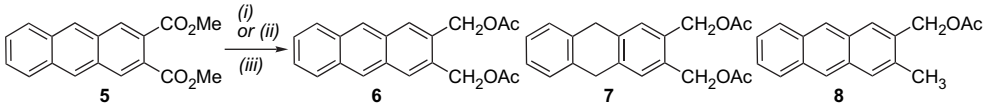

Figure 3. Reduction of the diester **5** followed by acetylation. (i) $LiAlH_4$; THF; low temperature (see Table 1). (ii) RedAl® 3.5 M in toluene; THF; low temperature (see Table 1). (iii) Ac_2O; pyridine.

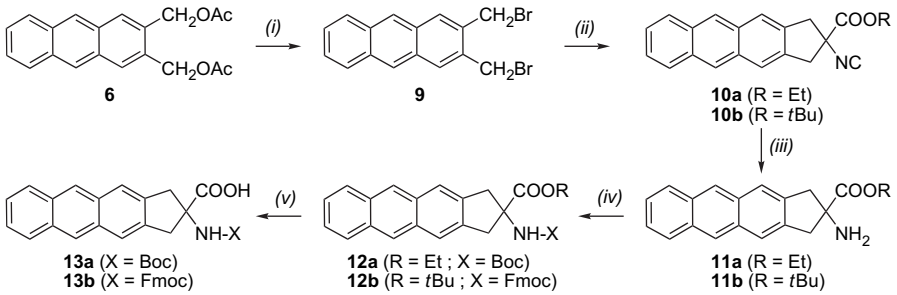

Figure 4. Synthesis of Boc–antAib–OH **13a** and Fmoc–antAib–OH **13b** from the diacetate **6**. (i) 33% HBr in AcOH; CH_2Cl_2; rt. (ii) CN–CH_2–COOEt (a series) or CN–CH_2–COO^{t}Bu (b series); K_2CO_3 or Cs_2CO_3; ^{n}Bu$_4$N$^+$, HSO_4^- (cat); CH_3CN; 80 °C. (iii) 10 N aq HCl; abs EtOH; 0 °C to rt. (iv) Boc_2O; CH_3CN; rt (a series) or Fmoc–OSu; CH_3CN/CH_2Cl_2; rt (b series). (v) 1 N NaOH; MeOH/THF; rt (a series) or TFA/CH_2Cl_2 1:2; 0 °C to rt (b series).

ester H–antAib–OEt **11a** in 28% yield after chromatography. It may be pointed out that this relatively moderate yield, due to unidentified side reactions of the dibromide **9**, is in agreement with the 29.7% yield[10] previously obtained by Kotha et al.[6b] in a similar synthesis of the parent anthraquinone-fused (instead of anthracene-fused) amino ester. Treatment of **11a** with di-*tert*-butyl dicarbonate in acetonitrile gave the fully protected derivative Boc–antAib–OEt (Boc, *tert*-butyloxycarbonyl; OEt, ethoxy) **12a** in only 37% yield after chromatography. In later runs we preferred not to isolate the α-amino ester **11a** but to perform *N*-Boc-protection directly on the crude product obtained after acid hydrolysis, thus reducing purification steps, which resulted in a slight increase in the overall yield in **12a** from **9** (18–25%). In a similar manner, the bis-alkylation of *tert*-butyl isocyanoacetate was conducted in acetonitrile at 80 °C, using cesium carbonate as a base and tetrabutylammonium hydrogen sulfate as the phase transfer catalyst, to afford 2-isocyano-2,3-dihydro-1*H*-cyclopenta[*b*]anthracene-2-carboxylic acid *tert*-butyl ester **10b** in 29% yield after chromatography. Remarkably, treatment of **10b** with a few drops of concentrated hydrochloric acid (ca. 10 N) in ethanol (EtOH)/dichloromethane, from 0 °C to room temperature for a few hours (monitored by TLC) allowed the selective acidolysis of the isonitrile function without cleavage of the *tert*-butyl ester, and furnished H–antAib–O^{t}Bu (O^{t}Bu, *tert*-butoxy) **11b** in 90% yield after chromatography. Protection of the amino function of **11b** by the Fmoc (9-fluorenylmethyloxycarbonyl) group was performed using Fmoc–OSu (OSu, 1-oxysuccinimide) in dichloromethane at room temperature to afford Fmoc–antAib–O^{t}Bu **12b** in 86% yield. Finally, *C*-deprotection of **12a** by saponification of the ethyl ester function with 1 N NaOH in MeOH/THF gave Boc–antAib–OH **13a** (89–96% yield), and *C*-deprotection of **12b** by acidolysis of the *tert*-butyl ester function with TFA (trifluoroacetic acid)/CH_2Cl_2 gave **13b** (92% yield), both suitable for use in peptide synthesis.

Solution synthesis of di- and tripeptides based on antAib was carried out in order to investigate the coupling ability at both C- and N-termini of such a structurally constrained residue. Coupling of Boc–antAib–OH and HCl·L-H–Ala–OMe was performed by EDC [*N*-ethyl, *N*′-(3-dimethylaminopropyl)-carbodiimide]/HOAt (7-aza-1-hydroxy-1,2,3-benzotriazole)[11] *C*-activation to furnish Boc–antAib–L-Ala–OMe **14a** (Fig. 5) in 50–60% yield. This dipeptide was N^{α}-deprotected with TFA/CH_2Cl_2 1:3 and the resulting TFA·H–antAib–L-Ala–OMe (not isolated) was coupled with the urethane-protected *N*-carboxyanhydride (UNCA)[12] Fmoc–L-Ala–NCA, to afford the tripeptide Fmoc–L-Ala–antAib–L-Ala–OMe **15a** in 83% overall yield. These two methods are known to be efficient in difficult cases involving sterically demanding C^{α}-tetrasubstituted α-amino acids.[13] It is also interesting to note that, as previously observed in a similar case,[14] coupling of N^{α}-deprotected **14a** with the more hindered Boc–Aib–NCA (Aib, α-aminoisobutyric acid) afforded the tripeptide Boc–Aib–antAib–L-Ala–OMe **16a** in only 14% overall yield, because of a competitive cyclization to the 2,5-dioxopiperazine *cyclo*-[antAib–L-Ala] **17a**, isolated in 48% yield. One can expect therefore that in general cases where antAib is at the N-terminal position of a peptide, but not of a dipeptide methyl ester (where a fast cyclization reaction is favored), coupling at its amino function with an additional C^{α}-tetrasubstituted α-amino acid, should occur efficiently.

We have recorded the UV absorption spectra of Boc–antAib–OEt **12a** and Boc–antAib–OH **13a** and their fluorescence spectral signatures in ethanol solution. The symmetry-allowed, intense, $S_0 \rightarrow S_1$ transition of the

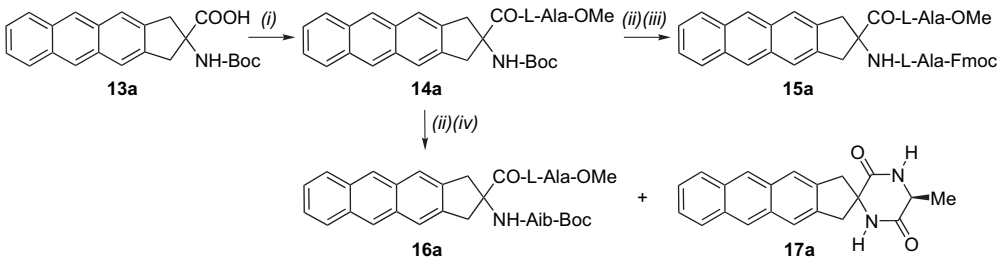

Figure 5. Solution synthesis of di- and tripeptides based on antAib. (i) HCl·H–L-Ala–OMe; NMM (*N*-methylmorpholine); EDC; HOAt; THF/CH_2Cl_2; rt. (ii) TFA/CH_2Cl_2 (1:3); 0 °C. (iii) Fmoc–L-Ala–NCA; DIEA (*N*,*N*,*N*-diisopropylethylamine); THF; 0 °C to rt. (iv) Boc–Aib–NCA; DIEA; THF; 60 °C.

anthracene chromophore is evident as a Frank–Condon vibronic progression with an origin (0,0) at 378 nm and additional peaks (0,1–0,4) separated by ca. 16 nm (λ_{max}= 359 nm)[15] (Fig. 6). The emission spectra (λ_{exc}=359 nm) have an origin at 384–385 nm, a maximum intensity at 407–408 nm, and two other peaks of the vibronic progression clearly observed at longer wavelengths (Fig. 7).

In conclusion, the syntheses of the protected derivatives Boc–antAib–OEt and Fmoc–antAib–O^{t}Bu were achieved in seven steps from 1,2,4-trimethylbenzene. Saponification of the ester function of Boc–antAib–OEt and acidolysis of Fmoc–antAib–O^{t}Bu afforded the corresponding *N*-protected amino acids, suitable for peptide elongation using either Boc or Fmoc strategies. Solution peptide syntheses of the tripeptides Fmoc–L-Ala–antAib–L-Ala–OMe and Boc–Aib–antAib–L-Ala–OMe demonstrated the coupling efficiency at both C- and N-termini of the structurally constrained antAib residue. The fluorescence spectra of Boc–antAib–OEt and Boc–antAib–OH suggest that the antAib residue may represent a novel useful spectroscopic probe in studies of peptide molecules by virtue of the appearance of its bands

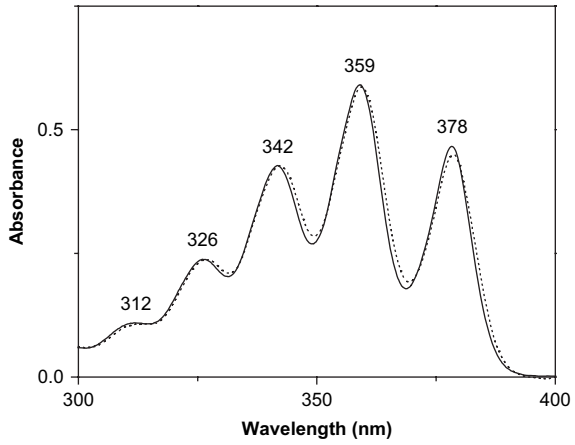

Figure 6. UV absorption spectrum (>300 nm region) of Boc–antAib–OEt **12a** (solid line) and Boc–antAib–OH **13a** (dotted line) in ethanol solution. Amino acid derivative concentration: 1×10^{-4} M.

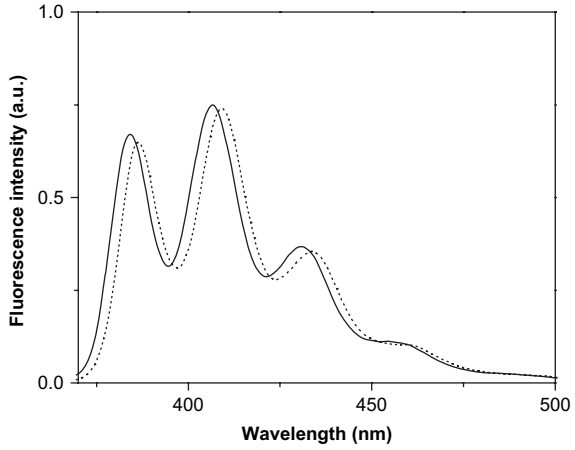

Figure 7. Fluorescence spectra in the 370–500 nm range of Boc–antAib–OEt **12a** (solid line) and Boc–antAib–OH **13a** (dotted line) in ethanol solution: λ_{exc}=359 nm. Amino acid derivative concentration: 1×10^{-6} M.

at much longer wavelengths than those typical of coded aromatic α-amino acids. To determine antAib preferred conformation, peptides based on this residue in combination with L-Ala to the hexamer level are currently being synthesized in our groups. Secondary structures involving β-turns/3_{10}-helices are expected to be efficiently induced by antAib as it is a member of the family of $C_i^{\alpha}\rightarrow C_i^{\alpha}$ cyclized, $C^{\alpha,\alpha}$-disubstituted glycines,[4] thus allowing its exploitation as a fluorophore in photophysical studies of rigid, folded, peptide architectures.

Altogether, we believe that the antAib residue will expand the scope of fluorescence analysis of peptide conformations and interactions in solution, as it represents a *unique label with frozen main-chain and side-chain rotational freedoms*, in contrast with all fluorescent probes proposed so far. That may be of main interest for the design of conformationally constrained bioactive peptide systems.[16]

3. Experimental

Melting points were measured on a Mettler apparatus with a final temperature raise of 3 °C/min or by means of a capillary tube immersed in an oil bath (Tottoli apparatus, Büchi) and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker WM300 spectrometer operating at 300 MHz and 77 MHz, respectively, the solvent being used as the internal standard: $CDCl_3$ (^{1}H: δ=7.27 ppm; ^{13}C: δ=77.00 ppm), CD_3COCD_3 (^{1}H: δ=2.05 ppm; ^{13}C: δ=29.80 ppm), CD_3SOCD_3 (^{1}H: δ=2.50 ppm; ^{13}C: δ= 39.50 ppm). Splitting patterns are abbreviated as follows: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet. Elemental analyses were performed by the C.N.R.S. Service of Microanalyses in Gif-sur-Yvette (France). Mass spectra (electrospray mode) were recorded by Vincent Steinmetz (Institut Lavoisier), and high-resolution mass spectra by Nicole Morin (Service of mass spectrometry, ENS, Paris). Analytical TLC and preparative column chromatography were performed on Kieselgel F 254 and Kieselgel 60 (0.040–0.063 mm) (Merck), respectively, with the following eluant systems: CH_2Cl_2 (I); 2.5% MeOH–97.5% CH_2Cl_2 (II); 2.5% EtOAc–97.5% CH_2Cl_2 (III); 5% EtOAc–95% CH_2Cl_2 (IV); 20% EtOAc–80% CH_2Cl_2 (V); 50% EtOAc–50% CH_2Cl_2 (VI); 5% MeOH–95% CH_2Cl_2 (VII); 10% MeOH–90% CH_2Cl_2 (VIII). UV light (λ=254 nm) allowed visualization of the spots after TLC runs for all compounds. Except when noted, all starting materials and solvents were obtained from commercial suppliers and were used as received. Fmoc–L-Ala–NCA and Boc–Aib–NCA were purchased from Fluka and Isochem, respectively.

3.1. 2,4,5-Trimethylbenzophenone (2)

A mixture of 1,2,4-trimethylbenzene **1** (20 g; 166.6 mmol) and $AlCl_3$ (23.4 g; 175 mmol) in CH_2Cl_2 (20 mL) was treated with benzoyl chloride (23.4 g; 166.5 mmol) as described in the literature,[7] to yield 24.9 g (67%) of pure **2** as a colorless liquid after vacuum distillation. Bp 152–155 °C/ca. 0.3 Torr (lit.[7] bp 130 °C/0.15 Torr). ^{1}H NMR ($CDCl_3$): δ 7.81 [m, 2H, ArH], 7.58 [m, 1H, ArH], 7.46 [m, 2H, ArH], 7.12 [s, 1H, ArH], 7.07 [s, 1H, ArH], 2.30 [s, 3H, $ArCH_3$], 2.29 [s, 3H, $ArCH_3$], 2.25 [s, 3H, $ArCH_3$].

^{13}C NMR ($CDCl_3$): δ 198.2 [C=O], 138.9, 138.0, 135.8, 134.2, 133.0, 132.5, 132.2, 129.9, 129.7, 129.1, 128.5, 128.1 [C_{Ar}], 19.35, 19.29, 18.8 [$ArCH_3$].

3.2. Benzophenone-2,4,5-tricarboxylic acid (3)

A mixture of 2,4,5-trimethylbenzophenone **2** (19.7 g; 88 mmol) and 20% HNO_3 (150 mL) was refluxed with stirring for 5 days. The resulting thick semisolid was decanted, rinsed with cold water (2×75 mL), dissolved in boiling 10% NaOH (200 mL), and treated with $KMnO_4$ (55.3 g; 350 mmol) as described in the literature,[7] to yield 17.0 g (61%) of pure **3** obtained as white crystals. Mp 224 °C (lit.[7] mp 281–283 °C). ^{1}H NMR (CD_3COCD_3): δ 8.47 [s, 1H, ArH], 7.76 [m, 2H, ArH], 7.75 [s, 1H, ArH], 7.67–7.46 [m, 3H, ArH]. ^{13}C NMR (CD_3COCD_3): δ 195.7 [C=O], 167.9, 167.4, 165.8 [COOH]; 145.5, 137.7, 137.6, 134.1, 133.8, 132.2, 131.7, 130.0, 129.4, 128.6 [C_{Ar}].

3.3. Anthraquinone-2,3-dicarboxylic acid (4)

The triacid **3** (4.20 g; 13.4 mmol) was treated with 98% H_2SO_4 (42 g) at 120–130 °C for 3 h as described in the literature,[7] to yield 3.49 g (88%) of pure **4** as a pale brown solid. Mp >300 °C (lit.[7] mp >310 °C). ^{1}H NMR (CD_3SOCD_3): δ 8.36 [s, 2H, ArH], 8.26–8.19 [m, 2H, ArH], 8.00–7.94 [m, 2H, ArH]. ^{13}C NMR (CD_3SOCD_3): δ 181.4 [C=O], 167.6 [COOH]; 137.5, 134.9, 134.2, 132.9, 127.0, 126.9 [C_{Ar}].

3.4. Dimethyl anthracene-2,3-dicarboxylate (5)

Following the literature procedure,[7] the diacid **4** (2.00 g; 6.76 mmol) was added by portions to a solution of 20% NH_4OH (100 mL) with stirring at room temperature. A clear red solution was obtained after ca. 15 min, to which was added by portions activated zinc dust (7.50 g). The resulting blood-red mixture was refluxed until the color was gone (ca. 2 h) and then filtered while hot. The solid residue was refluxed with 20% NH_4OH (50 mL) for 2 h and filtered while hot. The combined filtrates were cooled on an ice bath and then acidified to pH <1 with 10 N HCl, which resulted in the precipitation of a yellow colloidal solid which slowly settled overnight at room temperature. Attempted filtration on a Büchner was very slow and difficult. Therefore, the solid was collected by centrifugation, and then repeatedly dried by evaporation in vacuo at 50 °C after addition of methanol. The so-obtained crude anthracene-2,3-dicarboxylic acid (2.14 g) was pure by ^{1}H NMR (CD_3SOCD_3): δ 8.77 [s, 2H, ArH], 8.46 [s, 2H, ArH], 8.17–8.12 [m, 2H, ArH], 7.64–7.59 [m, 2H, ArH] with only the presence of NH_4Cl (δ 7.18 [t, J=51.0 Hz]) as contaminant. Ten similar runs starting with in total 19.25 g (65.0 mmol) of diacid **4** gave 21.80 g of such a mixture of anthracene-2,3-dicarboxylic acid and NH_4Cl. To this mixture (10.10 g) was added MeOH (400 mL) and 98% H_2SO_4 (10 mL). The resulting yellow suspension was stirred and refluxed. After ca. 1 h, a clear orange-brown solution was obtained, which was refluxed for 5 days to give a suspension again. The mixture was cooled to 0 °C, the solid was filtered, abundantly washed with MeOH, air dried (weight 6.27 g), and then dissolved in a mixture of H_2O (400 mL) and CH_2Cl_2 (600 mL) with stirring. The decanted CH_2Cl_2 solution was washed with 5% $NaHCO_3$ (100 mL), H_2O (2×400 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo to afford 5.76 g of crude diester **5** as a solid, which was pure by ^{1}H NMR and TLC. The original mother liquor (MeOH solution) was concentrated in vacuo at 40 °C to ca. 150 mL, H_2O (700 mL) added, and the mixture was extracted with CH_2Cl_2 (250 mL). The decanted CH_2Cl_2 solution was washed with 5% $NaHCO_3$ (100 mL) and H_2O (2×400 mL) as above, dried ($MgSO_4$), filtered, and evaporated in vacuo to afford 1.01 g of impure diester **5**. A second similar run starting from the remaining mixture of diacid **4** and NH_4Cl (11.70 g) gave 7.39 g of pure diester **5** after extraction of the filtered precipitate and 1.39 g of impure compound from the mother liquor. The combined pure samples of diester (13.14 g) were dissolved in boiling acetone (400 mL) and the solution concentrated to ca. 60 mL. Crystallization occurred from the boiling solution to which methanol (ca. 60 mL) was added by portions in order to increase the quantity of crystals. The mixture was concentrated again to ca. 60 mL, and then cooled to room temperature. The crystals were filtered, abundantly washed with methanol, and air dried (weight 11.96 g). More crystals (1.60 g) were obtained from the mother liquor and by repeated crystallization of the combined impure samples, to give a total of 13.56 g (71%) of analytically pure diester **5**, obtained as yellow-orange crystals. Mp 152 °C (lit.[7] mp 149–151 °C). R_f 0.18 (I). ^{1}H NMR ($CDCl_3$): δ 8.52 [s, 2H, ArH], 8.45 [s, 2H, ArH], 8.09–8.04 [m, 2H, ArH], 7.60–7.55 [m, 2H, ArH]. ^{13}C NMR ($CDCl_3$): δ 168.0 [C=O], 132.9, 131.1, 130.2, 128.2, 127.8, 127.4, 126.7 [C_{Ar}], 52.5 [OCH_3].

3.5. Reduction of dimethyl anthracene-2,3-dicarboxylate

(a) With lithium aluminum hydride: To a suspension of $LiAlH_4$ (0.190 g; 5 mmol) in THF (15 mL) magnetically stirred at 0 °C was added the diester **5** (0.294 g; 1 mmol) by portions under an argon atmosphere. The reaction mixture was stirred at room temperature for 2 h, cooled to 0 °C, and quenched by dropwise addition of saturated aqueous Na_2SO_4 (ca. 2 mL). After stirring at room temperature for 15 min, the mixture was filtered on glass wool and the filtrate was evaporated to dryness in vacuo at 40 °C. The residue was repeatedly evaporated again in vacuo after addition of methanol in order to remove water. The obtained crude product was dissolved in pyridine (10 mL) and acetic anhydride (1 mL) was added. The resulting solution was stirred at room temperature overnight and then evaporated to dryness in vacuo at 40 °C. The residue was taken up in ethyl acetate (100 mL) and 0.5 N aq HCl (100 mL) with stirring. The decanted organic phase was washed with 0.5 N aq HCl (100 mL), then with H_2O (2×100 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo. The crude product (0.175 g) presented a single spot on TLC with either eluant (I), (II), (III) or (IV), but contained compounds **6** and **7** in the ratio 30:70, determined by integration of their respective $ArCH_2O$ and $OCOCH_3$ singlets in ^{1}H NMR (vide infra). Preparative TLC on silica gel with eluant (III) gave 0.105 g of the same mixture **6** (30%) and **7** (70%). Fractional crystallization from a concentrated acetone solution (ca. 1 mL) gave 0.012 g (4%) of pure **6** as pale yellow crystals.

In another run, the diester **5** (0.294 g; 1 mmol) was treated by $LiAlH_4$ (0.190 g; 5 mmol) in THF (15 mL) in the same experimental conditions and workup as above, except that

the reaction was carried out at −14 °C to −2 °C for 1 h. The crude product obtained after acetylation (0.213 g) contained **6** and **7** in the ratio 82:18 by ^{1}H NMR. Fractional crystallization of this crude product from concentrated acetone (ca. 1 mL) gave 0.116 g (36%) of pure **6** as pale yellow crystals.

In another run, the diester **5** (1.470 g; 5 mmol) was treated by $LiAlH_4$ (0.950 g; 25 mmol) in THF (75 mL) in the same experimental conditions and workup as above, except that the reaction was carried out at −60 °C to −15 °C for 2 h. The crude product obtained after acetylation (1.486 g) contained compounds **6** and **7** in the ratio 85:15 by ^{1}H NMR. Fractional crystallization of this crude product from concentrated acetone (ca. 5 mL) gave 0.746 g (46%) of pure **6** as pale yellow crystals.

(b) With sodium bis(2-methoxyethoxy)aluminum hydride: To a solution of diester **5** (2.940 g; 10 mmol) in THF (100 mL) cooled to 0 °C and stirred under an argon atmosphere was added dropwise by syringe 16 mL (56 mmol) of ca. 3.5 M solution of RedAl® in toluene (Acros) over a period of 30 min. The resulting red-brown solution was stirred at 0 °C for 2 h, and then quenched by dropwise addition of aq 5% H_2SO_4 (100 mL). The mixture was poured into a separating funnel containing 5% H_2SO_4 (200 mL) and EtOAc (500 mL). After shaking and decantation, a large amount of foam at the interface between the milky yellow organic phase and the clear colorless aqueous acidic phase was present, due to the low solubility of the expected 2,3-bis(hydroxymethyl)-anthracene. The separated organic phase and the foam at the interface were washed with H_2O (500 mL), decanted, and then directly (without addition of drying agent) evaporated to dryness in vacuo. The residue was repeatedly evaporated again in vacuo after addition of methanol in order to remove water. The obtained crude product (2.454 g) was dissolved in pyridine (100 mL) and acetic anhydride (20 mL) was added. The resulting clear orange solution was magnetically stirred at room temperature for 20 h and then evaporated to dryness in vacuo at 40 °C. The residue was taken up in ethyl acetate (400 mL) and 0.5 N aq HCl (300 mL) with stirring. The decanted organic phase was washed with 0.5 N aq HCl (100 mL), then with brine (2×400 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo. The crude product (3.161 g) was chromatographed on a 2.3×54 cm column of silica gel with eluant (IV) to afford the desired pure 2,3-bis(acetoxymethyl)-anthracene **6** (1.752 g; 54%) and pure 2-acetoxymethyl-3-methyl-anthracene **8** (0.272 g; 10%) as a side product.

In another run, the diester **5** (2.940 g; 5 mmol) in THF (100 mL) was treated by a 3.5 M solution of RedAl® in toluene (16 mL; 56 mmol) in the same experimental conditions and workup as above, except that the reaction was conducted at 0 °C for 15 min (addition) and then for a further 1 h. Column chromatography of the crude product obtained after acetylation (3.198 g) gave **6** (2.354 g; 73%) and **8** (0.140 g; 5%).

In another run, the diester **5** (3.633 g; 12.4 mmol) in THF (125 mL) was treated by a 3.5 M solution of RedAl® in toluene (19 mL; 66 mmol) in the same experimental conditions and workup as above, except that the reaction was conducted at −15 °C to −13 °C for 15 min (addition) and then at −13 °C to +3 °C for a further 1 h. Column chromatography of the crude product obtained after acetylation (3.917 g) gave **6** (3.437 g; 86%) and **8** (0.114 g; 3%). The pure compound **6** (3.437 g) was dissolved in boiling CH_2Cl_2 (100 mL) (water bath at 80 °C) and MeOH (50 mL) was added. The solution was concentrated to ca. 50 mL upon boiling (crystallization started to occur from the boiling solution). Methanol (50 mL) was added, the mixture was concentrated again to ca. 30 mL upon boiling, and then cooled to room temperature. The resulting crystals were filtered, abundantly washed with MeOH, and air dried to give 3.203 g (81%) of analytically pure **6**. Similar crystallization of the side product **8** (0.553 g; combined samples from several runs) from boiling MeOH (CH_2Cl_2) gave 0.384 g of yellow crystals.

3.6. 2,3-Bis(acetoxymethyl)-anthracene (6)

Yellow crystals. Mp 185 °C. R_f 0.2–0.3 (I), 0.75 (II), 0.35 (III), 0.50 (IV). ^{1}H NMR ($CDCl_3$): δ 8.43 [s, 2H, ArH], 8.04 [s, 2H, ArH], 8.04–8.00 [m, 2H, ArH], 7.53–7.48 [m, 2H, ArH], 5.39 [s, 4H, $ArCH_2O$], 2.17 [s, 6H, $OCOCH_3$]. ^{13}C NMR ($CDCl_3$): δ 170.6 [C═O], 132.2, 131.3, 130.8, 129.7, 128.2, 126.4, 125.8 [C_{Ar}], 64.6 [$ArCH_2O$], 20.9 [$OCOCH_3$]. Anal. Calcd for $C_{20}H_{18}O_4$ (322.344): C, 74.52; H, 5.63. Found: C, 74.92; H, 5.65.

3.7. 2,3-Bis(acetoxymethyl)-9,10-dihydroanthracene (7)

Not isolated. Characterized by ^{1}H and ^{13}C NMR in mixtures with **6**. R_f 0.2–0.3 (I), 0.75 (II), 0.35 (III), 0.50 (IV). ^{1}H NMR ($CDCl_3$): δ 7.36 [s, 2H, ArH], 7.32–7.27 [m, 2H, ArH], 7.25–7.20 [m, 2H, ArH], 5.22 [s, 4H, $ArCH_2O$], 3.95 [s, 4H, $ArCH_2Ar$], 2.11 [s, 6H, $OCOCH_3$]. ^{13}C NMR ($CDCl_3$): δ 170.6 [C═O], 137.4–125.7 [C_{Ar}], 63.7 [$ArCH_2O$], 35.6 [$ArCH_2Ar$], 20.9 [$OCOCH_3$].

3.8. 2-Acetoxymethyl-3-methyl-anthracene (8)

Yellow crystals. Mp 194 °C. R_f 0.70 (IV). ^{1}H NMR ($CDCl_3$): δ 8.43 [s, 1H, ArH], 8.40 [s, 1H, ArH], 8.02–7.97 [m, 2H, ArH], 7.98 [s, 1H, ArH], 7.80 [s, 1H, ArH], 7.49–7.44 [m, 2H, ArH], 5.32 [s, 2H, $ArCH_2O$], 2.55 [s, 3H, $ArCH_3$], 2.18 [s, 3H, $OCOCH_3$]. ^{13}C NMR ($CDCl_3$): δ 170.9 [C═O], 133.9, 132.5, 132.1, 131.7, 131.5, 130.3, 128.5, 128.2, 128.1, 126.2, 125.5, 125.1, 125.0 [C_{Ar}], 65.2 [$ArCH_2O$], 21.0 [$OCOCH_3$], 19.5 [$ArCH_3$].

3.9. 2,3-Bis(bromomethyl)-anthracene (9)

To a solution of diacetate **6** (3.222 g; 10 mmol) in CH_2Cl_2 (200 mL) was added a 33 wt % solution of hydrogen bromide in acetic acid (Aldrich) (20 mL; ca. 110 mmol). The solution, which became more and more turbid, was stirred at room temperature for 24 h. Water (250 mL) was added, the reaction mixture was transferred to a separating funnel, and extracted with CH_2Cl_2 (800 mL necessary for complete solubilization). The separated CH_2Cl_2 solution was washed with 5% $NaHCO_3$ (150 mL) and then H_2O (2×150 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo. The obtained crystalline crude product (3.680 g) was stirred in boiling CH_2Cl_2 (200 mL) but could not be totally solubilized. Methanol (50 mL) was added and the mixture was

concentrated to ca. 30 mL with almost complete crystallization occurring from the boiling solution. After cooling to room temperature, the crystals were filtered, abundantly washed with MeOH, and air dried to afford 3.573 g (98%) of analytically pure dibromide **9** as yellow crystals. Mp 225 °C. R_f 0.85 (III). ^{1}H NMR ($CDCl_3$): δ 8.39 [s, 2H, ArH], 8.04 [s, 2H, ArH], 8.03–7.99 [m, 2H, ArH], 7.53–7.49 [m, 2H, ArH], 4.95 [s, 4H, $ArCH_2Br$]. ^{13}C NMR ($CDCl_3$): δ 133.1, 132.4, 131.2, 131.0, 128.2, 126.6, 126.1 [C_{Ar}], 31.6 [$ArCH_2Br$]. HRMS (DCI^+). Calcd $[M+H]^+$ for $C_{16}H_{13}$ $^{79}Br_2$: 362.9384. Found: 362.9388. Calcd $[M+H]^+$ for $C_{16}H_{13}$ $^{79}Br^{81}Br$: 364.9364. Found: 364.9373. Calcd $[M+H]^+$ for $C_{16}H_{13}$ $^{81}Br_2$: 366.9346. Found: 366.9350. Anal. Calcd for $C_{16}H_{12}Br_2$ (364.088): C, 52.78; H, 3.32. Found: C, 52.93; H, 3.29.

3.10. 2-Isocyano-2,3-dihydro-1*H*-cyclopenta[*b*]anthracene-2-carboxylic acid *tert*-butyl ester (C═antAib–O^{t}Bu 10b)

To a suspension of **9** (1.00 g; 2.75 mmol), tetrabutylammonium hydrogen sulfate (0.42 g; 1.24 mmol) and Cs_2CO_3 (5.38 g; 16.5 mmol) in CH_3CN (150 mL) was added *tert*-butyl isocyanoacetate (0.60 mL, 4.12 mmol). The mixture was stirred under argon at 75–80 °C for 48 h and then filtered through sintered glass. The filtrate was evaporated in vacuo and the residue dissolved in CH_2Cl_2 (150 mL). The organic layer was washed with water, dried over magnesium sulfate, and evaporated to dryness. The crude product was purified by column chromatography on silica gel using CH_2Cl_2 as eluant to give 0.275 g (29%) of pure **10b** as a pale yellow solid. Crystallization of an aliquot from cyclohexane/CH_2Cl_2 afforded analytically pure crystals (needles). Mp 203 °C. R_f 0.55 (I). ^{1}H NMR ($CDCl_3$): δ 8.37 [s, 2H, ArH], 8.00–7.97 [m, 2H, ArH], 7.85 [s, 2H, ArH], 7.48–7.45 [m, 2H, ArH], 3.81 and 3.61 [d, J=16.4 Hz, 2H and d, J=16.4 Hz, 2H, $ArCH_2$], 1.54 [s, 9H, CH_3 O^{t}Bu]. ^{13}C NMR ($CDCl_3$): δ 167.3 [C═O], 137.1, 131.9, 131.7, 128.3, 126.2, 125.6, 123.3 [C_{Ar}], 111.9 [C═N–], 84.3 [O–C O^{t}Bu], 77.4 [C$^\alpha$], 45.5 [$ArCH_2$], 28.0 [CH_3 O^{t}Bu]. Anal. Calcd for $C_{23}H_{21}NO_2$ (343.406): C, 80.44; H, 6.16; N, 4.08. Found: C, 80.43; H, 6.13; N, 3.88.

3.11. 2-Amino-2,3-dihydro-1*H*-cyclopenta[*b*]anthracene-2-carboxylic acid ethyl ester (H–antAib–OEt 11a)

To a suspension of **9** (0.182 g; 0.5 mmol), tetrabutylammonium hydrogen sulfate (0.068 g; 0.2 mmol) and K_2CO_3 (1.38 g; 10 mmol) in CH_3CN (25 mL) was added ethyl isocyanoacetate (0.55 mL; 5 mmol). The mixture was stirred under argon at 75–80 °C for 20 h, then cooled to room temperature, and filtered through sintered glass. The solid was abundantly washed with CH_2Cl_2 and the filtrate was evaporated in vacuo. To the residue (containing C═antAib–OEt **10a**, not isolated) was added CH_2Cl_2 (10 mL), abs EtOH (25 mL) and 10 N HCl (1 mL). The mixture was magnetically stirred at room temperature for 2 h and then diluted with CH_2Cl_2 (100 mL). Water (150 mL) was added, and the mixture was made alkaline by slow addition of $NaHCO_3$ with stirring. The separated CH_2Cl_2 solution was washed with H_2O (2×100 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo. The crude product was purified on a preparative TLC plate of silica gel with eluant (VI) to afford 0.043 g (28%) of α-amino ester **11a**. R_f 0.05 (IV); 0.20 (V). ^{1}H NMR ($CDCl_3$): δ 8.33 [s, 2H, ArH], 8.00–7.95 [m, 2H, ArH], 7.82 [s, 2H, ArH], 7.46–7.41 [m, 2H, ArH], 4.27 [q, J=7.1 Hz, 2H, CH_2 OEt], 3.71 and 3.08 [d, J=16.3 Hz, 2H, $ArCH_2$ and d, J=16.3 Hz, 2H, $ArCH_2$], 2.02 [br s, 2H, NH_2], 1.33 [t, J=7.1 Hz, 3H, CH_3 OEt]. ^{13}C NMR ($CDCl_3$): δ 172.5 [C═O], 139.8, 131.7, 131.4, 128.0, 125.5, 125.0, 122.8 [C_{Ar}], 65.4 [C$^\alpha$], 61.5 [CH_2 OEt], 45.4 [$ArCH_2$], 14.2 [CH_3 OEt]. HRMS (FAB^+) of **11a**·CF_3CO_2H. Calcd $[M+H]^+$ for $C_{20}H_{20}NO_2$: 306.1494. Found: 306.1487.

3.12. 2-Amino-2,3-dihydro-1*H*-cyclopenta[*b*]anthracene-2-carboxylic acid *tert*-butyl ester (H–antAib–O^{t}Bu 11b)

The isonitrile **10b** (0.103 g; 0.300 mmol) was dissolved in CH_2Cl_2 (20 mL) and ethanol (20 mL) was added. The solution was cooled to 0 °C and 10 N HCl (0.5 mL) was added. The reaction mixture was allowed to warm to room temperature and stirred for ca. 2 h, until total hydrolysis had occurred (TLC monitoring). Water (150 mL) and CH_2Cl_2 (150 mL) were added, and the mixture was made alkaline by slow addition of a large excess of $NaHCO_3$ (0.840 g; 10 mmol) with stirring. The separated CH_2Cl_2 solution was washed with water (2×100 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo. The crude product was chromatographed on a column of silica gel with eluant (V) to afford 0.090 g (90%) of pure **11b** as a pale yellow solid. Mp 227 °C. R_f 0.31 (V). ^{1}H NMR ($CDCl_3$): δ 8.33 [s, 2H, ArH], 8.00–7.96 [m, 2H, ArH], 7.81 [s, 2H, ArH], 7.47–7.42 [m, 2H, ArH], 3.66 and 3.05 [d, J=16.3 Hz, 2H, $ArCH_2$ and d, J=16.3 Hz, 2H, $ArCH_2$], 1.86 [br s, 2H, NH_2], 1.51 [s, 9H, CH_3 O^{t}Bu]. ^{13}C NMR ($CDCl_3$): δ 175.7 [C═O], 140.4, 131.9, 131.5, 128.2, 125.7, 125.1, 122.9 [C_{Ar}], 81.6 [O–C O^{t}Bu], 66.1 [C$^\alpha$], 45.6 [$ArCH_2$], 28.2 [CH_3 O^{t}Bu]. Anal. Calcd for $C_{22}H_{23}NO_2$ (333.412): C, 79.25; H, 6.95; N, 4.20. Found: C, 79.07; H, 6.98; N, 4.05.

3.13. 2-*tert*-Butyloxycarbonylamino-2,3-dihydro-1*H*-cyclopenta[*b*]anthracene-2-carboxylic acid ethyl ester (Boc–antAib–OEt 12a)

(a) A solution of the α-amino ester **11a** (0.034 g; 0.11 mmol) and Boc_2O (0.050 g; 0.22 mmol) in CH_3CN (2 mL) was stirred at room temperature for 3 days and then evaporated in vacuo. The crude product was chromatographed on a preparative TLC plate of silica gel with eluant (III) to afford 0.0167 g (37%) of pure **12a**.

(b) In other experiments on a larger scale, without isolation/characterization of the isonitrile and α-amino ester intermediates, a mixture of dibromide **9** (0.364 g; 1 mmol), tetrabutylammonium hydrogen sulfate (0.136 g; 0.4 mmol), K_2CO_3 (2.76 g; 10 mmol), and ethyl isocyanoacetate (0.30 mL; 2.75 mmol) in CH_3CN (50 mL) was reacted in the same experimental conditions and workup as above (in Section 3.10). The crude product was submitted to direct acidic hydrolysis with 10 N HCl (1 mL) in CH_2Cl_2 (10 mL) and abs EtOH (25 mL), also as above. The crude product obtained after extraction (containing the α-amino ester **11a**) was reacted with Boc_2O (0.347 g; 1.59 mmol) in CH_3CN (5 mL) and CH_2Cl_2

(10 mL) at room temperature for 5 days and then evaporated in vacuo. The crude product was chromatographed on a 2.3×27 cm column of silica gel with eluant (III), to afford 0.102 g (25%) of pure *N*-Boc-protected α-amino ester **12a**. In another run on a larger scale, a mixture of dibromide **9** (1.820 g; 5 mmol), tetrabutylammonium hydrogen sulfate (0.678 g; 2 mmol), K_2CO_3 (13.8 g; 100 mmol), and ethyl isocyanoacetate (1.5 mL; 2.75 mmol) in CH_3CN (250 mL) was reacted in the same experimental conditions and workup as above. The crude product was submitted to direct acidic hydrolysis with 10 N HCl (10 mL) in CH_2Cl_2 (75 mL) and abs EtOH (175 mL), also as above. The crude product obtained after extraction was reacted with Boc_2O (1.816 g; 8.33 mmol) in CH_3CN (25 mL) and CH_2Cl_2 (50 mL) at room temperature for 4 days, then diluted with CH_2Cl_2 (ca. 200 mL), filtered through Celite, and evaporated in vacuo. The crude product was dissolved in CH_2Cl_2 (75 mL) and chromatographed on a 3×42 cm column of silica gel with eluant (III) to afford 0.373 g (18%) of pure **12a**. Several other runs in the same experimental conditions and workup gave a similar yield. The pure compound **12a** from several runs (0.514 g) was dissolved in CH_2Cl_2 (25 mL) and EtOAc (5 mL) was added. The clear yellow solution was concentrated under a slight vacuum at 60 °C to ca. 4 mL where crystallization started to occur. Methanol (10 mL) was added and the mixture was concentrated again to ca. 2 mL, then cooled at +4 °C for 3 h. More MeOH (10 mL) was added, the crystals were filtered, abundantly washed with MeOH, and air dried to give 0.418 g (81%) of analytically pure **12a** as a yellow solid. Another similar crystallization process starting from pure **12a** (0.722 g) gave 0.598 g of analytically pure crystals. Mp 221 °C. R_f 0.30 (III); 0.90 (V). ^{1}H NMR ($CDCl_3$): δ 8.33 [s, 2H, ArH], 7.99–7.96 [m, 2H, ArH], 7.79 [s, 2H, ArH], 7.47–7.43 [m, 2H, ArH], 5.11 [br s, 1H, NH], 4.27 [q, J=7.1 Hz, 2H, CH_2 OEt], 3.77 and 3.42 [d, J=16.9 Hz, 2H, $ArCH_2$ and br d, J=16.7 Hz, 2H, $ArCH_2$], 1.45 [s, 9H, CH_3 Boc], 1.29 [t, J=7.1 Hz, 3H, CH_3 OEt]. ^{13}C NMR ($CDCl_3$): δ 173.2 [C=O], 155.0 [C=O Boc], 139.0, 131.6, 131.4, 128.0, 125.6, 125.1, 122.6 [C_{Ar}], 80.1 [C–O Boc], 66.4 [C^{α}], 61.6 [CH_2 OEt], 43.1 [$ArCH_2$], 28.2 [CH_3 Boc], 14.1 [CH_3 OEt]. ESI$^+$ MS m/z (relative intensity): 428.3 (100) $[M+Na]^+$, 833.5 (58) $[2M+Na]^+$. HRMS (FAB$^+$). Calcd $[M]^+$ for $C_{25}H_{27}NO_4$: 405.1940. Found: 405.1953. Calcd $[M+H]^+$ for $C_{25}H_{28}NO_4$: 406.2018. Found: 406.2014. Anal. Calcd for $C_{25}H_{27}NO_4$ (405.474): C, 74.05; H, 6.71; N, 3.45. Found: C, 73.87; H, 6.95; N, 3.71.

3.14. 2-(9-Fluorenylmethoxycarbonylamino)-2,3-dihydro-1*H*-cyclopenta[*b*]anthracene-2-carboxylic acid *tert*-butyl ester (Fmoc–antAib–O^{t}Bu 12b)

(a) A solution of the α-amino ester **11b** (0.120 g; 0.36 mmol) and Fmoc–OSu (0.146 g; 0.43 mmol) in CH_2Cl_2 (10 mL) was magnetically stirred at room temperature for 48 h, and then diluted with CH_2Cl_2 (100 mL). The CH_2Cl_2 solution was washed with 0.5 N HCl (100 mL), 5% $NaHCO_3$ (100 mL) and then H_2O (2×100 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo. The crude product was chromatographed on a column of silica gel with eluant (I) to afford 0.172 g (86%) of pure **12b** as a pale yellow solid.

(b) In other experiments on a larger scale, without isolation/characterization of the isonitrile and α-amino ester intermediates, a mixture of dibromide **9** (3.64 g; 10 mmol), tetrabutylammonium hydrogen sulfate (1.53 g; 4.5 mmol), Cs_2CO_3 (3.26 g; 50 mmol), and *tert*-butyl isocyanoacetate (1.75 mL; 12 mmol) in CH_3CN (250 mL) was reacted in the same experimental conditions and workup as in Section 3.9. The crude product (containing the isonitrile **10b**) was submitted to direct acidic hydrolysis with 10 N HCl (25 drops) in CH_2Cl_2 (150 mL) and abs EtOH (75 mL), in the same experimental conditions and workup as in Section 3.11. At this stage, the crude product (containing the α-amino ester **11b**) was combined with the crude products obtained from two other similar runs in which 2.27 g (6.24 mmol) and 1.14 g (3.12 mmol) of dibromide **9** were engaged, and the mixture was purified by column chromatography on silica gel with eluant (V) to afford 1.81 g (28%) of α-amino ester **11b** containing minor impurities. This sample was treated with Fmoc–OSu (3.10 g; 9.20 mmol) in CH_2Cl_2 (400 mL) in the same experimental conditions and workup as above in Section 3.13 (a). The crude product was chromatographed on a column of silica gel with eluant (I) to afford 1.84 g (17% overall from **9**) of pure **12b** as a pale yellow solid. Mp 243 °C. R_f 0.63 (III). ^{1}H NMR ($CDCl_3$): δ 8.35 [s, 2H, ArH], 8.01–7.96 [m, 2H, ArH], 7.79 [br s, 2H, ArH], 7.73 [br d, J∼7.5 Hz, 2H, ArH Fmoc], 7.58 [d, J=7.0 Hz, 2H, ArH Fmoc], 7.48–7.43 [m, 2H, ArH], 7.38–7.24 [m, 4H, ArH Fmoc], 5.40 [br s, 1H, NH], 4.41 [br m, 2H, CH_2 Fmoc], 4.22 [m (t-like), J∼6.7 Hz, 1H, CH Fmoc], 3.72 and 3.52 [br d, J∼17.3 Hz, 2H, $ArCH_2$ and br d, J∼17.3 Hz, 2H, $ArCH_2$], 1.41 [s, 9H, CH_3 O^{t}Bu]. ^{13}C NMR ($CDCl_3$): δ 172.3 [C=O], 155.7 [C=O Fmoc], 144.1, 141.5, 139.6, 131.8, 131.6, 128.2, 127.9, 127.3, 125.8, 125.3, 125.2, 122.6, 120.2 [C_{Ar}], 82.2 [O–C O^{t}Bu], 67.4 [CH_2 Fmoc], 66.9 [C^{α}], 47.4 [CH Fmoc], 43.1 [$ArCH_2$], 28.0 [CH_3 O^{t}Bu]. HRMS (FAB$^+$). Calcd $[M+Na]^+$ for $C_{37}H_{33}NO_4Na$: 578.2307. Found: 578.2313. Anal. Calcd for $C_{37}H_{33}NO_4$ (555.642): C, 79.97; H, 5.99; N, 2.52. Found: C, 80.45; H, 6.12; N, 2.42.

3.15. 2-*tert*-Butyloxycarbonylamino-2,3-dihydro-1*H*-cyclopenta[*b*]anthracene-2-carboxylic acid (Boc–antAib–OH 13a)

To a solution of **12a** (0.203 g; 0.5 mmol) in THF (20 mL) and MeOH (40 mL) was added a solution of 1 N NaOH (20 mL). The reaction mixture was magnetically stirred at room temperature for 24 h, cooled by addition of ice, acidified by addition of 0.5 N HCl (50 mL), and extracted with CH_2Cl_2 (2×250 mL). The CH_2Cl_2 solution was washed with H_2O (250 mL), filtered, and evaporated in vacuo. The residue was dried by repeated evaporation in vacuo at 40 °C after addition of methanol to afford 0.183 g (96%) of pure **13a** as a pale yellow crystalline powder. Several similar runs gave 89–96% yields. Mp >300 °C. R_f 0.03 (V); 0.10 (VI). ^{1}H NMR ($CDCl_3$/CD_3OD 4:1): δ 8.22 [s, 2H, ArH], 7.87 [m, 2H, ArH], 7.68 [s, 2H, ArH], 7.34 [m, 2H, ArH], 3.66 and 3.32 [d, J∼17.0 Hz, 2H and d, J∼17.0 Hz, 2H, Ar–CH_2], 1.32 [s, 9H, CH_3 Boc]. ^{13}C NMR ($CDCl_3$/CD_3OD 4:1): δ 175.5 [C=O], 155.6 [C=O Boc], 139.0, 131.2, 131.0, 127.5, 125.0, 124.5, 121.9 [C_{Ar}], 79.5 [C–O Boc], 65.7 [C^{α}], 42.4 [Ar–CH_2], 27.5 [CH_3 Boc]. ESI$^+$ MS m/z (relative intensity): 400.2 (100) $[M+Na]^+$, 416.2 (18) $[M+K]^+$, 777.4 (97) $[2M+Na]^+$. HRMS (FAB$^+$). Calcd $[M]^+$ for $C_{23}H_{23}NO_4$: 377.1627. Found: 377.1629. Calcd

$[M+H]^+$ for $C_{23}H_{24}NO_4$: 378.1705. Found: 378.1709. Anal. Calcd for $C_{23}H_{23}NO_4$ (377.422): C, 73.19; H, 6.14; N, 3.71. Found: C, 72.72; H, 6.55; N, 3.74.

3.16. 2-(9-Fluorenylmethoxycarbonylamino)-2,3-dihydro-1*H*-cyclopenta[*b*]anthracene-2-carboxylic acid (Fmoc–antAib–OH 13b)

To an ice-cold solution of **12b** (0.290 g; 0.52 mmol) in CH_2Cl_2 (10 mL) was added TFA (5 mL). The solution was magnetically stirred from 0 °C to room temperature for 4 h and then evaporated in vacuo. The solid residue was repeatedly taken up in CH_2Cl_2 and the suspension evaporated in vacuo at 40 °C. The crude product was dissolved in CH_2Cl_2 (150 mL necessary) with heating, the warm solution was filtered and then concentrated to ca. 50 mL under reduced pressure at 40 °C resulting in crystallization. Cyclohexane (ca. 30 mL) was added and the mixture was concentrated again to ca. 25 mL under reduced pressure. The crystals were triturated at room temperature, filtered, abundantly washed with a solution of cyclohexane/CH_2Cl_2 ca. 95:5 (v/v), and air dried to afford 0.240 g (92%) of pure **13b** as a pale yellow crystalline powder. Mp 244–246 °C. R_f 0.08 (VII), 0.42 (VIII). ^{1}H NMR ($CDCl_3$/CD_3OD 9:1 v/v): δ 8.29 [s, 2H, ArH], 7.94–7.91 [m, 2H, ArH], 7.75 [br s, 2H, ArH], 7.66 [br d, J∼7.3 Hz, 2H, ArH Fmoc], 7.51 [d, J=7.3 Hz, 2H, ArH Fmoc], 7.41–7.37 [m, 2H, ArH], 7.31 [m (t-like), J∼7.4 Hz, 2H, ArH Fmoc], 7.21 [m (t-like), J∼7.4 Hz, 2H, ArH Fmoc], 4.33 [br m, 2H, CH_2 Fmoc], 4.15 [m (t-like), J∼6.7 Hz, 1H, CH Fmoc], 3.74 and 3.46 [br d, J∼16.2 Hz, 2H, $ArCH_2$ and br d, J∼16.9 Hz, 2H, $ArCH_2$]. ^{13}C NMR ($CDCl_3$/CD_3OD 9:1 v/v): δ 175.2 [C=O], 156.0 [C=O Fmoc], 143.6, 141.1, 139.0, 131.5, 131.3, 127.9, 127.5, 126.9, 125.5, 125.0, 122.4, 119.8 [C_{Ar}], 67.1 [C^{α}], 66.6 [CH_2 Fmoc], 47.0 [CH Fmoc], 42.7 [$ArCH_2$]. ESI$^+$ MS *m/z* (relative intensity): 522.3 (100) $[M+Na]^+$, 538.3 (27) $[M+K]^+$. Anal. Calcd for $C_{33}H_{25}NO_4\cdot 0.5H_2O$ (508.546): C, 77.93; H, 5.15; N, 2.75. Found: C, 78.13; H, 5.39; N, 2.55.

3.17. Boc–antAib–L-Ala–OMe (14a)

To a suspension of **13a** (0.133 g; 0.35 mmol), HCl·L-H–Ala–OMe (0.148 g; 1.06 mmol), and HOAt (0.097 g; 0.71 mmol) in THF (3 mL) and CH_2Cl_2 (3 mL) was added NMM (0.160 mL; 1.45 mmol) and then EDC (0.102 g; 0.53 mmol). The reaction mixture was stirred at room temperature for 44 h and evaporated to dryness in vacuo. The residue was solubilized in several portions of EtOAc (total of ca. 150 mL) and 0.5 N HCl (total of ca. 100 mL) with stirring, and the solutions combined and transferred into a separatory funnel. The decanted organic phase was extracted again with 0.5 N HCl (2×50 mL), and then H_2O (100 mL), 5% $NaHCO_3$ (50 mL), and H_2O (2×100 mL), dried ($MgSO_4$), filtered and evaporated in vacuo. The crude product was chromatographed on a preparative TLC plate of silica gel with eluant (II) to afford 0.082 g (50%) of pure **14a** as a solid. Another run under similar experimental conditions and starting from 0.062 g (0.16 mmol) of **13a** gave 0.047 g (62%) of pure **14a**. Mp 226–228 °C. R_f 0.40 (II). ^{1}H NMR ($CDCl_3$): δ 8.33 [s, 2H, ArH], 7.99–7.96 [m, 2H, ArH], 7.81 [s, 1H, ArH], 7.79 [s, 1H, ArH], 7.46–7.43 [m, 2H, ArH], 7.15 [br m, 1H, NH Ala], 5.22 [s, 1H, NH antAib], 4.64 [dq, J∼7.2 Hz and 7.2 Hz, 1H, CH^{α} Ala], 3.85 [d, J∼16.7 Hz, 1H, $ArCH_A$ antAib], 3.76 [d (partly masked), J∼16.7 Hz, 1H, $ArC'H_A$ antAib], 3.52–3.36 [br m, 2H, $ArCH_B$ and $ArC'H_B$ antAib], 3.74 [s, 3H, OCH_3], 1.44 [s, 9H, CH_3 Boc], 1.43 [d (partly masked), J∼7.1 Hz, 3H, CH_3 Ala]. ^{13}C NMR ($CDCl_3$): δ 173.3, 172.3 [C=O Ala and antAib], 154.9 [C=O Boc], 139.1, 131.6, 131.4, 128.0, 125.6, 125.1, 122.9, 122.8 [C_{Ar}], 80.6 [C–O Boc], 67.5 [C^{α} antAib], 52.4 [OCH_3], 48.3 [C^{α} Ala], 42.6, 42.1 [$ArCH_2$ antAib], 28.2 [CH_3 Boc], 18.3 [CH_3 Ala]. $[\alpha]_{589}^{25}$ −16.7, $[\alpha]_{578}^{25}$ −17.3, $[\alpha]_{546}^{25}$ −19.3, $[\alpha]_{436}^{25}$ −34.0 (*c* 0.2; EtOAc). ESI$^+$ MS *m/z* (relative intensity): 485.3 (100) $[M+Na]^+$, 385.3 (40) $[M+Na\text{-}Boc]^+$, 947.6 (44) $[2M+Na]^+$. Anal. Calcd for $C_{27}H_{30}N_2O_5\cdot 0.5H_2O$ (471.534): C, 68.77; H, 6.63; N, 5.94. Found: C, 69.15; H, 6.85; N, 5.93.

3.18. Fmoc–L-Ala–antAib–L-Ala–OMe (15a)

To an ice-cold solution of dipeptide **14a** (0.109 g; 0.23 mmol) in CH_2Cl_2 (6 mL) was added TFA (2 mL). The solution was stirred at 0 °C for 3 h and evaporated in vacuo at 25 °C. The residue was repeatedly dissolved in CH_2Cl_2 and the solution evaporated in vacuo to yield crude TFA·H–antAib–L-Ala–OMe (not characterized). To this product was added THF (5 mL), the mixture was magnetically stirred at 0 °C for 10 min and DIEA (0.180 mL; 1.03 mmol) was added, followed by solid Fmoc–L-Ala–NCA (0.238 g; 0.71 mmol). The solution was stirred at room temperature for 66 h and evaporated in vacuo at 40 °C. The residue was dissolved in EtOAc by portions and the solutions combined and transferred to a separatory funnel. The organic solution (ca. 150 mL of EtOAc) was washed with 0.5 N HCl (2×75 mL), H_2O (2×100 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo. The crude product was chromatographed on a preparative TLC plate of silica gel with eluant (II) (three consecutive elutions) to afford 0.128 g (83%) of pure **15a** as a solid. Mp 210–212 °C. R_f 0.15 (II); 0.55 (VII). ^{1}H NMR ($CDCl_3$): δ 8.22 [br s, 1H, ArH antAib], 8.15 [s, 1H, ArH antAib], 7.92–7.85 [m, 2H, ArH antAib], 7.73 [br m, 2H, ArH Fmoc], 7.70 [s, 1H, ArH antAib], 7.66 [s, 1H, ArH antAib], 7.43–7.34 [m, 6H, 2 ArH antAib and 4 ArH Fmoc], 7.29 [m (partly masked), 1H, NH Ala–OMe], 7.30–7.22 [m, 2H, ArH Fmoc], 6.95 [br s, 1H, NH antAib], 5.34 [d, J∼6.4 Hz, 1H, NH Ala–Fmoc], 4.58 [dq, J∼7.2 Hz and 7.2 Hz, 1H, CH^{α} Ala–OMe], 4.18 [m (d-like), J∼6.9 Hz, 2H, CH_2O Fmoc], 4.06 [br dq, J∼6.8 Hz and 6.8 Hz, 1H, CH^{α} Ala–Fmoc], 3.95 [br m, t-like), 1H, Ar–CH Fmoc], 3.83 [d, J∼16.8 Hz, 1H, $ArCH_A$ antAib], 3.72 [d, J∼17.1 Hz, 1H, $ArC'H_A$ antAib], 3.60 [d, J∼16.9 Hz, 1H, $ArCH_B$ antAib], 3.47 [d, J∼16.7 Hz, 1H, $ArC'H_B$ antAib], 3.66 [s, 3H, OCH_3], 1.38 [d, J=7.1 Hz, 3H, CH_3 Ala–OMe], 1.30 [d, J=6.9 Hz, 3H, CH_3 Ala–Fmoc]. ^{13}C NMR ($CDCl_3$): δ 173.3, 171.8 [C=O Ala–OMe, Ala–Fmoc and antAib], 156.5 [C=O Fmoc], 143.6, 143.4, 141.1, 138.5, 131.4, 131.3, 128.7, 128.0, 127.7, 127.0, 126.9, 125.6, 125.1, 124.9, 122.8, 122.7, 119.9 [C_{Ar}], 67.8 [C^{α} antAib], 67.1 [CH_2–O Fmoc], 52.3 [OCH_3], 50.9, 48.5 [C^{α} Ala–OMe and Ala–Fmoc], 46.8 [Ar–CH Fmoc], 42.7, 41.5 [$ArCH_2$ and $ArC'H_2$ antAib], 17.8, 17.4 [CH_3 Ala–OMe and Ala–Fmoc]. $[\alpha]_{589}^{25}$ −39.4, $[\alpha]_{578}^{25}$ −40.4, $[\alpha]_{546}^{25}$ −45.9, $[\alpha]_{436}^{25}$ −84.9 (*c* 0.11, $CHCl_3$). ESI$^+$ MS *m/z* (relative intensity): 678.3 (100) $[M+Na]^+$.

Anal. Calcd for $C_{40}H_{37}N_3O_6\cdot 0.5H_2O$ (664.728): C, 72.27; H, 5.76; N, 6.32. Found: C, 72.46; H, 5.83; N, 5.91.

3.19. Synthesis of Boc–Aib–antAib–L-Ala–OMe (16a) and *cyclo*-[antAib–L-Ala] (17a)

The dipeptide **14a** (0.043 g; 0.09 mmol) was N^{α}-deprotected in CH_2Cl_2 (3 mL) and TFA (1 mL) as above (Section 3.18). To the obtained crude TFA·H–antAib–L-Ala–OMe was added THF (3 mL), the mixture was magnetically stirred at 0 °C for 10 min and DIEA (0.065 mL; 0.37 mmol) was added, followed by solid Boc–Aib–NCA (0.085 g; 0.37 mmol). The solution was stirred at 60 °C for 21 h and evaporated in vacuo at 40 °C. The residue was stirred in the presence of EtOAc (10 mL). The residual solid was filtered, washed with EtOAc (2×10 mL), then 0.5 N HCl (2×10 mL), then H_2O (2×10 mL), and air dried to afford 0.015 g (48%) of pure **17a** as a solid, sparingly soluble in the usual organic solvents. The filtrate (mixture of EtOAc and aqueous HCl) was diluted with more EtOAc (100 mL) and transferred to a separatory funnel. The separated organic phase was washed with 0.5 N HCl (2×50 mL), H_2O (2×100 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo. The crude product was chromatographed on a preparative TLC plate of silica gel with eluant (II) to afford 0.007 g (14%) of pure **16a** as a solid.

3.20. Boc–Aib–antAib–L-Ala–OMe (16a)

Mp 180–185 °C. R_f 0.25 (II). ^{1}H NMR ($CDCl_3$): δ 8.32 [s, 2H, ArH antAib], 7.99–7.96 [m, 2H, ArH antAib], 7.78 [m (s-like), 3H, 2 ArH antAib and NH Ala], 7.46–7.43 [m, 2H, ArH antAib], 6.65 [br s, 1H, NH antAib], 4.79 [s, 1H, NH Aib], 4.61 [dq, J∼7.1 Hz and 7.1 Hz, 1H, CH^{α} Ala], 4.02 [d, J∼17.0 Hz, 1H, $ArCH_A$ antAib], 3.80 [d, J∼17.0 Hz, 1H, $ArC'H_A$ antAib], 3.54 [d, J∼17.0 Hz, 1H, $ArCH_B$ antAib], 3.23 [d, J∼17.0 Hz, 1H, $ArC'H_B$ antAib], 3.73 [s, 3H, OCH_3], 1.47 [d (partly masked), J∼6.8 Hz, 3H, CH_3 Ala], 1.45 [s, 3H, CH_3 Aib], 1.42 [s, 12H, CH_3 Boc and CH_3 Aib]. ^{13}C NMR ($CDCl_3$): δ 173.5, 172.1 [C═O Ala, antAib and Aib], 155.2 [C═O Boc], 138.7, 131.5, 128.0, 125.6, 125.1, 122.7 [C_{Ar}], 81.2 [C–O Boc], 67.6 [C^{α} antAib], 57.1 [C^{α} Aib], 52.1 [OCH_3], 48.6 [C^{α} Ala], 41.2 [$ArCH_2$ antAib], 28.2 [CH_3 Boc], 26.3, 22.7 [CH_3 Aib], 17.2 [CH_3 Ala]. $[\alpha]^{25}_{589}$ −35.6, $[\alpha]^{25}_{578}$ −36.9, $[\alpha]^{25}_{546}$ −41.9, $[\alpha]^{25}_{436}$ −73.3 (*c* 0.12, $CHCl_3$). ESI$^+$ MS *m*/*z* (relative intensity): 570.4 (100) $[M+Na]^+$. Anal. Calcd for $C_{31}H_{37}N_3O_6$ (547.630): C, 67.99; H, 6.81. Found: C, 68.07; H, 7.46.

3.21. *cyclo*-[antAib–L-Ala] (17a)

Mp >300 °C. ^{1}H NMR (CD_3SOCD_3): δ 8.61 [s, 1H, NH antAib], 8.45 [s, 2H, ArH antAib], 8.27 [br s, 1H, NH Ala], 8.05–8.01 [m, 2H, ArH antAib], 7.84 [s, 1H, ArH antAib], 7.83 [s, 1H, ArH antAib], 7.48–7.44 [m, 2H, ArH antAib], 4.61 [m (q-like), J∼6.9 Hz, 1H, CH^{α} Ala], 3.72 [d, J∼17.0 Hz, 1H, $ArCH_A$ antAib], 3.61 [d, J∼17.0 Hz, 1H, $ArC'H_A$ antAib], 3.32 [d (partly masked), 1H, $ArCH_B$ antAib], 3.25 [d, J∼17.0 Hz, 1H, $ArC'H_B$ antAib], 1.34 [d, J∼6.9 Hz, 3H, CH_3 Ala]. ESI$^+$ MS *m*/*z* (relative intensity): 331.2 (100) $[M+H]^+$, 661.3 (35) $[2M+H]^+$. Anal. Calcd for $C_{21}H_{18}N_2O_2\cdot 0.3H_2O$ (335.775): C, 75.11; H, 5.58; N, 8.34. Found: C, 75.14; H, 5.58; N, 8.47.

3.22. Ultraviolet absorption and fluorescence

The electronic absorption spectra were recorded between 300 and 400 nm using a Shimadzu model UV-2501 PC spectrophotometer. The fluorescence spectra were measured between 370 and 500 nm (upon excitation at either 359 nm or 378 nm) using a Perkin Elmer model LS-50B spectrofluorimeter. Ethanol (99.8%) was purchased from Fluka.

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Tetrahedron 62 (2006) 6214–6221
Tetrahedron

Comparative studies for selective deprotection of the *N*-arylideneamino moiety from heterocyclic amides: kinetic and theoretical studies. Part 2☆

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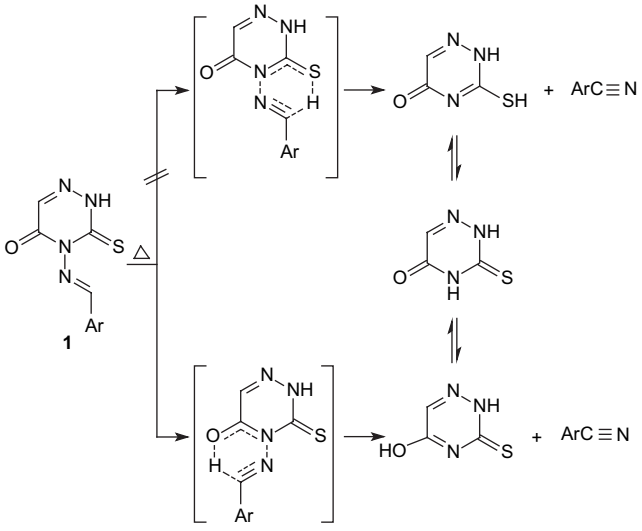
Received 22 February 2006; revised 30 March 2006; accepted 20 April 2006
Available online 15 May 2006

Abstract—4-Benzylideneamino-1,2,4-triazine-3,5(2*H*,4*H*)-diones (**2–5**), 6-styryl-1,2,4-triazine-3,5(2*H*,4*H*)-dione (**6**), and 6-styryl-2,3-dihydro-3-thioxo-1,2,4-triazin-5(4*H*)-one (**7**) were synthesized and pyrolyzed in the gas phase. The kinetic effect of changing the substituent on the triazine ring from hydrogen to methyl, phenyl, and styryl was measured. Analyses of the pyrolyzates of **2–5** showed the elimination products to be benzonitrile and the triazine fragment, while the pyrolyzates of **6** and **7** reveal the formation of *cis*- and *trans*-cinnamonitriles. Theoretical study of the pyrolysis reactions of **2–5** using an ab initio SCF method was investigated.

1. Introduction

Pyrolytic deprotection of 4-arylideneamino-3(2*H*)-thioxo-1,2,4-triazin-5(4*H*)-one (**1**) was carried out.[2] Based on the product analysis together with kinetic results and in the absence of theoretical studies two mechanistic pathways were suggested (Scheme 1). Theoretical studies showed that it is the carbonyl and not the thione bond that attacks the hydrogen atom of the arylideneamino groups.



Scheme 1.

In this study we have extended the investigation to include 4-benzylideneamino-1,2,4-triazine-3,5-dione derivatives **2–5**, 6-styryl-1,2,4-triazine-3,5(2*H*,4*H*)-dione (**6**), and 6-styryl-2,3-dihydro-3-thioxo-1,2,4-triazin-5(4*H*)-one (**7**).

2. Results and discussion

2.1. Product analysis

The substrates **2–7** were prepared and fully characterized by NMR and MS, as described in the Section 3. The products of static as well as flash vacuum pyrolysis of each substrate were analyzed; the constituents of pyrolyzate from **2–5** were ascertained to be benzonitrile[3] together with the heterocyclic fragments **10a**, **10b**, **10c**, and **6** while FVP of **6** and **7** results in *cis*- and *trans*-cinnamonitrile (Table 1).

2.2. Kinetic studies

For each substrate, first-order rate coefficients were obtained at regular temperature intervals. Each rate constant is an average of at least three independent measurements in agreement to within ±2%. The reactions for which the kinetic data were obtained have been ascertained to be homogeneous, unimolecular, non-catalytic, and non-radical processes.[4,5] Arrhenius plots of the data using first-order

☆ See Ref. 1.

Keywords: Arylideneamino; Pyrolysis; Kinetics; Reaction mechanism.

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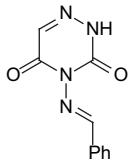
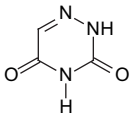
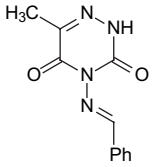
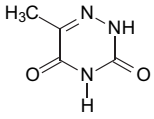
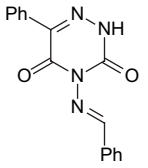
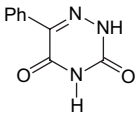
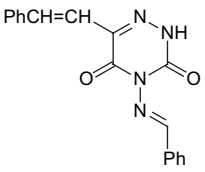
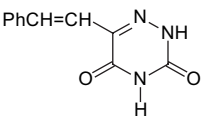
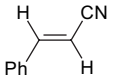
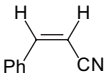
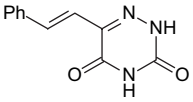
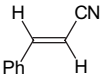
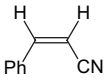
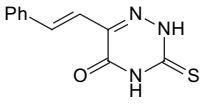
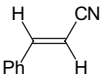
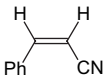
doi:10.1016/j.tet.2006.04.054

Table 1. Pyrolysis products and % yield of compounds **1–6** under static/FVP pyrolysis

Compound	Substrate	% Yield (static/FVP)			
		9	**10a–c, 5, 13**	**11**	**12**
1		Ph—C≡N (42/43)[3]	 **10a** (35/36)[12]	—	—
2		Ph—C≡N (32/48)[3]	 **10b** (53/39)[12]	—	—
3		Ph—C≡N (47/28)[3]	 **10c** (45/46)[12]	—	—
4		Ph—C≡N (34/38)[3]	 **5** (54/24)[10]	 **11** (19/13)[17]	 **12** (17/10)[17]
5		—	—	 **11** (18/9)[17]	 **12** (14/12)[17]
6		—	HS—CN **13**[18]	 **11** (38/23)[17]	 **12** (25/15)[17]

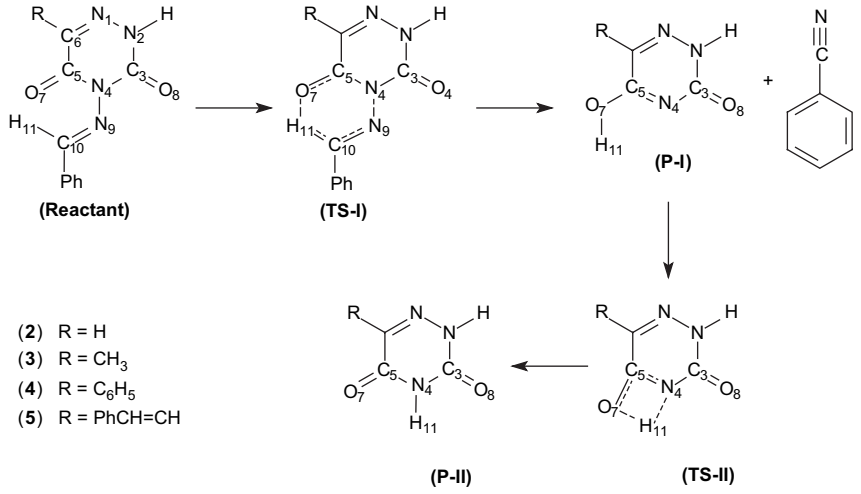
rate equation: $\log k\ (s^{-1}) = \log A - E_a\ kJ\ mol^{-1}\ (2.303RT)^{-1}$ were strictly linear over ≥95% reaction with correlation coefficient in the range 0.99±0.005. The log A, E_a values and the first-order rate constants of the six compounds under investigation are given in Table 2.

The main features of the kinetic results are as follows:

- Two alternative pathways could be postulated for the extrusion of PhC≡N from **2–5**. Pathway (A) involves nucleophilic attack of the C^5=O group on the hydrogen of the arylidene moiety (Scheme 2) while pathway (B) involves the nucleophilic attack of the C^3=O on the same hydrogen atom (Scheme 3). To make the choice between (A) and (B), we have examined both pathways theoretically for compounds **2–5**.
- Examination of the free energy of the two suggested pyrolytic pathways of compounds **2–5** shows that:
  1. For hydrogen, methyl, phenyl, and styryl substituted compounds (**2–5**), the final product P-II can be formed from either pathway **A** or **B** (Fig. 1).
  2. With the exception of compound **5**, there is no considerable difference between the TS-I in the two suggested pathways.
  3. For each compound, formation of P-I from TS-I requires relatively lower energy via pathway B than that in pathway A. This means that the reaction preferentially involves C^3=O rather than C^5=O in the transition state.
- The overall reactivities of compounds **2–5** are the result of electronic synergism involving bonds a, b, and c, and the stabilizing influences associated with the substituents (R) (Scheme 4).
- The reactivity of compound **3** exceeds that of compound **2** by a factor of 1.39×10^4 (Scheme 4), this rate enhancement is attributed to the influence that the methyl substituent has on the delocalization of the lone pair of electrons on N^2 onto the carbonyl oxygen atom of C^3=O group involved in the reaction pathway. This causes an increase in the protophilicity of the bond a, this effect of which the delocalization of this lone pair of electrons on N^2 is exerting is further revealed when

Table 2. Rate coefficients (k/s^{-1}), Arrhenius parameters of compounds **1–6**

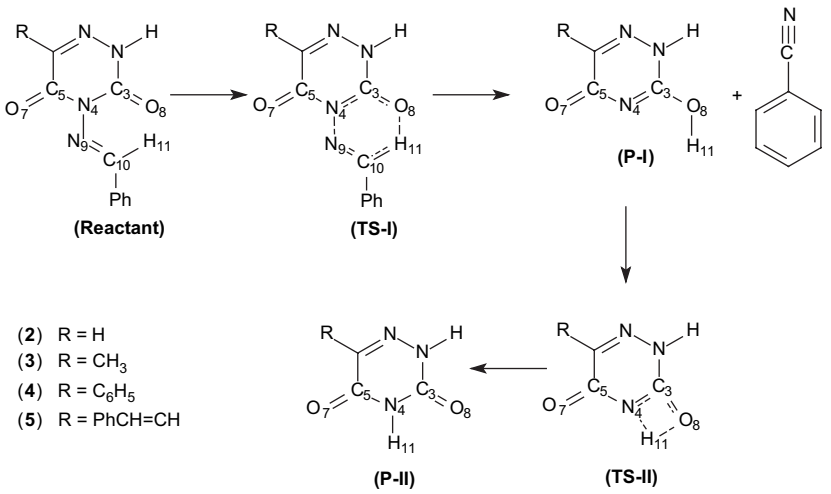
Compound	T/K	$10^4 k/s^{-1}$	$\log A/s^{-1}$	E_a/k J mol^{-1}	k_{500K}/s^{-1}
1	548.35	1.455	9.39±0.29	140.58±3.24	7.55×10^{-6}
	588.45	10.66			
	598.35	19.78			
	608.65	28.71			
	618.45	45.88			
2	399.45	0.790	11.35±0.68	119.36±5.44	1.05×10^{-1}
	409.55	1.942			
	419.05	4.582			
	428.85	11.23			
	444.25	26.41			
3	445.45	0.322	12.54±0.07	147.00±0.70	2.31×10^{-3}
	465.35	1.728			
	475.25	3.671			
	485.55	8.146			
	495.65	17.23			
4	456.75	1.332	6.13±0.10	88.572±1.02	9.69×10^{-4}
	471.25	2.643			
	514.55	18.07			
	519.35	20.77			
5	620.95	1.390	6.36±0.07	123.08±0.89	4.66×10^{-7}
	649.75	3.967			
	663.65	6.426			
	677.85	9.750			
	705.95	23.63			
	719.95	35.90			
6	639.85	1.125	6.02±0.33	124.25±4.41	1.56×10^{-7}
	661.55	1.947			
	683.05	3.573			
	704.75	8.391			
	726.45	17.46			
	748.10	29.84			
	770.15	46.17			



Scheme 2. Pathway A of the suggested mechanism.

the rate of compound **4** is compared with that of compound **3**.

- Changing (R) from methyl in compound **3** to phenyl in compound **4** resulted in reducing the molecular reactivity of compound **4** by a factor of 45.5, this is attributed to the competitive (opposing) delocalization of the lone pair of electrons on N^2 atom of the triazine ring by the phenyl group (Scheme 5).
- This effect explains also:
  (i) The relative reactivity factor of 304.5 of compound **4** over compound **2**.
  (ii) The comparable reactivities of compounds **4** and **5**.
- A reaction mechanism postulated in Scheme 6 is suggested for the formation of *cis*- and *trans*-cinnamonitrile from compounds **6** and **7** together with traces of HSCN from compound **7** (Table 1).



Scheme 3. Pathway B of the suggested mechanism.

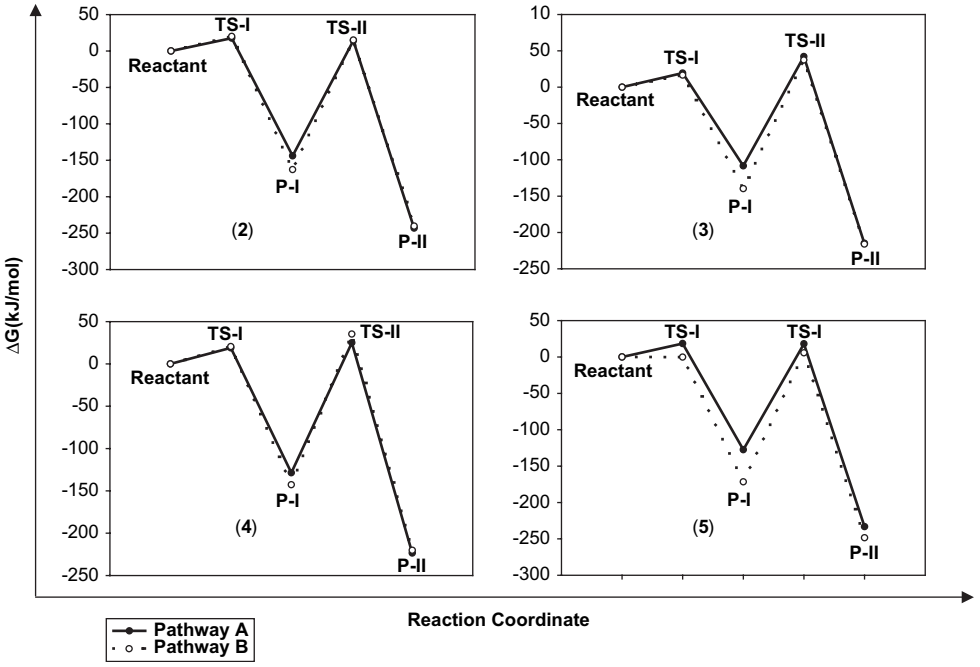
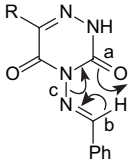


Figure 1. Free energy profile evaluated at the HF/6-31G* level for compounds **2**–**5**.



			k_{rel}
k_{500K}/s^{-1}=	(**3**) R = CH_3 1.05×10^{-1}	(**2**) R = H 7.55×10^{-6}	1.39×10^{4}
k_{500K}/s^{-1}=	(**3**) R = CH_3 1.05×10^{-1}	(**4**) R = Ph 2.30×10^{-3}	45.6
k_{500K}/s^{-1}=	(**4**) R = Ph 2.30×10^{-3}	(**2**) R = H 7.55×10^{-6}	304.6
k_{500K}/s^{-1}=	(**4**) R = Ph 2.30×10^{-3}	(**5**) R = PhCH=CH 9.70×10^{-4}	2.4

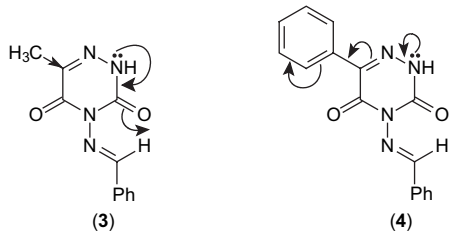
Scheme 4. Relative reactivities of compounds **2**–**5**.

This reaction mechanism accounts well for the absence of any electronic effect of changing X from oxygen in **6** to sulfur in **7**.

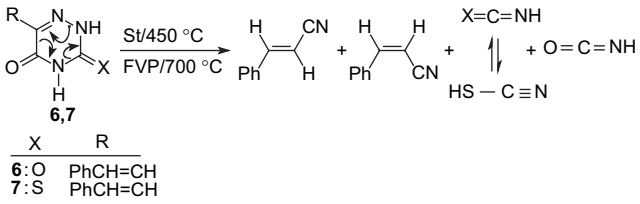
2.3. Computational studies

Ab initio molecular orbital studies on the thermolysis of four-substituted 1,2,4-triazine compounds **2**–**5**, were carried out in the gas phase using SCF method. Calculations were carried out to explore the nature of the reaction mechanism of the unimolecular decomposition of the studied reaction. Two competitive reaction pathways have been studied. Calculations have been performed using TITAN and SPARTAN packages.[6,7]

The geometric parameters of the reactants, the transition states (TS), and the products of the four studied reactions were fully optimized at the HF/6-31G* level to obtain the



Scheme 5. Opposing delocalization of the lone pair of electrons on N^2 atom by methyl (**3**) and phenyl (**4**) groups.



Scheme 6. Product analyses of compounds **6** and **7**.

energy profiles corresponding to the studied reactions. A scaling factor[8] of 0.9135 for the zero-point vibrational energies has been used. The main distances of the optimized structure of the two pathways of the two studied reactions is represented in Table 3. Electronic energies, zero-point vibrational energies, enthalpies, and entropies, evaluated at the HF/6-31G* level of theory, for all the reactants, transition states, and product involved in the two pathways of the studied reaction are collected in Table 4. The free energy

Table 3. Main distances (Å) of the reactants, TS-I, TS-II, TS-III, TS-IV, P-I, and P-II of the two pathways of the four-substituted compounds, calculated at the HF/6-31G* level

Species	C_3–O_8	C_5–O_7	O_7–H_{11}	C_{10}–H_{11}	C_{10}–N_9	N_4–H_{11}	N_4–N_9	O_8–H_{11}
R=H								
Path A								
Reactant	1.188	1.191	2.614	1.079	1.258	2.448	1.401	4.080
TS-I	1.185	1.193	2.080	1.071	1.260	2.617	1.382	4.586
P-I	1.190	1.322	0.948	—	—	3.028	—	5.271
TS-II	1.188	1.249	1.317	—	—	1.323	—	3.041
Path B								
Reactant	1.187	1.190	1.945	1.059	1.258	2.501	1.401	1.945
TS-I	1.195	1.184	2.078	1.071	1.260	2.629	1.382	2.078
P-I	1.316	1.191	0.953	—	—	2.281	—	0.953
TS-II	1.253	1.189	1.308	—	—	1.331	—	1.308
P-II	1.189	1.191	2.481	—	—	1.000	—	2.481
R=CH₃								
Path A								
Reactant	1.186	1.193	1.815	1.058	1.257	2.554	1.396	4.594
TS-I	1.186	1.195	2.079	1.071	1.260	2.627	1.382	4.589
P-I	1.191	1.323	0.947	—	—	3.026	—	5.262
TS-II	1.189	1.251	1.316	—	—	1.324	—	3.403
Path B								
Reactant	1.196	1.190	4.605	1.064	1.257	2.525	1.396	1.951
TS-I	1.196	1.185	4.581	1.071	1.259	2.628	1.382	2.072
P-I	1.317	1.193	4.492	—	—	2.279	—	0.953
TS-II	1.254	1.190	3.411	—	—	1.329	—	1.308
P-II	1.192	1.191	2.486	—	—	0.999	—	2.481
R=Phenyl								
Path A								
Reactant	1.181	1.180	1.744	1.038	1.258	2.542	1.403	4.601
TS-I	1.186	1.193	2.086	1.070	1.259	2.647	1.381	4.594
P-I	1.191	1.316	0.954	—	—	2.240	—	5.256
TS-II	1.189	1.253	1.307	—	—	1.321	—	3.390
Path B								
Reactant	1.198	1.187	4.504	1.064	1.258	2.490	1.403	1.909
TS-I	1.196	1.184	4.565	1.071	1.260	2.631	1.383	2.068
P-I	1.317	1.192	4.479	—	—	2.284	—	0.953
TS-II	1.254	1.189	3.386	—	—	1.331	—	1.308
P-II	1.192	1.190	2.455	—	—	1.000	—	2.481
R=PhCH=CH								
Path A								
Reactant	1.189	1.192	2.717	1.079	1.257	2.433	1.401	3.944
TS-I	1.186	1.194	2.097	1.071	1.259	2.654	1.381	4.595
P-I	1.191	1.320	0.949	—	—	3.013	—	4.492
TS-II	1.189	1.254	1.309	—	—	1.323	—	4.348
Path B								
Reactant	1.194	1.187	4.094	1.078	1.258	2.460	1.401	2.575
TS-I	1.196	1.185	4.569	1.070	1.260	2.627	1.384	2.058
P-I	1.313	1.198	4.457	—	—	2.276	—	0.954
TS-II	1.255	1.191	3.378	—	—	1.329	—	1.309
P-II	1.193	1.191	2.448	—	—	1.000	—	2.481

Table 4. Total energy, zero-point vibrational energy (ZPE) and thermal correction to enthalpy and entropy at HF/6-31G* for the two pathways of the four studied reactions

Species	Total energy (Hartree)	ZPE (kcal/mol)	Enthalpy (kcal/mol)	Entropy
R=H				
Pathway A				
R	−750.818948	120.113	127.63	111.907
TS-I	−750.816035	120.036	127.075	107.121
P-I	−428.390523	50.859	54.482	77.457
TS-II	−428.326590	47.769	51.182	76.326
Pathway B				
R	−750.819075	120.124	127.677	112.895
TS-I	−750.816123	120.011	127.06	107.211
P-I	−428.398918	51.036	54.652	77.529
TS-II	−428.327099	47.767	51.215	76.584
P-II	−428.428879	51.339	54.934	77.531
R=CH$_3$				
Pathway A				
R	−789.847659	138.917	146.924	114.956
TS-I	−789.859391	138.634	146.654	114.164
P-I	−467.432060	69.519	74.072	84.270
TS-II	−467.370078	66.306	70.756	84.052
Pathway B				
R	−789.847626	138.807	146.823	115.701
TS-I	−789.859867	138.607	146.644	114.384
P-I	−467.443538	69.632	74.203	84.584
TS-II	−467.371790	66.363	70.777	83.687
P-II	−467.472906	69.945	74.518	84.785
R=Phenyl				
Pathway A				
R	−980.323127	174.213	182.91	119.782
TS-I	−980.366412	174.408	184.171	129.535
P-I	−657.944562	105.496	111.736	100.159
TS-II	−657.880994	102.288	108.418	108.443
Pathway B				
R	−980.360354	174.604	184.363	130.6
TS-I	−980.366976	174.413	184.187	129.724
P-I	−657.950982	105.494	111.774	100.546
TS-II	−657.879006	102.236	108.357	99.479
P-II	−657.980931	105.843	112.109	100.544
R=PhCH=CH				
Pathway A				
R	−1057.258752	197.233	208.812	145.891
TS-I	−1057.25448	197.072	208.199	141.538
P-I	−734.828937	128.141	135.741	112.727
TS-II	−734.769220	124.933	132.416	112.019
Pathway B				
R	−1057.258991	197.102	207.682	136.564
TS-I	−1057.25538	197.093	208.241	142.02
P-I	−734.839730	128.205	135.847	113.125
TS-II	−734.767977	124.949	132.443	112.211
P-II	−734.869487	128.550	136.184	113.414

profile for the decomposition process of the two studied reactions is presented in Figure 1.

Examination of the free energy of the two suggested pathways of the four-substituted reactions studied shows that, for hydrogen, methyl, and phenyl substituted compounds the final product can be formed from either of the two pathways, since there is no large differences between the transition state one (TS-I) in the two pathways. Just in styryl, it appears clearly the formation for the final product (P-II) is favored via pathway B than pathway A, since the six-membered cyclic transition state (TS-I) is of lower energy in pathway A than in pathway B. The calculated activation energies of TS-I are 18.40 and −0.25 kJ/mol for the reaction via pathway A and pathway B, respectively. Although the formation of P-I is more favored for pathway B than pathway A. The difference between the activation energies of P-I in the two pathways is 44.18 kJ/mol.

3. Experimental

3.1. Synthesis

All melting points are uncorrected. IR spectra were recorded in KBr disk using Perkin–Elmer system 2000 FTIR spectrophotometer. ^{1}H NMR spectra were recorded on a BRUKER DPX 400 MHz Superconducting NMR spectrometer. Mass spectra were measured on Vg Auto-spes-q (high resolution, high performance, tri-sector GC/MS/MS) and with LCMS using Agilent 1100 series LC/MSD with an API-ES/APCI ionization mode. Microanalyses were performed on LECO CHNS-932 Elemental Analyzer.

3.1.1. 4-Benzylidineamino-1,2,4-triazine-3,5(2*H*,4*H*)-diones (2–5).

3.1.1.1. General procedure. A mixture of the appropriate 4-amino-1,2,4-triazine derivatives (5 mmol)[9] in acetic acid (10 mL) together with benzaldehyde (0.5 g, 5 mmol) and anhydrous sodium acetate (500 mg) was heated under reflux for 2 h. The solid that precipitated after cooling was collected and recrystallized from ethanol.

3.1.1.2. 4-Benzylidineamino-1,2,4-triazine-3,5(2*H*,4*H*)-dione (2). Colorless crystals from ethanol, yield 800 mg (74%), mp 205–207 °C. LCMS *m*/*z*=217 (M+1). IR: 3221, 3111, 3052, 2941, 1714, 1665, 1612, 1570, 1401, 1341, 1230, 1097, 976, 833, 743. ^{1}H NMR ($CDCl_3$) δ 9.75 (br, 1H, NH), 8.68 (s, 1H), 7.92 (d, 2H, *J* 8), 7.60 (m, 1H), 7.54 (s, 1H), 7.48 (t, 2H, *J* 7.6). ^{13}C NMR (DMSO) δ 172.6, 154.2, 148.1, 136.3, 134.1, 132.9, 130.2, 129.9. Anal. Calcd for $C_{10}H_8N_4O_2$ (216.2): C, 55.56; H, 3.73; N, 25.91. Found: C, 55.56; H, 3.67; N, 25.87.

3.1.1.3. 4-Benzylidineamino-6-methyl-1,2,4-triazine-3,5(2*H*,4*H*)-dione (3). Colorless crystals from ethanol, yield 900 mg (78%), mp 190–192 °C. LCMS *m*/*z*=231 (M+1). IR: 3252, 3189, 2927, 1733, 1684, 1660, 1615, 1423, 1373, 1280, 1239, 795, 760, 690. ^{1}H NMR ($CDCl_3$) δ 9.73 (br, 1H, NH), 8.66 (s, 1H), 7.91 (dd, 2H, *J* 8, 1.4), 7.56 (m, 1H), 7.44 (m, 2H), 2.31 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 169.9, 153.8, 148.1, 144.5, 133.4, 132.1, 129.7, 129.2, 17.3. Anal. Calcd for $C_{11}H_{10}N_4O_2$ (230.2): C, 57.39; H, 4.38; N, 24.34. Found: C, 57.35; H, 4.40; N, 24.38.

3.1.1.4. 4-Benzylidineamino-6-phenyl-1,2,4-triazine-3,5(2*H*,4*H*)-dione (4). Colorless crystals from ethanol, yield 1.20 g (82%), mp 230–232 °C. LCMS *m*/*z*=293 (M+1). IR: 3224, 3140, 3118, 3033, 2935, 1731, 1683, 1659, 1446, 1415, 1298, 1247, 1167, 768, 685. ^{1}H NMR ($CDCl_3$) δ 9.77 (br, 1H, NH), 8.70 (s, 1H), 7.99 (dd, 2H, *J* 8, 1.4), 7.94 (d, 2H, *J* 8.0), 7.57 (m, 1H), 7.53–7.45 (m, 5H). ^{13}C NMR (DMSO) δ 173.0, 153.8, 148.0, 142.4, 134.1, 133.7, 133.0, 130.6, 130.2, 129.9, 129.2, 129.0. Anal. Calcd for $C_{16}H_{12}N_4O_2$ (292.3): C, 65.75; H, 4.14; N, 19.17. Found: C, 65.55; H, 3.96; N, 19.10.

3.1.1.5. 4-Benzylidineamino-6-styryl-1,2,4-triazine-3,5(2*H*,4*H*)-dione (5). Colorless crystals from ethanol, yield 1.30 g (82%), mp 260–262 °C. LCMS *m*/*z*=319 (M+1). IR: 3322, 3236, 3081, 2945, 1723, 1653, 1562, 1418, 1317, 1199, 972, 941, 741. ^{1}H NMR (DMSO-d_6) δ 12.90 (br, 1H, NH), 8.79 (s, 1H), 7.92 (d, 2H, *J* 7.6), 7.74 (d, 1H, *J* 16.4), 7.67–7.57 (m, 5H), 7.43–7.33 (m, 3H), 7.14 (d, 1H, *J* 16.4). ^{13}C NMR (DMSO) δ 173.0, 153.9, 147.8, 140.9, 137.0, 135.0, 134.1, 133.0, 130.2, 130.0, 129.9, 129.1, 128.2, 121.0. Anal. Calcd for $C_{18}H_{14}N_4O_2$ (318.3): C, 67.92; H, 4.43; N, 17.60. Found: C, 67.88; H, 4.41; N, 17.58.

3.1.1.6. 6-Styryl-1,2,4-triazine-3,5(2*H*,4*H*)-dione (6). Prepared as reported,[10] mp 268–270 °C (lit.[9] 266 °C). ^{1}H NMR (DMSO-d_6) δ 12.41 (br, 1H, NH), 12.09 (br, 1H, NH), 7.69 (d, 1H, *J* 16.4), 7.60 (d, 2H, *J* 7.4), 7.39 (t, 2H, *J* 7.4), 7.33 (m, 1H), 7.05 (d, 1H, *J* 16.4).

3.1.1.7. 6-Styryl-2,3-dihydro-3-thioxo-1,2,4-triazine-5(4*H*)-one (7). Prepared as reported,[10] mp 278–280 °C (lit.[11] 264 °C). ^{1}H NMR (DMSO-d_6) δ 12.63 (br, 1H, NH), 12.23 (br, 1H, NH), 7.79 (d, 1H, *J* 16.4), 7.64 (d, 2H, *J* 7.4), 7.42–7.33 (m, 3H), 7.07 (d, 1H, *J* 16.4).

3.1.1.8. 1,2,4-Triazine-3,5(2*H*,4*H*)-dione (10a). Mp 282–283 °C (lit.[12] 283 °C). ^{1}H NMR (DMSO-d_6) δ 12.32 (br, 1H, NH), 11.96 (br, 1H, NH), 7.38 (s, 1H).

3.1.1.9. 6-Methyl-1,2,4-triazine-3,5(2*H*,4*H*)-dione (10b). Mp 215–216 °C (lit.[12] 216 °C). ^{1}H NMR (DMSO-d_6) δ 11.99 (br, 1H, NH), 11.88 (br, 1H, NH), 2.50 (s, 3H, CH_3).

3.1.1.10. 6-Phenyl-1,2,4-triazine-3,5(2*H*,4*H*)-dione (10c). Mp 262–263 °C (lit.[12] 262 °C). ^{1}H NMR (DMSO-d_6) δ 12.52 (br, 1H, NH), 12.11 (br, 1H, NH), 7.82 (d, 2H, *J* 7.4, Ar–H), 7.43 (m, 3H, Ar–H).

3.2. Pyrolysis

3.2.1. Static pyrolysis. Each of the substrates **2**–**7** (200 mg) was introduced into the reaction tube (1.5×12 cm Pyrex), cooled in liquid nitrogen, sealed under vacuum (0.06 mbar) and then placed in the pyrolyzer for 15 min at a temperature deemed necessary for complete pyrolysis of the substrate. The pyrolyzate was then separated by preparative HPLC using ABZ^+ column and an eluent of suitable composition (acetonitrile and water). The collected solutions of the pyrolyzate fractions were evaporated and each fraction was subjected to ^{1}H NMR, GC–MS, and LC–MS analyses (Table 1).

3.2.2. Flash vacuum pyrolysis (FVP). The apparatus used was similar to the one, which has been described in our recent publications.[5,13] The sample was volatilized from a tube in a Büchi Kugelrohr oven through a 30×2.5 cm horizontal fused quartz tube. This was heated externally by a Carbolite Eurotherm tube furnace MTF-12/38A to a temperature of 600 °C, the temperature being monitored by a Pt/Pt–13%Rh thermocouple situated at the center of the furnace. The products were collected in a U-shaped trap cooled in liquid nitrogen. The whole system was maintained at a pressure of 10^{-2} Torr by an Edwards Model E2M5 high-capacity rotary oil pump, the pressure being measured by a Pirani gauge situated between the cold trap and the pump. Under these conditions the contact time in the hot zone was estimated to be $\cong$10 ms. The different zones of the products collected in the U-shaped trap were analyzed by ^{1}H NMR, LC–MS, and GC–MS. Relative and percent yields were determined from ^{1}H NMR. Identities of compounds obtained were confirmed by comparison of their ^{1}H NMR with data of products separated from preparative HPLC (Table 1).

3.3. Kinetic runs and data analyses

A stock solution (7 mL) was prepared by dissolving 6–10 mg of the substrate in acetonitrile as solvent to give a concentration of 1000–2000 ppm. An internal standard was then added, the amount of which was adjusted to give the desired peak area ratio of substrate to standard (2.5:1). The solvent and the internal standard are selected because both are stable under the conditions of pyrolysis, and because they do not react with either substrate or product. The internal standard used in this study is chlorobenzene, 1,3-dichlorobenzene or 1,2,4-trichlorobenzene. Each solution was filtered to ensure

that a homogeneous solution is obtained. The weight ratio of the substrate with respect to the internal standard was calculated from the ratio of the substrate peak area to the peak area of the internal standard. The kinetic rate was obtained by tracing the rate of disappearance of the substrate with respect to the internal standard as follows: an aliquot part (0.2 mL) of each solution containing the substrate and the internal standard is pipetted into the reaction tube, which is then placed in the pyrolyzer for 6 min under non-thermal conditions. A sample is then analyzed using a Waters HPLC probe (pump model 515, UV detector model 2487), or a Metrohm HPLC (pump model 7091C, and SPD 10 AV Shimadzo UV detector) and UV detector at wavelength of 256 nm, and the standardization value (A_0) was then calculated. Several HPLC measurements were obtained with an accuracy of $\geq$2%. HPLC columns used for the analysis were Supelco (25 cm length, 4.6 mm ID) ABZ$^+$, LC-8, and LC-18. The temperature of the pyrolysis (aluminum) block is then raised and held for ca. 900 s to allow approximately 10% pyrolysis to take place at this temperature. This procedure is repeated after each 10–15 °C rise in the temperature of the pyrolyzer until $\geq$90% pyrolysis takes place. The relative ratios of the integration values of the sample and the internal standard (A) at the pyrolysis temperature are then calculated. A minimum of three kinetic runs were carried out at each reaction temperature, following every 10–15 °C rise in the temperature of the pyrolyzer, in order to ensure reproducible values of (A). Treatment of the kinetic data has been detailed elsewhere.[14–16]

Acknowledgements

The support of the University of Kuwait received through research grant (SC02/03) and the facilities of Analab/SAF (GS02/01, GS03/01) is gratefully acknowledged.

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Tetrahedron 62 (2006) 6222–6227
Tetrahedron

Synthesis of 1,4-dihydro-2-methyl-4-oxo-nicotinic acid: Ochiai's route failed

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Received 13 February 2006; revised 11 April 2006; accepted 20 April 2006
Available online 15 May 2006

Abstract—The synthesis of 1,4-dihydro-2-methyl- and 1,4-dihydro-1,2-dimethyl-4-oxo-nicotinic acids was accomplished following a route other than Ochiai's procedure, which yielded the isomer 1,6-dihydro-2-methyl-6-oxo-nicotinic acid ethyl ester, and not the 4-oxo-derivative, as reported. Analytical data confirmed the identity of the two isomer oxo-nicotinic acids. UV–vis and potentiometric preliminary data showed that Al(III) does not form complexes with 1,6-dihydro-2-methyl-6-oxo-nicotinic acid ethyl ester in solution, as expected, but with 1,4-dihydro-2-methyl-4-oxo-nicotinic acid.

1. Introduction

Therapy for metal overload pathologies usually involves administration of suitable chelating agents to remove the metal from the body selectively. Regarding aluminum(III) and iron(III), medical research constantly emphasizes the need for new safe, efficient, and orally effective chelators.[1–3]

Hydroxypyridinecarboxyl acids are a class of compounds, which have been considered for metal chelation therapy. Their structure suggests good binding capacity toward aluminum(III) and iron(III), and interactions between 2,3 and 3,2- and 3,4 and 4,3-hydroxypyridinecarboxyl acids with aluminum(III) and iron(III) have been studied.[4,5] The thermodynamic data and chelation efficiencies of these compounds are such that they cannot be considered in aluminum(III) chelation therapy. Synthesis of more lipophilic compounds was indicated and thus, in analogy with deferiprone or 1,2-dimethyl-3-hydroxy-pyridin-4-one, an effective oral chelation therapy, monomethyl- and dimethyl-derivatives of hydroxypyridinecarboxyl acids, was designed. Among these acids, new 1,4-dihydro-2-methyl-4-oxo-nicotinic acid **7** and the corresponding *N*-methyl derivative **8** were proposed for synthesis. At first, we adopted Ochiai's procedure, the only one reported in the literature,[6] which would have allowed us to prepare the methyl ester of the desired hydroxypyridinecarboxyl acid by only one reaction between aminomethylenemalonate and ethyl acetoacetate. However, in all conditions applied, we obtained the isomer 1,6-dihydro-2-methyl-6-oxo-nicotinic acid ethyl ester **1**. In the present work, we intend to demonstrate that the compound obtained using Ochiai's method is not the ethyl ester 1,4-dihydro-2-methyl-4-oxo-nicotinic acid but its isomer **1**. In this connection, we report a successful multi-step synthesis providing 1,4-dihydro-2-methyl-4-oxo-nicotinic acid (**7**) and its *N*-methyl derivative. Exhaustive analytical data (NMR, IR, UV, MS, mp) and preliminary potentiometric and UV–vis results on the complexation properties of the two ligands toward Al(III) are reported.

2. Results and discussion

In our first attempt at synthesizing 1,4-dihydro-2-methyl-4-oxo-nicotinic acid **7**, we adopted the one-step method described by Ochiai[6] for synthesis of its ethyl ester, by reacting diethyl aminomethylenemalonate and ethyl acetoacetate in the presence of bubbled HCl dry gas at room temperature (Scheme 1), and easily obtained the corresponding acid by alkaline hydrolysis. Ochiai conditions allowed us to isolate only the isomer 1,6-dihydro-2-methyl-6-oxo-pyridine-3-carboxyl acid ethyl ester (**1**) in 44% yield. Varying the molar ratio of the two reagents or increasing/decreasing the reaction temperature never isolated the desired ethyl ester of **7**.

Very recently, researchers have obtained identical results with Ochiai's reaction for 1,4-dihydro-2-methyl-4-oxo-pyridine-3-carboxyl acid ethyl ester and proposed a four-step pathway leading to the target.[7]

Analytical data for isomer ester **1** were consistent with those reported in the literature for the same compound (IR,

Keywords: Oxo-nicotinic acids; Chelating agents.
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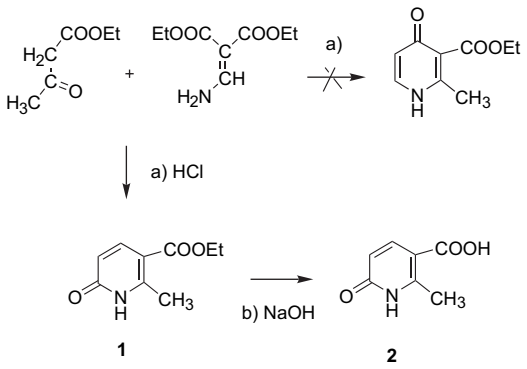
doi:10.1016/j.tet.2006.04.053

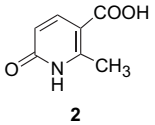
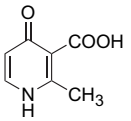


Scheme 1.

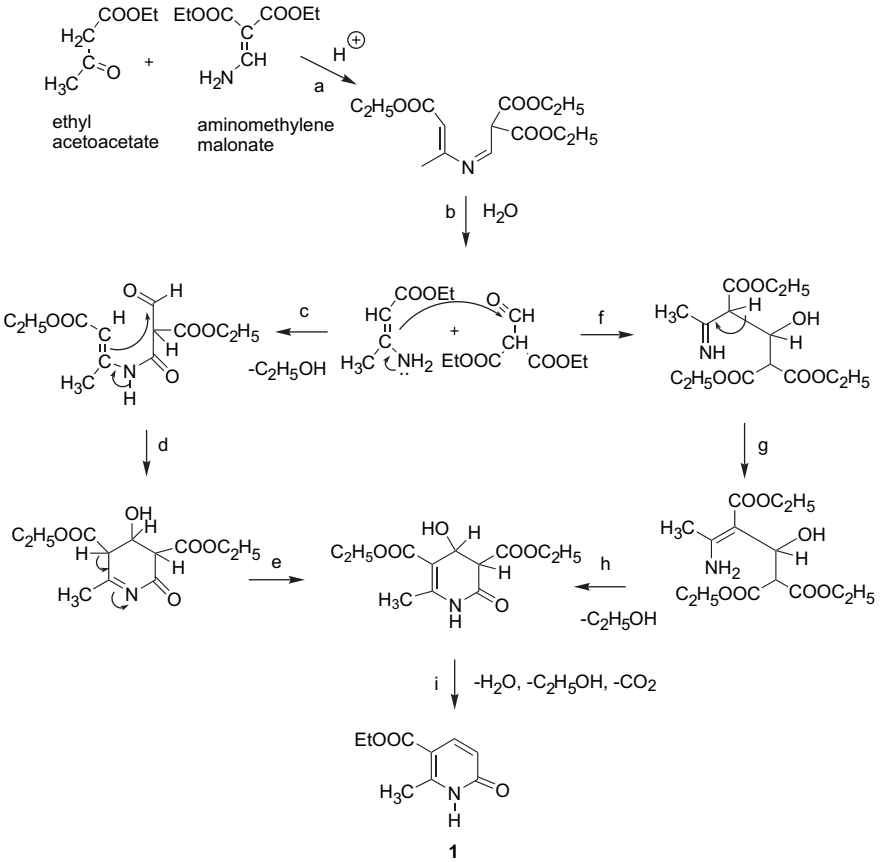
^{1}H NMR, mp) which, however, had been synthesized by means of other methods.[8–11] In particular, it has been obtained by cyclization of a dienamino ester, which resulted from the reaction between a β-amino-croton ester and a propiol ester.[8]

It is not the aim of this paper to speculate on the mechanism involved in the formation of 6-oxo-nicotinic derivative **1** by means of a reaction between aminomethylenmalonate and ethyl acetoacetate, but we suggest two similar possible mechanisms, described in Scheme 2. Briefly, after condensation between aminomethylenmalonate and ethyl acetoacetate (a), transamination may occur (a plus b), forming ethyl 3-amino-butenoate and formyl malonate, followed by amide formation (c) with one of the ester functions in the malonate derivative and ring closure (d, e). An alternative (which leads to the same thing) is first alkylation of ethyl 3-amino-butenoate by the formyl group in malonate (f) and then ring closure by amide formation (g, h). Lastly, water loss, ester hydrolysis, and carbon dioxide loss (i) affords compound **1**.

Table 1. Comparison of significant analytical data of the two isomer acids **2** and **7**

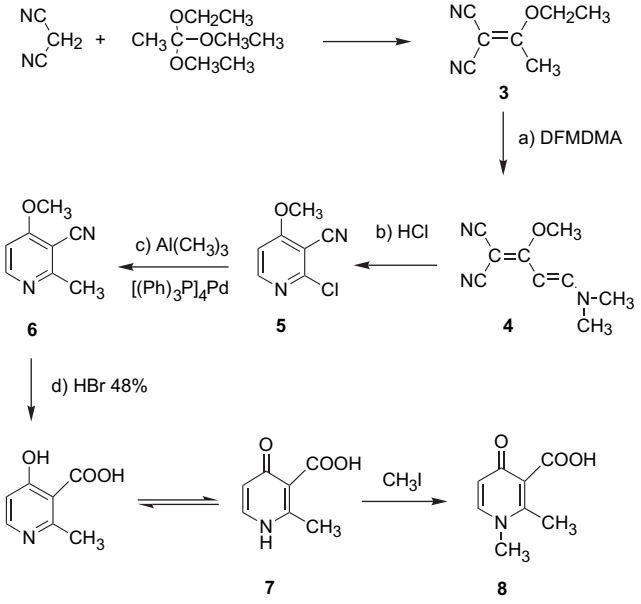
Analysis	 **2**	 **7**
UV (H_2O) (nm) pH 1	262 (14,209)	245 (6162)
IR (KBr) (cm^{-1})	1668 (carboxyl C=O), 1650 (amide C=O)	1721.27 (carboxyl C=O), 1646.41 (carbonyl C=O)
^{1}H NMR (δ)	6.16 (d, 1H, J=9.72 Hz, HC-5), 7.79 (d, 1H, J=9.72 Hz, HC-4)	6.66 (d, 1H, J=7.5 Hz, HC-5), 7.96 (d, 1H, J=7.5 Hz, HC-6)
^{13}C NMR (δ)	165.6 (carboxyl C=O), 155.9 (amide C=O)	167 (carboxyl C=O), 180 (carbonyl C=O)
Mp	153 °C dec	180 °C dec
HRMS	MH^+ 154	MH^+ 154

Subsequently, 5-carboxyl ethyl ester **1** is transformed by means of alkaline hydrolysis (NaOH 2 N) into the corresponding acid **2**, to yield a compound to compare with target acid **7** (Table 1).



Scheme 2.

The synthesis of definite 1,4-dihydro-2-methyl-4-oxo-nicotinic acid **7** was accomplished partly by taking advantage of a known method already used by us to prepare 4-oxo-nicotinic acid[5,12] (Scheme 3). We obtained a useful intermediate, 2-chloro-4-methoxy-nicotinonitrile **5**, starting from condensation of malodinitrile and triethyl orthoacetate to give 1,1-dicyano-2-ethoxypropene (**3**), which was reacted with DMF–DMA in methanol, yielding 1,1-dicyano-4-(*N*,*N*-dimethylamino)-2-methoxy-1,3-butadiene (**4**). The latter was cyclized to chloro-derivative **5** (70%), the key precursor for target acid **7**, which was obtained from it in two steps: (a) introduction of a methyl group by the cross-coupling reaction of **5** with $Al(CH_3)_3$ and $(Ph)_3P_4Pd$ as catalyst in dry dioxane to give 2-methyl-4-methoxy-nicotinonitrile (**6**) in optimum yield; (b) hydrolysis by HBr 48% to the final compound **7** (40% yield). Acid **7** was also N-methylated by the conventional method,[13] using CH_3I in DMF to furnish 1,2-dimethyl-4-oxo-nicotinic acid **8**.



Scheme 3.

Data on the characterization of the two isomer acids **2** and **7** by UV, IR, ^1H, and ^{13}C NMR, plus melting points, are listed in Table 1, so that, by comparing their analytical data, we can state that the Ochiai compound is the isomer 1,6-dihydro-6-oxo-pyridine derivative **1**. As shown, the IR and ^{13}C NMR values for the carbonyl C=O of **2**, 1665 cm^{-1} and δ 167, respectively, are consistent with an amide C=O, whereas for **7** the same signals occur at 1646.5 cm^{-1} and δ 180, according to a cyclic ketone structure. Moreover, ^1H NMR coupling constants for H-4 and H-5 (9.5 Hz) of **2** are higher than those for H-5 and H-6 (7.5 Hz) of **7**, in agreement with literature data on α-pyridinone.[8]

NOESY, HMQC, and HMBC 2D NMR experiments were also performed with **1** in order to confirm further the structure of the obtained compounds. In the NOESY spectrum, a weak correlation was observed between the doublet at δ 7.79 (H-4) and the triplet at δ 1.26 (methyl signal of the ethyl residue). Further evidence was represented by the diagnostic HMBC correlations between the proton signal

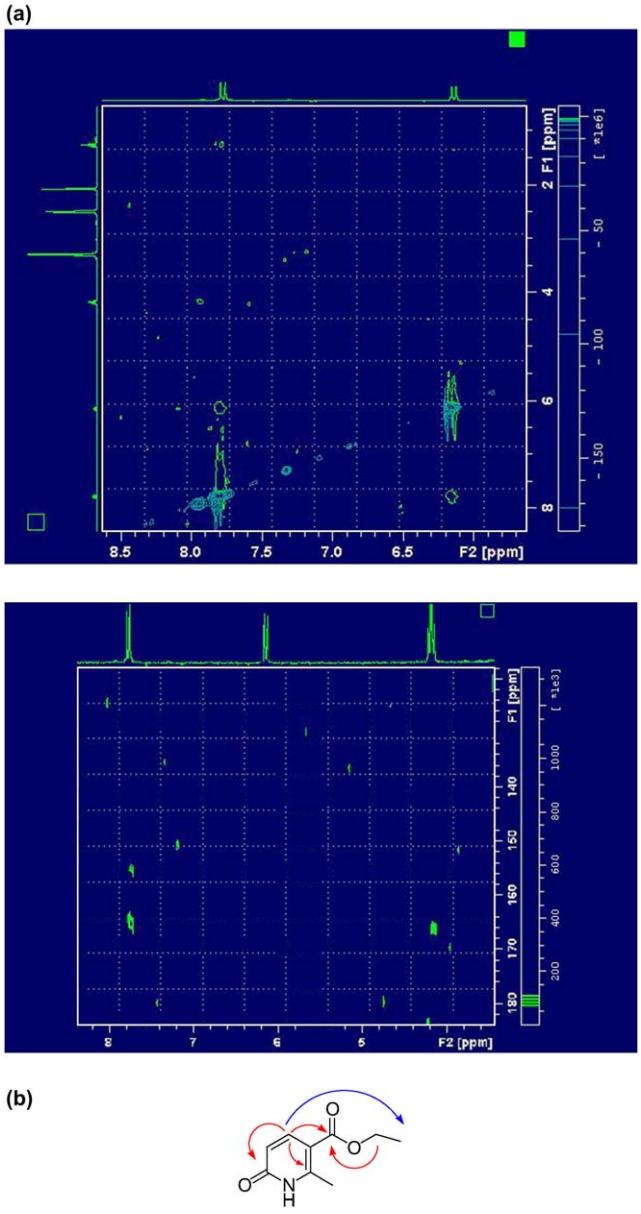


Figure 1. (a) NOESY and HMBC spectra of compound **1**; (b) Blue arrow: NOESY correlation; red arrows: diagnostic HMBC correlations described in text.

at δ 7.79 (H-4) and the carbon resonances at δ 155.9 (C-6) and 165.6 (carboxyl C=O) and between the CH_2 of the ethoxy group at δ 4.18 and the carbon resonance at δ 165.6 (C=O) (Fig. 1, a and b).

Lastly, as regards melting points, the breakdown of **2** at 153 °C is consistent with its decarboxylation, which occurs more readily than in **7** (180 °C), in which the carbonyl and carboxyl groups are next to each other.

Further evidence of the identity of the two isomers came from comparing their capacity to form complexes with a hard metal ion like aluminum(III). Hard metal ions can form reasonably stable complexes with hard functional groups, such as phenol and carboxyl oxygens, provided that a chelated complex forms, i.e., that two or more groups can bind simultaneously with the metal ion[14] to form a

five- or six-membered ring. If this is not the case then very weak monodentate complexes form (the only exceptions being phosphonate oxygens at acidic pH values, which form reasonably stable monodentate complexes). This property can be used to discriminate between isomers **2** and **7**, as only **7** has the carboxyl and phenol groups in *ortho*, and is thus able to chelate the metal ion.

The formation of complexes is easily detected by UV–vis analysis. Figure 2 shows UV–vis spectra obtained at the same pH value (4.1) for one solution containing **2** and Al(III) and another containing only **2**. A pH value of 4.1 was chosen to ensure reasonable complex formation (complexes may not form at more acidic values) and to avoid the precipitation of aluminum hydroxide (expected at pH>4.5–5 if complex stability constants are not sufficiently high). Nevertheless, the two spectra are practically identical, indicating that the ligand is not complexed.

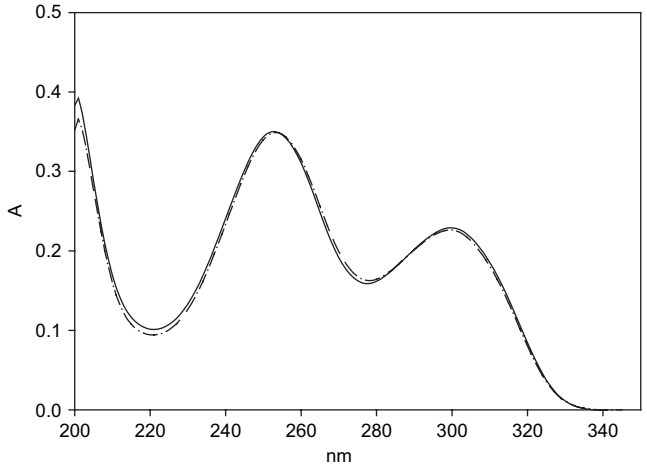


Figure 2. UV–vis spectra for one solution containing ligand alone, pH=4.08 (solid line) ($[L]_0=3.39\times10^{-4}$ m), and another containing ligand with metal ion, pH=4.13 (dash-dotted line) ($[L]_0=3.39\times10^{-4}$ m, $[Al^{3+}]_0=9.86\times10^{-5}$ m).

As regards potentiometric titrations, formation of the complexes is competitive with acid–base equilibria, so that the acidity constants of the ligand were determined first (the acid–base properties of Al^{3+} were known from previous data[15]). The results were $pK_1=0.308\pm0.008$ (OH), $pK_2=3.758\pm0.008$ (COOH), $pK_3=11.63\pm0.02$ (NH) (note that pK_a values alone cannot discriminate between **2** and **7**, as very similar values are expected from both isomers).

Figure 3 shows an experimental potentiometric titration for a solution containing **2** and Al^{3+}. The overlapping theoretical curve was calculated solely on the basis of the acid–base properties of the ligand and the metal ion, thus assuming no metal–ligand species. The theoretical curve almost coincides with the experimental data, demonstrating that, in this system, there is no (or very little) complexation at any pH (values higher than 4.5–5 could not be reached, due to precipitation of $Al(OH)_3$).

Different results were obtained for isomer **7**. The complete results and discussion will be provided elsewhere.[16] Figure 4 shows an experimental potentiometric titration for

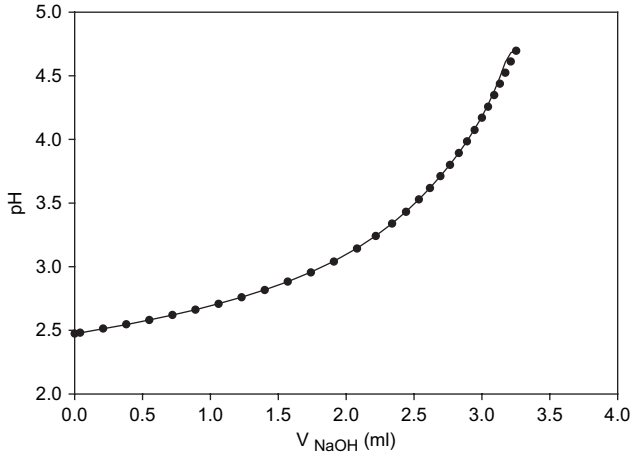


Figure 3. Theoretical (line) and experimental (circles) potentiometric titration curves for a solution containing Al^{3+} (3.81×10^{-4} m) and **2** (1.14×10^{-3} m).

a solution containing **7** and Al^{3+}. The overlapping theoretical curve was calculated presuming no complex formation. The differences between the two curves are large, especially at higher pH values. Assuming the formation of strong complexes of the type AlL, AlL_2, and AlL_3 leads to a far better match between the two curves.[16] Moreover, in solutions containing **7** and Al^{3+}, precipitation of $Al(OH)_3$ was only observed at [L]/[Al] ratios of less than 3.

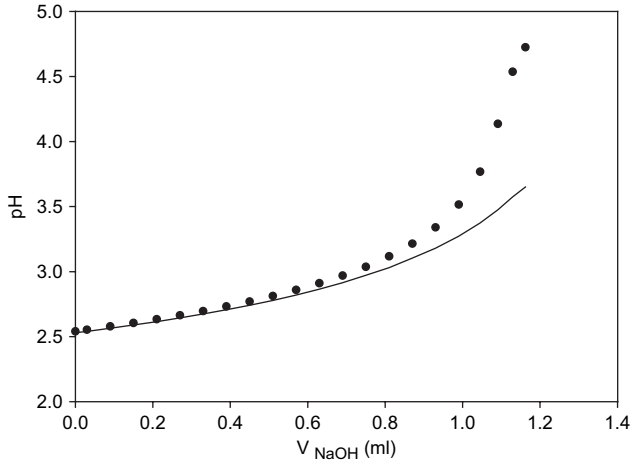


Figure 4. Theoretical (line) and experimental (circles) potentiometric titration curve for a solution containing Al^{3+} (3.11×10^{-3} m) and **7** (9.70×10^{-3} m).

These preliminary complexometric results undoubtedly support our structural assignments for **2** and **7**. The significant complexation ability of **7** toward Al^{3+} confirms that, in this compound, the two coordinating groups, COOH and OH, are in *ortho*, whereas this is not the case for **2**.

3. Experimental

3.1. General

Melting points were determined on a Gallenkamp MFB 595 010M/B capillary melting point apparatus, and are not corrected. Infrared spectra were recorded on a Perkin–Elmer

1760 FTIR spectrometer using potassium bromide pressed disks; all values are expressed in cm^{-1}. UV–vis spectra were recorded on a Perkin–Elmer Lambda UV/VIS spectrometer. ^1H NMR spectra were recorded on Varian Gemini (200 MHz) and Bruker (300 MHz) spectrometers, using the indicated solvents; chemical shifts are reported in δ (parts per million) downfield from tetramethylsilane as internal reference. Coupling constants are given in hertz. In the case of multiplets, chemical shift was measured starting from the approximate center. Integrals were satisfactorily in line with those expected on the basis of compound structure. Elemental analyses were performed in the Microanalytical Laboratory, Department of Pharmaceutical Sciences, University of Padova, using a Perkin–Elmer elemental analyzer model 240B; results fell in the range of calculated values ±0.4%. Mass spectra were obtained on a Mat 112 Varian Mat Bremen (70 eV) mass spectrometer and Applied Biosystems Mariner System 5220 LC/Ms (nozzle potential 250.00). Column flash chromatography was performed on Merck silica gels (250–400 mesh ASTM). Chemical reactions were monitored by analytical thin-layer chromatography (TLC) using Merck silica gel 60 F-254 glass plates with a 9:1 dichloromethane/methanol mixture as eluant, unless otherwise specified.

Solutions were concentrated in a rotary evaporator under reduced pressure. Starting materials were purchased from Aldrich Chimica and Acros, and solvents from Carlo Erba, Fluka and Lab-Scan. DMSO was made anhydrous by refluxing with CaO for 8 h and then by distillation under vacuum and storage on molecular sieves. Dioxane was dried by leaving it on KOH pellets overnight and then storing it on metallic Na.

3.1.1. Synthesis of 1,6-dihydro-2-methyl-6-oxo-nicotinic acid ethyl ester (1): Ochiai's procedure. A mixture of aminomethylenemalonic acid diethyl ester (1 g, 5.34 mmol) and ethyl acetoacetate (0.68 ml, 5.34 mmol) was stirred at room temperature to give a homogeneous suspension and then, after cooling at 0 °C, HCl gas was bubbled until saturation and complete solubilization. The yellow solution was then allowed to reach room temperature and left for 4–5 days. By this time, a yellow crystalline product had formed, which was collected and recrystallized from methanol/acetone 2:8, yielding 44% of pure compound. Mp 213 °C (lit.[6,7,10] 207 °C, lit.[10] 222 °C); R_f 0.34 (chloroform/methanol 95:5); IR (KBr) 3350 (NH), 1705 (ester CO), 1645 (amide CO) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.26 (t, 3H, CH_3), 2.52 (s, 3H, CH_3), 4.18 (q, 2H, J=7.0 Hz, CH_2O), 6.19 (d, 1H, $J_{4,5}$=9.66 Hz, H-5), 7.79 (d, 1H, $J_{5,4}$=9.59 Hz, H-4), 12.02 (s, 1H, H-1); ^{13}C NMR (DMSO-d_6) δ 165.6 (carboxyl C═O), 155.9 (C-6), 142.8 (C-4), 140.66 (C-2), 117.96 (C-5), 115.82 (C-3), 60.87 (CH_2), 15.96 (CH_3), 14.28 (CH_3); HRMS [MH^+] 182.12; Anal. Calcd for $C_9H_{11}NO_3$: C, 59.66; H, 6.12; N, 7.73; found: C, 59.45; H, 6.13; N, 7.64.

3.1.2. Synthesis of 1,6-dihydro-2-methyl-6-oxo-nicotinic acid (2). A solution of 1.2 g (6.62 mmol) of ethyl ester **1** in NaOH 0.5 M (3–4 ml) was heated at 70–80 °C for 3 h. After cooling, the solution was slowly acidified with HCl 2 N until precipitation was complete, and placed at 0 °C overnight. The white precipitate was collected, washed with water, and recrystallized from water. Yield 74%; mp 153 °C (dec); R_f 0.2 (methanol); UV–vis 262 (14,209) nm; IR (KBr) 3320 (NH), 1668 (carboxyl C═O), 1650 (amide C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.52 (s, 3H, CH_3), 6.1 (d, 1H, $J_{5,4}$=9.66 Hz, H-5), 7.81 (d, 1H, $J_{4,5}$=9.66 Hz, H-4), 11.96 (s, 1H, HN-1); ^1H NMR (MeOD) δ 2.63 (s, 3H, CH_3), 6.33 (d, 1H, $J_{4,5}$=9.70 Hz, HC-4), 8.04 (d, 1H, $J_{5,4}$=9.72 Hz, HC-5); ^{13}C NMR (DMSO-d_6) δ 170.37 (carboxyl C═O), 163.45 (C-6), 143.5 (C-2), 142.8 (C-4), 117.96 (C-5), 115.82 (C-3), 15.96 (CH_3); HRMS [MH^+] 154.129; Anal. Calcd for $C_7H_7NO_3$: C, 54.90; H, 4.61; N, 9.15; found: C, 54.78; H, 4.33; N, 9.00.

3.1.3. Synthesis of 2-chloro-4-methoxy-nicotinonitrile (5). Following the reported route,[12] 10 g (151.37 mmol) of malodinitrile were reacted with 27.1 g (167.05 mmol) of triethyl orthoacetate at 90–95 °C for 45 min. The mixture was then evaporated at 60 °C, giving a pink product of 1,1-dicyano-2-ethoxypropene (**3**). Yield 99%; mp 91–92 °C [lit.[17] 88.5–89.5 °C].

A solution obtained by heating a mixture of **3** (7.4 g, 54.35 mmol) in 17 ml of MeOH at 50 °C, was added with DMF–DMA (10.92 g, 82.05 mmol) and the resulting mixture was refluxed for 1.5 h. After cooling at room temperature, a dark red precipitate formed, which was collected, washed with cold MeOH, and dried, yielding 52% of **4**, mp 133 °C (lit.[12] 130 °C).

1,1-Dicyano-4-(*N*,*N*-dimethylamino)-2-methoxy-1,3-butadiene (**4**) (5 g, 28.21 mmol) in 100 ml of MeOH was submitted to cyclization in acid conditions by HCl gas, with vigorous stirring, at 15 °C. At the end of the reaction, water/ice (800 g) was added and a white precipitate separated from the solution. This was collected, washed with cold water, and dried. The filtrate was concentrated under vacuum and basified with NaOH 2 N, and a further product formed. Overall yield was 70% of almost pure **5**; mp 175–176 °C (methanol) (lit.[12] 168 °C).

3.1.4. Synthesis of 2-methyl-4-methoxy-nicotinonitrile (6). 2-Chloro-4-methoxy-nicotinonitrile (2 g, 11.86 mmol) and 85 ml of anhydrous dioxane (KOH, Na) were placed in a 250-ml two-necked round-bottomed flask. After dissolving by slight heating, 6 ml (56.84 mmol) of $(CH_3)_3Al$, 2 M *n*-hexane solution and then 0.224 g (0.1916 mmol) of $[(Ph_3)P]_4Pd$ as catalyst were added. The mixture was refluxed for 4 h under an inert atmosphere of N_2, checking the ongoing reaction by TLC analysis (chloroform/methanol 95:5). At the end, the cooled reaction mixture was acidified with HCl 2 N and the solvent evaporated off. The residue was treated with water, basified with NaOH 20%, and the mixture extracted with diethyl ether. The combined extracts, washed with water and dried over anhydrous Na_2SO_4, were evaporated until dry. Yield 99%; mp 117 °C; R_f ($CHCl_3$/MeOH 95:5); ^1H NMR (DMSO-d_6) δ 2.53 (s, 3H, CH_3), 4.05 (s, 3H, OCH_3), 7.39 (d, 1H, $J_{5,6}$=6.1 Hz, H-5), 8.56 (d, 1H, $J_{6,5}$=6.1 Hz, H-6); HRMS [MH^+] 147.15; Anal. Calcd for $C_8H_8N_2O$: C, 64.85; H, 5.44; N, 18.91; found: C, 64.69; H, 5.38; N, 18.86.

3.1.5. Synthesis of 1,4-dihydro-2-methyl-4-oxo-nicotinic acid (7). In a 50-ml flask, 1 g (6.84 mmol) of 2-methyl-4-methoxy-nicotinonitrile and 17 ml of HBr 48% water solution were heated at 70–80 °C for 1 h and then refluxed,

checking the course of reaction by TLC analysis (chloroform/methanol 9:1). Although refluxing lasted some days, the reaction had not finished, so refluxing was stopped, the mixture cooled, and worked up as follows. It was basified with NaOH 20% (pH 8–9), extracted with ethyl acetate in order to remove the starting material, and the aqueous phase acidified with HCl 2 N (pH 3). In this way, a fine white precipitate formed, which was left at 0–4 °C overnight. The next day, it was collected, washed with water, and dried. Yield 45%; mp 180 °C (dec); R_f 0.2 (methanol); UV–vis 245 nm (6162); IR (KBr) 3395 (NH), 1721 (carboxyl C═O), 1646.41 (carbonyl C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.76 (s, 3H, CH_3), 6.66 (d, 1H, $J_{5,6}$=7.06 Hz, H-5), 7.96 (d, 1H, $J_{6,5}$=7.25 Hz, H-6), 12.82 (s, 1H, HN-1), 16.15 (s, 1H, OH); ^{13}C NMR (DMSO-d_6) δ 180 (C-4), 167.1 (carboxyl C═O), 156.59 (C-2), 139.45 (C-6), 116.36 (C-5), 113.50 (C-3), 20.16 (CH_3); HRMS [MH^+] 154.12, [M−1] 152.0; Anal. Calcd for $C_7H_7NO_3$: C, 54.90; H, 4.61; N, 9.15; found: C, 54.95; H, 4.53; N, 9.02.

3.1.6. Synthesis of 1,4-dihydro-1,2-dimethyl-4-oxo-nicotinic acid (8). 2-Methyl-4-oxo-1,4-dihydro-pyridine-3-carboxyl acid (**7**) (0.900 g, 5.88 mmol) in 45 ml of DMF was added with 1.8 ml (29.1 mmol) of CH_3I and the mixture heated at 100 °C for 2 h. After cooling, the solvent was evaporated off and the residue was recrystallized with methanol. Yield 60%; mp 271 °C; R_f 0.2 (methanol); IR (KBr) 1718.6 (CO), 1655.6 (carbonyl); ^1H NMR (DMSO-d_6) δ 2.76 (s, 3H, CH_3), 3.45 (s, 3H, N–CH_3), 6.66 (d, 1H, $J_{5,6}$=7.06 Hz, H-5), 7.96 (d, 1H, $J_{6,5}$=7.25 Hz, H-6), 16.15 (s, 1H, COOH); ^{13}C NMR (DMSO-d_6) δ 180 (C-4), 170.1 (carboxyl C═O), 163.4 (C-2), 142.2 (C-6), 116.2 (C-5), 114.6 (C-3), 33.2 (CH_3), 19.3 (CH_3); HRMS [MH^+] 168.05; Anal. Calcd for $C_6H_9NO_3$: C, 57.48; H, 5.43; N, 8.38; found: C, 57.32; H, 5.41; N, 8.25.

4. Complexometric measurements

The experimental apparatus, reagents, and measurement methods were almost the same as those previously reported.[4] A summary follows, with details given only when they differ from those already described.

All analyte concentrations are expressed in the molality scale (mol/kg of water).

Potentiometric measurements were performed on a Radiometer ABU93 Triburette apparatus; UV–vis spectra were recorded on a Perkin–Elmer Lambda 25 instrument.

Working solutions of HCl (0.2 m), NaOH (0.2 m), and $AlCl_3$ (0.1 m, containing HCl 0.3 m) were prepared and standardized as previously described.[4] The water solubility of **2** and **7** is low ($<2\times10^{-3}$ m): working solutions at higher concentrations (4.4×10^{-3} m and 6.5×10^{-3} m) could be prepared upon slight basification. The ionic strength of all solutions was adjusted to 0.6 m (≈0.594 M) (Na)Cl.

Potentiometric titrations were carried out at 25±0.1 °C. Duplicate potentiometric measurements were carried out using two glass electrodes (Radiometer pHG201 and BDH 309/1015/02) and a Ag/AgCl/0.6 m NaCl reference electrode.

Solutions for UV–vis analysis were prepared in the same cell used for potentiometric titrations and, after fixing the pH, the solutions were transferred into a cuvette (1 cm path length). A 0.6 m NaCl solution was used as blank.

All stability constants were calculated using the PITMAP program.[18]

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Efficient halogen–lithium exchange reactions to functionalize poly(alkyl aryl ether) dendrimers

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Received 9 February 2006; revised 5 April 2006; accepted 20 April 2006
Available online 12 May 2006

Abstract—Poly(alkyl aryl ether) dendrimers were functionalized with bromophenyl groups at their peripheries, so as to have 3, 6, 12, and 24 groups in the zero, first, second, and third generation dendrimers, respectively. The new bromophenyl functionalized dendrimers were assessed for their reactivities in C–heteroatom and C–C bond forming reactions. For this purpose, the bromophenyl functionalized dendrimers were converted quantitatively to their polylithiated derivatives, using *n*-BuLi in benzene. The polylithiated dendrimers were reacted either with D_2O or with CO_2, so as to afford the corresponding deuterated and carboxylic acid functionalized dendrimers, respectively. The carboxylic acid functionalized dendrimers were modified further to the methyl esters during their characterization.

1. Introduction

Alkyl- and aryllithium derivatives are versatile intermediates in organic and organometallic synthesis.[1] Most often the lithium derivatives are derived by exchange reactions involving alkyl- or arylhalide and a lithium source.[2] Lithiation of an alkyl- or an aryl substrate is one of the important reaction in the course of many C–C bond forming, C–heteroatom bond forming and C–varied metal bond forming reactions.[3] Many theoretical and synthetic studies have been reported due to wide utility of organolithium compounds.[4] Polylithiation of macromolecules represents a system wherein multiple simultaneous lithiations are performed and such polylithiated intermediates are then used to prepare a variety of functionalized macromolecules.[5] Uniformly branched dendritic macromolecules[6] are recent additions in polylithiations. Carbosilane-based dendrimers were the only ones explored thus far in terms of their polylithiation and further functionalization.[7] As a strategy to functionalize poly(alkyl aryl ether) dendrimers,[8] polylithiations of the dendrimers were undertaken, in an effort to exploit the rich lithiation chemistry as applicable to this class of dendrimers. The polylithiated dendrimers, obtained by a halogen–metal exchange reaction, were subjected to reactions with a few electrophiles. The syntheses and reactions of polylithiated dendrimers up to the third generation, which exhibit 24 peripheral lithiated arenes, are presented herein.

2. Results and discussion

Poly(alkyl aryl ether) dendrimers are constituted with phloroglucinol as the core and branching component and a pentamethylene as the linker connecting the branches. *endo*- and *exo*-Receptor properties of these dendrimers have been explored and beneficial effects of the dendritic architectures have been previously identified.[9] The poly(alkyl aryl ether) dendrimers exhibit multiple phenolic hydroxyl groups at their peripheries and the zero, first, second, and third generation dendrimers present 3, 6, 12, and 24 such groups, respectively.[8] These phenolic hydroxyl groups were utilized to install functionalities necessary for polylithiations. Bromophenyl functionality was chosen for its subsequent bromide–lithium exchange reaction. Multiple phenolic O-alkylation of the dendrimer peripheries with a bromophenyl group containing monomer was thus conducted across different generations of the poly(alkyl aryl ether) dendrimers.

2.1. Synthesis of bromophenyl group functionalized monomer

The bromophenyl group containing derivative **2** was obtained upon reaction of 1,5-dibromopentane with 4-bromophenol (**1**) (Scheme 1). The mono-O-alkylated product **2** was utilized to alkylate the peripheral phenolic groups of the dendrimers.

Keywords: Alkylation; C–C bond formation; Dendrimers; Lithiation.
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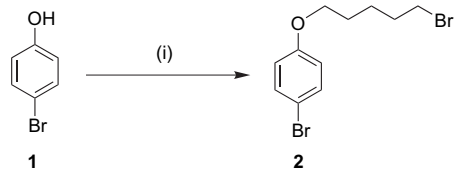
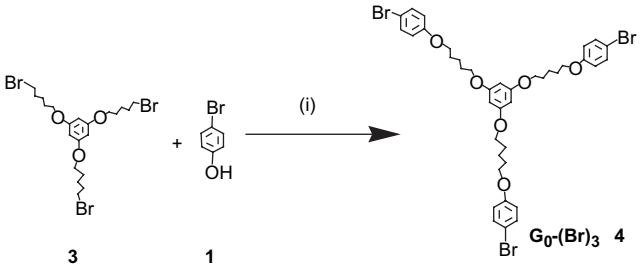
doi:10.1016/j.tet.2006.04.052



Scheme 1. (i) 1,5-Dibromopentane, K_2CO_3, 18-C-6, Me_2CO, 70 °C, 7 h, 80%.

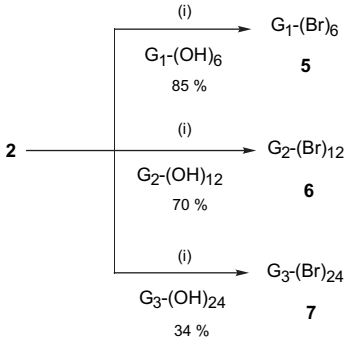
2.2. Synthesis of bromophenyl group functionalized dendrimer

The zero-generation dendrimer **4**, containing the bromophenyl group, was obtained by a three-fold alkylation of a tribromide **3** with 4-bromophenol, in good yields (Scheme 2).



Scheme 2. (i) K_2CO_3, 18-C-6, 2-butanone, 70 °C, 12 h, 86%.

The first, second, and third generation bromophenyl group containing dendrimers were obtained by multiple O-alkylation of the corresponding phenolic dendrimers with monomer **2**. The O-alkylations were performed in the presence of K_2CO_3 and 18-C-6 (cat.) in 2-butanone (DMF) and the bromophenyl terminated first (**5**), second (**6**), and third generation (**7**) dendrimers were obtained in moderate yields. The zero, first, second, and third generation dendrimers were thus functionalized with 3, 6, 12, and 24 bromophenyl groups at their peripheries, respectively (Scheme 3 and Fig. 1).



Scheme 3. (i) K_2CO_3, 18-C-6 (cat.), 2-butanone, DMF, reflux.

Dendrimers **4**–**7** were freely soluble in solvents such as, THF, PhMe, and MeOH whereas, their solubilities in MeOH, Et_2O, and hexane were moderate to poor. In terms of loading, the bromophenyl group constituted 3.53, 2.78, 2.52, and

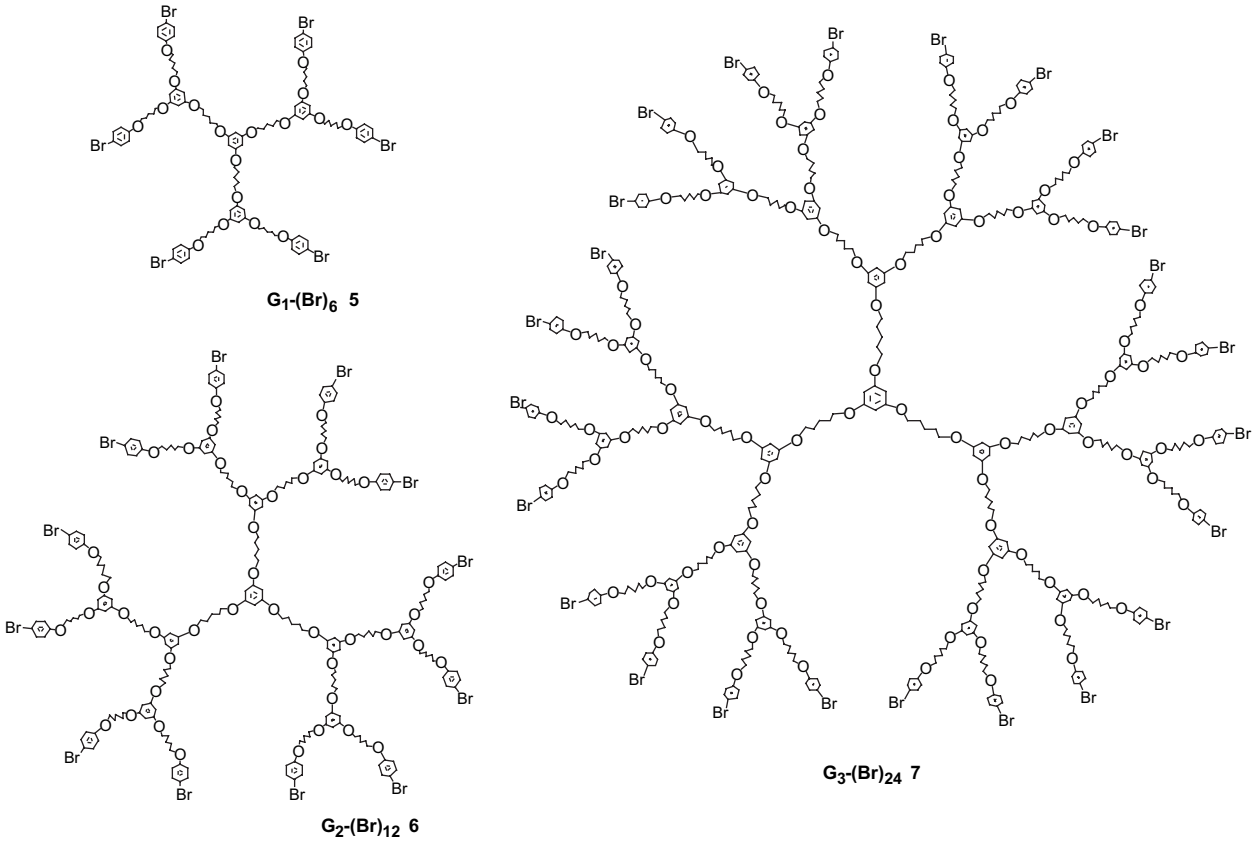


Figure 1. Molecular structures of bromophenyl group functionalized G_1-$(Br)_6$ (**5**), G_2-$(Br)_{12}$ (**6**), and G_3-$(Br)_{24}$ (**7**) dendrimers.

2.40 mmol/g in zero (**4**), first (**5**), second (**6**), and third (**7**) generation dendrimers, respectively.

2.3. Characterization

The constitutions and the structural homogeneities of the dendrimers were ascertained by ^{1}H and ^{13}C NMR spectroscopies and elemental analyses. Specifically, the phloroglucinol protons appeared as a singlet at 6.05 ppm and the bromophenyl moiety at ~6.70 and ~7.30 ppm, as a pair of doublets in the ^{1}H NMR spectra of the dendrimers. The CH (methine) and C (q) carbons of the phloroglucinol moiety appeared at ~93.5 and 160.5 ppm, respectively, in the ^{13}C NMR spectra. The resonances of CH (methine) and C (q) of the peripheral bromophenyl moieties were observed at ~116.0, ~132.0 and ~112.5, ~158.8 ppm, respectively. Mass spectral analysis by MALDI-TOF and ES methods, could not be accomplished even after several trials. Gel permeation chromatography was conducted to assess the GPC profiles of the dendrimers in THF as the eluant. The retention times of various generations were: **7**: 6.81 min; **6**: 7.37 min; **5**: 8.09 min; **4**: 9.13 min and monomer **2**: 10.63 min, consistent with the proposed M_r values.

2.4. Lithiations of bromophenyl functionalized dendrimers

Lithiation of the bromophenyl functionalized dendrimers was followed using lithium metal. The lithiated dendrimers would allow incorporation of various electrophiles in the course of carbon–heteroatom and carbon–carbon bond formation. Thus, lithiation reactions were conducted with the most widely used reagent and solvent, namely, *n*-BuLi and THF. Lithiation, followed by a subsequent reaction with an electrophile, was conducted across a series of dendrimers. In the event, it turned out that the electrophilic substituent on the product could not be identified. Rather, only the debromination of the bromophenyl functionality occurred. On the other hand, use of Et_2O as the solvent, in place of THF, allowed the reaction to proceed in the anticipated manner, leading to incorporation of the electrophile at the carbon subjected to the halogen–lithium exchange reaction. However, the successful reaction in Et_2O for the zero-generation dendrimer with three bromophenyl functionalities could not be extended to other dendrimers, due to the insolubility of the dendrimers in Et_2O. Benzene as the solvent of the reaction at ~50 °C was then identified for the lithiation reaction. Thus, the reaction of bromophenyl functionalized dendrimers **4**–**7**, in benzene at ~50 °C, afforded the lithiated derivatives **8**–**11** (Scheme 4).

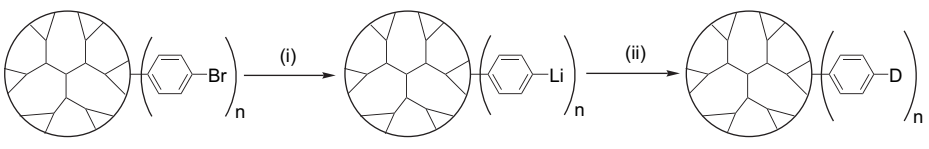
The lithiated derivatives precipitated as a white solid from the reaction mixture. Without isolation, lithiated products were reacted with electrophiles, so as to yield the C–heteroatom or C–C bond formed product. In one series, the lithiated derivatives were treated with D_2O (Scheme 4) and the deuterated dendrimers were obtained in good to excellent yields as the only isolable product of the reaction (Fig. 2).

Complete deuteration of the dendrimer peripheries was confirmed by the characteristic double doublet resonances, centered at 6.89 and 7.27 ppm, of the peripheral phenyl groups of the dendrimers in the ^{1}H NMR spectra. The corresponding ^{13}C NMR resonances for the deuterated phenyl group were observed at ~114.4, 129.3, 129.4, and 159.0 ppm. Elemental analyses of the deuterated products confirmed the structural homogeneities of the dendrimers.

Upon accomplishing deuteration of the lithiated dendrimers, efforts were focused to subject them to a C–C bond forming reaction. Specifically, the reaction mixture containing the lithiated product was treated with CO_2 (g), at 0 °C for ~45 min and left to stir further at room temperature for 15 h. Acidification of the reaction mixture with aq HCl afforded a precipitate, which was then triturated with ice-cold water. The precipitate was dried further to afford the carboxylic acid functionalized dendrimers **16**–**19** (Scheme 5 and Fig. 3).

Dendrimers **16**–**19** were viscous hygroscopic solids and thus were derivatized as the methyl ester derivatives (Scheme 5). Treatment of the carboxylic acid functionalized dendrimers with MeOH, in the presence of H_2SO_4 (cat.), refluxing the reaction mixture for ~24 h, work-up, and purification afforded the carboxylic acid methyl ester derivatives of the dendrimers **20**–**23** (Fig. 3). The constitutions of the methyl ester functionalized dendrimer were ascertained by spectroscopic methods and elemental analysis. Syntheses of a series of deuterated and carboxylic acid functionalized dendrimers thus illustrated the efficient formation of lithiated dendrimers from the corresponding bromide derivatives.

Only the polysilane dendrimers have thus far been explored for their lithiation reactions, followed by reactions with electrophiles.[7] Within the polysilane dendrimers, the largest dendrimer contained up to 12 lithiated centers at the peripheries of the dendrimers. The studies reported herein add to the



n = 3 **4**
n = 6 **5**
n = 12 **6**
n = 24 **7**

n = 3 **8**
n = 6 **9**
n = 12 **10**
n = 24 **11**

n = 3 **12**
n = 6 **13**
n = 12 **14**
n = 24 **15**

Scheme 4. (i) *n*-BuLi, PhH, 50 °C, 45 min and (ii) D_2O, rt, 15 h.

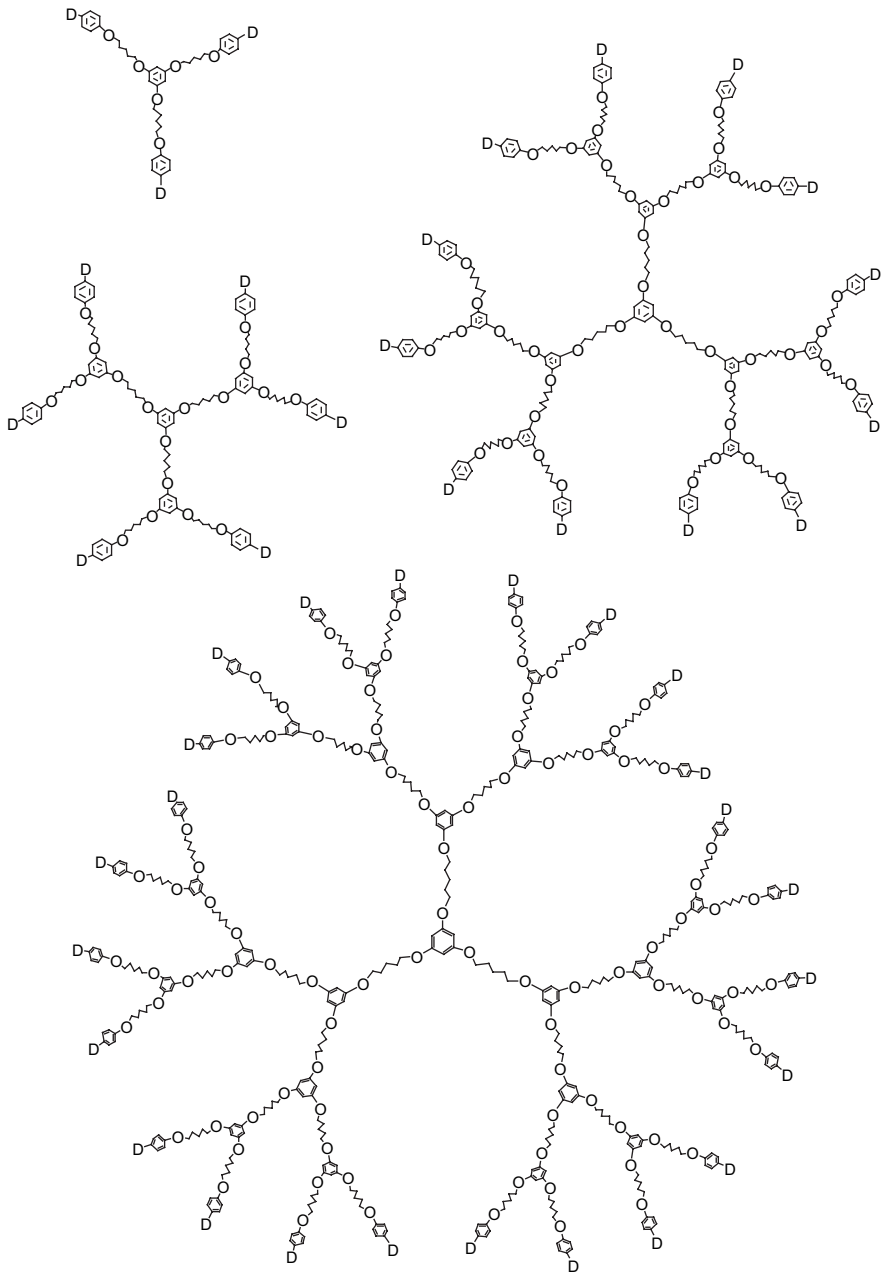
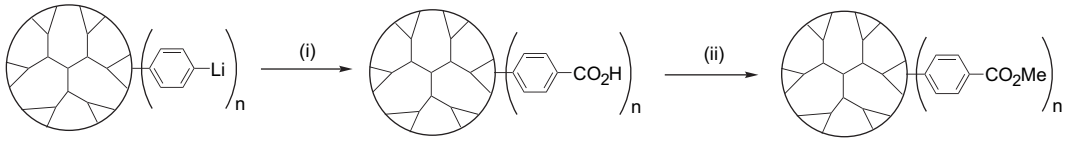


Figure 2. Molecular structures of deuterated G_0-$(D)_3$ (**12**) and G_1-$(D)_6$ (**13**) and G_2-$(D)_{12}$ (**14**) and G_3-$(D)_{24}$ (**15**) dendrimers.



n = 3 **8**
n = 6 **9**
n = 12 **10**
n = 24 **11**

n = 3 **16**
n = 6 **17**
n = 12 **18**
n = 24 **19**

n = 3 **20**
n = 6 **21**
n = 12 **22**
n = 24 **23**

Scheme 5. (i) CO_2 (gas), rt, 15 h and (ii) MeOH, H_2SO_4 (cat.), reflux, 24 h.

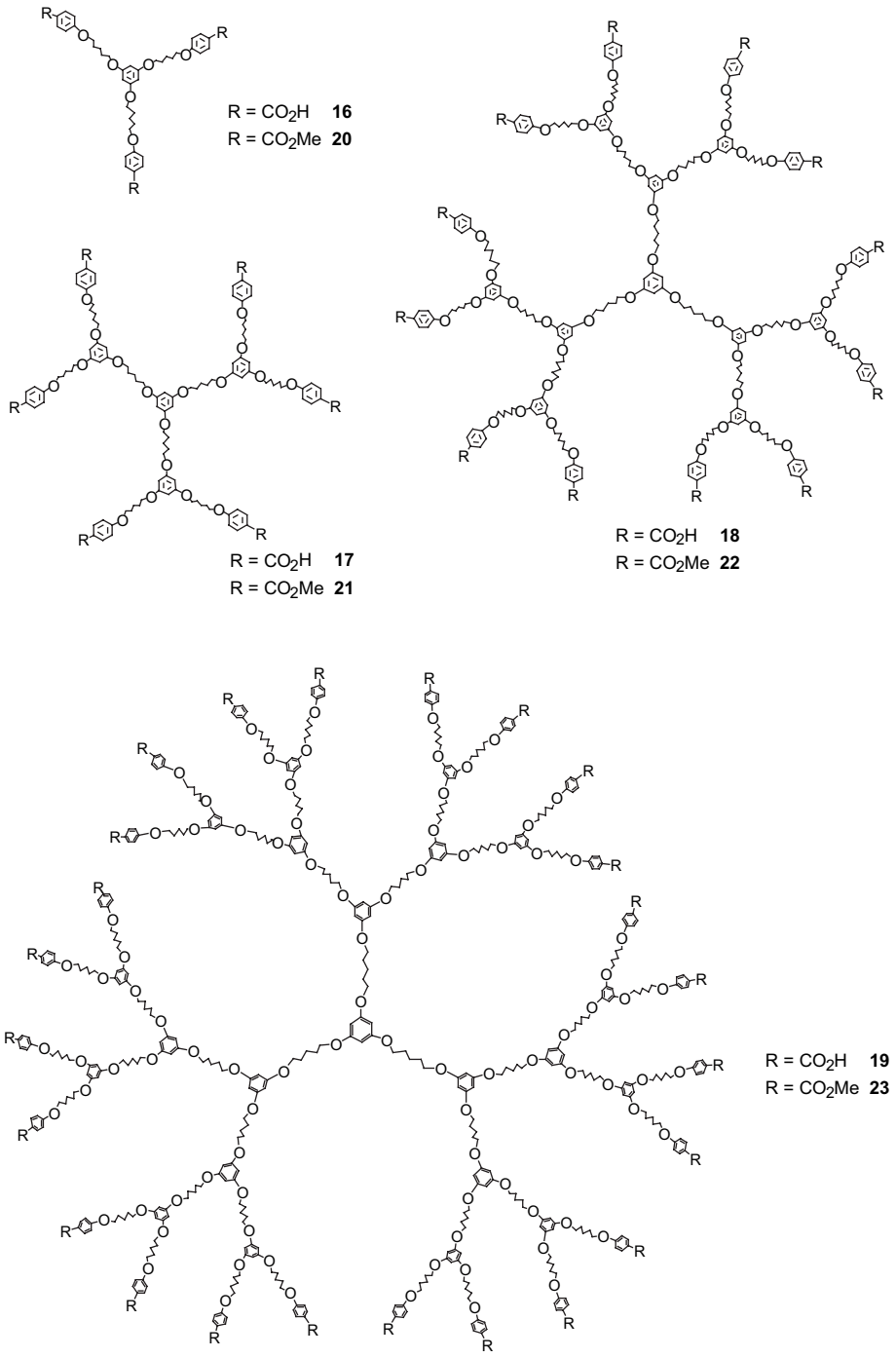


Figure 3. Molecular structures of carboxylic acid and methyl ester functionalized poly(alkyl aryl ether) dendrimers.

efforts targeting the widely practiced lithiations into the general area of dendrimer chemistry.

3. Conclusion

Poly(alkyl aryl ether) dendrimers, having up to 24 bromophenyl functionalities at their peripheries, were explored for their efficacies in lithiation reactions. The halide–lithium exchange reaction was found to be facile and the resulting lithiated dendrimers underwent reactions with deuterium and carbon electrophiles, leading, respectively, to deuterated and carboxylated dendrimers. Having established the formation of lithiated dendrimers, reaction of such dendrimers with varied electrophiles will be conducted further.

4. Experimental

4.1. General methods

Chemicals were purchased from commercial sources and were used without further purification. Powdered K_2CO_3 (Ar grade) was dried at 120 °C for 24 h before being used.

Solvents were dried and distilled according literature procedures.[10] Analytical TLC was performed on commercial Merck plates coated with silica gel GF_{254} (0.25 mm). Silica gel (230–400 mesh) was used for column chromatography. Melting points determined are uncorrected. Microanalyses were performed on an automated C, H, N analyzer. Benzene is a carcinogenic substance. Experiments involving this solvent were performed using safety gloves and in a well-ventilated fume hood. ^1H and ^{13}C NMR spectral analyses were performed on a 300 MHz or 400 MHz and 75 MHz spectrometer, respectively, with residual solvent signal acting as the internal standard. The following abbreviations are used to explain the multiplicities: s, singlet; d, doublet; t, triplet; m, multiplet; band, several overlapping signals, and br, broad. In addition, the following abbreviations are used to denote various carbon nuclei: Ar. (q) and Ar. (t) for phloroglucinol (quaternary) and phloroglucinol (tertiary), respectively; periphery Ar. (q) and periphery Ar. (t) for peripheral aromatic (quaternary) and aromatic (tertiary), respectively.

4.1.1. Synthesis of monomer 2. 4-Bromophenol (3.0 g, 17.3 mmol), 1,5-dibromopentane (8.44 g, 36.77 mmol), K_2CO_3 (2.4 g, 17.3 mmol), and 18-crown-6 (cat.) were dissolved in acetone (50 mL) and refluxed for 7 h. The reaction mixture was cooled, filtered, the solvents were removed under reduced pressure, and the residue was dissolved in $CHCl_3$ (100 mL) and washed with H_2O (2×100 mL). The organic layer was concentrated, dried (Na_2SO_4), and purified by column chromatography (100–200 mesh, SiO_2, petroleum ether/EtOAc=95:5) to afford the required mono-alkylated monomer **2** as a waxy solid (4.5 g, 80%, relative to 4-bromophenol). R_f 0.60 (petroleum ether/EtOAc=95:5). ^1H NMR (300 MHz, $CDCl_3$) δ: 1.58 (m, 2H), 1.78 (m, 2H), 1.91 (m, 2H), 4.32 (t, J=6.6 Hz, 2H), 3.91 (t, J=6.3 Hz, 2H), 6.75 (d, J=9.3 Hz, 2H), 7.34 (d, J=9.3 Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 24.8, 28.3, 32.4, 33.5, 67.8, 112.7 (periphery Ar. (q)), 116.3 (periphery Ar. (t)), 132.2 (periphery Ar. (t)), 158.1 (periphery Ar. (q)); Anal. Calcd for $C_{11}H_{14}OBr_2$: C, 41.03; H, 4.38. Found: C, 41.07; H, 4.57.

4.1.2. G_0-Br_3 (4). A mixture of **3** (0.618 g, 1.079 mmol), **1** (0.671 g, 3.88 mmol), K_2CO_3 (0.536 g, 3.88 mmol), and 18-C-6 (cat.) in 2-butanone (20 mL) was stirred at 70 °C for 12 h. The reaction mixture was filtered and solvents were then removed in vacuo, the resulting residue was dissolved in CH_2Cl_2, washed with water, dried, concentrated, and purified (SiO_2, PhMe/EtOAc=98:2) to afford **4**, as a white solid (0.84 g, 92%). Mp: 67–69 °C. TLC R_f 0.62 (PhMe/EtOAc=98:2). ^1H NMR (300 MHz, $CDCl_3$) δ: 1.63 (m, 6H), 1.82 (m, 12H), 3.92 (t, J=6.6 Hz, 12H), 6.05 (s, 3H), 6.75 (d, J=9.3 Hz, 6H), 7.34 (d, J=9.3 Hz, 6H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 22.7, 28.8, 28.9, 67.7, 67.9, 93.8 (Ar. (t)), 112.6 (periphery Ar. (q)), 116.2 (periphery Ar. (t)), 132.1 (periphery Ar. (t)), 158.1 (periphery Ar. (q)), 160.8 (Ar. (q)); GC–MS m/z: 850 $[M]^+$; Anal. Calcd for $C_{39}H_{45}Br_3O_6$: C, 55.14; H, 5.34. Found: C, 55.27; H, 5.34.

4.1.3. G_1-Br_6 (5). A mixture of G_1-$(OH)_6$ (0.3 g, 0.423 mmol), **2** (1.05 g, 3.04 mmol), K_2CO_3 (0.42 g, 3.04 mmol), and 18-C-6 (cat.) in 2-butanone (20 mL) and DMF (2 mL) was heated at 70 °C for 36 h. Solvents were then removed in vacuo and the resulting residue was dissolved in CH_2Cl_2, washed with water, dried (Na_2SO_4), and concentrated. Excess of **2** was removed by triturating the crude residue with Et_2O and the resulting residue was purified further (SiO_2, PhMe/EtOAc=95:5) to afford **5**, as a colorless oil (0.77 g, 85%). TLC R_f 0.71 (PhMe/EtOAc=96:4). ^1H NMR (300 MHz, $CDCl_3$) δ: 1.64 (m, 18H), 1.83 (m, 36H), 3.93 (t, J=6.3 Hz, 36H), 6.06 (s, 12H), 6.76 (d, J=9.3 Hz, 12H), 7.34 (d, J=9.3 Hz, 12H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 22.7, 28.9, 67.7, 68.0, 93.9 (Ar. (t)), 112.7 (periphery Ar. (q)), 116.3 (periphery Ar. (t)), 132.2 (periphery Ar. (t)), 158.2 (periphery Ar. (q)), 160.7 (Ar. (q)); Anal. Calcd for $C_{105}H_{126}Br_6O_{18}$: C, 58.51; H, 5.89. Found: C, 58.77; H, 6.19.

4.1.4. G_2-Br_{12} (6). A mixture of G_2-$(OH)_{12}$ (0.2 g, 0.423 mmol), **2** (0.527 g, 1.536 mmol), K_2CO_3 (0.21 g, 1.536 mmol), and 18-C-6 (cat.) in 2-butanone (15 mL) and DMF (5 mL) was heated at 70 °C for 36 h. Solvents were then removed in vacuo and the resulting residue was dissolved in CH_2Cl_2, washed with water, dried (Na_2SO_4), and concentrated. Excess of **2** was removed by triturating the crude residue with Et_2O and the resulting residue was purified further (SiO_2, PhMe/EtOAc=95:5) to afford **6**, as a colorless oil (0.374 g, 70%). TLC R_f 0.54 (PhMe/EtOAc=98:2). ^1H NMR (300 MHz, $CDCl_3$) δ: 1.60 (m, 42H), 1.80 (m, 84H), 3.92 (m, 84H), 6.06 (s, 30H), 6.75 (d, J=9.0 Hz, 24H), 7.34 (d, J=9.0 Hz, 24H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 15.2, 22.7, 65.8, 67.6, 67.9, 93.8 (Ar. (t)), 112.6 (periphery Ar. (q)), 116.2 (periphery Ar. (t)), 132.1 (periphery Ar. (t)), 158.1 (periphery Ar. (q)), 160.8 (Ar. (q)); Anal. Calcd for $C_{237}H_{288}Br_{12}O_{42}$: C, 59.71; H, 6.09. Found: C, 59.27; H, 5.92.

4.1.5. G_3-Br_{24} (7). A mixture of G_3-$(OH)_{24}$ (0.15 g, 0.036 mmol), **2** (0.353 g, 1.03 mmol), K_2CO_3 (0.143 g, 1.03 mmol), and 18-C-6 (cat.) in 2-butanone (15 mL) and DMF (2 mL) was heated at 100 °C for 96 h. Solvents were then removed in vacuo and the resulting residue was dissolved in CH_2Cl_2, washed with water, dried (Na_2SO_4), and concentrated. Excess of **2** was removed by triturating the crude residue with MeOH and the resulting residue was purified further (SiO_2, PhMe/EtOAc=95:5) to afford **7**, as a brown oil (0.12 g, 34%). TLC R_f 0.40 (PhMe/EtOAc=98:2). ^1H NMR (300 MHz, $CDCl_3$) δ: 1.61 (m, 90H), 1.81 (m, 180H), 3.92 (m, 180H), 6.05 (s, 66H), 6.74 (d, J=8.4 Hz, 48H), 7.33 (d, J=8.4 Hz, 48H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 22.7, 28.9, 29.0, 67.6, 67.9, 93.8 (Ar. (t)), 112.6 (periphery Ar. (q)), 116.2 (periphery Ar. (t)), 132.1 (periphery Ar. (t)), 158.1 (periphery Ar. (q)), 160.8 (Ar. (q)); Anal. Calcd for $C_{501}H_{612}Br_{24}O_{90}$: C, 60.22; H, 6.17. Found: C, 59.90; H, 6.29.

4.2. General procedure for the synthesis of deuterated dendrimers

Dendrimer (1.0 equiv) was dissolved in benzene (2 mL) and the system was charged with an inert atmosphere (N_2 atm). *n*-BuLi (2.0 equiv per bromophenyl group) was added and warmed to 50 °C for 45 min. The white precipitate was cooled to 0 °C and D_2O (99.9 atom %, Aldrich, 2.0 equiv per bromophenyl group) was added slowly over a period of 5 min and the reaction mixture was allowed to stir at room temperature for another 15 h. Solvents were removed under reduced pressure, washed with $CHCl_3$ (10 mL), and

H_2O (2×5 mL). The organic layer was dried (Na_2SO_4) and concentrated to afford the required deuterated dendrimers in good yield.

4.2.1. G_0-$(D)_3$ (12). G_0-$(Br)_3$ (62 mg, 0.073 mmol). G_0-$(D)_3$ (40 mg, 90%). TLC R_f 0.50 (PhMe/EtOAc=98:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.64 (m, 6H), 1.88 (m, 12H), 3.94 (m, 12H), 6.07 (s, 3H), 6.89 (d, J=8.7 Hz, 6H), 7.27 (d, J=8.7 Hz, 6H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 22.7, 29.0, 29.1, 67.6, 67.7, 93.8 (Ar. (t)), 114.4 (periphery Ar. (t)), 129.3 (periphery Ar. (t)), 129.4 (periphery Ar. (t)), 159.0 (periphery Ar. (q)), 160.8 (Ar. (q)); HRMS *m/z*: 850 $[M]^+$; Anal. Calcd for $C_{39}H_{45}D_3O_6$: C, 76.06; H, 8.35. Found: C, 76.00; H, 8.37.

4.2.2. G_1-$(D)_6$ (13). G_1-$(Br)_6$ (40 mg, 0.019 mmol). G_1-$(D)_6$ (27 mg, 86%). TLC R_f 0.55 (PhMe/EtOAc=96:4). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.64 (m, 18H), 1.82 (m, 36H), 3.97 (m, 36H), 6.06 (s, 12H), 6.89 (d, J=8.4 Hz, 12H), 7.26 (d, J=8.4 Hz, 12H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 22.7, 29.0, 67.6, 67.7, 68.0, 93.8 (Ar. (t)), 114.5 (periphery Ar. (t)), 129.4 (periphery Ar. (t)), 129.5 (br Ar. (t)), 159.0 (periphery Ar. (q)), 160.8 (Ar. (q)); Anal. Calcd for $C_{105}H_{126}D_6O_{18}$: C, 74.70; H, 8.24. Found: C, 74.39; H, 7.93.

4.2.3. G_2-$(D)_{12}$ (14). G_2-$(Br)_{12}$ (35 mg, 7.34 μmol). G_2-$(D)_{12}$ (20 mg, 71%). TLC R_f 0.51 (PhMe/EtOAc=98:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.64 (m, 42H), 1.84 (m, 84H), 3.94 (m, 84H), 6.06 (s, 30H), 6.88 (d, J=8.4 Hz, 24H), 7.26 (d, J=8.4 Hz, 24H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 22.7, 28.9, 29.0, 67.6, 67.7, 93.8 (Ar. (t)), 114.4 (periphery Ar. (t)), 129.3 (periphery Ar. (t)), 129.4 (br Ar. (t)), 159.0 (periphery Ar. (q)), 160.8 (Ar. (q)); Anal. Calcd for $C_{237}H_{288}D_{12}O_{42}$: C, 74.26; H, 8.20. Found: C, 74.10; H, 7.98.

4.2.4. G_3-$(D)_{24}$ (15). G_3-$(Br)_{24}$ (30 mg, 3.0 μmol). G_3-$(D)_{24}$ (18 mg, 75%). TLC R_f 0.35 (PhMe/EtOAc=98:2). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.62 (m, 90H), 1.81 (m, 180H), 3.91 (m, 180H), 6.05 (s, 66H), 6.78 (br d, 48H), 7.25 (br d, 48H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 22.7, 29.0, 67.6, 67.8, 93.8 (Ar. (t)), 114.6 (periphery Ar. (t)), 129.3 (periphery Ar. (t)), 129.4 (periphery Ar. (t)), 159.0 (periphery Ar. (q)), 160.8 (Ar. (q)); Anal. Calcd for $C_{501}H_{612}D_{24}O_{90}$: C, 74.08; H, 8.19. Found: C, 72.87; H, 7.96.

4.3. General procedure for the synthesis of carboxylic acid functionalized dendrimers

Dendrimer (1.0 molar equiv) was dissolved in benzene (2 mL) and the system was charged with inert atmosphere (N_2 atm). *n*-BuLi (2.0 molar equiv) was added and warmed to 50 °C for 45 min. The white precipitate was cooled to 0 °C and CO_2 (g) was passed over a period of 45 min and allowed to stir at room temperature for 15 h. The reaction mixture was cooled to 0 °C, ice was added, allowed to stir for 30 min, followed by the addition of concd HCl (0.5 mL). The pink color precipitate was separated by filtration, washed with ice-cold water (50 mL), co-evaporated with benzene (5×2 mL), and dried to afford the acid functionalized dendrimers, as a light pink color solid.

4.3.1. G_0-$(CO_2H)_3$ (16). G_0-$(Br)_3$ (60 mg, 0.071 mmol). G_0-$(CO_2H)_3$ (40 mg, 63%). Mp: 81.4–83.4 °C. IR (KBr, cm^{-1}): 3430.7, 1685.5, 1606.5; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.55 (m, 6H), 1.76 (m, 12H), 3.97-4.03 (m, 12H), 6.21 (s, 3H), 6.99 (d, J=8.0 Hz, 6H), 7.87 (d, J=8.0 Hz, 6H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ: 22.0, 22.1, 22.2, 28.1, 28.3, 67.7, 68.0, 92.1 (Ar. (t)), 114.1 (periphery Ar. (t)), 123.1 (periphery Ar. (q)), 131.3 (periphery Ar. (t)), 156.6 (Ar. (q)), 162.2 (periphery Ar. (q)), 167.1 (carbonyl).

4.3.2. G_1-$(CO_2H)_6$ (17). G_1-$(Br)_6$ (56 mg, 0.026 mmol). G_0-$(CO_2H)_3$ (45 mg, 90%). IR (KBr, cm^{-1}): 3436.5, 1685.5, 1604.5; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.64 (m, 18H), 1.82 (m, 36H), 4.03–4.06 (m, 36H), 6.26 (s, 12H), 7.01 (d, J=8.4 Hz, 12H), 7.94 (d, J=8.4 Hz, 12H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ: 21.7, 22.0, 22.2, 28.1, 28.2, 28.3, 67.7, 67.9, 92.1 (Ar. (t)), 114.2 (periphery Ar. (t)), 114.4 (periphery Ar. (t)), 122.9 (periphery Ar. (q)), 131.3 (periphery Ar. (t)), 156.6 (Ar. (q)), 162.2 (periphery Ar. (q)), 167.1 (carbonyl).

4.3.3. G_2-$(CO_2H)_{12}$ (18). G_2-$(Br)_{12}$ (50 mg, 0.011 mmol). G_2-$(CO_2H)_{12}$ (38 mg, 84%). IR (KBr, cm^{-1}): 3436.5, 1637.5, 1604.5; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.49 (m, 42H), 1.71 (m, 84H), 3.94 (m, 84H), 6.17 (s, 30H), 6.92 (br s, 24H), 7.85 (br s, 24H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ: 22.0, 22.1, 22.2, 28.2, 28.3, 67.7, 68.0, 92.1 (Ar. (t)), 114.2 (periphery Ar. (t)), 114.4 (periphery Ar. (t)), 123.2 (Ar. (q)), 131.4 (periphery Ar. (t)), 156.7 (Ar. (q)), 162.2 (periphery Ar. (q)), 167.2 (carbonyl).

4.3.4. G_3-$(CO_2H)_{24}$ (19). G_2-$(Br)_{12}$ (36 mg, 3.6 μmol). G_2-$(CO_2H)_{12}$ (20 mg, 60%). IR (KBr, cm^{-1}): 3424.8, 1683.6, 1606.4; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.62 (m, 90H), 1.81 (m, 180H), 3.91 (m, 180H), 6.05 (s, 66H), 6.78 (br d, 48H), 7.25 (br d, 48H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ: 21.9, 22.0, 28.2, 28.3, 67.7, 68.0, 92.2 (Ar. (t)), 114.2 (periphery Ar. (t)), 114.4 (periphery Ar. (t)), 123.1 (periphery Ar. (q)), 131.3 (periphery Ar. (t)), 156.6 (Ar. (q)), 162.2 (periphery Ar. (q)), 167.1 (carbonyl).

4.4. Esterification of carboxylic acid functionalized dendrimers

To a solution of acid functionalized dendrimer in methanol a catalytic amount of concd H_2SO_4 was added and refluxed for 24 h. Solvents were removed in vacuo, the crude reaction mixture was washed with $CHCl_3$ and H_2O. The organic layer was dried (Na_2SO_4), concentrated, and purified by column chromatography (SiO_2, 100–200 mesh) to afford the corresponding ester functionalized dendrimers as a glassy material.

4.4.1. G_0-$(CO_2Me)_3$ (20). Yield: 81%. TLC R_f 0.48 (PhMe/EtOAc=9:1). IR (KBr, cm^{-1}): 1724.1; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.67 (m, 6H), 1.87 (m, 12H), 3.88 (s, 9H), 3.94 (t, J=6.3 Hz, 6H), 4.03 (t, J=6.3 Hz), 6.06 (s, 3H), 6.90 (d, J=9.0 Hz, 6H), 7.98 (d, J=9.0 Hz, 6H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 22.7, 28.9, 51.80, 67.7, 67.9, 93.8 (Ar. (t)), 114.03 (periphery Ar. (t)), 122.4 (periphery Ar. (q)), 131.6 (periphery Ar. (t)), 160.9 (Ar. (q)), 162.8 (Ar. (q)), 166.9 (carbonyl); HRMS *m/z*: 809.1497 $[M+Na]^+$; Anal. Calcd for $C_{45}H_{54}O_{12}$: C, 68.68; H, 6.92. Found: C, 68.87; H, 7.04.

4.4.2. G_1-$(CO_2Me)_6$ (21). Yield: 90%. TLC R_f 0.37 (PhMe/EtOAc=8:2). IR (KBr, cm^{-1}): 1724.1, 1712.5; ^{1}H NMR

(300 MHz, $CDCl_3$) δ: 1.67 (m, 18H), 1.84 (m, 36H), 3.88 (s, 18H), 3.94 (m, 12H), 4.02 (m, 24H), 6.06 (s, 12H), 6.89 (d, J=9.3 Hz, 12H), 7.97 (d, J=9.3 Hz, 12H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 22.7, 28.8, 28.9, 51.80, 67.7, 67.9, 93.8 (Ar. (t)), 114.0 (periphery Ar. (t)), 122.4 (periphery Ar. (q)), 131.5 (periphery Ar. (t)), 160.8 (Ar. (q)), 162.8 (periphery Ar. (q)), 166.9 (carbonyl); Anal. Calcd for $C_{117}H_{144}O_{30}$: C, 69.21; H, 7.15. Found: C, 69.37; H, 7.08.

4.4.3. G_2-$(CO_2Me)_{12}$ (22). Yield: 84%. TLC R_f 0.45 (PhMe/EtOAc=7:3). IR (KBr, cm^{-1}): 1724.1, 1716.6; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.67 (m, 42H), 1.84 (m, 84H), 3.88 (s, 36H), 3.94 (m, 60H), 4.02 (m, 24H), 6.06 (s, 30H), 6.89 (d, J=9.3 Hz, 24H), 7.97 (d, J=9.3 Hz, 24H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 22.7, 28.9, 29.0, 51.9, 67.7, 67.9, 93.9 (Ar. (t)), 114.0 (periphery Ar. (t)), 122.4 (periphery Ar. (q)), 131.6 (periphery Ar. (t)), 160.9 (Ar. (q)), 162.8 (periphery Ar. (q)), 166.9 (carbonyl); Anal. Calcd for $C_{261}H_{324}O_{66}$: C, 69.39; H, 7.23. Found: C, 69.53; H, 7.07.

4.4.4. G_3-$(CO_2Me)_{24}$ (23). Yield: 76%. TLC R_f 0.41 (PhMe/EtOAc=7:3). IR (KBr, cm^{-1}): 1724.1, 1716.6; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.63 (m, 90H), 1.85 (m, 180H), 3.88 (s, 72H), 3.94 (m, 132H), 4.03 (t, J=6.3 Hz, 48H), 6.06 (s, 66H), 6.89 (d, J=10.8 Hz, 48H), 7.97 (d, J=10.8 Hz, 48H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 22.7, 28.9, 29.2, 29.5, 51.8, 67.9, 93.8 (Ar. (t)), 114.0 (periphery Ar. (t)), 120.1 (periphery Ar. (q)), 131.6 (periphery Ar. (t)), 160.9 (Ar. (q)), 162.8 (periphery Ar. (q)), 165.6 (carbonyl); Anal. Calcd for $C_{549}H_{684}O_{138}$: C, 69.47; H, 7.26. Found: C, 69.63; H, 7.06.

Acknowledgements

We are grateful to Council of Scientific and Industrial Research (CSIR), New Delhi, for their financial support. J.N. thanks CSIR for a research fellowship.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.04.052.

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Tetrahedron 62 (2006) 6236–6247
Tetrahedron

Photo- and electroluminescent properties of cyano-substituted styryl derivatives and synthesis of CN–PPV model compounds containing an alkoxy spacer for OLEDs

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Received 20 December 2005; revised 7 April 2006; accepted 20 April 2006
Available online 18 May 2006

Abstract—The series of cyano-substituted model compounds **18**–**20** for organic light emitting diodes (OLEDs) was prepared through Knoevenagel condensation reactions between the dialdehydes **3**,**6**,**9** and the differently substituted acetonitrile derivatives **11**,**16**,**17**. The influence of the different substituents on the optical properties of the resulting α-cyanostyryl compounds **18**–**20** was investigated. Another series of dimeric cyano-substituted styryl compounds **26**–**28** were prepared, in which a flexible, nonconjugated spacer is present between the two cyanostyryl moieties. The spacer isolates the π-conjugated portions of dimeric compounds **26**–**28**, improves their solubility in common organic solvents, and decreases their tendency to crystallize. These features are favorable for producing efficient luminescent films in OLEDs devices.

1. Introduction

During the last decade the application of organic materials in the area of optoelectronic technology has received great interest in academia and industry due to the easier fabrication in comparison to inorganic semiconductors and their easily tunable optical properties. The discovery of light emitting properties in electroluminescent (EL) devices based on poly(*p*-phenylenevinylene) (PPV) became a milestone in the development of organic light emitting materials,[1] and numerous efforts have been directed to the development of PPV derivatives in order to produce highly efficient organic EL devices as commercial products.[2,3] In particular, the introduction of electron-withdrawing groups on the polymer backbone (e.g., CN–PPV) results in a high electron affinity polymer, which can be used to manufacture organic light emitting diodes (OLEDs) with air-stable electrodes and produces a strong red-shift in the photo- and electroluminescent spectra.[4] The tuning of color emission in OLEDs can be accomplished by including functional groups in the polymer backbone or by changing the polymer repeat units.[5,6] Polymers in electronic applications show some disadvantages such as lack of efficient purification procedures, ill-defined structure, and generally scarce processability. For these reasons much interest arose for developing new materials in electronic applications with low molecular mass. In addition, short chain model compounds, which have electronic properties similar to those of their polymeric analogues were synthesized and studied in order to understand the mechanism of light emission in OLEDs.[7] Recently, a white-light-emitting electroluminescent device based on a single-emitting-component with a carbazyl moiety has been reported.[8]

Furthermore, a number of PPV derivatives containing nonconjugated blocks as spacer have been also studied.[9] The interruption of π-conjugation by spacers in the luminescent materials results in a blue-shift of their PL and EL spectra compared to fully conjugated materials and the nonconjugated spacer increases the solubility of the materials.

In this work, we present the synthesis and the characterization of CN–PPV model compounds **18**–**20** containing phenyl-, naphthyl-, and carbazyl units (Fig. 1). Upon variation of the substituents R, the influence on the photoluminescent and electroluminescent characteristics is investigated. Moreover, the synthesis and optical properties of CN–PPV model compounds **26**–**28** (Fig. 1) containing the alkoxy spacer, –O$(CH_2)_6$O–, was also accomplished in order to study their optical properties. The synthesis of **26**–**28** had the purpose of increasing the solubility and the processability of these compounds in the process of film preparation.

Keywords: Organic light emitting diodes; Photoluminescence; Electroluminescence; CN–PPV; Knoevenagel condensation; Spacer.
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doi:10.1016/j.tet.2006.04.051

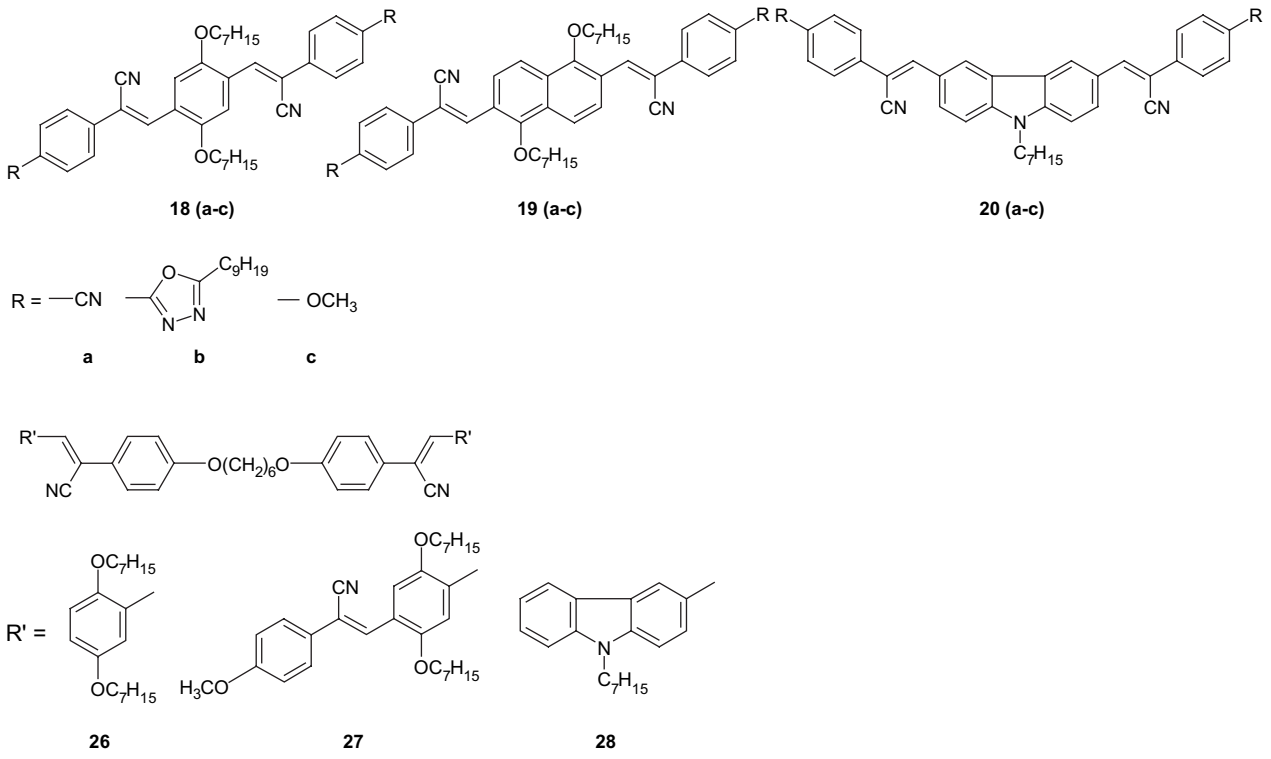
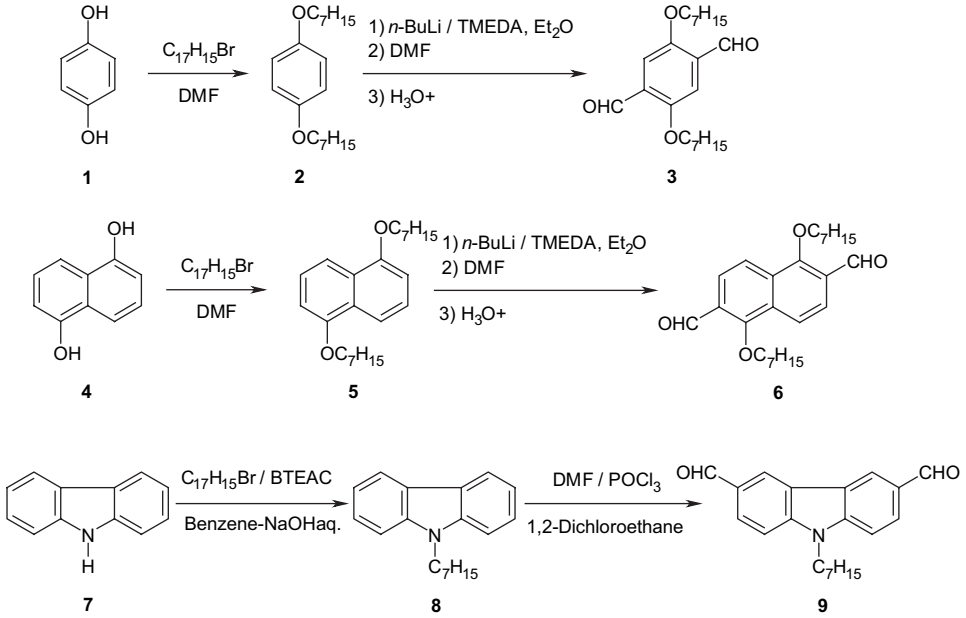


Figure 1. General structures of model compounds **18**–**20** and **26**–**28**.

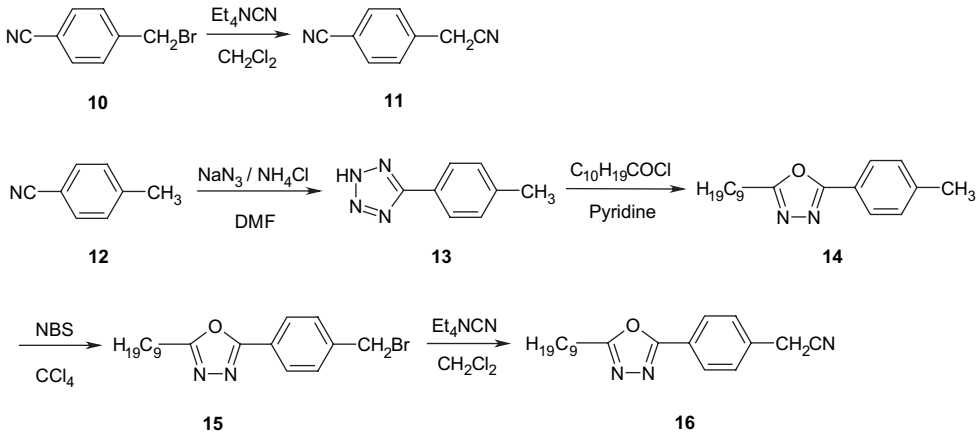
2. Results and discussion

The synthetic pathways for the precursors of the central units of the model compounds **18**–**20** are shown in Scheme 1. Dialdehydes **3** and **6** were prepared via dilithiation of aromatic ethers **2** and **5**, which were obtained by alkylation of commercially available hydroquinone and 1,5-dihydroxynaphthalene with *n*-bromoheptane in the presence of potassium carbonate in dimethyl formamide (DMF).[10] The dilithiated intermediate of **2** and **5** was allowed to react with DMF employing an excess of *n*-butyllithium/*N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) complex in diethyl ether to give the dialdehydes **3** and **6**.[11]

Compound **9** was obtained from **8** by Vilsmeier reaction with an excess of phosphorus oxychloride and DMF.[12] Alkylation of commercially available carbazole **7** was accomplished with *n*-bromoheptane in the presence of benzyltriethylammonium chloride (BTEAC) and aqueous sodium hydroxide in benzene to obtain **8**.[13]



Scheme 1. Syntheses of dialdehyde precursors **3**, **6**, and **9**.

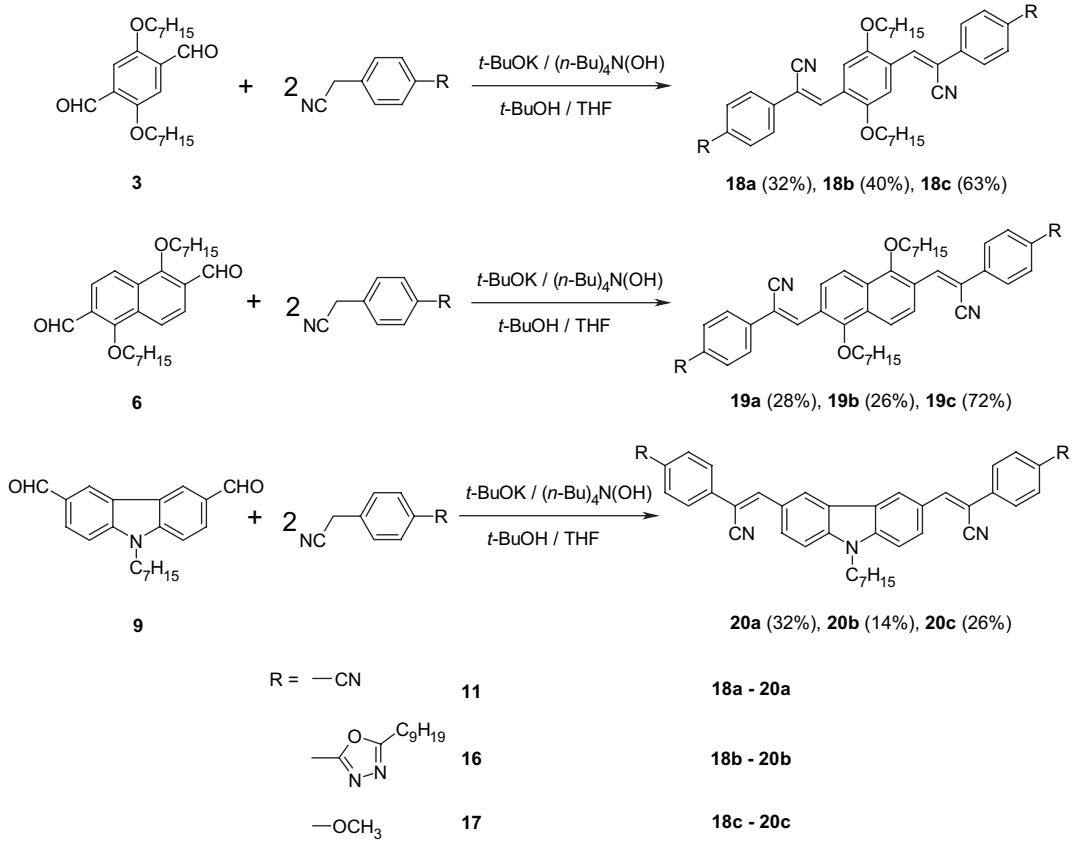


Scheme 2. Syntheses of acetonitrile precursors **11** and **16**.

The acetonitriles **11** and **16**, which contain various substituents were prepared as shown in Scheme 2. *p*-Bromomethylbenzonitrile readily reacted with tetraethylammonium cyanide (Et_4NCN) in dichloromethane to give acetonitrile **11** under mild conditions.[14]

Two synthetic routes to oxadiazoles are ring closure of bishydrazides with dehydrating agents[15] and intramolecular ring transformation of tetrazoles with acid chlorides.[16] In this work the oxadiazole **14** was obtained via tetrazole **13** as an intermediate. This synthetic route has some advantages, such as short reaction time and facile work-up procedure, in comparison to ring closure of bishydrazides with dehydrating agent. The tetrazole **13** was prepared by reacting 4-tolunitrile **12** with sodium azide in DMF.[17] Compound **13** reacted with decanecarboxylic acid chloride in pyridine under reflux to afford oxadiazole **14**, which was then converted to bromide **15** with *N*-bromosuccinimide (NBS) in CCl_4. Treatment of **15** with Et_4NCN in dichloromethane gave oxadiazole **16** using the same method as mentioned above.

The syntheses of model compounds **18**–**20** were accomplished using Knoevenagel condensation[18] between alkoxy- or alkyl-substituted dialdehydes **3**, **6**, **9** and acetonitriles **11**, **16**, the commercially available 4-methoxyphenylacetonitrile **17**, respectively, in *tert*-butyl alcohol and tetrahydrofuran (THF) at 50 °C (see Scheme 3). Potassium *tert*-butoxide and tetra-*n*-butylammonium hydroxide were used as bases.



Scheme 3. Synthesis of model compounds **18**–**20**.

The Knoevenagel reaction is stereoselective, resulting in the *E*,*E*-product. All compounds were characterized by ^{1}H NMR, ^{13}C NMR, MS, IR, and UV–vis spectroscopy (see Section 4).

The monoformylation[19] of **2** was accomplished by Friedel–Crafts reaction with titanium tetrachloride as a Lewis acid catalyst and dichloromethyl methyl ether to result in the formation of **21**. Compound **22** was prepared by Knoevenagel condensation between excess of **3** and 4-methoxyphenylacetonitrile **17** using potassium *tert*-butoxide as base in *tert*-butyl alcohol and THF. The monoformylation of **8** was carried out by using Vilsmeier reagent to afford compound **23** (Scheme 4).

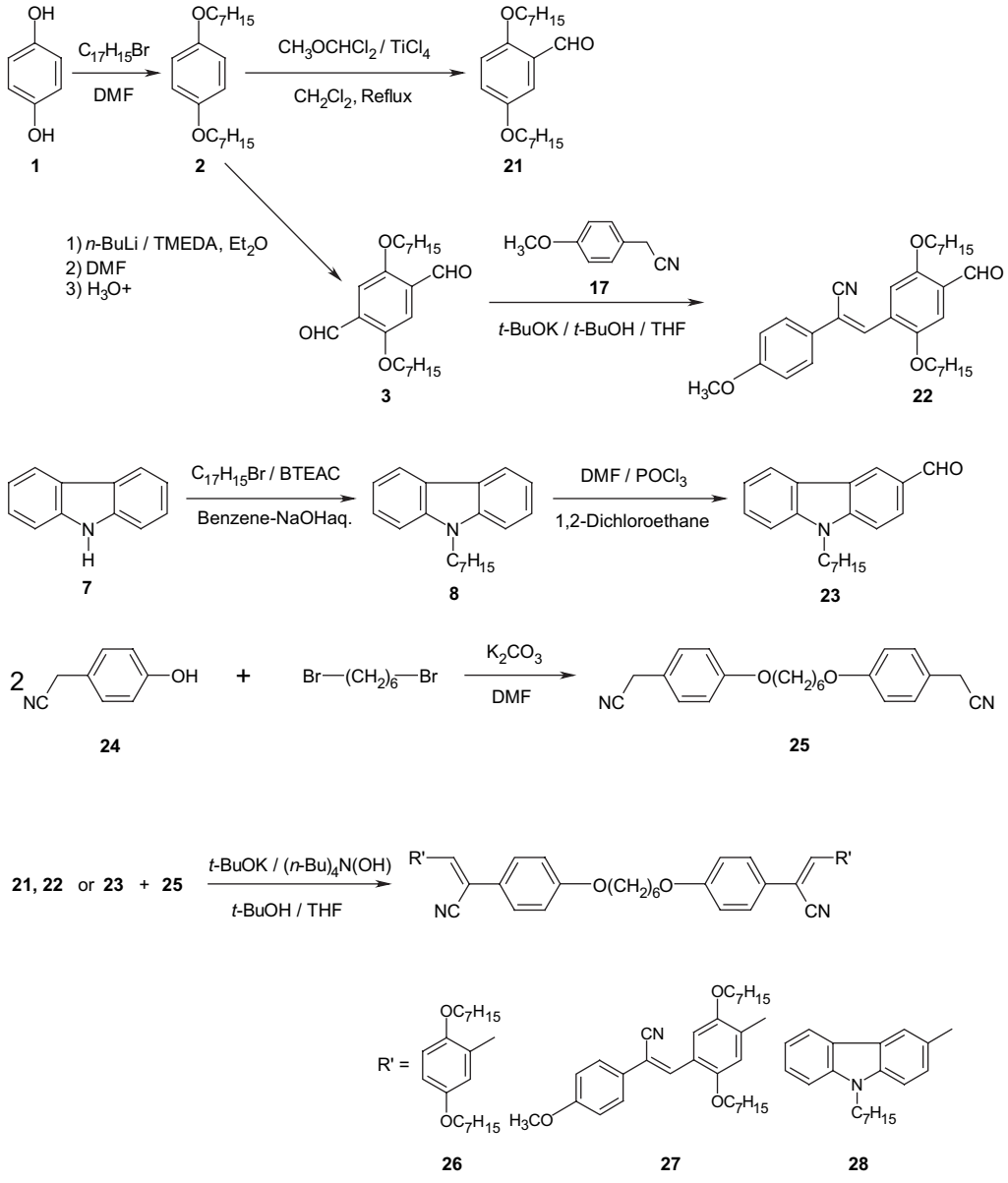
The reaction between 4-hydroxyphenylacetonitrile **24** and 1,6-dibromohexane was carried out in DMF with potassium carbonate as base leading to **25**, in which the two phenylacetonitriles are separated by the alkoxy spacer –O(CH$_2$)$_6$O–.

Compounds **26**–**28** were synthesized by Knoevenagel condensation between the monoaldehydes **21**–**23** and 1,6-bis(4-cyanomethylphenoxy)hexane **25**, respectively, in *tert*-butyl alcohol and THF at 50 °C with potassium *tert*-butoxide and tetra-*n*-butylammonium hydroxide as bases to result in the *E*,*E*-products. All compounds were characterized by ^{1}H NMR, ^{13}C NMR, MS, IR, and UV–vis-spectroscopy (see Section 4).

2.1. Optical properties

Figures 2 and 3 show the UV–vis absorption and photoluminescence (PL) spectra of **18**–**20** and **26**–**28**, respectively. The PL spectra were measured as thin films.

Optical and electrical properties of conjugated materials are dependent on the length of the conjugated π-system, interchain distance of the conjugated segment, and the influence of substituents (electron-donating or electron-withdrawing)



Scheme 4. Synthetic pathways for model compounds **26**–**28**.

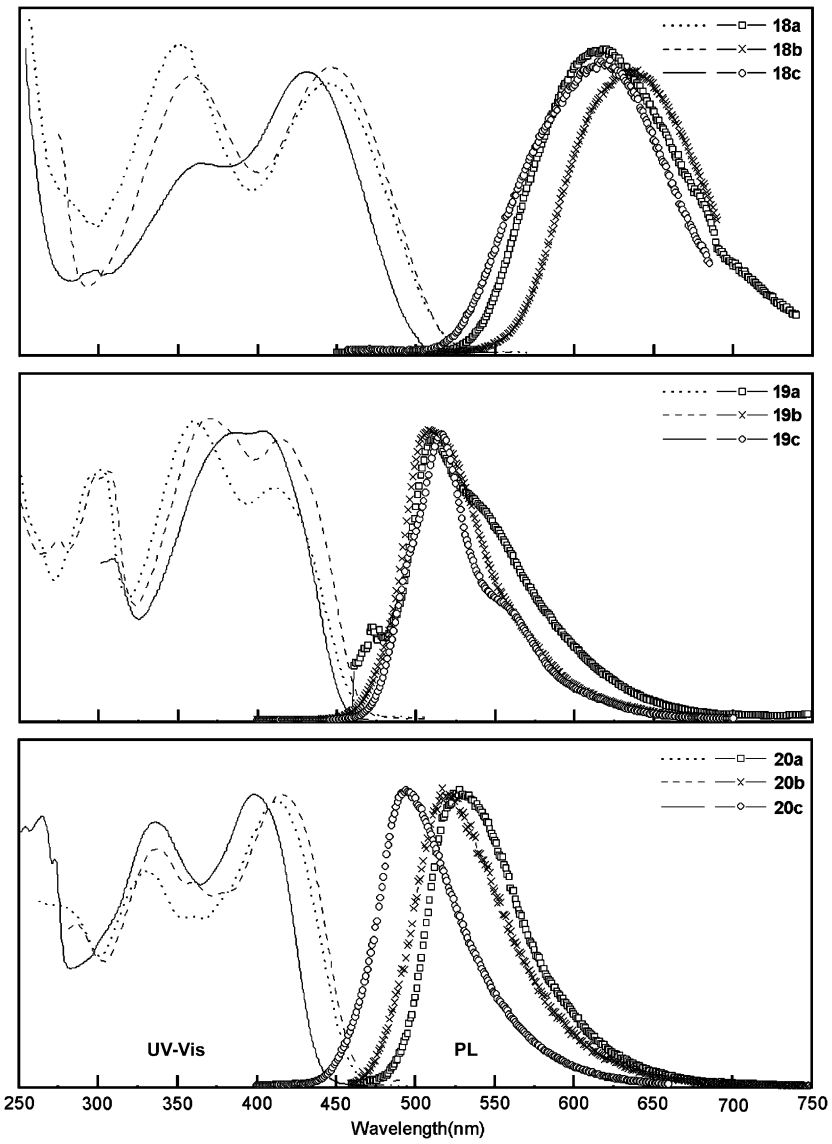


Figure 2. UV–vis absorption (CH_2Cl_2) and PL spectra (thin film) of **18**–**20**.

of the conjugated system. The influence of donor and acceptor substituents on the electronic structure of PPV derivatives has been studied.[20] Electron-donating substituents lead to an overall destabilization of the HOMO and LUMO levels. In contrast, electron-withdrawing substituents give a stabilization of the frontier levels. These substituents influence the location of HOMO and LUMO levels, respectively, to result in a decrease of the band gap.

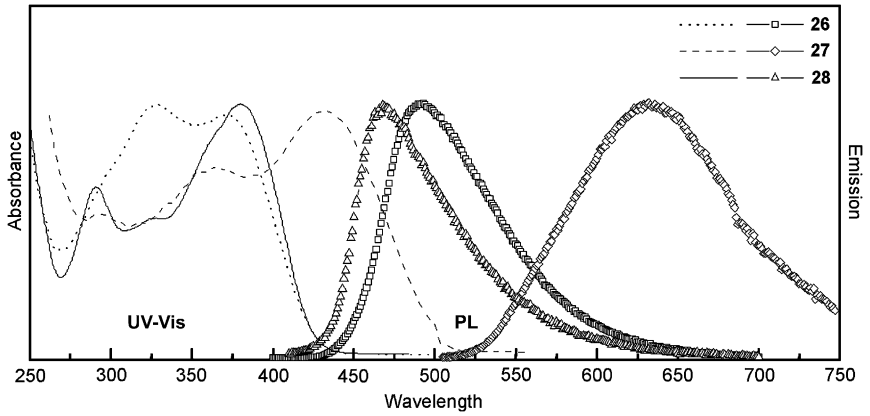


Figure 3. UV–vis and PL spectra of **26**–**28**.

Table 1. UV–vis absorption maxima, the calculated band gap energy, and PL maxima of **18–20** and **26–28**

Compound	UV–vis (nm)[a]	E_g (eV)[b]	PL (nm)[c]
18a	353, 442	2.36	618
18b	360, 446	2.35	636
18c	366, 432	2.45	615
19a	361, 412	2.65	511
19b	370, 416	2.63	508
19c	386, 404	2.71	515
20a	332, 412	2.65	528
20b	339, 417	2.65	522
20c	336, 399	2.79	495
26	330, 374	2.85	493
27	364, 433	2.45	632
28	291, 381	2.85	468

[a] UV–vis absorption in dichloromethane.
[b] Band gaps were calculated from the absorption spectra of the given model compound solutions.
[c] PL emission in solid state (thin film on glass).

In this work the band gap energy of compounds **18–20** were estimated from their UV–vis absorption spectra in solution as shown in Table 1.

Compounds **18a** and **18b** containing electron-withdrawing groups have smaller band gaps (π–π^* transition energy) than **18c** possessing electron-donating substituent (Table 1). This result can be explained by the effect of π-electron delocalization along the entire conjugated system. The introduction of the electron-withdrawing groups at the end phenyl rings in **18a** and **18b** increases the electron density of the chromophoric block due to the induction of strong permanent dipoles affecting the donor/acceptor strengths of the donating and withdrawing groups to result in a decrease of the energy in the excited state. This increasing electron density causes an enhanced delocalization of conjugated system. Although compound **18a** has smaller band gap than **18c**, both of them exhibit almost identical PL maximum in the solid state. It is assumed that the interchain distance of conjugated segment of **18a** and **18c** is similar in the solid state. Compound **18b** shows a red-shift in its PL spectrum compared to **18a** and **18c** due to the interchain interaction of oxadiazole moieties in the solid state.[21]

Earlier studies have shown that partial replacement of the phenylene unit in PPV by a naphthalene system linked through 1,4-position shows a significant effect in photoluminescence and electroluminescence emission maxima.[22] However, in the case of conjugated materials based on naphthalene unit linked through 2,6-positions, their optical properties are more dependent on the steric effects than their band structure.[23] The geometry optimization has been carried out by the Austin model 1 (AM 1) semiempirical technique to study the torsion and its effect on their structural and electronic properties. The bulky 1,5-dialkoxynaphthalene ring induces torsion of the main chain and, consequently, reduces the planarity of the backbone. The lack of planarity in conjugated materials based on 1,5-dialkoxynaphthalene unit linked through the 2,6-positions leads to a loss of conjugation and results in a blue-shift in their UV–vis spectra.[23] The band gaps were increased by replacing the central phenyl ring in cyano-substituted PPV derivatives with a bulky 1,5-diheptyloxynaphthalene ring (compounds **19**) compared to compounds **18** as shown in Table 1. Therefore, the PL emission maxima of compounds **19** show a significant blue-shift due to the loss of conjugation. The substituents at the *para*-position of end phenyl ring (electron-donating or -withdrawing) in compounds **19** do not affect the optical properties in PL emission spectra. It is assumed that the influence of substituents is compensated by an opposite effect due to predominant steric effects, therefore, compounds **19** exhibit similar PL maxima.

The carbazole unit is very well known as a hole transporting, electroluminescent material, and a wide band gap component.[24] Due to its wide band gap property conjugated systems based on carbazole units in compounds **20** exhibit a blue-shift in comparison to the system based on phenyl rings (compounds **18**). Compounds **20** exhibit PL emission maxima in the green or greenish yellow region (495–528 nm). The optical properties of these compounds show the same tendency as a result of their calculated band gap. The electron-withdrawing (cyano or alkyl oxadiazolyl) substituted **20a** and **20b** exhibit a red-shift compared to electron-donating (methoxy) substituted **20c**. The results show that the electron-withdrawing group affects more to reduce the band gap in conjugated system unit than the donating group on account of the effect of π-electron delocalization along the entire conjugated system as described above.

In general, OLEDs based on short chain PPV derivatives exhibit no detectable electroluminescence (EL) due to their electron conductive qualities. Therefore, an additional hole transporting layer, thermally stable copper phthalocyanine (PcCu) was used. The vacuum deposition of PcCu and model compounds **18–20** onto ITO covered glass substrates was accomplished, and the patterning of the devices were structured as described earlier.[25] The configuration of bilayer device is ITO/PcCu/model compound/Al as shown in Figure 4a.

EL spectra of **18a**, **18c**, and **20c** are almost identical to their PL spectra, indicating that the same excited state is involved in both EL and PL emission processes.[26] However, EL spectra of **19a** and **20a** exhibit a red-shift compared to their PL spectra (Fig. 4). It is assumed that interchain interactions are maximum in vapor deposited thin film of model compounds and interchain excitation is accelerated to result in the red-shift.[27]

In the case of **19c**, the EL spectrum shows a blue-shift compared to its PL spectrum due to the re-absorption effect of PcCu. Figure 4 shows the PL and EL spectra of **18–20**.

In compounds **26–28** the photoluminescent cyano-substituted PPV derivatives are separated by the nonconjugated segment, $-O(CH_2)_6O-$, as spacer group (Fig. 1). The UV–vis absorption and PL emission spectra were measured in dichloromethane and in solid state, respectively. The results are shown in Table 1.

The π-conjugated systems in **26–28** are isolated from each other through a flexible nonconjugated spacer. The introduction of the spacer improves the solubility in common organic solvents and decreases crystallinity, enhancing the properties of the film formation in OLED application. Furthermore, it is a convenient way to obtain a blue-shifted emission due

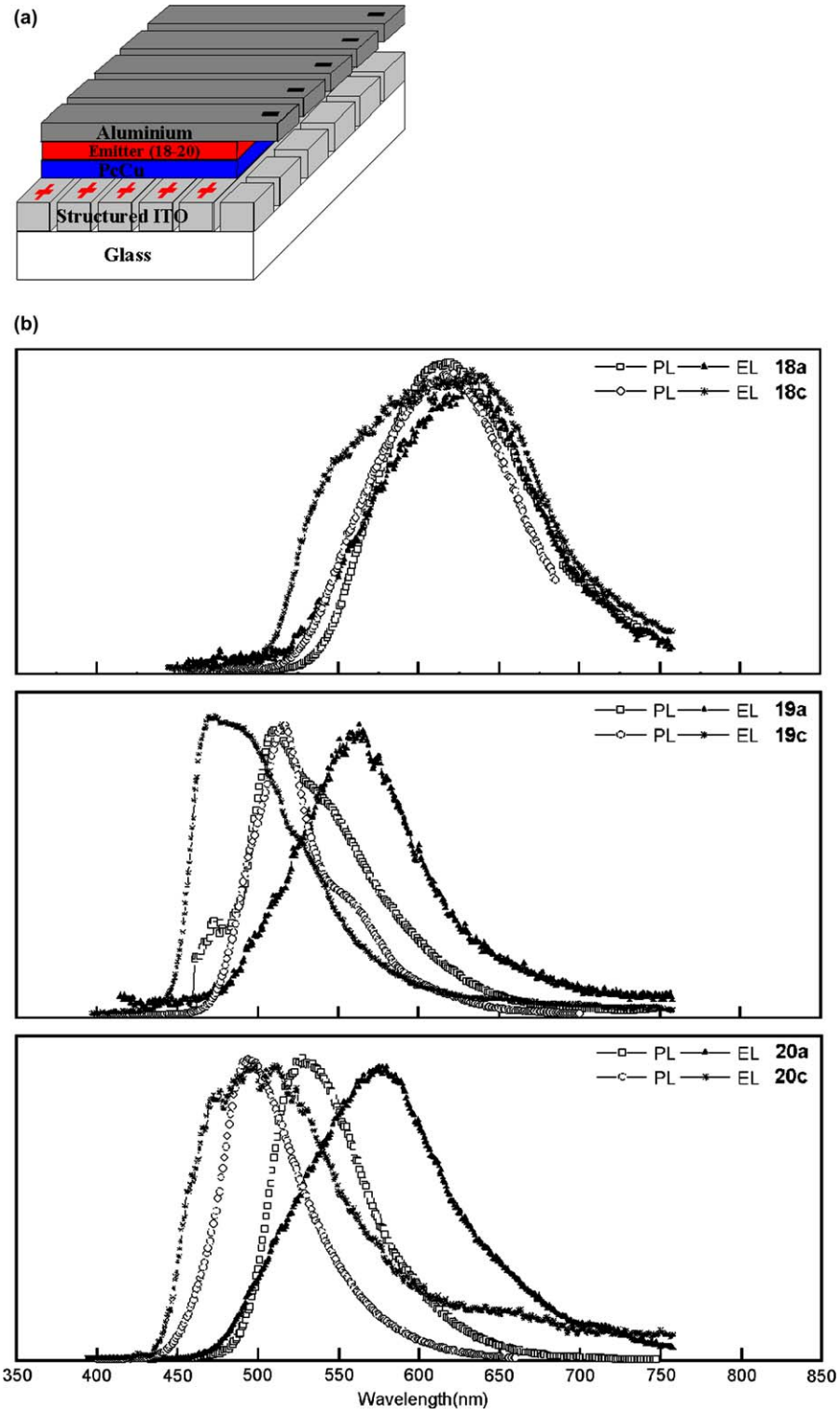


Figure 4. (a) Schematic setup of OLED, (b) PL and EL spectra of **18**–**20**.

to an increase in the band gap by interrupting conjugation in comparison with its fully conjugated system.

Concerning the end-groups of **26**–**28**, the band gap of **27** was decreased significantly compared to **26** to result in a red-shift in the UV–vis and PL spectra due to the extension of π-conjugated system. Compound **28** in which the carbazole ring is combined to cyano-substituted stilbene moiety exhibits a small blue-shift in its PL spectrum compared to **26** and **27** due to the influence of wide band gap component, carbazole.

3. Conclusions

The influence of different substituents on the α-cyanostyryl compounds **18**–**20** (phenyl, naphthalene or carbazole) was investigated by studying their UV–vis, PL, and EL spectra.

The introduction of electron-donating or electron-withdrawing groups into the conjugated system results in a red-shift in their PL spectra on account of the decrease in the band gap, and the effect is stronger in compounds containing electron-withdrawing substituent due to the effect of π-electron delocalization along the entire conjugated system. In the case of conjugated systems based on bulky 1,5-dialkoxynaphthalene ring linked through 2,6-positions, a similar PL maxima were observed. This is explained by predominant steric effect on their optical properties. The steric hindrance of the bulky 1,5-diheptyloxynaphthalene ring reduces the planarity of the backbone and results in a loss of conjugation, thus the influence of substituents are similar between electron-donating and electron-withdrawing substituents. The replacement of the central phenyl ring with a wide band gap carbazole results in a blue-shift in their UV–vis and PL spectra compared to α-cyanostyryl compound based on phenyl ring. The electron-withdrawing substituents affect more to reduce the band gap in the conjugated system based on carbazole unit than the electron-donating substituents due to the effect of π-electron delocalization along the entire conjugated system.

The electroluminescent properties of the model compounds **18**–**20** were studied in bilayer OLEDs with additional hole transporting layer (PcCu). In general, the PL and EL spectra are expected to be identical. However, the observed red- and blue-shift in the EL spectra compared to PL spectra are due to the film morphology of compounds and re-absorption effect of PcCu as hole transporting material, respectively.

The optical properties of model compounds **26**–**28** containing spacer group were studied by extending the cyano-substituted styryl moiety and replacing the phenyl ring with a carbazole ring (i.e., wide band gap component) resulting in a red- and blue-shift, respectively. The introduction of spacer moiety, $-O(CH_2)_6O-$, improves the solubility in common organic solvents and decreases crystallinity, enhancing the properties of the film formation with physical vapor deposition technique in OLED application. Furthermore, it is a convenient way to obtain a blue-shifted emission due to an increase in the band gap by interrupting conjugation, i.e., shortening of chromophore unit.

4. Experimental

4.1. General

Chemicals received from commercial sources (Aldrich, Merck, and Fluka) were used without further purification. All reactions were performed under a dry argon atmosphere. Solvents were dried according to standard procedures. The melting points are uncorrected.

Infrared spectra were taken as KBr pellets or with NaCl plate using a Bruker IFS 48 spectrometer. UV–vis spectra were recorded in CH_2Cl_2 solution with a Shimadzu UV-2102 PC. PL of evaporated films was measured with a SPEX fluorolog 112 in the 45° configuration. For EL measurements an HP 6030A voltage source was used together with a Keithley 171 DMM. The EL spectra were taken from devices with ITO/copper phthalocyanine (PcCu)/trimer **18**–**20**/Al configuration with a waveguide diode array setup in air at room temperature. NMR spectra were recorded on a Bruker AC 250 spectrometer at 250 MHz (^{1}H) and 62.9 MHz (^{13}C) in $CDCl_3$ and internally referenced to $CHCl_3$ (^{1}H: δ=7.24 ppm, ^{13}C: δ=77.00 ppm). Mass spectra were recorded on a Finnigan ISQ 70 and a Varian MAT 711 A. Elemental analysis of the products was carried out with a Carlo Erba Elemental Analyzer 1106.

4.2. Synthesis

The following compounds were prepared according to literature procedures: 1,4-di-*n*-heptyloxybenzene (**2**),[10] 1,5-di-*n*-heptyloxynaphthalene (**5**),[10] 9-*n*-heptylcarbazole (**8**),[13] 3,6-diformyl-9-*n*-heptylcarbazole (**9**),[12] *p*-cyanomethylbenzonitrile (**11**),[14] 5-(*p*-tolyl)tetrazole (**13**),[17] and 2,5-di-*n*-heptyloxybenzaldehyde (**21**).[19] *p*-Bromomethylbenzonitrile (**10**), 4-methoxyphenylacetonitrile (**17**), and 4-hydroxyphenylacetonitrile (**24**) are commercially available.

4.2.1. 2,5-Di-*n*-heptyloxyterephthaldialdehyde (3). A 2.5 M hexane solution of *n*-BuLi (24.6 ml, 61.5 mmol) was added dropwise to a solution of 1,4-di-*n*-heptyloxybenzene (**2**) (3.78 g, 12.3 mmol) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) (7.38 g, 63.5 mmol) in diethyl ether (190 ml) at −78 °C. The mixture was stirred at 0 °C for 1 h and DMF (7.3 g, 100 mmol) was added, which caused a yellow color to darken progressively. After refluxing for 16 h, the resulting pale brown suspension was placed in an ice bath. The mixture was stirred for 90 min at 0 °C and then 4 N HCl solution (37 ml) was added slowly under vigorous stirring. The resulting two-phase system was stirred for 30 min at room temperature. The organic layer was separated, washed with 0.5 N HCl solution (62 ml), saturated $NaHCO_3$ solution (62 ml), and brine (62 ml), and then dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to give a yellow solid. The crude product was purified on a silica gel column (dichloromethane/*n*-hexane). Yield: 1.1 g (25%). Yellow solid. Mp 72–74 °C. ^{1}H NMR ($CDCl_3$): 10.49 (s, 2H), 7.40 (s, 2H), 4.05 (t, *J*=6.4 Hz, 4H), 1.81 (m, 4H), 1.47 (m, 4H), 1.29 (m, 12H), 0.87 (t, *J*=7.0 Hz, 6H). ^{13}C NMR ($CDCl_3$): 189.34, 155.21, 129.28, 111.60, 69.24, 31.71, 29.21, 29.02, 25.95, 22.55, 14.01. IR (KBr): 2918, 2879, 1682, 1491, 1470, 1427, 1388, 1283, 1215, 1130, 1007, 696. MS: 362 (M^+), 264, 166, 57. Anal. Calcd: C, 72.89; H, 9.45. Found: C, 73.21; H, 9.52.

4.2.2. 1,5-Di-*n*-heptyloxynaphthalene-2,6-dicarbaldehyde (6). Compound **6** was prepared by the same method as **3** except for the starting material and the amount of reagents: 1,5-di-*n*-heptyloxynaphthalene (**5**) (5.3 g, 15 mmol), 2.5 M *n*-BuLi (75 mmol), TMEDA (75 mmol), DMF (8.8 g, 121 mmol), and diethyl ether (200 ml). The purification of the product was accomplished by chromatography on a silica gel column (dichloromethane/*n*-hexane). Yield: 1.0 g (16%). Yellowish white solid. Mp 88–90 °C. ^{1}H NMR ($CDCl_3$): 10.57 (s, 2H), 7.99 (d, *J*=8.8 Hz, 2H), 7.93 (*J*=8.8 Hz, 2H), 4.15 (t, *J*=6.4 Hz, 4H), 1.95 (m, 4H), 1.33 (m, 16H), 0.89 (t, *J*=6.7 Hz, 6H). ^{13}C NMR ($CDCl_3$): 189.48, 161.53, 133.13, 127.45, 123.63, 119.50, 79.47, 31.72, 30.24, 29.07, 25.90, 22.56, 14.03. IR (KBr): 2949, 2948, 2854, 1682, 1499, 1466, 1377, 1366, 1227,

1015, 822, 762. MS: 412 (M^+), 314, 216, 131, 57. Anal. Calcd: C, 75.69; H, 8.80, Found: C, 75.71; H, 8.55.

4.2.3. 5-(*p*-Tolyl)-2-*n*-nonyl-1,3,4-oxadiazole (14). To a solution of 5-(*p*-tolyl)tetrazole (**13**) (9.6 g, 60 mmol) in pyridine (95 ml) was added decanecarboxylic acid chloride (11.5 g, 60 mmol). After refluxing for 2 h under argon, the reaction mixture was cooled and poured into water containing crushed ice. The precipitate was collected in a sintered glass filter and then washed with water several times. The crude product was dried under vacuum for overnight. The purification was accomplished by chromatography on a silica gel column using chloroform as eluent. Yield: 14.1 g (82%). White solid. Mp 55–57 °C. ^{1}H NMR ($CDCl_3$): 7.88 (d, J=7.9 Hz, 2H), 7.29 (d, J=7.9 Hz, 2H), 2.88 (t, J=7.3 Hz, 2H), 2.40 (s, 3H), 1.81 (m, 2H), 1.24 (m, 12H), 0.85 (t, J=6.7 Hz, 3H). ^{13}C NMR ($CDCl_3$): 166.75, 164.78, 141.91, 129.66, 126.69, 121.37, 31.81, 29.34, 29.21, 29.10, 29.01, 26.60, 25.43, 23.63, 21.58, 14.06. IR (KBr): 2920, 2851, 1616, 1569, 1556, 1501, 1475, 1180, 1088, 1011, 959, 824, 727. MS: 286 (M^+), 257, 243, 229, 216, 201, 187, 174, 119, 117, 91, 55, 43, 41. Anal. Calcd: C, 75.48; H, 9.15; N, 9.78. Found: C, 75.15; H, 8.83; N, 9.11.

4.2.4. 5-(*p*-Bromomethylphenyl)-2-*n*-nonyl-1,3,4-oxadiazole (15). A mixture of 5-(*p*-tolyl)-2-*n*-nonyl-1,3,4-oxadiazole (**14**) (13.0 g, 45.4 mmol), *N*-bromosuccinimide (8.3 g, 46.6 mmol), dry carbon tetrachloride (250 ml), and catalytic amount of azobisisobutyronitrile was stirred at reflux under nitrogen for 10 h. The warm solution was filtered, and the filtrate was concentrated to give a light yellow solid. The crude product was purified by recrystallization from carbon tetrachloride. Yield: 8.4 g (51%). White solid. Mp 87–89 °C. ^{1}H NMR ($CDCl_3$): 8.00 (d, J=8.2 Hz, 2H), 7.51 (d, J=8.5 Hz, 2H), 4.49 (s, 2H), 2.90 (t, J=7.3 Hz, 2H), 1.82 (m, 2H), 1.24 (m, 12H), 0.85 (t, J=7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$): 167.17, 141.15, 129.63, 127.14, 124.02, 32.25, 31.80, 29.33, 29.18, 29.09, 28.99, 26.55, 25.43, 22.61, 14.06. IR (KBr): 2918, 2851, 1587, 1566, 1501, 1472, 1418, 1229, 1204, 1086, 1015, 854, 704. MS: 364 (M^+), 335, 321, 308, 294, 285, 265, 252, 228, 197, 186, 173, 131, 118, 116, 90, 43. Anal. Calcd: C, 59.18; H, 6.90; Br, 21.87; N, 7.67. Found: C, 58.98; H, 6.11; Br, 21.86; N, 7.60.

4.2.5. 5-(*p*-Cyanomethylphenyl)-2-*n*-nonyl-1,3,4-oxadiazole (16). A portion of tetraethylammonium cyanide (2.0 g, 12.8 mmol) was added to a stirred solution of 5-(*p*-bromomethylphenyl)-2-*n*-nonyl-1,3,4-oxadiazole (**15**) (3.0 g, 8.2 mmol) and dichloromethane (150 ml). The reaction mixture was stirred for 5 h at room temperature and then washed with water. After evaporation of the solvent, the product was purified by chromatography on a silica gel column using dichloromethane/ethyl acetate as eluent. Yield: 2.1 g (82%). White solid. Mp 93–95 °C. ^{1}H NMR ($CDCl_3$): 8.02 (d, J=8.2 Hz, 2H), 7.48 (d, J=8.5 Hz, 2H), 3.82 (s, 2H), 2.90 (t, J=7.3 Hz, 2H), 1.82 (m, 2H), 1.24 (m, 12H), 0.85 (t, J=6.7 Hz, 3H). ^{13}C NMR ($CDCl_3$): 167.28, 163.99, 133.33, 128.60, 127.49, 124.13, 117.01, 31.81, 29.34, 29.19, 29.09, 29.01, 26.57, 25.45, 23.64, 22.61, 14.06. IR (KBr): 2953, 2916, 2849, 2245, 1697, 1589, 1570, 1504, 1472, 1421, 1090, 918, 825, 723. MS: 311 (M^+), 282, 268, 241, 212, 199, 157, 144, 116, 89, 55, 41. Anal. Calcd: C, 73.28; H, 8.09; N, 13.49. Found: C, 72.40; H, 7.80; N, 13.06.

4.3. General procedure for the preparation of α-cyanostyryl compounds (18–20)

To a *tert*-butyl alcohol/THF solution of an aldehydes (**3**, **6** or **9**) and acetonitrile derivatives (**11**, **16** or **17**), respectively, at 50 °C under inert gas, a stoichiometric amount of potassium *tert*-butoxide and a methanolic solution of tetra-*n*-butylammonium hydroxide (catalytic amount) were added quickly (Scheme 3). The mixture was stirred vigorously for 20 min at 50 °C. After cooling, the mixture was poured into methanol. The precipitate (**18**–**20**) was collected using a sintered glass filter, washed with methanol, and dried under vacuum. The purification of **18**–**20** was accomplished by chromatography on a silica gel column using dichloromethane or chloroform as eluent.

4.3.1. 1,4-Bis(4-cyano-α-cyanostyryl)-2,5-di-*n*-heptyloxybenzene (18a). Yield: 32%. Orange solid. Mp 244–246 °C. ^{1}H NMR ($CDCl_3$): 8.12 (s, 2H), 7.89 (s, 2H), 7.77 (d, J=8.9 Hz, 4H), 7.75 (d, J=8.9 Hz, 4H), 4.11 (t, J=6.4 Hz, 4H), 1.84 (m, 4H), 1.29 (m, 16H), 0.86 (t, J=6.7 Hz, 6H). ^{13}C NMR ($CDCl_3$): 151.84, 138.83, 126.60, 125.85, 118.18, 117.47, 112.81, 111.37, 110.48, 69.56, 31.77, 29.09, 28.96, 26.10, 22.54, 14.06. IR (KBr): 2951, 2930, 2856, 2226, 2212, 1585, 1510, 1499, 1431, 1366, 1294, 1252, 1219, 1024, 835. MS: 610 (M^+). UV–vis (CH_2Cl_2): 353, 442. PL: 618. EL: 632. Anal. Calcd: C, 78.66; H, 6.93; N, 9.17. Found: C, 78.11; H, 6.89; N, 9.23.

4.3.2. 1,4-Bis[4-(2-*n*-nonyl-1,3,4-oxadiazolyl)-α-cyanostyryl]-2,5-di-*n*-heptyloxybenzene (18b). Yield: 40%. Pale red solid. Mp 165–167 °C. ^{1}H NMR ($CDCl_3$): 8.13 (s, 2H), 8.09 (d, J=8.9 Hz, 4H), 7.91 (s, 2H), 7.83 (d, J=8.6 Hz, 4H), 4.13 (t, J=6.4 Hz, 4H), 2.93 (t, J=7.3 Hz, 4H), 1.85 (m, 8H), 1.26 (m, 40H), 0.86 (t, J=6.7 Hz, 12H). ^{13}C NMR ($CDCl_3$): 167.37, 163.99, 151.77, 137.45, 137.19, 127.42, 126.58, 125.92, 124.66, 117.86, 111.42, 110.95, 69.54, 31.83, 31.78, 29.36, 29.21, 29.16, 29.12, 29.04, 29.01, 26.60, 26.14, 25.49, 22.64, 22.55, 14.06. IR (KBr): 2955, 2922, 2851, 2214, 1583, 1497, 1466, 1416, 1366, 1294, 1217, 1007, 847. MS: 949 (M^+). UV–vis (CH_2Cl_2): 360, 446. PL: 636. EL: 655. Anal. Calcd: C, 75.91; H, 8.49; N, 8.85. Found: C, 75.63; H, 8.15; N, 8.89.

4.3.3. 1,4-Bis(4-methoxy-α-cyanostyryl)-2,5-di-*n*-heptyloxybenzene (18c). Yield: 63%. Orange solid. Mp 158–160 °C. ^{1}H NMR ($CDCl_3$): 7.89 (s, 2H), 7.85 (s, 2H), 7.60 (d, J=8.9 Hz, 4H), 6.97 (d, J=8.9 Hz, 4H), 4.10 (t, J=6.4 Hz, 4H), 3.85 (s, 6H), 1.83 (m, 4H), 1.31 (m, 16H), 0.87 (t, J=6.7 Hz, 6H). ^{13}C NMR ($CDCl_3$): 160.50, 151.42, 134.03, 127.42, 125.76, 118.52, 114.48, 111.19, 69.46, 55.42, 31.80, 29.24, 29.02, 26.16, 22.53, 14.04. IR (KBr): 2955, 2930, 2854, 2208, 1607, 1514, 1466, 1431, 1367, 1300, 1250, 1217, 1180, 1036, 822. MS: 620 (M^+). UV–vis (CH_2Cl_2): 366, 432. PL: 615. EL: 633. Anal. Calcd: C, 77.39; H, 7.79; N, 4.51. Found: C, 77.36; H, 8.03; N, 4.47.

4.3.4. 2,6-Bis(4-cyano-α-cyanostyryl)-1,5-di-*n*-heptyloxynaphthalene (19a). Yield: 28%. Yellow solid. Mp

221–223 °C. ^{1}H NMR ($CDCl_3$): 8.37 (d, J=8.9 Hz, 2H), 8.20 (s, 2H), 8.02 (d, J=8.9 Hz, 2H), 7.83 (d, J=8.9 Hz, 4H), 7.78 (d, J=8.9 Hz, 4H), 4.02 (t, J=6.4 Hz, 4H), 1.91 (m, 4H), 1.32 (m, 16H), 0.88 (t, J=6.7 Hz, 6H). ^{13}C NMR ($CDCl_3$): 157.01, 139.02, 138.74, 132.94, 130.77, 126.51, 124.76, 119.15, 118.11, 117.15, 112.94, 111.10, 77.80, 31.78, 30.42, 29.13, 26.28, 22.57, 14.06. IR (KBr): 2961, 2928, 2854, 2228, 2214, 1591, 1506, 1467, 1412, 1371, 1250, 1186, 1063, 991, 841, 824. MS: 660 (M^+). UV–vis (CH_2Cl_2): 361, 412. PL: 511. EL: 563. Anal. Calcd: C, 79.97; H, 6.71; N, 8.48. Found: C, 79.54; H, 6.76; N, 8.44.

4.3.5. 2,6-Bis[4-(2-*n*-nonyl-1,3,4-oxadiazolyl)-α-cyanostyryl]-1,5-di-*n*-heptyloxynaphthalene (19b). Yield: 26%. Greenish yellow solid. Mp 165–167 °C. ^{1}H NMR ($CDCl_3$): 8.38 (d, J=9.2 Hz, 2H), 8.21 (s, 2H), 8.12 (d, J=8.9 Hz, 4H), 8.02 (d, J=9.2 Hz, 2H), 7.89 (d, J=8.9 Hz, 4H), 4.03 (t, J=6.7 Hz, 4H), 2.94 (t, J=7.6 Hz, 4H), 1.89 (m, 4H), 1.26 (m, 40H), 0.86 (t, J=7.0 Hz, 12H). ^{13}C NMR ($CDCl_3$): 167.40, 163.96, 156.71, 137.80, 137.32, 130.63, 127.48, 126.49, 124.88, 119.52, 117.56, 111.66, 31.83, 30.45, 29.36, 29.22, 29.18, 29.12, 29.04, 26.60, 26.31, 25.49, 22.64, 22.58, 14.06. IR (KBr): 2920, 2851, 2218, 1601, 1585, 1568, 1499, 1470, 1416, 1371, 1242, 1180, 1034, 1011, 851, 841. MS: 999 (M^+). UV–vis (CH_2Cl_2): 370, 416. PL: 508. EL: 500. Anal. Calcd: C, 76.92; H, 8.27; N, 8.41. Found: C, 76.61; H, 8.23; N, 8.32.

4.3.6. 2,6-Bis(4-methoxy-α-cyanostyryl)-1,5-di-*n*-heptyloxynaphthalene (19c). Yield: 72%. Greenish yellow solid. Mp 162–164 °C. ^{1}H NMR ($CDCl_3$): 8.31 (d, J=9.2 Hz, 2H), 7.98 (d, J=8.9 Hz, 2H), 7.97 (s, 2H), 7.65 (d, J=8.9 Hz, 4H), 7.00 (d, J=8.9 Hz, 4H), 4.00 (t, J=6.4 Hz, 4H), 3.86 (s, 6H), 1.90 (m, 4H), 1.31 (m, 16H), 0.88 (t, J=6.7 Hz, 6H). ^{13}C NMR ($CDCl_3$): 160.64, 155.88, 134.45, 130.25, 127.34, 125.02, 124.91, 119.15, 118.20, 114.57, 112.19, 77.08, 55.45, 31.81, 30.45, 29.19, 26.31, 22.58, 14.04. IR (KBr): 2955, 2928, 2854, 2218, 1609, 1510, 1458, 1408, 1369, 1288, 1261, 1182, 1072, 1034, 835. MS: 670 (M^+). UV–vis (CH_2Cl_2): 386, 404. PL: 515. EL: 473. Anal. Calcd: C, 78.77; H, 7.51; N, 4.18. Found: C, 78.72; H, 7.55; N, 4.25.

4.3.7. 2,6-Bis(4-cyano-α-cyanostyryl)-9-*n*-heptylcarbazole (20a). Yield: 32%. Yellow solid. Mp 225–227 °C. ^{1}H NMR ($CDCl_3$): 8.67 (d, J_1=1.5 Hz, 2H), 8.16 (dd, J_1=1.8 Hz, J_2=8.9 Hz, 2H), 7.79 (s, 2H), 7.77 (d, J=8.5 Hz, 4H), 7.72 (d, J=8.5 Hz, 4H), 7.51 (d, J=8.6 Hz, 2H), 4.32 (t, J=7.0 Hz, 2H), 1.89 (m, 2H), 1.24 (m, 8H), 0.85 (t, J=6.7 Hz, 3H). ^{13}C NMR ($CDCl_3$): 145.25, 142.66, 139.29, 132.75, 128.28, 126.13, 125.22, 123.31, 123.19, 118.33, 118.09, 112.00, 109.95, 106.40, 43.73, 31.65, 28.95, 27.18, 22.50, 13.98. IR (KBr): 2953, 2928, 2226, 2212, 1580, 1487, 1391, 1259, 1238, 1196, 1138, 839. MS: 569 (M^+). UV–vis (CH_2Cl_2): 332, 412. PL: 528. EL: 580. Anal. Calcd: C, 82.22; H, 5.48; N, 12.29. Found: C, 82.53; H, 4.43; N, 12.29.

4.3.8. 3,6-Bis[4-(2-*n*-nonyl-1,3,4-oxadiazolyl)-α-cyanostyryl]-9-*n*-heptylcarbazole (20b). Yield: 14%. Dark yellow solid. Mp 154–156 °C. ^{1}H NMR ($CDCl_3$): 8.64 (s, 2H), 8.16 (d, J=8.5 Hz, 2H), 8.06 (d, J=8.6 Hz, 4H), 7.79 (d, J=8.5 Hz, 4H), 7.78 (s, 2H), 7.48 (d, J=8.6 Hz, 2H), 4.30 (t, J=6.7 Hz, 2H), 2.92 (t, J=7.3 Hz, 4H), 1.85 (m, 6H), 1.26 (m, 32H), 0.87 (m, 9H). ^{13}C NMR ($CDCl_3$): 167.25, 164.06, 144.00, 142.37, 137.80, 127.89, 127.31, 126.11, 125.45, 123.94, 123.14, 118.47, 109.77, 107.02, 43.66, 31.36, 29.36, 29.21, 29.12, 29.04, 28.96, 27.18, 26.58, 25.48, 22.62, 22.52, 14.06. IR (KBr): 2955, 2926, 2854, 2210, 1585, 1566, 1499, 1420, 1308, 1236, 1204, 1138, 843. MS: 908 (M^+). UV–vis (CH_2Cl_2): 339, 417. PL: 522. Anal. Calcd: C, 78.02; H, 7.66; N, 10.80. Found: C, 77.14; H, 7.86; N, 10.56.

4.3.9. 3,6-Bis(4-methoxy-α-cyanostyryl)-9-*n*-heptylcarbazole (20c). Yield: 26%. Greenish yellow solid. Mp 119–121 °C. ^{1}H NMR ($CDCl_3$): 8.54 (s, 2H), 8.12 (d, J=8.5 Hz, 2H), 7.60 (d, J=8.9 Hz, 4H), 7.57 (s, 2H), 7.43 (d, J=8.9 Hz, 2H), 6.97 (d, J=8.9 Hz, 4H), 4.27 (t, J=7.0 Hz, 2H), 3.84 (s, 6H), 1.86 (m, 2H), 1.25 (m, 8H), 0.85 (t, J=6.7 Hz, 3H). ^{13}C NMR ($CDCl_3$): 160.05, 141.79, 141.02, 127.58, 127.04, 125.88, 123.06, 122.50, 119.06, 114.39, 109.46, 107.87, 55.41, 43.52, 31.65, 28.98, 27.18, 22.52, 14.00. IR (KBr): 2953, 2930, 2208, 1605, 1585, 1512, 1485, 1389, 1356, 1286, 1250, 1182, 1036, 829. MS: 579 (M^+). UV–vis (CH_2Cl_2): 336, 399. PL: 495. EL: 495. Anal. Calcd: C, 80.80; H, 6.43; N, 7.25. Found: C, 80.36; H, 5.76; N, 7.29.

4.3.10. 4-(4-Methoxy-α-cyanostyryl)-2,5-di-*n*-heptyloxybenzaldehyde (22). To a solution of 2,5-di-*n*-heptyloxyterephthaldialdehyde (**3**) (0.69 g, 1.90 mmol) and 4-methoxyphenylacetonitrile (**17**) (0.16 g, 1.09 mmol) in a mixture of *tert*-butyl alcohol (4 ml) and THF (7 ml) under inert gas was added potassium *tert*-butoxide (0.02 g, 0.18 mmol) in one portion at 40 °C. The mixture was stirred for 15 min at 40 °C, cooled, and poured into methanol. The precipitate was filtered off and dichloromethane (100 ml) was added to the filtrate. The solution was washed with water several times, dried with anhydrous Na_2SO_4, and concentrated under reduced pressure to give a crude product. The purification was accomplished by column chromatography on silica gel using dichloromethane/*n*-hexane as eluent. Yield: 0.14 g (26%). Yellow solid. Mp 60–62 °C. ^{1}H NMR ($CDCl_3$): 10.47 (s, 1H), 7.84–7.83 (ss, 2H), 7.63 (d, J=8.9 Hz, 2H), 7.32 (s, 1H), 6.97 (d, J=8.9 Hz, 2H), 4.13 (t, J=6.1 Hz, 2H), 4.01 (t, J=6.4 Hz, 2H), 3.84 (s, 3H), 1.81 (m, 4H), 1.29 (m, 16H), 0.87 (m, 6H). ^{13}C NMR ($CDCl_3$): 188.98, 160.78, 155.60, 151.31, 133.71, 130.13, 127.57, 126.84, 125.98, 118.05, 114.39, 113.42, 112.77, 109.69, 69.37, 69.21, 55.44, 31.75, 29.16, 29.09, 28.99, 28.96, 26.04, 22.57, 22.54, 14.04. IR (KBr): 2957, 2935, 2856, 2212, 1684, 1607, 1516, 1487, 1427, 1393, 1285, 1244, 1207, 1186, 1132, 1036, 831. MS: 491 (M^+). UV–vis (CH_2Cl_2): 355, 408. Anal. Calcd: C, 75.73; H, 8.41; N, 2.85. Found: C, 75.68; H, 8.52; N, 2.85.

4.3.11. 3-Formyl-9-*n*-heptylcarbazole (23). Phosphorus oxychloride (3.7 g, 24 mmol) was added dropwise to DMF (1.7 g, 23 mmol) at 0 °C. 9-*n*-Heptylcarbazole (**8**) (6.0 g, 22.6 mmol) in 1,2-dichloroethane (30 ml) was added to the reaction mixture at room temperature. The mixture was refluxed for 6 h. After cooling, the mixture was poured into water and extracted with dichloromethane. The solution was washed with water, dried with anhydrous Na_2SO_4, and concentrated under reduced pressure to give a crude product.

The purification was accomplished by chromatography on a silica gel column using ethyl acetate/*n*-hexane as eluent. The viscous pale yellow liquid was solidified on standing at room temperature. Yield: 2.7 g (41%). Pale yellow solid. Mp 60–62 °C. ^{1}H NMR ($CDCl_3$): 10.07 (s, 1H), 8.58 (d, J_1=1.2 Hz, 1H), 8.12 (d, J=7.6 Hz, 1H), 7.96 (dd, J_1=1.8 Hz, J_2=8.5 Hz, 1H), 7.46–7.30 (m, 4H), 4.30 (t, J=7.3 Hz, 2H), 1.86 (m, 2H), 1.22 (m, 8H), 0.84 (t, J=6.7 Hz, 3H). ^{13}C NMR ($CDCl_3$): 191.69, 144.03, 141.14, 128.48, 127.10, 126.66, 123.93, 123.03, 122.97, 120.69, 120.24, 109.36, 108.89, 43.40, 31.63, 28.98, 28.90, 27.19, 22.52, 13.98. IR (KBr): 2951, 2916, 2852, 1690, 1626, 1591, 1497, 1470, 1381, 1339, 1234, 1180, 1134, 810, 752. MS: 293 (M^+), 208, 180, 152, 84, 49. Anal. Calcd: C, 81.87; H, 7.90; N, 4.77. Found: C, 81.53; H, 7.80; N, 4.76.

4.3.12. 1,6-Bis(4-cyanomethylphenoxy)hexane (25). A solution of 4-hydroxyphenylacetonitrile (**24**) (1.9 g, 14.3 mmol), 1,6-dibromohexane (1.6 g, 6.6 mmol), and potassium carbonate (4.0 g, 29.0 mmol) in DMF (50 ml) was stirred at 70 °C overnight. The resulting mixture was poured into cold water. The precipitate was collected and dried under vacuum. The crude product was purified by chromatography on a silica gel column using dichloromethane as eluent. Yield: 1.8 g (79%). White solid. Mp 119–121 °C. ^{1}H NMR ($CDCl_3$): 7.18 (d, J=8.9 Hz, 4H), 6.88 (d, J=8.9 Hz, 4H), 3.94 (t, J=6.4 Hz, 4H), 3.66 (s, 4H), 1.80 (m, 4H), 1.52 (m, 4H). ^{13}C NMR ($CDCl_3$): 158.80, 129.01, 121.59, 118.18, 115.03, 67.89, 29.07, 25.76, 22.76. IR (KBr): 2937, 2872, 2245, 1614, 1585, 1514, 1475, 1408, 1254, 1180, 1111, 1028, 820, 804. MS: 348 (M^+), 216, 172, 146, 133, 116, 83, 67, 55. Anal. Calcd: C, 75.83; H, 6.94; N, 8.04. Found: C, 75.53; H, 6.20; N, 7.98.

4.4. General procedure for the preparation of α-cyanostyryl compounds with spacer group (26–28)

The model compounds **26–28** were prepared by reacting the aldehydes (**21**, **22** or **23**) with acetonitrile **25**, respectively, by the same method as model compounds **18–20**.

4.4.1. 1,6-Bis[(1,4-di-*n*-heptyloxyphenyl)-4-β-cyanostyryloxy]hexane (26). Yield: 76%. Greenish yellow solid. Mp 88–90 °C. ^{1}H NMR ($CDCl_3$): 7.84 (s, 2H), 7.72 (d, J=2.8 Hz, 2H), 7.57 (d, J=8.9 Hz, 4H), 6.95 (d, J=9.2 Hz, 4H), 6.90 (d, J=2.8 Hz, 2H), 6.84 (d, J=9.15 Hz, 2H), 3.97 (m, 12H), 1.77 (m, 12H), 1.29 (m, 36H), 0.87 (m, 12H). ^{13}C NMR ($CDCl_3$): 159.74, 152.92, 151.72, 135.16, 127.28, 123.81, 118.71, 118.42, 114.86, 113.42, 112.95, 110.69, 69.37, 68.77, 67.98, 31.77, 29.30, 29.13, 29.07, 28.99, 26.10, 26.01, 25.86, 22.60, 22.53, 14.06. IR (KBr): 2930, 2856, 2208, 1607, 1512, 1497, 1470, 1433, 1394, 1296, 1244, 1225, 1186, 1038, 824, 814. MS: 981 (M^+). UV–vis: 330, 374. PL: 493. Anal. Calcd: C, 79.36; H, 9.13; N, 2.99. Found: C, 78.25; H, 9.88; N, 2.81.

4.4.2. Oligomer (27). Yield: 21%. Orange solid. Mp 175–177 °C. ^{1}H NMR ($CDCl_3$): 7.89 (s, 4H), 7.83 (s, 4H), 7.59 (dd, J_1=2.4 Hz, J_2=8.9 Hz, 8H), 6.96 (dd, J_1=2.1 Hz, J_2=8.9 Hz, 8H), 4.09 (m, 12H), 3.85 (s, 6H), 1.79 (m, 12H), 1.30 (m, 36H), 0.86 (t, J=7 Hz, 12H). ^{13}C NMR ($CDCl_3$): 160.46, 159.99, 151.37, 134.01, 133.91, 127.40, 127.33, 127.16, 125.75, 125.69, 118.56, 114.97, 114.45, 111.19, 111.12, 69.42, 68.01, 31.80, 29.24, 29.13, 29.04, 26.16, 25.83, 22.57, 14.09. IR (KBr): 2932, 2854, 2208, 1607, 1514, 1431, 1300, 1248, 1217, 1182, 1034, 824. MS: 1295 (M^+). UV–vis: 364, 433. PL: 632. EL: 545, 641. Anal. Calcd: C, 77.86; H, 7.93; N, 4.32. Found: C, 77.64; H, 7.80; N, 4.21.

4.4.3. 1,6-Bis[(3-(9-*n*-heptylcarbazyl))-4-β-cyanostyryloxy]hexane (28). Yield: 39%. Pale yellow solid. Mp 126–128 °C. ^{1}H NMR ($CDCl_3$): 8.57 (d, J=1.2 Hz, 2H), 8.11 (d, J=7.9 Hz, 2H), 8.06 (dd, J_1=1.5 Hz, J_2=8.9 Hz, 2H), 7.60 (d, J=8.9 Hz, 4H), 7.58 (s, 2H), 7.42–7.26 (m, 8H), 6.97 (d, J=8.9 Hz, 4H), 4.27 (t, J=7.3 Hz, 4H), 4.02 (t, J=6.4 Hz, 4H), 1.85 (m, 8H), 1.56 (m, 4H), 1.25 (m, 16H), 0.85 (t, J=7.0 Hz, 6H). ^{13}C NMR ($CDCl_3$): 159.44, 141.47, 141.35, 140.96, 127.67, 126.98, 126.83, 126.26, 125.02, 123.16, 122.79, 122.14, 120.67, 119.62, 119.26, 114.91, 109.07, 108.98, 107.14, 67.96, 43.26, 31.66, 29.13, 29.01, 28.96, 27.22, 25.80, 22.54, 14.00. IR (KBr): 2928, 2860, 2208, 1605, 1589, 1512, 1493, 1475, 1389, 1348, 1288, 1248, 1182, 1028, 833, 748. MS: 898 (M^+). UV–vis: 291, 381. PL: 468. Anal. Calcd: C, 82.81; H, 7.40; N, 6.23. Found: C, 81.69; H, 6.99; N, 6.08.

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Tetrahedron 62 (2006) 6248–6257
Tetrahedron

Analogues of cytotoxic squamocin using reliable reactions: new insights into the reactivity and role of the α,β-unsaturated lactone of the annonaceous acetogenins

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Received 30 November 2005; revised 7 April 2006; accepted 20 April 2006
Available online 12 May 2006

Abstract—A small library of squamocin analogues has been prepared and screened biologically (cytotoxicity, inhibition of mitochondrial complex I and complex III). To centre diversity on a crucial part of the molecule (i.e., the α,β-unsaturated lactone), an original and reliable lactone opening reaction has been discovered and exploited among other efficient reactions.

1. Introduction

In the search for efficient principles to improve not only the number of compounds in libraries but also, and mainly, their quality in term of 'drug-likeness', new methodologies have to be designed. In this context, natural products with validated biological activities constitute ideal starting templates and drug researchers are increasingly embracing natural-product-like libraries. Concomitantly, among the new methodologies allowing a rapid access to original chemical entities developed in the recent years, 'click chemistry' constitutes an exciting direction.[1] As a way of making quickly large numbers of molecules, it offers an attractive route for developing compound libraries. In the search for original bioactive molecules, we believe that a high number of ready-to-test molecules can be prepared easily when combining the pre-existing complexity of natural products and selected reliable reactions. We want to use the set of these reactions, the essence of the concept, to synthesize natural-product-based libraries of molecules. The selectivity of the reactions is of first importance to enable the rapid assembly of molecules without the use of any protecting group.

In this paper, we wish to describe the first results of our explorations. We set on studying α,β-unsaturated lactones, a common substructure found in many natural products. Acetogenins from Annonaceae constitute interesting and privileged skeletons containing such a moiety because of their especially intense biological activities.[2] They are a structurally unique, broad group of polyketides, which are only found, so far, in several genuses of the Annonaceae. These compounds exhibit a wide range of potent biological activities (cytotoxic, antiparasitic, pesticidal) resulting from strong inhibition of the mitochondrial NADH–ubiquinone oxidoreductase (complex I) as the main target, with a dramatic drop of ATP production and cell death by apoptotic mechanisms. The exact structural basis of the interaction between the acetogenins and this respiratory target is unknown. They have also, recently, been suspected of being implicated in atypical Parkinsonism syndromes in conjunction with the consumption of annonaceous-derived edible products or traditional medicines.[3] Therefore, there is a great need for studies directed towards a better understanding of acetogenin molecular mechanisms of action.

In particular, the role of the terminal α,β-unsaturated γ-methylbutyrolactone as a quasi-ubiquitous moiety in the core of these inhibitors remains unclear. Since only a limited number of synthetic modifications have been described at this level (because of the scarcity of these compounds and

Keywords: Annonaceous acetogenins; Squamocin; Unsaturated lactone; Chemistry in water; Diels–Alder; Click chemistry.
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doi:10.1016/j.tet.2006.04.066

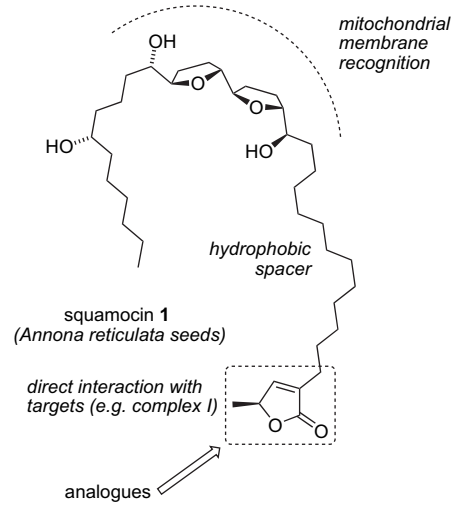


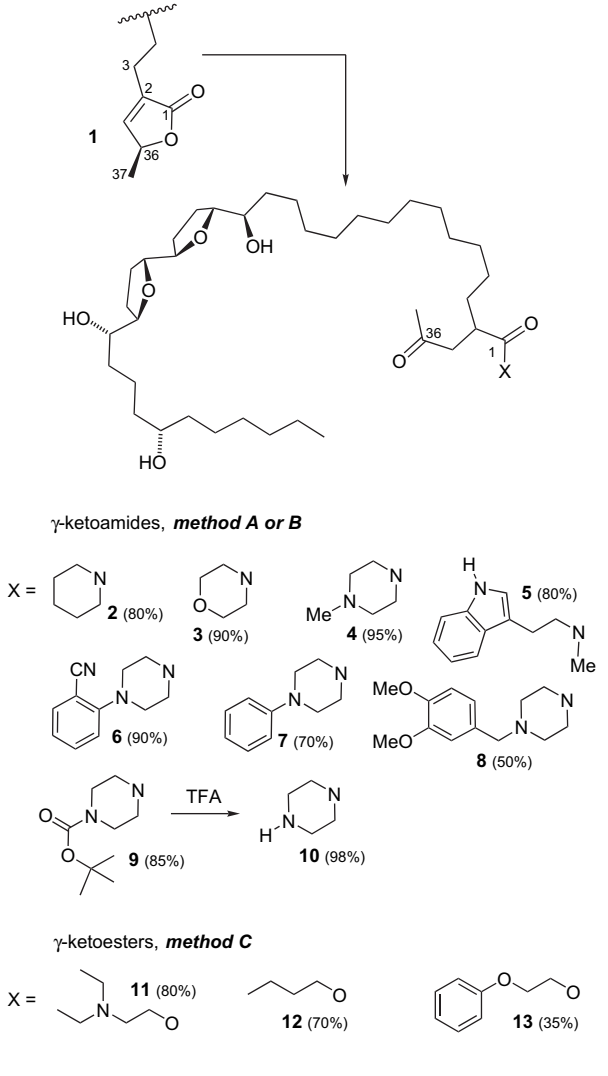
Figure 1. Squamocin **1** and fundamental pharmacophores.

the difficulties encountered in their total synthesis[4,5]), it is still uncertain whether this structural part has to be considered crucial for the inhibition.[6,7] Our aim was to develop simple, efficient and versatile transformation procedures of the lactone core of those rather complex natural products, allowing access to a certain degree of molecular diversity in this class of bioactive secondary metabolites. Isolated from *Annona reticulata*, squamocin **1** (Fig. 1) was selected as a lead compound for our studies for its exhibited properties. It also constituted a great underlying challenge to be able to modify **1**, considering the physicochemical issues inherent to the lipid-like behaviour of acetogenins, which make these molecules difficult to manipulate and purify.[†] Two types of modifications of the lactone will be described: a nucleophilic ring opening and a reduction into a furane suitable for Diels–Alder reactions.

2. Chemistry[‡]

2.1. An efficient lactone ring opening

With our natural substrate in hand, we explored its properties and discovered an original reactivity of the terminal butenolide with nucleophiles. Our conditions led to γ-ketoamides (**2**–**10**), γ-ketoesters (**11**–**13**) or γ-ketoacid (**14**) derivatives of squamocin,[§] these semisynthetic analogues being obtained in quasi-quantitative yields and high purity (Scheme 1). Few examples of such addition reactions even on simple α,β-unsaturated butyrolactones are described. Furthermore, in addition to an increase in the efficiency of those transformations, it must be mentioned that these have never been deliberately and widely applied to complex natural products.[8] A careful study of reaction conditions was carried out to understand these unusual transformations and define their scope and limitations.



Scheme 1. Lactone opening. Reagents and conditions: *Method A*, neat conditions, nucleophile (1.0 equiv to excess), 100–110 °C; *Method B*, boiling water, nucleophile (2.0–3.0 equiv); *Method C*, nucleophile+base, 100–110 °C. Average yields are indicated for compound purity >97% (simple work-up or purification if needed, see Section 5).

Reactions were especially practical when simple volatile secondary amines were used in excess and served as solvent but they ran well with only 1 equiv of nucleophile in neat conditions. Most interestingly, these reactions proceeded readily in boiling water despite the insolubility of the acetogenin (see below).[9] Apart from γ-ketoamides, the most striking example in this context corresponded to the high-yielding semisynthesis of a completely water-soluble sodium salt of acid **14** by simple dispersion of squamocin in boiling 1 N aqueous NaOH. As far as the obtention of γ-ketoesters is concerned, the reacting primary alcohol needed to be used as solvent for the reaction to occur, the amount of which being reducible to 3 equiv. Amines rarely required

[†] Concerning natural acetogenins, they are generally present in small to minute amounts as complex mixtures of isomers.

[‡] All compounds (**2**–**23**) were fully characterized by NMR (^{1}H, ^{13}C, HMQC, HMBC, NOESY and, when needed, HOHAHA experiments), IR and MS. See Section 5 for details.

[§] As can be expected, these adducts were obtained as equimolar mixtures of epimers, as evidenced by HPLC analysis. Most of the time, the diastereoisomers were undistinguishable by spectroscopic methods, this behaviour being similar to the one observed for γ-epimerized natural acetogenins (see Ref. 10).

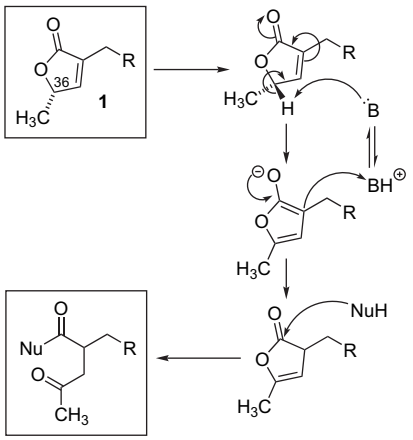


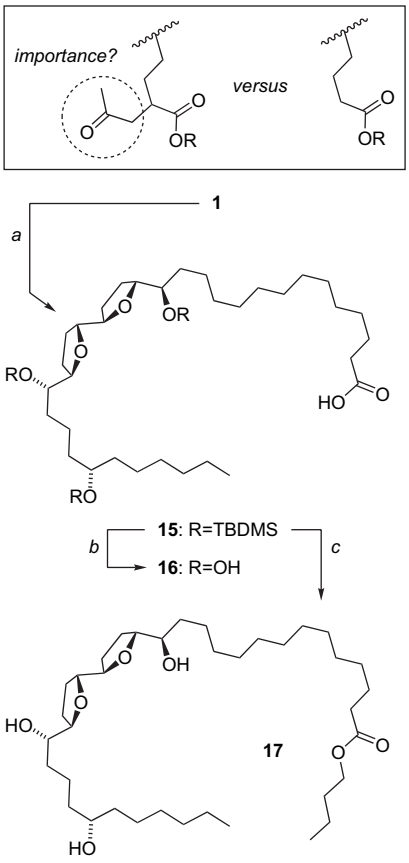
Figure 2. Plausible mechanism for the lactone opening.

any addition of base (easy to handle K_2CO_3 could be used efficiently, giving similar results with marked increase in reaction rates) whereas it was the case for primary alcohols. Among the numerous bases tested, DBU gave the best results. Importantly, it has to be mentioned here that secondary amines and primary alcohols of low melting points (below 100 °C) were also suitable for such reactions, allowing the introduction of more sophisticated substructures in the acetogenin skeleton (Schemes 1 and 3). Primary amines exhibited a different reactivity towards the butenolide nucleus and afforded Michael 1,4-adducts, while the tested secondary alcohols failed to furnish the desired γ-ketoesters.

On the basis of these results, we propose the following mechanistic scheme to explain this unusual nucleophilic opening of the lactone. A possible pathway might be initiated by a deprotonation of the acidic proton at C-36 on the butenolide system, leading to an increase of the electrophilicity of the lactone coupled to an irreversible ring opening (Fig. 2).[10]

As a way to evaluate the contribution of the α-acetonyl side chain to the biological activity of such derivatives, we synthesized two linear analogues of α-acetonylacid **14** and α-acetonyl-*n*-butylester **12**, respectively (Scheme 2). Acid **16** was obtained from its tri-*O*-TBDMS precursor **15** as described previously.[6a,6d,6e] On the other hand, *n*-butylester **17** was prepared by a one-pot desilylation/esterification reaction of **15** in a hot Amberlyst®-15/*n*-BuOH system, with an excellent yield.

The described reactions,[¶] involving only carbon–heteroatom bond formation, are highly selective with no or very few by-products. Having secured the reaction with various nucleophiles, our attention shifted to the possibility of gaining further complexity by constructing new molecular architectures. With this aim, dimeric structure **18** was targeted and obtained when reacting squamocin **1** with 0.5 equiv of dipiperidinyl propane, demonstrating as stated above the feasibility of the reactions with a stoichiometric amount of

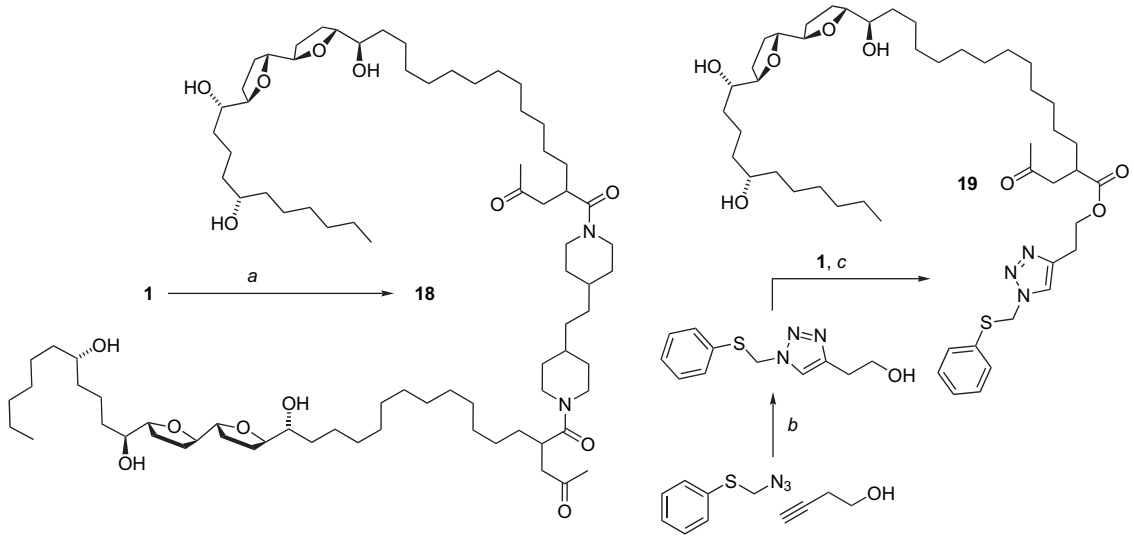


Scheme 2. Synthesis of acid **16** and ester **17** for SAR comparisons. Reagents and conditions: (a,b) Refs. 6a, 6d, 6e; (c) Amberlyst 15®, *n*-BuOH, 65 °C, 15 h (90%). TBDMS=*tert*-butyldimethylsilyl.

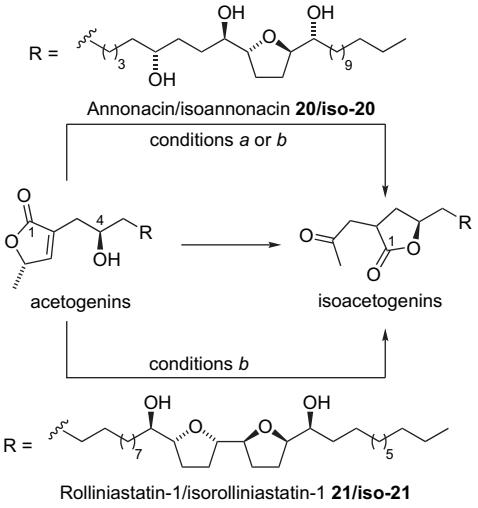
nucleophile in the absence of solvent (Scheme 3). Other developments were strongly influenced by a few of the seminal 'good reactions' in click chemistry, e.g., cycloadditions reactions to form triazoles. A two-step sequence was employed for the formation of derivative **19**. A copper(I)-catalyzed regioselective ligation of thiophenylazido-methane and butyn-1-ol via Huisgen dipolar cycloaddition in a simple Cu^{II}/ascorbate aqueous system[11] gave rise to an alcohol which was added to squamocin in the conditions described in Scheme 1, yielding highly functionalized compound **19** (Scheme 3).

Finally, the nucleophilic ring opening can be envisioned in an intramolecular way. Encouraged by our success in using water as a favourable medium for clean reactions between lipophilic partners, we discovered an original way to transform a type of natural compounds into another. Annonacin, a known annonaceous acetogenin from *Annona muricata*,[12a,b] was thus totally converted into isoannonacin *iso*-**20**[12c] by simple dispersion in boiling water. As in the case of the semisynthesis of γ-ketoacid salt of **14**, isoannonacin of high purity was simply recovered by extraction from water. Such a reaction failed in the case of rolliniastatin-1,[13] another 4-hydroxyacetogenin from *Rollinia mucosa*, probably because of its higher melting point. However, both acetogenins could be converted into their isoacetogenin counterparts by simple heating at 150 °C under argon (Scheme 4).

[¶] When using 1 equiv of nucleophile, the process is a 'fusion reaction', i.e., the combined formulae of the reactants equal the formula of the product.



Scheme 3. Two-step sequences to compounds **18** and **19**. Reagents and conditions: (a) 1,3 (4-piperidinyl)propane (0.5 equiv), K_2CO_3, 110 °C, 4 h (90%); (b) $CuSO_4$ (1 mol %), ascorbic acid (10 mol %), H_2O/*t*-BuOH (1:1), 20 °C, 48 h (90%); (c) 1 (0.3 equiv, excess alcohol easily recoverable), DBU (0.25 equiv), 110 °C, 4 h (70%). DBU=1,8-diazabicyclo[5.4.0]undec-7-ene.



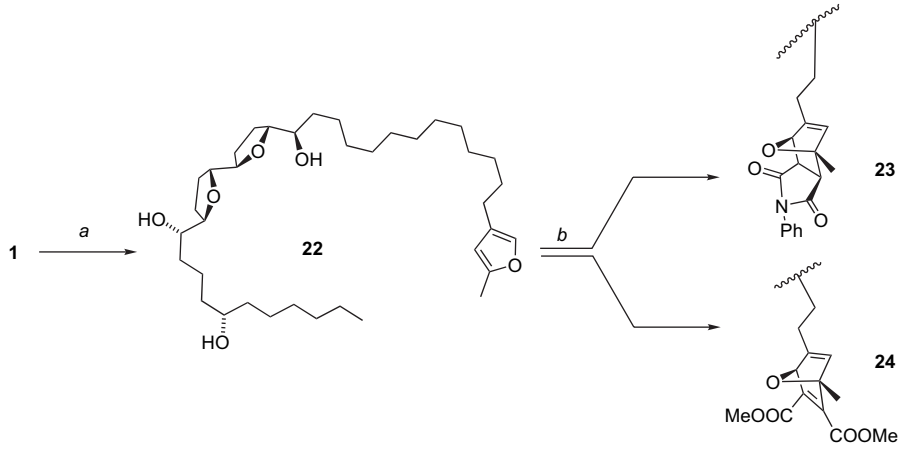
Scheme 4. Conversion of acetogenins into isoacetogenins: conditions (a) dispersion in boiling water, 12 h (*iso*-**20**: 90%); conditions (b) 150 °C, Ar (*iso*-**20**: 60%, *iso*-**21**: 40%).

2.2. Diels–Alder reactions

Reduction of squamocin **1** with DIBAL–H furnished, with an excellent yield, furane derivative **22** (Scheme 5), an ideal candidate for simple Diels–Alder reactions with symmetric dienophiles. Indeed, reaction with *N*-phenylmaleimide provided bicyclic compound **23** with 90% yield. A similar result was obtained with acetylenedicarboxylic dimethylester giving cycloadduct **24**. These reactions could alternatively be performed without any solvent or in boiling water (Scheme 5).

3. Biological activities

Compounds were evaluated for their cytotoxicity against KB 3-1 cells (human nasopharyngeal epidermoid carcinoma) in vitro (Table 1), as well as their complex I inhibitory activities (inhibition of the NADH oxidase and NADH-*n*-decylubiquinone oxidoreductase activity, measured on submitochondrial particles from sonicated beef heart mitochondria—Table 1). A selection of molecules was also tested as inhibitors of mitochondrial complex III.



Scheme 5. Reduction into furane and Diels–Alder reactions. Reagents and conditions: (a) DIBAL–H, CH_2Cl_2/$PhCH_3$, −78 °C (87%); (b) *N*-phenylmaleimide (1.0 equiv) or methyl acetylenedicarboxylate (1.0 equiv), 100–110 °C, no solvent or boiling water (**23**: 92%, **24**: 90%).

Table 1. Biological activities of the semisynthetic derivatives: complex I inhibition and cytotoxicity

Compound	Complex I inhibition[b] IC_{50} (nM)		Cytotoxicity IC_{50} (M)[a]
	NADH oxidase	NADH–DB oxidoreductase	
Squamocin **1**	0.9	1.3	1.8×10^{-13}
α-Acetonylpiperazinamide **2**	41	nt	4.0×10^{-7}
α-Acetonylmorpholinamide **3**	22		3.6×10^{-7}
α-Acetonyl-*N*-methylpiperazinamide **4**	nt[c]	nt	4.0×10^{-7}
α-Acetonyl-N_{ω}-methyltryptamide **5**	52	nt	3.9×10^{-7}
α-Acetonyl-*N*-phenylpiperazinamide **7**	nt	nt	1.5×10^{-8}
α-Acetonyl-*N*-(3′,4′-dimethoxybenzyl)-piperazinamide **8**	74	154	1.2×10^{-8}
α-Acetonyl-*N*-(Boc)piperazinamide **9**	nt	nt	4.1×10^{-8}
α-Acetonylpiperazinamide **10**	nt	nt	5×10^{-7}
α-Acetonyl-(*N*,*N*′-diethylamino)-2-ethyl ester **11**	13	nt	7.5×10^{-10}
α-Acetonyl-*n*-butylester **12**	17	nt	4.6×10^{-10}
α-Acetonyl-2-phenoxyethyl ester **13**	>3000	nt	8.1×10^{-8}
α-Acetonylacid **14**	12	nt	1.3×10^{-9}
Acid **16**	6.2	38	2.8×10^{-7}
n-Butylester **17**	2.3	9.2	6.8×10^{-8}
Bis(α-acetonyl-1,3-(4-piperidinamide)propane) **18**	26	nt	2.7×10^{-8}
Triazole ester **19**	12	nt	5.1×10^{-9}
Furane **22**	2.4	nt	3.2×10^{-8}
Cycloadduct **23**	30	nt	3.5×10^{-7}
Cycloadduct **24**	23	nt	3.0×10^{-8}
Doxorubicin[d]	—	—	2.4×10^{-9}
Rotenone[d]	4.9	28	—
DQA[d]	nt	4.9	—

[a] KB 3-1 cells.
[b] Mitochondrial complex I (NADH–ubiquinone oxidoreductase), submitochondrial particles from beef heart.
[c] Not tested.
[d] References; DQA=2-*n*-decyl-4-quinazolinyl amine.

Acetonylamides (**2–10**, **18**) showed collapsed, though still very significant, cytotoxic activities in comparison with squamocin **1**. Interestingly, all obtained α-acetonylamides exhibited very similar cytotoxicities despite the introduction of different and more or less complex moieties. Concerning α-acetonylesters (**11–13**) and α-acetonylacid (**14**), these analogues exhibited decreased though significantly more similar cytotoxicities relatively to squamocin **1** and compared to amide derivatives. The two linear analogues **16** and **17** exhibited a 100 times diminished cytotoxicity in comparison to their α-acetonyl counterparts, suggesting that the 1,4-dicarbonylated part of the above adducts from nucleophilic opening was responsible for some of their strong cytotoxic activity. Paradoxically, linear *n*-butylester **17** appeared to be a seven times more potent complex I inhibitor than its α-acetonylated counterpart **12**. Ester **17** is indeed a powerful mitochondrial inhibitor, exhibiting an activity close to the one of squamocin **1**. Furane analogue **22** exhibited a collapsed cytotoxicity in comparison with squamocin **1**, despite an important enzymatic inhibitory potency, which was only three times weaker than that of the natural acetogenin. This observation is an echo to the fact that the butenolide of squamocin **1** was completely substitutable by a benzimidazole nucleus,[2b] and by other electron-rich aromatic moieties.[6b] None of the compounds tested at concentrations of 1–3 μM (derivatives **5**, **8**, **16**, **17**, **19** and **21**) was found to inhibit the *n*-decylubiquinol/cytochrome *c* oxidoreductase activity of complex III from bovine heart mitochondria reconstituted into liposomes.

4. Conclusion

This study has led to the discovery of novel pharmacophores in the annonaceous acetogenins series (Fig. 3), both at the complex I inhibition and the cytotoxicity levels. But the results revealed obvious discrepancies between the cellular and enzymatic inhibitory activities of the squamocin analogues. Cytotoxicities are decreased but still in a 10^{-7} M range for the less active derivatives, placing the modified acetogenins on an average among cytotoxic agents used as antitumor drugs and giving new insights into the potential development of acetogenins for chemotherapy.

As far as synthetic organic chemistry is concerned, a single molecular skeleton was exposed to different reaction conditions to achieve original and efficient transformations. The α,β-unsaturated butyrolactone nucleus appeared to be a modular chemical block capable of driving spontaneous and irreversible linkage reactions. As part of chemically viable tools and methods, simple reaction conditions, especially the ones

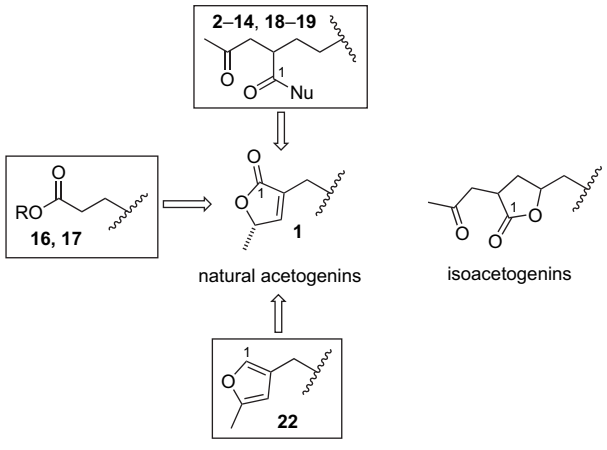


Figure 3. Novel terminal pharmacophores in the squamocin series.

ran in a boiling aqueous medium, allowed the easy preparation of original squamocin analogues. Moreover, our results demonstrated the usefulness of selecting reliable reactions for modifying with good to quantitative yields small amounts of natural products without the need for protecting groups.[‖] In this context, the set of powerful reactions proposed by Sharpless et al. has been an interesting source of inspiration. Although nucleophilic opening of a lactone was not part of the 'good reactions' toolbox,[1] this reaction was of great interest to us in the sense that it permitted, in one efficient operation, to considerably modify the pharmacophore of an active natural product while incorporating complexity. Moreover, Diels–Alder reactions and Huisgen cycloadditions are qualified as 'click reactions'. This is the first small discrete library of acetogenin derivatives with diversity centred on a crucial part of the natural substrate. Inherent reactivity combined with the efficiency of the selected reactions gave immediate access to the analogues in one step. As far as acetogenins are concerned, such efficient reactions as the ones described here may have important biological significance, as one can imagine a similar nucleophilic trapping and opening of the lactone ring by intracellular targets, explaining thereby part of their activity. This new postulate is currently being studied in our laboratory.

5. Experimental

5.1. General methods

^{1}H NMR spectra were recorded on a Bruker AM-400 (400 MHz) at room temperature unless specified otherwise, ^{13}C NMR spectra were recorded on a Bruker AC-200 (50 MHz) or on a Bruker AM-400 (100 MHz) spectrometer at room temperature and were calibrated with the residual undeuterated solvent as an internal reference. All compounds were fully characterized by NMR (^{1}H, ^{13}C, COSY, HMQC, HMBC and, when needed, NOESY and HOHAHA experiments). Mass spectra (MS or HRMS) were recorded at the 'Laboratoire de Spectrométrie de Masse' de l'Institut de Chimie des Substances Naturelles de Gif-sur-Yvette, France. Optical rotations were measured using a Schmidt–Haensch polarimeter E at 589 nm. IR spectra were recorded on a Perkin–Elmer 257 FTIR spectrometer and samples were analyzed as a film from a solution in the indicated solvent. All reagents were purchased at the highest commercial quality and used without further purification. Column chromatography was performed with silica gel 60 (9385 Merck) in the 'flash' mode or Sephadex® LH-20 (Pharmacia). Reactions were monitored by TLC carried out on aluminium plates coated with silica gel 60F254 (E Merck) using UV light as a visualizing agent and sulfuric vanillin and heat as a developing agent. Squamocin **1** was isolated from the seeds of *A. reticulata*, collected in Viet-Nam.

5.2. Lactone openings

5.2.1. General procedures (see Scheme 1 for individual yields). *Method A*: squamocin **1** (10 mg, 16 μmol) and the amine (1.1–20 equiv) were stirred at 100–110 °C until completion of the reaction followed by TLC (CH_2Cl_2/MeOH 9:1), dry K_2CO_3 (1 equiv *m*/*m*) could be added to increase reaction rate. The resulting mixture was evaporated or diluted with CH_2Cl_2 and washed 3 times with water. The organic phase was dried (Na_2SO_4) and concentrated under reduced pressure to give the desired compound after chromatography on silica gel (CH_2Cl_2/MeOH 95:5).

Method B: squamocin **1** (10 mg, 16 μmol) and the amine (2–3 equiv) were vigorously stirred in boiling water until completion of the reaction followed by TLC (CH_2Cl_2/MeOH 9:1). The cooled mixture was extracted with CH_2Cl_2 (3 times). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to give the desired compound after chromatography on silica gel (CH_2Cl_2/MeOH 95:5).

Synthesis of acid **14**: squamocin **1** (10 mg, 16 μmol) was dispersed under vigorous stirring in boiling 1 N aqueous NaOH (1 mL) for 15 h. After cooling of the reaction medium and elimination of the aqueous supernatant, the organic residue was partitioned between THF and brine, and the aqueous phase re-extracted with THF. The combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure. The crude product was triturated in ice-cold EtOAc then filtered, leading to the sodium salt of acid **14** as a white powder. The free acid form was obtained by acidification of an aqueous solution of sodium salt (1 N AcOH) and extraction by EtOAc followed by drying (Na_2SO_4) and evaporation under reduced pressure.

Method C: squamocin **1** (10 mg, 16 μmol) and the primary alcohol (1–3 equiv) were stirred at 100–110 °C with DBU (1 equiv) until completion of the reaction followed by TLC (CH_2Cl_2/MeOH 9:1). The mixture was then diluted with CH_2Cl_2 and washed with H_2O (3 times), dried (Na_2SO_4) and concentrated under reduced pressure after chromatography on silica gel (CH_2Cl_2/MeOH 95:5).

5.2.2. Data for compounds.

5.2.2.1. α-Acetonylpiperidinamide (2). Colourless resin; $C_{42}H_{77}NO_7$; R_f=0.3 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, J=6.5 Hz), 1.36 (m, 5H), 1.39 (m, 2H), 1.48 (m, 5H), 1.58 (m, 4H), 1.63 (m, 2H), 1.95 (m, 4H), 2.09 (s, 3H), 2.41 (dd, 1H, J=3.9 Hz, J=17.6 Hz), 2.98 (dd, 1H, J=9.5 Hz, J=17.6 Hz), 3.13 (m, 1H), 3.34 (m, 1H), 3.48 (dd, 2H, J=4.9 Hz, J=10.3 Hz), 3.50 (dd, 2H, J=4.9 Hz, J=10.3 Hz), 3.55 (m, 1H), 3.78 (m, 2H), 3.79 (m, 1H), 3.87 (m, 2H); ^{13}C NMR (CD_3OD, 50 MHz): δ 14.4, 23.1, 23.7, 25.6, 26.9, 27.5, 28.0, 29.2, 29.5, 29.7, 30.7, 33.0, 33.6, 34.2, 34.6, 38.4, 44.3, 72.3, 74.0, 74.9, 83.3, 83.7, 84.2, 175.9, 210.2; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 1715, 1620. ESIMS *m*/*z* 708 [M+H]$^+$, 730 [M+Na]$^+$, 746 [M+K]$^+$, 1438 [2M+Na]$^+$; HRMS (ES) *m*/*z* [M+Na]$^+$ calcd for $C_{42}H_{77}NO_7Na$ 730.5598, found 730.5602.

5.2.2.2. α-Acetonylmorpholinamide (3). Colourless resin; $C_{41}H_{75}NO_8$; R_f=0.35 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, J=6.9 Hz), 1.39 (m, 5H), 1.42 (m, 2H), 1.51 (m, 1H), 1.63 (m, 4H), 1.97 (m, 4H), 2.13 (s, 3H), 2.46 (dd, 1H, J=14.8 Hz, J=5 Hz), 3.09 (dd, 1H, J=14.8 Hz, J=9.8 Hz), 3.11 (m, 1H), 3.39

[‖] Typical scale for the described reactions was 10 mg of **1** with similar results when tested on 100 mg as in a few cases (**3**, **14**, **19**).

(m, 1H), 3.56 (m, 1H, H-38), 3.57 (m, 2H), 3.67 (m, 2H), 3.68 (m, 1H), 3.71 (m, 1H), 3.79 (m, 2H), 3.84 (m, 2H), 3.86 (m, 1H), 3.91 (m, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.1, 22.0, 22.6, 24.9, 25.6, 27.1, 28.4, 28.9, 29.6, 30.0, 31.8, 32.6, 33.2, 35.6, 37.2, 37.5, 42.3, 46.5, 46.4, 66.8, 66.9, 71.5, 71.7, 74.0, 82.1, 82.5, 82.8, 83.2, 174.1, 207.8; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 1715, 1635; ESIMS *m/z* 732 [M+Na]$^+$, 748 [M+K]$^+$; HRMS (ES) *m/z* [M+Na]$^+$ calcd for $C_{41}H_{75}NO_8Na$ 732.5390, found 732.5393.

5.2.2.3. α-Acetonyl-*N*-methylpiperazinamide (4). Colourless resin; $C_{42}H_{78}N_2O_7$; R_f=0.2 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.86 (t, 3H, *J*=7.0 Hz), 1.38 (m, 5H), 1.41 (m, 2H), 1.48 (m, 1H), 1.60 (m, 4H), 1.96 (m, 4H), 2.12 (s, 3H), 2.31 (s, 3H), 2.38 (m, 2H), 2.44 (dd, 1H, *J*=17.5 Hz, *J*=3.2 Hz), 2.52 (m, 2H), 3.06 (dd, 1H, *J*=9.7 Hz, *J*=17.5 Hz), 3.13 (m, 1H), 3.37 (m, 1H), 3.57 (m, 3H), 3.72 (m, 2H), 3.83 (m, 3H), 3.91 (m, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.6, 30.0, 35.6, 37.4, 45.5, 45.8, 46.0, 54.7, 55.0, 71.3, 71.6, 74.0, 82.1, 82.4, 82.7, 83.1, 173.7, 207.8; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 1715, 1638; ESIMS *m/z* 723 [M+H]$^+$, 745 [M+Na]$^+$, 761 [M+K]$^+$; HRMS (ES) *m/z* [M+Na]$^+$ calcd for $C_{42}H_{78}N_2O_7Na$ 745.5707, found 745.5701.

5.2.2.4. α-Acetonyl-*N*-methyltryptamide (5). Pale-yellow resin; $C_{48}H_{80}N_2O_7$; R_f=0.3 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (DMSO-d_6, 400 MHz, 390 K): δ 0.87 (t, 3H, *J*=6.7 Hz), 1.2–1.3 (m, ΣCH_2), 1.32 (m, 4H), 1.34 (m, 3H), 1.39 (m, 1H), 1.63 (m, 4H), 1.85 (m, 4H), 2.03 (s, 3H), 2.43 (dd, 1H, *J*=5.0 Hz, *J*=17.2 Hz), 2.75 (m, 1H), 2.89 (m, 2H), 2.95 (br s, 3H), 3.06 (m, 1H), 3.30 (m, 1H), 3.40 (m, 1H), 3.58 (m, 2H), 3.64 (m, 1H), 3.71 (m, 2H), 3.76 (m, 1H), 3.83 (m, 1H), 6.97 (dd, 1H, *J*=7.8 Hz, *J*=7.1 Hz), 7.05 (dd, 1H, *J*=7.1 Hz, *J*=8 Hz), 7.09 (s, 1H), 7.33 (d, 1H, *J*=8.0 Hz), 7.55 (d, 1H, *J*=7.8 Hz), 10.33 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz, equilibrium of rotamers): δ 14.1, 22.6, 23.2, 30.1, 34.0, 36.2, 36.3, 36.5, 37.3, 37.5, 45.8, 46.2, 49.2, 50.9, 71.5, 71.7, 74.1, 82.2, 83.3, 111.1, 111.3, 112.4, 113.1, 118.4, 118.8, 119.2, 119.4, 121.8, 122.0, 122.2, 122.3, 127.3, 127.5, 136.3, 136.4, 175.2, 175.4, 208.0, 208.1; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 3408, 2924, 2854, 1715, 1621; ESIMS *m/z* 797 [M+H]$^+$, 819 [M+Na]$^+$, 835 [M+K]$^+$; HRMS (ES) *m/z* [M+Na]$^+$ calcd for $C_{48}H_{80}N_2O_7Na$ 819.5863, found 819.5860.

5.2.2.5. α-Acetonyl-*N*-cyanophenylpiperazinamide (6). Pale-yellow resin; $C_{48}H_{79}N_3O_7$; R_f=0.4 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.86 (t, 3H, *J*=6.7 Hz), 1.15–1.45 (m, ΣCH_2), 1.45–1.70 (m, 8H), 1.75–2.0 (m, 6H), 2.13 (s, 3H), 2.47 (dd, 1H, *J*=17.4 Hz, *J*=2.4 Hz), 3.08 (dd, 1H, *J*=17.4 Hz, *J*=9 Hz), 3.18 (m, 2H), 3.29 (m, 2H), 3.37 (m, 1H), 3.56 (m, 2H), 3.70 (m, 1H), 3.82 (m, 2H), 3.90 (m, 2H), 3.97 (m, 1H), 4.09 (br d, 1H, *J*=13 Hz), 6.98–7.07 (m, 2H), 7.49 (t, *J*=7.8 Hz, 1H), 7.57 (d, *J*=7.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.0, 22.0, 22.6, 24.8, 25.6, 27.1, 28.3, 28.9, 29.3, 29.4, 29.6, 29.7, 30.0, 31.8, 32.5, 32.6, 33.2, 35.8, 37.2, 37.4, 41.9, 46.1, 46.2, 51.2, 52.1, 71.4, 71.7, 74.1, 81.7, 82.1, 82.5, 82.8, 83.3, 106.5, 118.1, 119.0, 122.4, 133.8, 134.3, 155.2, 174.0, 207.9; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 3420, 2246, 1717, 1632, 1600; ESIMS *m/z* 810 [M+H]$^+$, 832 [M+Na]$^+$; HRMS (ES) *m/z* [M+Na]$^+$ calcd for $C_{48}H_{79}N_3O_7Na$ 832.5816, found 832.5814.

5.2.2.6. α-Acetonyl-*N*-phenylpiperazinamide (7). Pale-yellow resin; $C_{47}H_{80}N_2O_7$; R_f=0.25 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, *J*=6.7 Hz), 1.2–1.3 (m, ΣCH_2), 1.38 (m, 2H), 1.40 (m, 1H), 1.42 (m, 4H), 1.50 (m, 1H), 1.62 (m, 4H), 1.94 (m, 4H), 2.14 (s, 3H), 2.48 (dd, 1H, *J*=17.7 Hz, *J*=2.5 Hz), 3.10 (dd, 1H, *J*=9.7 Hz, *J*=17.7 Hz), 3.16 (m, 2H), 3.19 (m, 2H), 3.20 (m, 1H), 3.39 (m, 1H), 3.59 (m, 1H), 3.71 (m, 2H), 3.82 (m, 1H), 3.84 (m, 2H), 3.87 (m, 2H), 3.92 (m, 2H), 6.89 (t, 1H, *J*=7.2 Hz), 6.93 (dd, 2H, *J*=8.0 Hz), 7.28 (dd, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.8, 30.0, 35.7, 37.2, 41.7, 45.7, 46.1, 49.2, 49.4, 71.4, 71.6, 74.0, 82.1–83.2, 116.4, 120.2, 129.1, 173.8, 207.8; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 3421, 2925, 2854, 1715, 1633, 1600; ESIMS *m/z* 785 [M+H]$^+$, 807 [M+Na]$^+$, 823 [M+K]$^+$; HRMS (ES) *m/z* [M+Na]$^+$ calcd for $C_{47}H_{80}N_2O_7Na$ 807.5863, found 807.5859.

5.2.2.7. α-Acetonyl-*N*-(dimethoxybenzyl)piperazinamide (8). Pale-yellow resin; $C_{50}H_{86}N_2O_9$; R_f=0.25 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, *J*=6.8 Hz), 1.2–1.3 (m, ΣCH_2), 1.38 (m, 7H), 1.50 (m, 1H), 1.60 (m, 4H), 1.95 (m, 4H), 2.12 (s, 3H), 2.41 (m, 2H), 2.43 (dd, 1H, *J*=2.6 Hz, *J*=17.5 Hz), 2.52 (m, 2H), 3.06 (dd, 1H, *J*=9.6 Hz, *J*=17.5 Hz), 3.13 (m, 1H), 3.38 (m, 1H), 3.45 (s, 2H), 3.51 (m, 2H), 3.55 (m, 1H), 3.69 (m, 2H), 3.83 (m, 2H), 3.86 (m, 1H), 3.86 (s, 3H), 3.87 (s, 3H), 3.90 (m, 2H), 6.78–6.83 (m, 2H), 6.89 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1, 22.0, 22.6, 24.8, 25.6, 27.1, 28.3, 28.9, 29.3, 29.4, 29.6, 29.7, 30.1, 31.8, 32.5, 32.6, 33.2, 33.4, 35.7, 37.2, 37.4, 41.8, 45.8, 46.1, 52.9, 53.1, 55.9, 62.5, 71.4, 71.7, 74.1, 82.2, 82.5, 82.8, 83.1, 83.3, 110.8, 112.1, 121.3, 130.3, 148.2, 148.9, 173.7, 208.0; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 2925, 2854, 1715, 1627, 1515, 1463; ESIMS *m/z* 859 [M+H]$^+$, 881 [M+Na]$^+$, 897 [M+K]$^+$; HRMS (ES) *m/z* [M+Na]$^+$ calcd for $C_{50}H_{86}N_2O_9Na$ 881.6231, found 881.6225.

5.2.2.8. α-Acetonyl-*N*-(Boc)piperazinamide (9). Colourless resin; $C_{46}H_{84}N_2O_9$; R_f=0.3 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.84 (t, 3H, *J*=6.9 Hz), 1.10–1.5 (m, ΣCH_2), 1.44 (s, 9H), 1.58 (m, 4H), 1.93 (m, 4H), 2.10 (s, 3H), 2.44 (d, 1H, *J*=15.0 Hz), 3.05 (m, 1H), 3.09 (m, 1H), 3.42 (m, 1H), 3.50 (m, 4H), 3.56 (m, 1H), 3.68 (m, 4H, H-39), 3.81 (m, 3H), 3.89 (m, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.9, 21.9, 22.5, 24.8, 25.5, 27.0, 28.3, 28.8, 29.2, 29.4, 29.9, 31.7, 32.5, 33.1, 35.7, 37.1, 41.6, 43.6, 45.6, 46.1, 71.3, 74.0 (C-15), 80.0, 82.1, 82.4, 82.7, 83.2, 154.5, 174.0, 207.7; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 2925, 2855, 1696, 1636, 1458, 1417; ESIMS *m/z* 831 [M+Na]$^+$, 848 [M+K]$^+$; HRMS (ES) *m/z* [M+Na]$^+$ calcd for $C_{46}H_{84}N_2O_9Na$ 831.6075, found 831.6074.

5.2.2.9. α-Acetonyl-piperazinamide (10). Compound **9** (20 mg, 29 μmol) was diluted with CH_2Cl_2 (1 mL). Trifluoroacetic acid (0.5 mL) was added dropwise to the solution at 0 °C. After 1 h of stirring, an aqueous saturated solution of $NaHCO_3$ (5 mL) was introduced and the resulting mixture stirred at rt for 2 h. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to yield

10 (21 mg, 98%). Colourless resin; $C_{41}H_{76}N_2O_9$; R_f=0.3 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.86 (t, 3H, J=6.5 Hz), 1.15–1.4 (m, ΣCH_2), 1.4–1.6 (m, 4H), 1.6–1.7 (m, 2H), 1.75–1.9 (m, 2H), 1.96 (br s, 4H), 2.11 (s, 3H), 2.51 (dd, 1H, J=23.6 Hz, J=8.4 Hz), 3.06 (m, 2H), 3.38 (m, 4H), 3.57 (m, 1H), 3.8 (m, 4H), 3.9–3.15 (m, 5H), 4.32 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.9, 21.5, 22.0, 22.5, 25.1, 25.5, 26.8, 28.6, 29.2, 29.6, 31.7, 32.4, 33.2, 33.5, 35.4, 37.0, 37.3, 43.3, 46.5, 71.5, 71.8, 74.3, 82.0, 82.6, 83.2, 174.4, 208.3; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 1714, 1620; ESIMS m/z 709 [M+H]$^+$, 731 [M+Na]$^+$; HRMS (ES) m/z [M+Na]$^+$ calcd for $C_{41}H_{76}N_2O_7Na$ 731.5550, found 731.5555.

5.2.2.10. α-Acetonyl-*N*,*N*′-diethylaminoethylester (11). Yellow resin; $C_{43}H_{81}NO_8$; R_f=0.35 (AcOEt/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, J=6.8 Hz), 1.20 (t, 6H, J=7.2 Hz), 1.20–1.35 (m, ΣCH_2), 1.39 (m, 2H), 1.41 (m, 4H), 1.47 (m, 1H), 1.56 (m, 1H), 1.61 (m, 4H), 1.95 (m, 4H), 2.14 (s, 3H), 2.53 (dd, 1H, J=3.7 Hz, J=17.7 Hz), 2.78 (m, 1H), 2.89 (dd, 1H, J=17.7 Hz, J=7 Hz), 2.93 (q, 4H, J=7 Hz), 3.05 (m, 2H), 3.38 (m, 1H), 3.59 (m, 1H), 3.83 (m, 3H), 3.92 (m, 2H), 4.27 (m, 1H), 4.42 (m, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 9.4, 13.9, 21.5, 21.9, 22.4, 24.8, 25.5, 26.9, 28.3, 28.8, 29.2, 29.4, 31.7, 32.3, 33.0, 37.1, 37.3, 39.9, 44.9, 46.5, 49.5, 59.9, 71.4, 71.6, 74.0, 82.0, 82.4, 82.7, 83.1, 175.2, 206.9; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 2923, 2854, 1718, 1459, 1403, 1370, 1266, 1160, 1058; ESIMS m/z 740 [M+H]$^+$, 762 [M+Na]$^+$; HRMS (ES) m/z [M+Na]$^+$ calcd for $C_{43}H_{81}NO_8Na$ 762.5860, found 762.5863.

5.2.2.11. α-Acetonyl-*n*-butylester (12). Amorphous white solid; $C_{41}H_{76}O_8$; R_f=0.4 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, J=6.8 Hz), 0.93 (t, 3H, J=7.4 Hz), 1.20–1.30 (m, ΣCH_2), 1.37 (m, 6H), 1.40 (m, 2H), 1.49 (m, 1H), 1.53 (m, 1H), 1.58 (m, 6H), 1.96 (m, 4H), 2.15 (s, 3H), 2.47 (dd, 1H, J=4.5 Hz, J=19.6 Hz), 2.84 (m, 1H), 2.89 (dd, 1H, J=19.6 Hz, J=9.6 Hz), 3.39 (m, 1H), 3.59 (m, 1H), 3.85 (m, 4H), 3.92 (m, 1H), 4.07 (m, 2H, J=6.6 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.4, 14.0, 19.1, 22.0, 22.6, 24.8, 25.6, 27.0, 28.4, 28.9, 29.4, 29.6, 30.0, 30.6, 31.0, 32.5, 33.3, 37.3, 37.5, 40.2, 45.0, 64.3, 71.4, 71.7, 74.1, 82.2, 82.5, 82.8, 83.3, 176.2, 206.8; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 2924, 2854, 1720, 1635, 1459, 1404, 1364; ESIMS m/z 720 [M+Na]$^+$, 736 [M+K]$^+$; HRMS (ES) m/z [M+Na]$^+$ calcd for $C_{41}H_{76}O_8Na$ 719.5483, found 719.5479.

5.2.2.12. α-Acetonyl-phenoxyethylester (13). Colourless resin; $C_{45}H_{76}O_9$; R_f=0.55 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.81 (t, 3H), 1.10–1.30 (m, ΣCH_2), 1.31 (m, 2H), 1.33 (m, 4H), 1.43 (m, 1H), 1.55 (m, 1H), 1.58 (m, 4H), 1.90 (m, 4H), 2.07 (s, 3H), 2.43 (dd, 1H, J=21.5 Hz, J=8.4 Hz), 2.82 (m, 2H), 3.33 (m, 1H), 3.52 (m, 1H), 3.78 (m, 2H), 3.80 (m, 1H), 3.85 (m, 2H), 4.09 (m, 2H), 4.36 (m, 2H), 6.83 (dd, 2H, J=8.7 Hz, J=1 Hz), 6.89 (br t, 1H, J=7.3 Hz), 7.21 (m, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.9, 21.9, 22.5, 24.7, 25.5, 26.8, 28.3, 28.9, 29.3, 29.5, 29.8, 31.8, 32.4, 33.1, 37.2, 37.4, 40.0, 44.9, 62.6, 65.7, 71.3, 71.6, 74.0, 76.3, 81.7, 82.1, 82.4, 87.7, 83.2, 114.5, 121.0, 129.3, 158.4, 175.2, 206.6; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 2927, 2855, 1736, 1653, 1541, 1492, 1254; ESIMS m/z 783 [M+Na]$^+$, 799 [M+K]$^+$; HRMS (ES) m/z [M+Na]$^+$ calcd for $C_{45}H_{76}O_9Na$ 783.5387, found 783.5390.

5.2.2.13. α-Acetonylacid 14. Colourless resin; $C_{37}H_{68}O_8$; R_f=0.25 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, J=7.0 Hz), 1.20–1.35 (m, ΣCH_2), 1.39 (m, 2H), 1.42 (m, 4H), 1.49 (m, 1H), 1.61 (m, 5H), 1.96 (m, 4H), 2.15 (s, 3H), 2.47 (dd, 1H, J=7.6 Hz, J=20.4 Hz), 2.86 (m, 3H), 3.40 (m, 1H), 3.59 (m, 1H), 3.84 (m, 3H), 3.93 (m, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.9, 21.9, 22.5, 24.7, 25.5, 26.8, 28.3, 28.8, 29.3, 29.5, 31.7, 32.3, 33.0, 37.1, 37.3, 39.8, 71.4, 71.7, 74.0, 76.3, 82.0, 82.4, 82.7, 83.1, 178.6, 207.7; IR (film, CH_2Cl_2, sodium carboxylate form) ν_{max} cm^{-1}: 2925, 2854, 1714, 1570, 1459, 1406, 1365, 1317, 1066; ESIMS m/z 663 [M+Na]$^+$, 679 [M+K]$^+$; HRMS (ES) m/z [M+Na]$^+$ calcd for $C_{37}H_{68}O_8Na$ 663.4812, found 663.4814.

5.2.2.14. Butylester 17. Compound **15** (100 mg, 0.109 mmol) was dissolved in *n*-BuOH (2 mL) with Amberlyst® 15 (500 mg). The reaction was stirred and heated at 65 °C for 15 h. After filtration, the solvent was evaporated under reduced pressure and the residue purified by flash chromatography (AcOEt) to afford **17** (65 mg, 90%). Colorless oil; $C_{37}H_{70}O_7$; R_f=0.25 (AcOEt); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.91 (t, 3H, J=6.8 Hz), 0.95 (t, 3H, J=8 Hz), 1.65 (m, 4H), 1.96 (m, 2H), 1.99 (m, 2H), 2.30 (t, 2H, J=8 Hz), 3.43 (m, 1H), 3.63 (m, 1H), 3.89 (m, 5H), 4.08 (t, 2H, J=7 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.5, 13.6, 17.1, 19.0, 21.9, 22.5, 24.7, 24.9, 25.5, 28.3, 28.8, 29.0, 29.1, 29.3, 29.5, 30.6, 31.7, 32.4, 33.2, 34.3, 37.2, 37.4, 63.9, 71.3, 71.6, 74.0, 82.1, 82.4, 82.7, 83.2, 174.0; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 1736; ESIMS m/z 649 [M+Na]$^+$, 665 [M+K]$^+$; HRMS (ES) m/z [M+Na]$^+$ calcd for $C_{37}H_{70}O_7Na$ 649.5019, found 649.5036.

5.2.2.15. Bis-[α-acetonyl-1,3-(4-piperidinamide)propane] (18). Colourless resin; $C_{87}H_{158}N_2O_{14}$; R_f=0.3 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.86 (t, 6H, J=7.0 Hz), 1.0–2.0 (m, ΣCH_2), 1.94 (m, 8H), 2.10 (s, 3H), 2.12 (s, 3H), 2.41 (dd, 2H, J=16.0 Hz, J=2.2 Hz), 2.53 (m, 2H), 3.03 (m, 14H), 3.15 (m, 2H), 3.39 (m, 2H), 3.57 (m, 2H), 3.75 (m, 4H), 3.84–3.94 (m, 10H), 4.02 (m, 2H), 4.55 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.0, 25.0, 25.3, 25.6, 27.0, 28.4, 28.8, 29.3, 29.4, 29.6, 29.7, 30.1, 31.8, 32.1, 32.6, 33.1, 33.4, 36.0, 36.5, 37.3, 37.5, 42.2, 42.5, 46.0, 46.1, 46.4, 71.6, 71.7, 74.1, 82.1, 82.4, 82.8, 83.3, 173.4, 173.5, 207.9; IR (film, CH_2Cl_2) ν_{max} cm^{-1}: 3428, 2922, 2853, 1716, 1619, 1458, 1366, 1266, 1165, 1058. ESIMS m/z 1478 [M+Na]$^+$.

5.2.2.16. Triazole 19. Colourless oil; $C_{41}H_{79}N_3O_7S$; R_f=0.4 (silica gel, CH_2Cl_2/MeOH 9:1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.9 (t, 3H, J=7 Hz), 1.15–2.05, 2.12 (s, 3H), 2.48 (dd, 1H, J=17 Hz, J=3.5 Hz), 2.8 (m, 1H), 2.87 (dd, 1H, J=17 Hz, J=9.7 Hz), 3.04 (t, 2H, J=6.5 Hz), 3.4 (m, 1H), 3.6 (m, 1H), 3.8–3.95 (m, 5H), 4.2–4.3 (m, 1H), 4.33–4.42 (m, 1H), 5.6 (s, 2H), 7.25–7.35 (m, 5H), 7.53 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.0, 22.0, 22.6, 25.0, 25.6, 27.0, 28.4, 28.9, 29.3, 29.4, 29.6, 29.7, 29.9, 31.8, 31.9, 32.6, 33.4, 37.3, 37.6, 40.2, 45.1, 53.7, 63.1, 71.6, 71.8, 74.1, 82.1, 82.4, 82.8, 83.2, 121.3, 128.6,

129.4, 132.1, 144.8, 175.2, 206.6; IR (film, $CHCl_3$) ν_{max} cm^{-1}: 3451, 2926, 2854, 2360, 2341, 1718, 1650, 1460, 1365, 1226; ESIMS *m/z* 864 $[M+Na]^+$; HRMS (ES) *m/z* $[M+Na]^+$ calcd for $C_{48}H_{79}N_3O_7SNa$ 864.5536, found 864.5531.

5.3. Diels–Alder adducts

5.3.1. Furane 22. To a vigorously stirred solution of **1** (116 mg, 0.186 mmol) in CH_2Cl_2 (2.5 mL) cooled to −78 °C a 1 M DIBAL–H solution in toluene (2.2 mL, 2.2 mmol) was added. The mixture was stirred for 4 h and then quenched by glacial AcOH (3.5 mL). The reaction mixture was allowed to warm up to rt, and stirring was continued for 1.5 h. The resulting gel was partitioned between EtOAc (50 mL) and water (35 mL). The organic phase was washed with water (2×30 mL), dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude product was purified over a short column of silica gel (toluene/EtOAc/EtOH 30:70:1 to 30:70:4), furnishing furane **22** (99 mg, 87%). White amorphous solid; $C_{37}H_{66}O_6$; R_f=0.45 (silica gel, CH_2Cl_2/MeOH 9:1); ^1H NMR ($CDCl_3$, 400 MHz): δ 0.91 (t, 3H, *J*=6 Hz), 1.2–1.35 (m, ΣCH_2), 1.63 (m, 4H), 1.97 (m, 4H), 2.27 (s, 3H), 2.36 (t, 2H, *J*=7.6 Hz), 3.43 (m, 1H), 3.63 (m, 1H), 3.89 (m, 5H), 5.86 (s, 1H), 7.04 (s, 1H); IR (film, CH_2Cl_3) ν_{max} cm^{-1}: 2926, 2855, 1764, 1458, 1065; ESIMS (16,25,29-tri-(*O*-TBDMS) derivative) *m/z* 971 $[M+Na]^+$, 966 $[M+NH_4]^+$; HRMS (ES) *m/z* $[M+Na]^+$ calcd for $C_{37}H_{66}O_6Na$ 729.4757, found 729.4756. $[\alpha]_D^{20}$ +2 (*c* 0.8, CH_2Cl_2).

5.3.2. Diels–Alder adducts 23 and 24. Furane **22** (15 mg, 25 μmol) and *N*-phenylmaleimide (4.3 mg, 25 μmol, 1 equiv) or methyl acetylenedicarboxylate (3.5 mg, 25 μmol, 1 equiv) were mixed in water (0.2 mL). The mixture was boiled for 6 h under vigorous stirring. Extraction with CH_2Cl_2 (2×10 mL), drying (Na_2SO_4) and concentration under reduced pressure yielded adduct **23** (17 mg, 92%) or **24** (16 mg, 90%). **23**: Colourless oil; $C_{47}H_{73}NO_8$; R_f=0.4 (CH_2Cl_2/MeOH 9:1); ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (t, 3H, *J*=7 Hz), 1.2–1.55 (m, ΣCH_2), 1.62 (m, 4H), 1.56–1.70 (m, 5H), 1.74 (s, 3H), 1.96 (m, 4H), 2.20 (m, 2H), 2.90 (d, 1H, *J*=6.5 Hz), 3.09 (d, 1H, *J*=6.5 Hz), 3.40 (m, 1H), 3.60 (m, 1H), 3.8–3.95 (m, 5H), 5.06 (s, 1H), 5.88 (s, 1H), 7.28–7.48 (m, 5H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.9, 15.9, 21.9, 22.5, 24.8, 25.5, 27.1, 27.3, 28.3, 28.8, 29.2, 29.5, 31.7, 32.4, 33.2, 37.2, 37.4, 50.3, 51.1, 71.4, 71.7, 74.0, 82.1, 82.4, 82.7, 83.2, 83.4, 89.3, 126.5, 128.5, 129.0, 131.8, 132.5, 152.7, 174.1, 175.5; IR (film, $CHCl_3$) ν_{max} cm^{-1}: 3439, 2924, 2853, 1711, 1500, 1458, 1382, 1189, 1058, 947 cm^{-1}; ESIMS *m/z* 802 $[M+Na]^+$; HRMS (MALDI) calcd for $C_{47}H_{73}NO_8Na$ $[M+Na]^+$ 802.5234, found 802.5240. **24**: Colourless oil; $C_{43}H_{72}O_{10}$; R_f=0.4 (CH_2Cl_2/MeOH 9:1); ^1H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, *J*=6.9 Hz), 1.2–1.3 (m, ΣCH_2), 1.35–1.55 (m, 17H), 1.55–1. 7 (m, 5H), 1.73 (s, 3H), 1.97 (m, 4H), 2.24 (m, 2H), 3.39 (m, 1H), 3.59 (m, 1H), 3.78 (s, 3H), 3.84 (s, 3H), 3.75–4.00 (m, 5H), 5.34 (s, 1H), 6.36 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.4, 15.8, 22.4, 23.0, 25.4, 26.0, 27.4, 28.8, 29.2, 29.4, 29.6, 29.7, 29.9, 30.0, 30.1, 32.2, 33.0, 33.8, 37.7, 37.9, 52.4, 52.5, 72.0, 72.2, 74.5, 82.5, 82.8, 83.2, 83.6, 86.3, 94.8, 136.3, 150.8, 157.9, 162.0; ESIMS *m/z* 771 $[M+Na]^+$; HRMS (ES) *m/z* $[M+Na]^+$ calcd for $C_{43}H_{72}O_{10}Na$ 771.5023, found 771.5029.

5.4. Biological activities

5.4.1. Cytotoxicity. Cytotoxic activities were colorimetrically evaluated on KB 3-1 cells according to previously described procedures.[6d]

5.4.2. Complex I inhibition. The inhibitory activities against bovine mitochondrial complex I from submitochondrial particles (SMP) (i.e., NADH oxidase and NADH/*n*-decylubiquinone oxidoreductase activities) of the described compounds were measured as described elsewhere.[14]

5.4.3. Complex III inhibition. Purification and reconstitution of complex III from bovine heart mitochondria, as well as measurement of *n*-decylubiquinol/cytochrome *c* oxidoreductase activity was performed as described earlier.[15]

Acknowledgements

Jean-Christophe Jullian is gratefully acknowledged for NMR assistance.

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Heteroatom directed photoannulation: synthesis of indoloquinoline alkaloids: cryptolepine, cryptotackieine, cryptosanguinolentine, and their methyl derivatives

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Received 28 October 2005; revised 7 April 2006; accepted 20 April 2006
Available online 11 May 2006

Abstract—A three-step synthesis of indoloquinoline alkaloids is described. The reaction of 2,3 and 4-substituted haloquinolines with anilines afforded the respective anilinoquinolines, which upon photocyclization gave the indoloquinolines. By regioselective methylation on quinoline nitrogen, furnished the alkaloids cryptotackieine, cryptosanguinolentine, cryptolepine, and the synthetic isomer isoneocryptolepine. Their methyl derivatives were also synthesized in search of new antiplasmodial drugs and DNA intercalating agents.

1. Introduction

Indoloquinoline alkaloids are receiving prominent attention in recent years as they are known to act as DNA intercalating agents[1] and exhibit antimalarial properties.[2,3] The World Health Organization placed malaria besides tuberculosis and AIDS as a major infectious disease. The roots of the West African plant *Cryptolepis sanguinolenta*,[4] a rich source of indoloquinoline alkaloids, have been used by Ghanaian healers to treat a variety of health disorders including malaria. Since 1974, a decoction of this plant has been used in the clinical therapy of rheumatism, urinary tract infections, malaria, and other diseases.[5,6] The linear indoloquinoline alkaloids cryptolepine (**1**) (5-methyl-5*H*-indolo[3,2-*b*]quinoline), cryptotackieine (**2**) (neocryptolepine, 5-methyl-5*H*-indolo[2,3-*b*]quinoline), and an angularly-fused alkaloid cryptosanguinolentine (**3**) (isocryptolepine, 5-methyl-5*H*-indolo[3,2-*c*]quinoline) are three of the characterized alkaloids, which behave as DNA intercalating agents, inhibiting DNA replication and transcription. These compounds also exhibit strong antiplasmodial activity. Cryptolepine binds 10-fold more tightly to DNA than other alkaloids and proves to be much more cytotoxic toward B16 melanoma cells.[7] It has been reported[8,9] that some methyl-substituted indoloquinolines act as cytotoxic agents, liposomally-formulated anticancer agents,[10] and DNA-Topoisomerase II inhibitors.[11]

Recent synthetic studies have detailed the preparation of indoloquinoline alkaloids by intramolecular reaction of iminophosphorane with isocyanate,[12,13] by the regioselective thermocyclization of the corresponding azide[14] or by *ortho*-metalation using a cross-coupling strategy.[15]

Recently, we have reported an efficient photochemical synthesis[16] and Fischer indole synthesis[17] toward a cryptosanguinolentine alkaloid. Here, we describe the synthesis of the titled alkaloids along with a synthetic indoloquinoline isomer, isoneocryptolepine (**4**), which also possesses promising in vitro antiplasmodial properties.[3] An approach based on a stepwise formation, starting from amination of the appropriate haloquinolines with anilines is discussed. The resultant intermediates on photochemical irradiation being treated in the presence of iodine catalyst for about 48–72 h undergo oxidative cyclization to afford the corresponding isomeric indoloquinolines.

In the last few decades, Schultz et al.[18,19,20] extensively investigated heteroatom directed-photoarylation[21,22] method to derive indoles,[23,24] benzothiophenes,[25] benzofurans,[25] and benzoselenophenes[22] by involving subsequent elimination of smaller molecules like H_2O, H_2, HCl, and MeOH. Several mechanisms have been considered for this diarylamine photocyclization.[26] The term 'heteroatom-directed photoarylation' characterizes photochemically-initiated electrocyclic reactions originating from the arrangements of an available electron pair in a heteroatom and those from at least one aromatic π-bond. An attractive feature of this technique is the regiospecificity of the aromatic substitution *ortho* to the heteroatom.

Keywords: Indoloquinoline alkaloids; Photoannulation; Cryptolepine; Cryptotackieine; Cryptosanguinolentine.
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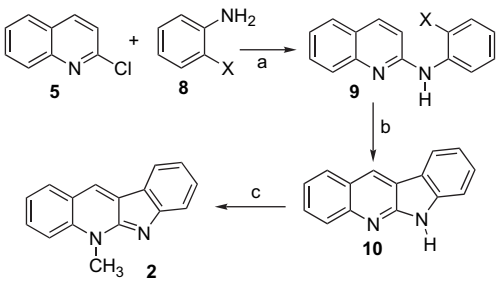
doi:10.1016/j.tet.2006.04.050

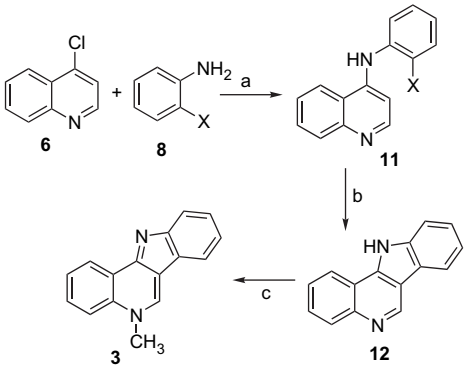
2. Results and discussion

To achieve our objective toward the synthesis of cryptotackieine (Scheme 1), cryptosanguinolentine (Scheme 2), and cryptolepine (Scheme 3), we have started from commercially available haloquinolines (**5**–**7**) and anilines (**8a**–**d**).



Where
8a) X = Cl, 8b) X = H, 8c) X = OH, 8d) X = OMe
a) 200 °C, 5h; b) hν, C_6H_6:CH_3OH:H_2SO_4 (60:30:1, v/v/v), I_2, rt.;
c) Me_2SO_4, CH_3CN, reflux, 6 h, K_2CO_3, 80%

Scheme 1.

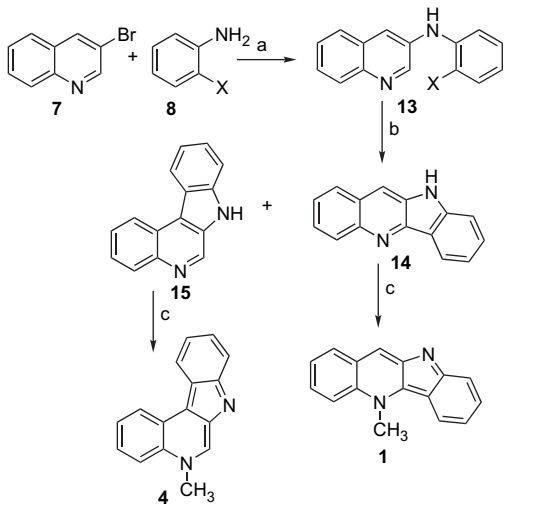


Where
8a) X = Cl, 8b) X = H, 8c) X = OH, 8d) X = OMe
a) 200 °C, 5h; b) hν, C_6H_6:CH_3OH:H_2SO_4 (60:30:1, v/v/v), I_2, rt.;
c) Me_2SO_4, CH_3CN, reflux, 6 h, K_2CO_3, 83%

Scheme 2.

It has been reported[27a–j] that aniline condenses with 2- and 4-chloroquinolines and many derivatives of haloquinolines on cautious heating at temperatures in the range of 100–200 °C. As a first step toward the synthesis of cryptotackieine (Scheme 1), regioselective amination of 2-chloroquinoline with 2-chloroaniline was carried out (**5**→**9a**). Several reports[28a–c] have appeared for such amination detailing the use of new catalysts. We have found the classical procedure[27a–h] more fruitful because the familiar procedure like Hartwig–Buchwald type amination requires expensive palladium catalysts.

In one approach, photochemical irradiation of anilinoquinolines in the absence of protic acid did not afford the desired products (**10** and **12**) when X=OH, and the yields were found to be ca. 55–60% when X=Cl, H, and OMe. Hence, we employed an acidic solvent system for the effective conversion to the desired photoproducts. Moreover, it should



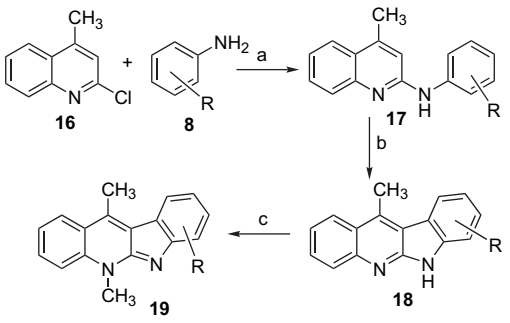
Where
8a) X = Cl, 8b) X = H;
a) 200 °C, 5h; b) hν, C_6H_6:CH_3OH:H_2SO_4 (60:30:1, v/v/v), I_2, rt.;
c) Me_2SO_4, CH_3CN, reflux, 6 h, K_2CO_3, 82-85%

Scheme 3.

be noted that the product yield for photocyclization in protic solvents was significantly higher than that in benzene alone. Consequently, all subsequent photoreactions were performed on **9**(**a**–**d**), **11**(**a**–**d**), **13**(**a**,**b**), **17**(**b**,**e**,**f**), and **21**(**b**,**e**,**f**) in benzene/methanol/sulfuric acid solution (60:30:1, v/v/v) in the presence of traces of iodine, which led via oxidative photocyclization to afford indoloquinolines **10**, **12**, **14**, **15**, **18**(**b**,**e**,**f**), and **22**(**b**,**e**,**f**). The cyclization of the resultant intermediates **9**(**a**–**d**) were found to occur at C-3 and not at C-1 position of the quinoline ring since the cyclization at the C-1 position requires carbon–nitrogen bond formation and would end with a net loss of aromaticity. In the final step, compound **4** was subjected to selective methylation on the quinoline nitrogen[8,12a,29] using $(CH_3)_2SO_4$ in CH_3CN or toluene refluxed for 6 h in the presence of K_2CO_3 to afford cryptotackieine (**2**). The same procedure was applied to compound **12** (Scheme 2), which afforded angularly-fused indoloquinoline alkaloid, cryptosanguinolentine (**3**).

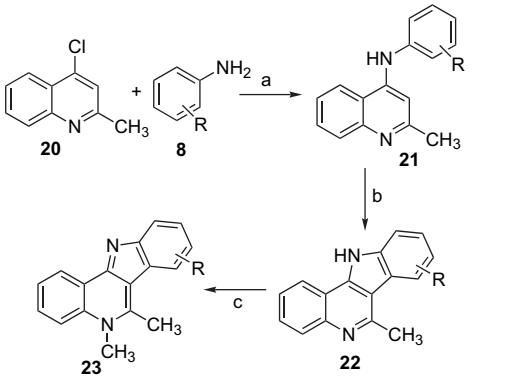
Interestingly, in the case of the reaction of 3-bromoquinoline with aniline (Scheme 3), the resulting intermediate **13** gave both linearly- and angularly-fused products. When irradiated, linear fusion provides quindoline as a minor product (16%), which on selective methylation on the quinoline nitrogen afforded the alkaloid, cryptolepine. On the other hand, the angularly-fused indoloquinoline (51%) **15** on methylation afforded isoneocryptolepine (**4**), which is a synthetic indoloquinoline alkaloid.[28a]

The methyl derivative of the alkaloids **2** and **3** was also prepared starting from 4-methyl-2-hydroxyquinoline and 2-methyl-4-hydroxyquinoline. On treatment with $POCl_3$ both compounds yielded their corresponding chloro-derivatives (**16** and **20**). Compounds **16** and **20**, on reaction with anilines (**8b**,**e**,**f**), followed by photochemical cyclization and regioselective methylation on the quinoline nitrogen, gave the newer derivatives **19**(**b**,**e**,**f**) and **23**(**b**,**e**,**f**), respectively (Schemes 4 and 5).



Where
8b) R = H, 8e) R = *o*-Me, 8f) R = *p*-Me,
a) Dry ethanol, reflux, 12h; b) hν, C_6H_6:CH_3OH:H_2SO_4 (60:30:1, v/v/v), I_2, rt.; c) Me_2SO_4, CH_3CN, reflux, 6 h, K_2CO_3, 81-82%

Scheme 4.



Where
8b) R = H, 8e) R = *o*-Me, 8f) R = *p*-Me,
a) Dry ethanol, reflux, 12h; b) hν, C_6H_6:CH_3OH:H_2SO_4 (60:30:1, v/v/v), I_2, rt.; c) Me_2SO_4, CH_3CN, reflux, 6 h, K_2CO_3, 80-83%

Scheme 5.

3. Conclusion

We have developed an efficient three-step synthesis of the alkaloids cryptotackieine, cryptosanguinolentine, and cryptolepine. Due to the easy availability of the starting materials and high yields realized in the different steps, this approach proves to be more attractive. We have followed the synthetically-useful method of 'heteroatom-directed photoannulation technique' for the construction of linearly, as well as angularly-fused indoloquinoline alkaloids.

4. Experimental

4.1. General methods

^{1}H NMR and ^{13}C NMR (400 and 100 MHz) spectra were recorded in $CDCl_3$ and TMS was used as an internal reference. Melting points were determined and were uncorrected. Chromatographic purification was conducted by column chromatography using 60–120 mesh silica gel. Reagent grade aniline and ethyl acetoacetate were used after usual purification methods (for the preparation of the compounds **16** and **20**). Reaction progress was monitored by thin layer chromatography. Mass spectra were recorded on a JMS-D-300 mass spectrometer. Elemental analyses were recorded on EL III, Elementar Analysen Systeme. 2-Chloroquinoline, 4-chloroquinoline, 2-chloroaniline, and 3-bromoquinoline were purchased and used as received. 2-Methyl-4-hydroxyquinoline and 4-methylquinolin-2(1*H*)-one were prepared by using reported procedures.[30a,b] Photolysis was carried out in a RPR-100 Photochemical Reactor, fitted with 16 RPR UV lamps and using quartz tube.

4.2. Preparation of anilinoquinolines and their derivatives: 9(a–d), 11(a–d), 13(a,b), 17(b,e,f), and 21(b,e,f)

4.2.1. Preparation of anilinoquinolines: 9(a–d), 11(a–d), and 13(a,b). Equimolar mixture (10 mmol) of the compounds 2-chloroquinoline (1.63 g) (**5**) and 2-chloroaniline (1.24 g) (**8a**) were heated in flame, and then a violent reaction occurred at 200 °C. The reaction mixture was cooled; crystals of the product (**9a**) were crashed out. This was heated in NaOH (dilute), cooled down, and recrystallized with alcohol (yield: 72%, 1.83 g). Similarly, other anilinoquinolines were prepared using appropriate haloquinolines and anilines.

4.2.2. Preparation of anilinoquinoline derivatives: 17(b,e,f) and 21(b,e,f). To 2-chloro-4-methylquinoline (1.77 g, 10 mmol) (**16**) dissolved in dry ethanol was added aniline (0.91 g, 10 mmol) (**8b**) and then the mixture was refluxed for about 12 h. Then the excess of ethanol was distilled off and the residue was then subjected to silica gel-column chromatography using petroleum ether/ethyl acetate (85:15, v/v) as eluants, yielded the product (**17b**) in 74%. Similarly, its derivatives **17**(**e**,**f**) and **21**(**b**,**e**,**f**) were prepared by treating 2-chloro-4-methylquinoline and 4-chloro-2-methylquinolines (**20**) with their respective anilines (**8b**,**e**,**f**). Since these methyl derivatives **16** and **20** were aminated using this procedure we did not opt the other procedures.

4.3. Preparation of isomeric indoloquinoline moieties: 10, 12, 14, 15, 18(b,e,f), and 22(b,e,f)

4.3.1. Photochemical cyclization of the compound 9b: 6*H*-indolo[2,3-*b*]quinoline. Irradiation of a solution of **9b** (1.1 g, 5 mmol) in the solvent system benzene/methanol/sulfuric acid (60:30:1, v/v/v) with iodine (25 mg, 0.1 mmol) was carried out in a RPR-100 Photochemical Reactor, fitted with 16 RPR UV lamps. The solution placed in a quartz flask was irradiated for 48 h. When the reaction controlled by TLC showed complete disappearance of **9b** the solvent was removed in vacuo and then the residue was subjected to column chromatography on silica gel using petroleum ether/ethyl acetate (80:20, v/v) as eluants, which afforded the pure photoproduct **10** (0.76 g); 70%, mp >300 °C. Likewise, **12** (78%, 0.85 g) (from **11a**), **14** (16%, 0.17 g) and **15** (51%, 0.56 g) (from **13b**), **18b** (70%, 0.83 g), **18e** (67%, 0.82 g), **18f** (68%, 0.83 g), **22b** (65%, 0.75 g), **22e** (63%, 0.77 g), **22f** (69%, 0.85 g) were prepared from their appropriate anilinoquinolines on photochemical irradiation.

4.4. Preparation of isomeric indoloquinoline alkaloids 2, 3, 1, and 4 and their derivatives 19(b,e,f) and 23(b,e,f)

4.4.1. Regioselective methylation of indoloquinolines. Regioselective methylation was done using the reported

procedure.[8] The methylated products **2** (80%, 0.37 g), **3** (83%, 0.38 g), **1** (82%, 0.094 g), **4** (84%, 0.19 g), **19b** (82%, 0.40 g), **19e** (81%, 0.42 g), **19f** (82%, 0.425 g), and **23b** (82%, 0.40 g), **23e** (80%, 0.42 g), **23f** (83%, 0.43 g) were obtained from **10** (2 mmol), **12** (2 mmol), **14** (0.5 mmol), **15** (1 mmol), **18**(**b**,**e**,**f**), and **22**(**b**,**e**,**f**) (2 mmol), respectively.

Compound **9a**. Yield: 72% (1.83 g); mp 137 °C; IR (KBr): 1046, 3215, 1610 cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.75 (1H, d, J=7.2 Hz, Quin–C_3–H), 7.05–7.42 (m, 4H, Ph–H), 7.66 (1H, t, J=7.8 Hz, C_6–H), 7.89 (1H, t, J=7.8 Hz, C_7–H), 7.99 (1H, d, J=7.2 Hz, C_5–H), 8.08 (1H, d, J=7.2 Hz, C_4–H), 8.19 (1H, d, J=8.2 Hz, C_8–H), 10.81 (1H, br s, NH); ^{13}C NMR ($CDCl_3$): C_3-118.23, C_4'-119.37, C_2'-119.52, C_3''-121.54, C_6-121.92, C_5-125.25, C_3'-125.32, C_7-126.45, C_4-127.43, C_8-128.05, C_{4a}-129.11, C_2''–Cl-135.71, C_1'-148.23, C_{8a}-148.61, C_2-159.24. EIMS (70 eV, *m/e*): 254 (M^+), 256 (M+2). Anal. Calcd for $C_{15}H_{11}N_2Cl$: C 70.73, H 4.35, N 11.00. Found: C 70.56, H 4.37, N 10.96.

Compound **10**. Yield: 70%, 0.761 g (from **9b**); mp >300 °C; IR (KBr): 1615, 3156 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.05–7.95 (m, 5H, Ar–H), 7.98 (1H, d, J=7.4 Hz, C_4–H), 8.11 (1H, d, J=7.8 Hz, C_1–H), 8.25 (1H, d, J=8.0 Hz, C_{10}–H), 9.05 (1H, s, C_{11}−H), 11.61 (1H, br s, NH); ^{13}C NMR ($CDCl_3$): C_7-111.13, C_4-118.97, C_9-119.94, C_{11a}-120.51, C_{10}-122.03, C_2-122.91, C_{10a}-123.92, C_1-127.33, C_{11}-127.74, C_{10b}-128.17, C_3-128.41, C_8-128.83, C_{4a}-141.53, C_{6a}-146.72, C_{5a}-154.40. EIMS (70 eV, *m/e*): 218 (M^+); Anal. Calcd for $C_{15}H_{10}N_2$: C 82.55, H 4.62, N 12.84. Found: C 82.41, H 4.63, N 12.80.

Compound **11a**. Yield: 74% (1.88 g); mp 142 °C; IR (KBr): 1045, 3220, 1610 cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.77 (1H, d, J=7.2 Hz, Quin–C_3–H), 7.04–7.43 (4H, m, Ph–H), 7.66 (1H, t, J=7.6 Hz, C_6–H), 7.90 (1H, t, J=8.0 Hz, C_7–H), 7.98 (1H, d, J=7.2 Hz, C_5–H), 8.09 (1H, d, J=7.6 Hz, C_8–H), 8.63 (1H, d, J=8.2 Hz, C_2–H), 10.71 (1H, br s, NH); ^{13}C NMR: C_3-107.11, C_3''-115.37, C_3'-119.71, C_5-120.33, C_1'-121.07, C_6-124.78, C_7-127.52, C_4'-127.76, C_2'-129.63, C_8-130.82, C_{4a}-132.13, C_2''–Cl-145.39, C_4-145.79, C_{8a}-149.01, C_2-151.32. EIMS (70 eV, *m/e*): 254 (M^+), 256 (M+2); Anal. Calcd for $C_{15}H_{11}N_2Cl$: C 70.73, H 4.35, N 11.00. Found: C 70.56, H 4.31, N 10.96.

Compound **12**. Yield: 78%, 0.85 g (from **11a**); mp >220 °C (decomp.). Spectral and analytical data of this compound coincide with our earlier reported one.[16]

Compound **13**. Yield: 70% (1.54 g); mp 116 °C; IR (KBr): 3276, 1611 cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.94–7.48 (5H, m, Ph–H), 7.66 (1H, t, J=7.8 Hz, C_6–H), 7.89 (1H, t, J=7.8 Hz, C_7–H), 7.99 (1H, d, J=8.0 Hz, C_5–H), 8.09 (1H, s, C_4–H), 8.23 (1H, d, J=8.2 Hz, C_8–H), 8.42 (1H, s, C_2–H), 10.44 (1H, br s, NH); ^{13}C NMR: C_4'-104.26, C_3'- and C_3''-114.27, C_2'- and C_2''-119.46 and 120.34, C_5-123.19, C_6-124.44, C_7-127.06, C_8-128.13, C_4-129.77, C_{4a}-131.31, C_1'-142.87, C_3-150.02, C_{8a}-150.98, C_2-151.51. EIMS (70 eV, *m/e*): 220 (M^+); Anal. Calcd for $C_{15}H_{12}N_2$: C 81.79, H 5.49, N 12.72. Found: C 81.61, H 5.51, N 12.69.

Compound **17b**. Yield: 74%, (1.73 g); mp 145 °C; IR (KBr): 3256, 2925, 2857, 1605 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.49 (3H, s, Ar–CH_3), 6.75 (1H, s, Quin–C_3–H), 7.05–7.38 (5H, m, Ph–H), 7.66 (1H, t, J=7.4 Hz, C_6–H), 7.89 (1H, t, J=8.0 Hz, C_7–H), 7.97 (1H, d, J=8.2 Hz, C_5–H), 8.08 (1H, d, J=8.2 Hz, C_8–H), 10.51 (1H, br s, NH); ^{13}C NMR: C_4–CH_3-20.01, C_3-10.38, C_4-119.60, C_4'-121.70, C_2'- and C_2''-124.70, C_3'- and C_3''-125.33, C_6-125.52, C_5-127.94, C_7-130.02, C_8-134.08, C_{4a}-135.00, C_1'-135.78, C_{8a}-152.15, C_2-153.94. EIMS (70 eV, *m/e*): 234 (M^+); Anal. Calcd for $C_{16}H_{14}N_2$: C 82.02, H 6.02, N 11.96. Found: C 82.07, H 6.03, N 11.95.

Compound **18b**. Yield: 72% (0.83 g); mp 206 °C; IR (KBr): 3196, 2921, 2845 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.50 (3H, s, C_{11}–CH_3), 7.25–8.00 (6H, m, Ar–H), 8.11 (1H, d, J=8.12 Hz, C_4–H), 8.36 (1H, d, J=8.10 Hz, C_{10}–H), 10.75 (1H, br s, NH); ^{13}C NMR ($CDCl_3$): C_{11}–CH_3-20.35, C_{10a}-103.65, C_9-121.67, C_8-122.48, C_{10b}-122.62, C_{10}-124.03, C_1-124.34, C_2-126.63, C_3-127.86, C_7-128.11, C_4-129.88, C_{11a}-130.07, C_{6a}-132.79, C_{11}-143.55, C_{4a}-149.47, C_{5a}-161.56. EIMS (70 eV, *m/e*): 232 (M^+). Anal. Calcd for $C_{16}H_{12}N_2$: C 82.73, H 5.21, N 12.06. Found: C 82.60, H 5.23, N 12.01.

Compound **19b**. Yield: 82% (0.40 g); mp >250 °C; IR (KBr): 2916, 2874, 1612 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.44 (3H, s, C_{11}–CH_3), 3.66 (3H, s, C_5–CH_3), 7.25–7.87 (7H, m, Ar–H), 8.41 (1H, d, J=8.24 Hz, C_{10}–H); ^{13}C NMR ($CDCl_3$): C_{11}–CH_3-21.63, C_5–CH_3-46.65, C_{10a}-105.39, C_9-121.07, C_{10b}-122.57, C_{10}-123.53, C_8-123.55, C_1-124.03, C_2-125.36, C_7-129.25, C_4-129.60, C_3-127.94, C_{11a}-130.14, C_{6a}-131.30, C_{11}-141.22, C_{4a}-147.23, C_{5a}-158.83. EIMS (70 eV, *m/e*): 278 (M^+); Anal. Calcd for $C_{19}H_{22}N_2$: C 81.97, H 7.97, N 10.06. Found: C 81.73, H 7.99, N 10.02.

Compound **21b**. Yield: 70% (1.64 g); mp 162 °C; IR (KBr): 3234, 2941, 2886 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.41 (3H, s, Ar–CH_3), 6.49 (1H, s, Quin–C_3–H), 7.08–7.48 (5H, m, Ph–H), 7.67 (1H, t, J=7.6 Hz, C_6–H), 7.89 (1H, t, J=7.6 Hz, C_7–H), 8.02 (1H, d, J=8.0 Hz, C_5–H), 8.12 (1H, d, J=8.2 Hz, C_8–H), 10.18 (1H, br s, NH); ^{13}C NMR: C_2–CH_3-27.34, C_3-110.78, C_3'- and C_3''-115.71, C_2'- and C_2''-119.42, C_4'-120.11, C_5-120.45, C_6-124.33, C_7-127.62, C_8-129.15, C_{4a}-131.77, C_1'-144.79, C_{8a}-149.34, C_2-155.13, C_4-157.50. EIMS (70 eV, *m/e*): 234 (M^+); Anal. Calcd for $C_{16}H_{14}N_2$: C 82.02, H 6.02, N 11.96. Found: C 81.95, H 6.03, N 11.89.

Compound **22b**. Yield: 65% (0.75 g); mp 235 °C; IR (KBr): 3207, 2936, 2885 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.43 (3H, s, C_6–CH_3), 7.33–7.88 (7H, m, Ar–H), 8.07 (1H, d, J=8.04 Hz, C_4–H), 10.51 (1H, br s, NH); ^{13}C NMR ($CDCl_3$): C_6–CH_3-23.14, C_{10}-118.64, C_9-119.54, C_7-120.29, C_{6a}-122.57, C_8-124.43, C_2-124.69, C_3-126.73, C_{6b}-126.78, C_1-127.41, C_4-129.44, C_{11b}-130.11, C_{10a}-135.67, C_{11a}-136.73, C_{4a}-150.17, C_6-154.75. EIMS (70 eV, *m/e*): 232 (M^+); Anal. Calcd for $C_{16}H_{12}N_2$: C 82.73, H 5.21, N 12.06. Found: C 82.66, H 5.22, N 12.10.

Compound **23b**. Yield: 82% (0.40 g); mp 197 °C; IR (KBr): 2922, 2857, 1606 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.43 (3H, s, C_6–CH_3), 3.75 (3H, s, C_5–CH_3), 7.36–7.92 (7H, m, Ar–H),

8.26 (1H, d, J=7.86 Hz, C_4–H); ^{13}C NMR ($CDCl_3$): C_6–CH_3-22.14, C_5–CH_3-41.12, C_{10}-117.23, C_9-118.49, C_7-121.34, C_8-123.45, C_{6a}-123.54, C_2-124.55, C_3-126.30, C_1-127.81, C_4-129.66, C_{11b}-130.05, C_{10a}-135.03, C_{11a}-135.41, C_{6b}-136.07, C_{4a}-149.69, C_6-157.61. EIMS (70 eV, *m*/*e*): 246 (M^+); Anal. Calcd for $C_{17}H_{14}N_2$: C 82.90, H 5.73, N 11.37. Found: C 82.67, H 5.74, N 11.41.

The spectral data for the compounds **2** (Yield 80%, 0.37 g), **3** (Yield 83%, 0.38 g), and **1** are identical with those reported[4d,4e,4a–c] for the natural products, respectively. The spectral data of **15** and **4** coincide with the earlier reported compound.[28a]

Acknowledgements

T.D. thanks CSIR, New Delhi for the award of Senior Research Fellowship. P.S.M. thanks CSIR-New Delhi for financial assistance (CSIR-Major project). Authors thank SIF, IISc, Bangalore, and IICT, Hyderabad for providing the spectral and analytical data.

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Stereoselective total synthesis of (±)-α-vetispirene, (±)-hinesol, and (±)-β-vetivone based on a Claisen rearrangement

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Received 7 March 2006; revised 17 April 2006; accepted 19 April 2006
Available online 19 May 2006

Abstract—The stereoselective total syntheses of (±)-α-vetispirene, (±)-hinesol, and (±)-β-vetivone were accomplished based on a Claisen rearrangement in an alkenyl bicyclic dihydropyran system. The most striking feature of this approach is that the Claisen rearrangement of bicyclic dihydropyran proceeds stereoselectively to provide a multi-functionalized spiro[4.5]decane, which is an efficient precursor for the synthesis of the vetivane sesquiterpenes.

1. Introduction

α-Vetispirene (**1**),[1] hinesol (**2**),[2] and β-vetivone (**3**)[3] are representative members of the vetivane sesquiterpene family, which possess a spiro[4.5]decane core and a branched three-carbon unit on a cyclopentane framework (Fig. 1). Related spirocyclic terpenes such as lubimin and gleenol, substituted with an oxy-functional group at a position adjacent to the spirocyclic carbon center, have been also isolated.[4] Some of these spirocyclic terpenes exhibit interesting biological activities. Especially, (−)-**2** is a relatively specific inhibitor of H^+, K^+-ATPase and an active ingredient of cerebral circulation and metabolism improvers.[2c] Because of their unique structures and biological activities, a number of synthetic approaches to these terpenes have been reported, including intramolecular alkylations, palladium-based cyclizations, and intermolecular cycloadditions.[5,6]

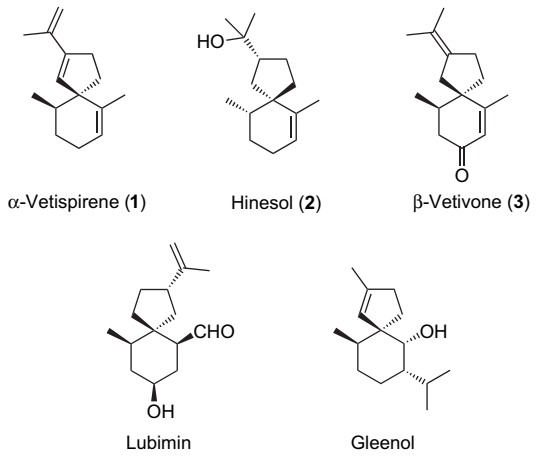
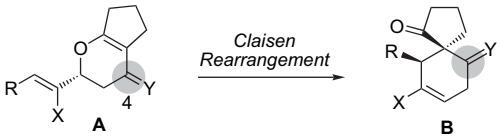


Figure 1. Terpenes with spiro[4.5]decane framework.

We have recently developed a new approach to multi-substituted spiro[4.5]decanes based on a Claisen rearrangement, in order to produce a more efficient general synthetic method for spirocyclic terpenes (Eq. 1).[7] Claisen rearrangement is one of the most reliable and efficient methods of introducing asymmetry and as such may be quite useful for the synthesis of the functionalized spiro[4.5]decane **B**.[8] The strategy involves a rearrangement of bicyclic dihydropyran **A**, substituted in the 4-position with a high-oxidation state group (Eq. 1, Y), thereby introducing a functional group at a position adjacent to the spirocyclic carbon center in **B**. By means of varying the groups R, X, and Y, this strategy could be applicable to the synthesis of multi-functionalized spirocyclic frameworks.



(1)

It is worth noting that the choice of the functional group Y is an important key to success in this rearrangement. Indeed, the expected rearrangement of alkenyl dihydroxypyrone (Y=O) did not proceed; instead the ring-opening product was mainly obtained (Eq. 2). On the other hand, the rearrangement of dihydropyrans, substituted in the 4-position

Keywords: Vetivane sesquiterpene; Spiro[4.5]decane; Claisen rearrangement.
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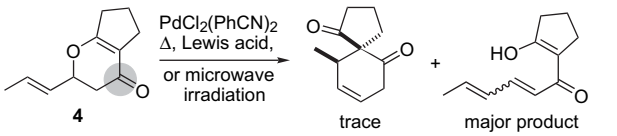
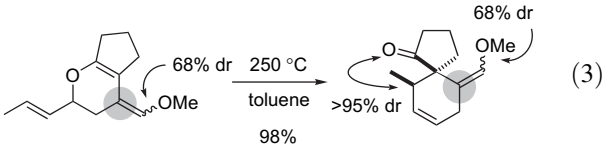
doi:10.1016/j.tet.2006.04.049

with a non-enolizable group such as a double bond or a siloxy group, proceeds in excellent yields and with high stereoselectivities (Eqs. 3 and 4).[7] This is the first report of successful Claisen rearrangement in bicyclic dihydropyran systems with a high-oxidation state functionality in the 4-position.[9]



(2)



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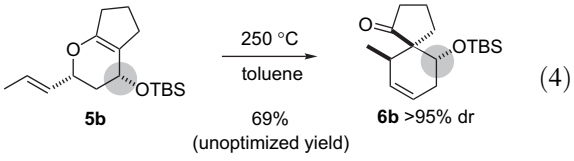
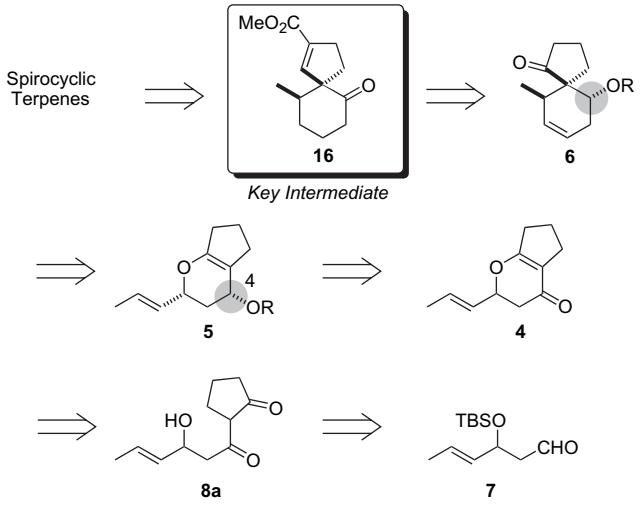


(4)

We focused our attention on the siloxy spiro[4.5]decane **6b** because this spirocycle **6b** would be a suitable precursor for the synthesis of vetivane sesquiterpenes. The present paper details optimization of the Claisen rearrangement of alkenyl bicyclic dihydropyran and the stereoselective total synthesis of (±)-α-vetispirene, (±)-hinesol, and (±)-β-vetivone.

2. Results and discussion

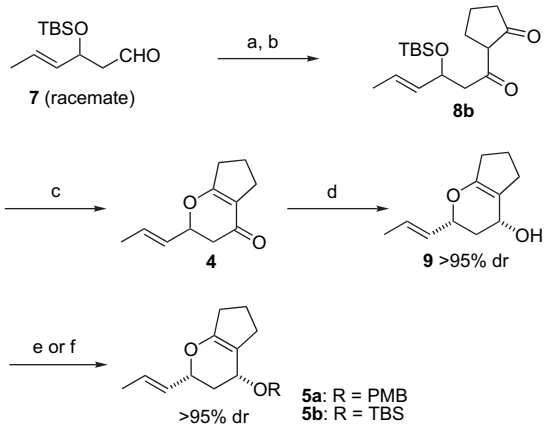
Our retrosynthetic analysis for the stereoselective synthesis of vetivane sesquiterpenes is outlined in Scheme 1. As shown, access to these spirocyclic terpenes was envisioned from a key intermediate **16**, which would be converted from spiro[4.5]decane **6**. The Claisen rearrangement of 4-oxy-functionalized alkenyl dihydropyran **5** would provide **6** in a stereoselective manner. Dihydropyran **5** should be obtainable from the corresponding dihydropyrone **4**, which would be readily available from a racemic hydroxy-1,3-diketone **8a** by acid-catalyzed cyclization. In turn, synthesis of diketone **8a** was envisaged from aldehyde **7** by means of several simple manipulations. As non-racemic **7** is easily prepared in high enantiomeric excess by several ways,[10] the present strategy would be applicable to the total synthesis of the optically active vetivane sesquiterpenes.



Scheme 1. Retrosynthetic analysis.

2.1. Stereoselective synthesis of alkenyl dihydropyranes 5

Our first step was the preparation of substrate **5** according to a modified version of our procedure (Scheme 2);[7] treatment of the known aldehyde **7** (racemate)[11] with the enolate derived from cyclopentanone afforded the aldol adduct as a mixture of diastereomers. This aldol adduct was converted to 1,3-diketone **8b** by DMSO/TFAA oxidation in good yield.[12] Notably, other oxidants, such as Dess–Martin periodinane, DMSO/$(COCl)_2$, and PCC were less effective. Next, removal of the TBS group of **8b** and acid-catalyzed cyclization proceeded in a single operation by an excess of TFA to afford alkenyl dihydropyrone **4** in 85% yield. Dihydropyrone **4** thus obtained was reduced by $LiAlH_4$ to afford **9**, followed by protection of the hydroxy group to give the requisite alkenyl dihydropyran **5a** (R=PMB) or **5b** (R=TBS) as single diastereomer. The relative stereochemistry shown for **5b** was determined by the NOESY correlation.

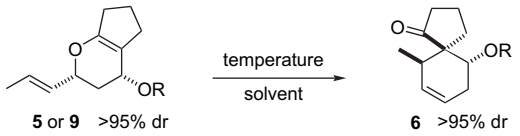


Scheme 2. Preparation of substrates **5a** and **5b** for the Claisen rearrangement. Reagents and conditions: (a) LDA, cyclopentanone, 91%; (b) DMSO, TFAA, then Et_3N, −78 °C, 87%; (c) TFA, CH_2Cl_2, 0 °C→rt, 85%; (d) $LiAlH_4$, Et_2O, 0 °C; (e) PMBCl, NaH, 57% (two steps); (f) TBSCl, imidazole, rt, 73% (two steps).

2.2. Optimization of the Claisen rearrangement

Next, we examined the construction of the spiro[4.5]decane framework by thermolytically induced Claisen rearrangement. After careful optimization, it was found that the choice of solvent, hydroxy protecting group, and temperature appears to be important for the success of this rearrangement (Table 1). The rearrangement of dihydropyran **5a** (R=PMB), in 1,2,4-trichlorobenzene (1,2,4-TCB) at 250 °C, provided a trace amount of the desired rearrangement product **6a**, along with an elimination product as the major component (entry 1). In contrast, the rearrangement of **5b**, which was protected by a bulkier group on the 4-hydroxy group, in the

Table 1. Claisen rearrangement of the alkenyl dihydropyrans



Entry	Substrate (R)	Solvent	Temperature (°C)	Product	Yield (%)
1	**5a** (PMB)	1,2,4-TCB	250	**6a**	Trace
2	**5b** (TBS)	1,2,4-TCB	250	**6b**	28
3	**5b**	Toluene	250	**6b**	87
4	**5b**	1,2,4-TCB	165	**6b**	Trace
5	**9** (H)	1,2,4-TCB	70–100	**6c**	0

same solvent, afforded rearrangement product **6b**. While this was a better result than the previous one it was still poor (28% yield, entry 2). In addition to changing the protective group, use of a less polar solvent provided much better results. Thus, alkenyl dihydropyran **5b** was heated at 250 °C in toluene to provide the desired spiro[4.5]decane **6b** in 87% yield as a single diastereomer (entry 3).[13] The rearrangement of **5b** or **9** (R=H) at lower temperature in 1,2,4-TCB afforded only elimination products; none of the desired products were obtained (entries 4 and 5).

The stereochemical assignment of **6b** was verified by NOE experiments on the tricycle, derived from **6b** in two steps [(1) $LiAlH_4$, Et_2O, 0 °C; (2) 2,2-dimethoxypropane, PPTS, CH_2Cl_2] (Fig. 2).[7] This stereochemical outcome suggests that the bicyclic dihydropyran **5b** undergoes the rearrangement through a boat-like transition state (Fig. 3).[14]

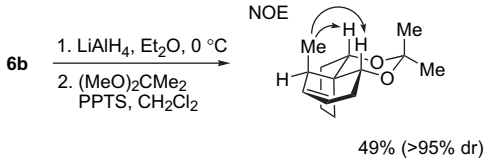


Figure 2. Stereochemical determination of **6b**.

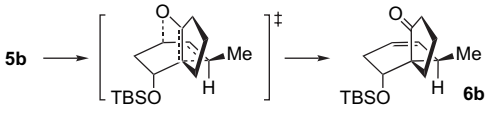
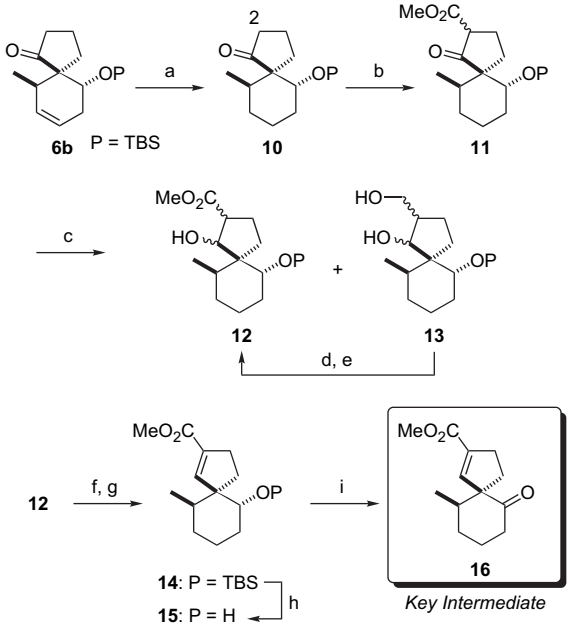


Figure 3. A possible transition state of Claisen rearrangement.

2.3. Synthesis of a key intermediate 16

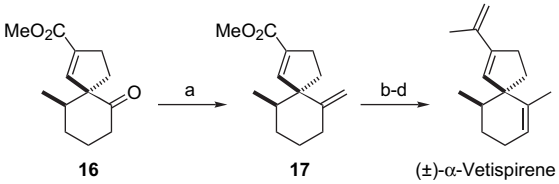
With the multi-functionalized spiro[4.5]decane framework in hand, we next synthesized **16**, a common key intermediate for the synthesis of vetivane sesquiterpenes, as shown in Scheme 3. The double bond in ketone **6b** could be saturated under 1 atm of hydrogen gas in the presence of 5% Pd on charcoal.[15] Although introduction of one-carbon unit at the C2-position of the spiro[4.5]decane framework has been achieved with difficulty,[6] exposure of ketone **10** to an excess of KH and dimethyl carbonate smoothly produced the desired keto ester **11** in good yield as a mixture of diastereomers. Next, the reduction with $NaBH_4$ was performed to give hydroxy ester **12** in moderate yield, along with the unexpected diol **13** in 20% yield. Diol **13** could be converted to hydroxy ester **12** by a two-step sequence. Chemoselective oxidation of the primary alcohol by the Merck method (TEMPO/$NaClO_2$/NaClO) to afford the corresponding carboxylic acid,[16] followed by methyl esterification (MeI, $KHCO_3$), led to **12** in 84% yield for the two steps. The hydroxy ester **12** was then mesylated and eliminated to provide the desired α,β-unsaturated ester **14**. Removal of the TBS group of **14** with an aqueous solution of hydrogen fluoride in CH_3CN, followed by Dess–Martin oxidation,[17] led to the key intermediate ketone **16**. This compound would be a versatile intermediate for the syntheses of a variety of spirocyclic terpenes.



Scheme 3. Preparation of the key intermediate **16**. Reagents and conditions: (a) Pd/C, H_2 (1 atm), EtOH; (b) KH, $(MeO)_2CO$, THF, 95 °C, 86% (two steps); (c) $NaBH_4$, MeOH, **12** 67% (based on the recovered S.M.: 74%), **13** 20%; (d) TEMPO, $NaClO_2$, NaClO; (e) MeI, $KHCO_3$, DMF, 84% (two steps); (f) MsCl, pyridine, 0 °C; (g) DBU, CH_2Cl_2, rt, 92% (two steps); (h) 48% HF aq, CH_3CN, rt; (i) Dess–Martin periodinane, CH_2Cl_2, rt, 88% (two steps).

2.4. Total synthesis of (±)-α-vetispirene

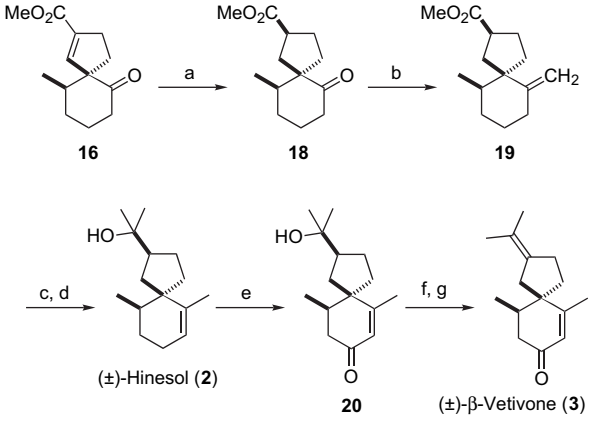
We next explored the final steps to (±)-α-vetispirene (**1**). Treatment of ketone **16** with excess MeLi, followed by acid-catalyzed dehydration with $TsOH\cdot H_2O$, led to (±)-**1**, along with inseparable byproducts. Thus, we chose an alternative stepwise route, whereby ketone **16** was first transformed into *exo*-methylene **17** under Nozaki's conditions ($TiCl_4$, CH_2I_2, and Zn) (Scheme 4).[2c,18] Acid-catalyzed isomerization of **17** provided the *endo*-isomer, which after treatment with an excess of MeLi, followed by dehydration led to (±)-α-vetispirene (**1**) in good overall yield.[19]



Scheme 4. Total synthesis of (±)-α-vetispirene (**1**). Reagents and conditions: (a) CH_2I_2, $TiCl_4$, Zn, THF–CH_2Cl_2, 72%; (b) cat. $TsOH\cdot H_2O$, PhH, reflux; (c) MeLi, THF; (d) cat. CSA, PhH, 60 °C, 87% (three steps).

2.5. Total synthesis of (±)-hinesol and (±)-β-vetivone

(±)-Hinesol (**2**) was also synthesized from the key intermediate **16** in a stereoselective manner (Scheme 5). Ketone **16** was subjected to hydrogenolysis to afford the desired ester **18** in 97% as an 87:13 mixture of separable diastereomers.[20] The keto carbonyl of **18** was converted to an *exo*-methylene group using Nozaki's conditions. Finally acid-catalyzed isomerization, to give *endo*-isomer followed by treatment with an excess of MeMgI, provided (±)-hinesol (**2**) in 90% yield for the two steps. (±)-β-Vetivone (**3**) was synthesized from (±)-hinesol by a reported procedure.[21] The regioselective allylic oxidation of (±)-hinesol (**2**) by means of CrO_3/3,5-dimethylpyrazole (3,5-DMP) afforded hinesolone **20** in moderate yield.[22] Then, treatment of **20** with acetic anhydride followed by $BF_3\cdot OEt_2$ yielded (±)-β-vetivone (**3**) contaminated with the *exo*-methylene isomer as an inseparable mixture (86:14 mixture of **3** and the *exo*-isomer) in 64% yield.[23]



Scheme 5. Total synthesis of (±)-hinesol (**2**) and (±)-β-vetivone (**3**). Reagents and conditions: (a) Pd/C, H_2 (1 atm), EtOH, 97% (87% dr), then separation; (b) CH_2I_2, $TiCl_4$, Zn, THF–CH_2Cl_2, 80%; (c) cat. TsOH·H_2O, PhH, reflux; (d) MeMgI, Et_2O, 0 °C to rt, 90% (two steps); (e) CrO_3, 3,5-DMP, CH_2Cl_2, 0 °C, 60%; (f) Ac_2O, NaOAc, 140 °C, 93%; (g) $BF_3\cdot OEt_2$, rt, 64% (based on the recovered S.M.: 84%, 86:14 mixture of **3** and the *exo*-isomer).

3. Conclusion

We were able to achieve the stereoselective total syntheses of (±)-α-vetispirene, (±)-hinesol, and (±)-β-vetivone based on a Claisen rearrangement of a functionalized alkenyl bicyclic dihydropyran system. Our strategy is unique and efficient, and could be applicable to other terpenes with a multi-functionalized spiro[4.5]decane framework. Further investigations into its application to the synthesis of optically active spirocyclic terpenes are in progress.

4. Experimental

4.1. General

All reactions sensitive to oxygen and moisture were performed in flame-dried glassware under a static argon atmosphere unless otherwise noted. ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-LD400 spectrometer operating at either 400 MHz (^1H) or 100 MHz (^{13}C) or on a JEOL AL-300 spectrometer operating at either 300 MHz (^1H) or 75 MHz (^{13}C). Chemical shifts are reported in δ units and are referenced to the solvent, i.e., 7.26/77.1 for $CDCl_3$. Multiplicities are indicated as br (broadened), s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), sept (septet), or m (multiplet). Coupling constants (J) are reported in Hertz (Hz). Infrared spectra were recorded on a Jasco FT-IR410 spectrometer. Electron impact mass spectra were performed on a HITACHI M-80B mass spectrometer. Electrospray ionization mass spectra were recorded on an Applied Biosystems API QSTAR pulsar i as high resolution, using poly(ethylene glycol) as internal standard. Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} (Merck 1.05715.0009) plates. Flash column chromatography was performed on a PSQ100B silica gel (Fuji Silysia Co., Ltd, Japan). THF and Et_2O were purchased from Wako Pure Chemical Industries Ltd, in anhydrous grade. CH_2Cl_2 was distilled from CaH_2 immediately before use. Diisopropylamine was distilled from CaH_2 and stored over KOH pellets.

4.1.1. 1,3-Diketone 8b. To the solution of diisopropylamine (0.982 mL, 7.05 mmol) in THF (14 mL) was added a solution of *n*-BuLi (4.6 mL of 1.52 M solution in hexane, 7.05 mmol) at 0 °C, then the resulting mixture was stirred at that temperature for 30 min and cooled to −78 °C. To this mixture was added cyclopentanone (0.567 mL, 6.41 mmol) at −78 °C, then this mixture was stirred at that temperature for 1 h. To this mixture was added a solution of aldehyde **7** (732 mg, 3.21 mmol) in THF (6 mL) at −78 °C, then this mixture was stirred at that temperature for 1 h 20 min. The reaction mixture was quenched with a saturated aqueous solution of NH_4Cl. The aqueous layer was extracted two times with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, filtrated, and concentrated under reduced pressure. Purification by silica-gel column chromatography (hexane/EtOAc=95:5→90:10→80:20) gave 908 mg (91% yield, a mixture of diastereomers by ^1H NMR analysis) of the aldol adduct: R_f 0.64, 0.52 (hexane/EtOAc=75:25); ^1H NMR (400 MHz, $CDCl_3$) δ 5.64–5.32 (m, 2H), 4.47–4.18 (m, 2H), 3.36 (br s, 0.8H), 3.21 (br s, 0.2H), 2.25 (br dd, J=16.3, 7.3 Hz, 1H), 2.13–1.95 (m, 4H), 1.76–1.59 (m, 7H), 0.851 (s, 6.3H), 0.840 (s, 2.7H), 0.0378 (s, 3H), 0.01 (s, 3H); IR (neat, cm^{-1}) 3504, 1726; HR-ESIMS calcd for $C_{17}H_{32}O_3NaSi$: 335.2018, found: 335.2018. To the solution of DMSO (1.24 mL, 17.4 mmol) in CH_2Cl_2 (10 mL) was added $(CF_3CO)_2O$ (1.21 mL, 8.72 mmol) at −78 °C, then the resulting mixture was stirred at that temperature for 40 min. To this mixture was added a solution of the aldol adduct (908 mg, 2.91 mmol) in CH_2Cl_2 (10 mL) at −78 °C, then this mixture was stirred at that temperature for 40 min. To this mixture was added Et_3N (3.65 mL, 26.2 mmol) at −78 °C, then this mixture was stirred at that temperature for 40 min. The reaction mixture was quenched with a saturated aqueous solution of NH_4Cl. The aqueous layer was extracted two times with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, filtrated, and concentrated under reduced pressure. Purification by silica-gel column chromatography (hexane/EtOAc=90:10) gave 820 mg of 1,3-diketone **8b** (91% yield, a mixture of diastereomers by ^1H NMR analysis): R_f 0.81 (hexane/EtOAc=75:25); ^1H NMR (400 MHz, $CDCl_3$) δ 13.6 (br s, 0.5H), 5.68–5.56 (m, 1H), 5.49–5.35

(m, 1H), 4.62–4.48 (m, 1H), 2.65–2.20 (m, 7H), 1.90–1.85 (m, 1.5H), 1.68–1.64 (m, 3H), 0.842 (s, 6.3H), 0.836 (s, 2.7H), 0.0311 (s, 0.6H), 0.0220 (s, 0.6H), 0.0085 (s, 0.6H), 0.001 (s, 2.7H), −0.014 (s, 1.5H); IR (neat, cm^{-1}) 1712, 1662, 1617; HR-ESIMS calcd for $C_{17}H_{30}O_3NaSi$: 333.1856, found: 333.1869.

4.1.2. Dihydropyrone 4. To a solution of 1,3-diketone **8b** (7.80 g, 25.1 mmol) in CH_2Cl_2 (200 mL) was added CF_3COOH (7.8 mL, 101.0 mmol) via a dropping funnel at 0 °C, then the resulting mixture was stirred at that temperature for 15 min. This mixture was warmed to rt and stirred at that temperature for 3 h. The reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$. The aqueous layer was extracted two times with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. Purification by silica-gel column chromatography (hexane/EtOAc=95:5→83:17) gave 3.779 g (85% yield) of dihydropyrone **4**: R_f 0.38 (hexane/EtOAc=75:25); ^1H NMR (300 MHz, $CDCl_3$) δ 5.88 (ddq, J=15.4, 0.72, 6.4 Hz, 1H), 5.66 (ddq, J=15.4, 7.2, 1.5 Hz, 1H), 4.88 (ddd, J=12.5, 7.2, 4.3 Hz, 1H), 2.60–2.50 (m, 5H), 2.41 (dd, J=16.9, 4.1 Hz, 1H), 1.92 (quint, J=7.5 Hz, 2H), 1.77 (ddd, J=6.4, 1.5, 0.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.7, 178.4, 131.4, 127.8, 114.0, 81.6, 40.9, 32.7, 25.4, 19.1, 17.7; IR (neat, cm^{-1}) 1779, 1666, 1613, 1426, 1154, 965; HR-EIMS calcd for $C_{11}H_{14}O_2$: 178.0994, found: 178.0987.

4.1.3. Dihydropyran 5b. To a solution of dihydropyrone **4** (153 mg, 0.859 mmol) in dry Et_2O was added $LiAlH_4$ (41 mg, 1.08 mmol) at 0 °C, then the resulting mixture was stirred for 25 min at that temperature. The reaction mixture was quenched with $Na_2SO_4\cdot 10H_2O$, and allowed to warm to rt. To this mixture was added hexane and dry Na_2SO_4, then stirred for 10 min. The resulting mixture was filtrated, and concentrated under reduced pressure to afford crude alcohol **9**. To a solution of crude alcohol **9** and imidazole (94 mg, 1.37 mmol) in CH_2Cl_2 (6 mL) was added TBSCl (194 mg, 1.29 mmol) at 0 °C, then the resulting mixture was allowed to warm to rt and stirred for 1.5 h at that temperature. The reaction was poured into a cold saturated aqueous solution of $NaHCO_3$. The aqueous layer was extracted two times with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4, filtrated, and concentrated under reduced pressure. Purification by silica-gel column chromatography (hexane/EtOAc=98:2→96:4) gave 184 mg (73% yield for the two steps, >95% dr by ^1H NMR analysis) of dihydropyran **5b** as a colorless clear oil. The relative stereochemistry was established by the NOESY correlation between α-oxymethyne protons: R_f 0.56 (hexane/EtOAc=90:10); ^1H NMR (300 MHz, $CDCl_3$) δ 5.70 (dq, J=15.3, 6.2 Hz, 1H), 5.55 (dd, J=15.3, 7.3 Hz, 1H), 4.38–4.27 (m, 2H), 2.42–2.08 (m, 5H), 1.94 (ddd, J=13.4, 6.5, 2.2 Hz, 1H), 1.81–1.72 (m, 2H), 1.63 (d, J=6.2 Hz, 3H), 0.815 (s, 9H), 0.00 (s, 3H), −0.0104 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.0, 130.4, 129.0, 110.6, 77.4, 64.7, 38.8, 31.4, 28.9, 25.8, 19.9, 18.2, 17.7, −4.6, −4.9; IR (neat, cm^{-1}) 1686; HR-ESIMS calcd for $C_{17}H_{30}O_2NaSi$: 317.1907, found: 317.1897.

4.1.4. Spiro[4.5]decane 6b. Degassed solutions of **5b** (47 mg, >95% dr) in dry toluene (1.7 mL) were heated at 250 °C for 14 h in sealed tubes. The resulting mixtures were cooled to rt, and concentrated. Purification by silica-gel column chromatography (hexane/EtOAc=100/1) gave 40 mg (87% yield, >95% dr by ^1H NMR analysis) of **6b** as a colorless clear oil. Multigram-scale synthesis (4.839 g, 250 °C, 12 h) was also performed to afford 3.623 g of **6b** (75% yield, >95% dr by ^1H NMR analysis). The stereochemical assignment to **6b** was verified by the NOE experiments on the tricycle.[7] R_f 0.51 (hexane/EtOAc=90:10); ^1H NMR (400 MHz, $CDCl_3$) δ 5.52–5.40 (m, 2H), 4.13 (dd, J=9.5, 6.1 Hz, 1H), 2.27–2.20 (m, 4H), 2.06–1.87 (m, 3H), 1.81–1.62 (m, 2H), 0.892 (d, J=7.4 Hz, 3H), 0.777 (s, 9H), 0.00 (s, 3H), −0.0281 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 220.5, 131.1, 123.1, 66.0, 56.4, 40.0, 37.2, 33.2, 29.1, 25.8, 18.7, 17.9, 17.6, −4.1, −5.3; IR (neat, cm^{-1}) 1734; HR-ESIMS calcd for $C_{17}H_{30}O_2NaSi$: 317.1907, found: 317.1903.

4.1.5. Keto ester 11. To a solution of spiro[4.5]decane **6b** (3.456 g, 11.7 mmol) in EtOH (234 mL) was added 10% Pd/charcoal (2.49 g) under argon atmosphere. The argon atmosphere was replaced by H_2 from a double balloon, the reaction mixture was stirred at rt for 5 h. After the H_2 atmosphere was replaced by argon, the resulting mixture was filtrated through a pad of Celite, and the solvent was concentrated to give 3.529 g of crude **10** (quantitative yield, >95% dr by ^1H NMR analysis) as a colorless clear oil: R_f 0.51 (hexane/EtOAc=90:10); ^1H NMR (400 MHz, $CDCl_3$) δ 3.97 (dd, J=9.8, 4.2 Hz, 1H), 2.34–2.20 (m, 2H), 2.12–2.02 (m, 1H), 1.93–1.73 (m, 5H), 1.61–1.25 (m, 5H), 0.910 (d, J=7.3 Hz, 3H), 0.831 (s, 9H), 0.0310 (s, 3H), −0.0044 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 221.3, 68.9, 57.7, 39.7, 33.5, 31.7, 30.0, 28.8, 25.8, 19.1, 19.0, 18.0, 15.4, −4.1, −5.1; IR (neat, cm^{-1}) 1733; HR-ESIMS calcd for $C_{17}H_{32}O_2NaSi$: 319.2069, found: 319.2083. To a suspension of KH (4.83 g, 60 wt % in mineral oil, 72.3 mmol) in THF (68 mL) was added a solution of crude ketone **10** in THF (50 mL) and dimethyl carbonate (9.92 mL, 118 mmol) at rt. The resulting mixture was heated at 95 °C, and stirred at that temperature for 9 h 15 min. The resulting mixture was cooled to rt, and the reaction mixture was quenched with a saturated aqueous solution of NH_4Cl. The aqueous layer was extracted two times with Et_2O. The combined organic layer was dried over Na_2SO_4, filtrated, and concentrated under reduced pressure. Purification by silica-gel column chromatography (hexane/EtOAc=20:1) gave 3.591 g of keto ester **11** (86% yield for the two steps, a mixture of diastereomers by ^1H NMR analysis) as a pink oil: R_f 0.47, 0.41 (hexane/EtOAc=10:1); ^1H NMR (400 MHz, $CDCl_3$) δ 4.03–4.00 (m, 1H), 3.733 (s, 1.35H), 3.730 (s, 1.65H), 3.27 (t, J=2.5 Hz, 0.45H), 3.07 (t, J=2.4 Hz, 0.55H), 2.34–1.24 (m, 11H), 0.907 (d, J=7.3 Hz, 1.65H), 0.886 (d, J=7.3 Hz, 1.35H), 0.859 (s, 4.05H), 0.823 (s, 4.95H), 0.0464 (s, 1.65H), 0.0409 (s, 1.35H), 0.0342 (s, 1.65H), 0.0134 (s, 1.35H); IR (neat, cm^{-1}) 1753, 1727; HR-ESIMS calcd for $C_{19}H_{34}O_4NaSi$: 377.2124, found: 377.2141.

4.1.6. Hydroxy ketone 12. To a solution of keto ester **11** (50 mg, 0.141 mmol) in dry MeOH (1.4 mL) was added $NaBH_4$ (53 mg, 1.41 mmol) at 0 °C, then the resulting mixture was stirred at that temperature for 35 min. The reaction mixture was quenched with an aqueous solution of NH_4Cl. The aqueous layer was extracted two times with Et_2O. The combined organic layer was dried over Na_2SO_4, filtrated,

and concentrated under reduced pressure. Purification by silica-gel column chromatography (hexane/EtOAc=10:1) gave 35 mg of hydroxy ketone **12** (67% yield, a mixture of diastereomers by ^{1}H NMR analysis) and 9 mg of diol **13** (20% yield, a mixture of diastereomers by ^{1}H NMR analysis). Hydroxy ketone **12**: R_f 0.25, 0.20; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.11–4.07 (m, 1H), 3.91 (t, J=9.5 Hz, 1H), 3.712 (s, 0.9H), 3.706 (s, 2.1H), 2.88–2.78 (m, 1H), 2.41 (br d, J=9.5 Hz, 1H), 2.14–2.02 (m, 1H), 1.95–1.26 (m, 10H), 1.13 (d, J=7.3 Hz, 2.1H), 1.05 (d, J=7.1 Hz, 0.9H), 0.902 (s, 6.3H), 0.886 (s, 2.7H), 0.113 (s, 2.1H), 0.110 (s, 2.1H), 0.0537 (s, 0.9H), 0.0495 (s, 0.9H); HR-ESIMS calcd for $C_{19}H_{36}O_4NaSi$: 379.2275, found: 379.2272. Diol **13**: R_f 0.13, 0.063 (hexane/EtOAc=10:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.08 (dd, J=11.5, 4.2 Hz, 1H), 3.87–3.42 (m, 4H), 2.59 (br d, J=11.2 Hz, 1H), 2.28–1.26 (m, 12H), 1.15 (d, J=7.3 Hz, 1.8H), 1.05 (d, J=7.6 Hz, 1.2H), 0.904 (s, 3.6H), 0.894 (s, 5.4H), 0.120 (s, 1.2H), 0.117 (s, 1.2H), 0.055 (s, 1.8H), 0.051 (s, 1.8H); HR-ESIMS calcd for $C_{18}H_{36}O_3NaSi$: 351.2325, found: 351.2317.

4.1.7. α,β-Unsaturated ester 14. To a solution of **12** (1.39 g, 3.90 mmol) in pyridine (40 mL) was added MsCl (2.7 mL, 35.1 mmol) at 0 °C, then the resulting mixture was stirred at that temperature for 15 h 40 min. The reaction mixture was quenched with H_2O. The aqueous layer was extracted two times with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4, filtrated, and concentrated under reduced pressure to give the crude mesylate. To a solution of the crude mesylate in CH_2Cl_2 (66.5 mL) was added DBU (5.0 mL, 33.00 mmol) at rt. After stirring for 2 h, the resulting mixture was concentrated under reduced pressure. Purification by silica-gel column chromatography (hexane/EtOAc=20:1) gave 1.227 g of α,β-unsaturated ester **14** (93% yield for the two steps, >95% dr by ^{1}H NMR analysis) as a colorless clear oil: R_f 0.63 (hexane/EtOAc=90:10); ^{1}H NMR (400 MHz, C_6D_6) δ 6.77 (t, J=2.0 Hz, 1H), 3.52–3.51 (m, 1H), 3.47 (s, 3H), 2.69 (dddd, J=16.8, 9.5, 5.1, 2.0 Hz, 1H), 2.61 (dddd, J=16.8, 8.6, 6.4, 2.0 Hz, 1H), 2.00 (ddd, J=13.5, 9.5, 6.1 Hz, 1H), 1.94–1.85 (m, 1H), 1.70 (ddd, J=13.5, 9.0, 5.0 Hz, 2H), 1.48–1.30 (m, 4H), 1.06–0.898 (m, 1H), 0.966 (s, 9H), 0.752 (d, J=6.8 Hz, 3H), 0.0079 (s, 3H), 0.00 (s, 3H); ^{13}C NMR (100 MHz, C_6D_6) δ 165.2, 145.8, 137.9, 73.8, 60.2, 51.0, 35.5, 32.6, 31.4, 31.4, 31.4, 26.1, 20.3, 18.3, 16.7, −4.1, −5.0; IR (neat, cm^{-1}) 1712, 1635; HR-ESIMS calcd for $C_{19}H_{34}O_3NaSi$: 361.2169, found: 361.2172.

4.1.8. Ketone 16. To a solution of silyl ether **14** (43 mg, 0.128 mmol) in CH_3CN (3 mL) was added aqueous solution of HF (46–48%, 30 drops via a pipette) at rt, then the resulting mixture was stirred at that temperature for 1 h. The reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$. The aqueous layer was extracted two times with EtOAc. The combined organic layer was dried over $MgSO_4$, filtrated, and concentrated under reduced pressure to afford crude alcohol **15**: R_f 0.27 (hexane/EtOAc=75:25); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.68 (t, J=1.9 Hz, 1H), 3.74 (s, 3H), 3.73–3.70 (m, 1H), 2.61 (dddd, J=16.9, 9.5, 5.4, 1.9 Hz, 1H), 2.56 (dddd, J=16.9, 8.8, 6.8, 1.9 Hz, 1H), 2.10 (ddd, J=13.5, 9.3, 6.9 Hz, 1H), 1.91–1.51 (m, 8H), 1.30–1.21 (m, 1H), 0.874 (d, J=7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 146.0, 137.5, 72.3, 59.4, 51.5, 35.9, 31.1 (br s), 31.0, 30.6, 19.8, 16.2; IR (neat, cm^{-1}) 3454, 1717, 1634. To a solution of crude alcohol **15** in CH_2Cl_2 (6 mL) was added Dess–Martin periodinane (275 mg, 0.648 mmol) at rt, then the resulting mixture was stirred at that temperature for 30 min. The reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$. The aqueous layer was extracted two times with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. Purification by silica-gel column chromatography (hexane/EtOAc=85:15) gave 25 mg of ketone **16** (88% yield for the two steps, >95% dr by ^{1}H NMR analysis) as a colorless clear oil: R_f 0.41 (hexane/EtOAc=75:25); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.77 (t, J=2.0 Hz, 1H), 3.74 (s, 3H), 2.66–2.40 (m, 5H), 2.08–2.03 (m, 1H), 1.88–1.62 (m, 5H), 0.940 (d, J=6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.2, 165.0, 141.2, 138.7, 69.8, 51.6, 42.8, 39.5, 30.9, 30.7, 28.6, 25.2, 16.8; IR (neat, cm^{-1}) 1713, 1630, 1299, 1260, 1213, 1154; HR-ESIMS calcd for $C_{13}H_{18}O_3Na$: 245.1148, found: 245.1144.

4.1.9. *exo*-Methylene 17. To a suspension of Zn (1.75 g, 26.8 mmol) in THF (20 mL) was added freshly distilled CH_2I_2 (1.19 mL, 14.74 mmol) at rt, then the resulting mixture was stirred at that temperature for 30 min. After the resulting mixture was cooled to 0 °C, to this mixture was added a solution of $TiCl_4$ in CH_2Cl_2 (1.0 M, 2.76 mL) at 0 °C, and stirred for 30 min. To this suspension was added a solution of **16** (318 mg, 1.42 mmol) in CH_2Cl_2 (17 mL) at rt, then the resulting mixture was stirred at that temperature for 30 min. The reaction mixture was quenched with a saturated aqueous solution of NH_4Cl, and Et_2O was added. The organic layer was washed with brine, dried over Na_2SO_4, filtrated, and concentrated under reduced pressure. Purification by silica-gel column chromatography (hexane/EtOAc=50:1) gave 225 mg (72% yield) of **17** as a colorless clear oil: R_f 0.55 (hexane/EtOAc=90:10); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.85 (t, J=1.8 Hz, 1H), 4.72 (br s, 1H), 4.57 (br s, 1H), 3.75 (s, 3H), 2.56 (td, J=7.6, 1.6 Hz, 2H), 2.27–2.17 (m, 2H), 2.05 (t, J=7.0 Hz, 2H), 1.82–1.43 (m, 5H), 0.916 (d, J=7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8, 151.0, 148.0, 135.1, 107.8, 59.8, 51.3, 39.3, 34.7, 33.6, 30.3, 30.2, 22.8, 16.0; IR (neat, cm^{-1}) 3077, 2933, 1719, 1636; HR-ESIMS calcd for $C_{14}H_{20}O_2Na$: 243.1360, found: 243.1369.

4.1.10. (±)-α-Vetispirene (1). To a solution of *exo*-isomer **17** (10 mg) in benzene (2.5 mL) was added $TsOH\cdot H_2O$ (1.6 mg, 0.0084 mmol) at rt, then the resulting mixture was heated at 95 °C for 14 h. The resulting mixture was cooled to rt, and the reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$. The aqueous layer was extracted two times with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, filtrated, and concentrated under reduced pressure to afford 12 mg of crude *endo*-isomer. To a solution of crude *endo*-isomer in THF (2 mL) was added a solution of MeLi (0.98 M, 1 mL, 0.98 mmol) at 0 °C, then the resulting mixture was warmed to rt over 30 min. The reaction mixture was quenched with H_2O. The aqueous layer was extracted two times with Et_2O. The combined organic layer was dried over $MgSO_4$, filtrated, and concentrated under reduced pressure to afford crude *tert*-alcohol. To a solution of this *tert*-alcohol in

benzene (2 mL) was added camphorsulfonic acid (2.3 mg, 0.0099 mmol) at rt. The resulting mixture was warmed to 60 °C and stirred at that temperature for 1 h 20 min. The resulting mixture was cooled to rt, and the reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$. The aqueous layer was extracted two times with hexane. The combined organic layer was washed with brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. Purification by silica-gel column chromatography (hexane) gave 8.6 mg of (±)-α-vetispirene (**1**) (87% yield for the three steps, >95% dr by ^1H NMR analysis) as a colorless clear oil: R_f 0.71 (hexane); ^1H NMR (400 MHz, $CDCl_3$) δ 5.51 (br s, 1H), 5.40–5.38 (m, 1H), 4.91 (br s, 1H), 4.88 (br s, 1H), 2.60–2.46 (m, 2H), 2.09–1.93 (m, 3H), 1.94 (s, 3H), 1.83 (ddd, J=13.7, 9.0, 5.8 Hz, 1H), 1.70–1.61 (m, 2H), 1.56 (d, J=1.5 Hz, 3H), 1.46–1.38 (m, 1H), 0.859 (d, J=6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.8, 140.3, 138.7, 132.7, 121.7, 112.3, 57.3, 37.8, 34.4, 32.3, 28.2, 23.8, 20.7, 19.9, 16.5; IR (neat, cm^{-1}) 3087, 2960, 1773, 1630, 1597, 1375, 1200, 1076, 881, 842, 796; HR-EIMS calcd for $C_{15}H_{22}$: 202.1721, found: 202.1717.

4.1.11. Ester 18. To a solution of α,β-unsaturated ester **16** (58 mg, 0.261 mmol) in EtOH (5.23 mL) was added 10% Pd/charcoal (56 mg) under argon atmosphere. The argon atmosphere was replaced by H_2 from a double balloon, the reaction mixture was stirred at rt for 2 h 20 min. After replaced by argon, the resulting mixture was filtrated through a pad of Celite, and the solvent was concentrated. Purification by silica-gel column chromatography (hexane/EtOAc=5:1) gave 57 mg (97% yield, 87% dr by ^1H NMR analysis) of **18**: R_f 0.32 (hexane/EtOAc=5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 3.67 (s, 3H), 2.74 (tt, J=8.7, 8.6 Hz, 1H), 2.42 (t, J=6.8 Hz, 2H), 2.34 (ddd, J=12.9, 7.6, 3.1 Hz, 1H), 2.12 (dd, J=13.7, 8.2 Hz, 1H), 1.98–1.67 (m, 7H), 1.60–1.46 (m, 2H), 0.940 (d, J=6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 213.5, 175.5, 60.6, 51.7, 44.0, 41.2, 38.3, 33.6, 33.1, 30.4, 29.1, 24.1, 16.0; IR (neat, cm^{-1}) 1735, 1704; HR-EIMS calcd for $C_{13}H_{20}O_3Na$: 247.1304, found: 247.1310.

4.1.12. (±)-Hinesol (2). To a solution of *exo*-isomer **19** (200 mg, 0.90 mmol) in benzene (45 mL) was added $TsOH\cdot H_2O$ (29.1 mg, 0.153 mmol) at rt, then the resulting mixture was heated at 95 °C for 12 h. The resulting mixture was cooled to rt, and the reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$. The aqueous layer was extracted two times with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4, filtrated, and concentrated under reduced pressure to afford the crude *endo*-isomer. To a solution of MeMgI (1 M, 15.7 mL) in dry Et_2O (120 mL) was added a solution of the crude *endo*-isomer in dry Et_2O (42 mL) at 0 °C, then the resulting mixture was stirred at that temperature for 30 min. The resulting mixture was allowed to warm to rt, then stirred at that temperature for 3 h. The reaction mixture was quenched with H_2O. The aqueous layer was extracted two times with Et_2O. The combined organic layer was washed with brine, dried over Na_2SO_4, filtrated, and concentrated under reduced pressure. Purification by silica-gel column chromatography (hexane/EtOAc=15:1) gave 181 mg of (±)-hinesol (**2**) (89% yield for the two steps, >95% dr by ^1H NMR analysis) as a colorless clear oil: R_f 0.34 (hexane/EtOAc=5:1); ^1H NMR (400 MHz, $CDCl_3$) δ 5.31 (br s, 1H), 2.00–1.90 (m, 3H), 1.79–1.25 (m, 13H), 1.20 (s, 6H), 0.922 (d, J=6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.0, 121.7, 72.0, 51.4, 48.7, 36.7, 35.7, 33.3, 28.4, 28.0, 27.9, 27.7, 24.2, 19.9, 16.2; IR (neat, cm^{-1}) 3400, 1659; HR-ESIMS calcd for $C_{15}H_{26}ONa$: 245.1881, found: 245.1876.

4.1.13. (±)-Hinesolone (20). To a suspension of CrO_3 (360 mg, 3.60 mmol; need to dry by heating under reduced pressure just before use) in CH_2Cl_2 (5 mL) was added 3,5-dimethylpyrrazole (346 mg, 3.60 mmol) at −20 °C, then the resulting mixture was stirred at that temperature for 30 min. After allowing to warm to 0 °C, to this mixture was added a solution of **2** (40 mg, 0.180 mmol) in CH_2Cl_2 (4 mL), then the resulting mixture was stirred for 2.5 h. The resulting mixture was filtrated with a pad of Florisil. The filtrate was washed with 1 N HCl and brine. The combined organic layer was dried over Na_2SO_4, filtrated, and concentrated under reduced pressure. Purification by silica-gel column chromatography (hexane/EtOAc=2:1) gave 26 mg (60%) of **20** as a colorless needle: mp: 112.5–115.0 °C; R_f 0.13 (hexane/EtOAc=2:1); ^1H NMR (400 MHz, $CDCl_3$) δ 5.76 (s, 1H), 2.44 (dd, J=16.6, 4.2 Hz, 1H), 2.22 (dd, J=16.6, 9.8 Hz, 1H), 2.14–2.02 (m, 2H), 1.97 (s, 3H), 1.90–1.81 (m, 4H), 1.68–1.61 (m, 1H), 1.50 (dd, J=13.3, 12.3 Hz, 1H), 1.25 (s, 3H), 1.24 (s, 3H), 1.02 (d, J=6.8 Hz, 3H), 0.867 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.3, 168.2, 125.9, 71.4, 50.8, 50.3, 42.8, 37.1, 34.8, 31.7, 28.7, 28.5, 27.6, 20.8, 16.4; IR (KBr, cm^{-1}) 3410, 1653; HR-EIMS calcd for $C_{15}H_{24}O_2Na$: 259.1673, found: 259.1682.

4.1.14. (±)-β-Vetivone (3). A mixture of **20** (28 mg, 0.118 mmol) and NaOAc (19 mg, 0.236 mmol) in Ac_2O (1.18 mL) was heated to 140 °C for 2 h in a sealed tube, cooled to rt. The reaction mixture was quenched with a saturated aqueous solution of $NaHCO_3$. The aqueous layer was extracted two times with CH_2Cl_2. The combined organic layer was washed with brine, dried over Na_2SO_4, filtrated, and concentrated under reduced pressure to afford a crude product. Purification by silica-gel column chromatography (hexane/EtOAc=83:17) gave 31 mg (93% yield) of the acetate as a colorless clear oil. To a solution of this acetate in Et_2O (2.19 mL) was added $BF_3\cdot OEt_2$ (0.104 mL, 0.822 mmol) at rt, then the mixture was stirred at that temperature for 6 h. The reaction mixture was quenched with a 5% aqueous solution of NaOH. The aqueous layer was extracted two times with Et_2O. The combined organic layer was washed with brine, dried over $MgSO_4$, filtrated, and concentrated under reduced pressure. Purification by silica-gel column chromatography (hexane/EtOAc=83:17) gave 15 mg of (±)-β-Vetivone (**3**) and the inseparable *exo*-isomer [64% yield (84%: based on the recovered S.M.), **3**/*exo*-isomer=86:14 by ^1H NMR analysis] and recovered acetate (24%): R_f 0.66 (hexane/EtOAc=2:1); ^1H NMR (400 MHz, $CDCl_3$) δ 5.79 (br s, 1H), 5.76 (br s, 0.17H), 4.74 (br s, 0.34H), 2.94–1.94 (m, 9.5H), 2.66 (dd, J=16.9, 4.8 Hz, 1H), 2.54 (dd, J=12.0, 5.4 Hz, 0.17H), 1.92 (d, J=1.2 Hz, 0.5H), 1.90 (d, J=1.2 Hz, 3H), 1.76 (s, 0.5H), 1.67 (s, 3H), 1.63 (s, 3H), 1.04 (d, J=6.6 Hz, 0.5H), 0.971 (d, J=7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.1, 167.15, 147.4, 133.8, 126.0, 122.6, 109.0, 51.1, 50.2, 47.0, 42.9, 42.9, 39.0, 38.1, 37.7, 35.4, 35.0, 32.1, 29.7, 29.4,

21.7, 21.3, 21.1, 21.0, 20.7, 16.6, 16.6; IR (KBr, cm^{-1}) 1668, 1613; HR-ESIMS calcd for $C_{15}H_{22}O_3Na$: 245.1562, found: 245.1564.

Acknowledgements

We thank Professor Istvan E. Markó (Université Catholique de Louvain) for providing the spectra of α-vetispirene. We also thank Shin-Etsu Chemical Co., Ltd, for the gift of silicone oil.

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Tetrahedron 62 (2006) 6272–6288
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Real light emitter in the bioluminescence of the calcium-activated photoproteins aequorin and obelin: light emission from the singlet-excited state of coelenteramide phenolate anion in a contact ion pair

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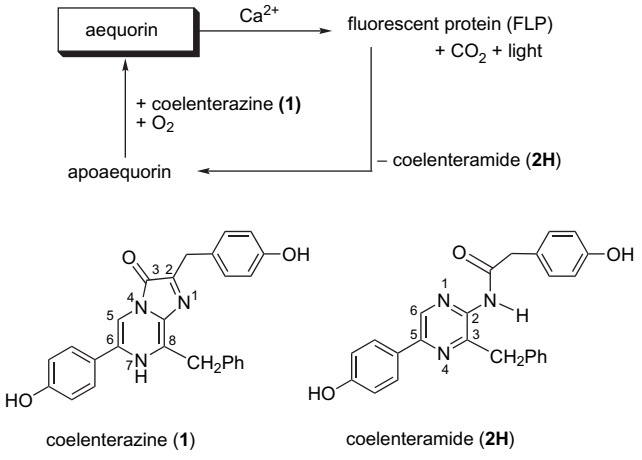
Received 22 March 2006; revised 8 April 2006; accepted 18 April 2006
Available online 19 May 2006

Abstract—Fluorescence of the phenolate anion (**3**(**O**)$^-$) and the amide anion (**5**(**N**)$^-$) of coelenteramide analogues in ion pairs with various counter cations was systematically investigated to elucidate the ionic structure of the light emitter in the bioluminescence of the calcium-activated photoproteins aequorin and obelin. The fluorescent properties of **3**(**O**)$^-$ in an ion pair with a conjugate acid of an organic base (BASE–H$^+$) were varied depending on the structural variation of the ion pair and the solvent polarity. In particular, the fluorescence of **3**(**O**)$^-$ in the ion pair with the conjugate acid of *n*-butylamine (NBA–H$^+$) indicates that the singlet-excited state of **3**(**O**)$^-$ (^{1}**3**(**O**)$^{-*}$) and NBA–H$^+$ make a contact ion pair in which the fluorescence emission maxima of **3**(**O**)$^-$ is sensitive to the solvent polarity and the fluorescence quantum yields of **3**(**O**)$^-$ increase in a less polar solvent. The results also confirm that ^{1}**3**(**O**)$^{-*}$ is a twisted intramolecular charge transfer state. By contrast, the fluorescence of **5**(**N**)$^-$ in an ion pair depends little on the BASE–H$^+$ or the solvent polarity. Based on these results, we conclude that the light emitter in aequorin and obelin bioluminescences is the singlet-excited state of coelenteramide phenolate anion **2**(**O**)$^-$ (^{1}**2**(**O**)$^{-*}$) in a contact ion pair with an imidazolium side chain of a histidine residue, which is located at the less polar active sites of the photoproteins. We also propose a mechanism for the bioluminescence reaction, including the chemiexcitation process to give ^{1}**2**(**O**)$^{-*}$.

1. Introduction

Calcium-activated photoproteins are light-generating molecules for several marine bioluminescent organisms.[1] The biochemical aspects of the photoproteins aequorin[2,3] and obelin,[4] isolated from the jellyfish *Aequorea* and *Obelia*, respectively, have been particularly well investigated. Chemical study on calcium-activated photoproteins was pioneered by Shimomura et al., who discovered aequorin.[2a] Aequorin is made from apoaequorin (apoprotein), coelenterazine (**1**) (substrate), and molecular oxygen (O_2). Chelation of Ca^{2+} with the EF-hands of aequorin initiates the bioluminescence reaction, which gives a fluorescent protein (FLP),[5] CO_2, and bluish light (Scheme 1). The crystal structure of aequorin helped to clarify its supramolecular structure, containing oxygenated coelenterazine in the active site.[6] Recent research on the crystal structures of the various forms of obelin has also yielded important information on the character of obelin's active site.[7]



Scheme 1.

The bioluminescence reaction of the calcium-activated photoproteins is based on the reaction of **1** with O_2, which

Keywords: Bioluminescence; Photoprotein; Aequorin; Obelin; Coelenteramide; Fluorescence; Solvent effect; Intramolecular charge transfer; Hydrogen bond; Contact ion pair.
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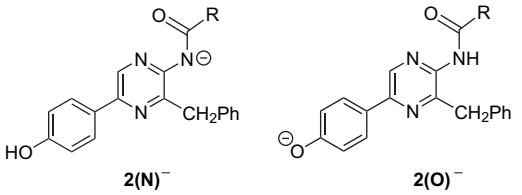
doi:10.1016/j.tet.2006.04.044

yields coelenteramide (**2H**), CO_2, and light.[8–10] Light emission occurs from the singlet-excited state of the anion species of **2H** (^{1}**2**$^{-*}$) in apoprotein, to give FLP, a complex of **2H** and apoprotein.[11] The structure elucidation of ^{1}**2**$^{-*}$ in apoprotein is one of the controversies in the reaction mechanism. Two structures are possible for ^{1}**2**$^{-*}$: the singlet-excited state of an amide anion **2**(**N**)$^-$ (^{1}**2**(**N**)$^{-*}$) and the singlet-excited state of a phenolate anion **2**(**O**)$^-$ (^{1}**2**(**O**)$^{-*}$) (Scheme 2). Both anions have an anionic center conjugating with the fluorescent core, the pyrazine ring.

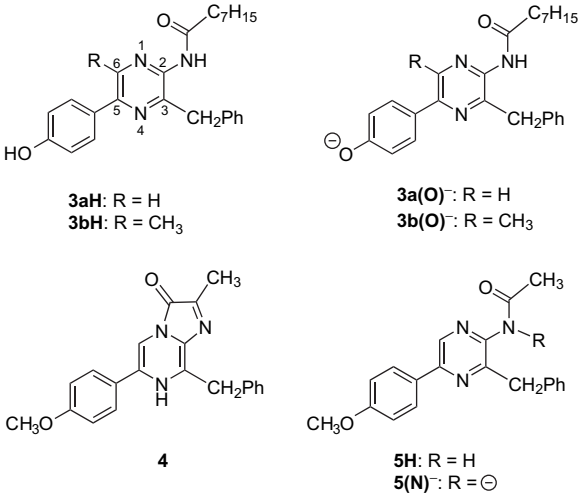


Scheme 2. R=(4-hydroxyphenyl)methyl or (4-oxidophenyl)methyl.

Excited amide anion ^{1}**2**(**N**)$^{-*}$ is the light emitter of a chemiluminescence reaction of **1** in an aprotic solvent such as *N*,*N*-dimethylformamide (DMF) and dimethylsulfoxide (DMSO).[12,13] Because the emission wavelengths of the chemiluminescence of **1** and the bioluminescence of aequorin are similar to each other, ^{1}**2**(**N**)$^{-*}$ has been believed as the light emitter in aequorin bioluminescence.[12–14] However, we noticed that this explanation cannot account for the fact that the fluorescence emission of FLP obtained from aequorin occurs from common ^{1}**2**$^{-*}$ to the light emission in aequorin bioluminescence.[11a,15] Thus, we are the first to propose that ^{1}**2**(**O**)$^{-*}$ is the light emitter in aequorin bioluminescence.[15–17] The fact suggests the generation of ^{1}**2**(**O**)$^{-*}$ in aequorin bioluminescence, because it is conceivable that **2H** eliminates a proton from the phenolic hydroxy group with a moderate acidity[18] rather than from the amide moiety with a weak acidity.[19–21] In addition, we found that **2**(**O**)$^-$ in benzene showed the fluorescence spectrum similar to the emission spectrum of aequorin bioluminescence,[17] supporting that the light emitter is ^{1}**2**(**O**)$^{-*}$ in apoaequorin, whose active site has a polarity similar to benzene.

The mechanism involving a light emission from ^{1}**2**(**O**)$^{-*}$ in aequorin is now applied to obelin bioluminescence.[7b,22] There is confusion in some reports, where ^{1}**2**(**O**)$^{-*}$ in polar and in less polar solvents has been categorized as a 'quinoid anion' and an 'ion pair,' respectively.[7b,22,23] However, the quinoid form, shown in Scheme 3c, is only one of the several resonance structures of ^{1}**2**(**O**)$^{-*}$. Similarly, we should not distinguish ^{1}**2**(**O**)$^{-*}$ in a less polar solvent as an ion pair, because ^{1}**2**(**O**)$^{-*}$ is only a component of the ion pair. To precisely understand the fundamental characteristics of ^{1}**2**(**O**)$^{-*}$, we have to further establish the spectroscopic properties of **2**(**O**)$^-$ in a chemically defined molecular environment, such as an ion pair with a counter cation.

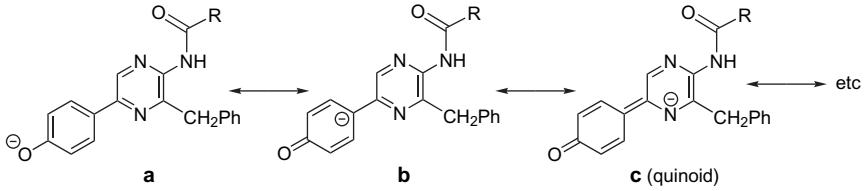
In the previous study we reported the florescence of **3a**(**O**)$^-$, an analogue of **2**(**O**)$^-$ having an octanoyl group for improving solubility in various solvents, though we did not clarify the influence of an ion pair of ^{1}**3a**(**O**)$^{-*}$ with a counter cation on its fluorescence.[17b] To confirm the mechanism for light emission from ^{1}**2**(**O**)$^{-*}$ in the photoprotein, here we reinvestigate the florescence of **3a**(**O**)$^-$ in ion pairs with conjugate acids of several organic bases as counter cations. The fluorescence of methyl analogue **3b**(**O**)$^-$ in an ion pair was also investigated to clarify steric effects of the methyl group at C6 on the π-conjugation between the pyrazine ring and the 4-oxidophenyl (4-O$^-$C$_6$H$_4$) group.[24] Furthermore, we investigated fluorescence emission from ^{1}**5**(**N**)$^{-*}$ in an ion pair generated by chemiluminescence reactions of coelenterazine analogue **4** in various solvents. Herein we report the spectroscopic characteristics of **3H** and those of **3**(**O**)$^-$ and **5**(**N**)$^-$ in an ion pair with a counter cation. We show that the fluorescence of **3**(**O**)$^-$ depends on a structural variation of an ion pair, while that of **5**(**N**)$^-$ does not. We also reconfirm that ^{1}**2**(**O**)$^{-*}$ is the light emitter in the bioluminescence of aequorin and obelin, and we propose a bioluminescence reaction mechanism involving a chemiexcitation process to give ^{1}**2**(**O**)$^{-*}$.



2. Results and discussion

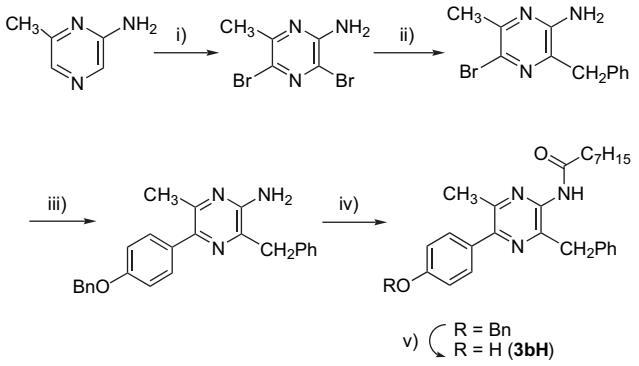
2.1. UV–vis absorption spectra of coelenteramide analogue 3H in the presence of organic bases

Coelenteramide analogue **3aH** was prepared by the procedure previously reported.[17b] Analogue **3bH** was prepared



Scheme 3. R=(4-hydroxyphenyl)methyl or (4-oxidophenyl)methyl.

from 6-methylpyrazinamine by the procedure shown in Scheme 4. Pyrazinamine precursor 3-benzyl-5-(4-benzyloxyphenyl)-6-methylpyrazinamine was prepared by bromination of 6-methylpyrazinamine with tetrabutylammonium tribromide followed by selective Stille and Suzuki couplings. Acylation of the pyrazinamine precursor and the following hydrogenolysis gave **3bH**.



Scheme 4. Reagent and conditions: (i) $(C_4H_9)_4NBr_3$, pyridine, $CHCl_3$, reflux, 46%; (ii) $Bn(C_4H_9)_3Sn$, $PdCl_2(PPh_3)_2$, DMF, 130 °C, 36%; (iii) $BnOC_6H_4B(OH)_2$, $Pd(PPh_3)_4$, 1,4-dioxane, 2 mol L^{-1} Na_2CO_3 aq, 110 °C, 94%; (iv) $C_7H_{15}COCl$, pyridine, CH_2Cl_2, 0 °C, 52%; (v) H_2, Pd–C, $C_2H_5OH/CH_3CO_2C_2H_5$, 67%.

UV–vis absorption spectra of **3H** were measured in *p*-xylene, toluene, benzene, benzene/chloroform (20:1), chlorobenzene, 1-chloropropane, benzene/chloroform (2:1), 1,2-dichlorobenzene, chloroform, dichloromethane, 1,2-dichloroethane, benzonitrile, *N*,*N*-dimethylacetamide (DMAc), DMF, propionitrile, DMSO, and acetonitrile in the absence and presence of an organic base. In this study, *n*-butylamine (NBA), 1,1,3,3-tetramethylguanidine (TMG), and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were used as an organic base (BASE). Thus, their conjugate acids ($BASE–H^+$) will become a counter cation for $\mathbf{3}(\mathbf{O})^-$ in an ion pair. Selected spectra are shown in Figure 1 and the spectral data are summarized in Tables 1 and 2, where the data are listed in order of the solvent polarity scale $E_T(30)$ (in kcal mol^{-1}).[25]

As reported previously,[17b] two types of characteristic spectral changes of the lowest energy band of **3H** were observed in the presence of BASE. One is type I, which caused the small shift ($\Delta\lambda<12$ nm) in a less polar solvent. Type II is the other, causing a large bathochromic shift ($\Delta\lambda>20$ nm) in a more polar solvent. Type I and II spectral changes were caused by the formation of a 1:1 hydrogen-bonded complex of **3H** and BASE ([BASE···**3H**]) and the formation of an ion pair of $\mathbf{3}(\mathbf{O})^-$ with $BASE–H^+$ ([$BASE–H^+$/$\mathbf{3}(\mathbf{O})^-$]), respectively (Scheme 5).

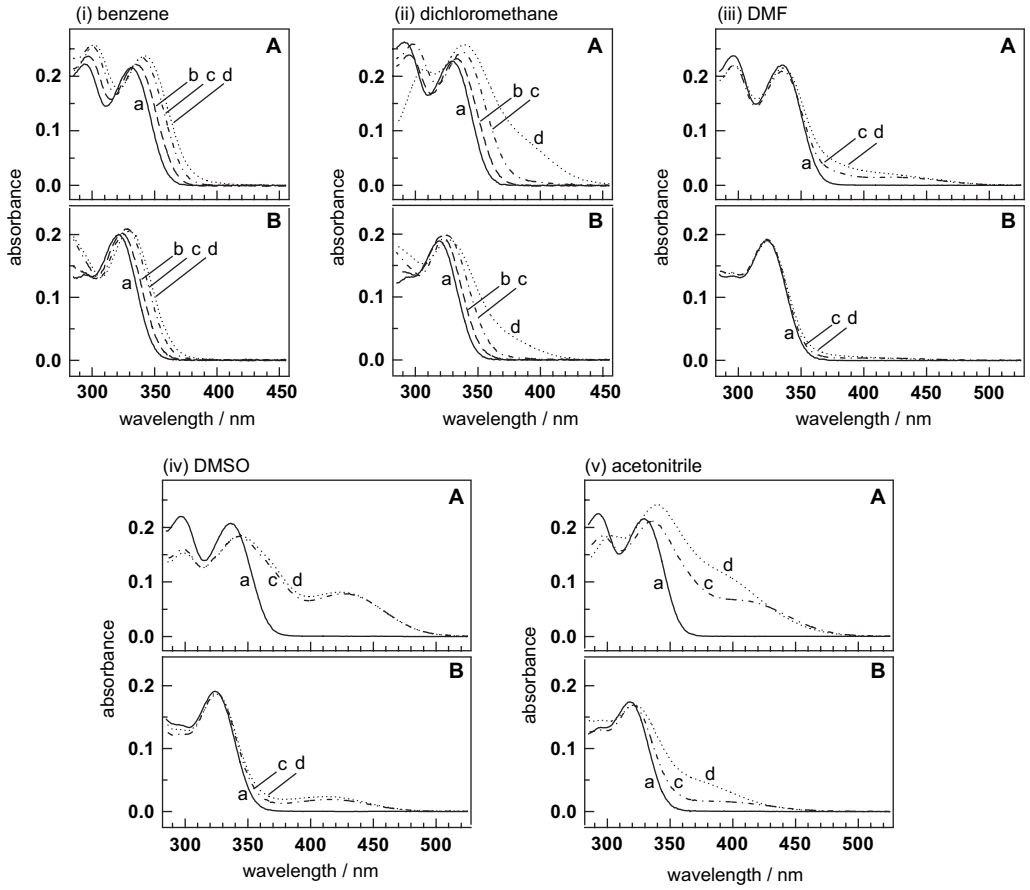


Figure 1. UV–vis absorption spectra of **3aH** (A, 1.5×10^{-5} mol L^{-1}) and **3bH** (B, 1.5×10^{-5} mol L^{-1}) in selected solvents in the absence and presence of organic bases (1.0×10^{-2} mol L^{-1}) at 25 °C; organic base: none (a), NBA (b), TMG (c), and DBU (d). The selected solvents are benzene (i), dichloromethane (ii), DMF (iii), DMSO (iv), and acetonitrile (v).

Table 1. UV–vis absorption data of **3aH** (1.5×10^{-5} mol L^{-1}) in various solvents in the absence and presence of organic bases (NBA, TMG, and DBU) at 25 °C

Solvent	$E_T(30)$/kcal mol^{-1}	λ_{max}/nm ($\varepsilon/10^4$)[a]	λ_{max}/nm (absorbance)		
		3aH	**3aH**+NBA[b]	**3aH**+TMG[b]	**3aH**+DBU[b]
p-Xylene	33.1	332 (1.05)	335 (0.164)	340 (0.170)	342 (0.188)
				300 (0.176)	302 (0.198)
Toluene	33.9	332 (1.24)	335 (0.212)	340 (0.228)	342 (0.222)
					302 (0.242)
Benzene	34.3	331 (1.44)	335 (0.224)	340 (0.233)	343 (0.237)
		294 (1.48)	297 (0.237)	299 (0.255)	301 (0.258)
Benzene/chloroform (20:1)	36.4	331 (1.46)	335 (0.228)	340 (0.239)	342 (0.242)
		295 (1.50)	297 (0.242)	300 (0.257)	302 (0.261)
Chlorobenzene	36.8	331 (1.16)	336 (0.186)	340 (0.212)	343 (0.218)
		291 (1.28)	298 (0.200)	300 (0.239)	304 (0.233)
1-Chloropropane	37.4	329 (1.16)	332 (0.185)	339 (0.212)	340 (0.221)
		293 (1.22)	295 (0.203)	299 (0.237)	301 (0.249)
Benzene/chloroform (2:1)	38.0	331 (1.49)	334 (0.221)	338 (0.228)	390 (0.035, sh)
		294 (1.56)	296 (0.230)	299 (0.243)	340 (0.228)
					302 (0.261)
1,2-Dichlorobenzene	38.0	332 (1.14)	339 (0.176)	342 (0.198)	343 (0.222)
				302 (0.221)	304 (0.249)
Chloroform	39.1	331 (1.33)	333 (0.231)	338 (0.234)	380 (0.072, sh)
		294 (1.33)	295 (0.237)	298 (0.246)	331 (0.227)
					302 (0.212)
Dichloromethane	40.7	330 (1.52)	333 (0.233)	338 (0.245)	380 (0.102, sh)
		291 (1.75)	295 (0.239)	298 (0.258)	339 (0.258)
					307 (0.213)
1,2-Dichloroethane	41.3	331 (1.43)	334 (0.219)	390 (0.014, sh)	375 (0.047, sh)
		293 (1.45)	295 (0.224)	338 (0.227)	338 (0.227)
				299 (0.242)	303 (0.227)
Benzonitrile	41.5	334 (1.36)	337 (0.204)	400 (0.010, sh)	390 (0.063, sh)
				338 (0.207)	341 (0.219)
DMAc	42.9	335 (1.54)	—[c]	336 (0.222)	400 (0.010, sh)
		296 (1.83)		298 (0.248)	337 (0.224)
					297 (0.276)
DMF	43.2	336 (1.47)	—[c]	430 (0.014, sh)	410 (0.024, sh)
		296 (1.58)		335 (0.209)	335 (0.215)
				296 (0.219)	297 (0.221)
Propionitrile	43.6	330 (1.46)	334 (0.224)	385 (0.023, sh)	385 (0.084, sh)
		292 (1.56)	296 (0.246)	333 (0.227)	337 (0.243)
		274 (1.52)	278 (0.224)	296 (0.233)	299 (0.218)
				276 (0.210)	
DMSO	45.1	337 (1.38)	—[c]	426 (0.078)	423 (0.081)
		297 (1.47)		343 (0.183)	344 (0.186)
				299 (0.159)	299 (0.153)
Acetonitrile	45.6	327 (1.44)	—[c]	410 (0.066, sh)	400 (0.105, sh)
		293 (1.50)		336 (0.212)	339 (0.242)
		272 (1.47)		296 (0.185)	302 (0.185)
				273 (0.177)	

[a] Extinction coefficient in L mol^{-1} cm^{-1}.
[b] Concentrations of the organic bases were 1.0×10^{-2} mol L^{-1}.
[c] Absorption spectra of **3aH** did not change in the presence of NBA.

The type I spectral change of **3H** was observed in the presence of BASE in solvents from *p*-xylene (top) to benzonitrile and propionitrile, listed in Tables 1 and 2. In the presence of DBU in benzene/chloroform (2:1), 1,2-dichlorobenzene, chloroform, dichloromethane, 1,2-dichloroethane, benzonitrile, and propionitrile, both the type I and II spectral changes were observed. Typical spectra are shown in Figure 1i and ii. Formation of [BASE···**3H**] was mainly observed in the solvents with low ionizing power. A partial generation of [DBU–H^+/**3**(**O**)$^-$] in these solvents was also observed, because DBU has high enough basicity to eliminate a proton from the phenolic hydroxy groups of **3H**. The order of $\Delta\lambda$, DBU>TMG>NBA, corresponds to the reported acidities (pK_a) of their conjugated acids, 24.1, 23.3, and 18.1, respectively, in acetonitrile.[26] The order of $\Delta\lambda$ also corresponds to the formation constants (K, Scheme 5) of [DBU···**3aH**], [TMG···**3aH**], and [NBA···**3aH**]: 7800, 1430, and 220[17b] mol^{-1} L, respectively, in benzene at 25 °C. Therefore, the more basic an organic base becomes, the stronger **3H** makes a hydrogen bond with the organic base and the more $\Delta\lambda$ and K increase.

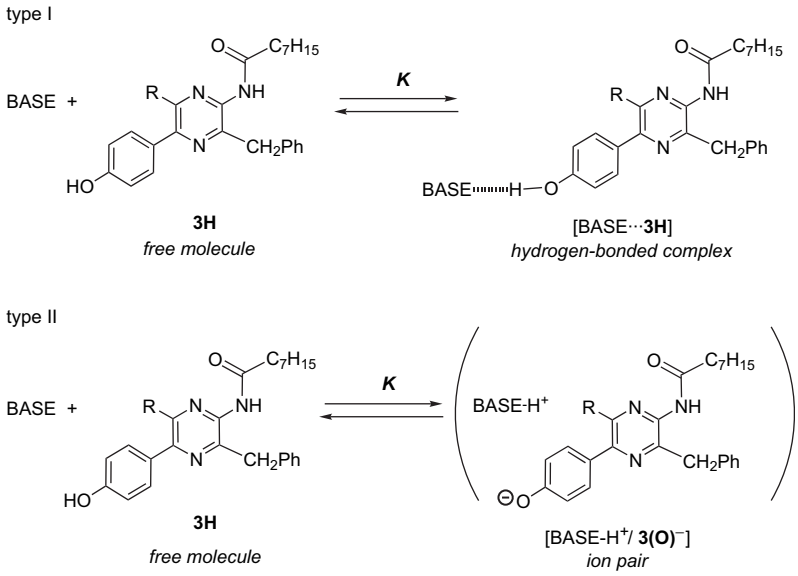
The type II spectral changes for **3H** were observed in the presence of TMG and DBU in DMAc, DMF, DMSO, and acetonitrile, while the addition of NBA did not affect the original spectra of **3H** (Fig. 1iii–v). These solvents have enough ionizing power to generate [BASE–H^+/**3**(**O**)$^-$]. In addition, TMG and DBU have high enough basicities to generate **3**(**O**)$^-$ from **3H**, but NBA does not. Absorption spectra of [TMG–H^+/**3**(**O**)$^-$] and [DBU–H^+/**3**(**O**)$^-$] were similar to each other, suggesting that the interaction of **3**(**O**)$^-$ with TMG–H^+ in the ion pair is similar to that of DBU–H^+. One of the characteristics of the spectral changes is that a growth of the absorption of [TMG–H^+/**3b**(**O**)$^-$] or [DBU–H^+/**3b**(**O**)$^-$]

Table 2. UV–vis absorption data of **3bH** (1.5×10^{-5} mol L^{-1}) in various solvents in the absence and presence of organic bases (NBA, TMG, and DBU) at 25 °C

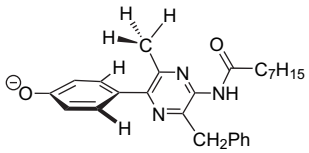
Solvent	$E_T(30)$/kcal mol^{-1}	λ_{max}/nm ($\varepsilon/10^4$)[a]	λ_{max}/nm (absorbance)		
		3bH	**3bH**+NBA[b]	**3bH**+TMG[b]	**3bH**+DBU[b]
p-Xylene	33.1	323 (1.35)	324 (0.201)	329 (0.206)	331 (0.207)
Toluene	33.9	322 (1.32)	324 (0.203)	328 (0.209)	329 (0.209)
Benzene	34.3	321 (1.33)	324 (0.203)	328 (0.210)	330 (0.206)
Benzene/chloroform (20:1)	36.4	321 (1.32)	323 (0.201)	327 (0.198)	329 (0.204)
Chlorobenzene	36.8	322 (1.43)	325 (0.218)	330 (0.225)	331 (0.225)
1-Chloropropane	37.4	320 (1.38)	322 (0.207)	327 (0.210)	328 (0.213)
				275 (0.206)	277 (0.216)
Benzene/chloroform (2:1)	38.0	321 (1.30)	324 (0.198)	327 (0.198)	380 (0.017, sh)
					331 (0.192)
1,2-Dichlorobenzene	38.0	321 (1.31)	325 (0.209)	330 (0.216)	331 (0.215)
Chloroform	39.1	319 (1.28)	322 (0.195)	326 (0.194)	370 (0.038, sh)
					322 (0.180)
Dichloromethane	40.7	319 (1.26)	321 (0.197)	325 (0.198)	370 (0.048, sh)
				274 (0.192)	328 (0.191)
					275 (0.192)
1,2-Dichloroethane	41.3	320 (1.31)	322 (0.197)	327 (0.203)	326 (0.192)
				273 (0.200)	276 (0.186)
Benzonitrile	41.5	323 (1.21)	325 (0.183)	390 (0.007, sh)	385 (0.029, sh)
				325 (0.183)	329 (0.185)
DMAc	42.9	323 (1.39)	—[c]	326 (0.197)	324 (0.198)
DMF	43.2	323 (1.27)	—[c]	410 (0.004, sh)	400 (0.005, sh)
		294 (0.89)		333 (0.192)	333 (0.189)
				294 (0.140)	295 (0.140)
Propionitrile	43.6	320 (1.24)	321 (0.188)	380 (0.008, sh)	375 (0.037, sh)
				321 (0.188)	323 (0.186)
DMSO	45.1	324 (1.27)	—[c]	416 (0.020)	412 (0.024)
				326 (0.188)	325 (0.186)
Acetonitrile	45.6	318 (1.16)	—[c]	390 (0.016, sh)	380 (0.045, sh)
		295 (0.89)		321 (0.170)	322 (0.170)
		263 (1.10)		294 (0.129)	271 (0.165)
				265 (0.156)	

[a] Extinction coefficient in L mol^{-1} cm^{-1}.
[b] Concentrations of the organic bases were 1.0×10^{-2} mol L^{-1}.
[c] Absorption spectra of **3bH** did not change in the presence of NBA.

was smaller than that of [TMG–H^+/**3a**(**O**)$^-$] or [DBU–H^+/**3a**(**O**)$^-$]. The *K* value for the formation of [TMG–H^+/**3b**(**O**)$^-$] in DMSO, 28 mol^{-1} L at 25 °C, is much smaller than that of [TMG–H^+/**3a**(**O**)$^-$], 480 mol^{-1} L at 25 °C,[17b] clearly indicating that the generation of **3b**(**O**)$^-$ from **3bH** is less favorable than that of **3a**(**O**)$^-$ from **3aH**. This is explained by the methyl group at C6 in **3b**(**O**)$^-$ sterically inhibiting the π-electronic conjugation between the 4-oxidophenyl and amidopyrazine moieties (vide infra) to decrease the molecular stability.



Scheme 5.



3b(O)$^-$

2.2. Fluorescent properties of phenolate anions 3(O)$^-$ in ion pairs with conjugate acids of organic bases

Growth of the fluorescence emission band of **3**(**O**)$^-$ was observed along with decreasing fluorescence intensity of the neutral molecule **3H** in the presence of NBA,[17b] TMG,[17b] and DBU, as shown in the selected spectra (Fig. 2). We also confirmed that fluorescence excitation spectra of **3**(**O**)$^-$ in the presence of organic bases correspond to their absorption spectra. Corresponding to the type I and II spectral changes in the UV–vis absorption, there are two possible processes to generate ^{1}**3**(**O**)$^{-*}$ in an ion pair with a BASE–H$^+$ (Scheme 6).[17] One is a stepwise process via [BASE$\cdots{}^1$**3H***] generated by electronic excitation of [BASE$\cdots$**3H**], as shown in Scheme 6a. The other is a direct electronic excitation process from [BASE–H$^+$/**3**(**O**)$^-$] (Scheme 6b). Both processes show the fluorescence from [BASE–H$^+$/^{1}**3**(**O**)$^{-*}$].

Fluorescence emission maxima (λ_{FL}) and quantum yields (Φ_f) are summarized in Tables 3 and 4. The Φ_f values of [BASE–H$^+$/**3**(**O**)$^-$] were obtained by subtracting the fluorescences of **3H** from the total spectra. Because the fluorescence emission from ^{1}**3**(**O**)$^{-*}$ in an ion pair has an intramolecular charge transfer (ICT) nature, energies E_{FL} (in kcal mol^{-1}) calculated from the λ_{FL} of [BASE–H$^+$/**3**(**O**)$^-$] show a linear correlation to $E_T(30)$.[17b] Thus, we made E_{FL}–$E_T(30)$ plots of **3**(**O**)$^-$ in the ion pairs with NBA–H$^+$, TMG–H$^+$, and DBU–H$^+$, as shown in Figure 3. The E_{FL}–$E_T(30)$ correlations were $E_{FL}=-1.04E_T(30)+97$ ($r=-0.96$), $E_{FL}=-0.89E_T(30)+88$ ($r=-0.98$), and $E_{FL}=-0.90E_T(30)+89$ ($r=-0.98$), for [NBA–H$^+$/**3a**(**O**)$^-$], [TMG–H$^+$/**3a**(**O**)$^-$], and [DBU–H$^+$/**3a**(**O**)$^-$], respectively.[27] Similarly, $E_{FL}=-1.40E_T(30)+111$ ($r=-0.99$), $E_{FL}=-1.00E_T(30)+92$ ($r=-0.97$), and $E_{FL}=-1.02E_T(30)+93$ ($r=-0.98$) were for [NBA–H$^+$/**3b**(**O**)$^-$], [TMG–H$^+$/**3b**(**O**)$^-$], and [DBU–H$^+$/**3b**(**O**)$^-$], respectively.

The E_{FL}–$E_T(30)$ correlations for [TMG–H$^+$/**3**(**O**)$^-$] and [DBU–H$^+$/**3**(**O**)$^-$] were similar to each other, while the slopes and intercepts of the linear E_{FL}–$E_T(30)$ correlations for [NBA–H$^+$/**3**(**O**)$^-$] were larger than those for [TMG–H$^+$/**3**(**O**)$^-$] or [DBU–H$^+$/**3**(**O**)$^-$]. Similarly, the Φ_f of [TMG–H$^+$/**3**(**O**)$^-$] and [DBU–H$^+$/**3**(**O**)$^-$] were similar to each other, while most of the Φ_f of [NBA–H$^+$/**3**(**O**)$^-$] were larger than those of [TMG–H$^+$/**3**(**O**)$^-$] or [DBU–H$^+$/**3**(**O**)$^-$]. These results indicate that there are two modes of interaction between ^{1}**3**(**O**)$^{-*}$ and a BASE–H$^+$, which may be caused by a structural variation of an ion pair: a solvent separated ion pair (SSIP) for [TMG–H$^+$/^{1}**3**(**O**)$^{-*}$] or [DBU–H$^+$/^{1}**3**(**O**)$^{-*}$], and a contact ion pair (CIP) for [NBA–H$^+$/^{1}**3**(**O**)$^{-*}$].[25,29] The selectivity to form an SSIP or a CIP is determined by the basicity of BASE. Because the SSIP of ^{1}**3**(**O**)$^{-*}$ and

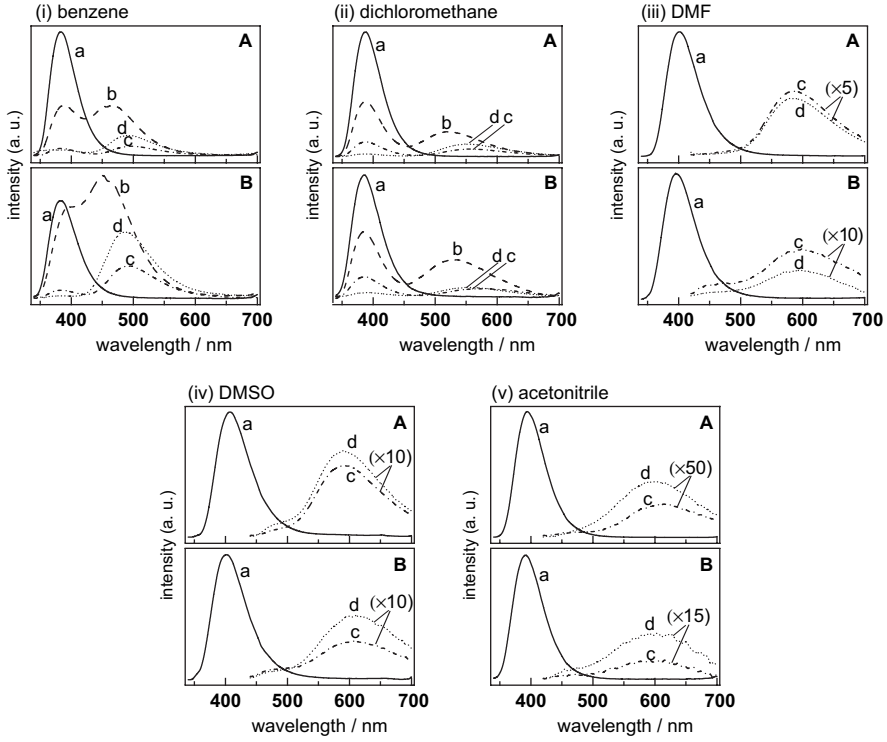
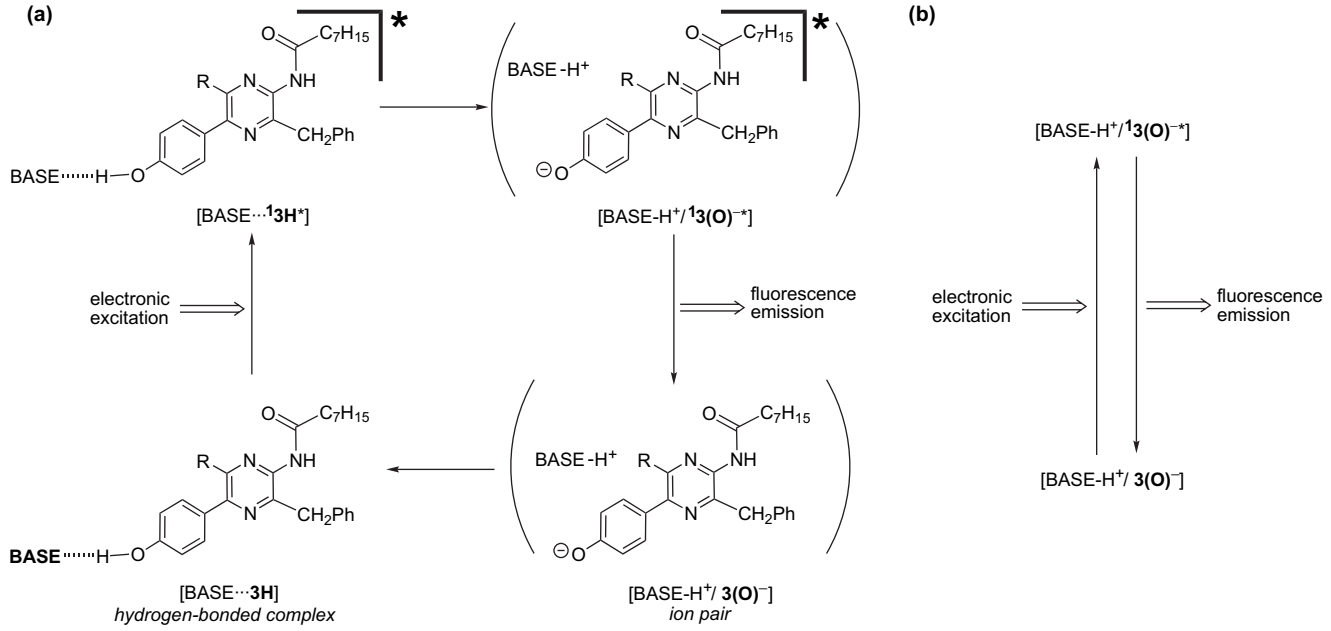


Figure 2. Fluorescence emission spectra of **3aH** and [BASE–H$^+$/**3a**(**O**)$^-$] (A) and **3bH** and [BASE–H$^+$/**3b**(**O**)$^-$] (B) in various solvents in the absence and presence of organic bases (1.0×10^{-2} mol L^{-1}) at 25 °C; organic base: none (a), NBA (b), TMG (c), and DBU (d). Selected solvents were benzene (i), dichloromethane (ii), DMF (iii), DMSO (iv), and acetonitrile (v). The initial concentrations of **3H** were 1.5×10^{-6} mol L^{-1}, except for those (1.5×10^{-5} mol L^{-1}) in DMF, DMSO, and acetonitrile containing TMG and DBU. Excitation wavelengths (λ_{ex}) were 320 nm in benzene and dichloromethane. The λ_{ex} in DMF, DMSO, and acetonitrile were also 320 nm in the absence of the organic bases. The λ_{ex} in DMF and acetonitrile containing bases and in DMSO containing bases were 400 and 425 nm, respectively.



Scheme 6.

TMG–H$^+$ or DBU–H$^+$ is a loose ion pair separated by solvent molecules, the Coulomb force between $^1\mathbf{3}(\mathbf{O})^{-*}$ and TMG–H$^+$ or DBU–H$^+$ is weak and the fluorescence of $\mathbf{3}(\mathbf{O})^-$ is not much affected by the choice of BASE–H$^+$. On the other hand, the CIP of $^1\mathbf{3}(\mathbf{O})^{-*}$ and NBA–H$^+$ holds together by an effective Coulomb force, and the degree of binding between $^1\mathbf{3}(\mathbf{O})^{-*}$ and NBA–H$^+$ varies with the solvent polarity. Therefore, the molecular stability of [NBA–H$^+$/$^1\mathbf{3}(\mathbf{O})^{-*}$] is sensitive to the solvent polarity, resulting in steep slopes of the E_{FL}–E_T(30) correlations for [NBA–H$^+$/$\mathbf{3}(\mathbf{O})^-$]. Among a variety of combinations of $\mathbf{3}(\mathbf{O})^-$ and BASE–H$^+$, [NBA–H$^+$/$\mathbf{3}(\mathbf{O})^-$] showed the highest Φ_f values (>0.1) in the solvent, with $E_T(30)<38$ kcal mol^{-1}. This indicates that [NBA–H$^+$/$^1\mathbf{3}(\mathbf{O})^{-*}$] in a less polar solvent is favorable for increasing the Φ_f of $\mathbf{3}(\mathbf{O})^-$.

The slopes of the E_{FL}–E_T(30) correlations for [BASE–H$^+$/$\mathbf{3b}(\mathbf{O})^-$] are larger than the corresponding correlations for [BASE–H$^+$/$\mathbf{3a}(\mathbf{O})^-$]. This indicates that the degree of ICT in methyl derivative $^1\mathbf{3b}(\mathbf{O})^{-*}$ is enhanced by the twisted structure between 4-oxidophenyl and amidopyrazine moieties, as shown in Scheme 7. The stability of the twisted $^1\mathbf{3b}(\mathbf{O})^{-*}$ is more sensitive to the solvent polarity than is that of $^1\mathbf{3a}(\mathbf{O})^{-*}$, giving a steeper slope for the E_{FL}–E_T(30) correlation for [BASE–H$^+$/$\mathbf{3b}(\mathbf{O})^-$] than for [BASE–H$^+$/$\mathbf{3a}(\mathbf{O})^-$]. On the other hand, the Φ_f values of $\mathbf{3a}(\mathbf{O})^-$ and

Table 3. Fluorescence of **3aH** and its phenolate anion $\mathbf{3a}(\mathbf{O})^-$ in ion pairs with NBA–H$^+$, TMG–H$^+$, and DBU–H$^+$ in various solvents at 25 °C

Solvent	E_T(30)/kcal mol^{-1}	λ_{FL}[a]/nm (Φ_f[b])			
		3aH[c]	**3aH**[c]+NBA[d] [NBA–H$^+$/**3a**(**O**)$^-$]	**3aH**[c]+TMG[d] [TMG–H$^+$/**3a**(**O**)$^-$]	**3aH**[c]+DBU[d] [DBU–H$^+$/**3a**(**O**)$^-$]
p-Xylene	33.1	383 (0.14)	452 (0.24)	490 (0.04)	486 (0.06)
Toluene	33.9	383 (0.17)	466 (0.23)	495 (0.03)	491 (0.06)
Benzene	34.3	383[e] (0.19)[e]	469[e] (0.27)[e]	498 (0.03)	490 (0.06)
Benzene/chloroform (20:1)	36.4	384 (0.19)	473 (0.23)	504 (0.03)	503 (0.05)
Chlorobenzene	36.8	385 (0.15)	501 (0.35)	542 (0.09)	531 (0.15)
Chloropropane	37.4	385 (0.17)	498 (0.19)	526 (0.10)	521 (0.12)
Benzene/chloroform (2:1)	38.0	387 (0.19)	499 (0.20)	527 (0.03)	522 (0.03)
1,2-Dichlorobenzene	38.0	385 (0.16)	505 (0.34)	537 (0.14)	530 (0.17)
Chloroform	39.1	389 (0.23)	500 (0.16)	542 (0.03)	542 (0.02)
Dichloromethane	40.7	388 (0.18)	518 (0.15)	557 (0.03)	553 (0.04)
1,2-Dichloroethane	41.3	388 (0.18)	520 (0.17)	554 (0.05)	544 (0.05)
Benzonitrile	41.5	394 (0.23)	546 (0.03)	562 (0.04)	554 (0.03)
DMAc	42.9	401 (0.22)	—[f]	575 (0.07)	575 (0.06)
DMF	43.2	402 (0.24)	—[f]	584[e] (0.04)[e]	583 (0.04)
Propionitrile	43.6	392 (0.20)	554 (0.02)	582 (0.02)	582 (0.01)
DMSO	45.1	408 (0.22)	—[f]	591[e] (0.02)[e]	590 (0.03)
Acetonitrile	45.6	394 (0.20)	—[f]	615[e] (0.01)[e]	598 (0.01)

[a] Fluorescence emission maxima. In the presence of the organic bases, only the λ_{FL} of the generated anion species are shown.
[b] Fluorescence quantum yields.
[c] The initial concentration of **3aH** was 1.5×10^{-6} mol L^{-1}.
[d] The initial concentration of the organic base was 1.0×10^{-2} mol L^{-1}.
[e] The value revised from the previous report (Ref. 28).
[f] No new emission band was observed in the presence of NBA.

Table 4. Fluorescence of **3bH** and its phenolate anion **3b(O)**$^-$ in ion pairs with NBA–H$^+$, TMG–H$^+$, and DBU–H$^+$ in various solvents at 25 °C

Solvent	$E_T(30)$/kcal mol^{-1}	λ_{FL}[a]/nm (Φ_f[b])			
		3bH[c]	**3bH**[c]+NBA[d] [NBA–H$^+$/**3b(O)**$^-$]	**3bH**[c]+TMG[d] [TMG–H$^+$/**3b(O)**$^-$]	**3bH**[c]+DBU[d] [DBU–H$^+$/**3b(O)**$^-$]
p-Xylene	33.1	383 (0.027)	444 (0.24)	480 (0.02)	479 (0.04)
Toluene	33.9	383 (0.031)	451 (0.30)	489 (0.02)	481 (0.04)
Benzene	34.3	384 (0.030)	451 (0.32)	494 (0.02)	488 (0.04)
Benzene/chloroform (20:1)	36.4	384 (0.029)	472 (0.16)	504 (0.02)	500 (0.03)
Chlorobenzene	36.8	384 (0.046)	480 (0.23)	522 (0.08)	515 (0.11)
1-Chloropropane	37.4	383 (0.026)	487 (0.09)	529 (0.04)	521 (0.05)
Benzene/chloroform (2:1)	38.0	385 (0.032)	504 (0.10)	540 (0.01)	534 (0.02)
1,2-Dichlorobenzene	38.0	385 (0.039)	501 (0.19)	542 (0.11)	531 (0.10)
Chloroform	39.1	385 (0.032)	514 (0.07)	560 (0.01)	556 (0.01)
Dichloromethane	40.7	386 (0.025)	530 (0.04)	578 (<0.005)	552 (<0.005)
1,2-Dichloroethane	41.3	384 (0.031)	527 (0.07)	562 (0.01)	552 (0.02)
Benzonitrile	41.5	394 (0.080)	557 (0.02)	570 (0.01)	557 (0.02)
DMAc	42.9	395 (0.052)	—[e]	572 (0.01)	582 (0.01)
DMF	43.2	396 (0.052)	—[e]	602 (0.01)	597 (0.01)
Propionitrile	43.6	391 (0.027)	566 (<0.005)	585 (0.01)	576 (0.01)
DMSO	45.1	402 (0.065)	—[e]	610 (0. 01)	607 (0.01)
Acetonitrile	45.6	391 (0.025)	—[e]	600 (<0.005)	595 (<0.005)

[a] Fluorescence emission maxima. In the presence of the organic bases, only the λ_{FL} of the generated anion species are shown.
[b] Fluorescence quantum yields.
[c] The initial concentration of **3bH** was 1.5×10^{-6} mol L^{-1}.
[d] The initial concentration of the organic base was 1.0×10^{-2} mol L^{-1}.
[e] No new emission band was observed in the presence of NBA.

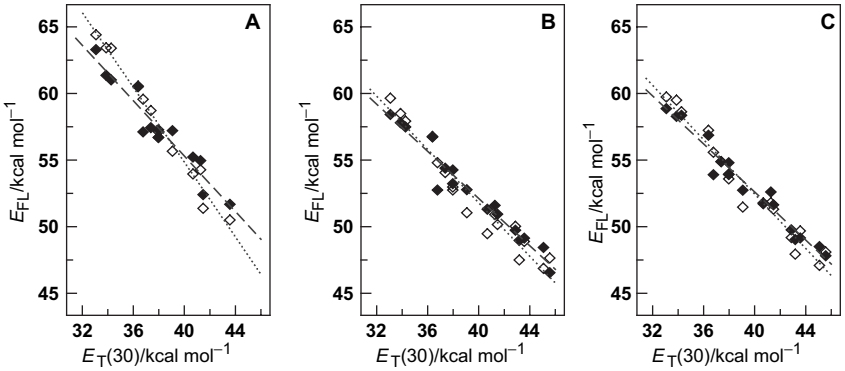
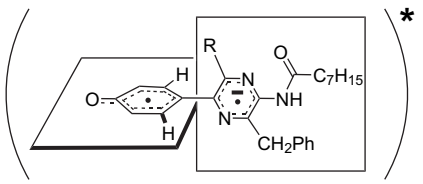


Figure 3. Plots of E_{FL} (in kcal mol^{-1}) for **3a(O)**$^-$ (◆) and **3b(O)**$^-$ (◇) in ion pairs with NBA–H$^+$ (A), TMG–H$^+$ (B), and DBU–H$^+$ (C) against $E_T(30)$ (in kcal mol^{-1}).

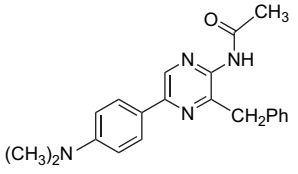
3b(O)$^-$ were similar to each other, while **3aH** and **3bH** had different Φ_f values. This result indicates that the methyl group at C6 in **3b(O)**$^-$ does not enhance a non-radiative process from the twisted ^{1}**3b(O)**$^{-*}$, which competes with the fluorescence emission process. Excited neutral molecules ^{1}**3H***, however, are affected by the methyl substitution, whose steric effect causes a decrease of Φ_f.



^{1}**3a(O)**$^{-*}$: R = H
^{1}**3b(O)**$^{-*}$: R = CH_3

Scheme 7.

Because the E_{FL}–$E_T(30)$ correlations for **3a(O)**$^-$ in the ion pairs were revised from the previous one,[27] we recompared the E_{FL}–$E_T(30)$ correlations for [BASE–H$^+$/**3a(O)**$^-$] with that for a dimethylamino analogue **6**, $E_{FL}=-0.64E_T(30)+85$ ($r=-0.98$).[16] The slopes of the E_{FL}–$E_T(30)$ correlations for [BASE–H$^+$/**3a(O)**$^-$] are all steeper than those for **6**, while the intercepts of the E_{FL}–$E_T(30)$ correlations for [TMG–H$^+$/**3a(O)**$^-$] or [DBU–H$^+$/**3a(O)**$^-$] and **6** are similar to each other. The different slopes for [BASE–H$^+$/**3a(O)**$^-$] and **6** are related to the electron-donating abilities of the oxido and dimethylamino groups, respectively. The modified Hammett constant σ_p^+ for the oxido group (-2.30) is smaller than that of the dimethylamino group (-1.70).[30,31] Therefore, the oxido group plays an important role as an electron donor for stabilizing the ICT state of ^{1}**3a(O)**$^{-*}$ (Scheme 7).

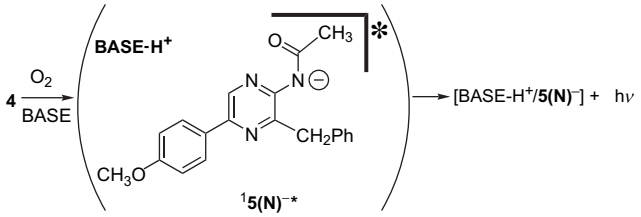


6

2.3. Luminescent properties of amide anion 5(N)$^-$: chemiluminescence of coelenterazine analogue 4 in various solvents

To consider the difference in the fluorescent properties between **2**(**O**)$^-$ and **2**(**N**)$^-$, we investigated the fluorescence of amide anion **5**(**N**)$^-$ generated from **5H** with a strong base, such as NaOH, in a polar solvent with strong ionizing power, such as DMSO.[32] Unfortunately, reproducible fluorescence data were not obtained for **5**(**N**)$^-$ in a solvent with a low ionizing power such as benzene, because of interferences from side reactions. The difficulty in observing **5**(**N**)$^-$ is caused by the low acidity of the amide moiety in **5H**.[19–21]

To evaluate the fluorescent properties of **5**(**N**)$^-$ in an ion pair in various solvents, we investigated the chemiluminescence of **4**, which showed the fluorescence emission from [BASE–H$^+$/^{1}**5**(**N**)$^{-*}$] (Scheme 8). Chemiluminescence of **4** was easily observed in aerated DMSO and DMF (Fig. 4), and the fluorescence emission maxima (λ_{FL}) of [DMSO–H$^+$/**5**(**N**)$^-$] and [DMF–H$^+$/**5**(**N**)$^-$] almost reproduced the reported data.[12,32–34] On the other hand, **4** did not react in aerated benzene, dichloromethane, or acetonitrile in the absence of a base. TMG was used as an organic base in these solvents to initiate a chemiluminescence reaction of **4**[35] and the fluorescence emission from [TMG–H$^+$/^{1}**5**(**N**)$^{-*}$] was observed, as shown in Figure 4. In benzene, an emission from excited neutral molecule ^{1}**5H*** also appeared around 400 nm. The λ_{FL} values and chemiluminescence quantum yields (Φ_{cl}) are summarized in Table 5. The Φ_{cl} values in benzene, dichloromethane, and acetonitrile were smaller than those in DMSO and DMF. The λ_{FL} values of [BASE–H$^+$/**5**(**N**)$^-$] were in the range of 464–476 nm, which is less than that of [BASE–H$^+$/**3a**(**O**)$^-$]. The λ_{FL} values of [BASE–H$^+$/**5**(**N**)$^-$] did not depend on the BASE–H$^+$, which were DMSO–H$^+$, DMF–H$^+$, and TMG–H$^+$. Therefore, the fluorescence of **5**(**N**)$^-$ was not significantly affected by the interaction with BASE–H$^+$ in the ion pair or the solvent polarity.



Scheme 8.

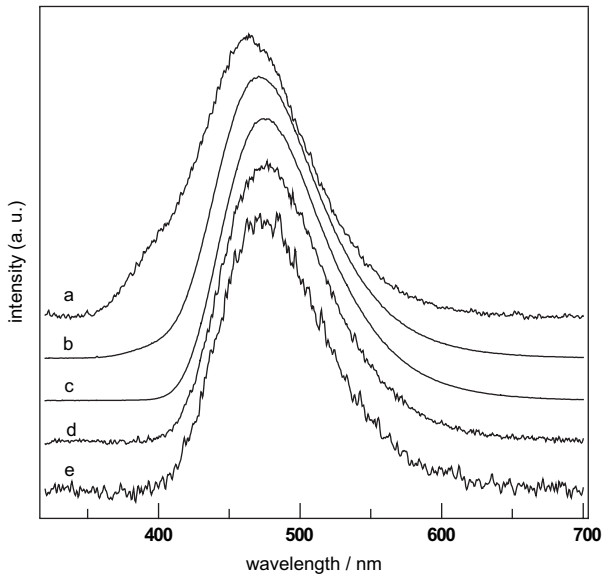


Figure 4. Chemiluminescence spectra of **4** (1.5×10^{-5} mol L^{-1}) in various solvents under air. Solvents were benzene containing TMG (a), dichloromethane containing TMG (b), DMF (c), DMSO (d), and acetonitrile containing TMG (e). Concentrations of TMG were 1.0×10^{-2} mol L^{-1}.

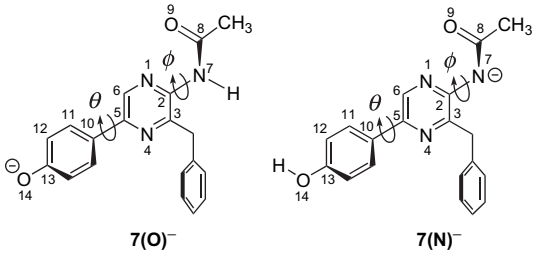
Table 5. Emission maxima (λ_{FL}) and quantum yields (Φ_{cl}) for the chemiluminescence of **4** in various solvents under air

Solvent	Additive	λ_{FL}/nm	$\Phi_{cl}/10^{-3}$
Benzene	TMG[a]	464	0.005
Dichloromethane	TMG[a]	470	0.02
DMF	None	471	1.5
DMSO	None	476	1.1
Acetonitrile	TMG[a]	473	0.06

[a] The concentration of TMG was 1.0×10^{-2} mol L^{-1}.

2.4. Molecular orbital calculations for the ground and the singlet-excited states of anion species of a coelenteramide analogue

To gain insight into the structural characteristics of **2**(**O**)$^-$ and **2**(**N**)$^-$, we carried out PM5 semi-empirical molecular orbital (MO) calculations for the ground and the singlet-excited states of **7**(**O**)$^-$ and **7**(**N**)$^-$, which are the anion species of an acetamide analogue (Tables 6 and 7). Because the stabilities of ionic molecules are affected by dielectric molecular environments, we performed the calculations with the COSMO method[36] using several dielectric constants (ε).[25]



In the ground state, it is reasonable that the heats of formation (ΔH_f) of **7**(**O**)$^-$ are smaller than those of **7**(**N**)$^-$ under various dielectric conditions, because the acidity of a phenolic hydroxy group is higher than that of an amide moiety in general.[18,19] The HOMO–LUMO energy gaps of **7**(**O**)$^-$ were predicted to be smaller than those of **7**(**N**)$^-$. The torsion angles C6–C5–C10–C11 (θ) and N1–C2–N7–C8 (ϕ) of **7**(**O**)$^-$ and **7**(**N**)$^-$ indicate that the conformations of the pyrazine rings with the 4-oxido- or 4-hydroxyphenyl group and with the amide moiety are twisted, but neither perpendicular nor coplanar. As the characteristic bond lengths in **7**(**O**)$^-$ and **7**(**N**)$^-$, the C13–O14 of **7**(**O**)$^-$ is shortened by the conjugation of the oxido group to the phenyl group, while the C8═O9 of **7**(**N**)$^-$ is elongated by the conjugation

Table 6. Calculated properties of **7**(**O**)$^-$ and **7**(**N**)$^-$ in the ground state with the PM5-COSMO method

Compounds	ε[a]	ΔH_f[b]/kJ mol^{-1}	HOMO/eV	LUMO/eV	$\Delta E_{HOMO-LUMO}$[c]/eV	θ[d]/°	ϕ[d]/°	r(C═O)[e]/Å	r(C–O)[e]/Å	r(C–C)[e]/Å
7(**O**)$^-$	2.27	−405	−6.06	0.61	6.67	155.5	113.1	1.235	1.271	1.444
	8.93	−593	−8.09	−0.73	7.26	142.7	42.4	1.247	1.298	1.461
	35.94	−664	−8.76	−1.14	7.62	140.5	37.8	1.253	1.309	1.464
	46.45	−669	−8.81	−1.17	7.64	141.0	39.7	1.254	1.309	1.465
7(**N**)$^-$	2.27	−396	−6.92	1.07	7.99	43.6	114.0	1.262	1.358	1.468
	8.93	−567	−8.53	−0.33	8.20	47.6	73.0	1.278	1.354	1.469
	35.94	−627	−8.95	−0.85	8.10	46.3	68.4	1.284	1.353	1.468
	46.45	−632	−9.01	−0.90	8.11	45.5	75.1	1.285	1.352	1.468

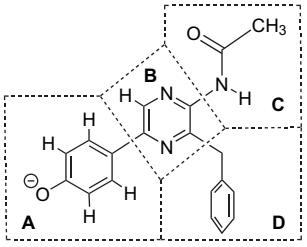
[a] Dielectric constants at 25 °C of benzene (2.27), dichloromethane (8.93), acetonitrile (35.94), and DMSO (46.45) from Ref. 25.
[b] Heat of formation.
[c] HOMO–LUMO energy gap.
[d] The angles θ and ϕ are torsion angles C6–C5–C10–C11 and N1–C2–N7–C8, respectively.
[e] The r(C═O), r(C–O), and r(C–C) are the bond lengths of C8–C9, C13–O14, and C5–C10, respectively.

of the amide anion. The C5–C10 of **7**(**O**)$^-$ and **7**(**N**)$^-$ are the lengths of typical single bonds. The calculated structures of **7**(**O**)$^-$ with a twisted conformation between the 4-oxidophenyl and the pyrazine moieties and the C5–C10 single bond predict that **2**(**O**)$^-$ has little proportion of a quinoid form (Scheme 3c) in the resonance structures.

The data of ^{1}**7**(**O**)$^{-*}$ and ^{1}**7**(**N**)$^{-*}$ calculated with the PM5-CI-COSMO method (Table 7) indicate that the ΔH_f values of ^{1}**7**(**O**)$^{-*}$ are much smaller than those of ^{1}**7**(**N**)$^{-*}$ under various dielectric conditions. The difference in ΔH_f between ^{1}**7**(**O**)$^{-*}$ and ^{1}**7**(**N**)$^{-*}$, 105–159 kJ mol^{-1}, is much larger than that between **7**(**O**)$^-$ and **7**(**N**)$^-$, 9–37 kJ mol^{-1}, suggesting that the difference in the acidities between the phenolic hydroxy group and the amide moiety in the singlet-excited state is much larger than that in the ground state. Thus, the phenolic hydroxy group is the most acidic moiety in ^{1}**2H***.[18] As structural characteristics, the θ values of ^{1}**7**(**O**)$^{-*}$ and ^{1}**7**(**N**)$^{-*}$ are close to 90°, except the θ value of ^{1}**7**(**N**)$^{-*}$ with ε=2.27 (benzene). The C8═O9 and C5–C10 of ^{1}**7**(**O**)$^{-*}$ and ^{1}**7**(**N**)$^{-*}$ show a small difference from those of the corresponding ground states, while their C13–O14 becomes slightly short. The calculated structure of ^{1}**7**(**O**)$^{-*}$ with the perpendicular conformation between the 4-oxidophenyl and the pyrazine moieties is consistent with the twisted structure of ^{1}**3**(**O**)$^{-*}$ (Scheme 7) concluded from the fluorescence observations. This result also indicates that ^{1}**2**(**O**)$^{-*}$ does not have the property of a quinoid form (Scheme 3c).

Table 8 shows the summations of the net atomic charge ($\sum q$) at the four parts of **7**(**O**)$^-$ and ^{1}**7**(**O**)$^{-*}$: the 4-oxidophenyl (**A**), pyrazine (**B**), acetamide (**C**), and benzyl (**D**). The negative charge in **7**(**O**)$^-$ is mainly localized at the 4-oxidophenyl group. On the other hand, the $\sum q$ values at the pyrazine and the acetamido parts of ^{1}**7**(**O**)$^{-*}$ were below −0.86 and −0.13, respectively, indicating that the negative

Table 8. Summations of the net atomic charges ($\sum q$) at the 4-oxidophenyl (A), pyrazine (B), acetamide (C), and benzyl (D) parts of **7**(**O**)$^-$ and ^{1}**7**(**O**)$^{-*}$, calculated with the PM5-COSMO and the PM5-CI-COSMO methods, respectively



7(**O**)$^-$ and ^{1}**7**(**O**)$^{-*}$

Compounds	ε[a]	Summations of the net atomic charges ($\sum q$)			
		A	B	C	D
7(**O**)$^-$	2.27	−0.814	−0.228	−0.082	+0.124
	8.93	−0.898	−0.199	−0.051	+0.148
	35.94	−0.917	−0.203	−0.039	+0.158
	46.45	−0.918	−0.204	−0.037	+0.159
^{1}**7**(**O**)$^{-*}$	2.27	−0.056	−0.863	−0.157	+0.076
	8.93	−0.045	−0.920	−0.137	+0.102
	35.94	−0.034	−0.949	−0.134	+0.117
	46.45	−0.028	−0.957	−0.133	+0.118

[a] Dielectric constants at 25 °C of benzene (2.27), dichloromethane (8.93), acetonitrile (35.94), and DMSO (46.45) from Ref. 25.

Table 7. Calculated properties of **7**(**O**)$^-$ and **7**(**N**)$^-$ in the singlet-excited states (^{1}**7**(**O**)$^{-*}$ and ^{1}**7**(**N**)$^{-*}$) with the PM5-CI-COSMO method

Compounds	ε[a]	ΔH_f[b]/kJ mol^{-1}	θ[c]/°	ϕ[c]/°	r(C═O)[d]/Å	r(C–O)[d]/Å	r(C–C)[d]/Å
^{1}**7**(**O**)$^{-*}$	2.27	−243	90.2	93.8	1.238	1.243	1.461
	8.93	−413	92.3	64.5	1.252	1.259	1.455
	35.94	−470	91.8	49.9	1.254	1.267	1.453
	46.45	−476	91.7	51.0	1.255	1.268	1.452
^{1}**7**(**N**)$^{-*}$	2.27	−84	26.5	44.9	1.250	1.357	1.437
	8.93	−261	92.8	114.6	1.283	1.295	1.453
	35.94	−363	94.1	114.9	1.287	1.291	1.457
	46.45	−371	94.0	115.4	1.287	1.290	1.457

[a] Dielectric constants at 25 °C of benzene (2.27), dichloromethane (8.93), acetonitrile (35.94), and DMSO (46.45) from Ref. 25.
[b] Heat of formation.
[c] The angles θ and ϕ are torsion angles C6–C5–C10–C11 and N1–C2–N7–C8, respectively.
[d] The r(C═O), r(C–O), and r(C–C) are the bond lengths of C8–C9, C13–O14, and C5–C10, respectively.

charge localized at the amidopyrazine moiety in the singlet-excited state. These results predict that the negative charge of **7**(**O**)$^-$ is transferred from the 4-oxidophenyl group to the amidopyrazine moiety upon electronic excitation, and strongly support that ^{1}**2**(**O**)$^{-*}$ is the twisted ICT state (Scheme 7). The amidopyrazine moiety of **2**(**O**)$^-$ is the center of the fluorescent chromophore and has a localized negative charge in the singlet-excited state. Therefore, a solvation of the amidopyrazine moiety of ^{1}**2**(**O**)$^{-*}$ by polar solvent molecules will effectively increase its molecular stability, resulting in a decreased transition energy from ^{1}**2**(**O**)$^{-*}$ to **2**(**O**)$^-$. This causes the solvent-dependent spectral shift of the fluorescence of **2**(**O**)$^-$, which is observed as the steep slopes of the E_{FL}–E_T(30) correlations for [BASE–H$^+$/**3**(**O**)$^-$].

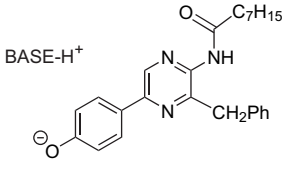
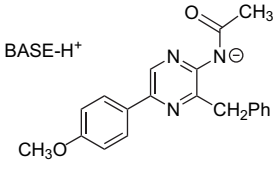
2.5. Bioluminescence light emitter and the reaction mechanism of the calcium-activated photoproteins

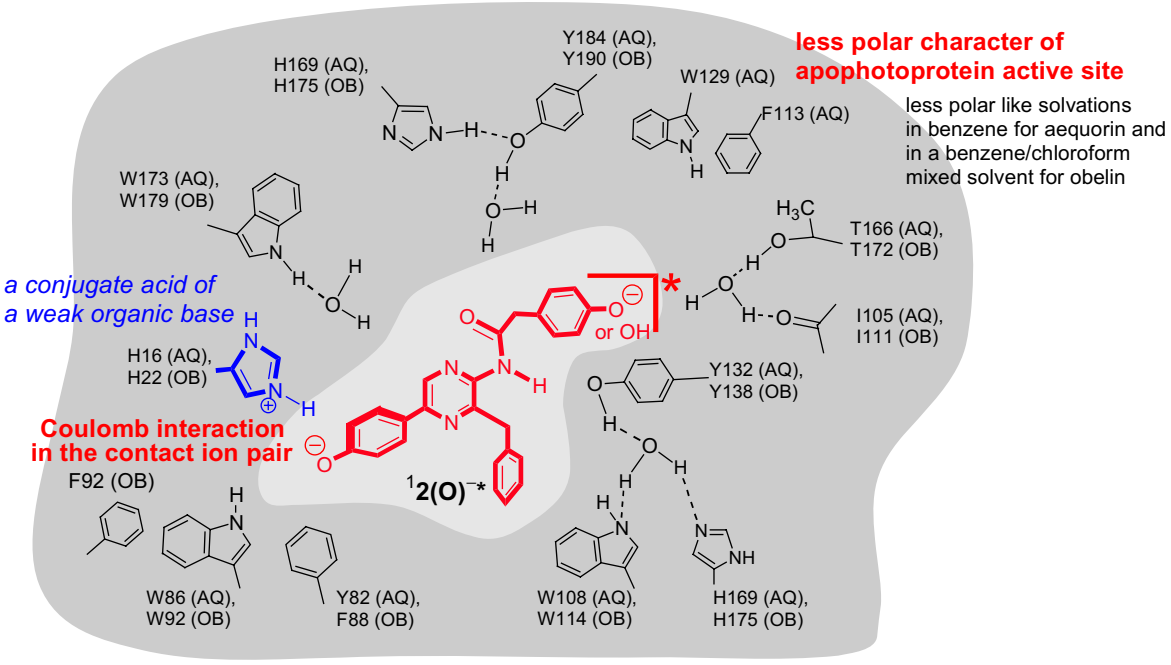
The fluorescent properties of [BASE–H$^+$/**3a**(**O**)$^-$] and [BASE–H$^+$/**5**(**N**)$^-$] are summarized in Table 9. The reported emission maxima (λ_{BL}) of the bioluminescence of aequorin and obelin are in the ranges of 465–470 nm[11a,37] and 485–495 nm[38], respectively. Both ranges are covered by the λ_{FL} range of [BASE–H$^+$/**3a**(**O**)$^-$], 452–615 nm, but not by that of [BASE–H$^+$/**5**(**N**)$^-$], 464–476 nm. The λ_{FL} range of [NBA–H$^+$/**3a**(**O**)$^-$], 452–554 nm, especially covers these λ_{BL} ranges, while that of [TMG–H$^+$/**3a**(**O**)$^-$] and [DBU–H$^+$/**3a**(**O**)$^-$], 486–615 nm, is red shifted compared with the λ_{BL} range of aequorin. These results support the fact that the real ionic structure of the excited coelenteramide in the bioluminescence of aequorin and obelin is ^{1}**2**(**O**)$^{-*}$ in a CIP, with a counter cation composed of a side chain of an amino acid. The large spectral variation in the fluorescence of **3a**(**O**)$^-$ is caused by the molecular stability change of ^{1}**3a**(**O**)$^{-*}$ at the twisted ICT state, depending on the ion pair structure and the solvent polarity.

The X-ray crystal structures of aequorin[6,39] and obelin[7] indicate that their active sites are composed of hydrophobic α-helices and the substrates are surrounded by tryptophans, tyrosines, histidines, and phenylalanines. Furthermore, the imidazolium side chains of His 16 in aequorin and His 22 in obelin are able to be counter cations for the 4-oxidophenyl group of ^{1}**2**(**O**)$^{-*}$. Because the imidazole part of the histidine side chain is a weak organic base (pK_a of the conjugated acid of imidazole: 14.2 in acetonitrile[40]), ^{1}**2**(**O**)$^{-*}$ and the imidazolium cation will make a CIP such as [NBA–H$^+$/^{1}**3**(**O**)$^{-*}$] at the active sites in aequorin and obelin. By applying the E_{FL}–E_T(30) correlation for [NBA–H$^+$/**3a**(**O**)$^-$] to evaluate the λ_{BL} of aequorin bioluminescence (465–470 nm), we can predict that the active site in aequorin has a polarity similar to benzene (E_T(30)=34.3), as predicted in previous reports.[17] The E_{FL}–E_T(30) correlation for [NBA–H$^+$/**3b**(**O**)$^-$] is also useful for evaluating the reported λ_{BL} of a semi-synthetic aequorin, *m*(5)-aequorin, prepared using 5-methylcoelenterazine analogue. The λ_{BL} of *m*(5)-aequorin (438–440 nm)[24] is similar to the λ_{FL} (444 nm) of [NBA–H$^+$/**3b**(**O**)$^-$] in *p*-xylene (E_T(30)=33.1), also supporting the less polar character of the active site in aequorin. The λ_{BL} of obelin bioluminescence (485–495 nm) predicts that the active site has a polarity intermediate between a mixed solvent of benzene/chloroform (20:1) (E_T(30)=36.4) and 1-chloropropane (E_T(30)=37.4). Thus, aequorin and obelin have less polar active sites, where the CIP of ^{1}**2**(**O**)$^{-*}$ and the imidazolium side chain show a high Φ_f. Based on these evaluations and the X-ray crystal structures of aequorin[6,39] and obelin,[7] we can draw a schematic representation of the important interactions of ^{1}**2**(**O**)$^{-*}$ with the active site in aequorin or obelin, shown in Scheme 9.

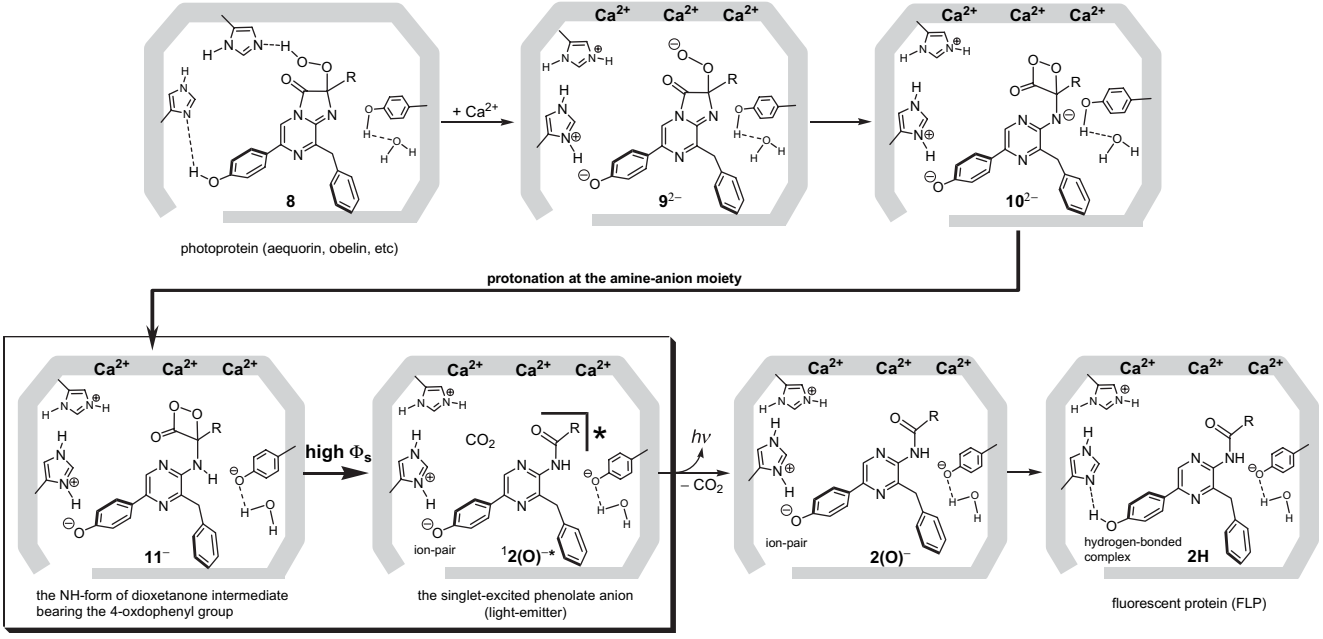
The conclusion that ^{1}**2**(**O**)$^{-*}$ in the CIP is the light emitter in aequorin and obelin bioluminescence leads us to propose a bioluminescence reaction mechanism (Scheme 10) including the active site structures of aequorin[6,39] and obelin.[7] Because ^{1}**2**(**O**)$^{-*}$ in the CIP is the primary excited product, we can propose that the chemiexcitation process is a thermal decomposition of the NH-form of dioxetanone intermediate **11**$^-$ possessing a 4-oxidophenyl group.[41] The chemiexcitation from **11**$^-$ to ^{1}**2**(**O**)$^{-*}$ in the CIP would be an important process to achieve the high quantum yield in the bioluminescence reaction. On the whole, a structural change of the active site due to chelation of Ca^{2+} ions with the EF-hands induces the conversion of coelenterazine 2-hydroperoxide **8** to a reactive peroxide anion (**9**$^{2-}$), followed by a cyclization to an unstable amine anion form of dioxetanone intermediate **10**$^{2-}$. Dioxetanone dianion **10**$^{2-}$ receives a proton from a neighboring 4-hydroxyphenyl group of a tyrosine residue (Y132 and Y138 for aequorin and obelin, respectively) in the active site to give **11**$^-$. Then, the chemiexciation from **11**$^-$ to ^{1}**2**(**O**)$^{-*}$ and the light emission from ^{1}**2**(**O**)$^{-*}$ lead to FLP, which contains the hydrogen-bonded complex

Table 9. Comparison of the fluorescent properties of [BASE–H$^+$/**3a**(**O**)$^-$] and [BASE–H$^+$/**5**(**N**)$^-$]

	 [BASE–H$^+$/**3a**(**O**)$^-$]	 [BASE–H$^+$/**5**(**N**)$^-$]
Character of the singlet-excited state of the anion	Twisted ICT state	Normal π–π* state
Variation of λ_{FL} depending on the solvent polarity (observed λ_{FL} range)	Large (452–615 nm)	Small (464–476 nm)
Variation of λ_{FL} depending on the ion pair structure	Large (SSIP vs CIP)	Small
High Φ_f condition	In a CIP with a conjugate acid of a weak base such as *n*-butylamine in a less polar solvent	0.2 in DMSO



Scheme 9. (AQ=aequorin, OB=obelin).



Scheme 10. R=(4-hydroxyphenyl)methyl or (4-oxidophenyl)methyl.

of **2H** with an imidazole side chain of a histidine residue (H16 and H22 for aequorin and obelin, respectively). Electronic excitation of FLP gives $^1\mathbf{2(O)}^{-*}$ in the CIP with the imidazolium side chain by the process illustrated in Scheme 6a, and shows a fluorescence emission spectrum identical to the bioluminescence spectrum. The molecular mechanism shown in Scheme 10 is common in the bioluminescence system of calcium-activated photoproteins.[1,42]

3. Conclusion

The fluorescent properties of [BASE–H$^+$/**3**(**O**)$^-$] and [BASE–H$^+$/**5**(**N**)$^-$] were systematically investigated to elucidate the ionic structure of the light emitter in aequorin and obelin bioluminescences. The fluorescence of [BASE–H$^+$/**3**(**O**)$^-$] was observed by an electronic excitation of [BASE–H$^+$/**3**(**O**)$^-$] or [BASE···**3H**] in a polar or a less polar solvent (Scheme 6). Because the fluorescence emission from $^1\mathbf{3(O)}^{-*}$ in an ion pair has an ICT nature, the λ_{FL} of [BASE–H$^+$/**3**(**O**)$^-$] was evaluated with linear E_{FL}–E_T(30) correlations. A comparison of the E_{FL}–E_T(30) correlations and the Φ_f values among the ion pairs of **3**(**O**)$^-$ with NBA–H$^+$, TMG–H$^+$, and DBU–H$^+$ clarified that the fluorescence of **3**(**O**)$^-$ was affected by a structural variation of an ion pair: an SSIP for [TMG–H$^+$/^{1}**3**(**O**)$^{-*}$] or [DBU–H$^+$/^{1}**3**(**O**)$^{-*}$], and a CIP for [NBA–H$^+$/^{1}**3**(**O**)$^{-*}$]. [NBA–H$^+$/^{1}**3**(**O**)$^{-*}$]

showed especially steep E_{FL}–$E_T(30)$ correlations and high Φ_f compared with those of [TMG–H^+/^{1}**3**(**O**)$^{-*}$] and [DBU–H^+/^{1}**3**(**O**)$^{-*}$]. Comparison of the fluorescent properties of **3a**(**O**)$^-$ and **3b**(**O**)$^-$ in ion pairs clarified that ^{1}**3**(**O**)$^{-*}$ is the twisted ICT state with a localized negative charge at the amidopyrazine moiety (Scheme 7). This was supported by PM5-CI-COSMO calculations for ^{1}**7**(**O**)$^{-*}$. The fluorescence emission from [BASE–H^+/^{1}**5**(**N**)$^{-*}$] generated by the chemiluminescence reactions of **4** indicates that the fluorescence of [BASE–H^+/**5**(**N**)$^-$] is not significantly affected by interaction with BASE–H^+ in the ion pair or the solvent polarity. These results confirm that ^{1}**2**(**O**)$^{-*}$ in a CIP is the light emitter in the bioluminescence of the representative calcium-activated photoproteins, aequorin and obelin. The E_{FL}–$E_T(30)$ correlations for [NBA–H^+/**3**(**O**)$^-$] were applied to evaluate the λ_{BL} values of aequorin and obelin bioluminescences, suggesting that the active sites of these photoproteins have a polarity similar to a less polar solvent such as benzene. The CIP of ^{1}**2**(**O**)$^{-*}$ with an imidazolium side chain of a histidine residue in the less polar active site is a good state for a high Φ_f of **2**(**O**)$^-$ in apoprotein. The predicted character of the active sites of aequorin and obelin is consistent with their structural characteristics established by X-ray crystallographic analyses of aequorin[6,39] and obelin[7] (Scheme 9). The conclusion that ^{1}**2**(**O**)$^{-*}$ in the CIP is the light emitter indicates that ^{1}**2**(**O**)$^{-*}$ is the primary product of the bioluminescence reaction. Therefore, we propose a bioluminescence reaction mechanism including the chemiexcitation from dioxetanone intermediate **11**$^-$ to ^{1}**2**(**O**)$^{-*}$ (Scheme 10). Further study to establish the reaction mechanism is now in progress in our lab.

4. Experimental

4.1. General

Melting points were obtained with a Yamato MP-21 apparatus and were uncorrected. IR spectra were measured with a Horiba FT-720 spectrometer. Electron ionization (EI) mass spectra were recorded with a JEOL JMS-600 mass spectrometer. High-resolution EI and electro-spray ionization (ESI) mass spectra were recorded with a JEOL HX-110 and a Bruker-Daltonics APEX III mass spectrometer, respectively, at the Research Centre for Giant Molecules, Graduate School of Science, Tohoku University. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL GX-270 instrument (270 and 67.8 MHz, respectively). UV–vis absorption spectra were measured with a Varian Cary 50 spectrophotometer (scan speed, 200 nm min^{-1}; data interval, 0.33 nm). Fluorescence spectra were measured with a JASCO FP-6500 fluorescence spectrophotometer (excitation and emission bandpasses, 3 nm; scan speed, 200 nm min^{-1}) and were corrected according to manufacturer's instructions. Fluorescence quantum yields were determined relative to quinine sulfate in 0.10 mol L^{-1} H_2SO_4 (Φ_F=0.55, λ_{ex} 366 nm) as the standard. Chemiluminescence spectra were measured with an ATTO AB-1850 spectrophotometer. Spectroscopic measurements were made in a quartz cuvette (1 cm path length) at 25±1 °C. Spectral-grade solvents were used for measurements of UV–vis absorption, fluorescence, and chemiluminescence emission spectra. The intensity of the total light (400–700 nm) emitted from a chemiluminescence reaction was monitored using a Hamamatsu R5929 photomultiplier tube powered by a Hamamatsu C4900 power supply. The signal from the photomultiplier was collected on a PC computer and the data were analyzed with the graphic program Igor Pro, Version 4.0.8.0 (Wave Metrics, Inc.). Semi-empirical MO calculations for ground and singlet-excited states were carried out with the PM5 and PM5-CI methods[43] and the COSMO model[36] of CAChe Ver. 5.04 for Windows (Fujitsu Ltd, Tokyo, Japan, 2002). The geometries of ground state molecules were fully optimized by the MM2 calculations before PM5 calculations. Geometries of singlet-excited molecules were optimized using the keywords excited singlet cisd c.i.=4 root=2.

4.2. Preparation of coelenteramide and coelenterazine derivatives

Coelenteramide analogue **3aH** [3-benzyl-5-(4-hydroxyphenyl)-2-octanamidopyrazine] and coelenterazine analogue **4** [8-benzyl-6-(4-hydroxyphenyl)-2-methylimidazo[1,2-*a*]-pyrazin-3(7*H*)-one] were prepared by reported procedures.[16,17b,44] Coelenteramide analogue **3bH** [3-benzyl-5-(4-hydroxyphenyl)-6-methyl-2-octanamidopyrazine] was synthesized from 6-methylpyrazinamine as follows (Scheme 4).

4.2.1. 3,5-Dibromo-6-methylpyrazinamine. To a solution of 6-methylpyrazinamine[45] (0.97 g, 8.8 mmol) in $CHCl_3$ (30 mL) and pyridine (3.5 mL) was added tetra-*n*-butylammonium tribromide (10.5 g, 0.21 mol) under Ar. The reaction mixture was heated under reflux for 2 h. After cooling, the mixture was concentrated under reduced pressure. The residue was diluted with water (100 mL) and the product was extracted with ether (200 mL×4). The ether phase was washed with saturated brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$), yielding 3,5-dibromo-6-methylpyrazinamine (1.10 g, 46%) as pale yellow cubes: mp 167–168 °C; ^{1}H NMR ($CDCl_3$) δ 4.93 (br s, 2H), 2.46 (s, 3H); IR (KBr) 3499, 3292, 3155, 1632, 1540, 1448 cm^{-1}; MS (EI, 70 eV) *m/z* (%) 269 (49), 267 (M^+, 100), 265 (51); HRMS (ESI) calcd for $C_5H_6Br_2N_3$ 265.8928; found 265.8923 ($M+H^+$).

4.2.2. 3-Benzyl-5-bromo-6-methylpyrazinamine. A solution of 3,5-dibromo-6-methylpyrazinamine (114 mg, 0.43 mmol), benzyltri-*n*-butyltin (195 mg, 0.51 mmol) and $PdCl_2(PPh_3)_2$ (15 mg, 5 mol %) in DMF (1 mL) was heated at 130 °C for 2.5 h under Ar. After cooling, the solution was concentrated under reduced pressure. The residue was dissolved in a saturated KF solution in methanol (7 mL) and the mixture was stirred for 1 h at room temperature. The reaction mixture was directly adsorbed on silica gel (5 g) and purified by silica gel column chromatography ($CHCl_3$/ethyl acetate=20:1), yielding 3-benzyl-5-bromo-6-methylpyrazinamine (43 mg, 36%) as pale yellow cubes: mp 94.5–95.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.20–7.36 (m, 5H), 4.28 (br s, 2H), 4.06 (s, 2H), 2.49 (s, 3H); IR (KBr) 3465, 3305, 3165, 1633, 1602, 1533, 1429 cm^{-1}; MS (EI, 70 eV) *m/z* (%) 279 (98), 277 (M^+, 100), 276 (41), 198 (25), 130 (26); HRMS (ESI) calcd for $C_{12}H_{13}BrN_3$ 278.0293; found 278.0287 ($M+H^+$).

4.2.3. 3-Benzyl-5-(4-benzyloxyphenyl)-6-methylpyrazinamine. A solution of 3-benzyl-5-bromo-6-methylpyrazinamine (64 mg, 0.23 mmol), 4-benzyloxyphenylboronic acid (78 mg, 0.34 mmol), and $Pd(PPh_3)_4$ (13 mg, 5 mol %) in 1,4-dioxane (1.2 mL) and 2 mol L^{-1} Na_2CO_3 aqueous solution (1.2 mL) was heated at 110 °C for 2 h under Ar. After cooling, the mixture was diluted with water and the product was extracted with ethyl acetate (20 mL×3). The organic phase was washed with saturated brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/ethyl acetate=5:1), yielding 3-benzyl-5-(4-benzyloxyphenyl)-6-methylpyrazinamine (58 mg, 94%) as pale yellow powder: mp 125.5–126.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.50 (AA′BB′, 2H), 7.21–7.45 (m, 10H), 7.06 (AA′BB′, 2H), 5.13 (s, 2H), 4.29 (br s, 2H), 4.19 (s, 2H), 2.45 (s, 3H); IR (KBr) 3478, 3303, 3168, 1633, 1610, 1449, 1425 cm^{-1}; MS (EI, 70 eV) *m/z* (%) 382 (19), 381 (M^+, 65), 290 (100); HRMS (ESI) calcd for $C_{25}H_{24}N_3O$ 382.1919; found 382.1914 ($M+H^+$).

4.2.4. 3-Benzyl-5-(4-benzyloxyphenyl)-6-methyl-2-octanamidopyrazine. To a solution of 3-benzyl-5-(4-benzyloxyphenyl)-6-methylpyrazinamine (45 mg, 0.12 mmol) and pyridine (0.2 mL) in dichloromethane (2 mL) was added octanoyl chloride (70 μL, 0.42 mmol) at 0 °C under Ar, and allowed to warm up to ambient temperature for 90 min. The reaction was quenched by the addition of saturated $NaHCO_3$ aqueous solution and the product was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/ethyl acetate=10:1), yielding 3-benzyl-5-(4-benzyloxyphenyl)-6-methyl-2-octanamidopyrazine (31 mg, 52%) as colorless powder: mp 156–157 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.54 (AA′BB′, 2H), 7.16–7.48 (m, 10H), 7.07 (AA′BB′, 2H), 5.14 (s, 2H), 4.23 (s, 2H), 2.55 (s, 3H), 2.36 (t, *J*=7.6 Hz, 2H), 1.30 (m, 10H), 0.89 (t, *J*=6.9 Hz, 3H); IR (KBr) 3274, 2926, 2854, 1668, 1608, 1567, 1496 cm^{-1}; MS (EI, 70 eV) *m/z* (%) 508 (38), 507 (M^+, 100), 290 (51); HRMS (ESI) calcd for $C_{33}H_{38}N_3O_2$ 508.2964; found 508.2962 ($M+H^+$).

4.2.5. 3-Benzyl-5-(4-hydroxyphenyl)-6-methyl-2-octanamidopyrazine (3bH). To a solution of 3-benzyl-5-(4-benzyloxyphenyl)-6-methyl-2-octanamidopyrazine (31 mg, 0.062 mmol) in 20 mL of ethanol/ethyl acetate (1:1) was added 12 mg of Pd/C powder. The suspension was stirred at room temperature overnight under H_2 and was filtered through Celite. The filtrate was concentrated in vacuo and the residue was purified by recrystallization, yielding 3-benzyl-5-(4-hydroxyphenyl)-6-methyl-2-octanamidopyrazine (**3bH**) (17 mg, 67%) as colorless cubes: mp 176.5–177 °C; ^{1}H NMR ($CDCl_3$) δ 7.41 (AA′BB′, 2H), 7.16–7.30 (m, 5H), 6.82 (AA′BB′, 2H), 6.12 (br s, 1H), 4.27 (s, 2H), 2.50 (s, 3H), 2.36 (t, *J*=7.8 Hz, 2H), 1.64 (quint, 2H), 1.28–1.31 (m, 8H), 0.89 (t, *J*=8.9 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 173.0 (s), 156.6 (s), 150.5 (s), 147.4 (s), 142.0 (s), 140.3 (s), 138.0 (s), 130.6 (d, 2C), 130.1 (s), 128.7 (d, 2C), 128.6 (d, 2C), 126.7 (d), 115.3 (d), 40.7 (t), 36.7 (t), 31.6 (t), 29.2 (t), 29.0 (t), 25.1 (t), 22.6 (q), 22.4 (t), 14.1 (q); IR (KBr) 3293, 2927, 2854, 1663, 1611, 1569, 1496, 1400 cm^{-1}; MS (EI, 70 eV) *m/z* (%) 418 (30), 417 (M^+, 100), 291 (72), 290 (44); HRMS (EI, 70 eV) calcd for $C_{26}H_{31}N_3O_2$ 417.2416; found 417.2424 (M^+).

4.3. Formation constant *K* of the 1:1 hydrogen-bonded complexes of 3aH with TMG and DBU in benzene

The equilibrium to form the 1:1 hydrogen-bonded complex [BASE···**3aH**] is shown in Scheme 5.[17b] To estimate the *K* of [TMG···**3aH**] and [DBU···**3aH**] in benzene, we measured UV–vis absorption spectra of **3aH** in benzene in the absence and the presence of various concentrations of TMG and DBU, as shown in Figure 5. The concentrations of TMG and DBU were in the ranges of 2.0×10^{-5}–0.050 and 1.0×10^{-5}–0.010 mol L^{-1}, respectively.

The observed spectral changes with clear isosbestic points were analyzed by Eq. 1 below.[46] The value A_0 is absorbance of **3aH** at a chosen wavelength in the absence of BASE. After addition of BASE, absorbance at the chosen wavelength is observed as A_{obs}. The difference between A_{obs} and A_0 is given by

$$A_{obs}-A_0=0.5\Delta\varepsilon\Big([\mathbf{3aH}]+[\mathrm{BASE}]+1/K-\{([\mathbf{3aH}]+[\mathrm{BASE}]+1/K)^2-4[\mathbf{3aH}][\mathrm{BASE}]\}^{1/2}\Big), \quad (1)$$

where [**3aH**] and [BASE] are the initial concentrations of **3aH** and BASE, respectively, and $\Delta\varepsilon$ is the difference in the molar absorptivity between **3aH** and [BASE···**3aH**] at the chosen wavelength. The ($A_{obs}-A_0$) values were plotted against [TMG] and [DBU], as shown in Figure 5B, D. The data were analyzed by a nonlinear least squares curve fitting Eq. 1, giving the *K* values 1430±40 and 7800±400 mol^{-1} L at 25 °C, respectively, accompanied by the $\Delta\varepsilon$ values 6680±40 (350 nm) and 8300±100 (355 nm) mol^{-1} L cm^{-1}, respectively.

4.4. Formation constant *K* of the ion pair of 3b(O)$^-$ with TMG–H$^+$ in DMSO

The formation constant *K* (Scheme 5) of [TMG–H^+/**3b(O)**$^-$] was estimated by an analysis of an absorption spectral change of **3bH** (1.5×10^{-5} mol L^{-1}) in DMSO in the absence and the presence of various concentrations of TMG (2.0×10^{-3}–0.25 mol L^{-1}), as shown in Figure 6.

The observed spectral change gave the differences between A_{obs} and A_0, which were analyzed by Eq. 2:

$$A_{obs}-A_0=0.5\Delta\varepsilon\Big([\mathbf{3bH}]+[\mathrm{TMG}]+1/K-\{([\mathbf{3bH}]+[\mathrm{TMG}]+1/K)^2-4[\mathbf{3aH}][\mathrm{TMG}]\}^{1/2}\Big), \quad (2)$$

where [**3bH**] and [TMG] are the initial concentrations and $\Delta\varepsilon$ is the difference in the molar absorptivity between **3bH** and [TMG–H^+/**3b(O)**$^-$] at a chosen wavelength.[46] The ($A_{obs}-A_0$) values were plotted against [TMG], as shown in Figure 6B, and were analyzed by a nonlinear least squares

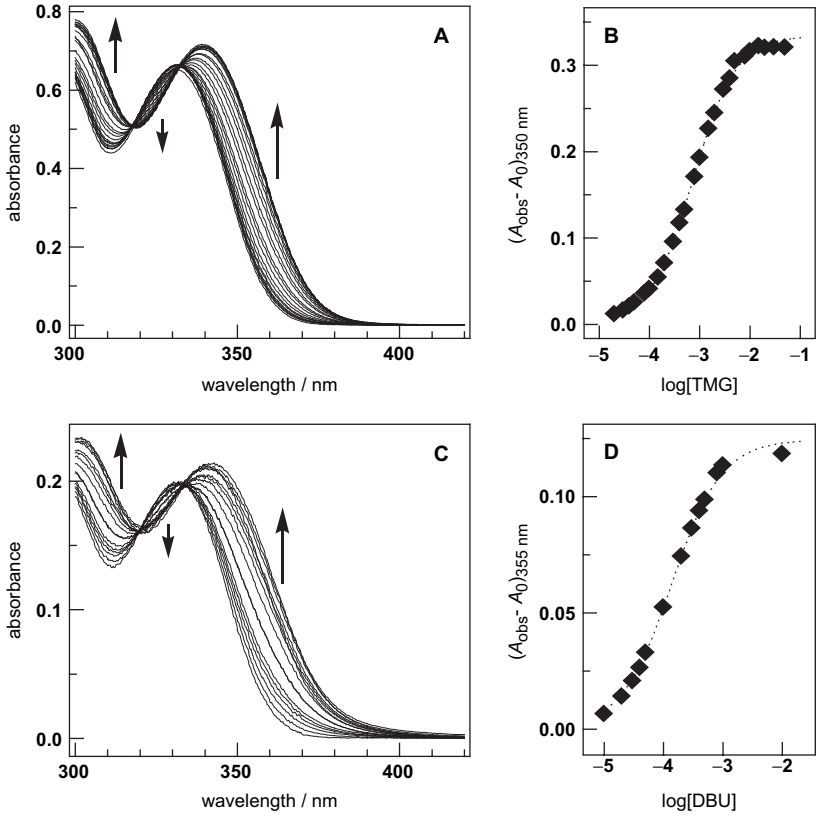


Figure 5. UV–vis absorption spectra of **3aH** in benzene containing various concentrations of TMG (A) and DBU (C) at 25 °C and plots of $(A_{obs}-A_0)$ at 350 nm versus log[TMG] (B) and $(A_{obs}-A_0)$ at 355 nm versus log[DBU] (D) for 1:1 hydrogen-bonded complexation. The initial concentrations of **3aH** were 5.0 and 1.5×10^{-5} mol L^{-1} for A and B, respectively. The concentration ranges of TMG and DBU were 2.0×10^{-5}–0.050 and 1.0×10^{-5}–0.010 mol L^{-1}, respectively. The dotted lines in B and D are the fitted curves corresponding to 1:1 complexation.

curve fitting Eq. 2, giving the K value 28±3 mol^{-1} L at 25 °C, accompanied with the $\Delta\varepsilon$ value 4480±160 (420 nm) mol^{-1} L cm^{-1}.

4.5. Quantum yields of the chemiluminescence reactions of 4

A small portion (10 or 20 μL) of a stock solution of **4** (2.0×10^{-3} mol L^{-1}) in methanol was put in a Pyrex cuvette and the methanol was removed in vacuo. The cuvette containing **4** was placed in a luminometer with a photomultiplier tube and an aerated organic solvent (2.0 mL) was injected to the cuvette at 25±1 °C. The chemiluminescence reactions of **4** were traced by monitoring intensity of the total emitted light. The Φ_{cl} values were determined as values relative to the Φ_{cl} value (0.013) of luminol in DMSO containing *t*-BuOK/*t*-BuOH under air.[47] The experimental errors of Φ_{cl} were within ±10%.

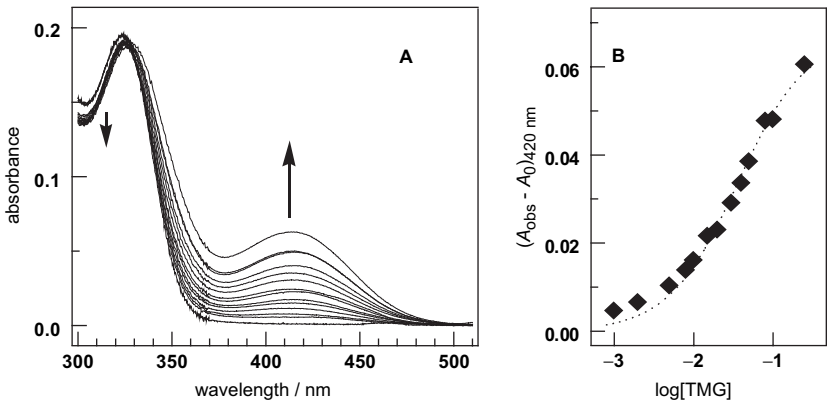


Figure 6. UV–vis absorption spectra of **3bH** (1.5×10^{-5} mol L^{-1}) in DMSO containing various concentrations of TMG (2.0×10^{-3}–0.25 mol L^{-1}) at 25 °C (A) and plot of $(A_{obs}-A_0)$ at 420 nm versus log[TMG] for ion pair generation (B). The dotted line in B is the fitted curve for ion pair generation.

Acknowledgements

We thank Mr Toshiteru Enomoto at ATTO Co. and Dr Yoshihiro Ohmiya at the National Institute of AIST for their excellent technical assistance in measuring chemiluminescence spectra and their kind discussions. We also thank Professors Mamoru Ohashi and Frederick I. Tsuji for their encouragement of this research. This work was supported by grants from the Ministry of Education, Culture, Sports, Science, and Technology [No. 15404009 for H.N. and No. 14050008 (Priority Area No. 417) for H.I.]. H.I. gratefully acknowledges financial supports from the Izumi Science and Technology Foundation and the Shorai Foundation. We thank Professor Minoru Ueda of Tohoku University for his generous considerations.

References and notes

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27. While we previously correlated the E_{FL} of **3a**(**O**)$^-$ in the ion pairs with NBA–H$^+$ and TMG–H$^+$ to $E_T(30)$ for making one plot,[17b] we had to revise the previous one to the E_{FL}–$E_T(30)$ correlations in the text.
28. The λ_{FL} and Φ_f values of **3aH** and/or **3a**(**O**)$^-$ were partially reported in the previous report.[17b] The previous data were collected with a spectrometer older than that used in this report. We carefully reinvestigated the fluorescence behaviors of **3aH** and **3a**(**O**)$^-$ in this work. Then, we revised the λ_{FL} and Φ_f values reported previously.
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41. Very recently, a reaction mechanism similar to ours was proposed based on evaluating the active site structure of FLP of obelin.[7d] Our mechanism contains the chemiexcitation process from dioxetanone intermediate **11**$^-$ possessing a 4-oxidophenyl group to ^{1}**2**(**O**)$^{-*}$, while their mechanism contains the chemiexcitation process from a neutral dioxetanone intermediate to neutral excited molecule ^{1}**2H***. Thus, there is an important difference in the chemiexcitation processes between the two mechanisms.
42. The authors thank one of the referees for his/her valuable comment on the other bioluminescence using coelenterazine as a luciferin. At present our molecular mechanism for calcium-activated photoproteins (Scheme 10) is not applicable to the bioluminescence of a deep-sea shrimp *Oplophorus*, because bisdeoxycoelenterazine, an analogue of coelenterazine, has a bioluminescent activity for the reactions using *Oplophorus* luciferase: Nakamura, H.; Wu, C.; Murai, A.; Inouye, S.; Shimomura, O. *Tetrahedron Lett.* **1997**, *38*, 6405–6406.
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A novel tridentate NHC–Pd(II) complex and its application in the Suzuki and Heck-type cross-coupling reactions

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Received 2 March 2006; revised 17 April 2006; accepted 18 April 2006
Available online 3 May 2006

Abstract—A novel tridentate NHC–Pd(II) complex derived from binaphthyl-2,2′-diamine (BINAM) has been synthesized and its structure has been characterized by single crystal X-ray diffraction. This NHC–Pd(II) complex was fairly effective in Suzuki and Heck-type cross-coupling reactions to give the products in good to excellent yields in most cases.

1. Introduction

Since the isolation and characterization of the stable free *N*-heterocyclic carbene (NHC) by Arduengo and co-workers in 1991,[1] much attention had been paid toward their properties and applications. During the past decade, numerous publications related to their metal complexes and catalytic reactions have been reported in a broad range of reactions.[2] Significantly, a number of Pd–NHC complexes have emerged as effective catalysts for a variety of coupling reactions.[3]

Previously, we reported the synthesis of a novel cis-chelated Pd(II)–NHC complex derived from binaphthyl-2,2′-diamine (BINAM) and a new dimeric bidentated NHC–Pd(II) complex from *trans*-cyclohexane-1,2-diamine and their applications in the Suzuki reaction and Heck reaction.[4] In this paper, we wish to report the synthesis of a novel tridentate NHC–Pd(II) complex derived from BINAM and its application in the Suzuki and Heck-type cross-coupling reactions.

2. Results and discussion

The synthesis of tridentate NHC–Pd(II) complex **4** is shown in Scheme 1. Using BINAM (1.0 equiv) as starting materials to react with *p*-toluenesulfonyl chloride (1.1 equiv) in dichloromethane, 2-(tosylamino)-2′-amino-1,1′-binaphthyl **1** was obtained in 76% yield at room temperature in the presence of pyridine as the base for 5 h. The reaction of **1** with each 2.5 equiv of glyoxal, paraformaldehyde, and ammonium chloride under these conditions reported by Crabtree[5] afforded 2-(tosylamino)-2′-(imidazol-1-yl)-1,1′-binaphthyl **2** in 72% yield. The corresponding imidazolium iodide **3** was obtained in 98% yield by stirring compound **2** with excess of iodomethane in acetonitrile at 80 °C for 5 h. Reaction of the NHC precursor **3** with $Pd(OAc)_2$ under reflux in THF for 6 h afforded the desired NHC–Pd(II) complex **4** in 56% yield. The metal complex was confirmed by elemental analysis, ^1H NMR spectroscopy and EIMS. Its crystal structure was also determined by X-ray diffraction (Fig. 1).[6] The complex is air and moisture stable in the solid state and it is also stable for several days in solution.

Structural features of NHC–Pd(II) complex **4**: The single crystals of this complex suitable for X-ray crystal structure analysis were grown from a saturated solution of PE/EtOAc=3/1. The structure of the compound, $C_{31}H_{24}IN_3O_2PdS$, is refined in space group *P*-1. The bite angle of C–Pd–N is slightly larger than 90° (95.2°). The bond lengths of Pd–C and Pd–N are 1.939(4) and 2.017(3) Å, respectively. The crystal structure shows that the O atom of sulfonyl group participates in chelating with Pd center. This is a tridentate NHC–Pd(II) complex. The bond distance of Pd–O is 2.219(3) Å. The angle of N–Pd–I is slightly less than 180° (170.6°), indicating that these three atoms extending as far as possible, which are almost on the same line. While the coordinated atoms of C (carbene), N, O, and I with Pd are effectively planar, with the maximum deviation form the best-squares plane of 0.0200 Å for atom Pd, and the mean deviation is 0.0080 Å.

Keywords: Tridentate NHC–Pd(II) complex; Binaphthyl-2,2′-diamine; Cross-coupling reactions; Suzuki–Miyaura cross-coupling reaction; Heck reaction.
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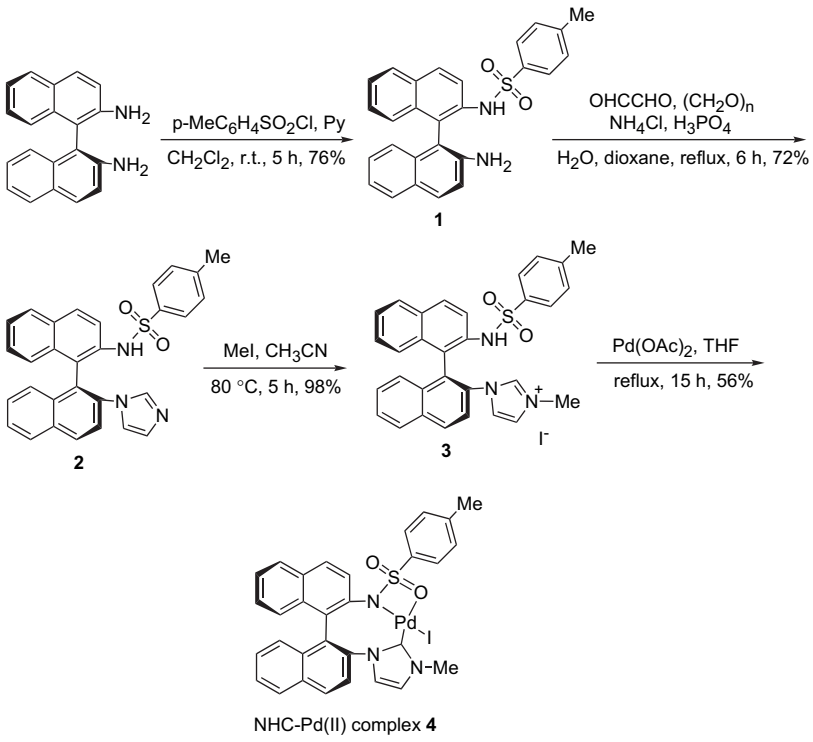
doi:10.1016/j.tet.2006.04.034



Scheme 1. Synthesis of the NHC–Pd(II) complex **4**.

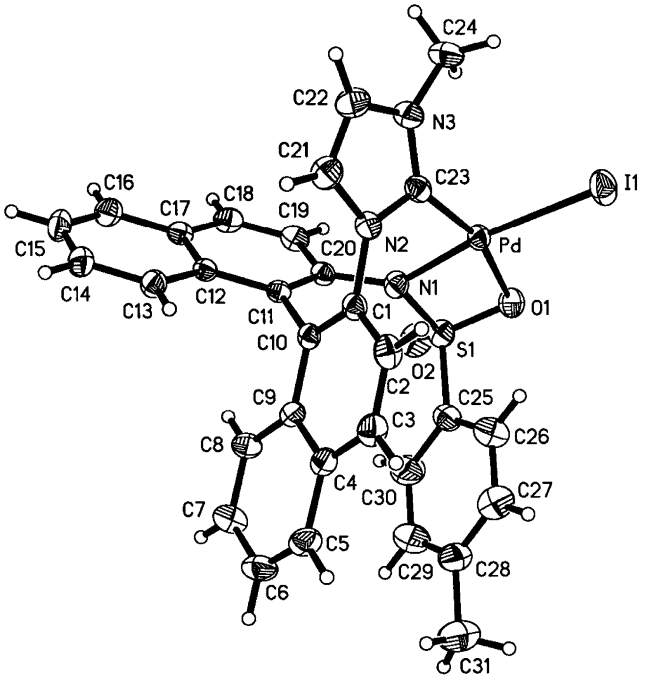
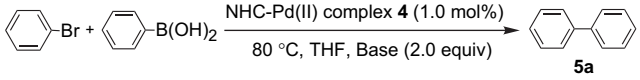


Figure 1. ORTEP drawing of NHC–Pd(II) complex **4**.

The application of NHC–Pd(II) complex **4** as a catalyst for the Suzuki–Miyaura cross-coupling reaction was examined where both base and solvent effects were carefully examined in the reaction of phenylboronic acid with bromobenzene under argon atmosphere. The results are summarized in Tables 1 and 2, respectively. We found that using Cs_2CO_3 as the base in tetrahydrofuran (THF) at 80 °C gave the coupled product biphenyl **5a** in 98% yield after 12 h (Table 1, entry 3). The other inorganic bases such as Na_2CO_3, K_2CO_3, $K_3PO_4\cdot 3H_2O$, and KO^{t}Bu were not as effective as Cs_2CO_3, only afforded moderate to low yields of coupling products (Table 1, entries 1, 2, 5, and 6). When $KF\cdot 2H_2O$, NaOAc, and DMAP were employed as the bases, no reaction occurred (Table 1, entries 4, 7, and 8). Then, we examined a variety of solvents at 80 °C with Cs_2CO_3 as the base. We found that using THF as the solvent gave the highest yield in 98% (Table 2, entries 1–8). Thus, Cs_2CO_3 was the base of choice and THF was the preferred solvent for this reaction.

Table 1. Screening for bases in the NHC–Pd(II) complex **4** catalyzed Suzuki cross-coupling reaction of bromobenzene (1.0 mmol) with phenylboronic acid (1.2 mmol) in THF (2.0 mL)

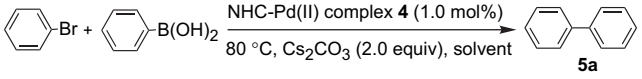


Entry	Base	Time (h)	Yield (%)[a]
			5a
1	Na_2CO_3	12	6
2	K_2CO_3	12	24
3	Cs_2CO_3	12	98
4	$KF\cdot 2H_2O$	12	N.R[b]
5	$K_3PO_4\cdot 3H_2O$	12	53
6	KO^{t}Bu	12	13
7	NaOAc	12	N.R[b]
8	DMAP	12	N.R[b]

[a] Isolated yields.
[b] No reaction occurred.

Using these optimized reaction conditions, the Suzuki–Miyaura reaction of a variety of aryl halides, including aryl bromides and phenyl iodide, with phenylboronic acid

Table 2. Screening for solvents in the NHC–Pd(II) complex **4** catalyzed Suzuki cross-coupling reaction of bromobenzene (1.0 mmol) with phenylboronic acid (1.2 mmol) in various solvents (2.0 mL)



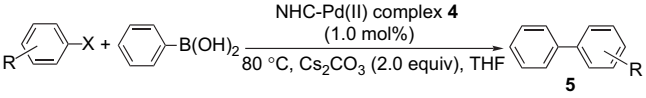
Entry	Solvent	Time (h)	Yield (%)[a]
			5a
1	THF	12	98
2	DMSO	12	24
3	DMF	12	55
4	DMA	12	67
5	Dioxane	12	96
6	$ClCH_2CH_2Cl$	12	21
7	CH_3CN	12	19
8	$PhCH_3$	12	54

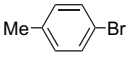
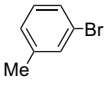
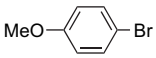
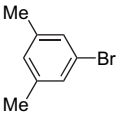
[a] Isolated yields.

was examined. The results are summarized in Table 3. As can be seen, aryl bromides and phenyl iodide afforded coupling products **5** in 71–90% yields within 12 h (Table 3, entries 1–6).

Heck reaction was examined in *N*,*N*-dimethylacetamide (DMA) by the reaction of bromobenzene with butyl acrylate in the presence of various bases (Table 4, entries 1–6). We found that Na_2CO_3 afforded the best results for this reaction and allowed the coupling product **6a** to be obtained in 62% under argon atmosphere at 160 °C after 18 h (Table 4, entry 1). In other organic solvents, the reactions were sluggish. Adding 1.0 and 20 mol % Bu_4NBr to the reaction system,

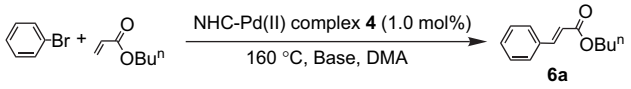
Table 3. NHC–Pd(II) complex **4** catalyzed Suzuki cross-coupling reaction between aryl halides (1.0 mmol) and phenylboronic acid (1.2 mmol) under optimized conditions



Entry	Ar-X	Time (h)	Yield (%)[a]
			5
1		12	**5b**, 74
2		12	**5c**, 71
3		12	**5d**, 90
4		12	**5e**, 81
5		12	**5f**, 72
6		12	**5a**, 88

[a] Isolated yields.

Table 4. NHC–Pd(II) complex **4** (1.0 mol %) catalyzed Heck coupling reaction of bromobenzene (1.0 equiv) with butyl acrylate (1.5 equiv)



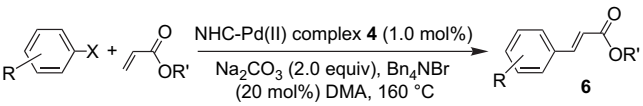
Entry	Base	Time (h)	Yield (%)[a]
			6a
1	Na_2CO_3	18	62
2	K_2CO_3	18	26
3	Cs_2CO_3	18	36
4	$KF\cdot 2H_2O$	18	21
5	$K_3PO_4\cdot 3H_2O$	18	33
6	NaOAc	18	24
7	Na_2CO_3[b]	18	84
8	Na_2CO_3[c]	18	97

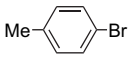
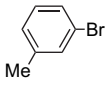
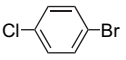
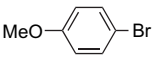
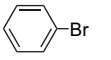
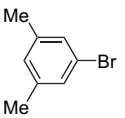
[a] Isolated yields.
[b] Bn_4NBr (1.0 mol %) was added.
[c] Bn_4NBr (20 mol %) was added.

the yields of **6a** were improved to 84 and 97%, respectively (Table 4, entries 7 and 8).

Using these reaction conditions, we next examined the Heck cross-coupling reaction of a variety of aryl halides with butyl and methyl acrylate. The results are summarized in Table 5. We found that the Heck reaction products **6** were obtained in good to high yields in most cases under argon atmosphere (Table 5, entries 1–3, 6, and 7). For electron-rich 4-bromoanisole, the reaction was sluggish and product **6e** was obtained in only 19% yield under the same conditions

Table 5. NHC–Pd(II) complex **4** (1.0 mol %) catalyzed Heck coupling reaction of aryl halides (1.0 equiv) with butyl acrylate (1.5 equiv)



Entry	Ar-X	R′	Time (h)	Yield (%)[a]
				6
1		Bu^n	18	**6b**, 81
2		Bu^n	18	**6c**, 91
3		Bu^n	18	**6d**, 87
4		Bu^n	18	**6e**, 19
5		Me	18	**6f**, 48
6		Bu^n	18	**6g**, 86
7		Bu^n	18	**6a**, 99

[a] Isolated yields.

(Table 5, entry 4). For methyl acrylate, the coupled product **6f** was obtained in moderate yield of 48% (Table 5, entry 5). Product conformations were determined by ^{1}H NMR spectroscopy (see Supplementary data).

In conclusion, we disclosed a novel tridentate NHC–Pd(II) complex **4** as an effective catalyst for Suzuki–Miyaura reaction and Heck cross-coupling reaction. The corresponding coupled products were obtained in good to high yields in most cases by this novel Pd(II)-catalyst under normal conditions. Efforts are underway to elucidate the mechanistic details of this C–C bond forming reaction catalyzed by Pd(II)–NHC complex and the use of **4** to catalyze other C–C bond forming transformations.

3. Experimental

3.1. General

The synthesis of ligands was performed in untreated solvents under ambient atmosphere. The preparation of Pd complex was performed under argon atmosphere. THF and toluene were distilled from sodium (Na) under argon (Ar) atmosphere; CH_3CN and 1,2-dichloroethane were distilled from CaH_2 under argon (Ar) atmosphere. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-300 spectrometer for solution in $CDCl_3$ with tetramethylsilane (TMS) as internal standard; *J*-values are in hertz. Mass spectra were recorded by EI, and HRMS was measured on a Finnigan MA$^+$ mass spectrometer. All of the solid compounds reported in this paper gave satisfactory carbon, hydrogen, and nitrogen microanalyses with a Carlo-Erba 1106 analyzer [**3** was characterized by high-resolution mass spectrometry (HRMS)]. Commercially obtained reagents were used without further purification. All reactions were monitored by thin layer chromatography with silica gel coated plates. Flash column chromatography was carried out using 300–400 mesh silica gel at increased pressure.

3.1.1. Synthesis of 2-(tosylamino)-2′-amino-1,1′-binaphthyl 1. To a mixture of 2,2′-diamino-1,1′-binaphthalene (a racemic compound, 569 mg, 2.0 mmol) and pyridine (2.0 mL, 24 mmol) in CH_2Cl_2 (15 mL) was added dropwise *p*-toluenesulfonyl chloride solution (420 mg, 2.2 mmol in 5.0 mL of CH_2Cl_2). The mixture was stirred at room temperature for 5 h. The reaction mixture was washed with 5% HCl and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by a silica gel flash column chromatography (eluent: PE/EtOAc=6/1) to give compound **1** as a white solid (667 mg, 76%). Mp 169–170 °C; IR (CH_2Cl_2): ν 3365, 3051, 1621, 1595, 1403, 1316, 1164, 1091, 977, 814, 666 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.31 (3H, s, CH_3), 3.30 (2H, br, NH_2), 6.41 (1H, d, J=8.1 Hz, ArH), 6.70 (1H, s, NH), 6.93–7.10 (5H, m, ArH), 7.19–7.24 (2H, m, ArH), 7.36–7.42 (3H, m, ArH), 7.77 (1H, d, J=8.1 Hz, ArH), 7.85 (2H, t, J=9.0 Hz, ArH), 8.03 (2H, dd, J_1=9.0 Hz, J_2=49.2 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 21.4, 117.9, 119.4, 121.5, 122.4, 123.2, 125.3, 125.6, 127.0, 127.1, 128.0, 128.1, 129.4, 129.7, 130.1, 131.2, 132.6, 136.0, 133.4, 133.5, 142.5, 143.6; EIMS *m*/*z* (%): 438 (75.20) [M$^+$], 283 (100), 267 (96.03), 91 (16.90); Anal. Calcd for $C_{27}H_{22}N_2O_2S$ requires: C, 73.95, H, 5.06, N, 6.39%. Found: C, 73.84, H, 5.00, N, 6.25%.

3.1.2. Synthesis of 2-(tosylamino)-2′-(imidazol-1-yl)-1,1′-binaphthyl 2. Compound **1** (876 mg, 2.0 mmol) and one drop of concentrated H_3PO_4 was added to 10 mL of de-ionized water. Then 40% aqueous glyoxal (726 mg, 5 mmol) and paraformaldehyde (150 mg, 5 mmol) as well as 10 mL of dioxane were added. The mixture was heated with stirring to 80 °C, ammonium chloride (267 mg, 5 mmol) was added and then the temperature of oil bath was elevated to 100 °C. After 6 h, the reaction mixture was cooled and a saturated aqueous solution of K_2CO_3 (20 mL) was added, and the mixture was extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by a silica gel flash column chromatography (eluent: PE/EtOAc=1/1–0/1) to give compound **2** as a white solid (704 mg, 72%). Mp 244–245 °C; IR (CH_2Cl_2): ν 3259, 3059, 2733, 1911, 1621, 1510, 1158, 1092, 981, 869, 523 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.35 (3H, s, CH_3), 6.35 (1H, br, CH), 6.70 (1H, s, NH), 6.75–6.76 (2H, m, CH), 6.88 (1H, d, J=8.7 Hz, ArH), 7.11–7.27 (5H, m, ArH), 7.33–7.40 (3H, m, ArH), 7.53–7.62 (2H, m, ArH), 7.80 (1H, d, J=7.5 Hz, ArH), 7.85–7.93 (2H, m, ArH), 8.00 (1H, d, J=8.4 Hz, ArH), 8.15 (1H, d, J=9.0 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 21.5, 117.6, 119.5, 119.7, 124.0, 124.6, 125.2, 125.7, 125.8, 127.0, 127.1, 127.5, 128.0, 128.3, 128.4, 128.0, 127.7, 130.3, 130.4, 131.3, 132.5, 132.9, 133.0, 133.3, 135.0, 136.0, 136.9, 144.0; EIMS *m*/*z* (%): 489 (30.77) [M$^+$], 421 (13.21), 334 (100), 266 (36.91), 91 (11.86); Anal. Calcd for $C_{30}H_{23}N_3O_2S$ requires: C, 73.60, H, 4.74, N, 8.58%. Found: C, 73.41, H, 4.60, N, 8.56%.

3.1.3. Synthesis of 2-(tosylamino)-2′-(3-methylimidazolium-1-yl)-1,1′-binaphthyl iodide 3. A mixture of **2** (98 mg, 0.2 mmol) and iodomethane (0.2 mL) were stirred in acetonitrile (5.0 mL) at 80 °C for 5 h. The volatiles were removed to give the crude product **3** as a yellow solid (124 mg, 98%). Mp 151–153 °C; IR (CH_2Cl_2): ν 3045, 2956, 2855, 1655, 1595, 1508, 1321, 1161, 1092, 816, 733, 550 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ 2.38 (3H, s, CH_3), 3.97 (3H, s, CH_3), 6.47 (1H, s, NH), 6.93 (1H, d, J=8.4 Hz, ArH), 7.01–7.06 (2H, m, CH), 7.15 (2H, d, J=8.1 Hz, ArH), 7.27–7.32 (2H, m, ArH), 7.38–7.45 (3H, m, ArH), 7.52 (2H, d, J=8.1 Hz, ArH), 7.59 (1H, t, J=7.8 Hz, ArH), 7.80–7.84 (2H, m, ArH), 7.97 (1H, d, J=8.4 Hz, ArH), 8.09 (2H, q, J=8.7 Hz, ArH), 9.48 (1H, s, CH); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ 21.3, 37.5, 121.3, 121.8, 122.7, 122.9, 123.2, 124.5, 125.9, 126.6, 126.7, 127.7, 127.8, 127.9, 128.2, 128.3, 128.4, 129.4, 130.5, 130.6, 131.3, 131.4, 132.3, 132.4, 133.3, 133.4, 136.1, 136.6, 143.6; HRMS (ESI) Calcd for $C_{31}H_{26}N_3O_2S$ (M$^+$−I) requires: 504.1746, Found: 504.1746.

3.1.4. Synthesis of NHC–Pd(II) complex 4. The compound **3** (126 mg, 0.2 mmol) and $Pd(OAc)_2$ (44.8 mg, 0.2 mmol) was refluxed in THF (10 mL) for 12 h. The volatiles were then removed under reduced pressure and the residue was purified by a silica gel flash column chromatography (eluent: PE/EtOAc=2/1) to give **4** as a red solid (82 mg, 56%). A single crystal suitable for X-ray crystal analysis was obtained by recrystallization from a saturated solution of PE/EtOAc=3/1. Mp >250 °C; IR (CH_2Cl_2): ν 3057,

2923, 1592, 1460, 1381, 1109, 1030, 878, 680, 553 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, TMS): δ 2.12 (3H, s, Me), 3.94 (3H, s, Me), 6.21 (1H, d, J=8.7 Hz, ArH), 6.46 (2H, d, J=8.4 Hz, CH), 6.68–6.76 (3H, m, ArH), 6.84 (1H, d, J=2.1 Hz, ArH), 7.07–7.09 (1H, m, ArH), 7.16 (2H, d, J=8.1 Hz, ArH), 7.30 (1H, d, J=7.2 Hz), 7.43 (1H, t, J=7.8 Hz, ArH), 7.68 (1H, d, J=8.4 Hz, ArH), 7.75 (2H, d, J=8.7 Hz, ArH), 7.86 (1H, d, J=8.7 Hz, ArH), 7.96 (1H, d, J=8.1 Hz, ArH), 8.19 (1H, d, J=8.7 Hz, ArH); EIMS m/z (%): 735 (1.66) [M^+], 579 (6.51), 502 (8.28), 438 (15.37), 422 (17.26), 347 (41.10), 332 (100), 278 (58.45), 91 (22.04); Anal. Calcd for $C_{31}H_{24}IN_3O_2PdS$ requires: C, 50.59, H, 3.29, N, 5.71%. Found: C, 50.76, H, 3.10, N, 5.52%.

3.2. General procedure for the Suzuki cross-coupling reaction of aryl halides with boronic acids

A typical procedure is given below for the reaction expressed in entry 3 of Table 1. An oven-dried Schlenk flask was evacuated and filled with argon (3 cycles), then charged with NHC–Pd(II) complex **4** (7.3 mg, 0.01 mmol), cesium carbonate (650 mg, 2.0 mmol), benzene bromide (105 μL, 1.0 mmol), phenylboronic acid (146 mg, 1.2 mmol), and THF (2.0 mL). The mixture was stirred at 80 °C for 12 h. The reaction mixture was diluted with H_2O (10 mL) and CH_2Cl_2 (10 mL), followed by extraction twice with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure to give crude product. The pure product was isolated by column chromatography on silica gel (eluent: petroleum ether) to give biphenyl (151 mg, 98%) as a white solid, which was analyzed by ^1H NMR spectroscopy.

3.2.1. Compound 5a. A white solid; ^1H NMR (300 MHz, $CDCl_3$, TMS): δ 7.37 (2H, m, ArH), 7.48 (4H, m, ArH), 7.65 (4H, m, ArH).

3.2.2. Compound 5b. A white solid; ^1H NMR (300 MHz, $CDCl_3$, TMS): δ 2.46 (3H, s, CH_3), 7.31–7.63 (2H, m, ArH), 7.36–7.41 (1H, m, ArH), 7.47–7.52 (2H, m, ArH), 7.56–7.58 (2H, m, ArH), 7.64–7.67 (2H, m, ArH).

3.2.3. Compound 5c. A colorless liquid; ^1H NMR (300 MHz, $CDCl_3$, TMS): δ 2.44 (3H, s, CH_3), 7.17–7.69 (1H, m, ArH), 7.32–7.47 (6H, m, ArH), 7.59–7.62 (2H, m, ArH).

3.2.4. Compound 5d. A white solid; ^1H NMR (300 MHz, $CDCl_3$, TMS): δ 7.37–7.60 (9H, m, ArH).

3.2.5. Compound 5e. A white solid; ^1H NMR (300 MHz, $CDCl_3$, TMS): δ 3.86 (3H, s, OCH_3), 6.98–7.01 (2H, m, ArH), 7.32–7.35 (1H, m, ArH), 7.41–7.46 (2H, m, ArH), 7.53–7.59 (4H, m, ArH).

3.2.6. Compound 5f. A white solid; ^1H NMR (300 MHz, $CDCl_3$, TMS): δ 2.39 (6H, s, CH_3), 7.01 (1H, s, ArH), 7.22 (2H, s, ArH), 7.30–7.36 (1H, m, ArH), 7.40–7.45 (2H, m, ArH), 7.57–7.60 (2H, m, ArH).

3.3. Typical reaction procedure for Heck reaction

A typical procedure is given below for the reaction expressed in entry 8 of Table 4. An oven-dried Schlenk flask was evacuated and filled with argon (3 cycles), then charged with aryl halide (1.0 mmol), butyl acrylate (1.5 mmol), sodium carbonate (212 mg, 2.0 mmol), cetyltrimethylammonium bromide (64.4 mg, 0.2 mmol), *N*,*N*-dimethylacetamide (DMA, 2.0 mL), and NHC–Pd(II) complex **4** (7.3 mg, 0.01 mmol). The reaction mixture was stirred at 160 °C for 18 h. The reaction mixture was diluted with H_2O (15 mL) and Et_2O (15 mL), followed by extraction twice with Et_2O. The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure to give crude product. A pure product (198 mg, 97%) was isolated by column chromatography (eluent: PE/EtOAc=30/1) on silica gel. The purified product was analyzed by ^1H NMR spectroscopy.

3.3.1. Compound 6a. A yellow liquid; ^1H NMR (300 MHz, $CDCl_3$, TMS): δ 0.97 (3H, t, J=7.2 Hz, CH_3), 1.40–1.48 (2H, m, CH_2), 1.65–1.72 (2H, m, CH_2), 4.21 (2H, t, J=6.6 Hz, OCH_2), 6.45 (1H, d, J=15.9 Hz, =CH), 7.37–7.40 (3H, m, ArH), 7.51–7.54 (2H, m, ArH), 7.69 (1H, d, J=15.9 Hz, =CH).

3.3.2. Compound 6b. A yellow liquid; ^1H NMR (300 MHz, $CDCl_3$, TMS): δ 0.96 (3H, t, J=7.2 Hz, CH_3), 1.40–1.47 (2H, m, CH_2), 1.63–1.71 (2H, m, CH_2), 2.37 (3H, s, CH_3), 4.20 (2H, t, J=6.9 Hz, OCH_2), 6.40 (1H, d, J=16.2 Hz, =CH), 7.18–7.44 (4H, m, ArH), 7.66 (1H, d, J=16.2 Hz, =CH).

3.3.3. Compound 6c. A yellow liquid; ^1H NMR (300 MHz, $CDCl_3$, TMS): δ 0.93 (3H, t, J=7.2 Hz, CH_3), 1.37–1.44 (2H, m, CH_2), 1.61–1.68 (2H, m, CH_2), 2.33 (3H, s, CH_3), 4.17 (2H, t, J=6.9 Hz, OCH_2), 6.40 (1H, d, J=16.2 Hz, =CH), 7.14–7.17 (1H, m, ArH), 7.21–7.24 (1H, m, ArH), 7.28–7.30 (2H, m, ArH), 7.62 (1H, d, J=16.2 Hz, =CH).

3.3.4. Compound 6d. A white solid; ^1H NMR (300 MHz, $CDCl_3$, TMS): δ 0.96 (3H, t, J=7.5 Hz, CH_3), 1.35–1.51 (2H, m, CH_2), 1.63–1.73 (2H, m, CH_2), 4.20 (2H, t, J=6.3 Hz, OCH_2), 6.41 (1H, d, J=15.9 Hz, =CH), 7.33–7.46 (4H, m, ArH), 7.62 (1H, d, J=15.9 Hz, =CH).

3.3.5. Compound 6e. A yellow liquid; ^1H NMR (300 MHz, $CDCl_3$, TMS): δ 0.96 (3H, t, J=7.2 Hz, CH_3), 1.37–1.49 (2H, m, CH_2), 1.63–1.73 (2H, m, CH_2), 3.83 (3H, s, OCH_3), 4.19 (2H, t, J=6.6 Hz, OCH_2), 6.31 (1H, d, J=15.9 Hz, =CH), 6.89–7.47 (4H, m, ArH), 7.64 (1H, d, J=15.9 Hz, =CH).

3.3.6. Compound 6f. A yellow liquid; ^1H NMR (300 MHz, $CDCl_3$, TMS): δ 3.81 (3H, s, OCH_3), 6.45 (1H, d, J=16.2 Hz, =CH), 7.37–7.40 (3H, m, ArH), 7.51–7.54 (2H, m, ArH), 7.70 (1H, d, J=16.2 Hz, =CH).

3.3.7. Compound 6g. A yellow liquid; ^1H NMR (300 MHz, $CDCl_3$, TMS): δ 0.96 (3H, t, J=7.5 Hz, CH_3), 1.38–1.50 (2H, m, CH_2), 1.64–1.73 (2H, m, CH_2), 2.33 (6H, s, CH_3), 4.20 (2H, t, J=6.9 Hz, OCH_2), 6.41 (1H, d, J=15.9 Hz, =CH), 7.02 (1H, s, ArH), 7.15 (2H, s, ArH), 7.62 (1H, d, J=15.9 Hz, =CH).

Acknowledgements

We thank the State Key Project of Basic Research (Project 973) (No. G2000048007), Shanghai Municipal Committee

of Science and Technology (04JC14083), and the National Natural Science Foundation of China (203900502, 20472096 and 20272069) for financial support.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.04.034.

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6. The crystal data of complex **4** has been deposited in CCDC with number 290551. Empirical formula: $C_{31}H_{24}IN_3O_2PdS$; formula weight: 735.89; crystal color, Habit: colorless, prismatic; crystal dimensions: 0.506×0.345×0.133 mm; crystal system: triclinic; lattice type: primitive; lattice parameters: a=9.9182(6) Å, b=12.4974(8) Å, c=13.4366(9) Å, α=114.8630(10)°, β=105.8190(10)°, γ=90.1450(10)°, V=1440.74(16) Å^3; space group: P-1; Z=2; D_{calcd}=1.696 g/cm^3; F_{000}=724; diffractometer: Rigaku AFC7R; residuals: R; Rw: 0.0461, 0.1268.

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Tetrahedron 62 (2006) 6295–6302
Tetrahedron

Metallo-phosphorylation of alkenes: a highly regioselective reaction of zirconocene–alkene complexes with chlorophosphate

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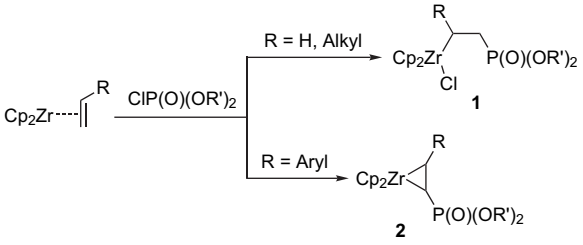
Received 27 January 2006; revised 12 April 2006; accepted 17 April 2006
Available online 8 May 2006

Abstract—Zirconocene–alkene complexes $Cp_2Zr(CH_2{=}CHR)$ reacted with chlorophosphate to form zircono-ethylphosphonate with high regioselectivity, which is versatile and could be converted into various functionalized organophosphonates.

1. Introduction

Phosphorylation of unsaturated substrates is an attractive reaction for the synthesis of organophosphonates $RP(O)(OR')_2$ that are useful intermediates in organic synthesis.[1] Particularly interesting and challenging in the reaction are the simultaneous introduction of phosphonate and other functional groups to multiple carbon–carbon bonds.[2] The metallo-phosphorylation is especially interesting, as it is a more versatile and elegant synthetic elaboration, although hydrophosphinylation of alkenes,[3] and hydrophosphorylation of alkenes,[4] and hydrophosphorylation of allenes[5] have been reported.

Zirconocene–alkene complexes have been attractive compounds in organic synthesis since they can be easily prepared by several methods such as (1) addition of alkenes to Cp_2ZrBu_2 (Negishi reagent),[6] (2) a β-hydrogen abstraction and an elimination of alkanes from zirconocenedialkyls,[7] (3) addition of alkenes to $Cp_2Zr(PMe_3)_2$,[8] and (4) replacement of alkenes in zirconocene–alkene complexes $Cp_2Zr(alkene)(PR_3)$.[9] A number of reactions of zirconocene–alkene complexes with unsaturated substrates such as alkenes[10] or aldehydes or ketones[11] have been studied. Recently, we reported a metallo-phosphorylation of ethylene based on the reaction of zirconocene–ethylene complex $Cp_2Zr(CH_2{=}CH_2)$ with chlorophosphate.[12] In the course of further investigations, we found a highly regioselective reaction of zirconocene–alkene complexes $Cp_2Zr(CH_2{=}CHR)$ with chlorophosphate to form zircono-ethylphosphonate **1** or three-membered zirconacycle **2** (Scheme 1). This result is useful for the preparation of variously functionalized organophosphonates $RP(O)(OR')_2$.



Scheme 1.

2. Results and discussion

To a solution of zirconocene–butene complex $Cp_2Zr(CH_2{=}CHEt)$,[6] generated by the reaction of Cp_2ZrCl_2 with 2 equiv of *n*-BuLi in THF, was added 1 equiv of diethyl chlorophosphate. The reaction mixture was kept at 0 °C for 6 h, and then it was quenched with 3 N HCl. Purification of crude product was carried out by column chromatography on silica gel. Diethyl butylphosphonate **3a** was exclusively obtained in ^{31}P NMR yield of 65% (isolated yield 58%) with excellent regioselectivity. Deuteriolysis and iodinolysis of the reaction mixture afforded deuterated compound **4a** in 57% yield with 95% deuterium incorporation and iodinated product **5a** in 51% yield, respectively (Scheme 2). The formation of **4a** and **5a** indicated that zirconium-containing complex **1a**

Keywords: Organophosphonate; Alkenes; Metallo-phosphorylation; Regioselectivity; Zirconocene–alkene.
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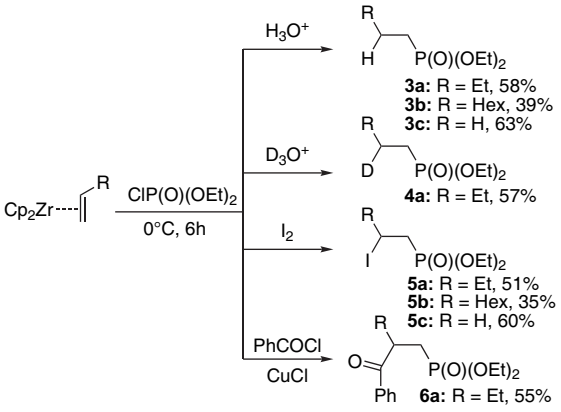
doi:10.1016/j.tet.2006.04.048

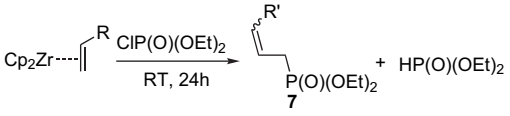
(R=Et) was formed as an intermediate. Moreover, addition of acyl chloride to the reaction mixture in the presence of CuCl afforded compound **6a** in 55% isolated yield, in which a new carbon–carbon bond was formed.



Scheme 2.

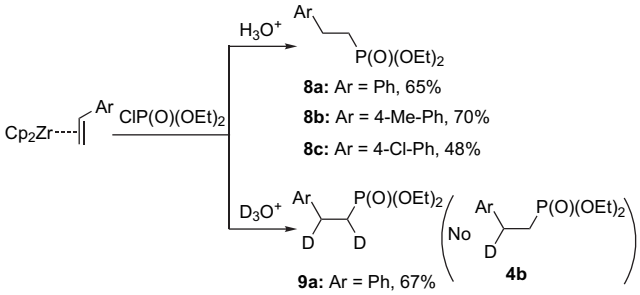
Similar types of products **3b**, **5b**, **3c**, and **5c** were obtained for reaction of zirconocene–octene complex $Cp_2Zr(CH_2{=}CHHex)$ or zirconocene–ethylene $Cp_2Zr(CH_2{=}CH_2)$[13] and chlorophosphate.

Moreover, when the reaction mixture of zirconocene–butene or zirconocene–octene complex with chlorophosphate was warmed to room temperature and kept for 24 h (Scheme 3), allylphosphonate **7** was obtained. This result further confirmed that the intermediate **1** was formed when reaction mixture was kept at 0 °C. Then, a β-hydrogen abstraction and elimination of Cp_2ZrHCl afforded allylphosphonate **7** slowly after the reaction mixture was warmed to room temperature. It was noteworthy that in this case $HP(O)(OEt)_2$ was obtained as indicated by ^{31}P NMR.



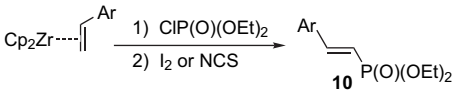
Scheme 3.

To extend the scope of the title reaction, we tested $Cp_2Zr(CH_2{=}CHAr)$ and chlorophosphonate under optimized reaction conditions (Scheme 4). Treatment of zirconocene–styrene complex, generated by addition of styrene to Negishi reagent[6] with diethyl chlorophosphonate at 5 °C for 14 h afforded diethyl 2-phenylethylphosphonate **8a** in 65% yield after hydrolysis. To our surprise, deuteriolysis of reaction mixture instead of hydrolysis afforded diethyl 1,2-deuterium-2-phenyl-ethylphosphonate **9a** in 67% yield with 85% deuterium incorporation. No formation of **4b** was observed. This result showed that the product of the reaction $Cp_2Zr(CH_2{=}CHPh)$ with chlorophosphate before hydrolysis contained two Zr–C bonds. It was noteworthy that in this case only small amount of product **8a** was observed.



Scheme 4.

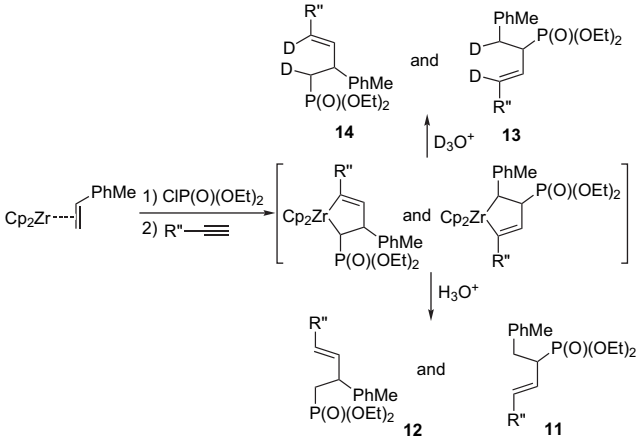
It is interesting to note that treatment of the reaction mixture of $Cp_2Zr(CH_2{=}CHAr)$ and chlorophosphate with iodine or NCS afforded diethyl *E*-2-arylethenylphosphonate in moderate yields (Scheme 5). No formation of halogenated organophosphonates was observed.



10a: Ar = Ph, 47%
10b: Ar = 4-Me-Ph, 49%
10c: Ar = 4-Cl-Ph, 30%

Scheme 5.

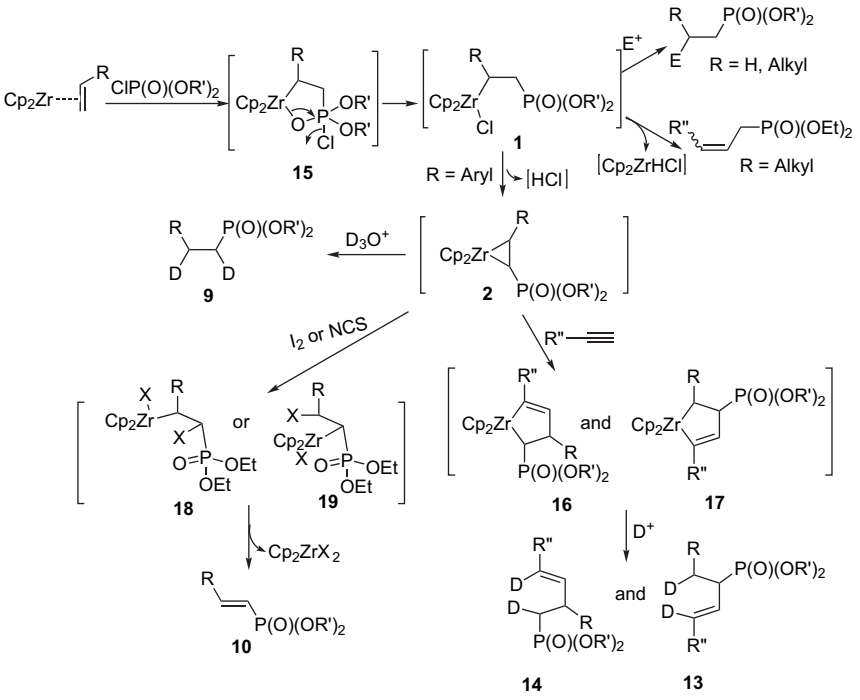
To further confirm the reaction intermediate, the reaction of $Cp_2Zr(CH_2{=}CHC_6H_4Me)$ and chlorophosphonate was carried out at 5 °C for 6 h and followed by addition of alkyne. The compounds **11** and **12** or **13** and **14** were formed after hydrolysis (or deuteriolysis) of the reaction mixture (Scheme 6).



the mixture of **11a** and **12a**: R" = C_4H_9, 38%, 11a/12a = 2:1
the mixture of **11b** and **12b**: R" = Ph, 35%, 11b/12b = 3:1
the mixture of **13a** and **14a**: R" = C_4H_9, 35%, 13a/14a = 2:1

Scheme 6.

On the basis of the results obtained above, a plausible reaction mechanism was shown in Scheme 7. In the first step, the zirconocene–alkene complex reacted with chlorophosphonate to form five-membered zirconacycle **15**. Then, elimination of chloride ion from zirconacycle took place to form zircono-ethylphosphonate **1**, which could be converted into



Scheme 7.

functionalized ethylphosphonate derivatives by coupling with various electrophiles when substituted R is hydrogen or alkyl groups. When R is aryl group, the conjugative effect of aryl group and electron-withdrawing effect of phosphate group favor β-H elimination to form intermediate **2**, which can be confirmed by further deuteriolysis to form compound **9** or coupling with terminal alkyne to produce compound **13** and **14** after deuteriolysis. The intermediate **2** could react with I_2 or NCS to form intermediate **18** or **19**, which would easily eliminate Cp_2ZrX_2 to form compound **10**.

3. Conclusion

In summary, we developed a versatile reaction to synthesize various functionalized organophosphonates $RP(O)(OR')_2$ via highly regioselective metallo-phosphorylation of alkenes. A detailed study on the reaction process is also presented.

4. Experimental

4.1. General

All manipulations were conducted in pre-dried Schlenk tube and under nitrogen with a slightly positive pressure. The reaction progress was monitored by ^{31}P NMR. The ^{31}P NMR yield of the products was obtained in proportion to the integral area of corresponding products. Unless otherwise noted, all starting materials were commercially available and were used without further purification. Tetrahydrofuran (THF) was refluxed and freshly distilled from dark purple solutions of sodium and benzophenone under a nitrogen atmosphere. ^1H NMR and ^{13}C NMR spectra were recorded on JOEL 300 NMR spectrometer with TMS as internal standard. ^{31}P NMR spectra were recorded on Bruker AC 200 NMR spectrometer at 81 MHz under ^1H decoupled conditions using 85% H_3PO_4 (δ_P=0) as an internal standard. Mass spectra were obtained using a Bruker Esquire iontrap mass spectrometer in positive ion mode.

4.2. A procedure for the reaction of $Cp_2Zr(CH_2{=}CHCH_2CH_3)$ with chlorophosphate: preparation of 2-zircono-*n*-butylphosphonate

To a solution of dibutylzirconocene, generated by the reaction of Cp_2ZrCl_2 with 2 equiv of BuLi (1.5 mL, 1.6 M in hexane solution) in THF was added 1.0 mmol diethyl chlorophosphate (144.6 μL) at −78 °C. The reaction mixture was warmed to 0–5 °C and stirred at the same temperature for 6 h (^{31}P NMR yield in 65%). ^{31}P NMR (81 MHz, THF, 85% H_3PO_4) δ 36.8 ppm.

4.2.1. Preparation of diethyl *n*-butylphosphonate (3a).[14] The resulting mixture of 2-zircono-*n*-butylphosphonate was treated with 3 N HCl. Product was extracted with ethyl acetate and the organic extract was dried over $MgSO_4$. Removal of the solvent and subsequent purification by column chromatography on silica gel (ethyl acetate/petroleum ether=3/1) afforded 113 mg of the title compound as a colorless liquid (isolated yield 58%). ^1H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 0.85 (t, $^3J_{HH}$=7.2 Hz, 3H), 1.25 (t, $^3J_{HH}$=6.9 Hz, 6H), 1.30–1.37 (m, 2H), 1.50–1.56 (m, 2H), 1.58–1.72 (m, 2H), 4.01–4.06 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 13.4, 16.3 (d, $^3J_{PC}$=5.7 Hz), 23.9 (d, $^3J_{PC}$=17.2 Hz), 24.3, 25.2 (d, $^1J_{PC}$=142.7 Hz), 61.2 (d, $^2J_{PC}$=6.5 Hz); ^{31}P NMR (81 MHz, $CDCl_3$, 85% H_3PO_4) δ 33.5. Positive ion ESI-MS, *m*/*z*=217.0 ($M+Na^+$). HRMS calcd for $C_8H_{19}O_3P$, 194.1072; found, 194.1075.

4.2.2. Preparation of diethyl 2-deuterium-*n*-butylphosphonate (4a). The reaction was carried out in a similar way to that described above using 20% DCl instead of 3 N HCl

to quench the reaction mixture and stirred for 3 h at room temperature (isolated yield: 57%). ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 0.92 (t, $^3J_{HH}$=7.2 Hz, 3H), 1.33 (t, $^3J_{HH}$=6.9 Hz, 6H), 1.37–1.42 (m, 2H), 1.48–1.62 (m, 1H), 1.72 (dd, $^3J_{HH}$=7.5 Hz, $^2J_{PH}$=18.0 Hz, 2H), 4.02–4.16 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 13.4, 16.4 (d, $^3J_{PC}$=5.7 Hz), 23.5 (d, $^3J_{PC}$=17.2 Hz), 23.7–24.2 (m), 25.2 (d, $^1J_{PC}$=139.8 Hz), 61.3 (d, $^2J_{PC}$=6.5 Hz); ^{31}P NMR (81 MHz, $CDCl_3$, 85% H_3PO_4) δ 33.6. Positive ion ESI-MS, *m*/*z*=196.0 (M+H$^+$), 217.9 (M+Na$^+$). HRMS calcd for $C_8H_{18}DO_3P$, 195.1135; found, 195.1139.

4.2.3. Preparation of diethyl 2-iodo-*n*-butylphosphonate (5a).[15] The reaction was carried out in a similar way to that described above using I_2 (1.0 mmol, 254 mg) instead of 3 N HCl and the reaction mixture was stirred for 6 h at room temperature. The resulting mixture was treated with 3 N HCl and $Na_2S_2O_3$ solution. Product was extracted with ethyl acetate and the organic extract was dried over $MgSO_4$. Removal of the solvent and subsequent purification by column chromatography on silica gel (ethyl acetate/petroleum ether=3/1) afforded 163 mg of the title compound as yellow oil (yield 51%). ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 1.04 (t, 3H, $^3J_{HH}$=7.0 Hz), 1.34 (t, $^3J_{HH}$=6.9 Hz, 6H), 1.72–2.00 (m, 2H), 2.50–2.70 (m, 2H), 4.01–4.14 (m, 4H), 4.30–4.40 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 14.0, 16.3 (d, $^3J_{PC}$=5.7 Hz), 27.9, 33.8 (d, $^3J_{PC}$=5.0 Hz), 38.4 (d, $^1J_{PC}$=130.3 Hz), 61.5 (d, $^2J_{PC}$=6.5 Hz), 61.7 (d, $^2J_{PC}$=6.5 Hz); ^{31}P NMR (81 MHz, $CDCl_3$, 85% H_3PO_4) δ 27.1. Positive ion ESI-MS: *m*/*z*=321.0 (M+H$^+$). HRMS calcd for $C_8H_{18}IO_3P$, 320.0038; found, 320.0035.

4.2.4. Preparation of diethyl 2-benzoyl-*n*-butylphosphonate (6a). After addition of CuCl (1.0 mmol, 99 mg) to the reaction mixture of zircono-2-butylphosphonate at room temperature, benzoyl chloride (1.0 mmol, 116 μL) was added and the reaction mixture was stirred at 50 °C for 12 h. The resulting mixture was treated with 3 N HCl at room temperature. Product was extracted with ethyl acetate and the organic extract was dried over $MgSO_4$. Removal of the solvent and subsequent purification by column chromatography on silica gel (ethyl acetate/petroleum ether=3/1) yielded 164 mg of the title compound as a colorless solid (yield 55%). ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 0.87 (t, $^3J_{HH}$=7.2 Hz, 3H), 1.34 (m, 6H), 1.57–1.97 (m, 2H), 2.38–2.52 (m, 1H), 3.44–3.90 (m, 2H) 3.93–4.04 (m, 4H), 7.45–7.60 (m, 3H), 8.00 (d, 2H, $^2J_{HH}$=8.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 11.0, 16.2 (d, $^3J_{PC}$=5.7 Hz), 26.6 (d, $^1J_{PC}$=139.9 Hz), 27.1 (d, $^3J_{PC}$=13.6 Hz), 41.4 (d, $^2J_{PC}$=3.6 Hz), 61.5 (d, $^2J_{PC}$=6.5 Hz), 61.7 (d, $^2J_{PC}$=6.5 Hz), 128.3, 128.6, 133.1, 136.7, 202.0 (d, $^3J_{PC}$=5.7 Hz); ^{31}P NMR (81 MHz, $CDCl_3$, 85% H_3PO_4) δ 31.5. Positive ion ESI-MS, *m*/*z*=305.1 (M+Li$^+$), 321.1 (M+Na$^+$). HRMS calcd for $C_{15}H_{23}O_4P$, 298.1334; found, 298.1338.

4.2.5. Preparation of diethyl 2-butenylphosphonate (7a). To a solution of dibutylzirconocene in THF was added 1.4 equiv of diethyl chlorophosphate (217 μL). The reaction mixture was warmed to room temperature and kept at the same temperature for 12 h. The resulting mixture was treated with 3 N HCl. Product was extracted with ethyl acetate and the organic extract was dried over $MgSO_4$. Removing the solvent and subsequent purification by column chromatography on silica gel (ethyl acetate/petroleum ether=2/1) afforded 109.5 mg of the title compound as a colorless liquid (yield 57%, *Z*/*E*=5:1). Major isomer—*diethyl (Z)-2-butenylphosphonate*:[16] ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 1.34 (m, 6H), 1.67 (dd, $^3J_{HH}$=10.2 Hz, $^4J_{HH}$=0.9 Hz, 3H), 2.62 (dd, $^2J_{PH}$=22.2 Hz, $^3J_{HH}$=7.5 Hz, 2H), 4.06–4.16 (m, 4H), 5.42–5.48 (m, 1H), 5.58–5.71 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 12.9 (d, $^4J_{PC}$=2.2 Hz), 16.5 (d, $^3J_{PC}$=6.2 Hz), 25.5 (d, $^1J_{PC}$=139.9 Hz), 61.9 (d, $^2J_{PC}$=6.8 Hz), 118.7 (d, $^2J_{PC}$=11.2 Hz), 128.7 (d, $^3J_{PC}$=14.3 Hz); ^{31}P NMR (81 MHz, $CDCl_3$, 85% H_3PO_4) δ 28.3. Positive ion ESI-MS: *m*/*z*=192.8 (M+H$^+$), 214.7 (M+Na$^+$). HRMS calcd for $C_8H_{17}O_3P$, 192.0915; found, 192.0913.

4.3. A procedure for the reaction of $Cp_2Zr(CH_2$=CHHex) with chlorophosphate: preparation of 2-zircono-*n*-octylphosphonate

To a solution of dibutylzirconocene, generated by the reaction of Cp_2ZrCl_2 with 2 equiv of BuLi (1.5 mL, 1.6 M in hexane solution) in THF was added 1.0 equiv of 1-octene (157 μL) at −78 °C. The reaction mixture was warmed to 0–5 °C and stirred at the same temperature for 6 h (^{31}P NMR yield 45%). ^{31}P NMR (81 MHz, THF, 85% H_3PO_4) δ 36.8 ppm.

4.3.1. Preparation of diethyl *n*-octylphosphonate (3b).[17] The resulting mixture of 2-zircono-*n*-octylphosphonate was treated with 3 N HCl. Product was extracted with ethyl acetate and the organic extract was dried over $MgSO_4$. Removing the solvent and subsequent purification by column chromatography on silica gel (ethyl acetate/petroleum ether=3/1) afforded 97 mg of the title compound as a colorless liquid (yield 39%). ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 0.88 (t, $^3J_{HH}$=6.3 Hz, 3H), 1.26–1.35 (m, 16H), 1.46–1.78 (m, 4H), 4.08–4.13 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 14.1, 16.5 (d, $^3J_{PC}$=5.7 Hz), 22.4 (d, $^3J_{PC}$=5.0 Hz), 22.6, 25.7 (d, $^1J_{PC}$=139.1 Hz), 29.1 (2C), 30.6 (d, $^3J_{PC}$=17.2 Hz), 31.8, 61.5 (d, $^3J_{PC}$=5.7 Hz); ^{31}P NMR (81 MHz, $CDCl_3$, 85% H_3PO_4) δ 33.7. Positive ion ESI-MS: *m*/*z*=251.2 (M+H$^+$), 273.2 (M+Na$^+$). HRMS calcd for $C_{12}H_{27}O_3P$, 250.1698; found, 250.1695.

4.3.2. Preparation of diethyl 2-iodo-*n*-octylphosphonate (5b). The reaction was carried out in a similar way to that described above using I_2(1.0 mol, 254 mg) instead of 3 N HCl and the reaction mixture was stirred for 6 h at room temperature. The resulting mixture was treated with 3 N HCl and $Na_2S_2O_3$ solution. Product was extracted with ethyl acetate and the organic extract was dried over $MgSO_4$. Removing the solvent and subsequent purification by column chromatography on silica gel (ethyl acetate/petroleum ether=2/1) afforded 132 mg of the title compound as a yellow oil (yield 35%). ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 0.84 (t, $^3J_{HH}$=8.4 Hz, 3H), 1.25–1.37 (m, 14H), 1.69–1.81 (m, 2H), 2.45–2.68 (m, 2H), 4.08–4.13 (m, 4H), 4.13–4.33 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 14.1, 16.5 (d, $^3J_{PC}$=5.9 Hz), 22.6, 26.2, 28.3, 29.7, 31.7, 38.9 (d, $^1J_{PC}$=139.1 Hz), 40.7 (d, $^3J_{PC}$=5.7 Hz), 62.0 (d, $^3J_{PC}$=8.2 Hz), 62.1 (d, $^3J_{PC}$=8.2 Hz); ^{31}P NMR (81 MHz,

$CDCl_3$, 85% H_3PO_4) δ 27.1. Positive ion ESI-MS: m/z=377.1 ($M+H^+$), 399.1 ($M+Na^+$). HRMS calcd for $C_{12}H_{26}IO_3P$, 376.0664; found, 376.0668.

4.3.3. Preparation of diethyl 2-octenylphosphonate (7b).[18] To a solution of dibutylzirconocene in THF was added 1.4 equiv of diethyl chlorophosphate (216.9 μL). The reaction mixture was warmed to room temperature and kept at the same temperature for 12 h. The resulting mixture was treated with 3 N HCl. Product was extracted with ethyl acetate and the organic extract was dried over $MgSO_4$. Removing the solvent and subsequent purification by column chromatography on silica gel (ethyl acetate/petroleum ether=2/1) afforded 75 mg of the title compound as a colorless liquid (yield 30%, *Z*/*E*=5:1). Major isomer—*diethyl (Z)-2-octenylphosphonate*: ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 0.87 (t, $^3J_{HH}$=7.2 Hz, 3H), 1.24–1.60 (m, 12H), 2.03 (m, 2H), 2.59 (dd, $^2J_{PH}$=21.9 Hz, $^3J_{HH}$=7.5 Hz, 2H), 4.00–4.11 (m, 4H), 5.36–5.42 (m, 1H), 5.55–5.61 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 14.2, 16.5 (d, $^3J_{PC}$=5.0 Hz), 22.6, 25.8 (d, $^1J_{PC}$=139.5 Hz), 27.4, 29.0, 31.6, 61.93 (d, $^2J_{PC}$=5.3 Hz), 117.6 (d, $^2J_{PC}$=10.5 Hz), 134.8 (d, $^3J_{PC}$=14.3 Hz); ^{31}P NMR (81 MHz, $CDCl_3$, 85% H_3PO_4) δ 28.8. Positive ion ESI-MS: m/z=249.2 ($M+H^+$), 271.1 ($M+Na^+$). HRMS calcd for $C_{12}H_{25}O_3P$, 248.1541; found, 248.1545.

4.4. A procedure for the reaction of $Cp_2Zr(CH_2{=}CH_2)$ with chlorophosphate: preparation of 2-zircono-ethylphosphonate

To a solution of diethylzirconocene, generated by the reaction of Cp_2ZrCl_2 with 2 equiv of EtMgBr (2.4 mL, 1 M in ether solution) in THF was added 1 equiv of diethyl chlorophosphate (144.6 μL). The reaction mixture was kept at 5 °C for 24 h or at room temperature for 12 h or at 40 °C for 3 h (^{31}P NMR yield 89%). ^{31}P NMR 34.7 (81 MHz, THF, 85% H_3PO_4).

4.4.1. Preparation of diethyl ethylphosphonate (3c).[19] The reaction mixture of zircono-ethylphosphonate was quenched with 3 N HCl solution and then extracted with ethyl acetate. The extract was washed with water and dried over $MgSO_4$. The solvent was evaporated in vacuo to give a light yellow liquid. Chromatography using a mixture of ethyl acetate and petroleum ether (2:1) as elute provided the product as a colorless liquid 104.6 mg (yield 63%). ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 1.16 (dt, $^3J_{PH}$=19.8 Hz, $^3J_{HH}$=7.8 Hz, 3H), 1.33 (t, J=7.0 Hz, 6H), 1.72 (q, $^2J_{PH}$=18.3 Hz, $^3J_{HH}$=7.8 Hz, 2H), 4.06–4.11 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 6.6 (d, $^2J_{PC}$=6.1 Hz), 16.5 (d, $^3J_{PC}$=7.2 Hz), 18.9 (d, $^1J_{PC}$=142.5 Hz), 61.6 (d, $^3J_{PC}$=6.2 Hz); ^{31}P NMR (81 MHz, THF, 85% H_3PO_4) δ 34.7. Positive ion ESI-MS: m/z=167.0 ($M+H^+$).

4.4.2. Preparation of diethyl 2-iodoethylphosphonate (5c).[20] To the reaction mixture of zircono-ethylphosphonate was added 1.2 equiv of I_2 (305 mg) and the reaction mixture was stirred at room temperature for 3 h. The above reaction mixture was quenched with 3 N HCl, and stirred at room temperature for 1 h and then extracted with ethyl acetate. The extract was washed with water and dried over $MgSO_4$. The solvent was evaporated in vacuo to a light brown liquid. Chromatography using a mixture of ethyl acetate and petroleum ether (2:1) as elute provided the product as a colorless liquid 175.2 mg (yield 60%). ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 1.29 (t, J=6.9 Hz, 6H), 2.31–2.43 (m, 2H), 3.19–3.28 (m, 2H), 4.05–4.10 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ −7.4 (d, $^2J_{PC}$=3.8 Hz), 16.4 (d, $^3J_{PC}$=6.3 Hz), 31.9 (d, $^1J_{PC}$=131.7 Hz), 62.0 (d, $^3J_{PC}$=6.8 Hz); ^{31}P NMR (81 MHz, THF, 85% H_3PO_4) δ 27.0. Positive ion ESI-MS: m/z=292.9 ($M+H^+$).

4.5. A procedure for the reaction of $Cp_2Zr(PhCH{=}CH_2)$ with chlorophosphate: preparation of diethyl 2-phenyl-1,2-zircono-ethylphosphonate

To a solution of dibutylzirconocene in THF was added 1.0 equiv of styrene and stirred for 1 h at room temperature. Chlorophosphate (1 mmol, 144.7 μL) was added to this solution and stirred for 14 h at 0–5 °C (^{31}P NMR yield 58%). ^{31}P NMR (81 MHz, THF, 85% H_3PO_4) δ 49.7 ppm.

4.5.1. Preparation of diethyl 2-phenylethylphosphonate (8a).[21] The resulting mixture of diethyl 2-phenyl-1,2-zircono-ethylphosphonate was treated with 3 N HCl and stirred for 1 h. Product was extracted with ethyl acetate and the organic extract was dried over $MgSO_4$. Removing the solvent and subsequent purification by column chromatography on silica gel (ethyl acetate/petroleum ether=2/1) afforded 157.3 mg of the title compound as a colorless liquid (yield 65%). ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 1.25–1.38 (m, 6H), 2.00–2.22 (m, 2H), 2.87–2.96 (m, 2H), 4.05–4.13 (m, 4H), 7.19–7.50 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 16.5 (d, $^3J_{PC}$=6.2 Hz), 27.6 (d, $^1J_{PC}$=139.3 Hz), 28.6 (d, $^2J_{PC}$=4.9 Hz), 61.6 (d, $^2J_{PC}$=6.2 Hz), 126.4, 128.1, 128.6, 140.8 (d, $^3J_{PC}$=15.0 Hz). ^{31}P NMR (81 MHz, $CDCl_3$, 85% H_3PO_4) δ 32.4. Positive ion ESI-MS: m/z=242.8 ($M+H^+$), 264.7 ($M+Na^+$). HRMS calcd for $C_{12}H_{19}O_3P$, 242.1072; found, 242.10726.

4.5.2. Preparation of diethyl 1,2-dideuterium-2-phenylethylphosphonate (9a). The reaction was carried out in a similar way to that described above using 20% DCl instead of 3 N HCl to quench the reaction mixture and stirred for 3 h at 5 °C (yield: 67% with 85% deuterium incorporation). ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 1.26–1.36 (m, 6H), 2.02–2.12 (m, 1.16H), 2.90–2.96 (m, 1.17H), 4.07–4.13 (m, 4H), 7.20–7.31 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 16.3 (d, $^3J_{PC}$=5.7 Hz), 27.2 (dt, $^1J_{PC}$=140.2 Hz, $^1J_{DC}$=19.4 Hz), 28.2 (dt, $^2J_{PC}$=4.6 Hz, $^1J_{DC}$=19.7 Hz), 61.4 (d, $^2J_{PC}$=6.5 Hz), 126.1, 127.8, 128.3, 140.7 (d, $^3J_{PC}$=15.8 Hz), ^{31}P NMR (81 MHz, $CDCl_3$, 85% H_3PO_4) δ 31.5. Positive ion ESI-MS: m/z=245.1 ($M+H^+$), 251.1 ($M+Li^+$). HRMS calcd for $C_{12}H_{17}D_2O_3P$, 244.1197; found, 244.1194.

4.5.3. Preparation of diethyl (*E*)-2-phenyl-ethenylphosphonate (10a).[2c,22] The resulting mixture of diethyl 2-phenyl-1,2-zircono-ethylphosphonate was treated with NCS (1 mmol, 133.5 mg) or I_2 (1 mmol, 254 mg) and stirred for 30 min at room temperature. Quenched with 3 N HCl and product was extracted with ethyl acetate and the organic extract was dried over $MgSO_4$. Removal of the solvent and subsequent purification by column chromatography on silica gel (ethyl acetate/petroleum ether=2/1) afforded 113 mg of

the title compound as a colorless liquid (yield 47%). ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 1.32 (t, $^3J_{HH}$=6.6 Hz, 6H), 4.03–4.16 (m, 4H), 6.28 (t, $^2J_{PH}$=17.4 Hz, $^3J_{HH}$=17.4 Hz, 1H), 7.28–7.58 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 16.5 (d, $^3J_{PC}$=5.6 Hz), 62.0 (d, $^2J_{PC}$=4.5 Hz), 114.1 (d, $^1J_{PC}$=193.4 Hz), 127.8, 128.9, 130.3, 134.8 (d, $^3J_{PC}$=23.0 Hz), 148.8 (d, $^2J_{PC}$=5.6 Hz); ^{31}P NMR (81 MHz, $CDCl_3$, 85% H_3PO_4) δ 20.2. Positive ion ESI-MS: m/z=241.0 (M+H$^+$), 263.0 (M+Na$^+$). HRMS calcd for $C_{12}H_{17}O_3P$, 240.0915; found, 240.0918.

4.6. A procedure for the reaction of Cp_2Zr(MePhCH=CH_2) with chlorophosphate: preparation of diethyl 2-(4-methylphenyl)-1,2-zircono-ethylphosphonate

To a solution of dibutylzirconocene in THF was added 1.0 equiv of 4-methyl-styrene and stirred for 1 h at room temperature. Chlorophosphate (1 mmol, 144.7 μL) was added to this solution and stirred for 6 h at 5 °C (^{31}P NMR yield 70%). ^{31}P NMR (81 MHz, THF, 85% H_3PO_4) δ 50.1 ppm.

4.6.1. Preparation of diethyl 2-(4-methylphenyl)ethylphosphonate (8b).[23] The resulting mixture of diethyl 2-(4-methylphenyl)-1,2-zircono-ethylphosphonate was treated with 3 N HCl and stirred for 1 h. Then it was carried out in a similar way to that described above to get 179 mg of the title compound as a colorless liquid (yield 70%). ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 1.32 (m, $^3J_{HH}$=6.9 Hz, 6H), 1.98–2.10 (m, 2H), 2.32 (s, 3H), 2.83–2.92 (m, 2H), 4.10 (t, $^3J_{HH}$=7.0 Hz, 4H), 7.10 (s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 16.4 (d, $^3J_{PC}$=5.9 Hz), 20.9, 27.6 (d, $^1J_{PC}$=138.4 Hz), 28.0 (d, $^2J_{PC}$=4.3 Hz), 61.5 (d, $^3J_{PC}$=5.7 Hz), 127.8, 129.1, 135.7, 137.8 (d, $^3J_{PC}$= 17.9 Hz) ; ^{31}P NMR (81 MHz, $CDCl_3$, 85% H_3PO_4) δ 31.6. Positive ion ESI-MS: m/z=257.1 (M+H$^+$), 279.0 (M+Na$^+$). HRMS calcd for $C_{13}H_{21}O_3P$, 256.1228; found, 256.1232.

4.6.2. Preparation of diethyl (*E*)-2-(4-methylphenyl) ethenylphosphonate (10b).[24] The resulting mixture of diethyl 2-(4-methylphenyl)-1,2-zircono-ethylphosphonate was treated with NCS (1 mmol, 133.5 mg) or I_2 (1 mmol, 254 mg) and stirred for 30 min at room temperature. Then it was carried out in a similar way to that described above to get 124.5 mg of the title compound as a colorless liquid (yield 49%). ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 1.25–1.35 (m, 6H), 2.37 (s, 3H), 4.03–4.12 (m, 4H), 6.20 (t, $^2J_{PH}$=17.5 Hz, $^3J_{HH}$=17.5 Hz), 7.18–7.47 (m, 5H), ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 16.4 (d, $^3J_{PC}$=5.8 Hz, 2C), 21.4, 61.9, 112.6 (d, $^1J_{PC}$=190.4 Hz), 127.7, 129.6, 130.0 (d, $^3J_{PC}$=23.3 Hz), 140.6, 148.8; ^{31}P NMR (81 MHz, $CDCl_3$, 85% H_3PO_4) δ 21.0. Positive ion ESI-MS: m/z=255.2 (M+H$^+$), 277.2 (M+Na$^+$). HRMS calcd for $C_{13}H_{19}O_3P$, 254.1072; found, 254.1075.

4.6.3. Preparation of diethyl 2-(*p*-tolyl)-oct-3-enylphosphonate (12a) and diethyl (4-methyl-benzyl)-hept-2-enylphosphonate (11a). The resulting mixture of diethyl 2-(4-methylphenyl)-1,2-zircono-ethylphosphonate was added 1.0 mmol 1-hexyne and stirred for 3 h at room temperature, then treated with 3 N HCl and stirred for 1 h. The product was extracted with ethyl acetate and the organic extract was dried over $MgSO_4$. Removing the solvent and subsequent purification by column chromatography on silica gel (ethyl acetate/petroleum ether=1/1) afforded 128 mg of the title compounds of the mixture as oil liquid (yield 38%, **12a/11a**=1:2).

4.6.3.1. Diethyl 2-(*p*-tolyl)-oct-3-enylphosphonate (12a). ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 0.73–0.83 (m, 3H), 1.12–1.24 (m, 4H), 1.25–1.30 (m, 6H), 1.98–2.03 (m, 2H), 2.09–2.26 (m, 2H), 2.26 (s, 3H), 3.50–3.59 (m, 1H), 3.63–4.00 (m, 4H), 5.31–5.59 (m, 2H), 6.94–7.02 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 13.8, 16.1–16.4 (m), 20.9, 22.1, 31.6, 32.0, 32.5 (d, $^1J_{PC}$=137.9 Hz), 42.4 (d, $^2J_{PC}$=3.0 Hz), 61.2 (d, $^3J_{PC}$=6.8 Hz), 61.3 (d, $^3J_{PC}$=6.8 Hz), 127.2, 129.0, 130.5, 132.9 (d, $^3J_{PC}$=11.3 Hz), 135.8, 141.05 (d, $^3J_{PC}$=9.8 Hz); ^{31}P NMR (81 MHz, $CDCl_3$, 85% H_3PO_4) δ 30.6. Positive ion ESI-MS: m/z=339.3 (M+H$^+$). HRMS calcd for $C_{19}H_{31}O_3P$, 338.2011; found, 338.2009.

4.6.3.2. Diethyl (4-methyl-benzyl)-hept-2-enylphosphonate (11a). ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 0.73–0.83 (m, 3H), 1.12–1.24 (m, 4H), 1.25–1.30 (m, 6H), 1.98–2.03 (m, 2H), 2.23 (s, 3H), 2.60–2.75 (m, 2H), 3.11–3.18 (m, 1H), 4.06–4.16 (m, 4H), 5.28–5.33 (m, 2H), 6.93–7.02 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 13.7, 16.1–16.4 (m), 20.9, 21.7, 31.1 (d, $^4J_{PC}$=3.0 Hz), 32.1, 34.5 (d, $^2J_{PC}$=3.0 Hz), 43.6 (d, $^1J_{PC}$=136.6 Hz), 61.7 (d, $^3J_{PC}$=6.8 Hz), 62.1 (d, $^3J_{PC}$=6.8 Hz), 123.5 (d, $^2J_{PC}$= 9.8 Hz), 128.7, 128.9, 135.4, 136.0 (d, $^3J_{PC}$=16.5 Hz), 136.1 (d, $^3J_{PC}$=13.5 Hz); ^{31}P NMR (81 MHz, $CDCl_3$, 85% H_3PO_4) δ 30.0. Positive ion ESI-MS: m/z=339.3 (M+H$^+$).

4.6.4. Preparation of diethyl 4-phenyl-2-(*p*-tolyl)-but-3-enylphosphonate (12b) and diethyl 1-(4-methyl-benzyl)-3-phenyl-allyl-phosphonate (11b). The resulting mixture of diethyl-2-(4-methylphenyl)-1,2-zircono-ethylphosphonate was added 1.0 mmol phenylacetylene and stirred for 3 h at room temperature, then treated with 3 N HCl and stirred for 1 h. The product was extracted with ethyl acetate and the organic extract was dried over $MgSO_4$. Removing the solvent and subsequent purification by column chromatography on silica gel (ethyl acetate/petroleum ether=1/1) afforded 132 mg of the title compounds of the mixture as a colorless solid (yield 37%, **12b/11b**=1:3).

4.6.4.1. Diethyl 1-(4-methyl-benzyl)-3-phenyl-allylphosphonate (11b). ^{1}H NMR ($CDCl_3$, Me_4Si) δ 1.26–1.36 (m, 6H), 2.29 (s, 3H), 2.83–3.00 (m, 2H), 3.22–3.30 (m, 1H), 4.00–4.20 (m, 4H), 6.00–6.13 (m, 1H), 6.27–6.39 (m, 1H) 7.03–7.32 (m, 9H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ 16.3–16.6 (m), 21.1, 34.8 (d, $^2J_{PC}$=3.8 Hz), 44.3 (d, $^1J_{PC}$=136.3 Hz), 62.2 (d, $^3J_{PC}$=7.5 Hz), 62.7 (d, $^3J_{PC}$=7.5 Hz), 124.2 (d, $^2J_{PC}$=10.8 Hz), 126.3, 127.6, 128.5, 129.0, 129.2, 134.6 (d, $^3J_{PC}$=14.3 Hz), 135.8, 135.9 (d, $^3J_{PC}$=13.5 Hz), 137.0 (d, $^4J_{PC}$=2.3 Hz); ^{31}P NMR (81 MHz, $CDCl_3$, 85% H_3PO_4) δ 28.9. Positive ion ESI-MS: m/z=359.1 (M+H$^+$), 381.1 (M+Na$^+$). HRMS calcd for $C_{21}H_{27}O_3P$, 358.1698; found, 358.1697.

4.6.5. Preparation of diethyl 1,4-dideuterium-2-(*p*-tolyl)-oct-3-enylphosphonate (14a) and diethyl 3-deuterium-1-(4-methylphenyl-deuterium-methyl)-hept-2-enylphosphonate (13a). The reaction was carried out in a similar way to that

described above using 20% DCl instead of 3 N HCl to quench the reaction mixture and stirred for 3 h at 5 °C (yield 35%, **14a/13a**=1:2).

4.6.5.1. Diethyl 1,4-dideuterium-2-(*p*-tolyl)-oct-3-enylphosphonate (14a). ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 0.78–0.93 (m, 3H), 1.11–1.24 (m, 4H), 1.26–1.32 (m, 6H), 1.91–2.02 (m, 2H), 2.08–2.22 (m, 1H), 2.29 (s, 3H), 3.66–3.72 (m, 1H), 3.83–4.00 (m, 4H), 5.56 (d, $^3J_{HH}$=6.9 Hz, 1H), 7.02–7.08 (m, 4H); ^{13}C NMR ($CDCl_3$, Me_4Si) δ 14.0, 16.1–16.4 (m), 21.1, 22.3, 31.5, 32.2, 33.3–30.9 (m), 42.5, 61.3 (d, $^2J_{PC}$=6.8 Hz), 61.4 (d, $^2J_{PC}$=6.8 Hz), 127.3, 129.3, 130.3 (t, $^1J_{CD}$=18.7 Hz), 133.0 (d, $^3J_{PC}$=11.3 Hz), 136.0, 141.2 (d, $^3J_{PC}$=10.8 Hz); ^{31}P NMR (81 MHz, $CDCl_3$, 85% H_3PO_4) δ 30.5. Positive ion ESI-MS: *m*/*z*=341.4 (M+H$^+$). HRMS calcd for $C_{19}H_{29}D_2O_3P$, 340.2136; found, 340.2139.

4.6.5.2. Diethyl 3-deuterium-1-(4-methylphenyl-deuterium-methyl)-hept-2-enylphosphonate (13a). ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 0.78–0.93 (m, 3H), 1.11–1.24 (m, 4H), 1.26–1.32 (m, 6H), 1.91–2.02 (m, 2H), 2.28 (s, 3H), 2.62–2.74 (ddd, $^3J_{HH}$=3.1 Hz, $^3J_{HH}$=9.3 Hz, $^2J_{PH}$=20.9 Hz, 1H), 3.12 (d, $^3J_{HH}$=9.6 Hz, 1H), 3.11–3.18 (m, 1H), 4.06–4.14 (m, 4H), 5.24 (t, $^3J_{HH}$=7.5 Hz, $^3J_{PH}$=7.5 Hz, 1H), 6.93–7.02 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 13.9, 16.3–16.6 (m), 21.1, 21.9, 31.3 (d, $^4J_{PC}$=2.9 Hz), 32.3, 34.3 (t, $^1J_{CD}$=17.9 Hz), 43.6 (d, $^1J_{PC}$=136.3 Hz), 61.8 (d, $^3J_{PC}$=7.5 Hz), 62.3 (d, $^3J_{PC}$=7.5 Hz), 123.6 (d, $^2J_{PC}$=9.8 Hz), 128.9, 129.1, 135.6, 136.2 (d, $^3J_{PC}$=16.5 Hz), 136.5–135.7 (m); ^{31}P NMR (81 MHz, $CDCl_3$, 85% H_3PO_4) δ 29.9. Positive ion ESI-MS: *m*/*z*=341.4 (M+H$^+$).

4.7. A procedure for the reaction of $Cp_2Zr(ClPhCH{=}CH_2)$ with chlorophosphate: preparation of diethyl-2-(4-chlorophenyl)-1,2-zircono-ethylphosphonate

To a solution of dibutylzirconocene in THF was added 1.0 equiv of 4-chloro-styrene and stirred for 1 h at room temperature. Chlorophosphate (1 mmol, 144.7 μL) was added to this solution and stirred for 24 h at 0–5 °C (^{31}P NMR yield 51%). ^{31}P NMR (81 MHz, THF, 85% H_3PO_4) δ 49.1 ppm.

4.7.1. Preparation of diethyl 2-(4-chlorophenyl)ethylphosphonate (8c).[25] The resulting mixture of diethyl 2-(4-chlorophenyl)-1,2-zircono-ethylphosphonate was treated with 3 N HCl and stirred for 1 h. Then it was carried out in a similar way to that described above to get 140 mg of the title compound as a colorless liquid (isolated yield 48%). ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 1.25–1.34 (m, 6H), 1.97–2.08 (m, 2H), 2.86–2.92 (m, 2H), 4.07–4.12 (m, 4H), 7.12–7.32 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 6.5 (d, $^3J_{PC}$=6.2 Hz), 27.5 (d, $^1J_{PC}$=139.1 Hz), 28.0 (d, $^2J_{PC}$=4.4 Hz), 61.7 (d, $^3J_{PC}$=6.8 Hz), 128.7, 129.5, 132.2, 140.5 (d, $^3J_{PC}$=18.3 Hz); ^{31}P NMR (81 MHz, $CDCl_3$, 85% H_3PO_4) δ 32.4. Positive ion ESI-MS: *m*/*z*=277.1, 279.0 (M+H$^+$). HRMS calcd for $C_{12}H_{18}ClO_3P$, 276.0682; found, 276.0685.

4.7.2. Preparation of diethyl (*E*)-2-(4-chlorophenyl) ethenylphosphonate (10c).[26] The resulting mixture of diethyl 2-(4-chlorophenyl)-1,2-zircono-ethylphosphonate was treated with NCS (1 mmol, 133.5 mg) or I_2 (1 mmol, 254 mg) and stirred for 30 min at room temperature. Then it was carried out in a similar way to that described above to get 82 mg of the title compound as a colorless liquid (yield 30%). ^{1}H NMR (300 MHz, $CDCl_3$, Me_4Si) δ 1.36 (t, $^3J_{HH}$=6.8 Hz, 6H), 4.08–4.18 (m, 4H), 6.23 (t, $^2J_{PH}$=17.3 Hz, $^3J_{HH}$=17.3 Hz), 7.13–7.52 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$, Me_4Si) δ 16.4 (d, $^3J_{PC}$=6.5 Hz), 61.9 (d, $^2J_{PC}$=6.0 Hz), 114.8 (d, $^2J_{PC}$=190.0 Hz), 128.9, 129.1, 133.4 (d, $^3J_{PC}$=23.7 Hz), 136.1, 147.2 (d, $^2J_{PC}$=6.5 Hz); ^{31}P NMR (81 MHz, $CDCl_3$, 85% H_3PO_4) δ 19.5. Positive ion ESI-MS: *m*/*z*=274.9, 276.9 (M+H$^+$), 296.9, 298.9 (M+Na$^+$). HRMS calcd for $C_{12}H_{16}ClO_3P$, 274.0526; found, 274.0522.

Acknowledgements

This work was supported by the National Natural Science Foundation of China 20372041 (20572058) and Beijing Department of Education (XK100030514).

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Tetrahedron 62 (2006) 6303–6311
Tetrahedron

Asymmetric syntheses of *N*-substituted α-amino esters via dynamic kinetic resolution of α-haloacyl diacetone-D-glucose

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Received 23 March 2006; revised 13 April 2006; accepted 14 April 2006
Available online 11 May 2006

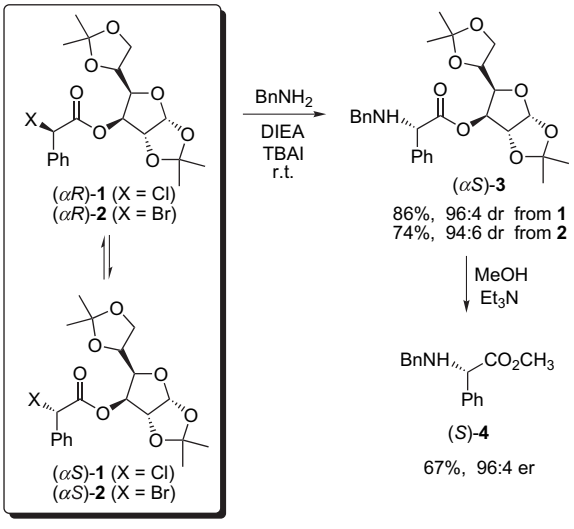
Abstract—Diacetone-D-glucose or D-allose mediated dynamic kinetic resolution of α-halo esters in nucleophilic substitution reaction has been investigated. Reactions with various amine nucleophiles in the presence of TBAI and DIEA can provide the *N*-substituted α-amino esters up to 99:1 dr. Stereochemical models of transition states, taking into account a hydrogen bonding, are proposed on the basis of the observed results. Also, application of this mild and simple methodology to stereoselective preparations of 1,1′-iminodicarboxylic acid derivatives is demonstrated.

1. Introduction

During the last decade, intensive efforts have been devoted to develop the chiral auxiliary mediated dynamic resolution of α-halo esters or α-halo amides in nucleophilic substitution.[1] For asymmetric syntheses of α-amino acid derivatives, recent studies in our laboratory have focused on the development of a practical chiral auxiliary for dynamic resolution of α-haloacyl compounds under mild and simple reaction conditions amenable to easily scalable processes. Carbohydrates are readily available and inexpensive natural products in which numerous functional groups and stereogenic centers are present in a molecule. Despite their stereochemical and structural complexities, a large number of carbohydrate based templates have been systematically developed and used as chiral auxiliaries and chiral ligands for various stereoselective reactions.[2,3] Earlier we outlined our preliminary results in dynamic kinetic resolution of α-chloro-α-aryl esters using diacetone-D-glucose as a chiral auxiliary.[4] Herein we describe our recent progress to extend the scope of the methodology to various α-halo esters and to understand the mechanism of the asymmetric nucleophilic substitution. Application of this methodology to highly stereoselective preparation of 1,1′-iminodicarboxylic acid derivatives is also presented.

2. Results and discussion

We have previously reported that the treatment of α-chloro-α-phenyl acetate **1** with benzylamine (1.2 equiv), tetrabutylammonium iodide (1.0 equiv, TBAI), and diisopropylethylamine (1.0 equiv, DIEA) in CH_2Cl_2 at room temperature provided the substitution product **3** in 86% yield with 96:4 diastereomeric ratio (dr, α*S*:α*R*) as summarized pictorially in Scheme 1.[4] Subsequent removal of the chiral auxiliary with MeOH and Et_3N gave *N*-benzyl phenylglycinate (*S*)-**4** in 67% yield with 96:4 er. The chiral information of D-glucose is transferred to the substitution at α-chloro carbon



Scheme 1. Reactions of α-halo-α-phenylacetates **1** and **2**.

Keywords: Dynamic kinetic resolution; Asymmetric syntheses; Nucleophilic substitution; Carbohydrate; Chiral auxiliary.
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doi:10.1016/j.tet.2006.04.045

center via dynamic kinetic resolution in the nucleophilic substitution with benzylamine. The α-chloro stereogenic center of **1** undergoes rapid epimerization in the presence of DIEA and TBAI, and (α*R*)-**1** reacts with benzylamine preferentially to provide (α*S*)-**3**.[5]

In order to assess the effect of leaving group, TBAI and DIEA on yield and stereoselectivity, a series of reactions were examined as shown in Table 1. When α-bromo acetate **2** was treated with benzylamine in the presence of both TBAI and DIEA, the substitution provided **3** in 74% yield with almost same diastereoselectivity (95:5 dr) compared to the reactions of α-chloro acetate **1** (entry 1). Significant decrease in stereoselectivity was observed in the absence of TBAI (entry 2), while mild drop in stereoselectivity was observed in the absence of DIEA (entry 3). We speculate that the lowering of dr in the absence of TBAI or DIEA probably results from the slower epimerization of α-bromo-α-phenyl acetate **2**. In addition, the result in entry 4 suggests that the presence of both TBAI and DIEA is important for highly stereoselective substitution. Unlike the cases of α-bromo acetate **2**, the reaction of α-chloro acetate **1** in the absence of TBAI gave the substitution product **3** with almost same stereoselectivity (95:5 dr) compared to the reaction of **1** in the presence of both TBAI and DIEA (entry 5 and Scheme 1). On the other hand, mild drop of selectivity was observed in the absence of DIEA (entry 6). In both the reactions of α-chloro acetate **1** as shown in entries 5 and 6, the rate of the substitution was substantially decreased and the product **3** was obtained in 33% and 34% yields, respectively, after 24 h stirring at room temperature. In addition, in the absence of both TBAI and DIEA, the reaction did not produce **3** and most of starting material was recovered (entry 7). These results seem to indicate that both TBAI and DIEA are crucial for rate acceleration of the substitution of α-chloro-α-phenyl acetate **1**.

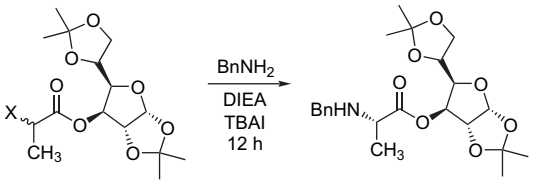
Table 1. Effects of leaving group, TBAI and DIEA in the reactions of **1** and **2**

Entry	S.M.[a]	Condition[b]	% Yield[c]	dr[d] (α*S*:α*R*)
1	**2**	TBAI, DIEA, $BnNH_2$	74 (**3**)	95:5
2	**2**	DIEA, $BnNH_2$	89 (**3**)	83:17
3	**2**	TBAI, $BnNH_2$	72 (**3**)	92:8
4	**2**	$BnNH_2$	67 (**3**)	74:26
5	**1**	DIEA, $BnNH_2$	34 (**3**)	95:5
6	**1**	TBAI, $BnNH_2$	33 (**3**)	92:8
7	**1**	$BnNH_2$	N.R.	—

[a] Initial drs of **1** and **2** were approximately 50:50.
[b] All reactions were carried out in CH_2Cl_2 for 24 h at rt.
[c] Isolated yields.
[d] The drs were determined by ^1H NMR of reaction mixture and confirmed by CSP-HPLC after removing the chiral auxiliary.

The scope of the observed dynamic kinetic resolution has been examined with various α-alkyl esters. Initial studies were carried out with the substitution reactions of α-chloro propionate **5** derived from diacetone-D-glucose and racemic α-chloro propionyl chloride. As shown in Table 2, entry 1, treatment of 69:31 diastereomeric mixture of **5** with $BnNH_2$ (1.2 equiv), TBAI (1.0 equiv), and DIEA (1.0 equiv) in CH_2Cl_2 for 24 h at room temperature gave **7** in 29% yield with 84:16 dr.[6] In an effort to improve the yield and the selectivity, we examined the substitutions of α-bromo propionate **6** under the same reaction condition. The reaction of α-bromo

Table 2. Reactions of α-halo-α-methyl acetates **5** and **6**



5 (X = Cl)
6 (X = Br)

7

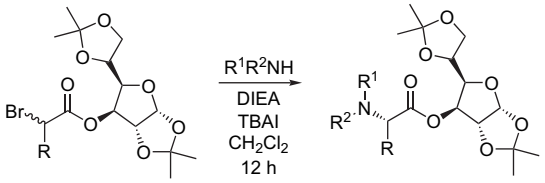
Entry[a]	X	dr of S.M.	Solvent	% Yield[b]	dr[c] (α*S*:α*R*)
1	Cl	69:31	CH_2Cl_2	29	84:16
2	Br	73:27	CH_2Cl_2	90	80:20
3	Br	55:45	CH_2Cl_2	91	80:20
4	Br	73:27	$CHCl_3$	99	76:24
5	Br	73:27	THF	87	80:20
6	Br	73:27	Ether	71	72:28
7	Br	73:27	DMF	73	72:28
8	Br	73:27	Hexane	93	69:31
9	Br	73:27	CH_3CN	99	76:24

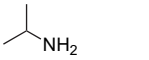
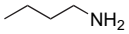
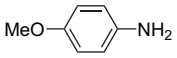
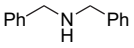
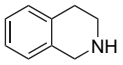
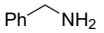
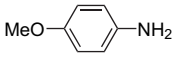
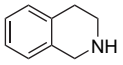
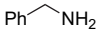
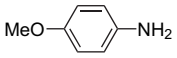
[a] All reactions were carried out in CH_2Cl_2 for 24 h at rt.
[b] Isolated yields.
[c] The drs were determined by ^1H NMR of reaction mixture and confirmed by CSP-HPLC after removing the chiral auxiliary.

propionate **6** (73:27 dr) gave **7** in a better yield with a slightly lower dr[6] (entry 2). When **6** of 55:45 dr was treated with benzylamine in the presence of TBAI and DIEA, the reaction gave the product **7** with 80:20 dr as shown in entry 3.[6] Thus, the dr of product **7** is not dependent on the starting ratio of two epimers of α-bromo propionate **6**, which indicates that the primary pathway of the asymmetric induction is a dynamic kinetic resolution. In the reactions of α-bromo propionate **6**, none of other solvents explored gave better selectivities than CH_2Cl_2. As shown in entries 4–9, the substitution product **7** was obtained with 76:24 dr in $CHCl_3$, 80:20 dr in THF, 72:28 dr in ether, 72:28 dr in DMF, 69:31 dr in *n*-hexane, and 76:24 dr in CH_3CN. The faster reactions at 50 °C in various solvents did not give better selectivities compared to the reactions at room temperature.

Next, we examined six different amine nucleophiles in the reactions of **6** as shown in Table 3. We were pleased to observe that this methodology is efficient for some aromatic and cyclic amine nucleophiles such as *p*-anisidine and 1,2,3,4-tetrahydroisoquinoline, affording amino acid derivatives **10** and **12** with 91:9 dr and 97:3 dr, respectively, as shown in entries 3 and 6. Reactions of α-bromo propionate **6** with isopropylamine, butylamine, dibenzylamine, and benzylmethylamine gave moderate stereoselectivities. Encouraged by the high asymmetric induction in the reactions of α-bromo-α-methyl ester **6** with some amine nucleophiles, we examined two other α-bromo α-alkyl acetates **8** and **9** as shown in entries 8–12. When α-bromo-α-ethyl acetate **8** was treated with 1,2,3,4-tetrahydroisoquinoline in the presence of TBAI and DIEA for 24 h, the substitution provided the corresponding amino ester **18** in 49% yield with 95:5 dr, while the reactions of benzylamine and *p*-anisidine gave moderate stereoselectivities (entries 8–10). The reactions of α-bromo-α-butyl acetate **9** were also carried out with benzylamine and *p*-anisidine for the asymmetric syntheses of α-butyl-α-amino acid derivatives **19** and **20** as shown in entries 11 and 12. Limited results in Table 3 indicate that the size and nature of the amine nucleophiles

Table 3. Reactions of α-bromo-α-alkyl acetates **6**, **7** and **8**



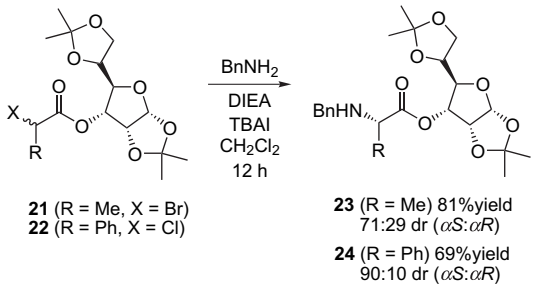
Entry[a]	R	R^1R^2NH	% Yield[b]	dr[c] (αS:αR)
1	Methyl (**6**)		70 (**10**)	80:20
2	Methyl (**6**)		80 (**11**)	77:23
3	Methyl (**6**)		85 (**12**)	91:9
4	Methyl (**6**)		48 (**13**)	69:31
5	Methyl (**6**)		91 (**14**)	75:25
6	Methyl (**6**)		92 (**15**)	97:3
8	Ethyl (**8**)		77 (**16**)	75:25
9	Ethyl (**8**)		99 (**17**)	70:30
10	Ethyl (**8**)		49 (**18**)	95:5
11	Butyl (**9**)		40 (**19**)	78:22
12	Butyl (**9**)		64 (**20**)	97:3

[a] All reactions were carried out in CH_2Cl_2 for 24 h at rt.
[b] Isolated yields.
[c] The drs were determined by ^1H NMR of reaction mixture.

significantly affect the stereoselectivity of the nucleophilic substitution. No noticeable size effects of R group attached to the reacting center were recognized.

Our next concern was to examine the reactivity and the stereocontrolling ability of D-allofuranose template for the dynamic kinetic resolution of α-halo esters in nucleophilic substitution. Nucleophilic substitutions of **21** and **22** with benzylamine were conducted under the same reaction condition as that used for D-glucose derivatives **1** and **6**. Treatment of α-bromo propionate **21** with benzylamine, TBAI, and DIEA gave the substitution product **23** in 81% isolated yield with 71:29 dr (α*S*:α*R*) as shown in Scheme 2. Also, the reaction of α-chloro acetate **22** took place with high stereoselectivity, affording **24** in 69% isolated yield with 90:10 dr (α*S*:α*R*). The ratio of the diastereomeric mixture obtained from each nucleophilic substitution was determined by ^1H NMR analysis. The absolute configurations were assigned after removal of chiral auxiliary by chiral HPLC analysis of enantioenriched methyl *N*-benzyl alaninate and methyl *N*-benzyl phenylglycinate.

A plausible mechanistic rationale for the stereochemical outcomes observed in the nucleophilic substitutions of



Scheme 2. Stereoselective reactions of diacetone-D-allose derivatives **21** and **22**.

D-glucose derivatives and D-allose derivatives is speculated in Figure 1. We propose two transition state structures in which the R group adopts cis conformation relatively to the carbonyl group and hydrogen atom at C-3 of furanose eclipses the carbonyl group, based on the previous mechanistic studies of analogous reaction systems.[1f,h,i] With the shown conformational alignment, the nucleophilic attack of an amine nucleophile to sterically more hindered face could be aided by hydrogen bonding to basic oxygen atom of chiral auxiliary, thus explaining *S*-configurations of products observed in both reactions of D-glucose derivatives and D-allose derivatives. The proposed model by relying on hydrogen bonding is consistent with the poor stereoselectivities of the reactions with thiol nucleophiles and metalated nucleophiles, relatively poor hydrogen bond donor nucleophiles.[7]

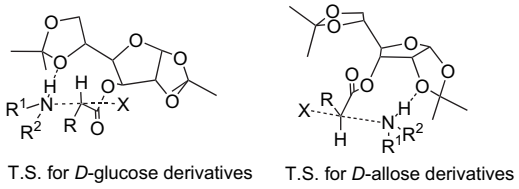
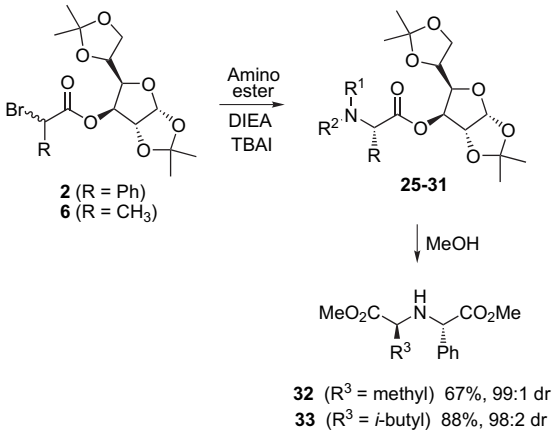


Figure 1. Proposed transition state structures.

Finally, we were pleased to demonstrate that this methodology is also efficient for the substitution with various amino ester nucleophiles, affording 1,1′-iminodicarboxylic acid derivatives **25–33** with high stereoselectivities as shown in Table 4. 1,1′-Iminodicarboxylic acid derivatives are pharmaceutically active as ACE-inhibitors and constitute interesting natural substance.[8] Substantial progress has been made toward the development of efficient methods for stereoselective preparation of these compounds.[9] As shown in Table 4, entry 1, treatment of α-bromo-α-phenyl acetate **2** with glycine methyl ester hydrochloride (1.2 equiv), TBAI (1.0 equiv), and DIEA (2.2 equiv) in CH_2Cl_2 at room temperature provided **25** in 36% yield with 92:8 dr (α*S*:α*R*). Interestingly, much higher stereoselectivities were obtained in the reactions with α-substituted amino ester nucleophiles to give the substituted products **26–31** as shown in entries 2–7. The reaction of **2** with L-alanine methyl ester afforded **26** in a ratio of 98:2 dr (α*S*:α*R*). When D-alanine methyl ester was used as a nucleophile, **27** was obtained in a 99:1 (α*S*:α*R*) ratio. Both L- and D-alanine ester nucleophiles gave the same chirality at the α-center (*S* configuration) and no notable double stereodifferentiation was observed. These results indicate

Table 4. Asymmetric syntheses of 1,1′-iminodicarboxylic acid derivatives



Entry[a]	Nucleophile	R	% Yield[b]	dr[c] (αS:αR)
1	MeO_2C NH_2	Ph	36 (**25**)	92:8
2	MeO_2C NH_2	Ph	55 (**26**)	98:2
3	MeO_2C NH_2	Ph	49 (**27**)	99:1
4	t-BuO_2C NH_2	Ph	67 (**28**)	98:2
5	MeO_2C NH_2	Ph	81 (**29**)	98:2
6	BnO_2C H N	Ph	60 (**30**)	99:1
7	BnO_2C H N	CH_3	32 (**31**)	99:1

[a] All reactions were carried out in CH_2Cl_2 for 24 h at rt.
[b] Isolated yields.
[c] The drs were determined by ^1H NMR of reaction mixture.

that the stereochemistry of the major product is dominated by the asymmetry of diacetone-D-glucose auxiliary and not that of the incoming amino ester nucleophile. Furthermore, we attempted the substitution reaction of α-bromo propionate **6** with proline benzyl ester and found that **31** was afforded in 32% yield with 99:1 dr. The absolute configurations of (αS)-**26** and (αS)-**29** were determined after removing chiral auxiliary with MeOH and Et_3N, by comparison to the ^1H NMR of authentic epimers of (*S*)-**32** and (*S*)-**33** individually prepared from the substitution of methyl (*R*)-α-bromo α-methyl acetate or methyl (*R*)-α-bromo α-isobutyl acetate with L-phenylglycine methyl ester on the basis of inversion mechanism (S_N2). Those of **28**, **30**, and **31** were assigned by analogy to the formation of **26** and **29**. This convenient approach for asymmetric syntheses of 1,1′-iminodicarboxylic acid derivatives appears to offer a substantial advantage over previous methodologies.[9]

3. Conclusion

We have developed a novel and practical method for the asymmetric syntheses of *N*-substituted amino esters via dynamic kinetic resolution of α-halo esters using carbohydrates as a chiral auxiliary. The present results indicate that stereoselectivity is very dependent on both substrate and nucleophile and is also significantly influenced by epimerizing agents. Although the precise mechanism is still unclear, we propose the presence of hydrogen bonding interaction between an amine nucleophile and the chiral auxiliary in the transition state. The methodology has also been successful for highly asymmetric syntheses of 1,1′-iminodicarboxylic acid derivatives. The simple and mild protocol requires no special precautions and can be run on a multigram scale. In the longer term, application to highly stereoselective nucleophilic substitution with a variety of nucleophiles should see more general development and mechanistic analysis.

4. Experimental

4.1. General procedure for the preparation of α-halo esters 1, 2, 5, 6, 8, 9, 21, and 22

*For α-chloro esters **1**, **5**, and **22***: chiral auxiliary (1.0 equiv), racemic α-chloro α-phenyl (or methyl) acetyl chloride (1.0 equiv), and Et_3N (2.2 equiv) were dissolved in CH_2Cl_2 and stirred at room temperature for 2 h. The mixture was treated with extractive work up and the organic phase was dried over $MgSO_4$. Filtration and concentration provided the crude product that was purified by column chromatography on silica gel.

*For α-bromo esters **2**, **6**, **8**, **9**, and **21***: chiral auxiliary (1.0 equiv), racemic α-bromo acid (1.0 equiv), DCC (1.0 equiv), Et_3N (2.2 equiv), and DMAP (0.2 equiv) were dissolved in CH_2Cl_2 and stirred at room temperature for 3 h. The precipitate was filtered off and the organic phase was washed with water. The organic phase was dried over $MgSO_4$, filtered and concentrated to provide the crude product that was purified by column chromatography on silica gel.

4.1.1. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) α-chloro-α-phenyl acetate (1). A colorless oil was obtained in 89% yield as a mixture of two diastereomers. ^1H NMR ($CDCl_3$, 400 MHz, two diastereomers) 7.51–7.36 (m, 5H), 5.85, 5.73 (d, J=3.6 Hz, 1H), 5.38, 5.37 (s, 1H), 5.34, 5.33 (d, J=2.9 Hz, 1H), 4.46, 4.32 (d, J=3.6 Hz, 1H), 4.04–4.13 (m, 2H), 3.88–3.91 (m, 2H), 1.50, 1.39, 1.34, 1.29, 1.26, 1.14 (s, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz, two diastereomers) 167.3, 167.2, 135.7, 135.6, 129.9, 129.3, 128.3, 128.2, 112.9, 109.8, 109.7, 105.5, 105.4, 83.4, 83.3, 80.5, 80.4, 78.1, 78.0, 72.5, 72.3, 67.8, 67.7, 59.5, 59.3, 27.2, 27.1, 27.0, 26.6, 25.5, 25.4; Anal. Calcd for $C_{20}H_{25}ClO_7$: C, 58.12; H, 6.05. Found: C, 58.18; H, 6.10.

4.1.2. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) α-bromo-α-phenyl acetate (2). A pale yellow oil was obtained in 54% yield as a mixture of two diastereomers. ^1H NMR ($CDCl_3$, 400 MHz, two diastereomers) 7.56–7.36 (m, 5H), 5.88, 5.83 (d, J=3.6 Hz, 1H), 5.38, 5.37 (s, 1H), 5.35, 5.34 (d, J=2.9 Hz, 1H), 4.48, 4.42 (d, J=3.6 Hz, 1H), 4.19–3.94 (m, 4H), 1.51, 1.39, 1.36, 1.30, 1.28, 1.26, 1.18 (s, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz, two diastereomers) 167.2, 135.5, 129.9, 129.3, 129.1, 112.9, 109.8, 105.5, 80.4, 78.1, 72.4, 67.7, 46.9, 28.2,

26.6, 25.5; Anal. Calcd for $C_{20}H_{25}BrO_7$: C, 52.53; H, 5.51. Found: C, 52.58; H, 5.64.

4.1.3. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) α-chloro propionate (5). A colorless oil was obtained in 74% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 5.91 (d, *J*=3.6 Hz, 1H), 5.33 (d, *J*=2.6 Hz, 1H), 4.51 (d, *J*=3.7 Hz, 1H), 4.43 (q, *J*=6.9 Hz, 1H), 4.24–4.20 (m, 2H), 4.14–4.10 (m, 1H), 4.00–3.97 (m, 1H), 1.72 (d, *J*=6.9 Hz, 3H), 1.53 (s, 3H), 1.37 (s, 3H), 1.32 (s, 3H), 1.31 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diasteromer) 168.9, 112.8, 109.8, 105.5, 83.4, 80.4, 77.8, 72.7, 67.9, 52.6, 27.2, 27.1, 26.6, 25.5, 21.7; Anal. Calcd for $C_{15}H_{23}ClO_{77}$: C, 51.36; H, 6.61. Found: C, 51.30; H, 6.72.

4.1.4. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) α-bromo propionate (6). A pale yellow oil was obtained in 61% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 5.91 (d, *J*=3.6 Hz, 1H), 5.33 (d, *J*=2.8 Hz, 1H), 4.50 (d, *J*=3.8 Hz, 1H), 4.40 (q, *J*=6.9 Hz, 1H), 4.26–4.22 (m, 2H), 4.15–4.12 (m, 1H), 3.99–3.96 (m, 1H), 1.86 (d, *J*=6.9 Hz, 3H), 1.56 (s, 3H), 1.40 (s, 3H), 1.31 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 169.0, 112.8, 109.8, 105.5, 83.4, 80.5, 80.1, 72.6, 67.9, 39.9, 27.2, 27.1, 26.6, 25.6, 21.8; Anal. Calcd for $C_{15}H_{23}BrO$: C, 45.58; H, 5.87. Found: C, 45.51; H, 5.97.

4.1.5. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) α-bromobutyrate (8). A pale yellow oil was obtained in 45% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 5.91 (m, 1H), 5.34 (m, 1H), 4.49 (m, 1H), 4.22 (m, 4H), 3.99 (m, 1H), 2.04 (m, 2H), 1.53 (s, 3H), 1.40 (s, 3H), 1.32 (s, 3H), 1.31 (s, 3H), 1.04 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 168.5, 112.8, 109.8, 105.5, 83.3, 80.4, 77.8, 72.6, 67.0, 47.5, 28.6, 27.2, 26.6, 25.5, 24.7, 12.2; Anal. Calcd for $C_{16}H_{25}BrO_7$: C, 46.95; H, 6.16. Found: C, 46.96; H, 6.24.

4.1.6. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) α-bromohexanoate (9). A pale yellow oil was obtained in 49% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 5.90 (d, *J*=2.2 Hz, 1H), 5.33 (m, 1H), 4.49 (m, 1H), 4.23 (m, 3H), 4.13 (m, 1H), 4.01 (m, 1H), 2.10 (m, 2H), 1.53 (s, 3H), 1.32 (m, 13H), 0.92 (t, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 168.6, 112.9, 109.8, 105.5, 83.3, 80.3, 77.7, 72.6, 67.9, 45.9, 34.8, 29.6, 27.2, 27.1, 26.6, 25.6, 22.4, 14.2; Anal. Calcd for $C_{18}H_{29}BrO_7$: C, 49.44; H, 6.68. Found: C, 49.52; H, 6.73.

4.1.7. (1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranos-3-*O*-yl) α-bromo propionate (21). A pale yellow oil was obtained in 98% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 5.86 (d, *J*=3.5 Hz, 1H), 4.87–4.86 (m, 2H), 4.43–4.41 (m, 1H), 4.33–4.32 (m, 1H), 4.19–4.18 (m, 1H), 4.11–4.07 (m, 1H), 3.95–3.92 (m, 1H), 1.86 (d, *J*=7.0 Hz, 3H), 1.55 (s, 3H), 1.43 (s, 3H), 1.36 (s, 3H), 1.34 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 169.6, 113.5, 110.4, 104.6, 78.0, 77.8, 75.3, 74.0, 66.0, 40.1, 29.2, 26.7, 25.4, 22.3, 21.9; Anal. Calcd for $C_{15}H_{23}BrO_7$: C, 45.58; H, 5.87. Found: C, 45.65; H, 5.81.

4.1.8. (1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranos-3-*O*-yl) α-chloro-α-phenyl acetate (22). A colorless oil was obtained in 99% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, two diastereomers) 7.52–7.37 (m, 5H), 5.83, 5.81 (d, *J*=3.8 Hz, 1H), 5.44–5.42 (s, 1H), 4.89–4.81 (m, 2H), 4.29–4.05 (m, 1H), 3.90–3.59 (m, 3H), 1.60–1.22 (s, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 167.7, 135.9, 129.7, 129.2, 128.7, 113.6, 110.4, 104.8, 78.3, 75.5, 75.0, 74.3, 66.2, 59.1, 27.3, 26.7, 26.5, 25.5, 21.4; Anal. Calcd for $C_{20}H_{25}ClO_7$: C, 58.18; H, 6.10. Found: C, 58.21; H, 6.05.

4.2. General procedure for the asymmetric preparation of 3, 7, 10–20, 23–31

To a solution of α-halo ester (**1**, **2**, **5**–**9**, **21**, and **22**) in CH_2Cl_2 (ca. 0.1 M) at room temperature were added DIEA (1.0 equiv), TBAI (1.0 equiv), and a nucleophile (1.2 equiv). After the resulting reaction mixture was stirred at room temperature for 12 h, the solvent was evaporated and the crude material was purified by column chromatography to give a α-amino ester. The dr of the product was determined by ^{1}H NMR integration of anomeric or α-hydrogens of two diastereomers.

4.2.1. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) *N*-benzyl-(*S*)-phenylglycinate (3). A colorless oil was obtained in 86% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 7.38–7.25 (m, 10H), 5.56 (d, *J*=3.6 Hz, 1H), 5.33 (d, *J*=2.6 Hz, 1H), 4.41 (s, 1H), 4.18–3.97 (m, 5H), 3.76 (d, *J*=4.0 Hz, 2H), 2.34 (br, 1H), 1.48 (s, 3H), 1.41 (s, 3H), 1.31 (s, 3H), 1.22 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 172.1, 139.8, 138.0, 129.2, 128.9, 128.8, 128.1, 127.8, 127.7, 112.8, 109.8, 105.4, 83.3, 80.3, 77.8, 76.8, 72.8, 67.7, 64.7, 51.8, 27.3, 27.2, 26.6, 25.7; HRMS (ESI) calcd for $C_{27}H_{34}NO_7$ (M^++1): 484.2335. Found: 484.2340.

4.2.2. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) *N*-benzyl-(*S*)-alaninate (7). A colorless oil was obtained in 90% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 7.33–7.25 (m, 5H), 5.86 (d, *J*=3.6 Hz, 1H), 5.39 (d, *J*=2.6 Hz, 1H), 4.42 (d, *J*=3.7 Hz, 1H), 4.21–4.18 (m, 2H), 4.11–4.07 (m, 1H), 4.03–4.00 (m, 1H), 3.81 (d, *J*=12.9 Hz, 1H), 3.68 (d, *J*=12.9 Hz, 1H), 3.44 (q, *J*=7.0 Hz, 1H), 1.81 (br, 1H), 1.53 (s, 3H), 1.42 (s, 3H), 1.32 (s, 3H), 1.31 (s, 3H), 1.29 (d, *J*=7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 174.8, 139.9, 128.9, 128.6, 127.6, 112.8, 109.9, 105.5, 83.9, 80.6, 76.4, 72.7, 67.9, 56.2, 52.3, 25.3, 27.2, 26.6, 25.7, 19.5; Anal. Calcd for $C_{22}H_{31}NO_7$: C, 62.69; H, 7.41; N, 3.32. Found: C, 62.86; H, 7.44; N; 3.12. For removal of chiral auxiliary, the mixture of **7** and Et_3N (15 equiv) in methanol (0.03 M) was stirred for 2 days. The solvent was evaporated and the crude material was purified by column chromatography to give methyl (*N*-benzyl) (*S*)-alaninate in 66% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) 7.32–7.23 (m, 5H), 3.80 (d, *J*=12.8 Hz, 1H), 3.72 (s, 3H), 3.67 (d, *J*=12.8 Hz, 1H), 3.39 (q, *J*=7.0 Hz, 1H), 1.85 (br, 1H), 1.32 (d, *J*=7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) 176.6, 140.1, 128.8, 128.6, 127.5, 56.3, 52.4, 52.2, 19.5. Chiral HPLC: 79:21 er, t_R (*S*)-major enantiomer, 5.1 min;

t_R (*R*)-minor enantiomer, 5.6 min (Chiralcel OD column; 10% 2-propanol in hexane; 0.5 mL/min).

4.2.3. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) *N*-isopropyl-(*S*)-alaninate (10). A colorless oil was obtained in 70% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 5.89 (d, *J*=3.5 Hz, 1H), 5.37 (d, *J*=2.0 Hz, 1H), 4.43 (d, *J*=3.7 Hz, 1H), 4.19–4.11 (m, 3H), 4.00 (m, 1H), 3.49 (q, *J*=7.0 Hz, 1H), 2.78 (m, 1H), 1.75 (br, 1H), 1.53 (s, 3H), 1.40 (s, 3H), 1.32 (m, 9H), 1.06, 1.02 (d, *J*=6.3 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 175.3, 112.8, 109.8, 105.5, 83.8, 80.6, 76.3, 72.7, 68.0, 54.3, 47.1, 27.2, 26.6, 25.6, 24.2, 22.4, 22.3, 20.0; Anal. Calcd for $C_{18}H_{31}NO_7$: C, 57.89; H, 8.37; N, 3.75. Found: C, 57.89; H, 8.50; N, 3.58.

4.2.4. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) *N*-butyl-(*S*)-alaninate (11). A colorless oil was obtained in 80% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 5.89 (d, *J*=3.5 Hz, 1H), 5.36 (d, *J*=2.6 Hz, 1H), 4.44 (d, *J*=3.7 Hz, 1H), 4.19 (m, 2H), 4.10 (m, 1H), 4.01 (m, 1H), 3.38 (q, *J*=7.0 Hz, 1H), 2.58 (m, 1H), 2.51 (m, 1H), 1.68 (br, 1H), 1.53 (s, 3H), 1.45 (m, 2H), 1.40 (s, 3H), 1.38–1.30 (m, 2H), 1.31 (s, 3H), 1.30 (s, 3H), 0.91 (t, *J*=7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 174.9, 112.8, 109.7, 105.6, 83.9, 80.5, 76.3, 72.7, 67.9, 57.0, 47.9, 32.7, 27.2, 27.1, 26.6, 25.6, 20.7, 19.4, 14.3; Anal. Calcd for $C_{19}H_{33}NO_7$: C, 58.90; H, 8.58; N, 3.61. Found: C, 58.88; H, 8.61; N, 3.51.

4.2.5. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) *N*-*p*-methoxyphenyl-(*S*)-alaninate (12). A colorless oil was obtained in 85% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 6.78 (d, *J*=8.8 Hz, 2H), 6.61 (d, *J*=8.9 Hz, 2H), 5.59 (d, *J*=3.6 Hz, 1H), 5.25 (d, *J*=1.8 Hz, 1H), 4.18 (m, 5H), 4.00 (m, 1H), 3.76 (m, 1H), 3.72 (s, 3H), 1.48 (m, 6H), 1.39 (s, 3H), 1.30 (s, 3H), 1.89 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 173.8, 153.4, 141.1, 115.7, 115.3, 112.7, 109.8, 105.5, 83.5, 80.2, 76.6, 72.8, 68.0, 56.1, 54.0, 27.2, 27.1, 26.4, 25.6, 19.1; Anal. Calcd for $C_{22}H_{31}NO_8$: C, 60.40; H, 7.14; N, 3.20. Found: C, 60.60; H, 7.09; N, 3.09.

4.2.6. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) *N*-dibenzyl-(*S*)-alaninate (13). A colorless oil was obtained in 48% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 7.39–7.23 (m, 10H), 5.84 (d, *J*=3.6 Hz, 1H), 5.35 (d, *J*=2.9 Hz, 1H), 4.42 (d, *J*=3.6 Hz, 1H), 4.20 (m, 2H), 4.09 (m, 1H), 4.05 (m, 1H), 3.84 (d, *J*=14.1 Hz, 2H), 3.68 (d, *J*=14.0 Hz, 2H), 3.55 (q, *J*=7.1 Hz, 1H), 1.53 (s, 3H), 1.44 (s, 3H), 1.24 (m, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 172.9, 140.1, 140.0, 129.0, 128.7, 128.6, 127.5, 127.4, 112.8, 109.8, 105.5, 84.0, 80.4, 76.5, 72.8, 68.1, 56.8, 56.6, 54.7, 27.4, 27.2, 26.5, 25.7, 15.5; Anal. Calcd for $C_{29}H_{37}NO_7$: C, 68.08; H, 7.29; N, 2.74. Found: C, 68.05; H, 7.15; N, 2.71.

4.2.7. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) *N*-benzyl-*N*-methyl-(*S*)-alaninate (14). A colorless oil was obtained in 91% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 7.34–7.24 (m, 5H), 5.87 (d, *J*=3.6 Hz, 1H), 5.34 (d, *J*=2.9 Hz, 1H), 4.45 (d, *J*=3.7 Hz, 1H), 4.21 (m, 2H), 4.11 (m, 1H), 4.01 (m, 1H), 3.75 (d, *J*=13.6 Hz, 1H), 3.64 (d, *J*=13.5 Hz, 1H), 3.49 (q, *J*=7.1 Hz, 1H), 2.30 (s, 3H), 1.53 (s, 3H), 1.41 (s, 3H), 1.35 (d, *J*=7.1 Hz, 3H), 1.34 (s, 3H), 1.27 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 172.5, 139.7, 129.0, 128.7, 127.4, 112.8, 109.8, 105.5, 84.0, 80.5, 76.4, 72.8, 68.0, 61.1, 58.7, 38.1, 27.2, 27.1, 26.6, 25.6, 15.4; Anal. Calcd for $C_{23}H_{33}NO_7$: C, 63.43; H, 7.64; N, 3.22. Found: C, 63.43; H, 7.68; N, 3.13.

4.2.8. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) (*S*)-α-(3,4-dihydro-2(1*H*)-isoquinolinyl)propionate (15). A pale yellow oil was obtained in 92% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 7.11–6.98 (m, 4H), 5.88 (d, *J*=3.7 Hz, 1H), 5.34 (d, *J*=2.9 Hz, 1H), 4.47 (d, *J*=3.7 Hz, 1H), 4.23–4.17 (m, 2H), 4.13–4.09 (m, 1H), 4.01–3.97 (m, 1H), 3.85–3.83 (m, 2H), 3.57 (q, *J*=7.0 Hz, 1H), 3.03–3.01 (m, 1H), 2.88–2.82 (m, 3H), 1.52 (s, 3H), 1.43 (d, *J*=7.0 Hz, 3H), 1.37 (s, 3H), 1.30 (s, 3H), 1.21 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 172.1, 135.1, 134.7, 129.2, 126.9, 126.5, 125.9, 112.8, 109.8, 105.5, 84.0, 80.5, 76.5, 72.8, 68.9, 62.5, 54.4, 47.6, 30.2, 27.2, 27.1, 26.6, 25.5, 15.4; Anal. Calcd for $C_{24}H_{33}NO_7$: C, 64.41; H, 7.43; N, 3.13. Found: C, 64.41; H, 7.54; N, 3.07.

4.2.9. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) α-(*N*-benzylamino)butyrate (16). A colorless oil was obtained in 77% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 7.34–7.25 (m, 5H), 5.85 (d, *J*=3.7 Hz, 1H), 5.40 (d, *J*=2.4 Hz, 1H), 4.41 (d, *J*=3.7 Hz, 1H), 4.21–4.17 (m, 2H), 4.11–4.08 (m, 1H), 4.03–4.00 (m, 1H), 3.83 (d, *J*=12.9 Hz, 1H), 3.63 (d, *J*=13.0 Hz, 1H), 3.23 (t, 1H), 1.70 (m, 2H), 1.53 (s, 3H), 1.42 (s, 3H), 1.32–1.26 (m, 6H), 0.94 (t, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 174.5, 140.2, 128.8, 128.6, 127.5, 112.9, 109.9, 105.7, 83.9, 80.6, 76.6, 72.8, 68.2, 62.4, 52.6, 27.3, 27.2, 27.1, 26.7, 25.7, 10.7; Anal. Calcd for $C_{23}H_{33}NO_7$: C, 63.43; H, 7.64; N, 3.22. Found: C, 63.66; H, 7.56; N, 3.01.

4.2.10. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) α-(*N*-*p*-methoxyphenylamino)butyrate (17). A colorless oil was obtained in 99% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 6.78 (d, *J*=8.9 Hz, 2H), 6.62 (d, *J*=8.9 Hz, 2H), 5.57 (d, *J*=3.6 Hz, 1H), 5.26 (d, *J*=2.1 Hz, 1H), 4.16–4.09 (m, 3H), 4.02–3.97 (m, 3H), 3.75 (br, 1H), 3.72 (s, 1H), 1.87–1.84 (m, 2H), 1.48 (s, 3H), 1.39 (s, 3H), 1.29 (m, 3H), 1.22 (s, 3H), 1.05 (t, *J*=7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 173.1, 153.4, 141.3, 115.3, 115.2, 112.7, 109.8, 105.6, 83.5, 80.3, 76.5, 72.8, 67.9, 60.1, 56.1, 27.2, 27.1, 26.6, 26.3, 25.6, 10.6; Anal. Calcd for $C_{23}H_{33}NO_8$: C, 61.18; H, 7.37; N, 3.10. Found: C, 61.17; H, 7.44; N, 3.39.

4.2.11. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) (*S*)-α-(3,4-dihydro-2(1*H*)-isoquinolinyl)butyrate (18). A colorless oil was obtained in 49% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major

diastereomer) 7.26–6.98 (m, 4H), 5.86 (d, J=3.7 Hz, 1H), 5.35 (d, J=2.9 Hz, 1H), 4.45 (d, J=3.6 Hz, 1H), 4.24–4.12 (m, 3H), 4.01–3.98 (m, 1H), 3.90–3.78 (m, 2H), 3.35 (m, 1H), 3.06–3.03 (m, 1H), 2.88–2.76 (m, 3H), 1.82 (m, 2H), 1.52 (s, 3H), 1.37 (s, 3H), 1.30 (s, 3H), 1.21 (s, 3H), 0.98 (t, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 171.4, 135.4, 134.8, 129.2, 126.9, 126.5, 126.0, 110.1, 112.9, 105.5, 84.0, 80.6, 76.4, 72.7, 69.4, 68.2, 52.6, 47.4, 30.3, 27.2, 26.6, 25.5, 23.2, 23.1, 11.2; HRMS (ESI) calcd for $C_{25}H_{36}NO_7$ (M^++1): 462.2492. Found: 462.2492.

4.2.12. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) (*S*)-α-(*N*-benzylamino)hexanoate (19). A colorless oil was obtained in 40% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 7.33–7.24 (m, 5H), 5.85 (d, J=3.6 Hz, 1H), 5.39 (d, J=2.3 Hz, 1H), 4.41 (d, J=3.6 Hz, 1H), 4.20 (m, 2H), 4.09 (m, 1H), 4.02 (m, 1H), 3.81 (d, J=12.9 Hz, 1H), 3.64 (d, J=12.9 Hz, 1H), 3.27 (t, 1H), 1.77–1.53 (m, 6H), 1.42–1.27 (m, 12H), 0.88 (t, J=7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 174.7, 140.2, 128.8, 128.7, 127.5, 112.8, 109.9, 105.5, 83.8, 80.5, 76.7, 72.8, 68.1, 61.1, 52.4, 33.7, 28.3, 27.3, 26.7, 25.7, 22.9, 14.3; HRMS (ESI) calcd for $C_{25}H_{38}NO_7$ (M^++1): 464.2648. Found: 464.2646.

4.2.13. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) (*S*)-α-(*N*-*p*-methoxyphenylamino)hexanoate (20). A pale yellow oil was obtained in 64% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 6.76 (d, J=8.8 Hz, 2H), 6.61 (d, J=8.8 Hz, 2H), 5.54 (d, J=3.6 Hz, 1H), 5.25 (d, J=2.2 Hz, 1H), 4.24–3.98 (m, 6H), 3.72 (s, 3H), 1.78 (m, 2H), 1.47–1.34 (m, 4H), 1.46 (s, 3H), 1.39 (s, 3H), 1.29 (s, 3H), 1.21 (s, 3H), 0.92 (t, J=7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 174.7, 153.4, 141.4, 115.6, 115.3, 112.7, 109.8, 105.5, 83.4, 80.3, 76.5, 72.8, 67.9, 58.9, 56.1, 33.2, 28.2, 27.2, 27.1, 26.4, 25.6, 22.8, 14.3; Anal. Calcd for $C_{25}H_{37}NO_8$: C, 62.61; H, 7.78; N, 2.92. Found: C, 62.58; H, 7.86; N, 2.63.

4.2.14. (1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranos-3-*O*-yl) *N*-benzyl-(*S*)-alaninate (23). A colorless oil was obtained in 81% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 7.36–7.25 (m, 5H), 5.84 (d, J=3.6 Hz, 1H), 4.90 (m, 2H), 4.30 (m, 1H), 4.17 (m, 1H), 4.07 (m, 1H), 3.90 (m, 2H), 3.70 (d, J=12.9 Hz, 1H), 3.47 (q, J=7.0 Hz, 1H), 1.92 (br, 1H), 1.58 (s, 3H), 1.47 (s, 3H), 1.35 (m, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 175.4, 140.2, 128.8, 128.7, 127.5, 113.4, 110.4, 104.6, 78.2, 75.5, 73.5, 66.2, 56.4, 55.8, 52.3, 27.2, 27.1, 26.7, 25.3, 19.2; Anal. Calcd for $C_{22}H_{31}NO_7$: C, 62.69; H, 7.41; N, 3.32. Found: C, 62.66; H, 7.57; N, 3.18.

4.2.15. (1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranos-3-*O*-yl) *N*-benzyl-(*S*)-phenylglycinate (24). A colorless oil was obtained in 69% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 7.40–7.25 (m, 10H), 5.83 (d, J=3.9 Hz, 1H), 4.92–4.81 (m, 2H), 4.53 (s, 1H), 4.15–4.06 (m, 2H), 3.81 (m, 3H), 3.38 (m, 1H), 2.34 (br, 1H), 1.56 (s, 3H), 1.34 (s, 3H), 1.24 (s, 3H), 1.13 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 172.6, 139.9, 138.0, 129.1, 128.8, 128.7, 128.4, 128.1, 127.5, 113.4, 110.2, 104.8, 78.1, 77.9, 75.6, 73.8, 65.6, 64.4, 51.7, 27.3, 26.7, 26.4, 25.5; Anal. Calcd for $C_{27}H_{33}NO_7$: C, 67.06; H, 6.88; N, 2.90. Found: C, 66.93; H, 7.08; N, 2.87.

4.2.16. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) *N*-[1-(methoxycarbonyl) methyl]-(*S*)-phenylglycinate (25). A colorless oil was obtained in 36% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 7.39–7.27 (m, 5H), 5.54 (d, J=3.7 Hz, 1H), 5.32 (d, J=2.4 Hz, 1H), 4.51 (s, 1H), 4.19–4.05 (m, 4H), 3.99–3.96 (m, 1H), 3.71 (s, 3H), 3.40 (d, J=3.6 Hz, 2H), 2.45 (br, 1H), 1.47 (s, 3H), 1.41 (s, 3H), 1.32 (s, 3H), 1.22 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 172.6, 171.3, 137.3, 129.3, 129.0, 128.0, 112.8, 109.8, 105.4, 83.3, 80.3, 76.9, 72.8, 67.7, 64.9, 52.3, 48.4, 27.2, 27.1, 26.6, 25.6; HRMS (ESI) calcd for $C_{23}H_{32}NO_9$ (M^++1): 466.2077. Found: 466.2054.

4.2.17. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) *N*-[(*S*)-1-(methoxycarbonyl)ethyl]-(*S*)-phenylglycinate (26). A colorless oil was obtained in 55% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 7.40–7.28 (m, 5H), 5.56 (d, J=3.6 Hz, 1H), 5.33 (d, J=2.2 Hz, 1H), 4.47 (s, 1H), 4.19–4.06 (m, 4H), 3.99–3.96 (m, 1H), 3.64 (s, 3H), 3.44 (q, J=6.4 Hz, 1H), 2.40 (br, 1H), 1.47 (s, 3H), 1.41 (s, 3H), 1.32 (m, 6H), 1.22 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 175.5, 171.6, 137.7, 129.2, 128.9, 127.9, 112.7, 109.8, 105.4, 83.3, 80.3, 76.8, 72.8, 67.7, 64.9, 54.9, 52.3, 27.2, 27.1, 26.6, 25.6, 19.2; HRMS (ESI) calcd for $C_{24}H_{34}NO_9$ (M^++1): 480.2234. Found: 480.2225.

4.2.18. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) *N*-[(*R*)-1-(methoxycarbonyl)ethyl]-(*S*)-phenylglycinate (27). A colorless oil was obtained in 49% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 7.38–7.29 (m, 5H), 5.48 (d, J=3.6 Hz, 1H), 5.26 (d, J=2.4 Hz, 1H), 4.46 (s, 1H), 4.19 (m, 2H), 4.07 (m, 2H), 3.95 (m, 1H), 3.71 (s, 3H), 3.23 (q, J=7.0 Hz, 1H), 2.65 (br, 1H), 1.47 (s, 3H), 1.40 (s, 3H), 1.33 (m, 6H), 1.21 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 175.7, 171.4, 137.8, 129.1, 128.8, 128.1, 112.8, 109.7, 105.3, 83.2, 80.1, 77.0, 72.8, 67.5, 63.8, 54.0, 52.3, 27.2, 27.1, 26.6, 25.6, 19.5; HRMS (ESI) calcd for $C_{24}H_{34}NO_9$ (M^++1): 480.2234. Found: 480.2220.

4.2.19. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) *N*-[(*S*)-1-(*tert*-butoxycarbonyl)ethyl]-(*S*)-phenylglycinate (28). A colorless oil was obtained in 67% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 7.40–7.28 (m, 5H), 5.57 (d, J=3.6 Hz, 1H), 5.33 (d, 1H), 4.47 (s, 1H), 4.18–4.06 (m, 4H), 3.99 (m, 1H), 3.30 (q, J=6.8 Hz, 1H), 2.36 (br, 1H), 1.47–1.37 (m, 15H), 1.32–1.26 (m, 6H), 1.21 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 174.4, 171.6, 138.0, 129.2, 128.8, 127.9, 112.7, 109.7, 105.4, 83.3, 81.5, 80.3, 76.7, 72.8, 67.6, 64.3, 55.5, 28.4, 27.2, 27.1, 26.6, 25.6, 19.3; HRMS (ESI) calcd for $C_{27}H_{40}NO_9$ (M^++1): 522.2703. Found: 522.2692.

4.2.20. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) *N*-[(*S*)-1-(methoxycarbonyl)-3-methylbutyl]-(*S*)-phenylglycinate (29). A colorless oil was obtained in 81%

yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 7.40–7.30 (m, 5H), 5.57 (d, J=3.6 Hz, 1H), 5.33 (d, J=2.0 Hz, 1H), 4.41 (s, 1H), 4.15 (m, 2H), 4.12 (m, 1H), 4.07 (m, 1H), 3.99 (m, 1H), 3.61 (s, 3H), 3.39 (t, J=7.1 Hz, 1H), 2.19 (br, 1H), 1.83 (m, 1H), 1.52–1.47 (m, 5H), 1.41 (s, 3H), 1.32 (s, 3H), 1.22 (s, 3H), 0.93 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 175.9, 171.5, 138.1, 129.1, 128.9, 128.0, 112.7, 109.8, 105.4, 83.3, 80.3, 76.7, 72.8, 67.6, 64.6, 58.5, 52.0, 43.0, 27.2, 27.1, 26.6, 25.5, 25.2, 23.2, 22.5; HRMS (ESI) calcd for $C_{27}H_{40}NO_9$ (M^++1): 522.2703. Found: 522.2687.

4.2.21. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) (*S*)-α-[(*S*)-2-(benzoxycarbonyl)-1-pyrrolidinyl]-α-phenylacetate (30). A colorless oil was obtained in 60% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 7.40–7.29 (m, 10H), 5.64 (d, J=3.6 Hz, 1H), 5.34 (d, J=2.8 Hz, 1H), 5.12 (d, J=12.6 Hz, 1H), 5.04 (d, J=12.3 Hz, 1H), 4.70 (s, 1H), 4.23 (d, J=3.6 Hz, 1H), 4.16 (m, 2H), 4.00 (m, 2H), 3.77 (m, 1H), 2.86 (m, 2H), 2.14 (m, 1H), 2.01 (m, 1H), 1.79 (m, 2H), 1.48 (s, 3H), 1.38 (s, 3H), 1.25 (s, 3H), 1.23 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 174.3, 170.6, 137.0, 136.3, 129.1, 129.0, 128.9, 128.7, 112.7, 109.7, 105.4, 83.4, 80.4, 76.5, 72.8, 68.5, 67.6, 66.7, 63.1, 49.9, 30.3, 27.2, 27.1, 26.6, 25.6, 24.0; HRMS (ESI) calcd for $C_{32}H_{40}NO_9$ (M^++1): 582.2703. Found: 582.2700.

4.2.22. (1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl) (*S*)-α-[(*S*)-2-(benzoxycarbonyl)-1-pyrrolidinyl]-propionate (31). A colorless oil was obtained in 32% yield as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz, major diastereomer) 7.38–7.31 (m, 5H), 5.86 (d, J=3.7 Hz, 1H), 5.29 (d, J=2.4 Hz, 1H), 5.19 (d, J=12.7 Hz, 1H), 5.12 (d, J=12.7 Hz, 1H), 4.44 (d, J=3.6 Hz, 1H), 4.14 (m, 2H), 3.97 (m, 2H), 3.78 (q, J=7.2 Hz, 1H), 3.72 (dd, J=5.4, 8.8 Hz, 1H), 3.14 (m, 1H), 2.86 (q, J=7.9 Hz, 1H), 2.09 (m, 1H), 1.98 (m, 1H), 1.85 (m, 2H), 1.52 (s, 3H), 1.39 (d, 3H), 1.38 (s, 3H), 1.30 (s, 3H), 1.23 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz, major diastereomer) 174.5, 172.3, 136.4, 129.0, 128.6, 128.5, 112.7, 109.8, 105.5, 83.9, 80.4, 76.4, 72.8, 67.9, 66.7, 63.1, 57.7, 47.5, 30.1, 27.2, 27.1, 26.6, 25.5, 24.2, 17.4; HRMS (ESI) calcd for $C_{27}H_{38}NO_9$ (M^++1): 520.2547. Found: 520.2551.

4.3. General procedure for the removal of chiral auxiliary

The mixture of diisopropylidene-α-D-glucofuranosyl *N*-substituted α-amino ester and Et_3N (15 equiv) in methanol (0.03 M) was stirred for 2 days. The solvent was evaporated and the crude material was purified by column chromatography to give a product.

4.3.1. Methyl (*N*-benzyl)-(*S*)-phenylglycinate (4). A colorless oil was obtained in 67% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) 7.39–7.25 (m, 1H), 4.40 (s, 1H), 3.73 (s, 2H), 3.68 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) 173.9, 139.9, 138.5, 129.1, 128.8, 128.7, 128.5, 128.0, 127.6. Chiral HPLC: 96:4 er t_R (*S*)-major enantiomer, 11.1 min; t_R (*R*)-minor enantiomer, 12.1 min (Chiralcel OD column; 10% 2-propanol in hexane; 0.5 mL/min).

4.3.2. *N*-[(*S*)-2-Methoxy-2-oxoethyl-1-phenyl]-(*S*)-alanine methyl ester (32). A colorless oil was obtained in 99% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) 7.39–7.27 (m, 5H), 4.45 (s, 1H), 3.70 (s, 3H), 3.64 (s, 3H), 3.39 (q, J=6.9 Hz, 1H), 2.45 (br, 1H), 1.34 (d, J=6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) 175.6, 173.4, 138.1, 129.2, 128.7, 128.1, 64.1, 54.9, 52.7, 52.3, 19.1. HRMS (ESI) calcd for $C_{13}H_{18}NO_4$ (M^++1): 252.1236. Found: 252.1236.

4.3.3. *N*-((*S*)-2-Methoxy-2-oxoethyl-1-phenyl)-(*S*)-leucine methyl ester (33). A colorless oil was obtained in 88% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) 7.39–7.26 (m, 5H), 4.39 (s, 1H), 3.69 (s, 3H), 3.59 (s, 3H), 3.36 (t, J=7.2 Hz, 1H), 2.24 (br, 1H), 1.80 (m, 1H), 1.48 (m, 2H), 0.92 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) 175.9, 173.3, 138.4, 129.1, 128.7, 128.1, 64.6, 58.5, 52.6, 52.1, 43.1, 25.2, 23.2, 22.6; HRMS (ESI) calcd for $C_{16}H_{24}NO_4$ (M^++1): 294.1705. Found: 294.1704.

Acknowledgements

This paper was supported by a grant from Korea Research Foundation (KRF-2004-F00019).

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4. Kim, H. J.; Shin, E.-k.; Chang, J.-y.; Kim, Y.; Park, Y. S. *Tetrahedron Lett.* **2005**, *46*, 4115.
5. The configurational stability of **3** (83:17 dr) was examined by the treatment with $BnNH_2$ (1.2 equiv), TBAI (1.0 equiv) and DIEA (1.0 equiv) in CH_2Cl_2 for 24 h. No epimerization was detected by ^1H NMR, which can rule out the possibility of epimerization after the nucleophilic substitution reaction.
6. When we prepared diacetone-D-glucose bound α-halo esters from racemic α-halo carboxylic acid derivatives, we obtained **5** and **6** with 69:31 dr and 73:27 dr, respectively. Formation of anything other than a 1:1 mixture of diastereomers can be explained by dynamic resolution of α-halo acids in the ester bond formation with chiral auxiliary. Alternatively, the in situ preparation of a ketene from α-halo acid halides and its treatment with a chiral auxiliary can afford diastereomerically enriched α-halo esters. For examples, see: (a) Durst, T.; Koh, K. *Tetrahedron Lett.* **1992**, *33*, 6799; (b) Camps, P.; Pérez, F.; Soldevilla, N. *Tetrahedron: Asymmetry* **1997**, *11*, 1877; (c) Cardillo, G.; Fabbroni, S.; Gentilucci, L.; Perciaccante, R.; Tolomelli, A. *Tetrahedron: Asymmetry* **2004**, *15*, 593. The 55:45 diastereomeric mixture of **6** is prepared by column chromatography with fractional collection.
7. When nucleophiles such as tritylthiol, sodium malonate, sodium 3,5-dimethoxyphenoxides, and potassium 6-flavonoxide were used for the reaction with **1** or **2**, the corresponding substitution products were obtained in 76–94% yields with diastereomeric ratios from 69:31 to 54:46.
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Tetrahedron 62 (2006) 6312–6320
Tetrahedron

Sequential addition reaction of lithium acetylides and Grignard reagents to thioiminium salts from thiolactams leading to 2,2-disubstituted cyclic amines

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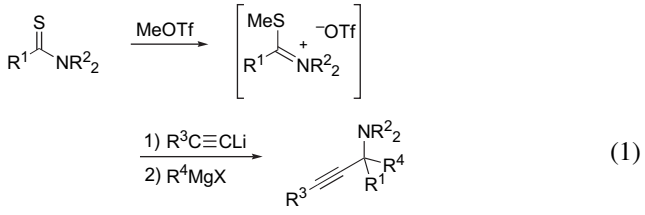
Received 20 March 2006; revised 13 April 2006; accepted 14 April 2006
Available online 12 May 2006

Abstract—The reaction of thioiminium salts derived from γ- and δ-thiolactams with lithium acetylides and Grignard reagents proceeded sequentially to give 2,2-disubstituted pyrrolidines and piperidines in moderate to high yields. In the initial step of the reaction, 2-(methylthio)-pyrrolidines and -piperidines may be formed. The use of lithium (trimethylsilyl)acetylide gave the products most effectively. Aryl-, alkyl-, and allylmagnesium halides were used as Grignard reagents. Silylcarbocyclization of *N*-allyl 2-ethynyl cyclic amines with $HSiMe_2Ph$ in the presence of a catalytic amount of $Rh_4(CO)_{12}$ was carried out to give trisubstituted hexahydro-1*H*-pyrrolizines and octahydroindolizines.

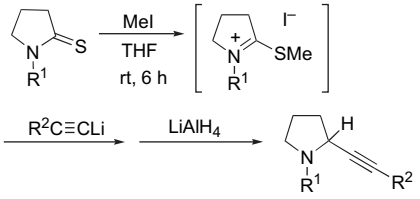
1. Introduction

The development of new carbon–carbon bond-forming reactions, in which multiple components are coupled in one operation, is needed.[1] In this context, we have recently reported the sequential addition reactions of lithium acetylides and Grignard reagents to thioiminium salts derived from acyclic thioamides[2] (Eq. 1) during the course of our studies on thioamides.[3]



(1)

In those studies, two different carbon nucleophiles were selectively introduced to thioiminium salts, and no products, to which the same carbon nucleophiles were doubly introduced, were observed. Cyclic thioamides, i.e., γ- and δ-thiolactams, can be used as a substrate in Eq. 1. In fact, the methylation of γ-thiolactams with methyl iodide has been reported to take place.[4] Alkynylation of the resulting thioiminium salts followed by reduction gives 2-alkynyl pyrrolidines (Eq. 2).[4a]



(2)

In this case, about 6 h was necessary for methylation of the thiolactams. Due to the higher reactivity of MeOTf, the methylation of thiolactams is expected to be complete within a shorter reaction time. Furthermore, the introduction of two different carbon nucleophiles to thioiminium salts derived from thiolactams can lead to 2-alkynyl-2-substituted pyrrolidines and piperidines, which are not readily available by ordinary synthetic methods. Several examples of the alkynylation of cyclic imines and iminium salts leading to 2-alkynyl pyrrolidines[5] and piperidines[6] are known, but reactions leading to 2-alkynyl-2-substituted pyrrolidines[7] and piperidines[7a,8] are rare. We report here sequential addition reactions of lithium acetylides and Grignard reagents to thioiminium salts derived from γ- and δ-thiolactams. Additionally, silylcarbocyclization was applied to the obtained *N*-allyl 2-ethynyl-2-substituted cyclic amines.

2. Results and discussion

Initially, methylation of *N*-methyl γ-thiolactam **1a** with methyl triflate (MeOTf) was carried out (Eq. 3). This reaction went to completion almost instantly to give thioiminium salt **2a**. Lithium (trimethylsilyl)acetylide (**3a**) (1.5 equiv) and phenylmagnesium bromide (**4a**) (2 equiv) were then added to an Et_2O solution of **2a** to give

Keywords: Thiolactams; Thioiminium salts; Lithium acetylides; Grignard reagents; 2,2-Disubstituted cyclic amines; Silylcarbocyclization.
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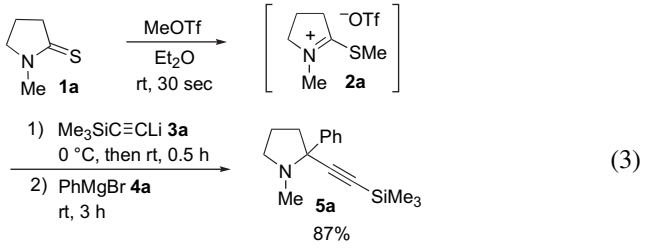
doi:10.1016/j.tet.2006.04.065

N-methyl-2-alkynyl-2-phenylpyrrolidine **5a** in 87% yield. 2-(Methylthio)pyrrolidine **6a**[9] is probably formed in the step of the addition of **3a**. In the next step, the substitution reaction occurs at the carbon atom bearing nitrogen and sulfur atoms with Grignard reagent **4a**. As expected, products derived from 2 equiv of the identical carbon nucleophiles were not observed, and 2 equiv or one more equiv of Grignard reagent **4a** was enough to efficiently substitute the MeS group in **6a**. In the first step, thioiminium salt **2a** appears to react with more ionic organolithium reagents, whereas in the second step, the coordination of the sulfur atom to the Lewis acidic magnesium metal may facilitate the reaction of Grignard reagents.

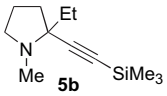
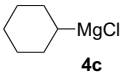
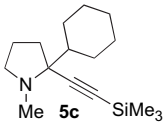
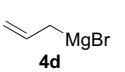
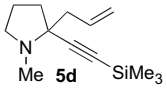
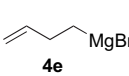
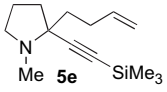
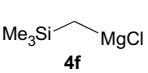
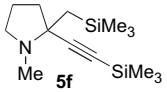


(3)

Next, a variety of Grignard reagents **4** were used for the sequential reaction. The results are summarized in Table 1. Ethyl-, cyclohexyl-, 2-propenyl-, 3-butenyl-, and silylmethylmagnesium halides **4b**–**4f** were used as aliphatic Grignard reagents. In all cases, the reaction proceeded smoothly to give the corresponding 2-alkynyl-2-alkylpyrrolidines **5b**–**5f** in good to high yields. The usual aqueous workup of the reaction mixture followed by concentration of the organic layers gave products **5** with high purity except for the reaction with **4f**.

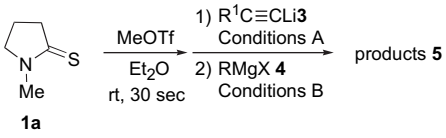
Lithium acetylides **3b**–**3e** derived from phenylacetylene, 2-methyl-1-butene-3-yne, 1-hexyne, and 1-ethynylcyclohexene were also used for the sequential addition reaction to **2a** (Eq. 4). The results are summarized in Table 2. As in the reaction with lithium silylacetylide **3a**, thioiminium salt **2a** was stirred with lithium phenylacetylide (**3b**) at 0 °C–room temperature for 30 min. To the reaction mixture was then added Grignard reagents **4a**, **4b**, and **4f**, and the mixture was stirred at room temperature for 3 h, but the desired products **5g**–**5i** were obtained in at most 40% yield (entries 1, 3, and 4). To confirm the efficiency of the generation of 2-(methylthio)pyrrolidine **6b** from **2a** and **3b**, the reduction of in situ-generated **6b** was carried out with $LiAlH_4$ to give 2-phenylethynylpyrrolidine (**5j**) in 59% yield (entry 5). Thus, the addition of **3b** to **2a** occurs efficiently, and lower yields in entries 1, 3, and 4 may be due to a less efficient reaction of **6b** with Grignard reagents **4**. Attempts to enhance the yields of **5g**–**5i** by raising the reaction temperature or by using a large excess of Grignard reagents **4** were not successful, but the reaction with **4a** under reflux in THF gave the product **5g** in slightly better yield (entry 2). The use of lithium acetylides **3c**–**3e** gave 2-alkynyl-2-alkyl or -aryl

Table 1. Sequential reaction of thiolactam **1a** with MeOTf, lithium silylacetylide **3a**, and Grignard reagents **4**[a]

Grignard reagent **4**	Product **5**	Yield (%)[b]
EtMgBr, **4b**		58
 **4c**		70
 **4d**		96[c]
 **4e**		88[c]
 **4f**		51[d]

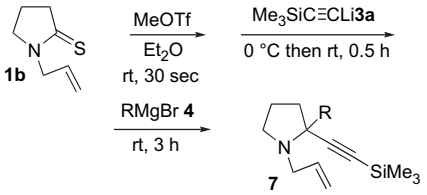
[a] Reaction was carried out as follows, unless otherwise noted: to thiolactam **1a** (1 mmol) in Et_2O (5 mL) were added MeOTf (1 equiv), lithium silylacetylide **3a** (1.5 equiv), and Grignard reagents **4** (2 equiv).
[b] Yields of crude product **5** with purity higher than 95%.
[c] Thiolactam **1a** (5 mmol) was used.
[d] Product **5f** was purified by column chromatography on silica gel, and the isolated yield is shown.

pyrrolidines **5k**–**5m** and **5o**, albeit in low to moderate yields (entries 6–8 and 10). The sequential reaction of **2a** with **3d** and $LiAlH_4$ gave the product **5n** in 59% yield, which suggested the efficient formation of **6d** and the lower efficiency of the reaction of **6d** with Grignard reagents **4**.



(4)

Instead of γ-thiolactam **1a**, *N*-allyl γ-thiolactam **1b** was chosen as a starting material (Eq. 5). As in the reaction of **1a**, the corresponding pyrrolidines **7** were obtained in good to high yields by reacting with lithium acetylide **3a** and Grignard reagents **4**.

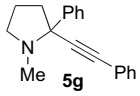
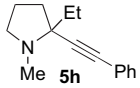
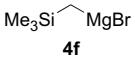
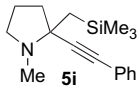
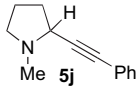
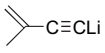
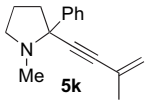
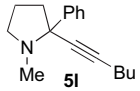
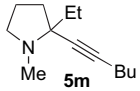
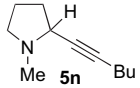
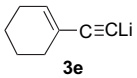
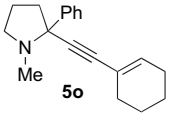


(5)

7a R = Ph 94% **7c** R = Et 83%
7b R = Me 60% **7d** R = C_6H_4OMe-4 55%[a]

[a]after the purification by column chromatography on silica gel

Table 2. Sequential reaction of thiolactam **1a** with MeOTf, lithium acetylides **3**, and Grignard reagents **4** or $LiAlH_4$[a]

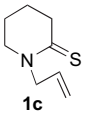
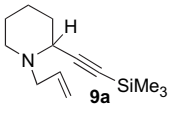
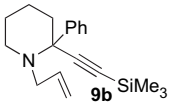
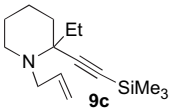
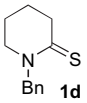
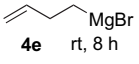
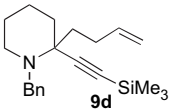
Entry	$R^1C{\equiv}CLi$ **3**	Conditions A	RMgBr **4** or $LiAlH_4$	Conditions B	Product **5**	Yield (%)[b]
1	PhC≡CLi, **3b**	0 °C, then rt, 0.5 h	PhMgBr, **4a**	rt, 3 h	 **5g**	30
2[c]				Reflux, 12 h		44
3	**3b**	0 °C, then rt, 0.75 h	EtMgBr, **4b**	rt, 3 h	 **5h**	40
4	**3b**	0 °C, then rt, 0.5 h	 **4f**	rt, 3 h	 **5i**	36
5[d]	**3b**	0 °C, then rt, 0.5 h	$LiAlH_4$	−10 °C, 3 h	 **5j**	59
6	 **3c**	0 °C, then rt, 0.5 h	**4a**	rt, 3 h	 **5k**	18
7	BuC≡CLi, **3d**	−78 °C, 1 h	**4a**	−78 °C, then rt, 3 h	 **5l**	40
8	**3d**	−30 °C, 0.5 h	**4b**	Reflux, 3 h	 **5m**	40
9[d]	**3d**	0 °C, 0.5 h	$LiAlH_4$	−10 °C, 3 h	 **5n**	59
10	 **3e**	−78 °C, 1 h	**4a**	−78 °C, then rt, 3 h	 **5o**	41

[a] Reaction was carried out as follows, unless otherwise noted: to thiolactam **1a** (1 mmol) in Et_2O (5 mL) were added MeOTf (1 equiv), lithium acetylide (1.5 equiv), and Grignard reagent **4** (2 equiv). Products **5** were purified by column chromatography on silica gel.
[b] Isolated yields.
[c] Lithium phenylacetylide **3b** (3 equiv) and phenylmagnesium bromide **4a** (4 equiv) were used in THF.
[d] $LiAlH_4$ (2.6 equiv) was used.

The above results clearly show that the 2-(methylthio)pyrrolidine **6a** derived from **2a** and lithium silylacetylide **3a** undergoes a substitution reaction at the carbon atom adjacent to the nitrogen atom with Grignard reagents **4** with better efficiency. This may be partly due to the electron-withdrawing ability of the silicon atom enabling the carbon atom bearing the sulfur and nitrogen atoms in **6a** more electropositive.

δ-Thiolactams **1c** and **1d** were also used as a starting material. The results are summarized in Table 3. To confirm the efficient generation of 2-(methylthio)piperidine **8**,[10] the reaction mixture obtained from δ-thiolactam **1c**, MeOTf, and lithium silylacetylide **3a** was treated with $LiAlH_4$ to give 2-silylethynylpiperidine **9a** in good yield (entry 1). The use of phenylmagnesium bromide (**4a**) produced the corresponding product **9b** in low yield (entry 2), whereas the

Table 3. Sequential reaction of thiolactam **1c** and **1d** with MeOTf, lithium silylacetylide **3a**, and $LiAlH_4$ or Grignard reagents **4**[a]

Entry		$LiAlH_4$ or R′MgBr **4** Conditions[b]	Product **9**	Yield (%)[c]
1	 **1c**	$LiAlH_4$, −10 °C, 3 h	 **9a**	67[d]
2	**1c**	PhMgBr **4a**, rt, 5 h	 **9b**	21
3	**1c**	EtMgBr **4b**, rt, 8 h	 **9c**	77
4	 **1d**	 **4e** rt, 8 h	 **9d**	48

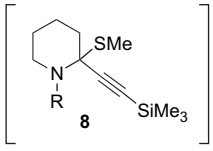
[a] Reaction was carried out unless otherwise noted: to thiolactam **1** (1 mmol) in Et_2O (5 mL) were added MeOTf (1 equiv), lithium silylactylide **3a** (1.5 equiv), and Grignard reagents **4** (2 eqiuv). Products **9** were purified by column chromatography on silica gel.

[b] Reaction conditions of the reaction with $LiAlH_4$ or Grignard reagents **4** are shown.

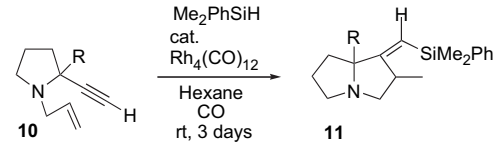
[c] Isolated yields.

[d] $LiAlH_4$ (2.6 equiv) was used.

reaction with ethylmagnesium bromide (**4b**) led to the product **9c** in better yield (entry 3).



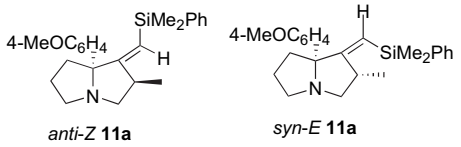
Finally, 2-alkynyl-2-substituted pyrrolidines and piperidines obtained above were subjected to silylcarbocyclization developed by Ojima and his co-workers.[11] *N*-Allyl-2-ethynylpyrrolidines **10**, obtained by the desilylation of cyclic amines **7** and **9**, were reacted with $HSiPhMe_2$ in the presence of $Rh_4(CO)_{12}$ under a CO atmosphere (Eq. 6). The silylcarbocyclization of acyclic amines has been reported to be complete within 1 min, but in the reaction of **10a**, only a starting cyclic amine **10a** was observed after 1 min. To efficiently complete the cyclization, the reaction was carried out under various reaction conditions. Consequently, the reaction at room temperature for 3 days gave 1,2,7a-trisubstituted hexahydro-1*H*-pyrrolizine[12] **11a** as a mixture of four diastereomers (67:26:6:1) in 85% yield. The stereochemistry of the two major diastereomers was estimated to be *anti-Z*-**11a**[15] and *syn-E*-**11a** by phase-sensitive NOESY spectroscopy. A similar reaction of **10b** took place to give the corresponding pyrrolizine **11b**, whereas 2-ethynyl-2-alkylpyrrolidines **10c** and **10d** did not react at all under identical reaction conditions. In contrast, the silylcarbocyclization of 2-ethynyl-2-ethylpiperidine **12** proceeded in a similar manner to give 1,2,8a-trisubstituted octahydroindolizine[12] **13** as a mixture of two diastereomers.[16]

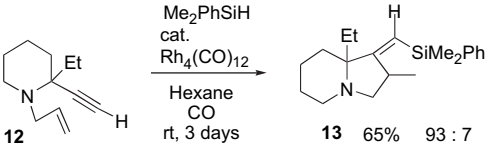


(6)

10a R = C_6H_4-OMe-4
10b R = Ph
10c R = Me
10d R = Et

11a 85% (67 : 26 : 6 : 1)
11b 88% (70 : 26 : 3 : 1)





(7)

In summary, we have demonstrated the sequential reaction of γ- and δ-thiolactams with MeOTf, lithium acetylides, and Grignard reagents to give 2-alkynyl-2-substituted pyrrolidines and piperidines in low to high yields. The synthesis of trisubstituted pyrrolizines and indolizines was achieved by $Rh_4(CO)_{12}$-catalyzed silylcarbocyclization.

3. Experimental

3.1. General methods

The IR spectra were obtained on a JASCO FTIR 410 spectrophotometer. ^1H (399.7 MHz) and ^{13}C (100.4 MHz) NMR spectra were measured on a JEOL α-400 spectrometer. The ^1H and the ^{13}C chemical shifts are reported in δ values referred to Me_4Si and $CHCl_3$ as an internal standard, respectively. All spectra were acquired in the proton-decoupled mode. Phase-sensitive NOESY spectra were measured with a Varian Inova 500 NMR spectrometer. The mass spectra (MS) were taken on SHIMADZU GCMS QP1000 and JEOL MStation 700 spectrometers. The high-resolution mass spectra (HRMS) were measured on JEOL GC-mate II and JEOL MStation 700 mass spectrometers.

3.2. General procedure for the sequential addition reaction of lithium acetylides and Grignard reagents to thioiminium salts derived from γ-thiolactams

In a typical example, to an Et_2O (5 mL) solution of 1-methyl-2-pyrrolidinethione (0.115 g, 1.0 mmol) was added methyl triflate (0.115 mL, 1.0 mmol) at room temperature, and the mixture was stirred at this temperature for 30 s. To this was added an Et_2O solution (5 mL) of the alkynyllithium

prepared from (trimethylsilyl)acetylene (0.21 mL, 1.5 mmol) and BuLi (1.6 M solution in hexane, 0.94 mL, 1.5 mmol) at 0 °C, and this was stirred for 0.5 h at room temperature. To this was added phenylmagnesium bromide (1.0 M solution in THF, 2.0 mL, 2.0 mmol) at room temperature, and the mixture was stirred at this temperature for 3 h. The resulting mixture was poured into a saturated aqueous solution of NH_4Cl, and extracted with Et_2O. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give the amine **5a** (0.206 g, 87%) as an orange oil.

3.2.1. 1-Methyl-2-phenyl-2-(2-trimethylsilyl)ethynylpyrrolidine (5a). IR (neat) 3060, 3026, 2959, 2902, 2878, 2843, 2817, 2787, 2156, 1621, 1601, 1447, 1250, 880, 843, 759 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.22 (s, 9H), 1.89–2.02 (m, 3H), 2.09 (s, 3H), 2.23–2.29 (m, 1H), 2.61 (q, J=8.4 Hz, 1H), 3.13–3.18 (m, 1H), 7.02 (t, J=6.3 Hz, 1H), 7.10 (t, J=6.8 Hz, 2H), 7.45 (d, J=7.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 0.41, 21.4, 35.8, 44.5, 53.6, 68.9, 92.1, 104.0, 127.0, 127.4, 128.3, 142.5; MS (EI) m/z 257 (M^+); HRMS Calcd for $C_{16}H_{23}NSi$: 257.1600 (M^+). Found: 257.1582.

3.2.2. 1-Methyl-2-ethyl-2-(2-trimethylsilyl)ethynylpyrrolidine (5b). Orange oil: IR (neat) 2966, 2880, 2844, 2811, 2787, 2153, 1460, 1378, 1306, 1251, 1082, 1031, 971, 920, 879, 842, 760, 689 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.10 (s, 9H), 0.93 (t, J=7.6 Hz, 3H), 1.22–1.31 (m, 1H), 1.59–1.75 (m, 4H), 1.93–1.99 (m, 1H), 2.18 (s, 3H), 2.38–2.44 (m, 1H), 2.89–2.94 (m, 1H); ^{13}C (NMR) δ 0.39, 9.8, 20.3, 31.4, 35.5, 37.4, 54.1, 65.1, 89.6, 105.7; MS (EI) m/z 180 ($M^+-CH_2CH_3$); HRMS Calcd for $C_{12}H_{23}NSi$: 209.1600 (M^+). Found: 209.1585.

3.2.3. 1-Methyl-2-cyclohexyl-2-(2-trimethylsilyl)ethynylpyrrolidine (5c). Orange oil: IR (neat) 2927, 2852, 2788, 2671, 2153, 1649, 1578, 1449, 1408, 1345, 1299, 1250, 1213, 1164, 1105, 1045, 1000, 877, 842, 760, 689 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.08 (s, 9H), 0.84–0.92 (m, 2H), 1.05–1.21 (m, 4H), 1.44–1.45 (m, 1H), 1.57–1.74 (m, 7H), 1.93–1.96 (m, 1H), 2.10 (s, 3H), 2.33–2.38 (m, 1H), 2.87 (dt, J=8.5, 2.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 0.45, 20.4, 25.5, 26.4, 26.9, 27.2, 29.8, 32.8, 35.2, 42.8, 53.9, 67.6, 88.8, 107.1; MS (EI) m/z 263 (M^+), 180 ($M^+-C_6H_{11}$); HRMS Calcd for $C_{16}H_{29}NSi$: 263.2069 (M^+). Found: 263.2082.

3.2.4. 1-Methyl-2-(2-propenyl)-2-(2-trimethylsilyl)ethynylpyrrolidine (5d). Orange oil: IR (neat) 3077, 2960, 2907, 2845, 2813, 2788, 2677, 2154, 1641, 1449, 1416, 1347, 1251, 1201, 1167, 1110, 1045, 994, 949, 913, 843, 760, 698 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.16 (s, 9H), 1.71–1.84 (m, 3H), 1.92–1.98 (m, 1H), 2.16 (dd, J=13.5, 7.6 Hz, 1H), 2.27 (s, 3H), 2.44–2.52 (m, 2H), 2.97 (dt, J=8.5, 3.2 Hz, 1H,), 5.01–5.13 (m, 2H), 5.90 (dddd, J=17.1, 10.3, 7.8, 6.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 0.36, 20.3, 35.5, 37.5, 42.9, 54.0, 63.6, 89.8, 105.7, 117.4, 134.3; MS (EI) m/z 221 (M^+); HRMS Calcd for $C_{13}H_{23}NSi$: 221.1600 (M^+). Found: 221.1620.

3.2.5. 1-Methyl-2-(3-butenyl)-2-(2-trimethylsilyl)ethynylpyrrolidine (5e). Orange oil: IR (neat) 3078, 2959, 2847, 2788, 2153, 1641, 1451, 1346, 1250, 1165, 1109, 1031, 910, 843, 760, 698 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.11 (s, 9H), 1.35 (dt, J=12.7, 4.9 Hz, 1H), 1.66–1.82 (m, 4H), 1.99–2.08 (m, 2H), 2.21–2.26 (m, 4H), 2.39–2.43 (m, 1H), 2.89–2.94 (m, 1H), 4.91 (d, J=10.3 Hz, 1H), 4.99 (d, J=17.1 Hz, 1H), 5.82 (dddd, J=17.0, 12.7, 10.2, 6.3 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 0.35, 20.4, 29.8, 35.5, 37.8, 38.0, 53.9, 64.1, 89.9, 105.6, 114.3, 138.7; MS (EI) m/z 235 (M^+); HRMS Calcd for $C_{14}H_{25}NSi$: 235.1756 (M^+). Found: 235.1750.

3.2.6. 1-Methyl-2-(1-trimethylsilyl)methyl-2-(2-trimethylsilyl)ethynylpyrrolidine (5f). Yellow oil: IR (neat) 2958, 2903, 2843, 2813, 2785, 2153, 1450, 1415, 1345, 1250, 1207, 1138, 1101, 1018, 986, 925, 842, 760, 697 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.09 (s, 9H), 0.15 (s, 9H), 0.67 (d, J=14.2 Hz, 1H), 1.29 (d, J=14.2 Hz, 1H), 1.66–1.76 (m, 3H), 2.06–2.10 (m, 1H), 2.24 (s, 3H), 2.37–2.43 (m, 1H), 2.91–2.95 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 0.21, 0.23, 20.2, 27.4, 34.9, 39.8, 52.8, 62.3, 89.0, 106.5; MS (EI) m/z 267 (M^+); HRMS Calcd for $C_{14}H_{29}NSi$: 267.1839 (M^+). Found: 267.1841.

3.2.7. 1-Methyl-2-phenyl-2-(2-phenyl)ethynylpyrrolidine (5g). Yellow oil: IR (neat) 3060, 3031, 2963, 2906, 2816, 2787, 1947, 1599, 1489, 1445, 1414, 1260, 1027, 865, 796, 756, 699 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.88–2.08 (m, 3H), 2.13 (s, 3H), 2.28–2.34 (m, 1H), 2.67 (q, J=8.8 Hz, 1H), 3.13–3.18 (m, 1H), 7.16–7.69 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 21.5, 36.0, 44.6, 53.8, 68.9, 87.9, 88.3, 123.5, 126.8, 127.2, 127.3, 128.2, 128.3, 131.8, 142.8; MS (EI) m/z 261 (M^+), 184 ($M^+-C_6H_5$); HRMS Calcd for $C_{19}H_{19}N$: 261.1517 (M^+). Found: 261.1488.

3.2.8. 1-Methyl-2-ethyl-2-(2-phenyl)ethynylpyrrolidine (5h). Yellow oil: IR (neat) 3055, 2968, 2877, 2786, 1600, 1573, 1489, 1443, 1345, 1231, 1147, 1031, 914, 755, 596 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.08 (t, J=7.6 Hz, 3H,), 1.40–1.49 (m, 1H), 1.78–1.95 (m, 4H), 2.12–2.17 (m, 1H), 2.36 (s, 3H), 2.57–2.64 (m, 1H), 3.03–3.08 (m, 1H), 7.26–7.32 (m, 3H), 7.40–7.44 (m, 2H); ^{13}C NMR δ 9.9, 20.4, 31.7, 35.7, 37.6, 54.4, 65.2, 86.2, 89.2, 123.6, 127.7, 128.2, 131.7; MS (EI) m/z 184 ($M^+-CH_2CH_3$); HRMS Calcd for $C_{13}H_{14}N$: 184.1126 ($M^+-CH_2CH_3$). Found: 184.1114.

3.2.9. 1-Methyl-2-(1-trimethylsilyl)methyl-2-(2-phenyl)-ethynylpyrrolidine (5i). Yellow oil: IR (neat) 3080, 3056, 3033, 2952, 2904, 2841, 2810, 2784, 1945, 1742, 1598, 1573, 1545, 1490, 1248, 846, 755, 691 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.10 (s, 9H), 0.73 (d, J=13.9 Hz, 1H), 1.37 (d, J=13.9 Hz, 1H), 1.73–1.80 (m, 3H), 2.13–2.18 (m, 1H), 2.30 (s, 3H), 2.45–2.53 (m, 1H), 2.95–3.00 (m, 1H), 7.22–7.27 (m, 3H), 7.34–7.37 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 0.2, 20.3, 27.6, 35.1, 40.0, 53.1, 62.4, 85.8, 90.1, 123.6, 127.7, 128.2, 131.5; MS (EI): m/z 271 (M^+); HRMS Calcd for $C_{17}H_{25}NSi$: 271.1756 (M^+). Found: 271.1779.

3.2.10. 1-Methyl-2-(2-phenyl)ethynylpyrrolidine (5j). Yellow oil: IR (neat) 2975, 2877, 2838, 2777, 1739, 1599, 1490, 1444, 1355, 1241, 1046, 757, 691 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.77–1.83 (m, 1H), 1.90–2.06 (m, 2H), 2.15–2.24 (m, 1H), 2.39 (q, J=8.8 Hz, 1H), 2.48 (s, 3H), 2.94 (dt, J=8.8, 3.9 Hz, 1H), 3.30 (t, J=7.1 Hz, 1H), 7.26–7.29 (m, 3H), 7.41–7.45 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 22.4,

32.2, 39.9, 54.8, 57.0, 84.1, 88.8, 123.3, 127.8, 128.1, 131.6; MS (EI) *m/z* 185 (M^+); HRMS Calcd for $C_{13}H_{15}N$: 185.1204 (M^+). Found: 185.1179.

3.2.11. 1-Methyl-2-phenyl-2-(3-methyl-3-buten-1-ynyl)-pyrrolidine (5k). Yellow oil: IR (neat) 3423, 3060, 3030, 2969, 2843, 2816, 2787, 2514, 2346, 1946, 1794, 1600, 1483, 1446, 1299, 1173, 895, 738, 699 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.82–2.01 (m, 6H), 2.05 (s, 3H), 2.18–2.24 (m, 1H), 2.58 (q, J=8.6 Hz, 1H), 3.08–3.13 (m, 1H), 5.15 (quint, J=1.23 Hz, 1H), 5.26 (quint, J=1.23 Hz, 1H), 7.15–7.19 (m, 1H), 7.24–7.28 (m, 2H), 7.59–7.61 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 21.5, 24.1, 35.9, 44.5, 53.7, 68.7, 86.8, 89.5, 121.1, 126.8, 127.2, 128.1, 128.8, 142.9; MS (EI) *m/z* 225 (M^+); HRMS Calcd for $C_{16}H_{19}N$: 225.1518 (M^+). Found: 225.1530.

3.2.12. 1-Methyl-2-phenyl-2-hexynylpyrrolidine (5l). Yellow oil: IR (neat) 2934, 2861, 2785, 1601, 1487, 1447, 1299, 1244, 1172, 1055, 913, 756, 699 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (t, J=7.3 Hz, 3H), 1.36–1.53 (m, 4H), 1.80–1.98 (m, 3H), 2.02 (s, 3H), 2.12–2.18 (m, 1H), 2.26 (t, J=6.8 Hz, 2H), 2.55 (q, J=8.7 Hz, 1H), 3.06–3.11 (m, 1H), 7.16 (t, J=7.3 Hz, 1H), 7.25 (t, J=7.3 Hz, 2H), 7.62 (d, J=7.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 13.7, 18.5, 21.5, 22.0, 31.4, 35.8, 44.7, 53.6, 68.4, 77.7, 88.3, 126.9, 127.0, 128.0, 143.4; MS (EI) *m/z* 241 (M^+); HRMS Calcd for $C_{17}H_{23}N$: 241.1830 (M^+). Found: 241.1831.

3.2.13. 1-Methyl-2-ethyl-2-hexynylpyrrolidine (5m). Yellow oil: IR (neat) 2964, 2934, 2875, 2786, 1460, 1378, 1260, 1231, 1083, 1031, 909, 806, 404 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (t, J=7.3 Hz, 3H), 0.95 (t, J=7.6 Hz, 3H), 1.21–1.31 (m, 1H), 1.35–1.46 (m, 4H), 1.62–1.75 (m, 4H), 1.91–1.96 (m, 1H), 2.16 (t, J=6.8 Hz, 2H), 2.20 (s, 3H), 2.43 (q, J=8.6 Hz, 1H), 2.90–2.95 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 9.9, 13.7, 18.3, 20.2, 21.9, 31.4, 31.8, 35.5, 37.7, 54.2, 64.6, 78.9, 85.8; MS (EI) *m/z* 164 ($M^+-CH_2CH_3$); HRMS Calcd for $C_{11}H_{18}N$: 164.1439 ($M^+-CH_2CH_3$). Found: 164.1406.

3.2.14. 1-Methyl-2-hexynylpyrrolidine (5n). Yellow oil: IR (neat) 2958, 2873, 2775, 2235, 1641, 1458, 1353, 1321, 1219, 1159, 1115, 1039, 904, 733, 407 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.84 (t, J=7.3 Hz, 3H), 1.30–1.46 (m, 4H), 1.62–1.71 (m, 1H), 1.75–1.84 (m, 2H), 1.98–2.05 (m, 1H), 2.14 (td, J=7.3, 1.8 Hz, 2H), 2.19–2.25 (m, 1H), 2.33 (s, 3H), 2.79–2.85 (m, 1H), 2.94 (t, J=6.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 13.6, 18.4, 21.9, 22.2, 31.0, 32.4, 39.8, 54.8, 56.8, 79.2, 84.2; MS (EI) *m/z* 165 (M^+); HRMS Calcd for $C_{11}H_{19}N$: 165.1517 (M^+). Found: 165.1493.

3.2.15. 1-Methyl-2-phenyl-2-(1-cyclohexenyl)ethynylpyrrolidine (5o). Yellow oil: IR (neat) 3060, 2935, 2840, 2785, 2204, 1948, 1810, 1741, 1600, 1488, 1446, 1346, 1297, 1241, 1173, 1055, 918, 756, 699 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.51–1.63 (m, 4H), 1.82–1.89 (m, 2H), 1.94–2.07 (m, 6H), 2.12–2.23 (m, 3H), 2.58 (dd, J=17.1, 8.8 Hz, 1H), 3.08–3.13 (m, 1H), 6.08 (quint, J=2.0 Hz, 1H), 7.16 (t, J=7.3 Hz, 1H), 7.25 (t, J=7.8 Hz, 2H), 7.62 (d, J=7.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 21.5, 21.6, 22.4, 25.6, 29.9, 35.9, 44.6, 53.7, 68.8, 84.7, 90.2, 120.7, 126.8, 127.1, 128.1, 134.0, 143.1; MS (EI) *m/z* 265 (M^+); HRMS Calcd for $C_{19}H_{23}N$: 265.1830 (M^+). Found: 265.1810.

3.2.16. 1-(2-Propenyl)-2-phenyl-2-(2-trimethylsilyl)ethynylpyrrolidine (7a). Orange oil: IR (neat) 3389, 3063, 2959, 2815, 2155, 1737, 1598, 1447, 1249, 1167, 1098, 916, 841, 759, 699 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.15 (s, 9H), 1.78–1.92 (m, 3H), 2.13–2.19 (m, 1H), 2.38–2.44 (m, 1H), 2.65 (dd, J=13.7, 7.8 Hz, 1H), 2.94–2.98 (m, 1H), 3.20 (dt, J=8.8, 3.1 Hz, 1H), 4.94 (d, J=10.3 Hz, 1H), 5.09 (d, J=17.1 Hz, 1H), 5.76 (dddd, J=17.6, 10.3, 7.8, 4.9 Hz, 1H), 7.16 (t, J=7.3 Hz, 1H), 7.24 (t, J=7.6 Hz, 2H), 7.63 (d, J=8.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 0.39, 21.4, 44.4, 50.7, 53.5, 68.6, 91.6, 104.6, 115.8, 126.7, 127.2, 128.1, 136.9, 143.0; MS (EI) *m/z* 206 ($M^+-C_6H_5$); HRMS Calcd for $C_{18}H_{25}NSi$: 283.1756 (M^+). Found: 283.1741.

3.2.17. 1-(2-Propenyl)-2-methyl-2-(2-trimethylsilyl)ethynylpyrrolidine (7b). Orange oil: IR (neat) 3080, 2961, 2812, 2155, 1718, 1644, 1418, 1350, 1251, 1098, 1020, 843, 760 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.13 (s, 9H), 1.33 (s, 3H), 1.68–1.79 (m, 3H), 2.02–2.07 (m, 1H), 2.30–2.36 (m, 1H), 2.79 (dd, J=13.2, 7.8 Hz, 1H), 2.98–3.04 (m, 1H), 3.30 (dd, J=13.2, 5.9 Hz, 1H), 5.05 (d, J=9.8 Hz, 1H), 5.18 (d, J=17.1 Hz, 1H), 5.89 (dddd, J=17.6, 9.8, 7.8, 5.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 0.37, 20.2, 25.6, 40.4, 51.5, 53.5, 60.1, 88.2, 107.2, 116.6, 136.9; MS (EI) *m/z* 221 (M^+), 206 (M^+-CH_3); HRMS Calcd for $C_{13}H_{23}NSi$: 221.1600 (M^+). Found: 221.1569.

3.2.18. 1-(2-Propenyl)-2-ethyl-2-(2-trimethylsilyl)ethynylpyrrolidine (7c). Orange oil: IR (neat) 3079, 2967, 2879, 2811, 2153, 1643, 1460, 1417, 1378, 1350, 1250, 1144, 995, 842, 760 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.14 (s, 9H), 0.96 (t, J=7.6 Hz, 3H), 1.32–1.41 (m, 1H), 1.66–1.81 (m, 4H), 1.97–2.01 (m, 1H), 2.31–2.37 (m, 1H), 2.77 (dd, J=13.2, 7.8 Hz, 1H), 3.02–3.07 (m, 1H), 3.31 (dd, J=13.7, 5.4 Hz, 1H), 5.04 (d, J=10.2 Hz, 1H), 5.18 (d, J=17.1 Hz, 1H), 5.88 (dddd, J=17.6, 10.3, 8.3, 5.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 0.41, 9.6, 20.3, 31.7, 37.4, 51.8, 53.5, 64.8, 89.1, 106.6, 116.3, 137.1; MS (EI) *m/z* 206 ($M^+-CH_2CH_3$); HRMS Calcd for $C_{12}H_{20}NSi$: 206.1365 ($M^+-CH_2CH_3$). Found: 206.1354.

3.2.19. 1-(2-Propenyl)-2-(4-methoxyphenyl)-2-(2-trimethylsilyl)ethynylpyrrolidine (7d). Yellow oil: IR (neat) 3076, 2957, 2813, 2155, 1643, 1611, 1582, 1509, 1464, 1415, 1349, 1250, 1172, 1038, 843, 760, 699 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.21 (s, 9H), 1.84–1.96 (m, 3H), 2.16–2.22 (m, 1H), 2.41–2.47 (m, 1H), 2.67 (dd, J=13.7, 7.8 Hz, 1H), 2.98–3.03 (m, 1H), 3.24 (dt, J=8.3, 2.9 Hz, 1H), 3.79 (s, 3H), 5.00 (d, J=10.2 Hz, 1H), 5.15 (dd, J=17.1, 0.98 Hz, 1H), 5.80 (dddd, J=17.6, 10.2, 7.8, 4.9 Hz, 1H), 6.85 (d, J=8.8 Hz, 2H), 7.59 (d, J=8.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 0.41, 21.3, 44.2, 50.6, 53.4, 55.3, 68.1, 91.5, 104.7, 113.4, 115.8, 127.9, 134.9, 136.9, 158.8; MS (EI) *m/z* 313 (M^+); HRMS Calcd for $C_{19}H_{27}NOSi$: 313.1862 (M^+). Found: 313.1836.

3.2.20. 1-(2-Propenyl)-2-(2-trimethylsilyl)ethynylpiperidine (9a). Yellow oil: IR (neat) 3080, 2937, 2861, 2814, 2157, 1745, 1643, 1442, 1420, 1319, 1250, 1124, 955, 920, 843, 760 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.11 (s, 9H), 1.36–1.61 (m, 4H), 1.66–1.70 (m, 2H), 2.39–2.44 (m, 2H), 2.96 (dd, J=7.3, 5.9 Hz, 1H), 3.06–3.11 (m, 1H), 3.50 (s,

1H), 5.07 (d, J=10.2 Hz, 1H), 5.15 (d, J=17.1 Hz, 1H), 5.78 (dddd, J=17.1, 13.2, 10.3, 6.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 0.25, 20.6, 25.6, 31.2, 48.9, 52.1, 59.3, 90.6, 103.5, 118.0, 135.2; MS (EI) m/z 221 (M^+); HRMS Calcd for $C_{13}H_{23}NSi$: 221.1600 (M^+). Found: 221.1569.

3.2.21. 1-(2-Propenyl)-2-phenyl-2-(2-trimethylsilyl)ethynylpiperidine (9b). Yellow oil: IR (neat) 3062, 2935, 2860, 2817, 2724, 2156, 1642, 1601, 1488, 1446, 1347, 1250, 1222, 1131, 1012, 915, 843, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.19 (s, 9H), 1.47–1.71 (m, 6H), 2.28–2.38 (m, 2H), 2.84–2.90 (m, 2H), 4.94 (d, J=10.3 Hz, 1H), 5.04 (d, J=17.1 Hz, 1H), 5.65 (dddd, J=17.6, 10.2, 7.8, 4.4 Hz, 1H), 7.16 (t, J=7.3 Hz, 1H), 7.24 (t, J=7.6 Hz, 2H), 7.67 (d, J=7.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 0.48, 22.3, 25.9, 43.3, 48.2, 55.8, 65.5, 93.4, 103.6, 116.0, 126.6, 127.1, 128.1, 136.8, 145.6; MS (EI) m/z 297 (M^+); HRMS Calcd for $C_{19}H_{27}NSi$: 297.1913 (M^+). Found: 297.1888.

3.2.22. 1-(2-Propenyl)-2-ethyl-2-(2-trimethylsilyl)ethynylpiperidine (9c). Orange oil: IR (neat) 2935, 2810, 2154, 1641, 1444, 1250, 1161, 1085, 992, 905, 859, 842, 760, 698 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.11 (s, 9H), 0.87 (t, J=7.8 Hz, 3H), 1.19–1.73 (m, 8H), 2.18 (dt, J=11.7, 2.4 Hz, 1H), 2.50 (dd, J=13.7, 7.8 Hz, 1H), 2.70 (d, J=12.2 Hz, 1H), 3.34 (dt, J=14.2, 2.4 Hz, 1H), 5.00 (d, J=10.2 Hz, 1H), 5.09 (d, J=17.1 Hz, 1H), 5.78 (dddd, J=17.5, 10.3, 8.3, 4.9 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 0.46, 8.0, 21.6, 25.8, 32.4, 35.4, 48.6, 54.1, 59.3, 89.8, 106.7, 116.4, 137.1; MS (EI) m/z 220 ($M^+-CH_2CH_3$); HRMS Calcd for $C_{13}H_{22}NSi$: 220.1522 ($M^+-CH_2CH_3$). Found: 220.1528.

3.2.23. 1-Phenylmethyl-2-(3-butenyl)-2-(2-trimethylsilyl) ethynylpiperidine (9d). Yellow oil: IR (neat) 3063, 3028, 2935, 2861, 2804, 2153, 1640, 1603, 1495, 1452, 1360, 1250, 1162, 1067, 991, 842, 728, 640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.14 (s, 9H), 1.25–1.41 (m, 2H), 1.50–1.64 (m, 4H), 1.73–1.85 (m, 2H), 2.13–2.23 (m, 3H), 2.47 (d, J=11.7 Hz, 1H), 2.94 (d, J=14.2 Hz, 1H), 3.93 (d, J=14.2 Hz, 1H), 4.85 (d, J=10.3 Hz, 1H), 4.93 (d, J=17.1 Hz, 1H), 5.74 (dddd, J=17.0, 12.7, 10.2, 6.3 Hz, 1H), 7.11–7.28 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 0.53, 21.8, 25.9, 27.7, 36.1, 39.2, 48.6, 55.0, 58.8, 89.9, 106.6, 114.3, 126.5, 128.2, 128.6, 138.9, 140.5; MS (EI) m/z 325 (M^+); HRMS Calcd for $C_{21}H_{31}NSi$: 325.2226 (M^+). Found: 325.2215.

3.3. General procedure for the desilylation of cyclic amines 7 and 9 with K_2CO_3 in MeOH

In a typical experiment, a mixture of 1-(2-propenyl)-2-phenyl-2-(2-trimethylsilyl)ethynylpyrrolidine **7a** (0.603 g, 2.1 mmol) and K_2CO_3 (0.290 g, 2.1 mmol) in MeOH (10 mL) was stirred at room temperature for 5 h, and then treated with an aqueous solution of NH_4Cl, extracted with ether, washed with brine, dried over $MgSO_4$ and concentrated in vacuo to give the amine **10b** (0.415 g, 93%) as a yellow oil.

3.3.1. 1-(2-Propenyl)-2-(4-methoxyphenyl)-2-ethynylpyrrolidine (10a). Yellow oil: IR (neat) 3296, 3075, 2955, 2815, 1643, 1609, 1581, 1509, 1463, 1416, 1349, 1249, 1172, 1037, 919, 831, 794 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.87–2.00 (m, 3H), 2.20–2.26 (m, 1H), 2.44–2.51 (m, 1H), 2.56 (s, 1H), 2.72 (dd, J=13.7, 8.3 Hz, 1H), 3.01–3.06 (m, 1H), 3.27 (dt, J=8.5, 3.2 Hz, 1H), 3.79 (s, 3H), 5.01 (dt, J=11.2, 1.5 Hz, 1H), 5.17 (dq, J=17.1, 1.5 Hz, 1H), 5.81 (dddd, J=17.1, 10.2, 8.3, 4.9 Hz, 1H), 6.85 (d, J=8.8 Hz, 2H), 7.62 (d, J=8.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 21.2, 44.3, 50.5, 53.2, 55.2, 67.5, 75.1, 82.5, 113.4, 115.9, 127.8, 134.6, 136.7, 158.8; MS (EI) m/z 241 (M^+); HRMS Calcd for $C_{16}H_{19}NO$: 241.1467 (M^+). Found: 241.1458.

3.3.2. 1-(2-Propenyl)-2-phenyl-2-ethynylpyrrolidine (10b). Yellow oil: IR (neat) 3300, 3062, 3025, 2977, 2878, 2816, 1642, 1600, 1488, 1446, 1418, 1350, 1262, 1098, 918, 758, 699 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.88–2.03 (m, 3H), 2.24–2.30 (m, 1H), 2.48–2.55 (m, 1H), 2.57 (s, 1H), 2.76 (dd, J=13.7, 8.3 Hz, 1H), 3.04–3.09 (m, 1H), 3.31 (dt, J=8.6, 3.1 Hz, 1H), 5.03 (d, J=10.3 Hz, 1H), 5.19 (dq, J=17.1, 1.5 Hz, 1H), 5.84 (dddd, J=17.1, 10.2, 7.8, 4.4 Hz, 1H), 7.25 (t, J=7.3 Hz, 1H), 7.33 (t, J=7.6 Hz, 2H), 7.72 (d, J=7.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 21.3, 44.5, 50.6, 53.4, 68.0, 75.1, 82.4, 116.0, 126.6, 127.3, 128.2, 136.7, 142.7; MS (EI) m/z 211 (M^+), 170 ($M^+-CH_2CH{=}CH_2$); HRMS Calcd for $C_{15}H_{17}N$: 211.1361 (M^+). Found: 211.1375.

3.3.3. 1-(2-Propenyl)-2-methyl-2-ethynylpyrrolidine (10c). Yellow oil: IR (neat) 3303, 2975, 2814, 1643, 1443, 1370, 1351, 1260, 1143, 995, 919, 636 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 3H), 1.67–1.77 (m, 3H), 2.02–2.06 (m, 1H), 2.23 (s, 1H), 2.26–2.33 (m, 1H), 2.77 (dd, J=13.2, 7.8 Hz, 1H), 2.97–3.02 (m, 1H), 3.28 (dd, J=13.2, 4.9 Hz, 1H), 5.00 (d, J=9.8 Hz, 1H), 5.15 (d, J=17.1 Hz, 1H), 5.83 (dddd, J=17.6, 9.8, 7.8, 5.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 20.1, 25.6, 40.4, 51.4, 53.3, 59.5, 72.1, 84.7, 116.6, 136.7; MS (EI) m/z 149 (M^+); HRMS Calcd for $C_9H_{12}N$: 134.0970 (M^+-CH_3). Found: 134.0937.

3.3.4. 1-(2-Propenyl)-2-ethyl-2-ethynylpyrrolidine (10d). Yellow oil: IR (neat) 3303, 3079, 2971, 2936, 2880, 2813, 2362, 2095, 1643, 1460, 1351, 1260, 1143, 997, 918, 635 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.97 (t, J=7.3 Hz, 3H), 1.34–1.43 (m, 1H), 1.68–1.81 (m, 4H), 1.97–2.02 (m, 1H), 2.26 (s, 1H), 2.31–2.37 (m, 1H), 2.77 (dd, J=13.2, 7.8 Hz, 1H), 3.03–3.08 (m, 1H), 3.31 (dd, J=13.2, 5.4 Hz, 1H), 5.02 (d, J=9.8 Hz, 1H), 5.17 (dd, J=17.1, 0.98 Hz, 1H), 5.85 (dddd, J=17.1, 10.2, 8.3, 5.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 9.4, 20.2, 31.7, 37.3, 51.7, 53.3, 64.1, 72.8, 84.1, 116.4, 136.8; MS (EI) m/z 134 ($M^+-CH_2CH_3$); HRMS Calcd for $C_9H_{12}N$: 134.0970 ($M^+-CH_2CH_3$). Found: 134.0956.

3.3.5. 1-(2-Propenyl)-2-ethyl-2-ethynylpiperidine (12). Yellow oil: IR (neat) 3305, 2935, 2812, 2095, 1643, 1443, 1347, 1261, 1166, 1019, 918, 805, 637 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, J=7.8 Hz, 3H), 1.37–1.78 (m, 8H), 2.23 (td, J=11.7, 2.9 Hz, 1H), 2.30 (s, 1H), 2.56 (dd, J=14.2, 8.3 Hz, 1H), 2.77 (dt, J=12.2, 2.9 Hz, 1H), 3.41 (d, J=14.2 Hz, 1H), 5.04 (d, J=10.2 Hz, 1H), 5.14 (d, J=17.1 Hz, 1H), 5.81 (dddd, J=17.5, 10.3, 8.3, 4.9 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 7.9, 21.5, 25.7, 32.6, 35.4, 48.6, 54.0, 58.8, 73.4, 84.1, 116.5, 136.9; MS (EI) m/z 148

($M^+-CH_2CH_3$); HRMS Calcd for $C_{12}H_{19}N$: 177.1517 (M^+). Found: 177.1507.

3.4. General procedure for the silylcarbocyclization of cyclic amines 10 and 12

In a typical example, a two-necked flask equipped with a stirring bar and a CO inlet was charged with $Rh_4(CO)_{12}$ (1.9 mg, 0.0025 mmol). After the flask was purged with CO, hexane (1 mL) was added to dissolve the catalyst. Me_2PhSiH (0.038 mL, 0.25 mmol) was added via a syringe. After the reaction mixture was stirred for 5 min at room temperature, it was then cannulated into a flask containing a solution of 1-(2-propenyl)-2-phenyl-2-ethynylpyrrolidine **10b** (0.106 g, 0.5 mmol), Me_2PhSiH (0.077 mL, 0.5 mmol) in hexane (1 mL) via CO pressure without stirring. The resulting mixture was stirred for 72 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified by column chromatography (SiO_2, hexane/EtOAc/Et_3N=3:1:1 vol %) to give a mixture of four diastereomers of the bicyclic compound **11b** (0.153 g, 88%, 70:26:3:1) as a yellow oil.

3.4.1. *anti*-1-[*Z*-(Dimethylsilyl)methylidene]-7a-(4-methoxyphenyl)-2-methylhexahydro-1*H*-pyrrolizine (11a). Yellow oil: IR (neat) 3067, 2955, 2833, 1609, 1508, 1462, 1427, 1375, 1302, 1247, 1176, 1114, 1038, 836, 774, 639 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.27 (s, 3H), 0.29 (s, 3H), 1.03 (d, J=6.8 Hz, 3H), 1.62–1.71 (m, 1H), 1.87–1.96 (m, 1H), 2.00–2.06 (m, 1H), 2.24–2.30 (m, 1H), 2.38 (dd, J=10.3, 6.8 Hz, 1H), 2.68–2.73 (m, 1H), 2.76–2.82 (m, 1H), 2.90–2.96 (m, 1H), 3.24 (dd, J=10.3, 8.3 Hz, 1H), 3.73 (s, 3H), 5.36 (d, J=2.4 Hz, 1H), 6.79 (d, J=8.8 Hz, 2H), 7.22–7.29 (m, 3H), 7.37–7.40 (m, 4H); ^{13}C NMR ($CDCl_3$) δ −0.52, 22.0, 24.8, 37.3, 38.6, 53.5, 55.2, 60.2, 82.2, 113.2, 117.9, 127.7, 127.8, 128.7, 133.7, 138.8, 139.9, 158.0, 173.4; MS (EI) m/z 377 (M^+); HRMS Calcd for $C_{24}H_{31}NOSi$: 377.2175 (M^+). Found: 377.2165.

3.4.2. *syn*-1-[*E*-(Dimethylsilyl)methylidene]-7a-(4-methoxyphenyl)-2-methylhexahydro-1*H*-pyrrolizine. ^1H NMR ($CDCl_3$) δ 0.35 (t, J=4.6 Hz, 6H), 0.84 (d, J=6.8 Hz, 3H), 1.63–1.72 (m, 1H), 1.74–1.84 (m, 1H), 1.97–2.03 (m, 1H), 2.08–2.15 (m, 1H), 2.58–2.65 (m, 1H), 2.68–2.78 (m, 3H), 3.00–3.06 (m, 1H), 3.73 (s, 3H), 5.56 (s, 1H), 6.77 (d, J=9.3 Hz, 2H), 7.28–7.30 (m, 2H), 7.43–7.50 (m, 4H), 7.54–7.56 (m, 1H); ^{13}C NMR ($CDCl_3$) δ −0.82, −0.66, 21.4, 24.8, 38.6, 40.2, 53.8, 55.2, 59.0, 80.7, 113.2, 117.3, 127.2, 127.8, 128.9, 133.7, 139.2, 139.8, 158.0, 171.7.

3.4.3. *anti*-1-[*Z*-(Dimethylsilyl)methylidene]-7a-phenyl-2-methylhexahydro-1*H*-pyrrolizine (11b). Yellow oil: IR (neat) 3066, 2956, 1620, 1487, 1444, 1427, 1248, 1113, 1028, 836, 762, 730, 700, 641 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.26 (s, 3H), 0.29 (s, 3H), 1.03 (d, J=7.3 Hz, 3H), 1.61–1.71 (m, 1H), 1.87–1.96 (m, 1H), 2.03–2.10 (m, 1H), 2.27–2.33 (m, 1H), 2.41 (dd, J=10.7, 6.8 Hz, 1H), 2.67–2.74 (m, 1H), 2.77–2.84 (m, 1H), 2.93–2.99 (m, 1H), 3.26 (dd, J=10.7, 8.3 Hz, 1H), 5.39 (d, J=2.4 Hz, 1H), 7.09–7.16 (m, 1H), 7.20–7.29 (m, 5H), 7.36–7.39 (m, 2H), 7.46–7.49 (m, 2H); ^{13}C NMR ($CDCl_3$) δ −0.49, 22.0, 25.0, 37.8, 38.8, 53.9, 60.4, 82.6, 118.1, 126.2, 126.7, 127.7, 127.9, 128.7, 133.7, 139.9, 146.9, 173.2; MS (EI) m/z 347 (M^+); HRMS Calcd for $C_{23}H_{29}NSi$: 347.2069 (M^+). Found: 347.2041.

3.4.4. *syn*-1-[*E*-(Dimethylsilyl)methylidene]-7a-phenyl-2-methylhexahydro-1*H*-pyrrolizine. ^1H NMR ($CDCl_3$) δ 0.33 (s, 3H), 0.35 (s, 3H), 0.81 (d, J=6.8 Hz, 3H), 1.62–1.71 (m, 1H), 1.74–1.84 (m, 1H), 1.97–2.04 (m, 1H), 2.10–2.17 (m, 1H), 2.61–2.67 (m, 1H), 2.72–2.76 (m, 3H, CH_2), 3.01–3.07 (m, 1H), 5.60 (s, 1H), 7.10–7.13 (m, 1H), 7.19–7.24 (m, 2H), 7.27–7.29 (m, 3H), 7.45–7.48 (m, 2H), 7.54–7.56 (m, 2H); ^{13}C NMR ($CDCl_3$) δ −0.81, −0.65, 21.3, 25.0, 39.0, 40.6, 54.1, 59.2, 80.9, 117.4, 126.0, 127.8, 127.9, 128.9, 133.8, 139.8, 147.4, 171.5.

3.4.5. Major isomer of 1-[*E*-(dimethylsilyl)methylidene]-8a-ethyl-2-methyloctahydroindolizine (13). Yellow oil: IR (neat) 3067, 2927, 2797, 1619, 1483, 1427, 1372, 1329, 1248, 1113, 974, 832, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.29 (s, 3H), 0.31 (s, 3H), 0.71 (t, J=7.3 Hz, 3H), 0.96 (t, J=6.8 Hz, 3H), 1.08–1.11 (m, 2H), 1.30 (sext, J=7.3 Hz, 1H), 1.42–1.65 (m, 4H), 1.95 (sext, J=7.3 Hz, 1H), 2.49 (sext, J=7.3 Hz, 1H), 2.65–2.73 (m, 2H), 2.78–2.84 (m, 1H), 2.91 (t, J=8.3 Hz, 1H), 5.15 (d, J=2.4 Hz, 1H), 7.24–7.26 (m, 3H), 7.45–7.48 (m, 2H); ^{13}C NMR ($CDCl_3$) δ −0.63, −0.45, 7.8, 18.9, 21.2, 21.5, 24.0, 30.8, 36.6, 43.9, 56.2, 67.0, 113.2, 127.7, 128.7, 133.8, 140.1; MS (EI) m/z 313 (M^+); HRMS Calcd for $C_{20}H_{31}NSi$: 313.2226 (M^+). Found: 313.2249.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research on Priority Area (No. 18037024, 'Advanced Molecular Transformations of Carbon Resources') from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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15. For example, in phase-sensitive NOESY spectroscopy of *anti*-*Z*-**11a**, cross peaks were observed among the protons at the *ortho*-positions of 4-methoxyphenyl group and at the proton attached to the carbon atom bearing a methyl group.
16. The stereochemistry of **13** was not determined, but the geometry of the carbon–carbon double bond was estimated to be *E* on the basis of phase-sensitive NOESY spectroscopy.

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Tetrahedron 62 (2006) 6321–6331

The effect of boronic acid-positioning in an optical glucose-sensing ensemble

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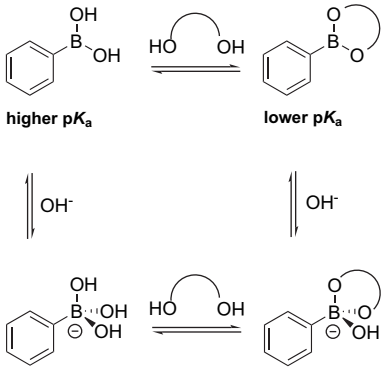
Received 24 February 2006; revised 11 April 2006; accepted 13 April 2006
Available online 12 May 2006

Abstract—The quenching of the anionic dye 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (pyranine) with three different boronic acid-substituted benzyl viologens was determined, and the fluorescence signal modulation obtained upon addition of glucose to the dye/quencher system was also studied. The benzyl viologen that contains boronic acids in the *ortho*-position (*o*-BBV) was found to display unique behavior, which can be rationalized by a charge neutralization mechanism facilitated by an intramolecular interaction between sp^3 boronate and the quaternary nitrogen of the viologen. Potentiometric titration and ^{11}B NMR spectroscopy were used to generate pH profiles for the boronic acids, which provide additional evidence for the proposed mechanism.

1. Introduction

In recent years, much research has been focused on the development of boronic acid-based chemosensors for the detection of glucose.[1] Such sensors are particularly useful to those suffering from diabetes, since accurate monitoring of blood glucose concentrations is essential for the proper management of this disease.[2–5] Boronic acids have proven to be excellent synthetic receptors for glucose due to their ability to reversibly bind 1,2- and 1,3-diols in aqueous media (Scheme 1).[6–10] In many boronic acid-based sensing systems, the saccharide recognition event is monitored using fluorescence spectroscopy.[11] In such systems, the boronic acid receptor is usually directly attached to the fluorophore, and formation of the cyclic boronate ester upon glucose binding results in fluorescence modulation.[12]



Scheme 1. Equilibria between boronic acids and generic diols.

In contrast to boronic acid-based glucose recognition systems that consist of a single detector molecule, we have developed a two-component sensing system[13] comprising an anionic fluorescent dye (**1**), and a boronic acid-appended cationic viologen (**2**) that dually serves as a fluorescence quencher and a glucose receptor (Scheme 2).[14–17] In the proposed mechanism, the electrostatic association of **1** and **2** results in ground-state complex formation facilitating electron transfer from the dye to the viologen, which leads to a decrease in fluorescence.[18] When glucose is added to the system, formation of two anionic boronate esters effectively neutralizes the dicationic viologen, thus greatly diminishing its quenching efficiency, and an increase in the fluorescence intensity of the dye is observed. Fluorescence modulation is therefore directly correlated with glucose concentration.

In terms of system optimization, the two-component nature of this sensing ensemble allows for a more facile probing of its multivariate space. For example, we have demonstrated that glucose sensing can be carried out using excitation and emission wavelengths across the visible spectrum solely by varying the dye component.[19] Similarly, by modification of the quencher/receptor component, we have investigated the dependence of cationic charge on the sensing

Keywords: Boronic acid; Glucose detection; Fluorescence; pK_a; Viologen; Pyranine; Saccharide sensor.
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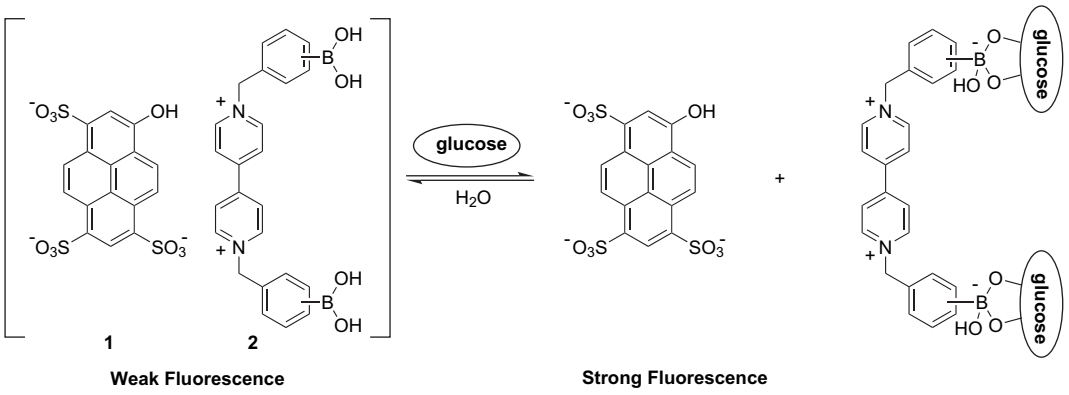
doi:10.1016/j.tet.2006.04.047



Scheme 2. Proposed mechanism of glucose detection: glucose-induced dissociation of ground-state complex results in fluorescence increase.

mechanism,[20] and also explored the use of phenanthrolinium-based receptors in lieu of viologens (4,4′-bipyridiniums).[21] In the present study, we further probe the system to better understand how positioning of the boronic acid on benzyl viologen affects fluorescence quenching as well as the ability of the quencher/receptor to sense saccharides when used in combination with 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (pyranine, **1**). To help rationalize the observed differences in quenching and glucose sensing, the apparent pK_a values for each of the quencher/receptors were determined by ^{11}B NMR and potentiometric titration. The results of this study provide some insight into the quenching and saccharide sensing mechanisms involved in our two-component system. Such information can be used to aid in the design and optimization of future glucose-sensing systems.

2. Results and discussion

2.1. Description of the system

Three quencher/receptors, *N*,*N*′-4,4′-bis(benzyl-2-boronic acid)-bipyridinium dibromide (*o*-BBV), *N*,*N*′-4,4′-bis(benzyl-3-boronic acid)-bipyridinium dibromide (*m*-BBV), and *N*,*N*′-4,4′-bis(benzyl-4-boronic acid)-bipyridinium dibromide (*p*-BBV), were used to study how positioning of the boronic acid receptor around the benzyl ring affects the system in terms of quenching ability and glucose response (Fig. 1). The non-boronic acid-containing analog, benzyl viologen (BV), was used as a control compound.

The compounds were prepared using a one-step procedure by reacting a twofold excess of bromomethylphenylboronic acid with 4,4′-dipyridyl in DMF. Using acetone, the compounds were precipitated from the reaction mixture in analytically pure form. Recrystallization from water and/or methanol provided single crystals suitable for X-ray analysis. The X-ray crystal structures for *o*-, *m*-, and *p*-BBV are shown in Figure 2.

We anticipated that *o*-BBV would exhibit unique behavior because, in the *ortho*-position, the boronic acid is capable of interacting with the quaternary nitrogen. At pH values above the apparent pK_a of the boronic acid or boronate ester, the boron moiety will exist in its anionic tetrahedral form and a ‘charge neutralization–stabilization mechanism’, previously described by Lakowicz and co-workers for quinolinium benzyl boronic acids[22,23] can occur (Scheme 3). Shinaki and co-workers[16,24–26] have also suggested that an electrostatic interaction is taking place in certain porphyrin-based boronic acids. Both groups agree that the proposed interaction facilitates the formation of boronate ester complexes with saccharides, and have thus integrated this structural motif into glucose sensor designs. The present study provides a systematic investigation in order to verify the

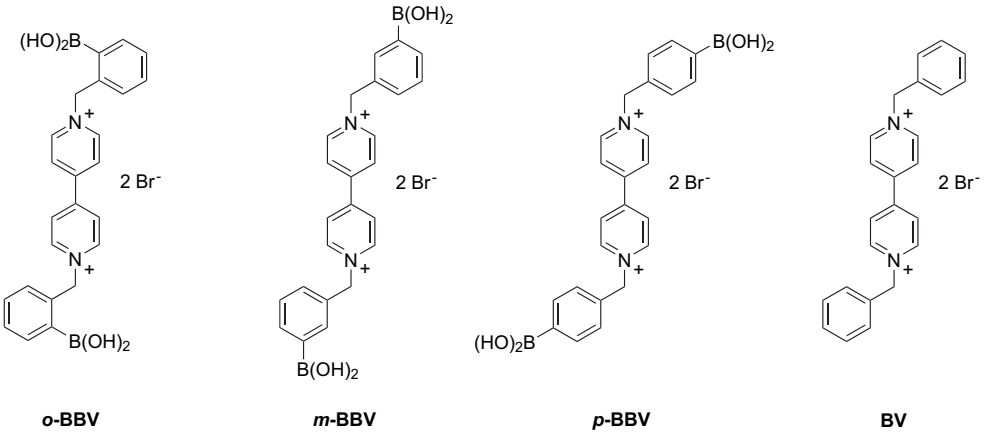


Figure 1. Structures of boronic acid-substituted benzyl viologens, *o*-BBV, *m*-BBV, and *p*-BBV, and their non-boronic acid counterpart, BV.

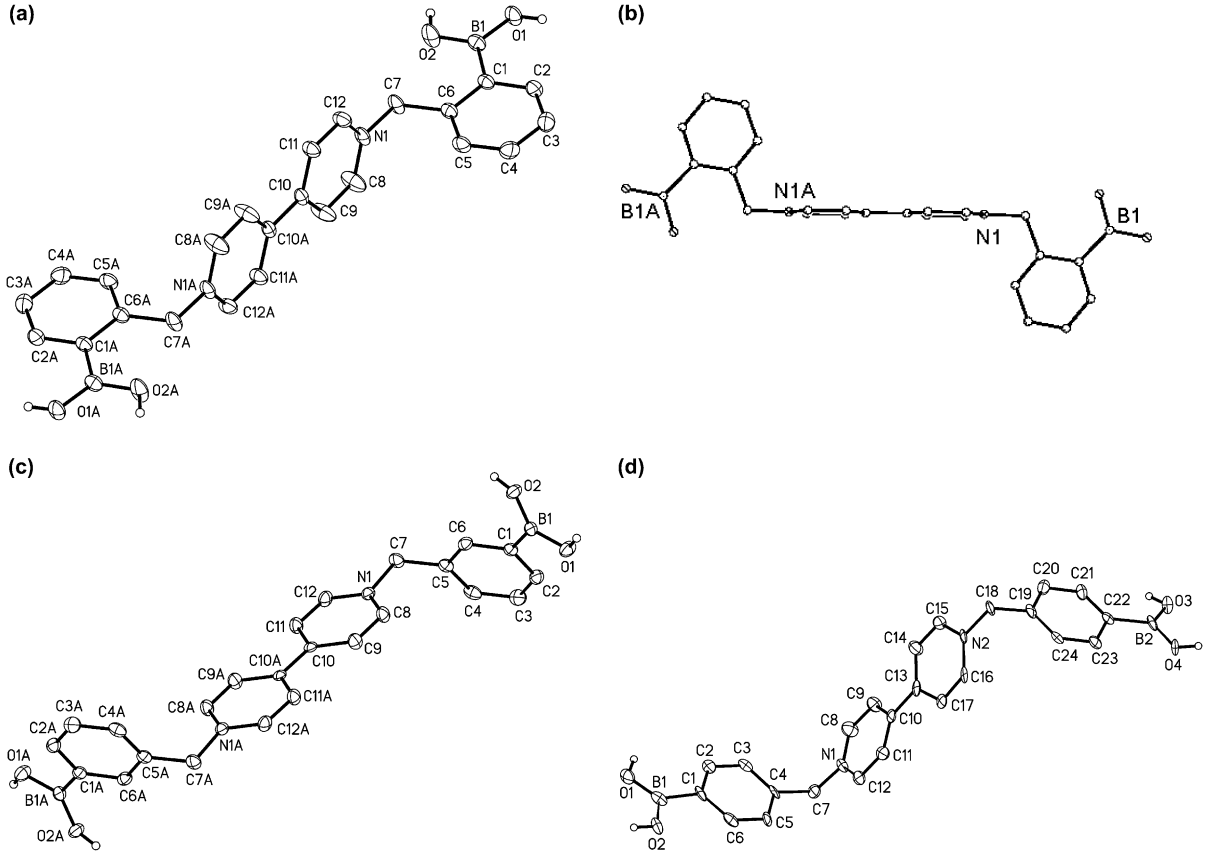
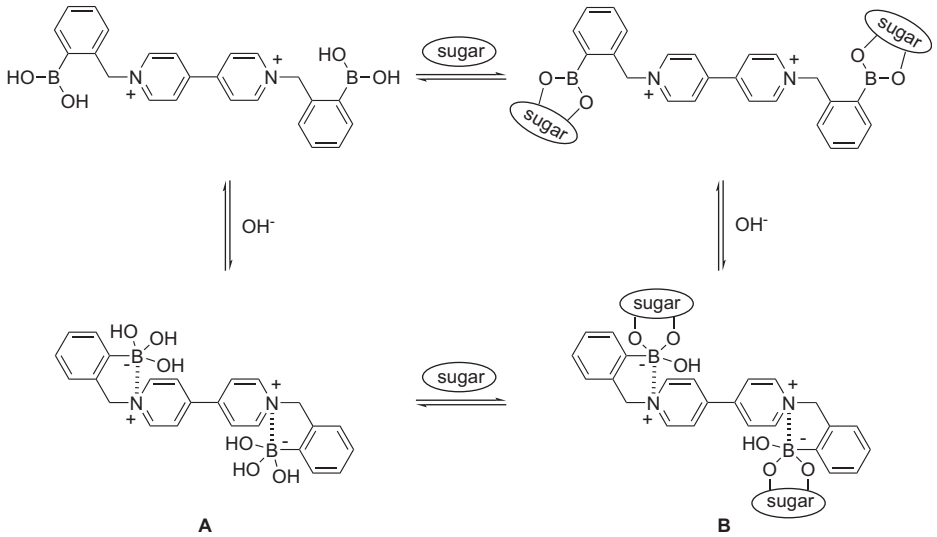


Figure 2. X-ray crystal structures of (a) *o*-BBV, (b) side view of *o*-BBV, (c) *m*-BBV, and (d) *p*-BBV. Thermal ellipsoids are drawn at the 50% probability level.



Scheme 3. The charge neutralization–stabilization mechanism of *o*-BBV. In structures **A** and **B**, dashed lines represent a charge interaction between the boronate and quaternary nitrogen.

occurrence of the proposed intramolecular electrostatic interaction for *o*-BBV.

2.2. Quenching of pyranine with the BBVs

For spectroscopic measurements to determine the relative quenching abilities of *o*-, *m*-, and *p*-BBV, a solution of pyranine (4×10^{-6} M in pH 7.4 phosphate buffer) was titrated with increasing amounts of the BBVs. The changes in the UV–vis absorbance spectra of pyranine upon addition of each of the viologens is shown in Figure 3. The bands at 404 and 454 nm represent the protonated and deprotonated forms of pyranine, respectively (due to the phenolic group). The addition of either *m*- or *p*-BBV to pyranine caused

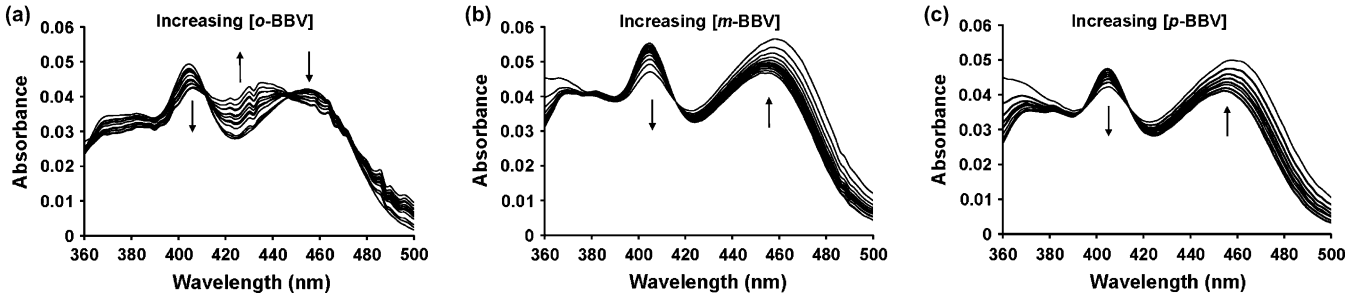


Figure 3. UV–vis absorbance spectra of pyranine (4×10^{-6} M in pH 7.4 phosphate buffer) with increasing concentrations of (a) *o*-BBV, (b) *m*-BBV, and (c) *p*-BBV.

a decrease in the 404 nm band and an increase in the 454 nm band. The addition of *o*-BBV, however, affected the absorbance of pyranine differently in that both the 404 and 454 nm bands decreased and a new band arose at 436 nm. These results indicate that *o*-BBV interacts with pyranine in a unique way.

The quenching efficiencies of each of the BBVs with pyranine were quantified using fluorescence measurements. The decrease in the fluorescence emission of pyranine (λ_{em}= 510 nm, λ_{ex}=460 nm) upon addition of *o*-BBV at pH 7.4 is demonstrated in Figure 4. Fluorescence quenching studies

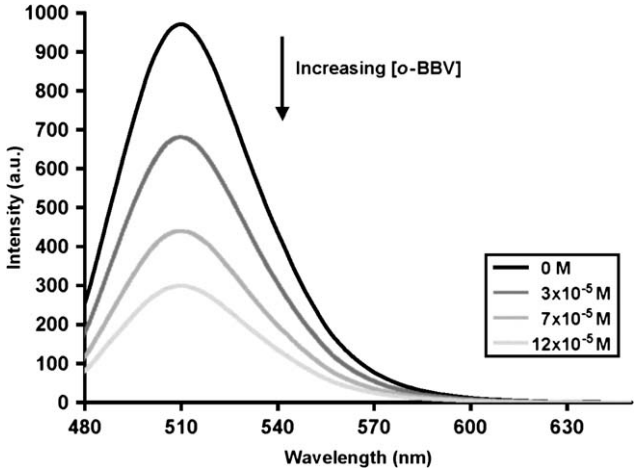


Figure 4. Fluorescence emission spectra of pyranine (4×10^{-6} M in pH 7.4 phosphate buffer, λ_{ex}=460 nm and λ_{em}=510 nm) with increasing concentrations of *o*-BBV.

were carried out at pH 3, 7.4, and 10. Stern–Volmer plots, which give graphical representations of the fluorescence data, are shown in Figure 5. From these plots, the static and dynamic quenching constants for BV and each of the BBVs were calculated using Eq. 1 (see Section 4), and are summarized in Table 1.

At pH 7.4 and 3, the BBVs are quite effective quenchers, giving static quenching constants around K_s~8000 M^{-1}. At pH 10 however, where the boronic acids are sp^3 hybridized and therefore anionic, the degree of quenching observed for all of the BBVs is greatly diminished (K_s~1500 M^{-1}) compared to that at pH 3 and 7.4. This is because formation of anionic boronates at pH 10 causes the BBVs to become zwitterionic, and a loss in electrostatic affinity for the anionic dye pyranine results. Conversely, for the non-boronic acid-containing compound, BV, the quenching constants were greatest at pH 10. This type of behavior for non-boronic acid-containing viologens has been observed in previous studies,[21] and is expected since Coulombic attraction in the ground-state is maximized at high pH.[18g]

When comparing each of the quenchers within a given pH range, we observe that at neutral or low pH values, the quenching efficiencies of *o*-, *m*-, and *p*-BBV are of similar magnitudes. But, at pH 10, the quenching efficiency of *o*-BBV is less than half that of *m*- and *p*-BBV. This dissimilarity of *o*-BBV from *m*- and *p*-BBV at high pH can be explained by evoking the charge neutralization–stabilization mechanism previously described (Scheme 3). For *o*-BBV at high pH, an intramolecular charge interaction can occur between the anionic boron and the quaternary nitrogen

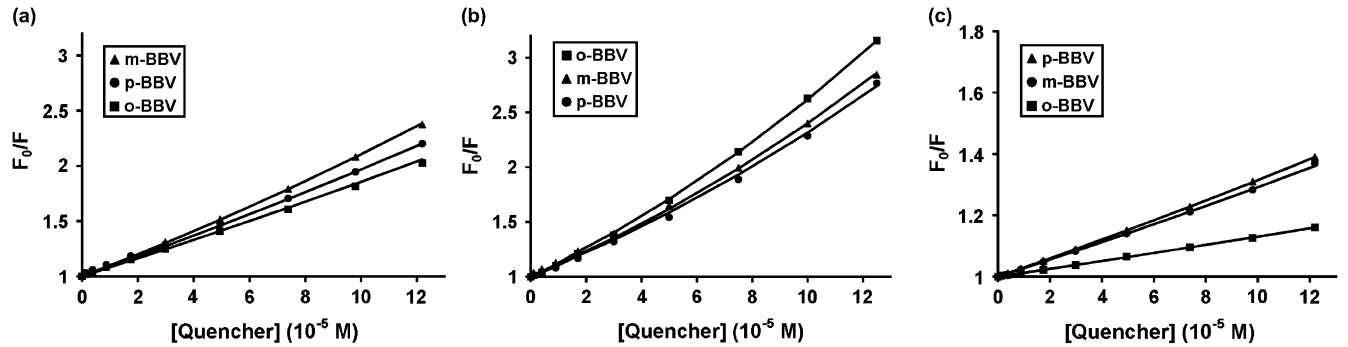


Figure 5. Stern–Volmer plots of pyranine (4×10^{-6} M) with increasing concentrations of *o*-BBV (■), *m*-BBV (▲), and *p*-BBV (●) at (a) pH 3, (b) pH 7.4, and (c) pH 10. For pH 3, λ_{ex}=416 nm and λ_{em}=510 nm. For pH 7.4 and 10, λ_{ex}=460 nm and λ_{em}=510 nm.

Table 1. Static (K_s) and dynamic (V) quenching constants for BV, *o*-BBV, *m*-BBV, and *p*-BBV with pyranine (4×10^{-6} M) in buffered 0.1 ionic strength solutions at different pH values

	pH 3		pH 7.4		pH 10	
	K_s (M^{-1})	V (M^{-1})	K_s (M^{-1})	V (M^{-1})	K_s (M^{-1})	V (M^{-1})
o-BBV	7300±100	750±100	8900±200	2900±200	670±80	570±60
m-BBV	8200±100	1400±150	7800±100	3100±100	2000±100	750±100
p-BBV	8000±100	890±180	7600±250	2700±150	2100±100	800±100
BV	10,000±200	1100±200	14,000±200	2500±200	25,000±200	3000±200

(structure A, Scheme 3).[†] This interaction allows for more effective neutralization of the cationic viologen, which results in less effective quenching of pyranine with *o*-BBV at pH 10 relative to *m*- and *p*-BBV.

2.3. pK_a Determination methods

It has been shown that the pK_a values for arylboronic acids are dependent upon the substitution pattern and nature of the substituents on the aromatic ring.[27–35] We therefore expected to see a lowered pK_a for the BBVs relative to phenylboronic acid (pK_a=8.8)[36] due to the methylpyridinuim substituent. Several different methods have been used to measure boronic acid pK_a values, such as UV–vis spectroscopy,[22,23,27–29] fluorescence spectroscopy,[12,30,31,37] cyclic voltammetry,[38] ^{11}B NMR,[21,35,39] and potentiometric titration.[16,39b,40] Determinations of pK_a values using UV–vis spectroscopy are carried out by monitoring the change in absorbance of the arylboronic acid upon increasing pH, which occurs as a result of a differential molar absorbtivity between sp^2 boronic acid and the sp^3 boronate. For our benzyl viologen-based boronic acids, however, the UV–vis titration method did not produce clean pH profiles. The use of fluorescence spectroscopy to measure boronic acid pK_a values is usually carried out by monitoring the change in fluorescence emission of a fluorophore-containing boronic acid upon increasing pH, which often occurs due to a photo-induced electron transfer (PET) mechanism brought about upon conversion to the sp^3 boronate. But, since the benzyl viologen-based boronic acids used in this study do not fluoresce, only indirect methods of measuring their apparent pK_a values via fluorescence are possible. Such analyses, however, are beyond the scope of this study.

For most systems, the use of ^{11}B NMR spectroscopy to determine boronic acid pK_a values is a reliable technique. We were able to generate pH profiles for the BBVs using ^{11}B NMR by observing the chemical shift dependence of the boron moiety on pH (Fig. 6). At low pH values, the boronic acids exist in their neutral sp^2 form and display a chemical shift of ~30 ppm. Increasing addition of base resulted in conversion to the anionic sp^3 form, causing a gradual upfield shift to lower parts per million values (Fig. 7). The apparent pK_a values for *m*- and *p*-BBV obtained by this method correlated fairly well with values previously reported for similar compounds.[‡] However, the value obtained for *o*-BBV (pK_a=8.8) seemed unreasonably high. We suspect that the mechanism proposed in Scheme 3 is

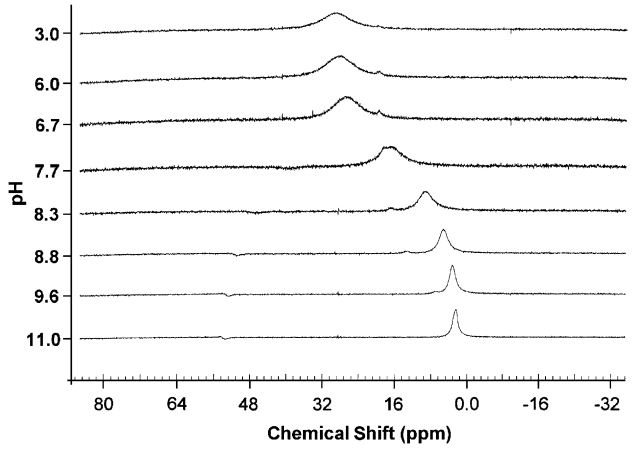


Figure 7. Changes in ^{11}B NMR spectrum of *m*-BBV with increasing pH (30 mM *m*-BBV in 0.15 M KCl titrated with 3 M KOH).

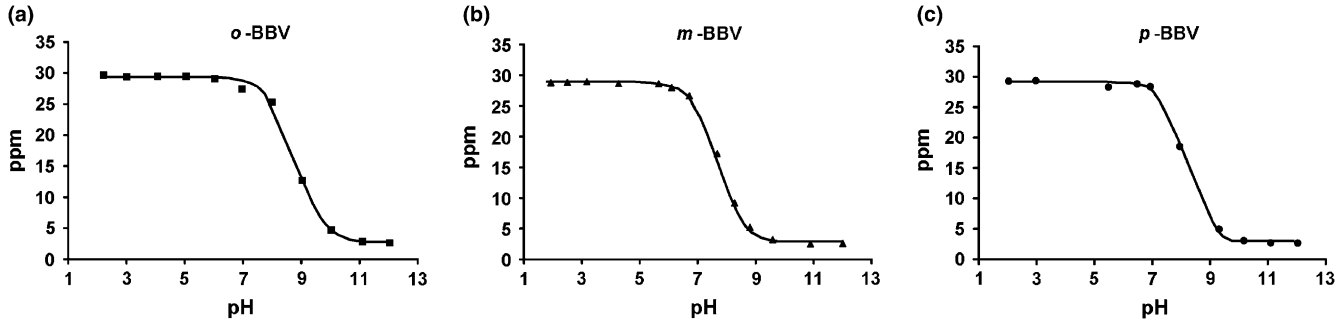


Figure 6. Determination of pK_a by ^{11}B NMR (30 mM BBV in 0.15 M KCl titrated with 3 M KOH). The pH profiles of (a) *o*-BBV, (b) *m*-BBV, and (c) *p*-BBV plotted as chemical shift versus pH.

[†] Structure **A** was optimized at the semi-empirical AM1 level using Spartan. The molecular modeling data indicate B–N interaction (see Supplementary data).

[‡] See Figure 9 and references listed in Table 2.

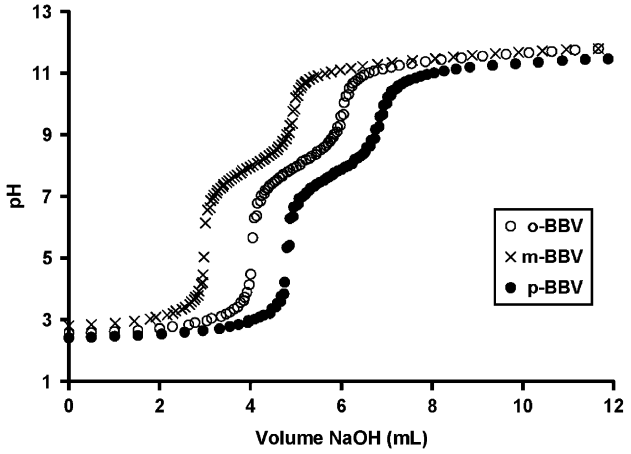


Figure 8. pH profile of *o*-BBV (○), *m*-BBV (×), and *p*-BBV (●) generated via potentiometric titration (4 mM BBV and HCl titrated with 0.1 M NaOH in 0.15 M NaCl; the pH at the halfway point equals the pK_a).

responsible for this misleading result, since neutralization of the negative charge on sp^3 boron by the quaternary nitrogen can cause higher chemical shift values than normal for the boronate by minimizing shielding effects.

Of the possible pK_a determination methods, we found potentiometric titration to be most suitable for the particular boronic acids under investigation, specifically *o*-BBV. Figure 8 shows the pH profiles for *o*-, *m*-, and *p*-BBV generated by potentiometric titration. Table 2 summarizes the pK_a results for both methods employed and, for comparison, lists pK_a values found in the literature for compounds containing a similar boronic acid-substituted benzyl pyridinium motif (Fig. 9). In contrast to the results obtained by ^{11}B NMR, the pK_a of *o*-BBV determined by potentiometric titration (8.0) was very similar to those determined for *m*- and *p*-BBV (7.9 and 7.7, respectively). These values were found to correlate quite well with pK_as reported in the literature for structurally similar boronic acids.

Table 2. Comparison of apparent pK_a values for the BBVs determined via ^{11}B NMR and potentiometric titration with literature values reported for structurally similar (Fig. 9) compounds

	^{11}B NMR	Potentiometric titration	Literature values[a]		
			Ref. 23[b]	Ref. 22[b]	Ref. 16[c]
o-BBV	8.8	8.0	7.9 (3*ortho*)	6.70 (4*ortho*)	7.73 (5*ortho*)
m-BBV	7.8	7.9	7.7 (3*meta*)	7.75 (4*meta*)	
p-BBV	8.1	7.7	7.9 (3*para*)	7.80 (4*para*)	7.92 (5*para*)

[a] See Figure 9.
[b] Determined by UV–vis absorbance spectroscopy.
[c] Determined by potentiometric titration.

2.4. Saccharide sensing

As described in Scheme 2, signal modulation in response to varying glucose concentrations occurs when glucose is added to a solution of pyranine that has been quenched by a BBV. When the glucoboronic ester forms, the Lewis acidity of boron is increased, facilitating 'ate' complex formation, and the BBV becomes partially zwitterionic at pH 7.4. This change in hybridization of the boron moiety from sp^2 to sp^3 upon glucose addition can be seen by ^{11}B NMR (Fig. 10), where the signal for trigonal boron at ~30 ppm decreases and a new signal at ~8 ppm for the tetrahedral glucoboronate ester arises.

This change in charge of the BBV decreases its ability to effectively quench the fluorescence of pyranine via static quenching. Since the structure of *o*-BBV is such that a through-space neutralization of the quaternary nitrogen by the anionic glucoboronate is feasible (structure B, Scheme 3), we rationalized that it should be the least effective quencher in the presence of glucose relative to *m*- or *p*-BBV. Accordingly, a comparison of the Stern–Volmer quenching constants determined for *o*-, *m*-, and *p*-BBV in the presence of 20 mM glucose revealed this to be the case. Figure 11 shows a Stern–Volmer plot of pyranine with *o*- and *m*-BBV in the absence and presence of glucose, where it can be seen that the quenching ability of *o*-BBV decreases most dramatically in the presence of glucose. Table 3 summarizes these data and gives the difference in quenching constants (ΔK_s and ΔV) with and without glucose. The presence of glucose did not affect the quenching ability of the control compound, BV.

According to our proposed glucose-sensing mechanism (Scheme 2), the fluorescence increase obtained when glucose is added to the system results from a decrease in the quenching efficiency of the glucoboronate ester compared to that of the free boronic acid. Therefore, since *o*-BBV is the weakest quencher in the presence of glucose (Table 3), we expected the *o*-BBV/pyranine complex to display the

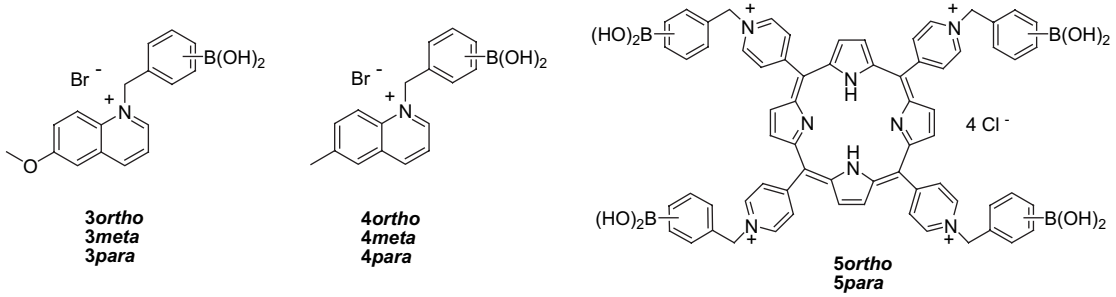


Figure 9. Structures of compounds **3**,[23] **4**,[22] and **5**[16] containing a boronic acid-substituted benzyl pyridinium motif for which pK_a values have been previously reported (Table 2).

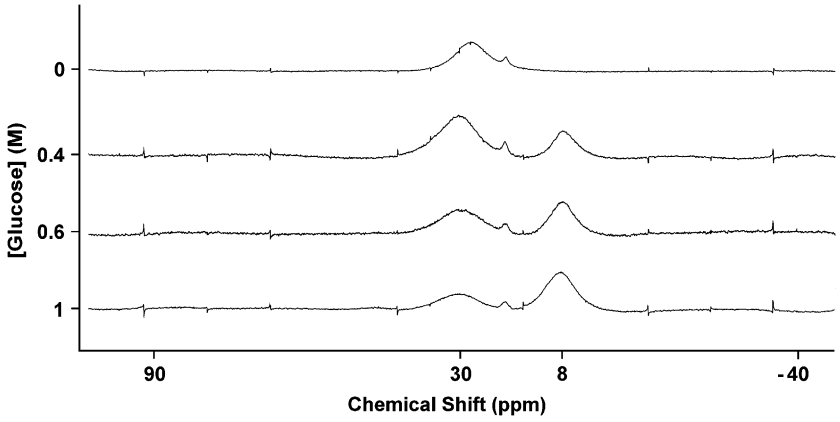


Figure 10. Changes in ^{11}B NMR spectrum of *m*-BBV (30 mM in pH 7.4 phosphate buffer) with increasing concentrations of glucose. High concentrations of glucose were used in order to achieve [quencher]/[glucose] ratios similar to those used in fluorescence studies. The small peak at 19 ppm is due to boric acid.

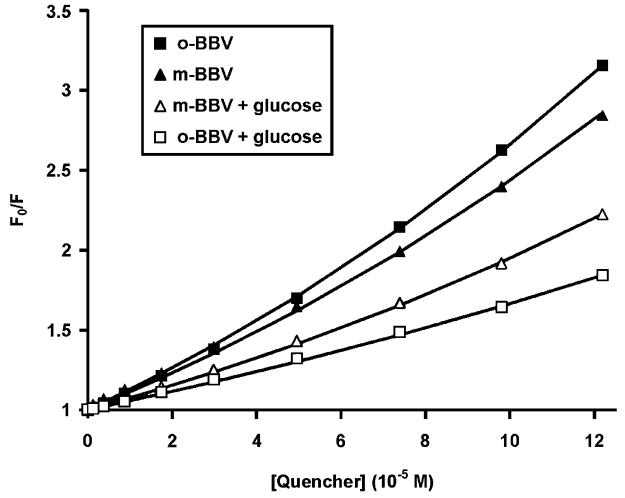


Figure 11. Stern–Volmer plot for the quenching of pyranine (4×10^{-6} M in pH 7.4 phosphate buffer, λ_{ex}=460 nm and λ_{em}=510 nm) with *m*-BBV (▲), *m*-BBV in the presence of 20 mM glucose (△), *o*-BBV (■), and *o*-BBV in the presence of 20 mM glucose (□).

largest fluorescence modulation upon glucose addition relative to *m*- and *p*-BBV/pyranine. For glucose-sensing experiments, BBV is added to a solution of pyranine, and the quenched fluorescence intensity is recorded. Increasing concentrations of glucose are then added to the BBV/pyranine complex and enhanced fluorescence intensities are observed (Fig. 12a). Figure 12b shows the degree of fluorescence enhancement obtained for each of the quencher/dye complexes plotted as a function of glucose concentration, where it can be seen that *o*-BBV exhibits the highest fluorescence modulation out of the three receptors. Importantly, all the BBVs displayed considerable fluorescence enhancements

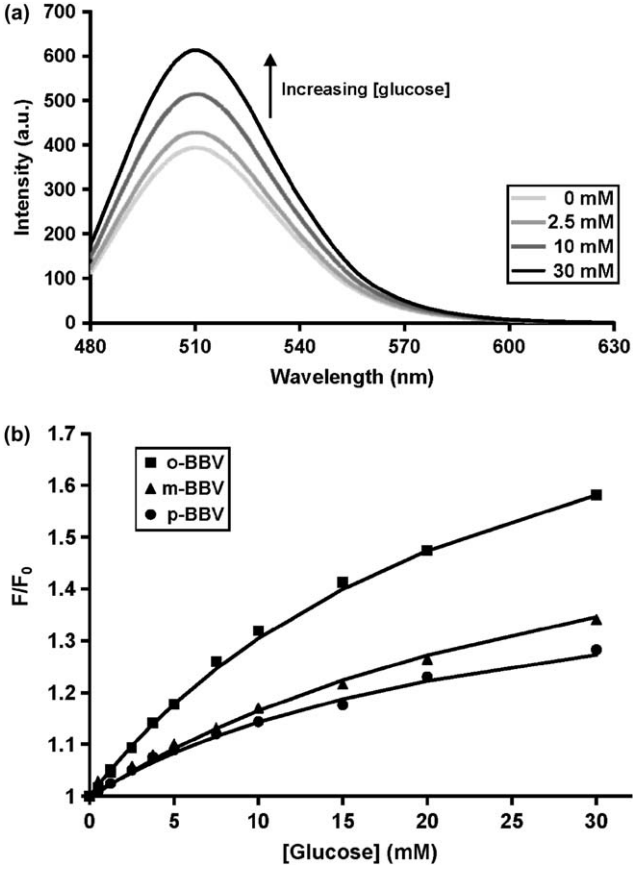


Figure 12. Glucose sensing of BBVs (1.2×10^{-4} M) with pyranine (4×10^{-6} M in pH 7.4 phosphate buffer, λ_{ex}=460 nm and λ_{em}=510 nm). (a) Change in fluorescence spectrum of pyranine in the presence of *o*-BBV upon addition of glucose. (b) Binding isotherms: fluorescence increase of pyranine in the presence of *o*-BBV (■), *m*-BBV (▲), and *p*-BBV (●) as a function of glucose concentration.

Table 3. Static (K_s) and dynamic (V) quenching constants of *o*-BBV, *m*-BBV, *p*-BBV, and BV with pyranine (4×10^{-6} M in pH 7.4 phosphate buffer, λ_{ex}=460 nm and λ_{em}=510 nm) in the absence and presence of glucose (20 mM)

	Absence of glucose		Presence of 20 mM glucose		Change	
	K_s (M^{-1})	V (M^{-1})	K_s (M^{-1})	V (M^{-1})	ΔK_s	ΔV
o-BBV	8900±200	2900±200	3000±100	2400±100	5900	500
m-BBV	7800±100	3100±100	4000±100	3300±100	3800	200
p-BBV	7600±250	2700±150	3600±100	2900±100	4000	200
BV	14,000±200	2500±200	14,000±200	2100±200	0	400

in response to glucose concentrations within the clinically relevant range of 45–360 mg dL^{-1} (2.5–20 mM).

The calculated apparent binding constants (K_b) listed in Table 4 show that *o*-BBV also has the largest binding constant, which is consistent with an enhanced stabilization of the glucoboronate ester. Previously, BV was shown to be unresponsive to glucose in this system.[13]

The BBVs were also tested for their ability to bind other monosaccharides such as fructose and galactose (Fig. 13). The saccharide selectivity for all of the quencher/receptors was found to be of the order fructose>galactose>glucose. This binding order is parallel to that observed for monoboronic acids, indicating that cooperative binding is not taking place.[6,9] Table 4 lists the calculated apparent binding constants (K_b) for each of the BBVs with the different monosaccharides.

Finally, the pK_a values for *o*-, *m*-, and *p*-BBV in the presence of glucose were obtained by potentiometric titration (Fig. 14). *o*-BBV displayed the largest drop in pK_a in the presence of glucose (ΔpK_a) relative to *m*- or *p*-BBV (Table 5), again indicating an enhanced stabilization of the boronate ester. The results further substantiate that the proposed charge neutralization–stabilization mechanism is operative for *o*-BBV.

Table 4. Apparent saccharide binding constants (K_b) determined for the BBVs (1.2×10^{-4} M) with pyranine (4×10^{-6} M in phosphate buffer, λ_{ex}=460 nm and λ_{em}=510 nm)

	Glucose	Galactose	Fructose
	K_b (M^{-1})	K_b (M^{-1})	K_b (M^{-1})
o-BBV	45±5	150±5	2000±100
m-BBV	30±3	85±5	1200±100
p-BBV	33±3	70±5	800±50

Table 5. pK_a values, determined by potentiometric titration, for the BBVs (4 mM) in the absence and presence of 0.3 M glucose

	pK_a without glucose	pK_a with 0.3 M glucose	ΔpK_a
o-BBV	8.0	6.3	1.7
m-BBV	7.9	6.5	1.4
p-BBV	7.7	6.5	1.2

3. Conclusions

For the glucose-sensing ensemble comprising a boronic acid-substituted benzyl viologen and the anionic dye pyranine, a systematic investigation of the effect of boronic acid-positioning on both the quenching and glucose-sensing mechanisms was performed. Under conditions where the anionic boronate is formed, such as at pH 10 and in the presence of glucose at pH 7.4, the benzyl viologen that contains *ortho*-substituted boronic acids, *o*-BBV, stood out from the other two (*m*- and *p*-BBV) presumably as a result of the intramolecular electrostatic interaction between the anionic boronate and the quaternary nitrogen. Apparent pK_a data obtained from ^{11}B NMR studies and potentiometric titration helped confirm the occurrence of this mechanism in *o*-BBV. From the standpoint of sensor design, in this type of two-component system, having the boron in a position where the negative charge resulting from sugar complexation

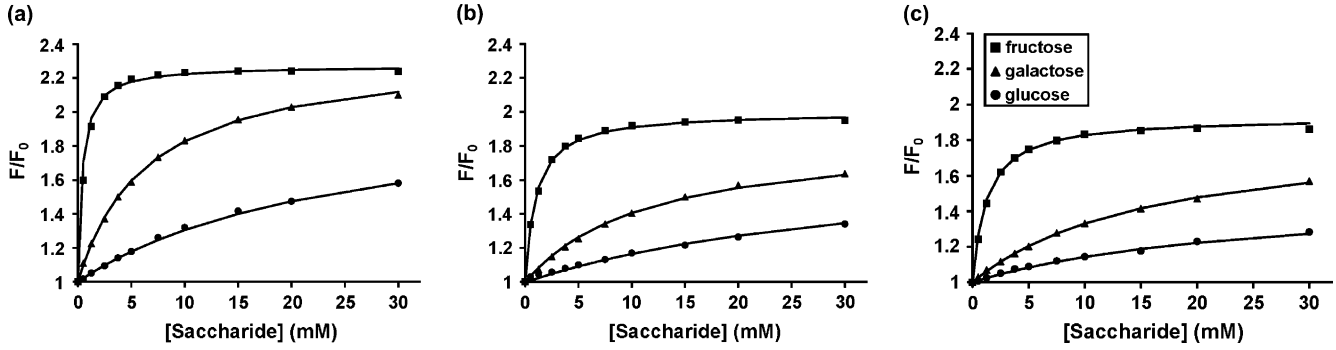


Figure 13. Fluorescence increase of pyranine (4×10^{-6} M in phosphate buffer, λ_{ex}=460 nm and λ_{em}=510 nm) in the presence of (a) *o*-BBV, (b) *m*-BBV, and (c) *p*-BBV (1.2×10^{-4} M) upon addition of different monosaccharides.

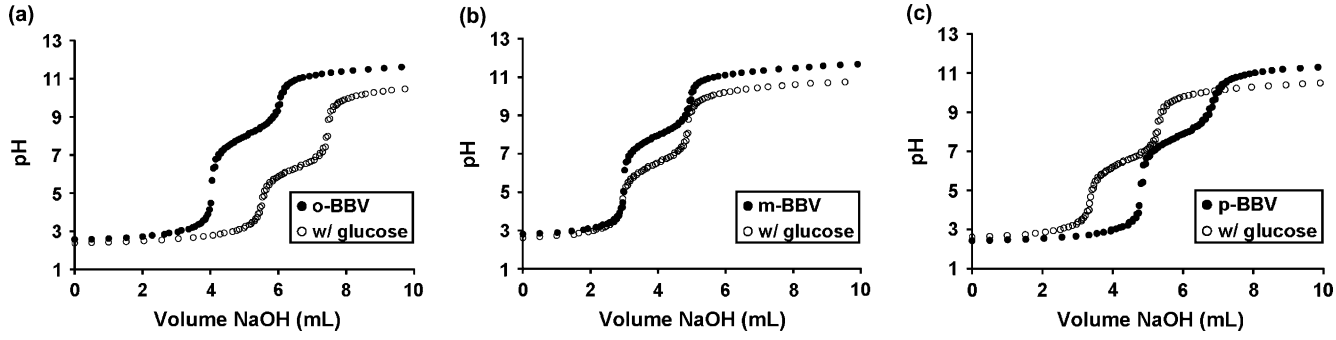


Figure 14. pH profile of (a) *o*-BBV, (b) *m*-BBV, and (c) *p*-BBV in the absence (●) and presence (○) of 0.3 M glucose generated via potentiometric titration (4 mM BBV titrated with 0.1 M NaOH in 0.15 M NaCl; the pH at the halfway point equals the pK_a).

can more effectively neutralize the positive charge of the viologen is most desirable.

4. Experimental

4.1. Synthesis

The syntheses of *o*-BBV[13] and *m*-BBV[20] have been previously reported.

4.1.1. General. Reactions were performed using standard syringe techniques, and carried out in an oven-dried glassware under an argon atmosphere. Dimethylformamide (DMF) was dried over CaH_2 prior to use. 4-Bromomethyl phenylboronic acid was purchased from Lancaster and used as received. Benzyl bromide and 4,4′-dipyridyl were purchased from Aldrich and used as received. ^{1}H NMR spectra were recorded on a Varian spectrometer at 500 MHz and are reported in parts per million with respect to TMS (δ=0). Proton decoupled ^{13}C NMR spectra were recorded on a Varian at 125 MHz and are reported in parts per million.[§] ^{11}B NMR spectra were recorded on a Bruker at 80.25 MHz and are reported in parts per million with respect to $BF_3\cdot OEt_2$ (δ=0). High-resolution mass measurements were obtained on a bench-top Mariner ESITOF mass spectrometer.

4.1.2. *N*,*N*′-4,4′-Bis(benzyl-4-boronic acid)-bipyridinium dibromide (*p*-BBV). To a solution of 4-(bromomethyl)-phenylboronic acid (1.74 g, 8.1 mmol) in DMF (15 mL) was added 4,4′-dipyridyl (0.5 g, 3.2 mmol), and the reaction mixture was stirred at 55 °C for 48 h. The orange precipitate was collected by centrifugation, washed with DMF, then acetone, and dried under a stream of argon to yield *p*-BBV (1.3 g, 69% yield). ^{1}H NMR (D_2O, 500 MHz) δ 6.09 (s, 4H), 7.67 (d, J=8.0 Hz, 4H), 7.98 (d, J=7.5 Hz, 4H), 8.68 (d, J=7.0 Hz, 4H), 9.30 (d, J=7.0 Hz, 4H); ^{13}C NMR (D_2O, 125 MHz) δ 66.1, 128.6, 130.0, 136.08, 136.2, 147.05, 147.12, 151.8; ^{11}B NMR (80 MHz, D_2O) δ 28.9. HRMS–ESI *m*/*z* calcd for $C_{24}H_{23}B_2N_2O_4$ $[M-H]^+$: 425.18301, found 425.18385.

4.1.3. *N*,*N*′-4,4′-Bis(benzyl)-bipyridinium dibromide (BV). To a solution of benzyl bromide (0.45 mL, 3.75 mmol) in DMF (5 mL) was added 4,4′-dipyridyl (0.23 g, 1.5 mmol), and the reaction mixture was stirred at 70 °C for 16 h. The yellow precipitate was collected by centrifugation, washed with acetone, and dried under a stream of argon to yield pure BV (0.67 g, 90% yield). Spectroscopic data were in accord with the literature values.[41]

4.2. X-ray structure determinations

X-ray single crystal diffraction experiments were carried out on a Bruker SMART 1000 for [*o*-BBV]Br_2 and [*m*-BBV]$Br_2\cdot H_2O$ and a Bruker SMART Apex for [*p*-BBV]$Br_2\cdot 5H_2O$ with the use of Mo Kα radiation (λ=0.71073 Å). The data for *o*-BBV were collected at 166(2) K due to cracking of the crystals at temperatures lower than this. Data for *m*-BBV and *p*-BBV were collected at 90(2) K. Solution and refinement software were SAINT for data reduction, SHELXS97 for solution, and SHELXL97 for refinement. In the structures of [*o*-BBV]Br_2 and [*m*-BBV]$Br_2\cdot H_2O$ the cations reside on centers of symmetry, and this requires the bipyridine planes to be coplanar. In the structure of [*p*-BBV]$Br_2\cdot 5H_2O$, there are two independent molecules in the asymmetric unit, and the normals to the bipyridine planes subtend dihedral angles of 16.5° and 23.9° for the two different molecules. Disordered water molecules of crystallization caused the *R* value to be somewhat elevated for this latter structure. Crystallographic data for the structures in this paper have been deposited with the Cambridge Crytallographic Data Centre as supplementary publication numbers CCDC 298628-298630. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

4.2.1. [*o*-BBV]Br_2. $C_{24}H_{24}B_2Br_2N_2O_4$, M=585.89, monoclinic, a=9.0541(7) Å, b=9.6817(8) Å, c=14.3909(12) Å, β=107.574(2)°, V=1202.62(17) Å^3, space group $P2_1/c$, Z=2, T=166(2) K, D_{calcd}=1.618 mg m^{-3}, μ(Mo Kα)=3.405 mm^{-1}, 13,306 reflections measured, 2771 unique (R_{int}=0.039) used in all calculations. The final *wR*2 was 0.0779 (all data) and $R_1(2165I>2\sigma(I))$=0.0306. Residual electron density was 0.444 and −0.346 eÅ^{-3}.

4.2.2. [*m*-BBV]$Br_2\cdot H_2O$. $C_{24}H_{26}B_2Br_2N_2O_5$, M=603.91, monoclinic, a=14.3954(11) Å, b=14.0371(9) Å, c=12.7590(9)Å, β=104.299(2)°, V=2498.3(3) Å^3, space group $P2_1/c$, Z=4, T=90(2) K, D_{calcd}=1.606 mg m^{-3}, μ(Mo Kα)=3.283 mm^{-1}, 25,461 reflections measured, 5733 unique (R_{int}=0.045) used in all calculations. The final *wR*2 was 0.0717 (all data) and $R_1(4372I>2\sigma(I))$=0.0293. Residual electron density was 0.490 and −0.329 eÅ^{-3}.

4.2.3. [*p*-BBV]$Br_2\cdot 5H_2O$. $C_{24}H_{34}B_2Br_2N_2O_9$, M=675.97, monoclinic, a=16.932(5) Å, b=28.674(8) Å, c=12.118(3) Å, β=103.963(5)°, V=5710(3) Å^3, space group $P2_1/c$, Z=8, T=90(2) K, D_{calcd}=1.573 mg m^{-3}, μ(Mo Kα)=2.892 mm^{-1}, 53,403 reflections measured, 10,494 unique (R_{int}=0.151) used in all calculations. The final *wR*2 was 0.2178 (all data) and $R_1(6197I>2\sigma(I))$=0.0827. Residual electron density was 1.784 and −1.363 eÅ^{-3}.

4.3. Fluorescence emission and UV–vis absorption measurements

4.3.1. General. 8-Hydroxypyrene-1,3,6-trisulfonic acid, trisodium salt (pyranine), D-glucose, D-glactose, and D-fructose were purchased from Aldrich. All solutions were prepared with water that was purified via a Barnstead NANOpure system (17.7 MΩ/cm). Buffers (0.1 ionic strength) were freshly prepared before use (pH 3: KH_2PO_4, H_3PO_4; pH 7.4: KH_2PO_4, Na_2HPO_4; pH 10: Na_2CO_3, $NaHCO_3$). pH measurements were taken on a Denver Instrument UB-10 pH/mV meter and calibrated with standard buffer solutions (pH 4, 7, and 10 from Fisher). Absorption spectra were taken on a Hewlett Packard 8452A Diode Array Spectrophotometer. Fluorescence spectra were taken on a Perkin–Elmer LS50-B luminescence spectrometer, and were carried out at 25 °C. Standard quartz fluorescence cuvettes were used in all studies. Pyranine was excited at two different wavelengths depending on the pH. For samples run at pH 3, the

[§] Due to relaxed ^{13}C–^{11}B spin–spin coupling, signals for carbons directly attached to boron are not observed.

excitation was 416 nm; for samples run at pH 7.4 and 10, the excitation was 460 nm. The emission was collected from 470 to 650 nm. Pyranine is known to undergo an excited state proton transfer reaction in bulk water where water acts as the proton acceptor.[42] Thus, the emission collected at pH 3, 7.4, or 10 corresponds to the emission of the deprotonated form of pyranine. For fluorescence titration experiments, the added volume did not exceed 3% of the total volume and the absorbance for all fluorescence measurements was below 0.1.[43] All experiments were carried out in triplicate, and the errors in the reported binding constants are based on the standard deviation of three independent determinations. All data were analyzed using the Solver (nonlinear least-squares curve fitting) in Microsoft Excel.

4.3.2. Absorbance measurements. Measurements were done in situ by taking the absorbance spectra of pyranine with each of the quenchers. The emission of pyranine (2 mL of 4×10^{-6} M in buffer) was first obtained, then aliquots of quencher (0.5–10 μL aliquots of 5 mM in buffer) were added, the solution was shaken for 30 s, and the absorbance was measured after each quencher addition.

4.3.3. Fluorescence measurements for quenching studies. Fluorescence measurements were done in situ by taking the emission spectrum of pyranine at a series of quencher concentrations. The emission spectrum of pyranine (2 mL of 4×10^{-6} M in buffer) was first obtained, quencher was added (0.5–10 μL aliquots of 5 mM in buffer), the solution was shaken for 30 s, and the new emission was measured after each quencher addition. For quenching studies carried out in the presence of glucose, the emission spectrum of pyranine (2 mL of 4×10^{-6} M in buffer) was first obtained, glucose was added (40 μL of 1.0 M in buffer), the emission measured, then quencher was added (0.5–10 μL aliquots of 5 mM in buffer), the solution was shaken for 30 s, and the new emission was measured after each quencher addition.

Data analysis: Fluorescence intensity was taken as the area under the curve between 470 and 650 nm for all studies. Stern–Volmer quenching constants were calculated by fitting the data with Eq. 1:

$$F_0/F = (1 + K_s[Q])e^{V[Q]} \quad (1)$$

where F_0 is the initial fluorescence intensity, F is the fluorescence intensity after the addition of quencher, V is the dynamic quenching constant, K_s is the static quenching constant, and $[Q]$ is the quencher concentration.[18a,44]

4.3.4. Fluorescence measurements for saccharide sensing studies. The emission spectrum of pyranine (2 mL of 4×10^{-6} M in buffer) was taken, quencher was added (50 μL of 5 mM in buffer) to obtain a quencher/pyranine ratio of 31:1, the emission measured, then sugar solution was added (0.5–10 μL aliquots of 1 M in buffer), the solution was shaken for 30 s, and the new emission was measured after each addition of sugar.

Data analysis: Fluorescence intensity was taken as the area under the curve between 470 and 650 nm for all studies. Apparent sugar binding constants were calculated by fitting the data with Eq. 2:

$$F/F_0 = (F_0 + F_{max}K_b[S])/(1 + K_b[S]) \quad (2)$$

where F_0 is the fluorescence intensity of the quenched dye, F is the fluorescence intensity after the addition of sugar, F_{max} is the intensity at which the fluorescence increase reaches its maximum, K_b is the apparent binding constant, and $[S]$ is the concentration of sugar (glucose, fructose, or galactose).[37b]

4.4. ^{11}B NMR titrations

A 2 mL solution quencher (0.03 M) containing 0.15 M KCl was lowered to pH 2 by the addition of 3 M HCl. A 5-mm quartz NMR tube (Norell) was charged with 0.75 mL of this solution and a spectrum was recorded. The contents of the tube were added to the original 2 mL solution, and the pH was raised in increments of 0.5–1 pH unit by adding 3 M KOH. The ^{11}B NMR spectrum was taken after each pH adjustment until pH 12. The pK_as were calculated using Eq. 3:

$$PPM_{calc} = \left(PPM_{min} + PPM_{max}10^{pK_a}\left[H^+\right]\right)/\left(1 + 10^{pK_a}\left[H^+\right]\right) \quad (3)$$

where PPM_{calc} is the calculated chemical shift in parts per million, PPM_{min} is the initial chemical shift, PPM_{max} is the final chemical shift, pK_a is the acidity constant, and $[H^+]$ is the hydrogen ion concentration.[37b]

4.5. Potentiometric titrations

Potentiometric studies were conducted with a KEM AT-500N automatic titrator equipped with a combined pH glass electrode (Ag/AgCl reference electrode) containing 3.3 M KCl internal filling solution. Measurements were taken at 25 °C. About 100 data points were collected for each titration and imported into Excel using the AT-Win software package. Solutions of boronic acid (4 mM in deionized water) were adjusted to pH ~2 with a drop of concd HCl, then titrated with 0.1 M NaOH in 0.15 M NaCl. Two endpoints were detected (one for HCl and one for the boronic acid). For the weak acid–strong base titration curve of the boronic acid, the pK_a was determined from the halfway point in the titration.

Acknowledgements

We thank the BioSTAR Project and the Industry–University Cooperative Research Program (grant bio04-10458) with GluMetrics, Inc. for their financial support.

Supplementary data

Molecular modelling data for structure A (Scheme 3). ^{1}H and ^{13}C NMR spectra for *p*-BBV. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.04.047.

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Synthesis and reactions of 1-methyl-5-tributylstannyl-4-trifluoromethylpyrazole

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Received 28 March 2006; revised 10 April 2006; accepted 12 April 2006
Available online 18 May 2006

Abstract—N-Methylation at the pyrazole ring by sequential treatment of 5-tributylstannyl-4-trifluoromethylpyrazole with LDA and iodomethane regioselectively provided the title compound in high yield. The addition reaction of 5-lithiated-4-trifluoromethylpyrazole with a wide range of electrophiles allowed easy and high-yielding introduction of substituents on position 5.

1. Introduction

Much effort has been exerted to introduce fluorine into organic compounds due to its dramatic effects on structure, stability, reactivity, and biological activity.[1] These features have been exploited to design and prepare new, more selective, and more potent pharmaceuticals and agrochemicals, for instance, and consequently the number of new fluorinated compounds burgeons out in recent days. Among these, trifluoromethylated molecules display remarkable applications in the pharmaceutical field as exemplified by Celecoxib[2] and Efavirenz,[3] and additional developments of useful trifluoromethylated building blocks are much being sought.

In our study on the development of new fluorinated building blocks, we have recently reported the synthesis and some cross-coupling reactions of 5-tributylstannyl-4-trifluoromethylpyrazole.[4] The wide range of physical and biological activities of trifluoromethylated pyrazoles has thus made them significant synthetic targets.[5] Moreover, the *N*-methylpyrazole unit is part of several agrochemicals and pharmaceuticals.[6] For instance, pyrazosulfon-ethyl is a successful rice herbicide,[6l] and the *N*-methylpyrazole group is found in a structural element present in antibacterial 4-pyrrolidinythiocarbapenems.[6g]

As an extension of our work, the title trifluoromethylated pyrazole bearing a tributylstannyl group on position 5 can be considered as a promising candidate to build up more complex 1-methyl-4-trifluoromethylpyrazole compounds. Indeed transmetallation of the stannylpyrazole with ^{n}BuLi to generate the corresponding organolithium species and reaction with suitable electrophiles should give rise to the corresponding 1-methyl-4-trifluoromethyl-5-substituted-pyrazoles. However, to the best of our knowledge, there are no reports concerning the generation of the corresponding organolithium species from stannylpyrazoles.[5c,6h,7] We herein report the synthesis and reactions of the title compound including an application to the synthetic trifluoromethylated analogue of the analgesic Cizolirtine.

2. Results and discussion

We previously reported the preparation of 5-tributylstannyl-4-trifluoromethyl-1*H*-pyrazole **3** from 3,3,3-trifluoro-2-bromopropene **1** in two steps.[4] However, the moderate overall yield (40% in two steps) prompted us to improve the reaction procedure. Careful examination of the reaction conditions led to two improvements: (i) bis(tributylstannyl)-oxide [$(Bu_3Sn)_2O$] is a better electrophile than chlorotributylstannane (Bu_3SnCl) in the reaction of 3,3,3-trifluoro-1-propynyllithium generated in situ. (ii) No isolation of tributyl(3,3,3-trifluoro-1-propynyl)stannane **2** is necessary for the preparation of **3**. Thus, to a freshly prepared solution of **2** [from sequential treatment of **1** with LDA and $(Bu_3Sn)_2O$] was successively added an excess of ethereal diazomethane solution at −30 °C, and the mixture was gradually warmed to 0 °C to give the desired product **3** in 64% overall yield (Scheme 1).

Keywords: 1-Methyl-5-tributylstannyl-4-trifluoromethylpyrazole; 1-Methyl-5-substituted-4-trifluoromethylpyrazole; Cizolirtine CF_3-analogue; Tributyl(3,3,3-trifluoro-1-propynyl)stannane.
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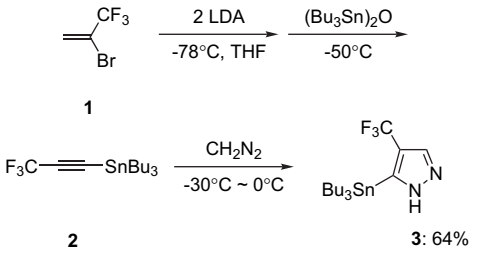
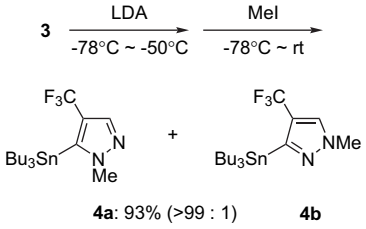
doi:10.1016/j.tet.2006.04.043



Scheme 1. One-pot synthesis of **3**.

With the precursor of the title compound produced, we examined methylation at the pyrazole ring nitrogen. It is well-accepted that the reaction of unsymmetrical pyrazoles affords products in which the less hindered nitrogen is preferentially substituted. In a literature report, for example, 1-methyl-3,4-bis(trimethylsilyl)pyrazole was regioselectively prepared from 3,4-bis(trimethylsilyl)-1*H*-pyrazole under mild conditions.[6n] According to a slightly modified procedure, the preliminary reaction of **3** with iodomethane was conducted in acetone at 40 °C using potassium carbonate as a base to give a 62/38 mixture of regioisomers in 84% yield. Attempt to overcome this problem involved the preparation of the corresponding anionic nitrogen species with a strong base. Thus, deprotonation of pyrazole **3** was carried out using LDA in THF at −78 °C, the resultant mixture being gradually warmed up to −50 °C over 20 min, and then cooled again to −78 °C. Treatment with a slight excess iodomethane at −78 °C followed by a slow warming up to room temperature overnight afforded, after work-up, a crude product whose GC–MS analysis indicated the presence of a single regioisomer. Although the exact regioselectivity was not determined at this point, we tentatively assigned this *N*-methylpyrazole to 1-methyl-5-tributylstannyl-4-trifluoromethylpyrazole **4a** and continued our study (Scheme 2).



Scheme 2. N-Methylation of **3**.

The alkylation reaction of **4a** with benzaldehyde as a model compound was initially examined. It is well-known that transmetallation of tributylstannyl compound with ^{n}BuLi at low temperature gives the corresponding lithiated species in high yield.[8] The transmetallation of **4a** was performed by addition of ^{n}BuLi at −78 °C, and then warming up to −50 °C in THF. The resultant solution containing the corresponding 5-lithiated 4-trifluoromethylpyrazole was then reacted with benzaldehyde at −78 °C. The mixture was gradually warmed up to room temperature while being stirred to yield the desired product in 93% yield (Table 1, entry 1). Under similar conditions, the reaction of **4a** with other aromatic aldehydes bearing either an electron donating substituent or an electron withdrawing one on the ring gave the corresponding adducts in high yields (entries 2–6).

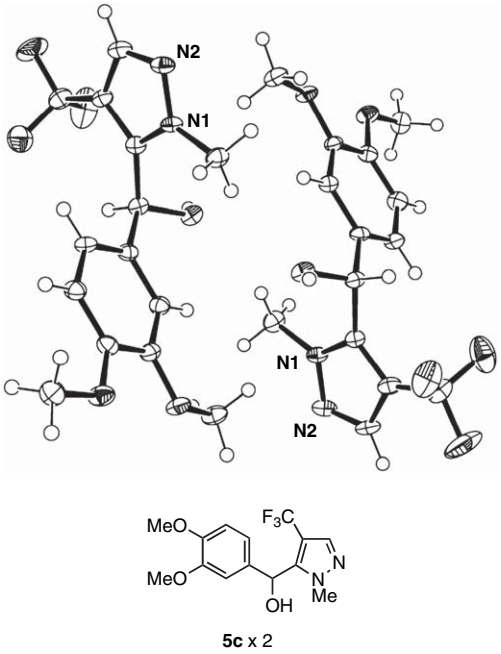


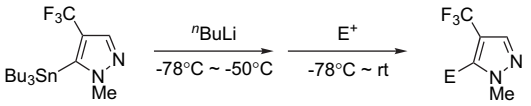
Figure 1. X-ray crystal structure of **5c** with thermal ellipsoids shown at the 50% probability level (hydrogen atoms are omitted for clarity).

To our delight, product **5c** gave single crystals suitable for X-ray crystallographic analysis. A crystal drawing of **5c** (two molecules) is shown in Figure 1. The result of this X-ray analysis revealed unambiguous proof of the regiochemistry of 1-methyl-5-alkylated-4-trifluoromethylpyrazoles and definitely assigned the structure of **4a** as 1-methyl-5-tributylstannyl-4-trifluoromethylpyrazole. For the findings stated above, our procedure turned out to be useful for the complete C-5-lithiation of 1-methylpyrazole.[6c,e,h,k–m,9]

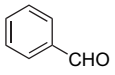
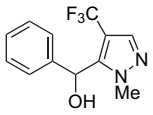
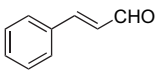
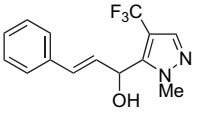
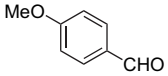
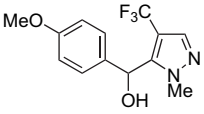
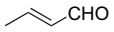
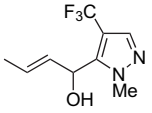
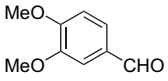
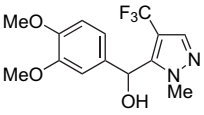
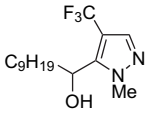
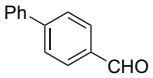
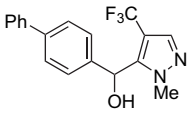
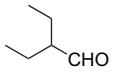
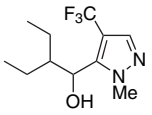
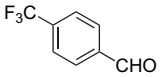
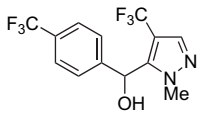
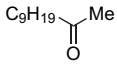
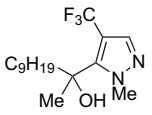
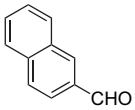
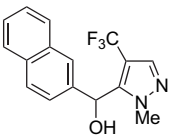
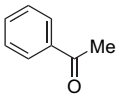
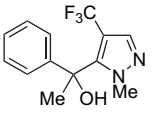
α,β-Unsaturated aldehydes such as cinnamaldehyde and crotonaldehyde also proved to be good electrophiles and delivered the adducts resulting from 1,2-addition fashion. No conjugated addition products were observed (entries 7 and 8). Linear and branched aliphatic aldehydes successfully underwent addition reaction to give the corresponding adducts in high yields (entries 9 and 10). Although 2-undecanone as an aliphatic ketone also functioned satisfactorily (entry 11), acetophenone as an aromatic counterpart led to a lower yield (46%) maybe due to either enolization or steric hindrance (entry 12). The compound **4a** was very capable of nucleophilic addition with a variety of aldehydes and aliphatic ketones.

Application of the above methodology to the preparation of potentially bioactive compounds was next considered. According to Hueso-Rodriguez's report, adduct **5a** was transformed into the corresponding *N*,*N*-dimethylaminoethyl ether derivative to yield the trifluoromethylated analogue of Cizolirtine.[6] Thus, reaction of **5a** with (2-chloroethyl)dimethylamine hydrochloride using 40% aqueous sodium hydroxide solution in the presence of a catalytic amount of tetrabutylammonium bromide in refluxing toluene for 8 h smoothly proceeded to give the desired product **6** in 78% yield (Scheme 3).

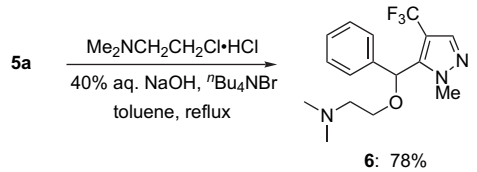
Table 1. Reaction of **4a** with various electrophiles[a]



4a **5**

Entry	E^+	Product yield[b] (%)	Entry	E^+	Product yield[b] (%)
1		 **5a**: 93	7		 **5g**: 96
2		 **5b**: 89	8		 **5h**: 93
3		 **5c**: 94	9	C_9H_{19}–CHO	 **5i**: 79
4		 **5d**: 93	10		 **5j**: 79
5		 **5e**: 85	11		 **5k**: 80
6		 **5f**: 86	12[c]		 **5l**: 46

[a] Aldehyde or ketone, 1.5 equiv.
[b] Isolated yield.
[c] Ketone, 3.0 equiv.

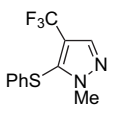


6: 78%

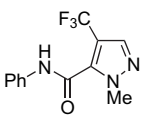
Scheme 3. Synthesis of Cizolirtine analogue **6**.

Further expansion of the synthetic value of the new methodology for the preparation of 1-methyl-5-substituted-4-trifluoromethylpyrazole involved the use of other electrophiles such as *S*-phenyl benzenethiosulfate, phenyl isocyanate, *N*,*N*-dimethylformamide, and methyl cyanoacetate. The former two electrophiles afforded the desired 1-methyl-5-functionalized-4-trifluoromethylpyrazoles in good yields; however, no adducts were obtained from the latter two electrophiles with despite a number of reaction conditions (temperature control, use of additives, and change of solvents; Fig. 2).

In conclusion, we have developed a facile method for the preparation of a variety of 1-methyl-4-trifluoromethyl-5-substituted-pyrazoles. The important key trifluoromethylated building block, 1-methyl-5-tributylstannyl-4-trifluoromethylpyrazole **4a**, was prepared from the *N*-unsubstituted



7: 63%



8: 69%

Figure 2. Structure of pyrazoles **7** and **8**.

precursor, 5-tributylstannyl-4-trifluoromethylpyrazole **3**, through completely regioselective N-methylation using LDA and MeI at low temperature.

3. Experimental

3.1. General

Melting points are uncorrected. Infrared (IR) spectra are reported in inverse centimeter. ^{1}H, ^{19}F, and ^{13}C NMR spectra were measured in $CDCl_3$ solutions. Chemical shifts were given by δ relative to that of an internal Me_4Si (TMS) for ^{1}H NMR and ^{13}C NMR spectra and benzylidyne trifluoride ($CF_3C_6H_5$) for ^{19}F NMR spectra.

3.1.1. Preparation of 5-tributylstannyl-4-trifluoromethylpyrazole (3). A 100 mL two-necked flask equipped with a magnetic stir bar, a stopcock, and a three-way stopcock, were charged with diisopropylamine (1.6 mL, 11.4 mmol) and 10 mL of THF under argon atmosphere. To the stirring mixture was added dropwise ^{n}BuLi (2.67 M in hexane solution, 4.4 mL, 11.8 mmol) via syringe at 0 °C. After the addition was completed, the mixture was cooled to −78 °C, and then 2-bromo-3,3,3-trifluoropropene (0.54 mL, 5.21 mmol) was slowly added to the mixture. After the mixture was gradually warmed to −50 °C, $(Bu_3Sn)_2O$ (2.6 mL, 4.87 mmol) was added dropwise at this temperature. The mixture was gradually warmed to −30 °C while being stirred and an excess of ethereal CH_2N_2 solution was added to the mixture at this temperature. The whole mixture was gradually warmed to 0 °C, and stirring was continued for several hours until the reaction was complete. The reaction was quenched with hexane (ca. 10 mL) and sodium sulfate decahydrate ($Na_2SO_4\cdot 10H_2O$, ca. 10 g). After the mixture was dried over sodium sulfate and filtered through a short sodium sulfate column (ether as an eluent), the solution was concentrated in vacuo. The resulting oily residue was purified by column chromatography (silica gel, hexane/ethyl acetate/triethylamine=200/40/1) to give the desired compound (**3**) as a colorless oil (1.42 g, 64%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.89 (9H, t, J=7.3 Hz), 1.16–1.21 (6H, m), 1.27–1.39 (6H, m), 1.48–1.58 (6H, m), 7.87 (1H, s), 10.22 (1H, br s).[4]

3.1.2. Preparation of *N*-methyl-5-tributylstannyl-4-trifluoromethylpyrazole (4a). To a solution containing 889.5 mg (2.09 mmol) of pyrazole (**3**) in THF (8 mL) was added LDA (0.38 M in THF solution, 7.0 mL, 2.66 mmol) by means of double-ended needle at −78 °C. The reaction mixture was gradually warmed to −50 °C, and then again cooled to −78 °C. At this temperature 0.17 mL (2.72 mmol) of MeI was added to the mixture, and the temperature of the stirring mixture was allowed to warm to room temperature overnight. The reaction was quenched with a saturated aqueous $NaHCO_3$ solution and extracted with hexane/ether=3/1. The combined organic layer was dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate/triethylamine=200/40/1) to give the desired compound (**4a**) as a colorless oil (853.1 mg, 93%, >99/1): IR (neat) 2958, 1529, 1221 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.89 (9H, t, J=7.3 Hz), 1.17–1.22 (6H, m), 1.29–1.36 (6H, m), 1.46–1.57 (6H, m), 3.96 (3H, s), 7.72 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.9, 13.3, 27.0, 28.6, 41.1, 121.4 (q, J=35.5 Hz), 124.0 (q, J=265.9 Hz), 137.2 (q, J=3.1 Hz), 143.1 (d, J=3.1 Hz); ^{19}F NMR ($CDCl_3$, 283 MHz) δ −55.14 (s); GC–MS *m*/*z* 382 (3.4, M$^+$−Bu), 250 (21), 248 (14), 104 (15), 84 (100), 83 (40); Anal. Calcd for $C_{17}H_{31}F_3N_2Sn$: C, 46.50; H, 7.12; N, 6.38. Found: C, 46.53; H, 7.11; N, 6.37.

3.1.3. Preparation of *N*-methyl-5-[(hydroxy)(phenyl)]-methyl-4-trifluoromethylpyrazole (5a). To a solution containing 112.3 mg (0.256 mmol) of pyrazole (**4a**) in THF (1 mL) was added ^{n}BuLi (2.67 M in hexane solution, 0.125 mL, 0.332 mmol) by means of syringe at −78 °C. The reaction mixture was gradually warmed to −50 °C, and then again cooled to −78 °C. At this temperature 40 mL (0.384 mmol) of benzaldehyde was added to the mixture, and the temperature was allowed to warm to room temperature with stirring for 30 min. The reaction was quenched with a saturated aqueous $NaHCO_3$ solution and extracted with ethyl acetate. The combined organic layer was dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate=10/1 then 3/1) to give the desired compound (**5a**) as a white solid (61.2 mg, 93%): mp 112.5–114.2 °C; IR (KBr) 3252, 1574, 759, 708 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.59 (1H, d, J=4.4 Hz), 3.66 (3H, s), 6.31 (1H, d, J=4.4 Hz), 7.28–7.41 (5H, m), 7.66 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 38.3, 65.2 (d, J=1.3 Hz), 111.8 (q, J=37.4 Hz), 122.8 (q, J=266.6 Hz), 125.3, 127.8, 128.5, 136.0 (q, J=3.7 Hz), 138.7, 142.0 (q, J=2.5 Hz); ^{19}F NMR ($CDCl_3$, 283 MHz) δ −55.8 (s); GC–MS *m*/*z* 255 (9.3, M$^+$), 105 (50), 103 (32), 79 (90), 78 (73), 77 (100), 51 (36); Anal. Calcd for $C_{12}H_{11}F_3N_2O$: C, 56.25; H, 4.33; N, 10.93. Found: C, 56.35; H, 4.32; N, 10.90.

3.1.4. *N*-Methyl-5-[(hydroxy)(4′-methoxyphenyl)]-methyl-4-trifluoromethylpyrazole (5b). White solid; yield 89%; mp 109.0–110.9 °C; IR (KBr) 3194, 2957, 1574, 877 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.46 (1H, d, J=3.1 Hz), 3.67 (3H, s), 3.81 (3H, s), 6.26 (1H, d, J=3.1 Hz), 6.90 (2H, d, J=7.9 Hz), 7.22 (2H, d, J=7.9 Hz), 7.65 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 38.5, 55.3, 65.4, 111.8 (q, J=36.8 Hz), 114.0, 122.4 (q, J=266.6 Hz), 130.9, 136.1 (q, J=3.7 Hz), 142.3 (q, J=2.5 Hz), 159.2; ^{19}F NMR ($CDCl_3$, 283 MHz) δ −55.7 (s); GC–MS *m*/*z* 285 (4.2, M$^+$), 109 (100), 108 (64), 77 (11); Anal. Calcd for $C_{13}H_{13}F_3N_2O_2$: C, 54.55; H, 4.58; N, 9.79. Found: C, 54.62; H, 4.58; N, 9.72.

3.1.5. *N*-Methyl-5-[(hydroxy)(3′,4′-dimethoxyphenyl)]-methyl-4-trifluoromethylpyrazole (5c). White solid; yield 94%; mp 133.8–135.9 °C; IR (KBr) 3203, 2938, 1574, 869 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.55 (1H, d, J=4.4 Hz), 3.70 (3H, s), 3.84 (3H, s), 3.88 (3H, s), 6.25 (1H, d, J=4.4 Hz), 6.77 (1H, ddd, J=8.3, 1.8, 0.9 Hz), 6.84 (1H, d, J=8.3 Hz), 6.90 (1H, d, J=1.8 Hz), 7.66 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 38.9, 56.2, 56.3, 65.8, 109.3, 111.4, 112.0 (q, J=37.4 Hz), 118.2, 123.3 (q, J=266.6 Hz), 131.8, 136.5 (q, J=3.7 Hz), 142.6 (q, J=2.5 Hz), 149.0, 149.5; ^{19}F NMR ($CDCl_3$, 283 MHz) δ −55.7 (s); GC–MS *m*/*z* 315 (24, M$^+$), 264 (21), 139

(100), 138 (75), 108 (12), 77 (13); Anal. Calcd for $C_{14}H_{15}F_3N_2O_3$: C, 53.17; H, 4.78; N, 8.86. Found: C, 53.26; H, 4.82; N, 8.77.

3.1.6. *N*-Methyl-5-[(hydroxy)(4′-biphenyl)]methyl-4-trifluoromethylpyrazole (5d). White solid; yield 93%; mp 146.8–149.1 °C; IR (KBr) 3252, 2956, 1574, 1402, 876 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.63 (1H, d, J=3.9 Hz), 3.71 (3H, s), 6.35 (1H, d, J=3.9 Hz), 7.30–7.48 (5H, m), 7.56–7.63 (4H, m), 7.68 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 38.6, 65.4, 111.8 (q, J=37.4 Hz), 123.0 (q, J=266.6 Hz), 126.0, 127.0, 127.4, 127.5, 128.8, 136.2 (q, J=3.4 Hz), 137.7, 140.3, 140.9, 141.9 (q, J=2.5 Hz); ^{19}F NMR ($CDCl_3$, 283 MHz) δ −55.8 (s); GC–MS m/z 331 (17, M^+), 155 (100), 154 (73), 152 (43), 151 (31), 77 (33); Anal. Calcd for $C_{18}H_{15}F_3N_2O_2$: C, 65.06; H, 4.55; N, 8.43. Found: C, 65.08; H, 4.62; N, 8.48.

3.1.7. *N*-Methyl-5-[(4′-trifluoromethylphenyl)(hydroxy)]-methyl-4-trifluoromethylpyrazole (5e). White solid; yield 85%; mp 130.0–132.5 °C; IR (KBr) 3223, 2957, 1576, 866 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.34 (1H, d, J=3.5 Hz), 3.62 (3H, s), 6.33 (1H, d, J=3.5 Hz), 7.46 (2H, d, J=8.1 Hz), 7.64 (2H, d, J=8.1 Hz), 7.64 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 38.5, 64.9, 112.3 (q, J=37.4 Hz), 122.9 (q, J=266.6 Hz), 123.9 (q, J=264.8 Hz), 125.7 (q, J=3.7 Hz), 126.0, 130.2 (q, J=32.4 Hz), 136.3 (q, J=3.1 Hz), 141.2, 142.7; ^{19}F NMR ($CDCl_3$, 283 MHz) δ −55.9 (s), −64.1 (s); GC–MS m/z 323 (1.9, M^+), 234 (52), 159 (66), 158 (81), 145 (27), 130 (20), 127 (100), 103 (66); Anal. Calcd for $C_{13}H_{10}F_6N_2O_2$: C, 48.16; H, 3.11; N, 8.64. Found: C, 48.24; H, 3.07; N, 8.70.

3.1.8. *N*-Methyl-5-[(hydroxy)(2′-naphthyl)]methyl-4-trifluoromethylpyrazole (5f). White solid; yield 86%; mp 169.1–170.0 °C; IR (KBr) 3241, 3064, 2956, 1574, 1403 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.67 (1H, d, J=4.2 Hz), 3.65 (3H, s), 6.47 (1H, d, J=4.2 Hz), 7.32 (1H, dd, J=8.6, 1.5 Hz), 7.48–7.54 (2H, m), 7.70 (1H, s), 7.81–7.87 (4H, m); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.0, 66.1, 112.5 (q, J=37.4 Hz), 123.5 (q, J=266.6 Hz), 123.8, 124.8, 126.9, 127.0, 128.1, 128.6, 129.1, 133.3, 133.5, 136.5, 136.6 (q, J=3.7 Hz), 142.2 (q, J=2.5 Hz); ^{19}F NMR ($CDCl_3$, 283 MHz) δ −55.7 (s); GC–MS m/z 306 (0.01, M^+), 304 (11), 130 (11), 129 (59), 128 (100), 127 (67), 103 (11), 102 (18), 101 (10); Anal. Calcd for $C_{16}H_{13}F_3N_2O_2$: C, 62.74; H, 4.28; N, 9.15. Found: C, 62.79; H, 4.31; N, 9.19.

3.1.9. *N*-Methyl-5-[(cinnamyl)(hydroxyl)]methyl-4-trifluoromethylpyrazole (5g). White solid; yield 96%; mp 95.5–96.8 °C; IR (KBr) 3234, 3030, 2953, 1572 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.33 (1H, d, J=4.0 Hz), 3.99 (3H, s), 6.47 (1H, d, J=4.0 Hz), 6.32 (1H, dd, J=15.9, 5.1 Hz), 6.66 (1H, dd, J=15.9, 1.7 Hz), 7.26–7.40 (5H, m), 7.64 (1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.1, 65.8, 111.2 (q, J=37.4 Hz), 121.6 (q, J=266.6 Hz), 126.9, 127.0, 128.8, 129.2, 132.3, 136.1, 136.7 (q, J=3.7 Hz), 141.6 (q, J=2.5 Hz); ^{19}F NMR ($CDCl_3$, 283 MHz) δ −56.0 (s); GC–MS m/z 281 (0.76, M^+), 105 (100), 104 (85), 103 (66), 91 (69), 77 (38); Anal. Calcd for $C_{14}H_{13}F_3N_2O_2$: C, 59.57; H, 4.64; N, 9.92. Found: C, 59.40; H, 4.61; N, 9.97.

3.1.10. *N*-Methyl-5-[(crotyl)(hydroxy)]methyl-4-trifluoromethylpyrazole (5h). Colorless oil; yield 93%; IR (neat) 3331, 3034, 2957, 1573 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.74 (3H, d, J=4.8 Hz), 2.25 (1H, br s), 3.95 (3H, s), 5.53–5.79 (3H, m), 7.59 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 17.5, 38.4, 65.3, 110.5 (q, J=38.0 Hz), 122.0 (q, J=265.9 Hz), 128.65, 128.73, 136.0 (q, J=3.7 Hz), 142.1 (q, J=3.1 Hz); ^{19}F NMR ($CDCl_3$, 283 MHz) δ −55.9 (s); GC–MS m/z 219 (0.63, M^+), 199 (50), 184 (63), 176 (52), 158 (88), 156 (82), 130 (83), 103 (87), 102 (100); HRMS (EI) m/z calcd for $C_9H_{11}F_3N_2O$ 221.0902, found 221.0883.

3.1.11. *N*-Methyl-5-[(hydroxy)(nonyl)]methyl-4-trifluoromethylpyrazole (5i). Colorless oil; yield 79%; IR (neat) 3228, 2957, 1573 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.87 (3H, t, J=6.2 Hz), 1.26 (12H, br s), 1.74–1.79 (2H, m), 1.89–1.94 (2H, m), 2.09 (1H, d, J=4.1 Hz), 4.02 (3H, s), 5.06 (1H, d, J=4.1 Hz), 7.58 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.0, 22.6, 25.7, 29.1, 29.2, 29.371, 29.429, 31.8, 35.9, 38.6, 65.4, 110.5 (q, J=36.7 Hz), 122.0 (q, J=266.6 Hz), 136.2 (q, J=3.7 Hz), 143.3 (q, J=2.5 Hz); ^{19}F NMR ($CDCl_3$, 283 MHz) δ −55.9 (s); GC–MS m/z 306 (0.17, M^+), 179 (23), 178 (22), 159 (100); HRMS (EI) m/z calcd for $C_{15}H_{25}F_3N_2O$ 307.1997, found 307.2015.

3.1.12. *N*-Methyl-5-[(1′-ethylpropyl)(hydroxy)]methyl-4-trifluoromethylpyrazole (5j). White solid; yield 79%; mp 71.0–72.3 °C; IR (KBr) 3225, 2940, 1573 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.75 (3H, t, J=7.4 Hz), 0.91 (3H, t, J=7.4 Hz), 0.94–1.05 (1H, m), 1.10–1.26 (1H, m), 1.55–1.75 (2H, m), 1.75–1.90 (1H, m), 3.01 (1H, d, J=4.2 Hz), 4.86 (1H, dd, J=9.5, 4.2 Hz), 7.54 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 11.5, 14.5, 21.3, 21.5, 39.3, 45.2, 67.6, 111.0 (q, J=36.7 Hz), 123.0 (q, J=265.9 Hz), 136.9 (q, J=3.7 Hz), 141.6 (q, J=2.5 Hz); ^{19}F NMR ($CDCl_3$, 283 MHz) δ −55.7 (s); GC–MS m/z 249 (1.4, M^+), 180 (25), 179 (50), 178 (26), 159 (86), 158 (100), 55 (20); Anal. Calcd for $C_{11}H_{17}F_3N_2O$: C, 52.79; H, 6.85; N, 11.19. Found: C, 53.02; H, 6.77; N, 11.26.

3.1.13. *N*-Methyl-5-[(hydroxy)(methyl)(nonyl)]methyl-4-trifluoromethylpyrazole (5k). Colorless oil; yield 80%; IR (neat) 3349, 2927, 1548 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 0.87 (3H, t, J=6.3 Hz), 1.15–1.35 (14H, m), 1.68 (3H, s), 1.70–1.98 (2H, m), 2.09 (1H, s), 4.06 (3H, s), 7.61 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.5, 23.0, 24.1, 29.6, 29.84, 29.87, 30.0, 32.2, 32.2, 41.4, 42.5, 73.3, 111.5 (q, J=36.7 Hz), 123.6 (q, J=266.6 Hz), 138.0 (q, J=5.0 Hz), 146.8 (q, J=2.5 Hz); ^{19}F NMR ($CDCl_3$, 283 MHz) δ −53.9 (s); GC–MS m/z 193 (13), 192 (28), 173 (100), 172 (80), 131 (26); Anal. Calcd for $C_{16}H_{27}F_3N_2O$: C, 59.98; H, 8.49; N, 8.74. Found: C, 59.95; H, 8.45; N, 8.64.

3.1.14. *N*-Methyl-5-[(hydroxy)(methyl)(phenyl)]methyl-4-trifluoromethylpyrazole (5l). Colorless oil; yield 46%; IR (neat) 3312, 3063, 3027, 2988, 1552 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.02 (3H, s), 2.67 (1H, s), 3.50 (3H, s), 7.27–7.38 (5H, m), 7.66 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 30.8 (q, J=3.7 Hz), 39.7, 73.1, 111.6 (q, J=37.4 Hz), 122.3 (q, J=224.2 Hz), 124.9, 127.8, 128.7,

137.2 (q, J=5.0 Hz), 144.3, 145.9 (q, J=3.1 Hz); ^{19}F NMR ($CDCl_3$, 283 MHz) δ −53.6 (s); GC–MS m/z 269 (1.7, M^+), 234 (91), 105 (58), 78 (43), 77 (100), 51 (45); HRMS (FAB) m/z calcd for $C_{13}H_{13}F_3N_2O$ 271.1058 (M^++1), found 271.1056.

3.1.15. *N*-Methyl-5-[(*N*′,*N*′-dimethylaminoethoxy)(phenyl)]methyl-4-trifluoromethylpyrazole (6). To a mixture containing 49.8 mg (0.196 mmol) of pyrazole **5a**, 69.9 mg (0.485 mmol) of (2-chloroethyl)dimethylamine hydrochloride, and 8.4 mg (0.026 mmol) of tetrabutylammonium bromide in toluene (1 mL) was added 40% aqueous sodium hydroxide solution (0.12 mL, 1.18 mmol) by means of syringe. After the whole reaction mixture was refluxed for 8 h, the reaction was quenched with water and extracted with hexane/ethyl acetate=3/1 solution. The combined organic layer was dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (methanol/dichloromethane/triethylamine=200/200/1) to give the desired compound (**6**) as colorless oil (49.7 mg, 69%): IR (neat) 2946, 1571, 1397, 1232, 1114, 982, 760, 720 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.27 (6H, s), 2.57 (1H, dt, J=12.8, 5.7 Hz), 2.63 (1H, dt, J=12.8, 5.7 Hz), 3.57 (1H, dt, J=9.9, 5.7 Hz), 3.63 (3H, s), 3.70 (1H, dt, J=9.9, 5.7 Hz), 5.89 (1H, s), 7.27–7.37 (5H, m), 7.68 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 38.5, 45.9 (2C), 58.6, 68.2, 73.1, 113.7 (q, J=37.1 Hz), 123.1 (q, J=264.5 Hz), 125.7 (2C), 127.8, 128.5 (2C), 136.1 (q, J=3.1 Hz), 137.8, 140.1 (q, J=2.5 Hz); ^{19}F NMR ($CDCl_3$, 283 MHz) δ −55.7 (s); GC–MS m/z 238 (0.5), 73 (7), 72 (6), 58 (100), 57 (33); HRMS (FAB) calcd for $C_{16}H_{20}F_3N_3O$: 328.1637 ($M+H^+$), found 328.1634.

3.1.16. *N*-Methyl-5-*S*-phenyl-4-trifluoromethylpyrazole (7). Colorless oil; yield 63%; IR (neat) 3067, 2953, 1583, 1228, 1121 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.84 (3H, s), 7.08–7.31 (5H, m), 7.80 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 37.7, 118.6 (q, J=36.7 Hz), 124.3 (q, J=266.6 Hz), 127.6, 128.5, 129.9, 131.3 (q, J=3.1 Hz), 133.7, 137.6 (q, J=3.1 Hz); ^{19}F NMR ($CDCl_3$, 283 MHz) δ −58.3 (s); GC–MS m/z 258 (8.7, M^+), 257 (100), 91 (25), 77 (63), 69 (24), 51 (41); Anal. Calcd for $C_{11}H_9F_3N_2S$: C, 51.16; H, 3.51; N, 10.85. Found: C, 51.30; H, 3.58; N, 10.78.

3.1.17. *N*-Methyl-5-(*N*-phenylcarbamoyl)-4-trifluoromethylpyrazole (8). White solid; yield 79%; mp 92.8–94.0 °C; IR (KBr) 3256, 3142, 2951, 1652, 1558, 795 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.13 (3H, s), 7.23 (1H, t, J=7.5 Hz), 7.39 (2H, t, J=7.5 Hz), 7.57 (2H, d, J=7.5 Hz), 7.72 (1H, s), 7.87 (1H, br s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.5, 111.7 (q, J=37.4 Hz), 120.4, 122.4 (q, J=267.2 Hz), 125.7, 129.3, 135.0 (q, J=2.5 Hz), 136.4 (q, J=3.7 Hz), 136.5, 156.2; ^{19}F NMR ($CDCl_3$, 283 MHz) δ −55.8 (s); GC–MS m/z 268 (13, M^+), 177 (23), 176 (100), 102 (26), 77 (6), 64 (13); Anal. Calcd for $C_{12}H_{10}F_3N_3O$: C, 53.54; H, 3.74; N, 15.61. Found: C, 53.83; H, 3.80; N, 15.57.

3.1.18. Crystal data for 5c. $C_{14}H_{15}F_3N_2O_3$: M_r=316.28, T=93(2) K, orthorhombic, space group $Pbn21$, a=11.062(7) Å, b=11.683(17) Å, c=22.423(7) Å, V=2898(5) Å^3, Z=8, D_x=1.450 Mg m^{-3}, m=0.127 mm^{-1}, l=0.71073 Å, q_{max}=27.48°, 25,011 measured reflection, 3400 independent reflections, 405 refined parameters, GOF=1.069, $R[F^2>2s(F^2)]$=0.0419, $wR(F^2)$=0.1103. The intensity data were collected on a Rigaku RAXIS-RAPID diffractometer. The structure was solved by direct methods (SIR2002[10]) and the non-hydrogen atoms were refined anisotropically by full-matrix least-squares procedures on F^2 for all reductions (SHELXL-97[11]). All hydrogen atoms were positioned geometrically and refined as riding. CCDC-297123 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by e-mailing to data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

Acknowledgements

We greatly thank F-Tech Co. Ltd for a gift of 2-bromo-3,3,3-trifluoropropene.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.04.043.

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Tetrahedron 62 (2006) 6339–6342
Tetrahedron

Asymmetric synthesis of homo-apioneplanocin A from D-ribose

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Received 4 March 2006; revised 12 April 2006; accepted 12 April 2006
Available online 8 May 2006

Abstract—Homo-apioneplanocin A was efficiently synthesized via stereoselective hydroxymethylation, regio- and chemoselective hydroboration, and chemoselective oxidation as key steps from D-ribose.

1. Introduction

(−)-Neplanocin A (NPA), originally isolated from the culture filtrate of the soil fungus *Ampullariella regularis*,[1] is a carbocyclic nucleoside in which a methylene group replaces the oxygen atom in the furanose ring and one of the most potent *S*-adenosylhomocysteine (AdoHcy) hydrolase inhibitors.[2] NPA has been reported to possess a significant antiviral effect[3] correlating with the inhibition of AdoHcy hydrolase, but it was too cytotoxic to be a clinically useful antiviral agent.[2b,4]

Moreover, it is known to undergo deamination by adenosine deaminase to the biologically inactive compound, the inosine congener.[5]

Apionucleoside has a unique sugar moiety, in that the 4′-hydroxymethyl group of the sugar is shifted to the C3′-position.[6–9] Apio-ddA has been reported to show potent anti-AIDS activity comparable to that of the parent nucleoside, 2′,3′-dideoxyadenosine (ddA) and better stability of the glycosidic bond under acidic conditions than ddA.[8b] In order to search for potent inhibitors of AdoHcy hydrolase, we have recently synthesized the apioneplanocin A (apio-NPA),[10] which combines the structural characteristics of NPA and apionucleoside, but this compound showed neither a significant antiviral activity nor inhibitory activity against AdoHcy hydrolase.

However, since another NPA-modified compound, 6′-homoneplanocin A[11] was reported to have a significant inhibitory activity against AdoHcy hydrolase, we designed the homo-apioneplanocin A (HANPA, **1**), which combined apioneplanocin A and 6′-homoneplanocin A as a potential inhibitor of AdoHcy hydrolase (Fig. 1). Herein, we wish to describe the asymmetric synthesis of HANPA (**1**) from D-ribose via stereoselective hydroxymethylation, regio- and chemoselective hydroboration, and chemoselective oxidation as key steps.

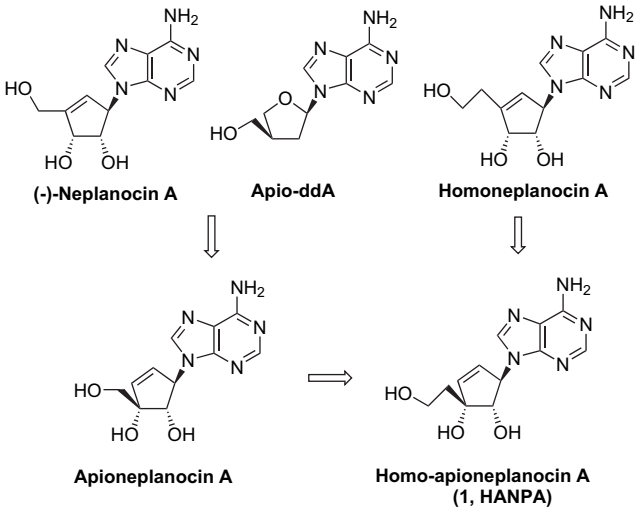

Figure 1. The rational for the design of the desired nucleoside, HANPA (**1**).

2. Results and discussion

The synthetic strategy of the target compound **1** is outlined in Scheme 1. It was envisioned that dienyl diols **4**[10] could

Keywords: Homo-apioneplanocin A; Stereoselective hydroxymethylation; Chemoselective hydroboration; Chemoselective oxidation.
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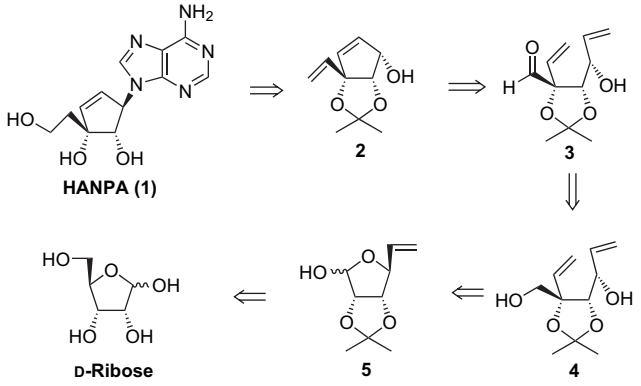
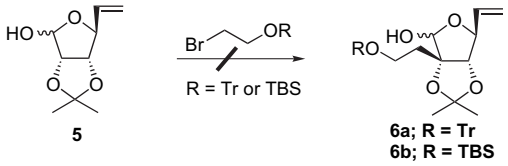
doi:10.1016/j.tet.2006.04.042

be an appropriate intermediate, efficiently synthesized by our laboratory, for the final compound.



Scheme 1. Retrosynthetic analysis of the desired nucleoside, HANPA (**1**).

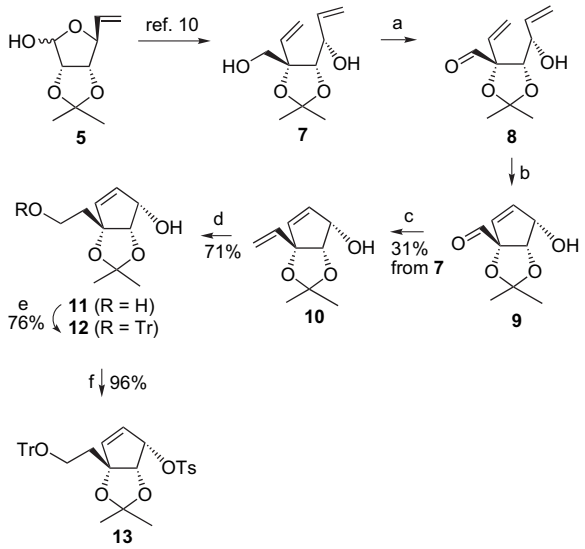
Our initial approach was to introduce the hydroxyethyl group at C3-position of **5** directly in the stereoselective manner, as depicted in Scheme 2. Treatment of **5** with 2-trityloxyethyl bromide and 2-*tert*-butyldimethylsilyloxyethyl bromide in the presence of various bases such as LDA, LiHMDS, and K_2CO_3 at various temperatures such as −78 and 80 °C in THF failed to produce the desired product **6** or afforded **6** in less than 5% yield.



Scheme 2. Attempted methods for the direct introduction of hydroxyethyl functional group.

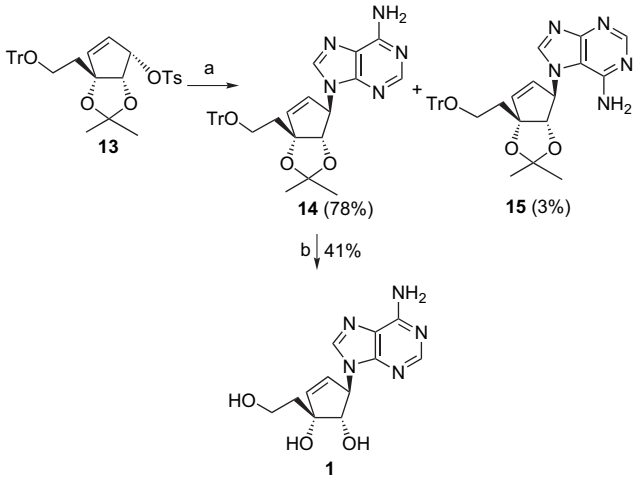
Thus, another route including stereoselective hydroxymethylation, regioselective oxidation, Wittig reaction, and chemoselective hydroboration was employed to introduce hydroxyethyl group stereoselectively at C3-position (Scheme 3). Lactol **5** was converted to diol **7** as a single diastereoisomer according to our previously reported procedure.[10]

Chemoselective oxidation of diol **7** to aldehyde **8** was achieved by treating with TEMPO, TBACl, and NCS under pH 8.6.[12] Ring-closing metathesis (RCM)[13] of **8** with second generation Grubbs catalyst afforded cyclopentenal **9**. However, RCM reaction of **7** gave the cyclopentene diol in good yield, but chemoselective oxidation of the diol failed to give the desired product **9**. Wittig reaction of **9** under the standard conditions gave vinylcyclopentenol **10**. Various hydroboration reagents were attempted to accomplish regio- and chemoselective hydroboration. Hydroboration of **10** using catecholborane and Wilkinson catalyst $Rh(PPh_3)_3Cl$ (rhodium-catalyzed olefin hydroboration) in THF[14] or 9-BBN in THF did not give the desired alcohol **11**, while treatment with Sia_2BH gave the desired product **11** in good yield (71%) after oxidation with sodium perborate. Selective protection of the primary hydroxyl group of **11** with a bulky trityl group followed by tosylation of the resulting **12** gave the glycosyl donor **13**.



Scheme 3. Reagents and conditions: (a) TEMPO, TBACl, NCS, aqueous $NaHCO_3$ and K_2CO_3 (pH=8.6), CH_2Cl_2, rt, 3 h; (b) second generation Grubbs catalyst, CH_2Cl_2, rt, 5 h; (c) $MePh_3PBr$, KO*t*-Bu, THF, rt, 12 h; (d) (i) Sia_2BH, THF, from 0 °C to rt, 16 h; (ii) $NaBO_3 \cdot H_2O$, rt, 12 h; (e) TrCl, pyridine, DMAP, rt, 24 h; (f) TsCl, DMAP, CH_2Cl_2, rt, 24 h.

Condensation of **13** with adenine in the presence of K_2CO_3 and 18-crown-6 in DMF afforded the N-9 isomer **14** as a major product (78%) along with its N-7 isomer **15** (3%) after the separation by silica gel column chromatography (Scheme 4). The regio-isomers **14** and **15** were easily assigned based on the comparison with UV literature data.[15] Removal of the protecting groups of **14** under acidic conditions produced the desired product **1**.



Scheme 4. Reagents and conditions: (a) adenine, K_2CO_3, 18-crown-6, DMF, 80 °C, 12 h; (b) 1% HCl, MeOH, rt, 12 h.

Inhibitory activity of AdoHcy hydrolase by compound **1** was measured using pure recombinant enzyme obtained from human placenta. Compound **1** did not exhibit significant inhibitory activity against AdoHcy hydrolase, although we expected the hydroxyethyl side chain of **1** to induce the favorable binding to AdoHcy hydrolase. This lack of enzyme inhibitory activity might be attributed to the presence of the tertiary hydroxyl group at the C3-position, which should be oxidized by cofactor-bound NAD^+.

3. Conclusions

Asymmetric synthesis of homo-apioneplanocin A (**1**, HANPA) was efficiently achieved using stereoselective hydroxylation, chemoselective oxidation, and regio- and chemoselective hydroboration as key steps from D-ribose. To our best knowledge, homo-apioneplanocin A is the first example of carbocyclic nucleosides with unnatural homo-apio carbasugar.

4. Experimental

4.1. General

Melting points are uncorrected. NMR data were recorded on a 300, 400, and 500 MHz NMR spectrometer using tetramethylsilane (TMS) as an internal standard, and the chemical shifts are reported in parts per million (δ). Coupling constants are reported in Hertz. The abbreviations used are as follows: s (singlet), d (doublet), m (multiplet), dd (doublet of doublet), and br s (broad singlet). All the reactions described below were performed under argon or nitrogen atmosphere and monitored by thin layer chromatography (TLC, Silica gel 60 F_{254}). All anhydrous solvents were distilled over CaH_2 or Na/benzophenone prior to use.

4.1.1. (−)-(1*S*,2*S*,3*R*)-2,3-*O*-Isopropylidene-3-vinyl-4-cyclopentenol (10). A solution of diol **7** (3.09 g, 14.47 mmol), TEMPO (226 mg, 1.45 mmol), and tetrabutylammonium chloride (403 mg, 1.45 mmol) in methylene chloride (100 mL) and an aqueous solution (100 mL) of 0.5 M $NaHCO_3$ and 0.05 M K_2CO_3 were vigorously stirred at room temperature. After adding *N*-chlorosuccinimide (2.125 g, 15.9 mmol) to the mixture, the mixture was stirred at room temperature for 3 h. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried ($MgSO_4$), and evaporated. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate=3:1) to give **8** as a colorless oil, which was immediately used for the next step. To a stirred solution of **8** in CH_2Cl_2 (25 mL) was added second generation Grubbs catalyst (7 mg, 0.07 mmol) at room temperature and the reaction mixture was stirred at room temperature for 5 h and evaporated to give a brown residue. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate=1:3) to give cyclopentenal **9** as a colorless oil, which was immediately used for the next step. To a stirred suspension of CH_3PPh_3Br (6.058 g, 16.62 mmol) in THF (80 mL) was added potassium *tert*-butoxide (16.6 mL, 16.60 mmol, 1 M solution in THF) at 0 °C and the mixture was stirred at room temperature for 1 h . To this stirred solution was added a solution of cyclopentenal **9** in THF (30 mL) through a cannula at 0 °C, and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was partitioned between water and ethyl acetate and the organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate=3:1) to give diene **10** (826 mg, 31% over three steps) as a colorless oil: $[\alpha]_D^{25}$ −32.9 (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.94 (dd, 1H, *J*=10.6, 17.3 Hz), 5.8 (td, 1H, *J*=0.8, 5.7 Hz), 5.75 (ddd, 1H, *J*=0.6, 1.6, 5.7 Hz), 5.24 (dd, 1H, *J*=1.1, 17.3 Hz), 5.15 (dd, 1H, *J*=1.1, 10.6 Hz), 4.61 (tdd, 1H, *J*=1.6, 5.1, 10.6 Hz), 4.43 (d, 1H, *J*=5.1 Hz), 2.64 (d, 1H, *J*=10.6 Hz), 1.42 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 137.9, 135.6, 134.0, 115.6, 112.6, 94.0, 82.6, 74.6, 27.8, 27.5; LRMS (ESI) *m/z* 205 $[M+Na]^+$; Anal. Calcd for $C_{10}H_{14}O_3$: C, 65.91; H, 7.74. Found: C, 66.04; H, 7.60.

4.1.2. (+)-(1*S*,2*S*,3*R*)-2,3-*O*-Isopropylidene-3-(2-hydroxy)-ethyl-4-cyclopentenol (11). 2-Methyl-2-butene (9.2 mL, 18.4 mmol, 2 M solution in THF) was added to borane–dimethyl sulfide (4.6 mL, 9.2 mmol, 2 M solution in THF) and the reaction mixture was stirred at 0 °C for 2.5 h. The resulting solution was added dropwise through a cannula to a stirred solution of **10** (712 mg, 3.91 mmol) in THF (5 mL) at 0 °C and the mixture was stirred at room temperature for 16 h. $NaBO_3 \cdot H_2O$ (1.512 g, 15.15 mmol) and H_2O (8 mL) were added carefully, and the mixture was stirred at room temperature for 12 h. The mixture was partitioned between water and CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate=1:3) to give **11** (559 mg, 71%) as a white solid; mp 81.9 °C; $[\alpha]_D^{25}$ +20.1 (*c* 1.52, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.84 (d, 1H, *J*=5.9 Hz), 5.81 (d, 1H, *J*=5.9 Hz), 4.62 (s, 1H), 4.49 (d, 1H, *J*=4.9 Hz), 3.79 (m, 1H), 3.71 (m, 1H), 2.87 (s, 1H), 2.74 (s, 1H), 1.97 (m, 2H), 1.44 (s, 3H), 1.41 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 135.0, 134.7, 111.9, 93.8, 82.1, 74.3, 59.2, 38.2, 27.9, 27.9; IR (KBr): 3395, 2922, 1646, 1372, 1105, 893, 615 cm^{-1}; LRMS (FAB) *m/z* 201 $[M+H]^+$; HRMS calcd for $C_{10}H_{17}O_4$ $[M+H]^+$: 201.1129, found: 201.1126; Anal. Calcd for $C_{10}H_{16}O_4$: C, 59.98; H, 8.05. Found: C, 59.61; H, 8.19.

4.1.3. (+)-(1*S*,2*S*,3*R*)-2,3-*O*-Isopropylidene-3-(2-triphenylmethyloxy)ethyl-4-cyclopentenol (12). A solution of cyclopentenol **11** (482 mg, 2.4 mmol), trityl chloride (1.338 g, 4.8 mmol), and 4-(dimethylamino)pyridine (61 mg, 0.5 mmol) in pyridine (15 mL) was stirred at room temperature for 24 h. The reaction mixture was partitioned between water and ethyl acetate and the organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate=5:1) to give **12** (807 mg, 76%) as a white solid; mp 135.8 °C; $[\alpha]_D^{25}$ +17.1 (*c* 2.57, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.30 (m, 15H), 5.64 (d, 1H, *J*=5.8 Hz), 5.58 (d, 1H, *J*=5.8 Hz), 4.55 (d, 1H, *J*=5.0 Hz), 4.47 (tdd, 1H, *J*=1.3, 5.0, 10.4 Hz), 3.23 (td, 1H, *J*=5.8, 11.4 Hz), 2.97 (m, 1H), 2.62 (d, 1H, *J*=10.4 Hz), 2.12 (ddd, 1H, *J*=6.0, 7.9. 14.1 Hz), 1.97 (td, 1H, *J*=5.6, 14.2 Hz), 1.33 (s, 3H), 1.31 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 144.0, 135.1, 135.1, 128.5, 127.8, 127.0, 111.4, 96.1, 93.3, 87.2, 82.1, 74.5, 60.0, 36.6, 28.0, 28.0; IR (KBr) 3543, 2986, 2931, 1489, 1448, 1376, 1221, 1066 cm^{-1}; LRMS (ESI) *m/z* 465 $[M+Na]^+$; Anal. Calcd for $C_{29}H_{30}O_4$: C, 78.71; H, 6.83. Found: C, 78.45; H, 6.81.

4.1.4. (−)-(1*S*,2*S*,3*R*)-2,3-*O*-Isopropylidene-3-(2-triphenylmethyloxy)ethyl-4-cyclopentenyl *p*-toluenesulfonate (13). To a stirred solution of **12** (473 mg, 1.07 mmol) and 4-(dimethylamino)pyridine (395 mg, 3.23 mmol) in CH_2Cl_2 (15 mL) was added TsCl (416 mg, 2.18 mmol). After being stirred at room temperature for 24 h, the reaction mixture was partitioned between water and CH_2Cl_2. The

organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate=3:1) to give tosylate **13** (614 mg, 96%) as a white solid; mp 133.4 °C; $[\alpha]_D^{25}$ −0.6 (*c* 1.18, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.41 (m, 19H), 5.67 (dd, 1H, *J*=1.6, 5.8 Hz), 5.42 (dd, 1H, *J*=0.9, 5.8 Hz), 5.16 (td, 1H, *J*=1.6, 5.4 Hz), 4.71 (d, 1H, *J*=4.9 Hz), 3.22 (td, 1H, *J*=4.9, 9.9 Hz), 2.80 (dt, 1H, *J*=3.9, 9.5 Hz), 2.48 (s, 3H), 2.15 (qd, 1H, *J*=4.6, 14.4 Hz), 1.89 (td, 1H, *J*=4.1, 14.4 Hz), 1.28 (s, 3H), 1.27 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 144.3, 143.7, 138.4, 129.7, 129.5, 128.4, 128.0, 127.9, 127.0, 111.8, 93.1, 87.3, 81.5, 81.5, 59.7, 36.1, 28.0, 27.6, 21.6; IR (KBr): 2927, 1597, 1449, 1368, 1179, 1073, 997 cm^{-1}; LRMS (ESI) *m/z* 619 $[M+Na]^+$.

4.1.5. (−)-(3*R*,4*S*,5*S*)-3,4-*O*-Isopropylidene-3-(2-triphenylmethyloxy)ethyl-5-(adenin-9-yl)-cyclopentene (14) and its N-7 isomer (15). A stirred suspension of adenine (36 mg, 0.26 mmol), 18-crown-6 (69 mg, 0.26 mmol), and K_2CO_3 (54 mg, 0.39 mmol) in DMF (2 mL) was heated at 80 °C for 30 min. To this clear solution was added a solution of tosylate **13** (77 mg, 0.13 mmol) in DMF (3 mL) at 80 °C and the reaction mixture was heated at 80 °C for 12 h. The reaction mixture was cooled to room temperature and partitioned between water and CH_2Cl_2. The organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (methylene chloride/methanol=30:1) to give **14** (57 mg, 78%) as a white solid and **15** (2 mg, 3%).

Compound **14**: mp 77.1 °C; $[\alpha]_D^{25}$ −52.9 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} 260 nm; ^1H NMR ($CDCl_3$) δ 8.44 (s, 1H), 7.62 (s, 1H), 7.24 (m, 15H), 6.29 (dd, 1H, *J*=1.2, 5.7 Hz), 5.94 (dd, 1H, *J*=2.0, 5.7 Hz), 5.64 (br s, 2H), 5.57 (s, 1H), 4.49 (s, 1H), 3.29 (t, 2H, *J*=6.4 Hz), 2.05 (t, 2H, *J*=6.5 Hz), 1.39 (s, 3H, CH_3), 1.22 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 155.3, 153.4, 150.0, 143.9, 143.2, 138.1, 128.5, 127.7, 127.0, 126.7, 112.2, 93.8, 87.5, 87.2, 65.5, 59.7, 37.9, 28.0, 27.8; IR (KBr) 2922, 1728, 1642, 1462, 1285, 1072, 615 cm^{-1}; LRMS (ESI) *m/z* 560 $[M+H]^+$; Anal. Calcd for $C_{34}H_{33}N_5O_3$: C, 72.97; H, 5.94; N, 12.51. Found: C, 72.74; H, 5.87; N, 12.81.

Compound **15**: UV ($CHCl_3$) λ_{max} 279 nm; ^1H NMR ($CDCl_3$) δ 8.17 (s, 1H), 7.89 (s, 1H), 7.22 (m, 15H), 6.41 (d, 1H, *J*=5.0 Hz), 5.93 (m, 2H), 4.56 (s, 1H), 3.29 (t, 2H, *J*=6.2 Hz), 1.99 (t, 2H, *J*=6.0 Hz), 1.25 (s, 6H).

4.1.6. (−)-(1*R*,2*S*,3*S*)-1-(2-Hydroxy)ethyl-3-(adenin-9-yl)-4-cyclopentene-1,2-diol (1). A solution of **14** (59 mg, 0.11 mmol) in a mixture of MeOH (15 mL) and acetyl chloride (0.2 mL) was stirred at room temperature for 12 h. The reaction mixture was neutralized with Et_3N and evaporated. The resulting residue was purified by silica gel column chromatography (methylene chloride/methanol=5:1) to give **1** (12 mg, 41%) as a white solid; mp 198.5 °C; $[\alpha]_D^{25}$ −25.8 (*c* 0.1, MeOH); UV ($CHCl_3$) λ_{max} 260 nm; ^1H NMR (MeOH-d_4) δ 8.17 (s, 1H), 8.14 (s, 1H), 6.22 (dd, 1H, *J*=2.4, 6.3 Hz), 6.06 (dd, 1H, *J*=1.7, 6.3 Hz), 5.50 (td, 1H, *J*=2.0, 6.4 Hz), 4.28 (d, 1H, *J*=6.5 Hz), 3.83 (t, 2H, *J*=6.6 Hz), 1.97 (m, 2H); ^{13}C NMR (MeOH-d_4) δ 41.8, 59.3, 66.9, 80.9, 82.5, 120.8, 132.4, 139.8, 141.6, 150.9, 153.8, 157.5; LRMS (EI) *m/z* 277 $[M]^+$; HRMS calcd for $C_{12}H_{12}N_5O_3$: 277.1175, found: 277.1178.

Acknowledgements

This work was supported by a grant from Korea Research Foundation (KRF-2005-005-J01502).

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Tetrahedron 62 (2006) 6343–6348
Tetrahedron

Synthesis of pyrrolo[3,2,1-*hi*]indazoles from indole-7-ketoximes

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Received 6 February 2006; revised 26 March 2006; accepted 11 April 2006
Available online 19 May 2006

Abstract—Treatment of the 2,4-dinitrophenyl ethers of some indole-7-ketoximes with base results in a cyclisation reaction to yield pyrrolo[3,2,1-*hi*]indazoles.

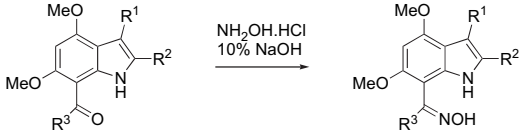
1. Introduction

We have previously reported that indole-7-carbaldehydes can be converted into the corresponding indole-7-carbonitriles by treatment of carbaldoxime 2,4-dinitrophenyl ether intermediates with base.[1] The related ketoximes can be derived from 7-acyl-, 7-aroyl- and 7-glyoxyloyl-indoles, and we wished to investigate the behaviour of their 2,4-dinitrophenyl ethers under similar conditions. One possible outcome could in principle be a Beckmann rearrangement leading to a 7-amidoindole, an extremely useful addition to the range of indole functionality. Two other possibilities might be cyclisation processes either directly onto an intermediate electron-deficient nitrogen atom arising from the loss of a 2,4-dinitrophenolate anion or radical, or alternatively onto an electron-deficient carbon atom generated by a Beckmann rearrangement process. In any cyclisation process, the most likely event would be bond formation with the indole nitrogen atom, leading to formation of either a pyrazole or an imidazole ring, respectively, in the above two scenarios.

2. Results and discussion

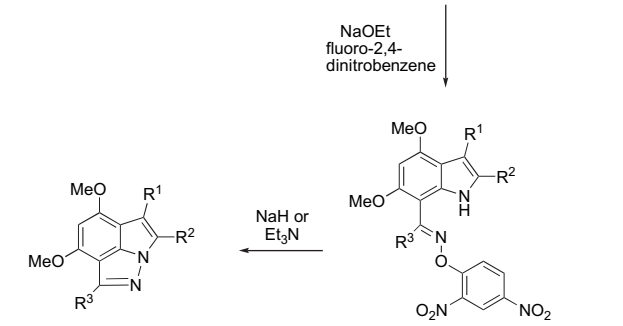
2.1. Formation of indole-7-ketoximes and ketoxime ethers

The various 7-acyl-, 7-aroyl- and 7-glyoxyloyl-indoles **1** and **3–6** have already been reported.[2,3] The 7-acetylindole **2** is detailed here for the first time, although closely related to several known compounds.[4,5] Treatment of these compounds with hydroxylamine hydrochloride and sodium hydroxide generally gave high yields (78–99%) of the indole-7-ketoximes **7–12**, except for the glyoxylic amide **6**, which gave only a 51% yield of the oxime **12** (Scheme 1). The reactions were considerably slower than those for the related aldehydes,[1] requiring approximately two days instead of 2 h for completion. In each case only one isomer was observed, and the structures were confirmed by their spectroscopic data as the respective *anti*-isomers. In particular the imine infrared stretching frequencies[5] were consistent with that



	R^1	R^2	R^3
1	4-BrC_6H_4	Me	Me
2	4-ClC_6H_4	H	Me
3	Ph	Me	4-ClC_6H_4
4	4-BrC_6H_4	Me	CF_3
5	4-BrC_6H_4	Me	CO_2Et
6	4-BrC_6H_4	Me	$CONMe_2$

	R^1	R^2	R^3	Yield %
7	4-BrC_6H_4	Me	Me	81
8	4-ClC_6H_4	H	Me	80
9	Ph	Me	4-ClC_6H_4	78
10	4-BrC_6H_4	Me	CF_3	85
11	4-BrC_6H_4	Me	CO_2Et	99
12	4-BrC_6H_4	Me	$CONMe_2$	51



	R^1	R^2	R^3	Yield %
19	4-BrC_6H_4	Me	Me	63
20	4-ClC_6H_4	H	Me	69
21	Ph	Me	4-ClC_6H_4	10
22	4-BrC_6H_4	Me	CF_3	29
23	4-BrC_6H_4	Me	CO_2Et	21
24	4-BrC_6H_4	Me	$CONMe_2$	30

	R^1	R^2	R^3	Yield %
13	4-BrC_6H_4	Me	Me	49
14	4-ClC_6H_4	H	Me	64
15	Ph	Me	4-ClC_6H_4	78
16	4-BrC_6H_4	Me	CF_3	100
17	4-BrC_6H_4	Me	CO_2Et	100
18	4-BrC_6H_4	Me	$CONMe_2$	100

Scheme 1.

Keywords: Indoles; Oxime ethers; Pyrazoles; Pyrroles; Pyrrolo-indazoles.
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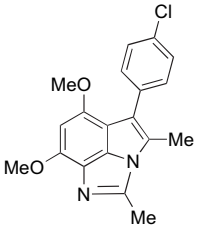
doi:10.1016/j.tet.2006.04.041

assignment, which was further supported by ^{1}H NMR spectroscopic data showing hydrogen bonding of the indole NH to the oxime N atom. For example, in oxime **7**, the two resonances at 10.49 and 10.89 ppm were unequivocally assigned to the indole NH and oxime OH protons, respectively, on the basis of NOE experiments. Irradiation of the C2-methyl protons affected the indole NH and C3-aryl proton resonances; irradiation of the ketoxime methyl protons affected the methoxy, H5, NH and OH resonances; irradiation of the 6-methoxy protons affected the methyl ketoxime and H5 resonances.

Treatment of the indole-7-ketoximes **7–12** with sodium in absolute ethanol, followed by addition of fluoro-2,4-dinitrobenzene at room temperature, yielded the oxime ethers **13–18** in 49–100% yield (Scheme 1). Except for the amido-substituted compound **18**, all of these oxime ethers showed only one isomer with an *anti*-configuration, based on infrared[6] and ^{1}H NMR spectroscopic data. This assignment is also logical for steric reasons as the bulky ether group would probably tend to be away from the NH of the indole ring.



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2.2. Formation of pyrrolo-indazoles

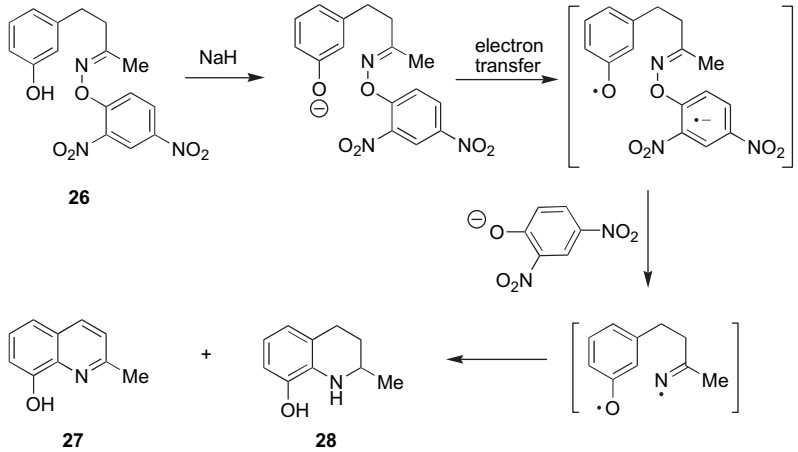
Treatment of the indole ketoxime ether **13** with triethylamine in tetrahydrofuran under reflux for 6 h gave a white solid as the major product after chromatography. The mass spectrum of the product showed a molecular ion at m/e 384 (^{79}Br, 23%), while its infrared spectrum showed that there was no NH stretching frequency present. The ^{1}H NMR spectrum displayed two singlet resonances at 2.67 and 2.75 ppm for protons of the two methyl groups and a singlet at 6.29 ppm corresponding to H5 of the starting indole. It was clear that the elimination of the dinitrophenoxy group had occurred and some kind of cyclisation had taken place onto the indole N atom. Two options were considered. Direct cyclisation would result in bond formation between the oxime and indole nitrogen atoms to give the pyrrolo-indazole **19**. On the other hand, if a Beckmann rearrangement preceded cyclisation, the pyrrolo-benzimidazole **25** could be formed.

The pyrrolo-indazole structure **19** was confirmed by NMR spectroscopy. Initial analysis with NOESY ^{1}H NMR spectroscopy failed to give any useful information, and HMBC ^{1}H–^{13}C NMR spectra could only differentiate between the resonances of the two methyl groups. However, analysis with ^{1}H–^{15}N correlations (HSQC-HMBC NMR spectra) gave clear evidence that the methyl group of the ketoxime ether has a correlation with its nitrogen atom at 216 ppm, while the C2-methyl group protons correlate with the nitrogen atom of the indole. These data are consistent with the structure **19**, but not with structure **25**, in which the ketoxime ether methyl group would be expected to correlate with each of the nitrogen atoms. The yield of compound **19** was 63%.

The other oxime ethers **14–18** were also treated with base and converted into the pyrrolo-indazoles **20–24** in somewhat variable yields ranging from 10–69%. It was observed that different bases could be utilised for the cyclisation reaction. If an electron-donating R^3 substituent was present on the oxime ether, cyclisation occurred readily with the use of a weak base such as triethylamine. If an electron-withdrawing group was present, cyclisation required a stronger base such as sodium hydride. It was found that the indole oxime ether **15** could only be converted into its geometrical *syn*-isomer when heated under reflux with triethylamine for two days, while treatment with sodium hydride gave a low yield of the pyrrolo-indazole **21** together with the *syn*-isomer.

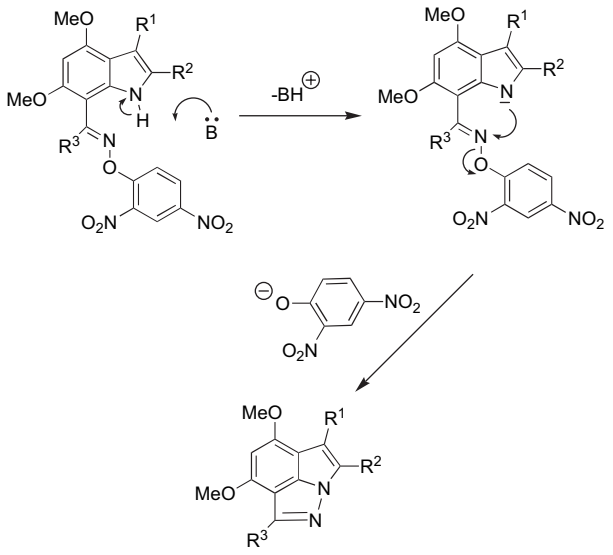
The pyrrolo-indazoles **19–24** all exhibited a bright yellow fluorescence under long-wavelength ultraviolet light.

Narasaka and co-workers have investigated various cyclisation reactions of oxime ethers.[7,8] The cyclisation of either the *syn*- or *anti*-isomer of the 2-(3-hydroxyphenyl)ethyl ketone *O*-2,4-dinitrophenyloxime **26** on treatment with sodium hydride gave a mixture of the quinoline **27** and the tetrahydroquinoline **28**, proceeded via a radical mechanism involving an alkylideneaminyl radical (Scheme 2). While



Scheme 2.

such a radical mechanism is possible for the conversion of indole oxime ethers **13**–**18** into the pyrrolo-indazoles **19**–**24**, the presence of the *anti*-configuration supports a displacement mechanism, with attack of the indole anion on the nitrogen atom of the oxime ether with simultaneous release of the dinitrophenoxide anion (Scheme 3).



Scheme 3.

3. Conclusions

The treatment of 2,4-dinitrophenyl ethers to a range of indole-7-ketoximes with base provides an effective method for the synthesis of the novel pyrrolo[3,2,1-*hi*]indazoles.

4. Experimental

4.1. General

Melting points were measured using a Mel-Temp melting point apparatus, and are uncorrected. Microanalyses were performed on a Carlo Erba Elemental Analyser EA 1108 at the Campbell Microanalytical Laboratory, University of Otago, New Zealand. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker DPX300 spectrometer. Mass spectra were recorded on either a Bruker FT-ICR MS (EI) or a Micromass ZQ2000 (ESI) at UNSW, or a Shimadzu LCMS QP 8000 (ESI) at the University of Otago, New Zealand. Infrared spectra were recorded with a Thermo Nicolet 370 FTIR Spectrometer using KBr discs. Ultraviolet–visible spectra were recorded using a Varian Cary 100 Scan Spectrometer. Column chromatography was carried out using Merck 230–400 mesh ASTM silica gel, whilst preparative thin layer chromatography was performed using Merck silica gel 7730 60GF$_{254}$.

4.1.1. 1-[3-(4-Chlorophenyl)-4,6-dimethoxyindol-7-yl]-ethanone (2). To a solution of 3-(4-chlorophenyl)-4,6-dimethoxyindole[9] (0.50 g, 1.74 mmol) at 0 °C in *N*,*N*-dimethylacetamide (1 mL) was slowly added phosphoryl chloride (1.1 mL, 11.8 mmol). The mixture was heated to 50–60 °C, stirred at this temperature overnight and quenched with ice/water. Aqueous sodium hydroxide (2 M) was added until a pH of 14 was reached, followed by 3 h of stirring at room temperature. The precipitate was filtered off and purified by chromatography (dichloromethane) yielding compound **2** (0.30 g, 52%) as a white solid, mp 202–204 °C. (Found: C, 65.5; H, 4.9; N, 4.3. $C_{18}H_{16}ClNO_3$ requires C, 65.6; H, 4.9; N, 4.3%). ν_{max}: 3328, 2942, 2844, 1624, 1585, 1561, 1343, 1272, 1216, 1093, 965, 796 cm^{-1}. λ_{max}: 232 nm (ε 22,300 cm^{-1} M^{-1}), 250 (24,500), 323 (13,200). ^{1}H NMR spectrum (300 MHz, $CDCl_3$): δ 2.69 (3H, s, Me), 3.90 (3H, s, OMe), 4.01 (3H, s, OMe), 6.23 (1H, s, H5), 7.08 (1H, d, *J* 1.9 Hz, H2), 7.32, 7.49 (4H, AA′BB′, Ar), 11.06 (1H, br s, NH). ^{13}C NMR spectrum (75 MHz, $CDCl_3$): δ 33.1 (Me), 55.1 (OMe), 56.2 (OMe), 87.3 (C5), 121.7 (C2), 127.6, 130.6 (ArCH), 104.8, 110.2, 117.1, 131.6, 134.2, 139.0, 159.3, 161.0 (ArC), 198.6 (CO).

4.1.2. 1-[3-(4-Bromophenyl)-4,6-dimethoxy-2-methyl-indol-7-yl]ethanone oxime (7). The 7-acetylindole **1**[2] (0.88 g, 2.28 mmol), hydroxylamine hydrochloride (0.25 g, 3.60 mmol) and potassium hydroxide (0.5 g, 8.93 mmol) in 95% ethanol (50 mL) were heated under reflux for 45 h. After cooling, cold water was added and the mixture was acidified with 1 M HCl. The solution was cooled and the resulting white precipitate was filtered off and dried. Column chromatography (dichloromethane/ethyl acetate, 95:5) yielded the oxime **7** (0.74 g, 81%) as a white solid, mp 221–222 °C. (Found: C, 56.9; H, 4.8; N, 6.9. $C_{19}H_{19}BrN_2O_3$ requires C, 56.6; H, 4.7; N, 6.9%). ν_{max}: 3380, 1596, 1289, 1140, 1001, 912, 824, 782 cm^{-1}. λ_{max}: 231 nm (ε 27,100 cm^{-1} M^{-1}), 313 (9250), 398 (4150). ^{1}H NMR spectrum (300 MHz, DMSO-d_6): δ 2.12 (3H, s, Me), 2.23 (3H, s, MeC=N), 3.69, 3.79 (6H, 2s, 2×OMe), 6.36 (1H, s, H5), 7.24, 7.48 (4H, AA′BB′, ArH), 10.49 (1H, s, NH), 10.88 (1H, s, OH). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ 12.1 (Me), 16.1 (*Me*C=N), 55.5, 57.3 (OMe), 89.8 (C5), 130.2, 132.9 (ArCH), 103.7, 111.4, 111.5, 118.6, 131.4, 135.1, 135.8, 152.3, 153.5 (ArC), 153.9 (C=N). Mass spectrum (EI): *m*/*z* 405 (M+1, ^{81}Br, 17%), 404 (99), 403 (M+1, ^{79}Br, 15), 402 (100), 371 (23), 276 (25), 275 (28).

4.1.3. 1-[3-(4-Chlorophenyl)-4,6-dimethoxyindol-7-yl]-ethanone oxime (8). This was prepared as described for the oxime **7** from the 7-acetylindole **2** (0.50 g, 1.52 mmol), hydroxylamine hydrochloride (0.17 g, 2.45 mmol) and potassium hydroxide (0.33 g, 5.88 mmol) in 95% ethanol (30 mL) under reflux for 45 h. After filtration the product was chromatographed (dichloromethane/ethyl acetate, 95:5) to yield oxime **8** (0.52 g, 80%) as a white solid, mp 171 °C. (Found: C, 62.1; H, 4.8; N, 8.1. $C_{18}H_{17}ClN_2O_3 \cdot 0.2H_2O$ requires C, 62.1; H, 5.0; N, 8.0%). ν_{max}: 3418, 3353, 1585, 1349, 1285, 1213, 1089, 994 cm^{-1}. λ_{max}: 230 nm (ε 24,700 cm^{-1} M^{-1}). ^{1}H NMR spectrum (300 MHz, DMSO-d_6): δ 2.15 (3H, s, Me), 3.80, 3.84 (6H, 2s, 2×OMe), 6.44 (1H, s, H5), 7.16 (1H, d, *J* 2.6 Hz, H2), 7.34, 7.51 (4H, AA′BB′, ArH), 10.67 (1H, br, NH), 10.88 (1H, s, OH). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ 15.9 (Me), 55.5, 57.3 (OMe), 89.9 (C5), 123.4 (C2), 127.8, 130.9 (ArCH), 103.9, 110.2, 115.8, 130.1, 135.4, 136.6, 152.5, 154.4 (ArC), 154.7 (C=N). Mass spectrum (EI): *m*/*z* 346 (M, ^{37}Cl, 35%), 344 (M, ^{35}Cl, 100), 313 (23), 311 (34).

4.1.4. 1-[4,6-Dimethoxy-2-methyl-3-phenylindol-7-yl]-1-[4-chlorophenyl]methanone oxime (9). Indole **3**[2] (0.31 g, 0.76 mmol), hydroxylamine hydrochloride (0.11 g, 1.58 mmol), pyridine (3 mL) and absolute ethanol (3 mL) were heated under reflux for 24 h. After cooling, the solvent was evaporated off under reduced pressure. The residue was dissolved in dichloromethane, acidified with 1 N HCl, washed with water and dried. The solvent was evaporated off and the residue was chromatographed (dichloromethane/ethyl acetate, 95:5) to give the oxime **9** (0.25 g, 78%) as a light brown solid, mp 128 °C. (Found: C, 68.1; H, 5.1; N, 6.8. $C_{24}H_{21}ClN_2O_3 \cdot 0.1H_2O$ requires C, 68.2; H, 5.1; N, 6.6%). ν_{max}: 3422, 1599, 1574, 1289, 1213, 1148, 1091, 991 cm^{-1}. λ_{max}: 247 nm (ε 38,650 $cm^{-1}\,M^{-1}$), 314 (10,700). ^1H NMR spectrum (300 MHz, DMSO-d_6): δ 2.18, 2.22 (3H, 2s, *syn* and *anti* Me), 3.55, 3.63, 3.67, 3.70 (6H, 4s, *syn* and *anti* OMe), 6.33, 6.39 (1H, 2s, *syn* and *anti* H5), 7.18–7.58 (9H, m, *syn* and *anti* ArH), 10.43, 10.64 (1H, 2s, *syn* and *anti* NH), 11.27, 11.40 (1H, 2s, *syn* and *anti* OH). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ 12.2 (Me), 55.5, 57.1 (OMe), 89.4 (C5), 125.3, 127.4, 128.2, 128.5, 130.9 (ArCH), 98.5, 111.7, 112.7, 131.3, 133.3, 134.6, 136.0, 136.5, 150.4, 152.4 (ArC), 154.1 (C=N). Mass spectrum (EI): *m/z* 422 (M, ^{37}Cl, 32%), 420 (M, ^{35}Cl, 100), 404 (24), 389 (28), 387 (65), 291 (21), 267 (44).

4.1.5. 1-[3-(4-Bromophenyl)-4,6-dimethoxy-2-methylindol-7-yl]-2,2,2-trifluoroethanone oxime (10). This was prepared as described for oxime **7** from 7-trifluoroacetylindole **4**[2] (0.50 g, 1.13 mmol), hydroxylamine hydrochloride (0.10 g, 1.44 mmol) and pyridine (5 mL) in absolute ethanol (2.5 mL) under reflux for 24 h. After extraction and concentration, the residue was chromatographed (dichloromethane/ethyl acetate, 95:5) to give the oxime **10** (0.44 g, 85%) as a white solid, mp 212–214 °C. (Found: C, 50.2; H, 3.6; N, 6.1. $C_{19}H_{16}BrF_3N_2O_3$ requires C, 49.9; H, 3.5; N, 6.1%). ν_{max}: 3516, 3361, 1594, 1370, 1347, 1194, 1139, 988 cm^{-1}. λ_{max}: 231 nm (ε 24,200 $cm^{-1}\,M^{-1}$), 319 (10,300). ^1H NMR spectrum (300 MHz, DMSO-d_6): δ 2.22 (3H, 2s, Me), 3.72, 3.78 (6H, 2s, 2×OMe), 6.40 (1H, s, H5), 7.26, 7.49 (4H, AA′BB′, ArH), 10.75 (1H, s, NH), 12.37 (1H, s, OH). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ 12.1 (Me), 55.5, 57.1 (OMe), 88.8 (C5), 92.3 (CF_3), 130.3, 132.9 (ArCH), 111.2, 111.5, 131.8, 134.6, 135.6, 142.3, 142.8, 153.8 (ArC), 155.1 (C=N). Mass spectrum (EI): *m/z* 459 (M+1, ^{81}Br, 16%), 458 (100), 457 (M+1, ^{79}Br, 13), 456 (96), 372 (24), 370 (23), 362 (21), 345 (24), 276 (49), 261 (41).

4.1.6. Ethyl 2-[3-(4-bromophenyl)-4,6-dimethoxy-2-methylindol-7-yl]-2-(hydroxyimino)acetate (11). This was prepared as described for oxime **7** from indole **5**[2] (0.40 g, 0.90 mmol), hydroxylamine hydrochloride (0.13 g, 1.87 mmol) and pyridine (4 mL) in absolute ethanol (2.5 mL) under reflux for 16 h. After extraction and concentration, the residue was chromatographed (dichloromethane/ethyl acetate, 95:5) to give the oxime **11** (0.41 g, 99%) as a yellow solid, mp 182–184 °C (dec). (Found: C, 54.6; H, 4.6; N, 6.1. $C_{21}H_{21}BrN_2O_5$ requires C, 54.7; H, 4.6; N, 6.1%). ν_{max}: 3400, 3330, 1732, 1595, 1357, 1210, 998, 911, 787 cm^{-1}. λ_{max}: 231 nm (ε 26,350 $cm^{-1}\,M^{-1}$), 252 (24,800), 329 (14,100). ^1H NMR spectrum (300 MHz, DMSO-d_6): δ 1.24 (3H, m, *syn* and *anti* CH_2–*Me*), 2.21, 2.28 (3H, 2s, *syn* and *anti* Me), 3.73 (6H, m, *syn* and *anti* OMe), 4.23 (2H, m, *syn* and *anti* OCH_2), 6.35, 6.39 (1H, 2s, *syn* and *anti* H5), 7.27–7.51 (4H, 2d, *J* 8.7 Hz, *syn* and *anti* ArH), 10.29, 10.39 (1H, 2s, *syn* and *anti* NH), 11.35, 12.17 (1H, 2s, *syn* and *anti* OH). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ 12.1, 12.2 (Me), 14.4, 14.5 (CH_2–*Me*), 55.5, 55.6, 57.0, 57.9 (OMe), 60.8, 60.9 (OCH_2), 88.9, 90.0 (C5), 130.3, 130.4, 132.9, 133.0 (ArCH), 96.7, 96.9, 111.2, 111.3, 111.9, 112.3, 118.6, 119.0, 131.2, 131.4, 133.7, 134.5, 135.3, 135.8, 145.7, 149.4, 153.6, 154.6 (ArC), 155.3, 155.7 (C=N), 164.1, 164.4 (C=O). Mass spectrum (EI): *m/z* 463 (M+1, ^{81}Br, 17%), 462 (100), 461 (M+1, ^{79}Br, 13), 460 (95), 444 (29), 430 (41), 371 (52), 357 (89), 276 (91), 261 (85).

4.1.7. *N,N*-Dimethyl-2-[3-(4-bromophenyl)-4,6-dimethoxy-2-methylindol-7-yl]-2-(hydroxyimino)acetamide (12). This was prepared as described for the oxime **7** from indole **6**[2] (0.50 g, 1.1 mmol), hydroxylamine hydrochloride (0.14 g, 2.01 mmol) and pyridine (5 mL) in absolute ethanol (5 mL) under reflux for 48 h. After extraction and concentration, the residue was chromatographed (dichloromethane/methanol, 95:5) to give the oxime **12** (0.26 g, 51%) as a white solid, mp 256–257 °C. (Found: C, 54.5; H, 4.8; N, 9.2. $C_{21}H_{22}BrN_3O_4$ requires C, 54.8; H, 4.8; N, 9.1%). ν_{max}: 3386, 3153, 1636, 1585, 1213, 1127, 1002 cm^{-1}. λ_{max}: 231 nm (ε 29,000 $cm^{-1}\,M^{-1}$), 326 (13,250). ^1H NMR spectrum (300 MHz, DMSO-d_6): δ 2.27 (3H, s, Me), 2.88, 2.93 (6H, 2s, NMe_2), 3.71, 3.73 (6H, 2s, 2×OMe), 6.36 (1H, s, H5), 7.26, 7.50 (4H, 2d, *J* 8.7 Hz, ArH), 10.34 (1H, br, NH), 11.17 (1H, s, OH). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ 12.2 (Me), 33.6, 36.7 (NMe_2), 55.6, 58.1 (OMe), 90.2 (C5), 130.3, 133.1 (ArCH), 98.4, 111.8, 112.1, 118.9, 131.0, 134.3, 135.4, 151.9, 155.2 (ArC), 155.4 (C=N), 165.5 (C=O). Mass spectrum (EI): *m/z* 462 (M+1, ^{81}Br, 18%), 461 (100), 460 (M+1, ^{79}Br, 16), 459 (97), 443 (26), 372 (62), 371 (45), 370 (58), 276 (45), 261 (59).

4.1.8. 1-[3-(4-Bromophenyl)-4,6-dimethoxy-2-methylindol-7-yl]ethanone *O*-2,4-dinitrophenyloxime (13). Indole oxime **7** (0.20 g, 0.49 mmol) was dissolved in absolute ethanol (20 mL) and sodium (18 mg, 0.78 mmol) was added. The solution became clear and was stirred at room temperature for 30 min. The mixture was cooled in an ice-bath, and fluoro-2,4-dinitrobenzene (0.1 mL, 0.83 mmol) was added dropwise. The mixture was stirred for another 2 h and the resulting precipitate was filtered off, washed with absolute ethanol and dried to yield the oxime ether **13** (0.14 g, 49%) as an orange solid, which could not be fully purified. ^1H NMR spectrum (300 MHz, $CDCl_3$): δ 2.67, 2.74 (6H, 2s, 2×Me), 3.92, 4.03 (6H, 2s, 2×OMe), 6.29 (1H, s, H5), 7.32 (1H, d, *J* 9.4 Hz, ArH), 7.46–7.56 (4H, m, ArH), 8.44 (1H, dd, *J* 9.4, 2.6 Hz, ArH), 9.06 (1H, d, *J* 2.6 Hz, ArH), 11.0 (1H, br, NH). The sample was not soluble enough for ^{13}C NMR measurement.

4.1.9. 1-[3-(4-Chlorophenyl)-4,6-dimethoxyindol-7-yl]-ethanone *O*-2,4-dinitrophenyloxime (14). This was prepared as described for indole **13** from indole oxime **8** (0.18 g, 0.54 mmol), absolute ethanol (20 mL), sodium (18 mg, 0.78 mmol) and fluoro-2,4-dinitrobenzene (0.1 mL, 0.83 mmol). After filtration, washing and chromatography (dichloromethane) it was dried to yield the oxime ether **14** (0.17 g, 64%) as an orange solid, mp 102 °C (dec).

(Found: C, 49.0; H, 3.1; N, 9.3. $C_{24}H_{19}ClN_4O_7 \cdot 1.3CH_2Cl_2$ requires C, 48.9; H, 3.5; N, 9.0%). ν_{max}: 3396, 1607, 1530, 1462, 1340, 1214 cm^{-1}. ^1H NMR spectrum (300 MHz, $CDCl_3$): δ 2.74 (3H, s, Me), 4.04, 4.06 (6H, 2s, 2×OMe), 6.35 (1H, s, H5), 7.36, 7.47 (4H, AA′BB′, ArH), 7.67 (1H, s, H2), 7.80 (2H, d, *J* 8.7 Hz, ArH and NH), 8.44 (1H, dd, *J* 9.4, 2.6 Hz, ArH), 9.06 (1H, d, *J* 2.6 Hz, ArH). The sample was not soluble enough for ^{13}C NMR measurement. Mass spectrum (EI): *m/z* 345 (100%), 327 (37).

4.1.10. 1-(4-Chlorophenyl)-1-[4,6-dimethoxy-2-methyl-3-phenylindole]methanone *O*-2,4-dinitrophenyloxime (15). This was prepared as described for indole **13** from indole oxime **9** (0.24 g, 0.57 mmol), absolute ethanol (25 mL), sodium (24 mg, 1.04 mmol) and fluoro-2,4-dinitrobenzene (0.1 mL, 0.83 mmol). After filtration, washing and chromatography (dichloromethane) it was dried to yield the oxime ether **15** (0.25 g, 78%) as an orange solid, mp 158–160 °C. (Found: C, 51.3; H, 3.3; N, 7.9. $C_{30}H_{23}ClN_4O_7 \cdot 1.8CH_2Cl_2$ requires C, 51.6; H, 3.6; N, 7.6%). ν_{max}: 3419, 1600, 1541, 1526, 1469, 1341, 1264, 1215, 1149, 924 cm^{-1}. λ_{max}: 228 nm (ε 32,250 $cm^{-1}\,M^{-1}$), 242 (32,100), 311 (24,650). ^1H NMR spectrum (300 MHz, $CDCl_3$): δ 2.27 (3H, s, Me), 3.66, 3.79 (6H, 2s, 2×OMe), 6.31 (1H, s, H5), 7.28–7.68 (10H, m, ArH and NH), 8.01 (1H, d, *J* 9.4 Hz, ArH), 8.44 (1H, dd, *J* 9.4, 2.6 Hz, ArH), 8.76 (1H, d, *J* 2.6 Hz, ArH). ^{13}C NMR spectrum (75 MHz, $CDCl_3$): δ 12.0 (Me), 55.2, 56.3 (OMe), 88.3 (C5), 118.3, 121.6, 125.6, 127.1, 128.7, 128.9, 129.4, 130.9 (ArCH), 95.5, 112.2, 114.3, 130.6, 132.5, 134.4, 135.5, 137.1, 137.2, 141.2, 154.3, 156.3, 157.0, 160.3 (ArC). Mass spectrum (EI): *m/z* 589 (M+1, ^{37}Cl, 2%), 588 (6), 587 (M+1, ^{35}Cl, 6), 586 (17), 513 (26), 294 (100), 293 (45).

4.1.11. 1-[3-(4-Bromophenyl)-4,6-dimethoxy-2-methylindol-7-yl]-2,2,2-trifluoroethanone *O*-2,4-dinitrophenyloxime (16). This was prepared as described for indole **13** from indole oxime **10** (0.15 g, 0.33 mmol), absolute ethanol (15 mL), sodium (0.02 g, 1.07 mmol) and fluoro-2,4-dinitrobenzene (0.06 mL, 0.33 mmol). After filtration and washing, it was dried to yield the oxime ether **16** (0.20 g, 100%) as an orange solid, mp 189 °C (dec). (Found: C, 48.3; H, 2.8; N, 8.7. $C_{25}H_{18}BrF_3N_4O_7$ requires C, 48.2; H, 2.9; N, 9.0%). ν_{max}: 3420, 1603, 1541, 1469, 1345, 1221, 1153, 1123, 933 cm^{-1}. λ_{max}: 233 nm (ε 35,750 $cm^{-1}\,M^{-1}$). ^1H NMR spectrum (300 MHz, $CDCl_3$): δ 2.33 (3H, s, Me), 3.79, 3.88 (6H, 2s, OMe), 6.28 (1H, s, H5), 7.28, 7.49 (4H, AA′BB′, ArH), 7.92 (1H, d, *J* 9.0 Hz, ArH), 7.94 (1H, br, NH), 8.48 (1H, dd, *J* 9.0, 2.6 Hz, ArH), 8.78 (1H, d, *J* 2.6 Hz, ArH). The sample was not soluble enough for ^{13}C NMR measurement. Mass spectrum (EI): *m/z* 624 (M, ^{81}Br, 1%), 622 (M, ^{79}Br, 1), 440 (95), 371 (100).

4.1.12. Ethyl 2-[3-(4-bromophenyl)-4,6-dimethoxy-2-methylindol-7-yl]-2-(*O*-2,4-dinitrophenyloxyimino)-acetate (17). This was prepared as described for indole **13** from indole oxime **11** (0.20 g, 0.43 mmol), absolute ethanol (20 mL), sodium (27 mg, 1.17 mmol) and 2,4-dinitrofluorobenzene (0.08 mL, 0.66 mmol). After filtration and washing, it was dried to yield the oxime ether **17** (0.27 g, 100%) as an orange solid, mp 179–180 °C. (Found: C, 51.4; H, 3.6; N, 8.7. $C_{27}H_{23}BrN_4O_9$ requires C, 51.7; H, 3.7; N, 8.9%). ν_{max}: 3434, 1743, 1591, 1542, 1342, 1208, 1159 cm^{-1}. λ_{max}: 230 nm (ε 39,900 $cm^{-1}\,M^{-1}$), 257 (38,400). ^1H NMR spectrum (300 MHz, $CDCl_3$): δ 1.39 (3H, t, *J* 7.2 Hz, CH_2*Me*), 2.37 (3H, s, Me), 3.78, 3.86 (6H, 2s, OMe), 4.41 (2H, q, *J* 7.2 Hz, OCH_2), 6.23 (1H, s, H5), 7.28, 7.49 (4H, 2d, *J* 8.6 Hz, ArH), 8.00 (1H, d, *J* 9.4 Hz, ArH), 8.42 (1H, s, NH), 8.47 (1H, dd, *J* 9.4, 2.6 Hz, ArH), 8.79 (1H, d, *J* 2.6 Hz, ArH). The sample was not soluble enough for ^{13}C NMR measurement. Mass spectrum (EI): *m/z* 629 (M+1, ^{81}Br, 26%), 627 (M+1, ^{79}Br, 26) 583 (12), 446 (100), 371 (55).

4.1.13. *N*,*N*-Dimethyl-2-[3-(4-bromophenyl)-4,6-dimethoxy-2-methylindol-7-yl]-2-(*O*-2,4-dinitrophenyloxyimino)acetamide (18). This was prepared as described for indole **13** from indole oxime **12** (0.15 g, 0.33 mmol), absolute ethanol (15 mL), sodium (0.02 g, 0.87 mmol) and fluoro-2,4-dinitrobenzene (0.06 mL, 0.50 mmol). After filtration and washing, it was dried to yield the oxime ether **18** (0.20 g, 100%) as an orange solid, mp 162 °C (dec). (Found: C, 50.5; H, 3.8; N, 10.3. $C_{27}H_{24}BrN_5O_8 \cdot 1H_2O$ requires C, 50.3; H, 4.0; N, 10.8%). ν_{max}: 3428, 1646, 1585, 1515, 1343, 1296, 1215 cm^{-1}. λ_{max}: 230 nm (ε 45,650 $cm^{-1}\,M^{-1}$), 255 (42,800), 364 (18,850). ^1H NMR spectrum (300 MHz, $CDCl_3$): δ 2.32, 2.37 (3H, s, *syn* and *anti* Me), 2.99–3.18 (6H, m, *syn* and *anti* NMe_2), 3.75–3.88 (6H, m, *syn* and *anti* OMe), 6.20, 6.23 (1H, s, *syn* and *anti* H5), 7.46–7.52 (4H, m, *syn* and *anti* ArH), 7.83, 7.86 (1H, d, *J* 9.4 Hz, *syn* and *anti* ArH), 8.39–8.50 (1H, m, *syn* and *anti* ArH), 8.72, 8.90 (1H, d, *J* 2.6 Hz, ArH), 9.66 (1H, s, *syn* and *anti* NH). The sample was not soluble enough for ^{13}C NMR measurement. Mass spectrum (EI): *m/z* 509 (12%), 463 (46), 461 (41), 445 (100), 443 (98), 289 (47).

4.1.14. 5-(4-Bromophenyl)-6,8-dimethoxy-1,4-dimethylpyrrolo[3,2,1-*hi*]indazole (19). A mixture of indole oxime ether **13** (0.14 g, 0.24 mmol) and triethylamine (0.75 mL) in dry tetrahydrofuran (15 mL) was heated under reflux for 6 h with stirring. The solvent was evaporated off and the residue was dissolved in dichloromethane. The organic layer was washed with 2 M NaOH and water, dried and concentrated. Column chromatography (dichloromethane) yielded compound **19** (0.06 g, 63%) as a white solid, mp 180–181 °C. (Found: C, 59.1; H, 4.5; N, 7.4. $C_{19}H_{17}BrN_2O_2$ requires C, 59.2; H, 4.5; N, 7.3%). ν_{max}: 1682, 1603, 1511, 1234, 1122, 1000 cm^{-1}. λ_{max}: 229 nm (ε 25,500 $cm^{-1}\,M^{-1}$), 270 (13,450), 329 (15,900). ^1H NMR spectrum (300 MHz, $CDCl_3$): δ 2.67 (3H, s, Me), 2.75 (3H, s, MeC=N), 3.91, 4.03 (6H, 2s, 2×OMe), 6.29 (1H, s, H7), 7.48, 7.55 (4H, AA′BB′, ArH). ^{13}C NMR spectrum (75 MHz, $CDCl_3$): δ 10.7 (Me), 15.2 (*Me*C=N), 56.2, 56.7 (OMe), 92.1 (C7), 131.0, 131.2 (ArCH), 101.4, 103.5, 118.4, 119.9, 129.8, 134.1, 142.9, 151.1, 156.3, 158.4 (ArC). Mass spectrum (EI): *m/z* 387 (M+1, ^{81}Br, 20%), 386 (99), 385 (M+1, ^{79}Br, 23), 384 (100), 369 (55), 275 (90).

4.1.15. 5-(4-Chlorophenyl)-6,8-dimethoxy-1-methylpyrrolo[3,2,1-*hi*]indazole (20). This was prepared as described for indole **19** from indole oxime ether **14** (0.15 g, 0.29 mmol) and triethylamine (0.75 mL) in dry tetrahydrofuran (15 mL) under reflux for 6 h. After extraction and concentration, the residue was chromatographed (dichloromethane) to give the pyrrolo-indazole **20** (0.07 g, 69%) as a white solid, mp 198–200 °C. (Found: C, 66.0; H,

4.6; N, 8.7. $C_{18}H_{15}ClN_2O_2$ requires C, 66.2; H, 4.6; N, 8.6%). ν_{max}: 3102, 1675, 1604, 1505, 1333, 1220, 1176, 1117, 1067 cm^{-1}. λ_{max}: 230 nm (ε 20,200 $cm^{-1}\,M^{-1}$), 274 (12,400). ^1H NMR spectrum (300 MHz, $CDCl_3$): δ 2.74 (3H, s, Me), 4.03, 4.05 (6H, 2s, 2×OMe), 6.34 (1H, s, H7), 7.67 (1H, s, H2), 7.36, 7.80 (4H, AA′BB′, ArH). ^{13}C NMR spectrum (75 MHz, $CDCl_3$): δ 15.2 (Me), 56.3, 56.5 (OMe), 91.7 (C7), 118.6 (C2), 128.4, 128.6 (ArCH), 100.5, 103.4, 123.9, 132.0, 133.2, 144.4, 151.7, 157.2, 159.2 (ArC). Mass spectrum (EI): *m/z* 328 (M, ^{37}Cl, 31%), 326 (M, ^{35}Cl, 100), 311 (57), 261 (32).

4.1.16. 1-(4-Chlorophenyl)-6,8-dimethoxy-4-methyl-5-phenylpyrrolo[3,2,1-*hi*]indazole (21). This was prepared as described for indole **19** from indole oxime ether **15** (0.30 g, 0.51 mmol) and sodium hydride (0.12 g, 80% dispersion in oil, 4.0 mmol) in dioxane (7.5 mL) under reflux for 1 h. After extraction and concentration, the residue was chromatographed (dichloromethane) to give the pyrrolo-indazole **21** (20 mg, 10%) as a yellow solid, mp 190–192 °C. (Found: C, 70.5; H, 4.9; N, 6.7. $C_{24}H_{19}ClN_2O_2\cdot 0.3H_2O$ requires C, 70.6; H, 4.8; N, 6.9%). ν_{max}: 1675, 1600, 1518, 1325, 1299, 1234, 1216, 1134, 1013 cm^{-1}. λ_{max}: 231 nm (ε 31,350 $cm^{-1}\,M^{-1}$), 246 (31,200), 318 (12,100). ^1H NMR spectrum (300 MHz, $CDCl_3$): δ 2.75 (3H, s, Me), 3.92, 4.06 (6H, 2s, 2×OMe), 6.37 (1H, s, H7), 7.30–7.66 (7H, m, ArH), 8.35 (1H, part of AA′BB′, ArH). ^{13}C NMR spectrum (75 MHz, $CDCl_3$): δ 10.6 (Me), 56.2, 56.8 (OMe), 92.8 (C7), 126.3, 127.9, 128.6, 129.4, 129.7 (ArCH), 101.6, 120.5, 129.6, 131.7, 134.4, 134.8, 142.7, 151.7, 155.4, 158.4 (ArC). Mass spectrum (EI): *m/z* 404 (M, ^{37}Cl, 35%), 402 (M, ^{35}Cl, 100), 387 (43), 291 (20), 220 (22).

4.1.17. 5-(4-Bromophenyl)-6,8-dimethoxy-4-methyl-1-trifluoromethylpyrrolo[3,2,1-*hi*]indazole (22). This was described as prepared for indole **19** from indole oxime ether **16** (0.20 g, 0.32 mmol) and sodium hydride (97 mg, 80% dispersion in oil, 3.23 mmol) in dioxane (5 mL) under reflux for 1 h. After extraction and concentration, the residue was chromatographed (dichloromethane) to give the pyrrolo-indazole **22** (41 mg, 29%) as a pale yellow solid, which could not be fully purified. ^1H NMR spectrum (300 MHz, $CDCl_3$): δ 2.68 (3H, s, Me), 3.95, 4.05 (6H, 2s, 2×OMe), 6.37 (1H, s, H7), 7.47, 7.58 (4H, AA′BB′, ArH).

4.1.18. Ethyl 5-(4-bromophenyl)-6,8-dimethoxy-4-methylpyrrolo[3,2,1-*hi*]indazole-1-carboxylate (23). This was prepared as described for indole **19** from indole oxime ether **17** (0.27 g, 0.43 mmol) and triethylamine (1 mL) in dry tetrahydrofuran (20 mL) under reflux overnight. After extraction and concentration, the residue was chromatographed (dichloromethane) to give the pyrrolo-indazole **23** (40 mg, 21%) as a yellow solid, which could not be fully purified. ^1H NMR spectrum (300 MHz, $CDCl_3$): δ 1.49 (3H, t, *J* 7.1 Hz, OCH_2Me), 2.70 (3H, s, Me), 3.94, 4.08 (6H, 2s, 2×OMe), 4.54 (2H, q, *J* 7.1 Hz, OCH_2), 6.38 (1H, s, H5), 7.48, 7.57 (4H, AA′BB′, ArH). ^{13}C NMR spectrum (75 MHz, $CDCl_3$): δ 10.5 (Me), 14.3 (OCH_2*Me*), 56.6, 56.7 (OMe), 61.6 (OCH_2), 93.9 (C5), 131.1, 131.2 (ArCH), 100.5, 102.7, 120.8, 122.5, 129.9, 133.1, 142.1, 142.6, 156.6, 158.8 (ArC), 162.0 (C═O). Mass spectrum (EI): *m/z* 444 (M, ^{81}Br, 35%), 442 (M, ^{79}Br, 28), 372 (23), 371 (100), 369 (96).

4.1.19. *N*,*N*-Dimethyl-5-(4-bromophenyl)-6,8-dimethoxy-4-methylpyrrolo[3,2,1-*hi*]indazole-1-carboxamide (24). This was prepared as described for indole **19** from indole oxime ether **18** (0.20 g, 0.32 mmol) and sodium hydride (0.12 g, 80% dispersion in oil, 4.0 mmol) in dioxane (4 mL) under reflux for 1 h. After extraction and concentration, the residue was chromatographed (dichloromethane) to give the pyrrolo-indazole **24** (42 mg, 30 %) as a pale yellow solid, mp 230–232 °C. (Found: C, 55.3; H, 4.4; N, 9.2. $C_{21}H_{20}BrN_3O_3\cdot 0.7H_2O$ requires C, 55.4; H, 4.7; N, 9.2%). ν_{max}: 1677, 1627, 1596, 1515, 1327, 1225, 1132, 1077, 1005 cm^{-1}. λ_{max}: 231 nm (ε 27,900 $cm^{-1}\,M^{-1}$), 329 (8250). ^1H NMR spectrum (300 MHz, $CDCl_3$): δ 2.67 (3H, s, Me), 3.21 (6H, s, NMe_2), 3.92, 4.04 (6H, 2s, OMe), 6.36 (1H, s, H5), 7.48, 7.56 (4H, 2d, *J* 8.3 Hz, ArH). ^{13}C NMR spectrum (75 MHz, $CDCl_3$): δ 10.5 (Me), 35.4, 38.7 (NMe_2), 56.7, 56.9 (OMe), 94.0 (C5), 131.1, 131.2 (ArCH), 100.7, 101.8, 120.5, 120.7, 129.6, 133.5, 141.6, 145.8, 156.3, 158.8 (ArC), 164.1 (C═O). Mass spectrum (EI): *m/z* 444 (M+1, ^{81}Br, 27%), 443 (100), 442 (M+1, ^{79}Br, 20), 441 (90), 372 (38), 371 (68), 370 (22), 369 (71), 275 (32).

Acknowledgements

Financial support from the Australian Research Council is gratefully acknowledged. T.D.W. and K.P. acknowledge receipt of an Indonesian Government Quality of Undergraduate Education (QUE) Scholarship and an International Postgraduate Scholarship, respectively.

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Tetrahedron
Tetrahedron 62 (2006) 6349–6354

Cohaerins C–F, four azaphilones from the xylariaceous fungus *Annulohypoxylon cohaerens*

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Received 2 February 2006; revised 10 April 2006; accepted 12 April 2006
Available online 11 May 2006

Dedicated to the celebration of the 65th birthday of Professor Yoshinori Asakawa

Abstract—Four azaphilones named cohaerins C–F, along with 4,5,4′,5′-tetrahydroxy-1,1′-binaphthyl were isolated from the methanolic extract of the stromata of *Annulohypoxylon cohaerens* (Ascomycetes, Xylariaceae). Cohaerins C–E constitute typical azaphilones, bearing a γ-lactone ring, while cohaerin F has an unprecedented carbon skeleton, lacking the lactone ring of the azaphilones and with an aliphatic side chain being attached directly to the azaphilone backbone by a C–C bond. Their structures were determined by 2D NMR, IR, UV, and CD spectroscopy. They showed moderate inhibitory activity of nitric oxide production in RAW cells, and strong and nonselective antimicrobial effects.

1. Introduction

The new genus *Annulohypoxylon* (Ascomycota, Xylariaceae) has recently been recognized from a comparison of morphological features and molecular taxonomy and segregated from the related genus *Hypoxylon*.[1] *Annulohypoxylon* had until recently been treated as *Hypoxylon* sect. *Annulata*[2] and is believed to have evolved from the same evolutionary lineage as *Hypoxylon* and *Daldinia*. The fruit bodies (stromata) of all these genera are usually associated with—and commonly encountered on—woody angiosperms.

This study relates to our ongoing projects on the pigments and other secondary metabolites produced in abundance by *Hypoxylon* and its relatives, which have been known to be chemotaxonomically significant for a long time.[3,4] In fact, the characteristic pigments of certain *Annulohypoxylon* spp. [multiformins from *Hypoxylon* (=*Annulohypoxylon*) *multiforme*[5] and cohaerins from *Hypoxylon* (=*Annulohypoxylon*) *cohaerens*][6] were only recently isolated and identified. Using HPLC profiling based on diode array and ESIMS data, a broad range of species, including type and other authentic material, were examined in the latter study.[6] These chemotaxonomic results also confirmed the new generic concept:[1] *Annulohypoxylon* and *Hypoxylon* as understood today (i.e., sect. *Annulata* and *Hypoxylon* in Refs. 2 and 5) significantly differ in the distribution of stromatal secondary metabolites. Several hundreds of their specimens, including representatives of most currently accepted species, were already studied by HPLC profiling. According to these results, no azaphilone type contained in *Hypoxylon* was ever found in *Annulohypoxylon* and vice versa. Further, the metabolites that are frequently encountered in *Annulohypoxylon* are BNT (**5**, ubiquitous in all of its species and many other Xylariaceae), daldinone A (also present in a few other genera), truncatone[6,7] (apparently restricted to *Annulohypoxylon*[2,6]), and spider sex pheromones that were hitherto only found in *Annulohypoxylon annulatum*.[8] The genus *Hypoxylon* is instead characterized by the occurrence of, e.g., mitorubrins, entonaemins, rubiginosins, and daldinin type azaphilones, and by the lack of truncatone, cohaerins, and multiformins.[4]

The above HPLC-based study[6] was rather conclusive with respect to the general occurrence of chemical types of

Keywords: Fungi; *Annulohypoxylon cohaerens*; Azaphilone; Cohaerin; Xylariaceae.
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doi:10.1016/j.tet.2006.04.040

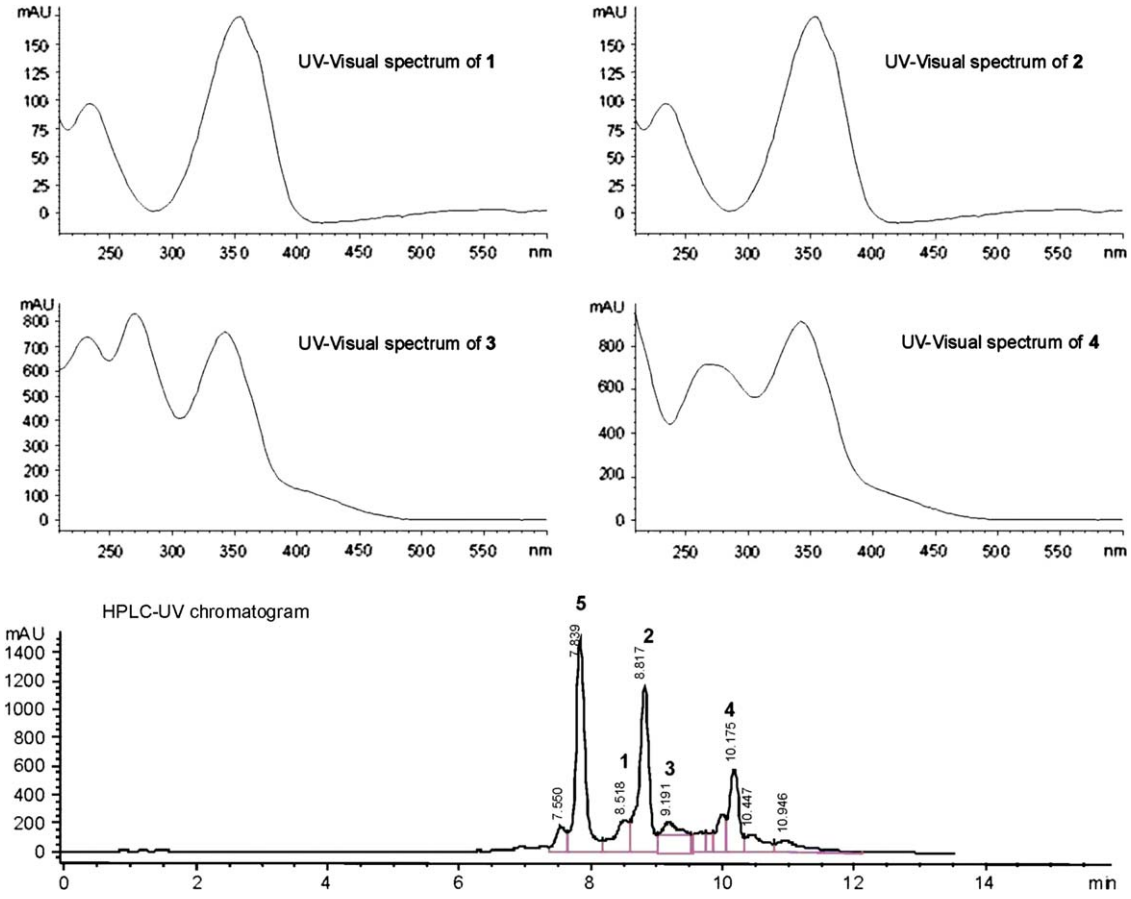

Figure 1. HPLC–UV chromatogram (210 nm) of the crude stromatal MeOH extract of *A. cohaerens* STMA 05296 and UV–vis spectra of cohaerins C–F (**1**–**4**). Besides the new compounds, BNT (**5**), another major component, is also indicated in the chromatogram trace; other peaks relate to unknown minor components. Column: Merck Lichrospher C18 (5 mm, 125×4 mm); mobile phase: 0.1% H_3PO_4 (A):acetonitrile (B); linear gradient from 0 to 100% B in 10 min; thereafter continuing at 100% B (for details on method see Refs. 6,9, and 10).

pigments in these fungi, and their characteristic metabolites were still detectable in type specimens over 200 years old. Nonetheless, little is known about the aspects such as the host-specific metabolite production in the species of *Annulohypoxylon*, or on the occurrence of chemical races in certain geographic regions. HPLC profiling is being used as a routine procedure in our floristic and chemotaxonomic studies of *Hypoxylon* and allied genera,[9–12] and so far all materials referable to *A. cohaerens* showed the same HPLC profile, with BNT (**5**) and cohaerins A (**6**) and B[6] as prevailing components. However, we recently noted that some specimens of *A. cohaerens* from Central and Southeastern Europe showed a deviating HPLC profile (see Fig. 1). Despite their typical morphology, cohaerins A (**6**) and B[6] lacked in their HPLC chromatograms, and other components with similar UV–vis spectra were observed. One of these specimens was therefore obtained in sufficient quantities, which allowed us to study its chemical constituents, resulting in the identification of four new azaphilones named cohaerins C–F (**1**–**4**). We would like to report here their isolation and biological activities.

2. Results and discussion

The MeOH extract of *A. cohaerens* STMA 04158 was subjected to conventional purification procedures, resulting in the isolation of four new azaphilone derivatives (**1**–**4**), along with the known compound, 4,5,4′,5′-tetrahydroxy-1,1′-binaphthyl (BNT, **5**).[11]

Compound **1** gave a molecular ion peak at *m*/*z* 505 $(M+Na)^+$ in the FABMS, which, is in accordance with the data obtained from the NMR spectra, corresponded to the molecular formula $C_{28}H_{34}O_7$, which was determined by HRFABMS. Its IR and UV–vis spectra showed the presence of a γ-lactone (1784 cm^{-1}), a conjugated ketone (1717 cm^{-1}; 269 and 341 nm), and an olefinic functional group (1637 cm^{-1}). The ^1H NMR spectrum of **1** displayed the typical pattern of an azaphilone skeleton with three olefinic protons attributed to H-1, H-4, and H-5. In addition, a singlet methyl was located at C-9. Interpretation of 2D NMR spectrum indicated the presence of two subunits linked to the main azaphilone backbone at C-3 and C-18. The spectral data of the first subunit were identical to those of 4-hydroxy-2-methyl-6-oxocyclohex-1-enyl, i.e., a partial structure of cohaerin B.[6] This unit was also connected with the main azaphilone structure at C-3 by HMBC correlations between H-4 and C-10, and H-16 and C-3. Interpretation of ^1H–^1H COSY spectrum of **1** suggested that the second unit was an aliphatic 2-methyl-octanoyl side chain. The connection of this unit and the main azaphilone backbone was determined at C-18, based on HMBC correlations between H-18 and C-19, and H-20 and C-18 and C-19. Furthermore, H-8 coupled to C-17 and

C-18, and H-9 coupled to C-7, C-8, and C-17 in the HMBC spectrum, which confirmed the presence of a γ-lactone in the molecule. The relative stereochemistries of H-8, H-9, and H-18 were established to be α-oriented, due to their NOESY correlations between H-8 and H-18, and H-9 and H-18, respectively. However, the relative stereochemistry of the hydroxyl group at C-13 and the secondary methyl at C-20 remained unclear. In addition, the absolute configuration at C-7 was found to be *R* based on its CD spectrum, which showed positive (354 and 230 nm) and negative (257 nm) Cotton effects.[13] Based on the above spectral evidence, cohaerin C (**1**) was determined to be 3-(4-hydroxy-2-methyl-6-oxocyclohexenyl)-6*a*(*R*)-methyl-9-(2-methyloctanoyl)-9(*S*),9*a*(*S*)-dihydro-6*aH*-furo[2,3-*h*]isochromene-6,8-dione as shown in Fig. 2.

Cohaerin D (**2**) has the molecular formula $C_{28}H_{32}O_7$ based on HRFABMS with two hydrogen atoms less than cohaerin C (**1**). Its spectral data are similar to those of **1**, except for the presence of a double bond at C-8 and C-18 to form an unsaturated lactone, which was revealed by IR absorption band at 1764 cm^{-1} and the downfield shifts of C-8 and C-18 compared with those of **1** in ^{13}C NMR. The absolute configuration at C-7 was also determined to be *R* based on the CD spectrum with positive (353 nm) and negative (280 nm) Cotton effects.[13] Thus, cohaerin D (**2**) was elucidated to be 3-(4-hydroxy-2-methyl-6-oxocyclohexenyl)-6*a*(*R*)-methyl-9-(2-methyloctanoyl)-6*aH*-furo[2,3-*h*]isochromene-6,8-dione.

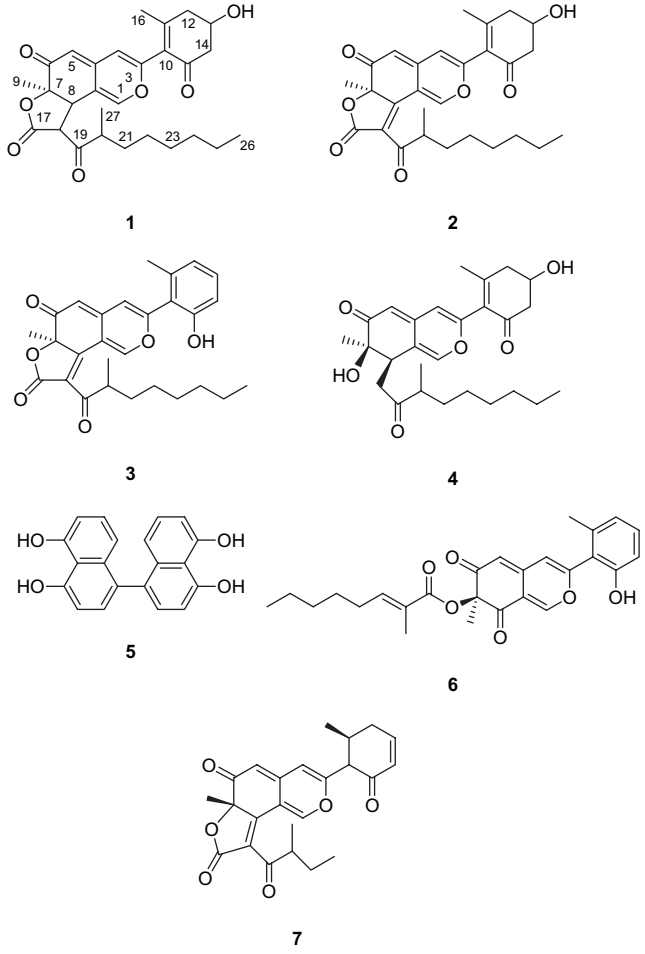

Figure 2. Structures of **1**–**7**.

A molecular formula of $C_{28}H_{30}O_6$ was determined for cohaerin E (**3**) on the basis of the observed molecular ion peak at *m*/*z* 463.2148 [Calcd for $C_{28}H_{31}O_6$ $(M+H)^+$, 463.2121] in its HRFABMS. The NMR spectra of **3** resembled those of **2**. The only notable difference was observed in the signals of the partial structure at C-3, which was found to be 2-hydroxy-6-methylphenyl as deduced from its 2D NMR spectra and comparison with the data for cohaerin A (**6**).[6] The absolute configuration at C-7 was also found to be *R* by comparing its CD spectrum (see Section 3) with those of cohaerins C and D (**1**–**2**). From the above spectral evidence, cohaerin E (**3**) was determined to be 3-(2-hydroxy-6-methylphenyl)-6*aR*-methyl-9-(2-methyloctanoyl)-6*aH*-furo[2,3-*h*]isochromene-6,8-dione.

Cohaerin F (**4**) exhibited a *quasi*-molecular ion peak at *m*/*z* 479.2442 $(M+Na)^+$ corresponding to a molecular formula of $C_{27}H_{36}O_6Na$ as determined by HRFABMS [Calcd for $C_{27}H_{36}O_6Na$ $(M+Na)^+$, 479.2410]. Its IR spectrum showed only the absorption bands of ketones (1711 and 1678 cm^{-1}). Further interpretation of its NMR spectral data revealed that **4** contained the same cyclohexadienone group at C-3 as found in **1**. In comparison with the structure of cohaerin C (**1**), the lactone group had disappeared, but signals for a methylene group at C-18 were noted and correlated with C-8 and C-19 in its HMBC spectrum (Fig. 3). Taking into account the 2D NMR spectra and comparing with those of **1**–**3**, we could deduce the structure of **4** as shown in Figure 2. Furthermore, the absolute configuration of C-7 was established to be *R* by comparing its CD spectrum (see Section 3) with those of **1**–**3**. In addition, both H-8 and H-9 were in α-face, since they correlated in NOESY spectrum. Consequently, cohaerin F (**4**) is 7-hydroxy-3-(4-hydroxy-2-methyl-6-oxocyclohexenyl)-7(*R*)-methyl-8-(3-methyl-2-oxononyl)-7,8-dihydro-isochromen-6-one.

Recent studies have revealed that the Xylariaceae azaphilones possess broad-spectral activities in biological systems.[5,13,14] Especially, they exhibited nonselective antimicrobial activities, which suggested their role for protection of the fungal stromata from feeding enemies in the environment.[2,6,15] Some of their pigments are also potent nitric oxide inhibitors in macrophages.[16] In this paper, we continued to screen the inhibitory activity of nitric oxide production in RAW 264.7 cells by the cohaerins C–F, which showed moderate activity with their IC_{50} values of 30.2 (**1**), 19.6 (**2**), 26.1 (**3**), and 41.2 μM (**4**), respectively. These

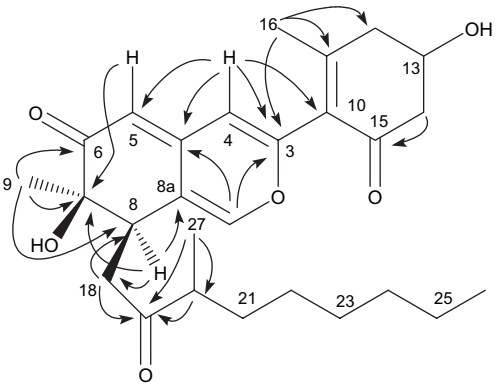

Figure 3. Important HMBC correlations of **4**.

Table 1. Antimicrobial activities of metabolites from *Annulohypoxylon* and two standard antibiotics in the agar diffusion assay against *Bacillus subtilis* (NB medium, after 18 h of incubation) and *Yarrowia lipolytica* (YMG medium, after 24 h of incubation)

Compound	*B. subtilis*	*Y. lipolytica*
Cohaerin C (**1**)	**13**	**11**
Cohaerin D (**2**)	**12**	**12**
Cohaerin E (**3**)	**14**	**12**
Cohaerin F (**4**)	**14**	**12**
From previous work		
Cohaerin A (**6**)	**12**	**10**
Multiformin B (**7**)	**11**	**9**
Standards		
Penicillin G	**23**	—
Actinomycin D	—	**17**

Inhibitory concentrations are given in millimeter for diameter of inhibition zone for concentrations of 50 mg/paper disk (diameter of paper disk; 6 mm). (—) Indicates lack of activity.

results again suggested that the presence of a lactone ring in the molecule increased the activity, but the activities were substantially weaker than those of some azaphilones from *Hypoxylon*, such as rutilins and rubiginosin A.[16] By contrast, the antimicrobial activities (Table 1) did not deviate much from other compounds previously evaluated, including cohaerin A (**6**) and multiformin B (**7**), which were tested for comparison. No particular selectivity against the yeast *Yarrowia lipolytica* or the bacterium *Bacillus subtilis* was observed.

Previous work on the related genera *Daldinia*[10] and *Hypoxylon*[11,12] had revealed rather constant secondary metabolite profiles in a given species, and this was confirmed by examination of hundreds of specimens. Nonetheless, so far there is no evidence that the specimens used for isolation of cohaerins C–F belong to a new morphological species or variety, despite it yielded four unprecedented azaphilones and lacked the cohaerins A and B that were previously attributed to this species.[6] Neither the morphological features (asci, ascospores, perithecia, ostioles, etc.) of the teleomorph nor the conidiogenous structures of the anamorph deviated from typical *A. cohaerens.* All of them were even collected from *Fagus*, as typical for this highly host-specific species. Since the stromata of the specimens STMA 04158, STMA 05161, and STMA 05296 also gave the typical olivaceous color in KOH that is useful to distinguish *A. cohaerens* from morphologically similar, related species, including *A. multiforme*[2] (as *Hypoxylon* spp.), it is not possible to distinguish the 'chemical races' of *A. cohaerens* without the aid of HPLC or, possibly, TLC.

A comparison of the structural features of **1**–**4** with those of other known azaphilones of *Annulohypoxylon* suggests that their biogenesis maybe quite different from those of the cohaerins A and B, and multiformins. Compounds **1**–**3** and the multiformins[5] possess a tricyclic ring attached by an ester bond to a branched unsaturated C-8 fatty acid and an additional lactone ring is present. In contrast, **4** remains tricyclic but lacks the ester bond of the cohaerins. The multiformins differ from all cohaerins in having a shorter side chain attached to their tetracyclic ring. All of them indeed have carbon skeletons quite different from those of the daldinins or the orsellinic acid containing azaphilones such as mitorubrin that are found in the related genera *Daldinia* and *Hypoxylon*, respectively.[7,10–12] Molecular taxonomy, including studies on polyketide synthases and other enzymes of secondary metabolite biogenesis, may eventually show whether the current fungus constitutes a cryptic species within *A. cohaerens.* For such purposes, a culture of the fungus was deposited in a public collection.

Trace amounts of mitorubrin derivatives[10] were also isolated from the stromatal extract, which was later shown to be due to the fact that the substrate contained some small stromata of *Hypoxylon fragiforme*, another fungus commonly encountered on *Fagus* in Europe. Stromata of *A. cohaerens* from the voucher specimens were found devoid of mitorubrins by HPLC–MS. This observation relates to the problem that different Xylariaceae species may frequently colonize the same substrate, with their fruit bodies becoming intermingled.[11] Special care must therefore be taken in the examination of such material to be extracted.

3. Experimental

3.1. General

Optical rotations were measured on a JASCO DIP-1000 polarimeter in $CHCl_3$. IR spectra were measured on a Perkin–Elmer Spectrum One FTIR spectrometer. UV–vis spectra were obtained on a Shimadzu UV-1650PC in MeOH. CD spectra were measured on a JASCO J-725 spectrometer in MeOH. Mass spectra were recorded on a JEOL JMS AX-500 spectrometer. NMR spectra were recorded on a Varian Unity 600 (600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR). Column chromatography was carried out on silica gel 60 (0.2–0.5 and 0.04–0.063 mm, Merck), reverse-phase C_{18} silica gel (Merck) and Sephadex LH-20 (Amersham Pharmacia Biotech, $CHCl_3$–MeOH, 1:1). HPLC analyses were carried out as described earlier.[6]

3.2. Fungal material

Stromata of *A. cohaerens* was collected by N. Radulovíc at Mt. Suva Planina, near Niš city, Serbia and Montenegro, from decaying tree trunks of *Fagus sylvatica* in September 2004 (STMA 04158, used for preparative work) and again on the same site in September 2005 (STMA 05296, see Fig. 4). Another specimen (STMA 05161) that showed the same chemotype was collected by M.S. on 17 July 2005 from *F. sylvatica* in Austria, Lower Austria Prov., Mauerbach, Nature reserve Kartause, in the course of the mycological excursion of the 16th International Botanical Congress (Vienna). The material was identified by the authors. Voucher specimens bearing the above STMA numbers are deposited at the mycological herbarium, Staatliches Museum für Naturkunde, Karlsruhe, Germany. A culture obtained from specimen STMA 05296 has been deposited with CBS, Utrecht, The Netherlands as Strain nr CBS 119311.

3.3. Extraction and isolation

The crude extract (4.9 g from 47 g of dry stromata) was chromatographed by Sephadex LH-20 column chromatography using $CHCl_3$–MeOH (1:1) to give six fractions.

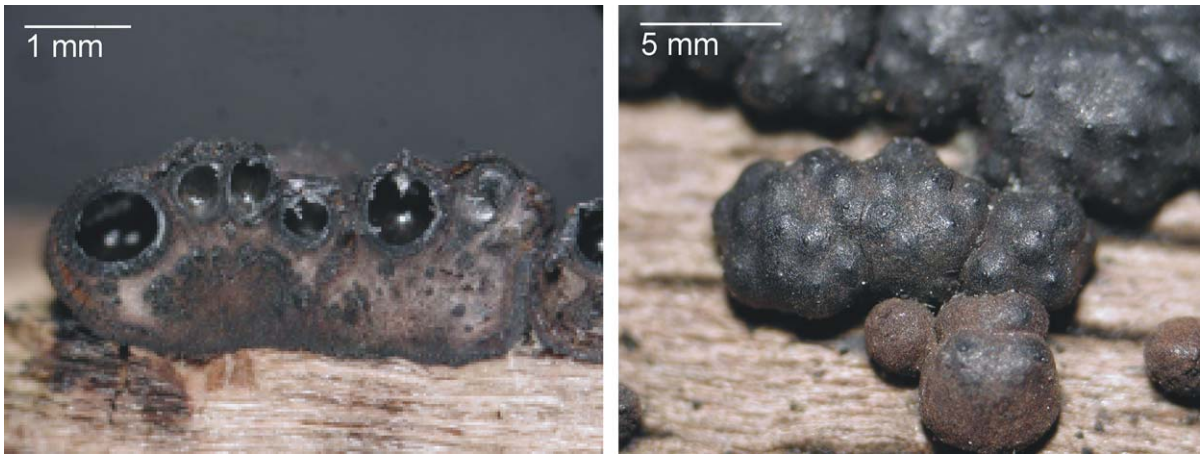

Figure 4. Stromata of *A. cohaerens* STMA 05296 on decorticated wood of *F. sylvatica*. Left: section through stromata. Right: stromatal habit. Scale is indicated by bars.

The first fraction (1330.5 mg) was purified by SiO_2 column chromatography ($CHCl_3$–MeOH, 30:1) to afford **1** (95.3 mg), **2** (176.2 mg), and two sub-fractions 1–1 (154.1 mg) and 1–2 (114.2 mg), which were further separated by reversed-phase column chromatography, using MeOH–H_2O (4:1) as mobile phase to yield **3** (27.9 mg) and **4** (12.0 mg), respectively. Second fraction (1079.7 mg) was subjected to SiO_2 column chromatography, using a $CHCl_3$–MeOH gradient from 0 to 10% MeOH, to give **1** (54.8 mg), **2** (142.3 mg), and a mixture (164.0 mg) that was purified by reversed-phase column chromatography (MeOH–H_2O, 4:1) to obtain **3** (22.2 mg). Sixth fraction (216.3 mg) was BNT (**5**) in pure state.

3.3.1. Cohaerin C (1). $[\alpha]_D^{20}$ +3.7 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1}: 3425, 1784, 1717, 1637, 1530, 1458, 1380, 1188, 1066, 976; UV λ_{max} nm (log ε): 353 (4.1), 233 (3.9); CD (MeOH) λ_{ext} nm ($\delta\varepsilon$): 354 (+3.0), 257 (−1.0), 230 (+1.0); FABMS *m/z* 505 $(M+Na)^+$; HRFABMS *m/z* 505.2206 [Calcd for $C_{28}H_{34}O_7Na$ $(M+Na)^+$, 505.2202]; ^1H and ^{13}C NMR ($CDCl_3$) data, Tables 2 and 3.

3.3.2. Cohaerin D (2). $[\alpha]_D^{20}$ −2.2 (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1}: 3427, 1764, 1683, 1627, 1537, 1456, 1379, 1248, 1157, 1068, 754; UV λ_{max} nm (log ε): 341 (4.2), 269 (3.3); CD (MeOH) λ_{ext} nm ($\delta\varepsilon$): 353 (+3.3), 280 (−2.8); FABMS *m/z* 503 $(M+Na)^+$; HRFABMS *m/z* 503.2031 [Calcd for $C_{28}H_{32}O_7Na$ $(M+Na)^+$, 503.2046]; ^1H and ^{13}C NMR (CD_3OD) data, Tables 2 and 3.

3.3.3. Cohaerin E (3). $[\alpha]_D^{20}$ −1.6 (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1}: 3314, 1766, 1683, 1624, 1532, 1466, 1167, 1013, 875; UV λ_{max} nm (log ε): 344 (3.1), 267 (3.0); CD (MeOH) λ_{ext} nm ($\delta\varepsilon$): 359 (+12.8), 291 (−6.4), 261 (−7.1); FABMS *m/z* 463 $(M+H)^+$; HRFABMS *m/z* 463.2148 [Calcd for $C_{28}H_{31}O_6$ $(M+H)^+$, 463.2121]; ^1H and ^{13}C NMR ($CDCl_3$) data, Tables 2 and 3.

3.3.4. Cohaerin F (4). $[\alpha]_D^{20}$ +1.2 (*c* 0.3, $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1}: 3415, 1711, 1678, 1615, 1548, 1456, 1379, 1167, 1070, 782; UV λ_{max} nm (log ε): 354 (3.0), 232 (2.9); CD (MeOH) λ_{ext} nm ($\delta\varepsilon$): 370 (+1.0), 318 (+0.8), 254 (−0.7), 228 (+0.9); FABMS *m/z* 479 $(M+Na)^+$; HRFABMS *m/z* 479.2442 [Calcd for $C_{27}H_{36}O_6Na$ $(M+Na)^+$, 479.2410); ^1H and ^{13}C NMR ($CDCl_3$) data, Tables 2 and 3.

3.3.5. Bioassays. Inhibition of nitric oxide production of **1–4** in RAW 264.7 cells stimulated by lipopolysaccharide was evaluated by the same method as previously reported.[16]

Table 2. ^1H NMR data for cohaerins C–F (**1–4**)

Position	1	2	3	4
1	6.87 (s)	8.75 (s)	8.91 (d, 0.6)	6.83 (t, 1.4)
4	6.15 (s)	6.47 (s)	6.49 (s)	6.08 (s)
5	5.41 (d, 1.1)	5.39 (s)	5.42 (d, 1.1)	5.45 (d, 1.1)
8	3.85 (dt, 1.9, 7.4)			3.32 (td, 1.9, 9.9)
9	1.40 (s)	1.70 (s)	1.75 (s)	1.13 (s)
12	2.80 (m), 2.62 (m)	2.89 (dd, 4.1, 18.4), 2.61 (dd, 5.8, 18.4)	6.84 (d, 7.4)	2.62 (overlapped), 2.80 (overlapped)
13	4.39 (m)	4.29 (m)	7.24 (t, 8.0)	4.38 (m)
14	2.80 (m), 2.62 (m)	2.77 (dd, 3.6, 16.2), 2.56 (dd, 7.4, 16.2)	6.84 (d, 8.0)	2.80 (overlapped), 2.60 (overlapped)
16	2.06 (s)	2.09 (s)	2.31 (s)	2.05 (s)
18	4.13 (d, 12.6)			3.22 (dd, 1.9, 17.6), 2.79 (m)
20	3.22 (m)	3.56 (q, 6.6)	3.62 (m)	2.67 (q, 6.9)
21	1.76 (m), 1.34 (m)	1.58 (m), 1.33 (m)	1.55 (m), 1.28 (m)	1.34 (m), 1.68 (m)
22	1.28 (m), 1.20 (m)	1.13 (m), 1.02 (m)	1.12 (m), 1.18 (m)	1.22 (m), 1.27 (m)
23	1.24 (m)	1.30 (m)	1.25 (m)	1.25 (m)
24	1.22 (m)	1.11 (m)	1.15 (m)	1.24 (m)
25	1.23 (m)	1.16 (m)	1.19 (m)	1.26 (m)
26	0.87 (t, 7.1)	0.82 (t, 7.1)	0.81 (t, 7.1)	0.87 (t, 7.1)
27	1.23 (d, 7.1)	1.12 (d, 6.6)	0.88 (d, 7.4)	1.15 (d, 6.9)

Table 3. ^{13}C NMR data for cohaerins C–F (**1**–**4**)

Position	**1**	**2**	**3**	**4**
1	143.8	155.0	153.8	145.2
3	153.3	155.8	154.5	153.0
4	111.8	114.4	113.1	111.8
5	107.1	106.3	105.5	105.3
6	192.0	192.9	190.9	199.3
7	82.5	89.1	87.7	73.0
8	43.5	166.5	165.4	40.2
9	19.1	26.0	26.4	21.4
10	130.6	130.9	118.5	130.8
11	160.7	164.5	155.7	160.3
12	40.8	41.7	113.9	40.9
13	65.3	66.1	131.8	65.4
14	46.1	46.7	122.8	46.2
15	194.0	196.5	139.0	193.9
16	23.0	23.0	20.2	23.0
17	169.0	169.6	167.9	
18	51.2	125.7	124.2	40.2
19	206.0	202.1	201.4	213.3
20	45.7	44.7	43.7	46.3
21	31.7	34.6	33.4	32.9
22	27.0	27.7	26.9	27.2
23	29.3	30.3	29.1	29.3
24	31.6	32.8	31.6	31.6
25	22.5	23.5	22.4	22.6
26	14.0	14.4	14.0	14.0
27	17.2	15.2	14.8	16.8
4a	144.9	146.5	144.6	146.9
8a	116.3	112.5	111.4	120.5

Antimicrobial activities were tested as reported concurrently[17] (in slight modification of the method reported in Ref. 15).

Acknowledgements

Financial support of JSPS (Japan Society for the Promotion of Science) for granting a postdoctoral fellowship to D. N. Quang No. P04162 is greatly acknowledged. Thanks are also due to Masami Tanaka and Yasuko Okamoto (TBU, Japan) for recording NMR and mass spectra, respectively. We greatly acknowledge Mr. N. Radulovic for his collection of fungal material. Our special thanks are due to Dr. Takashi Nishizawa and Dr. Gen-Ichiro Soma (TBU, Japan) for their help in examination of nitric oxide production.

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Tetrahedron 62 (2006) 6355–6360

Transition-metal-catalyzed carbonylation of allenes with carbon monoxide and thiols

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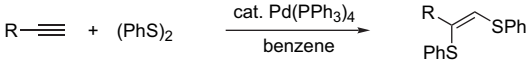
Received 21 January 2006; revised 11 April 2006; accepted 12 April 2006
Available online 12 May 2006

Abstract—In the presence of a catalytic amount of tetrakis(triphenylphosphine)platinum(0), allenes undergo carbonylative thiolation with carbon monoxide and thiols to provide the corresponding α,β- and β,γ-unsaturated thioesters in good yields. In contrast, the use of rhodium(I) catalysts such as $RhH(CO)(PPh_3)_3$ in place of $Pt(PPh_3)_4$ leads to copolymerization of allenes and carbon monoxide without incorporation of thio groups.

1. Introduction

The transition-metal-catalyzed carbonylation of carbon–carbon unsaturated compounds involving the simultaneous manipulation of heteroatom functions has received a great deal of attention, because synthetically useful heterofunctionalized carbonyl compounds can be easily obtained in one portion.[1] Along this line, carbonylative silylation,[2] amination,[3] and alkoxylation[4] of carbon–carbon unsaturated compounds have been studied intensively. In contrast, the corresponding carbonylative thiolation of unsaturated compounds has been largely unexplored, because organosulfur compounds are generally believed to be catalyst poisons.[5]

Recently, we have developed a series of transition-metal-catalyzed addition/carbonylative addition of organic disulfides and thiols to acetylenes in the absence/presence of carbon monoxide, as depicted in Eqs. 1–4. For example, $Pd(PPh_3)_4$ catalyzes bisthiolation and carbonylative thiolation of acetylenes with disulfides in the absence/presence of carbon monoxide with excellent stereo- and regioselectivities (Eqs. 1 and 2).[6]



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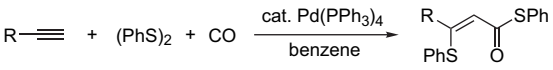
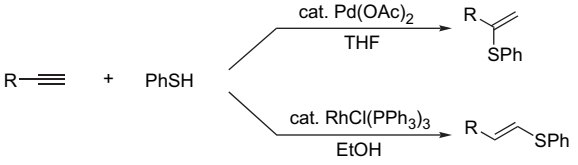


(2)

As to the addition of thiols to acetylenes, the regioselectivity of the addition can be controlled easily by the selection of the catalyst: the $Pd(OAc)_2$-catalyzed reaction of acetylenes with thiols provides the corresponding Markovnikov adducts,[7] whereas the use of Wilkinson's catalyst ($RhCl(PPh_3)_3$) leads to the formation of *anti*-Markovnikov adducts, regioselectively (Eq. 3).[8]



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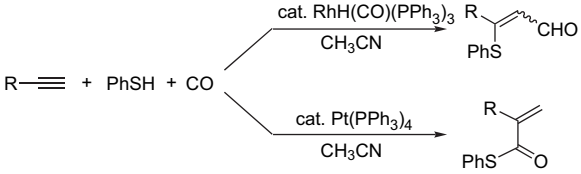

(4)

Furthermore, highly selective thioformylation[9] and hydrothiocarbonylation[10] of acetylenes with carbon monoxide and thiols are able to take place successfully in the presence of rhodium(I) and platinum(0) catalysts, respectively (Eq. 4).

Keywords: Carbonylative thiolation; Thiol; Allene; Carbon monoxide; Copolymerization.
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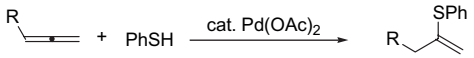
0040–4020/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2006.04.039

Compared to the transition-metal-catalyzed reactions with acetylenes, transition-metal-catalyzed addition of organosulfur compounds to carbon–carbon double bonds is little known.[11] We have found that the addition of thiols to allenes successfully proceeds regioselectively at the internal double bonds by the action of palladium acetate catalyst (Eq. 5).[12]



(5)

In the course of our studies on the transition-metal-catalyzed carbonylative thiolation, we have investigated the transition-metal-catalyzed carbonylation of allenes as activated carbon–carbon double bond compounds.[13–15]

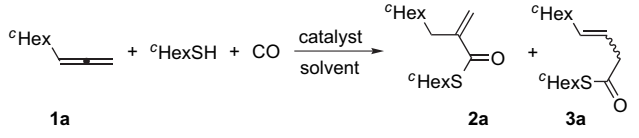
2. Results and discussion

2.1. Platinum-catalyzed carbonylative thiolation of allenes with thiols and carbon monoxide

We initiated the reaction of cyclohexanethiol (^{c}HexSH) with cyclohexylallene under the pressure of carbon monoxide by varying the transition-metal catalysts and solvents (Table 1).

$Pd(PPh_3)_4$ in benzene exhibited no catalytic activity towards the desired carbonylative thiolation, and a simple thiol addition product to the internal double bond of the allene (^{c}HexCH$_2$C(S^{c}Hex)=CH$_2$) was obtained as the major product in 40% yield (Entry 1). $Pd(OAc)_2$ in THF, $RhCl(PPh_3)_3$ in EtOH, $RhH(CO)(PPh_3)_3$ in CH_3CN, and $Co_2(CO)_8$ in CH_3CN also did not catalyze the carbonylative thiolation (Entries 2–5). On the other hand, platinum catalysts such as $PtCl_2(PPh_3)_2$ and $Pt(PPh_3)_4$ in CH_3CN play a good catalyst for the carbonylative thiolation of the allene. For example, the $PtCl_2(PPh_3)_2$-catalyzed carbonylative thiolation of cyclohexylallene with carbon monoxide and ^{c}HexSH proceeded regioselectively at the terminal double bond of the allene (Entry 7). Whereas, in the case of zerovalent platinum catalyst ($Pt(PPh_3)_4$), the thiolative carbonylation took place at the both terminal and central double bonds (Entry 6). Although $Pt(cod)Me_2$, which was expected to generate Pt(0) species in situ, could not catalyze this reaction, the addition of phosphine ligands gave **2a** and **3a**. This result suggests that Pt catalyst requires phosphine ligands (Entries 8 and 9).

Table 1. Transition-metal-catalyzed carbonylative thiolation



1a **2a** **3a**

Entry	Catalyst	Solvent	Yield[a] (%)		
			2a	**3a** [*E*/*Z*]	Total
1	$Pd(PPh_3)_4$	Benzene	0	0	0
2	$Pd(OAc)_2$	THF	0	0	0
3	$RhCl(PPh_3)_3$	EtOH	0	0	0
4	$RhH(CO)(PPh_3)_3$	CH_3CN	0	0	0
5	$Co_2(CO)_8$	CH_3CN	0	0	0
6	$Pt(PPh_3)_4$	CH_3CN	39	48 [80/20]	87
7	$PtCl_2(PPh_3)_2$	CH_3CN	Trace	35 [85/15]	35
8[b]	$Pt(cod)Me_2$	CH_3CN	0	0	0
9[c]	$Pt(cod)Me_2$	CH_3CN	7	17 [77/23]	24
10[d]	None	CH_3CN	0	0	0

Reaction conditions: allene (3.8 mmol), thiol (0.5 mmol), CO (3 MPa), catalyst (15 mol %), solvent (1 mL), 120 °C, and 4 h.
[a] Determined by ^{1}H NMR.
[b] Allene (2 mmol), thiol (0.5 mmol), and catalyst (3 mol %).
[c] Allene (1.2 mmol), thiol (1.0 mmol), catalyst (3 mol %), and PPh_3 (9 mol %).
[d] Allene (2 mmol) and thiol (1.0 mmol).

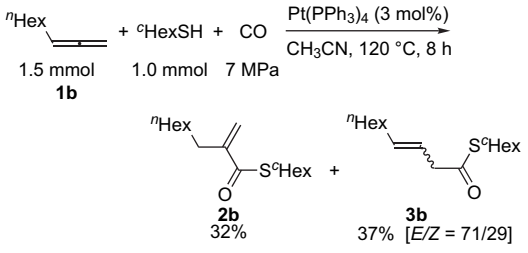
Table 2. Influence of solvents for platinum(0)-catalyzed carbonylative thiolation

Entry	Solvent	Yield[a] (%)		
		2a	**3a** [*E*/*Z*]	Total
1	THF	12	14 [72/28]	26
2	Benzene	10	17 [72/28]	27
3	EtOH	3	3 [24/76]	6
4[b]	CH_3CN	39	48 [80/20]	87

Reaction conditions: allene (2 mmol), thiol (0.5 mmol), CO (3 MPa), $Pt(PPh_3)_4$ (3 mol %), solvent (1 mL), 120 °C, and 4 h.
[a] Determined by ^{1}H NMR.
[b] Allene (3.8 mmol), thiol (0.5 mmol), and catalyst (15 mol %).

Table 2 summarizes the results of the platinum-catalyzed carbonylative thiolation of cyclohexylallene (**1a**) in various solvents. Ethereal solvent like THF, nonpolar solvent like benzene and protic solvent like EtOH were ineffective for the desired carbonylative thiolation (Entries 1–3). CH_3CN, which has both suitable polarity and coordinating property, worked as a good solvent (Entry 4).

The carbonylative thiolation of some other substituted allenes are shown in Eqs. 6–8. Monosubstituted allenes underwent the carbonylative thiolation to provide the corresponding α,β- and β,γ-unsaturated thioesters successfully (Eqs. 6 and 7). In the case of a cyclic allene, β,γ-unsaturated thioester (**3d**) was obtained preferentially (Eq. 8).



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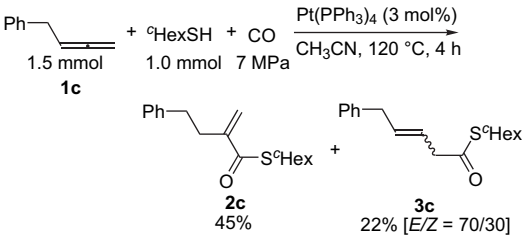

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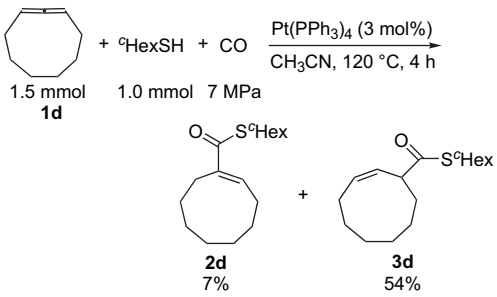
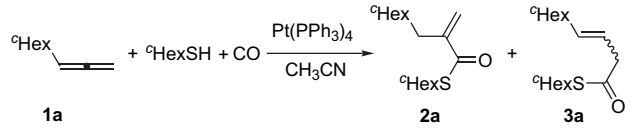


(8)

To get insight into the reaction pathway for this carbonylative thiolation, influence of the pressure of carbon monoxide on the product selectivity was studied. Interestingly, the ratios of **2a/3a** (i.e., the ratio of internal addition/terminal addition) were increased dramatically, with increase in the pressure of carbon monoxide (Table 3 and Fig. 1).

In the case of low pressure (1 MPa), β,γ-unsaturated thioester (**3a**) was obtained preferentially in 61% yield (Entry 1). Under 7 MPa of carbon monoxide, the yield of β,γ-unsaturated thioester (**3a**) was decreased to 22%, but the yield of α,β-unsaturated thioester (**2a**) was increased to 48% (Entry 4).

Table 3. Influence of carbon monoxide pressure for $Pt(PPh_3)_4$-catalyzed carbonylative thiolation



Entry	CO (MPa)	Yield[a] (%)		
		2a	**3a** [*E*/*Z*]	Total
1	1	30	61 [82/18]	91
2	3	39	48 [80/20]	87
3	5	44	33 [80/20]	77
4[b]	7	48	22 [80/20]	70
5[c]	7	50	24 [80/20]	74

Reaction conditions: allene (3.8 mmol), thiol (0.5 mmol), $Pt(PPh_3)_4$ (15 mol %), CH_3CN (1 mL), 120 °C, and 4 h.
[a] Determined by ^1H NMR.
[b] $Pt(PPh_3)_4$ (3 mol %) and allene (2.0 mmol).
[c] 8 h.

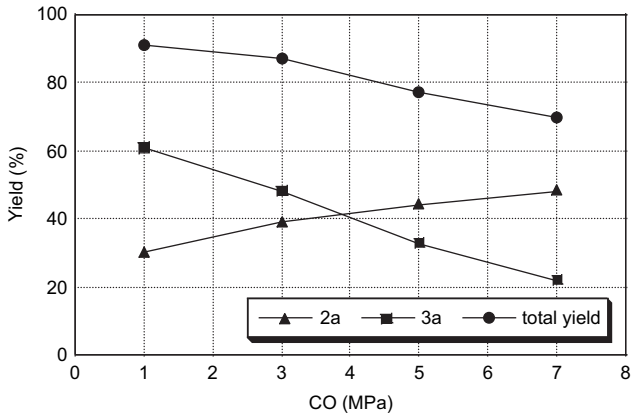
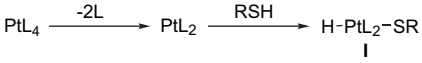
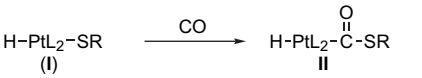


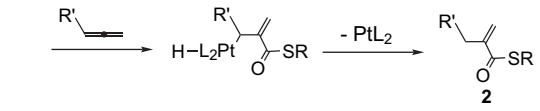
Figure 1. Influence of carbon monoxide pressure for $Pt(PPh_3)_4$-catalyzed carbonylative thiolation.

The high pressure of carbon monoxide resulted in the low total yield. Most probably, the coordination sites of platinum complex may be occupied by dissolved carbon monoxide to inhibit the following processes (Eqs. 9–11).

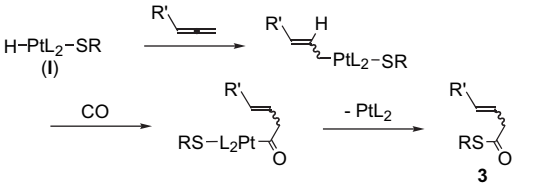


(9)





(10)



(11)

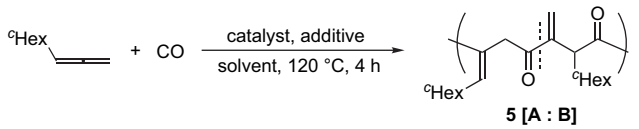
Eqs. 9–11 show possible pathways for this carbonylative thiolation: (1) oxidative addition of thiol (RSH) to $Pt(PPh_3)_4$ forms H–Pt–SR species (**I**) (Eq. 9); (2) coordination of carbon monoxide and subsequent insertion into the Pt–S bond takes place when the pressure of carbon monoxide is high; (3) coordination of allenes and subsequent acylplatination proceeds at the internal carbon–carbon double bond of the allene to give α,β-unsaturated thioester **2** after reductive elimination (Eq. 10). In the case of high pressure of CO, Pt undergoes coordination of CO to generate acylplatinum complex (**II**), the electron density of which is probably very low due to both the electron abstraction by the acyl groups and the back donation of CO ligands. Accordingly, acylplatination species (**II**) coordinate the relatively electron-rich double bonds (i.e., inner double bond of the allene). In contrast, when the pressure of carbon monoxide is low, coordination of allenes takes place prior to insertion of carbon monoxide. Probably, Pt still bears phosphine ligands, which act as electron-donating ligands and enhance the electron density on Pt. Thus, the hydroplatination takes place at the relatively electron-poor terminal carbon–carbon double bond of the allene, and then carbon monoxide insert into the Pt–C bond. Reductive elimination takes place sequentially to form β,γ-unsaturated thioester **3** (Eq. 11).[16,17]

2.2. Rhodium-catalyzed copolymerization of allenes and carbon monoxide

As mentioned in Table 1, $RhCl(PPh_3)_3$ in EtOH and $RhH(CO)(PPh_3)_3$ in CH_3CN did not exhibit catalytic activity toward the desired carbonylative thiolation (Table 1, Entries 3 and 4). Interestingly, however, the copolymerization product derived from cyclohexylallene and carbon monoxide was obtained as the major product (Table 4).

This polyketone is composed of the units **A** and **B** formed via the addition to the terminal double bond (**A**) and the internal double bond (**B**) of the allene, respectively.[18]

Table 4. Transition-metal-catalyzed copolymerization of allene with CO in the presence/absence of thiol or disulfide



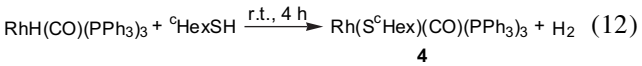
5 [A : B]

Entry	Catalyst	Additive (mmol)	Solvent	Yield[a] (%)	[A:B]
1	$RhHCO(PPh_3)_3$	—	CH_3CN	85	[99:1]
2	$RhHCO(PPh_3)_3$	^{c}HexSH (0.5)	CH_3CN	89	[97:3]
3	$RhHCO(PPh_3)_3$	$(PhS)_2$ (0.5)	CH_3CN	92	[82:18]
4	$RhCl(PPh_3)_3$	—	EtOH	63	[95:5]
5	$RhCl(PPh_3)_3$	^{c}HexSH (0.5)	EtOH	43	[100:0]

Reaction conditions: catalyst (2.5–15 mol %), solvent (1 mL), allene (2.0–3.8 mmol), thiol (0.5 mmol), and CO (3 MPa).
[a] Determined by ^{1}H NMR; based on allene employed.

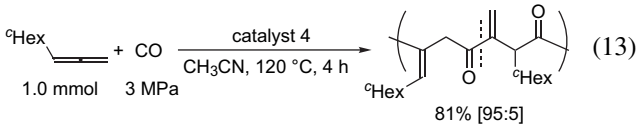
In the presence of cyclohexanethiol, the $RhH(CO)(PPh_3)_3$-catalyzed copolymerization took place in high yield with good selectivity of unit **A/B** (Entry 2). In the absence of thiols, the yield of **5** was slightly low, but the ratios of **A/B** were increased. The molecular weight of the polymer is as follows: M_n=22,000; M_w/M_n=2.2 (Entry 1).

To explore the effect of the cyclohexanethiol on this copolymerization, the reaction of the $RhH(CO)(PPh_3)_3$ with cyclohexanethiol was examined. The reaction of the $RhH(CO)(PPh_3)_3$ (0.075 mmol) with cyclohexanethiol (0.5 mmol) at room temperature in CH_3CN (1 mL) under nitrogen atmosphere afforded a yellow solid (**4**) with the evolution of hydrogen (Eq. 12).[9a]



(12)

The reaction of cyclohexylallene and carbon monoxide in the presence of complex **4** as a catalyst afforded the corresponding product **5** in good yield.



(13)

These results strongly suggest that rhodium thiolate complex exhibits a catalytic activity towards the copolymerization of allenes and carbon monoxide.

3. Conclusion

We have developed a highly selective carbonylation of allenes with carbon monoxide and thiols catalyzed by transition-metal catalysts. By the selection of the catalysts, carbonylative thiolation of allenes and copolymerization of allenes and carbon monoxide can take place selectively. These results mentioned in this paper suggest that novel combination of organic sulfur compounds and transition-metal catalysts is synthetically useful.

4. Experimental

4.1. General procedure for the $Pt(PPh_3)_4$-catalyzed carbonylative thiolation of allenes with carbon monoxide and thiols

In a 50 mL stainless steel autoclave with a magnetic stirring bar under nitrogen atmosphere were placed $Pt(PPh_3)_4$ (3–15 mol %), solvent (1 mL), allene (1.2–3.8 mmol), and thiol (0.5–1.0 mmol). Carbon monoxide was purged for three times and then charged at 3–7 MPa. The reaction was conducted with magnetic stirring for 4 h upon heating at 120 °C. After carbon monoxide was purged, the resulting mixture was filtered through Celite and concentrated in vacuo. Purification of the product was carried out by a preparative TLC eluted by hexane/ethyl acetate (0:10 for Eq. 6; 4:1 for Eq. 7; 1:9 for Eq. 8) and/or a recycling preparative HPLC (Japan Analytical Industry, Model LC-908), equipped with JAIGEL-1H and -2H columns (GPC) with $CHCl_3$ as eluent.

4.1.1. 2-(Cyclohexylthiocarbonyl)-3-cyclohexyl-1-propene (2a). A colorless liquid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.81–0.93 (m, 2H), 1.10–1.34 (m, 4H), 1.34–1.48 (m, 5H), 1.51–1.99 (m, 10H), 1.83–1.98 (m, 2H), 2.20 (d, J=6.6 Hz, 2H), 3.43–3.60 (m, 1H), 5.47 (s, 1H), 6.06 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.6, 26.0, 26.2, 26.4, 33.0, 36.6, 39.9, 42.2, 122.8, 147.3, 194.0; IR (NaCl) 2926, 2853, 1661, 1624, 1448, 1263, 980, 928 cm^{-1}; HRMS calcd for $C_{16}H_{26}OS$: 266.1704. Found 266.1705; Anal. Calcd for $C_{16}H_{26}OS$: C, 72.12; H, 9.84; S, 12.03. Found: C, 72.09; H, 9.74; S, 11.91.

4.1.2. 2-(Cyclohexylthiocarbonyl)-1-nonane (2b). A colorless liquid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80–0.93 (m, 3H), 1.20–1.34 (m, 11H), 1.34–1.52 (m, 3H), 1.51–1.80 (m, 4H), 1.83–2.01 (m, 2H), 2.30 (t, J=6.6 Hz, 2H), 3.40–3.60 (m, 1H), 5.49 (s, 1H), 6.02 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 19.1, 22.6, 25.6, 26.0, 28.2, 29.1, 29.2, 31.8, 33.1, 42.2, 121.5, 148.9, 193.9; IR (NaCl) 2928, 2855, 1661, 1625, 1448, 1263, 974, 930 cm^{-1}; HRMS calcd for $C_{16}H_{28}OS$: 268.1861. Found 268.1858; Anal. Calcd for $C_{16}H_{28}OS$: C, 71.58; H, 10.51; S, 11.94. Found: C, 71.32; H, 10.28; S, 11.29.

4.1.3. 2-(Cyclohexylthiocarbonyl)-4-phenyl-1-butene (2c). A colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21–1.34 (m, 1H), 1.37–1.53 (m, 4H), 1.53–1.66 (m, 1H), 1.66–1.80 (m, 2H), 1.85–2.02 (m, 2H), 2.58–2.68 (m, 2H), 2.72–2.81 (m, 2H), 3.47–3.64 (m, 1H), 5.47 (s, 1H), 6.06 (s, 1H), 7.14–7.23 (m, 3H), 7.24–7.32 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.6, 26.0, 33.1, 33.8, 34.7, 42.3, 122.4, 126.0, 128.3, 128.5, 141.2, 147.8, 193.4; IR (NaCl) 3026, 2930, 2853, 1659, 1626, 1497, 1448, 1263, 999, 972, 930 cm^{-1}; Anal. Calcd for $C_{17}H_{22}OS$: C, 74.40; H, 8.08; S, 11.68. Found: C, 74.21; H, 8.24; S, 11.85.

4.1.4. Cyclohexyl 4-cyclohexyl-3-butenethioate (3a). Isolated as a mixture of (*E*-) and (*Z*-) isomers (73/27); a colorless liquid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97–1.20 (m, 3H), 1.20–1.36 (m, 8H), 1.36–1.51 (m, 6H), 1.52–1.80 (m, 2H), 1.83–2.04 (m, 2H), 3.17 (d, J=6.3 Hz, 1.64H, *E*-isomer), 3.27 (d, J=6.3 Hz, 0.41H, *Z*-isomer), 3.41–3.56 (m, 1H), 5.36–5.58 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$)

δ 25.4, 25.7, 25.8, 26.0, 32.6, 32.8, 32.9, 36.5, 40.5, 42.1, 47.8, 118.7, 119.0, 140.5, 142.0, 198.5; IR (NaCl) 2926, 2851, 1682, 1448, 1263, 968, 887 cm^{-1}; HRMS calcd for $C_{16}H_{26}OS$: 266.1704. Found 266.1705; Anal. Calcd for $C_{16}H_{26}OS$: C, 72.12; H, 9.84; S, 12.03. Found: C, 71.85; H, 9.47; S, 11.94.

4.1.5. Cyclohexyl 3-decenethioate (3b). Isolated as a mixture of (*E*-) and (*Z*-) isomers (73/27); a colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84–0.92 (m, 3H), 1.18–1.33 (m, 8H), 1.33–1.51 (m, 6H), 1.52–1.63 (m, 2H), 1.63–1.77 (m, 2H), 1.79–1.98 (m, 2H), 1.98–2.11 (m, 2H), 3.18 (d, *J*=6.3 Hz, 1.46H, *E*-isomer), 3.26 (d, *J*=6.3 Hz, 0.54H, *Z*-isomer), 3.43–3.56 (m, 1H), 5.42–5.69 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.6, 25.5, 25.9, 28.7, 29.0, 31.7, 32.5, 33.0, 42.2, 42.7, 47.8, 120.5, 121.3, 134.7, 136.3, 198.1; IR (NaCl) 2928, 2855, 1690, 1448, 1263, 997, 966, 887 cm^{-1}; HRMS calcd for $C_{16}H_{28}OS$: 268.1861. Found 268.1855; Anal. Calcd for $C_{16}H_{28}OS$: C, 71.58; H, 10.51; S, 11.94. Found: C, 71.51; H, 10.30; S, 11.67.

4.1.6. Cyclohexyl 5-phenyl-3-pentenethioate (3c). Isolated as a mixture of (*E*-) and (*Z*-) isomers. It is difficult to determine the ratio of *E*/*Z* based on ^{1}H NMR. The ratio of *E*/*Z* is determined tentatively by^{13}C NMR as 70/30; a colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20–1.33 (m, 1H), 1.33–1.51 (m, 4H), 1.51–1.63 (m, 1H), 1.63–1.80 (m, 2H), 1.85–2.0 (m, 2H), 3.23 (d, *J*=6.8 Hz, 2H), 3.39 (d, *J*=6.3 Hz, 1.4H, *E*-isomer), 3.44 (d, *J*=7.3 Hz, 0.6H, *Z*-isomer), 3.46–3.57 (m, 1H), 5.56–5.68 (m, 1H), 5.68–5.85 (m, 2H), 7.11–7.24 (m, 3H), 7.24–7.35 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.5, 26.0, 33.0, 33.7, 38.9, 42.3, 42.7, 47.6, 121.6, 123.0, 126.1, 128.4, 128.5, 128.6, 132.6, 134.3, 140.0, 197.7; IR (NaCl) 3026, 2930, 2853, 1688, 1494, 1450, 1263, 997, 970 cm^{-1}; HRMS calcd for $C_{17}H_{22}OS$: 274.1391. Found 274.1395.

4.1.7. Cyclohexyl 1-cyclononene-1-carbothiolate (2d) and cyclohexyl 2-cyclononene-1-carbothiolate (3d). Isolated as a mixture of α,β- and β,γ-isomers. The ratio of **2d/3d** isomer was determined by ^{1}H NMR as 10/90; a colorless liquid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.06–1.79 (m, 18H), 1.79–2.05 (m, 2H), 2.05–2.53 (m, 2H), 3.42–3.57 (m, 1H), 3.63 (ddd, *J*=3.3, 10.2 Hz, 0.9H, **3d**), 5.52 (t, *J*=10.2 Hz, 0.9H, **3d**), 5.72 (ddd, *J*=2.4, 7.5, 8.1 Hz, 0.9H, **3d**), 6.84 (t, *J*=8.7 Hz, 0.1H, **2d**); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.0, 25.1, 25.3, 25.3, 25.6, 25.7, 25.9, 26.1, 26.3, 26.6, 30.4, 30.6, 33.1, 33.3, 37.7, 38.0, 42.1, 52.2, 127.6 (**3d**), 131.9 (**3d**), 140.5 (**2d**), 154.5 (**2d**), 193.8 (**2d**), 201.6 (**3d**); IR (NaCl) 3014, 2927, 2852, 1693, 1682, 1651, 1446, 1346, 1263, 999 cm^{-1}; HRMS calcd for $C_{16}H_{26}OS$: 266.1704. Found 266.1705; Anal. Calcd for $C_{16}H_{26}OS$: C, 72.12; H, 9.84; S, 12.03. Found: C, 72.11; H, 9.56; S, 11.82.

4.2. General procedure for the rhodium-catalyzed copolymerization of allenes and carbon monoxide

In a 50 mL stainless steel autoclave with a magnetic stirring bar under nitrogen atmosphere were placed Rh catalyst (3–15 mol %), solvent (1 mL), allene (2.0–3.8 mmol), and thiol or disulfide (0.5 mmol). Carbon monoxide was purged for three times and then charged at 3 MPa. The reaction was conducted with magnetic stirring for 4 h upon heating at 120 °C. After carbon monoxide was purged, the resulting mixture was filtered through Celite and concentrated in vacuo. Purification of the product was carried out by reprecipitation ($CHCl_3$/MeOH). The polymers were characterized by NMR spectroscopy, IR, and GPC measurements (polystyrene standards, eluent: THF).

4.2.1. Polyketone 5. A pale yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95–1.20 (m, 3H), 1.20–1.41 (m, 2H), 1.45–1.85 (m, 6H), 2.11–2.34 (m, 1H), 3.67 (s, 2H), 5.45 (d, *J*=6.0 Hz, unit B =CH_2), 6.64 (d, *J*=9.8 Hz, unit A =CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.2, 31.9, 34.6, 38.2, 134.0, 149.9, 198.9. Other signals of the minor structural unit (unit B) are not assigned unambiguously. IR (NaCl) 2924, 2851, 1738, 1668, 1448, 1373, 1317, 1240, 1124, 1028, 972, 901, 842 cm^{-1}.

Acknowledgements

This work was supported in part by The Sumitomo Foundation.

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16. In this reaction, reductive elimination selectively takes place after insertion of CO. Indeed, the formation of allylic sulfides (derived from allylic platinum species by the reductive elimination) was not detected at all.
17. This platinum-catalyzed carbonylative thiolation involves two key platinum species, i.e., H–Pt–SR (I) and H–Pt–C(O)SR (II). Allenes may insert preferentially into Pt–X bonds (X=H, SR, and C(O)SR) by the order of Pt–C(O)SR>H–Pt>Pt–SR, although the real reason to explain this tendency should wait for further detailed mechanistic study. On the other hand, the regioselectivity of the catalysis suggests terminal attachment of the bulkier platinum moiety at the insertion stage. Also, the stability between allylic platinum species (formed by terminal attack) and vinylic platinum species (formed by inner attack) may contribute to the reaction course. Cf., (a) Sugoh, K.; Kuniyasu, H.; Sugae, T.; Ohtaka, A.; Takai, Y.; Tanaka, A.; Machino, C.; Kambe, N.; Kurosawa, H. *J. Am. Chem. Soc.* **2001**, *123*, 5108; (b) Hirai, T.; Kuniyasu, H.; Kambe, N. *Chem. Lett.* **2004**, *33*, 1148; (c) Hirai, T.; Kuniyasu, H.; Kambe, N. *Tetrahedron Lett.* **2005**, *46*, 117.
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Tetrahedron 62 (2006) 6361–6369
Tetrahedron

Acid-catalyzed rearrangement of 1-benzyl-2-methyl-3-piperidone to 1-benzyl-2-acetylpyrrolidine

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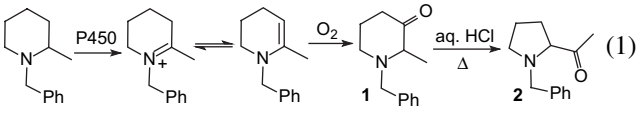
Received 15 January 2006; revised 8 April 2006; accepted 11 April 2006
Available online 19 May 2006

Abstract—We report that 1-benzyl-2-methyl-3-piperidone, conveniently prepared from 3-hydroxy-2-methylpyridine, undergoes rearrangement to 1-benzyl-2-acetylpyrrolidine in aqueous 6 N HCl at reflux. Studies showing that the 2,2-dimethyl analog is inert under the same conditions support a mechanism of reversible tautomeric equilibria via ring-opened intermediates, one of which was independently synthesized and shown to be a kinetically competent intermediate to product.

1. Introduction

Piperidine and pyrrolidine rings are ubiquitous structural features of many alkaloid natural products[1] and drug candidates,[2] and their derivatives are important intermediates in organic synthesis. We have been interested in the chemistry of reactive intermediates generated in the oxidative metabolism of the *N*-alkyl tertiary amine ring systems. In particular, we have been interested in the oxygenation of endocyclic enamines in equilibrium with the initial endocyclic iminium forms that result from cytochrome P450-mediated metabolism (e.g., Eq. 1). During an investigation into the chemistry of *N*-benzyl-3-piperidones,[3,4] resulting from autoxidation of the corresponding *N*-benzyl-Δ^2-tetrahydropyridines, we found that the 2-methyl compound **1** rearranges in hot aqueous HCl to 2-acetylpyrrolidine **2**.



(1)

A number of nonoxidative[5] and oxidative[6] examples of rearrangements of α-amino ketones have been observed, but the exact type of rearrangement we observed has not, to our knowledge, been reported before. A possibly related rearrangement was observed more than 50 years ago by Leonard and co-workers in Clemmensen reduction of cyclic amine 3-ones, exemplified in Eq. 2. The rearrangement proceeded smoothly for 2-unsubstituted, mono 2-alkyl, and 2,2-dialkyl reactants **3a**–**d**.[7] The mechanism of Clemmensen reduction had only been postulated at that time, and Leonard rationalized the rearrangement he observed in terms of carbonyl O-protonation followed by 1,2-shift of nitrogen to the adjacent carbonyl carbon, with concomitant[8] or subsequent[9] delivery of a zinc-derived hydride equivalent to the migration origin (Eq. 3). Such mechanism would not accommodate the *nonreductive* rearrangement we are now reporting.

Although there may be no relationship between the rearrangements observed by Leonard and by us, two conceivable mechanisms that could be considered for both are shown in Eqs. 4 and 5, assuming that under Clemmensen conditions, these 3-piperidones underwent rearrangement *prior to zinc-mediated reduction*. The mechanism in Eq. 4 would explain our observed conversion of **1** to **2**, as well as Leonard's observations for **3a** and **3b**, whereas for the 2,2-dialkyl reactants **3c** and **3d**, reduction would have to occur at the level of intermediate **6**. Another possible mechanism (Eq.5) would involve two consecutive 1,2-alkyl shifts, based on the aza equivalent of the α-ketol rearrangement,[10] namely the interconversion between α-hydroxy imines and α-amino ketones,[11] which has been observed (α-amino aldehyde) to be catalyzed by Lewis acids.[12] Again, the mechanism in Eq. 5 would explain our observed conversion of **1** to **2** and Leonard's observations for **3a** and **3b**, whereas in the 2,2-dialkyl reactants **3c** and **3d** an additional rapid 1,2-alkyl shift would be needed, with reduction occurring only after generation of an intermediate **6**.

Keywords: Rearrangement; Piperidone; Pyrrolidone; Clemmensen reduction.
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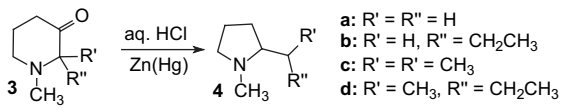
doi:10.1016/j.tet.2006.04.038



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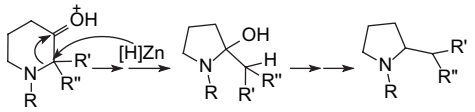

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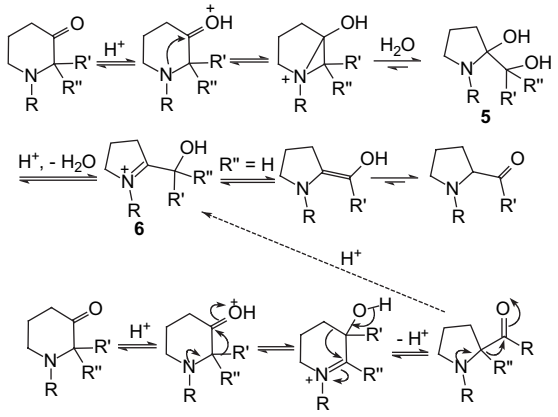


(4)

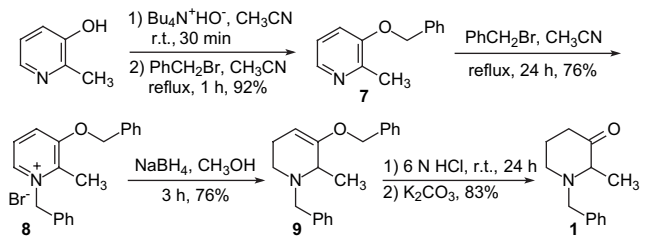
(5)

The purpose of this study was to clarify the mechanism of the nonreductive rearrangement responsible for conversion of **1** to **2**. Based on these results, we further propose a mechanism for the rearrangement observed under Clemmensen conditions that is consistent with recent views on the chemistry of this reduction process.

2. Results and discussions

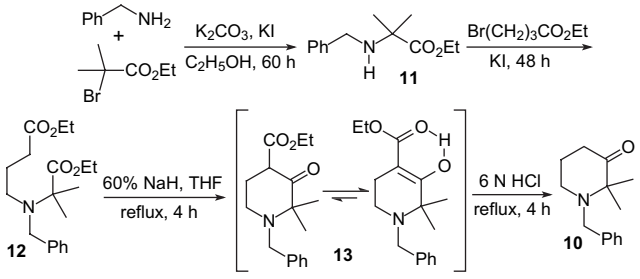
2.1. Preparation of 1-benzyl-3-piperidones 1 and 10

The required 2-methyl-3-piperidone **1** was prepared from commercially available 3-hydroxy-2-methylpyridine in four steps (Scheme 1). The key *N*,*O*-dibenzyl-protected intermediate **8**[13] was most conveniently obtained by the two-step benzylation sequence as shown. Yield of **8** obtained without isolation of **7** was inferior. Reduction ($NaBH_4$) of **8** and *O*-deprotection of the resulting **9**[14] by treatment with 6 N HCl at room temperature gave **1**[15] in 83% yield. This scheme might be adapted to offer a useful synthesis of other 3-piperidone analogs.



Scheme 1.

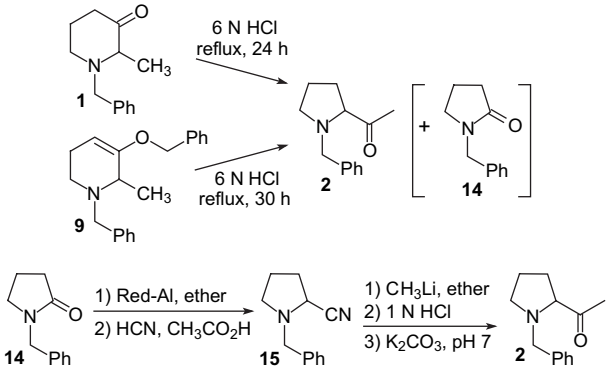
Synthesis of the 2,2-dimethyl-3-piperidone **10** was achieved according to the route shown in Scheme 2.[16] Heating benzylamine with ethyl 2-bromoisobutyrate in ethanol in the presence of a catalytic amount of KI gave **11** in 52% yield. Conversion of secondary amine **11** to tertiary amine **12** with ethyl 4-bromobutyrate under the same conditions was more sluggish, even in refluxing *n*-butanol, but could be achieved by heating in solvent-free conditions. Dieckmann condensation of diester **12** with sodium ethoxide in THF gave keto ester **13** (as the enol) in a low yield, but a more satisfactory result (95% yield) was obtained using 60% sodium hydride. Hydrolytic decarboxylation (6 N HCl) afforded **10** in 89% yield.



Scheme 2.

2.2. Rearrangement of 1-benzyl-2-methyl-3-piperidone (1) to 1-benzyl-2-acetylpyrrolidine (2)

Heating **1** in 6 N aqueous HCl at reflux for 24 h under argon resulted in its rearrangement in 90% yield (remaining is recovered **1**) to 2-acetylpyrrolidine **2** (Scheme 3), accompanied by a trace amount of 1-benzyl-2-pyrrolidine **14**, representing oxidation of rearrangement product **2**.[17] Compound **2** was also obtained directly from **9** by heating the latter in 6 N HCl at reflux for 30 h. The identity of **2** was verified by comparison with an authentic sample prepared from **14** by reduction of the latter with Red-Al followed by treatment with aqueous HCN solution, and reaction of the resulting nitrile **15**[18] with methyllithium in benzene, followed by hydrolytic workup.



Scheme 3.

Various reaction conditions were tested in order to evaluate the ease of rearrangement of **1**. Compound **1** was generated from **9** by exposure to 6 N HCl at *room temperature* for 24 h, and we verified that **1** is stable under these conditions. Heating **1** at reflux in 6 N HCl for periods of time shorter

than 24 h resulted in a lower yield of conversion to **2** (e.g., 55% in 6 h). The importance of acid concentration in this rearrangement was easily recognized by the finding that heating **1** at reflux in 0.5 N HCl for 6 h gave **2** in only 38% yield.

2.3. Attempted rearrangement of 1-benzyl-2,2-dimethyl-3-piperidone (10)

The mechanism considered in Eq. 5 would predict that 2,2-dimethyl substitution would still allow rearrangement to a 2-acylpyrrolidine. According to Eq. 4, whereas 2,2-dimethyl substitution would prevent rearrangement to a 2-acylpyrrolidine, the reaction could still proceed to the stage of intermediates **5/6**. Thus, according to these mechanisms, one might predict that 1-benzyl-2,2-dimethyl-3-piperidone (**10**) would undergo, in refluxing aqueous 6 N HCl, conversion to either 2-acetyl-2-methylpyrrolidine or to a product derived from **5/6**, possibly the ring-opened amino ketone. However, heating **10** in 6 N HCl at reflux for 24 h resulted in total recovery of starting material. The inertness of **10** is inconsistent with Eq. 4 or 5 as the mechanism of the nonreductive rearrangement, and suggests consideration of a mechanism relying on an initial enolization step. At the same time, we found that the unsubstituted parent compound 1-benzyl-3-piperidone also does not undergo the rearrangement.

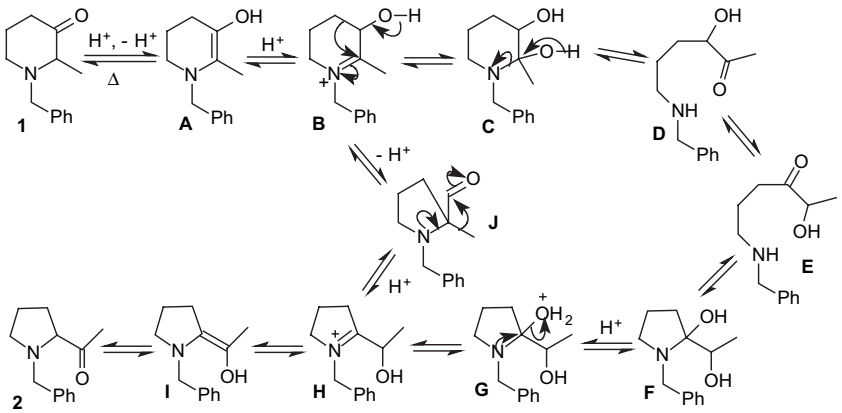
2.4. Mechanism of rearrangement

Two plausible mechanisms that would explain the rearrangement of **1** to **2** but the inertness of **10** are outlined in Scheme 4. In acid, the 3-piperidone would exist in equilibrium with enol–enamine **A** and iminium form **B**. The latter would further be in equilibrium with the hydrate **C** and ring-opened ketone **D**, with the overall conversion of **1** to **D** corresponding to the reverse of the well-known Amadori rearrangement that occurs following condensation of reducing sugars with amines.[19] If ketol **D** could be interchanged in acid with ketol **E**,[20] then the latter would be in equilibrium with **2** through the reverse sequence of analogous intermediates containing a five- rather than a six-membered ring. This would suggest that the conversion of **1** to **2** merely represents an equilibrium process and the thermodynamic predominance of **2**. Precedent for these types of proposed keto–enol tautomerizations has been reported.[21] Why the unsubstituted parent 1-benzyl-3-piperidone would not undergo rearrangement according to this mechanism may reflect the insufficient enol/iminium stability of the intermediates, or the instability of the aldehyde equivalents of **D** and **2**.

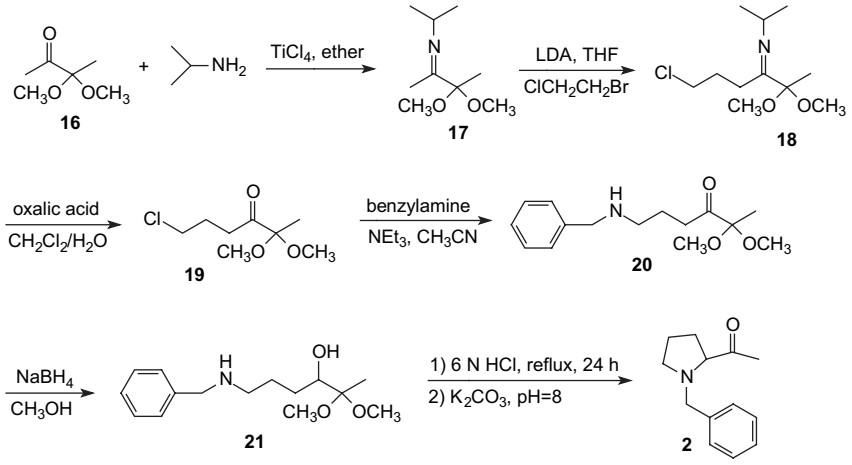
Another possible pathway, based on the aza equivalent of the α-ketol rearrangement,[11,12] is that α-hydroxy iminium intermediate **B** could undergo two subsequent 1,2-alkyl shifts, first a ring contraction to give **J** and then a methyl shift to give **H**. Although this mechanism would involve a lesser number of steps, we believe that the sequence of tautomerizations through **D** and **E** provides a more likely overall low energy pathway.

To determine whether the sequence of tautomerizations through **D** and **E** provides at least a kinetically competent mechanism for the rearrangement of **1** to **2**, it would be important to show that one of the two proposed ring-opened intermediates could convert to **2** at least as rapidly as **1** converts to **2** under the same reaction conditions. We chose to prepare intermediate **D**, which could be obtained by deprotection of the dimethyl ketal **21**, independently synthesized as shown in Scheme 5. On the basis of a previous report,[22] γ-chloroketone **19** was selected as the key synthetic intermediate, and was obtained by alkylation with 1-bromo-2-chloroethane of the lithium derivative formed from deprotonation of the *N*-isopropylimine of butane-2,3-dione monoketal **16**. Without isolation of the intermediate **18** following workup, selective hydrolysis of the imino function in the presence of the ketal function was conveniently achieved using aqueous oxalic acid. Alkylation of benzylamine with **19**, and $NaBH_4$ reduction of the resulting amino ketone **20** without its isolation, afforded **21**. An attempt to prepare **21** by first reducing **19** and then reacting with benzylamine was abandoned due to sluggish and low yield reactions. Heating **21** in 6 N HCl at reflux was indeed found to give **2**, presumably through initial hydrolysis to intermediate **D** and rearrangement of the latter.

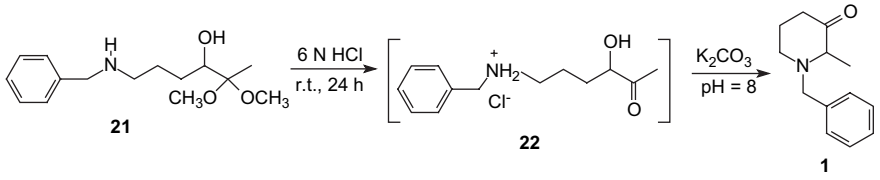
On the other hand, deprotection of **21** by treatment with 6 N HCl for 24 h at *room temperature* followed by neutralization gave **1** (Scheme 6). Assuming Scheme 4 is operative, this result suggests that the interconversion between intermediate **D** (six-membered ring manifold) and intermediate **E** (five-membered ring manifold) is mainly rate-limiting in the rearrangement of **1** that requires higher temperature. At the same time, this result also suggests the possibility that in acid, **21** first undergoes conversion to **1**, which at



Scheme 4.



Scheme 5.



Scheme 6.

elevated temperature then proceeds to **2** along a route that is distinct from that shown in Scheme 4. However, evidence that **D** is a kinetically competent intermediate on the lowest energy pathway from **1** to **2**, was provided by following the time course of conversion of both **1** and **21** to **2** under the conditions of refluxing 6 N HCl. As shown in Figure 1, the fraction of **2** (remainder is **1**), as determined by neutralization and extraction, was significantly greater at early time (1–3 h) when starting with **21** than with **1**. After 24 h, however, the amount of **2** (90%) was the same, and this appears to be close to the equilibrium distribution between **1** and **2** under the conditions of refluxing 6 N HCl.

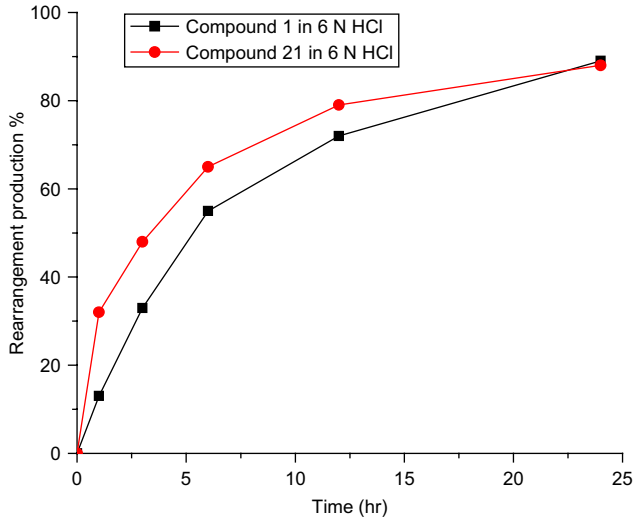


Figure 1. The relative amount of rearrangement product **2** formed from 3-piperidone **1** or compound **21** in 6 N HCl with heating at reflux for different reaction times, followed by neutralization and product analysis by ^{1}H NMR spectroscopy.

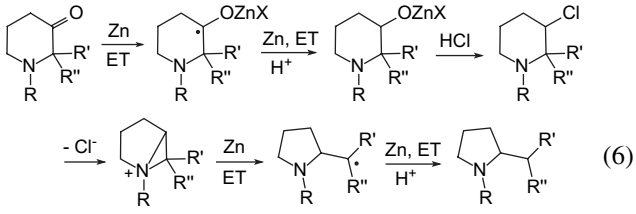
Additional information on the mechanism was provided by following the fate of **21** in 6 N deuterium chloride–deuterium oxide in an NMR tube. At room temperature, deprotection occurred immediately to reveal signals expected for the protonated form of **22**. Signals at δ 2.1 (CH_3) and δ 4.4 (CH) gradually disappeared over the course of 24 h due to deuterium exchange, but otherwise no other changes were observed. Although rapid neutralization and extraction at this point affords **1**, this result suggests that in the strongly acidic medium at room temperature, ring-opened amino ketone **D** does not interconvert with **E** or any of the ring-closed amino ketone forms.

In the same manner, the solution forms of both the six- and five-membered amino ketones were investigated, in these cases most readily by ^{13}C NMR spectroscopy in DCl–D_2O. 1-Benzyl-3-piperidone·HCl is known to exist as its hydrate in aqueous solution,[23] as verified in this study, where the free base form exhibited a carbonyl signal at 207.1 ppm, whereas for the DCl salt in D_2O, this signal was replaced by one at 91.6 ppm assigned to the carbon bearing two OH groups. The ^{13}C NMR spectrum of the 2-methyl analog **1** shows that it also exists mainly as the hydrate in 6 N DCl–D_2O, as apparent from the lack of a carbonyl signal and presence of a geminal dihydroxy ^{13}C signal at 93.8 ppm. The 2,2-dimethyl analog **10** in 6 N DCl–D_2O exists as a mixture of ketone and hydrate forms (^{13}C NMR signals are seen at both 207.6 and 95.3 ppm). On the other hand, 1-benzyl-2-acetylpyrrolidine **2** exists as such in 6 N DCl–D_2O (^{13}C NMR carbonyl signal at 205.0 ppm). The tendency of the 3-piperidones but not **2** to form the hydrate likely reflects

the well-known tendency of six-membered rings to become entirely sp^3 tetrahedral to minimize torsional strain,[24,25] with the lack of hydration of **2** indicating that there is no intrinsic electronic preference for protonated α-amino ketones to exist as their hydrates.

As expected from these results, when the NMR tube containing **21** in 6 N DCl–D_2O was heated at 95 °C, the ^{13}C NMR spectrum after 5 h revealed a mixture of **1** (as its hydrate) and **2**, whereas continued heating resulted in mainly **2**, as is seen when starting with **1**. The main importance of these experiments is the demonstration that equilibration between the ring-opened and ring-closed forms of both the six- and five-membered ring manifolds shown in Scheme 4 occurs only at elevated temperature. Thus, although the **D**⇌**E** interconversion has been ascribed to be the main rate-limiting step in the rearrangement, other steps in the overall mechanism may additionally be partially rate-limiting.

Over the years, although the mechanism of Clemmensen reduction has still not been totally elucidated, the most recent studies on this subject suggest a stepwise electron/proton transfer mechanism via radical and probably alkyl chloride intermediates,[26] with the additional possible intermediacy of an organozinc species. It is known that the corresponding carbinols are not intermediates, though dimeric pinacol side-products are sometimes observed, suggestive of coupling of a α-oxycarbon radical intermediate. A possible mechanism that follows this line of reasoning and that would explain the ring-contractions for **3a**–**d** and the racemization of **3d** observed by Leonard and co-workers[9] is given in Eq. 6. Since the first of the four electron reduction steps would, according to this mechanism, occur prior to any rearrangement step, this would then clearly be a distinct process from the *nonreductive* rearrangement we proposed in Scheme 4.



(6)

3. Conclusions

In summary, we have developed an efficient route to 1-alkyl-2-methyl-3-piperidones and demonstrated that the 1-benzyl compound undergoes rearrangement to 1-benzyl-2-acetylpyrrolidine in refluxing 6 N HCl. The reaction reaches an equilibrium state at ~90% conversion, with the driving force apparently being the thermodynamic preference of a five-membered ring over a sp^2 carbon-containing six-membered ring because of torsional strain in the latter.[24,25] A mechanism has been proposed for the rearrangement involving a series of acid-catalyzed tautomerization and hydration/dehydration steps that is distinct from a seemingly similar rearrangement known to accompany Clemmensen reduction of the same starting materials.

4. Experimental

4.1. General

All melting points are uncorrected. ^1H NMR spectra were obtained with Varian Gemini 200 MHz (^{13}C NMR at 50 MHz) or 300 MHz (^{13}C NMR at 75 MHz) instruments. In all cases, tetramethylsilane (TMS) or the solvent peak served as internal standard for reporting chemical shifts, which are expressed as parts per million downfield from TMS (δ scale). In the ^{13}C NMR line listings, attached proton test designations are given as (+) or (−) following the chemical shifts. High-resolution mass spectra (HRMS) using electron impact (EI) or fast atom bombardment (FAB), were obtained at 20–40 eV on a Kratos MS-25A instrument. TLC was performed on glass plates precoated with silica gel $60GF_{254}$. Compounds on the developed plate were visualized by short-wavelength UV light (λ=254 nm) or by spraying with either ninhydrin or 2,4-dinitrophenylhydrazine solutions. All evaporations were conducted at reduced pressure using a rotary evaporator. All new compounds were determined to be >95% pure by ^1H NMR spectroscopy.

4.2. 3-Benzyloxy-2-methylpyridine (7)

A mixture of 2-methyl-3-pyridinol (2.0 g, 0.018 mol) and tetra-*n*-butylammonium hydroxide (40% solution in water, 11.68 g, 0.018 mol) in 50 mL of CH_3CN was stirred at room temperature for 30 min and then evaporated to dryness, and the resulting ammonium salt was dried in vacuo. Benzyl bromide (2.14 mL, 0.018 mol) was added to a solution of the ammonium salt in 100 mL of CH_3CN, and the mixture was heated at reflux for 1 h. The reaction mixture was cooled and filtered, and the filtrate was evaporated to afford a yellowish oil. The crude product was purified by silica gel flash chromatography using EtOAc–CH_2Cl_2 (1:4) to give **7** as a pale yellowish oil (3.36 g, 92%): ^1H NMR ($CDCl_3$) δ 2.54 (s, 3H), 5.06 (s, 2H), 7.08 (m, 2H), 7.32–7.43 (5H), 8.09 (d, 1H, *J*=4.6 Hz); ^{13}C NMR ($CDCl_3$) δ 19.6 (−), 64.9 (+), 117.8 (−), 121.7 (−), 127.2 (−), 128.1 (−), 128.7 (−), 136.6 (+), 140.7 (−), 149.3 (+), 153.0 (+); EI HRMS *m/z* calcd for $C_{13}H_{13}NO$ $(M+H)^+$ 199.0998, found 199.0997.

4.3. 1-Benzyl-3-benzyloxy-2-methylpyridinium bromide (8)

Benzyl bromide (2.4 mL, 0.02 mol) was added to a solution of **7** (3.36 g, 0.017 mol) in 100 mL of CH_3CN. The reaction mixture was heated at reflux for 24 h under argon. The solvent was then evaporated, and the residue was washed with diethyl ether (3×10 mL) and then dried in vacuo to give pyridinium salt **8** (4.8 g, 76%): mp 186–188 °C; ^1H NMR ($CDCl_3$) δ 2.72 (s, 3H), 5.28 (s, 2H), 6.20 (s, 2H), 7.24–7.35 (10H), 7.88 (dd, 1H, *J*=6.2 and 8.6 Hz), 8.22 (d, 1H, *J*=8.6 Hz), 9.16 (d, 1H, *J*=6.2 Hz); ^{13}C NMR ($CDCl_3$, two carbons overlapped) δ 14.3 (−), 62.4 (+), 72.6 (+), 126.2 (−), 127.1 (−), 127.9 (−, 2C), 128.9 (−, 2C), 129.3 (−), 129.5 (−), 132.2 (+), 134.1 (+), 138.1 (−), 147.2 (−), 156.0 (+); FAB HRMS *m/z* calcd for $C_{20}H_{20}NO$ (M^+) 290.1546, found 290.1548.

4.4. 1-Benzyl-3-benzyloxy-2-methyl-1,2,5,6-tetrahydropyridine (9)

To a solution of **8** (3.0 g, 8.11 mmol) in 50 mL of methanol at 0 °C was added $NaBH_4$ (0.613 g, 16.2 mmol) over 15 min. The reaction mixture was stirred at room temperature for 3 h and then methanol was evaporated. To the solid residue were added 50 mL of ether and 25 mL of saturated aqueous K_2CO_3 solution. The reaction mixture was stirred vigorously at room temperature for 1 h and separated, and the aqueous layer was extracted with diethyl ether (3×20 mL). The combined ether extract was dried (Na_2SO_4) and evaporated to furnish **9** as a brownish oil (1.8 g, 76%) that was of satisfactory purity (NMR) to be used directly in the next step: ^{1}H NMR ($CDCl_3$) δ 1.36 (d, 3H, J=6.6 Hz), 2.03–2.09 (m, 1H), 2.25–2.35 (m, 1H), 2.19 (m, 1H), 3.24 (q, 1H, J=6.6 Hz), 3.67 (d, 1H, J=13.5 Hz), 3.87 (d, 1H, J=13.5 Hz), 4.73–4.83 (m, 3H), 7.14–7.46 (10H); ^{13}C NMR ($CDCl_3$) δ 16.2 (−), 22.4 (+), 44.2 (+), 55.4 (−), 58.0 (+), 68.6 (+), 92.3 (−), 126.9 (−), 127.4 (−), 127.7 (−), 128.2 (−), 128.5 (−), 128.9 (−), 137.7 (+), 139.5 (+), 156.5 (+); EI HRMS m/z calcd for $C_{20}H_{30}NO$ $(M+H)^+$ 293.1781, found 293.1781.

4.5. 1-Benzyl-2-methyl-3-piperidone (1)

A solution of **9** (1.0 g, 3.7 mmol) in 25 mL of 6 N HCl was stirred at room temperature for 24 h. The reaction mixture was extracted with ether. The aqueous phase was separated and neutralized with K_2CO_3 to pH 8. The solution was then extracted with diethyl ether (3×20 mL). The combined ether layer was dried (Na_2SO_4) and evaporated to give **1** as an oil (0.58 g, 83%): ^{1}H NMR ($CDCl_3$) δ 1.29 (d, 3H, J=6.8 Hz), 1.87–1.96 (2H), 2.30–2.38 (m, 1H), 2.40–2.57 (2H), 2.88–2.96 (m, 1H), 3.18 (q, 1H, J=6.8 Hz), 3.47 (d, 1H, J=13.5 Hz), 3.87 (d, 1H, J=13.5 Hz), 7.23–7.39 (5H); ^{13}C NMR ($CDCl_3$) δ 12.2 (−), 24.2 (+), 37.8 (+), 47.6 (+), 57.8 (+), 66.2 (−), 127.2 (−), 128.3 (−), 128.8 (−), 138.6 (+), 210.2 (+); EI HRMS m/z calcd for $C_{13}H_{17}NO$ $(M+H)^+$ 203.1311, found 203.1310.

4.6. 1-Benzyl-2-acetylpyrrolidine (2)

A solution of **1** (0.5 g, 2.5 mmol) in 25 mL of 6 N HCl was heated at reflux for 24 h under argon. The reaction mixture was extracted with diethyl ether. The aqueous phase was separated, neutralized with K_2CO_3 to pH 8, and extracted with diethyl ether (3×15 mL). The combined ether layer was dried (Na_2SO_4) and evaporated, and the residue was fractionated by flash silica gel chromatography with CH_2Cl_2–EtOAc (4:1) as eluent to yield **2** as an oil (0.39 g, 78%): ^{1}H NMR ($CDCl_3$) δ 1.76–1.88 (3H), 2.03–2.11 (m, 1H), 2.14 (s, 3H), 2.29 (dd, 1H, J=8.3 and 12.0 Hz), 3.04–3.13 (2H), 3.44 (d, 1H, J=13.1 Hz), 3.81 (d, 1H, J=13.1 Hz), 7.23–7.32 (5H); ^{13}C NMR ($CDCl_3$) δ 23.6 (+), 25.3 (−), 28.8 (+), 53.9 (+), 59.4 (+), 73.6 (−), 127.2 (−), 128.4 (−), 129.0 (−), 138.6 (+), 212.1 (+); EI HRMS m/z calcd for $C_{13}H_{17}NO$ $(M)^+$ 203.1311, found 203.1309. A trace of the known 1-benzyl-2-pyrrolidone (**14**) was also obtained in this experiment: ^{1}H NMR ($CDCl_3$) δ 1.96 (m, 2H), 2.42 (t, 2H), 3.24 (t, 2H), 4.43 (s, 2H), 7.27 (5H); ^{13}C NMR ($CDCl_3$) δ 17.8 (+), 31.0 (+), 46.6 (+), 46.7 (+), 127.6 (−), 128.2 (−), 128.7 (−), 136.6 (+), 175.0 (+).

4.7. 1-Benzyl-2-cyanopyrrolidine (15)[18]

To a solution of 1-benzyl-2-pyrrolidone **14** (ICN Pharmaceuticals, 15.0 g, 0.0856 mL) in 300 mL of dry ether was added 15 mL of Red-Al (3.4 M solution in toluene, 0.051 mol) at 0 °C. The reaction mixture was stirred at 0 °C under argon for 3 h. A cold solution of NaCN (12.0 g) in 8 mL of H_2O was slowly added to the reaction mixture, and the pH of the reaction mixture was adjusted to 5 with glacial acetic acid. The solution was stirred for 1 h at room temperature and then extracted with diethyl ether (3×50 mL). The combined ether extract was dried (Na_2SO_4) and evaporated to yield an oily crude product that was purified by silica gel flash chromatography using CH_2Cl_2–EtOAc (4:1) to give **15** as an oil (10.5 g, 66%): ^{1}H NMR ($CDCl_3$) δ 1.88–2.20 (4H), 2.59 (dd, 1H, J=8.2 and 10.0 Hz), 2.95 (m, 2H), 3.70 (d, 1H, J=13.1 Hz), 3.93 (d, 1H, J=13.1 Hz), 7.35 (5H); ^{13}C NMR ($CDCl_3$) δ 22.0 (+), 29.6 (+), 51.3 (+), 53.3 (−), 56.6 (+), 118.0 (+), 127.6 (−), 128.56 (−), 128.57 (−), 128.60 (−), 128.9 (−), 137.7 (+); EI HRMS m/z calcd for $C_{12}H_{14}N_2$ (M^+) 186.1158, found 186.1157.

4.8. 1-Benzyl-2-acetylpyrrolidine (2, alternate method)

To a solution of 1-benzyl-2-cyanopyrrolidine **15** (0.62 g, 3.33 mmol) in 30 mL of dry benzene at 0 °C was added 2.84 mL of methyllithium (1.4 M solution in ether, 3.98 mmol) under argon. The reaction mixture was allowed to warm to room temperature and was then heated at reflux for 2 h. The reaction solution was hydrolyzed with 1 N HCl, and then extracted with diethyl ether. The aqueous phase was separated and neutralized with aqueous K_2CO_3 solution to pH 7. The solution was then extracted with CH_2Cl_2. The combined organic extract was dried (Na_2SO_4) and evaporated, and the crude oily product was purified by silica gel column chromatography using CH_2Cl_2–EtOAc (4:1) to give **2** as an oil (0.473 g, 70%). The NMR data were identical with the data obtained for the rearrangement product.

4.9. Ethyl 2-benzylamino-2-methylpropionate (11)[27]

A solution of benzylamine (10.7 g, 0.1 mol), ethyl 2-bromoisobutyrate (12.5 mL, 0.085 mol), potassium carbonate (13.8 g, 0.1 mol), and potassium iodide (166 mg, 1 mmol) in 120 mL of ethanol was stirred at reflux for 60 h. After filtration of ethanol to remove suspended salts, the filtrate was evaporated under reduced pressure, and the residue was subjected to silica gel column chromatography using hexane–ethyl acetate (6:1) as eluent to give the known amino ester **11** (9.5 g, 43%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.31 (t, 3H), 1.38 (s, 6H), 3.63 (s, 2H), 4.18 (q, 2H), 7.33 (5H).

4.10. Ethyl 4-[*N*-benzyl-*N*-(1-ethoxycarbonyl-1-methylethyl)]aminobutyrate (12)

A mixture of compound **11** (4.4 g, 20 mmol), ethyl 4-bromobutyrate (3.3 mL, 22 mmol), and potassium iodide (166 mg, 1 mmol) was stirred at 120–130 °C for 48 h. The mixture was then subjected to silica gel column chromatography using hexane–ethyl acetate (8:1) as eluent to give the product **12** (3.41 g, 51%): ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H,

J=7.0 Hz), 1.29 (t, 3H, J=7.0 Hz), 1.35 (s, 6H), 1.53 (m, 2H), 2.17 (t, 2H, J=7.2 Hz), 2.62 (t, 2H, J=7.5 Hz), 3.82 (s, 2H), 4.03 (q, 2H, J=7.0 Hz), 4.14 (q, 2H, J=7.0 Hz), 7.35 (5H); ^{13}C NMR ($CDCl_3$) δ 14.3, 14.4, 25.2, 25.4, 31.8, 51.5, 55.8, 60.2, 60.5, 63.8, 126.4, 127.7, 128.1, 142.8, 173.7, 176.2; FAB HRMS m/z calcd for $C_{19}H_{30}NO_4$ $(M+H)^+$ 336.2175, found 336.2182.

4.11. 1-Benzyl-2,2-dimethyl-4-carbethoxy-3-piperidone (13)

Sodium hydride (240 mg, 6.6 mmol, 60%) was suspended in a solution of **12** (2.0 g, 6.0 mmol) in 50 mL of dry THF. The mixture was heated at reflux for 4 h. After evaporation of the solvent, the viscous product was treated with H_2O and the resulting mixture was acidified to pH 5, maintaining the temperature under 10 °C with an ice bath. The aqueous solution was neutralized with solid K_2CO_3 and extracted with Et_2O. Evaporation of the dried (Na_2SO_4) organic extract yielded **13** (1.27 g, 74%) as an oil that NMR showed to exist as the enol tautomer: ^{1}H NMR ($CDCl_3$) δ 1.30 (t, 3H, J=7.0 Hz), 1.41 (s, 6H), 2.18 (t, 2H, J=5.8 Hz), 2.52 (t, 2H, J=5.8 Hz), 3.62 (s, 2H), 4.21 (q, 2H, J=7.0 Hz), 7.35 (5H); ^{13}C NMR ($CDCl_3$) δ 14.4 (−), 21.4 (−), 23.2 (+), 42.6 (+), 53.2 (+), 58.4 (+), 60.4 (+), 95.6 (+), 126.8 (−), 128.3 (−), 140.7 (+), 172.9 (+), 175.5 (+). FAB HRMS m/z calcd for $C_{17}H_{24}NO_3$ $(MH)^+$ 290.1756, found 290.1756.

4.12. 1-Benzyl-2,2-dimethyl-3-piperidone (10)

A solution of **13** (0.58 g, 2.0 mmol) in 10 mL of 6 N HCl was heated at reflux for 3 h. The reaction mixture was cooled to room temperature, neutralized with solid K_2CO_3 to pH 8.0, and extracted with ether. The organic layer was washed, filtered, and evaporated to give pure piperidone **10** (0.39 g, 90%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 6H), 1.82 (m, 2H), 2.50 (t, 2H, J=7.1 Hz), 2.65 (t, 2H, J=6.1 Hz), 3.63 (s, 2H), 7.31 (5H); ^{13}C NMR ($CDCl_3$) δ 20.0 (−), 23.9 (+), 36.2 (+), 44.9 (+), 53.7 (+), 66.6 (+), 126.9 (−), 128.2 (−), 128.3 (−), 140.5 (+), 212.2 (+); FAB HRMS m/z calcd for $C_{14}H_{20}NO$ $(M+H)^+$ 218.1545, found 218.1545.

4.13. *N*-(3,3-Dimethoxy-2-butylidene)isopropylamine (17)[22]

The preparation of **17** was carried out exactly according to the literature report.[22] The final residual oil was distilled in vacuum to afford the product **17** (15.3 g, 89%) as a colorless liquid: bp 70–76 °C/25 mmHg (lit.[22] 60–63 °C/11 mmHg); ^{1}H NMR ($CDCl_3$) δ 1.09 (d, 3H, J=6.1 Hz), 1.12 (d, 3H, J=6.2 Hz), 1.33 (s, 3H), 1.81 (s, 3H), 3.17 (s, 3H), 3.19 (s, 3H), 3.65 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 13.0 (+), 20.9 (+), 23.1 (+), 49.2 (+), 50.9 (+), 102.3 (−), 165.4 (−).

4.14. 6-Chloro-2,2-dimethoxy-3-hexanone (19)

A solution of lithium diisopropylamide (6 mL of 2.0 M, 0.012 mol) in 30 mL of dry tetrahydrofuran at 0 °C was treated dropwise with 1.73 g (0.01 mol) of *N*-(3,3-dimethoxy-2-butylidene)isopropylamine, dissolved in 3 mL of dry tetrahydrofuran. The mixture was stirred for 2 h at 0 °C after which 1.72 g (0.012 mol) of 1-bromo-2-chloroethane was added dropwise. The solution was stirred for 20 h at ambient temperature. The reaction mixture was poured into 100 mL of 0.05 N sodium hydroxide and extracted three times with ether. The combined organic extracts were dried with K_2CO_3, filtered, and evaporated to afford *N*-(6-chloro-2,2-dimethoxy-3-hexylidene)isopropylamine (**18**, 2.1 g, 89%) as an oil. The compound was used without further purification in the next step.

To a solution of imine **18** (2.1 g, 9 mmol) in 120 mL of CH_2Cl_2 was added oxalic acid dihydrate (1.7 g, 13 mmol) in 100 mL of water. The mixture was heated at reflux with vigorous stirring for 1 h and extracted three times with CH_2Cl_2. The combined organic layer was dried (Na_2SO_4) and filtered, and the resulting oil obtained upon evaporation of solvent was subjected to silica gel flash chromatography using hexane–ethyl acetate (15:1) as eluent to give the keto acetal **19** (0.81 g, 42% overall for two steps) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3H), 2.05 (quintuplet, 2H), 2.80 (t, 2H, J=7.0 Hz), 3.24 (s, 6H), 3.59 (t, 2H, J=6.4 Hz); ^{13}C NMR ($CDCl_3$) δ 19.9 (−), 26.0 (+), 35.0 (+), 44.4 (+), 49.7 (−), 102.6 (+), 206.1 (+); FAB HRMS calcd for $C_7H_{12}^{35}ClO_2$ (M^+-OCH_3) m/z 163.0526, found 163.0518.

4.15. 6-Benzylamino-2,2-dimethoxy-3-hexanol (21)

A solution of 6-chloro-2,2-dimethoxy-3-hexanone **19** (0.35 g, 1.8 mmol), potassium iodide (300 mg, 1.8 mmol), benzylamine (0.29 g, 2.7 mmol), and triethylamine (0.4 mL, 2.9 mmol) in 50 mL of acetonitrile was heated at reflux for 20 h under argon. The reaction mixture was filtered and evaporated to dryness. The residue was extracted with CH_2Cl_2, and the organic extract was washed with water, dried (Na_2SO_4), and concentrated. The residue was purified by chromatography using CH_2Cl_2–methanol (30:1) as eluent to give 6-benzylamino-2,2-dimethoxy-3-hexanone (**20**, 185 mg, 38%) as an oil, which has a low stability and was used quickly for the next step: ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 3H), 1.78 (quintuplet, 2H), 2.61 (m, 4H), 3.19 (s, 6H), 3.77 (s, 2H), 7.29 (5H); ^{13}C NMR ($CDCl_3$) δ 19.9, 23.2, 35.6, 48.4, 49.6, 53.5, 102.7, 127.1, 128.3, 128.4, 139.5, 209.1.

To a solution of **20** (270 mg, 1 mmol) in 10 mL of methanol, sodium borohydride (57 mg, 1.5 mmol) was added portionwise at 0 °C. The reaction mixture was stirred at room temperature for 3 h. After removal of methanol, the viscous product was treated with H_2O and then extracted with ether. The organic layer was dried (Na_2SO_4) and evaporated to dryness. The residue was purified by chromatography with CH_2Cl_2–methanol (15:1) as eluent to afford the product **21** (201 mg, 76%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.29 (s, 3H), 1.25 (m, 2H), 1.78 (m, 2H), 2.61 (m, 1H), 2.86 (m, 1H), 3.18 (s, 3H), 3.23 (s, 3H), 3.61 (d, 1H, J=9.8 Hz), 3.87 (s, 1H), 7.34 (5H); ^{13}C NMR ($CDCl_3$) δ 16.3 (−), 26.7 (+), 30.3 (+), 48.2 (−), 48.5 (−), 48.6 (+), 53.0 (+), 72.3 (−), 102.7 (+), 127.8 (−), 128.7 (−), 128.9 (−), 137.0 (+); FAB HRMS m/z calcd for $C_{15}H_{26}NO_3$ $(M+H)^+$ 268.1912, found 268.1915.

4.16. The reactions of compound 21 in 6 N HCl

A solution of compound **21** (50 mg, 0.19 mmol) in 5 mL of 6 N HCl solution was heated at reflux for 24 h under argon.

The reaction mixture was extracted with ether. The aqueous phase was separated and neutralized with K_2CO_3 to pH 8. The solution was then extracted with ether, which was dried (Na_2SO_4) and evaporated to give **2** (33 mg, 87%) as an oil. A solution of compound **21** (50 mg, 0.18 mmol) in 5 mL of 6 N HCl solution was stirred at room temperature for 24 h under argon. The reaction mixture was neutralized with K_2CO_3 to pH 8. The solution was extracted with ether. The combined ether layer was dried (Na_2SO_4) and evaporated to give **1** (30 mg, 79%) as an oil.

4.17. Time profile for conversion of compounds 1 or 21 to compound 2 in 6 N HCl solution

A solution of compound **1** or **21** in 25 mL of 6 N HCl solution was heated at reflux under argon. Aliquots were taken at 1, 3, 6, 12, and 24 h. The aliquots were neutralized with K_2CO_3 to pH 8 and extracted with ether. The ether layers were dried (Na_2SO_4) and evaporated to give mixtures of **1** and **2** (oils) that were analyzed by ^{1}H NMR spectroscopy. The fraction of rearrangement was calculated from the integral ratio of the ketone methyl group in **2** (δ 2.1 ppm) and the piperidine ring 2-methyl group in **1** (δ 1.3 ppm).

4.18. NMR spectral data of compounds in DCl–D_2O

4.18.1. 1-Benzyl-3-piperidone (free base). ^{1}H NMR ($CDCl_3$) δ 1.94 (m, 2H), 2.36 (t, 2H), 2.65 (t, 2H), 3.01 (s, 2H), 3.58 (s, 2H), 7.29 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 24.04 (+), 38.81 (+), 51.60 (+), 62.60 (+), 64.63 (+), 127.41 (−), 128.42 (−), 129.08 (−), 137.26 (+), 207.12 (+).

4.18.1.1. 1-Benzyl-3-piperidone·HCl. ^{13}C NMR (6 N DCl–D_2O) δ 20.5 (+), 34.2 (+), 53.1 (+), 58.9 (+), 61.1 (+), 91.6 (+), 128.8 (+), 130.1 (−), 131.1 (−), 132.1 (−).

4.18.2. 1-Benzyl-2-methyl-3-piperidone·HCl. ^{13}C NMR (6 N DCl–D_2O, mixture of cis and trans isomers) δ 7.1 (−), 10.1 (−), 19.6 (+), 23.5 (+), 29.2 (+), 35.2 (+), 47.3 (+), 51.5 (+), 61.3 (−), 65.7 (−), 92.6 (+), 93.7 (+), 128.6 (+), 129.2 (+), 129.7 (−), 130.0 (−), 130.5 (−), 130.7 (−), 132.3 (−).

4.18.3. 1-Benzyl-2,2-dimethyl-3-piperidone·HCl. ^{13}C NMR (6 N DCl–D_2O) δ 16.07 (−), 19.18 (−), 19.52 (−), 20.13 (+), 20.59 (−), 20.85 (+), 31.33 (+), 35.31 (+), 46.93 (+), 47.67 (+), 55.89 (+), 55.93 (+), 71.15 (+), 73.01 (+), 95.29 (+), 130.08 (+), 130.21 (−), 130.29 (+), 130.48 (−), 131.15 (−), 131.33 (−), 132.21 (−), 132.81 (−), 207.57 (+).

4.18.4. 1-Benzyl-2-acetylpyrrolidine·HCl. ^{1}H NMR (6 N DCl–D_2O) δ 1.94 (m, 2H), 2.16 (s, 3H), 2.22 (m, 1H), 2.67 (m, 1H), 3.38 (m, 1H), 3.68 (m, 1H), 4.37 (d, 2H), 4.75 (t, 1H), 7.49 (m, 5H); ^{13}C NMR (6 N DCl–D_2O) δ 23.34 (+), 27.20 (−), 28.30 (+), 56.12 (+), 59.37 (+), 72.96 (−), 129.96 (−), 130.22 (+), 130.87 (−), 131.34 (−), 205.03 (+).

4.18.5. 6-Benzylamino-2,2-dimethoxy-3-hexanol·HCl. ^{1}H NMR (6 N DCl–D_2O) δ 1.8 (m, 4H), 2.15 (s, 3H, exchangeable), 3.10 (m, 2H), 4.21 (s, 2H), 4.40 (m, 1H, exchangeable), 7.45 (m, 5H); ^{13}C NMR (6 N DCl–D_2O) δ 22.05 (+), 26.15 (−), 29.57 (+), 47.21 (+), 51.55 (+), 76.44 (−), 129.64 (−), 130.10 (−), 130.35 (−), 130.84 (+), 215.16 (+).

Acknowledgements

We are grateful for support of the early stages of this work by the National Institutes of Health grants NS 22688 and GM 48812. We thank Drs. Ke-Qing Ling and Yuming Zhang for helpful discussion.

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Tetrahedron 62 (2006) 6370–6378
Tetrahedron

α-Aminoisobutyric acid modified protected analogues of β-amyloid residue 17–20: a change from sheet to helix

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Received 3 January 2006; revised 29 March 2006; accepted 11 April 2006
Available online 6 May 2006

Abstract—The strong intermolecular interactions mediated by short hydrophobic sequences (e.g., 17–20, -L-Leu-L-Val-L-Phe-L-Phe-) in the middle of Aβ are known to play a crucial role in the neuropathology of Alzheimer's disease. FTIR, TEM and Congo red binding studies indicated that a series of L-Ala substituted terminally protected peptides related to the sequence 17–20 of the β-amyloid peptide, adopted β-sheet conformations. However, the Aib-modified analogues disrupt the β-sheet structure and switch over to a 3_{10} helix with increasing number of Aib residues. X-ray crystallography shed some light on the change from sheet to helix at atomic resolution.

1. Introduction

The molecular self-assembly of synthetic peptides demands special interest in advanced medicine because such molecules can serve as bioactive extra cellular materials. Self-aggregation of proteins or protein fragments is particularly important for studying pathogenesis of certain age-related diseases.[1] Though there is no similarity in native structures, sequences, length and composition of different proteins or peptides, subsequent formation of insoluble amyloid plaque is the key factor for several neurodegenerative diseases including Alzheimer's disease,[2] Huntington's disease[3] and prion protein diseases.[4] The fibrillar deposits of amyloid plaque with diameters ranging from 60 to 120 Å appeared in vivo by the transition of a misfolded protein or a peptide from its native structure to a supramolecular β-sheets arrangement.[5] For Alzheimer's disease, the Aβ peptide is generated by the proteolytic processing of a type-1 glycoprotein APP by successive β-cleavage at the N-terminus of Aβ and γ-cleavage (in the trans membrane domain) either at position 40 or 42. Moreover, APP is more frequently cleaved between amino acid 16 and 17 of the Aβ region (α-cleavage) (Fig. 1).[6] The fibrillar aggregation due to the strong intermolecular interaction of the resultant hydrophobic peptide fragments may be the direct or indirect cause of the pathological conditions associated with the amyloid diseases.[7] Some recent results demonstrate that not the matured fibrils but their precursor is pathogenic.[8] Hence, the therapeutic target is to prohibit the fibrillogenesis process.

In order to design therapeutic drugs against amyloid diseases, one of the popular approaches is the modification of amyloidogenic proteins to prevent their ability to adopt a β-sheet conformation. Previously, numerous studies have been performed using β-sheet breaking elements into short recognition sequence of amyloid protein to develop inhibitory drugs.[9] Proline and α-aminoisobutyric acid (Aib)[10] have been widely used for this purpose. Gazit et al. have

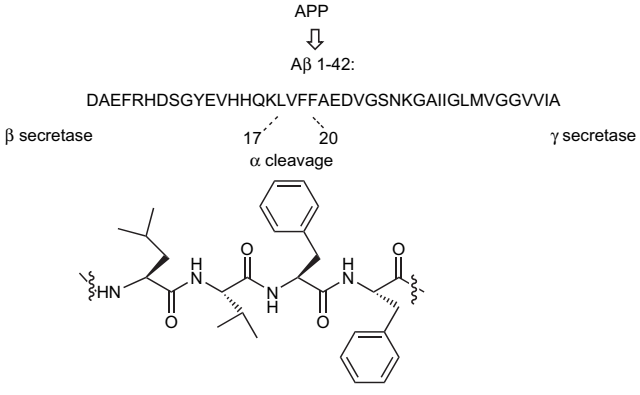

Figure 1. The sequence of amyloid β-peptide ($Aβ^{42}$) and schematic presentation of the residue 17–20 of $Aβ^{42}$. The proteolytic processing of a type-1 gycloprotein APP to generate Aβ peptide. APP is first cleaved at the N-terminus of Aβ (β-cleavage) and then in the trans membrane domain (γ-cleavage), either at position 40 or 42. Moreover, APP is more frequently cleaved between amino acid 16 and 17 of the Aβ region (α-cleavage).

Keywords: Amyloid β-peptide; Amyloid-like fibril; Aib; Supramolecular β-sheet; β-Turn; 3_{10} Helix.
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doi:10.1016/j.tet.2006.04.036

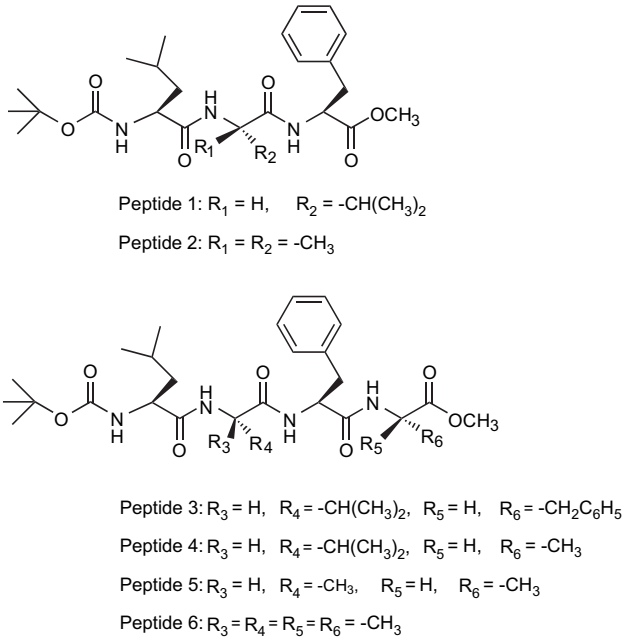
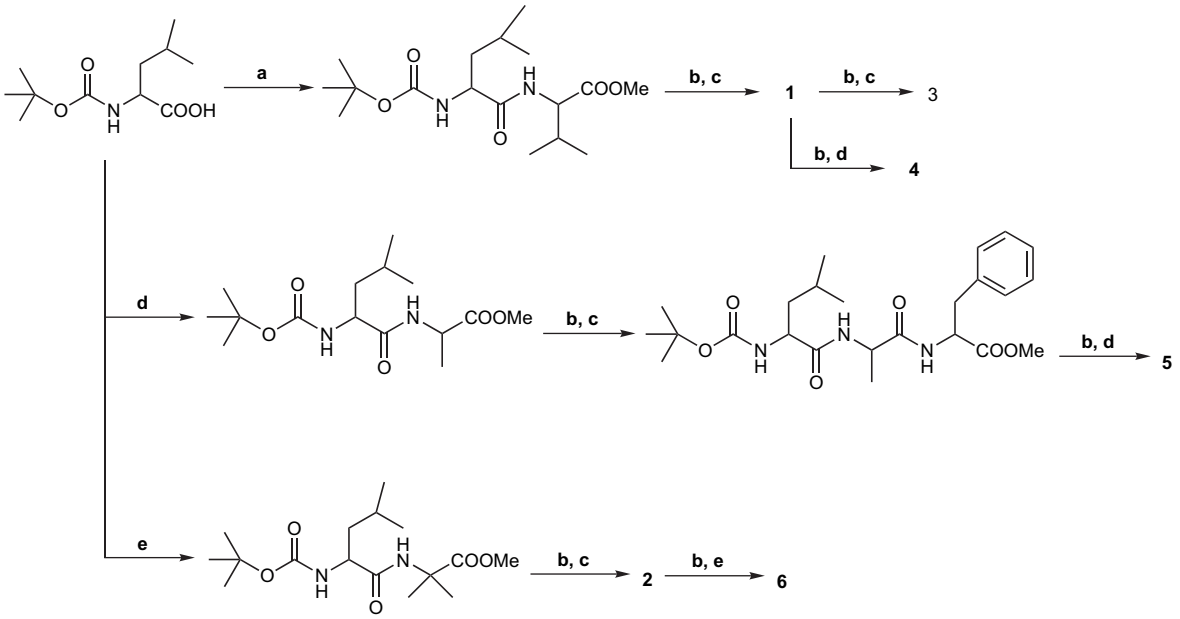


Figure 2. The schematic presentation of peptides **1**–**6**.

reported that the Aib modification of 13–20 residue of human islet amyloid polypeptide (hIPP) generates significant inhibition.[11] Formaggio and co-workers have demonstrated that the incorporation of an Aib residue into a fully protected Alzheimer's β-amyloid fragment (Aβ 17–21) can change the backbone conformation in organic solvents.[12] But all these studies depend on conventional methods like TEM and FTIR and the finer structural detail at the atomic level is still elusive.[11,12] In this context, we have synthesized some terminally protected L-Ala and Aib[13] modified analogs of Aβ 17–20 (LVFF) peptide sequences (Fig. 2). The L-Ala substituted peptides (peptides **4** and **5**) LVFA and LAFA which also have existence in the β-sheet region of corresponding protein structures (NCBI GI 5915735 (60–63) and GI 23019621 (300–303), Protein Data Bank) retain their β-sheet conformations and form amyloid-like fibrils. However, from X-ray crystallography, the Aib-modified analogues (peptide **2** and peptide **6**) disrupt the β-sheet structure and switch over to a 3_{10} helix conformation in the solid state.

2. Results and discussion

The peptides reported in this study have been synthesized using conventional solution phase methodology (Scheme 1).[14] FTIR spectra were recorded to determine the internal conformation of the reported peptides in the solid state (KBr matrix). The most informative frequency ranges are (i) 3500–3200 cm^{-1}, corresponding to the N–H stretching vibrations of the peptide and (ii) 1800–1500 cm^{-1}, corresponding to the stretching band of amide I and bending peak of amide II.[15] Figure 3 shows that molecules of the fully protected Aβ 17–20 peptides Boc-Leu-Val-Phe-OMe (**1**) and Boc-Leu-Val-Phe-Phe-OMe (**3**) have strong intermolecular H-bonds in the solid state. An intense band at 3295 cm^{-1} (peptide **1**) and 3291 cm^{-1} (peptide **3**) and a shoulder at 3328 cm^{-1} were observed for the reported peptides, indicating the presence of strongly hydrogen-bonded NH groups.[16] No band was observed at around 3400 cm^{-1}, indicating that all NH groups are involved in intermolecular hydrogen bonding.[16] The CO stretching band at 1628, 1648 cm^{-1} (amide I) and the NH bending peak at 1554, 1544 cm^{-1} (amide II) corresponding to peptides **1** and **3** suggest the presence of a β-sheet conformation in the solid state.[17] Modification of the native peptides with L-Ala produced no significant change in the infrared spectra. Peptides **4** (Boc-Leu-Val-Phe-Ala-OMe) and **5** (Boc-Leu-Ala-Phe-Ala-OMe) have peaks at 3316 and 3289 cm^{-1}, which might indicate the presence of intermolecular hydrogen-bonded NH groups (Fig. 4). Moreover, the characteristic IR absorption bands at about 1642 and 1640 cm^{-1} (amide I)



Scheme 1. Reagents and conditions: (a) DMF, H-Val-OMe, DCC, HOBt, 0 °C, 90% yield; (b) MeOH, 2 M NaOH, 85% yield; (c) DMF, H-Phe-OMe, DCC, HOBt, 0 °C, 80% yield; (d) DMF, H-Ala-OMe, DCC, HOBt, 0 °C, 80% yield; (e) DMF, H-Aib-OMe, DCC, HOBt, 0 °C, 90% yield.

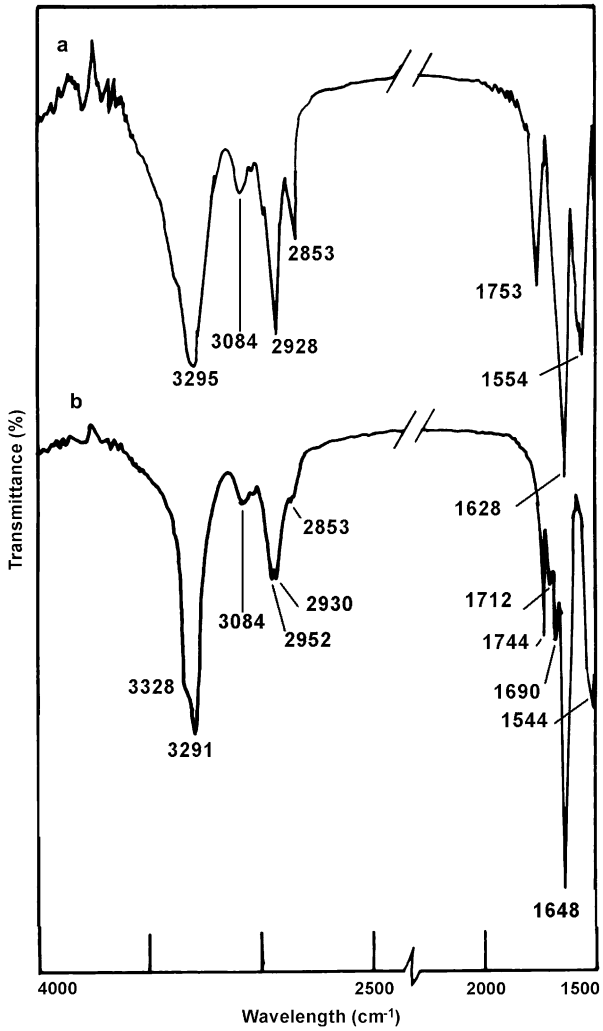


Figure 3. Solid state FTIR spectra of (a) peptide **1** and (b) peptide **3** at the region 2500–4000 cm^{-1} and 1500–2000 cm^{-1}.

and 1530 and 1546 cm^{-1} (amide II) of peptides **4** and **5**, respectively, suggest that the peptides have spectra typical of β-sheet structures.[15,17] Therefore, this very minor chemical modification (replacement of amino acid residues 2 and 4 of peptide **3** by L-Ala) results in little effect on the amyloidogenic potential of the native peptide. But the Aib-modified peptides produce significant changes (1659 and 1661 cm^{-1} (amide I) for peptide **2** and **6**), which are consistent with the previous report.[11,18]

Further, we examined the ability of the L-Ala modified peptides to form amyloid-like fibrils, compared to that of native peptides. Although the overall changes in the chemical structures are minor, we observed no differences between the abilities of the native and modified peptides to form amyloid-like fibrils. The amyloidogenic nature of the peptides was determined by transmission electron microscopy (TEM). The TEM was performed on both transmission mode and diffraction mode. From the TEM images of these peptides, it is evident that they share a common morphological property irrespective of the difference in sequences. Figure 5 clearly shows that peptide **4** exhibits amyloid-like tangles with multiple branching nodes, a very common feature of amyloid plaque obtained from many neurodegenerative diseases. From the transmission

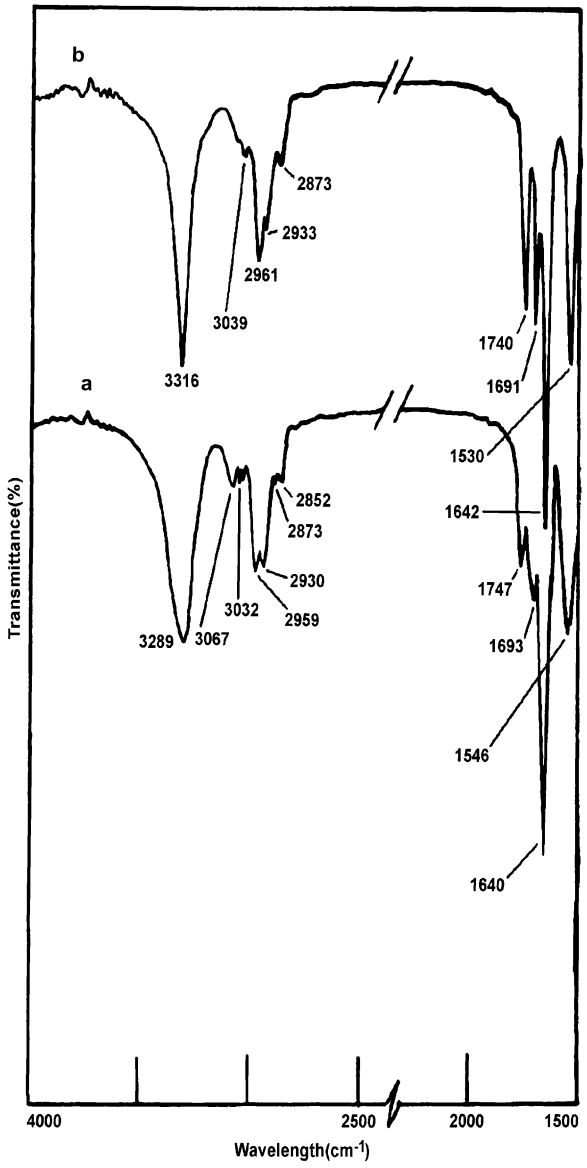


Figure 4. FTIR spectra at the region 2500–4000 cm^{-1} and 1500–2000 cm^{-1} of (a) peptide **5** and (b) peptide **4** in the solid state.

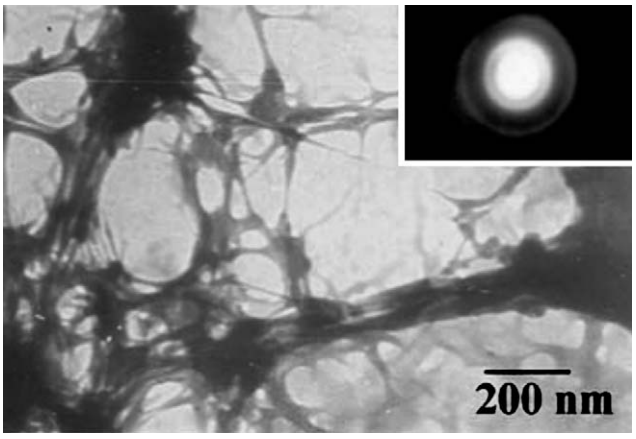


Figure 5. Electron microscopy and electron diffraction (inset) (0.5% w/v) of peptide **4**. Transmission electron micrograph of peptide **4** showing amyloid-like tangles and multiple branching nodes.

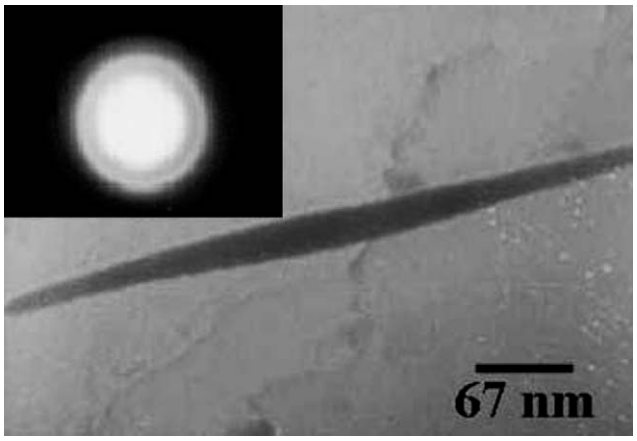


Figure 6. Transmission electron microscopy and electron diffraction (inset) (0.5% w/v) of peptide **5**. The TEM image exhibits filamentous fibrillar morphology.

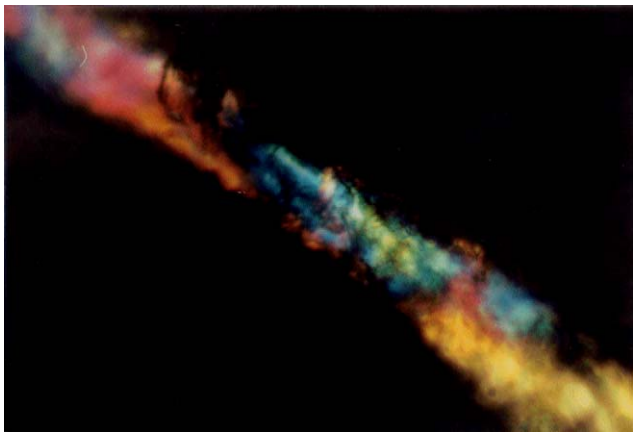


Figure 8. Congo red stained peptide **5** fibrils observed through crossed polarizer showing green-gold birefringence, a characteristic feature of amyloid fibrils.

electron micrograph, peptide **5** aggregates like a bundle of long filaments, reminiscent of amyloid fibrils (Fig. 6). However, the transmission electron micrograph of the corresponding Aib analogue (peptide **6**) shows nanorod[19]-like structures with diameters ranging from 60 to 70 nm in the solid state (Fig. 7).

The morphological resemblance of these peptide fibrils with amyloid plaque was also studied by Congo red staining. It has been reported that Congo red binds with amyloid fibrils responsible for various neurodegenerative diseases like Alzheimer's disease and shows a distinct birefringence under polarized light. To determine the similarity with Alzheimer's β-amyloid fibrils, the aggregated fibrils obtained from these peptides were stained with Congo red and observed through a cross polarizer. Figure 8 exhibits the typical green-gold birefringence of Congo red bound fibrils of peptide **5** under cross polarizer, similar to the native peptides. These results are consistent with Congo red binding to an amyloid β-sheet fibrillar structure.[7a,b,d] We conclude that there is almost no difference between native peptides, which are highly amyloidogenic and their L-Ala containing analogues. But the aggregate obtained from Aib-modified analogue peptide **6** does not exhibit typical birefringence when stained with Congo red and viewed through cross polarizers under same conditions (Fig. 9).

However, from X-ray crystallography, the modification of the native peptides to Aib-containing peptides produced a significant change in the peptide backbone conformations.[20] Most of the ϕ and ψ values of the constituent amino acid residues of peptide **2**, an Aib analogue of native peptide **1**, fall within the helical region of the Ramachandran map. The torsion angles ϕ_1 (−62.91) and ψ_1 (−41.20) are in the right-handed helical region whereas the ϕ_2 (58.15) and ψ_2 (46.24) are in the left-handed helical region. There is also a distortion for ϕ_3 (−55.3) and ψ_3 (141.4), which prevents the peptide from forming any intramolecular hydrogen-bonded folded structures. Hence, the overall backbone conformation is a helical one in comparison with the native peptides (Fig. 10a).[20a] However, with an increasing number of Aib residues, the peptide backbone completely switches over into a 3_{10} helical conformation. Peptide **6** contains two alternating Aib residues and forms a consecutive β-turn (10 member intramolecular hydrogen-bonded ring) structure where the Aib(2) occupies the *i*+2th position of first turn and *i*+1th position of the second turn (Fig. 10b). From Figure 10b, there are two intramolecular hydrogen bonds N4–H4···O11 (2.21 Å, 2.94 Å, 143°) and N7–H7···O14 (2.41 Å, 3.26 Å, 169°) resulting in a consecutive double bend conformation for individual peptide (**6**) molecules in the solid state. Most of the backbone torsion angles [ϕ_1(−70.2°), ψ_1(−16.9°),

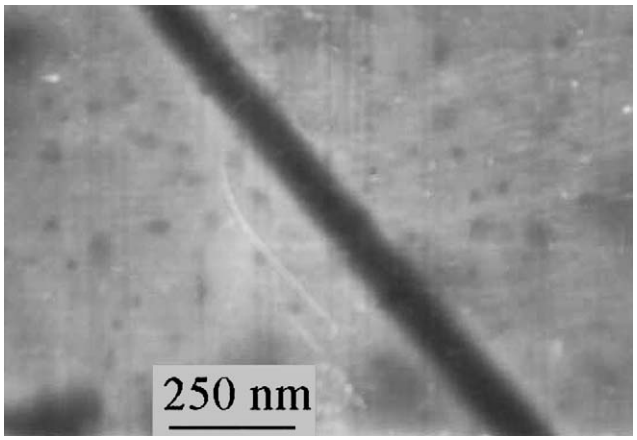


Figure 7. Transmission electron micrographs of peptide **6** showing nanorod-like morphology in the solid state.

Figure 9. Peptide **6** stained with Congo red dye fails to show any birefringence observed between cross polarizer.

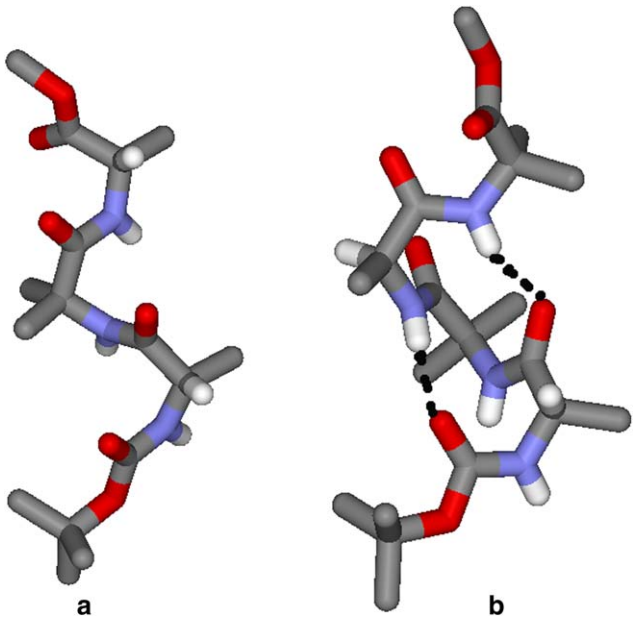


Figure 10. The molecular backbone conformation of (a) peptide **2** and (b) peptide **6** showing a 3_{10} helical switch over from peptide **2** to **6**. Intramolecular hydrogen bonds are shown as dotted line. The side chains for Leu and Phe residues and other non-hydrogen-bonded hydrogen atoms are omitted for clarity. Nitrogen atoms are blue, oxygen atoms are red and carbon atoms are gray.

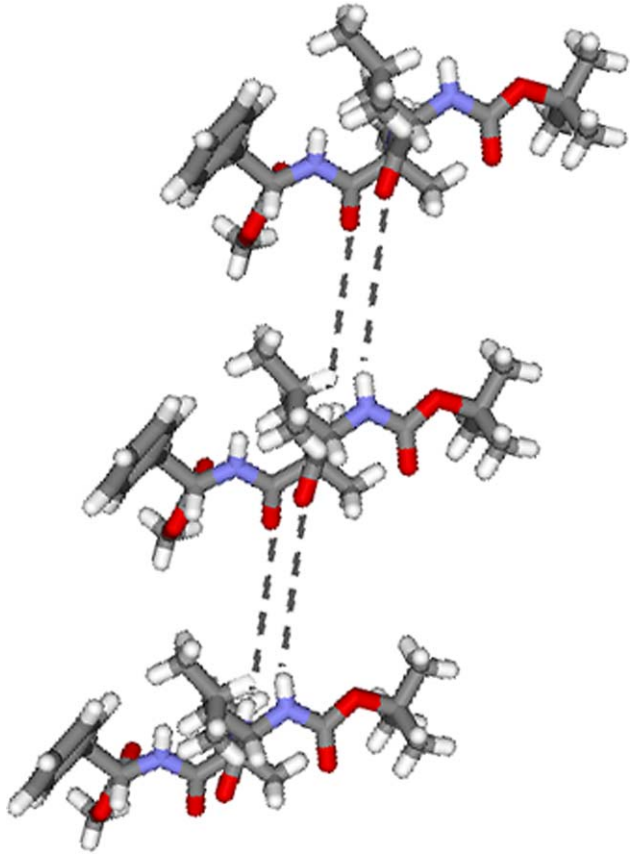


Figure 11. The packing of peptide **2** along crystallographic *a*-axis showing multiple intermolecular hydrogen bonds that connect individual molecules to form parallel sheet-like structure. Hydrogen bonds are shown as dotted line.

$\phi_2(-57.8°)$, $\psi_2(-21.3°)$, $\phi_3(-56.4°)$ and $\psi_3(-35.9°)$] of peptide **6** are in the 3_{10} helical region of Ramachandran diagram [except $\phi(51.3°)$ and $\psi(-135.7°)$ values of Aib(4)].[20b] Thus, there is a distinct conformational change from the native peptide to its Aib analogues. The peptide backbone changes from a β-sheet structure of the native peptide to the 3_{10} helical conformations in the Aib-modified peptide.

Moreover, in higher order packing, two intermolecular hydrogen bonds N4–H4···O3 (2.27 Å, 3.08 Å, 158°) and N6–H62···O5 (2.10 Å, 2.98 Å, 147°) connect individual molecules of peptide **2** to form a parallel sheet-like structure (Fig. 11) along crystallographic *a*-axis. Therefore, peptide **2** preserves some characteristics of the native peptides instead of the incorporation of an Aib residue. But, the individual sub-units of the 3_{10} helical peptide **6** are themselves regularly inter-linked via multiple intermolecular hydrogen bonds N10–H10···O5 (2.20 Å, 2.99 Å, 152°) and N13–H13···O8 (2.30 Å, 3.15 Å, 170°) and thereby form a supramolecular helix along the crystallographic *b*-direction (Fig. 12). This very minor chemical modification results in such a dramatic effect on the molecular backbone

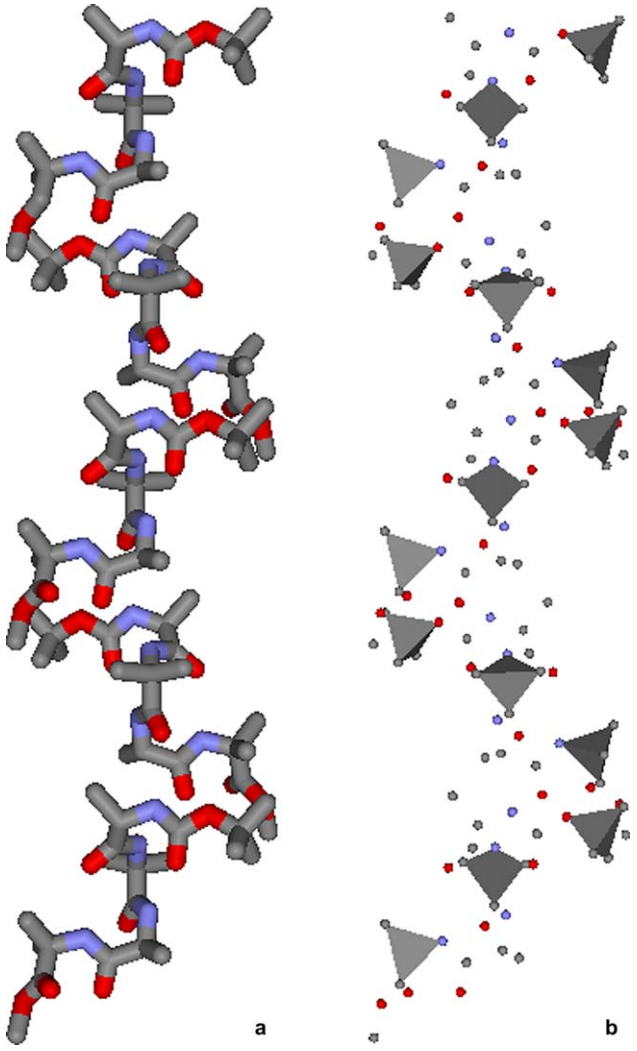


Figure 12. The packing diagram of peptide **6** showing the intermolecular hydrogen bonded supramolecular helix along the crystallographic *b*-direction in (a) stick and (b) polyhedron representation.

Table 1. Crystal and data collection parameters of peptides **2** and **6**

	Peptide **2**	Peptide **6**
Empirical formula	$C_{25}H_{39}N_3O_6$	$C_{29}H_{45}N_4O_7$
Crystallizing solvent	Dimethyl sulfoxide	Ethyl acetate
Crystal system	Orthorhombic	Orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$
a (Å)	6.023(3)	13.253(17)
b (Å)	10.311(3)	15.694(19)
c (Å)	43.051(7)	15.768(19)
α (°)	90	90
β (°)	90	90
γ (°)	90	90
v (Å^3)	2673.6(16)	3280(7)
μ (Mo Kα)/mm	0.085	0.081
Z	4	4
Mol. wt.	477.59	561.69
Density (calcd, mg/mm^3)	1.186	1.137
F(000)	1032	1212
T (°)	293	293
Tot., uniq. data	4509, 4179	3474, 3474
Observed reflns. $I>2\sigma(I)$	2887	1716
R	0.0577	0.0624
wR	0.1983	0.1800
S	1.22	0.98
λ (Å) (Mo Kα)	0.71073	0.71073
No. of param.	340	372

conformation and nature of the native peptides. Crystal data for peptides **2** and **6** are listed in Table 1.

3. Conclusion

In conclusion, it is clearly established that the L-Ala substituted analogue of the fully protected peptides related to the sequence 17–20 of amyloid β peptide have almost similar characteristics in comparison to the native peptides, which are highly amyloidogenic. Moreover, the β-sheet structure of the native peptides is completely disrupted by the incorporation of a conformationally constrained α-aminoisobutyric acid. From X-ray crystallography, it is unambiguously demonstrated that there is a distinct change from β-sheet to 3_{10} helix conformations in the Aib modification of the native peptides. These results will be useful in efforts to design peptide-based inhibitors of amyloid fibrillogenesis to prevent various neurodegenerative diseases.

4. Experimental

4.1. Synthesis of the peptides

4.1.1. Boc-Leu-OH 7. See Ref. 21.

4.1.2. Boc-Leu-Val-OMe 8. See Ref. 22.

4.1.3. Boc-Leu-Val-OH 9. See Ref. 22.

4.1.4. Boc-Leu-Val-Phe-OMe 1. See Ref. 23.

4.1.5. Boc-Leu-Val-Phe-OH 10. To Boc-Leu-Val-Phe-OMe (9.82 g, 20 mmol), methanol (50 mL) and 2 M NaOH (20 mL) were added and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred at room temperature. After 10 h, methanol was removed under vacuum, the residue was taken in 50 mL of water, washed with diethyl ether (2×50 mL). Then, pH of the aqueous layer was adjusted to 2 by adding 1 M HCl and it was extracted with ethyl acetate (3× 40 mL). The extracts were pooled, dried over anhydrous sodium sulfate and evaporated under vacuum to yield a white waxy solid.

Yield=80% (7.64 g, 16 mmol). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.37 (br, 1H), 8.27 (d, J=7.5 Hz, 1H), 7.46 (d, J=9.1 Hz, 1H), 7.19–7.27 (m, 5H), 7.05 (d, J=8.5 Hz, 1H), 4.42 (m, 1H), 4.20 (m, 1H), 3.94 (m, 1H), 3.02 (m, 2H), 2.88 (m, 1H), 1.92 (m, 2H), 1.36 (s, 9H), 0.87 (d, J= 6.9 Hz, 6H), 0.76–0.84 (m, 7H) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz): δ 11.24, 11.47, 18.18, 22.37, 22.93, 22.98, 24.82, 28.14, 31.17, 35.03, 41.38, 52.23, 52.26, 58.43, 80.16, 127.14, 128.47, 129.15, 135.81, 170.18, 170.81, 171.69, 176.25 ppm. $[\alpha]_D^{27.8}$ −19.9 (c 2.10, $CHCl_3$). Elemental Anal. Calcd for $C_{25}H_{39}N_3O_6$ (477): C, 62.89; H, 8.18; N, 8.80. Found: C, 63.21; H, 8.36; N, 7.94.

4.1.6. Boc-Leu-Val-Phe-Phe-OMe 3. The mixture of **10** (4.77 g, 10 mmol) in DMF (20 mL) was cooled in an ice-water bath. H-Phe-OMe was isolated from the corresponding methyl ester hydrochloride (4.33 g, 20 mmol) by neutralization with saturated $NaHCO_3$ solution, subsequent extraction with ethyl acetate and the ethyl acetate extract was concentrated to 20 mL. This was added to the reaction mixture, followed immediately by DCC (2 g, 10 mmol) and HOBt (1.4 g, 10 mmol). The reaction mixture was stirred for 3 days. The residue was taken in ethyl acetate (50 mL) and DCU was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine, 1 M sodium carbonate (3× 50 mL), brine (2×50 mL), dried over anhydrous sodium sulfate and evaporated in vacuum to get a white solid.

Yield=80% (5.1 g, 8 mmol). Mp 153–154 °C. IR (KBr): 1544, 1648, 1690, 1744, 2853, 2930, 2952, 3084, 3291, 3328 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.22–7.26 (m, 10H), 7.02 (d, J=6.9 Hz, 1H), 6.62 (d, J=7.8 Hz, 1H), 6.53 (d, J=7.1 Hz, 1H), 4.83 (d, J=8.3 Hz, 1H), 4.78 (m, 1H), 4.74 (m, 1H), 4.14 (m, 1H), 4.03 (m, 1H), 3.66 (s, 3H), 2.94–3.13 (m, 4H), 2.14 (m, 1H), 1.94 (m, 2H), 1.44 (s, 9H), 0.96 (d, J=6.6 Hz, 6H), 0.74–0.85 (m, 7H) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz): δ 11.23, 11.49, 18.23, 22.35, 22.91, 22.99, 24.83, 28.14, 31.13, 35.07, 36.88, 41.33, 51.71, 52.33, 52.19, 58.47, 80.18, 127.14, 128.47, 129.15, 135.81, 170.20, 170.80, 171.69, 171.84, 173.35 ppm. $[\alpha]_D^{27.8}$ −35.7 (c 2.10, $CHCl_3$). Mass spectral data $(M+Na)^+$=661.60, M_{calcd}=638. Elemental Anal. Calcd for $C_{35}H_{50}N_4O_7$ (638): C, 65.83; H, 7.84; N, 8.78. Found: C, 65.34; H, 7.51; N, 10.16.

4.1.7. Boc-Leu-Val-Phe-Ala-OMe 4. The Boc-Leu-Val-Phe-OH (2.40 g, 5 mmol) in DMF (10 mL) was cooled in an ice-water bath. H-Ala-OMe was isolated from the corresponding methyl ester hydrochloride (1.4 g, 10 mmol) by neutralization with saturated $NaHCO_3$ solution, subsequent extraction with ethyl acetate and the ethyl acetate extract was then concentrated to 7 mL. This was added to the reaction mixture, followed immediately by DCC (1 g, 5 mmol) and HOBt (0.7 g, 5 mmol). The reaction mixture was stirred for 3 days. The residue was taken in ethyl acetate (50 mL) and DCU was filtered off. The organic layer was washed

with 2 M HCl (3×50 mL), brine, 1 M sodium carbonate (3×50 mL), brine (2×50 mL), dried over anhydrous sodium sulfate and evaporated under vacuum to get 1.97 g of white solid.

Yield=70% (1.97 g, 3.5 mmol). Mp 144–145 °C. IR (KBr): 1530, 1642, 1691, 1740, 2933, 2961, 3039, 3316 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 7.22–7.26 (m, 5H), 7.19 (d, J=6.9 Hz, 1H), 6.75 (d, J=8.4 Hz, 1H), 6.70 (d, J=7.5 Hz, 1H), 4.91 (d, J=6.0 Hz, 1H), 4.80 (m, 1H), 4.50 (m, 1H), 4.16 (m, 1H), 4.06 (m, 1H), 3.71 (s, 3H), 3.01 (m, 2H), 2.15 (m, 1H), 1.93 (m, 2H), 1.44 (s, 9H), 1.37 (d, J=7.2 Hz, 3H), 0.95 (m, 6H), 0.74–0.87 (m, 7H) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz): δ 11.16, 11.29, 18.99, 22.33, 22.82, 22.97, 24.79, 24.83, 28.14, 31.13, 35.07, 41.35, 51.71, 52.13, 52.19, 58.47, 80.17, 127.14, 128.47, 129.15, 135.81, 170.24, 170.87, 171.73, 171.81, 172.25 ppm. $[\alpha]_D^{27.8}$ −42.2 (c 2.10, $CHCl_3$). Mass spectral data (M+Na+2H)$^+$=587.20, M_{calcd}=562. Elemental Anal. Calcd for $C_{29}H_{46}N_4O_7$ (562): C, 61.92; H, 8.18; N, 9.96. Found: C, 61.49; H, 7.81; N, 10.26.

4.1.8. Boc-Leu-Ala-OMe 11. See Ref. 24.

4.1.9. Boc-Leu-Ala-OH 12. See Ref. 25.

4.1.10. Boc-Leu-Ala-Phe-OMe 13. See Ref. 26.

4.1.11. Boc-Leu-Ala-Phe-OH 14. To Boc-Leu-Ala-Phe-OMe (3.70 g, 8 mmol), methanol (50 mL) and 2 M NaOH (20 mL) were added and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred at room temperature. After 10 h, methanol was removed under vacuum, the residue was taken in water (50 mL), washed with diethyl ether (2×50 mL). Then, pH of the aqueous layer was adjusted to 2 by adding 1 M HCl and it was extracted with ethyl acetate (3×40 mL). The extracts were pooled, dried over anhydrous sodium sulfate and evaporated under vacuum to yield a waxy solid.

Yield=87% (3.14 g, 7 mmol). ^1H NMR (DMSO-d_6, 300 MHz): δ 12.1 (br, 1H), 7.20–7.27 (m, 5H), 8.02 (d, J=7.5 Hz, 1H), 7.85 (d, J=7.9 Hz, 1H), 6.80 (d, J=8.8 Hz, 1H), 4.40 (m, 1H), 3.97 (m, 1H), 3.86 (m, 1H), 3.10 (m, 2H), 2.88 (d, J=4.4 Hz, 3H), 2.50 (m, 2H), 1.36 (s, 9H), 0.80–0.90 (m, 7H) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz): δ 11.35, 11.57, 18.19, 22.37, 22.93, 24.79, 24.82, 28.14, 31.17, 41.38, 52.23, 52.26, 58.43, 80.16, 127.14, 128.47, 129.15, 135.81, 170.18, 170.80, 171.71, 176.35 ppm. $[\alpha]_D^{27.8}$ −15.3 (c 2.10, $CHCl_3$). Elemental Anal. Calcd for $C_{23}H_{35}N_3O_6$ (449): C, 61.47; H, 7.79; N, 9.35. Found: C, 61.85; H, 8.16; N, 8.89.

4.1.12. Boc-Leu-Ala-Phe-Ala-OMe 5. The mixture of **14** (2.24 g, 5 mmol) in DMF (10 mL) was cooled in an ice-water bath. H-Ala-OMe was isolated from the corresponding methyl ester hydrochloride (1.40 g, 10 mmol) by neutralization with saturated $NaHCO_3$ solution, subsequent extraction with ethyl acetate and the ethyl acetate extract was concentrated to 7 mL. This was added to the reaction mixture, followed immediately by DCC (1 g, 5 mmol) and HOBt (0.70 g, 5 mmol). The reaction mixture was stirred for 3 days. The residue was taken in ethyl acetate (50 mL) and DCU was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine, 1 M sodium carbonate (3×50 mL), brine (2×50 mL), dried over anhydrous sodium sulfate and evaporated in vacuum to get 2.14 g (4 mmol) of white solid.

Yield=80% (2.14 g, 4 mmol). Mp 161–162 °C. IR (KBr): 1546, 1640, 1693, 1747, 2930, 2959, 3032, 3289 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 7.24–7.27 (m, 5H), 7.18 (d, J=8.4 Hz, 1H), 6.72 (d, J=9.4 Hz, 1H), 6.66 (d, J=9.4 Hz, 1H), 4.99 (d, J=7.1 Hz, 1H), 4.63 (m, 1H), 4.00 (m, 2H), 3.80 (m, 1H), 3.72 (s, 3H), 3.17 (m, 2H), 3.03 (m, 2H), 1.44 (s, 9H), 1.36 (d, J=7.2 Hz, 6H), 0.89–0.96 (m, 7H) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz): δ 11.23, 11.49, 18.95, 22.36, 22.80, 22.99, 24.74, 24.80, 28.16, 31.23, 35.09, 41.25, 51.69, 52.11, 52.20, 58.47, 80.18, 127.14, 128.47, 129.15, 135.81, 170.24, 170.85, 171.73, 171.80, 172.25 ppm. $[\alpha]_D^{27.8}$ −52.7 (c 2.10, $CHCl_3$). Mass spectral data (M+Na)$^+$=557.70, M_{calcd}=534. Elemental Anal. Calcd for $C_{27}H_{42}N_4O_7$ (534): C, 60.67; H, 7.86; N, 10.49. Found: C, 60.43; H, 7.25; N, 10.07.

4.1.13. Boc-Leu-Aib-OMe 15. See Ref. 27.

4.1.14. Boc-Leu-Aib-OH 16. See Ref. 27.

4.1.15. Boc-Leu-Aib-Phe-OMe 2. Boc-Leu-Aib-OH (6.06 g, 20 mmol) in DMF (20 mL) was cooled in an ice-water bath and H-Phe-OMe was isolated from the corresponding methyl ester hydrochloride (8.62 g, 40 mmol) by neutralization with saturated $NaHCO_3$ solution, subsequent extraction with ethyl acetate and the ethyl acetate extract was concentrated to 10 mL. It was added to the reaction mixture, followed immediately by DCC (4.12 g, 20 mmol) and HOBt (2.70 g, 20 mmol). The reaction mixture was stirred for 3 days. The residue was taken in ethyl acetate (60 mL) and the DCU was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine, 1 M sodium carbonate (3×50 mL), brine (2×50 mL), dried over anhydrous sodium sulfate and evaporated under *vacuum* to yield 8.11 g (17 mmol) of white solid. Purification was achieved by silica gel column (100–200 mesh) using ethyl acetate and toluene mixture (1:2) as an eluent. Single crystals were grown from dimethyl sulfoxide (DMSO) mixture by slow evaporation.

Yield=81% (8.11 g, 17 mmol). Mp 115–117 °C. IR (KBr): 1659, 1697, 3321, 3421 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): δ 7.22–7.30 (m, 5H), 7.13 (d, J=7.8 Hz, 1H), 6.89 (d, J=6.8 Hz, 1H), 6.61 (s, 1H), 4.81 (m, 1H), 3.98 (m, 1H), 3.70 (s, 3H), 3.11 (m, 2H), 1.60 (m, 2H), 1.49 (s, 9H), 1.44 (s, 6H), 1.25 (m, 1H), 0.94 (m, 6H) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz): δ 11.31, 11.49, 15.33, 18.18, 22.37, 24.38, 24.82, 28.04, 31.17, 37.03, 51.23, 56.26, 58.43, 80.24, 127.54, 129.06, 129.15, 136.81, 170.60, 170.80, 171.84, 173.58 ppm. $[\alpha]_D^{27.8}$ +5.3 (c 2.10, $CHCl_3$). Mass spectral data (M+Na)$^+$=500, M_{calcd}=477. Elemental Anal. Calcd for $C_{25}H_{39}N_3O_6$ (477): C, 62.89; N, 8.80; H, 8.17. Found: C, 63.06; N, 8.87; H, 7.90.

4.1.16. Boc-Leu-Aib-Phe-OH 17. To Boc-Leu-Aib-Phe-OMe (4.77 g, 10 mmol), methanol (50 mL) and 2 M NaOH (20 mL) were added and the progress of saponification was

monitored by thin layer chromatography (TLC). The reaction mixture was stirred at room temperature. After 10 h methanol was removed under vacuum, the residue was taken in water (50 mL), washed with diethyl ether (2×50 mL). Then, the pH of the aqueous layer was adjusted to 2 by adding 1 M HCl and it was extracted with ethyl acetate (3×40 mL). The extracts were pooled, dried over anhydrous sodium sulfate and evaporated under vacuum to yield a waxy solid.

Yield=70% (3.24 g, 7 mmol). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.56 (br, 1H), 7.85 (s, 1H), 7.47 (d, J=5.4 Hz, 1H), 7.23–7.13 (m, 5H), 6.90 (d, J=7.56 Hz, 1H), 4.36 (m, 1H), 3.85 (m, 1H), 2.86 (m, 2H), 1.56 (m, 2H), 1.34 (s, 9H), 1.26 (s, 6H), 0.84–0.80 (m, 7H) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz): δ 11.23, 11.47, 15.31, 18.16, 22.37, 24.38, 24.82, 28.04, 31.17, 37.03, 56.26, 58.43, 80.24, 127.54, 129.06, 129.15, 136.81, 170.69, 170.78, 171.81, 176.25 ppm. $[\alpha]_D^{27.8}$ −5.7 (c 2.10, $CHCl_3$). Elemental Anal. Calcd for $C_{24}H_{37}N_3O_6$ (463): C, 62.20; H, 7.99; N, 9.07. Found: C, 62.05; H, 8.16; N, 8.89

4.1.17. Boc-Leu-Aib-Phe-Aib-OMe 6. Boc-Leu-Aib-Phe-OH (2.32 g, 5 mmol) in DMF (10 mL) was cooled in an ice-water bath. H-Aib-OMe was isolated from the corresponding methyl ester hydrochloride (1.53 g, 10 mmol) by neutralization with saturated $NaHCO_3$, subsequent extraction with ethyl acetate and the ethyl acetate extract was then concentrated to 7 mL. This was added to the reaction mixture, followed immediately by DCC (1 g, 5 mmol) and HOBt (0.7 g, 5 mmol). The reaction mixture was stirred for 3 days. The residue was taken in ethyl acetate (50 mL) and DCU was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine, 1 M sodium carbonate (3×50 mL), brine (2×50 mL), dried over anhydrous sodium sulfate and evaporated under vacuum to get white solid. Purification was done by silica gel column (100–200 mesh) using ethyl acetate as an eluent. Single crystals were obtained from ethyl acetate solution by slow evaporation.

Yield=80% (2.25 g, 4 mmol). Mp 141–142 °C. IR (KBr): 1661, 1728, 3273, 3341 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.19–7.26 (m, 5H), 7.17 (s, 1H), 6.74 (d, J=8.1 Hz, 1H), 6.49 (s, 1H), 4.79 (d, J=5.1 Hz, 1H), 4.75 (m, 1H), 3.87 (m, 1H), 3.72 (s, 3H), 3.43 (m, 2H), 2.94 (m, 2H), 1.52 (s, 6H), 1.48 (s, 6H), 1.45 (s, 9H), 0.93 (m, 7H) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz): δ 11.49, 11.57, 15.47, 18.18, 22.37, 24.38, 24.82, 28.04, 31.17, 37.03, 52.03, 56.26, 57.43, 80.24, 128.54, 129.06, 129.15, 155.82, 170.37, 171.80, 173.84, 174.89 ppm. $[\alpha]_D^{27.8}$ −17.6 (c 2.10, $CHCl_3$). Mass spectral data $(M+Na)^+$=585.4, M_{calcd}=462. Elemental Anal. Calcd for $C_{29}H_{46}N_4O_7$ (562): C, 61.81; H, 8.35; N, 9.95. Found: C, 61.03; H, 8.57; N, 9.19.

4.2. NMR experiments

All NMR studies were carried out on Brüker DPX 300 and DRX 500 MHz spectrometer at 300 K in $CDCl_3$ and DMSO-d_6. Peptide concentrations were in the range 1–10 mM.

4.3. Mass spectrometry

Mass spectra of peptides were recorded on a Micromass Zabspec Hybrid Sector-TOF by positive mode electronspray ionization using a 1% solution of acetic acid in methanol/water as liquid carrier.

4.4. FTIR spectroscopy

The FTIR spectra were taken using Shimadzu (Japan) model FTIR spectrophotometer. The solid-state FTIR measurements were performed using the KBr disk technique.

4.5. Morphological studies

Morphologies of the reported compounds were investigated using transmission electron microscopy (TEM). The transmission electron microscopic studies of these peptides were performed using a small amount of the solution of the corresponding compound on carbon-coated copper grids (200 mesh) by slow evaporation and allowed to dry in vacuum at 30 °C for 2 days. Images were taken at an accelerating voltage of 100 kV both in the transmission mode and diffraction mode (0.5% w/v, camera length 0.8 m). TEM was done by a Hitachi 600 electron microscope.

4.6. Congo red binding study

An alkaline saturated Congo red solution was prepared. The peptide fibrils were stained by alkaline Congo red solution (80% methanol/20% glass distilled water containing 10 mL of 1% NaOH) for 2 min and then the excess stain (Congo red) was removed by rinsing the stained fibril with 80% methanol/20% glass distilled water solution for several times. The stained fibrils were dried in vacuum at room temperature for 24 h, then visualized at 100× or 500× magnification and birefringence was observed between crossed polarizer.

4.7. Single crystal X-ray diffraction studies

For peptide **2**, intensity data of orthorhombic colorless crystals 0.48×0.52×0.65 of space group $P2_12_12_1$ were collected with Mo Kα radiation, ω scan using graphite-monochromated Siemens P4 diffractometer. The crystal was positioned at 70 mm from the image plate. One hundred frames were measured at 2° intervals with a counting time of 2 min to give 4179 independent reflections of which 2887 had $I>2\sigma(I)$. The structure was solved by direct methods (SHELXS-97)[28] and refined against F(obs)×2 by full-matrix least squares (SHELXL-97).[29] Hydrogen atoms were placed at calculated positions and allowed to ride on their parent atoms. Terminal reliability indices were $R1$=0.058 [$I>2\sigma(I)$], $wR2$=0.198 for 340 refined parameters, S=1.21, min./max. res. 0.19/−0.19 eÅ^{-3}.

For peptide **6**, intensity data were collected with Mo Kα radiation using the MAR research Image Plate System. The crystal was positioned at 70 mm from the image plate. One hundred frames were measured at 2° intervals with a counting time of 5 min to give 3474 independent reflections. Data analysis was carried out with the XDS program.[30] The structure was solved using direct methods with the SHELX-86 program.[31] The non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms were included in geometric positions and given thermal parameters equivalent to 1.2 times those of

the atom to which they were attached. The structure was refined on F^2 using SHELXL.[32] The final R values were $R1=0.0610$ and $wR2=0.1779$ for 1716 data with $I>2\sigma(I)$. The largest peak and hole in the final difference Fourier were 0.20 and -0.20 eÅ^{-3}.

Crystallographic data have been deposited at the Cambridge Crystallographic Data Center reference CCDC-176329 for peptide **2** and CCDC 184602 for peptide **6**.

Acknowledgements

We thank EPSRC and the University of Reading, UK for funds for the Image Plate System. A.B. acknowledges Department of Science and Technology, New Delhi, India for the grant No. SR/S5/OC-29/2003.

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Tetrahedron 62 (2006) 6379–6387

Three different fluorescent responses to transition metal ions using receptors based on 1,2-bis- and 1,2,4,5-tetrakis-(8-hydroxyquinolinoxymethyl)benzene

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Received 12 December 2005; revised 25 March 2006; accepted 11 April 2006
Available online 8 May 2006

Abstract—The dipod 1,2-bis(8-hydroxyquinolinoxymethyl)benzene (**3**) and tetrapod 1,2,4,5-tetrakis(8-hydroxyquinolinoxymethyl)benzene (**5**) have been synthesized through nucleophilic substitution of respective 1,2-bis(bromomethyl)benzene (**2**) and 1,2,4,5-tetra(bromomethyl)benzene (**4**) with 8-hydroxyquinoline (**1**). For comparison, 1,3,5-tris(8-hydroxyquinolinoxymethyl)benzene derivatives (**7a** and **7b**) have been obtained. The complexation behavior of these podands towards Ag^+, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} metal ions has been investigated in acetonitrile by fluorescence spectroscopy. The sterically crowded 1,2,4,5-tetrapod **5** displays unique fluorescence 'ON–OFF–ON' switching through fluorescence quenching (λ_{max} 395 nm, switch OFF) with <1.0 equiv of Ag^+ and fluorescence enhancement (λ_{max} 495 nm, switch ON) with >3 equiv Ag^+ and can be used for estimation of two different concentrations of Ag^+ at two different wavelengths. The addition of Cu^{2+}, Ni^{2+}, and Co^{2+} metal ions to tetrapod **5** causes fluorescence quenching, i.e., 'ON–OFF' phenomena at λ_{max} 395 nm for <10 μM (1 equiv) of these ions but addition of Zn^{2+} and Cd^{2+} to tetrapod **5** results in fluorescence enhancement with a gradual shift of λ_{em} from 395 to 432 and 418 nm, respectively. Similarly, dipod **3** behaves as an 'ON–OFF–ON' switch with Ag^+, an 'ON–OFF' switch with Cu^{2+}, and an 'OFF–ON' switch with Zn^{2+}. The placement of quinolinoxymethyl groups at the 1,3,5-positions of benzene ring in tripod **7a**–**b** leads to simultaneous fluorescence quenching at λ_{max} 380 nm and enhancement at λ_{max} 490 nm with both Ag^+ and Cu^{2+}. This behavior is in parallel with 8-methoxyquinoline **8**. The rationalization of these results in terms of metal ion coordination and protonation of podands shows that 1,2 placement of quinoline units in tetrapod **5** and dipod **3** causes three different fluorescent responses, i.e., 'ON–OFF–ON', 'ON–OFF', and 'OFF–ON' due to metal ion coordination of different transition metal ions and 1, 3, and 5 placement of three quinolines in tripod **7**, the protonation of quinolines is preferred over metal ion coordination. In general, the greater number of quinoline units coordinated per metal ion in **5** compared with the other podands points to organization of the four quinoline moieties around metal ions in the case of **5**.

1. Introduction

The design and synthesis of target selective receptors with luminescent signaling systems for direct measurement of changes in emission intensities or wavelength, arising due to perturbation upon ion or molecular recognition, have attained a central position in supramolecular chemistry.[1–2]

These recognition phenomena depend primarily on multiple host-guest interactions. Locking of conformations of both the host and the guest make negative contributions to total free energies of the system.[3] So, in addition to the complementarity of binding sites, the correct spatial placement of subunits[4] constitutes a major criterion for designing new receptors.

8-Hydroxyquinoline (oxine) and its derivatives are known to be the best chelaters after EDTA and its derivatives due to their guest modulated chromogenic and fluorescent behavior. Accordingly, they have attained prime significance and have been used in chromatography,[5] detection of metal ions,[6] in organic light emitting diode devices,[7] and in electrochemiluminescence[8] etc. In the case of planar platforms, the contribution of molecular architectures arising due to placement of two or three subunits at 1,3- or 1,3,5-positions on a benzene ring has been well studied in molecular recognition.[9] However, the supramolecular behavior of receptors possessing two or four such functional groups placed symmetrically at 1,2- and 1,2,4,5-positions of a benzene ring has been scarcely studied.[10]

In continuation of our work[11] on syntheses of receptors possessing 8-hydroxyquinoline moieties as the only binding sites, in the present work[12] a fluorescent dipod **3** and a tetrapod **5** possessing two and four 8-hydroxyquinoline units at 1,2- and 1,2,4,5-positions of benzene ring, respectively, have been synthesized and their binding features

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doi:10.1016/j.tet.2006.04.035

towards transition metal ions have been evaluated by fluorescence studies in CH_3CN. For comparison the behavior of 1,3,5-tris(8-hydroxyquinolinoxymethyl)benzene derivatives **7** and 8-methoxyquinoline **8** has been also studied. Dipod **3** and tetrapod **5** show unique switch-like 'ON–OFF–ON' behavior towards Ag^+. Dipod **3** and tetrapod **5** provide three different fluorescent responses to transition metal ions as 'ON–OFF–ON' switch with Ag^+, 'ON–OFF' switch with Cu^{2+}, Co^{2+}, and Ni^{2+} and 'OFF–ON' switch with Zn^{2+} and Cd^{2+}. On moving to 1,3,5-tripods **7** and 8-methoxyquinoline **8** due to preferred protonation over metal ion coordination the occurrence of these phenomena is lowered. To the best of our knowledge, this constitutes the first example of a benzene-based tetrapod organization.

2. Results and discussion

2.1. Synthesis of podands 3, 5, and 7

The nucleophilic substitution of 1,2-bis(bromomethyl)benzene (**2**) with 8-hydroxyquinoline under phase transfer catalyzed conditions provided a white solid. NMR spectroscopy and COSY experiments allowed the spectral assignment. For assigning the signals in the multiplet region, the ^1H NMR spectrum of **3** after addition of 0.1 equiv $AgNO_3$ was recorded (Fig. 1). These spectral data along with ^{13}C NMR spectral data corroborated the structure **3** for this compound.

Similarly, the nucleophilic substitution of 1,2,4,5-tetrakis-(bromomethyl)benzene (**4**) with 8-hydroxyquinoline (**1**) under phase transfer catalyzed conditions provided **5** as a white solid. The positions of all the protons have been assigned by decoupling experiment. These spectral data and ^1H–^1H COSY and ^{13}C NMR spectral data confirmed the structure **5** for this compound. The reactions of tribromides **6a** and **6b** with **1** gave tripods **7a** and **7b**, respectively (Scheme 1).

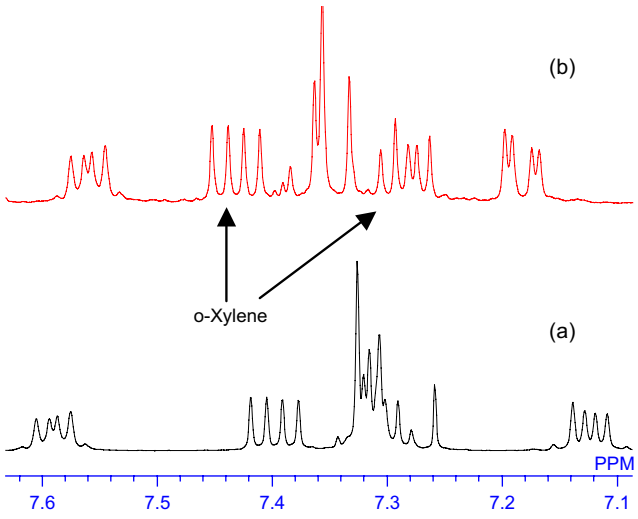
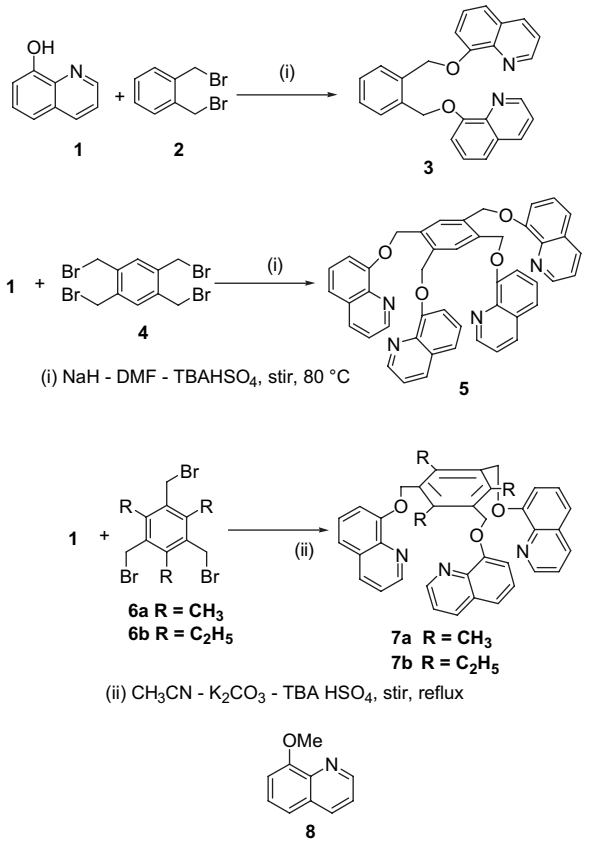


Figure 1. ^1H NMR of (a) dipod **3** (b) dipod **3**+0.1 equiv $AgNO_3$.



Scheme 1.

2.2. Photophysical behavior of podands 3, 5, 7, and 8 towards transition metal ions

Podands **3**, **5**, **7**, and **8** (10 μM, CH_3CN) in their UV–vis spectra exhibit broad absorption bands at λ_{max} 305 nm due to the 8-alkoxyquinoline moiety. The solutions of podands **3**, **5**, **7**, and **8** on excitation at λ_{max} 305 nm exhibit fluorescence spectra typical of the 8-hydroxyquinoline moieties with λ_{max} at 385 nm, and remain stable within λ_{ex} 305–360 nm. In this work, all the studies have been performed using λ_{ex} 345 nm except in cases when stated otherwise. In the concentration range 1–10 μM, the fluorescence of **3**, **5**, **7**, and **8** is directly proportional to their concentration. This linear increase in fluorescence with concentration indicates that these podands are not susceptible to self quenching and to aggregation processes in this concentration range.

2.2.1. Photophysical behavior of tetrapod 5 towards transition metal ions. In the preliminary investigations, tetrapod **5** (10 μM, CH_3CN) on addition of 50 μM concd of Co^{2+}, Cu^{2+}, and Ni^{2+}, respectively, shows >90% quenching of fluorescence at λ_{max} 385 nm whereas Zn^{2+} and Cd^{2+} ions cause >10 times fluorescence enhancement with a gradual bathochromic shift of λ_{max} from 395 to 432 nm. However on addition of Ag^+, up to 10 μM to tetrapod **5** shows fluorescence quenching at λ_{max} 395 nm and at higher concentration of Ag^+ a delayed emission band appears at λ_{max} 495 nm. Therefore **5** undergoes 'ON–OFF' switching with Co^{2+}, Cu^{2+}, and Ni^{2+} ions, 'OFF–ON' switching with Zn^{2+} and Cd^{2+} and 'ON–OFF–ON' switching with Ag^+.

A solution of tetrapod **5** (10 μM, CH_3CN) on the addition of $AgNO_3$ showed fluorescence quenching, which gradually increased with increasing concentration of $AgNO_3$. A plot of concentration of $AgNO_3$ versus fluorescence at 395 nm showed a linear decrease with up to 15 μM $AgNO_3$ (1.5 equiv) and then a plateau was achieved. On addition of further $AgNO_3$, a new fluorescence band emerged at λ_{max} 495 nm. The fluorescence intensity at 495 nm gradually increased with increased concentration of $AgNO_3$ (Figs. 2–4).

The spectral fitting of the data showed the formation of ML, M_2L, and M_4L complexes with $\log \beta_{ML}=6.8\pm0.2$, $\log \beta_{M_2L}=12.2\pm0.3$, and $\log \beta_{M_4L}=17.3\pm0.6$ (Table 1). Analysis of the distribution of the different species showed that their concentration varied significantly with the concentration of Ag^+. In a 1:1 mixture of **5** and $AgNO_3$, an almost maximum concentration of the 1:1 complex (>75%) is observed. At this concentration nearly 10% of 2:1 $2AgNO_3$ tetrapod **5** is formed. On further increase in the concentration of Ag^+ to 200 μM, the formation of M_2L increases to 97% along with <1% of a 4:1 $4AgNO_3$ tetrapod **5** complex.

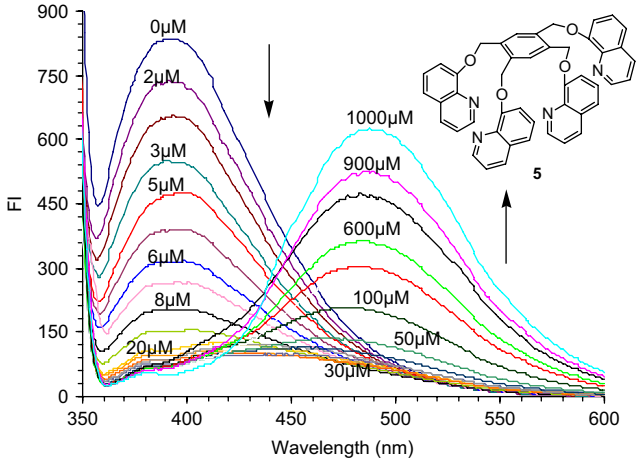


Figure 2. The effect of Ag^+ on the fluorescence spectrum of tetrapod **5** (10 μM).

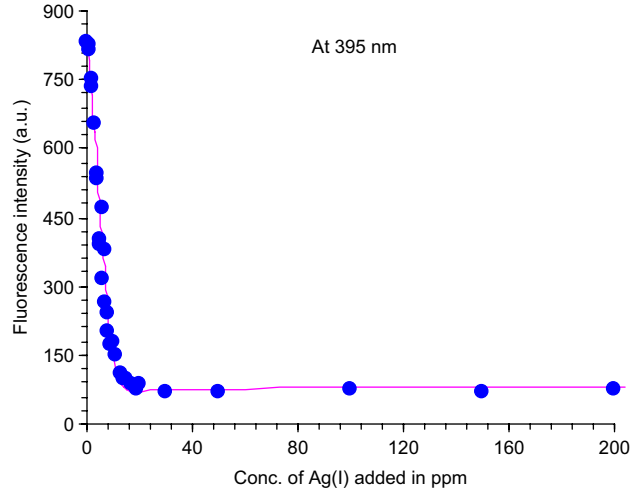


Figure 3. The curve fitting of fluorescence spectral data of **5** at 395 nm on addition of $AgNO_3$. (●) Experimental points, (—) fitted line.

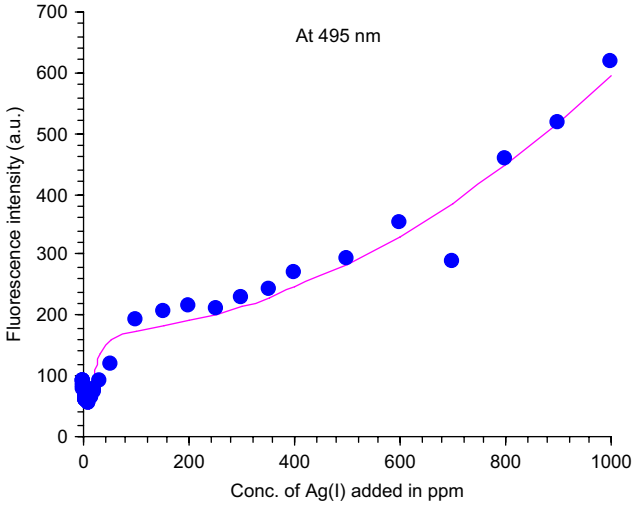


Figure 4. The curve fitting of fluorescence spectral data of **5** (10 μM in CH_3CN) at 495 nm on addition of $AgNO_3$. (●) Experimental points, (—) fitted line.

Even at 1000 μM $AgNO_3$, the 4:1 $4AgNO_3$ tetrapod **5** complex is only formed to 22% extent along with 78% of the 2:1 complex. Due to the existence of number of different stoichiometric complexes of **5** with Ag^+, isosbestic points were not observed (Fig. 5).

The UV–vis spectrum of tetrapod **5** (50 μM, CH_3CN) shows a gradual bathochromic shift from 305 to 314 nm on the addition of 50 μM $AgNO_3$. However, the absorbance between 300–350 nm shows a small (<5%) enhancement in contrast to quenching in fluorescence. Therefore, in tetrapod **5**, the lack of any change in the UV–vis spectrum and 'ON–OFF–ON' switching behavior in fluorescence on addition of $AgNO_3$ point to multiple interactions of Ag^+ with the excited state of **5**.

A solution of tetrapod **5** (10 μM, CH_3CN) on addition of $Cu(NO_3)_2$ showed fluorescence quenching, which gradually increased with increasing concentration of $Cu(NO_3)_2$. On addition of >15 μM $Cu(NO_3)_2$ no further change in fluorescence spectrum was observed. Therefore, in contrast to 'ON–OFF–ON' behavior towards Ag^+, tetrapod **5** with Cu(II) shows 'ON–OFF' behavior. The plot of concentration of $Cu(NO_3)_2$ versus fluorescence at 395 nm shows a linear decrease up to 10 μM $Cu(NO_3)_2$ (1 equiv) and then a plateau is achieved (Fig. 6). The spectral fitting of the data showed the formation of only ML complex with $\log \beta_{ML}=6.6\pm0.1$. In a 1:1 mixture of **5** and $Cu(NO_3)_2$, formation of 1:1 complex (>90%) was observed. Similarly, other paramagnetic ions Co(II) and Ni(II) with **5** showed 'ON–OFF' switch with $\log \beta_{ML}$ value 6.6±0.1 and 5.77±0.06, respectively (Table 1).

Tetrapod **5** on the addition of Zn^{2+} and Cd^{2+} shows fluorescence enhancement. On using λ_{ex} 345 nm, on addition of Zn^{2+} and Cd^{2+} (0.3 equiv) to a solution of **5**, the fluorescence went off scale. So, all studies of Zn^{2+} and Cd^{2+} were performed at λ_{ex} 360 nm. The plot of fluorescence intensity of **5** against concentration of Zn^{2+} shows a gradual fluorescence enhancement using 1–30 μM Zn^{2+} concentration with a gradual red shift of λ_{max} from 385 to 432 nm and between

Table 1. Fluorescence behavior and log β_{MXL} values for podands **5**, **3**, and **7a–b** towards transition metal ions

Serial no.	Metal ion	Fq/Fe[a]	λ_{max} (nm)	log β_{ML_2}	log β_{ML}	log β_{M_2L}	log β_{M_4L}
Tetrapod ***5***							
1	Ag^{+}	'ON–OFF–ON'	$385–385_{fe}–495_{fe}$		6.8±0.2	12.2±0.3	17.3±0.6
2	Cu^{2+}	'ON–OFF'	$385–385_{fq}$		6.6±0.1		
3	Ni^{2+}	'ON–OFF'	$385–385_{fq}$		5.7±0.1		
4	Co^{2+}	'ON–OFF'	$385–385_{fq}$		6.5±0.1		
5	Zn^{2+}	'OFF–ON'	$385–432_{fe}$		5.1±0.1		
6	Cd^{2+}	'OFF–ON'	$385–423_{fe}$		6.6±0.1	11.8±0.5	
Dipod ***3***							
7	Ag^{+}	'ON–OFF–ON'	$385–385_{fe}–495_{fe}$		6.3±0.2	9.5±0.2	
8	Cu^{2+}	'ON–OFF'	$385–385_{fq}$		4.9±0.2		
9	Zn^{2+}	'OFF–ON'	$385–432_{fe}$	14.1±0.4	7.9±0.3		
					log β_{ML}	log β_{M_2L}	log β_{M_3L}
Tripod ***7a***							
10	Ag^{+}	Set on 'OFF-ON'	$385_{fq}–490_{fe}$			11.00±0.06	15.3±0.2
11	Ag^{+}	From UV–vis	$301_{Al}–360_{Ae}$		6.30±0.3	10.50±0.48	15.3±0.3
12	Cu^{2+}	Set on 'OFF–ON'	$385_{fq}–490_{fe}$		5.36±0.7		14.9±0.1
Tripod ***7b***							
13	Ag^{+}	Set on 'OFF–ON'	$385_{fq}–490_{fe}$			10.6±0.1	14.6±0.3
14	Ag^{+}	From UV–vis	$301_{Al}–360_{Ae}$		6.1±0.2	10.6±0.3	15.1±0.3

[a] Fq=fluorescence quenching; Fe=fluorescence enhancement; Al=absorbance lowering; Ae=absorbance enhancement.

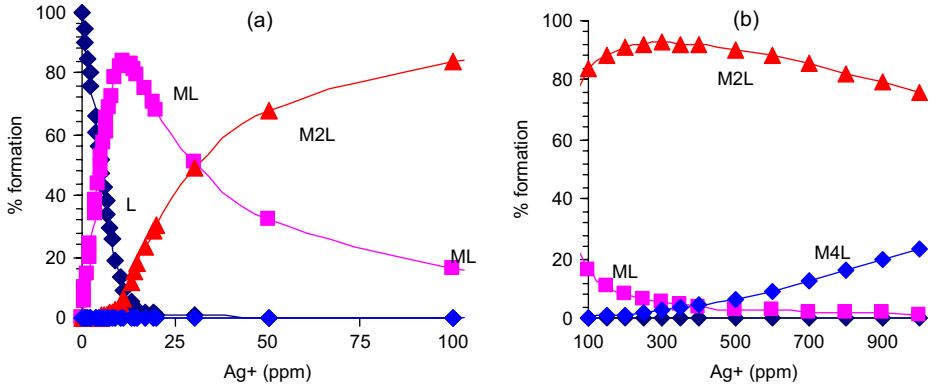


Figure 5. The distribution of ML, M_2L, and M_4L species on addition of $AgNO_3$ to **5** (10 μM in CH_3CN) (a) 0–100 μM $AgNO_3$ (b) 100–1000 μM $AgNO_3$.

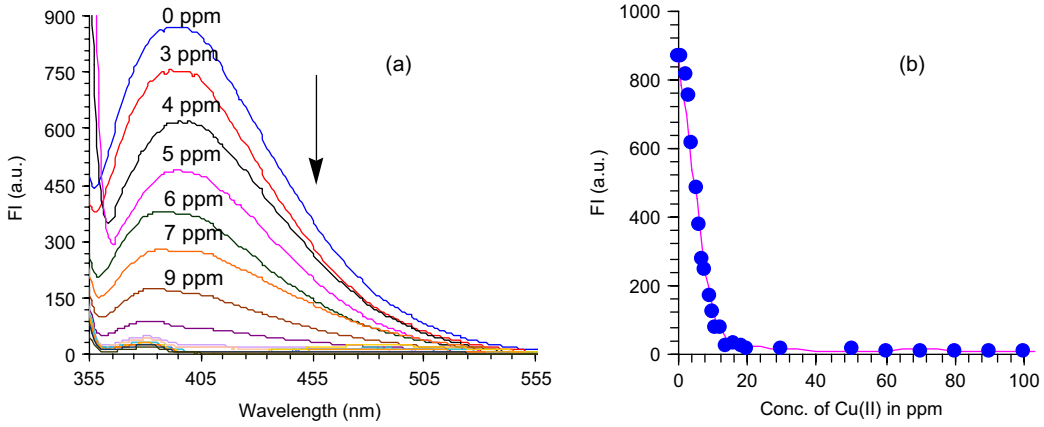


Figure 6. (a) The effect of Cu^{2+} on the fluorescence spectrum of tetrapod **5** in acetonitrile and (b) curve fitting of change in FI of tetrapod **5** (10 μM) at 395 nm on addition of $Cu(NO_3)_2$. (●) Experimental points, (—) fitted line.

30 and 100 μM Zn^{2+}, a plateau is achieved. The spectral fitting of the data showed the formation of ML with log β_{ML}=5.11±0.05. The formation of >80% Zn^{2+}:**5** complex is observed at 50 μM Zn^{2+} (5 equiv).

Similarly, the addition of $Cd(NO_3)_2$ to a solution of **5** caused fluorescence enhancement with a gradual red shift in λ_{max} from 385 to 418 nm (Fig. 7). These data showed the formation of M_2L and ML complexes with log β_{M_2L}=11.83±0.5

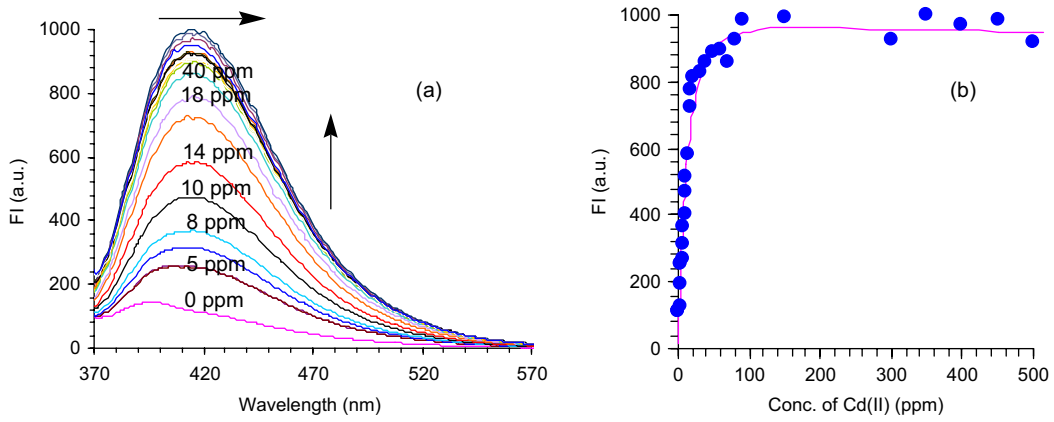


Figure 7. (a) The effect of Cd^{2+} on the fluorescence spectrum of tetrapod **5** and (b) curve fitting of change in FI of tetrapod **5** (10 μM) at 418 nm on addition of $Cd(NO_3)_2$. (●) Experimental points, (—) fitted line.

and log β_{ML}=6.7±0.1. In a 1:2 mixture of **5** and $Cd(NO_3)_2$, an almost maximum concentration of the 1:1 complex (47%) along with small amounts <5% of M_2L was observed. On further increasing the **5**:$Cd(NO_3)_2$ ratio to 1:10, the formation of M_2L increased to nearly 70% along with 30% of ML complex.

2.2.2. Photophysical behavior of dipod 3 towards transition metal ions. A solution of dipod **3** on excitation at λ_{ex} 345 nm showed a gradual decrease in fluorescence on addition of $AgNO_3$, which gradually increased on increasing the concentration of $AgNO_3$.

A plot of concentration of $AgNO_3$ versus fluorescence at 385 nm shows a linear decrease with up to 50 μM $AgNO_3$ (5 equiv) and then a plateau is achieved. During addition of $AgNO_3$, between 10–30 μM (1–3 equiv), no significant change in fluorescence at 395 nm or 500 nm was observed. However, on further addition of $AgNO_3$ a fluorescence band at λ_{max} 500 nm appeared and its intensity gradually increased with an increase in the concentration of $AgNO_3$. The spectral fitting of the titration data of dipod **3** with $AgNO_3$ shows the formation of ML and M_2L complexes with log β_{ML}=6.3±0.2 and log β_{M_2L}=9.5±0.2, respectively (Fig. 8).

Analysis of the distribution of different species showed that their concentration varied significantly with the concentration of Ag^+. In a 1:2 mixture of **3** and $AgNO_3$, an almost nearly maximum concentration of a 1:1 complex (94%) was observed. At this concentration nearly 1% of the 2:1 $2AgNO_3$ dipod **3** was observed. On further increasing the concentration of Ag^+ to 1000 μM, the formation of M_2L increases to 60% along with 40% of the ML complex (Fig. 9).

Dipod **3** (10 μM, CH_3CN) on addition of $Cu(NO_3)_2$ showed fluorescence quenching, which gradually increased with increasing concentration of $Cu(NO_3)_2$. A plot of concentration of $Cu(NO_3)_2$ versus fluorescence at 385 nm shows a fluorescence decrease up to 50 μM and converges for the formation of the ML complex.

Dipod **3**, on addition of $Zn(NO_3)_2$ showed fluorescence enhancement, which goes out of scale at λ_{ex} 343 nm. So, further studies were carried out at λ_{ex} 350 nm. The plot of, fluorescence intensity of **3** against concentration of Zn^{2+} shows gradual fluorescence enhancement from 1 to 50 μM Zn^{2+} concentration with a gradual red shift of the λ_{max} from 385 to 432 nm, and between 50 and 100 μM Zn^{2+} the fluorescence achieved a plateau (Fig. 10). The spectral fitting of the data showed the formation of only ML_2 and ML

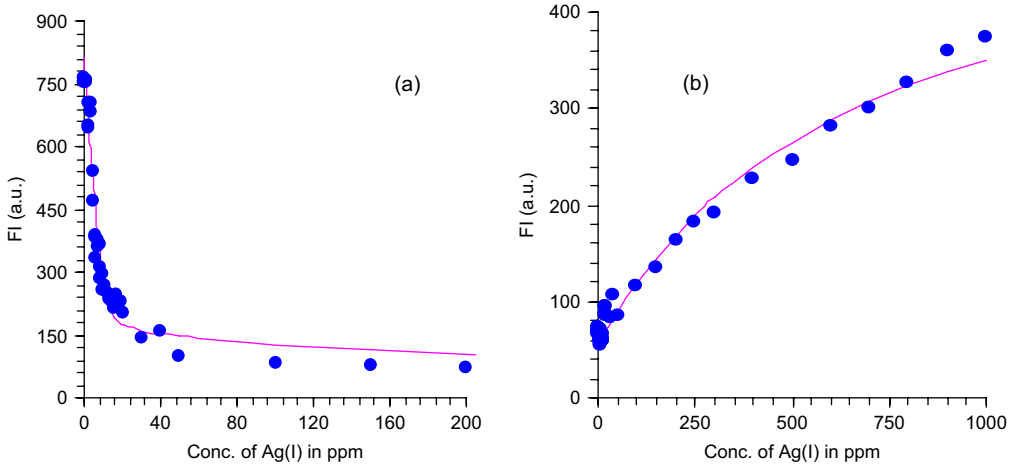


Figure 8. The curve fitting of fluorescence spectral data of **3** on addition of Ag^+: (a) at 385 nm and (b) at 495 nm, respectively. (●) Experimental points, (—) fitted line.

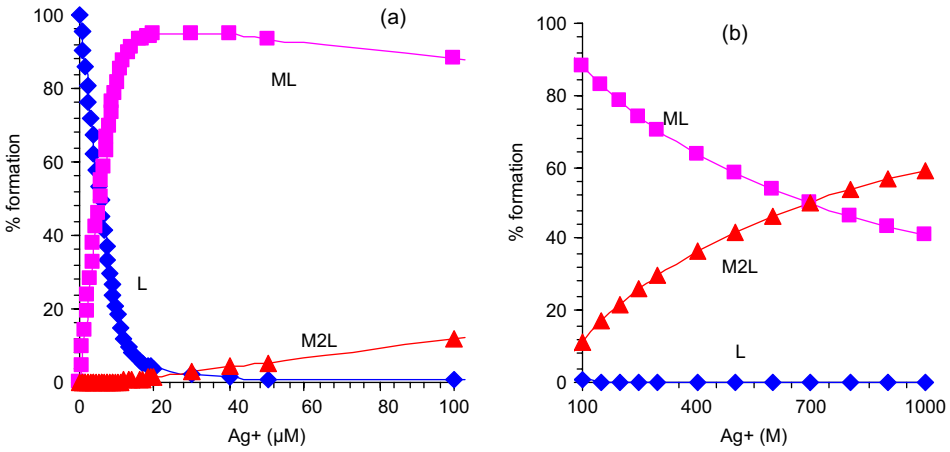


Figure 9. The distribution of ML and M_2L species on addition of $AgNO_3$ to **3** (10 μM in CH_3CN): (a) 0–100 μM $AgNO_3$ (b) 100–1000 μM $AgNO_3$.

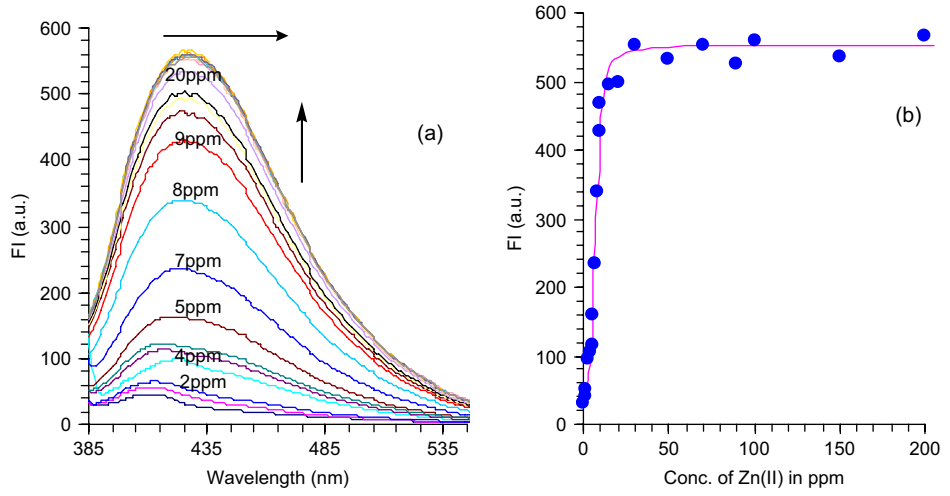


Figure 10. (a) The effect of Zn^{2+} on the fluorescence spectrum of dipod **3** and (b) curve fitting of change in FI of dipod **3** (10 μM) at 428 nm on addition of $Zn(NO_3)_2$. (●) Experimental points, (—) fitted line.

complexes with $\log \beta_{ML_2}=14.10\pm0.48$ and $\log \beta_{ML}=7.9\pm0.3$. In a 1:0.5 mixture of **3** and $Zn(NO_3)_2$, formation of ML_2 complex (>34%) is observed. At this concentration nearly 15% of 1:1 $Zn(NO_3)_2$ dipod **3** is formed. On further increasing the concentration of Zn^{2+} to 50 μM, the formation of ML_2 completely vanishes and 99% ML complex is achieved.

Dipod **3** (10 μM, CH_3CN) in its UV–vis spectrum showed a gradual bathochromic shift from 301 to 315 nm upto addition of 500 μM $AgNO_3$, $Cu(NO_3)_2$, and $Zn(NO_3)_2$. The absorbance between 340 and 360 nm showed small (<5%) enhancement in contrast to >90% fluorescence quenching (with Ag^+ and Cu^{2+}) or >10 times fluorescence enhancement (with Zn^{2+}). Therefore, dipod **3** in parallel with tetrapod **5** on addition of metal ions shows both fluorescence enhancement and fluorescence quenching without any significant change in absorbance in their UV–vis spectra.

2.2.3. Photophysical behavior of tripod 7a–b and 8 towards transition metal ions. Tripods **7a–b** on addition of 100 μM concd of Ag^+ and Cu^{2+}, respectively, showed simultaneous quenching of fluorescence at λ_{max} 380 nm and fluorescence enhancement at λ_{max} 490 nm, and with Zn^{2+} showed erratic behavior both in terms of λ_{max} and fluorescence intensity (FI).

Tripods **7a** and **7b** showed quite similar results and replacement of methyl by ethyl groups on the benzene ring does not have any effect on their metal ion complexation behavior. Tripods **7a–b** (1 μM, CH_3CN) showed fluorescence quenching at 380 nm with simultaneous enhancement at 490 nm on addition of up to 10 μM $AgNO_3$. On further addition of $AgNO_3$ the fluorescence at 380 nm trailed to a small residual value and at 490 nm the emission showed a gradual increase. The fluorescence spectra had two isosbestic points at 431 and 450 nm for both **7a** and **7b** with the formation of M_2L and M_3L complexes (Table 1). In a 1:10 mixture of **7a/7b** and $AgNO_3$ (10 μM), an almost maximum concentration of the M_2L complex (>74%) is observed. At this concentration nearly 13% of M_3L is formed. On further increasing the concentration of $AgNO_3$ to 100 μM, the formation of M_3L increases to 67% along with 33% of M_2L complex.

The UV–vis spectrum of tripod **7a/7b** (10 μM, CH_3CN) showed a simultaneous decrease in absorbance at λ_{max}

301 nm and an increase in the absorbance at λ_{max} 360 nm on gradual addition of $AgNO_3$ between 1 and 1000 μM (Fig. 11). All the spectral lines passed through an isosbestic point at 334 nm. These data converge to the formation of ML, M_2L, and M_3L complexes (Table 1). The similar stability constants arising from both UV–vis and fluorescence spectra data (Table 1, entries 10, 11, 13, and 14) confirm their concomitant presence.

Similarly, addition of $Cu(NO_3)_2$ to **7a/7b** showed simultaneous fluorescence quenching at λ_{max} 380 nm and enhancement at λ_{max} 490 nm with two isosbestic points at 431 and 450 nm and converged to the formation of only ML and M_3L complexes (Table 1). In a 1:10 mixture of **7a** and $Cu(NO_3)_2$, the formation of ML (58%) and M_3L complexes (16%) was observed and at 100 μM Cu^{2+} only formation of M_3L was observed.

Characteristically, like tripods **7a/7b**, 8-methoxyquinoline **8** with both $AgNO_3$ and $Cu(NO_3)_2$ showed simultaneous fluorescence quenching at λ_{max} 395 nm and enhancement at λ_{max} 500 nm due to the formation of ML complex with $\log \beta_{ML}=5.2\pm0.05$. Also, like tripods **7**, UV–vis spectrum of 8-methoxyquinoline **8** on gradual addition of $AgNO_3$ or $Cu(NO_3)_2$ showed simultaneous decrease in absorbance at λ_{max} 301 nm and increase in absorbance at λ_{max} 360 nm.

Therefore, podands **3** and **5**, where quinoline units are placed at adjacent carbons of benzene ring, show differential fluorescence phenomena with transition metal ions without any significant change in their UV–vis spectra but, tripods **7a/7b** and 8-methoxyquinoline **8**, exhibit a fluorescence change, which is parallel to the change in their UV–vis spectra.

A plot of the number of quinoline units/Ag^+ cation versus concentration of Ag^+ shows that both dipod **3** and tetrapod **5** organize higher numbers of quinoline units around Ag^+ than in the case of 8-methoxyquinoline **8** and tripod **7** (Fig. 12). At less than 5 equiv of $AgNO_3$, the tetrapod **5** shows significantly higher numbers of quinoline units around Ag^+ than in the case of **3**, which in turn shows a higher number of quinolines per Ag^+ than in the cases of **7** and **8**.

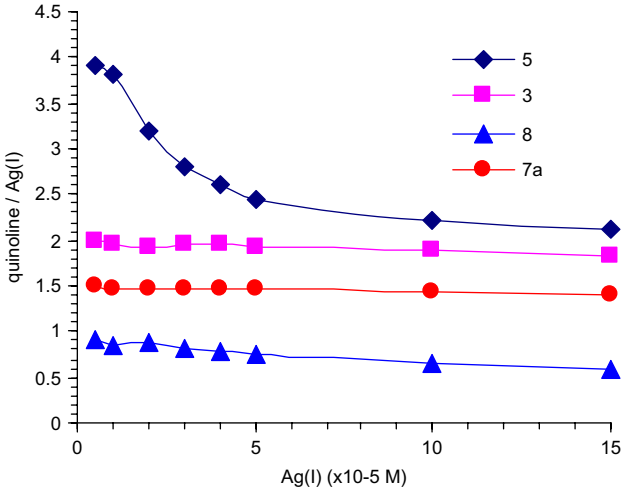


Figure 12. The plot of number of quinoline units per Ag^+ in podands **3**, **5**, **7**, and **8**.

The formation of only ML complexes by **5** with other transition metal ions further exhibits the organization of four quinoline moieties around each metal ion. All these results clearly point to the organization of four quinoline units around metal ions in the case of **5**.

In order to rationalize if some of these metal ion induced fluorescence phenomenon arise due to protonation[13] of the quinoline moieties, the podands **3**, **5**, **7a**, and **8** were titrated against perchloric acid. It is found that in case of dipod **3** and tetrapod **5**, the protonation with $HClO_4$ has much stronger effect on 'ON–OFF–ON' phenomenon. Therefore, dipod **3** and tetrapod **5** on addition of metal ions show change in fluorescence due to metal ion coordination. However, in case of tripods **7a** and **7b** and monopod **8**, the changes in fluorescence behavior on addition of metal ions are in parallel with protonation and could be due to metal salt mediated protonation of tripod **7** and monopod **8**.

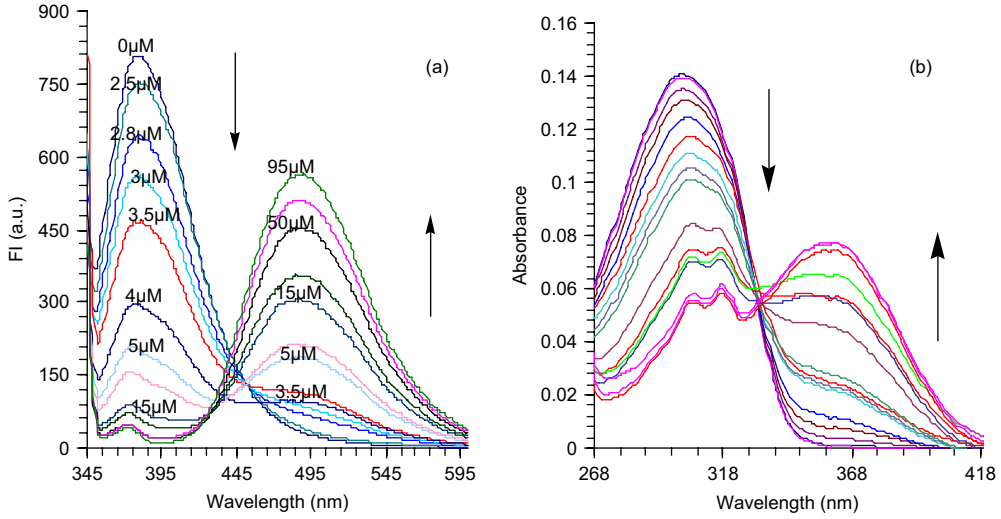


Figure 11. The effect of Ag^+ on: (a) fluorescence spectrum and (b) UV–vis spectrum of **7a**.

3. Experimental

3.1. General details

Melting points were determined in capillaries and are uncorrected. ^{1}H NMR spectra were recorded on JEOL Al 300 MHz instrument using $CDCl_3$ solution containing tetramethylsilane as an internal standard. The chemical shifts are reported in δ values relative to TMS and coupling constants (J) are expressed in hertz. ^{13}C NMR spectra were recorded at 75 MHz and values are reported relative to $CDCl_3$ signal at δ 77.0. Chromatography was performed with silica gel 100–200 mesh and the reactions were monitored by thin layer chromatography (TLC) with glass plates coated with silica gel HF-254. 1,2-Bis(bromomethyl)benzene[14] (**2**), 1,2,4,5-tetrakis(bromomethyl)benzene[15] (**4**), 1,3,5-trimethyl-2,4,6-tris(bromomethyl)benzene[16] (**6a**), 1,3,5-triethyl-2,4,6-tris(bromomethyl)benzene[17] (**6b**), and 8-methoxyquinoline[18] were synthesized according to reported procedures.

3.1.1. 1,2-Bis(8-hydroxyquinolinoxymethyl)benzene (3). A solution of 8-hydroxyquinoline (1.38 g, 9.5 mmol), NaH (pre-washed with hexane) (380 mg, 15.8 mmol), and tetrabutylammonium hydrogen sulfate (20 mg) (catalyst) in DMF (30 ml) was stirred at 80 °C. After 30 min, 1,2-bis(bromomethyl)benzene (**2**) (1.00 g, 3.8 mmol) was added and stirring was continued at 80 °C. After completion of the reaction (tlc, 24 h), the solid residue was filtered off and was washed with ethyl acetate. The combined filtrate was evaporated under vacuum and the solid residue was purified by column chromatography over silica gel (60–120 mesh) using a mixture of CH_2Cl_2/ethyl acetate/MeOH (80:17:3, v/v) to obtain pure **5**, 716 mg, 48%, white solid, mp 112–115 °C (CH_3CN), FAB mass m/z 392 (M^++H); ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.62 (s, 2×OCH_2, 4H), 7.12 (dd, J_1=5.8 Hz, J_2=3.0 Hz, 2H, ArH), 7.28–7.34 (m, 6H, 2×HQ-H6, 5, 7), 7.40 (dd, J_1=8.4 Hz, J_2=3.9 Hz, 2H, 2×HQ-H3), 7.59 (dd, J_1=5.7 Hz, J_2=3.3 Hz, 2H, ArH), 8.09 (dd, J_1=8.4 Hz, J_2=1.8 Hz, 2H, 2×HQ-H4), 8.92 (dd, J_1=4.2 Hz, J_2=1.8 Hz, 2H, 2×HQ-H2); ^{13}C NMR ($CDCl_3$) (75 MHz) (normal/DEPT-135): δ 69.1 (−ve, CH_2), 110.0 (+ve, ArCH), 119.8 (+ve, ArCH), 121.5 (+ve, ArCH), 126.5 (+ve, ArCH), 128.2 (+ve, ArCH), 128.7 (+ve, ArCH), 129.4 (ab, ArC), 134.9 (ab, ArC), 135.7 (+ve, ArCH), 140.5 (ab, ArC), 149.2 (+ve, ArCH), 154.2 (ab, ArC). Found C, 79.3; H, 5.4; N, 6.9%. $C_{26}H_{20}N_2O_2$ requires C, 79.57; H, 5.14; N, 7.14%.

3.1.2. 1,2,4,5-Tetrakis(8-hydroxyquinolinoxymethyl)-benzene (5). The reaction of 8-hydroxyquinoline with 1,2,4,5-tetrakis(bromomethyl)benzene (**4**) using the above procedure provided **5**, 633 mg, 40%, white solid, mp 233–237 °C (CH_3CN/$CHCl_3$), FAB mass m/z 707 (M^++H); ^{1}H NMR ($CDCl_3$) (300 MHz): δ 5.62 (s, 4×CH_2, 8H), 7.00 (d, J=7.2 Hz, 4H, 4×HQ-H7), 7.19–7.30 (m, 8H, 4×HQ-H6, 5), 7.37 (dd, J_1=8.1 Hz, J_2=4.2 Hz, 4H, 4×HQ-H3), 7.80 (s, 2H, ArH), 8.06 (d, J=6.9 Hz, 4H, 4×HQ-H4), 8.86 (d, J=2.7 Hz, 4H, 4×HQ-H2); ^{13}C NMR ($CDCl_3$/DMSO-d_6) (75 MHz) (normal/DEPT-135): δ 67.36 (−ve, CH_2), 112.5 (+ve, ArCH), 119.0 (+ve, ArCH), 119.1 (ab, ArC), 121.1 (+ve, ArCH), 128.2 (ab, ArC), 128.3 (+ve, ArCH), 128.5 (+ve, ArCH), 133.4 (ab, ArC), 143.2 (+ve, ArCH), 144.5 (+ve, ArCH), 147.2 (ab, ArC). Found C, 77.93; H, 4.7; N, 7.9%. $C_{46}H_{34}N_4O_4$ requires C, 78.17; H, 4.85; N, 7.93%.

3.1.3. 1,3,5-Tris(8-hydroxyquinolinoxymethyl)-2,4,6-trimethylbenzene (7a). The solution of 2,4,6-trimethyl-1,3,5-tris(bromomethyl)benzene (**6a**) (1.00 g, 2.5 mmol), K_2CO_3 (1.03 g, 7.5 mmol), 8-hydroxyquinoline (1.08 g, 7.5 mmol), and TBA·HSO_4 (20 mg) in acetonitrile (30 ml) was refluxed with stirring for 10 h. The solvent was distilled off. The crude mixture was diluted with water and was extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and solvent was distilled off. The residue was column chromatographed over silica gel column to isolate **7a**, 430 mg, 34%, white solid, mp 200–202 °C (acetonitrile), FAB mass m/z 592 (M+H); ^{1}H NMR ($CDCl_3$): δ 2.51 (s, 9H, 3×CH_3), 5.33 (s, 6H, 3×CH_3), 7.21 (d, 3H, J_1=8 Hz, J_2=2 Hz, 3×HQ-H7), 7.32–7.49 (m, 9H, 3×HQ-H3, 5, 6), 8.08 (dd, 3H, J_1=10 Hz, J_2=4 Hz, 2×HQ-H4), 8.88 (dd, 3H, J_1=8 Hz, J_2=4 Hz, 3×HQ-H2); ^{13}C NMR ($CDCl_3$): δ 16.38 (+ve, CH_3), 66.72 (−ve, OCH_2), 109.96 (+ve, CH), 120.05 (+ve, CH), 121.59 (+ve, CH), 126.73 (+ve, CH), 129.56 (ab, C), 131.48 (ab, C), 135.69 (+ve, CH), 140.04 (ab, C), 140.67 (ab, C), 149.29 (+ve, CH), 155.20 (ab, C). Found C, 79.2; H, 5.6; N, 7.1%. $C_{39}H_{33}N_3O_3$ requires C, 79.19; H, 5.58; N, 7.12%.

3.1.4. 1,3,5-Tris(8-hydroxyquinolinoxymethyl)-2,4,6-triethylbenzene (7b). The reaction of **6b** with 8-hyroxyquinoline (**1**) by the above procedure gave **7b**, 440 mg, 30%, thick transparent liquid, FAB mass 634 [M+H]; ^{1}H NMR ($CDCl_3$): δ 1.29 (t, J=7.2 Hz, 9H, 3×CH_3), 2.96 (q, J=7.2 Hz, 6H, 3×CH_2), 5.35 (s, 6H, 3×OCH_2), 7.31 (dd, J_1=7.5 Hz, J_2=1.2 Hz, 3H, 3×HQ-H7), 7.35–7.54 (m, 9H, 3×HQ-H3, 5, 6), 8.13 (dd, 3H, J_1=8.4 Hz, J_2=1.8 Hz, 3H, 3×HQ-H4), 8.91 (dd, J_1=4.2 Hz, J_2=1.8 Hz, 3H, 3×HQ-H2); ^{13}C NMR ($CDCl_3$): δ 16.57 (+ve, CH_3), 23.37 (−ve, CH_2), 65.71 (−ve, OCH_2), 109.72 (+ve, CH), 119.99 (+ve, CH), 121.56 (+ve, CH), 126.70 (+ve, CH), 129.55 (ab, C), 130.69 (ab, C), 135.87 (+ve, CH), 140.52 (ab, C), 146.88 (ab, C), 149.02 (+ve, CH), 155.22 (ab, C). Found C, 79.54; H, 6.15; N, 7.1%. $C_{39}H_{33}N_3O_3$ requires C, 79.59; H, 6.20; N, 7.08%.

3.1.5. UV–vis and fluorescence experiments. UV–vis absorption and fluorescence spectra were recorded on Shimadazu UV-1601-PC spectrophotometer and Shimadazu RF1501 spectrofluorophotometer with a 1 cm quartz cell at 25±0.1 °C. The solutions of **3**, **5**, **7a**, and **8** and metal nitrates were prepared in double distilled acetonitrile. The number of solutions containing **3/5/7a/8** (10 μM) and different concentrations of metal nitrates were prepared and were kept at 25±1 °C for 2 h before recording their absorption or fluorescence spectra. The spectra obtained were analyzed through curve fitting procedures by using SPECFIT 3.0.36 to determine the stability constants and the distribution of various species.

Acknowledgements

We thank UGC, New Delhi for SAP program; DST, New Delhi for financial assistance and FIST program and CDRI, Lucknow for CHN and mass spectral data.

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Tetrahedron 62 (2006) 6388–6391
Tetrahedron

Palladium-catalyzed C–N bond formation: synthesis of 1-aryl-1*H*-pyrazoles from β-bromovinyl aldehydes and arylhydrazines

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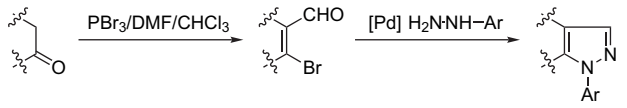
Received 10 March 2006; revised 4 April 2006; accepted 7 April 2006
Available online 4 May 2006

Abstract—Cyclic and acyclic β-bromovinyl aldehydes are cyclized with an array of arylhydrazines in toluene at 125 °C in the presence of a palladium catalyst and a phosphorus chelating ligand together with NaO^{t}Bu to give 1-aryl-1*H*-pyrazoles in moderate to good yields.

1. Introduction

Palladium-catalyzed sp^2-carbon–nitrogen bond forming reaction by the cross-coupling of aryl halides (or triflate) with primary or secondary amines has been recognized as an attractive tool in synthetic organic chemistry.[1] In connection with this report, it is known that several *N*-heterocycles can be synthesized by palladium-catalyzed coupling protocol of aryl halides with hydrazones.[2–9] Buchwald et al. have reported on the synthesis of indoles by palladium-catalyzed coupling of aryl bromides with benzophenone hydrazone followed by the treatment of ketones under $TsOH\cdot H_2O$.[4] Song and Yee have reported a palladium-catalyzed intramolecular amination of *N*-aryl-*N*′-(*o*-bromobenzyl)hydrazines and *N*-aryl-*N*-(*o*-bromobenzyl)hydrazines leading to 1-aryl-1*H*-indazoles and 2-aryl-2*H*-indazoles, respectively.[5,6] It is also reported by Haddad et al. that indazoles can be synthesized by the palladium-catalyzed coupling of aryl halides with benzophenone hydrazone and subsequent reaction with 1,3-bifunctional substrates.[7,8] Such an intrinsic palladium-catalyzed C–N bond formation was also applied to the synthesis of 1-aryl-1*H*-indazoles by us through the coupling between 2-bromobenzaldehydes and arylhydrazines.[9,10] The indazole protocol led us to extend to the reaction with β-bromovinyl aldehydes, which are readily prepared from ketones via the bromo analogue of Vilsmeier reaction (Scheme 1).[11] Herein, this report describes a palladium-catalyzed cyclization of β-bromovinyl aldehydes with arylhydrazines leading to pyrazoles via an intrinsic C–N bond formation.[12,13]



Scheme 1.

2. Results and discussion

Based on our recent report on palladium-catalyzed synthesis of 1-aryl-1*H*-indazoles from 2-bromobenzaldehydes and arylhydrazines,[9] the results of several attempted cyclizations of 2-bromocyclohex-1-enecarbaldehyde (**1a**) with phenylhydrazine (**2a**) are listed in Table 1. Treatment of equimolar amount of **1a** and **2a** in toluene under the catalytic system of $Pd(OAc)_2$ combined with 1,3-bis(diphenylphosphino)propane (dppp) along with NaO^{t}Bu at 100 °C for 24 h afforded 1-phenyl-4,5,6,7-tetrahydro-1*H*-indazole (**3a**) in 34% yield (entry 1). This result indicates that the present reaction, nevertheless the use of more amount of a palladium catalyst, is slower than that of 2-bromobenzaldehyde with **2a** leading to 1-phenyl-1*H*-indazole.[9] Higher reaction temperature resulted in an elevated yield of **3a** (entry 2). Similar catalytic activity was observed with $PdCl_2$ combined with dppp (entry 3). Among the catalytic systems of $Pd(OAc)_2$ combined with mono- and bidentate phosphorus ligands such as PPh_3, 1,1′-bis(diphenylphosphino)ferrocene (dppf), and 1,1′-bis(di-*i*-propylphosphino)ferrocene (dipf) examined dppf revealed to be the ligand of choice and monodentate PPh_3 did not work at all for the present reaction (entries 4–6). On the other hand, when the reaction was carried out under a dilute concentration, **3a** was more effectively formed (entry 7). However, the addition of molecular sieves, 4 Å (0.2 g) under the condition of entry 7 did not give any significant change in

Keywords: β-Bromovinyl aldehydes; C–N bond formation; Arylhydrazines; Palladium catalyst; Pyrazoles.
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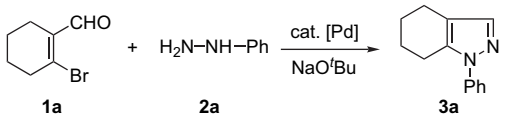
doi:10.1016/j.tet.2006.04.031

Table 1. Optimization of conditions for the reaction of **1a** with **2a**



1a **2a** **3a**

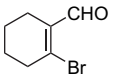
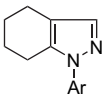
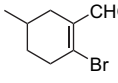
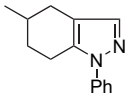
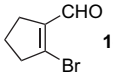
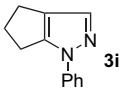
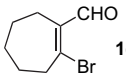
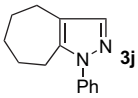
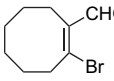
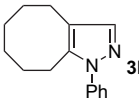
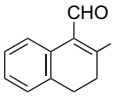
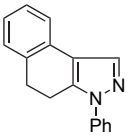
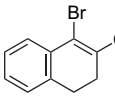
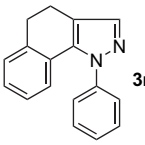
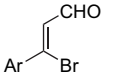
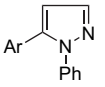
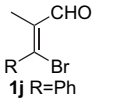
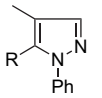
Entry	Ligands	Solvents (mL)	Temperature (°C)	Yield (%)
1	dppp	Toluene (5)	100	34
2	dppp	Toluene (5)	125	59
3[a]	dppp	Toluene (5)	125	57
4	dppf	Toluene (5)	125	67
5	dipf	Toluene (5)	125	60
6	PPh_3	Toluene (5)	125	1
7	dppf	Toluene (10)	125	79
8	(*S*)-(−)-BINAP	Toluene (10)	125	77
9	dppf	THF (10)	125	62
10	dppf	MeCN (10)	125	64

Reaction conditions: **1a** (1 mmol), **2a** (1 mmol), $Pd(OAc)_2$ (0.05 mmol), NaO^{t}Bu (2 mmol), bidentate ligand (0.075 mmol), monodentate ligand (0.15 mmol), for 24 h.
[a] $PdCl_2$ was used in place of $Pd(OAc)_2$.

the yield of **3a** (78%). The catalytic system using (*S*)-(−)-BINAP was revealed to be as effective as that using dppp (entry 8). From the solvents examined THF and MeCN could be alternatively used, but the yield of **3a** was lower than that when toluene was used (entries 9 an 10). As a result, the best result in terms of both yield and complete conversion of **1a** was accomplished by the standard set of reaction conditions shown in entry 7 of Table 1.

Having established reaction conditions, various β-bromovinyl aldehydes **1** were subjected to react with various arylhydrazines **2** in order to investigate the reaction scope and several representative results are summarized in Table 2. Cyclic β-bromovinyl aldehyde **1a** was readily cyclized with an array of arylhydrazines (**2a**–**g**) having electron donating and withdrawing substituents to give the corresponding 1-aryl-1*H*-pyrazoles (**3a**–**g**) in the range of 42–79% yields. The product yield was not significantly affected by the electronic nature of the substituent on the aromatic ring of **2a**–**g**, whereas the position of that had some relevance to the pyrazole yield. With arylhydrazines having *ortho*-substituent, the pyrazole yield was generally lower than that when arylhydrazines having *meta*- and *para*-substituents were used. 2-Bromo-5-methylcyclohex-1-enecarbaldehyde (**1b**) reacts similarly with **2a** to afford 5-methyl-1-phenyl-4,5,6,7-tetrahydro-1*H*-indazole (**3h**) in 76% yield. From the reactions between several cyclic β-bromovinyl aldehydes (**1d** and **1e**) and **2a**, the corresponding pyrazoles (**3j** and **3k**) were produced in similar yields. However, lower reaction yield was observed with five-membered cyclic β-bromovinyl aldehyde **1c**. To test the effect of the position of formyl group and bromide on cyclic β-bromovinyl aldehydes, **1f** and **1g** were employed. However, the cyclization took place similarly irrespective of the position. Next, we carried out the reaction with trans/cis mixture (trans/cis=ca. 1/10 on GLC) of acyclic β-bromovinyl aldehydes (**1h**–**k**), which could not be separated by chromatography.[14] Similar treatment of acyclic β-bromovinyl aldehydes (**1h** and **1i**), which have no substituent at α-position with **2a** under the employed conditions afforded the corresponding 1,5-disubstituted pyrazoles (**3n** and **3o**). Acyclic β-bromovinyl aldehydes such as 3-bromo-2-methyl-3-phenylpropenal (**1j**) and 3-bromo-2-methylpent-2-enal (**1k**), which have a substituent at α-position were also reacted with **2a** to give 4-methyl-1,5-dimethyl-1*H*-pyrazole (**3p**) and 5-ethyl-4-methyl-1-phenyl-1*H*-pyrazole (**3q**) in 56 and 40% yields, respectively.

Table 2. Palladium-catalyzed synthesis of 1-aryl-1*H*-pyrazoles

β-Bromovinyl aldehyde **1**	Arylhydrazine **2**	Product **3**	Yield (%)
 **1a**	H_2N–NH–Ar	 **3a**	
	2a Ar=Ph		79
	2b Ar=2-MeC_6H_4	**3b**	42
	2c Ar=3-MeC_6H_4	**3c**	56
	2d Ar=4-MeC_6H_4	**3d**	68
	2e Ar=4-$MeOC_6H_4$	**3e**	54
	2f Ar=2-$CF_3C_6H_4$	**3f**	45
	2g Ar=4-$NO_2C_6H_4$	**3g**	56
 **1b**	**2a**	 **3h**	76
 **1c**	**2a**	 **3i**	20
 **1d**	**2a**	 **3j**	65
 **1e**	**2a**	 **3k**	77
 **1f**	**2a**	 **3l**	54
 **1g**	**2a**	 **3m**	46
 **1h** Ar=Ph	**2a**	 **3n**	48
1i Ar=2-naphthyl	**2a**	**3o**	48
 **1j** R=Ph	**2a**	 **3p**	56
1k R=Et	**2a**	**3q**	40

Reaction conditions: **1** (1 mmol), **2** (1 mmol), $Pd(OAc)_2$ (5 mol %), dppf (7.5 mol %), NaO^{t}Bu (2 mmol), toluene (10 mL), 125 °C, for 24 h.

In summary, we have demonstrated that cyclic and acyclic β-bromovinyl aldehydes are cyclized with an array of arylhydrazines in the presence of a palladium catalyst and a phosphorus chelating ligand to afford pyrazoles via an

intrinsic C–N bond protocol. The present reaction is a new route for the synthesis of pyrazoles from ketones.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR (400 and 100 MHz) spectra were recorded on a Bruker Avance Digital 400 spectrometer using TMS as an internal standard. Melting points were determined on a Thomas–Hoover capillary melting point apparatus and were uncorrected. The isolation of pure products was carried out via thin layer (silica gel 60 GF_{254}, Merck) chromatography. β-Bromovinyl aldehydes **1** were prepared by the reported methods.[11] Commercially available organic and inorganic compounds were used without further purification except for toluene, THF, and MeCN, which were distilled by known methods before use.

3.2. Typical procedure for palladium-catalyzed synthesis of pyrazoles from β-bromovinyl aldehydes and arylhydrazines

A mixture of 2-bromocyclohex-1-enecarbaldehyde (**1a**) (0.189 g, 1 mmol), phenylhydrazine (**2a**) (0.108 g, 1 mmol), $Pd(OAc)_2$ (0.011 g, 0.05 mmol), dppf (0.042 g, 0.075 mmol), and NaO^{t}Bu (0.192 g, 2 mmol) in toluene (10 mL) was placed in a 50 mL pressure vessel. After the system was flushed with argon, the mixture was stirred at 125 °C for 24 h. The reaction mixture was passed through a short silica gel column (ethyl acetate) to eliminate inorganic salts. Removal of the solvent left a crude mixture, which was separated by thin layer chromatography (silica gel, ethyl acetate/hexane=1/10) to give 1-phenyl-4,5,6,7-tetrahydro-1*H*-indazole (**3a**) (0.157 g, 79%). All new compounds prepared by the above procedure were characterized spectroscopically as shown below.

3.2.1. 1-(2-Methylphenyl)-4,5,6,7-tetrahydro-1*H*-indazole (3b). Oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.75–1.80 (m, 4H), 2.08 (s, 3H), 2.35–2.37 (m, 2H), 2.58–2.60 (m, 2H), 7.23–7.34 (m, 4H), 7.44 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.44, 20.68, 21.90, 22.88, 23.14, 115.95, 126.29, 127.43, 128.71, 130.93, 135.97, 137.90, 138.74, 139.29; Anal. Calcd for $C_{14}H_{16}N_2$: C 79.21; H 7.60; N 13.20; found: C 79.04; H 7.88; N 13.00.

3.2.2. 1-(3-Methylphenyl)-4,5,6,7-tetrahydro-1*H*-indazole (3c). Oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.74–1.82 (m, 4H), 2.39 (s, 3H), 2.56–2.59 (m, 2H), 2.70–2.72 (m, 2H), 7.10 (d, J=7.5 Hz, 1H), 7.24–7.34 (m, 3H), 7.45 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.14, 21.80, 23.22, 23.57, 24.08, 118.00, 120.33, 124.25, 127.76, 129.10, 138.54, 139.03, 139.49, 140.43; Anal. Calcd for $C_{14}H_{16}N_2$: C 79.21; H 7.60; N 13.20; found: C 78.98; H 7.85; N 13.13.

3.2.3. 1-(4-Methylphenyl)-4,5,6,7-tetrahydro-1*H*-indazole (3d). Oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.74–1.82 (m, 4H), 2.37 (s, 3H), 2.56–2.59 (m, 2H), 2.67–2.70 (m, 2H), 7.22 (d, J=8.0 Hz, 2H), 7.34–7.38 (m, 2H), 7.44 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.74, 20.99, 22.84, 23.16, 23.55, 117.45, 122.99, 129.56, 136.43, 137.70, 138.11, 138.47; Anal. Calcd for $C_{14}H_{16}N_2$: C 79.21; H 7.60; N 13.20; found: C 79.08; H 7.81; N 13.06.

3.2.4. 1-(4-Methoxyphenyl)-4,5,6,7-tetrahydro-1*H*-indazole (3e). Oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.74–1.86 (m, 4H), 2.56–2.59 (m, 2H), 2.64–2.67 (m, 2H), 3.83 (s, 3H), 6.93–6.97 (m, 2H), 7.36–7.40 (m, 2H), 7.42 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.12, 23.26, 23.50, 23.69, 55.90, 114.54, 117.58, 125.10, 133.79, 138.56, 138.63, 158.73; Anal. Calcd for $C_{14}H_{16}N_2O$: C 73.66; H 7.06; N 12.27; found: C 73.51; H 7.31; N 12.24.

3.2.5. 1-(2-Trifluoromethylphenyl)-4,5,6,7-tetrahydro-1*H*-indazole (3f). Oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.72–1.81 (m, 4H), 2.33–2.36 (m, 2H), 2.57–2.59 (m, 2H), 7.34 (d, J=7.5 Hz, 1H), 7.46 (s, 1H), 7.57 (t, J=7.5 Hz, 1H), 7.62–7.66 (m, 1H), 7.79–7.81 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.92, 21.98, 23.09, 23.36, 116.73, 123.31 (q, J=272.4 Hz), 127.72 (q, J=4.8 Hz), 128.68 (q, J=31.2 Hz), 129.65, 130.56, 132.87, 138.01 (q, J=1.9 Hz), 139.01, 140.82; Anal. Calcd for $C_{14}H_{13}F_3N_2$: C 63.15; H 4.92; N 10.52; found: C 63.41; H 5.04; N 10.54.

3.2.6. 1-Phenyl-1,4,5,6-tetrahydrocyclopentapyrazole (3i). Oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.57–2.69 (m, 4H), 2.97–3.01 (m, 2H), 7.20–7.24 (m, 1H), 7.38 (s, 1H), 7.39–7.43 (m, 2H), 7.62–7.65 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.15, 26.85, 31.37, 119.41, 125.98, 129.64, 129.74, 135.20, 140.88, 148.90; Anal. Calcd for $C_{12}H_{12}N_2$: C 78.23; H 6.57; N 15.21; found: C 77.91; H 6.81; N 15.06.

3.2.7. 1-Phenyl-1,4,5,6,7,8-hexahydrocycloheptapyrazole (3j). Solid (hexane/chloroform); mp 90 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.61–1.72 (m, 4H), 1.82–1.87 (m, 2H), 2.61–2.64 (m, 2H), 2.75–2.78 (m, 2H), 7.33–7.38 (m, 4H), 7.43–7.46 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.05, 27.58, 27.63, 28.94, 32.23, 122.37, 125.86, 127.83, 129.30, 140.12, 140.41, 142.54; Anal. Calcd for $C_{14}H_{16}N_2$: C 79.21; H 7.60; N 13.20; found: C 79.22; H 7.72; N 12.99.

3.2.8. 1-Phenyl-4,5,6,7,8,9-hexahydro-1*H*-cyclooctapyrazole (3k). Oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46–1.55 (m, 4H), 1.64–1.73 (m, 4H), 2.61–2.64 (m, 2H), 2.72–2.75 (m, 2H), 7.34–7.47 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.90, 23.52, 25.42, 25.71, 28.87, 30.44, 119.71, 125.25, 127.54, 128.96, 139.59, 140.18, 140.36; Anal. Calcd for $C_{15}H_{18}N_2$: C 79.61; H 8.02; N 12.38; found: C 79.40; H 8.16; N 12.22.

3.2.9. 5-Naphthalen-2-yl-1-phenyl-1*H*-pyrazole (3o). Oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.60 (d, J=1.5 Hz, 1H), 7.22–7.34 (m, 6H), 7.45–7.50 (m, 2H), 7.70–7.80 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 108.62, 125.59 (×2), 126.75, 126.94, 127.05, 127.85, 128.11, 128.34, 128.46, 128.57, 129.36, 133.17, 133.50, 140.59, 140.83, 143.37; Anal. Calcd for $C_{19}H_{14}N_2$: C 84.42; H 5.22; N 10.36; found: C 84.54; H 5.33; N 9.91.

3.2.10. 5-Ethyl-4-methyl-1-phenyl-1*H*-pyrazole (3q). Oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 (t, J=7.5 Hz, 3H), 2.08

(s, 3H), 2.67 (q, J=7.5 Hz, 2H), 7.17–7.20 (m, 1H), 7.37–7.41 (m, 2H), 7.60–7.62 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.47, 13.47, 20.06, 115.84, 118.40, 125.35, 125.81, 129.27, 140.35, 154.99; Anal. Calcd for $C_{12}H_{14}N_2$: C 77.38; H 7.58; N 15.04; found: C 77.00; H 7.88; N 14.94.

Acknowledgements

C.S.C. gratefully acknowledges a Research Professor Grant of Kyungpook National University (2005).

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Tetrahedron 62 (2006) 6392–6397
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Reactivity difference between diphosgene and phosgene in reaction with (2,3-*anti*)-3-amino-1,2-diols

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Received 14 March 2006; revised 5 April 2006; accepted 6 April 2006
Available online 11 May 2006

Abstract—In reactions of (2,3-*anti*)-3-amino-1,2-diols with diphosgene and phosgene and their conversion into 1,3-oxazolidin-2-ones, some differences in the stereochemistry of the reactions have been found with these two reagents. The reactions with phosgene afforded the expected *cis*-oxazolidinones, and in the reaction with diphosgene under the same reaction conditions, the *trans*-oxazolidinones were also obtained.

1. Introduction

The synthesis of chiral 4,5-disubstituted 1,3-oxazolidin-2-ones has received special attention, because these structural units are present in molecules with pharmaceutical interest such as cytoxazone[1] and they are also useful chiral auxiliaries for asymmetric synthesis.[2]

Different methods described in the literature for the preparation of 1,3-oxazolidin-2-ones use *N*-Boc derivatives of 1,2-amino alcohols as starting materials. The reaction with sodium hydride,[3] for example, affords oxazolidin-2-ones without changes in the configuration of the carbon supporting the hydroxyl group. By contrast, the conversion of the alcohol function into a good leaving group, reaction with methanesulfonyl chloride[4] or reaction with triphenylphosphine-DEAD,[5] affords the cyclisation products with inverted configuration.

There are recent reports in the literature related with synthesis of 1,3-oxazolidin-2-ones from *N*-acylamino alcohols with unexpected stereochemical results. One example is the reaction of *N*-Boc amino alcohols with mesyl chloride, which not only gave oxazolidinones with inversion of the configuration as expected, but also gave some oxazolidinones with retention of configuration due to competition between S_N1 and S_N2 mechanisms.[6] Other exception with unexpected stereochemical results is the carboxylation of 1,2-amino alcohols followed by Mitsunobo reaction. The reaction is reported[7] to be substituent dependent affording oxazolidinones with retention, when the N atom is substituted with hydrogen, or inversion when it is substituted with carbon. Other examples of retention of configuration in intramolecular versions of Mitsunobo reaction are attributed to steric congestion at the hydroxyl reaction centre.[8]

1,3-Oxazolidin-2-ones can also be prepared from 1,2-amino alcohols as starting materials using reagents such as phosgene,[9] diphosgene (trichloromethyl chloroformate),[10] carbonyl diimidazol,[11] etc. In all these reactions, the configuration of the stereocentres of the starting amino alcohols is retained in the oxazolidinone. The formation of these cyclic carbamates is a procedure used to establish the configuration of the stereocentres of 1,2-amino alcohols,[12] because the stereochemical assignment is easier in the cyclic derivatives.

As a part of our current interest in the reactivity of amino alcohols,[13–17] we have studied in this work the reaction of different compounds containing the 1,2-amino alcohol unit with diphosgene and phosgene and we have found some exceptions to the general rule that establish that the reaction of 1,2-amino alcohols with both reagents affords the same stereochemical results and proceeds with retention of configuration. These exceptions have been found working with some compounds containing the (2,3-*anti*)-3-amino-1,2-diol moiety.

Keywords: 1,3-Oxazolidin-2-ones; Stereochemistry; Diphosgene; Phosgene; 3-Amino-1,2-diols.
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doi:10.1016/j.tet.2006.04.033

2. Results and discussion

In the course of a work on reactivity of polyfunctionalized molecules, we studied the reaction of 3-amino-1,2-diol **1a**[18] with diphosgene and triethylamine. The result was similar to the same reaction reported in the literature[19] for other aminodiols and *cis*-oxazolidinone **2a** (70%) was obtained. The reaction of **1a** with phosgene afforded a similar result with the formation of **2a** (75%) (Scheme 1) (Table 1, entry 1).

When similar reactions were attempted with 3-amino-1,2-diol **1b**,[13] different stereochemical results were observed with these two reagents. In the reaction of aminodiol **1b** with diphosgene, the *trans*-oxazolidinone **3b**[17] (45%) was obtained, along with the cis isomer **2b** (16%) and a small amount of the six-membered oxazinone **4b**[17] (8%). However, when aminodiol (**1b**) reacted with phosgene, instead of diphosgene, *cis*-oxazolidinone **2b** (70%) was obtained, with no traces of the *trans*-oxazolidinone (Table 1, entry 2).

Other examples of inversion on the oxygen-bearing centre were observed in the reactions of the *N*-carbon substituted 3-amino-1,2-diols **1c**[13] and **1d**.[21] In the reaction of aminodiol (**1c**) with diphosgene in triethylamine, *trans*-oxazolidinone (**3c**) was obtained as major product (30%), along with *cis*-oxazolidinone **2c** (16%) and a small amount of oxazinone **4c** (12%) (Table 1, entry 3). In the reaction aminodiol (**1d**) with diphosgene in triethylamine, *trans*-oxazolidinone **3d** (18%) was obtained along with oxazinone **4d** (11%) and carbonate **5d** (40%) (Table 1, entry 4).

In contrast with the reaction of aminodiols **1c** and **1d** with diphosgene, in the reaction with phosgene, the exceptional inversion was not observed. The reaction of **1c** with phosgene afforded cis isomer **2c** (20%) and carbonate **6c** (46%) as a major product (Table 1, entry 3) and in the reaction of **1d** with phosgene only carbonate **5d** (70%) was obtained (Table 1, entry 4). The isolation of carbonate **6c** as a carbamoyl chloride derivative was probably due to the presence of an excess of phosgene in these experiments.[20]

This trend in the formation of the carbonate with increasing steric hindrance on the *N*-substituent was observed for aminodiol **1e**.[21] In this case with both reagents, diphosgene and phosgene, carbonate **5e** was the only isolated product from the reactions (Table 1, entry 5).

The reactions of the 1,2-amino alcohols, (1*R*,2*S*)-(−)-norephedrine and (1*R*,2*S*)-(−)-ephedrine, molecules without the primary hydroxyl group of our previous examples were also studied. The reaction with diphosgene in triethylamine afforded, in both cases, the corresponding *cis*-oxazolidinones reported in the literature,[22] without stereochemical inversion in the oxygen-bearing centres. This result induced us to think that the presence of a vicinal primary hydroxyl group was necessary for the unexpected stereochemical inversions observed in the cases of **1b–d**. The hypothesis of the necessary presence of the vicinal primary hydroxyl group was confirmed when we studied the reaction of the partially protected aminodiols **1f**, **1g**[23] and **1h** with diphosgene in triethylamine. Here again there was not observed any inversion on the oxygen-bearing centre and *cis*-oxazolidinones **2f** (80%), **2g** (73%) and **2h** (71%) were obtained, respectively.

Table 1. Reaction of aminodiols **1a–e** with diphosgene and phosgene

Entry	Starting material	Diphosgene/Et_3N	Phosgene/Et_3N
1	**1a**	**2a** (70%)	**2a** (75%)
2	**1b**	**2b** (16%)+**3b** (45%)+**4b** (8%)	**2b** (70%)
3	**1c**	**2c** (16%)+**3c** (30%)+**4c** (12%)	**2c** (20%)+**6c** (46%)
4	**1d**	**3d** (18%)+**4d** (11%)+**5d** (40%)	**5d** (70%)
5	**1e**	**5e** (96%)	**5e** (97%)

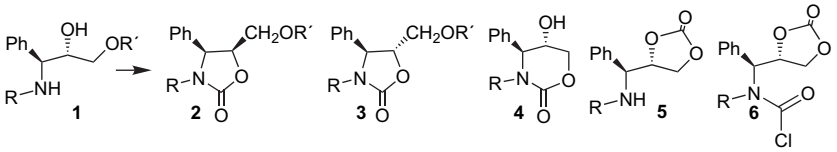
The proposed mechanism (Scheme 2) accounts for the stereochemical differences in the reaction of (2,3-*anti*)-3-amino-1,2-diols (**1b–d**) with diphosgene and the formation of *trans*-oxazolidinones (**3**) and *cis*-oxazolidinones **2**, through the intermediates (**7–11**).

After the initial attack of the amino group of 3-amino-1,2-diol (**1**) to diphosgene and formation of carbamate (**7**), an intramolecular attack of the secondary hydroxyl group (path 1) to the carbamate with the elimination of phosgene would explain the formation of *cis*-oxazolidinones (**2**).

For the formation of *trans*-oxazolidinones (**3**), we suggest an initial acid–base equilibrium (path 2) between **7** and **8** in the presence of Et_3N. The transfer of the trichloromethyl group from the carbamate to the primary alcoxide function would afford intermediate (**9**), which could be converted into epoxide (**10**) by attack of the secondary hydroxyl group and extrusion of phosgene. The intramolecular attack of the carbamate to the epoxide at C2 would afford the corresponding alcoxide (**11**) with inverted configuration at C2, whose protonated species are *trans*-oxazolidinones (**3**).

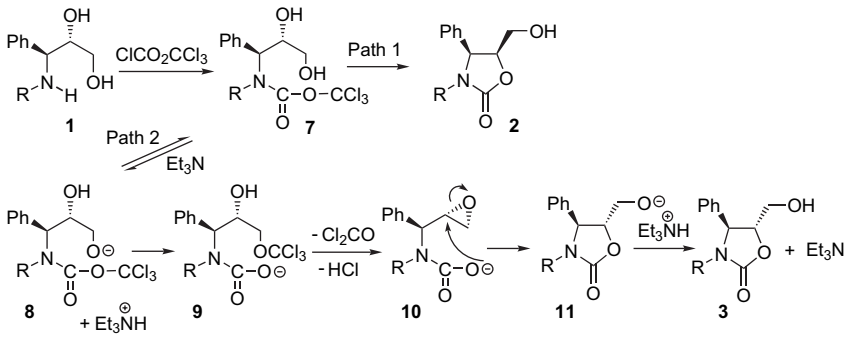
The formation of oxazinones (**4**), in experiments where *trans*-oxazolidinones (**3**) were isolated, could be explained through intermediate (**10**), by an intramolecular attack of the carbamate to the epoxide at C1. This fact would be an additional support to the proposed mechanism for the reaction of 3-amino-1,2-diols with diphosgene.

The use of diphosgene and the presence of a primary hydroxy group are essential conditions for the observed



a R=H, R´=H; **b** R=CH_3, R´=H; **c** R=Et, R´=H; **d** R=CH_2Ph, R´=H; **e** R=Ph_2CH, R´=H; **f** R=H, R´=TBDMS; **g** R=CH_3, R´=TBDMS; **h** R=Ph_2CH, R´=TBDMS

Scheme 1.



Scheme 2.

inversion to take place, according to the mechanism. The amino substitution looks not necessary. The fact that the presence of oxazolidinone **3a** (path 2) could not be detected in the reaction of **1a** (R=H) with diphosgene, can be due to the low concentration of this product as a result of a more favourable process through path 1 in relation with path 2, or the existence of a competitive mechanism through an isocyanate.[7]

3. Conclusion

In conclusion, we have presented for the first time examples with different stereochemical behaviour in reactions between diphosgene and phosgene with (2,3-*anti*)-3-amino-1,2-diols affording 1,3-oxazolidin-2-ones. These results must be considered when the configuration of stereocentres in oxazolidinones had to be established in relation with the stereochemistry of the starting amino alcohols.

4. Experimental

4.1. General

Unless otherwise specified, materials were purchased from commercial suppliers and used without further purification. Solvents were distilled prior to use. Thin-layer chromatography was performed on Merck 60F254 sheets. Preparative column chromatography was performed on Merck Kieselgel 60 (230–240 mesh) silica gel. IR spectra were recorded on a FTIR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded with an Avance DPX Bruker 500 MHz or an Avance 400 MHz Bruker or an Avance DRX Bruker 300 MHz spectrometers, in $CDCl_3$ solutions. Chemical shifts were recorded in parts per million (ppm), downfield from internal Me_4Si. The carbon multiplicity was determined by edited HSQC and DEPT experiments. High-resolution mass spectral data were obtained on a VG Autospec, TRIO 1000 (Fisons) instrument. FAB or EI at 70 eV was used as ionisation mode in mass spectra.

The structure of all the compounds and their stereochemistry was determined spectroscopically and by comparison with the data of compounds of similar structure reported in the literature. Every ^{1}H and ^{13}C NMR signals have been assigned by single and multiple bond ^{1}H–^{13}C and ^{1}H–^{1}H NMR correlations. When required, 1D and 2D NOESY experiments were performed in order to determine relative configurations.

4.2. Preparation of the starting materials 1f, 1g and 1h

A solution of the corresponding aminodiol **1a**,[18] **1b**[13] or **1e**[21] (6.0 mmol), *tert*-butyldimethylsilyl chloride (0.9 g, 6.5 mmol), imidazole (1.0 g, 14.9 mmol) in dichloromethane (12 mL) was stirred at room temperature for 24 h. The reaction mixture was washed with water, dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography on silica gel eluting with hexane/ethyl acetate (1:1) to afford compounds **1f**, **1g**[23] and **1h**, respectively. In the reaction with aminodiol (**1a**) in the addition of compound **1f**, the disilylated product was also obtained.

4.2.1. 3-(*tert*-Butyldimethylsilyloxy)-1-amino-1-phenylpropan-2-ol (1f). Yield 30% (28% of the disilyl derivative was also isolated from the reaction mixture). Colourless oil. IR (KBr): ν_{max} 3370, 2928, 2857, 1254, 1114 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.15 (s, 6H), 0.91 (s, 9H), 3.70 (m, 2H), 3.96 (m, 1H), 4.28 (d, 1H, J=5.5 Hz), 7.25 (m, 5H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ −4.7 (q), −4.2 (q), 18.2 (s), 25.9 (q), 58.1 (d), 64.3 (t), 75.0 (d), 127.2 (d), 127.6 (d), 128.6 (d), 142.7 (s). HRMS (MH^+) 282.1907. Calculated for $C_{15}H_{28}NO_2Si$ 282.1889.

4.2.2. 3-(*tert*-Butyldimethylsilyloxy)-1-benzhydrylamino-1-phenylpropan-2-ol (1h). Yield 90%. White solid. Mp 80–81 °C. IR (KBr): ν_{max} 3478, 2928, 2857, 1255, 1100 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.31 (br s, 1H), 3.47 (dd, 1H, J=10.5, 5.0 Hz); 3.61 (dd, 1H, J=10.5, 3.5 Hz); 3.77 (br s, 1H), 3.83 (d, 1H, J=6.0 Hz), 7.33 (m, 15H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ −5.4 (q), −5.3 (q), 18.3 (s), 26.0 (q), 62.8 (d), 63.7 (d), 64.9 (t), 73.9 (d), 127.3 (d), 127.4 (d), 127.5 (d), 127.6 (d), 128.0 (d), 128.1 (d), 128.4 (d), 128.6 (d), 128.7 (d), 140.2 (s), 143.9 (s), 144.2 (s); HRFAB *m/z* calcd for $[M+1]^+$ $C_{28}H_{38}NO_2Si$ 448.2671, found: 448.2680.

4.3. General procedure for the reactions of amino alcohols (1) with diphosgene or phosgene[19]

Trichloromethyl chloroformate (0.17 mL, 1.4 mmol) in CH_2Cl_2 (0.15 mL) or a solution of phosgene in toluene (0.87 mL, 8.28 mmol) was slowly added to the corresponding aminodiol **1a–h** (2.76 mmol) in CH_2Cl_2/Et_3N (1:1) mixture (35 mL) at −20 °C. After stirring for 3 h, the reaction mixture was quenched by the addition of H_2O (15 mL). After decantation and CH_2Cl_2 extraction (3×25 mL), the combined organic layers were washed with brine, dried

(Na_2SO_4) and concentrated to dryness. The complex mixtures were purified by silica gel column chromatography using hexane/EtOAc mixtures as eluent.

4.3.1. From 3-amino-3-phenyl-1,2-propanediol[18] (1a). The reaction with diphosgene afforded 1,3-oxazolidin-2-one (**2a**) (70%); the reaction with phosgene 1,3-oxazolidin-2-one (**2a**) (75%).

4.3.1.1. *cis*-5-Hydroxymethyl-4-phenyl-1,3-oxazolidin-2-one (2a). Colourless oil. IR (KBr): ν_{max} 3336, 1745 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.17 (dd, 1H, J=12.5, 4.0 Hz, CH_2–O), 3.31 (dd, 1H, J=12.5, 4.0 Hz, CH_2–O), 4.87 (td, 1H, J=8.5, 4.0 Hz, *H*-5), 5.03 (d, 1H, J=8.5 Hz, *H*-4), 6.31(br s, N*H*), 7.26 (m, 2H), 7.36 (m, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 57.8 (d), 62.0 (t), 80.8 (d), 127.4 (d), 128.9 (d), 129.0 (d), 135.9 (s), 159.9 (s); HREI-MS *m/z* calcd for $[M]^+$ $C_{10}H_{11}NO_3$ 193.0738, found: 193.0719.

4.3.2. From 3-methylamino-3-phenyl-1,2-propanediol[13] (1b). The reaction with diphosgene afforded a mixture of *cis*-1,3-oxazolidin-2-one (**2b**) (16%), *trans*-1,3-oxazolidin-2-one[17] (**3b**) (45%) and tetrahydro-1,3-oxazin-2-one[17] (**4b**) (8%); the reaction with phosgene afforded *cis*-oxazolidinone (**2b**) (70%).

4.3.2.1. *cis*-5-Hydroxymethyl-3-methyl-4-phenyl-1,3-oxazolidin-2-one (2b). White solid. Mp 69–70 °C. IR: ν_{max} 3502, 1744 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.77 (s, 3H), 3.18 (dd, 1H, J=12.0, 3.0 Hz, CH_2–O), 3.32 (m, 1H, CH_2–O), 4.82 (m, 2H, *H*-4+*H*-5), 7.17 (m, 2H), 7.39 (m, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 29.6 (q), 62.1 (t), 63.4 (d), 77.8 (d), 127.6 (d), 129.1 (d), 129.4 (d), 133.6 (s), 158.6 (s); HREI-MS *m/z* calcd for $[M]^+$ $C_{11}H_{13}NO_3$ 207.0853, found: 207.0895.

4.3.3. From 3-ethylamino-3-phenyl-1,2-propanediol[13] (1c). The reaction with diphosgene afforded a mixture of *cis*-1,3-oxazolidin-2-one (**2c**) (16%), *trans*-1,3-oxazolidin-2-one (**3c**) (30%) and tetrahydro-1,3-oxazin-2-one (**4c**) (12%); the reaction with phosgene afforded 1,3-oxazolidin-2-one (**2c**) (20%) and carbamoyl chloride **6c** (46%).

4.3.3.1. *cis*-5-Hydroxymethyl-3-ethyl-4-phenyl-1,3-oxazolidin-2-one (2c). Colourless oil. IR (KBr): ν_{max} 3430, 1742 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.19 (t, 3H, J=7.0 Hz), 2.75 (dq, 1H, J=14.0, 7.0 Hz, CH_2–N), 3.10 (dd, 1H, J=12.5, 4.5 Hz, CH_2–O), 3.10 (dd, 1H, J=12.5, 8.0 Hz, CH_2–O), 3.53 (dq, 1H, J=14.0, 7.0 Hz, CH_2–N), 4.76 (m, 1H, *H*-5), 4.84 (d, 1H, J=8.5, *H*-4), 7.05 (m, 2H), 7.40 (m, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 12.7 (q), 37.3 (t), 60.8 (d), 62.5 (t), 78.1 (d), 127.8 (d), 129.3 (d), 129.5 (d), 133.9 (s), 158.1 (s); HREI-MS *m/z* calcd for $[M]^+$ $C_{12}H_{15}NO_3$ 221.1051, found: 221.1040.

4.3.3.2. *trans*-5-Hydroxymethyl-3-ethyl-4-phenyl-1,3-oxazolidin-2-one (3c). Colourless oil. IR (KBr): ν_{max} 3419, 1740 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.09 (t, 3H, J=7.0 Hz), 2.61 (br s, 1H, O*H*), 2.89 (dq, 1H, J=14.0, 7.0 Hz, CH_2–N), 3.52 (dq, 1H, J=14.0, 7.0 Hz, CH_2–N), 3.70 (dd, 1H, J=13.0 and 3.5 Hz, CH_2–O), 3.97 (dd, 1H, J=13.0, 3.0 Hz, CH_2–O), 4.34 (ddd, 1H, J=7.0, 3.5, 3.0 Hz, *H*-5), 4.76 (d, 1H, J=7.0 Hz, *H*-4), 7.34 (m, 2H), 7.42 (m, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 12.4 (q), 37.4 (t), 60.7 (d), 61.9 (t), 82.3 (d), 127.3 (d), 129.3 (d), 129.6 (d), 138.0 (s), 157.9 (s). HREI-MS *m/z* calcd for $[M]^+$ $C_{12}H_{15}NO_3$ 221.1051, found: 221.1033.

4.3.3.3. 5-Hydroxy-3-ethyl-4-phenyltetrahydro-1,3-oxazin-2-one (4c). White solid. Mp 132–133 °C. IR (KBr): ν_{max} 3380, 3272, 1672 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.17 (t, 3H, J=7.0 Hz), 2.87 (dq, 1H, J=14.0, 7.0 Hz, CH_2–N), 3.77 (dq, 1H, J=14.0, 7.0 Hz, CH_2–N), 4.02 (d, 1H, J=2.0 Hz, *H*-5), 4.15 (dt, 1H, J=12.0, 2.0 Hz, *H*-6eq), 4.21 (dd, 1H, J=11.8, 1.3 Hz, *H*-6ax), 4.62 (br s, 1H, *H*-4), 7.28 (m, 2H), 7.37 (m, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 12.2 (q), 43.6 (t), 65.3 (d), 66.8 (t), 67.2 (d), 126.5 (d), 128.4 (d), 129.1 (d), 138.5 (s), 153.7 (s); HRFAB-MS *m/z* calcd for $[M+1]^+$ $C_{12}H_{16}NO_3$ 222.1130, found: 222.1120.

4.3.3.4. Ethyl-[(2-oxo-1,3-dioxolan-4-yl)(phenyl)methyl]-carbamoyl chloride (6c). White solid. Mp 85–86 °C (hexane/chloroform). IR (KBr): ν_{max} 1814, 1728 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.12 (t, 3H, J=7.0 Hz, CH_2–CH_3), 3.30 (dq, 1H, J=21.0, 7.0 Hz, CH_2–CH_3), 3.61 (dq, 1H, J=21.0, 7.0 Hz, CH_2–CH_3), 4.33 (dd, 1H, J=8.5, 7.0 Hz, *H*-5), 4.66 (t, 1H, J=8.5 Hz, *H*-5), 4.87 (d, J=8.5 Hz, 1H, Ph-C*H*-N), 5.52 (m, 1H, *H*-4), 7.42 (s, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0 (q), 45.9 (t), 65.4 (d), 67.3 (t), 76.7 (d), 126.5 (d), 128.4 (d), 129.4 (d), 134.8 (s), 150.9 (s), 154.1 (s); HREI-MS *m/z* calcd for $[M]^+$ $C_{13}H_{14}NO_4Cl$ 283.0611, found: 283.0590.

4.3.4. From 3-benzylamino-3-phenyl-1,2-propanediol[21] (1d). The reaction with diphosgene afforded a mixture of *trans*-1,3-oxazolidin-2-one (**3d**) (18%), tetrahydro-1,3-oxazin-2-one (**4d**) (11%) and 1,3-dioxolan-2-one (**5d**) (40%); the reaction with phosgene afforded 1,3-dioxolan-2-one (**5d**) (70%).

4.3.4.1. *trans*-5-Hydroxymethyl-3-benzyl-4-phenyl-1,3-oxazolidin-2-one (3d). Yield 18%. Oil. IR (KBr): ν_{max} 3422, 1744 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.50 (dd, 1H, J=12.7, 3.7 Hz, CH_2–O), 3.55 (d, 1H, J=15.0 Hz, CH_2–N), 3.75 (dd, 1H, J=12.5, 3.0 Hz, CH_2–O), 4.26 (m, 1H, *H*-5), 4.39 (d, 1H, J=7.0 Hz, *H*-4), 4.74 (d, 1H, J=15.0 Hz, CH_2–N), 7.04 (m, 2H), 7.15 (m, 6H), 7.30 (m, 2H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 46.1 (t), 60.3 (d), 61.8 (t), 82.5 (d), 127.4 (d), 127.8 (d), 128.1 (d), 128.5 (d), 128.9 (d), 129.5 (d), 135.3 (s), 137.5 (s), 158.2 (s); HREI-MS *m/z* calcd for $[M]^+$ $C_{17}H_{17}NO_3$ 283.1208, found: 283.1211.

4.3.4.2. 3-Benzyl-5-hydroxy-4-phenyl-1,3-oxazinan-2-one (4d). White solid. Mp 200–201 °C. IR (KBr): ν_{max} 3442, 1704 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.65 (d, 1H, J=15.5 Hz, CH_2N), 4.16 (dt, 1H, J=13.0, 2.0 Hz, *H*-6eq), 4.34 (dd, 1H, J=13.0, 1.6 Hz, *H*-6ax), 4.59 (br s, 1H, *H*-4), 4.68 (m, 1H, *H*-5), 5.47 (d, 1H, J=15.5 Hz, CH_2–N), 7.30 (m, 5H, *Ph*–CH_2N), 7.46 (m, 5H, *Ph*-C4); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 50.7 (t), 60.8 (d), 64.1 (t), 73.1 (d), 126.7, 128.1 (d), 128.3 (d), 129.1 (d), 129.3 (d), 129.9 (d), 136.1 (s), 136.3 (s), 152.8 (s); HREI-MS *m/z* calcd for $[M]^+$ $C_{17}H_{17}NO_3$ 283.1208, found: 283.1114.

4.3.4.3. 4-[(Benzylamino)(phenyl)methyl]-1,3-dioxolan-2-one (5d). White solid. Mp 90–91 °C. IR (KBr): ν_{max} 3324, 1775, 1177 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.58 (d, 1H, J=13.0 Hz, CH_2–Ph), 3.80 (d, 1H, J=13.0 Hz, CH_2–Ph), 3.96 (d, 1H, J=6.0 Hz, Ph–CH-NH), 4.34 (t, 1H, J=8.5 Hz, H-5), 4.50 (t, 1H, J=8.5 Hz, H-5), 4.83 (ddd, 1H, J=13.0, 8.5, 6.0 Hz, H-4), 7.29 (m, 3H), 7.35 (m, 4H), 7.44 (m, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 51.1 (t), 62.6 (d), 66.7 (t), 79.2 (d), 127.5 (d), 127.8 (d), 127.9 (d), 128.5 (d), 128.8 (d), 129.4 (d), 136.9 (s), 139.6 (s), 155.0 (s); HRFAB-MS m/z calcd for $[M+H]^+$ $C_{17}H_{18}NO_3$ 284.1286, found: 284.1288.

4.3.5. From 3-benzhydrylamino-3-phenyl-1,2-propanediol[21] (1e). The reaction with diphosgene afforded 1,3-dioxolan-2-one (**5e**) (96%); the reaction with phosgene afforded 1,3-dioxolan-2-one (**5e**) (97%).

4.3.5.1. 4-[(Benzhydrylamino)(phenyl)methyl-1,3-dioxolan-2-one (5e). White solid. Mp 69–70 °C (hexane/chloroform). IR (KBr): ν_{max} 1753 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.76 (d, 1H, J=6.0 Hz, Ph–CH–NH), 4.39 (t, 1H, J=8.0 Hz, H-5), 4.46 (t, 1H, J=8.0 Hz, H-5), 4.68 (s, 1H, CH–Ph_2), 4.91 (dd, 1H, J=8.0, 6.0 Hz, H-4), 7.25 (m, 4H), 7.32 (m, 8H), 7.42 (m, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 61.7 (d), 63.6 (d), 67.2 (t), 79.1 (d), 127.2 (d), 127.4 (d), 127.5 (d), 127.6 (d), 127.9 (d), 128.0 (d), 128.1 (d), 128.3 (d), 128.6 (d), 128.7 (d), 128.9 (d), 129.0 (d), 129.3 (d), 136.7 (s), 142.2 (s), 143.7 (s), 155.1 (s); HREI-MS m/z calcd for $[M]^+$ $C_{23}H_{21}NO_3$ 359.1521, found: 359.1531.

4.3.6. From 3-(*tert*-butyldimethylsilyloxy)-1-amino-1-phenylpropan-2-ol (1f). The reaction with diphosgene afforded 1,3-oxazolidin-2-one (**2f**) (80%).

4.3.6.1. *cis*-5-(*tert*-Butyldimethylsilyloxymethyl)-4-phenyl-1,3-oxazolidin-2-one (2f). White solid. Mp 103–104 °C. IR: ν_{max} 3226, 1737 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ −0.14 (s, 3H), −0.09 (s, 3H), 0.82 (s, 9H), 3.28 (dd, 1H, J=11.0, 6 Hz, CH_2–O), 3.46 (dd, 1H, J=11.0, 6.0 Hz, CH_2–O), 4.84 (m, 1H, H-5), 4.98 (d, 1H, J=8.5 Hz, H-4), 6.3 (br s, 1H, NH), 7.28 (m, H), 7.36 (m, H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ −5.5 (q), −5.1 (q), 18.4 (s), 25.9 (q), 58.7 (d), 61.8 (t), 80.5 (d), 127.5 (d), 128.9 (d), 129.0 (d), 136.4 (s), 159.7 (s); HRFAB-MS m/z calcd for $[M+H]^+$ $C_{16}H_{26}NO_3Si$ 308.1681, found: 308.1686.

4.3.7. From 3 (*tert*-butyldimethylsilyloxy)1-methylamino-1-phenyl-propan-2-ol[23] (1g). The reaction with diphosgene afforded *cis*-1,3-oxazolidin-2-one (**2g**) (73%).

4.3.7.1. *cis*-5-(*tert*-Butyldimethylsilyloxymethyl)-3-methyl-4-phenyl-1,3-oxazolidin-2-one (2g). White solid. Mp 117–118 °C. IR: ν_{max} 3417, 1753, 1445 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ −0.17 (s, 3H), −0.10 (s, 3H), 0.79 (s, 9H), 2.75 (s, 3H), 3.23 (dd, 1H, J=11.0, 6.0 Hz, CH_2–O), 3.48 (dd, 1H, J=11.0, 5.0 Hz, CH_2–O), 4.75 (m, 2H, H-4+H-5), 7.16 (m, 2H), 7.35 (m, 3H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ −4.9 (q), −4.8 (c), 18.3 (s), 25.9 (q), 29.7 (q), 61.6 (t), 64.2 (d), 77.4 (d), 127.9 (d), 128.9 (d), 129.2 (d), 133.9 (s), 158.5 (s); HREI-MS m/z calcd for $[M]^+$ $C_{17}H_{27}NO_3Si$ 321.1760, found: 321.1783.

4.3.8. From 3-(*tert*-butyldimethylsilyloxy)-1-benzhydrylamino-1-phenylpropan-2-ol (1h). The reaction with diphosgene afforded *cis*-1,3-oxazolidin-2-one (**2h**) (71%).

4.3.8.1. *cis*-3-Benzhydrylamino-5-(*tert*-butyldimethylsilyloxy)-4-phenyl-1,3-oxazolidin-2-one (2h). Colourless oil. IR (KBr): ν_{max} 3484, 1756, 1105, 837 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ −0.17 (s, 3H); −0.11 (s, 3H); 3.22 (dd, 1H, J=11.0, 7.0 Hz, CH_2–O), 3.51 (dd, 1H, J=11.0, 6.0 Hz, CH_2–O), 4.79 (d, 1H, J=8.0 Hz, H-4), 4.89 (m, 1H, H-5); 7.01 (m, 10H), 7.27 (m, 3H), 7.34 (m, 2H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ −5.8 (q), −5.7 (q), 18.1 (s), 25.9 (q), 45.9 (d), 61.3 (t), 62.3 (d), 78.3 (d), 126.3 (d), 126.8 (d), 127.1 (d), 127.2 (d), 127.3 (d), 127.7 (d), 128.0 (d), 128.1 (d), 128.3 (d), 128.4 (d), 128.7 (d), 128.8 (d), 128.9 (d), 129.0 (d), 129.7 (d), 135.1 (s), 137.7 (s), 139.5 (s), 157.7 (s); HRFAB m/z calcd for $[M+H]^+$ $C_{29}H_{36}NO_3Si$ 474.2464, found: 474.2486.

4.4. General procedure for the deprotection of the silyl ethers with KF

A mixture of 5-(*tert*-butyldimethylsilyloxymethyl)-1,3-oxazolidin-2-one **2f**, **2g** or **2h** (4.7 mmol), potassium fluoride (800 mg, 14.0 mmol) and methanol (10 mL) was heated to reflux for 3 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel, eluting with hexane/ethyl acetate mixture to afford the corresponding 1,3-oxazolidin-2-one (**2a**) (90%), (**2b**) (94%) or **2e** (96%).

4.4.1. *cis*-3-Benzhydrylamino-5-hydroxymethyl-4-phenyl-1,3-oxazolidin-2-one (2e). From the deprotection of **2h**. Colourless oil. IR (KBr): ν_{max} 3430, 1744 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.18 (dd, 1H, J=12.0, 7.5 Hz, CH_2–O), 3.23 (br s, 1H, OH), 3.35 (dd, 1H, J=12.0, 7.5 Hz, 1H, CH_2–O), 4.84 (d, 1H, J=8.0 Hz, H-4), 4.94 (m, 1H, H-5), 5.93 (s, 1H), 7.07 (m, 4H), 7.23 (m, 4H), 7.30 (m, 7H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 52.4 (d), 61.7 (t), 61.9 (d), 62.3 (d), 78.7 (d), 127.3 (d), 127.4 (d), 127.9 (d), 128.1 (d), 128.2 (d), 128.3 (d), 128.7 (d), 129.7 (d), 134.9 (s), 137.7 (s), 139.3 (s), 157.8 (s); HREI-MS m/z calcd for $[M]^+$ $C_{23}H_{21}NO_3$ 359.1521, found: 359.1481.

Acknowledgements

A.H. thanks AECI (Agencia Española de Cooperación Internacional) for a Grant. This work was supported by research funds provided by the Ministerio de Educación y Cultura of the Spanish Government (projects BQU2003-01756 and BQU2002-01032), and by the Agencia Valenciana de Ciencia y Tecnología of the Generalitat Valenciana (reference GRUPOS03/176).

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Access to substituted thiapyrrolizidinones and fused pyridones using the domino *N*-acyliminium-thionium equilibrium/1,3-dipolar cycloaddition/desulfurization cyclization cascade

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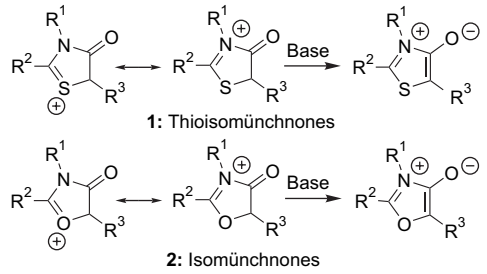
Received 10 March 2006; revised 5 April 2006; accepted 6 April 2006
Available online 6 May 2006

Abstract—Substituted thiapyrrolizidinones and fused pyridones, and quinolizinones were reported efficaciously from suitable thioamides in yields ranging from 30% to 65%. The reaction proceeded in a one-pot procedure as cascade process by the intramolecular 1,3-dipolar cycloaddition of thioisomünchnones followed by desulfurization of the adducts. During these investigations, the mechanistic aspects of the process were also discussed.

1. Introduction

Thioisomünchnones conventionally named 1,3-thiazolium-4-olates (**1**) and belonging to a five-membered mesoionic systems are receiving less attention compared to their oxygen homologues as isomünchnones (1,3-oxazolium-4-olates (**2**)), (Scheme 1).[1] Since the pioneering work by Potts and co-workers given on their syntheses and reactivity,[2] it has been demonstrated now that these species constitute powerful intermediates in the synthesis of complex nitrogen-containing heterocycles.[3] In particular, they offer rapid access to different heterocyclic compounds containing a pyridone nucleus useful in natural products syntheses[4] as well as β-lactams, polyhydrothiophenes in chiral form or not, thiiranes, thiophenes, etc.[4,5]



Scheme 1. Mesoionic five-membered heterocyclic mesomeric betaines.

These mesoionic ring systems, which are easily prepared by the reaction of *N*-monosubstituted thioamides with α-haloacyl halides in the presence of 2 equiv of triethylamine are stabilized by the conjugation effect as shown in Scheme 1 (thionium ion ↔ *N*-acylimiunim cation ↔ mesoionic specie). In addition, some extra stabilization could arise from the conjugation with an exocyclic electron rich aromatic or heteroaromatic system when R^2 is an aryl or heteroaryl group. As a consequence, these mesomeric betaines exhibit an interesting synthetic potential, which could be attributed in addition to (a) the interesting physical properties they possess,[6] and importantly (b) the propensity of its thio-carbonyl ylide dipole to undergo 1,3-dipolar cycloaddition with a range of double and triple-bond dipolarophiles.

Due to the high number of natural and unnatural biological active molecules containing the thiapyrrolizinone[7] and quinolizinone[8] subunits, the use of that approach is still of continuous interest in organic synthetic chemistry. Among the most known naturally occurring alkaloids, oxogambirtannine (**3**)[9] and sempervilam (**4**)[10] belonging to the yohimboid alkaloids, constitute one of the major subgroups of the indole class. If these structures have not yet shown any biological properties, mitragynaline (**5a**, R=OMe) as an indole alkaloid,[11] was isolated from the Malaysian *Mitragyna speciosa korth* plant. This is used in the Malay Peninsula as a stimulant like coca or as substitute from opium.[12] Moreover other tricyclic benzo- and thieno[*a*]quinazolines developed by investigators at Hoffman La-Roche, Ltd have been shown to be excellent alternatives to molecules with benzodiazepines scaffolds for the treatment of anxiety and sleep disorders.[13]

Keywords: Thioisomünchnone; 1,3-Dipolar cycloaddition; Thioamide; Heterocycle; Domino process; Lawesson's; Thionation; One-pot procedure.
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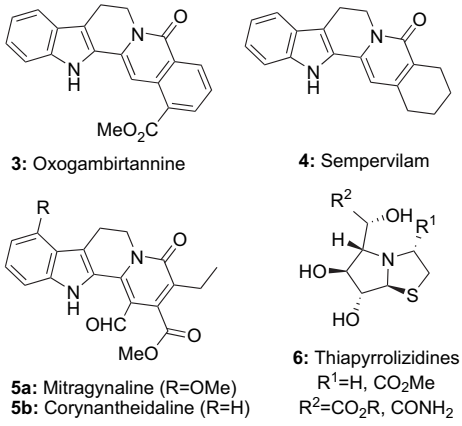
0040–4020/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.tet.2006.04.032

On the other hand, pyrrolo[2,1-*b*]thiazoles are rare scaffolds since only few reports are done in the literature. In this sense, whatever, two related structures were reported by the Padwa group using that strategy[4,14] while others described some thia-analogues of the glycosidase inhibitors polyhydroxylated pyrrolizidinones (**6**, R^1=H and CO_2Me; R^2=CO_2R and $CONH_2$).[15,16] The strategy used in these cases was based on the cyclodehydration of thiazolidines bearing an hydroxymethyl branched group[15] and an unexpected ring contraction of 7,5-fused bicyclic thiazolidine-lactams,[16] respectively.

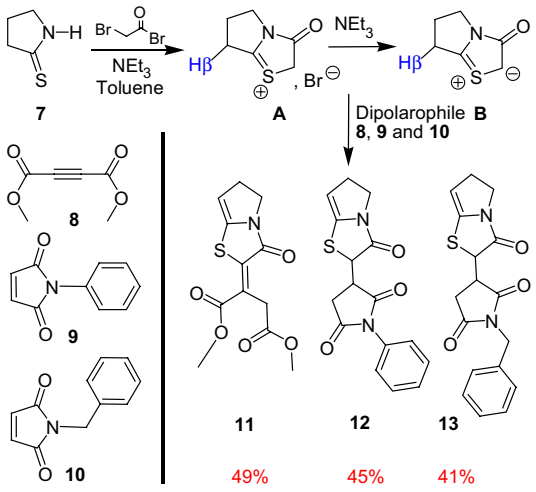
2. Results and discussion

As part of a long-term project dealing with our search for a simple synthetic route to heterocyclic homologues of the above structures (Scheme 2), which could be applicable to the synthesis of different condensed five and six-membered azacycles, we explore in this paper the intramolecular 1,3-dipolar cycloaddition of new thioisomünchnones with systematically three different dipolarophiles. This process proceeds in a one-pot procedure and result in the formation of new substituted 1,3-thiazolidinones and fused pyridones, heterocyclic scaffolds with promising biological activity.



Scheme 2. Representative structures containing thiapyrrolizinone and quinolizinone subunits.

Because of the use of thioisomünchnone of type **B** as a dipole, in cycloaddition reactions remains unreported, we first investigated the behavior of this thioisomünchnone, derived from pyrrolidine-2-thione (**7**), in the intramolecular 1,3-dipolar cycloaddition conditions as shown in Scheme 3. For this purpose, the requisite pyrrolidine-2-thione (**7**) was obtained in one step in quantitative yield by thionation of pyrrolidine-2-one with 1 equiv of Lawesson's reagent in dry toluene at reflux for 3 h[17] and the dipolarophile chosen as the reaction partner for optimizing conditions was the methyl acetylenedicarboxylate (**8**). So, after intensive screening of reaction conditions,[18] the use of 1.1 equiv of 2-bromoacetyl bromide, 2.0 equiv of dry triethylamine as a base, 1.5 equiv of suitable dipolarophile, and anhydrous toluene as solvent was found to be the most effective combination for obtaining representative results. Under these conditions, pyrrolidine-2-thione (**7**) with 2-bromoacetyl bromide and triethylamine led to thionium salt **A** then mesoionic five-membered **B**, which after reaction with dipolarophile **9**, led to the structure containing thiapyrrolizin-one ring **11** in 49% yield (Scheme 3).

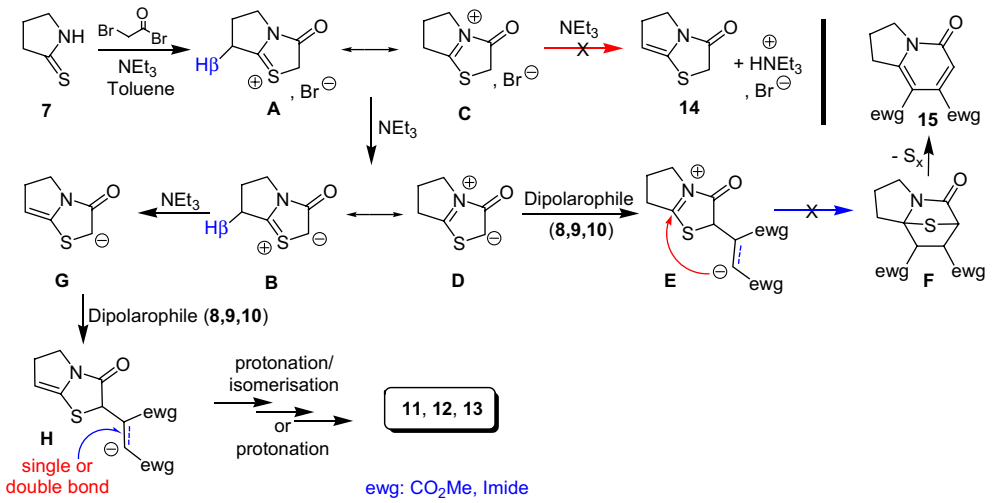


Scheme 3. Scheme leading to new structures containing thiapyrrolizinone nucleus as **11**, **12**, and **13** from betaine **B**.

Having the best conditions in hand, we examined next the reaction of the same substrate as above with 2-bromoacetyl bromide and other dipolarophiles in the presence of triethylamine (Scheme 3). In this context, the commercially available *N*-phenylmaleimide (**10**) and *N*-benzylmaleimide (**11**) were found to be employable efficaciously without the change of the course of the cascade process as well as the reaction yields. Indeed, the reaction products, identified as thiapyrrolizinones **12** and **13**, were isolated in 45% and 41% yields, respectively, comparables to that obtained for the thiapyrrolizinone derivative **11**.

As shown in Scheme 4, the suggested reaction mechanism illustrates three pathways that appear possible. Initially formed thionium cation **A**, in equilibrium with corresponding *N*-acyliminium one **C**, would lead with triethylamine to either the thiapyrrolizinone **14** or the carbanion **G** via mesoionic five-membered heterocyclic mesomeric betaines **B** and **D**, in equilibrium. No traces of product **14** were found in the reaction mixture examined by TLC before addition of a second equivalent of triethylamine, a dipolarophile as well as at the end of the reaction process before any purification. Also, no trace of pyridone component **15** as well as corresponding thia-bridged products **F**, which would be formed by the intramolecular 1,3-dipolar cycloaddition of 3-thiazolium-4-olate salt (**B** or **D**, see Scheme 4), was observed. Therefore, these thioisomünchnones species afforded the thiapyrrolizinones **11**, **12**, and **13** upon abstraction of the hydrogen atom at β-position of nitrogen and/or sulfur atom(s) in the intermediate (**A**↔**C**) or (**B**↔**D**) followed in an ultimate stage by addition of a dipolarophile and protonation of the resulting carbanion. Interestingly, the process seems to be general and the key step seems to be the hydrogen abstraction.

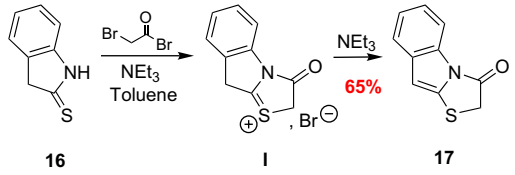
The ^{1}H NMR spectra of thiapyrrolizinones **11**, **12**, and **13** are characterized by having the olefinic proton of the dihydropyrrole nucleus in the aromatic region (from δ=6.98 up to 7.35 ppm). This was coupled with methylene protons at β-position of the dihydropyrrole ring and in the case of



Scheme 4. Mechanistic considerations leading to the formation of substituted thiapyrrolizinone scaffolds **11**, **12**, and **13**.

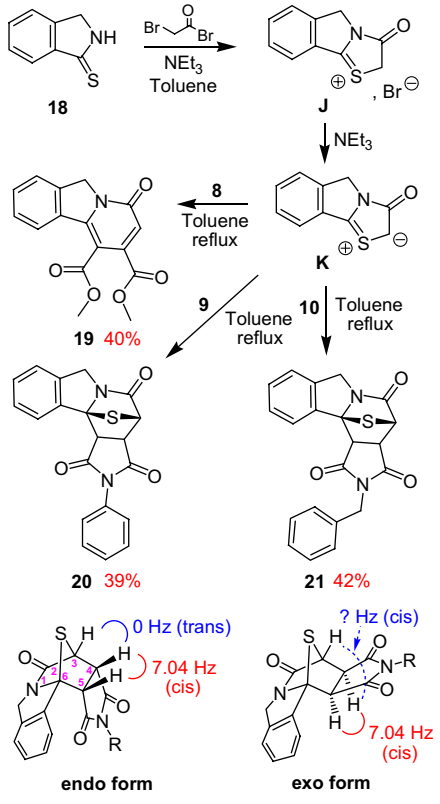
product **11**, they appear as a triplet at δ=6.98 ppm with coupling constant of J=5.48 Hz. This proton became singlet when the methylene protons outlined above were irradiated. Also, the ^{1}H NMR spectra of **11** showed the presence of three methylene groups CH_2 demonstrating the carbon–carbon double-bond migration. As a consequence, the resulting thiapyrrolizinone system bearing an exocyclic double-bond in the conjugation with either the sulfur atom or the carbonyl function constitutes the thermodynamic isomer.

Consistent with the above remark, isoindoline-1-thione (**16**) (Scheme 5), obtained from oxindole by known procedure[19] was subjected to our standard protocol outlined above. Surprisingly, whatever change operated in the experimental procedure only product namely, thiazolo[3,2-*a*]indol-3-one (**17**), was obtained as the sole reaction product in yields near 65%.[20] Also, when the reaction was repeated using more than 2 equiv of triethylamine (up to 4 equiv), the reaction profile remains unchanged. The change of dipolarophile in combination of an excess of triethylamine had also little effect on the course of the reaction as well as on the yield of the cyclized product **17**. From these considerations, the benzylic proton abstraction leading to the more stable fused indole **17** appears to be largely responsible for the differences observed in the formation of heterocyclic systems **11**, **12**, **13**, and **17**, respectively.[21]



Scheme 5. One-pot protocol leading to thiazolo[3,2-*a*]indol-3-one (**17**).

We next directed our attention to explore the behavior of 2,3-dihydroisoindole-1-thione (**18**)[22] under the precedent well established protocol. The choice of this thioamide is due to the absence of a proton at α-position of the thio-carbonyl function. Thus, treatment of **18** in the presence of 1 equiv of methyl acetylenedicarboxylate (**9**) according to conditions outlined above, gave exclusively after chromatography purification methyl 4-oxo-4,6-dihydropyrido[2,1-*a*]isoindole-1,2-dicarboxylate (**19**) as a brown solid in 40% yield (Scheme 6).



Scheme 6. One-pot procedure leading to dihydropyrido[2,1-*a*]isoindole **19** and thiadiazacyclopenta[*c*]fluorenetriones **20** and **21**.

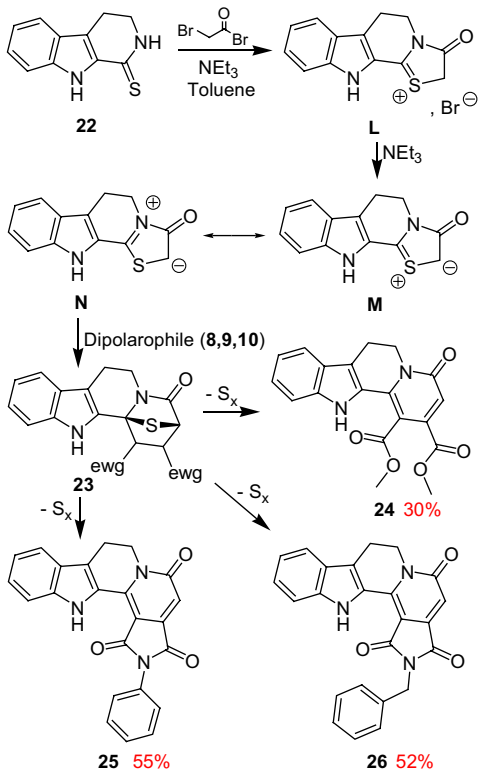
As highlight in Scheme 6, the formation of that product seems to proceed by a cascade process. This implies the formation of thionium ion **J**, its transformation with NEt_3 into corresponding thioisomünchnone salt **K** followed by the intramolecular 1,3-dipolar cycloaddition with **8** and desulfurization of the adduct in an ultimate step. This mechanistic paradigm was extended efficaciously to other dipolarophiles as **9** and **10**, but surprisingly the process stopped at the

bridgehead sulfur components **20** and **21**. Indeed, reaction of **18** with **9** and **10** furnished thiadiazacyclopenta[*c*]fluorenetriones **20** and **21** in 39% and 42% yields, respectively. From these results, the reaction appears tolerant with respect to the nature of dipolarophile and, importantly, it proceeds without any significant changes in the reaction yields.

The structure of these products was assigned on the basis on their IR, NMR (^{1}H and ^{13}C experiments including NOE difference and DEPT program, respectively) as well as elemental analyses. For instance, the ^{1}H NMR spectra of **19** showed a non-conventional doublet of doublet with J=17.12 Hz centered at δ=3.71 ppm, one singlet at δ=6.02 ppm characteristics of non-equivalent[23] methylene protons (CH_2–N) and a pyridone proton, respectively. On the other hand, the ^{1}H NMR spectra of fluorenetrione derivative **20** showed besides nine aromatic protons four aliphatic proton blocks. They consist of two doublets at δ=3.16 ppm and δ=3.42 ppm with coupling constant of about J=7.04 Hz (cis coupling between H_4 and H_5, see Scheme 6), a doublet of doublet for methylene protons (CH_2–N as an AB system, which appeared at δ=3.62 and 3.80 ppm with coupling constant J=16.43 Hz), and one singlet at δ=5.68 ppm attributed to H_3.[24] Interestingly, product **21** exhibits same profile with, in addition, an AB system at down field centered at δ=4.77 ppm with J=14.09 Hz. These data are consistent with the data described earlier by Potts et al.[2b,25] for the mesoionic *N*-phenylmaleimide adduct in the *endo* configuration. In these cases, the coupling constant between the 3 and 4 protons in the *endo* form was of about 1 up to 1.5 Hz indicating a trans coupling, and between the protons 4 and 5 it was of J=7 Hz consistent with a cis coupling. Our observations were in accordance with these values, indicating the formation of the thiadiazacyclopenta[*c*]fluorinetriones **20** and **21** in only the *endo* configuration.

For the generalization and additional demonstration of our tandem protocol, another class of thioisomünchnones fused to six-membered azacyclic system was investigated (Scheme 7). In the first step, the requisite 2,3,4,9-tetrahydro-β-carboline-1-thione (**22**) was prepared by two pathways from tryptamine. In fact, the reaction of tryptamine with phosgene in dry toluene gave 2,3,4,9-tetrahydro-β-carboline-1-one in 78% yield. The thioamide **22** was obtained in an ultimate step by thionation of the amide with Lawesson's reagent in toluene in 82% yield.[26] This product was also obtained directly in one step, with however very modest yield of 29%, when tryptamine was treated with the NEt_3/$ClCO_2Et$ combination in the presence of CS_2 used as reactant and solvent.

As showed in Scheme 7, the reaction of the above 2,3,4,9-tetrahydro-β-carboline-1-thione (**22**) with 2-bromoacetyl bromide (1.1 equiv) and triethylamine (1 equiv) in toluene gave the corresponding thionium cation **L**. By its heating with additional 1 equiv of triethylamine the mesoionic salt **M** was generated and this underwent an external 1,3-dipolar cycloaddition and desulfurization with methyl acetylenedicarboxylate (**8**) to give after chromatography purification methyl 4-oxo-4,6,7,12-tetrahydroindolo[2,3-*a*]quinolizine-1,2-dicarboxylate (**24**) as red-orange solid in 30% yield. Here, the tandem route evoked above was evidenced since the desulfurization of the key adduct intermediate **23**



Scheme 7. Tandem process giving *N*-substituted indoloquinolizinone products **24**, **25**, and **26**.

(ewg=CO_2Me) was effective without additional heating. In this case, all the tentative made for isolation of the intermediate **23** failed.

With *N*-phenylmaleimide (**9**) and *N*-benzylmaleimide (**10**) as dipolarophiles, the betaine **M**[27] under reflux in toluene gave an extremely colorless solution, which after a classical work up, indicated the presence of only one product identified as indolopyrroloquinolizinones **25** and **26** (Scheme 7). These results demonstrate the effectiveness of the betaine formation → intramolecular 1,3-dipolar cycloaddition → desulfurization tandem process and the products were isolated in appreciable yields of 55% and 52%, respectively, more better than the ones indicated for related structures as above. This could be explained by the fact that 1,3-thiazolium-4-olates salts in β-carboline series were more stable in thermal conditions than betaines formed above in pyrrolidinone, isoindolinone, and indolinone heterocycles.

Again, the structure of these products was secured by the NMR study. Thus, the ^{1}H NMR spectra of **24**, **25**, and **26** showed two triplets characteristics of –N–CH_2–CH_2– functionality from δ=3.11 to 3.19 ppm and from δ=4.47 to 4.89 ppm, respectively, with coupling constants of about J=7.03–7.04 Hz. Especially diagnosis was made by the presence of a singlet proton at δ=6.91, 7.02, and 6.92 ppm for structures **24**, **25**, and **26**, respectively, characteristic of the formation of the pyridone nucleus. These values were in accordance with those reported for compounds containing the pyridone ring bearing an hydrogen atom at the α-position of the thiolactam function.[8a,28] In the concomitant fashion, the ^{13}C NMR spectra of these products also showed two methine carbons, which inverted in the DEPT-135

experiments. In the case of product **26**, an additional methine carbon corresponding to the benzyl group also appeared at δ=6.92 ppm in the ^{13}C NMR spectra but inverted in the ^{13}C NMR, recorded using DEPT-135 technique. These values, as well as elemental analyses are in accordance with the proposed structures.

3. Conclusion

In summary, we have demonstrated the utility of 1,3-thiazolium-4-olates salts, derived from pyrrolidine-2-thione (**7**) and isoindoline-1-thione (**16**), to create with dipolarophiles a set of diverse and new thiapyrrolizidinones. The reaction seems to proceed with a hydrogen abstraction as well as addition of the dipolarophile. This consequently induces the interruption of the 1,3-dipolar cycloaddition process in the intramolecular fashion.

Further, with thioisomünchnones in benzene and indole series not possessing a hydrogen atom at β-position of the sulfur atom, the tandem betaine formation/intramolecular 1,3-dipolar cycloaddition/desulfurization process was effective and led to fused pyridones and quinolizinones products **19** and **24**–**26**. Elsewhere, in the case of 2,3-dihydroisoindole-1-thione (**18**) as a betaine precursor, thiadiazacyclopenta[*c*]fluorenetrione derivatives **20** and **21** exclusively in *endo* configuration were formed in an interruption of the tandem process as outlined above. Finally, the yields associated with obtaining these products were generally ranged from 30% to 65%. The latter were higher in betaines derived from β-carboline.

Finally, these processes might find applications as valuable strategies in syntheses of potentially active and bioactive compounds. Studies along this line are currently underway in our laboratory, and the results will be published soon.

4. Experimental

4.1. General

All melting points were measured on a Boetius micro hot-stage and are uncorrected. The infrared spectra of solids (potassium bromide) and liquids (neat) were recorded on a Perkin–Elmer FTIR paragon 1000 spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 (300 MHz) instrument in deuteriochloroform unless other indicated solvent and chemical shifts (δ) are expressed in parts per million relative to TMS as internal standard. Ascending thin layer chromatography was performed on precoated plates of silica gel 60 F_{254} (Merck) and the spots were visualized using an ultraviolet lamp or an iodine vapor. Mass spectral measurements were recorded on a AEI MS 902 S spectrophotometer. The elemental analyses were carried out by the microanalysis laboratory of INSA, F-76130 Mont-Saint-Aignan, France.

4.2. General procedure for the reaction of thioisomünchnones with dipolarophiles

To a solution of thioamide (4.94 mmol) in 30 mL of dry toluene was added 0.47 mL (5.39 mmol) of 2-bromoacetyl bromide at room temperature under an atmosphere of nitrogen. The reaction mixture was stirred at room temperature for 30 min and then heated at 100 °C for 3 h. After cooling, 1.39 mL (9.89 mmol) of triethylamine was added to the solution at room temperature followed 10 min after by dropwise addition of a solution of 7.39 mmol of the expected dipolarophile dissolved in 10 mL of dry toluene. The mixture was heated at reflux for an additional 12 h, cooled to room temperature, concentrated under reduced pressure, and chromatographed on a silica gel column using a cyclohexane/AcOEt (4/1) as eluting mixture to give solids in yields ranging from 30% to 65%.

4.2.1. Dimethyl (*Z*)-2-(3-oxo-(5,6-dihydropyrrolo[2,1-*b*]thiazol-2(3*H*)-ylidene)succinate (11). This product was isolated as a yellow solid in 49% yield and melted at 147–149 °C; IR (KBr) $\bar{\nu}_{max}$=2977 (CH), 1738 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.53 (dt, 2H, *J*=7.82 and 5.48 Hz, CH_2–N), 3.71 (s, 2H, CH_2), 3.73 (s, 3H, CH_3), 3.74 (s, 3H, CH_3), 3.91 (dt, 2H, *J*=7.04 Hz, CH_2–CH=), 6.98 (t, 1H, *J*=5.48 Hz, CH=); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 26.6 (CH_2), 31.2 (CH_2), 48.2 (CH_2), 52.1 (CH_3), 52.3 (CH_3), 131.3 (C^q), 138.9 (CH=), 152.4 (C^q), 155.2 (C^q), 168.0 (C=O), 168.3 (C=O), 173.0 (C=O); Anal. Calcd for $C_{12}H_{13}NO_5S$ (283.31): C, 50.88%; H, 4.63%; N, 4.94%. Found: C, 50.65%; H, 4.44%; N, 4.81%.

4.2.2. 3-(3-Oxo-2,3,5,6-tetrahydropyrrolo[2,1-*b*]thiazol-2-yl)-1-phenylpyrrolidine-2,5-dione (12). This product was isolated as an orange solid in 45% yield and melted at 152–154 °C; IR (KBr) $\bar{\nu}_{max}$=3000 (CH), 2985 (CH), 1717 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.19–2.51 (m, 2H, CH_2), 2.55–2.80 (m, 1H, CH), 3.12–3.27 (m, 1H, CH), 3.45–3.63 (m, 2H, 2CH), 3.75–3.78 (m, 1H, CH), 4.36 (d, 1H, *J*=2.35 Hz, CH–S), 7.21–7.24 (m, 3H, CH_{aro}+CH=), 7.25–7.46 (m, 3H, CH_{aro}); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 22.6 (CH_2), 29.1 (CH_2), 42.7 (CH_2), 47.7 (CH), 55.4 (CH), 58.4 (CH), 86.1 (C^q), 126.5 (2×CH), 129.2 (CH), 129.4 (2×CH), 131.6 (C^q), 170.4 (C=O), 173.1 (C=O), 173.7 (C=O); Anal. Calcd for $C_{16}H_{12}N_2O_3S$ (314.07): C, 61.13%; H, 4.49%; N, 8.91%. Found: C, 61.00%; H, 4.31%; N, 8.69%.

4.2.3. 1-Benzyl-3-(3-oxo-2,3,5,6-tetrahydropyrrolo[2,1-*b*]thiazol-2-yl)-1-pyrrolidine-2,5-dione (13). This product was isolated as a yellow solid in 41% yield and melted at 158–160 °C; IR (KBr) $\bar{\nu}_{max}$=3011 (CH), 2982 (CH), 1709 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.15–2.45 (m, 2H, CH_2), 2.55–2.72 (m, 1H, CH), 3.08–3.23 (m, 1H, CH), 3.31 (d, 1H, *J*=7.04 Hz, CH), 3.40–3.57 (m, 1H, CH), 3.67 (dd, 1H, *J*=6.34 and 1.57 Hz, CH), 4.23 (d, 1H, *J*=7.04 Hz, CH–S), 4.64 (d, 2H, *J*=2.35 Hz, CH_2–N), 7.25–7.35 (m, 6H, CH_{aro}+CH=); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 26.5 (CH_2), 28.9 (CH_2), 42.6 (CH_2), 43.1 (CH_2), 47.7 (CH), 55.5 (CH), 57.9 (CH), 85.6 (C^q), 128.1 (CH), 128.7 (2×CH), 129.0 (2×CH), 135.1 (C^q), 173.7 (C=O), 174.2 (C=O), 178.4 (C=O); Anal. Calcd for $C_{17}H_{14}N_2O_3S$ (328.09): C, 62.18%; H, 4.91%; N, 8.53%. Found: C, 62.01%; H, 4.76%; N, 8.42%.

4.2.4. Thiazolo[3,2-*a*]indol-3-one (17). This product was isolated as a yellow solid in 65% yield and melted at 136–138 °C (lit.[20] 141–143 °C); IR (KBr) $\bar{\nu}_{max}$=3009 (CH),

2965 (CH), 1718 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.20 (s, 2H, CH_2), 6.85 (s, 1H, CH), 7.15–7.31 (m, 2H, CH_{aro}), 7.36–7.44 (m, 1H, CH_{aro}), 8.09 (dd, 1H, J=6.26 and 3.91 Hz, CH_{aro}); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 38.4 (CH_2), 100.4 (CH), 113.0 (CH), 119.6 (CH), 122.9 (CH), 122.9 (CH), 124.8 (CH), 132.0 (C^q), 135.7 (C^q), 136.8 (C^q), 166.6 (C=O); Anal. Calcd for $C_{10}H_7NOS$ (189.02): C, 63.47%; H, 3.73%; N, 7.40%. Found: C, 63.21%; H, 3.65%; N, 7.12%.

4.2.5. Methyl 4-oxo-4,6-dihydropyrido[2,1-*a*]isoindole-1,2-dicarboxylate (19). This product was isolated as a brown solid in 40% yield and melted at 104–106 °C; IR (KBr) $\bar{\nu}_{max}$=3004 (CH), 2942 (CH), 1767 (C=O), 1732 (C=O), 1700 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.71 (dd, 2H, J=17.12 Hz, CH_2), 3.79 (s, 3H, CH_3), 5.57 (s, 3H, CH_3), 6.02 (s, 1H, CH), 7.03–7.19 (m, 2H, $2CH_{aro}$), 7.39 (d, 1H, J=7.05 Hz, CH_{aro}), 7.50 (d, 1H, J=6.26 Hz, CH_{aro}); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 38.1 (CH_2), 52.9 (CH_3), 53.0 (CH_3), 113.0 (CH), 121.3 (CH), 122.1 (CH), 126.6 (CH), 126.9 (CH), 146.2 (C^q), 146.9 (C^q), 150.6 (C^q), 153.1 (C^q), 154.5 (C^q), 161.6 (C=O), 162.1 (C=O), 163.6 (C=O); Anal. Calcd for $C_{16}H_{13}NO_5$ (299.08): C, 64.21%; H, 4.38%; N, 4.68%. Found: C, 64.04%; H, 4.15%; N, 4.43%.

4.2.6. 3*a*,7,11*b*,11*c*-Tetrahydro-2-phenyl-4,11*b*-thia-2,6-diazacyclopenta[*c*]fluorene-1,3,5(4*H*)-trione (20). This product was isolated as a brown solid in 39% yield and melted at 218–220 °C; IR (KBr) $\bar{\nu}_{max}$=3019 (CH), 2936 (CH), 1718 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.16 (d, 1H, J=7.04 Hz, CH), 3.42 (d, 1H, J=7.04 Hz, CH), 3.62 (d, 1H, J=16.43 Hz, CH_2–N), 3.80 (d, 1H, J=16.43 Hz, CH_2–N), 5.68 (s, 1H, CH), 7.22–7.36 (m, 4H, H_{aro}), 7.38–7.55 (m, 5H, H_{aro}); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.5 (CH_2), 51.3 (CH), 55.0 (CH), 59.9 (CH), 80.4 (C^q), 120.4 (CH), 121.1 (CH), 125.6 (2CH), 128.5 (CH), 128.7 (CH), 129.1 (CH), 129.4 (2×CH), 131.4 (CH), 143.6 (C^q), 145.5 (C^q), 168.1 (C=O), 171.4 (C=O), 173.4 (C=O); Anal. Calcd for $C_{20}H_{14}N_2O_3S$ (362.07): C, 66.28%; H, 3.89%; N, 7.73%. Found: C, 66.02%; H, 3.64%; N, 7.55%.

4.2.7. 2-Benzyl-3*a*,7,11*b*,11*c*-tetrahydro-4,11*b*-thia-2,6-diazacyclopenta[*c*]fluorene-1,3,5(4*H*)-trione (21). This product was isolated as a brown solid in 42% yield and melted at 208–210 °C; IR (KBr) $\bar{\nu}_{max}$=3003 (CH), 2976 (CH), 1710 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.98 (d, 1H, J=7.04 Hz, CH), 3.26 (d, 1H, J=7.04 Hz, CH), 3.46 (s, 2H, CH_2–N), 4.66 (d, 1H, J=14.09 Hz, CH_2–N), 4.77 (d, 1H, J=14.09 Hz, CH_2–N), 5.57 (s, 1H, CH), 7.15–7.44 (m, 9H, H_{aro}); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.3 (CH_2), 43.3 (CH_2), 51.3 (CH), 55.0 (CH), 59.4 (CH), 80.4 (C^q), 120.2 (CH), 120.9 (CH), 128.2 (CH), 128.3 (CH), 128.7 (CH), 128.9 (2×CH), 130.4 (2×CH), 135.4 (C^q), 143.5 (C^q), 145.9 (C^q), 167.1 (C=O), 171.9 (C=O), 173.9 (C=O); Anal. Calcd for $C_{21}H_{16}N_2O_3S$ (376.09): C, 67.00%; H, 4.28%; N, 7.44%. Found: C, 66.87%; H, 4.03%; N, 7.21%.

4.2.8. Methyl 4-oxo-4,6,7,12-tetrahydroindolo[2,3-*a*]-quinolizine-1,2-dicarboxylate (24). This product was isolated as a red-orange solids in 30% yield and melted at 164–166 °C; IR (KBr) $\bar{\nu}_{max}$=3006 (CH), 2965 (CH), 1784 (C=O), 1760 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.11 (t, 2H, J=7.04 Hz, CH_2), 3.86 (s, 3H, CH_3), 3.91 (s, 3H, CH_3), 4.47 (t, 2H, J=7.04 Hz, CH_2), 6.91 (s, 1H, CH), 7.17 (t, 1H, J=7.04 Hz, CH_{aro}), 7.29 (t, 1H, J=7.05 Hz, CH_{aro}), 7.43 (d, 1H, J=7.82 Hz, CH_{aro}), 7.61 (d, 1H, J=7.83 Hz, CH_{aro}), 9.94 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 19.3 (CH_2), 41.5 (CH_2), 53.2 (CH_3), 53.5 (CH_3), 106.0 (C^q), 112.3 (CH), 118.8 (C^q), 119.1 (CH), 119.7 (CH), 120.9 (CH), 124.5 (C^q), 125.8 (CH), 128.5 (C^q), 137.8 (C^q), 138.9 (C^q), 140.9 (C^q), 161.5 (C=O), 165.9 (C=O), 169.7 (C=O); Anal. Calcd for $C_{19}H_{16}N_2O_5$ (352.11): C, 64.77%; H, 4.58%; N, 7.95%. Found: C, 64.45%; H, 4.33%; N, 7.76%.

4.2.9. 7,8-Dihydro-2-phenylindolo[2,3-*a*]pyrrolo[3,4-*a*]quinolizine-1,3,5(13*H*)-trione (25). This product was isolated as a red-orange solid in 55% yield and melted at 234–236 °C; IR (KBr) $\bar{\nu}_{max}$=3010 (CH), 2954 (CH), 1756 (C=O), 1742 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 3.24 (t, 2H, J=7.03 Hz, CH_2), 4.59 (t, 2H, J=7.03 Hz, CH_2), 7.02 (s, 1H, CH), 7.12–7.30 (m, 2H, CH_{aro}), 7.32–7.69 (m, 7H, CH_{aro}), 12.04 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 18.7 (CH_2), 20.8 (CH_2), 111.4 (CH), 113.2 (CH), 119.0 (C^q), 119.4 (CH), 120.6 (CH), 123.9 (C^q), 124.3 (C^q), 124.7 (CH), 126.2 (C^q), 127.6 (2×CH), 128.6 (CH), 128.9 (2×CH), 131.5 (C^q), 138.0 (C^q), 139.8 (C^q), 140.3 (C^q), 161.8 (C=O), 164.6 (C=O), 167.0 (C=O); Anal. Calcd for $C_{23}H_{15}N_3O_3$ (381.39): C, 72.43%; H, 3.96%; N, 11.02%. Found: C, 72.21%; H, 3.77%; N, 10.86%.

4.2.10. 2-Benzyl-7,8-dihydroindolo[2,3-*a*]pyrrolo[3,4-*a*]quinolizine-1,3,5(13*H*)-trione (26). This product was isolated as a red-orange solid in 52% yield and melted at 230–232 °C; IR (KBr) $\bar{\nu}_{max}$=3017 (CH), 2948 (CH), 1757 (C=O), 1749 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.19 (t, 2H, J=7.04 Hz, CH_2), 4.52 (t, 2H, J=7.04 Hz, CH_2), 4.89 (s, 2H, CH_2–N), 6.92 (s, 1H, CH), 7.14–7.59 (m, 9H, CH), 12.10 (s, 1H, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 19.0 (CH_2), 41.4 (CH_2), 41.8 (CH_2), 112.3 (CH), 118.7 (C^q), 119.6 (CH), 120.4 (CH), 123.6 (C^q), 123.8 (C^q), 123.9 (C^q), 124.5 (C^q), 126.2 (CH), 127.6 (CH), 128.1 (2×CH), 128.3 (2×CH), 128.5 (CH), 135.1 (C^q), 138.1 (C^q), 139.7 (C^q), 162.0 (C=O), 164.8 (C=O), 167.3 (C=O); Anal. Calcd for $C_{24}H_{17}N_3O_3$ (395.13): C, 72.90%; H, 4.33%; N, 10.63%. Found: C, 72.78%; H, 4.14%; N, 10.46%.

Acknowledgements

The authors are grateful to the Libyan Government for a Graduate Fellowship (2002–2005) attributed to A.H. We are also grateful for many fruitful discussions throughout the course of this work with Prof. B. Decroix.

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Synthesis of 1,4,5-trisubstituted-1,2,3-triazoles by copper-catalyzed cycloaddition-coupling of azides and terminal alkynes

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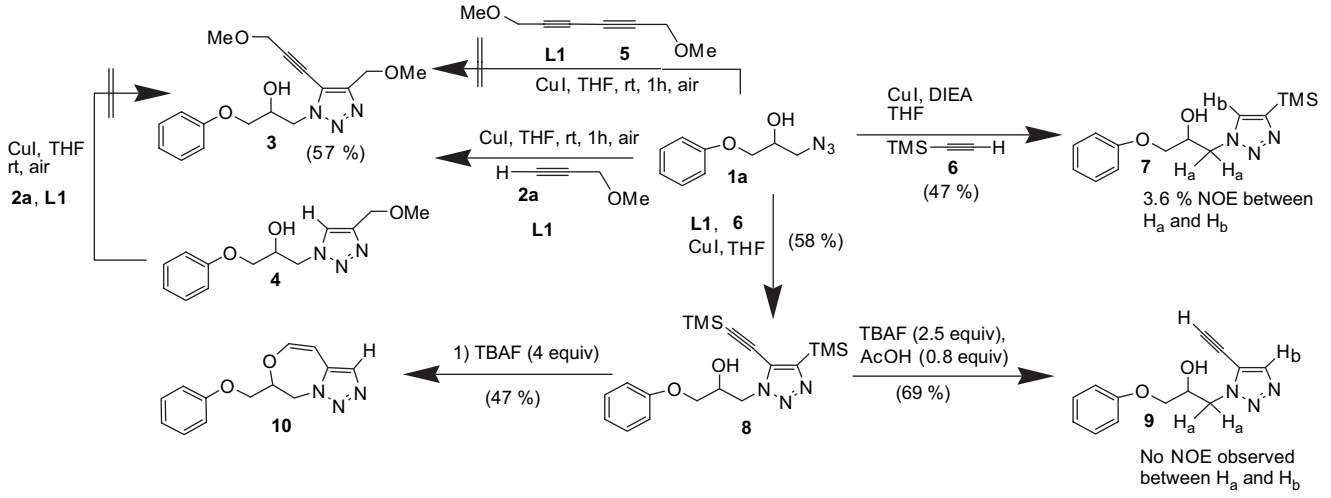
Received 10 February 2006; revised 1 April 2006; accepted 6 April 2006
Available online 12 May 2006

Abstract—Primary, secondary, and aromatic azides undergo 1,3 dipolar cycloaddition-coupling with an excess of alkyne in the presence of $Cu(CH_3CN)_4PF_6$ as catalyst, *N*,*N*,*N*′-trimethylethylenediamine as ligand, molecular oxygen, and 4-methoxymorpholine *N*-oxide (NMO) as co-oxidant to afford 1,4,5-trisubstituted-1,2,3-triazoles.

1. Introduction

1,2,3-Triazoles have received significant attention as biologically important heterocycles.[1] These compounds are typically prepared by thermal cycloaddition of azides and alkynes to afford a mixture of 1,4- and 1,5-disubstituted isomers.[2] Alternative methods have been developed to gain regioselectivity such as the use of push–pull alkenes containing a leaving group.[3] Recent publications from Sharpless and co-workers,[4] Meldal and co-workers,[5] and others[6] have reported copper(I) catalysis for regioselective cycloaddition of terminal alkynes and azides to afford exclusively 1,4-disubstituted-1,2,3-triazoles. Our interest in this methodology involved applications to diversify resin-bound alkynes.[7] Based on previous studies, we employed amine ligands in order to increase the solubility of copper(I).[8] However, treatment of hydroxy azide **1a** and alkyne **2a** (Scheme 1) with CuI and *N*,*N*-dimethylethylenediamine **L1** (Fig. 1) as ligand led unexpectedly to the production of 5-alkynyl-1,2,3-triazole **3**. Sharpless and co-workers have also observed these derivatives in the direct synthesis of trisubstituted triazoles via addition of bromomagnesium acetylides to azides.[9] In this communication, we report the regioselective formation of 1,4,5-trisubstituted-1,2,3-triazoles via copper-catalyzed cycloaddition-coupling and our initial studies concerning the scope and limitation of the process.



Scheme 1.

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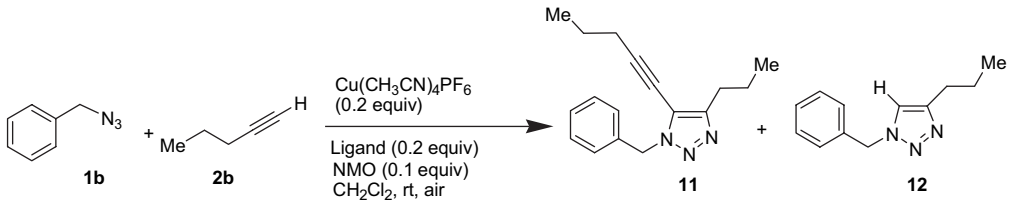
doi:10.1016/j.tet.2006.04.025



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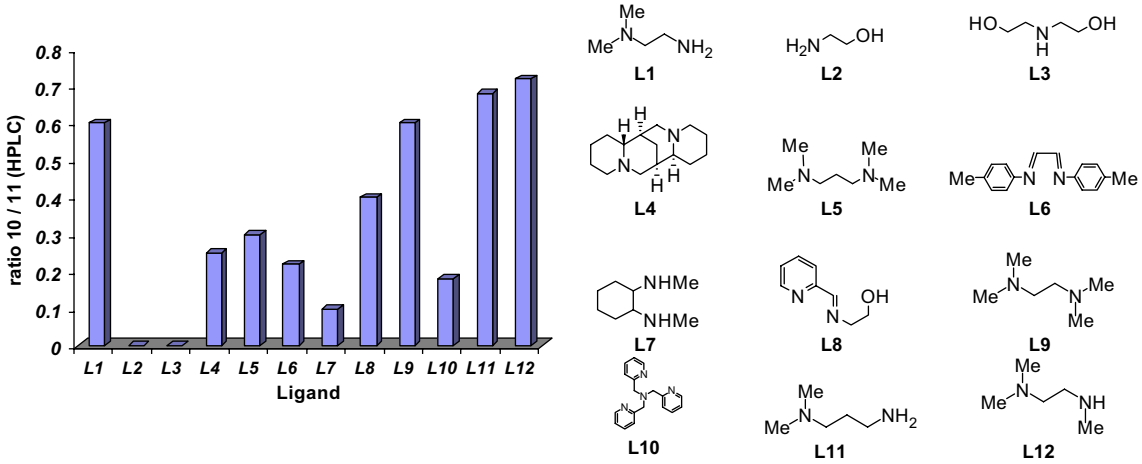
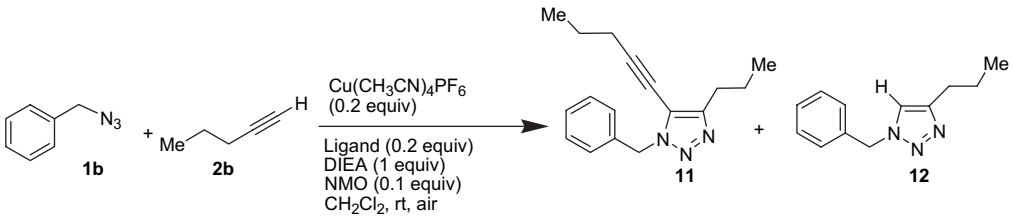


Figure 1. Parallel ligand evaluation. Ratio of **11/12** determined by HPLC analysis (gradient: 70:30 to 30:70 water/acetonitrile in 10 min) using evaporative light scattering detection (ELSD).

2. Results and discussion

We first conducted control experiments to define the requirements for copper source and alkyne coupling partner. Initial studies found that Cu(II) sources (e.g., $CuCl_2$) were not effective for cycloaddition-coupling of **1a** and **2a**. In addition, when Cu(I) or both Cu(I) and Cu(II) catalysts were used under oxygen-free conditions, only 1,4-disubstituted triazole **4** was observed. These results indicate that the presence of both Cu(I) and Cu(II) in an oxygen atmosphere maybe necessary for the production of **3**. Because the oxidative coupling of terminal alkynes (Glaser reaction) is also conducted using similar conditions (Hay's conditions),[10] we reasoned that the product **3** maybe formed by an initial Glaser coupling and subsequent 1,3-dipolar cycloaddition. However, treatment of 1,6-dimethoxyhexa-2,4-diyne[11] **5** and hydroxy azide **1a** with CuI and *N*,*N*-dimethylethylenediamine **L1** led to no reaction.[12] An alternative pathway may involve initial formation of 1,4-disubstituted triazole **4** and subsequent insertion of a second equivalent of alkyne at C5. Treatment of disubstituted triazole **4** with alkyne **2a** in the presence of CuI and *N*,*N*-dimethylethylenediamine **L1** led to no reaction. This result indicates that 1,4-disubstituted triazole **4** is not likely a discrete intermediate en route to 1,4,5-trisubstituted triazole **3**. The regioselectivity of the cycloaddition-coupling process was verified by the experiments outlined in Scheme 1. Copper(I)-mediated cycloaddition[4] of hydroxy azide **1a** and trimethylsilyl acetylene **6** afforded 1,4-triazole **7** (47%). The regiochemistry of **7** was confirmed using NOE difference NMR spectroscopy (3.6% NOE from the C5 vinyl hydrogen to the indicated methylene). Treatment of **1a** with **6**, **L1**, and CuI (THF, rt, and 8 h) afforded 1,4,5-trisubstituted triazole **8** (58%). Desilylation of **8** with tetrabutylammonium fluoride (TBAF) (1 equiv) buffered with acetic acid afforded 1,2,3-triazole **9** (69%), which did not have a corresponding NOE between the vinylic and methylene hydrogens. Interestingly, desilylation of **8** with TBAF (4 equiv, unbuffered) produced triazole oxepin **10** through 7-*endo*-*dig* cyclization.[13]

Further optimization of reaction conditions indicated that the copper source and oxygen diffusion were important factors for the reaction.[14] Tetrakisacetonitrile copper(I)hexafluorophosphate was found to be the most effective copper catalyst examined; use of an amine oxide as co-oxidant minimized the complication of direct delivery of oxygen[15] possibly by facilitating formation of high valent copper intermediates.[16] Reaction optimization (data not shown) indicated that a 2:1 ratio of Cu(I)/*N*-methylmorpholine oxide (NMO) afforded 1,4,5-trisubstituted triazole **3** in optimal yield (64%). Parallel evaluation of amino ligands[8] was next conducted in order to define structural requirements for formation of trisubstituted versus disubstituted triazoles using benzyl azide **1b** and pentyne **2b** as substrates (Scheme 2). We found that the nature of the diamine ligand had a pronounced effect on the ability to facilitate the formation of triazole **11** (Fig. 1). Interestingly, the presence of N–H functionality on the ligand favored **11** as the major product (ligands **L11** and **L12**), whereas peralkylated ligands increased selectivity toward the disubstituted product **12** (**L4** and **L10**) and hydroxyl amine ligands **L2** and **L3** led to no reaction. Further optimization using ligands **L5**, **L9**, **L11**, and **L12** was performed (Scheme 2 and Fig. 2). We found that addition of 1 equiv of Hünig's base (DIEA) at rt noticeably improved the ratio of **11/12**, optimally using **L12** (*N*,*N*,*N*′-trimethylethylenediamine) as ligand (Scheme 3). Additionally, a control experiment was



Scheme 3.

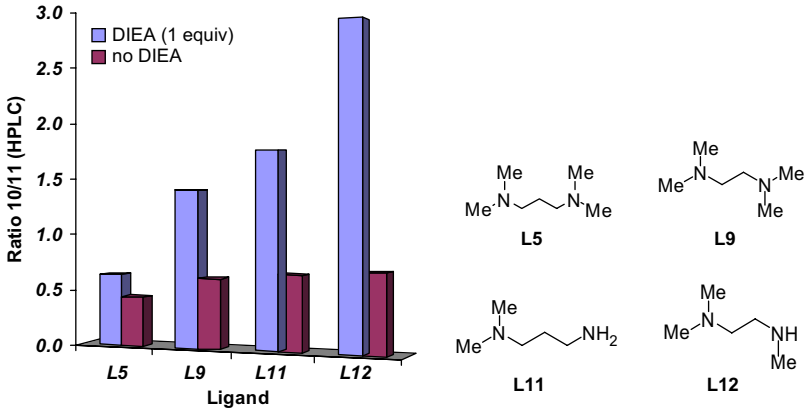


Figure 2. Parallel evaluation of selected ligands in presence of Hünig's base (DIEA, 1 equiv). Ratio of **11/12** determined by HPLC analysis (gradient: 70:30 to 30:70 water/acetonitrile in 10 min) using evaporative light scattering detection (ELSD).

performed without diamine ligand **L12**, reasoning that it might be possible that the added DIEA may also serve as a ligand. Although, the reaction went to completion under these conditions, the ratio of trisubstituted/disubstituted triazole for this reaction was significantly decreased (1:1).

We next investigated the cycloaddition-coupling using a number of azide and alkyne substrates using the optimized conditions in which DIEA (1 equiv) was included (Table 1 and Scheme 4). In many cases, lower isolated yields were likely due to competing Glaser coupling (diyne formation, entries 5, 6, and 7) and cycloaddition to afford 1,4-disubstituted triazoles without further alkynylation. Higher selectivity for the formation of trisubstituted triazoles was observed using a β-hydroxy azide (entries 1 and 2). Stabilization of a putative vinyl copper intermediate by hydroxyl chelation may account for this selectivity (Fig. 3).[17]

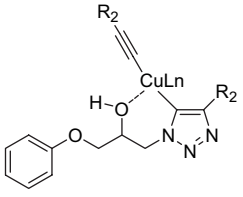


Figure 3.

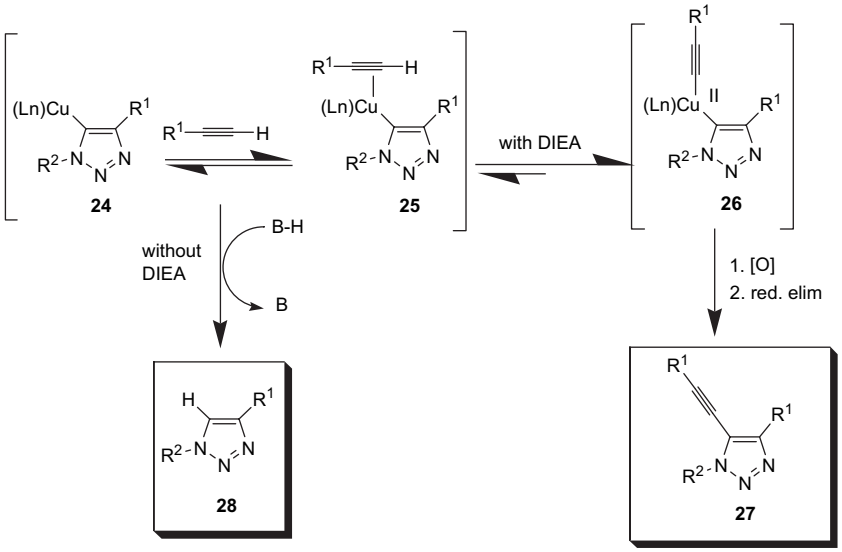
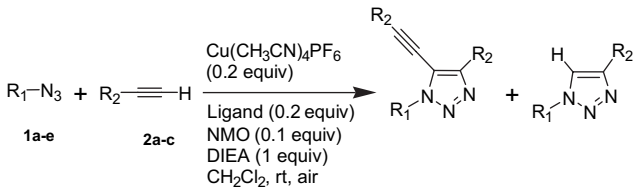


Figure 4.

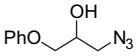
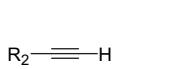
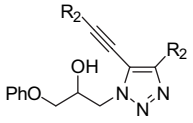
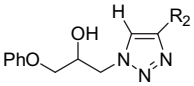
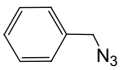
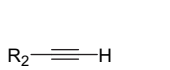
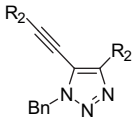
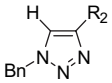
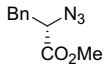
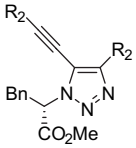
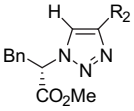
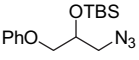
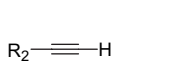
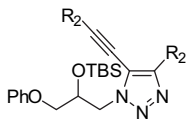
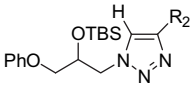
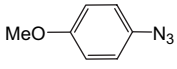
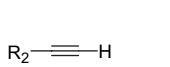
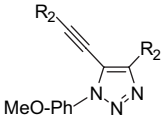
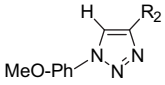


Scheme 4.

Blocking accessibility of the oxygen by silylation resulted in a decreased yield of 1,4,5-trisubstituted triazole (entry 7) reinforcing the lower selectivity generally observed for azides lacking a β-chelating group (entries 1 and 7).

Although it is not possible to define a detailed reaction mechanism at this stage,[18] the cycloaddition-coupling likely proceeds via formation of a Cu(II) intermediate such as **24** (Fig. 4). The role of the amine oxide maybe to oxidize **24** to a Cu(III) species to facilitate reductive elimination and regeneration of Cu(I).[19] The addition of Hünig's base may help the deprotonation step of a putative π Cu–acetylide complex **25** to σ-Cu–acetylide intermediate **26**. As described in a recent mechanistic study, the general mechanism shown in Figure 4 may involve binuclear or multinuclear copper complexes. Additional mechanistic studies to determine the nature of the copper acetylide undergoing cycloaddition and possible involvement of dicopper–dioxo complexes[20] are currently under investigation.

Table 1. Synthesis of 1,4,5-trisubstituted-1,2,3-triazoles by copper-catalyzed cycloaddition-coupling of azide (**1a–e**) and alkynes (**2a–c**)

Entry	Azide	Alkyne	Product (yield (%))[a]	
				
1	**1a**	R_2=MeOCH$_2$ **2a**	**3** (68)[b]	**4** (32)
2	**1a**	R_2=*n*Pr **2b**	**13** (57)[b]	**14** (25)
				
3	**1b**	R_2=*n*Pr **2b**	**11** (61)	**12** (23)
4	**1b**	R_2=Ph **2c**	**15** (42)[a]	**16** (46)
				
5	**1c**	R_2=MeOCH$_2$ **2a**	**17** (55)	**18** (24)
6	**1c**	R_2=*n*Pr **2b**	**19** (55)	**20** (31)
				
7	**1d**	R_2=MeOCH$_2$ **2a**	**21** (31)	**22** (45)
				
8	**1e**	R_2=MeOCH$_2$ **2a**	**23** (49)[a]	**24** (32)

[a] Reactions were conducted with 0.4 equiv of Cu(CH$_3$CN)$_4$PF$_6$ and 0.4 equiv of *N*,*N*,*N*′-trimethylethylenediamine **L12**.
[b] Reactions were conducted with 0.3 equiv of Cu(CH$_3$CN)$_4$PF$_6$ and 0.3 equiv of *N*,*N*,*N*′-trimethylethylenediamine **L12**.

3. Conclusion

In summary, we have developed a dipolar cycloaddition-coupling process, which affords 1,4,5-trisubstituted-1,2,3-triazoles in a regioselective manner. The selectivity for trisubstituted triazole synthesis was shown to be dependent on the nature of the diamine ligand. Preliminary results indicate that a broad scope of azides and terminal alkynes maybe employed to prepare highly functionalized 1,4,5-trisubstituted-1,2,3-triazoles.[21] Further applications of the cycloaddition-coupling process are currently under investigation and will be reported in due course.

4. Experimental

4.1. General

Column chromatography was performed on silica gel (60–120 mesh size). Infrared resonance spectra were recorded on a Nicolet Impact 400 FTIR. ^{1}H (400 MHz) NMR spectra were recorded in $CDCl_3$ on a Varian spectrometer. HRMS were obtained on a Finnegan MAT-90 spectrometer. Optical rotations were recorded on an AUTOPOL III digital polarimeter at 589 nm, and are recorded as $[\alpha]_D$ (concentration in grams/100 mL solvent).

4.1.1. Preparation of two-layer Hydromatrix columns. To a 1 mL Isolute HM-N cartridge (Biotage, 800-0100-CM) was added 1 mL of 1 M EDTA and the cartridge was allowed to stand for 10 min. Volume of loose Celite® (1 mL) (C566 18/60, Celite Corporation) was then conditioned with 1 mL of 1 N HCl and allowed to stand for 10 min. The acidic material was layered onto the pre-packed Isolute cartridge and used for workup as described in the general procedure for the preparation of 1,4,5-trisubstituted-1,2,3-triazoles.

4.2. Experimental procedures

4.2.1. Attempted cycloaddition using dimethoxyhexa-2,4-diyne. To a solution of azido alcohol **1a** (100 mg, 0.53 mmol) in THF was added 21 mg of CuI (0.11 mmol) followed by 14 μL (0.11 mmol) of *N*,*N*,*N*′-trimethylethylenediamine. To the stirred solution was added 109 mg (0.795 mmol) of dimethoxyhexa-2,4-diyne **5**. The reaction was stirred at rt for 6 h. No conversion of starting material was observed.

4.2.2. Attempted alkynylation of 1,4-disubstituted-1,2,3-triazole (4). To a solution of disubstituted triazole **4** (50 mg, 0.19 mmol) in THF was added CuI (7 mg, 0.03 mmol) followed by 4 μL (0.03 mmol) of *N*,*N*,*N*′-trimethylethylenediamine. To the stirred solution was added 32 μL (27 mg, 0.38 mmol) of **2a**. The reaction was stirred at rt for 9 h. No conversion of starting material was observed.

4.2.3. Synthesis of 1-phenoxy-3-(4-trimethylsilyl-[1,2,3]-triazol-1-yl)-propan-2-ol (7). To a solution of azido alcohol **1a** (100 mg, 0.53 mmol) in THF was added 21 mg of CuI (0.11 mmol) followed by 460 μL (2.65 mmol) of diisopropylethylamine. To the stirred solution was added 82 μL (0.58 mmol) of trimethylsilyl acetylene **6**. The solution became yellow and a slight precipitate formed after 30 min. The reaction was stirred at rt for 12 h, the mixture was filtered, and the solvent was removed in vacuo. Flash chromatography (silica, 60:40 hexane/EtOAc) afforded 75 mg (0.26 mmol, 48%) of **7** as an amorphous solid (mp=80–83 °C). IR ν_{max} (film) 3216.1, 2955.9, 1599.4, 1587.9, 1496.1, 1247.7 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (1H, s), 7.28 (2H, t, J=7.6 Hz), 6.98 (1H, t), 6.86 (2H, d, J=8.4 Hz), 4.65 (1H, dd, J=3.6, 10.4 Hz), 4.54 (1H, dd, J=6.8, 7.2 Hz), 4.41 (1H, m), 3.98 (2H, d, J=5.6 Hz), 0.23 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.6, 131.2, 130.0, 121.9, 114.9, 69.2, 69.1, 52.8, −0.93; HRMS (CI/NH_3) m/z calculated for $C_{14}H_{21}N_3O_2Si$, 291.1403; found, 291.1393.

4.2.4. Synthesis of 1-phenoxy-3-(4-trimethylsilanyl-5-trimethylsilanylethynyl-[1,2,3]triazol-1-yl)-propan-2-ol (8). Prepared according to the general procedure using 100 mg (0.53 mmol) of azido alcohol **1a**, 152 μL (1.5 mmol) of trimethylsilyl acetylene **6**, 41 mg (0.11 mmol) of tetrakisacetonitrile copper(I)hexafluorophosphate, 14 μL (0.11 mmol) of *N*,*N*,*N*′-trimethylethylenediamine, and 6 mg (0.053 mmol) of NMO. Flash chromatography (silica, 70:30 hexane/EtOAc) afforded 120 mg (0.3 mmol, 58%) of triazole **8** as an amorphous solid (mp=73–75 °C). IR ν_{max} (film) 3263.7, 2958.3, 2166.8, 1599.8, 1588.1, 1496.3, 1413.2, 1249.9 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (2H, t, J=8.4 Hz), 6.95 (1H, t, J=7.6 Hz), 6.87 (2H, d, J=8.4 Hz), 4.67–4.50 (3H, overlap), 4.08–3.96 (2H, m), 3.39 (1H, d, J=5.2 Hz), 0.34 (3H, s), 0.24 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.7, 130.1, 121.9, 114.9, 70.2, 69.7, 69.3, 69.0, 68.7, 53.7, 51.4, 46.3, −0.33, −1.4; HRMS (CI/NH_3) m/z calculated for $C_{19}H_{29}N_3O_2Si_2$, 387.1798; found, 388.1865 (M+H).

4.2.5. Preparation of 1-(5-ethynyl-[1,2,3]triazol-1-yl)-3-phenoxy-propan-2-ol (9). To a solution of 40 mg (0.03 mmol) of bis-TMS triazole **8** in 0.5 mL of THF was added 6 μL of AcOH followed by 300 μL (0.075 mmol) of 1.0 M tetrabutylammonium fluoride in THF. The reaction was stirred for 6 h at rt and the solvent was removed in vacuo. Flash chromatography (silica, 50:50 hexane/EtOAc) afforded 20 mg (0.082 mmol, 69%) of **9** as a colorless oil. IR ν_{max} (film) 3279.9, 2919.1, 2850.4, 1599.3, 1588.1, 1496.7, 1244.6 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (1H, s), 7.28 (2H, t, J=8.8 Hz), 6.98 (1H, t, J=7.2 Hz), 6.89 (2H, d, J=8.4 Hz), 4.73–4.62 (2H, m), 4.53 (1H, dd, J=6, 5.2 Hz), 3.65 (1H, s), 3.00 (1H, d, J=6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.6, 132.7, 125.1, 117.3, 110.0, 94.5, 71.6, 62.5, 48.5; HRMS (CI/NH_3) m/z calculated for $C_{13}H_{13}N_3O_2$, 243.1008; found, 244.1105 (M+H).

4.2.6. Preparation of 7-phenoxymethyl-4,5,7,8-tetrahydro-6-oxa-1,2,8a-triaza-azulene (10). Bis-TMS triazole **7** (60 mg, 0.18 mmol) was dissolved in 1 mL of THF. To this was added 700 μL of 1.0 M tetrabutylammonium fluoride in THF. The reaction was stirred for 12 h at rt and the solvent was removed in vacuo. Flash chromatography (silica, 70:30 hexane/EtOAc) afforded 24 mg (0.099 mmol, 53%) of bicyclic triazole **10** as a colorless oil. IR ν_{max} (film) 2928.8, 1656.1, 1650.1, 1598.8, 1587.8, 1494.6, 1304.2, 1243.5 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (1H, s), 7.30 (2H, t, J=8.0 Hz), 6.99 (1H, t, J=7.2 Hz), 6.91 (2H, d, J=8.4 Hz), 6.59 (1H, d, J=7.6 Hz), 5.56 (1H, d, J=7.6 Hz), 5.52 (1H, m), 4.53–4.45 (3H, overlap),

4.31 (1H, dd, J=3.6, 6.4 Hz), 4.17 (1H, dd, J=4.8, 8.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.4, 141.8, 127.5, 125.2, 117.3, 110.0, 84.5, 71.6, 62.5, 48.5; HRMS (CI/NH_3) m/z calculated for $C_{13}H_{13}N_3O_2$, 243.1008; found, 243.1018.

4.3. General procedure for the preparation of 1,4,5-trisubstituted-1,2,3-triazoles

Tetrakisacetonitrile copper(I)hexafluorophosphate (0.05 mmol) was placed in a 110 mL miniblock XT reaction vessel (Mettler Toledo Autochem) and dissolved in 2 mL of CH_2Cl_2 (15×45 mm) with the vessel open to the atmosphere. *N*,*N*,*N*′-Trimethylethylenediamine (6.3 μL, 0.05 mmol) was then added to afford a purple solution. A solution of azide **1a–e** (0.26 mmol) and alkyne **2a–c** (0.55 mmol) in 1 mL of CH_2Cl_2 was then added. After 5 min NMO (0.025 mmol) was added and the resulting green solution was stirred for 20 min at rt. The solution was transferred to a two-layer Hydromatrix column (see Section 4.1.1). After washing the Hydromatrix column with CH_2Cl_2 (5 mL), the solution was concentrated in vacuo to afford a yellow oil. Purification via flash chromatography (30:70 hexane/EtOAc) afforded trisubstituted triazole as a colorless oil.

4.4. Characterization data

4.4.1. 1-[4-Methoxymethyl-5-(3-methoxy-prop-1-ynyl)-[1,2,3]-triazol-1-yl]-3-phenoxy-propan-2-ol (3). Colorless oil; IR ν_{max} (film) 3367.0, 2924.3, 2819.4, 1600.0, 1491.3, 1448.5, 1359.2, 1293.2, 1246.6, 1099.1, 834.9, 761.2 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (1H, d, J=8 Hz), 7.27 (1H, d, J=7.4 Hz), 6.97 (1H, t, J=7.4 Hz), 6.87 (2H, d, J=8 Hz), 4.60–4.70 (2H, m), 4.55 (2H, s), 4.49–4.51 (1H, m), 4.39 (2H, s), 4.29 (2H, s), 3.90–4.10 (1H, m), 3.40 (3H, s), 3.38 (3H, s), 3.10–3.11 (1H, d, J=6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.6, 142.3, 125.1, 117.0, 116.6, 109.9, 94.1, 66.0, 64.2, 63.9, 60.1, 55.5, 53.8, 46.9; HRMS (CI/NH_3) m/z calculated for $C_{17}H_{21}N_3O_4$, 331.1532; found, 332.1580 (M+H).

4.4.2. 1-(4-Methoxymethyl-[1,2,3]-triazol-1-yl)-3-phenoxy-propan-2-ol (4). Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (1H, s), 7.28 (1H, d, J=8.4 Hz), 7.26 (1H, d, J=7.2 Hz), 6.98 (1H, t, J=7.2 Hz), 6.87 (2H, d, J=8.4 Hz), 4.68–4.69 (1H, dd, J=3.2, 14 Hz), 4.56 (2H, s), 4.55–4.49 (1H, dd, J=6.8, 14 Hz), 4.45–4.43 (1H, m), 4.00–3.96 (1H, dd, J=5.2, 9.6 Hz), 3.94–3.90 (1H, dd, J=6, 9.6 Hz), 3.39 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.8, 140.7, 125.1, 119.4, 117.1, 110.2, 64.4, 64.3, 61.3, 53.6, 48.3; HRMS (CI/NH_3) m/z calculated for $C_{13}H_{17}N_3O_3$, 263.1270; found, 264.1362 (M+H).

4.4.3. 1-Benzyl-4-methoxymethyl-5-(3-methoxy-prop-1-ynyl)-1*H*-[1,2,3]-triazole (11). Colorless oil; IR ν_{max} (film) 2996.9, 2926.1, 2823.3, 1731.9, 1565.4, 1501.6, 1459.1, 1363.4, 1186.2, 1097.6, 895.7 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (5H, s), 5.56 (2H, s), 4.56 (2H, s), 4.35 (2H, s), 3.40 (3H, s), 3.37 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.7, 129.9, 124.4, 124.1, 123.9, 123.5, 93.9, 66.5, 60.2, 55.6, 53.8, 53.3, 48.3; HRMS (CI/NH_3) m/z calculated for $C_{15}H_{17}N_3O_2$, 271.1321; found, 272.1428 (M+H).

4.4.4. 1-(5-Pent-1-ynyl-4-propyl-[1,2,3]-triazol-1-yl)-3-phenoxy-propan-2-ol (13). Colorless oil; IR ν_{max} (film) 3246.6, 2959.2, 2920.4, 2862.1, 1592.3, 1580.6, 1495.1, 1444.6, 1242.7, 1048.6, 831.1, 741.8, 687.4 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (1H, t, J=8.4 Hz), 7.26 (1H, d, J=7.2 Hz), 6.97 (1H, t, J=7.2 Hz), 6.88 (2H, d, J=8.4 Hz), 4.46–4.62 (3H, m), 4.02–4.05 (1H, dd, J=5.6, 9.6 Hz), 3.97–3.93 (1H, dd, J=5.6, 9.6 Hz), 3.17 (1H, d, J=5.2 Hz), 2.66 (2H, t, J=7.6 Hz), 2.40 (2H, t, J=7.2 Hz), 1.71 (2H, sextet, J=7.6 Hz), 1.61 (2H, sextet, J=7.2 Hz), 1.02 (3H, t, J=7.6 Hz), 0.93 (3H, t, J=7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.7, 145.4, 125.1, 116.9, 116.1, 109.9, 98.8, 64.3, 63.9, 61.3, 46.5, 22.8, 17.5, 17.1, 16.9, 9.1, 8.8; HRMS (CI/NH_3) m/z calculated for $C_{19}H_{25}N_3O_2$, 327.1947; found, 328.2018 (M+H).

4.4.5. 1-Benzyl-5-pent-1-ynyl-4-propyl-1*H*-[1,2,3]-triazole (15). Colorless oil; IR ν_{max} (film) 3033.2, 3962.1, 2933.4, 2872.2, 1558.4, 1496.9, 1456.2, 1429.5, 1338.7, 731.9 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.2–7.4 (5H, m), 5.48 (1H, s), 2.66 (2H, t, J=7.2 Hz,), 2.41 (2H, t, J=7 Hz), 1.71 (2H, sextet, J=7.2 Hz), 1.59 (2H, sextet, J=7 Hz), 0.98 (3H, t, J=7.2 Hz), 0.93 (3H, t, J=7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.8, 130.7, 124.2, 123.7, 123.4, 98.4, 61.8, 47.8, 22.9, 17.6, 17.1, 16.9, 9.1, 8.8; HRMS (CI/NH_3) m/z calculated for $C_{17}H_{21}N_3$, 267.1735; found, 267.1719.

4.4.6. 2-[4-Methoxymethyl-5-(3-methoxy-prop-1-ynyl)-[1,2,3]-triazol-1-yl]-3-phenyl-propionic acid methyl ester (17). Colorless oil; $[\alpha]_D^{22}$ −36.0 (c 0.1, $CHCl_3$); IR ν_{max} (film) 2954.4, 2926.1, 2844.5, 1746.1, 1629.1, 1498.1, 1448.4, 1264.2, 1264.2, 1214.6, 1101.2 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17–7.18 (3H, m), 7.02–7.04 (2H, m), 5.46–5.42 (1H, q, J=5.3 Hz), 4.51 (2H, s), 4.30 (2H, s), 3.75 (3H, s), 3.62–3.64 (2H, m), 3.38 (3H, s), 3.30 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.5, 130.9, 124.4, 124.2, 123.5, 122.8, 65.9, 60.0, 58.6, 55.5, 53.5, 53.3, 48.6, 32.4; HRMS (CI/NH_3) m/z calculated for $C_{18}H_{21}N_3O_4$, 343.1532; found, 344.1606 (M+H).

4.4.7. 2-(5-Pent-1-ynyl-4-propyl-[1,2,3]-triazol-1-yl)-3-phenyl-propionic acid methyl ester (19). Colorless oil; $[\alpha]_D^{22}$ −53.5 (c 0.5, $CHCl_3$); IR ν_{max} (film) 3060.2, 3025.2, 2951.5, 2928.1, 2846.6, 2217.5, 1452.4, 1429.1, 1335.9, 1277.7, 1262.1, 1219.5, 986.4, 772.8, 749.5, 687.4 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16–7.27 (3H, dd, J=1.6, 7.6 Hz), 7.00–7.02 (2H, dd, J=1.6, 7.6 Hz), 5.36–5.40 (1H, dd, J=6, 9.2 Hz), 3.73 (3H, s), 3.62–3.66 (2H, m), 2.61 (2H, t, J=7.4 Hz), 2.38 (2H, t, J=7.6 Hz), 1.66 (2H, sextet, J=7.4 Hz), 1.58 (2H, sextet, J=7.6 Hz), 0.99 (3H, t, J=7.4 Hz), 0.86 (3H, t, J=7.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.9, 145.2, 131.3, 124.5, 124.3, 124.1, 122.6, 98.9, 61.3, 58.2, 48.4, 32.4, 22.6, 17.5, 17.1, 16.9, 8.9, 8.8; HRMS (CI/NH_3) m/z calculated for $C_{20}H_{25}N_3O_2$, 339.1947; found, 340.1997 (M+H).

4.4.8. 1-[2-(*tert*-Butyl-dimethyl-silanyloxy)-3-phenoxy-propyl]-4-methoxymethyl-5-(3-methoxy-prop-1-ynyl)-1*H*-[1,2,3]-triazole (21). Colorless oil; IR ν_{max} (film) 2928.2, 2850.5, 1592.2, 1487.4, 1452.4, 1246.6, 1095.1, 834.9, 772.8 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (1H, d, J=8.4 Hz), 7.26 (1H, d, J=7.2 Hz), 6.96 (1H, t, J=7.2 Hz), 6.87

(2H, d, J=8.4 Hz), 4.66–4.62 (1H, dd, J=4.4, 13.2 Hz), 4.56 (2H, s), 4.50–4.45 (1H, dd, J=7.6, 13.2 Hz), 4.32 (2H, s), 3.99–3.92 (3H, m), 3.42 (3H, s), 3.38 (3H, s), 0 (3H, s), −0.2 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.8, 142.3, 125.1, 116.7, 109.9, 93.7, 72.8, 65.4, 65.0, 60.1, 55.6, 53.6, 53.3, 47.9, 21.0, 20.9, 132, −9.6, −10.1; HRMS (CI/NH_3) m/z calculated for $C_{23}H_{35}N_3O_4Si$, 445.2397; found, 446.2462 (M+H).

4.4.9. 4-Methoxymethyl-5-(3-methoxy-prop-1-ynyl)-1-(4-methoxy-phenyl)-1*H*-[1,2,3]-triazole (23). Colorless oil; IR ν_{max} (film) 2932.1, 1751.5, 1592.2, 1518.3, 1258.3, 1099.1, 834.9, 737.9, 675.7 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (2H, d, J=9.2 Hz), 7.00 (2H, d, J=9.2 Hz), 4.63 (2H, s), 4.31 (2H, s), 3.86 (3H, s), 3.45 (3H, s), 3.36 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.9, 143.1, 120.6, 120.5, 109.9, 93.4, 88.4, 67.1, 60.2, 55.6, 53.9, 53.3, 51.0; HRMS (CI/NH_3) m/z calculated for $C_{15}H_{17}N_3O_3$, 287.1270; found, 288.1319 (M+H).

Acknowledgements

We thank Professors Sean Elliott, Scott Schaus (Boston University), and Valery Fokin (Scripps Research Institute) for helpful discussions. Financial support for this project from Boston University and Boehringer Ingelheim Pharmaceuticals, Inc. (Ridgefield, CT) is gratefully acknowledged. We thank Bristol-Myers Squibb for an unrestricted Grant in Synthetic Organic Chemistry (J.A.P.), Novartis Pharma AG for research support, and Mettler Toledo Autochem for assistance with reaction blocks.

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Tetrahedron

Operationally convenient, efficient asymmetric synthesis of enantiomerically pure 4-aminoglutamic acids via methylene dimerization of chiral glycine equivalents with dichloromethane

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Received 7 February 2006; revised 31 March 2006; accepted 6 April 2006
Available online 11 May 2006

Abstract—This paper presents a practical and efficient asymmetric synthesis of enantiomerically pure 4-aminoglutamic acids using a quite unusual methylene dimerization of chiral nucleophilic glycine equivalents with dichloromethane under phase-transfer catalysis (PTC) conditions. From a synthetic standpoint, the reported procedure is highly operationally convenient and scalable as it does not require any chromatographic purification of the intermediate products.

1. Introduction

Bis-α-amino acids (α,α′-diamino-dicarboxylic acids) (bis-AA) are naturally occurring compounds found in various microorganisms and higher plants.[1] For instance, 2,6-diaminopimelic acid (DAP) (**1b**) (Fig. 1) is a metabolic precursor of (*S*)-lysine in Gram-positive bacteria,[2] and serves as the key cross-linking unit in the peptidoglycan layers of the cell wall in most pathogenic bacteria.[3] In general the cross-linking property of **1b** and bis-AA was shown to be of enormous potential for an application in the design and synthesis of conformationally constrained cyclic peptides[4] and peptidomimetics, as aliphatic linkage providers between two C^{α} sites.[5] Thus, the ability of natural and tailor-made[6,7] bis-AA to support peptide secondary structures (turns, sheets, and helices) and to serve as dicarba cystine isosteres [2,7-diaminosuberic acid (**1c**)] has generated a great deal of interest in the development of stereoselective methods for preparing these amino acids.[8,9]

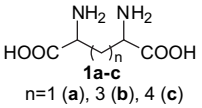

Figure 1. (a) Bis-AA: 4-aminoglutamic acid, (b) 2,6-diaminopimelic acid, (c) 2,7-diaminosuberic acid.

The interest of our group in bis-AA is concerned with syntheses of conformationally constrained amino acids and peptides.[10] In particular, for a systematic analysis of a series of cyclic bis-AA-based peptides, currently under study in our laboratories, we needed a reliable and operationally convenient and a simple access to (2*S*,4*S*)- and (2*R*,4*R*)-2,4-diaminoglutaric acid (4-aminoglutamic acid) (**1a**). Our interest in 4-aminoglutamic acid **1a**, as an example of substituted glutamic acid family, was also stimulated by our recent success in the design[11] and development[12] of the first truly practical methodology for stereocontrolled synthesis of sterically/configurationally constrained glutamic/pyroglutamic acids.[10–12] Moreover, an additional bonus for the development of an optimized synthetic approach to enantiomerically pure 4-aminoglutamic acid **1a** might be extended further and assist in a systematic study of its intriguing multifold biological activity.[13]

According to the relevant literature,[14] most of the methods for the asymmetric synthesis of bis-AA **1a** are based on an elaborate multistep (seven steps or more) transformation of enantiomerically pure naturally occurring amino acids, such as γ-hydroxyproline[14a] or serine,[14b,c] and all of these methods are unappealing from a preparative standpoint. More straightforward approaches include Michael addition reactions between chiral nucleophilic glycine equivalents

Keywords: Asymmetric synthesis; Bis-amino acid; Chiral glycine equivalents; Methylene dimerization.
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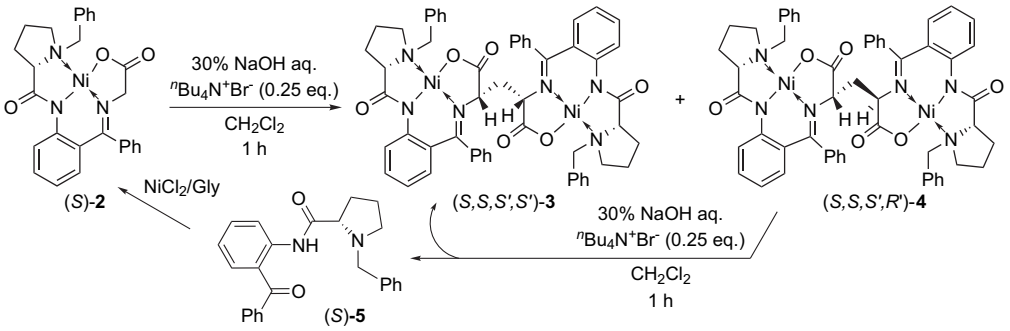
doi:10.1016/j.tet.2006.04.023



Scheme 1.

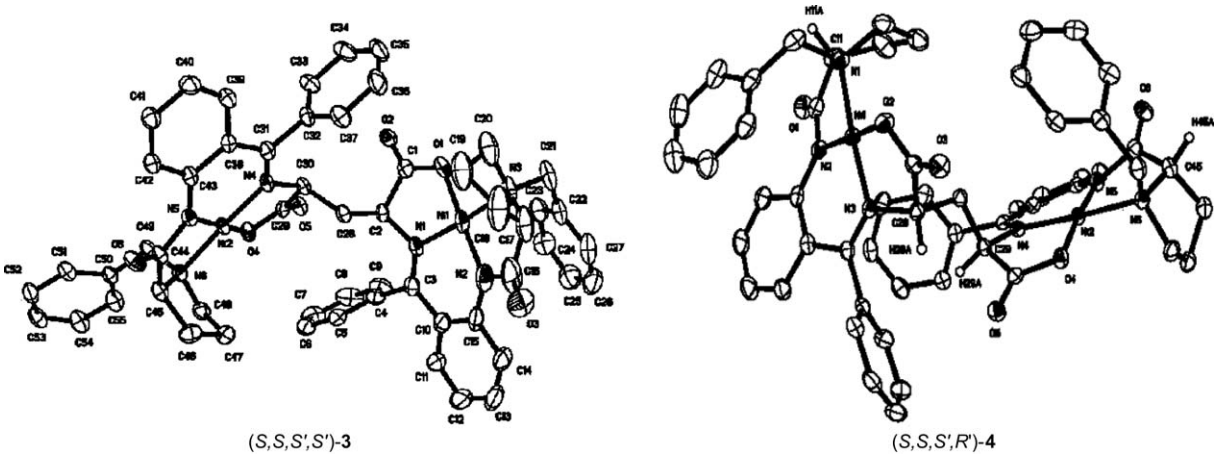


Figure 2. X-ray structures of both Ni(II) Complexes (*S*,*S*,*S*′,*S*′)-**3** and (*S*,*S*,*S*′,*R*′)-**4**.

and corresponding derivatives of α-aminoacrylic acids.[15] However, the major drawback of these Michael additions is a low stereoselectivity, close to a 1:1 mixture of diastereomers, requiring tedious chromatographic separations.

The most concise and direct method for preparation of enantiomerically pure bis-AA **1a** is the methylene dimerization[16] of glycine equivalents with CH_2Br_2. The asymmetric version of this reaction was reported by Belokon et al.[17] According to the corresponding protocol, the treatment of a chiral glycine equivalent, Ni(II)-complex (*S*)-**2** (Scheme 1) in dry acetonitrile with 0.5 equiv of CH_2Br_2 and powdered NaOH resulted in the formation of Ni-complex **3** as a major product, which was easily converted into (2*S*,4*S*)-**1a**. Regardless of the need for a dry solvent and inert atmosphere, as well as the necessity for purification of complex **3** by column chromatography, the simplicity of this method seemed very attractive. Unfortunately, our attempts to reproduce this protocol failed. First, we found that 0.5 equiv of CH_2Br_2 is not quite enough for complete consumption of the starting complex (*S*)-**2**. Second, we also observed the formation of the diastereomeric product (*S*,*S*,*S*′,*R*′)-**4**[18] in a variable ratio to (*S*,*S*,*S*′,*S*′)-**3**, depending on the reaction time. Furthermore, we found that the major problem of these reaction conditions is the formation of a substantial amount of decomposition products. Our numerous attempts to improve these reaction conditions by varying the ratios of the starting compounds, reaction temperature, solvent, and nature of a base gave us little improvement; the target product (*S*,*S*,*S*′,*S*′)-**3** was obtained in about 50% yield after painstaking column purification.

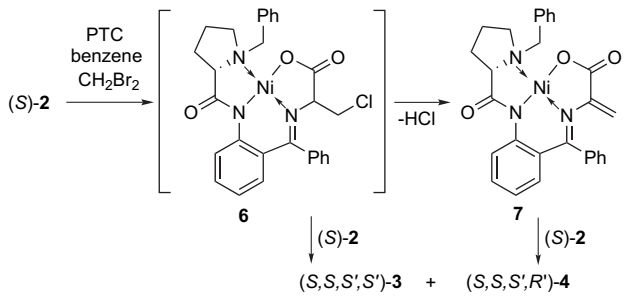
Fortunately, an unrelated research project in our laboratory gave us an unexpected solution to the synthesis of the product **3** and target amino acid **1a**. The alkylation of complex (*S*)-**2** under PTC in CH_2Cl_2 with sterically constrained alkyl halides usually requires prolonged reaction times. By checking the content of the reaction mixtures through TLC we noticed the formation of some byproducts, which were concluded to be (*S*,*S*,*S*′,*S*′)-**3** and (*S*,*S*,*S*′,*R*′)-**4** after isolation and characterization. The absolute configuration of each complex was determined by X-ray analysis (Fig. 2).

This unexpected finding showed us the possibility of preparing compounds (*S*,*S*,*S*′,*S*′)-**3** and (*S*,*S*,*S*′,*R*′)-**4** under PTC using dichloromethane as a solvent and a reagent at the same time. Our next goal was to optimize the reaction conditions of the methylene dimerization of complex (*S*)-**2** by screening various bases, solvents, phase-transfer catalysts, and concentration of the reagents.[19]

2. Results and discussions

After an extensive series of experiments we finally found the following most efficient reaction conditions: 25 mol % of tetrabutylammonium bromide (TBAB), 30% aqueous NaOH, and 0.2 M solution of complex (*S*)-**2** in CH_2Cl_2. Under these conditions the reaction was completed within 1 h and resulted in a mixture of products (*S*,*S*,*S*′,*S*′)-**3** and (*S*,*S*,*S*′,*R*′)-**4** (Scheme 1) in a ratio of 1:1 (95% in combined yield).

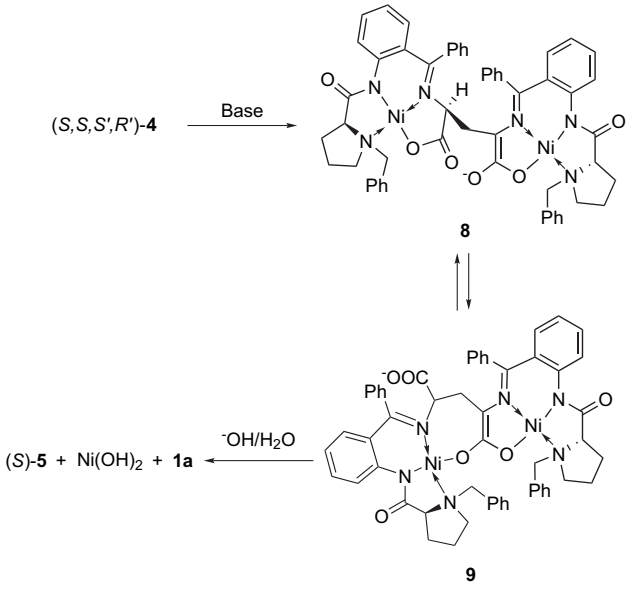
From a mechanistic standpoint, the reaction obviously proceeds through the formation of an intermediate mono-alkylated complex **6** (Scheme 2), which transformed into the products of dimerization via two possible pathways. In the first scenario, the intermediate **6** can be engaged in the direct alkylation of the starting complex **2**, resulting in the products **3** and **4**. Alternatively, the complex **6** can undergo dehydrochlorination, leading to the formation of an unsaturated derivative **7**. The complex **7**, which can act as a Michael acceptor, might react with the starting glycine complex (*S*)-**2** furnishing the reaction products **3** and **4**. Although, neither the mono-alkylated complex **6** nor the Michael acceptor **7** were observed at any point of the reactions that were conducted under our standard phase-transfer conditions, we were able to detect and isolate the intermediate Michael acceptor **7** (13% yield) in a reaction conducted in benzene with the application of CH_2Br_2 as an alkylating reagent. With the intermediate **7** in hand, we studied the reaction with (*S*)-**2** under the standard PTC conditions. The Michael addition between complexes **7** and (*S*)-**2** successfully gave rise to a mixture of products (*S*,*S*,*S*′,*S*′)-**3** and (*S*,*S*,*S*′,*R*′)-**4** in a ratio similar to that observed in the direct methylene dimerization of (*S*)-**2** in CH_2Cl_2.



Scheme 2.

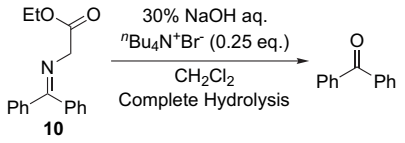
A very important and unexpected observation was made when the methylene dimerization under PTC condition was allowed to continue further. After complete consumption of the starting complex (*S*)-**2**, the initial 1:1 ratio of the two products gradually changed; a preference for the formation of (*S*,*S*,*S*′,*S*′)-**3** was observed while the amount of (*S*,*S*,*S*′,*R*′)-**4** was gradually decreasing (Scheme 1), besides a gradually increasing formation of ligand **5**. Complete disappearance of the complex (*S*,*S*,*S*′,*R*′)-**4** was observed after 24 h leaving complex (*S*,*S*,*S*′,*S*′)-**3** as a sole diastereomer in a mixture with the corresponding ligand (*S*)-**5**.

With isolated and diastereomerically pure (*S*,*S*,*S*′,*S*′)-**3** and (*S*,*S*,*S*′,*R*′)-**4** in hand, we subjected each complex to the original reaction conditions of the methylene dimerization separately. Surprisingly, the diastereomers (*S*,*S*,*S*′,*S*′)-**3** and (*S*,*S*,*S*′,*R*′)-**4** showed completely different reactivity. While the product (*S*,*S*,*S*′,*S*′)-**3** was found to be chemically and stereochemically intact, the diastereomer (*S*,*S*,*S*′,*R*′)-**4** underwent partial epimerization giving rise to the complex (*S*,*S*,*S*′,*S*′)-**3** and decomposition furnishing the ligand (*S*)-**5**. Complete consumption of the complex (*S*,*S*,*S*′,*R*′)-**4** was observed after about 24 h. Further experiments demonstrated that the diastereomer (*S*,*S*,*S*′,*R*′)-**4** can be obtained as the major product when the methylene dimerization of (*S*)-**2** was conducted at 0 °C. These results suggested that (*S*,*S*,*S*′,*S*′)-**3** is the thermodynamically controlled product and (*S*,*S*,*S*′,*R*′)-**4** is the kinetically controlled product. While the epimerization of (*S*,*S*,*S*′,*R*′)-**4** to the thermodynamic product (*S*,*S*,*S*′,*S*′)-**3** under basic conditions can be easily explained, the total difference in chemical reactivity between the diastereomers (*S*,*S*,*S*′,*S*′)-**3** and (*S*,*S*,*S*′,*R*′)-**4** was quite puzzling. Since the only difference between (*S*,*S*,*S*′,*S*′)-**3** and (*S*,*S*,*S*′,*R*′)-**4** is their absolute configuration, we based our rational on the different arrangement of the Ni(II)-coordinated planes in space. According to the crystallographic data, the carboxylic acid moieties in (*S*,*S*,*S*′,*R*′)-**4** are in close proximity to each other. Therefore, one may suggest that the mono-enolate **8**, generated under basic conditions, can substitute the carboxylic acid moiety in the second Ni(II)-coordination plane giving rise to complex **9** (Scheme 3). The uncoordinated carboxylic group in the complex **9** might render the complex **9** as polar and relatively soluble in the aqueous phase were it can undergo complete hydrolysis producing $Ni(OH)_2$, amino acid **1a**, and chiral ligand (*S*)-**5**.



Scheme 3.

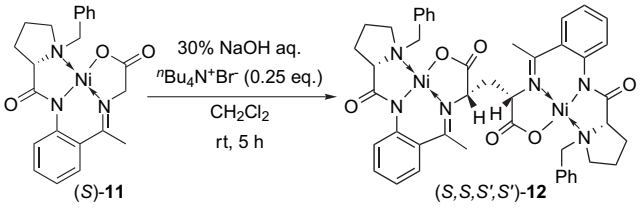
With these results in hand, we thought it would be interesting to explore the generality of this methylene dimerization using other nucleophilic glycine equivalents. First, we tried the commercially available O'Donnell's-type[20] nucleophilic glycine equivalent **10** under various reaction conditions, which include our standard PTC conditions. Under any conditions, we observed only complete hydrolysis of the compound **10** resulting in the formation of benzophenone (Scheme 4) and the corresponding methylene dimerization product was never detected in the reaction mixtures.



Scheme 4.

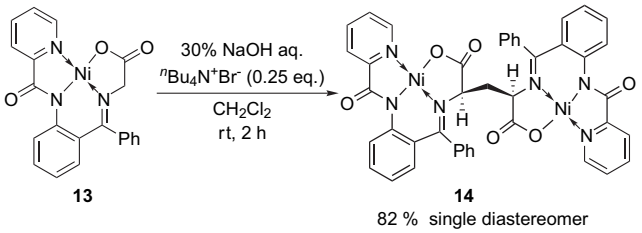
Next, with a goal to improve the stereochemical outcome of the reactions, we studied the methylene dimerization of

the acetophenone-derived chiral Ni(II)-complex (*S*)-**11** (Scheme 5).[21] Surprisingly, (*S*)-**11** showed very different reactivity compared with that of (*S*)-**2**. Thus, the application of our standard reaction conditions using 25 mol % of TBAB resulted in very fast consumption of the starting complex (*S*)-**11** and formation of a complex mixture of various unidentified byproducts. We found that an application of 5 mol % of the catalyst TBAB allowed to slow down the reaction rate and isolate a single reaction product (*S*,*S*,*S*′,*S*′)-**12** in 37% yield. The ^{1}H NMR spectrum of the product (*S*,*S*,*S*′,*S*′)-**12** showed a simple pattern of peaks, similar to those of (*S*,*S*,*S*′,*S*′)-**3**; in particular, only one singlet (at 2.46 ppm) of the acetophenone moiety was observed in the ^{1}H NMR spectrum, suggesting that the compound possesses a C_2-symmetry and therefore (*S*,*S*,*S*′,*S*′) absolute configuration. The observed remarkable difference in the reactivity between glycine equivalents (*S*)-**2** and (*S*)-**11** can be explained by considerably high C–H acidity of the acetophenone methyl group in the starting complex (*S*)-**11** as well as in the product of its methylene dimerization (*S*,*S*,*S*′,*S*′)-**12**.[22] Despite the formation of a single diastereomer in the methylene dimerization of (*S*)-**11**, the low chemical yield (37%) rendered this reaction unpractical for preparation of the target amino acid **1a**.



Scheme 5.

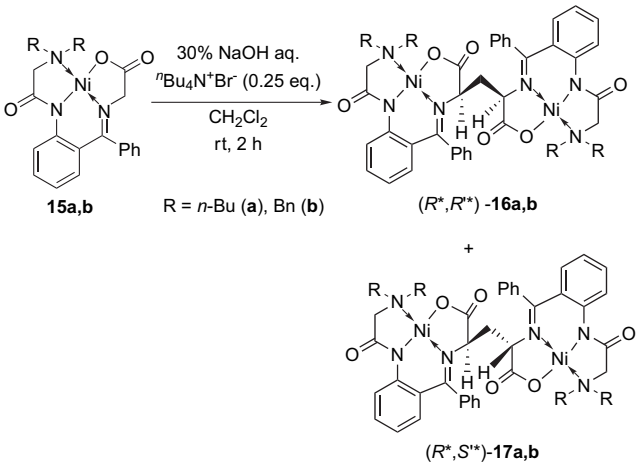
To further investigate the generality of this unusual methylene dimerization of glycine derivatives with dichloromethane under PTC conditions, we studied the reactions using a series of achiral Ni(II) complexes. First, we investigated the methylene dimerization of the picolinic acid derived Ni(II)-complex of glycine **13**[23] (Scheme 6). Surprisingly, the stereochemical outcome of this reaction was different from that of both chiral complexes (*S*)-**2** and (*S*)-**11**. This reaction was completed within 2 h giving rise to a single diastereomeric product **14**, isolated in 82% yield. As discussed above, the relative configuration of the product **14** was assigned (*R**,*R*′*) based on the symmetrical pattern of its ^{1}H NMR spectrum. The product (*R**,*R*′*)-**14** was found to be stable under the PTC conditions, as no decompositions of **14** were observed in the reaction even after 24 h. To rationalize the obtained high diastereoselectivity[24] in this reaction, we can suggest that the difference in geometry between



Scheme 6.

the flat picolinic acid moiety in **13** and the nonflat chiral (*N*-benzyl)prolyl moieties in (*S*)-**2** and (*S*)-**11**, may play an important role in determining the stereochemical outcome in these reactions.

Finally, we investigated the methylene dimerization of a new generation of modular nucleophilic glycine equivalents **15a**,**b** (Scheme 7), recently introduced by our group.[25]



Scheme 7.

Under the standard conditions, the starting complexes **15a**,**b** were completely consumed after 5 h of the reaction giving rise to a mixture of two diastereomers **16a**,**b** and **17a**,**b** in a ratio close to 1:1. The relative configuration of the symmetric complex **16b** was determined by X-ray analysis (Fig. 3). Considering the stereochemical outcome in these reactions, one may assume that it is similar to that observed in the reactions of complexes (*S*)-**2** and (*S*)-**11**. However, in sharp contrast to the diastereomers **3**, **4**, **12**, and **14**, both products **16a**,**b** and **17a**,**b** were found to be highly unstable under the

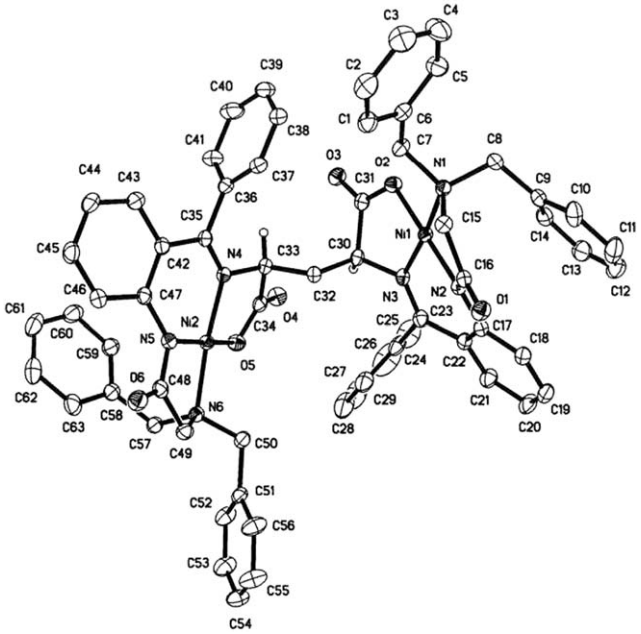
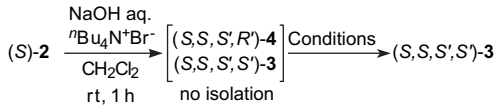


Figure 3. X-ray structure of Ni(II)-complex **16b**.

PTC conditions, undergoing complete decomposition within 24 h. Again, structural features of complexes **16a**,**b** and **17a**,**b**, such as nonflat and a more flexible arrangement of the chelating rings, maybe a reason for their instability.

With these results in hand, we decided to focus next on the development of an optimized and synthetically useful procedure for the preparation of enantiomerically pure 4-aminoglutamic acids. Considering the data obtained, it is clear that the methylene dimerization of complex (*S*)-**2** gave the most promising results furnishing a mixture of diastereomeric products **3** and **4** (Scheme 1) in high chemical yield. To make this reaction synthetically useful, we needed to find a way for selective epimerization of the unwanted isomer **4** to the symmetrical dimer **3**. To this end we conducted a series of experiments investigating the effect of various bases and solvents on the epimerization of diastereomer **4** to the target product **3** (Table 1).

Table 1. Synthesis of (*S*,*S*,*S*′,*S*′)-**3** from chiral Ni(II)-complex (*S*)-**2**[a]

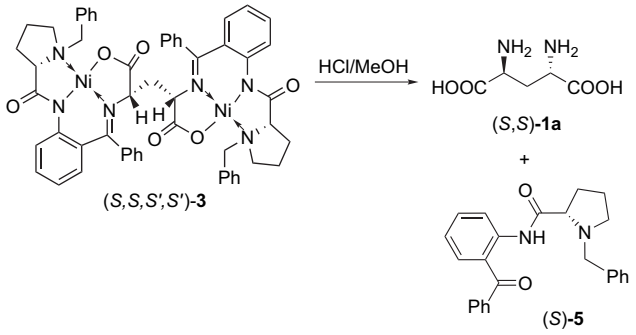


Entry	Base	Solvent	Time (h)	Yield[b] (%)
1	NaOMe	MeOH	0.5	57
2	NaOH	DMF	0.33	[c]
3	Cs_2CO_3, TBTA	CH_2Cl_2	24	0
4	TEA	CH_2Cl_2	24	0
5	DBU	CH_2Cl_2	6	80
6	Guanidine[d]	CH_2Cl_2	1	80

[a] All reactions were conducted at rt in the indicated solvent.
[b] Isolated yield of the pure product **3**.
[c] No products were isolated.
[d] 2,3,4,6,7,8-Hexahydro-1*H*-pyrimido[1,2-*a*]pyrimidine.

The treatment of a 1:1 mixture of **3** and **4** with NaOMe in MeOH solution resulted in a relatively fast epimerization of diastereomer **4** to target product **3** (entry 1). However, substantial decomposition of both diastereomers allowed isolation of **3** only in 57% yield. Under more basic conditions, NaOH in DMF, resulted in almost complete decomposition of both **3** and **4** (entry 2). Cesium carbonate, under PTC conditions (entry 3), and NEt_3 in dichloromethane (entry 4), were found to be inefficient for the target transformation. However, we found that treatment of a 1:1 mixture of **3** and **4** with DBU in dichloromethane resulted in a clean and relatively fast epimerization of **4** to product **3**, which was isolated in 80% yield (entry 5). This conditions were applied to the isolated diastereomerically pure (*S*,*S*,*S*′,*R*′)-**4**, and the desired quantitative epimerization to product **3** was found without decomposition of **4** to ligand **5**. Encouraged by these results, we conducted reaction using guanidine (entry 6), which is a stronger base than DBU. Guanidine-catalyzed reaction occurred at a higher reaction rate (1 h) producing compound **3** as a sole product in 80% isolated yield. These reaction conditions were found to be scalable to 10 g scale.

Thus, prepared product **3** without any chromatographic purification was disassembled under the standard conditions (MeOH/3 N HCl, 30 min, and 60 °C) to give bis-AA **1a** and ligand (*S*)-**5**. The recovered ligand (*S*)-**5** was used for the preparation of starting complex (*S*)-**2** (Scheme 8).



Scheme 8.

In summary, we have developed a practical and efficient asymmetric synthesis of enantiomerically pure 4-aminoglutamic acid **1a** using quite unusual methylene dimerization reaction under PTC conditions. From a synthetic standpoint the reported procedure is highly operationally convenient and scalable as it does not require any chromatographic purification of the intermediate products.

3. Experimental

3.1. General

Unless otherwise noted, all reagents and solvents were obtained from commercial suppliers and used without further purification. Unless indicated, ^1H and ^{13}C NMR spectra were taken in $CDCl_3$ solutions at 300 and 75 MHz, respectively, on an instrument in the University of Oklahoma NMR Spectroscopy Laboratory. Chemical shifts refer to TMS and $CDCl_3$ as the internal standards.

Yields refer to isolated yields of products of greater than 95% purity as estimated by ^1H NMR spectrometry. All new compounds were characterized by ^1H and ^{13}C NMR.

3.2. General procedure for methylene dimerization of Ni-complex

NaOH (1 mL, 30%) and $^nBu_4N^+Br^-$ (16.2 mg, 0.05 mmol) were added to a solution of Ni-complex (0.20 mmol) in CH_2Cl_2 (1 mL) under N_2 atmosphere. The reaction mixture was stirred at room temperature, quenched with H_2O, and then extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated to get a red solid. Silica gel chromatography ($CHCl_3$/acetone=10:1 then 5:1) of the crude product gave the corresponding Ni-complex dimer.

3.2.1. (*S*,*S*,*S*′,*S*′)-3. Mp 225.3–226.9 °C; R_f 0.41 ($CHCl_3$/acetone=4:1); $[\alpha]_D^{23}$ +991.4 (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 8.07–8.02 (m, 6H), 7.59–7.26 (m, 4H), 7.39–7.32 (m, 2H), 7.32–7.22 (m, 6H), 7.14–7.00 (m, 6H), 6.65–6.59 (m, 2H), 6.53–6.50 (m, 2H), 4.40 (d, *J*=12.6 Hz, 2H), 4.26 (dd, *J*=8.4, 5.4 Hz, 2H), 3.90–3.68 (m, 2H), 3.56–3.40 (m, 4H), 3.39 (d, *J*=12.6 Hz, 2H), 2.73–2.42 (m, 6H), 2.39–2.23 (m, 2H), 2.19–2.02 (m,

2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.5, 177.0, 171.3, 142.2, 133.7, 133.5, 133.2, 132.2, 131.4, 131.2, 130.1, 129.9, 128.9, 128.6, 128.1, 126.4, 126.1, 123.5, 120.7, 70.0, 66.1, 63.2, 57.4, 42.7, 30.7, 25.0 ppm; HRMS (TOF) $[M+Na]^+$, calcd for $[C_{55}H_{50}N_6Ni_2O_6+Na]^+$: 1029.2397, found: 1029.2396.

3.2.2. (*S*,*S*,*S*′,*R*′)-4. Mp 132.9–134.1 °C; R_f 0.17 ($CHCl_3$/acetone=4:1); $[\alpha]_D^{23}$ +4.9 (*c* 3.3, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.61–8.57 (m, 1H), 8.32–8.29 (m, 1H), 8.03 (d, *J*=7.2 Hz, 2H), 7.82 (d, *J*=7.5 Hz, 2H), 7.57–7.02 (m, 18H), 6.72–6.56 (m, 3H), 6.47 (dd, *J*=8.1, 1.2 Hz, 1H), 4.37 (d, *J*=12.6 Hz, 1H), 4.35 (d, *J*=12.9 Hz, 1H), 4.22–4.11 (m, 1H), 3.92 (br s, 1H), 3.83–3.76 (m, 1H), 3.75–3.59 (m, 1H), 3.53–3.33 (m, 5H), 3.22 (m, 1H), 2.84–1.98 (m, 10H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 181.8, 180.3, 177.6, 177.3, 171.4, 170.8, 143.2, 142.6, 134.1, 133.6, 133.5, 133.4, 133.2, 132.7, 132.4, 132.0, 131.4, 130.9, 129.8, 129.4, 129.2, 128.9, 128.8, 128.8, 127.6, 127.3, 127.1, 126.0, 125.6, 123.3, 123.2, 120.5, 70.3, 68.9, 66.6, 66.5, 62.9, 60.9, 58.9, 57.3, 44.5, 31.0, 30.4, 24.3, 23.0 ppm; HRMS (TOF) $[M+Na]^+$, calcd for $[C_{55}H_{50}N_6Ni_2O_6+Na]^+$: 1029.2397, found: 1029.2396.

3.2.3. Synthesis of key intermediate 7.[17] To a solution of Ni-complex (*S*)-**2** (500 mg, 1.0 mmol) in benzene (50 mL), KOH (489 mg, 8.7 mmol), ^{n}Bu$_4$N$^+$Br$^-$ (81 mg, 0.25 mmol), and CH_2Br_2 (70 mg, 0.4 mmol) were added in N_2 atmosphere. The reaction mixture was stirred at room temperature for 2 h, quenched with H_2O, and then extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried with $MgSO_4$, and concentrated to get a red solid. Silica gel chromatography ($CHCl_3$/acetone=10:1 then 5:1) of the crude product gave pure product **7** (71 mg, 13% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.11 (m, 3H), 7.52–7.13 (m, 9H), 6.86–6.83 (m, 1H), 6.72–6.67 (m, 1H), 5.63 (s, 1H), 4.36 (d, *J*=12.3 Hz, 1H), 4.15 (s, 1H), 3.70–3.45 (m, 3H), 3.45 (d, *J*=12.6 Hz, 1H), 2.77–2.62 (m, 1H), 2.61–2.41 (m, 1H), 2.26–1.90 (m, 1H) ppm.

3.2.4. (*S*,*S*,*S*′,*S*′)-12. Yield 37%; mp 342.7 °C (decomp.); $[\alpha]_D^{23}$ +1872 (*c* 0.29, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.09 (m, 4H), 7.91 (dd, *J*=8.6, 1.2 Hz, 2H), 7.56 (dd, *J*=8.3, 1.6 Hz, 2H), 7.30–7.35 (m, 4H), 7.14–7.21 (m, 4H), 6.93 (ddd, *J*=8.3, 7.0, 1.2 Hz, 2H), 4.75 (dd, *J*=8.7, 5.8 Hz, 2H), 4.28 (d, *J*=12.5 Hz, 2H), 3.58–3.30 (m, 6H), 3.48 (d, *J*=12.6 Hz, 2H), 2.96 (dd, *J*=8.7, 5.8 Hz, 2H), 2.69 (m, 2H), 2.59–2.40 (m, 2H), 2.46 (s, 6H), 2.00–2.09 (m, 2H), 2.08–1.97 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 179.8, 178.7, 168.7, 141.1, 133.4, 131.7, 131.3, 129.0, 128.9, 126.5, 124.3, 121.3, 70.5, 65.9, 63.5, 57.3, 42.6, 30.5, 24.2, 17.9 ppm; HRMS (TOF) $[M+Na]^+$, calcd for $[C_{45}H_{46}N_6Ni_2O_6+Na]^+$: 905.2083, found: 905.2145.

3.2.5. (*R,*R*′*)-14.** Yield 82%; mp 301.5 °C (decomp.); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.89 (d, *J*=8.6 Hz, 2H), 8.20 (d, *J*=5.2 Hz, 2H), 7.80 (m, 4H), 7.47–7.22 (m, 14H), 7.05 (d, *J*=8.1 Hz, 2H), 6.86 (m, 2H), 4.15 (m, 2H), 2.02 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 179.6, 177.4, 170.0, 152.4, 147.4, 142.6, 139.9, 135.4, 134.4, 133.7, 130.1, 129.6, 128.8, 128.3, 126.9, 126.8, 125.9, 123.7, 123.2, 122.0, 68.2, 35.1 ppm; HRMS (TOF) $[M+H]^+$, calcd for $[C_{43}H_{30}N_6Ni_2O_6+H]^+$: 843.1, found: 843.2.

3.2.6. 16a and 17a. Yield 72%; **16a/17a**=35:65. *Compound* **17a**: mp 301.3 °C (decomp.); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.63 (d, *J*=8.6 Hz, 2H), 7.47–7.22 (m, 10H), 7.17 (d, *J*=7.4 Hz, 2H), 6.73 (m, 2H), 6.64 (dd, *J*=8.0, 1.3 Hz, 2H), 4.08–3.91 (m, 2H), 4.02 (d, *J*=16.2 Hz, 2H), 3.28 (quint, *J*=7.0 Hz, 1H), 3.02 (d, *J*=16.2 Hz, 2H), 2.96–2.83 (m, 3H), 2.60–2.32 (m, 10H), 2.22 (m, 2H), 1.98–1.82 (m, 2H), 1.54–1.30 (m, 8H), 1.00 (t, *J*=7.7 Hz, 6H), 0.98 (t, *J*=7.7 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.9, 170.8, 142.7, 133.9, 133.2, 132.7, 130.0, 129.1, 129.0, 127.5, 127.2, 126.4, 123.3, 120.8, 66.6, 62.5, 60.0, 56.7, 53.7, 43.3, 29.0, 26.8, 20.7, 20.6, 13.8 ppm. *Compound* **16a**: mp 275.6 °C (decomp.); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.56 (dd, *J*=8.7, 1.2 Hz, 2H), 7.52–7.18 (m, 10H), 7.02 (d, *J*=7.2 Hz, 2H), 6.76 (m, 2H), 6.69 (dd, *J*=8.0, 1.7 Hz, 2H), 4.07 (dd, *J*=8.3, 6.1 Hz, 2H), 3.96 (d, *J*=16.3 Hz, 2H), 3.14 (d, *J*=16.3 Hz, 2H), 3.03 (m, 2H), 2.72 (m, 2H), 2.68–2.20 (m, 10H), 2.02–1.90 (m, 2H), 1.74–1.60 (m, 2H), 1.58–1.36 (m, 8H), 1.03 (t, *J*=7.3 Hz, 6H), 0.99 (t, *J*=7.5 Hz, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.8, 176.7, 171.4, 142.5, 133.9, 133.2, 132.8, 130.1, 129.7, 128.7, 127.5, 126.6, 126.3, 123.4, 121.0, 66.4, 63.8, 58.5, 56.9, 53.7, 43.2, 29.4, 24.7, 20.9, 20.8, 13.9 ppm. HRMS (TOF) $[M+Na]^+$, calcd for $[C_{51}H_{62}N_6Ni_2O_6+Na]^+$: 993.3335, found: 993.3293.

3.2.7. 16b and 17b. Yield 72%; **16b/17b**=44:56. *Compound* **17b**: mp 313.5 °C (decomp.); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.63 (d, *J*=8.6 Hz, 2H), 8.13 (d, *J*=7.7 Hz, 4H), 7.66 (d, *J*=7.7 Hz, 2H), 7.52–7.02 (m, 26H), 6.60 (m, 2H), 6.43 (d, *J*=8.2 Hz, 2H), 4.56 (d, *J*=12.3 Hz, 2H), 4.30 (d, *J*=12.7 Hz, 2H), 4.14 (d, *J*=16.4 Hz, 2H), 3.81 (s, 2H), 3.61 (d, *J*=12.3 Hz, 2H), 3.40 (m, 1H), 3.26 (d, *J*=16.3 Hz, 2H), 3.08 (d, *J*=16.6 Hz, 2H), 2.98 (m, 1H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 177.0, 170.9, 142.4, 133.6, 133.1, 132.8, 132.6, 132.3, 132.0, 131.9, 129.8, 129.5, 129.2, 129.0, 128.9, 128.9, 128.8, 128.3, 128.1, 127.4, 127.1, 126.9, 125.5, 123.2, 120.4, 66.5, 64.6, 61.6, 59.6, 53.0, 44.0, 29.6 ppm. *Compound* **16b**: mp 342.2 °C (decomp.); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.88–8.22 (m, 4H), 7.94 (d, *J*=8.31 Hz, 2H), 7.89–7.82 (m, 4H), 7.58–7.21 (m, 16H), 7.13–6.98 (m, 6H), 6.84 (br d, *J*=7.6 Hz, 2H), 6.56 (m, 2H), 6.44 (dd, *J*=8.2, 1.5 Hz, 2H), 4.58 (d, *J*=13.9 Hz, 2H), 4.35 (dd, *J*=8.7, 6.2 Hz, 2H), 4.19 (d, *J*=12.2 Hz, 2H), 4.07 (d, *J*=16.9 Hz, 2H), 3.90 (d, *J*=13.7 Hz, 2H), 3.20 (d, *J*=12.0 Hz, 2H), 2.97 (d, *J*=16.9 Hz, 2H), 2.87 (dd, *J*=8.7, 6.2 Hz, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.6, 176.4, 170.9, 141.7, 133.7, 133.0, 132.7, 131.8, 131.2, 131.0, 130.7, 129.6, 129.4, 129.1, 128.8, 128.6, 128.2, 127.6, 126.3, 126.0, 123.2, 120.3, 65.9, 64.3, 63.6, 61.7, 41.5 ppm. HRMS (TOF) $[M+H]^+$, calcd for $[C_{63}H_{55}N_6Ni_2O_6+H]^+$: 1107.2890, found: 1107.2972.

3.3. Synthesis of 4-aminoglutamic acid (*S*,*S*)-1a[17]

Compound (*S*,*S*,*S*′,*S*′)-**3** was decomposed following the procedure by Belokon and his co-workers, see Ref. 17. Yield 61%; $[\alpha]_D^{25}$ +20.1 (*c* 1.35, as HCl salt in D_2O).

Acknowledgements

This work was supported by the Department of Chemistry and Biochemistry, University of Oklahoma. The authors

gratefully acknowledge generous financial support from Central Glass Company (Tokyo, Japan) and Ajinomoto Company (Tokyo, Japan).

Supplementary data

X-ray crystallographic files for the complexes (*S*,*S*,*S*′,*S*′)-**3**, (*S*,*S*,*S*′,*R*′)-**4**, and **16b** in cif format. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.04.023.

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24. Considering the significant high diastereoselectivity obtained in these reactions, it was of interest to study the asymmetric version of this methylene dimerization using achiral complex **15** and chiral phase-transfer catalyst. Investigation of this catalytic asymmetric methylene dimerization is currently under study and the results obtained will be reported in due course.
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Tetrahedron

On the reactivity of some 2-methyleneindolines with β-nitroenamines, α-nitroalkenes, and 1,2-diaza-1,3-butadienes

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Received 11 January 2006; revised 21 March 2006; accepted 6 April 2006
Available online 11 May 2006

Abstract—A study of the behaviour of some electron-rich 2-methyleneindolines (**1**–**3**) with different electron-poor reagents (formation of new carbon–carbon and nitrogen–carbon bonds) has furnished interesting results from both synthetic and the mechanistic viewpoints. Enamines **1**–**3** have been reacted with the β-nitroenamines **4**–**7** (reaction $CeCl_3 \cdot 7H_2O$ promoted), giving the polymethine dyes **14**–**23**. The same bases **1**–**3** have been nitroalkylated with the nitroolefins **8**–**10**, furnishing the indolines **24**–**32**, and the diastereoselectivity of the reaction has been thoroughly investigated. The most unexpected results derived from the first example of reaction of Fischer's bases with 1,2-diaza-1,3-butadienes. In fact, with **11**–**13**, the 'unknown' indoline spirodihydropyrroles **33**–**40** were formed. Their structures were unambiguously assigned, and we determined, as an example, that of **33** by X-ray analysis.

1. Introduction

Fischer's base[1] **1** (1,3,3-trimethyl-2-methyleneindoline) has been frequently used in dye chemistry for the synthesis of polymethine dyes, a class of compounds that contain an electron donor and an electron acceptor at the opposite ends of the methine chain.[2] Thus, chiral monomethine cyanine dyes,[3] chiral arylazomethyleneindoline dyes[4] and chiral trimethine cyanine dyes[2c] were synthesized using chiral 2-methyleneindolines as key intermediates.

Also interesting are the reactions of Fischer's base with 2-hydroxybenzaldehyde derivatives[5] and 1-nitroso-2-hydroxyaryl derivatives,[6] which afford spiropyran and [1,4]-spirooxazine derivatives, respectively, whereas [1,2]-spirooxazine derivatives can be obtained using nonaromatic nitrosohydroxy compounds.[7] These spirocompounds are a class of photochromic organic compounds that have been extensively studied since the first report by Fischer and Hirshberg.[8] The photochromism of spiropyran[9] and spirooxazine[6,9c,10] is based on the reversible colour change between the closed spiro-structure and the open planar merocyanine structure. Permanent open forms of spirooxazines can be also synthesized.[11]

In accordance with previous considerations and in the framework of our interest on the use of nitroalkenylation reactions in organic synthesis, we have addressed our attention to the behaviour of bases **1**–**3** (electron-rich substrates) with the β-nitroenamines **4**–**7**[12–16] (electron-poor reagents) (Fig. 1) with the aim of obtaining new polymethine dyes. For the sake of comparison and continuing our studies on nitroalkylation reactions[16] of 2-methyleneindolines, we investigated the reactivity of bases **1**–**3** with the α-nitroalkenes **8**–**10**,[17–18] also to verify the diastereoselectivity of this reaction on the chiral racemic substrates **2** and **3**. Furthermore, for the first time, the study of the reactivity study of nucleophiles such as **1**–**3** has been extended to the 1,2-diaza-1,3-butadienes **11**–**13**,[19] electrophiles that, because of their polyfunctionalized structure, could show unexpected development in the reaction.

Keywords: Polymethine cyanine dyes; Nitroalkenylation; Nitroalkylation; Diastereoselection; Spiroindolinedihydropyrroles.
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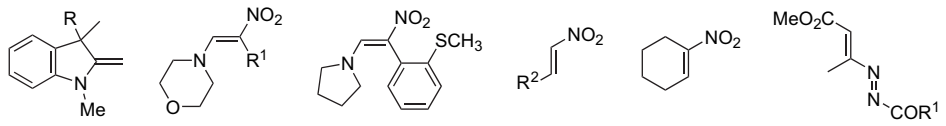
doi:10.1016/j.tet.2006.04.027



1: R = Me
2: R = Et
3: R = Ph

4: R^1 = H
5: R^1 = Me
6: R^1 = Ph

7

8: R^2 = Me
9: R^2 = Ph

10

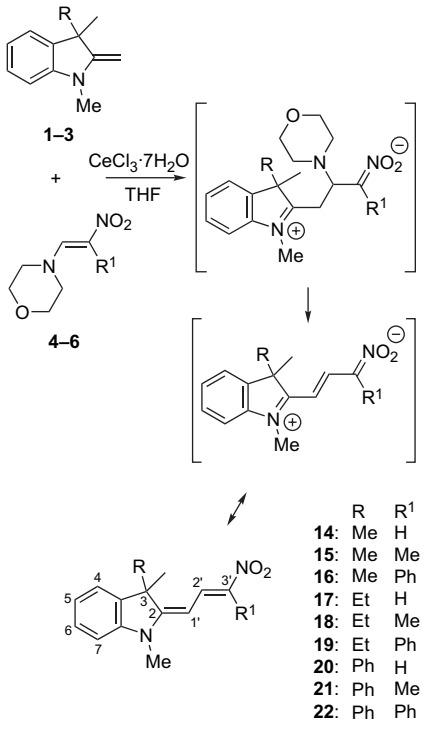
11: R^1 = NH_2
12: R^1 = OMe
13: R^1 = O-*t*-Bu

Figure 1. 2-Methyleneindolines **1**–**3**, β-nitroenamines **4**–**7**, nitroolefins **8**–**10** and 1,2-diaza-1,3-butadienes **11**–**13**.

2. Results and discussion

2.1. Reactivity of indolines with β-nitroenamines

2.1.1. Nitroalkenylation reactions of 2-methyleneindolines 1–3 with nitroenamines 4–6. The enamines **1**–**3** (2 equiv) reacted with the nitroenamines **4**–**6** (1 equiv) in dichloromethane, in the presence of 1 equiv of $CeCl_3 \cdot 7H_2O$,[20] to yield the corresponding nitroalkenylated products **14**–**22** (Scheme 1), with formation of a new carbon–carbon bond between two sp^2-hybridized carbon atoms, showing nucleophilic (in **1**–**3**) and electrophilic (in **4**–**6** or in **7**, see subsequently) characters, respectively. In such a way compounds containing the interesting diene system having at the two ends an electron-donating and an electron-withdrawing group have been built-up, that is, polymethine dye systems.

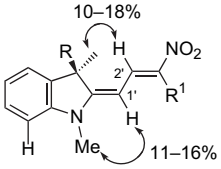


Scheme 1. Nitroalkenylation products of 2-methyleneindolines **1**–**3**.

The presence of Ce(III) chloride promotes the reaction, with time varying from 4 to 15 days. Yields of purified products ranged between 26% and 56% (Table 2). The geometry of the two conjugated double bonds in the products **16**–**22** (compounds **14** and **15** were already known[16]) was established as (1′*E*,2′*E*) by difference NOE measurements performed on **16**, **19**, **20**, **21** and **22** (Table 1) and by comparison of the resonances of their vinyl protons (Table 2). In all compounds examined, irradiation of the methyl group on nitrogen caused the enhancement of the H-1′ vinyl proton signal, whereas by irradiating the H-2′ vinyl proton either the methyl group (in **16**, **19**, **20**, **21**, and **22**) or the methylene group of the ethyl chain (in **19**) was enhanced, thus demonstrating the *s*-trans geometry of the butadiene moiety.

2.1.2. Nitroalkenylation reaction of Fischer's base 1 with nitroenamine 7. In this case, $CeCl_3 \cdot 7H_2O$ alone was not able to promote the reaction. On the contrary, when a mixture of $CeCl_3 \cdot 7H_2O$ (0.2 equiv) and NaI (0.1 equiv)[21] was used, the nitroalkenylated product **23** was isolated in 10% yield. The lower yield found in this case, in which the nitroolefin phenyl ring bears a methylthio group, when compared with that found for the nitroolefin **6** with the same substrate (Table 2) would suggest a preferred coordination of cerium with sulfur, owing to its known great affinity for oxygen and sulfur. $InCl_3$ was also used as a Lewis acid, however, after 7 days only traces of the product **23** could be detected in the ^{1}H NMR spectrum of the crude reaction mixture (Fig. 2). This result is difficult to explain, as in some cases $InCl_3$ has been found to be more efficient than $CeCl_3 \cdot 7H_2O$.[22] When $Zn(CF_3SO_3)_2$ was used, the same product **23** was isolated in 30% yield. The ability of zinc triflate to promote carbon–carbon bond formation in the indole chemistry has

Table 1. Difference NOE data for compounds **16**, **19**, **20**, **21**, and **22**

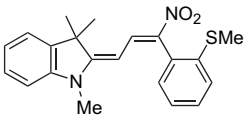
	Compound	Irradiated nucleus: CH_3 at N c.s. (ppm)	Enhanced nucleus: H-1′ c.s. (ppm)	η	Irradiated nucleus: H-2′ c.s. (ppm)	Enhanced nucleus: CH_3 at C-3 c.s. (ppm)	η
	**16**	3.09	5.23	0.14	8.74	1.71	0.18
	19	3.09	5.30	0.15	8.71	1.69	0.12
						2.31[a]	0.14
	20	3.40	5.41	0.11	7.70	1.93	0.10
	21	3.40	5.25	0.13	7.82	1.93	0.13
	22	3.20	5.20	0.16	8.07	1.99	0.18

16, 19, 20, 21, 22

[a] Enhancement of the methylene of the ethyl group.

Table 2. Reaction times, reaction yields and the most meaningful ^{1}H NMR data for the nitrodiene derivatives **14–23**

Product	Reaction time (d)	Yield (%)	^{1}H NMR	
			H-1′ ppm, mult., *J* (Hz)	H-2′ ppm, mult., *J* (Hz)
14	14	56	5.46, d, 13.2	8.38, dd, 13.2, 12.1
15	14	53	5.30, d, 13.2	8.50, d, 13.2
16	6	45	5.23, d, 13.5	8.74, d, 13.5
17	15	38	5.51, d, 13.1	8.34, t, 13.1
18	4	42	5.34, d, 13.5	8.46, d, 13.5
19	7	44	5.30, d, 13.5	8.71, d, 13.5
20	8	31	5.41, d, 12.9	7.70, t, 12.9
21	4	42	5.25, d, 13.0	7.82, d, 13.0
22	15	26	5.20, d, 13.2	8.07, d, 13.2
23	12	30	4.93, d, 13.5	8.74, d, 13.5



23

Figure 2. Compound **23**.

already been evidenced to be superior to other heavy-metal salts and lanthanide salts as well.[23]

2.1.3. UV spectra of trimethines 16–23. All the nitrodiene derivatives **16–23** exhibited an intense absorption band in the visible region. Their electronic spectra were recorded (see data in Table 3) in three solvents with very different properties. In fact, cyclohexane, acetonitrile and methanol were different from one another, as shown by the values of their empirical parameters of solvent polarity. They differed not only in their dielectric properties, evaluated by using the E_T^N (that is, the normalized parameter of solvatochromic solvent polarity: 0.006, 0.460 and 0.762, respectively) values,[24] but also for their different aptitude to participate in hydrogen bond formation, as evaluated by the (A_j+B_j) parameter[25] (0.09, 1.22 and 1.25, respectively).

It is noteworthy that the position of band 4 was particularly affected by solvent polarity, being significantly shifted from 434–452 nm in cyclohexane to longer wavelengths (bathochromic shift) in acetonitrile (473–489 nm) and in methanol (477–491 nm). Moreover, for all compounds, in the apolar solvent cyclohexane, a further intense absorption band (band 3) appeared around 417–436 nm.

2.2. Reactivity of indolines with α-nitroolefins

2.2.1. Nitroalkylation reactions of 2-methyleneindolines 1–3 with α-nitroolefins 8–10. The nitroalkylation reactions of 2-methyleneindolines **1–3** with the α-nitroolefins **8–10** were performed in diethyl ether and furnished the corresponding products **24–32** (Scheme 2) in good yields, with the exception of the products derived from **3**, which were obtained in a much lower yield. Evidently, the steric hindrance of the phenyl group greatly affected the approach of the reagents. Compound **26** had already been synthesized,[16] and it is included only for comparison.

Once more a new carbon–carbon bond between carbon atoms initially sp^2-hybridized is formed, but, in this case, the absence of a leaving group in **8–10** causes the formation of an alkenic instead of an alkadienic system.

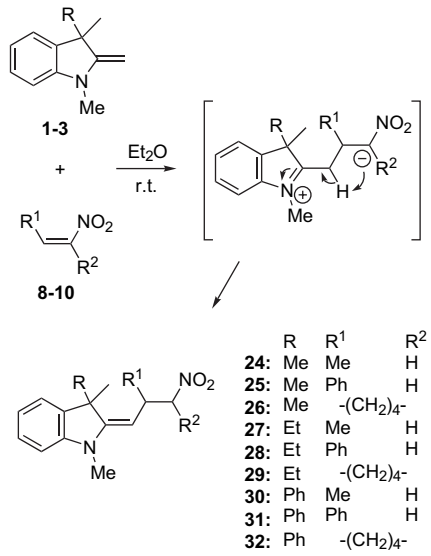
Moreover the higher electrophilic character of **8–10**, caused by the absence of the amino moiety, makes unnecessary the presence of the Lewis acid promoters.

2.2.2. Reactions of 2-methyleneindolines 1–3 with 1-nitropropene 8 and β-nitrostyrene 9. The reaction of Fischer's base **1** with (*E*)-1-nitropropene **8** gave compound **24** as a single diastereomer. By contrast, in the reaction of

Table 3. Electronic absorption spectra of compounds **16–23**: λ_{max} [nm] (log ε)

	Solvent	Band 1	Band 2	Band 3	Band 4
16	CH_3OH	208 (4.29)	275 (4.12)		490 (4.42)
	CH_3CN	193 (4.76)	277 (4.05)		485 (4.46)
	CyH[a]	195 (4.65)	251 (3.71), 279 (4.09)	434 (3.93)	450 (3.94)
17	CH_3OH	209 (3.97)	277 (3.90)		483 (4.41)
	CH_3CN	195 (4.60)	280 (4.13)		477 (4.59)
	CyH[a]	212 (4.01)	247 (3.68), 276 (3.94)	420 (4.22)	440 (4.26)
18	CH_3OH	209 (4.01)	283 (3.99)		490 (4.48)
	CH_3CN	192 (4.94)	280 (4.35)		480 (4.65)
	CyH[a]	216 (4.15)	248 (4.06), 280 (4.20)	428 (4.51)	448 (4.53)
19	CH_3OH	208 (4.25)	284 (3.95)		491 (4.57)
	CH_3CN	193 (3.91)	276 (4.16)		488 (4.56)
	CyH[a]	195 (4.68)	279 (4.21)	436 (4.45)	452 (4.46)
20	CH_3OH	208 (4.41)	275 (3.94)		477 (4.15)
	CH_3CN	194 (5.02)	277 (4.24)		473 (4.55)
	CyH[a]	197 (4.66)	271 (4.11)	417 (4.43)	434 (4.45)
21	CH_3OH	207 (4.29)	276 (4.28)		483 (4.46)
	CH_3CN	194 (4.72)	280 (4.07)		477 (4.49)
	CyH[a]	210 (4.21)	248 (3.77)	421 (3.86)	440 (3.85)
22	CH_3OH	209 (4.60)	263 (4.12)		487 (4.35)
	CH_3CN	193 (4.98)	260 (4.17)		484 (4.40)
	CyH[a]	210 (4.49)	254 (3.98)	429 (4.15)	443 (4.13)
23	CH_3OH	209 (4.50)	254 (4.08), 281 (3.97)		489 (4.42)
	CH_3CN	203 (4.46), 209 (4.47)	259 (4.10), 288 (3.99)		489 (4.48)
	CyH[a]	201 (4.24), 210 (4.41)	259 (4.09), 281 (3.99)	432 (4.35)	451 (4.36)

[a] CyH, cyclohexane.



Scheme 2. Nitroalkylation products of 2-methyleneindolines **1**–**3** with nitroalkenes **8**–**10**.

1 with (*E*)-β-nitrostyrene **9**, two isomers, **25a** and **25b**, were obtained in a 9:1 ratio. The *E* geometry was assigned to compounds **24** and **25a** on the basis of NOE measurements. In fact, irradiation of the respective vinyl protons (3.96 ppm for **24** and 4.39 ppm for **25a**) enhanced the signal of the singlet relative to the methyl group at nitrogen (2.95 ppm for **24** and 3.00 ppm for **25a**) for 6% and 13%, respectively. As a consequence, **25b** was assigned the *Z* configuration.

The reactions of 2-methyleneindolines **2** and **3** with 1-nitropropene **8** and β-nitrostyrene **9** gave pairs of inseparable diastereomers **27a**,**b**, **28a**,**b**, **30a**,**b** and **31a**,**b** at ratios of 9:1, 3:2, 3:1 and 1:1, respectively (Table 4). The **a** and **b** isomers were assigned the *E* configuration because the chemical shifts of their vinyl proton H-1′ were practically the same, differing by 0.01–0.04 ppm. In Table 4 we also report the chemical shifts of (*E*)-**24**, (*E*)-**25a** and (*Z*)-**25b**. NOE experiments were performed on compounds **24**–**28, 30** and **31**, in order to confirm their geometries. Irradiation of the nitrogen methyl group caused enhancement of the respective vinyl proton signal for amounts ranging from 5% to 13%.

It is interesting to point out that only in the reactions of **2** and **3** with 1-nitropropene **8** it was possible to envisage a 1,4-asymmetric induction for the formation of the new stereocentre. In fact, the diastereomeric excess (de) was 80% for the reaction of enamine **2**, and it was only 50% for the reaction of the 2-methyleneindoline **3** with the same α-nitroolefin. The relative stereochemistry of the nitroalkyl chain was tentatively assigned as 2′*R** and 2′*S** by analysis of the ^{1}H NMR data for compounds **30a** and **30b** (Fig. 3).

Table 4. Relative yields and chemical shift values of vinyl proton signals for compounds **24**, **25**, **27**, **28**, **30** and **31**

Entry	Product	Yield (%)	H-1′ (ppm)
1	(*E*)-**24**	100	3.96
2	(*E*)-**25a**	90	4.39
	(*Z*)-**25b**	10	4.25
3	(*E*)-**27a**	90	4.02
	(*E*)-**27b**	10	4.03
4	(*E*)-**28a**	60	4.46
	(*E*)-**28b**	40	4.47
5	(*E*)-**30a**	75	3.99
	(*E*)-**30b**	25	3.95
6	(*E*)-**31a**	50	4.40
	(*E*)-**31b**	50	4.36

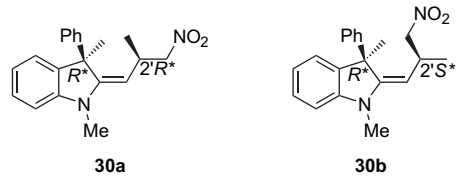


Figure 3. Structures of compounds **30a** and **30b**.

Although a rotation around the C1′–C2′ single bond is possible, the average positions of the methyl group and the nitromethylenic group are influenced differently by the presence of the phenyl group. Thus, the methyl doublet at C-2′ resonated at 0.50 ppm for the major component **30a** and at 1.04 ppm for the minor component **30b**, whereas the resonances of the respective nitromethylenic protons appeared at 4.19 ppm for **30a**, as an AB part of an ABXY3 system, and at higher field (3.64 and 3.36 ppm, two double doublets) for **30b**. Therefore, the (3*R**,2′*R**) configuration was assigned to **30a**, for which the methyl group at C-2′ is more shielded by the phenyl group, and the (3*R**,2′*S**) configuration to **30b**, for which the nitromethylenic protons are more shielded. These assignments agree with the *R**,*Si** topological approach of α-nitroolefins to the enamines, and is similar to that proposed by Seebach et al.[26] (Fig. 4). In a similar manner, the diastereomers **27a** and **27b** were assigned the (3*R**,2′*R**) and (3*R**,2′*S**) configurations.

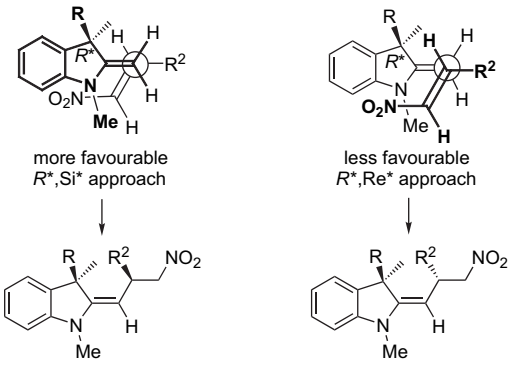


Figure 4. The proposed topological approach.

In the reaction of 2-methyleneindoline **3** with β-nitrostyrene **9**, the isomers **31a** and **31b** were formed in a 1:1 ratio. Since the nitromethylenic protons resonated as two double doublets at 4.56 and 4.48 ppm for **31a** and at 4.14 and 3.60 ppm for **31b**, owing to the C.I.P. configurational rules, in this case the (3*R**,2′*S**) configuration was assigned to **31a** and the (3*R**,2′*R**) configuration to **31b**, the former being generated by an *R**,*Si** approach and the latter by an *R**,*Re** approach. In this case the two approaches were equally probable.

2.2.3. Reactions of 2-methyleneindolines 1–3 with nitrocyclohexene 10. The reaction of Fischer's base **1** with nitrocyclohexene **10** gave two diastereomeric Michael-type adducts **26a** and **26b**, both of which were in *E* geometry and differed in the orientation of the nitro group.[16]

In the reaction of 2-methyleneindoline **2** with nitrocyclohexene **10**, two isomers, **29a** and **29b**, were formed in a 3:1 ratio. The same *E* geometry was assigned to both diastereomers by comparison of their ^{1}H NMR data with those of the known compounds **26**.[16] Since the axial and equatorial orientations of the nitro group were easily recognizable from the positions and patterns of the respective nitromethine proton signals, the *cis* and *trans* geometries were assigned to **29a** and **29b**, respectively. In fact, in the *cis* isomer **29a**, the equatorial nitromethine proton resonated at lower field than the same proton in the *trans* isomer **29b** (4.62 ppm vs 4.22 ppm). The two signals also exhibited different patterns: a double triplet with J_1=J_2=4.3, J_3=8.8 Hz and W_H=16 Hz for **29a** and a multiplet with W_H=26 Hz for **29b**, in accordance with equatorial and axial orientations, respectively, of the nitromethine protons. On standing, the *cis* diastereomer **29a** slowly converted into the more stable *trans* isomer **29b**, thus confirming the assignments made.

The ^{1}H NMR analysis of the crude reaction mixture obtained from the enamine **3** and nitrocyclohexene **10** indicated the presence of three diastereomers of *E* configuration: *cis*-**32a**, *trans*-**32b** and *cis*-**32c** in 60%, 25% and 15% yields, respectively. Unfortunately, purification by column chromatography did not allow a complete separation of the products. In fact, *cis*-**32a** transformed in large amount into *trans*-**32b**, whereas *cis*-**32c** converted completely into *trans*-**32d**.

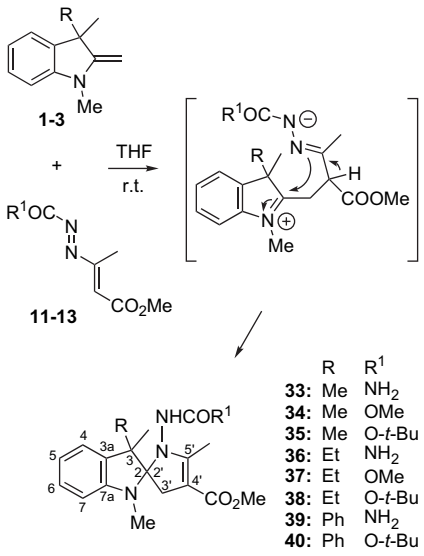
The *cis* configuration assigned to **32a** and **32c** on the basis of their lower thermodynamic stability was confirmed by an analysis of the signals of their respective nitromethine protons when they were compared with those of the corresponding *trans* isomers **32b** and **32d**. In fact, the nitromethine protons resonated at 4.48 ppm (W_H=18.4 Hz) for **32a** and at 4.48 ppm (W_H=18.4 Hz) for **32c**, whereas the same signal appeared at 4.10 ppm (W_H=30.0 Hz) for **32b** and at 3.94 ppm (W_H=29.0 Hz) for **32d**.

In accordance with the above stereochemical considerations and the type of proposed topological approach described in Figure 4, the same (3*R**,2′*R**) configuration was assigned to **32a** and **32b**, whereas the (3*R**,2′*S**) configuration was assigned to **32c** and **32d**.

2.3. Reactions of 2-methyleneindolines 1–3 with 1,2-diaza-1,3-butadienes 11–13

The reactions between the indolines **1–3** and the 1,2-diaza-1,3-butadienes **11–13**[19a,b] were performed in THF at room temperature, which produced the tricyclic addition compounds **33–40** in good yields (81–96%) (Scheme 3) with formation of two new bonds (carbon–carbon and nitrogen–carbon, respectively).

The interesting spiro-structure of compound **33** has been unambiguously determined by X-ray diffraction study



Scheme 3. The spirocompounds **33–40**.

(Fig. 5).[27] Indoline spirodihydropyrroles have not been reported in the literature, and only few cases of spiroindolepyrrolidinones are known.[28] Interestingly, whereas the ^{1}H and ^{13}C NMR spectra of **33** in $CDCl_3$ showed the presence of a single product, in DMSO-d_6 each peak was split into two signals, thus indicating the presence of two conformers **a** and **b** in a 60:40 ratio. They remained stable even when the temperature was increased. The relative signals did not coalesce even at 110 °C. However, after recovering the product from DMSO-d_6, its spectrum again in $CDCl_3$ showed the signals of the parent isomer. DIFNOE measurements were performed on the two conformers **33a** and **33b** with the aim of understanding the origin of this isomerism. Irradiating the methyl group linked to nitrogen in the major component **33a** at 2.60 ppm an enhancement was observed for the signal of the NH group at 6.94 ppm (4%), whereas, in the minor component **33b**, the same signal (at 7.22 ppm) was enhanced by irradiating the methyl group at C-3 at 1.23 ppm. These results suggest that, in the major isomer **33a**, the NH group of the chain pointed towards the nitrogen of the indole moiety, as shown by the X-ray structure, whereas, in the minor isomer, the same group pointed towards C-3 of the indole moiety. This could be consistent with an inversion (flip-flap) at the pyrroline nitrogen.

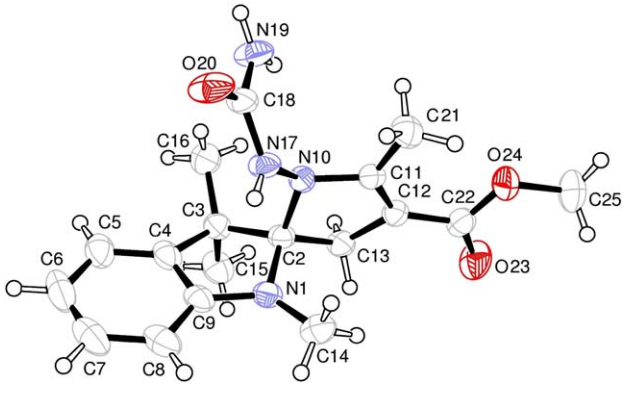


Figure 5. X-ray structure of compound **33**.

The structure of the minor isomer **33b** was optimized with the Cornell version of the Amber force field,[29] which showed a relative energy difference of 4 kcal/mol with respect to **33a**. In the invertomer **33b**, the proximity of the NH group to the methyl group at C-3 is evident. No other rotamer of **33a** would account for the NOE effect observed for **33b**. Figure 6 presents a better representation of the two isomers showing the distance between the protons involved in the NOE effects observed.

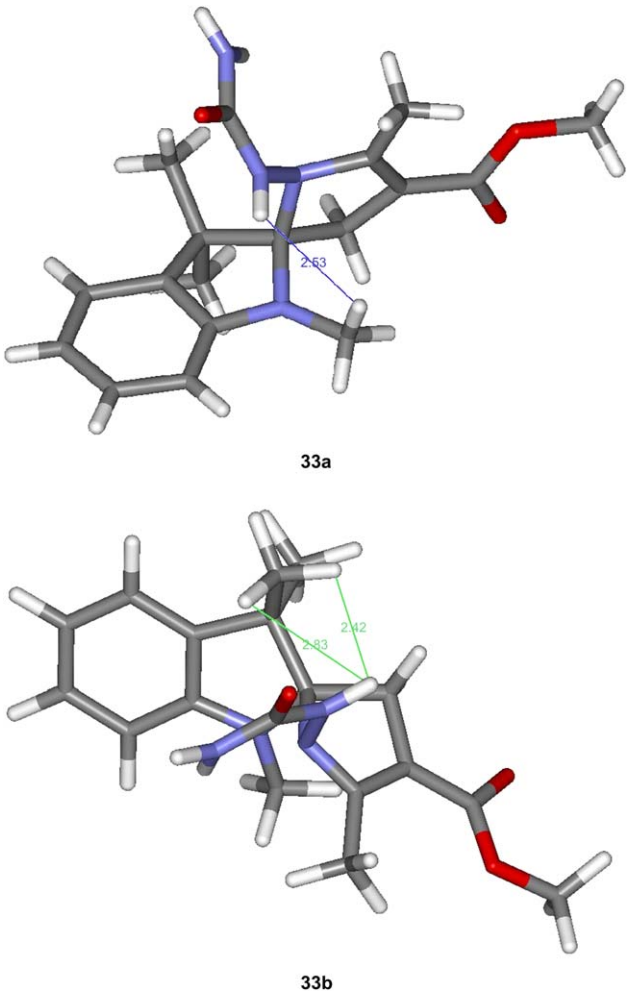


Figure 6. Optimized geometries of **33a** and **33b**.

NMR analysis of all the other products **34–40** showed that they were mixtures of isomers **a** and **b** even in $CDCl_3$. If this result can be attributed to the presence of conformers for compounds **34** and **35**, which possess a single stereocentre, the same conclusion cannot be drawn immediately for compounds **36–40**, which possess two stereocentres. However, a comparison between the spectra of the same spirocompounds in $CDCl_3$ and in DMSO-d_6 revealed that they simply differed in the composition of **a** and **b**, as shown in Table 5. This could suggest that **a** and **b** are conformers and not diastereomers. In that case the preferred approach of the enamines **2** and **3** onto the 1,2-diaza-1,3-butadienes would occur from the less sterically demanding side namely the one that contains the methyl group at C-3.

Table 5. Chemical compositions of compounds **33–40**

Compound	*a*:*b* $CDCl_3$	*a*:*b* DMSO-d_6
33	100:0	60:40
34	60:40	55:45
35	60:40	50:50
36	75:25	60:40
37	55:45	50:50
38	60:40	50:50
39	40:60	65:35
40	40:60	55:45

3. Conclusion

By reaction of 2-methyleneindoline derivatives **1–3** with β-nitroenamines **4–7**, new deeply coloured trimethine dyes containing the nitro function as the electron-acceptor group were obtained. These syntheses were promoted by $CeCl_3 \cdot 7H_2O$, although in most cases long reaction times were required. Studies on the optical properties of these dyes are under investigation.

In the nitroalkylation reactions of **1** and **2**, (*E*)-1-nitropropene **8** proved more diastereoselective than (*E*)-β-nitrostyrene **9**, as it has already been observed for enolates,[14a] whereas the nitrocyclohexene **10** was the most diastereoselective. The nitroalkylation reactions of the 2-methyleneindoline **3** were less satisfactory as far as yields and diastereoselectivity are concerned. This was probably due to the severe steric hindrance carried on both sides of the enamine system by the phenyl group.

The reactions of 2-methyleneindolines with 1,2-diaza-1,3-butadienes **11–13** gave rise to unknown indoline spiropyrrolines. It is noteworthy that neither spirotetrahydropyridazines deriving from the possible [4+2] cycloaddition, nor the simple Michael addition products were detected. The new reaction observed provides a route to interesting, partially reduced benzocondensed pyrrole derivatives that are intermediates in natural product synthesis.[28,30] Experiments of ring opening under thermal and photochemical conditions are in progress, to verify whether a ring open-chain equilibration is possible to modulate the absorption wavelength of the molecules.

4. Experimental

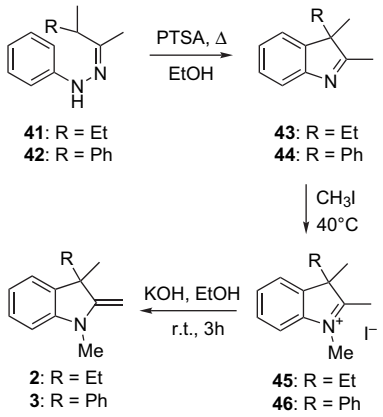
4.1. General

IR spectra were recorded on a Jasco FT/IR 200 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were run on a Jeol EX-400 spectrometer (400 MHz for proton, 100 MHz for carbon) and a Jeol EX-270 spectrometer (270 MHz for proton, 68 MHz for carbon), using deuteriochloroform as a solvent and tetramethylsilane as the internal standard. Coupling constants are given in Hertz. GLC analyses were run on a Carlo Erba GC 8000 instrument, the capillary column being OV 1701 (25 m×0.32 mm) (carrier gas He, 40 kPa, split 1:50). Mass spectra were recorded on an ion trap FINNIGAN GCQ (70 eV) spectrometer, HRMS were recorded on a FINNIGAN MAT95XP apparatus. UV spectra were recorded on a HELIOS β-UNICAM spectrophotometer. TLCs were performed on Polygram® Sil G/UV254

silica gel pre-coated plastic sheets (eluant: light petroleum–ethyl acetate). Flash chromatography was run on silica gel 230–400 mesh ASTM (Kieselgel 60, Merck). Light petroleum refers to the fraction with bp 40–70 °C. All solvents were distilled over appropriate drying agents and maintained over molecular sieves. 2,3-Dihydro-2-methylene-1,3,3-trimethyl-1*H*-indole **1**, 3-methyl-2-pentanone and *trans*-β-nitrostyrene **9** were purchased from Sigma–Aldrich. Phenylhydrazine was purchased from Carlo Erba; 4-(2-nitroethenyl)morpholine **4**,[12] 4-(2-nitro-1-propenyl)morpholine **5**,[13] 4-(2-phenylethenyl-2-nitro)morpholine **6**,[14] 1-[(1*E*)-2-[2-(methylthio)phenyl]-2-nitroethenylpyrrolidine **7**,[15] 3-phenyl-2-butanone,[31a] 1-nitropropene,[17] 1-nitrocyclohexene[18] and 1,2-diaza-1,3-butadienes **11**–**13**[19,32] were synthesized according to the literature.

4.2. Synthesis of 2-methyleneindoline derivatives 2 and 3

2-Methyleneindoline derivatives **2** and **3** were prepared according to the procedure of Brunner[33] and Ferratini,[34] as indicated in Scheme 4. Fischer's indolization[35] of 3-methyl-2-pentanone phenylhydrazone **41** and 3-phenyl-2-butanone phenylhydrazone **42**[31b] furnished the corresponding 3*H*-indoles **43**[36] and **44**[37] that were alkylated with iodomethane providing salts **45**[2c] and **46**, respectively. Their treatment with KOH afforded 3-ethyl-1,3-dimethyl-2-methyleneindoline **2** and 2-methylene-1,3-dimethyl-3-phenylindoline **3**, respectively.



Scheme 4. Synthesis of 2-methyleneindolines **2** and **3**.

4.3. Synthesis of substrates

4.3.1. 3-Methyl-2-pentanone phenylhydrazone (41). To a solution of phenylhydrazine (7.8 ml, 80 mmol) in ethanol (28.8 ml) 3-methyl-2-pentanone (9.8 ml, 80.0 mmol) was added. After refluxing the solution for 5 h and evaporation of the solvent the phenylhydrazone **41** was obtained as a yellow oil (99% yield). IR (cm^{-1}, film) 3350 (NH), 1602 (C═N), 1502 (Ph); ^1H NMR (δ, ppm, $CDCl_3$) 7.23 (2H, t, Ph-H, J=7.9 Hz), 7.05 (2H, d, Ph-H, J=8.4 Hz), 6.81 (1H, t, Ph-H, J=7.3 Hz), 2.43 (1H, m, $CHCH_3$), 2.13 (1H, s, NH), 1.82 (3H, s, CH_3C), 1.57 (1H, m, *H*$CHCH_3$), 1.44 (1H, m, *H*$CHCH_3$), 1.10 (3H, d, C*H*$_3$CH, J=7.0 Hz), 0.89 (3H, t, C*H*$_3$CH$_2$, J=7.3 Hz); ^{13}C NMR (δ, ppm, $CDCl_3$) 146.0 (s, C═N), 129.2 (2d, Ph), 119.7 (d, Ph), 113.2 (2d, Ph), 43.8 (d, *C*HCH_3), 27.3 (t, *C*H_2CH_3), 17.8 (q, CH_3), 12.1 (q, CH_3), 12.0 (q, CH_3).

4.3.2. 3-Phenyl-2-butanone phenylhydrazone (42).[31a] To a solution of phenylhydrazine (2.5 ml, 25 mmol) in ethanol (9 ml) 3-phenyl-2-butanone[31a] (3.70 g, 25 mmol) was added. The orange solution obtained was refluxed for 7 h and after removal of the solvent the phenylhydrazone **42** was obtained in 91% yield. Mp 70–72 °C; IR (cm^{-1}, Nujol) 3350 (NH), 1601 (C═N), 1498 (Ph); ^1H NMR (δ, ppm, $CDCl_3$) 7.40–7.12 (9H, m, Ar-H), 6.84 (1H, t, Ar-H, J=7.3 Hz), 3.70 (1H, m, CH), 2.05 (1H, s, NH), 1.68 (3H, s, CH_3C), 1.51 (3H, d, CH_3CH, J=7.0 Hz); ^{13}C NMR (δ, ppm, $CDCl_3$) 148.0 (s), 146.1 (s), 143.8 (s), 129.3 (d), 128.6 (d), 127.8 (d), 126.6 (d), 119.7 (d), 113.1 (d), 48.3 (d, C-3), 18.9 (q, CH_3), 13.6 (q, CH_3).

4.3.3. 3-Ethyl-2,3-dimethyl-3*H*-indole (43).[36] To a solution of **41** (15.02 g, 79.0 mmol) in ethanol (28.8 ml) *p*-toluenesulfonic acid monohydrate (PTSA, 30.43 g, 160.0 mmol) was added and the mixture refluxed for 5 h. After evaporation of the solvent the oil obtained was dissolved in CH_2Cl_2, washed with a saturated solution of $NaHCO_3$, brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave compound **16** as a brown oil in 85% yield. IR (cm^{-1}, film) 1577 (C═N); ^1H NMR (δ, ppm, $CDCl_3$) 7.53 (1H, d, Ar-H, J=7.7 Hz), 7.29 (1H, t, Ar-H, J=7.5 Hz), 7.21 (2H, m, Ar-H), 2.23 (3H, s, CH_3C═N), 1.90 (1H, m, *H*$CHCH_3$), 1.79 (1H, m, *H*$CHCH_3$), 1.28 (3H, s, CH_3C), 0.39 (3H, t, C*H*$_3$CH$_2$, J=7.5 Hz); ^{13}C NMR (δ, ppm, $CDCl_3$) 187.0 (s, C═N), 154.5 (s), 143.4 (s), 127.4 (d), 125.0 (d), 121.4 (d), 119.6 (d), 58.3 (s, C-3), 30.0 (t, *C*H_2CH_3), 22.3 (q, *C*H_3C═N), 15.6 (q, *C*H_3C), 8.4 (q, *C*H_3CH_2); HRGC (OV1701) t_R=13.72 (10 min at 100 °C, 3 °C/min up to 200 °C).

4.3.4. 2,3-Dimethyl-3-phenyl-3*H*-indole (44).[37] Compound **44** was obtained in 94% yield following the same procedure described for the synthesis of **43**. Mp, IR and ^1H NMR are in accordance with those reported in the literature.[37] ^{13}C NMR (δ, ppm, $CDCl_3$) 187.1 (s), 154.5 (s), 146.9 (s), 139.2 (s), 128.9 (d), 128.0 (d), 127.3 (d), 126.1 (d), 125.9 (d), 122.6 (d), 120.1 (d), 61.8 (s), 20.4 (q, CH_3), 15.9 (q, CH_3).

4.3.5. 3-Ethyl-1,2,3-trimethyl-3*H*-indolium iodide (45).[2c] Methyl iodide (12.6 ml, 0.20 mol) was added to compound **43** (11.72 g, 68.0 mmol), and the solution was warmed at 40 °C until a precipitate was formed. The white solid was filtered and washed with diethyl ether. Compound **45** was obtained in 72% yield (15.33 g, 49.0 mmol). Mp 240–242 °C. All spectroscopic data were identical to those reported in the literature.[2c]

4.3.6. 1,2,3-Trimethyl-3-phenyl-3*H*-indolium iodide (46). Compound **46** was obtained in 34% yield by the same procedure described for the synthesis of **45**. Mp 227–229 °C; IR (cm^{-1}, Nujol) 1633, 1610, 1590; ^1H NMR (δ, ppm, $CDCl_3$) 7.78 (1H, d, Ar-H, J=8.0 Hz), 7.64 (1H, t, Ar-H, J=7.7 Hz), 7.57 (1H, t, Ar-H, J=7.5 Hz), 7.41–7.33 (4H, m, Ar-H), 7.10–7.06 (2H, m, Ar-H), 4.42 (3H, s, CH_3N^+), 2.94 (3H, s, CH_3C═N), 1.25 (3H, s, CH_3C); ^{13}C NMR (δ, ppm, $CDCl_3$) 194.6 (s, C-2), 142.3 (s), 142.2 (s), 133.8 (s), 130.6 (d), 129.9 (2d), 129.7 (d), 129.4 (d), 126.5 (2d), 124.1 (d), 115.7 (d), 62.0 (s, C-3), 38.1 (q, CH_3N^+), 20.6 (q, CH_3), 17.5 (q, CH_3).

4.3.7. 3-Ethyl-2,3-dihydro-1,3-dimethyl-2-methylene-1*H*-indole (2).[2c] Compound **45** (9.48 g, 30.1 mmol) in anhydrous ethanol (150 ml) was treated with KOH (3.38 g, 60.2 mmol). The solution was stirred for 3 h at room temperature. After removal of the solvent, water was added and the aqueous solution was extracted four times with diethyl ether. The combined organic phases were dried over anhydrous Na_2SO_4 and after evaporation of the solvent compound **2** was obtained as a yellowish oil in 92% yield. ^{1}H NMR and ^{13}C NMR spectra were reported in the literature.[2c] IR (cm^{-1}, film) 1649, 1608, 1492, 1462; EIMS (*m/z*) 187 (M^+, 64), 158 (100); HRGC (OV1701) t_R=15.17 (10 min at 100 °C, 3 °C/min up to 200 °C).

4.3.8. 2,3-Dihydro-1,3-dimethyl-2-methylene-3-phenyl-1*H*-indole (3).[38] 2-Methyleneindoline **3** was obtained as an orange oil in 92% yield by the same procedure described for the synthesis of **2**. IR (cm^{-1}, film) 1650, 1606, 1495, 1460; ^{1}H NMR (δ, ppm, $CDCl_3$) 7.29–7.17 (5H, m, Ar-H), 7.15 (1H, t, H-6, *J*=7.3 Hz), 6.91 (1H, d, H-4, *J*=7.3 Hz), 6.71 (1H, t, H-5, *J*=7.3 Hz), 6.61 (1H, d, H-7, *J*=7.7 Hz), 3.93 (1H, d, H-1′, *J*=1.8 Hz), 3.73 (1H, d, H-1′, *J*=1.8 Hz), 3.09 (3H, s, CH_3N), 1.74 (3H, s, CH_3C); ^{13}C NMR (δ, ppm, $CDCl_3$) 162.5 (s, C-2), 146.7 (s), 146.6 (s), 137.5 (s), 128.1 (d), 127.7 (d), 126.4 (d), 126.1 (d), 123.3 (d), 118.7 (d), 105.1 (d, C-7), 76.2 (d, C-1′), 52.0 (s, C-3), 28.8 (q, CH_3), 28.0 (q, CH_3).

4.4. Nitroalkenylation reactions

4.4.1. General procedure. To a solution of the nitroenamines **4–6** (0.29 mmol) in CH_2Cl_2 (1.8 ml), a solution of 2-methyleneindolines **1–3** (0.58 mmol) in CH_2Cl_2 (0.9 ml) and $CeCl_3\cdot 7H_2O$ (0.108 g, 0.29 mmol) was added. The reaction mixture was stirred at room temperature monitoring the course of the reaction by ^{1}H NMR. At the end of the reaction, water was added, and the organic phase was dried over anhydrous Na_2SO_4. After evaporation of the solvent the crude reaction mixture was purified by flash chromatography (light petroleum–ethyl acetate, 85:15) and products **14–22** were isolated.

4.4.2. 2,3-Dihydro-1,3,3-trimethyl-2-[(3-nitro) propenylidene]-1*H*-indole (14). After 14 days, compound **14** was obtained in 56% yield. All spectroscopic data are in accordance with those reported in the literature.[16] Mp 161–162 °C. ^{1}H NMR (δ, ppm, $CDCl_3$) 8.38 (1H, dd, H-2′, J_1=13.2 Hz, J_1=12.1 Hz), 7.27 (2H, t+d, H-6 and H-4), 7.10 (1H, d, H-3′, *J*=12.1 Hz), 7.06 (1H, t, H-5), 6.85 (1H, d, H-7), 5.46 (1H, d, N–C=CH, *J*=13.2 Hz), 3.30 (3H, s, NCH_3), 1.64 (6H, s, *gem*-CH_3).

4.4.3. 2,3-Dihydro-1,3,3-trimethyl-2-[(3-nitro) but-2-enylidene]-1*H*-indole (15). After 14 days, compound **15** was obtained as a purple solid in 53% yield. All spectroscopic data are in accordance with those reported in the literature.[16] Mp 191–192 °C. ^{1}H NMR (δ, ppm, $CDCl_3$) 8.50 (1H, d, H-2′, *J*=13.2 Hz), 7.27 (1H, t, H-6), 7.24 (1H, d, H-4), 7.03 (1H, t, H-5), 6.84 (1H, d, H-7), 5.30 (1H, d, H-1′, *J*=13.2 Hz), 3.32 (3H, s, NCH_3), 2.25 (3H, s, CH_3), 1.64 (6H, s, *gem*-CH_3).

4.4.4. 2,3-Dihydro-1,3,3-trimethyl-2-[(3-nitro-3-phenyl)-propenylidene]-1*H*-indole (16). After 6 days, compound **16** was obtained in 45% yield. Reddish solid; mp 188–190 °C, IR (cm^{-1}, Nujol) 1616, 1568, 1489; UV (nm, CH_3OH) (log ε) 208 (4.29), 275 (4.12), 490 (4.42); UV (nm, CH_3CN) (log ε) 193 (4.76), 277 (4.05), 485 (4.46); UV (nm, cyclohexane) (log ε) 195 (4.65), 251 (3.71), 279 (4.09), 434 (3.93), 450 (3.94); ^{1}H NMR (δ, ppm, $CDCl_3$) 8.74 (1H, d, H-2′, *J*=13.5 Hz), 7.50–7.36 (5H, m, Ph), 7.27–7.22 (2H, m, H-6 and H-4) 7.04 (1H, t, H-5, *J*=7.4 Hz), 6.77 (1H, d, H-7, *J*=8.2 Hz), 5.23 (1H, d, H-1′, *J*=13.5 Hz), 3.09 (3H, s, CH_3N), 1.71 (6H, s, *gem*-CH_3). ^{13}C NMR (δ, ppm, $CDCl_3$) 168.6 (s, C-2), 143.5 (s, C-7a), 139.6 (s), 139.5 (s), 135.5 (d, C-2′), 131.4 (s), 130.9 (2d, Ph), 128.4 (2d, Ph), 128.1 (d), 122.4 (d), 121.9 (d), 107.8 (d, C-7), 90.8 (d, C-1′), 47.4 (s, C-3), 29.5 (q, CH_3N), 28.7 (2q, CH_3C); EIMS (*m/z*) 320 ($M^{+\bullet}$, 100); HRMS calcd for $C_{20}H_{20}N_2O_2$ 320.1525, found 320.1522.

4.4.5. (1′*E*,2′*E*)-3-Ethyl-2,3-dihydro-1,3-dimethyl-2-[(3′-nitro)propenylidene]-1*H*-indole (17). After 15 days, compound **17** was obtained in 38% yield. Orange-brown solid; mp 110–113 °C. IR (cm^{-1}, Nujol) 1618, 1579, 1491; UV (nm, CH_3OH) (log ε) 209 (3.97), 277 (3.90), 483 (4.41); UV (nm, CH_3CN) (log ε) 195 (4.60), 280 (4.13), 477 (4.59); UV (nm, cyclohexane) (log ε) 212 (4.01), 247 (3.68), 276 (3.94), 420 (4.22), 440 (4.26); ^{1}H NMR (δ, ppm, $CDCl_3$) 8.34 (1H, t, H-2′, *J*=13.1 Hz), 7.25 (2H, m, H-6 and H-4), 7.08 (1H, d, H-3′, *J*=12.1 Hz), 7.06 (1H, t, H-5, *J*=7.5 Hz), 6.83 (1H, d, H-7, *J*=8.05 Hz), 5.51 (1H, d, H-1′, *J*=13.1 Hz), 3.30 (3H, s, CH_3N), 1.92 (1H, m, *H*CHCH$_3$), 1.80 (1H, m, *H*CHCH$_3$), 1.30 (3H, s, CH_3C), 0.47 (3H, t, C*H*$_3$CH$_2$, *J*=7.3 Hz). ^{13}C NMR (δ, ppm, $CDCl_3$) 167.8 (s, C-2), 144.4 (s, C-7a), 138.3 (d, C-2′), 137.4 (s, C-3a), 129.1 (d), 128.1 (d), 122.5 (d), 121.9 (d), 107.7 (d, C-7), 90.1 (d, C-1′), 52.5 (s, C-3), 35.0 (t, CH_2CH_3), 29.6 (q), 28.1 (q), 8.8 (q, *C*H$_3$CH$_2$); EIMS (*m/z*) 258 ($M^{+\bullet}$, 100); HRMS calcd for $C_{15}H_{18}N_2O_2$ 258.1368, found 258.1363.

4.4.6. (1′*E*,2′*E*)-3-Ethyl-2,3-dihydro-1,3-dimethyl-2-[(3′-nitro)but-2-enylidene]-1*H*-indole (18). After 4 days compound **18** was obtained in 42% yield. Purple solid; mp 168–169 °C. IR (cm^{-1}, Nujol) 1620, 1595, 1572, 1489; UV (nm, CH_3OH) (log ε) 209 (4.01), 283 (3.99), 490 (4.48); UV (nm, CH_3CN) (log ε) 192 (4.94), 280 (4.35), 480 (4.65); UV (nm, cyclohexane) (log ε) 216 (4.15), 248 (4.06), 280 (4.20), 428 (4.51), 448 (4.53); ^{1}H NMR (δ, ppm, $CDCl_3$) 8.46 (1H, d, H-2′, *J*=13.5 Hz), 7.26 (1H, t, H-6, *J*=7.7 Hz), 7.20 (1H, d, H-4, *J*=7.3 Hz), 7.04 (1H, t, H-5, *J*=7.3 Hz), 6.81 (1H, d, H-7, *J*=8.0 Hz), 5.34 (1H, d, H-1′, *J*=13.5 Hz), 3.31 (3H, s, CH_3N), 2.26 (3H, s, CH_3CNO_2), 2.22 (1H, m, *H*CHCH$_3$), 2.05 (1H, m, *H*CHCH$_3$), 1.64 (3H, s, CH_3C), 0.47 (3H, t, C*H*$_3$CH$_2$, *J*=7.3 Hz). ^{13}C NMR (δ, ppm, $CDCl_3$) 165.9 (s, C-2), 144.6 (s, C-7a), 137.3 (s), 136.0 (s), 133.3 (d, C-2′), 128.0 (d, C-6), 122.1 (d, Ar), 121.9 (d, Ar), 107.3 (d, C-7), 90.8 (d, C-1′), 52.2 (s, C-3), 34.9 (t, *C*H$_2$CH$_3$), 29.5 (q), 28.1 (q), 12.0 (q, C-4′), 8.9 (q, CH$_3$CH$_2$); EIMS (*m/z*) 272 ($M^{+\bullet}$, 100); HRMS calcd for $C_{16}H_{20}N_2O_2$ 272.1525, found 272.1520.

4.4.7. (1′*E*,2′*E*)-3-Ethyl-2,3-dihydro-1,3-dimethyl-2-[(3′-nitro-3′-phenyl)propenylidene]-1*H*-indole (19). After 7

days compound **19** was obtained in 44% yield. Red solid; mp 126–128 °C. IR (cm^{-1}, Nujol) 1616, 1586, 1570, 1491; UV (nm, CH_3OH) (log ε) 208 (4.25), 284 (3.95), 491 (4.57); UV (nm, CH_3CN) (log ε) 193 (3.91), 276 (4.16), 488 (4.56); UV (nm, cyclohexane) (log ε) 195 (4.68), 279 (4.21), 436 (4.45), 452 (4.46); ^1H NMR (δ, ppm, $CDCl_3$) 8.71 (1H, d, H-2′, J=13.5 Hz), 7.60–7.40 (6H, m, Ph and H-6), 7.23 (1H, m, H-4), 7.04 (1H, t, H-5, J=7.3 Hz), 6.77 (1H, d, H-7, J=7.9 Hz), 5.30 (1H, d, H-1′, J=13.5 Hz), 3.09 (3H, s, CH_3N), 2.31 (1H, m, *H*$CHCH_3$), 2.09 (1H, m, *H*$CHCH_3$), 1.69 (3H, s, CH_3C), 0.49 (3H, t, CH_3CH_2, J=7.3 Hz). ^{13}C NMR (δ, ppm, $CDCl_3$) 167.0 (s, C-2), 144.5 (s, C-7a), 139.3 (s), 137.5 (s), 135.2 (d, C-2′), 131.5 (s), 131.0 (2d), 128.4 (2d), 128.1 (d), 122.4 (d), 121.9 (d), 107.6 (d, C-7), 91.3 (d, C-1′), 52.5 (s, C-3), 35.1 (t, *C*H_2CH_3), 29.5 (q), 28.2 (q), 9.0 (q, *C*H_3CH_2); EIMS (*m*/*z*) 334 ($M^{+\bullet}$, 53), 273 (31), 260 (100); HRMS calcd for $C_{21}H_{22}N_2O_2$ 334.1681, found 334.1680.

4.4.8. (1′*E*,2′*E*)-2,3-Dihydro-1,3-dimethyl-2-[(3′-nitro)-propenylidene]-3-phenyl-1*H*-indole (20). After 8 days compound **20** was obtained in 31% yield. Red solid; mp 152–159 °C. IR (cm^{-1}, film) 1620, 1574, 1491; UV (nm, CH_3OH) (log ε) 208 (4.41), 275 (3.94), 477 (4.15); UV (nm, CH_3CN) (log ε) 194 (5.02), 277 (4.24), 473 (4.55); UV (nm, cyclohexane) (log ε) 197 (4.66), 271 (4.11), 417 (4.43), 434 (4.45); ^1H NMR (δ, ppm, $CDCl_3$) 7.70 (1H, t, H-2′, J=12.9 Hz), 7.46–7.22 (6H, m), 6.94–6.88 (4H, m), 5.41 (1H, d, H-1′, J=12.9 Hz), 3.40 (3H, s, CH_3N), 1.93 (3H, s, CH_3C). ^{13}C NMR (δ, ppm, $CDCl_3$) 169.2 (s, C-2), 143.5 (s), 142.8 (s), 140.0 (s), 138.7 (d), 129.9 (d), 129.0 (2d), 128.1 (d), 127.4 (d), 126.0 (2d), 123.2 (d), 122.6 (d), 107.9 (d, C-7), 89.3 (d, C-1′), 54.5 (s, C-3), 29.7 (q), 27.1 (q); EIMS (*m*/*z*) 306 ($M^{+\bullet}$, 25), 259 (49), 244 (13), 237 (70), 235 (30), 234 (22), 222 (100); HRMS calcd for $C_{19}H_{18}N_2O_2$ 306.1368, found 306.1365.

4.4.9. (1′*E*,2′*E*)-2,3-Dihydro-1,3-dimethyl-3-phenyl-2-[(3′-nitro)but-2-enylidene]-1*H*-indole (21). After 4 days compound **21** was obtained in 42% yield. Red solid; mp 174–176 °C. IR (cm^{-1}, Nujol) 1616, 1597, 1574, 1487; UV (nm, CH_3OH) (log ε) 207 (4.29), 276 (4.28), 483 (4.46); UV (nm, CH_3CN) (log ε) 194 (4.72), 280 (4.07), 477 (4.49); UV (nm, cyclohexane) (log ε) 210 (4.21), 248 (3.77), 421 (3.86), 440 (3.85); ^1H NMR (δ, ppm, $CDCl_3$) 7.82 (1H, d, H-2′, J=13.0 Hz), 7.33–7.20 (5H, m), 6.92–6.86 (4H, m), 5.25 (1H, d, H-1′, J=13.0 Hz), 3.40 (3H, s, CH_3N), 2.12 (3H, s, CH_3CNO_2), 1.93 (3H, s, CH_3C). ^{13}C NMR (δ, ppm, $CDCl_3$) 167.4 (s, C-2), 143.7 (s), 143.0 (s), 139.9 (s), 136.7 (s), 133.6 (d, C-2′), 128.8 (2d), 128.0 (d), 127.2 (d), 126.1 (2d), 123.2 (d), 122.2 (d), 107.5 (d, C-7), 89.9 (d, C-1′), 54.3 (s, C-3), 29.7 (q), 26.9 (q), 12.0 (q); EIMS (*m*/*z*) 320 ($M^{+\bullet}$, 88), 303 (15), 289 (14), 273 (56), 272 (20), 258 (41), 243 (22), 241 (16), 237 (29), 234 (33), 231 (16), 221 (100); HRMS calcd for $C_{20}H_{20}N_2O_2$ 320.1525, found 320.1523.

4.4.10. (1′*E*,2′*E*)-2,3-Dihydro-1,3-dimethyl-3-phenyl-2-[(3′-nitro-3′-phenyl)propenylidene]-1*H*-indole (22). After 15 days compound **22** was obtained in 26% yield. Red solid; mp 179–180 °C. IR (cm^{-1}, Nujol) 1618, 1572, 1491; UV (nm, CH_3OH) (log ε) 209 (4.60), 263 (4.12), 487 (4.35); UV (nm, CH_3CN) (log ε) 193 (4.98), 260 (4.17), 484 (4.40); UV (nm, cyclohexane) (log ε) 210 (4.49), 254 (3.98), 429 (4.15), 443 (4.13); ^1H NMR (δ, ppm, $CDCl_3$) 8.07 (1H, d, H-2′, J=13.2 Hz), 7.66–6.82 (14H, m), 5.20 (1H, d, H-1′, J=13.2 Hz), 3.20 (3H, s, CH_3N), 1.99 (3H, s, CH_3C). ^{13}C NMR (δ, ppm, $CDCl_3$) 168.4 (s, C-2), 143.6 (s), 143.0 (s), 140.1 (s), 135.6 (d, C-2′), 131.4 (s), 130.9 (2d), 129.0 (2d), 128.4 (d), 128.3 (2d), 128.1 (d), 127.4 (d), 126.1 (2d), 123.2 (d), 122.5 (d), 107.7 (d, C-7), 90.4 (d, C-1′), 54.5 (s, C-3), 29.6 (q), 27.1 (q); an aromatic singlet was hidden under other signals; EIMS (*m*/*z*) 382 ($M^{+\bullet}$, 75), 335 (54), 334 (24), 320 (20), 273 (23), 262 (53), 258 (16), 247 (56), 246 (40), 244 (27), 232 (47), 231 (31), 230 (23), 221 (100); HRMS calcd for $C_{25}H_{22}N_2O_2$ 382.1681, found 382.1686.

4.4.11. (1′*E*,2′*E*)-2,3-Dihydro-2-[(3′-(2-methylthiophenyl)-3′-nitro)propenylidene]-1,3,3-trimethyl-1*H*-indole (23). To a solution of the nitroenamine **7** (0.038 g, 0.14 mmol) and 2-methyleneindoline **1** (0.05 g, 0.29 mmol) in CH_2Cl_2 (2 ml), $Zn(OTf)_2$ (0.105 g, 0.29 mmol) was added. The reaction mixture was stirred at room temperature. After 12 days water was added, and the organic phase was dried over Na_2SO_4 anhydrous. After evaporation of the solvent the crude reaction mixture was purified by flash chromatography (light petroleum–ethyl acetate, 85:15) to give compound **23** (0.015 g, 30% yield). Red oil; IR (cm^{-1}, Nujol) 1580; UV (nm, CH_3OH) (log ε) 209 (4.50), 254 (4.08), 281 (3.97), 489 (4.42); UV (nm, CH_3CN) (log ε) 203 (4.46), 209 (4.47), 259 (4.10), 288 (3.99), 489 (4.48); UV (nm, cyclohexane) (log ε) 201 (4.24), 210 (4.41), 259 (4.09), 281 (3.99), 432 (4.35), 451 (4.36); ^1H NMR (δ, ppm, $CDCl_3$), 8.74 (1H, d, H-2′, J=13.5 Hz), 7.45–7.15 (6H, m), 7.04, (1H, t, J=7.7 Hz), 6.77 (1H, d, H-7, J=8.0 Hz), 4.93 (1H, d, H-1′, J=13.5 Hz), 3.06 (3H, s, CH_3N), 2.42 (3H, s, CH_3S), 1.72 (3H, s, *gem*-CH_3), 1.70 (3H, s, *gem*-CH_3); ^{13}C NMR (δ, ppm, $CDCl_3$) 168.8 (s, C-2), 143.5 (s), 140.4 (s), 139.6 (s), 138.0 (s), 136.6 (d), 131.7 (d), 130.3 (s), 129.6 (d), 128.0 (d), 126.0 (d), 125.1 (d), 122.4 (d), 121.8 (d), 107.8 (d, C-7), 90.7 (d, C-1′), 47.5 (s, C-3), 29.6 (q, *C*H_3N), 28.8 (q, *gem*-CH_3), 28.7 (q, *gem*-CH_3), 15.8 (q, SCH_3); EIMS (*m*/*z*) 366 ($M^{+\bullet}$, 100); HRMS calcd for $C_{21}H_{22}N_2O_2S$ 366.1402, found 366.1405.

4.5. Nitroalkylation reactions

4.5.1. General procedure. To a solution of 2-methylenindolines **1–3** (3 mmol) in diethyl ether (7 ml), a solution of nitroolefins **8–10** (3 mmol) in diethyl ether (3.5 ml) was added at −15/−5 °C. The solution was allowed to warm to room temperature and the course of the reaction was monitored by ^1H NMR. After 2–3 days the products obtained **24–32** were purified by flash chromatography (light petroleum–ethyl acetate, 95:5).

4.5.2. (*E*)-2,3-Dihydro-1,3,3-trimethyl-2-[(2-methyl-3-nitro)propylidene]-1*H*-indole (24). 62% Yield; yellow oil; IR (cm^{-1}, film) 1658, 1604, 1547, 1496, 1454; UV (nm, CH_3OH) (log ε) 210 (4.29), 280 (4.13); UV (nm, CH_3CN) (log ε) 195 (4.58), 204 (4.54), 282 (4.26); UV (nm, cyclohexane) (log ε) 194 (4.73), 207 (4.61), 281 (4.42); ^1H NMR (δ, ppm, $CDCl_3$) 7.10 (1H, t, H-6, J=7.7 Hz), 7.04 (1H, d, H-4,

J=7.0 Hz), 6.73 (1H, t, H-5, J=7.3 Hz), 6.47 (1H, d, H-7, J=7.7 Hz), 4.30 (2H, m, CH_2NO_2), 3.96 (1H, d, H-1′, J=11.0 Hz), 3.61 (1H, m, H-2′), 2.95 (3H, s, CH_3N), 1.50 (3H, s, CH_3C), 1.49 (3H, s, CH_3C), 1.17 (3H, d, CH_3CH, J=6.6 Hz); ^{13}C NMR (δ, ppm, $CDCl_3$) 155.0 (s, C-2), 145.5 (s, C-7a), 137.4 (s, C-3a), 127.4 (d, C-6), 121.0 (d, C-4), 118.0 (d, C-5), 104.5 (d, C-7), 93.5 (d, C-1′), 82.3 (t, C-3′), 44.2 (s, C-3), 31.2 (d, C-2′), 28.5 (q, CH$_3$N), 28.0 (q, CH$_3$C), 27.8 (q, CH$_3$C), 20.3 (q, CH$_3$CH); EIMS (*m*/*z*) 260 ($M^{+\bullet}$, 90), 230 (13), 214 (15), 200 (35), 198 (15), 185 (24), 184 (22), 175 (50), 160 (100); HRMS calcd for $C_{15}H_{20}N_2O_2$ 260.1525, found 260.1524.

4.5.3. (*E*) and (*Z*)-2,3-Dihydro-1,3,3-trimethyl-2-[(3-nitro-2-phenyl)propylidene]-1*H*-indole (25a,b). The two compounds (*E*)-**25a** and (*Z*)-**25b** in ratio 9:1, respectively, were inseparable by flash chromatography. 75% Yield; yellow oil; IR (cm^{-1}, film) 1653, 1604, 1550, 1491, 1456; UV (nm, CH_3OH) (log ε) 210 (4.48), 283 (4.47); UV (nm, CH_3CN) (log ε) 193 (5.02), 206 (4.62), 285 (4.54). UV (nm, cyclohexane) (log ε) 197 (4.42), 210 (4.50), 284 (4.38). EIMS (*m*/*z*) 322 ($M^{+\bullet}$, 22), 276 (16), 262 (100); HRMS calcd for $C_{20}H_{22}N_2O_2$ 322.1681, found 322.1682. For clarity sake the NMR values are given separately for each isomer. Compound (*E*)-**25a**: ^{1}H NMR (δ, ppm, $CDCl_3$) 7.41 (5H, m, Ph), 7.09 (1H, t, H-6, J=7.7 Hz), 7.03 (1H, d, H-4, J=7.0 Hz), 6.73 (1H, t, H-5, J=7.3 Hz), 6.47 (1H, d, H-7, J=8.1 Hz), 4.77 (1H, m, CHPh), 4.68 (1H, dd, $CHNO_2$, J_1=7.7 Hz, J_2=11.2 Hz), 4.51 (1H, dd, $CHNO_2$, J_1=7.8 Hz, J_2=11.2 Hz), 4.39 (1H, d, H-1′, J=10.6 Hz), 3.00 (3H, s, CH_3N), 1.59 (3H, s, CH_3C), 1.41 (3H, s, CH_3C). ^{13}C NMR (δ, ppm, $CDCl_3$) 156.2 (s, C-2), 145.8 (s), 141.8 (s), 138.0 (s), 129.2 (2d), 127.7 (d, C-6), 127.4 (d), 127.1 (2d), 121.4 (d, C-4), 118.5 (d, C-5), 105.0 (d, C-7), 90.8 (d, C-1′), 82.3 (t, C-3′), 44.7 (s, C-3), 41.7 (d, CHPh), 29.2 (q, CH_3N), 28.3 (q, CH$_3$C), 28.1 (q, CH$_3$C). Compound (*Z*)-**25b**, only a few signals were identified. ^{1}H NMR (δ, ppm, $CDCl_3$) 6.56 (1H, d, H-7), 4.98 (1H, m, CHPh), 4.65 (1H, dd, $CHNO_2$, J_1=6.2 Hz, J_2=11.3 Hz), 4.50 (1H, m, CHNO$_2$), 4.25 (1H, d, H-1′, J=9.9 Hz), 3.32 (3H, s, CH_3N), 1.31 (3H, s, CH_3), 1.30 (3H, s, CH_3); ^{13}C NMR (δ, ppm, $CDCl_3$) 121.8 (d, C-4), 119.2 (d, C-5), 105.5 (d, C-7), 88.6 (d, C-1′), 41.2 (d, CHPh) 33.0 (q, CH_3N).

4.5.4. (*E*)-2,3-Dihydro-1,3,3-trimethyl-2-[(2-nitrocyclohexyl)methylidene]-1*H*-indole (26a,b). Compounds **26a**,**b** were reported in the literature.[16] Compound **26a**. ^{1}H NMR (δ, ppm, $CDCl_3$) 7.08 (1H, t, H-6), 7.01 (1H, d, H-4), 6.71 (1H, t, H-5), 6.45 (1H, d, H-7), 4.76 (1H, dt, $CHNO_2$, J_1=J_2=4.8 Hz, J_3=9.5 Hz, W_H=16.5 Hz), 4.34 (1H, d, H-1′, J=11.4 Hz), 3.51 (1H, m, CHCHNO$_2$, W_H=21.5 Hz), 2.95 (3H, s, NCH_3), 2.26 (1H, m, annular H), 2.0 (3H, m, annular H), 1.78 (1H, m, annular H), 1.45 (3H, s, CH_3 at C-3), 1.41 (3H, s, CH_3 at C-3), 1.40 (1H, m, annular H), 1.20 (1H, m, annular H). Compound **26b**. ^{1}H NMR (δ, ppm, $CDCl_3$) 7.07 (1H, t, H-6), 7.01 (1H, d, H-4), 6.70 (1H, t, H-5), 6.43 (1H, d, H-7), 4.25 (1H, ddd, $CHNO_2$, J_1=11.7 Hz, J_2=10.6 Hz, J_3=3.7 Hz, W_H=27.5 Hz), 4.05 (1H, d, H-1′, J=10.6 Hz), 3.05 (1H, dq, CHCHNO$_2$, J_1=J_2=J_3=10.6 Hz, J_4=10.6 Hz, J_3=3.7 Hz), 2.92 (3H, s, NCH_3), 2.26 (1H, m, annular H), 2.01 (1H, dq, annular H), 1.96 (3H, m, annular H), 1.76 (2H, m, annular H), 1.50 (1H, m, annular H), 1.46 (4H, s+m, CH_3 at C-3, annular H), 1.42 (3H, s, CH_3 at C-3).

4.5.5. (*E*)-3-Ethyl-2,3-dihydro-1,3-dimethyl-2-[(2-methyl-3-nitro)propylidene]-1*H*-indole (27a,b). The isomers **27a** and **27b** (80% yield) were obtained in 9:1 ratio (determined by HRGC) and were not separable by flash chromatography. Yellow oil; IR (cm^{-1}, film) 1655, 1606, 1551, 1496, 1460; UV (nm, CH_3OH) (log ε) 211 (4.34), 282 (4.37); UV (nm, CH_3CN) (log ε) 192 (4.53), 204 (4.46), 282 (4.30). UV (nm, cyclohexane) (log ε) 216 (4.00), 280 (4.30); EIMS (*m*/*z*) 274 ($M^{+\bullet}$, 56), 245 (13), 228 (21), 214 (40), 198 (80), 184 (33), 183 (21), 182 (16), 174 (100); HRMS calcd for $C_{16}H_{22}N_2O_2$ 274.1681, found 274.1683; HRGC (OV1701) t_R=45.00 min for **27b**; t_R=45.44 min for **27a** (10 min at 100 °C, 3 °C/min up to 200 °C). For clarity sake the NMR values are given separately for each isomer. Compound **27a**: ^{1}H NMR (δ, ppm, $CDCl_3$) 7.10 (1H, t, H-6, J=7.5 Hz), 6.99 (1H, d, H-4, J=7.3 Hz), 6.73 (1H, t, H-5, J=7.3 Hz), 6.46 (1H, d, H-7, J=7.5 Hz), 4.30 (2H, m, CH_2NO_2), 4.02 (1H, d, H-1′, J=11.0 Hz), 3.57 (1H, m, H-2′), 2.94 (3H, s, CH_3N), 1.92 (1H, m, HCHCH$_3$), 1.80 (1H, m, HCHCH$_3$), 1.50 (3H, s, CH_3C), 1.16 (3H, d, CH_3CH, J=6.6 Hz), 0.56 (3H, t, CH_3CH$_2$, J=7.3 Hz); ^{13}C NMR (δ, ppm, $CDCl_3$) 152.9 (s, C-2), 146.7 (s, C-7a), 135.3 (s, C-3a), 127.5 (d, C-6), 121.3 (d, C-4), 118.1 (d, C-5), 104.4 (d, C-7), 93.8 (d, C-1′), 82.7 (t, CH$_2$NO$_2$), 49.4 (s, C-3), 33.8 (t, CH$_2$CH$_3$), 31.2 (d, C-2′), 28.8 (q, CH$_3$N), 27.4 (q, CH$_3$C), 20.4 (q, CH$_3$CH), 9.3 (q, CH$_3$CH$_2$). Compound **27b**: ^{1}H NMR (δ, ppm, $CDCl_3$) 6.78 (1H, t, H-5, J=7.7 Hz), 6.54 (1H, d, H-7, J=7.7 Hz), 4.03 (1H, d, H-1′, J=10.6 Hz), 1.16 (3H, d, CH_3CH, J=6.2 Hz), 0.44 (3H, t, CH_3CH_2, J=7.3 Hz); ^{13}C NMR (δ, ppm, $CDCl_3$) 153.4 (s, C-2), 148.2 (s, C-7a), 121.9 (d, C-4), 118.8 (d, C-5), 105.2 (d, C-7), 94.0 (d, C-1′), 82.5 (t, CH$_2$NO$_2$), 34.0 (t, CH$_2$CH$_3$), 31.4 (d, C-2′), 20.8 (q, CH$_3$CH), 8.7 (q, CH$_3$CH$_2$).

4.5.6. (*E*)-3-Ethyl-2,3-dihydro-1,3-dimethyl-2-[(3-nitro-2-phenyl)propylidene]-1*H*-indole (28a,b). The isomers **28a** and **28b** (94% yield) were obtained in 3:2 ratio and were not separable by flash chromatography. Yellow solid; mp 73–81 °C; IR (cm^{-1}, Nujol) 1650, 1605, 1550, 1495; UV (nm, CH_3OH) (log ε) 211 (4.48), 285 (4.48); UV (nm, CH_3CN) (log ε) 192 (4.96), 208 (4.60), 288 (4.50). UV (nm, cyclohexane) (log ε) 216 (4.27), 284 (4.57); EIMS (*m*/*z*) 336 ($M^{+\bullet}$, 33), 276 (45), 260 (26), 247 (31), 246 (17), 232 (13), 202 (48), 174 (100); HRMS calcd for $C_{21}H_{24}N_2O_2$ 336.1838, found 336.1833. For clarity sake the NMR values are given separately for each isomer. Compound **28a**: ^{1}H NMR (δ, ppm, $CDCl_3$) 7.33 (5H, m, Ph), 7.09 (1H, t, H-6, J=7.7 Hz), 6.98 (1H, d, H-4, J=7.0 Hz), 6.73 (1H, t, H-5, J=7.3 Hz), 6.46 (1H, d, H-7, J=8.0 Hz), 4.74–4.61 (2H, m, CHPh+$CHNO_2$), 4.54–4.46 (2H, m, $CHNO_2$+H-1′), 3.00 (3H, s, CH_3N), 1.92 (1H, m, HCHCH$_3$), 1.80 (1H, m, HCHCH$_3$), 1.58 (3H, s, CH_3C), 0.25 (3H, t, CH_3CH$_2$, J=7.3 Hz); ^{13}C NMR (δ, ppm, $CDCl_3$) 153.8 (s, C-2), 146.6 (s), 141.1 (s), 135.4 (s), 128.9 (d), 127.6 (d, C-6), 127.1 (3d, Ph), 121.3 (d, C-4), 118.4 (d, C-5), 104.6 (d, C-7), 91.1 (d, C-1′), 82.3 (t, C-3′), 49.6 (s, C-3), 41.5 (d, CHPh), 33.9 (t, CH$_2$CH$_3$), 28.9 (q, CH_3N), 27.5 (q, CH$_3$C), 8.9 (q, CH$_3$CH$_2$). Compound **28b**: ^{1}H NMR (δ, ppm, $CDCl_3$) 6.78 (1H, t, H-5, J=7.3 Hz), 6.52 (1H, d, H-7, J=8.0 Hz), 3.01 (3H, s, CH_3N), 1.38 (3H, s, CH_3C), 0.55 (3H, t, CH_3CH$_2$, J=7.3 Hz). ^{13}C NMR (δ, ppm, $CDCl_3$) 153.9 (s, C-2), 141.4 (s, C-3a), 91.0 (d, C-1′), 49.6 (s, C-3), 9.3 (q, CH$_3$CH$_2$).

4.5.7. (*E*)-3-Ethyl-2,3-dihydro-1,3-dimethyl-2-[(2-nitrocyclohexyl)methylidene]-1*H*-indole (29a,b). In the crude reaction mixture the isomers *cis*-**29a** and *trans*-**29b** were detected in 3:1 ratio. After purification on flash chromatography fractions of different composition were obtained (45% yield). Yellow solid; mp 110–115 °C (for 1:9 ratio of *cis*-**29a** and *trans*-**29b**, respectively); IR (cm^{-1}, Nujol) 1658, 1606, 1550, 1496; UV (nm, CH_3OH) (log ε) 210 (4.39), 282 (4.41); UV (nm, CH_3CN) (log ε) 192 (4.82), 204 (4.55), 284 (4.48); UV (nm, cyclohexane) (log ε) 216 (4.20), 284 (4.51). EIMS (*m*/*z*) 314 ($M^{+\bullet}$, 100); HRMS calcd for $C_{19}H_{26}N_2O_2$ 314.1994, found 314.1995. For clarity sake the NMR values are given separately for each isomer. Compound **29a**: ^1H NMR (δ, ppm, $CDCl_3$) 7.07 (1H, t, H-6, J=7.7 Hz), 6.94 (1H, d, H-4, J=6.2 Hz), 6.70 (1H, t, H-5, J=7.1 Hz), 6.43 (1H, d, H-7, J=7.7 Hz), 4.62 (1H, dt, $CHNO_2$, J_1=J_2=4.3 Hz, J_3=8.8 Hz, W_H=16 Hz), 4.39 (1H, d, H-1′, J=11.0 Hz), 3.42 (1H, m, C*H*$CHNO_2$, W_H=24 Hz), 2.93 (3H, s, CH_3N), 2.23 (1H,m), 1.86 (3H, m), 1.78 (2H, m, CH_2CH_3), 1.65 (2H, m), 1.45 (2H, m), 1.40 (3H, s, CH_3C), 0.53 (3H, t, CH_3CH_2, J=7.1 Hz). ^{13}C NMR (δ, ppm, $CDCl_3$) 153.1 (s, C-2), 146.7 (s, C-7a), 135.4 (s, C-3a), 127.5 (d, C-6), 121.2 (d, C-4), 117.9 (d, C-5), 104.3 (d, C-7), 89.6 (d, C-1′), 87.6 (d, $CHNO_2$), 49.3 (s, C-3), 35.8 (d, *C*H$CHNO_2$), 34.0 (t, *C*H_2CH_3), 31.8 (t, CH_2), 28.9 (q, CH_3N), 27.8 (q, *C*H_3C), 26.0 (t, CH_2), 22.5 (t, CH_2), 21.3 (t, CH_2), 9.4 (q, *C*H_3CH_2). Compound **29b**: ^1H NMR (δ, ppm, $CDCl_3$) 7.06 (1H, t, H-6, J=7.1 Hz), 6.93 (1H, d, H-4, J=7.3 Hz), 6.68 (1H, t, H-5, J=7.3 Hz), 6.41 (1H, d, H-7, J=8.0 Hz), 4.22 (1H, m, $CHNO_2$, W_H=26 Hz), 4.09 (1H, d, H-1′, J=10.6 Hz), 3.01 (1H, dq, C*H*$CHNO_2$, J_1=J_2=J_3=10.8 Hz, J_4=3.8 Hz), 2.90 (3H, s, CH_3N), 2.23 (1H, m), 1.95 (1H, m), 1.87 (2H, m), 1.77 (2H, m, CH_2CH_3), 1.75 (1H, m), 1.42 (3H, s, CH_3C), 1.35 (2H, m), 1.22 (1H, m), 0.53 (3H, t, CH_3CH_2, J=7.3 Hz); ^{13}C NMR (δ, ppm, $CDCl_3$) 152.9 (s, C-2), 146.7 (s, C-7a), 135.4 (s, C-3a), 127.4 (d, C-6), 121.2 (d, C-4), 117.9 (d, C-5), 104.3 (d, C-7), 94.3 (d, C-1′), 92.3 (d, $CHNO_2$), 49.4 (s, C-3), 40.0 (d, *C*H$CHNO_2$), 34.3 (t, *C*H_2CH_3), 34.2 (t, CH_2), 31.0 (t, CH_2), 28.8 (q, CH_3N), 27.7 (q, *C*H_3C), 24.8 (t, CH_2), 24.0 (t, CH_2), 9.4 (q, *C*H_3CH_2).

4.5.8. (*E*)-2,3-Dihydro-1,3-dimethyl-2-[(2-methyl-3-nitro)propylidene]-3-phenyl-1*H*-indole (30a,b). The isomers **30a** and **30b** (30% yield) inseparable by flash chromatography, were obtained in 3:1 ratio, respectively (determined by HRGC). Yellow oil; IR (cm^{-1}, film) 1650, 1600, 1550, 1495, 1460; UV (nm, CH_3OH) (log ε) 208 (4.24), 276 (4.17); UV (nm, CH_3CN) (log ε) 194 (4.73), 207 (4.56), 281 (4.07); UV (nm, cyclohexane) (log ε) 195 (4.62), 207 (4.52), 280 (4.18); EIMS (*m*/*z*) 322 ($M^{+\bullet}$, 12), 276 (15), 262 (37), 246 (15), 238 (52), 236 (23), 222 (100); HRMS calcd for $C_{20}H_{22}N_2O_2$ 322.1681, found 322.1680; HRGC (OV1701) t_R=39.97 min for **30a**, t_R=40.97 min for **30b** (200 °C isotherm). For clarity sake the ^1H NMR values are given separately for each isomer. Compound **30a**: ^1H NMR (δ, ppm, $CDCl_3$) 7.32–7.23 (4H, m, Ph), 7.19–7.15 (1H, m, Ph), 7.08 (1H, t, H-6, J=7.5 Hz), 6.71 (1H, d, H-4, J=7.0 Hz), 6.62 (1H, t, H-5, J=7.0 Hz), 6.55 (1H, d, H-7, J=7.3 Hz), 4.19 (2H, AB part of an ABX system, CH_2NO_2, J_{AB}=7.3 Hz), 3.99 (1H, d, H-1′, J=10.6 Hz), 3.07 (3H, s, CH_3N), 2.95 (1H, m, C*H*CH_3, J=7.0 Hz), 1.81 (3H, s, CH_3C), 0.50 (3H, d, CH_3CH, J=6.2 Hz). Compound **30b**, only some signals were identified. ^1H NMR (δ, ppm, $CDCl_3$) 3.95 (1H, d, H-1′, J=10.6 Hz), 3.64 (1H, dd, C*H*NO_2, J_1=9.1 Hz, J_2=11.3 Hz), 3.36 (1H, dd, C*H*NO_2, J_1=4.6 Hz, J_2=11.6 Hz), 3.24 (3H, s, CH_3N), 1.79 (3H, s, CH_3C), 1.04 (3H, d, CH_3CH, J=6.6 Hz); ^{13}C NMR (δ, ppm, $CDCl_3$) 156.4 (s, C-2), 145.6 (s), 145.5 (s), 138.3 (s), 134.8 (s), 128.5 (d), 128.4 (d), 128.2 (d), 128.1 (d), 127.7 (d), 127.2 (d), 126.6 (d), 126.3 (d), 122.8 (d), 122.7 (d), for **30a**: 118.6 (d, C-5), 104.9 (d, C-7), 94.0 (d, C-1′), 82.6 (t, C-3′), 51.9 (s, C-3), 31.7 (d, C-2′), 29.0 (q, CH_3N), 25.9 (q, *C*H_3C), 18.7 (q, *C*H_3CH); for **30b**: 118.7 (d, C-5), 105.0 (d, C-7), 93.5 (d, C-1′), 80.6 (t, C-3′), 52.1 (s, C-3), 30.6 (d, C-2′), 26.5 (q), 25.4 (q), 23.7 (q).

4.5.9. (*E*)-2,3-Dihydro-1,3-dimethyl-2-[(3-nitro-2-phenyl)propylidene]-3-phenyl-1*H*-indole (31a,b). The isomers **31a** and **31b** (13% yield) inseparable by flash chromatography, were obtained in 1:1 ratio. Orange oil; IR (cm^{-1}, film) 1651, 1606, 1552, 1491, 1458; UV (nm, CH_3OH) (log ε) 209 (4.50), 285 (4.15); UV (nm, CH_3CN) (log ε) 193 (4.93), 206 (4.66), 286 (4.23); UV (nm, cyclohexane) (log ε) 196 (4.69), 205 (4.63), 285 (4.32); EIMS (*m*/*z*) 384 ($M^{+\bullet}$, 31), 338 (18), 324 (91), 310 (32), 308 (19), 246 (13), 231 (22), 230 (18), 223 (100); HRMS calcd for $C_{25}H_{24}N_2O_2$ 384.1838, found 384.1838; ^1H NMR (δ, ppm, $CDCl_3$) 7.35–7.06 (10H, m, Ph), 6.70–6.40 (4H, m, Ar), 4.56 (0.5H, dd, C*H*NO_2, J_1=7.10 Hz, J_2=11.2 Hz, for **31a**), 4.48 (0.5H, m, C*H*NO_2 for **31a**), 4.40 and 4.36 (1H, d, H-1′, J=11.0 Hz), 4.14 (0.5H, dd, C*H*NO_2, J_1=8.8 Hz, J_2=11.3 Hz, for **31b**), 4.11 (1H, m, C*H*Ph, for **31a** and **31b**), 3.60 (0.5H, dd, C*H*NO_2, J_1=4.9 Hz, J_2=11.2 Hz, for **31b**), 3.10 (3H, s, CH_3N for **31a** and **31b**), 1.91 and 1.78 (3H, s, CH_3C); ^{13}C NMR (δ, ppm, $CDCl_3$) 156.9 and 156.7 (s, C-2), 144.9 (2s), 141.2 (s), 139.9 (s), 138.4 (2s), 128.8 (d), 128.6 (d), 128.4 (d), 128.3 (d), 127.8 (d), 127.7 (d), 127.1 (2d), 126.7 (2d), 126.6 (2d), 126.4 (d), 122.8 (2d) (d, C-4), 119.0 and 118.9 (d, C-5), 105.1 and 105.2 (d, C-7), 91.7 and 91.3 (d, C-1′), 81.7 and 79.9 (t, C-3′), 52.2 and 52.1 (s, C-3), 42.1 and 41.0 (d, CHPh), 29.1 (2q, CH_3N for **31a** and **31b**), 26.0 and 25.4 (q, *C*H_3C).

4.5.10. 2,3-Dihydro-1,3-dimethyl-2-[(2-nitrocyclohexyl)methylidene]-3-phenyl-1*H*-indole (32a,b,c). ^1H NMR analysis of the crude reaction mixture indicated the presence of three isomers, *cis*-**32a**, *trans*-**32b** and *cis*-**32c** in 60%, 25% and 15%, respectively. After purification on flash chromatography fractions of different composition in *cis*-**32a**, *trans*-**32b** and *trans*-**32d** were isolated in only 6% yield. Oil; IR (cm^{-1}, film) 1660, 1601, 1551, 1507; UV (nm, CH_3OH) (log ε) 211 (4.34), 280 (4.09); UV (nm, CH_3CN) (log ε) 196 (4.36), 212 (4.22), 281 (4.01); UV (nm, cyclohexane) (log ε) 201 (4.27), 212 (4.32), 279 (4.08); EIMS (*m*/*z*) 362 ($M^{+\bullet}$, 28), 316 (10), 237 (15), 234 (14), 222 (100); HRMS calcd for $C_{23}H_{26}N_2O_2$ 362.1994, found 362.1990. For clarity sake the NMR values are given separately for each isomer. Compound *cis*-**32c**: ^1H NMR (δ, ppm, $CDCl_3$) 7.28–7.16 (5H, m, Ph), 7.08 (1H, t, Ar, J=7.4 Hz), 6.67–6.53 (3H, m, Ar), 4.48 (1H, dt, $CHNO_2$, J_1=J_2=4.2 Hz, J_3=8.6 Hz, W_H=18.4 Hz), 4.33 (1H, d, H-1′, J=11.2 Hz), 3.07 (3H, s, CH_3N), 2.78 (1H, m), 2.12 (1H, m), 1.9–0.5 (7H, m), 1.72 (3H, s, CH_3C); ^{13}C NMR (δ, ppm, $CDCl_3$) 156.6 (s, C-2), 146.3 (s), 145.8 (s), 138.6 (s), 128.1 (d), 127.5 (d), 126.8 (d), 126.2 (d), 122.7 (d, C-4), 118.4 (d, C-5), 104.9 (d,

C-7), 89.9 (d, C-1′), 87.7 (d, $CHNO_2$), 51.8 (s, C-3), 36.3 (d, *C*HCHNO$_2$), 29.7 (t, CH_2), 29.5 (t, CH_2), 29.2 (q, CH_3N), 26.4 (t, CH_2), 26.2 (q, *C*H$_3$C), 22.4 (t, CH_2). Compound *trans*-**32b**: ^{1}H NMR (δ, ppm, $CDCl_3$) 4.10 (1H, dt, C*H*NO$_2$, J_1=J_2 11.2 Hz, J_3=3.5 Hz, W_H=30 Hz), 4.01 (1H, d, H-1′, J=10.6 Hz), 3.03 (3H, s, CH_3N), 2.49 (1H, m), 2.12 (1H, m), 1.9–0.5 (7H, m), 1.74 (3H, s, CH_3C); ^{13}C NMR (δ, ppm, $CDCl_3$) 156.6 (s, C-2), 146.3 (s), 145.9 (s), 139.1 (s), 128.0 (d), 127.6 (d), 127.4 (d), 126.2 (d), 122.7 (d, C-4), 118.4 (d, C-5), 104.8 (d, C-7), 94.2 (d, C-1′), 92.0 (d, $CHNO_2$), 51.9 (s, C-3), 40.2 (d, *C*HCHNO$_2$), 32.7 (t, CH_2), 31.0 (t, CH_2), 29.1 (q, CH_3N), 25.7 (q, *C*H$_3$C), 24.6 (t, CH_2), 24.1 (t, CH_2). Compound *cis*-**32c**: ^{1}H NMR (δ, ppm, $CDCl_3$) 4.48 (1H, m, C*H*NO$_2$), 4.21 (1H, d, H-1′, J=11.0 Hz), 3.05 (3H, s, CH_3). Compound *trans*-**32d**: ^{1}H NMR (δ, ppm, $CDCl_3$) 4.24 (1H, d, H-1′, J=10.2 Hz), 3.94 (1H, dt, C*H*NO$_2$, J_1=J_2=10.0 Hz, J_3=3.5 Hz, W_H=29.0 Hz), 3.07 (3H, s, CH_3), 2.49 (1H, m), 1.9–0.8 (8H, m), 1.78 (3H, s, CH_3C); ^{13}C NMR (δ, ppm, $CDCl_3$) 155.9 (s, C-2), 145.7 (s), 145.2 (s), 138.6 (s), 128.4 (d), 127.6 (d), 126.3 (d), 126.1 (d), 122.6 (d, C-4), 118.4 (d, C-5), 104.9 (d, C-7), 95.2 (d, C-1′), 90.5 (d, $CHNO_2$), 51.7 (s, C-3), 39.3 (d, *C*HCHNO$_2$), 34.6 (t, CH_2), 29.9 (t), 29.2 (q, CH_3N), 26.3 (q, *C*H$_3$C), 24.1 (t, CH_2), 23.7 (t, CH_2).

4.6. Reactions of 2-methyleneindolines (1–3) with 1,2-diaza-1,3-butadienes (11–13)

4.6.1. General procedure. To a stirred solution of the appropriate 1,2-diaza-1,3-butadienes **11**–**13**[32] (0.85 mmol) in THF (5 ml) the substrates **1**–**3** (0.94 mmol) was added. The mixture was allowed to stand at room temperature for 2 h and then the solvent was evaporated under reduced pressure. The resulting products **33**–**40** were isolated by chromatography on silica gel column with cyclohexane–ethyl acetate (90:10 v/v) and then purified by crystallization from diethyl ether.

4.6.2. 2,2′,3,3′-Tetrahydro-1,3,3,5′-tetramethyl-4′-methoxycarbonyl-1′-ureidospiro[1*H*-indole-2,2′-pyrrole] (33). Yield 88%; pale pink solid; mp 203–206 °C; IR (cm^{-1}, Nujol) 3465, 3281, 3201, 1693, 1661, 1486, 1321, 1143, 892, 747. ^{1}H NMR (δ, ppm, $CDCl_3$) 7.09 (1H, t, J=7.6 Hz, H-6), 6.97 (1H, d, J=7.6 Hz, H-4), 6.73 (1H, t, J=7.6 Hz, H-5), 6.34 (1H, d, J=7.6 Hz, H-7), 5.24 (1H, s, NH), 4.79 (2H, br s, NH_2), 3.75 (3H, s, OCH_3), 3.03 (1H, dq, H-3′, J_1=16.5 Hz, J_2=2.2 Hz), 2.76 (1H, br d, H-3′, J=16.5 Hz), 2.68 (3H, s, NCH_3), 2.23 (3H, br s, CH_3 at C-3′), 1.40 (3H, s, CH_3 at C-3), 1.21 (3H, s, CH_3 at C-3); ^{13}C NMR (δ, ppm, $CDCl_3$) 166.0 (s), 158.9 (s), 157.1 (s), 147.6 (s), 136.3 (s), 128.0 (d, C-6), 120.9 (d, C-4), 119.0 (d, C-5), 103.6 (d, C-7), 99.2 (s), 93.4 (s, C-2), 50.9 (q, OCH_3), 45.1 (s, C-3), 31.7 (t, CH_2), 29.7 (q, NCH_3), 28.6 (q, CH_3 at C-3), 20.2 (q, at C-3), 10.8 (q, CH_3 at C-5′); EIMS (*m*/*z*) 344 (M$^{+\bullet}$, 65), 285 (100); Anal. Calcd for $C_{18}H_{24}N_4O_3$: C, 62.77; H, 7.02; N, 16.27. Found: C, 62.54; H, 7.13; N, 16.30. The isomers **33a** and **33b** were obtained in 60:40 ratio by using DMSO-d_6 as a solvent (determined by ^{1}H NMR). For clarity sake the NMR values are given separately for each isomer. Major component **a**: ^{1}H NMR (δ, ppm, DMSO-d_6) 6.96 (1H, t, H-6, J=7.6 Hz), 6.94 (1H, s, NH), 6.87 (1H, d, H-4, J=7.6 Hz), 6.55 (1H, t, H-5, J=7.6 Hz), 6.34 (1H, d, H-7, J=7.6 Hz), 5.72 (2H, br s, NH_2), 3.59 (3H, s, OCH_3), 2.97 (1H, br d, H-3′, J=16.0 Hz), 2.60 (3H, s, NCH_3), 2.54 (1H, br d, H-3′, J=16.0 Hz), 2.06 (3H, br s, CH_3 at C-5′), 1.34 (3H, s, CH_3 at C-3), 1.06 (3H, s, CH_3 at C-3); ^{13}C NMR (δ, ppm, DMSO-d_6) 165.4 (s), 160.1 (s), 156.4 (s), 148.0 (s), 136.8 (s), 126.8 (d, C-5), 120.0 (d, C-4), 117.3 (d, C-6), 103.9 (d, C-7), 97.6 (s), 89.5 (s, C-2), 50.1 (q, OCH_3), 44.9 (s, C-3), 31.4 (t, CH_2), 28.4 (q, NCH_3), 28.1 (q, CH_3 at C-3), 18.7 (q, at C-3), 10.5 (q, CH_3 at C-5′); minor component **b**: 7.22 (1H, s, NH), 6.93 (1H, d, H-4, J=7.6 Hz), 6.91 (1H, t, H-6, J=7.6 Hz), 6.52 (1H, t, H-5, J=7.6 Hz), 6.16 (1H, d, H-7, J=7.6 Hz), 5.36 (2H, br s, NH_2), 3.56 (3H, s, OCH_3), 2.94 (1H, br d, H-3′, J=16.0 Hz), 2.72 (3H, s, NCH_3), 2.70 (1H, br d, H-3′, J=16.0 Hz), 1.99 (3H, s, CH_3 at C-5′), 1.21 (3H, s, CH_3 at C-3), 1.04 (3H, s, CH_3 at C-3); ^{13}C NMR (δ, ppm, DMSO-d_6) 165.7 (s), 159.8 (s), 157.2 (br s), 148.2 (s), 135.9 (s), 127.0 (d, C-6), 120.1 (d, C-4), 116.5 (d, C-5), 103.3 (d, C-7), 96.7 (s), 93.9 (s, C-2), 49.8 (q, OCH_3), 45.9 (s, C-3), 29.2 (t, CH_2), 28.6 (q, NCH_3), 28.0 (q, CH_3 at C-3), 19.3 (q, at C-3), 11.4 (q, CH_3 at C-5′).

4.6.3. 2,2′,3,3′-Tetrahydro-1,3,3,5′-tetramethyl-4′-methoxycarbonyl-1′ methoxycarbonylaminospiro[1*H*-indole-2,2′-pyrrole] (34). Yield 93%; pink solid; mp 182–185 °C; IR (cm^{-1}, Nujol) 3280, 1753, 1645, 1604, 1463, 1388, 1197, 1135, 999, 893, 749; EIMS (*m*/*z*) 359 (M$^{+\bullet}$, 82), 285 (100); Anal. Calcd for $C_{19}H_{25}N_3O_4$: C, 63.49; H, 7.01; N, 11.69. Found: C, 63.26; H, 7.18; N, 11.53. The isomers **34a** and **34b** were obtained in 55:45 ratio by using DMSO-d_6 as a solvent (determined by ^{1}H NMR). For clarity sake the NMR values are given separately for each isomer. Major component **a**: ^{1}H NMR (δ, ppm, DMSO-d_6) 8.24 (1H, s, NH), 6.95 (1H, t, H-6, J=7.6 Hz), 6.86 (1H, d, H-4, J=7.6 Hz), 6.54 (1H, t, H-5, J=7.6 Hz), 6.33 (1H, d, H-7, J=7.6 Hz), 3.59 (3H, s, OCH_3), 3.32 (3H, s, OCH_3), 2.98 (1H, br d, J=16.4 Hz, H-3′), 2.64 (3H, s, NCH_3), 2.59 (1H, br d, J=16.4 Hz, H-3′), 2.02 (3H, br s, CH_3 at C-5′), 1.30 (3H, s, CH_3 at C-3), 1.05 (3H, s, CH_3 at C-3); ^{13}C NMR (δ, ppm, DMSO-d_6) 165.4 (s), 158.9 (s), 155.6 (s), 147.8 (s), 136.5 (s), 126.9 (d, C-5), 120.0 (d, C-4), 117.2 (d, C-6), 103.9 (d, C-7), 97.8 (s), 90.8 (s, C-2), 51.8 (q, OCH_3), 50.3 (q, OCH_3), 45.2 (s, C-3), 30.9 (t, CH_2), 28.1 (q, NCH_3), 28.0 (q, CH_3 at C-3), 18.2 (q, at C-3), 10.4 (q, CH_3 at C-5′); minor component **b**: ^{1}H NMR (δ, ppm, DMSO-d_6) 8.55 (1H, s, NH), 6.91 (1H, d, H-4, J=7.6 Hz), 6.90 (1H, t, H-6, J=7.6 Hz), 6.51 (1H, t, H-5, J=7.6 Hz), 6.14 (1H, d, H-7, J=7.6 Hz), 3.57 (3H, s, OCH_3), 3.39 (3H, s, OCH_3), 2.95 (1H, br d, H-3′, J=16.4 Hz), 2.71 (3H, s, NCH_3), 2.69 (1H, br d, H-3′, J=16.4 Hz), 1.98 (3H, s, CH_3 at C-5′), 1.24 (3H, s, CH_3 at C-3), 1.03 (3H, s, CH_3 at C-3); ^{13}C NMR (δ, ppm, DMSO-d_6) 165.6 (s), 158.6 (s), 156.0 (br s), 148.2 (s), 136.2 (s), 126.8 (d, C-6), 120.1 (d, C-4), 116.7 (d, C-5), 102.9 (d, C-7), 96.9 (s), 94.1 (s, C-2), 51.8 (q, OCH_3), 50.0 (q, OCH_3), 45.8 (s, C-3), 29.3 (t, CH_2), 28.5 (q, NCH_3), 27.9 (q, CH_3 at C-3), 18.8 (q, at C-3), 11.0 (q, CH_3 at C-5′).

4.6.4. 2,2′,3,3′-Tetrahydro-1,3,3,5′-tetramethyl-4′-methoxycarbonyl-1′-*tert*-butoxycarbonylaminospiro[2*H*-indole-2,2′-pyrrole] (35). Yield 85%; pale pink solid; mp 164–167 °C; IR (cm^{-1}, Nujol) 3253, 1744, 1643, 1605, 1453, 1377, 1133, 989, 892, 741; EIMS (*m*/*z*) 401 (M$^{+\bullet}$, 73), 301 (31), 285 (100); Anal. Calcd for $C_{22}H_{31}N_3O_4$: C, 65.81; H, 7.78; N, 10.47. Found: C, 65.97; H, 7.57; N,

10.32. The isomers **35a** and **35b** were obtained in 50:50 ratio by using DMSO-d_6 as a solvent (determined by ^{1}H NMR). For clarity sake the NMR values are given separately for each isomer. Component **a**: ^{1}H NMR (δ, ppm, DMSO-d_6) 7.80 (1H, s, NH), 6.96 (1H, t, H-6, J=7.6 Hz), 6.85 (1H, d, H-4, J=7.6 Hz), 6.51 (1H, t, H-5, J=7.6 Hz), 6.34 (1H, d, H-7, J=7.6 Hz), 3.58 (3H, s, OCH_3), 2.96 (1H, br d, H-3′, J=16.4 Hz), 2.63 (3H, s, NCH_3), 2.58 (1H, br d, H-3′, J=16.4 Hz), 2.01 (3H, br s, CH_3 at C-5′), 1.32 (3H, s, CH_3 at C-3), 1.19 (9H, s, $C(CH_3)_3$), 1.04 (3H, s, CH_3 at C-3); ^{13}C NMR (δ, ppm, DMSO-d_6) 165.4 (s), 159.1 (s), 154.4 (s), 148.2 (s), 136.5 (s), 126.8 (d, C-5), 119.8 (d, C-4), 117.2 (d, C-6), 103.9 (d, C-7), 97.9 (s), 90.5 (s, C-2), 79.1 (s, $C(CH_3)_3$), 50.1 (q, OCH_3), 45.8 (s, C-3), 30.8 (t, CH_2), 28.2 (q, NCH_3), 28.0 (q, CH_3 at C-3), 27.9 (q, $C(CH_3)_3$), 18.6 (q, at C-3), 10.5 (q, CH_3 at C-5′); component **b**: ^{1}H NMR (δ, ppm, DMSO-d_6) 8.29 (1H, s, NH), 6.90 (1H, d, H-4, J=7.6 Hz), 6.89 (1H, t, H-6, J=7.6 Hz), 6.51 (1H, t, H-5, J=7.6 Hz), 6.13 (1H, d, H-7, J=7.6 Hz), 3.56 (3H, s, OCH_3), 2.93 (1H, br d, H-3′, J=16.4 Hz), 2.71 (3H, s, NCH_3), 2.68 (1H, br d, H-3′, J=16.4 Hz), 1.96 (3H, s, CH_3 at C-5′), 1.22 (3H, s, CH_3 at C-3), 1.19 (9H, s, $C(CH_3)_3$), 1.02 (3H, s, CH_3 at C-3); ^{13}C NMR (δ, ppm, DMSO-d_6) 165.6 (s), 158.8 (s), 154.0 (s), 147.8 (s), 136.2 (s), 126.7 (d, C-6), 119.9 (d, C-4), 116.4 (d, C-5), 103.1 (d, C-7), 96.7 (s), 93.6 (s, C-2), 79.2 (s, $C(CH_3)_3$), 49.8 (q, OCH_3), 45.2 (s, C-3), 29.4 (t, CH_2), 28.4 (q, NCH_3), 27.9 (q, CH_3 at C-3), 27.8 (q, $C(CH_3)_3$), 18.1 (q, at C-3), 11.0 (q, CH_3 at C-5′).

4.6.5. 2,2′,3,3′-Tetrahydro-3-ethyl-1,3,5′-trimethyl-4′-methoxycarbonyl-1′-ureidospiro[2*H*-indole-2,2′-pyrrole] (36). Yield 87%; white solid; mp 171–174 °C; IR (cm^{-1}, Nujol) 3431, 3340, 3284, 1673, 1621, 1463, 1446, 1333, 1145, 887, 743; EIMS (*m*/*z*) 358 ($M^{+\bullet}$, 58), 299 (100), 262 (100); Anal. Calcd for $C_{19}H_{26}N_4O_3$: C, 63.67; H, 7.31; N, 15.63. Found: C, 63.74; H, 7.19; N, 15.79. The isomers **36a** and **36b** were obtained in 60:40 ratio by using DMSO-d_6 as a solvent (determined by ^{1}H NMR). For clarity sake the NMR values are given separately for each isomer. Major component **a**: ^{1}H NMR (δ, ppm, DMSO-d_6) 6.97 (1H, t, H-6, J=7.6 Hz), 6.84 (1H, s, NH), 6.80 (1H, d, H-4, J=7.6 Hz), 6.54 (1H, t, H-5, J=7.6 Hz), 6.33 (1H, d, H-7, J=7.6 Hz), 5.63 (2H, br s, NH_2), 3.59 (3H, s, OCH_3), 2.97 (1H, br d, H-3′, J=16.0 Hz), 2.59 (1H, br d, H-3′, J=16.0 Hz), 2.58 (3H, s, NCH_3), 2.05 (3H, br s, CH_3 at C-5′), 1.42 (2H, q, CH_2 at C-3, J=7.6 Hz), 1.27 (3H, s, CH_3 at C-3), 0.59 (3H, t, CH_3 at C-3); ^{13}C NMR (δ, ppm, DMSO-d_6) 165.4 (s), 159.9 (s), 156.4 (s), 148.6 (s), 133.6 (s), 126.9 (d, C-5), 121.5 (d, C-4), 116.7 (d, C-6), 103.7 (d, C-7), 98.3 (s), 89.4 (s, C-2), 50.1 (q, OCH_3), 48.3 (s, C-3), 31.1 (t, CH_2), 28.7 (t, CH_2), 28.4 (q, NCH_3), 16.7 (q, at C-3), 10.4 (q, CH_3 at C-5′), 8.6 (q, CH_3 at C-3); minor component **b**: ^{1}H NMR (δ, ppm, DMSO-d_6) 7.19 (1H, s, NH), 6.92 (1H, t, H-6, J=7.6 Hz), 6.86 (1H, d, H-4, J=7.6 Hz), 6.51 (1H, t, H-5, J=7.6 Hz), 6.14 (1H, d, H-7, J=7.6 Hz), 5.33 (2H, br s, NH_2), 3.55 (3H, s, OCH_3), 2.93 (1H, br d, H-3′, J=16.0 Hz), 2.73 (1H, br d, H-3′, J=16.0 Hz), 2.68 (3H, s, NCH_3), 1.98 (3H, s, CH_3 at C-5′), 1.41 (2H, q, CH_2 at C-3, J=7.6 Hz), 1.14 (3H, s, CH_3 at C-3), 0.55 (3H, t, CH_3 at C-3); ^{13}C NMR (δ, ppm, DMSO-d_6) 165.7 (s), 159.6 (s), 157.1 (s), 148.8 (s), 132.5 (s), 127.1 (d, C-6), 121.5 (d, C-4), 115.8 (d, C-5), 102.9 (d, C-7), 97.0 (s), 93.8 (s, C-2), 49.7 (q, OCH_3), 49.2 (s, C-3), 31.1 (t, CH_2), 30.8 (t, CH_2), 27.9 (q, NCH_3), 16.3 (q, at C-3), 11.4 (q, CH_3 at C-5′), 8.6 (q, CH_3 at C-3).

4.6.6. 2,2′,3,3′-Tetrahydro-3-ethyl-1,3,5′-trimethyl-4′-methoxycarbonyl-1′-methoxycarbonylaminospiro[1*H*-indole-2,2′-pyrrole] (37). Yield 96%; pale pink solid; mp 168–171 °C; IR (cm^{-1}, Nujol) 3262, 1751, 1648, 1612, 1484, 1368, 1224, 1140, 1013, 889, 761; EIMS (*m*/*z*) 373 ($M^{+\bullet}$, 91), 358 (21), 312 (20), 299 (100); Anal. Calcd for $C_{20}H_{27}N_3O_4$: C, 64.32; H, 7.29; N, 11.25. Found: C, 64.44; H, 7.18; N, 11.19. The isomers **37a** and **37b** were obtained in 50:50 ratio by using DMSO-d_6 as a solvent (determined by ^{1}H NMR). For clarity sake the NMR values are given separately for each isomer. Component **a**: ^{1}H NMR (δ, ppm, DMSO-d_6) 8.14 (1H, s, NH), 6.92 (1H, t, H-6, J=7.6 Hz), 6.84 (1H, d, H-4, J=7.6 Hz), 6.54 (1H, t, H-5, J=7.6 Hz), 6.32 (1H, d, H-7, J=7.6 Hz), 3.59 (3H, s, OCH_3), 3.32 (3H, s, OCH_3), 2.95 (1H, br d, H-3′, J=16.0 Hz), 2.64 (1H, br d, H-3′, J=16.0 Hz), 2.63 (3H, s, NCH_3), 1.99 (3H, br s, CH_3 at C-5′), 1.41 (2H, q, CH_2 at C-3, J=7.6 Hz), 1.18 (3H, s, CH_3 at C-3), 0.59 (3H, t, CH_3 at C-3); ^{13}C NMR (δ, ppm, DMSO-d_6) 165.3 (s), 158.7 (s), 155.5 (s), 148.4 (s), 133.2 (s), 126.8 (d, C-5), 121.5 (d, C-4), 116.5 (d, C-6), 103.8 (d, C-7), 98.7 (s), 90.9 (s, C-2), 51.8 (q, OCH_3), 50.2 (q, OCH_3), 48.6 (s, C-3), 30.7 (t, CH_2), 28.8 (t, CH_2), 28.0 (q, NCH_3), 15.9 (q, at C-3), 10.4 (q, CH_3 at C-5′), 8.6 (q, CH_3 at C-3); component **b**: ^{1}H NMR (δ, ppm, DMSO-d_6) 8.51 (1H, s, NH), 6.98 (1H, t, H-6, J=7.6 Hz), 6.80 (1H, d, H-4, J=7.6 Hz), 6.51 (1H, t, H-5, J=7.6 Hz), 6.13 (1H, d, H-7, J=7.6 Hz), 3.58 (3H, s, OCH_3), 3.38 (3H, s, OCH_3), 2.95 (1H, br d, H-3′, J=16.0 Hz), 2.74 (1H, br d, H-3′, J=16.0 Hz), 2.68 (3H, s, NCH_3), 2.03 (3H, s, CH_3 at C-5′), 1.42 (2H, q, CH_2 at C-3, J=7.6 Hz), 1.26 (3H, s, CH_3 at C-3), 0.57 (3H, t, CH_3 at C-3); ^{13}C NMR (δ, ppm, DMSO-d_6) 165.6 (s), 158.4 (s), 155.9 (s), 148.7 (s), 132.9 (s), 127.0 (d, C-6), 121.6 (d, C-4), 116.0 (d, C-5), 102.6 (d, C-7), 97.4 (s), 94.2 (s, C-2), 51.7 (q, OCH_3), 49.9 (q, OCH_3), 49.1 (s, C-3), 30.9 (t, CH_2), 30.4 (t, CH_2), 27.9 (q, NCH_3), 15.8 (q, at C-3), 11.0 (q, CH_3 at C-5′), 8.5 (q, CH_3 at C-3).

4.6.7. 1,2′,3,3′-Tetrahydro-3-ethyl-1,3,4′-trimethyl-5′-methoxycarbonyl-1′-*tert*-butoxycarbonylaminospiro[2*H*-indole-2,2′-pyrrole] (38). Yield 85%; pink solid; mp 138–141 °C; IR (cm^{-1}, Nujol) 3248, 1738, 1650, 1602, 1458, 1367, 1271, 1135, 891, 783; EIMS (*m*/*z*) 415 ($M^{+\bullet}$, 63), 315 (31), 299 (100); Anal. Calcd for $C_{20}H_{27}N_3O_4$: C, 64.32; H, 7.29; N, 11.25. Found: C, 64.44; H, 7.18; N, 11.19. The isomers **38a** and **38b** were obtained in 50:50 ratio by using DMSO-d_6 as a solvent (determined by ^{1}H NMR). For clarity sake the NMR values are given separately for each isomer. Component **a**: ^{1}H NMR (δ, ppm, DMSO-d_6) 7.67 (1H, s, NH), 6.98 (1H, t, H-6, J=7.6 Hz), 6.79 (1H, d, H-4, J=7.6 Hz), 6.55 (1H, t, H-5, J=7.6 Hz), 6.33 (1H, d, H-7, J=7.6 Hz), 3.59 (3H, s, OCH_3), 2.95 (1H, br d, H-3′, J=16.0 Hz), 2.66 (1H, br d, H-3′, J=16.0 Hz), 2.62 (3H, s, NCH_3), 2.01 (3H, br s, CH_3 at C-5′), 1.40 (2H, q, CH_2 at C-3, J=7.6 Hz), 1.27 (3H, s, CH_3 at C-3), 1.20 (9H, s, $C(CH_3)_3$), 0.59 (3H, t, CH_3 at C-3); ^{13}C NMR (δ, ppm, DMSO-d_6) 165.3 (s), 159.0 (s), 154.3 (s), 148.8 (s), 133.3 (s), 126.8 (d, C-5), 121.5 (d, C-4), 116.5 (d, C-6), 103.7 (d, C-7), 98.8 (s), 93.8 (s, C-2), 79.0 (s, $C(CH_3)_3$), 50.1 (q, OCH_3), 49.1 (s, C-3), 30.7 (t, CH_2), 30.2 (t, CH_2), 28.0 (q, NCH_3), 27.8 (q, $C(CH_3)_3$), 15.6 (q, at C-3), 10.4 (q, CH_3

at C-5′), 8.6 (q, CH_3 at C-3); component **b**: ^{1}H NMR (δ, ppm, DMSO-d_6) 8.26 (1H, s, NH), 6.90 (1H, t, H-6, J=7.6 Hz), 6.84 (1H, d, H-4, J=7.6 Hz), 6.51 (1H, t, H-5, J=7.6 Hz), 6.12 (1H, d, H-7, J=7.6 Hz), 3.57 (3H, s, OCH_3), 2.94 (1H, br d, H-3′, J=16.0 Hz), 2.72 (1H, br d, H-3′, J=16.0 Hz), 2.69 (3H, s, NCH_3), 1.97 (3H, s, CH_3 at C-5′), 1.41 (2H, q, CH_2 at C-3, J=7.6 Hz), 1.20 (9H, s, $C(CH_3)_3$), 1.16 (3H, s, CH_3 at C-3), 0.56 (3H, t, CH_3 at C-3); ^{13}C NMR (δ, ppm, DMSO-d_6) 165.5 (s), 158.7 (s), 154.0 (s), 148.4 (s), 132.8 (s), 126.8 (d, C-6), 121.5 (d, C-4), 115.8 (d, C-5), 102.8 (d, C-7), 97.1 (s), 90.6 (s, C-2), 79.2 (s, $C(CH_3)_3$), 49.8 (q, OCH_3), 48.6 (s, C-3), 30.8 (t, CH_2), 28.9 (t, CH_2), 28.0 (q, NCH_3), 27.9 (q, $C(CH_3)_3$), 15.9 (q, at C-3), 11.0 (q, CH_3 at C-5′), 8.5 (q, CH_3 at C-3).

4.6.8. 2,2′3,3′-Tetrahydro-1,3,5′-trimethyl-4′-methoxycarbonyl-3-phenyl-1′-ureidospiro[1*H*-indole-2,2′-pyrrole] (39). Yield 94%; white solid; mp 144–147 °C; IR (cm^{-1}, Nujol) 3428, 3285, 3173, 1666, 1606, 1493, 1440, 1363, 1319, 1200, 890, 782; EIMS (*m/z*) 406 ($M^{+\bullet}$, 74), 347 (100); Anal. Calcd for $C_{23}H_{26}N_4O_3$: C, 67.96; H, 6.45; N, 13.78. Found: C, 67.81; H, 6.50; N, 13.84. The isomers **39a** and **39b** were obtained in 65:35 ratio by using DMSO-d_6 as a solvent (determined by ^{1}H NMR). For clarity sake the NMR values are given separately for each isomer. Major component **a**: ^{1}H NMR (δ, ppm, DMSO-d_6) 7.25-6.73 (8H, m, Ph+NH+H-4+H-6), 6.57 (1H, t, H-5, J=7.6 Hz), 6.50 (1H, d, H-7, J=7.6 Hz), 5.92 (2H, br s, NH_2), 3.46 (3H, s, OCH_3), 2.62 (3H, s, NCH_3), 2.43 (1H, br d, H-3′, J=16.0 Hz), 2.09 (3H, br s, CH_3 at C-5′), 1.82 (1H, br d, H-3′, J=16.0 Hz), 1.75 (3H, s, CH_3 at C-3); ^{13}C NMR (δ, ppm, DMSO-d_6) 165.2 (s), 160.1 (s), 156.6 (s), 149.3 (s), 145.2 (s), 136.3 (s), 127.8 (s), 127.7 (s), 127.2 (s), 126.2 (d, C-5), 121.8 (d, C-4), 117.7 (d, C-6), 103.6 (d, C-7), 98.0 (s), 90.0 (s, C-2), 52.7 (q, OCH_3), 50.0 (s, C-3), 33.9 (t, CH_2), 28.4 (q, NCH_3), 19.3 (q, CH_3 at C-5′), 10.6 (q, CH_3 at C-3); minor component **b**: ^{1}H NMR (δ, ppm, DMSO-d_6) 7.49 (1H, s, NH), 7.25–6.73 (7H, m, Ph+H-4+H-6), 6.49 (1H, t, H-5, J=7.6 Hz), 6.34 (1H, d, H-7, J=7.6 Hz), 5.53 (2H, br s, NH_2), 3.45 (3H, s, OCH_3), 2.71 (3H, s, NCH_3), 2.36 (1H, br d, H-3′, J=16.0 Hz), 2.17 (1H, br d, H-3′, J=16.0 Hz), 2.01 (3H, s, CH_3 at C-5′), 1.65 (3H, s, CH_3 at C-3); ^{13}C NMR (δ, ppm, DMSO-d_6) 165.5 (s), 159.5 (s), 157.6 (br s), 149.9 (s), 144.7 (s), 135.7 (s), 127.9 (s), 127.7 (s), 127.1 (s), 126.2 (d, C-6), 121.7 (d, C-4), 117.0 (d, C-5), 104.3 (d, C-7), 97.2 (s), 94.7 (s, C-2), 54.2 (q, OCH_3), 49.7 (s, C-3), 31.3 (t, CH_2), 28.2 (q, NCH_3), 18.0 (q, CH_3 at C-5′), 11.5 (q, CH_3 at C-3).

4.6.9. 2,2′3,3′-Tetrahydro-1,3,5′-trimethyl-4′-methoxycarbonyl-3-phenyl-1′-*tert*-butoxycarbonylaminospiro[1*H*-indole-2,2′-pyrrole] (40). Yield 81%; pink solid; mp 184–187 °C; IR (cm^{-1}, Nujol) 3241, 1763, 1656, 1605, 1366, 1276, 1161, 1135, 999, 752; EIMS (*m/z*) 463 ($M^{+\bullet}$, 56), 347 (62), 221 (100); Anal. Calcd for $C_{27}H_{33}N_3O_4$: C, 69.95; H, 7.18; N, 9.06. Found: C, 70.04; H, 7.01; N, 9.25. The isomers **40a** and **40b** were obtained in 55:45 ratio by using DMSO-d_6 as a solvent (determined by ^{1}H NMR). For clarity sake the NMR values are given separately for each isomer. Major component **a**: ^{1}H NMR (δ, ppm, DMSO-d_6) 8.18 (1H, s, NH), 7.23–7.00 (6H, m, Ph+H-6), 6.98 (1H, d, H-4, J=7.6 Hz), 6.58 (1H, t, H-5, J=7.6 Hz), 6.50 (1H, d, H-7, J=7.6 Hz), 3.46 (3H, s, OCH_3), 2.63 (3H, s, NCH_3), 2.46 (1H, br d, H-3′, J=16.0 Hz), 2.05 (3H, br s, CH_3 at C-5′), 1.88 (1H, br d, H-3′, J=16.0 Hz), 1.71 (3H, s, CH_3 at C-3), 1.28 (9H, s, $C(CH_3)_3$). ^{13}C NMR (δ, ppm, DMSO-d_6) 165.1 (s), 158.6 (s), 154.9 (s), 149.6 (s), 144.8 (s), 135.9 (s), 127.7 (s), 127.6 (s), 127.3 (s), 126.2 (d, C-5), 121.8 (d, C-4), 117.6 (d, C-6), 104.4 (d, C-7), 98.1 (s), 94.4 (s, C-2), 79.5 (s, $C(CH_3)_3$), 53.7 (q, OCH_3), 50.0 (s, C-3), 33.2 (t, CH_2), 28.4 (q, NCH_3), 27.9 (q, $C(CH_3)_3$), 19.0 (q, CH_3 at C-3), 10.5 (q, CH_3 at C-5′); minor component **b**: ^{1}H NMR (δ, ppm, DMSO-d_6) 8.55 (1H, s, NH), 7.23–7.00 (6H, m, Ph+H-6), 6.77 (1H, d, H-4, J=7.6 Hz), 6.53 (1H, t, H-5, J=7.6 Hz), 6.31 (1H, d, H-7, J=7.6 Hz), 3.32 (3H, s, OCH_3), 2.70 (3H, s, NCH_3), 2.43 (1H, br d, H-3′, J=16.0 Hz), 2.10 (1H, br d, H-3′, J=16.0 Hz), 1.99 (3H, s, CH_3 at C-5′), 1.67 (3H, s, CH_3 at C-3), 1.27 (9H, s, $C(CH_3)_3$); ^{13}C NMR (δ, ppm, DMSO-d_6) 165.4 (s), 159.1 (s), 154.4 (br s), 149.6 (s), 144.6 (s), 135.9 (s), 127.9 (s), 127.8 (s), 127.1 (s), 126.2 (d, C-6), 121.6 (d, C-4), 117.0 (d, C-5), 103.4 (d, C-7), 97.1 (s), 91.1 (s, C-2), 79.5 (s, $C(CH_3)_3$), 53.0 (q, OCH_3), 49.8 (s, C-3), 31.4 (t, CH_2), 28.1 (q, NCH_3), 28.0 (q, $C(CH_3)_3$), 17.3 (q, CH_3 at C-3), 11.2 (q, CH_3 at C-5′).

Acknowledgements

Financial supports from M.I.U.R. (Rome), PRIN 2003-2004, and the Universities of Urbino, Trieste and Bologna are gratefully acknowledged. Thanks are also given to Dr. Federico Berti of the Dipartimento di Scienze Chimiche, Università di Trieste, for molecular modelling calculations.

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Tetrahedron 62 (2006) 6435–6442
Tetrahedron

Pseudoguaiane-type sesquiterpenes and inhibitors on nitric oxide production from *Dichrocephala integrifolia*

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Received 30 January 2006; accepted 22 March 2006
Available online 11 May 2006

Abstract—Three new pseudoguaiane-type sesquiterpenes, dichrocepholides A–C, and two new pseudoguaiane-type sesquiterpene dimers, dichrocepholides D and E, were isolated from the aerial part of *Dichrocephala integrifolia*. Their stereostructures were determined on the basis of chemical and physicochemical evidence. In addition, the extract and its principal sesquiterpene constituent, parthenin, showed an inhibitory activity on nitric oxide (NO) production and on induction of inducible NO synthase.

1. Introduction

The Asteraceae plant, *Dichrocephala integrifolia* (L. f.) O. Kuntze, is a sparingly branched annual herb, which is widely distributed in African, Middle Eastern, and Asian countries.[1,2] Many genus of *Dichrocephala* have been commonly used as folk medicines for pneumonia, hypertension, fever, and ulcers. The leaves of *D. integrifolia* have also been used for the treatment of malaria and hepatitis.[2] The composition of the essential oil from the leaves and flowers of *D. integrifolia* was reported.[2] However, the studies on the pharmacologic activity and biological constituents of *D. integrifolia* are left uncharacterized. Previously, we have reported on bioactive constituents from several Egyptian and Yemeni natural medicines such as *Cyperus longus*,[3,4] *Anastatica hierochuntica*,[5,6] *Nigella sativa*,[7,8] and *Crinum yemens*.[9] As a continuing study, we found that the aqueous methanolic extract from the aerial parts of *D. integrifolia* showed potent inhibitory effect on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in mouse peritoneal macrophages. From the aqueous methanolic extract, three new pseudoguaiane-type sesquiterpenes, dichrocepholides A (**1**), B (**2**), and C (**3**) and two new pseudoguaiane-type sesquiterpene dimers, dichrocepholides D (**4**) and E (**5**), were isolated together with six known constituents, parthenin (**6**),[10–12] **7**,[13] 6-methoxy-3-*O*-methylkaempferol (**8**),[14] quercetagetin 3,7-dimethyl ether (**9**),[15] 3,5,4′-trihydroxy-6,7,3′-trimethoxyflavone (**10**),[16] and kaempferol 3-*O*-β-D-glucopyranoside (**11**).[17] In this paper, we describe the isolation and structure elucidation of five new sesquiterpenes (**1**–**5**) from the aerial parts of *D. integrifolia* as well as inhibitory effects of the isolated constituents on NO production and induction of NO synthase inhibitory activities.

2. Results and discussion

The 80% aqueous methanolic extract of the aerial part of *D. integrifolia* was found to show NO production inhibitory activity on LPS-activated mouse peritoneal macrophages (IC_{50}=15 μg/mL). From the active fraction (the EtOAc-soluble fraction, IC_{50}=4.2 μg/mL), three new pseudoguaiane-type sesquiterpenes, dichrocepholides A (**1**, 0.0013%), B (**2**, 0.0014%), and C (**3**, 0.0007%), and two new pseudoguaiane-type sesquiterpene dimers, dichrocepholides D (**4**, 0.0055%) and E (**5**, 0.0047%), were isolated together with six known constituents, **6** (0.75%), **7** (0.0070%), **8** (0.0007%), **9** (0.0033%), **10** (0.0004%), and **11** (0.0015%) (Table 1).

Dichrocepholide A (**1**) was isolated as colorless fine crystals, mp 151–153 °C, with positive optical rotation ($[\alpha]_D^{24}$ +23.0). The EIMS spectrum of **1** showed a molecular ion peak at *m/z* 280 (M^+) and the molecular formula $C_{15}H_{20}O_5$ of **1** was elucidated by HREIMS measurement. The UV spectrum of **1**

Keywords: Dichrocepholide; Pseudoguaiane-type sesquiterpene; *Dichrocephala integrifolia*; Nitric oxide inhibitor.
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0040–4020/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.tet.2006.03.091

Table 1. Inhibitory effects of the 80% aqueous methanolic extract and EtOAc-, *n*-BuOH-, and H_2O-soluble fractions from *D. integrifolia* on NO production in LPS-activated mouse peritoneal macrophages

	Inhibition (%)						IC_{50} (μg/mL)
	0 μg/mL	1 μg/mL	3 μg/mL	10 μg/mL	30 μg/mL	100 μg/mL	
80% Aqueous methanolic extract	0.0±2.8	0.2±3.5	2.9±2.1	35.3±4.2**	100.0±0.2**	101.0±0.2**,[a]	15
EtOAc-soluble fraction	0.0±5.8	9.2±4.1	24.0±1.8**	91.0±2.5**	100.4±0.1**,[a]		4.2
n-BuOH-soluble fraction	0.0±4.9	−2.1±2.8	1.1±3.6	3.5±3.2	0.0±6.3	31.5±3.2**	
H_2O-soluble fraction	0.0±5.0	1.3±2.9	4.7±1.8	5.0±1.5	3.6±1.3	7.0±1.2	

Each value represents the mean±SEM, (N=4).
Significantly different from the control, *p<0.05, **p<0.01.
[a] Cytotoxic effect was observed.

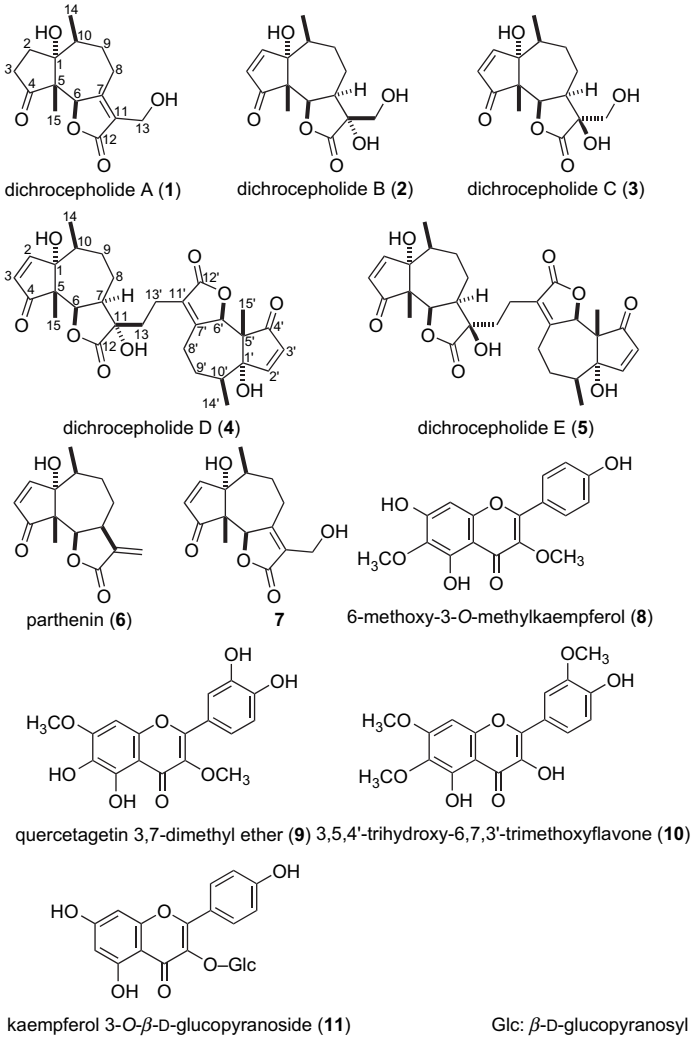


Chart 1.

showed an absorption maximum at 219 (log ε 3.13) and the IR spectrum of **1** showed absorption bands at 3565, 1762, and 1740 cm^{-1} ascribable to hydroxyl, carbonyl, and α,β-unsaturated γ-lactone functions. The ^1H (CD_3OD, Table 2) and ^{13}C NMR (Table 2) spectra of **1** showed signals due to two methyl [δ 0.86 (3H, s, H_3-15), 1.10 (3H, d, J=7.0 Hz, H_3-14)], four methylenes [δ 1.45 (1H, ddd, J=6.9, 10.1, 13.5 Hz, Hβ-9), 2.33 (1H, ddd, J=3.5, 6.9, 13.5 Hz, Hα-9), 1.82, 2.56 (1H each, both m, H_2-2), 2.41 (2H, m, H_2-3), 2.97 (2H, d-like, H_2-8)], a methine [δ 2.16 (1H, m, H-10)] together with a methylene and a methine bearing an oxygen function [δ 4.28 (2H, s, H_2-13), 5.43 (1H, s, H-6)]. The proton and carbon signals in the ^1H and ^{13}C NMR spectra of **1** were found to be similar to those of **7**, except for the 2- and 3-positions. The 1,13-dihydroxy-4-oxo-7(11)-pseudoguaien-12,6-olide structure in **1** was constructed on the basis of homo- and heterocorrelation spectroscopy (^1H–^1H, ^{13}C–^1H COSY), distortionless enhancement by polarization transfer (DEPT), and heteronuclear multiple bond connectivity (HMBC) experiments. Thus, the ^1H–^1H COSY experiment on **1** indicated the presence of partial structures shown by bold lines in Figure 1. In the HMBC experiment of **1**, long-range correlations were observed between the following proton and carbon pairs: H_2-2 and C-1, 4, 5; H_2-3

Table 2. ^{1}H and ^{13}C NMR data of dichrocepholides A–C (**1**–**3**) in CD_3OD

	1		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		84.0		85.0		85.0
2	1.82 m 2.56 m	32.4	7.61 d (6.0)	166.2	7.60 d (6.0)	166.2
3	2.41 m (2H)	34.2	6.13 d (6.0)	131.6	6.12 d (6.0)	131.6
4		218.5		213.7		213.6
5		59.0		60.0		60.4
6	5.43 s	83.7	5.01 d (6.3)	80.6	4.93 d (7.3)	80.2
7		170.3	2.96 ddd (2.3, 6.3, 6.3)	52.9	3.04 ddd (3.9, 7.3, 7.3)	48.6
8	2.97 d-like (2H)	24.0	1.79 m 2.07 m	21.5	1.80 m 1.90 m	20.5
9α	2.33 ddd (3.5, 6.9, 13.5)	31.0	2.21 ddd (3.6, 6.7, 13.2)	32.7	2.17 ddd (4.1, 7.2, 14.1)	32.1
9β	1.45 ddd (6.9, 10.1, 13.5)		1.68 ddd (6.7, 9.8, 13.2)		1.75 ddd (7.2, 10.2, 14.1)	
10	2.16 m	43.3	2.33 m	41.3	2.34 m	41.6
11		127.1		78.7		78.1
12		176.1		177.9		180.0
13	4.28 s (2H)	53.6	3.70 d (12.0) 3.80 d (12.0)	63.2	3.57 d (11.0) 3.70 d (11.0)	67.8
14	1.10 d (7.0)	18.3	1.12 d (6.8)	18.0	1.11 d (7.7)	18.0
15	0.86 s	11.3	1.31 s	20.4	1.31 s	19.8

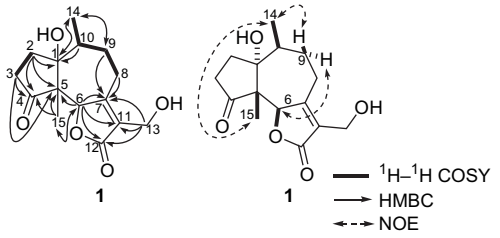
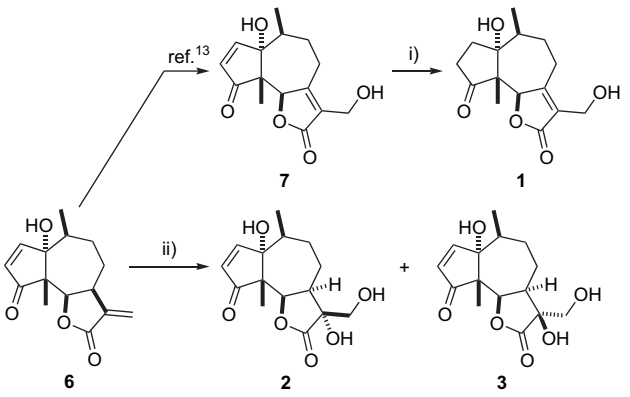


Figure 1.

and C-4, 5; H-6 and C-5, 7, 11, 12, 15; H_2-8 and C-7; H_2-9 and C-7, 14; H-10 and C-1; H_2-13 and C-7, 11, 12; H_3-14 and C-1, 9; H_3-15 and C-4, 5, 6 (Fig. 1). The relative stereostructure of **1** was determined by nuclear Overhauser enhancement spectroscopy (NOESY) experiment in which NOE correlations were observed as shown in Figure 1. To clarify the absolute stereostructure of **1**, we carried out the chemical correlation of **1** to **6**, whose absolute stereostructure was elucidated previously.[18] That is, hydrogenation of **7**, which was derived from **6**,[13] with 10% palladium–carbon (Pd–C) under a hydrogen atmosphere furnished **1** as shown in Scheme 1. This evidence led us to designate the absolute stereostructure of dichrocepholide A (**1**) as shown.



Scheme 1. Reagents and conditions: (i) 10% Pd–C, H_2/MeOH, rt, 30 min, 97%; (ii) MC-OsO_4, NMO/CH_3CN–acetone–H_2O (1:1:1, v/v/v), rt, 48 h, **2** (59%), **3** (6%), recover **6** (26%).

Dichrocepholides B (**2**) and C (**3**) were obtained as colorless fine crystals with positive optical rotation (**2**: $[\alpha]_D^{24}$ +26.0, **3**: $[\alpha]_D^{24}$ +42.5) and the molecular formulas of **2** and **3** were determined by EIMS and HREIMS measurements to be $C_{15}H_{20}O_6$. The UV spectra of **2** and **3** showed an absorption maximum [**2**: 215 nm (log ε 3.78); **3**: 214 (3.70)], while their IR spectra showed absorption bands due to hydroxyl, γ-lactone, and α,β-unsaturated carbonyl functions (**2**: 3550, 1755, and 1742 cm^{-1}; **3**: 3550, 1755, and 1741 cm^{-1}). The proton and carbon signals in the ^{1}H and ^{13}C NMR spectra of **2** and **3** were superimposable on those of **6**, except for the *exo*-methylene part in **6**. Namely, ^{1}H (CD_3OD, Table 2) and ^{13}C NMR (Table 2) spectra of **2** and **3** showed signals assignable to two methyls, two methylenes, two methines, a methylene and a methine bearing an oxygen function, and two olefin protons. As shown in Figure 2, the ^{1}H–^{1}H COSY experiment on **2** and **3** indicated the presence of partial structures drawn in bold lines. In the HMBC experiment, long-range correlations were observed between the following proton and carbon pairs of **2** and **3** (H-2 and C-1, 4, 5; H-3 and C-4, 5; H-6 and C-5, 11, 12, 15; H-7 and C-11, 12; H_2-9 and C-14; H-10 and C-1; H_2-13 and C-7, 11, 12; H_3-14 and C-1, 9; H_3-15 and C-4, 5, 6). Those findings led us to confirm the planar structures of **2** and **3** to be the same. Next, the relative structure of **2** was clarified by the NOESY experiment, which showed NOE correlations between the following protons (H-6 and H-7, Hα-9; Hβ-9 and H_3-14; H_3-15 and H_3-13, H_3-14), while NOE correlations were observed between the following protons of **3** (H-6 and H-7, Hα-9,

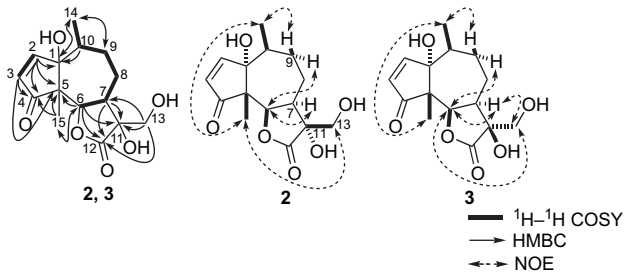


Figure 2.

H_2-13; H-7 and H_2-13; H_3-14 and Hβ-9, H_3-15). Furthermore, osmium oxidation of **6** with microcapsuled osmium tetraoxide (MC-OsO_4) and *N*-methylmorpholine *N*-oxide (NMO) yielded **2** and **3** (ca. 10:1 ratio). Consequently, the absolute stereostructures of **2** and **3** were determined as shown.

Dichrocepholide D (**4**) was obtained as colorless needles, mp 174–176 °C, with positive optical rotation ($[\alpha]_D^{24}$ +49.0) and its UV spectrum showed an absorption maximum at 216 nm (log ε 4.58). The IR spectrum of **4** showed absorption bands at 3575, 1765, and 1742 cm^{-1}. The EIMS spectrum of **4** showed a molecular ion peak at *m/z* 540 (M^+) and the molecular formula $C_{30}H_{36}O_9$ of **4** was elucidated by HREIMS measurement. The proton and carbon signals due to the sesquiterpene part (C-1–C-15) having a saturated lactone moiety in the ^1H and ^{13}C NMR data of **4** were found to be very similar to those of **2**, whereas the proton and carbon signals of the sesquiterpene part (C-1′–C-15′) having an unsaturated lactone moiety were superimposable on those of **7**, except for the signals due to the 13- and 13′-positions. That is, the ^1H (CD_3OD, Table 3) and ^{13}C NMR (Table 3) spectra of **4** showed signals assignable to four methyls [δ 0.94 (3H, s, H_3-15′), 1.07 (3H, d, *J*=7.2 Hz, H_3-14′), 1.13 (3H, d, *J*=7.0 Hz, H_3-14), 1.31 (3H, s, H_3-15)], six methylenes {δ 1.41 (1H, ddd, *J*=6.8, 9.5, 13.5 Hz, Hβ-9′), 2.34 (1H, ddd, *J*=3.7, 6.8, 13.5 Hz, Hα-9′), 1.68 (2H, m, H_2-13), 1.72 (1H, ddd, *J*=6.8, 10.0, 13.4 Hz, Hβ-9), 2.18 (1H, ddd, *J*=3.4, 6.8, 13.4 Hz, Hα-9), 1.90 (2H, m, H_2-8), 2.30, 2.70 (1H each, both m, H_2-13′), [2.85 (1H, m), 3.05 (1H, dd-like), H_2-8′]}, three methines [δ 2.30 (1H, m, H-10), 2.34 (1H, m, H-10′), 2.98 (1H, m, H-7)], two methines bearing an oxygen function [δ 5.12 (1H, d, *J*=5.2 Hz, H-6), 5.43 (1H, s, H-6′)], and four olefinic protons [δ 6.09, 7.70 (1H each, both d, *J*=5.8 Hz, H-3′ and H-2′), 6.14, 7.63 (1H each, both d, *J*=5.8 Hz, H-3 and H-2)]. The ^1H–^1H COSY experiment on **4** indicated the presence of five partial structures shown by bold lines in Figure 3 (C-2–C-3, C-6–C-10–C-14, C-13–C-13′, C-2′–C-3′, and C-8′–C-10′–C-14′). In the HMBC experiment, long-range correlations were observed

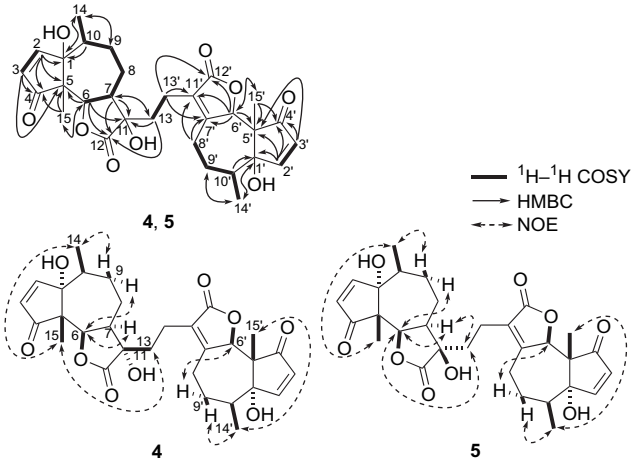


Figure 3.

Table 3. ^1H and ^{13}C NMR data of dichrocepholides D (**4**) and E (**5**) in CD_3OD

	4		**5**	
	δ_H	δ_C	δ_H	δ_C
1		85.6		85.6
2	7.63 d (5.8)	166.3	7.63 d (5.8)	166.3
3	6.14 d (5.8)	131.5	6.14 d (5.8)	131.5
4		213.5		213.5
5		60.0		60.3
6	5.12 d (5.2)	80.7	4.85 d (6.6)	79.4
7	2.98 m	53.0	2.93 ddd (2.7, 5.5, 6.6)	51.9
8	1.90 m (2H)	31.9	1.44 m (2H)	38.0
9α	2.18 ddd (3.4, 6.8, 13.4)	33.0	2.18 ddd (3.0, 6.6, 13.3)	32.3
9β	1.72 ddd (6.8, 10.0, 13.4)		1.78 ddd (6.6, 10.2, 13.3)	
10	2.30 m	41.3	2.34 m	41.4
11		78.5		77.0
12		178.9		179.8
13	1.68 m (2H)	21.3	1.87 m (2H)	20.3
14	1.13 d (7.0)	18.2	1.13 d (7.0)	18.2
15	1.31 s	20.9	1.32 s	21.0
1′		84.5		84.5
2′	7.70 d (5.8)	163.9	7.70 d (5.8)	163.9
3′	6.09 d (5.8)	130.8	6.09 d (5.8)	130.8
4′		211.4		211.4
5′		57.4		57.4
6′	5.43 s	83.6	5.43 s	83.6
7′		166.5		166.5
8′	2.85 m	25.1	2.85 m	25.1
	3.05 dd-like		3.05 dd-like	
9′α	2.34 ddd (3.7, 6.8, 13.5)	33.1	2.34 ddd (4.0, 7.1, 14.2)	33.1
9′β	1.41 ddd (6.8, 9.5, 13.5)		1.41 ddd (7.1, 10.3, 14.2)	
10′	2.34 m	41.2	2.34 m	41.2
11′		128.7		127.9
12′		176.8		176.8
13′	2.30 m	17.8	2.50 m (2H)	18.4
	2.70 m			
14′	1.07 d (7.2)	18.7	1.07 d (7.2)	18.7
15′	0.94 s	15.2	0.94 s	15.2

between the following protons and carbons of **4** (H-2 and C-1, 4, 5; H-3 and C-4, 5; H-6 and C-5, 11, 12, 15; H-7 and C-11, 12, 13; H_2-9 and C-14; H-10 and C-1; H_2-13 and C-7, 11, 12; H_3-14 and C-1, 9; H_3-15 and C-4, 5, 6; H-2′ and C-1′, 4′, 5′; H-3′ and C-4′, 5′; H-6′ and C-5′, 7′, 11′, 12′, 15′; H_2-8′ and C-7′, 11′; H_2-9′ and C-14′; H-10′ and C-1′; H_2-13′ and C-7′, 11′, 12′; H_3-14′ and C-1′, 9′; H_3-15′ and C-4′, 5′, 6′). The stereostructure of **4** was determined by the NOESY experiment, which showed NOE correlations between the following proton pairs (H-6 and H-7, Hα-9; H_2-13 and H_3-15; H_3-14 and Hβ-9, H_3-15; H-6′ and Hα-9′; H_3-14′ and Hβ-9′, H_3-15′). Those findings led us to indicate the dimeric sesquiterpene structure composed of **2** and **7**. Finally, the stereostructure of **4** was confirmed by the X-ray crystallographic analysis as shown in Figure 4.

Dichrocepholide E (**5**) was also obtained as colorless needles, mp 169–171 °C, with negative optical rotation ($[\alpha]_D^{24}$ −3.0) and its molecular formula $C_{30}H_{36}O_9$, which was the same as that of **4**, was determined from the EIMS and HREIMS measurements. The UV spectrum of **5** showed an absorption maximum at 217 nm (log ε 4.26), whereas the IR spectrum showed absorption bands at 3560, 1760, and 1740 cm^{-1}, which was similar to those of **4**. The proton and carbon signals in the ^1H (CD_3OD, Table 3) and ^{13}C NMR (Table 3) spectra of **5** were found to be superimposable on those of **4** and indicated the presence of the same functional groups. In the ^1H–^1H COSY and the HBMC experiments on **5**, the planar structure was constructed to be the same dimeric sesquiterpene skeleton as that of **4**. The stereostructure of **5** was elucidated by the NOESY experiments, which showed NOE correlations were observed between the following proton pairs (H-6 and H-7, Hα-9, H_2-13; H-7 and H_2-13; H_3-14 and Hβ-9, H_3-15; H-6′ and Hα-9′; H_3-14′ and Hβ-9′, H_3-15′). Consequently, the stereostructure of **5** was elucidated as shown.

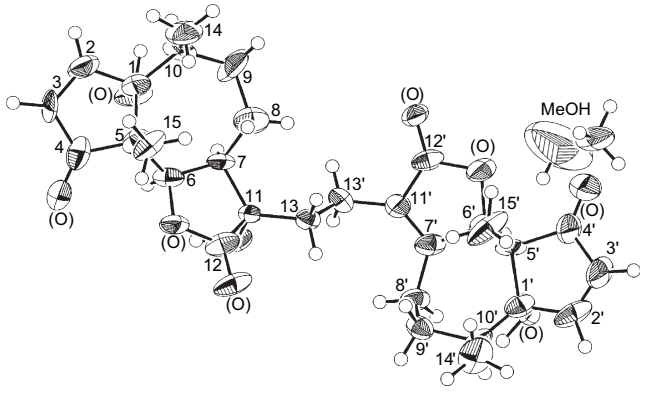


Figure 4. X-ray crystallographic analysis of dichrocepholide D (**4**).

The inorganic free radical NO has been implicated in physiologic and pathologic processes, such as vasodilation, nonspecific host defense, ischemia-reperfusion injury, and chronic or acute inflammation. NO is produced by the oxidation of L-arginine by NO synthase (NOS). In the family of NOS, inducible NOS (iNOS) is specifically involved in pathologic aspects with the overproduction of NO and can be expressed in response to proinflammatory agents such as interleukin-1β, tumor necrosis factor-α, and LPS in various cell types including macrophages, endothelial cells, and smooth muscle cells. As a part of our studies to characterize the bioactive components of natural medicines, we have investigated various NO production inhibitors, i.e., higher unsaturated fatty acids,[19] polyacetylenes,[20–22] coumarins,[20,22,23] flavonoids,[21,24] stilbenes,[25,26] lignans,[6,27,28] sesquiterpenes,[29–35] diterpenes,[36,37] triterpenes,[38–41] diarylheptanoids,[42–44] cyclic peptides,[40] alkaloids,[9,45] and phenylpropanoids.[28,46,47] Continuing of these studies, the effects of the sesquiterpene constituents (**1**–**7**) from the aerial parts of *D. integrifolia* on NO production from LPS-activated macrophages were examined, and the results were summarized in Table 4. Among them, parthenin (**6**, IC_{50}=1.4 μM) and **7** (29 μM) exhibited inhibitory activity. The inhibitory activity of **6** was more potent than that of guanidinoethyldisulfide (GED), a selective inducible nitric oxide synthase (iNOS) inhibitor (IC_{50}=7.4 μM).[9]

Next, the effects of the two sesquiterpenes (**6**, **7**) on iNOS induction were examined. iNOS was detected at 130 kDa after a 20 h incubation with LPS by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE)-Western blotting [reference compound, caffeic acid phenethyl ester (CAPE, an inhibitor of NF-κB activation)].[9,47] As shown in Figure 5, iNOS inductions of LPS-activated macrophages were suppressed by **6** and **7** closely related to their

Table 4. Inhibitory effects of constituents from *D. integrifolia* on NO production in LPS-activated mouse peritoneal macrophages

	Inhibition (%)						IC_{50} (μM)
	0 μM	1 μM	3 μM	10 μM	30 μM	100 μM	
Dichrocepholide A (**1**)	0.0±3.6	−6.7±3.4	−0.1±1.4	−0.1±3.6	−0.3±4.1	10.2±5.7	
Dichrocepholide B (**2**)	0.0±2.0	−2.2±1.3	−3.3±2.1	3.8±4.0	4.2±6.1	65.6±1.6**	
Dichrocepholide C (**3**)	0.0±1.9	−2.0±4.1	1.9±3.2	3.2±2.8	10.2±2.2	11.3±1.8*	
Dichrocepholide D (**4**)	0.0±3.2	0.3±2.1	−0.6±9.7	−6.0±7.7	18.5±1.5	36.1±10.6**	
Dichrocepholide E (**5**)	0.0±5.5	−5.2±5.2	−17.2±14.5	−9.5±9.1	−0.1±2.5	−3.1±10.4	
7	0.0±2.2	−2.5±3.4	−7.1±3.7	−1.9±2.5	51.0±1.6**	95.9±1.1**	29
L-NMMA	0.0±4.0	5.9±0.9	10.3±3.7	15.0±1.6**	34.1±3.2**	63.1±1.2**	57[9]
CAPE	0.0±0.7	3.8±0.1	1.4±0.1	68.2±0.0**	93.7±0.2**	99.6±0.0**,[a]	15[9]
GED	0.0±0.0	6.2±0.1	24.4±0.1**	57.9±0.1**	89.7±0.2**	97.9±0.0**	7.4[9]
	0 μM	0.03 μM	0.1 μM	0.3 μM	1 μM	3 μM	
Parthenin (**6**)	0.0±1.4	1.8±3.7	−5.9±5.3	9.9±5.8	34.8±0.5**	92.7±2.0**	1.4

Each value represents the mean±SEM, (N=4).
Significantly different from the control, *p<0.05, **p<0.01.
[a] Cytotoxic effect was observed.

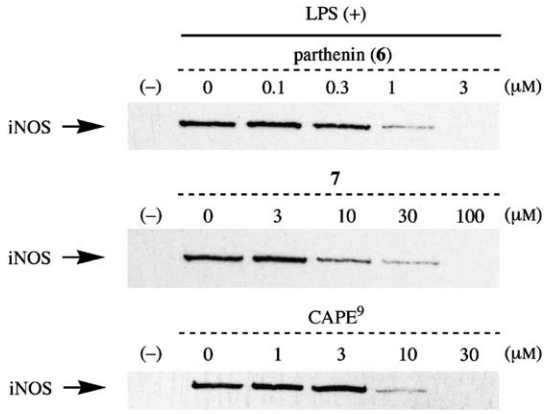


Figure 5. Effects of **6**, **7**, and CAPE on iNOS induction in LPS-activated mouse macrophages.

inhibitions of NO. These results suggested that the two sesquiterpenes (**6**, **7**) inhibited NO production due to their inhibitory activities against iNOS induction in LPS-activated macrophages.

3. Experimental

3.1. General

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; EIMS and HREIMS, JEOL JMS-GCMATE mass spectrometer, JEOL JMS-SX 102A mass spectrometer; ^{1}H NMR spectra, JNM-LA500 (500 MHz); ^{13}C NMR spectra, JNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard; Combustion analysis was done on a Perkin–Elmer Series II CHNS/O Analyzer 2400; HPLC detector, Shimadzu RID-6A refractive index detector; HPLC column, YMC-Pack ODS-A (250×4.6 mm i.d.) and (250×20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: ordinary-phase column chromatography; Silica gel BW-200 (Fuji Silysia Chemical, Ltd, 150–350 mesh), reversed-phase column chromatography; Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd, 100–200 mesh); TLC, pre-coated TLC plates with Silica gel 60F_{254} (Merck, normal-phase) and Silica gel RP-18 F_{254S} (Merck, reversed-phase); HPTLC, pre-coated TLC plates with Silica gel 60F_{254} (Merck, normal-phase), Silica gel RP-18 WF_{254S} (Merck, reversed-phase) and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

3.2. Plant material

The aerial parts of *D. integrifolia* was collected in Ibb province, Yemen in July 2000, and was identified by one of the authors, Dr. Osama B. Abdel-Halim (Professor of Pharmacognosy of Mansura University, Egypt). A voucher specimen (No. Y-02) of this natural medicine is on file in our laboratory.

3.3. Extraction and isolation

The dried aerial parts of *D. integrifolia* (1.6 kg) was finely cut and extracted three times with 80% aqueous methanol under reflux for 3 h. Evaporation of the solvent under reduced pressure gave the aqueous methanolic extract (213 g, 13.3% from dried aerial parts). The aqueous methanolic extract (213 g) was partitioned in an EtOAc–H_2O (1:1, v/v) mixture. The aqueous layer was extracted with *n*-BuOH and removal of the solvent in vacuo from the EtOAc-, *n*-BuOH-, and H_2O-soluble portions yielded 60 g (3.7%), 99 g (6.2%), and 54 g (3.4%) of the residue, respectively. The EtOAc-soluble fraction (50.0 g) was subjected to ordinary-phase silica gel column chromatography [1.5 kg, *n*-hexane–EtOAc (10:1–5:1–1:1–1:10, v/v)–$CHCl_3$–MeOH–H_2O (10:3:1–7:3:1, lower layer)–MeOH] to afford 10 fractions [Fr. 1 (4.1 g), Fr. 2 (2.7 g), Fr. 3 (7.8 g), Fr. 4 (4.4 g), Fr. 5 (4.1 g), Fr. 6 (9.5 g), Fr. 7 (5.7 g), Fr. 8 (2.2 g), Fr. 9 (3.5 g), Fr. 10 (6.0 g)]. Fraction 4 (4.4 g) was separated by reversed-phase silica gel column chromatography [125 g, MeOH–H_2O (20:80–50:50–70:30, v/v)–MeOH] to give four fractions {Fr. 4-1 (650 mg), 4-2 [=parthenin (**6**, 1200 mg, 0.75%)], 4-3 (851 mg), 4-4 (1685 mg)}. Fraction 6 (9.5 g) was separated by reversed-phase silica gel column chromatography [280 g, MeOH–H_2O (30:70–40:60–60:40–70:30, v/v)–MeOH] to furnish five fractions [Fr. 6-1 (82 mg), 6-2 (3500 mg), 6-3 (1500 mg), 6-4 (750 mg), 6-5 (2800 mg)]. Fraction 6-2 (1.0 g) was further subjected to HPLC [MeOH–H_2O (25:75, v/v)] to give **7** (110 mg, 0.0070%), **1** (21 mg, 0.0013%), **2** (23 mg, 0.0014%), and **3** (11 mg, 0.0007%). Fraction 6-3 (1.0 g) was further purified by HPLC [MeOH–H_2O (55:45, v/v)] to give **4** (88 mg, 0.0055%), **5** (76 mg, 0.0047%), and **9** (53 mg, 0.0033%). Fraction 6-4 (750 mg) was further separated by HPLC [MeOH–H_2O (55:45, v/v)] to give **8** (11 mg, 0.0007%) and **10** (7 mg, 0.0004%). Fraction 8 (2.2 g) was separated by reversed-phase silica gel column chromatography [65 g, MeOH–H_2O (30:70–55:45–70:30, v/v)–MeOH] to give four fractions [Fr. 8-1 (296 mg), 8-2 (422 mg), 8-3 (536 mg), 8-4 (946 mg)]. Fraction 8-2 (422 mg) was subjected to HPLC [MeOH–H_2O (45:55, v/v)] to give **11** (24 mg, 0.0015%).

3.3.1. Dichrocephalide A (1). Colorless fine crystals (from MeOH), mp 151–153 °C, $[\alpha]_D^{24}$ +23.0 (c 1.10, MeOH). HREIMS, calcd for $C_{15}H_{20}O_5$ (M^+): 280.1311. Found: 280.1314. UV [MeOH, nm (log ε)]: 219 (3.13). IR (KBr): 3565, 1762, 1740 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD) δ_H: given in Table 2. ^{13}C NMR (125 MHz, CD_3OD) δ_C: given in Table 2. EIMS: m/z 280 (M^+, 11), 262 (M^+-H_2O, 61), 244 (22), 189 (100), 161 (88), 91 (58), 55 (95).

3.3.2. Dichrocephalide B (2). Colorless fine crystals (from MeOH), mp 177–179 °C, $[\alpha]_D^{24}$ +26.0 (c 1.40, MeOH). HREIMS, calcd for $C_{15}H_{20}O_6$ (M^+): 296.1260. Found: 296.1264. UV [MeOH, nm (log ε)]: 215 (3.78). IR (KBr): 3550, 1755, 1742 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD) δ_H: given in Table 2. ^{13}C NMR (125 MHz, CD_3OD) δ_C: given in Table 2. EIMS: m/z 296 (M^+, 11), 278 (M^+-H_2O, 16), 260 (16), 91 (67), 55 (100). Anal. requires C, 60.80; H, 6.80%. Found C, 60.65; H, 6.86%.

3.3.3. Dichrocephalide C (3). Colorless fine crystals (from MeOH), mp 166–168 °C, $[\alpha]_D^{24}$ +42.5 (c 0.60, MeOH).

HREIMS, calcd for $C_{15}H_{20}O_6$ (M^+): 296.1260. Found: 296.1265. UV [MeOH, nm (log ε)]: 214 (3.70). IR (KBr): 3550, 1755, 1741 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ_H: given in Table 2. ^{13}C NMR (125 MHz, CD_3OD) δ_C: given in Table 2. EIMS: *m/z* 296 (M^+, 13), 278 (M^+-H_2O, 21), 260 (11), 91 (61), 55 (100). Anal. requires C, 60.80; H, 6.80%. Found C, 60.96; H, 6.77%.

3.3.4. Dichrocepholide D (4). Colorless needles (from MeOH), mp 174–176 °C, $[\alpha]_D^{24}$ +49.0 (*c* 2.70, MeOH). HREIMS, calcd for $C_{30}H_{36}O_9$ (M^+): 540.2359. Found: 540.2355. UV [MeOH, nm (log ε)]: 216 (4.58). IR (KBr): 3575, 1765, 1742 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ_H: given in Table 3. ^{13}C NMR (125 MHz, CD_3OD) δ_C: given in Table 3. EIMS: *m/z* 540 (M^+, 8), 496 (10), 478 (15), 303 (18), 231 (55), 79 (100).

3.3.5. Dichrocepholide E (5). Colorless needles (from MeOH), mp 169–171 °C, $[\alpha]_D^{24}$ −3.0 (*c* 2.30, MeOH). HREIMS, calcd for $C_{30}H_{36}O_9$ (M^+): 540.2359. Found: 540.2357. UV [MeOH, nm (log ε)]: 217 (4.26). IR (KBr): 3560, 1760, 1740 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ_H: given in Table 3. ^{13}C NMR (125 MHz, CD_3OD) δ_C: given in Table 3. EIMS: *m/z* 540 (M^+, 7), 496 (12), 478 (11), 303 (15), 231 (62), 79 (100).

3.3.6. Hydrogenation of 7. A solution of **7** (10.0 mg) in MeOH (1.0 mL) was treated with 10% palladium–carbon (Pd–C, 10.0 mg) and the whole mixture was stirred at room temperature under an H_2 atmosphere for 30 min. The catalyst was filtered off, and the solvent from the filtrate was evaporated under reduced pressure to give a residue, which was purified by ordinary-phase silica gel column chromatography [0.5 g, *n*-hexane–acetone (3:2, v/v)] to give **1** (9.8 mg, 97%).

3.3.7. Osmium tetraoxide oxidation of 6. A solution of **6** (9.8 mg) in CH_3CN–acetone–H_2O (1:1:1, v/v/v, 1.0 mL) was treated with microcapsuled osmium tetraoxide (MC-OsO_4, ca. 10 mg) in the presence of *N*-methylmorpholine *N*-oxide (NMO, 6.6 mg) and the whole mixture was stirred at room temperature for 48 h. The MC-OsO_4 was filtered off, and the solvent from the filtrate was evaporated under reduced pressure to give a residue, which was purified by HPLC [MeOH–H_2O (25:75, v/v)] to give **2** (6.5 mg, 59%), **3** (0.7 mg, 6%), and **6** (2.5 mg, 26%).

3.4. Crystal data for 4

Colorless needles, mp 174–176 °C (from MeOH); $C_{30}H_{36}O_9 \cdot CH_3OH$; M=572.65; crystal dimensions: 0.06×0.03×0.25 mm; crystal system: orthorhombic; lattice type: primitive; lattice parameters: a=13.189(3), b=29.814(2), c=7.432(2) Å, V=2922(1) $Å^3$; space group: $P2_12_12_1$ (#19), Z=4, D_{calc}=1.301 g/cm^3, F_{000}=1224.00, μ (Cu Kα)=8.03 cm^{-1}; temperature: 23.0 °C; structure solution: Direct methods (SHELEXS-86); residuals: R=0.257, R_w=0.251, R_1=0.096; goodness of fit indicator: 1.42. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated Cu Kα (λ=1.54178 Å) radiation and a rotating anode generator. The data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC265768.

3.5. Bioassay methods

3.5.1. Reagents. Lipopolysaccharide (LPS, from *Salmonella enteritidis*), N^G-monomethyl-L-arginine (L-NMMA), and RPMI 1640 medium were purchased from Sigma; 3-(4,5-dimethyl-2-thiazolyl) 2,5-diphenyl tetrazolium bromide (MTT) was from Dojindo Laboratories; protease inhibitor cocktail (Complete Mini) was from Roche Diagnostics GmbM; fetal calf serum (FCS) was from Gibco; thioglycolate (TGC) medium was from Nissui Seiyaku; other reagents was from Wako Pure Chemical.

3.5.2. Effects on production of NO in LPS-stimulated macrophages. Screening test for NO production using TGC-induced mouse peritoneal macrophages was performed as described previously.[9,28,46,47] Briefly, peritoneal exudate cells (5×10^5 cells/well) were collected from the peritoneal cavities of male ddY mice and were suspended in 100 μL of RPMI 1640 supplemented with 5% fetal calf serum (FCS), penicillin (100 units/mL), and streptomycin (100 μg/mL), and pre-cultured in 96-well microplates at 37 °C in 5% CO_2 in air for 1 h. Nonadherent cells were removed by washing with PBS, and the adherent cells were cultured in 200 μL of fresh medium containing 10 μg/mL LPS and various concentrations of test compound for 20 h. NO production in each well was assessed by measuring the accumulation of nitrite (NO_2^-) in the culture medium using Griess reagent. Cytotoxicity was determined by the MTT colorimetric assay, after 20 h incubation with test compounds. Each test compound was dissolved in DMSO and then the solution was added to the medium (final DMSO concentration was 0.5%). Inhibition (%) was calculated using the following formula and IC_{50} was determined graphically (N=4).

$$\text{Inhibition}\,(\%) = [(A-B)/(A-C)] \times 100 \quad A-C:\ NO_2^- \text{ concentration}\,(\mu M)$$

[A: LPS (+), sample (−); B: LPS (+), sample (+); C: LPS (−), sample (−)]

3.5.3. Detection of iNOS. In this experiment, TGC-induced peritoneal exudate cells (7.5×10^6 cells/3 mL/dish) from male ddY mice were pre-cultured in culture dishes (6 cm i.d.) for 1 h, and the adherent cells were obtained as described previously.[9,47] After washing, the culture medium was then exchanged for fresh medium containing 5% FCS, 20 μg/ml LPS, and test compound for 20 h. Cells were collected in lysis buffer [100 mM NaCl, 10 mM Tris, protease inhibitor cocktail (1 tab/10 mL), 0.1% Triton X-100, 2 mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), pH 7.4] and sonicated. After determination of the protein concentration of each suspension by the BCA method (BCA™ Protein Assay Kit, Pierce), the suspension was boiled in Laemmli buffer. For SDS-PAGE, aliquots of 40 μg of protein from each sample were subjected to electrophoresis in 7.5% polyacrylamide gels. Following electrophoresis, the proteins were transferred electrophoretically onto nitrocellulose membranes. The membranes were incubated with 5% nonfat dried milk in Tris-buffered saline (T-TBS, 100 mM NaCl, 10 mM Tris, 0.1% Tween 20, pH 7.4) and probed with mouse monoclonal IgG (dilution of 1:1000) against iNOS. The blots were washed in T-TBS

and probed with the secondary antibody, anti-mouse IgG antibody conjugated with horseradish peroxidase (dilution of 1:5000). Detection was performed using an ECL™ and X-ray film (Hyperfilm-ECL™, Amersham).

3.5.4. Statistics. Values are expressed as means±SEM. One-way analysis of variance followed by Dunnett's test was used for statistical analysis.

Acknowledgements

This research was supported by the 21st COE Program, Academic Frontier Project, and a Grant-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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