ELSEVIER

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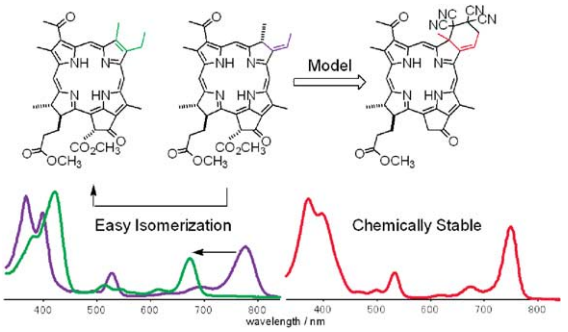
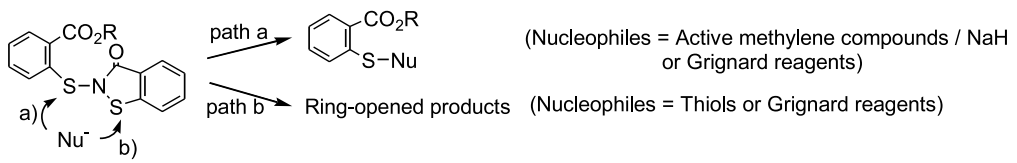
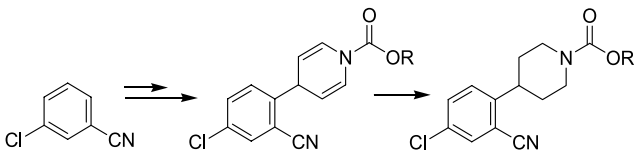
REPORT

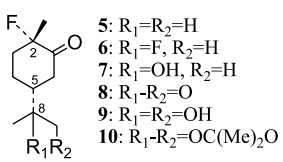
ARTICLES

Chemically stable 8-alkylidene-bacteriochlorins were systematically prepared as models of natural bacteriochlorophylls-*b*/*g* easily isomerizing to the corresponding chlorins, and their optical properties were investigated in detail.

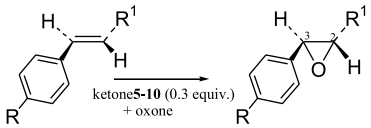








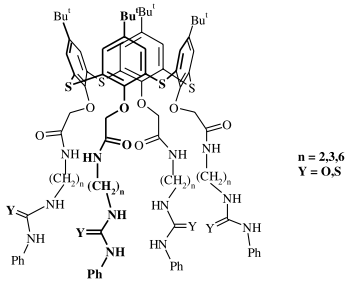
5: R_1=R_2=H
6: R_1=F, R_2=H
7: R_1=OH, R_2=H
8: R_1-R_2=O
9: R_1=R_2=OH
10: R_1-R_2=$OC(Me)_2O$



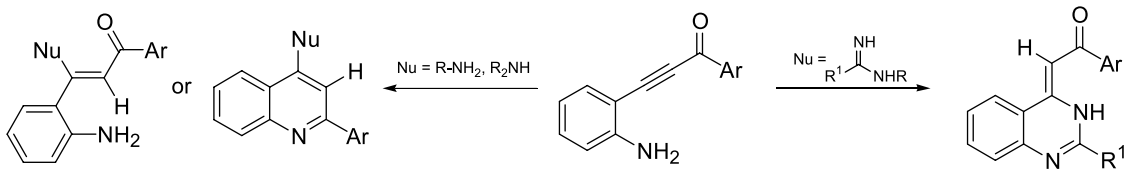
12: R=H; R^1=Ph; **13**: R=MeO; R^1=CO_2Me
14: R=H; R^1=CH_2OH; **15**: R=H; R^1=CH_2OCOCH_3
16: R=H; R^1=CH_2OSiMe_2tBu

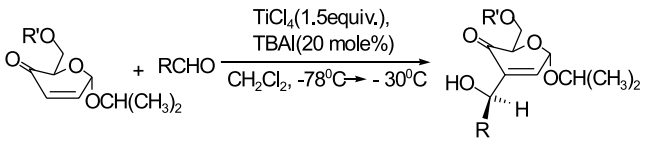
R_1 different from H provides higher ee%; absolute configuration at C8 has no effect on the ee%

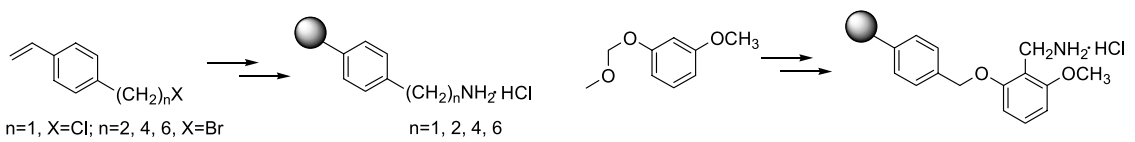
The thiacalix[4]arene-tetraacetates or diacetates (*cone*) serve as a starting point for novel thiacalixarenes derivatives bearing urea or thiourea units on the lower rim. As revealed by NMR titration experiments, these compounds are preorganised for anion recognition in organic solvents.

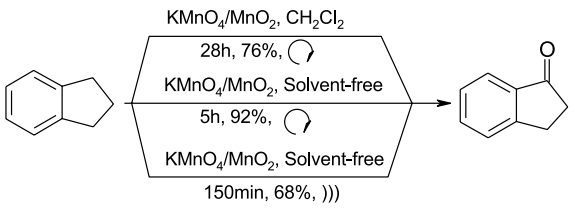


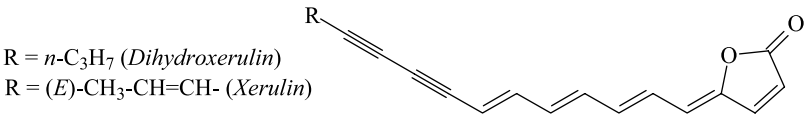
n = 2,3,6
Y = O,S



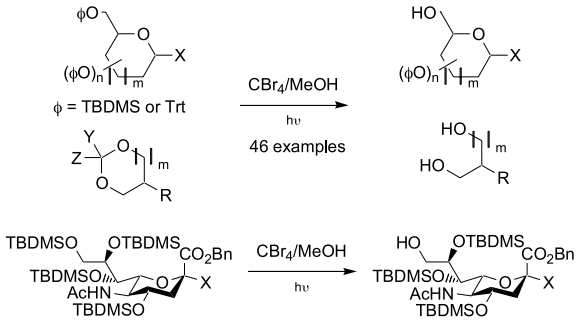


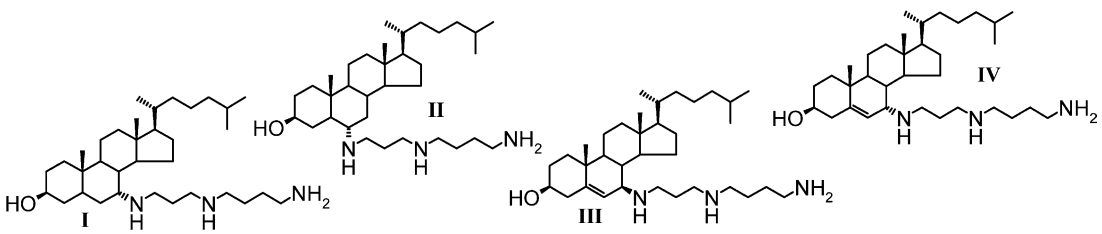


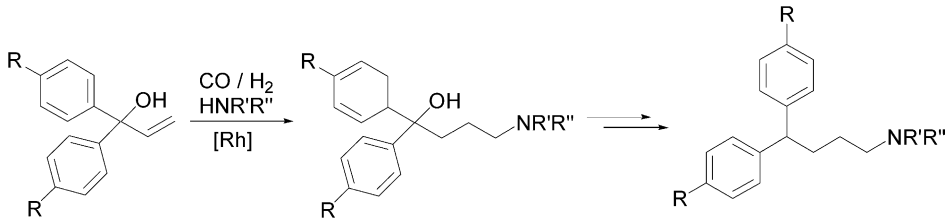


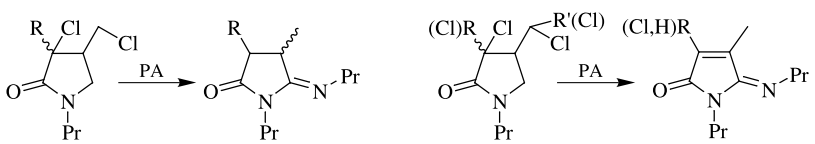


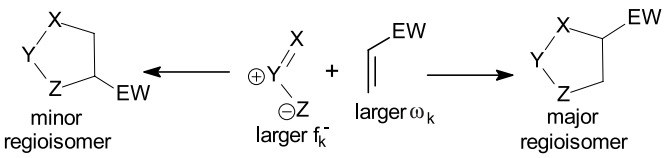
A new stereoselective methodology has been devised for the total synthesis of both dihydroxerulin and xerulin.

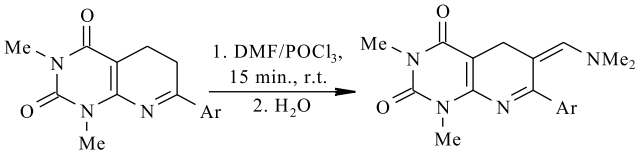




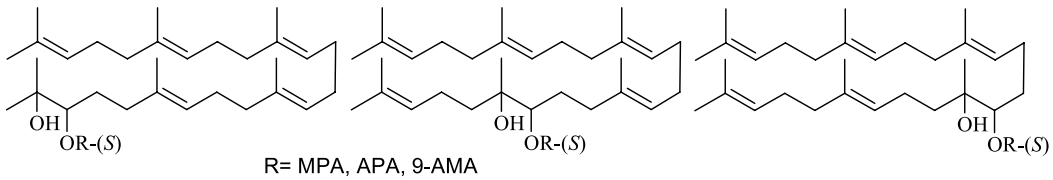




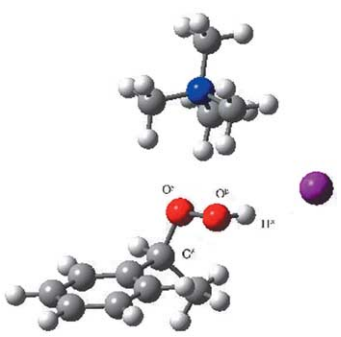


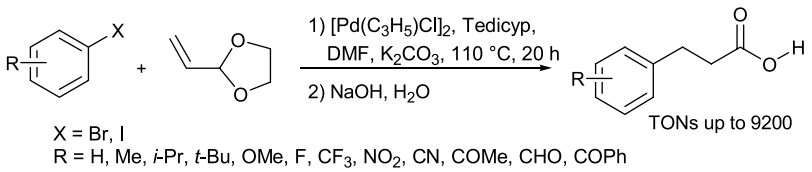


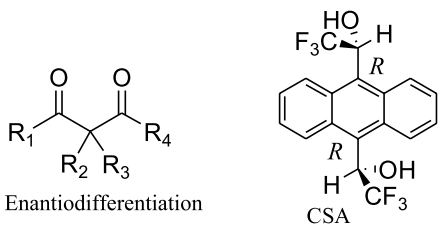
The reaction of 7-aryl-5,6-dihydropyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-diones with phosphorous oxychloride and *N*,*N*-dimethylformamide affords either the shown 6-dimethylaminomethylidene substituted heterocycle or the corresponding pyridopyrimidine(1*H*,3*H*)-2,4-dione bearing a carboxaldehyde function. These compounds are suitable structures for further modifications by introducing pharmacologically relevant substituents.

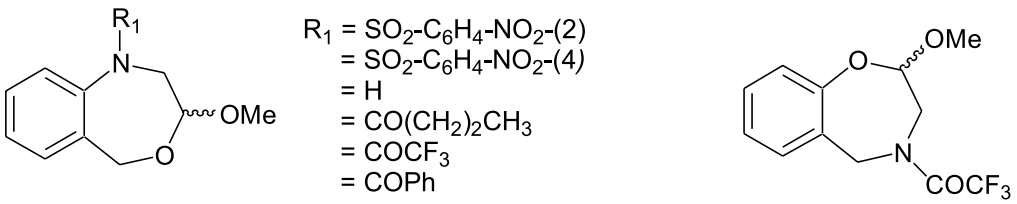


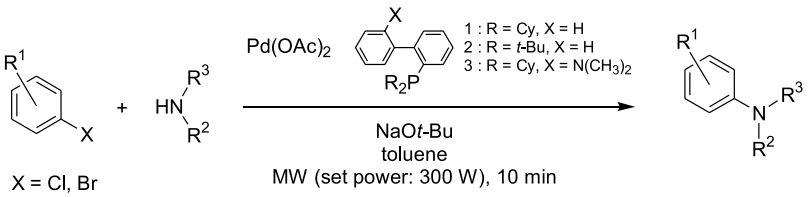
Analysis of the electronic properties of the structures formed between reactants and catalysts offers a better understanding of the mechanism of ethylbenzene oxidation reactions. The atoms-in-molecules approach enables a rigorous description of atomic charges and bond properties that can be qualitatively related to the experimental data.

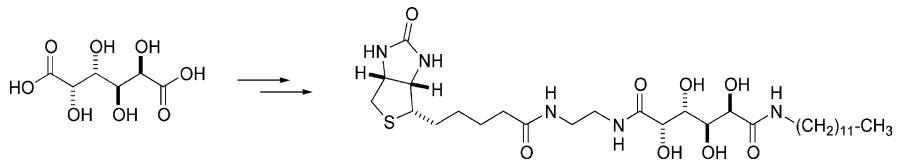


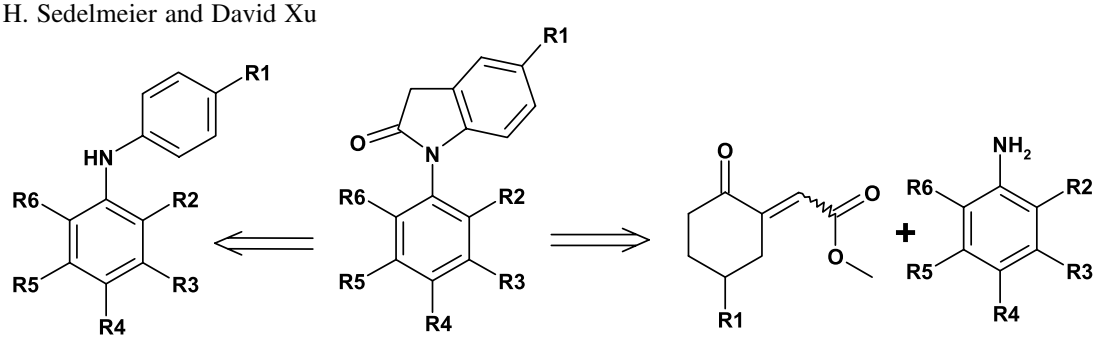


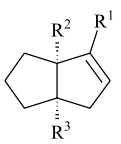






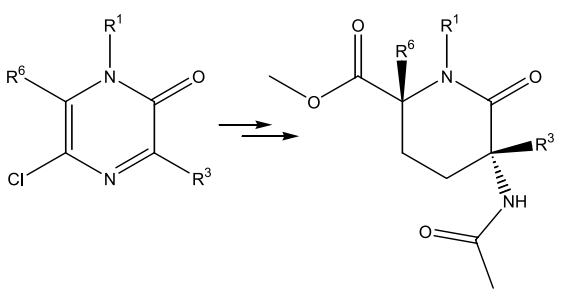


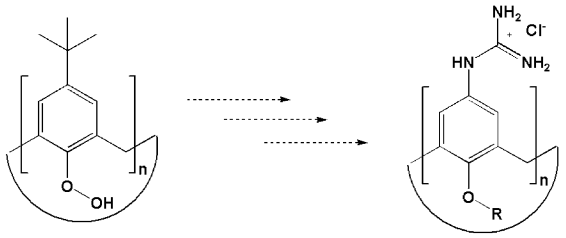


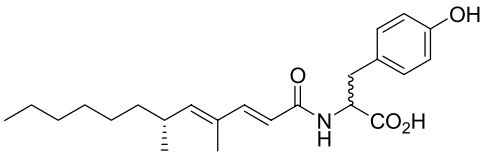


	R^1	R^2	R^3
42	CH_3	H	H
43	CH_3	H	CH_3
44	CH_3	CH_3	CH_3
45	CH_3	CH_3	H

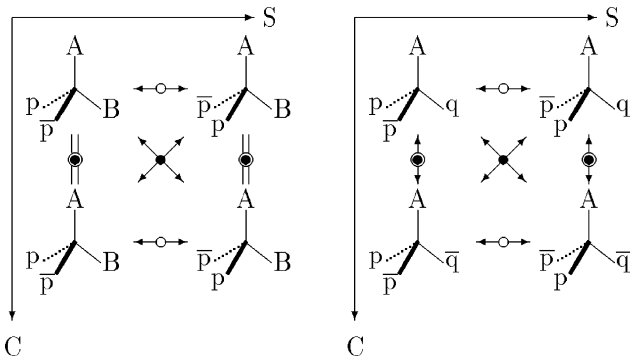
Mono- to trimethylated [1,1′-bicyclobutyl]-1-ols and spiro[3.4]octan-5-ols have been synthesized and rearranged to yield a single bicyclo[3.3.0]octene in each case. Examples are **42**, **43**, **44** and **45**.

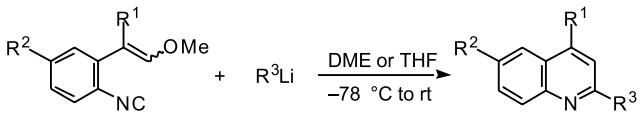




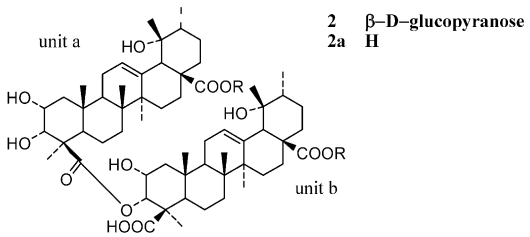


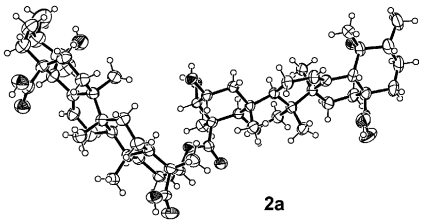
Gymnastatin N



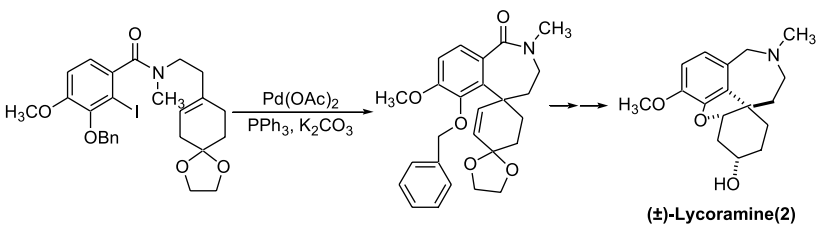


R1 = Ph, Me; R2 = H, Cl; R3 = alkyl, aryl, heteroaryl, R4 2N, PhS





This article discloses the isolation and structural elucidation of four new triterpene glycosides (**1**–**4**) from the roots of *Sanguisorba officinalis*. Alkaline hydrolysis of them led to the dimeric triterpenes (**1a**, **2a** and **3a**), their intramolecular transesterification isomers (**1b** and **2b**), and a new triterpene monomer (**1c**). The structure of **2a** is further confirmed by the X-ray diffraction analysis.



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Supplementary data available via ScienceDirect

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Approaches to the preparation of sphinganines

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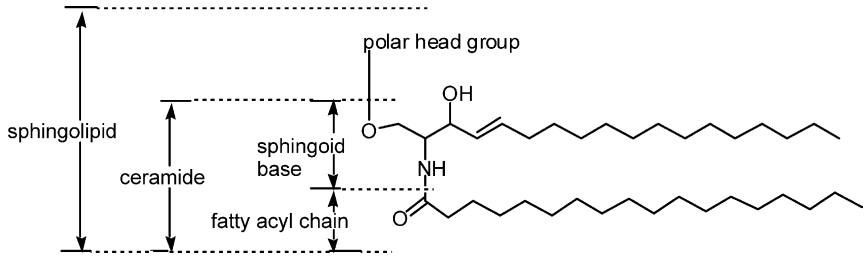


Figure 1.

1. Introduction

Sphingolipids (Fig. 1) are widely found in nature, having been identified in and isolated from mammals, marine organisms, plants, fungi and yeast. Structurally, sphingolipids are composed of three distinct subunits (Fig. 1): a polar head group (sugar, phosphate or sulfate) and a fatty acyl chain, which is linked to the sphingoid base by an amide bond. Glycosphingolipids,[1] containing a sugar head group, are the most widespread of the subclasses of sphingolipid, and these can be further subdivided into cerebrosides (a single sugar residue), neutral glycosphingolipids (one or more uncharged sugar residues) and gangliosides (at least one sugar residue is neuraminic acid).

Sphingolipids are anchored in biomembranes by the hydrophobic nature of the ceramide chains, leaving the polar head group exposed at the membrane surface where its primary function appears to involve molecular recognition and cellular signaling. The biological roles of sphingolipids are complex and diverse. Gangliosides have been identified as the influenza virus receptor,[2] and they are capable of modulating protein kinase activity.[3–6] In addition, they act as immunosuppressants[7,8] and modulate neuronal proliferation and differentiation.[9,10] Ganglioside composition and expression are also different on the cell surface of glioma, melanoma, sarcoma and renal cancer cells, and because of this there is interest in the therapeutic applications of ganglioside antibodies in cancer therapy.[11–13] Cerebrosides, such as the agelasphins, have been found to have antitumor and immunostimulatory activity,[14] while others have shown modest antifungal, antiulcerogenic[15] and Ca^{2+} ionophoretic activity.[16] Other sphingolipids have been shown to have diverging effects on the immune system. For example, the β-galactosyl ceramide, plakoside A, is an immunosuppressant[17] whereas the α-galactosyl ceramide, KRN 7000, has immunostimulatory properties (Fig. 2).

An overview of glycosphingolipid research reveals an increasing diversity of structure. The polar sugar can be a simple monosaccharide or an oligosaccharide composed of different sugars. The fatty acid residue may be composed of a simple alkyl chain, or it may contain alkenyl, hydroxyl or cyclic residues. Sphingoid bases fall into three main classes (Fig. 3). Sphingosines, which are the most prevalent, contain an aminodiol and a C4–C5-double bond. Phytosphingosines are 2-amino-1,3,4-triols, and sphinganines (dihydrosphingosines) are aminodiols saturated at C4–C5. Biogenetically, sphingosines are derived from enzymatic oxidation of sphinganine (Fig. 4).[18,19]

The biological importance of sphingolipids has meant that methods for their preparation have been the subject of frequent publications. Some of the more recently isolated sphingolipids, e.g. plakoside A, have substituents in the fatty acyl chain and the sugar, and it is likely that these two regions will have to be more carefully addressed in future syntheses. However, the main synthetic challenges have been, until recently, the preparation of the sphingoid base and the construction of the glycosidic bond with the correct stereochemistry. As the most prevalent naturally occurring sphingoid bases are sphingosines[20] and phytosphingosines,[21] there has been a considerable body of work detailing their preparation, and this has been recently reviewed. Approaches to the preparation of sphinganines are covered here.

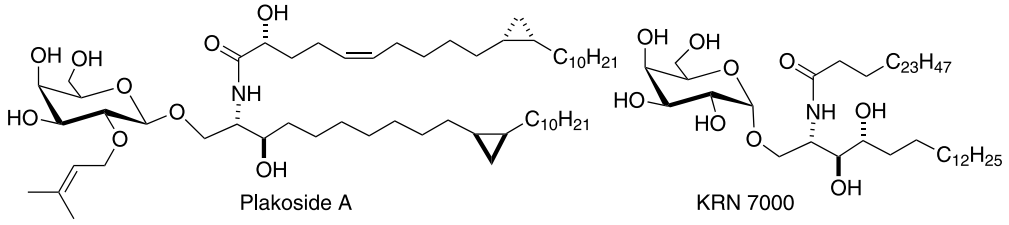


Figure 2.

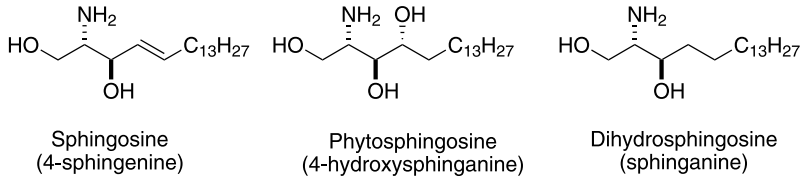


Figure 3.

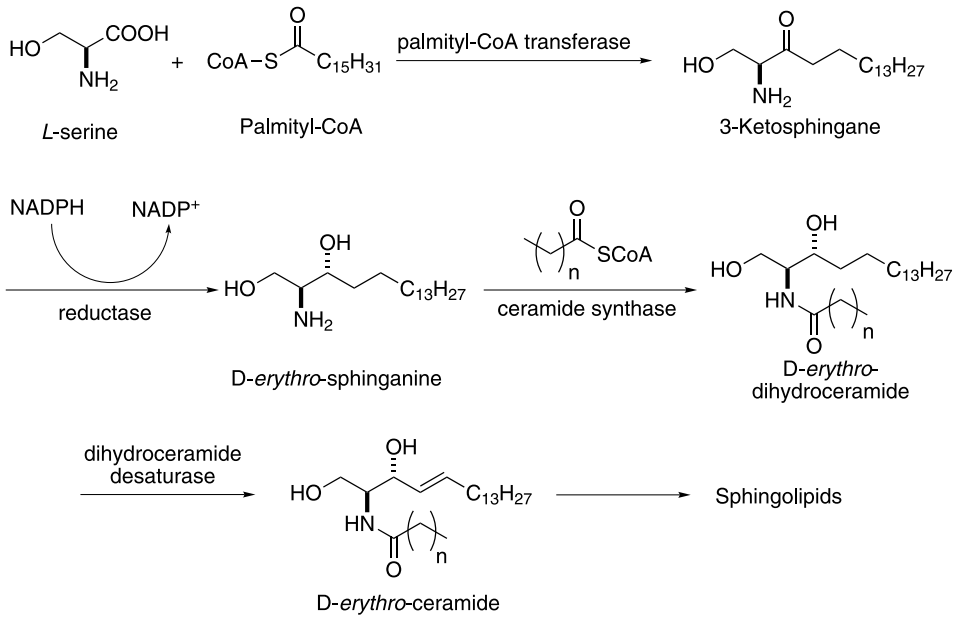
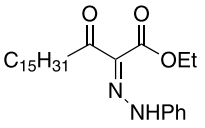


Figure 4.

2. Early syntheses of sphinganines

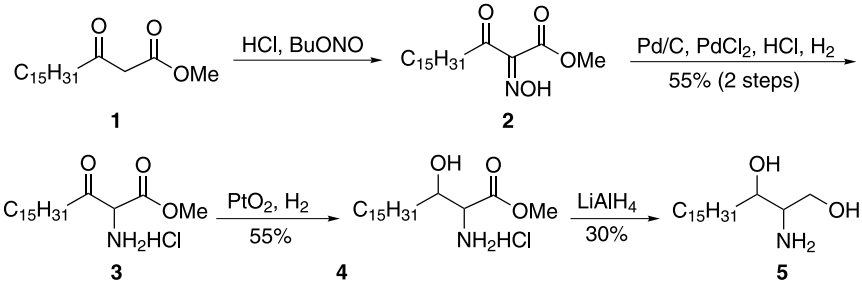
Several groups working independently reported the first syntheses of sphinganines in a flurry of activity fifty years ago. In 1951 Gregory and Malkin[22] (Scheme 1) started by oximinating the β-ketoester **1** to give **2**. Attempts to directly reduce the latter with lithium aluminum hydride to sphinganine (**5**) gave a mixture that could not be purified. Consequently, a stepwise approach was adopted. This involved reduction of oxime **2** to amine **3** with a palladium on carbon/palladium chloride mixture, subsequent reduction of ketone **3** to secondary alcohol **4** and final reduction of the ester to give **5**. In a paper published in 1952 Fisher[23] reported that a product corresponding to **5**, formed from a successful reduction of **2** with lithium aluminum hydride, was formed and characterized as the tribenzoyl derivative. A later paper by Shapiro et al. used a similar procedure involving reduction of the hydrazone **6**,[24] and this was used by a number of other groups, including that of Kulkarni,[25,26] to prepare other derivatives.



6

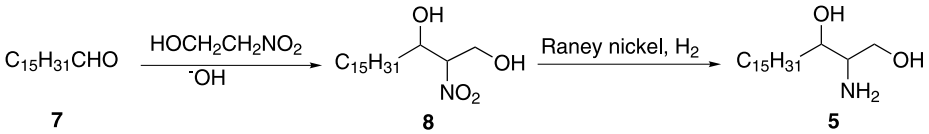
In the second paper published in 1951, Grob et al. reported (Scheme 2) that Henry reaction of 2-nitroethanol with hexadecanal (**7**) gave the nitrodiol **8**. This was reduced to **5** with Raney nickel.[27]

In the papers cited above there was no discussion or determination of the stereochemical outcome of the transformations reported. However, in each case a mixture of isomers must have been produced. In 1952 Grob and Jenny resolved (+)- and (−)-sphinganine from the racemate by crystallization with glutamic acid.[28] In 1953 Carter et al. prepared separately the two racemic mixtures of 2-amino-3-hydroxyoctadecanoic acid and characterized



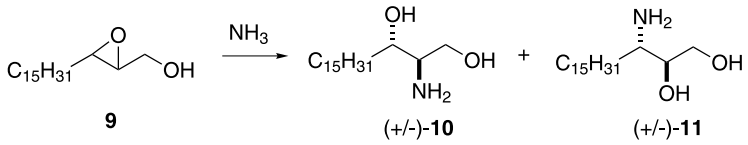
1 **2** **3** **4** **5**

Scheme 1.



7 **8** **5**

Scheme 2.



Scheme 3.

them as the *erythro* and *threo* isomers by comparison with threonine and *allo*-threonine derivatives. Conversion of the acid to the ester and reduction with lithium aluminum hydride or Raney nickel gave the corresponding aminodiols. Determination of the configuration of natural sphinganine as the *erythro* isomer was confirmed by comparison of the reaction of the natural material and the synthetic isomers with benzoyl chloride.[29] Racemic *erythro*-sphinganine **10** was also prepared from ring opening of the *trans* epoxy-alcohol **9** with ammonia.[30] Ring opening, however, was not regioselective and gave a mixture of **10** and **11** (Scheme 3). Shapiro and Sheradsky reported that racemic *erythro*-sphinganine could be separated from the *threo* isomer by crystallization of the corresponding dichloroacetamide.[31]

Since these pioneering efforts, various strategies have been developed for the synthesis of sphinganines. These strategies can be divided into four broad categories: the use of the chiral pool, such as (a) serine and (b) sugars; (c) the use of asymmetric methods, such as the Sharpless asymmetric dihydroxylation (AD) or epoxidation (AE) reactions or the use of chiral auxillaries; and (d) other miscellaneous methods.

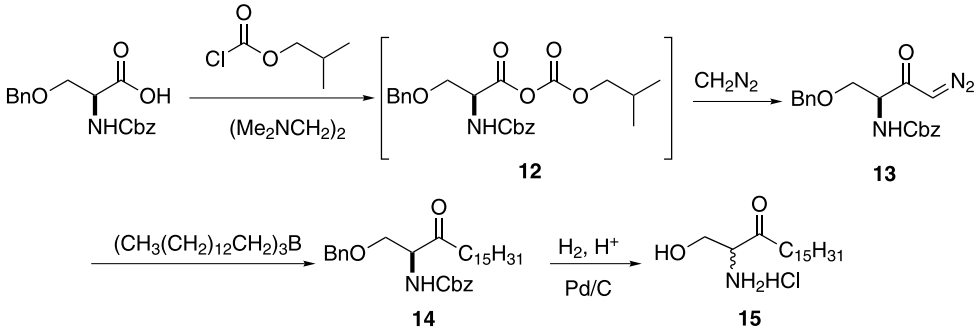
3. Sphinganines from serine

The 2-amino-1,3-dioxygenated skeleton of serine makes it, not surprisingly, the most widely used starting material for sphinganine synthesis. There are several advantages in using this amino acid as a template. Serine is: (a) inexpensive and both enantiomers are commercially available; (b) each of the three carbon atoms bears a functionality that can be further elaborated and (c) a variety of transformations can be carried out while its optical integrity is maintained. The use of serine and its derivatives as templates for organic synthesis has been reviewed.[32]

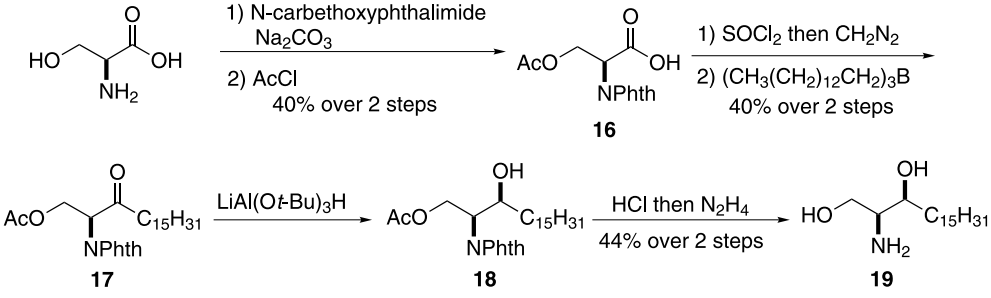
3.1. From serine-derived diazoketones

Two early examples of the utility of serine were reported by Newman in 1974 and rely on the addition of trialkylboranes to α-diazoketones. In the first, L-*O*-benzyl-*N*-carbobenzyloxy(Cbz)-serine was converted to the mixed anhydride **12** and then to α-diazoketone **13** (Scheme 4). Reaction of **13** with *tri*-tetradecylborane gave ketone **14**. Cleavage of the Cbz and Bn groups via hydrogenolysis in the presence of acid gave 3-ketosphinganine **15**. Unfortunately, racemization had occurred during the deprotection step.[33]

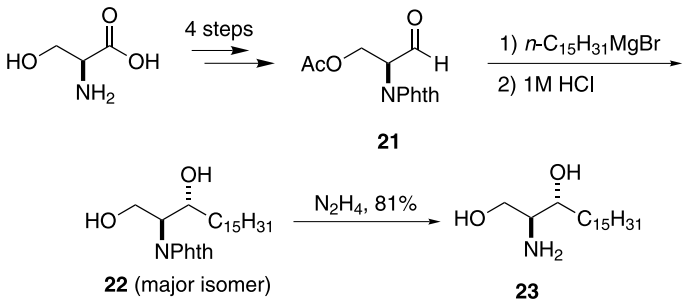
In the second paper Newman modified the protecting group strategy, with a more successful outcome (Scheme 5). L-Serine was doubly protected with phthaloyl and acetate groups. The product **16** was converted to protected 3-keto-sphinganine **17** as in Scheme 4 but via the acid chloride. Stereoselective reduction of this with lithium tri-*tert*-butoxyaluminum hydride gave the *threo* product **18** from which the protecting groups were easily removed to give



Scheme 4.

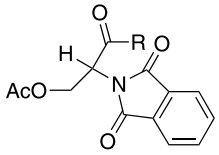


Scheme 5.



Scheme 6.

D-*threo*-sphinganine **19**. The diastereoselectivity of the reduction was explained on the basis of conformation **20**, where the carbonyl and phthalimido groups of **17** are antiparallel to each other, thus minimizing dipole–dipole interactions. Attack by the bulky hydride donor then occurred from the more accessible top face.[34]



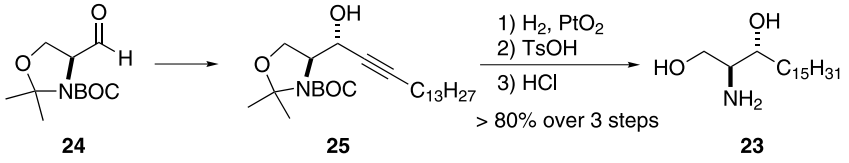
20

3.2. From serine-derived aldehydes

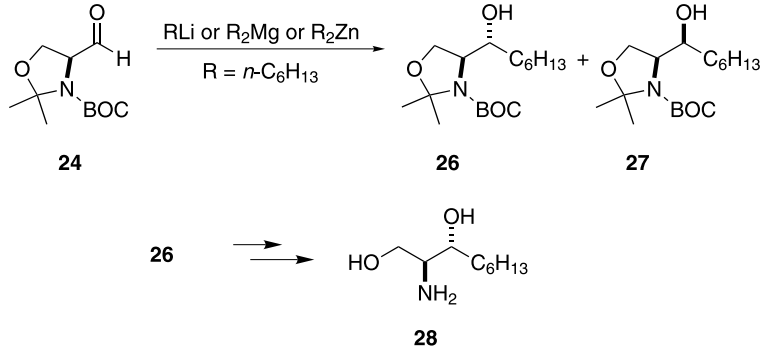
3.2.1. From phthalimido aldehyde. Saitoh in 1980 reported the synthesis of sphinganine from phthalimido protected serine aldehyde **21** (Scheme 6). The latter, prepared using Newman's procedure[35] in four steps from L-serine, was reacted with pentadecylmagnesium bromide. The product mixture was subjected to acid hydrolysis of the acetate to give a separable mixture of the desired *erythro*-**22** and *threo* isomers in 13 and 1% yields from **21**. Lastly, hydrazinolysis provided *erythro*-sphinganine **23**.[36]

3.2.2. From Garner's aldehyde. In the late 1980's, Garner described the first synthesis of the aldehyde **24**, now frequently referred to as Garner's aldehyde.[37] Others since then have optimized the synthesis.[38] Garner's aldehyde may now be the most commonly used single starting material for the preparation of 1,2-aminoalcohols and 2-amino-1,3-diols, including sphinganines. There are many methods reported for the diastereoselective addition of alkyl, alkenyl and alkynyl nucleophiles to Garner's aldehyde. In one example, used for the synthesis of sphinganine (Scheme 7), aldehyde **24** was converted to propargylic alcohol **25**, which was reduced to the corresponding alkane. D-*erythro*-Sphinganine (**23**) was available by double deprotection.[39]

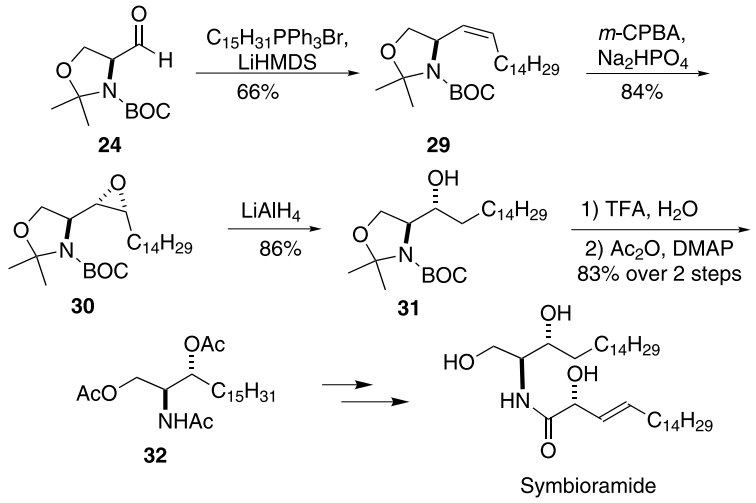
In 1998 Villard completed the synthesis of two diastereomeric, chain-shortened sphinganines via the highly diastereoselective addition of an *n*-hexyl group to aldehyde **24** under various conditions (Scheme 8). In a non-coordinating solvent, toluene, dihexylzinc gave the *anti* diastereoisomer **26** (67% yield) with 1:9 selectivity. However the addition of zinc chloride reversed the selectivity in favor of the *syn* isomer **27** (79% yield, *syn*/*anti* 83:17). These observations were explained by invoking the Felkin–Ahn and Cram-chelate models, respectively. Hexylmagnesium bromide in ether also gave, preferentially, the *syn* isomer (90%, *syn*/*anti* 95:5), again attributed to a chelation controlled transition state. The addition of zinc chloride did not increase the *syn* selectivity. Surprisingly, use of a more coordinating solvent (THF) or of additives,



Scheme 7.



Scheme 8.



Scheme 9.

such as HMPA, both of which might be expected to disrupt the chelate, did not alter the diastereoselectivity to any significant extent. Lastly, *n*-hexyllithium in THF gave the *anti* product only, but in a much reduced 23% yield.[40]

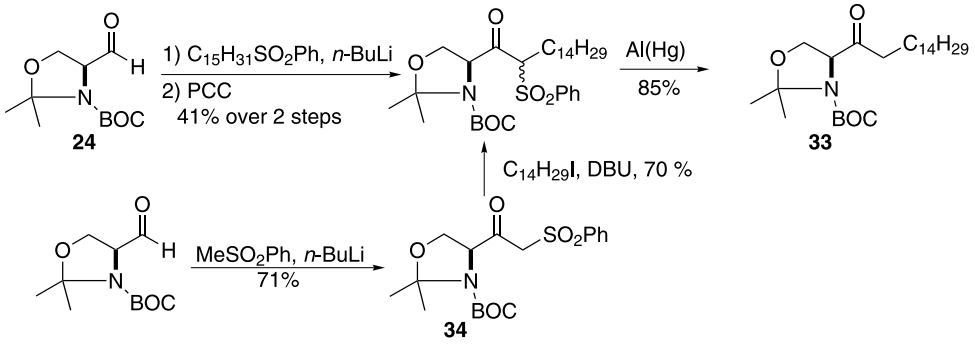
In closely related methodology, Azuma reported the total synthesis of *erythro*- and *threo*-sphinganine (Scheme 9). The *erythro* isomer was converted to the sphingoid base of symbioramide, which exhibited antileukemic activity against L-1210 murine leukemia cells and also increased the sarcoplasmic reticulum Ca^{2+}-ATPase activity. The key step was the selective (*Z*/*E*, 9:1) formation of *Z*-alkene **29**, which was achieved by a modification of a procedure developed by Dondoni et al. using lithium hexamethyldisilazide as base. Epoxidation of **29** with *m*-chloroperbenzoic acid (*m*-CPBA) in phosphate buffered THF occurred selectively from the bottom face (*de* 84%) to give **30**. Reduction with lithium aluminum hydride was highly regioselective and gave **31** in 86% yield. Deprotection with trifluoroacetic acid (TFA) gave the aminodiol, which was characterized as the triacetate **32**. The *threo* isomer was prepared in an analogous manner from the minor epoxide formed along with **30**.[41]

Bittman and co-workers prepared D-*erythro*-sphinganine from Garner's aldehyde **24**, as shown in Scheme 10. Addition of pentadecylphenylsulfone to **24** gave the β-hydroxysulfone which was oxidized to the corresponding ketone with PCC. The sulfone was removed with aluminum amalgam to give ketone **33**, which had previously been converted to D-*erythro*-sphinganine. An alternative pathway involved alkylation of ketosulfone **34**.[42]

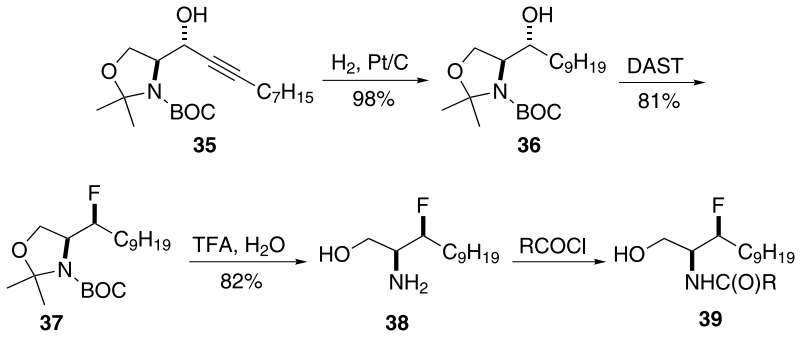
Recently, Herdewijn and co-workers prepared short chain analogs of sphinganine in which the C3 alcohol had been replaced with a C3 fluoride (Scheme 11). These compounds were designed as inhibitors of dihydroceramide desaturase and ceramide synthase (see Fig. 4), two enzymes involved in the biosynthesis of sphingolipids. *erythro*-Alkynol **35**, derived from Garner's aldehyde and lithium nonynolide, was reduced to give saturated alcohol **36**. The alcohol was fluorinated with diethylaminosulfur trifluoride (DAST), to give **37**, deprotected to **38** and acylated to give *threo*-fluoroceramide **39**. The corresponding *erythro* isomer was obtained from the *threo* isomer of **35**.[18] The same group had earlier used a similar procedure to prepare non-fluorinated, short chain sphinganine analogues.[43]

3.3. From serine derived esters

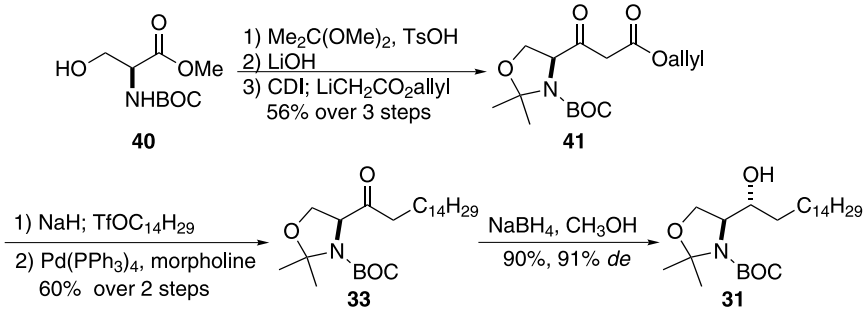
There are other methods in which serine was utilized as the starting material for the synthesis of sphinganines. Recently, Hoffman demonstrated the use of commercially available serine ester **40** for the synthesis of the biomimetic precursor ketosphinganine **33** as well as *erythro*-sphinganine **31** in protected form (Scheme 12). The protected serine ester **40** was converted to the corresponding oxazolidine β-keto-ester **41**. Alkylation with 1-tetradecyl triflate, followed



Scheme 10.



Scheme 11.

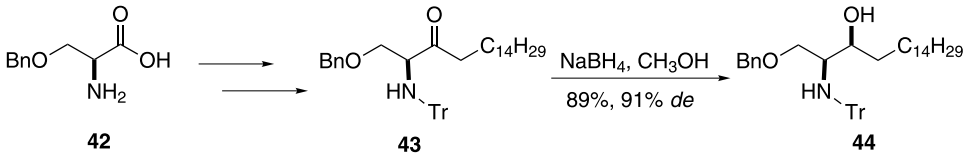


Scheme 12.

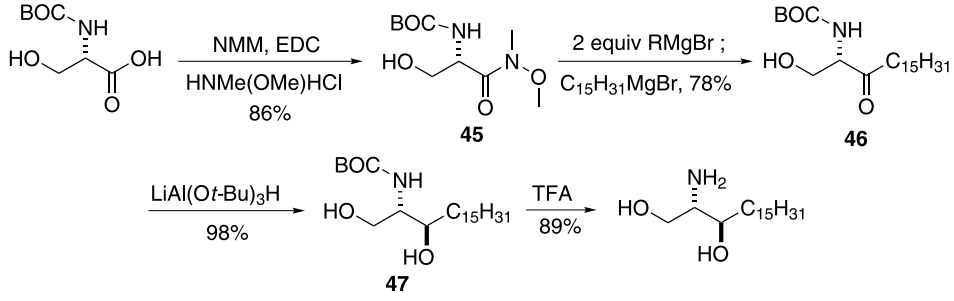
by palladium catalyzed deallylation and in situ decarboxylation, gave protected 3-ketosphinganine **33**. Reduction gave sphinganine derivative **31**. Although the alkylation could be achieved using 1-bromotetradecane, refluxing with THF/HMPA (5:1) for 6 h in the presence of NaI was required for effective conversion. The triflate was preferred because there was less epimerization during the reaction. The use of the allyl ester was a significant improvement over other possibilities, such as a *tert*-butyl ester, as palladium not only removed the allyl group under mild conditions but also catalyzed the decarboxylation at room temperature.[44] In another paper by the same group, acid **42** was converted to **43** by a similar procedure. Reduction with sodium borohydride reduction was *syn*-selective and gave protected L-*threo*-sphinganine **44** (Scheme 13).[45] These two results demonstrated that, by starting from L- or D-serine and choosing appropriate reduction conditions, any one of the four stereoisomers of sphinganine can be obtained selectively.

3.4. From serine-derived Weinreb amide

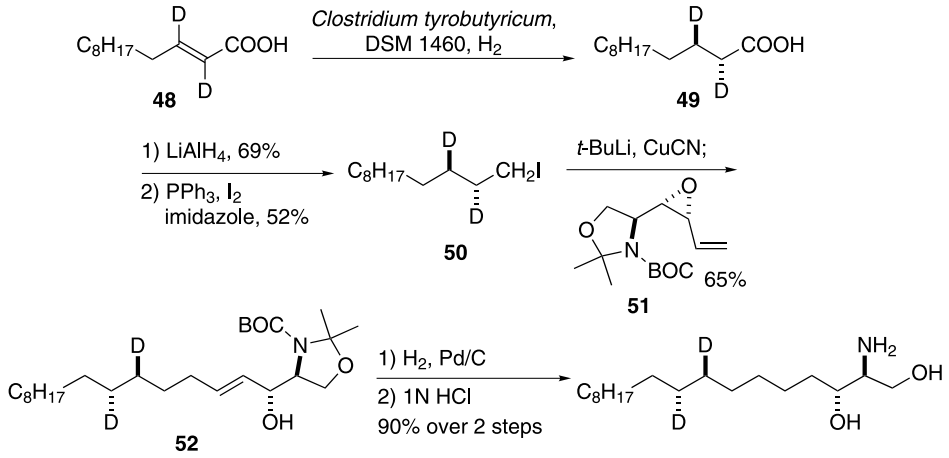
Howell and co-workers prepared D-*erythro*-sphinganine in only four steps from commercially available *N*-BOC-L-serine (Scheme 14). In the first step condensation with *N*,*O*-dimethylhydroxylamine gave Weinreb amide **45**. Efficient conversion of **45** to ketone **46** required more than 3 equiv of pentadecylmagnesium bromide because of the presence of acidic NH and OH sites. This was wasteful of the Grignard reagent, which was not commercially available.



Scheme 13.



Scheme 14.



Scheme 15.

Subsequently, 2 equiv of a sacrificial base (RMgX) were employed to deprotonate the acidic sites prior to the addition of pentadecylmagnesium bromide. Selective (*anti*) reduction of the ketone, according to the method of Hoffman, gave aminodiol **47**, which was deprotected to give the target.[46]

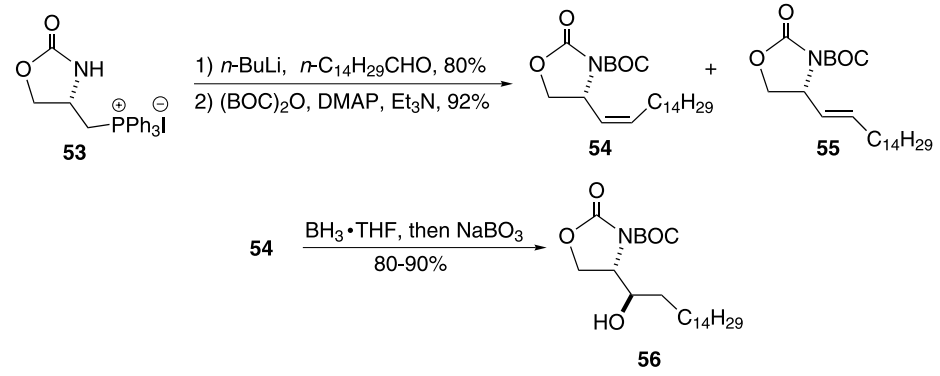
3.5. From serine-derived vinyloxirane

Deuterated *erythro*-sphinganine was prepared from serine-derived vinyloxirane **51** as shown in Scheme 15. Biocatalytic reduction of (*E*)-[2,3-$^2H_2$]dodec-2-enoate **48** with broken cells of *Clostridium tyrobutyricum*, DSM 1460 in the presence of hydrogen gas as the ultimate electron donor gave stereospecifically labeled (2*R*,3*R*)-[2,3-$^2H_2$]-dodecanoic acid **49** by a formal *anti* addition of H_2. Reduction, followed by iodination, gave **50**. In situ lithium-iodide exchange, followed by alkylation with **51**, provided **52** as a single (*E*) isomer by S_N2' alkylation. Hydrogenation, followed by double deprotection, gave deuterium labeled C-18 *erythro*-sphinganine.[47]

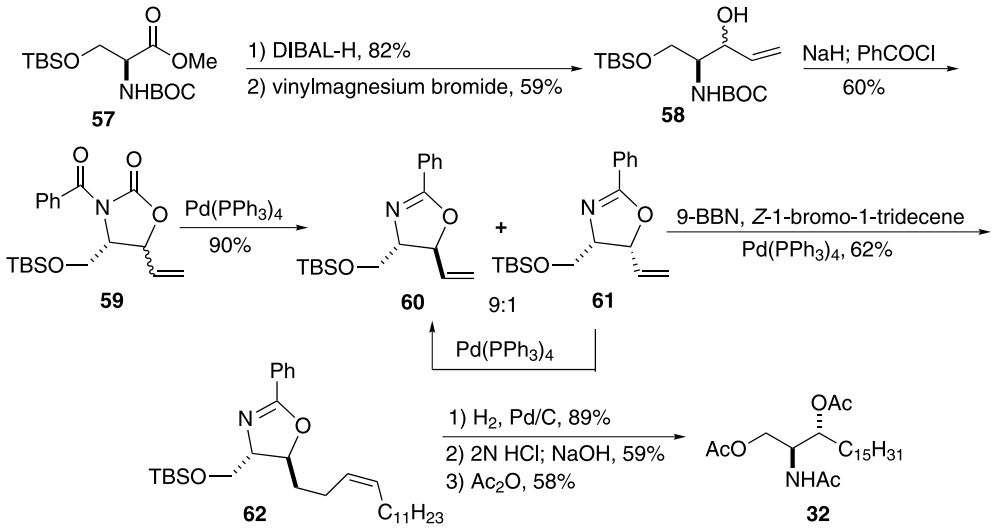
3.6. From serine-derived heterocycles

3.6.1. From serine-derived oxazolidinone. Sibi and co-workers had previously demonstrated the preparation of a nucleophilic alaninol synthon **53** from serine. In a later paper, Wittig reaction of this with pentadecanal gave alkenes **54** and **55** in a 1:1 ratio (Scheme 16). In a study of the hydroboration of these alkenes with $BH_3 \cdot THF$, it was found that the regio- and stereoselectivity of the outcome was highly dependent on the geometry of the double bond, the presence or absence of a protecting group on nitrogen, the reaction solvent and the temperature. The highest selectivities for the desired *erythro*-product were obtained using BOC protected *cis* alkene **54** at 0 °C in THF, which gave **56** with 19:1 regioselectivity and 16:1 stereoselectivity. The observed regioselectivity, giving the 1,2,3-trisubstituted system was explained by the presence of the allylic heteroatom directing the boron atom to the alpha position. The stereoselectivity was explained by the effect of $A^{1,3}$ strain on the ground state conformation of the *cis* alkene which was accentuated by the bulky BOC protecting group.[48]

3.6.2. From serine-derived oxazolines. In another example, Cook and Pararajasingham prepared sphinganines from serine-derived ester **57** (Scheme 17). Reduction to the aldehyde followed by the addition of vinylmagnesium bromide gave alcohol **58** as a 6:4 mixture of *syn* to *anti* diastereoisomers. Cyclization followed by in situ acylation gave **59** as a mixture of *syn* and *anti* isomers in the same ratio. Palladium-catalyzed isomerization gave the *trans* isomer **60**, which could be separated from the corresponding *cis* isomer **61**, in 90% yield (*trans*/*cis*, 91:9). The minor isomer **61** could be recycled under the same reaction conditions to give the same product ratio. Hydroboration of **60** with 9-BBN, followed by Suzuki coupling with 1-bromo-1-tridecene, gave **62**. Reduction of the double bond followed by hydrolysis gave D-*erythro*-sphinganine,

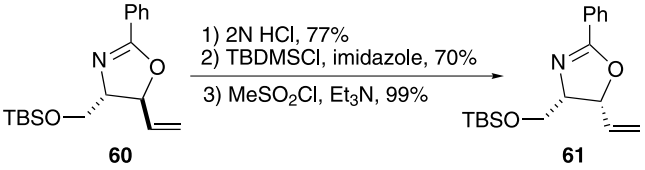


Scheme 16.



Scheme 17.

which was isolated and characterized as the triacetate **32**. L-*threo*-Sphinganine was obtained by the same protocol from **61**, the *cis* isomer of **60**. However, as **61** was only available in small quantities from the palladium catalyzed isomerization of **59**, it was prepared separately from **60** by partial hydrolysis, reprotection and cyclization with inversion (Scheme 18).[49]



Scheme 18.

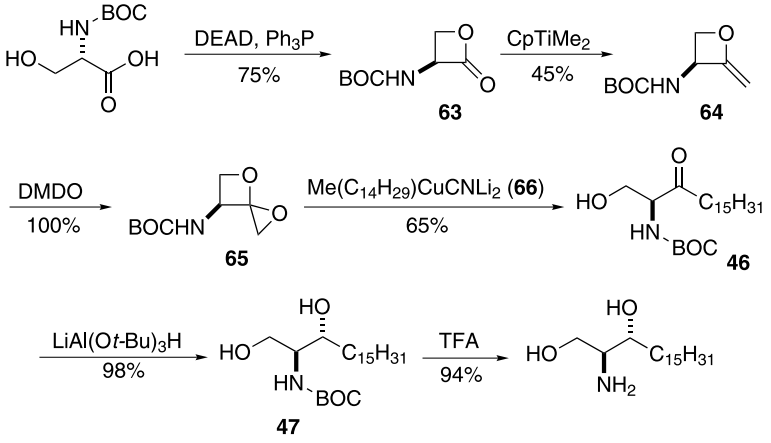
3.6.3. From serine-derived 1,5-dioxaspiro[3.2]hexane. Howell and co-workers have exploited their novel templates, 2-methyleneoxetanes and 1,5-dioxaspiro[3,2]hexanes, for the synthesis of D-*erythro*-sphinganine, as shown in Scheme 19. *N*-BOC-L-Serine was converted into β-lactone **63** under Mitsunobu conditions. Methylenation using the Petasis reagent gave 2-methyleneoxetane **64**, which was converted quantitatively to dioxaspirohexane **65** with dimethyldioxirane (DMDO). Considerable experimentation was required to promote the ring opening of **65** to **46**. The use of Grignard or organolithium reagents gave, not surprisingly, complex mixtures. The use of thienylcuprates represented an improvement, but the best result was realized with a mixed alkyl cuprate **66**, which gave **46** in 65% yield. Reduction under Hoffman conditions gave *erythro* adduct **47**. This was deprotected to give D-*erythro*-sphinganine.[50]

4. Sphinganines from sugars

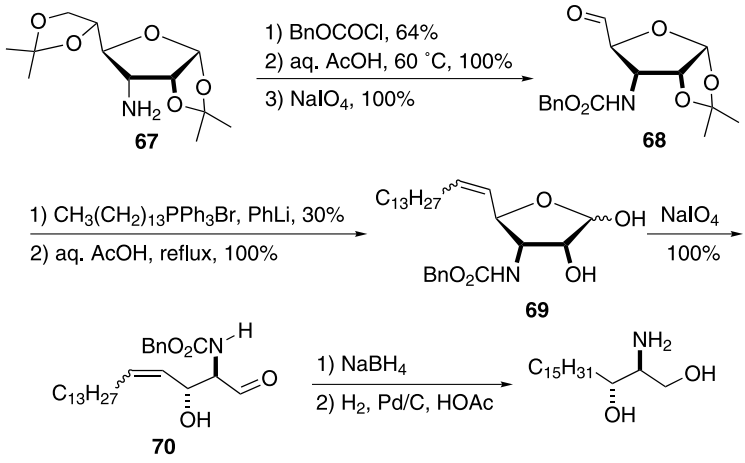
Sugars are commonly used as chiral templates for the synthesis of biologically active compounds. The inherent advantages of sugars as starting materials include: (1) their high degree of functionality; (2) the ability to selectively manipulate each position; (3) the extensive stereodiversity and (4) perhaps most importantly, their optical purity. Given this, for sphinganine synthesis it is logical that sugars have been utilized as a source for the aminodiol portion, to which the hydrophobic tail becomes attached.

4.1. From allofuranose

Reist and Christie reported in 1970 an example of a stereoselective synthesis of *erythro*-shinganine from a derivative of protected α-allofuranose (Scheme 20).



Scheme 19.



Scheme 20.

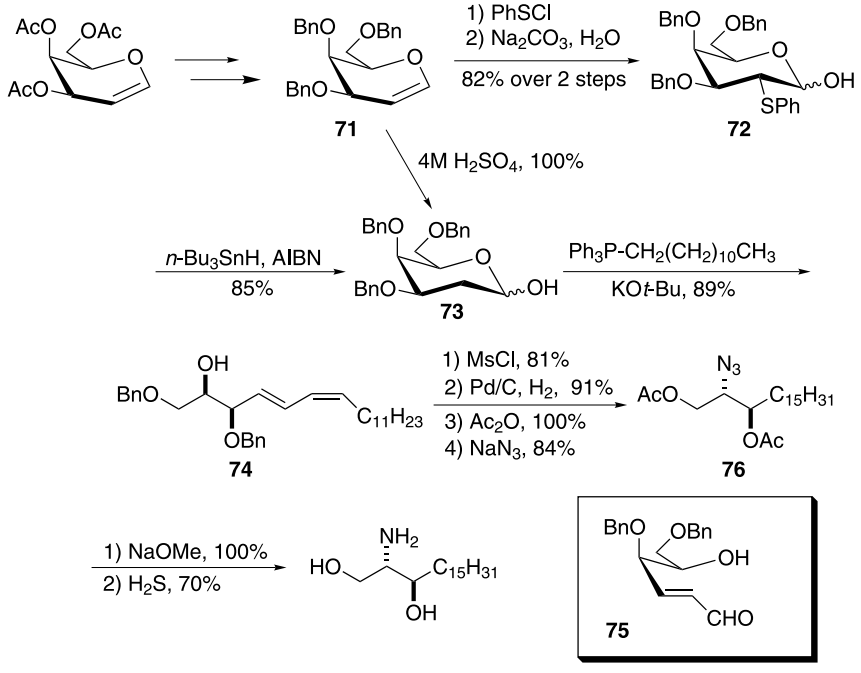
Protection of the amino group, selective deprotection of the most accessible isopropylidene group and oxidative cleavage of **67** provided aldehyde **68**. Wittig reaction gave a mixture of the *cis* and *trans* isomers, which was not separated but hydrolyzed to give **69**. Oxidative cleavage of the diol gave aldehyde **70**. Reduction of the carbonyl group, followed by simultaneous hydrogenation and deprotection, gave the desired sphinganine, which was characterized as the triacetate.[51]

4.2. From 3,4,6-*tri*-*O*-acetyl-D-galactal

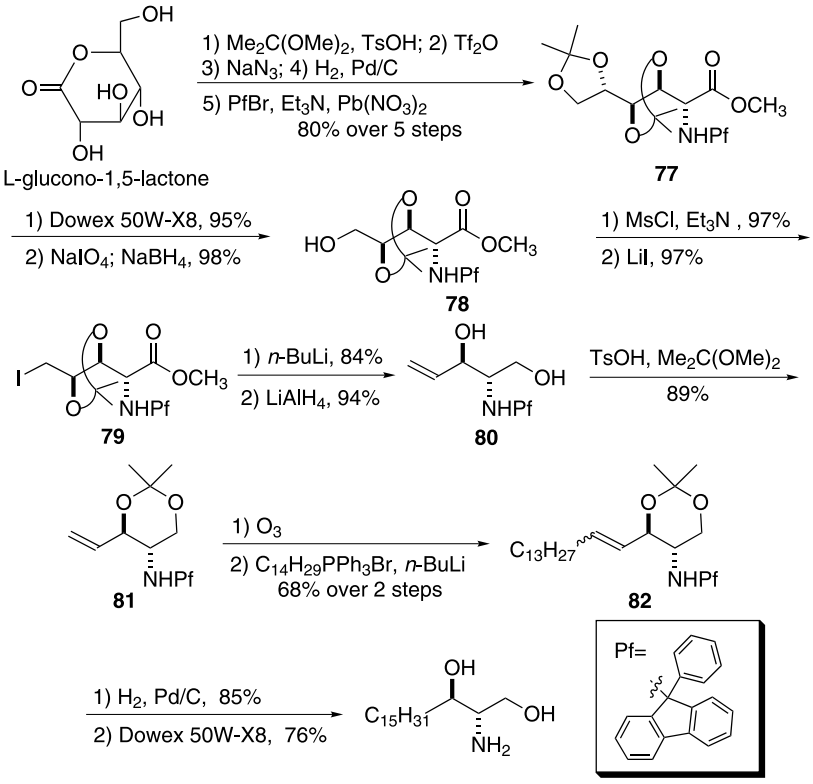
In the mid-1990's Wild and Schmidt reported the synthesis of sphinganine from commercially available 3,4,6-*tri*-*O*-acetyl-D-galactal (Scheme 21). Known conversion to tribenzyl derivative **71**, followed by addition of phenylsulfenyl chloride and hydrolysis, gave **72**. The phenylthio group was then removed under radical conditions to give **73**. Alternatively, it was found that treatment of **71** with sulfuric acid gave **73** in one step. Using potassium *tert*-butoxide as base, Wittig reaction with dodecyltriphenylphosphonium bromide gave diene **74**. The outcome was explained by initial base promoted ring opening of the sugar and benzyl alcohol elimination to give **75**, prior to alkene formation. Mesylation of the free alcohol, reduction/deprotection, acetylation and azide formation gave **76**. Deprotection and reduction of the azide with hydrogen sulphide gave D-*erythro*-sphinganine, which was characterized as the triacetate.[52]

4.3. From glucose-derived lactones

Recently, Park and co-workers utilized a carbohydrate template to prepare both the D-*erythro* and L-*threo* isomers of sphinganine (Scheme 22). The synthesis of the *erythro* isomer commenced with known diisopropylidene ester **77**, which was synthesized from L-glucono-1,5-lactone in 5 steps. A 9-phenyl-9-fluorenyl protecting group was chosen because of its ability to inhibit deprotonation at the adjacent position. Selective deprotection of the terminal



Scheme 21.



Scheme 22.

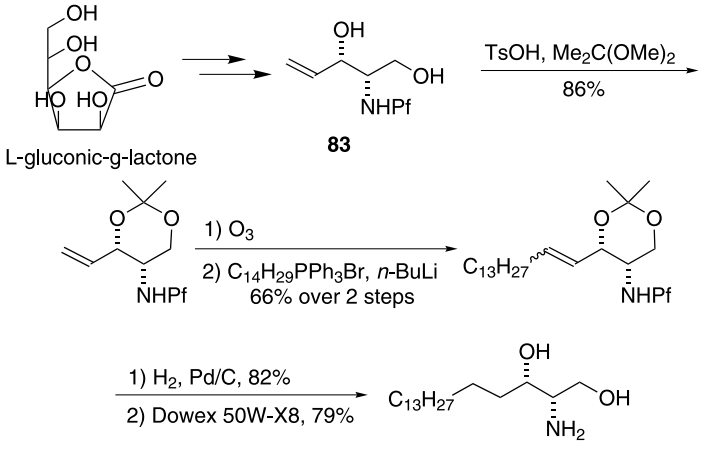
isopropylidene with a cationic resin was followed by oxidative cleavage to give **78**. The terminal alcohol was converted to iodide **79** by mesylation, followed by treatment with lithium iodide. Treatment of **79** with *n*-BuLi resulted in metal/iodide exchange, followed by 1,2-elimination, to introduce the terminal alkene. Subsequent reduction of the ester provided aminodiol **80**. Protection and ozonolysis, followed by Wittig reaction, gave olefin **82** as a 16:1 mixture of *Z* and *E* isomers. Simultaneous reduction and deprotection of the amine followed by hydrolysis of the acetonide gave L-*erythro*-sphinganine.[53]

The *threo* isomer of sphinganine was synthesized by Park, as shown in Scheme 23. L-Gluconic acid γ-lactone was converted to diol **83**, which is the *syn* isomer of **80**. Conversion of this to L-*threo*-sphinganine followed that of **80** in Scheme 22.[53]

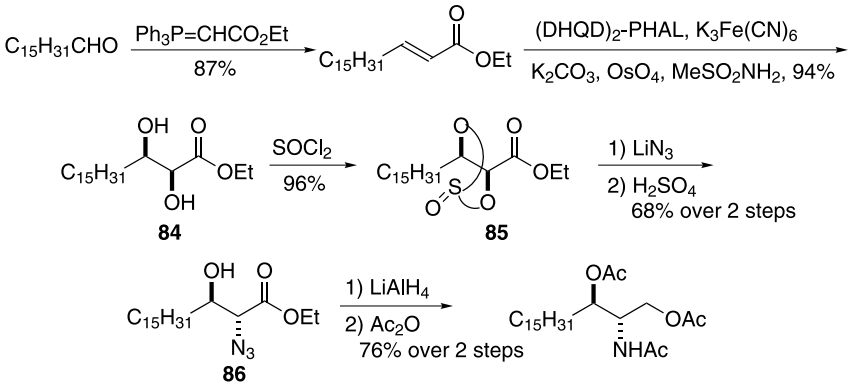
5. Sphinganines using asymmetric methods

5.1. Using Sharpless asymmetric dihydroxylation

A number of asymmetric transformations have been employed for the syntheses of sphinganines. Simple olefins have been transformed into useful homochiral building blocks through Sharpless asymmetric dihydroxylation (AD) and Sharpless asymmetric epoxidation (AE) reactions. For example, Sharpless AD was used to access the



Scheme 23.



Scheme 24.

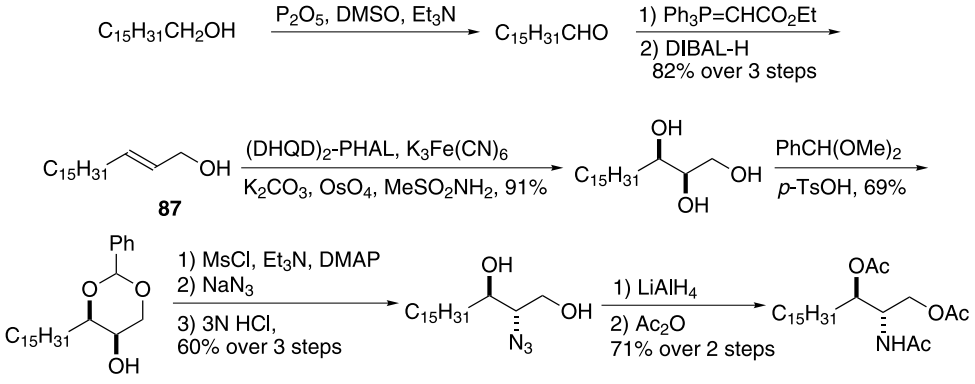
2-amino-1,3-diol moiety of sphinganine in two similar papers published at almost the same time by the groups of Kumar and Bittman. Kumar's procedure is shown in Scheme 24. Starting with hexadecanal, Wittig olefination, followed by Sharpless AD, gave diol **84** in excellent yield with 99% ee. Treatment of **84** with thionyl chloride afforded the cyclic sulfite **85**. Regioselective ring opening with lithium azide, followed by sulfite hydrolysis gave azido alcohol **86**. Reduction gave D-*erythro*-sphinganine, which was characterized as the triacetate.[54] Bittman's procedure differed mainly in that oxidation of the cyclic sulfite **85** to the corresponding cyclic sulfate preceded ring opening with azide.[55]

In a similar, slightly later paper from Kumar's group allylic alcohol **87** (Scheme 25), was used as the substrate for the AD reaction. Further transformation along largely conventional lines gave the same triacetate.[56]

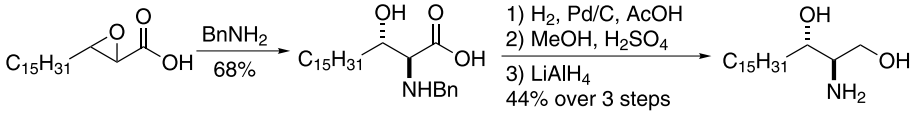
5.2. Using Sharpless asymmetric epoxidation

As shown earlier by Grob's racemic synthesis (Scheme 3), ring opening of an epoxide can be a useful method for the synthesis of sphinganines. Unfortunately, ring opening of oxirane **9** via aminolysis gave regioisomeric mixtures of aminodiols. Umemura and Mori obtained a similar result.[57,58] However, Sisido et al. reported that when *trans*-2,3-epoxyoctadecanoic acid was treated with benzylamine in water, regioselective ring opening occurred to give *dl-erythro*-2-benzylamino-3-hydroxyoctadecanoic acid in 68% yield after recrystallization. Presumably, as for the cyclic sulfite **85**, the ester group directs attack to the α position. Debenzylation, Fischer esterification, and reduction gave racemic *erythro*-sphinganine (Scheme 26).[59]

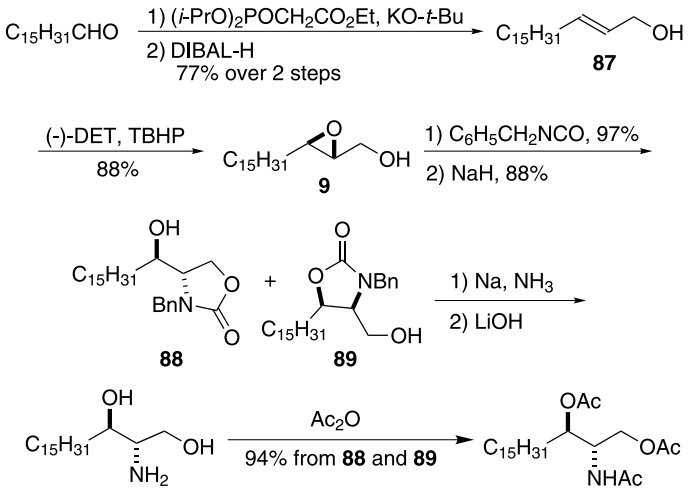
In order to use this approach to obtain homochiral material, a procedure involving an asymmetric epoxidation followed by regioselective ring opening must be employed. Roush solved both of these problems using the Sharpless AE reaction, followed by a directed intramolecular aminolysis, to prepare D-*erythro*-sphinganine (Scheme 27). Known allylic alcohol **87**, was subjected to Sharpless AE to give homochiral epoxide **9** in 88% yield and >95% optical purity. Reaction with benzylisocyanate, followed by cyclization under basic conditions, provided carbamates **88** and **89** (1:1 ratio). Deprotection of this mixture gave the desired sphinganine, which was characterized as the corresponding triacetate.[60]



Scheme 25.



Scheme 26.



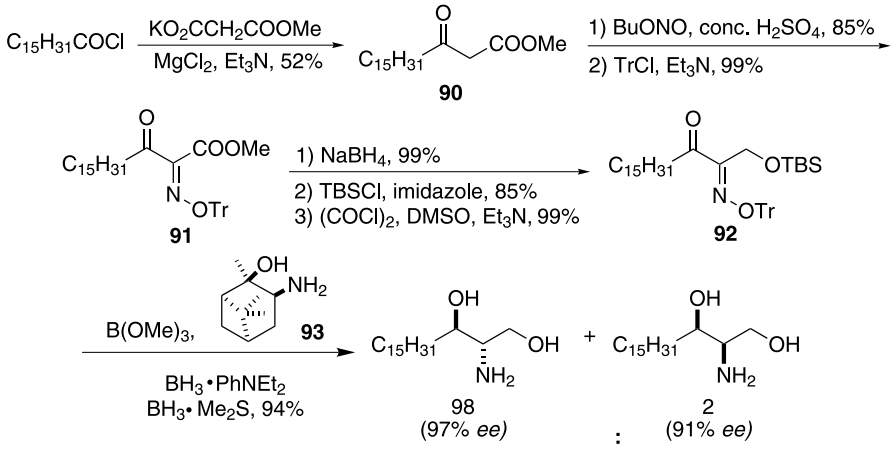
Scheme 27.

5.3. Using asymmetric reduction

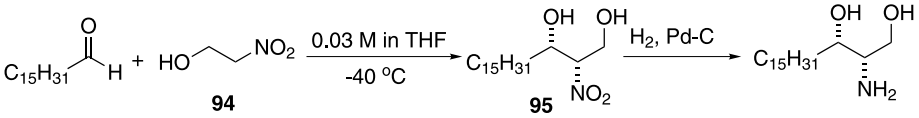
Other asymmetric transformations have been used for the synthesis of sphinganines. Masui reported a stereoselective synthesis of sphinganine isomers in which the key step involved the stereoselective reduction of α-oxoketoximes with a borane in the presence of a catalyst prepared from chiral aminoalcohols and trimethylborate (Scheme 28). The synthesis started with the condensation of hexadecanoyl chloride with methyl malonate monopotassium salt to give β-ketoester **90**. Nitrosation, followed by tritylation, gave ester **91**, which was further transformed by reduction of both carbonyl groups. Selective silylation of the primary alcohol and Swern oxidation of the secondary one gave ketone **92**. After screening a number of catalysts and reducing agents, the best *erythro*/*threo* selectivity (98:2) was obtained using a mixture of borane diethylaniline complex/trimethylborate and amino alcohol **93**, followed by the addition of borane-dimethylsulfide. Both diasteriomers were obtained in >90% ee. Under similar conditions reduction of **91** gave predominantly the *threo* isomer, although with lower selectivity (*erythro*/*threo* 1:4). Again both isomers were obtained in high (>93%) ee. Thus, this method would allow selective access to all four stereoisomers of the 2-amino-1,3-diol by changing the combination of substrate and the chiral amino alcohol **93**.[61]

5.4. Using asymmetric Henry reaction

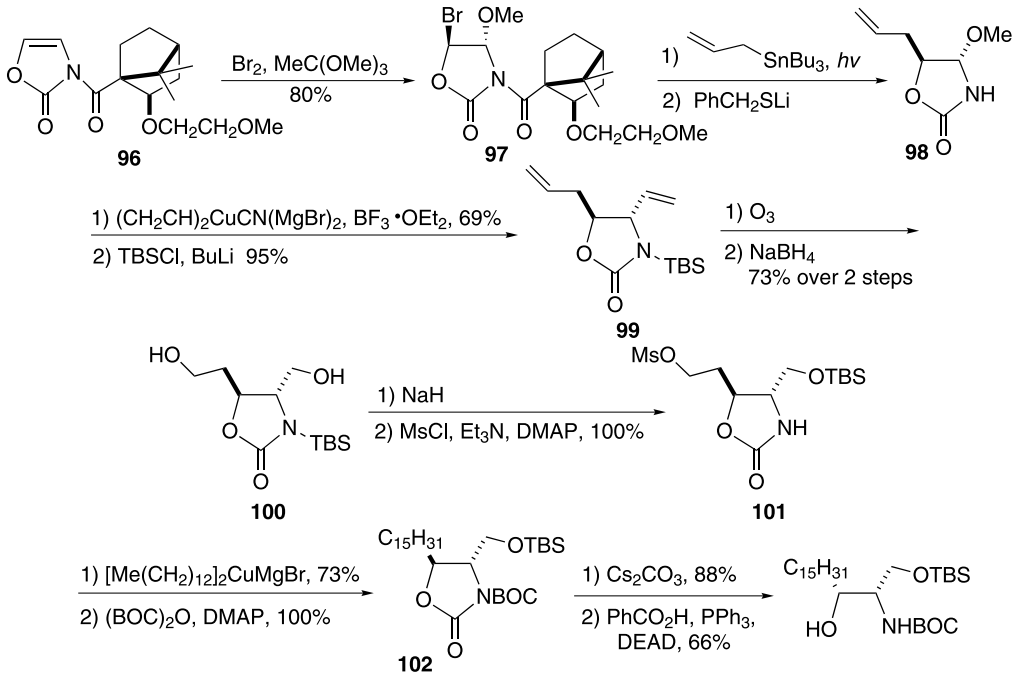
An enantio- and diastereoselective nitroaldol reaction was used to prepare *threo*-sphinganine. Reaction of hexadecanal with nitroethane **94** in the presence of 10 mol % of a lanthanum *tris*-BINOL catalyst gave adduct **95** in 78% yield with high enantio- and diastereoselectivity (*syn*/*anti* 91:9, *syn* 97% ee). Reduction gave *threo*-sphinganine (Scheme 29).[62]



Scheme 28.



Scheme 29.



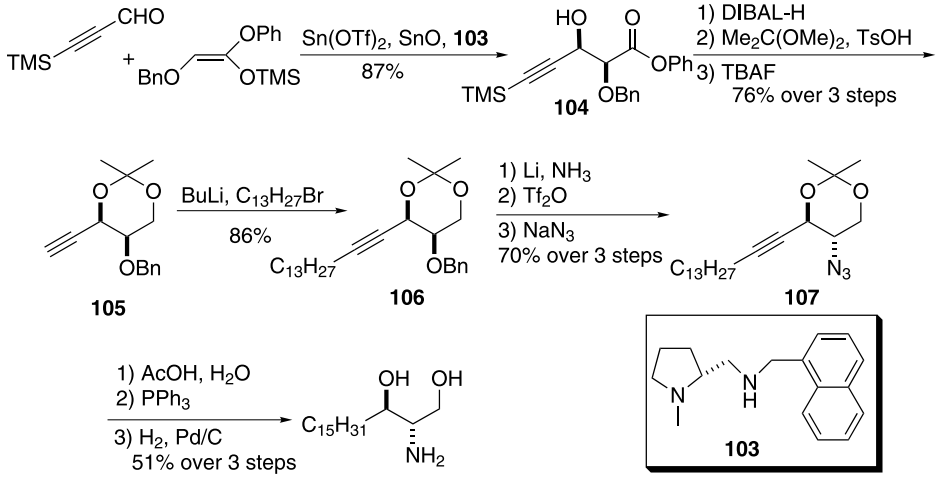
Scheme 30.

5.5. Using chiral auxiliaries

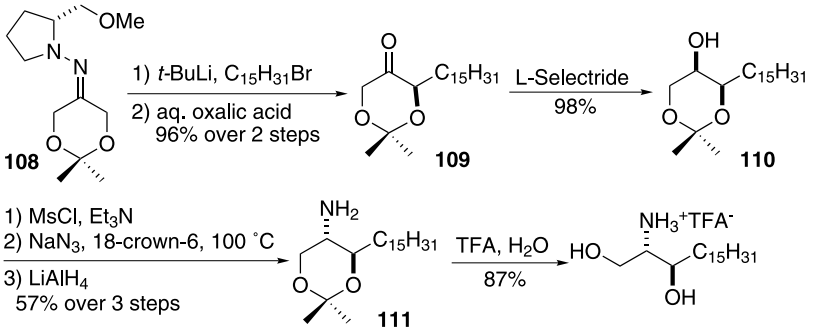
Chiral auxiliaries have also been used to establish the stereochemistry of the aminodiol portion of sphinganines. Addition of bromine and methanol to **96** gave the intermediate bromomethoxyoxazolidinone **97** with high regio- and stereoselectivity. Radical allylation with retention of configuration, followed by deprotection, gave **98** in 96% *de*. Lewis acid assisted iminium ion formation, followed by vinylation and silylation, provided **99**. Ozonolysis and then reduction afforded diol **100**. Treatment of this with NaH resulted in an intramolecular migration of the silyl group from nitrogen to the proximal alcohol. This allowed differentiation between the two primary alcohols. Mesylation then provided **101**. Chain elongation gave protected *threo*-sphinganine **102**. This was smoothly converted to the *erythro* isomer by hydrolysis followed by inversion of the hydroxyl group under Mitsunobu conditions (Scheme 30).[63]

An asymmetric aldol reaction was used to set the key sphinganine stereochemistry in a paper published by Kobayashi and Furuta (Scheme 31). The reaction between trimethylsilylpropynal and (*Z*)-2-benzyloxy-1-phenoxy-1-trimethylsilyloxyethene in the presence of tin(II) triflate, tin(II) oxide and the chiral diamine **103** gave the aldol adduct **104** (*syn*/*anti* 97:3, *syn* isomer 91% ee). The ester was reduced with DIBAL-H to give the diol, which was protected as the acetonide and desilylated to give **105**. The enantiomer of chiral diamine **103** can be used to make *ent*-**104**. Deprotonation of the alkyne, followed by alkylation with tridecyl bromide, gave **106** from which the benzyl group was removed and the free alcohol converted to azide **107** via the triflate. The acetonide was deprotected, and the azide and alkyne groups were reduced successively to give D-*erythro*-sphinganine.[64]

Enders has used his well-known SAMP/RAMP hydrazones to prepare D-*erythro*-sphinganine with excellent yields and



Scheme 31.



Scheme 32.

stereocontrol (Scheme 32). RAMP hydrazone **108** was alkylated with pentadecyl bromide, and the hydrazone cleaved with oxalic acid. Ketone **109** was obtained in 96% yield with an *ee* >96%. The carbonyl group was reduced with L-selectride to give *syn* alcohol **110** in 98% yield with 96% ee. Inversion of configuration and conversion to the amine was achieved via the mesylate and azide, followed by reduction to **111**. Finally, deprotection gave the sphinganine TFA salt in 47% overall yield with *de* and *ee* both >96%.[65]

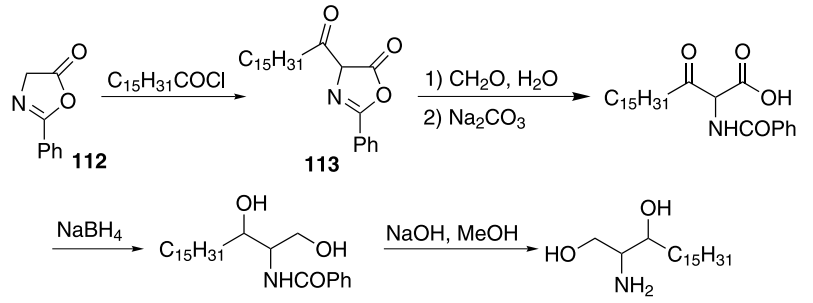
6. Sphinganines from miscellaneous methods

6.1. From crossed Claisen condensation

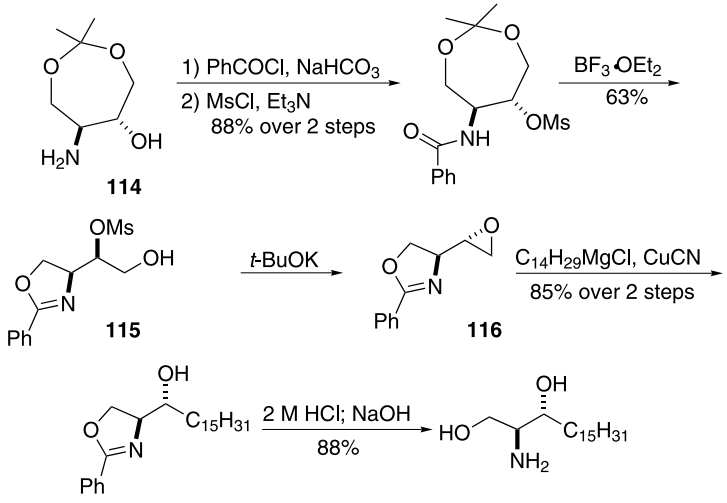
In the early 1970's racemic sphinganine was prepared as a mixture of the *erythro* and *threo* diastereomers via a crossed Claisen reaction from oxazolinone derivative **112** (Scheme 33). Deprotection of product **113**, followed by reduction and hydrolysis, gave the sphinganine mixture. The diastereoselectivity of the reduction was not specified. *erythro*-Sphinganine was isolated by selective crystallization of the dichloacetamide derivatives of the product mixture.[66]

6.2. From aminoalcohol derivatives

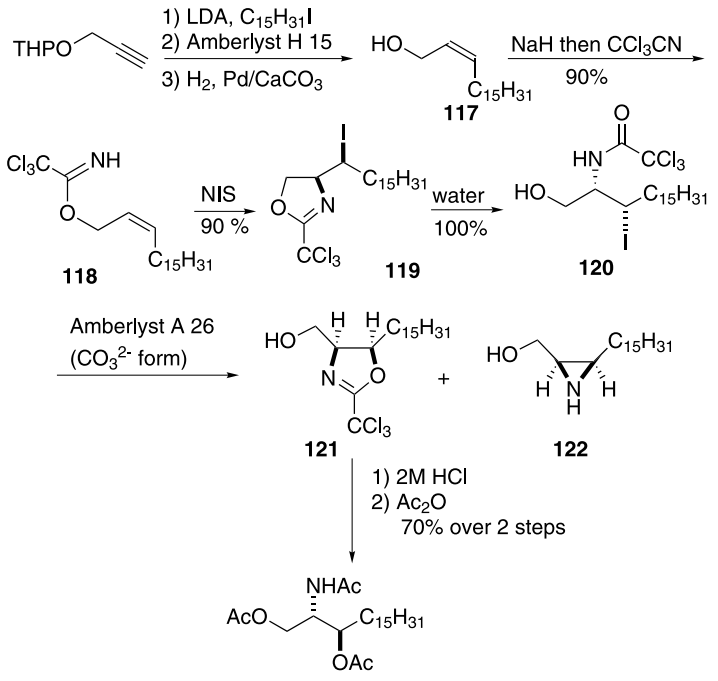
Hertweck and co-workers recently used chiral, inexpensive industrial intermediates for the synthesis of D-*erythro*-sphinganine (Scheme 34). 2-Aminobutane-1,3,4-triol derivative **114** and its enantiomer were available enantiomerically pure in bulk quantities by Ti-BINOL-catalyzed asymmetric aminolysis of the corresponding *meso*-epoxide. N-Acylation, mesylation and Lewis acid mediated cyclization/deprotection gave oxazoline **115**. Treatment of this



Scheme 33.



Scheme 34.



Scheme 35.

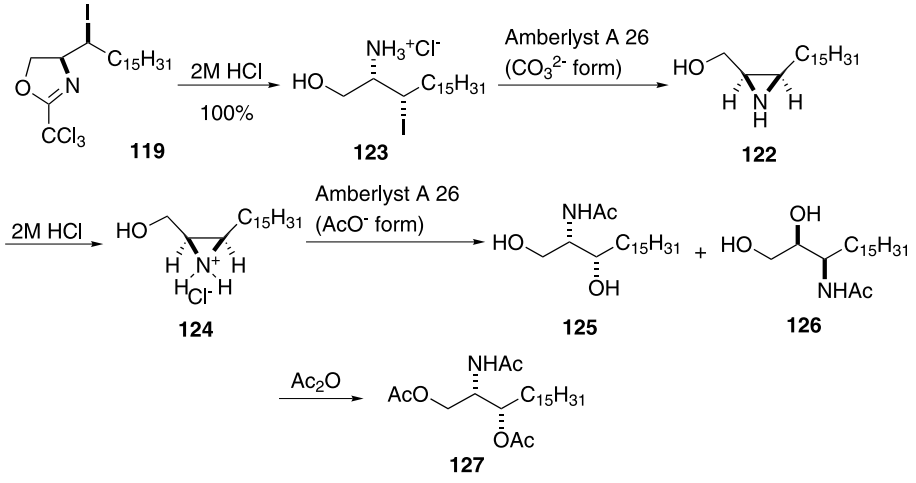
with potassium *tert*-butoxide gave epoxide **116**, which was not purified but exposed directly to an organocuprate reagent. Ring opening occurred regiospecifically, and, following acid and basic hydrolysis, D-*erythro*-sphinganine was obtained. The enantiomer of **114** gave L-*erythro*-sphinganine.[67]

6.3. From iodocyclization of unsaturated trichloroacetimidates

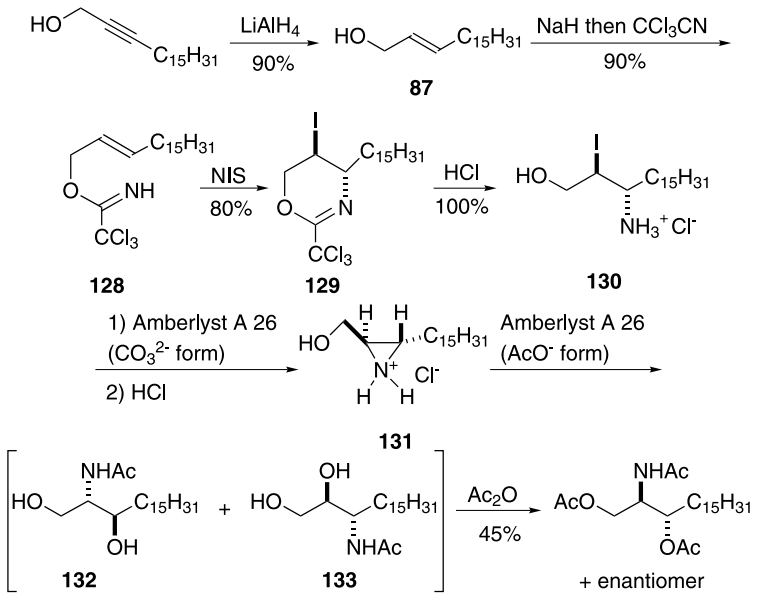
Another novel approach to sphinganines involved a diastereoselective iodocyclization of trichloroacetimidates, as demonstrated by Cardillo and co-workers in two papers. In the first (Scheme 35), THP protected propargyl alcohol was deprotonated with LDA and alkylated with pentadecyl iodide. The THP group was removed and the alkyne reduced to *cis* alkene **117**. The alcohol was converted to the trichloroacetimidate **118**, which, in the presence of N-iodosuccinimide (NIS), gave racemic oxazoline **119** in 90% yield. Hydrolysis under mild conditions (aq. acetone at reflux) gave amide **120**. Exposure to a basic ion exchange resin gave **121** and **122** (70:30). These were easily separable, and **121** hydrolyzed to racemic *erythro*-sphinganine, which was characterized as the triacetate.[68]

In the same paper, racemic *threo*-sphinganine was prepared from **119** (Scheme 36). Hydrolysis with 2 M HCl gave the *threo* salt **123** in quantitative yield. The remaining transformation required a double inversion at C3 with conversion of the iodide to an alcohol. This was achieved by base promoted formation of the intermediate aziridine **122** and protonation to the aziridinium ion **124**. Ring opening of the latter favored the desired regioisomer (**125**:**126**, 70:30). The mixture was separated after acetylation to give racemic *threo*-sphinganine **127**.[68]

In the second paper by the Cardillo group *trans* alkene **87**, obtained by reduction of octadec-2-yn-1-ol with lithium



Scheme 36.



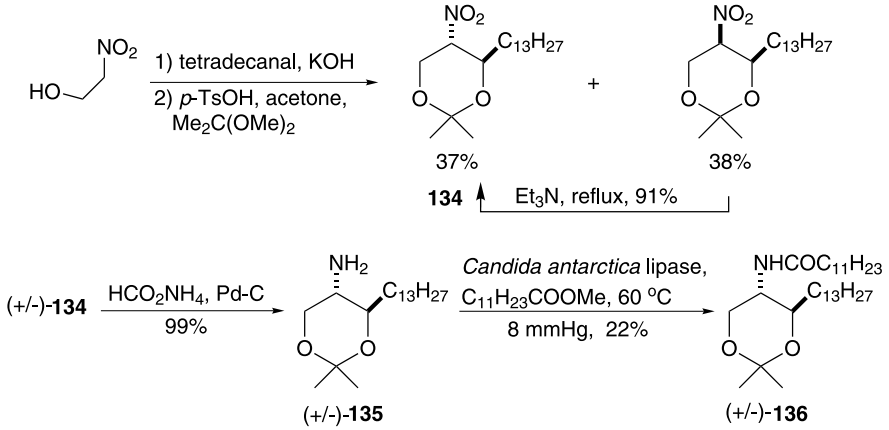
Scheme 37.

aluminum hydride, was used as the starting material instead of *cis* alkene **117** (Scheme 37). Compound **87** was converted to trichloroacetimidate **128** as described above. Electrophilic cyclization with NIS gave oxazine **129**, instead of the previously observed oxazoline. Hydrolysis of **129** gave ring opened ammonium salt **130**. Neutralization resulted in cyclization to the aziridine, which was protonated to give **131**. This underwent ring opening by acetate in a regioselective manner to give a 70:30 mixture of **132** and **133**. Acetylation gave a mixture of two compounds from which racemic *erythro*-sphinganine was separated. Alternatively, treatment of iodide **130** with Amberlyst A 26 (AcO^- form) gave the same mixture of **132** and **133** directly.[69]

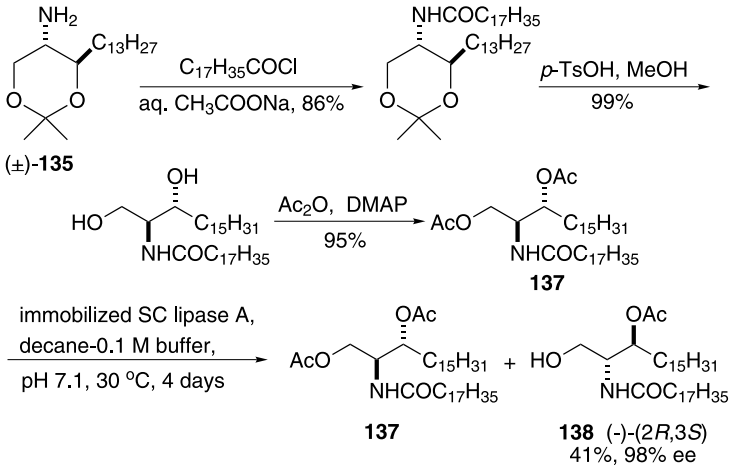
6.4. From enzymatic methods

Sugai and co-workers attempted an enzyme catalyzed enantiomeric kinetic resolution of dihydroceramides (Scheme 38). The reaction started with racemic **134**, prepared via Grob's nitroaldol synthesis, followed by acetonide formation. Reduction gave amine **135**. Acylation using *Candida antarctica* lipase was slow, even under forcing conditions of elevated temperatures and reduced pressure, and gave compound **136** in low yield as the racemic mixture.[70]

With the failure of enzyme catalyzed enantiomeric kinetic resolution described above, the Sugai group developed an alternative strategy (Scheme 39). Acylation, deprotection and acetylation of **135** provided racemic **137**. Compound **137** was subjected to a number of lipases. After screening, *Burkholderia cepacia* lipase (SC lipase) was shown to be the most promising candidate. Although three products, the monoacetate, diol and unreacted ceramide, were obtained, SC lipase predominantly hydrolyzed the unnatural form of the ceramide acetate (2*R*,3*S*-**137**) to give monoacetate **138**. However, the hydrophobic and crystalline nature of **137** meant that a two phase decane/buffer system was required, and, as the reaction progressed, an intractable emulsion formed. To solve this problem, an immobilized form of the enzyme was used. Under these conditions, the reaction stopped at the monoacetate stage and gave **138** in 41% yield and 98% ee.[70]



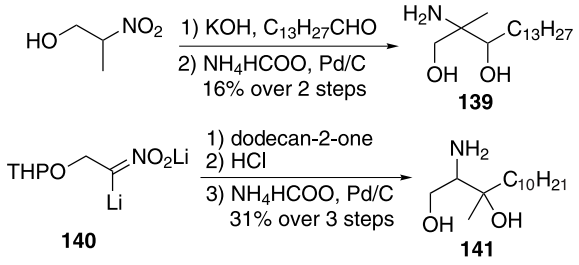
Scheme 38.



Scheme 39.

6.5. From nitroaldol condensation (racemic)

A nitroaldol reaction was also used to prepare sphinganines modified in the aminodiol region (Scheme 40). For example, addition of 2-nitro-1-propanol to tetradecanal followed by reduction using ammonium formate gave **139**. Alternatively, nitronate salt **140** added to ketones to give, following deprotection and reduction, **141**.[71] Use of the nitroaldol reaction has in fact been used several times to prepare sphinganines. For example, Umemura and Mori used a similar procedure to prepare *erythro*- and *threo*-aplidiasphingosine,[72] and much earlier Majhofer–Orescanin and Prostenik prepared mixtures of all four isomers of C-20 sphinganine. Glutamic acid was then used to resolve the enantiomers.[73]



Scheme 40.

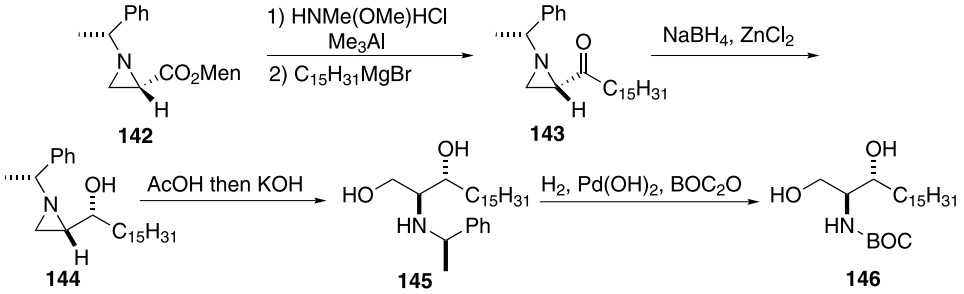
6.6. From chiral aziridine

Lee and Ha and co-workers prepared *N*-BOC-D-*erythro*-sphinganine from commercially available aziridine menthyl ester **142** (Scheme 41). Weinreb amide formation using trimethylaluminum was followed by reaction with pentadecylmagnesium bromide to give ketone **143**. Chelate controlled reduction by sodium borohydride in the presence of zinc chloride gave *erythro* alcohol **144** in 84% yield and 88% *ee*. Ketones with shorter side chains gave higher yields and stereoselectivities. This was attributed to the poor solubility of **143**. Ring opening of the aziridine occurred regiospecifically at the most accessible position to give **145**. Finally, exchange of protecting groups to give **146** was achieved by hydrogenolysis in the presence of BOC_2O. L-*threo*-Sphinganine was prepared in the same way following *threo* selective reduction of **143** with L-selectride (Scheme 42).[74]

Scheme 42.

7. Summary

Sphinganine synthesis has received increasing attention in recent years because of the growing recognition of the biological importance of a range of sphingolipids. Not surprisingly, the majority of reported approaches to sphinganines rely on the chiral pool, particularly serine. What is apparent is that, among the many elegant approaches to sphinganines, some of the newer serine-based



Scheme 41.

methodologies and some of the asymmetric strategies provide efficient and highly stereoselective access to diverse sphinganine structures.

Acknowledgements

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Biographical sketch

Amy Howell was born in Cleveland, OH and grew up in Wilmore, KY. She graduated with a B.Sc. from Wheaton College, IL and then taught for three years at high schools in Farmington, NM and Liberia, West Africa. She then returned to graduate school at the University of Kentucky, where she obtained a Ph.D. working with Professor W. T. Smith. Following postdoctoral studies at Northwestern University (Professor A. G. M. Barrett) and the University of Nottingham, England (Professor G. Pattenden), she worked at Glaxo in the U.K. In 1994 she moved to the University of Connecticut.

Regina So received her BS degree in Chemistry from De La Salle University in Manila, Philippines in 1996. She was an assistant lecturer for almost two years at De La Salle University before joining Professor Howell's group at the University of Connecticut. Her graduate work at the University of Connecticut focused on the synthesis of sphinganine-containing glycosphingolipids. After obtaining her Ph.D degree in 2003, she joined Professor Mukund P. Sibi's group as a postdoctoral research associate where she is now working on the synthesis on P, N ligands and their application in asymmetric catalysis.

Stewart Richardson grew up in Tholthorpe, North Yorkshire, England. He received his undergraduate and Ph.D Degrees from Sheffield Hallam University, where he worked with Dr. Alan Hewson. He did postdoctoral work at the University of Kentucky (Professor David Watt) and the University of Notre Dame (Professor Marvin Miller). Before coming to the University of Connecticut in 2002 he worked at what was then SmithKline Beecham in England and later at NitroMed Inc., in Bedford, MA.

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Syntheses and optical properties of stable 8-alkylidene-bacteriochlorins mimicking the molecular structures of natural bacteriochlorophylls-*b* and *g*

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Abstract—We prepared bacteriochlorophyll(BChl)-*b* and *g* models by Diels–Alder reactions of 8-vinyl-chlorophylls with tetracyanoethylene. The resulting 8-alkylidene-bacteriochlorins with various substituent groups at the 3-position had the same π-conjugate as BChls-*b*/*g*. While the natural pigments isomerized by addition of an acid to afford the corresponding chlorins, the synthetic models were stable under the acidic conditions due to dialkylation at the 7-position. These BChl-*b*/*g* models are useful for investigating the optical properties of relatively unstable BChls-*b*/*g*.

1. Introduction

Bacteriochlorophyll(BChl)s-*b* and *g* are found in some purple bacteria[1] and heliobacteria,[2] respectively, and their molecular structures including the absolute configurations have already been determined (left drawing of Fig. 1).[3,4] They function as photosynthetic pigments in natural light-harvesting, energy transfer and charge separation systems as do chlorophyll(Chl)s-*a*/*d* in higher plants or cyanobacteria and (Zn-)BChl-*a* in photosynthetic bacteria.[5] As compared with any other photosynthetic (B)Chls (Chls-*a*/*b*/*c*/*d*, BChls-*a*/*c*/*d*/*e*), BChls-*b* and *g* are quite unique in their molecular structures possessing an ethylidene group at the 8-position, and have the same bacteriochlorin π-conjugate (doubly saturated porphyrin moiety at 7–8 and 17–18 bonds). BChl-*b* possesses an acetyl group (R^3) at the 3-position, which is

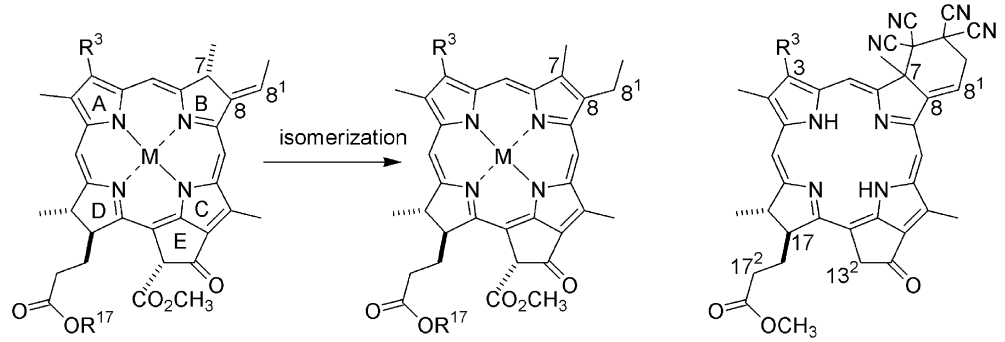

Figure 1. Molecular structures of natural BChls- and BPhes-*b*/*g* (left), their isomerized products (center) and synthetic stable model compounds **1**–**5** (right).

Keywords: Bacteriochlorin; Diels–Alder reaction; Isomerization; Tetracyanoethylene.
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the same function group as in BChl-*a*. BChl-*g* possesses a vinyl group (R^3) at the 3-position, which is the same substituent as in Chl-*a*. Only a few reports are available on in vitro investigation of BChls-*b*/*g*[6,7] because of their instabilities, i.e. the easy isomerization of the bacteriochlorin moiety (two reduced pyrroles at rings B and D) to the corresponding chlorin (one reduced pyrrole at ring D) even under ambient conditions as shown in Figure 1.[8,9] Stable bacteriochlorin models possessing a C8=C8^1 double bond are thus necessary to elucidate the optical properties of naturally occurring BChls-*b*/*g*. In this paper, we intended to synthesize chemically stable 8-alkylidene-bacteriochlorins **1**–**5** (right drawing of Fig. 1) as the BChl-*b*/*g* models.

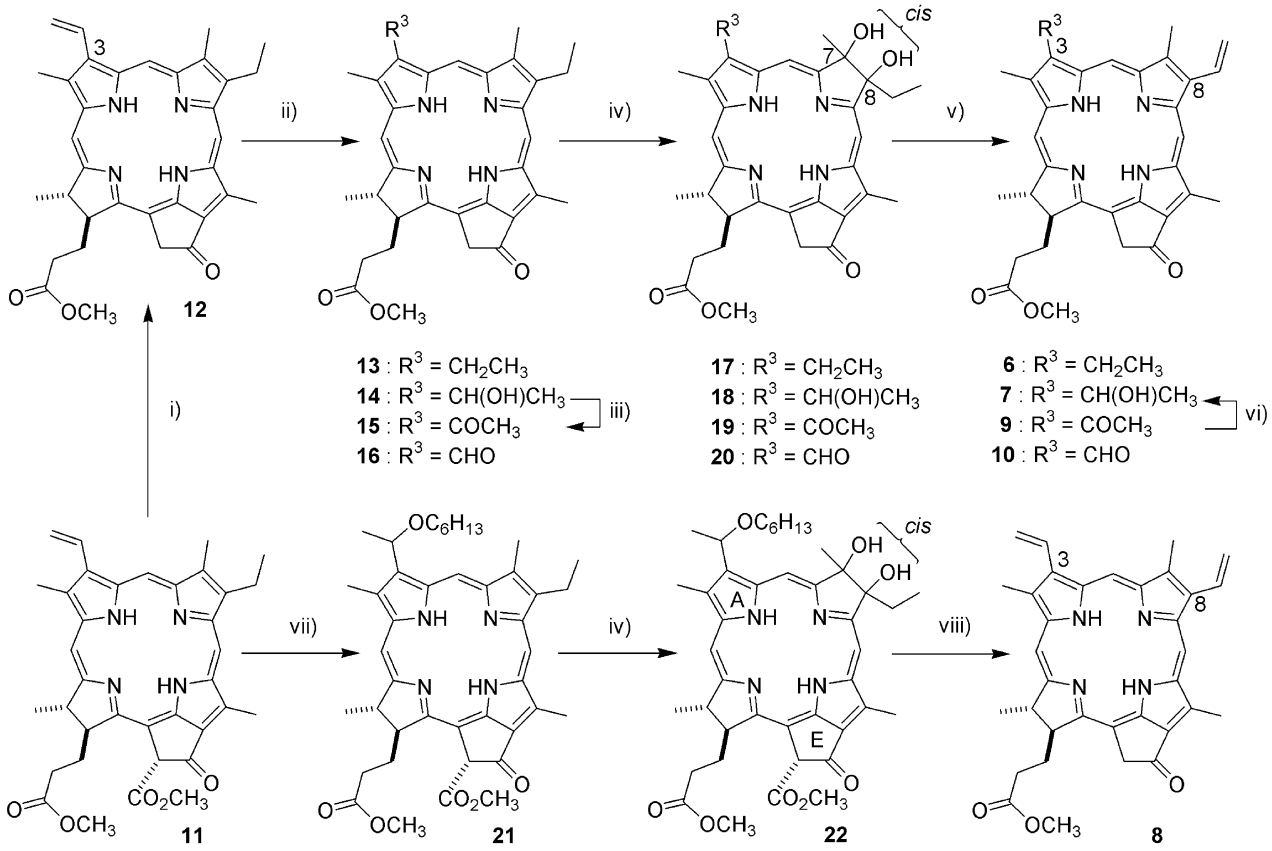
To form the C8=C8^1 double bond, we employed Diels–Alder reaction of 8-vinyl-chlorin with tetaracyanoethylene (TCNE). Various Diels–Alder reactions of cyclic tetrapyrroles have been studied.[10–15] In most cases, β-vinylporphyrins or chlorins, C(β′)=C(β)–CH=CH_2, reacted with a dienophile to give the corresponding [4+2] cycloadduct with one more reduced π-conjugate, –C(β′)–C(β)=CH–CH_2–. The resulting Diels–Alder products had a six-membered ring attached to the β,β′-positions and also an *exo*-double bond on the β-position similar to the 8-ethylidene group of BChls-*b*/*g*. In the chlorin π-conjugate system, the C=C double bond on the pyrrole ring opposite the reduced pyrolidine is more reactive than any other double bonds in the skeletal π-conjugate.[16] The Diels–Alder reaction of 8-vinyl-chlorophyll derivatives with a dienophile thus tended to give bacteriochlorin molecules bearing a C8=C8^1 double bond as models of BChls-*b*/*g* as in the right drawing of Figure 1. Additionally, the stereochemistry of the double bond was fixed as an *E*-form in the same stereoisomer with the natural BChls-*b*/*g*,[3,4] due to the C7–C8 fused six-membered ring. BChls-*b* and *g* have a phytyl, farnesyl or geranylgeranyl group as the 17-propionate ester and also have a methoxycarbonyl group at the 13^2-position. These moieties do not play any significant roles in their monomeric absorption spectra,[17–19] and are here changed to 17^2-$COOCH_3$ and 13^2-H_2 for preparing simple and stable model compounds.

Such Diels–Alder reactions would allow us to prepare a series of 8-alkylidene-BChl analogues possessing various substituents at the 3-position. Since the synthetic bacteriochlorins have two alkyl groups as peripheral substituents at the 7-position, undesired rearrangement of the bacteriochlorin moiety to the chlorin can no longer take place. Here, we report syntheses of stable bacteriochlorins possessing a similar π-conjugate system with natural BChls-*b*/*g* as well as their chemical stabilities and optical properties.

2. Results and discussion

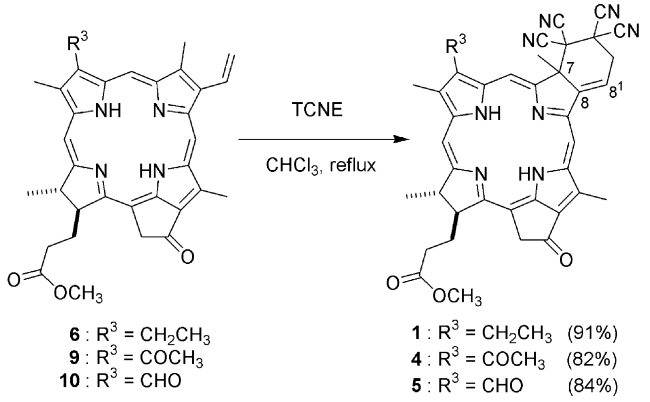
2.1. Synthesis of 8-vinyl-chlorophyll derivatives

As diene molecules, we prepared 8-vinyl-chlorins **6**–**10**



Scheme 1. Synthesis of 3-substituted 8-vinyl-chlorins: (i) collidine, reflux; (ii) H_2–Pd/C for **13**, 30% HBr–AcOH, H_2O, CH_2N_2 for **14**, cat. OsO_4–$NaIO_4$ in AcOH–THF for **16**; (iii) *N*-methyl-morphorine-*N*-oxide–Pr_4RuO_4; (iv) OsO_4-pyridine, H_2S; (v) TsOH, CH_2Cl_2–benzene (rt to reflux), CH_2N_2; (vi) $NaBH_4$–MeOH; (vii) 30% HBr–AcOH, 1-hexanol, CH_2N_2; (viii) 1,2-dichlorobenzene, 170 °C.

possessing various substituent groups at the 3-position as follows (Scheme 1). The 3-vinyl group of methyl pyropheophorbide-*a* (**12**)[20] was converted to ethyl, 1-hydroxyethyl and formyl groups (R^3) as in chlorins **13**,[20] **14**[21] and **16**,[20] respectively, according to reported procedures. Oxidation of 1-hydroxyethyl group in **14** produced 3-acetyl-chlorin **15**.[22] Reaction of chlorins **13–16** with OsO_4 in the presence of pyridine followed by treatment of H_2S gas[23–25] afforded the corresponding 7,8-*cis*-diols **17–20** in moderate yields. The resulting diols **17**, **19** and **20** were double-dehydrated by treatment of *p*-toluenesulfonic acid at room temperature followed by reflux[23,26,27] to give 3-ethyl, 3-acetyl and 3-formyl-8-vinyl-chlorins **6**, **9** and **10** as a major product. After the similar acidic treatment of **18**, 3-(1-hydroxyethyl)-8-vinyl-chlorin **7** and 3,8-divinyl-chlorin **8** were produced in ~1 and 3%, respectively, with some degradation products. Desired **7** was successively prepared by reduction of the 3-acetyl group in **9**. To synthesize 3,8-divinyl-chlorin **8**, we utilized reported thermal degradation of **21**[28] derivatized from methyl pheophorbide-*a* **11**, because the β-keto ester moiety on the E-ring promoted an elimination reaction at the 3-position of the A-ring.[29] As described above, a series of 3-substituted 8-vinyl-chlorins **6–10** were prepared.

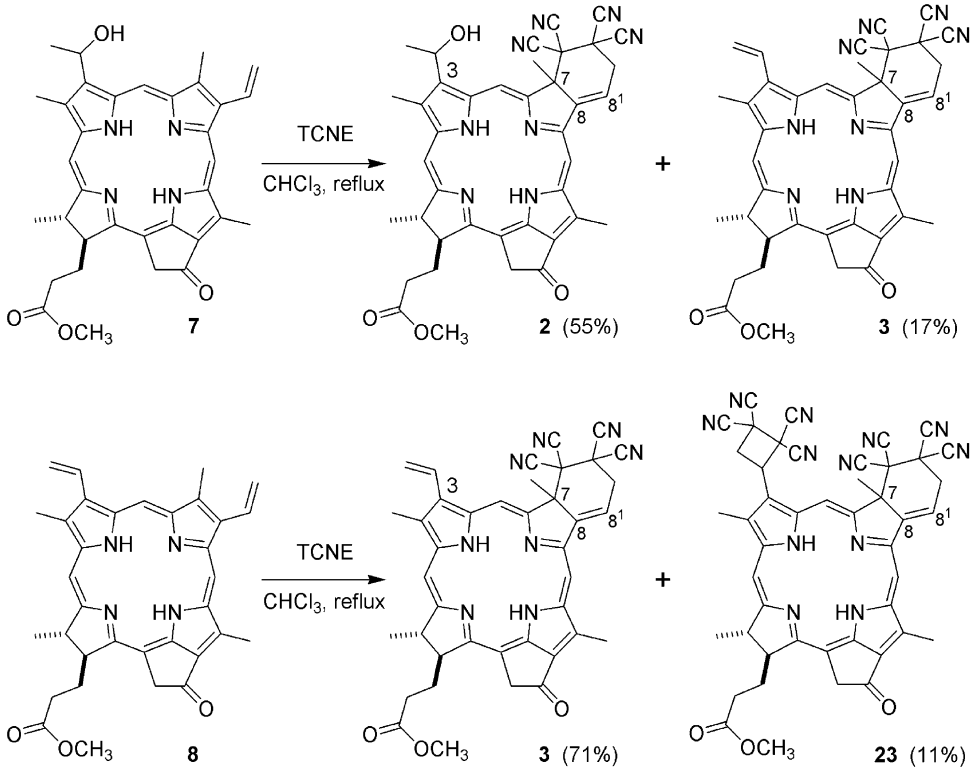


Scheme 2. Diels–Alder reactions of 3-ethyl-, 3-acetyl- and 3-formyl-8-vinyl-chlorins **6**, **9** and **10** with TCNE.

2.2. Diels–Alder reaction for preparing 8-alkylidene-BChl derivatives

Diels–Alder reactions of 8-vinyl-chlorins **6–10** as dienes with TCNE were achieved by the following procedures. A slight excess of TCNE (ca. 1.2 equiv) was added to **6–10** in dry chloroform and refluxed for 30 min under nitrogen. The Diels–Alder reaction was monitored by ultraviolet–visible–near infrared (UV–VIS–NIR) spectral analysis; a specific red-shift of the Qy absorption maximum based on a change of π-conjugate moieties from chlorin to bacteriochlorin. All the reactions afforded the corresponding 8-alkylidene-bacteriochlorins **1–5** as a 1:1 7-epimeric mixture. Molecular structures of all the Diels–Alder products **1–5** were determined by their 1D ^{1}H NMR, 2D ^{1}H–^{1}H correlation and rotating-frame Overhauser effect spectroscopies (COSY/ROESY) and high resolution mass (HR-MS, ionized by fast atomic bombardment (FAB)) spectral analyses: the specific proton signals of the 8-alkylidene group were situated around 7 and 4 ppm for 8-CH and 8^1-CH_2, respectively, and the main mass ion peak was observed at the position of one-to-one adduct.

In the reactions of 3-ethyl, 3-acetyl and 3-formyl-8-vinyl-chlorins **6**, **9** and **10** with TCNE, no significant side-reaction occurred and desired [4+2] cycloadducts **1**, **4** and **5** were



Scheme 3. Reactions of 3-(1-hydroxyethyl)-8-vinyl- and 3,8-divinyl-chlorins **7** and **8** with TCNE.

isolated in good yields after purification of flash column chromatography (FCC) and recrystallization (Scheme 2).

Diels–Alder reactions of **7** and **8** afforded the corresponding [4+2] cycloadducts **2** and **3**, respectively, and additional by-products (Scheme 3). Complete consumption of 3-(1-hydroxyethyl)-8-vinyl-chlorin **7** led to the formation of desired **2** (55%) and its dehydrated product **3** (17%). In refluxing chloroform, neither 3-(1-hydroxyethyl)-8-ethyl-chlorin **14** in the presence of TCNE nor 3-(1-hydroxyethyl)-8-vinyl-chlorin **7** in the absence of TCNE afforded their dehydrated chlorins **12** and **8**, but **2** gave the corresponding dehydrated **3**, indicating that thermal dehydration of 3-(1-hydroxyethyl) group occurred more easily in **2** than in **7** and **14**. Under the above reaction conditions, such a bacteriochlorin π-conjugate promoted elimination of a water molecule on the 3-position.

In the case of 3,8-divinyl-chlorin **8**, desired Diels–Alder adduct **3** was prepared predominantly after 30-min reflux. When the reaction mixture was worked up by a standard procedure, an undesired product was isolated besides **3** (71%). The over-reaction of **3** with TCNE occurred during evaporation of the reaction mixture in the dark to produce [2+2] cycloadduct **23** (11%).[11] Addition of excess TCNE (5 equiv) to the chloroform solution of **3** resulted in exclusive formation of **23**, but no [4+2] cycloadducts at around the 3-position could be isolated. In the chlorin chromophore (17,18-dihydroporphyrin), the 3-vinyl group essentially acted as an ene and the 8-vinyl group could function as a diene accompanying the C8═C7.

2.3. Chemical stabilities of synthetic models 3 and 4 in comparison with BPhes-*b*/*g*

Molecular structures and skeletal π-conjugates of synthetic **1–5** were quite similar to those of natural BChls-*b*/*g*, all of which had the C8═C8^1 double bond, so bacteriochlorins **1–5** might be model compounds for BChls-*b*/*g*. In this section, we will compare the chemical stabilities between the synthetic **3/4** and natural metal-free bacteriopheophytin(BPhe)s-*b*/*g* (see left drawing of Fig. 1).

Figure 2 shows UV–VIS–NIR spectral change of BPhes-*b*/*g* (upper) and the corresponding models **3/4** (lower) in acidic acetone in the dark. After acidic treatment, absorption peaks characteristic of BPhe-*b* at 777, 527 and 367 nm (red line of Fig. 2A) decreased with a concomitant increase in new peaks (blacks in Fig. 2A), and finally changed to peaks typical of chlorin chromophore at 674 and 421 nm (blue in Fig. 2A), which were similar to peaks of 3-acetyl-chlorin **15**. Normal-phase HPLC analysis showed that the products were a simple isomerized chlorin, 3-Ac-pheophytin(Phe)-*a* as a major product and its 8^1-oxidized chlorins (8^1-OH or 8^1-OOH, not determined)[6] as a minor product. These results indicated that the acidic treatment of metal-free BPhe-*b* promoted isomerization of C7H–C8═C8^1 to C7═C8–C8^{1}H moiety to give 3-Ac-Phe-*a* (Fig. 1) as in the

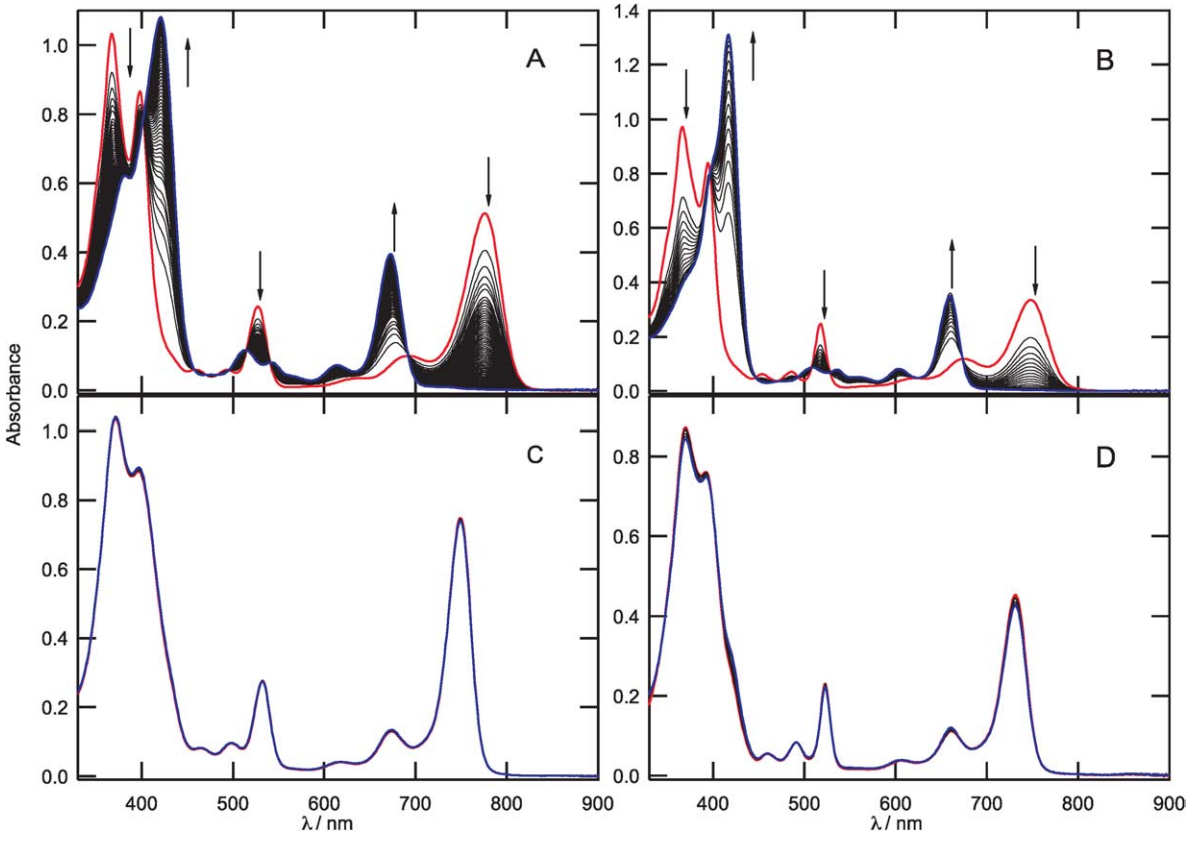


Figure 2. UV–VIS–NIR spectral changes of BPhe-*b* (A) and *g* (B), and the corresponding models **4** (C) and **3** (D) in acetone (3.0 ml) by acidic treatment (aqueous 3.5% HCl, 10 μl) at room temperature. Spectra were recorded at 3/2/30/30-min intervals for A/B/C/D, respectively. The red and blue lines are initial (before acid treatment) and final spectra (after 213/38/300/300-min treatment), respectively.

magnesium complex BChl-*b* (to 3-Ac-Chl-*a*) previously reported.[6,8]

Acidic treatment also induced isomerization of BPhe-*g* (absorption peaks at 748, 515 and 366 nm, red line of Fig. 2B) to a chlorin chromophore (660 and 417 nm, blue in Fig. 2B), resembling 3-vinyl-chlorin **12**. HPLC analyses supported that the major product was a chlorin chromophore possessing 660 nm as the Qy maxima. Therefore, BPhe-*g* isomerized by an action of an acid to give Phe-*a* (Fig. 1) as in BChl-*g* to Chl-*a*.[6,9] The isomerization of bacteriochlorin to chlorin chromophores was completed in 213 and 38 min for BPhes-*b* and *g*, respectively, and their half lifetimes were estimated to be 25 and 6 min, respectively, showing that the isomerization of BPhe-*g* was catalyzed by an acid more efficiently than that of BPhe-*b*, similar to the reported isomerization of BChls-*b*/*g*.[8,9]

We examined chemical stabilities of synthetic models **3** and **4** under the same acidic conditions (Fig. 2C and D). Almost no spectral changes were observed for 5 h. HPLC analyses after acidic treatment did not show any other peaks than **3/4**, indicating that synthetic models **3/4** were quite inactive for the acidic treatment compared to natural BPhes-*b*/*g*. Dialkylation of the 7-position and/or the fused ring on the 7,8-positions led to **3/4** being highly chemically stable.

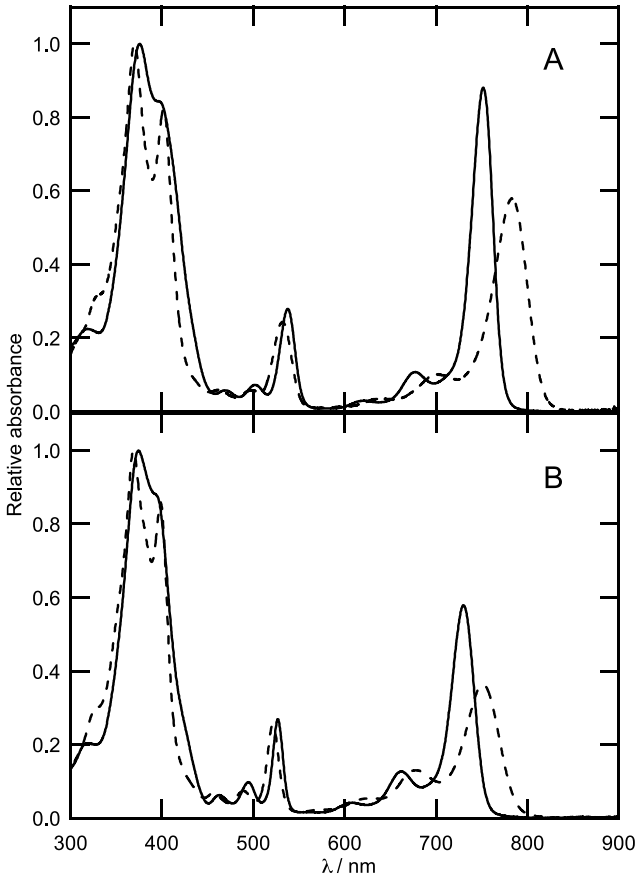


Figure 3. UV–VIS–NIR spectra of BPhe-*b* and **4** (A), and BPhe-*g* and **3** (B) in dichloromethane. Solid and broken lines were synthetic **4/3** and BPhes-*b*/*g*, respectively. All the spectra were normalized at the Soret peak.

2.4. Electronic absorption properties of BPhes-*b*/*g* and their synthetic stable models 1–5

Figure 3A shows UV–VIS–NIR spectra of 3-acetyl forms BPhe-*b* and **4** (broken and solid lines, respectively) in dichloromethane, and broken and solid lines in Figure 3B are spectra of 3-vinyl forms BPhe-*g* and **3**, respectively. Synthetic **4/3** had similar spectra to BPhes-*b*/*g* except for slight blue-shifted Qy maxima. In the Qy region (600–850 nm), all four bacteriochlorins had an intense Qy(0,0) band at a longer wavelength with association of a small Qy(1,0) band at a shorter wavelength. The Qy(0,0) maxima λ_{max}[Qy(0,0)]s of semi-natural BPhes-*b*/*g* were situated at 783/751 nm with full widths at half maximum (FWHMs) of 610/720 cm^{-1}, and those of synthetic **4/3** were situated at 752/730 nm with FWHMs of 430/500 cm^{-1}, respectively (see Table 1). BPhe-*b* and **4** possessing the 3-acetyl group had more red-shifted and sharper Qy(0,0) bands than BPhe-*g* and **3** which possessed the 3-vinyl group, respectively. The λ_{max}[Qy(1,0)]s of BPhes-*b*/*g* and **4/3** were located at 700/677 and 677/662 nm, respectively. Energetic difference Δ between Qy(0,0) and Qy(1,0) peaks of BPhes-*b*/*g* and **4/3** was 1490/1450 and 1460/1410 cm^{-1}, respectively, showing the Δs were slightly larger in BPhe-*b* and **4** than in BPhe-*g* and **3**. Relative intensities I_{rel}s (based on the Soret peak intensity) in Qy(0,0) peaks of BPhe-*b*/*g* and **4/3** were 0.58/0.36 and 0.88/0.58, and their Qy(1,0) peak intensities were 0.10/0.13 and 0.11/0.13, showing that BPhe-*b* and **4** had more intense Qy(0,0) and less intense Qy(1,0) bands than BPhe-*g* and **3**, respectively.

In the Qx absorption region (460–550 nm) of all the above four bacteriochlorins, large Qx(0,0) bands were associated with small Qx(1,0) bands at shorter wavelength. The λ_{max}[Qx(0,0)]s of BPhe-*b*/*g* and **4/3** situated at 532/521 and 538/527 nm with FWHMs of 850/570 and 620/460 cm^{-1}, respectively, while I_{rel}s in Qx(0,0) peaks of BPhes-*b*/*g* and **4/3** were 0.24/0.25 and 0.28/0.27. BPhe-*b* and **4** had more red-shifted and broadened Qx(0,0) bands than BPhe-*g* and **3**. The Δs between Qx(0,0) and Qx(1,0) of BPhe-*b* and **4** (1310 and 1330 cm^{-1}) were slightly larger than those of BPhe-*g* and **3** (1260 and 1250 cm^{-1}) as observed in Qy bands. The same substituent effect on 3-acetyl/vinyl groups was thus clearly observed in both Qy and Qx bands of semi-natural BPhes-*b*/*g* and synthetic **4/3**. As described above, BPhes-*b*/*g* were easily transferred to their chlorin chromophores, so their visible spectra were sensitive to the presence of a small amount of such chlorin impurities; typically, small Qy(1,0) peaks of BPhes-*b*/*g* are situated at around intense Qy(0,0) peaks of their altered chlorins. It is noteworthy that the present spectral analyses can be achieved in detail by using stable models containing no chlorin-type impurities.

Next, we measured UV–VIS–NIR spectra of other bacteriochlorins **1/2/5** possessing the same skeletal π-conjugate as BPhes-*b*/*g*. The λ_{max}[Qy(0,0)]s of **1–5** as well as their λ_{max}[Qx(0,0)]s were red-shifted in the order of **1**<**2**<**3**<**4**<**5** (see Table 1). These observed orders were reproduced by model calculation using ZINDO/S[30–32] (Table 2). All the synthetic bacteriochlorins **1–5** are applicable to the investigation of optical properties of a series of 3-substituted 8-alkylidene-bacteriochlorins as described above; they

Table 1. UV–VIS–NIR peak data of **1–5** and BPhes-*b*/*g* in CH_2Cl_2

Compound	λ_{max}/nm (I_{rel}[a]) [FWHM/cm^{-1}]		Δ[Qy(0,0)–(1,0)][b] /cm^{-1}	λ_{max}/nm (I_{rel}[a]) [FWHM/cm^{-1}]		Δ[Qx(0,0)–(1,0)][c] /cm^{-1}
	Qy(0,0)	Qy(1,0)		Qx(0,0)	Qx(1,0)	
1	715 (0.50) [490]	651 (0.13)	1370	522 (0.25) [450]	490 (0.10)	1250
2	722 (0.56) [480]	656 (0.13)	1410	524 (0.27) [460]	492 (0.10)	1260
3	730 (0.58) [500]	662 (0.13)	1410	527 (0.27) [460]	494 (0.10)	1250
4	752 (0.88) [430]	677 (0.11)	1460	538 (0.28) [620]	502 (0.07)	1330
5	760 (1.30) [340]	686 (0.09)	1430	543 (0.39) [520]	507 (0.08)	1290
BPhe-b	783 (0.58) [610]	701 (0.10)	1490	532 (0.24) [850]	497 (0.06)	1310
BPhe-g	751 (0.36) [720]	677 (0.13)	1450	521 (0.25) [570]	489 (0.07)	1260

[a] Relative peak intensity I_{rel} was based on the Soret peak intensity.
[b] $\Delta[\mathrm{Qy}(0,0)-(1,0)] = (1/\lambda_{max}[\mathrm{Qy}(1,0)] - 1/\lambda_{max}[\mathrm{Qy}(0,0)]) \times 10^7$.
[c] $\Delta[\mathrm{Qx}(0,0)-(1,0)] = (1/\lambda_{max}[\mathrm{Qx}(1,0)] - 1/\lambda_{max}[\mathrm{Qx}(0,0)]) \times 10^7$.

Table 2. Absorption maxima (nm) observed for **1–5** in CH_2Cl_2 (exp) and estimated by ZINDO/S calculation (calcd)

Compound	Qy(0,0)		Qx(0,0)	
	Exp	Calcd	Exp	Calcd
1	715	727	522	551
2	722	727	524	552
3	730	739	527	560
4	752	745	538	562
5	760	751	543	563

would also be useful to elucidate optical properties of semi-natural BPhes-*b*/*g*, as well as natural BChls-*b*/*g* in the distorted forms in the polypeptide environment where the 3-substituents were conformationally restricted for rotation around the 3–3[1] bond.[33,34]

3. Experimental

3.1. General

UV–VIS–NIR spectra were measured in air-saturated solvents at room temperature on a Hitachi U-3500 spectrophotometer. ^{1}H NMR spectra in chloroform-d were recorded at room temperature with a JEOL JNM-A400 Fourier transform NMR spectrometer; tetramethylsilane was used as an internal standard. ^{1}H–^{1}H COSY and ^{1}H–^{1}H ROESY (τ_m=400 ms) were recorded to determine the molecular structure of synthetic compounds. FAB-MS spectra were recorded on a JEOL GCmate II spectrometer; FAB-MS samples were dissolved in chloroform and *m*-nitrobenzyl alcohol and polyethylene glycol were used as a matrix and an internal standard, respectively. HPLC was carried out with a Shimadzu LC-10AD pump and an SPD-M10A photodiode array detector. A packed silica gel column (Cosmosil 5SL-II, Nacalai Tesque, 6.0ϕ 250 mm) was used for normal-phase HPLC. Dichloromethane and acetone for UV–VIS–NIR spectra were purchased from Nacalai Tesque (grade for spectroscopy). FCC was carried out on silica gel (Merck Kieselgel 60, 9358). All procedures including syntheses and spectral measurements were performed in the dark.

Methyl pheophorbide-*a* (**11**),[35] methyl pyropheophorbide-*a* (**12**),[20] methyl 3-devinyl-3-ethyl-pyropheophorbide-*a* (**13**),[20] methyl bacteriopheophorbide-*d* (**14**),[21] methyl 3-acetyl-3-devinyl-pyropheophorbide-*a* (**15**)[22] and methyl pyropheophorbide-*d* (**16**)[20] were prepared according to the reported procedures.

3.2. Estimation of electronic absorption peaks in 1–5 using ZINDO/S

Initial molecular structures of bacteriochlorins **1–5** were made using MM+ and PM3 in HYPERCHEM version 6.0 according to the literature.[30–32] In the calculation, configurations of the 7-methyl group in **1–5** were fixed in the same direction as natural BChls- and BPhes-*b*/*g*. Their energy minimized structures were made by repeating ZINDO/S and MM+ calculations until the MM+ calculation was finished with one cycle.[30] In the ZINDO/S calculation, were used HOMO and LUMO whose energy gaps were less than 7 eV. Their electronic absorption peaks were estimated from the ZINDO/S calculation based on their energy minimized structures.

3.3. Oxidation of C7–C8 double bond[23,25]

1.4 Equivalent of OsO_4 and a small amount of pyridine were added to a dichloromethane solution of chlorin (1 equiv). After stirring at room temperature for 16 h under nitrogen, the reaction was quenched by addition of methanol and 10-min bubbling of H_2S gas. The resulting OsS_4 was removed and the filtrate was evaporated in vacuo. The residue was purified by FCC and recrystallization from dichloromethane and hexane to give the corresponding 7,8-*cis*-dihydroxy-chlorin.

Oxidation of **13** gave **17** in 69% yield (lit.,[23] 52%). Oxidation of **14** gave **18** in 57% yield (lit.,[25] 56%). Oxidation of **15** gave **19** in 75% yield (lit.,[27] 57%). Oxidation of **16** gave **20** in 71% yield (lit.,[24] 74%). Oxidation of **21** gave **22**[28] and the crude product without FCC was used for the following pyrolysis due to instability

of **22** which possessed the 13^2-methoxycarbonyl group for FCC.

3.4. Preparation of 3-substituted 8-vinyl-chlorins

3.4.1. Double dehydration of 7,8-*cis*-diol[23,27]. To a dichloromethane and benzene solution (1:4) of 7,8-*cis*-diol, *p*-toluenesulfonic acid was added and the reaction mixture was stirred at room temperature for 2 h under nitrogen. After consumption of the diol was completed to give singly dehydrated 8-(1-hydroxyethyl)-chlorin by monitoring blue-shifted Qy maximum to 650–690 from 700–760 nm, the reaction mixture was refluxed for 30 min. The solution was poured into ice-cold water and extracted with dichloromethane. The organic phase was washed with 4% $KHSO_4$ and water, and dried over Na_2SO_4. After evaporation in vacuo, the residue was dissolved in a small amount of dichloromethane and subsequently an ethereal diazomethane solution was added and stirred for 30 min. After removal of the solvents in vacuo, the residue was purified with FCC and recrystallization from dichloromethane and hexane to give pure 8-vinyl-chlorin.

Dehydration of **17** gave **6** in 60% yield (lit.,[23] 40%). Dehydration of **19** gave **9** in 28% yield (lit.,[27] 44%). The other double dehydration of **22** including removal of hexanol and hydrolysis-de(carbon dioxide) of methoxycarbonyl group by heating in 1,2-dichlorobenzene at 160 °C was done according to the reported procedures[28] to give **8** in 40% total yield based on **21** (lit.,[28] 45%).

Compound **10**. Dehydration of **20** (52.0 mg) in dichloromethane and benzene (8/24 ml) afforded methyl 8-deethyl-8-vinyl-pyropheophorbide-*d* (**10**, 11.7 mg, 24%) as dark brown solid after FCC (3–4% Et_2O/CH_2Cl_2) and recrystallization (CH_2Cl_2/hexane); UV–VIS–NIR (CH_2Cl_2) $\lambda_{max}=693$ (relative intensity, 0.60), 631 (0.07), 557 (0.09), 526 (0.14), 436 (1.00), 390 nm (0.60); ^{1}H NMR ($CDCl_3$) $\delta=11.57$ (1H, s, 3-CHO), 10.41 (1H, s, 5-H), 9.80 (1H, s, 10-H), 8.85 (1H, s, 20-H), 7.97 (1H, dd, $J=11$, 18 Hz, 8-CH), 6.65 (1H, d, $J=11$ Hz, 8^1-CH-*cis*-to 8-CH), 6.20 (1H, d, $J=18$ Hz, 8^1-CH_2-*trans*-to 8-CH), 5.35, 5.20 (each 1H, d, $J=19$ Hz, 13^1-CH_2), 4.59 (1H, dq, $J=2$, 8 Hz, 18-H), 4.49 (1H, dt, $J=8$, 2 Hz, 17-H), 3.79 (3H, s, 2-CH_3), 3.73 (3H, s, 12-CH_3), 3.62 (3H, s, $COOCH_3$), 3.46 (3H, s, 7-CH_3), 2.69–2.78, 2.56–2.65, 2.25–2.39 (1H+1H+2H, m, 17-CH_2CH_2), 1.85 (3H, d, $J=8$ Hz, 18-CH_3), −0.19 and −2.05 (each 1H, s, NH). MS (FAB) found: *m/z* 548.2417. Calcd for $C_{33}H_{32}N_4O_4$: M^+, 548.2424.

3.4.2. Methyl 8-deethyl-8-vinyl-bacteriopheophorbide-*d* (7).[27] To a dichloromethane solution (15 ml) of 3-acetyl-chlorin **8** (13.3 mg), a methanol solution (200 µl) saturated with $NaBH_4$ was added and the reaction mixture was stirred for 10 min under nitrogen. The reaction mixture was poured into ice-cold water and the separated organic phase was washed with 2% HCl and water, and dried over Na_2SO_4. After removal of the solvent in vacuo, the residue was purified with FCC (8% Et_2O/CH_2Cl_2) and recrystallization from dichloromethane and hexane to give pure **7** (10.7 mg) in 80% yield (lit.,[27] 82%).

3.5. Diels–Alder reaction

TCNE (1.2 equiv) was added to a 8-vinyl-chlorin (ca. 50 µmol) in dry chloroform (25 ml) and refluxed for 30 min under nitrogen. The solution was poured into water, washed twice, extracted with dichloromethane and dried over Na_2SO_4. After evaporation, the residue was purified by FCC (Et_2O/CH_2Cl_2) and recrystallization from dichloromethane and hexane to give the corresponding bacteriochlorin as a 1:1 7-epimeric mixture.

3.5.1. TCNE adduct of 3-ethyl-8-vinyl-chlorin (1). Diels–Alder reaction of 3-ethyl-8-vinyl-chlorin **6** with TCNE afforded bacteriochlorin **1** as a dark green solid in 91% yield (3–4% Et_2O/CH_2Cl_2 for FCC); UV–VIS–NIR (CH_2Cl_2) $\lambda_{max}=715$ (rel. 0.50), 651 (0.13), 598 (0.04), 522 (0.25), 490 (0.10), 460 (0.06), 393 (0.85), 370 nm (1.0); ^{1}H NMR ($CDCl_3$) $\delta=8.76/75$ (1H, s, 10-H), 8.62/61 (1H, s, 5-H), 8.27/26 (1H, s, 20-H), 6.74–6.79 (1H, m, 8-CH), 5.05/4.89, 5.02/4.86 (each 1H, d, $J=20$ Hz, 13^1-CH_2), 4.26 (1H, m, 18-H), 4.08 (1H, m, 17-H), 3.82–3.99 (2H, m, 8^1-CH_2), 3.64–3.68 (2H, m, 3-CH_2), 3.63/62 (3H, s, $COOCH_3$), 3.43 (3H, s, 12-CH_3), 3.17 (3H, s, 2-CH_3), 2.48–2.58, 2.17–2.33 (each 2H, m, 17-CH_2CH_2), 2.24/23 (3H, s, 7-CH_3), 1.67–1.74 (3H+3H, m, 3^1-CH_3, 18-CH_3), 0.64/62 and −0.69/71 (each 1H, s, NH). MS (FAB) found: *m/z* 676.2920. Calcd for $C_{40}H_{36}N_8O_3$: M^+, 676.2910.

3.5.2. TCNE adduct of 3-(1-hydroxyethyl)-8-vinyl-chlorin (2). Diels–Alder reaction of 3-(1-hydroxyethyl)-8-vinyl-chlorin **7** with TCNE afforded bacteriochlorin **2** as a 3^1/7-diastereomeric mixture as a green solid in 55% yield (5–6% Et_2O/CH_2Cl_2 for FCC); UV–VIS–NIR (CH_2Cl_2) $\lambda_{max}=722$ (rel. 0.56), 656 (0.13), 598 (0.04), 524 (0.27), 492 (0.10), 461 (0.06), 394 (0.87), 371 nm (1.0); ^{1}H NMR ($CDCl_3$) $\delta=9.33/32/19/15$ (1H, s, 5-H), 8.82/81 (1H, s, 10-H), 8.36/35/34/33 (1H, s, 20-H), 6.71–6.80 (1H, m, 8-CH), 6.23–32 (1H, m, 3-CH), 5.02–5.14, 4.87–4.93 (each 1H, m, 13^1-CH_2), 4.29 (1H, m, 18-H), 4.11 (1H, m, 17-H), 3.81–3.99 (2H, m, 8^1-CH_2), 3.63/62 (3H, s, $COOCH_3$), 3.49/48/45 (3H, s, 12-CH_3), 3.30/29/27/26 (1H, s, 2-CH_3), 2.49–2.63, 2.17–2.32 (each 2H, m, 17-CH_2CH_2), 2.25/24/23 (3H, s, 7-CH_3), 2.08–2.15 (3H, m, 3^1-CH_3), 1.67–1.75 (3H, m, 18-CH_3), 0.36/33 and −0.96/99 (each 1H, s, NH). MS (FAB) found: *m/z* 692.2888. Calcd for $C_{40}H_{36}N_8O_4$: M^+, 692.2860.

3.5.3. TCNE adduct of 3,8-divinyl-chlorin (3). Diels–Alder reaction of 3,8-divinyl-chlorin **8** with TCNE afforded bacteriochlorin **3** as a dark green solid in 71% yield (3–4% Et_2O/CH_2Cl_2 for FCC); UV–VIS–NIR (CH_2Cl_2) $\lambda_{max}=730$ (rel. 0.58), 662 (0.13), 608 (0.04), 527 (0.27), 494 (0.10), 463 (0.06), 394 (0.88), 374 nm (1.0); ^{1}H NMR ($CDCl_3$) $\delta=$ 8.83 (1H, s, 5-H), 8.82 (1H, s, 10-H), 8.40 (1H, s, 20-H), 7.75–7.80 (1H, m, 3-CH), 6.77 (1H, m, 8-CH), 6.19–6.27 (2H, m, 3^1-CH_2), 5.03–5.10, 4.88–4.94 (each 1H, m, 13^1-CH_2), 4.30 (1H, m, 18-H), 4.12 (1H, m, 17-H), 3.82–3.98 (2H, m, 8^1-CH_2), 3.63/62 (3H, s, $COOCH_3$), 3.46 (3H, s, 12-CH_3), 3.29 (3H, s, 2-CH_3), 2.50–2.66, 2.21–2.40 (each 2H, m, 17-CH_2CH_2), 2.24/2.23 (3H, s, 7-CH_3), 1.74/71 (3H, d, $J=8$ Hz, 18-CH_3), 0.34/32 and −0.91/93 (each 1H, s, NH). MS (FAB) found: *m/z* 674.2765. Calcd for $C_{40}H_{34}N_8O_3$: M^+, 674.2754.

3.5.4. TCNE adduct of 3-acetyl-8-vinyl-chlorin (4). Diels–Alder reaction of 3-acetyl-8-vinyl-chlorin **9** with TCNE afforded bacteriochlorin **4** as a dark brown solid in 82% yield (3–4% Et_2O/CH_2Cl_2 for FCC); UV–VIS–NIR (CH_2Cl_2) λ_{max}=751 (rel. 0.88), 677 (0.11), 620 (0.03), 538 (0.28), 502 (0.07), 469 (0.06), 376 nm (1.0); ^{1}H NMR ($CDCl_3$) δ=9.63 (1H, s, 5-H), 8.99/98 (1H, s, 10-H), 8.71/70 (1H, s, 20-H), 6.83–6.88 (1H, m, 8-CH), 5.13–5.21, 4.98–5.05 (each 1H, m, 13^1-CH_2), 4.41 (1H, m, 18-H), 4.22 (1H, m, 17-H), 3.83–4.02 (2H, m, 8^1-CH_2), 3.63/62 (3H, s, $COOCH_3$), 3.57 (3H, s, 2-CH_3), 3.53 (3H, s, 12-CH_3), 3.28 (3H, s, 3-$COCH_3$), 2.52–2.68, 2.18–2.34 (each 2H, m, 17-CH_2CH_2), 2.29 (3H, s, 7-CH_3), 1.74–1.79 (3H, d, J=8 Hz, 18-CH_3), −0.37/41 and −1.54/56 (each 1H, s, NH). MS (FAB) found: m/z 690.2696. Calcd for $C_{40}H_{34}N_8O_4$: M^+, 690.2703

3.5.5. TCNE adduct of 3-formyl-8-vinyl-chlorin (5). Diels–Alder reaction of 3-formyl-8-vinyl-chlorin **10** with TCNE afforded bacteriochlorin **5** as a dark brown solid in 84% yield (3–5% Et_2O/CH_2Cl_2 for FCC); UV–VIS–NIR (CH_2Cl_2) λ_{max}=760 (rel. 1.30), 686 (0.09), 627 (0.03), 543 (0.39), 507 (0.08), 474 (0.07), 381 nm (1.0); ^{1}H NMR ($CDCl_3$) δ=11.44 (1H, s, 3-CHO), 9.98/97 (1H, s, 5-H), 9.04/03 (1H, s, 10-H), 8.79/78 (1H, s, 20-H), 6.89–6.93 (1H, m, 8-CH), 5.17–5.25, 5.03–5.10 (each 1H, m, 13^1-CH_2), 4.44 (1H, m, 18-H), 4.26 (1H, m, 17-H), 3.87–4.04 (2H, m, 8^1-CH_2), 3.70 (3H, s, 2-CH_3), 3.65/63 (3H, s, $COOCH_3$) 3.56 (3H, s, 12-CH_3), 2.56–2.70, 2.18–2.30 (each 2H, m, 17-CH_2CH_2), 2.32 (3H, s, 7-CH_3), 1.77/80 (3H, d, J=8 Hz, 18-CH_3), −0.50/54 and −1.58/60 (each 1H, s, NH). MS (FAB) found: m/z 676.2564. Calcd for $C_{39}H_{32}N_8O_4$: M^+, 676.2547.

3.5.6. [2+2] TCNE adduct at the 3-vinyl group of 3 (23). The title compound **23** was isolated in 11% yield as a by-product in the Diels–Alder reaction of 3,8-divinyl-chlorin **8** with TCNE described above. Alternative preparation of **23** as a major product was achieved as follows. TCNE (3.0 equiv) was added to 3,8-divinyl-chlorin **8** (50 μmol) in dry chloroform (20 ml) and refluxed for 30 min under nitrogen. The solution was repeatedly evaporated in vacuo after addition of chloroform (each 15 ml). When the Qy maximum was completely red-shifted (730–740 nm), the reaction mixture was worked up in a similar manner with the above Diels–Alder reaction to give pure **23** as a 3^1/7-diastereomeric mixture as a dark brown solid in 78% yield (6% Et_2O/CH_2Cl_2 for FCC). The mixture was separated into two fractions on normal-phase silica gel HPLC (acetone/hexane=3:7) to afford each 3^1-epimerically pure sample as a 7-epimeric mixture, but the absolute configurations could not be determined. Fraction 1 (first elution): UV–VIS–NIR (CH_2Cl_2) λ_{max}=743 (rel. 1.23), 671 (0.14), 614 (0.06), 529 (0.36), 497 (0.12), 465 (0.09), 397 (1.0), 374 nm (0.91); ^{1}H NMR ($CDCl_3$) δ=9.10 (1H, s, 10-H), 8.96/92 (1H, s, 5-H), 8.77/76 (1H, s, 20-H), 6.92–6.99 (1H, m, 8-CH), 6.04–6.11 (1H, m, 3-CH), 5.17–5.29, 5.03–5.12 (each 1H, m, 13^1-CH_2), 4.94–5.04, 4.06–4.15 (each 1H, m, 3^1-CH_2), 4.47 (1H, m, 18-H), 4.27 (1H, m, 17-H), 3.88–4.07 (2H, m, 8^1-CH_2), 3.65/64 (3H, s, 2-CH_3), 3.62 (3H, s, $COOCH_3$) 3.57 (3H, s, 12-CH_3), 2.54–2.72, 2.16–2.38 (each 2H, m, 17-CH_2CH_2), 2.30 (3H, s, 7-CH_3), 1.81/77 (3H, d, J=7 Hz, 18-CH_3), −0.76/78 and −1.78/80 (each 1H, s, NH). Fraction 2 (second elution): UV–VIS–NIR (CH_2Cl_2) λ_{max}=743 (rel. 1.23), 671 (0.14), 614 (0.06), 529 (0.36), 497 (0.12), 465 (0.09), 397 (1.0), 374 nm (0.91); ^{1}H NMR ($CDCl_3$) δ=9.10 (1H, s, 10-H), 8.99/98 (1H, s, 5-H), 8.76 (1H, s, 20-H), 6.94–7.01 (1H, m, 8-CH), 6.28–6.38 (1H, m, 3-CH), 5.18–5.27, 5.04–5.13 (each 1H, m, 13^1-CH_2), 4.77–4.85, 4.02–4.07 (each 1H, m, 3^1-CH_2), 4.45 (1H, m, 18-H), 4.27 (1H, m, 17-H), 3.78–4.09 (2H, m, 8^1-CH_2), 3.67 (3H, s, 2-CH_3), 3.64/63 (3H, s, $COOCH_3$) 3.57 (3H, s, 12-CH_3), 2.53–2.71, 2.18–2.36 (each 2H, m, 17-CH_2CH_2), 2.37/36 (3H, s, 7-CH_3), 1.82/77 (3H, d, J=7 Hz, 18-CH_3), −0.58/61 and −1.62/64 (each 1H, s, NH). MS (FAB) found: m/z 802.2884 Calcd for $C_{46}H_{34}N_{12}O_3$: M^+, 802.2877.

3.6. Preparation of BPhes-*b*/*g*

An acetone solution (10 ml) of extracted pigments from cultured *Blastochloris viridis*[1] was demetallated by 5-min stirring with an aqueous diluted HCl solution (3 ml). The reaction mixture was poured into ice-cold water and extracted by dichloromethane. After evaporation in vacuo, the crude mixture including BPhe-*b* and some carotenoids was separated and purified by normal-phase HPLC (acetone/hexane=1/4) to give pure BPhe-*b*.

Pure BPhe-*g* was also prepared by the above procedures except that pigment extracts from *Heliobacterium modesticaldum* were used.[36]

3.7. Acidic treatment of natural BPhes-*b*/*g* and synthetic 4/3

To an acetone solution (3 ml) of sample (ca. 10 μmol), an aqueous 3.5% HCl solution (10 μl) was added allowed to stand in the dark. UV–VIS–NIR spectra were recorded at 2/3 min intervals for BPhes-*b*/*g* and every 30 min for **4** and **3**. When Qy maxima of BPhes-*b*/*g* had completely disappeared, the acidic acetone solution was diluted with water and extracted with dichloromethane. In **4/3**, the same work-up was done after 5-h standing. After evaporation in vacuo, the residue was analyzed by normal-phase HPLC (acetone/hexane=1/4 for BPhes-*b*/*g* and 3/7 for **4/3**). Chromatograms given from BPhe-*b* or *g* showed some chlorin chromophores, 3-Ac-Phe-*a* or Phe-*a* at 11 or 9 min, respectively, which was eluted faster than BPhe-*b* or *g* at 13 or 11 min, and a small amount of 8^1-oxidized chlorin at over 50 min. HPLC from **4** or **3** showed a single peak at 10 or 8 min, which was consistent with the elution time of **4** or **3**.

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Reactions of *N*-sulfenyl-1,2-benzisothiazolin-3-ones with nucleophiles

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Abstract—Reactions of *N*-[2-(alkoxycarbonyl)benzenesulfenyl]-1,2-benzisothiazolin-3-ones (**1**) with various nucleophiles were examined. Anions of active methylene compounds attacked the sulfur atoms of the sulfenyl moieties of **1** to afford sulfide compounds, while thiols attacked the sulfur atoms of the benzisothiazolinone moieties of **1** to afford ring-opened products. Grignard reagents attacked both the above sulfur atoms to give ring-opened products along with sulfide compounds.

1. Introduction

The transformation of organosulfur compounds to other sulfur-containing compounds is of importance in various applications.[1] Several organosulfur compounds have been used as intermediates for synthesis of other sulfur-containing compounds.[1b,2] Among them, sulfenamides are useful sulfenylating reagents, and they react with various nucleophiles such as amines,[2–4] active methylene compounds,[4–6] and thiols[2,4,7,8] to afford corresponding sulfenylated products in high yields. In particular, the introduction of heterocyclic leaving groups into sulfenamides leads to a remarkable increase in the reactivity of sulfenamides in substitution reactions.[3,4,6,8] Exceptional examples are reactions of *N*-sulfenylated phthalimides and succinimides with primary amines to give ring-opened products.[9] In a previous paper,[10] we reported that the substitution reaction of *N*-[2-(alkoxycarbonyl)benzenesulfenyl]-1,2-benzisothiazolin-3-ones (**1**) with amines efficiently afforded *N*-substituted 2-sulfenamoylbenzoates. In these reactions, nucleophiles selectively attacked the sulfur atom of the sulfenyl moieties of **1**, and the 1,2-benzisothiazolin-3-one group behaved as an excellent leaving group. During our ongoing investigation of the substitution reactions of **1** with other nucleophiles, we found that there are two reaction sites on **1**: anions of active methylene compounds attacked the sulfur atoms of the sulfenyl moieties of **1** to afford sulfide compounds, while thiols attacked the sulfur atoms of the benzisothiazolinone moieties of **1** to give ring-opened products. Grignard reagents attacked both the above sulfur atoms to produce the mixture of sulfides and ring-opened products.

2. Results and discussion

2.1. Substitution reaction of 1a with active methylene compounds

Initially, we examined the influence of the bases triethylamine, pyridine, and sodium hydride in a model reaction of *N*-[2-(methoxycarbonyl)benzenesulfenyl]-1,2-benzisothiazolin-3-one (**1a**) with diethyl malonate in THF. When the reaction was carried out in the presence of triethylamine or pyridine, none of the desired product, diethyl 2-[2-(methoxycarbonyl)benzenesulfenyl]malonate (**2a**), was obtained, even when the reaction mixture was refluxed for several hours, and only starting materials were recovered. However, **2a** was obtained in 78% yield when the reaction was carried out in the presence of sodium hydride (1.5 equiv) at room temperature for 1 h (Table 1, entry 1). We next examined the substitution reaction of various active methylene compounds with **1a** under the same reaction conditions (Table 1, entries 2–6). Reactions of **1a**

Keywords: 1,2-Benzisothiazolin-3-one; Nucleophiles; Active methylene compounds; Grignard reagents; Thiols; Substitution reactions; Ring-opening reactions.
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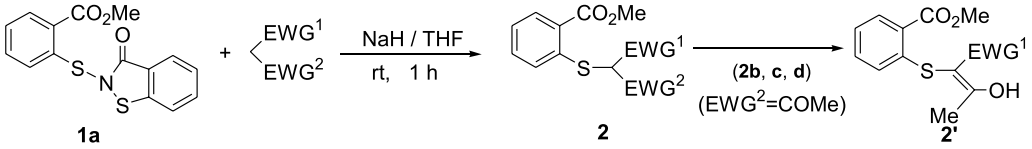
Table 1. Substitution reaction of *N*-[2-(methoxycarbonyl)benzenesulfenyl]-1,2-benzisothiazolin-3-one (**1a**) with active methylene compounds



Entry	EWG^1	EWG^2	Yield (%) of **2**[a]	
1	CO_2Et	CO_2Et	**2a**	78
2	CO_2Et	COMe	**2b**	88
3	COPh	COMe	**2c**	73
4	COMe	COMe	**2d**	81
5	CN	CO_2Et	**2e**	74
6	CN	SO_2Ph	**2f**	91

[a] Isolated product.

with active methylene compounds having an acetyl group proceeded smoothly: ethyl acetoacetate, 1-benzoylacetone, and acetylacetone provided ethyl 2-[2-(methoxycarbonyl)-benzenesulfenyl]-2-acetylacetate (**2b**), 1-phenyl-2-[2-(methoxycarbonyl)benzenesulfenyl]butan-1,3-dione (**2c**), and 3-[2-(methoxycarbonyl)benzenesulfenyl]pentan-2,4-dione (**2d**) in good yields (88, 73, and 81%, respectively; entries 2–4). The NMR spectra of **2b**, **2c**, and **2d** showed that they existed as the enol isomers **2′** in $CDCl_3$. For example, the ^1H NMR spectrum of **2d** in $CDCl_3$ displayed a singlet peak at low field (δ_H 17.4), which was assigned to the O–*H* of the enol isomer of **2d**; the ^{13}C NMR signal of the S–*C* of **2d** shifted downfield (δ_C 101.5), which indicated that the carbon transformed to sp^2 carbon. This observation is in agreement with the isomerization found for α-sulfenyl-β-dicarbonyl compounds, which exist in the enol form in $CDCl_3$.[11] Reactions of **1a** with ethyl cyanoacetate and (phenylsulfonyl)acetonitrile proceeded smoothly and provided ethyl 2-[2-(methoxycarbonyl)benzenesulfenyl]-2-cyanoacetate (**2e**) and 2-[2-(methoxycarbonyl)benzenesulfenyl]-2-(phenylsulfonyl)acetonitrile (**2f**) in good yields (74 and 91%, respectively; entries 5 and 6).

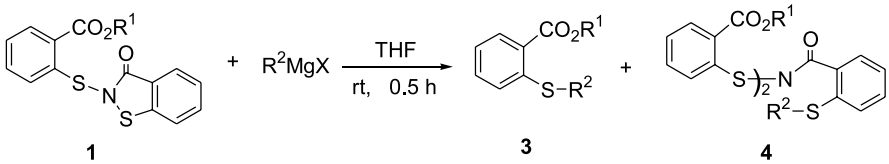
2.2. Substitution reactions of 1 with Grignard reagents

When the reaction of *N*-[2-(ethoxycarbonyl)benzenesulfenyl]-1,2-benzisothiazolin-3-one (**1b**) with a small excess of methylmagnesium bromide was carried out in THF at room temperature for 0.5 h, the desired product, sulfide derivative **3a**, was obtained in only 13% yield. Interestingly, an unexpected product, *N*,*N*-disulfenyl-2-(methylthio)benzamide derivative **4a**, was isolated in 45% yield as a major product (Table 2, entry 1). The structure of **4a** was determined by IR and NMR spectroscopy and elemental analysis. No N–H absorption was observed in the IR spectrum. The resonances for CH_2 and CH_3 of the OCH_2CH_3 group were observed at δ_H 4.35 and 1.34, respectively, and a single resonance for the S–CH_3 group was observed at δ_H 2.55 in the ^1H NMR spectrum. Two resonances were observed in the O=*C* region at δ_C 175.8 and 166.6 in the ^{13}C NMR spectrum and were assigned to the carbonyl groups of the amide and ester moieties, respectively. The similar ring-opened products, **4b** (41%, entry 2) and **4c** (47%, entry 3), were also obtained as major products when **1b** was treated with benzylmagnesium chloride or phenylmagnesium bromide, respectively. Reaction of **1a** with phenylmagnesium bromide gave **4d** in 32% yield along with **3d** in 40% yield (entry 4). To further explore the structure of **4**, a single-crystal X-ray diffraction study of **4b** was performed. The molecular structure of **4b** is shown in Figure 1, and the structure was consistent with that of *N*,*N*-disulfenyl-2-(methylthio)benzamide.

A plausible mechanism for the formation of **4** is shown in Scheme 1. In this mechanism, the Grignard reagent attacks the sulfur atom of the 1,2-benzisothiazolin-3-one moiety of **1** to generate an amide anion. The amide anion then attacks the sulfur atom of the sulfenamide moiety of another molecule of **1** to form **4** along with 1,2-benzisothiazolin-3-one as a leaving group.

The foregoing results clearly showed that reactions of

Table 2. Reaction of *N*-sulfenyl-1,2-benzisothiazolin-3-ones **1** with Grignard reagents



Entry	**1**	R^1	R^2	X	Product			
					3	Yield[a] (%)	**4**	Yield[a] (%)
1	**1b**	Et	Me	Br	**3a**	13	**4a**	45
2	**1b**	Et	$PhCH_2$	Cl	**3b**	30	**4b**	41
3	**1b**	Et	Ph	Br	**3c**	26	**4c**	47
4	**1a**	Me	Ph	Br	**3d**	40	**4d**	32

[a] Isolated product.

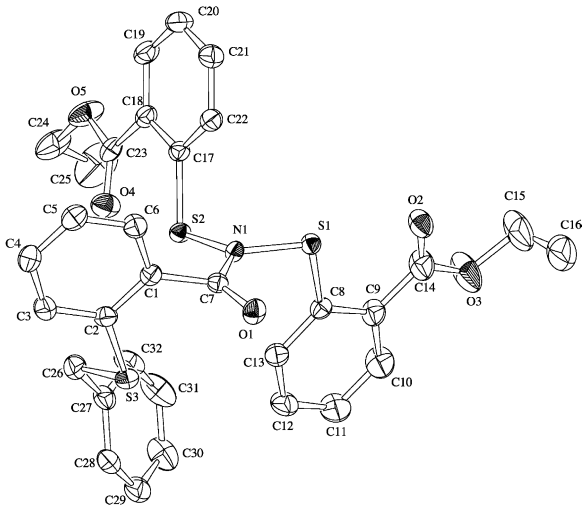
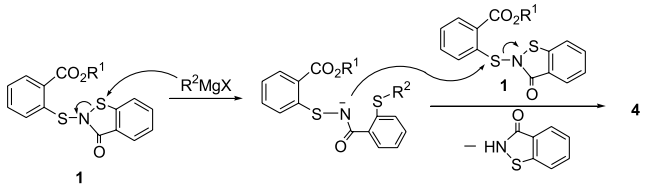


Figure 1. Crystal structure of **4b**.



Scheme 1. A plausible mechanism for the formation of **4**.

N-sulfenylated 1,2-benzisothiazolin-3-ones with Grignard reagents occurred on the sulfur atom in the ring. However, in 1952 it was reported that Grignard reagents added to the carbonyl group of *N*-aryl-substituted 1,2-benzisothiazolin-3-ones to afford hydroxyl compounds **6′**.[12] To verify our results, we re-examined the reactions of *N*-aryl- and *N*-aralkyl-substituted 1,2-benzisothiazolin-3-ones with Grignard reagents (Table 3). The reaction of *N*-phenyl-1,2-benzisothiazolin-3-one (**5a**) with 1.4 equiv of methylmagnesium bromide did not give hydroxyl compound **6′a**, but afforded a ring-opened product, *N*-phenyl-2-(methylthio)benzamide (**6a**), with the same melting point as reported in the literature[12] (entry 1). The structure of **6a** was established by IR and NMR spectroscopy. A sharp absorption at $\nu = 3300\ \mathrm{cm}^{-1}$ in the IR spectrum was assigned to the N–H bond. The resonance for O=*C* of the amide group was observed at δ_C 166.0 in the ^{13}C NMR spectrum. The agreement between the melting point of **6a** and that reported for *N*-phenyl-2-(methylthio)benzamide obtained by the reaction of 2-(methylthio)benzoyl chloride with aniline[13] confirmed the structure. Reactions of **5a–c** with phenylmagnesium bromide or methylmagnesium bromide also provided ring-opened products, **6b–e**, in moderate to high yields (entries 2–5).

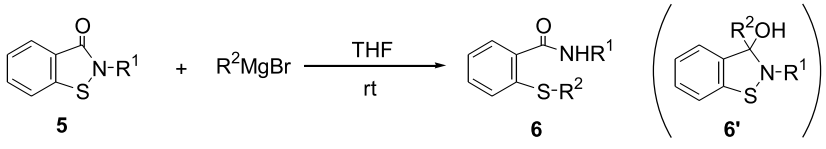
2.3. Substitution reaction of 1 with thiols

Reaction of **1a** with 1 equiv of 1-dodecanethiol in methanol at room temperature for 0.5 h gave a ring-opened product, *N*-[2-(methoxycarbonyl)benzenesulfenyl]-2-(dodecyldithio)benzamide (**7a**), in 92% yield (Table 4, entry 1). Ring-opened products, *N*-[2-(ethoxycarbonyl)benzenesulfenyl]-2-(dodecyldithio)benzamide (**7b**) and *N*-[2-(ethoxycarbonyl)benzenesulfenyl]-2-(octyldithio)benzamide (**7c**), were also obtained in excellent yields from the reactions of **1b** with 1-dodecanethiol and 1-octanethiol under the same reaction conditions (Table 4, entries 2 and 3). When **7a** was treated with 1 equiv of 1-dodecanethiol in methanol at 40 °C for 1 h, two kinds of unsymmetrical disulfides, methyl 2-(dodecyldithio)benzoate (**8**) and 2-(dodecyldithio)benzamide (**9**), were obtained in 70 and 77% yields, respectively (Scheme 2, Eq. (1)). The foregoing results suggested that thiols could react with 1,2-benzisothiazolin-3-one (**10**) and *N*-alkyl-substituted derivatives to afford ring-opened products.[14] Indeed, when **10** and **11** were treated with 1-dodecanethiol in methanol at room temperature for 0.5 h, respectively, the corresponding products **9** and **12** were obtained in good yields (98 and 76%, respectively; Scheme 2, Eq. (2)).

3. Conclusion

Reactions of *N*-[2-(alkoxycarbonyl)benzenesulfenyl]-1,2-benzisothiazolin-3-ones (**1**) with various nucleophiles were examined. Anions of active methylene compounds, like amines,[10] attacked the sulfur atoms of the sulfenyl moieties of **1** to afford sulfide compounds. However, in the case of thiols, reaction occurred on the benzisothiazolinone ring moieties of **1** to give ring-opened products. Grignard reagents attacked both the above sulfur atoms to produce the mixtures of sulfides and ring-opened products. These results

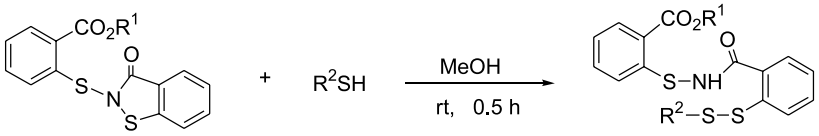
Table 3. Reaction of 1,2-benzisothiazolin-3-ones **5** with Grignard reagents



Entry	5	R^1	R^2	Time (h)	6	Yield[a] (%)	Mp (°C)		
							Observed	6, reported[b]	6′, reported[c]
1	**5a**	Ph	Me	3	**6a**	41	146.7–147.5	148–149	150
2	**5a**	Ph	Ph	1	**6b**	79	118.7–119.0	—	170
3	**5b**	*p*-MeC_6H_4	Me	3	**6c**	67	144.1–145.5	145–146	—
4	**5c**	$PhCH_2CH_2$	Me	1.5	**6d**	55	118.1–118.6	—	—

[a] Isolated product.
[b] Uchida, Y.; Kozuka, S. *Bull. Chem. Soc. Jpn* **1982**, *55*, 1183–1187.
[c] Mustafa, A.; Hilmy, M. K. *J. Chem. Soc.* **1952**, 1339–1342.

Table 4. Reaction of *N*-sulfenyl-1,2-benzisothiazolin-3-ones **1** with thiols



Entry	**1**	R^1	R^2	Yield (%) of **7**[a]	
1	**1a**	Me	$CH_3(CH_2)_{11}$	**7a**	92
2	**1b**	Et	$CH_3(CH_2)_{11}$	**7b**	97
3	**1b**	Et	$CH_3(CH_2)_7$	**7c**	99

[a] Isolated product.

indicated that the reaction pathway of **1** can be controlled by using different nucleophiles for different purposes: hard nucleophiles such as amines and sodium salts of active methylene compounds can be used to synthesize sulfenylated compounds; soft nucleophiles such as thiols can be employed to obtain ring-opened products.

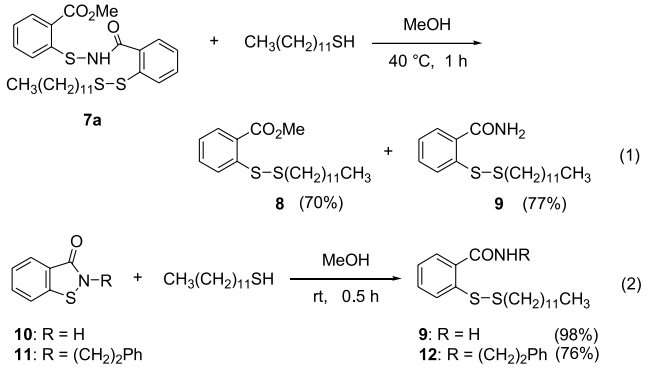
4. Experimental

4.1. General

Melting points were determined on a Mettler FP90 microscopic plate and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained with a JEOL LA-500 spectrometer, and chemical shifts (δ) are reported in parts per million relative to internal tetramethylsilane and $CDCl_3$, respectively. IR spectra were recorded on a JASCO FT IR-5300 spectrophotometer. Analytical TLC was performed on a Merck precoated TLC plate (silica gel 60 F254, 0.25 mm). Silica gel column chromatography was carried out on Merck silica gel 60 (0.063–0.200 mm). Elemental analysis and HRMS analysis were performed by the Analytical Center at the National Institute of Advanced Industrial Science and Technology. *N*-[2-(Alkoxycarbonyl)benzenesulfenyl]-1,2-benzisothiazolin-3-ones (**1**) were prepared by the method described in our previous paper.[15]

4.2. General procedure for the substitution reaction of 1a with active methylene compounds

To a suspension of NaH (0.75 mmol, 18.0 mg) in THF (5 mL) at room temperature under a nitrogen atmosphere was added a solution of active methylene compound (0.75 mmol) in 5 mL of THF. The mixture was stirred for 0.5 h. A solution of **1a** (0.50 mmol, 158.5 mg) in 5 mL of THF was then added, and after the reaction mixture was stirred for 0.5 h, water was added. Product was extracted with dichloromethane, and the organic layer was washed with water and dried over magnesium sulfate. The solvent was removed under reduced pressure, and the crude product was purified by silica gel column chromatography (eluent: dichloromethane).



Scheme 2.

4.2.1. Diethyl 2-[2-(methoxycarbonyl)benzenesulfenyl]-malonate (2a). Colorless oil; R_f=0.23 (hexane/ethyl acetate=4:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.25 (6H, t, J=7.0 Hz), 3.93 (3H, s), 4.24 (4H, q, J=7.0 Hz), 4.82 (1H, s), 7.29 (1H, ddd, J=7.9, 7.0, 1.2 Hz), 7.45 (1H, ddd, J=8.2, 7.0, 1.5 Hz), 7.50 (1H, dd, J=8.2, 1.2 Hz), 7.92 (1H, dd, J=7.9, 1.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.9, 52.4, 54.1, 62.5, 126.4, 129.8, 130.6, 131.1, 132.4, 136.7, 166.4, 166.9; IR (neat): 2984, 1730, 1466, 1437, 1368, 1256, 1146, 1061, 1028, 747 cm^{-1}; HRMS: calcd for $C_{15}H_{18}O_6S$ 326.0824, found 326.0827.

4.2.2. Ethyl 3-hydroxy-2-[2-(methoxycarbonyl)benzenesulfenyl]-2-butenecarboxylate (2′b). Colorless crystal with mp 77.6–79.0 °C (from CH_2Cl_2–hexane); R_f=0.50 (dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.13 (3H, t, J=7.0 Hz), 2.29 (3H, s), 3.95 (3H, s), 4.19 (2H, q, J=7.0 Hz), 7.06 (1H, dd, J=8.2, 1.2 Hz), 7.14 (1H, ddd, J=7.6, 7.3, 1.2 Hz), 7.38 (1H, ddd, J=8.2, 7.3, 1.5 Hz), 8.02 (1H, dd, J=7.6, 1.5 Hz), 13.96 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.0, 20.8, 52.1, 61.6, 91.8, 123.9, 124.7, 126.1, 131.5, 132.4, 143.0, 166.8, 173.0, 185.0; IR (KBr): 2984, 2957, 1717, 1628, 1589, 1333, 1250, 745 cm^{-1}; Anal. calcd for $C_{14}H_{16}O_5S$: C, 56.74; H, 5.44. Found: C, 57.04; H, 5.32.

4.2.3. 3-Hydroxy-2-[2-(methoxycarbonyl)benzenesulfenyl]-1-phenyl-2-buten-1-one (2′c). Yellow crystal with mp 141.2–142.5 °C (from CH_2Cl_2–hexane); R_f=0.38 (hexane/ethyl acetate=4:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.37 (3H, s), 3.90 (3H, s), 7.19 (1H, t, J=8.2 Hz), 7.26–7.30 (3H, m), 7.39 (1H, t, J=7.7 Hz), 7.48 (1H, td, J=7.7, 1.8 Hz), 7.61 (2H, dd, J=8.2, 1.8 Hz), 8.02 (1H, dd, J=7.3, 1.8 Hz), 17.84 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.7, 52.1, 100.9, 124.3, 124.6, 126.2, 127.8, 128.4, 131.2,

131.9, 133.0, 135.4, 143.4, 166.5, 190.6, 203.6; IR (KBr): 3063, 3003, 2951, 1715, 1588, 1535, 1460, 1435, 1269, 1252, 745, 694 cm^{-1}; Anal. calcd for $C_{18}H_{16}O_4S$: C, 65.84; H, 4.91. Found: C, 65.85; H, 4.71.

4.2.4. 4-Hydroxy-3-[2-(methoxycarbonyl)benzenesulfenyl]-3-penten-2-one (2′d). Colorless crystal with mp 116.0–117.0 °C (from CH_2Cl_2–hexane); R_f=0.50 (dichloromethane); ^1H NMR (500 MHz, $CDCl_3$): δ 2.29 (6H, s), 3.96 (3H, s), 7.06 (1H, dd, J=8.2, 1.2 Hz), 7.19 (1H, ddd, J=7.9, 7.3, 1.2 Hz), 7.42 (1H, ddd, J=8.2, 7.3, 1.5 Hz), 8.07 (1H, dd, J=7.9, 1.5 Hz), 17.38 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.3, 52.2, 101.5, 124.0, 124.3, 126.2, 132.0, 133.0, 142.5, 166.7, 198.6; IR (KBr): 2957, 1713, 1593, 1562, 1462, 1437, 1275, 1256 cm^{-1}; Anal. calcd for $C_{13}H_{14}O_4S$: C, 58.63; H, 5.30. Found: C, 58.63; H, 5.15.

4.2.5. Ethyl 2-cyano-2-[2-(methoxycarbonyl)benzenesulfenyl]acetate (2e). Colorless crystal with mp 67.3–68.3 °C (from CH_2Cl_2–hexane); R_f=0.37 (dichloromethane); ^1H NMR (500 MHz, $CDCl_3$): δ 1.31 (3H, t, J=7.0 Hz), 3.95 (3H, s), 4.30 (2H, qd, J=7.0, 0.9 Hz), 4.86 (1H, s), 7.42 (1H, ddd, J=7.6, 7.3, 1.2 Hz), 7.56 (1H, ddd, J=8.2, 7.3, 1.5 Hz), 7.63 (1H, dd, J=8.2, 1.2 Hz), 7.98 (1H, dd, J=7.6, 1.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.9, 39.0, 52.7, 63.9, 114.1, 128.1, 131.4, 131.5, 131.6, 132.9, 133.5, 163.2, 166.8; IR (KBr): 2988, 2955, 2907, 1746, 1709, 1289, 1260, 745 cm^{-1}; Anal. calcd for $C_{13}H_{13}NO_4S$: C, 55.90; H, 4.69; N, 5.01. Found: C, 55.98; H, 4.51; N, 4.91.

4.2.6. 2-(Benzenesulfonyl)-2-[2-(methoxycarbonyl)benzenesulfenyl]acetonitrile (2f). Yellow oil; R_f=0.36 (dichloromethane/ethyl acetate=4:1); ^1H NMR (500 MHz, $CDCl_3$): δ 5.34 (1H, s), 7.49 (1H, td, J=7.6, 1.2 Hz), 7.55 (1H, td, J=7.6, 1.8 Hz), 7.66 (2H, td, J=7.6, 1.2 Hz), 7.76 (1H, dd, J=7.6, 1.2 Hz), 7.79 (1H, td, J=7.6, 1.2 Hz), 7.92 (1H, dd, J=7.6, 1.8 Hz), 8.09 (2H, dd, J=7.6, 1.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 52.9, 60.5, 112.2, 129.5, 129.8, 130.4, 131.0, 131.3, 132.8, 133.8, 134.8, 135.0, 135.6, 166.8; IR (neat): 3067, 2957, 2924, 1711, 1586, 1449, 1337, 1290, 1262, 1159, 748, 685, 588 cm^{-1}; HRMS: calcd for $C_{16}H_{13}NO_4S_2$ 347.0286, found 347.0250.

4.3. General procedure for reaction of 1 with Grignard reagents

To a solution of **1** (0.5 mmol) in THF (10 mL) at room temperature was added a Grignard reagent (1.0 M in THF, 0.7 mL) under a nitrogen atmosphere. The mixture was stirred for 2 h, and then water was added to the reaction mixture. Product was extracted with dichloromethane, and the organic layer was washed with water and dried over magnesium sulfate. The solvent was removed under reduced pressure, and the crude product was purified by silica gel column chromatography (eluent: 1:1 dichloromethane/hexane, dichloromethane, 100:5:1 dichloromethane/acetone/methanol).

4.3.1. Ethyl 2-(methylthio)benzoate (3a). Colorless oil;[15] R_f=0.67 (dichloromethane); ^1H NMR (500 MHz, $CDCl_3$): δ 1.40 (3H, t, J=7.3 Hz), 2.45 (3H, s), 4.39 (2H, q, J=7.3 Hz), 7.15 (1H, ddd, J=8.5, 7.6, 0.9 Hz), 7.27 (1H, d, J=7.9 Hz), 7.47 (1H, ddd, J=8.5, 7.9, 1.5 Hz), 8.01 (1H, dd, J=7.6, 1.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.0, 15.3, 60.7, 123.1, 124.0, 126.8, 130.9, 132.1, 142.8, 166.1; IR (KBr): 1709, 1246, 1062, 742 cm^{-1}.

4.3.2. *N*,*N*-Bis[2-(ethoxycarbonyl)benzenesulfenyl]-2-(methylthio)benzamide (4a). Colorless crystal with mp 165.4–166.9 °C (from dichloromethane–hexane); R_f=0.50 (dichloromethane); ^1H NMR (500 MHz, $CDCl_3$): δ 1.34 (6H, t, J=7.0 Hz), 2.55 (3H, s), 4.35 (4H, q, J=7.0 Hz), 6.99 (1H, td, J=7.6, 1.2 Hz), 7.17(1H, dd, J=7.6, 1.2 Hz), 7.26 (4H, t, J=6.1 Hz), 7.40 (1H, d, J=7.3 Hz), 7.66 (2H, t, J=7.3 Hz), 7.80 (1H, brs), 8.01 (2H, d, J=7.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.2, 18.5, 61.6, 123.4, 124.0, 124.9, 125.0, 125.9, 129.9, 130.4, 131.0, 133.4, 134.9, 137.5, 166.6, 175.8; IR (KBr): 1688, 1310, 1176, 1275, 1101, 749 cm^{-1}; Anal. calcd for $C_{26}H_{25}NO_5S_3$: C, 59.18; H, 4.78; N, 2.65. Found: C, 59.40; H, 4.60; N, 2.59.

4.3.3. Ethyl 2-(benzylthio)benzoate (3b). Colorless crystal with mp 69.6–70.2 °C (from hexane) (lit.[16] 68.5–69 °C); R_f=0.67 (dichloromethane); ^1H NMR (500 MHz, $CDCl_3$): δ 1.38 (3H, t, J=7.2 Hz), 4.16 (2H, s), 4.37 (2H, q, J=7.2 Hz), 7.16 (1H, ddd, J=7.8, 7.0, 1.2 Hz), 7.25 (1H, ddd, J=7.6, 7.0, 1.5 Hz), 7.29–7.34 (3H, m), 7.39 (1H, dd, J=7.6, 1.5 Hz), 7.40–7.44 (2H, m), 7.97 (1H, dd, J=7.8, 1.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.4, 37.5, 61.3, 124.2, 126.1, 127.5, 128.1, 128.7, 129.2, 131.3, 132.4, 136.3, 141.9, 166.6; IR (neat): 2978, 1705, 1277, 1250, 1148, 1063, 734, 712 cm^{-1}.

4.3.4. *N*,*N*-Bis[2-(ethoxycarbonyl)benzenesulfenyl]-2-(benzylthio)benzamide (4b). Colorless crystal with mp 150.2–151.6 °C (from ethyl acetate–hexane); R_f=0.50 (dichloromethane); ^1H NMR (500 MHz, $CDCl_3$): δ 1.32 (6H, t, J=7.0 Hz), 4.21 (2H, s), 4.26–4.33 (4H, m), 7.02 (1H, td, J=7.6, 0.9 Hz), 7.13–7.34 (13H, m), 7.63 (1H, brs), 8.00 (2H, d, J=5.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.3, 41.2, 61.6, 123.5, 124.0, 124.8, 124.9, 127.2, 127.3, 128.5, 129.4, 129.6, 131.0, 132.1, 133.4, 134.1, 137.1, 140.0, 143.6, 166.6, 176.0; IR (KBr) 1687, 1312, 1277, 1104, 747 cm^{-1}; Anal. calcd for $C_{32}H_{29}NO_5S_3$: C, 63.66; H, 4.84; N, 2.32. Found: C, 63.82; H, 4.71; N, 2.34.

4.3.5. Ethyl 2-(phenylthio)benzoate (3c). Colorless oil;[17] R_f=0.67 (dichloromethane); ^1H NMR (500 MHz, $CDCl_3$): δ 1.42 (3H, t, J=7.2 Hz), 4.42 (2H, q, J=7.2 Hz), 6.82 (1H, dd, J=8.1, 1.1 Hz), 7.13 (1H, td, J=7.6, 1.1 Hz), 7.24 (2H, ddd, J=8.5, 7.3, 1.5 Hz), 7.41–7.44 (3H, m), 7.56 (2H, dt, J=7.9, 2.4 Hz), 7.99 (1H, dd, J=7.9, 1.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.3, 61.3, 124.3, 127.2, 127.5, 129.0, 129.7, 131.0, 132.2, 132.7, 135.5, 143.0, 166.5; IR (KBr): 1711, 1250, 742 cm^{-1}.

4.3.6. *N*,*N*-Bis[2-(ethoxycarbonyl)benzenesulfenyl]-2-(phenylthio)benzamide (4c). Colorless crystal with mp 197.0–200.0 °C (from dichloromethane–hexane); R_f=0.50 (dichloromethane); ^1H NMR (500 MHz, $CDCl_3$): δ 1.34 (6H, t, J=7.2 Hz), 4.32 (4H, q, J=7.0 Hz), 7.07 (1H, td, J=7.5, 1.2 Hz), 7.02–7.34 (11H, m), 7.41 (2H, dd, J=7.0, 1.5 Hz), 7.58 (1H, brs), 8.00 (2H, d, J=7.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 61.5, 123.3, 124.0, 124.9, 125.5, 127.1, 129.2, 130.1, 130.7, 131.0, 132.6, 133.4, 133.5,

135.7, 138.6, 166.6, 175.5; IR (KBr): 2980, 1687, 1462, 1278, 1059, 744 cm^{-1}; Anal. calcd for $C_{31}H_{27}NO_5S_3$: C, 63.13; H, 4.61; N, 2.38. Found: C, 62.81; H, 4.50; N, 2.23.

4.3.7. Methyl 2-(phenylthio)benzoate (3d). Colorless oil; (lit.[18] bp 140 °C/120 Pa); R_f=0.67 (dichloromethane); ^1H NMR (500 MHz, $CDCl_3$): δ 3.95 (3H, s), 6.82 (1H, d, J=8.2 Hz), 7.12 (1H, ddd, J=7.9, 7.3, 1.2 Hz), 7.23 (1H, ddd, J=8.2, 7.3, 1.5 Hz), 7.41–7.43 (3H, m), 7.55–7.57 (2H, m), 7.97 (1H, dd, J=8.2, 1.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 52.1, 124.3, 126.7, 127.4, 129.0, 129.7, 131.0, 132.2, 132.5, 135.5, 143.1, 166.8; IR (neat): 1717, 1435, 1250, 1059, 743 cm^{-1}.

4.3.8. *N*,*N*-Bis[2-(methoxycarbonyl)benzenesulfenyl]-2-(phenylthio)benzamide (4d). Colorless crystal with mp 197.7–199.3 °C (from ethyl acetate–hexane); R_f=0.50 (dichloromethane); ^1H NMR (500 MHz, $CDCl_3$): δ 3.86 (6H, s), 7.07 (1H, td, J=7.5, 1.2 Hz), 7.20–7.32 (11H, m), 7.41 (2H, d, J=7.3 Hz), 7.58 (1H, brs), 7.98 (2H, d, J=7.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 52.5, 123.4, 123.7, 125.0, 125.5, 127.1, 129.2, 130.2, 130.7, 131.1, 132.7, 133.6, 133.6, 135.8, 138.6, 175.5, 178.2, 190.5, 196.9; IR (KBr): 1680, 1314, 1281, 1424, 747 cm^{-1}; Anal. calcd for $C_{29}H_{23}NO_5S_3$: C, 62.01; H, 4.13; N, 2.49. Found: C, 62.21; H, 3.92; N, 2.51.

4.4. X-ray crystallographic analysis of 4b

X-ray crystallographic analysis was carried out on a Rigaku AFC7R diffractometer using a rotating anode with graphite monochromated Mo Kα radiation (λ=0.7107 Å). Crystal data for **4b**: $C_{32}H_{29}NO_5S_3$, M=603.76, monoclinic, space group $P2_1/n$, a=11.138(2), b=16.692(2), c=16.453(3) Å, β=98.99(2)°, V=3021.3(9) $Å^3$, T=173.2 K, Z=4, D_{calc}=1.327 g cm^{-3}, μ=0.286 mm^{-1}; goodness of fit=1.005; $R1$ [$I>2\sigma(I)$]=0.038, $wR2$=0.145 (all data).

Selected bond distances (Å) and angles (°) are shown as follows: S(1)–N(1) 1.722(1), S(1)–C(8) 1.777(2), S(2)–N(1) 1.714(1), S(2)–C(17) 1.774(2), O(1)–C(7) 1.203(2), N(1)–C(7) 1.404(2), C(1)–C(7) 1.512(2); S(1)–N(1)–S(2) 117.76(7), S(1)–N(1)–C(7) 120.9(1), C(8)–S(1)–N(1) 101.50(6), S(2)–N(1)–C(7) 120.3(1), C(17)–S(2)–N(1) 101.02(7), O(1)–C(7)–N(1) 121.9(1), O(1)–C(7)–C(1) 123.3(1). Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 243897. 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].

4.5. General procedure for the reaction of 5 with Grignard reagents

To a solution of **5** (0.5 mmol) in THF (10 mL) at room temperature was added a Grignard reagent (1 M in THF, 0.7 mL) under nitrogen atmosphere. The mixture was stirred for 2 h, and then water was added to the reaction mixture. Product was extracted with dichloromethane, and the organic layer was washed with water and dried over magnesium sulfate. The solvent was removed under reduced pressure, and the crude product was purified by silica gel column chromatography (eluent: dichloromethane, 10:1 dichloromethane/ethyl acetate).

4.5.1. *N*-Phenyl-2-(methylthio)benzamide (6a). Colorless crystal with mp 146.7–147.5 °C (from ethyl acetate–hexane) (lit.[13] 148–149 °C); R_f=0.70 (dichloromethane/ethyl acetate=10:1); ^1H NMR (500 MHz, $CDCl_3$): δ 2.50 (3H, s), 7.16 (1H, t, J=7.3 Hz), 7.28 (2H, t, J=7.6 Hz), 7.36–7.45 (4H, m), 7.66 (2H, d, J=7.3 Hz), 7.74 (1H, d, J=7.6 Hz), 8.31 (1H, brs); ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.9, 120.0, 124.5, 125.6, 127.9, 129.0, 129.3, 131.0, 132.3, 136.6, 137.9, 166.0; IR (KBr): 3300, 1649, 1601 cm^{-1}.

4.5.2. *N*-Phenyl-2-(phenylthio)benzamide (6b). Colorless crystal with mp 118.7–119.0 °C (from ethyl acetate–hexane); R_f=0.45 (dichloromethane); ^1H NMR (500 MHz, $CDCl_3$): δ 7.13 (1H, tt, J=7.3, 1.2 Hz), 7.26–7.37 (10H, m), 7.52 (2H, d, J=7.9 Hz), 7.78 (1H, d, J=8.5 Hz), 8.20 (1H, brs); ^{13}C NMR (125 MHz, $CDCl_3$): δ 120.0, 124.6, 127.5, 127.8, 129.0, 129.6, 131.2, 131.6, 132.7, 134.1, 134.4, 136.9, 137.7, 165.6; IR (KBr): 3354, 1664, 1597 cm^{-1}; Anal. calcd for $C_{19}H_{15}NOS$: C, 74.72; H, 4.95; N, 4.59. Found: C, 74.70; H, 4.82; N, 4.69.

4.5.3. *N*-(*p*-Methylphenyl)-2-(methylthio)benzamide (6c). Colorless crystal with mp 144.1–145.5 °C (from ethyl acetate–hexane) (lit.[13] 145–146 °C); R_f=0.60 (dichloromethane); ^1H NMR (500 MHz, $CDCl_3$): δ 2.33 (3H, s), 2.47 (3H,s), 7.16 (2H, d, J=8.1 Hz), 7.25 (1H, td, J=7.3, 1.2 Hz), 7.36 (1H, dd, J=7.9, 1.2 Hz), 7.41 (1H, ddd, J=7.9, 7.3, 1.5 Hz), 7.53 (2H, d, J=8.1 Hz), 7.69 (1H, d, J=7.3 Hz), 8.29 (1H, brs); ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.0, 20.9, 120.0, 125.7, 128.0, 129.1, 129.5, 130.9, 134.2, 135.3, 135.3, 136.5, 165.8; IR (KBr): 3281, 1649, 1602 cm^{-1}.

4.5.4. *N*-(2-Phenylethyl)-2-(methylthio)benzamide (6d). Colorless crystal with mp 118.1–118.6 °C (from ethyl acetate–hexane); R_f=0.33 (dichloromethane/ethyl acetate=20:1); ^1H NMR (500 MHz, $CDCl_3$): δ 2.39 (3H, s), 2.94 (2H, t, J=7.2 Hz), 3.70 (2H, td, J=7.2, 5.8 Hz), 6.49 (1H, brs), 7.13 (1H, ddd, J=8.2, 7.3, 1.2 Hz), 7.21–7.36 (8H, m), 7.44 (1H, dd, J=7.8, 1.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.6, 35.5, 41.1, 125.1, 126.5, 127.0, 128.3, 128.6, 128.8, 130.6, 135.0, 137.0, 138.9, 168.1; IR (KBr): 3308, 1632, 1541 cm^{-1}; Anal. calcd for $C_{16}H_{17}NOS$: C, 70.81; H, 6.31; N, 5.16. Found: C, 70.90; H, 6.24; N, 5.30.

4.6. General procedure for reaction of 1 with thiols

To a solution of **1** (0.5 mmol) in methanol (10 mL) at room temperature was added thiol (0.5 mmol). The mixture was stirred for 0.5 h, and then the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (eluent: dichloromethane).

4.6.1. *N*-[2-(Methoxycarbonyl)benzenesulfenyl]-2-(dodecyldithio)benzamide (7a). Colorless crystal with mp 79.5–80.3 °C (from ethyl acetate–hexane); R_f=0.38 (dichloromethane); ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, J=6.7 Hz), 1.24–1.36 (18H, m), 1.64–1.70 (2H, m), 2.75 (2H, t, J=7.4 Hz), 3.95 (3H, s), 7.20–7.32 (3H, m),

7.47–7.53 (3H, m), 7.69 (1H, s), 8.00–8.04 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 22.7, 28.5, 28.9, 29.2, 29.3, 29.5, 29.58, 29.63, 31.9, 38.8, 52.5, 122.5, 124.3, 124.7, 126.6, 128.0, 128.7, 131.1, 131.6, 133.1, 133.5, 138.2, 144.5, 167.1, 169.3; IR (KBr): 3268, 2955, 2922, 2851, 1701, 1632, 1470, 1422, 1318, 1292, 1246, 739 cm^{-1}; Anal. calcd for $C_{27}H_{37}NO_3S_3$: C, 62.39; H, 7.17; N, 2.69. Found: C, 62.56; H, 7.19; N, 2.61.

4.6.2. *N*-[2-(Ethoxycarbonyl)benzenesulfenyl]-2-(dodecyldithio)benzamide (7b). Colorless crystal with mp 47.2–48.5 °C (from hexane); R_f=0.38 (dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.87 (3H, t, J=7.0 Hz), 1.23–1.35 (18H, m), 1.41 (3H, t, J=7.3 Hz), 1.66 (2H, quint, J=7.3 Hz), 2.74 (2H, t, J=7.3 Hz), 4.40 (2H, q, J=7.0 Hz), 7.20 (1H, ddd, J=8.2, 6.7, 1.5 Hz), 7.29–7.34 (2H, m), 7.47–7.50 (3H, m), 7.69 (1H, brs), 8.00–8.01 (1H, m), 8.03 (1H, d, J=7.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 14.3, 22.7, 28.5, 28.9, 29.2, 29.3, 29.5, 29.6, 29.6, 29.6, 31.9, 38.8, 61.6, 122.4, 124.6, 124.7, 126.5, 128.6, 131.1, 131.6, 133.0, 133.5, 138.3, 144.4, 166.7, 169.4; IR (KBr): 3235, 1696, 1661, 1420, 1275, 1150, 1103, 1057, 749 cm^{-1}; Anal. calcd for $C_{28}H_{39}NO_3S_3$: C, 63.00; H, 7.36; N, 2.62. Found: C, 63.39; H, 7.43; N, 2.57.

4.6.3. *N*-[2-(Ethoxycarbonyl)benzenesulfenyl]-2-(octyldithio)benzamide (7c). Colorless oil; R_f=0.38 (dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.87 (3H, t, J=7.3 Hz), 1.24–1.31 (8H, m), 1.35–1.37 (2H, m), 1.40 (3H, t, J=7.0 Hz), 1.63–1.69 (2H, m), 2.73 (2H, t, J=7.3 Hz), 4.39 (2H, q, J=7.0 Hz), 7.19 (1H, td, J=7.9, 1.5 Hz), 7.27 (1H, m), 7.40–7.49 (4H, m), 7.68 (1H, brs), 7.99–8.01 (1H, m), 8.02 (1H, d, J=7.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.0, 14.3, 22.6, 28.4, 28.8, 29.1, 31.7, 38.7, 61.5, 122.4, 124.5, 124.6, 126.4, 128.4, 131.0, 131.5, 133.0, 133.3, 133.3, 144.5, 166.6, 169.3; IR (neat): 3266, 1696, 1466, 1433, 1273, 1103, 741 cm^{-1}; HRMS: calcd for $C_{24}H_{31}NO_3S_3$ 477.1466. Found 477.1422.

4.7. Procedure for the reaction of 7a with 1-dodecanethiol

To a solution of **7a** (0.5 mmol, 259.9 mg) in methanol (10 mL) was added 1-dodecanethiol (0.5 mmol, 101.2 mg). The mixture was stirred at 40 °C for 0.5 h, and then the solvent was removed under reduced pressure. The crude products were purified by silica gel column chromatography (eluent: 1:1 dichloromethane/hexane).

4.7.1. Methyl 2-(dodecyldithio)benzoate (8). Colorless oil;[19] R_f=0.70 (dichloromethane/hexane=1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, J=6.8 Hz), 1.24–1.36 (18H, m), 1.63–1.69 (2H, m), 2.71 (2H, t, J=7.6 Hz), 3.94 (3H, s), 7.21–7.26 (1H, m), 7.55 (1H, ddd, J=8.9, 7.3, 1.5 Hz), 8.01 (1H, dd, J=7.8, 1.5 Hz), 8.18 (1H, d, J=8.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 22.7, 28.6, 29.0, 29.2, 29.3, 29.5, 29.57, 29.63, 31.9, 38.5, 52.2, 125.0, 125.7, 127.0, 131.4, 132.7, 142.2, 166.8; IR (KBr): 2926, 2853, 1715, 1460, 1435, 1269, 1252, 1144, 1101, 1053, 745 cm^{-1};

4.7.2. 2-(Dodecyldithio)benzamide (9). Colorless crystal with mp 120.0–121.4 °C (from ethyl acetate–hexane); R_f=0.45 (dichloromethane/acetone/methanol=100:10:2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (3H, t, J=7.0 Hz), 1.28–1.37 (18H, m), 1.66 (2H, quint, J=7.3 Hz), 2.73 (2H, t, J=7.3 Hz), 7.49 (1H, ddd, J=8.2, 7.3, 1.2 Hz), 7.58 (1H, dd, J=7.6, 1.2 Hz), 8.04 (1H, dd, J=8.2, 0.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 22.7, 28.6, 29.0, 29.2, 29.4, 29.5, 29.59, 29.64, 31.9, 38.8, 126.0, 127.9, 128.3, 131.4, 132.9, 138.5, 169.5; IR (KBr): 3372, 3185, 2953, 2920, 2851, 1649, 1616, 1464, 1406, 1103 cm^{-1}; Anal. calcd for $C_{19}H_{31}NOS_2$: C, 64.54; H, 8.84; N, 3.96. Found: C, 64.74; H, 8.91; N, 3.86.

4.8. Procedure for the reactions of 10 or 11 with 1-dodecanethiol

To a solution of **10** or **11** (0.5 mmol) in methanol (10 mL) at room temperature was added 1-dodecanethiol (0.5 mmol, 101.2 mg). The mixture was stirred for 0.5 h, and then the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (eluent: dichloromethane).

4.8.1. *N*-(2-Phenylethyl)-2-(dodecyldithio)benzamide (12). Colorless crystal with mp 71.4–72.3 °C (from dichloromethane–hexane); R_f=0.50 (dichloromethane); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (3H, t, J=6.7 Hz), 1.24–1.35 (18H, m), 1.61–1.67 (2H, m), 2.70 (2H, t, J=7.4 Hz), 2.96 (2H, t, J=6.7 Hz), 3.71–3.75 (2H, m), 6.08 (1H, brs), 7.18–7.44 (8H, m), 7.96 (1H, d, J=8.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 22.7, 28.5, 28.9, 29.2, 29.3, 29.5, 29.59, 29.63, 31.9, 35.6, 38.8, 41.2, 126.1, 126.6, 127.7, 128.7, 128.9, 130.8, 134.5, 137.6, 138.8, 167.7; IR (KBr): 3318, 3065, 3032, 2920, 2851, 1630, 1537, 1456, 1313, 1194, 745, 696 cm^{-1}; Anal. calcd for $C_{27}H_{39}NOS_2$: C, 70.85; H, 8.59; N, 3.06. Found: C, 71.02; H, 8.48; N, 2.85.

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An efficient synthesis of a highly functionalized 4-arylpiperidine

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Abstract—In this manuscript, an efficient synthesis of a functionalized 4-arylpiperidine is disclosed. Several synthetic approaches towards formation of the key aryl-piperidine sp3 carbon–carbon bond are discussed, including a scalable route to the piperidine via reaction of acyl pyridinium ions with aryl Grignard reagents to form the corresponding dihydropyridines. Methods to access the BOC protected piperidine through dihydropyridine intermediates are described.

1. Introduction

4-Arylpiperidines and their derivatives exhibit a diverse array of biological activity and as such are useful pharmacophores. 4-Arylpiperidines have been investigated as *N*-methyl-D-aspartate (NMDA) receptor antagonists, renin inhibitors, α_1 adrenergic receptor antagonists, beta-secretase inhibitors, and serotonin reuptake inhibitors, encompassing therapeutic areas including epilepsy and Parkinson's disease,[1a] heart and kidney insufficiency,[1b,c] hypertension and benign prostatic hyperplasia,[1d] Alzheimer's disease,[1e] and depression.[1f] The broad applicability of 4-arylpiperidines as structural scaffolds has generated considerable interest in general methods for their preparation.

We recently required a synthesis of 4-arylpiperidines containing multiple functional groups. A representative example, **1**, contains both aryl halide and benzonitrile functional groups. The nitrile is a versatile handle which can be further transformed to generate 4-arylpiperidines containing ketones, amines, aldehydes, and amides. Additionally, the aryl chloride moiety can provide functionalization through carbon–carbon or carbon–heteroatom bond formations accessed by various transition-metal-catalyzed cross-coupling reactions. The juxtaposition of both of these functional groups in the same molecule represents a challenge to existing methodology for preparing members of this class of compounds. While the nitrile is reactive to highly nucleophilic reagents, the halide is reactive towards reducing conditions which are frequently employed as a means of obtaining the saturated piperidine ring. In this manuscript, we disclose several routes towards the desired 4-aryl piperidines including a scalable route which utilizes the reaction of acyl pyridinium ions with aryl Grignard reagents to form the corresponding dihydropyridines.

1.1. Retrosynthetic analysis

Literature precedence suggested several possible approaches towards introducing the piperidine ring (Scheme 1). The piperidine ring could be accessed directly by coupling of a piperidyl zinc halide with an aryl bromide[2] or by stepwise reaction of an aryllithium or aryl Grignard species with a protected piperidinone followed by elimination and hydrogenation or deoxygenation.[1a,3] Alternatively, we envisioned formation of the aryl–piperidine bond either by Suzuki coupling of the aryl bromide (or aryl boronate) and suitable partner[4] or by the reaction of the aryl Grignard with an acyl pyridinium ion.[5] Access to the piperidine ring by these methods would further involve reduction of the resulting double-bond(s). Commonly performed via hydrogenation, such reductions could become complicated by dehalogenation. In each case, initial exploration of the route hinged on preparation of 2-bromo-5-chlorobenzonitrile **2b**, preferably by selective bromination of inexpensive chlorobenzonitrile **2a** at the 6 position (Scheme 2). In just 2–4 steps, these methods, if successful, would afford nitrile and halogen-containing 4-arylpiperidines ready for further functionalization.

2. Results and discussion

2.1. Bromination of 3-chlorobenzonitrile

In each of the proposed routes to arylpiperidines **1**, 2-bromo-5-chloro benzonitrile **2b** is a key intermediate.

Keywords: Arylpiperidine; Dihydropyridine; Catalytic hydrogenation.
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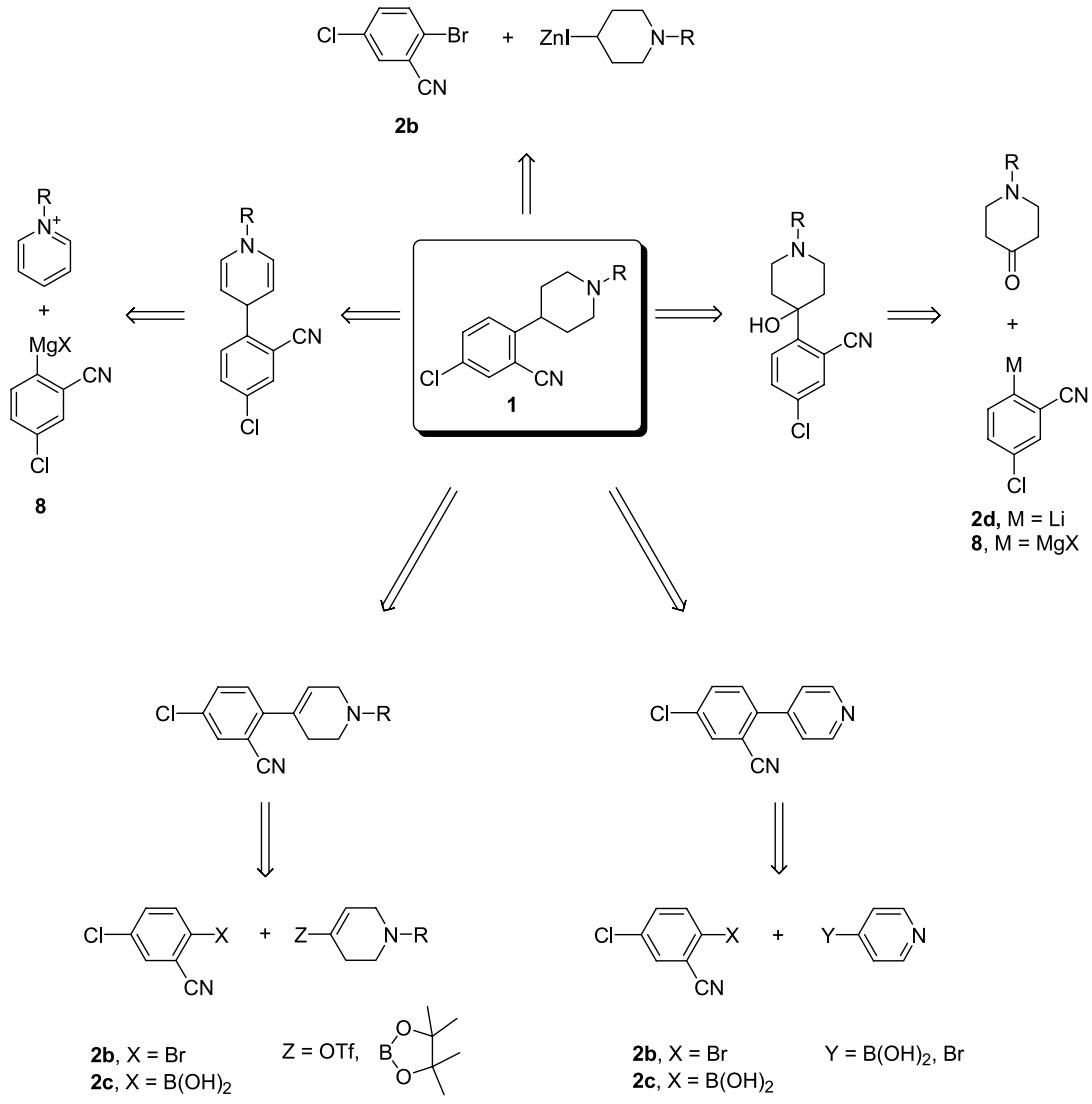
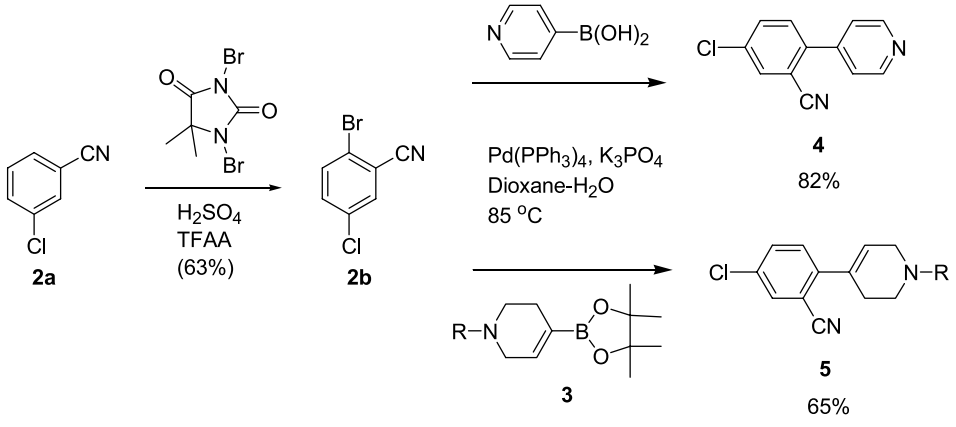


Scheme 1. Retrosynthetic analysis.



Scheme 2. Preparation and coupling of bromochlorobenzonitrile **2b**.

Preparations of this compound from 2-amino-5-chlorobenzonitrile via Sandmeyer reaction (formation of the diazonium salt and reaction with CuBr)[6] and from 2-bromo-5-chlorobenzoic acid via amide formation and dehydration[7] have been described. We desired a more direct synthesis using a readily available starting material and therefore decided to examine selective bromination of chlorobenzonitrile **2a**.[8] Using 1,3-dibromo-5,5-dimethylhydantoin (DBH) and H_2SO_4 in refluxing CH_2Cl_2, we obtained a 50% isolated yield of bromobenzonitrile **2b** after

crystallization. Attempts to improve the yield by varying solvent and brominating agent gave generally poor results, indicating that selective bromination could be affected only under a narrow range of conditions. Neat acids, however, emerged as the best solvents for the DBH bromination. The major by-products formed during the bromination were two mono-brominated regioisomers each occurring in approximately 10% and three dibrominated compounds, occurring in 3–4% each.[†]

An improved yield of 63% was obtained from reaction in trifluoroacetic acid with 1.3 equiv H_2SO_4.

2.2. Initial routes to arylpiperidine 1: Negishi and Suzuki coupling approaches

With the arylbromide in hand, we began investigation of the four potential routes to arylpiperidines **1**. We first attempted the most direct route: coupling of the arylbromide with 4-iodo-*N*-BOC-piperidyl zinc. Billotte has reported that coupling of a protected iodopiperidine with an unhindered aryl iodide provided 47% of the 4-arylpiperidine.[2] Unfortunately, employing these conditions did not provide the desired product. However, we demonstrated by direct quench with DCl/MeOD that the active zinc species was formed in solution. In addition, employing the commercially available cyclohexyl zincate with **2b** under the literature conditions provided the coupled product in 65% yield. Thus, we concluded that while the zinc reagent had been formed, and the conditions were competent with known zincate nucleophiles, the coupling was not a viable route to the desired compound.

An alternate route would be condensation of the aryllithium or aryl Grignard with a protected piperidinone. However, with our substrate, the reported literature methods for these condensations resulted in complex reaction mixtures and only trace amounts of desired product.[‡,1a,3]

We subsequently investigated the Suzuki reaction of the arylbromide **2b** with appropriate coupling partners including 4-pyridylboronic acid and boronate ester **3** (Scheme 2).[9] Coupling of bromide **2b** with pyridylboronic acid using $Pd(PPh_3)_4$ and K_3PO_4 in dioxane–H_2O at 85 °C resulted in a 82% yield of **4**. Under the same conditions, reaction of the boronate ester **3** with bromide **2b** afforded **5** in 65% yield. Due to the limited availability of the pyridyl boronic acid and boronate ester **3**,[10] we decided to switch the coupling partners (Scheme 3). Synthesis of boronic acid **2c** was performed by adding *n*-BuLi dropwise to a −70 °C solution of bromobenzonitrile **2b** and $B(O\text{-}i\text{-}Pr)_3$ in THF.[11] The isolated solid (69% yield) was used without further purification in coupling reactions with both 4-bromopyridine HCl and triflate **6**.[12] The bromopyridine coupling proved problematic as incomplete consumption of the arylbromide was observed even in the presence of 3 equiv of boronic acid. Reaction with triflate **6** exhibited similar limitations and gave the desired product in only moderate conversion under the unoptimized conditions.

Turning our attention to the hydrogenation of arylpyridine **4** and tetrahydropyridine **5**, we found that using the literature protocols[13] (PtO_2, Pd/C, Pt/C, Rh/C, $RhClPPh_3$ in solvents including MeOH, EtOH, AcOH/H_2O, with and without acid) 5–15% of the dechlorinated product **7** was always detected. The presence of this by-product in the reaction mixture complicated purification of the piperidines **1**. In addition, controlling the levels of these impurities as a function of increasing scale could become problematic. These results made clear to us the need for a method of installing the piperidine ring that would avoid highly reducing conditions and subsequent dechlorination.

2.3. Comins approach to arylpiperidine 1

Given the issues associated with the Suzuki approach outlined above, we next focused on construction of the dihydropyridine by reaction of an acyl pyridinium ion with aryl Grignard **8** (Scheme 4). The preparation of dihydropyridines by this method has been well documented by Comins[5,14] and precedent existed for reduction to the piperidine under milder conditions than needed for substrates **4** and **5**.[15] We envisioned that using milder reducing conditions might decrease or eliminate formation of the dechlorinated by-product and thereby facilitate recovery of **1**.

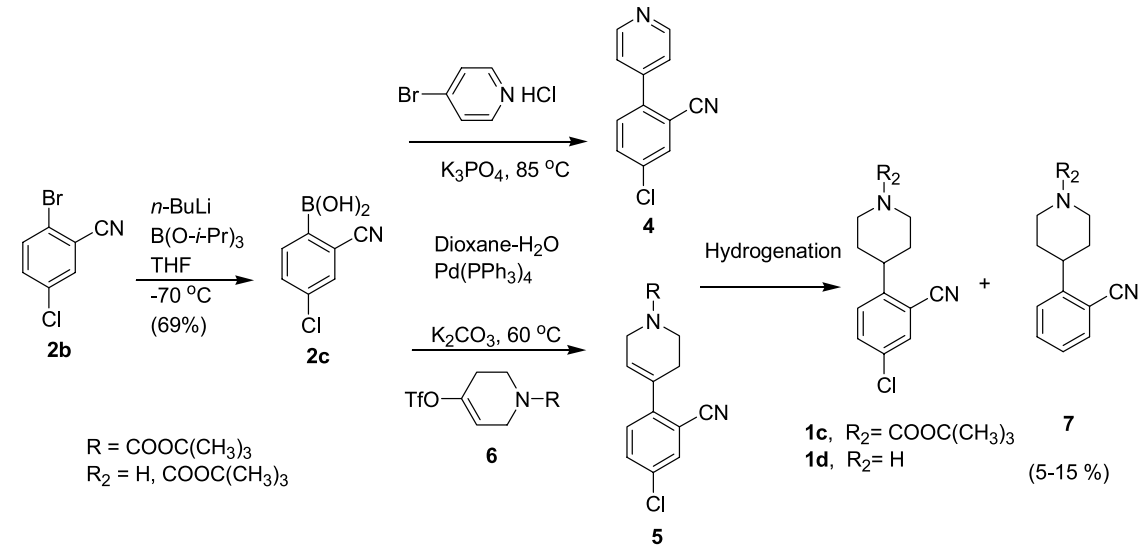
Formation of the aryl Grignard **8** was accomplished by addition of *i*-PrMgCl to bromobenzonitrile **2b** in THF at −20 °C. In parallel, the acyl pyridinium was formed by addition of either benzyl or phenyl chloroformate to a solution of pyridine and CuI (2 mol%) in THF at −10 °C. The aryl Grignard **8** was then transferred to the acyl pyridinium mixture and the reaction was allowed to warm to rt. In the course of probing this reaction, we found that Grignard **8** decomposes at temperatures above 0 °C. We also observed that maintaining an inert atmosphere was critical in preventing the formation of Ullmann dimerization product **10**. The resulting dihydropyridine **9** was formed in good yields although recovery via crystallization was not optimized (assay yield 70–80%, isolated yield 45–57%).[§]

Hydrogenation of the dihydropyridine to the piperidine proved to be highly sensitive to the reaction conditions. A screen of catalysts including Pd/C, PtO_2, Pt/C, $Pd/CaCO_3$, Ru/C activated, Rh/C, $Pd/BaSO_4$, and Rh/alumina resulted in either dechlorination or incomplete reaction. Interestingly, the intermediate isolated from incomplete reactions was monohydrogenated product **11** and we did not observe any isomerization of the double-bond to the more substituted position in conjugation with the aryl ring. Eventually we were able to reduce the dihydropyridine to the desired product **1** in 80–85% yield by using Wilkinson's catalyst ($RhCl(PPh_3)_4$) (10 mol%) in toluene at 70 °C,

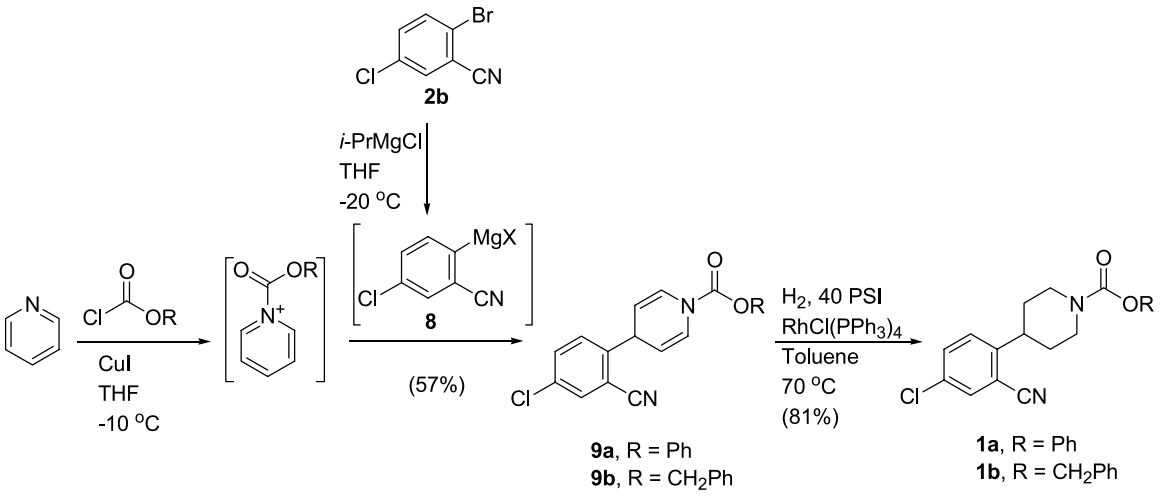
[†] Percents reported are liquid chromatograph area percents (LCAP).

[‡] Reaction conditions included use of aryl Grignard and aryllithium in THF and toluene, temperatures from −70 to 0 °C, and additives including TMEDA. We believe the complex mixtures are due in part to reaction at the nitrile as evidenced by absence of the nitrile signal in IR and ^{13}C NMR.

[§] The isolated yields are obtained using direct crystallization from the crude organic layer after the aqueous work-up. Higher yields can be obtained if column chromatography is performed after the aqueous extraction.



Scheme 3. Preparation and coupling of boronic acid **2c**; hydrogenation of coupling products **4** and **5**.



Scheme 4. Formation and hydrogenation of dihydropyridines **9** to prepare 4-arylpiperidines **1a**,**b**.

40 psi H_2. Under these conditions, the dechlorinated product was not detected. With this method, the phenyl and CBZ carbamate protected piperidines (**1a** and **1b**, respectively) could be obtained as crystalline solids in good yields after a simple silica gel filtration of the reaction mixture to remove $O(PPh_3)_3$ (Fig. 1).

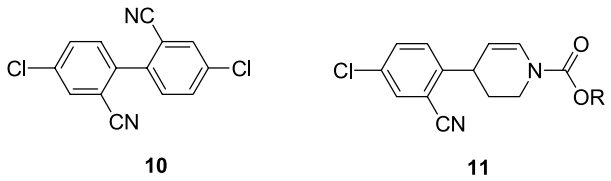
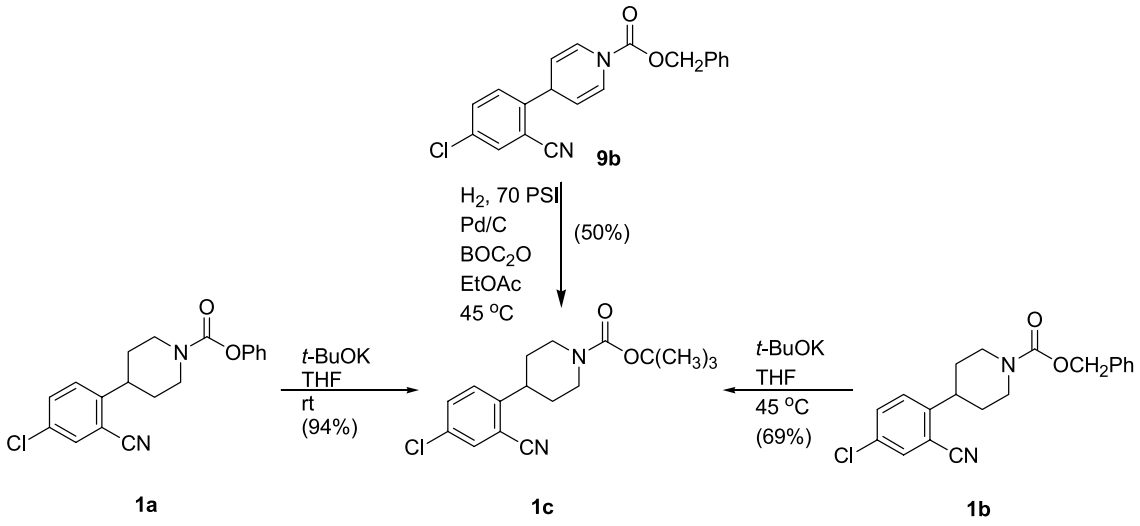


Figure 1. Dimer and monohydrogenated piperidine.

To increase the synthetic potential of **1**, we also needed access to the BOC protected piperidine. As the acyl pyridinium chemistry cannot be performed directly incorporating the BOC moiety, we synthesized the desired BOC protected piperidine from the CBZ and phenyl carbamates (Scheme 5). While CBZ to BOC transformation under hydrogenation conditions (H_2/Pd–C, $(BOC)_2O$) is well known,[16] it resulted in significant amounts of dechlorination and other by-products when attempted with piperidine **1b**. We next contemplated a stepwise approach, with combined hydrogenation of the dihydropyridine and removal of the CBZ group preceding subsequent BOC protection. Unfortunately, when we submitted dihydropyridine **9b** to hydrogenation (Pd/C(10%), H_2, EtOAc) the reaction gave almost exclusively dechlorination.

However, in the presence of BOC_2O, hydrogenation of dihydropyridine **9b** with Pd/C 10 wt%) 50% wet, H_2, in EtOAc gave minimal dechlorination and yielded BOC piperidine **1c** as the major product. The major by-product was monohydrogenated BOC compound. Screens of pressure, temperature, solvent, and catalyst loading failed to drive the reaction to completion, as did resubmission of



Scheme 5. Preparation of BOC protected 4-arylpiperidine **1c**.

the product mixture to reaction conditions.[¶] Under optimized conditions (1 equiv BOC_2O, 15 wt% catalyst, 70 psi H_2, 45 °C) **1c** was obtained in 50% yield after crystallization.

While this procedure was effective to replace the CBZ protecting group of the dihydropyridine, a different method was needed for the phenyl carbamate protecting group. In the literature, phenyl carbamate protected dihydropyridines have been treated with *t*-BuOK in THF to affect the desired protecting group switch.[17] However, in our hands, the dihydropyridine quickly oxidized to the corresponding pyridine when treated with *t*-BuOK. We found instead that the protective group switch could be efficiently performed on the fully hydrogenated piperidine. Adding 1.1 equiv of *t*-BuOK (1 M in THF) to a rt solution of phenyl carbamate protected piperidine **1a** in THF afforded BOC protected piperidine **1c** in 94% yield. We were delighted to find that by modifying the procedure to run at 45 °C in THF/hexanes we were also able to transform CBZ piperidine **1b** to BOC piperidine **1c** in 69% yield.

3. Conclusion

In conclusion, several routes towards the desired 4-aryl piperidines were investigated. The optimum route (Scheme 4) is a 3-step synthesis of **1** which involves selective bromination of 3-chlorobenzonitrile **2a** at the 6-position followed by reaction of the corresponding aryl Grignard with an acyl pyridinium under Cu(I) catalysis to form an *N*-protected dihydropyridine. The dihydropyridine can then be hydrogenated under mild conditions to produce the desired 4-arylpiperidines in 29% overall yield. Introduction of a BOC protecting group is possible in good yields in one step from either piperidines **1a**,**b** or dihydropyridine **9b**. The synthesis utilizes readily available and inexpensive starting materials (pyridine and 3-chlorobenzonitrile) and eliminates the dechlorination side product which often accompanies hydrogenation of unsaturated compounds **9**.

[¶] This suggests that reduction of the double bonds is occurring prior to BOC protection and we believe that steric hindrance prevents the reduction of the final double bond.

4. Experimental

4.1. General methods

Reagents and solvents were obtained from commercial suppliers and were used without further purification or drying unless otherwise noted. Chromatography was done on silica gel (70–230 mesh). ^1H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2,-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate **3**,[11] *tert*-butyl 4 {[(trifluoromethyl)sulfonyl]oxy}-3,6-dihydropyridine-1(2*H*)-carboxylate **6**,[13] were previously described in the literature.

4.1.1. Phenyl 4-(4-chloro-2-cyanophenyl)piperidine-1-carboxylate (1a). To a solution of **9a** (12.0 g, 35.6 mmol) in toluene (160 mL) was added $RhCl(PPh_3)_3$ (3.3 g, 3.6 mmol). The reaction mixture was submitted to H_2 (40 psi) and heated to 70 °C. After 8 h, the reaction stream was filtered through silica gel (100 g) and the silica gel was washed with 1:2 EtOAc/hexanes (800 mL). The filtrate was concentrated and solvent switched to toluene. Crystallization from 2:1 toluene/heptane followed by a wash of the filtercake (1:1 toluene/heptane) afforded a white solid (9.82 g, 81%). Mp 157–159 °C; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.64 (d, $J=2.2$ Hz, 1H), 7.57 (dd, $J=8.5$, 2.3 Hz, 1H), 7.40–7.32 (m, 3H), 7.23 (app t, $J=7.4$ Hz, 1H), 7.14 (dd, $J=8.4$, 1.2 Hz, 2H), 4.49 (br s, 2H), 3.24–2.93 (m, 3H), 1.96 (app d, $J=12.5$ Hz, 2H), 1.76 (dq, $J=12.4$, 4.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 153.5, 151.3, 147.1, 133.5, 132.8, 132.5, 129.2, 127.8, 125.2, 121.6, 116.5, 113.4, 44.7, 44.4, 40.2, 32.5, 31.9; IR (thin film) 3065, 2934, 2858, 2228, 1722 cm^{-1}. Anal. Calcd for $C_{19}H_{17}ClN_2O_2$: C, 66.96; H, 5.03; N, 8.22. Found: C, 67.08; H, 4.86; N, 8.15.

4.1.2. Benzyl 4-(4-chloro-2-cyanophenyl)piperidine-1-carboxylate (1b). To a solution of **9b** (9.18 kg, 27.2 mol)

in toluene (55.3 kg) was added $RhCl(PPh_3)_3$ (2.42 kg, 2.5 mol) as a slurry in toluene (20 kg). The reaction mixture was submitted to H_2 (40 psi) and heated to 70 °C. After 6 h, the reaction stream was filtered through silica gel (27.5 kg) and the silica gel was washed with 1:9 EtOAc/toluene (84 L). The filtrate was solvent switched to toluene and concentrated to a volume of 18 L. Heptane (7.5 kg) was added to the rt solution. The solution was seeded and aged overnight. Additional heptane (41.8 kg) was added over 2 h. The resulting slurry was cooled to 0 °C, filtered, and the product cake washed with 1:4 toluene/heptane affording a yellow solid (7.1 kg, 81%). Mp 85–87 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.61 (d, J=2.2 Hz, 1H), 7.54 (dd, J=8.5, 2.2 Hz, 1H), 7.39–7.31 (m, 5H), 7.27 (d, J=8.5 Hz, 1H), 5.17 (s, 2H), 4.37 (br s, 2H), 3.14 (tt, J=12.1, 3.5 Hz, 1H), 2.95 (br s, 2H), 1.87 (br d, J=12.3 Hz, 2H), 1.62 (br s, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.1, 147.3, 136.6, 133.4, 132.7, 132.4, 128.4, 128.0, 127.9, 127.8, 116.5, 113.3, 67.1, 44.2, 40.3, 32.2; IR (thin film) 3065, 2934, 2844, 2221, 1695 cm^{-1}. Anal. Calcd for $C_{20}H_{19}ClN_2O_2$: C, 67.70; H, 5.40; N, 7.89. Found: C, 67.56; H, 5.22; N, 7.71.

4.1.3. *tert*-Butyl 4-(4-chloro-2-cyanophenyl)piperidine-1-carboxylate (1c). *Method A*. To a solution of **1b** (3.27 kg, 9.2 mol) in hexanes (3 L) and THF (3 L) was added *t*-BuOK (1 M in THF, 28.0 L, 28.0 mol). The reaction was heated to 45 °C, aged 4 h, and then cooled to 5 °C. Water (14 L) and hexanes (28 L) were added and the mixture was stirred for 30 min. The aqueous layer was cut and the organic layer was washed twice with water (2×24 L). The organic layer was treated with Ecosorb C-941 (1.14 kg). After aging overnight, the mixture was filtered through Solka Floc followed by hexanes (3 L). The resulting solution was solvent switched to EtOH and concentrated to 14 L. Water (4.5 L) was added slowly and the resulting slurry was cooled to −10 °C and filtered. The filtercake was washed with 0 °C 2:1 EtOH/H_2O to afford, after drying, a yellow solid (2.22 kg, 69%).

Method B. To a heterogeneous mixture of **1a** (5.10 g, 15.0 mmol) in THF (17 mL) was added *t*-BuOK (1.0 M in THF, 15.0 mL, 15.0 mmol). After an overnight age and assay for completeness, *t*-BuOK (2.0 mL, 2.0 mmol) was added. After 5 h, 1 M NaOH (30 mL) was added and the resulting layers were separated. The organic layer was washed with 9 wt% NaCl (aq) (30 mL), dried over Na_2SO_4, and concentrated to afford an oil that solidified upon standing (4.54 g, 94%).

Method C. To a slurry of **9b** (5.0 g, 14.2 mmol) in EtOAc (150 mL) was added BOC anhydride (3.4 g, 15.5 mol). 50% wet 10% Pd/C (0.78 g, 15.6 wt%) was added and the mixture was aged at 45 °C under 70 psi of H_2 for 8 h. The solution was cooled to rt, filtered through Solka Floc, and solvent switched to MeOH. After addition of 2:1 MeOH/H_2O and an overnight age the resulting slurry was filtered. The solid was recrystallized from heptane yielding 2.26 g (50%). Mp 97–99 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.61 (d, J=2.2 Hz, 1H), 7.55 (dd, J=8.6, 2.2 Hz, 1H), 7.29 (d, J=8.6 Hz, 1H), 4.28 (br s, 2H), 3.11 (tt, J=12.2, 3.5 Hz, 1H), 2.87 (app t, J=12.0 Hz, 2H), 1.85 (app d, J=13.1 Hz, 2H), 1.66–1.54 (m, 2H), 1.49 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.5, 147.5, 133.3, 132.5, 132.4, 127.8, 116.5, 113.3, 79.6, 43.9, 40.4, 32.2, 28.3; IR (thin film) 3072, 2975, 2929, 2858, 2235, 1681 cm^{-1}. Anal. Calcd for $C_{17}H_{21}ClN_2O_2$: C, 63.64; H, 6.60; N, 8.73. Found: C, 63.85; H, 6.49; N, 8.60.

4.1.4. 2-Bromo-5-chlorobenzonitrile (2b). To a solution of 3-chlorobenzonitrile (50 g, 360 mmol) in trifluoroacetic acid (180 mL) was added sulfuric acid (24 mL) and then 1,3-dibromo-5,5-dimethylhydantoin (67 g, 234 mmol) in portions over 8 min. The reaction temperature was allowed to reach 31 °C and then cooling was applied to bring the temperature to 24 °C. After a 6 h age the heterogeneous reaction was cooled to 10 °C and water (250 mL) was added. Following a 10 min ages, the reaction was filtered and the product cake was washed twice with water (250 and 100 mL) to afford a white solid (52.4 g, 63%). Mp 137–140 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.64–7.62 (m, 2H), 7.44 (dd, J=8.6, 2.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 134.2, 134.1, 133.9, 133.8, 123.3, 117.2, 115.8; IR (thin film) 3086, 3052, 2228 cm^{-1}. Anal. Calcd for C_7H_3BrClN: C, 38.84; H, 1.40; N, 6.47. Found: C, 38.64; H, 1.18; N, 6.35.

4.1.5. (4-Chloro-2-cyanophenyl)boronic acid (2c). To a −72 °C heterogeneous mixture of **2b** (1.5 g, 6.9 mmol) and triisopropyl borate (1.9 mL, 8.3 mmol) in THF (40 mL) was added *n*-BuLi (2.5 M in hexanes, 2.9 mL, 7.2 mmol) at a rate such that the temperature <-69 °C. The reaction was aged 20 min, allowed to warm to −20 °C and quenched with 1 M HCl (40 mL). EtOAc was added (20 mL), the aqueous layer was cut and the organic layer extracted with 1.25 M NaOH (2×50 mL). The aqueous extracts were combined and EtOAc (75 mL) was added. HCl (2 M) was added until the pH=4.8. The layers were separated and the organic was concentrated to give a pale yellow solid (0.86 g, 69%). The solid decomposes above 285 °C. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.77 (d, J=1.8 Hz, 1H), 7.70 (d, J=8.1 Hz, 1H), 7.65 (dd, J=8.2, 1.9 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 135.5, 135.3, 132.2, 131.7, 117.8, 117.1, the boron bearing carbon is not observed due to signal broadening; IR (thin film) 3356, 2927, 2234 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_7H_5BClNO_2$ 181.0216 (M+H), found 181.0219 (M+H).

4.1.6. 5-Chloro-2-pyridin-4-ylbenzonitrile (4). A flask under N_2 was charged with 2-bromo-5-chlorobenzonitrile **2b** (2.1 g, 9.6 mmol), pyridine-4-boronic acid (2.0 g, 16.3 mmol), K_3PO_4 (4.7 g, 22.1 mmol), $Pd(PPh_3)_4$ (0.55 g, 0.5 mmol), dioxane (90 mL), and H_2O (18 mL). The reaction mixture was heated to 85 °C. A second charge of pyridine-4-boronic acid (0.1 g, 0.8 mmol) was added after 5 h. After 6 h, the reaction mixture was cooled to rt. Saturated $NaHCO_3$(aq) (80 mL) and EtOAc (90 mL) were added and the resulting layers were separated. The aqueous layer was extracted with EtOAc (40 mL) and the combined organic layers were dried over Na_2SO_4. The organic layer was concentrated to a golden oil. Purification by silica gel chromatography (2:1 EtOAc/hexanes to 100% EtOAc) afforded the product as an off-white solid (1.69 g, 82%). Mp 143–145 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.75 (dd, J=4.5, 1.7 Hz, 2 H), 7.79 (d, J=2.1 Hz, 1H), 7.68 (dd, J=8.4, 2.1 Hz, 1H), 7.49–7.46 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.2, 144.4, 140.7, 135.3, 133.50, 133.48,

131.0, 123.0, 116.5, 112.5; IR (thin film) 3350, 3065, 2228, 1598 cm^{-1}. Anal. Calcd for $C_{12}H_7ClN_2$: C, 67.15; H, 3.29; N, 13.05. Found: C, 67.04; H, 2.96; N, 12.86.

4.1.7. *tert*-Butyl 4-(4-chloro-2-cyanophenyl)-3,6-dihydropyridine-1(2*H*)-carboxylate (5). A flask under N_2 was charged with 2-bromo-5-chlorobenzonitrile **2b** (0.9 g, 4.2 mmol), boronate **3** (1.7 g, 5.6 mmol), K_3PO_4 (1.8 g, 8.4 mmol), $Pd(PPh_3)_4$ (0.24 g, 0.2 mmol), dioxane (60 mL), and H_2O (15 mL). The reaction mixture was heated to 85 °C. After 21 h, the reaction mixture was cooled to rt. Purification of the concentrated reaction mixture by silica gel chromatography (1:3 CH_2Cl_2/Hex) afforded the product as a pale yellow oil that solidified upon standing (0.87 g, 65%). Mp 93–95 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.64 (d, J=2.2 Hz, 1H), 7.52 (dd, J=8.4, 2.4 Hz, 1H), 7.27 (d, J=8.4, 1H), 6.00 (br s, 1H), 4.10 (d, J=2.8 Hz, 2H), 3.66 (t, J=5.6 Hz, 2H), 2.53–2.48 (m, 2H), 1.50 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.6, 144.4, 133.2, 133.1, 132.93, 132.90, 129.4, 126.9, 117.2, 111.7, 79.9, 43.5, 39.5, 28.8, 28.4; IR (thin film) 2968, 2228, 1688 cm^{-1}. Anal. Calcd for $C_{17}H_{19}ClN_2O_2$: C, 64.05; H, 6.01; N, 8.79. Found: C, 64.33; H, 5.88; N, 8.61.

4.1.8. Phenyl 4-(4-chloro-2-cyanophenyl)pyridine-1(4*H*)-carboxylate (9a). General procedure for dihydropyridines. To a −30 °C solution of **2b** (835 g, 3.86 mol) in THF (10 L) was added *i*-PrMgCl (1.71 M in THF, 2.5 L, 4.25 mol) at a rate such that the temperature <-20 °C. Meanwhile, to a −10 °C solution of CuI (36 g, 190 mmol) in THF (10 L) was added pyridine (624 mL, 7.72 mol) and then phenyl chloroformate (532 mL, 4.25 mmol) such that the temperature <0 °C. To this heterogeneous mixture was added the previously formed Grignard at a rate such that temperature <0 °C. The resulting solution was aged at 0 °C for 30 min and allowed to warm up to rt. The reaction was then quenched with 10% aqueous NH_4Cl (20 L). EtOAc (20 L) was added and the blue aqueous layer was removed. The organic layer was washed with 10% aqueous NH_4Cl (20 L), 1 N HCl (20 L), and finally an aqueous 20% NaCl solution (20 L). The organic layer was then concentrated, solvent switched to MeOH and crystallized. The slurry was filtered and the filtercake washed with MeOH, yielding an off-white solid (584 g, 43%). Mp 128–130 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.63–7.59 (m, 2H), 7.50 (d, J=8.4 Hz, 1H), 7.41 (app t, J=7.8 Hz, 2 H), 7.27 (t, J=7.2 Hz, 1H), 7.20–7.11 (m, 4H), 5.05 (br d, J=15.5 Hz, 2H), 4.70 (br s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.5, 149.7, 146.9, 133.7, 133.1, 132.3, 131.2, 129.5, 126.1, 123.7, 123.4, 121.4, 116.3, 112.3, 107.9, 107.3, 36.9; IR (thin film) 3065, 2228, 1736, 1688 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{19}H^{13}ClN_2O_2$ 337.0744 (M+H), found 337.0740 (M+H).

4.1.9. Benzyl 4-(4-chloro-2-cyanophenyl)pyridine-1(4*H*)-carboxylate (9b). The general procedure for dihydropyridines was followed, using **2b** (5.71 kg, 26.4 mol), THF (114 L) and *i*-PrMgCl (1.74 M in THF, 23.9 L, 41.6 mol), CuI (250 g, 1.31 mol), THF (114 L), pyridine (4.25 L, 52.5 mol) and benzylchloroformate (5.83 L, 40.9 mol), 10% aqueous NH_4Cl (55 L), MTBE (55 L), 10% aqueous NH_4Cl (55 L), 1 N HCl (55 L), and finally an aqueous 5% $NaHCO_3$/5% NaCl solution (55 L). The organic layer was then concentrated, solvent switched to MeOH, and further concentrated to 60 L. The solution was aged and the resulting slurry was cooled to 0 °C, filtered and the filtercake washed with MeOH to afford an off-white solid (5.31 kg, 57%). Mp 98–100 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.60 (d, J=2.2 Hz, 1H), 7.57 (dd, J=8.5, 2.2 Hz, 1H), 7.44–7.35 (m, 6H), 7.01 (br d, J=44.4 Hz, 2H), 5.27 (s, 2H), 4.93 (br d, J=36.4 Hz, 2H), 4.65–4.63 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.0, 147.2, 135.2, 133.6, 133.0, 132.1, 131.2, 128.6, 128.5, 128.3, 123.8, 123.3, 116.3, 112.1, 107.0, 106.5, 68.4, 36.8; IR (thin film) 3058, 2955, 2221, 1729, 1681 cm^{-1}. Anal. Calcd for $C_{20}H_{15}ClN_2O_2$: C, 68.48; H, 4.31; N, 7.99. Found: C, 68.32; H, 4.12; N, 7.89.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2004.09.092

References and notes

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Cyclohexanones derived from dihydrocarvone as precursor of chiral dioxiranes for epoxidation of olefins

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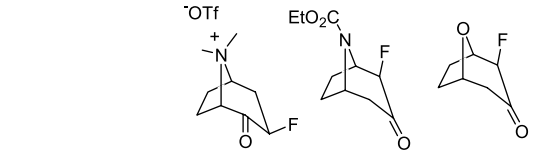
Abstract—New ketones having an axial α-fluorine atom and substituents other than fluorine at C8, derived from commercially available (+)-dihydrocarvone, have been prepared and used for epoxidations of *trans* stilbene, *trans* methyl *p*-methoxy cinnamate, *trans* cinnamyl alcohol and derivatives. It was found that replacement of the H at C8 by a substituent containing an oxygen atom increases the enantioselectivities in all cases. It was also shown that protic substituents (hydroxyl groups) provide a decrease in enantioselectivity in the case of cinnamates probably because of H-bonding dioxirane-substrate. It is noted that the absolute configurations of the various epoxides obtained hold with the usual model involving a spiro-approach on the dioxirane conformation **C1** having the α-fluorine axial. Moreover, sub-stoichiometric amounts (0.3 equiv) of ketone can be used in all cases as these ketones do not undergo Baeyer-Villiger oxidation and are recovered.

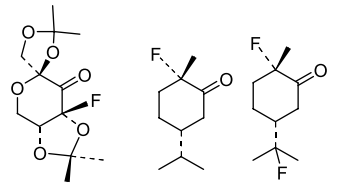
1. Introduction

Enantioselective oxygen transfer through dioxiranes generated in situ from chiral ketones and oxone has received, in recent years, greater attention for the asymmetric epoxidation of *trans* olefins[1,2] and a significant activating effect by α-fluorine substitution has been observed.[3–8]

Among the enantiopure α-fluoro ketones involving a six-membered ring **1**–**6**[3–8] designed for epoxidation of *trans* olefins, the monocyclic ketone **6** is the most efficient with stilbene. Moreover, compared to ketone **5**, ketone **6**,[7] due to the fluorine at the C8 position, led to higher enantioselectivities with stilbene and methyl *p*-methoxycinnamate.



	1	**2**	**3**
Stilbene	35% ee[3]	76% ee[4]	83% ee[5a]
p-MeO-cinnamate	-	-	-



	4	**5**	**6**
Stilbene	11% ee[6]	60% ee[7]	90% ee[8]
p-MeO-cinnamate	-	40% ee	60% ee

Ketones **7**–**10**, having an axial α-fluorine atom, but with substituents other than fluorine at C8 have thus been envisaged. We present here the synthesis of these ketones from commercially available (+)-dihydrocarvone and their

Keywords: Chiral cyclohexanones; Fluoro ketones; Asymmetric epoxidation; Chiral dioxiranes.
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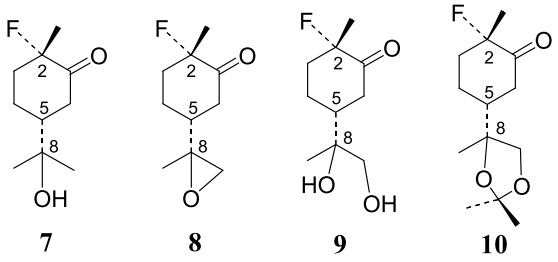
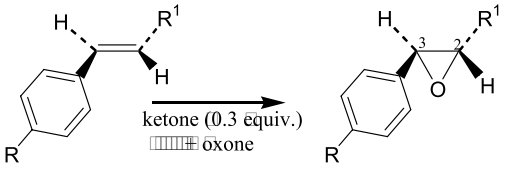
use for epoxidations of *trans* stilbene, methyl *p*-methoxy cinnamate, *trans* cinnamyl alcohol and derivatives.



7 **8** **9** **10**



12: R=H; R^1=Ph
13: R=MeO; R^1=CO_2Me
14: R=H; R^1=CH_2OH
15: R=H; R^1=CH_2OCOCH_3
16: R=H; R^1=CH_2OSiMe_2tBu

17 (+)-(*S*,*S*)
18: (−)-(2*R*,3*S*)
19: (−)-(2*S*,3*S*)
20: (−)-(2*S*,3*S*)
21: (−)-(2*S*,3*S*)

Scheme 2.

2. Results and discussion

2.1. Synthesis of ketones 7–10

All four ketones **7**–**10** were prepared from the common intermediate 2-fluoro-5-isopropenyl-2-methylcyclohexanone **11** (Scheme 1), which was obtained from (+)-dihydrocarvone, as previously described.[9] The diastereomer (−)-(2*S*,5*R*)-**11**, having the α-fluorine atom axial according to ^{1}H NMR,[10] was isolated pure after chromatography. From ketone **11**, acid (H_2SO_4) hydration[11] afforded **7**, while epoxidation using dimethyldioxirane[12] and dihydroxylation using OsO_4[13] gave **8** and **9**, as 57:43 and 65:35 diastereomers mixtures, respectively (Scheme 1). Ketone **10** was then obtained from **9** via acidic protection in acetone (as a 1:1 diastereomers mixture). Separation of diastereomers of ketones **8** and **9** could not be performed by chromatography, but it has been possible to isolate both diastereomers of ketone **10** through repeated chromatography.

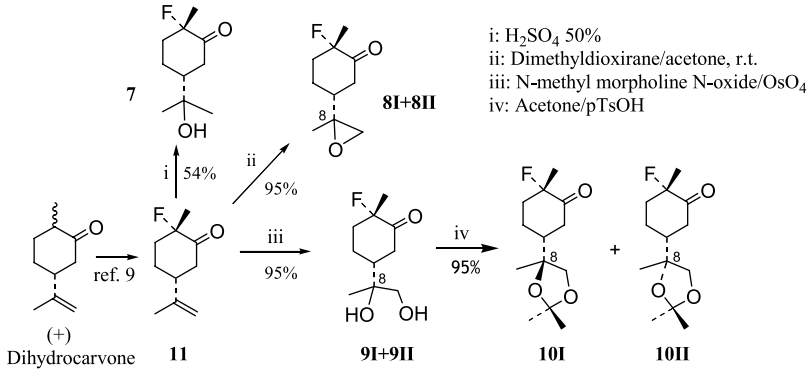
2.2. Epoxidation of olefins

The results of asymmetric epoxidation of stilbene **12**, methyl *p*-methoxy cinnamate **13**, cinnamyl alcohol **14** and derivatives **15**, **16** (Scheme 2) with these ketones (**7**–**10**)[14] are gathered in Table 1. The percentages of conversion have been determined by combining weights and ^{1}H NMR (300 and/or 400 MHz) of solvent-free crude products; then epoxides were isolated by flash chromatography on silica gel. The *ee*s% were determined by ^{1}H NMR using Eu(hfc)$_3$ in $CDCl_3$ and optical rotations. All *trans*-epoxides being known, the absolute configurations were determined using the sign of the optical rotations[15] ($[\alpha]_D$ measured under identical conditions: same solvent, same concentration). None of the ketones studied here underwent Baeyer-Villiger oxidation. Used in sub-stoichiometric amounts (0.3 equiv) they were recovered almost quantitatively after reaction (through chromatography) and no lactones were detected by NMR (400 MHz) of crude products in none of the reactions.

Conducted in dioxane/H_2O mixture at 25 °C, epoxidation of *trans* stilbene **12** (Entries 1–4) and methyl *p*-methoxycinnamate **13** (entries 5–9) went smoothly to completion and the *ee* of the corresponding epoxides ranged between 82–88% (epoxide **17**) and 26–66% (epoxide **18**). In the case of cinnamate **13** the enantioselectivities were slightly higher but conversions significantly lower (entries 10 and 11) when the reaction was performed in DME/H_2O at 0 °C.

The epoxidation of cinnamyl alcohol and derivatives (performed in DME/CH_3CN/H_2O mixture at room temperature) gave conversions from 80 to 99% with all ketones and *ee* ranging between 60 and 75%.

For substrates **12**, **13** and **15**, ketone **8** was more efficient than the others with higher yields and enantioselectivities (entries 2, 6 and 16). For substrates **14** and **16**, the highest enantioselectivities were achieved by ketone **10** (even used as 1:1 diastereoisomeric mixture), with slightly higher yields (entries 14 and 20). It must be noted that ketone **10** is as efficient as ketone **8** in the case of substrate **15** (compare entries 16 and 17).



Scheme 1.

Table 1. Epoxidation of *trans* stilbene **12**, methyl *p*-methoxy cinnamate **13**, cinnamyl alcohol **14** and derivatives **15**, **16**

Entry	Olefin	Ketone	Prod.	Solvent[a]	React. time (h)	Conv. (%)[b]	e.r. (%)[c]	Absol. Config.[d]	*ee* (%)
1	**12**	**7**	**17**	Diox/H_2O	4	100	92:8	(−)-(*S*,*S*)	84
2	**12**	**8**	**17**	Diox/H_2O	4	100	94:6	(−)-(*S*,*S*)	88
3	**12**	**9**	**17**	Diox/H_2O	2	100	93:7	(−)-(*S*,*S*)	86
4	**12**	**10(I+II)**[e]	**17**	Diox/H_2O	3	100	91:9	(−)-(*S*,*S*)	82
5	**13**	**7**	**18**	Diox/H_2O	6	100	63:37	(−)-(2*R*,3*S*)	26
6	**13**	**8**	**18**	Diox/H_2O	6	100	83:17	(−)-(2*R*,3*S*)	66
7	**13**	**9**	**18**	Diox/H_2O	6	100	77:33	(−)-(2*R*,3*S*)	44
8	**13**	**10I**	**18**	Diox/H_2O	6	100	80:20	(−)-(2*R*,3*S*)	60
9	**13**	**10II**	**18**	Diox/H_2O	6	100	80:20	(−)-(2*R*,3*S*)	60
10	**13**	**8**	**18**	DME/H_2O	8	50	84:16	(−)-(2*R*,3*S*)	68
11	**13**	**10(I+II)**[e]	**18**	DME/H_2O	8	53	83:17	(−)-(2*R*,3*S*)	66
12	**14**	**5**	**19**	DME/CH_3CN/H_2O	1	85	80:20	(−)-(2*S*,3*S*)	60
13	**14**	**8**	**19**	DME/CH_3CN/H_2O	1	90	80:20	(−)-(2*S*,3*S*)	60
14	**14**	**10(I+II)**[e]	**19**	DME/CH_3CN/H_2O	1	99	88:12	(−)-(2*S*,3*S*)	75
15	**15**	**5**	**20**	DME/CH_3CN/H_2O	1	80	80:20	(−)-(2*S*,3*S*)	60
16	**15**	**8**	**20**	DME/CH_3CN/H_2O	1	80	82:18	(−)-(2*S*,3*S*)	65
17	**15**	**10(I+II)**[e]	**20**	DME/CH_3CN/H_2O	1	99	82:18	(−)-(2*S*,3*S*)	65
18	**16**	**5**	**21**	DME/CH_3CN/H_2O	1	95	80:20	(−)-(2*S*,3*S*)	60
19	**16**	**8**	**21**	DME/CH_3CN/H_2O	1	95	82:18	(−)-(2*S*,3*S*)	65
20	**16**	**10(I+II)**[e]	**21**	DME/CH_3CN/H_2O	1	99	88:12	(−)-(2*S*,3*S*)	75

[a] Diox/H_2O 2:1 (3 equiv of Oxone), rt; DME/H_2O 2:1 (1.4 equiv of Oxone), 0 °C; DME/CH_3CN/H_2O 1:1:1 (3 equiv of Oxone), rt.
[b] Determined by ^1H NMR (300 and/or 400 MHz) on the crude products of the reactions.
[c] Enantiomeric ratios have been determined by ^1H NMR (400 MHz) using Eu(hfc)$_3$ in $CDCl_3$: the precision is $\pm 2\%$.
[d] Absolute configurations have been determined from the sign of specific rotation as compared with literature results (cf. Ref. 15).
[e] 1:1 Diastereoisomeric mixture.

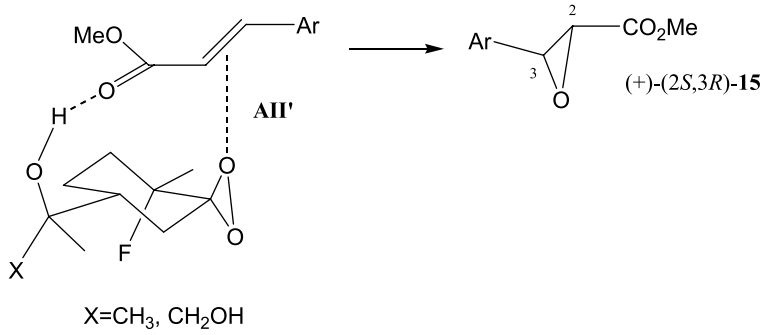


Figure 1.

It is worth noting that, under identical conditions, diastereomers **10I** and **10II** provided identical results for epoxidation of methyl *p*-methoxycinnamate (entries 8 and 9). Therefore, it seems that the chiral center C8, being remote from the reacting dioxirane site, has no effect on the asymmetric induction of epoxidation and one can extrapolate that it will be such also in the cases of ketones **8** and **9**.

Replacement of the H at C8 by a substituent containing an oxygen atom (ketones **7**–**10**) increases the enantioselectivities in the case of epoxidation of stilbene: compare the 82–88% *ee* (Table 1, lines 1–4) with the 60% *ee* obtained with ketone **5**. However, a fluorine atom at this C8 position (ketone **6**) remains the most efficient one, with 90% *ee*.

The same trend holds for epoxidation of methyl *p*-methoxycinnamate with ketones **8** and **10** (entries 6, 8 and 9), compare the 66 and 60% *ee* with the 40% *ee* observed with unsubstituted ketone **5**. Moreover, in this case, ketone **8** provides higher enantioselectivities (66% *ee*) than ketone **6** (60% *ee*).

However, protic substituents (ketones **7** and **9**) provide a decrease in enantioselectivity (26 and 44% *ee* compared to 60–66% *ee*) with methyl cinnamate **13**. And, according to previous results, which had led to the conclusion that axial approaches contributed to the reactions,[8] one can envisaged that, because of possible formation of H-bond, Figure 1, between the olefin and the dioxirane, approach **AII′**, Figure 2, is less disfavored than with aprotic substituents and will provide larger amount of the epoxide of opposite configuration.

Replacement of the H at C8 by a substituent containing an oxygen atom (ketones **8** and **10**) also increases the enantioselectivities in the case of epoxidation of cinnamyl alcohol (**14**) and derivatives (**15** and **16**): compare the 60% *ee* obtained with **5** to the 65–75% obtained with **8** and **10**.

3. Conclusion

It is thus clear that substitution at C8 (on the isopropyl group located at C5), even with substituent different from halogens, has a significant influence on the enantioselectivity of epoxidation of *trans* olefins which has also been

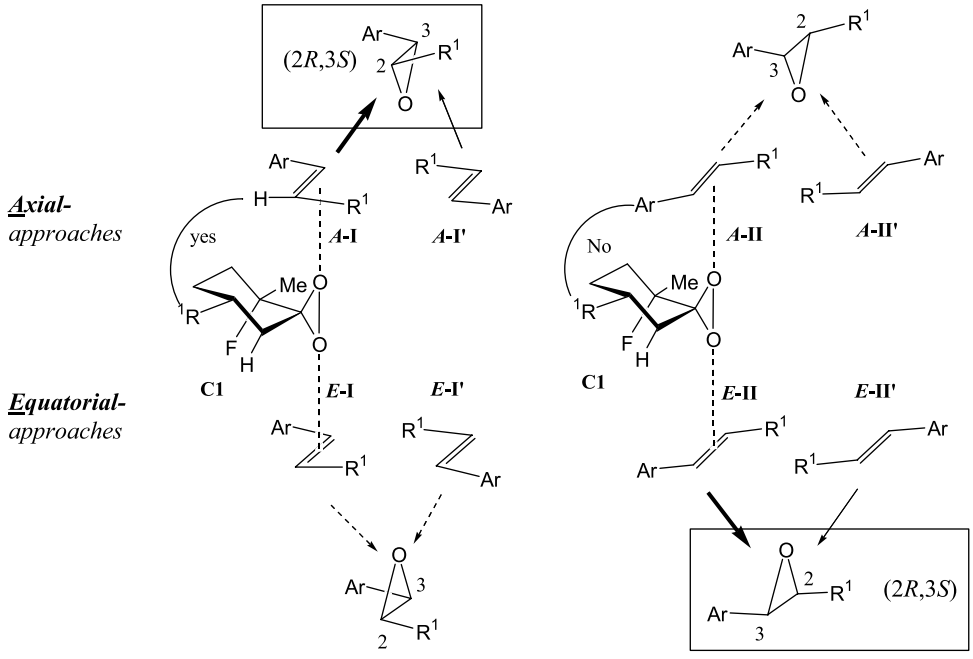


Figure 2. Epoxidation of *trans*-cinnamate ($R^1=CO_2Me$) and *trans* cinnamyl derivatives ($R^1=CH_2OH$, CH_2OAc, $CH_2OSiTBDM$) by dioxiranes derived from chiral cyclohexanones **7**–**10**.

observed by Yang et al. with α-chloro cyclohexanones.[16] It appears also that the absolute configuration of the new chiral center formed (C8) has no effect on the enantioselectivity. However, protic substituents at C8 are not suitable for epoxidation of cinnamate esters (probably because of unfavorable H-bond during the reagents approaches, Fig. 1).

It is worth noting that the absolute configurations of the various epoxides obtained hold with the model (Fig. 2) involving the spiro Model[17] and both equatorial[18–20] and axial[8] approaches.

Considering the configuration of ketones **7**–**10** and using both equatorial and axial approaches on the more reactive[21] and more populated[22] conformation **C1** of the dioxiranes having the α-fluorine atom axial one could expect the enantiomers (−)-(*S*,*S*)-**17**, (−)-(2*R*,3*S*)-**18**, (−)-(2*S*,3*S*)-**19**, (−)-(2*S*,3*S*)-**20** and (−)-(2*S*,3*S*)-**21** to be obtained which is indeed observed (Table 1). Equatorial **E-II** (and/or **E-II′**) approach with no $n\cdot\pi$ repulsion between F and the phenyl is expected to be favored over the **E-I** (and/or **E-I′**) approach, which involves such a $n\cdot\pi$ repulsion. Similarly, in the case of axial approaches **A-I** (and/or **A-I′**) which involves no R·Ph repulsive interaction[16] is favored over **A-II** (and/or **A-II′**). And **E-II**, **E-II′**, **A-I** and **A-I′** provides the same epoxide enantiomer.

4. Experimental

4.1. General

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 300 and Avance 400 spectrometers with $CDCl_3$ or C_6D_6 as solvents. Chemical shifts (δ) are given in ppm downfield from TMS as an internal standard. Optical rotation were determined on a Perkin–Elmer 241 MC polarimeter. TLC was performed on Merck's glass plates with silica gel 60 F_{254}. Silica gel Si 60 (40–60 μm) from Merck was used for the chromatographic purifications and a Chiralcel OD column was used for *ee* determination of epoxides. (+)-Dihydrocarvone (99% *R*-configured) was purchased from Fluka.

4.1.1. (2*S*,5*R*)-2-Fluoro-5-(1-hydroxy-1-methyl-ethyl)-2-methyl-cyclohexanone 7. To ketone **11** (200 mg, 1.17 mmol) was added dropwise, at −5 to 0 °C, a solution of H_2SO_4 (0.75 mL) in distilled H_2O (2.25 mL). Stirring was maintained for one night at 0 °C (the temperature must not rise above 0 °C). The reaction mixture was then extracted four times with Et_2O (10 mL) and four times with CH_2Cl_2 (10 mL). The joined organic phases were washed with a saturated $NaHCO_3$ solution, dried over Na_2SO_4, filtered and concentrated. A chromatographic purification over silica gel (hexane/Et_2O=4:6 to 1:9) provide 116 mg of ketone **7** as a white solid (54% yield). F. 36 °C. Anal. calcd for $C_{10}H_{17}FO_2$: C, 63.80; H, 9.10. Found: C, 63.42; H, 9.22. ^{1}H NMR (400 MHz C_6D_6) δ 2.58 (td, $J=J=12$ Hz, $J_{HF}=6$ Hz, 1H), 2.42 (broad d, $J=12$ Hz, 1H), 1.88 (dddd, $^2J=15$ Hz, $J_{HF}=10$ Hz, $J=4.5$ Hz, $J=2$ Hz, 1H), 1.48 (qd, $^2J=^3J=^3J=13.5$ Hz, $^3J=5$ Hz, 1H), 1.35 (d, $J_{HF}=22$ Hz, 3H, Me), 1.28 (m, 1H), 1.11 (m, 1H), 0.99 (dddd, $^2J=15$ Hz, $J_{HF}=40$ Hz, $J=10$ Hz, $J=3$ Hz, 1H), 0.80 (s, 3H, Me), 0.79 (s, 3H, Me). ^{13}C NMR (100 MHz C_6D_6) δ 206.6 (d, $^2J_{CF}=25$ Hz), 95.2 (d, $^1J_{CF}=172$ Hz), 71.2, 50.7, 39.9 (d, $^3J_{CF}=3$ Hz), 38.3 (d, $^2J_{CF}=23$ Hz), 27.2, 26.8, 21.8 (d, $^3J_{CF}=2$ Hz), 20.2 (d, $^2J_{CF}=24$ Hz).

4.1.2. (2*S*,5*R*)-2-Fluoro-2-methyl-5-(2-methyl-oxiranyl)-cyclohexanone 8. To a solution of **11** (144 mg, 0.847 mmol) in acetone (2 mL), under stirring, at 0 °C, 0.08 M dimethyldioxirane solution in acetone (20 mL, 16 mmol) was added. Upon stirring to completion, as judged by TLC (about 1 h 30 min), the reaction mixture was concentrated. Water was added and the crude extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, filtered and concentrated, to give **8** as colorless oil (149 mg, 95%); 57:43 mixture of diastereomer **8I** and **8II**. Anal. calcd for $C_{10}H_{15}FO_2$: C, 64.49; H, 8.11. Found: C, 64.10; H, 8.02. IR: 3050, 1727, 1458; ^{1}H NMR (400 MHz C_6D_6) δ 2.57 (td, $J=J=12.5$ Hz, $J=6.0$ Hz, 1H, **8I**, 57%), 2.51 (td, $J=J=13$ Hz, $J=6.0$ Hz, 1H, **8II**, 43%), 2.34 (bd, $J=12.5$ Hz, 1H, **8I**, 57%), 2.20 (bd, $J=13.0$ Hz, 1H, **8II**, 43%), 2.11 (d, $J=5.0$ Hz, 1H, **8I**, 57%), 2.07 (d, $J=4.5$ Hz, 1H, **8II**, 43%), 2.02 (d, $J=5.0$ Hz, 1H, **8I**, 57%), 2.01 (d, $J=4.5$ Hz, 1H, **8II**, 43%), 1.79 (m, 2H, **8I** and **8II** overlapped), 1.63 (qd, $J=J=J=12.5$ Hz, $J=3.5$ Hz, 1H, **8II**, 43%), 1.54 (qd, $J=J=J=13.0$ Hz, $J=4.0$ Hz, 1H, **8I**, 57%), 1.29 (d, $J=22.5$ Hz, 3H, **8I**, 57%), 1.54 (d, $J=22.0$ Hz, 3H, **8II**, 43%), 1.26 (m, 2H, **8II**, 43%), 1.17 (m, 2H, **8I**, 57%), 1.00 (m, 1H, **8II**, 43%), 0.90 (m, 1H, **8I**, 57%), 0.86 (s, 6H, **8I** and **8II** overlapped); ^{13}C NMR (100 MHz C_6D_6) signals of both diastereomers not assigned δ 205.3 (d, $J=25.5$ Hz), 205.1 (d, $J=25.5$ Hz), 95.6 (d, $J=172.3$ Hz), 95.5 (d, $J=173.1$ Hz), 57.6, 57.5, 52.2, 52.5, 45.9, 45.5, 40.8 (d, $J=36$ Hz), 40.7 (d, $J=36$ Hz), 37.9 (d, $J=6.5$ Hz), 37.8 (d, $J=6.5$ Hz), 22.8 (d, $J=1.5$ Hz), 22.7 (d, $J=1.5$ Hz), 20.3 (d, $J=24$ Hz), 20.1 (d, $J=24$ Hz), 18.1, 17.6. MS (*m*/*z* %) 178 (2), 153 (4), 130 (4), 109 (19), 99 (2), 75 (19), 48 (100).

4.1.3. (2*S*,5*R*)-5-(1,2-Dihydroxy-1-methyl-ethyl)-2-fluoro-2-methyl-cyclohexanone 9. To a solution of **11** (100 mg, 0.59 mmol), in 9 mL of a mixture H_2O/acetone 1:8, were added *N*-methylmorpholine *N*-oxide (138 mg, 1.18 mmol) and a solution of OsO_4 in *t*-butanol (0.05 equiv in 1.5 mL).Upon stirring at room temperature to completion as judged by TLC (about 2 h) the reaction mixture was quenched with a saturated solution of $NaHSO_3$. After 10 min the mixture was extracted with EtOAc, filtered, concentrated and purified by flash chromatography to give the diol **9** as a colorless oil (105 mg, 95%). The ratio of diastereomer was determined by NMR as **9I/9II**=65:35. Anal. calcd for $C_{10}H_{17}FO_3$: C, 58.80; H, 8.38. Found: C, 58.12; H, 8.03. IR: 3445, 2985, 1726, 1408; ^{1}H NMR (400 MHz C_6D_6) δ 3.35 (d, $J=11$ Hz, 1H, **9II**, 35%), 3.32 (d, $J=11$ Hz, 1H, **9I**, 65%), 3.25 (d, $J=11$ Hz, 1H, **9I**, 65%), 3.19 (d, $J=11$ Hz, 1H, **9I**, 65%), 3.13 (bs, 1H, **9II**, 35%), 3.05 (bs, 1H, **9I**, 65%), 2.98 (bs, 1H, **9II**, 35%), 2.86 (bs, 1H, **9I**, 65%), 2.77 (td, $J=J=12.5$ Hz, $J=6.5$ Hz, 1H, **9II**, 35%), 2.64 (bd, $J=12.5$ Hz, 1H, **9II**, 35%), 2.61 (td, $J=J=13.0$ Hz, $J=6.5$ Hz, 1H, **9I**, 65%), 2.37 (bd, $J=13.0$ Hz, 1H, **9I**, 65%), 1.87 (m, 6H, **9I** and **9II** overlapped), 1.60 (m, 2H, **9I** and **9II** overlapped), 1.34 (d, $J=22$ Hz, **9II**, 35%), 1.33 (d, $J=22$ Hz, **9I**, 65%), 1.20 (m, 2H, **9I** and **9II** overlapped); ^{13}C NMR (100 MHz C_6D_6) signals of both

diastereomers not assigned δ 207.8 (d, $J=25$ Hz), 207.1 (d, $J=25$ Hz), 95.8 (d, $J=173$ Hz), 95.6 (d, $J=173$ Hz), 74.1, 74.0, 68.5, 68.0, 46.4, 46.1, 40.3 (d, $J=2.5$ Hz), 39.1 (d, $J=2.5$ Hz), 38.6, 38.3, 22.3 (d, $J=2.5$ Hz), 21.1 (d, $J=2.5$ Hz), 20.3 (d, $J=24$ Hz), 20.1 (d, $J=24$ Hz), 20.2, 19.8. MS (*m/z* %) 179 (4), 158 (17), 130 (10), 109 (42), 81 (12), 75 (31), 48 (100).

4.1.4. (2*S*,5*R*)-2-Fluoro-2-methyl-5-[(4*R*)-2,2,4-trimethyl11,3-dioxolan-4-yl]cyclohexanone 10. To a solution of **9** (381 mg, 2.016 mmol) in acetone (previously distilled on K_2CO_3) was added pTsOH (20 mg, 0.105 mmol). Upon stirring under argon for 4 days at room temperature, NEt_3 (0.015 mL, 0.105 mmol) was added. The reaction mixture was concentrated and purified by flash chromatography (treated with 1% NEt_3) to give ketone **10** (440 mg, 95%) as a colorless oil in a 50:50 ratio of diastereomers **10I/10II**. Anal. calcd for $C_{13}H_{21}FO_3$: C, 63.91; H, 8.66. Found: C, 63.13; H, 8.11. Each diastereomers was separated by repeated silica gel chromatography (hexane 9/ethyl acetate 1) **10I**: $[\alpha]_D^{20}=-0.5$ ($c=4.15$, $CHCl_3$); IR: 2990, 1727, 1269, 1239; ^{1}H NMR (400 MHz C_6D_6) δ 3.45 (d, $J=9.0$ Hz, 1H), 3.28 (d, $J=9.0$ Hz, 1H), 2.53 (dt, $J=J=13.0$ Hz, $J=6.5$ Hz, 1H), 2.15 (bd, $J=13.0$ Hz, 1H), 1.87 (m, 1H), 1.60 (m, 3H), 1.34 (s, 3H), 1.28 (s, 3H), 1.26 (d, $J=22.0$ Hz, 3H), 1.01 (m, 1H), 0.93 (s, 3H); ^{13}C NMR (100 MHz C_6D_6) δ 205.5 (d, $J=25$ Hz), 109.4, 95.3 (d, $J=172.5$ Hz), 82.2, 72.8, 48.2, 40.6 (d, $J=2.5$ Hz), 38.2 (d, $J=23$ Hz), 27.2 (d, $J=9.5$ Hz), 21.9 (d, $J=24.5$ Hz), 21.6, 20.3, 20.1; **10II**: $[\alpha]_D^{20}=-2.8$ ($c=3.40$, $CHCl_3$); IR: 2990, 1727, 1269, 1239; ^{1}H NMR (400 MHz C_6D_6) δ 3.47 (d, $J=8.5$ Hz, 1H), 3.32 (d, $J=8.5$ Hz, 1H), 2.63 (m, 2H), 1.82 (m, 1H), 1.55 (m, 2H), 1.31 (d, $J=22.5$ Hz, 3H), 1.29 (s, 3H), 1.24 (s, 3H), 1.00 (m, 3H), 0.92 (s, 3H), 0.89 (m, 1H); ^{13}C NMR (100 MHz C_6D_6) δ 205.9 (d, $J=25$ Hz), 109.6, 95.7 (d, $J=173.5$ Hz), 82.1, 72.9, 48.2, 39.8 (d, $J=2.5$ Hz), 38.1 (d, $J=23$ Hz), 27.5, 27.4, 22.7 (d, $J=31.5$ Hz), 21.5, 20.2 (d, $J=31.5$ Hz).

4.2. General procedure for the epoxidation of stilbene, methyl *p*-methoxycinnamate and cinnamyl alcohol derivatives

In dioxane/water. To a solution of 1 mmol of the desired olefin and 0.3 mmol of the desired ketone in dioxane (20 mL) are added successively and under stirring a solution (4 mL) of a buffer (made from addition of 0.5 mL of AcOH in 100 mL of an aqueous solution of K_2CO_3, 0.1 M), and then 6 mL of H_2O. The mixture is cooled to the desired temperature, and a solution of oxone (1.850 g, 3 mmol) in distilled water (7 mL) is added dropwise over 6 h. During addition of oxone, the pH is controlled and regulated (8.5–9) by addition of a 1 M solution of K_2CO_3. The reaction is quenched by addition of CH_2Cl_2 (30 mL) and water (10 mL). The mixture is extracted with CH_2Cl_2, dried over Na_2SO_4, and analysed by NMR.

In DME/water. To a solution of 1 mmol of the desired olefin and 0.3 mmol of the desired ketone in DME (10 mL) is added under stirring a solution (6 mL) of a buffer (made from addition of 0.5 mL of AcOH in 100 mL of an aqueous solution of K_2CO_3, 0.1 M). The mixture is cooled to the desired temperature, and a solution of oxone (1.4 mmol) in distilled water (3.5 mL) is added dropwise over 8 h. During addition of oxone, the pH is controlled and regulated (8.5–9) by addition of 1.4 M solution of K_2CO_3. The reaction is quenched by addition of CH_2Cl_2 (30 mL) and water (10 mL). The mixture is extracted with CH_2Cl_2, dried over Na_2SO_4, and analysed by NMR.

In DME/CH$_3$CN/water. To a solution of 0.5 mmol of the desired olefin and 0.15 mmol of the ketone in DME (6 mL) under stirring, a solution (7.5 mL) of a 0.05 M $Na_2B_4O_7$ buffer, 0.5 mL of tetrabutyl ammonium hydrogensulfate and 6 mL of CH_3CN are added. A solution of oxone (1.5 mmol) in distilled water (4 mL) is added dropwise within 1 h to the mixture at room temperature. During the addition of oxone, the pH is controlled and regulated (8.5–9.0) by addition of a 1 M solution of K_2CO_3. The reaction is quenched by addition of Et_2O (40 mL) and water (10 mL). After extraction with CH_2Cl_2, the mixture is dried over Na_2SO_4 and analysed by NMR.

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Novel anion receptors based on thiacalix[4]arene derivatives

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Abstract—Starting from the thiacalix[4]arene tetraacetate, novel derivatives bearing four ureido or thioureido functions on the lower rim have been prepared. As proven by NMR titrations, these compounds can bind anions via hydrogen bonding interactions and represent the first example of anion receptors in the thiacalixarene series.

1. Introduction

Anion recognition, complexation and transport were recognised only rather recently as a very important part of supramolecular chemistry.[1] Despite many indispensable roles in the biochemical processes and pathways, where anions are frequently engaged in enzymatic reactions as substrates and/or cofactors, the supramolecular chemistry of anions is still quite unexplored if compared with that of cations.

There are several different strategies used for anion complexation so far.[2] Basically, the receptors can be divided into two main groups: (i) charged systems exploring electrostatic interactions with positively charged species (polyammonium[3] and guanidinium[4] salts, quaternary ammonium salts); (ii) neutral systems[5] using other kind of interactions, such as donor–acceptor interactions (receptors based on the Lewis acids), hydrogen bonds, hydrophobic effects etc. Significant progress has been made, especially in the design and synthesis of neutral receptors based on the hydrogen bonding interactions. Among them, systems employing amidic and (thio)urea moieties[6] have been proven especially efficient in the design of anion receptors.

Calix[*n*]arenes are very well-known building blocks[7] in supramolecular chemistry, frequently used as molecular scaffolds for the synthesis of more elaborated structures. There are many examples in the literature, where substituted calixarenes were used for anion complexation.[8] Many different systems exploiting the HB interactions,[9] Lewis acids,[10] or charged receptors[11] have been introduced for anion recognition. Another interesting group of anion receptors is represented by compounds capable of ion-pair recognition (ditopic receptors[12]), where the calixarene skeleton holds both cation- and anion-complexing functions.[13]

There is much evidence that ureido functions appended to calixarene can form a suitably pre-organized cavity (depending on the calixarene conformation used) with pronounced anion recognition capability. We[14] and others[15] have reported the *cone* and the *1,3-alternate* calixarenes bearing urea or thiourea functions on the upper/lower rim that can bind anions such as halogenides and carboxylates.

Thiacalixarenes[16] have appeared recently as novel members of the calixarene[7] family. The presence of sulfur atoms instead of CH_2 groups induces many novel features[17] compared with 'classical' calixarenes. Thiacalix[4]arenes exhibit a broad range of interesting functions such as different size and conformational behaviour, different complexation ability, easy oxidation of –S– bridges etc., which makes these compounds good candidates for many applications in supramolecular chemistry.

In this paper we report on the synthesis of thiacalix[4]arene derivatives bearing ureido or thioureido functions on the lower rim, which can serve as anion receptors. To the best of our knowledge, this is the first example of anion recognition in thiacalixarene series.

2. Results and discussion

2.1. Synthesis of receptors

The synthesis started from parent thiacalixarene **1**, which

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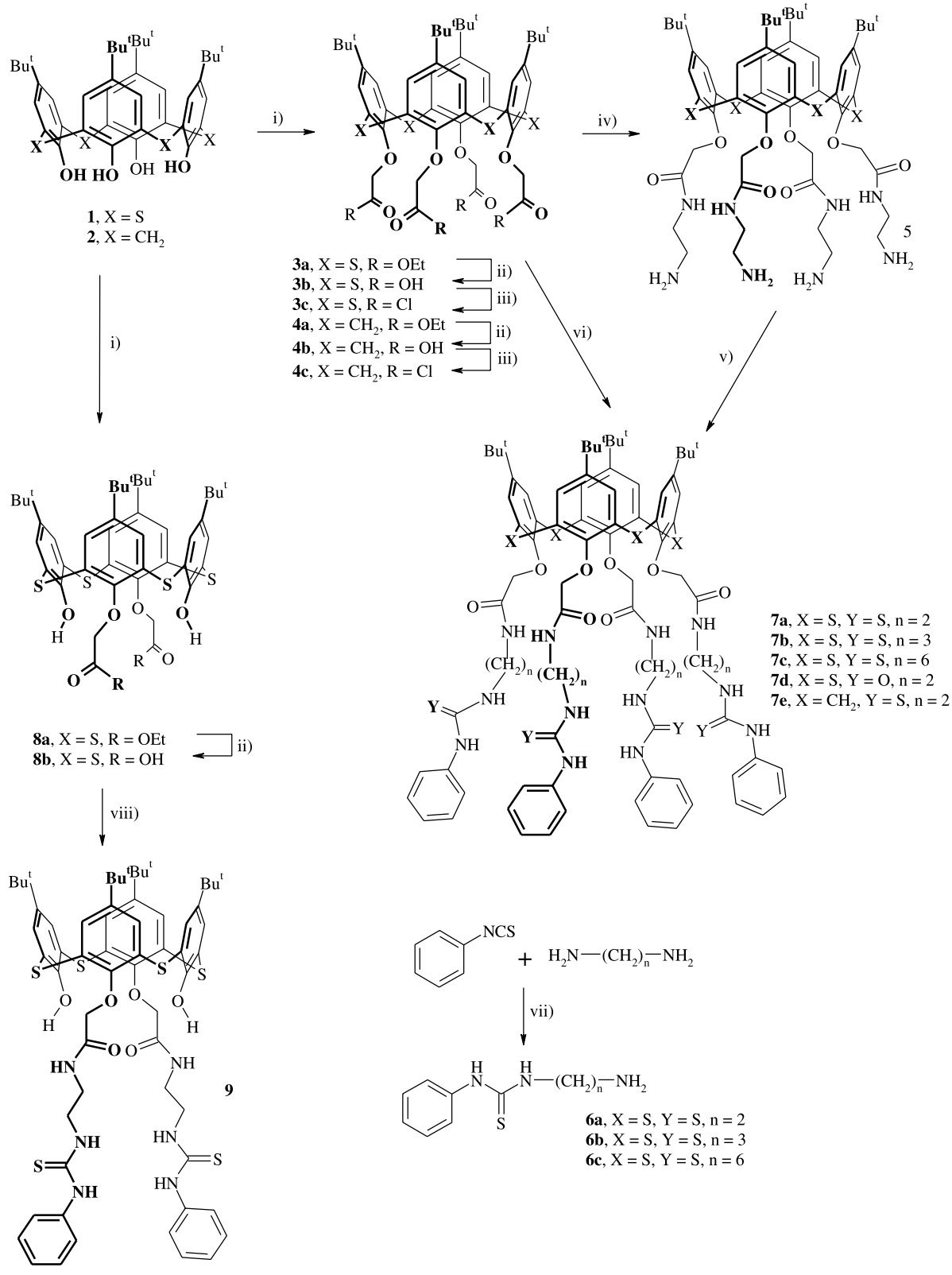
were transformed into the *cone* tetraacetate **3a** (75% yield) by alkylation with ethyl bromoacetate in refluxing acetone[18] using Na_2CO_3 as a base. Ester **3a** was hydrolysed (NaOH, aqueous ethanol, reflux) to yield the carboxylic acid **3b** in almost quantitative yield (Scheme 1). The carboxylic acid **3b** was then reacted with oxalyl chloride to yield the corresponding acyl chloride[19] **3c**. The crude chloride was used without purification for subsequent condensation



Scheme 1. (i) $BrCH_2COOEt/Na_2CO_3$, acetone, reflux (**3a**, 75%, **4a**, 55%, **8a**, 51%); (ii) NaOH, EtOH/water, reflux (**3b**, 99%, **4b**, 99%, **8b**, 99%); (iii) oxalyl chloride/CCl_4, reflux (**3c**, **4c**, quant.); (iv) ethylenediamine/THF, rt; (v) phenyl isocyanate/*i*-PrOH, reflux (8% from **3a**); (vi) **6a**–**6c**/Et_3N/THF, rt (**7a**, 23%; **7b**, 26%; **7c**, 22%; **7e**, 7%); (vii) *i*-PrOH, rt (**6a**, 46%; **6b**, 30%; **6c**, 17%); (viii) **6a**/CDI/DMF, rt (**9**, 54%).

(THF/EtN_3, rt) with the amino component **6a–6c**. The corresponding tetraamides **7a–7c** immobilised in the *cone* conformation were obtained after purification by preparative TLC (silica gel) in 23, 26 and 22% yields, respectively.

The reaction of tetraacetate **3a** with an excess of 1,2-ethanediamine led to an intractable mixture of tetraamine **5**, partly substituted and bridged compounds, that was used without purification in the next step-reaction with phenyl isocyanate. This procedure gave the appropriate urea derivative **7d** in 8% yield (overall yield from **3a**). Interestingly, attempts at the direct aminolysis of tetraester **3a** with amines **6a–6b** were unsuccessful as only very complicated reaction mixtures were obtained.

To compare the complexation properties of novel thiacalix[4]arene derivatives with classical calixarene series, tetrathioureido receptor **7e** was prepared using essentially the same strategy as described for **7a–7c**. Another model compound, dithioureido receptor **9**, was synthesised in order to evaluate the role of preorganisation and multiplicity of ureido functions on the lower rim of thiacalixarene. The synthesis followed the above strategy with one exception-the amidic bond in **9** was constructed using the reaction of free dicarboxylic acid **8b** with amine **6a** after activation with *N*,*N*′-carbonyl diimidazole in 54% yield.

2.2. Structure assignments and the NMR complexation study

The structures of novel ureido/thioureido calixarenes were proven using the combination of ^{1}H NMR spectroscopy and mass spectroscopy (ESI-MS or FAB MS). The full assignment of ^{1}H and ^{13}C NMR spectra was done using gCOSY, gHSQC and gHMBC methods. As the synthetic pathway started from the conformationally immobilised *cone* tetraacetates **3a** and **4a** of known structures,[18a] the conformational assignment of products was not necessary. The conformational preferences of thiacalixarene diacetate **8a** are also known,[20] anyhow the structure of derivative **9** was proved independently by one-dimensional DPFGSE-NOE experiments. The ESI MS spectra of all receptors usually showed the two most intense signals corresponding to the molecular peak $[M+Na]^+$ and to the doubly charged species $[M+2Na]^{2+}$. Thus, derivative **7e** exhibits two peaks at $m/z=817.64$ $[M+2Na]^{2+}$ (65% int.) and 1611.4 $[M+Na]^+$ (100% int.), respectively.

As the ^{1}H NMR spectra are almost uninfluenced by the change of solvent polarity ($CDCl_3$, $CDCl_3$–$CD_3CN=4:1$, DMSO-d_6) we can conclude that the possible intra-/intermolecular hydrogen bonds are very week or absent under the measuring conditions.

The complexation abilities of receptors **7** and **9** towards Cl^- anion were studied by ^{1}H NMR titrations in $CDCl_3$–$CD_3CN=4:1$ (v/v) mixture. The addition of Bu_4NCl resulted in the complexation-induced shift of NH signals ($\Delta\approx200$ Hz). All titration experiments were performed with constant concentration of receptor (approx. 2×10^{-4} M) and an increasing concentration of anion added. These experiments led to well reproducible binding isotherms corresponding to 1:1 binding (Fig. 1) that were analysed using an original nonlinear curve-fitting program[21] and statistically treated for several various signals. To confirm the stoichiometry of anion complexation, Job plots were constructed from ^{1}H NMR titration data. In all cases studied, the formation of the 1:1 complexes was clearly substantiated (Fig. 2).

The highest complexation-induced NMR shifts within the receptor molecules are those of the NH signals. As shown in Figure 3, both ureido NH protons (Nr. 12 and 14) in **7a** are the most downfield shifted signals after the addition of 5 equiv of chloride. This clearly indicates that the ureido/thioureido groups create a binding site, which is responsible for anion binding via synchronous hydrogen bonding interactions. The comparison with classical calix[4]arene derivative **7e** shows that the binding modes are identical in both systems.

As shown in Table 1, the corresponding complexation constants depend on the length of the lower rim alkylene chains. The lower is the rigidity of the system (the longer

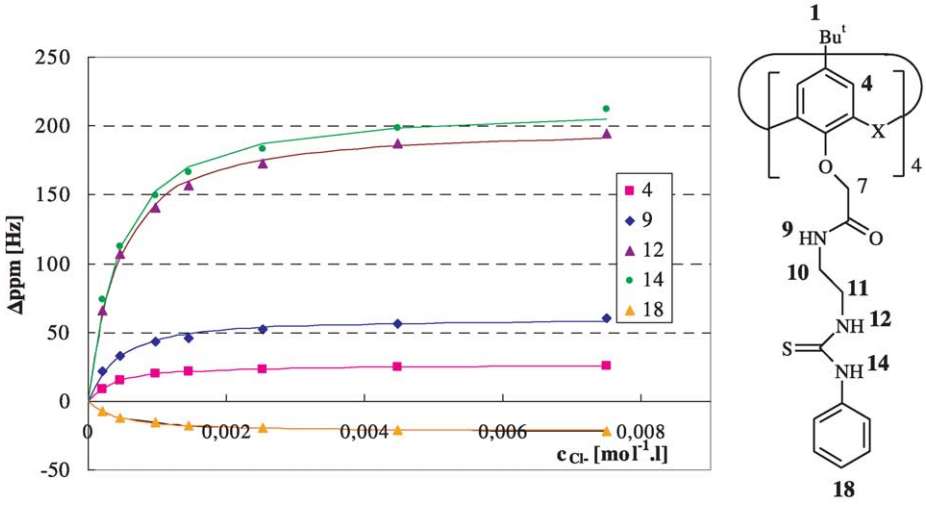


Figure 1. ^{1}H NMR titration curves of **7a** with $Bu_4^+NCl^-$ (300 MHz, $CDCl_3$–$CD_3CN=4:1$v/v, 298 K), several various ^{1}H signals used (see the numbering scheme).

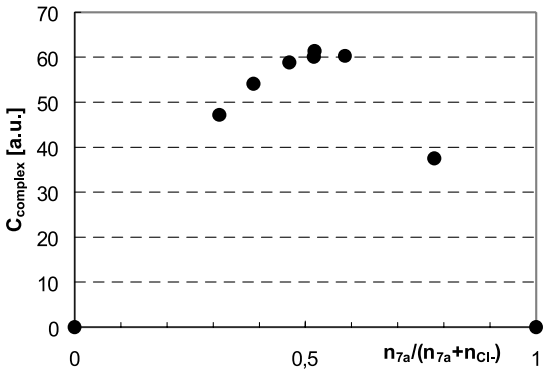


Figure 2. Job plot for the **7a**+$Bu_4N^+Cl^-$ system (^{1}H NMR, 300 MHz, $CDCl_3$–CD_3CN=4:1v/v).

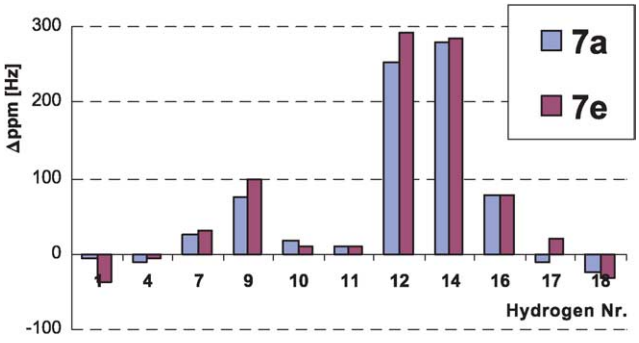


Figure 3. Complexation induced ^{1}H NMR chemical shifts in **7a** and **7e** after addition of 5 equiv of $Bu_4N^+Cl^-$ (300 MHz, $CDCl_3$–CD_3CN=4:1 v/v).

Derivatives **7** could, in principle, operate as ditopic receptors for ion-pair complexation. These molecules contain a second binding site at the thiacalix[4]arene lower rim formed by four amidic groups potentially capable of synchronous binding of alkaline metal cations. The complexation ability for sodium cations in $CDCl_3$ was determined by ^{1}H NMR titrations with Kobayashi reagent $NaB[3,5\text{-}(CF_3)_2C_6H_3]_4$ (sodium salt well soluble in apolar solvents). It was found that complexation constants of **7a** ($K_{Na}=1960\ M^{-1}$) and **7d** ($K_{Na}=520\ M^{-1}$) are very small if compared with classical calixarene derivative **7e** ($K_{Na}>10^5\ M^{-1}$).

To test the influence of cation complexation on the anion recognition the ^{1}H NMR titrations of preformed sodium complex was carried out. Unfortunately, as the NH signals are invisible or extensively broadened under the conditions used, they cannot be used for the construction of titration curves. The addition of Bu_4NCl to the mixture of **7a** and excess of Kobayashi reagent (5 equiv) led to only minor changes in chemical shifts of aromatic or CH_2 signals. As shown in Figure 4 the titration curves do not correspond to simple 1:1 binding. Rather than synchronous complexation of cation and anion they reflect more complex process. As the separately obtained complexation constants for Na^+ and Cl^- are of the same order ($K_{Na}=1960\ M^{-1}$ vs $K_{Cl}=3480\ M^{-1}$), it seems highly probable that both processes (cation vs anion complexations) are competitive rather than cooperative. The same phenomenon was found for thiacalixarene **7d** (Fig. 5).

In conclusion, easily accessible thiacalix[4]arene tetraacetate in the *cone* conformation was transformed into the

Table 1. Complexation constants K of receptors **7** and **9** towards selected anions (^{1}H NMR, $CDCl_3$–CD_3CN=4:1 v/v, 25 °C, 300 MHz)

Anion	K_C [mol^{-1} l]					
	7a	**7b**	**7c**	**7d**	**7e**	**9**
Cl^-	3480	1900	1790	4300	2670	220
Br^-	830	—	—	990	780	—
I^-	100	—	—	240	250	—
CN^-	1740	—	—	2510	5140	—

—=not measured.

chain) the weaker is the binding (compare the complexation constants K for chloride: **7a** (3480 M^{-1}), **7b** (1900 M^{-1}), **7c** (1790 M^{-1})). The comparison of **7a** and **7d** shows that thioureido derivative is slightly worse receptor for chloride if compared with the corresponding thiacalixarene bearing ureido functions. On the other hand, both thiacalix[4]arene receptors **7a** and **7d** exhibit higher complexation abilities for chloride than classical calix[4]arene derivative **7e**, while the situation with binding of cyanide anion is just reversed.

The relationship between the number of hydrogen bonds and the strength of anion complexation can be evaluated by comparing the binding affinity of **7a** ($K_{Cl}=3480\ M^{-1}$) with that of model derivative **9** ($K_{Cl}=220\ M^{-1}$) possessing only two thiourea moieties. The considerable difference (15×) in the complexation constants clearly demonstrates the importance of multiple hydrogen bonds and preorganisation for efficient anion binding.

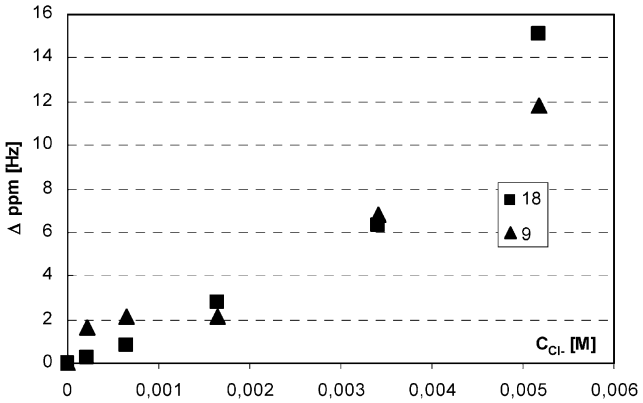


Figure 4. Complexation induced ^{1}H NMR chemical shifts in **7a**/Kobayashi agent system after addition of Bu_4NCl (300 MHz, $CDCl_3$–CD_3CN=4:1 v/v), for numbering see Figure 1.

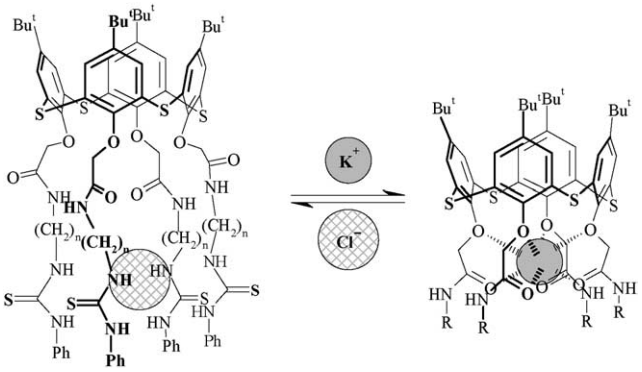


Figure 5. Competitive cation/anion complexation in derivative **7a**.

corresponding derivatives bearing four ureido or thioureido functions on the lower rim. The NMR titrations revealed that these compounds can interact with anions in apolar solvents via hydrogen bonding interactions and represent the first example of anion receptors in the thiacalixarene series.

3. Experimental

3.1. General

Melting points were determined on a Boetius block (Carl Zeiss Jena, Germany) and are not corrected. The IR spectra were measured on an FT-IR spectrometer Nicolet 740 in $CHCl_3$ and/or in KBr. ^{1}H NMR spectra were recorded on a Varian Gemini 300 spectrometer. Dichloromethane and CCl_4 used for the reaction were dried with CaH_2 and P_2O_5, respectively, and stored over molecular sieves. The purity of the substances and the course of reactions was monitored by TLC using TLC aluminium sheets with Silica gel 60 F_{254} (Merck). Preparative TLC chromatography was carried out on 20×20 cm glass plates covered by Silica gel 60 GF_{254} (Merck).

Starting esters **3a** (75%), **4a** (77%), and **8a** were prepared according to procedures known[20] by the reaction of **1** or **2** with ethyl bromoacetate in boiling acetone in the presence of Na_2CO_3 or Cs_2CO_3.

3.1.1. Preparation of acyl chlorides. *Synthesis of 5,11,17,23-tetra-tert-butyl-25,26,27,28-tetrakis[(chlorocarbonyl)methoxy]-thiacalix[4]arene* (**3c**). Tetraacid **3b** (0.4 g, 0.42 mmol) was dissolved in anhydrous tetrachloromethane (20 ml) and oxalyl chloride (3 ml, 0.034 mol) was added. The reaction mixture was refluxed for 3 h and the volatile part of the reaction was distilled off. Fresh tetrachloromethane (5 ml) was then added and the distillation was repeated under reduced pressure. The product was immediately used in the next step.

Synthesis of 5,11,17,23-tetra-tert-butyl-25,26,27,28-[(chlorocarbonyl)methoxy]calix[4]arene (**4c**). Calixarene **4b** (0.4 g, 0.45 mmol) was reacted with oxalyl chloride (3 ml, 0.035 ml) following the same procedure as described above for **3c**. The product was obtained quantitatively, and was used immediately in the next step.

3.1.2. Synthesis of 5,11,17,23-tetra-*tert*-butyl-25,26,27,28-tetrakis[((*N*-2-aminoethyl)aminocarbonyl)-methoxy]thiacalix[4]arene (5). A solution of tetraester **3a** (1.1 g, 1.04 mmol) in 5 ml of absolute THF was added to 40 ml of ethylene diamine (598 mmol) and the mixture was stirred for four days at rt. The remaining ethylene diamine was distilled off at a reduced pressure, the residue was suspended in water, and the precipitate was filtered off. The crude product (0.92 g) was used without further purification in the next step. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.33 (broad peak, 4H, NH), 8.10 (b.s., 4H, NH), 7.34 (s, 8H, H-arom), 4.88 (s, 8H, O–*CH_2*–CO–), 3.43 (m, 8H, –NH–*CH_2*–CH_2–NH_2), 2.92 (m, 8H, NH–CH_2–*CH_2*–NH_2), 1.12 (s, 36H, Bu^t).

3.1.3. Synthesis of thioureas. *N-(2-Aminoethyl)-N′-phenylthiourea* (**6a**). Phenyl isothiocyanate (2.4 ml, 0.02 mol) in 5 ml of diethyl ether was added dropwise to the solution of 1.2 ml of ethylene-1,2-diamine (0.02 mol) in 30 ml of isopropyl alcohol over a period of 40 min. The reaction mixture was then stirred for 2 h at rt and quenched by addition of 80 ml of diluted HCl (40:1). The solvents were evaporated, the residue was suspended in hot water and the resulting precipitate was filtered off. The filtrate was basified by the addition of solid NaOH, and the product (1.8 g, 46%) crystallised in the form of white crystals. Mp: 135–136 °C (lit.[22] 135–137 °C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.43 (t, 2H, J=7.7 Hz, H-arom), 7.28 (m, 3H, H-arom), 6.75 (b.s., 2H, NH) 3.67 (m, 2H, NH–CH_2–*CH_2*–NH), 2.92 (t, 2H, J=5.8 Hz, NH–*CH_2*–CH_2–NH).

N-(3-Aminopropyl)-N′-phenylthiourea (**6b**). Phenyl isothiocyanate (2.4 ml, 0.02 mol) in diethyl ether (5 ml) was added dropwise to the solution of 1.7 ml (0.02 mol) of propan-1,3-diamine in 30 ml of isopropyl alcohol during 40 min. The reaction mixture was stirred for 2 h at rt and quenched by addition of 80 ml of diluted HCl (40:1). The solvents were evaporated, the residue was suspended in hot water and the resulting precipitate was filtered off. The filtrate was basified by NaOH to approx. pH=10. After standing at rt the product crystallised to give 1.23 g of white crystals (30%). Mp: 103–105 °C (lit.[23] 106–107 °C).^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.77 (b.s., 1H, NH), 7.67 (b.s., 1H, NH), 7.38 (t, 2H, J=7.4 Hz, H-arom), 7.24 (d, 3H, J=6.6 Hz, H-arom), 3.74 (m, 2H, NH–*CH_2*–CH_2–CH_2–NH_2), 2.80 (m, 2H, NH–CH_2–CH_2–*CH_2*–NH_2), 1.66 (m, 2H, NH–CH_2–*CH_2*–CH_2–NH_2), 1.10 (b.s., 2H, NH_2).

N-(6-aminohexyl)-N′-phenylthiourea (**6c**). Phenyl isothiocyanate, 2.4 ml (0.02 mol) in diethyl ether (5 ml) was added dropwise to the solution of 2.32 ml (0.02 mol) hexane-1,6-diamine in 30 ml of isopropyl alcohol during 40 min. After addition of all the isothiocyanate, the reaction mixture was stirred for 2 h at rt and quenched with 80 ml of dilute HCl (40:1). The solvents were evaporated, the residue was suspended in hot water and the resulting solid was filtered off. The filtrate was basified by NaOH to pH=10 and the product crystallised from water to give 0.84 g of colourless crystals (17%). Mp: 90–92 °C (lit.[22] 89–92 °C. ^{1}H NMR ($CDCl_3$, 300 Hz) δ (ppm): 7.73 (b.s., 1H, NH), 7.44 (t, 2H, J=7.4 Hz, H-arom), 7.31 (t, 1H, J=7.7 Hz, H-arom), 7.19 (d, 2H, J=7.1 Hz, H-arom), 6.03 (b.s., 1H, NH), 3.62 (m,

2H, –NH–CH_2–CH_2–CH_2–), 2.66 (m, 2H, –CH_2–CH_2–NH_2), 1.57 (m, 2H, NH–CH_2–CH_2–CH_2–), 1.42 (m, 2H, –CH_2–CH_2–CH_2–NH_2), 1.32 (m, 4H, –NH–CH_2–CH_2–CH_2–CH_2–CH_2–CH_2–NH_2), 1.02 (b.s., 4H, NH_2).

3.1.4. Synthesis of receptors 7a–7e. *Receptor* **7a**. A solution of acyl chloride **3c** (0.23 g, 0.21 mmol) in 10 ml of absolute THF was added dropwise to a stirred solution of *N*-(2-aminoethyl)-*N′*-phenylthiourea (0.5 g, 2.56 mmol) in THF (10 ml) under the nitrogen atmosphere. Triethylamine (0.4 ml) was then added and the mixture was stirred for three days at rt. Reaction mixture was quenched by addition of 1 M aqueous HCl and the product was extracted with chloroform. The organic layer was dried on $MgSO_4$, evaporated to dryness and the solid residue was purified by preparative TLC (petroleum ether–acetone=2:1) to give 80 mg of title compound (23%) as a white solid. Mp: 142–146 °C. ^{1}H NMR ($CDCl_3$–CD_3CN=4:1, 300 MHz) δ (ppm): 8.46 (s, 4H, –NH), 8.30 (b.s., 4H, –NH), 7.35 (s, 8H, H-arom), 7.32 (d, 8H, J=7.7 Hz, H-arom), 7.30 (t, 8H, J=7.8 Hz, H-arom), 7.23 (b.s., 4H, –CH_2–CO*NH*–), 7.15 (t, 4H, J=7.1 Hz, H-arom), 4.87 (s, 8H, O–CH_2–CONH–), 3.80 (m, 8H, –NH–CH_2–CH_2–NH–), 3.54 (m, 8H, –NH–CH_2–CH_2–NH–), 1.10 (s, 36H, Bu^t). IR ($CHCl_3$) ν_{max} (cm^{-1}): 1663 (CO), 3306 (NH). MS-ES *m/z* (rel. int.) 853.10 $[M+2Na]^{2+}$ (33), 1683.27 $[M+Na]^+$ (52). EA calcd for $C_{84}H_{100}N_{12}O_8S_8$: C, 60.69; H, 6.06; N, 10.11%. Found: C, 60.23; H, 6.21; N, 10.01%.[24]

Receptor **7b**. Triethylamine (0.4 ml) was added to the solution of *N*-3-aminopropyl-*N′*-phenylthiourea **6b** 0.53 g (2.5 mmol) in anhydrous THF (10 ml) under nitrogen atmosphere. Then the solution of acyl chloride **3c** (0.23 g, 0.21 mmol) in THF (10 ml) was added and the reaction mixture was stirred for five days at rt. An aqueous 1 M solution of HCl was added and the product was extracted with chloroform. The organic layer was dried over $MgSO_4$, evaporated to dryness, and the crude product was purified using the preparative TLC on silica gel (20×20 cm, chloroform–acetone=2:1). The title compound (0.08 g, 26%) was obtained as a white solid. Mp: 136–139 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.23 (b.s., 4H, NH), 7.97 (b.s., 4H, NH), 7.35 (m, 8H, H-arom), 7.33 (s, 8H, H-arom), 7.25 (m, 8H, H-arom), 7.20 (m, 4H, H-arom), 6.99 (b.s., 4H, NH), 4.79 (s, 8H, O–CH_2–CONH–), 3.62 (m, 8H, NH–CH_2–CH_2–CH_2–NH), 3.31 (m, 8H, NH–CH_2–CH_2–CH_2–NH), 1.81 (m, 8H, NH–CH_2–CH_2–CH_2–NH_2), 1.11 (s, 36H, Bu^t). IR ($CHCl_3$) ν_{max} (cm^{-1}): 1663 (C=O), 3317 (NH). MS-ESI *m/z* (rel int.) 881.63 $[M+2Na]^{2+}$ (66), 1740.28 $[M+Na]^+$ (100). EA calcd for $C_{88}H_{108}N_{12}O_8S_8$: C, 61.51; H, 6.33; N, 9.78%. Found: C, 61.13; H, 6.56; N, 9.84%.[24]

Receptor **7c**. *N*-6-Aminohexyl-*N′*-phenylthiourea **6c** (0.44 g, 1.753 mmol) was dissolved in anhydrous THF (10 ml) under nitrogen atmosphere and treated with triethylamine (0.3 ml) and the solution of **3c** (0.16 g, 0.14 mmol) in THF (5 ml) was added. The reaction mixture was then stirred for five days at rt and purified as described above for **7b** to give 0.062 g of product (22%) as a white solid. Mp: 96–99 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.06 (s, 4H, SH), 7.98 (b.s., 4H, NH), 7.36 (s, 8H, Ar-H), 7.36 (m, 8H, H-arom), 7.29 (m, 8H, H-arom), 7.19 (t, 4H, J=7.2 Hz, H-arom), 6.58 (b.s., 4H, NH), 4.79 (s, 8H, O–CH_2–CONH–), 3.59 (m, 8H, NH–CH_2–$(CH)_5$–NH), 3.32 (m, 8H, NH–$(CH)_5$–CH_2–NH), 1.57 (m, 16H, NH–CH_2–CH_2–$(CH_2)_2$–CH_2–CH_2–NH), 1.34 (m, 16H, NH–$(CH_2)_2$–CH_2–CH_2–$(CH_2)_2$–NH), 1.12 (s, 36H, Bu^t). IR ($CHCl_3$) ν_{max} (cm^{-1}): 1665 (CO), 3326 (NH). MS-ESI *m/z* (rel. int.) 965.67 $[M+2Na]^{2+}$ (98), 1908.2 $[M+Na]^+$ (100). EA calcd for $C_{100}H_{132}N_{12}O_8S_8$: C, 63.66; H, 7.05; N, 8.91%. Found: C, 62.93; H, 7.11; N, 8.71%.[24]

Receptor **7d**. A solution of phenyl isocyanate (0.72 ml, 6.6 mmol) in ether (10 ml) was added to derivative **5** (0.92 g, 0.82 mmol) dissolved in 15 ml of isopropyl alcohol. The reaction mixture was stirred at rt for 2 h, then 20 ml of water was added and the product was extracted with chloroform. The organic layer was dried over $MgSO_4$, evaporated to dryness and the residue was purified using column chromatography on silica gel ($CHCl_3$–ethyl acetate=100:1) to yield 0.108 g of the product (8% overall from **3a**) as a yellowish solid. Mp: 162–166 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 8.51 (b.s., 8H, 2NH), 7.37 (s, 8H, H-arom), 7.34 (d, 8H, J=7.1 Hz, H-arom), 7.18 (t, 8H, J=7.2 Hz, H-arom), 6.86 (t, 4H, J=7.0 Hz, H-arom), 6.27 (t, 4H, J=4.9 Hz, NH), 4.84 (s, 8H, O–CH_2–CONH–), 3.29 (m, 8H, NH–CH_2–CH_2–NH), 3.22 (m, 8H, NH–CH_2–CH_2–NH), 1.06 (s, 36H, Bu^t). IR ($CHCl_3$) ν_{max} (cm^{-1}): 1659 (CO), 3321 (NH). MS-ESI *m/z* 1619.3 $[M+Na]^+$. EA calcd for $C_{84}H_{100}N_{12}O_{12}S_4$: C, 63.13; H, 6.31; N, 10.52%. Found: C, 62.63; H, 6.02; N, 10.11%.[24]

Receptor **7e**. *N*−2-aminoethyl-*N′*-phenylthiourea **6a** (0.7 g, 0.0035 mol) and 0.4 ml of triethylamine were dissolved in anhydrous THF (10 ml) under a nitrogen atmosphere. A solution of acyl chloride **4c** (0.43 g, 0.45 mmol) in 10 ml of anhydrous THF was added and the mixture was stirred for three days at rt. The reaction mixture was then quenched by aqueous 1 M HCl (10 ml) and the product was extracted to chloroform. The organic layer was dried over $MgSO_4$, solvent was removed under a reduced pressure, and the residue was purified by preparative TLC ($CHCl_3$–methanol=10:1) to yield 50 mg (7%) of product as a white solid. Mp: 136–142 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.35 (b.s., 4H, NH); 8.20 (b.s., 4H, NH); 7.28–7.19 (m, 24H, H-arom, NH); 6.77 (s, 8H, H-arom); 4.52 (s, 8H, O–CH_2–CO); 4.43 (d, 4H, J=13.2 Hz, Ar-CH_2-Ar ax.); 3.77 (m, 8H, –CH_2–CH_2–); 3.52 (m, 8H, –CH_2–CH_2–); 3.23 (d, 4H, J=13.2 Hz, Ar-CH_2-Ar eq.); 1.06 (s, 36H, Bu^t). MS-ESI *m/z* (rel. int.) 817.64 $[M+2Na]^{2+}$ (65), 1611.4 $[M+Na]^+$ (100). EA calcd for $C_{88}H_{108}N_{12}O_8S_4$: C, 66.47; H, 6.85; N, 10.57%. Found: C, 65.98; H, 6.88; N, 10.17%.[24]

3.1.5. Synthesis of receptor 9. Di-acid **8b** (0.117 g, 0.14 mmol) was dissolved in 20 ml of dry DMF under the nitrogen atmosphere, the *N*,*N′*-carbonyl diimidazole (0.05 g, 0.294 mmol) was added and the mixture was stirred for 1 h at rt. A solution of *N*-(2-aminoethyl)-*N′*-phenylthiourea **6a** (0.11 g, 0.56 mmol) in DMF was added and the mixture was heated to 70 °C overnight. The solvents were distilled off under a reduced pressure and the remaining solid was purified by the preparative TLC on silica gel ($CHCl_3$–acetone=10:1) to give 90 mg of product (54%) as a white solid. Mp: 181–183 °C. ^{1}H NMR ($CDCl_3$–CD_3CN=4:1) δ (ppm): 8.45 (s, 2H, OH), 8.27 (b.s., 2H, NH), 8.12 (b.s., 2H, NH), 7.53 (s, 4H, H-arom), 7.16 (s, 4H,

H-arom), 7.12 (m, 8H, H-arom), 6.98 (m, 2H, H-arom), 6.91 (b.s., 2H, NH), 4.77 (s, 4H, O–CH_2–CONH–), 3.76 (m, 4H, NH–CH_2–CH_2–NH), 3.56 (m, 4H, NH–CH_2–CH_2–NH), 1.17 (s, 18H, Bu^t), 0.83 (s, 18H, Bu^t). IR ($CHCl_3$) ν_{max} (cm^{-1}): 1671 (CO), 3340 (NH). MS ES *m/z* (rel. int.) 1191.09 $[M+H]^+$ (22), 1213.1 $[M+Na]^+$ (100). EA calcd for $C_{62}H_{74}N_6O_6S_6$: C, 62.49; H, 6.26; N, 7.05%. Found: C, 61.88; H, 6.31; N, 6.87%.

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24. The EA usually resulted in C values about 1 to 2% lower than the calculated values. This fact is well documented in calixarene chemistry and there are two widely accepted explanations: (i) cavity of calixarenes contains the molecules of solvents/reagents, which are extremely difficult to eliminate; (ii) the incomplete combustion of these high-melting compounds under the standardized conditions of the elemental analysis. We believe that the structures of the calixarenes are sufficiently documented by the spectral evidence.

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Divergent sequential reactions of β-(2-aminophenyl)-α,β-ynones with nitrogen nucleophiles

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Abstract—β-(2-Aminophenyl)-α,β-ynones readily react with nitrogen nucleophiles to give three major types of products, depending on reaction conditions and variation in the nucleophiles. The reaction may lead to simple 1,2-nucleophilic adducts or, at higher temperatures, to a divergent sequential cyclisation giving rise to 2-aryl-4-aminoquinolines by reaction with amines, or to substituted 2-aryl or 2-alkyl-4-alkylidene quinazolines by reaction with amidines. The latter could also be synthesised by reaction of β-(2-aminophenyl)-α,β-ynones with iminochlorides.

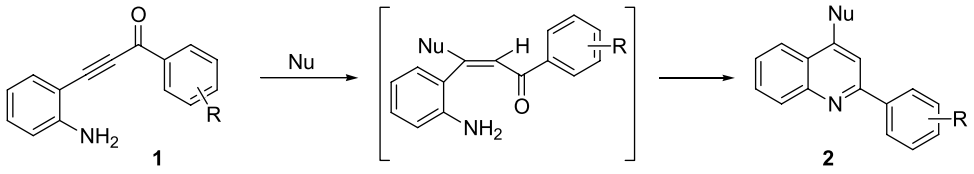
1. Introduction

The domino addition/annulation reactions of β-(2-aminoaryl)-α,β-ynones **1** provide an interesting synthetic tool for building quinoline and fused quinoline derivatives.[1–7] Indeed, we reported that domino [2+2] cycloaddition/annulation reactions of β-(2-aminophenyl)-α,β-ynones **1** with enamines accomplished the synthesis of quinolines *c*-fused with different sized carbocyclic rings.[1] Moreover, the sequential 1,3-dipolar cycloaddition/annulation reactions of **1** with *p*-nitrophenyl azide and phenyl nitrile oxide can lead respectively to the synthesis of triazolo[4,5-*c*]-quinoline and isoxazolo[4,5-*c*]quinoline derivatives.[2]

In a recent paper, Zhu and Fayol reported the synthesis of furo[2,3-*c*]quinoline derivatives by a multicomponent domino process involving β-(2-aminoaryl)-α,β-ynones as starting material.[3]

So far, the palladium-catalysed transfer hydrogenation/cyclisation of ynones **1** afforded 2-vinyl and 2-arylquinolines in good yields.[4] 2,3-Disubstituted quinolines have been synthesised in high yields by nucleophile-induced intramolecular cyclisation of *o*-alkynylisocyanobenzenes.[5] Other known syntheses of quinolines from acetylenic ketones include the reaction of secondary amines with α,β-ynones generated in situ through the carbonylative coupling of *o*-ethynylaniline with aryl iodides,[6] and the reaction of 2-amino thiophenol with α,β-ynones bearing an acetal group.[7] These last two methods both provide 2,4-disubstituted quinolines in good yields, but are restricted to the synthesis of 4-*N*,*N*-dialkylamino- and 4-formyl quinolines respectively.

Finally, we recently reported the reaction of substrates **1** with nucleophilic partners as a versatile, new approach to 2,4-disubstituted quinolines **2** through a conjugate addition/cyclisation domino reaction.[2a] The oxygen, sulfur, halo and carbonucleophile addition reactions proceeded with high stereoselectivity and the stereochemical outcome resulted in the subsequent cyclisation reaction (Scheme 1).



Scheme 1.

Keywords: α-β-ynones; Quinolines; Quinazolines; Sequential reactions; Nitrogen nucleophiles.
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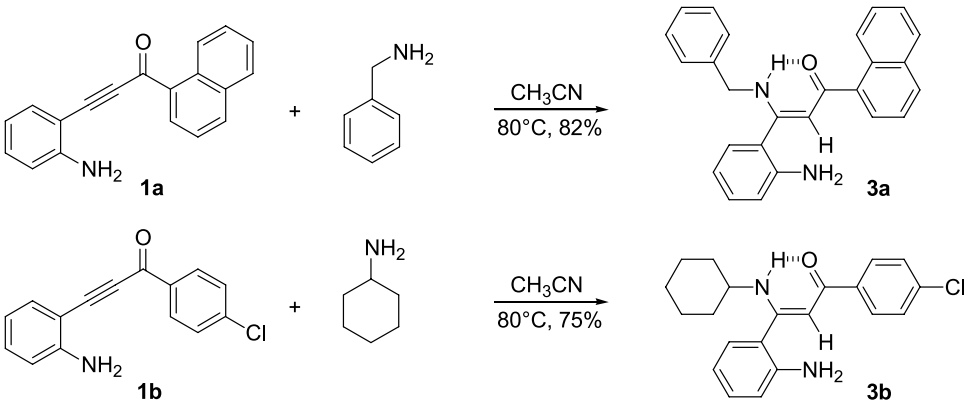


Scheme 2.

Considering that the ynone-mediated approach to these heterocyclic compounds is one of the most efficient procedures particularly because compounds **1** can be easily prepared through a high yielding carbonylative coupling reaction of *o*-trimethylsilylethynylaniline with aryl iodides,[4] and in connection with the previous results we wish to report herein a study of the chemical behaviour of β-(2-amino-aryl)-α,β-ynones **1** with nitrogen nucleophiles.

2. Results and discussion

In contrast with our previously reported results with non-nitrogen nucleophiles, the reaction of α,β-ynone **1a** with benzylamine, performed in boiling acetonitrile, failed to afford the corresponding quinoline derivative, and the *Z*-3-(2-amino-phenyl)-3-benzylamino-1-naphthalen-1-yl-propenone **3a** was isolated as the main reaction product[2a] (Scheme 2). Analogously when the ynone **1b** was reacted, under the same reaction conditions, with cyclohexylamine, the *Z*-3-(2-amino-phenyl)-1-(4-chloro-phenyl)-3-cyclohexylamino-propenone **3b** was isolated in 75% yield (Scheme 2).

β-Amino-α,β-unsaturated ketones (enaminones) **3** are typical push–pull ethylenes, which can exist in several configurational and conformational isomeric forms (Fig. 1).

These compounds are well suited for spectral and theoretical studies and have been subjected to extensive investigations.[8] Mono-, bi-, and dynamic NMR spectra analysis generally allows the detection of different isomers and the study of their isomeric equilibria. ^{1}H NMR and NOESY experiments, performed for **3a–b** in deuterochloroform solution at room temperature and at a concentration of about 2×10^{-2} M, demonstrated the presence, under these conditions, of a single *Z*-isomer (Fig. 1) with a strong hydrogen bond between the oxygen of the carbonyl group and the hydrogen of the secondary amino group which lowers both the electron density around the proton on nitrogen and the speed of proton exchange. In fact, ^{1}H NMR spectra of **3a–b** show single signals for each type of proton, the protons of the secondary amino group resonate respectively at 11.6 ppm (**3a**) and at 11.4 ppm (**3b**) and the signals are clearly split into a double doublet with vicinal coupling constants of 6.4 and 5.1 Hz for **3a** and into a doublet with a vicinal coupling constant of 9.5 Hz for **3b**. Moreover, the olefinic proton on double bond shows positive NOE interactions with the *ortho*-hydrogens of the aryl substituents in position 1 and 3 of the enaminone skeleton (Fig. 1). In particular, the interaction with the *orhto*-hydrogen of the aryl substituent in position 3 is diagnostic for the proposed *Z*-geometry around the double bond.

It is well known that enaminones bearing at least one hydrogen atom on nitrogen can undergo $Z\rightarrow E$ isomerisation when heated or melted. Thus, dynamic ^{1}H NMR experiments performed for **3b** in DMSO-d_6 at a concentration of about 2×10^{-2} M, clearly showed, at 90 °C, the presence of two signal patterns, which can be easily attributed to **3b** and to a new compound characterised by a singlet at 6.94 ppm and a broad exchangeable singlet at 6.38 ppm. These new signals, however, are incompatible

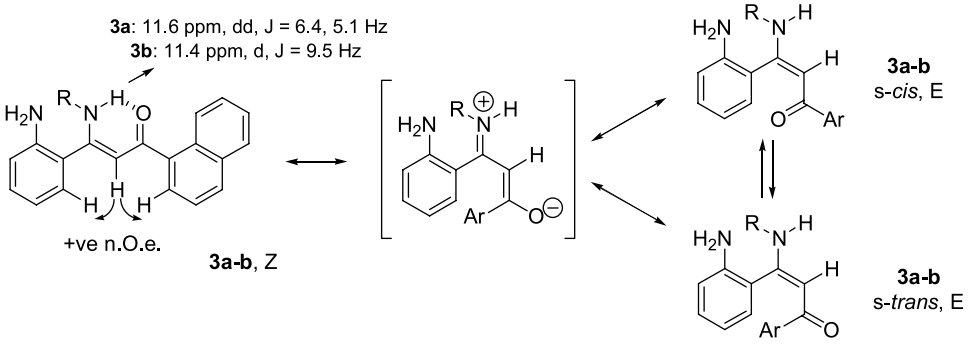


Figure 1.

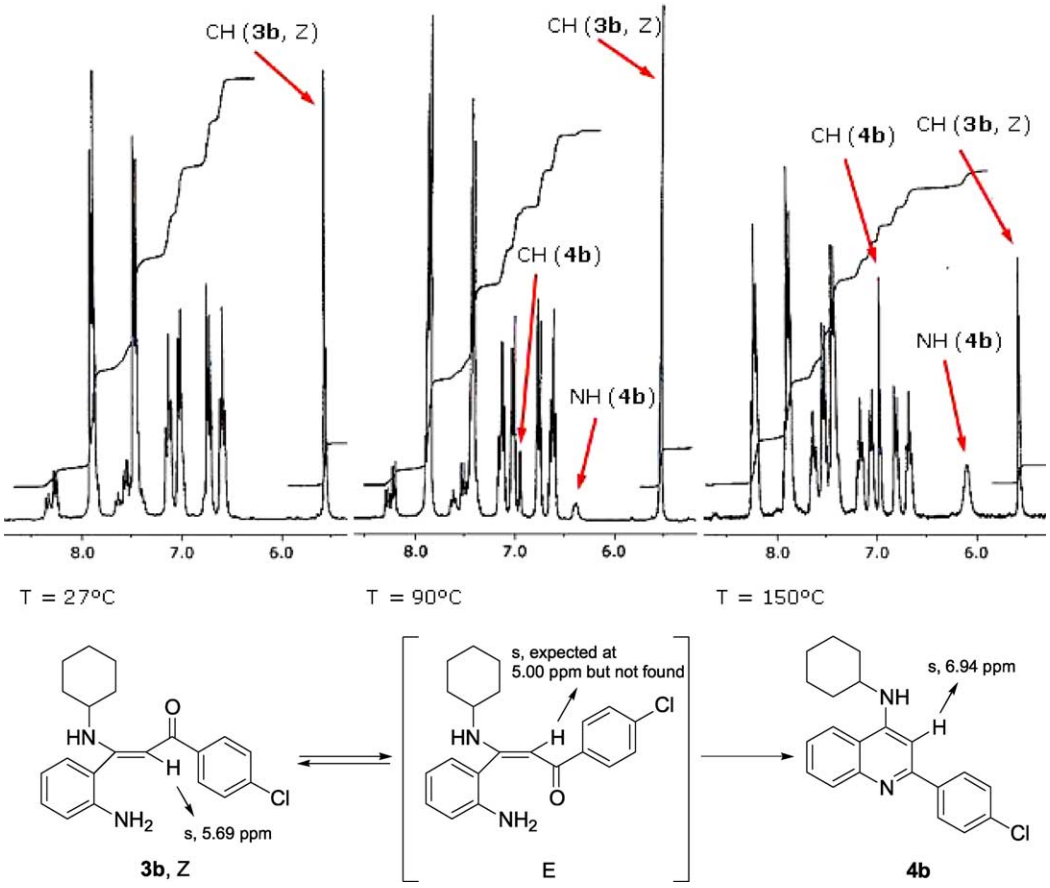
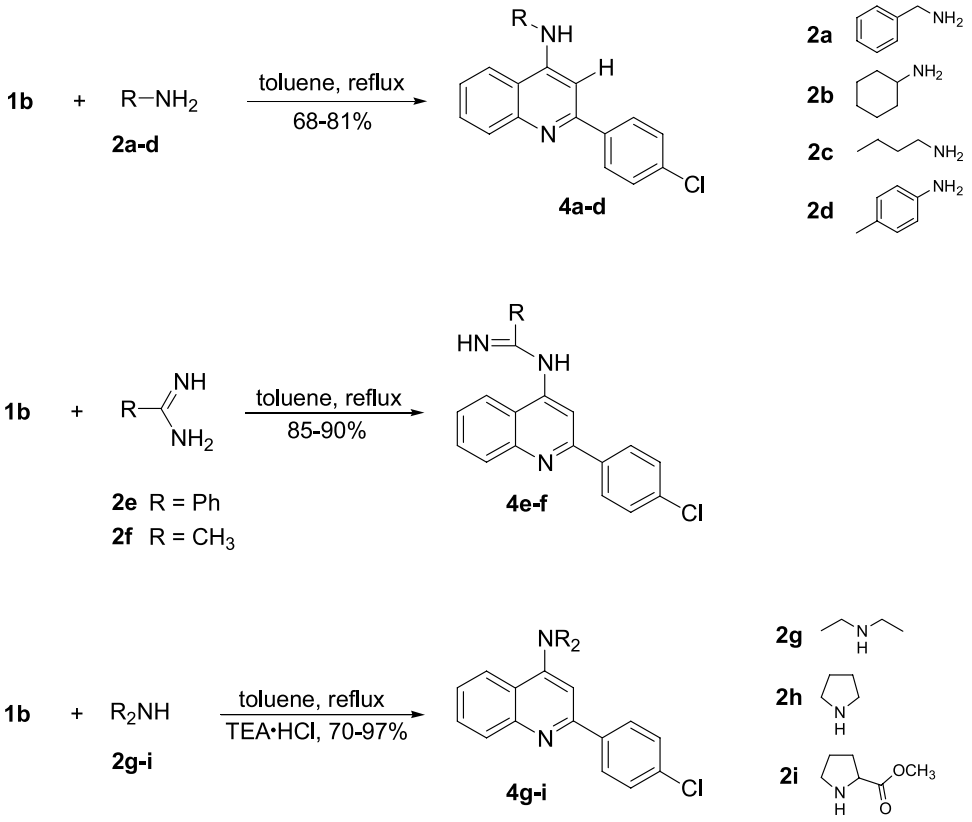
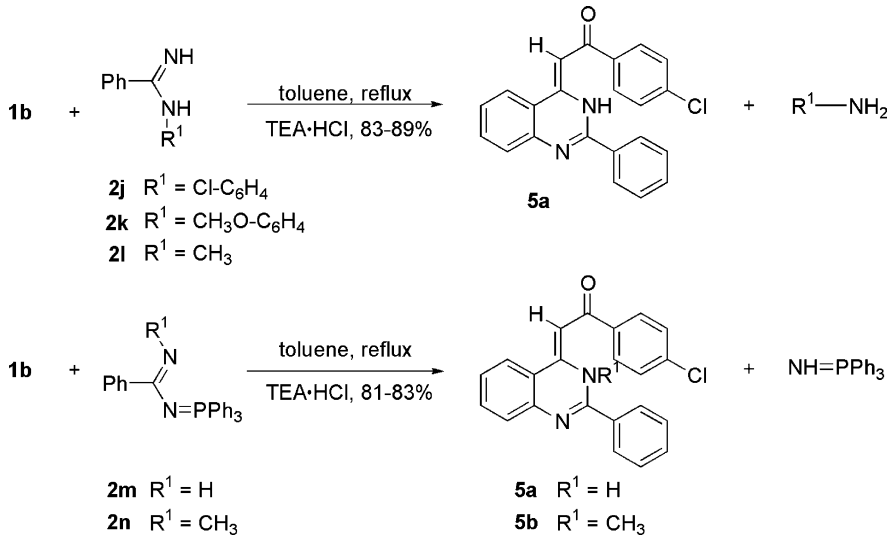


Figure 2.



Scheme 3.



Scheme 4.

with the *E*-isomer of **3b**, which olefinic proton could appear at a calculated value of about 5.00 ppm, and have been attributed to the quinoline nucleus **4b** (Fig. 2).

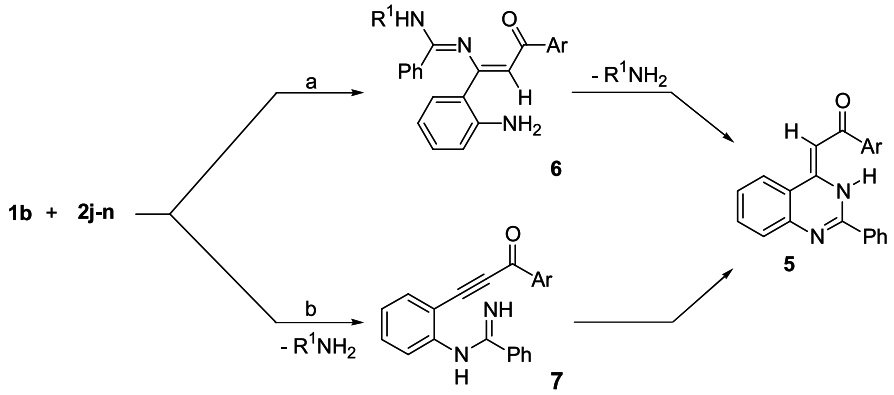
On the basis of this experimental evidence we increased the temperature in the reaction between the ynone **1b** and primary amines **2a–d** by using toluene at reflux whereby the targeted 2-aryl-4-aminoquinolines **4a–d** were isolated in good yields (Scheme 3).

Then, to better define the scope and limitations of these addition/annulation reactions we tested the reactivity of **1b** with more complex nitrogen-containing nucleophiles. Ynone **1b** reacts with N,N-unsubstituted amidines **2e–f**, and with secondary amines **2g–i** giving rise to the corresponding quinolines **4e–i** in excellent yields under fairly acidic catalysis.[9] By contrast, in the presence of primary amines bearing an electronwithdrawing group in α-position, such as aminoacetonitrile and methyl esters of glycine, phenyl glycine or valine, typical reaction conditions were ineffective and ynone **1b** was recovered unreacted even after prolonged reaction times.

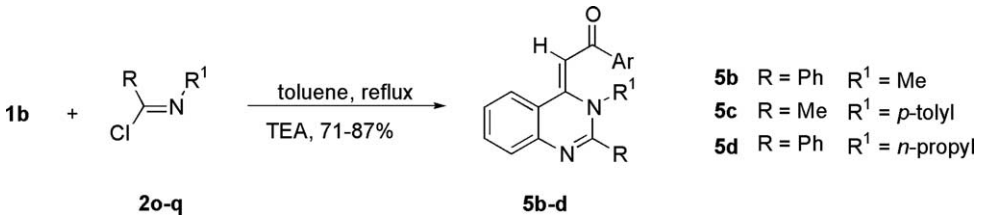
Moreover, a different behaviour was observed when ynone **1b** was reacted in the usual reaction conditions with N-alkyl or N-aryl substituted benzamidines **2j–l**, *N*-imidoylimino-triphenylphophorane **2m** and *N*-methylimidoyliminotriphenylphosphorane **2n**. Also in this case the reactions proceed in the presence of triethylamine hydrochloride giving rise to the vinylidenequinazolines **5a** and **5b** in 81–89% yield, Scheme 4. The structures of compounds **5** were assigned on the basis of analytical (C, H, N) and spectral data (NMR, MS). In particular NOESY experiments clearly demonstrated the geometry of the exocyclic double bond. Moreover, both **5a** and **5b** showed deuterium exchange for the vinylic proton.

The geometry and the structure of the isolated products could be explained by the initial formation of the intermediate **6** (Scheme 5, path a) arising from a stereoselective *trans*-addition of the amidine moiety over the triple bond followed by a transamination reaction between the amino group and the amidine function, giving rise to the formation of compounds **5**. Nevertheless, an initial transamination step, giving rise to the intermediate **7**, cannot be ruled out (Scheme 5, path b).

To shed a light on the mechanism pathway we envasiged that intermediate **7** could be derived through the reaction of **1** with iminochlorides. Indeed, the reaction of



Scheme 5.



Scheme 6.

iminochlorides **2o–q** with the ynone **1b** afforded the expected quinazolines **5b–d** (Scheme 6). Interestingly, these results clearly suggests that simple and cheap reactants such iminochlorides can be used instead of amidines giving rise to the same reaction products with an increase of the efficiency in terms of atom-economy.

Compounds **5c–e** showed the usual stereochemical arrangement for the exocyclic double bond and deuterium exchange for the vinylic proton.

In conclusion, β-(2-aminoaryl)-α,β-ynones **1** easily react with different nitrogen nucleophiles and reaction conditions and substitution pattern in the nucleophilic moiety directed the reactions towards the formation of simple nucleophilic adducts or toward a divergent cyclisation step giving rise either to the formation of 2-aryl-4-aminoquinolines or to substituted 2-aryl or 2-alkyl-4-alkylidene-quinazolines.

3. Experimental

3.1. General

All reagents and solvents are commercially available. Fluka silica gel TLC plates (2–25 μm, 60 Å, F_{254}) were employed for thin layer chromatography. Merck or Davisil silica gel 60 (40–63 μm) was employed for flash column chromatography. Melting points, measured with a Stuart Scientific SMP3 apparatus, are uncorrected. Infrared spectra were recorded on a FT-IR Perkin–Elmer Spectrum One spectrophotometer using KBr tablets for solids or NaCl dishes for liquids. Proton and ^{13}C NMR spectra were recorded at room temperature on Varian-Gemini 200 at 200 MHz. The APT or DEPT sequences were used to distinguish the methine and methyl carbon signals from those due to methylene and quaternary carbons. Two-dimensional NMR experiments (NOESY) were used, where appropriate, to aid in the assignment of signals in the proton spectra. Low-resolution mass spectra were run on a Fisons MD-800 spectrometer. α,β-Ynones **1a–b**,[4] N-substituted benzamidines **2j–l**[10] and iminotriphenylphosphoranes **2m–n**[11] were prepared according to the described methods. 'PE' refers to the fraction of petroleum ether with boiling point of 40–60 °C. 'EtOAc' means ethyl acetate and 'TEA' means triethylamine.

3.1.1. (*Z*)-3-(2-Amino-phenyl)-1-(4-chlorophenyl)-3-cyclohexylamino-propenone (3b). Prepared as described for **3a** in Ref. 2a. Eluent for chromatography: PE/EtOAc (95:5). Yield: 146.0 mg, 75%. Pale yellow oil. IR (NaCl, neat) ν=3458, 3340, 1586, 1481, 1450 cm^{-1}. ^1H NMR ($CDCl_3$): δ=1.18–1.92 (m, 10H, CH_2), 3.18–3.23 (m, 1H, CH-N), 3.93 (bs, 2H, NH_2, exchange with D_2O), 5.69 (s, 1H, C=CH), 6.80 (m, 2H, arom.), 7.09 (dd, J=7.5, 1.4 Hz, 1H, arom.), 7.24 (dt, J=7.3, 1.4 Hz, 1H, arom.), 7.35 (d, J=8.8 Hz, 2H, arom.), 7.83 (d, J=8.8 Hz, 2H, arom.), 11.47 (bd, J=9.5 Hz, 1H, exchange with D_2O) ppm. ^{13}C NMR (DMSO): δ=24.4, 24.6, 25.5, 33.2, 34.7, 52.8, 92.7, 115.8, 116.6, 119.9, 129.0, 129.3, 130.7, 136.1, 139.4, 145.5, 165.1, 186.0 ppm (one signal obscured). $C_{21}H_{23}ClN_2O$ (354.15): calcd C 74.07, H 6.53, N 7.89; found C 74.31, H 6.50, N 7.85.

3.2. Reactions of ynone 1b with primary amines 2a–d

Under a nitrogen atmosphere, a solution of **1b** (100 mg, 0.391 mmol) and the appropriate primary amine **2a–d** (0.587 mmol) in dry toluene (5 mL) was stirred under reflux for 6–16 h, until no more α,β-ynone was detectable by TLC. The solvent was then removed in vacuum, the residue taken up in EtOAc (30 mL) and washed with a solution of HCl 0.1 N (30 mL). Finally, the organic layer was dried over anhydrous sodium sulfate and freed from solvent under reduced pressure at 40 °C. The crude product was purified by flash chromatography over silica gel column.

3.2.1. Benzyl-[2-(4-chloro-phenyl)-quinolin-4-yl]-amine (4a). Reaction time: 6 h. Eluent for chromatography: PE/EtOAc (9:1). Yield: 94.4 mg, 70%. Yellow solid. Mp: 166–167 °C. IR (KBr) ν=3330, 1587, 1533, 1431 cm^{-1}. ^1H NMR ($CDCl_3$): δ=4.62 (d, J=4.7 Hz, 2H, CH_2), 5.38 (bs, 1H, NH, exchange with D_2O), 6.89 (s, 1H, H-3 quinoline), 7.29 (m, 1H, arom.), 7.39–7.49 (m, 7H, arom.), 7.64–7.87 (m, 2H, arom.), 8.00 (dt, J=8.4, 2.5 Hz, 2H, arom.), 8.10 (dd, J=7.3, 1.8 Hz, 1H, arom.) ppm. ^{13}C NMR: δ=29.9, 97.1, 118.1, 119.3, 125.0, 127.9, 128.2, 128.9, 129.0, 129.2, 129.6, 130.6, 135.2, 137.7, 139.5, 148.8, 150.3, 157.3 ppm. $C_{22}H_{17}ClN_2$ (344.84): calcd C 76.63, H 4.97, N 8.12; found C 76.38, H 4.92, N 8.18.

3.2.2. [2-(4-Chloro-phenyl)-quinolin-4-yl]-cyclohexyl-amine (4b). Reaction time: 6 h. Eluent for chromatography: PE/EtOAc (9:1). Yield: 106.7 mg, 81%. Yellow solid. Mp: 141–142 °C. IR (KBr) ν=3340, 1631, 1583, 1555, 1533 cm^{-1}. ^1H NMR ($CDCl_3$): δ=1.25–2.24 (m, 10H, CH_2), 3.63 (m, 1H, CH-N), 4.97 (m, 1H, NH, exchange with D_2O), 6.48 (s, 1H, H-3 quinoline), 7.40–7.52 (m, 3H, arom.), 7.62–7.74 (m, 2H, arom.), 8.00–8.08 (m, 3H, arom.) ppm. ^{13}C NMR: δ=25.1, 25.2, 33.0, 51.4, 96.7, 118.1, 119.3, 124.7, 128.9, 129.1, 129.5, 130.5, 135.1, 139.8, 149.0, 149.4, 157.4 ppm. $C_{21}H_{21}ClN_2$ (336.86): calcd C 74.88, H 6.28, N 8.32; found C 74.59, H 6.31, N 8.35.

3.2.3. Butyl-[2-(4-chloro-phenyl)-quinolin-4-yl]-amine (4c). Reaction time: 6 h. Eluent for chromatography: PE/EtOAc (95:5). Yield: 82.6 mg, 68%. Yellow solid. Mp:

129–130 °C. IR (KBr) ν=3449, 1588, 1555, 1535 cm^{-1}. ^1H NMR ($CDCl_3$): δ=1.06 (t, J=7.3 Hz, 3H, CH_3), 1.67 (m, 2H, CH_2), 1.83 (m, 2H, CH_2), 3.40 (m, 2H, CH_2), 5.00 (m, 1H, NH, exchange with D_2O), 6.83 (s, 1H, H-3 quinoline), 7.46–7.50 (m, 3H, arom.), 7.67–7.75 (m, 2H, arom.), 8.04–8.08 (m, 3H, arom.) ppm. ^{13}C NMR: δ=14.1, 20.6, 31.3, 43.3, 96.5, 118.1, 119.3, 124.8, 128.9, 129.1, 129.6, 130.3, 135.2, 139.5, 148.7, 150.7, 157.2 ppm. $C_{19}H_{19}ClN_2$ (310.82): calcd C 73.42, H 6.16, N 9.01; found C 73.58, H 6.08, N 8.96.

3.2.4. [2-(4-Chloro-phenyl)-quinolin-4-yl]-*p*-tolyl-amine (4d). Reaction time: 16 h. Eluent for chromatography: PE/EtOAc (95:5). Yield: 101.1 mg, 75%. Yellow solid. Mp: 182–183 °C. IR (KBr) ν=3412, 1617, 1584, 1551, 1513 cm^{-1}. ^1H NMR ($CDCl_3$): δ=2.43 (s, 3H, CH_3), 6.63 (bs, 1H, NH, exchange with D_2O), 7.28 (m, 4H, arom.), 7.36 (s, 1H, H-3 quinoline), 7.42–7.56 (m, 2H, arom.), 7.52 (dt, J=1.5, 6.9 Hz, 1H, arom.), 7.72 (dt, J=1.5, 6.9 Hz, 1H, arom.), 7.91–7.99 (m, 3H, arom.), 8.12 (d, J=8.4 Hz, 1H, arom.) ppm. ^{13}C NMR: δ=21.2, 99.5, 118.8, 119.6, 123.4, 125.4, 128.9, 129.0, 129.9, 130.6, 135.0, 135.3, 137.3, 139.1, 148.9, 149.4, 157.1 ppm (one signal obscured). $C_{22}H_{17}ClN_2$ (344.84): calcd C 76.63, H 4.97, N 8.12; found C 76.65, H 4.99, N 8.10.

3.3. Reactions of ynone 1b with N,N-unsubstituted amidines 2e–f

Under a nitrogen atmosphere, an equimolar solution of **1b** (100 mg, 0.391 mmol) and the appropriate amidine (free base) **2e–f** (0.391 mmol) in dry toluene (5 mL) was stirred under reflux for 3 h, until no more α,β-ynone was detectable by TLC. The solvent was then removed in vacuum, the residue taken up in EtOAc (30 mL) and washed with a saturated solution of $NaHCO_3$ (30 mL). Finally, the organic layer was dried over anhydrous sodium sulfate and freed from solvent under reduced pressure at 40 °C. The crude product was purified by flash chromatography over silica gel column.

3.3.1. *N*-[2-(4-Chloro-phenyl)-quinolin-4-yl]-benzamidine (4e). Eluent for chromatography: PE/EtOAc (98:2). Yield: 118.9 mg, 85%. Yellow solid. Mp: 151–152 °C. IR (KBr) ν=3438, 3271, 1613, 1587, 1564, 1521, 1491 cm^{-1}. ^1H NMR ($CDCl_3$): δ=6.20 (bs, 2H, NH_2, exchange with D_2O), 6.86 (t, J=7.1 Hz, 1H, arom.), 7.23–7.36 (m, 2H, arom.), 7.49–7.61 (m, 5H, arom.), 7.76 (d, J=7.3 Hz, 1H, arom.), 7.93 (s, 1H, H-3 quinoline), 8.25 (d, J=8.4 Hz, 2H, arom.), 8.54–8.58 (m, 2H, arom.) ppm. ^{13}C NMR: δ=111.3, 117.6, 117.7, 119.4, 128.4, 128.6, 128.7, 129.2, 129.5, 130.8, 131.9, 136.1, 137.0, 138.1, 148.0, 163.2, 163.5, 167.3 ppm. $C_{22}H_{16}ClN_3$ (357.84): calcd C 73.84, H 4.51, N 11.74; found C 75.01, H 4.55, N 11.67.

3.3.2. *N*-[2-(4-Chloro-phenyl)-quinolin-4-yl]-acetamidine (4f). Eluent for chromatography: PE/EtOAc (95:5). Yield: 104.1 mg, 90%. Yellowish solid. Mp: 134–136 °C. IR (KBr) ν=3439, 3270, 1614, 1582, 1526, 1488 cm^{-1}. ^1H NMR ($CDCl_3$): δ=2.84 (s, 3H, CH_3), 6.10 (bs, 2H, NH_2, exchange with D_2O), 6.82 (m, 2H, arom.), 7.27 (dt, J=7.5, 1.4 Hz 1H, arom.), 7.52 (d, J=8.5 Hz 2H, arom.), 7.72 (d, J=8.1 Hz, 1H, arom.), 7.84 (s, 1H, H-3 quinoline), 8.09 (d, J=8.5 Hz, 2H, arom.) ppm. ^{13}C NMR: δ=26.6, 110.6, 117.7, 117.9, 119.0, 128.8, 129.3, 129.5, 132.0, 136.3, 137.0, 148.3, 163.4, 167.2 ppm (one signal obscured). $C_{17}H_{14}ClN_3$ (295.77): calcd C 69.03, H 4.77, N 14.21; found C 68.94, H 4.81, N 14.28.

3.4. Reactions of ynone 1b with secondary amines 2g–h

Under a nitrogen atmosphere, a solution of **1b** (100 mg, 0.391 mmol), TEA hydrochloride (0.586 mmol) and the appropriate secondary amine **2g–h** (0.586 mmol) in dry toluene (5 mL) was stirred under reflux for 4–16 h, until no more α,β-ynone was detectable by TLC. The solvent was then removed in vacuum, the residue taken up in EtOAc (30 mL) and washed with a saturated solution of $NaHCO_3$ (30 mL). Finally, the organic layer was dried over anhydrous sodium sulfate and freed from solvent under reduced pressure at 40 °C. The crude product was purified by flash chromatography over silica gel column or by crystallisation.

3.4.1. [2-(4-Chloro-phenyl)-quinolin-4-yl]-diethyl-amine (4g). Reaction time: 16 h. Eluent for chromatography: PE/EtOAc (9:1). Yield: 110.6 mg, 91%. Yellow solid. Mp: 84–85 °C. IR (KBr) ν=1578, 1547, 1493 cm^{-1}. ^1H NMR ($CDCl_3$): δ=1.22 (t, J=6.9 Hz, 6H, CH_3), 3.43 (q, J=6.9 Hz, 4H, CH_2), 7.26 (s, 1H, H-3 quinoline), 7.46–7.52 (m, 3H, arom.), 7.62–7.68 (m, 1H, arom.), 8.05–8.10 (m, 4H, arom.) ppm. ^{13}C NMR: δ=12.5, 46.7, 108.3, 123.9, 124.3, 125.0, 129.1, 129.2, 129.4, 130.4, 135.3, 139.3, 150.3, 156.4, 156.8 ppm. $C_{19}H_{19}ClN_2$ (310.82): calcd C 73.42, H 6.16, N 9.01; found C 73.40, H 6.17, N 9.01.

3.4.2. 2-(4-Chloro-phenyl)-4-pyrrolidin-1-yl-quinoline (4h). Reaction time: 4 h. Crystallised from isopropyl ether. Yield: 117.1 mg, 97%. Yellow solid. Mp: 136–137 °C. IR (KBr) ν=1571, 1530, 1513, 1490 cm^{-1}. ^1H NMR ($CDCl_3$): δ=2.09 (m, 4H, CH_2), 3.78 (m, 4H, CH_2), 6.88 (s, 1H, H-3 quinoline), 7.34 (dt, J=6.9, 1.1 Hz, 1H, arom.), 7.45–7.50 (m, 2H, arom.), 7.62 (dt, J=6.1, 1.1 Hz, 1H, arom.), 8.05–8.10 (m, 4H, arom.) ppm. ^{13}C NMR: δ=26.2, 54.4, 100.5, 120.6, 120.7, 123.3, 125.2, 128.9, 129.1, 130.1, 135.1, 139.6, 150.5, 153.7, 156.3 ppm. $C_{19}H_{17}ClN_2$ (308.80): calcd C 73.90, H 5.55, N 9.07; found C 73.68, H 5.58, N 9.11.

3.4.3. 1-[2-(4-Chloro-phenyl)-quinolin-4-yl]-pyrrolidine-2-carboxylic acid methyl ester (4i). Under a nitrogen atmosphere, a solution of **1b** (100 mg, 0.391 mmol), TEA (0.081 mL, 0.586 mmol) and pyrrolidine-2-carboxylic acid methyl ester hydrochloride **2i** (96 mg, 0.586 mmol) in dry toluene (5 mL) was stirred under reflux for 4 h. The solvent was then removed in vacuum, the residue taken up in EtOAc (30 mL) and washed with a saturated solution of $NaHCO_3$ (30 mL). Finally, the organic layer was dried over anhydrous sodium sulfate and freed from solvent under reduced pressure at 40 °C. The crude product was purified by flash chromatography over silica gel column. Eluent for chromatography: PE/EtOAc (8:2). Yield: 100.4 mg, 70%. Yellow solid. Mp: 151–153 °C. IR (KBr) ν=1747, 1530, 1575, 1514, 1542 cm^{-1}. ^1H NMR ($CDCl_3$): δ=2.17–2.28 (m, 3H, CH_2), 2.47–2.59 (m, 1H, CH_2), 3.69 (s, 3H, CH_3), 3.79–3.88 (m, 1H, CH_2-N), 4.15–4.27 (m, 1H, CH_2-N), 4.78

(t, J=6.9 Hz, 1H, CH), 6.95 (s, 1H, H-3 quinoline), 7.35–7.49 (m, 3H, arom.), 7.60–7.68 (m, 1H, arom.), 7.99–8.05 (m, 3H, arom.), 8.18 (dt, J=8.4, 1.1 Hz, 1H, arom.) ppm. ^{13}C NMR: δ=25.5, 31.2, 52.6, 54.3. 63.4, 101.9, 120.8, 120.9, 124.1, 124.7, 129.0, 129.3, 130.4, 135.5, 139.3, 150.5, 153.0, 156.3, 173.7 ppm. $C_{21}H_{19}ClN_2O_2$ (366.84): calcd C 68.76, H 5.22, N 7.64; found C 68.58, H 5.28, N 7.61.

3.5. Reactions of ynone 1b with N-substituted benzamidines 2j–l

Under a nitrogen atmosphere, an equimolar solution of **1b** (100 mg, 0.391 mmol), TEA hydrochloride (0.391 mmol) and the appropriate N-substituted benzamidine (free base) **2j–l** (0.391 mmol) in dry toluene (5 mL) was stirred under reflux for 16 h. The solvent was then removed in vacuum, the residue taken up in EtOAc (30 mL) and washed with a saturated solution of $NaHCO_3$ (30 mL). Finally, the organic layer was dried over anhydrous sodium sulfate and freed from solvent under reduced pressure at 40 °C. The crude product was purified by flash chromatography over silica gel column.

3.6. Reactions of ynone 1b with iminotriphenyl-phosphoranes 2m–n

Under a nitrogen atmosphere, an equimolar solution of **1b** (100 mg, 0.391 mmol), TEA hydrochloride (0.391 mmol) and the appropriate iminotriphenylphosphorane **2m–n** (0.391 mmol) in dry toluene (5 mL) was stirred under reflux for 3 h. The solvent was removed in vacuum, the residue taken up in EtOAc (30 mL) and washed with a saturated solution of $NaHCO_3$ (30 mL). Finally, the organic layer was dried over anhydrous sodium sulfate and freed from solvent under reduced pressure at 40 °C. The crude product was purified by flash chromatography over silica gel column.

3.6.1. 1-(4-Chloro-phenyl)-2-(2-phenyl-3*H*-quinazolin-4-ylidene)-ethanone (5a). Eluent for chromatography: PE/EtOAc (97:3). Yield: 113.6–124.86 mg, 81–89%. Yellow solid. Mp: 188–189 °C. IR (KBr) ν=3434, 1607, 1555, 1478, 1466 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ=6.80 (s, 1H, CH), 7.40–8.20 (bs, 1H, NH, exchange with D_2O), 7.48 (d, J=8.8 Hz, 2H, arom.), 7.56 (d, J=8.1 Hz, 1H, arom.), 7.61–7.64 (m, 3H, arom.), 7.79–7.87 (m, 2H, arom.), 7.95 (d, J=8.8 Hz, 2H, arom.), 8.06 (d, J=8.1 Hz, 1H, arom.), 8.34–8.38 (m, 2H, arom.) ppm. ^{13}C NMR: δ=85.7, 118.7, 123.5, 127.0, 127.1, 128.6, 128.8, 128.9, 129.2, 131.8, 132.7, 134.0, 137.6, 138.2, 147.2, 150.1, 153.7, 187.5 ppm. EI-MS m/z (%): 360 [M$^+$+2] (46), 358 [M$^+$] (100), 329 (27), 247 (57), 147 (30), 139 (47). $C_{22}H_{15}ClN_2O$ (358.82): calcd C 73.64, H 4.21, N 7.81; found C 73.78, H 4.25, N 7.86.

3.6.2. 1-(4-Chloro-phenyl)-2-(3-methyl-2-phenyl-3*H*-quinazolin-4-ylidene)-ethanone (5b). Eluent for chromatography: PE/EtOAc (9:1). Yield: 120.8 mg, 83%. Dark yellow solid. Mp: 195–197 °C. IR (KBr) ν=1614, 1588, 1515, 1445, cm^{-1}. ^{1}H NMR ($CDCl_3$): δ=3.49 (s, 3H, CH_3), 6.93 (s, 1H, CH), 7.41–7.57 (m, 6H, arom.), 7.62–7.74 (m, 2H, arom.), 7.87–7.90 (m, 2H, arom.), 7.98–8.09 (m, 3H, arom.) ppm. ^{13}C NMR: δ=47.1, 93.4, 122.7, 122.9, 127.6, 128.1, 128.8, 129.1, 129.2, 129.5, 131.0, 132.7, 135.7, 137.7, 139.6, 143.9, 154.0, 157.0, 184.7 ppm. EI-MS m/z (%): 374 [M$^+$+2] (4), 372 [M$^+$] (12), 139 (100), 111 (44), 77 (34). $C_{23}H_{17}ClN_2O$ (372.10): calcd C 74.09, H 4.60, N 7.51; found C 73.95, H 4.59, N 7.56.

3.7. Reactions of ynone 1b with iminochlorides 2o–q

Under a nitrogen atmosphere, an equimolar solution of **1b** (100 mg, 0.391 mmol), TEA (0.469 mmol) and the appropriate iminochloride **2o–q** (0.469 mmol) in dry toluene (5 mL) was stirred under reflux for 4–16 h. The solvent was removed in vacuum, the residue taken up in EtOAc (30 mL) and washed with a solution of HCl 0.1 N (30 mL). Finally, the organic layer was dried over anhydrous sodium sulfate and freed from solvent under reduced pressure at 40 °C. The crude product was purified by flash chromatography over silica gel column.

3.7.1. 1-(4-Chloro-phenyl)-2-(3-methyl-2-phenyl-3*H*-quinazolin-4-ylidene)-ethanone (5b). Reaction time: 16 h. Eluent for chromatography: PE/EtOAc (95:5). Yield: 103.3 mg, 71%. Orange solid. Mp: 192–194 °C. For spectroscopic detail see above.

3.7.2. 1-(4-Chloro-phenyl)-2-(2-methyl-3-*p*-tolyl-3*H*-quinazolin-4-ylidene)-ethanone (5c). Reaction time: 4 h. Eluent for chromatography: PE/EtOAc (9:1). Yield: 113.4 mg, 75%. Orange solid. Mp: 131 °C. IR (KBr) ν=1632, 1528 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ=2.14 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 5.45 (s, 1H, CH), 7.19 (d, J=8.1 Hz, 2H, arom.), 7.30 (d, J=8.1 Hz, 2H, arom.), 7.36–7.65 (m, 7H, arom.), 8.62 (d, J=8.1 Hz 1H, arom.) ppm. ^{13}C NMR: δ=21.6, 25.3, 98.6, 119.6, 125.7, 126.6, 128.0, 128.6, 129.4, 130.3, 131.9, 133.9, 137.6, 140.0, 140.2, 144.9, 152.9, 154.4, 187.1 ppm (one signal obscured). EI-MS m/z (%): 388 [M$^+$+2] (18), 386 [M$^+$] (37), 250 (56), 139 (100). $C_{24}H_{19}ClN_2O$ (386.87): calcd C 74.51, H 4.95, N 7.24; found C 74.59, H 4.95, N 7.27.

3.7.3. 1-(4-Chloro-phenyl)-2-(2-phenyl-3-propyl-3*H*-quinazolin-4-ylidene)-ethanone (5d). Reaction time: 4 h. Eluent for chromatography: PE/EtOAc (95:5). Yield: 136.4 mg, 87%. Yellow solid. Mp: 169–171 °C. IR (KBr) ν=1589, 1526 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ=0.53 (t, J=7.3 Hz, 3H, CH_3), 1.34 (m, 2H, CH_2), 4.15 (t, J=7.0 Hz, 2H, CH_2), 7.00 (s, 1H, CH), 7.40–7.98 (m, 8H, arom.), 8.03 (m, 5H, arom.) ppm. ^{13}C NMR: δ=11.2, 22.3, 59.5, 96.3, 122.7, 123.5, 127.4, 127.7, 129.0, 129.1, 129.3, 129.4, 131.0, 132.6, 135.9, 137.8, 139.7, 143.9, 152.9, 159.0, 184.3 ppm. EI-MS m/z (%): 402 [M$^+$+2] (11), 400 [M$^+$] (26), 330 (34), 139 (100). $C_{25}H_{21}ClN_2O$ (400.90): calcd C 74.90, H 5.28, N 6.99; found C 74.81, H 5.25, N 7.02.

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A substrate controlled, very highly diastereoselective Morita–Baylis–Hillman reaction: a remote activation of the diastereofacial selectivity in the synthesis of C-3-branched deoxysugars☆

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Abstract—The Morita–Baylis–Hillman (MBH) reaction of *p*-nitrobenzaldehyde with C (6) acyl protected enuloside **1** in the presence of $TiCl_4$/TBAI yielded highly diastereoenriched C-3-branched deoxysugar derivative or MBH adduct **1′a** in high yield, while reactions of unprotected enuloside **2a** and C (6) alkyl protected enulosides **2d–e** with *p*-nitrobenzaldehyde under the same conditions afforded the adducts **2′a** and **2′d–e**, respectively, in low yield with moderate selectivity. Several representative aromatic and aliphatic aldehydes were selected to undergo MBH reaction with **1** to give their respective adducts in very good yield with a very high diastereoselectivity. A plausible mechanism based on the assumption of a Zimmerman–Traxler-type transition state was proposed to explain the excellent selectivity observed with adducts derived from **1**. The synthetic application of these adducts were shown by their stereoselective reduction to corresponding *threo* isomers in very good yield.

1. Introduction

Deoxysugars with a branched carbon skeleton occur abundantly in nature and have been found in many antibiotics. The appendage at the branching carbon can be various side chains besides methyl, formyl, hydroxymethyl, 1-hydroxyethyl, acetyl, 2-hydroxyacetyl, 1,3-dimethylpropyl and other side chains.[1,2] There are number of antibiotics that contain C-branched sugars as the glycosidic components[3] and they have been characterized from various natural products.[4] In synthetic organic chemistry C-branched sugars have been identified as useful chiral synthons for the total synthesis of natural products.[2b,3a] Their use has also been explored in the synthesis of non-carbohydrate compounds[5] and carbohydrate mimetics.[3a,c] Some of the most useful methods for the synthesis of C-branched sugars involving the formation of new C–C bond at the branching point take the advantage of the reactivity of carbonyl group of uloses.[6–8] Thus, the literature reports on C-branched sugars for their synthetic importance[6–9] as well as biological significance[1,10] prompted us to develop a convenient strategy for the synthesis of C-3-branched deoxysugars in preparative scale.

The branching of a carbon skeleton by construction of C–C bonds is one of the most challenging tasks in the field of synthetic organic chemistry.[11–13] During the last few years Morita–Baylis–Hillman (MBH) chemistry[14,15] has been recognized as one of the most versatile and more economically feasible C–C bond forming reactions to generate multifunctionalized allylic alcohols generally called MBH or BH adducts. These find various applications as chiral building blocks in organic synthesis.[16] Highly diastereoselective BH reactions have been extensively studied during the past few years.[17] The syntheses of biologically active important molecules involving asymmetric MBH reactions have also been reported.[18] However, the application of the asymmetric MBH reaction in carbohydrate chemistry is very limited.[19,20] Herein, we wish to describe an efficient strategy for an almost completely diastereoselective synthesis of C-3 branched deoxysugars involving the protocols of the asymmetric MBH reaction. To our knowledge, this present study represents an unprecedented substrate controlled/directed, highly diastereoselective MBH reaction, catalyzed by $TiCl_4$/TBAI in carbohydrate chemistry. The various aromatic and aliphatic aldehydes as electrophiles, and sugar-derived chiral-activated alkenes as one of the chiral sources were selected for the present study.

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Keywords: Diastereoselective; Morita–Baylis–Hillman reaction; 2,3-Dideoxy sugar derivatives.

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2. Results and discussion

The required alkene **1** was prepared from the readily 3,4,6,tri-*O*-acetyl-D-glucal by modification of literature methods.[21] First, we attempted this reaction in CH_2Cl_2 with three-components, *p*-nitrobenzaldehyde (*p*nb), sugar-enone **1** and $TiCl_4$[22] at 0 °C. The adduct was obtained in a very low yield. After several trial experiments using various combinations of substrate and $TiCl_4$ at different temperatures, it was found that stirring a mixture of 2 equiv aldehyde and 1 equiv alkene with 1.5 equiv $TiCl_4$ in CH_2Cl_2 at −78 °C yielded the product (45%) with moderate diastereoselectivity after 10 h, beyond that no appreciable change was noticed in the course of the reaction. However, the yield of the product was increased by 6% when an additive (TBAI or Me_2S) was added. Several research groups have reported the $TiCl_4$ mediated MBH reaction in the presence of additives like TBAI[23,24] or Me_2S.[25–27] Recently, we reported that the $TiCl_4/Me_2S$ mediated BH reaction can be successfully conducted on carbohydrate enals with methyl vinyl ketone, extending the chain of acyclic deoxysugars, where the unsaturation in the enals did not interfere with the electrophilicity of the aldehydic carbon.[28,29] After exploring the combination of $TiCl_4$/TBAI or $TiCl_4/Me_2S$ in different ratios, it was found that 20 mol% of additive with 1.5 equiv $TiCl_4$ turned out to be an effective combination at −78 °C. The loading of 20 mol% of either of the two additives resulted the desired adduct from *p*nb in almost same yield (51%) after 10 h. However, when the temperature of the reaction was gradually increased to −30 °C (−78 °C→−30 °C), remarkable change was observed in the yield of the chromatographically pure adducts (85% with TBAI and 62% with Me_2S). Only one isomer was isolated from each combination. The ^1H NMR and ^{13}C NMR spectra of the crude reaction mixture surprisingly showed a very high degree of diastereoselectivity (>99%). Encouraged by these results, we opted for a representative selection of aromatic and aliphatic aldehydes (Table 1). The reactions were performed at −78 °C→−30 °C. The efficacy of the reactivity of the reagents $TiCl_4$/TBAI and $TiCl_4/Me_2S$ with almost all the aldehydes used in this study showed that $TiCl_4$/TBAI was the superior (Scheme 1).

Table 1. $TiCl_4$/TBAI mediated MBH reaction of enuloside **1** with aldehydes at −78 to −30 °C

Entry	RCHO	Time (h)	Product	dr[a]	Yield (%)[b]
1	*p*-NO_2PhCHO	8	**1′a**	>99/1	85
2	*p*-FPhCHO	48	**1′b**	>99/1	68
3	$CH_3(CH_2)_2CHO$	48	**1′c**	>99/1	82
4	$CH_3(CH_2)_8CHO$	30	**1′d**	>99/1	81
5	*m*-NO_2PhCHO	10	**1′e**	>99/1	64
6	*o*-NO_2PhCHO	6	**1′f**	>99/1	80
7	*p*-CF_3PhCHO	10	**1′g**	>99/1	86
8	PhCHO	92	**1′h**	>99/1	26

[a] Determined by ^1H NMR and ^{13}C NMR of the crude material.
[b] Isolated yield of pure isomer after column chromatography on silica gel.

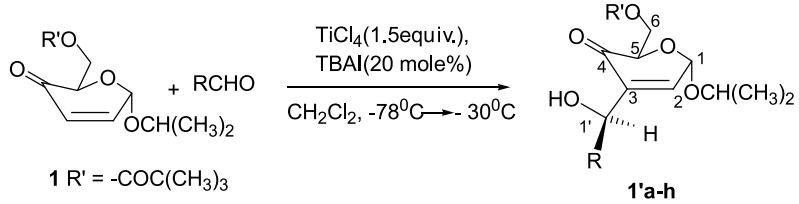
Therefore, herein we wish to highlight our synthetic strategy developed for a very high diastereoselective synthesis of C-3-branched deoxysugar derivatives by taking the advantage of MBH chemistry performed in the presence of $TiCl_4$/TBAI at −78 °C→−30 °C and the plausible mechanism for this reaction. Both aromatic as well as aliphatic aldehydes formed the products in good to very good yield (Table 1) with almost complete diastereoselectivity. As expected the benzaldehyde derived adduct was obtained in low yield (Table 1, entry 8) in 92 h. The MBH reaction of **1** with less reactive aldehyde like *p*-methoxybenzaldehyde did not work under this condition. Once the reaction conditions had been established, its generality with unprotected enuloside **2a** was examined. The free 6-OH in the adduct could be replaced by an amino group, an H atom or a carbon appendage by adopting standard synthetic protocols. This could form novel 2,3,6-trideoxy branched sugar derivatives which exist in important antibiotics.[30] Therefore, when a mixture of enuloside **2a**, *p*nb and $TiCl_4$/TBAI was stirred at −78 °C→−30 °C for 33 h, the expected product formed was in 40% (Table 2, entry 1) with moderate diastereoselectivity.

Table 2. $TiCl_4$/TBAI mediated MBH reaction of enuloside **2a–e**

Entry	R′	Time (h)	Product	dr[a]	Yield (%)[b]
1	H	33	**2′a**	82/18	40
2	CH_3CO	5	**2′b**	>99/1	64
3	*p*-NBz[c]	6	**2′c**	>99/1	88
4	TBDPS	10	**2′d**	83/17	28
5	TBDMS	10	**2′e**	66/34	44

[a] Determined by ^1H NMR and ^{13}C NMR of the crude material.
[b] Isolated yield of pure isomer after column chromatography on silica gel.
[c] *p*-NBz=*p*-nitrobenzoyl group.

The difference in the reactivity of the two enulosides **1** and **2a** with *p*-nitrobenzaldehyde in our MBH reaction can be argued on the basis of the mechanistic aspects of the reaction, as well as the geometry of the proposed transition states (Scheme 4). Both the active alkenes follow the same multiple mechanistic pathways taking place successively. It can be presumed that the reaction starts with nucleophilic attack of iodide from the TBAI to enuloside (Michael acceptor), with concomitant shifting of the 2,3-double bond to 3,4. This generates the oxyanion from the C-4 carbonyl which in turn attacks the carbonyl carbon of the titanium-coordinated pivaloyl group. By virtue of this conjugative



1 R' = -COC(CH3)3

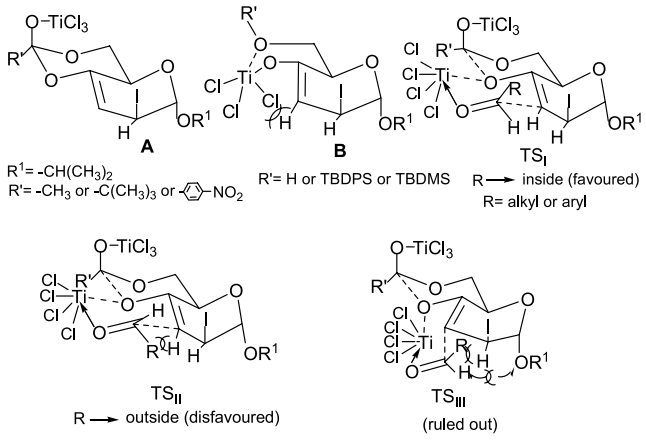
1'a-h

Scheme 1.

effect the formation of an orthoester intermediate[31] 'A' could therefore be predicted. This resembles a *trans* decalin (pseudo) conformation that can activate the site of reaction (C-3) of the enolate. The conjugate addition of iodide to the unprotected enuloside **2a** in the presence of the lewis acid could generate an unfavorable intermediate 'B', where conformation of one of the rings is in a twist-boat form that brings steric crowding near the site of the reaction. This mechanism can justify the low yield of adduct and its moderate selectivity from enuloside **2a**. The reaction of **2a** with *p*nb in presence of $TiCl_4/Me_2S$ did not form any adduct.

In order to justify the above mechanistic argument, the MBH reactions between other acyl (acetate and benzoyl, **2b–c**) and alkyl protected *tert*-butyldiphenyllsilyl and *tert*-butyldimethylsilyl (TBDPS and TBDMS, **2d–e**) enulosides, and *p*nb in the presence of $TiCl_4$/TBAI at $-78\,^{\circ}C \rightarrow -30\,^{\circ}C$ were carried out. The acetyl and *p*-nitrobenzoyl protected enulosides furnished the respective adducts in good to very good yield with same selectivity. The alkyl protected enulosides (**2d–e**) formed their respective adducts in low to moderate yield with moderate selectivity (Scheme 2) (Table 2).

A very high diastereoselective MBH chemistry described herein may be explained on the basis of the assumption that Zimmermann–Traxler-type transition-state[32] TS-I, involving A and the titanium coordinated aldehyde, was formed. Among the other possible chelated transition-states TS-II or TS-III, the latter can be completely ruled out by considering severe steric repulsion arising due to 1,3-diaxial type interactions between the α-faced *O*-isopropyl group at C-1, and the approaching aldehyde from the same face during the reaction as depicted in Scheme 4. The transition-state TS-II is also unfavorable due to steric repulsion between the R-group of the approaching aldehyde, and the hydrogen at C-3 of the enuloside, therefore, giving preference to TS-I over TS-II. Thus, consideration of the



Scheme 4.

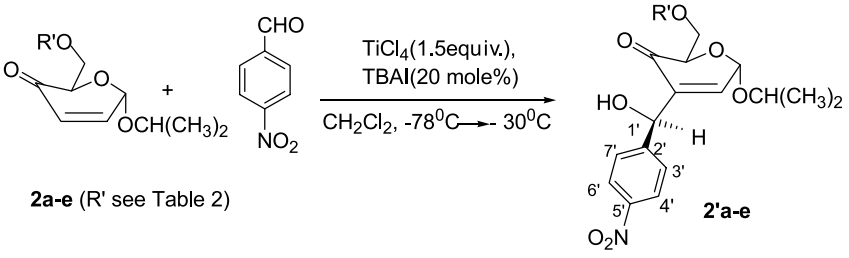
stereochemical model TS-I revealed that one of the enantiotopic faces of the aldehyde, playing a key role in the diastereoface selectivity, was well disposed to C-3 of the enuloside, resulting in its intermolecular diastereoselective *re*-face attack at the electrophilic carbonyl carbon of the approaching aldehyde.

In order to show the synthetic utility of these adducts, the C-4 keto groups of **1′a**, **1′d** and **1′f** were subjected to stereoselective reduction with $NaBH_4$ in the presence of $CeCl_3 \cdot 7H_2O$ (Scheme 3) to obtain their *threo* derivatives **3′a**, **3′d** and **3′f** in 78, 68 and 74%, respectively (Table 3).

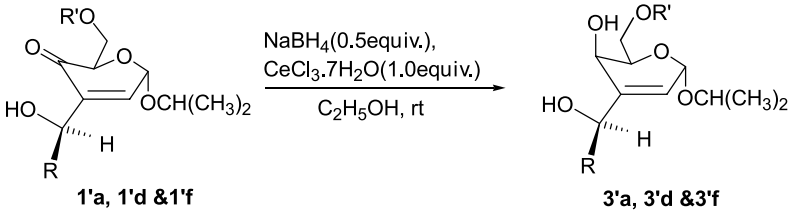
Table 3. Diastereoselective reduction of Baylis–Hillman adducts (**1′a**, **1′d** and **1′f**) with $NaBH_4$ and $CeCl_3 \cdot 7H_2O$

Entry	B–H adducts	Time (h)	Product	dr[a]	Yield (%)[b]
1	**1′a**	2	**3′a**	>99/1	78
2	**1′d**	5	**3′d**	>99/1	68
3	**1′f**	5	**3′f**	>99/1	74

[a] Determined by ^1H NMR and ^{13}C NMR of the crude material.
[b] Isolated yield of pure isomer after column chromatography on silica gel.



Scheme 2.



Scheme 3.

3. Conclusion

In summary, we have developed and disclosed herein a new and convenient strategy for the almost complete diastereoselective synthesis of new multifunctionalized unsaturated C-3-alkylated 2,3-dideoxy sugars[1] or derivatives of pyran-3-ones in a good to very good yield. This involves $TiCl_4$/TBAI-mediated asymmetric Morita–Baylis–Hillman reaction performed at $-78\,^{\circ}C \rightarrow -30\,^{\circ}C$ involving a readily available sugar-derived enuloside and various aldehydes.[33] On the basis of the experimental results, it was postulated that a plausible chelated transition-state TS-I played a key role in the formation of a very high diastereoenriched MBH adduct involving multiple mechanistic pathways. The energetically favored orthoester intermediate A governed the remote activation[34] of reaction site C-3 of the enuloside, resulting the most favorable approach for the incoming titanium-coordinated aldehyde to be from *re*-face, making the whole reaction a substrate controlled diastereoselective MBH reaction.[35] The low yield of adducts **2a′**, **2d′**, **2e′** from enulosides **2a**, **2d**, **2e** is attributed to an energetically unfavored twist-boat intermediate B. The resulting unprecedented C-3 branched deoxy sugar derivatives (or pyran-3-ones) may be as useful as other versatile key intermediates like Corey's lactone, the Wieland–Miescher ketone, and the Prelog–Djerassi lactone which will find numerous applications as chiral building blocks for the construction of medicinally important molecules. These may exhibit activities[9,36] such as anti-microbial anti-viral, anti-fungal, anti-coccidial, anti-inflammatory, anti-cancer, etc. through skeleton rearrangement and functional group transformation/manipulation or structural diversification (Fig. 1). Our efforts to make this synthetic strategy more versatile to obtain novel highly functionalized MBH adducts as chiral building blocks from other active sugar-enones are underway.

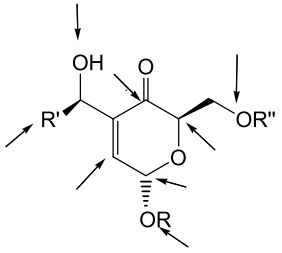


Figure 1. Arrow shows the site for diversification.

4. Experimental

4.1. General

All the reactions were monitored by warming the $CeSO_4$ (1% in 1 M H_2SO_4) sprayed precoated silica gel TLC plates at 100 °C. NMR spectra were recorded on Bruker Avance DPX 200 FT, Bruker Robotics and Bruker DRX 300 Spectrometers at 200, 300 MHz (^1H) and 50, 75 MHz (^{13}C). For ^{13}C NMR reference $CDCl_3$ appeared at 77.4 ppm, unless otherwise stated. Mass spectra were recorded on a JEOL SX 102/DA 6000 mass spectrometer using argon/xenon (6 kV, 100 mA) as the FAB gas. Organic solvents were dried by standard methods. Aldehydes were purchased from Aldrich and Fluka chemical co. Enones **1** and **2a–e** were synthesized in the lab. IR spectra were recorded on Perkin–Elmer 881 and FTIR-8210 PC Shimadzu Spectrophotometers. Optical rotations were determined on an Autopol III polarimeter using a 1 dm cell at 28 °C in methanol or chloroform as the solvent; concentrations mentioned are in g/100 mL. Elemental analyses were carried out on Carlo-Erba-1108 and Vario EL III instruments.

4.2. Typical reaction procedure for the preparation of MBH adducts

To a stirred solution of tetrabutylammonium iodide, TBAI, (0.2 mmol) in dry CH_2Cl_2 (5 mL) at $-78\,^{\circ}C$ was added $TiCl_4$ (1.5 mmol) dropwise. After stirring for two minutes, a mixture containing, **1** (1 mmol) and *p*-nitrobenzaldehyde (2 mmol) in dry CH_2Cl_2 (5 mL) was added. The reaction mixture was slowly warmed to $-30\,^{\circ}C$ and stirred for the specified time depending on the enulosides and aldehydes used. A saturated aqueous solution of sodium bicarbonate was added, followed by filtration through a celite pad. The organic layer from the filtrate was separated, and the aqueous layer was again extracted with ethyl acetate. The organic layers were then combined, washed with brine solution, and dried over sodium sulfate. The crude product obtained after evaporation of the solvent was chromatographed to yield pure compounds.

4.2.1. Isopropyl-6-*O*-trimethylacetyl-3-[hydroxy (4-nitrophenyl) methyl]-2,3-dideoxy-α-D-*glycero*-hex-2-enopyranoside-4-ulose (1′a). Oil. Eluent for column chromatography (20% ethyl acetate/hexane); [Found: C, 59.32; H, 6.72; N, 3.09. $C_{21}H_{27}NO_8$ requires C, 59.85; H, 6.46; N, 3.32%]; R_f (25% ethyl acetate/hexane) 0.39; $[\alpha]_D = -1.33$ (*c* 0.30, CH_3OH); ν_{max} (neat) 3483, 1724, 1684 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.22 (2H, d, $J_{4',3' \text{ or } 6',7'} = 8.7$ Hz, H-4′ and H-6′), 7.57 (2H, d, $J_{3',4' \text{ or } 7',6'} = 8.7$ Hz, H-3′ and H-7′), 6.60 (1H, d, $J_{2,1} = 3.6$ Hz, H-2), 5.68 (1H, d, $J_{1',OH} = 4.2$ Hz, H-1′), 5.40 (1H, d, $J_{1,2} = 3.6$ Hz, H-1), 4.67 (1H, dd, $J_{5,6a} = 2.4$ Hz, $J_{5,6b} = 5.6$ Hz, H-5), 4.52 (1H, dd, $J_{6a,5} = 2.4$ Hz, $J_{6a,6b} = 11.9$ Hz, H-6a), 4.40 (1H, dd, $J_{6b,5} = 5.7$ Hz, $J_{6b,6a} = 12.0$ Hz, H-6b), 4.05 (1H, sept., $J = 6.3$ Hz, –OC*H* $(CH_3)_2$), 3.13 (1H, d, $J_{OH,1'} = 4.8$ Hz, –O*H*), 1.24 (3H, d, $J = 6.0$ Hz, $-OCH(CH_3)_2$), 1.19 (3H, d, $J = 6.0$ Hz, $-OCH(CH_3)_2$),1.14 (9H, s, $-OCOC(CH_3)_3$); δ_C (50 MHz, $CDCl_3$) 194.9 (C-4), 178.5 ($-OCOC(CH_3)_3$), 148.0 (C-3 and C-5′), 141.2 (C-2), 138.9 (C-2′), 128.0 (C-4′ and C-6′), 124.1 (C-3′ and C-7′), 92.1 (C-1), 72.9 (C-5), 71.8 ($-OCH(CH_3)_2$), 70.6 (C-1′), 63.0 (C-6), 39.1 ($-OCOC(CH_3)_3$), 27.4 ($-OCH(CH_3)_2$), 23.5, 22.1 ($-OCH(CH_3)_2$); *m/z* (FABMS) 422 $[M+H]^+$, 362 $[M-OCH(CH_3)_2]^+$, 260, 243, 232, 217, 154, 136.

4.2.2. Isopropyl-6-*O*-trimethylacetyl-3-[hydroxy (4-fluorophenyl) methyl]-2,3-dideoxy-α-D-*glycero*-hex-2-enopyranoside-4-ulose (1′b). Oil. Eluent for column chromatography (12% ethyl acetate/hexane); [Found: C, 63.50; H, 7.52. $C_{21}H_{27}O_6F$ requires C, 63.94; H, 6.89%]; R_f (25% ethyl acetate/hexane) 0.45; $[\alpha]_D = -8.48$ (*c* 0.33, CH_3OH); ν_{max} (neat) 3481, 1726, 1686 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 7.34–7.04 (4H, m, Ph *H*), 6.56 (1H, d, $J_{2,1} = 3.6$ Hz, H-2), 5.57 (1H, s, H-1′), 5.38 (1H, d, $J_{1,2} =$

3.6 Hz, H-1), 4.66 (1H, dd, $J_{5,6a}$=2.6 Hz, $J_{5,6b}$=5.6 Hz, H-5), 4.53 (1H, dd, $J_{6a,5}$=2.6 Hz, $J_{6a,6b}$=11.9 Hz, H-6a), 4.39 (1H, dd, $J_{6b,5}$=5.7 Hz, $J_{6b,6a}$=11.9 Hz, H-6b), 4.02 (1H, sept., J=6.2 Hz, –OC*H*$(CH_3)_2$), 3.00 (1H, br s, –OH), 1.24 (3H, d, J=6.1 Hz, –OCH(C*H*$_3)_2$), 1.19 (3H, d, J=6.3 Hz, –OCH(C*H*$_3)_2$), 1.16 (9H, s, –OCOC(C*H*$_3)_3$); δ_C (50 MHz, $CDCl_3$) 195.2 (C-4), 178.5 (–OCOC$(CH_3)_3$), 160.4 (C-5′), 140.6 (C-2), 139.6 (C-3), 136.4 (C-2′), 129.0 (C-4′ and C-6′), 128.9 (C-3′ and C-7′), 92.2 (C-1), 72.9 (C-5), 71.6 (–O*C*H$(CH_3)_2$), 70.9 (C-1′), 63.1 (C-6), 39.1 (–OCO*C*$(CH_3)_3$), 27.5 (–OCOC(*C*H$_3)_3$), 23.6, 22.1 (–OCH(*C*H$_3)_2$); *m/z* (FAB MS) 394 $[M]^+$, 376 $[M-H_2O]^+$, 335 $[M-OCH(CH_3)_2]^+$.

4.2.3. Isopropyl-6-*O*-trimethylacetyl-3-(1-hydroxybutyl)-2,3-dideoxy-α-D-*glycero*-hex-2-enopyranoside-4-ulose (1′c). Oil. Eluent for column chromatography (12% ethyl acetate/hexane); [Found: C, 62.79; H, 8.07. $C_{18}H_{30}O_6$ requires C, 63.13; H, 8.83%]; R_f (30% ethyl acetate/hexane) 0.55; $[\alpha]_D$=+44 (*c* 0.20, CH_3OH); ν_{max} (neat) 3424, 1726, 1686 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 6.73 (1H, d, $J_{2,1}$=3.7 Hz, H-2), 5.39 (1H, d, $J_{1,2}$=3.7 Hz, H-1), 4.68 (1H, dd, $J_{5,6a}$=2.6 Hz, $J_{5,6b}$=5.7 Hz, H-5), 4.54 (1H, dd, $J_{6a,5}$=2.6 Hz, $J_{6a,6b}$=11.9 Hz, H-6a), 4.41 (2H, dd, $J_{6b,5}$=5.7 Hz, $J_{6b,6a}$=11.9 Hz, H-6b and H-1′), 4.04 (1H, sept., J=6.2 Hz, –OC*H*$(CH_3)_2$), 1.66–1.33 (4H, m, C-2′ and C-3′), 1.26 (3H, d, J=6.2 Hz, –OCH(C*H*$_3)_2$), 1.21 (3H, d, J=6.3 Hz, –OCH(C*H*$_3)_2$), 1.18 (9H, s, –OCOC(C*H*$_3)_3$), 0.93 (3H, t, J=7.2 Hz, H-4′); δ_C (50 MHz, $CDCl_3$) 195.4 (C-4), 178.5 (–O*C*OC$(CH_3)_3$, 140.0 (C-2), 139.4 (C-3), 92.2 (C-1), 72.9 (C-5), 71.5 (–O*C*H$(CH_3)_2$), 69.7 (C-1′), 63.2 (C-6), 39.2 (–OCO*C*$(CH_3)_3$), 38.3 (C-2′), 27.5 (–OCOC(*C*H$_3)_3$), 23.6, 22.2 (–OCH(*C*H$_3)_2$), 19.3 (C-3′), 14.2 (C-4′); *m/z* (FABMS) 343 $[M+H]^+$, 324 $[M-H_2O]^+$, 283 $[M-OCH(CH_3)_2]^+$, 265, 223, 181.

4.2.4. Isopropyl-6-*O*-trimethylacetyl-3-(1-hydroxydecyl)-2,3-dideoxy-α-D-*glycero*-hex-2-enopyranoside-4-ulose (1′d). Oil. Eluent for column chromatography (10% ethyl acetate/hexane); [Found: C, 67.51; H, 9.24. $C_{24}H_{42}O_6$ requires C, 67.57; H, 9.92%]; R_f (15% ethyl acetate/hexane) 0.33; $[\alpha]_D$=+32.5 (*c* 0.24, CH_3OH); ν_{max} (neat) 3449, 1726, 1687 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 6.66 (1H, d, $J_{2,1}$=3.6 Hz, H-2), 5.32 (1H, d, $J_{1,2}$=3.6 Hz, H-1), 4.60 (1H, dd, $J_{5,6a}$=2.6 Hz, $J_{5,6b}$=5.7 Hz, H-5), 4.48 (1H, dd, $J_{6a,5}$=2.6 Hz, $J_{6a,6b}$=11.9 Hz, H-6a), 4.33 (1H, dd, $J_{6b,5}$=5.7 Hz, $J_{6b,6a}$=11.9 Hz, H-6b), 4.30 (1H, t, J=3.1 Hz, H-1′), 4.05 (1H, sept., J=6.2 Hz, –OC*H*$(CH_3)_2$), 1.55 (2H, m, H-2′), 1.16 (20H, m, –OCH(C*H*$_3)_2$ and H-3′ to H-9′), 1.11 (9H, s, (–OCOC(C*H*$_3)_3$), 0.80 (3H, t, J=6.6 Hz, H-10′); δ_C (50 MHz, $CDCl_3$) 195.4 (C-4); 178.5 (–OCOC$(CH_3)_3$), 140.0 (C-2), 139.4 (C-3), 92.2 (C-1), 72.9 (C-5), 71.5 (–O*C*H$(CH_3)_2$), 70.0 (C-1′), 63.2 (C-6), 39.2 (–OCO*C*$(CH_3)_3$), 36.2, 32.3, 30.1, 30.0, 29.8, 29.7 (C-2′ to C-7′), 27.5 (–OCOC(*C*H$_3)_3$), 26.2 (C-8′), 23.6 (C-9′), 23.0, 22.2 (–OCH(*C*H$_3)_2$), 14.5 (C-10′); *m/z* (FABMS) 426 $[M]^+$, 408 $[M-H_2O]^+$, 367 $[M-OCH(CH_3)_2]^+$, 265.

4.2.5. Isopropyl-6-*O*-trimethylacetyl-3-[hydroxy (3-nitrophenyl) methyl]-2,3-dideoxy-α-D-*glycero*-hex-2-enopyranoside-4-ulose (1′e). Oil. Eluent for column chromatography (14% ethyl acetate/hexane); [Found: C, 59.57; H, 5.92; N, 3.25. $C_{21}H_{27}NO_8$ requires C, 59.85; H, 6.46; N, 3.32%] R_f (25% ethyl acetate/hexane) 0.42; $[\alpha]_D$=+4.95 (*c* 0.20, $CHCl_3$); ν_{max} (neat) 3483, 1728, 1689 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 8.26 (1H, s, H-3′), 8.14 (1H, d, $J_{5',6'}$=7.2 Hz, H-5′), 7.73 (1H, d, $J_{7',6'}$=7.3 Hz, H-7′), 7.53 (1H, t, $J_{6',5' \text{ or } 7'}$=7.8 Hz, H-6′), 6.71 (1H, d, $J_{2,1}$=3.5 Hz, H-2), 5.42 (1H, d, $J_{1,2}$=3.6 Hz, H-1), 5.68 (1H, s, H-1′), 4.66 (1H, dd, $J_{5,6a}$=2.6 Hz, $J_{5,6b}$=5.4 Hz, H-5), 4.50 (1H, dd, $J_{6a,5}$=2.4 Hz, $J_{6a,6b}$=11.8 Hz, H-6a), 4.37 (1H, dd, $J_{6b,5}$=5.6 Hz, $J_{6b,6a}$=11.9 Hz, H-6b), 4.06 (1H, sept., J=6.2 Hz, –OC*H*$(CH_3)_2$), 1.24 (3H, d, J=6.2 Hz, –OCH(C*H*$_3)_2$), 1.20 (3H, d, J=6.2 Hz, –OCH(C*H*$_3)_2$), 1.13 (9H, s, (–OCOC(C*H*$_3)_3$); δ_C (50 MHz, $CDCl_3$) 195.0 (C-4), 178.0 (–OCOC$(CH_3)_3$), 148.8 (C-3), 143.1 (C–4′), 141.2 (C-2), 138.8 (C-2′), 133.1 (C-3′), 129.8 (C-5′), 123.3 (C-7′), 122.1 (C-6′), 92.0 (C-1), 72.9 (C-5), 71.8 (–O*C*H$(CH_3)_2$), 70.6 (C-1′), 63.0 (C-6), 39.1 (–OCO*C*$(CH_3)_3$), 27.4 (–OCOC(*C*H$_3)_3$), 23.5, 22.1 (–OCH(*C*H$_3)_2$); *m/z* (FABMS) 421 $[M]^+$, 404 $[M-OH]^+$, 362 $[M-OCH(CH_3)_2]^+$, 278, 260, 243, 218, 154.

4.2.6. Isopropyl-6-*O*-trimethylacetyl-3-[hydroxy (2-nitrophenyl) methyl]-2,3-dideoxy-α-D-*glycero*-hex-2-enopyranoside-4-ulose (1′f). Oil. Eluent for column chromatography (15% ethyl acetate/hexane); [Found: C, 59.70; H, 6.71; N, 3.71. $C_{21}H_{27}NO_8$ requires C, 59.85; H, 6.46; N, 3.32%]; R_f (25% ethyl acetate/hexane) 0.40; $[\alpha]_D$=−6.13 (*c* 0.23, $CHCl_3$); ν_{max} (neat) 3478, 1727, 1687 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 7.97 (1H, d, J=8.0 Hz, H-4′), 7.68 (2H, m, H-5′ and H-6′), 7.46 (1H, t, J=7.3 Hz, H-7′), 6.66 (1H, d, $J_{2,1}$=3.6 Hz, H-2), 6.17 (1H, d, $J_{1',OH}$=4.0 Hz, H-1′), 5.39 (1H, d, $J_{1,2}$=3.6 Hz, H-1), 4.69 (1H, dd, $J_{5,6a}$=2.4 Hz, $J_{5,6b}$=5.8 Hz, H-5), 4.52 (1H, dd, $J_{6a,5}$=2.4 Hz, $J_{6a,6b}$=11.9 Hz, H-6a), 4.36 (1H, dd, $J_{6b,5}$=5.8 Hz, $J_{6b,6a}$=11.9 Hz, H-6b), 4.06 (1H, sept., J=6.1 Hz, –OC*H*$(CH_3)_2$), 3.41 (1H, d, $J_{OH,1'}$=4.9 Hz, O*H*), 1.25 (3H, d, J=6.1 Hz, –OCH(C*H*$_3)_2$), 1.19 (3H, d, J=6.1 Hz, –OCH(C*H*$_3)_2$), 1.14 (9H, s, –OCOC(C*H*$_3)_3$); δ_C (50 MHz, $CDCl_3$) 194.4 (C-4), 178.4 (–OCOC$(CH_3)_3$), 148.6 (C-3), 141.2 (C-2), 138.0 (C-3′), 136.0 (C-2′), 133.9 (C-4′), 129.2 (C-5′ and C-6′), 125.0 (C-7′), 92.1 (C-1), 72.7 (C-5), 71.6 (–O*C*H$(CH_3)_2$), 66.4 (C-1′), 63.0 (C-6), 39.1 (–OCO*C*$(CH_3)_3$), 27.4 (–OCOC(*C*H$_3)_3$), 23.5, 22.1 (–OCH(*C*H$_3)_2$); *m/z* (FABMS) 420 $[M-1]^+$, 404 $[M-OH]^+$, 362 $[M-OCH(CH_3)_2]^+$, 278, 260, 218, 154.

4.2.7. Isopropyl-6-*O*-trimethylacetyl-3-[hydroxy (4-trifluoromethylphenyl) methyl]-2,3-dideoxy-α-D-*glycero*-hex-2-enopyranoside-4-ulose (1′g). Oil. Eluent for column chromatography (14% ethyl acetate/hexane); [Found: C, 58.45; H, 5.93. $C_{22}H_{27}F_3O_6 \cdot 1/4H_2O$ requires C, 58.85; H, 6.17%]; R_f (20% ethyl acetate/hexane) 0.40; $[\alpha]_D$=+3.87 (*c* 0.28, $CHCl_3$); ν_{max} (neat) 3467, 1728, 1690 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 7.62 (2H, d, $J_{4',3' \text{ or } 6',7'}$=8.2 Hz, H-4′ and H-6′), 7.50 (2H, d, $J_{3',4' \text{ or } 7',6'}$=8.2 Hz, H-3′ and H-7′), 6.59 (1H, d, $J_{2,1}$=3.7 Hz, H-2), 5.62 (1H, s, H-1′), 5.39 (1H, d, $J_{1,2}$=3.6 Hz, H-1), 4.66 (1H, dd, $J_{5,6a}$=2.6 Hz, $J_{5,6b}$=5.4 Hz, H-5), 4.51 (1H, dd, $J_{6a,5}$=2.6 Hz, $J_{6a,6b}$=11.9 Hz, H-6a),), 4.40 (1H, dd, $J_{6b,5}$=5.4 Hz, $J_{6b,6a}$=11.9 Hz, H-6b), 4.03 (1H, sept., J=6.1 Hz, –OC*H*$(CH_3)_2$ 1.24 (3H, d, J=6.1 Hz, –OCH(C*H*$_3)_2$), 1.19 (3H, d, J=6.1 Hz, –OCH(C*H*$_3)_2$), 1.13 (9H, s, –OCOC(C*H*$_3)_3$); δ_C (75 MHz, $CDCl_3$) 194.6 (C-4); 177.9 (–OCOC$(CH_3)_3$), 144.1 (C-3), 140.4 (C-2), 138.6 (C-2′ and C-5′), 127.0 (*C*F_3), 126.9 (C-4′ and

C-6′), 125.3 (C-3′ and C-7′), 91.5 (C-1), 72.3 (C-5), 71.2 (–O*C*H(CH$_3$)$_2$), 70.5 (C-1′), 62.4 (C-6), 38.5 (–OCO*C*(CH$_3$)$_3$), 26.8 (–OCOC(*C*H$_3$)$_3$), 23.0, 21.5 (–OCH(*C*H$_3$)$_2$); *m/z* (FAB MS) 443 [M−1]$^+$, 427 [M−OH]$^+$, 385 [M−OCH(CH$_3$)$_2$]$^+$, 368, 301, 283, 266, 240.

4.2.8. Isopropyl-6-*O*-trimethylacetyl-3-[hydroxy (phenyl) methyl]-2,3-dideoxy-α-D-*glycero*-hex-2-enopyranoside-4-ulose (1′h). Oil. Eluent for column chromatography (12% ethyl acetate/hexane); [Found: C, 64.94; H, 7.19. C$_{21}$H$_{28}$O$_6$·1/2H$_2$O requires C, 65.43; H, 7.58%]; R_f (20% ethyl acetate/hexane) 0.49; $[\alpha]_D=-10.0$ (*c* 0.08, CHCl$_3$); ν_{max} (neat) 3430, 1727, 1687 cm^{-1}; δ_H (200 MHz, CDCl$_3$) 7.38–7.33 (5H, m, Ph*H*), 6.56 (1H, dd, $J_{2,1}=3.7$ Hz, and $J_{2,1'}=1.2$ Hz, H-2), 5.38 (1H, d, $J_{1,2}=3.6$ Hz, H-1), 5.58 (1H, s, H-1′), 4.66 (1H, dd, $J_{5,6a}=2.6$ Hz, $J_{5,6b}=5.7$ Hz, H-5), 4.53 (1H, dd, $J_{6a,5}=2.6$ Hz, $J_{6a,6b}=11.9$ Hz, H-6a), 4.40 (1H, dd, $J_{6b,5}=5.7$ Hz, $J_{6b,6a}=11.9$ Hz, H-6b), 4.02 (1H, sept., $J=6.2$ Hz, –OC*H*(CH$_3$)$_2$), 1.24 (3H, d, $J=6.2$ Hz, –OCH(C*H*$_3$)$_2$), 1.18 (3H, d, $J=6.2$ Hz, –OCH(C*H*$_3$)$_2$), 1.15 (9H, s, –OCOC(C*H*$_3$)$_3$); δ_C (50 MHz, CDCl$_3$) 195.2 (C-4), 178.4 (–OCOC(CH$_3$)$_3$), 140.6 (C-2 and C-2′), 139.7 (C-3), 128.9 (C-3′ and C-7′), 128.4 (C-5′), 127.2 (C-4′ and C-6′), 92.2 (C-1), 72.9 (C-5), 71.5 (C-1′ and –O*C*H(CH$_3$)$_2$), 63.1 (C-6), 39.1 (–OCO*C*(CH$_3$)$_3$), 27.4 (–OCOC(*C*H$_3$)$_3$), 23.5, 22.1 (–OCH(*C*H$_3$)$_2$); *m/z* (FABMS) 375 [M−1]$^+$, 359 [M−OH]$^+$, 317 [M−OCH(CH$_3$)$_2$]$^+$, 233, 215, 198, 173, 154.

4.2.9. Isopropyl-6-hydroxy-3-[hydroxy (4-nitrophenyl) methyl]-2,3-dideoxy-α-D-*glycero*-hex-2-enopyranoside-4-ulose (2′a). Oil. Eluent for column chromatography (30% ethyl acetate/hexane); [Found: C, 54.29; H, 6.07; N, 3.87. C$_{16}$H$_{19}$NO$_7$·H$_2$O requires C, 54.01; H, 5.95; N, 3.94%]; R_f (50% ethyl acetate/hexane) 0.39; $[\alpha]_D=-10.86$ (*c* 0.09, CHCl$_3$); ν_{max} (neat) 3405, 1686 cm^{-1}; δ_H (200 MHz, CDCl$_3$) 8.21 (2H, d, $J_{4',3' \text{ or } 6',7'}=8.6$ Hz, H-4′ and H-6′), 7.57 (2H, d, $J_{3',4' \text{ or } 7',6'}=8.7$ Hz, H-3′ and H-7′), 6.63 (1H, d, $J_{2,1}=3.4$ Hz, H-2), 5.71 (1H, s, H-1′), 5.42 (1H, d, $J_{1,2}=3.7$ Hz, H-1), 4.46 (1H, t, $J=3.9$ Hz, H-5), 4.13–3.96 (2H, m, H-6a and OC*H*(CH$_3$)$_2$), 3.89 (1H, dd, $J_{6b,5}=3.9$ Hz, $J_{6b,6a}=11.9$ Hz, H-6b), 1.23 (3H, d, $J=6.3$ Hz, –OCH(C*H*$_3$)$_2$), 1.19 (3H, d, $J=6.2$ Hz, –OCH(C*H*$_3$)$_2$); δ_C (50 MHz, CDCl$_3$) 196.7 (C-4) 148.0 (C-3 and C-5′), 141.3 (C-2), 139.3 (C-2′), 128.0 (C-4′ and C-6′), 124.1 (C-3′ and C-7′), 92.2 (C-1), 74.5 (C-5), 71.9 (–O*C*H(CH$_3$)$_2$), 70.0 (C-1′), 62.0 (C-6), 23.5, 22.1 (–OCH(*C*H$_3$)$_2$); *m/z* (FABMS) 338 [M+1]$^+$, 320 [M−OH]$^+$, 307 [M−32]$^+$, 278 [M−OCH (CH$_3$)$_2$]$^+$, 218, 154, 136.

4.2.10. Isopropyl-6-*O*-acetyl-3-[hydroxy (4-nitrophenyl) methyl]-2,3-dideoxy-α-D-*glycero*-hex-2-enopyranoside-4-ulose (2′b). Oil. Eluent for column chromatography (18% ethyl acetate/hexane); [Found: C, 57.01; H, 5.36; N, 3.83. C$_{18}$H$_{21}$NO$_8$ requires C, 56.98; H, 5.58; N, 3.69%]; R_f (30% ethyl acetate/hexane) 0.39; $[\alpha]_D=-7.93$ (*c* 0.12, CHCl$_3$). ν_{max} (neat) 3483, 1740, 1692 cm^{-1}; δ_H (200 MHz, CDCl$_3$) 8.22 (2H, d, $J_{4',3' \text{ or } 6',7'}=8.7$ Hz, H-4′ and H-6′) 7.58 (2H, d, $J_{3',4' \text{ or } 7',6'}=8.7$ Hz, H-3′and H-7′), 5.69 (1H, s, H-1′), 6.65 (1H, d, $J_{2,1}=3.7$ Hz, H-2), 5.42 (1H, d, $J_{1,2}=3.6$ Hz, H-1), 4.65 (1H, t, $J_{5,6a\&6b}=4.5$ Hz, H-5), 4.46 (2H, d, $J_{6a\&6b,\ 5}=4.5$ Hz, H-6), 4.02 (1H, sept., $J=6.1$ Hz, –OCH (CH$_3$)$_2$), 3.13 (1H, br s, –OH), 2.03 (3H, s, OCOCH$_3$), 1.24 (3H, d, $J=6.1$ Hz, –OCH(C*H*$_3$)$_2$), 1.19 (3H, d, $J=6.1$ Hz, –OCH(C*H*$_3$)$_2$); δ_C (50 MHz, CDCl$_3$) 194.7 (C-4), 171.0 (–O*C*OCH$_3$), 148.0 (C-3 and C-5′), 141.1 (C-2), 138.9 (C-2′), 128.0 (C-4′ and C-6′), 124.0 (C-3′ and C-7′), 92.4 (C-1), 72.5 (C-5), 72.2 (–O*C*H(CH$_3$)$_2$), 70.3 (C-1′), 62.8 (C-6), 23.4, 22.3 (–OCH(*C*H$_3$)$_2$), 21.0 (CO*C*H$_3$); *m/z* (FABMS) 380 [M+1]$^+$, 362 [M−OH]$^+$, 320 [M−OCH (CH$_3$)$_2$]$^+$, 307, 289, 260, 232, 154.

4.2.11. Isopropyl-6-*O*-4-nitrobenzoyl-3-[hydroxy (4-nitrophenyl) methyl]-2,3-dideoxy-α-D-*glycero*-hex-2-enopyranoside-4-ulose (2′c). Amorphous solid. Eluent for column chromatography (16% ethyl acetate/hexane); [Found: C, 56.27; H, 4.98; N, 5.79. C$_{23}$H$_{22}$N$_2$O$_{10}$ requires C, 56.79; H, 4.55; N, 5.75%]; R_f (25% ethyl acetate/hexane) 0.50; $[\alpha]_D=-39.13$ (*c* 0.05, CHCl$_3$); ν_{max} (neat) 3453, 1727, 1688 cm^{-1}; δ_H NMR (300 MHz, CDCl$_3$) 8.27 (2H, d, $J_{4'',3'' \text{ or } 6'',7''}=9.0$ Hz, H-4″ and H-6″), 8.19 (2H, d, $J_{4',3' \text{ or } 6',7'}=9.0$ Hz, H-4′ and H-6′), 8.13 (2H, d, $J_{3'',4'' \text{ or } 7'',6''}=9.0$ Hz, H-3″ and H-7″), 7.59 (2H, d, $J_{3',4' \text{ or } 7',6'}=9.0$ Hz, H-3′ and H-7′), 6.68 (1H, d, $J_{2,1}=3.7$ Hz, H-2), 5.44 (1H, d, $J_{1,2}=3.9$ Hz, H-1), 5.74 (1H, s, H-1′), 4.83–4.78 (2H, m, H-5 and H-6a), 4.73 (1H, dd, $J_{6b,5}=5.7$ Hz, $J_{6b,6a}=12.0$ Hz, H-6b), 4.02 (1H, sept., $J=6.3$ Hz, –OC*H*(CH$_3$)$_2$), 1.24 (3H, d, $J=6.0$ Hz, –OCH(C*H*$_3$)$_2$), 1.20 (3H, d, $J=6.0$ Hz, –OCH(C*H*$_3$)$_2$); δ_C (50 MHz, CDCl$_3$) 194.4 (C-4) 164.6 (C-1″), 151.0 (C-5″), 148.0 (C-5′), 147.8 (C-3), 141.3 (C-2), 138.9 (C-2′), 135.4 (C-2″), 131.1 (C-4″ and C-6″), 128.0 (C-4′ andC-6′), 124.1 (C-3′ and C-7′), 123.9 (C-3″ and C-7″), 92.5 (C-1), 72.5 (C-5), 72.4 (–O*C*H(CH$_3$)$_2$), 70.2 (C-1′), 64.1 (C-6), 23.5, 22.3 (–OCH(*C*H$_3$)$_2$); *m/z* (FABMS) 487 [M+1]$^+$, 469 [M−OH]$^+$, 427 [M−OCH(CH$_3$)$_2$]$^+$, 410, 392, 307, 289, 278, 154.

4.2.12. Isopropyl-6-*O*-trimethyldiphenylsilyl-3-[hydroxy (4-nitrophenyl)methyl]-2,3-dideoxy-α-D-*glycero*-hex-2-enopyranoside-4-ulose (2′d). Oil. Eluent for column chromatography (10% ethyl acetate/hexane); [Found: C, 65.82; H, 6.26; N, 2.12. C$_{32}$H$_{37}$NO$_7$Si·1/2H$_2$O requires C, 65.73; H, 6.55; N, 2.39%]; R_f (20% ethyl acetate/hexane) 0.50; $[\alpha]_D=+9.0$ (*c* 0.20, CHCl$_3$); ν_{max} (neat) 3464, 1688 cm^{-1}; δ_H (200 MHz, CDCl$_3$) 8.17 (2H, d, $J_{4',3' \text{ or } 6',7'}=8.7$ Hz, H-4′ and H-6′), 7.66–7.63 (4H, m, Ph*H*), 7.56 (2H, d, $J_{3',4' \text{ or } 7',6'}=8.7$ Hz, H-3′ and H-7′), 7.42–7.36 (6H, m, PhH), 6.58 (1H, dd, $J_{2,1}=3.6$ Hz, $J_{2,1'}=1.0$ Hz, H-2), 5.62 (1H, s, H-1′), 5.47 (1H, d, $J_{1,2}=3.5$ Hz, H-1), 4.50 (1H, dd, $J_{5,6a}=2.8$ Hz, $J_{5,6b}=4.6$ Hz, H-5), 4.09–3.99 (3H, m, H-6a, H-6b and OC*H*(CH$_3$)$_2$), 1.23 (3H, d, $J=6.3$ Hz, –OCH(C*H*$_3$)$_2$), 1.19 (3H, d, $J=6.3$ Hz, –OCH(C*H*$_3$)$_2$), 0.98 (9H, s, –C(C*H*$_3$)$_3$); δ_C (50 MHz, CDCl$_3$) 196.1 (C-4), 148.1 (C-5′), 148.0 (C-3), 141.6 (C-2), 139.3 (C-2′), 136.0 (Ph*C*), 133.0 (Ph*C*), 130.1 (C-4′ and C-6′), 128.0 (Ph*C*), 124.1 (C-3′ and C-7′), 92.1 (C-1), 76.1 (C-5), 71.6 (–O*C*H(CH$_3$)$_2$), 71.2 (C-1′), 63.7 (C-6), 27.0 (–C(*C*H$_3$)$_3$), 23.6, 22.1 (–OCH(*C*H$_3$)$_2$), 19.6 (–*C*(CH$_3$)$_3$); *m/z* (FABMS) 574 [M−1]$^+$, 558 [M−OH]$^+$, 518 [M−(CH$_3$)$_3$C]$^+$, 498, 458, 438, 365, 335, 307, 241.

4.2.13. Isopropyl-6-*O*-trimethyldimethylsilyl-3-[hydroxy (4-nitrophenyl)methyl]-2,3-dideoxy-α-D-*glycero*-hex-2-enopyranoside-4-ulose (2′e). Oil. Eluent for column chromatography (12% ethyl acetate/hexane); [Found: C, 58.42; H, 7.26; N, 2.81. C$_{22}$H$_{33}$NO$_7$Si requires C, 58.52; H,

7.36; N, 3.10%]; R_f (30% ethyl acetate/hexane) 0.30; $[\alpha]_D = +8.33$ (*c* 0.24, $CHCl_3$); ν_{max} (neat) 3467, 1687 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 8.20 (2H, d, $J_{4',3' \text{ or } 6',7'} = 8.7$ Hz, H-4′ and H-6′), 7.56 (2H, d, $J_{3',4' \text{ or } 7',6'} = 8.4$ Hz, H-3′ and H-7′), 6.57 (1H, d, $J_{2,1} = 3.6$ Hz, H-2), 5.63 (1H, s, H-1′), 5.45 (1H, d, $J_{1,2} = 3.6$ Hz, H-1), 4.44 (1H, t, $J = 3.6$ Hz, H-5), 4.08–4.01 (3H, m, H-6a, H-6b and –OC*H*$(CH_3)_2$), 1.24 (3H, d, $J = 6.0$ Hz, –OCH(C*H*$_3)_2$), 1.19 (3H, d, $J = 6.0$ Hz, –OCH(C*H*$_3)_2$), 0.84 (9H, s, –C(C*H*$_3)_3$), 0.03 (6H, s, –Si(C*H*$_3)_2$); δ_C (50 MHz, $CDCl_3$) 196.1 (C-4), 147.7 (C-3 and C-5′), 141.1 (C-2), 138.6 (C-2′), 127.5 (C-4′ and C-6′), 123.6 (C-3′ and C-7′), 91.7 (C-1), 75.7 (C-5), 71.2 (–O*C*H$(CH_3)_2$), 70.6 (C-1′), 62.5 (C-6), 25.7 (–C(*C*H$_3)_3$) 23.1, 21.7 (–OCH(*C*H$_3)_2$), 18.2 (–*C*$(CH_3)_3$), −5.46 (–Si(*C*H$_3)_2$; *m/z* (FABMS) 452 $[M+1]^+$, 434 $[M-OH]^+$, 392 $[M-OCH(CH_3)_2]^+$, 334, 318, 289, 266.

4.2.14. Isopropyl-6-*O*-(trimethylacetyl)-3-[hydroxy (4-nitrophenyl) methyl]-2,3-dideoxy-α-D-*threo*-hex-2-enopyranoside (3′a). Solid, mp 122–24 °C. Eluent for column chromatography (30% ethyl acetate/hexane); [Found: C, 59.62; H, 6.41; N, 3.34. $C_{21}H_{29}NO_8$ requires C, 59.56; H, 6.90; N, 3.30%]; R_f (40% ethyl acetate/hexane) 0.47; $[\alpha]_D = +36.84$ (*c* 0.03, $CHCl_3$); ν_{max} (KBr) 3425, 3027, 2978, 1728 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 8.19 (2H, d, $J_{4',3' \text{ or } 6',7'} = 8.7$ Hz, H-4′ and H-6′), 7.57 (2H, d, $J_{3',4' \text{ or } 7',6'} = 8.5$ Hz, H-3′ and H-7′), 5.66 (1H, d, $J_{2,1} = 2.8$ Hz, H-2), 5.44 (1H, d, $J_{1',OH} = 6.5$ Hz, H-1′) 5.12, (1H, d, $J_{1,2} = 2.8$ Hz, H-1), 4.39 (1H, dd, $J_{6b,5} = 4.9$ Hz, $J_{6b,6a} = 12.2$ Hz, H-6b), 4.27 (1H, d, $J = 7.2$ Hz, H-4), 4.17 (1H, dd, $J_{6a,5} = 2.1$ Hz, $J_{6a,6b} = 12.2$ Hz, H-6a), 4.01 (1H, sept., $J = 6.0$ Hz, –OC*H*$(CH_3)_2$), 3.87 (1H, m, H-5), 1.23 (3H, d, $J = 6.1$ Hz, –OCH(C*H*$_3)_2$), 1.16 (3H, d, $J = 6.1$ Hz, –OCH(C*H*$_3)_2$), 1.11 (9H, s, –OCOC(C*H*$_3)_3$); δ_C (50 MHz, $CDCl_3$) 180.4 (–OCOC$(CH_3)_3$), 149.4 (C-5′), 147.7 (C-3), 141.6 (C-2′), 127.3 (C-4′ and C-6′), 126.8 (C-2), 123.9 (C-3′ and C-7′), 93.0 (C-1), 76.4 (C-5), 70.7 (–O*C*H$(CH_3)_2$), 70.4 (C-1′), 65.2 (C-4), 64.3 (C-6), 39.2 (–OCO*C*$(CH_3)_3$), 27.4 (–OCOC(*C*H$_3)_3$), 24.0, 22.1 (–OCH(*C*H$_3)_2$); *m/z* (FABMS) 422 $[M-1]^+$, 406 $[M-OH]^+$, 364 $[M-OCH(CH_3)_2]^+$, 346 $[M-18-OCH(CH_3)_2]^+$, 262, 244, 154.

4.2.15. Isopropyl-6-*O*-(trimethylacetyl)-3-(1-hydroxy decyl)-2,3-dideoxy-α-D-*threo*-hex-2-enopyranoside (3′d). Solid, mp 55–56 °C. Eluent for column chromatography (18% ethyl acetate/hexane); [Found: C, 65.31; H, 10.55. $C_{24}H_{44}O_6 \cdot 1/2H_2O$ requires C, 65.86; H, 10.36%]; R_f (20% ethyl acetate/hexane) 0.37; $[\alpha]_D = +33.50$ (*c* 0.14, $CHCl_3$); ν_{max} (KBr) 3440, 2934, 1724, 1460, 1373 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 5.60 (1H, d, $J_{2,1} = 2.3$ Hz, H-2), 5.08 (1H, d, $J_{1,2} = 2.8$ Hz, H-1), 4.35–4.33 (2H, m, H-1′ and H-5), 4.21 (1H, d, $J = 6.3$ Hz, H-4), 4.20 (1H, m, H-6a), 4.01 (1H, dd, $J_{6b,5} = 6.0$ Hz, $J_{6b,6a} = 12.1$ Hz, H-6b), 3.99 (1H, m, –OC*H*$(CH_3)_2$), 1.71 (2H, m, H-2′), 1.26–1.22 (26H, m, –OCH(C*H*$_3)_2$, –OCOC(C*H*$_3)_3$ and H-3′ to H-9′), 1.17 (3H, d, $J = 6.0$ Hz, –OCH(C*H*$_3)_2$), 0.88 (3H, t, $J = 6.7$ Hz, H-10′); δ_C (50 MHz, $CDCl_3$) 179.6 (–OCOC$(CH_3)_3$), 142.4 (C-3), 124.5 (C-2), 92.8 (C-1), 76.7 (C-5), 71.0 (–O*C*H$(CH_3)_2$), 70.1 (C-1′), 65.7 (C-4), 64.5 (C-6), 39.3 (–OCO*C*$(CH_3)_3$), 35.1, 32.3, 30.0, 29.8, 29.7 (C-2′ to C-7′), 27.6 (–OCOC(*C*H$_3)_3$), 26.2 (C-8′), 24.1 (C-9′), 23.0, 22.1 (–OCH(*C*H$_3)_2$), 14.5 (C-10′); *m/z* (FABMS) 427 $[M-H]^+$, 411 $[M-H_2O+1]^+$, 369 $[M-OCH(CH_3)_2]^+$, 351, 267, 249.

4.2.16. Isopropyl-6-*O*-(trimethylacetyl)-3-[hydroxy (2-nitrophenyl) methyl]-2,3-dideoxy-α-D-*threo*-hex-2-enopyranoside (3′f). Sticky oil. Eluent for column chromatography (25% ethyl acetate/hexane); [Found: C, 59.70; H, 6.88; N, 2.90. $C_{21}H_{29}NO_8$ requires C, 59.56; H, 6.90; N, 3.30%]; R_f (40% ethyl acetate/hexane) 0.49; $[\alpha]_D = -38.89$ (*c* 0.09, $CHCl_3$); ν_{max} (neat) 3428, 2925, 1721, 1349 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 7.88 (1H, d, $J_{4',5'} = 7.5$ Hz, H-4′), 7.87 (1H, d, $J_{7',6'} = 7.3$ Hz, H-7′), 7.67 (1H, t, $J_{6',7' \text{ or } 5'} = 7.3$ Hz, H-6′), 7.45 (1H, t, $J_{5',4' \text{ or } 6'} = 7.5$ Hz, H-4′), 6.03 (1H, brd, $J_{1',OH} = 5.8$ Hz, H-1′), 5.52 (1H, br s, H-2), 5.05 (1H, d, $J_{1,2} = 2.8$ Hz, H-1), 4.43 (1H, dd, $J_{6a,5} = 4.7$ Hz, $J_{6a,6b} = 12.1$ Hz, H-6a), 4.19 (1H, dd, $J_{6b,5} = 2.0$ Hz, $J_{6b,6a} = 12.2$ Hz, H-6b), 4.12 (1H, d, $J = 7.1$ Hz, H-4), 4.06–3.89 (2H, m, H-5 and –OC*H*$(CH_3)_2$), 1.24 (3H, d, $J = 6.4$ Hz, –OCH(C*H*$_3)_2$), 1.20 (3H, d, $J = 6.3$ Hz, –OCH(C*H*$_3)_2$), 1.14 (9H, s, –OCOC(C*H*$_3)_3$); δ_C (50 MHz, $CDCl_3$) 180.2 (–O*C*OC$(CH_3)_3$), 148.7 (C-3), 140.5 (C-3′), 136.5 (C-2′), 133.5 (C-4′), 129.6 (C-6′), 129.0 (C-5′), 126.3 (C-2), 125.0 (C-7′), 92.9 (C-1), 72.2 (C-5), 70.4 (C-1′ and –O*C*H$(CH_3)_2$), 65.1 (C-4), 64.3 (C-6), 39.3 (–OCO*C*$(CH_3)_3$), 27.5 (–OCOC(*C*H$_3)_3$), 24.0, 22.1 (–OCH(*C*H$_3)_2$); *m/z* (FABMS) 424 $[M+1]^+$, 364 $[M-OCH(CH_3)_2]^+$, 346 $[M-H_2O-OCH(CH_3)_2]^+$, 265, 244, 202.

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Monomers containing substrate or inhibitor residues for copper amine oxidases and their hydrophilic beaded resins designed for enzyme interaction studies

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Abstract—Five styrenic monomers, four with aminoalkyl residues typical of copper containing amine oxidase substrates and one with a 2,6-dialkoxybenzylamine residue which mimics previously prepared selective substrate-like benzylamine oxidase inhibitors, have been synthesized and transformed into radical homopolymers, copolymers with *N*,*N*-dimethylacrylamide (DMAA), and hydrophilic beaded resins, designed for enzyme interaction studies aimed in finding new materials for highly biospecific chromatographic separations. The five monomers have given beaded resins of 125–500 μm swellable in water with a volume increase of 1200–1500%. The four aminoalkyl monomers have given water soluble copolymers some of which are good substrates of benzylamine oxidase (BAO), diamine oxidase (DAO) and lysyl oxidase (LO), up to 9.7 times better than elastin for LO.

1. Introduction

Copper amine oxidases (CAOs)[1] are a large family of enzymes (EC 1.4.3.6) present in prokaryotes and eukaryotes including man, that control important cellular processes such as the removal of biogenic amines, the cross-linking of elastin and collagen, the regulation of intracellular polyamines. The enzymatic reaction consists of the oxidative deamination of aminomethyl substrates to produce aldehydes, hydrogen peroxide and ammonia. In the last decade, X-ray structure determinations[2] of CAOs obtained from microorganisms and plants have allowed important progress in the knowledge of cofactors,[3] enzymatic reaction mechanism,[4] role of the copper etc. evidencing significant differences among them. Nevertheless, no crystallographic data are available at present for the mammalian members of CAOs that suffer from laborious purification protocols.[5–8]

With the final aim of improving and shortening the enzyme purification procedures through the synthesis of new materials for biospecific chromatographic separations, we want to report in this work the preparation and characterization of highly hydrophilic *N*,*N*-dimethylacrylamide-based resins of the type **R1** and **R2** (Fig. 1), both conceived as macromolecular tools for biospecific interactions with mammalian CAOs. The structure of **R1** is characterized by aminoalkyl residues typical of the CAO substrates, while **R2** contains 2,6-dialkoxybenzylamine residues which mimic previously prepared selective benzylamine oxidase inhibitors[9] with IC_{50}(M) up to 6.6×10^{-8}.

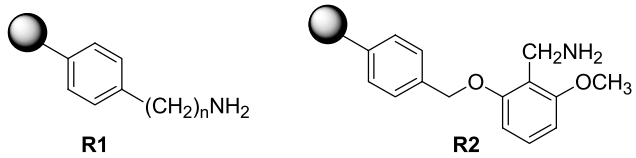

Figure 1.

The preparation of macromolecular systems for specific interactions with enzymes usually active on small molecule substrates is expected to be quite a demanding task that requires the careful choice and optimization of many parameters such as type of the active functions, nature and length of the linker, physical and chemical stability, degree of hydrophilicity, flow properties and accessibility of the

Keywords: Monomers; Polymers; Hydrophilic beaded resins; Copper amine oxidases; Reverse suspension polymerization.
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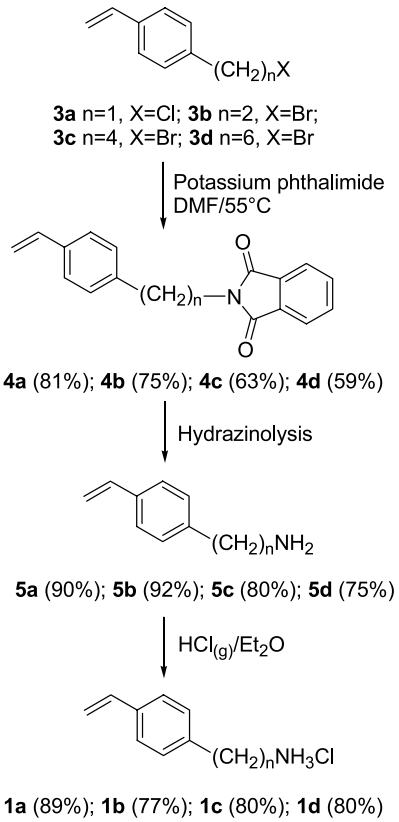
active functions. Consequently, we rejected synthetic procedures entailing functionalization of preformed commercial matrices[10] preferring the synthesis ab initio of functionalized monomers and copolymers to gain advantages such as the control of structure, purity, and loading of the active monomer, and flexibility in tailoring important properties for the interaction process with the enzyme such as the swellability and porosity of the macromolecular system.[11]

2. Results and discussion

2.1. Synthesis of substrate-like monomers 1a–d

Monomers **1a–d** were synthesized according to the Scheme 1. The necessary ω-haloalkylstyrenes were purchased (**3a**), or obtained through the copper halide coupling reaction[12,13] of α,ω-dibromoalkanes with styryl Grignard reagents[14] with minor modifications[15] (**3c** and **3d**), or prepared (**3b**)[16] by an alternative route based on the acylation of 2-bromoethylbenzene, since 1,2-dibromoethane forms ethylene when it reacts with Grignard reagents.[13] The purification of **3c** and **3d** was preferentially performed by column chromatography to avoid occasional polymerization during distillation at reduced pressure.

The successive reaction of ω-haloalkylstyrenes with potassium phthalimide in DMF afforded Gabriel adducts **4a–d** which were purified and characterized before submission to hydrazinolysis. The obtained ω-aminoalkylstyrenes **5a–d** were promptly transformed into the corresponding hydrochlorides **1a–d** for better purification and storage.

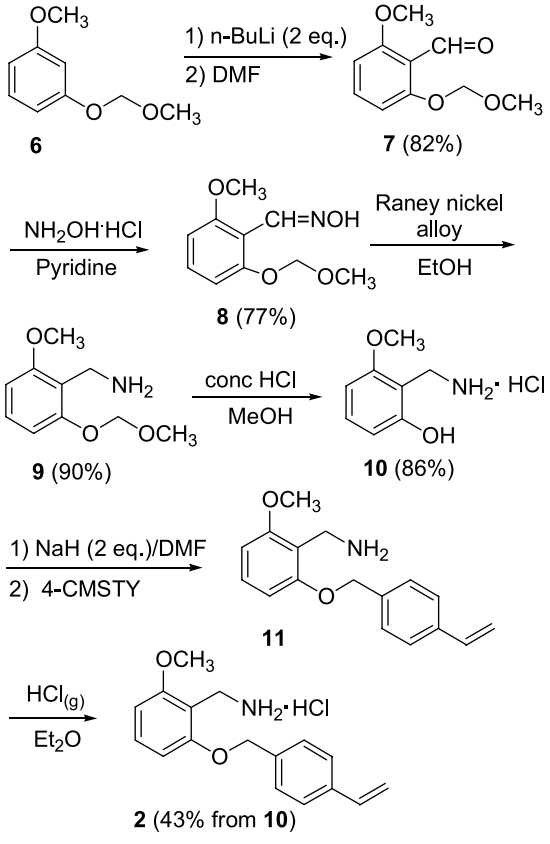


Scheme 1.

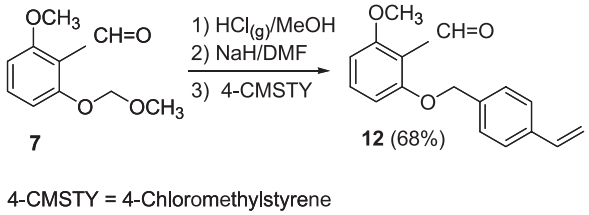
2.2. Synthesis of the inhibitor-like monomer 2

The multistep synthesis of **2**, performed according to Scheme 2, is based on the *ortho*-directed metallation of 3-methoxymethoxyanisole (**6**)[17] for which we recently highlighted some very interesting results, producing the desired aldehyde in good yield, or substituting the methoxymethoxy moiety with formation of tri- and tetrasubstituted benzenes, or affording quite unusually stable doubly lithiated intermediates.[18] The aldehyde **7**, after transformation into its oxime **8** and reduction, afforded **9** as an easily distillable liquid. The deprotection of **9** afforded **10** in good yield, but the successive alkylation with 4-chloromethylstyrene to produce the crude free base **11**, then monomer **2** with an overall 26% yield from **7** was not as good.

Following an alternative route (Scheme 3), **7** was transformed into 2-methoxy-6-hydroxybenzaldehyde[19] which was alkylated with 4-chloromethylstyrene to afford 2-methoxy-6-[(4-vinyl)benzyloxy]benzaldehyde (**12**) in good yield, but **12** proved difficult to transform into **11** either by reducing its oxime derivative with the Raney nickel alloy or by treating it with sodium cyanoborohydride and ammonium acetate. If **11** in the form of its hydrochloride **2** is demonstrated to be very effective in the



Scheme 2.



Scheme 3.

biological tests, its synthesis and the obstacles to the accomplishment of its structure will be further examined.

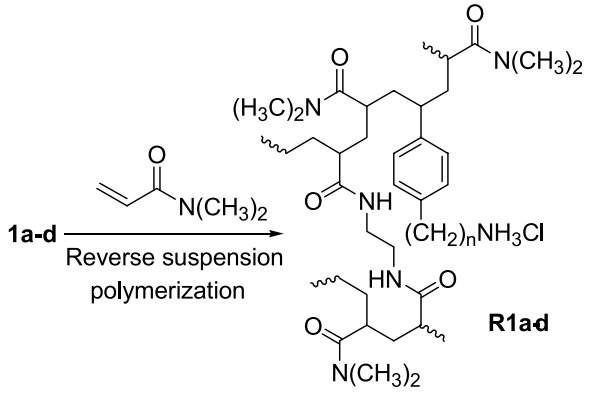
2.3. Polymers and resins

Preliminary polymerization studies showed that the monomers **1a–d** and **2** homopolymerized and copolymerized easily with *N*,*N*-dimethylacrylamide, a good hydrophilic comonomer for polar supports,[20] in water with ammonium persulfate and in methanol or DMF with AIBN as radical initiators affording conversions in the range 20–94%. In the IR spectra of the homopolymers, intense broad absorptions around 3000 cm^{-1} due to NH_3^+ groups were present, while the IR spectra of the copolymers showed the amide band of the comonomer around 1620 cm^{-1}.

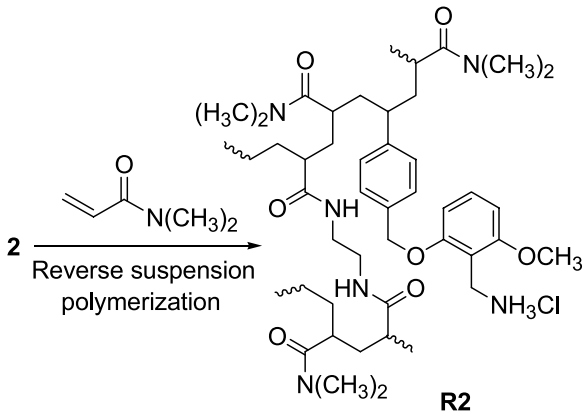
All the copolymers with DMAA were soluble in water and methanol and insoluble in petroleum ether, benzene, diethyl ether, dioxane, acetone. DMF proved to be a good solvent for polymers containing units of **2** and a poor solvent for those containing units of **1a–d**.

Preliminary biological tests showed that some of the prepared polymeric materials were good to excellent substrates for different CAOs.

After these positive indications, the transformation of **1a–d** and **2** into cross-linked resins was achieved by suspending the aqueous solution of the monomers in a mixture of CCl_4/hexane and using SPAN 85 and APS/TMEDA as anticoagulant and initiator respectively[21] (Schemes 4 and 5).



Scheme 4.



Scheme 5.

The conditions applied afforded very good conversions (92–95%) for all the monomers except for **1d** (51%). Probably, the longer hydrophobic methylene sequence in **1d** makes it more soluble in the organic phase thus slowing the polymerization process. This fact and other undesired properties of the resins **R1d** (see below) made us lose interest in this monomer.

The resins **R1a–c** and **R2**, with the exclusion of **R1d**, appear as microspherular beads with the bulk of material (>96%) in the size range 125–500 μm. They are endowed with high hydrophilicity, as estimated by observing the increase of volume of the dry material after overnight swelling in water (Table 1), and show good flow properties. Fig. 2 shows a typical sample of a dry and a swollen resin.

The NH_2 content of the resins was estimated following the method of Gaur and Gupta[22] based on the labeling of the amino groups with 4-O-(4,4′-dimethoxytriphenylmethyl)-butyryl residues and the quantitative determination through UV-Vis spectroscopy of the 4,4′-dimethoxytriphenylmethyl cation ($\varepsilon = 70{,}000$ at 498 nm) released from the resin after treatment with $HClO_4$. The values of NH_2 loading (Table 1) for all the prepared resins appear to be suitable for enzyme interaction studies, which for their specificity deserve a separate paper. Nevertheless, keeping in mind that among CAOs only lysyl oxidase (LO) is naturally devoted to oxidize lysine residues in macromolecular structures like elastin and collagen while all the other enzymes of the same class oxidize small molecules, some remarkable results highlighting the bioactivity levels of some of our polymeric materials are briefly anticipated in Table 2.

The soluble copolymers **P1a** and **P1c**, and resin **R1a** are very good substrates of LO and benzylamine oxidase (BAO), with **P1c** also active towards diamine oxidase (DAO). As far as LO is concerned, it is noteworthy that resin **R1a** is as active as the natural substrate elastin, and copolymer **P1c** is 9.7 times more active than the elastin itself.

3. Conclusions

Since the first essential step for setting up a biospecific separation of CAOs is the synthesis of polymeric materials

Table 1. Data of some representative reverse-phase suspension copolymerizations of **1a–c** and **2** with *N,N*-dimethylacrylamide and *N,N′*-ethylenebisacrylamide

Monomers g (mole fraction)	H_2O mL	Span 85 µl	CCl_4/Hexane mL/mL	APS g	TMEDA µl	Time min	Resin, g (%)	Volume Increase[a]%	NH_2 µmol/g
1a 2.96 (0.140) DMAA 9.91 (0.799) EBA 1.29 (0.061)	89	521	154/264	0.25	510	90	**R1a**, 12.98 (92)	1160	696
1b 3.00 (0.187) DMAA 6.47 (0.748) EBA 0.95 (0.065)	120	680	215/360	0.22	780	105	**R1b**, 9.54 (92)	1520	932
1c 4.08 (0.138) DMAA 12.3 (0.797) EBA 1.72 (0.065)	122	680	205/360	0.34	680	90	**R1c**, 17.84 (98)	1260	894
2 1.00 (0.138) DMAA 1.80 (0.762) EBA 0.40 (0.100)	H_2O/DMF, (1.2/1.0), 34	110	64/128	0.16	320	120	**R2**, 3.21 (99)	1340	305

[a] Calculated from the formula $100(V_s - V_d)/V_d$ where V_s and V_d are the volumes of the swollen and dry resin, respectively.

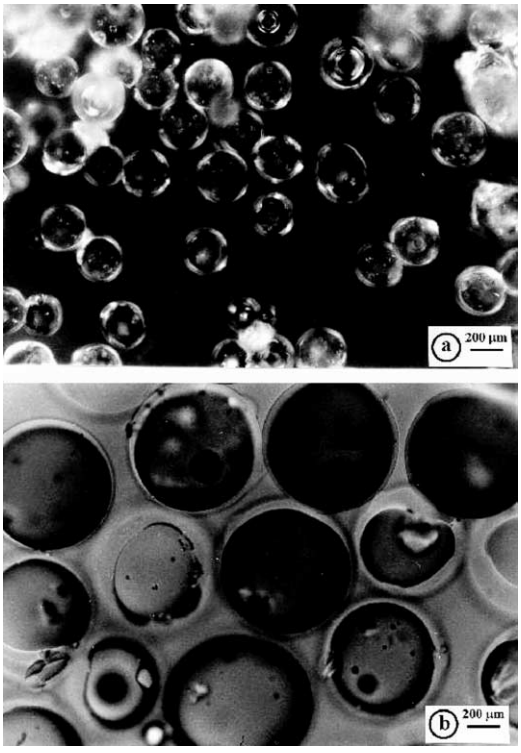


Figure 2. Optical microphotographs of resin **1b**/DMAA/EBA: (a) dry; (b) swollen in water.

Table 2. Substrate activities of some of the prepared polymeric materials as percentage of the activity of the best substrate for each enzyme

Copolymer or resin	Enzyme (substrate)	Substrate activity (%)
P1a	LO (a)	64
P1a	BAO (b)	63
P1c	LO (a)	970
P1c	BAO (b)	89
P1c	DAO (c)	30
R1a	LO (a)	100
R1a	BAO (b)	62

Enzymes: LO=lysyl oxidase from porcine aorta; BAO=benzylamine oxidase from porcine serum; DAO=diamine oxidase from porcine kidney. Best substrates: a=elastin; b=benzylamine; c=puterescine.

able to interact with the enzyme active site either as substrate or as reversible not denaturing inhibitor, we prepared four styrenic monomers with aminoalkyl residues (**1a–d**) having alkyl chains of different length designed as substrates and one styrenic monomer with a 2,6-dialkoxybenzylamine residue (**2**) designed as inhibitor.

The four monomers **1a–d** were transformed into soluble copolymers and microspherular water swellable resins with DMAA as comonomer, rejecting the materials from **1d** due to inadequate properties. Copolymers and resins obtained from **1a** and **1c** proved reactive as substrate of LO, BAO and DAO beyond all expectations.

For the monomer **2** which mimics selective substrate-like inhibitor of BAO, a multistep synthesis, based on the formylation of 3-methoxymethoxyanisole and flexible to structural modifications regarding type and length of the

linker, was performed. Monomer **2** easily afforded after copolymerization with DMAA microspherular beaded swellable resins whose biological behavior with CAOs is under study.

4. Experimental

4.1. Instruments and methods

Melting points and boiling points are uncorrected. FTIR spectra were recorded as films or KBr pellets on a Perkin Elmer System 2000 instrument. ^{1}H and ^{13}C-NMR spectra were acquired on a Bruker DPX spectrometer at 300 and 75.5 MHz respectively with tetramethylsilane as internal reference. Mass spectra were obtained with a GC-MS Ion Trap Varian Saturn 2000 instrument (EI or CI mode; filament current: 10 μA) equipped with a DB-5MS (J&W) capillary column. UV–Vis spectra were recorded with a Varian Cary 18 spectrometer. Microanalyses were obtained from the Laboratorio di Microanalisi (Faculty of Pharmacy, University of Pisa).

HPLC analyses were performed at room temperature, constant flow rate (1 mL/min) and UV detection (254 nm) using a 25×0.46 cm Hypersil ODS 5 μm column using a mixture acetonitrile/water=6/4 as eluent. GC-FID analyses were performed on Perkin Elmer Autosystem using a DB-5, 30 m, i.d. 0.32 mm, film 1 μm capillary column. Column chromatographies were performed on Merck silica gel (70–230 mesh). TLCs were obtained on Merck F_{254} silica gel plates.

Optical microphotographs were obtained with a Zeiss Axioskop instrument. Sieving was performed with a 2000 Basic Analytical Sieve Shaker-Retsch apparatus.

4.2. Materials

4-Chloromethylstyrene (**3a**) and all the other reagents and solvents were from Sigma-Aldrich and were purified by standard procedures. Azobisisobutyronitrile (AIBN) was crystallized from methanol. 4-(2-Bromoethyl)styrene (**3b**),[16] *N*,*N*′-ethylenebisacrylamide[20] (EBA), and *N*-succinimidyl-4-O-(4,4′-dimethoxytriphenylmethyl)butyrate[23] were prepared by known procedures.

Further acronyms and registered trademarks of commercial products used are: APS=ammonium persulfate; DMAA=*N*,*N*-dimethylacrylamide; DMF=*N*,*N*-dimethylformamide; TMEDA=*N*,*N*,*N*′,*N*′-tetramethylethylenediamine; SPAN 85=sorbitan trioleate.

4.3. 4-(ω-Bromoalkyl)styrenes 3c and 3d

A mixture of the α,ω-dibromoalkane (50 mmol), dry THF (20 mL) and a solution of $LiCuBr_2$[15] (1.5 mL) in dry THF was cooled to 0 °C, treated dropwise with 0.68 M 4-vinylphenyl magnesium chloride (18 mL, 12.2 mmol) in THF and stirred at room temperature for 5 h. The reaction mixture was then treated with an iced aqueous solution of NaCN (0.80 g) and NH_4Cl (5.00 g) dissolved in water (35 mL) and extracted with peroxide-free ethyl ether (4×30 mL). The extracts were dried over anhydrous $MgSO_4$, treated at reduced pressure to remove most of the unreacted dibromide and purified by column chromatography using petroleum ether 40–60 °C as eluent to afford monomers **3c** and **3d** as colorless liquids.

4.3.1. 4-(4-Bromobutyl)styrene (3c). 1.87 g (64%); bp=100 °C/0.15 torr, [lit.[24]: 92–93 °C/0.1 torr]. IR (film, ν, cm^{-1}) 990, 906 (CH_2=CH). ^{1}H NMR ($CDCl_3$, ppm) 1.76 (m, 2H); 1.86 (m, 2H); 2.61 (t, 2H, J=7.4 Hz); 3.39 (t, 2H, J=6.6 Hz); 5.19 (dd, 1H, J_1=1.0 Hz, J_{cis}=10.9 Hz); 5.70 (dd, 1H, J_1=1.0 Hz, J_{trans}=17.6 Hz); 6.68 (dd, 1H, J_{cis}=10.9 Hz, J_{trans}=17.6 Hz); 7.11–7.34 (m, 4H). ^{13}C NMR 29.74; 32.18; 33.60; 34.66; 113.05; 126.24; 128.54; 135.36; 136.61; 141.50. GC-MS (EI, *m*/*z*, %): 240 (M^+ [^{81}Br], 37); 238 (M^+ [^{79}Br], 34); 117 (100).

4.3.2. 4-(6-Bromohexyl)styrene (3d). 2.25 g (77%); bp=80 °C/0.06 torr. IR (film, ν, cm^{-1}) 990, 905 (CH_2=CH). ^{1}H NMR ($CDCl_3$, ppm) 1.29–1.51 (m, 4H); 1.62 (m, 2H); 1.85 (m, 2H); 2.59 (t, 2H, J=7.5 Hz); 3.39 (t, 2H, J=6.8 Hz); 5.18 (dd, 1H, J_1=1.0 Hz, J_{cis}=10.9 Hz); 5.70 (dd, 1H, J_1=1.0 Hz, J_{trans}=17.6 Hz); 6.69 (dd, 1H, J_{cis}=10.9 Hz, J_{trans}=17.6 Hz); 7.11–7.34 (m, 4H). ^{13}C NMR 28.02; 28.34; 31.14; 32.73; 33.88; 35.51; 112.88; 126.17; 128.55; 135.18; 136.71; 142.32. GC–MS (EI, *m*/*z*, %): 268 (M^+ [^{81}Br], 59); 266 (M^+ [^{79}Br], 57); 117 (100).

4.4. *N*-[(4-Vinylphenyl)alkyl]phthalimides 4a–d

A mixture of 4-haloalkylstyrene (**3a–d**) (26.6 mmol), potassium phthalimide (27.4 mmol) and dry DMF (25 mL) was heated at 55 °C under nitrogen and mechanical stirring for 17 h. After removal of the solvent at reduced pressure the solid residue was taken with chloroform (40 mL), filtered and washed with chloroform (3×10 mL). All the organic extracts were combined, washed with 0.2 M NaOH (15 mL), water (2×15 mL) and dried over anhydrous Na_2SO_4. The removal of the solvent at reduced pressure afforded **4a–d** as a crude solid which was crystallized from methanol (**4a–b**) or column chromatographed using benzene as eluent.

4.4.1. *N*-(4-Vinylbenzyl)phthalimide (4a). Reagent **3a**. Yield 81%. White flakes. Mp 107 °C; [lit.[25]: 107–108 °C]. Purity 99% by HPLC. IR (KBr, ν, cm^{-1}) 1704 (C=O), 995, 914 (CH_2=CH). ^{1}H NMR ($CDCl_3$, ppm) 4.82 (s, 2H); 5.21 (dd, 1H, J_{gem}=0.9 Hz; J_{cis}=10.9 Hz); 5.70 (dd, 1H, J_{gem}=0.9 Hz; J_{trans}=17.6 Hz); 6.66 (dd, 1H, J_{cis}=10.9 Hz; J_{trans}=17.6 Hz); 7.36 (m, 4H); 7.67–7.82 (m, 4H). ^{13}C NMR 41.31, 114.13, 123.31, 126.47, 128.84, 132.10, 133.96, 135.86, 136.31, 137.18, 167.98. GC-MS (CI, *m*/*z*, %): 264 (M^++1, 100). Anal. Calcd. for $C_{17}H_{13}NO_2$: C, 77.55; H, 4.98; N, 5.32. Found: C, 77.32; H, 5.00; N, 5.32.

4.4.2. *N*-[2-(4-Vinylphenyl)ethyl]phthalimide (4b). Reagent **3b**. Yield 75%. White flakes. Mp 135–137 °C. Purity 98% by HPLC. IR (KBr, ν, cm^{-1}) 1703 (C=O), 990, 907 (CH_2=CH). ^{1}H NMR ($CDCl_3$ ppm) 2.98 (t, 2H, J=7.8 Hz); 3.91 (t, 2H, J=7.8 Hz); 5.20 (dd, 1H, J_{gem}=1.0 Hz; J_{cis}=10.9 Hz); 5.70 (dd, 1H, J_{gem}=1.0 Hz; J_{trans}=17.6 Hz); 6.67 (dd, 1H, J_{cis}=10.9 Hz; J_{trans}=17.6 Hz); 7.21–7.32 (m, 4H); 7.69–7.82 (m, 4H). ^{13}C NMR 34.28,

39.14, 113.42, 123.22, 126.40, 129.01, 132.06, 133.90, 136.01, 136.54, 137.63, 168.14. GC-MS (CI, *m/z*, %): 278 ($M^+ + 1$, 100). Anal. Calcd. for $C_{18}H_{15}NO_2$: C, 77.96; H, 5.45; N, 5.05. Found: C, 77.86; H, 5.47; N, 5.04.

4.4.3. *N*-[4-(4-Vinylphenyl)butyl]phthalimide (4c). Reagent **3c**. Yield 63%. White grains. Mp 117–119 °C. Purity 98% by HPLC. IR (KBr, ν, cm^{-1}) 1703 (C=O), 992, 913 (CH_2=CH). ^1H NMR ($CDCl_3$ ppm) 1.69 (m, 4H); 2.64 (t, 2H, J=7.0 Hz); 3.71 (t, 2H, J=7.0 Hz); 5.18 (dd, 1H, J_{gem}=1.0 Hz; J_{cis}=10.9 Hz); 5.69 (dd, 1H, J_{gem}=1.0 Hz; J_{trans}=17.6 Hz); 6.68 (dd, 1H, J_{cis}=10.9 Hz; J_{trans}=17.6 Hz); 7.13–7.36 (m, 4H); 7.67–7.85 (m, 4H). ^{13}C NMR 28.14, 28.53, 35.07, 37.76, 112.94, 123.18, 126.20, 128.59, 132.14, 133.87, 135.27, 136.66, 141.73, 168.43. GC-MS (EI, *m/z*, %): 305 (M^+, 100). Anal. Calcd. for $C_{20}H_{19}NO_2$: C, 78.66; H, 6.27; N, 4.59. Found: C, 78.62; H, 6.26; N, 4.58.

4.4.4. *N*-[6-(4-Vinylphenyl)hexyl]phthalimide (4d). Reagent **3d**. Yield 59%. White needles. Mp 75–77 °C. Purity 99% by HPLC. IR (KBr, ν, cm^{-1}) 1707 (C=O), 988, 966 (CH_2=CH). ^1H NMR ($CDCl_3$, ppm) 1.30–1.72 (m, 8H); 2.58 (t, 2H, J=7.5 Hz); 3.67 (t, 2H, J=7.5 Hz); 5.18 (dd, 1H, J_{gem}=1.0 Hz; J_{cis}=10.9 Hz); 5.69 (dd, 1H, J_{gem}=1.0 Hz; J_{trans}=17.6 Hz); 6.68 (dd, 1H, J_{cis}=10.9 Hz; J_{trans}=17.6 Hz); 7.11 (m, 2H); 7.31 (m, 2H); 7.71 (m, 2H); 7.83 (m, 2H). ^{13}C NMR 26.72, 28.53, 28.79, 31.18, 35.54, 38.02, 112.81, 123.17, 126.13, 128.55, 132.19, 133.85, 135.10, 136.73, 142.43, 168.47. Anal. Calcd. for $C_{22}H_{23}NO_2$: C, 79.25; H, 6.95; N, 4.20. Found: C, 79.20; H, 6.93; N, 4.18.

4.5. Hydrazinolysis of Phthalimides 4a–d

Phthalimide **4a**–**d** (38.3 mmol) was dissolved in 95% ethanol (50 mL) and treated under nitrogen and stirring at reflux with a solution of hydrazine hydrate (2.74 g, 54.7 mmol) in 95% ethanol (5 mL) for 2.5 h up to the disappearance of **4a**–**d** (TLC, eluent benzene). After removal of the solvent at reduced pressure the solid residue was taken with chloroform (50 mL) and treated with 20% aqueous NaOH (50 mL). The aqueous phase was separated, extracted with chloroform (3×50 mL) and the extracts combined and dried over Na_2SO_4. The removal of chloroform afforded the free bases **5a** (90%), **5b** (92%), **5c** (80%) and **5d** (75%) as oils which were transformed into their hydrochlorides without distillation. The free base **5a** was vacuum distilled and characterized. Bp 58–60 °C/1 torr, [lit.[26]: 58–60 °C/0.7 torr]. ^1H NMR ($CDCl_3$, ppm) 1.76 (bs, 2H); 3.84 (s, 2H); 5.22 (dd, 1H, J_{gem}=0.9 Hz; J_{cis}=10.9 Hz); 5.72 (dd, 1H, J_{gem}=0.9 Hz; J_{trans}=17.6 Hz); 6.70 (dd, 1H, J_{cis}=10.9 Hz; J_{trans}=17.6 Hz); 7.24–7.39 (m, 4H).

4.6. Hydrochlorides 1a–d

A solution of the amine **5a**–**d** (25 mmol) in dry diethyl ether (500 ml) was cooled to 0 °C and treated under stirring up to saturation with dry gaseous hydrochloric acid. The white precipitate was filtered, washed with fresh ether, dried and crystallized to afford the hydrochloride derivative **1a**–**d**.

4.6.1. 4-Aminomethylstyrene hydrochloride (1a). Yield 89%. Mp 180 °C (dec.; 2-propanol); [lit.[27]: 160–170 °C (dec.)]. IR (KBr, ν, cm^{-1}) 989 and 901 (CH_2=CH). ^1H NMR (CD_3OD, ppm) 4.11 (s, 2H); 5.29 (dd, 1H, J_{gem}=0.90 Hz; J_{cis}=10.9 Hz); 5.83 (dd, 1H, J_{gem}=0.90 Hz; J_{trans}=17.6 Hz); 6.76 (dd, 1H, J_{cis}=10.9 Hz; J_{trans}=17.6 Hz); 7.42–7.52 (m, 4H). ^{13}C NMR 44.09, 115.36, 127.91, 130.35, 133.80, 137.37, 139.93. Anal. Calcd. for $C_9H_{12}ClN$: C, 63.72; H, 7.13; N, 8.26; Cl, 20.90. Found: C, 63.75; H, 7.12; N, 8.22; Cl, 20.88.

4.6.2. 4-Aminoethylstyrene hydrochloride (1b). Yield 77%. Mp 210 °C (ethanol); [lit.[16]: 210 °C]. IR (KBr, ν, cm^{-1}) 994 and 912 (CH_2=CH). ^1H NMR (CD_3OD, ppm) 2.92–3.03 (m, 2H); 3.12–3.23 (m, 2H); 5.21 (dd, 1H, J_{gem}=1.0 Hz; J_{cis}=10.9 Hz); 5.76 (dd, 1H, J_{gem}=1.0 Hz; J_{trans}=17.6 Hz); 6.72 (dd, 1H, J_{cis}=10.9 Hz; J_{trans}=17.6 Hz); 7.24–7.44 (m, 4H). ^{13}C NMR 34.22, 41.89, 114.08, 127.75, 130.03, 137.52, 137.70, 138.04. Anal. Calcd. for $C_{10}H_{14}ClN$: C, 65.39; H, 7.68; N, 7.63; Cl, 19.30. Found: C, 65.42; H, 7.69; N, 7.65; Cl, 19.30.

4.6.3. 4-Aminobutylstyrene hydrochloride (1c). Yield 80%. Mp 207–210 °C (acetonitrile); IR (KBr, ν, cm^{-1}) 985 and 905 (CH_2=CH). ^1H NMR (CD_3OD, ppm) 1.63–1.75 (m, 4H); 2.57–2.69 (m, 2H); 2.90–2.95 (m, 2H); 5.16 (dd, 1H, J_{gem}=1.1 Hz; J_{cis}=10.9 Hz); 5.71 (dd, 1H, J_{gem}=1.1 Hz; J_{trans}=17.6 Hz); 6.69 (dd, 1H, J_{cis}=10.9 Hz; J_{trans}=17.6 Hz); 7.10–7.36 (m, 4H). ^{13}C NMR 28.08, 29.16, 35.89, 40.70, 113.26, 117.32, 129.70, 136.97, 137.99, 142.63. Anal. Calcd. for $C_{12}H_{18}ClN$: C, 68.07; H, 8.57; N, 6.62; Cl, 16.74. Found: C, 68.05; H, 8.58; N, 6.64; Cl, 16.78.

4.6.4. 4-Aminohexylstyrene hydrochloride (1d). Yield 80%. Mp 150–153 °C (acetonitrile); IR (KBr, ν, cm^{-1}) 990 and 900 (CH_2=CH). ^1H NMR (CD_3OD, ppm) 1.30–1.50 (m, 4H); 1.55–1.75 (m, 4H); 2.61 (t, 2H, J=7.4 Hz); 2.90 (t, 2H, J=7.4 Hz); 5.15 (dd, 1H, J_{gem}=1.1 Hz; J_{cis}=10.9 Hz); 5.70 (dd, 1H, J_{gem}=1.1 Hz; J_{trans}=17.6 Hz); 6.69 (dd, 1H, J_{cis}=10.9 Hz; J_{trans}=17.6 Hz); 7.12–7.33 (m, 4H). ^{13}C NMR 27.31, 28.53, 29.69, 32.27, 36.42, 40.79, 113.05, 117.20, 129.63, 136.69, 138.08, 143.53. Anal. Calcd. for $C_{14}H_{22}ClN$: C, 70.13; H, 9.25; N, 5.84; Cl, 14.78. Found: C, 70.09; H, 9.26; N, 5.82; Cl, 14.81.

4.7. 2-Methoxy-6-hydroxybenzylamine hydrochloride (10)

A solution of 2-methoxy-6-methoxymethoxybenzaldehyde (**7**)[19] (1.1425 g; 5.8 mmol) in 95% ethanol (12 mL) was treated with a solution of hydroxylamine hydrochloride (0.4864 g, 7.0 mmol) in dry pyridine (2.3 mL) under stirring at room temperature for 90 min and at 0 °C for 30 min to facilitate the oxime precipitation. The white solid was filtered, dried, weighed (0.7210 g) and used without further purification. The mother liquors were concentrated to afford additional 0.2208 g of 2-methoxy-6-methoxymethoxybenzaldoxime (**8**) for an overall yield of 77%. Mp 131–135 °C; IR (ν, cm^{-1}) 3178, 1626, 1596, 1582, 1478, 1071.

A solution of **8** (3.13 g; 14.8 mmol) in 95% ethanol (43 mL) was treated with an equal volume of 2 M NaOH followed by

Raney nickel alloy (4.67 g) under stirring at room temperature for 90 min. The Raney nickel alloy was removed by filtration and washed with fresh ethanol. Filtrate and washings were combined, acidified with 0.8 M HCl (230 ml) and extracted with CH_2Cl_2 (30 mL). The aqueous phase was treated with solid KOH up to pH=14 and extracted with diethyl ether (3×30 mL). The extracts after drying over anhydrous Na_2SO_4 and removal of the solvent afforded 2-methoxy-6-methoxymethoxybenzylamine (**9**) (2.64 g, 90%). Bp 70 °C/0.02 torr.. Purity 98% by GC-FID. IR (ν, cm^{-1}) 3378, 3310, 1596, 1474, 1069. ^1H NMR ($CDCl_3$ ppm) 1.62 (bs, 2H); 3.48 (s, 3H); 3.82 (s, 3H); 3.89 (s, 2H); 5.20 (s, 2H); 6.58 (d, 1H, J_o=8.0 Hz); 6.73 (d, 1H, J_o=8.0 Hz); 7.14 (t, 1H, J_o=8.0 Hz).

A mixture of **9** (2.93 g, 14.9 mmol), methanol (150 mL) and hydrochloric acid (5 mL) was heated at 62 °C under stirring for 20 min up to the disappearance of **9** (TLC, eluent benzene/ethyl acetate=90/10). After removal of the solvent at reduced pressure the solid residue was dissolved in the minimum amount of DMF and precipitated in chloroform to afford **10** in the form of pearly flakes (2.03 g). The mother liquor after concentration and cooling afforded additional 0.39 g of **10** for an overall yield of 86%. Mp 211–214 °C. IR (ν, cm^{-1}) 3197, 3026, 1602, 1573, 1503, 1473, 1120. ^1H NMR (CD_3OD, ppm) 3.86 (s, 3H); 4.16 (s, 2H); 4.88 (s, 4H); 6.54 (m, 2H); 7.19 (m, 1H). ^{13}C NMR 33.66, 56.30, 103.12, 108.60, 109.21, 131.99, 158.21, 160.33.

4.7.1. 2-Methoxy-6-[(4-vinyl)benzyloxy]benzylamine hydrochloride (2). Sodium hydride (1.0112 g, 25.3 mmol) as a 60% dispersion in mineral oil was washed three times with pentane under nitrogen and suspended in dry DMF (84 mL). The suspension was added with a solution of **10** (2.40 g, 12.6 mmol) in dry DMF (24 mL), stirred for 90 min and treated with **3a** (1.9292 g, 12.6 mmol) under stirring at 40 °C for 23 h. The reaction mixture was hydrolyzed with 10% aqueous NaOH (40 mL) and extracted with peroxide-free diethyl ether. The extracts after drying over anhydrous Na_2SO_4 and removal of the last traces of DMF under vacuum afforded **11** as crude oil (2.3056 g) soon converted into its hydrochloride **2** as described for **1a–d**. Yield 43%. Mp 215–218 °C (acetonitrile). Purity 99% by HPLC. IR (KBr, ν, cm^{-1}) 990 and 924 (CH_2=CH). ^1H NMR (CD_3OD, ppm) 3.90 (s, 3H), 4.20 (s, 2H); 5.16 (s, 2H); 5.23 (dd, 1H, J_{gem}=1.0 Hz; J_{cis}=10.9 Hz); 5.82 (dd, 1H, J_{gem}=1.0 Hz; J_{trans}=17.6 Hz); 6.70–6.80 (m, 3H); 7.33–7.45 (m, 5H). ^{13}C NMR 33.41, 56.45, 71.50, 105.06, 106.50, 110.26, 114.46, 127.43, 128.95, 132.46, 137.70, 137.73, 138.95, 159.05, 160.28. Anal. Calcd. for $C_{17}H_{20}ClNO_2$: C, 66.77; H, 6.59; N, 4.58; Cl, 11.59. Found: C, 66.80; H, 6.58; N, 4.60; Cl, 11.62.

4.7.2. 2-Methoxy-6-[(4-vinyl)benzyloxy]benzaldehyde (12). A solution of 2-hydroxy-6-methoxybenzaldehyde[19] (0.130 g, 0.84 mmol) in dry DMF (1 mL) was treated with NaH (0.033 g, 1.10 mmol) as an 80% mineral oil dispersion at rt for 1 h. The suspension was cooled to 0 °C, **3a** (0.160 g, 1.10 mmol) was added and the mixture was heated at 40 °C for 48 h checking the progress of the reaction by TLC (benzene/ethyl acetate 70/30 as eluent). The mixture was hydrolyzed with 1 M HCl (4 mL), extracted with diethyl ether (3×8 mL) and dried over Na_2SO_4. After removal of the solvent at reduced pressure, the crude oil was purified by column chromatography (eluent chloroform) to afford 12 (0.150 g, 75%). Purity 94% by HPLC. IR (ν, cm^{-1}) 2773 (aldehydic CH), 1686 (C=O), 1596, 1475, 1109. ^1H NMR ($CDCl_3$, ppm) 3.90 (s, 3H); 5.16 (s, 2H); 5.26 (dd, 1H, J_{gem}=0.8 Hz; J_{cis}=10.8 Hz); 5.76 (dd, 1H, J_{gem}=0.8 Hz; J_{trans}=17.6 Hz); 6.61 (m, 2H); 6.72 (dd, 1H, J_{cis}=10.8 Hz; J_{trans}=17.6 Hz); 7.40–7.50 (m, 5H); 10.59 (s, 1H). GC-MS (EI, *m/z*, %): 268 (M^+, 4), 117 (100).

4.8. General procedure for solution polymerizations of 1a–d or 2 and copolymerizations with DMAA

Degassed monomers, solvent and initiator were introduced in the desired ratios under nitrogen in the polymerization flask and magnetically stirred. After a suitable period the mixture was poured into diethyl ether and the polymer was filtered, submitted to two dissolution/precipitation cycles with methanol/diethyl ether and vacuum-dried at room temperature.

4.9. General procedure for reverse-phase suspension copolymerizations

A mixture of hexane and CCl_4 was placed in a round-bottom cylindrical flanged reactor equipped with an anchor-type mechanical stirrer and nitrogen inlet, thermostated at 35 °C and deoxygenated by nitrogen bubbling for 30 min. A solution obtained by dissolving under nitrogen the monomer **1a–d** or **2**, DMAA, EBA, and APS in deoxygenated water distilled over $KMnO_4$, was siphoned into the reaction vessel. The density of the organic phase was adjusted by addition of CCl_4 so that the aqueous phase sank slowly when the stirring was stopped. The polymerization was started by setting the mechanical stirring at 900 rpm, introducing SPAN 85 and after 10 min TMEDA, and continuing the polymerization for 90 min. The resin was filtered, washed with 2-propanol, chloroform, water, absolute ethanol, chloroform, 2-propanol and acetone in order, then dried at reduced pressure and room temperature for 16–20 h and sieved. Table 1 collects data of some representative experiments.

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Green oxidations. The use of potassium permanganate supported on manganese dioxide

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Abstract—Permanganate supported on active manganese dioxide can be used effectively for the oxidation of organic compounds under heterogeneous or solvent-free conditions. The residue that remains after extraction of the organic products, manganese dioxide, can be recycled, making the process infinitely sustainable, in theory. The use of this approach for the oxidation of arenes, alcohols and sulfides is described.

1. Introduction

During the past few decades, alternative experimental methods with reduced environmental impact have been developed for many reactions. However, similar progress has not been made on the modification of organic oxidations since the removal of electrons from stable molecules is relatively difficult, requiring vigorous reagents and/or forcing conditions. There is, therefore, little likelihood that it will ever be possible to use mild reagents and conditions for most oxidation reactions.

As an alternative to seeking gentle reactions that have low environmental impact, we have been searching for reactions that are easily insulated from the environment and which consume less material. For example, previously reported work from our laboratories and others has indicated that carrying out oxidation reactions under solvent-free conditions decreases environmental impact by reducing the need to deal with contaminated solvents.[1] When oxidation reactions are carried out under homogeneous conditions in polar solvents, such as aqueous acetone, the solvents must be cleaned up and disposed of or, preferably, recycled at the conclusion of the reactions. However, when the reactions are conducted under solvent-free conditions, no solvents are contaminated during the reaction. (Some solvent is usually required to separate the organic products from the spent oxidant; however, the amount of solvent required is reduced and it usually contains fewer contaminants.)

Homogeneous permanganate oxidations must be carried out in acidic or basic aqueous solutions,[2] and the corresponding heterogeneous reactions require the use of chemically inert solvents such as methylene chloride.[3–5] However, under solvent-free conditions, oxidations are done in the absence of solvent by using a reagent that consists of potassium permanganate and an approximately equal amount of a solid support such as alumina or silica. A minimum amount of organic solvent is then required to extract the products from the spent oxidant. In addition, solvents such as diethyl ether can be employed to extract the products, thus reducing the need to use chlorinated hydrocarbons.

It has recently been reported that the co-product obtained when organic compounds are oxidized, manganese dioxide, can be recycled under industrial conditions to regenerate permanganate.[6] Manganese dioxide is regenerated in a two-stage process that involves air oxidation to potassium manganate(VI) in a concentrated potassium hydroxide solution, followed by electrochemical oxidation to potassium permanganate.[7]

In previous descriptions of solvent-free permanganate reactions, procedures for separating manganese dioxide from solid supports, such as alumina or silica, have not been reported. Our experience with these reactions suggests that such a separation will not be easily achieved and we have, consequently, begun to investigate other approaches. The

Keywords: Manganese dioxide; Potassium permanganate; Solid support; Arenes.
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most successful of our attempts, to date, has been realized by substituting manganese dioxide as the solid support.

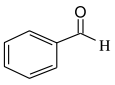
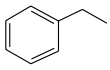
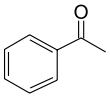
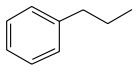
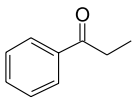
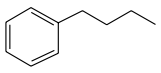
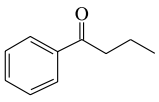
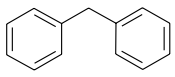
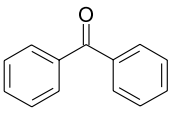
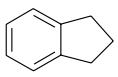
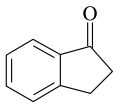
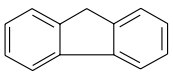
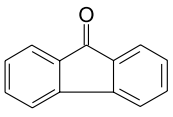
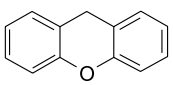
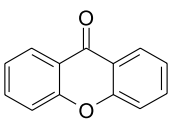
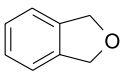
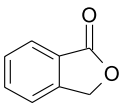
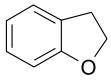
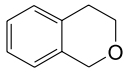
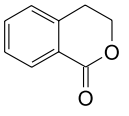
The combination of potassium permanganate and active manganese dioxide produces a reagent that can be used effectively for the oxidation of a variety of organic compounds. When organic compounds are oxidized by this reagent under solvent-free conditions, the solid coproduct remaining after the organic products have been separated is manganese dioxide containing residual amounts of potassium permanganate. This coproduct can then be recycled, making the process infinitely sustainable, in theory.

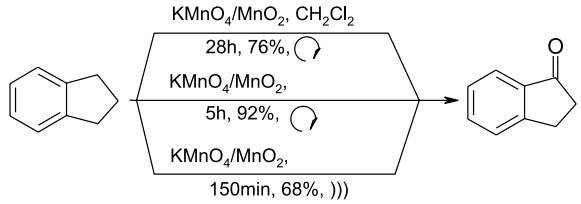
While recognizing that this approach may require further refining before it can be used commercially, we wish, at this time, to report the results that have been obtained for the oxidation of arenes, alcohols and sulfides. For comparison purposes, the results obtained for oxidation of the corresponding compounds under heterogeneous conditions is included.

2. Results and discussion

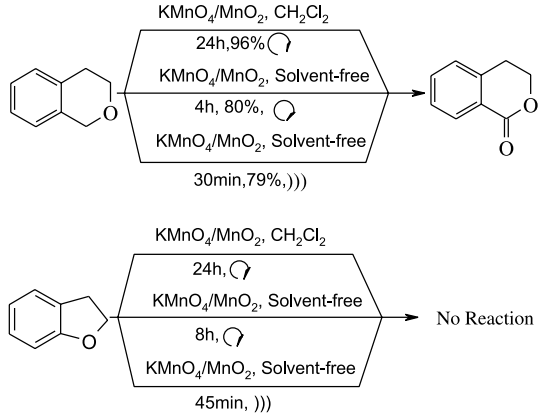
As can be seen from results reported in Table 1, alkyl arenes are converted into the corresponding α-ketones in good yields at room temperature. The products obtained under solvent-free conditions are identical to those obtained from heterogeneous reactions where the oxidant is dispersed in methylene chloride. In addition, the reaction times are reduced from a day or more to a few hours for many of the reactions, as summarized in Scheme 1.

Table 1. Oxidation of arenes to the corresponding carbonyl compounds by $KMnO_4/MnO_2$ under heterogeneous conditions (A), under conventional solvent-free conditions (B) and under solvent-free conditions assisted by ultrasonic irradiation (C)

Entry	Reactant	Product	Yield (%)		
			(A) (time, h)	(B) (time, h)	(C) (time, min)
1			25 (48)	21 (30)	0 (42)
2			89 (23)	56 (5.5)	62 (50)
3			85 (24)	62 (6)	65 (58)
4			92 (27)	70 (8)	78 (85)
5			93 (22)	85 (5)	85 (38)
6			76 (28)	92 (5)	68 (150)
7			96 (22)	85 (6)	82 (75)
8			93 (16)	94 (6)	80 (75)
9			81 (16)	80 (4)	70 (68)
10		No reaction	0 (24)	0 (8)	0 (45)
11			96 (24)	80 (4)	89 (30)



Scheme 1.



Scheme 2.

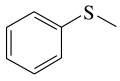
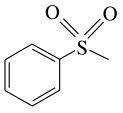
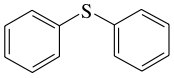
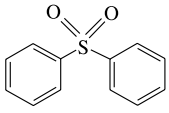
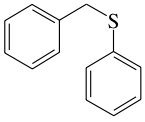
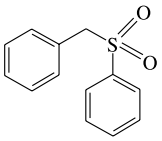
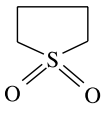
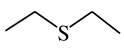
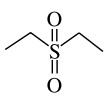
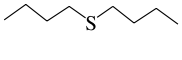
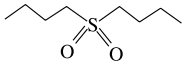
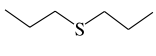
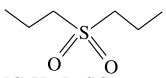
As indicated in Scheme 2, the reactions display a selectivity similar to that previously observed when oxygen is part of the bicyclic system. When oxygen is in the β-position, lactones are obtained in good yields; when it is in the α-position the reaction is completely inhibited.[4,5]

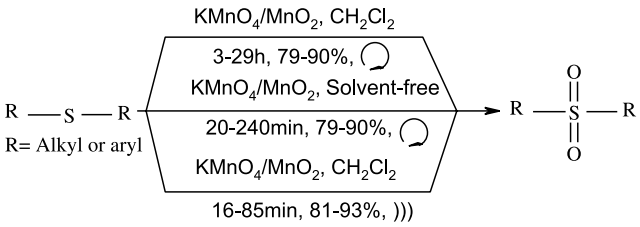
Application of ultrasonic irradiation in these reactions decreases the time required to obtain good yields by a factor of about 10. As a consequence, it is possible to select the most appropriate conditions for a particular application. If time is of little importance, the reaction can be completed without expenditure of additional energy; however, if time is an important factor, energy can be applied in the form of ultrasonic irradiation.

As can be seen from Table 2, aryl and alkyl sulfides are converted into the corresponding sulfones in good yields by $KMnO_4$ supported on MnO_2, with slightly shorter times being required when the reaction is subjected to ultrasonic irradiation.

This is a highly useful reaction for the preparation of sulfones which are important intermediates in the synthesis of many organic compounds.[8] The observation that benzyl phenyl sulfide (entry 3) is oxidized to the corresponding sulfone suggests that the reaction likely proceeds by way of an oxygen transfer mechanism. If the reaction involved electron transfer instead of oxygen transfer, substantial

Table 2. Oxidation of sulfides to the corresponding sulfones by $KMnO_4/MnO_2$ under heterogeneous conditions (A), under conventional solvent-free conditions (B) and under solvent-free conditions with the assistance of ultrasonic irradiation (C)

Entry	Reactant	Product	Yield (%)		
			(A) (time, h)	(B) (time, min)	(C) (time, min)
1			93 (3)	83 (25)	91 (24)
2			94 (8)	90 (60)	81 (45)
3			72 (29)	82 (240)	86 (85)
4			90 (4.5)	83 (25)	93 (25)
5			90 (3.5)	84 (20)	90 (16)
6			92 (4)	90 (45)	86 (38)
7			86 (4.5)	79 (30)	83 (27)
8	$[C_8H_{17}]_2$ S	$[C_8H_{17}]_2$ SO_2	89 (4)	85 (30)	93 (28)



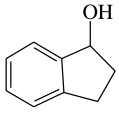
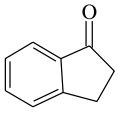
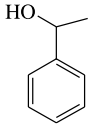
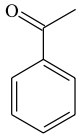
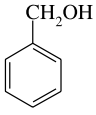
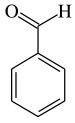
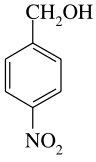
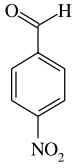
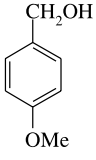
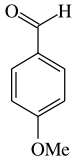
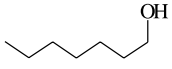
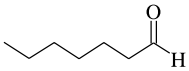
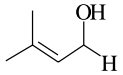
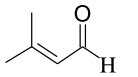
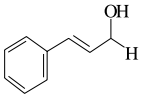
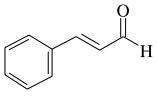
Scheme 3.

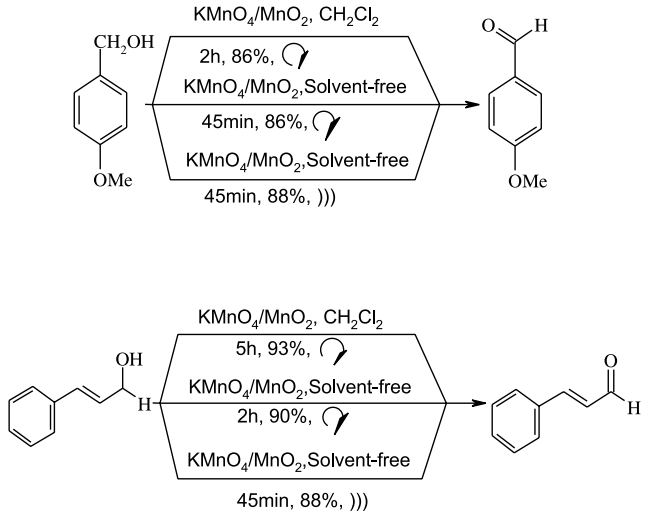
amounts of benzaldehyde would have been formed.[9,10] Most standard oxidations of sulfides under solvent-free conditions result in the formation of sulfoxides;[11–13] however, sulfoxides were not produced in these reactions.

Both manganese dioxide and potassium permanganate will oxidize sulfides. However, the reaction of active manganese dioxide with sulfides is known to give only sulfoxides. Consequently, the observation that only sulfones are obtained in these reactions, as summarized in Scheme 3, suggests that the products are produced from the reaction of sulfides with $KMnO_4$, and not by a reaction of sulfides with MnO_2.

As indicated from the results reported in Table 3, secondary alcohols are efficiently converted into the corresponding ketones in good yields and aldehydes are selectively obtained from the oxidation of primary alcohols. The preparation of both aliphatic and aromatic aldehydes from the corresponding primary alcohols has not previously been easily achieved using permanganate or other strong oxidants because aldehydes are readily converted to carboxylic acids under oxidizing conditions. In addition, it was found that both primary and secondary α,β-unsaturated alcohols were oxidized to the corresponding carbonyl compounds without disruption of the double bond (entries 8–10). These results are summarized in Scheme 4.

Table 3. Oxidation of alcohols to the corresponding carbonyl compounds by $KMnO_4/MnO_2$ under heterogeneous conditions (A), under conventional solvent-free conditions (B) and under solvent-free conditions assisted by ultrasonic irradiation (C)

Entry	Reactant	Product	Yield (%)		
			(A) (time, h)	(B) (time)	(C) (time, min)
1			83 (4)	94 (50 min)	90 (43)
2			92 (5)	91 (35 min)	86 (30)
3			74 (5)	78 (60 min)	94 (63)
4			52 (14)	67 (3 h)	46 (150)
5			86 (2)	86 (45 min)	88 (45)
6			93 (24)	90 (6 h)	68 (120)
8			82 (17)	79 (4 h)	64 (150)
9			87 (8)	90 (2.5 h)	72 (45)
10			93 (5)	90 (2 h)	95 (105)



Scheme 4.

Active manganese dioxide in the absence of permanganate is able to oxidize primary and secondary alcohols to the corresponding aldehydes and ketones under solvent-free conditions.[14] However, the time required to complete the reaction is often a few days instead of 1 h or less as observed when permanganate is present.

3. Conclusions

1. A mixture of potassium permanganate (1 g) and activated manganese dioxide (3 g) can be used as an effective oxidant for arenes, sulfides and alcohols under both heterogeneous and solvent-free conditions. Removal of the organic products by extraction leaves a residue that consists primarily of manganese dioxide containing small amounts of potassium permanganate.
2. Ultrasound irradiation increases the rate at which products are formed.

Table 4. Characterization of products

Product	^{1}H NMR (ppm)	IR (cm^{-1})	Melting points
Benzaldehyde	7.20–7.79 (m, 5H), 9.94 (s, 1H)	3030, 2778, 2703, 1695, 1667, 1587, 1449, 1389, 1299, 1205, 1163, 1075	2,4-DNP derivative 234–236 °C (lit. 237 °C)[15]
Acetophenone	2.43 (s, 3H), 7.15–7.80 (m, 5H)	3100, 1700, 1600, 1580, 1450, 1360, 1300, 950	2,4-DNP derivative 247–250 °C (lit. 250 °C)[16]
Propiophenone	1.18 (t, $^3J_{HH}$=5.1 Hz, 3H) 2.94 (q, $^3J_{HH}$=5.2 Hz, 2H) 7.47–7.92 (m, 5H)	2980, 1690, 1595, 1450, 1215, 950	2,4-DNP derivative 187–188 °C (lit. 187–189 °C)[15]
Butyrophenone	0.95 (t, $^3J_{HH}$=5.1 Hz, 3H) 1.41–1.96 (m, 2H) 2.82 (t, $^3J_{HH}$=5.1 Hz, 2H) 7.12–7.87 (m, 5H)	2960, 1700, 1610, 1455, 1220, 1010, 700	2,4-DNP derivative 198–200 °C (lit. 200 °C)[16]
Benzophenone	7.20–7.78 (m, 10H)	3060, 1655, 1600, 1450, 1270, 800	2,4-DNP derivative 237–238 °C (lit. 239 °C)[16]
1-Indanone	2.63 (t, $^3J_{HH}$=6.1 Hz, 2H) 3.09 (t, $^3J_{HH}$=6.1 Hz, 2H) 7.08–7.80 (m, 4H)	2925, 1710, 1600, 1450, 1270, 750	2,4-DNP derivative 253–256 °C (lit. 258 °C)[16]
9-Fluorenone	7.10–7.70 (m, 8H)	3050, 1700, 1600, 1440, 1290, 860	81–82 °C (lit. 84 °C)[17]
Xanthone	7.36–8.32 (m, 8H)	1655, 1610, 1480, 1450, 1340, 1140, 760	172–173 °C (lit. 174 °C)[17]
Phthalide	5.32 (s, 2H) 7.35–7.86 (m, 4H)	1760, 1460, 1440, 1310, 1050, 745	73–74 °C (lit. 75 °C)[17]
1-Isochromaone	3.10 (t, $^3J_{HH}$=8.5 Hz, 2H) 4.56 (t, $^3J_{HH}$=8.5 Hz, 2H) 7.20–8.20 (m, 4H)	2945, 1716, 1602, 1455, 1389, 1289, 1237, 1115	175–176 °C (lit. 176 °C)[15]
Methyl phenyl sulfone	3.05 (s, 3H) 7.61–7.94 (m, 5H)	3091, 3066, 2928, 1585, 1480, 1449	85–87 °C (lit. 86 °C)[18]
Diphenyl sulfone	7.94 (m, 4H) 7.56–7.41 (m, 6H)	1450, 1310, 1150, 1110, 1000	128–129 °C (lit. 128 °C)[17]
Benzy phenyl sulfone	4.32 (s, 2H) 7.08–7.68 (m, 10H)	3088, 3006, 2969, 1606, 1495	144–145 °C (lit. 146 °C)[17]
Tetramethylene sulfone	2.20 (m, 4H) 3.01 (m, 4H)	3436, 2973, 2882, 1669, 1464, 1309	—
Diethyl sulfone	1.42 (t, $^3J_{HH}$=7.50 Hz, 4H) 3.00 (q, $^3J_{HH}$=7.47 Hz, 6H)	3241, 2996, 1672, 1479, 1378, 1286	73–75 °C (lit. 74 °C)[15]
Dibutyl sulfone	0.97 (t, $^3J_{HH}$=6.7 Hz, 6H) 1.17–1.96 (m, 8H) 2.95 (t, $^3J_{HH}$=10.1 Hz, 4H)	2960, 2880, 1460, 1410, 1300, 1130	45–46 °C (lit. 46 °C)[17]
Dipropyl sulfone	1.09 (t, $^3J_{HH}$=6.5 Hz, 6H) 1.46–2.11 (m, 4H) 2.95 (t, $^3J_{HH}$=6.7 Hz, 4H)	2960, 2880, 1460, 1410, 1280, 1125	29–30 °C (lit. 30 °C)[15]
Dioctyl sulfone	0.89 (t, $^3J_{HH}$=5.93 Hz, 6H) 1.20–1.92 (m, 24H) 2.88–3.00 (m, 4H)	2910, 1463, 1313, 1264, 1122, 771	74–76 °C (lit. 74 °C)[17]
4-Nitrobenzaldehyde	8.09–8.39 (m, 4H) 10.18 (s, 1H)	2850, 2720, 1705, 1600, 1540, 1350, 1200	104–105 °C (lit. 106 °C)[15]
4-Methoxybenzaldehyde	3.83 (s, 3H) 6.92–7.72 (m, 2H) 9.80 (s, 1H)	2850, 2750, 1700, 1610, 1510, 1255	2,4-DNP derivative 190–191 °C (lit. 191 °C)[16]
Cyclohexanone	1.79–2.25 (m, 10H)	2940, 1710, 1450, 1310, 1220, 1120	2,4-DNP derivative 160–161 °C (lit. 162 °C)[19]
3-Methyl-2-butenal	9.96 (d, 1H) 5.87 (m, 1H) 2.18 (m, 3H) 1.99 (m, 3H)	3360, 3302, 2855, 2722, 1890, 1693	—
Acrolein	6.11–6.68 (m, 3H) 9.54 (m, 1H)	3360, 3060, 2800, 1690, 1420, 1360	—
Cinnamaldehyde	6.29–6.91 (m, 1H) 7.30–7.58 (m, 1H) 9.67 (d, $^3J_{HH}$=6.7 Hz, 1H)	3025, 2805, 2740, 1680, 1630, 1450	2,4-DNP derivative 252–254 °C (lit. 255 °C)[16]
Heptanal	0.90 (t, $^3J_{HH}$=5.1 Hz, 3H) 1.34–2.50 (m, 8H) 4.60–4.90 (m, 2H) 9.72 (t, $^3J_{HH}$=5.1 Hz, 1H)	2950, 2725, 1750, 1460	2,4-DNP derivative 106–107 °C (lit. 108 °C)[16]

3. Since industrial processes for recycling and reoxidizing manganese dioxide to permanganate are well established, the reactions are infinitely sustainable, in theory.

4. Experimental

4.1. Preparation of oxidant

The oxidant was prepared by grinding potassium permanganate (1.0 g, 6.3 mmol) and active manganese dioxide (3.00 g, 34.5 mmol) in a mortar until a homogeneous powder was obtained. This reagent was used for all of the oxidations described herein. Active manganese dioxide, obtained commercially, is a reagent that is produced by the Carus Chemical Company of La Salle, Il, USA.

4.2. Procedure A. The oxidation of organic compounds by $KMnO_4/MnO_2$ under heterogeneous conditions

Substrate (2.0 mmol) was dissolved in CH_2Cl_2 (25 mL) and placed in a round-bottomed flask fitted with a magnetic stirrer. The oxidant, finely grounded $KMnO_4/MnO_2$ reagent (4.0 g), was added in small portions over a period of 15 min. The mixture was stirred vigorously at room temperature while the progress of the reaction was monitored by TLC. Upon completion of the reaction, the product was filtered through a sintered glass funnel to remove spent oxidant. The residue was then washed successively with CH_2Cl_2 ($2\times$ 20 mL). Evaporation of the solvent gave product that was characterized by the use of spectroscopy and melting points, as described below.

4.3. Procedure B. The oxidation of organic compounds by $KMnO_4/MnO_2$ under solvent-free conditions

Substrate (2.0 mmol) was added to the oxidant, a mixture of $KMnO_4$ and MnO_2 (4.0 g), in a 25 mL round bottomed flask. These reactants were then mixed by magnetic stirring at room temperature. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the residue was washed with a minimum amount of methylene chloride or diethyl ether. After filtration to remove any spent oxidant, the solvent was evaporated. Product of acceptable purity for most purposes was obtained. If greater purity is required, the product can be distilled or recrystallized. The products were characterized as described below.

4.4. Procedure C. The oxidation of organic compounds by $KMnO_4/MnO_2$ under solvent-free conditions and assisted by ultrasound irradiation

Substrate (2.0 mmol) and oxidant, a mixture of $KMnO_4$ and MnO_2 (4.0 g), were thoroughly mixed together and the reaction mixture was irradiated in a 25 mL beaker for an appropriate period at room temperature. The progress of the reaction was monitored by TLC. When complete, the reaction mixture was washed with methylene chloride or diethyl ether (2×20 mL). After filtration to separate spent oxidant, the solvent was evaporated. Relatively pure product was obtained and characterized as described below.

4.5. Characterization of products

The products of these reactions were characterized from their ^1H NMR and IR spectra and by comparison of their melting points (or those of their derivatives) with known compounds, as indicated in Table 4.

Acknowledgements

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New stereoselective methodology for the synthesis of dihydroxerulin and xerulin, potent inhibitors of the biosynthesis of cholesterol

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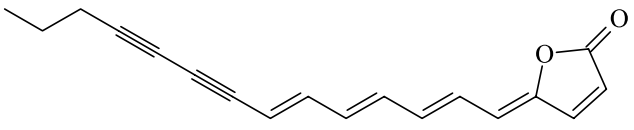
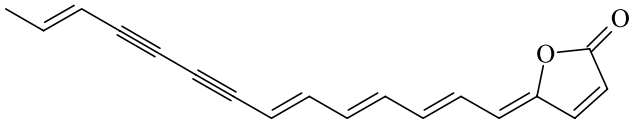
Abstract—A new stereoselective methodology for the synthesis of both dihydroxerulin and xerulin has been devised. The key step required cross-coupling reactions between the bromo trienyne, (1*E*,3*E*,5*E*)-1-bromo-8-trimethylsilyl-1,3,5-octatrien-7-yne and the appropriate conjugated diynes. The palladium-catalyzed tandem cross-coupling/cyclization reaction of the resulting polyenynes with (*Z*)-3-iodo-2-propenoic acid led directly to dihydroxerulin or xerulin with a high degree of stereoselectivity.

1. Introduction

A large number of natural products of medicinal and biological interest contain the butenolide moiety.[1] In particular, a wide variety of stereodefined γ-alkylidene butenolides have been isolated from natural sources and have shown interesting biological activities such as free-lingyne[2] and tetrenolin[3] which display antibiotic activity, or dihydroxerulin **1** and xerulin **2**, isolated in 90:10–65:35 mixtures from cultures of *Xerula melanotricha*,[4] potent noncytotoxic inhibitors of the biosynthesis of cholesterol. Recently, Siegel and Brückner reported the first total synthesis of dihydroxerulin[5] and of xerulin[6] employing Wittig olefination as a final step. The same olefination approach has been followed by Rossi and co-workers[7] for the synthesis of dihydroxerulin, whereas a different methodology has been reported by Negishi and co-workers[8] for the synthesis of xerulin.



1 *Dihydroxerulin*



2 *Xerulin*

In our previous studies on the synthesis of stereodefined compounds[9–12] in which we succeeded in synthesizing a series of natural compounds[10] with a conjugated unsaturated structure, starting from unsaturated bis-silyl derivatives, we have also reported an efficient and stereoselective approach to a variety of silylated polyunsaturated γ-alkylidene butenolides.[11] Herein, we now report a new stereoselective methodology for the total synthesis of both dihydroxerulin and xerulin.

2. Results and discussion

Our overall retrosynthesis is summarized in Scheme 1.

The xerulins differ in the terminal R group, a *n*-propyl group for the dihydroxerulin **1** or an (*E*)-propenyl group for the xerulin **2**. Thus, the disconnection of the butenolide moiety leads to the fragments **3** and **4**. A further disconnection of the C_8–C_9 bond of the polyunsaturated compounds **3** leads to the conjugated diynes **5** and to silylated bromo trienyne **6**.

Accordingly, the synthesis of both the xerulins employing the same methodology can be realized starting with a

Keywords: Silicon and compounds; Stereoselective synthesis; Diynes; Xerulins.
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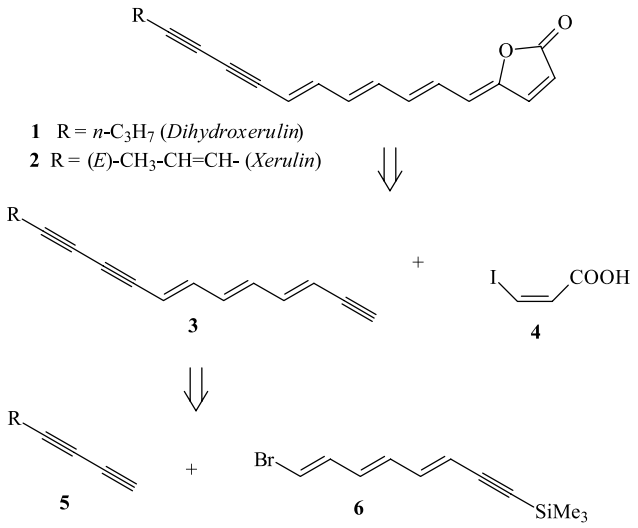


Scheme 1.

Sonogashira[13] coupling reaction between the appropriate diynes **5** and the readily available silylated bromo trienyne **6**,[14,15] which leads to the polyunsaturated compounds **3**. The tandem cross-coupling/cyclisation reaction[11,16] between the compounds **3** and (*Z*)-3-iodo-2-propenoic acid[17] can directly give both the xerulins **1** and **2**.

The synthesis of the diynes **5a**[5] and **5b**[8] was performed as depicted in Scheme 2 and based upon our recently published procedure for the synthesis of conjugated diynes.[12]

To synthesize both compounds **5a** and **5b** it was sufficient to start with a common intermediate, the commercially available 1,4-bis-trimethylsilyl-1,3-butadiyne **7a**. To obtain compound **5a**, the diyne **7a** was selectively desilylated with MeLi–LiBr complex affording the lithium salt of the mono-silylated terminal diyne[18] which was coupled with *n*-propylbromide to give the mono-silylated heptadiyne **8a** in 60% yield. A further desilylation reaction of **8a** with KF in MeOH led to compound **5a** (95% yield). In a similar manner compound **5b** was obtained from **7a** that was selectively desilylated with MeLi–LiBr complex. The mono-silylated diyne **7b**[12,19] was isolated in 79% yield and was reacted with (*E*)-1-bromopropene in the presence of a Pd(II) catalyst leading to compound **8b** in 82% yield. After the usual desilylation reaction with KF in MeOH compound **5b** was obtained in 93% yield.

The bromo trienyne **6** was prepared by a literature procedure[14] as an ≥85:15 mixture of *E*, *E*, *E* and *E*, *E*, *Z* isomers by reaction of all *E*-bromo dienal **9**[15] and the ylide deriving from commercially available phosphonium salt **10** (Scheme 3).

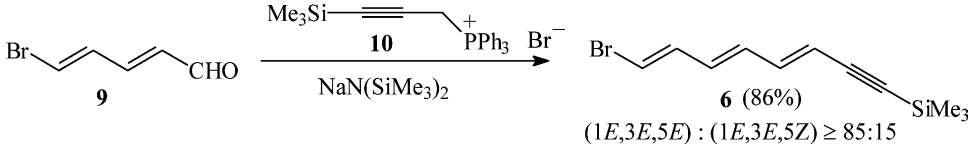
Therefore, the synthesis of xerulins **1** and **2** was performed as outlined in Scheme 4.

Both the xerulins **1** and **2** were synthesized starting from bromo trienyne **6**. In the case of dihydroxerulin **1**, the coupling reaction of 1-trimethylsilyl-1,3-heptadiyne **5a** in THF with compound **6** in the presence of a Pd(II) catalyst led to mono-silylated polyunsaturated product **11a** in 73% yield. It is noteworthy that, notwithstanding the ≥85:15 mixture of compound **6**, essentially all *E* product was formed, as evidenced by ^{1}H NMR spectroscopy. The desilylation reaction of **11a** with a TBAF solution in THF afforded product **3a** in 90% yield. Finally, the palladium catalyzed tandem coupling/cyclisation reaction[11,16] of **3a** with (*Z*)-3-iodo-2-propenoic acid **4** led to dihydroxerulin **1** in 68% yield (97% diastereoisomeric purity).

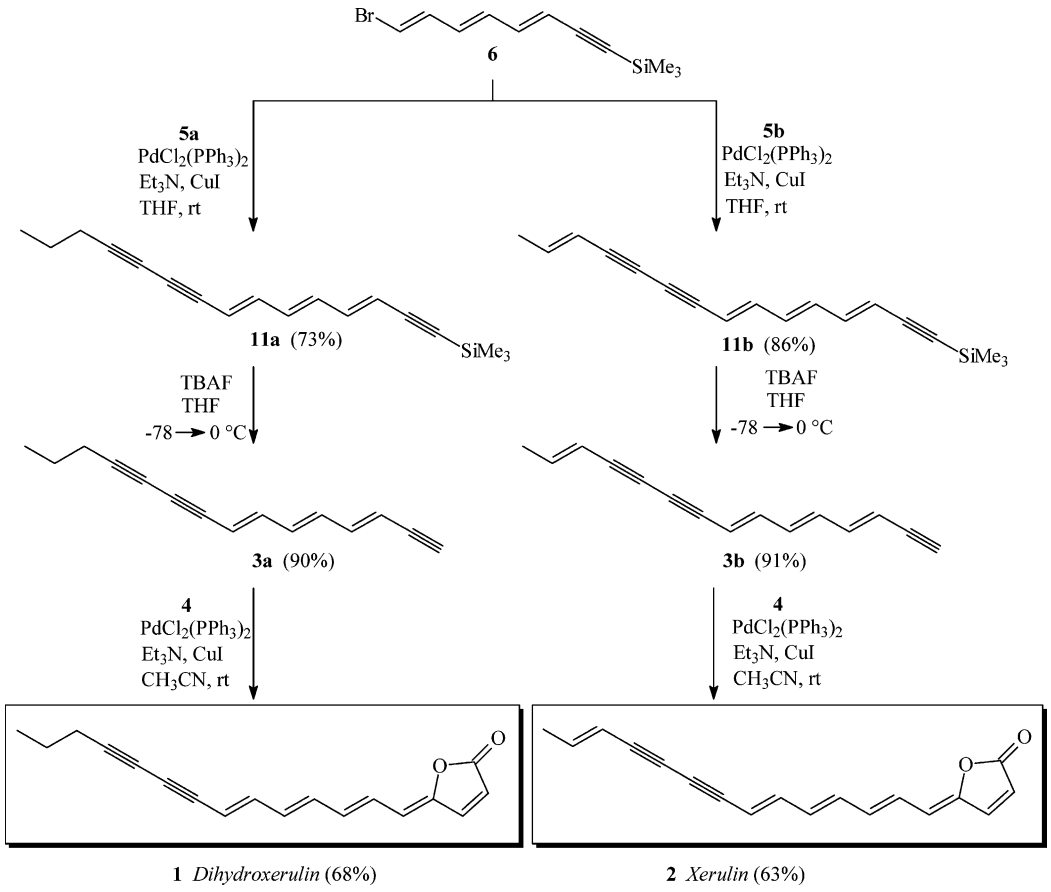
Starting from the enediyne **5b**, the same reaction sequence was followed for the synthesis of xerulin **2**, that was obtained in 63% yield (98% diastereoisomeric purity).

In conclusion, our synthetic approach to the xerulins compares favourably with other procedures. A special advantage of our strategy is represented by the possibility of synthesizing different xerulins starting from a common intermediate and following the same reaction sequence. Moreover, the ready availability of the silyl derivatives employed and the simplicity of the operations involved are additional features making the procedure very useful.

Scheme 2. Reagents and conditions: (i) MeLi–LiBr complex (1.5 M in Et_2O, 1.1 equiv), rt; (ii) *n*-propyl bromide (1.1 equiv) at −78 °C in HMPA (2 equiv) then to rt; (iii) KF (10 equiv) in MeOH at 50 °C; (iv) aq NH_4Cl, 79% diyne **7b** (1-trimethylsilyl-1,3-butadiyne); (v) (*E*)-1-bromopropene (0.85 equiv), Et_3N (1.3 equiv), CuI (0.033 equiv), $PdCl_2(PPh_3)_2$ (0.016 equiv) in THF, rt.



Scheme 3.



Scheme 4.

3. Experimental

Macherey-Nagel silica gel (60, particle size 0.040–0.063 mm) for column chromatography and Macherey-Nagel aluminum sheets with silica gel 60 F_{254} for TLC were used. GC analysis was performed on a Hewlett–Packard 5890 series II gas chromatograph equipped with a SE-30 (methylsilicone, 30 m×0.25 mm i.d.) capillary column. GC/mass-spectrometry analysis was performed on a Shimadzu GCMS-QP5000 gas chromatograph-mass spectrometer equipped with a MDN-1 capillary column (methylsilicone, 30 m×0.25 mm i.d.). IR spectra were recorded on a Perkin–Elmer FT-IR 1710 spectrometer. ^{1}H NMR spectra were recorded in deuterochloroform on a Bruker AM 500 spectrometer at 500 MHz. ^{13}C NMR spectra were recorded in deuterochloroform on a Bruker AM 500 spectrometer at 125.7 MHz. Elemental analyses were recorded on a Carlo Erba EA 1108 elemental analyzer. Solvents were dried before use as follows: acetonitrile was distilled over molecular sieves, tetrahydrofuran was distilled from sodium.

3.1. Synthesis of conjugated diynes 5

3.1.1. 1-Trimethylsilyl-1,3-heptadiyne (8a). MeLi–LiBr complex (1.5 M) in ether (5.67 mL, 8.50 mmol) was added to a THF solution (15 mL) of 1,4-bis-trimethylsilyl-1,3-butadiyne **7a** (1.5 g, 7.72 mmol) at room temperature. After complete desilylation (3 h), the reaction mixture was cooled to −78 °C, a solution of *n*-propylbromide (1.04 g, 8.5 mmol) in HMPA (2.7 mL, 15.51 mmol) was added and the mixture was slowly brought to room temperature. After reaction completion (12 h), the mixture was quenched with a saturated aqueous solution of NH_4Cl (10 mL), and extracted with ethyl acetate (3×10 mL). The organic extracts were washed with water (3×10 mL), dried over Na_2SO_4 and concentrated under vacuum. Purification by percolation on florisil column (petroleum ether as eluent) led to the title compound **8a** (0.76 g, 60% yield) as a yellow oil. [Found: C, 73.00; H, 9.88. $C_{10}H_{16}Si$ requires C, 73.10; H, 9.81%]; ν_{max} (neat) 2964, 2937, 2902, 2875, 2227, 2109, 1460, 1251, 1181, 846, 761, 630 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 2.23 (2H, t, J=7.2 Hz), 1.53 (2H, sextet like J=7.2 Hz) 0.97 (3H, t, J=7.2 Hz), 0.16 (9H, s); δ_C (125.7 MHz, $CDCl_3$) 88.4, 83.0, 80.1, 65.5, 21.6, 21.2, 13.5, −0.3; MS *m/z* 164 (M^+, 7), 150 (15), 149 (100), 121 (6), 120 (7), 107 (7), 105 (7), 93 (5), 91 (7), 83 (11), 79 (9), 77 (6), 73 (3), 69 (4), 67 (7), 53 (10), 43 (25%).

3.1.2. 1,3-Heptadiyne (5a).[5] KF (0.99 g, 17.04 mmol) was added to a MeOH solution (3 mL) of compound **8a** (0.28 g, 1.70 mmol). The reaction mixture was stirred for 1 h at 50 °C, then, after cooling at room temperature, quenched with water (10 mL) and extracted with ethyl ether (3×10 mL). The organic extracts were dried over Na_2SO_4 and concentrated under vacuum. The title compound **5a** (0.15 g, 95% yield) was obtained as a colorless oil, pure for the next reaction. The spectral data were in agreement with those reported.[5]

3.1.3. (5*E*)-1-Trimethylsilyl-hept-5-en-1,3-diyne (8b). MeLi–LiBr complex (1.5 M) in ether (15.1 mL, 22.65 mmol) was added to an ether solution (40 mL) of 1,4-bis-trimethylsilyl-1,3-butadiyne **7a** (4 g, 20.57 mmol) at room temperature. After reaction completion (3 h), the mixture was quenched with a saturated aqueous solution of NH_4Cl (20 mL), and extracted with ethyl ether (3×20 mL). The organic extracts were washed with water (3×20 mL), dried over Na_2SO_4 and concentrated under vacuum. The mono-silylated diyne 1-trimethylsilyl-1,3-butadiyne **7b**[19] was purified by distillation (2.0 g, 79% yield). A THF solution (20 mL) of diyne **7b** (1.96 g, 16.03 mmol) was added at room temperature, under nitrogen, to a stirred mixture of (*E*)-1-bromopropene (1.67 g, 13.6 mmol), Et_3N (2.15 g, 21.25 mmol), CuI (0.103 g, 0.54 mmol) and $Pd(PPh_3)_2Cl_2$ (0.182 g, 0.26 mmol) in THF (20 mL). After reaction completion (12 h), the mixture was quenched with a saturated aqueous solution of NH_4Cl (20 mL), and extracted with ethyl acetate (3×20 mL). The organic extracts were washed with water (3×20 mL), dried over Na_2SO_4 and concentrated under vacuum. Purification by column chromatography (petroleum ether) led to the title compound **8b** as a yellow oil (1.82 g, 82% yield). [Found: C, 73.93; H, 8.81. $C_{10}H_{14}Si$ requires C, 74.00; H, 8.69%]; ν_{max} (neat) 3030, 2961, 2915, 2851, 2200, 2101, 1627, 1443, 1410, 1252, 1212, 947, 936, 860, 845, 761, 634 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 6.33 (1H, dq, J=15.7, 6.9 Hz), 5.51 (1H, dq, J=15.7, 1.8 Hz), 1.81 (3H, dd, J=6.9, 1.8 Hz), 0.17 (9H, s); δ_C (125.7 MHz, $CDCl_3$) 144.3, 109.4, 89.0, 88.1, 75.9, 72.6, 18.8, −0.5; MS *m*/*z* 162 (M^+, 20), 148 (15), 147 (100), 131 (4), 119 (4), 117 (4), 107 (4), 105 (7), 93 (7), 79 (6), 77 (9), 73 (4), 69 (5), 67 (9), 55 (6), 53 (21), 43 (36%).

3.1.4. (5*E*)-Hept-5-en-1,3-diyne (5b).[8] KF (0.99 g, 17.04 mmol) was added to a MeOH solution (3 mL) of compound **8b** (0.28 g, 1.73 mmol). The reaction mixture was stirred for 1 h at 50 °C, then, after cooling at room temperature, quenched with water (10 mL) and extracted with ethyl ether (3×20 mL). The organic extracts were dried over Na_2SO_4 and concentrated under vacuum. The title compound **5b** (0.14 g, 93% yield) was obtained as a brown oil, pure for the next reaction. The spectral data were consistent with those reported.[8]

3.2. General procedure for the synthesis of dihydroxerulin 1 and xerulin 2

A solution of diyne **5a** or **5b** (1 equiv) in THF (1 M) was added dropwise at room temperature, under nitrogen, to a stirred mixture of bromo trienyne **6** (0.67 equiv), Et_3N (18 equiv), CuI (0.026 equiv) and $PdCl_2(PPh_3)_2$ (0.014 equiv) in THF (0.32 M). After reaction completion (12 h), the mixture was quenched with a saturated aqueous solution of NH_4Cl (20 mL), and extracted with ethyl acetate (3×20 mL). The organic extracts were washed with water (3×20 mL), dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography leading to the diynes **11a** or **11b**. To a solution of the diyne **11a** or **11b** (1 equiv) in THF (0.23 M), was added 1 equiv of TBAF (1 M in THF) at −78 °C and the mixture was stirred for 10 min at the same temperature. The mixture was slowly brought to 0 °C, stirred for additional 10 min, quenched with water (10 mL) and extracted with ethyl ether (3×20 mL). The organic extracts were dried over Na_2SO_4 and concentrated under vacuum. Purification by column chromatography led to compound **3a** or **3b**. A CH_3CN solution (0.23 M) of polyyne **3a** or **3b** (1 equiv) was added at room temperature, under nitrogen, to a stirred mixture of **4** (0.67 equiv), Et_3N (18 equiv), CuI (0.025 equiv) and $PdCl_2(PPh_3)_2$ (0.013 equiv) in CH_3CN (0.15 M). After reaction completion (2–3 h), the mixture was quenched with a saturated aqueous solution of NH_4Cl (20 mL), and extracted with ethyl acetate (3×20 mL). The organic extracts were washed with water (3×20 mL), dried over Na_2SO_4 and concentrated under vacuum. Purification by column chromatography led to the title compounds **1** or **2**.

3.3. Synthesis of dihydroxerulin 1

3.3.1. (3*E*,5*E*,7*E*)-1-Trimethylsilyl-pentadeca-3,5,7-trien-1,9,11-triyne (11a). Compound **11a** was prepared from **5a** (0.087 g, 0.95 mmol) and bromo trienyne **6** (0.18 g, 0.63 mmol) in accordance with general procedure. Purification by column chromatography (silica gel, petroleum ether) gave **11a** (0.122 g, 73% yield, 98% isomeric purity, determined by ^1H NMR spectroscopy) as a yellow solid (mp 139–141 °C). [Found: C, 81.05; H, 8.35. $C_{18}H_{22}Si$ requires C, 81.13; H, 8.32%]; ν_{max} (KBr) 3025, 2961, 2930, 2872, 2157, 2111, 1459, 1260, 1249, 1073, 1019, 985, 843, 801 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 6.70–6.56 (2H, m), 6.35–6.24 (2H, m), 5.67 (1H, d, J=15.5 Hz), 5.65 (1H, d, J=15.3 Hz), 2.31 (2H, t, J=7.2 Hz), 1.56 (2H, sextet like, J=7.2 Hz), 0.98 (3H, t, J=7.2 Hz), 0.18 (9H, s); δ_C (125.7 MHz, $CDCl_3$) 143.5, 141.9, 134.5, 133.9, 113.3, 111.9, 104.5, 99.8, 86.9, 79.0, 74.7, 65.4, 21.7, 21.6, 13.4, −0.1; MS *m*/*z* 266 (M^+, 28), 251 (8), 237 (6), 221 (10), 209 (9), 207 (8), 205 (8), 195 (11), 193 (8), 191 (14), 183 (12), 179 (19), 178 (14), 165 (20), 155 (8), 152 (8), 139 (4), 125 (6), 115 (6), 105 (7), 97 (8), 91 (8), 83 (13), 77 (7), 73 (87), 59 (100), 53 (18), 45 (38), 43 (50%).

3.3.2. (3*E*,5*E*,7*E*)-Pentadeca-3,5,7-trien-1,9,11-triyne (3a). Compound **3a** was prepared from **11a** (0.11 g, 0.41 mmol) in accordance with general procedure and was purified by column chromatography (5% ethyl acetate/hexane). Compound **3a** [0.072 g (90% yield, 98% stereoisomeric purity)] as a red solid (mp 57–59 °C) was obtained. [Found: C, 92.78; H, 7.22. $C_{15}H_{14}$ requires C, 92.73; H, 7.26%]; ν_{max} (KBr) 3274, 3024, 2962, 2930, 2872, 2220, 1462, 1283, 1259, 1092, 1019, 993, 800 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 6.70–6.60 (2H, m), 6.35–6.27 (2H, m), 5.67 (1H, d, J=15.5 Hz), 5.63 (1H, dd, J=15.3, 2.2 Hz), 3.13 (1H, d, J=2.2 Hz), 2.31 (2H, t, J=7.2 Hz), 1.56 (2H, sextet like, J=7.2 Hz), 0.98 (3H, t, J=7.2 Hz); δ_C (125.7 MHz, $CDCl_3$) 143.4, 142.6, 134.1, 112.2, 112.1, 86.9, 83.0, 81.6, 79.1, 74.5, 65.4, 60.4, 21.7, 21.6, 13.5.

3.3.3. (Dihydroxerulin). (5*Z*)-5-[(2*E*,4*E*,6*E*)-Tetradeca-2,4,6-trien-8,10-diynylidene]furan-2(5*H*)-one (1).[5,7] The title compound **1** was prepared from **3a** (0.07 g, 0.36 mmol) and (*Z*)-3-iodo-2-propenoic acid **4** (0.047 g, 0.24 mmol) in accordance with general procedure. Purification by column chromatography, (30% ethyl acetate/hexane) led to 0.043 g (68% yield, 97% stereoisomeric purity) of **1** as an orange solid (mp 135–137 °C). [Found: C, 81.70; H, 6.13.

$C_{18}H_{16}O_2$ requires C, 81.79; H, 6.10%]; ν_{max} (KBr) 3102, 2960, 2923, 2854, 2215, 1764, 1739, 1528, 1455, 1324, 1259, 1092, 1021, 934, 873, 799, 700, 675 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 7.35 (1H, d, $J=5.3$ Hz), 6.83–6.69 (2H, m), 6.53–6.35 (3H, m), 6.16 (1H, d, $J=5.3$ Hz), 5.87 (1H, d, $J=11.8$ Hz), 5.68 (1H, d, $J=15.5$ Hz), 2.32 (2H, t, $J=7.2$ Hz), 1.56 (2H, sextet like, $J=7.2$ Hz), 0.98 (3H, t, $J=7.2$ Hz); δ_C (125.7 MHz, $CDCl_3$) 169.3, 149.4, 143.6, 142.5, 137.8, 135.2, 135.1, 127.6, 118.8, 114.7, 112.3, 87.4, 79.7, 74.8, 65.4, 21.7, 13.5.

3.4. Synthesis of xerulin 2

3.4.1. (3*E*,5*E*,7*E*,13*E*)-1-Trimethylsilyl-pentadeca-3,5,7,13-tetraen-1,9,11-triyne (11b). Compound **11b** was prepared from **5b** (0.085 g, 0.94 mmol) and bromo trienyne **6** (0.18 g, 0.63 mmol) in accordance with general procedure. Purification by column chromatography (silica gel, petroleum ether) gave **11b** (0.144 g, 86% yield, 98% isomeric purity determined by ^1H NMR spectroscopy) as a yellow orange solid (mp 121–123 °C). [Found: C, 81.70; H, 7.65. $C_{18}H_{20}Si$ requires C, 81.75; H, 7.62%]; ν_{max} (KBr) 2961, 2917, 2849, 2155, 2110, 1260, 1072, 1019, 987, 842, 800, 758, 661 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 6.72–6.57 (2H, m), 6.36–6.25 (3H, m), 5.71 (1H, d, $J=14.5$ Hz), 5.68 (1H, d, $J=15.0$ Hz), 5.58 (1H, d, $J=16.0$ Hz), 1.82 (3H, d, $J=6.6$ Hz), 0.20 (9H, s); δ_C (125.7 MHz, $CDCl_3$) 143.8, 143.7, 141.8, 134.8, 133.8, 113.4, 111.7, 109.9, 104.4, 100.0, 82.9, 80.5, 78.7, 72.5, 19.2, −0.1; MS *m/z* 264 (M^+, 19), 249 (5), 234 (4), 233 (9), 219 (12), 205 (12), 203 (8), 191 (14), 189 (17), 183 (6), 179 (6), 165 (14), 152 (5), 143 (4), 123 (7), 117 (5), 97 (5), 83 (10), 77 (5), 73 (51), 63 (7), 59 (100), 55 (14), 53 (26), 45 (42), 43 (65%).

3.4.2. (3*E*,5*E*,7*E*,13*E*)-Pentadeca-3,5,7,13-tetraen-1,9,11-triyne (3b).[8] Compound **3b** was prepared from **11b** (0.11 g, 0.42 mmol) in accordance with general procedure and was purified by column chromatography, (5% ethyl acetate/hexane). Compound **3b** [0.073 g (91% yield, 98% stereoisomeric purity)] was obtained. The spectral data were consistent with those reported.[8]

3.4.3. (Xerulin) (5*Z*)-5-[(2*E*,4*E*,6*E*,12*E*)-Tetradeca-2,4,6,12-tetraen-8,10-diynylidene]furan-2(5*H*)-one (2).[6,8] The title compound **2** was prepared from **3b** (0.07 g, 0.364 mmol) and (*Z*)-3-iodo-2-propenoic acid **4** (0.048 g, 0.242 mmol) in accordance with general procedure. Purification by column chromatography, (30% ethyl acetate/hexane) led to 0.04 g (63% yield, stereoisomeric purity 98%) of **1** as an orange solid (mp 166–168 °C). [Found: C, 82.50; H, 5.32. $C_{18}H_{14}O_2$ requires C, 82.42; H, 5.38%]; ν_{max} (KBr) 3091, 3025, 2957, 2923, 2853, 2183, 2117, 1780, 1745, 1532, 1443, 1340, 1261, 1104, 1073, 997, 936, 877, 851, 815, 768 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 7.35 (1H, d, $J=5.3$ Hz), 6.84–6.70 (2H, m), 6.54–6.40 (3H, m), 6.32 (1H, dq, $J=15.8$, 6.9 Hz), 6.16 (1H, d, $J=5.3$ Hz), 5.88 (1H, d, $J=11.8$ Hz), 5.74 (1H, d, $J=15.3$ Hz), 5.58 (1H, d, $J=15.8$ Hz), 1.82 (3H, dd, $J=6.9$, 1.5 Hz); δ_C (125.7 MHz, $CDCl_3$) 169.3, 149.5, 143.9, 143.8, 142.5, 137.8, 135.5, 135.1, 127.7, 118.9, 114.7, 112.1, 109.9, 83.3, 80.6, 79.4, 72.5, 19.0.

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Identification of the biosynthetic units of Cypridina luciferin in *Cypridina* (*Vargula*) *hilgendorfii* by LC/ESI-TOF-MS

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Abstract—In a luminous ostracod *Cypridina* (*Vargula*) *hilgendorfii*, Cypridina luciferin with an imidazopyrazinone structure (3,7-dihydroimidazopyrazin-3-one) is utilized for the luminescence reaction. To identify the biosynthetic units of Cypridina luciferin, the stable isotope labeled compounds were examined by feeding experiments with living *Cypridina* specimens. The incorporation of the labeled compounds into Cypridina luciferin was identified by the method of LC/ESI-TOF-MS analyses and these results suggested that L-tryptophan, L-arginine and L-isoleucine are structural units of Cypridina luciferin.

1. Introduction

In bioluminescent marine organisms, Cypridina luciferin (**1**) and coelenterazine (**2**) having an imidazopyrazinone structure (3,7-dihydroimidazopyrazin-3-one, Fig. 1) are used for the luminescence reaction as follows;

$$\text{Luciferin} + O_2 \xrightarrow{\text{Luciferase}} \text{Oxyluciferin} + CO_2 + \text{Light}$$

Cypridina luciferin (**1**) is isolated from a luminous ostracod crustacean, *Cypridina hilgendorfii* (presently *Vargula hilgendorfii*), living near the Japanese coast.[1] It is also utilized for the luciferase reaction in certain kinds of bioluminescent fishes including *Apogon*, *Parapriacanthus and Porichthys*.[2] On the other hand, coelenterazine (**2**) is widely distributed in luminous and non-luminous coelenterates, fishes, shrimps and squids,[3] and is known as *Watasenia* preluciferin, *Renilla* luciferin and *Oplophorus* luciferin.[4] Further, **2** serves as the chromogenic compound of photoproteins including aequorin and obelin.[5]

The luminescence system of *Cypridina* has been investigated extensively, since Harvey reported the luciferin-luciferase reaction with extracts of the specimens in 1917.[6] When *Cypridina* specimens are stimulated physically or electronically, it expels Cypridina luciferin (**1**) and luciferase directly into the seawater to produce a brilliant bluish luminescence ($\lambda_{max}=460$ nm). After the luminescent reaction, **1** is converted to oxyluciferin (**3**), which is then hydrolyzed to etioluciferin (**4**) (Fig. 2).[7] The chemical mechanism of the luminescence reaction catalyzed by

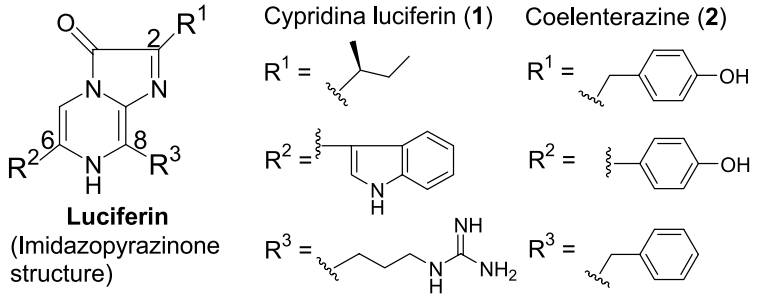

Figure 1. Structures of imidazopyrazinone, Cypridina luciferin (**1**) and coelenterazine (**2**).

Keywords: Bioluminescence; Stable isotope; Imidazopyrazinone; Luciferase; Biosynthesis.
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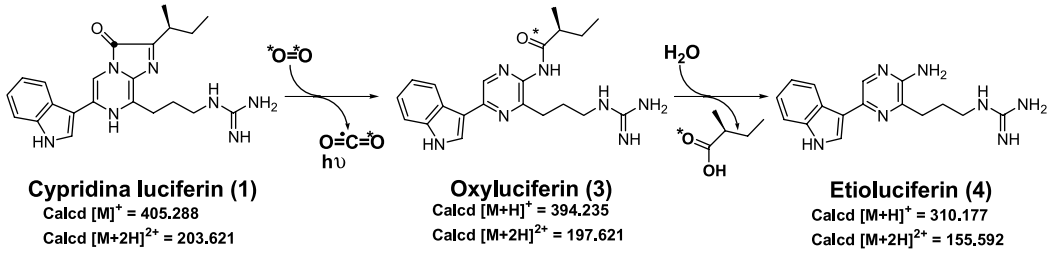

Figure 2. Luminescence reaction of Cypridina luciferin by Cypridina luciferase and the hydrolysis of oxyluciferin. Oxygen atoms derived from O_2 and a carbon atom at the C3 position of Cypridina luciferin are represented by asterisks and dot, respectively.

Cypridina luciferase was proposed.[8] Its luciferase gene was isolated[9] and has been utilized as a reporter protein.[10] The isolation, structural determination and total synthesis of Cypridina luciferin (**1**) was achieved[1] and Kishi et al. proposed that **1** should be biosynthesized from three amino acids or their equivalents: L-arginine, L-isoleucine and L-tryptophan (or tryptamine).[1]

Recently, we reported that L-tryptophan is one of the structural units of Cypridina luciferin (**1**) in its biosynthesis.[11] After feeding of the deuterium labeled L-tryptophan as bait to *Cypridina* specimens for 6 days, the ethanol extract from living specimens was analyzed by LC/ESI-TOF-MS and the incorporation of the deuterium labeled L-tryptophan into Cypridina luciferin was identified. In this study, other possible amino acids for the biosynthetic units of Cypridina luciferin (**1**) were examined and we concluded that natural amino acids of L-arginine, L-isoleucine and L-tryptophan are structural units, but not D-tryptophan and tryptamine.

Table 1. Monovalent and divalent ions of natural and synthetic Cypridina luciferin (**1**) by ESI-TOF-MS

Ions	Ion state	*m/z* (relative intensity, %)		Calculated mass (%)
		Natural[a]	Synthetic[b]	
Monovalent	$[M]^+$	405.227 (100)	405.226 (100)	405.228 (100)
	$[M+1]^+$	406.233 (39.6)	406.227 (51.6)	406.230 (27.4)
Divalent	$[M+2H]^{2+}$	203.619 (100)	203.614 (100)	203.621 (100)
	$[M+1+2H]^{2+}$	204.120 (27.5)	204.120 (29.8)	204.123 (27.4)

[a] Extracted from *C. hilgendorfii*.
[b] Chemical synthesized.[12]

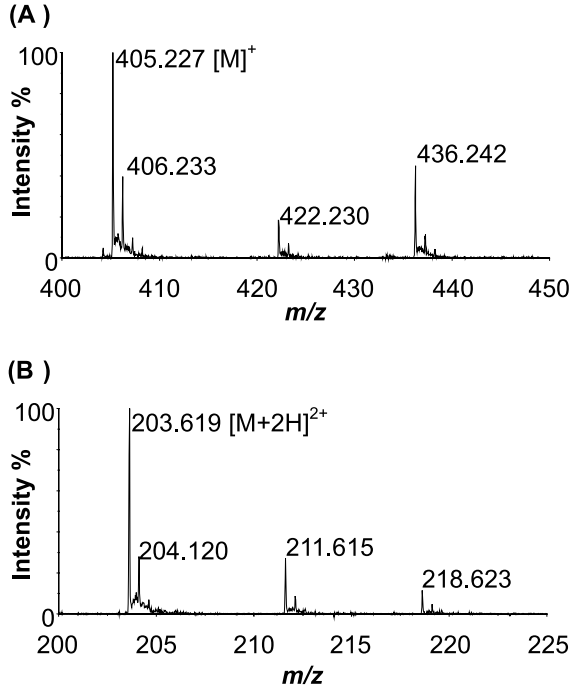

Figure 3. Mass spectra of Cypridina luciferin (**1**) extracted from *C. hilgendorfii* by ESI-TOF-MS (+). (A) monovalent ions, (B) divalent ions.

2. Results and discussion

2.1. ESI-TOF-MS analyses of natural and synthetic Cypridina luciferin (1)

Before identifying the incorporation of stable isotopic compounds into Cypridina luciferin (**1**) by LC/ESI-TOF-MS, **1** was analyzed by the infusion method with ESI-TOF-MS. As the authentic compounds, chemically synthesized *dl*-Cypridina luciferin (**1**) and natural **1** were used and the peaks of the monovalent and divalent ions were detected in the positive mode (Table 1 and Fig. 3). The mass value of monovalent ion corresponding to Cypridina luciferin (**1**) was mainly observed at *m/z* 405.2 as $[M]^+$, but not at *m/z* 406.2 as $[M+H]^+$. Similar peak patterns were observed by FD-MS[12] and MALDI-TOF-MS (data not shown). The intensities of *m/z* 406.2 $[M+1]^+$ as an isotopic peak for natural and synthetic Cypridina luciferin (**1**) were 39.6 and 51.6%, respectively. These intensities were inconsistent with the calculated value of 27.4%. On the other hand, the intensity of the isotopic peak (*m/z* 204.1) of the divalent ion was good agreement with the calculated value. Thus, the divalent ion was chosen for determining the number of isotope atoms in Cypridina luciferin (**1**). In ESI-TOF-MS analysis of Cypridina luciferin (**1**), the monovalent peaks at *m/z* 422.2 and *m/z* 436.2 were also detected and these mass

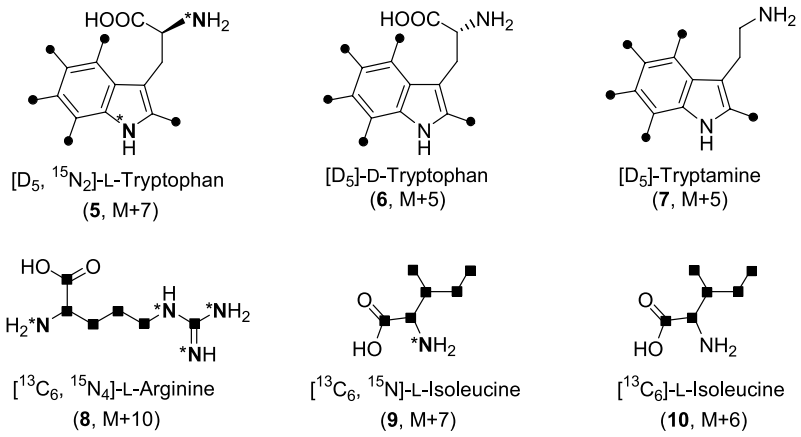

Figure 4. Stable isotope labeled compounds for the feeding experiments in *C. hilgendorfii*. Positions of stable isotopes are indicated as ^{13}C (■), ^{15}N (*N) and D (●).

values were corresponded to that of luciferinol and luciferyl methyl ether,[13] respectively. They might be generated during measuring with ESI-TOF-MS in our conditions.

2.2. Preparation of [D_5,$^{15}N_2$]-L-tryptophan (5), [D_5]-D-tryptophan (6) and [D_5]-tryptamine (7)

The deuterium labeled tryptophan (**5** and **6**) and tryptamine (**7**) (Fig. 4) were prepared by the deuterium-exchange method as previously reported.[11] Briefly, [D_5,$^{15}N_2$]-L-tryptophan (**5**) was prepared from [$^{15}N_2$]-L-tryptophan by heating with DCl in D_2O. [D_5]-D-Tryptophan (**6**) and [D_5]-tryptamine (**7**) were prepared from D-tryptophan and tryptamine by the same method, respectively. The purity of the deuterium labeled compounds was determined to be >99% by HPLC analysis.[11] The optical purity of these compounds was >95% ee, and slight decrease of optical purity was observed during the labeling procedures. To confirm the deuteration efficiency of the labeled compounds, [D_5,$^{15}N_2$]-L-tryptophan (**5**), [D_5]-D-tryptophan (**6**) and [D_5]-tryptamine (**7**) were analyzed by ESI-TOF-MS (Table 2). The peak ratios of [D_5]:[D_4] for L-tryptophan (**5**), D-tryptophan (**6**) and tryptamine (**7**) were 100: 23.3, 100: 53.0 and 100: 21.6, respectively (Table 2). The labeling efficiency of these compounds was suitable for the feeding experiments (Table 2, Fig. 4).

2.3. Identification of biosynthetic units in Cypridina luciferin (1) by LC/ESI-TOF-MS analysis

To identify the biosynthetic units in Cypridina luciferin (**1**), the feeding experiments were performed as described in Section 4.5. After feeding with isotope labeled compounds (Fig. 4) for 15 days, 2–5 specimens were extracted with ethanol and the incorporation of the stable isotopes into Cypridina luciferin (**1**) was identified by LC/ESI-TOF-MS. Cypridina luciferin (**1**) on HPLC was confirmed using *dl*-synthetic **1** (Fig. 5). Oxyluciferin (**3**) and etioluciferin (**4**)[1,7] were also detected in ethanol extracts. The mass spectral data (Fig. 6) indicated that [D_5,$^{15}N_2$]-L-tryptophan (**5**), [$^{13}C_6$,$^{15}N_4$]-L-arginine (**8**), [$^{13}C_6$,^{15}N]-L-isoleucine (**9**) and [$^{13}C_6$]-L-isoleucine (**10**) were incorporated into Cypridina luciferin as biosynthetic units (Table 3). Based on the peak intensity of mass spectra, the incorporation efficiency of [D_5,$^{15}N_2$]-L-tryptophan (**5**), [$^{13}C_6$,$^{15}N_4$]-L-arginine (**8**), [$^{13}C_6$,^{15}N]-L-isoleucine (**9**) and [$^{13}C_6$]-L-isoleucine (**10**) into **1** in living animals were estimated to 36.4%, 19.2%, 29.4% and 10.1%, respectively. The difference of the incorporation efficiency between [$^{13}C_6$,^{15}N]-L-isoleucine (**9**) and [$^{13}C_6$]-L-isoleucine (**10**) might be due to the animal conditions. On the other hand, Cypridina luciferin (**1**) obtained by feeding with [D_5]-D-tryptophan (**6**) and [D_5]-tryptamine (**7**) did not show the signification incorporation of stable isotopes. Thus, Cypridina luciferin (**1**) is

Table 2. Relative intensity of mass ion peaks in stable isotope labeled compounds

Numbers of stable isotope atom	Relative intensity (%)					
	[D_5,$^{15}N_2$]-L-Trp (**5**)	[D_5]-D-Trp (**6**)	[D_5]-Tryptamine (**7**)	[$^{13}C_6$,$^{15}N_4$]-L-Arg (**8**)	[$^{13}C_6$,^{15}N]-L-Ile (**9**)	[$^{13}C_6$]-L-Ile (**10**)
+0	3.3					
+1						
+2		3.2				
+3	2.0	13.9	3.3			
+4	2.4	53.0	21.6			
+5	5.2	100.0	100.0		2.0	6.6
+6	23.3	11.9	9.4		13.8	100.0
+7	100.0				100.0	1.1
+8	16.3					
+9	2.8			5.1		
+10				100.0		
+11						

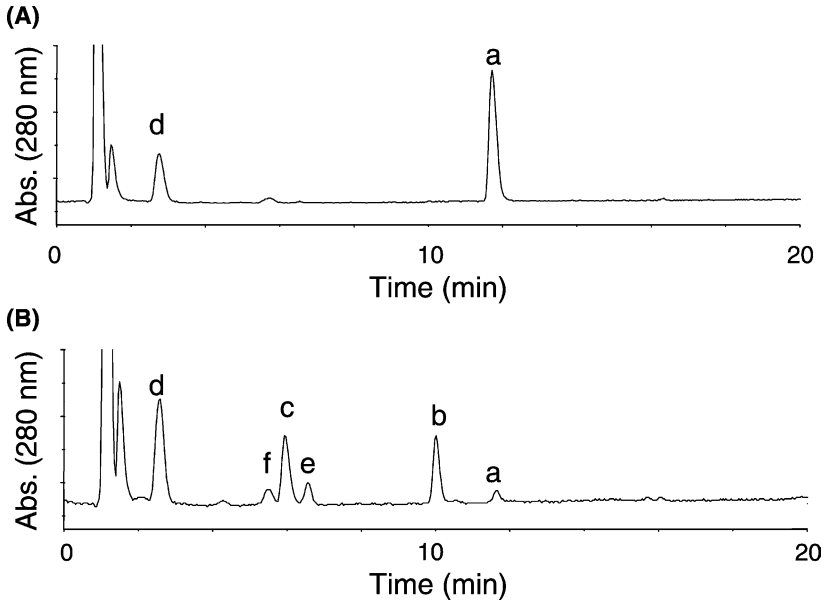

Figure 5. HPLC chromatogram of ethanol extracts from *C. hilgendorfii* (A) and its air oxidation products (B). a, Cypridina luciferin (**1**); b, oxyluciferin (**3**); c, etioluciferin (**4**); d, tryptophan; e, unknown (*m/z*=311); f, unknown (*m/z*, not detected).

biosynthesized from L-tryptophan, L-arginine and L-isoleucine in living animals, and tryptamine and D-tryptophan do not take part in the structural units. As previously reported,[1–4] Cypridina luciferin (**1**) and coelenterazine (**2**) are used for luciferase reaction in many marine organisms. In a luminous fish *Porichthys notaus*, the recycling system of Cypridina luciferin (**1**) was suggested.[15] However, the biosynthetic pathway and the animal species of Cypridina luciferin synthesis have not been demonstrated. Thus, the present study is the first demonstration of the biosynthesis of Cypridina luciferin (**1**) from three amino acids and suggests that coelenterazine (**2**) may be synthesized from two moles of L-tyrosine and one mole of phenylalanine in some marine organisms.

In the feeding experiments with [D_5,$^{15}N_2$]-L-tryptophan (**5**, M+7) and [$^{13}C_6$,^{15}N]-L-isoleucine (**9**, M+7), the mass spectral data of Cypridina luciferin (**1**) (Table 3, Fig. 6) indicated that the relative peak intensity of [M+7]-luciferin was decreased, by comparing the ratio of the relative intensity for [M+7]: [M+6] in [D_5,$^{15}N_2$]-L-tryptophan (**5**) and [$^{13}C_6$,^{15}N]-L-isoleucine (**9**) (Table 3, Fig. 6). To explain mass decrease in these labeled Cypridina luciferins, the labeled amino acids used for the feeding experiments were recovered from the animals and were analyzed by LC/ESI-TOF-MS (Table 4). The mass data indicated that the ratios of [M+6]:[M+7] for the labeled tryptophan and isoleucine from the animals were 100: 57.1 and 100: 33.6, respectively, and both intensities of [M+7] were decreased (Table 4). Thus, one stable isotope atom of D, ^{15}N or ^{13}C in the labeled amino acids was exchanged by a non-isotope atom after feeding. As previously reported,[11] the replacement of deuterium atoms on the indol ring of labeled tryptophan with hydrogen atoms from H_2O could not occur in living animals. When [$^{13}C_6$]-L-isoleucine (**10**, M+6) was used as bait, the loss of a ^{13}C atom was not detected (Table 3). These results suggested that the ^{15}N-atom of amino group in [D_5,$^{15}N_2$]-L-tryptophan (**5**) and [$^{13}C_6$,^{15}N]-L-isoleucine (**9**) were replaced with a ^{14}N-atom in living animals. Regarding [$^{13}C_6$,$^{15}N_4$]-L-arginine (**8**, M+10), the signal peak intensity of recovered arginine was lower and the significant exchange in four ^{15}N-atoms was not detected (Table 4). The replacement of amino group in L-tryptophan and L-isoleucine could occurred enzymatically, catalyzing by an enzyme like aminotransferase (EC 2.6.1.-) or dehydrogenase (EC 1.4.1.9).[16] From above results, we summarized the biosynthetic structural units of Cypridina luciferin (**1**) in Fig. 7, even if the biosynthetic pathway of Cypridina luciferin (**1**) from three amino acids in *C. hilgendorfii* is not clear.

2.4. Air oxidation of Cypridina luciferin (1) extracted from *C. hilgendorfii*

It is known that Cypridina luciferin (**1**) decomposes non-enzymatically into oxyluciferin (**3**) and CO_2 by air oxidation, and oxyluciferin (**3**) is further hydrolyzed to etioluciferin (**4**) with release of 2-methyl butyric acid, as similar to the Cypridina luciferase reaction[1,7] (Fig. 2). In our experiments with air oxidation procedures of labeled Cypridina luciferin, the formation of oxyluciferin (**3**) and etioluciferin (**4**)[1,7] was identified by LC/ESI-TOF-MS. As previously reported by Shimomura and Johnson,[17] the formation of CO_2 in the Cypridina luciferin-luciferase reaction was determined by incorporation of one oxygen atom from $^{18}O_2$ into CO_2. Thus, the carbon atom of CO_2 is considered to be the carbonyl carbon in the isoleucine moiety. The elimination of one carbon atom from Cypridina luciferin (**1**) was confirmed using [$^{13}C_6$]-Cypridina luciferin labeled with [$^{13}C_6$]-L-isoleucine (**10**). After air oxidation, [$^{13}C_6$]-Cypridina luciferin gave [$^{13}C_5$]-Cypridina oxyluciferin which was then converted to non-labeled etioluciferin (Table 3). On the other hand, the air oxidation products of Cypridina luciferin obtained from [D_5,$^{15}N_2$]-L-tryptophan

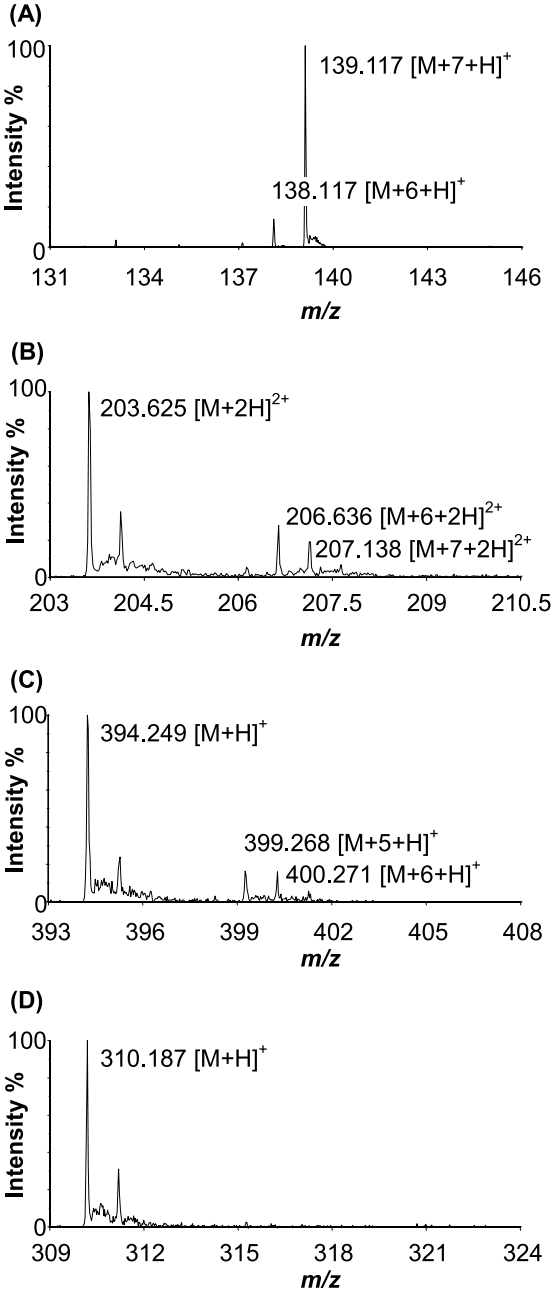

Figure 6. Mass spectra of Cypridina luciferin (**1**) labeled with [$^{13}C_6$,^{15}N]-L-isoleucine (**9**), its oxyluciferin and etioluciferin by LC/ESI-TOF-MS. (A) [$^{13}C_6$,^{15}N]-L-isoleucine (**9**), (B) Cypridina luciferin (**1**), (C) oxyluciferin (**3**), (D) etioluciferin (**4**).

(**5**) or [$^{13}C_6$,$^{15}N_4$]-L-arginine (**8**) retained the labeled isotopes in their molecules (Table 3).

3. Conclusion

After feeding experiments of the stable isotope labeled compounds to *C. hilgendorfii*, Cypridina luciferin (**1**) extracted from the living specimens was analyzed by LC/ESI-TOF-MS. From the mass spectral analysis, we concluded that Cypridina luciferin (**1**) is biosynthesized from three amino acids, L-tryptophan, L-arginine and L-isoleucine in *C. hilgendorfii*.

Table 3. Incorporation of the isotope labeled amino acids into Cypridina luciferin (**1**), oxyluciferin (**3**) and etioluciferin (**4**)

Numbers of stable isotope atom	Relative intensity (%)											
	[D_5,$^{15}N_2$]-L-Trp (**5**)			[$^{13}C_6$,$^{15}N_4$]-L-Arg (**8**)			[$^{13}C_6$,^{15}N]-L-Ile (**9**)			[$^{13}C_6$]-L-Ile (**10**)		
	1	**3**	**4**	**1**	**3**	**4**	**1**	**3**	**4**	**1**	**3**	**4**
+0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
+1	25.0	27.8	20.4	24.5	28.2	19.8	35.3	23.9	31.1	25.5	21.4	17.4
+2	8.2	8.4		8.6	8.4		7.6	5.6		7.0	7.0	
+3								3.0				
+4							5.4	16.4			7.8	
+5	9.5	7.2					28.3	16.0		11.5	3.7	
+6	24.3	37.7	43.0				19.2	5.6		3.4		
+7	30.5	36.4	40.1				6.7					
+8	11.9	17.7	21.6									
+9				5.1								
+10				19.8	16.9	23.0						
+11				6.8								

Table 4. Replacement of amino group in labeled amino acid after feeding experiments

Numbers of stable isotope atom	Relative intensity (%)		
	[D_5,$^{15}N_2$]-L-Trp (**5**)	[$^{13}C_6$,$^{15}N_4$]-L-Arg (**8**)	[$^{13}C_6$,^{15}N]-L-Ile (**9**)
+0	79.3	100.0	58.9
+1	22.1	24.3	14.3
+2			
+3			
+4	3.6		5.3
+5	26.6		10.3
+6	100.0		100.0
+7	57.1		33.6
+8	8.3		
+9	3.7	2.4	
+10		14.1	
+11			

4. Experimental

4.1. Materials

[$^{13}C_6$,^{15}N]-L-Isoleucine (**9**)(L-[U–$^{13}C_6$,U–^{15}N; >98%]isoleucine; chemical purity >95%) was purchased from Spectra Stable Isotopes (Columbia, MD, USA). [$^{15}N_2$]-L-Tryptophan (L-[U–$^{15}N_2$; 96–99%]tryptophan, chemical purity >98%), [$^{13}C_6$,$^{15}N_4$]-L-arginine (**8**)(L-[U–$^{13}C_6$; >99%, U–$^{15}N_4$; >99%]arginine), [$^{13}C_6$]-L-Isoleucine (**10**)(L-[U–$^{13}C_6$; >98%]isoleucine, chemical purity >98%) and D_2O (99.9 atom % D) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). DCl (20 wt% solution, 99.5 atom% D) and mercaptoacetic acid were from Aldrich. (1-Fluoro-2,4-dinitrophenyl)-5-L-alaninamide was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). *dl*-Cypridina luciferin as an authentic compound was chemically synthesized.[12]

4.2. Preparation of deuterium labeled indol compounds, [D_5,$^{15}N_2$]-L-tryptophan (5), [D_5]-D-tryptophan (6) and [D_5]-tryptamine (7)

The procedures for deuterium exchange at the indol ring in [$^{15}N_2$]-L-tryptophan, D-tryptophan and tryptamine were carried out as previously described.[11] A compound (20–40 mg/ml) was dissolved in 2 ml of D_2O containing 4% DCl and 5% of mercaptoacetic acid, and the mixture was degassed three times in a methanol-dry ice bath. The reaction mixture was heated at 110 °C for 3–4 h. After removing the reagents and solvent by evaporation, this labeling procedure was repeated one times. The resultant solution was concentrated, resolved in H_2O and dried up again. The deuterium labeled compound was obtained quantitatively as hydrochloride salt. The purity was confirmed by HPLC with a multi-wavelength UV detector and any other products were not detected. The labeling efficiency was determined by ESI-TOF-MS analysis and the peak ratios of [D_5]:[D_4] for [$^{15}N_2$]-L-tryptophan, D-tryptophan and tryptamine were 100:23.3, 100:53.0 and 100:21.6, respectively. The absolute configuration was determined by the Marfey's method to be >95% ee for both [D_5,$^{15}N_2$]-L-tryptophan (**5**) and [D_5]-D-tryptophan (**6**).

4.3. Determination of enantio excess of amino acids by the Marfey's method

Absolute configuration of the deuterium labeled tryptophan was determined by the Marfey's method.[14] Fifty microliters of 50 mM [D_5,$^{15}N_2$]-L- or [D_5]-D-tryptophan was added to 20 µl of 1 M $NaHCO_3$, and then the mixture was incubated with 100 µl of 1% (w/w) (1-fluoro-2,4-dinitrophenyl)-5-L-alaninamide in acetone for 1 h at 37 °C. The reaction was terminated by adding 20 µl of 1 N HCl and the mixture was subjected to reversed-phase HPLC analysis with a Develosil ODS-SR-5 (4.6×250 mm) column by a linear gradient of 30–60% acetonitrile in 0.1 M ammonium acetate (pH 3) in 45 min at a flow rate of 0.8 ml/min. The elution was monitored at 340 nm using a multiwave-length detector (MD-2010 plus, JASCO). Retention times of L- and D-derivatives were 25.8 and 28.3 min, respectively.

4.4. LC/ESI-TOF-MS analyses

The ethanol extracts from frozen specimens of *C. hilgendorfii* were analyzed by LC/ESI-TOF-MS with an Agilent 1100 HPLC system (Hewlett–Packard) connected to a Mariner Biospectrometry Workstation (Applied Biosystems). The column was Cadenza CD-C18 (2.0×75 mm, Imtakt) and the mobile phase was water–methanol containing 0.1% of formic acid, from 25 to 65% for 20 min at a flow rate of 0.2 ml/min. The monitoring was performed at 280 nm. The ESI-TOF-MS was carried out in the positive mode at the sprit ratio of 1:40 (5 µl/min). The calibration of

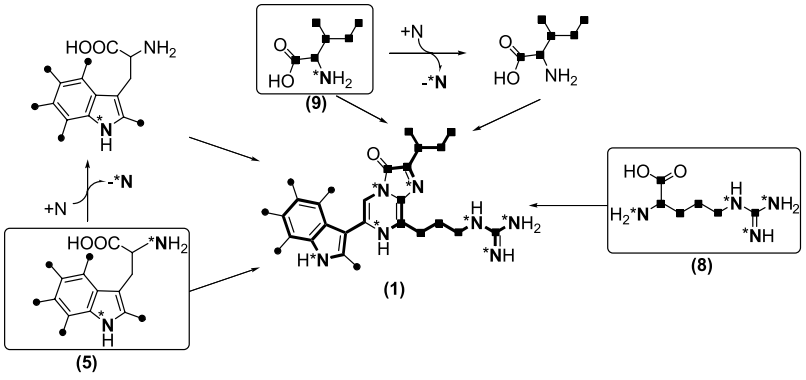

Figure 7. Biosynthesis of Cypridina luciferin (**1**) from L-tryptophan, L-arginine and L-isoleucine, and plausible nitrogen exchange of amino group in *C. hilgendorfii*.

mass value was performed using 1 μM each of angiotensin I (m/z=324.9272, 432.9003), bradykinin (m/z=354.1949) and neurotensin (m/z=558.3111) in 50% acetonitrile containing 1% acetic acid as external standards. The labeling efficiency was calculated from peak intensity of labeled ions in comparison with that of non-labeled luciferin ions.

4.5. Feeding experiments and extraction of Cypridina luciferin (1) from specimens

(i) Cypridina specimens. The specimens of *C. hilgendorfii* were collected at night using a bottle trap with a porcine liver as bait, at Mukaishima, Hiroshima in Japan, on 20 Dec. 2001, 13 Sept. 2002, 26 Dec. 2002, 7 Aug. 2003 and 7 Apr. 2004. The living specimens were kept in an aquarium (12 L) with aeration under the control of temperature at 20 °C.

(ii) Preparation of feeding gel for incorporation experiments. The preparation of the feeding gel containing a stable isotopic labeled amino acid was as follows; two grams of well-washed porcine liver were soaked in 15 ml distilled water for 15 min. The soaked extract was mixed with a labeled amino acid at the concentration of 50 mg/ml, except for 25 mg/ml of [$^{13}C_6$]-L-isoleucine (**10**) and [$^{13}C_6$,^{15}N]-L-isoleucine (**9**). The mixture was melt in 3% agarose (type VII, Sigma) at 75 °C and then was gelled in a plastic dish.

(iii) Feeding experiments. A feeding gel containing a labeled amino acid was cut into 7 mm cubic and fed to 2–5 specimens (2.0–3.0 mm in body size) in a small Petri dish (ϕ35 mm) for 1 h once a day. The feeding was monitored by blue staining of the stomach with trypan blue dye in feeding gel.

(iv) Ethanol extraction of Cypridina luciferin (**1**) from the specimens. After feeding for 15 days, the specimens were collected and immediately frozen in liquid nitrogen. The frozen specimens (24.8 and 27.8 mg wet weight/4 specimens for [D_5,$^{15}N_2$]-L-tryptophan (**5**) and [$^{13}C_6$,^{15}N]-L-isoleucine (**9**), respectively; 39.5 mg/5 specimens and 7.5 mg/2 specimens for [$^{13}C_6$,$^{15}N_4$]-L-arginine (**8**) and [$^{13}C_6$]-L-isoleucine (**10**), respectively) were homogenized with 3 times weight volume of ethanol by a plastic pestle on dry ice. The homogenate was sonicated (UT-105, Sharp) for 30 s, centrifuged at 12,000*g* for 10 min, and filtrated using an Ultrafree-MC filter (pore size 0.45 μm, Amicon). Two microliters of the filtrate were served to LC/ESI-TOF-MS analysis.

4.6. LC/ESI-TOF-MS analysis of stable isotope labeled amino acid extracted from *C. hilgendorfii*

Tryptophan in the ethanol extracts from *C. hilgendorfii* was analyzed by LC/ESI-TOF-MS as described in Section 4.4. Arginine and isoleucine in the extracts were analyzed by the same procedures except for the mobile phase and the detection wavelength at 210 nm on HPLC conditions. For arginine, the mobile phase was 40% methanol–water containing 0.05% heptafluorobutyric acid (HFBA) and arginine was detected at 3.6 min in the mass chromatogram. For isoleucine, the elution was performed stepwise with 35% methanol–water containing 0.05% HFBA (0–3 min) and then 100% methanol containing HFBA (3–10 min), and isoleucine was detected at 6.1 min in the mass chromatogram.

4.7. Air oxidation and hydrolysis of Cypridina luciferin (1) extracted from *C. hilgendorfii*

For air oxidation of Cypridina luciferin (**1**), five microliters of ethanol extracts from *C. hilgendorfii* in a plastic tube were left at room temperature (20–25 °C) for 3 days, and then analyzed by LC/ESI-TOF-MS as described in Section 4.4.

Acknowledgements

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Effects of aldehyde or dipyrromethane substituents on the reaction course leading to *meso*-substituted porphyrins

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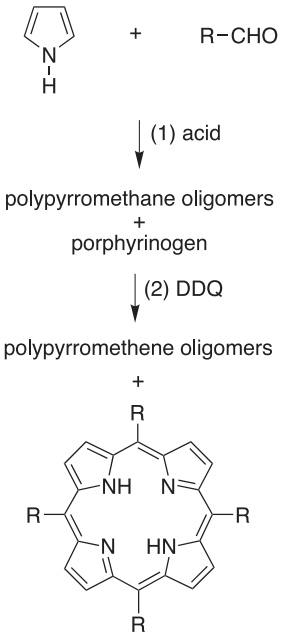
Abstract—To better understand the effects of diverse substituents on reactions leading to porphyrins, pyrrole + aldehyde condensations and related reactions of dipyrromethanes were examined. The course of pyrrole + aldehyde condensations was investigated by monitoring the yield of porphyrin (by UV–Vis spectroscopy), reaction of aldehyde (by TLC), and changes in the composition of oligomers (by laser desorption mass spectrometry). Reaction reversibility was examined via exchange experiments. Reversibility of reactions leading to porphyrin was further probed with studies of dipyrromethanes. The reaction course was found to depend on the nature of the substituent and the acid catalyst. Alkyl or electron-donating substituents displayed levels of reversibility (exchange/scrambling) on par or greater than that of the phenyl substituent, whereas electron-withdrawing or sterically bulky substituents exhibited little to no reversibility. The results obtained provide insight into the electronic and steric effects of different substituents and should facilitate the design of synthetic plans for preparing porphyrinic macrocycles.

1. Introduction

The one-flask reaction of pyrrole and an aldehyde provides a direct approach for the synthesis of *meso*-substituted porphyrins.[1] The reaction is performed in two stages: (1) acid catalyzed condensation of pyrrole + aldehyde forming predominantly polypyrromethane oligomers and the cyclic porphyrinogen; (2) addition of an oxidant (e.g., DDQ or *p*-chloranil) to give the corresponding polypyrromethenes and porphyrin (Scheme 1). We previously carried out a series of experiments to gain insight into the course of the condensation.[2–5] A battery of analytical techniques was employed, which allowed determination of the yields of porphyrinic macrocycles such as the porphyrin, *N*-confused porphyrin and sapphyrin (by UV–Vis and HPLC),[6] consumption of the aldehyde (by TLC),[7] change in the composition of the mixture of oligomers (by laser desorption mass spectrometry, LD-MS),[2,3] and formation of dipyrrin chromophores (by UV–Vis).[8] These studies, performed with the benchmark reaction of benzaldehyde and pyrrole under a variety of conditions, revealed the complexity of the overall reaction.

A key finding was that the condensation entails a combination of reversible and irreversible reactions, thereby often causing the yield of porphyrinogen to pass through a maximum and then decline. The overall rate of reaction and



Scheme 1.

Keywords: Porphyrin; Porphyrinogen; Dipyrromethane; Polypyrromethane; Pyrrole; Laser desorption mass spectrometry.
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ultimate yield of porphyrin is dependent on the concentration of the reactants, the nature of the acid catalyst, and on the concentration of the acid. Obtaining a maximal yield of porphyrin requires careful monitoring of the reaction 'trajectory' so that oxidation, the second step of the porphyrin-forming process (which terminates all condensation processes), can be initiated at the appropriate time. The decline in porphyrinogen yield that occurs under some conditions is accompanied by a shift of the oligomer composition to shorter species ('oligomer truncation').

In related experiments, we examined the reversibility of dipyrromethane + aldehyde condensations leading to *trans*-A_2B_2-porphyrins. Dipyrromethanes are valuable building blocks in diverse routes to porphyrins.[9–13] However, the successful use of dipyrromethanes requires the reaction to proceed without acidolysis of the dipyrromethane and recombination of the resulting dipyrromethane fragments yielding undesired porphyrin products (i.e., scrambling).[14] Again, the phenyl group was the predominant substituent employed.[4,5]

A present objective has been to determine the generality of the observations for substituents that differ markedly from that of the phenyl group. Of particular interest are the effects of electron-withdrawing, electron-releasing, sterically bulky, and non-aryl groups on the catalytic requirements, the trajectory of the reaction, the oligomer composition and its change over time, and the reversibility of the reaction. Improved understanding of these issues is important for the rational refinement of reaction conditions, for suppressing undesired reversible processes, and for the design of synthetic plans so as to avoid potentially problematic reactions.

In this paper, we report studies of condensations of a representative set of aldehydes with pyrrole, and condensations involving dipyrromethanes. Diverse aryl substituents were employed (electron donating, electron withdrawing, and sterically bulky) as well as alkyl groups. The studies first identified appropriate catalytic conditions with TFA or BF_3-etherate for reaction with 10 mM each of aldehyde and pyrrole in CH_2Cl_2 at room temperature. Under refined conditions, the consumption of aldehyde, changes in the oligomer composition, and the yield of porphyrin were investigated as a function of time for each aldehyde. The reversibility of the condensation was investigated via exchange experiments. Studies of a set of dipyrromethanes examined the propensity for reversible processes leading to scrambling. Taken together, these studies provide a foundation for understanding the effects of structural and electronic changes in the aldehyde or dipyrromethane moiety on the course of reaction leading to porphyrins.

2. Results and discussion

2.1. Reactions of aldehydes

Two specific aldehydes of a given type were selected for comparison to benzaldehyde in reactions leading to *meso*-substituted porphyrins. In each case, the two aldehydes that comprise a pair have similar structures but different molecular weights in anticipation of the exchange experiments. The selected aldehydes are as follows: benzaldehyde and *p*-tolualdehyde (benchmark case), *p*-anisaldehyde and 4-ethoxybenzaldehyde (electron donating), pentafluorobenzaldehyde and 2,3,5,6-tetrafluorobenzaldehyde (electron withdrawing), mesitaldehyde and 2,6-dimethylbenzaldehyde (sterically bulky), and hexanal and heptanal (alkyl). Three general types of experiments were performed: investigation of acid catalyst requirements, reaction time course studies, and examination of reaction reversibility.

2.1.1. Acid catalysis requirements. The catalytic requirements for each aldehyde were examined by reacting an aldehyde and pyrrole (10 mM each) at room temperature in CH_2Cl_2 over a range of concentrations of TFA or BF_3-etherate. After condensation for 15 min, 1 h, or 4 h, an aliquot of the reaction mixture was oxidized with DDQ and the yield of porphyrin was determined

Table 1. The acid catalysis conditions identified for each aldehyde[a]

Entry	Substituent type	R group in RCHO	Acid	[Acid], mM[b]	% Yield of porphyrin[c]
1	Benchmark	Phenyl	TFA	22	41
2	Benchmark	Phenyl	BF_3-etherate	1.0	23
3	Benchmark	*p*-Tolyl	TFA	22	41
4	Benchmark	*p*-Tolyl	BF_3-etherate	1.0	17
5	e-rich	4-MeO-phenyl	TFA	22	29
6	e-rich	4-MeO-phenyl	BF_3-etherate	—[d]	ND[d]
7	e-rich	4-EtO-phenyl	TFA	22	28
8	e-rich	4-EtO-phenyl	BF_3-etherate	—[d]	ND[d]
9	e-deficient	C_6F_5	TFA	215	11
10	e-deficient	C_6F_5	BF_3-etherate	10	23
11	e-deficient	2,3,5,6-F_4-phenyl	TFA	215	13
12	e-deficient	2,3,5,6-F_4-phenyl	BF_3-etherate	10	22
13	Alkyl	Pentyl	TFA	10	14
14	Alkyl	Pentyl	BF_3-etherate	1.0	8
15	Alkyl	Hexyl	TFA	10	16
16	Alkyl	Hexyl	BF_3-etherate	1.0	12

[a] The reactions were performed with 10 mM each of pyrrole and aldehyde at a 10 ml scale in CH_2Cl_2 at room temperature. The reactions were monitored at 15 min, 1 h, and 4 h.
[b] The optimal acid concentration in terms of yield of porphyrin and reaction time.
[c] The highest yield (UV–Vis) at any of the three timepoints is reported.
[d] No porphyrin was detected at any concentration of BF_3-etherate (limit of detection is 0.5%).

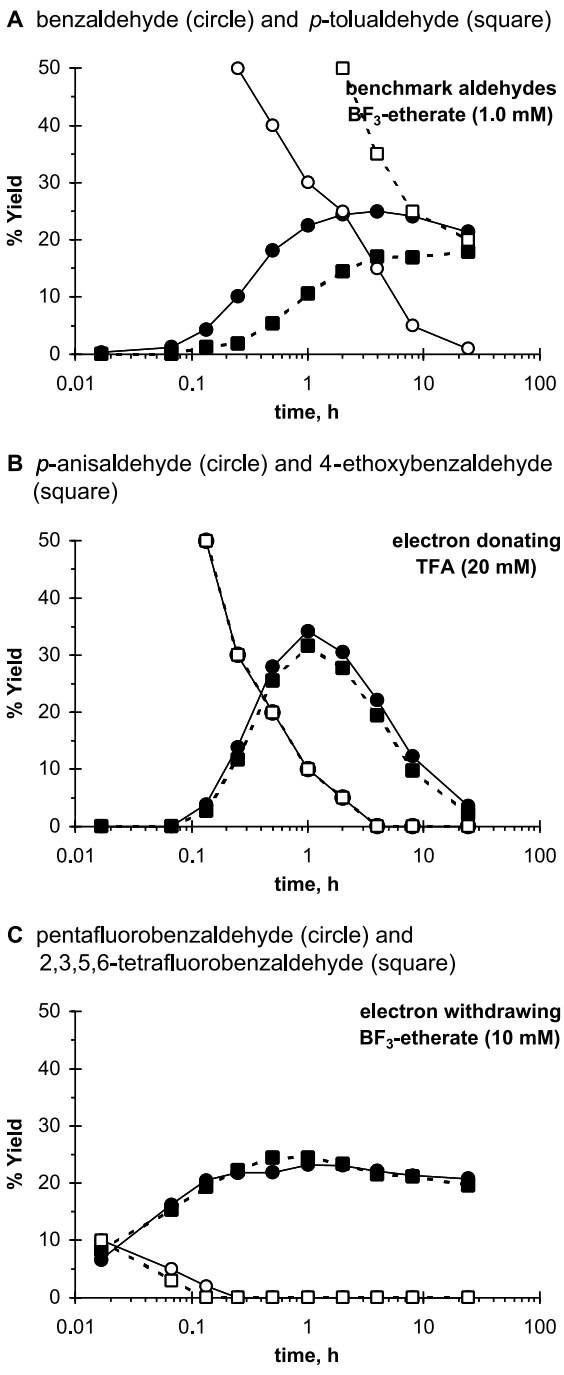


Figure 1. Percent yield of porphyrin (filled symbols) and percent unreacted aldehyde (open symbols) as a function of condensation time for reactions of pyrrole + aldehyde (10 mM each) in CH_2Cl_2 at room temperature. Reactions were monitored by removing an aliquot of the reaction mixture, oxidizing with DDQ, and analyzing spectrophotometrically for yield of porphyrin and by TLC for unreacted aldehyde. Note the log scale for time.

spectrophotometrically. All aldehydes were examined in this manner with the exception of mesitaldehyde and 2,6-dimethylbenzaldehyde, which are known not to react under these conditions and were instead condensed with pyrrole using the established conditions of BF_3-etherate/ethanol cocatalysis.[15]

The rate of formation (and possible decay) of the porphyrinogen is dependent on the concentration of a

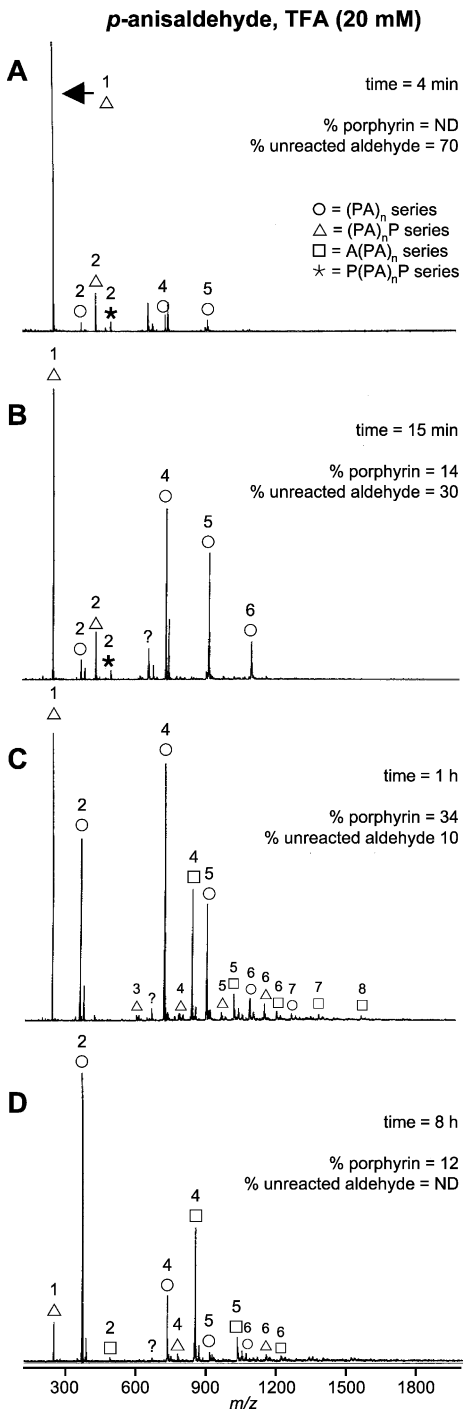


Figure 2. LD-MS spectra showing the oligomer composition at selected timepoints for the reaction of pyrrole + *p*-anisaldehyde (10 mM each) under TFA catalysis (20 mM) in CH_2Cl_2 at room temperature. Reactions were monitored by removing an aliquot of the reaction mixture, oxidizing with DDQ, and directly analyzing the crude, oxidized reaction mixture. The percent yield of porphyrin (UV–Vis) and percent unreacted aldehyde (TLC) are reported for each timepoint. Three oligomer series are defined based on the groups present at the oligomer chain termini: (1) a pyrrole and an aldehyde terminus, $(PA)_n$ series, ○; (2) pyrrole units at both termini, $(PA)_nP$ series, △; and (3) aldehyde units at both termini, $A(PA)_n$ series, □. A fourth oligomer series has an internal bipyrrole group, and pyrrole groups at both termini, $P(PA)_nP$ series, ★. Significant peaks that could not be assigned to one of the four oligomer series are denoted with a '?'. The number (*n*) of repeating pyrrole-aldehyde (PA) units is given by the number above the symbol.

given type of acid. Such a phenomenon, which is well documented for the reaction of benzaldehyde and pyrrole, was observed to varying degrees with the aldehydes examined herein (see the Supplementary data for plots of the yield of porphyrin as a function of acid concentration). For example, for *p*-anisaldehyde, the reaction with 46 mM TFA afforded a yield of 26% at 15 min, 5% after 1 h, and 0% after 4 h. However, the reaction with 22 mM TFA provided a yield of 29% after 1 h and ~17% after 4 h. For the reaction at 10 mM TFA, the reaction at 4 h afforded the highest yield (24%), with less than a 10% yield obtained at the earlier timepoints.

These results concerning the interplay of acid concentration and reaction rate illustrate the complexity of identifying a single best set of conditions for a given aldehyde. A range of acid concentration afforded a good yield of porphyrin at one of the three timepoints for each type of aldehyde. This range varied from the extremes of ~50-fold for reactions of aldehydes with electron-withdrawing groups under BF_3-etherate catalysis to ~5-fold for aldehydes with electron-donating groups under TFA catalysis. In addition, aldehydes bearing electron-withdrawing groups required ~10 times higher acid concentration to obtain the maximum yield of porphyrin than the other aldehydes examined herein. It is noteworthy that despite the subtleties of the conditions affording the highest yield of porphyrin, application of one or both of the standard reaction conditions [20 mM TFA or 1.0 mM BF_3-etherate] to all aldehydes (except those bearing bulky substituents in the 2- and 6-positions) provided a yield of porphyrin at some point in the reaction that was close to the maximum yield of porphyrin obtained under any acid concentration. Nearly identical yields of porphyrin were obtained from each member of a pair of aldehydes under a given reaction condition. The lone exception involved benzaldehyde and *p*-tolualdehyde. Under BF_3-etherate catalysis, *p*-tolualdehyde provided less porphyrin (1.4 to five times lower) than did benzaldehyde at equivalent reaction times. The acid catalyst concentrations identified for subsequent use herein are summarized in Table 1. Note that the yields obtained are not fully optimized. Investigation of other reaction conditions known to give improved yields in some cases such as use of clays,[16–20] cocatalysis (BF_3-etherate + salt,[7] BF_3-etherate + TFA[21]), or other catalysts,[22–24] as well as variation in the concentration of the aldehyde and pyrrole were not within the scope of the present study.

2.1.2. Reaction time course. The time course for the pyrrole + aldehyde reactions was examined in greater detail by following the formation of the porphyrin (UV–Vis), disappearance of the aldehyde (TLC), and change in oligomer composition (LD-MS) as a function of time from 1 min to 24 h. The reactions were performed under the catalytic conditions identified from the acid catalysis examination, as well as BF_3-ethanol cocatalysis conditions previously reported for mesitaldehyde.[15] The latter conditions also were employed for *p*-anisaldehyde, as *p*-alkoxybenzaldehydes are known to condense poorly with pyrrole in the presence of BF_3-etherate alone.[8] Representative plots of the percent yield of porphyrin and percent unreacted aldehyde as a function of condensation

Table 2. Effects of aldehyde structure on the reaction course leading to porphyrin[a]

Entry	R in RCHO	Acid	[Acid], mM	Time[b]	% Porphyrin[c]	% Unreacted aldehyde[d]	Yield turnover[e]	Oligomer truncation[f]
1	Phenyl	TFA	20	1–4 h	40	<5	High	High
2	Phenyl	BF_3-etherate	1.0	1–8 h	25	30	Low	Trace
3	*p*-Tolyl	TFA	20	30 min–1 h	39	15	High	High
4	*p*-Tolyl	BF_3-etherate	1.0	4–24 h	17	35	None	None
5	4-MeO-phenyl	TFA	20	1 h	34	10	Near total	High
6	4-MeO-phenyl	BF_3/EtOH	3.3/130	15 min–1 h	38	<5	Near total	Low
7	4-EtO-phenyl	TFA	20	1 h	32	10	Near total	High
8	4-EtO-phenyl	BF_3/EtOH	3.3/130	15 min–1 h	36	ND[g]	Near total	Low
9	C_6F_5	TFA	215	8 min–4 h	12	5	Low	Low[h]
10	C_6F_5	BF_3-etherate	10	8 min–24 h	23	<5	Low	None
11	2,3,5,6-F_4-phenyl	TFA	215	4 min–8 h	13	10	Low	Low[h]
12	2,3,5,6-F_4-phenyl	BF_3-etherate	10	15 min–8 h	25	ND[g]	Low	Trace
13	2,4,6-Me_3-phenyl	BF_3/EtOH	3.3/130	1–4 h	19	10	Low	Low[h]
14	2,6-Me_2-phenyl	BF_3/EtOH	3.3/130	1–8 h	19	10	Low	Low[h]
15	Pentyl	TFA	10	15 min–8 h	16	—[i]	Low	None[i]
16	Pentyl	BF_3-etherate	1.0	8 min–24 h	9	—[i]	None	None[j]
17	Hexyl	TFA	10	15 min–8 h	16	—[i]	Low	None[j]
18	Hexyl	BF_3-etherate	1.0	8 min–24 h	9	—[i]	None	None[j]

[a] The reactions were performed with 10 mM each of pyrrole and aldehyde at a 15 ml scale at room temperature in CH_2Cl_2 (for mesitaldehyde and 2,6-dimethylbenzaldehyde, ethanol cocatalysis was carried out in $CHCl_3$ stabilized with amylenes or in CH_2Cl_2). The reactions were monitored at 1 min, 4 min, 8 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, and 24 h.
[b] The range of time during the condensation reaction where the yield of porphyrinogen was maximal (as inferred from the yield of porphyrin in aliquots of the oxidized reaction mixture).
[c] The maximum yield of porphyrin (UV–Vis).
[d] The level of unreacted aldehyde at the time that the maximum yield of porphyrin was first obtained.
[e] Yield turnover refers to a decline in the yield of porphyrin after the maximum has been obtained.
[f] Oligomer truncation refers to disappearance of larger oligomers, and appearance of shorter oligomers that were not present at early reaction times.
[g] Below the limits of detection (<1%).
[h] The changes in oligomer composition occurred between the 8 h and 24 h timepoints.
[i] Alkyl aldehydes could not be detected readily by TLC analysis.
[j] The intensity and resolution of peaks of higher *m/z* than the porphyrin were poor for all spectra; nevertheless, the appearance of the spectra remained consistent once the maximum yield of porphyrin was obtained.

time are provided in Figure 1 (see the Supplementary data for the complete set of plots for all aldehydes). The quantity of unreacted aldehyde was not determined for the alkyl aldehydes, which are poorly detected upon TLC analysis. A representative set of LD-MS spectra showing changes in the oligomer composition as a function of time are shown in Figure 2 (see the Supplementary data for the complete set of spectra). Observations from the time course experiments are summarized in Table 2.

Two key features concerning the trajectory of the pyrrole + aldehyde reaction are (1) the extent to which the yield of porphyrinogen declines after reaching a maximum, and (2) the extent to which the oligomers undergo truncation. The turnover in porphyrinogen yield is readily established by monitoring the condensation over time. In some cases, the turnover was near total while in other cases the porphyrinogen yield reached a maximum and then plateaued. Reactions of aldehydes with electron-donating substituents showed a substantial turnover in the yield of porphyrinogen under TFA or BF_3-etherate/EtOH catalysis (Fig. 1B), much more than previously observed with benzaldehyde under TFA or BF_3-etherate catalysis. Examination of crude, oxidized reaction samples by LD-MS reveals the composition of the oligomers.[2] Studies of the reaction with benzaldehyde + pyrrole under TFA catalysis showed that the oligomeric composition continues changing over a period well beyond the peak in porphyrinogen yield. The composition becomes enriched in shorter oligomers that were not originally present in the LD-MS spectra, a phenomenon we have referred to as oligomer truncation.[2] Oligomer truncation can result from reactions giving reversible formation/disassembly of polypyrromethanes in conjunction with irreversible side reactions, and/or irreversible side reactions that cause fragmentation of polypyrromethanes. In the present work, reactions of aldehydes with electron-donating substituents showed significant levels of oligomer truncation (Fig. 2), while other reactions provided little to no truncation. It is noteworthy that continued formation of long oligomers at prolonged reaction times was not observed, even for aldehydes that react poorly or in a non-reversible manner.

The two members of a given pair of aldehydes generally provided identical results. The lone exception was the benchmark pair of benzaldehyde and *p*-tolualdehyde. Benzaldehyde was consumed faster under BF_3-etherate or TFA catalysis and provided a higher yield of porphyrin under BF_3-etherate than *p*-tolualdehyde (Fig. 1A). There were no sharp differences in the oligomer compositions provided by the two aldehydes. The disparities are not due to inappropriate acid concentrations, as the acid catalysis study found both aldehydes to require similar acid concentrations.

2.1.3. Reaction reversibility. The reversibility of the condensations was examined by double-labeling crossover (i.e., 'exchange') studies.[7,8] Reactions of two aldehydes of a given type (e.g., *p*-anisaldehyde and 4-ethoxybenzaldehyde) were performed side-by-side under the conditions used in the reaction course studies. After a defined 'premixing time', an aliquot from each reaction was transferred to a common flask and the exchange processes were allowed to occur. Three premixing times were examined: a time close to when the maximum yield of porphyrinogen is initially attained and the concentration of unreacted aldehyde is $<10\%$ of the original concentration, a later timepoint when porphyrinogen yield was still high, and a long timepoint when the porphyrinogen yield had in some cases declined. (The objective of $<10\%$ unreacted aldehyde was achieved in every case with the exception of *p*-tolualdehyde under BF_3-etherate catalysis.) Duplicate mixtures were prepared at each premixing time. One mixture was allowed to react with no additional acid catalyst, and the second mixture was

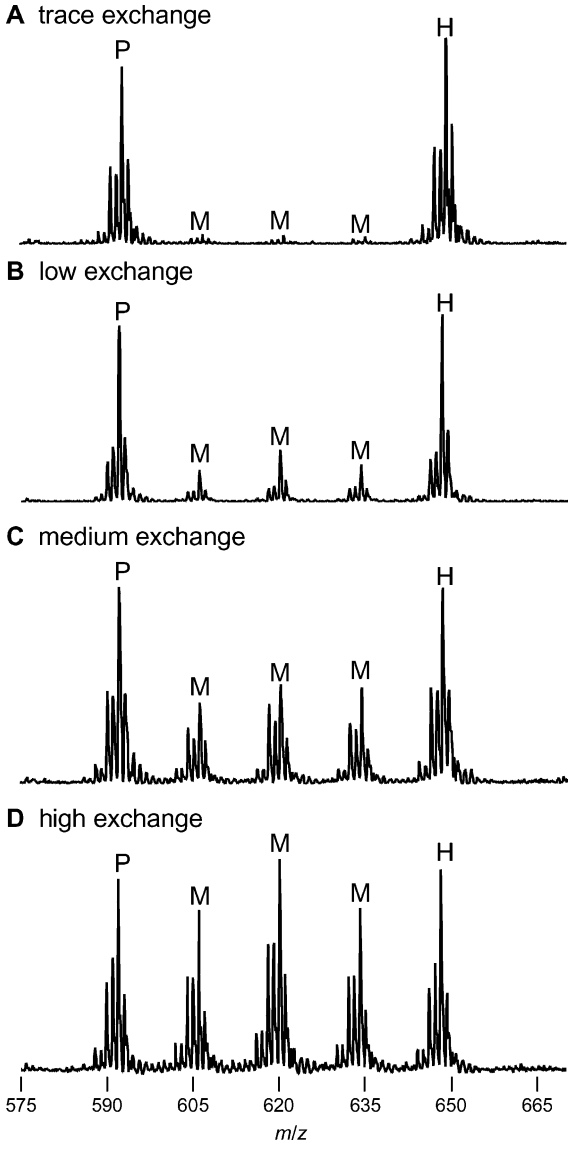


Figure 3. LD-MS spectra of crude, oxidized reaction mixtures from exchange experiments involving hexanal (R = pentyl) and heptanal (R = hexyl) that illustrate the assignment of the level of exchange. The region corresponding to the *m/z* ratio of the possible porphyrin products is shown. The peaks are labeled as follows: P = oligomer containing only pentyl substituents, H = oligomer containing only hexyl substituents, and M = oligomers containing a mixture of pentyl and hexyl substituents. The conditions that provide each spectrum are as follows: (A) BF_3-etherate (1.0 mM), no acid pulse, premixing = 15 h, postmixing = 4 h; (B) BF_3-etherate (1.0 mM), no acid pulse, premixing = 15 min, postmixing = 1 h; (C) BF_3-etherate (1.0 mM), acid pulse, premixing = 4 h, postmixing = 4 h; (D) BF_3-etherate (1.0 mM), acid pulse, premixing = 15 min, postmixing = 4 h.

treated with fresh acid catalyst (acid pulse) at the time of mixing. At the time of mixing, the oligomer composition (LD-MS) and the level of unreacted aldehyde (TLC analysis) were examined. After mixing, the porphyrin yield and oligomer composition of the combined reactions were monitored from 4 min to 4 h. As the two aldehydes of a given pair are of different mass, oligomers containing the two different substituents could be distinguished by LD-MS. The peak assignments were made as described previously.[2] In the limit of no reversibility, the LD-MS spectrum would be identical to the superimposed spectra of separate reactions of pyrrole with each aldehyde. In the limit of total reversibility, the oligomer composition (and thus the LD-MS spectrum) would be identical to that obtained from a mixed condensation of pyrrole and the two aldehydes. Illustrative LD-MS spectra for the case of hexanal and heptanal are shown in Figure 3. The results of all of the exchange experiments are summarized in Table 3.

The results led to an approximate rank ordering of aldehyde substituents in terms of reversibility in the pyrrole + aldehyde condensation: phenyl/*p*-tolyl ≥ 4-alkoxyphenyl > alkyl ≫ pentafluorophenyl/tetrafluorophenyl > mesityl/2,6-dimethylphenyl. The rank order is somewhat dependent on the conditions of the exchange experiment as evidenced by entries 1–4 in Table 3 that pertain to the benchmark aldehydes. Previously, we reported observing a low level of exchange in reactions involving alkyl aldehydes and concluded that reactions involving such aldehydes are largely irreversible.[8] Those previous experiments were restricted to a single reaction condition and premixing period, and only exchange manifested in the distribution of porphyrins upon oxidation and TLC analysis was detected. The present experiments investigated wider reaction conditions and mixing times, and the LD-MS analysis allows detection of exchange in all oligomer species produced in the reaction. Thus, it is now clear that at least some aspects of condensations of alkyl aldehydes exhibit reversibility under some reaction conditions.

There was no strong correlation between yield of porphyrin and extent of exchange, indicating that porphyrinogen formation does not depend on reaction reversibility (recovery from unproductive oligomers). The extent of exchange was greatest at the shortest premixing times, regardless of the addition of an acid pulse. Thus, in all cases there appears to be gradual, irreversible damage to the oligomers that prevents exchange processes. In all cases where exchange was detected, the exchange occurred gradually upon mixing the individual reactions and did not reach a statistical level. These results indicate that while initial oligomer formation is reversible to varying degrees depending on the aldehyde and acid, the reversibility is sluggish, declines over time, and cannot be overcome by adding fresh acid catalyst.

2.2. Reactions of dipyrromethanes

5-Substituted dipyrromethanes possessing substituents analogous to those employed in the reactions of aldehydes were selected: phenyl (benchmark case), 4-methoxyphenyl (electron donating), pentafluorophenyl (electron withdrawing), mesityl (sterically hindered), and pentyl (alkyl). Two general types of experiments were performed: acidolysis and oligomerization of the dipyrromethane in the absence of

Table 3. Results of porphyrinogen exchange experiments[a]

Entry	Substituent type	Acid[b]	Premixing times[c]	Acid pulse[d]	Level of exchange[e]		
					Short premixing time	Intermediate premixing time	Long premixing time
1	Benchmark	TFA	1, 4, 15 h	No	Trace-low	Trace	Trace
2	Benchmark	TFA	1, 4, 15 h	Yes	Medium-high	Low-medium	Trace
3	Benchmark	BF_3-etherate	1, 4, 15 h	No	High	Low	Trace-low
4	Benchmark	BF_3-etherate	1, 4, 15 h	Yes	High-statistical	Medium-high	Medium
5	e-rich	TFA	1, 2, 6 h	No	Medium	Low	Trace
6	e-rich	TFA	1, 2, 6 h	Yes	High-statistical	Medium-high	Trace
7	e-rich	BF_3/EtOH	0.5, 2, 6 h	No	Medium	Low	Trace
8	e-rich	BF_3/EtOH	0.5, 2, 6 h	Yes	Medium-high	Medium	Trace
9	e-deficient	TFA	0.25, 2, 15 h	No	Trace	Not detected	Not detected
10	e-deficient	TFA	0.25, 2, 15 h	Yes	Trace	Trace	Not detected
11	e-deficient	BF_3-etherate	0.25, 2, 15 h	No	Low	Trace	Not detected
12	e-deficient	BF_3-etherate	0.25, 2, 15 h	Yes	Low	Trace	Not detected
13	Bulky	BF_3/EtOH	2, 4, 24 h	No	Trace	Not detected	Not detected
14	Bulky	BF_3/EtOH	2, 4, 24 h	Yes	Low	Trace-low	Not detected
15	Alkyl	TFA	0.5, 4, 15 h	No	Low	Trace	Trace
16	Alkyl	TFA	0.5, 4, 15 h	Yes	Medium	Trace	Trace
17	Alkyl	BF_3-etherate	0.25, 4, 15 h	No	Medium	Trace-low	Trace
18	Alkyl	BF_3-etherate	0.25, 4, 15 h	Yes	High	Medium	Low

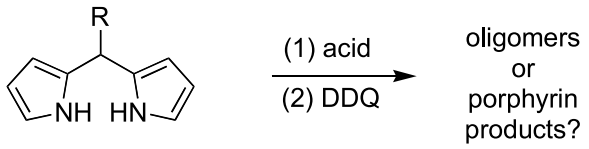
[a] The reactions were performed with 10 mM each of pyrrole and aldehyde at room temperature in CH_2Cl_2 (for mesitaldehyde and 2,6-dimethylbenzaldehyde, $CHCl_3$ stabilized with amylenes was used instead of CH_2Cl_2). At the conclusion of the premixing time, an equal volume (7.5 mL) of each pair of reaction mixtures was mixed in a common flask. The combined mixture was monitored for exchange at 4 min, 15 min, 1 h, and 4 h by LD-MS.

[b] Refer to Table 2 for acid concentrations.

[c] The three values refer to the short, intermediate, and long premixing times listed in the final three columns of this table. A level of unreacted aldehyde of $<10\%$ was present in each exchange experiment except with benzaldehyde and *p*-tolualdehyde under BF_3-etherate (1.0 mM) catalysis. Both aldehydes react slowly under BF_3-etherate catalysis. The level of unreacted aldehyde at the three premixing times are as follows: 1 h: $\sim$30% benzaldehyde and $\sim$70% *p*-tolualdehyde, 4 h: $\sim$15% benzaldehyde and $\sim$35% *p*-tolualdehyde, and 15 h: $<5\%$ benzaldehyde and $\sim$20% *p*-tolualdehyde.

[d] The acid pulse provided a quantity of fresh acid catalyst equal to that initially present (i.e., the overall acid concentration is doubled).

[e] The level of exchange by 4 h after mixing ranges from not detected/trace/low/medium/high/statistical.



Scheme 2.

added aldehyde, and reaction of a dipyrromethane and an aldehyde.

2.2.1. Acidolysis and oligomerization. Each dipyrromethane (10 mM) was treated with acid catalysis conditions in the absence of any added aldehyde (Scheme 2). The acid catalysis conditions include (a) those identified herein for porphyrin formation in the corresponding pyrrole + aldehyde reaction, (b) the standard condition of TFA (20 mM), (c) the standard condition of BF_3-etherate (1.0 mM), and (d) our previously reported 'low scrambling' condition for the condensation of sterically unhindered dipyrromethanes and an aldehyde leading to *trans*-A_2B_2-porphyrins (1.0 mM BF_3-etherate, 100 mM NH_4Cl, in MeCN at 0 °C).[14] The latter condition was employed to better discriminate among those dipyrromethanes that are particularly susceptible toward acidolysis. The reactions were monitored from 4 min to 4 h for yield of porphyrin (UV–Vis) and oligomer composition (LD-MS). Observation of peaks in the LD-MS spectrum due to the porphyrin and to oligomers of mass greater than the dipyrromethane imply scrambling processes, as their formation requires acidolysis of the dipyrromethane and further reaction of the resulting fragments. The results of the experiments are summarized in Table 4, and representative LD-MS spectra are provided in the Supplementary data.

5-Mesityldipyrromethane was found to be stable towards all conditions investigated (Table 4, entries 13–15), and 5-pentafluorophenyldipyrromethane only underwent significant, albeit slow reaction under BF_3-etherate catalysis (entries 9 and 12). The other dipyrromethanes generally underwent rapid reaction (except under the low scrambling conditions). Interestingly, 5-(4-methoxyphenyl)dipyrromethane underwent significant reaction in the presence of 1.0 mM BF_3-etherate (entry 5) even though the same condition fails to cause pyrrole + *p*-anisaldehyde to produce detectable porphyrin. Accordingly, the failure of the *p*-anisaldehyde + pyrrole reaction with 1.0 mM BF_3-etherate to give porphyrin must originate in the initial condensation rather than subsequent steps of oligomer or porphyrinogen formation. With the exception of 5-mesityldipyrromethane, each dipyrromethane provided a yield of porphyrin and oligomer composition similar to the reaction of pyrrole + aldehyde under at least one of the conditions investigated. Thus, the extent of reversibility from the starting point of pure dipyrromethane is greater than that obtained from the mixing of diverse species in the exchange experiments (where statistical exchange was not observed).

2.2.2. Reaction of a dipyrromethane and an aldehyde. Reactions of a dipyrromethane with an aldehyde bearing a complementary substituent (e.g., 5-pentafluorophenyldipyrromethane + 2,3,5,6-tetrafluorobenzaldehyde; 5 mM each; Scheme 3) were performed under conditions identified for the corresponding pyrrole + aldehyde reaction. The

Table 4. Results upon treatment of dipyrromethanes with acid (in the absence of added aldehyde)

Entry	R in R-dipyrromethane	Reaction condition[a]	Detection of porphyrin[b]	Maximum porphyrin[c]	% Porphyrin[d]	Oligomer formation[e]
1	Phenyl	TFA, 20 mM	15 min	1 h	35	Yes
2	Phenyl	BF_3-etherate, 1.0 mM	30 min	4 h	33	Yes
3	Phenyl	Low scrambling	ND[f]	ND[f]	ND[f]	Trace
4	4-MeO-phenyl	TFA, 20 mM	<4 min	15 min	36	Yes
5	4-MeO-phenyl	BF_3-etherate, 1.0 mM	15 min	1 h	41	Yes
6	4-MeO-phenyl	Low scrambling	4 h	4 h	6	Yes
7	4-MeO-phenyl	BF_3-etherate/EtOH, 3.3/130 mM	<4 min	15 min	38	Yes
8	C_6F_5	TFA, 20 mM	ND[f]	ND[f]	ND[f]	Trace
9	C_6F_5	BF_3-etherate, 1.0 mM	1 h	4 h	15	Yes
10	C_6F_5	Low scrambling	ND[f]	ND[f]	ND[f]	Yes
11	C_6F_5	TFA, 215 mM	30 min	4 h	3	Yes
12	C_6F_5	BF_3-etherate, 10 mM	30 min	4 h	13	Yes
13	Mesityl	TFA, 20 mM	ND[f]	ND[f]	ND[f]	Trace
14	Mesityl	BF_3-etherate, 1.0 mM	ND[f]	ND[f]	ND[f]	Trace
15	Mesityl	BF_3-etherate/EtOH, 3.3/130 mM	ND[f]	ND[f]	ND[f]	Trace
16	Pentyl	TFA, 20 mM	<4 min	15 min	23	Yes
17	Pentyl	BF_3-etherate, 1.0 mM	<4 min	4 h	25	Yes
18	Pentyl	TFA, 10 mM	<4 min	30 min	25	Yes
19	Pentyl	Low scrambling	1 h	4 h	10	Yes

[a] The reactions were performed with 10 mM of dipyrromethane. With the exception of the low scrambling condition, reactions were performed at room temperature in CH_2Cl_2 (for mesitaldehyde and 2,6-dimethylbenzaldehyde, $CHCl_3$ stabilized with amylenes was used instead of CH_2Cl_2). The low scrambling conditions are as follows: BF_3-etherate (1.0 mM), NH_4Cl (100 mmol l^{-1}), in MeCN at 0 °C. The reactions were monitored at 4 min, 15 min, 30 min, 1 h, and 4 h.

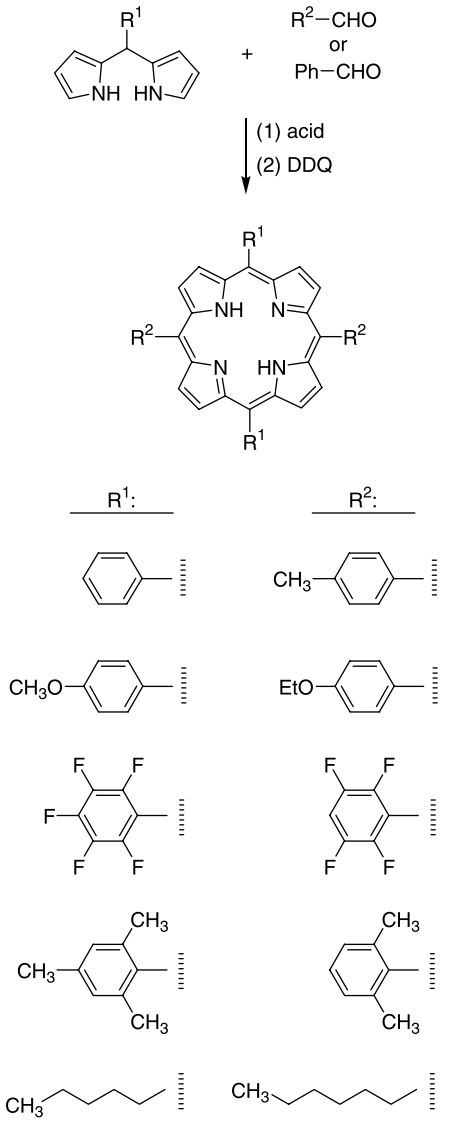
[b] The time at which porphyrin was first detected (UV–Vis) in an oxidized aliquot of the reaction mixture (limit of detection is 0.5%).

[c] The time at which the highest yield of porphyrin was observed.

[d] The highest yield of porphyrin observed. The yield is based on the stoichiometry of 1 equiv of porphyrinogen per 4 equiv of dipyrromethane.

[e] Detection of oligomers (LD-MS) of *m/z* greater than that of the dipyrromethane. 'Trace' means that only a small number of peaks of intensity close to the limit of detection were observed.

[f] No porphyrin was detected at any timepoint (limit of detection is 0.5%).



Scheme 3.

reactions were monitored from 4 min to 8 h for yield of porphyrin (UV–Vis) and oligomer composition (LD-MS). Acidolysis and scrambling were assessed by examination of the LD-MS spectra,[4] where peaks due to expected oligomers, oligomers formed by acidolysis, and oligomers produced by scrambling are readily identified. The results of these experiments are summarized in Table 5, and illustrative LD-MS spectra are provided in Figure 4. Similar reactions and analyses were performed for the reaction of each dipyrromethane + benzaldehyde (see Supplementary data).

With the exception of 5-mesityldipyrromethane and 5-pentafluorophenyldipyrromethane, condensations performed in the presence of a complementary aldehyde or benzaldehyde generally exhibited scrambling very early in the reaction and the level of scrambling reached statistical levels by the time the maximum yield of porphyrinogen was obtained. 5-(4-Methylphenyl)dipyrromethane was found to undergo scrambling more rapidly than 5-phenyldipyrromethane, even under the 'low scrambling' conditions (see Supplementary data for LD-MS spectra). Thus, differences between benzaldehyde and *p*-tolualdehyde observed during the studies of pyrrole + aldehyde condensations may also stem from different stability of oligomers bearing phenyl vs. *p*-tolyl substituents. Although in this study aldehydes of related structure generally behaved similarly, the contrasting behavior of benzaldehyde and *p*-tolualdehyde does show that small electronic differences can alter the course and outcome of the reaction.

3. Outlook

The ability to prepare porphyrinic macrocycles bearing diverse substituents is essential for a broad range of applications. The objectives of this study were to examine the catalytic requirements of a range of aldehydes, to better understand the effects of diverse substituents on the reaction course in terms of yield of porphyrin, reaction of aldehyde, composition of the oligomers, and reversibility of polypyrromethane and porphyrinogen formation, and to better

Table 5. Results of reactions of dipyrromethanes with their partner aldehydes[a]

Entry	R in R-dipyrromethane	Aldehyde substituent	Reaction condition	Time[b]	% Porphyrin	Scrambling[c]	Onset of scrambling[d]
1	Phenyl	*p*-Tolyl	TFA, 20 mM	15 min	45	Statistical	<4 min
2	Phenyl	*p*-Tolyl	BF_3-etherate, 1.0 mM	30 min	33	Statistical	15 min
3	4-MeO-phenyl	4-EtO-phenyl	TFA, 20 mM	15 min	34	Statistical	<4 min
4	4-MeO-phenyl	4-EtO-phenyl	BF_3-etherate/EtOH, 3.3/130 mM	4 min	38	Statistical	<4 min
5	C_6F_5	2,3,5,6-F_4-phenyl	TFA, 215 mM	4 min	15	Low level	<4 min
6	C_6F_5	2,3,5,6-F_4-phenyl	BF_3-etherate, 10 mM	15 min	20	Statistical	<4 min
7	Mesityl	2,6-Me_2-phenyl	BF_3-etherate/EtOH, 3.3/130 mM	4 h	35	None[e]	NA
8	Pentyl	Hexyl	TFA, 10 mM	4 min	22	Statistical	<4 min
9	Pentyl	Hexyl	BF_3-etherate, 1.0 mM	15 min	31	Statistical	<4 min

[a] The reactions were performed with 5 mM each of dipyrromethane and aldehyde at the 15 ml scale at room temperature in CH_2Cl_2 (for 5-mesityldipyrromethane and 2,6-dimethylbenzaldehyde, $CHCl_3$ stabilized with amylenes was used instead of CH_2Cl_2). The reactions were monitored at 4 min, 15 min, 30 min, 1 h, 4 h, and 8 h.
[b] The time at which the yield of porphyrin reached its maximum value.
[c] Level of scrambling (LD-MS) at the time of maximum yield of porphyrin. Refer to Figure 4 for representative LD-MS spectra.
[d] The time at which scrambling was first detected (LD-MS).
[e] Peaks were observed that may be assigned to oligomers produced by acidolysis, but no scrambling was detected.

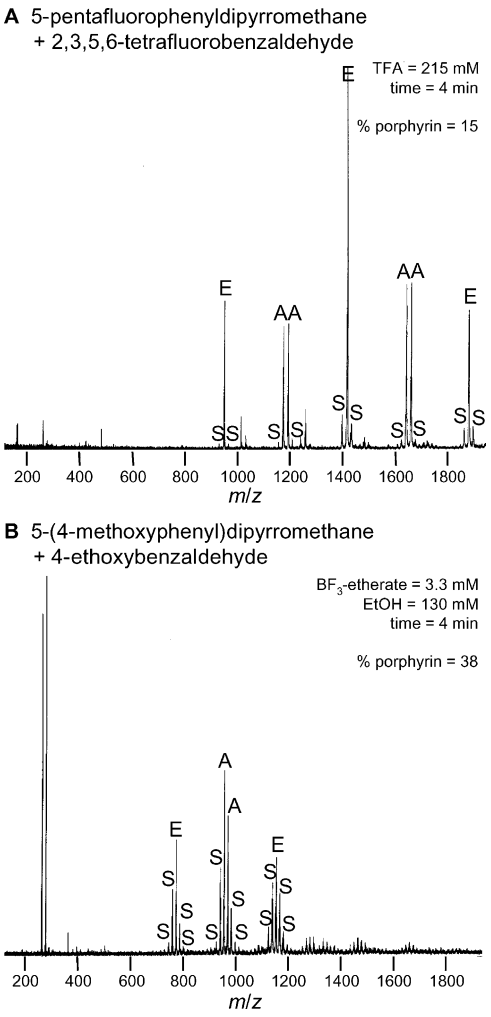


Figure 4. LD-MS spectra of crude, oxidized reaction mixtures from condensation of a dipyrromethane (R^1-DPM) with an aldehyde bearing a complementary substituent (R^2-CHO). The yield of porphyrin was determined by UV–Vis analysis. The panels illustrate the following: (A) a low level of scrambling, and (B) statistical scrambling. The peaks are labeled as follows: E=expected oligomers, A=oligomers formed by acidolysis, and S=oligomers produced by scrambling. The peaks were assigned as described previously.[4]

delineate the propensity for reversible processes leading to scrambling in reactions of dipyrromethanes.

Examination of the catalytic requirements of the pyrrole+aldehyde condensations revealed that quite a broad range of acid concentrations can be employed as long as the differing reaction trajectories resulting from the various aldehydes are taken into account. Application of one or both of the standard catalysis conditions [TFA (20 mM) or BF_3-etherate (1.0 mM)] results in reasonable yields of porphyrin for most aldehydes (except sterically bulky aldehydes such as mesitaldehyde) provided that the condensation is monitored so that the oxidant is added at the time of maximum yield of porphyrinogen.

The reaction course of the pyrrole+aldehyde condensations showed dependence on reaction conditions and aldehyde substituent. The reactions of the phenyl, *p*-tolyl, and alkyl aldehydes using BF_3-etherate generally afforded little turnover in porphyrinogen, little oligomer truncation, and a medium to high level of reversibility. The same aldehydes with TFA catalysis generated a higher level of turnover in porphyrinogen with a commensurate increase in level of oligomer truncation. Turnover in porphyrinogen level and extent of oligomer truncation were not always correlated. For example, the reaction of *p*-anisaldehyde gave near-total turnover ($\sim$35% yield of porphyrin at 1 h; $<$4% at 24 h) with both TFA and BF_3-ethanol catalysis, but the oligomer truncation was pronounced in the former yet low in the latter. The condensations with mesitaldehyde and pentafluorobenzaldehyde were generally irreversible, as evidenced by the lack of porphyrinogen and oligomer exchange. This finding illustrates that reversibility is not a prerequisite for achieving a good yield of the porphyrin.

The level of scrambling observed with various 5-substituted dipyrromethanes is somewhat dependent on the reaction conditions. Nevertheless, dipyrromethanes bearing phenyl, alkyl, and electron-donating aryl groups were generally prone to scrambling, while 5-pentafluorophenyldipyrromethane was very insensitive and 5-mesityldipyrromethane was essentially inert toward scrambling. This order is largely in agreement with the results of the porphyrinogen exchange experiments, although the extent of scrambling was often much greater than the level of exchange produced in the corresponding pyrrole+aldehyde reaction. These observations provide guidance for the design of synthetic plans that minimize the potential for undesired scrambling.

Acknowledgements

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Supplementary data

Complete experimental section, plots of the yield of porphyrin as a function of acid concentration, and plots of the yield of porphyrin and the percent unreacted aldehyde as a function of condensation time for all aldehydes examined; LD-MS spectra showing the oligomer composition at each timepoint for all reactions of pyrrole+aldehyde examined; data pertaining to studies of 5-substituted dipyrromethanes bearing diverse substituents.

Supplementary data associated with this article can be found at doi:10.1016/j.tet.2004.09.081

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Selective trifluorination of alkyl aryl sulfides using IF_5

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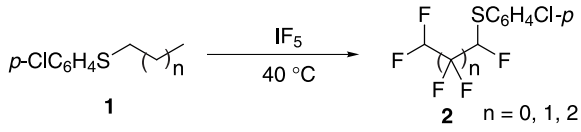
Abstract—In the reaction of IF_5 with alkyl aryl sulfides in heptane under reflux conditions, the arylthio group migrated once and three fluorine atoms were selectively introduced on the alkyl chain. In order to find the reason why the reaction stopped at the trifluorination step, we examined the oxidation potentials of the starting material, a reaction intermediate, and the product, and the time course of the reactions.

1. Introduction

Oxidative fluorination of sulfur compounds has been conveniently used to introduce one or several fluorine atoms into organic molecules under mild conditions.[1] Recently, we have found that an arylthio group of alkyl aryl sulfides migrated on the carbon chain successively to the terminal carbon by the reaction with IF_5 at 40 °C in a tight-screw capped vessel, and the fluorination took place on the carbon where the arylthio group was attached.[2] Finally, 3–7 fluorine atoms could be introduced into the alkyl chains depending on the alkyl chain length (Scheme 1). During our continuous study of fluorination using IF_5, we found that three fluorine atoms can be selectively introduced into the alkyl chain of the alkyl aryl sulfides regardless of the alkyl chain length by carrying out the reaction in heptane under reflux conditions.



Scheme 1.

2. Results and discussion

When *p*-chlorophenyl propyl sulfide (**1a**) was allowed to react with 1.2 equiv of IF_5 in heptane under reflux condition for 1 h, the arylthio group migrated once and a mixture of 1,1-difluoro-2-(*p*-chlorophenylthio)propane (**3a**) and 1,1,2-trifluoro-2-(*p*-chlorophenylthio)propane (**4a**) was obtained. By using 2.4 equiv of IF_5, **4a** could be selectively obtained and the formation of **3a** was not observed. When the reaction was carried out at 40 °C in a tight-screw capped vessel, the arylthio group migrated twice and 1,2,2,3,3-pentafluoro-1-(*p*-chlorophenylthio)propane (**2a**) was obtained as the main product. However, under reflux in heptane, the main product was **4a** even after 6 h, and **2a** was formed only as a minor product (5%) (Table 1).

When isolated **4a** was subjected to the reaction with IF_5 at 40 °C in the tight-screw capped vessel for 28 h, further fluorination and migration of the arylthio group took place to give **2a** as the main product (Scheme 2).

Various alkyl aryl sulfides were used for the reaction with IF_5 under reflux condition in heptane (Table 2). When an electron-donating group was attached at the *p*-position of a phenyl group (**1b**), 1.2 equiv of IF_5 was enough to obtain the trifluorinated product (**4b**). On the other hand, the presence of a strong electron-withdrawing group (**1c**) retarded the reaction, and longer reaction time and large excess of IF_5 were required to complete the reaction. When phenyl propyl sulfide was used, a competitive iodination by in situ-generated IF[3] at the *p*-position of the phenyl group took place,[4] and a mixture of 1,1,2-trifluoro-2-phenylthiopropane and 1,1,2-trifluoro-2-(*p*-iodophenylthio)propane was formed. In all cases, the arylthio group migrated only once and three fluorine atoms were introduced into the sulfides regardless of the alkyl chain length. The functional groups such as ester (**1h**) or amide (**1i**), can tolerate the reaction conditions.

As previously proposed,[2] the fluorination and migration of the arylthio group proceed as follows. In the first step, oxidation of the sulfur in **1a** takes place to give a sulfonium intermediate which gives mono-fluorinated product **5a**,[5] or a

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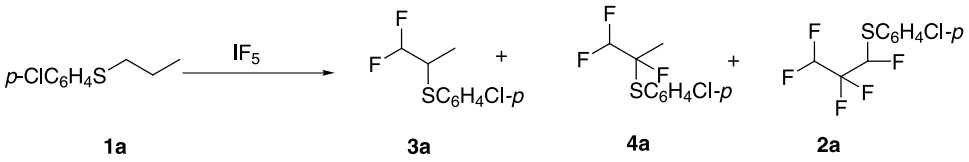
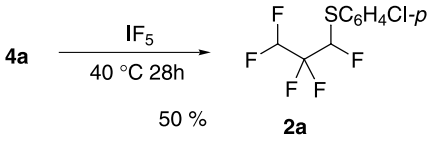
Table 1. Reaction of **1a** with IF_5 under various conditions[a]



1a **3a** **4a** **2a**

Amount of IF_5 (eq to **1a**)	Reaction time (h)	Yield (%)[b]		
		3a	**4a**	**2a**
1.2	1	43	17	0
2.4	1	0	74	0
2.4	6	0	72	5
2.4[c]	48	0	0	72

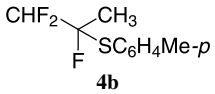
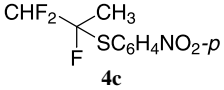
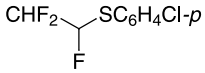
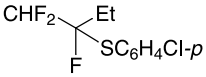
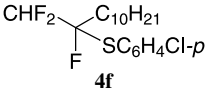
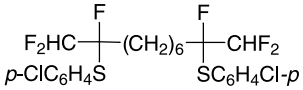
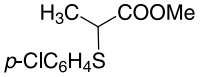
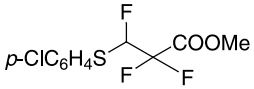
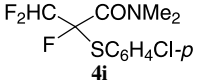
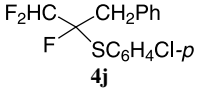
[a] If otherwise not mentioned, the reaction was carried out in heptane under reflux.
[b] Isolated yield based on **1a**.
[c] The reaction was carried out in hexane at 40 °C in a tight-screw capped vessel.



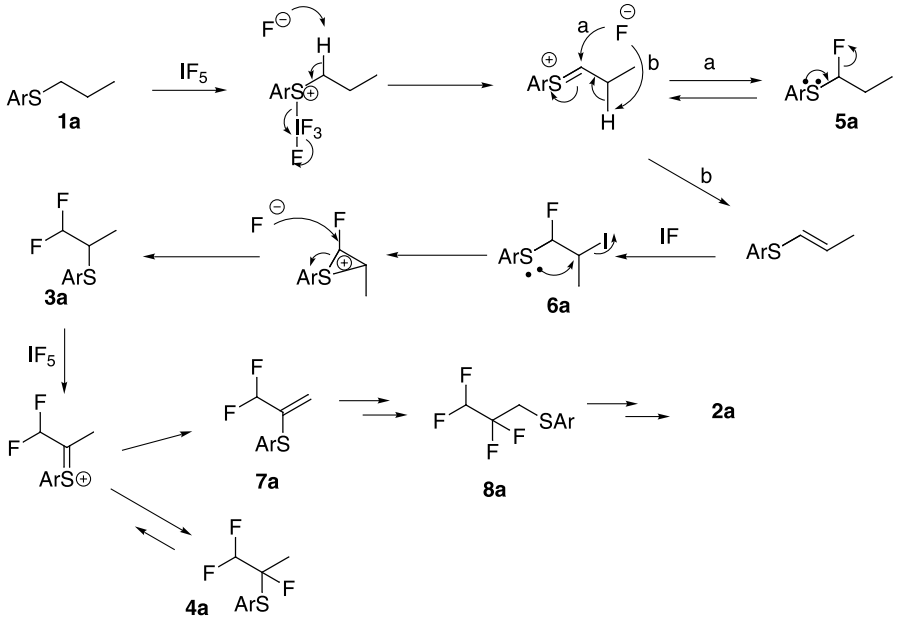
Scheme 2.

vinylic sulfide.[6] Addition of in situ-generated IF to the vinylic sulfide gives 1-arylthio-1-fluoro-2-iodopropane (**6a**).[3] Then elimination of an iodide, migration of the arylthio group, and fluorination at the terminal carbon take place successively to give 1,1-difluoro-2-arylthiopropane **3a**. Finally, the oxidative fluorination of **3a** takes place at the α-carbon of the sulfur to give **4a**. The next step,

Table 2. Fluorination of alkyl aryl sulfides using IF_5[a]

Substrate	IF_5/**1**	Product	Yield (%)[b]
p-MeC_6H_4SPr **1b**	1.2	 **4b**	51
p-$NO_2C_6H_4SPr$ **1c**	4.8	 **4c**	66[c]
p-ClC_6H_4SEt **1d**	2.4	 **4d**	66
p-ClC_6H_4SBu **1e**	2.4	 **4e**	70
p-$ClC_6H_4SC_{12}H_{25}$ **1f**	3.6	 **4f**	83
p-$ClC_6H_4S(CH_2)_{10}SC_6H_4Cl$-*p* **1g**	7.2	 **4g**	75
 **1h**	4.8	 **4h**	71[d]
p-$ClC_6H_4S(CH_2)CONMe_2$ **1i**	2.4	 **4i**	71
p-$ClC_6H_4S(CH_2)_3Ph$ **1j**	3.6	 **4j**	71

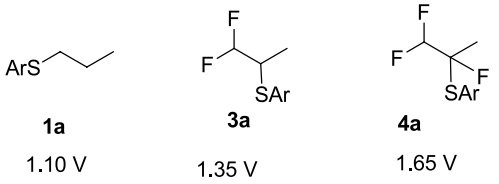
[a] If otherwise not mentioned, the reaction was carried out in heptane under reflux for 1 h.
[b] Isolated yields based on substrates.
[c] The reaction was carried out for 24 h.
[d] The reaction was carried out for 6 h.



Scheme 3.

elimination of HF from **4a** to a vinylic sulfide **7a**, is slow and **4a** can be selectively obtained (Scheme 3).

The oxidation potentials of **1a**, **3a**, and **4a** were measured by an electrochemical method to find the reason why the reaction stopped at **4a** (Scheme 4). As expected, the starting material **1a** has low oxidation potential (1.10 V) and, therefore, the oxidative fluorination step of **1a** proceeded fast under the reaction conditions. 1,1-Difluoro-2-arylthiopropane **3a**, which was isolated as the main product when the reaction was carried out using 1.2 equiv of IF_5 to **1a**, has higher oxidation potential than **1a** due to the electron-withdrawing effect of the difluoromethyl group, and the oxidative fluorination of **3a** to **4a** proceeds relatively slowly. Trifluorinated product **4a** has the highest oxidation potential of 1.65 V which indicates that a lone-paired electron density on the sulfur is the lowest because of the strong electron-withdrawing effect of an attached trifluoropropyl group. Generally, α-fluorosulfides are unstable due to the lone-paired electrons on the sulfur and a facile elimination of fluoride takes place to cause their decomposition,[8] and isolation of 1-fluoro-1-arylthiopropane (**5a**) was unsuccessful. Though **4a** is also the α-fluorosulfide, **4a** was stable enough to isolate. As the lone-paired electron density on the sulfur is low in **4a**, the arylthio group did not destabilize **4a**. Consequently, elimination of HF from **4a** to the formation of **7a** is slow.



Scheme 4.

Then, we investigated the time courses of the reactions at 40 °C in the tight-screw capped vessel to find the reason why the reaction does not stop at **4a** and proceeds to **2a** under the reaction conditions. The yields of **3a** increase up to 1 h and then gradually decrease with an increase in the yields of **4a**. The amount of **4a** gradually increases up to 3 h and then begins to decrease with an increase in the yields of **2a**. After 24 h, **2a** is almost the sole product and the yields of **3a** and **4a** are less than 2%. During the reaction, the formation of 1,1,2,2-tetrafluoro-3-(*p*-chlorophenylthio)propane (**8a**) is observed but the yield is low (less than 6%) (Fig. 1). When the reaction is carried out under reflux in heptane, **3a** and **4a** are formed as main products in 15 min

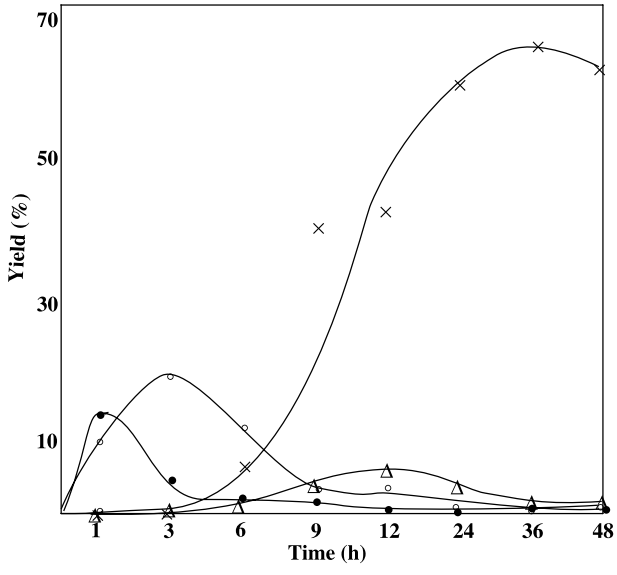


Figure 1. Time dependence of the product distributions in the reaction of IF_5 with **1a** at 40 °C in a tight-screw capped vessel (●; **3a**, ○; **4a**, △; **8a**, ×; **2a**).

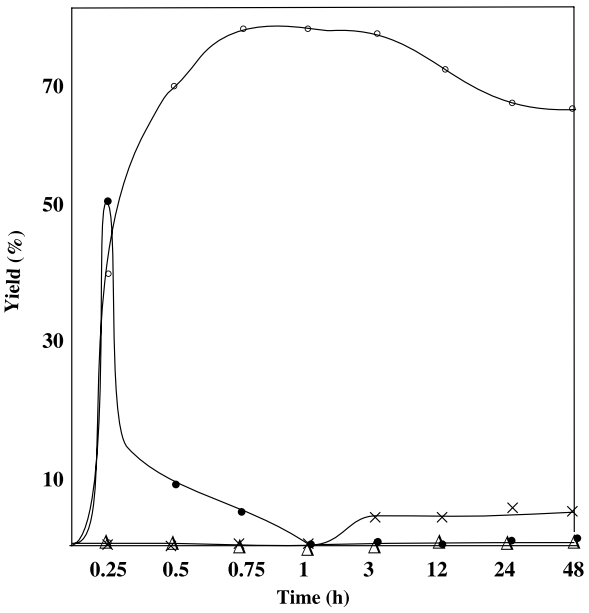


Figure 2. Time dependence of the product distributions in the reaction of IF_5 with **1a** under reflux in heptane (●; **3a**, ○; **4a**, △; **8a**, ×; **2a**).

and then the yields of **3a** decrease with an increase in the yields of **4a**. After 1 h, **3a** disappears and **4a** becomes the sole main product. The formation of **2a** is observed but the yield is low (less than 6%) (Fig. 2). These results suggest that IF_5 quickly decomposes at the higher temperature (98 °C) before **4a** changes to **2a**. However, such a possibility is ruled out because an extra addition of IF_5 to the reaction mixture after 1 h does not cause an increase of **2a**. Another possibility is the generation of a volatile material which is necessary to transform **4a** to **2a**. When the reaction is carried out in the tight-screw capped vessel, it stays in the reaction mixture and the transformation from **4a** to **2a** proceeds. On the other hand, it escapes from the reaction mixture under reflux conditions and the reaction stopps at **4a**. In order to examine the possibility, we carried out the reaction at 98 °C in the tight-screw capped vessel. Under the reaction conditions, the generated volatile material stays in the reaction mixture and, therefore, the reaction must proceed to give **2a**. As expected, the reaction is completed in 1 h and **2a** is formed as the main product with a trace amount of **8a** (Fig. 3). The reaction proceeds more quickly than that at 40 °C, and **3a** and **4a** disappear in 30 min. These results indicate that volatile material which is necessary to convert **4a** to **2a**, may be formed during the reaction. When the reaction is carried out under reflux without tight-screw cap, it escapes from the reaction mixture and the further conversion of **4a** does not take place. A volatile material such as HF (bp 19.5 °C) or IF (1.0 °C) is generated during the reaction. However, we could not identify the material which actually plays an important role to convert **4a** to **2a**.

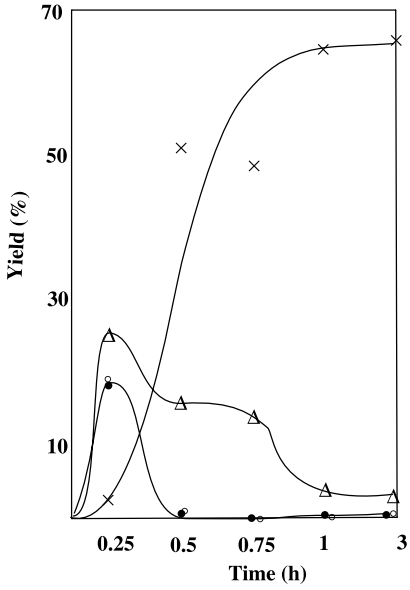


Figure 3. Time dependence of the product distributions in the reaction of IF_5 with **1a** at 98 °C in a tight-screw capped vessel (●; **3a**, ○; **4a**, △; **8a**, ×; **2a**).

3. Conclusion

We have succeeded in introducing three fluorine atoms into sulfides selectively by the reaction with IF_5 in heptane under reflux conditions. During the reaction, the migration of the arylthio group takes place only once.

4. Experimental

4.1. General

The IR spectra were recorded using a JASCO FT/IR-410. The ^1H NMR (400 MHz), ^{19}F NMR (376 MHz), and ^{13}C NMR (100 MHz) spectra were recorded in $CDCl_3$ on a JEOL JNM-A400II FT NMR and the chemical shift, δ, are referred to TMS (^1H, ^{13}C) and $CFCl_3$ (^{19}F), respectively. The EI-low and high-resolution mass spectra were measured on a JEOL JMS-700TZ, JMS-FABmate or JMS-HX110. IF_5 in a stainless-steel cylinder was supplied by Daikin Industries, Ltd. IF_5 decomposes in air emitting HF fume, and, therefore, it should be carefully handled in a bench hood with rubber-gloved hands. Due to its low viscosity and high density, it is difficult to transfer IF_5 from the cylinder to a reaction vessel with a pipette; therefore, IF_5 was used as a CH_2Cl_2 solution. From the cylinder, IF_5 was transferred through a Teflon™ tube into a Teflon™ FEP bottle under an N_2 atmosphere. After measuring the amount of IF_5 in the bottle, CH_2Cl_2 was added to make a 16.7 mol% solution. IF_5 in CH_2Cl_2 was kept in the Teflon™ FEP bottle and transferred quickly from the bottle to the reaction vessel using a Teflon™ pipette in open air. The sulfides other than **1i** were prepared from the corresponding aryl mercaptans and alkyl halides under basic conditions.[9] The sulfide **1i** was prepared from *p*-chlorophenylthiol with *N,N*-diethylacrylamide.[10] Et_3N-5HF was prepared by the addition of Et_3N to anhydrous HF.[11]

4.2. Fluorination of sulfides 1 using IF_5

4.2.1. General procedure. The reaction was carried out in an 8 ml-Teflon™ FEP bottle. IF_5 in CH_2Cl_2 (0.776 g of 16.7 mol% solution, 1.2 mmol), heptane (1 ml) and substrate (0.5 mmol) were introduced into a reaction vessel and a Teflon™ tube with a diameter of 10 mm and a length of

1000 mm was attached to the top of the bottle. A water-jacket was attached to the Teflon™ tube for cooling and the reaction mixture was stirred under reflux for 1 h. After consumption of the starting material was confirmed by GC, the reaction mixture was poured into ice–water and the product was extracted three times with ether. The combined ethereal phases were washed with aqueous $Na_2S_2O_3$, $NaHCO_3$, and brine, successively and dried over $MgSO_4$. After concentration under reduced pressure, the product was isolated by column chromatography (silica gel/hexane–ether as eluent).

4.2.2. 1,1-Difluoro-2-(*p*-chlorophenylthio)propane 3a. Colorless liquid: IR (neat) 1477, 1455, 1390, 1150, 1095, 1051, 1033, 1013, 823 cm^{-1}. ^1H NMR δ 1.37 (d, J=7.1 Hz, 3H), 3.24–3.37 (m, 1H), 5.74 (dt, J=56.6, 3.7 Hz, 1H), 7.30 (d, J=8.4 Hz, 2H), 7.42 (d, J=8.4 Hz, 2H). ^{19}F NMR δ −124.56 (ddd, J=276.0, 56.5, 16.3 Hz, 1F), −118.34 (ddd, J=276.0, 56.2, 9.8 Hz, 1F). ^{13}C NMR δ 13.40 (t, J=4.1 Hz), 45.76 (t, J=21.9 Hz), 116.59 (t, J=246.0 Hz), 129.25 (2C, s), 131.09 (s), 134.43 (s), 134.68 (2C, s). MS: 224 (M^++2, 25), 222 (M^+, 69), 173 (37), 172 (10), 171 (100), 145 (17), 144 (12), 143 (45), 136 (18), 109 (10), 108 (28), 59 (11). HRMS (EI) Calcd for $C_9H_9ClF_2S$: (M^+) 222.0081. Found: 222.0075.

4.2.3. 1,1,2-Trifluoro-2-(*p*-chlorophenylthio)propane 4a. Colorless liquid: IR (neat) 1574, 1477, 1448, 1389, 1225, 1092, 1015, 938, 852, 825 cm^{-1}. ^1H NMR δ 1.67 (dm, J=19.0 Hz, 3H), 5.50 (ddd, J=56.6, 54.4, 2.0 Hz, 1H), 7.36 (d, J=8.5 Hz, 2H), 7.54 (d, J=8.5 Hz, 2H). ^{19}F NMR δ −135.96 to −135.73 (m, 1F), −135.62 (dddd, J=283.5, 56.8, 14.6, 1.2 Hz, 1F), −127.88 (dddd, J=283.5, 54.3, 12.2, 1.2 Hz, 1F). ^{13}C NMR δ 18.79 (dd, J=23.2, 2.5 Hz), 101.83 (dm, J=221.4 Hz), 112.12 (ddd, J=253.5, 246.9, 40.5 Hz), 125.53 (s), 129.49 (2C, s), 136.72 (s), 137.71 (2C, s). MS: 242 (M^++2, 37), 241 (M^++1, 11), 240 (M^+, 100), 191 (28), 189 (76), 146 (32), 145 (18), 144 (87), 143 (32), 134 (11), 109 (23), 108 (33). HRMS (EI) Calcd for $C_9H_8ClF_3S$: (M^+) 239.9987. Found: 239.9980.

4.2.4. 1,1,2-Trifluoro-2-(*p*-methylphenylthio)propane 4b. Colorless liquid: IR (neat) 2993, 1494, 1386, 1096, 1072, 813 cm^{-1}. ^1H NMR δ 1.66 (d, J=19.0 Hz, 3H), 2.38 (s, 3H), 5.34–5.64 (m, 1H), 7.18 (d, J=8.5 Hz, 2H), 7.48 (d, J=8.5 Hz, 2H). ^{19}F NMR δ −136.58 to –135.53 (m, 2F), −128.56 to −127.60 (m, 1F). ^{13}C NMR δ 18.37–18.76 (m), 21.25 (s), 101.79 (ddd, J=222.17, 27.9, 23.8 Hz), 112.13 (ddd, J=253.5, 246.0, 41.4 Hz), 123.36 (s), 129.97 (2C, s), 136.50 (2C, s), 140.38 (s). MS: 221 (M^++1, 12), 220 (M^+, 100), 200 (13), 169 (62), 149 (17), 124 (41), 123 (63), 121 (10), 92 (11), 91 (47), 79 (12), 77 (16). HRMS (EI) Calcd for $C_{10}H_{11}F_3S$: (M^+) 220.0533. Found: 220.0526.

4.2.5. 1,1,2-Trifluoro-2-(*p*-nitrophenylthio)propane 4c. Colorless liquid: IR (neat) 3104, 2996, 1600, 1523, 1347, 1093, 854 cm^{-1}. ^1H NMR δ 1.74 (d, J=19.3 Hz, 3H), 5.58 (ddd, J=56.1, 54.2, 2.4 Hz, 1H), 7.79 (d, J=8.8 Hz, 2H), 8.23 (d, J=9.0 Hz, 2H). ^{19}F NMR δ −135.97 to −135.74 (m, 1F), −133.53 (ddd, J=285.0, 55.8, 14.3 Hz, 1F), −127.29 (ddd, J=285.0, 54.3, 12.8 Hz, 1F). ^{13}C NMR δ 19.29 (d, J=23.2 Hz), 102.06 (ddd, J=225.8, 27.3, 24.8 Hz), 112.11 (ddd, J=253.5, 248.5, 39.3 Hz), 123.93 (2C, s), 124.17 (s), 136.23 (2C, s), 148.57 (s). MS: 251 (M^+, 84), 201 (10), 200 (100), 155 (32), 154 (12), 125 (14), 109 (16), 108 (17), 97 (11), 69 (11). HRMS (EI) Calcd for $C_9H_8O_2NF_3S$: (M^+) 251.0228. Found: 251.0217.

4.2.6. 1,1,2-Trifluoro-2-(*p*-chlorophenylthio)ethane 4d. Colorless liquid: IR (neat) 1571, 1477, 1378, 1151, 1095, 1013, 826 cm^{-1}. ^1H NMR δ 5.67 (ddt, J=25.2, 9.0, 3.9 Hz, 1H), 5.80 (tt, J=54.8, 3.7 Hz, 1H), 7.36 (d, J=8.1 Hz, 2H), 7.51 (d, J=8.1 Hz, 2H). ^{19}F NMR δ −168.00 (ddd, J=50.7, 19.2, 3.7 Hz, 1F), −128.76 (dddd, J=293.0, 54.9, 18.9, 9.8 Hz, 1F), −126.85 (dddd, J=292.3, 54.3, 23.8, 9.2 Hz, 1F). ^{13}C NMR δ 97.63 (dt, J=226.6, 28.1 Hz), 111.48 (ddd, J=248.9, 246.5, 34.7 Hz), 127.62 (s), 129.70 (2C, s), 135.32 (2C, s), 136.06 (s). MS: 228 (M^++2, 37), 226 (M^+, 100), 177 (33), 175 (90), 143 (47), 108 (29). HRMS (EI) Calcd for $C_8H_6ClF_3S$: (M^+) 225.9831. Found: 225.9829.

4.2.7. 1,1,2-Trifluoro-2-(*p*-chlorophenylthio)butane 4e. Colorless liquid: IR (neat) 1574, 1477, 1389, 1187, 1093, 1015, 891, 825 cm^{-1}. ^1H NMR δ 1.16 (t, J=7.6 Hz, 3H), 1.88–2.19 (m, 2H), 5.49 (ddd, J=56.4, 54.5, 2.3 Hz, 1H), 7.35 (d, J=8.5 Hz, 2H), 7.53 (d, J=8.5 Hz, 2H). ^{19}F NMR δ −144.89 to −144.72 (m, 1F), −135.67 (ddd, J=283.8, 56.5, 14.3 Hz, 1F), −127.94 (ddd, J=283.8, 53.9, 13.0 Hz, 1F). ^{13}C NMR δ 7.49 (d, J=4.1 Hz), 25.43 (dd, J=22.3, 2.5 Hz), 104.35 (ddd, J=226.2, 26.9, 23.2 Hz), 112.44 (ddd, J=253.9, 247.3, 41.4 Hz), 125.53 (s), 129.43 (2C, s), 136.55 (s), 137.76 (2C, s). MS: 256 (M^++2, 26), 254 (M^+, 70), 205 (11), 203 (29), 146 (38), 145 (19), 144 (100), 143 (31), 109 (21), 108 (27). HRMS (EI) Calcd for $C_{10}H_{10}ClF_3S$: (M^+) 254.0144. Found: 254.0160.

4.2.8. 1,1,2-Trifluoro-2-(*p*-chlorophenylthio)dodecane 4f. Colorless liquid: IR (neat) 2925, 2855, 1574, 1476, 1093, 1015, 824 cm^{-1}. ^1H NMR δ 0.89 (t, J=6.8 Hz, 3H), 1.27 (bs, 14H), 1.53–1.63 (m, 2H), 1.83–2.09 (m, 2H), 5.47 (ddd, J=58.3, 56.4, 2.0 Hz, 1H), 7.35 (d, J=8.3 Hz, 2H), 7.53 (d, J=8.3 Hz, 2H). ^{19}F NMR δ −143.15 to −142.98 (m, 1F), −135.88 (ddd, J=282.6, 55.5, 14.0 Hz, 1F), −127.76 (ddd, J=283.8, 54.3, 12.2 Hz, 1F). ^{13}C NMR δ 14.11 (s), 22.69 (s), 22.93 (d, J=2.5 Hz), 29.29 (s), 29.32 (s), 29.47 (s), 29.56 (s), 29.65 (s), 31.86–31.91 (m), 32.06 (d, J=1.7 Hz), 104.06 (ddd, J=225.8, 27.3, 24.0 Hz), 112.30 (ddd, J=253.1, 247.3, 41.0 Hz), 125.61 (s), 129.39 (2C, s), 136.52 (s), 137.69 (2C, s). MS: 368 (M^++2, 31), 367 (M^++1, 17), 366 (M^+, 81), 146 (37), 145 (13), 144 (100), 143 (12), 57 (12), 43 (18). HRMS (EI) Calcd for $C_{18}H_{26}ClF_3S$: (M^+) 366.1396. Found: 366.1404.

4.2.9. 2,9-Bis(*p*-chlorophenylthio)-1,1,2,9,10,10-hexafluorodecane 4g. Yellow solid: mp 59–62 °C; IR (neat) 2938, 2858, 1745, 1573, 1475, 1092, 825 cm^{-1}. ^1H NMR δ 0.88–2.13 (m, 12H), 5.46 (ddd, J=56.4, 54.2, 2.0 Hz, 2H), 7.36 (d, J=8.5 Hz, 4H), 7.53 (d, J=8.5 Hz, 4H). ^{19}F NMR δ −143.25 to −143.07 (m, 2F), −136.01 (ddd, J=282.6, 56.2, 14.6, Hz, 2F), −127.76 (ddd, J=283.2, 53.7, 12.8 Hz, 2F). ^{13}C NMR δ 22.78 (2C, s) 29.25 (2C, s), 31.80 (2C, d, J=20.7 Hz), 101.83 (2C, dm, J=221.4 Hz), 112.12 (2C, ddd, J=253.5, 246.9, 40.5 Hz), 125.53 (2C, s), 129.49 (4C, s), 136.72 (2C, s), 137.71 (4C, s). MS: 538 (M^++4,

16), 537 ($M^+ + 3$, 17), 536 ($M^+ + 2$, 68), 535 ($M^+ + 1$, 24), 534 (M^+, 91), 390 (12), 146 (37), 145 (27), 144 (100), 143 (41), 109 (13), 108 (15). HRMS (EI) Calcd for $C_{22}H_{22}Cl_2F_6S_2$: (M^+) 534.0444. Found: 534.0433.

4.2.10. Methyl 3-(*p*-chlorophenylthio)-2,2,3-trifluoropropanoate 4h. Colorless liquid: IR (neat) 2961, 1785, 1575, 1478, 1442, 1391, 1312, 1220, 1096, 1074, 1014 cm^{-1}. ^1H NMR δ 3.95 (s, 3H), 6.00 (ddd, $J=50.0$, 12.2, 9.3 Hz, 1H), 7.36 (d, $J=8.5$ Hz, 2H), 7.49 (d, $J=8.5$ Hz, 2H). ^{19}F NMR δ −167.38 (ddd, $J=50.0$, 22.0, 20.1 Hz, 1F), −115.63 (ddd, $J=268.6$, 20.1, 12.2 Hz, 1F), −114.27 (ddd, $J=268.6$, 22.0, 9.3 Hz, 1F). ^{13}C NMR δ 53.91 (s), 99.23 (ddd, $J=230.5$, 29.7, 27.3 Hz), 111.13 (ddd, $J=259.7$, 258.9, 30.6 Hz), 128.47 (s), 129.61 (2C, s), 134.74 (2C, s), 135.85 (s), 161.78 (t, $J=31.0$ Hz). MS: 286 ($M^+ + 2$, 23), 284 (M^+, 57), 177 (37), 175 (100), 145 (20), 143 (53), 108 (46). HRMS (EI) Calcd for $C_{10}H_8F_3O_2ClS$: (M^+) 283.9885. Found: 283.9880.

4.2.11. *N*,*N*-Dimethyl 2-(*p*-chlorophenylthio)-2,3,3-trifluoropropanamide 4i. White solid: mp 37–39 °C; IR (KBr) 3056, 2942, 1651, 1476, 1402, 1153, 1085, 1015, 926, 825 cm^{-1}. ^1H NMR δ 2.81 (s, 3H), 2.85 (d, $J=7.1$ Hz, 3H), 6.43 (ddd, $J=54.6$, 53.4, 11.0 Hz, 1H), 7.37 (d, $J=8.3$ Hz, 2H), 7.56 (d, $J=8.3$ Hz, 2H). ^{19}F NMR δ −152.70 to −152.54 (m, 1F), −135.99 (ddd, $J=223.4$, 54.9, 17.7 Hz, 1F), −125.32 (ddd, $J=225.2$, 53.1, 20.1 Hz, 1F). ^{13}C NMR δ 37.12 (d, $J=15.7$ Hz), 37.30 (s), 103.29 (dt, $J=256.4$, 22.3 Hz), 113.48 (ddd, $J=254.4$, 249.8, 24.0 Hz), 124.69 (s), 129.23 (2C, s), 137.29 (s), 138.14 (2C, s), 162.24 (dd, $J=20.7$, 4.1 Hz). MS: 299 ($M^+ + 2$, 10), 297 (M^+, 27), 72 (100). HRMS (EI) Calcd for $C_{11}H_{11}F_3ONClS$: (M^+) 297.0202. Found: 297.0202.

4.2.12. 1,1,2-Trifluoro-2-(*p*-chlorophenylthio)-3-phenylpropane 4j. Colorless liquid: IR (neat) 3033, 2988, 1476, 1092, 1015, 984, 825, 700 cm^{-1}. ^1H NMR δ 3.17 (ddd, $J=26.8$, 14.9, 2.2 Hz, 1H), 3.44 (t, $J=15.6$ Hz, 1H), 5.45 (ddd, $J=55.9$, 54.2, 1.5 Hz, 1H), 7.31–7.47 (m, 7H), 7.49 (d, $J=8.3$ Hz, 2H). ^{19}F NMR δ −142.14 to −141.95 (m, 1F), −135.12 (dddd, $J=283.8$, 56.2, 14.3, 1.2 Hz, 1F), −128.92 (dddd, $J=283.8$, 54.3, 12.8, 2.4 Hz, 1F). ^{13}C NMR δ 38.43 (d, $J=20.7$ Hz), 103.42 (ddd, $J=228.3$, 26.5, 24.0 Hz), 112.11 (ddd, $J=258.1$, 248.1, 41.4 Hz), 125.50 (s), 127.67 (s), 128.40 (2C, s), 129.39 (2C, s), 130.82 (2C, s), 132.53 (s), 136.51 (s), 137.64 (2C, s). MS: 318 ($M^+ + 2$, 38), 317 ($M^+ + 1$, 18), 316 (M^+, 100), 225 (16), 173 (64), 157 (21), 153 (12), 146 (10), 144 (26), 143 (13), 133 (12), 127 (19), 122 (15), 109 (17), 108 (14), 91 (95). HRMS (EI) Calcd for $C_{15}H_{12}ClF_3S$: (M^+) 316.0301. Found: 316.0300.

4.3. Oxidation potentials of the sulfides 1a, 3a, and 4a.[11]

The oxidation potentials of the sulfides **1a**, **3a**, and **4a** (0.25 mmol) were measured in Et_3N-5HF (6 ml) using an undivided cell (30 ml) made of Teflon™ PFA, a smooth Pt wire (1 mm×10 mm) as a working electrode, and a smooth Pt sheet (20 mm×20 mm) as a counter electrode. The reference electrode was $Ag^+/AgClNO_3$ (0.01 M) in MeCN containing Et_4NBF_4 (0.1 M). The potential was scanned with a potential scanner (Nichia ES 512A) connected to a potentio/galvanostat (Nichia NP-100M).

4.3.1. Fluorination of 4a with IF_5. Hexane (2 ml), **4a** (0.118 g, 0.5 mmol), and IF_5 in CH_2Cl_2 (0.776 g of 16.7 mol% solution, 1.2 mmol) were introduced into a reaction vessel made of Teflon™ FEP with a tight screw cap and the mixture was stirred at 40 °C for 28 h. After consumption of the starting material was confirmed by GC, the reaction mixture was poured into ice–water and the product was extracted three times with ether. The combined etheral phases were washed with aqueous $Na_2S_2O_3$, $NaHCO_3$, and brine successively and dried over $MgSO_4$. After concentration under reduced pressure, the product was isolated by column chlomatography (silica gel/hexane–ether as eluent).

4.3.2. 1,2,2,3,3-Pentafluoro-1-(*p*-chlorophenylthio)propane 2a. Colorless liquid: IR (neat) 1478, 1391, 1215, 1096, 1013, 829, 744 cm^{-1}. ^1H NMR δ 5.87 (ddd, $J=66.8$, 16.1, 6.1 Hz, 1H), 6.02 (dt, $J=52.7$, 6.4 Hz, 1H), 7.37 (d, $J=8.5$ Hz, 2H), 7.52 (d, $J=8.5$ Hz, 2H). ^{19}F NMR δ −167.19 (ddt, $J=50.7$, 18.9, 9.2 Hz, 1F), −140, 65 (dddd, $J=305.2$, 53.1, 8.9, 5.8 Hz, 1F), −137.08 (ddt, $J=304.9$, 52.8, 9.5 Hz, 1F), −128.50 (dm, $J=276.5$ Hz, 1F), −125.77 (dm, $J=276.5$ Hz, 1F). ^{13}C NMR δ 98.60 (ddd, $J=227.4$, 33.1, 25.6 Hz), 108.70 (tm, $J=251.5$ Hz), 112.74 (tm, $J=255.4$ Hz), 128.35 (s), 129.79 (2C, s), 134.81 (2C, s), 136.18 (s). MS: 278 ($M^+ + 2$, 37), 276 (M^+, 100), 175 (81), 143 (48), 108 (27). HRMS (EI) Calcd for $C_9H_6ClF_5S$: (M^+) 275.9799. Found: 275.9797.

4.3.3. The time course of the reaction of 1a with IF_5 at 40 °C in the tight-screw capped vessel. The reaction was carried out at 40 °C using hexane (2 ml), **1a** (0.093 g, 0.5 mmol), and IF_5 in CH_2Cl_2 (0.776 g of 16.7 mol% solution, 1.2 mmol) in the tight-screw capped vessel as described in Section 4.3.1 and the yields were obtained by GC using undecane as an internal standard.

4.3.4. The time course of the reaction of 1a with IF_5 in heptane under reflux condition. The reaction was carried out using heptane (1 ml), **1a** (0.093 g, 0.5 mmol), and IF_5 in CH_2Cl_2 (0.776 g of 16.7 mol% solution, 1.2 mmol) as described in Section 4.2.1 and the yields were obtained by GC using undecane as an internal standard.

4.3.5. The time course of the reaction of 1a with IF_5 at 98 °C in the tight-screw capped vessel. The reaction was carried out at 98 °C using heptane (2 ml), **1a** (0.093 g, 0.5 mmol), and IF_5 in CH_2Cl_2 (0.776 g of 16.7 mol% solution, 1.2 mmol) in a tight-screw capped vessel as described in Section 4.3.1 and the yields were obtained by GC using undecane as an internal standard.

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Substituent effects on the reaction mode between 2-hydroxybenzyl alcohol derivatives and MEM chloride: synthesis and mechanistic aspects of seven- and ten-membered benzo-fused *O*,*O*-acetals

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Abstract—The synthesis of (*RS*)-2- or (*RS*)-3-methoxy-2,3-dihydro-5*H*-1,4-benzodioxepins and (*RS*)-5- or (*RS*)-3-methoxy-2,3,5,6-tetrahydro-8*H*-benzo-[1,4,7]-trioxecins has been developed. The mechanism of such a reaction via the boron trifluoride etherate-promoted transformation of 2-(methoxyethoxymethoxy)benzyloxyacetaldehyde dimethyl acetals or 2-(methoxyethoxymethoxymethyl)phenyloxy-acetaldehyde dimethyl acetals has been proposed. Transannular versions of the reaction results in the facile ring contraction of 12-membered intermediates to the 10- and to 7-membered benzene-fused *O*,*O*-acetals. The characterization of the by-products strongly supports the mechanisms proposed.

1. Introduction

Selective protection and deprotection of functional groups is one of the major issues in multistep synthetic strategies of organic compounds. In particular, hydroxyl groups are targets for selective protection, because selectively accessible OH-groups are often required for the following reaction. Many OH-protecting groups are known and the ability to protect a primary hydroxyl group in the presence of a secondary one was found with a variety of protecting reagents.[1,2] It has lately been shown that hydroxyalkyl phenols undergo selective protection either at the hydroxyl or at the phenol group by simply choosing the protecting reagent under essentially the same reaction conditions.[3] A literature survey revealed no reports on the regioselective protection of 2-hydroxybenzyl alcohol derivatives as a function of the electronic nature of the substituents at positions 3 or 5 of the aromatic ring. Accordingly, we decided to fill this gap in scientific literature and, at the same time, to use this synthetic tool for the preparation of isomeric seven-membered benzo-fused *O*,*O*-acetals, and isomeric ten-membered benzo-fused analogues.

We have recently reported the synthesis and biological activities of 2,3-dihydro-5*H*-1,4-benzodioxepin derivatives condensed with the 5-fluorouracil (5-FU) moiety on position 3 (**1**).[4] For these compounds the starting materials were the 2,3-dihydro-5*H*-1,4-benzodioxepin synthons **2a**–**c**. We embarked on a programme of synthesis and study of the biological properties of 2,3-dihydro-5*H*-1,4-benzodioxepin fragments that have the pyrimidine base linked in all the possible sites of the seven-membered ring, and directed our efforts in a second phase to the preparation of the cyclic *O*,*O*-acetal **3a**,**d**,**e**, with the acetalic methoxy group on position 2. The mechanistic aspects of the reaction between the acyclic *O*,*O*-acetals **4a**–**g** or the cyclic ones **2a**–**c** and 5-fluorouracil (5-FU) have been reported.[5] In the course of our present studies, the benzo-fused ten-membered *O*,*O*-acetals **5a**,**d**,**e** were also obtained. Here we report the three-step synthesis of **3a**,**d**,**e** and **5a**,**d**,**e**, together with their mechanisms. When the 2-hydroxybenzyl alcohol has a 5-OMe group or a 3-OMe substituent, the final compounds are the seven-membered *O*,*O*-acetals **2b**,**c**, together with **6c**. The importance of the ten-membered *O*,*O*-acetals **5a**,**d**,**e** and **6c** lies in the following: (a) These unreported structures could be the starting synthons for the preparation of the

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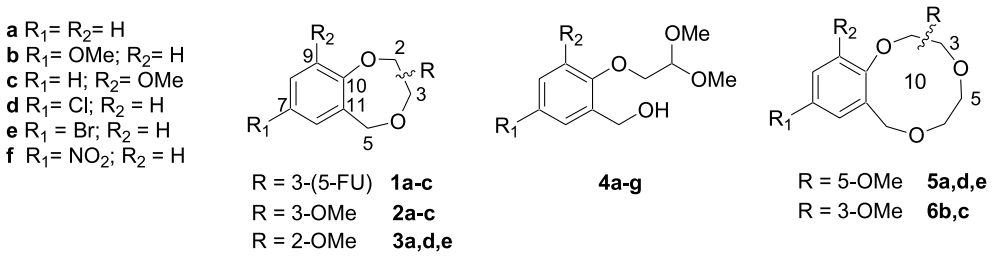


Chart 1.

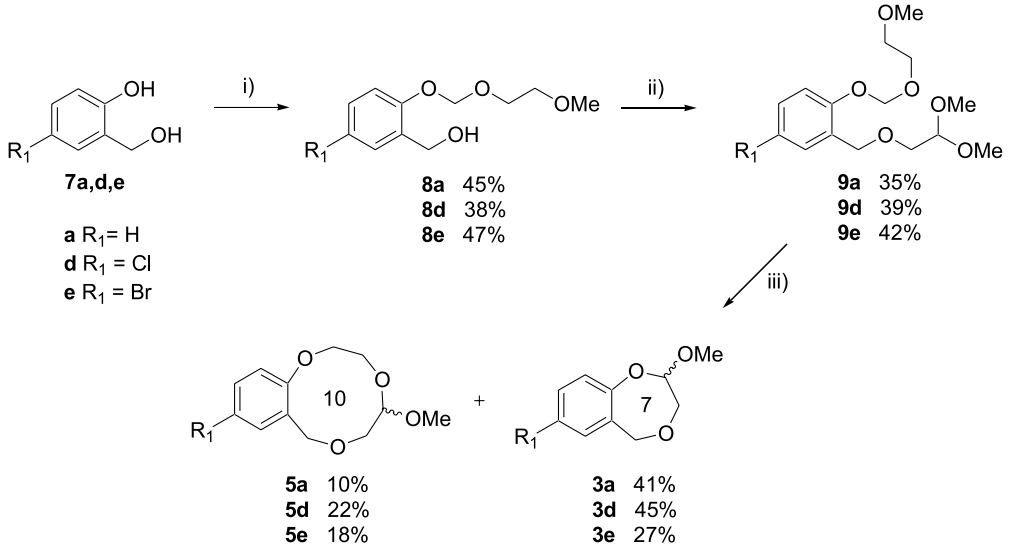
corresponding ten-membered *O*,*N*-acetals that, in a similar way to what was reported for the fourteen-membered bis(5-FU *O*,*N*-acetals), could exhibit notable biological activities against breast cancer cells; and (b) their formation sheds light on the mechanism of reaction in which the neighbouring-group participation plays a pivotal role (Chart 1).

2. Results and discussion

2.1. Reaction between 2-hydroxybenzyl alcohols 7a,d,e and 2-methoxyethoxymethyl chloride

Before carrying out the synthesis of **3a**,**d**,**e** it is necessary to protect the phenolic hydroxy group of the 2-hydroxybenzyl alcohol **7a**. Among other functionalities, the 2-methoxyethoxylmethyl (MEM) group was developed as a protective group of alcohols[6] and phenols.[7] Nevertheless, this protective group does not present enough selectivity and also leads to the blocking of the benzylic alcohol. Accordingly, the protection reaction with MEMCl has been carried out under several conditions, with the object of improving its modest selectivity in favour of **8a** and to the detriment of 2-(methoxyethoxymethoxymethyl)phenol, by using several bases and solvents. Such a study was performed on 2-hydroxybenzyl alcohol (salicyl alcohol) **7a**. We have studied three experimental conditions: (a) acetone and potassium carbonate; (b) sodium hydride and tetrahydrofuran (THF); and (c) diisopropylethylamine (DIPEA) and methylene chloride. The better yield in compound **6a** was obtained using conditions (a) (See Section 4).

Both MEM ethers [2-(methoxy-2-ethoxymethoxymethyl) phenol and **8a**] possess similar polarities (very close R_f, 0.3 and 0.2, respectively, using diethyl ether/hexane: 3/1 as eluant) and spectroscopic properties. Both compounds show the same molecular-ion peak of M^+ (calculated for $C_{11}H_{16}O_4Na$ $(M+Na)^+$ 235.0946, found 235.0946) in their high resolution liquid secondary ion mass spectrum (HR LSIMS) spectra, confirming that both have incorporated the MEM moiety into their structures. We thought that in the corresponding ^1H NMR spectra the chemical shift of the –O–CH_2–O– group could serve as a probe to decide the identity of both isomers: in compound **8a** such a group should appear at a lower field (δ 5.34 ppm) than in compound 2-(methoxy-2-ethoxymethoxymethyl)phenol (δ 4.85 ppm), due to the electron-withdrawing effect originated by the phenoxy moiety. Once the structure of **8a** had been demonstrated we decided to extend the reaction starting with 2-hydroxybenzyl alcohols with different substituents on the aromatic ring (**8d**,**e**). The synthesis of the cyclic *O*,*O*-acetals was carried out in a three-step process: (a) the formation of MEM ethers **8a**,**d**,**e** using MEMCl (1.5 equiv), K_2CO_3 (1.1 equiv), the salicyl alcohols (1 equiv) in acetone as solvent at 0 °C, under an inert atmosphere; (b) preparation of the intermediate acyclic *O*,*O*-acetals **9a**,**d**,**e** by alkylation of the benzylic hydroxy group with bromoacetaldehyde dimethyl acetal, using sodium hydride as a base and anhydrous



Scheme 1. Reagents: (i) K_2CO_3, anhydrous acetone, MEMCl; (ii) $BrCH_2CH(OMe)_2$, NaH, anhydrous DMF; (iii) $BF_3 \cdot OEt_2$ in anhydrous Et_2O.

dimethylformamide (DMF) as solvent; and (c) the cleavage of the MEM moiety and subsequent cyclization to yield the target molecules **3a**,**d**,**e**. In the original paper, which introduced the MEM group as a protective group for the hydroxyl function,[6] the advantages of using anhydrous $ZnBr_2$ or $TiCl_4$ over other Lewis acids were highlighted. We have reported the $BF_3 \cdot OEt_2$-mediated seven-membered cyclization of acyclic *O*,*O*-acetals[4,8,9] and accordingly, we supposed that the use of such a catalyst could lead to the target molecules **3a**,**d**,**e** in a one-step/pot reaction, as a consequence of the simultaneous deblocking/cyclization process. The experimental results confirmed our hypothesis but, in addition to the expected benzofused seven-membered *O*,*O*-acetals **3a**,**d**,**e**, the ten-membered *O*,*O*-acetals **5a**,**d**,**e** were also produced (Scheme 1).

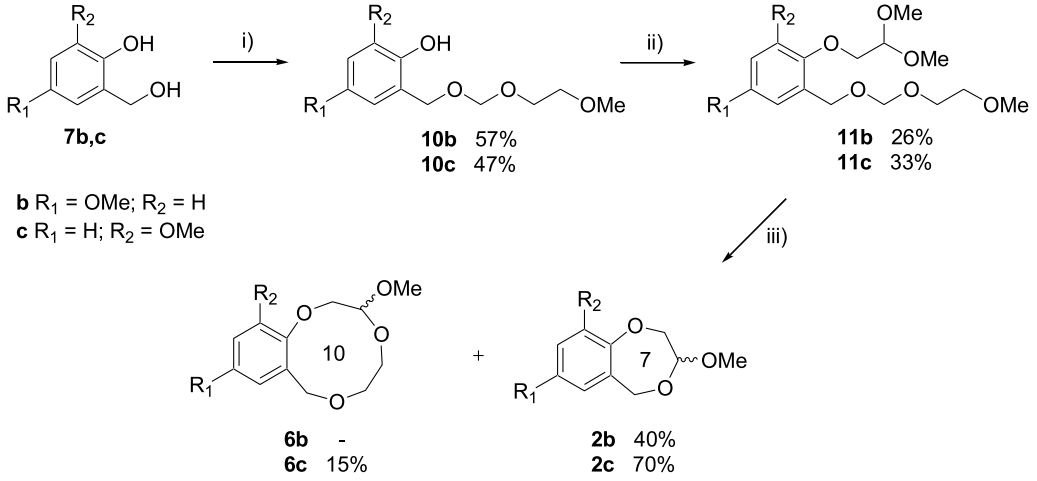
In order to confirm the structures of the compounds, we focused our attention on the NMR chemical shift of the benzylic carbon atoms and found that in the case of **8a**,**d**,**e**, the range covers a narrow interval of ≈ 1 ppm (in $CDCl_3$): δ 61.58 ppm (**9a**), δ 60.62 ppm (**9d**), and δ 60.41 ppm (**9e**).

2.2. Reaction between 2-hydroxybenzyl alcohols 7b,c and 2-methoxyethoxymethyl chloride

Nevertheless, when we tried to extend this series of reactions with the aim of obtaining **8b**,**c**, starting from the salicyl alcohols **7b**,**c**, their ^{13}C NMR chemical behaviour was not compatible with such structures on the basis of the chemical shifts of the benzylic carbon atoms, that is, δ 66.80 ppm when the benzene ring had a 5-OMe group or δ 64.55 ppm when the aromatic substituent was the 3-OMe moiety. These two low field chemical shifts, in relation to the corresponding values of **9a**,**d**,**e** cannot be explained by the field/inductive effects of the aromatic methoxy fragments because the distance between the two centres involved is very high in both cases. However, such a chemical shift difference could be justified should the oxygen atom of the benzylic alcohol be alkylated by the MEM moiety, instead of the oxygen atom of the phenol group. Should this be the case, the sequence of reactions (Scheme 2) would lead to the previously reported seven-membered *O*,*O*-acetals **2b**,**c** (with the acetalic –OMe fragment in position 3), together with **6c** in the case of starting from **7c**. Scheme 2 depicts the synthetic route followed whose difference with respect to Scheme 1 is based on the different alkylation site by MEMCl.

Another key point is the chemical shift of the benzylic carbon atoms of both target molecules **3a**,**d**,**e** and **2b**,**c**. For compounds **2b**,**c**, such carbons are located γ (an 1,3-relationship) in relation to the acetalic methoxy groups, their ^{13}C chemical shifts being very sensitive to the steric compression. As a rule, it is found that the ^{13}C NMR chemical shifts of carbon atoms in spatially crowded alkyl groups are more upfield than similar carbon atoms in unperturbed systems. Therefore, such an effect is negligible for compounds **3a**,**d**,**e** because the proximity relationship between both groups is even higher (delta or an 1,4-relationship). Table 1 shows the ^{13}C chemical shifts of the corresponding seven-membered moieties of the cyclic *O*,*O*-acetals.

In spite of the accurate ^{13}C NMR reasoning carried out to prove the structures of **2b**,**c**, the confirmation of such compounds needed to be corroborated because this point is



Scheme 2. Reagents: (i) K_2CO_3, anhydrous acetone, MEMCl; (ii) $BrCH_2CH(OMe)_2$, NaH, anhydrous DMF; (iii) $BF_3 \cdot OEt_2$ in anhydrous Et_2O.

Table 1. ^{13}C NMR chemical shifts[a] (ppm) for the 2,3-dihydro-5*H*-1,4-dioxepin moiety in **3a**,**d**,**e** and **2b**,**c** for $CDCl_3$ solutions

	3a	3d	3e	2b[b]	2c[b]
C-2	103.99	103.99	104.04	73.00	72.37
C-3	74.86	74.85	74.85	101.54	101.25
C-5	72.87	72.37	72.32	63.23	62.85
C-10	154.30	152.85	153.45	152.91	147.90
C-11	133.26	134.80	135.27	131.15	130.49

[a] Each reading was quoted to the nearest 0.05 ppm.
[b] See Ref. 4.

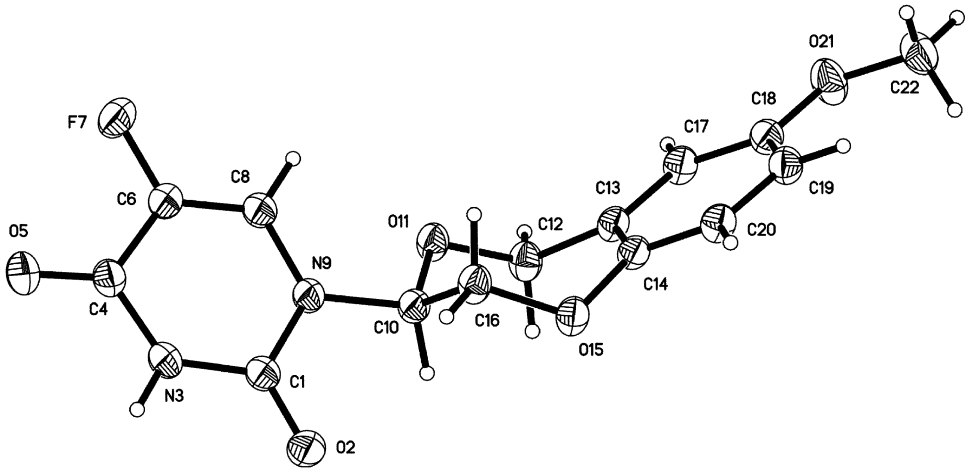


Figure 1. Molecular structure of (*RS*)-1-(7-methoxy-2,3-dihydro-5*H*-1,4-benzodioxepin-3-yl)-5-fluorouracil (ORTEP drawing at 50% probability).

critical for the confirmation of the alkylation site of **7b** by MEMCl. There is always the chance that the structure of **2b** with the acetalic –OMe group at position 3 could have been mistaken for the corresponding analogue having the acetalic –OMe group at position 2 (the hypothetical molecule **3b**) because their ^{1}H and ^{13}C NMR data are very close. Accordingly, we decided to unequivocally elucidate the structure of the acetal (**2b** or **3b**) by its reaction with 5-fluorouracil, 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and trimethylchlorosilane (TCS), under acid catalysis ($SnCl_4$) in acetonitrile during 144 h. Such a process led to (*RS*)-1-(7-methoxy-2,3-dihydro-5*H*-1,4-benzodioxepin-3-yl)-5-fluorouracil,[4] whose structure was unambiguously determined by X-ray crystallography (Fig. 1). Therefore, the regioselective protection of the primary hydroxy group of the corresponding salicyl alcohol was finally proved by a synthetic method, which made secure our previous structural assignments.

The explanation of the different chemical behaviour (see Schemes 1 and 2) is very simple: the acidity of phenolic compounds is modulated by electronic effects. *ortho* and *para* electron-donating groups in relation to the phenol group decrease acidity, whilst electron-withdrawing groups at the same position act in the opposite manner. As a result of both resonance and field/inductive effects, charge concentration leads to lesser stability of phenoxy anions and to a decrease in acidity.[10] Accordingly, the electronic properties of the *ortho* and *para* substituents to the hydroxy phenoxy group modifies the selectivity of the alkylation site by MEMCl.

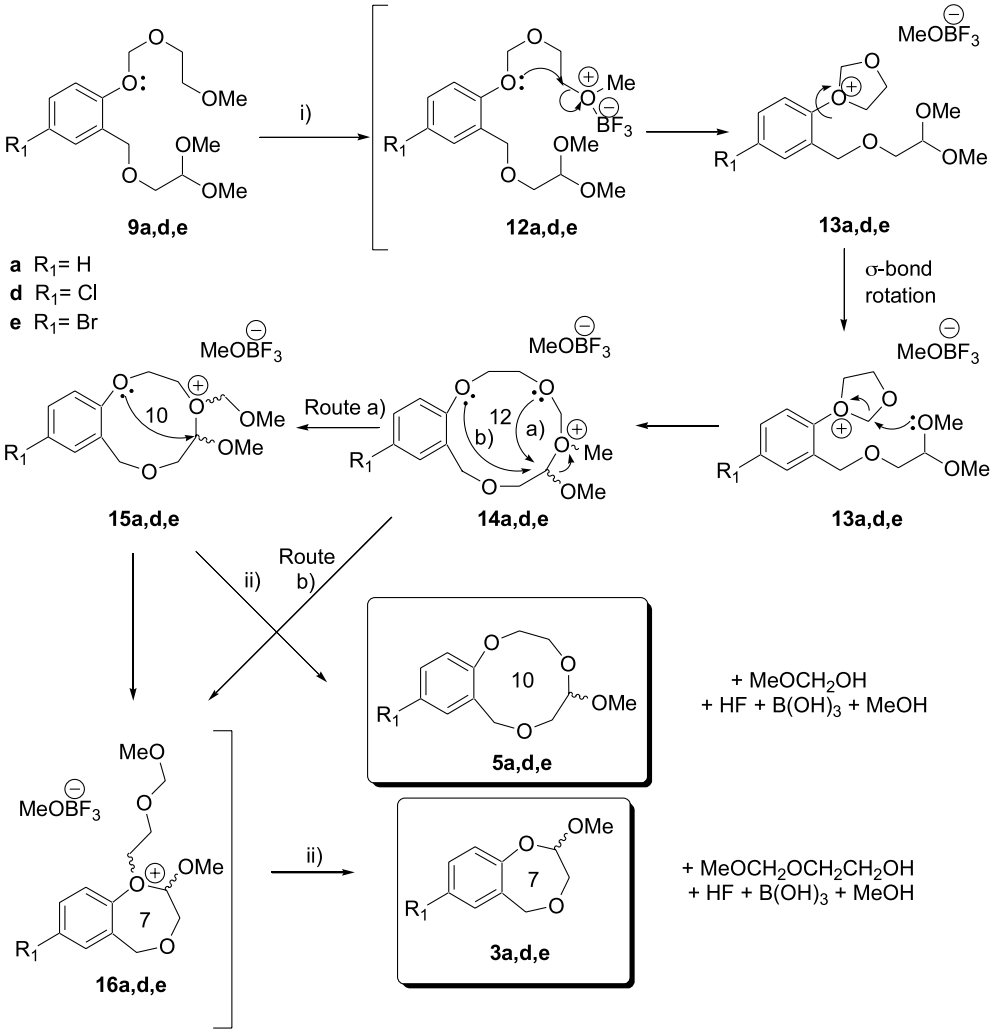
2.3. Mechanistic aspects of the synthesis of (*RS*)-2-methoxy-2,3-dihydro-5*H*-1,4-benzodioxepins 3a,d,e and (*RS*)-5-methoxy-2,3,5,6-tetrahydro-8*H*-benzo-[1,4,7]-trioxecins 5a,d,e

This process is effected by the reaction of **9a**,**d**,**e** (1 equiv) in tetrahydrofuran (THF) at 0 °C under an inert atmosphere with 0.5 equiv of $BF_3 \cdot OEt_2$. If the structures of the starting material **9a**,**d**,**e** and of the final compounds **3a**,**d**,**e** and **5a**,**d**,**e** are compared, one comes to the conclusion that the MEM moiety of **9a**,**d**,**e** should suffer two different cleavage processes from a formal point of view: (a) on one hand, the breaking of the methoxyethoxymethyl moiety, then the nucleophilic attack of the phenoxy group to the acetalic functionality with the concomitant cyclization process should give rise to the seven-membered acetal **3a**,**d**,**e**; and (b) formation of the ten-membered acetal **5a**,**d**,**e** is not so obvious: the terminal methyl ether and the internal methylene-oxy group of the MEM fragment should be eliminated before or after the corresponding cyclization step takes place. Such processes are likely to occur through concerted processes and rearrangements on common intermediates. It must be emphasized that outside the protective group arena, MEMCl has been used to alkylate enolates[11] and aryllithium reagents in the presence of Ph_2TlBr.[12] MEM ethers have also proved to be a good one-carbon source for the preparation of isochromans[13] and seven- and eight-membered oxacyclic rings.[14]

Scheme 3 shows a possible mechanism for the formation of both cyclic *O*,*O*-acetals. First of all, the complexation of the ethereal oxygen atom of the methoxy group of the MEM moiety takes place with the concomitant O-*5*[†] participation of the ethereal phenoxy atom and formation of a 1,3-dioxolane-1-ylium cation. The intermediate **13a**,**d**,**e** might undergo σ-bond rotation about the C_{Ph}–O bond, and then its highly electrophilic carbon atom of the methylenedioxy fragment could be attacked by one of the acetalic –OMe groups. This would give rise to the 12-membered transition state **14a**,**d**,**e**, which could suffer a reduction of the ring size to the 10-membered intermediate **15a**,**d**,**e** by means of an intramolecular reaction and the later leaving of the methoxymethanol fragment. An O-*5* participation of the oxygen atom at position 1 and the acetalic carbon of **15a**,**d**,**e** gives rise to a ring contraction leading to **3a**,**d**,**e** through the intermediacy of the seven-membered oxonium ion **16a**,**d**,**e**.

On one hand, it could have been supposed that, rather than the formation of **16a**,**d**,**e** through the intermediates **12a**,**d**,**e**–**15a**,**d**,**e**, the synthesis of **3a**,**d**,**e** could be considered more directly and simply from the open acetals **9a**,**d**,**e** by nucleophilic attack of the phenoxy oxygen to the acetalic

[†] When describing nucleophilic participation it is frequently convenient to use the symbol G-*n*, where G is the participating group and *n* the size of the ring that is formed in the transition state.



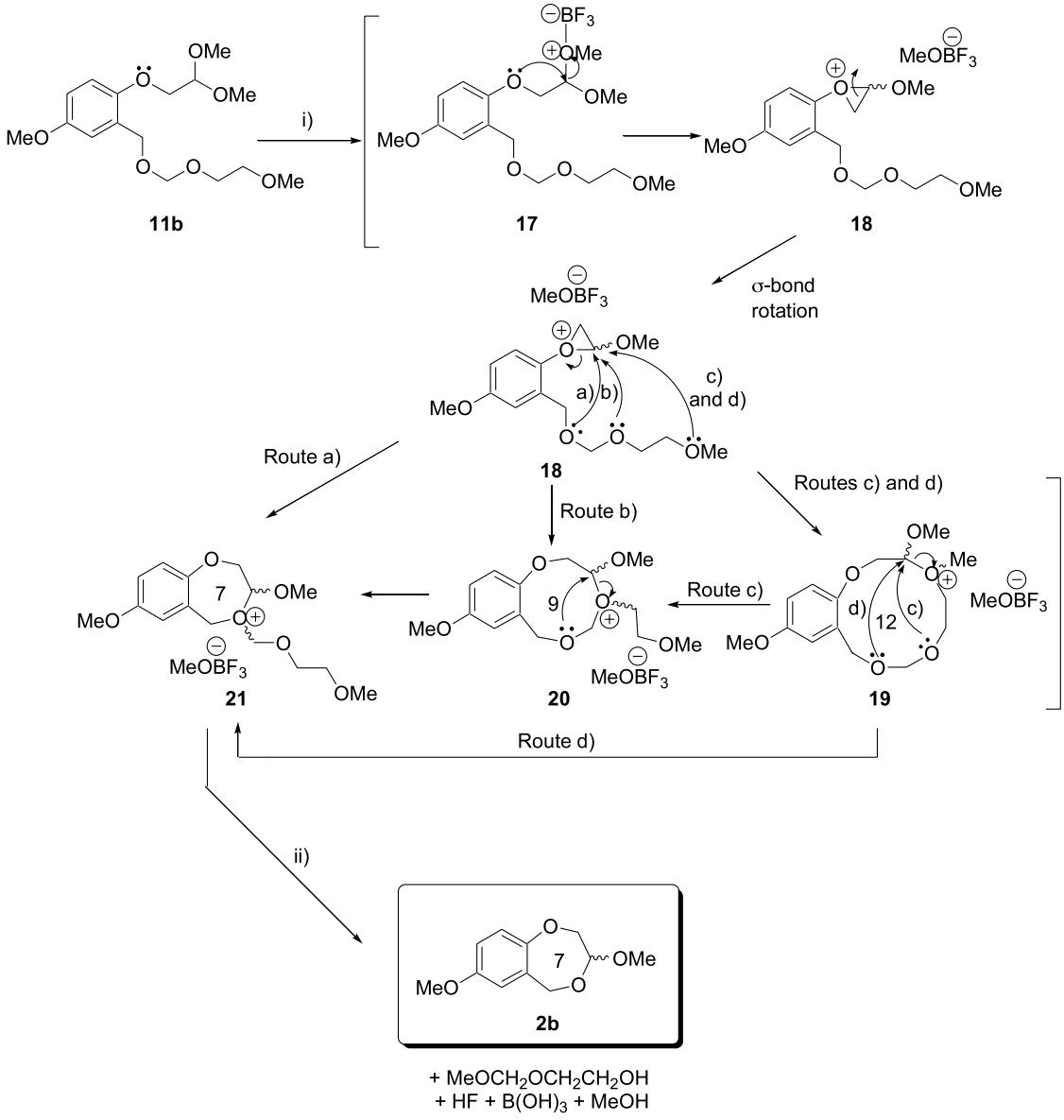
Scheme 3. Reagents: (i) $BF_3 \cdot OEt_2$, THF; (ii) H_2O.

functionality, after complexation by BF_3 of one of the acetalic oxygens. Then the intermediate analogous to **16a**,**d**,**e** should arise, but in this case substituted on the oxonium oxygen by a 2-methoxyethoxymethyl group. Cleavage of this group should also deliver **3a**,**d**,**e**. Nevertheless, the proof of the presence of the by-product 2-(methoxymethoxy)ethanol[15] (See Section 4), formed through **12a**,**d**,**e**–**15a**,**d**,**e**, and the absence of methoxyethoxymethanol, arising directly from **9a**,**d**,**e**, allow us to settle the proposed mechanism. On the other hand, it has been checked that the seven-membered rings **3a**,**d**,**e** (major products of the rearrangements) do not arise from the ten-membered rings **5a**,**d**,**e**, upon treatment of the latter with boron trifluoride diethyl etherate under the conditions of the rearrangement.

2.4. Mechanistic aspects on the synthesis of (*RS*)-3-methoxy-2,3-dihydro-5*H*-1,4-benzodioxepins 2b,c and (*RS*)-3-methoxy-2,3,5,6-tetrahydro-8*H*-benzo-[1,4,7]-trioxecins 6c

When the starting materials are **11b** and **11c**, both the nature and the yields of the final compounds, are determining factors to shed light on the two different mechanisms that could explain the course of the cyclization/contraction reaction. We believe that the mechanism of the transformation **11b**→**2b** is best represented as in Scheme 4. The aromatic –OMe substituent has an influence on the course of the reaction: the phenolic oxygen atom (O-1), whose nucleophilicity may be strongly influenced by the electronic character of the 4-OMe moiety, should intervene as a neighbouring group. We have previously reported a similar feature.[4] According to this hypothesis, the intermediate **17** suffers the neighbouring group attack to give the oxyranium ion **18**, much more reactive than its predecessor. After a σ-bond rotation through the $C–O^+$ bond of this highly reactive species, the acetalic-like carbon atom could be attacked by either of the three oxygen atoms of the adjacent lateral chain [routes (a), (b), or (c) and (d)]. Through any of the twelve- or nine-membered intermediates (**19** and **20**, respectively), the final destiny is the seven-membered intermediate **21**, which after work up leads to **2b**. The characterization of the by-product 2-(methoxymethoxy)-ethanol justifies the proposed mechanism (See Section 4).

The most important feature of this mechanism is the electrophilic character of the acetalic carbon atom. Nevertheless, the course of the reaction that leads to **2c** and **6c** is

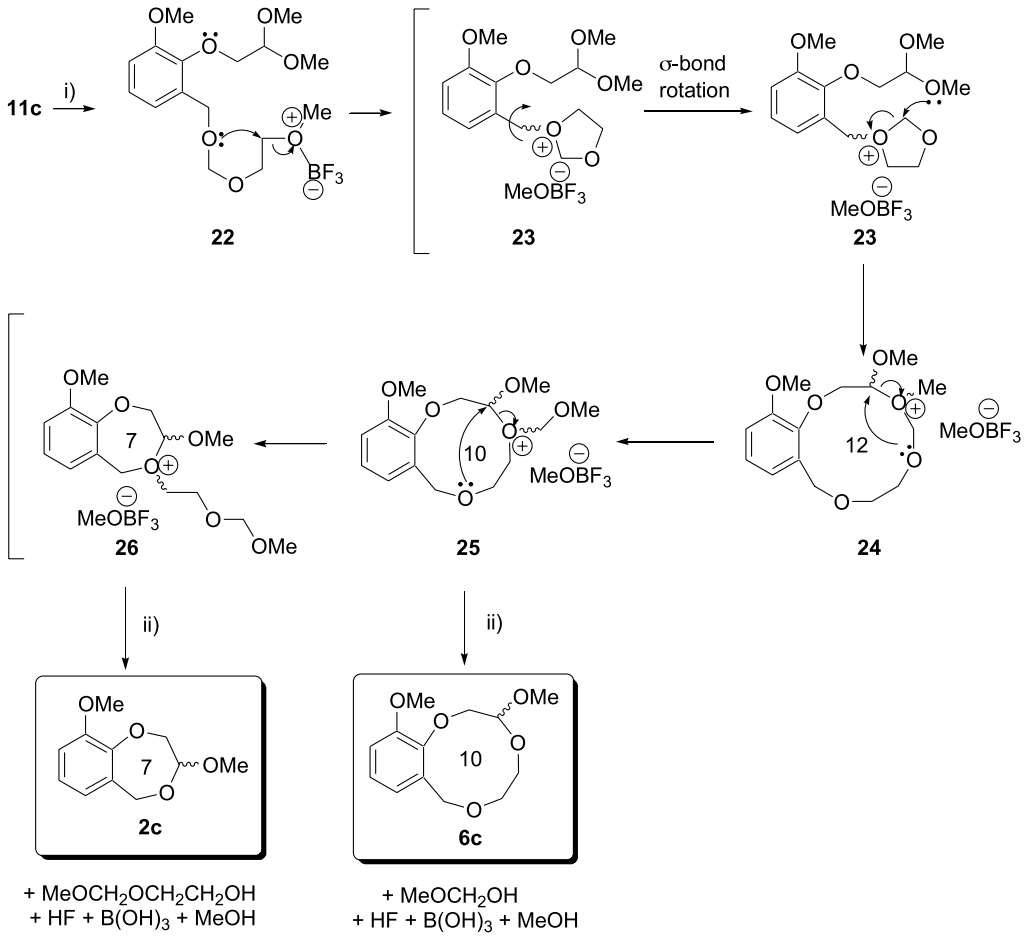


Scheme 4. Reagents: (i) $BF_3 \cdot OEt_2$, THF; (ii) H_2O.

different (Scheme 5). In this case and via a different mechanism, closely related to the one shown in Scheme 3, the acetalic –OMe group acts as a nucleophile and the MEM-derived chain as a good electrophile through the 1,3-dioxolane-1-ylium cation **23**. Again, the proof of the presence of 2-(methoxymethoxy)ethanol[15] and methoxymethanol[16] strongly supports the mechanism.

An important question that needs to be answered is the following: Why this different behaviour if in **11b** and in **11c** the aromatic –OMe groups are *para* and *ortho*, respectively, in relation to the phenolic oxygen atom that carries the acetaldehyde dimethyl acetal moiety? Although the electronic effects of the –OMe group in both positions are composed of field/inductive and resonance effects, the latter is far more important and, in principle, the mechanisms of the transformations **11b**→**2b** (Scheme 4) and **11c**→**2c**+**6c** (Scheme 5) should have been the same. Should this be the case, Chart 2 shows the two key intermediates, one of them (**27**) is highly unstable due to the closeness of both positive charges and accordingly very unlikely.

In short, when in the doubly protected salicyl alcohol the substituent R_1, *para* in relation to the phenolic oxygen atom, is electronical neutral (H), electron-withdrawing (Cl, Br) or electron-releasing groups the phenolic *O*-linked moiety acts as an electrophile and the alcoholic *O*-linked fragment acts as a nucleophile (Schemes 3 and 4). Nevertheless, the differences in nucleophilicity and electrophilicity of such groups are so subtle that the presence of an electron-releasing group *ortho* in relation to the phenolic *O*-linked fragment can invert the reactivity of both lateral chains: that is to say, the unstability of the intermediate **27** makes the upper *O*-phenolic fragment to act as electrophile and, accordingly the lower alcoholic *O*-linked moiety to work as nucleophile (Scheme 5). Such behaviour can be confirmed after the structural proofs of the by-products methoxymethanol and methoxymethoxyethanol.



Scheme 5. Reagents: (i) $BF_3 \cdot OEt_2$, THF; (ii) H_2O.

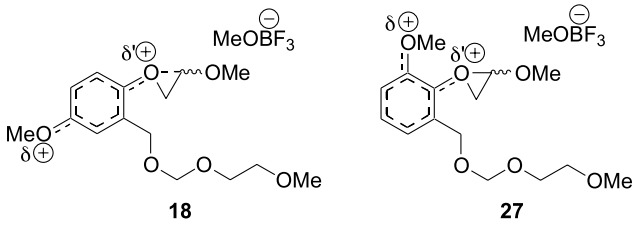


Chart 2.

2.5. Structural characteristics of the seven-membered *O*,*O*-acetals (3a,d,e and 2b,c), and the ten-membered ones (5a,d,e and 6c)

The structures of all derivatives were ascertained by their spectroscopic data (^{1}H, ^{13}C NMR, MS) and elemental analyses. In compounds **3a**,**d**,**e** the acetalic hydrogen atom (H-2) appears between δ 4.65–4.89 ppm as double of doublets (dd), whilst the H-3 atoms resonate between δ 4.00–4.22 ppm as dd with a J_{gem} in the range of 12.5–13.00 Hz. The H-5 atoms are in all cases diastereotopic and compounds **3d**,**e** show a $J_{gem} \approx 14$ Hz. Nevertheless, in the case of **2a**–**c** their chemical shifts are nearly equivalent giving an aspect of a doublet (d) with a small coupling constant ($J = 1.50$–2.10 Hz). It is noteworthy that the chemical shifts of the acetalic –OMe group of compounds **3d**,**e** are located upfield (δ 3.46–3.47 ppm) in relation to the same signals of **2a**–**c** (δ 3.56–3.62 ppm). Regarding the ^{13}C NMR spectra, the acetalic C-2 atoms of **3d**,**e** (δ 101.32–101.40 ppm) are upfield in relation to the same signals of **2a**–**c** (δ 103.99–104.04 ppm). This tendency is also observed with C-3 [**3d**,**e** (δ 72.43–72.80 ppm) and **2a**–**c** ($\delta \approx 74.8$ ppm)] and C-5 [**3d**,**e** (δ 62.93–63.05 ppm) and **2a**–**c** (δ 72.87–72.32 ppm)].

The 10-membered cycloacetals **5a**,**d**,**e** show the following characteristics: (a) The resonance of the acetalic proton H-5 appears between δ 4.77–4.81 ppm as dd with coupling constants of 1.7 and 6.6 Hz; (b) the signals of H-8 resonate as dd at a δ value between 4.56–4.65 ppm with $J = 1.2$–2.6 Hz and $J_{gem} \approx 13.3$–14.1 Hz; (c) the protons of the methylene groups H-2, H-3 and H-6 appear as multiplets; and (c) the singlet of the acetalic –OMe group presents a chemical shift close in all cases to δ 3.4 ppm. The most interesting aspects of the ^{13}C NMR spectra of the 10-membered moiety of **5a**,**d**,**e** are the following: (a) The acetalic C-5 atom is the most deshielded one and appears at δ 100.47–103.22 ppm, followed by C-6 at δ 72.19–74.92 ppm, C-2 (δ 71.85–72.72 ppm), C-8 (δ 67.20–71.58 ppm) and C-3 (δ 63.05–68.25 ppm); (b) the acetalic –OMe moiety appears close to δ 59.10 ppm, except in the case of **6c** that resonates at δ 56.14 ppm; (c) the chemical shift values of **6c** appear generally slightly upfield with regard to the rest of the compounds **5a**,**d**,**e**.

3. Conclusion

Three main conclusions can be drawn from our results: (1) It has been found that the substituents on 2-hydroxybenzylic alcohols affect the protection mode with MEMCl of the two different hydroxyl groups. The 5-methoxy *O*-alcoholic-MEM-protected phenol structure was demonstrated on the following basis: (a) by ^{1}H and ^{13}C NMR assignments, and (b) by an X-ray crystallographic determination of (*RS*)-1-(7-methoxy-2,3-dihydro-5*H*-1,4-benzodioxepin-3-yl)-5-fluorouracil, which unambiguously proved the nature of the starting material. (2) The mild reaction conditions can be of particular interest for the preparation of seven- and ten-membered benzo-fused acetals, which are otherwise difficult to prepare, although the latter ones are obtained with low yields. (3) The formation of the ten-membered *O*,*O*-acetals **5a**,**d**,**e** and **6b**,**c** and characterization of the by-products throw light on the course of the $BF_3 \cdot OEt_2$-promoted reaction on **9a**,**d**,**e** and **11b**,**c**, respectively.

4. Experimental

All moisture-sensitive reactions were performed in flame-dried glassware equipped with rubber septa under a positive pressure of dry argon. Organic extracts were dried over $MgSO_4$. Thin layer chromatography was performed on Merck Kieselgel 60 F_{254}, the spots being developed at the UV light. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker at 300.13 and 400.1 MHz, and at 75.78 and 100.03 MHz, respectively in $CDCl_3$ solutions. Chemical shifts were measured in δ and referenced to $CDCl_3$ (7.25 ppm for ^{1}H NMR and 77.20 ppm for ^{13}C NMR). The accurate mass determination was carried out in an AutoSpec-Q mass spectrometer arranged in an EBE geometry (Micromass Instruments, Manchester, UK) and equipped with a FAB (LSIMS) source. The instrument was operated at 8 kV of accelerating voltage and Cs^+ cations were used as primary ions. The GC/MS was carried out on a Platfom II mass spectrometer (Micromass Instruments, Manchester, UK) coupled with a Carlo Erba gas chromatograph (ThermoInstruments, CA, USA) and equipped with an EI source at 70 eV. The analysis was performed on a HP-5MS capillary column (30 m×0.25 mm×0.25 μm) in a splitless mode, inserted directly into the ion source. The temperature programme was the following: 60 °C, 10 °C/min to 300 °C, then isothermal for 10 min. The carrier gas was helium with a flow rate of 1 mL/min. Solvents were obtained dry as follows: tetrahydrofuran (THF) was distilled from benzophenone ketyl, CH_2Cl_2 was refluxed over, and distilled from CaH_2 and then stored over molecular sieves (3 Å), CH_3OH from Mg. 2-Hydroxybenzyl alcohols were kept at 40 °C and 0.1 mm Hg for 48 h. $BF_3 \cdot OEt_2$ was distilled prior to use, in an all-glass apparatus with calcium hydroxide to remove volatile acids and to reduce bumping.

4.1. Reaction between 3- or 5-substituted-2-hydroxybenzyl alcohols and 2-methoxyethoxymethyl chloride (MEMCl)

The general procedure is exemplified with the case of 2-hydroxybenzyl alcohol. Synthesis of 2-(methoxyethoxymethoxymethyl)phenol and 2-(methoxyethoxymethoxy)benzyl alcohol **8a**. K_2CO_3 (5.6 g, 40.8 mmol) were added to a solution of 2-hydroxybenzyl alcohol **7a** (5.6 g, 45.2 mmol) in anhydrous acetone (65 mL), and the suspension was left at room temperature under stirring for 30 min. After this time, the temperature of the suspension had to fall to 0 °C before the addition of MEMCl (6.85 mL, 60 mmol) and the suspension was left under stirring at 0 °C for 6 h. K_2CO_3 was filtrated and the resulting solution was concentrated in vacuo. The resulting residue was purified by flash chromatography (diethyl ether/hexane: 1/2) and the following two fractions were obtained: the first one was identified as 2-(methoxyethoxymethoxymethyl)phenol (2.58 g, 27% yield) and the second one identified as **8a** (4.31 g, 45% yield). When other conditions were used, the corresponding yields were the following: NaH and THF [2-(methoxyethoxymethoxymethyl)phenol 17%, and **8a** 17%], and DIPEA and CH_2Cl_2 [2-(methoxyethoxymethoxymethyl)phenol 50%, and **8a** 29%]. When 5-chloro-2-hydroxybenzyl alcohol **7d**, and 5-bromo-2-hydroxybenzyl alcohol **7e** were used, the 2-[(2-methoxyethoxymethoxy)]benzyl alcohols **8d**,**e** were the only compounds obtained, the corresponding 2-(methoxyethoxymethoxymethyl)phenols were not detected. When 5-methoxy-2-hydroxybenzyl alcohol **7b**, and 3-methoxy-2-hydroxybenzyl alcohol **7c** were used, the 2-(methoxy-2-ethoxymethoxymethyl)phenols **10b**,**c** were the only compounds obtained.

4.1.1. 2-(Methoxyethoxymethoxymethyl)phenol. Compond **7a** was used as starting material. Yield: 27%. R_f (diethyl ether/hexane: 3/1): 0.3. ^{1}H NMR (300 MHz) δ 7.22 (dt, 1H, H-5; $J=1.7$, 7.7 Hz); 7.06 (dd, 1H, H-6; $J=1.5$, 7.4 Hz); 6.87 (m, 2H, H-3 and H-4); 4.85 (s, 2H, OCH_2O); 4.76 (s, 2H, PhCH_2O); 3.65 (m, 4H, OCH_2CH_2O); 3.41 (s, 3H, O*Me*). HR LSIMS calcd for $C_{11}H_{16}O_4Na$ $(M+Na)^+$ 235.0946, found 235.0946. Anal. for $C_{11}H_{16}O_4$: Calcd: C 62.25; H 7.60. Found: C 62.48; H 8.00.

4.1.2. 2-(Methoxyethoxymethoxy)benzyl alcohol 8a. Yield: 45%. R_f (diethyl ether/hexane: 3/1): 0.2. ^{1}H NMR (300 MHz,) δ 7.33 (dd, 1H, H-3 or H-6, $J=1.8$, 7.4 Hz); 7.26 (dt, 1H, H-5 or H-4, $J=1.8$, 7.9 Hz); 7.14 (dd, 1H, H-6 or H-3, $J=1.0$, 7.9 Hz); 7.02 (dt, 1H, H-4 or H-5, $J=1.0$, 7.4 Hz); 5.34 (s, 2H, OCH_2O); 4.72 (s, 2H, CH_2OH); 3.87 (m, 2H, AA′ part of the ethylenedioxy fragment); 3.56 (m, 2H, BB′ part of the ethylenedioxy fragment); 3.37 (s, 3H, O*Me*). ^{13}C NMR (75 MHz) δ 155.07 (C-2); 130.00 (C-1); 128.98, 128.93 (C-6 and C-4); 121.96 (C-5); 114.12 (C-3); 93.57 (O*C*H$_2$O); 71.62 (*C*H$_2$OMe); 68.04 (*C*H$_2$CH$_2$OMe); 61.58 (*C*H$_2$OH); 59.04 (O*Me*). HR LSIMS calcd for $C_{11}H_{16}O_4Na$ $(M+Na)^+$ 235.0946, found 235.0946. Anal. for $C_{11}H_{16}O_4$: Calcd: C 62.25; H 7.60. Found: C 62.48; H 8.00.

4.1.3. [5-Chloro-2-(methoxyethoxymethoxy)]benzyl alcohol 8d. Compond **7d** was used as starting material. Yield: 38%. R_f (diethyl ether/hexane: 4/1): 0.3. ^{1}H NMR (300 MHz) δ 7.28 (d, 1H, H-6, $J=2.6$ Hz); 7.15 (dd, 1H, H-4, $J=2.6$, 8.7 Hz); 7.01 (d, 1H, H-3, $J=8.7$ Hz); 5.24 (s, 2H, OCH_2O); 4.60 (d, 2H, CH_2OH, $J=6.1$ Hz); 3.77 (m, 2H, AA′ part of the ethylenedioxy fragment); 3.50 (m, 2H,

BB′ part of the ethylenedioxy fragment); 3.31 (s, 3H, O*Me*). ^{13}C NMR (75 MHz) δ 153.29 (C-2); 131.85 (C-5); 128.36 (C-4); 128.32 (C-6); 126.86 (C-1); 115.33 (C-3); 93.66 (O*C*H$_2$O); 71.56 (*C*H$_2$OMe); 68.06 (*C*H$_2$CH$_2$OMe); 60.62 (*C*H$_2$OH); 59.02 (O*Me*). HR LSIMS calcd for $C_{11}H_{15}O_4$NaCl $(M+Na)^+$ 269.0556, found 269.0553. Anal. for $C_{11}H_{15}O_4Cl$: Calcd: C 53.56; H 6.13. Found: C 53.85; H 5.83.

4.1.4. [5-Bromo-2-(methoxyethoxymethoxy)]benzyl alcohol 8e. Compound **7e** was used as starting material. Yield: 47%. R_f (diethyl ether/hexane: 2/1): 0.26. ^{1}H NMR (400 MHz) δ 7.42 (d, 1H, H-6, $J=2.5$ Hz); 7.28 (dd, 1H, H-4, $J=2.5$, 8.7 Hz); 6.95 (d, 1H, H-3, $J=8.7$ Hz); 5.23 (s, 2H, OC*H*$_2$O); 4.59 (d, 1H, 2H, C*H*$_2$OH, $J=3.5$ Hz); 3.76 (m, 2H, AA′ part of the ethylenedioxy fragment); 3.49 (m, 2H, BB′ part of the ethylenedioxy fragment); 3.30 (s, 3H, O*Me*). ^{13}C NMR (100 MHz) δ 153.76 (C-2); 132.36 (C-1); 131.23, 131.16 (C-4, C-6); 115.77 (C-3); 114.30 (C-5); 93.61 (O*C*H$_2$O); 71.55 (*C*H$_2$OMe); 68.05 (*C*H$_2$CH$_2$OMe); 60.41 (*C*H$_2$OH); 58.95 (O*Me*). HR LSIMS calcd for $C_{11}H_{15}O_4$NaBr $(M+Na)^+$ 313.0051, found 313.0049. Anal. for $C_{11}H_{15}O_4Br$: Calcd: C 45.38; H 5.19. Found: C 44.99; H 4.84.

4.1.5. [2-(Methoxyethoxymethoxymethyl)]-4-methoxyphenol 10b. Compound **7b**[17] was used as starting material. Yield: 57%. R_f (diethyl ether/hexane: 2/1): 0.4. ^{1}H NMR (300 MHz) δ 6.75 (d, 1H, H-3, $J=7.5$ Hz); 6.70 (d, 1H, H-6, $J=3.0$ Hz); 6.68 (dd, 1H, H-4, $J=3.0$, 7.5 Hz); 4.75 (s, 2H, OC*H*$_2$O); 4.65 (s, 2H, C*H*$_2$OH); 3.69 (s, 3H, C-5-O*Me*); 3.69 (m, 2H, AA′ part of the ethylenedioxy fragment); 3.52 (m, 2H, BB′ part of the ethylenedioxy fragment); 3.35 (s, 3H, O*Me*). ^{13}C NMR (75 MHz) δ 153.02 (C-5); 149.28 (C-2); 123.32 (C-1); 117.01 (C-3); 114.70, 114.51 (C-4, C-6); 94.44 (O*C*H$_2$O); 71.60 (*C*H$_2$OCH$_3$); 67.13 (*C*H$_2$CH$_2$-OMe); 66.80 (*C*H$_2$OH); 58.85 (O*Me*); 55.61 (C-5-O*Me*). HR LSIMS calcd for $C_{12}H_{18}O_5$ $(M+Na)^+$ 265.1051, found 265.1050. Anal. for $C_{12}H_{18}O_5$: Calcd: C 59.49; H 7.49. Found: C 59.76; H 7.22.

4.1.6. [2-(Methoxyethoxymethoxymethyl)]-6-methoxyphenol 10c. Compound **7c** was used as starting material. Yield: 47%. R_f (diethyl ether/hexane: 4/1): 0.42. ^{1}H NMR (400 MHz) δ 6.9 (dd, 1H, H-5, $J=4.3$, 5.2 Hz); 6.25 (d, 2H, H-4, H-6, $J=5.2$ Hz); 6.17 (s, 1H, OH); 4.81 (s, 2H, OC*H*$_2$O); 4.70 (s, 2H, C*H*$_2$OH); 3.85 (s, 3H, C-3-O*Me*); 3.75 (m, 2H, AA′ part of the ethylenedioxy fragment); 3.56 (m, 2H, BB′ part of the ethylenedioxy fragment); 3.39 (s, 3H, O*Me*). ^{13}C NMR (100 MHz) δ 146.66 (C-3); 144.02 (C-2); 123.48 (C-1); 121.73 (C-5); 119.36 (C-6); 110.47 (C-4); 94.76 (O*C*H$_2$O); 71.70 (*C*H$_2$OMe); 66.78 (*C*H$_2$-CH$_2$OMe); 64.55 (*C*H$_2$OH); 58.85 (CH$_2$O*Me*); 55.94 (C-3-O*Me*). HR LSIMS calcd for $C_{12}H_{18}O_5$Na $(M+Na)^+$ 265.1051, found 265.1050. Anal. for $C_{12}H_{18}O_5$: Calcd: C 59.49; H 7.49. Found: C 59.22; H 7.76.

4.2. Synthesis of 2-(methoxyethoxymethoxy)benzyloxyacetaldehyde dimethyl acetals 9a,d,e

The general procedure is exemplified with the case of **9a**: **8a** (1 g, 4.7 mmol) was added to a suspension of NaH (0.42 g of a 80% dispersion in mineral oil) in anhydrous DMF (12.5 mL) while cooling in an ice bath and the resulting mixture was left under stirring at room temperature for 2 h. After this time, bromoacetaldehyde dimenthyl acetal (1.1 mL, 9.4 mmol) was added while cooling with ice, and the resulting mixture was left under stirring at room temperature at 50 °C for 5 h. The reaction mixture was distilled under diminished pressure and the resulting residue was diluted with water, the pH was adjusted to 2–3 by adding an aqueous solution of HCl 2N and was then extracted (EtOAc). The extract was washed with brine and dried with Na_2SO_4, filtrated, and concentrated in vacuo. The resulting residue was purified by flash chromatography (diethyl ether/hexane: 1/1.5) and **9a** was obtained (0.5 g, 35%). R_f (diethyl ether/hexane: 3/1): 0.38. ^{1}H NMR (300 MHz) δ 7.41 (dd, 1H, H$_{Ar}$; $J=1.6$, 7.4 Hz); 7.25 (dt, 1H, H$_{Ar}$; $J=1.6$, 7.4 Hz); 7.15 (d, 1H, H$_{Ar}$; $J=1.0$, 7.4 Hz); 7.02 (dt, 1H, H$_{Ar}$; $J=1.0$, 7.4 Hz); 5.41 (s, 2H, OC*H*$_2$O); 4.65 (s, 2H, C-1-C*H*$_2$O); 4.57 (t, C*H*(OMe)$_2$); $J=5.2$ Hz); 3.86 (m, 2H, AA′ part of the ethylenedioxy fragment); 3.56 (m, 4H, BB′ part of the ethylenedioxy fragment and O–C*H*$_2$–CH); 3.41 (s, 6H, (O*Me*)$_2$); 3.39 (s, 3H, O*Me*). ^{13}C NMR (75 MHz) δ 154.88 (C-2); 129.18, 128.84 (C-6 and C-4); 127.00 (C-1); 121.73 (C-5); 114.04 (C-3); 102.74 (*C*H(OMe)$_2$); 95.46 (O*C*H$_2$O); 71.61, 69.93, 68.28, 67.71 (*C*H$_2$OMe), (*C*H$_2$CH$_2$OMe), (C-1-*C*H$_2$O), (O–*C*H$_2$–CH); 59.05 (O*Me*); 53.85 (O*Me*)$_2$. HR LSIMS calcd for $C_{15}H_{23}O_6$Na $(M+Na-1)^+$ 299.1494, found 299.1494. Anal. for $C_{15}H_{24}O_6$: Calcd: C 59.98; H 8.05. Found: C 59.95; H 8.23.

4.2.1. [5-Chloro-2-(methoxyethoxymethoxy)benzyloxyacetaldehyde dimethyl acetal 9d. Compound **8d** was used as starting material. Yield: 39%. R_f (ethyl acetate/hexane: 4/1): 0.54. ^{1}H NMR (400 MHz) δ 7.37 (d, 1H, H-6, $J=2.6$ Hz); 7.16 (dd, 1H, H-4, $J=2.6$, 8.7 Hz); 7.06 (d, 1H, H-3, $J=8.76$ Hz); 5.25 (s, 2H, OC*H*$_2$O); 4.57 (s, 2H, C-1-C*H*$_2$O); 4.55 (t, 1H, H-1′, $J=5.2$ Hz); 3.79 (m, 2H, H-2′); 3.54 (m, 4H, AA′BB′ system); 3.40 (s, 3H, O*Me*); 3.36 (s, 6H, (O*Me*)$_2$). ^{13}C NMR (100 MHz) δ 153.11 (C-2); 129.08 (C-5); 128.47 (C-4); 128.22 (C-6); 126.82 (C-1); 115.25 (C-3); 102.74 (*C*H(OMe)$_2$); 93.60 (O*C*H$_2$O); 71.53, 70.30, 67.79, 67.71 (O–*C*H$_2$–CH) (*C*H$_2$OMe), (C-1-*C*H$_2$O), (*C*H$_2$CH$_2$OMe); 58.99 (O*Me*); 53.92 (O*Me*)$_2$. HR LSIMS calcd for $C_{15}H_{23}O_6$NaCl $(M+Na)^+$ 357.1080, found 357.1078. Anal. for $C_{15}H_{23}O_6Cl$: Calcd: C 53.81; H 6.92. Found: C 53.48; H 7.23.

4.2.2. [5-Bromo-2-(methoxyethoxymethoxy)benzyloxyacetaldehyde dimethyl acetal 9e. Compound **8e** was used as starting material. Yield: 42%. R_f (ethyl acetate/hexane: 4/1): 0.38. ^{1}H NMR (400 MHz) δ 7.47 (d, 1H, H-6, $J=2.5$ Hz); 7.27 (dd, 1H, H-4, $J=2.5$, 8.7 Hz); 6.97 (d, 1H, H-3, $J=8.7$ Hz); 5.21 (s, 2H, OC*H*$_2$O); 4.53 (s, 2H, C-1-C*H*$_2$O); 4.51 (t, 1H, H-1′, $J=5.2$ Hz); 3.75 (m, 2H, H-2′); 3.50 (m, 4H, AA′BB′ system); 3.35 (s, 3H, O*Me*); 3.31 (s, 6H, (O*Me*)$_2$). ^{13}C NMR (100 MHz) δ 153.61 (C-2); 131.34 (C-4); 131.17 (C-6); 129.51 (C-1); 115.67 (C-3); 114.22 (C-5); 102.73 (*C*H(OMe)$_2$); 93.51 (O*C*H$_2$O); 71.50, 70.31, 67.78, 67.67 (O–*C*H$_2$–CH), (*C*H$_2$OMe), (C-1-*C*H$_2$O), (*C*H$_2$CH$_2$-OMe); 58.94 (O*Me*); 53.88 (O*Me*)$_2$. HR LSIMS calcd for $C_{15}H_{23}O_6$NaBr $(M+Na)^+$ 401.0575 $C_{15}H_{23}O_6$NaBr $(M+Na)^+$ 401.0575, found 401.0583. Anal. for $C_{15}H_{23}O_6Br$: Calcd: C 47.51; H 6.11. Found: C 47.9; H 5.76.

4.3. Synthesis of (*RS*)-2-methoxy-2,3-dihydro-5*H*-1,4-benzodioxepins 2a,d,e and (*RS*)-5-methoxy-2,3,5,6-tetrahydro-8*H*-benzo-[1,4,7]-trioxecin 5a,d,e

The general procedure is exemplified with the case of **2a** and **5a**: **9a** (1.5 g, 4.9 mmol) was dissolved in anhydrous THF (15 mL), and $BF_3 \cdot OEt_2$ (0.3 mL) was added at 0 °C and the mixture was kept at this temperature under stirring for 24 h. After this time the solution was washed with an aqueous solution of K_2CO_3 (10%) and extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The residue was purified by flash chromatography with diethyl ether/hexane (1/3) yielding 0.36 g of **3a** (41%), and 0.12 g of **5a** (6%). After concentrating the organic layer, the residue was subjected to a simple combination of directly-coupled gas chromatography-mass spectrometry (GC-MS): t_R of methoxymethanol: 4.14 min; t_R of methoxymethoxyethanol: 6.76 min. The identity of methoxymethanol[14] and methoxymethoxyethanol[15] were confirmed by comparison of their retention times with pure authentic samples.

Compound **3a**. R_f (diethyl ether/hexane: 2/1): 0.61. ^{1}H NMR (300 MHz) δ 7.31 (dt, 1H, H_{Ar}, J=1.7, 7.7 Hz); 7.20 (dd, 1H, H_{Ar}, J=1.7, 7.7 Hz); 7.17 (dd, 1H, H_{Ar}, J=1.3, 7.7 Hz); 7.09 (dt, 1H, H_{Ar}, J=1.3, 7.7 Hz); 4.70 (dd, 1H, H-2, J=1.6, 6.3 Hz); 4.68 (d, 2H, H-5, 1.6); 4.07 (dd, 1H, H-3, J=1.6, 12.5 Hz); 3.81 (dd, 1H, H-3, J=6.3, 12.5 Hz); 3.62 (s, 3H, O*Me*). ^{13}C NMR (75 MHz) δ 154.30 (C-10); 133.26 (C-11); 129.49, 129.04 (C-6 and C-8); 124.00 (C-9); 121.48 (C-7); 103.99 (C-2); 74.86 (C-3); 72.87 (C-5); 56.50 (O*Me*). HR LSIMS calcd for $C_{10}H_{12}O_3Na$ $(M+Na)^+$ 203.0694, found 203.0683. Anal. for $C_{10}H_{12}O_3$: Calcd: C 66.65; H 6.71. Found: C 66.19; H 6.85.

Compound **5a**. R_f (diethyl ether/hexane: 4/1): 0.39. ^{1}H NMR (400 MHz) δ 7.29 (dt, 1H, H-11 or H-10, J=1.7, 7.8 Hz); 7.17 (dd, 1H, H-9 or H-12, J=1.7, 7.4 Hz); 7.13 (dd, 1H, H-12 or H-9, J=1.1, 7.8 Hz); 7.07 (dt, 1H, H-10 or H-11, J=1.1, 7.4 Hz); 4.81 (dd, 1H, H-5, J=1.7, 6.8 Hz); 4.65 (dd, 2H, H-8, J=2.6, 13.3 Hz); 4.14 (m, 2H, H-6, H-2); 3.81 (m, 2H, H-6, H-2); 3.61 (m, 2H, H-3, H-3); 3.39 (s, 3H, O*Me*). ^{13}C NMR (100 MHz) δ 154.44 (C-13); 133.43 (C-14); 129.52 (C-11); 129.05 (C-9); 124.05 (C-10); 121.40 (C-12); 103.16 (C-5); 74.91 (C-6); 72.72 (C-2); 71.58 (C-8); 68.08 (C-3); 59.12 (O*Me*). HR LSIMS calcd for $C_{12}H_{16}O_4Na$ $(M+Na)^+$ 247.0946, found 247.0945. Anal. for $C_{12}H_{16}O_4 \cdot 0.1H_2O$: Calcd: C 64.27; H 7.19. Found: C 64.00; H 7.20.

4.3.1. (*RS*)-7-Chloro-2-methoxy-2,3-dihydro-5*H*-1,4-benzodioxepin 3d and (*RS*)-10-chloro-5-methoxy-2,3,5,6-tetrahydro-8*H*-benzo-[1,4,7]-trioxecin 5d.

Compound **9d** was used as a starting material. **3d**: Yield: 45%. R_f (ethyl acetate/hexane: 4/1): 0.7. ^{1}H NMR (400 MHz) δ 7.23 (dd, 1H, H-8, J=2.5, 8.4 Hz); 7.15 (d, 1H, H-6, J=2.5 Hz); 7.07 (d, 1H, H-9, J=8.4 Hz); 4.68 (dd, 1H, H-2, J=1.5, 6.0 Hz); 4.60 (d, 2H, H-5, J=1.7 Hz); 4.03 (dd, 1H, H-3, J=1.5, 12.6 Hz); 3.80 (dd, 1H, H-3, J=6.0, 12.6 Hz); 3.59 (s, 3H, O*Me*). ^{13}C NMR (100 MHz) δ 152.85 (C-10); 134.80 (C-11); 134.79 (C-7); 129.20 (C-8); 128.83 (C-6); 122.94 (C-9); 103.99 (C-2); 74.85 (C-3); 72.37 (C-5); 56.61 (O*Me*). HR LSIMS calcd for $C_{10}H_{11}O_3NaCl$ $(M+Na)^+$ 237.0294, found 237.0299. Anal. for $C_{10}H_{11}O_3Cl$: Calcd: C 55.96; H 5.17. Found: C 56.22; H 4.87.

Compound **5d**. Yield: 22%. R_f (ethyl acetate/hexane: 4/1): 0.6. ^{1}H NMR (400 MHz) δ 7.23 (dd, 1H, H-11, J=2.5, 8.4 Hz); 7.15 (d, 1H, H-9, J=2.5 Hz); 7.05 (d, 1H, H-12, J=8.4 Hz); 4.8 (dd, 1H, H-5, J=1.6, 6.6 Hz); 4.59 (dd, 2H, H-8, J=1.2, 13.6 Hz); 4.11 (m, 2H, H-6, H-2); 3.8 (m, 2H, H-6, H-2); 3.6 (m, 2H, H-3, H-3); 3.39 (s, 3H, O*Me*). ^{13}C NMR (100 MHz) δ 153.00 (C-13); 134.97 (C-10); 129.22 (C-11); 128.92 (C-9); 128.85 (C-14); 122.87 (C-12); 103.18 (C-5); 74.91 (C-6); 72.22 (C-2); 71.54 (C-8); 68.20 (C-3); 59.15 (O*Me*). HR LSIMS calcd for $C_{12}H_{15}O_4NaCl$ $(M+Na)^+$ 281.0556, found 281.0563. Anal. for $C_{12}H_{15}O_4Cl$: Calcd: C 55.71; H 5.84. Found: C 55.83; H 6.03.

4.3.2. (*RS*)-7-Bromo-2-methoxy-2,3-dihydro-5*H*-1,4-benzodioxepin 3e and (*RS*)-10-bromo-5-methoxy-2,3,5,6-tetrahydro-8*H*-benzo-[1,4,7]-trioxecin 5e.

Compound **9e** was used as a starting material. **3e**: Yield: 27%. R_f (diethyl ether/hexane: 2/1): 0.6. ^{1}H NMR (400 MHz) δ 7.35 (dd, 1H, H-8, J=2.4, 8.4 Hz); 7.27 (d, 1H, H-6, J=2.4 Hz); 6.99 (d, 1H, H-9, J=8.4 Hz); 4.65 (dd, 1H, H-2, J=1.6, 6.1 Hz); 4.57 (d, 2H, H-5, J=2.1 Hz); 4.00 (dd, 1H, H-3, J=1.6, 12.6 Hz); 3.76 (dd, 1H, H-3, J=6.1, 12.6 Hz); 3.56 (s, 3H, O*Me*). ^{13}C NMR (100 MHz) δ 153.45 (C-10); 135.27 (C-11); 132.25 (C-8); 131.76 (C-6); 123.32 (C-9); 116.46 (C-7); 104.04 (C-2); 74.85 (C-3); 72.32 (C-5); 56.63 (O*Me*). HR LSIMS calcd for $C_{10}H_{12}O_3Br$ $(M^+ + 1)$ 258.9969, found 258.9976. Anal. for $C_{10}H_{11}O_3Br$: Calcd: C 46.36; H 4.28. Found: C 45.97; H 4.11.

Compound **5e**. Yield: 18%. R_f (diethyl ether/hexane: 2/1): 0.46. ^{1}H NMR (400 MHz) δ 7.34 (dd, 1H, H-11, J=2.4, 8.4 Hz); 7.26 (d, 1H, H-9, J=2.4 Hz); 6.97 (d, 1H, H-12, J=8.4 Hz); 4.77 (dd, 1H, H-5, J=1.7, 6.6 Hz); 4.56 (dd, 2H, H-8, J=1.5, 14.1 Hz); 4.07 (m, 2H, H-6, H-2); 3.76 (m, 2H, H-6, H-2); 3.56 (m, 2H, H-3, H-3); 3.35 (s, 3H, O*Me*). ^{13}C NMR (100 MHz) δ 153.60 (C-13); 135.45 (C-10); 132.28 (C-11); 131.82 (C-9); 123.35 (C-12); 116.53 (C-14); 103.22 (C-5); 74.92 (C-6); 72.19 (C-2); 71.61 (C-8); 68.25 (C-3); 59.17 (O*Me*). HR LSIMS calcd for $C_{12}H_{15}O_4NaBr$ $(M+Na)^+$ 325.0051, found 325.0053. Anal. for $C_{12}H_{15}O_4Br$: Calcd: C 47.54; H 4.99. Found: C 47.81; H 5.13.

4.4. Synthesis of 2-(methoxyethoxymethoxymethyl)-phenyloxyacetaldehyde dimethyl acetals 11b and 11c

The procedure was similar to the one used in 4.3, but changing **9a** by **10b**, and **9a** by **10c**.

4.4.1. [4-Methoxy-2-(methoxyethoxymethoxymethyl)]-phenyloxyacetaldehyde dimethyl acetal 11b.

Compound **10b** was used as starting material. Yield: 26%. R_f (ethyl acetate/hexane: 2/1): 0.4. ^{1}H NMR (300 MHz) δ 6.95 (d, 1H, H-3, J=2.8 Hz); 6.76 (t, 1H, H-6, J=8.8 Hz); 6.73 (dd, 1H, H-5, J=2.8, 8.8 Hz); 4.82 (s, 2H, OCH_2O); 4.66 (t, 1H, H-1′, J=5.2 Hz); 4.63 (s, 2H, C-2-CH_2O); 3.94 (d, 2H, H-2′, J=5.2 Hz); 3.73 (s, 1H, C-4-OMe); 3.73 (m, 2H, AA′ part of the ethylenedioxy fragment); 3.54 (m, 2H, BB′ part of the ethylenedioxy fragment); 3.41 (s, 6H, $(OMe)_2$); 3.37

(s, 3H, O*Me*). ^{13}C NMR (75 MHz) δ 154.05 (C-4); 150.20 (C-1); 128.22 (C-2); 114.75 (C-6); 113.16 (C-5); 113.12 (C-3); 102.37 (*C*H(OMe)$_2$); 95.29 (O*C*H$_2$O); 71.82, 68.97, 66.86, 64.55 (O-*C*H$_2$–CH), (*C*H$_2$OMe), (C-2-*C*H$_2$O), (*C*H$_2$-CH$_2$OMe); 59.03 (O*Me*); 55.72 (C-4-O*Me*); 54.17 (O*Me*)$_2$. HR LSIMS calcd for $C_{16}H_{26}O_7$ $(M+Na)^+$ 353.1576, found 353.1577. Anal. for $C_{16}H_{26}O_7$: Calcd: C 58.17; H 7.93. Found: C 57.82; H 8.27.

4.4.2. [6-Methoxy-2-(methoxyethoxymethoxymethyl)-phenyloxyacetaldehyde dimethyl acetal 11c. Compond **10c** was used as starting material. Yield: 33%. R_f (diethyl ether/hexane: 2/1): 0.35. ^{1}H NMR (300 MHz) δ 7.00 (d, 1H, H-4, $J=7.7$ Hz); 6.96 (dd, 1H, H-3 or H-5, $J=1.9$, 7.7 Hz); 6.82 (dd, 1H, H-5 or H-3, $J=1.9$, 7.7 Hz); 4.80 (s, 2H, OC*H*$_2$O); 4.70 (t, 1H, H-1′, $J=5.4$ Hz); 4.67 (s, 2H, C-2-C*H*$_2$O); 4.01 (d, 2H, H-2′, $J=5.4$ Hz); 3.81 (s, 3H, C-6-O*Me*); 3.72 (m, 2H, AA′ part of the ethylenedioxy fragment); 3.55 (m, 2H, BB′ part of the ethylenedioxy fragment); 3.40 (s, 6H, (O*Me*)$_2$); 3.37 (s, 3H, O*Me*). ^{13}C NMR (75 MHz) δ 152.31 (C-6); 145.84 (C-1); 131.84 (C-2); 124.06 (C-4); 121.24 (C-3); 111.95 (C-5); 102.47 (*C*H(OMe)$_2$); 95.15 (O*C*H$_2$O); 71.87, 71.82, 66.87, 64.50 (O-*C*H$_2$–CH), (*C*H$_2$OMe), (C-1-*C*H$_2$O), (*C*H$_2$CH$_2$OMe); 59.05 (O*Me*); 55.77 (C-6-O*Me*); 53.85 (O*Me*)$_2$. HR LSIMS calcd for $C_{16}H_{26}O_7Na$ $(M+Na)^+$ 353.1576, found 353.1573. Anal. for $C_{16}H_{26}O_7$: Calcd: C 58.17; H 7.93. Found: C 57.85; H 8.02.

4.5. Synthesis of (*RS*)-3-methoxy-2,3-dihydro-5*H*-1,4-benzodioxepins 2b,c and (*RS*)-3,12-dimethoxy-2,3,5,6-tetrahydro-8*H*-benzo-[1,4,7]-trioxecin 6c

The procedure is similar to the one used in 4.3, but changing **9a** by **11b**, and **9a** by **11c**.

4.5.1. (*RS*)-2,7-Dimethoxy-2,3-dihydro-5*H*-1,4-benzodioxepin 2b. Compond **11b** was used as a starting material, yielding 40% of **2b**, whose spectroscopical characteristics were identical to the ones previously described.[4] The organic residue was subjected to a simple combination of directly-coupled gas chromatography-mass spectrometry (GC-MS): t_R of methoxymethoxyethanol: 6.76 min.

4.5.2. (*RS*)-3,9-Dimethoxy-2,3-dihydro-5*H*-1,4-benzodioxepin 2c and (*RS*)-3,12-dimethoxy-2,3,5,6-tetrahydro-8*H*-benzo-[1,4,7]-trioxecin 6c. Compond **11c** was used as a starting material, yielding 70% of **2c** and 15% of **6c**. The spectroscopic characteristics of **2c** were identical to the ones previously described.[5] The aqueous layer was freeze-dried and the residue was subjected to a simple combination of directly-coupled gas chromatography-mass spectrometry (GC-MS): t_R of methoxymethanol: 4.14 min; t_R of methoxymethoxyethanol: 6.76 min.

Compound **6c**. Yield: 15%. R_f (2/1, diethyl ether/hexane): 0.27. ^{1}H NMR (300 MHz) δ 6.88 (d, 1H, H-10, $J=7.6$ Hz); 6.80 (d, 1H, H-9, $J=1.5$ Hz); 6.64 (dd, 1H, H-11, $J=1.5$, 7.6 Hz); 5.24 (d, 1H, H-8, $J=14.5$ Hz); 5.05 (dd, 1H, H-5, $J=3.4$, 7.6 Hz); 4.38 (dd, 1H, H-8, $J=14.5$ Hz); 4.29 (dd, 1H, H-6, $J=3.4$, 13.1 Hz); 4.13 (dd, 1H, H-6, $J=7.6$, 13.1 Hz); 3.95 (m, 1H, H-2); 3.83 (s, 3H, C-12-O*Me*); 3.69 (m, 1H, H-2); 3.56 (m, 2H, H-3, H-3); 3.38 (s, 3H, O*Me*). ^{13}C NMR (75 MHz) δ 150.47 (C-12); 147.87 (C-13); 130.12 (C-14); 122.48 (C-10); 120.18 (C-9); 111.38 (C-11); 100.47 (C-5); 72.19 (C-6); 71.85 (C-2); 67.20 (C-8); 63.05 (C-3); 59.16 (C-12-O*Me*); 56.14 (C-5-O*Me*). HR LSIMS calcd for $C_{13}H_{18}O_5Na$ $(M+Na)^+$ 277.1052, found 277.1055. Anal. for $C_{13}H_{18}O_5$: Calcd: C 61.4; H 7.13. Found: C 61.02; H 6.88.

4.6. X-ray crystallographic study of (*RS*)-1-(7-methoxy-2,3-dihydro-5*H*-1,4-benzodioxepin-3-yl)-5-fluorouracil

A colourless crystal was mounted on a glass fibre and used for data collection. Crystal data were collected at 298(2) K, using a Bruker SMART CCD 1000 diffractometer. Graphite monochromated Mo Kα radiation ($\lambda=0.71073$ Å) was used throughout. The data were processed with SAINT[18] and corrected for absorption using SADABS (transmissions factors: 0.976–0.971).[19] The structure was solved by direct methods using the programme SHELXS-97[20] and refined by full-matrix least-squares techniques against F^2 using SHELXL-97.[21] Positional and anisotropic atomic displacement parameters were refined for all nonhydrogen atoms. Hydrogen atoms were located in difference maps and included as fixed contributions riding on attached atoms with isotropic thermal parameters 1.2 times those of their carrier atoms. Criteria of a satisfactory complete analysis were the ratios of rms shift to standard deviation less than 0.001 and no significant features in final difference maps. Atomic scattering factors from 'International Tables for Crystallography'.[22] Molecular graphics and geometrical calculatioons from PLATON[23] and SHELTXL.[24] Relevant crystal data: formula $C_{14}H_{13}FN_2O_5$, formula weight 308.26, $T=298(2)$ K, crystal system triclinic, space group *P*-1, unit cell dimensions $a=6.491(2)$, $b=7.588(3)$ and $c=14.037(2)$ Å, and $\alpha=89.41(2)°$, $\beta=87.70(2)$ and $\gamma=73.72(2)$, $Z=2$, $D=1.544$ Mg m^{-3}, μ(Mo Kα)$=0.127$ mm^{-1}, measured/unique reflections 7805/3007 [R(int) 0.0191], refined parameters 200, final R_1 ($I>2\sigma(I)$)$=0.0408$ and w$R_2=0.1119$, and GOF$=1.053$. CCDC reference number 236010. 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@ccd.cam.ac.uk].

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Chemoselective deprotection of acid labile primary hydroxyl protecting groups under CBr_4-photoirradiation conditions

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Abstract—The CBr_4-photoirradiation in methanol generates a controlled source of HBr, which can chemoselectively deprotect commonly used hydroxyl-protecting groups in saccharides and nucleosides, such as *tert*-butyldimethylsilyl, isopropylidene, benzylidene and triphenyl ethers in the presence of other acid-labile functional groups.

1. Introduction

A protective and deprotective strategy is a critical part of many synthetic scheme that involve carbohydrate and nucleoside chemistry. According, an impressive array of protective groups have been developed to protect the hydroxyl group, along with methods for removing them.[1] Additionally, the efficient synthesis of polyfunctional target molecules relies on the development of a protection–deprotection strategy with fewer steps than implemented at present. This, in principle, can be achieved using a single group to protect all functional groups and then chemoselectively deprotecting the desired functional group, rather than using different groups to protect each functional group and then deprotecting them individually.[2] The development of chemoselectively deprotecting reagents is critical to the success of this strategy. Our earlier results showed that a catalytic amount of carbon tetrabromide under photoirradiation in methanol can chemoselectively deprotect *tert*-butyldimethylsilyl (TBDMS), isopropylidenyl, benzylidenyl and triphenylmethyl groups on the primary hydroxyl groups of saccharides and nucleosides.[3] This study reports the scope of this mild and efficient protocol.

CBr_4 has been used as a catalyst and reagent to perform various interesting transformations.[4] It has also been successfully used to cleave acetals/ketals,[4b] tetrahydropyranyl ethers,[4a] trialkylsilyl ethers,[5] methoxymethyl ethers, methoxyethoxymethyl ethers,[6] trityl ethers,[7] *p*-methoxybenzyl (PMB)[8] and β-(trimethylsilyl)ethoxymethyl ethers[9] in only catalytic amounts, but heating in methanol to the reflux temperature is required. The success of this deprotection protocol depends on the in situ generation of HBr, which provides mild but sufficiently anhydrous and acidic reaction conditions.[4d,6] This fact motivated us to investigate the use of this protocol in carbohydrate and nucleoside chemistry, eventually leading to identifying the mild reaction conditions.[3]

Among the various protecting groups, commonly used groups, such as TBDMS, acetonide and trityl, were used in this deprotection study. Silyl ethers such as TBDMS are particularly useful because they are stable in the presence of various reagents and conditions.[10] TBDMS ethers can be deprotected under various conditions, including the prescence of fluoride ions, protic acids and Lewis acids.[11–13] In other cases the primary TBDMS group can be selectively removed under acidic conditions if the secondary silyl group is sterically hindered.[14,15] However, this procedure normally exhibits poor selectivity. Apart from silyl ethers, acetonide has been used widely in carbohydrate chemistry to selectively mask the hydroxyls of many sugars. Although normal acidic catalysts such as HCl, HBr, TFA and AcOH have been satisfactory reagents[1,4b,16] for selectively hydrolyzing primary–secondary hydroxyl groups in simple diacetonide derivatives, their strong acidity and free protons make them undesirable hydrolyzing derivatives with acid-sensitive groups.[17]

Keywords: CBr_4-photoirradiation; Deprotection.
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Triphenylmethyl (trityl) ethers are also extensively used as selective protecting groups for primary alcohols, particularly in carbohydrate and nucleoside chemistry, but few procedures exist for selectively cleaving them.[18] Trityl ethers are generally cleaved under strong protic or Lewis acid conditions, such as in formic acid, 80% acetic acid, mineral acids, zinc bromide, trifluoro acetic acid, iodine–methanol and BCl_3.[7,18] Some methods of detritylation under mild conditions have been reported to avoid the cleavage of glycosidic bond and the hydrolysis of acetate.[18] Numerous such methods suffer from the requirement of the use of strongly acidic conditions, incompatibility with other acid sensitive groups, unsatisfactory yields, corrosive/expensive reagents, longer reaction times and the need for anhydrous conditions.[18]

Here, the CBr_4/MeOH system was applied to cleave selectively *tert*-butyldimethylsilyl, isopropylidene acetals, benzylidine and trityl groups in carbohydrate and nucleoside chemistry.

2. Results and discussion

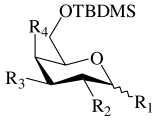
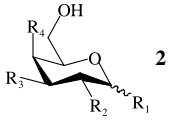
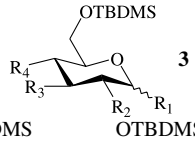
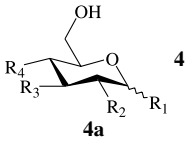
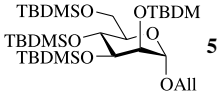
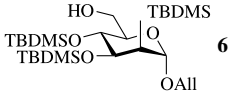
2.1. Deprotection of *tert*-butyldimethylsilyl ethers

Table 1 presents the results of our initial investigation of the regioselective deprotection of primary TBDMS ether in the presence of secondary TBDMS ethers. The primary TBDMS ethers of pyranose sugar,[19] **1**, **3** and **5**, are removed in excellent yields (71–94%) by treatment with a catalytic amount of CBr_4 in methanol under photochemical reaction conditions.[3] The results in Table 1 indicate that other protecting groups on saccharides affected the extent of deprotecting rates. When an anomeric hydroxyl group was protected as methyl ether, the rate of desilylation of the primary silyl ether normally exceeded faster than other types of protecting groups on anomeric center (Table 1, entries 3 and 7). Interestingly, the presence of the free hydroxyl group near the primary silyl ether accelerated the deprotection (Table 1, entry 2 and 11). Notably, no silyl group migration was observed in any cases.

Various substrates **7a–d** with primary and secondary TBDMS groups were prepared[20] and their cleavage investigated to elucidate the utility of the CBr_4/MeOH photoirradiation conditions for deprotecting TBDMS-protected nucleotides (Table 2, entries 1–4). In the presence of the amino group of adenosine **7c** and guanosine **7d**, only moderate yields were obtained after a reaction over 3 days (62 and 54%, respectively) even when the amount of CBr_4 was increased to 50% mole equivalent. In the presence of basic amine on the nucleoside base, the base is expected to trap the catalytic amount of HBr, making it unavailable for deprotection. Contrary to this expectation, however, the deprotection of primary TBDMS in nucleosides proceeded smoothly.

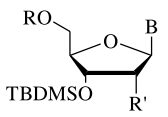
An interesting application of this method was demonstrated by the synthesis of sialic acid derivatives with a free primary

Table 1. Selective deprotection of TBDMS ethers with 5 mol% CBr_4/MeOH

Entry	Substrate[a]				Product	Time[b] (h)	Yield (%)[c]
	 **1**				 **2**		
	R_1	R_2	R_3	R_4			
1	β-SPh-*p*-Me	OTBDMS	OTBDMS	OTBDMS	**2a**	20	75
2	β-SPh-*p*-Me	OTBDMS	OTBDMS	OH	**2b**	1	87
3	α-Ome	OTBDMS	OTBDMS	OTBDMS	**2c**	4	94
4	α-All	OTBDMS	OTBDMS	OTBDMS	**2d**	28	90
5	β-SPh-*p*-Me	Obn	OBn	OTBDMS	**2e**	12	93
6	β-SPh-*p*-Me	OTol[b]	OTol	OTBDMS	**2f**	39	71
7	α-Ome	Obn	OBn	OTBDMS	**2g**	5	85
8	β-SPh-*p*-Me	N_3	OBz	OTBDMS	**2h**	12	89
9	α-SePh	N_3	OTBDMS	OTBDMS	**2i**	20	90
	 **3**				 **4**		
10	β-SPh-*p*-Me	OTBDMS	OTBDMS	OTBDMS	**4a**	28	83
11	β-SPh-*p*-Me	OTBDMS	OTBDMS	OH	**4b**	2	95
12	α-Ome	OTBDMS	OTBDMS	OTBDMS	**4c**	23	87
13	β-SPh-*p*-Me	Obn	OBn	OTBDMS	**4d**	19	90
14	β-SPh-*p*-Me	Otol	OTol	OTBDMS	**4e**	10	92
15	β-SPh-*p*-Me	NHTroc	OTBDMS	OTBDMS	**4f**	6	82
16	 **5**				 **6**	13	83

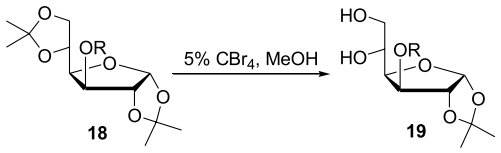
[a] TBDMS, *tert*-butyldimethylsilyl; Tol, *p*-methylbenzoyl; All, allyl; Troc, 2,2,2-trichloroethoxycarbonyl.
[b] Irradiated for 0.5 h then stirred at room temperature.
[c] The isolated yield after chromatographic purification.

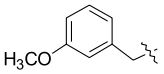
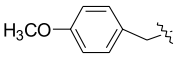
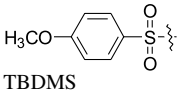
Table 2. Selective deprotection of TBDMS-protected nucleotides with 5 mol% CBr_4/MeOH

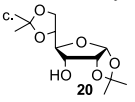
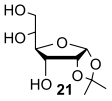
Entry	Substrate			Product	Time (h)[a]	Yield (%)[b]
						
	7 R=TBDMS			**8** R=H		
		B	R′			
1	**7a**	Thymine	H	**8a**	68	85
2	**7b**	Uracil	OTBDMS	**8b**	68	92
3	**7c**	Adenine	OTBDMS	**8c**	72	62 (33)[c]
4	**7d**	Guanosine	OTBDMS	**8d**	72	54 (43)[c]

[a] Irradiated for 0.5 h then stirred at room temperature.
[b] The isolated yield after chromatographic purification.
[c] The number in parenthesis indicates the yield of recovery of the starting material.

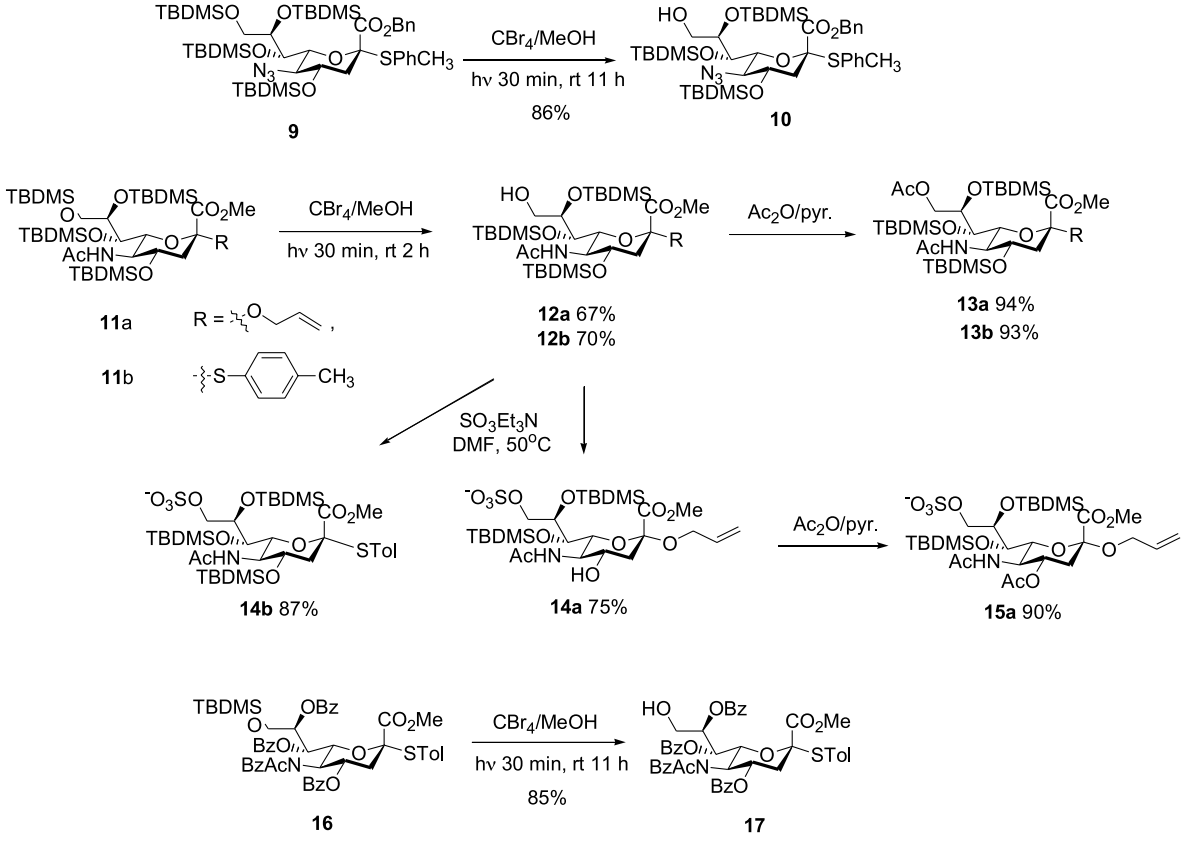
Table 3. Deprotection of isopropylidene from furanose substrates



	R	Reaction time (h)[a]	Yield (%)[b]
a	H[c]	19	95
b		36	86
c	Ac	24	89
d	Bz	24	82
e	Bn	48	84
f		42	85
g		35	83
h		48	88
i	TBDMS	45	81

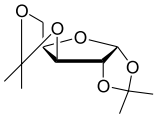
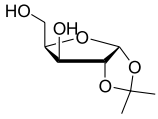
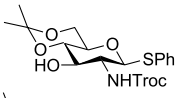
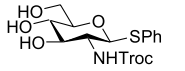
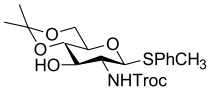
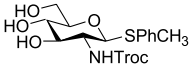
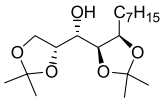
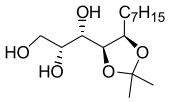
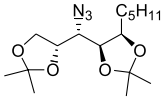
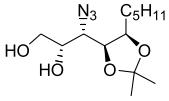
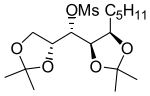
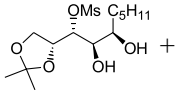
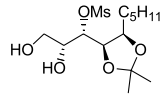
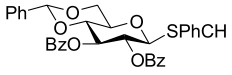
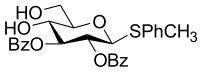
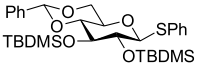
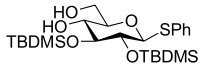
[a] Irradiated for 0.5 h then stirred at room temperature.
[b] The isolated yield after chromatographic purification.
[c]  was also deprotected to get  in 15 h (90%).

hydroxyl group in the C-9 position (Scheme 1). Sialic acids are the terminal sugars of various glycoproteins and glycolipids and participate in masking cell surface antigens, bacterial cell surface activity, cell-to-cell recognition and mitogenic–receptor activity with some lectins.[21] Therefore, the synthesis of analogs of sialic acids has attracted significant interest in studies of relationships between the structure and function and their inhibitory activity.[22] Access to these synthetic analogs depends primarily on the use of appropriate protection–deprotection schemes, since the sialic acid has many hydroxyl groups. As shown in Scheme 1, sialic acid derivative **9**, protected as silyl ether at positions C-4, 7, 8 and 9 underwent the optimized deprotection protocol, producing compound **10** in very high yield. Similarly, compounds **11a** and **11b** gave satisfactory yields of **12a** and **12b**. Compounds **12a** and **12b** were



Scheme 1.

Table 4. Deprotection of isopropylidene and benzylidene groups from saccharides with 5 mol% CBr_4

Entry	Substrate[a]		Time (h)[b]	Product		Yield (%)[c]
1		**22**	8		**23**	86
2		**24**	5		**25**	93
3		**26**	3		**27**	92
4		**28**	43		**29**	72
5		**30**	36		**31**	62
6		**32**	36		**33a**	70 (4:1)
					**33b**	
7[d]		**34**	41		**35**	83
8		**36**	32		**37**	86

[a] Troc, 2,2,2-trichloroethoxycarbonyl; Ms, methanesulfonyl; TBDMS, *tert*-butyldimethylsilyl.
[b] Irradiated for 0.5 h then stirred at room temperature.
[c] The isolated yield after chromatographic purification.
[d] Cosolvent was used to dissolve the substrate ($CH_3OH/CH_2Cl_2=1:1$).

subjected to the standard acetylation protocol to yield compound **13a** and **13b**, confirming that the C-9 hydroxy group had indeed undergone deprotection. The sulfation of **12a** and **12b** in DMF at 50 °C with SO_3/Et_3N gave the sulfated derivatives **14a** (75%) and **14b** (87%). Notably, the TBDMS protecting group at C-4 position of **12a** was cleaved under sulfation conditions. The deprotection of C-4 TBDMS was further verified by acetlyation of **14a** to give **15a**. Consistent with the above results, compound **17** was obtained from **16**. Interestingly, the substituent of amine function group in position C-5 in sialic acid affected the rate of deprotection of C-9 TBDMS. In the presence of the proton at the nitrogen atom, the cleavage of C-9 TBDMS of compounds **11a** and **11b** proceeded more quickly than that of compounds **9** and **16**.

2.2. Deprotection of isopropylidene and benzylidene

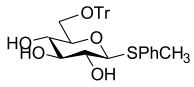
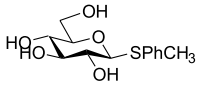
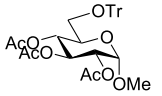
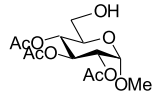
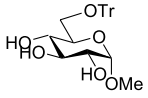
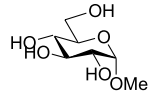
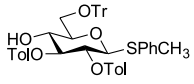
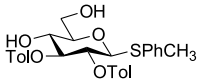
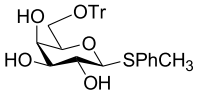
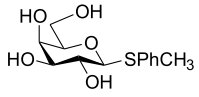
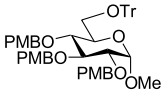
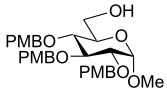
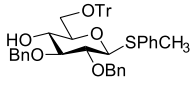
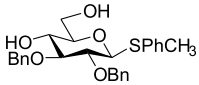
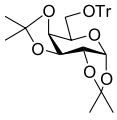
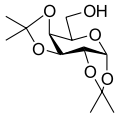
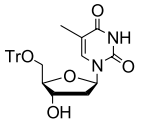
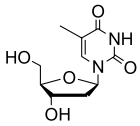
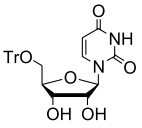
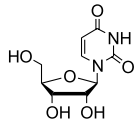
Following the success of the selective cleavage of TBDMS ethers, whether the protocol could be effective in selectively cleaving the isoproylidene group in sugar derivatives was examined (Table 3). Selectively deprotecting of the five-membered 5,6-O-isopropylidene group in **18** using a catalytic amount of CBr_4 in MeOH formed **19**[23] in good yields (81–95%). As shown in Table 3, Ac, Bz, Bn and acid-sensitive protecting groups, such as PMB, MMB, Ts, TBDMS, and allyl ethers, were unaffected under the reaction conditions. The PMB group, which undergoes cleavage in the presence of CBr_4 under methanol reflux conditions, survived under photoirradiation (Entry 7).

According to Table 4, many acid labile acetonide protecting groups were selectively hydrolyzed by the CBr_4-photo-irradiation protocol (Entries 1–6).[24] Notably, in the deprotection of isopropylidene of compound **32**, the internal isopropylidene was more easily cleaved than the terminal isopropylidene. The difference in reactivity may follow from electron withdrawal properties of the Ms group. As expected, the benzylidene group was deprotected with this protocol, yielding **35** (83%) and **37** (86%) from **34** and **36**.

2.3. Deprotection of trityl protecting group

The cleavage of trityl-protected saccharides **38–56**[25] and nucleotides **39–52** by the same method also proceeded smoothly with high yields (Table 5). Other protecting

Table 5. Deprotection of trityl group from saccharides and nucleotides with 5 mol% CBr_4/MeOH

Entry	Substrate[a]		Time (h)[b]	Product[26]		Yield (%)[c]
1		**38**	8		**39**	90
2		**40**	6		**41**	86
3		**42**	5		**43**	93
4		**44**	9		**45**	88
5		**46**	8		**47**	95
6		**48**	38		**49**	91
7		**50**	12		**51**	91
8		**52**	9		**53**	90
9		**54**	13		**55**	97
10		**56**	12		**57**	94

[a] Tr, triphenylmethyl; Tol , *p*-methylbenzoyl; PMB, *p*-methoxybenzyl.
[b] Irradiated for 0.5 h then stirred at room temperature.
[c] The isolated yield after chromatographic purification.

groups present on the substrates were unaffected and no protecting group migrated during the reaction.

3. Conclusion

This work described a novel and efficient method for chemoselectively removing TBDMS, isopropylidene, benzylidine, trityl groups using CBr_4 in MeOH. The method provides several advantages, including operational simplicity, mild reaction conditions, compatibility with other acid sensitive functional groups, low cost of the reagents and high yields of the deprotected products. This method should, therefore, have broad applications in carbohydrate chemistry, in which the selective deprotection of acid labile ethers is an important requirement. The authors believe that this elegant method of deprotection method will also have many applications in natural product synthesis.

4. Experimental

4.1. General

The ^{1}H NMR (proton nuclear magnetic resonance) spectra were recorded at 300 MHz (Bruker-AC300P) with deuteriochloroform ($CDCl_3$, Aldrich 99.8 atom% D) as the solvent and the internal standard. The ^{13}C NMR (carbon nuclear magnetic resonance) spectra were recorded at 75.5 MHz (Bruker-AC300P) with $CDCl_3$ as the solvent and the internal standard. Chemical shifts are reported in parts per million and resonance patterns are reported with the notations of either s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). Coupling constant (*J*) are reported in hertz (Hz). MeOH was distilled from magnesium and recirculated prior to use. Hexane and ethyl acetate were distilled from calcium hydride. Thin-layer-chromatography

(TLC) analysis was performed on a plastic plate (or glass plate) precoated with silica gel (Merck, 5554 Silica gel 60 F_{254}). Visualization was accomplished by UV light or developed by spraying with a 10% phosphomolybdic acid ethanol solution. Column chromatography was performed using silica gel (Merck, 230–400 mesh) and ethyl/hexane mixture as the eluent.

4.2. General deprotection procedure using CBr_4/MeOH photoirradiation conditions

A solution of protected saccharide (1.0 equiv), CBr_4 (0.05 equiv) and anhydrous MeOH (10 mL/1.0 equiv saccharide) in a pyrex round bottom was irradiated by a TLC-lamp (Uvltec Limited, 245 nm, 8 W) for 0.5 h, followed by stirring without irradiation at room temperature. After the reaction was complete (by TLC), the organic solvent was removed directly under reduced pressure. Further purification was achieved on a flash chromatograph with silica gel and ethyl acetate/hexane.

4.2.1. *p*-Methylphenylsulfinyl 2,3,4-tri-*O*-*tert*-butyldimethylsilyl-β-D-galactopyranoside (2a). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.09 (s, 3H), 0.10 (s, 3H), 0.11 (s, 3H), 0.14 (s, 3H), 0.15 (s, 3H), 0.16 (s, 3H), 0.91 (s, 9H), 0.93 (s, 9H), 0.98 (s, 9H), 2.02 (br s, 1H), 2.32 (s, 3H), 3.78–3.83 (m, 2H), 3.85–3.88 (m, 1H), 4.02–4.05 (m, 1H), 4.13 (dd, J=9.0, 2.8 Hz, 1H), 4.54 (d, J=2.8 Hz, 1H), 5.01 (d, J=9.0 Hz, 1H), 7.11 (d, J=7.9 Hz, 2H), 7.39 (d, J=7.9 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ−4.9 (2C), −4.6 (2C), −4.4 (2C), 17.9, 18.3, 18.4, 21.0, 25.8, 25.9, 26.0, 61.9, 68.1, 74.4, 74.9, 78.3, 87.2, 129.9, 130.4, 133.6, 136.8. HRMS (EI) Calcd for $C_{31}H_{61}O_5SSi_3$ $[M+H]^+$: 629.3548. Found: 629.3567.

4.2.2. *p*-Methylphenylsulfinyl 2,3-di-*O*-*tert*-butyldimethylsilyl-β-D-galactopyranoside (2b). ^{1}H NMR (300 MHz, $CDCl_3$) δ0.09 (s, 3H), 0.10 (s, 3H), 0.13 (s, 3H), 0.16 (s, 3H), 0.92 (s, 9H), 0.94 (s, 9H), 0.98 (s, 9H), 1.90 (br s, 2H), 2.31 (s, 3H), 3.46 (dd, J=8.8, 2.8 Hz, 1H, H-3), 3.50–3.54 (m, 1H, H-5), 3.57 (dd, J=11.0, 4.1 Hz, 1H, H-6), 3.77 (dd, J=9.0, 8.8 Hz, 1H, H-2), 3.88 (dd, J=11.0, 7.8 Hz, 1H, H-6), 3.97 (d, J=2.8 Hz, 1H, H-4), 4.47 (d, J=9.0 Hz, 1H, H-1), 7.08 (d, J=7.9 Hz, 2H), 7.38 (d, J=7.9 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.7, −4.2 (2C), −4.0, 18.3, 18.4, 21.0, 25.9, 26.0, 62.9, 70.4, 71.6, 76.5, 79.7, 88.9, 129.6, 130.5, 131.4, 137.0. HRMS (EI) Calcd for $C_{25}H_{47}O_5SSi_2$ $[M+H]^+$: 515.2683. Found: 515.2696.

4.2.3. *O*-Methyl 2,3,4-tri-*O*-*tert*-butyldimethylsilyl-α-D-galactopyranoside (2c). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.05 (s, 3H), 0.07 (s, 3H), 0.08 (s, 3H), 0.09 (s, 3H), 0.11 (s, 6H), 0.87 (s, 9H), 0.88 (s, 9H), 0.89 (s, 9H), 2.00 (br s, 1H), 3.39 (s, 3H), 3.58–3.62 (m, 2H), 3.76–3.82 (m, 3H), 4.12–4.16 (m, 1H), 4.65 (d, J=2.9 Hz, 1H, H-1). ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.7, −4.6, −4.5, −3.9, −3.7, −3.2, 17.9, 18.0, 18.4, 25.9, 26.0, 26.1, 55.0, 62.8, 72.1, 73.0, 73.6, 75.9, 98.0. HRMS (EI) Calcd for $C_{25}H_{57}O_6Si_3$ $[M+H]^+$: 537.3463. Found: 537.3478.

4.2.4. *O*-Allyl 2,3,4-tri-*O*-*tert*-butyldimethylsilyl-β-D-galactopyranoside (2d). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.05 (s, 3H), 0.06 (s, 3H), 0.07 (s, 3H), 0.10 (s, 3H), 0.12 (s, 3H), 0.13 (s, 3H), 0.90 (s, 9H), 0.91 (s, 9H), 0.94 (s, 9H), 1.94 (br s, OH, 1H), 3.62 (dd, J=6.3, 2.0 Hz, 1H), 3.86–3.91 (m, 2H), 3.94–3.99 (m, 4H), 4.22 (dd, J=13.2, 4.9 Hz, 1H), 4.85 (d, J=4.9 Hz, 1H), 5.15 (dd, J=10.4, 1.3 Hz, 1H), 5.31 (dd, J=14.0, 1.3 Hz, 1H), 5.87–6.00 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.9, −4.6, −4.3, −4.1, −4.0, −3.7, 18.3, 18.4, 18.6, 26.0, 26.1, 26.4, 62.3, 68.8, 70.6, 72.3, 73.8, 83.2, 90.8, 116.4, 134.4. HRMS (EI) Calcd for $C_{27}H_{59}O_6Si_3$ $[M+H]^+$: 563.3619. Found: 563.3633.

4.2.5. *p*-Methylphenylsulfinyl 2,3-di-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-β-D-galactopyranoside (2e). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.05 (s, 3H), 0.07 (s, 3H), 0.90 (s, 9H), 2.19 (br s, 1H, OH), 2.33 (s, 3H), 3.45 (dd, J=9.4, 2.5 Hz, 1H, H-3), 3.48 (dd, J=7.8, 4.3 Hz, 1H, H-5), 3.60 (dd, J=11.1, 4.3 Hz, 1H, H-6), 3.80 (t, J=9.4 Hz, 1H, H-2), 3.91 (dd, J=11.1, 7.8 Hz, 1H, H-6), 4.04 (d, J=2.5 Hz, 1H, H-4), 4.55 (d, J=9.4 Hz, 1H, H-1), 4.71 (s, 2H), 4.72 (d, J=10.3 Hz, 1H), 4.76 (d, J=10.3 Hz, 1H), 7.09 (d, J=8.0 Hz, 2H), 7.26–7.40 (m, 10H), 7.48(d, J=8.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.0, −3.9, 18.5, 21.1, 26.0, 62.9, 69.2, 73.4, 75.2, 76.6, 80.0, 83.6, 87.3, 127.5, 127.6, 127.8, 128.1, 128.2, 128.3, 129.6, 129.7, 132.3, 137.3, 138.0, 138.3. HRMS (EI) Calcd for $C_{33}H_{45}O_5SSi$ $[M+H]^+$: 581.2757. Found: 581.2774.

4.2.6. *p*-Methylphenylsulfinyl 2,3-di-*O*-*p*-methylbenzyl-4-*O*-*tert*-butyldimethylsilyl-β-D-galactopyranoside (2f). ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.20 (s, 3H), −0.07 (s, 3H), 0.83 (s, 9H), 2.32 (s, 3H), 2.33 (s, 3H), 2.34 (s, 3H), 3.62–3.65 (m, 1H), 3.82 (dd, J=7.8, 4.0 Hz, 1H), 3.92 (dd, J=10.7, 7.8 Hz, 1H), 4.33 (d, J=2.5 Hz, 1H, H-4), 4.83 (d, J=10.1 Hz, 1H, H-1), 5.16 (dd, J=10.0 Hz, 2.5, 1H, H-3), 5.66 (dd, J=10.1, 10.0 Hz, 1H), 7.09–7.18 (m, 6H), 7.41 (d, J=8.0 Hz, 2H), 7.80 (d, J=8.2 Hz, 2H), 7.87 (d, J=8.2 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.9, −4.6, 18.0, 21.2, 21.5, 21.6, 25.6, 62.6, 67.3, 68.6, 76.5, 79.9, 85.4, 126.6, 126.8, 127.0, 129.0, 129.1, 129.5, 129.7, 129.9, 134.4, 138.4, 143.7, 144.0, 165.2, 166.4. HRMS (EI) Calcd for $C_{35}H_{45}O_7SSi$ $[M+H]^+$: 637.2655. Found: 637.2676.

4.2.7. *O*-Methyl 2,3-di-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-β-D-galactopyranoside (2g). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.09 (s, 3H), 0.11 (s, 3H), 0.92 (s, 9H), 2.66 (br d, 1H, OH), 3.40 (s, 3H), 3.72–3.89 (m, 5H), 4.04–4.07 (m, 1H), 4.67–4.86 (m, 5H), 7.28–7.42 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.9, −3.9, 18.5, 26.0, 55.2, 62.9, 70.5, 71.7, 73.4, 73.8, 75.6, 78.0, 98.9, 127.5(2C), 127.8, 128.0, 128.2, 128.4, 138.4, 138.5. HRMS (EI) Calcd for $C_{27}H_{41}O_6Si$ $[M+H]^+$: 489.2672. Found: 489.2690.

4.2.8. *O*-*tert*-Butyldiphenylsilyl-2-azido-3-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-β-D-galactopyranoside (2h). ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.18 (s, 3H), −0.08 (s, 3H), 0.91 (s, 9H), 1.14 (s, 9H), 2.20 (br d, 1H, OH), 3.20 (dd, J=7.6, 6.1 Hz, 1H, H-5), 3.27 (dd, J=10.9, 6.1 Hz, 1H, H-6), 3.50 (dd, J=10.8, 7.6 Hz, 1H, H-2), 3.99 (dd, J=10.9, 7.6 Hz, 1H, H-2), 4.06 (d, J=2.7 Hz, 1H, H-4), 4.68 (d, J=7.6 Hz, 1H, H-1), 4.75 (dd, J=10.8, 2.7 Hz, 1H, H-3), 7.36–7.50 (m, 8H), 7.57–7.60 (m, 1H), 7.70–7.80 (m, 4H), 8.05–8.08 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.8, −5.7,

18.1, 19.2, 22.6, 25.7, 63.2, 63.8, 67.6, 73.0, 74.6, 97.2, 127.4, 128.2, 128.4, 129.4, 129.6, 129.7, 129.9, 132.7, 133.2, 133.3, 135.8, 135.9, 165.7. HRMS (EI) Calcd for $C_{35}H_{48}N_3O_6Si_2$ $[M+H]^+$: 662.3082. Found: 662.3100.

4.2.9. Phenylselenyl 2-azido-3,4-di-*O*-*tert*-butyldimethylsilyl-α-D-galactopyranoside (2i). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.08 (s, 3H), 0.17 (s, 3H), 0.19 (s, 3H), 0.22 (s, 3H), 0.90 (s, 9H), 0.98 (s, 9H), 1.56 (br s, 1H), 3.53 (dd, J=11.3, 4.2 Hz, 1H, H-6), 3.73 (dd, J=11.3, 7.9 Hz, 1H, H-6), 3.86 (dd, J=9.8, 2.1 Hz, 1H, H-3), 3.94 (d, J=2.1 Hz, 1H, H-4), 4.12–4.18 (m, 2H), 6.01 (d, J=4.8 Hz, 1H, H-1), 7.24–7.29 (m, 3H), 7.61–7.64 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.9, −4.5, −3.9, −3.6, 18.4, 18.5, 26.0, 26.3, 62.4, 62.7, 71.7, 73.4, 75.2, 84.8, 128.0, 129.1, 131.5, 135.2. HRMS (EI) Calcd for $C_{24}H_{44}N_3O_4SeSi_2$ $[M+H]^+$: 574.2036. Found: 574.2051.

4.2.10. *p*-Methylphenylsulfinyl 2,3,4-tri-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside (4a). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.06 (s, 3H), 0.08 (s, 3H), 0.10 (s, 3H), 0.11 (s, 3H), 0.13 (s, 6H), 0.88 (s, 9H), 0.89 (s, 9H), 0.91 (s, 9H), 2.04 (br s, 1H), 2.32 (s, 3H), 3.68–3.79 (m, 3H), 3.80–3.89 (m, 3H), 4.90 (d, J=6.7 Hz, 1H), 7.09 (d, J=8.1 Hz, 2H), 7.37 (d, J=8.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.9, −4.4, −4.3, −4.2, −4.1, −4.0, 17.9, 18.0, 18.1, 21.0, 25.8, 25.9, 26.0, 64.0, 71.2, 75.4, 77.2, 82.8, 87.5, 129.6, 131.0, 131.7, 136.8. HRMS (EI) Calcd for $C_{31}H_{61}O_5SSi_3$ $[M+H]^+$: 629.3547. Found: 629.3564.

4.2.11. *p*-Methylphenylsulfinyl 2,3-di-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside (4b). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.06 (s, 3H), 0.10 (s, 3H), 0.13 (s, 3H), 0.19 (s, 3H), 0.84 (s, 9H), 0.90 (s, 9H), 1.80 (br s, 2H), 2.28 (s, 3H), 3.38–3.46 (m, 4H), 3.59 (dd, J=11.8, 6.0 Hz, 1H), 3.87 (dd, J=11.8, 2.7 Hz, 1H), 4.51 (d, J=9.0 Hz, 1H), 7.06 (d, J=7.9 Hz, 2H), 7.31 (d, J=7.9 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.9, −4.0, −3.8, −3.6, 18.2, 18.4, 21.1, 25.9, 26.1, 62.5, 71.2, 74.5, 79.9, 80.1, 89.1, 129.7, 130.6, 131.7, 137.6. HRMS (EI) Calcd for $C_{25}H_{47}O_5SSi_2$ $[M+H]^+$: 515.2682. Found: 515.2701.

4.2.12. *O*-Methyl 2,3,4-tri-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside (4c). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.06 (s, 3H), 0.07 (s, 3H), 0.08 (s, 3H), 0.09 (s, 3H), 0.11 (s, 3H), 0.14 (s, 3H), 0.88 (s, 9H), 0.89 (s, 9H), 0.91 (s, 9H), 3.38 (s, 3H), 3.56–3.64 (m, 2H), 3.77–3.84 (m, 3H), 3.92 (m, 1H), 4.65 (d, J=3.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.7, −4.6, −4.5, −3.9, −3.7, −3.2, 18.0, 18.1, 18.4, 25.9, 26.0, 26.1, 55.0, 62.9, 72.1, 73.0, 73.5, 75.2, 98.0. HRMS (EI) Calcd for $C_{25}H_{57}O_6Si_3$ $[M+H]^+$: 537.3463. Found: 537.3478.

4.2.13. *p*-Methylphenylsulfinyl 2,3-di-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside (4d). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.01 (s, 3H), 0.10 (s, 3H), 0.89 (s, 9H), 1.96 (br s, 1H), 2.36 (s, 3H), 3.33–3.37 (m, 1H), 3.46–3.56 (m, 2H), 3.62 (dd, J=6.2, 3.2 Hz, 1H), 3.69 (dd, J=5.9, 5.9 Hz, 1H), 3.88 (dd, J=11.6, 2.7 Hz, 1H), 4.66 (d, J=10.1 Hz, 1H), 4.69–4.72 (m, 1H), 4.81 (d, J=11.6, 1H), 4.93 (d, J=10.1, 1H), 5.00 (d, J=11.6, 1H), 7.14 (d, J=8.2 Hz, 2H), 7.26–7.34 (m, 10H), 7.54 (d, J=8.9 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.8, −3.9, 17.9, 21.0, 25.8, 62.3, 68.1, 70.6, 75.2, 80.7, 81.5, 86.6, 87.8, 126.7, 127.1, 127.8, 128.1, 128.3, 128.8, 129.2, 129.8, 130.8, 132.3, 137.8, 138.7. HRMS (EI) Calcd for $C_{33}H_{45}O_5SSi$ $[M+H]^+$: 581.2757. Found: 581.2776.

4.2.14. *p*-Methylphenylsulfinyl 2,3-di-*O*-*p*-methylbenzyl-4-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside (4e). ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.20 (s, 3H), 0.05 (s, 3H), 0.75 (s, 9H), 2.33 (s, 3H), 2.34 (s, 6H), 3.52–3.58 (m, 1H), 3.73–3.79 (m, 1H), 3.93–3.99 (m, 2H), 4.90 (d, J=9.8, 1H), 5.26 (dd, J=9.7, 9.7 Hz, 1H), 5.58 (dd, J=9.8, 9.7 Hz, 1H), 7.09–7.13 (m, 6H), 7.32–7.35 (m, 2H), 7.76–7.81 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.8, −4.3, 17.8, 21.1, 21.6, 22.6, 25.5, 61.7, 68.8, 70.9, 76.7, 80.9, 86.3, 126.6, 127.0, 128.4, 129.0(2C), 129.7(2C), 129.8, 133.2, 138.4, 143.6, 143.8, 165.3, 165.8. HRMS (EI) Calcd for $C_{35}H_{45}O_7SSi$ $[M+H]^+$: 637.2655. Found: 637.2676.

4.2.15. *p*-Methylphenylsulfinyl 3,4-tri-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-(2,2,2-trichloro ethoxycarbonylamino)-β-D-glucopyranoside (4f). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.07 (s, 3H), 0.10 (s, 3H), 0.12 (s, 3H), 0.87 (s, 9H), 0.89 (s, 9H), 1.99 (br s, 1H), 2.34 (s, 3H), 3.35–3.48 (m, 2H), 3.51 (dd, J=9.2, 9.2 Hz, 1H), 3.63–3.67 (m, 1H), 3.67–3.71 (m, 1H), 4.74–4.78 (m, 3H), 4.85 (dd, J=9.2, 2.6 Hz, 1H), 5.23 (br d, J=12.5 Hz, NH, 1H), 7.11 (d, J=8.1 Hz, 2H), 7.36 (d, J=8.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.9, −3.9, −2.7, −1.8, 18.2, 21.0, 21.1, 22.6, 25.8, 53.4, 60.4, 62.2, 71.8, 74.8, 76.8, 80.6, 95.3, 129.9, 130.6, 133.0, 137.3, 156.0. HRMS (EI) Calcd for $C_{28}H_{49}Cl_3NO_6SSi_2$ $[M+H]^+$: 688.1885. Found: 688.1902.

4.2.16. *O*-Allyl 2,3,4-tri-*O*-*tert*-butyldimethylsilyl-β-D-mannoside (6). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.04 (s, 3H), 0.07 (s, 3H), 0.09 (s, 3H), 0.10 (s, 3H), 0.13 (s, 3H), 0.14 (s, 3H), 0.88 (s, 9H), 0.90 (s, 9H), 0.94 (s, 9H), 1.82 (br s, 1H, OH), 3.61–3.80 (m, 5H), 3.89 (dd, J=4.2, 1.8 Hz, 1H), 3.99–4.02 (m, 1H), 4.20 (dd, J=12.8, 5.0 Hz, 1H), 4.66 (d, J=5.0 Hz, 1H), 5.15 (dd, J=10.4, 1.5 Hz, 1H), 5.27 (dd, J=15.3, 1.5 Hz, 1H), 5.90 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.3, −4.8, −4.5, −4.2, −3.8, −3.7, 17.9, 18.1, 18.2, 25.7, 25.8, 26.0, 62.7, 68.6, 72.0, 73.0, 75.3, 83.2, 100.0, 116.4, 134.5. HRMS (EI) Calcd for $C_{27}H_{59}O_6Si_3$ $[M+H]^+$: 563.3619. Found: 563.3632.

4.2.17. 3-*O*-*tert*-Butyldimethylsilyl-2-deoxy-β-D-thymidine (8a). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.05 (s, 3H), 0.06 (s, 3H), 0.86 (s, 9H), 2.01 (s, 3H), 2.19–2.30 (m, 2H), 3.02 (br s, 1H, OH), 3.72 (dd, J=12.6, 3.6 Hz, 1H), 3.86–3.91 (m, 2H), 4.46 (dd, J=6.5, 2.6 Hz, 1H), 6.15 (dd, J=6.7, 6.5 Hz, 1H), 7.42 (d, J=1.0 Hz, 1H), 9.62 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.9, −4.8, 12.4, 17.9, 25.7, 40.5, 61.8, 71.5, 86.4, 87.6, 110.8, 137.0, 150.5, 164.2. HRMS (EI) Calcd for $C_{16}H_{29}N_2O_5Si$ $[M+H]^+$: 357.1845. Found: 357.1868.

4.2.18. 2,3-di-*O*-*tert*-Butyldimethylsilyl-β-D-uridine (8b). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.04 (s, 3H), 0.06 (s, 3H), 0.08 (s, 3H), 0.09 (s, 3H), 0.87 (s, 9H), 0.90 (s, 9H), 2.93 (br s, 1H, OH), 3.72 (d, J=11.0 Hz, 1H, H-5′), 3.94 (d, J=11.0 Hz, 1H, H-5′), 4.08 (br s, 1H, H-4′), 4.16 (dd, J=4.1, 4.0 Hz, 1H, H-3′), 4.50 (dd, J=4.9, 4.0 Hz, 1H, H-2′), 5.50

(d, J=4.9 Hz, 1H, H-1), 5.72 (d, J=7.6 Hz, 1H), 7.69 (d, J=7.6 Hz, 1H), 9.35 (br s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ −4.8 (2C), −4.7, −4.4, 17.9, 18.0, 25.7, 25.8, 61.4, 71.5, 73.9, 85.8, 93.4, 102.0, 142.9, 150.4, 163.7. HRMS (EI) Calcd for $C_{21}H_{41}N_2O_6Si_2$ $[M+H]^+$: 473.2503. Found: 473.2520.

4.2.19. 2,3-di-*O*-*tert*-Butyldimethylsilyl-β-D-adenosine (8c). ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.58 (s, 3H), −1.13 (s, 3H), 0.11 (s, 3H), 0.12 (s, 3H), 0.75 (s, 9H), 0.94 (s, 9H), 3.71 (dd, J=12.4, 1.2 Hz, 1H, H-5′), 3.95 (dd, J=12.4, 1.5 Hz, 1H, H-5′), 4.16 (m, 1H, H-4′), 4.33 (d, J=4.5 Hz, 1H, H-3′), 4.97 (dd, J=7.5 Hz, 4.5, 1H, H-2′), 5.80 (d, J=7.5 Hz, 1H, H-1′), 6.48 (br s, 2H, NH_2), 7.94 (s, 1H), 8.33 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.4, −5.1, −4.7, −4.4, 17.8, 18.5, 25.8, 26.0, 62.3, 72.0, 75.7, 85.4, 88.3, 120.1, 139.6, 150.0, 152.8, 155.5. HRMS (EI) Calcd for $C_{22}H_{42}N_5O_4Si_2$ $[M+H]^+$: 496.2775. Found: 496.2768.

4.2.20. 2,3-di-*O*-*tert*-Butyldimethylsilyl-β-D-guanosine (8d). ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.28 (s, 3H), 0.00 (s, 3H), 0.11 (s, 3H), 0.13 (s, 3H), 0.84 (s, 9H), 0.93 (s, 9H), 3.73 (dd, J=12.6, 1.9 Hz, 1H, H-5′), 4.11 (dd, J=12.6, 1.0 Hz, 1H, H-5′), 4.20 (m, 1H, H-4′), 4.28 (dd, J=4.7, 2.8 Hz, 1H, H-3′), 4.57 (dd, J=5.9, 4.7 Hz, 1H, H-2′), 6.00 (d, J=5.9 Hz, 1H, H-1′), 6.35 (br s, 2H, NH_2), 6.75 (br s, 1H, NH_2), 7.97 (s, 1H), 8.33 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.5, −5.0, −4.5, −4.3, 17.9, 18.3, 25.6, 26.1, 61.9, 71.9, 74.5, 81.0, 83.2, 120.1, 138.0, 149.0, 151.0, 155.2. HRMS (EI) Calcd for $C_{22}H_{42}N_5O_5Si_2$ $[M+H]^+$: 512.2724. Found: 512.2738.

4.2.21. Benzyl (*p*-tolyl 5-azido-4,7,8-tri-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-2-thio-D-glycero-α-galacto-non-2-ulpyranosid)onate (10). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.00 (s, 6H), 0.01 (s, 3H), 0.07 (s, 3H), 0.13 (s, 3H), 0.20 (s, 3H), 0.87 (s, 9H), 0.88 (m, 1H, H-3), 0.88 (s, 9H), 0.98 (s, 9H), 1.73 (dd, J=11.6, 9.2 Hz, 1H, H-3), 2.34 (s, 3H), 2.69 (dd, J=7.6, 3.5 Hz, 1H, H-5), 3.09 (dd, J=9.5, 1.8 Hz, 1H), 3.30 (dd, J=7.2, 1.8 Hz, 1H), 3.51–3.59 (m, 3H), 3.83 (m, 1H), 4.03 (br s, 1H, OH), 5.03 (d, J=2.2 Hz, 2H), 7.10 (d, J=7.8 Hz, 2H), 7.23–7.27 (m, 3H), 7.34–7.38 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.8, −5.1, −5.0, −4.8, −4.7, −4.1, −3.4, −3.0, 14.4, 17.8, 18.1, 18.4, 25.6, 25.7, 26.1, 26.3, 31.6, 41.0, 62.8, 65.8, 67.6, 71.7, 77.8, 87.5, 125.8, 128.5, 128.6, 129.6, 134.9, 136.3, 139.6, 168.3. HRMS (EI) Calcd for $C_{41}H_{70}N_3O_7SSi_3$ $[M+H]^+$: 832.4242. Found: 832.4267.

4.2.22. Methyl (*O*-allyl 5-acetamido-4,7,8,9-tetra-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-D-glycero-α-galacto-non-2-ulpyranosid)onate (11a). To a solution of sialic acid (280 mg, 0.77 mmol) in CH_2Cl_2 (5 mL) and lutidine (720 μL, 6.16 mmol) was added TBDMSOTf (1 mL, 4.62 mmol), and the mixture was stirred at room temperature for overnight. The solution was evaporated in vacuo, and the residue was purified by column chromatography (EA/Hexane=1/4) to yield product (505 mg, 80%). R_f 0.5 (EA/Hexane=1/4). ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.08 (s, 3H), −0.03 (s, 3H), 0.01 (s, 6H), 0.03 (s, 3H), 0.07 (s, 3H), 0.13 (s, 3H), 0.21 (s, 3H), 0.88 (s, 9H), 0.90 (s, 9H), 0.91 (s, 9H), 0.93 (s, 9H), 1.72 (t, J=12.4 Hz, 1H, H-3*ax*), 1.91 (s, 3H, Ac), 2.59 (dd, J_{3eq-4}=4.8 Hz, $J_{3eq-3ax}$=12.4 Hz, 1H, H-3*eq*), 3.28–3.35 (m, 1H, H-5), 3.54 (dd, J_{9a-8}=7.6 Hz, J_{9a-9b}=10.4 Hz, 1H, H-9a), 3.80 (s, 3H, Me), 3.90–3.93 (m, H-7, 2H, H-9b), 4.02 (dddd, J=1.6, 2.8, 6.0, 12.4 Hz, 1H, All), 4.02–4.06 (m, 2H, H-4, H-8), 4.09 (dd, 1H, J_{6-7}=2.0 Hz, J_{6-5}=10.8 Hz, H-6), 4.27 (dddd, 1H, All, J=1.6, 2.8, 5.2, 12.4 Hz), 5.14 (dddd, 1H, J=1.6, 2.8, 4.8, 10.4 Hz, All), 5.25 (dddd, 1H, J=1.6, 2.8, 4.8, 17.2 Hz, All), 5.45 (d, 1H, J=7.6 Hz, NH), 5.86 (dddd, 1H, J=5.2, 6.0, 10.4, 17.2 Hz, All). ^{13}C NMR (100 MHz, CD_3OD): δ −5.4, −5.3, −5.1, −5.0, −4.6, −4.6, −4.6, −3.9, 17.8, 18.2, 23.8, 25.6, 25.6, 25.6, 26.0, 26.0, 26.0, 26.0, 26.0, 26.0, 26.1, 26.1, 26.1, 41.2, 52.3, 55.5, 64.8, 65.0, 67.7, 74.2, 76.1, 76.8, 98.6, 112.3, 134.0, 168.9, 169.6. HRMS (FAB) calcd for $C_{39}H_{82}NO_9Si_4$ $[M+H]^+$: 820.5066 Found: 820.5081.

4.2.23. Methyl (*p*-tolyl 5-acetamido-4,7,8,9-tetra-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-2-thio-D-glycero-α-galacto-non-2-ulpyranosid)onate (11b). ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.10 (s, 3H), −0.04 (s, 3H), 0.00 (s, 6H), 0.05 (s, 3H), 0.08 (s, 3H), 0.14 (s, 3H), 0.21 (s, 3H), 0.84 (s, 9H), 0.91 (s, 9H), 0.93 (s, 9H), 0.96 (s, 9H), 1.62 (dd, J_{3ax-4}=11.6 Hz, $J_{3ax-3eq}$=12.8 Hz, 1H, H-3*ax*), 1.88 (s, 3H, Ac), 2.36 (s, 3H, CH_3), 2.60 (dd, J_{3eq-4}=4.8 Hz, $J_{3eq-3ax}$=12.8 Hz, 1H, H-3*eq*), 3.21–3.24 (m, 1H, H-5), 3.57 (dd, J_{9a-8}=7.2 Hz, J_{9a-9b}=10.4 Hz, 1H, H-9a), 3.62 (s, 3H, Me), 3.90 (dd, J_{7-8}=1.6 Hz, J_{7-6}=3.2 Hz, 1H, H-7), 3.94 (ddd, J_{8-7}=1.6 Hz, J_{8-9b}=4.0 Hz, J_{8-9a}=7.2 Hz, 1H, H-8), 4.00–4.04 (m, 1H, H-4), 4.01 (dd, J_{6-7}=3.2 Hz, J_{6-5}=10.4 Hz, 1H, 1H, H-6), 4.16 (dd, J_{9b-8}=4.0 Hz, J_{9b-9a}=10.4 Hz, 1H, H-9b), 5.52 (d, J=7.6 Hz, 1H, NH), 7.11 (d, J=8.0 Hz, 2H, Ar), 7.42 (d, J=8.0 Hz, 2H, Ar). ^{13}C NMR (100 MHz, CD_3OD) δ −5.2, −5.2, −5.0, −4.8, −4.6, −4.5, −4.5, −4.1, 17.8, 18.2, 18.6, 18.6, 21.3, 23.9, 25.6, 25.6, 25.6, 26.1, 26.1, 26.1, 26.1, 26.1, 26.1, 26.2, 26.2, 26.2, 41.9, 52.3, 55.4, 65.0, 68.4, 76.2, 76.4, 76.6, 126.2, 129.4, 129.4, 136.4, 136.4, 139.6, 169.2, 169.5. HRMS (FAB) calcd for $C_{43}H_{84}NO_8SSi_4$ $[M+H]^+$: 886.4995 Found: 886.5009.

4.2.24. Methyl (*O*-allyl 5-acetamido-4,7,8-tri-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-D-glycero-α-galacto-non-2-ulpyranosid)onate (12a). A solution of saccharide (100 mg, 0.12 mmol), CBr_4 (6 mg, 0.018 mmol) and anhydrous MeOH (1.2 mL) in a pyrex round bottom was irradiated by a TLC-lamp (Uvltec Limited, 245 nm, 8 W) for 0.5 h, followed by stirring without irradiation at room temperature. After the reaction was completed (by TLC about 2 h), the organic solvent was removed directly under reduced pressure. Further purification was achieved on a flash chromatography with silica gel and ethyl acetate/hexane(1/2) to get product (57.9 mg, 67%). R_f 0.4 (EA/Hexane=1/4). ^{1}H NMR (400 MHz, $CDCl_3$) δ−0.08 (s, 3H), 0.01 (s, 6H), 0.05 (s, 3H), 0.10 (s, 3H), 0.20 (s, 3H), 0.87 (s, 9H), 0.93 (s, 9H), 0.94 (s, 9H), 1.75 (dd, J_{3ax-4} 11.6 Hz, $J_{3ax-3eq}$=12.8 Hz, 1H, H-3*ax*), 1.93 (s, 3H, Ac), 2.60 (dd, J_{3eq-4}=4.4 Hz, $J_{3eq-3ax}$=12.8 Hz, 1H, H-3*eq*), 3.35–3.42 (m, 1H, H-5), 3.60 (dd, J_{9a-8}=4.8 Hz, J_{9a-9b}= 11.2 Hz, 1H, H-9a), 3.83 (s, 3H, Me), 3.84–3.87 (m, 1H, OH), 3.93 (dd, J_{9b-8}=5.6 Hz, J_{9b-9a}=11.2 Hz, 1H, H-9b), 3.97 (m, 3H, H-4, H-8, H-7), 4.01 (dddd, J=1.2, 2.8, 6.0, 12.8 Hz, 1H, All), 4.14 (dd, J_{6-7}=0.8 Hz, J_{6-5}=10.4 Hz, 1H, H-6), 4.28 (dddd, J=1.2, 3.2, 5.6, 12.8 Hz, 1H, All),

5.15 (dddd, J=1.2, 2.8, 4.8, 10.4 Hz, 1H, All), 5.21 (d, J=7.6 Hz, 1H, NH), 5.27 (dddd, J=1.2, 3.2, 4.8, 17.2 Hz, 1H, All), 5.82 (dddd, J=5.6, 6.0, 10.4, 17.2 Hz, 1H, All). ^{13}C NMR (100 MHz, CD_3OD) δ −5.2, −5.0, −4.8, −4.5, −4.5, −3.9, 17.8, 18.3, 18.5, 23.7, 25.6, 25.6, 25.6, 26.0, 26.0, 26.0, 26.0, 26.0, 26.0, 41.1, 52.6, 55.6, 63.3, 65.1, 67.3, 74.2, 74.7, 77.2, 98.8, 117.2, 133.8, 168.9, 169.7. HRMS (FAB) calcd for $C_{33}H_{68}NO_9Si_3$ $[M+H]^+$: 706.4202 Found: 706.4214.

4.2.25. Methyl (*p*-tolyl 5-acetamido-4,7,8-tri-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-2-thio-D-glycero-α-galacto-non-2-ulpyranosid)onate (12b). ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.06 (s, 3H), 0.01 (s, 3H), 0.03 (s, 3H), 0.07 (s, 3H), 0.10 (s, 3H), 0.20 (s, 3H), 0.86 (s, 9H), 0.91 (s, 9H), 0.98 (s, 9H), 1.70 (dd, J_{3ax-4}=11.6 Hz, $J_{3ax-3eq}$=12.8 Hz, 1H, H-3*ax*), 1.90 (s, 3H, Ac), 2.36 (s, 3H, CH_3), 2.72 (dd, J_{3eq-4}=4.8 Hz, $J_{3eq-3ax}$=12.8 Hz, 1H, H-3*eq*), 3.27–3.34 (m, 1H, H-5), 3.50 (dd, J_{9a-8}=4.8 Hz, J_{9a-9b}=11.6 Hz, 1H, H-9a), 3.64 (s, 3H, Me), 3.71–3.74 (m, 1H, H-8), 3.91 (dd, J_{9b-8}=6.0 Hz, J_{9b-9a}=11.6 Hz, 1H, H-9b), 3.95–4.03 (m, 3H, H-4, H-6, H-7), 5.19 (d, J=7.6 Hz, 1H, NH), 7.14 (d, J=8.0 Hz, 2H, Ar), 7.43 (d, J=8.0 Hz, 2H, Ar). ^{13}C NMR (100 MHz, CD_3OD) δ −5.2, −5.0, −4.9, −4.6, −4.5, −3.9, 17.8, 18.3, 18.6, 23.7, 25.6, 25.6, 25.6, 26.0, 26.0, 26.0, 26.1, 26.1, 26.1, 41.7, 52.5, 55.7, 63.3, 67.8, 74.5, 76.1, 77.7, 87.4, 125.3, 129.5, 129.5, 136.8, 136.8, 140.2, 169.0, 169.7. HRMS (FAB) calcd for $C_{37}H_{70}NO_8S_1Si_3$ $[M+H]^+$: 772.4130 Found: 772.4127.

4.2.26. Methyl (*O*-allyl 5-acetamido-9-*O*-acetyl-4,7,8-tri-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-D-glycero-α-galacto-non-2-ulpyranosid)onate (13a). To a solution of sialic acid (50 mg, 0.07 mmol) in pyridine (2 mL) was added Ac_2O (0.5 mL) at 0 °C, and the mixture was stirred at room temperature for overnight. The solution was evaporated in vacuo, and the residue was purified by column chromatography (EA/Hexane=1/4) to yield product (50 mg, 94%). R_f 0.3 (EA/Hexane=1/4). ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.10 (s, 3H), 0.00 (s, 3H), 0.02 (s, 3H), 0.07 (s, 3H), 0.14 (s, 3H), 0.20 (s, 3H), 0.87 (s, 9H), 0.91 (s, 9H), 0.94 (s, 9H), 1.70 (dd, J_{3ax-4}=11.6 Hz, $J_{3ax-3eq}$=12.8 Hz, 1H, H-3*ax*), 1.92 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.63 (dd, J_{3eq-4}=4.8 Hz, $J_{3eq-3ax}$=12.8 Hz, 1H, H-3*eq*), 3.21–3.28 (m, 1H, H-5), 3.81 (s, 3H, Me), 3.94–3.95 (m, 1H, H-7), 3.97 (dddd, J=1.2, 2.8, 5.6, 12.4 Hz, 1H, All), 4.03 (dd, J_{9a-8}=4.4 Hz, J_{9a-8}=10.4 Hz, 1H, H-9a), 4.05–4.14 (m, 2H, H-4, H-8), 4.19 (dd, J_{6-7}=1.2 Hz, J_{6-5}=10.4 Hz, 1H, H-6), 4.27 (dddd, J=1.6, 3.2, 5.2, 12.4 Hz, 1H, All), 4.55 (d, J=10.4 Hz, 1H, H-9b), 5.14 (dddd, J=1.2, 2.8, 4.8, 10.4 Hz, 1H, All), 5.23–5.28 (m, 1H, All), 5.26 (dddd, J=1.6, 3.2, 4.8, 17.2 Hz, 1H, All), 5.85 (dddd, J=5.2, 5.6, 10.4, 17.2 Hz, 1H, All). ^{13}C NMR (100 MHz, CD_3OD) δ −5.3, −5.0, −4.9, −4.6, −4.5, −3.8, 17.8, 18.1, 18.4, 21.0, 23.7, 25.6, 25.6, 25.57, 25.8, 25.8, 25.8, 26.0, 26.0, 26.0, 41.6, 52.4, 55.9, 65.2, 66.9, 67.1, 73.8, 74.8, 75.2, 98.6, 117.0, 134.0, 168.8, 169.8, 170.8. HRMS (FAB) calcd for $C_{35}H_{70}NO_{10}Si_3$ $[M+H]^+$: 748.4307 Found: 748.4297.

4.2.27. Methyl (*p*-tolyl 5-acetamido-9-acetyl-4,7,8-tri-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-2-thio-D-glycero-α-galacto-non-2-ulpyranosid)onate (13b). ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.09 (s, 3H), −0.01 (s, 3H), 0.02 (s, 3H), 0.05 (s, 3H), 0.12 (s, 3H), 0.19 (s, 3H), 0.87 (s, 9H), 0.89 (s, 9H), 0.98 (s, 9H), 1.71 (dd, J_{3ax-4}=11.6 Hz, $J_{3ax-3eq}$=12.8 Hz, 1H, H-3*ax*), 1.90 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.34 (s, 3H, CH_3), 2.75 (dd, J_{3eq-4}=4.4 Hz, $J_{3eq-3ax}$=12.8 Hz, 1H, H-3*eq*), 3.15–3.22 (m, 1H, H-5), 3.59 (s, 3H, Me), 3.91–3.93 (m, 2H, H-7, H-9a), 4.01–4.07 (m, 2H, H-4, H-6), 4.24–4.33 (m, 1H, H-8), 4.31 (dd, J_{9b-8}=7.6 Hz, J_{9b-9a}=12.0 Hz, 1H, H-9b), 5.25 (d, J=7.6 Hz, 1H, NH), 7.09 (d, J=8.0 Hz, 2H, Ar), 7.39 (d, J=8.0 Hz, 2H, Ar). ^{13}C NMR (100 MHz, CD_3OD) δ −5.6, −5.2, −5.0, −4.5, −3.8, −3.6, 17.8, 18.0, 18.5, 21.1, 21.3, 23.7, 25.6, 25.6, 25.6, 25.8, 25.8, 25.8, 26.0, 26.0, 26.0, 42.0, 52.2, 56.0, 66.6, 67.8, 74.7, 75.0, 76.0, 87.4, 125.7, 129.4, 129.4, 136.7, 136.7, 139.8, 169.1, 169.7, 170.6. HRMS (FAB) calcd for $C_{39}H_{72}NO_9S_1Si_3$ $[M+H]^+$: 814.4235 Found: 814.4241.

4.2.28. Methyl (*O*-allyl 5-acetamido-7,8-di-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-9-sulfate-D-glycero-α-galacto-non-2-ulpyranosid)onate (14a). To a solution of sialic acid (50.0 mg, 0.07 mmol) in dry DMF (5 mL) was added $(Et_3)_3\cdot SO_3$ (192.5 mg, 1.06 mmol) and the mixture was stirred overnight at 50 °C under Ar. Then, TLC showed the disappearance of starting material and the formation of a new spot. After the addition of MeOH (5 mL), stirring was continued for 15 min. The mixture was concentrated and a solution of residue in MeOH (10 mL) was stirred with Dowex 50 (Na^+) for 1 h, the solution was filtrated and concentrated under reduced pressure. Further purification was achieved on a flash chromatography with silica gel and MeOH/CH_2Cl_2 (1/4) to get product (44.6 mg, 75%). R_f 0.3 (MeOH/CH_2Cl_2=1/4). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00 (s, 3H), 0.05 (s, 3H), 0.12 (s, 3H), 0.19 (s, 3H), 0.97 (s, 9H), 0.98 (s, 9H), 1.69 (t, 1H, J=12.4 Hz, H-3*ax*), 1.99 (s, 3H, Ac), 2.63 (dd, J_{3eq-4}=4.4 Hz, $J_{3eq-3ax}$=12.4 Hz, 1H, H-3*eq*), 3.51–3.56 (m, 1H, H-4), 3.76–3.81 (m, 2H, H-5, H-7), 3.83 (s, 3H, Me), 4.01–4.09 (m, 4H, H-6, H-8, H-9a, All), 4.37 (dddd, J=1.2, 3.2, 5.2, 13.2 Hz, 1H, All), 4.65 (d, J=10.4 Hz, 1H, H-9b), 5.09 (m, 1H, All), 5.26–5.31 (m, 1H, All), 5.85–5.91 (m, 1H, All). ^{13}C NMR (100 MHz, CD_3OD) δ −4.7, −4.2, −3.9, −3.2, 19.3, 19.5, 23.4, 26.8, 26.9, 41.7, 53.1, 54.5, 66.7, 70.0, 71.7, 76.3, 77.0, 77.8, 100.3, 116.8, 135.7, 170.6, 173.8. HRMS (FAB) calcd for $C_{27}H_{54}NO_{12}SSi_2$ $[M+H]^+$: 672.2905 Found: 672.2890.

4.2.29. Methyl (*p*-tolyl 5-acetamido-4,7,8-tri-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-9-sulfate-2-thio-D-glycero-α-galacto-non-2-ulpyranosid)onate (14b). ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.04 (s, 3H), 0.00 (s, 3H), 0.03 (s, 3H), 0.10 (s, 3H), 0.12 (s, 3H), 0.20 (s, 3H), 0.85 (s, 9H), 0.93 (s, 9H), 1.00 (s, 9H), 1.60 (dd, J_{3ax-4}=11.2 Hz, $J_{3ax-3eq}$=12.4 Hz, 1H, H-3*ax*), 1.90 (s, 3H, Ac), 2.35 (s, 3H, CH_3), 2.60 (dd, J_{3eq-4}=3.2 Hz, $J_{3eq-3ax}$=12.4 Hz, 1H, H-3*eq*), 3.50–3.53 (m, 1H, H-5), 3.53 (s, 3H, Me), 3.60–3.64 (m, 2H, H-4, H-9a), 3.91–3.96 (m, 2H, H-8, H-6), 4.10 (dd, J_{9b-8}=9.2 Hz, J_{9b-9a}=10.0 Hz, 1H, H-9b), 4.54–4.55 (d, 1H, H-7), 7.20 (d, J=8.0 Hz, 2H, Ar), 7.45 (d, J=8.0 Hz, 2H, Ar). ^{13}C NMR (100 MHz, CD_3OD): −4.5, −4.3, −4.2, −4.1, −3.8, −3.2, 18.8, 19.4, 19.6, 21.5, 23.7, 23.6, 26.3, 26.9, 27.1, 43.0, 53.2, 71.4, 77.3, 78.1, 89.2, 127.4, 130.9, 137.8, 141.5, 170.9, 173.0, 173.1. HRMS (FAB)

calcd for $C_{37}H_{68}NO_{11}S_2Si_3Na_2$ $[M+Na]^+$: 896.3337 Found: 896.3350.

4.2.30. Methyl (*O*-allyl 5-acetamido-4-acetyl-7,8-di-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-9-sulfate-D-glycero-α-galacto-non-2-ulpyranosid)onate (15a). ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.03 (s, 3H), 0.01 (s, 3H), 0.10 (s, 3H), 0.18 (s, 3H), 0.93 (s, 9H), 0.95 (s, 9H), 1.77 (t, J=12.0 Hz, 1H, H-3*ax*), 1.88 (s, 3H, Ac), 2.01 (s, 3H, Ac), 2.60 (dd, J_{3eq-4}=4.4 Hz, $J_{3eq-3ax}$=12.0 Hz, 1H, H-3*eq*), 3.86–4.07 (m, 5H, H-6, H-7, H-8, H-9a, All), 4.36 (dddd, J=1.6, 2.4, 5.2, 11.2 Hz, 1H, All), 4.62 (d, J=9.2 Hz, 1H, H-9b), 4.90 (ddd, J_{4-3eq}=4.4 Hz, J_{4-5}=7.2 Hz, J_{4-3ax}=12.0 Hz, 1H, H-4), 5.06–5.09 (m, 1H, All), 5.23–5.28 (m, 1H, All), 5.85 (dddd, J=5.2, 6.0, 10.8, 17.2 Hz, 1H, All). ^{13}C NMR (100 MHz, CD_3OD) δ −5.0, −4.2, −3.9, −2.9, 19.3, 19.5, 21.0, 23.1, 26.8, 26.8, 26.8, 26.9, 26.9, 26.9, 38.5, 51.8, 53.3, 66.8, 71.5, 71.5, 76.2, 76.9, 77.6, 100.1, 116.8, 135.8, 170.4, 172.1, 173.5. HRMS (FAB) calcd for $C_{29}H_{56}NO_{13}SSi_2$ $[M+H]^+$: 714.3011 Found: 714.3031.

4.2.31. Methyl [*p*-tolyl 5-(*N*-acetyl-*N*-benzoyl)amido-4,7,8-tri-*O*-benzoyl-9-*O*-tert-butyldimethylsilyl-3,5-dideoxy-2-thio-D-glycero-α-galacto-non-2-ulpyranosid]-onate (16). ^{1}H NMR (400 MHz, CD_3OD) δ 0.72 (s, 9H), 1.93 (t, J_{3ax-4}=$J_{3ax-3eq}$=12.0 Hz, 1H, H-3*ax*), 2.39 (s, 3H, Ac), 3.15 (dd, J_{3eq-4}=4.8 Hz, $J_{3eq-3ax}$=12.0 Hz, 1H, H-3*eq*), 3.29 (s, 3H, CH_3), 3.87 (dd, J_{9a-8}=3.2 Hz, J_{9a-9b}=11.6 Hz, 1H, H-9a), 4.15 (dd, J_{9b-8}=7.6 Hz, J_{9b-9a}=11.6 Hz, 1H, H-9b), 4.87–4.98 (m, 2H, H-5, H-6), 5.50–5.52 (m, 1H, H-8), 5.82–5.87 (m, 2H, H–4, H-7), 7.14–7.22 (m, 3H, Ar), 7.31–7.76 (m, 15H, Ar), 7.81–7.92 (m, 3H, Ar), 8.13–8.20 (m, 3H, Ar). ^{13}C NMR (100 MHz, CD_3OD) δ −5.7, 18.1, 21.4, 25.6, 28.0, 39.0, 52.6, 55.8, 60.8, 68.0, 69.4, 72.4, 72.6, 87.4, 125.6, 126.0, 128.2, 128.4, 128.9, 129.1, 129.6, 129.7, 129.8, 123.0, 130.2, 132.8, 133.1, 133.6. HRMS (FAB) calcd for $C_{53}H_{58}NO_{12}SSi$ $[M+H]^+$: 960.3449 Found: 960.3457.

4.2.32. Methyl [*p*-tolyl 5-(*N*-acetyl-*N*-benzoyl)amido-4,7,8-tri-*O*-benzoyl-3,5-dideoxy-2-thio-D-glycero-α-galacto-non-2-ulpyranosid]onate (17). ^{1}H NMR (400 MHz, CD_3OD) δ 2.01 (dd, 1H, J_{3ax-4}=11.2 Hz, $J_{3ax-3eq}$=12.4 Hz, H-3*ax*), 2.41 (s, 3H, Ac), 3.21 (s, 3H, Me), 3.21–3.30 (m, 1H, H-3*eq*), 3.74–3.80 (m, 1H, H-9a), 4.10–4.23 (m, 1H, H-9b), 5.04–5.05 (m, 2H, H-5, H-6), 5.43–5.45 (m, 1H, H-8), 5.68–5.72 (2H, H-4, H-7), 7.16–7.21 (m, 4H), 7.36–7.48 (m, 5H), 7.51–7.78 (m, 10H), 7.91 (d, J=7.6 Hz, 1H, Ar), 8.02–8.04 (m, 2H, Ar), 8.14–8.21 (m, 2H, Ar). ^{13}C NMR (100 MHz, CD_3OD) δ 21.4, 27.9, 39.2, 52.7, 55.8, 60.6, 68.2, 68.5, 72.9, 73.0, 87.5, 125.4, 126.0, 128.3, 128.5, 128.7, 128.9, 129.0, 129.3, 129.7, 129.9, 130.1, 132.7, 133.0, 133.3, 133.8, 136.1, 136.7, 140.2, 164.9, 165.5, 167.1, 168.3, 173.3, 174.0. HRMS (FAB) calcd for $C_{47}H_{44}NO_{12}S$ $[M+H]^+$: 846.2584 Found: 846.2592.

4.2.33. 1,2-Isopropylidene-3-*O*-(*m*-methoxyphenyl)-β-D-galactofuranose (19f). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (s, 3H), 1.47 (s, 3H), 2.72 (br s, 2H, OH), 3.68 (dd, J=11.5, 5.4 Hz, 1H, H-6), 3.79 (s, 3H), 3.80 (dd, J=11.5, 3.3 Hz, 1H, H-6), 4.01 (m, 1H), 4.08–4.13 (m, 2H), 4.53 (d, J=11.9 Hz, 1H), 4.60 (d, J=3.7 Hz, 1H, H-2), 4.67 (d, J=11.9 Hz, 1H), 4.68 (d, J=12.0 Hz, 1H), 5.91 (d, J=3.7 Hz, 1H, H-1). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.2, 26.6, 55.2, 64.2, 69.1, 71.9, 79.9, 81.9, 82.1, 105.1, 111.7, 113.4, 119.9, 129.7, 138.8, 160.0. HRMS (EI) Calcd for $C_{17}H_{25}O_7$ $[M+H]^+$: 341.1600. Found: 341.1612.

4.2.34. 1,2-Isopropylidene-3-*O*-(*p*-toluenesuphonyl)-β-D-galactofuranose (19h). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (s, 3H), 1.46 (s, 3H), 2.45 (s, 3H), 2.57 (br s, 2H), 3.65–3.68 (m, 1H), 3.84–3.88 (m, 1H), 4.11–4.17 (m, 2H), 4.56 (d, J=3.7 Hz, 1H, H-2), 5.00 (d, J=2.6 Hz, 1H, H-3), 5.86 (d, J=3.7 Hz, 1H, H-1), 7.37 (d, J=8.6 Hz, 2H), 7.83 (d, J=8.6 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.6, 21.7, 26.5, 64.0, 68.0, 79.0, 81.9, 82.8, 104.9, 112.6, 128.1, 130.1, 132.4, 145.7. HRMS (EI) Calcd for $C_{16}H_{23}O_8S$ $[M+H]^+$: 375.1113. Found: 375.1120.

4.2.35. 1,2-Isopropylidene-β-D-arabinofuranose (23). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (s, 3H), 1.46 (s, 3H), 3.64 (br s, 2H, OH), 3.96 (dd, J=12.3, 3.7 Hz, 1H, H-5), 4.03 (dd, J=12.3, 4.1 Hz, 1H, H-5), 4.13–4.16 (m, 1H, H-4), 4.27 (br d, J=2.6 Hz, 1H, H-3), 4.50 (br d, J=3.6 Hz, 1H, H-2), 5.95 (d, J=3.6 Hz, 1H, H-1). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.1, 26.6, 60.7, 76.4, 79.1, 85.5, 104.7, 111.8. HRMS (EI) Calcd for $C_8H_{15}O_5$ $[M+H]^+$: 191.0919. Found: 191.0914.

4.2.36. (2*R*,3*S*,4*R*,5*R*)-4,5-*O*-Isopropylidene-1,2,3,4,5-dodecanepentol (29). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J=6.8 Hz, 3H), 1.28–1.36 (m, 10H), 1.36 (s, 6H), 1.73–1.76 (m, 2H), 3.56 (ddd, J=10.6, 8.1, 2.4 Hz, 1H), 3.62–3.70 (m, 3H), 3.80–3.87 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 22.6, 25.0, 26.8, 26.9, 29.3, 29.6, 31.8, 34.4, 63.9, 73.0, 73.2, 80.2, 82.8, 109.2. HRMS (EI) Calcd for $C_{15}H_{31}O_5$ $[M+H]^+$: 291.2171. Found: 291.2175.

4.2.37. (2*R*,3*S*,4*R*,5*R*)-3-Azido-4,5-*O*-isopropylidene-1,2,4,5-decanetetrol (31). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, J=6.6 Hz, 3H), 1.33–1.40 (m, 12H), 1.38 (s, 3H), 1.50 (s, 3H), 1.86 (br s, 2H), 3.21–3.24 (m, 1H), 3.68–3.75 (m, 2H), 3.81–3.84 (m, 1H), 3.99–4.07 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0 (2C), 22.5, 26.2, 26.7, 31.0, 31.5, 48.2, 61.6, 63.8, 72.8, 81.8, 104.5. HRMS (EI) Calcd for $C_{13}H_{26}N_3O_4$ $[M+H]^+$: 288.1923. Found: 288.1931.

4.2.38. (2*R*,3*S*,4*R*,5*R*)-1,2-*O*-Isopropylidene-3-*O*-methanesulfonyl-1,2,3,4,5-decanepentol (33a). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J=6.7 Hz, 3H), 1.28–1.50 (m, 8H), 1.36 (s, 3H), 1.42 (s, 3H), 1.91 (br s, 2H), 3.16 (s, 3H), 3.53–3.62 (m, 2H), 3.98 (dd, J=8.8, 7.3 Hz, H-1, 1H), 4.13 (dd, J=8.8, 6.4 Hz, 1H, H-1), 4.32–4.36 (m, 1H), 5.12 (d, J=4.3 Hz, 1H, H-3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0 (2C), 22.6, 25.1, 26.5, 31.8, 32.9, 38.8, 65.7, 70.4, 73.7, 75.9, 79.1, 109.2. HRMS (EI) Calcd for $C_{14}H_{29}O_7S$ $[M+H]^+$: 341.1634. Found: 341.1644.

4.2.39. (2*R*,3*S*,4*R*,5*R*)-4,5-*O*-Isopropylidene-3-*O*-methanesulfonyl-1,2,3,4,5-decanepentol (33b). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, J=6.7 Hz, 3H), 1.27–1.51 (m, 8H), 1.38 (s, 3H), 1.42 (s, 3H), 1.90 (br s, 2H), 3.15 (s, 3H), 3.82–3.88 (m, 2H), 4.18–4.25 (m, 2H), 4.32 (dd, J=6.0, 4.3 Hz, 1H, H-4), 4.76 (d, J=4.3 Hz, 1H, H-3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1 (2C), 23.6, 26.0, 26.5, 32.5, 34.6, 38.7, 63.8, 71.1, 72.8, 74.1, 82.3, 109.2. HRMS (EI) Calcd for $C_{14}H_{29}O_7S$ $[M+H]^+$: 341.1634. Found: 341.1645.

4.2.40. *p*-Methylphenylsulfinyl 2,3-di-*O*-*p*-methylbenzyl-β-D-glucopyranoside (45). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.04 (br s, 1H, OH), 2.33 (s, 3H), 2.34 (s, 3H), 2.36 (s, 3H), 3.41 (br s, 1H, OH), 3.58 (m, 1H), 3.82–3.90 (m, 2H), 4.00 (dd, $J=11.8$, 3.7 Hz, 1H), 4.86–4.90 (m, 1H), 5.33–5.40 (m, 2H), 7.09–7.19 (m, 6H), 7.35 (dd, $J=6.6$, 1.7 Hz, 2H), 7.83 (d, $J=8.2$ Hz, 2H), 7.85 (d, $J=8.2$ Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.1, 21.5, 21.6, 62.4, 69.8, 70.0, 78.2, 80.0, 86.2, 126.0, 126.5, 128.1, 129.0, 129.1, 129.7, 129.8, 130.0, 133.4, 138.5, 144.0, 144.3, 165.2, 167.6. HRMS (EI) Calcd for $C_{29}H_{31}O_7S$ $[M+H]^+$: 523.1790. Found: 523.1802.

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Synthesis of spermidinylcholestanol and spermidinylcholesterol, squalamine analogues

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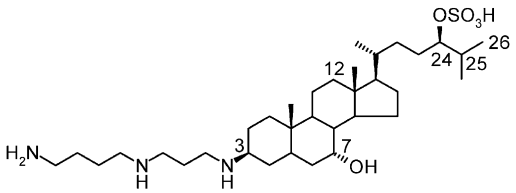
Abstract—Several novel squalamine-related polyaminosterols are reported. The synthesis of 7α-*N*-[3*N*-(4-aminobutyl)aminopropyl]aminocholestanol **I**, 6α-*N*-[3*N*-(4-aminobutyl)aminopropyl]aminocholestanol **II**, 7α and 7β-*N*-[3*N*-(4-aminobutyl)aminopropyl]aminocholesterol (**III** and **IV**), was accomplished from cholesterol, they provide the first examples in which spermidine is introduced in the B steroidal ring. These molecules showed comparable antibacteria and fungi activities to squalamine, and were cytotoxic on a human non-small cell bronchopulmonary carcinoma line (NSCLC-N6). Therefore, these molecules with antibiotic and cytotoxic activities are promising for immune-compromised patients in cancer chemotherapy.

1. Introduction

Squalamine is a novel aminosterol isolated from the tissues of the dog fish shark, *Squalus acanthias*. This compound has an unusual chemical structure, its a 5α-cholestane with 7α-hydroxy, 3β-spermidinyl and 24*R*-sulfate groups (Fig. 1).[1] Squalamine was the first example of a natural product which is a steroïdal polyamine (spermidine). Squalamine exhibits antimicrobial activity against a wide variety of microorganisms (Gram negative bacteria, Gram positive bacteria and fungi).[2] Its also demonstrates promise as an antiangiogenic agent, preventing the formation of new blood vessels in developing malignant tumors.[3] Squalamine also inhibits tumor growth in multiple animal models and is currently in phase II clinical trial for treatment of advanced non-small cell lung cancer.[4] The combination of squalamine and cisplatin has shown high activity against human cancer cells.[5] More recently, seven new aminosterols related to squalamine were discovered from the liver of the dog fish shark *S. acanthias*. These compounds exhibited a relatively invariant cholestane skeleton with a trans A/B ring junction, with spermidine or spermine attached equatoriallly at C3. The side chain had several variations: The sterol side chain had a hydroxyl or a hydroxymethyl group at the C24 position, and a sulfate group at either the C24 or C26 position. One of these aminosterols was dessulfated, but had a ketone function at C24 and a double bond Δ25–26, while another had a hydroxyl group at the C12 position. These compounds had a broad spectrum of antimicrobial activity comparable to squalamine.[6]



squalamine

Figure 1. Squalamine.

However, only trace amounts of these molecules were present in the different tissues of the shark. Chemical syntheses of squalamine has been accomplished from the expensive starting materials, 3β-acetoxy-5-cholenic acid,[7] 5-cholenic acid[8] and desmosterol.[9] Inexpensive starting materials used include stigmasterol,[10–12] methyl 3-keto-5α-chenodeoxy cholonate,[13] 3-keto-23,24-bis-norchol-4-en-22-ol[14] and methyl chenodeoxycholonate.[15] Fourteen to seventeen steps are needed to synthesize this compound, but these syntheses are accomplished with a low overall yield (1.9–19%). For these reasons, several squalamine analogues

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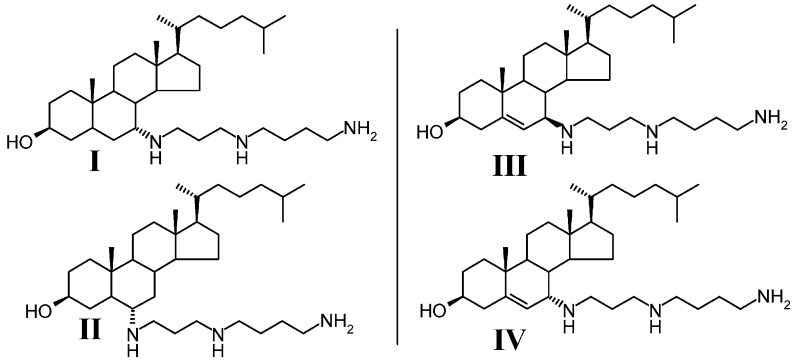
were synthesized. Only one analogue has been described with a spermidine moiety at the side chain,[16–17] but the other analogues have spermidine attached at the C3 position.[18–19] Selinsky's groups has synthesized squalamine analogues with a 24*R*,*S*-hydroxy or 24*R*,*S*-amino, with or without the 7-hydroxy group. Their aim was to explore the relationship between the functional groups and antimicrobial activity. Some of these analogues exhibit antimicrobial activity comparable to squalamine.[20]

Biological studies of squalamine and analogues led to the following conclusions. The sterol side chain could be dessulfated. The 7α-hydroxyl group could be suppressed. The structure of the polyamine on the steroid could be varied. The steroid can be have other functions on the side chain.

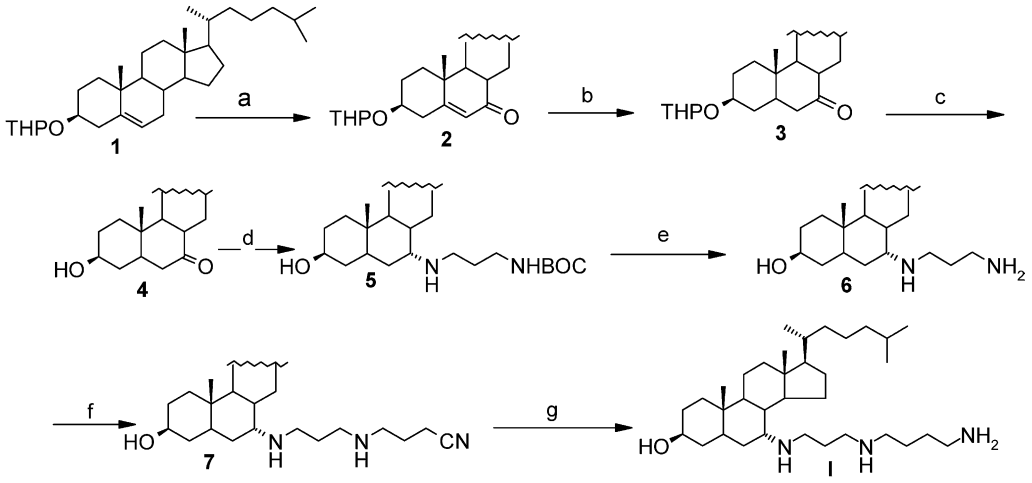
In this way, our approach was to develop new analogues of squalamine. Squalamine has a spermidine unit at C3 and a hydroxyl at the C7 position of the steroidal skeleton. We describe analogues with spermidine at C7 and a hydroxyl group at C3 position. These analogues were synthesized from cholesterol, an inexpensive starting material. The hydroxyl group at the C3 position of this steroid was conserved and the asymmetric polyamine (spermidine) was introduced at the C7 position. Therefore, the synthesis steps were minimized. 7α-Polyaminocholestanol (**I**), 6α-polyaminocholestanol (**II**) 7α- and 7β-polyaminocholesterols (**III** and **IV**) were synthesized (Scheme 1).

2. Results and discussion

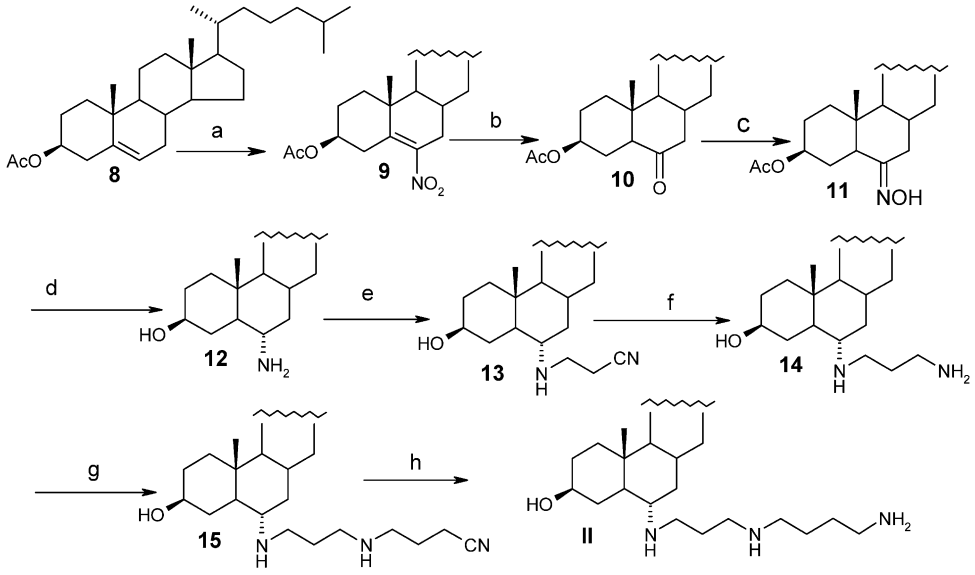
7α-*N*-[3*N*-(4-Aminobutyl)aminopropyl]aminocholestanol **I** was prepared from cholesterol. As depicted in Scheme 2, the hydroxyl group of cholesterol was protected with a tetrahydropyranyl group **1** (99%). Oxidation with chromium trioxide–pyridine complex afforded the allylic ketone **2** in 62% yield. Selective reduction of the Δ5 double bond under Birsh conditions, with lithium/ammonia at −78 °C, gave the A/B trans junction in 82% yield (compound **3**). Alcohol functionality of this compound was deprotected to give compound **4**. The intermediate **5** was obtained in 84% yield via a reductive amination of ketone **4** with 3*N*-(tertiobutylcarbonyl)aminopropane utilizing sodium cyanoborohydride (pH 5–6 was ajusted with acetic acid) as the reducing agent. Probably due to steric hinderance, only the



Scheme 1. Analogues of squalamine.



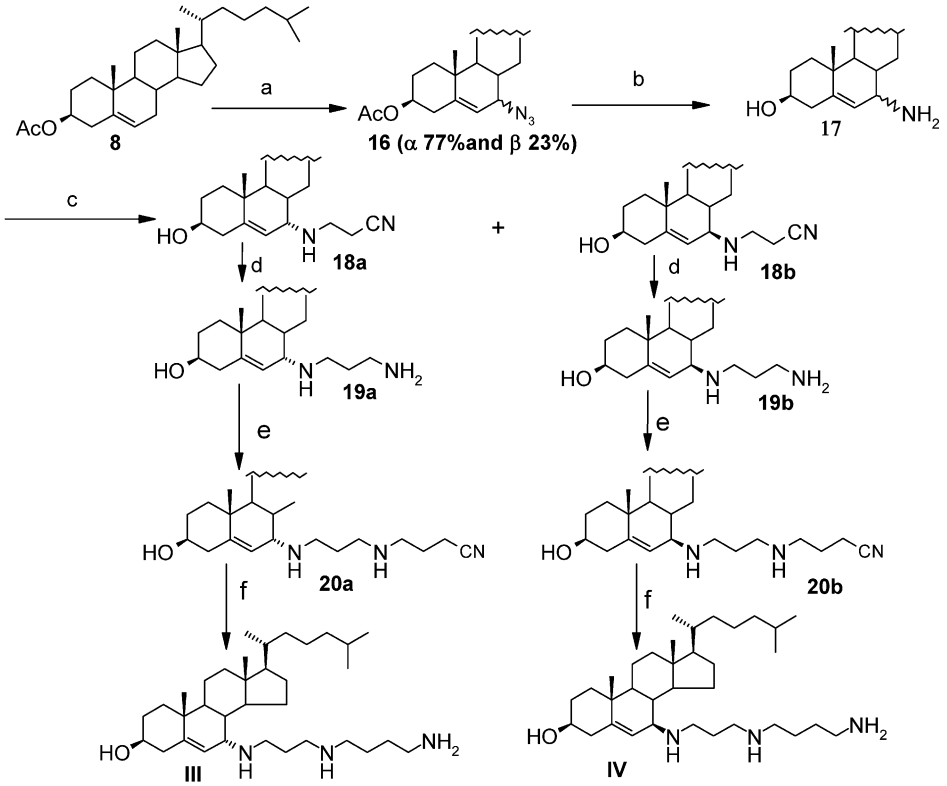
Scheme 2. Synthesis of 7α-spermidinylcholestanol. Reagents and conditions: (a) CrO_3-2py, CH_2Cl_2, RT; (b) Li/NH_3, THF, −78 °C; (c) PPTS, ethanol, reflux; (d) $NH_2(CH_2)_3NHBOC$, $NaBH_3CN$, AcOH pH 5–6; (e) TFA, CH_2Cl_2; (f) Br–$(CH_2)_3$–CN, DMF, 60 °C; (g) $LiAlH_4$, $NiCl_2$, $6H_2O$ THF, reflux.



Scheme 3. Synthesis of 6α-spermidinylcholestanol. Reagents and conditions: (a) CH_3COOH, HNO_3; (b) CH_3COOH/Zn, reflux; (c) $HONH_2.HCl$, py, reflux; (d) $LiAlH_4$, THF, reflux; (e) acrylonitrile, MeOH; (f) $LiAlH_4/NiCl_2,6H_2O$, THF, reflux; (g) $Br–(CH_2)_3–CN$, DMF, 60 °C; (h) $LiAlH_4/NiCl_2$, $6H_2O$, THF, reflux.

α epimer was observed. The amine function was deprotected **6** (79%) and alkylated by 4-bromobutyronitrile to give compound **7** in 51% yield. Nitrile reduction of **7** was achieved with lithium aluminium hydride in presence of nickel chloride hexahydrate giving the polyaminosterol **I** in 30% yield.

6α-*N*-[3*N*-(4-Aminobutyl)aminopropyl]aminocholestanol **II** was prepared as shown in Scheme 3 cholesteryl acetate was treated with high density ($d=1.53$) nitric acid in anhydrous acetic acid to give the 6-nitro derivative **9** in 74% yield. Ketone **10** was treated with zinc in acetic acid. Oxime derivative **11** was obtained from the ketone by reaction with hydroxylamine hydrochloride in pyridine and reduced by lithium aluminium hydride in tetrahydrofuran, to 6α-aminocholestanol (**12**). Probably due to steric hinderance, only the α epimer was observed. Acrylonitrile was reacted in methanol with aminosterol (**12**) giving the



Scheme 4. Synthesis of 7α- and 7β-spermidinylcholesterol. Reagents and conditions. (a) Lead IV acetate, $(CH_3)_3SiN_3$, CH_2Cl_2; (b) $LiAlH_4$, THF reflux; (c) CH_2═CH–CN, MeOH; (d) $LiAlH_4$,/$NiCl_2$, $6H_2O$, THF, reflux; (e) $Br–(CH_2)_3–CN$, DMF, 60 °C, 72 h, (f) $LiAlH_4$,/$NiCl_2$, $6H_2O$, THF, reflux.

6α-*N*(2-cyanoethyl)aminocholestanol (**13**). The nitrile function was reduced by lithium aluminium hydride (compound **14**) and the amine obtained was alkylated by 4-bromobutyronitrile to give compound **15** in 51% yield. Nitrile reduction of **15** was achieved with lithium aluminium hydride in presence of nickel chloride hexahydrate giving the polyaminosterol **II** in 35% yield.

The polyaminosterols **III** and **IV** were synthesized steroselectively from 7α and 7β-aminocholesterol as a key intermediate[21]. A convenient novel synthetic route was developped. Polyaminocholesterols (**III**) and (**IV**) were easily prepared from epimeric mixture, 7α,β-aminocholesterol. This epimeric mixture was obtained in two steps from cholesteryl acetate (Scheme 4): cholesterol was acetylated by using acetic anhydride and pyridine to give the acetate **8** (90%), and the azido group was introduced directly by trimethylsilyl azide action in presence of lead IV acetate on allylic position C-7 of cholesteryl acetate. α/β Epimeric mixture **16** (77%α and 23%β) was obtained in 68% yield. Reduction of epimeric azides **16** was acheived with lithium aluminium hydride to give amine **17**. Acrylonitrile was reacted in methanol with amine epimeric mixture **17** required the 7α- and 7β-*N*(2-cyanoethyl)aminocholesterol (**18a** and **18b**) easily separated by chromatography on silica gel column. Each nitrile function was reduced by lithium aluminium hydride in presence of nickel chloride hexahydrate (**19a** and **19b**) and the amine obtained was alkylated by 4-bromobutyronitrile to give compounds **20a** and **20b**. Finally, each nitrile was reduced with lithium aluminium hydride in presence of nickel chloride hexahydrate to give squalamine analogue **III** in 46% yield and **IV** in 48% yield.

Minimum inhibitory concentrations (MIC) of these aminosterols (**I**, **II**, **III** and **IV**) were determined against fungi (*S. cerevisiaie* and *C. albicans*), and both Gram-positive (*S. aureus* and *E. hirae*) and Gram-negative (*E. coli*) bacteria (Table 1).

The activity on *C. albicans* was compared to clinical references products (Amphotericin B, Econazole, Nystatin and 5-fluorocytosine, Table 2).

The antiproliferative activity of these squalamine analogues was studied in vitro on a human non-small cell bronchopulmonary (NSCLC-N6). The cytotoxicity determinations (inhibitory concentration: IC_{50}) showed clearly strong antiproliferative properties on this cell line ($IC_{50} <$ 3.3 μg/mL; significative activity when $IC_{50} < 10$ μg/mL).

3. Conclusion

There are two a key elements in our synthesis of squalamine analogues. Its the first example, to our knowledge, in which spermidine is introduced into the B steroid ring. Cholesterol was chosen as the starting material in the synthesis of these squalamine analogues. An inexpensive material allowing preparation of significant quantities of material following a multistep synthetic process. Analogues **I** and **II** were prepared in seven and eight steps, respectively. Aminosterols (**III**) and (**IV**) were easily prepared from epimeric mixture, 7α,β-aminocholesterol in six steps.

The results of these studies clearly show the antiproliferative effect on the cloned NSCLC-N6 cell line and the antimicrobial activity against a broad spectrum of microorganisms. 7α- and 7β-aminospermidinylcholesterol were active against Gram negative bacteria. No activity with analogues **I** and **II** was observed on Gram negative bacteria. The stereochemistry of spermidinyl moiety at the steroid seemed to have small affect on antimicrobial activity.

Our results suggest that introduction of spermidine at B steroid ring is a key target of biological activites for polyaminosterols. Therefore, these molecules with antibiotic and cytotoxic properties are promising for immune-compromised patients in cancer chemotherapy.

4. Experimental

4.1. General procedure

All melting points are uncorrected The IR spectra were recorded on a Perkin Elmer 1600 FT-IR spectrometer. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were obtained on a Brucker DPX 250 MHz or a Jeol JNM 400 MHz. The chemical shift values (parts per million) are relative to tetramethylsilane as solvent reference and coupling constant (*J*) values are expressed in Hertz. The mass spectra were recorded Jeol-GC mate (GC–MS system). The optical rotations were measured with Perkin Elmer 343 polarimeter. Thin-layer chromatography was run on Merck silica gel 60 F254. Developed plates were

Table 1. Determination of MIC (μg/mL) values after 48 h incubation. NI: no inhibition with MIC > 50 μg/mL

Strain	*S. cerevisiaie* ATCC28383	*C. albicans* CIP1180-79	*S. aureus* CIP 54127	*E. hirae* CIP 5855	*E. coli* 54127
Analogue **I**	6.25	25	6.25	6.25	NI
Analogue **II**	1.56	6.25	3.12	1.56	NI
Analogue **III**	3.12	6.25	3.12	3.12	6.25
Analogue **IV**	3.12	3.12	6.25	6.25	3.12
Squalamine	—	4–8	1–2	—	1–2

Table 2. Determination of MIC (μg/mL) values after 48 h incubation

Compounds	Amp B	Econazole	Nystatin	5-Fc	**I**	**II**	**III**	**IV**
C. albicans CIP1180-79	0.4	1.5	6	> 50	25	6.25	12.5	3.12

visualized by upon spraying with sulfuric acid/ethanol 2:8 and heating.

4.1.1. 3β-[(Tetrahydropyran-2*R*,*S*-yl)oxy]cholest-5-ene 1. To a solution of cholesterol (10 g, 24 mmol) in methylene chloride (50 mL) was added 3,4-dihydropyrane freshly distilled (3.18 mL, 36 mmol) and pyridinium *para*-toluene sulfonate (0.59 g, 2.4 mmol). The mixture was stirred at room temperature and under nitrogen atmosphere for 1 h 30 min. The solution was washed with water (50 mL), and the organic layer was dried over sodium sulfate and evaporated to give the crude product, which was purified by chromatography on silica gel column (hexane/ethyl acetate 9.5:0.5) to afford compound **1** as a white solid (11.29 g, 99%).

^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.68 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.92 (d, 3H, J=6.5 Hz, Me 21), 0.97 (s, 3H, Me 19), 3.49–3.54 (m, 2H, H-6′ of THP), 3.92 (m, 1H, H3α), 4.71 (br s, 1H, H-2′ of THP), 5.33 (td, 1H, J=1.01, 6.8 Hz, H6). ^{13}C NMR ($CDCl_3$): δ: 12.1, 18.7, 19.4, 19.7, 21.2, 22.7, 23.9, 24.1, 25.4, 28.1, 28.3, 29.6, 31.2, 32.0, 32.1, 35.9, 36.2, 37.5, 38.0, 39.1, 39.6, 40.0, 42.4, 50.3, 56.0, 56.8, 63.0, 77.3, 96.8, 122.0, 140.5. MS-EI: m/z=470 (10%, $M^{+\cdot}$), 386 (14%), $M^{+\cdot}$−tetrahydropyranyl), 368 (28%, 386 –H_2O), 85 (100%).

4.1.2. 3β-[(Tetrahydropyran-2*R*,*S*-yl)oxy]-7-oxo-cholest-5-ene 2. Pyridine (16.2 mL, 200 mmol) was added to a solution of chromium anhydride (10.61 g, 106 mmol) in methylene chloride (100 mL). The solution was stirred at room temperature and under nitrogen atmosphere for 2 h. The protected cholesterol **1** (5 g, 10 mmol) was added, and the mixture was stirred for 12 h. The solution was filtered, washed with 0.1 N HCl (100 mL), 5% $NaHCO_3$ (100 mL) and with water 100 mL). The organic layer was dried over sodium sulfate and evaporated to give the crude product, which was purified by chromatography on silica gel column (hexane/ethyl acetate 8:2) to afford pure compounds **2** (3 g, 62%). IR: ν: 2944 (CH_2), 1673 (C=O allylic ketone), 1651 (C=C alkene). ^{1}H NMR($CDCl_3$, 250 MHz), δ: 0.68 (s, 3H, Me 18), 0.85 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.90 (d, 3H, J=6.5 Hz, Me 21), 0.93 (s, 3H, Me 19), 3.45–3.49 (m, 2H, H-6′ of THP), 3.55–3.64 (m, 1H, H3α), 4.72 (br s, 1H, H-2′ of THP), 5.68 (d, 1H, J=1.02 Hz, H6). ^{13}C NMR ($CDCl_3$): δ: 12.3, 17.7, 19.2, 20.2, 21.5, 22.7, 24.1, 25.7, 26.7, 28.1, 28.3, 28.9, 29.7, 31.5, 36.0, 36.5, 38.8, 39.1, 39.8, 40.4, 43.4, 45.; 50.3, 55.1, 63.2, 75.3, 97.4, 126.4, 165.9, 202.5. MS-EI: m/z=400 (19%, $M^{+\cdot}$−tetrahydropyranyl), 344 (75%), 85 (100%).

4.1.3. 3β-[(Tetrahydropyran-2*R*,*S*-yl)oxy]cholestan-7-one 3. Tetrahydrofuran (10 mL) was cooled to −78 °C with dry ice/acetone, and ammonia was then collected to a total volume of approximately 20 mL. Lithium wire (0.2 g, 28 mmol) was added in small pieces to the solution with vigorous stirring. A deep blue solution was obtained after the lithium was completely dissolved. Ketone **2** (3.5 g, 7.22 mmol) was dissolved in THF (50 mL) and added to the flask in a steady stream from an addition funnel. The solution was stirred for 40 min and then quenched by the addition of ammonium chloride and ethanol unitil the blue coloration dissipated. The ammonia was allowed to evaporate at room temperature, and the residue was dissoved in ethyl ether (30 mL) and washed with 0.1 N HCl (25 mL) and water (25 mL). The organic layer was dried over sodium sulfate and evaporated. The crude product was purified by chromatography on silica gel column (hexane/ethyl acetate 8:2). The desired product **3** (2.89 g, 82%) was obtained as a white solid. IR: ν: 2940 (CH_2), 1707 (C=O ketone). ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.65 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.90 (d, 3H, J=6.4 Hz, Me 21), 0.93 (s, 3H, Me 19), 3.46–3.48 (m, 1H, H-6′ of THP), 3.57–3.59 (m, 1H, H3α), 3.88–3.90 (m, 1H, H-6′ of THP), 4.68 (br s, 1H, H-2′ of THP). ^{13}C NMR ($CDCl_3$): δ: 12.1, 18.8, 19.7, 21.7, 22.7, 22.9, 23.8, 25.0, 25.4, 26.8, 28.1, 28.5, 33.2, 34.8, 35.1, 35.7, 36.2, 36.5, 39.6, 42.5, 44.2, 45.1, 45.7, 48.9, 52.1, 56.1, 63.0, 77.6, 96.5, 211.5. MS-EI: m/z=486 (24%, $M^{+\cdot}$), 385 (10%, $M^{+\cdot}$−tetrahydropyranyl), 367 (32%, 385 –H_2O).

4.1.4. 7-Oxo-cholestan-3β-ol 4. Pyridinium *para*-toluene sulfonate (0.6, 2.4 mmol) was added to a solution of compound **3** (1.2 g, 2.4 mmol) in ethanol (25 mL) and heated under reflux for 12 h. The solvent was evaporated and the residue was dissolved in ethyl ether/ethyl acetate (5:5). The mixture was washed with brine, dried over sodium sulfate and evaporated to give the crude product, which was purified by chromatography (hexane/methylene chloride 5:5) and crystallized from ethanol/ethyl ether (5:5) to afford product **4** (0.89 g, 85%) as a white solid. Mp 140 °C. IR: ν: 3419 (OH alcohol), 2948 (CH_2), 1706 (C=O ketone). ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.65 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.89 (d, 3H, J=6.4 Hz, Me 21), 0.91 (s, 3H, Me 19), 3.57–3.59 (m, 1H, H3α). ^{13}C NMR ($CDCl_3$): δ: 12.1, 19.1, 22.2, 22.7, 24.1, 25.3, 28.3, 28.7, 29.7, 31.3, 36.0, 36.3, 36.4, 36.5, 38.2, 39.1, 39.8, 42.8, 46.4, 47.2, 49.2, 50.3, 55.4, 55.6, 70.9, 212.5. MS-EI: m/z=402 (18%, $M^{+\cdot}$), 385 (100%, $M^{+\cdot}-{}^{\cdot}OH$).

4.1.5. 7α-*N*-(3*N*-tert-Butoxycarbonyl-aminopropyl)aminocholestan-3β-ol 5. To a solution of 3*N*-(tertiobutylcarbonyl)aminopropane (0.921 g, 5.29 mmol) and compound **4** (1.29 g, 2.64 mmol) in anhydrous methanol (10 mL) was added sodium borohydride (0.25 g, 3.96 mmol) and the pH was ajusted with acetic acid to 5–6. The mixture was stirred at room temperature and under argon atmosphere for 48 h. The solution was treated with 0.1 N HCl (30 mL), 5% $NaHCO_3$ (30 mL) and extracted with methylene chloride. The organic layer was washed with, brine and dried over sodium sulfate. The solution was evaporated and the crude product was purified by chromatography (ethyl acetate/hexane 5:5 and methylene chloride/methanol 9:1) to afford product **5** (1.467 g, 84%) as amorphous solid. ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.65 (s, 3H, Me 18), 0.84 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.4 Hz, Me 21), 1.02 (s, 3H, Me 19), 1.45 (s, 9H, HN–CO_2–C(CH_3)$_3$), 2.48 (m, 1H, St–HN–$CH_aH_{a'}$–$(CH_2)_2$–NH–), 2.57 (m, 1H, H7β of α epimer), 2.72 (m, 1H, HN–$CH_aH_{a'}$–$(CH_2)_2$–NH–), 3.21 (t, 2H, J=5.1 Hz, –CH_2–NH–BOC), 3.63 (m, 1H, H3α), 5.80 (br s, 1H, HN–CO_2–C(CH_3)$_3$, D_2O exchange). ^{13}C NMR ($CDCl_3$): δ: 12.1, 15.1, 18.8, 21.8, 22.7, 23.9, 26.3, 27.1, 28.1, 28.2, 28.3, 29.7, 33.6, 33.8, 35.2, 35.9, 36.3, 37.1, 39.5, 39.6, 39.8, 41.0, 42.9, 43.0, 46.5, 47.6, 54.4, 56.3, 61.9, 71.6, 78.8, 155.9. MS-EI: m/z=560 (25%,

$M^{+\cdot}$), 416 (25%, $M^{+\cdot}-CH_2$=CH–NHBoc), 402 (64%, 416 $-{}^{\cdot}CH_3$), 387 (22%, St^+), 293 (100%).

4.1.6. 7α-*N*-(3*N*-Aminopropyl)aminocholestan-3β-ol 6. To a solution of compound **5** (1.55 g, 2.75 mmol) in methylene chloride (20 mL) was added dropwise trifluoroacetic acid (1.92 mL, 24.8 mmol). The mixture was stirred at room temperature for 3 h. The mixture was treated with 5% $NaHCO_3$ (20 mL) and washed with water (20 mL). The organic layer was dried over sodium sulfate and evaporated. The crude product was purified by chromatography (methylene chloride/methanol/ammoniaque 8:1:1) to give product **6** (1.0 g, 79%) as amorphous solid. ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.65 (s, 3H, Me 18), 0.85 (d, 6H, J= 6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.4 Hz, Me 21), 1.02 (s, 3H, Me 19), 2.63 (m, 1H, H7β of α epimer), 2.74 (m, 1H, St–HN–CH_aH$_{a'}$–$(CH_2)_2$–NH_2), 2.87 (m, 1H, St–HN–$CH_aH_{a'}$–$(CH_2)_2$–NH_2), 3.25 (t, 2H, J=7.5 Hz, –HN–$(CH_2)_2$–CH_2–NH_2), 3.66 (m, 1H, H3α), 8.91 (br s, 1H, St–N*H*–$(CH_2)_2$, D_2O exchange). ^{13}C NMR ($CDCl_3$): δ: 12.1, 15.2, 19.0, 21.5, 22.7, 24.0, 26.3, 28.1, 28.3, 31.7, 33.6, 33.8, 36.0, 36.2, 36.4, 36.9, 37.7, 38.2, 39.2, 39.7, 39.9, 42.9, 43.2, 46.6, 47.6, 51.1, 56.3, 61.9, 71.4. MS-EI: m/z=443 (16%, $M^{+\cdot}-{}^{\cdot}CH_3$), 428 (12%, $M^{+\cdot}-{}^{+}CH_2$–NH_2), 416 (47%, St–NH–CH_2^+), 402 (7%, St–NH^+), 387 (100%, St^+).

4.1.7. 7α-*N*-[3*N*-(3-Cyanopropyl)aminopropyl]aminocholestan-3β-ol 7. To a solution of amine **6** (0.330 g, 0.71 mmol) in DMF (5 mL) was added sodium hydrogenocarbonate (0.09 g, 1.07 mmol). After stirring at room temperature for 15 min, 4-bromobutyronitrile (0.14 mL, 1.43 mmol) was added and the mixture was heated at 60 °C for 36 h. The solution was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was purified by chromatography (hexane/ethyl acetate 5:5) afforded product **7** (0.190 g, 51%) as a oil. IR: ν: 2932 (CH_2), 2254 (CN). ^{1}H NMR ($CDCl_3$, 250 MHz): δ: 0.65 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 1.1 (s, 3H, Me 19), 1.19 (t, 2H, J=6.7 Hz, –NH–CH_2–CH_2–CH_2–CN), 2.47–2.53 (t, 4H, J_1=7.06 Hz, J_2=7.13 Hz, –CH_2–CN and –NH–CH_2–$(CH_2)_2$–CN), 2.60 (ddd, 1H, $J_{7\beta-8}$=4.3 Hz, $J_{7\beta-6}$= 5 Hz, H7β of α epimer), 2.68 (m, 1H, St–HN–CH_aH$_{a'}$–$(CH_2)_2$–NH–), 2.82 (m, 1H, St–HN–$CH_aH_{a'}$–$(CH_2)_2$–NH–), 3.38 (m, 1H, H3α), 3.75 (t, 2H, J=5.9 Hz, St–HN–$(CH_2)_2$–CH_2–NH–), 8.98 (br s, 1H, St–N*H*–, D_2O exchange). ^{13}C NMR ($CDCl_3$): δ: 12.0, 14.1, 15.2, 18.8, 21.5, 22.7, 24.0, 24.6, 25.6, 26.3, 28.1, 28.4, 31.6, 36.0, 36.2, 36.4, 36.9, 37.6, 38.0, 39.1, 39.6, 39.8, 42.9, 43.0, 43.2, 46.5, 51.0, 54.4, 56.3, 56.5, 60.5, 71.3, 120.0. MS-EI: m/z=487 (2%, St–NH–$(CH_2)_3$–NH–CH_2–CH_2^+), 416 (46%, St–NH–CH_2^+), 402 (7%, St–NH^+), 387 (8%, St+), 369 (5%, 386 – H_2O), 169 (100%).

4.1.8. 7α-*N*-[3*N*-(4-Aminobutyl)-3-aminopropyl]aminocholestan-3β-ol I. A solution of nitrile **7** (0.500 g, 0.9 mmol) in dry THF (5 mL) was added dropwise to a suspension of lithium aluminium hydride (0.21 g, 5.6 mmol) and nickel chloride hexahydrate (0.213 g, 0.9 mmol) in dry THF (20 mL) under argon. The mixture was refluxed for 6 h. The reaction mixture quenched with $Na_2SO_4.10H_2O$, filtered through a pad of celite, and washed with ethyl ether/n butanol (6:4). Removal of the solvent in vacuo and purification by flash chromatography (dichloromethane/methanol/ammoniaque 8:1.5:0.5) to afford a pure product **I** as amorphous solid (0.15 g, 30%). ^{1}H NMR ($CDCl_3$, 250 MHz): δ: 0.68 (s, 3H, Me 18), 0.85 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 1.03 (s, 3H, Me 19), 2.44 (m, 1H, St–HN–CH_aH$_{a'}$–$(CH_2)_2$–NH–), 2.60 (m, 1H, H7β of α epimer), 2.67 (m, 1H, St–HN–$CH_aH_{a'}$–$(CH_2)_2$–NH–), 2.81 (t, 2H, J=5.7 Hz, –HN–$(CH_2)_3$–CH_2–NH_2), 3.11–3.23 (t, 4H, J_1=9.5 Hz, J_2=7.6 Hz, St–HN–$(CH_2)_2$–CH_2–NH– and –NH–CH_2–$(CH_2)_3$–NH_2), 3.58 (m, 1H, H3α), 7.15 (br s, 2H, –NH2, D_2O exchange), 8.10 (br s, 1H, –NH–, D_2O exchange), 8.32 (br s, 1H, –NH–, D_2O exchange). ^{13}C NMR ($CDCl_3$): δ: 12.1, 15.2, 18.8, 21.8, 22.7, 23.9, 25.6, 25.7, 26.3, 28.1, 28.2, 29.5, 29.8, 33.6, 33.8, 35.2, 35.9, 36.3, 37.1, 39.6, 39.7, 40.0, 41.0, 42.9, 43.0, 46.1, 46.6, 47.6, 47.7, 54.2, 56.3, 62.0, 71.6. MS-EI: m/z=473 (3%, St–NH–$(CH_2)_3$–NH–CH_2^+), 430 (5%, St–NH–CH_2–CH_2^+), 416 (50%, St–NH–CH_2^+), 402 (100%, St–NH^+), 387 (8%, St^+), 369 (5%, 387 –H_2O). Anal. Calcd for $C_{34}H_{65}N_3O$: C 76.77; H 12.32; N 7.90. Found: C 76.75; H 12.39; N 7.92.

4.1.9. 3β-Acetoxy-6-nitro cholest-5-ene 9. To a solution of nitric acid (9.6 mL, 197 mmol) in acetic acid (36 mL) was added dropwise cholesteryl acetate (12.5 g, 29 mmol). The mixture was stirred for 2 h. The ice was added to the solution. The product was filtered, washed with water and crystallized from ethanol to afford product **9** (10.3 g, 74%) as a yellow solid. Mp 110 °C. ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.68 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 1.03 (s, 3H, Me 19), 2.17 (s, 3H, CH_3–CO), 4.6–4.68 (m, 1H, H3α). ^{13}C NMR ($CDCl_3$): δ: 11.9, 18.7, 19.2, 21.0, 22.7, 23.2, 23.9, 24.4, 27.8, 28.1, 28.3, 30.4, 30.5, 33.7, 35.4, 35.9, 36.2, 38.7, 39.6, 39.8, 42.1, 51.8, 55.8, 56.4, 72.8, 130.2, 131.3, 171.2. MS-EI: m/z=473 (27%, $M^{+\cdot}$), 426 (20%, $M^{+\cdot}-HNO_2$), 398 (46%, $M^{+\cdot}-(CH_3COOH+.CH_3)$), 383 (100%).

4.1.10. 3β-Acetoxy-cholestan-6-one 10. To a solution of compound **9** (0.890 g, 1.87 mmol) in acetic acid (20 mL) was added zinc (2 g, 30 mmol) and the solution was refluxed for 4 h 30 min. The solution was filtered, diluted with water and extracted with methylene chloride. The organic layer was dried over sodium sulfate and evaporated. The crude product was purified by chromatography (Hexane/ethyl acetate 9.8:0.2) and crystallized from ethanol (0.60 g, 72%). Mp 136 °C.

IR: ν: 1730 (C=O/ester), 1718 (C=O/ketone). ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.66 (s, 3H, Me 18), 0.76 (d, 6H, J= 6.5 Hz, Me 27 and Me 26), 0.90 (d, 3H, J=6.5 Hz, Me 21), 0.95 (s, 3H, Me 19), 2.02 (s, 3H, CH_3–COO), 4.67 (m, 1H, H3α). ^{13}C NMR ($CDCl_3$): δ: 12.3, 13.4, 19.0, 21.7, 21.8, 24.1, 24.3, 26.4, 27.2, 28.3, 28.4, 36.0, 36.4, 36.7, 38.3, 39.2, 39.8, 41.3, 43.2, 43.3, 47.0, 54.1, 56.4, 56.8, 73.2, 170.0, 210.7. MS-EI: m/z=444 (25%, $M^{+\cdot}$), 384 (100%, $M^{+\cdot}-CH_3COOH$), 369 (27%, 384 $-{}^{\cdot}CH_3$).

4.1.11. 3β-Acetoxy-6*N*-hydroxyiminocholestane 11. Ketone **10** was added to a solution of hydroxylamine chloride (0.751 g, 10 mmol) in pyridine (8 mL). The

mixture was refluxed under argon atmosphere for 5 h. The solution was diluted with water and extracted with methylene chloride. The organic layer was treated with 0.1 N HCl (15 mL), 5% $NaHCO_3$ (15 mL) and washed with water. The solution was dried over sodium sulfate and evaporated. The product was purified by chromatography (hexane/ethyl acetate 9:1) to afford product **11** (2.38 g, 76%) as a white solid. IR: ν: 2950 (O–H alcohol), 1734 (C=O/ester), 1712 (C=N–OH). ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.65 (s, 3H, Me 18), 0.85 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 0.99 (s, 3H, Me 19), 2.02 (s, 3H, CH_3–COO), 3.32 (dd, 1H, J=9.0 Hz, C_5–*H*), 4.67 (m, 1H, H3α), 7.73 (br s, 1H, –C=N–O–*H*). ^{13}C NMR ($CDCl_3$): δ: 12.4, 18.9, 19.0, 21.7, 22.9, 24.2, 24.4, 24.5, 24.8, 27.4, 27.9, 28.3, 28.5, 30.0, 36.1, 36.2, 36.3, 36.5, 39.3, 39.8, 40.0, 43.3, 49.7, 56.5, 57.0, 73.6, 159.9, 171.0. MS-EI: m/z=459 (11%, $M^{+\cdot}$), 442 (17%, $M^{+\cdot}-{}^{\cdot}OH$), 399 (79%, $M^{+\cdot}-CH_3COOH$), 384 (100%, 399 $-{}^{\cdot}CH_3$).

4.1.12. 6α-Aminocholestanol 12. Under nitrogen atmosphere, solution of **11** (1.50 g, 3.27 mmol) in tetrahydrofuran (8 mL) was added dropwise over 10 min to a stirred suspension of lithium aluminium hydride (0.744 g, 19 mmol) in tetrahydrofuran (20 mL) at 0 °C. The mixture was refluxed for 4 h, after which it was cooled and quenched by careful addition of saturated aqueous sodium sulfate. The solution was filtered, dried and evaporated under reduced pressure. The crude product was purified by column chromatography (methylene chloride/methanol/triethylamine (8:1.8:0.2) crystallized from ethanol/ethyl ether (8:2) to give amine **12** (0.900 g, 70%) as a white solid. Mp 127–128 °C. IR: ν: 3500–3000 (OH alcohol and NH_2 amine). ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.69 (s, 3H, Me 18), 0.86 (d, 6H, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 1.02 (s, 3H, Me 19), 2.32 (br s, 2H, NH_2, D_2O exchange), 2.95 (dd, 1H, H6β of α epimer, 3.64 (m, 1H, H3α). ^{13}C NMR ($CDCl_3$): δ: 12.5, 16.9, 19.0, 21.4, 22.9, 24.2, 24.6, 28.4, 28.5, 30.0, 30.4, 31.8, 35.9, 36.1, 36.3, 36.5, 39.5, 39.9, 40.3, 43.0, 47.0, 52.4, 54.7, 56.4, 56.7. MS-EI: m/z=403 (16%, $M^{+\cdot}$), 386 (24%, $M^{+\cdot}-NH_3$), 248 (100%).

4.1.13. 6α-*N*-(2*N*-Cyanoethyl)aminocholestanol 13. A solution of amine **12** (0.72 g, 1.8 mmol) and acrylonitrile (1.1 mL, 16 mmol) in methanol (10 mL) was stirred at room temperature for 24 h. The solvent was evaporated giving the crude product which was purified by chromatography (hexane/ethyl acetate 8:2) and crystallized from ethanol/ethyl ether (8:2) to afford (0.760 g, 93%) of nitrile **13** (β) as a white solid. Mp=136 °C. IR: ν: 3391–3000 (OH alcohol and NH amine), 2248 (nitrile). ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.68 (s, 3H, Me 18), 0.84 (dd, 6H, J_1=6.6 Hz, J_2=0.8 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 1.04 (s, 3H, Me 19), 2.44 (m, 2H, –HN–CH_2–CH_2–CN), 2.62 (m, 1H, H6β of α epimer), 2.74 (m, 1H, St–HN–C$H_a$$CH_{a'}$–$CH_2$–CN), 2.92 (m, 1H, St–HN–$CH_a$$H_{a'}$–$CH_2$–CN), 3.6–3.7 (m, 1H, H3α). ^{13}C NMR ($CDCl_3$): δ: 11.6, 18.6, 18.7, 19.7, 22.5, 22.8, 23.9, 28.0, 28.2, 31.4, 35.8, 36.2, 36.9, 37.4, 39.5, 42.1, 43.0, 43.8, 49.5, 52.8, 55.8, 58.2, 58.3, 58.9, 59.0, 59.3, 59.9, 71.6, 118.9. MS-EI: m/z=456 (12%, $M^{+\cdot}$), 416 (30%, St–NH–CH_2^+), 301 (100%).

4.1.14. 6α-*N*-(3*N*-Aminopropyl)aminocholestanol 14. A solution of nitrile **11a** (0.40 g, 0.87 mmol) in dry THF (5 mL) was added dropwise to a suspension of lithium aluminium hydride (0.19 g, 5.25 mmol) and nickel hexahydrate (0.206, 0.87 mmol) in dry THF (10 mL) under argon. The mixture was refluxed for 2 h. The reaction mixture quenched with $Na_2SO_4\cdot10H_2O$, filtered through a pad of celite, and washed with ethyl ether/*n*-butanol (6:4). Removal of the solvent in vacuo and purification by flash chromatography (methylene chloride/methanol/ammoniaque 7:2.5:0.5) to afford a pure product **12a** (0.18 g, 41%) as amorphous solid. ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.68 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 1.04 (s, 3H, Me 19), 2.70 (m, 2H, St–NH–CH_2–CH_2–), 2.74 (m, 1H, St–NH–C$H_a$$H_{a'}$–$CH_2$–), 2.80 (m, 1H, H6β of α epimer), 2.87 (m, 1H, St–HN–$CH_a$$H_{a'}$–$(CH_2)_2$–), 3.00 (t, 2H, J=12 Hz, St–HN–$(CH_2)_2$– CH_2–NH_2), 3.66 (m, 1H, H3α). ^{13}C NMR ($CDCl_3$): δ: 12.1, 15.3, 18.7, 20.9, 22.7, 23.9, 24.1, 28.1, 28.2, 30.3, 33.4, 33.6, 34.1, 35.0, 35.8, 36.2, 36.5, 39.6, 40.0, 40.6, 44.4, 45.6, 49.5, 52.0, 56.3, 56.9, 60.2, 73.1. MS-EI: m/z=460 ($M^{+\cdot}$, 13%), 443 (12%, $M^{+\cdot}-NH_3$), 402 (100%, St–NH^+).

4.1.15. 6α-*N*-[3*N*-(3-Cyanopropyl)aminopropyl]aminocholestanol 15. To a solution of amine **14** (0.270 g, 0.580 mmol in DMF (4 mL) was added sodium hydrogenocarbonate (0.073 g, 0.871 mmol). After stirring at room temperature for 35 min, 4-bromobutyronitrile (0.115 mL, 1.161 mmol) was added and the mixture was heated at 60 °C for 36 h. The solution was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was purified by chromatography (hexane/ethyl acetate 6:4) afforded product **15** (0.180 g, 59%) as a oil. IR: ν: 3500–3000 (OH alcohol and NH amine); 2254 (nitrile). ^{1}H NMR ($CDCl_3$, 250 MHz), δ: 0.65 (s, 3H, Me 18), 0.85 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.90 (d, 3H, J=6.5 Hz, Me 21), 1.08 (s, 3H, Me 19), 2.49 (t, 2H, J=7.2 Hz, –HN–$(CH_2)_2$–CH_2–CN), 2.59 (td, 1H, J_1=6.1 Hz, J_2=3.9 Hz, H6β of α epimer), 2.64 (m, 1H, St–HN–C$H_a$$H_{a'}$–$(CH_2)_2$–NH), 2.85 (m, 1H, St–HN–$CH_a$$H_{a'}$–$(CH_2)_2$–NH), 3.44 (m, 1H, H3α), 3.61 (t, 2H, J=5.7 Hz, –HN–CH_2–$(CH_2)_2$–CN), 4.30 (t, 2H, J=6.2 Hz, St–NH–$(CH_2)_2$–CH_2–NH–), 8.69 (br s, 1H, St–N*H*–, D_2O exchange). ^{13}C NMR ($CDCl_3$): δ: 12.5, 15.4; 16.7, 19.0; 20.9, 22.9, 23.2, 24.1, 24.6, 25.6, 28.4, 28.2, 30.8, 33.4, 33.6, 35.0, 35.8, 36.0, 36.5, 39.9, 43.0, 44.8, 46.1, 46.6, 48.3, 49.5, 52.0, 56.3, 56.9, 60.2, 72.2, 120.3. MS-EI: m/z=487 (20%, St–NH–$(CH_2)_3$–NH–CH_2–CH_2^+), 473 (4%, St–NH–$(CH_2)_3$–NH–CH_2^+), 416 (29%, St–NH–CH_2 $^+$), 402 (14%, St–NH^+), 315 (100%).

4.1.16. 6α-*N*-[3*N*-(4-Aminobutyl)aminopropyl]aminocholestanol II. Nitrile **15** (0.070 g, 0.132 mmol) was reduced in the same manner as **7** to yield (0.025 g, 35%).^{1}H NMR ($CDCl_3$, 250 MHz): δ: 0.67 (s, 3H, Me 18), 0.84 (d, 6H, J=6.6 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 0.95 (s, 3H, Me 19), 2.45 (m, 1H, St–HN–C$H_a$$H_{a'}$–$(CH_2)_2$–NH–), 2.54 (m, 1H, H6β of α epimer), 2.69 (m, 1H, St–HN–$CH_a$$H_{a'}$–$(CH_2)_2$–NH–), 2.79 (t, 2H, J=6.3 Hz, –HN–$(CH_2)_3$–CH_2–NH_2), 3.15 (t, 2H, J=9.2 Hz, St–HN–$(CH_2)_2$–CH_2–NH–), 3.62 (m, 1H, H3α), 7.12 (br s, 2H, –NH2, D_2O exchange), 7.89 (br s, 1H, –NH–,

D_2O exchange), 8.25 (br s, 1H, –NH–, D_2O exchange). ^{13}C NMR ($CDCl_3$): δ: 12.0, 16.2, 18.6, 21.0, 22.5, 22.8, 23.8, 24.4, 24.8, 28.0, 28.2, 30.4, 30.5, 31.6, 35.6, 35.8, 36.0, 36.1, 38.7, 38.9, 39.5, 40.0, 42.5, 42.6, 47.3; 47.8, 48.9, 50.6, 51.1, 54.8, 56.3, 59.2, 71.9. MS-EI: m/z (%)=473 (4%, St–NH–$(CH_2)_3$–NH–CH_2^+), 444 (5%, St–NH–$(CH_2)_2$–CH_2^+), 430 (10%, St–NH–CH_2–CH_2^+), 416 (40%, St–NH–CH_2^+), 402 (100%, St–NH^+), 387 (72%, St^+). Anal. Calcd for $C_{34}H_{65}N_3O$: C 76.77; H 12.32; N 7.90. Found: C 76.72; H 12.38; N 7.93.

4.1.17. 3β-Acetyl-7α,β-azidocholesterol 16. Trimethylsilyl azide (15 mL, 104.9 mmol) was added dropwise to a solution of cholesteryl acetate (4.5, 10.5 mmol) and lead (IV) acetate (9.45 g, 21 mmol) in methylene chloride (40 mL). The mixture was stirred for 2 h. the solution was diluted with water and the precipitate lead (II) azide was removed by fitration, and decomposed with sodium nitrite/dilute hydrochloric acid. The organic layer was dried over sodium sulfate and evaporated. The crude product was purified by chromatography (hexane/ethyl acetate 8:2) to give 3.4 g (68% of epimeric mixture: α epimer 77% and β epimer 23%) of **16** as colorless oil. IR: ν: 2103 (N_3 azide) and 1727 (C=O ester). ^1H NMR ($CDCl_3$, 250 MHz): δ_H=0.67 (s, 3H, 18-Me), 0.87 (dd, 6H, J=6.6, 1.92 Hz, $CH(CH_3)_2$), 0.92 (d, 3H, J=6.6 Hz, 21-Me), 1.03 (s, 3H, 19-Me), 2.04 (s, 3H, CH_3–COO), 3.27 (ddd, 0.23H, $J_{7\alpha-8}$=8.5 Hz, $J_{7\alpha-6}$=1 Hz and $J_{7\alpha-4}$=1.5 Hz, H-7α of β epimer), 3.5 (ddd, 0.77H, $J_{7\beta-8}$=4.5 Hz, $J_{7\beta-6}$=5 Hz and $J_{7\beta-4}$=1.5 Hz, H-7β of α epimer), 4.66 (m, 1H, 3-H), 5.30 (dd, 0.23H, $J_{6-7\alpha}$<1 Hz and J_{6-4}=1 Hz, 6-H of β epimer), 5.56 (dd, 0.77H, $J_{6-7\beta}$=5.1 Hz and J_{6-4}=1.6 Hz, 6-H of α epimer). ^{13}C NMR ($CDCl_3$): δ: 12.4, 12.7, 19.0, 21.8, 22.9, 23.2, 24.2, 24.5, 28.0, 28.4, 28.5, 36.1, 36.2, 36.5, 39.2, 39.9, 40.0, 43.2, 49.7, 54.6, 54.3, 58.1, 72.3, 120.2, 149.3, 171.0. MS-EI: m/z=469 (5%, $M^{+\cdot}$), 441 (100%, $M^{+\cdot}-N_2$).

4.1.18. 7α,β-Aminocholesterol 17. Under nitrogen atmosphere, solution of **16** (1 g, 2.13 mmol) in tetrahydrofuran (10 mL) was added dropwise over 10 min to a stirred suspension of lithium aluminium hydride (1 g, 2.13 mmol) in tetrahydrofuran (20 mL) at 0 °C. The mixture was refluxed for 4 h, after which it was cooled and quenched by careful addition of saturated aqueous sodium sulfate. The solution was filtered, dried and evaporated under reduced pressure. The crude product was purified by column chromatography (methylene chloride/methanol/ammoniaque 8:1.8:0.2) to give 7α,β-aminocholesterol (0.93 g, 70%) as amorphous white solid. IR: ν: 3445–3000 (OH alcohol and NH_2 amine). ^1H NMR ($CDCl_3$, 400 MHz), δ: 0.69 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.92 (d, 3H, J=6.5 Hz, Me 21), 1.05 (s, 3H, Me 19), 3.49 (m, 1H, H3),, 3.54 (ddd, 1H, $J_{7\alpha-8}$=8.5 Hz, $J_{7\alpha-6}$=1.2 Hz, $J_{7\alpha-4}$=1.5 Hz, H7α of β epimer), 3.58 (ddd, 1H, $J_{7\beta-8}$=4.8 Hz, $J_{7\beta-6}$=5.3 Hz, $J_{7\beta-4}$=1.2 Hz, H7β of α epimer), 4.62 (br s, 2H, NH_2, D_2O exchange), 5.55 (dd, 1H, $J_{6-7\beta}$=5.2 Hz, J_{6-4}<1 Hz, H6 of α epimer), 5.26 (dd, 1H, $J_{6-7\alpha}$=1.2 Hz, J_{6-4}<1 Hz, H6 of β epimer). ^{13}C NMR ($CDCl_3$): δ: 12.1, 15.2, 18.8, 21.8, 22.7, 23.9, 26.2, 28.1, 28.3, 29.8, 35.9, 36.2, 37.1, 37.4, 39.6, 39.8, 41.8, 42.9, 43.5, 46.5, 52.2, 49.6; 53.5; 56.3, 71.6, 128.6, 141.0. MS-EI: m/z=401 (71%, $M^{+\cdot}$), 384 (4%, $M^{+\cdot}-NH_3$), 351 (29%), 289 (100%). Anal. Calcd for $C_{27}H_{47}NO$ (401.68): C 80.7, H 11.7, N 3.5. Found: C 80.3, H 11.3, N 3.2.

4.1.19. 7α-*N*-(2-Cyanoethyl)aminocholesterol 18a and 7β-*N*-(2-cyanoethyl)aminocholesterol 18b. A solution of amine **17** (0.714 g, 1.77 mmol) and acrylonitrile (0.8 mL, 15 mmol) in methanol (5 mL) was stirred at room temperature for 24 h. The solvent was evaporated giving the crude product which was purified by chromatography (hexane/ethyl acetate) to afford 0.5 g (66%) of **18a** and **18b**, respectively, 70% (α) 30% (β) as amorphous solid.

Compound **18a**. IR: ν: 3481–3314 (OH alcohol and NH amine); 2254 (CN). ^1H NMR ($CDCl_3$, 250 MHz): δ: 0.68 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 1.05 (s, 3H, Me 19), 2.40 (m, 2H, St–NH–$CH_aH_{a'}$–CH_2–CN), 2.69 (dd, 1H, $J_{7\beta-6}$=7.25 Hz, $J_{7\beta-8}$=4.2 Hz, H7β of α epimer), 2.77 (m, 1H, St–NH–$CH_aH_{a'}$–CH_2–CN), 3.08 (m,1H, St–NH–$CH_aH_{a''}$–CH_2–CN), 3.49 (m, 1H, H3α), 5.55 (dd, 1H, $J_{6-7\beta}$=5 Hz, J_{6-4}<1 Hz, H6 of α epimer). ^{13}C NMR ($CDCl_3$): δ: 12.1, 19.1, 19.4, 19.8, 22.9, 23.2, 24.1, 26.7, 28.1, 28.3, 29.9, 35.9, 36.3, 37.1, 39.6, 39.8, 41.0, 42.9, 43.0, 44.2, 47.6, 54.4, 56.3, 56.4, 60.2, 71.6, 119.2, 124.0, 143.5. MS-EI: m/z=454 (22%, $M^{+\cdot}$), 385 (100%, St^+).

Compound **18b**. IR: ν: 2934 (CH_2), 2249 (CN). ^1H NMR ($CDCl_3$, 250 MHz): δ: 0.68 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.93 (d, 3H, J=6.5 Hz, Me 21), 1.02 (s, 3H, Me 19), 2.39 (m, 2H, St–NH–CH_2–CH_2–CN), 2.66 (m, 1H, St–NH–$CH_aH_{a'}$–CH_2–CN), 2.77 (ddd, 1H, $J_{7\alpha-8}$=8.3 Hz, $J_{7\alpha-6}$=1.2 Hz, H7α of β epimer), 2.91 (m,1H, St–NH–$CH_aH_{a'}$–CH_2–CN), 3.46 (m, 1H, H3α), 5.21 (d, 1H, $J_{6-7\alpha}$=3 Hz, H6 of β epimer). ^{13}C NMR ($CDCl_3$): δ: 13.7, 18.6, 18.8, 20.8, 21.3, 22.7, 24.3, 25.1, 28.0, 29.7, 33.4, 37.4, 37.7, 37.9, 38.5, 39.7, 40.7, 41.2, 43.7, 45.0, 51.3, 54.2, 54.8, 58.5, 73.1, 120.6, 125.5, 144.7. MS-EI: m/z=454 (14%, $M^{+\cdot}$), 385 (100%, St^+).

4.1.20. 7α-*N*-(3-Aminopropyl)aminocholesterol 19a. A solution of nitrile **18a** (0.2 g, 0.44 mmol) in dry THF (5 mL) was added dropwise to a suspension of lithium aluminium hydride (0.14 g, 2.64 mmol) and nickel chloride hexahydrate (0.105 g, 0.44 mmol) in dry THF (15 mL) under argon. The mixture was refluxed for 1 h. The reaction mixture quenched with $Na_2SO_4.10H_2O$, filtered through a pad of celite, and washed with ethyl ether/n butanol (6:4). Removal of the solvent in vacuo and purification by flash chromatography (methylene chloride/methanol/ammoniaque 7:2:1) to afford a pure product **19a** (0.4 g, 69%) as amorphous solid. IR: ν: 3434 (NH_2 amine). ^1H NMR ($CDCl_3$, 250 MHz): δ: 0.66 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 0.99 (s, 3H, Me 19), 2.17 (m, 1H, St–NH–$CH_aH_{a'}$–$(CH_2)_2$–NH_2), 2.30 (m, 2H, St–NH–CH_2–CH_2–CH_2–NH_2), 2.55 (m, 1H, St–NH–$CH_aH_{a'}$–$(CH_2)_2$–NH_2), 2.74 (m, 1H, H7β of α epimer), 2.81 (t, 2H, J=5.29 Hz, St–NH–$(CH_2)_2$–CH_2–NH_2), 3.56 (m, 1H, H3α), 5.66 (dd, 1H, $J_{6-7\beta}$=4.85 Hz, J_{6-4}<1 Hz, H6 of α epimer). ^{13}C NMR ($CDCl_3$): δ: 12.0, 19.0, 19.7, 21.0, 22.9, 23.1, 24.2, 27.0, 28.3, 31.6, 34.6, 35.9, 36.5, 37.0, 37.4, 39.6, 39.8, 40.0, 42.1, 42.4, 42.6, 43.6, 47.6, 54.3, 56.3, 61.4, 71.2, 125.3, 142.9.

4.1.21. 7β-*N*-(3-Aminopropyl)aminocholesterol 19b. Nitrile **18b** (0.250 g, 0.55 mmol) was reduced in the same manner as **18a** to yield (0.180 g, 72%) of product **19b** as

amorphous solid. IR: ν: 3500–3430 (OH alcohol and NH_2 amine), 2961 (CH_2). ^{1}H NMR ($CDCl_3$, 250 MHz): δ: 0.68 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.90 (d, 3H, J=6.5 Hz, Me 21), 1.03 (s, 3H, Me 19), 2.63 (m, 1H, St–HN–CH_aH$_{a'}$–$(CH_2)_2$–NH_2–), 2.74 (m, 1H, H7α of β epimer), 2. 87 (m, 1H, St–NH–$CH_aH_{a'}$–$(CH_2)_2$–NH_2), 3.25 (t, 2H, J=5.8 Hz, St–NH–$(CH_2)_2$–CH_2–NH_2), 3.66 (m, 1H, H3α), 5.63 (br s, 1H, St–NH–, D_2O exchange). ^{13}C NMR ($CDCl_3$): δ: 13.7, 18.6, 18.8, 20.8, 21.3, 22.7, 24.3, 25.1, 28.0, 29.7, 33.4, 33.8, 37.4, 37.7, 37.9, 38.5, 39.7, 40.6, 40.7, 41.2, 43.7, 45.6, 51.3, 54.2, 59.0, 58.5, 73.1, 120.6, 122.8, 139.0.

4.1.22. 7α-*N*-[3*N*-(3-Cyanopropyl)aminopropyl]aminocholesterol 20a. Compound **20a** was obtained from amine **19a** (0.46 g, 1 mmol) as described in the preparation of compound **7** to yield (0.22 g, 42%) as a oil. IR: ν: 2935 (CH_2), 2249 (CN). ^{1}H NMR ($CDCl_3$, 250 MHz): δ: 0.68 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.92 (d, 3H, J=6.5 Hz, Me 21), 0.99 (s, 3H, Me 19), 2.40 (t, 2H, J=7.7 Hz, –CH_2–CN), 2.50 (m, 1H, St–NH–CH_aH$_{a'}$–$(CH_2)_2$–NH–), 2.62 (ddd, 1H, $J_{7\beta-8}$=4.3 Hz, $J_{7\beta-6}$=5 Hz, H7β of α epimer), 2.95 (m, 1H, St–NH–$CH_aH_{a'}$–$(CH_2)_2$–NH–), 3.5 (m, 1H, H3α), 5.62 (dd, 1H, $J_{6-7\beta}$=1.8 Hz, J_{6-4}<1 Hz, H6 of α epimer). ^{13}C NMR ($CDCl_3$): δ: 12.0, 15.2, 19.0, 19.1, 21.2, 22.9, 23.2, 24.1, 24.3, 27.2, 28.1, 28.3, 36.2, 36.6, 37.3, 37.7, 37.8, 39.4, 39.7, 40.7, 40.8, 43.0, 44.5, 46.1, 46.3, 46.8, 53.4, 56.3, 65.4, 72.0, 119.9, 121.1, 143.0. MS-EI: m/z=471 (6%, St–NH–$(CH_2)_3$–NH–CH_2^+), 428 (16%, St–NH–CH_2–CH_2^+), 400 (12%, St–NH^+), 385 (100%, St^+).

4.1.23. 7β-*N*-[3*N*-(3-Cyanopropyl)aminopropyl]aminocholesterol 20b. Amine **19b** (0.180 g, 0.40 mmol) was alkylated in the same manner as **19a** to yield 0.10 g (50%) of product **20b** as a oil. IR: ν: 2935 (CH_2), 2249 (CN). ^{1}H NMR ($CDCl_3$, 250 MHz): δ: 0.68 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.90 (d, 3H, J=6.5 Hz, Me 21), 0.93 (s, 3H, Me 19), 2.45 (t, 2H, J=7.0 Hz, St–HN–$(CH_2)_3$–NH–$(CH_2)_2$–CH_2–CN), 2.53 (m, 1H, St–NH–CH_aH$_{a'}$–CH_2–), 2.74 (ddd, 1H, $J_{7\alpha-8}$=8.7 Hz, $J_{7\alpha-6}$=1.2 Hz, H7α of β epimer), 2.86 (m, 1H, St–NH–$CH_aH_{a'}$–CH_2–), 3.53 (m, 1H, H3α), 5.31 (d, 1H, $J_{6-7\alpha}$=3.0 Hz, H6 of β epimer). ^{13}C NMR ($CDCl_3$): δ: 12.4, 15.4, 19.1, 19.4, 21.7, 22.9, 24.1, 26.8, 27.0, 28.4, 28.8, 28.9, 30.0, 32.0, 36.1, 36.4, 36.5, 37.4, 39.8 40.1, 42.4, 43.6, 44.6, 46.8, 48.3, 48.8, 55.7, 57.2, 60.4, 71.8, 120.2, 124.6, 142.6. MS-EI: m/z=471 (3%, St–NH–$(CH_2)_3$–NH–CH_2^+), 428 (26%, St–NH–CH_2–CH_2^+); 400 (21%, St–NH^+).

4.1.24. 7α-*N*-[3*N*-(4-Aminobutyl)aminopropyl]aminocholesterol III. Compound **III** (0.09 g, 0.16 mmol) was prepared in the same manner as **I** to yield (0.04 g, 46%) as amorphous solid. ^{1}H NMR ($CDCl_3$, 250 MHz): δ: 0.68 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.91 (d, 3H, J=6.5 Hz, Me 21), 0.99 (s, 3H, Me 19), 2.66 (m, 1H, St–NH–CH_aH$_{a'}$–$(CH_2)_2$–NH–), 2.74 (ddd, 1H, $J_{7\beta-8}$=4.3 Hz, $J_{7\beta-6}$=5.0 Hz, H7β of α epimer), 2.80 (t 2H, J=5.4 Hz, –HN–$(CH_2)_3$–CH_2–NH_2), 2.92 (m, 1H, St–NH–$CH_aH_{a'}$–$(CH_2)_2$–NH–), 3.00–3.48 (m, 4H, St–NH–$(CH_2)_2$–CH_2–NH– and NH–CH_2–$(CH_2)_3$–NH_2), 3.63 (m, 1H, H3α), 5.66 (dd, 1H, $J_{6-7\beta}$=1.8 Hz, J_{6-4}<1.0 Hz, H6 of α epimer), 7.23 (br s, 2H, –NH2, D_2O exchange), 8.15 (br s, 1H, –NH–, D_2O exchange), 8.44 (br s, 1H, –NH–, D_2O exchange). ^{13}C NMR ($CDCl_3$): δ: 12.1, 18.6, 18.8, 21.7, 22.7, 23.9, 25.4, 25.6, 26.3, 28.1, 28.2, 29.5, 31.7 35.9, 36.3, 37.0, 37.4, 39.6, 39.7, 40.0, 40.7, 40.8, 43.0, 46.1, 46.6, 46.8, 47.6, 54.2, 56.3, 65.4, 71.5, 122.2, 139. MS-EI (m/z)= 471 (3%, St–NH–$(CH_2)_3$–NH–CH_2^+), 442 (2%, St–NH–$(CH_2)_2$–CH_2^+), 400 (100%, St–NH^+), 385 (24%, St^+). $[\alpha]_D^{20}$ −41° (C=0.2 M in MeOH). Anal. Calcd for $C_{34}H_{63}N_3O$: C 77.35, H 11.66, N 7.96. Found: C 77.29, H 11.69, N 7.95.

4.1.25. 7β-*N*-[3*N*-(4-Aminobutyl)aminopropyl]aminocholesterol IV. Compound **IV** (0.040 g, 0.78 mmol) was prepared in the same manner as **I** to yield (0.02 g, 48%) as amorphous solid. ^{1}H NMR ($CDCl_3$, 250 MHz): δ: 0.69 (s, 3H, Me 18), 0.86 (d, 6H, J=6.5 Hz, Me 27 and Me 26), 0.90 (d, 3H, J=6.5 Hz, Me 21), 0.93 (s, 3H, Me 19), 2.37 (m, 1H, St–NH–CH_aH$_{a'}$–$(CH_2)_2$–), 2.42–2.47 (m, 2H, –HN–$(CH_2)_3$–CH_2–NH_2 and –HN–CH_2–$(CH_2)_3$–NH_2), 2.70 (m, 1H, St–NH–$CH_aH_{a'}$–$(CH_2)_2$–), 2.75 (ddd, 1H, $J_{7\beta-8}$= 4.3 Hz, $J_{7\beta-6}$=5 Hz, H7α of β epimer), 2.79 (t, 2H, J= 8.3 Hz, St–NH–$(CH_2)_2$–CH_2–NH–), 3.5 (m, 1H, H3α), 5.30 (d, 1H, J=3.0 Hz, H6 of β epimer), 7.19 (br s, 2H, –NH_2, D_2O exchange), 8.13 (br s, 1H, –NH–, D_2O exchange), 8.39 (br s, 1H, –NH–, D_2O exchange). ^{13}C NMR ($CDCl_3$): δ: 12.1, 18.6, 18.8, 21.7, 22.7, 23.9, 25.4, 25.6, 26.3 28.1, 28.2, 29.5, 31.7, 35.9, 36.3, 37.0, 37.4, 39.6, 39.7, 40.0, 40.7, 40.8, 43.0, 46.1, 46.6, 46.8, 47.6, 54.2, 56.3, 59.0, 71.5, 122.8, 139.0. MS-EI: m/z=471 (1%, St–NH–$(CH_2)_3$–NH–CH_2^+), 400 (33%, St–NH^+), 385 (100%, St^+). $[\alpha]_D^{20}$ +41° (C=0.2 M in MeOH). Anal. Calcd for $C_{34}H_{63}N_3O$: C 77.35, H 11.66, N 7.96. Found: C 77.27, H 11.71, N 7.94.

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Regioselective hydroaminomethylation of 1,1-diaryl-allyl-alcohols: a new access to 4,4-diarylbutylamines

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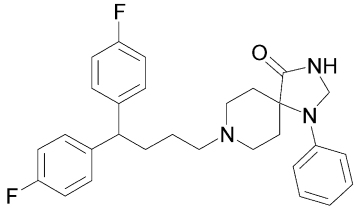
Abstract—Pharmacologically active 4,4-diarylbutylamines like Fluspirilene and 4-amino-1,1-diarylbutan-1-ols like Difenidol were prepared in high yields via rhodium catalysed hydroaminomethylation of 1,1-diaryl-allylalcohols. Conversion of these olefins with carbon monoxide, hydrogen and secondary amines proceeds with complete regioselectivity. This group can easily be removed under acidic and hydrogenating conditions, enabling the transformation of 4-amino-1,1-diarylbutan-1-ols to 4,4-diarylbutylamines in high yields. Thus Fluspirilene was synthesised in 88% yield in four steps starting from commercially available materials.

1. Introduction

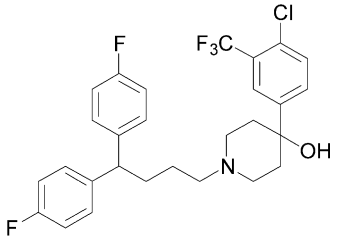
Numerous tertiary 4,4-diarylbutylamines possess therapeutical activity and are commercially obtainable therapeutic agents. Especially amines with the 4,4-bis(*p*-fluorophenyl)butyl group have been synthesised and successfully tested for pharmacological activity, among them some older neuroleptics such as Fluspirilene (**1**)[1] and Penfluridol (**2**)[2] (Fig. 1).

Most synthetic strategies leading to these 4,4-diarylbutylamines proceed via connection of a 4,4-diarylbutylhalide with the amino group.[3] The synthesis of these halides requires several steps with low overall yields, but recently one of us presented a new approach to 4,4-bis(*p*-fluorophenyl)butylbromide via hydroformylation of 1,1-bis(*p*-fluorophenyl)prop-3-en-1-ol.[4] The oxo-aldehyde obtained was transformed to the desired halide in only few steps. An earlier published route towards the halide starting from 3,3-diaryl-1-propene required several steps for the synthesis of the olefin and the hydroformylation of this olefin proceeded with poor regioselectivities.[5]

On the other hand we have presented a very short and efficient synthesis of the homologues 3,3-diarylpropylamines, which are normally prepared similarly to the 4,4-diarybutylamines.[6] In this route the amines are formed via hydroaminomethylation of 1,1-diarylethenes.[7] This tandem reaction[8] enables the synthesis of the desired 3,3-diarylpropylamines in one step with high yields by a direct reductive amination of the oxo-aldehyde formed without isolation of any intermediates. Here now we report a new synthetic route to 4,4-diarylbutylamines via direct hydroaminomethylation of 1,1-diaryl-2-propen-1-ols.



Fluspirilene
1



Penfluridol
2

Figure 1. Pharmacologically active 4,4-diarylbutylamines.

Keywords: Hydroformylation; Hydroaminomethylation; Rhodium; Homogeneous catalysis.
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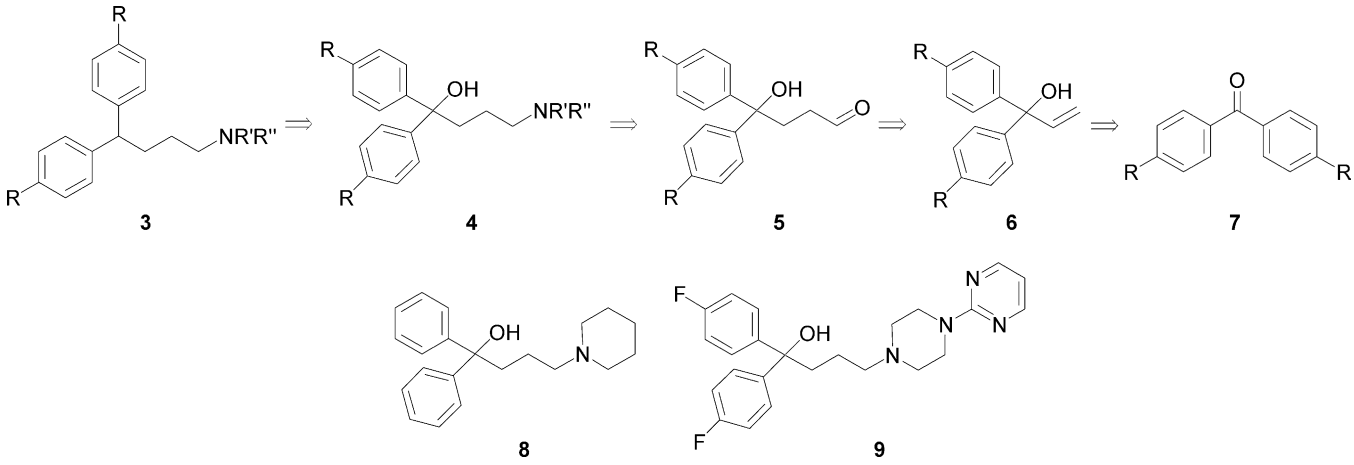


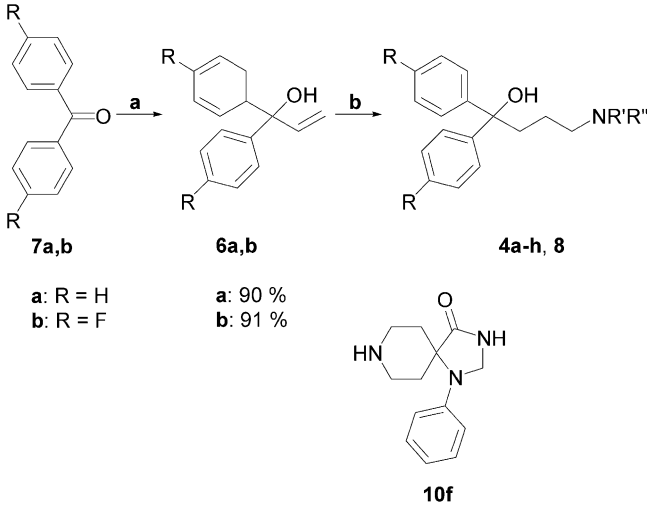
Scheme 1. Retrosynthetical analysis of 4,4-diarylbutylamines **3** and 4-amino-1,1-diaryl-1-butanols **4**.

2. Results and discussion

Based on the previously described stepwise synthesis of Fluspirilene (**1**)[4] we developed a new synthetic strategy to obtain the 4,4-diarylbutylamines **3** via the 4-amino-1,1-diarylbutan-1-ols **4**, which can be formed by reductive amination of the aldehyde **5**. This aldehyde is prepared by hydroformylation of the corresponding allylic alcohol **6**, which is obtained from the commercially available diarylketones **7** (Scheme 1).

Under hydroaminomethylation conditions[8] the aldehyde **5** is not isolated but directly converted to the amines **4**. These intermediates of our synthetic route are also interesting targets, since various 4-amino-1,1-diarylbutan-1-ols are known for pharmacological activity, like the antihistaminic agent Difenidol (**8**)[9] or the piperazine derivative **9**.[10] These aminoalcohols are normally prepared similarly to the 4,4-diarylbutylamines by reacting the heterocyclic amine with the corresponding butyl chloride.[11]

For model investigations we used both the unsubstituted benzophenone (**7a**) and *p*,*p*′-difluorobenzophenone (**7b**).



Scheme 2. Synthesis of 4-amino-1,1-diaryl-1-butanols. Reagents and reaction conditions: (a) vinyl magnesium chloride, THF, reflux; (b) amine **10a–f**, $[Rh(cod)Cl]_2$, 50 bar CO/H_2 (3:2), 120 °C, 1,4-dioxane, 45–65 h.

Both ketones were converted to the allylic alcohols **6a** resp. **6b** by reaction with the vinyl magnesium chloride solution in THF in 90% resp. 91% yield (Scheme 2). The alcohols were subjected to rhodium catalysed hydroaminomethylation with various secondary amines **10** at 120 °C and 50 bar syngas ($CO/H_2=3:2$) using the $[Rh(cod)Cl]_2$ catalyst precursor. Under these conditions all hydroaminomethylation reactions proceed with quantitative yields, giving exclusively the linear products **4**.

The high regioselectivity of the oxo-reaction[12] can be explained by the catalyst directing effect of the bulky trisubstituted carbon in the starting olefin **6**, as it was observed with similar tertiary and secondary allylic alcohols.[13] Also the high reaction tem-perature generally causes a higher *n*-selectivity of the hydroformylation with unmodified rhodium catalysts.[14] Thus small amounts of branched oxo-aldehydes or decomposition products derived thereof were observed if using unmodified rhodium catalysts at lower reaction temperatures during the hydroformylation process.[4] Phosphorous ligands are not necessary for a regioselective hydroaminomethylation reaction, probably due to the presence of the amines, which can support the catalyst directing effect of the starting olefin.

Both cyclic (**10a–10c**, **10f**) and acyclic amines (**10d**, **10e**) are tolerated (Table 1). If using 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**10f**), only the amino functionality at N-8 reacts as a nucleophile, the amide functionality at N-3 undergoes neither any condensation with the oxo-aldehyde nor any hydrogenation. Also the aminal functionality, formed by N-1 and N-3 is stable under hydroformylation conditions. Thus the aminoalcohol **4h**, a precursor of Fluspirilene (**1**) is obtained in 99% yield.

If using piperidine (**10c**) as amine, Difenidol (**8**) is formed in 99% yield. Starting from benzophenone (**6a**), Difenidol is accessible in two steps with an overall yield of 89% using only commercially available and inexpensive chemicals. This novel synthesis of Difenidol (**8**) demonstrates the applicability of the hydroaminomethylation for the synthesis of 4-amino-1,1-diarylbutan-1-ols.

For the defunctionalisation of the aminoalcohols **4** to the amines **3** several methods are described, like refluxing with

Table 1. Hydroaminomethylation of allyl alcohols **6a**,**b**[a]

Olefin		Secondary amine			Time (h)	Product	Yield, %
	R		R′	R″			
6a	H	**10a**	$-(CH_2)_2-O-(CH_2)_2-$		65	**4a**	100
6a	H	**10b**	$-(CH_2)_6-$		45	**4b**	99
6a	H	**10c**	$-(CH_2)_5-$		65	**8**	99
6a	H	**10d**	Me	Me	65	**4d**	100
6a	H	**10e**	Bzl	Bzl	65	**4e**	100
6a	H	**10f**	cf. Scheme 2		65	**4f**	100
6b	F	**10a**	$-(CH_2)_2-O-(CH_2)_2-$		45	**4g**	99
6b	F	**10f**	cf. Scheme 2		65	**4h**	99

[a] Reaction conditions: 120 °C, 50 bar syngas ($CO/H_2=3:2$), 1 equiv olefin **6**, 1–1.3 equiv amine **10**, 0.5 mol% $[Rh(cod)Cl]_2$, 10 mL 1,4-dioxane.

hydroiodic acid in the presence of red phosphorous[15] or hydrogenation with palladium on charcoal in ethanol.[4,16] Furthermore it is possible to perform the transformation in two steps by isolating the dehydration product, which can be hydrogenated by homogeneous and heterogeneous catalysts.

We decided to investigate the defunctionalisation of the model morpholine derivative **4a** under acidic and hydrogenation conditions, since this reaction can be performed at room temperature in neutral or weakly acidic media. Using ethanol as the solvent no reaction occurred, but addition of hydrochloric acid enables a selective defunctionalisation in high yields (Scheme 3). In summary, 4-(4,4-diphenyl)morpholin (**11**) is available starting from benzophenone (**7a**) in three steps with an overall yield of 87%.

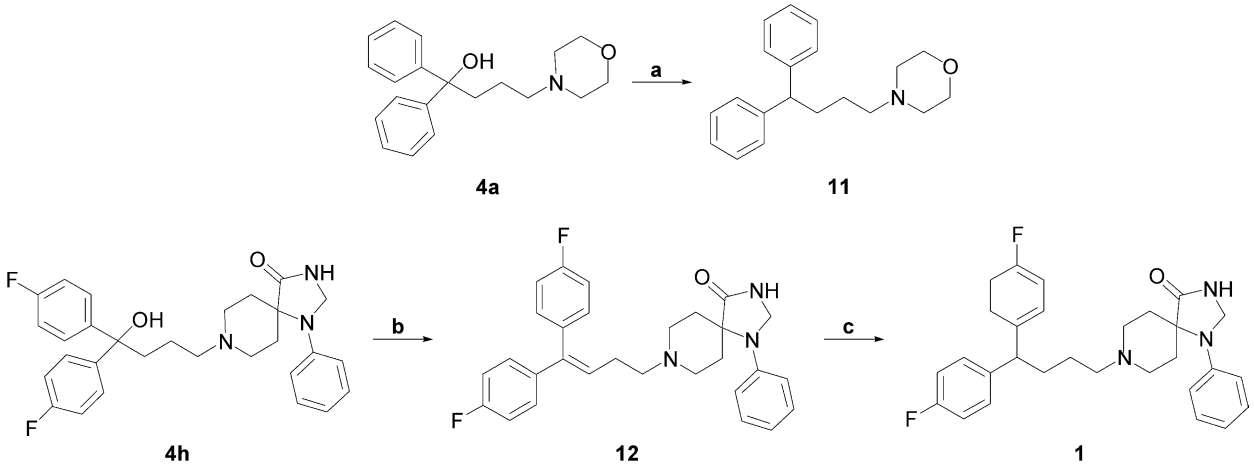
For the preparation of Fluspirilene (**1**) we tried to apply the same reaction conditions for the defunctionalisation of the intermediate **4h**. Surprisingly no conversion was observed, further optimisation also failed, but the dehydration to the olefin **12** was easily achieved. Thus we executed the reaction in two steps by dehydration of the alcohol **4h** with catalytic amounts of hydrochlorid acid in refluxing ethanol to obtain **12** in quantitative yields, followed by hydrogenation in ethanol at room temperature. This protocol enables to synthesise Fluspirilene (**1**) in a new four step reaction sequence starting from *p*,*p*′-difluorobenzo-phenone (**7b**) with an overall yield of 88%.

3. Conclusions

We have developed a convenient method for the synthesis of 4,4-diarylbutylamines via a hydroaminomethylation sequence. The use of easily obtainable 1,1-diaryl allyl alcohols allows the regioselective formation of the desired linear hydroaminomethylation products. Both the 4-amino-1,1-diarylbutan-1-ols and the 4,4-diarylbutylamines, which can be formed by dehydration of the aminoalcohol and hydrogenation of the unsaturated amine, are interesting classes of compounds. As demonstrated for the synthesis of Difenidol (**8**) and Fluspirilene (**1**) this synthetic route enables the preparation of pharmaceutical agents in excellent yields, in which both the aromatic substituents and the substituents of the amino groups can be varied. Thus our new method represents an interesting alternative to the hitherto applied methods. Upon upscaling further optimisations of catalyst effectivity (reaction times, catalyst amounts, TOF) can be achieved by use of appropriate ligands.[14,22]

4. Experimental

All chemicals were purchased from commercial sources, all solvents were dried by standard methods if necessary. The catalyst precursor $[Rh(cod)Cl]_2$ was prepared as previously described.[17] Unless otherwise noted ^1H and ^{13}C NMR spectra were recorded at room temperature with Bruker DRX 400 and DRX 500 spectrometer using $CDCl_3$ as



Scheme 3. Defunctionalisation reactions. Reagents and conditions: (a) H_2, Pd–C, ethanol/HCl, 97%; (b) HCl, ethanol, reflux, 100%; (c) H_2, Pd–C, ethanol, 98%.

solvent and TMS as internal standard. The signals were assigned using DEPT techniques. Infrared spectra were recorded with a Nicolet Impact 400 D spectrometer using neat compounds as films between NaCl plates or as disks with KBr. Mass spectra were recorded with a Finnigan CA 5 spectrometer (Electron Impact, 70 eV) and with a Finnigan ThermoQuest TSQ (ESI). Elemental analyses were performed. Analytical gas chromatography was performed with a Fisons 8130 gas chromatograph with 30-m CP sil-5 capillaries.

Pressure reactions were carried out in autoclaves (250 mL, PTFE insert) from Berghof, Eningen. After charging the autoclave with the starting material, the catalyst precursor, and the solvent, the reactor was flushed with argon, then pressurised with hydrogen and carbon monoxide at room temperature, and heated to the required reaction temperature.

4.1. Synthesis of 1,1-diphenylprop-2-en-1-ol (6a)

A solution of vinyl magnesium chloride (25 mL, 15 wt%, 40 mmol) in THF was added to a solution of benzophenone (**7a**; 3.62 g, 20 mmol) in 20 mL dry THF. After refluxing for 3 h the solution was stirred overnight at room temperature. 25 g ice were added and the mixture was diluted with a saturated solution of NH_4Cl. The phases were separated, the aqueous phase was extracted with ether. The combined etheral solution was dried over $MgSO_4$ and evaporated at reduced pressure. 3.77 g (18 mmol; 90% yield) of alcohol **6a** were obtained.[18]

4.2. Synthesis of 1,1-bis(*p*-fluorophenyl)prop-2-en-1-ol (6b)

A solution of vinyl magnesium chloride (19 mL, 15 wt%, 30 mmol) in THF was added to a solution of 4,4′-difluorobenzophenone (**7b**; 3.62 g, 20 mmol) in 20 mL dry THF. After refluxing for 3 h the solution was stirred overnight at room temperature. 25 g ice were added and the mixture was diluted with a saturated solution of NH_4Cl. The phases were separated, the aqueous phase was extracted with ether. The combined etheral solution was dried over $MgSO_4$ and evaporated at reduced pressure. 3.35 g (14 mmol; 91% yield) of alcohol **6b** were obtained as a yellow oil.[4]

4.3. General procedure for the hydroaminomethylation of allylic alcohols 6

A mixture of the olefin **6**, the corresponding amine **10** and $[Rh(cod)Cl]_2$ (6 mg, 12 μmol) in 10 mL anhydrous 1,4-dioxane was heated at 120 °C for the reaction time given below in an autoclave under 30 bar carbon monoxide and 20 bar hydrogen. Following the reaction, the catalyst was filtered off by passage through a small pad of basic alumina (activity II–III) and the solvent was removed by rotary evaporation. The crude product was crystallised from ethanol.

4.3.1. 4-Morpholin-4-yl-1,1-diphenylbutan-1-ol (4a). Hydroaminomethylation of olefin **6a** (421 mg, 2.0 mmol) with morpholin (**10a**; 0.2 mL, 2.3 mmol) gave 620 mg (2.0 mmol, 100% yield) of aminoalcohol **4a**.[19] ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.63 (quint, 2H, ^3J=5.5 Hz), 2.27 (br s, 4H), 2.39 (dd, 2H, ^3J=5.5, 5.5 Hz), 2.48 (dd, 2H, ^3J=5.5, 5.5 Hz), 3.70 (t, 4H, ^3J=4.8 Hz), 7.16 (m, 2H), 7.27 (m, 4H), 7.49 (d, 4H, ^3J=7.3 Hz), 8.15 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=21.1, 42.5, 53.1, 59.5, 66.3, 76.6, 126.1, 126.2, 127.9, 148.1.

4.3.2. 4-Azepan-1-yl-1,1-diphenylbutan-1-ol (4b). Hydroaminomethylation of olefin **6a** (315 mg, 1.5 mmol) with hexamethylenimine (**10b**; 0.23 mL, 2.0 mmol) gave 482 mg (1.5 mmol, 99% yield) of aminoalcohol **4b**. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.54–1.75 (m, 12H), 2.42–2.48 (m, 6H), 7.15 (t, 2H, ^3J=7.3 Hz), 7.26 (m, 4H), 7.50 (d, 4H, ^3J=8.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ=22.9, 26.4, 26.7, 42.7, 55.7, 59.1, 76.4, 126.0, 126.2, 127.7, 148.6. MS (ESI): *m/z* (%)=324 (100, M^++1).

4.3.3. 1,1-Diphenyl-4-piperidin-1-ylbutan-1-ol (Difenidol, 8). Hydroaminomethylation of olefin **6a** (315 mg, 1.5 mmol) with piperidin (**10c**; 0.2 mL, 2.0 mmol) gave 460 mg (1.5 mmol, 99% yield) of aminoalcohol **8**[20] as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.43–1.62 (m, 8H), 2.22 (br s, 4H), 2.31 (dd, 2H, ^3J=5.5, 5.5 Hz), 2.47 (dd, 2H, ^3J=5.5, 5.5 Hz), 7.13–7.38 (m, 6H), 7.50 (dd, 4H, ^3J=8.6 Hz, ^4J=1.3 Hz), 7.96 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=21.7, 24.1, 25.2, 42.7, 54.1, 59.8, 76.4, 126.0, 126.2, 127.8, 148.5. MS (ESI): *m/z* (%)=310 (100, M^++1).

4.3.4. 4-Dimethylamino-1,1-diphenylbutan-1-ol (4d). Hydroaminomethylation of olefin **6a** (430 mg, 2.0 mmol) with a solution of dimethylamine (**10d**) in ethanol (1 mL, *c*=5 mol/L, 2.0 mmol) gave 550 mg (2.0 mmol, 100% yield) of aminoalcohol **4d**[21] as a white solid. Mp 102 °C. IR (disk): ν [cm^{-1}]=3423, 3058, 2954, 2942, 2785, 2638, 1658, 1597, 1487, 1447, 1252, 1179, 1065, 1017, 777, 744, 707. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.56 (quint, 2H, ^3J=5.8 Hz), 2.09 (s, 6H), 2.32 (dd, 2H, ^3J=5.8, 5.8 Hz), 2.48 (dd, 2H, ^3J=5.8, 5.8 Hz), 5.81 (s, 1H), 7.13–7.38 (m, 6H), 7.49 (d, 4H, ^3J=7.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ=22.5, 42.2, 44.7, 59.9, 76.2, 126.0, 126.1, 127.8, 128.4. MS (ESI): *m/z* (%)=310 (100, M^++1).

4.3.5. 4-Dibenzylamino-1,1-diphenylbutan-1-ol (4e). Hydroaminomethylation of olefin **6a** (315 mg, 1.5 mmol) with dibenzylamine (**10e**; 0.38 mL, 2.0 mmol) gave 630 mg (1.5 mmol, 100% yield) of aminoalcohol **4e** as a yellow solid. Mp 90 °C. IR (disk): ν [cm^{-1}]=3435, 3060, 3028, 2936, 2817, 1599, 1495, 1450, 1383, 1376, 1247, 1168, 1103, 1055, 1027, 990, 734, 698. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.60 (quint, 2H, ^3J=6.3 Hz), 2.25 (t, 2H, ^3J=6.3 Hz), 2.42 (t, 2H, ^3J=6.3 Hz), 3.48 (s, 4H), 7.15–7.44 (m, 20H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=21.6, 41.4, 53.1, 58.0, 77.3, 126.3, 126.9, 127.1, 127.9, 128.4, 129.6, 140.2, 147.9. MS (EI, 70 eV): *m/z* (%)=421 (10, M^+), 287 (8), 210 (100), 196 (9), 183 (12), 161 (3), 148 (7), 118 (3), 105 (20), 91 (87), 77 (8). $C_{30}H_{31}NO$ (421.6): calcd. C 85.5, H 7.4, N 3.8; found C 84.9, H 7.5, N 3.8.

4.3.6. 8-(4-Hydroxy-4,4-diphenylbutyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (4f). Hydroaminomethylation of olefin **6a** (315 mg, 1.5 mmol) with 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**10f**; 0.38 mL, 2.0 mmol)

gave 680 mg (1.5 mmol, 100% yield) of aminoalcohol **4f** as a white solid. Mp 80 °C. IR (disk): ν [cm^{-1}]=3391, 3204, 3958, 2926, 2848, 1705, 1600, 1502, 1371, 1308, 1264, 1189, 748, 698. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.60–1.77 (m, 4H), 2.48 (dd, 2H, ^3J=5.1, 5.2 Hz), 2.54 (dd, 2H, ^3J=5.2, 5.3 Hz), 2.64–2.91 (m, 6H), 3.70 (s, 1H), 4.72 (s, 2H), 6.87–7.60 (m, 15 H), 9.05 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=22.1, 28.3, 42.4, 49.3, 58.8, 58.9, 59.2, 76.2, 114.3, 118.5, 125.9, 126.0, 127.9, 129.4, 142.9, 148.8, 177.9. MS (ESI): *m/z* (%)=456 (100, M^+).

4.3.7. 1,1-Bis-(*p*-fluorophenyl)-4-morpholin-4-yl-butan-1-ol (4g). Hydroaminomethylation of olefin **6b** (407 mg, 1.7 mmol) with morpholine (**10a**; 0.15 mL, 1.7 mmol) gave 585 mg (1.7 mmol, 99% yield) of aminoalcohol **4g**. IR (film): ν [cm^{-1}]=3410, 3068, 2956, 2921, 2856, 2818, 1662, 1601, 1506, 1457, 1446, 1305, 1268, 1223, 1158, 1117, 1013, 837. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.62 (quint, 2H, ^3J=5.5 Hz), 2.29 (br s, 4H), 2.40 (dd, 2H, ^3J=5.5, 5.5 Hz), 2.43 (dd, 2H, ^3J=5.5, 5.5 Hz), 3.69 (t, 4H, ^3J=4.8 Hz), 6.97 (td, 4H, ^3J=2.0, 8.8 Hz), 7.43 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=21.1, 42.8, 53.0, 59.4, 66.2, 76.0, 114.7 ($J_{C,F}$=21 Hz), 127.7 ($J_{C,F}$=8 Hz), 143.8 ($J_{C,F}$=3 Hz), 161.4 ($J_{C,F}$=243 Hz). MS (ESI): *m/z* (%)=348 (100, M^++1).

4.3.8. 8-[4,4-Bis(-*p*-fluorophenyl)-4-hydroxybutyl]-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (4h). Hydroaminomethylation of olefin **6b** (369 mg, 1.5 mmol) with 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**10f**; 400 mg, 1.7 mmol) gave 730 mg (1.5 mmol, 99% yield) of aminoalcohol **4h** as a white solid. Mp 203 °C. IR (disk): ν [cm^{-1}]=3381, 3118, 3072, 2973, 2952, 2930, 2857, 1707, 1599, 1502, 1369, 1223, 1155, 822, 748, 558. ^{1}H NMR (500 MHz, $CDCl_3$): δ=1.63–1.69 (m, 4H), 2.48 (m, 4H), 2.65–2.85 (m, 6H), 3.71 (s, 1H), 4.74 (s, 2H), 6.88–7.06 (m, 7H), 7.36–7.39 (m, 3H), 7.50–7.52 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$): δ=22.1, 28.3, 42.7, 49.3, 58.8, 58.8, 59.2, 75.6, 114.5, 114.7 ($J_{C,F}$=20 Hz), 118.7, 127.5 ($J_{C,F}$=9 Hz), 129.4, 142.9, 144.5, 161.3 ($J_{C,F}$=233 Hz), 177.9. MS (ESI): *m/z* (%)=492 (100, M^+). $C_{29}H_{31}F_2N_3O_2$ (491.6): calcd. C 70.9, H 6.4, N 8.6; found C 70.4, H 6.6, N 8.4.

4.4. Direct synthesis of 4-(4,4-diphenylbutyl)-morpholin (11) from 4a

A suspension of aminoalcohol **4a** (65 mg, 0.21 mmol) and palladium on charcoal (200 mg, 10 wt%) in 50 mL ethanol and 1 mL concentrated hydrochlorid acid was stirred for 3 days under a hydrogen atmosphere (1.5 bar). The catalyst was removed by suction, the filtrate was neutralised with a diluted solution of $NaHCO_3$ and extracted with ether. The etheral solution was dried over $MgSO_4$ and evaporated at reduced pressure to obtain 60 mg (0.20 mmol, 97% yield) of amine **11**. ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.47 (quint, 2H, ^3J=7.8 Hz), 2.07 (q, 2H, ^3J=7.8 Hz), 2.35–2.41 (m, 6H), 3.69 (t, 4H, ^3J=4.5 Hz), 3.89 (t, 1H, ^3J=7.8 Hz), 7.14–7.29 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$): δ=24.9, 33.4, 51.3, 53.6, 58.8, 66.8, 126.1, 127.8, 128.4, 144.9.

4.5. Synthesis of Fluspirilene (1)

A solution of **4h** (305 mg, 0.62 mmol) and 1 mL concentrated hydrochlorid acid in 50 mL ethanol was refluxed for 4 h. The solution was neutralised with a diluted solution of NaOH and extracted with dichloromethane. The extraction was dried over $MgSO_4$ and evaporated at reduced pressure to obtain 293 mg (0.62 mmol, 100% yield) of 8-[4,4-bis-(4-fluorophenyl)-but-3-enyl]-1-phenyl-1,3,8-triazaspiro[4.5]-decan-4-one (**12**).

The olefin **12** (280 mg, 0.59 mmol) was dissolved in 50 mL ethanol, palladium on charcoal (150 mg, 10 wt%) was added and the suspension was stirred for 3 days at room temperature under a hydrogen atmosphere (1.5 bar). The catalyst was removed by suction and the filtrate was evaporated at reduced pressure to obtain 275 mg (0.58 mmol, 98% yield) of Fluspirilene (**1**).[4]

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Functional rearrangement of polychlorinated pyrrolidin-2-ones to 5-imino-lactams promoted by *n*-propylamine

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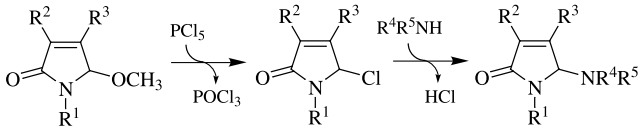
Abstract—The reaction of 4-methyl-pyrrolidin-2-ones, chlorinated at the C(3) and C(6) positions, with *n*-propylamine constitutes a new method for the preparation of 5-propylimino-pyrrolidin-2-ones or 3-pyrrolin-2-ones in generally good yields. The transformation involves a series of eliminations, substitutions and double bond shifts. This constitutes a remarkable example of a functional rearrangement.

1. Introduction

Pyrrolidin-2-ones (γ-lactams) and 3-pyrrolin-2-ones incorporating a nitrogen substituent at the C(5) carbon are important structural motifs, which are found in numerous biologically active compounds. This includes herbicides and plant growth regulators,[1–10] nootropic agents,[11] cognition enhancing pharmaceuticals,[12] antidepressants[13,14] and azamitosane systems.[15,16] Moreover, some γ-lactams have found application in the assembly of polymers with semiconducting properties.[17]

In spite of the current interest in these types of compounds, only a few preparative routes have been described in the literature. The most common method exploits the cyclization of succinic or maleic acid derivatives, typically succinic or maleic monoamides,[18–20] β-cyanoamides,[21] succinic diamides or amidines,[22,23] succinonitriles,[24,25] 3-cyanopropanoates[26] and, for analogy, 4-oxobutanoate esters.[11] Other interesting approaches to these compounds involve a three-component reaction using α-aminoalkenes, isonitriles and isocyanates,[27] and the rearrangement of *N*-acyl-*N*′-enylhydrazines.[28] Alternatively, the nitrogen appendage can be introduced at the C(5) position through the manipulation of preexisting functional groups, as in the case of alkylimino-de-oxo-bisubstitution of succinic or maleic imides,[13,15] and autoxidation of pyrrole systems.[29] Of particular promise, in this context, is the nitrogenation of γ-lactams that carry a C(5)-halogen atom by reaction with *N*-nucleophiles. This approach was originally investigated by Foucaud[30,31] and has recently been re-evaluated by Nikitin and Andryukhova.[32] Interestingly, the Russian researchers obtained 5-amino-1,5-dihydro-2*H*-pyrrol-2-ones in only two steps from the 5-methoxy analogues. This involved conversion of the 5-methoxy analogues into the corresponding 5-chloro intermediates (Scheme 1).[33]



Scheme 1.

During our recent studies on the synthesis and reactivity of 4-methyl-pyrrolidin-2-ones (**A**) chlorinated at the C(3) and C(6) positions, we disclosed that when treated with a solution of alkaline methoxide (in methanol under mild conditions), these compounds were converted into the corresponding *N*-substituted 5-methoxy or 5,5-dimethoxy-4-methyl-3-pyrrolin-2-ones (**B**) (Scheme 2).[34] The transformation involves a series of eliminations/substitutions that give rise to a remarkable functional rearrangement (FR) in which the oxidation state of the starting molecule is preserved and the functionalities repositioned. In practice, two or three of the C–Cl groups at the C(3) and C(6) positions of **A** are replaced by a double bond at C(3)–C(4) and, respectively, one or two methoxy groups at C(5). The

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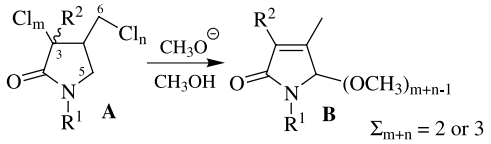
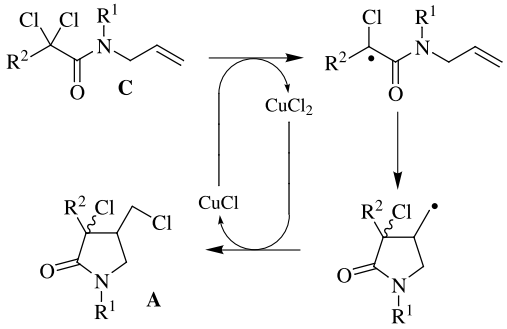


Scheme 2.

starting polyhalogenated pyrrolidin-2-ones **A** can be easily prepared, as a mixture of *cis*- and *trans*-isomers, by the atom transfer radical cyclization (ATRC) of *N*-allyl α-perchloro amides **C** (Scheme 3).[35] This reaction is typically mediated by redox catalysts, such as the complex between CuCl and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA).[36,37] The C(3) stereocentre of the lactams is configurationally unstable under the reaction conditions and the thermodynamically more stable diastereoisomer predominates. This diastereoisomer has the larger substituents at the C(3) and C(4) positions on the opposite faces of the ring.[35]



Scheme 3.

The effectiveness and generality of the FR of polychlorinated pyrrolidin-2-ones with alkaline alcoholates prompted us to investigate if alternative nucleophilic/basic systems could be equally effective. Of particular interest was the incorporation of nitrogen nucleophiles in the functional rearrangement as this could allow the direct introduction of a nitrogen atom at the unfunctionalized C(5) position. This would avoid the need to start from C(5)-oxygen substituted γ-lactams.[13,15,30–32] Previous studies of alcohols with polychlorinated pyrrolidinones showed the importance of the alcohol structure (on increasing the size of the alkyl group in the alcohol from methyl to ethyl, yields decrease to some extent) and reaction conditions in the FR (e.g., no FR occurred when methanol was combined with a weak base, like carbonate).[34] Based on this work, amines appeared to be the most appropriate nitrogen nucleophiles with which to study the functional rearrangement of polychlorinated pyrrolidin-2-ones.

Herein we report, for the first time, our findings on the reaction of various chlorinated γ-lactams with *n*-propylamine (PA). The choice of PA was made on the basis of the low boiling point (48 °C) of this amine. It can easily be evaporated from reaction mixtures and so can play a combined role as reagent and solvent. As a consequence of the FR using PA, the 5-imino functional group was, in each case considered, introduced on the γ-lactam ring in generally good yield.

2. Results and discussion

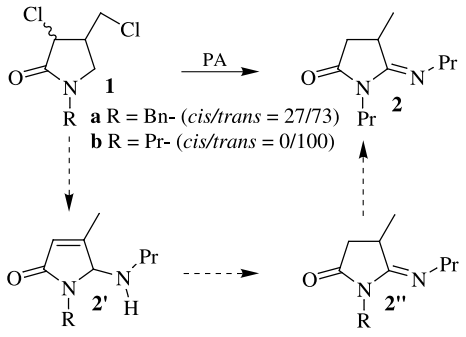
Our preliminary studies on the viability of the FR involved reaction of *N*-benzyl-3-chloro-4-chloromethyl-pyrrolidin-2-one (**1a**) with PA at different temperatures and concentrations (Table 1). Unexpectedly, in all cases, *N*-propyl-4-methyl-5-propylimino-pyrrolidin-2-one (**2**) was recovered as the main product (Scheme 4). This result contrasts with the reaction of **1a** with sodium methoxide in methanol, which led to 5-methoxy-4-methyl-3-pyrrolin-2-one.[34] For reaction with PA, the double bond of the FR product is located between C(5) and the exocyclic nitrogen and not, as foreseen, between the C(3) and C(4) positions.

The discrepancy may be explained by the higher valency of nitrogen compared to oxygen which, when an NHPr group residues at C(5), should permit the double bond to move from C(3)–C(4) to form an imine bond at C(5). We believe that FR of **1a** with PA should then provide the predicted *N*-benzyl-4-methyl-5-propylamino-3-pyrrolin-2-one (**2′a**), but as a transient product, which immediately undergoes double bond isomerisation. Indeed, when **1a** was reacted with $(CH_3CH_2)_2NH$ (DEA), a secondary amine, *N*-benzyl-4-methyl-5-diethylamino-3-pyrrolin-2-one (**3**) was obtained

Table 1. Reaction of **1a** with PA[a]

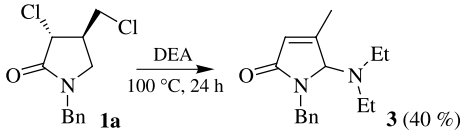
Entry	PA (mL)	*T* (°C)	Conv. (%)[b]	**2** (%)[c]
1	2.5	60	100	28
2	2.5	80	100	40
3	2.5	120	100	47
4	2.5	140	100	48
5	5	140	100	54

[a] 1 mmol of substrate was used; reaction time 24 h.
[b] GC values.
[c] Yield determined on isolated material.

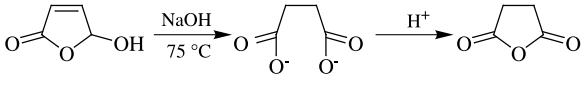


Scheme 4.

as the main product (Scheme 5). In this case, as the exocyclic nitrogen in the intermediate pyrrolin-2-one is unable to form a double bond at C(5), so the unsaturation has to remain inside the ring. Further evidence that the FR of **1a** with PA involves intermediate **2′a** comes from the related, and recently observed, rearrangement of 5-hydroxy-2(5H)-furanone to succinic anhydride under basic conditions (Scheme 6).[38] In this reaction, double bond isomerisation was observed to form an intermediate dicarboxylate.



Scheme 5.



Scheme 6.

Another remarkable feature of this reaction is replacement of the *N*-benzyl group in **1a** with a propyl appendage in product **2**. Evidently, during the course of the reaction, most likely following formation of the 5-imino lactam **2″a**, substitution of the benzylamine moiety with *n*-propylamine takes place (Scheme 4).[39,40] As a consequence, two different primary amines come to share the same reaction mixture. This resulted in the formation of a variety of byproducts that contained the benzylic group (as indicated by GC-MS), unless forcing conditions, such as high temperature (140 °C) and relatively large volumes of *n*-propylamine (5 mL/mmol) were employed.

As a consequence of the facile substitution of the *N*-benzyl group in **1a**, we decided to study the FR of polychlorinated pyrrolidin-2-ones bearing an *N*-propyl chain. In this way, any exchange of the *N*-substituent with PA would not generate a new product. This tactic allowed us to investigate the FR under mild reaction conditions. Indeed, the FR of pyrrolidin-2-one **1b** with PA (Scheme 4) was observed at temperatures as low as 40 °C (Table 2). Moreover, when using 2 mL of PA to 1 mmol of **1b**, the 5-imino pyrrolidin-2-one **2** was isolated in a respectable 57% yield (Table 2, entry 7).

Gratified by these preliminary results and with a view to assessing the scope of the method, the four chlorinated pyrrolidin-2-ones **4**–**7** were prepared in high yield through the HATRC of the respective *N*-allyl amides with CuCl/TMEDA.

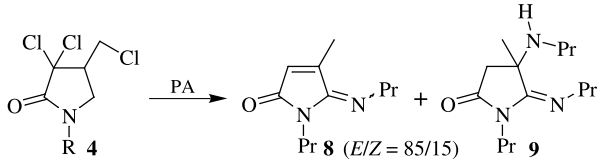
The FR of pyrrolidin-2-one **4** at 40 °C with 2 mL of *n*-propylamine per mmol of substrate, gave the 5-imino pyrrolidin-2-one **9** as the main product together with a small amount of the expected γ-lactam **8** (Scheme 7; Table 3, entry 5). It is likely that lactam **9** is formed by a highly regioselective Michael-type addition of PA onto the C=C bond of **8**. When the temperature of the reaction was raised, and the mixture heated at 80 °C, only the Michael-type adduct was isolated (Table 3, entry 6). In order to avoid the Michael-type addition, the reaction was carried out at lower temperatures. Pleasingly, following reaction of **4** with PA at −13 °C for 4 h, only the 3-pyrrolin-2-one **8** was isolated in a respectable yield of 63% (Table 3, entry 1).

When substrate **5** was heated with PA (2 mL/mmol of substrate) for 16 h at 40 °C, some starting material remained and a variety of products were formed. From the crude reaction mixture we isolated the FR adduct **10**, in low yield (6%), and *N*-propyl-3-methyl-4-(propylamino)methyl-3-pyrrolin-2-one (**11**) as the main product (Scheme 8; Table 3, entry 7). On increasing the reaction temperature to 80–100 °C, it was possible to obtain complete conversion of starting material and to improve the yield of **10** to 29% (Table 3, entry 8 and 9), whereas the yield of **11** remained well above 50%. The unwanted product **11** could derive from one, or more, of the three possible paths outlined in Scheme 9, wherein an amino-de-halogenation step is variously combined with a dehydrohalogenation step.

Table 2. Reaction of **1b** with PA[a]

Entry	PA (mL)	*t* (h)	*T* (°C)	Conv. (%)[b]	**2** (%)[c]
1	1	24	20	73	28
2	1	24	40	100	48
3	1	16	40	97	45
4	1	24	60	100	42
5	1	24	80	100	38
6	1	24	100	100	43
7	2	24	40	99	57

[a] 1 mmol of **1b** was used.
[b] GC values.
[c] Yield determined on isolated material.



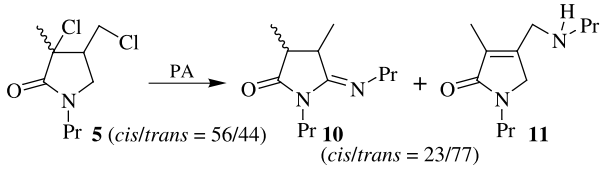
Scheme 7.

As far as the γ-lactam **6** is concerned, the FR with *n*-propylamine (2 mL/mmol of substrate) at 40 °C was disappointing. The rearranged product, *N*-propyl-3,4-dimethyl-5-propylimino-3-pyrrolin-2-one (**12**), was isolated

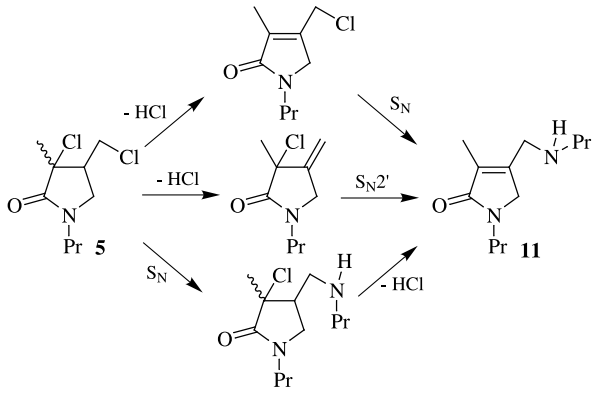
Table 3. Reaction of pyrrolidin-2-ones **4–7** with PA[a]

Entry	Substrate	*t* (h)	*T* (°C)	Conv. (%)[b]	Products (%)[c]
1[d]	**4**	4	−13	100	**8** (63); **9** (0)
2	**4**	16	−13	100	**8** (63); **9** (6)
3	**4**	16	0	100	**8** (61); **9** (15)
4	**4**	16	20	100	**8** (53); **9** (41)
5	**4**	16	40	100	**8** (7); **9** (59)
6	**4**	16	60	100	**9** (56)
7	**5**	16	40	85	**10** (6); **11** (64)
8	**5**	16	80	100	**10** (28); **11** (68)
9	**5**	16	100	100	**10** (29); **11** (59)
10	**6**	16	40	100	**12** (5)
11	**6**	16	60	100	**12** (9)
12	**6**	16	80	100	**12** (22)
13	**6**	16	100	100	**12** (25)
14	**6**	16	120	100	**12** (15)
15[e]	**7**	0.5	−13	100	**13** (68); **14** (0)
16[e]	**7**	1	−13	100	**13** (63); **14** (0)
17	**7**	16	−13	100	**13** (13); **14** (0)
18	**7**	16	0	100	**13** (7); **14** (0)
19	**7**	16	40	100	**13** (0); **14** (23)
20	**7**	16	60	100	**13** (0); **14** (37)
21	**7**	16	80	100	**13** (0); **14** (44)

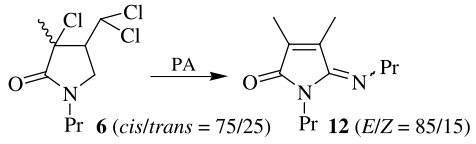
[a] Substrate (1 mmol), PA (2 mL).
[b] GC values.
[c] Yield determined on isolated material.
[d] The substrate was thermostated at −13 °C before addition of PA.
[e] The substrate was thermostated at −13 °C before addition of PA and 1 mL of diethyl ether was added to prevent its solidification.



Scheme 8.



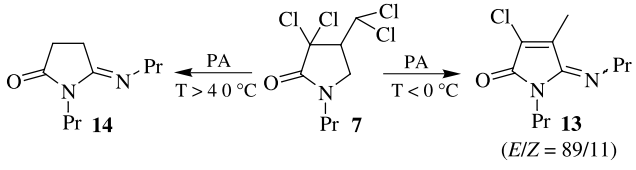
Scheme 9.



Scheme 10.

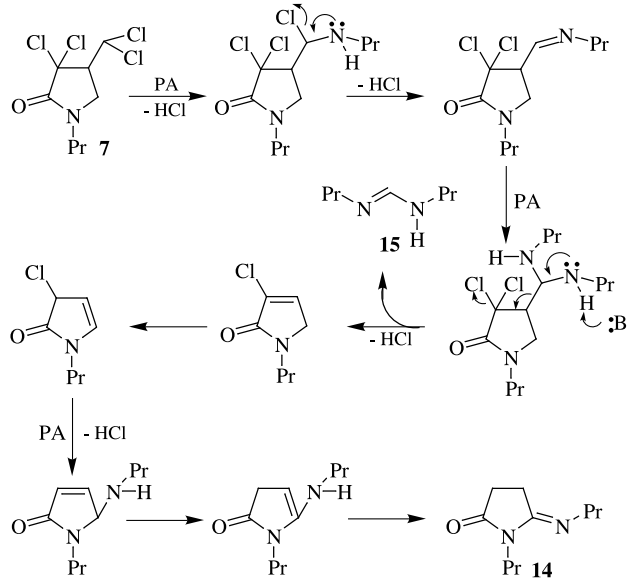
in only 5% yield (Scheme 10; Table 3, entry 10). In this case, heating the reaction mixture to 100 °C was also beneficial and the yield of **12** increased to 25% (Table 3, entry 13).

Finally, treatment of the polychlorinated pyrrolidin-2-one **7** with PA (at 40 °C) surprisingly afforded the degradation product **14** as the main component of the reaction crude (23%). No trace of the rearranged lactam **13** was detected (Scheme 11; Table 3, entry 19). As observed for the previous rearrangements, temperature proved to be a critical reaction parameter. In fact, on mixing the reagents at −13 °C for no more than 0.5 h (Scheme 11; Table 3, entry 15), the FR proceeded efficiently, providing the rearranged lactam **13** in good yield (68%). Temperatures higher than 40 °C caused, on the contrary, a progressive increase in the amount of imine **14**. Interestingly, from the MS analysis of the crude reaction mixtures containing *N*-propyl-5-propylimino-pyrrolidin-2-one (**14**) it was possible to detect a peak, which can be assigned to *N*,*N*′-dipropylformamidine (**15**). This was confirmed after comparison of the spectrum with an authentic sample of **15**, synthesized by a literature procedure.[41] The simultaneous formation of amidine **15** together with lactam **14** sheds some light on the mechanism of degradation of substrate **7**. The degradation is likely to start by substitution of the two *exo*-Cl atoms in **7** by two PA units. Then, from the resulting *N*,*N*-acetal, a base catalyzed fragmentation should afford amidine **15** and the intermediate *N*-propyl-3-chloro-3-pyrrolin-2-one, which in



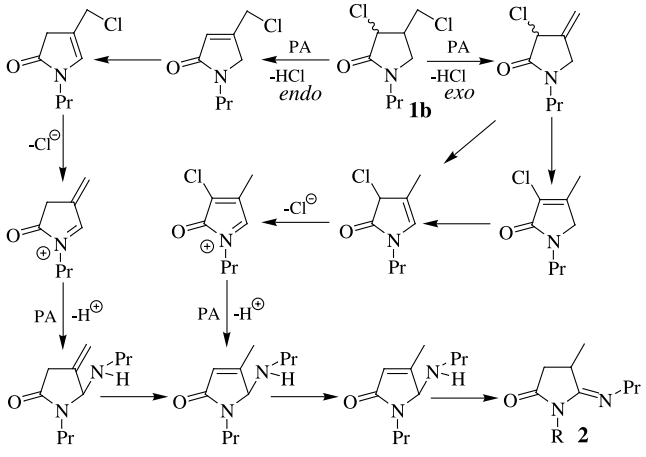
Scheme 11.

a few further steps could be transformed into **14** (Scheme 12).



Scheme 12.

The results from all these experiments clearly show that similarities can be drawn between the FR of polychlorinated pyrrolidin-2-ones using PA or CH_3OH/CH_3ONa. Therefore, it is reasonable to assume that the same type of mechanism is occurring in both transformations. In agreement with our previous proposal[34] and taking **1b** as a model pyrrolidin-2-one, we believe that the reaction starts with a base-promoted dehydrohalogention. This is an *exo* or *endo* elimination according to the ease with which the required *anti-periplanar* conformation is attained. The double bond then shifts from the C(4)–C(5) position to afford an intermediate Δ^4-pyrrolin-2-one. This can react further to afford, via a solvolysis step, a stable *N*-acyliminium cation that, after condensation with PA, is converted into the final product **2** (Scheme 13).



Scheme 13.

A few years ago we reported that *N*-substituted-3-benzyl-imino-pyrrolidin-2-ones could be prepared by heating *N*-substituted-3-chloro-4-chloromethyl-pyrrolidin-2-ones with benzylamine (3 equiv) and NaI in THF at reflux.[42] Given that these are similar reaction conditions to the ones described for the FR, it is surprising that different products are formed. Consequently, the reaction with the *N*-benzyl-3-chloro-4-chloromethyl-pyrrolidin-2-one was repeated and, as expected from the results of the present work, the structure of the reaction product was, without doubt, defined as *N*-benzyl-5-benzylimino-4-methyl-pyrrolidin-2-ones. This means that our former product characterization was erroneous and that the same functional rearrangement is operative.

3. Structural characterizations of the (*E*)- and (*Z*)-5-iminolactams 2, 8–10, 12–14

For all the 5-iminolactams, the (*E*) configuration largely or exclusively prevails. For adducts **2**, **9**, **10** and **14** only the (*E*)-isomer was isolated, while for products **8**, **12** and **13**, 11–15% of the minor (*Z*)-isomer (in equilibrium with the (*E*)-counterpart) was observed.

The stereochemistry of the 5-propylimino group was derived from ^{1}H NMR two-dimensional nuclear Overhauser enhancement experiments in the phase sensitive mode (NOESY-TPPI),[43] that were run after a complete assignment of the ^{1}H and ^{13}C NMR spectra, through inverse detection techniques (HMQC).[44] This was used in order to unambiguously assign all proton and carbon signals, in particular, those of the different propyl chains. NOESY-TPPI experiments permit the detection of protons close to one another in space and, at the same time, to evidence exchange processes which are slow enough on the NMR timescale.[45] The two situations can be distinguished from the sign of the relevant cross-peaks with respect to that of diagonal peaks: cross-peaks denoting proximity of two protons have opposite sign, whereas cross-peaks deriving from exchange phenomena have the same sign with respect to the diagonal ones (which are set as negative). From NOESY-TPPI spectra it was readily seen that the signals of methylene protons bonded to the imino moiety share positive cross-peaks with the signals of groups at the C(4) position in derivatives **2**, **9**, **10** and **14**, and this indicates an (*E*)-type configuration. In the case of compounds **8**, **12** and **13**, ^{1}H NMR spectra showed the presence of two sets of signals, and negative correlations were found between the corresponding signals of the two forms in NOESY-TPPI spectra, evidencing that an exchange process between two isomeric species is present.[42] Moreover, positive cross-peaks were found, for the major isomer only, among the signals of the iminopropyl appendage and those of the groups at the C(4) position, indicating that the (*E*)-type configuration also prevails in these products. It is interesting to note that the tendency to have a small amount of the (*Z*)-isomer of the iminopropyl group (in equilibrium with the prevailing (*E*)-isomer) is only observed for the γ-lactams with a $C(4)_{sp2}$ carbon. The (*Z*)-isomer is not observed for those compounds with a $C(4)_{sp3}$ carbon, even when two substituents are present, for example, as in the case of the pyrrolidin-2-one **9**. The preponderance of the (*E*)-isomers, then, is attributed to the higher thermodynamic stability of these frameworks.

4. Conclusion

The reaction of 4-methyl-pyrrolidin-2-ones chlorinated at the C(3) and C(6) positions with PA establishes a new method for the preparation, generally in good yield, of 5-propylimino pyrrolidin-2-ones or 3-pyrrolin-2-ones. This FR is similar to that observed when the same substrates are treated with CH_3OH/CH_3ONa. In order to obviate the problem of substitution of the *N*-substituent on the γ-lactam ring, the FR with PA requires the use of polychlorinated pyrrolidin-2-ones bearing an *N*-propyl group. In this way, notwithstanding that the exchange still occurs, it cannot be noticed owing to the degeneracy of the phenomenon. This structural prerequisite, however, restricts the versatility of the method. Current studies are investigating the application of the methodology to the synthesis of roccellic acid[46] and the results will be reported in due course.

5. Experimental

5.1. General

^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions with a Bruker Avance 400 Spectrometer and a Bruker DPX 200 spectrometer, and the chemical shifts are reported in ppm relative to tetramethylsilane as external standard. Conditions for HMQC spectra were: evolution delay = 50 ms, spectral width = 10 ppm with 2048 complex points in *f*2; 256 *t*1 values and 64 scans for *t*1 value. A squared sine function (SSB = 2) in *f*2 and *f*1 was applied before Fourier transformation. Conditions for NOESY phase-sensitive spectra by time-proportional phase incrementation (TPPI) were: mixing time of 600 ms, spectral width 8.16 ppm with 2048 complex points in *f*2; 128 *t*1 values and 64 scans for *t*1 value. A squared sine function (SSB = 2) in *f*2 and gaussian multiplication (LB = −1, GB = 0.01) in *f*1 were applied before Fourier transformation. IR spectra were obtained with a Perkin–Elmer 1600 Series FTIR. Mass spectra were acquired with a combined HP 5890 GC/HP 5989A MS Engine. Reagents and solvents were standard grade commercial products, purchased from Acros, Aldrich or Fluka, and used without further purification. Acetonitrile (for the radical cyclizations) was dried over three batches of 3 Å sieves (5% w/v, 12 h). The silica gel used for flash chromatography was 60 Merck (0.040–0.063 mm). *N*-propyl-2-propenylamine and *N*-propyl-3-chloro-2-propenylamine were prepared for *N*-alkylation of *n*-propylamine with allyl bromide or 1,3-dichloropropene. The *N*-allyl-*N*-propyl α-perchloro amide precursors of polychlorinated pyrrolidin-2-ones **1b** and **4**–**7** were obtained by amino-de-chlorination of the appropriate acyl chlorides.[35] *N*-Benzyl-3-chloro-4-chloromethyl-pyrrolidin-2-one (**1a**) was prepared according to the literature.[47]

5.2. Preparation of polychlorinated pyrrolidin-2-ones

5.2.1. Typical procedure for the preparation of polychlorinated pyrrolidin-2-ones: *N*-propyl-3-chloro-4-chloromethyl-pyrrolidin-2-one (1b). CuCl (0.20 g, 2 mmol) and the *N*-allyl-*N*-propyl-dichloroacetamide (4.20 g, 20 mmol) were weighted in a Schlenk tube, then dry acetonitrile (10 mL) and TMEDA (0.6 mL, 4 mmol) were added under argon. The mixture was stirred at 80 °C and after 24 h diluted with HCl (5% w/v, 20 mL) and extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried with toluene (10 mL) through azeotropic distillation. Flash-chromatography of the crude product on silica, eluting with a petroleum ether (bp 40/60 °C)/diethyl ether gradient, afforded the pyrrolidin-2-one **1b** (3.40 g, 81%) as an unseparable mixture of *cis*/*trans* diastereoisomers (27/73); orange oil; [found: C, 45.59; H, 6.31; N, 6.76. $C_8H_{13}Cl_2NO$ requires C, 45.73; H, 6.24; N, 6.67]; IR (liquid film) 1707 (C=O) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): *trans* diastereoisomer (73%) δ 0.91 (3H, t, *J* = 7.4 Hz, $CH_2CH_2CH_3$), 1.57 (2H, m, $CH_2CH_2CH_3$), 2.65–3.05 (1H, m, C(4)*H*), 3.05–3.91 (6H, m, CH_2NCH_2-C(4)HCH_2), 4.33 (1H, d, *J* = 7.6 Hz, C(3)*H*); *cis* diastereoisomer (27%) δ 0.91 (3H, t, *J* = 7.4 Hz, $CH_2CH_2CH_3$), 1.57 (2H, m, $CH_2CH_2CH_3$), 2.65–3.05 (1H, m, C(4)*H*), 3.05–3.91 (6H, m, $CH_2NCH_2C(4)HCH_2$), 4.44 (1H, d, *J* = 7.6 Hz, C(3)*H*); MS (EI, 70 eV) *m*/*z*: 209 (17, M^+), 194 (35), 180 (100), 174 (52), 152 (22), 42 (17).

5.2.2. *N*-Propyl-3,3-dichloro-4-chloromethyl-pyrrolidin-2-one (4). According to the general procedure, cyclization of *N*-allyl-*N*-propyl-trichloroacetamide (4.90 g, 20 mmol) at 25 °C for 20 h gave the pyrrolidin-2-one **4** (4.35 g, 89%) as yellow oil; [found: C, 39.41; H, 5.01; N, 5.86. $C_8H_{12}Cl_3NO$ requires C, 39.29; H, 4.95; N, 5.73]; IR (liquid film) 1729 (C=O) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 0.92 (3H, t, *J* = 7.4 Hz, $CH_2CH_2CH_3$), 1.61 (2H, m, $CH_2CH_2CH_3$), 2.87–4.17 (7H, m, $CH_2NCH_2C(4)HCH_2$); MS (EI, 70 eV) *m*/*z*: 243 (13, M^+), 228 (47), 214 (100), 208 (55), 180 (28), 42 (47).

5.2.3. *N*-Propyl-3-chloro-4-chloromethyl-3-methyl-pyrrolidin-2-one (5). According to the general procedure, cyclization of *N*-allyl-*N*-propyl-2,2-dichloropropanamide (4.48 g, 20 mmol) at 25 °C for 20 h gave the pyrrolidin-2-one **5** (4.30 g, 96%) as an unseparable mixture of *cis*/*trans* diastereoisomers (56/44); orange oil; [found: C, 48.34; H, 6.68; N, 6.12. $C_9H_{15}Cl_2NO$ requires C, 48.23; H, 6.75; N, 6.25]; IR (liquid film) 1706 (C=O) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): *cis* diastereoisomer (56%) δ 0.91 (3H, t, *J* = 7.4 Hz, $CH_2CH_2CH_3$), 1.58 (2H, m, $CH_2CH_2CH_3$), 1.76 (3H, s, C(3)CH_3), 2.36–2.68 (1H, m, C(4)*H*), 3.04–4.00 (6H, m, $CH_2NCH_2C(4)HCH_2$); *trans* diastereoisomer (44%) δ 0.91 (3H, t, *J* = 7.4 Hz, $CH_2CH_2CH_3$), 1.58 (2H, m, $CH_2CH_2CH_3$), 1.63 (3H, s, C(3)CH_3), 2.80–3.04 (1H, m, C(4)*H*), 3.04–4.00 (6H, m, $CH_2NCH_2C(4)HCH_2$); MS (EI, 70 eV) *m*/*z*: 223 (17, M^+), 208 (25), 194 (100), 188 (48), 160 (18), 42 (22).

5.2.4. *N*-Propyl-3-chloro-4-dichloromethyl-3-methyl-pyrrolidin-2-one (6). According to the general procedure, cyclization of *N*-(3-chloroallyl)-*N*-propyl-2,2-dichloropropanamide (5.17 g, 20 mmol) at 25 °C for 20 h gave the pyrrolidin-2-one **6** (4.71 g, 91%) as an unseparable mixture of *cis*/*trans* diastereoisomers (75/25); light yellow oil; [found: C, 41.84; H, 5.39; N, 5.36. $C_9H_{14}Cl_3NO$ requires C, 41.81; H, 5.46; N, 5.42]; IR (liquid film) 1711 (C=O) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): *cis* diastereoisomer (75%) δ 0.92 (3H, t, *J* = 7.4 Hz, $CH_2CH_2CH_3$), 1.59 (2H, m, $CH_2CH_2CH_3$), 1.91 (3H, s, C(3)CH_3), 2.89 (1H, td, *J* = 7.4, 9.5 Hz, C(4)*H*), 3.03–3.56 (4H, m, C(5)$H_2NCH_2CH_2$), 6.04

(1H, d, J=9.5 Hz, C(4)C*H*); *trans* diastereoisomer (25%) δ 0.94 (3H, t, J=7.4 Hz, $CH_2CH_2CH_3$), 1.59 (2H, m, $CH_2CH_2CH_3$), 1.78 (3H, s, C(3)CH_3), 3.03–3.56 (4H, m, CH_2NC(5)*H*C(4)*H*), 3.77 (1H, dd, J=7.6, 11.4 Hz, C(5)*H*), 5.93 (1H, d, J=4.5 Hz, C(4)C*H*); MS (EI, 70 eV) *m/z*: 257 (15, M^+), 242 (33), 228 (100), 222 (52), 194 (23), 42 (25).

5.2.5. *N*-Propyl-3,3-dichloro-4-dichloromethyl-pyrrolidin-2-one (7). According to the general procedure, cyclization of *N*-(3-chlorallyl)-*N*-propyl-trichloroacetamide (5.58 g, 20 mmol) at 25 °C for 20 h gave the pyrrolidin-2-one **7** (5.08 g, 91%) as pale yellow oil; [found: C, 34.53; H, 4.04; N, 5.09. $C_8H_{11}Cl_4NO$ requires C, 34.44; H, 3.97; N, 5.02]; IR (liquid film) 1733 (C=O) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.94 (3H, t, J=7.4 Hz, $CH_2CH_2CH_3$), 1.62 (2H, m, $CH_2CH_2CH_3$), 3.22–3.51 (4H, m, CH_2-NC(5)*H*C(4)*H*), 3.64 (1H, dd, J=6.8, 9.6 Hz, C(5)*H*), 6.09 (1H, d, J=7.9 Hz, C(4)C*H*); MS (EI, 70 eV) *m/z*: 277 (10, M^+), 264 (58), 250 (100), 242 (48), 214 (27), 159 (20), 130 (47), 109 (20), 42 (30).

5.3. Functional rearrangement of polychlorinated pyrrolidin-2-ones

5.3.1. Preparation of (*E*)-*N*-propyl-4-methyl-5-propylimino-pyrrolidin-2-one (2). The polychlorinated pyrrolidin-2-one **1a** (0.26 g, 1 mmol) was weighted in a Schlenk tube, then *n*-propylamine (5 mL) was added under argon. The mixture was stirred at 140 °C for 24 h, then evaporated under vacuum to remove *n*-propylamine, and finally diluted with H_2O (4 mL) and extracted with CH_2Cl_2 (2×5 mL). Flash-chromatography of the crude product on silica, eluting with a petroleum ether (bp 40/60 °C)/diethyl ether gradient, afforded **2** (106 mg, 54%) as an orange yellow oil; [found: C, 67.40; H, 10.21; N, 14.19. $C_{11}H_{20}N_2O$ requires C, 67.31; H, 10.27; N, 14.27]; IR (liquid film) 1660 cm^{-1} (C=N), 1737 cm^{-1} (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (3H, t, J=7.4 Hz, N(1)$CH_2CH_2CH_3$), 0.94 (3H, t, J=7.4 Hz, C=N$CH_2CH_2CH_3$), 1.21 (3H, d, J=7.2 Hz, C(4)CH_3), 1.58 (2H, m, C=NCH_2CH_2), 1.61 (2H, m, N(1)CH_2CH_2), 2.15 (1H, dd, J=1.9, 17.5 Hz, C(3)*H*), 2.76 (1H, dd, J=8.8, 17.5 Hz, C(3)*H*), 3.09 (1H, m, C(4)*H*), 3.28 (1H, m, C=NC*H*), 3.35 (1H, m, C=NC*H*), 3.50 (2H, m, N(1)CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.27 (N(1)$CH_2CH_2CH_3$), 11.84 (C=N$CH_2CH_2CH_3$), 19.28 (C(4)CH_3), 20.46 (N(1)CH_2CH_2), 24.87 (C=NCH_2CH_2), 29.01 (*C*(4)), 37.88 (*C*(3)), 40.47 (N(1)CH_2), 50.97 (C=NCH_2), 161.80 (*C*(5)), 175.64 (*C*(2)); MS (EI, 70 eV) *m/z*: 196 (100, M^+), 181 (40), 167 (68), 153 (40), 139 (83), 126 (57), 113 (25), 97 (20), 41 (22).

5.3.2. Preparation of *N*-benzyl-5-diethylamino-4-methyl-3-pyrrolin-2-one (3). According to the general procedure, the rearrangement of **1a** (0.26 g, 1 mmol) with diethylamine (2.5 mL) at 100 °C for 24 h gave **3** (0.10 g, 40%) as a yellowish oil; [found: C, 74.34; H, 8.62; N, 10.77. $C_{16}H_{22}N_2O$ requires C, 74.38; H, 8.58; N, 10.84]; IR (liquid film) 1696 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.97 (6H, t, J=7.1 Hz, N(CH_2CH_3)$_2$), 1.93 (3H, d, J=1.4 Hz, C(4)CH_3), 2.60 (4H, broad m, N(CH_2CH_3)$_2$), 4.02 (1H, d, J=15.1 Hz, N(1)C*H*Ar), 4.53 (1H, s, C(5)*H*), 5.07 (1H, d, J=15.1 Hz, N(1)C*H*Ar), 5.88 (1H, q, J=1.4 Hz, C(3)*H*), 7.19–7.30 (5H, m, Ar(*H*)); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.91 (C(4)CH_3), 15.04 (N(CH_2CH_3)$_2$), 43.0 (br, N(CH_2CH_3)$_2$), 43.27 (CH_2Ar), 79.15 (*C*(5)N), 123.36 (*C*(3)), 127.07 (*Ar*), 127.75 (*Ar*), 128.44 (*Ar*), 138.18 (*Ar*), 158.55 (*C*(4)), 170.29 (*C*=O); MS (EI, 70 eV) *m/z*: 258 (5, M^+), 186 (67), 91 (100).

5.3.3. Preparation of *N*-propyl-4-methyl-5-propylimino-3-pyrrolin-2-one (8). According to the general procedure, the rearrangement of **4** (0.24 g, 1 mmol) with *n*-propylamine (2 mL) at −13 °C for 4 h gave **8** (0.12 g, 63%) as an unseparable mixture of *E*/*Z* diastereoisomers (85/15); yellow oil; [found: C, 68.10; H, 9.41; N, 14.47. $C_{11}H_{18}N_2O$ requires C, 68.01; H, 9.34; N, 14.42]; IR (liquid film) 1654 cm^{-1} (C=N), 1724 cm^{-1} (C=N); ^{1}H NMR (400 MHz, $CDCl_3$): (*E*)-diastereoisomer (85%) δ 0.84 (3H, t, J=7.5 Hz, N(1)$CH_2CH_2CH_3$), 0.96 (3H, t, J=7.4 Hz, C=N$CH_2CH_2CH_3$), 1.55 (2H, m, N(1)CH_2CH_2), 1.67 (2H, m, C=NCH_2CH_2), 2.26 (3H, d, J=1.6 Hz, C(4)CH_3), 3.50 (2H, t, J=7.3 Hz, N(1)CH_2), 3.71 (2H, t, J=6.8 Hz, C=NCH_2), 6.13 (1H, q, J=1.6 Hz, C(3)*H*); (*Z*)-diastereoisomer (15%) δ 0.87 (3H, t, J=7.4 Hz, N(1)$CH_2CH_2CH_3$), 0.98 (3H, t, J=7.2 Hz, C=N$CH_2CH_2CH_3$), 1.55 (2H, m, N(1)CH_2CH_2), 1.67 (2H, m, C=NCH_2CH_2), 2.01 (3H, d, J=1.5 Hz, C(4)CH_3), 3.66 (2H, t, J=7.6 Hz, N(1)CH_2), 3.67 (2H, t, J=6.8 Hz, C=NCH_2), 6.06 (1H, q, J=1.5 Hz, C(3)*H*); ^{13}C NMR (100 MHz, $CDCl_3$): (*E*)-diastereoisomer (85%) δ 11.27 (N(1)$CH_2CH_2CH_3$), 11.72 (C=N$CH_2CH_2CH_3$), 17.43 (C(4)CH_3), 21.77 (N(1)CH_2CH_2), 25.31 (C=NCH_2CH_2), 39.61 (N(1)CH_2), 51.08 (C=NCH_2), 129.09 (*C*(3)), 140.63 (*C*(4)), 152.76 (*C*(5)), 169.96 (*C*(2)); (*Z*)-diastereoisomer (15%) δ 10.97 (N(1)$CH_2CH_2CH_3$), 11.72 (C=N$CH_2CH_2CH_3$), 11.99 (C(4)CH_3), 23.76 (N(1)CH_2CH_2), 25.20 (C=NCH_2CH_2), 42.79 (N(1)CH_2), 50.27 (C=NCH_2), 122.78 (*C*(3)), 150.40 (*C*(4)), 150.72 (*C*(5)), 172.54 (*C*(2)); MS (EI, 70 eV) *m/z*: 194 (100, M^+), 179 (28), 165 (58), 151 (58), 137 (37), 123 (35), 111 (22), 94 (67), 41 (22).

5.3.4. Preparation of (*E*)-*N*-propyl-4-methyl-4-propylamino-5-propylimino-pyrrolidin-2-one (9). According to the general procedure, the rearrangement of **4** (0.24 g, 1 mmol) with *n*-propylamine (2 mL) at 40 °C for 16 h gave **9** (0.15 g, 59%) as a yellowish oil; [found: C, 66.45; H, 10.76; N, 16.48. $C_{14}H_{27}N_3O$ requires C, 66.36; H, 10.74; N, 16.58]; IR (liquid film) 1668 cm^{-1} (C=N), 1733 cm^{-1} (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84 (3H, t, J=7.5 Hz, N(1)$CH_2CH_2CH_3$), 0.89 (3H, t, J=7.4 Hz, NH$CH_2CH_2CH_3$), 0.93 (3H, t, J=7.4 Hz, C=N$CH_2CH_2CH_3$), 1.40–1.50 (2H, m, NHCH_2CH_2), 1.46 (3H, s, C(4)CH_3), 1.55 (2H, m, N(1)CH_2CH_2), 1.58 (2H, m, C=NCH_2CH_2), 2.26 (1H, dt, J=7.1, 10.5 Hz, NHC*H*), 2.34 (1H, d, J=18.0 Hz, C(3)H_2), 2.43 (1H, dt, J=7.1, 10.5 Hz, NHC*H*), 2.74 (1H, d, J=18.0 Hz, C(3)H_2), 3.48 (2H, t, J=7.3 Hz, N(1)CH_2), 3.59 (2H, t, J=6.8 Hz, C=NCH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.30 (N(1)$CH_2CH_2CH_3$), 11.75 (NH$CH_2CH_2CH_3$), 11.82 (C=N$CH_2CH_2CH_3$), 20.44 (N(1)CH_2CH_2), 23.54 (NHCH_2CH_2), 25.22 (C=NCH_2CH_2), 26.60 (C(4)CH_3), 40.38 (N(1)CH_2), 42.77 (*C*(3)), 45.34 (NHCH_2), 48.58 (C=NCH_2), 59.77 (*C*(4)), 157.68 (*C*(5)), 173.69 (*C*(2)); MS (EI, 70 eV) *m/z*: 253 (1, M^+), 238 (3), 224 (8), 194 (100), 179 (22), 126 (45), 84 (23), 42 (15).

5.3.5. Preparation of (*E*)-*N*-propyl-3,4-dimethyl-5-propylimino-pyrrolidin-2-one (10). According to the general procedure, the rearrangement of **5** (0.22 g, 1 mmol) with *n*-propylamine (2 mL) at 100 °C for 16 h gave **10** (61 mg, 29%) as an unseparable mixture of *cis*/*trans* diastereoisomers (23/77); yellow oil; [found: C, 68.64; H, 10.59; N, 13.33. $C_{12}H_{22}N_2O$ requires C, 68.53; H, 10.54; N, 13.32]; IR (liquid film) 1671 cm^{-1} (C=N), 1736 cm^{-1} (C=O); ^1H NMR (400 MHz, $CDCl_3$): *trans* diastereoisomer (77%) δ 0.84 (3H, t, J=7.5 Hz, N(1)$CH_2CH_2CH_3$), 0.90 (3H, t, J=7.4 Hz, C=N$CH_2CH_2CH_3$), 1.18 (3H, d, J=7.2 Hz, C(4)CH_3), 1.19 (3H, d, J=7.4 Hz, C(3)CH_3), 1.56 (2H, m, N(1)CH_2CH_2), 1.58 (2H, m, C=NCH_2CH_2), 2.22 (1H, m, J=1.9, 7.4 Hz, C(3)*H*), 2.61 (1H, m, J=1.9, 7.2 Hz, C(4)*H*), 3.20–3.53 (4H, m, CH_2NC=NCH_2); *cis* diastereoisomer (23%) δ 0.83 (3H, t, J=7.4 Hz, N(1)$CH_2CH_2CH_3$), 0.91 (3H, t, J=7.4 Hz, C=N$CH_2CH_2CH_3$), 1.02 (3H, d, J=7.3 Hz, C(4)CH_3), 1.15 (3H, d, J=7.4 Hz, C(3)CH_3), 1.55 (2H, m, N(1)CH_2CH_2), 1.57 (2H, m, C=NCH_2CH_2), 2.71 (1H, m, J=7.8 Hz, C(3)*H*), 3.12 (1H, m, J=7.7 Hz, C(4)*H*), 3.20–3.53 (4H, m, CH_2-NC=NCH_2); ^{13}C NMR (100 MHz, $CDCl_3$): *trans* diastereoisomer (77%) δ 11.18 (N(1)$CH_2CH_2CH_3$), 11.77 (C=N$CH_2CH_2CH_3$), 17.57 (C(3)CH_3), 18.57 (C(4)CH_3), 20.43 (N(1)CH_2CH_2), 24.85 (C=NCH_2CH_2), 37.67 (*C*(4)), 40.32 (N(1)CH_2), 44.50 (*C*(3)), 50.96 (C=NCH_2), 161.03 (*C*(5)), 179.06 (*C*(2)); *cis* diastereoisomer (23%) δ 9.44 (C(3)CH_3), 11.23 (N(1)$CH_2CH_2CH_3$), 11.82 (C=NCH_2-CH_2CH_3), 13.27 (C(4)CH_3), 20.47 (N(1)CH_2CH_2), 24.83 (C=NCH_2CH_2), 33.11 (*C*(4)), 38.89 (*C*(3)), 40.36 (N(1)CH_2), 51.05 (C=NCH_2), 161.63 (*C*(5)), 178.35 (*C*(2)); MS (EI, 70 eV) *m*/*z*: 210 (65, M^+), 195 (43), 181 (45), 167 (35), 153 (100), 140 (52), 111 (32), 55 (20), 41 (18).

5.3.6. Preparation of *N*-propyl-3-methyl-4-(propylamino)methyl-3-pyrrolin-2-one (11). According to the general procedure, the rearrangement of **5** (0.22 g, 1 mmol) with *n*-propylamine (2 mL) at 80 °C for 16 h gave **11** (0.14 g, 68%) as a pale yellow oil; [found: C, 68.51; H, 10.58; N, 13.40. $C_{12}H_{22}N_2O$ requires C, 68.53; H, 10.54; N, 13.32]; IR (liquid film) 1681 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.80 (3H, t, J=7.5 Hz, N(1)$CH_2CH_2CH_3$), 0.81 (3H, t, J=7.5 Hz, NH$CH_2CH_2CH_3$), 1.40 (2H, m, NHCH_2-CH_2), 1.48 (2H, m, N(1)CH_2CH_2), 1.73 (3H, s, C(3)CH_3), 2.45 (2H, m, NHCH_2), 3.29 (2H, t, J=7.3 Hz, N(1)CH_2), 3.47 (2H, s, C(4)CH_2), 3.77 (2H, d, J=1.4 Hz, C(5)H_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 8.79 (C(3)CH_3), 11.18 (N(1)$CH_2CH_2CH_3$), 11.58 (NH$CH_2CH_2CH_3$), 21.78 (N(1)CH_2CH_2), 22.96 (NHCH_2CH_2), 43.72 (N(1)CH_2), 46.08 (C(3)CH_2), 51.58 (NHCH_2), 52.01 (*C*(5)), 130.08 (*C*(3)), 147.63 (*C*(4)), 172.18 (*C*(2)); MS (EI, 70 eV) *m*/*z*: 210 (1, M^+), 181 (4), 151 (100).

5.3.7. Preparation of *N*-propyl-3,4-dimethyl-5-propylimino-3-pyrrolin-2-one (12). According to the general procedure, the rearrangement of **6** (0.26 g, 1 mmol) with *n*-propylamine (2 mL) at 100 °C for 16 h gave **12** (52 mg, 25%) as an unseparable mixture of *E*/*Z* diastereoisomers (85/15); yellow oil; [found: C, 69.08; H, 9.71; N, 13.40. $C_{12}H_{20}N_2O$ requires C, 69.19; H, 9.68; N, 13.45]; IR (liquid film) 1656 cm^{-1} (C=N), 1718 cm^{-1} (C=O); ^1H NMR (400 MHz, $CDCl_3$): (*E*)-diastereoisomer (85%) δ 0.85 (3H, t, J=7.4 Hz, N(1)$CH_2CH_2CH_3$), 0.97 (3H, t, J=7.4 Hz, C=N$CH_2CH_2CH_3$), 1.55 (2H, m, N(1)CH_2CH_2), 1.68 (2H, m, C=NCH_2CH_2), 1.89 (3H, q, J=1.1 Hz, C(3)CH_3), 2.16 (3H, q, J=1.1 Hz, C(4)CH_3), 3.52 (2H, t, J=7.2 Hz, N(1)CH_2), 3.72 (2H, t, J=6.8 Hz, C=NCH_2); (*Z*)-diastereoisomer (15%) δ 0.88 (3H, t, J=7.4 Hz, N(1)CH_2CH_2-CH_3), 0.98 (3H, t, J=7.3 Hz, C=N$CH_2CH_2CH_3$), 1.57 (2H, m, N(1)CH_2CH_2), 1.70 (2H, m, C=NCH_2CH_2), 1.87 (3H, m, C(3)CH_3), 1.93 (3H, q, J=1.1 Hz, C(4)CH_3), 3.66 (2H, m, C=NCH_2), 3.67 (2H, m, N(1)CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): (*E*)-diastereoisomer (85%) δ 8.18 (C(3)CH_3), 11.33 (N(1)$CH_2CH_2CH_3$), 11.76 (C=NCH_2-CH_2CH_3), 14.40 (C(4)CH_3), 21.87 (N(1)CH_2CH_2), 25.45 (C=NCH_2CH_2), 39.70 (N(1)CH_2), 51.05 (C=NCH_2), 132.53 (*C*(3)), 136.65 (*C*(4)), 152.93 (*C*(5)), 171.01 (*C*(2)); (*Z*)-diastereoisomer (15%) δ 8.70 (C(3)CH_3), 9.51 (C(4)CH_3), 11.33 (N(1)$CH_2CH_2CH_3$), 11.76 (C=NCH_2-CH_2CH_3), 22.66 (N(1)CH_2CH_2), 25.35 (C=NCH_2CH_2), 43.11 (N(1)CH_2), 50.25 (C=NCH_2), 130.55 (*C*(3)), 139.42 (*C*(4)), 151.02 (*C*(5)), 170.81 (*C*(2)); MS (EI, 70 eV) *m*/*z*: 208 (100, M^+), 193 (43), 179 (73), 165 (53), 151 (83), 138 (48), 123 (22), 108 (48).

5.3.8. Preparation of *N*-propyl-3-chloro-4-methyl-5-propylimino-3-pyrrolin-2-one (13). According to the general procedure, the rearrangement of **7** (0.28 g, 1 mmol) with *n*-propylamine (2 mL) at −13 °C for 0.5 h gave **13** (0.15 g, 68%) as an unseparable mixture of *E*/*Z* diastereoisomers (89/11); orange oil; [found: C, 57.64; H, 7.56; N, 12.17. $C_{11}H_{17}ClN_2O$ requires C, 57.77; H, 7.49; N, 12.25]; IR (liquid film) 1657 cm^{-1} (C=N), 1734 cm^{-1} (C=O); ^1H NMR (400 MHz, $CDCl_3$): (*E*)-diastereoisomer (89%) δ 0.84 (3H, t, J=7.4 Hz, N(1)$CH_2CH_2CH_3$), 0.96 (3H, t, J=7.4 Hz, C=N$CH_2CH_2CH_3$), 1.56 (2H, m, N(1)CH_2CH_2), 1.68 (2H, m, C=NCH_2CH_2), 2.27 (3H, s, C(4)CH_3), 3.56 (2H, t, J=7.2 Hz, N(1)CH_2), 3.72 (2H, t, J=6.8 Hz, C=NCH_2); (*Z*)-diastereoisomer (11%) δ 0.88 (3H, t, J=7.4 Hz, N(1)$CH_2CH_2CH_3$), 0.97 (3H, t, J=7.3 Hz, C=N$CH_2CH_2CH_3$), 1.56 (2H, m, N(1)CH_2CH_2), 1.68 (2H, m, C=NCH_2CH_2), 2.01 (3H, s, C(4)CH_3), 3.67 (2H, t, J=6.8 Hz, C=NCH_2), 3.70 (2H, t, J=7.7 Hz, N(1)CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): (*E*)-diastereoisomer (89%) δ 11.18 (N(1)$CH_2CH_2CH_3$), 11.66 (C=N$CH_2CH_2CH_3$), 14.40 (C(4)CH_3), 21.72 (N(1)CH_2CH_2), 25.15 (C=NCH_2CH_2), 40.22 (N(1)CH_2), 51.31 (C=NCH_2), 132.55 (*C*(3)), 134.22 (*C*(4)), 149.71 (*C*(5)), 164.20 (*C*(2)); (*Z*)-diastereoisomer (11%) δ 9.89 (C(4)CH_3), 10.89 (N(1)$CH_2CH_2CH_3$), 11.66 (C=N$CH_2CH_2CH_3$), 23.56 (N(1)CH_2CH_2), 25.09 (C=NCH_2CH_2), 43.81 (N(1)CH_2), 50.58 (C=NCH_2), 127.0 (*C*(3)), 143.5 (*C*(4)), 147.5 (*C*(5)), 164.20 (*C*(2)); MS (EI, 70 eV) *m*/*z*: 228 (100, M^+), 213 (20), 199 (77), 185 (47), 171 (35), 158 (42), 151 (72), 145 (27), 128 (53).

5.3.9. Preparation of (*E*)-*N*-propyl-5-propylimino-pyrrolidin-2-one (14). According to the general procedure, the rearrangement of **7** (0.28 g, 1 mmol) with *n*-propylamine (2 mL) at 80 °C for 16 h gave **14** (80 mg, 44%) as an orange-yellow oil; [found: C, 65.78; H, 9.88; N, 15.41. $C_{10}H_{18}N_2O$ requires C, 65.90; H, 9.95; N, 15.37]; IR (liquid film) 1671 cm^{-1} (C=N), 1739 cm^{-1} (C=O); ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (3H, t, J=7.4 Hz, N(1)CH_2-CH_2CH_3), 0.92 (3H, t, J=7.4 Hz, C=N$CH_2CH_2CH_3$), 1.59

(2H, m, N(1)CH_2CH_2), 1.60 (2H, m, C=NCH_2CH_2), 2.53–2.57 (2H, m, C(3)H_2), 2.60–2.65 (2H, m, C(4)H_2), 3.22 (2H, t, J=6.9 Hz, C=NCH_2), 3.52 (2H, t, J=7.4 Hz, N(1)CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.29 (N(1)$CH_2CH_2CH_3$), 11.83 (C=N$CH_2CH_2CH_3$), 20.45 (N(1)CH_2CH_2), 21.80 (*C*(4)), 24.27 (C=NCH_2CH_2), 28.29 (*C*(3)), 40.79 (N(1)CH_2), 51.47 (C=NCH_2), 158.73 (*C*(5)), 176.47 (*C*(2)); MS (EI, 70 eV) *m/z*: 182 (78, M^+), 167 (48), 153 (100), 139 (73), 125 (92), 112 (62), 99 (30), 83 (47), 68 (45), 54 (63), 41 (83).

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A theoretical study on the regioselectivity of 1,3-dipolar cycloadditions using DFT-based reactivity indexes

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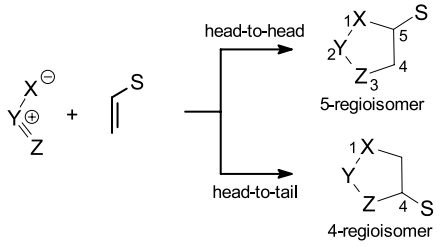
Abstract—The regioselectivity for a series of four 1,3-dipolar cycloaddition reactions has been studied using global and local reactivity indexes. The results of the theoretical analysis suggest that for asynchronous cycloadditions associated to polar processes, the regioselectivity is consistently explained by the most favorable two-center interactions between the highest nucleophilic and electrophilic sites of the reagents.

1. Introduction

Cycloaddition reactions are one of the most important processes with both synthetic and mechanistic interest in organic chemistry. Current understanding of the underlying principles in the Diels–Alder (DA) and the 1,3-dipolar cycloaddition (13DC) reactions has grown from a fruitful interplay between theory and experiment.[1] The usefulness of these cycloaddition reactions arises from their versatility and from their remarkable stereochemistry.[2] By varying the nature of the reagents many different types of carbocyclic structures can be built up. The interaction between unsymmetrical reagents in a 13DC reaction can give two isomeric adducts depending on the relative position of the substituent in the cycloadduct, head-to-head, 5-regioisomer, or head-to-tail, 4-regioisomer (see Scheme 1).



Regioisomeric pathways for a 1,3-dipolar cycloaddition

Scheme 1.

It is well known that one of the preferred theoretical devices of synthetic organic chemists to discuss reactivity and selectivity of many organic reactions is the frontier molecular orbital (FMO) model introduced by Fukui.[3] In the case of cycloaddition reactions, there are several examples that illustrate well the usefulness of this approach to classifying the 13DC reactions.[4–6] Within the FMO frame, selectivity is usually described in terms of the square of the FMO coefficients, or in other cases by this same term weighted by a resonance integral, a model proposed by Houk,[6] based on the orbital control term in the Salem,[7] Klopman,[8] and other equations.

There is however some relationships between the FMO approach and the one framed on the density functional theory (DFT) based reactivity indexes.[9] They are the following: (a) the square of the coefficients, which is used to describe positional selectivity in a molecule within the FMO model represents in DFT a good approximation to the Fukui functions condensed to atoms in their valence state;[10,11] (b) the model using the square of the coefficients weighted by a resonance integral on the other hand, accounts for just a piece of the interaction energy between the dipole and the dipolarophile localized in specific regions

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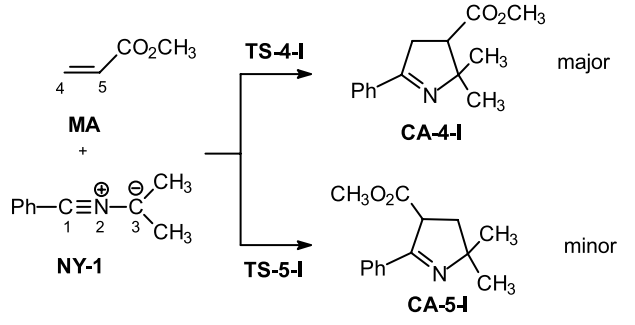
of the interacting pair, describing charge transfer stabilization effects in the FMO framework. Charge transfer may also be quantitatively described within DFT in terms of the electronic chemical potential and the chemical hardness throughout Pearson's equations,[12] and also within the concept of global electrophilicity introduced by Parr et al.[13] For instance, using the DFT reactivity model, the regioselectivity in the cycloaddition reactions has been described in terms of a local hard and soft acid and bases (HSAB) principle, and some empirical rules have been proposed to rationalize the experimental regioselectivity pattern observed in some DA[14] and 13DC[15] reactions. Recent DFT studies devoted to the DA[16] and 13DC[17] reactions on the other hand, have shown that the classification of reagents within a unique scale of electrophilicity, is a useful tool for predicting the reaction mechanism and regioselectivity of a cycloaddition process.[16] Even though in the case of the 13DC reactions the global analysis resulted more complex, substitution effects on the dipole and dipolarophile were correctly assessed.[17] In a DA reaction, diene/dienophile pairs located at the ends of the electrophilicity scale usually display a polar reactivity characterized by a large charge transfer (CT) at the transition structure (TS) involved in the mechanism of this particular cycloaddition reaction.[16a] For these polar cycloadditions the most favorable regioisomeric pathway corresponds to the bond-formation at the more electrophilic and nucleophilic sites of unsymmetrical reagents. In this context, we have shown that the analysis based on the local electrophilicity index ω_k,[18] at the more electrophile DA reagent together with the analysis of the nucleophilic Fukui functions,[19] f_k^-, at the less electrophilic one, allows the prediction of the regioselectivity in these cycloadditions.[16c–g]

There is not a single criterion however to explain most of the experimental evidence accumulated in cycloaddition processes involving four center interactions. Recent studies devoted to regioselective 13DC reactions have questioned the feasibility of the FMO approach to explain this kind of selectivity.[20] An excellent source for the discussion of regioselectivity in concerted pericyclic reactions is given in reference.[15d] In this context, the model based on DFT global and local descriptors is a reliable alternative to discuss reactivity and selectivity based on absolute scales, which have been already validated against experimental scales of reactivity and selectivity.

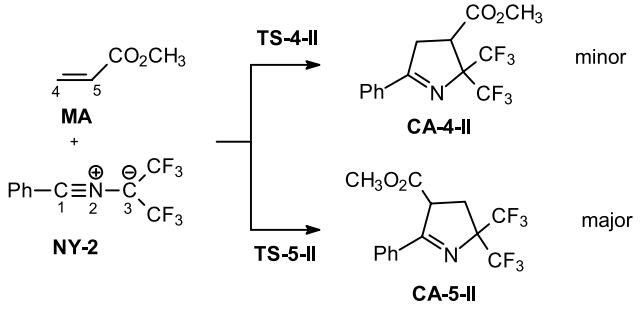
In this work we extend the local analysis to the regioselectivity of the 13DC processes. We have chosen as model reactions a series of four regioselective 13DC reactions for which experimental data are available. They are the reactions between the nitrile ylides **NY-1**[1d] and **NY-2**[21] with methyl acrylate (**MA**), models **I** and **II** in Schemes 2 and 3, and the 13DC reactions of the azomethine ylide **AY**[22] and the nitrile imine **NI**[23] with methyl propiolate (**MP**), models **III** and **IV** in Schemes 4 and 5, respectively. The comparative analysis will be made on the basis of the relative energies, asynchronicity and CT at the TSs on one hand, and the analysis based on the reactivity indexes defined in the context of DFT[9c] evaluated at the ground state (GS) of reactants.

2. Computational methods and models

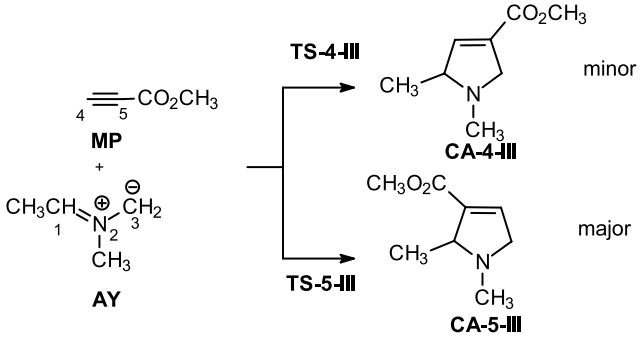
In recent years, theoretical methods based on the DFT have emerged as an alternative to traditional ab initio methods in the study of structure and reactivity of chemical systems. DA and 13DC reactions have been the object of several DFT studies showing that functionals that include gradient corrections and hybrid functionals for exchange and correlation, such as B3LYP,[24] together with the standard



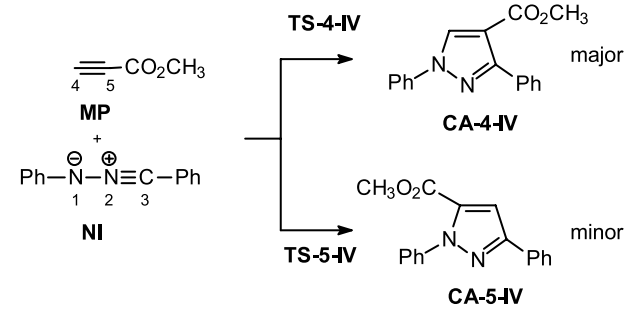
Scheme 2.



Scheme 3.



Scheme 4.



Scheme 5.

6-31G* basis set,[25] yield potential energy barriers in good agreement with the experiment.[26] Thus, in the present study geometrical optimizations of the stationary points were carried out using this methodology. The optimizations were performed using the Berny analytical gradient optimization method.[27] The stationary points were characterized by frequency calculations in order to verify that the TSs had one and only one imaginary frequency. The intrinsic reaction coordinate (IRC)[28] path was traced in order to check the energy profiles connecting each TS to the two associated minima of the proposed mechanism by using the second order González–Schlegel integration method.[29] The electronic structures of TSs were analyzed by the natural bond orbital (NBO) method.[30] All calculations were carried out using the Gaussian 98 suite of programs.[31] Thermal corrections to enthalpy and entropy values were evaluated at 298.15 K. The computed values of enthalpies, entropies and free energies were estimated by means of the B3LYP/6-31G* potential energy barriers, along with the gas-phase harmonic frequencies.[25]

The global electrophilicity index ω,[13] which measures the stabilization in energy when the system acquires an additional electronic charge ΔN from the environment, has been given the following simple expression,[13] $\omega=(\mu^2/2\eta)$, in terms of the electronic chemical potential μ and the chemical hardness η. Both quantities may be approached in terms of the one electron energies of the frontier molecular orbital HOMO and LUMO, ε_H and ε_L, as $\mu\approx(\varepsilon_H+\varepsilon_L)/2$ and $\mu\approx(\varepsilon_L-\varepsilon_H)$, respectively.[32]

The local electrophilicity index, ω_k,[18] can be expressed as $\omega_k=\omega f_k^+$ where f_k^+ is the Fukui function for a nucleophilic attack. This expression shows that the maximum electrophilicity power in a molecule will be developed at the site where the Fukui function for a nucleophilic attack displays its maximum value, i.e. at the active site of the electrophile. Electrophilic and nucleophilic Fukui functions[32] condensed to atoms have been evaluated from single point calculations performed at the GS of molecules at the same level of theory, using a method described elsewhere.[11] This method evaluates the Fukui functions using the coefficients of the frontier molecular orbitals involved in the reaction and the overlap matrix.

3. Results and discussion

We first evaluated the activation energies and the geometrical parameters of TSs for the four 13DC reactions. The population analysis at the TSs in terms of bond orders and natural charges was performed, together with an analysis based on the global and local reactivity indexes of the reactants involved in these cycloadditions.

3.1. Analysis of activation energies, geometries, bond orders, and charge transfer at the transition state geometries

An analysis of the gas-phase results indicates that these 13DC reactions take place along asynchronous concerted processes. Therefore eight TSs, **TS-4-I**, **TS-5-I**, **TS-4-II**, **TS-5-II**, **TS-4-III**, **TS-5-III**, **TS-4-IV** and **TS-5-IV**, and their corresponding cycloadducts associated with the two regioisomeric channels of the four cycloadditions were located and characterized. These acronyms are related to the formation of the 4- or 5-regioisomers, hereafter structures **4** and **5**, the 13DCs between **NY-1** and **MA**, named model **I**, the cycloaddition between **NY-2** and **MA**, named model **II**, the cycloaddition between **AY** and **MP**, named model **III**, and the cycloaddition between **NI** and **MP**, named model **IV**. The stationary points corresponding to these 13DC reactions are presented in Schemes 2–5 together with atom numbering. The total and relative energies are compiled in Table 1. The geometries of the TSs are presented in Figure 1.

These 13DC reactions present very low activation enthalpies. For instance, for the reaction model **III**, **TS-5-III** is located 7.6 kcal/mol below the reactants. However,

Table 1. Relative[a] energies (ΔE, in kcal/mol), enthalpies (ΔH, in kcal/mol), entropies (ΔS, in kcal/mol K) and free energies (ΔG, in kcal/mol) energies computed at 25 °C of the stationary points involved in the 13DC reaction models **I–IV**

	ΔE	ΔH	ΔS	ΔG
Model **I** (**NY-1**+**MA**)				
TS-4-I	1.04	0.72	−43.97	13.83
TS-5-I	3.20	2.66	−48.30	17.06
CA-4-I	−58.76	−60.20	−55.60	−43.62
CA-5-I	−57.57	−59.05	−56.09	−42.33
Model **II** (**NY-2**+**MA**)				
TS-4-II	1.75	1.72	−46.06	15.45
TS-5-II	1.52	1.41	−47.83	15.67
CA-4-II	−60.56	−61.52	−57.79	−44.29
CA-5-II	−61.25	−62.20	−56.35	−45.40
Model **III** (**AY**+**MP**)				
TS-4-III	−6.41	−6.48	−39.08	5.18
TS-5-III	−7.57	−7.67	−40.48	4.40
CA-4-III	−87.29	−88.45	−48.48	−74.00
CA-5-III	−86.96	−88.16	−48.88	−73.58
Model **IV** (**NI**+**MP**)				
TS-4-IV	6.08	5.75	−44.64	19.06
TS-5-IV	5.74	5.47	−44.12	18.63
CA-4-IV	−98.65	−100.07	−55.61	−83.50
CA-5-IV	−96.93	−98.28	−53.93	−82.20

[a] Relative to the corresponding reagents.

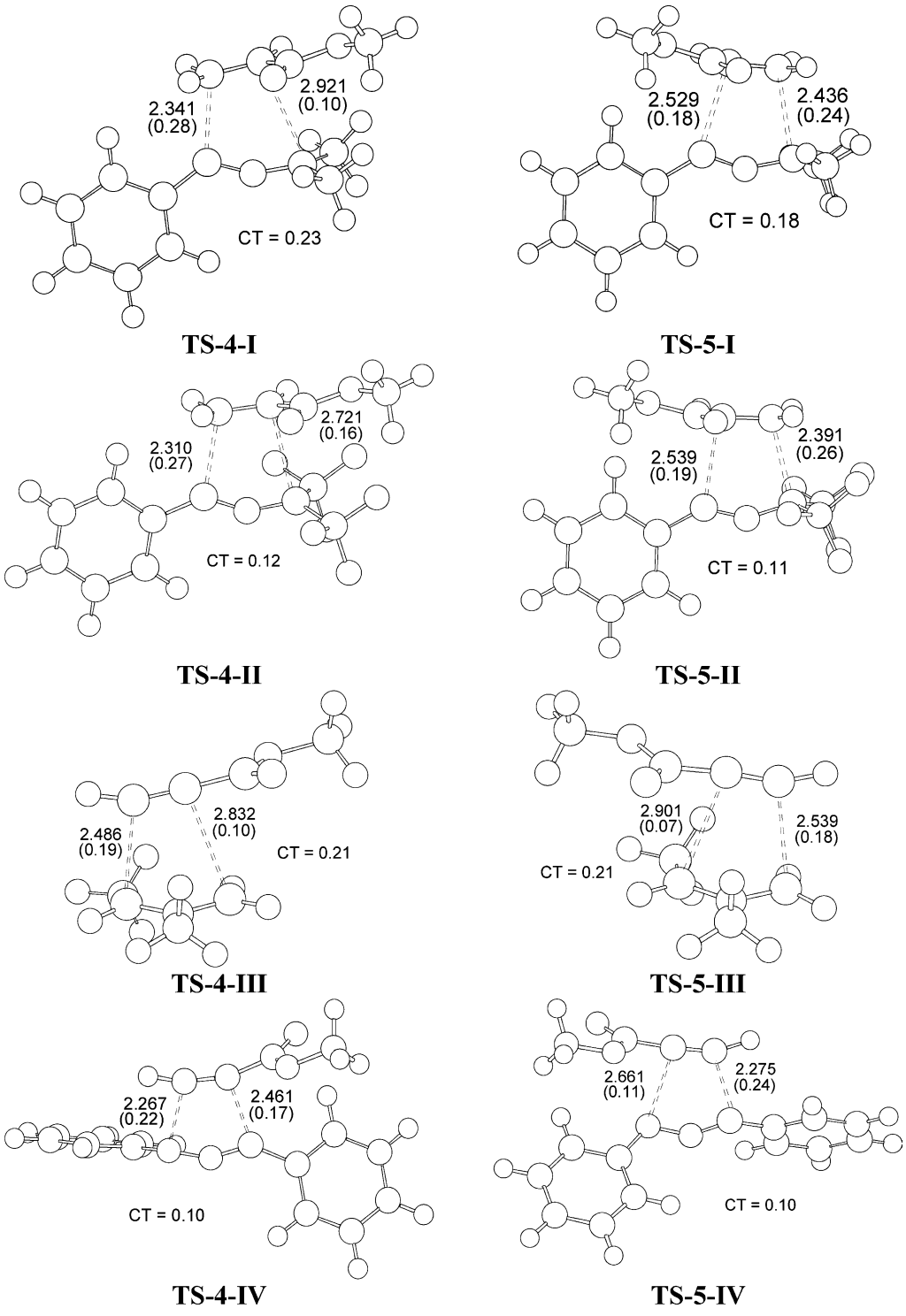


Figure 1. Transition structures corresponding to the regiosomeric path of the 13DC reaction models **I–IV**. The bond lengths directly involved in the reaction are given in angstroms. The bond orders are given in parenthesis. The charge transfers (CT) are given in a.u.

inclusion of the activation entropies to the free energies brings the activation free energy associated to **TS-5-III** to 4.4 kcal/mol. These cycloadditions are very exothermic by about −60 to −100 kcal/mol. The analysis of the regioselectivity for these 13DC reactions measured as the difference of activation enthalpies between the TSs leading to different regioisomers shows that they fall in a narrow range between 0.3 and 1.9 kcal/mol. While for the reaction models **I**, **II** and **III** the B3LYP/6-31G* calculations predict the regioselectivity experimentally observed, for the model **IV** it fails. Furthermore, previous theoretical studies on 13DC reactions have pointed out that the regioselectivity of this type of cycloadditions is strongly dependent on the computational level used.[33]

An analysis of the geometries at the TSs given in Figure 1 indicates that they correspond to asynchronous bond-formation processes. While for the reaction models **I**, **III**

and **IV** the more asynchronous TSs correspond to less energetic one, **TS-4-I**, **TS-5-III** and **TS-5-IV**, for model **II** the result is different. In this case, the hindrance that appears between the carboxylate group present in **MA** and the two trifluoromethyl groups present in the dipole **NY-2** may account for the large asynchronicity found at **TS-4-II**.

The extent of bond-formation along a reaction pathway may be provided by the quantitative concept of bond order (BO).[34] The BO values of the C–C and N–C σ-bond that are being formed along these 13DC reactions are shown in parenthesis in Figure 1. These values are within the range of 0.07 to 0.28. Therefore, it may be suggested that these TSs correspond to early processes. In general, the asynchronicity shown by the geometrical data is accounted for by the BO values. The reaction models **I** and **III**, which present larger regioselectivity, also present the more asynchronous TSs along the more favorable reactive channels **TS-4-I** and **TS-5-III**, respectively.

Finally, the CT was evaluated at the corresponding TSs. The natural charges at the TSs appear shared between the dipole framework, **NY-1**, **NY-2**, **AY** and **NI**, and the dipolarophile moiety, **MA** and **MP**, respectively. The resulting values are presented in Figure 1. The CT ranges from 0.10 e for model **IV** to 0.23 e for model **I**. For all cases the CT flows from the dipole to the dipolarophile. For models **II–IV** both regioisomeric TSs present the same CT pattern. Only model **I** presents a larger charge transfer at the more favorable reactive channel; 0.23 e at **TS-4-I** compared to 0.18 e at **TS-5-I**.

3.2. Analysis based on the global and local electrophilicity at the ground state of reagents

In Table 2 are displayed the electronic chemical potential μ, chemical hardness η, global electrophilicity ω. Also included in Table 2 are the values of local electrophilicity and the electrophilic and nucleophilic Fukui functions for the dipoles **NY-1**, **NY-2**, **AY** and **NI**, and the dipolarophiles **MA** and **MP**. The electronic chemical potential, μ, of the four dipoles with values between −0.0585 a.u. and −0.1408 a.u., are higher than those for the dipolarophiles **MA** and **MP**, −0.1586 a.u. and −0.1624 a.u., respectively. Therefore the CT at these 13DC reactions will take place from the dipole to the dipolarophile, in complete agreement with the CT analysis performed at the TSs (see Section 3.1). The two dipolarophiles present similar electrophilicity values, 1.51 eV (**MA**) and 1.52 eV (**MP**). According to the absolute scale of electrophilicity based on the ω index,[16a] these compounds may be classified as strong electrophiles. On the other hand, the four dipoles present electrophilicity values in a wider range: 0.31 eV for **AY**, 1.15 eV for **NY-1**, 1.43 eV for **NI** and 1.95 eV for **NY-2**. Thus, while **AY** is classified as a marginal electrophile (and probably a good nucleophile), **NY-2** is classified as a strong electrophile. Note that even though **NY-2** presents a larger electrophilicity value than **MA**, the later has a lower chemical potential, which is the index that determines the direction of the electronic flux along the cycloaddition. Therefore, along this series of 13DC reactions the more favorable interaction will take place between the less electrophilic specie, **AY**, namely the dipoles in the present case, and the electrophilic dipolarophile **MP**. Note that for the 13DC reaction between **AY** and **MP**, **TS-4-III** and **TS-5-III** are located below reactants on potential energy surface. In addition, the lower CT found for models **II** and **IV** can be related to the similar electrophilicity values displayed by the dipoles and dipolarophiles (see Table 2). In these cases, along the cycloaddition pathway any of them have the same trend to supply or accept electron density from each other.

Recent studies devoted to regioselective DA reactions have shown that the analysis of the local electrophilicity,[18] ω_k, at the electrophile together with the analysis of the nucleophilic Fukui functions, f_k^-,[19] at the nucleophile, allows the prediction of the regioselectivity in these competitive cycloadditions.[16b,c,g] The local functions are also summarized in Table 2. For the two dipolarophiles **MA** and **MP**, classified as strong electrophiles, the C4 carbon atom (the β-position) presents a larger local electrophilicity value than the C5 site.[35] Therefore, the C4 will be the preferred position for a nucleophilic attack by a dipole. This fact is in agreement with the asynchronicity shown at all the TSs. The C(N)1–C4 or C(N)3–C4 bonds formed at **TS-4-X** or **TS-5-X**, respectively, are shorter and more advanced than the C(N)3–C5 or C(N)1–C5 ones. This fact that has also been observed in other cycloaddition reactions suggests that the most electrophilic reagents control the asynchronicity of the process by a larger bond-formation process at the most electrophilic site of the molecule.

A different local picture is found for the four dipoles where the values of the nucleophilic Fukui functions, f_k^- depend on both the structure of the dipole and the substitution pattern. For instance, for the nitrile ylide **NY-1** the C1 carbon atom has a larger f_k^- value than the C3 one, 0.53 and 0.29, respectively, while for the nitrile ylide **NY-2** there is a change on the local activation: now the C3 carbon atom presents the larger f_k^- value (see Table 2). This result allows to explain the change of regioselectivity for these 13DC reactions. Substitution of the two electron-releasing methyl groups present in **NY-1** by two electron-withdrawing CF_3 groups in **NY-2** produces a change in the polarization of the $HOMO_{dipole}$ which acts as a nucleophile, thereby reverting the regioselectivity pattern.

For the azomethyne ylide **AY**, the presence of the electron-releasing methyl group on the C1 position polarizes the $HOMO_{dipole}$ through the C3 carbon atom. As a result, the unsubstituted C3 carbon atom presents a larger f_k^- value compared to that at the C1 site. Therefore, along the cycloaddition reaction the more favorable reactive channel takes place through the C3–C4 bond-formation by the nucleophilic attack of the C3 carbon atom of **AY** to the more electrophilic C4 site of **MP**. Note that at the highly asynchronous **TS-5-III** the covalent interactions between the C1 and C5 are negligible.

Finally, for the 1,3-diphenylnitrile imine **NI** the N1 nitrogen atom presents a larger nucleophilic activation than the C3 carbon atom (see Table 2). Therefore, the local analysis predicts a more favorable interaction between the N1 nitrogen of **NI** and the C4 carbon atom of **MP** along the **TS-4-IV**, in agreement with the regioselectivity

Table 2. Global and local properties of dipoles **NY-1**, **NY-2**, **NI** and **AY** and dipolarophiles **MA** and **MP**. *k* defines de site in the molecule where the property is being evaluated

	Global properties					Local properties			
	HOMO	LUMO	μ (a.u.)	η (a.u.)	ω (eV)	*k*	f^+	f^-	ω_k (eV)
NY-2	−0.2097	−0.0718	−0.1408	0.1379	1.95	1	0.1819	0.2232	0.36
						2	0.1452	0.0160	0.28
						3	0.0714	0.4488	0.14
MP	−0.2806	−0.0442	−0.1624	0.2363	1.52	4	0.3055	0.2328	0.46
						5	0.1168	0.1655	0.18
MA	−0.2720	−0.0452	−0.1586	0.2268	1.51	4	0.4154	0.0048	0.62
						5	0.1969	0.0714	0.29
NI	−0.1827	−0.0520	−0.1173	0.1307	1.43	1	0.0759	0.2685	0.11
						2	0.1159	0.0104	0.17
						3	0.0662	0.1613	0.10
NY-1	−0.1771	−0.0392	−0.1081	0.1379	1.15	1	0.2100	0.5339	0.24
						2	0.1208	0.0274	0.14
						3	0.0089	0.2909	0.01
AY	−0.1341	0.0171	−0.0585	0.1512	0.31	1	0.3165	0.4379	0.10
						2	0.2481	0.0118	0.08
						3	0.3460	0.4937	0.11

experimentally observed. Note that the analysis based on the energetic results predicts a reverse regioselectivity.

In summary, the present model based on the local electrophilicity index appears as a reliable approach to discuss regioselectivity. For instance, electrophilic activation/deactivation promoted by chemical substitution is correctly assessed within this framework. All this information is easily available from the properties of the GS electron density, and its first derivative with respect to the number of electrons which defines the Fukui function of the system. The electrophilic Fukui function in turn, permits the projection of the global electrophilicity into atoms, or group of atoms (i.e. functional groups) in the molecule. The polarization pattern at the dipoles induced by substituent effects may be assessed from a static model based on the difference in electrophilicity of the dipole/dipolarophile interacting pair.

4. Conclusions

The regioselectivity for a series of four 13DC reactions has been studied using DFT methods at the B3LYP/6-31G* level. The analysis of the activation enthalpies, asynchronicity and charge transfer at the TSs, as well as the analysis of the global and local electrophilicity index at the GS of reactants have been performed in order to rationalize the regioselectivity experimentally observed in these cycloadditions. We found that for this series of 13DCs the observed regioselectivity is well explained by reactivity analysis performed using global and local electrophilicity indexes. While the analysis of the global indexes allows to anticipate the polar character of the reaction as well the shift of the CT along the cycloaddition process, the local analysis allows to identify the more electrophilic and nucleophilic centers of the two reactants. Along an asynchronous cycloaddition associated to a polar process the most favorable two-center interaction between the highest nucleophilic and electrophilic sites of the reagents is responsible for the regioselectivity observed on these 13DC reactions.

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Synthesis of 6-substituted 7-aryl-5,6-dihydropyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-diones using the Vilsmeier reaction☆

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Abstract—The reaction of 6-amino-1,3-dimethyluracil with equimolar amounts of arylalkanone Mannich bases under optimized reaction conditions leads to 7-aryl-5,6-dihydropyrido[2,3-*d*]pyrimidines in a yield of 50–80%. Functionalization of these dihydropyridopyrimidine(1*H*,3*H*)-2,4-diones with the Vilsmeier reagent affords, depending on the reaction conditions, either 6-dimethylaminomethylidene substituted 5*H*-pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-diones or the corresponding pyridopyrimidine(1*H*,3*H*)-2,4-diones bearing a carboxaldehyde function in position 6 of the heterocycle. Some further transformations of the aldehyde function demonstrate the synthetic potential of the synthesized structures, introducing pharmacologically relevant basic substituents into the side chain of these pyrido[2,3-*d*]pyrimidine derivatives.

1. Introduction

Among the methods for the synthesis of 1,3-dimethylpyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-diones,[2–8] the condensation of 6-amino-1,3-dimethyluracil with suitable electrophiles is a straightforward and often used approach. The substitution pattern of the annelated pyridine ring formed is determined in these reactions by the structure of the biselectrophile. When an arylalkanone Mannich base is employed, formation of mixtures of dihydropyridopyrimidine(1*H*,3*H*)-2,4-dione **5** and pyridopyrimidine(1*H*,3*H*)-2,4-dione **6** has been reported.[3] However, the formation of the regioisomeric 5-aryl substituted pyridopyrimidines is not observed. The reaction mechanism is outlined in Scheme 1, in cyclocondensations performed with 6-aminouracil analogs and Mannich bases the postulated intermediates **3** und **4** could be isolated.[8]

We considered position 6 of the pyridopyrimidine **5** an interesting target for further modifications and employed the Vilsmeier reaction, as the structures obtainable are of interest regarding their pharmacological properties. The biological activity of this class of compounds, that is, their antitumor, antifolate and antibacterial properties are referenced elsewhere.[8] There are also only a few 6- or 7-substituted 5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidines used as drugs described in the literature,[9,10] for example, the antineoplastic lometrexol sodium.[10]

2. Results and discussion

6-Amino-1,3-dimethyluracil (**1**) was reacted with an equimolar amount of the Mannich bases **2a–c** according to the reported procedure.[3] We performed this reaction under an atmosphere of nitrogen in order to prevent oxidation to **6**, thus it was not necessary to purify the reaction mixture by column chromatography. The pyridopyrimidines **6** can be obtained directly, when the cyclocondensation is performed in acetic acid as the solvent.[3] The 5,6-dihydropyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-diones **5** were then converted to the red colored dimethylaminomethylidene derivatives **7** by treatment with the Vilsmeier mixture of phosphorous oxychloride and *N*,*N*-dimethylformamide at room temperature and subsequent hydrolysis (Scheme 1). Crystals of **7** decolorized with formation of the carbaldehyde **8**, which was also the main product, when the Vilsmeier reaction was performed at 80 °C followed by hydrolysis. The position 6 of the carbaldehyde group is evidenced by a H,H long-range coupling observed in the two dimensional spectrum with the proton in position 5 and

☆ See Ref. 1.

Keywords: Cyclization; Cyclocondensation; 6-Amino-1,3-dimethyluracil; Mannich bases; Vilsmeier reaction; Pyrido[2,3-*d*]pyrimidines.
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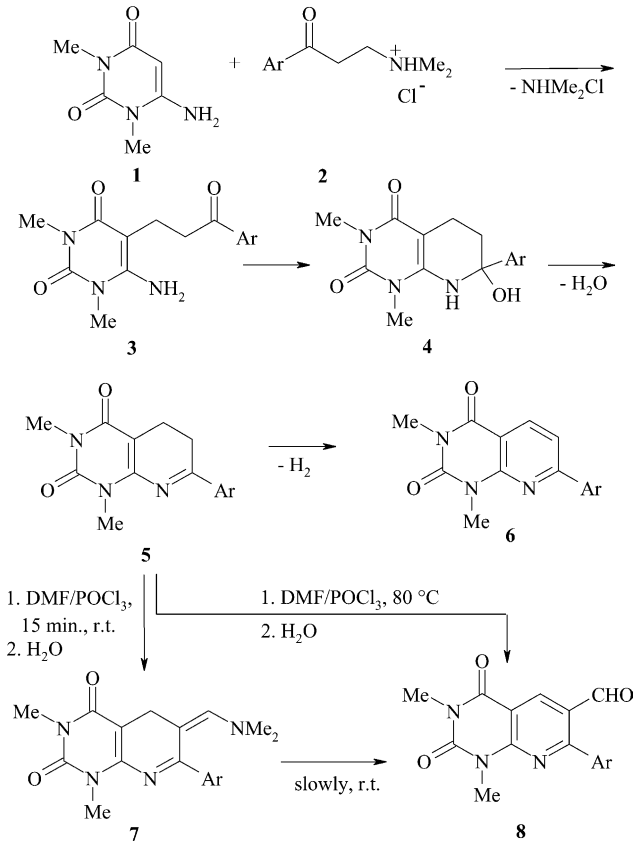
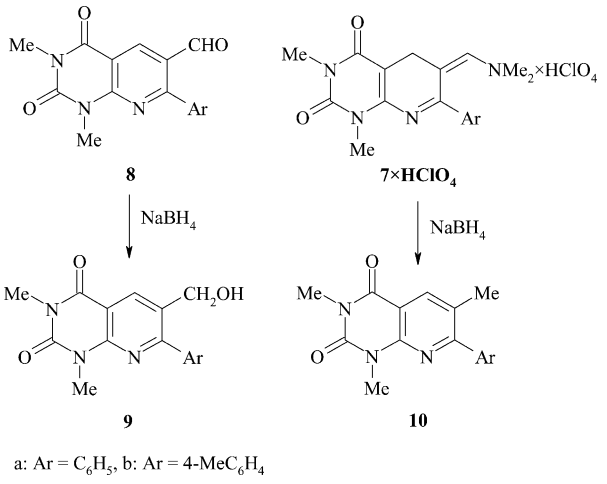


Scheme 1.

also a weak cross peak in the NOESY spectrum which showed additionally a strong cross peak with the protons in *ortho* position of the phenyl group. Two dimensional C,H-correlations aided also in the assignment of the ^{13}C NMR data of all the structures prepared.

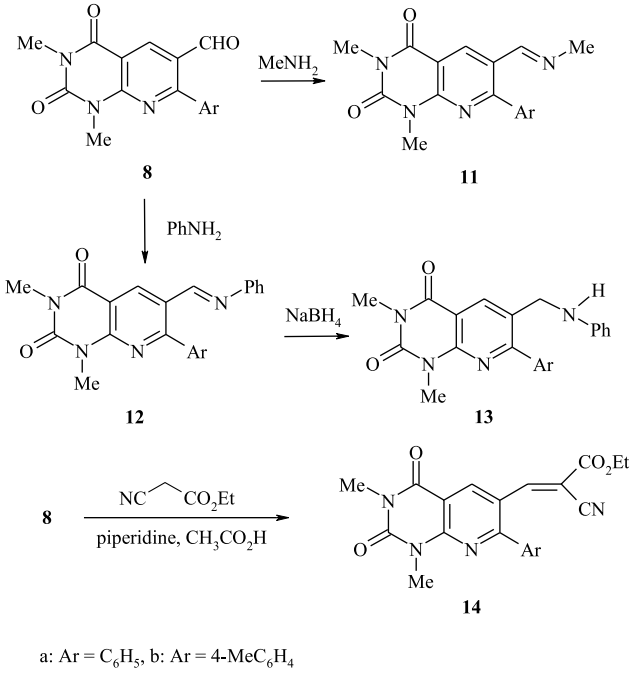
Thus, oxidation of the dihydropyridine moiety is occurring together with hydrolysis. The red dimethylaminomethylidene derivatives **7**, which are very sensitive towards air and water, can be precipitated as yellow hydroperchlorates, however, the salts do show only a slightly higher stability towards hydrolysis and, for example, completely decompose during the recording of the ^{13}C NMR spectra under routine conditions. The UV/vis spectra of the hydroperchlorates **7**×$HClO_4$ show an absorption band at about 465–470 nm with only a small bathochromic shift compared with the bases **7** of about 5 nm. Upon reduction of the carbaldehydes **8** with sodium borohydride, the expected hydroxymethylderivatives **9** are obtained. However, when performing the reduction of the hydroperchlorates **7** under the same reaction conditions, a complex mixture was obtained, the major product being the pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-diones **10**. The dimethylaminomethyl derivative is not formed, instead cleavage of the amino group and reduction to the methyl residue is observed, the pyridine moiety being unaffected (Scheme 2).

Another approach to pharmacological relevant structures is the introduction of a basic center into the side chain of the heterocycle, presenting the characteristic string of aromatic



Scheme 2.

moiety connected via an aliphatic side chain to a basic function.[11] In order to modify the carbaldehydes **8**, reactions with a primary amine afforded the imines **11** and **12**, the reduction of which allowed access to the anilinomethyl derivative **13**, again without reduction of the pyridine nucleus (Scheme 3).



Scheme 3.

Finally, under the reaction of the carbaldehydes **8** with numerous C–H acidic compounds we describe here only the one with ethyl cyano acetate under Knoevenagel conditions, affording the expected acrylate derivatives in about 60–70% yield.

3. Conclusions

The synthetic approach to 6-substituted pyrido[2,3-*d*]-pyrimidine(1*H*,3*H*)-2,4-diones presented here using the

Vilsmeier reaction of 5,6-dihydropyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-diones, allows access to a great number of structures and introduction of functionalities via the intermediate carboxaldehydes.

4. Experimental

4.1. General

Electron impact (EI) mass spectra were obtained with an ionization energy of 70 eV using a HP 5989A mass spectrometer and a direct inlet probe with a tungsten wire; *m*/*z* values are reported followed by the relative intensity in parentheses. APCI mass spectra were obtained on a Bruker Esquire LC. Nuclear Magnetic Resonance (^{1}H and ^{13}C) spectra were recorded on a Bruker instrument ARX300. In all cases DMSO-d_6 was used as the solvent. Chemical shifts are reported in parts per million (δ) downfield from an internal TMS reference. Coupling constants (*J*) are reported in Hertz (Hz), and spin multiplicities are indicated by the following symbols s (singlet), d (doublet), t (triplet), q (quartet), quint. (quintet), m (multiplet), br (broad signal). Multiplets were analyzed according to first order coupling. Elemental analyses were either performed by the Mikroanalytisches Labor Beetz or at the Institute of Inorganic Chemistry of the University of Kiel. High resolution mass spectra were performed by the Institute or Organic Chemistry at the University of Kiel. UV/vis spectra were recorded with a HP 8542A spectrophotometer in MeOH as the solvent. The wavelengths of the three or four strongest absorption bands together with the logarithm of the extinction coefficient ε in l/(mol×cm) are given. IR spectra were recorded as KBr pellets on a Perkin–Elmer 1600 PC FT-IR machine. Melting points were recorded with a Thiele (Büchi SMP-20) melting point apparatus and are not corrected. For preparative column chromatography silica gel 60 (Merck) was used.

4.2. 5,6-Dihydropyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-diones 5a–c and pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-diones 6a–c

A solution of 620 mg (4 mmol) of 4-amino-1,3-dimethyluracil (**1**) (Merck, Darmstadt, Germany) and 4 mmol of the corresponding arylalkanone Mannich bases **2a–c** was heated to reflux in 20 mL of deoxygenated water for 90 min under an atmosphere of nitrogen. After cooling to rt the yellow crystals were filtered and recrystallized from ethanol. In order to obtain pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-diones **6a–c**, the reaction was performed on the same scale as described above for **5**, acetic acid was employed as the solvent and air was not excluded. After cooling to rt the slightly yellow crystals were filtered and recrystallized from ethanol.

4.2.1. 1,3-Dimethyl-7-phenyl-5,6-dihydropyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-dione (5a). 540 mg (2.0 mmol, 50%), mp 180 °C, mp[3] 180 °C. UV/vis λ: 218 (4.1), 276 (4.2), 368 (3.8). IR ν: 1688, 1644/1640. MS *m*/*z*: 269 (M^+, 84), 268 (100), 192 (38), 154 (19), 81 (40). ^{1}H NMR data in accordance with Ref. 5. ^{13}C NMR δ: 16.3 (C-5), 24.2 (C-6), 28.2 (1-CH_3), 29.9 (3-CH_3), 94.2 (C-4a), 128.0 (C-3′), 128.8 (C-2′), 132.7 (C-4′), 136.8 (C-1′), 149.4 (C-8a), 152.1 (C-2), 163.0 (C-4), 174.6 (C-7).

4.2.2. 1,3-Dimethyl-7-(4-tolyl)-5,6-dihydropyrido[2,3-*d*]-pyrimidine(1*H*,3*H*)-2,4-dione (5b). 690 mg (2.4 mmol, 61%), mp 181 °C. UV/vis λ: 218 (4.2), 276 (4.2), 362 (3.9). IR ν: 1692, 1660/1650. MS *m*/*z*: 283 (M^+, 71), 282 (100), 281 (21), 192 (26), 81 (40). ^{1}H NMR δ: 2.44 (s, 3H, 4′-CH_3), 2.68/2.89 (2×t, J=9.0 Hz, 2×2H, 5-H, 6-H), 3.42/3.56 (2×s, 2×3H, 1-CH_3, 3-CH_3), 7.30/7.98 (2×d, J=8.3 Hz, 2×2H, 2′-H, 3′-H). ^{13}C NMR δ: 16.4 (C-5), 21.7 (4′-CH_3), 24.1 (C-6), 28.2 (1-CH_3), 30.0 (3-CH_3), 94.4 (C-4a), 128.2 (C-2′), 129.6 (C-3′), 134.2 (C-1′), 43.7 (C-4′), 149.7 (C-8a), 152.2 (C-2), 163.1 (C-4), 174.5 (C-7). HRMS Calcd for $C_{15}H_{12}N_3O_2$: 283.1321, found: 283.1319.

4.2.3. 7-(4-Bromophenyl)-1,3-dimethyl-5,6-dihydropyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-dione (5c). 1.09 g (3.1 mmol, 78%), mp 178–180 °C. UV/vis λ: 218 220 (4.2), 284 (4.3), 370 (3.9). IR ν: 1692, 1638 (br). MS *m*/*z*: 349 (M^+, ^{81}Br, 76), 348 (100), 347 (M^+, ^{79}Br, 88), 346 (85), 345 (23), 192 (59), 81 (64). ^{1}H NMR δ: 2.69/2.88 (2×t, J=9 Hz, 2×2H, 5-H, 6-H), 3.41/3.64 (2×s, 2×3H, 1-CH_3, 3-CH_3), 7.64/7.95 (2×d, J=8.7 Hz, 2×2H, 2′-H, 3′-H). ^{13}C NMR δ: 16.3 (C-5), 24.0 (C-6), 28.3 (1-CH_3), 30.0 (3-CH_3), 94.5 (C-4a), 127.8 (C-1′), 129.4 (C-2′), 132.1 (C-3′), 135.6 (C-4′), 149.3 (C-8a), 152.0 (C-2), 163.0 (C-4), 173.5 (C-7).

4.2.4. 1,3-Dimethyl-7-phenylpyrido[2,3-*d*]pyrimidine-(1*H*,3*H*)-2,4-dione (6a). 570 mg (2.2 mmol, 54%), mp 184 °C, mp[3] 156–157 °C. UV/vis λ: 220 (4.5), 252 (4.1), 272 (4.0), 332 (4.2). IR ν: 1706, 1658, 1602. MS *m*/*z*: 267 (M^+, 100), 239 (47), 238 (42), 155 (44), 154 (19). ^{1}H NMR data in accordance with Ref. 3. ^{13}C NMR δ: 28.4 (1-CH_3), 29.4 (3-CH_3), 109.0 (C-4a), 115.0 (C-6), 127.5 (C-3′), 128.9 (C-2′), 130.7 (C-4′), 137.4 (C-1′), 138.3 (C-5), 150.7 (C-8a), 151.6 (C-2), 161.1 (C-7), 161.3 (C-4).

4.2.5. 1,3-Dimethyl-7-(4-tolyl)-pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-dione (6b). 550 mg (2.0 mmol, 49%), mp 183 °C, mp[3] 173–174 °C. UV/vis λ: 220 (4.6), 254 (4.1), 336 (4.1), 336 (4.4). IR ν: 1704, 1660, 1596. MS *m*/*z*: 281 (M^+, 100), 253 (46), 252 (43), 169 (34). ^{1}H NMR δ: 2.44 (s, 3H, 4′-CH_3), 3.50/3.82 (2×s, 2×3H, 1-CH_3, 3-CH_3), 7.31/8.02 (2×d, J=8.0 Hz, 2×2H, 2′-H, 3′-H), 7.62/8.46 (2×d, J=8.2 Hz, 2×1H, 6-H, 5-H). ^{13}C NMR δ: 21.4 (4′-CH_3), 28.4 (1-CH_3), 29.3 (3-CH_3), 108.7 (C-4a), 114.7 (C-6), 127.4 (C-2′), 129.6 (C-3′), 134.6 (C-1′), 138.1 (C-5), 141.4 (C-4′), 150.6 (C-8a), 151.6 (C-2), 161.1 (C-7), 161.3 (C-4).

4.2.6. 7-(4-Bromophenyl)-1,3-dimethylpyrido[2,3-*d*]-pyrimidine(1*H*,3*H*)-2,4-dione (6c). 910 mg (2.6 mmol, 66%), mp 216 °C. UV/vis λ: 222 (4.5), 256 (4.2), 276 (4.2), 336 (4.3). IR ν: 1704, 1656, 1598. MS *m*/*z*: 347 (M^+, ^{81}Br, 100), 319 (36), 318 (35), 317 (36), 316 (29), 235 (35), 233 (36). ^{1}H NMR δ: 3.51/3.81 (2×s, 2×3H, 1-CH_3, 3-CH_3), 7.60/8.47 (2×d, J=8.1 Hz, 2×1H, 6-H, 5-H), 7.63/7.97 (2×d, J=8 Hz, 2×2H, 2′-H, 3′-H). ^{13}C NMR δ: 28.5 (1-CH_3), 29.4 (3-CH_3), 109.3 (C-4a), 114.8 (C-6), 125.4 (C-1′), 129.0 (C-3′), 132.2 (C-2′), 136.3 (C-4′), 138.6 (C-5), 150.8 (C-8a), 151.6 (C-2), 160.0 (C-7), 161.2 (C-4).

HRMS Calcd for $C_{15}H_{12}N_3O_2^{79}Br$ 345.0113, found: 345.0113.

4.3. 6-(Dimethylaminomethylidene)-5*H*-pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-diones 7a–c, hydroperchlorates 7a–c×HClO$_4$, and 1,3-dimethyl-2,4-dioxo-(1*H*,3*H*)-pyrido[2,3-*d*]pyrimidine-6-carbaldehydes 8a–c

To a suspension of 1.0 mmol of **5** in 2 mL of DMF was added dropwise 200 µl (0.33 g, 2.1 mmol) of $POCl_3$ while cooling with an ice/water bath. The reaction mixture was stirred for 15 min at rt, followed by the addition of 10 mL of cold water. Solid sodium bicarbonate was added to the yellow solution until alkaline. The red solution was extracted with three 10 mL portions of dichloromethane, the combined organic layers were washed with water, dried over anhydrous sodium sulfate, filtered, and evaporated. The remainder was treated with diethyl ether and the solids of **7a–c** obtained were filtered and dried in vacuo. The corresponding hydroperchlorates were obtained by dissolving 1 mmol of **7** in 25 mL of methanol and 0.2 mL of $HClO_4$ (70%) was added while cooling with an ice/water bath. After about 15 min a precipitate was formed, which was filtered off and dried in vacuo. In all cases the yield was quantitative. The carbaldehydes **8a–c** were obtained when performing the reaction on the same scale as described above for **7a–c**, after stirring the reaction mixture for 30 min at rt, then the reaction mixture was heated to 80 °C for 3 h. After cooling 15 g of ice was added and the mixture was stirred vigorously. The yellow precipitate was filtered, dried, and recrystallized.

4.3.1. 1,3-Dimethyl-6-(dimethylaminomethylidene)-7-phenyl-5*H*-pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-dione (7a). 165 mg (0.51 mmol, 51%), mp 141 °C, decomposition upon recrystallization. UV/vis λ: 260 (3.9), 324 (3.8), 464 (3.6). IR ν: 2910 (w), 1662, 1636 (w). MS *m/z*: 324 (M^+, 21), 323 (17), 309 (53), 280 (38), 166 (35), 58 (100). ^{1}H NMR δ: 3.16 (s, 6H, $N(CH_3)_2$), 3.38/3.56 (2×s, 2×3H, 1-CH_3, 3-CH_3), 3.87 (br s, 2H, 5-H), 6.69 (t, J=1 Hz, 1H, 6=CH), 7.30–7.60 (m, 5H, phenyl-H). ^{13}C NMR δ: 22.9 (C-5), 27.9 (1-CH_3), 29.4 (3-CH_3), 43.9 (N-CH_3), 89.9 (C-6), 100.2 (C-4a), 128.1 (C-3′), 130.1 (C-2′), 130.3 (C-4′), 140.3 (C-1′), 151.5 (C-2), 152.5 (C-8a), 155 (6=CH), 162.9 (C-4), 176.9 (C-7).

4.3.2. 1,3-Dimethyl-6-(dimethylaminomethylidene)-7-(4-tolyl)-5*H*-pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-dione (7b). 210 mg (0.61 mmol, 61%), mp 166 °C, decomposition upon recrystallization. UV/vis λ: 276 (4.1), 326 (4.1), 466 (3.9). IR ν: 1684, 1644 (s), 1610. MS *m/z*: 338 (M^+, 54), 337 (51), 323 (94), 294 (82), 180 (48), 58 (100). ^{1}H NMR δ: 2.38 (s, 3H, 4′-CH_3), 3.16 (s, 6H, $N(CH_3)_2$), 3.38/3.57 (2×s, 2×3H, 1-CH_3, 3-CH_3), 3.83 (br s, 2H, 5-H), 6.72 (t, J=1 Hz, 1H, 6=CH), 7.21/7.46 (2×d, J=8.1 Hz, 2×2H, 2′-H, 3′-H). ^{13}C NMR δ: 21.4 (4′-CH_3), 22.9 (C-5), 27.9 (1-CH_3), 29.4 (3-CH_3), 43.8 (N-CH_3), 89.9 (C-6), 100.1 (C-4a), 128.9 (C-2′), 130.2 (C-3′), 137.4 (C-1′), 140.8 (C-4′), 151.6 (C-2), 152.5 (C-8a), 155.2 (6=CH), 163.0 (C-4), 176.9 (C-7).

4.3.3. 7-(4-Bromophenyl)-1,3-dimethyl-6-(dimethylaminomethylidene)-5*H*-pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-dione (7c). 250 mg (0.63 mmol, 63%), mp 143 °C, decomposition upon recrystallization. UV/vis λ: 218 (4.4), 276 (4.3), 368 (4.1), 466 (3.8). IR ν: 1686, 1640 (s). MS *m/z*: 402 (M^+, ^{79}Br, 8), 387 (21), 279 (55), 58 (100). ^{1}H NMR δ: 3.17 (s, 6H, $N(CH_3)_2$), 3.37/3.54 (2×s, 2×3H, 1-CH_3, 3-CH_3), 3.86 (br s, 2H, 5-H), 6.64 (br s, 1H, 6=CH), 7.42/7.54 (2×d, J=8.4 Hz, 2×2H, 2′-H, 3′-H). ^{13}C NMR δ: 22.9 (C-5), 27.9 (1-CH_3), 29.4 (3-CH_3), 43.9 (N-CH_3), 90.0 (C-6), 100.1 (C-4a), 124.8 (C-1′), 131.6 (C-3′), 131.9 (C-2′), 139.2 (C-4′), 151.3 (C-2), 152.4 (C-8a), 155.0 (6=CH), 162.9 (C-4), 175.6 (C-7).

4.3.4. 1,3-Dimethyl-6-(dimethylaminomethylidene)-7-phenyl-5*H*-pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-dione hydroperchlorate (7a×HClO$_4$). Mp 206–208 °C, decomposition upon recrystallization. UV/vis λ: 204 (4.4), 268 (4.2), 316 (4.1), 456 (3.9). IR ν: 3200 (br), 1690, 1664, 1090 (s). MS *m/z*: 325 [M+H$^+$] in MeOH/10 mM NH_4OAc by APCI. ^{1}H NMR δ: 3.20/3.47 (2×s, 2×3H, 1-CH_3, 3-CH_3), 3.50 (br s, 6H, $N(CH_3)_2$), 3.73 (br s, 2H, 5-H), 7.55–7.75 (m, 6H, phenyl-H, 6=CH), 10.31 (br s, 1H, NH). ^{13}C NMR δ: 22.2 (C-5), 27.8 (1-CH_3), 30.3 (3-CH_3), 43–49 (very br, N-CH_3), 86.9 (C-6), 102.6 (C-4a), 128.9 (C-3′), 131.3 (C-2′), 132.4 (C-1′), 132.8 (C-4′), 143.0 (C-8a), 150.5 (C-2), 160.7 (C-4), 160.8 (C-7), 166.4 (6=CH).

4.3.5. 1,3-Dimethyl-6-(dimethylaminomethylidene)-7-(4-tolyl)-5*H*-pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-dione hydroperchlorate (7b×HClO$_4$). Mp 192–194 °C, decomposition upon recrystallization. UV/vis λ: 278 (4.2), 322 (4.1), 462 (4.0). IR ν: 3200–2800 (w), 1694, 1664, 1092 (br, s). MS *m/z*: 339 [M+H$^+$] in MeOH/10 mM NH_4OAc by APCI. ^{1}H NMR δ: 2.43 (s, 3H, 4′-CH_3), 3.19/3.47 (2×s, 2×3H, 1-CH_3, 3-CH_3), 3.49 (br s, 6H, $N(CH_3)_2$), 3.70 (br s, 2H, 5-H), 7.52 (m, 5H, phenyl-H, 6=CH), 10.26 (br s, 1H, NH). ^{13}C NMR δ: 21.1 (4′-CH_3), 22.2 (C-5), 27.8 (1-CH_3), 30.2 (3-CH_3), 43-47 (very br, N-CH_3), 87.0 (C-6), 102.1 (C-4a), 129.4 (C-2′), 129.7 (C-1′), 131.3 (C-3′), 143.3 (C-8a), 143.9 (C-4′), 150.6 (C-2), 160.7 (C-4), 161.0 (C-7), 166.2 (6=CH).

4.3.6. 7-(4-Bromophenyl)-1,3-dimethyl-6-(dimethylaminomethylidene)-5*H*-pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-dione hydroperchlorate (7c×HClO$_4$). Mp 195–197 °C, decomposition upon recrystallization. UV/vis λ: 220 (4.3), 274 (4.3), 326 (4.1), 470 (4.0). IR ν: 3400–2900 (w), 1694, 1666, 1090 (br, s). MS *m/z*: 404/406 [M+H$^+$] in MeOH/10 mM NH_4OAc by APCI. ^{1}H NMR δ: 3.19/3.45 (2×s, 2×3H, 1-CH_3, 3-CH_3), 3.50 (br s, 6H, $N(CH_3)_2$), 3.72 (br s, 2H, 5-H), 7.60-7.80 (m, 5H, phenyl-H, 6=CH), 10.25 (br s, 1H, NH). ^{13}C NMR δ: 22.2 (C-5), 27.8 (1-CH_3), 30.2 (3-CH_3), 43-50 (very br, N-CH_3), 86.8 (C-6), 103.0 (C-4a), 126.6 (C-1′), 131.6 (C-4′), 131.8 (C-3′), 132.1 (C-2′), 143.9 (C-8a), 150.5 (C-2), 159.7 (C-7), 160.7 (C-4), 166.2 (6=CH).

4.3.7. 1,3-Dimethyl-2,4-dioxo-7-phenyl-(1*H*,3*H*)-pyrido[2,3-*d*]pyrimidine-6-carbaldehyde (8a). 210 mg (0.71 mmol, 71%), mp 189 °C (ethyl acetate). UV/vis λ: 222 (4.4), 256 (4.0), 276 (4.1), 336 (4.2). IR ν: 1718, 1680, 1658. MS *m/z*: 295 (M^+, 95), 294 (100), 267 (17), 239 (27), 238 (24). ^{1}H NMR δ: 3.52/3.81 (2×s, 2×3H, 1-CH_3,

3-CH_3), 7.50–7.80 (m, 5H, phenyl-H), 9.06 (s, 1H, 5-H), 10.05 (s, 1H, CHO). ^{13}C NMR δ: 28.6 (1-CH_3), 30.0 (3-CH_3), 109.6 (C-4a), 125.5 (C-6), 128.8 (C-3′), 130.5 (C-2′), 130.8 (C-4′), 136.1 (C-1′), 139.3 (C-5), 151.3 (C-2), 152.3 (C-8a), 160.5 (C-4), 166.2 (C-7), 189.2 (6-CHO). Anal. Calcd: C: 65.08, H: 4.44, N: 14.23, found: C: 65.40, H: 4.48, N: 14.12.

4.3.8. 1,3-Dimethyl-2,4-dioxo-7-(4-tolyl)-(1*H*,3*H*)-pyrido[2,3-*d*]pyrimidine-6-carbaldehyde (8b). 220 mg (0.70 mmol, 70%), mp 194 °C (ethyl acetate). UV/vis λ: 220 (4.4), 274 (4.3), 326 (4.2). IR ν: 1718, 1686, 1662. MS *m/z*: 309 (M^+, 100), 308 (82), 294 (61), 253 (33), 252 (31), 169 (34). ^{1}H NMR δ: 2.49 (s, 3H, 4′-CH_3), 3.51/3.80 (2×s, 2×3H, 1-CH_3, 3-CH_3), 7.38/7.57 (2×d, J=7.9 Hz, 2×2H, 2′-H, 3′-H), 9.05 (s, 1H, 5-H), 10.06 (s, 1H, CHO). ^{13}C NMR δ: 21.5 (4′-CH_3), 28.6 (1-CH_3), 29.9 (3-CH_3), 109.4 (C-4a), 125.4 (C-6), 129.6 (C-2′), 130.6 (C-3′), 133.3 (C-1′), 139.3 (C-5), 141.3 (C-4′), 151.4 (C-2), 152.2 (C-8a), 160.6 (C-4), 166.2 (C-7), 189.4 (6-CHO). Anal. Calcd: C: 66.01, H: 4.89, N: 13.58, found: C: 65.82, H: 5.14, N: 13.41.

4.3.9. 7-(4-Bromophenyl)-1,3-dimethyl-2,4-dioxo-(1*H*,3*H*)-pyrido[2,3-*d*]pyrimidine-6-carbaldehyde (8c). 250 mg (0.67 mmol, 67%), mp 203 °C (toluene). UV/vis λ: 220 (4.4), 258 (4.3), 324 (4.1). IR ν: 1720, 1684, 1660. MS *m/z*: 375 (M^+, ^{81}Br, 96), 374 (71), 373 (M^+, ^{79}Br, 100), 372 (53), 294 (47), 293 (35). ^{1}H NMR δ: 3.52/3.79 (2×s, 2×3H, 1-CH_3, 3-CH_3), 7.54/7.72 (2×d, J=8.6 Hz, 2×2H, 2′-H, 3′-H), 9.05 (s, 1H, 5-H), 10.04 (s, 1H, CHO). ^{13}C NMR δ: 28.7 (1-CH_3), 30.0 (3-CH_3), 109.9 (C-4a), 125.4 (C-6), 125.7 (C-1′), 131.9 (C-3′), 132.1 (C-2′), 135.0 (C-4′), 139.6 (C-5), 151.3 (C-2), 152.4 (C-8a), 160.4 (C-4), 164.9 (C-7), 188.7 (6-CHO). Anal. Calcd: C: 51.36, H: 3.23, N: 11.23, found: C: 51.07, H: 3.13, N: 11.56.

4.4. 6-Hydroxymethyl-1,3-dimethylpyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-diones 9a–b and 1,3,6-trimethylpyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-diones 10a–b

0.5 mmol of **8** and 100 mg (2.65 mmol) of sodium borohydride were suspended in 10 mL of ethanol and stirred for 45 min at rt. The mixture was concentrated in vacuo and vigorously stirred with 20 mL of cold water. Then the mixture was acidified by addition of diluted HCl, the precipitate was filtered, washed with water, and recrystallized from ethanol. The trimethyl derivatives **10a–b** were obtained by heating 1 mmol of **7**×$HClO_4$ and 100 mg (2.65 mmol) of sodium borohydride in 25 mL of ethanol to 50 °C for 90 min. After cooling the reaction mixture was concentrated in vacuum and 20 mL of cold water was added to the remainder. The mixture was stirred vigorously and the precipitate was filtered off, dried in vacuum and subjected to column chromatography on silica gel (cyclohexane/ethyl acetate 6/4) to afford the colorless derivatives **10**.

4.4.1. 6-Hydroxymethyl-1,3-dimethyl-7-phenylpyrido-[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-dione (9a). 120 mg (0.41 mmol, 81%), mp 193 °C. UV/vis λ: 220 (4.5), 250 (4.3), 326 (4.1). IR ν: 3438 (br), 1714, 1670, 1608. MS *m/z*: 297 (M^+, 100), 296 (77), 268 (19), 239 (16), 220 (15). ^{1}H NMR δ: 2.57 (br s, 1H, OH), 3.47/3.72 (2×s, 2×3H, 1-CH_3, 3-CH_3), 4.76 (s, 2H, 6-CH_2), 7.50/7.69 (2×m, 5H, phenyl-H), 8.66 (s, 1H, 5-H). ^{13}C NMR δ: 28.5 (1-CH_3), 29.5 (3-CH_3), 61.7 (6-CH_2), 109.1 (C-4a), 128.4 (C-3′), 129.3 (C-2′), 129.6 (two overlapping signals, C-6, C-4′), 138.3 (C-1′), 138.8 (C-5), 149.5 (C-8a), 151.6 (C-2), 161.4 (C-4), 162.5 (C-7). HRMS Calcd for $C_{16}H_{15}N_3O_3$ 297.1133, found: 297.1114.

4.4.2. 6-Hydroxymethyl-1,3-dimethyl-7-(4-tolyl)-pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-dione (9b). 140 mg (0.45 mmol, 90%), mp 198 °C. UV/vis λ: 220 (4.6), 254 (4.2), 328 (4.2). IR ν: 3400 (br), 1700, 1640, 1605. MS *m/z*: 311 (M^+, 100), 310 (76), 296 (17), 282 (16). ^{1}H NMR δ: 2.44 (s, 3H, 4′-CH_3), 2.61 (br s, 1H, OH), 3.46/3.71 (2×s, 2×3H, 1-CH_3, 3-CH_3), 4.76 (s, 2H, 6-CH_2), 7.31/7.59 (2×d, J=8.0 Hz, 2×2H, 2′-H, 3′-H), 8.47 (s, 1H, 5-H). ^{13}C NMR δ: 21.4 (4′-CH_3), 28.5 (1-CH_3), 29.5 (3-CH_3), 61.8 (6-CH_2), 108.8 (C-4a), 129.2 (two overlapping signals C-2′, C-3′), 129.5 (C-6), 135.5 (C-1′), 138.7 (C-5), 139.9 (C-4′), 149.5 (C-8a), 151.7 (C-2), 161.4 (C-4), 162.5 (C-7). Anal. Calcd: C: 65.58, H: 5.50, N: 13.50, found: C: 65.46, H: 5.42, N: 13.55.

4.4.3. 1,3,6-Trimethyl-7-phenylpyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-dione (10a). 56 mg (0.20 mmol, 20%), mp 165 °C, mp[3] 162–163 °C. UV/vis λ: 218 (4.5), 248 (4.2), 330 (4.1). IR ν: 1706, 1670, 1606. MS *m/z*: 281 (M^+, 98), 280 (100), 253 (23), 252 (32), 169 (37). ^{1}H NMR δ: 2.45 (s, 3H, 6-CH_3), 3.50/3.73 (2×s, 2×3H, 1-CH_3, 3-CH_3), 7.49/7.62 (2×m, 3H/2H, phenyl-H), 8.33 (s, 1H, 5-H). ^{13}C NMR δ: 19.2 (6-CH_3), 27.3 (1-CH_3), 28.9 (3-CH_3), 108.5 (C-4a), 125.8 (C-6), 127.8 (C-3′), 128.6 (two overlapping signals C-2′,C-4′), 138.8 (C-1′), 139.1 (C-5), 148.2 (C-8a), 151.2 (C-2), 161.0 (C-4), 162.3 (C-7). HRMS Calcd for $C_{16}H_{15}N_3O_2$ 281.1164, found: 281.1164.

4.4.4. 1,3,6-Trimethyl-7-(4-tolyl)-pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-dione (10b). 44 mg (0.15 mmol, 15%), mp 166 °C. UV/vis λ: 220 (4.6), 252 (4.1), 332 (4.1). IR ν: 1702, 1654 (s), 1604. MS *m/z*: 295 (M^+, 92), 294 (100), 267 (16), 266 (22), 183 (18). ^{1}H NMR δ: 2.44 (s, 6H, 6-CH_3, 4′-CH_3), 3.49/3.71 (2×s, 2×3H, 1-CH_3, 3-CH_3), 4.76 (s, 2H, 6-CH_2), 7.30/7.59 (2×d, J=8.0 Hz, 2×2H, 2′-H, 3′-H), 8.29 (s, 1H, 5-H). ^{13}C NMR δ: 19.6 (6-CH_3), 21.3 (4′-CH_3), 28.3 (1-CH_3), 29.3 (3-CH_3), 108.6 (C-4a), 126.2 (C-6), 128.9 (C-2′), 129.0 (C-3′), 136.4 (C-1′), 139.1 (C-4′), 139.4 (C-5), 148.6 (C-8a), 151.6 (C-2), 161.4 (C-4), 162.7 (C-7). HRMS Calcd for $C_{17}H_{17}N_3O_2$ 295.1321, found: 295.1320.

4.5. Substituted 6-iminomethylpyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-diones 11a–b, 12a–b, and substituted 6-aminomethylpyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-diones 13a–b

2 mmol of **8** and 4 mmol of aniline or the corresponding amount of a solution of methyl amine in ethanol were suspended in 20 mL of ethanol and heated to reflux for 30 min. The solids **11a–b**, **12a–b** formed upon cooling of the mixture were filtered off, dried in vacuo, and used as such for further reactions. **13a–b** was obtained by reaction of 0.5 mmol of **12** and 100 mg (2.65 mmol) of sodium borohydride in 25 mL of ethanol, the mixture was stirred overnight at rt. The solvent was evaporated in vacuum,

25 mL of cold water was added and the mixture was stirred vigorously. The precipitate which formed was filtered off, dried, and subjected to column chromatography on silica gel (ethyl acetate/cyclohexane, 3:7).

4.5.1. 1,3-Dimethyl-6-(*N*-methyliminomethyl)-7-phenyl-pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-dione (11a). 350 mg (1.1 mmol, 57%), mp 208 °C (ethanol). UV/vis λ: 206 (4.4), 228 (4.6), 332 (4.3). IR ν: 1712, 1676, 1600.MS *m/z*: 308 (M^+, 20), 307 (100), 250 (11), 193 (8). ^{1}H NMR δ: 3.50 (br s, 6H, 1-CH_3, N-CH_3), 3.76 (s, 3H, 3-CH_3), 7.52/7.62 (2× m, 5H, phenyl-H), 8.31 (q, J=1.6 Hz, 1H, 6-CH), 9.08 (s, 1H, 5-H). ^{13}C NMR δ: 28.5 (1-CH_3), 29.6 (C-3-CH_3), 48.4 (N-CH_3), 109.7 (C-4a), 125.7 (C-6), 128.5 (C-3′), 129.8 (C-4′), 130.2 (C-2′), 137.5 (C-1′), 138.0 (C-5), 150.6 (C-8a), 151.5 (C-2), 158.7 (C-6-CH), 160.9 (C-4), 162.7 (C-7). Anal. Calcd: C: 66.22, H: 5.23, N: 18.17, found: C: 65.97, H: 5.11, N: 18.05.

4.5.2. 1,3-Dimethyl-6-(*N*-methyliminomethyl)-7-(4-tolyl)-pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-dione (11b). 390 mg (1.2 mmol, 60%), mp 190 °C (ethanol). UV/vis λ: 228 (4.5), 268 (4.4), 334 (4.1). IR ν: 1710, 1684, 1600. MS *m/z*: 322 (M^+, 22), 321 (100), 264 (7), 132 (7). ^{1}H NMR δ: 2.46 (s, 3H, 4′-CH_3), 3.49 (s, 3H, N-CH_3) 3.51/3.76 (2×s, 2×3H, 1-CH_3, 3-CH_3), 7.33/7.52 (2×d, J=8.0 Hz, 2×2H, phenyl-H), 8.32 (q, J=1.6 Hz, 1H, 6-CH), 9.06 (s, 1H, 5-H). ^{13}C NMR δ: 21.4 (4′-CH_3), 28.5 (1-CH_3), 29.6 (3-CH_3), 48.3 (N-CH_3), 109.4 (C-4a), 125.6 (C-6), 129.2 (C-2′), 130.0 (C-3′), 134.7 (C-1′), 137.9 (C-5), 140.1 (C-4′), 150.5 (C-8a), 151.5 (C-2), 158.9 (6-CH), 160.9 (C-4), 162.8 (C-7). Anal. Calcd: C: 67.07, H: 5.63, N: 17.38, found: C: 66.93, H: 5.39, N: 17.13.

4.5.3. 1,3-Dimethyl-7-phenyl-6-(*N*-phenyliminomethyl)-pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-dione (12a). 460 mg (1.26 mmol, 63%), mp 210 °C (ethanol). UV/vis λ: 232 (4.3), 254 (4.2), 300 (4.1), 334 (4.2). IR ν: 1718, 1670 (s), 1605. MS *m/z*: 370 (M^+, 30), 369 (100), 312 (4), 278 (8), 255 (5). ^{1}H NMR δ: 3.51/3.79 (2×s, 2×3H, 1-CH_3, 3-CH_3), 7.10–7.70 (m, 10H, phenyl-H, aniline-H), 8.52 (s, 1H, 5-H), 9.33 (s, 1H, 6-CH). ^{13}C NMR δ: 28.6 (1-CH_3), 29.7 (3-CH_3), 109.8 (C-4a), 121.1 (aniline-C2), 125.7 (C-6), 128.6 (aniline-C3), 129.2 (C-3′), 130.1 (C-4′), 130.2 (C-2′), 137.4 (C-1′), 138.4 (C-5), 138.4 (aniline-C4), 151.0 (C-8a), 151.2 (aniline-C 1), 151.5 (C-2), 156.0 (6-CH), 160.9 (C-4), 163.7 (C-7). HRMS Calcd for $C_{23}H_{17}N_4O_2$ $(M\text{-}H)^+$ 369.1352, found: 369.1351.

4.5.4. 1,3-Dimethyl-6-(*N*-phenyliminomethyl)-7-(4-tolyl)-pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-dione (12b). 445 mg (1.16 mmol, 58%), mp 209 °C (ethanol). UV/vis λ: 232 (4.6), 270 (4.6), 336 (4.5). IR ν: 1712, 1664, 1600. MS *m/z*: 384 (M^+, 30), 383 (100), 326 (3), 292 (7). ^{1}H NMR δ: 2.45 (s, 3H, 4′-CH_3), 3.51/3.78 (2×s, 2×3H, 1-CH_3, 3-CH_3), 7.18-7.57 (m, 9H, phenyl-H, aniline-H), 8.53 (s, 1H, 5-H), 9.30 (s, 1H, 6-CH). ^{13}C NMR δ: 21.4 (4′-CH_3), 28.5 (1-CH_3), 29.6 (3-CH_3), 109.5 (C-4a), 121.0, (aniline-C2), 125.5 (C-6), 129.1 (aniline-C3), 129.3 (C-2′), 130.1 (C-3′), 134.5 (C-1′), 138.3 (C-5), 138.4 (aniline-C4), 140.4 (C-4′), 150.9 (C-8a), 151.2 (aniline-C1), 151.7 (C-2), 156.2 (6-CH), 161.0 (C-4), 163.7 (C-7). Anal. Calcd: C: 71.86, H: 5.24, N: 14.57, found: C: 71.78, H: 5.24, N: 14.58.

4.5.5. 1,3-Dimethyl-7-phenyl-6-(*N*-phenylaminomethyl)-pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-dione (13a). 37 mg (0.10 mmol, 20%), mp 131 °C (ethyl acetate). UV/vis λ: 218 (4.6), 248 (4.4), 328 (4.0). IR ν: 3350 (m), 1708, 1652, 1640 (s). MS *m/z*: 372 (M^+, 19), 281 (25), 280 (100), 223 (51). ^{1}H NMR δ: 2.44 (s, 1H, NH), 3.48/3.73 (2×s, 2×3H, 1-CH_3, 3-CH_3), 4.39 (s, 2H, 6-CH_2), 6.48 (d, J=7.7 Hz, 2H, aniline-2H), 6.71 (t, J=7.3 Hz, 1H, aniline-4H), 7.12 (br t, J=7.8 Hz, 2H, aniline-3H), 7.50/7.63 (2×m, 5H, phenyl-H), 8.60 (s, 1H, 5-H). ^{13}C NMR δ: 28.5 (1-CH_3), 29.5 (3-CH_3), 45.3 (6-CH_2), 109.3 (C-4a), 113.2 (aniline-C2), 118.3 (aniline-C4), 128.2 (C-6), 128.5 (C-3′), 129.0 (C-2′), 129.3 (aniline-C3), 129.5 (C-4′), 138.6 (C-5), 139.6 (C-1′), 146.9 (aniline-C1), 149.4 (C-8a), 151.6 (C-2), 161.3 (C-7), 162.8 (C-4). HRMS Calcd for $C_{22}H_{20}N_4O_2$ 372.1586, found: 372.1584.

4.5.6. 1,3-Dimethyl-6-(*N*-phenylaminomethyl)-7-(4-tolyl)-pyrido[2,3-*d*]pyrimidine(1*H*,3*H*)-2,4-dione (13b). 60 mg (0.16 mmol, 32%), mp 195 °C (ethanol). UV/vis λ: 220 (4.6), 250 (4.4), 328 (4.1). IR ν: 3408, 1702, 1666, 1602. MS *m/z*: 384 (M^+, 22), 295 (20), 294 (100), 237 (36). ^{1}H NMR δ: 2.46 (s, 3H, 4′-CH_3), 3.47/3.72 (2×s, 2×3H, 1-CH_3, 3-CH_3), 4.07 (br s, 1H, NH), 4.38 (br s, 2H, 6-CH_2), 6.48 (d, J=7.9 Hz, 2H, aniline-2H), 6.68 (t, J=7.3 Hz, 1H, aniline-4H), 7.12 (t, J=7.8 Hz, 2H, aniline-3H), 7.31/7.59 (2×d, 2×2H, 4H, phenyl-H), 8.56 (s, 1H, 5-H). ^{13}C NMR δ: 21.8 (4′-CH_3), 28.4 (1-CH_3), 29.4 (3-CH_3), 45.2 (6-CH_2), 109.0 (C-4a), 113.0 (aniline-C2), 118.1 (aniline-C4), 128.0 (C-6), 129.0 (C-2′), 129.2 (C-3′), 129.2 (aniline-C3), 135.7 (C-1′), 138.6 (C-4′), 139.8 (C-5), 147.1 (aniline-C1), 149.4 (C-8a), 151.6 (C-2), 161.3 (C-7), 162.9 (C-4), Anal. Calcd: C: 71.48, H: 5.74, N: 14.50, found: C: 71.41, H: 5.81, N: 14.43.

4.6. Ethyl 2-cyano-3-(pyrido[2,3-*d*]pyrimidin-6-yl)acrylates 14a–b

2 mmol of **8** was dissolved together with 2.5 mmol of ethyl cyano acetate in 20 mL of toluene, three drops of piperidine and eight drops of acetic acid were added and the reaction mixture was heated to reflux until no more water condensed. After cooling the mixture was washed with brine, dried, and concentrated in vacuo. The precipitate was treated with diethyl ether, filtered, and recrystallized from ethyl acetate to afford yellow crystals of **14**.

4.6.1. Ethyl 2-cyano-3-(1,3-dimethyl-2,4-dioxo-7-phenyl-(1*H*,3*H*)-pyrido[2,3-*d*]pyrimidine-6-yl)acrylate (14a). 490 mg (1.26 mmol, 63%), mp 171 °C (ethyl acetate). UV/vis λ: 262 (4.3), 338 (4.3). IR ν: 2210, 1720, 1680, 1600. MS *m/z*: 390 (M^+, 21), 361 (8), 318 (26), 317 (100). ^{1}H NMR δ: 1.28 (t, J=7.1 Hz, 3H, OCH_2CH_3), 3.51/3.79 (2×s, 2×3H, 1-CH_3, 3-CH_3), 4.37 (q, J=7.1 Hz, 2H, OCH_2CH_3), 7.56 (m, 5H, phenyl-H), 8.27 (d, J=0.6 Hz, 1H, 5-H), 9.28 (d, J=0.6 Hz, 1H, 6-CH). ^{13}C NMR δ: 14.1 (CH_3), 28.6 (1-CH_3), 29.8 (3-CH_3), 62.9 (CH_2), 105.8 (C≡N), 109.3 (C-4a), 114.6 (C=C), 121.0 (C-6), 128.8 (C-3′), 130.4 (C-2′), 130.9 (C-4′), 137.1 (C-1′), 139.0 (C-5), 151.3 (C-2), 151.6 (C-8a), 151.8 (C=CH), 160.2 (C-7), 161.7 (C-4), 164.5 (C=O). Anal. Calcd: C: 64.61, H: 4.65, N: 14.35, found: C: 64.76, H: 4.65, N: 14.04.

4.6.2. Ethyl 2-cyano-3-(1,3-dimethyl-2,4-dioxo7-(4-tolyl)-(1*H*,3*H*)-pyrido[2,3-*d*]pyrimidine-6-yl)acrylate (14b). 525 mg (1.30 mmol, 65%), mp 188 °C (ethyl acetate). UV/vis λ: 284 (4.3), 342 (4.4). IR ν: 2239, 1718, 1714, 1670, 1590. MS *m/z*: 404 (M^+, 6), 332 (14), 331 (53), 45 (100). ^{1}H NMR δ: 1.39 (t, $J=7.1$ Hz, 3H, OCH_2CH_3), 2.46 (s, 3H, 4′-CH_3), 3.52/3.79 (2×s, 2×3H, 1-CH_3, 3-CH_3), 4.37 (q, $J=7.1$ Hz, 2H, OCH_2CH_3), 7.34/7.49 (2× d, $J=8.0$ Hz, 2×2H, phenyl-H), 8.28 (s, 1H, 5-H), 9.26 (s, 1H, 6-CH). ^{13}C NMR δ: 14.1 (CH_3), 21.4 (4′-CH_3), 28.6 (1-CH_3), 29.7 (3-CH_3), 62.9 (CH_2), 105.4 (C≡N), 109.0 (C-4a), 114.6 (C=C), 120.9 (C-6), 129.5 (C-2′), 130.4 (C-3′), 134.3 (C-1′), 139.0 (C-5), 141.5 (C-4′), 151.3 (C-2), 151.6 (C-8a), 152.0 (C=CH), 160.2 (C-7), 161.8 (C-4), 164.5 (C=O). HRMS Calcd for $C_{22}H_{20}N_4O_4$, 404.1485, found: 404.1483.

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Arylacetic acid derivatization of 2,3- and internal *erythro*-squalene diols. Separation and absolute configuration determination

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Abstract—We have studied a new approach for the resolution and absolute configuration determination of the enantiomers of squalene diols as intermediate precursors in the chemical synthesis of different squalene oxides (SOs); (3*R*)- and (3*S*)-2,3-SO, (6*R*,7*R*)- and (6*S*,7*S*)-6,7-SO, and (10*R*,11*R*)- and (10*S*,11*S*)-10,11-SO. Monoderivatization of the corresponding racemic squalene diol intermediates with pure stereoisomers of (*S*)-(+)-methoxyphenyl acetic acid ((*S*)-(+)-MPA), (*S*)-(+)-9-anthrylmethoxyacetic acid ((*S*)-(+)-9-AMA) and (*S*)-(+)-acetoxyphenylacetic acid ((*S*)-(+)-APA) afforded the diastereomeric esters which could be easily separated by column flash chromatography with silica gel. In addition, the absolute configuration for these diastereoisomers of the derivatized diols was advantageously inferred from ^{1}H NMR data according to the models depicted for these derivatizing chiral agents. In order to demonstrate the absolute configuration assignment of the different stereoisomers, (*S*)-(+)-AMA showed the larger $\Delta\delta$ by ^{1}H NMR, however, (*S*)-(+)-MPA esters were much more stable derivatives.

1. Introduction

In the past years, we developed a route to synthesize and determine the absolute configuration of the different enantiomers of the squalene oxides using 2,3- and *erythro*−6,7- and 10,11-squalene diols (Sdiols) as intermediates. In this case, the resolution of the corresponding Sdiols was achieved by derivatization with MTPA (Mosher's acid, α-methoxy-α-trifluoromethylphenylacetic acid). In addition, we demonstrated that these enantiomers of the internal SOs were valuable substrates for inhibitory studies of squalene epoxidase (SE) leading to the epoxidation at both terminal double bonds of the squalene chain but affording regioselective and asymmetric ratios of enantiomers of squalene dioxides.[1] Likewise, we studied the activity and interactions of the resulting squalene dioxides with oxidosqualene-lanosterol cyclase (OSLC), another key enzyme in the cholesterol biosynthesis.[1]

In this paper, we report a practical new approach to resolve both enantiomers of the different squalene diols with high enantiomeric excesses by simple flash chromatography using as derivatizing agents chiral aryl acetic acids currently applied to determine the absolute configuration of secondary alcohols. For this purpose, the (*S*)-(+)-MPA, (*S*)-(+)-APA, and (*S*)-(+)-9-AMA diastereomeric esters from the above squalene diols were prepared (Fig. 1) and separated by flash chromatography with silica gel. In addition, we present here the ^{1}H NMR data needed for the easy assignment of the absolute configuration of the derivatized secondary carbinols present in 2,3- and *erythro*-squalene diols and we compare these results with the more cumbersome assignation from data previously obtained for the above squalene diols derivatized with MTPA.[1]

2. Results and discussion

2.1. Preparation, derivatization and separation of the 2,3- and *erythro*-squalene diols

So far, there have been a great number of studies for the synthesis of chiral squalene epoxides. In most of these syntheses, 2,3-squalene diols were used as versatile key intermediates in the preparation of enantiomerally pure 2,3-squalene oxides. Other approaches were reported by using chiral synthons,[2] by enantioselective reactions[3] or by chemical resolution of the diols.[1,4] among others. Previously, we had performed the chemical resolution

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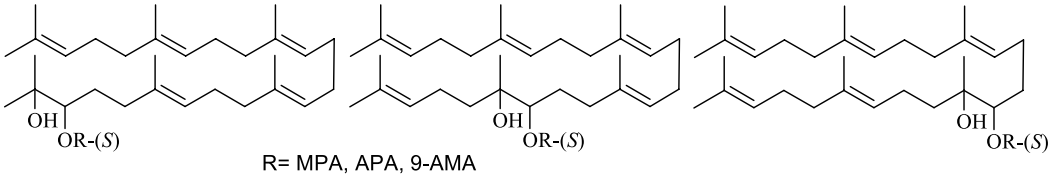


Figure 1. Arylacetic esters of 2,3- and *erythro*-6,7- and 10,11-squalene diols.

strategy involving the formation of the (*R*)-MTPA esters of the corresponding enantiomers of 2,3-, *erythro*-6,7- and *erythro*−10,11-squalene diols. The obtained esters were submitted to HPLC chromatographic resolution and used for further determination of the absolute configuration of the stereogenic centers present at the squalene skeleton for each diastereoisomer. But HPLC chromatographic resolution by reverse phase HPLC was very tedious and difficult.

Our ongoing interest in resolving secondary alcohols with lipases[5] has led us to undertake the search for a new enzymatic strategy. In this context, specific lipases could be able to remove selectively the arylacetic group from the derivatized diasteromeric (*S*)-(+)-esters of the corresponding squalene diols. As a result, we would be able to separate both enantiomers of the racemic mixture facilitating the stereochemical studies of the resolved diol intermediate and determining the absolute configuration of both diastereomeric esters. Consequently, we decided to prove this strategy for arylacetic derivatized compounds which could be potential substrates with acylases or lipases and had been utilized in determining the absolute configuration of secondary alcohols, such as (*S*)-(+)-MPA, (*S*)-(+)-APA, and (*S*)-(+)-9-AMA esters. In this context, some representative model secondary alcohols were derivatized and enzymatically hydrolyzed but results were not so good as anticipated and this approach was disregarded.

2,3-Squalene diol, *erythro*-6,7- and *erythro*−10,11-squalene diol were prepared according to procedures previously reported[1] and derivatization with (*S*)-(+)-MPA, (*S*)-(+)-APA, and (*S*)-(+)-9-AMA, was achieved by one of these two methods: (1) By previous preparation of the proper acid chloride and then reaction with the squalene diol in presence of DMAP and NEt_3[6] or (2) by using a carbodiimide in presence of DMAP.[5] Apparently, no important racemizations have been reported under the derivatization conditions described for these two methods and yields used to be higher than 90%.

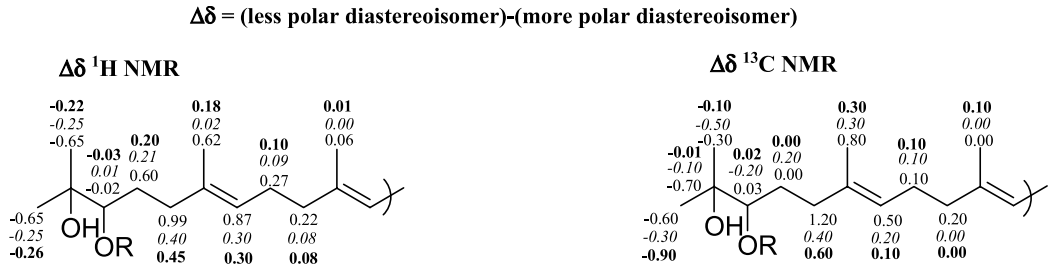
Surprisingly, when we analyzed the reaction mixture by TLC we observed high differences of R_f in the TLC plate for all the squalene arylacetic esters prepared. As an example, TLC separation of diasteromeric esters was better for the derivatizing acid in the order 9-AMA> APA≈MPA and for the derivatized diol in the order 10,11->6,7->2,3-squalene diol. As we expected, stability of products followed the order MPA >> APA > 9-AMA, since after 1 year of storage only 4% of MPA derivatized product was isomerized. ^{1}H, ^{13}C NMR and HPLC-MS-FIA data allowed us to determine that the two major products formed corresponded to the monoderivatized diastereoismers at the secondary carbinol of the squalene diol.

2.2. Absolute configuration determination of the 2,3-, *erythro*-6,7- and *erythro*-10,11-squalene diols

Since Mosher[7] proposed ^{1}H NMR to determine the absolute configuration of stereogenic centers of secondary alcohols derivatized with MTPA, many other researchers have reported similar methodologies and derivatizing agents. Most of these methodologies try to visualize important ^{1}H NMR chemical shifts changes due to the diamagnetic effect of the aromatic ring(s) on certain protons of the derivatized molecule. Obviously, the success to determine the absolute configuration of a derivatized alcohol by ^{1}H NMR depends on the $\Delta\delta$ value calculated from the different chemical shifts for the same hydrogen of both diastereoisomers. The greater is this anisotropy effect in both diastereomers, the easier is the proton chemical shifts differentiation and therefore the absolute configuration determination. When we applied this technique to determine the absolute configuration of the squalene diols derivatized with (*R*)-MTPA by NMR, the chemical shifts signals of the diasteromeric derivatives were too close with only small $\Delta\delta$ values among 0.15–0.05 ppm, complicating the assignment process. However, the recent availability of acids like MPA, APA and 9-AMA has facilitated the application of the corresponding stereochemical models for the assignment of absolute configurations. In order to extend the use of this methodology, we present here a comparative study of the $\Delta\delta$ values obtained for the assignment of the absolute configuration of the squalene diols and we compare these results with the previously studied derivatives obtained with Mosher's acid (MTPA).

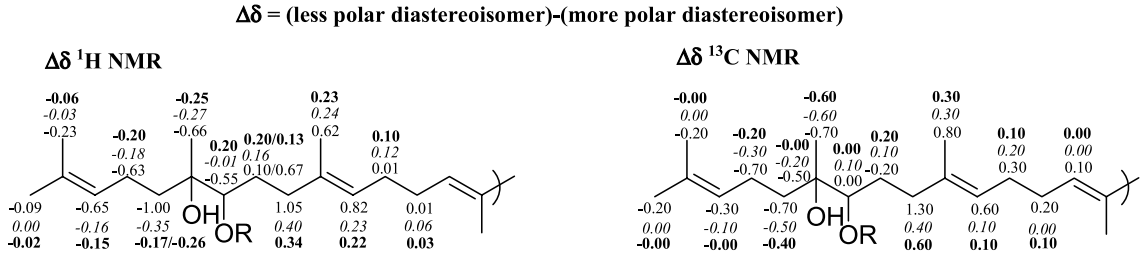
To this aim, ^{1}H, ^{13}C NMR, DQFCOSY and XH-CORFE spectra for the different arylacetic esters were registered, and interpretation of COSY and HETCOR data allowed the assignments of ^{1}H and ^{13}C NMR signals, which readily demonstrated that another difference between them was the asymmetric diamagnetic effect of the aromatic ring(s) on the ^{1}H NMR chemical shifts for the hydrogens with respect to each diastereoisomer.

The ^{1}H and ^{13}C NMR chemical shift increments ($\Delta\delta$) in each pair of diastereomeric esters are shown in Schemes 1–3. ^{1}H NMR $\Delta\delta$ calculations showed a similar sign behavior for the squalene diols substituents in all arylacetic esters. As we had checked previously for aliphatic chains, hydrogens at β position from the carbinol exhibited the highest shielding effect.[8] As expected, (*S*)-(+)-9-AMA showed the most important shielding values for both diastereomeric esters (i.e., 1 ppm for hydrogens at β position). However, results using APA were comparable to those obtained with MPA (i.e., 0.4 ppm for hydrogens at β position), but much better than those reported with MTPA (i.e, 0.15 ppm for hydrogens at β position). Likewise, ^{13}C NMR chemical shift assignments showed a ^{13}C NMR $\Delta\delta$ behavior similar to that



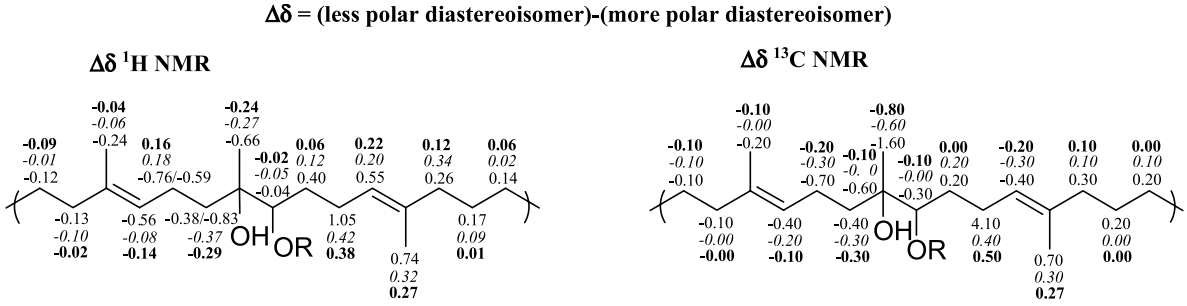
Plain R= (*S*)-(+)-9-AMA, *italic*=(*S*)-(+)-APA, **Bold**= (*S*)-(+)-MPA

Scheme 1. ^{1}H and ^{13}C NMR chemical shift differences (in ppm) for the positions closest to the substituted carbinolic center for the aryl acetic esters of the 2,3-squalene diol skeleton.



Plain R= (*S*)-(+)-9-AMA, *italic*=(*S*)-(+)-APA, **Bold**= (*S*)-(+)-MPA

Scheme 2. ^{1}H and ^{13}C NMR chemical shift differences (in ppm) for the positions closest to the substituted carbinolic center for the aryl acetic esters of the 6,7-*erythro*-squalene diol skeleton.



Plain R= (*S*)-(+)-9-AMA, *italic*=(*S*)-(+)-APA, **Bold**= (*S*)-(+)-MPA

Scheme 3. ^{1}H and ^{13}C NMR chemical shift differences (in ppm) for the positions closest to the substituted carbinolic center for the aryl acetic esters of the 10,11-*erythro*-squalene diol skeleton.

observed for ^{1}H NMR[9] and the highest anisotropy effect was observed for the carbons located at β position from the carbinol substitution. (cf Schemes 1–3).

Comparison with the corresponding stereochemical models for (*S*)-(+)-MPA,[10] (*S*)-(+)-APA,[11] and (*S*)-(+)-9-AMA[12] allowed us to determine the absolute configuration of the stereocenter of each diastereoisomer in the derivatized carbinol resulting in the "*S*" configuration for the less polar diastereoisomer (ca R_f 0.4, hexane–MTBE 7:3).

Reduction or saponification of individual isomers of Sdiols esters with $LiAlH_4$/Diethyl Ether or K_2CO_3 in MeOH, respectively, gave rise to the enantiomeric diols. As expected, sign of the optical rotation of the released squalene diol confirmed the absolute configuration assignment. Since resolution has been so good, we expected that the optical purity of the enantiomerically pure squalene diols were in agreement with the enantiomeric purity of the ester precursors. However, rederivatization with (*S*)-(+)-MPA of each one of the resulting squalene diols and TLC and HPLC analysis of the resulting esters showed that there was 6-8% of isomerized alcohol. This isomerization was much more important (up to 12%) for squalene diols previously derivatized with (*S*)-(+)-APA and by the acyl chloride method. These results are somewhat worse than those observed for the derivatized squalene diols obtained after rederivatization with MTPA that showed 5% of isomerization. In spite of the higher degree of isomerization, any of the recovered enantiomerically enriched squalene diols could be useful as squalene oxides intermediates to perform any biological experiment.

In conclusion, we have improved the methodology for

the separation and absolute configuration determination of the different squalene diol stereoisomers that afford the biologically active squalene oxides. In this context, MPA esters of squalene diols are the most appropriate and stable derivatized intermediates to separate and determine the absolute configuration of the enantiomers of the squalene diols.

3. Experimental

General methods. The liquid chromatography-mass spectrometry analyses (HPLC-MS-FIA) were performed with an apparatus with a chemical ionization interface at atmospheric pressure and diode array detector (CH_3CN/H_2O (80:20) at 1 mL/min; positive mode). All ^1H NMR spectra were acquired at 300 MHz, and ^{13}C NMR spectra, at 75 MHz in freshly neutralized $CDCl_3$ solutions, and chemical shifts are given in ppm using as internal standards $Si(CH_3)_4$ for ^1H, and $CDCl_3$ for ^{13}C. The standard ^1H DQFCOSY and XH-CORFE spectra for the determination of the absolute configuration of (*S*)-(+)-9-AMA, (*S*)-(+)-MPA and (*S*)-(+)-APA esters were recorded at 25 °C using the same concentration for both diastereomeric pairs as described elsewhere.[1] All IR spectra were run as film.

Unless otherwise stated, organic solutions obtained from workup of crude reaction mixtures were dried over $MgSO_4$. The purification procedures were carried out by flash chromatography on silica gel (230–400 mesh) and products were obtained as oils unless otherwise specified. Visualization of UV-inactive materials was accomplished by soaking the TLC plates in an ethanolic solution of anisaldehyde and sulfuric acid (v/v/v, 96:2:2). Optical rotations were determined at 25° in $CHCl_3$ solution at the specified concentration (g/100 mL). Enantiomeric and diastereomeric excesses (ee and de) values of the corresponding (*S*)-(+)-arylacetic diastereomeric esters were calculated by NMR or by HPLC analyses using Spherisorb ODS-2 (5 μm) columns (10×0.6 cm and 15×1 cm, respectively) and eluting with CH_3CN–H_2O mixtures at 1 mL/min. All TLC plates were eluted with hexanes–MTBE 7:3.

3.1. Preparation of esters from the acid chloride. General procedure

Oxalyl chloride (370 μL, 4.2 mmol) was added to a mixture of the corresponding acid [(S)-(+)-APA, -MPA, -9-AMA] (0.7 mmol) and DMF (10 μL, 0.12 mmol) in 30 mL of hexane at room temperature. After 2 days, solvent was evaporated to dryness at reduced pressure to afford the acid chloride. The freshly prepared acid chloride [APA-Cl, MPA-Cl, 9-AMA-Cl] was dissolved in 4 mL of CH_2Cl_2 and added to a solution of the squalene diol (250 mg, 0.56 mmol), Et_3N (240 μL, 1.7 mmol) and DMAP (60 mg, 0.55 mmol) in 20 mL of CH_2Cl_2. After 30 min, water was added (10 mL), decanted and the organic solution was dried, concentrated at reduced pressure to a residue which was purified by flash chromatography on silica gel using a gradient of 0–30% MTBE (methyl tert-butyl ether) in hexane (83–89% yield).

3.2. Preparation of esters by the carbodiimide methodology. General procedure

A mixture of DMAP (0.2 mmol), arylacetic acid (0.32 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (0.3 mmol) and squalene diol (0.2 mmol) in 2 mL of CH_2Cl_2 was stirred for 1 h at RT, washed with $NaHCO_3$ solution, concentrated at reduced pressure and purified by flash chromatography on silica gel using a gradient of 0–30% MTBE in hexane (85–92% yield).

3.2.1. (*S*)-(+)-α-Methoxy-α-(9-anthryl)-acetate of (3*S*)-2,3-squalene diol (higher R_f in TLC). R_f=0.22; IR 3450, 2965, 2925, 2855, 1750 (CO), 1450, 1380, 1190, 1115, 730 cm^{-1}; ^1H NMR δ 8.61 (d, J=8 Hz, 2H), 8.47 (s, 1H), 8.02 (dd, J_1=8 Hz, J_2=1 Hz, 2H), 7.60–7.40 (4H), 6.33 (s, 1H), 5.20–5.00 (5H), 4.73 (dd, J_1=10 Hz, J_2=2.5 Hz, 1H), 3.46 (s, 3H), 2.20–1.92 (18H), 1.68 (s, 3H), 1.60 (bs, 12H), 1.58 (s, 3H), 1.60–1.20 (4H), 0.58 (s, 1H), 0.46 (s, 3H), 0.41 (s, 3H); ^{13}C NMR δ 170.7, 135.1, 135.0, 134.9, 133.9, 131.3, 131.2, 130.4, 129.3, 129.2, 127.1, 126.8, 125.1, 124.9, 124.4, 124.3, 124.2, 80.8, 77.2, 71.8, 57.4, 39.7, 39.7, 39.7, 36.0, 28.3, 27.9, 26.7, 26.6, 25.7, 25.3, 23.9, 17.7, 16.0, 16.0; HPLC-MS-FIA *m/z* 710 ($M^{\cdot+}$+18), 692 ($M^{\cdot+}$), 677 ($M^{\cdot+}$−18+3), 427 ($M^{\cdot+}$−265); $[\alpha]_D$=+43.4 (c=1, 98% de).

3.2.2. (*S*)-(+)-α-Methoxy-α-(9-anthryl)-acetate of (3*R*)-2,3-squalene diol (lower R_f in TLC). R_f=0.08; IR 3450, 2965, 2925, 2855, 1750 (CO), 1450, 1380, 1190, 1115, 730 cm^{-1}; ^1H NMR δ 8.61 (d, J=8 Hz, 2H), 8.47 (s, 1H), 8.02 (d, J=8 Hz, 2H), 7.60–7.40 (4H), 6.35 (s, 1H), 5.20–5.00 (4H), 4.73 (dd, J_1=10 Hz, J_2=2 Hz, 1H), 4.23 (m, 1H), 3.44 (s, 3H), 2.14–1.92 (12H), 1.79 (s, 1H), 1.78 (s, 3H), 1.68 (d, J=1 Hz, 3H), 1.60 (bs, 9H), 1.54 (d, J=1 Hz, 3H), 1.32 (m, 2H), 1.11 (s,3H), 1.06 (s,3H), 0.98 (d, J=1 Hz, 3H), 1.10–0.7 (2H); ^{13}C NMR δ 171.4, 135.1, 134.9, 133.3, 131.5, 131.2, 130.5, 129.2, 129.2, 127.1, 126.6, 125.0, 124.4, 124.4, 124.4, 124.2, 124.1, 80.6, 77.3, 72.5, 57.4, 39.7, 39.7, 39.5, 34.8, 28.2, 27.9, 26.7, 26.6, 26.6, 25.7, 24.2, 17.7, 16.0, 15.2; HPLC-MS-FIA *m/z* 710 ($M^{\cdot+}$+18), 691 ($M^{\cdot+}$−1), 677 ($M^{\cdot+}$−18+3), 427 ($M^{\cdot+}$−265); $[\alpha]_D$=+77.6 (c=1, 98% de).

3.2.3. (*S*)-(+)-α-Methoxy-α-(9-anthryl)-acetate of (6*R*,7*S*)-6,7-squalene diol (higher R_f in TLC). R_f=0.47; IR 3450, 2965, 2925, 2855, 1750 (CO), 1450, 1380, 1190, 1115, 730 cm^{-1}; ^1H NMR δ 8.59 (bb, 2H), 8.47 (s, 1H), 8.01 (d, J=8 Hz, 2H), 7.62–7.42 (4H), 6.32 (s, 1H), 5.22–5.02 (4H), 4.76 (dd, J_1=10 Hz, J_2=2.5 Hz, 1H), 4.23 (t, J=10 Hz, 1H), 3.46 (s, 3H), 2.16–1.90 (12H), 1.68 (d, J=1 Hz, 3H), 1.62 (d, J=1 Hz, 3H), 1.60 (bs, 12H), 1.59 (s, 3H), 1.56 (s, 3H), 1.60–1.10 (3H), 1.37 (s, 3H), 0.87 (m, 1H), 0.63 (m, 1H), 0.45 (m, 1H), 0.39 (s, 3H); ^{13}C NMR δ 170.6, 135.2, 134.8, 134.1, 131.3, 131.3, 131.2, 130.4, 129.3, 129.2, 127.1, 126.7, 125.1, 124.8, 124.3, 124.2, 124.1, 123.9, 80.1, 77.2, 73.5, 57.3, 39.7, 39.7, 36.5, 36.2, 28.3, 28.2, 27.5, 26.7, 26.6, 25.6, 25.5, 22.1, 21.2, 17.6, 17.4, 16.0, 16.0, 16.0; HPLC-MS-FIA *m/z* 710 ($M^{\cdot+}$+18), 692 ($M^{\cdot+}$), 675 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot+}$−265); $[\alpha]_D$=+43.4 (c=1, 98% de).

3.2.4. (*S*)-(+)-α-Methoxy-α-(9-anthryl)-acetate of (6*S*,7*R*)-6,7-squalene diol (lower R_f in TLC). R_f=0.15; IR 3450, 2965, 2925, 2855, 1750 (CO), 1450, 1375, 1190, 1115, 730 cm^{-1}; ^1H NMR δ 8.59 (bb, 2H), 8.47 (s, 1H), 8.01 (d, J=8 Hz, 2H), 7.60–7.40 (4H), 6.35 (s, 1H), 5.18–4.98 (4H), 4.78 (dd, J_1=10 Hz, J_2=2 Hz, 1H), 4.28 (t, J=6.5 Hz, 1H), 3.43 (s, 3H), 2.16–1.90 (10H), 1.90–1.64 (4H), 1.68 (s, 6H), 1.59 (s, 6H), 1.56 (s, 3H), 1.60–1.08 (6H), 1.05 (s, 3H), 0.98 (s, 3H), 0.84 (m, 2H); ^{13}C NMR δ 171.2, 134.9, 134.8, 133.6, 131.9, 131.4, 131.2, 130.5, 129.2, 129.1, 127.1, 126.5, 125.0, 124.3, 124.3, 124.2, 80.1, 77.3, 74.0, 57.4, 39.7 37.2, 34.9, 28.0, 28.0, 27.7, 26.7, 26.6, 25.7, 23.7, 21.9, 17.6, 17.6, 16.0, 15.2; HPLC-MS-FIA *m/z* 710 ($M^{\cdot+}$+18), 692 ($M^{\cdot+}$), 675 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot+}$−265); $[\alpha]_D$=+50.8 (c=1, 98% de).

3.2.5. (*S*)-(+)-α-Methoxy-α-(9-anthryl)-acetate of (10*R*,11*S*)-10,11-squalene diol (higher R_f in TLC). R_f=0.48; IR 3530, 2975, 2935, 2860, 1750 (CO), 1445, 1380, 1180, 1110, 725 cm^{-1}; ^1H NMR δ 8.62 (bb, 2H), 8.47 (s, 1H), 8.01 (d, J=8 Hz, 2H), 7.62–7.42 (4H), 6.32 (s, 1H), 5.20–5.00 (4H), 4.79 (dd, J_1=10 Hz, J_2=2 Hz, 1H), 4.48 (t, J=7 Hz, 1H), 3.46 (s, 3H), 2.16–1.90 (12H), 1.85 (m, 2H), 1.68 (s, 6H), 1.62 (s, 3H), 1.60 (bs, 9H), 1.70–1.10 (4H), 1.37 (s, 3H), 0.66 (s, 1H), 0.64 (m, 1H), 0.43 (m,1H), 0.39 (s, 3H); ^{13}C NMR δ 170.6, 136.0, 135.0, 131.3, 131.3, 131.2, 130.4, 129.3, 129.2, 127.1, 126.7, 125.1, 124.3, 124.2, 124.1, 123.8, 123.3, 80.0, 77.1, 73.5, 57.4, 39.7, 39.7, 39.6, 36.4, 29.4, 26.7, 26.6, 26.6, 25.7, 24.7, 22.2, 21.1, 17.7, 16.1, 16.0, 15.7; HPLC-MS-FIA *m/z* 710 ($M^{\cdot+}$+18), 692 ($M^{\cdot+}$), 675 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot+}$−265); $[\alpha]_D$=+41.1 (c=1, 98% de).

3.2.6. (*S*)-(+)-α-Methoxy-α-(9-anthryl)-acetate of (10*S*,11*R*)-10,11-squalene diol (lower R_f in TLC). R_f=0.20; IR 3530, 2975, 2935, 2855, 1745 (CO), 1445, 1380, 1180, 1110, 725 cm^{-1}; ^1H NMR δ 8.60 (bb, 2H), 8.46 (s, 1H), 8.00 (d, J=8 Hz, 2H), 7.60–7.40 (4H), 6.34 (s, 1H), 5.16–4.92 (4H), 4.85 (dd, J_1=10 Hz, J_2=2 Hz, 1H), 4.59 (t, J=7 Hz, 1H), 3.42 (s, 3H), 2.14–1.85 (12H), 1.74 (m, 2H), 1.68 (d, J=1 Hz, 3H), 1.66 (d, J=1 Hz, 3H), 1.61 (s, 3H), 1.58 (bs, 6H), 1.54 (d, J=1 Hz, 3H), 1.70–1.10 (6H), 1.06 (s, 3H), 0.88 (s, 3H), 1.10–0.80 (2H); ^{13}C NMR δ 171.3, 135.5, 135.5, 134.8, 131.4, 131.4, 130.8, 130.5, 129.2, 129.1, 127.1, 126.5, 125.0, 124.3, 124.2, 124.1, 124.0, 122.7, 80.3, 77.4, 74.1, 57.4, 39.7, 39.4, 37.1, 29.2, 26.7, 26.6, 26.4, 25.7, 23.8, 23.6, 21.8, 17.7, 17.6, 15.9, 15.9, 15.3; HPLC-MS-FIA *m/z* 710 ($M^{\cdot+}$+18), 692 ($M^{\cdot+}$), 675 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot+}$−265); $[\alpha]_D$=+57.4 (c=1, 98% de).

3.2.7. (*S*)-(+)-α-Methoxy-α-(phenyl)-acetate of (3*S*)-2,3-squalene diol (higher R_f). R_f=0.22; IR 3510, 2965, 2925, 2855, 1750 (CO), 1455, 1380, 1190, 1115, 730 cm^{-1}; ^1H NMR δ 7.52–7.42 (2H), 7.30–7.42 (3H), 5.22–5.00 (5H), 4.80 (s, 1H), 4.79 (dd, J_1=10 Hz, J_2=3 Hz, 1H), 3.44 (s, 3H), 2.14–1.86 (18H), 1.68 (d, J=1 Hz, 3H), 1.60 (bs, 12H), 1.56 (d, J=1 Hz, 3H), 1.60–1.20 (5H), 0.94 (s, 3H), 0.91 (s, 3H); ^{13}C NMR δ 170.4, 136.5, 135.1, 135.0, 134.9, 133.8, 131.2, 129.0, 128.7, 127.2, 125.0, 124.4, 124.3, 124.2, 82.6, 80.5, 72.3, 57.3, 39.7, 39.7, 39.6, 35.9, 28.3, 28.0, 26.7, 26.7, 26.6, 25.9, 25.7, 24.6, 17.7, 16.0, 16.0; HPLC-MS-FIA *m/z* 610 ($M^{\cdot+}$+18), 593 ($M^{\cdot+}$+1), 575 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot+}$−165); $[\alpha]_D$=+25.0 (c=1, 98% de).

3.2.8. (*S*)-(+)-α-Methoxy-α-(phenyl)-acetate of (3*R*)-2,3-squalene diol (lower R_f in TLC). R_f=0.15; IR 3510, 2965, 2925, 2855, 1750 (CO), 1455, 1380, 1190, 1115, 730 cm^{-1}; ^1H NMR δ 7.52–7.42 (2H), 7.30–7.42 (3H), 5.20–5.05 (4H), 4.81 (s, 1H), 4.78 (dd, J_1=10 Hz, J_2=2.5 Hz, 1H), 4.76 (t, J=6 Hz, 1H), 3.44 (s, 3H), 2.14–1.86 (18H), 1.68 (d, J=1 Hz, 3H), 1.60 (bs, 9H), 1.58 (d, J=1 Hz, 3H), 1.60–1.20 (5H), 1.38 (d, J=1 Hz, 3H), 1.16 (s, 3H), 1.16 (s, 3H); ^{13}C NMR δ 170.8, 136.3, 135.1, 135.0, 134.9, 133.5, 131.2, 128.8, 128.6, 127.1, 124.9, 124.4, 124.2, 82.7, 80.3, 72.4, 57.3, 39.7, 39.7, 39.6, 35.3, 28.2, 28.1, 26.7, 26.6, 25.7, 24.7, 17.6, 16.0, 16.0, 15.7; HPLC-MS-FIA *m/z* 610 ($M^{\cdot+}$+18), 593 ($M^{\cdot+}$+1), 575 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot+}$−165); $[\alpha]_D$=+28.8 (c=1, 98% de).

3.2.9. (*S*)-(+)-α-Methoxy-α-(phenyl)-acetate of (6*R*,7*S*)-6,7-squalene diol (higher R_f in TLC). R_f=0.30; IR 3525, 2965, 2925, 2855, 1750 (CO), 1455, 1375, 1200, 1175, 1115, 1000, 730, 695 cm^{-1}; ^1H NMR δ 7.52–7.42 (2H), 7.30–7.42 (3H), 5.20–5.02 (4H), 4.94 (m, 1H), 4.83 (dd, J_1=10 Hz, J_2=3 Hz, 1H), 4.78 (s, 3H), 3.43 (s, 3H), 2.14–1.80 (18H), 1.80–1.54 (4H), 1.68 (d, J=1 Hz, 3H), 1.66 (d, J=1 Hz, 3H), 1.60 (bs, 9H), 1.56 (bs, 6H), 1.26 (s, 1H), 1.18 (m, 2H), 0.87 (s, 3H); ^{13}C NMR δ 170.3, 136.5, 135.2, 134.9, 134.1, 131.9, 131.3, 128.9, 128.7, 127.2, 124.9, 124.4, 124.2, 124.2, 124.1, 82.6, 79.8, 74.0, 57.2, 39.7, 39.7, 37.1, 36.0, 28.3, 28.2, 27.6, 26.7, 26.7, 25.7, 22.9, 21.8, 17.7, 17.6, 16.0, 16.0; HPLC-MS-FIA *m/z* 610 ($M^{\cdot+}$+18), 593 ($M^{\cdot+}$+1), 575 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot+}$−165); $[\alpha]_D$=+21.2 (c=1, 98% de).

3.2.10. (*S*)-(+)-α-Methoxy-α-(phenyl)-acetate of (6*S*,7*R*)-6,7-squalene diol (lower R_f in TLC). R_f=0.25; IR 3505, 2965, 2925, 2855, 1750 (CO), 1455, 1375, 1200, 1175, 1115, 1000, 730, 695 cm^{-1}; ^1H NMR δ 7.52–7.42 (2H), 7.30–7.42 (3H), 5.20–5.02 (4H), 4.82 (dd, J_1=10 Hz, J_2=3 Hz, 1H), 4.80 (s, 3H), 4.81 (m, 1H), 3.43 (s, 3H), 2.14–1.80 (18H), 1.78–1.30 (5H), 1.68 (bs, 6H), 1.62 (d, J=1 Hz, 3H), 1.60 (bs, 9H), 1.37 (d, J=1 Hz, 3H), 1.12 (s, 3H); ^{13}C NMR δ 170.7, 136.3, 135.0, 134.9, 133.8, 132.0, 131.2, 128.8, 128.6, 127.1, 124.8, 124.4, 124.2, 124.2, 82.7, 79.8, 74.0, 57.3, 39.7, 39.7, 37.4, 35.4, 28.2, 28.1, 27.8, 26.7, 26.6, 25.7, 23.5, 22.0, 17.6, 16.0, 16.0, 15.7; HPLC-MS-FIA *m/z* 610 ($M^{\cdot+}$+18), 593 ($M^{\cdot+}$+1), 575 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot+}$−265); $[\alpha]_D$=+26.0 (c=1, 98% de).

3.2.11. (*S*)-(+)-α-Methoxy-α-(phenyl)-acetate of (10*R*,11*S*)-10,11-squalene diol (higher R_f in TLC). R_f=0.35; IR 3520, 2965, 2925, 2855, 1750 (CO), 1455, 1375, 1200, 1175, 1115, 1000, 730, 695 cm^{-1}; ^1H NMR δ 7.52–7.42 (2H), 7.42–7.28 (3H), 5.18–5.02 (4H), 4.95 (m, 1H), 4.86 (dd, J_1=9.5 Hz, J_2=3.5 Hz, 1H), 4.78 (s, 1H), 3.43 (s, 3H), 2.14–1.80 (18H), 1.80–1.60 (2H), 1.68 (bs, 6H), 1.60 (bs, 9H), 1.56 (bs, 6H), 1.27 (s, 1H), 1.18 (m, 2H), 0.87 (s, 3H); ^{13}C NMR δ 170.3, 136.5, 136.0, 135.5, 135.0, 131.4, 131.2, 128.9, 128.7, 127.3, 124.3, 124.2, 124.1, 124.0, 82.6, 79.8, 74.0, 57.3, 39.7, 39.7, 37.1, 29.3, 26.7, 26.6, 25.7, 24.6, 22.8, 21.7, 17.7, 16.0, 16.0, 15.9; HPLC-MS-FIA *m/z* 610 ($M^{\cdot+}$+18), 593 ($M^{\cdot+}$+1), 575 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot+}$−265); $[\alpha]_D$=+27.0 (c=1, 98% de).

3.2.12. (*S*)-(+)-α-Methoxy-α-(phenyl)-acetate of (10*S*, 11*R*)-10,11-squalene diol (lower R_f in TLC). R_f=0.27; IR 3520, 2965, 2925, 2855, 1750 (CO), 1455, 1375, 1200, 1175, 1115, 1000, 730, 695 cm^{-1}; ^1H NMR δ 7.52–7.42 (2H), 7.40–7.28 (3H), 5.18–5.02 (4H), 4.92 (m, 1H), 4.86 (ca, 1H), 4.81 (s, 1H), 3.44 (s, 3H), 2.16–1.80 (18H), 1.80–1.30 (5H), 1.68 (bs, 6H), 1.60 (bs, 9H), 1.58 (d, J=1 Hz, 3H), 1.33 (d, J=1 Hz, 3H), 1.26 (s, 1H), 1.11 (s, 3H); ^{13}C NMR δ 170.7, 136.3, 136.0, 135.7, 134.9, 131.4, 131.2, 128.8, 128.6, 127.0, 124.3, 124.2, 124.1, 124.0, 123.0, 82.8, 79.9, 74.1, 57.3, 39.7, 39.7, 39.6, 37.4, 29.3, 26.7, 26.6, 26.5, 25.7, 24.1, 23.6, 21.9, 17.7, 16.0, 16.0, 15.8; HPLC-MS-FIA *m/z* 610 ($M^{\cdot+}$+18), 593 ($M^{\cdot+}$+1), 575 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot}$ −265); $[\alpha]_D$=+26.6 (*c*=1, 98% de).

3.2.13. (*S*)-(+)-α-Acetoxy-α-(phenyl)-acetate of (3*S*)-2,3-squalene diol (higher R_f in TLC). R_f=0.30; IR 3510, 2970, 2925, 2855, 1750 (CO), 1450, 1380, 1200, 1175, 1115, 730, 695 cm^{-1}; ^1H NMR δ 7.58–7.46 (2H), 7.44–7.32 (3H), 5.93 (s, 1H), 5.24–5.02 (5H), 4.79 (dd, J_1=10 Hz, J_2=2.5 Hz, 1H), 2.21 (s, 3H), 2.14–1.84 (18H), 1.68 (d, J=1 Hz, 3H), 1.60 (bs, 12H), 1.57 (bs, 3H), 1.60–1.20 (5H), 0.95 (s, 6H); ^{13}C NMR δ 170.5, 168.6, 135.1, 135.1, 134.9, 133.9, 133.8, 131.2, 129.4, 128.8, 127.6, 125.0, 124.4, 124.3, 124.2, 81.2, 74.8, 72.2, 39.7, 39.7, 39.6, 35.6, 28.3, 28.2, 26.7, 26.6, 25.7, 24.6, 20.8, 17.7, 16.0, 16.0; HPLC-MS-FIA *m/z* 638 ($M^{\cdot+}$+18), 621 ($M^{\cdot+}$+1), 603 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot}$−193); $[\alpha]_D$=+26.0 (*c*=1, 98% de).

3.2.14. (*S*)-(+)-α-Acetoxy-α-(phenyl)-acetate of (3*R*)-2,3-squalene diol (lower R_f in TLC). R_f=0.16; IR 3510, 2970, 2925, 2855, 1750 (CO), 1450, 1380, 1200, 1175, 1115, 730, 695 cm^{-1}; ^1H NMR δ 7.58–7.46 (2H), 7.44–7.32 (3H), 5.86 (s, 1H), 5.22–5.02 (4H), 4.80 (dd, J_1=10 Hz, J_2=2 Hz, 1H), 4.74 (t, J=6 Hz, 1H), 2.20 (s, 3H), 2.14–1.85 (18H), 1.70–1.20 (5H), 1.68 (d, J=1 Hz, 3H), 1.60 (bs, 9H), 1.58 (d, J=1 Hz, 3H), 1.36 (d, J=1 Hz, 3H), 1.21 (s, 6H); ^{13}C NMR δ 170.7, 168.9, 135.1, 135.0, 134.9, 133.5, 133.4, 131.2, 129.4, 128.8, 128.8, 127.6, 124.8, 124.4, 124.2, 80.9, 75.0, 72.3, 39.7, 39.7, 39.6, 35.2, 28.2, 28.0, 26.7, 26.6, 26.0, 25.7, 25.1, 20.7, 17.6, 16.0, 16.0, 15.7; HPLC-MS-FIA *m/z* 638 ($M^{\cdot+}$+18), 621 ($M^{\cdot+}$+1), 603 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot}$−193); $[\alpha]_D$=+27.4 (*c*=1, 98% de).

3.2.15. (*S*)-(+)-α-Acetoxy-α-(phenyl)-acetate of (6*R*,7*S*)-6,7-squalene diol (higher R_f in TLC). R_f=0.40; IR 3520, 2965, 2925, 2855, 1750 (CO), 1455, 1375, 1230, 1175, 1055, 740, 695 cm^{-1}; ^1H NMR δ 7.56–7.44 (2H), 7.43–7.35 (3H), 5.91 (s, 1H), 5.20–5.05 (5H), 4.94 (m, 1H), 4.84 (dd, J_1=10 Hz, J_2=2.5 Hz, 1H), 2.21 (s, 3H), 2.14–1.80 (18H), 1.68 (d, J=1 Hz, 3H), 1.66 (d, J=1 Hz, 3H), 1.60 (bs, 9H), 1.60–1.20 (2H), 1.57 (bs, 6H), 1.40 (s, 1H), 1.28–1.13 (3H), 0.91 (s, 3H); ^{13}C NMR δ 170.5, 168.5, 135.1, 134.9, 134.1, 133.9, 131.9, 131.2, 129.4, 128.8, 127.6, 124.9, 124.4, 124.2, 124.1, 80.6, 74.8, 73.9, 39.7, 37.1, 35.7, 28.3, 28.2, 27.9, 26.7, 26.6, 25.7, 22.7, 21.7, 20.7, 17.6, 17.6, 16.0; HPLC-MS-FIA *m/z* 638 ($M^{\cdot+}$+18), 621 ($M^{\cdot+}$+1), 603 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot}$−193); $[\alpha]_D$=+38.6 (*c*=1, 98% de).

3.2.16. (*S*)-(+)-α-Acetoxy-α-(phenyl)-acetate of (6*S*,7*R*)-6,7-squalene diol (lower R_f in TLC). R_f=0.26; IR 3520, 2965, 2925, 2855, 1750 (CO), 1455, 1375, 1230, 1175, 1055, 740, 695 cm^{-1}; ^1H NMR δ 7.56–7.44 (2H), 7.43–7.35 (3H), 5.85 (s, 1H), 5.18–5.05 (5H), 4.85 (dd, J_1=10 Hz, J_2=2.5 Hz, 1H), 4.77 (m, 1H), 2.21 (s, 3H), 2.16–1.80 (18H), 1.68 (bs, 6H), 1.62 (d, J=1 Hz, 3H), 1.60 (bs, 9H), 1.70–1.20 (4H), 1.35 (s, 3H), 1.26 (s, 1H), 1.18 (s, 3H); ^{13}C NMR δ 170.6, 168.8, 135.1, 134.9, 133.8, 133.5, 132.1, 131.2, 129.4, 128.8, 127.6, 124.8, 124.4, 124.2, 124.2, 80.5, 75.0, 74.1, 39.7, 37.5, 35.3, 28.2, 28.1, 27.8, 26.7, 26.6, 25.7, 23.3, 22.0, 20.7, 17.7, 16.0, 15.7; HPLC-MS-FIA *m/z* 638 ($M^{\cdot+}$+18), 621 ($M^{\cdot+}$+1), 603 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot}$−193); $[\alpha]_D$=+29.0 (*c*=1, 98% de).

3.2.17. (*S*)-(+)-α-Acetoxy-α-(phenyl)-acetate of (10*R*,11*S*)-10,11-squalene diol (higher R_f in TLC). R_f=0.40; IR 3530, 2965, 2925, 2855, 1750 (CO), 1455, 1375, 1230, 1180, 1060, 740, 695 cm^{-1}; ^1H NMR δ 7.55–7.44 (2H), 7.43–7.34 (3H), 5.93 (s, 1H), 5.15–5.03 (4H), 4.95 (m, 1H), 4.84 (dd, J_1=9 Hz, J_2=3.5 Hz, 1H), 2.21 (s, 3H), 2.14–1.76 (18H), 1.68 (bs, 6H), 1.60 (bs, 9H), 1.56 (bs, 6H), 1.70–1.20 (2H), 1.37 (s, 1H), 1.28–1.13 (3H), 0.91 (s, 3H); ^{13}C NMR δ 170.5, 168.5, 136.1, 135.6, 134.9, 133.9, 131.5, 131.3, 129.4, 128.8, 127.6, 124.4, 124.2, 123.9, 123.2, 80.6, 74.8, 73.9, 39.7, 39.7, 37.1, 29.6, 26.7, 26.6, 26.6, 25.7, 24.4, 22.7, 21.6, 20.8, 17.7, 16.0; HPLC-MS-FIA *m/z* 638 ($M^{\cdot+}$+18), 621 ($M^{\cdot+}$+1), 603 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot}$−193); $[\alpha]_D$=+31.2 (*c*=1, 98% de).

3.2.18. (*S*)-(+)-α-Acetoxy-α-(phenyl)-acetate of (10*S*,11*R*)-10,11-squalene diol (lower R_f in TLC). R_f=0.30; IR 3520, 2965, 2925, 2855, 1750 (CO), 1455, 1375, 1230, 1180, 1060, 740, 695 cm^{-1}; ^1H NMR δ 7.56–7.44 (2H), 7.43–7.33 (3H), 5.86 (s, 1H), 5.17–5.01 (4H), 4.95–4.85 (2H), 2.20 (s, 3H), 2.18–1.82 (14H), 1.68 (bs, 6H), 1.62 (d, J=1 Hz, 3H), 1.60 (bs, 6H), 1.57 (d, J=1 Hz, 3H), 1.72–1.20 (2H), 1.27 (d, J=1 Hz, 3H), 1.18 (s, 3H); ^{13}C NMR δ 170.6, 168.9, 135.9, 135.7, 134.9, 133.4, 131.4, 131.2, 129.4, 128.8, 127.6, 124.3, 124.2, 124.1, 122.9, 80.6, 75.0, 74.1, 39.7, 39.6, 37.4, 29.3, 26.7, 26.6, 26.5, 25.7, 24.0, 23.3, 21.9, 20.7, 17.7, 16.0, 16.0, 15.8; HPLC-MS-FIA *m/z* 638 ($M^{\cdot+}$+18), 621 ($M^{\cdot+}$+1), 603 ($M^{\cdot+}$−18+1), 427 ($M^{\cdot}$−193); $[\alpha]_D$=+36.0 (*c*=1, 98% de).

3.3. Preparation of squalene diols by reduction of arylacetic esters. General procedure

A solution of the corresponding arylacetic ester in Et_2O, maintained under nitrogen atmosphere and at room temperature, was treated with 4.5 molar equiv of $LiAlH_4$. The mixture was stirred until the reaction was completed. After the usual workup, the residue was purified by flash chromatography using as eluent hexanes–MTBE 85:15 to afford the corresponding enantiomeric diol (87–93% yield).

Squalene diols reobtained by reduction with $LiAlH_4$ of the corresponding (*R*)-(−)-MPA derivatives showed the following optical rotations: (3*S*)-2,3-Dihydroxy-2,3-dihydrosqualene (from higher R_f) $[\alpha]_D$=−11.8 (*c*=1, 97% ee); (3*R*)-2,3-Dihydroxy-2,3-dihydrosqualene (from lower R_f) $[\alpha]_D$=+11.4 (*c*=1, 97% ee); (6*R*,7*S*)-6,7-Dihydroxy-6,7-dihydrosqualene (from higher Rf) $[\alpha]_D$=−10.0 (*c*=1, 97% ee); (6*S*,7*R*)-6,7-Dihydroxy-6,7-dihydrosqualene (from lower R_f) $[\alpha]_D$=+10.2 (*c*=1, 97% ee); (10*R*,11*S*)-10,11-Dihydroxy-10,11-dihydrosqualene

(from higher R_f) $[\alpha]_D = -6.6$ ($c=1$, 97% ee); (10*S*,11*R*)-10,11-Dihydroxy-10,11-dihydrosqualene (from lower R_f) $[\alpha]_D = +6.5$ ($c=1$, 97% ee). These data are in agreement with the previously reported optical rotations for these compounds.[1]

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An experimental and theoretical study of the catalytic effect of quaternary ammonium salts on the oxidation of hydrocarbons

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Abstract—The enhancement in the autoxidation of ethylbenzene by molecular oxygen in the presence of quaternary ammonium salts (QAS) was investigated from the experimental and theoretical points of view. The primary effect of the addition of QAS to the reaction medium was an increase in ethylbenzene conversion. Quantum chemical calculations, using B3LYP hybrid functional, revealed a weakening of C–O and O–H bonds of the hydroperoxide. These effects favor the formation of ethylbenzenyl and peroxyl radicals, respectively, both of which are involved in the propagation reaction that leads to the formation of hydroperoxide, the desired final product.

1. Introduction

Autoxidation reactions are acquiring increasing importance in industrial oxidation processes for several reasons: they use the most abundant and cheapest oxidizing reagent, and usually mild temperatures and pressures are required. The use of molecular oxygen as an oxidant avoids the generation of pollutants, as occurs with stoichiometric oxidants. However, hydrocarbon autoxidation occurs by means of a free radical chain mechanism,[1,2] that hinders control of selectivity. One major drawback of the complex radical mechanism of these reactions, however, is the lack of a good understanding of the activity of the catalytic systems employed in the reactions. Under these circumstances, quantum chemical calculations are a powerful tool that can help to explain the catalytic effect observed in these processes.

The susceptibility of any substrate to autoxidation is determined by the $k_p/(2k_t)^{1/2}$ ratio, where k_p and k_t are, respectively, the specific rate constants of the propagation and termination reactions, which determine the length of the propagating chain and hence the reaction rate (see Scheme 1a). Alkylperoxyl radicals, which are indeed the active propagating species in liquid phase, play a pivotal role within this scheme. Along the reaction, the hydroperoxide species formed acts as a radical initiator, which subsequently decomposes mainly into alkylperoxyl radicals, or alkyl radicals (Scheme 1b). The alkyl radical species reacts very quickly in presence of molecular oxygen to alkylperoxyl radicals; and hence both species could produce an increase in the selectivity to hydroperoxide product, because it favors the propagation step.

The most interesting products of hydrocarbon autoxidation

(a)

Initiation
$In_2 \xrightarrow{R_i} 2In\bullet$
$In\bullet + RH \longrightarrow R\bullet + InH$

Propagation
$R\bullet + O_2 \longrightarrow ROO\bullet$
$ROO\bullet + RH \xrightarrow{k_p} R\bullet + ROOH$

Termination
$2\,ROO\bullet \xrightarrow{k_t}$ non-radical products

(b)

$ROOH \longrightarrow$ $ROO\bullet + \bullet H$; $RO\bullet + \bullet OH$; $R\bullet + \bullet OOH$

Scheme 1. (a) Standard autoxidation mechanism. (b) Different radical products of the decomposition of hydroperoxide.

Keywords: Autoxidation; Ethylbenzene hydroperoxide; Quaternary ammonium salts; Density-functional calculations; Bond activation.
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are hydroperoxides. Hydroperoxides are used as oxidizing agents of olefins, and as important precursors for the synthesis of phenols and other organic by-products. Ethylbenzene hydroperoxide is obtained by oxidizing ethylbenzene with air in a liquid phase, and it is used in the epoxidation step of the industrial process to obtain propylene oxide.[3]

A very broad variety of homogeneous catalytic systems has been described in the literature for this reaction.[4–7] It has been also reported that quaternary ammonium halides (and other onium salts, i.e., sulphonium and phosphonium) enhance the solubility of oxygen in the liquid phase, and at the same time show catalytic activity in free radical oxidation reactions, but their role in these processes is not clear.[5,7] In some cases, the explanations invoked to account for the catalytic activity of these compounds point to the possibility to produce free radicals, which is related with the donor effect of the alkyl chains and/or the halide atom of the salt, whereas in other cases the catalytic activity is ascribed to oxygen activation.

According to the above, this work was undertaken with the aim to relate the experimental data on catalytic activity to quantum chemical calculations of the system involved in the reaction. The combination of both studies should afford a better understanding of the factors affecting hydrocarbon oxidation reactions. This work will focus mainly on the study of the influence of catalysts on the desired final product, ethylbenzene hydroperoxide, which also plays an important role in the initiation and propagation steps. Due to the high complexity of autoxidation mechanism and the high number of species present in the reaction medium, one can take this approach as a preliminary step to understand the effect of quaternary ammonium salts on reaction mechanism. The usefulness of computational catalyst relays on its ability to provide a simple and efficient tool to relate catalyst structure with its reactivity. Further studies, involving transition state structures for both initiation and propagation steps, will be needed to completely identify the effects of these salts on reaction mechanism. The analysis of bond properties, by means of atoms-in-molecules methodology, offers a descriptor for the stability of the O–O bond, which accounts for the selectivity of the reaction.

2. Results and discussion

Figure 1 shows the dependence of ethylbenzene conversion with reaction time in the presence of quaternary ammonium salt (QAS) catalysts. For the sake of comparison, the time dependence of ethylbenzene conversion is also included for the QAS-free reaction. The incorporation of QAS to the reaction medium led to an increase in ethylbenzene conversion with respect to the QAS-free reaction. This increase was much more pronounced with the iodide salt than with the hydroxide. However, the increase in ethylbenzene conversion was accompanied by a parallel increase in the EBHP concentration upon addition of TMAI, but never in the presence of TMAOH (Fig. 2). The plot of EBHP selectivity versus ethylbenzene conversion revealed that TAMOH elicits a drop in EBHP selectivity whereas the EBHP selectivity profile of the reference experiment did not

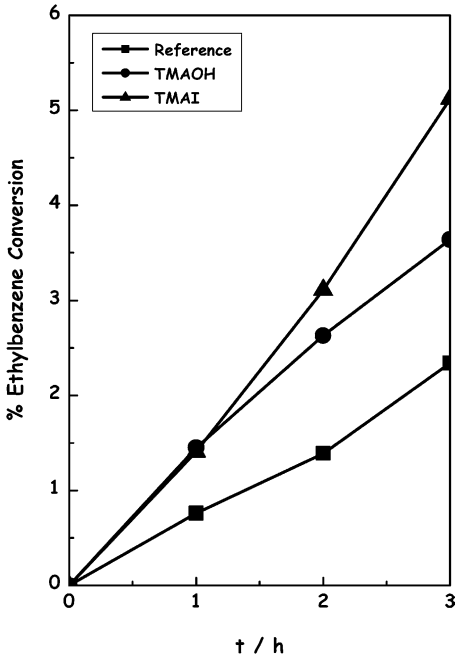


Figure 1. Experimental ethylbenzene conversion during autoxidation with air at 506 K.

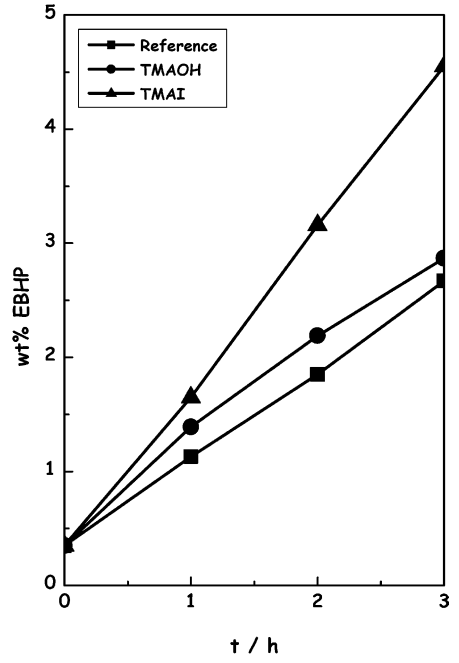


Figure 2. EBHP concentration versus reaction time during autoxidation with air at 506 K.

change to any significant extent in the presence of TMAI (Fig. 1). This indicates that EBHP selectivity remains essentially unchanged when the reaction rate is increased, and hence the hydroperoxide yield is enhanced (Fig. 3).

In order to explain this selectivity pattern, theoretical calculations of the interaction between the hydroperoxide and QAS were made. The systems investigated comprise the individual hydroperoxide, the interaction between EBHP and QAS salts, and also the interaction between EBHP and the separate cations and anions (Scheme 2).

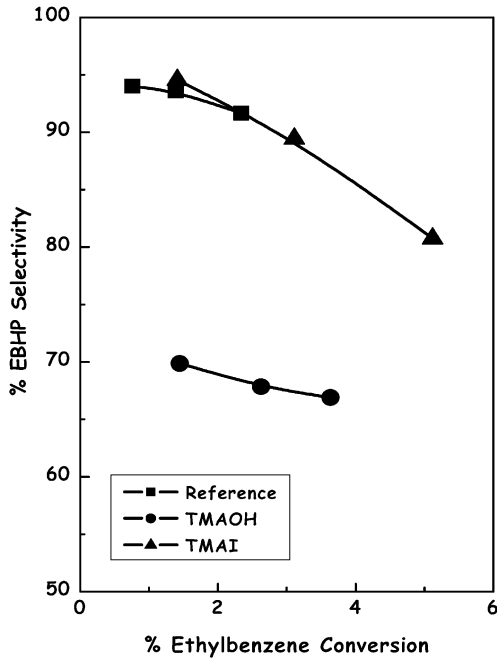
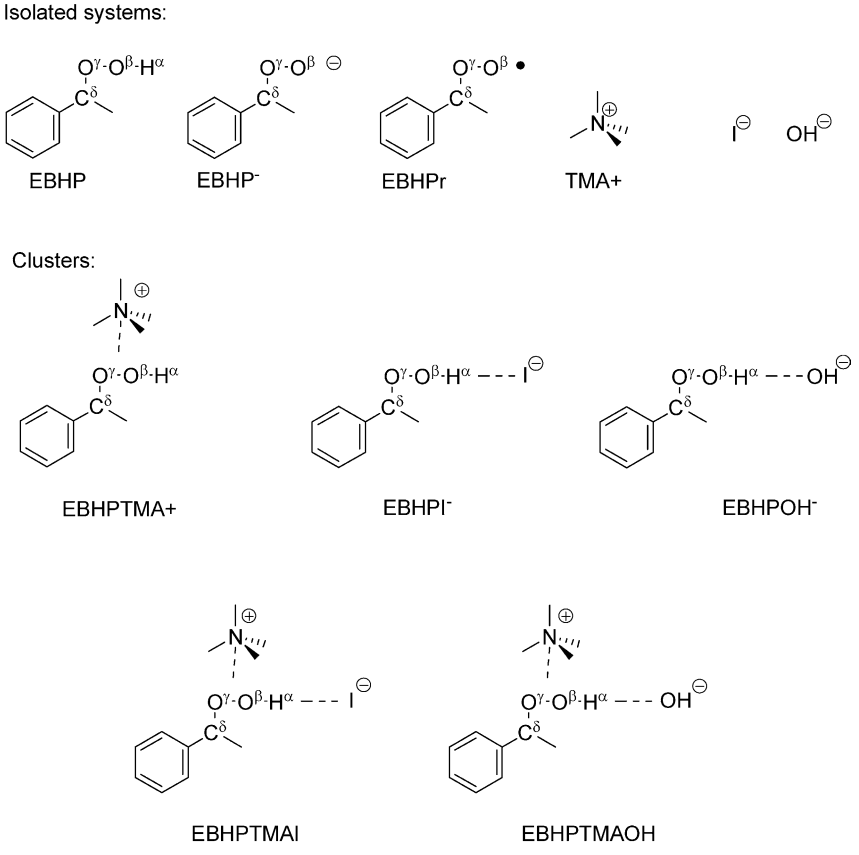


Figure 3. Selectivity to EBHP versus ethylbenzene conversion.

The energies and molecular properties involved in the calculations at B3LYP/631G(d,p) level of theory are shown in Table 1. First, it should be noted that the formation of any catalyst–hydroperoxide complex proves to be energetically favorable; that is, the incorporation of QAS or each of its components leads to the formation of new species in the bulk reaction mixture. Study of the properties of these new species in comparison with when they are absent from the system should allow us to understand the observed changes in catalytic performance. The bond distance ant the electron density at the bond critical point[8] (obtained by AIM methodology) are used to estimate bond strength.

The study focused on the bonds involved in the reaction mechanism, which were mainly C^{δ}–O^{γ}, O^{γ}–O^{β} and O^{β}–H^{α}. As reported in the Introduction, the oxidation mechanism of ethylbenzene occurs via radical intermediates produced by homolytic rupture of these bonds. Each bond weakening observed in the structures was considered to favor radical formation. In other words, homolytic bond scission is favored by bond weakening in presence of radicals.

First, we shall address the properties of the neutral EBHP system and then we shall study the properties of the anionic, radical and cluster systems. Ethylbenzene hydroperoxide is a highly polarized system, as can be seen from its charge distribution (see Table 1). The excess of positive charge on H^{α} explains the acidity of the hydroperoxide. One remarkable fact, well established in the literature, is that the oxygen atoms of the peroxide group are not electronically equivalent; that is, the O^{β} is more electronegative than the inner one.[5] The peroxyl radical system (EBHPr) showed a dramatic reinforcement of the O^{γ}–O^{β} bond. The opposite effect was observed for the anionic system ($EBHP^{-}$), in which the O^{γ}–O^{β} bond is weakened. This effect implies that the formation of the anionic system favors the alkoxyl



Scheme 2. Molecular structures of the isolated systems and the clusters under study.

Table 1. Results of the calculations: electronic energy (with zero point correction) in kcal/mol, net charges over the atoms in atomic units, bond distance in angstroms, and electronic density at the bond critical point in atomic units with the 6-31G(d,p) basis set

Structure	$E+E_0$ (kcal/mol)	q C^{δ}	q O^{γ}	q O^{β}	q H^{α}	C^{δ}–O^{γ} bond		O^{γ}–O^{β} bond		O^{β}–H^{α} bond	
						R_{C-O} (Å)	$\rho_{(b.c.p).}$	r_{O-O} (Å)	$\rho_{(b.c.p).}$	r_{O-H} (Å)	$\rho_{(b.c.p)}$
EBHP	0	0.5135	−0.5555	−0.5986	0.5787	1.436	0.2519	1.461	0.2733	0.971	0.3664
$EBHP^{-}$	—	0.6385	−0.6941	−0.6332	—	1.403	0.2848	1.487	0.2472	—	—
EBHPr	—	0.3994	−0.3614	−0.1836	—	1.492	0.2245	1.319	0.3917	—	—
EBHPT-MA+	−16.29	0.4564	−0.5277	−0.6436	0.6002	1.463	0.2339	1.458	0.2751	0.972	0.3626
$EBHPI^{-}$	−7.14	0.5501	−0.5812	−0.6397	0.5965	1.425	0.2618	1.450	0.2752	0.990	0.3360
EBH-POH^{-}	−74.68	0.6686	−0.6816	−0.6315	0.6361	1.400	0.2844	1.480	0.2537	1.539	0.0733
EBHPT-MAI	−86.65	0.4884	−0.5926	−0.6434	0.5876	1.451	0.2414	1.451	0.2749	1.000	0.3259
EBHPT-MAOH	−176.96	0.5623	−0.6970	−0.6264	0.6339	1.448	0.2616	1.480	0.2547	1.626	0.0584

radical, and this radical participates in the reactions to afford ketone, acid and alcohol products instead hydroperoxide, implying a reduction in the selectivity to EBHP.

Once the systems have been analyzed in isolation, the effects of the ammonium salts are discussed. The main effect of the addition of the tetramethylammonium cation is a weakening of the C^{δ}–O^{γ} bond, reflected in an increase in bond distance and a decrease in electronic density at the bond critical point. This favors C^{δ}–O^{γ} bond scission and, consequently, the formation of the desired ethylbenzene radical. Changes in the O^{γ}–O^{β} and O^{β}–H^{α} bonds are much less pronounced. The inclusion of the iodide anion causes an interaction between the halogen anion and the acid H^{α}. The I^{-} attracts the positively charged hydrogen and causes the O^{β}–H^{α} bond to weaken. Accordingly, formation of the peroxyl radical is favored. The O^{γ}–O^{β} bond remains practically unchanged, whereas the C^{δ}–O^{γ} bond is reinforced.

The effect of the hydroxide anion is much more significant. First, the high stabilization energy indicates the presence of a very strong interaction in the structure. As a strong base, the OH^{-} reacts with EBHP by catching the H^{α} to give water and $EBHP^{-}$. The data in Table 1 clearly show that the charge distribution and bond parameters for the oxygen atoms are quite close to those of the anion. The O^{β}–H^{α} bond distance and electronic density reveal the change in the nature of the bond, which passes from a covalent interaction to a hydrogen bond. On this basis, the formation of anionic species ($EBHP^{-}$) is strongly favored by OH^{-}. The hydroperoxo anion favors the formation of the alkoxyl radical. At the same time, the C^{δ}–O^{γ} bond is reinforced and the O^{γ}–O^{β} bond is weakened. Consequently, the selectivity of the oxidation reaction is switched to the generation of undesired products.

Having analyzed the effect of the cation and the anion separately, the effect of the whole salt on the EBHP system was studied. First, the high stabilization energies for both salts indicate the presence of stronger interactions in these systems than in the former ones. TMAI produced a weakening of the C^{δ}–O^{γ} and O^{β}–H^{α} bond, whereas the O^{γ}–O^{β} was reinforced (see Table 1). These changes in the molecular properties favor the reaction mechanism to the formation of EBHP. The key bond for the selectivity of the reaction between the oxygen atoms is slightly reinforced, whereas the formation of the radicals responsible for the reaction is favored. The iodide salt has all the desired effects on the hydroperoxide system and this explains its high catalytic activity. TMAOH exerts similar effects to the hydroxide anion. It weakens or almost breaks the O^{β}–H^{α} bond but the effects on the C^{δ}–O^{γ} and O^{γ}–O^{β} bonds are not so beneficial for the reaction. Since the presence of the anion favors the formation of alkoxyl radicals (which yields ketone, acid and alcohol products instead hydroperoxide), the hydroxide salt decreases selectivity to EBHP.

In order to check the accuracy of these results a larger basis set, which included diffuse functions such as 6-31++G(d,p), was employed to analyze the salt/EBHP cluster. These new data are summarized in Table 2. The optimized geometry of EBHPTMAI and EBHPTMAOH clusters is shown in Figure 6. If the results obtained with the 6-31G++(d,p) basis set are compared with that obtained previously with the 6-31G(d,p) basis set, the following observations can be made: (i) the stabilization energy for the cluster EBHPTMAI is quite similar for both basis sets. That implies that the basis set employed for I atom is large enough to take into consideration the anionic character of iodide; (ii) both the bond distances and electronic densities at bond critical point change slightly with the addition of

Table 2. Results of the calculations: electronic energy (with zero point correction) in kcal/mol, bond distance in angstroms, and electronic density at the bond critical point in atomic units with the 6-31++G(d,p) basis set

Structure	$E+E_0$ (kcal/mol)	C^{δ}–O^{γ} bond		O^{γ}–O^{β} bond		O^{β}–H^{α} bond	
		R_{C-O} (Å)	$\rho_{(b.c.p).}$	r_{O-O} (Å)	$\rho_{(b.c.p).}$	r_{O-H} (Å)	$\rho_{(b.c.p)}$
EBHP	0	1.442	0.2521	1.464	0.2734	0.971	0.3667
EBHPTMAI	−86.26	1.448	0.2413	1.456	0.2758	0.994	0.3373
EBHPTMAOH	−140.38	1.430	0.2599	1.474	0.2592	1.525	0.0734

diffuse functions. As it was accomplished previously, the O^{γ}–O^{β} bond is strengthened whereas the C^{δ}–O^{γ} and O^{β}–H^{α} bonds are weakened in this cluster; (iii) with respect to EBHPTMAOH cluster a drastic increase in stabilization energy is observed when employing the 6-31++G(d,p) basis set. Due to the strong anionic character of EBHP in this cluster, the addition of diffuse functions is mandatory for its correct description; (iv), geometrical and bond parameters also change noticeably with this basis set. Nevertheless, the global effect of the TMAOH salt over the EBHP species remains unchanged. The O^{β}–H^{α} bond is almost broken to infer anionic character to EBHP. The O^{γ}–O^{β} bond is enlarged and the C^{δ}–O^{γ} is shortened, as it was achieved for the 6-31G(d,p) basis set (Fig. 4).

To sum up, the employment of the 6-31G++(d,p) basis set does not change the effect of the catalyst on the EBHP but reveals the need of diffuse functions when a rigorous description of anionic character is required.

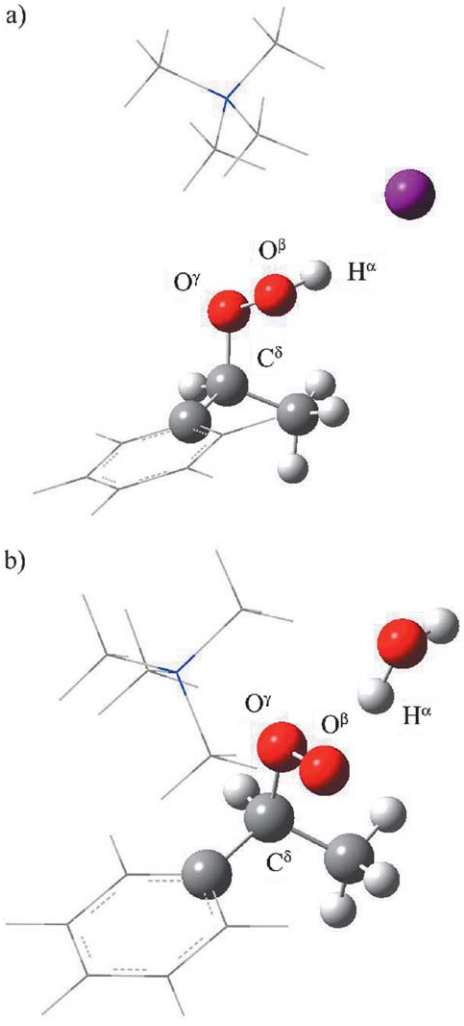


Figure 4. 3-D structure at B3LYP/6-31G++(d,p) level for: (a) EBHPTMAI cluster, (b) EBHPTMAOH cluster.

3. Conclusions

Analysis of the electronic properties of the structures formed between reactants and catalysts offers a better understanding of the mechanism of oxidation reactions. (i) Quantum chemical calculations, particularly the B3LYP hybrid functional, proved to be a valuable tool for the study of the catalytic activity of the ammonium salts on the oxidation of ethylbenzene. Owing to the considerable complexity of the radical mechanism for this reaction, further studies will be necessary in order to gain a deeper understanding of such process and the effect of catalysts on reaction mechanisms. Thus, the DFT methodology is a promising technique for explaining the changes in activity due to the addition of catalysts. (ii) The strong catalytic activity and high selectivity to the hydroperoxide of TMAI is explained in terms of the weakening of C^{δ}–O^{γ} and O^{β}–H^{α} bonds. These effects favor the formation of ethylbenzenyl and peroxyl radicals, respectively, which are involved in the propagation reaction that leads to hydroperoxide formation. (iii) The lower selectivity to EBHP reached in the presence of TMAOH is due to the weakening of O^{β}–H^{α} bond, inducing the formation of the anionic species. The presence of the anionic species favors the formation of alkoxyl radicals, which yield ketone, acid and alcohol products instead the hydroperoxide. Consequently, a reduction of the selectivity to EBHP is observed.

4. Experimental

A 150 ml cylindrical thermostatable glass vessel provided with a glass stirrer and a gas inlet system, a type K thermocouple, and a reflux condenser cooled by water were used for the experiments. Temperature was controlled by a heated circulating bath; the stirrer was powered by a variable-speed engine and the gases were fed to the reactor through mass flow controllers. In a typical oxidation with air run, 50 g of ethylbenzene (EB) containing about 0.4 wt% of ethylbenzene hydroperoxide (EBHP), kindly provided by Repsol-YPF, and 10^{-4} M of quaternary ammonium salt (QAS) were loaded into the reactor. The temperature of the bath and stirring speed were set at the desired values, typically 403 K and 1000 rpm, respectively. The QAS compounds selected in this work were tetramethylammonium iodide (TMAI) and tetramethylammonium hydroxide (TMAOH), both from Aldrich Chemie. Then, an air flow of 10 L h^{-1} was fed. In order to reduce the reaction time along hydrocarbon oxidation, a common practice is to add small amounts of hydroperoxides, which act as radical initiators and eliminates the induction period typical of liquid phase oxidations with molecular oxygen. A reference experiment was carried out without the addition of ammonium salt. Once the temperature had reached a constant value, the gas flow was set and kept constant during the experiment. Aliquots of the reaction mixture were taken at 0, 60, 120 and 180 min of reaction. The total amount extracted was less than 10 wt% of the total mixture inside the reactor. The concentration of EBHP was measured by standard iodometric titration. The remaining organic compounds were analyzed by GC-FID on a Hewlett Packard 6890-plus gas chromatograph equipped with an HP-WAX capillary column. Before GC analysis, these samples were pre-treated with triphenylphosphine to decompose the EBHP quantitatively to 1-phenylethanol.

Caution

As the autoxidation of hydrocarbons with molecular oxygen entrails some risk, an important safety issue was considered in all the experiments. The experimental conditions described in the preceding paragraphs indicate that the reaction is conducted above the explosion limit of the reaction mixture. To avoid this hazard two actions were undertaken: (i) a flow of nitrogen was fed just on the surface of the liquid, and (ii) the effluent oxygen concentration was continuously monitored with an oxygen sensor and kept almost constant at approximately 1 vol%. In any case, the laboratory glassware was used behind safety screens, and the volume of reaction was limited to 50 ml solutions.

4.1. Computational methods

All calculations shown were performed with the Gaussian 98[9] program. The hybrid functional B3LYP was chosen, since it had been previously shown to describe systems containing peroxyl radicals or ionic species accurately.[10–12] For H, C, N and O (atoms from the first and second rows) a 6-31G (d,p) or a 6-31++G(d,p) basis set was chosen. For iodine[13] a 6-311G++(3df) basis set was obtained from the EMSL[14] (the supplementary diffuse and polarization functions of reference[13] were added manually). All structures presented are full-geometry optimizations at the B3LYP theoretical level. Frequency analyses were carried out on each system and it was verified that all were true minima. Stabilization energies were estimated as the difference between the isolated system (molecule or ion) and the structure under study (Scheme 2). All energies reported include zero-point energy correction. An estimation of the basis set superposition error (BSSE) can be worked out on the energy difference between the two basis sets employed. Spin contamination was checked for the radical analyzed and no significant deviation from the pure spin state was detected. The atoms-in-molecules[15] (AIM) approach was used, as implemented in the AIMPAC[16] suite of programs, to estimate atomic charges and bond strengths.

Cartesian coordinates, thermochemical data for all optimized structures and basis set input for iodine are provided in the Supplementary information.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2004.09.060

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Direct synthesis of 3-arylpropionic acids by tetraphosphine/palladium catalysed Heck reactions of aryl halides with acrolein ethylene acetal

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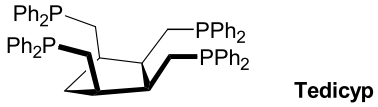
Abstract—Through the use of $[PdCl(C_3H_5)]_2$/*Cis*,*cis*,*cis*-1,2,3,4-tetrakis(diphenylphosphinomethyl)cyclopentane as a catalyst, a range of aryl bromides undergoes Heck reaction with acrolein ethylene acetal. With this acetal, the selective formation of 3-arylpropionic acids/esters was observed. The functional group tolerance on the aryl halide is remarkable; substituents such as fluoro, methyl, methoxy, acetyl, formyl, benzoyl, nitro or nitrile are tolerated. Furthermore, this catalyst can be used at low loading, even for reactions of sterically hindered aryl bromides.

1. Introduction

Arylpropionic acids are important building blocks in organic synthesis and their preparation is an important industrial goal.[1] Cacchi et al. have described recently that the palladium-catalysed Heck reaction[2] between aryl halides and acrolein acetals using $Pd(OAc)_2$ as catalyst is a very powerful method for the direct synthesis of 3-arylpropionic esters. However, this procedure needs a relatively high catalyst loading (3%) due to the absence of ligand on the palladium catalyst.[3] In recent years, several thermally stable palladium catalysts have been successfully used for Heck reactions,[4] but these catalysts have not been tested for the coupling of aryl halides with acrolein acetals. To our knowledge, only five phosphine ligands have been used for the reaction between aryl halides or vinyl halides and acrolein acetals: PPh_3, P(*o*-Me-$C_6H_4)_3$, P(*p*-Cl-$C_6H_4)_3$, P(2,4,6-triMeO-$C_6H_2)_3$ and dppf. In the presence of these phosphine ligands, the formation of the corresponding arylated or vinylated acrolein acetal (or aldehyde) derivatives[5a–c,e] or mixtures of products were generally obtained.[3,5d] We found only one example of a selective formation of a 3-arylpropionic acids using a Pd–phosphine catalyst.[6]

In order to find more efficient palladium catalysts, we have prepared the tetrapodal phosphine ligand, Tedicyp[7] (Fig. 1). We have reported recently several results obtained in allylic substitution,[7] Suzuki cross-coupling,[8] Sonogashira reactions[9] and Heck reaction[10] using Tedicyp as ligand. Here, in order to further establish the requirements for a successful Heck reaction, we wish to report on the reaction of aryl bromides with acrolein diethyl acetal or acrolein ethylene acetal using Tedicyp as the ligand.



Tedicyp

Figure 1.

2. Results and discussion

For this study, based on previous results,[10] DMF was chosen as the solvent for polarity reasons and potassium carbonate as the base. The reactions were performed at 110 °C under argon in the presence of a 1/2 ratio of $[Pd(C_3H_5)Cl]_2$/Tedicyp as catalyst.

First, we have investigated the Heck reactions of a few *para*- and *ortho*-substituted arylbromides with acrolein diethyl acetal. The results presented in the Table 1 disclose a low selectivity of the reaction. In all cases the formation of mixtures of cinnamaldehyde derivatives **1a–7a** and

Keywords: Palladium; Catalysis; Heck reaction; 3-Arylpropionic acids; Aryl bromides; Acrolein ethylene acetal.
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Table 1. Palladium–Tedicyp catalysed Heck reactions with acrolein diethyl acetal (Scheme 1)

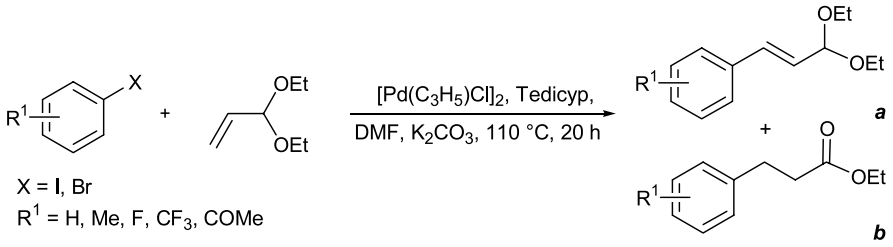
Entry	Aryl halide	Ratio substrate/catalyst	Ratio **a/b**	Products numbers	Yield (%)
1	Iodobenzene	1000	28/72	**1a**, **1b**	100
2	4-Bromoacetophenone	1000	21/79	**2a**, **2b**	100
3	4-Trifluoromethylbromobenzene	1000	27/73	**3a**, **3b**	95
4	4-Fluorobromobenzene	100	27/73	**4a**, **4b**	100
5	4-Methylbromobenzene	1000	28/72	**5a**, **5b**	100
6	2-Methylbromobenzene	250	40/60	**6a**, **6b**	80
7	2-Trifluoromethylbromobenzene	250	20/80	**7a**, **7b**	75

Conditions: Pd–Tedicyp catalyst, ArX (1 equiv), acrolein diethyl acetal (2 equiv), K_2CO_3 (2 equiv), DMF, 110 °C, 20 h, ratio **a/b** determined by NMR, yield in products **a**+**b**, GC and NMR yields.

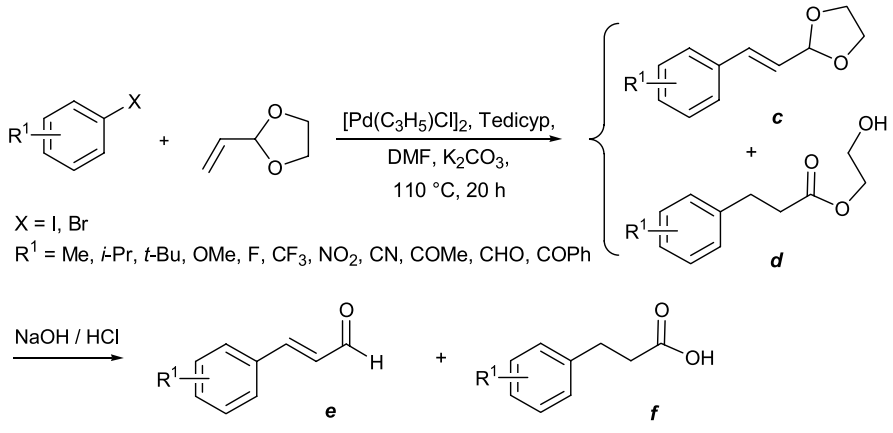
3-arylpropanoates **1b**–**7b** were observed (Table 1, entries 1–7). Similar mixtures of products had been obtained by Cacchi in the presence of PPh_3, P(*o*-Me-C_6H_4)$_3$, P(*p*-Cl-C_6H_4)$_3$ and P(2,4,6-triMeO-C_6H_2)$_3$ as ligands when acrolein diethyl acetal was used. The formation of these mixtures is due to the involvement of both the available β hydrogen atoms of the PdCH(CH_2Ar)[CH(OEt$_2$)] intermediate in the elimination step of the catalytic cycle (Scheme 1).

In order to improve the selectivity of this reaction, we studied several reactions conditions and we found that the use of acrolein ethylene acetal instead of acrolein diethyl acetal led to much higher selectivities in favour of the formation of 3-arylpropanoates (Scheme 2). For example, iodobenzene with acrolein diethyl acetal led to a mixture of phenylpropanoate and cinnamaldehyde in a ratio 72/28 (Table 1, entry 1). The same reaction performed with acrolein ethylene acetal gave the mixture of products in a ratio 97/3 (Table 2, entry 1). Moreover, the reaction using acrolein ethylene acetal can be performed with as little as 0.01% [Pd(C_3H_5)Cl]$_2$/Tedicyp catalyst.

Then, the reaction with acrolein ethylene acetal was applied to several aryl bromides (Table 2) and heteroaryl bromides (Table 3). We observed that in most cases the reaction performed with acrolein ethylene acetal proceeds very smoothly with high regioselectivity. First, we studied the reactivity of *para*-substituted aryl bromides. We observed that electron-withdrawing groups in the aryl bromide support the reaction, while electron-donation groups are unfavourable. Turnover numbers of 700–8400 can be achieved with this catalyst for activated substrates such as 4-bromoacetophenone, 4-bromobenzaldehyde, 4-bromobenzophenone, 4-bromobenzonitrile and 4-fluorobromobenzene (Table 2, entries 2–13). With the deactivated aryl bromides: 4-bromoanisole and 4-dimethylaminobromobenzene lower TONs of 100 and 45 were obtained respectively (Table 2, entries 16–19). A higher selectivity in favour of the formation of 3-arylpropanoates was observed with electron-poor aryl bromides than with electron-rich aryl bromides. For example, the reaction performed with 4-trifluoromethylbromobenzene led to 3-(4-trifluoro-methylphenyl)propionic acid in 99% selectivity. On the other hand, with 4-dimethylaminobromobenzene a lower selectivity of



Scheme 1.



Scheme 2.

Table 2. Palladium–Tedicyp catalysed Heck reactions with acrolein ethylene acetal (Scheme 2)

Entry	Aryl halide	Ratio substrate/catalyst	Ratio **c**/(**d**+**f**)[a]	Products numbers[b]	Yield in product **e** or **f** (%)[c]
1	4-Iodobenzene	10000	3/97	**8f**	92
2	4-Bromoacetophenone	1000	6/94	**9f**	88
3	4-Bromobenzophenone	1000	3/97	**10f**	91
4	4-Bromobenzophenone	10000	3/97	**10f**	(60)
5	4-Bromobenzaldehyde	1000	7/93	**11f**	(93)
6	4-Bromobenzaldehyde	10000	7/93	**11f**	84
7	4-Trifluoromethylbromobenzene	1000	1/99	**12f**	92
8	4-Trifluoromethylbromobenzene	10000	1/99	**12f**	(10)
9	4-Bromobenzonitrile	1000	3/97	**13f**	36[d]
10	3,5-Bis(trifluoromethyl)bromobenzene	1000	3/97	**14f**	89
11	4-Nitrobromobenzene	250	3/97	**15f**	83
12	4-Fluorobromobenzene	250	11/89	**16f**	79
13	4-Fluorobromobenzene	1000	10/90	**16f**	(70)
14	4-*t*-Butylbromobenzene	250	7/93	**17f**	(93)
15	4-*t*-Butylbromobenzene	1000	7/93	**17f**	84
16	4-Bromoanisole	100	13/87	**18f**	69
17	4-Bromoanisole	250	8/92	**18f**	(40)
18	4-Dimethylaminobromobenzene	25	14/86	**19f**	80
19	4-Dimethylaminobromobenzene	100	10/90	**19f**	(45)
20	3-Bromoacetophenone	1000	4/96	**20f**	87
21	3-Bromobenzaldehyde	1000	3/97	**21f**	89
22	3-Trifluoromethylbromobenzene	1000	1/99	**22f**	93
23	6-Methoxy-2-bromonaphthalene	1000	6/94	**23f**	90
24	2-Trifluoromethylbromobenzene	1000	2/98	**24f**	93
25	2-Fluorobromobenzene	100	10/90	**25f**	82
26	2-Fluorobromobenzene	250	8/92	**25f**	(30)
27	2-Bromotoluene	100	3/97	**26f**	87
28	2-Bromotoluene	250	3/97	**26f**	(82)
29	1-Bromonaphthalene	1000	2/98	**27f**	94
30	2-Bromoanisole	250	14/86	**28f**	60
31	2,6-Difluorobromobenzene	250	6/94	**29f**	78
32	9-Bromoanthracene	100	2/98	**30f**	82
33	2,4,6-Trimethylbromobenzene	25	75/25	**31e**	64
34	2,4,6-Trimethylbromobenzene	100	79/21	**31e**	(60)
35	2,6-Diethyl-4-methylbromobenzene	50	72/28	**32e**	62
36	2,4,6-Triisopropylbromobenzene	50	15/85	**33f**	78

Conditions: (1) Pd–Tedicyp catalyst, ArX (1 equiv), acrolein ethylene acetal (2 equiv), K_2CO_3 (2 equiv), DMF, 110 °C, 20 h; (2) NaOH, 50–80 °C, 1–4 h, isolated yields.
[a] Ratio of products **c**/(**d**+**f**) obtained before treatment by NaOH and HCl, calculated with ^{1}H NMR spectra of the crude mixture.
[b] Major product obtained after treatment by NaOH or HCl.
[c] Yield in the major product of the reaction, yields in parentheses are GC and NMR conversions.
[d] Product **13f** directly obtained without treatment by NaOH. The ester **13d** was also obtained in 43% yield.

86–90% in favour of the formation of 3-(4-dimethylaminophenyl)propionic acid was observed (Table 2, entries 7, 8, 18 and 19).

Then, we studied the influence of the presence of *meta* and *ortho* substituents on the aryl bromide on the reaction rate. As expected very similar TONs were obtained with *meta*-substituted aryl bromides than with the *para*-substituted (Table 2, entries 20–23). *Ortho*-substituents on the aryl bromides have a more important effect on the reactions rates. We observed that the coupling of 2-trifluoromethylbromobenzene or 1-bromonaphthalene with acrolein ethylene acetal proceeds in the presence of 0.1% catalyst, moreover a very high selectivity of 98% in favour of the formation of 3-arylpropanoates was observed (Table 2, entries 24 and 29). Next, we tried to evaluate the difference

Table 3. Palladium–Tedicyp catalysed Heck reactions with acrolein ethylene acetal and heteroaryl bromides (Scheme 3)

Entry	Aryl halide	Ratio substrate/catalyst	Ratio **c**/(**d**+**f**)[a]	Products number[b]	Yield in product **f** (%)
1	3-Bromopyridine	1000	6/94	**34f**	79
2	4-Bromopyridine	250	8/92	**35f**	84
3	3-Bromoquinoline	1000	6/94	**36f**	80
4	4-Bromoisoquinoline	250	5/95	**37f**	87
5	2-Bromothiophene	50	6/94	**38f**	75
6	2-Bromothiophene	100	6/94	**38f**	80[c]
7	3-Bromothiophene	100	7/93	**39f**	70
8	3-Bromofurane	100	5/95	**40f**	81[d]

Conditions: (1) Pd–Tedicyp catalyst, ArX (1 equiv), acrolein ethylene acetal (2 equiv), K_2CO_3 (2 equiv), DMF, 110 °C, 20 h; (2) NaOH, 50–80 °C, 1–4 h, isolated yields.
[a] Ratio of products **c**/(**d**+**f**) obtained before treatment by NaOH and HCl, calculated by ^{1}H NMR of the crude mixture.
[b] Major product obtained after treatment by NaOH.
[c] GC and NMR conversion.
[d] Reaction temp.: 90 °C.

of reaction rate between mono- and di-*ortho*-substituted aryl bromides, and we observed that even very hindered aryl bromides could be coupled efficiently with acrolein ethylene acetal,. For example, with 9-bromoanthracene and 1-bromo-2,4,6-triisopropylbenzene the 3-arylpropanoates were also obtained in 98% and 85% selectivities respectively in the presence of 1–2% catalyst (Table 2, entries 32 and 36). The coupling reactions also proceeds in the presence of 2,4,6-trimethylbromobenzene and 2,6-diethyl-4-methylbromobenzene, but with these substrates the selectivity of the reaction was completely reversed and the cinnamaldehyde derivatives were reproducibly obtained in 72–79% selectivity (Table 2, entries 33–35). Presumably, the steric demand of the two *ortho* alkyl groups disfavours one of the two possible β-hydride eliminations to generate either the cinnamaldehyde derivatives or the 3-arylpropanoates.

In most cases, mixtures of 3-arylpropanoates esters **d** and 3-arylpropionic acids **f** were obtained after the Pd catalysed reaction due to partial K_2CO_3-catalysed hydrolysis of the esters. In order to obtain selectively the 3-arylpropionic acids **f**, the hydrolysis of the esters **d** was performed with a NaOH solution. Aldehydes **31e** and **32e** were obtained by deprotection of acetals **31c** and **32c** using an HCl (1 M) solution.

Finally, we have investigated the Heck reaction of seven heteroaryl bromides. The results are summarized in Table 3. Pyridines or quinolines are π-electron deficient. Thiophenes or furanes are π-electron excessive. If the oxidative addition of the aryl halides to the palladium complex is the rate-limiting step of the reaction with this catalyst, the reactions should be slower with thiophenes or furanes than with pyridines or quinolines. In the presence of 3-bromopyridine, 4-bromopyridine, 3-bromoquinoline and 4-bromoisoquinoline the reactions were performed with 0.4–0.1% catalyst (Table 3, entries 1–4). Slower reactions were observed with 2-bromothiophene and 3-bromothiophene and 1% catalyst were necessary in order to obtain high conversions (Table 3, entries 5–7). The reaction in the presence of 3-bromofurane also led to the corresponding adduct (Table 3, entry 8). With all these heteroaryl bromides high selectivities in favor of the formation of 3-heteroaryl propionic acids were observed. These results seems also to indicate that the oxidative addition of the heteroaryl bromides to palladium is the rate-limiting step of this reaction.

The synthesis of 1,4-phenylenedipropionic acid **41f** from 1,4-dibromobenzene using 4 equiv of acrolein ethylene acetal in the presence of 1% catalyst also proceeds in good yield.

In summary, we have established that the Tedicyp–palladium system provides an efficient catalyst for the selective synthesis of 3-arylpropionic acids from acrolein ethylene acetal and aryl bromides. The use of acrolein ethylene acetal led to much higher selectivities in favour of the formation of 3-arylpropanoates (up to 99% selectivity) than the reactions performed with acrolein diethyl acetal. Moreover the uses of this Pd-tetraphosphine catalyst allow the use of low-catalyst loading. This reaction can be performed with as little as 0.01% catalyst with some of the aryl bromides. Due to the high price of palladium, the practical advantage of such low catalyst loadings can become increasingly important for industrial processes. In all cases, the formation of homocoupling products from aryl halides was not observed. A wide range of functions such as methoxy, fluoro, acetyl, formyl, benzoyl, nitro or nitrile on the aryl bromide are tolerated. The steric hindrance of the aryl bromide has an important effect on the reaction rates and on the selectivity of the reactions. Lower TONs were obtained for the coupling with sterically hindered aryl bromides such as 9-bromoanthracene. Several heteroaromatic substrates have also been used successfully. Moreover, acrolein acetals are commercially available and this is a practical advantage of this reaction.

3. Experimental

General remarks. All reactions were run under argon in Schlenk tubes using vacuum lines. DMF analytical grade was not distilled before use. Some of the aryl halides were distilled before use. Potassium carbonate (99+) was used without drying. Commercial acrolein diethyl acetal (95%) and acrolein ethylene acetal (99%) were used without purification. The reactions were followed by GC and NMR for high boiling point substrates and by GC for low boiling point substrates. ^1H and ^{13}C spectrum were recorded with a Bruker 300 MHz spectrometer in $CDCl_3$ solutions. Chemical shift are reported in ppm relative to $CDCl_3$ (7.25 for ^1H NMR and 77.0 for ^{13}C NMR). Flash chromatography were performed on silica gel (230–400 mesh) GC and NMR yields in the tables are conversions of the aryl halides into the product calculated with GC and ^1H NMR spectrum of the crude mixtures.

3.1. Preparation of the Pd–Tedicyp catalyst

An oven-dried 40-mL Schlenk tube equipped with a magnetic stirring bar under argon atmosphere, was charged with $[Pd(\eta^3\text{-}C_3H_5)Cl]_2$ (30 mg, 81 μmol) and Tedicyp (140 mg, 162 μmol). 10 mL of anhydrous DMF were added, then the solution was stirred at room temperature for ten minutes. The appropriate catalyst concentration was obtained by successive dilutions. ^{31}P NMR (162 MHz, $CDCl_3$) δ 25 (w=80 Hz), 19.4 (w=110 Hz).

3.2. General procedure

In a typical experiment, the aryl halide (1 mmol), acrolein ethylene acetal (0.200 g, 2 mmol) and K_2CO_3 (0.276 g, 2 mmol) were dissolved in DMF (3 mL) under an argon atmosphere. The prepared Pd–Tedicyp catalyst complex (see tables) was then transferred to the reaction flask via cannula. The reaction mixture was stirred at 110 °C for 20 h. The solution was diluted with H_2O (2 ml) and NaOH was added (0.200 g). The reaction mixture was stirred at 50–80 °C for 1–4 h. The mixture was acidified with an HCl solution (pH 2–4) then the product was extracted three times with CH_2Cl_2. For compounds **19**, **35**–**38**, the mixture was acidified with an HCl solution (pH 6–7) then the product was extracted three times with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and the solvent was removed in vacuo. The crude product was purified by silica gel column chromatography.

3.2.1. 3-Phenylpropionic acid 8f. From iodobenzene (0.204 g, 1 mmol), product **8f** was obtained in 92% (0.138 g) yield. Before hydrolysis **8d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (t, 2H, J=7.6 Hz), 7.23–7.18 (m, 3H), 4.18 (t, 2H, J=4.6 Hz), 3.75 (t, 2H, J=4.6 Hz), 2.96 (t, 2H, J=7.6 Hz), 2.68 (t, 2H, J=7.6 Hz).

3.2.2. 3-(4-Acetylphenyl)propionic acid 9f. From 4-bromoacetophenone (0.199 g, 1 mmol), product **9f** was obtained in 88% (0.169 g) yield. Before hydrolysis **9d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (d, 2H, J=8.2 Hz), 7.29 (d, 2H, J=8.2 Hz), 4.20 (t, 2H, J=4.6 Hz), 3.79 (t, 2H, J=4.6 Hz), 3.02 (t, 2H, J=7.6 Hz), 2.70 (t, 2H, J=7.6 Hz), 2.56 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.7, 172.7, 146.0, 135.5, 128.7, 128.5, 66.2, 61.1, 35.1, 30.8, 26.5.

3.2.3. 3-(4-Benzoylphenyl)propionic acid 10f. From 4-bromobenzophenone (0.261 g, 1 mmol), product **10f** was obtained in 91% (0.231 g) yield. Before hydrolysis **10d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, 2H, J=7.5 Hz), 7.73 (d, 2H, J=8.2 Hz), 7.57 (t, 1H, J=7.5 Hz), 7.46 (t, 2H, J=7.5 Hz), 7.31 (d, 2H, J=8.3 Hz), 4.21 (t, 2H, J=4.6 Hz), 3.79 (t, 2H, J=4.6 Hz), 3.05 (t, 2H, J=7.6 Hz), 2.73 (t, 2H, J=7.6 Hz).

3.2.4. 3-(4-Formylphenyl)propionic acid 11f. From 4-bromobenzaldehyde (0.185 g, 1 mmol), product **11f** was obtained in 84% (0.150 g) yield. Before hydrolysis **11d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.93 (s, 1H), 7.78 (d, 2H, J=8.2 Hz), 7.34 (d, 2H, J=8.2 Hz), 4.18 (t, 2H, J=4.6 Hz), 3.76 (t, 2H, J=4.6 Hz), 3.02 (t, 2H, J=7.6 Hz), 2.69 (t, 2H, J=7.6 Hz).

3.2.5. 3-(4-Trifluoromethylphenyl)propionic acid 12f. From 4-trifluoromethylbromobenzene (0.225 g, 1 mmol), product **12f** was obtained in 92% (0.201 g) yield. Before hydrolysis **12d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, 2H, J=8.1 Hz), 7.30 (d, 2H, J=8.1 Hz), 4.19 (t, 2H, J=4.6 Hz), 3.77 (t, 2H, J=4.6 Hz), 3.01 (t, 2H, J=7.6 Hz), 2.68 (t, 2H, J=7.6 Hz).

3.2.6. 3-(4-Cyanophenyl)propionic acid 13f. From 4-bromobenzonitrile (0.182 g, 1 mmol), product **13f** was obtained in 36% (0.063 g) yield. This compound was not treated with an NaOH solution, and ester **13d** was also isolated in 43% (0.094 g) yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, 2H, J=8.5 Hz), 7.29 (d, 2H, J=8.5 Hz), 4.17 (t, 2H, J=4.6 Hz), 3.76 (t, 2H, J=4.6 Hz), 2.99 (t, 2H, J=7.6 Hz), 2.67 (t, 2H, J=7.6 Hz).

3.2.7. 3-[3,5-Bis(trifluoromethyl)phenyl]propionic acid 14f. From 3,5-bis(trifluoromethyl)bromobenzene (0.293 g, 1 mmol), product **14f** was obtained in 89% (0.255 g) yield. Before hydrolysis **14d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.67 (s, 2H), 4.21 (t, 2H, J=4.6 Hz), 3.80 (t, 2H, J=4.6 Hz), 3.09 (t, 2H, J=7.6 Hz), 2.73 (t, 2H, J=7.6 Hz).

3.2.8. 3-(4-Nitrophenyl)propionic acid 15f. From 4-bromonitrobenzene (0.202 g, 1 mmol), product **15f** was obtained in 83% (0.162 g) yield. Before hydrolysis **15d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (d, 2H, J=8.7 Hz), 7.36 (d, 2H, J=8.7 Hz), 4.21 (t, 2H, J=4.6 Hz), 3.80 (t, 2H, J=4.6 Hz), 3.07 (t, 2H, J=7.6 Hz), 2.72 (t, 2H, J=7.6 Hz).

3.2.9. 3-(4-Fluorophenyl)propionic acid 16f. From 4-fluorobromobenzene (0.175 g, 1 mmol), product **16f** was obtained in 79% (0.133 g) yield. Before hydrolysis **16d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (m, 2H), 6.85 (t, 2H, J=8.5 Hz), 4.09 (t, 2H, J=4.6 Hz), 3.67 (t, 2H, J=4.6 Hz), 2.83 (t, 2H, J=7.6 Hz), 2.55 (t, 2H, J=7.6 Hz).

3.2.10. 3-(4-*tert*-Butylphenyl)propionic acid 17f. From 4-*tert*-butylbromobenzene (0.213 g, 1 mmol), product **17f** was obtained in 84% (0.173 g) yield. Before hydrolysis **17d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (d, 2H, J=8.3 Hz), 7.13 (d, 2H, J=8.3 Hz), 4.19 (t, 2H, J=4.6 Hz), 3.75 (t, 2H, J=4.6 Hz), 2.94 (t, 2H, J=7.6 Hz), 2.67 (t, 2H, J=7.6 Hz), 1.30 (s, 9H).

3.2.11. 3-(4-Methoxyphenyl)propionic acid 18f. From 4-bromoanisole (0.187 g, 1 mmol), product **18f** was obtained in 69% (0.124 g) yield. Before hydrolysis **18d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08 (d, 2H, J=8.5 Hz), 6.80 (d, 2H, J=8.5 Hz), 4.15 (t, 2H, J=4.6 Hz), 3.75 (t, 2H, J=4.6 Hz), 3.74 (s, 3H), 2.87 (t, 2H, J=7.6 Hz), 2.61 (t, 2H, J=7.6 Hz).

3.2.12. 3-(4-Dimethylaminophenyl)propionic acid 19f. From 4-bromo-*N*,*N*-dimethylaniline (0.200 g, 1 mmol), product **19f** was obtained in 80% (0.155 g) yield. Before hydrolysis **19d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.06 (d, 2H, J=8.1 Hz), 6.67 (d, 2H, J=8.1 Hz), 4.16 (t, 2H, J=4.6 Hz), 3.74 (t, 2H, J=4.6 Hz), 2.90 (s, 6H), 2.87 (t, 2H, J=7.6 Hz), 2.62 (t, 2H, J=7.6 Hz).

3.2.13. 3-(3-Acetylphenyl)propionic acid 20f. From 3-bromoacetophenone (0.199 g, 1 mmol), product **20f** was obtained in 87% (0.167 g) yield. Colourless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (m, 2H), 7.40 (m, 2H), 2.99 (t, 2H, J=7.6 Hz), 2.65 (t, 2H, J=7.6 Hz), 2.57 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.2, 172.3, 140.7, 137.3, 133.1, 128.7, 128.0, 126.6, 35.3, 30.4, 26.6; $C_{11}H_{12}O_3$: Calcd C 68.74, H 6.29; Found C 68.58, H 6.34. Before hydrolysis **20d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 (m, 2H), 7.40 (m, 2H), 4.20 (t, 2H, J=4.6 Hz), 3.78 (t, 2H, J=4.6 Hz), 3.02 (t, 2H, J=7.6 Hz), 2.70 (t, 2H, J=7.6 Hz), 2.57 (s, 3H).

3.2.14. 3-(3-Formylphenyl)propionic acid 21f. From 3-bromobenzaldehyde (0.185 g, 1 mmol), product **21f** was obtained in 89% (0.159 g) yield. Before hydrolysis **21d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.99 (s, 1H), 7.73 (m, 2H), 7.48 (m, 2H), 4.20 (t, 2H, J=4.6 Hz), 3.78 (t, 2H, J=4.6 Hz), 3.05 (t, 2H, J=7.6 Hz), 2.72 (t, 2H, J=7.6 Hz).

3.2.15. 3-(3-Trifluoromethylphenyl)propionic acid 22f. From 3-trifluoromethylbromobenzene (0.225 g, 1 mmol), product **22f** was obtained in 93% (0.203 g) yield. Before hydrolysis **22d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (m, 2H), 7.39 (m, 2H), 4.20 (t, 2H, J=4.6 Hz), 3.79 (t, 2H, J=4.6 Hz), 3.02 (t, 2H, J=7.6 Hz), 2.70 (t, 2H, J=7.6 Hz).

3.2.16. 3-(6-Methoxynaphthalen-2-yl)propionic acid 23f. From 1-bromo-6-methoxynaphthalene (0.237 g, 1 mmol), product **23f** was obtained in 90% (0.207 g) yield. Before hydrolysis **23d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67 (d, 2H, J=8.3 Hz), 7.56 (s, 1H), 7.29 (d, 1H, J=8.5 Hz), 7.12 (d, 1H, J=8.3 Hz), 7.10 (s, 1H), 4.19 (t, 2H, J=4.6 Hz), 3.90 (s, 3H), 3.74 (t, 2H, J=4.6 Hz), 3.09 (t, 2H, J=7.6 Hz), 2.75 (t, 2H, J=7.6 Hz).

3.2.17. 3-(2-Trifluoromethylphenyl)propionic acid 24f. From 2-trifluoromethylbromobenzene (0.225 g, 1 mmol), product **24f** was obtained in 93% (0.203 g) yield. Before hydrolysis **24d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (d, 1H, J=7.8 Hz), 7.47 (t, 1H, J=7.2 Hz), 7.35 (d, 1H, J=7.8 Hz), 7.31 (t, 1H, J=7.2 Hz), 4.22 (t, 2H, J=4.6 Hz), 3.80 (m, 2H), 3.14 (t, 2H, J=7.6 Hz), 2.67 (t, 2H, J=7.6 Hz).

3.2.18. 3-(2-Fluorophenyl)propionic acid 25f. From 2-fluorobromobenzene (0.175 g, 1 mmol), product **25f** was obtained in 82% (0.138 g) yield. Before hydrolysis **25d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22–7.15 (m, 2H), 7.07–6.97 (m, 2H), 4.19 (t, 2H, J=4.6 Hz), 3.77 (m, 2H), 2.98 (t, 2H, J=7.6 Hz), 2.68 (t, 2H, J=7.6 Hz).

3.2.19. 3-(2-Methylphenyl)propionic acid 26f. From 2-bromotoluene (0.171 g, 1 mmol), product **26f** was obtained in 87% (0.143 g) yield. Before hydrolysis **26d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20–7.05 (s, 4H), 4.20 (t, 2H, J=4.6 Hz), 3.79 (t, 2H, J=4.6 Hz), 2.95 (t, 2H, J=7.6 Hz), 2.64 (t, 2H, J=7.6 Hz), 2.32 (s, 3H).

3.2.20. 3-(Naphthalen-1-yl)propionic acid 27f. From 1-bromonaphthalene (0.207 g, 1 mmol), product **27f** was obtained in 94% (0.188 g) yield. Before hydrolysis **27d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (d, 1H, J=8.3 Hz), 7.86 (d, 1H, J=8.3 Hz), 7.73 (d, 1H, J=8.3 Hz), 7.54 (t, 1H, J=7.8 Hz), 7.48 (t, 1H, J=7.8 Hz), 7.40 (t, 1H, J=7.8 Hz), 7.35 (d, 1H, J=7.0 Hz), 4.20 (t, 2H, J=4.6 Hz), 3.75 (t, 2H, J=4.6 Hz), 3.44 (t, 2H, J=7.6 Hz), 2.81 (t, 2H, J=7.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 136.2, 133.8, 131.5, 128.9, 127.2, 126.1, 125.9, 125.6, 125.5, 123.3, 66.1, 61.1, 35.0, 28.0.

3.2.21. 3-(2-Methoxyphenyl)propionic acid 28f. From 2-bromoanisole (0.187 g, 1 mmol), product **28f** was obtained in 60% (0.108 g) yield. Before hydrolysis **28d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 (t, 1H, J=7.5 Hz), 7.14 (d, 1H, J=7.1 Hz), 6.87 (t, 1H, J=7.5 Hz), 6.84 (d, 1H, J=7.9 Hz), 4.18 (t, 2H, J=4.6 Hz), 3.82 (s, 3H), 3.76 (t, 2H, J=4.6 Hz), 2.95 (t, 2H, J=7.6 Hz), 2.66 (t, 2H, J=7.6 Hz).

3.2.22. 3-(2,6-Difluorophenyl)propionic acid 29f. From 2,6-difluorobromobenzene (0.193 g, 1 mmol), product **29f** was obtained in 78% (0.145 g) yield. Before hydrolysis **29d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (m, 1H), 6.85 (t, 2H, J=7.8 Hz), 4.21 (t, 2H, J=4.6 Hz), 3.80 (t, 2H, J=4.6 Hz), 3.02 (t, 2H, J=7.6 Hz), 2.65 (t, 2H, J=7.6 Hz).

3.2.23. 3-(Anthracen-9-yl)propionic acid 30f. From 9-bromoanthracene (0.257 g, 1 mmol), product **30f** was obtained in 82% (0.205 g) yield. Before hydrolysis **30d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 8.25 (d, 1H, J=8.5 Hz), 8.01 (d, 1H, J=8.3 Hz), 7.53 (t, 1H, J=6.8 Hz), 7.47 (t, 1H, J=6.8 Hz), 4.23 (t, 2H, J=4.6 Hz), 3.98 (t, 2H, J=7.6 Hz), 3.77 (t, 2H, J=4.6 Hz), 2.84 (t, 2H, J=7.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4, 132.1, 131.6, 129.5, 129.3, 126.5, 126.0, 125.0, 123.8, 66.3, 61.2, 35.2, 23.2.

3.2.24. *E*-3-(2,4,6-Trimethylphenyl)propenal 31e. From bromomesitylene (0.199 g, 1 mmol) and after HCl hydrolysis aldehyde **31e** was obtained in 64% (0.111 g) yield. Product **31d** was also observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.84 (s, 2H), 4.23 (t, 2H, J=4.6 Hz), 3.81 (t, 2H, J=4.6 Hz), 2.95 (t, 2H, J=7.6 Hz), 2.48 (t, 2H, J=7.6 Hz), 2.34 (s, 6H), 2.29 (s, 3H).

3.2.25. *E*-3-(2,6-Diethyl-4-methylphenyl)propenal 32e. From 2,6-diethyl-4-methylbromobenzene (0.227 g, 1 mmol) and after HCl hydrolysis aldehyde **32e** was obtained in 62% (0.125 g) yield. Oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.72 (d, 1H, J=7.8 Hz), 7.72 (d, 1H, J=16.5 Hz), 6.94 (s, 2H), 6.36 (dd, 1H, J=16.5, 7.8 Hz), 2.65 (q, 4H, J=7.6 Hz), 2.33 (s, 3H), 1.19 (t, 6H, J=7.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.1, 152.0, 142.7, 139.5, 134.3, 129.4, 127.6, 26.9, 21.3, 15.5; $C_{14}H_{18}O$: Calcd C 83.12, H 8.97; Found C 82.96, H 9.07. Product **32d** was also observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.86 (s, 2H), 4.24 (t, 2H, J=4.6 Hz), 3.82 (t, 2H, J=4.6 Hz), 2.61 (q, 4H, J=7.6 Hz), 2.96 (t, 2H, J=7.6 Hz), 2.50 (t, 2H, J=7.6 Hz), 2.28 (s, 3H), 1.21 (t, 6H, J=7.6 Hz).

3.2.26. 3-(2,4,6-Triisopropylphenyl)propionic acid 33f. From 2,4,6-triisopropylbromobenzene (0.283 g, 1 mmol), product **33f** was obtained in 78% (0.215 g) yield. White solid mp 106 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (s, 2H), 3.13 (sept, 2H, J=6.8 Hz), 3.01 (t, 2H, J=8.6 Hz), 2.85 (sept, 1H, J=6.8 Hz), 2.49 (t, 2H, J=8.6 Hz), 1.24 (d, 18H, J=6.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 146.9, 146.6, 131.1, 35.8, 34.1, 29.2, 24.5, 24.0, 22.9; $C_{18}H_{28}O_2$: Calcd C 78.21, H 10.21; Found C 78.00, H 10.31. Before hydrolysis **33d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (s, 2H), 4.25 (t, 2H, J=4.6 Hz), 3.84 (t, 2H, J=4.6 Hz), 3.12 (sept, 2H, J=6.8 Hz), 2.97 (t, 2H, J=7.6 Hz), 2.85 (sept, 1H, J=6.8 Hz), 2.50 (t, 2H, J=7.6 Hz), 1.25 (m, 18H).

3.2.27. 3-(Pyridin-3-yl)propionic acid 34f. From 3-bromopyridine (0.158 g, 1 mmol), product **34f** was obtained in 79% (0.119 g) yield. Before hydrolysis **34d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (m, 2H), 7.54 (d, 1H, J=7.7 Hz), 7.24 (dd, 1H, J=7.7 and 5.1 Hz), 4.20 (t, 2H, J=4.6 Hz), 3.79 (t, 2H, J=4.6 Hz), 2.97 (t, 2H, J=7.6 Hz), 2.80 (t, 2H, J=7.6 Hz).

3.2.28. 3-(Pyridin-4-yl)propionic acid 35f. From 4-bromopyridine (0.158 g, 1 mmol), product **35f** was obtained in 84% (0.127 g) yield. Before hydrolysis **35d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.44 (d, 2H, J=5.8 Hz), 7.09 (d, 2H, J=5.8 Hz), 4.17 (t, 2H, J=4.6 Hz), 3.76 (t, 2H, J=4.6 Hz), 2.91 (t, 2H, J=7.6 Hz), 2.64 (t, 2H, J=7.6 Hz).

3.2.29. 3-(Quinolin-3-yl)propionic acid 36f. From 3-bromoquinoline (0.208 g, 1 mmol), product **36f** was obtained in 80% (0.161 g) yield. Before hydrolysis **36d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.73 (s, 1H), 8.02 (d, 1H, J=8.5 Hz), 7.95 (s, 1H), 7.71 (d, 1H, J=8.3 Hz), 7.60 (t, 1H, J=7.5 Hz), 7.48 (t, 1H, J=7.5 Hz), 4.20 (t, 2H, J=4.6 Hz), 3.78 (t, 2H, J=4.6 Hz), 3.05 (t, 2H, J=7.6 Hz), 2.70 (t, 2H, J=7.6 Hz).

3.2.30. 3-(Isoquinolin-4-yl)propionic acid 37f. From 4-bromoisoquinoline (0.208 g, 1 mmol), product **37f** was obtained in 87% (0.175 g) yield. White solid mp 145 °C; ^{1}H NMR (300 MHz, DMSO) δ 9.18 (s, 1H), 8.38 (s, 1H), 8.12 (d, 1H, J=8.5 Hz), 8.09 (d, 1H, J=8.5 Hz), 7.82 (t, 1H, J=7.5 Hz), 7.68 (t, 1H, J=7.5 Hz), 3.27 (t, 2H, J=7.6 Hz), 2.66 (t, 2H, J=7.6 Hz); ^{13}C NMR (75 MHz, DMSO) δ 173.7, 151.5, 142.5, 134.0, 130.9, 130.0, 128.4, 128.1, 127.3, 122.9, 34.5, 24.8. $C_{12}H_{11}NO_2$: Calcd C 71.63, H 6.96; Found C 71.24, H 7.01. Before hydrolysis **37d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.06 (s, 1H), 8.33 (s, 1H), 7.95 (d, 1H, J=8.5 Hz), 7.89 (d, 1H, J=8.5 Hz), 7.66 (t, 1H, J=7.7 Hz), 7.54 (t, 1H, J=7.7 Hz), 4.20 (t, 2H, J=4.6 Hz), 3.78 (t, 2H, J=4.6 Hz), 3.30 (t, 2H, J=7.6 Hz), 2.72 (t, 2H, J=7.6 Hz).

3.2.31. 3-(Thiophen-2-yl)propionic acid 38f. From 2-bromothiophene (0.163 g, 1 mmol), product **38f** was obtained in 75% (0.117 g) yield. Before hydrolysis **38d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10 (d, 1H, J=5.1 Hz), 6.87 (dd, 1H, J=5.1, 3.4 Hz), 6.77 (m, 1H), 4.19 (t, 2H, J=4.6 Hz), 3.76 (t, 2H, J=4.6 Hz), 3.15 (t, 2H, J=7.6 Hz), 2.70 (t, 2H, J=7.6 Hz).

3.2.32. 3-(Thiophen-3-yl)propionic acid 39f. From 3-bromothiophene (0.163 g, 1 mmol), product **39f** was obtained in 70% (0.109 g) yield. Before hydrolysis **39d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.00–6.96 (m, 2H), 6.95 (dd, 1H, J=5.1, 1.1 Hz), 4.20 (t, 2H, J=4.6 Hz), 3.78 (t, 2H, J=4.6 Hz), 2.99 (t, 2H, J=7.6 Hz), 2.68 (t, 2H, J=7.6 Hz).

3.2.33. 3-(Furan-3-yl)propionic acid 40f. From 3-bromofurane (0.147 g, 1 mmol), product **40f** was obtained in 81% (0.113 g) yield. Before hydrolysis **40d** was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (s, 1H), 7.23 (s, 1H), 6.26 (s, 1H), 4.20 (t, 2H, J=4.6 Hz), 3.79 (t, 2H, J=4.6 Hz), 2.76 (t, 2H, J=7.6 Hz), 2.59 (t, 2H, J=7.6 Hz).

3.2.34. 1,4-Phenylenedipropionic acid 41f. From 1,4-dibromobenzene (0.236 g, 1 mmol), acrolein ethyene acetal (0.400 g, 4 mmol) and K_2CO_3 (0.552 g, 4 mmol), Pd–Tedicyp (0.01 mmol) dissolved in DMF (3 mL) under an argon atmosphere. Product **41f** was obtained in 74% (0.164 g) yield. Before hydrolysis the diester was observed: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (s, 4H), 4.11 (t, 4H, J=4.6 Hz), 3.69 (t, 4H, J=4.6 Hz), 2.83 (t, 4H, J=7.6 Hz), 2.58 (t, 4H, J=7.6 Hz).

Registry No.: **8f**, 114-84-1; **9f**, 39105-51-6; **10f**, 71388-83-5; **11f**, 34961-64-3; **12f**, 53473-36-2; **13f**, 42287-94-5; **14f**, 181772-16-7; **15f**, 16642-79-8; **16f**, 459-31-4; **17f**, 1208-64-6; **18f**, 1929-29-9; **19f**, 73718-09-9; **21f**, 56030-19-4; **22f**, 585-50-2; **23f**, 3453-40-5; **24f**, 94022-99-8; **25f**, 1643-26-1; **26f**, 22084-89-5; **27f**, 3243-42-3; **28f**, 6342-77-4; **29f**, 167683-63-8; **30f**, 41034-83-7; **31e**, 131534-70-8; **34f**, 3724-19-4; **35f**, 6318-43-0; **36f**, 67752-28-7; **38f**, 5928-51-8; **39f**, 16378-06-6; **40f**, 90048-04-7; **41f**, 4251-21-2.

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(*R*,*R*)-α,α′-Bis(trifluoromethyl)-9,10-anthracenedimethanol: a chiral solvating agent for enantiomeric resolution of β-dicarbonyl compounds

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Abstract—Commercially available (*R*,*R*)-α,α′-bis(trifluoromethyl)-9,10-anthracenedimetanol is a very efficient solvating agent for the enantiodifferentiation of a series of chiral Michael adducts and related β-dicarbonyl compounds.

1. Introduction

Chiral solvating agents (CSA's) have been extensively used for the determination of enantiomeric composition of chiral substrates by NMR analysis.[1] Some of us have described the preparation and uses of α,α′-bis(trifluoromethyl)-9,10-anthracenedimethanol, **1**, (Fig. 1) as a very efficient and commercially available CSA.[2] Other difunctional compounds have also been tested with satisfactory results.[3] Moreover, (*R*,*R*)-**1** has *C*2 symmetry and presents a clear NMR spectra. Recently we have reported the formation of bidentate complexes of **1** with benzenedimethanols that have been studied by low temperature NMR.[4] The equilibrium of Figure 1 shows that compound **1** exists in two conformations where the two functional groups are distributed in a 'cisoid' or in a 'transoid' position with respect to the anthracene ring. The 'cisoid' conformer ought to be responsible for the important enantiodistinction capacity since it forms bidentate associates with polyfunctional compounds. Main features of these associates are their stability and their geometry. The stability (binding constant) depends principally on the weak bonds (hydrogen bonds, π,π-stacking…) that are very similar for both enantioisomers. The difference between the tridimensional geometry of the complexes of each enantiomer with the same enantiopure CSA, that is, the relative positions of the two components in the multipoint associates determine the differentiation of the chemical shifts of nuclei active in NMR. The difference in the relative positions of the anisotropic aromatic ring and its proximity to heteroatoms are the principal causes of the enantiodifferentiation.

Some of us have extensively worked on the functionalization of β-dicarbonyl compounds through metal promoted alkylations[5] and conjugate additions.[6] When carrying out asymmetric reactions at the intercarbonylic position, we faced the problem of determining the enantiomeric excesses obtained in the reactions. There is no general method described in the literature for the very common family of α-substituted β-dicarbonyl compounds.

In this paper, we describe the results obtained by NMR analysis using CSA (*R*,*R*)-**1** with a series of racemic α-substituted β-dicarbonyl compounds.

2. Results and discussion

We first studied ethyl *N*-*t*-Boc-3-acetyl-4-oxonorvalinate, **2**, which was prepared as previously described from ethyl 2-bromo-*N*-*t*-Boc-glycinate and $Co(acac)_2$ following a well established methodology.[7] The experiments were carried out adding increasing quantities of CSA (*R*,*R*)-**1** at different temperatures to a solution ($CDCl_3$) of the racemic **2** until a maximum of 1.5 equiv. The experiment is presented in Figure 2 where fragments of the ^1H NMR spectra at 280 K are compared for several molar ratios **1**:**2**. Significant upfield shifts were registered for all protons of **2** and high enantiodifferentiation was observed for most of them. The greater enantiodifferentiation was observed for protons H5 (or H7) and H3 in the diketone moiety.

Keywords: Chiral solvating agents; NMR; Dicarbonyl compounds; Enantiomeric resolution.
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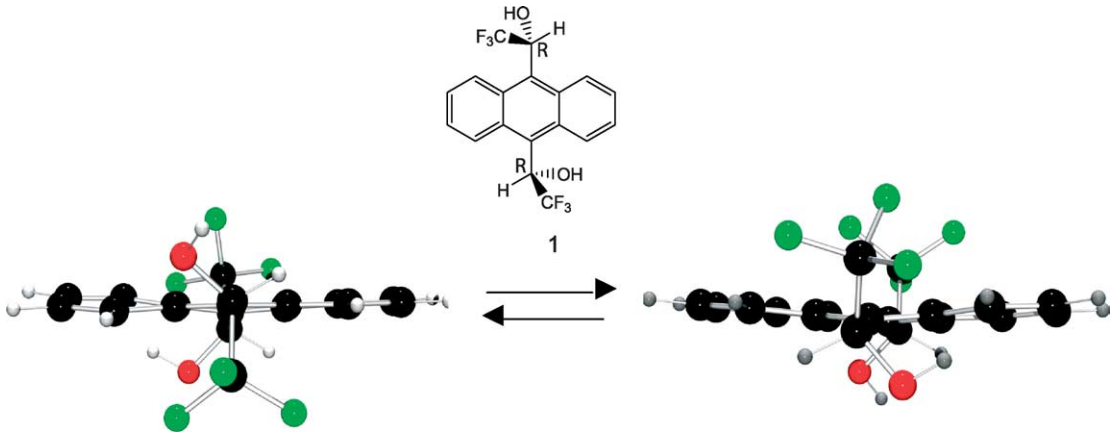


Figure 1. Structure and conformational equilibrium of CSA (*R*,*R*)-**1**.

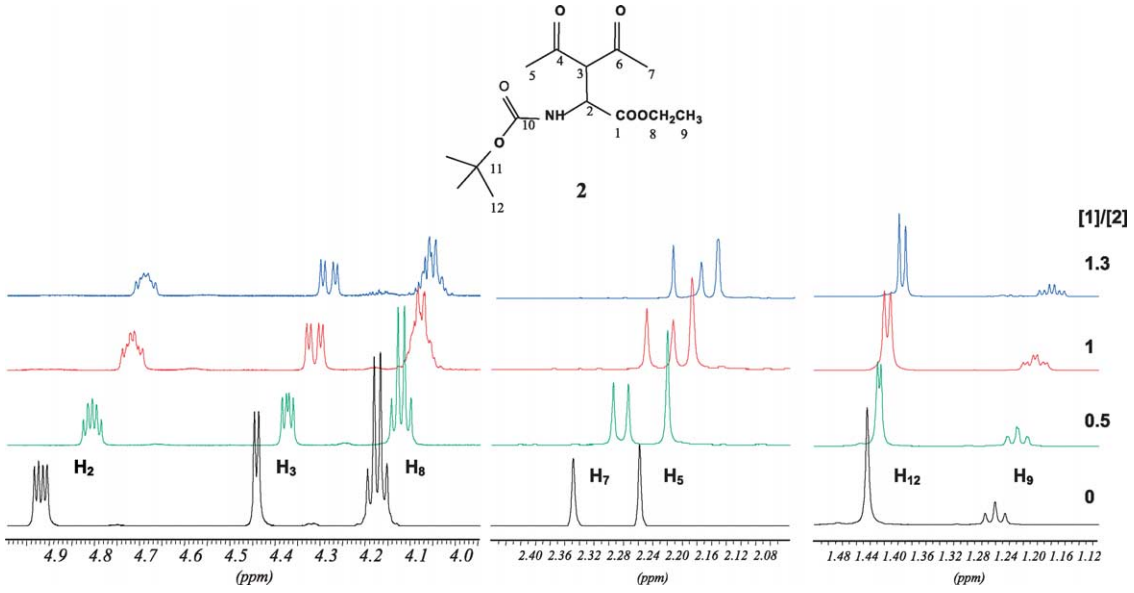


Figure 2. Evolution of the NMR spectra (280 K) of **2** when increasing quantities of (*R*,*R*)-**1** were added.

Differences in chemical shifts for both enantiomers at three temperatures are listed in Table 1. Best results were obtained at low temperatures (280 K), corresponding to a higher value of the binding constant, that is, to an increment of the stability of the complex.

Figure 3 shows a hypothetical geometry of the associated complex of **1** with (*R*)-**2**, where two types of hydrogen bonds have been formed: the first between one hydroxyl group of **1** and both ketone groups of **2**, and the second between the second hydroxyl group of **1** and the carbonyl of the amide of **2**. In this arrangement H3 and one of the acetyl methyl groups are directly under the influence of the anisotropy of anthracene.

Table 1. Differences in chemical shifts of some protons for enantiomers of **2**

[**1**]/[**2**]	*T* (K)	ΔΔδ (ppm)			
		H_5 or H_7	H_3	H_9	H_{12}
0.5	300	0.013	0.010	—	—
1	300	0.024	0.019	0.004	0.006
1.3	300	0.034	0.025	0.007	0.008
0.5	280	0.020	0.015	0.002	0.005
1	280	0.036	0.026	0.006	0.009
1.3	280	0.038	0.028	0.007	0.009
0.5	265	0.028	0.021	—	0.007
1	265	0.052	0.036	0.006	0.011

Other functionalized β-dicarbonilic compounds substituted at Cα were studied (Fig. 4). Compounds **3**–**7** possessing two carbonyl groups four bonds separated, were prepared by Michael addition under catalysis by copper(II) species.[8] In all cases the NMR experiments were carried out adding increasing quantities of CSA (*R*,*R*)-**1** to a solution of the racemic substrate in $CDCl_3$ up to a maximum molar ratio (*R*,*R*)-**1**/**3**-**7** of 1.5. A summary of the results are in Table 2.

Enantiodifferentiation for structurally similar compounds **3**–**5** (Fig. 4) was best observed for one of the diastereotopic H10 protons, next to carbonyl (C11) or cyano group (C11). The presence of a carbonyl or cyano at this position enhances the complexation with (*R*,*R*)-**1**. Thus, this arrangement is optimal for stereodifferentiation at H10.

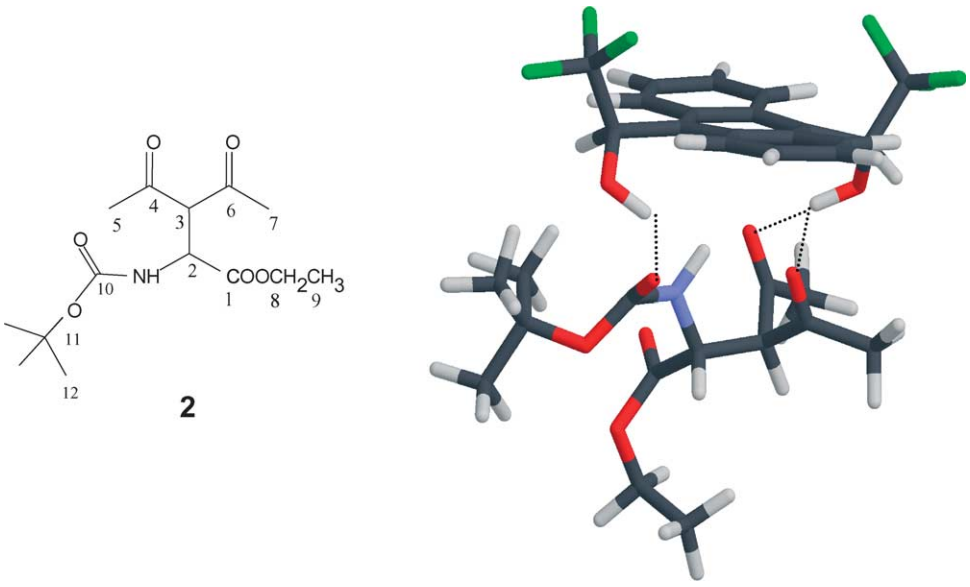


Figure 3. Structure of ethyl *N*-*t*-Boc-3-acetyl-4-oxonorvalinate and hypothetical model for the association of (*R*)-**2** with (*R*,*R*)-**1**.

Compound **6** gave excellent results and significant differences of chemical shifts between both enantiomers are observed until a maximum of $\Delta\Delta\delta = 0.053$ ppm for the intercarbonylic proton H3.

Enantiodifferentiation for compound **7** was best observed for H9 protons (H10 protons for **3**–**5**) and for the methyl group H11, both next to carbonyl (C10). Moreover, compound **7** shows very good stereodifferentiation for the aromatic protons, a possible effect of a π–π-stacking of both aromatic systems (Table 3 and Fig. 5).

Thereby, we have shown that CSA (*R*,*R*)-**1** can be used as chiral discriminator for a series of 1,3-dicarbonyl compounds. It seems that the presence of a third coordinating group (carbonyl or cyano) at 1,5 relative positions increases the enantiodifferentiation. To determine the influence of the third coordinating point we have studied the effect of CSA (*R*,*R*)-**1** on simple 1,3-dicarbonyl compounds **8** and **9** (Fig. 6).

The studies were carried out in the same experimental conditions. The results show that two carbonyl groups are enough to observe enantiodifferentiation (Table 4), although best results have been obtained when an additional carbonyl or cyano group was present. For compounds **8** and **9** the intercarbonylic proton H2 is the one most affected, until a maximum of $\Delta\Delta\delta = 0.0199$ ppm in the case of compound **8**.

In summary, we have found that CSA (*R*,*R*)-**1** is and excellent enantiodifferentiating agent for Michael addition

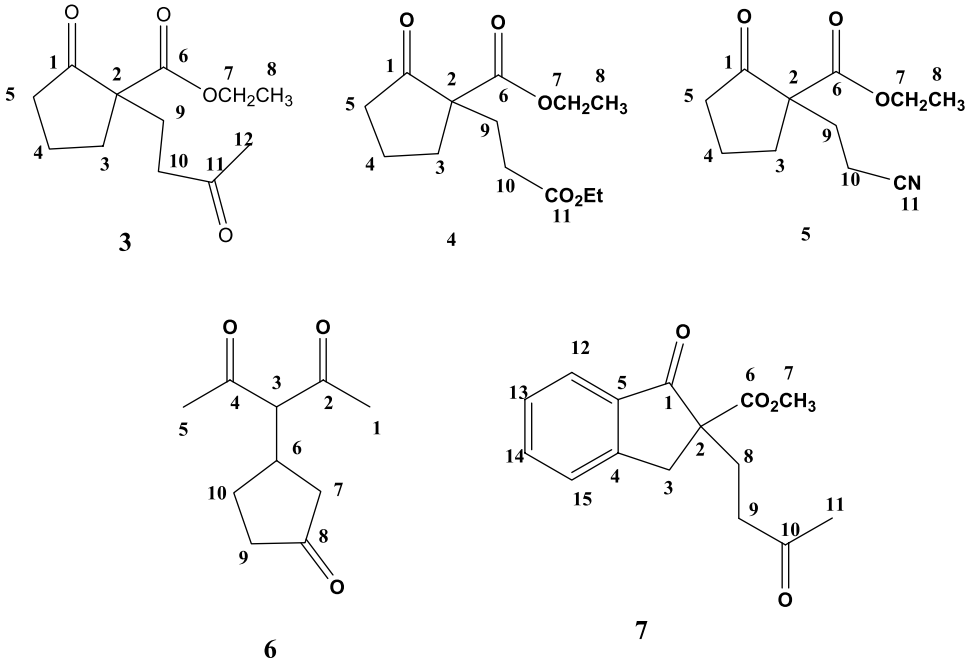


Figure 4. Formulae and arbitrary numbering of the dicarbonyl compounds **3**–**7**.

Table 2. ^{1}H NMR (500 MHz, $CDCl_3$) enantiodifferentiations ($\Delta\Delta\delta$, ppm): difference between the chemical shifts of corresponding protons of the two enantiomers for protons of **3–6** in the presence of CSA (*R*,*R*)-**1**

Compound	$\delta_{R,S}$ (ppm)	[CSA]/[**3–6**]	$\Delta\Delta\delta$ (ppm)		
			$T=265$ K	$T=280$ K	$T=300$ K
3	H8, 1.2179	0.50	0	0	0
		1.00	0.003	0.0012	0.0013
		1.50	—	—	0
	H12, 2.1062	0.50	0	0	0
		1.00	0.0024	0.002	0
		1.50	—	—	0.0038
	H10, 2.6717	0.50	0.0073	0.0067	0.0055
		1.00	0.0114	0.011	0.0099
		1.50	—	—	0.0117
4	H10, 2.2039	0.50	0.0038	0.0038	0.0029
		1.00	0.0109	0.0076	0.0067
		1.50	—	—	0.0074
5	H10, 2.2035	0.50	0.004	0.0034	0.0025
		1.00	0.0059	0.0055	0.0048
		1.50	—	0.0059	0.0054
6	H7, 1.7760	0.50	0.0364	0.0236	0.0158
		1.00	0.0577	0.0422	0.0242
	H1 or H5, 2.1829	0.50	0.0034	0.0017	0
		1.00	0.0059	0	0.0008
	H5 or H1, 2.2231	0.50	0.0028	0.0017	0
		1.00	0.0056	0	0.0028
	H3, 3.6212	0.50	0.0342	0.0221	0.0156
		1.00	0.0526	0.0403	0.0258

products and related β-dicarbonylic compounds. The intercarbonylic proton is particularly sensitive and therefore, useful for separate integration. Moreover, for compounds possessing a quaternary center at C-α where the intercarbonylic proton is missing, other protons can be separately integrated, namely protons in the carbon atoms next to the activating group of the Michael acceptor.

Table 3. ^{1}H NMR (500 MHz, $CDCl_3$) enantiodifferentiations ($\Delta\Delta\delta$, ppm): difference between the chemical shifts of corresponding protons of the two enantiomers for protons of **7** in the presence of CSA (*R*,*R*)-**1**

$\delta_{R,S}$ (ppm)	[CSA]/[**7**]	$\Delta\Delta\delta$ (ppm)		
		$T=265$ K	$T=280$ K	$T=300$ K
H12, 7.7708	0.50	0.0084	0.0062	0
	1.00	0.0147	0.0104	0.0066
	1.50	—	—	0.0085
H14, 7.6337	0.50	0.0059	0.0038	0
	1.00	0.0094	0.006	0.0028
	1.50	—	—	0.0057
H15, 7.4699	0.50	0.0031	0	0
	1.00	0.0088	0.0063	0
	1.50	—	—	0
H13, 7.4108	0.50	0.0080	0.0059	0
	1.00	0.0143	0.0112	0.0070
	1.50	—	—	0.0084
H3, 3.6678	0.50	0.0069	0.0053	0
	1.00	0.0123	0.0091	0.0072
	1.50	—	—	0.0089
H3′, 3.0383	0.50	0	0	0
	1.00	0.0042	0	0
	1.50	—	—	0
H9, 2.6258	0.50	0.0127	0.0118	0.0091
	1.00	0.0198	0.0163	0.0122
	1.50	—	—	0.0161
H9′, 2.5111	0.50	0.0113	0.0084	0.0061
	1.00	0.0197	0.0143	0.0096
	1.50	—	—	0.0118
H11, 2.1241	0.50	0.0117	0.0095	0.0070
	1.00	0.0182	0.0153	0.0117
	1.50	—	—	0.0143

3. Experimental

3.1. General

The nuclear magnetic resonance recognizing experiments were carried out at 11.75 T magnetic field strength instrument, operating at ^{1}H frequency of 500 MHz in solution of $CDCl_3$ (concentration ca. 0.033 mM). The temperature was controlled to ± 0.1 K. Chemical shifts are reported in parts per million relative to internal TMS.

3.1.1. Ethyl *N-t*-Boc-3-acetyl-4-oxonorvalinate 2. Mp 70–71 °C (Lit.[7] mp 70–71 °C) IR (KBr): [cm^{-1}] 1158, 1265, 1290, 1362, 1517, 1679, 1708, 1728, 1745, 3356. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 1.26 (t, $J=7.2$, Hz, 3H, H9), 1.43 (s, 9H, H12), 2.23 (s, 3H, H5 or H7), 2.32 (s, 3H, H7 or H5), 4.17 (q, $J=7.0$ Hz, 2H, H8), 4.43 (d, $J=4.7$ Hz, 1H, H3), 4.92 (dd, $J=4.6$, 9.4 Hz, 1H, H2), 5.54 (d, $J=9.2$ Hz, 1H, N*H*). (Anal. found: C, 55.77; H,7.70; N, 4.66. Calcd for $C_{14}H_{23}NO_6$: C, 55.80; H, 7.69; N, 4.65.

Compounds **3–7** were prepared by copper-catalyzed Michael addition.[8]

3.1.2. 2-Ethoxycarbonyl-2-(3-oxobutyl)cyclopentanone 3.[8] Bp 155 °C/1 mmHg; IR: [cm^{-1}] 1166, 1718, 1747, 2976. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.22 (t, $J=7.1$ Hz, 3H, H8), 1.82–2.02 (m, 4H, (H3, H9, H4 and H4′)), 2.07 (ddd, $J=5.9$, 9.8, 15.4 Hz, 1H, H9′), 2.11 (s, 3H, H12), 2.27 (ddd, $J=7.2$, 7.9, 19.0 Hz, 1H, H5), 2.34–2.47 (m, 3H, (H5′, H3′and H10)), 2.67 (ddd, $J=5.8$, 9.7, 17.9 Hz, 1H, H10′), 4.13 (q, $J=7.2$ Hz, 2H, H7); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 14.5 (C8), 20.0 (C4), 27.4 (C9), 30.3 (C12), 34.8 (C3), 38.4 (C5), 39.3 (C10), 59.4 (C2), 61.8 (C7), 171.8 (C6), 208.2 (C11), 215.3 (C1).

3.1.3. 2-(2-(Ethoxycarbonyl)ethyl)-2-ethoxycarbonylcyclopentanone 4.[8] IR (ATR): [cm^{-1}] 1160, 1180, 1722.

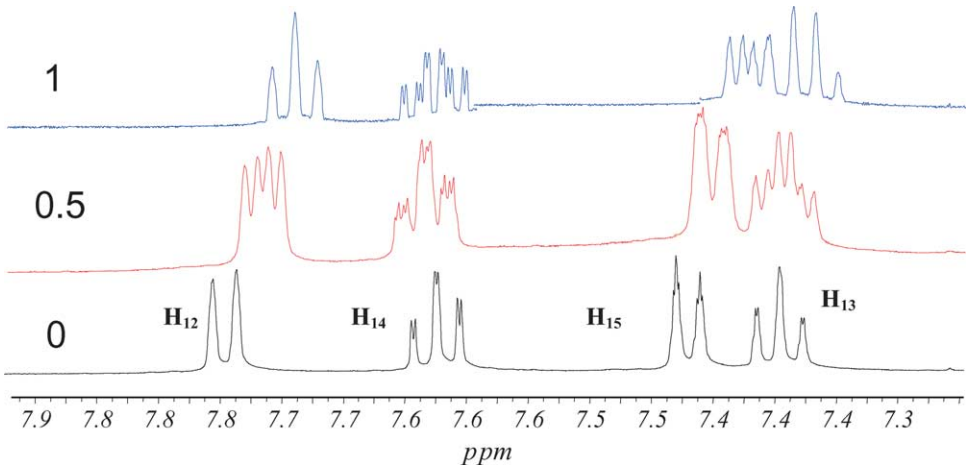
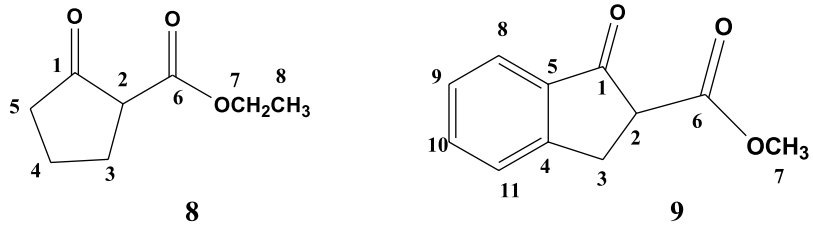


Figure 5. Evolution of the aromatic part of NMR spectra (265 K) of compound **7** when two portions of 0.5 equiv of **1** were added.



8 **9**

Figure 6. Formulae and arbitrary numbering of 1,3-dicarbonyl compounds **8**, **9**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.24 (t, J=7.2 Hz, 6H), 1.85–2.07 (m, 4H), 2.20 (ddd, J=5.4, 10.5, 15.8 Hz, 1H), 2.26–2.34 (m, 2H), 2.42 (ddd, J=6.3, 8.4, 19.1 Hz, 1H), 2.46–2.53 (m, 2H), 4.11 (q, J=7.1 Hz, 2H), 4.16 (q, J=7.0 Hz, 2H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 13.8, 13.9, 19.3, 28.1, 29.6, 33.3, 37.6, 59.0, 60.2, 61.2, 170.7, 172.7, 214.1.

3.1.4. 2-(2-Cyanoethyl)-2-ethoxycarbonylcyclopentanone 5.[8] Bp 165 °C/3 mmHg; IR (ATR): [cm^{-1}] 1717, 1746, 2247. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.26 (t, J=7.2 Hz, 3H), 1.90–2.13 (m, 4H), 2.20 (ddd, J=5.4, 9.8, 14.6 Hz, 1H), 2.32 (ddd, J=8.1, 8.4, 18.6 Hz, 1H), 2.43–2.56 (m, 3H), 2.61 (ddd, J=5.5, 9.9, 16.8 Hz, 1H), 4.19 (q, J=7.2 Hz, 2H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 12.6, 13.6, 19.2, 28.9, 33.3, 37.4, 58.3, 61.4, 119.0, 170.0, 213.4.

3.1.5. 3-(3-Oxocyclopentyl)pentane-2,4-dione 6.[8] IR (ATR): [cm^{-1}] 1159, 1357, 1694, 1720, 1739, 2914, 2963. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.50 (m, 1H, H10), 1.77 (dd, J=10.9, 18.2 Hz, 1H, H7), 2.10–2.35 (m, 3H, (H10′, H9 and H9′)), 2.18 (s, 3H, H1 or H5), 2.22 (s, 3H, H5 or H1), 2.40 (dd, J=7.2, 18.4 Hz, 1H, H7′), 2.94 (m, 1H, H6), 3.62 (d, J=10.5 Hz, 1H, H3); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 27.4 (C10), 29.3 (C1 or C5), 29.6 (C5 or C1), 36.2 (C6), 37.9 (C9), 42.6 (C7), 74.8 (C3), 202.5 (C2 or C4), 202.7 (C4 or C2), 216.4 (C8).

3.1.6. 2-Methoxycarbonyl-2-(3-oxobutyl)indan-1-one 7.[8] IR (KBr): [cm^{-1}] 1713, 1734. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.12 (s, 3H, H11), 2.22–2.26 (m, 2H, H8 and H8′), 2.51 (ddd, J=6.1, 9.5, 17.6 Hz, 1H, H9), 2.62 (ddd, J=6.4, 9.3, 17.6 Hz, 1H, H9′), 3.04 (d, J=17.3 Hz, 1H, H3), 3.67

Table 4. ^{1}H NMR (500 MHz, $CDCl_3$) enantiodifferentiations ($\Delta\Delta\delta$, ppm): difference between the chemical shifts of corresponding protons of the two enantiomers for protons of **8–9** in the presence of CSA (*R*,*R*)-**1**

Compound	$\delta_{R,S}$ (ppm)	[CSA]/[**3–6**]	$\Delta\Delta\delta$ (ppm)		
			T=265 K	T=280 K	T=300 K
8	H2, 3.1433	0.50	0.0111	0.0096	0.0086
		1.00	0.0196	0.0186	0.0172
		1.50	—	0.0199	0.0188
	H8, 1.2833	0.50	0.0034	0.0027	0.0025
		1.00	0.0066	0.0059	0.0049
		1.50	—	0.0062	0.0056
9	H9, 7.4021	0.50	0	0	0
		1.00	0.0064	0.0057	0.0043
		1.50	—	—	0.0060
	H2, 3.7465	0.50	0.0042	0.0042	0.0041
		1.00	0.0077	0.0076	0.0076
		1.50	—	—	0.0081
	H3, 3.5747	0.50	0	0	0
		1.00	0.0073	0.0069	0.0060
		1.50	—	—	0.0066

(d, J=17.5 Hz, 1H, H3′), 3.69 (s, 3H, H7), 7.41 (t, J=7.5 Hz, 1H, H13), 7.47 (d, J=7.7 Hz, 1H, H15), 7.63 (t, J=7.6 Hz, 1H, H14), 7.77 (d, J=7.7 Hz, 1H, H12); ^{13}C NMR ($CDCl_3$) δ 28.9 (C8), 30.3 (C11), 38.2 (C3), 39.2 (C9), 53.1 (C7), 59.5 (C2), 125.2 (C12), 126.8 (C15), 128.4 (C13), 135.4 (C14), 135.9 (C5), 152.9 (C4), 171.9 (C6), 202.6 (C1), 207.7 (C10).

3.1.7. Ethyl 2-oxocyclopentanecarboxylate 8. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.28 (t, J=7.2 Hz, 3H), 1.86 (m, 1H), 2.14 (m, 1H), 2.30 (m, 4H), 3.13 (t, J=9.0 Hz, 1H), 4.20 (t, J=7.2 Hz, 2H).

3.1.8. 2-Methoxycarbonyl-1-indanone 9. Mp 56–60 °C (Lit.[9] mp 51–60) °C IR (ATR): [cm^{-1}] 1731, 1703, 1206, 1157, 766. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.38 (dd, J=8.3, 17.3 Hz, 1H), 3.57 (dd, J=3.8, 17.2 Hz, 1H), 3.75 (dd, J=4.1, 8.3 Hz, 1H), 3.80 (s, 3H), 7.40 (t, J=7.7 Hz, 1H), 7.51 (d, J=7.6 Hz, 1H), 7.63 (t, J=7.6 Hz, 1H), 7.78 (d, J=7.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 30.6, 53.2, 53.5, 125.1, 127.0, 128.2, 135.6, 135.9, 154.0, 170.0, 199.8.

Acknowledgements

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Synthesis of tetrahydrobenzoxazepine acetals with electron-withdrawing groups on the nitrogen atom. Novel scaffolds endowed with anticancer activity against breast cancer cells

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Abstract—Synthetic approaches that have led to (*RS*)-3-methoxy-*N*-substituded-1,2,3,5-tetrahydro-4,1-benzoxazepines with different electron-withdrawing groups, and (*RS*)-2-methoxy-*N*-trifluoroacetyl-2,3,4,5-tetrahydro-1,4-benzoxazepine are described. These novel synthons that were designed to be used as scaffolds for the preparation of new *O*,*N*-acetals as anticancer agents, unexpectedly proved to show antiproliferative activity against the MCF-7 breast cancer cell line. It has been found that substituents on the nitrogen atom have an influence on biological activity. In particular, the presence of a trifluoroacetyl moiety on the nitrogen atom leads to amides displaying interesting in vitro antitumour activities.

1. Introduction

3-Methoxy-2,3-dihydro-5*H*-1,4-benzodioxepins (**1**–**3**) have been used as starting synthons for the preparation of 5-FU derivatives (**4**–**6**, Fig. 1).[1] On the other hand, the reaction between 2-(hydroxymethyl)phenyloxyacetaldehyde dimethyl acetals with 5-FU was subsequently studied.[2] In contrast to 5-FU,[3] the benzannelated 5-FU *O*,*N*-acetals[1] and corresponding open analogues[2] have proved to be non-toxic. Moreover, the bioisosteric benzannelated seven-membered *O*,*N*-acetal **7** (Fig. 1) is particularly useful in stimulating the apoptotic process in breast cancer cells.[1] This was an outstanding biological result because there are few commonly used agents which elicit apoptosis in breast cancer cells, these including paclitaxel (Taxol®), cyclophosphamide, doxorubicin and cytosine arabinoside.[4–6] Nevertheless, their toxicity implies a serious drawback for their therapeutic use and the search of new derivatives possessing even better apoptotic properties and fewer toxic side effects are being diligently sought throughout the scientific community.

Over the years isosteric replacement of oxygen by the nitrogen atom has proved to be one of the most useful tools for medicinal chemistry.[7] The presence of a nitrogen atom provides a new substitution position, the modulation of lipophilicity being possible by the appropriate selection of the nitrogen group. Eleven years ago we described the preparation of acyclic nucleoside analogues,[8] by the tin(IV) chloride-catalysed ring opening of alkoxy-1,4-diheteroepanes with several silylated 5-substituted pyrimidine bases generated in situ. In the case of 7-isopropoxy-1,4-oxazepane **8** (Fig. 1) we noticed that better yields were obtained when the nitrogen atom was substituted by an electron-withdrawing group (such as a tosyl group).

With all this background and as part of an Anticancer Drug Programme we are interested in the preparation of the heterocycles **9a**–**f** and **10** (Fig. 1) that can be useful intermediates for the synthesis of novel bioactive

Keywords: Acetals; Antitumour compounds; Medium-ring heterocycles; Mitsunobu reactions.

Abbreviations: DEAD, diethyl azodicarboxylate; DIAD, diisopropyl azodicarboxylate; DMF, *N*,*N*-dimethylformamide; 5-FU, 5-fluorouracil; MTBD, 7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene; TBAF, tetrabutylammonium fluoride; THF, tetrahydrofuran; TEA, triethylamine; TFAA, trifluoroacetic anhydride.

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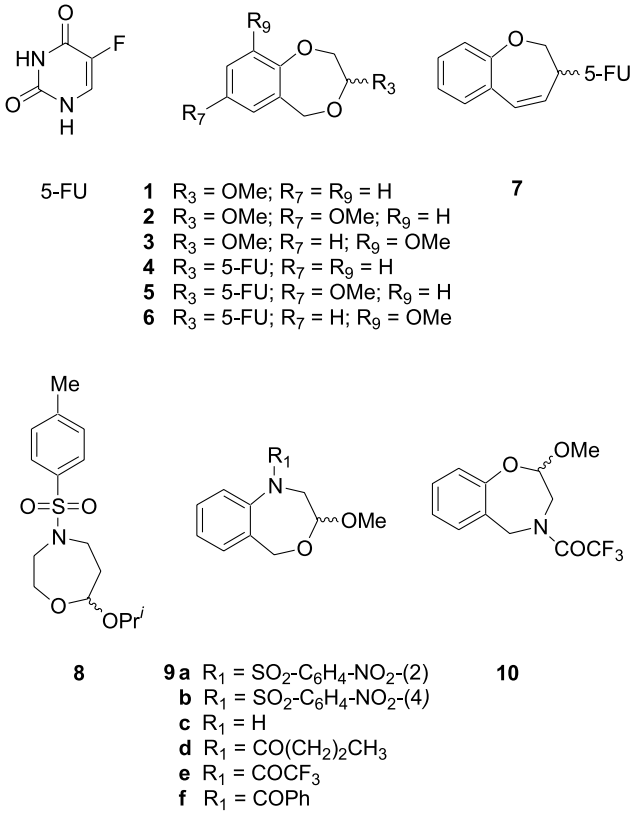


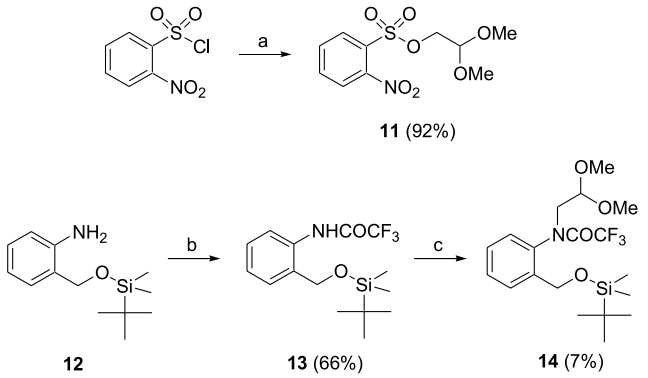
Figure 1.

compounds. In fact, these intermediates were also explored for antitumour activity against the MCF-7 breast cancer cell line. The biological properties of the target molecules **9a–f** and **10** were compared with that of their bioisostere analogue **1** to emphasize the significance of the *N*-substituted fragment in the benzannelated seven-membered acetalic scaffold.

2. Results and discussion

2.1. Synthesis of (*RS*)-3-methoxy-*N*-substituted-1,2,3,5-tetrahydro-4,1-benzoxazepines (9a–f)

All the attempts to alkylate the nitrogen atom of the 2-(hydroxymetyl)aniline with the bromoacetaldehyde dimethyl acetal failed, even when the alcohol group was protected (data not shown). Accordingly, another alkylating strategy was designed (Scheme 1). The formation of 2,2-dimethoxyethyl-2-nitrobenzenesulfonate **11** makes the carbon bearing the sulfonate group adequately electrophilic, which facilitates the attack by a not excessively strong nucleophile. The protection of the hydroxy group of 2-aminobenzyl alcohol with the *tert*-butyldimethylsilanyl group gave **12**,[9] which was trifluoroacetylated by 1-(trifluoroacetyl)benzotriazole[10] to yield **13** (66%). The attack by **11** on the conjugate base of the trifluoroacetanilide moiety could give rise to **14** in the presence of the non-nucleophilic guanidine-like base MTBD. The reaction proceeded neither at rt nor at 40 °C. Compound **14** was obtained when the temperature was raised to 100 °C in a low yield (7%). We think this may be due to an elimination process leading to 1,1-dimethoxyethene and concomitant

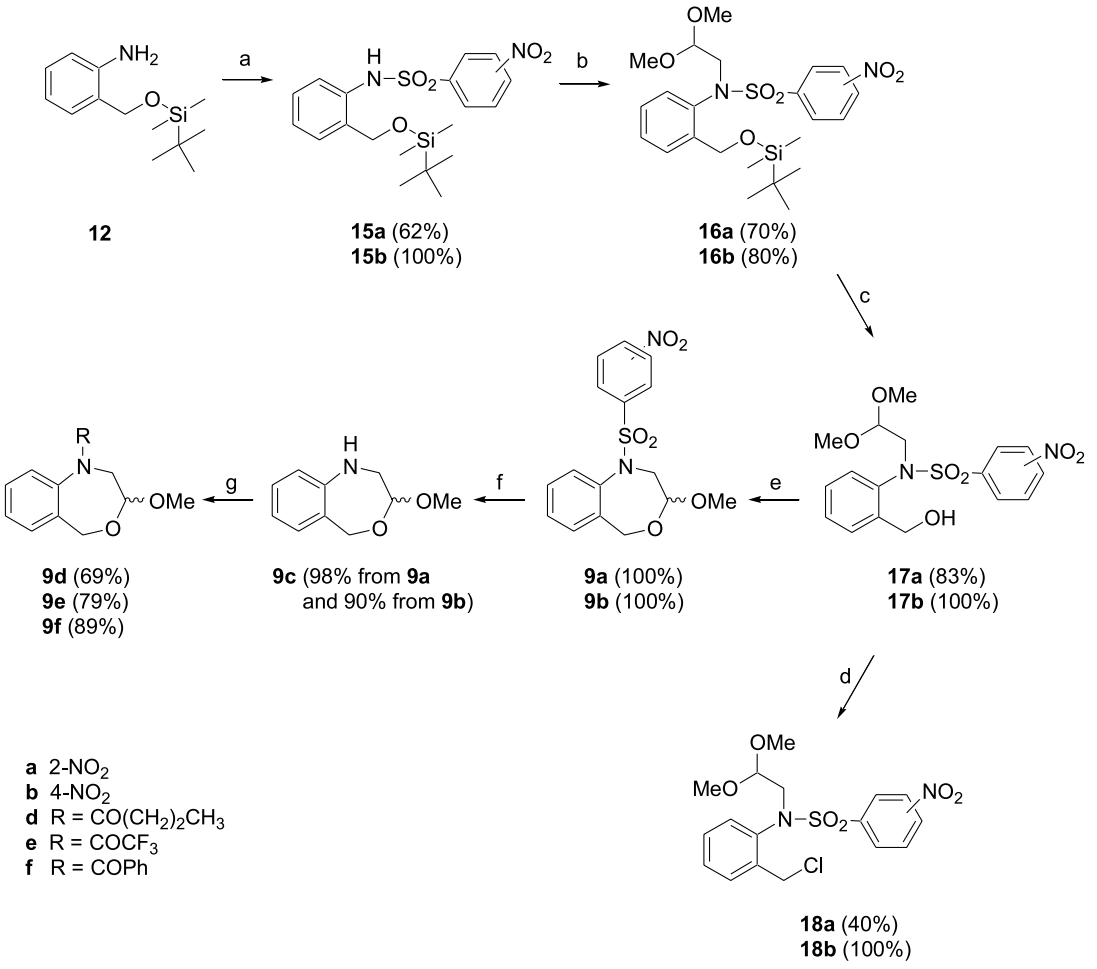


Scheme 1. Reagents and conditions: (a) hydroxyacetaldehyde dimethyl acetal (1 equiv), TEA (1 equiv), anhydrous CH_2Cl_2, 0 °C→rt, 3 h under argon; (b) 1-(trifluoroacetyl)benzotriazole (2.5 equiv), anhydrous THF, rt, 3 h; (c) **11** (1 equiv), MTBD (1 equiv), anhydrous DMF, 100 °C, 24 h under argon.

formation of the sulfonate salt of the base. Due to this low yield, a new synthetic approach needed to be investigated.

The following third strategy was the most rewarding and the synthesis of derivatives **9a–f** was accomplished as outlined in Scheme 2. The Mitsunobu reaction is a versatile method for the conversion of aliphatic alcohols into alkylating agents in situ and under mild conditions.[11] It has been demonstrated that a successful Mitsunobu displacement depends not on the nucleophilicity of the incoming nucleophile but rather on the pK_a associated with the N–H bond.[12] Thus, an 'activating group' (a powerful electron-withdrawing moiety) for the amino fragment is needed, such as the 2- or 4-nitrobenzenesulfonyl function. Attempts of amide alkylation such as the trifluoroacetamide derivative failed. The incapacity of the trifluoroacetamide **13** to participate in the Mitsunobu reaction could be explained by its insufficient acidity, although several alkylation reactions on aromatic trifluoroacetamides have been reported by means of the Mitsunobu reaction.[13–16] The silanyl protecting group probably sterically influenced the outcome of the reaction. Herein we have protected the aniline nitrogen atom as 2- and 4-nitrobenzenesulfonamides. A benzannelated seven-membered secondary amine was obtained (**9c**) after alkylation, deprotection and subsequent cyclization. This synthon could be transformed to a wide range of terciary amides. After protection of the hydroxyl group by the *tert*-butyldimethylsilanyl group (**12**), the synthesis of sulfonamides **15a** and **15b** was accomplished under the conditions of Fukuyama et al.[17] The Mitsunobu conditions were applied to **15a** and hydroxyacetaldehyde dimethyl acetal[18] to give **16a** in a 70% yield. It is worth emphasizing that the yield of **16a** depends greatly on the temperature of the reaction (Scheme 2). When the optimized temperature conditions were applied to **15b**, **16b** was obtained in a 80% yield. After deprotection of the silanyl group of **16a** (and **16b**) with TBAF in THF, **17a** was obtained in a 83% yield (and **17b** in a 100% yield). Compounds **17a** and **17b** quantitatively afforded the cyclic compounds **9a** and **9b**, respectively, using boron trifluoride diethyl etherate as previously reported.[19]

Other conditions were also used for the synthesis of **9a** and **9b**. Such seven-membered acetals were formed after a



Scheme 2. Reagents and conditions: (a) (2)-O_2N-C_6H_4-SO_2Cl (1.1 equiv), TEA (1.5 equiv), CH_2Cl_2, reflux, 24 h, for **15a**; (4)-O_2N-C_6H_4-SO_2Cl (0.5 equiv), CH_2Cl_2, rt, 3 h, for **15b**; (b) $HOCH_2CH(OMe)_2$ (1 equiv), DIAD (1.1 equiv), PPh_3 (1.2 equiv), anhydrous THF, 21 h; the yield of **16a** depends greatly on the reaction temperature: at rt it is 19%, increases up to 70% at 30 °C and goes down to 40% when the temperature is 40 °C; (c) TBAF (1 equiv), THF, rt, 1 h; (d) PhP_3 (1 equiv), CCl_4, 110 °C, 30 min; (e) $BF_3 \cdot OEt_2$ (2 equiv), anhydrous Et_2O, rt, 7 days for **9a**; when these conditions were used to obtain **9b**, the yield was 67%; *p*-H_3C-C_6H_4-SO_3H (0.03 equiv), anhydrous toluene, 110 °C, 2 h under argon for **9a** and **9b**; (f) PhSH (1.1 equiv), K_2CO_3 (3 equiv), DMF, rt, 1 h; (g) $CH_3(CH_2)_2COCl$ (2 equiv), TEA (3 equiv), anhydrous CH_2Cl_2, rt, 17 h under argon, for **9d**; TFAA (2 equiv), TEA (3 equiv), anhydrous CH_2Cl_2, rt, 18 h under argon for **9e**; PhCOCl (2 equiv), TEA (3 equiv), anhydrous CH_2Cl_2, 0–5 °C, 3 h under argon for **9f**.

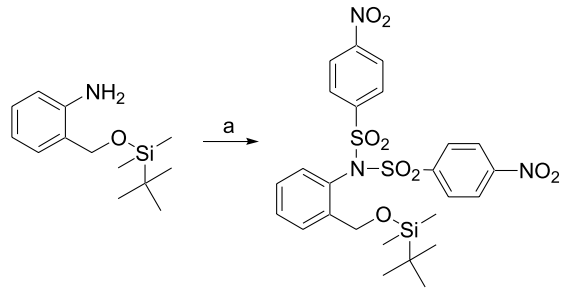
p-toluenesulfonic acid-mediated cyclization from the acyclic acetals **17a** and **17b**, using anhydrous toluene as solvent. We tried to carry out the cyclization process on **17a** (and on **17b**) under the neutral and mild conditions mediated by the triphenylphosphine/carbon tetrachloride system[20] but the process failed and the substitution of the hydroxy group by the chlorine took place to afford **18a** (and **18b**). **17a** and **17b** present bulky groups that limit their conformational motions, making them rigid structures. The oxygen atoms of the acetalic groups cannot act as nucleophiles against the benzylic position (with the triphenylphosphine ether) due to steric hindering.[†]

Finally, the elimination of the sulfonamide group was carried out by treatment with thiophenol[17] and potassium carbonate in DMF to yield the secondary cyclic amine **9c** (98% from **9a**, and 90% from **9b**). Amine **9c** seems to be rather unreactive towards non-activated anhydrides. Acyl chlorides or TFAA were used to conduct the acetylation process that afforded amides **9d** (69%), **9e** (79%), and **9f** (89%). The three amides **9d–f** showed duplicity of all the signals in their ^1H NMR spectra. Such a phenomenon has been studied at length and considered to be due to the existence of a barrier to rotation in amides.[21]

Product **15b** (Scheme 2) was obtained using a two-fold excess of the 2-aminobenzyl silanyl ether **12**. The conditions under which this reaction was carried out were most important for the preparation of **15b**. In fact, the disubstituted derivative **19** was isolated when the reaction is conducted using TEA as a hydrochloride acid scavenger or 1.1 equiv of the sulfonyl chloride was added (Scheme 3).

A plausible explanation implies the previous ionization of the sulfonamide hydrogen atom of **15b** to give a $^-NSO_2$ anion which reacts more rapidly than the starting amine to give **19**. The disulfonimides have been stated[22] to be by-products in reactions of sulfonyl halides with primary amines and ammonia. The 2-nitro group might sterically hinder the $^-NSO_2$ anion and the analogous side product was not isolated.

[†] Hydrogen atoms of the methylene groups of both compounds (**17a** and **17b**) are diastereotopic protons (J_{gem}=12.6–14.1 Hz).

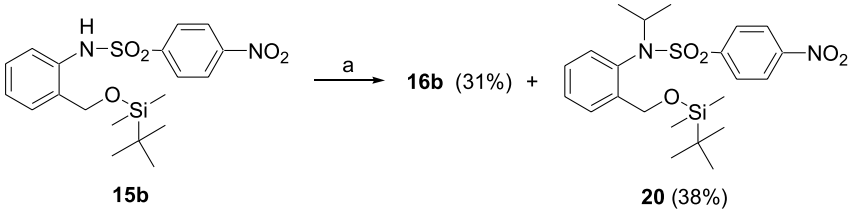


Scheme 3. Reagents and conditions: (a) (4)-O_2N-C_6H_4-SO_2Cl (1.1 equiv), TEA (1.5 equiv), anhydrous CH_2Cl_2, rt, 5 h. When, 0.5 equiv of (4)-O_2N-C_6H_4-SO_2Cl was used, see Scheme 2 (conversion **12**→**15b**).

value is similar to the barrier to rotation around the C_{CO-N} bond in *N*,*N*-dimethylacetamide.[21]

2.3. Structural characteristics of the seven-membered acetals 9a–f and 10

The structures of all derivatives were ascertained by their spectroscopic data (^1H, ^{13}C NMR, MS) and elemental analyses. In compounds **9a–f** ($CDCl_3$ solutions) the acetalic hydrogen atom (H-3) appears between δ 4.67–4.78 ppm as double of doublets (dd). The H-5 atoms are in all cases diastereotopic and compounds **9a–f** show a J_{gem} in the range 13.70–14.30 Hz. Regarding the ^{13}C NMR spectra, the acetalic C-3 atoms of **9a–f** appear at δ 99.03–102.28 ppm. In the case



Scheme 4. Reagents and conditions: (a) $HOCH_2CH(OMe)_2$ (4.3 equiv), DIAD (1.2 equiv), PPh_3 (1.2 equiv), anhydrous THF, rt, 18 h; when 1.2 equiv of $HOCH_2CH(OMe)_2$ were used, see Scheme 2 (conversion **15b**→**16b**).

Alternative conditions to obtain compound **16b** were also investigated (Scheme 4). Thus, addition of an excess of hydroxyacetaldehyde dimethyl acetal (4.3 equiv) afforded the expected acetal **16b** (31%) but the isopropyl alkylated derivative **20** (38%) was also formed. Such a compound could be interpreted by the transesterification reaction of hydroxyacetaldehyde dimethyl acetal with DIAD with the concomitant leaving of isopropanol. A similar process had been previously reported for DEAD but not when DIAD was used.[23]

2.2. Synthesis of (*RS*)-*N*-trifluoroacetyl-2-methoxy-2,3,4,5-tetrahydro-1,4-benzoxazepine (10)

Compound **10** was synthesized as depicted in Scheme 5. Secondary amine **21** was obtained by a reductive alkylation from 2-hydroxybenzaldehyde and aminoacetaldehyde dimethyl acetal. The secondary amine **21** was transformed into the trifluoroacetamide **22** (72%) under the neutral conditions supplied by trifluoroacetylbenzotriazol.[10] Finally, the cyclization process was carried out as previously reported[19] and yielded **10**. The acyclic and cyclic compounds **22** and **10** showed duplicity in their ^1H NMR signals. The hydrogen atoms of the benzyl group had a $\Delta G_c^{\ddagger}$ value of 18.5 kcal/mol using the Eyring equation[24] at a coalescence temperature (T_c) of 80 °C for **22**.[‡] Such a

[‡] The rate constant (k_c) and the free energy of activation ($\Delta G_c^{\ddagger}$) at the coalescence temperature (T_c) were calculated using Gutowsky (1) and Eyring (2) equations, respectively. $\Delta\nu$ is the limiting frequency separation. For Eqs. 1 and 2, see Ref. 24.

$$k_c = \frac{\pi(\Delta\nu)}{\sqrt{2}} \qquad \text{Gutowsky equation (1)}$$

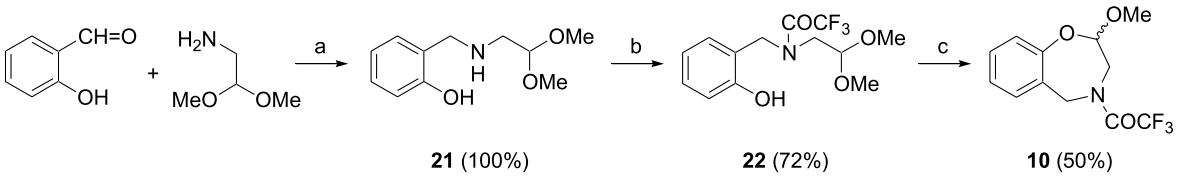
$$\Delta G_c^{\ddagger} = 191.2T_c(10.32 + \log T_c - \log k_c) \qquad \text{Eyring equation (2)}$$

of **10** ($CDCl_3$ solutions) the acetalic proton appears at δ 4.73 ppm as a dd (J=7.8, 2.3 Hz) for one isomer (which represents the 63% of the mixture) and δ 4.79 ppm as a dd (J=7.1, 2.2 Hz) for the other one (37%). Moreover, exactly as it occurred to **9a–f**, the benzilic protons are diastereotopic and resonate at δ 4.91 and 4.35 ppm as doublets (J=14.4 Hz) for the major isomer and at δ 4.73 and 4.57 ppm as doublets (J=15.5 Hz) for the minor one.

2.4. Antiproliferative activity against the MCF-7 human breast cancer cell line for compounds 9a–f,10 and 1

Breast cancer is the second most frequent cancer in the world (1.05 million cases), and is by far the most common malignant disease in women (22% of all new cancer cases). The ratio of mortality to incidence is about 36% worldwide, and breast cancer ranks fifth as a cause of death from cancer overall (although it is the leading cause of cancer mortality in women - the 370 000 annual deaths represent 13.9% of cancer death in women).[25] The MCF-7 human breast cancer cell line had been used as an excellent experimental model to improve the efficacy of different therapies before its use in patients.[26,27] Compounds **9a–f**,**10** and **1** were assayed for their in vitro antiproliferative activity against the MCF-7 cell line and the results are summarized in Table 1. The two most potent compounds are **9e** (IC_{50}=27.39±0.71 μM) and **10** (IC_{50}=27.85±0.64 μM), which bear a trifluoroacetyl group on their nitrogen atom. On the other hand, it is worth pointing out that all the nitrogen-containing acetals (**9a–f**,**10**) are better anticancer agents than the oxygen-containing acetal **1**. This seems to emphasize the importance of the presence of the nitrogen atom on the benzannelated seven-membered rings (biophore).[§]

[§] A biophore may consist of a single feature or a family of chemically similar features.



Scheme 5. Reagents and conditions: (a) (i) EtOH, 78 °C, 5 h, (ii) $NaBH_4$ (2.5 equiv), anhydrous MeOH, 65 °C, 75 min; (b) 1-(trifluoroacetyl)benzotriazole (2.5 equiv), THF, rt, 3 h; (c) $BF_3 \cdot OEt_2$ (2 equiv), anhydrous Et_2O, rt, 5 days.

Table 1. Antiproliferative activities for the compounds against the MCF-7 breast cancer cell line

	9a	9b	9c	9d	9e	9f	10	1
IC_{50} (μM)[a]	51.78±0.21	44.80±0.37	81.05±2.86	39.84±5.35	27.39±0.71	36.70±0.78	27.85±0.64	151.28±16.3

[a] See Ref. 29. The data are means±SEM of three independent determinations.

3. Conclusion

We have developed a new synthetic strategy for the preparation of previously unreported seven-membered acetals such as *N*-substituted-3-methoxy-1,2,3,5-tetrahydro-4,1-benzoxazepines and 2-methoxy-*N*-trifluoroacetyl-2,3,4,5-tetrahydro-1,4-benzoxazepine. These new bicyclic cores may be promising intermediates in the synthesis of new anticancer agents. **9c** is appropriate for the synthesis of an array of target structures for structure–activity relationship studies. An additional point of diversity can now be rapidly introduced on the nitrogen atom of the benzannelated nitrogen-containing acetals utilizing readily available building blocks.

4. Experimental

4.1. Chemistry

Melting points (mp) were taken in open capillaries on an Electrothermal melting point apparatus and are uncorrected. All moisture-sensitive reactions were performed in flame-dried glassware equipped with rubber septa under a positive pressure of dry argon. Organic extracts were dried over $MgSO_4$ and Na_2SO_4. Thin layer chromatography (TLC) was performed on Merck Kieselgel 60 F_{254}, the spots being developed at the UV light. The FLASH 40 chromatography module and the prepacked cartridge systems were supplied by Biotage UK Limited, 15 Harforde Court. Foxholes Business Park, John Tate Rd. Hertford, England SG13 7NM. ^1H and ^{13}C NMR spectra were recorded on a Bruker at 300.13 and 400.13 MHz, and at 75.78 and 100.03 MHz, respectively in $CDCl_3$ and in DMSO-d_6 solutions. Chemical shifts were measured in δ and referenced to $CDCl_3$ (7.25 ppm for ^1H NMR and 77.20 ppm for ^{13}C NMR) and to DMSO-d_6 (2.50 ppm for ^1H NMR and 39.50 ppm for ^{13}C NMR). The mass spectra (MS) were obtained using a Micromass Platform II spectrometer at 70 eV, carrying out injection through a Carlo Erba GC 8000 chromatograph in a splitless mode for capillary columns. The accurate mass determination was carried out in an AutoSpec-Q mass spectrometer arranged in an EBE geometry (Micromass Instruments, Manchester, UK) and equipped with a liquid secondary ion mass spectra (LSIMS) source. The instrument was operated at 8 kV of accelerating voltage and Cs^+ cations were used as primary ions. Solvents were obtained dry as follows: THF was distilled from benzophenone ketyl, CH_2Cl_2 was refluxed over, and distilled from P_2O_5 and then stored over molecular sieves (3 Å), CH_3OH from Mg. Anhydrous DMF was purchased from Sigma-Aldrich Quimica S. A.

4.1.1. 2,2-Dimethoxyethyl-2-nitrobenzenesulfonate (11). A solution of hydroxyacetaldehyde dimethyl acetal (1 equiv) in dried CH_2Cl_2 (3 mL/mmol of hydroxyacetaldehyde dimethyl acetal) was prepared under argon. After cooling to 0 °C, 2-nitrobenzenesulfonile chloride (1 equiv) was added as a solid in one portion, followed by TEA (1 equiv). The reaction mixture was then allowed to warm at rt and stirred under argon for 3 h after which CH_2Cl_2 was added and the solution was washed with water. The final organic layer was dried (Na_2SO_4) and the solvent evaporated under vacuum. Purification by flash chromatography (elution with CH_2Cl_2) afforded **11** as a yellow liquid (92% yield). ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.12 (dd, J= 7.9 Hz, 1H), 7.84–7.70 (m, 3H), 4.60 [t, J_{CH-CH2}=5.3 Hz, 1H, C*H*$(OCH_3)_2$], 4.22 (d, 2H, CH_2), 3.36 [s, 6H, $(OCH_3)_2$]. ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 134.93, 132.38, 131.43, 124.93 (C_3, C_4, C_5, C_6), 129.72 (C_1), 101.09 [*C*H$(OCH_3)_2$], 69.62 (*C*H_2), 54.73 [$(OCH_3)_2$]. LSIMS *m/z* (relative intensity) 316 (7), 315 (14), 314 $[M+Na)^+$, 100], 307 (8), 289 (7), 279 (5), 260 (25), 237 (46), 226 (7), 215 (4), 214 (10). HR LSIMS *m/z* calcd for $C_{10}H_{13}NO_7SNa$ $(M+Na)^+$: 314.0310, found: 314.0309.

4.1.2. *N*-[2-(*tert*-Butyldimethylsilanyloxymethyl)phenyl]-2,2,2-trifluoroacetamide (13). A solution of **12**[9] (1 equiv) and 1-(trifluoroacetyl)benzotriazole[10] (2.5 equiv) in THF (3 mL/mmol of **12**) was prepared and allowed to stir at rt for 3 h. The solvent was then removed under vacuum and the residue purified by flash chromatography (elution mixture EtOAc/hexane 1/100 in flash 40 chromatography). **13** was obtained as a yellow liquid in 66% yield. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 10.15 (bs, 1H, N*H*), 8.22, (d, J=8.12 Hz, 1H), 7.36 (m, 1H), 7.17–7.11 (m, 2H), 4.80 (s, 2H, CH_2), 0.89 [s, 9H, $(CH_3)_3C$], 0.10 (s, 6H, $(CH_3)_2Si$]. ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 135.77, 129.43 (C_1, C_2), 128.98, 127.92, 125.50, 121.76 (C_5, C_6, C_7, C_8), 115.69 (q, J_{C-F}=290.0 Hz, *C*F_3), 65.61 (*C*H_2), 25.66 [$(CH_3)_3C$], 18.26 [$(CH_3)_3C$], −5.44 [$(CH_3)_2Si$]. LSIMS *m/z* (relative intensity) 358 (8), 357 (18), 356 $[M+Na)^+$, 100], 355 (7). HR LSIMS *m/z* calcd for $C_{15}H_{22}NO_2F_3SiNa$ $(M+Na)^+$: 356.1270, found: 356.1268.

4.1.3. *N*-[2-(*tert*-Butyldimethylsilanyloxymethyl)phenyl]-*N*-(2,2-dimethoxyethyl)-2,2,2-trifluoroacetamide (14). The base MTBD (1 equiv) was added dropwise, at rt, to a solution of **13** (1 equiv) in dried DMF (3 mL/mmol of **13**) prepared under argon. The mixture was stirred at rt for 1 h, after which a solution of sulfonate **11** (1 equiv) in dried DMF (3 mL/mmol of **11**) was added. The temperature was then raised to 100 °C. After 24 h stirring under argon, water and CH_2Cl_2 were added to the cooled reaction mixture and the organic layer was subjected to several washes with water. The final organic layer was dried (Na_2SO_4) and the solvent removed under vacuum. The residue was chromatographed on silica flash by gradient elution mixtures (EtOAc/hexane 1/150→1/100) to afford **14** as a yellow liquid in 7% yield. ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.18 (t, 1H), 7.01 (d, 1H), 6.65 (m, 2H), 4.66 (s, 2H, OCH_2), 4.62 [t, 1H, $CH(OCH_3)_2$], 3.41 [s, 6H, $(OCH_3)_2$], 3.28 (d, 2H, NCH_2), 0.89 [s, 9H, $(CH_3)_3C$], 0.06 [s, 6H, $(CH_3)_2Si$].

4.1.4. *N*-[2-(*tert*-Butyldimethylsilanyloxymethyl)phenyl]-2-nitrobenzenesulfonamide (15a). To a solution of **12**[9] in CH_2Cl_2 (3 mL/mmol of **12**) at rt, was added solid 2-nitrobenzenesulfonyl chloride (1.1 equiv) followed by TEA (1.5 equiv). After stirring for 24 h at 30 °C, the mixture was diluted with CH_2Cl_2 and the resulting organic layer was washed with water, dried on Na_2SO_4 and the solvent evaporated under vacuum. **15a** was purified by flash chromatography (gradient elution EtOAc/hexane 1/100→1/8) and obtained as a yellow, low melting point solid (mp 83.0–83.9 °C) in 62% yield. ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.70 (bs, 1H, N*H*), 7.90 (dd, J=7.8, 1.5 Hz, $1H_{sulfonamide}$), 7.82 (dd, J=7.9, 1.4 Hz, $1H_{sulfonamide}$), 7.68 (ddd, $J_1=J_2$=7.7 Hz, J_3=1.5 Hz, $1H_{sulfonamide}$), 7.61–7.54 (m, 2H), 7.28–7.23 (m, 1H), 7.12–7.08 (m, 2H), 4.58 (s, 2H, CH_2), 0.91 [s, 9H, $(CH_3)_3C$], 0.07 (s, 6H, $(CH_3)_2Si$]. ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 148.04, 135.58 ($C_{2\ sulfonamide}$, C_{1}), 133.81, 132.45 ($C_{4\ sulfonamide}$, $C_{5\ sulfonamide}$), 133.63, 132.61 ($C_{1\ sulfonamide}$, C_2), 131.26, 128.71, 128.28 125.54, 125.14 ($C_{3\ sulfonamide}$, $C_{6\ sulfonamide}$, C_3, C_4, C_5, C_6), 64.32 (CH_2), 25.87 [$(CH_3)_3C$], 18.36 [$(CH_3)_3C$], −5.30 [$(CH_3)_2Si$]. LSIMS *m/z* (relative intensity) 448 (2), 447 (13), 446 (27), 445 [$(M+Na)^+$, 100], 423 [$(M+H)^+$, 14], 421 (6), 367 (6), 366 (12), 365 (50), 329 (17), 313 (11), 292 (11), 291 (70). HR LSIMS *m/z* calcd for $C_{19}H_{26}N_2O_5SSiNa$ $(M+Na)^+$: 445.1229, found: 445.1230.

4.1.5. *N*-[2-(*tert*-Butyldimethylsilanyloxymethyl)phenyl]-4-nitrobenzenesulfonamide (15b). Small portions 4-nitrobenzenesulfonyl chloride (0.5 equiv) were added to a solution of **12**[9] (1 equiv) in dried CH_2Cl_2 (3 mL/mmol of **12**). After 3 h stirring at rt the reaction mixture was washed 3 times with water. The final organic layer was dried on Na_2SO_4 and evaporated under vacuum. Purification by flash chromatography (gradient elution mixtures EtOAc/hexane 1/50→1/20) afforded **15b** as a white solid (mp 103.6–103.8 °C) in quantitative yield. ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.69 (s, 1H, N*H*), 8.27 (d, $J_{6-5\ sulfonamide}=J_{3-2\ sulfonamide}$= 8.8 Hz, 2H, $H_{3,5\ sulfonamide}$), 7.95 (d, 2H, $H_{2,6\ sulfonamide}$), 7.56 (dd, J=8.1, 0.9 Hz, 1H), 7.29 (ddd, $J_1=J_2$=7.7 Hz, J_3= 1.6 Hz, 1H), 7.08 (ddd, $J_1=J_2$=7.5 Hz, J_3=1.2 Hz, 1H), 7.00 (dd, J=7.6, 1.6 Hz, 1H), 4.36 (s, 2H, CH_2), 0.92 [s, 9H, $(CH_3)_3C$], 0.08 [s, 6H, $(CH_3)_2Si$]. ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 150.23 ($C_{4\ sulfonamide}$), 146.14, 136.18 ($C_{1\ sulfonamide}$, C_1), 130.54 (C_2), 129.18, 128.24, 125.36, 122.22 (C_3, C_4, C_5, C_6), 128.24 ($C_{2\ sulfonamide}$, $C_{6\ sulfonamide}$), 124.27 ($C_{3\ sulfonamide}$, $C_{5\ sulfonamide}$), 65.25 (CH_2), 25.80 [$(CH_3)_3C$], 18.19 [$(CH_3)_3C$], −5.38 [$(CH_3)_2Si$]. LSIMS *m/z* (relative intensity) 448 (12), 447 (17), 446 (22), 445 [$(M+Na)^+$, 100], HR LSIMS *m/z* calcd for $C_{19}H_{26}N_2O_5SSiNa$ $(M+Na)^+$: 445.1229, found: 445.1229.

4.1.6. *N*-[2-(*tert*-Butyldimethylsilanyloxymethyl)phenyl]-*N*-(2,2-dimethoxyethyl)-2-nitrobenzenesulfonamide (16a). A solution of **15a** (1 equiv), hydroxyacetaldehyde dimethyl acetal (1 equiv) and triphenylphosphine (1.2 equiv) in dried THF (5 mL/mmol of **15a**) was prepared under argon atmosphere. To this solution, DIAD (1.1 equiv) was added dropwise at −20 °C and temperature was then allowed to rise to 5 °C before heating to 30 °C. Stirring under argon atmosphere was maintained for 21 h after which the solvent was removed in vacuum and the residue purified by flash chromatography (gradient elution mixtures EtOAc/hexane 1/20→1/8) to afford **16a** (70% yield) as a pale yellow, low melting point solid (mp 83.5–84.5 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.68–7.57 (m, 3H), 7.48–7.34 (m, 3H), 7.16 (ddd, $J_1=J_2$=7.7 Hz, J_3=1.7 Hz, 1H), 6.98 (dd, J=8.0 Hz, J=1.3 Hz, 1H), 4.85 (d, $J_{gem\ OCH2}$= 14.3 Hz, 1H, OCH_2), 4.56 (d, 1H, OCH_2), 4.42 [dd, J_{CH-CH2}=5.8 Hz, J_{CH-CH2}=5.3 Hz, 1H, $CH(OCH_3)_2$], 3.92 (dd, $J_{gem\ NCH2}$=14.6 Hz, J_{CH-CH2}=5.8 Hz, 1H, NCH_2), 3.71 (dd, J_{CH-CH2}=5.3 Hz, 1H, NCH_2), 3.30 (s, 3H, OCH_3), 3.23 (s, 3H, OCH_3), 0.91 [s, 9H, $(CH_3)_3C$], 0.06 [s, 3H, $(CH_3)_2Si$], 0.05 [s, 3H, $(CH_3)_2Si$]. ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 148.16 ($C_{2\ sulfonamide}$), 142.56, 135.11 (C_1, $C_{1\ sulfonamide}$), 133.80 ($C_{5\ sulfonamide}$), 132.19 ($C_{4\ sulfonamide}$), 131.74 (C_2), 131.08, 129.86, 129.26, 128.21, 127.44, 123.73 ($C_{3\ sulfonamide}$, $C_{6\ sulfonamide}$, C_3, C_4, C_5, C_6), 101.58 [$CH(OCH_3)_2$], 60.69 (OCH_2), 53.58, 52.72 [$(OCH_3)_2$], 53.13 (NCH_2), 25.99 [$(CH_3)_3C$], 18.41 [$(CH_3)_3C$], −5.32 [$(CH_3)_2Si$]. LSIMS *m/z* (relative intensity) 536 (2), 535 (14), 534 (31), 533 [$(M+Na)^+$, 100], 481 (3), 480 (7), 479 (22), 455 (2), 454 (6), 453 (18), 349 (1), 348 (3), 347 (8), 295 (4), 294 (12), 293 (50), 269 (1), 268 (2), 267 (8). HR LSIMS *m/z* calcd for $C_{23}H_{34}N_2O_7SSiNa$ $(M+Na)^+$: 533.1754, found: 533.1758.

4.1.7. *N*-[2-(*tert*-Butyldimethylsilanyloxymethyl)phenyl]-*N*-(2,2-dimethoxyethyl)-4-nitrobenzenesulfonamide (16b). Compound **16b** was obtained from **15b** following the procedure described for **15a**, as a white, low melting point solid (mp 92.0–93.0 °C) eluted with gradient elution mixtures EtOAc/hexane (1/50→1/20) by flash chromatography (80% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.32 (d, $J_{6-5\ sulfonamide}=J_{3-2\ sulfonamide}$=8.8 Hz, 2H, $H_{3,5\ sulfonamide}$), 7.83 (d, 2H, $H_{2,6\ sulfonamide}$), 7.69 (d, J= 7.7 Hz, 1H), 7.38 (t, J=7.5 Hz, 1H), 7.10 (t, J=7.4 Hz, 1H), 6.42 (d, J=7.5 Hz, 1H), 4.99 (d, $J_{gem\ OCH2}$=14.4 Hz, 1H, OCH_2), 4.93 (d, 1H, OCH_2), 4.36 [dd, J_{CH-CH2}=5.5, 5.7 Hz, 1H, $CH(OCH_3)_2$], 3.84 (dd, $J_{gem\ NCH2}$=14.0 Hz, J_{CH-CH2}=5.5 Hz, 1H, NCH_2), 3.41 (dd, J_{CH-CH2}=5.7 Hz, 1H, NCH_2), 3.32 (s, 3H, OCH_3), 3.15 (s, 3H, OCH_3), 0.96 [s, 9H, $(CH_3)_3C$], 0.14 [s, 3H, $(CH_3)_2Si$], 0.13 [s, 3H, $(CH_3)_2Si$]. ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 150.26 ($C_{4\ sulfonamide}$), 143.97, 143.36 ($C_{1\ sulfonamide}$, C_1), 135.70 (C_2), 129.48 ($C_{2\ sulfonamide}$, $C_{6\ sulfonamide}$), 129.29, 128.48, 127.30, 127.10 (C_3, C_4, C_5, C_6), 124.03 ($C_{3\ sulfonamide}$,

$C_{5\ sulfonamide}$), 101.26 [*C*H(OCH$_3$)$_2$], 61.05 (O*C*H$_2$), 53.77, 52.50 [(O*C*H$_3$)$_2$], 52.79 (N*C*H$_2$), 26.04 [(*C*H$_3$)$_3$C], 18.46 [(CH$_3$)$_3$*C*], −5.25 [(*C*H$_3$)$_2$Si]. LSIMS *m/z* (relative intensity) 536 (3), 535 (15), 534 (34), 533 [(M+Na)$^+$, 100], 482 (1), 481 (3), 480 (5), 479 (15), 455 (4), 454 (9), 453 (33), 349 (2), 348 (3), 347 (7), 331 (3), 330 (9), 329 (30), 311 (40), 295 (4), 294 (13), 293 (52), 284 (21), 262 (14), 254 (6). HR LSIMS *m/z* calcd for $C_{23}H_{34}N_2O_7SSiNa$ (M+Na)$^+$: 533.1754, found: 533.1753.

4.1.8. *N*-(2,2-Dimethoxyethyl)-*N*-(2-hydroxymethylphenyl)-2-nitrobenzenesulfonamide (17a). To a stirred solution of **16a** (1 equiv) in THF (6 mL/mmol of **16a**) monohydrated TBAF (1 equiv) was added at rt. The reaction mixture was stirred for 1 h (until no starting material could be visualised on TLC). The solvent was then removed in vacuum, the residue solved in CH_2Cl_2 and washed with water. The organic layer, dried on Na_2SO_4, was evaporated and purified by flash chromatography (gradient elution mixtures EtOAc/hexane 1/10→1/1). **17a** was obtained as a yellow oil (83% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.71–7.59 (m, 3H), 7.47 (ddd, $J_1=J_2=7.6$ Hz, $J_3=$ 1.4 Hz, 1H), 7.41–7.35 (m, 2H), 7.14 (ddd, $J_1=J_2=7.7$ Hz, $J_3=1.6$ Hz, 1H), 6.74 (dd, $J=8.0$, 1.1 Hz, 1H), 4.89 (d, $J_{gem\ OCH2}=12.4$ Hz, 1H, OC*H*$_2$), 4.65 (d, 1H, OC*H*$_2$), 4.45 [dd, $J_{CH-CH2}=5.9$, 5.3 Hz, 1H, C*H*(OCH$_3$)$_2$], 4.29 (dd, $J_{gem\ NCH2}=14.5$ Hz, $J_{CH-CH2}=5.9$ Hz, 1H, NC*H*$_2$), 3.46 (dd, $J_{CH-CH2}=5.3$ Hz, 1H, NC*H*$_2$), 3.37 (s, 3H, OC*H*$_3$), 3.14 (s, 3H, OC*H*$_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 148.46 ($C_{2\ sulfonamide}$), 142.95, 136.78 (C_1, $C_{1\ sulfonamide}$), 134.11 ($C_{5\ sulfonamide}$), 132.52 ($C_{4\ sulfonamide}$), 130.86 (C_2), 131.94, 131.03, 129.81, 128.77, 128.38, 123.86 ($C_{3\ sulfonamide}$, $C_{6\ sulfonamide}$, C_3, C_4, C_5, C_6), 101.20 [*C*H(OCH$_3$)$_2$], 61.02 (O*C*H$_2$), 53.23, 52.22 [(O*C*H$_3$)$_2$], 53.13 (N*C*H$_2$). LSIMS *m/z* (relative intensity) 420 (20), 419 [(M+Na)$^+$, 100], 413 (11), 369 (6), 334 (8), 333 (37), 326 (11). HR LSIMS *m/z* calcd for $C_{17}H_{20}N_2O_7SNa$ (M+Na)$^+$: 419.0889, found: 419.0889.

4.1.9. *N*-(2,2-Dimethoxyethyl)-*N*-(2-hydroxymethylphenyl)-4-nitrobenzenesulfonamide (17b). Compound **17b** was prepared from **16b** following the procedure described for **17a** and obtained as a yellow solid (mp 139.5–140.7 °C) eluted by flash chromatography (gradient elution mixtures EtOAc/hexane 1/10→1/2) (90% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.34 (d, $J_{3-2\ sulfonamide}=J_{6-5\ sulfonamide}=8.9$ Hz, 2H, $H_{3,5\ sulfonamide}$), 7.81 (d, 2H, $H_{2,6\ sulfonamide}$), 7.64 (dd, $J=7.7$, 1.6 Hz, 1H), 7.39 (ddd, $J_1=J_2=7.6$ Hz, $J_3=$ 1.1 Hz, 1H), 7.16 (ddd, $J_1=J_2=7.7$ Hz, $J_3=1.6$ Hz, 1H), 6.36 (dd, $J=8.0$, 0.9 Hz, 1H), 4.94 (dd, $J_{gem\ OCH2}=$ 12.4 Hz, $J_{CH2\text{-}OH}=6.7$ Hz, 1H, OC*H*$_2$), 4.69 (dd, $J_{CH2\text{-}OH}=7.2$ Hz, 1H, OC*H*$_2$), 4.43 [dd, $J_{CH-CH2}=5.9$, 5.6 Hz, 1H, C*H*(OCH$_3$)$_2$], 4.01 (dd, $J_{gem\ NCH2}=14.0$ Hz, $J_{CH-CH2}=$ 5.9 Hz, 1H, NC*H*$_2$), 3.38 (s, 3H, OC*H*$_3$), 3.35 (dd, 1H, O*H*), 3.24 (dd, $J_{CH-CH2}=5.6$ Hz, 1H, NC*H*$_2$), 3.09 (s, 3H, OC*H*$_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 150.43 ($C_{4\ sulfonamide}$), 143.02, 142.99 ($C_{1\ sulfonamide}$, C_1), 136.95 (C_2), 132.10, 129.82, 128.77, 126.48 (C_3, C_4, C_5, C_6), 129.48 ($C_{2\ sulfonamide}$, $C_{6\ sulfonamide}$), 124.25 ($C_{3\ sulfonamide}$, $C_{5\ sulfonamide}$), 100.62 [*C*H(OCH$_3$)$_2$], 61.14 (O*C*H$_2$), 53.38, 51.75 [(O*C*H$_3$)$_2$], 52.22 (N*C*H$_2$). LSIMS *m/z* (relative intensity) 421 (9), 420 (24), 419 [(M+Na)$^+$, 100], 405 (9), 403 (6), 365 (7), 335 (4), 334 (6), 333 (27), 331 (6), 330 (23), 329 (88), 325 (10). HR LSIMS *m/z* calcd for $C_{17}H_{20}N_2O_7SNa$ (M+Na)$^+$: 419.0889, found: 419.0889.

4.1.10. *N*-(2-Chloromethylphenyl)-*N*-(2,2-dimethoxyethyl)-2-nitrobenzenesulfonamide (18a). A solution of **17a** (1 equiv) and triphenylphosphine (1 equiv) in CCl_4 was suddenly heated to 110 °C and then stirred at this temperature for 30 min. After this time, the reaction mixture was allowed to cool at rt, the solvent was removed in vacuum and the residue solved in CH_2Cl_2. Purification by flash chromatography eluting with CH_2Cl_2 gave **18a** as a liquid (40% yield). After standing it solidified giving a white amorphous solid (mp 65.4–66.4 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.69–7.59 (m, 3H), 7.48–7.35 (m, 3H), 7.20 (ddd, $J_1=J_2=7.4$ Hz, $J_3=1.6$ Hz, 1H), 6.99 (dd, $J=$ 8.0, 1.2 Hz, 1H), 4.86 (d, $J_{gem\ CH2Cl}=12.6$ Hz, 1H, C*H*$_2$Cl), 4.59 (d, 1H, C*H*$_2$Cl), 4.47 [dd, $J_{CH-CH2}=5.8$, 5.2 Hz, 1H, C*H*(OCH$_3$)$_2$], 4.05 (dd, $J_{gem\ NCH2}=14.6$ Hz, $J_{CH-CH2}=$ 5.8 Hz, 1H, NC*H*$_2$), 3.69 (dd, $J_{CH-CH2}=5.2$ Hz, 1H, NC*H*$_2$), 3.34 (s, 3H, OC*H*$_3$), 3.28 (s, 3H, OC*H*$_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 148.18 ($C_{2\ sulfonamide}$), 139.04, 136.39 (C_1, $C_{1\ sulfonamide}$), 134.05 ($C_{5\ sulfonamide}$), 132.28 ($C_{4\ sulfonamide}$), 131.40 (C_2), 131.20, 131.04, 129.64, 128.96, 123.99 ($C_{3\ sulfonamide}$, $C_{6\ sulfonamide}$, C_3, C_4, C_5, C_6), 101.76 [*C*H(OCH$_3$)$_2$], 53.71 (N*C*H$_2$), 53.66, 53.28 [(O*C*H$_3$)$_2$], 41.56 (*C*H$_2$Cl). LSIMS *m/z* (relative intensity) 441 (3), 440 (7), 439 (40), 438 (21), 437 [(M+Na)$^+$, 100], 413 (6), 385 (8), 384 (3), 383 (20). HR LSIMS *m/z* calcd for $C_{17}H_{19}ClN_2O_6SNa$ (M+Na)$^+$: 437.0550, found: 437.0557.

4.1.11. *N*-(2-Chloromethylphenyl)-*N*-(2,2-dimethoxyethyl)-4-nitrobenzenesulfonamide (18b). The same procedure that led to **18a** was applied to **17b** to afford **18b** as a liquid in quantitative yield (purification by flash chromatography, elution with CH_2Cl_2) which solidified on standing as a white solid (mp 100.0–101.0 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.33 (d, $J_{3-2\ sulfonamide}=J_{6-5\ sulfonamide}=8.8$ Hz, 2H, $H_{3,5\ sulfonamide}$), 7.80 (d, 2H, $H_{2,6\ sulfonamide}$), 7.73 (dd, $J=7.8$, 1.3 Hz, 1H), 7.41 (ddd, $J_1=J_2=7.7$ Hz, $J_3=1.1$ Hz, 1H), 7.18 (ddd, $J_1=J_2=$ 7.7 Hz, $J_3=1.5$ Hz, 1H), 6.46 (dd, $J=8.0$, 1.0 Hz, 1H), 5.04 (d, $J_{gem\ CH2Cl}=12.8$ Hz, 1H, C*H*$_2$Cl), 4.73 (d, 1H, C*H*$_2$Cl), 4.43 [dd, $J_{CH-CH2}=5.3$, 5.8 Hz, 1H, C*H*(OCH$_3$)$_2$], 3.93 (dd, $J_{gem\ NCH2}=14.1$ Hz, $J_{CH-CH2}=5.3$ Hz, 1H, NC*H*$_2$), 3.42 (dd, $J_{CH-CH2}=5.8$ Hz, 1H, NC*H*$_2$), 3.36 (s, 3H, OC*H*$_3$), 3.19 (s, 3H, OC*H*$_3$). LSIMS *m/z* (relative intensity) 440 (5), 439 (37), 438 (20), 437 [(M+Na)$^+$, 100], 435 (7), 433 (5), 427 (7), 426 (6), 425 (10), 424 (6), 423 (9), 422 (5), 421 (7), 419 (6), 413 (35), 412 (11), 411 (16), 410 (9), 409 (14), 408 (6), 407 (9). HR LSIMS *m/z* calcd for $C_{17}H_{19}ClN_2O_6SNa$ (M+Na)$^+$: 437.0550, found: 437.0548.

4.1.12. *N*-[2-(*tert*-Butyldimethylsilanyloxymethyl)phenyl]bis(4-nitrobenzenesulfonyl)imide (19). Compound **19** is obtained as sole product when the initial amine **12** is treated with 4-nitrobenzenesulfonyl chloride (1.1 equiv) and TEA (1.5 equiv) in CH_2Cl_2 (3 mL/mmol of **12**) as described for the preparation of **15a**. After 5 h stirring at rt, the reaction mixture was washed with water and the final organic layer dried (Na_2SO_4) and evaporated under vacuum. Purification by flash chromatography (gradient elution mixtures EtOAc/hexane 1/50→1/10) afforded **19** as a

white solid (mp 178.9–179.9 °C) in quantitative yield. When the same reaction was carried out using only 1 equiv of 4-nitrobenzenesulfonyl chloride (1 equiv **12**, 1.36 equiv NEt_3), stirring at rt for 1 h it leads to a mixture of **19** (53% yield) and **15b** (23% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.42 (2d, $J_{3-2\ \text{sulfonylimide}}=J_{6-5\ \text{sulfonylimide}}=8.9$ Hz, 4H, $H_{3,5\ \text{sulfonylimide}}$), 8.17 (2d, 4H, $H_{2,6\ \text{sulfonylimide}}$), 7.67 (dd, $J=7.8$, 1.1 Hz, 1H), 7.55 (ddd, $J_1=J_2=7.6$ Hz, $J_3=$ 1.1 Hz, 1H), 7.27 (ddd, $J_1=J_2=7.6$ Hz, $J_3=1.6$ Hz, 1H), 6.83 (dd, $J=8.0$, 1.1 Hz, 1H), 4.32 (s, 2H, OCH_2), 0.87 [s, 9H, $(CH_3)_3C$], −0.02 [s, 6H, $(CH_3)_2Si$]. ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 151.10 ($C_{4\ \text{sulfonylimide}}$), 144.27 ($C_{1\ \text{sulfonylimide}}$), 143.23 ($C_1$), 131.54, 129.07, 127.89 (C_3, C_4, C_5, C_6), 130.49, 124.42 ($C_{2\ \text{sulfonylimide}}$, $C_{3\ \text{sulfonylimide}}$, $C_{5\ \text{sulfonylimide}}$, $C_{6\ \text{sulfonylimide}}$), 129.92 ($C_2$), 60.59 ($OCH_2$), 25.86 [$(CH_3)_3C$], 18.36 [$(CH_3)_3C$], −5.40 [$(CH_3)_2Si$]. LSIMS (relative intensity) 632 (16), 631 (23), 630 [$(M+Na)^+$, 50], 617 (7), 616 (19), 615 (16), 614 (11), 608 (10), 606 (8), 552 (18), 551 (33), 550 (100), 536 (3), 535 (7), 534 (15), 477 (4), 476 (23), 467 (12), 445 (8), 443 (6), 421 (9), 386 (14), 385 (21), 364 (20), 351 (5), 350 (7), 349 (43), 327 (24), 291 (39), 290 (26), 279 (67). HR LSIMS *m/z* calcd for $C_{25}H_{29}N_3O_9S_2SiNa$ $(M+Na)^+$: 630.1012, found: 630.1010.

4.1.13. (*RS*)-3-Methoxy-1-(2-nitrobenzenesulfonyl)-1,2,3,5-tetrahydro-4,1-benzoxazepine (9a). *Method A*. $BF_3\cdot OEt_2$ (2 equiv) was added dropwise to a stirred solution of **17a** (1 equiv) in dried ether (5 mL/mmol of **17a**) at rt under argon. The mixture was kept in a dark place maintaining inert atmosphere, until no initial material was visualised on TLC (4–7 days). Solvent was then evaporated under vacuum and the residue dissolved in CH_2Cl_2 and washed with distilled water. The organic layer was dried (Na_2SO_4), evaporated and chromatographed on silica flash by elution with CH_2Cl_2, to give **9a** as a white solid (mp 128.7–129.3 °C), in quantitative yield. ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.92 (dd, $J=7.9$, 1.3 Hz, 1H), 7.75–7.59 (m, 3H), 7.32–7.17 (m, 3H), 7.07 (d, $J=7.7$ Hz, 1H), 4.86 (d, $J_{gem\ 5-5}=13.7$ Hz, 1H, H_5), 4.74 (dd, 1H, H_3), 4.42 (d, 1H, H_5), 3.90 (bs, 1H, H_2), 3.75 (bs, 1H, H_2), 3.39 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 148.02 ($C_{2\ \text{sulfonamide}}$), 139.34, 138.28 ($C_{9a}$, $C_{1\ \text{sulfonamide}}$), 134.25 ($C_{5a}$), 133.86, 132.08, 131.42, 129.63, 128.81, 128.54, 128.28, 124.24 ($C_{3\ \text{sulfonamide}}$, $C_{4\ \text{sulfonamide}}$, $C_{5\ \text{sulfonamide}}$, $C_{6\ \text{sulfonamide}}$, C_6, C_7, C_8, C_9), 101.00 (bs, C_3), 64.00 (bs, C_5), 55.58 (OCH_3), 54.36 (C_2). LSIMS *m/z* (relative intensity) 389 (25), 388 (13), 387 [$(M+Na^+$, 100], 386 (6), 385 (44), 371 (10), 369 (13), 367 (11), 349 (5), 345 (12), 329 (10), 327 (44), 311 (21), 301 (12). HR LSIMS *m/z* calcd for $C_{16}H_{16}N_2O_6SNa$ $(M+Na)^+$: 387.0627, found: 387.0626. Anal. for $C_{16}H_{16}N_2O_6S$: calcd C 52.75; H 4.40; N 7.69. Found: C 52.69; H 4.51; N 7.73.

Method B. Compound **17a** (1 equiv) was dissolved in toluene (30 mL/mmol of **17a**) under argon atmosphere. A catalytic amount of *p*-toluenesulfonic acid (0.03 equiv) was added at rt and the mixture was then heated to 110 °C for 2 h. After cooling, neutralisation with an excess of K_2CO_3 under argon atmosphere was followed by filtration washing with ether. The filtrate was again neutralised with an excess of K_2CO_3 and after a second filtration the solvent was removed under vacuum. Flash chromatography (gradient elution mixtures EtOAc/hexane 1/10→1/2) gave **9a** in quantitative yield.

4.1.14. (*RS*)-3-Methoxy-1-(4-nitrobenzenesulfonyl)-1,2,3,5-tetrahydro-4,1-benzoxazepine (9b). *Method A*. The procedure described for the preparation of **9a** (method A), was applied to **17b** to obtain **9b** as a white solid (mp 171.8–172.6 °C, purification by flash chromatography with CH_2Cl_2 (67% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.24 (d, $J_{3-2\ \text{sulfonamide}}=J_{6-5\ \text{sulfonamide}}=8.9$ Hz, 2H, $H_{3,5\ \text{sulfonamide}}$), 7.79 (d, 2H, $H_{2,6\ \text{sulfonamide}}$), 7.67 (d, $J=$ 7.8 Hz, 1H), 7.37 (ddd, $J_1=J_2=7.7$ Hz, $J_3=1.7$ Hz, 1H), 7.30 (ddd, $J_1=J_2=7.4$ Hz, $J_3=1.4$ Hz, 1H), 7.18 (dd, $J=$ 7.4, 1.7 Hz, 1H), 4.67 (dd, 1H, H_3), 4.25 (d, $J_{gem\ 5-5}=$ 13.7 Hz, 1H, H_5), 4.17 (bdd, 1H, H_2), 3.99 (d, 1H, H_5), 3.68 (bdd, $J_{gem\ 2-2}=13.4$ Hz, 1H, H_2), 3.29 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 149.94 ($C_{4\ \text{sulfonamide}}$), 145.95, 138.94, 136.87 ($C_{1\ \text{sulfonamide}}$, C_{9a}, C_{5a}), 129.36, 129.13, 129.00, 128.49 (C_6, C_7, C_8, C_9), 129.00, 123.54 ($C_{2\ \text{sulfonamide}}$, $C_{3\ \text{sulfonamide}}$, $C_{5\ \text{sulfonamide}}$, $C_{6\ \text{sulfonamide}}$), 99.36 (bs, C_3), 62.75 (bs, C_5), 54.79 (OCH_3), 53.73 (C_2). LSIMS *m/z* (relative intensity) 389 (10), 388 (6), 387 [$(M+$ Na)+, 100], 386 (10), 385 (66), 383 (5). HR LSIMS *m/z* calcd for $C_{16}H_{16}N_2O_6SNa$ $(M+Na)^+$: 387.0627, found: 387.0627. Anal. for $C_{16}H_{16}N_2O_6S$: calcd C 52.75; H 4.40; N 7.69. Found: C 52.70; H 4.39; N 8.03.

Method B. The procedure described for the preparation of **9a** was applied to **17b** to obtain **9b** in quantitative yield (purification by flash chromatography with gradient elution mixtures EtOAc/hexane from 1/20→1/9).

4.1.15. (*RS*)-3-Methoxy-1,2,3,5-tetrahydro-4,1-benzoxazepine (9c). To a solution of **9a** or **9b** (1 equiv) in DMF (5 mL/mmol of **9a** or **9b**) at rt, was added K_2CO_3 (3 equiv) followed by PhSH (1.1 equiv). Stirring was maintained for 1 h after which the reaction was treated by addition of EtOAc and distilled water. The aqueous layer was extracted three times with EtOAc. The combined organic layers were dried (Na_2SO_4) and concentrated under vacuum. Purification by flash chromatography (gradient elution mixtures EtOAc/hexane 1/50→1/15) yielded the free amine **9c** in 98% from **9a** and 90% yield from **9b** (white solid, mp 89.5–90.5 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.10–7.02 (m, 2H), 6.79 (ddd, $J_1=J_2=7.4$ Hz, $J_3=1.0$ Hz, 1H), 6.67 (dd, $J=7.8$, 0.8 Hz, 1H), 5.20 (d, $J_{gem\ 5-5}=14.3$ Hz, 1H, H_5), 4.78 (dd, $J_{3-2}=7.2$, 3.5 Hz, 1H, H_3), 4.33 (d, 1H, H_5), 3.48 (s, 3H, OCH_3), 3.41 (dd, $J_{gem\ 2-2}=14.0$ Hz, $J_{3-2}=$ 7.2 Hz, 1H, H_2), 3.25 (dd, $J_{3-2}=3.5$ Hz, 1H, H_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 148.48 (C_{9a}), 128.88, 128.23, 119.94, 117.64 (C_6, C_7, C_8, C_9), 126.84 (C_{5a}), 102.28 (C_3), 63.99 (C_5), 55.28 (OCH_3), 50.38 (C_2). Anal. for $C_{10}H_{13}NO_2$: calcd C 67.02; H 7.31; N 7.82. Found: C 67.09; H 7.13; N 7.98.

4.1.16. (*RS*)-1-Butyryl-3-methoxy-1,2,3,5-tetrahydro-4,1-benzoxazepine (9d). A solution of **9c** (1 equiv) in dried CH_2Cl_2 (3 mL/mmol of **9c**) was prepared under argon and then cooled to 0 °C. First TEA (3 equiv) and then butyryl chloride (2 equiv), were added in dropwise fashion. The mixture was then allowed to warm at rt and stirred for 17 h. After dilution with CH_2Cl_2, the organic layer was washed with water, dried (Na_2SO_4) and concentrated under

vacuum. Purification by flash chromatography with gradient elution using mixtures EtOAc/hexane (1/20→1/5), afforded the final amide **9d** as a colourless liquid (69% yield). In $CDCl_3$ at rt two isomers are observed: isomer A (60%) (numbers without primes), isomer B (40%) (numbers with primes): ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.39–7.10 (m, 4H, 4H′), 5.15 (d, J_{gem}=13.0 Hz, 1H′), 5.05 (d, J_{gem}=14.0 Hz, 1H′), 4.82 (d, $J_{gem\ 2\text{–}2}$=13.6 Hz, 1H, H_2), 4.76–4.67 (m, 2H, 1H′), 4.49 (d, $J_{gem\ 5\text{–}5}$=14.1 Hz, 1H, H_5), 4.15 (d, J_{gem}=13.0 Hz, 1H′), 3.49 (s, 3H, OCH_3), 3.43 (s, 3H, OCH'_3), 2.85 (d, J_{gem}=14.0 Hz, 1H′), 2.70 (dd, $J_{3\text{–}2}$=8.8 Hz, 1H, H_2), 2.26 (m, 2H, COCH_2, COCH'_2), 2.06 (m, 2H, COCH_2, COCH'_2), 1.58 (m, 4H, CH_3CH_2, $CH_3CH'_2$), 0.83 (t, $J_{CH2\text{–}CH3}$=$J_{CH2'\text{–}CH3'}$=7.4 Hz, 6H, CH_3, CH'_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 173.01 (*C*O), 143.60, 142.00, 136.70, 136.27 ($C_{9a'}$, C_{9a}, $C_{5a'}$, C_{5a}), 129.81–127.24 (C_6, $C_{6'}$, C_7, $C_{7'}$, C_8, $C_{8'}$, C_9, $C_{9'}$), 102.67 (C_3), 99.03 ($C_{3'}$), 66.05 (C_5), 61.58 ($C_{5'}$), 56.28 (O*C*H$_3$), 55.09 (O*C*H′$_3$), 50.71 (C_2), 48.94 ($C_{2'}$), 36.07 (CO*C*H$_2$, CO*C*H′$_2$), 19.83 (CH_3CH_2, $CH_3CH'_2$), 13.85 (*C*H$_3$, *C*H′$_3$). LSIMS *m/z* (relative intensity) 274 (13), 273 (17), 272 [(M+Na)$^+$, 66], 266 (16), 265 (50), 264 (25), 263 (34), 260 (19), 251 (11), 250 [(M+H)$^+$, 47], 249 (20), 248 (23), 247 (30), 246 (14), 245 (9), 244 (17), 243 (27), 237 (42), 236 (19), 235 (21), 234 (15), 233 (29), 232 (18), 231 (35), 230 (21), 229 (30), 228 (17), 227 (38), 226 (16), 225 (8), 222 (37), 221 (100), 220 (27), 219 (49), 218 (53). HR LSIMS *m/z* calcd for $C_{14}H_{19}NO_3Na$ (M+Na)$^+$: 272.1263, found: 272.1260. Anal. for $C_{14}H_{19}NO_3$: calcd C 67.45; H 7.68; N 5.62. Found: C 67.56; H 7.43; N 5.60.

4.1.17. (*RS*)-3-Methoxy-1-trifluoroacetyl-1,2,3,5-tetrahydro-4,1-benzoxazepine (9e). Reaction of **9c** with TFAA according to the procedure described for the preparation of **9d**. After 18 h stirring at rt, the reaction mixture was diluted with CH_2Cl_2 and the organic layer washed with water, dried (Na_2SO_4) and concentrated under vacuum. Purification was performed by flash chromatography (gradient elution mixtures EtOAc/hexane 1/50→1/16), to yield the final product **9e** as a white solid, mp 76.0–77.0 °C (79% yield). In $CDCl_3$ at rt two isomers are observed: isomer A (45%) (numbers without primes), isomer B (55%) (numbers with primes): ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.39–7.18 (m, 4H′, 4H), 5.21 (d, $J_{gem\ 5'\text{–}5'}$=13.2 Hz, 1H, $H_{5'}$), 4.91 (dd, $J_{gem\ 2'\text{–}2'}$=13.8 Hz, $J_{3'\text{–}2'}$=1.4 Hz, 1H, $H_{2'}$), 4.75–4.68 (m, 1H′, 3H), 4.56 (d, $J_{gem\ 5\text{–}5}$=14.0 Hz, 1H, H_5), 4.18 (d, 1H, $H_{5'}$), 3.51 (s, 3H, OCH_3), 3.44 (s, 3H, OCH'_3), 3.06 (d, 1H, $H_{2'}$), 2.85 (dd, $J_{gem\ 2\text{–}2}$=13.0 Hz, $J_{3\text{–}2}$=8.2 Hz, 1H, H_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 156.74 (q, $J_{CO\text{–}F}$=36.0 Hz, *C*O), 156.65 (q, $J_{CO'\text{–}F}$=36.0 Hz, *C*O′), 140.40 ($C_{9a'}$), 138.85 (C_{9a}), 136.55 ($C_{5a'}$), 136.42 (C_{5a}), 129.77–126.75 ($C_{6'}$, C_6, $C_{7'}$, C_7, $C_{8'}$, C_8, $C_{9'}$, C_9), 116.44 (q, $J_{C'\text{–}F}$=287.0 Hz, *C*F′$_3$), 116.25 (q, $J_{C\text{–}F}$=287.0 Hz, *C*F$_3$), 102.24 (C_3), 98.56 ($C_{3'}$), 66.32 (C_5), 61.30 ($C_{5'}$), 56.44 (O*C*H$_3$), 55.24 (O*C*H′$_3$), 52.90 (C_2), 51.27 ($C_{2'}$). LSIMS *m/z* (relative intensity) 331 (7), 330 (26), 329 (100), 308 (8), 307 (35), 299 (12), 299 (12), 298 [(M+Na)$^+$, 51], 289 (21), 277 (9), 276 [(M+H)$^+$, 34], 274 (15), 273 (15), 259 (13), 257 (11), 245 (22), 244 (61), 243 (13), 239 (9), 217 (14), 216 (18), 215 (29). HR LSIMS *m/z* calcd for $C_{12}H_{12}NO_3F_3Na$ (M+Na)$^+$: 298.0670, found: 298.0673. Anal. for $C_{12}H_{12}FNO_3$: calcd C 52.37; H 4.39; N 5.09. Found: C 52.41; H 4.22; N 5.21.

4.1.18. (*RS*)-1-Benzoyl-3-methoxy-1,2,3,5-tetrahydro-4,1-benzoxazepine (9f). A solution of **9c** (1 equiv) in dried CH_2Cl_2 (3 mL/mmol **9c**) was prepared under argon and cooled to 0 °C. At this temperature, TEA (3 equiv) and then benzoyl chloride (2 equiv) were added dropwise. The mixture was stirred between 0 and 5 °C for 3 h and afterwards diluted with CH_2Cl_2. The organic layer was washed with water, dried (Na_2SO_4) and concentrated under vacuum. The final product carrying the benzoyl moiety was purified by flash chromatography (gradient elution mixtures EtOAc/hexane 1/20→1/4) as a white solid, mp 104.5–105.0 °C (quantitative yield). $CDCl_3$, rt: Isomer A (86%) (numbers without primes), isomer B (14%) (numbers with primes): ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.10 (d, 2H′), 7.59 (t, 1H′), 7.47 (t, 2H′), 7.38–7.04 (m, 7H, 4H′), 6.93 (t, 1H), 6.61 (d, 1H), 5.39–5.06 (bs), 4.87 (bs, 1H, 1H′), 4.70–4.33 (bs), 3.51 (s, 6H, OCH_3, OCH'_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.11 (*C*O), 135.59, 133.55, 130.18, 130.01, 129.17, 128.65, 128.49, 128.07, 127.86, 126.68, 126.34 ($C_{aromatic}$), 101.54 (C_3), 64.60 (C_5), 55.74 (O*C*H$_3$), 50.47 (C_2). DMSO-d_6, 80°C: Isomer A (90%) (numbers without primes), isomer B (10%) (numbers with primes): ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.91 (m, 2H′), 7.56 (dddd, J_1=J_2=7.4 Hz, J_3=J_4=1.4 Hz, 1H′), 7.45–7.38 (m, 3H′), 7.32 (dd, J=7.5, 1.4 Hz, 2H), 7.30–7.17 (m, 4H, 3H′), 7.09 (ddd, J_1=J_2=7.5 Hz, J_3=1.2 Hz, 1H), 6.97 (ddd, J_1=J_2=7.6 Hz, J_3=1.5 Hz, 1H), 6.69 (d, J=7.6 Hz, 1H), 5.07 (d, J_{gem}=13.9 Hz, 2H, $H_{5,5'}$), 4.77 (dd, J=5.0, 2.9 Hz, 2H, $H_{3,3'}$), 4.56 (d, J_{gem}=13.9 Hz, 2H, $H_{5,5'}$), 3.90 (bs), 3.40 (s, 6H, OCH_3, OCH'_3), 3.29 (m). LSIMS *m/z* (relative intensity) 285 (15), 284 [(M+H)$^+$, 100], 283 (M$^+$, 30), 282 (12). HR LSIMS *m/z* calcd for $C_{17}H_{18}NO_3$ (M+H)$^+$: 284.1287, found: 284.1282. Anal. for $C_{17}H_{17}NO_3$: calcd C 72.07; H 6.05; N 4.94. Found: C 71.92; H 6.00; N 4.99.

4.1.19. *N*-[2-(*tert*-Butyldimethylsilanyloxymethyl)phenyl]-*N*-isopropyl-4-nitrobenzenesulfonamide (20). Compound **20** was obtained from **15b** with the same procedure described for the preparation of **16b**, but using an excess of hydroxyacetaldehyde dimethyl acetal (4.3 equiv). Flash chromatography purification with a gradient elution mixture EtOAc/hexane (1/50→1/20) afforded **20** (38% yield) as a white solid (mp 178.0–179.0 °C) and **16b** (31% yield). *Compound* **20**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.33 (d, $J_{6\text{–}5\ sulfonamide}$=$J_{3\text{–}2\ sulfonamide}$ 8.8 Hz, 2H, $H_{3,5\ sulfonamide}$), 7.89 (d, 2H, $H_{2,6\ sulfonamide}$), 7.73 (d, J=7.2 Hz, 1H), 7.44 (ddd, J_1=J_2=7.6 Hz, J_3=0.8 Hz, 1H), 7.16 (ddd, J_1=J_2=7.7 Hz, J_3=1.5 Hz, 1H), 6.58 (dd, J=7.9, 0.8 Hz, 1H), 4.90 (d, $J_{gem\ CH2}$=15.0 Hz, 1H, CH_2), 4.85 (d, 1H, CH_2), 4.61 [m, 1H, C*H*(CH$_3$)$_2$], 1.13 [d, $J_{CH\text{–}CH3}$=6.7 Hz, 3H, CH(CH_3)$_2$], 1.00 [d, $J_{CH\text{–}CH3}$=6.8 Hz, 3H, CH(CH_3)$_2$], 0.96 [s, 9H, (*C*H$_3$)$_3$C], 0.14 [2s, 6H, (*C*H$_3$)$_2$Si]. LSIMS *m/z* (relative intensity) 490 (4), 489 (15), 488 (36), 487 [(M+Na)$^+$, 100], 468 (1), 467 (5), 466 (12), 465 [M+H)$^+$, 39], 410 (3), 409 (11), 408 (25), 407 (87), 393 (2), 392 (3), 391 (7), 336 (1), 335 (5), 334 (13), 333 (65), 331 (2), 330 (3), 329 (15), 282 (1), 281 (2), 280 (4), 279 (18). HR LSIMS *m/z* calcd for $C_{22}H_{32}N_2O_5SSiNa$ (M+Na)$^+$: 487.1699, found: 487.1702.

4.1.20. 2-(2,2-Dimethoxyethylaminomethyl)phenol (21). A solution of 2-hydroxybenzaldehyde (1 equiv) in EtOH

(0.2 mL/mmol of 2-hydroxybenzaldehyde) was prepared under argon atmosphere. Aminoacetaldehyde dimethyl acetal (1 equiv) was added at rt and the mixture was heated at reflux. Stirring continued for 5 h until the initial aldehyde disappeared on TLC. The mixture was then allowed to return to rt and the solvent eliminated under vacuum. The resulting residue was dissolved in anhydrous methanol under argon atmosphere and added dropwise on a mixture of $NaBH_4$ (2.5 equiv) and anhydrous methanol (1 mL/mmol of $NaBH_4$) at rt. After stirring 30 min at rt, the mixture was heated at 65 °C for 2 h. Water was then added and when bubbling ceased, methanol was eliminated under vacuum. The basic pH aqueous layer was extracted with CH_2Cl_2 and the organic layer dried ($MgSO_4$) and concentrated. Purification was performed by flash chromatography and the final product (**21**) eluted with EtOAc/hexane (3/10) as a yellow oil in quantitative yield. ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.16 (ddd, $J_1=J_2=7.7$ Hz, $J_3=1.6$ Hz, 1H), 6.98 (dd, $J=7.4$, 1.2 Hz, 1H), 6.83 (dd, $J=8.1$, 0.9 Hz, 1H), 6.77 (ddd, $J_1=J_2=7.4$ Hz, $J_3=1.1$ Hz, 1H), 4.48 [t, $J_{CH-CH2}=5.4$ Hz, 1H, C*H*$(OCH_3)_2$], 4.00 (s, 2H, PhC*H*$_2$), 3.38 [s, 6H, $(OCH_3)_2$], 2.78 (d, 2H, CHC*H*$_2$N). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 158.19 (C_1), 128.87, 128.46, 119.14, 116.47 (C_3, C_4, C_5, C_6), 122.35 (C_2), 103.20 [*C*H$(OCH_3)_2$], 54.33 [$(OCH_3)_2$], 52.59, 49.73 (Ph*C*H$_2$, CH*C*H$_2$NH). LSIMS *m/z* (relative intensity) 234 [$(M+Na)^+$, 61], 233 (8), 213 (9), 212 [$(M+H)^+$, 100], 211 (M^+, 58), 210 (5). HR LSIMS *m/z* calcd for $C_{11}H_{17}NO_3Na$ $(M+Na)^+$: 234.1106, found: 234.1107.

4.1.21. *N*-(2,2-Dimethoxyethyl)-2,2,2-trifluoro-*N*-(2-hydroxybenzyl)acetamide (22). A solution of **21** (1 equiv) and 1-(trifluoroacetyl)benzotriazole[10] (2.5 equiv) in THF (2 mL/mmol of **21**) is stirred for 3 h at rt. The solvent was then evaporated under vacuum and the residue purified by flash chromatography [gradient elution mixtures with EtOAc/hexane (1/15 → 1/13) mixtures], to give **22** as a yellow oil in 72% yield. $CDCl_3$, rt: isomer A (89%) (numbers without primes), isomer B (11%) (numbers with primes): ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.80 (bs, 1H, OH), 7.30–7.15 (m, 2H, 1H′), 7.05 (dd, 1H′), 6.95–6.80 (m, 2H, 2H′), 4.76 (s, 4H, PhC*H*$_2$, PhC*H*′$_2$), 4.60 [t, 1H, C*H*′$(OCH_3)_2$], 4.50 [t, $J_{CH-CH2}=5.1$ Hz, 1H, C*H*$(OCH_3)_2$], 3.53 (2d, 4H, CHC*H*$_2$N, CHC*H*′$_2$N), 3.46, 3.43 [2s, 12H, $(OCH_3)_2$, $(OCH'_3)_2$]. ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 155.65 (C_2), 132.14, 130.80, 120.19, 117.71 (C_3, C_4, C_5, C_6), 129.76, 129.24, 121.33, 116.62 ($C_{3'}$, $C_{4'}$, $C_{5'}$, $C_{6'}$), 114.92 (C_1), 104.43 [*C*H$(OCH_3)_2$], 102.67 [*C*H′$(OCH_3)_2$], 55.44 [$(OCH_3)_2$, $(OCH'_3)_2$], 48.25, 47.36 (Ph*C*H$_2$, CH*C*H$_2$N), 47.08, 46.44 (Ph*C*H′$_2$, CH*C*H′$_2$N). LSIMS *m/z* (relative intensity) 331 (15), 330 [$(M+Na)^+$, 100], 329 (12), 281 (8), 277 (11), 276 (76), 275 (22), 244 (6). HR LSIMS *m/z* calcd for $C_{13}H_{16}NO_4F_3Na$ $(M+Na)^+$: 330.0929, found: 330.0927.

4.1.22. (*RS*)-2-Methoxy-*N*-trifluoroacetyl-2,3,4,5-tetrahydro-1,4-benzoxazepine (10). A solution of **22** (1 equiv) in dried Et_2O (4 mL/mmol) was prepared under argon. $BF_3\cdot OEt_2$ (2 equiv) was then added dropwise at rt. The resulting mixture was kept away from light, under argon, until no initial product could be visualized on TLC (5 d). Et_2O was then added and the solution washed with water. The final organic phase was dried (Na_2SO_4), concentrated and purified by flash chromatography. The cyclic product **10** was eluted (with gradient elution mixtures EtOAc/hexane 1/50 → 1/25) as a colourless oil (49% yield). $CDCl_3$, rt: Isomer A (63%) (numbers without primes), isomer B (37%) (number with primes): ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.33–7.09 (m, 4H, 4H′), 4.91 (d, $J_{gem\ 5-5}=14.4$ Hz, 1H, H_5), 4.79 (dd, $J_{3'-2'}=2.2$, 7.1 Hz, 1H, $H_{2'}$), 4.73 (d, 1H, $H_{5'}$), 4.73 (dd, $J_{3-2}=2.3$, 7.8 Hz, 1H, H_2), 4.57 (d, $J_{gem\ 5'-5'}=15.5$ Hz, 1H, $H_{5'}$), 4.40 (dd, $J_{gem\ 3'-3'}=13.9$ Hz, $J_{3'-2'}=2.2$ Hz, 1H, $H_{3'}$), 4.35 (d, 1H, H_5), 4.04 (dd, $J_{gem\ 3-3}=15.1$ Hz, $J_{3-2}=2.3$ Hz, 1H, H_3), 3.61 (s, 3H, O*C*H_3), 3.59 (s, 3H, O*C*H'_3), 3.56–3.48 (m, 2H, $H_{3,3'}$). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 156.06 (2q, $J_{CO-F}=J_{CO'-F}=36.0$ Hz, *C*O, *C*O′), 153.53 ($C_{9a'}$), 153.33 (C_{9a}), 130.25–122.34 ($C_{aromatic}$), 116.46 (q, $J_{C'-F}=286.3$ Hz, *C*F′$_3$), 116.31 (q, $J_{C-F}=286.0$ Hz, *C*F_3), 102.93 (C_2), 102.39 ($C_{2'}$), 56.63 (O*C*H′$_3$), 56.53 (O*C*H_3), 51.51, 49.85 (C_3, C_5), 51.19, 50.42 ($C_{3'}$, $C_{5'}$). LSIMS *m/z* (relative intensity) 300 (2), 299 (14), 298 [$(M+Na)^+$, 100], 290 (7), 289 (17), 277 (7), 276 [$(M+H)^+$, 60], 275 (M^+, 34). HR LSIMS *m/z* calcd for $C_{12}H_{12}NO_3F_3Na$ $(M+Na)^+$: 298.0667, found: 298.0666. Anal. for $C_{12}H_{12}F_3NO_3$: calcd C 52.37; H 4.39; N 5.09. Found: C 52.30; H 4.46; N 5.01.

4.2. Biological activity

4.2.1. Cell culture. The human breast cancer MCF-7 cell line, used for treatment with the drugs, was kindly provided by Dr. N. Olea of the Sánchez Mora Tumour Biology Institute, University Hospital of Granada. MCF-7 cells were grown at 37 °C in an atmosphere containing 5% CO_2, with Dubelcco's modified Eagle Medium (DMEM) (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco), 2% L-glutamine, 2.7% sodium bicarbonate, 1% Hepes buffer, 40 mg/L gentamicin and 500 mg/L ampicillin.

4.2.2. Drugs and drug treatments. After the synthesis and purification of the final compounds stock solutions were prepared. The drugs were dissolved in DMSO or water and stored at −20 °C. For each experiment, the stock solutions were further diluted in medium to obtain the desired concentrations. The final solvent concentration in cell culture was ≤0.5% v/v of DMSO, a concentration without effect on cell replication.[28] Parallel cultures of MCF-7 cells in medium with DMSO were used as controls.

4.2.3. Cytotoxicity assays in vitro. The effect of anticancer drugs on cell viability was assessed using the sulforhodamine-B (SRB) colorimetric assay. Aliquots of MCF-7 cells suspension (30×10^3 cells/well) were seeded onto 24-well plates and incubated for 24 h. The cells were then treated with different concentrations of drugs in the culture medium. Three days later, the wells were aspirated, fresh medium was added, and cells were maintained for 3 additional days. Thereafter, cells were processed as described previously,[29] using a Titertek Multiscan apparatus (Flow, Irvine, California) at 492 nm. We evaluated the linearity of the SRB assay with the cell number for each MCF-7 cell stock before each cell growth experiment. The IC_{50} values were calculated from semilogarithmic dose–response curves by linear interpolation. All of the

experiments were plated in triplicate wells and were carried out at least twice.

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Rapid palladium-catalyzed aminations of aryl chlorides with aliphatic amines under temperature-controlled microwave heating

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Abstract—Rapid Buchwald–Hartwig amination of electron neutral and rich aryl chlorides and bromides have been achieved using temperature-controlled microwave heating. Primary and secondary aliphatic amines can be coupled with these substrates in good yields within a reaction time of only 10 min.

In the last decade, the impact of homogeneous palladium catalysis on the field of organic syntheses has increased exponentially. Within this diverse research area the palladium-catalyzed amination, in the mid nineties independently discovered by Buchwald and Hartwig, has currently established itself as the most powerful method available for the C(sp^2)–N bond formation as it is applicable for both activated and non-activated aryl halogenides.[1] This is exemplified by its use in the synthesis of several natural products such as the alkaloids makaluvamine C,[2] damirones A and B,[2] lavendamycin[3] and isocryptolepine.[4] Besides natural products also drug analogues and drug metabolites have been prepared by a Buchwald–Hartwig reaction. For instance, raloxifene (launched by Eli Lilly to prevent osteoporosis) analogues were synthesized via an alternative route by Schmid and co-workers using 2-bromobenzo-[*b*]thiophenes as the amination substrates.[5] Another example is the synthesis of hydroxyitraconazole, an active metabolite of the antifungal drug itraconazole launched by Janssen Pharmaceutica, in which one of the arylpiperazine moieties is synthesized via a palladium-catalyzed C–N bond formation.[6] Norastemizole, another example of an active metabolite, was smoothly obtained via the selective coupling of 2-chloro-1-(4-fluorobenzyl)benzimidazole with the primary amino group of 4-aminopiperidine.[7] It is a metabolite of the antiallergic drug Astemizole of Janssen Pharmaceutica which has reduced side effects. All these examples clearly indicate the large potential of this C–N bond formation methodology for its use in the preparation of biologically active compounds. However, for incorporation in lead discovery and optimization processes the Buchwald–Hartwig amination reaction still has the major drawback that the reaction times are usually in the order of hours to one day. This might be too long for high-throughput medicinal chemistry programs in which large libraries of compounds have to be made within a short period of time.[8] Moreover, in such programs aryl chlorides, the least reactive substrates of the aryl halogenides, are more preferred compounds than aryl bromides and aryl iodides because of their lower cost and wider availability.[1g] Recently, our laboratory reported a procedure to perform Buchwald–Hartwig aminations of aryl chlorides with anilines within 10 min using temperature-controlled microwave heating.[9,10] The ligand as well as the palladium source reported in this short communication are air stable and commercially available products which are important aspects when aiming for a user-friendly protocol (without the necessity of a glovebox) as required for chemistry in a high-throughput program. As an extension of the published work, we now report the more challenging rapid palladium-catalyzed microwave-assisted amination of aryl chlorides with aliphatic amines.[9,11]

First, we investigated the Buchwald–Hartwig amination of morpholine with chlorobenzene, 4-chlorotoluene and 4-chloroanisole under microwave irradiation since secondary cyclic amines are known to be the easiest coupling partners of the aliphatic amines (Table 1, entries 1–3). We selected the same catalyst, $Pd(OAc)_2$ and 2-(dicyclohexylphosphanyl)biphenyl (DCPB),[12] as we previously found to be optimal for rapid aminations of aryl chlorides with

Keywords: Palladium; Homogeneous catalysis; Buchwald–Hartwig amination; Aryl chlorides; Aryl bromides; Microwave irradiation.
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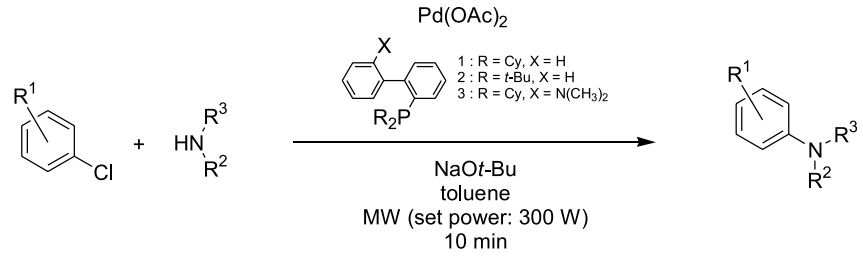
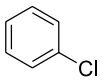
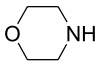
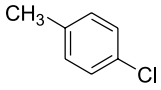
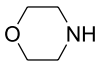
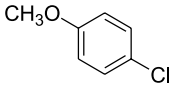
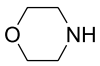
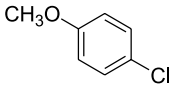
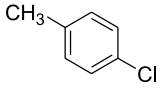
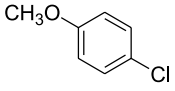
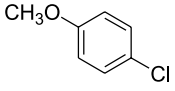
Table 1. Rapid microwave-assisted Buchwald–Hartwig amination of aryl chlorides (CEM Discover apparatus)[22]



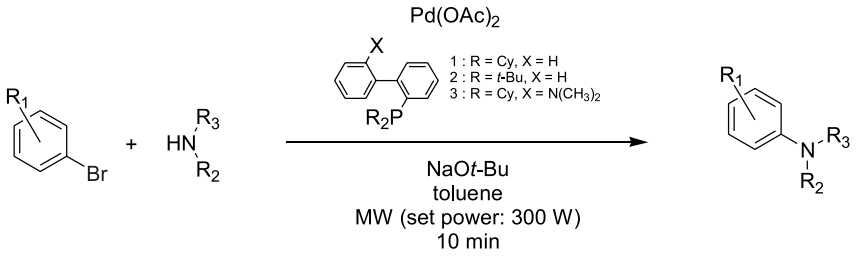
Entry	Aryl Chloride	Amine	Ligand	Pd loading (mol%)	Temp (°C)	Yield[a,b] MW (%)	Yield[a,b] oil bath (%)
1			1	1	150	91	91
2			1	1	150	83	85
3			1	1	150	76	76
4		Bu_2NH	1	1	150	15	
5		Bu_2NH	1	1	200	28	
6		Bu_2NH	1	2	200	35	
7		Bu_2NH	2	1	150	50	47
8		Bu_2NH	3	1	150	54[c]	
9		Bu_2NH	2	2	110	48[d]	43[d]
10		Bu_2NH	2	1	150	70	63
11		$BnNH_2$	2	1	150	81[e]	87[e]
12		$BnNH_2$	2	1	150	85[f]	
13		$BnNH_2$	1	1	150	42[e]	
14		*n*-$HexNH_2$	2	1	150	86[e]	85[e]
15		*n*-$HexNH_2$	2	1	150	87[f]	
16		*n*-$HexNH_2$	1	1	150	20[e]	

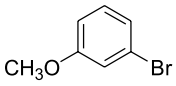
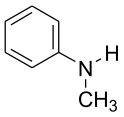
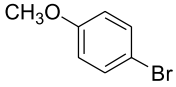
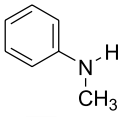
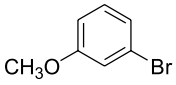
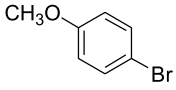
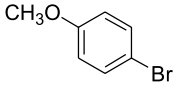
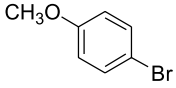
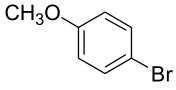
[a] Reaction conditions: $Pd(OAc)_2$ (X mol%), DCPB or DTPB or DCPAB (2 X mol%), aryl chloride (1 mmol), amine (1.2 mmol), NaO*t*-Bu (1.4 mmol), toluene (1 mL).
[b] Yields of isolated products are an average of two runs.
[c] $Pd_2(dba)_3$ was used instead of $Pd(OAc)_2$, a Pd/L ratio of 1.5 was used.
[d] The reaction time was 20 min instead of 10 min.
[e] 1.5 mmol of amine were used instead of 1.2 mmol.
[f] 3 mmol of amine were used instead of 1.2 mmol.

anilines.[9] Interestingly, with a catalyst loading of only 1 mol% and a temperature of 150 °C good results were obtained in a reaction time of only 10 min. The isolated yield decreased as the substrate became more electron rich (Table 1, compare entries 1–3). Nevertheless, even for 4-chloroanisole (Table 1, entry 3), the most electron rich aryl chloride substrate of the three used, 4-(4-methoxyphenyl)morpholine could be obtained in 76% yield.

Next, we tried to couple acyclic secondary and primary amines with aryl chlorides. We selected 4-chloroanisole as our test substrate since electron rich aryl chlorides are more difficult substrates than electron neutral ones.[1g] This can be explained by taking into account that the fundamental processes oxidative addition as well as reductive elimination are disfavoured by decreasing the electrophilicity of the aryl group.[1c,g] In addition, the selectivity of reductive elimination versus β-H elimination from a palladium amide complex is better for electron neutral than for electron rich substrates.[1c] As a test case, we have chosen the coupling of 4-chloroanisole with dibutylamine which is one among the most challenging combinations. Disappointingly, only 15%

Table 2. Rapid microwave-assisted Buchwald–Hartwig amination of aryl bromides (CEM Discover apparatus)[22]



Entry	Aryl bromide	Amine	Ligand	Pd loading (mol%)	Temp (°C)	Yield[a,b] MW (%)	Yield[a,b] oil bath (%)
1			1	1	150	90	91
2			1	1	150	89	87
3			1	1	150	87	88
4			1	1	150	80	83
5		Bu_2NH	1	1	150	49	60
6		Bu_2NH	2	1	150	36	
7		Bu_2NH	3	1	150	54[c]	
8		$BnNH_2$	1	1	150	61[d]	
9		$BnNH_2$	2	1	150	87[d]	93[d]
10		*n*-$HexNH_2$	2	1	150	79[d]	79[d]

[a] Reaction conditions: $Pd(OAc)_2$ (X mol%), DCPB or DTPB or DCPAB (2 X mol%), aryl bromide (1 mmol), amine (1.2 mmol), NaO*t*-Bu (1.4 mmol), toluene (1 mL).
[b] Yields of isolated products are an average of two runs.
[c] $Pd_2(dba)_3$ was used instead of $Pd(OAc)_2$, a Pd/L ratio of 1.5 was used.
[d] 3 mmol of amine were used instead of 1.2 mmol.

of *N*,*N*-dibutyl-4-methoxyaniline and an incomplete reaction was obtained in 10 min under the standard conditions (1 mol% catalyst, 150 °C) (Table 1, entry 4). Neither increasing the temperature to 200 °C (Table 1, entry 5) nor additional increasing of the catalyst loading to 2 mol% (Table 1, entry 6) provided us with acceptable yields and complete conversion of starting material. Finally, we discovered that the use of 2-(di-*t*-butylphosphanyl)biphenyl (DTPB)[12] as the ligand instead of 2-(dicyclohexylphosphanyl)biphenyl (DCPB) gave an acceptable result since a complete conversion of starting material was observed and 50% of *N*,*N*-dibutyl-4-methoxyaniline could be isolated (Table 1, entry 7). An attempt to further increase the yield by performing the reaction at a lower temperature using a double loading of catalyst was unsuccessful (Table 1, entry 9). In this case a reaction time of 20 min was necessary to get a full conversion of starting material. Interestingly, the use 2-dicyclohexylphosphanyl-2′-(*N*,*N*-dimethylamino)biphenyl (DCPAB)[12] another electron rich biphenyl based phosphane, gave a similar result as we obtained with DTPB (Table 1, compare entries 7 and 8). We decided to continue our studies with the latter since DCPAB is substantially more expensive than DTPB.[13] As could be expected the coupling of the less electron rich 4-chlorotoluene with dibutylamine gave a higher yield than 4-chloroanisole (Table 1, compare entries 7 and 10). Under the same conditions as used in entry 7 (1 mol% catalyst, 150 °C, 10 min) we also tried to couple benzylamine and hexylamine with 4-chloroanisole. Since primary aliphatic amines are known to give diarylation, we used a larger excess of amine (1.5 equiv of amine instead of 1.2 equiv) to suppress the formation of undesired diarylated compound. Gratifyingly, *N*-benzyl-4-methoxyaniline and *N*-hexyl-4-methoxyaniline could be obtained in excellent yields (81 and 86%, respectively) (Table 1, entries 11 and 14). The use of 3 equiv of amine did not further significantly increase the yields (Table 1, entries 12 and 15). Reinvestigation of the ligand DCPB for the coupling of 4-chloroanisole with

benzylamine and hexylamine, respectively, showed inferior results in comparison with DTBP as the ligand (entries 13 and 16). Interestingly, a literature search revealed that hitherto for the synthesis of *N*-benzyl-4-methoxyaniline (93%, 1 mol% catalyst, 4 h) and *N*-hexyl-4-methoxyaniline (92%, 2 mol% catalyst, 18 h) from 4-chloroanisole only one palladium-catalyzed amination protocol has been reported using pentaphenylferrocenyl di-*tert*-butylphosphine (PPFDTBP) as the ligand for the catalyst.[14] Although, we obtained somewhat lower yields than Hartwig's group our protocol has the important advantage that high conversion rates are obtained for the synthesis of *N*-benzyl-4-methoxyaniline and *N*-hexyl-4-methoxyaniline. For the former, we observed a moderate speed-up by a factor 24 while the conversion time of the latter increases more than 100 times. Moreover, in this last mentioned case we used only 1 mol% Pd/2 DTPB catalyst while 2 mol% Pd/2 PPFDTBP was used in the literature procedure.

Finally, we investigated if the same protocol developed for aryl chlorides could also be used for aryl bromides.[15,16] This is an important aspect when aiming a generally applicable protocol for incorporation in high-throughput medicinal chemistry programs since aryl bromide substrates are also easily accessible. This would seriously expand the scope of our microwave amination method. Again electron rich substrates (3- and 4-bromoanisole) were selected as test substrates. First, we investigated the amination of 3- and 4-bromoanisole with *N*-methylaniline. Gratifyingly, we found that 90% of 3-methoxy-*N*-methyl-*N*-phenylaniline and 89% of 4-methoxy-*N*-methyl-*N*-phenylaniline were obtained under the standard conditions used for aryl chlorides (1 mol% Pd/2 DCPB, 150 °C, 10 min) (Table 2, entries 1 and 2). Under these conditions also morpholine could be efficiently coupled with 3- and 4-bromoanisole (Table 2, entries 3 and 4). Next, we investigated the amination of 4-bromoanisole with dibutylamine. Interestingly, in this case the DCPB ligand gave a better result than DTPB (Table 2, entries 5 and 6), while the reverse was observed in the related coupling starting from 4-chloroanisole (Table 1, entries 4 and 7). The use of DCPAB as ligand gave a similar yield as obtained with DCPB (Table 2, compare entries 5 and 7). We also tried to couple primary aliphatic amines (benzylamine and hexylamine) with 4-bromoanisole. As mentioned before a larger excess of amine is required for primary amines in order to suppress the formation of undesired diarylated compound. For the coupling of primary aliphatic amines with 4-bromoanisole we immediately used 3 equiv of amine without investigating if a smaller excess would be sufficient. Amination of 4-bromoanisole with benzylamine using DTPB (87%) gave a higher yield than when DCPB (61%) was used (Table 2, entries 9 and 8). Finally, Buchwald–Hartwig amination of 4-bromoanisole with hexylamine, using DTPB as ligand for the catalyst, gave 79% of *N*-hexyl-4-methoxyaniline (Table 2, entry 10).

To investigate a possible existence of non-thermal MW effects,[10h] we executed all the coupling reactions optimized for MW irradiation also in an oil bath at the same temperature as the set temperature of the MW experiments. To allow a reliable comparison the same vessels as used in the MW runs were used. To mimic the flash heating rate of the MW experiments the loaded vessels were immersed in a preheated oil bath. Rapid cooling of the vessels was done in the MW cavity using a propelled air flow. All the oil bath experiments performed, clearly revealed a similar yield as obtained under MW irradiation in the same reaction time (Tables 1 and 2). These results indicate that, for this type of reaction, no specific MW effects have to be taken into account to explain the rapid aminations.[23] The studied MW-assisted reactions are only governed by thermal effects (Arrhenius). Nevertheless, from a practical point of view, the microwave-assisted procedure is still more convenient than classical heating.

In conclusion, we have described general Pd-catalyzed amination conditions for the high-speed coupling of electron rich and neutral aryl chlorides with all types of aliphatic amines under temperature-controlled MW heating at 150 °C with DCPB (secondary cyclic aliphatic amines) or DTBP (acyclic secondary and primary aliphatic amines) as ligand using a low loading of catalyst in only 10 min. Interestingly, the method developed for aryl chlorides can also be applied for the amination of aryl bromides.

1. Experimental

1.1. General

^{1}H NMR spectra were recorded on a Varian Unity 400 spectrometer in the solvent indicated with TMS as the internal standard. All chemical shifts are given in ppm and coupling constants are given in Hz. For column chromatography Kieselgel 60 (ROCC, 0.040–0.063 mm) was used. $Pd(OAc)_2$ (Acros), $Pd_2(dba)_3$ (Acros), DCPB (Strem Chemicals or Acros), DTPB (Strem Chemicals or Acros), DCPAB (Strem Chemicals or Acros), toluene (Acros, extra dry <30 ppm water) as well as all the amines and aryl halides were obtained from commercial sources and used as such.

1.2. General procedure for the rapid palladium-catalyzed amination of aryl halides

A pressure vial of 10 mL was charged with aryl halide (1 mmol), amine (1.2, 1.5 or 3 mmol) and NaO*t*-Bu (0.1345 g, 1.4 mmol) in air. Subsequently the vial was flushed with Ar for 1 min. Then, 1 mL of a stock solution[†] of catalyst was added via a syringe and the resulting mixture

[†] Preparation of the stock solutions of catalyst: the stock solutions of catalyst (Pd/2 L or Pd/1.5 L) were prepared using $Pd(OAc)_2$ or $Pd_2(dba)_3$ as Pd(0) source and DCPB [=2-(dicyclohexylphosphanyl)biphenyl], DTPB [=2-(di-*t*-butylphosphanyl)biphenyl] or DCPAB [=2-dicyclohexylphosphanyl-2′-(*N*,*N*-dimethylamino)biphenyl] as ligand. The stock solutions were stored under an Ar atmosphere. When DTPB was used, the catalyst solution was stirred for 16 h prior to its use.[19]

$Pd(OAc)_2$/DCPB or DTPB. 1 mol% catalyst solution: $Pd(OAc)_2$ (0.0225 g, 0.1 mmol), ligand (0.2 mmol) and toluene (10 mL) were used. 2 mol% catalyst solution: $Pd(OAc)_2$ (0.0449 g, 0.2 mmol), ligand (0.4 mmol) and toluene (10 mL) were used.

$Pd_2(dba)_3$/DCPAB stock solution. 1 mol% catalyst solution: $Pd_2(dba)_3$ (0.0458 g, 0.05 mmol), DCPAB (0.0590 g, 0.15 mmol) and toluene (10 mL) were used.

stirred and flushed with Ar for an additional 2 min. Next, the vial was sealed with an Al crimp cap with septum and heated at the desired temperature (Tables 1 and 2) in a CEM Discover microwave apparatus. The set power for all experiments was 300 W. The total heating time for all reactions was 10 min. After the reaction vial was cooled down to room temperature using a propelled air flow, it was opened and filtered over Celite and rinsed well with 100 mL dichloromethane. The filtrate was subsequently evaporated under reduced pressure and the residue purified by flash column chromatography on silica gel.

The following compounds were prepared in this manner.

1.2.1. 4-Phenylmorpholine (Table 1, entry 1). Eluent for flash column chromatography: CH_2Cl_2. The characterization data obtained for 4-phenylmorpholine are identical to those previously reported in the literature.[20] For comparison, we report here our ^{1}H NMR data: δ_H ($CDCl_3$): 7.28 (dd, J=8.8, 7.3 Hz, 2H), 6.92 (d, J=8.1 Hz, 2H), 6.88 (t, J=7.3 Hz, 1H), 3.86 (br t, J=4.7 Hz, 4H), 3.16 (br t, J=4.7 Hz, 4H).

1.2.2. 4-(4-Methylphenyl)morpholine (Table 1, entry 2). Eluent for flash column chromatography: CH_2Cl_2/heptane (90/10). The characterization data obtained for 4-(4-methylphenyl)morpholine are identical to those previously reported in the literature.[17] For comparison, we report here our ^{1}H NMR data: δ_H ($CDCl_3$): 7.08 (d, J=8.3 Hz, 2H), 6.83 (d, J=8.3 Hz, 2H), 3.85 (br t, J=4.8 Hz, 4H), 3.11 (br t, J=4.8 Hz, 4H), 2.27 (s, 3H).

1.2.3. 4-(4-Methoxyphenyl)morpholine (Table 1, entry 3; Table 2, entry 4). Eluent for flash column chromatography: CH_2Cl_2/heptane (90/10). The characterization data obtained for 4-(4-methoxyphenyl)morpholine are identical to those previously reported in the literature.[17] For comparison, we report here our ^{1}H NMR data: δ_H ($CDCl_3$): 6.91–6.83 (m, 4H), 3.86 (br t, J=4.8 Hz, 4H), 3.77 (s, 3H), 3.05 (br t, J=4.8 Hz, 4H).

1.2.4. *N*,*N*-Dibutyl-4-methoxyaniline (Table 1, entry 7; Table 2, entry 5). Eluent for flash column chromatography: CH_2Cl_2/heptane (90/10). The characterization data obtained for *N*,*N*-dibutyl-4-methoxyaniline are identical to those previously reported in the literature.[14] For comparison, we report here our ^{1}H NMR data: δ_H ($CDCl_3$): 6.80 (d, J=9.0 Hz, 2H), 6.65 (d, J=9.0 Hz, 2H), 3.75 (s, 3H), 3.17 (br t, J=7.5 Hz, 4H), 1.51 (p, J=7.5 Hz, 4H), 1.33 (sx, J=7.4 Hz, 4H), 0.93 (t, J=7.3 Hz, 6H).

1.2.5. *N*,*N*-Dibutyl-4-methylaniline (Table 1, entry 10). Eluent for flash column chromatography: CH_2Cl_2/heptane (1/1). The characterization data obtained for *N*,*N*-dibutyl-4-methylaniline are identical to those previously reported in the literature.[17] For comparison, we report here our ^{1}H NMR data: δ_H ($CDCl_3$): 6.99 (d, J=8.7 Hz, 2H), 6.57 (d, J=8.7 Hz, 2H), 3.22 (br t, J=7.5 Hz, 4H), 2.23 (s, 3H), 1.54 (p, J=7.3 Hz, 4H), 1.33 (sx, J=7.5 Hz, 4H), 0.94 (t, J=7.3 Hz, 6H).

1.2.6. *N*-Benzyl-4-methoxyaniline (Table 1, entry 11; Table 2, entry 9). Eluent for flash column chromatography: CH_2Cl_2/heptane (7/3). The characterization data obtained for *N*-benzyl-4-methoxyaniline are identical to those previously reported in the literature.[14] For comparison, we report here our ^{1}H NMR data: δ_H ($CDCl_3$): 7.38–7.31 (m, 4H), 7.27 (t, J=7.0 Hz, 1H), 6.77 (d, J=9.0 Hz, 2H), 6.61 (d, J=9.0 Hz, 2H), 4.28 (s, 2H), 3.8 (br s, 1H), 3.74 (s, 3H).

1.2.7. *N*-Hexyl-4-methoxyaniline (Table 1, entry 14; Table 2, entry 10). Eluent for flash column chromatography: CH_2Cl_2/heptane (8/2). The characterization data obtained for *N*-hexyl-4-methoxyaniline are identical to those previously reported in the literature.[14] For comparison, we report here our ^{1}H NMR data: δ_H ($CDCl_3$): 6.77 (d, J=9.0 Hz, 2H), 6.56 (d, J=9.0 Hz, 2H), 3.74 (s, 3H), 3.3 (br s, 1H), 3.06 (t, J=7.1 Hz, 2H), 1.59 (p, J=7.4 Hz, 2H), 1.43–1.1.29 (m, 6H), 0,90 (t, J=7.0 Hz, 3H).

1.2.8. 3-Methoxy-*N*-methyl-*N*-phenylaniline (Table 2, entry 1). Eluent for flash column chromatography: CH_2Cl_2/heptane (6/4). The characterization data obtained for 3-methoxy-*N*-methyl-*N*-phenylaniline are identical to those previously reported in the literature.[21] For comparison, we report here our ^{1}H NMR data: δ_H ($CDCl_3$): 7.28 (dd, J=8.4, 7.3 Hz, 2H), 7.15 (t, J=8.1 Hz, 1H), 7.06 (dd, J=8.7, 1.1 Hz, 2H), 6.99 (tt, J=7.3, 1.1 Hz, 1H), 6.58 (ddd, J=8.1, 2.3, 0.8 Hz, 1H), 6.53 (t, J=2.3 Hz, 1H), 6.49 (ddd, J=8.1, 2.3, 0.8 Hz, 1H), 3.75 (s, 3H), 3.30 (s, 3H)

1.2.9. 4-Methoxy-*N*-methyl-*N*-phenylaniline (Table 2, entry 2). Eluent for flash column chromatography: CH_2Cl_2/heptane (1/1). The characterization data obtained for 4-methoxyphenyl-*N*-methyl-*N*-phenylaniline are identical to those previously reported in the literature.[18] For comparison we report here our ^{1}H NMR data: δ_H ($CDCl_3$): 7.19 (dd, J=9.0, 7.2 Hz, 2H), 7.08 (d, J=9.0 Hz, 2H), 6.88 (d, J=9.0 Hz, 2H), 6.79 (d, J=7.2 Hz, 2H), 6.78 (t, J=6.8 Hz, 1H), 3.80 (s, 3H), 3.25 (s, 3H).

1.2.10. 4-(3-Methoxyphenyl)morpholine (Table 2, entry 3). Eluent for flash column chromatography: CH_2Cl_2. The characterization data obtained for 4-(3-methoxyphenyl)-morpholine are identical to those previously reported in the literature.[14] For comparison, we report here our ^{1}H NMR data: δ_H ($CDCl_3$): 7.19 (t, J=8.2 Hz, 1H), 6.53 (br d, J=8 Hz, 1H), 6.46 (br s, 1H), 6.45 (br d, J=8 Hz, 1H), 3.85 (t, J=4.8 Hz, 4H), 3.80 (s, 3H), 3.15 (t, J=4.8 Hz, 4H).

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22. In control microwave experiments the amination reactions studied were also executed without the use of Pd-catalyst under otherwise identical reaction conditions. The obtained results clearly indicate that these reactions require the use of transition metal catalyst.
23. It was not attempted to check whether the studied microwave-assisted aminations were not already finished before 10 minutes. Theoretically, the possibility of a specific microwave effect can therefore not be ruled out. Nevertheless, if there is such a microwave effect, it is surely very small.

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Synthesis of a biotinated amphiphile

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Abstract—The synthesis of a biotinated amphiphile assembled from D-(+)-biotin, ethylene diamine as spacer, galactaric acid and 1-dodecylamine was achieved in six steps. The key step was the synthesis of a bisacetonide protected galactaric ester, the structure of which was determined by X-ray analysis. Aminolysis, spacer attachment, coupling with biotin and deprotection led to the amphiphilic galactaramide.

1. Introduction

The controlled self-assembly of specific amphiphilic molecules, leading to defined and stable bilayer systems and micelles, represents a challenge for fundamental research and has potential nanotechnological applications. To attain this goal a tailor-made design of amphiphilic building blocks containing encoded structural information on their potential specific supramolecular assembly is needed. In the course of our investigations in the field of artificial amphiphils and liposomes,[1–3] the preparation of the biotinated amphiphilic galactaramide **8** was designed. Similar to long-chain alkylaldonamides, which form micellar fibrous aggregates,[4,5] the *N*-dodecylgalactaramide with its hydrophobic aliphatic dodecyl chain serves as the amphiphilic scaffold. The hydrophilic aldaric acid moiety is expected to induce specific aggregation motifs within micelles or liposomes. The biotin unit, attached by a spacer to the second carboxylic group of galactaric acid (mucic acid), should act as a transmembrane anchor in intercalation experiments into synthetic lecitin bilayer membranes[2a] and act as a biological marker towards streptavidin and avidin.[6]

2. Results and discussion

Galactaric acid (**1**) was transformed into dimethyl galactarate (**2**) with a mixture of methanol and concd sulfuric acid under reflux conditions[7] in 89% yield (Scheme 1). The resulting dimethyl ester **2** was further protected as dimethyl (2,3:4,5-di-*O*-isopropylidene)galactarate (**3**) by heating in dry acetone with anhydrous ferric chloride[8] obtaining a brown oil after flash chromatography on silica gel with chloroform/methanol 1:1, and methanol. This brown oil could be transformed into colourless crystals when washed with diethylether, methanol and chloroform in 27% yield (Scheme 1). The application of anhydrous HCl[7] instead of anhydrous ferric chloride for this protection reaction was not successful.

In general, five-membered 1,3-dioxolanes are preferred over six-membered 1,3-dioxanes,[9] when acyclic sugar compounds are protected with acetone as their isopropylidene derivatives. The structure of **3** was unambiguously proven by X-ray single crystal analysis (Fig. 1) and is in contrast with the report of Butler et al., who claimed to have isolated the 2,4:3,5-diisopropylidenegalactarate isomer from the reaction with acetone and anhydrous HCl.[7]

The subsequent aminolysis of dimethyl ester **3** with 1-dodecylamine in the presence of sodium methanolate/methanol afforded a mixture of dicarboxamide diisopropylidenegalactardidodecylamide (**5**) and of racemic monocarboxamide methyl (2,3:4,5-di-*O*-isopropylidene) galactar-*N*-dodecylamide (**4**) (Scheme 2). The major product **4** was isolated as colourless oil by flash chromatography on silica gel with hexane/acetone 4:1 as eluent in 26% yield. The compound froze at −20 °C affording a white solid. The minor product **5** was obtained in 14% yield.

To attach a spacer unit, **4** was subsequently heated with ethylene diamine in dry dichloromethane for 25 h (Scheme 3) and purified by flash chromatography on silica gel with acetone/methanol 4:1 to obtain (2,3;4,5)-di-*O*-isopropylidenegalactardi-*N*-(2-aminoethyl)-*N′*-dodecylamide (**6**) in 62% yield from a yellow oil which slowly

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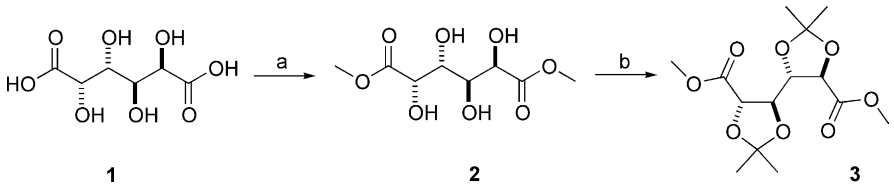


Scheme 1. Synthesis of the (2,3;4,5)bisacetonide protected dimethyl galactarate **3**. (a) CH_3OH, H_2SO_4, reflux, 3 days; (b) acetone, $FeCl_3$, reflux, 2.5 h.

crystallizes into a slightly yellow solid. Product **6** and D-(+)-biotin were coupled with *N*,*N*′-carbonyldiimidazole (CDI) in dimethyl formamide at ambient temperature (Scheme 3).[10] After separation by flash chromatography, the resulting oil was dissolved in chloroform, the solution extracted with water, and a diastereomeric mixture of *N*-[2-({5-[(3a*S*,4*R*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazole-4-yl]pentanoyl}amino)ethyl]-*N*′-dodecyl (2,3;4,5-di-*O*-isopropylidene)galactaric diamide (**7**) was obtained in 79% yield as light yellow crystals. Finally, the deprotection of **7** succeeded with a mixture of trifluoroacetic acid/water 10:1 and the target molecule *N*-[2-({3a*S*,4*R*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazole-4-yl]pentanoyl}amino)ethyl]-*N*′-dodecyl galactaric diamide (**8**) precipitated by addition of diethylether in 70% yield (Scheme 3).

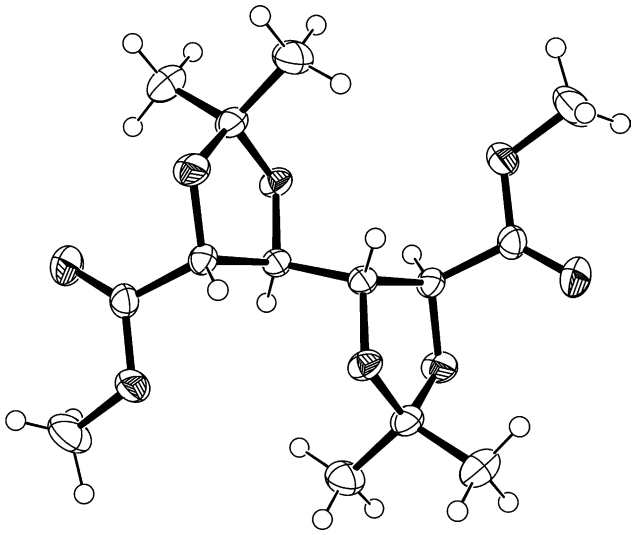
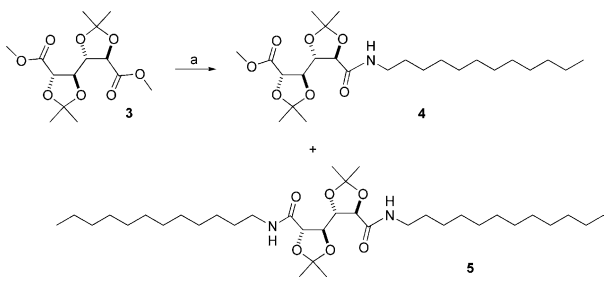


Figure 1. ORTEP-plot of dimethyl (2,3:4,5-di-*O*-isopropylidene)galactarate (**3**).



Scheme 2. Aminolysis of **3** to *N*-dodecyl monoamide **4** and *N*,*N*′-dodecyl diamide **5** (only one of the two enantiomers of **4** is given in the scheme). (a) NH_2–$(CH_2)_{11}$–CH_3, $NaOCH_3$, CH_3OH, room temperature, 21 h.

3. Conclusion

The synthesis of the biotinated glycolipid **8** was accomplished in six steps. This compound is an example of a new class of possible liposome forming substances that could be employed in the delivery of drugs in the body either by using them alone or as highly functionalized additives to conventional micelle or liposome producing amphiphiles such as phosphorglycerides. The chiral information in the sugar scaffold might be able to induce the formation of chiral assemblies such as micelles, vesicles, and even fibre-like structures with interesting properties due to the flexibility these molecules have in terms the biotin anchor.

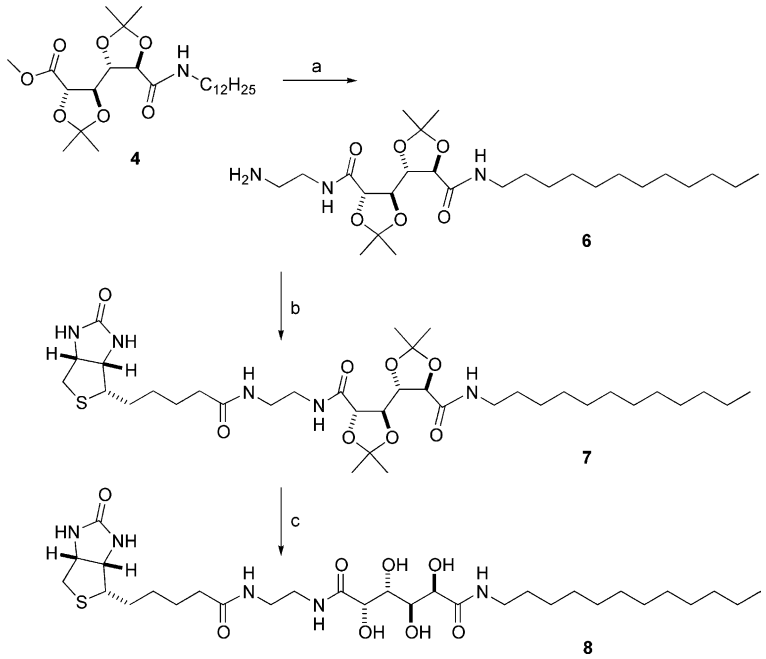
4. Experimental

4.1. General

All chemicals were distilled or dried prior to use. Chromatography was performed on Cellulose MN 2100ff from Macherey-Nagel (Düren). Differential Scanning Calorimetry was done with the DSC821^e, Mettler Toledo (Gießen). NMR spectra were determined with JNM EX 400, JNM GX 400, and ALPHA 500 spectrometers, JEOL (Tokyo, Japan). X-ray analysis was performed with the X-ray diffractometer Kappa CCD, Nonius (Solingen). Melting points were determined with an IA 9100, Electrothermal (Southend-on-Sea, UK) and are uncorrected.

4.1.1. Galactaric acid dimethyl ester (2). Galactaric acid (**1**) (40.0 g, 190 mmol) was suspended in methanol (190 mL), concd sulfuric acid (3.0 mL) was added under vigorous stirring and the mixture heated to reflux. After 3 days at this temperature, the mixture was stirred at room temperature for another 1.5 days. Finally, the mixture was cooled to 0 °C for two days. The white precipitate was filtered off and washed several times with water. After drying on a high vacuum pump **2** was yielded as a white solid (40.3 g, 169 mmol, 89%). Mp 189 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.63 (s, 6H, OMe), 3.77 (m, 2H, O*H*), 4.30 (d, J=8.1 Hz, 2H, C*H*–OH), 4.80 (m, 2H, O*H*), 4.91 (d, 2H, J=8.1 Hz, C*H*–OH); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 51.43, 70.27, 71.20, 174.11; IR (KBr) 3345, 3265, 3120, 2966, 2923, 1726, 1384, 1288, 1119, 1052 cm^{-1}; EI-MS *m*/*z* 239 (M^++H), 221 (M^++H−H_2O). Anal. Calcd for $C_8H_{14}O_8$: C 40.34, H 5.92. Found: C 40.15, H 5.91.

4.1.2. Dimethyl (2,3:4,5-di-*O*-isopropylidene)galactarate (3). Galactaric acid dimethyl ester (**2**) (20.0 g, 84.0 mmol) was suspended in dry acetone (1 L). To this mixture anhydrous ferric chloride (4.54 g, 28.0 mmol) was added, whereupon the mixture immediately turned yellow. The colour darkened while heating to reflux and the starting



Scheme 3. Final steps in the synthesis of **8** (only one of the possible enantiomers or diastereomers of **6**, **7** and **8** is drawn in the scheme). (a) NH_2–CH_2–CH_2–NH_2, CH_2Cl_2, reflux, 25 h; (b) D-(+)-biotin, CDI, DMF, room temperature, 24 h; (c) TFA/H_2O 10:1, room temperature, 4 h.

material dissolved within 45 min. The reaction mixture was refluxed 2.5 h then cooled to room temperature. Treatment with a 10% aqueous potassium carbonate solution (200 mL) gave a dark residue. The mixture was then evaporated in vacuo from acetone and extracted with chloroform (3×100 mL). The combined organic layers were washed with water (3×50 mL), dried by passing the solution through a magnesium sulfate column and the solvent removed in vacuo. The resulting brown oil was purified by flash chromatography (silica gel; (1) chloroform/methanol 1:1, (2) methanol 100%) to give a thin brown oil from which **3** was obtained as colourless crystals (7.27 g, 22.8 mmol, 27%) by washing with diethylether, methanol and chloroform. Mp 98 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.38 (s, 6H, Me), 1.44 (s, 6H, Me), 3.76 (s, 6H, OMe), 4.44 (m, 2H, C*H*–O), 4.56 (m, 2H, C*H*–O); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 25.84, 26.89, 52.52, 75.69, 78.90, 112.22, 171.36; IR (KBr) 3498 (w), 3029, 2988, 2959, 2941, 1759, 1375, 1219, 1126, 854 cm^{-1}; FAB-MS (NBA) *m/z* 319 (M^++H), 261 (M^++H−acetone). Anal. Calcd for $C_{14}H_{22}O_8$: C 52.82, H 6.97. Found: C 52.46, H 6.94.

4.1.3. (2,3:4,5-Di-*O*-isopropylidene)galactaric acid methyl ester *N*-dodecyl amide (4), *N*,*N*′-didodecyl (2,3:4,5-di-*O*-isopropylidene)galactaric acid diamide (5). Under an atmosphere of nitrogen sodium metal (80.0 mg, 3.48 mmol) was dissolved in dry methanol (5 mL), and 1-dodecylamin (582 mg, 3.14 mmol) was added. Upon dissolution, this mixture was added dropwise to a solution of dimethyl (2,3:4,5-di-*O*-isopropylidene) galactarate (**3**) (1.00 g, 3.14 mmol) in dry methanol (10 mL) within 1 h. After 21 h stirring at room temperature under an atmosphere of nitrogen the reaction mixture was 50% evaporated in vacuo. After 1.5 days in total the reaction was stopped to avoid formation of the disubstituted product. Evaporation of the solvent in vacuo was followed by separation of the light orange oil by flash chromatography (silica gel; hexane/acetone 4:1). Monoamide **4** was recovered first as colourless oil (387 mg, 0.821 mmol, 26%). The compound froze at −20 °C affording a white solid. Diamide **5** eluted second as white solid (283 mg, 0.453 mmol, 14%).

4.1.3.1. (2,3:4,5-Di-*O*-isopropylidene)galactaric acid methyl ester *N*-dodecyl amide (4). Mp (DSC) 38 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (t, J=6.8 Hz, 3H, Me), 1.23–1.27 (m, 20H, CH_2), 1.40 (s, 3H, Me), 1.41 (s, 3H, Me), 1.486 (s, 3H, Me), 1.488 (s, 3H, Me), 3.25 (m, 2H, CH_2–NH), 3.77 (s, 3H, OMe), 4.47 (m, 2H, C*H*–O), 4.56 (dd, J=6.8, 2.2 Hz, 1H, C*H*–O), 4.93 (d, J=6.8 Hz, 1H, C*H*–O), 6.59 (t, J=5.7 Hz, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 14.08, 22.65, 25.59, 26.05, 26.65, 26.78, 26.87, 29.21, 29.32, 29.54, 29.59, 31.87, 39.04, 52.46, 74.68, 75.19, 78.37, 79.01, 111.11, 111.79, 170.69, 171.66; IR (KBr) 3427, 3356, 2989, 2926, 2855, 1763, 1736, 1671, 1530, 1383, 1211, 1103, 866 cm^{-1}; FAB-MS (NBA) *m/z* 472 (M^+), 414 (M^+−acetone), 356 (M^+−2 acetone). Anal. Calcd for $C_{25}H_{45}NO_7$: C 63.67, H 9.62, N 2.97. Found: C 63.20, H 9.55, N 2.95.

4.1.3.2. *N*,*N*′-Didodecyl (2,3:4,5-di-*O*-isopropylidene)-galactaric acid diamide (5). Mp 89 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (t, J=6.8 Hz, 6H, Me), 1.23–1.28 (m, 40H, CH_2), 1.40 (s, 6H, Me), 1.48 (s, 6H, Me), 3.23 (m, 4H, CH_2–NH), 4.48 (d, J=7.1 Hz, 2H, C*H*–O), 4.72 (d, J=7.1 Hz, 2H, C*H*–O), 6.62 (t, J=5.7 Hz, 2H, N*H*); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 14.10, 22.67, 25.95, 26.74, 26.89, 29.24, 29.32, 29.55, 29.61, 31.89, 38.96, 74.95, 78.66, 110.69, 170.65; IR (KBr) 3374, 2989, 2921, 2852, 1654, 1533, 1209, 872 cm^{-1}; FAB-MS (NBA) *m/z* 1250 (2(M^++H)), 625 (M^++H), 567 (M^++H−acetone), 509 (M^++H−2 acetone). Anal. Calcd for $C_{36}H_{68}N_2O_6$: C 69.19, H 10.97, N 4.48. Found: C 69.04, H 10.93, N 4.41.

4.1.4. *N*-(2-Aminoethyl)-*N′*-dodecyl (2,3:4,5-di-*O*-isopropylidene)galactaric acid diamide (6). Ethylene diamine (0.61 mL, 549 mg, 9.13 mmol) was dissolved in dry dichloromethane (5 mL). (2,3:4,5-Di-*O*-isopropylidene) galactaric acid methyl ester *N*-dodecyl amide (**4**) (287 mg, 0.609 mmol) in dry dichloromethane (5 mL) was added and the reaction mixture was stirred under an atmosphere of nitrogen at reflux for 25 h (TLC-control: silica gel; acetone/methanol 4:1). The solvent was removed in vacuo. Purification of the orange oil by flash chromatography (silica gel; acetone/methanol 4:1) gave a yellow oil (187 mg, 0.374 mmol, 62%), turning to a yellow solid **6**. Mp 59 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.85 (t, J=6.9 Hz, 3H, Me), 1.21–1.27 (m, 20H), 1.39 (s, 3H, Me), 1.42 (s, 3H, Me), 1.47 (s, 3H, Me), 1.48 (s, 3H, Me), 2.97 (t, J=5.8 Hz, 2H, C*H*$_2$–NH$_2$), 3.23 (m, 2H, C*H*$_2$–NH), 3.40 (m, 1H, C*H*$_2$–NH), 3.50 (m, 1H, C*H*$_2$–NH), 3.82 (br, 2H, N*H*$_2$), 4.46 (dd, J=7.5, 2.0 Hz, 1H, C*H*–O), 4.50 (dd, J=7.5, 2.0 Hz, 1H, C*H*–O), 4.67 (d, J=7.0 Hz, 1H, C*H*–O), 4.73 (d, J=7.3 Hz, 1H, C*H*–O), 6.66 (t, J=5.7 Hz, 1H, N*H*), 7.34 (t, J=6.2 Hz, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 14.10, 22.66, 25.94, 26.01, 26.76, 26.90, 29.26, 29.32, 29.49, 29.51, 29.56, 29.60, 29.62, 31.89, 39.02, 39.86, 40.76, 74.95, 75.06, 78.59, 78.71, 110.83, 110.94, 170.81, 171.83; IR (film, NaCl) 3424, 3338, 2986, 2926, 2855, 1666, 1529, 1382, 1213, 1087, 877 cm^{-1}; FAB-MS (NBA) *m*/*z* 540 (M+H+K$^+$), 500 (M$^+$). Anal. Calcd for $C_{26}H_{49}N_3O_6$: C 62.50, H 9.88, N 8.41, O 19.21. Found: C 62.16, H 9.94, N 8.98, O 18.93.

4.1.5. *N*-[2-({5-[(3a*S*,4*R*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazole-4-yl]pentanoyl}amino)ethyl]-*N′*-dodecyl (2,3:4,5-di-*O*-isopropylidene)galactaric acid diamide (7). D-(+)-Biotin (68.5 mg, 0.280 mmol) was dissolved in dimethyl formamide (2.5 mL) at 60 °C. After cooling to room temperature, *N*,*N′*-carbonyldiimidazole (45.4 mg, 0.280 mmol) was added under an atmosphere of nitrogen and the mixture was stirred until CO_2 evolution stopped (7 h). To the cloudy mixture was added *N*-(2-aminoethyl)-*N′*-dodecyl (2,3:4,5-di-*O*-isopropylidene) galactaric acid diamide (**6**) (140.0 mg, 0.280 mmol) in dimethyl formamide (3.5 mL) and the clear solution was stirred for 24 h at room temperature. After removal of dimethyl formamide in vacuo at 60 °C, the yellow oil was separated by flash chromatography (silica gel; acetone/methanol 4:1). The resulting oil was dried, and the imidazole sublimed at 50 °C under high vacuum. The yellow oil was dissolved in chloroform (5 mL) and extracted with water (3×5 mL). Evaporation of the solvent in vacuo and drying under high vacuum provided light yellow crystals (164 mg, 0.223 mmol, 79%) of **7**. Mp 158 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.84 (t, J=6.8 Hz, 3H, Me), 1.22–1.26 (m, 20H, C*H*$_2$), 1.38 (s, 3H, Me), 1.39 (s, 3H, Me), 1.42–1.48 (m, 2H, C*H*$_2$), 1.45 (s, 3H, Me), 1.46 (s, 3H, Me), 1.60–1.66 (m, 4H, C*H*$_2$), 2.18 (t, J=7.3 Hz, 2H, C*H*$_2$), 2.70 (d, J=12.9 Hz, 1H, C*H*$_2$–S), 2.85 (dd, J=12.8, 4.8 Hz, 1H, C*H*$_2$–S), 3.09 (m, 1H, C*H*–S), 3.21 (m, 2H, C*H*$_2$–NH), 3.36 (m, 4H, C*H*$_2$–NH), 4.28 (m, 1H, C*H*–NH), 4.43 (m, 2H, C*H*–O), 4.48 (m, 1H, C*H*–NH), 4.67 (m, 2H, C*H*–O), 6.16 (br, 1H, N*H*), 6.66 (t, J=6.0 Hz, 0.5H, N*H*), 6.70 (t, J=6.0 Hz, 0.5H, N*H*), 6.97 (br, 0.5H, N*H*), 7.05 (br, 0.5H, N*H*), 7.21 (m, 1H, N*H*), 7.26 (m, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 101 MHz) (values in brackets indicate chemical shifts of the minor diastereomer) δ 14.06, 22.61, 25.61, 25.66, 25.81, 25.88, 25.95, 26.67, 26.70, 26.87, 28.02, 28.17, 29.22, 29.28, 29.48, 29.52, 29.57, 31.84, 35.82, 38.98, 39.11, 39.18, 40.50, 55.75, 60.32, 61.64, 74.84, 74.92, 78.66, 78.72, 110.87, 110.98, 164.37 (164.42), 170.76, 171.93 (171.99), 174.14 (174.18); IR (KBr) 3419, 3322, 3089, 2986, 2927, 2855, 1701, 1663, 1535, 1262, 1089, 803 cm^{-1}; FAB-MS (NBA) *m*/*z* 726 (M$^+$). Anal. Calcd for $C_{36}H_{64.2}N_5O_{8.6}S$ (**7**+0.6H_2O): C 58.69, H 8.78, N 9.51, S 4.35. Found: C 58.58, H 8.51, N 9.45, S 4.40.

4.1.6. *N*-[2-({5-[(3a*S*,4*R*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazole-4-yl]pentanoyl}amino)ethyl]-*N′*-dodecyl galactaric acid diamide (8). *N*-[2-({5-[(3a*S*,4*R*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazole-4-yl]pentanoyl}amino)ethyl]-*N′*-dodecyl-(2,3:4,5-di-*O*-isopropylidene)galactaric acid diamide (**7**) (70 mg, 0.096 mmol) was dissolved in trifluoroacetic acid/water 10:1 (1.1 mL) and stirred for 4 h at room temperature. After evaporation of trifluoroacetic acid in vacuo, diethylether was added whereupon a white solid formed which was centrifuged and washed with diethylether (2×) and chloroform (3×). Drying at 85 °C for 30 min in the drying oven and 1.5 days under high vacuum resulted in a white powder (45 mg, 0.067 mmol, 70%) of **8** containing water. Mp 167 °C (decomp.); ^{1}H NMR (DMSO-d_6, 400 MHz, 60 °C) δ 0.85 (t, J=6.8 Hz, 3H, Me), 1.27 (s, 20H, C*H*$_2$), 1.36 (m, 2H, C*H*$_2$), 1.44 (m, 2H, C*H*$_2$), 1.54 (m, 2H, C*H*$_2$), 2.08 (t, J=7.2 Hz, 2H, C*H*$_2$), 2.62 (d, J=12.2 Hz, 1H, C*H*$_2$–S), 2.85 (dd, J=12.3, 5.2 Hz, 1H, C*H*$_2$–S), 3.05 (m, 1H, C*H*–S), 3.12 (m, 2H, C*H*$_2$–NH), 3.19 (m, 4H, C*H*$_2$–NH), 3.80 (br, 2H, O*H*), 4.15 (m, 1H, C*H*–NH; 4H, C*H*–OH), 4.32 (m, 1H, C*H*–NH), 4.84 (br, 2H, O*H*), 6.05 (br, 2H, N*H*), 7.33 (br, 1H, N*H*), 7.49 (br, 1H, N*H*), 7.52 (br, 1H, N*H*); ^{13}C NMR (DMSO-d_6, 101 MHz, 90 °C) δ 13.19, 21.43, 24.63, 25.89, 27.63, 28.06, 28.23, 28.41, 28.68, 30.71, 34.90, 38.02, 38.17, 38.88, 39.08, 39.92, 54.74, 59.03, 60.78, 70.49, 70.56, 70.71, 162.15, 171.94, 172.43, 173.00; IR (KBr) 3310, 3100, 2923, 2853, 1689, 1648, 1544, 1466, 1266, 1114, 1044, 722, 648 cm^{-1}; MALDI-TOF-MS *m*/*z* 669 (M+Na$^+$); FAB-MS (NBA) *m*/*z* 646 (M$^+$). Anal. Calcd for $C_{30}H_{57.8}N_5O_{9.4}S$ (**8**+1.4H_2O): C 53.69, H 8.68, N 10.44, S 4.78. Found: C 53.53, H 8.13, N 10.93, S 4.83.

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Synthesis of new *N*-aryl oxindoles as intermediates for pharmacologically active compounds

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Abstract—Various new *N*-aryl oxindoles were synthesized as intermediates for the preparation of pharmacologically active 2-(*N*-arylamino)-phenylacetic acids. Two novel approaches were explored for the construction of diarylamine and *N*-aryl oxindole core structures, in addition to Buchwald-arylamination and Smiles rearrangement. Condensation of anilines with 2-oxo-cyclohexylidene-acetic acid derivatives and subsequent dehydrogenation is a new and viable method for the preparation of *N*-aryl oxindoles.

1. Introduction

Since the discovery of anti-inflammatory effects of salicylic acid many decades ago, a number of much more powerful non-steroidal anti-inflammatory drugs (NSAID's) have been discovered and commercialized. The structures of a number of these drugs including Naproxen®, Ibuprofen®, Diclofenac®, Celecoxib®, Rofecoxib® and Lumiracoxib® are shown in Figure 1. Voltaren® (diclofenac)[1] and Prexige® (lumiracoxib)[2] are prominent representatives of the 2-(*N*-arylamino)-phenylacetic acids, a class of compounds exhibiting anti-inflammatory properties. *N*-Aryl oxindoles can be converted to the corresponding 2-(*N*-arylamino)-phenylacetic acids in one hydrolysis step and are thus useful intermediates for the preparation of these compounds. *N*-Aryl oxindoles themselves can be prepared from corresponding diarylamines by N-acetylation with chloroacetyl-chloride followed by intramolecular Friedel–Crafts alkylation (ring closure) to *N*-aryl oxindoles. This route is of particular usefulness because of the availability of various methods for the preparation of diarylamines, the most frequently used methods being the Ullmann coupling[3] and the Buchwald–Hartwig coupling[4] of aryl halides with aryl amines. The Smiles rearrangement[5] is yet another useful method utilizing phenols and *N*-chloroacetyl anilines as starting materials for the synthesis of diaryl amines. In this paper, the synthesis of a number of new *N*-aryl oxindoles will be described, using new methods developed in our laboratories as well as utilizing known literature methods mentioned above. The new methods will be discussed in detail: (i) nitrile groups can be used as masked methyl equivalents to activate aryl halides towards nucleophilic aromatic substitution and can subsequently be converted to methyl groups, to afford 4-methyl-diarylamines and (ii) the condensation of 2-oxo-cyclohexylidene acetic acid derivatives with anilines affords dihydro-*N*-aryl-oxindoles, which can be dehydrogenated to obtain the corresponding *N*-aryl oxindoles.

2. Results and discussion

2.1. Diarylamines and *N*-chloroacetyl-diarylamines from Buchwald-coupling

In a first set of experiments, 4-alkyl-substituted anilines (**1**–**2**) and aryl bromides (**3**–**4**) were coupled with the *ortho*-halogenated aryl bromides (**5**–**6**) and anilines (**7**–**12**) respectively, utilising the Pd(0) catalyzed Buchwald–Hartwig reaction (Scheme 1 and Table 1). Numerous reports on the Pd(0)-catalyzed amination appeared in recent years, indicating the subtle influence of the ligand to reaction rate, conversion and yield.[6] Therefore, $Pd(dba)_2$ was used as Pd-source and the influence of ligands on the reaction rate, conversion, and yield was investigated in detail for the coupling of toluidine (**1**) with 2-chloro-6-fluoro-bromobenzene (**5**) to diaryl-amine **13a**. The results of

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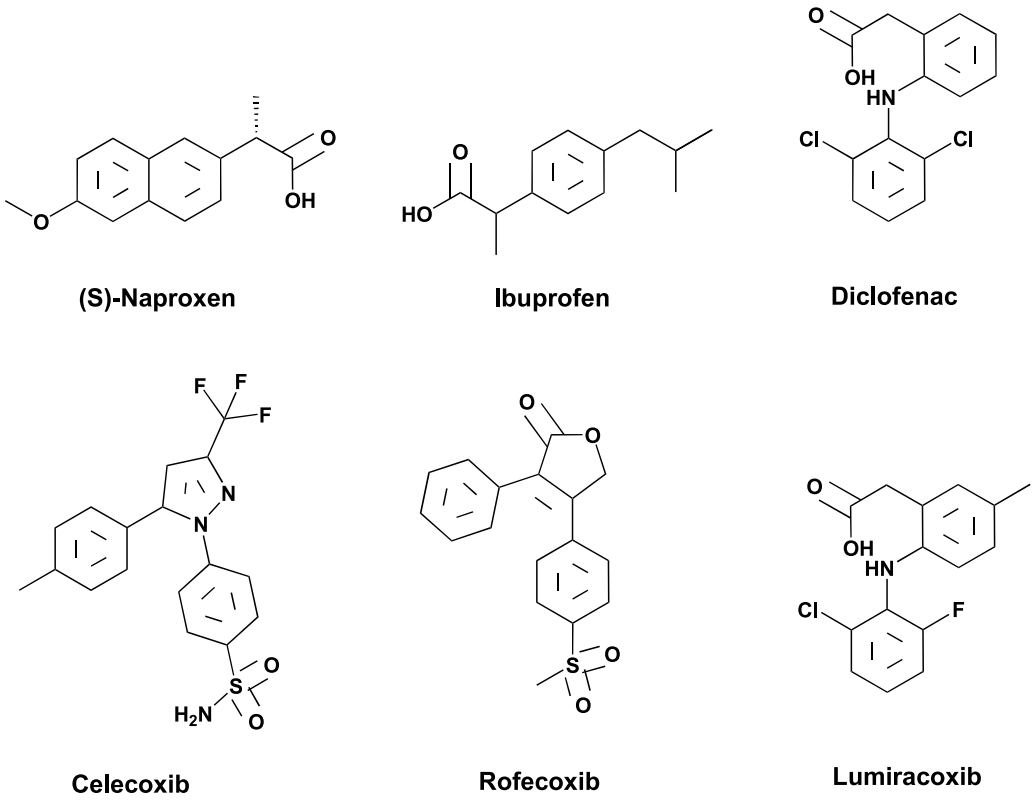
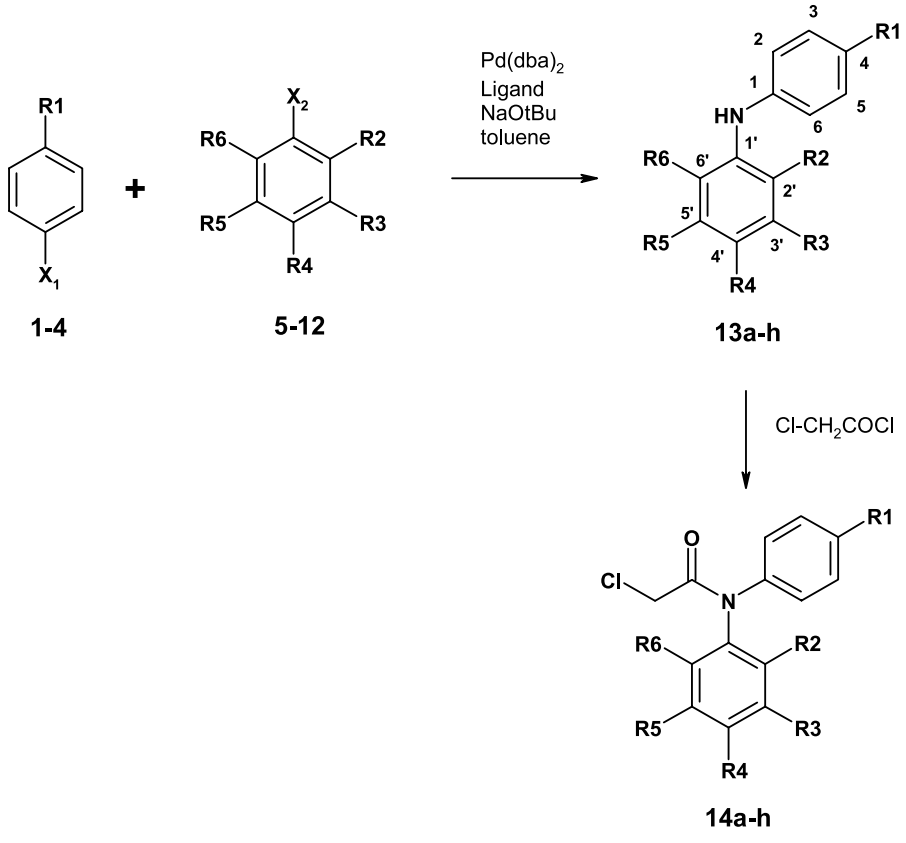


Figure 1. A selection of marketed non-steroidal anti-inflammatory drugs (NSAID's).



Scheme 1.

Table 1. Starting materials for the Buchwald–Hartwig coupling

Compound	X1	X2	R1	R2	R3	R4	R5	R6
1	NH2	—	Me	—	—	—	—	—
2	NH2	—	Et	—	—	—	—	—
3	Br	—	Me	—	—	—	—	—
4	Br	—	Et	—	—	—	—	—
5	—	Br	—	Cl	H	H	H	F
6	—	Br	—	F	F	H	H	F
7	—	NH2	—	Cl	H	H	H	F
8	—	NH2	—	Cl	H	Cl	H	Me
9	—	NH2	—	F	F	F	H	F
10	—	NH2	—	F	F	H	F	F
11	—	NH2	—	Cl	H	F	H	Me
12	—	NH2	—	Cl	H	H	H	Me

Table 2. Ligand screening for the Buchwald-coupling of toluidine (**1**) with 2-chloro-6-fluoro-bromobenzene (**5**)

Ligand	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**
Cat. (mol%)	2	2	2	4	0.7	1.0	1.1	1.8	0.2
Reaction time (h)	20	20	18	18	14	4	20	5	14
Conversion (%)	0	0	15	30	100	80	100	100	100

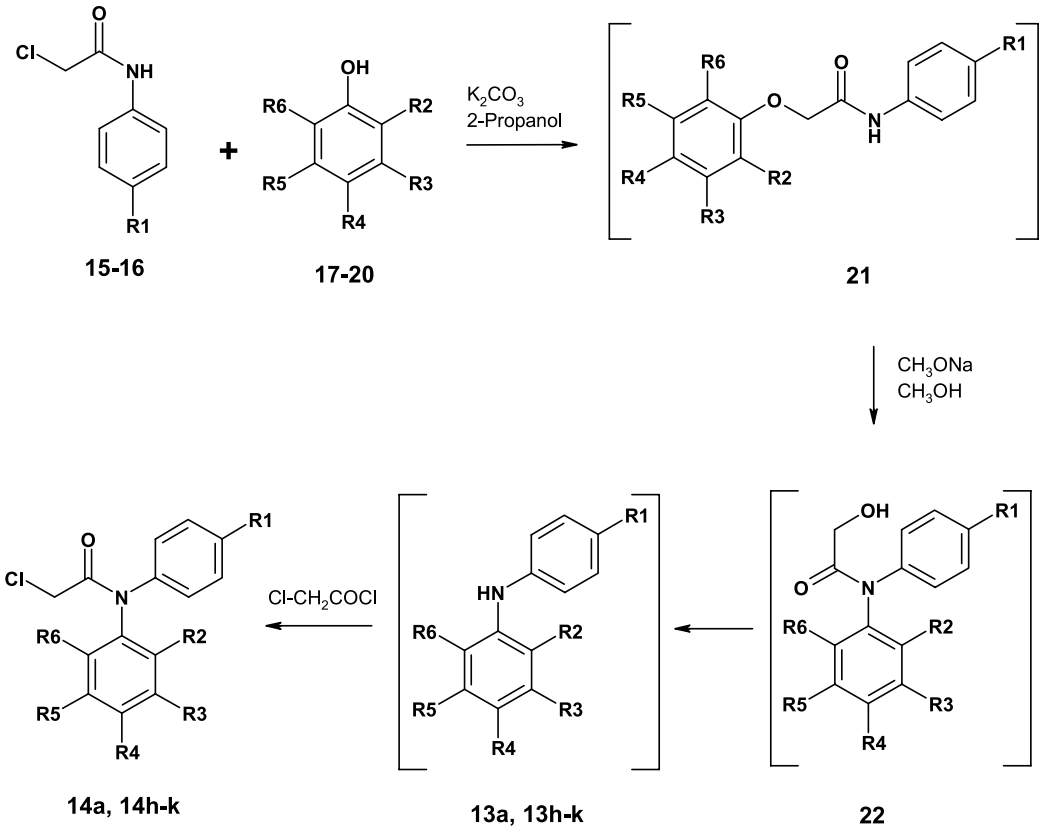
the ligand screening are summarized in Table 2. Catalysts with simple triaryl-phosphines as ligands, such as triphenyl-phosphine (**I**) and tri-(*ortho*-toluyl)phosphine (**II**) did not show any catalytic activity for the coupling of **1** and **5** to form **13a**. Tricyclohexyl-phosphine (**III**) and 2-(2-diphenyl-phosphanyl-phenyl)-4-isopropyl-4,5-dihydro-oxazole (**IV**) showed low activity with 15 and 30% conversion, respectively, after 18 h reaction time. The bidentate ligands BINAP (**V**), DPE-Phos (**VI**) and DPPF (**VII**) gave good conversions and yields. The best results were obtained with tri-*t*-butyl-phosphine (TTBP, **VIII**) as ligand. TTBP was also the ligand of choice for the inverse coupling strategy: using $Pd(dba)_2$ with TTBP (**VIII**) as ligand, *p*-bromo toluene (**3**) and 4-ethyl-phenyl-bromide (**4**) were coupled with the *ortho*-halogen substituted anilines **7**–**12** (Scheme 1 and Table 3), to obtain the corresponding diaryl amines **13a**–**g** in good yields. Under optimal conditions, the catalyst load could be reduced to 0.2 mol% with TTBP (**VIII**) as ligand. Similar results were obtained with 2-(*N*,*N*-dimethyl-amino)-2′-(dicyclohexylphosphino)biphenyl[7] (**IX**) as monodentate ligand. When BINAP (**V**) was used as ligand, a higher load of catalyst and longer reaction times were necessary as compared to TTBP (**VIII**). Under the optimized Buchwald–Hartwig coupling conditions with $Pd(dba)_2$/TTBP, excess aryl bromides reacted rapidly with the products (diarylamines **13a**–**h**) to form the corresponding triarylamines as byproducts. Thus, the purity of the crude products depended on the catalyst and on the excess arylbromide used. On small scale (0.5–5 g), the diaryl-amines could be separated from triarylamines and other byproducts by simple Kugelrohr-distillation. On large scale (5–100 g), short path distillation resulted in polymerization with only low recovery of the products. Alternatively, the products could be purified by chromatography on silica gel with cyclohexane/toluene as eluent. However, when a 1:1 ratio of the arylamine and the arylbromide components were used, only negligible amounts of the triarylamines were formed and a purification at this stage was not necessary. The crude products **13a**–**g** were then acylated with 2-chloro-acetyl-chloride to afford the mainly crystalline compounds **14a**–**g** (Scheme 1 and Table 3).

2.2. Diarylamines and *N*-chloroacetyl-diarylamines from Smiles rearrangement

The Buchwald-coupling is among the most useful methods for the preparation of diarylamines using aryl halides and anilines as starting materials. However, on a large scale and for commercial production, the cost of palladium catalysts and ligands may still account for a major part of the total production costs. Thus, the Smiles rearrangement[8] utilizing phenols as starting materials is a complementary and valuable alternative for the preparation of diarylamines. Smiles rearrangement of substituted aryloxy-acetamides has been reported for the synthesis of 2,6-dichoro-diphenyl-amine, an intermediate of diclofenac, using KOH/toluene[9]

Table 3. Diaryl-amines (**13**) and *N*-(chloroacetyl)-diarylamines (**14**) from Buchwald–Hartwig coupling

Product	Aniline	Aryl-bromide	Yield (%)	Product	Yield (%)
13a	**1**	**5**	70	**14a**	82
13a	**7**	**3**	90	**14a**	82
13b	**8**	**4**	56	**14b**	92
13c	**9**	**3**	82	**14c**	92
13d	**10**	**4**	75	**14d**	70
13e	**11**	**3**	73	**14e**	60
13f	**12**	**3**	89	**14f**	78
13g	**12**	**4**	58	**14g**	88
13h	**2**	**6**	86		



Scheme 2.

or MeONa/*n*-BuOH.[10] The Smiles-rearrangement works well only with sufficiently activated phenoxy components bearing at least one halogen substituent. 2-Chloro- and 2,4-dichloro-phenolethers are less reactive and the cleavage of the amide bond predominates under similar reaction conditions.[11] Smiles rearrangement of 2-chloro-6-fluoro- and 2,6-difluoro-phenoxy-derivatives proceeds smoothly. The rearrangement is usually accompanied by a major side reaction, arising from intramolecular nucleophilic substitution of the fluorine atom by nitrogen. This side reaction can be minimized by the proper choice of the reaction conditions, MeONa/*i*-PrOH being much better than KOH/toluene. Thus, compounds **13a** and **13h–k** were synthesized by this method using a simple one pot procedure: the 2-chloro(4-alkylphenyl)acetamides **15** and **16** (Scheme 2 and Table 4) were coupled with the 2,6-dihalogenated phenols **17–20** (Table 4) in boiling 2-propanol in the presence of potassium carbonate. The resulting phenol ethers **21** were treated with methanolic sodium methylate and heated to 85 °C to effect the rearrangement. Fast transesterification of the *N*-(2-hydroxyacetyl)-diarylamine intermediates **22** afforded the desired diarylamines **13a** and **13h–k**. The crude diarylamines were converted into the corresponding *N*-(2-chloroacetyl)-diarylamines **14a** and **14h–k** by heating with pure, excess chloroacetyl chloride. After work-up, the products **14a** and **14h–k** were isolated as crystalline compounds (Table 5). The overall yields from the corresponding phenols were: **14a** (76% from **17**), **14h** (45% from **18**), **14i** (66% from **19**), **14j** (78% from **17**) and **14k** (89% from **20**).

Table 5. *N*-(Chloroacetyl)-diarylamines (**14**) from Smiles-rearrangement

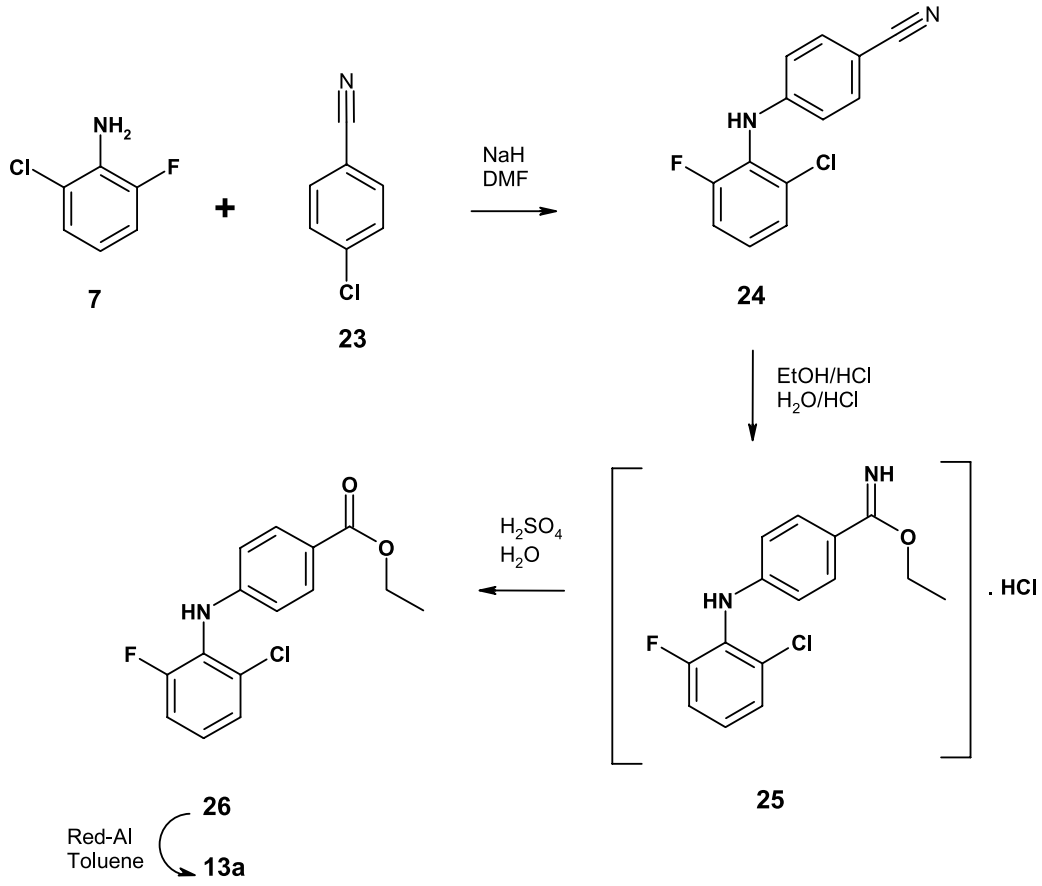
Product	*N*-(Chloroacetyl)-aniline	Phenol	Yield (%)
14a	**15**	**17**	76
14h	**16**	**18**	45
14i	**15**	**19**	66
14j	**16**	**17**	78
14k	**15**	**20**	89

2.3. Diarylamines from nucleophilic aromatic substitution

Yet another generally applicable method for the synthesis of certain diarylamines is the utilization of an electron-withdrawing group (EWG) for a nucleophilic aromatic

Table 4. Starting materials for the Smiles rearrangement

	R1	R2	R3	R4	R5	R6
15	Me	—	—	—	—	—
16	Et	—	—	—	—	—
17	—	Cl	H	H	H	F
18	—	F	F	H	H	F
19	—	Cl	H	Me	H	Cl
20	—	Cl	H	H	H	Cl



Scheme 3.

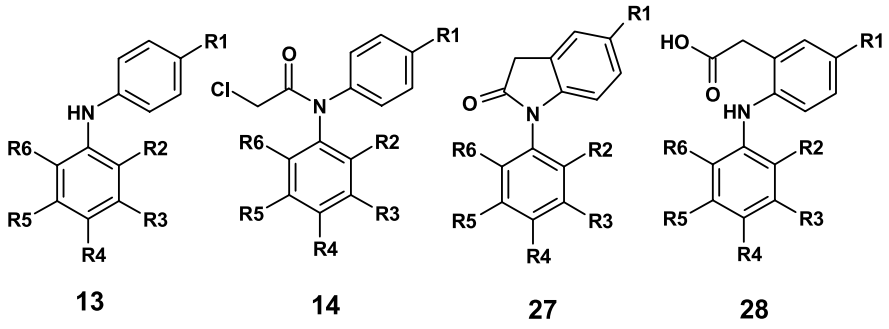
substitution and subsequent conversion of the EWG to a given substituent. For this purpose, nitrile groups[12] and hindered carboxylic ester groups[13] can serve as EWG. This strategy was successfully applied for the synthesis of compound **13a** by using a nitrile substituent as a masked methyl equivalent (Scheme 3). The reaction of 2-chloro-4-fluoro-aniline (**7**) with 4-chloro-benzonitrile (**23**) in DMF or DMPU as solvent and NaH as base afforded **24** in 91% yield. Excess base (2 equiv) had to be applied in this case, since the product **24** was more acidic than the aniline **7** and was readily deprotonated by the anilide-salt. Attempts to achieve a direct catalytic hydrogenation of the nitrile function into a methyl group failed due to de-chlorination side reaction in all cases. Different Ni- and Pd-catalysts were tested for this purpose without success. Therefore, the nitrile function was converted into the imido-ester hydrochloride intermediate **25**, which on treatment with H_2SO_4 in EtOH/H_2O afforded the ethyl ester derivative **26** in 80% yield over two steps. The imido-ester intermediate **25** was very sensitive to heat and humidity and the corresponding amide was formed as by-product at temperatures >40 °C. Heating the wet crystalline product **25** to 90 °C resulted in complete conversion to the amide within a few minutes. Finally, the ethyl ester function in **26** could be reduced to the desired methyl substituent with Red-Al in toluene to obtain compound **13a** in 83% yield. Reductive de-fluorination to the corresponding 2-chloro-diarylamine was a significant side reaction (ca. 5–10%), as could be detected by GC/MS. In addition, dimeric condensation products (from **26** with **13a**) were also formed in smaller quantities. Other reducing agents like $NaBH_4$ and $LiAlH_4$ in the presence or absence of $AlCl_3$ in different solvents were unsuccessful for the reduction of the ethyl ester, even in refluxing toluene. In many cases, the ester was completely inert to reduction and de-fluorination occurred. This unusual inertness of the ethyl ester function towards reduction might reflect its structural feature as a vinylogous aromatic carbamate. Finally, the diaryl-amine **13a** was converted into its 2-chloro-acetyl derivative **14a** in 92% yield (55.6% overall yield from 2-chloro-6-fluoro-aniline (**7**)).

2.4. Synthesis of *N*-aryl oxindoles and phenylacetic acid derivatives

A selection of *N*-chloroacetyl-diarylamines (compounds **14a**, **14e–k**) were subjected to Friedel–Crafts alkylation conditions to obtain the corresponding *N*-aryl oxindoles **27a**, and **27e–k** (Scheme 4, see also Table 6). The cyclizations were performed by heating with $AlCl_3$ at 150–170 °C without solvent or in chlorobenzene at reflux. A major side reaction was the migration of the *p*-alkyl substituent to the *meta* position. The amount of the *meta* isomer was ca. 3–5% in case of the methyl-substituent and 10–20% in case of the ethyl-substituent. The migration of the alkyl substituents is a known[14] side reaction. It is catalyzed by $AlCl_3$ and HCl, which is formed during the Friedel–Crafts alkylation.

Finally, the *N*-aryl oxindoles **27a**, **27f**, **27g** and **27i** were hydrolysed with NaOH in EtOH/H_2O to obtain the

Table 6. Substitution pattern of the product series **13**, **14**, **27** and **28**



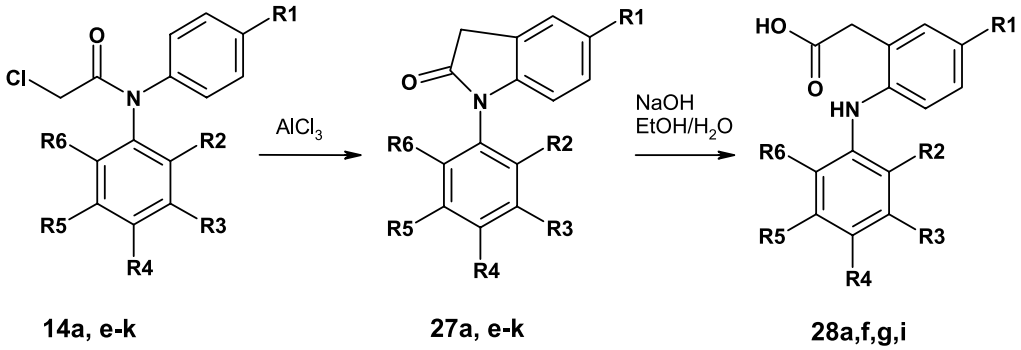
	R1	R2	R3	R4	R5	R6
a	Me	Cl	H	H	H	F
b	Et	Cl	H	Cl	H	Me
c	Me	F	F	F	H	F
d	Et	F	F	H	F	F
e	Me	Cl	H	F	H	Me
f	Me	Cl	H	H	H	Me
g	Et	Cl	H	H	H	Me
h	Et	F	F	H	H	F
i	Me	Cl	H	Me	H	Cl
j	Et	Cl	H	H	H	F
k	Me	Cl	H	H	H	Cl

physiologically active phenylacetic acid derivatives **28a** (lumiracoxib),[2] **28f**, **28g** and **28i**, respectively (Scheme 4). The substitution pattern of all products is summarized in Table 6. The sequence **13a**, **14a**, **27a** and **28a** corresponds to a total synthesis of lumiracoxib, a new NSAID.

During our search for new synthetic routes to *N*-Aryl-oxindoles, the condensation of anilines with 2-oxo-cyclohexylidene-acetic acid derivatives was found to be a viable method for the preparation of dehydro-*N*-aryl-oxindoles: 2-ethyl-cyclohexanone (**29**) was condensed (Scheme 5) with morpholine (**30**) to obtain enamine **31**. Condensation[15] of crude **31** with gyloxylic acid ethyl ester gave **32** in 25% yield over two steps. Compound **32** was stable and could be distilled and fully characterized. Subsequent hydrolysis of **32** afforded the 2-oxo-cyclohexylidene acetic acid derivative **33**, which was condensed with compound **12** to obtain the dihydro-*N*-aryl-oxindole **34** (22.8% over two steps). The conversion of **34** to **27g** was performed by dehydrogenation on Pd/C. The dehydrogenation on Pd/C proved to be a clean but very slow reaction and gave only 21% yield of **27g** after 72 h reaction time in refluxing xylene. The analogous reaction sequence starting from 4-methyl-cyclohexanone (**35**) and morpholine (**30**) gave the enamine **36**, which was condensed with glyoxylic acid ethyl ester to obtain compound **37** (25% yield over both steps). Hydrolysis of crude **37** to **38** and condensation of **38** with 2-chloro-6-fluoro-aniline (**7**) gave the nicely crystalline dehydro-*N*-aryl oxindole **39** in 57.8% yield over two steps. Finally, the oxidation of **39** was accomplished with I_2 in the presence of DBU in refluxing xylene, to obtain the *N*-aryl oxindole **27a** in 76% yield. The overall yields from 4-methylcyclohexanone varied from 11% for **27a** to 1.2% for **27g**. However, the overall yields were significantly higher with regard to the more expensive anilines: 58% for **27a** from 2-chloro-6-fluoroaniline (**7**) and 5.4% for **27g** from 2-chloro-6-methyl-aniline (**12**).

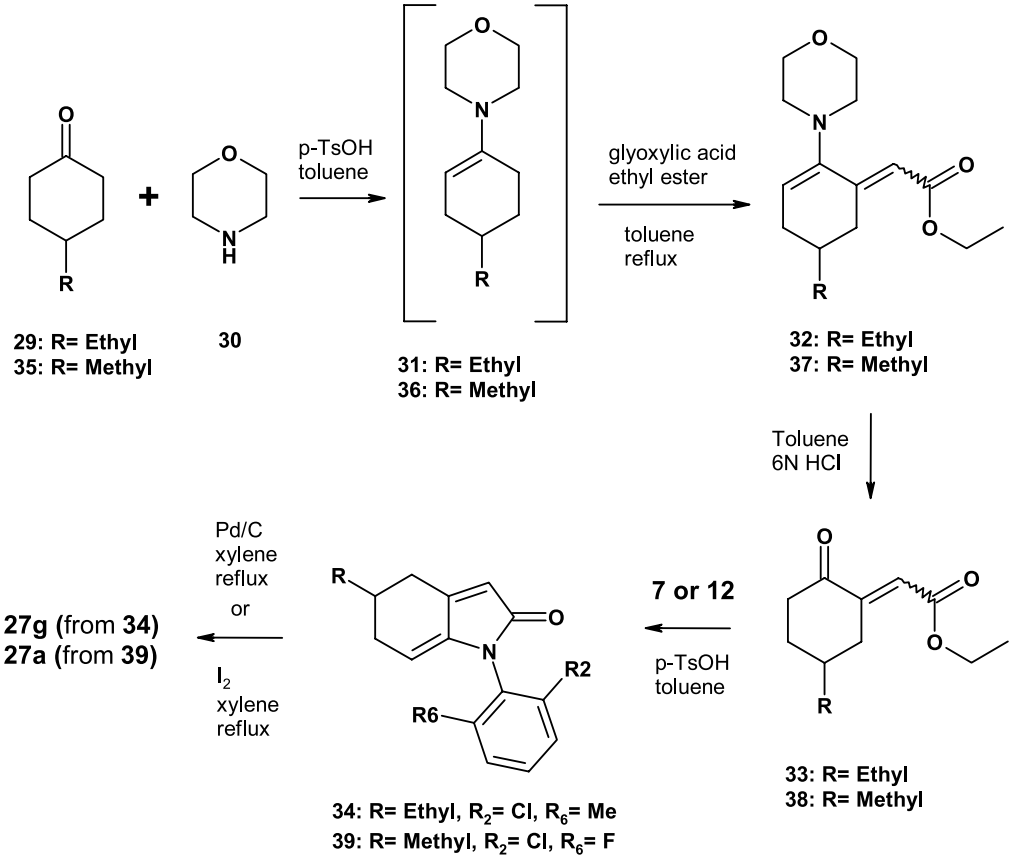
3. Conclusions

N-Aryl oxindoles are useful intermediates for the synthesis of pharmacologically active phenylacetic acid derivatives. Their diarylamine core structures can be synthesized by different methods, including Buchwald–Hartwig arylamination, Smiles-rearrangement and coupling of anilines with



Scheme 4.

4-chloro-benzonitrile derivatives. Whilst the Buchwald–Hartwig arylamination is generally applicable for the synthesis of diarylamines, the applicability of the Smiles-rearrangement is limited to *ortho*-halogene substituted phenols as starting materials. Nitrile groups can be used as masked methyl equivalents to activate aryl halides towards nucleophilic aromatic substitution and can subsequently be converted to methyl groups, to afford 4-methyl-diaryl-amines. It is obvious that only *ortho*- and/or *para*-methyl substituted diarylamines can be prepared by this method. The condensation of 2-oxo-cyclohexylidene acetic acid derivatives with anilines and subsequent oxidation of the formed tetrahydroindol-2-ones represent another, novel route for the synthesis of *N*-aryl oxindoles. Lumiracoxib (**28a**) can be synthesized by any of the methods described above.



Scheme 5.

4. Experimental

4.1. General

Reagents and solvents were obtained from commercial sources and were used as received. All reactions were carried out under an atmosphere of nitrogen unless otherwise stated. Temperatures are internally measured unless otherwise stated. Chromatography was performed using silica gel (E. Merck, Grade 60, particle size 0.040–0.063 mm, 230–400 mesh ASTM) with the eluent indicated. Melting points were determined on a Leitz Kofler hot-stage apparatus and are uncorrected. IR spectra were measured on a BRUKER IFS66 or BRUKER IFS88 instrument. Mass spectra were measured on a Fisons VG Quattro II (ESI) or Finnigan TSQ 7000 (ApCI) or Finnigan 8430 (EI) instrument. High Resolution Mass Spectra were measured on a Bruker Daltonics, 9.4T APEX-III FT-MS instrument. NMR spectra were recorded on a BRUKER DPX300 or BRUKER AMX 400 or BRUKER DRX500 spectrometer at the indicated temperature in the given solvent.

4.2. Diarylamines from Buchwald–Hartwig coupling

Typical procedure A. A mixture of the aniline (27.5 mmol), arylbromide (27.5 mmol), sodium *tert*-butylate (49.4 mmol), and toluene (55 mL) was stirred at 25 °C under nitrogen for 30 min. To this mixture, a solution of palladium-bis-(dibenzylidenacetone) (55 μmol) and tri-*tert*-butylphosphine (82 μmol) in toluene (5 mL) was added and the resulting suspension was stirred at 110 °C for 14 h. The mixture was then cooled to 30 °C. Water (30 mL), concentrated hydrochloric acid (10 mL), charcoal and cellite (1 g each) were added and stirring was continued for 1 h. The mixture was filtered to obtain two clear phases. The organic phase was separated, washed with water (3× 10 mL) and the solvent was evaporated under reduced pressure to obtain the crude diaryl-amine **13**. The crude product was purified by Kugelrohr-distillation or was used directly for the next step without purification.

Typical procedure B. A mixture of the aniline (0.153 mol), arylbromide (0.153 mol), sodium *tert*.butylat (0.286 mol),

(+)-BINAP [2,2′-bis-(diphenylphosphino)-1,1′-binaphthalin, (1.1 mmol, 0.7 mol%) and toluene (250 mL) was stirred under nitrogen for about 30 min. After the addition of palladium-bis-(dibenzylideneaceton) (0.8 g, 1 mmol), the mixture was heated to 110 °C (slight reflux) for 14–20 h. The mixture was then cooled to 30 °C, water (60 mL), concentrated hydrochloric acid (60 mL) as well as charcoal and cellite (5 g each) were added and stirring was continued for 1 h. The mixture was filtered to obtain two clear phases. The organic phase was washed with water (3×70 mL) and the solvent was evaporated under reduced pressure to obtain the crude diaryl-amine **13**. The crude product was purified by Kugelrohr-distillation or by chromatography. Alternatively, the crude product was used directly for the next step.

4.2.1. *N*-(2-Chloro-6-fluoro-phenyl)-4-methylaniline (13a). Synthesis from aryl bromide **3** and aniline **7** according to typical procedure A (14 h reaction time at 110 °C, 90% yield) or from aryl bromide **5** and aniline **1** according to procedure B (70% yield). ^{1}H NMR (DMSO-d_6, 500 MHz, 300 K) δ 2.17 (s, 3H, H3C), 6.53 (dd, J=8.5 Hz, J_{H-F}=1.5 Hz, 2H, H–C(2) and H–C(6)), 6.94 (d, J=8.0 Hz, 2H, H–C(3) and H–C(5)), 7.16 (ddd, J=8.0, 6.0 Hz, 1H, H–C(4′)), 7.25 (ddd, J=8.0, 1.5 Hz, J_{H-F}=8.0 Hz, 1H, H–C(5′)), 7.34 (ddd, J=8.0, 1.5 Hz, J_{H-F}=1.5 Hz, 1H, H–C(3′)), 7.63 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 125 MHz, 300 K) 20.6 (s, 1C, CH3), 114.9 (s, 2C, C(2,6)), 115.7 (d, J_{C-F}=20.7 Hz, 1C, C(5′)), 125.35 (d, J_{C-F}=9 Hz, 1C, C(4′)), 126.2 (s, 1C, C(3′)), 127.8 (s, 1C, C(4)), 128.4 (d, J_{C-F}=14.6 Hz, 1C, C(1′)), 129.0 (s, 2C, C(3,5)), 130.8 (d, J_{C-F}=3.7 Hz, 1C, C(2′)), 142.6 (s, 1C, C(1)), 158 (d, J_{C-F}=248 Hz, 1C, C(6′)).

4.2.2. *N*-(2′,4′Dichloro-6′-methylphenyl)-4-ethylaniline (13b). In analogy to typical procedure B, 2,4-dichloro-6-methylaniline (**8**) (1 equiv), 4-ethyl bromobenzene (**4**) (1.06 equiv), sodium *tert*-butoxide (1.86 equiv), racemic BINAP (0.02 equiv) and bis-dibenzylideneacetone-palladium(0) (0.02 equiv) were heated at reflux in toluene for 22 h to afford pure **13b** after aqueous workup and chromatography (silica gel, toluene as eluent). Yield: 56%. HRMS: 280.0655 (M^++H); $C_{15}H_{15}Cl_2N$ requires 280.0654 (M^++H). ^{1}H NMR (DMSO-d_6, 500 MHz, 300 K) δ 1.11 (t, J=7.6 Hz, 3H, H_3C–CH_2–), 2.15 (s, 3H, H_3C–C(6′)), 2.46 (q, J=7.6 Hz, 2H, CH_2–CH_3), 6.40 (d, J=8.4 Hz, 2H, H–C(2,6)), 6.94 (d, J=8.4 Hz, 2H, H–C(3,5)), 7.36 (d, J=2.1 Hz, 1H, H–C(3′)), 7.43 (s, 1H, NH), 7.50 (d, J=2.1 Hz, 1H, H–C(5′)).

The reaction proceeded much faster even at 85 °C when BINAP was replaced by tri-*tert*-butylphosphin; however, *N*,*N*-di-(4-ethylphenyl)-2′,4′-dichloro-6′-methylaniline was formed as a byproduct in considerable amounts when excess of 4-ethyl bromobenzene was used. This byproduct can be isolated as a solid, mp: 74–75 °C. HRMS: 384.12801 (M^++H); $C_{23}H_{23}Cl_2N$ requires 384.12803 (M^++H). ^{1}H NMR (400 MHz, $CDCl_3$, 300 K): δ 1.24 (t, J=7.5 Hz, 6H, CH_3–C–C(4)), 2.09 (s, 3H, CH_3–C(6′)), 2.61 (q, J=7.5 Hz, 4H, Me–CH_2–C(4)), 6.89 (d, J=8.5 Hz, 4H, H–C(2,6)), 7.06 (d, J=8.5 Hz, 4H, H–C(3,5)), 7.20 (s, 1H, H–C(5′)), 7.36 (s, 1H, H–C(3′)); MS (EI): *m*/*z* 387 (12%, M^+, ^{35}Cl, ^{37}Cl), 385 (70%, M^+, ^{35}Cl, ^{37}Cl), 383 (100%, M^+, ^{35}Cl, ^{35}Cl), 368/370 (50/30%, M−CH_3), 354 (6%, M−CH_2CH_3).

4.2.3. *N*-(2′,3′,4′,6′-Tetrafluorophenyl)-4-methylanilin (13c). In analogy to typical procedure A, 2,3,4,6-tetrafluoroaniline (**9**) (1 equiv), 4-bromotoluene (**3**) (0.8 g, 4.7 mmol), sodium *tert*-butoxide (1.9 equiv), tri-*tert*-butylphosphin (0.14 equiv) and bis-dibenzylideneacetone-palladium(0) (0.04 equiv) in toluene (70 mL/g **9**) were allowed to react at 85 °C for 3 h. Aqueous workup and chromatography (silica gel, heptane/toluene 2:1) afforded **13c** as a solid, mp 64–65 °C. Yield: 82%. HRMS: 254.05971 $(M-H)^-$, $C_{13}H_9F_4N$ requires 254.05984 $(M-H)^-$. ^{1}H NMR (400 MHz, DMSO-d_6 300 K): δ 2.20 (s, 3H, CH_3–C(4)), 6.63 (d, J=8.2 Hz, 2H, H–C(2)), 6.99 (d, J=8.2 Hz, 2H, H–C(3)), 7.56 (tdd, $^3J_{HF}$=10.9 Hz, $^4J_{HF}$=7.3 Hz, $^5J_{HF}$=2.4 Hz, 1H, H–C(5′)), 7.84 (s, 1H, NH). *N*,*N*-Bis-*p*-tolyl-2,3,4,6-tetrafluoroaniline was isolated as a byproduct, mp: 94–96 °C.

4.2.4. *N*-(2′,3′,5′,6′-Tetrafluorophenyl)-4-ethylanilin (13d). In analogy to typical procedure A, 2,3,5,6-tetrafluoroaniline (**10**) (1 equiv), 4-ethylbromobenzene (**4**) (0.99 equiv), sodium *tert*-butoxide (1.76 equiv), tri-*tert*-butylphosphin (0.039 equiv) and bis-dibenzylideneacetone-palladium(0) (0.016 equiv) were stirred in toluene at 85 °C for 15.5 h. Aqueous workup and chromatography (silica gel, hexane/toluene 9:1 to 3:1) afforded **13d** as a liquid. Yield: 75%. HRMS: 268.07534 $(M-H)^-$, $C_{14}H_{11}F_4N$ requires 268.07549 $(M-H)^-$. ^{1}H NMR (400 MHz, $CDCl_3$, 300 K) δ 1.26 (t, J=7.5 Hz, 3H, CH_3–C–C(4)), 2.65 (q, J=7.5 Hz, 2H, Me–CH_2–C(4)), 5.65 (br s, 1H, NH), 6.73 (tt, $^3J_{HF}$=10 Hz, $^4J_{HF}$=7 Hz, 1H, H–C(4′)), 6.88 (d, J=7.8 Hz, 2H, H–C(2,6)), 7.15 (d, J=7.8 Hz, 2H, H–C(3,5)); MS(EI) *m*/*z* 268 (M−H), 248 (M−HF).

4.2.5. *N*-(2′-Chloro-4′-fluoro-6′-methylphenyl)-4-methylaniline (13e). 2-Chloro-4-fluoro-6-methyl-aniline (**11**) (prepared from *N*-acetyl-4-fluoro-2-methylaniline by chlorination followed by hydrolysis) was coupled with 4-bromotoluene (**3**) according to the typical procedure A. Reaction time was 40 min at 90 °C. Aqueous workup and chromatography afforded **13e** as an oil, yield: 73%. HRMS: 250.07945 (M^++H); $C_{14}H_{13}ClFN$ requires 250.07933 (M^++H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.13 and 2.16 (each s, each 3H, CH_3–C(4) and CH_3–C(6′)), 6.32 (d, J=8 Hz, 2H, H–C(2,6)), 6.88 (d, J=8 Hz, 2H, H–C(3,5)), 7.16 (dd, $^3J_{HF}$=9.25 Hz, $^4J_{HH}$=2.4 Hz, 1H, H–C(5′)), 7.28 (s, 1H, NH), 7.31 (dd, $^3J_{HF}$=8.4 Hz, $^4J_{HH}$=3 Hz, 1H, H–C(3′)).

4.2.6. *N*-(2′-Chloro-6′-methylphenyl)-4-methylaniline (13f). 2-Chloro-6-methylaniline (**12**) and 4-bromotoluene (**3**) were coupled according to typical procedure A. Reaction time: 20 min at 90 °C and 16 h at 60 °C. Aqueous workup and chromatography (silica gel, heptan/toluene 4:1) afforded pure **13f** as an oil, yield: 89%. HRMS: 232.0889 (M^++H), $C_{14}H_{14}ClN$ requires 232.0888 (M^++H). ^{1}H NMR (400 MHz, $CDCl_3$, 300 K): δ 2.21 (s, 3H, CH_3–C(6′)), 2.29 (s, 3H, CH_3–C(4)), 5.61 (br s, 1H, NH), 6.57 (d, J=8.4 Hz, 2H, H–C(2,6)), 7.04 (d, J=8.4 Hz, 2H, H–C(3,5)), 7.07 (t, J=8.0 Hz, 1H, H–C(4′)), 7.16 (d, J=8.0 Hz, 1H, H–C(5′)), 7.33 (d, J=8.0 Hz, 1H, H–C(3′)). MS (EI): *m*/*z* 232 (80%, M+H), 197 (100%, M+H−Cl), 140 (55%, M−C_7H_7).

4.2.7. *N*-(2′-Chloro-6′-methylphenyl)-*N*-4-ethylaniline (13g). In analogy to typical procedure A, 2-chloro-6-methylaniline (**12**), 4-etyhl-bromobenzene (**4**), sodium *tert*-butoxide, tri-*tert*-butylphosphin and bis-dibenzyl-ideneacetone-palladium(0) (1.4 mol%) were heated in toluene (90 °C, 3 h) followed by aqueous workup and chromatography (silica gel, *n*-heptane) to afford **13g** as an oil. Yield: 58%. HRMS: 246.1044 ($M^+ + H$), $C_{14}H_{14}ClN$ requires 246.1044 ($M^+ + H$). ^1H NMR (300 MHz, $CDCl_3$, 300 K): δ 1.10 (t, $J=7.5$ Hz, 3H, CH_3–C–C(4)); 2.10 (s, 3H, CH_3–C(6′)), 2.50 (q, $J=7.5$ Hz, 2H, Me–CH_2–C(4)), 5.54 (br s, 1H, NH), 6.48 (d, $J=8.3$ Hz, 2H, H–C(2,6)), 7.93 (t, $J=8.0$ Hz, 1H, H–C(4′)), 7.95 (d, $J=8.3$ Hz, 2H, H–C(3,5)), 7.05 (d, $J=8.0$ Hz, 1H, H–C(5′)), 7.22 (d, $J=8.0$ Hz, 1H, H–C(3′)).

4.2.8. *N*-(2′3′6′-Trifluorophenyl)-4-ethylanilin (13h). In analogy to typical procedure B, 4-ethylaniline (**2**) (2 equiv), 2,3,6-trifluoro-bromobenzene (**6**) (1 equiv), sodium *tert*-butoxide, BINAP and bis-dibenzylideneaceton-palladium (10 mol%) were allowed to react in refluxing toluene for 6 h. Aqueous workup and chromatography (silica gel, toluene) afforded pure **13h** as an oil. Yield: 86%. HRMS: 250.08465 ($M-H)^-$, $C_{14}H_{12}F_3N$ requires 250.08491 ($M-H)^-$. ^1H NMR (400 MHz, $CDCl_3$, 300 K): δ 1.14 (t, $J=7.7$ Hz, 3H, CH_3–C–C(4)); 2.53 (q, $J=7.7$ Hz, 2H, Me–CH_2–C(4)); 5.29 (br s, 1H, NH); 6.7–6.81 (m, 2H, H–C(4′,5′)); 6.75 (d, $J=8.5$ Hz, 2H, H–C(2,6)); 7.02 (d, $J=8.5$ Hz, 2H, H–C(3,5)).

4.3. *N*-(2-Chloroacetyl)-derivatives 14a–g of from Buchwald coupling-products 13a–g

Typical acetylation procedure A. Diarylamine **13** (350 mmol) was heated to about 80 °C and 2-chloroacetyl-chloride (425 mmol) was added within 45–90 min during which time the temperature of the exothermic reaction was maintained below 90 °C (caution: gaseous HCl is being formed). After the addition was complete, the mixture was stirred for an additional hour at 80–85 °C and then 145 mL of 2-propanol was added within 30 min at a 70–80 °C. The mixture was then cooled to 35 °C and seeded with crystalline product. After cooling to 0–5 °C, the product was isolated by filtration, washed with cold 2-propanol and dried under reduced pressure to obtain product **14**.

Typical acetylation procedure B. Reaction in analogy to typical acetylation procedure A. For workup, the reaction mixture was diluted with toluene and extracted with aqueous sodium-bicarbonate. After evaporation of the solvent, the crude product was purified by column chromatography on silicagel (toluene as eluent) and crystallized to afford product **14**.

4.3.1. 2-Chloro-*N*-(2-chloro-6-fluoro-phenyl)-*N*-*p*-tolyl-acetamide (14a). Preparation from **13a** according to acetylation procedure A. Yield 82%, mp 80–82 °C. Analytical data see Section 4.4.

4.3.2. 2-Chloro-*N*-(2,4-dichloro-6-methyl-phenyl)-*N*-*p*-ethylphenyl-acetamide (14b). Preparation from **13b** according to acetylation procedure B. Yield 92%, mp 83–84 °C. HRMS: 356.0369 ($M^+ + H$); $C_{17}H_{16}Cl_3NO$ requires 356.0370 ($M^+ + H$). ^1H NMR (DMSO-d_6, 400 MHz, 393 K) δ 1.20 (t, $J=7.5$ Hz, 3H, H_3C–C–C(4)), 2.23 (br s, 3H, H_3C–C(6′)), 2.62 (q, $J=7.5$ Hz, 2H, H_2C–C(4)), 4.17 (br s, 2H, H_2C–C(O)), 7.21 (m, 4H, H–C(2,3,5,6)), 7.40 (m, 1H, H–C(3′)), 7.54 (m, 1H, H–C(5′)).

4.3.3. 2-Chloro-*N*-(2,3,4,6-tetrafluoro-phenyl)-*N*-*p*-tolyl-acetamide (14c). Preparation from **13c** according to acetylation procedure B. Yield: 92%. Viscous oil. ^1H NMR (400 MHz, DMSO-d_6, 300 K): spectrum comprised signals of several rotamers. δ 2.33 (br s, 3H, H_3C), 4.30–4.42 (m, 2H, H_2C), 7.10–7.60 (m, 4H, arom. H), 7.70 (m, 1H, arom. H). HRMS: 332.04601 ($M^+ + H$); $C_{15}H_{10}ClF_4NO$ requires 332.04598 ($M^+ + H$).

4.3.4. 2-Chloro-*N*-(2,3,5,6-tetrafluoro-phenyl)-*N*-*p*-ethyl-phenyl-acetamide (14d). Preparation from **13d** according to acetylation procedure B. Crystallization from *n*-heptane. Yield 70%. Mp 72 °C. HRMS: 346.06148 ($M^+ + H$); $C_{16}H_{12}ClF_4NO$ requires 346.06163 ($M^+ + H$). ^1H NMR (400 MHz, DMF-d_7, 413 K): δ 1.25 (t, $J=7.5$ Hz, 3H, CH_3); 2.70 (q, $J=7.5$ Hz, 2H, CH_2); 4.28 (s, 2H, CH_2–CO); 7.35 (d, $J=8.1$ Hz, 2H, H–C(3,5)); 7.43 (d, $J=8.1$ Hz, 2H, H–C(2,6)); 7.65 (tt, $^3J_{HF}=10.5$ Hz, $^4J_{HF}=7.6$ Hz, 1H, H–C(4′)). Anal. Calcd for $C_{16}H_{12}ClF_4NO$: C, 55.59; H, 3.50; Cl, 10.25; F, 21.98; N, 4.05%. Found: C, 55.42; H, 3.58; Cl, 10.24; F, 22.35; N, 4.07%.

4.3.5. 2-Chloro-*N*-(2-chloro-4-fluoro-6-methyl-phenyl)-*N*-*p*-tolyl-acetamide (14e). Preparation from **13e** according to acetylation procedure B. Crystallization from *n*-heptane/2-propanol 9:1). Yield 60%. Mp 96–97 °C. ^1H NMR (400 MHz, DMSO-d_6, 394 K): δ 2.24 (s, 3H, H_3C), 2.29 (s, 3H, H_3C), 4.15 (br s, 2H, H_2C), 7.10–7.24 (m, 5H, arom. H), 730–7.40 (m, 1H, arom. H). Anal. Calcd for $C_{16}H_{14}Cl_2FNO$: C, 58.91; H, 4.33; N, 4.29; Cl, 21.74; F, 5.82. Found: C, 58.82; H, 4.27; N, 4.37; Cl, 21.84; F, 5.85%.

4.3.6. 2-Chloro-*N*-(2-chloro-6-methyl-phenyl)-*N*-*p*-tolyl-acetamide (14f). Preparation from **13f** according to acetylation procedure B. Crystallization from *n*-heptane. Yield 78%. Mp 113–114 °C. ^1H NMR (400 MHz, DMSO-d_6, 394 K): spectrum comprised signals of two rotameres. Main rotamer: δ 2.20 (s, 3H, H_3C), 2.29 (s, 3H, H_3C), 4.12 (m, 2H, H_2C), 7.08–7.25 (m, 4H, arom. H), 7.25–7.37 (m, 2H, arom. H), 7.40–7.46 (m, 1H, arom. H). Anal. Calcd. for $C_{16}H_{15}Cl_2NO$: C, 62.35; H, 4.91; N, 4.54; Cl, 23.01%. Found: C, 62.61; H, 5.03; N, 4.43; Cl, 22.97%.

4.3.7. 2-Chloro-*N*-(2-chloro-6-methyl-phenyl)-*N*-*p*-ethyl-phenyl-acetamide (14g). Preparation from **13g** according to acetylation procedure B. Yield 88%. Viscous liquid. HRMS 322.0760 ($M^+ + H$), 344.0580 ($M^+ + Na$), 360.0319 ($M^+ + K$), $C_{17}H_{17}Cl_2NO$ requires 322.0760 ($M^+ + H$), 344.0579 ($M^+ + Na$), 360.0319 ($M^+ + K$). ^1H NMR (400 MHz, DMF-d_7, 413 K): δ 1.22 (t, $J=7.5$ Hz, 3H, CH_3–C–C(4)), 2.32 (s, 3H, CH_3–C(6′)), 2.65 (q, $J=7.5$ Hz, 2H, Me–CH_2–C(4)), 4.15 (m, 2H, CH_2–Cl), 7.22 (d, $J=8.5$ Hz, 2H, H–C(2,6)), 7.31 (d, $J=8.5$ Hz, 2H, H–C(3,5)), 7.3–7.4 (m, 2H, H–C(4′,5′)), 7.47 (dd, $J=6.8$, 2.4 Hz, 1H, H–C(3′)).

4.4. *N*-(2-Chloroacetyl)-diaryl amines (14a and 14h–k) from Smiles-rearrangement

General procedure for the preparation of diaryl-amines **13** *by Smiles-rearrangement and subsequent acylation to N-(2-chloroacetyl)-diaryl amines* **14**.

Potassium carbonate (10.5 g, 76 mmol) and the anilide **15** or **16** (70 mmol) were added to a solution of the phenol-component (67 mmol) in 2-propanol (25 mL) and the mixture was heated for 4 h at reflux, until the formation of intermediate **21** was completed. Then a solution of sodium methylate (30% m/m in methanol, 13.2 g, 73.3 mmol) was added over 30 min at 70 °C and the temperature was increased to about 85 °C by distilling 25 mL of the solvent. The mixture was stirred for additional 2 h at this temperature to complete the rearrangement. For work-up, water (25 mL) was added at 70 °C and the mixture was stirred for 5 min. The organic layer was separated, diluted with heptanes (20 mL), washed with water (2×25 mL) and the solvent was evaporated under reduced pressure to obtain the corresponding diaryl-amine **13** as an oil. The crude diaryl-amine **13** was heated to 90 °C and treated slowly with chloroacetyl chloride (9.2 g, 81 mmol). The reaction mixture was stirred for an additional hour at this temperature and cooled to 70 °C. Isopropanol (40 mL) was added at 70 °C, the formed solution was cooled to 40 °C and seeded. Crystallization occurred. The suspension was cooled to 0–5 °C, diluted with heptanes (20 mL) at 0 °C, stirred for an additional hour at this temperature and the crystals were collected by filtration to obtain the crystalline product **14**.

4.4.1. 2-Chloro-*N*-(2′-chloro-6′-fluoro-phenyl)-*N*-*p*-tolyl-acetamide (14a). Same procedure starting from 2-chloro-*N*-(4-methylphenyl)acetamide (**15**) and 2-chloro-6-fluoro-phenol (**17**). Yield 76% from **17**. Mp 80–82 °C. MS (APCI) 312 (MH^+). ^1H NMR (DMF-d_7, 393 K, 400 MHz) δ 2.4 (s, 3H, CH_3), 4.3 (s, 2H, CH_2), 7.35 (d, $J=8.0$ Hz, 2H, HC(3) and HC(5)), 7.43 (ddd, $J=8.0$, 2.0 Hz, $J_{H-F}=8.0$ Hz, 1H, HC(5′)), 7.48 (d, $J=8.0$ Hz, 2H, HC(2) and HC(6)), 7.55 (d, $J=8.0$ Hz, 1H, HC(3′)), 7.6 (ddd, $J=8.0$ Hz, $J_{H-F}=5.5$ Hz, 1H, HC(4′)). ^{13}C NMR (DMF-d_7, 100 MHz, 300 K) δ 15.4 (s, 1C, CH_3), 37.1 (s, 2C, CH_2), 111.0 (d, $J_{C-F}=21$ Hz, C(5′)), 121.7 (d, 1C, $J_{C-F}=3.5$ Hz, C(3′)), 122 (s, 2C, C(2,6)), 124 (d, 1C, $J_{C-F}=16$ Hz, C(1′)), 125.3 (s, 2C, C(3,5)), 126.1 (d, 1C, $J_{C-F}=9$ Hz, C(4′)), 129.6 (s, 1C, C(2′)), 133.3 (s, 1C, C(4)), 133.6 (s, 1C, C(1)), 154.9 (d, 1C, $J_{C-F}=251$ Hz, C(6′)), 161.2 (s, 1C, carbonyl C). IR (film): characteristical absorbtions: 1699, 1512, 1470, 1452, 782 cm^{-1}. Anal. Calcd for $C_{15}H_{12}Cl_2FNO$: C, 57.71; H, 3.87; N, 4.49; Cl, 22.71; F, 6.09. Found: C, 57.73; H, 3.70; N, 4.15; Cl, 22.59; F, 6.13%.

4.4.2. 2-Chloro-*N*-(4-ethyl-phenyl)-*N*-(2′,3′,6′-trifluoro-phenyl)-acetamide (14h). Same procedure starting from 2-chloro-*N*-(4-ethylphenyl)acetamide (**16**) and 2,3,6-trifluorophenol (**18**). The crude intermediate *N*-(2,3,6-trifluorophenyl)-4-ethylaniline was filtered on silicagel using toluene as eluent before chloroacetylation. Yield 45% from **18**.

Mp 49–50 °C. MS (APCI) 328 (MH^+). ^1H NMR (DMF-d_7, 413 K, 400 MHz) δ 1.24 (t, $J=7.55$ Hz, 3H, CH_3), 2.70 (q, $J=7.61$ Hz, 2H, CH_2–CH_3), 4.25 (s, 2H, CH_2–Cl), 7.20 (m, 1H, HC(5′)), 7.34 (d, $J=8.17$ Hz, 2H, HC(3) and HC(5)), 7.42 (d, $J=8.30$ Hz, 2H, HC(2) and HC(6)), 7.46 (m, 1H, HC(4′)). IR (film): characteristical absorbtions: 1699, 1504, 815 cm^{-1}. Anal. Calcd for $C_{16}H_{13}ClF_3NO$: C, 58.64; H, 4.00; N, 4.27; Cl, 10.82; F, 17.39. Found: C, 58.60; H, 3.84; N, 4.07; Cl, 10.91; F, 17.45%.

4.4.3. 2-Chloro-*N*-(2′,6′-dichloro-4′-methyl-phenyl)-*N*-*p*-tolyl-acetamide (14i). Preparation from 2-chloro-*N*-(4-methylphenyl)acetamide (**15**) and 2,6-dichloro-4-methyl-phenol (**19**). Yield 66% from **19**. Mp 140–141 °C. MS (APCI) 342 (MH^+). ^1H NMR (DMF-d_7, 413 K, 400 MHz) δ 2.33 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 4.18 (s, 2H, CH_2), 7.22 (d, $J=8.29$ Hz, 2H, HC(5) and HC(3)), 7.38 (d, $J=8.40$ Hz, 2H, HC(2) and HC(6)), 7.42 (s, 2H, HC(3′)and HC(5′)). IR (film): characteristical absorbtions: 1694, 1509, 1469, 811 cm^{-1}. Anal. Calcd for $C_{16}H_{14}Cl_3NO$: C, 56.08; H, 4.12; N, 4.09; Cl, 31.04. Found: C, 56.02; H, 3.93; N, 3.84; Cl, 30.93%.

4.4.4. 2-Chloro-*N*-(2′-chloro-6′-fluoro-phenyl)-*N*-(4-ethyl-phenyl)-acetamide (14j). Same procedure starting from 2-chloro-*N*-(4-ethylphenyl)acetamide (**16**) and 2-chloro-6-fluorophenol (**17**). Yield 78% from **17**. Mp 67–68 °C. MS (APCI) 326 (MH^+). ^1H NMR (DMF-d_7, 413 K, 400 MHz) δ 1.23 (t, $J=7.55$ Hz, 3H, CH_3), 2.68 (q, $J=7.53$ Hz, 2H, CH_2–CH_3), 4.20 (s, 2H, CH_2–Cl), 7.29 (d, 2H, HC(3) and HC(5)), 7.31 (m, 3H, HC(5), HC(3) and HC(5′)), 7.47 (m, 4H, HC(2), HC(6), HC(3′) and HC(4′)). IR (film): characteristical absorbtions: 1701, 1506, 1469, 1451, 783 cm^{-1}. Anal. Calcd for $C_{16}H_{14}Cl_2FNO$: C, 58.91; H, 4.33; N, 4.29; Cl, 21.74; F, 5.82. Found: C, 58.82; H, 4.12; N, 4.03; Cl, 21.90; F, 5.77%.

4.4.5. 2-Chloro-*N*-(2′,6′-dichloro-phenyl)-*N*-*p*-tolyl-acetamide (14k). Same procedure starting from 2-chloro-*N*-(4-methylphenyl)acetamide (**15**) and 2,6-dichlorophenol (**20**). At the end of the chloroacetylation the reaction mixture was diluted with a small amount of toluene (0.2 part) to prevent solidification before dilution with isopropanol. Yield 89% from **20**. Mp 129–130 °C. MS (APCI) 328 (MH^+). ^1H NMR(DMF-d_7, 393 K, 400 MHz) δ 2.40 (s, 3H, CH_3), 4.28 (s, 2H, CH_2–Cl), 7.30 (d, $J=7.43$ Hz, 2H, HC(3) and HC(5)), 7.46 (d, $J=7.63$ Hz, 2H, HC(2) and HC(6)), 7.54 (d, $J=7.63$ Hz, 1H, HC(4′)), 7.67 (d, $J=7.63$ Hz, 2H, HC(3′) and HC(5′)). IR (film): characteristical absorbtions: 1704, 1509, 1440, 793 cm^{-1}. Anal. Calcd for $C_{15}H_{12}Cl_3NO$: C, 54.82; H, 3.68; N, 4.26; Cl, 32.36. Found: C, 54.90; H, 3.54; N, 4.14; Cl, 32.28%.

4.5. Synthesis of diaryl-amine 13a from 2-chloro-6-fluoro aniline (7) and 4-chloro-benzonitrile (23)

4.5.1. Synthesis of 4-(2-chloro-6-fluoro-phenylamino)-benzonitrile (24). DMF (190 mL) was placed in a dry 4-necked, round bottomed flask equipped with a mechanical stirrer, thermometer, and argon inlet/outlet and was cooled to 0–4 °C. NaH (12.09 g, ca. 60% m/m, corresponding to ca. 7.254 g 100% pure NaH, 302 mmol) was added at 0–4 °C, followed by slow addition of a solution of 2-chloro-6-fluoro-aniline (20 g, 137.4 mmol) in DMF (114 mL) at this

temperature. Evolution of hydrogen gas started immediately. The reaction mixture is stirred for additional 1 h at 0–4 °C, until the gas evolution was ceased and a dark brown suspension was formed. A solution of 4-chlorobenzonitrile (19.49 g, 141.7 mmol) in DMF (100 mL) was added during 5 min at 0–4 °C and the reaction mixture was heated to 50–55 °C for 20 h. For work-up, the suspension was cooled to 0–4 °C and was diluted with ethyl acetate (400 mL) and water (300 mL). After intense stirring for additional 5 min, the layers were separated and the aqueous layer was extracted with ethyl acetate (2×250 mL). The combined organic layers were washed with brine (2×350 mL), dried over anhydrous sodium sulfate (20 g) and the solvent was evaporated at reduced pressure. The residue was dissolved in ethanol (100 mL) and the solvent was evaporated. The residue was re-dissolved in ethanol (100 mL) and the solution was heated to 55–60 °C. Water (100 mL) was added at this temperature over a time period of 1.5 h, during which time crystallization occurred. The suspension was stirred at rt over night and for another 2 h at 0 °C and the product was collected by filtration. The product was dried in vacuo at 50 °C to obtain 30.87 g (91%) of 4-(2-chloro-6-fluoro-phenylamino)-benzonitrile (**24**) as red-brown solid, mp 135.5–139 °C. MS (EI): *m/z* 246 (M^+, 100%), 226 (M−HF), 211 (M−HCl). IR (KBr): strong absorbtions at 3342 (NH), 2217 (CN), 1613, 1599, 1576, 1523, 1480, 1467, 1447, 1414, 1331 (Ar–N), 1288, 1249, 1175, 906, 877, 827, 774, 749 cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz, 300 K): δ 6.66 (d, J=8.7 Hz, 2H, H–C(2,6)), 7.34 (m, 2H, H–C(4′,5′)), 7.45 (m, 1H, H–C(3′), 7.55 (d, J=8.7 Hz, 2H, H–C(3,5)), 8.68 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 125 MHz, 300 K): δ 99.6 (s, 1C, C(4)), 114.1 (s, 2C, C(2,6)), 116 (d, J_{C-F}=20.6 Hz, 1C, C(5′)), 120.3 (s, 1C, CN), 125.9 (d, J_{C-F}=15.1 Hz, 1C, C(1′)), 126.5 (d, J_{C-F}=2.8 Hz, 1C, C(3′)), 128.2 (d, J_{C-F}=9.2 Hz, 1C, C(4′)), 132.6 (s, 1C, C(2′)), 133.9 (s, 2C, C(3,5)), 149.5 (s, 1C, C(1)), 158.8 (d, J_{C-F}=248.9 Hz, 1C, C(6′)). Assignments according to diarylamine numbering given in Scheme 5. Anal. Calcd for $C_{13}H_8ClFN_2$: C, 63.30; H, 3.27; N, 11.36; Cl, 14.37, F, 7.70. Found: C, 63.39; H, 3.32; N, 11.34; Cl, 14.20; F, 7.67.

4.5.2. Synthesis of 4-(2-chloro-6-fluoro-phenylamino)-benzimidic acid ethyl ester hydrochloride (25). 4-(2-Chloro-6-fluoro-phenylamino)-benzonitrile (**24**) (10 g, 40.54 mmol) was dissolved in EtOH (100 mL) and ethyl acetate (100 mL). The solution was cooled down to −5 °C and HCl-gas (135 g) was bubbled into the solution during 1.5 h. The reaction mixture was stirred for 1 h at 0 to −5 °C and water (5 mL) was added, maintaining the temperature at 0 °C. The mixture was allowed to warm up to room temperature and stirring was continued at room temperature. Water (5 mL) was added after 10 and 26 h reaction time, maintaining the temperature at 20–25 °C. HPLC analysis after 36 h reaction time indicated the disappearance of compound **24** and the formation of the imino-ester **25** as main product. The reaction mixture was poured onto ice-water (300 g) and stirred for 30 min. Then, ethyl acetate (650 mL) was added, followed by the addition of water (500 mL) to obtain two clear phases. The organic layer was separated and was extracted twice with aq HCl (250 mL of a 2 M solution). The aqueous phases were combined and the solvent was evaporated at T<40 °C under reduced pressure to a final volume of 500 mL. Precipitation/crystallization occurred during evaporation. The crude precipitate was isolated by filtration and the wet product was used directly for the next step. An analytical sample was dried in vacuo at room temperature and was characterized as following: MS (EI): *m/z* 292 (M^+), 264 (M−C_2H_4), 248 (100%), 211, 185. IR (KBr): 3397 (NH), 2982 (NH^+), 1679 (C=N), 1612, 1594, 1529, 1499, 1470, 1437, 1386, 1335, 1291, 1257, 1229, 1195, 1156, 1138, 1119, 1064, 1002, 905, 881, 842, 771, 749 cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz, 300 K): δ 1.44 (t, J=7 Hz, 3H, H_3C), 4.54 (q, J=7 Hz, 2H, H_2C), 6.72 (d, J=8.4 Hz, 2H, H–C(2,6)), 7.39 (m, 2H, H–C(4′,5′)), 7.48 (m, 1H, H–C(3′)), 7.95 (d, J=9 Hz, 2H, H–C(3,5)), 9.07 (s, 1H, NH), 11.01 (br s, 2H, H_2N^+). ^{13}C NMR (DMSO-d_6, 125 MHz, 300 K): δ 13.6 (s, 1C, CH_3), 68.4 (s, 1C, CH_2), 113.0 (s, 2C, C(2,6)), 113.8 (s, 1C, C(4)), 115.7 (d, J_{C-F}=20.7 Hz, 1C, C(5′)), 125.1 (d, J_{C-F}=15.2 Hz, 1C, C(1′)), 126.2 (s, 1C, C(3′)), 128.1 (d, J_{C-F}=9.2 Hz, 1C, C(4′)), 131.1 (s, 2C, C(3,5)), 132.2 (d, J_{C-F}=2.5 Hz, 1H, C(2′)), 151.6 (s, 1C, C(1)), 158.4 (d, J_{C-F}=249 Hz, 1C, C(6′)), 169.6 (s, 1C, O–C=N). Assignments according to diaryl-amine numbering is given in Scheme 5.

4.5.3. Synthesis of 4-(2-chloro-6-fluoro-phenylamino)-benzoic acid ethyl ester (26). The crude product **24** from the previous step was suspended in ethanol (77 mL). Water (38.5 mL) and concd H_2SO_4 (3.85 mL) were added and the suspension was heated to 66–70 °C to obtain a clear solution. The reaction mixture was stirred at this temperature for 4 h until the conversion was completed according to HPLC. The reaction mixture was then cooled down to 60 °C and water (4.8 mL) was added at this temperature. The cloudy solution was allowed to cool down slowly to room temperature. Crystallization occurred. The suspension was stirred for 16 h at room temperature and the product was isolated by filtration. The filter cake was washed with ice cold water/ethanol (9:1) and dried in vacuo at 55 °C to obtain 9.5 g (80%) of 4-(2-chloro-6-fluoro-phenylamino)-benzoic acid ethyl ester (**26**), mp 113–115.5 °C. MS (EI): *m/z* 293 (M^+), 265, 250, 248 (100%), 221, 185. IR (KBr): strong absorbtions at 3353 (NH), 2958, 1684 (CO), 1610, 1594, 1522, 1500, 1476, 1450, 1366, 1339 (Ar–N), 1311, 1279 (CO), 1251, 1175, 1130, 1211, 1108, 1025, 905, 880, 844, 783, 770 cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz, 300 K): δ 1.262 (t, J=7.0 Hz, 3H, CH_3), 4.222 (q, J=7.0 Hz, 2H, CH_2), 6.638 (d, J=8.5 Hz, 2H, H–C(2,6)), 7.32 (m, 2H, H–C(4′,5′)), 7.43 (m, 1H, H–C(3′)), 7.75 (d, 2H, J=8.5 Hz, H–C(3,5)), 8.51 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 125 MHz, 300 K): δ 14.7 (s, 1C, H_3C), 60.3 (s, 1C, H_2C), 113.4 (s, 2C, C(2,6)), 115.9 (d, J_{C-F}=20.7 Hz, 1C, C(5′)), 119.7 (s, 1C, C(4)), 126.5 (m, 2C, C(1′,3′)), 127.7 (d, J_{C-F}=9.2 Hz, 1C, C(4′)), 131.2 (s, 2C, C(3,5)), 132.4 (d, J_{C-F}=2.6 Hz, 1C, C(2′)), 149.8 (s, 1C, C(1)), 158.8 (d, J_{C-F}=248.8 Hz, 1C, C(6′)), 166.0 (s, 1C, COOEt). Assignments according to diaryl-amine numbering given in Scheme 5. Anal. Calcd for $C_{15}H_{13}ClFNO_2$: C, 61.34; H, 4.46; N, 4.77; Cl, 12.07; F, 6.47. Found: C, 61.37; H, 4.60; N, 4.55; Cl, 11.77; F, 6.29.

4.5.4. Reduction of 4-(2-chloro-6-fluoro-phenylamino)-benzoic acid ethyl ester (26) to diaryl-amine 13a. Red-Al (14.75 g of a 70% (w/w) solution in toluene, 10.325 g 100%, 51.07 mmol) was dissolved in toluene (25 mL) and the solution was heated to 60–62 °C. A solution of the ester **26**

(5 g, 17.02 mmol)) in toluene (50 mL) was added during 1 h and the reaction mixture was stirred for additional 1.5 h at this temperature. The reaction mixture is then cooled down to 30–40 °C and was poured onto a stirred mixture of ethyl acetate (100 mL) and sulfuric acid (150 mL of a 20% aqueous solution) at 0–4 °C. The two-phases mixture was stirred for 30 min at 0–4 °C, the layers were separated and the aqueous layer was extracted with ethyl acetate (100 mL). The organic layers were combined, washed with aq sodium bicarbonate (150 mL), water (150 mL) and the solvent was evaporated under reduced pressure to afford 4.0 g of crude product as an oil. The crude product was purified by column chromatography on silica gel with hexanes/ethyl acetate (8:2) as eluent to obtain 3.35 g (83%) of **13a** as an oil. The product was converted into its *N*-(2-chloroacetyl) derivative **14a** according to acetylation procedure A (see Section 4.3). Analytical data of the products **13a** and **14a** were identical with those described above.

4.6. Synthesis of *N*-aryl oxindoles (27) from *N*-(2-chloroacetyl)-diaryl amines (14)

General procedures for the Friedel–Crafts cyclisation of N-(2-chloroacetyl)-diaryl-amines **14** *to N-aryl-oxindoles* **27**.

Friedel–Crafts procedure A. A mixture of the *N*-(chloroacetyl)-diarylamine (**14**) (20 mmol) and aluminium trichloride (26 mmol) was heated slowly to 170 °C and was stirred at this temperature for 3 h. During this time, nitrogen was continuously bubbled into the melt. The mixture was cooled to 100–110 °C, diluted with toluene (20 mL) and was poured onto warm water (20 mL). The organic layer was separated, washed with water and evaporated. The residue was crystallized from 2-propanol (20 mL) to obtain the crystalline *N*-aryl-oxindole **27**.

Friedel–Crafts procedure B. A mixture of the *N*-(chloroacetyl)-diarylamine (**14**) (2 mmol) and aluminium trichloride (2.6 mmol) in chlorobenzene (2 mL) was heated at 155 °C (oil bath temperature) for 5 h. The reaction mixture was diluted with toluene (30 mL) and water (20 mL) and the phases were separated. The organic layer was washed with aq 2 N HCl and water. Evaporation of the solvent and crystallization afforded the corresponding *N*-aryl oxindole.

4.6.1. *N*-(2′-Chloro-6′-fluorophenyl)-5-methyloxindole (27a). Preparation according to Friedel–Crafts procedure A. Yield 80% from **14a**. Mp 137–138 °C. MS (APCI) 276 (MH^+). ^{1}H NMR (DMSO-d_6, 500 MHz, 300 K) δ 2.27 (s, 3H, CH_3), 3.83 (s, 2H, CH_2); 6.35 (d, J=8.0 Hz, 1H, HC(7)), 7.01 (d, J=8.0 Hz, 1H, HC(6)), 7.19 (s, 1H, HC(4)), 7.52 (ddd, J=8.5, 2.0 Hz, J_{H-F}=10.0 Hz, 1H, HC(5′)), 7.60 (ddd, J=8.5, 2.0 Hz, J_{H-F}=1.5 Hz, 1H, HC(3′)), 7.63 (ddd, J=8.5 Hz, J_{H-F}=1.5 Hz, 1H, HC(4′)). ^{13}C NMR (DMSO-d_6, 125 MHz, 300 K) δ 21.0 (s, 1C, CH_3), 35.5 (s, 1C, CH_2), 108.8 (s, 1C, C(7)), 116.3 (d, J_{C-F}= 20.2 Hz, 1C, C(5′)), 121 (d, J_{C-F}=16.1 Hz, 1C, C(1′)), 125 (s, 1C, C(3a)) 126 (s, 1C, C(4)), 126.7 (d, J_{C-F}=2.2 Hz, 1C, C(3′)), 128.4 (s, 1C, C(6)), 132.2 (d, J_{C-F}=9.3 Hz, 1C, C(4′)), 132.4 (s, 1C, C(5)), 134.2 (s, 1C, C(2′)), 141.2 (s, 1C, C(7a)), 159.4 (d, J_{C-F}=252.3 Hz, 1C, C(6′)), 173.9 (s, 1C, C(2)). IR (film): characteristical absorbtions: 1726, 1497, 1477, 1457, 780 cm^{-1}. Anal. Calcd for $C_{15}H_{11}ClFNO$: C, 65.35; H, 4.02; N, 5.08; Cl, 12.86; F, 6.89. Found: C, 65.51; H, 3.90; N, 4.96; Cl, 12.92; F, 6.90%.

4.6.2. *N*-(2′-Chloro-4′-fluoro-6′-methylphenyl)-5-methyloxindole (27e). Preparation according to Friedel–Crafts procedure B. The crude product was purified by column chromatography on silicagel (toluene/ethylacetate 19:1 to 4:1) and crystallized from *n*-hexane. Yield 11%. Mp 111–112 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 2.10 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 3.80 (m, 2H, HC(3)), 6.23 (d, J= 7.9 Hz, 1H, HC(7)), 6.99 (d, J=7.9 Hz, 1H, HC(6)), 7.19 (s, 1H, HC(4)), 7.38 (dd, J=9.3, 2.6 Hz, 1H, HC(5′)), 7.56 (dd, J=8.4, 2.7 Hz, 1H, HC(3′). Anal. Calcd for $C_{16}H_{13}ClFNO$: C, 66.33; H, 4.52; N, 4.83; Cl, 12.24; F, 6.56. Found: C, 66.16; H, 4.57; N, 4.72; Cl, 12.28; F, 6.43%.

4.6.3. *N*-(2′-Chloro-6′-methylphenyl)-5-methyloxindole (27f). Preparation according to Friedel–Crafts procedure B. Crystallization from 2-propanol/hexane. Yield 47%. Mp 158–159 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 2.10 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 3.80 (m, J=7.3 Hz, 2H, HC(3)), 6.18 (d, J=7.9 Hz, 1H, HC(7)), 6.98 (d, J=7.9 Hz, 1H, HC(6)), 7.19 (s, 1H, HC(4)), 7.44 (m, J=7.2 Hz, 2H, HC(3′) and HC(4′)), 7.53 (dd, 1H, HC(5′)).

Anal. Calcd for $C_{16}H_{14}ClNO$: C, 70.72; H, 5.19; N, 5.15; Cl, 13.05. Found: C, 70.64; H, 5.23; N, 4.97; Cl, 12.99%.

4.6.4. *N*-(2′-Chloro-6′-methylphenyl)-5-ethyloxindole (27g). Preparation according to Friedel–Crafts procedure A. The crude product was purified by column chromatography on silicagel (toluene/ethylacetate 19:1 to 4:1) and crystallized from *n*-hexane to afford **27g**. Yield: 59%, Mp: 99–101 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.17 (t, J= 7.6 Hz, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.58 (q, J=7.6 Hz, 2H, CH_2), 3.81 (m, 2H, HC(3)), 6.20 (d, J=7.9 Hz, 1H, HC(7)), 7.01 (d, J=7.9 Hz, 1H, HC(6)), 7.23 (s, 1H, HC(4)), 7.44 (m, 2H, HC(4′) and HC(5′)), 7.53 (m, 1H, HC(3′)). Anal. Calcd for $C_{17}H_{16}ClNO$: C, 71.45; H, 5.64; N, 4.90; Cl, 12.41. Found: C, 71.34; H, 5.72; N, 4.79; Cl, 12.41%.

4.6.5. *N*-(2′,3′,6′-Trifluorophenyl)-5-ethyloxindole (27h). Preparation according to Friedel–Crafts procedure A. After 4 h reaction time, 10% more aluminium trichloride was added and the mixture was stirred for another 2 h. Yield from **14h**: 48% after two re-crystallizations.

Mp 171–172 °C. MS (APCI) 292 (MH^+). ^{1}H NMR (DMSO-d_6, 400 MHz, 300 K) δ 1.18 (t, J=7.63 Hz, 3H, CH_3), 2.60 (q, J=7.63 Hz, 2H, CH_2–CH_3), 3.89 (s, 2H, CH_2–CO), 6.62 (d, J=8.02 Hz, 1H, HC(7)), 7.09 (d, J= 8.02 Hz, 1H, HC(6)), 7.25 (s, 1H, HC(4)), 7.46 (m, 1H, HC(5′)), 7.76 (m, 1H, HC(4′)). ^{13}C NMR ($CDCl_3$, 125 MHz, 300 K) δ 16.4 (s, 1C, CH_3), 28.3 (s, 1C, CH_2), 35.5 (s, 1C, C(3)), 109 (s, 1C, C(7)), 112,7 (m, 1C, C(5′)), 113.2 (m, 1C, C(1′)), 118.7 (m, 1C, C(4′)), 124.9 (s, 1C, C(4)), 125.2 (s, 1C, C(3a)), 127.3 (s, 1C, C(6)), 139.3 (s, 1C, C(5)), 141.0 (s, 1C, C(7a)), 146.1 (m, 1C, C(2′)), 148.1 (m, 1C, C(3′)), 154.5 (d, J_{C-F}=249 Hz, 1C, C(6′)), 173.8 (s, 1C, C(2)). IR (film): characteristical absorptions: 1729, 1503, 1485, 822 cm^{-1}. Anal. Calcd for $C_{16}H_{12}F_3NO$: C, 65.98; H,

4.15; N, 4.81; F, 19.57. Found: C, 65.99; H, 4.20; N, 4.59; F, 19.67%.

4.6.6. *N*-(2′,6′-Dichloro-4′-methylphenyl)-5-methyl-oxindole (27i). Preparation according to Friedel–Crafts procedure A. Yield: 55% from **14i**. Mp 153–154 °C. MS (APCI) 306 (MH^+). ^{1}H NMR (DMSO-d_6, 400 MHz, 300 K) δ 2.29 (s, 3H, CH_3–C(5)), 2.41 (s, 3H, CH_3–C(4′)), 3.81 (s, 2H, CH_2), 6.27 (d, J=8.02 Hz, 1H, HC(7)), 7.00 (d, J=7.83 Hz, 1H, HC(6)), 7.19 (s, 1H, HC(4)), 7.58 (s, 2H, HC(3′) and HC(5′)). ^{13}C NMR ($CDCl_3$, 125 MHz, 300 K) δ 20.8 (s, 1C, CH_3), 21.1 (s, 1C, CH_3), 35.5 (s, 1C, C(3)), 108.6 (s, 1C, C(7)), 125.0 (s, 1C, C(3a)), 126,1 (s, 1C, C(4)), 127.7 (s, 1C, C(1′)), 128.4 (s, 1C, C(6)), 130.1 (s, 2C, C(3′,5′)), 132.2 (s, 1C, C(5)), 134.3 (s, 2C, C(2′,6′)), 141.2 (s, 1C, C(7a)), 142.9 (s, 1C, C(4′)); 173.7 (s, 1C, C(2)). IR (film): characteristical absorptions: 1734, 1497, 816, 801 cm^{-1}. Anal. Calcd for $C_{16}H_{13}Cl_2NO$: C, 62.76; H, 4.28; N, 4.57; Cl, 23.16. Found: C, 62.61; H, 4.33; N, 4.26; Cl, 22.89%.

4.6.7. *N*-(2′-Chloro-6′-fluorophenyl)-5-ethyloxindole (27j). Preparation according to Friedel–Crafts procedure A. After 4 h reaction time, 10% more aluminium trichloride was added and the mixture was stirred for another 2 h. Yield from **14j**: 38% after two recrystallizations. Mp 129–130 °C. MS (APCI) 290 (MH^+). ^{1}H NMR (DMSO-d_6, 300 K, 400 MHz) δ 1.18 (t, J=7.43 Hz, 3H, CH_3), 2.59 (q, J=7.43 Hz, 2H, CH_2–CH_3), 3.86 (s, 2H, CH2–CO), 6.39 (d, J=8.02 Hz, 1H, HC(7)), 7.05 (d, J=8.22 Hz, 1H, HC(6)), 7.24 (s, 1H, HC(4)), 7.53 (m, 1H, HC(5′)), 7.64 (m, 2H, HC(3′) and HC(4′)). ^{13}C NMR ($CDCl_3$, 125 MHz, 300 K) δ 116.4 (s, 1C, CH_3), 28.3 (s, 1C, CH_2), 35.6 (s, 1C, C(3)), 108.8 (s, 1C, C(7)), 116.3 (d, J_{C-F}=19.7 Hz, 1C, C(5′)), 121 (d, J_{C-F}=16.2 Hz, 1C, C(1′)), 124.9 (s, 1C, C(4)), 125.1 (s, 1C, C(3a)), 126.7 (s, 1C, C(3′)), 127.3 (s, 1C, C(6)), 132.2 (d, J_{C-F}=8.5 Hz, 1C, C(4′)), 134.2 (s, 1C, C(2′)), 139 (s, 1C, C(5)), 141.8 (s, 1C, C(7a)), 159.4 (d, J_{C-F}=253 Hz, 1C, C(6′)), 173.9 (s, 1C, C(2)). IR (film): characteristical absorbtions: 1727, 1499, 1477, 1456, 783 cm^{-1}. Anal. Calcd for $C_{16}H_{13}ClFNO$: C, 66.33; H, 4.52; N, 4.83; Cl, 12.24; F, 6.56. Found: C, 66.42; H, 4.57; N, 4.69; Cl, 12.31; F, 6.65%.

4.6.8. *N*-(2′,6′-Dichlorophenyl)-5-methyloxindole (27k). Preparation according to Friedel–Crafts procedure A. Yield from **14k**: 70%.

Mp 152–153 °C. MS (EI) *m/z* 291 (MH^+), 256, 228 (100%), 193. ^{1}H NMR (DMSO-d_6, 400 MHz, 300 K) δ 2.30 (s, 3H, CH_3), 3.85 (s, 2H, CH_2), 6.29 (d, J=8.02 Hz, 1H, HC(7)), 7.02 (d, J=8.02 Hz, 1H, HC(6)), 7.22 (s, 1H, HC(4)), 7.62 (dd, J=8.02 Hz, 1H, HC(4′)), 7.76 (d, J=8.20 Hz, 2H, HC(3′) and HC(5′)). ^{13}C NMR ($CDCl_3$, 125 MHz, 300 K) δ 21.1 (s, 1C, CH_3), 35.6 (s, 1C, C(3)), 108.7 (s, 1C, C(7)), 125.0 (s, 1C, C(3a), 126.1 (s, 1C, C(4)), 128.4 (s, 1C, C(6)), 129.8 (s, 2C, C(3′,5′)), 130.5 (s, 1C, C(1′)), 132.2 (s, 1C, C(4′)), 132.3 (s, 1C, C(5)), 134.9 (s, 2C, C(2′,6′)), 141.0 (s, 1C, C(7a)), 173.6 (s, 1C, C(2)). IR (film): characteristical absorbtions: 1731, 1493, 1463, 819, 783 cm^{-1}. Anal. Calcd for $C_{15}H_{11}Cl_2NO$: C, 61.67; H, 3.79; N, 4.79; Cl, 24.27. Found: C, 61.70; H, 3.91; N, 4.61; Cl, 24.21%.

4.7. Synthesis of phenylacetic acid derivatives 28 from *N*-aryl oxindoles 27

General procedure for the hydrolysis of N-aryl-oxindoles (**27**) *to phenylacetic acid derivatives* **28**.

A solution of the *N*-aryl-oxindole **27** (4.9 mmol), in ethanol (18 mL) and water (1 mL) was heated to reflux. Sodium hydroxide solution (1.9 g of a 30%, w/w solution) was slowly added and reflux was continued for 4–5 h. The solution was cooled to about 40 °C and treated slowly with a solution of concentrated hydrochloric acid (1.5 g) in water (12 mL) up to a pH of 3–4. The obtained suspension was cooled to 20 °C. The crystals were collected by filtration, washed with water and dried to obtain the phenylacetic acid derivative **28**.

4.7.1. 5-Methyl-2-(2′-chloro-6′-fluoroanilino)phenylacetic acid (28a). Yield from **27a**: 90%. Mp 152–154 °C. MS(EI) *m/z* 293 (M^+), 275, 240, 212 (100%). ^{1}H NMR(DMSO-d_6, 500 MHz, 300 K) δ 2.21 (s, 3H, CH_3), 3.64 (s, 2H, CH_2), 6.42 (dd, J=8.0 Hz, J_{H-F}=3.0 Hz, 1H, HC(3)), 6.90 (dd, J=8.0, 2.0 Hz, 1H, HC(4)), 7.01 (d, J=2.0 Hz, 1H, HC(6)), 7.09 (s, 1H, NH), 7.09 (ddd, J=8.5 Hz, J_{H-F}=5.5 Hz, 1H, HC(4′)), 7.23 (ddd, J=8.5, 1.5 Hz, J_{H-F}=11 Hz, 1H, HC(5′)), 7.34 (ddd, J=8.5, 1.5 Hz, J_{H-F}=1.5 Hz, 1H, HC(3′)), 12.67 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6, 125 MHz, 300 K) δ 20.55 (s, 1C, CH_3), 38.2 (s, 1C, CH_2), 115.7 (d, J_{C-F}=20 Hz, 1C, C(5′)), 117.2 (s, 1C, C(3)), 123.75 (d, J_{C-F}=8 Hz, 1C, C(4′)), 124.8 (s, 1C, C(1)), 126.1 (s, 1C, C(3′)), 127.7 (d, J_{C-F}=4 Hz, 1C, C(2′)), 128.3 (s, 1C, C(4)), 129.5 (d, J_{C-F}=14 Hz, 1C, C(1′)), 130.4 (s, 1C, C(5)), 131.7 (s, 1C, C(6)), 140.3 (s, 1C, C(2)), 156 (d, J_{C-F}=247 Hz, 1C, C(6′)), 173.9 (s, 1C, CO_2H). IR (film): characteristical absorbtions: 3359, 1672, 1511, 1479, 1255 cm^{-1}. Anal. Calcd for $C_{15}H_{13}ClFNO_2$: C, 61.34; H, 4.46; N, 4.77; Cl, 12.07; F, 6.47. Found: C, 61.38; H, 4.54; N, 4.65; Cl, 12.12; F, 6.54%.

4.7.2. 5-Methyl-2-(2′-chloro-6′-methylanilino)-phenylacetic acid (28f). Hydrolysis of *N*-(2′-chloro-6′-methylphenyl)-5-methyloxindole **27f** in refluxing aqueous ethanol in the presence of at least 2 equiv of sodium hydroxide followed by precipitation by adding hydrochloric acid gave **28f**. Mp 116–120 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 2.02 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 3.67 (s, 2H, CH_2), 6.07 (d, J=8.1 Hz, 1H, HC(3)), 6.78 (s, 1H, HN), 6.83 (dd, J=8.2, 1.4 Hz, 1H, HC(5)), 6.99 (d, J=1.5 Hz, 1H, HC(6)), 7.07 (t, J=7.8 Hz, 1H, HC(4′)); 7.21 (d, J=7.5 Hz, 1H, HC(5′)), 7.35 (d, J=7.5 Hz, 1H, HC(3′)), 12.59 (s, 1H, COOH). Anal. Calcd for $C_{16}H_{16}ClNO_2$: C, 66.32; H, 5.57; N, 4.83; Cl, 12.24. Found: C, 66.50; H, 5.53; N, 4.69; Cl, 12.22%.

4.7.3. 5-Ethyl-2-(2′-chloro-6′-methylanilino)-phenylacetic acid (28g). A mixture of 0.89 g of *N*-(2′-chloro-6′-methylphenyl)-5-ethyloxindole (**27g**), 11 g of ethanol and 3 g of water was degassed with nitrogen under vigorous stirring for 15 min. The mixture was then treated with 1.8 g of 30% aqueous sodium hydroxide and heated to reflux for 36 h. After adding 1 N hydrochloric acid (about 10 g to reach a pH of 4), the mixture was cooled to room temperature and extracted with dichloromethane. The organic phase was washed with water and evaporated to

dryness to afford crude 5-ethyl-2-(2′-chloro-6′-methyl-anilino)-phenylacetic acid (**28**) as a 92:8 mixture with the *meta*-ethyl isomer. The crude product was purified by chromatography on silica gel with toluene/ethylacetate 9:1 as eluent and crystallized from hexanes. Mp 108–110 °C. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 1.13 (t, J=7.6 Hz, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.49 (q, J=7.6 Hz, 2H, $CH_2(CH_3)$), 3.68 (s, 2H, CH_2(COOH)), 6.09 (d, J=8.2 Hz, 1H, HC(3)), 6.79 (s, 1H, NH), 6.86 (dd, J=8.1, 1.8 Hz, 1H, HC(4)), 7.02 (d, J=1.8 Hz, 1H, HC(6)), 7.07 (t, J=7.7 Hz, 1H, HC(4′)), 7.21 (d, J=7.5 Hz, 1H, HC(5′)), 7.36 (d, J=7.9 Hz, 1H, HC(3′)), 12.59 (s, 1H, COOH). Anal. Calcd for $C_{17}H_{18}ClNO_2$: C, 67.21; H, 5.97; N, 4.61; Cl, 11.67. Found: C, 67.10; H, 5.98; N, 4.41; Cl, 11.62%.

4.7.4. 5-Methyl-2-(2′,6′-dichloro-4′-methylanilino)-phenylacetic acid (28i). Yield from **27i**: 82%. Mp 179–182 °C. MS (EI) *m/z* 323 (M^+), 305, 270, 242 (100%). ^{1}H NMR (DMSO-d_6, 300 K, 400 MHz) δ 2.22 (s, 3H, CH_3–C(5)), 2.32 (s, 3H, CH_3–C(4′)), 3.67 (s, 2H, CH_2), 6.18 (d, J=8.40 Hz, 1H, HC(3)), 6.87 (dd, J=8.40, 1.50 Hz, 1H, HC(4)), 6.97 (s, 1H, NH), 7.02 (s, 1H, HC(6)), 7.36 (s, 2H, HC(3′) and HC(5′)), 12.68 (br s, 1H, COOH). ^{13}C NMR ($CDCl_3$, 125 MHz, 300 K) δ 20.3 (s, 1C, CH_3), 20.5 (s, 1C, CH_3), 38.2 (s, 1C, CH_2), 116.3 (s, 1C, C(3)), 124.2 (s, 1C, C(1)), 128.3 (s, 1C, C(4)), 129.8 (s,1C, C(5)), 129.9 (s, 2C, C(3′,5′)), 130.1 (s, 2C, C(2′, 6′)), 131.8 (s, 1C, C(6)), 135.2 (s, 1C, C(1′)), 135.9 (s, 1C, C(4′)), 141 (s, 1C, C(2)), 173.8 (s, 1C, CO_2H). IR (film): characteristical absorptions: 3336, 1696, 1510, 1486, 1305 cm^{-1}. Anal. Calcd for $C_{16}H_{15}Cl_2NO_2$: C, 59.28; H, 4.66; N, 4.32; Cl, 21.87. Found: C, 59.11; H, 4.92; N, 4.30; Cl, 21.71%.

4.7.5. Synthesis of [5-ethyl-2-morpholin-4-yl-cyclohex-2-en-(*E*/*Z*)-ylidene]-acetic acid ethyl ester (32). 4-Ethyl-cyclohexanone (88.85 g, 704 mmol), morpholine (73.6 g, 845 mmol) and *p*-toluene-sulfonic acid monohydrate (2 g, 21 mmol) were dissolved in toluene (400 mL). The mixture was heated to reflux and the water formed was removed by a water separator. After 24 h reaction time, the reaction mixture was cooled to 100 °C and *p*-toluene-sulfonic acid mono hydrate (2 g, 21 mmol) was added, followed by the addition of glyoxylic acid ethyl ester (78.61 g, 770 mmol) during 30 min. The mixture was heated again to reflux for 5 h and allowed to cool down to 22 °C. The solvent was evaporated under reduced pressure and the crude product was distilled at 140–150 °C/9.5^{-2} mbar to obtain [5-ethyl-2-morpholin-4-yl-cyclohex-2-en-(*E*/*Z*)-ylidene]-acetic acid ethyl ester (**32**) as an oil. Yield: 48.3 g (25%). HRMS: 280.19076 (M^++H); $C_{16}H_{25}NO_3$ requires 280.19072 (M^++H). ^{1}H NMR($CDCl_3$, 500 MHz, 277 K) δ 0.896 (t, J=7 Hz, 3H, H_3C(17)), 1.277 (t, J=7 Hz, 3H, H_3C(10)), 1.20–1.45 (m, 2H, H_2C(16)), 1.50–1.62 (m, 1H, H–C(4)), 1.876 (ddd, J_1=18 Hz, J_2=9 Hz, J_3=3 Hz, 1H, H–C(3)), 2.13 (m, 1H, H–C(5)), 2.35 (dt, J_1=17 Hz, J_2=5 Hz, 1H, H–C(3)), 2.55–2.65 (m, 2H, H–C(12) and H–C(15)), 2.72–2.80 (m, 2H, H–C(12) and H–C(15)), 3.55 (dm, J=15 Hz, 1H, H–C(5)), 3.74 (m, 4H, H_2C(13) and H_2C(14)), 4.152 (q, J=7 Hz, 2H, H_2C(9)), 5.46 (dd, J_1=5 Hz, J_2=3 Hz, 1H, H–C(2)), 6.17 (br s, 1H, H–C(7)). Assignments according to numbers given in the formula. IR (film): strong absorptions at 2960, 1710, 1624, 1609, 1191, 1156 and 1120 cm^{-1}. MS (EI): *m/z* 279 (M^+), 250 ($M-C_2H_5$)$^+$, 234, 206 ($M-CO_2C_2H_5$)$^+$, 176, 164, 135, 84.

4.7.6. Synthesis of (5-ethyl-2-oxo-cyclohexylidene)-acetic acid ethyl ester (33). [5-Ethyl-2-morpholin-4-yl-cyclohex-2-enylidene]-acetic acid ethyl ester (10 g, 35.79 mmol) was dissolved in toluene (20 mL). HCl (12 mL of a 6 M solution) was added dropwise under rigorous stirring and the reaction mixture was stirred for additional 60 min at 22 °C. The organic layer was separated and was washed with water (2×25 mL). The water layers were combined and were extracted with toluene (25 mL). The toluene layers were combined, dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure to yield 6.72 g (89%) of [5-ethyl-2-oxo-cyclohexylidene]-acetic acid ethyl ester as an oil. HRMS: 211.13287 (M^++H); $C_{12}H_{18}O_3$ requires 211.13287 (M^++H). ^{1}H NMR ($CDCl_3$, 500 MHz, 277 K) δ 0.935 (t, J=7 Hz, 3H, H_3C(12)), 1.259 (t, J=7 Hz, 3H, H_3C(10)), 1.31–1.45 (m, 2H, H_2C(11)), 1.46–1.55 (m, 1H, H–C(5)), 1.59–1.69 (m, 1H, H–C(4)), 1.97–2.04 (m, 1H, H–C(5)), 2.296 (ddd, J=17, 11, 3 Hz, 1H, H–C(3)), 2.383 (m, 1H, H–C(6)), 2.615 (dt, J=17, 4 Hz, 1H, H–C(6)), 3.57 (dm, J=17 Hz, 1H, H–C(3)), 4.17 (q, J=7 Hz, 2H, H_2C(9)), 6.42 (m, 1H, H–C(7)). Assignments according to numbers given in the formula. ^{13}C NMR (DMSO-d_6, 100 MHz, 300 K) δ 11.41, 14.05, 27.99 (2C), 33.75, 35.54, 38.98, 60.12, 120.43, 150.34, 164.88, 199.93 ppm. IR (film): strong absorptions at 1719, 1698 and 1200 cm^{-1}. MS (EI): *m/z* 210 (M^+), 164 ($M-C_2H_5OH$)$^+$, 135.

4.7.7. Synthesis of 1-(2-chloro-6-methyl-phenyl)-5-ethyl-1,4,5,6-tetrahydro-indol-2-one (34). 2-Chloro-6-methyl-aniline (3.45 g, 23.9 mmol) was dissolved in toluene (26 mL). *p*-Toluene-sulfonic acid mono hydrate (0.227 g, 1.2 mmol) was added and the mixture was heated to reflux. A solution of (5-ethyl-2-oxo-cyclohexylidene)-acetic acid ethyl ester (**33**) (5.0 g, 23.9 mmol) in toluene (13 mL) was added dropwise during 75 min and the water formed was collected by the means of a water separator. The reaction mixture was stirred under reflux for 15 h, during which time the solvent was frequently removed and was replaced with fresh toluene. For work-up, the mixture was cooled to 22 °C and was treated with saturated aq sodium hydrogen carbonate solution (70 mL) under rigorous stirring. The layers were separated and the toluene phase was washed with a 5% aqueous solution of citric acid and finally with a 10% solution of sodium chloride in water. The aqueous phases were extracted with toluene (70 mL) and the toluene phases were combined. The solvent was evaporated under reduced pressure to yield 7.1 g of a highly viscose liquid as crude product. The crude product was purified by chromatography on silica gel with toluene/ethyl acetate (9:1) as eluent to yield 1.76 g (25.6%) of pure 1-(2-chloro-6-methyl-phenyl)-5-ethyl-1,4,5,6-tetrahydro-indol-2-one (**34**). HRMS: 288.11506 (M^++H); $C_{17}H_{18}ClNO$ requires 288.11497 (M^++H). ^{1}H NMR (DMSO-d_6, 400 MHz, 300 K) δ 0.894 ppm (t, J=7 Hz, 3H, H_3C(11)), 1.34–1.43 (m, 2H, H_2C(10)), 1.70–1.82 (m, 1H, H–C(5)), 1.90–2.02 (m, 1H, H–C(6)), 2.038 (s, 3H, H_3C(6′)), 2.28–2.40 (m, 2H, H–C(4) and H–C(6)), 2.87 (dd, J_1=17 Hz, J_2=4 Hz, 1H, H–C(4)), 5.14 (m, 1H, H–C(7)), 5.96 (br s, 1H, H–C(3)), 7.3–7.5 (m, 3H, H–C(3′), H–C(4′), H–C(5′)). Assignments

according to the numbers given in the formula. ^{13}C NMR (DMSO-d_6, 125 MHz, 300 K) δ 11.3, 17.6, 27.6, 29.7, 37.2, 54.9, 110.1, 115.3, 127.5, 129.4, 130.0, 131.2, 133.4, 138.2, 140.0, 149.0, 168.3 ppm. IR (film): strong absorptions at 1703, 1660 and 1476 cm^{-1}. MS (EI): *m/z* 287 (M^+), 272 $(M-CH_3)^+$, 258 $(M-C_2H_5)^+$, 252 $(M-Cl)^+$.

4.7.8. Synthesis of *N*-(2-chloro-6-methyl-phenyl)-5-ethyl-oxindole (27g). A mixture of 1-(2-chloro-6-methyl-phenyl)-5-ethyl-1,4,5,6-tetrahydro-indol-2-one (**34**) (1 g, 3.5 mmol) and 10% palladium on charcoal (0.1 g) in xylene (20 mL) was heated under reflux for 72 h. The palladium-catalyst was removed by filtration and the solvent was evaporated. The product was purified by column chromatography on silica gel (toluene/ethylacetate 19:1 to 4:1) and the product was crystallized from *n*-hexane to obtain 0.21 g (21%) of **27g**. Analytical data see Section 4.6.

4.7.9. Synthesis of [5-methyl-2-morpholin-4-yl-cyclohex-2-en-(*E/Z*)-ylidene]-acetic acid ethyl ester (37). Compound **37** was prepared from sequential condensations of 4-methyl-cyclohexanone (**35**), morpholine (**30**) and glyoxylic acid ethyl ester according to the procedures given above for the preparation of compound **32**. Crude **37** was directly used for the preparation of compound **38**.

4.7.10. Synthesis of (5-methyl-2-oxo-cyclohexylidene)-acetic acid ethyl ester (38). Compound **37** was hydrolyzed using the same procedure as described above for the preparation of compound **32**. The product **38** was purified either by chromatography on silica gel or by distillation. The yield after chromatography was higher (76%) as compared to the yield from purification by distillation (68.5%), presumably due to decomposition of the product during distillation. Bp 80–85 °C at $8.0–9.0\times10^{-2}$ bar. HRMS: 197.11716 ($M^+ + H$); $C_{11}H_{16}O_3$ requires 197.11722 ($M^+ + H$). ^1H NMR ($CDCl_3$, 400 MHz, 300 K): δ (ppm) 1.10 (d, 3H), 1.30 (t, 3H), 1.52–1.68 (br m, 1H), 1.87–2.02 (br m, 2H), 2.24–2.33 (ddd, 1H), 2.38–2.50 (m, 1H), 2.61–2.70 (m, 1H), 3.62 (d, 1H), 4.22 (q, 2H), 6.45 (m, 1H).

4.7.11. Synthesis of 1-(2-chloro-6-fluoro-phenyl)-5-methyl-1,4,5,6-tetrahydro-indol-2-one (39). 2-Chloro-6-fluoroaniline (76.84 g, 0.528 mol) was dissolved in toluene (400 mL). *p*-Toluene-sulfonic acid mono hydrate (5.02 g, 260 mmol) was added and the mixture was heated to reflux. A solution of (5-methyl-2-oxo-cyclohexylidene)-acetic acid ethyl ester (**38**) (103.6 g, 528 mmol) in toluene (200 mL) was added dropwise during 4 h and the water formed was collected by the means of a water separator. The reaction mixture was heated to reflux for 18 h, during which time the condensing solvent was frequently removed and was replaced with fresh toluene. For work-up, the mixture was cooled to 22 °C and was treated with saturated aq sodium hydrogen carbonate solution (250 mL) under rigorous stirring. The layers were separated and the toluene phase was washed with 5% aqueous solution of citric acid (250 mL) and finally with water (250 mL). The aqueous phases were extracted with toluene (250 mL) and the toluene phases were combined. The solvent was evaporated under reduced pressure to yield 175 g of a highly viscose liquid as crude product. The crude product was treated with 200 mL of a mixture of hexane/toluene (10:1) for crystallization. The slurry was stirred at 0 °C for several hours, the product was isolated by filtration and washed with hexane to yield 111.45 g (76.0%) of 1-(2-chloro-6-fluoro-phenyl)-5-methyl-1,4,5,6-tetrahydro-indol-2-one (**39**) as beige crystals, mp 103–105 °C. ^1H NMR ($CDCl_3$, 400 MHz, 300 K): δ 1.10 (d, $J=6.5$ Hz, 3H), 1.98–2.18 (br m, 2H), 2.30–2.44 (br m, 2H), 2.86 (d, $J=16$ Hz, 1H), 5.28 (br m, 1H), 5.93 (br s, 1H), 7.11–7.18 (br m, 1H), 7.31–7.38 (br m, 2H). ^{13}C NMR (DMSO-d_6, 125 MHz, 300 K) δ 20.6, 30.4, 31.7, 110.8, 115.1, 115.6, 120.8, 126.0, 131.3, 134.4, 138.0, 150.0, 159.4 (d, $J_{C-F}=252$ Hz), 168.2. Anal. Calcd for $C_{15}H_{13}ClFNO$: C, 64.87; H, 4.72; N, 5.04; Cl, 12.77; F, 6.84. Found: C, 64.83; H, 4.97; N, 5.04; Cl, 12.78; F, 6.87%.

4.7.12. Oxidation of 39 to *N*-(2′-chloro-6′-fluorophenyl)-5-methyloxindole (27a). Compound **39** (2.77 g, 10 mmol), iodine (0.254 g, 1 mmol) and DBU (1.52 g, 10 mmol) were dissolved in 35 mL of xylenes and heated under reflux at an external temperature of 155 °C for 36 h. After almost complete conversion the reaction mixture was worked up by extraction with 2 N HCl and an aqueous solution of sodium thiosulfate to remove DBU and iodine. The solvent was evaporated under reduced pressure to obtain a crude crystalline product (2.8 g). Re-crystallization of the crude product from toluene/hexane afforded 2.1 g (76.0%) of almost pure product **27a** which contained traces of starting material and an isomeric analogue of the starting material. An analytically pure sample was obtained by re-crystallization from ethyl acetate/hexane. Analytical data see Section 4.6.

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Synthesis and rearrangement of [1,1′-bicyclobutyl]-1-ols and spiro[3.4]octan-5-ols: a general access to bicyclo[3.3.0]octenes (hexahydropentalenes)☆

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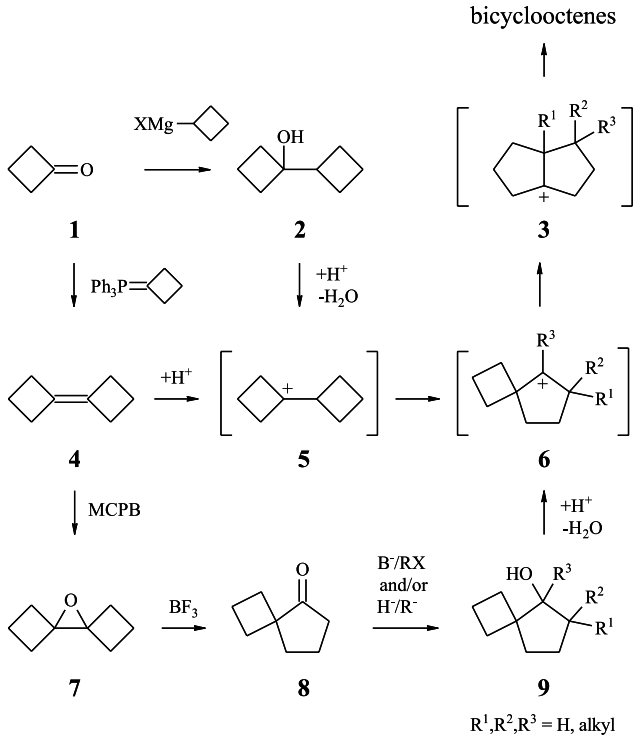
Abstract—Several new Grignard reagents based on substituted cyclobutanes have been generated and added to cyclobutanones to yield mono- to trimethylated [1,1′-bicyclobutyl]-1-ols. Mono- to trimethylated spiro[3.4]octan-5-ols have been prepared from the parent ketone via alkylation and/or addition reactions. Upon treatment with acid, all [1,1′-bicyclobutyl]-1-ols and spiro[3.4]octan-5-ols rearrange to yield a single bicyclo[3.3.0]octene.

1. Introduction

As shown for the parent compounds, the acid catalyzed rearrangement of [1,1′-bicyclobutyl]-1-ols and bicyclobutylidenes [**2**(**4**)–**5**–**6**–**3**)] is a potentially useful method for the construction of bicyclo[3.3.0]octenes.[2,3] However, any substituent to be established in the bicyclooctene must already be present in the cyclobutanone and/or the Wittig or Grignard reagent used for the synthesis of the educts required [**1**–**2**(**4**)] (Scheme 1). While cyclobutanones of greatest structural diversity are readily accessible,[4] Wittig and Grignard reagents based on substituted cyclobutanes are rare.[5,6] Therefore, substituents in that part of a bicyclooctene originating from such a reagent may be difficult to establish.

A possible resort from this dilemma is the synthesis and use of new Grignard reagents based on substituted cyclobutanes and/or a sequential transformation of a bicyclobutylidene to a bicyclooctene with intermediate introduction of substituents. In this last case the bicyclobutylidene must first be epoxidized and rearranged to give a cyclopentanone (**4**–**7**–**8**), and subsequently be modified via alkylation and/or addition reactions (**8**–**9**) such, that after a second rearrangement (**9**–**6**–**3**) the substitution pattern fits (Scheme 1). We herein report on both possibilities. In the first part, we describe the preparation of the new Grignard reagents **11a**,**b**, **12a**,**b** and **13** (Scheme 2), and their use, together with the previously described **10**,[6] for the synthesis of differently



Scheme 1.

☆ Cascade Rearrangements, Part 24. For Part 23, see Ref. 1.

Keywords: Grignard reagents; Cyclobutanes; Spiro compounds; Rearrangements; Bicyclic aliphatic compounds.

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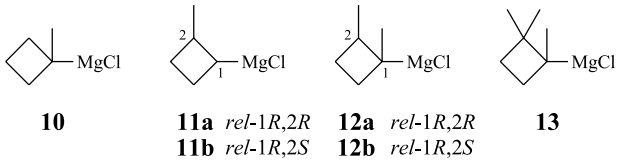


Scheme 2.

methylated [1,1′-bicyclobutyl]-1-ols. In the second part, we describe the introduction of up to three methyl groups to spiro[3.4]octan-5-one (**8**), and in the third part, we will show that acid catalyzed rearrangements of [1,1′-bicyclobutyl]-1-ols and spiro[3.4]octan-5-ols provide efficient entries to differently substituted bicyclo[3.3.0]octenes.

2. Results

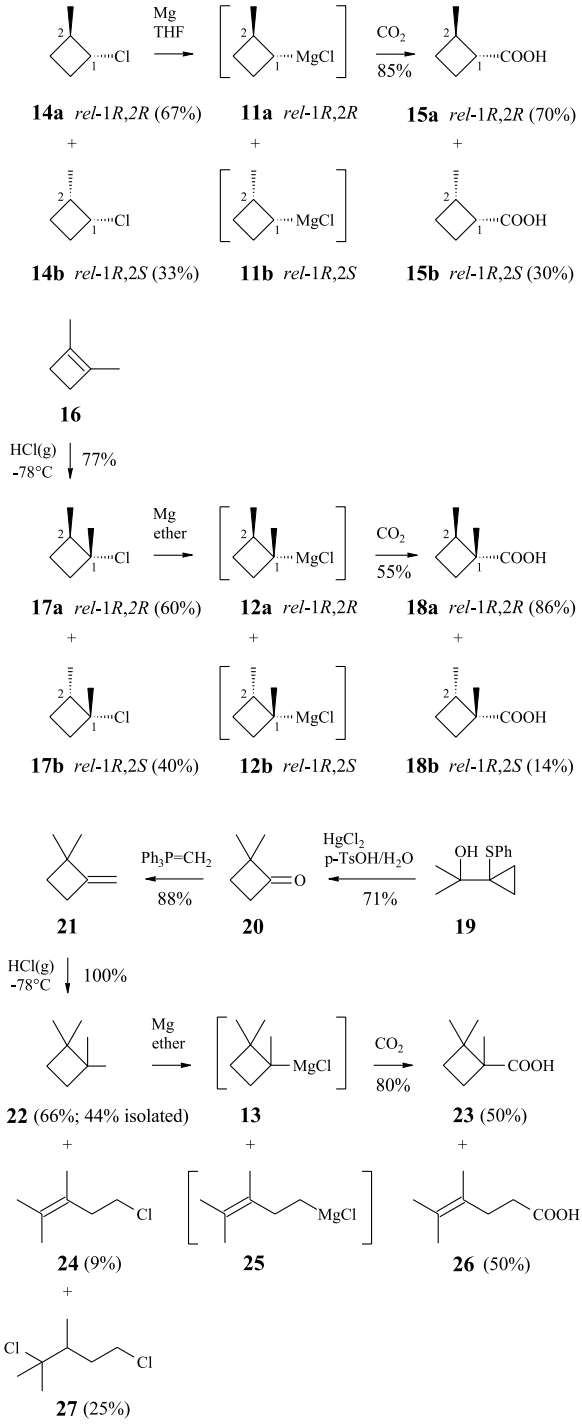
2.1. Synthesis of [1,1′-bicyclobutyl]-1-ols

For the preparation of the new Grignard reagents **11a**,**b**, **12a**,**b** and **13**, we needed the corresponding cyclobutyl chlorides **14a**,**b**, **17a**,**b** and **22**. Of these, **14a**,**b**[7] were prepared according to published procedures, while **17a**,**b**[8] and **22** were obtained by low temperature hydrochlorination of 1,2-dimethylcyclobutene (**16**)[9] and 1,1-dimethyl-2-methylene-cyclobutane (**21**), respectively. **21** was prepared by acid catalyzed rearrangement of the β-hydroxy sulfide **19**[10] to 2,2-dimethylcyclobutanone (**20**)[11] and subsequent methylenation.[12] Upon hydrochlorination, partial ring opening with formation of the homoallylic chloride **24**[13] and the dichloride **27**[14] was observed (Scheme 3).

To generate the Grignard reagents **11a**,**b**, **12a**,**b** and **13**, the corresponding cyclobutyl chlorides were reacted with magnesium in tetrahydrofurane (**14a**,**b**) and ether (**17a**,**b** and **22**), respectively. Upon carboxylation, **11a**,**b** and **12a**,**b** yielded the cyclobutane carboxylic acids **15a**,**b**[15] and **18a**,**b**, respectively, while **13** partially ring opened to **25** to give a mixture of **23**[16] and **26**[17] (Scheme 3). While the configuration of **15a**,**b** was known,[15e] the configuration of **18a**,**b** was deduced from the known γ-*gauche* effect,[18] i.e. the upfield shift of the ^{13}C NMR resonances of 1,2-*cis* oriented substituents. This technique had formerly been applied to **17a**,**b**[8] and other methylated cyclobutanes[19] and was also used to determine the configuration of the [1,1′-bicyclobutyl]-1-ols **29a**,**b** and **30a**,**b** and the spiro[3.4]octan-5-ols **34a**,**b**[20] and **38a**,**b** described below.

In practice, we first identified the methyl groups as bound to tertiary and quaternary carbon atoms, respectively, and then used the ^{13}C chemical shifts of those methyl groups showing pure 1,2-*cis* or 1,2-*trans* relationships to other groups (CH_3, COOH, *c*-C_4H_6OH, OH) as stereochemical indicators (the corresponding shifts are given in bold). In all cases, and regardless of a geminal substituent eventually present, the shift differences between the stereoisomers proved large enough to allow an unambiguous assignment (Scheme 4).

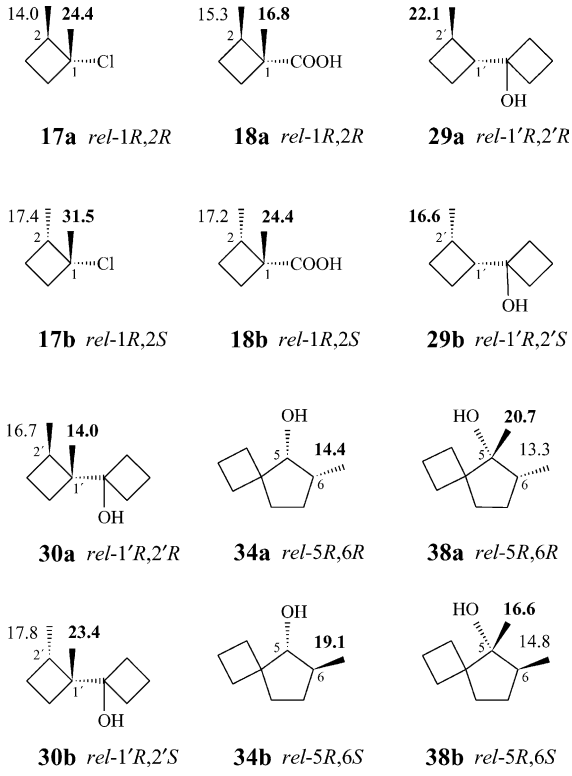
Having established the structures of the new Grignard reagents as **11a**,**b**, **12a**,**b** and **13**, we examined their usefulness for the synthesis of [1,1′-bicyclobutyl]-1-ols. This time, the Grignard reagent **10**[6] was included. Catalyzed



Scheme 3.

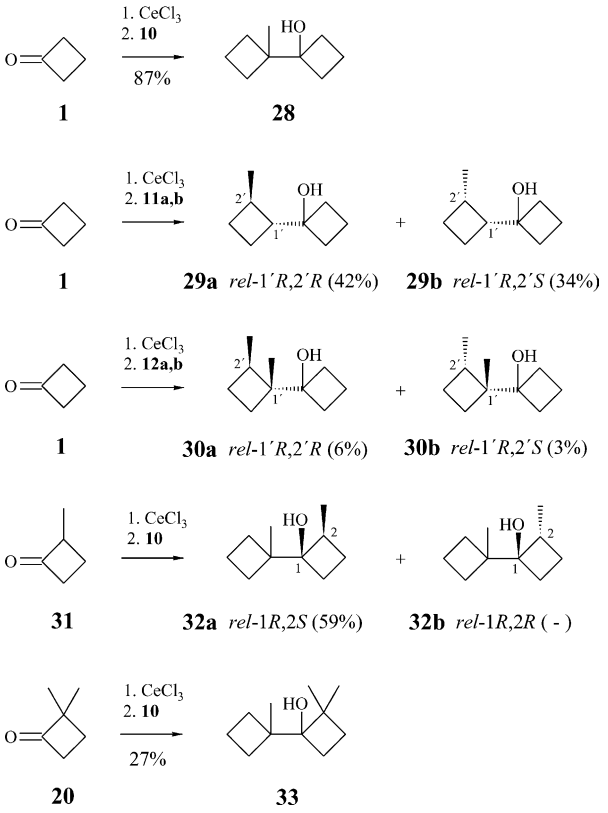
by anhydrous $CeCl_3$,[21] this reagent adds to cyclobutanone (**1**) with formation of the 1′-methyl-[1,1′-bicyclobutyl]-1-ol (**28**)[6] (Scheme 5).

Of the new Grignard reagents, **11a**,**b** and **12a**,**b** reacted with cyclobutanone (**1**) to yield the diastereoisomeric mono- and dimethylated [1,1′-bicyclobutyl]-1-ols **29a**,**b** and **30a**,**b**, respectively. On the contrary, attempted additions of **13** to **1**, and of **12a**,**b** to 2-methyl-cyclobutanone (**31**)[22] failed. However, **10**[6] reacted with **31** to give a single dimethylated [1,1′-bicyclobutyl]-1-ol, thought to be **32a**,[23] and with 2,2-dimethylcyclobutanone (**20**) to give the trimethylated



Scheme 4.

[1,1′-bicyclobutyl]-1-ol **33** (Scheme 5). The stereochemistry of **29a**,**b** and **30a**,**b** was determined as detailed above, and the results are given in Scheme 4. From a preparative point of view, the yields of **30a**,**b** and **33** are unsatisfactory.

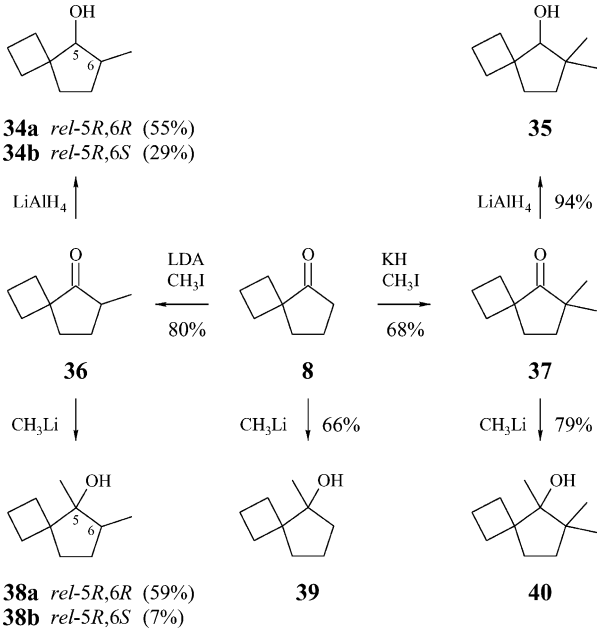


Scheme 5.

Obviously, steric hindrance in the Grignard reagent and/or the cyclobutanone employed is the principal factor that impedes or prevents an addition.

2.2. Synthesis of spiro[3.4]octan-5-ols

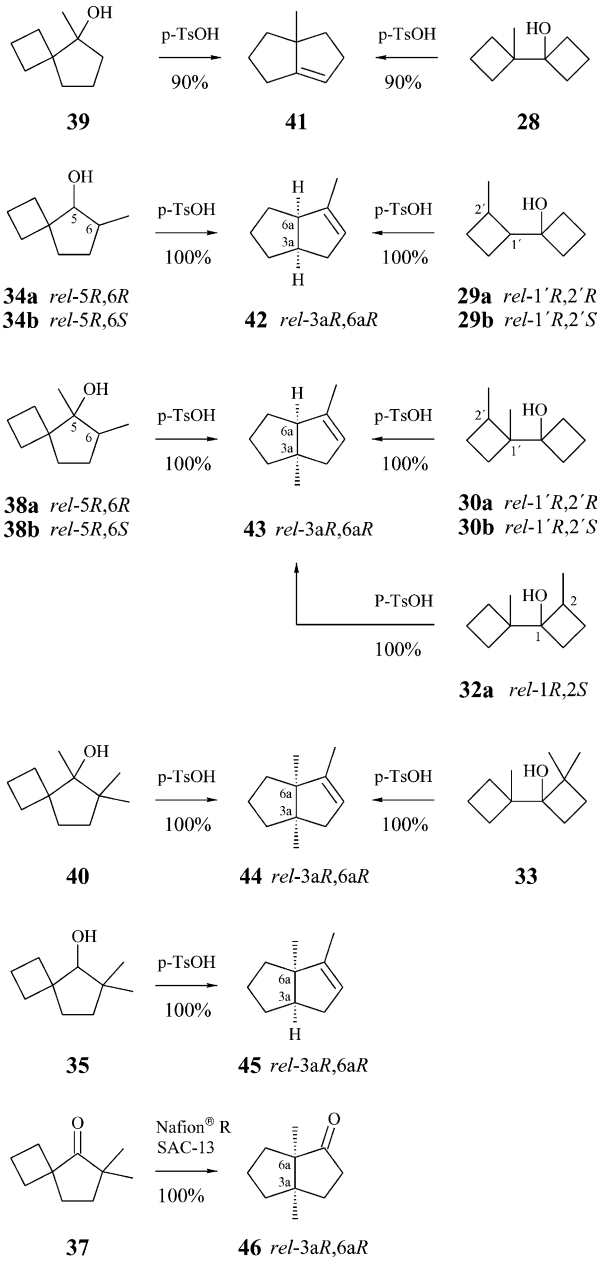
As described earlier,[24] epoxidation of bicyclobutylidene (**4**) and in situ rearrangement of the resulting oxaspirohexane **7** is a productive route to spiro[3.4]octan-5-one (**8**). In this case, the introduction of up to three methyl groups was easy to achieve: mono- and dimethylation, respectively, yielded the ketones **36** and **37**, and subsequent reduction with lithium aluminium hydride and addition of methyllithium, respectively, yielded the secondary alcohols **34a**,**b** and **35**, and the tertiary alcohols **38a**,**b** and **40**. The last reaction was also performed with **8** and yielded the tertiary alcohol **39** (Scheme 6). As with **18a**,**b**, **29a**,**b** and **30a**,**b**, the stereochemistry of **34a**,**b**[20] and **38a**,**b** resulted from an analysis of their ^{13}C methyl shifts (Scheme 4).



Scheme 6.

2.3. Rearrangements

Upon treatment with an equimolar amount of a 0.074 mol solution of anhydrous *p*-toluenesulfonic acid in benzene at 70 °C, all [1,1′-bicyclobutyl]-1-ols and all spiro[3.4]octan-5-ols underwent clear-cut rearrangements to yield a single bicyclo[3.3.0]octene. All stereoisomers were rearranged separately. The results were as follows: of the monomethylated alcohols, **39** rearranged to **41**, previously obtained from **28**,[6] and **34a** and **34b** rearranged to **42**,[25] which was also formed from **29a** and **29b**. Of the dimethylated alcohols, **35** rearranged to **45**, and **38a** and **38b** rearranged to **43**,[26] which was also formed from **30a**, **30b** and **32a**. The trimethylated alcohols **33** and **40** rearranged to **44**, and the dimethylated ketone **37** underwent a ketone to ketone rearrangement to yield **46**[27] (Scheme 7). Of the products formed, **41**,[6] **42**[25a–c,e] and **43**[26a,c] were identified by their known ^{1}H and/or ^{13}C NMR data, while the structures of **44**,

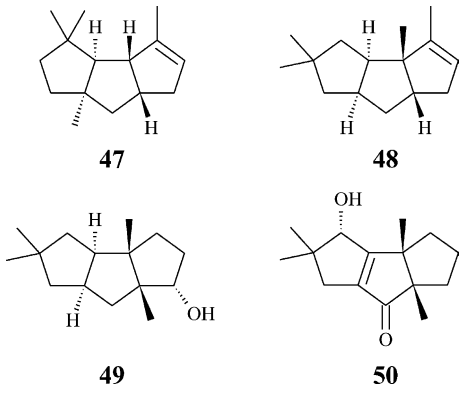


Scheme 7.

45 and **46** followed from their ^{1}H and ^{13}C NMR spectra together with APT, HETCOR and COSY measurements.

Mechanistically, all rearrangements benefit from the pronounced relief of strain associated with cyclobutylmethyl to cyclopentyl rearrangements.[28] Once a bicycloctyl cation has been formed, 1,2-methyl- and/or 1,2-hydride shifts with eventual intermediate deprotonations and reprotonations occur until the thermodynamically most stable bicyclooctene is formed. During this process, only energetically favoured tertiary cations are involved. For the mechanism of the rearrangement of **37**, we refer to a closely related example.[24]

From a synthetic point of view it is interesting to note that **42**, **45** and **46** represent partial structures of the linear triquinanes *endo*-hirsutene (**47**),[29] *endo*-capnellene (**48**)[30]



Scheme 8.

and both ceratopicanol (**49**)[31] and cucumin-H (**50**),[27,32] respectively (Scheme 8). While examples for successful syntheses of angular triquinanes via cyclobutylmethyl to cyclopentyl rearrangements are known,[33] their potential for a synthesis of linear triquinanes remains to be explored.

In summary, we describe the synthesis and rearrangement of mono- to trimethylated [1,1′-bicyclobutyl]-1-ols and spiro[3.4]octan-5-ols to yield a single bicyclo[3.3.0]octene in all cases. To note, that stereoisomeric educts yield the same product and hence mixtures of stereoisomers may be employed. With regard to the short and productive syntheses of the educts required, we recommend the route over spiro[3.4]octan-5-ols.

3. Experimental

3.1. General

IR spectra were obtained with a Perkin–Elmer 457 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR 200 or a Bruker AMX 300 spectrometer. For standards other than TMS the following chemical shifts were used: δ_H ($CHCl_3$)=7.24, δ_H (C_6D_5H)=7.15, δ_C ($CDCl_3$)=77.00, δ_C (C_6D_6)=128.00. ^{13}C multiplicities were studied by APT and/or DEPT measurements. Mass spectra were obtained with a Finnigan MAT 95 spectrometer (EI, CI and HREI) operated at 70 eV. Analytical and preparative GC was carried out on a Carlo Erba 6000 Vega 2 instrument using a thermal conductivity detector and hydrogen as carrier gas. The following columns were used: (A): 3 m×1/4″ all glass system, 15% OV 101 on Chromosorb W AW/DMCS 60–80 mesh; (B): 3 m×1/4″ all glass system, 15% FFAP on Chromosorb W AW/DMCS 60–80 mesh. Product ratios were not corrected for relative response. R_f values are quoted for Macherey and Nagel Polygram SIL G/UV_{254} plates. Colourless substances were detected by oxidation with 3.5% alcoholic 12-molybdophosphoric acid (Merck) and subsequent warming. Melting points were observed on a Reichert microhotstage. Boiling and melting points are not corrected. Microanalytical determinations were done at the Microanalytical Laboratory of the Institute of Organic and Bioorganic Chemistry, Göttingen. For the preparation of anhydrous $CeCl_3$, finely powdered $CeCl_3 \cdot 7H_2O$ was heated at 140 °C/0.1 Torr to constant weight. Nafion® R SAC-13 was purchased from

Aldrich Chemical Company, Inc. Some of the stereoisomers could also have been described using the *cis*/*trans* convention. However, in view of a consistent notation, the *R*,*S* convention was used throughout.

3.1.1. *rel*-(1*R*,2*R*)-1-Chloro-1,2-dimethyl-cyclobutane (17a) and *rel*-(1*R*,2*S*)-1-chloro-1,2-dimethyl-cyclobutane (17b)

At −78 °C, hydrogen chloride was bubbled through a solution of 1,2-dimethyl-cyclobutene (**16**)[9] (5.88 g, 72 mmol) in pentane (2 ml) until GC analysis [column A, 7 min 50 °C, 20 °C/min to 140 °C; retention times: 2.69 (**16**), 8.21 (**17a**), 8.53 (**17b**)] indicated that the addition was complete (1.5 h). The solution was diluted with pentane (20 ml), washed with water (20 ml), saturated sodium bicarbonate (2×25 ml) and dried ($MgSO_4$). Fractional distillation yielded 5.91 g (77%) of a 60:40 mixture of **17a** and **17b** as colourless liquid, bp 73–76 °C/140 Torr. The ^1H and ^{13}C NMR data were in accord with literature data.[8]

3.1.2. 2,2-Dimethyl-cyclobutanone (20)

To a solution of the β-hydroxy sulfide **19**[10] (24.8 g, 120 mmol) in tetralin (40 ml) was added $HgCl_2$ (19.0 g, 70 mmol), water (2.48 g, 140 mmol) and *p*-toluenesulfonic acid monohydrate (2.51 g, 10 mmol) and the mixture heated to 70 °C. After 2 h, more water (3.72 g, 210 mmol) was added, the temperature was raised to 140 °C and the distillate collected. The phases were separated and the aqueous phase was saturated with sodium chloride and extracted with dichloromethane (2×10 ml). The combined organic phases were dried ($MgSO_4$) and fractionated over a 10 cm Vigreux column to yield 8.5 g (72%) of **20** as colourless liquid, bp 106–110 °C (lit.[11a] bp 108 °C). The ^1H[11b] and ^{13}C NMR data[34] were in accord with literature data.

3.1.3. 1,1-Dimethyl-2-methylene-cyclobutane (21)

To a suspension of methyltriphenylphosphonium bromide (37.5 g, 105 mmol) in dry xylene (100 ml) was added under nitrogen with stirring sodium hydride (2.52 g, 105 mmol) and the mixture heated to 90 °C. After 3.5 h, the mixture was cooled to 45 °C, 2,2-dimethyl-cyclobutanone (**20**) (8.5 g, 87 mmol) was added slowly, and after additional 3 h at 60 °C direct distillation over a 30 cm Vigreux column yielded 6.66 g (88%) of pure **21** as colourless liquid, bp 83–90 °C (lit.[12] bp 80–95 °C). The ^1H NMR data were in accord with literature data.[12] The ^{13}C NMR data have not yet been reported and were as follows: ^{13}C NMR (50 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=26.62$ (t), 27.44 (q), 31.97 (t), 44.20 (s), 101.58 (t), 160.24 (s).

3.1.4. 1-Chloro-1,2,2-trimethyl-cyclobutane (22), 5-chloro-2,3-dimethyl-pent-2-ene (24) and 1,4-dichloro-3,4-dimethyl-pentane (27)

At −78 °C, hydrogen chloride was bubbled through a solution of 1,1-dimethyl-2-methylene-cyclobutane (**21**) (6.66 g, 69 mmol) in pentane (2 ml) until GC analysis [column A, 5 min 100 °C, 20 °C/min to 190 °C; retention times (min): 1.22 (**21**), 3.38 (**22**) (66%), 5.76 (**24**) (9%), 9.17 (**27**) (25%)] indicated that the addition was complete (3 h). The solution was diluted with pentane (20 ml), washed with water (2×20 ml), saturated sodium bicarbonate (3×20 ml) and dried ($MgSO_4$). Fractional distillation yielded 4.03 g (44%) of **22** as colourless liquid, bp 95–105 °C/180 Torr, which solidified on cooling; mp 27–30 °C. Preparative GC of the remaining material delivered pure samples of **24** and **27** as colourless liquids. **22**: ^1H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=0.99$ (s, 3H), 1.24 (s, 3H), 1.60 (ddd, $J=10.5, 10.5, 9$ Hz, 1H), 1.61 (s, 3H), 1.73 (ddd, $J=10.5, 10.5, 3$ Hz, 1H), 2.08 (ddd, $J=12, 9, 3$ Hz, 1H), 2.44 (ddd, $J=12, 10.5, 10.5$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=23.36$ (q), 26.90 (q), 27.38 (q), 30.45 (t), 36.25 (t), 44.72 (s), 72.99 (s); MS (EI): $m/e=56$ (100). $C_7H_{13}Cl$ requires C, 63.39; H, 9.88. Found: C, 63.15; H, 9.84. **24**: ^1H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=1.66$ (s, 6H), 1.68 (s, 3H), 2.48 (symm m, 2H), 3.46 (symm m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=18.35$ (q), 20.26 (q), 20.61 (q), 37.99 (t), 42.82 (t), 123.64 (s), 127.88 (s); MS (EI): $m/e=132$ (30, M^+), 83 (100). $C_7H_{13}Cl$ requires C, 63.39; H, 9.88. Found: 61.70; H, 9.62. **27**: The ^1H NMR data were in accord with literature data.[14] The ^{13}C NMR data have not yet been reported and were as follows: ^{13}C NMR (50 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=14.50$ (q), 30.04 (q), 30.86 (q), 35.11 (t), 42.68 (d), 43.63 (t), 74.48 (s).

3.1.5. Carboxylation of the Grignard reagents 11a,b, 12a,b and 13 generated from 14a,b, 17a,b and 22

Mg turnings (304 mg, 12.5 mmol) were covered with ether (**14a**,**b**: THF) (1.5 ml), a solution of the appropriate cyclobutyl chloride(s) (10.0 mmol) in ether (**14a**,**b**: THF) (0.5 ml) and a drop of Br_2 were added under argon with stirring, and the reaction was started by gentle to strong heating until additional ether (**14a**,**b**: THF) (10 ml) was added and the mixture was heated to reflux. After 2 h (**14a**,**b**: 3 h), GC analysis on column A [retention times (min): 2.01 (**14b**) and 2.51 (**14a**) at 90 °C; 2.64 (**17b**) and 2.82 (**17a**) at 90 °C; 3.38 (**22**) at 100 °C] indicated that the cyclobutyl chloride(s) had been consumed. The solution was cooled to 0 °C and a stream of dry carbon dioxide was passed through. After 2 h, the mixture was hydrolyzed with 0.5 N HCl (20 ml), and the aqueous phase was adjusted to pH 1 and extracted with ether (7×20 ml). The combined organic phases were dried ($MgSO_4$), and most of the solvent was distilled off over a 20 cm Vigreux column. Final concentration on a rotary evaporator (bath temperature 20 °C/14 Torr) yielded the cyclobutanecarboxylic acids. Pure samples were obtained by preparative GC on column B.

3.1.5.1. *rel*-(1*R*,2*R*)-2-Methyl-cyclobutanecarboxylic acid (15a) and rel-(1*R*,2*S*)-2-methyl-cyclobutanecarboxylic acid (15b). Yield 970 mg (85%) of a 70:30 mixture of **15a** and **15b**. Pure samples were obtained by preparative GC [column B, 160 °C; retention times (min): 4.90 (**15b**), 5.70 (**15a**)]. Colourless liquids. The ^1H and ^{13}C NMR data were in accord with literature data.[15e]

3.1.5.2. *rel*-(1*R*,2*R*)-1,2-Dimethyl-cyclobutanecarboxylic acid (18a) and *rel*-(1*R*,2*S*)-1,2-dimethyl-cyclobutanecarboxylic acid (18b). Yield 705 mg (55%) of a 86:14 mixture of **18a** and **18b**. Pure samples were obtained by preparative

GC [column B, 175 °C; retention times (min): 3.08 (**18b**), 3.61 (**18a**)]. Colourless liquids. **18a**: ^{1}H NMR (200 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=0.97$ (d, $J=7$ Hz, 3H), 1.27 (s, 3H), 1.45–1.70 (m, 2H), 1.85–2.05 (m, 1H), 2.24–2.45 (m, 1H), 2.72 (symm m, 1H), 10.7–11.7 (br s, 1H, COO*H*); ^{13}C NMR (50 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=15.28$ (q), 16.83 (q), 23.92 (t), 28.63 (t), 35.86 (d), 45.39 (s), 184.49 (s); MS (EI): *m/e*=128 (7, M^+), 87 (100). $C_7H_{12}O_2$ requires C, 65.60; H, 9.44. Found: C, 65.56; H, 9.62. **18b**: ^{1}H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=1.06$ (d, $J=7$ Hz, 3H), 1.38 (s, 3H), 1.50–1.72 (m, 2H), 2.00–2.15 (m, 1H), 2.20–2.37 (m, 1H), 2.45–2.58 (m, 1H), COO*H* not detected; ^{13}C NMR (75 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=17.15$ (q), 23.66 (t), 24.39 (q), 27.36 (t), 40.68 (d), 47.55 (s), 182.66 (s); MS (EI): *m/e*=128 (8, M^+), 87 (100). $C_7H_{12}O_2$ requires C, 65.60; H, 9.44. Found: C, 65.25; H, 9.70.

3.1.5.3. 1,2,2-Trimethyl-cyclobutanecarboxylic acid (23) and 4,5-dimethyl-hex-4-enoic acid (26). Yield 1.14 g (80%) of a 1:1-mixture of **23** and **26**. Pure samples were obtained by preparative GC [column B, 6 min 180 °C, 20 °C/min to 220 °C; retention times (min): 4.68 (**23**), 8.85 (**26**)]. **23**: colourless solid, mp 115–117 °C. ^{1}H NMR (200 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=1.03$ (s, 3H), 1.12 (s, 3H), 1.33 (s, 3H), 1.44–1.54 (m, 2H), 1.75 (ddd, $J=$ 10, 10, 9 Hz, 1H), 2.50 (ddd, $J=10$, 10, 9 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=19.90$ (q), 23.85 (q), 25.40 (t), 25.53 (q), 30.07 (t), 40.51 (s), 48.58 (s), 182.83 (s); MS (EI): *m/e*=142 (4, M^+), 56 (100). $C_8H_{14}O_2$ requires C, 67.35; H, 9.92. Found: C, 67.35; H, 9.77. **26**: colourless liquid.[17] Spectral data have not yet been reported and were as follows: ^{1}H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=$ 1.63 (s, 6H), 1.65 (s, 3H), 2.36 (s, 4H); ^{13}C NMR (50 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=17.98$ (q), 20.08 (q), 20.63 (q), 29.60 (t), 32.87 (t), 125.34 (s), 125.94 (s), 180.14 (s); MS (EI): *m/e*=142 (98, M^+), 83 (100).

3.1.6. Synthesis of the [1,1′-bicyclobutyl]-1-ols 29a,b, 30a,b, 32a and 33

A suspension of finely powdered dry $CeCl_3$ (4.93 g, 20 mmol) in THF (85 ml) was stirred under argon overnight. After addition of the appropriate cyclobutanone (10 mmol), stirring was continued for 2 h until the mixture was cooled to −78 °C and the appropriate Grignard reagent (15 mmol) was added. After 15 min at −78 °C and 4 h at room temperature the mixture was hydrolyzed with 2 N HCl (50 ml). The aqueous phase was extracted with ether (4×40 ml), and the combined organic phases were washed with saturated sodium bicarbonate (60 ml), brine (60 ml) and dried ($MgSO_4$). The solvents were distilled off on a rotary evaporator (bath temperature 20 °C/14 Torr) and the residue was chromatographed on silica gel (0.05–0.20 mm) in pentane/ether 3:1 (**32a**) and 5:1 (**29a**,**b**, **30a**,**b**, **33**) respectively.

3.1.6.1. *rel*-(1′*R*,2′*R*)-2′-Methyl-[1,1′-bicyclobutyl]-1-ol (29a) and *rel*-(1′*R*,2′*S*)-2′-methyl-[1,1′-bicyclobutyl]-1-ol (29b). Yield 297 mg (42%) **29a** and 237 mg (34%) **29b**; R_f 0.14 (**29a**) and 0.23 (**29b**). Colourless liquids. **29a**: ^{1}H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=1.07$ (d, $J=7$ Hz, 3H), 1.30–1.55 (m, 2H), 1.60 (s, 1H, OH), 1.55–2.16 (m, 9H), 2.16–2.30 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=11.96$ (t), 18.69 (t), 22.06 (q), 25.74 (t), 31.47 (d), 33.82 (t), 34.08 (t), 50.72 (d), 75.87 (s); MS (CI): *m/e*=140 (100, $M+NH_4-H_2O]^+$). $C_9H_{16}O$ requires C, 77.09; H, 11.50. Found: C, 77.15; H, 11.33. **29b**: ^{1}H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=1.07$ (d, $J=7$ Hz, 3H), 1.40–1.60 (m, 2H), 1.72 (br s, 1H, OH), 1.70–1.85 (m, 1H), 1.85–2.06 (m, 6H), 2.06–2.18 (m, 1H), 2.46–2.52 (m, 2H); ^{13}C NMR (50 MHz, C_6D_6, C_6D_6 int): $\delta=12.85$ (t), 17.00 (q), 20.04 (t), 26.80 (t), 32.63 (d), 36.03 (t), 36.41 (t), 44.20 (d), 76.37 (s, hidden in $CDCl_3$); MS (CI): *m/e*=140 (93, $M+NH_4-H_2O]^+$), 123 (100). $C_9H_{16}O$ requires C, 77.09; H, 11.50. Found: C, 77.05; H, 11.29.

3.1.6.2. *rel*-(1′*R*,2′*R*)-1′,2′-Dimethyl-[1,1′-bicyclobutyl]-1-ol (30a) and *rel*-(1′*R*,2′*S*)-1′,2′-dimethyl-[1,1′-bicyclobutyl]-1-ol (30b). Yield 90 mg (6%) **30a** and 50 mg (3%) **30b**; R_f 0.22 (**30a**) and 0.29 (**30b**). Colourless liquids. **30a**: ^{1}H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=0.92$ (d, $J=$ 7 Hz, 3H), 1.00 (s, 3H), 1.30–1.56 (m, 3H), 1.46 (s, 1H, OH), 1.76–1.94 (m, 5H), 2.16–2.42 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=12.37$ (t), 14.01 (q), 16.67 (q), 23.64 (t), 26.28 (t), 31.56 (t), 31.58 (t), 31.76 (d), 45.14 (s), 80.85 (s); MS (EI): *m/e*=154 (1, M^+), 84 (100). HRMS *m/e* (M^+) calcd 154.1358, obsd 154.1357. **30b**: ^{1}H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=1.03$ (s, 3H), 1.04 (d, $J=$ 7 Hz, 3H), 1.30–1.45 (m, 1H), 1.50–1.85 (m, 5H), 1.90–2.20 (m, 5H), 2.55–2.67 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=14.78$ (t), 17.77 (q), 23.26 (q), 23.62 (t), 27.92 (t), 32.29 (t), 33.52 (t), 39.35 (d), 45.30 (s), 81.58 (s); MS (EI): *m/e*=154 (2, M^+), 84 (100). HRMS *m/z* (M^+) calcd 154.1358, obsd 154.1357.

3.1.6.3. *rel*-(1*R*,2*S*)-1′,2-Dimethyl-[1,1′-bicyclobutyl]-1-ol (32a). Yield 841 mg (59%); R_f 0.24. Colourless liquid. ^{1}H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=1.00$ (d, $J=$ 7 Hz, 3H), 1.07 (s, 3H), 1.42 (br s, 1H, OH), 1.45–1.57 (m, 3H), 1.60–2.20 (m, 7H), 2.40 (symm m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=14.09$ (t), 15.37 (q), 21.36 (q), 22.40 (t), 28.27 (t), 28.43 (t), 28.47 (t), 33.57 (d), 43.76 (s), 80.99 (s); MS (EI): *m/e*=154 (60, M^+), 137 (100). $C_{10}H_{18}O$ requires C, 77.87; H, 11.76. Found: C, 78.15; H, 11.81.

3.1.6.4. 1′,2,2-Trimethyl-[1,1′-bicyclobutyl]-1-ol (33). Yield 460 mg (27%); R_f 0.32. Colourless liquid. ^{1}H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=0.98$ (s, 3H), 1.02 (s, 3H), 1.19 (s, 3H), 1.40 (s, 1H, OH), 1.30–1.48 (m, 2H), 1.53–2.04 (m, 6H), 2.30 (symm m, 1H), 2.40 (symm m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=15.32$ (t), 20.82 (q), 24.47 (q), 24.99 (q), 27.37 (t), 29.35 (t), 30.00 (t), 31.52 (t), 43.00 (s), 44.24 (s), 82.96 (s); MS (EI): *m/e*=168 (16, M^+), 151 (100). $C_{11}H_{20}O$ requires C, 78.77; H, 12.01. Found: C, 78.51; H, 11.98.

3.1.7. 6-Methyl-spiro[3.4]octan-5-one (36)

To a solution of diisopropylamine (8.1 g, 80 mmol) in THF (160 ml) was added at 5–10 °C under nitrogen with stirring a 1.6 M solution of n-butyllithium in hexane (50 ml, 80 mmol) followed by neat **8**[24] (9.9 g, 80 mmol). The mixture was stirred for 0.5 h at room temperature, until it was cooled to −78 °C and methyl iodide (56.8 g, 400 mmol) was added. Afterwards, the mixture was held

at −25 °C until GC analysis [column A, 110 °C; retention times (min): 9.19 (**8**) (8%), 11.94 (**36**) (89%), 12.93 (**37**) (3%)] indicated that the reaction was complete (2.5 h). The mixture was hydrolyzed with sat NH_4Cl (20 ml), the organic phase was decanted, the residue was extracted with pentane (3×75 ml), and the combined organic phases were concentrated by distillation over a 30 cm Vigreux column. The residue was diluted with pentane (20 ml) and extracted with 1 N HCl (10 ml). The organic phase was washed with water (2×10 ml), dried ($MgSO_4$) and distilled to yield 8.8 g (80%) of **36** as colourless liquid, bp 85–87 °C/30 Torr. IR (neat): 1730 cm^{-1} (C═O); ^1H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=1.06$ (d, $J=7$ Hz, 3H), 1.25–1.40 (m, 1H), 1.70–1.85 (m, 3H), 1.85–2.00 (m, 2H), 2.00–2.20 (m, 4H), 2.30 (symm m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=15.27$ (q), 15.78 (t), 27.97 (t), 28.66 (t), 32.11(t), 35.04 (t), 42.93 (d), 50.70 (s), 222.98 (s); MS (EI): $m/e=$ 138 (61, M^+), 67 (100). $C_9H_{14}O$ requires C, 78.21; H, 10.21. Found: C, 78.44; H, 10.48.

3.1.8. 6,6-Dimethyl-spiro[3.4]octan-5-one (37)

To a suspension of potassium hydride (3.48 g, 87 mmol) in ether (100 ml) was added at 0 °C under argon **8**[24] (3.61 g, 29 mmol). After the hydrogen evolution had ceased (30 min), methyl iodide (12.4 g, 87 mol) was added and the reaction progress monitored by GC [column A, 130 °C; retention times (min): 4.97 (**8**), 6.08 (**36**), 6.62 (**37**)]. After 45 min at 0 °C the reaction was complete. The mixture was hydrolyzed with sat NH_4Cl (5 ml), the organic phase was decanted, the residue was extracted with ether (2×20 ml), and the combined organic phases were dried ($MgSO_4$) and distilled to yield 3.00 g (68%) of **37** as colourless liquid, bp 55 °C/5 Torr. IR (neat): 1730 cm^{-1} (C═O); ^1H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=0.97$ (s, 6H), 1.64 (t, $J=$ 7 Hz, 2H), 1.72–1.85 (m, 2H), 1.85–2.00 (m, 2H) 1.95 (t, $J=7$ Hz, 2H), 2.17–2.30 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=15.86$ (t), 24.61 (q), 30.58 (t), 33.42 (t), 34.73 (t), 44.45 (s), 50.75 (s), 224.44 (s); MS (EI): $m/e=$ 152 (68, M^+), 68 (100). $C_{10}H_{16}O$ requires C, 78.89; H, 10.59. Found: C, 79.16; H, 10.59.

3.1.9. Addition of methyllithium to 8, 36 and 37

To a 0.5 M solution of methyllithium in ether were added at 0 °C under argon with stirring within 10 min 0.5 equiv of a 0.5 M solution of the selected ketone in ether. After additional 15 min at 0 °C, GC analysis indicated that the reaction was complete. The mixture was hydrolyzed with sat NH_4Cl, and the organic phase was separated and dried ($MgSO_4$). The solvent was distilled off on a rotary evaporator (bath temperature 20 °C/15 Torr), and the residue was chromatographed on silica gel (0.05–0.20 mm) in pentane/ether (8:2; column 30×3.5 cm).

3.1.9.1. *rel*-(5*R*,6*R*)-5,6-Dimethyl-spiro[3.4]octan-5-ol (38a) and *rel*-(5*R*,6*S*)-5,6-dimethyl-spiro[3.4]octan-5-ol (38b). From **36** (490 mg, 3.5 mmol); retention times (min): 4.59 (**36**), 8.25 (**38a**), 9.81 (**38b**) at 140 °C on column B; $R_f=0.79$ (**36**), 0.33 (**38a**), 0.28 (**38b**). Yield: 317 mg (59%) of pure **38a**, 137 mg (25%) of a 4:1 mixture of **38a** and **38b**, and 37 mg (7%) of pure **38b**. Colourless liquids. **38a**: ^1H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=0.91$ (d, $J=7$ Hz, 3H), 1.18 (s, 3H), 1.20–1.30 (m, 1H), 1.50–1.95 (m, 10H), 2.10 (symm m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=13.26$ (q), 15.18 (t), 20.70 (q), 26.34 (t), 28.63 (t), 31.77 (t), 36.57 (t), 40.86 (d), 52.79 (s), 81.19 (s); MS (EI): $m/e=154$ (18, M^+), 111 (100). $C_{10}H_{18}O$ requires C, 77.87; H, 11.76. Found: C, 76.97; H, 11.76. **38b**: ^1H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=0.88$ (d, $J=7$ Hz, 3H), 0.90 (s, 3H), 1.05 (symm m, 1H), 1.22 (br s, 1H), 1.45–2.20 (m, 10H); ^{13}C NMR (50 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=14.79$ (q), 15.19 (t), 16.60 (q), 27.45 (t), 27.55 (t), 28.47 (t), 34.68 (t), 41.25 (d), 52.23 (s), 80.37 (s); MS (EI): $m/e=154$ (13, M^+), 111 (100). $C_{10}H_{18}O$ requires C, 77.87; H, 11.76. Found: C, 77.72; H, 11.77.

3.1.9.2. 5-Methyl-spiro[3.4]octan-5-ol (39). From **8**[24] (1.00 g, 8.05 mmol); retention times (min): 1.95 (**8**), 2.33 (**39**) at 150 °C on column A; $R_f=0.21$ (**39**). Yield: 740 mg (66%) of pure **39** as colourless liquid. ^1H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=1.21$ (s, 3H), 1.36 (br s, 1H), 1.45–2.30 (m, 12H); ^{13}C NMR (50 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=$ 14.98 (t), 16.72 (t), 22.59 (q), 26.18 (t), 29.10 (t), 37.00 (t), 37.66 (t), 52.03 (s), 80.31 (s); MS (EI): $m/e=140$ (6, M^+), 97 (100). $C_9H_{16}O$ requires C, 77.09; H, 11.50. Found: C, 76.95; H, 11.37.

3.1.9.3. 5,6,6-Trimethyl-spiro[3.4]octan-5-ol (40). From **37** (460 mg, 3.0 mmol); retention times (min): 2.38 (**37**), 4.16 (**40**) at 150 °C on column A; $R_f=0.35$ (**40**). Yield: 399 mg (79%) of pure **40** as colourless oil. ^1H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=0.78$ (s, 3H), 0.92 (s, 3H), 1.06 (s, 3H), 1.14 (s, 1H), 1.40 (symm m, 1H), 1.50–1.70 (m, 4H), 1.82–2.07 (m, 3H), 2.07–2.22 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=15.75$ (t), 18.23 (q), 23.46 (q), 26.09 (q), 29.89 (t), 33.90 (t), 36.70 (t), 37.10 (t), 44.84 (s), 52.92 (s), 83.08 (s); MS (EI): $m/e=168$ (7, M^+), 97 (100). $C_{11}H_{20}O$ requires C, 78.75; H, 11.98. Found: C, 78.76; H, 11.89.

3.1.10. Reduction of 36 and 37

To a suspension of $LiAlH_4$ (607 mg, 16.0 mmol) in ether (32 ml) was added under argon with stirring a solution of the selected ketone (8.0 mmol) in ether (8 ml) and the mixture heated to reflux until GC analysis indicated that the reaction was complete (1 h). Water (0.6 ml), 15% aqueous NaOH (0.6 ml) and water (1.8 ml) were added, the liquid was decanted and the residue was extracted with ether (2×20 ml). The combined organic layers were concentrated on a rotary evaporator (bath temperature 20 °C/15 Torr) and the residue chromatographed on silica gel (0.05–0.20 mm) in pentane/ether (8:2; column 60×2 cm).

3.1.10.1. *rel*-(5*R*,6*R*)-6-Methyl-spiro[3.4]octan-5-ol (34a) and *rel*-(5*R*,6*S*)-6-methyl-spiro[3.4]octan-5-ol (34b). From **36** (1.11 g, 8.0 mmol); retention times (min) 6.90 (**36**), 7.78 (**34a**,**b**) at 130 °C on column A; $R_f=0.34$ (**34a**), 0.25 (**34b**). Yield 620 mg (55%) of pure **34a**, 100 mg (9%) of a 1:1 mixture of **34a** and **34b**, and 320 mg (29%) of pure **34b** as colourless liquids. **34a**: ^1H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=0.98$ (d, $J=7$ Hz, 3H), 1.18–1.36 (m, 2H), 1.65–1.90 (m, 8H), 1.98 (symm m, 1H), 2.10–2.20 (m, 1H), 3.65 (d, $J=4$ Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=14.39$ (q), 15.80 (t), 26.97 (t), 29.20 (t), 33.99 (t),

36.00 (t), 36.19 (d), 50.75 (s), 82.19 (d); MS (EI): $m/e=140$ (3, M^+), 97 (100). $C_9H_{16}O$ requires C, 77.09; H, 11.50. Found: C, 76.99; H, 11.40. **34b**: ^1H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=1.00$ (d, $J=7$ Hz, 3H), 1.02–1.10 (m, 1H), 1.55–2.00 (m, 10H), 2.08–2.20 (m, 1H), 3.22 (d, $J=6$ Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=16.44$ (t), 19.08 (q), 27.12 (t), 28.62 (t), 30.61 (t), 35.41 (t), 40.12 (d), 49.31 (s), 85.33 (d); MS (EI): $m/e=140$ (3, M^+), 97 (100). $C_9H_{16}O$ requires C, 77.09; H, 11.50. Found: C, 76.87; H, 11.47.

3.1.10.2. 6,6-Dimethyl-spiro[3.4]octan-5-ol (35). From **37** (1.22 g, 8.0 mmol); retention times (min) 2.38 (**37**), 3.52 (**35**) at 150 °C on column A; $R_f=0.30$ (**35**). Yield 1.17 g (94%) of pure **35** as colourless liquid. ^1H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=0.82$ (s, 3H), 0.96 (s, 3H), 1.30–1.61 (m, 4H), 1.65–1.80 (m, 3H), 1.80–1.97 (m, 2H), 2.01–2.13 (m, 1H), 2.26 (symm m, 1H), 3.26 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=16.72$ (t), 22.62 (q), 28.46 (q), 29.03 (t), 33.13 (t), 35.74 (t), 36.79 (t), 41.23 (s), 49.34 (s), 86.66 (d); MS (EI): $m/e=154$ (6, M^+), 139 (100). $C_{10}H_{18}O$ requires C, 77.87; H, 11.76. Found: C, 77.72; H, 11.77.

3.1.11. Rearrangement of 29a, 29b, 30a, 30b, 32a, 33, 34a, 34b, 35, 38a, 38b, 39 and 40

The selected bicyclobutyl-1-ol or spiro[3.4]octane-5-ol (1.0 mmol; **30a**, **30b**, **38b**: 0.2 mmol) was heated with an equimolar amount (13.5 ml; **30a**, **30b**, **38b**: 1.35 ml) of a 0.074 M solution of anhydrous *p*-toluenesulfonic acid in benzene to 70 °C. After 3 h, the mixture was diluted with pentane, washed with saturated sodium carbonate, dried over molecular sieves 3 Å and concentrated by distillation over a 20 cm Vigreux column (bath temperature 100°). According to GC on column A, the residue contained a single product in all cases. Analytically pure samples were isolated by preparative GC.

3.1.11.1. 3a-Methyl-1,2,3,3a,4,5-hexahydro-pentalen (41). From **39**; retention time (min): 2.42 at 120 °C. Colourless liquid. The ^1H[6] and ^{13}C NMR data[6] were in accord with literature data.

3.1.11.2. *rel*-(3a*R*,6a*R*)-6-Methyl-1,2,3,3a,4,6a-hexahydro-pentalene (42). From **29a**, **29b**, **34a** and **34b**; retention time (min): 2.81 at 130 °C. Colourless liquid. The ^1H[25b,c,e] and ^{13}C NMR data[25a,e] were in accord with literature data.

3.1.11.3. *rel*-(3a*R*,6a*R*)-3a,6-Dimethyl-1,2,3,3a,4,6a-hexahydro-pentalene (43). From **30a**, **30b**, **32a**, **38a** and **38b**; retention time (min): 3.09 at 120 °C. Colourless liquid. The ^{13}C NMR data[26a,c] were in accord with literature data. The ^1H NMR data have not yet been reported and were as follows: ^1H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=1.12$ (s, 3H), 1.32–1.72 (m, 6H), 1.62 (br s, 3H), 2.15 (symm m, 2H), 2.34 (m_c, 1H), 5.10 (br s, 1H).

3.1.11.4. *rel*-(3a*R*,6a*R*)-3a,6,6a-Trimethyl-1,2,3,3a,4,6a-hexahydro-pentalene (44). From **33** and **40**; retention time (min): 5.22 at 120 °C. Colourless liquid. ^1H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=0.91$ (s, 3H), 0.98 (s, 3H), 1.15–1.50 (m, 4H), 1.50–1.75 (m, 2H), 1.56 (br s, 3H), 2.11 (symm m, 2H), 5.13 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=13.03$ (q), 20.86 (q), 23.68 (t), 24.80 (q), 37.96 (t), 44.05 (t), 47.38 (t), 49.57 (s), 58.16 (s), 121.81 (d), 145.44 (s); MS (CI): $m/e=168$ (100, $M+NH_4]^+$). $C_{11}H_{18}$ requires C, 87.92; H, 12.08. Found: C, 88.10; H, 12.00.

3.1.11.5. *rel*-(3a*R*,6a*R*)-6,6a-Dimethyl-1,2,3,3a,4,6-hexahydro-pentalene (45). From **35**; retention time (min): 1.60 at 150 °C. Colourless liquid. ^1H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=1.05$ (s, 3H), 1.18–1.55 (m, 5H), 1.57 (symm m, 3H), 1.74–1.88 (m, 2H), 2.12 (symm m, 1H), 2.52 (symm m, 1H), 5.10 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=12.69$ (q), 25.84 (q), 26.00 (t), 36.05 (t), 37.98 (t), 38.51 (t), 49.11 (d), 58.18 (s), 122.30 (d), 145.33 (s); MS (EI): $m/e=136$ (26, M^+), 107 (100). $C_{10}H_{16}$ requires C, 88.16; H, 11.84. Found: C, 88.18; H, 11.65.

3.1.12. *rel*-(3a*R*,6a*R*)-3a,6a-Dimethyl-hexahydro-pentalen-1-one (46)

To a suspension of Nafion® R SAC-13 (100 mg) in dry benzene (1.5 ml) was added under argon with stirring **37** (76 mg, 0.50 mmol). Afterwards, the mixture was heated to 70 °C until GC analysis [column B, 140 °C, retention times (min): 4.00 (**37**), 8.97 (**46**)] indicated, that the rearrangement was complete (45 h). The mixture was filtered, the residue was washed with ether (2×2.5 ml) and the combined organic phases were concentrated. An analytically pure sample was isolated by preparative GC. Colourless solid, m.p. 96 °C. IR (KBr): 1735 cm^{-1} (C=O); ^1H NMR (300 MHz, $CDCl_3$, $CHCl_3$ int): $\delta=0.88$ (s, 3H), 1.00 (s, 3H), 1.48 (m_c, 2H), 1.54–1.70 (m, 4H), 1.80 (symm m, 1H), 1.98 (symm m, 1H), 2.30 (symm m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$, $CDCl_3$ int): $\delta=17.33$ (q), 22.52 (q), 22.72 (t), 31.92 (t), 35.61 (t), 37.05 (t), 39.82 (t), 49.63 (s), 58.69 (s), 224.96 (s); MS (EI): $m/e=152$ (38, M^+), 95 (100). HRMS m/z (M^+) calcd 152.1201, obsd 152.1201.

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Design and synthesis of novel type VI-like β-turn mimetics. Diversity at the $i+1$ and the $i+2$ position

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Abstract—In this paper, a synthetic approach for functionalised 5-aminopiperidinone-2-carboxylate (APC) systems as non-pro *cis*-peptide bond containing external β-turn mimics is presented. The scope and limitations of the synthetic method are discussed and the potential turn inducing properties of a model compound are evaluated by means of molecular modelling and NMR analysis.

1. Introduction

Analysis of a non-redundant set of proteins from the Brookhaven Protein Data Bank has revealed that non-Pro *cis*-peptide bonds might occur more often in proteins than previously thought.[1] Moreover, they appear at or near functionally important sites (such as metal binding sites, dimerisation domain, active center, cofactor/substrate binding domain) and are very likely involved in the function of the molecules. Because *cis*-peptide bonds can cause reversal of the peptide chain propagation, they can be involved in the formation of turn structures.

A turn or loop is one of the three major motifs of peptide and protein secondary structure. It plays a key role in many molecular recognition events including interactions between antigens and antibodies, peptide hormones and their receptors, and enzymes and their corresponding substrates. Different types of turns have been recognised by now, including δ-, γ-, β-, α- and π-turns corresponding to loops involving two to six residues, respectively.[2] Among the naturally occurring turns, the β-turn in which the peptide reverses direction over four residues (amino acids i, $i+1$, $i+2$ and $i+3$) is the most common one (Fig. 1). In this type of turns, an intramolecular hydrogen bond is usually observed between residue i and $i+3$ giving rise to a pseudo ten membered ring, though this is not a prerequisite.

The development of new turn stabilising structures (β-turn mimetics) has been the subject of numerous research papers.[3] Ball subdivides the different turn mimics in two classes: the ones with an internal support and those with an

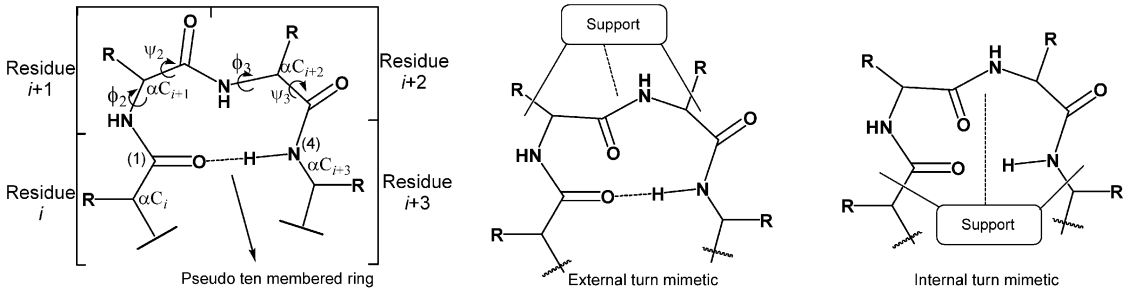

Figure 1. β-Turn mimics with internal and external support.

Keywords: Turn mimics; Pyrazinone; Diels–Alder reaction.
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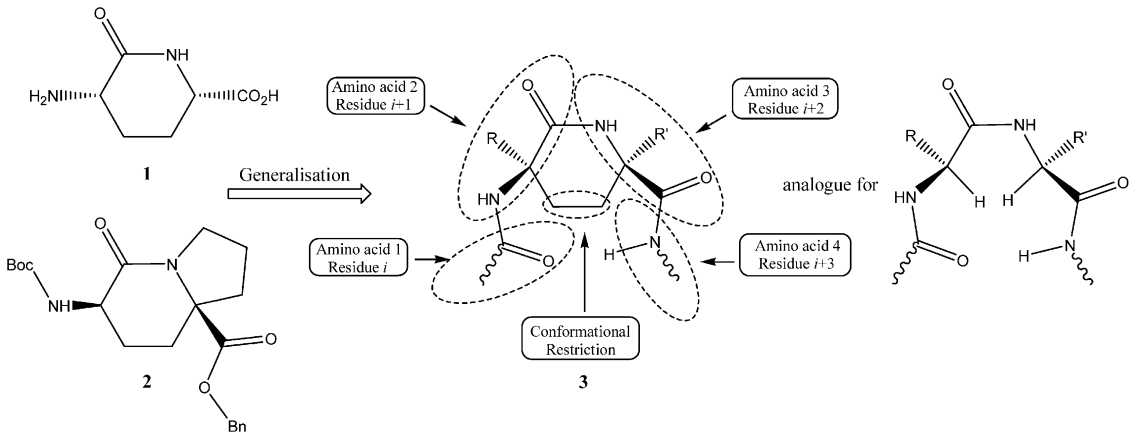


Figure 2. Design hypothesis based on known systems **1** and **2**.

external support (Fig. 1).[4] In internal support turn mimics, the emphasis of the system is on the pseudo ten membered ring of the hydrogen bonded tetrapeptides involved in the turn. The main focus in these systems is on generating scaffolds containing the side chain functionality of the β-turns they are mimicking.

In external turn mimetics on the other hand, the conformational flexibility of the peptide is diminished by an (isosteric) skeleton that is located outside the pseudo-10-membered structure: these mimics usually replace the central $i+1$ and $i+2$ residues in a turn. In these systems, incorporation of side chain functionality is less evident because the external skeleton is often not allowing this.

This is not really a problem if the turn is merely a structural prerequisite not involved in binding to the receptor.[5] However, in cases where binding to the receptor occurs at the turning position, the side chains might be involved. Hence the development of functionalised external turn mimetics, which is the subject of this paper, is an interesting topic.

2. Design hypothesis

In order to stabilise *cis*-peptide bond containing turns ('type VI-like' turns because they do not contain proline), we have to devise a synthesis for molecules which can replace the $i+1$ and the $i+2$ positions of a β-turn and which display the side chain functionality of the parent dipeptide mimicked.

Interesting examples of *cis*-peptide bond containing constrained dipeptides that have been developed as β-turn mimetics are the system **1** reported by Kemp[6] and a similar system **2** described by Robinson[7] and Germanas[8] (Fig. 2). Unfortunately, the compounds are not so easily functionalised using the synthetic strategies presented by the authors. As a consequence displaying side chain functionality at the $i+1$ and the $i+2$ position is somewhat difficult.

Based on the structural resemblance between the compounds **1** and **2**, we hypothesised that the turn inducing properties of these compounds were due to the *cis* 5-aminopiperidinone-2-carboxylate (APC) unit **3** (Fig. 2). A functionalised system of this type probably would meet the criteria we set ourselves (*cis*-peptide bond, functionalised and turn inducing). The relationship between the APC system **3** and the dipeptide mimicked is shown in Figure 2.

3. Molecular modelling

In order to verify our design hypothesis, a molecular modelling analysis was performed on a system **4** (Fig. 3). This compound **4** serves as a model for a tetrapeptide. The i and $i+3$ residues in the system are simplified to an *N*-methyl amide and an acyl amide. In this system, the hydrogen bond present in most β-turns can still be formed.

The general procedure for the computational analysis is outlined in Figure 3.[9] A conformational search was performed starting from 2000 random starting conformations (Macromodel, MCMM search, AMBER* force field, solvation model water) and all structures were energy minimised to 0.05 kcal/mol Å.[10] All conformations found within 3 kcal/mol of the global minimum conformation were checked for the accepted indicators for β-turn properties: this includes checking whether the distance between the α carbon atoms of residue 1 (residue i) and residue 4 (residue $i+3$) is smaller than 7 Å. Another criterion is the virtual dihedral angle β as defined by Ball[11] which has to be between -30 and $+30°$.[12] Finally, the presence of a hydrogen bond is also considered as indicative for a β-turn (though open turns without this hydrogen bond also exist).

Including the global minimum conformation, 11 conformations were found within 3 kcal/mol of this global minimum. The global minimum conformation (depicted in Fig. 4) fulfils all of the turn criteria. Also the other local minima within 3 kcal/mol are good turn inducing candidates. In order to get an idea about the stability of the turn conformation in the global minimum, a 1000 ps molecular dynamics analysis was performed on this structure. 10,000 snapshots were taken which were further analysed for the properties mentioned above. The results are summarised in Figure 5. According to this molecular dynamics analysis, this conformation is stable and the hydrogen bond is

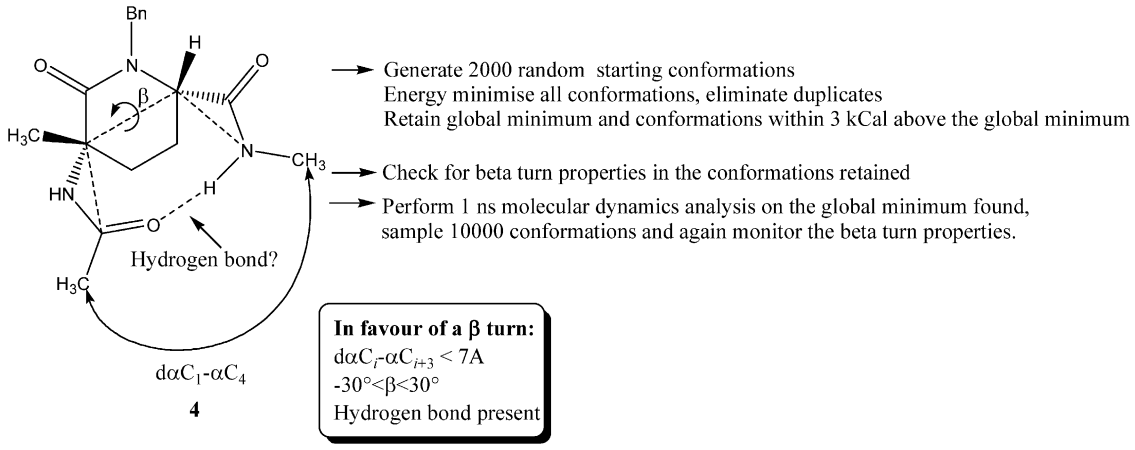


Figure 3. Analysis of model compound **4**.

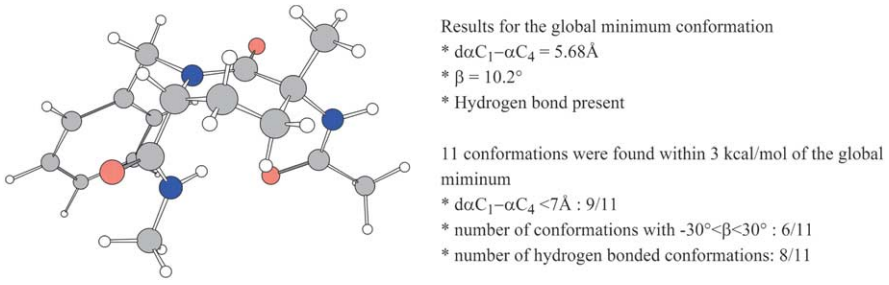


Figure 4. Global minimum conformation and its β-turn indicators.

retained in 89.4% of the samples. A narrow distribution in the sampled conformers is observed both for the distance between the α-carbon atoms and for the virtual dihedral angle β. The mean values for the distance between the α-carbon atoms of 5.68 Å and a mean dihedral angle of 9.4° in the samples are close to the observed values for the global minimum starting conformation. This also proves this conformation is a stable one.

According to this molecular modelling analysis, compound **4**, which is taken as a model for the target systems, shows good turn inducing properties in this model system. This is in agreement with our design hypothesis and urged us to start the synthesis of these compounds.

4. Synthesis

The development of a synthetic method starting from cheap commercially available starting materials was our main objective. Retrosynthetic analysis (Fig. 6) shows that the functionalised APC-systems **8** can be derived from bicyclic precursors **7**. These bicyclic lactams can be considered as the reaction product of an intermolecular Diels–Alder reaction of a functionalised 5-chloropyrazinone **6** with ethene followed by hydrolysis. A very efficient synthesis for these easily functionalisable pyrazinones has been developed in our laboratory starting from simple amino nitriles.[13]

Hence, the 4 steps in the synthesis of these compounds can

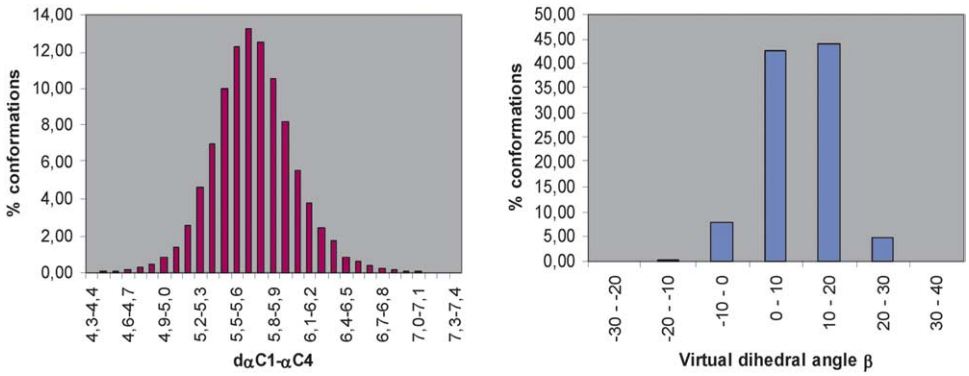


Figure 5. Results of the molecular dynamics analysis.

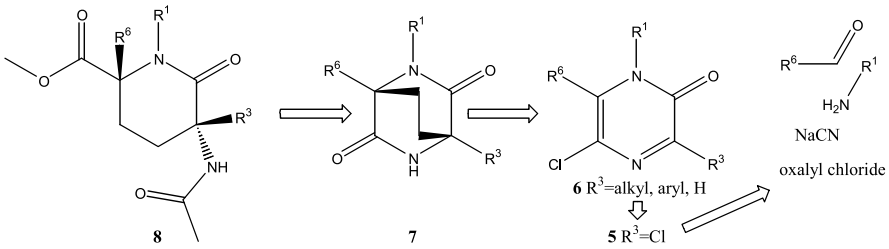


Figure 6. Retrosynthetic analysis of the APC systems.

be summarised as follows: (A) pyrazinone synthesis, (B) functionalisation of the 3-position of the pyrazinone, (C) Diels–Alder reaction, (D) methanolysis. These steps will further be discussed in more detail.[14]

4.1. Synthesis of dichloropyrazinones, introduction of the $i+2$ functionality in the target compound

In our procedure for the synthesis of 3,5-dichloropyrazinones,[13] the functional groups at positions 1 and 6 of the pyrazinone originate, respectively, from a primary amine R^1NH_2 and an aldehyde R^6CHO which are converted to an amino nitrile (or the corresponding HCl salt) via Strecker synthesis. Upon treatment with oxalyl chloride, this amino nitrile smoothly converts to a dichloropyrazinone in chlorobenzene as the solvent; in this reaction triethyl ammonium chloride is added to drive the reaction to completion by acting as an additional chloride source (Scheme 1). In the case where R^6=isopropyl, the yields of dichloropyrazinone were originally very low (10–15%). Mass spectral analysis of the crude reaction mixture revealed that the conversion of the intermediate **9** to the corresponding pyrazinone was not very efficient (Scheme 1). Addition of a catalytic amount of DMF at this stage in the reaction proved to solve this problem by increasing the yield of product **5d** up to 85%. In the ^{1}H NMR spectrum of **5d** run at room temperature, some peaks are doubled because of hindered rotation of the isopropyl and the *p*-methoxybenzyl groups. At 213 K all peaks are doubled while at 303 K all peaks are coalesced and only one compound is observed.

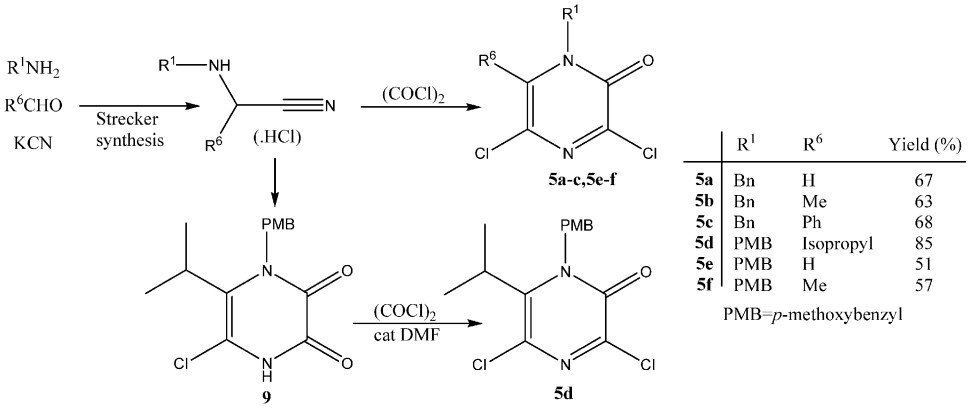
Throughout the years this pyrazinone synthesis has proven to be very robust and many substituents have been introduced on the dichloropyrazinones.[15]

At this stage of the synthesis, the functionality R^6 of what will become the $i+2$ residue of the target APC system is introduced as well as the Bn or PMB protective group (Fig. 2).[16] In the development stage of this work, we have chosen a number of 'random' substituents to prove the methodology. All yields of the compounds obtained were acceptable to good (Scheme 1). In this respect, we introduced the side chains of Gly (R^6=H), Ala (R^6=Me), Val (R^6=*iso*-propyl) and the unnatural Phg (phenylglycine, R^6=Ph). In theory, the functionality at this position is only limited by the availability of the corresponding aldehyde and its compatibility with the acidic cyclisation conditions to form a pyrazinone.

4.2. Functionalisation of the 3-position of pyrazinones, introduction of the $i+1$ functionality in the target compound

As discussed in the retrosynthetic approach (Fig. 6), the next step in the synthesis of the APC turn mimics is the functionalisation of the 3-position of the pyrazinones.

Different methods have been developed by us to functionalise these 3,5-dichloropyrazinones at the 3-position. Both carbon–heteroatom bonds[15] and carbon–carbon bonds can be formed. In our current approach towards APC systems, we are interested in forming new carbon–carbon bonds. Common organometallic reactions can be used to achieve this goal. Successful functionalisation of the 3-position is possible using Grignard reactions, Stille couplings, Suzuki reactions and Heck reactions (Scheme 2).[15] The palladium catalysed reactions are all carried out at reflux temperature of the appropriate solvent (see Section 7) while the substitution by a Grignard reagent needs deep cooling to −78 °C in order to get the compounds in a reasonable yield



	R^1	R^6	Yield (%)
5a	Bn	H	67
5b	Bn	Me	63
5c	Bn	Ph	68
5d	PMB	Isopropyl	85
5e	PMB	H	51
5f	PMB	Me	57

PMB=*p*-methoxybenzyl

Scheme 1. Synthesis of dichloropyrazinones.

(if addition of the Grignard reagent is performed at room temperature, tarry side products are formed which impede isolation of the target compound). The Stille reactions proceed smoothly, but it is sometimes difficult to get rid of the last traces of organotin impurities. We tried to overcome this problem by stirring the crude extract of the reaction with KF in ethyl acetate. Most of the organotin impurities are removed by this procedure. Reductive dechlorination using $Pd(PPh_3)_4$ and sodium formate in DMF or via H_2/Pd/C is also an option (compound **6c**).

In this step of the synthesis, the functionality R^3 of what will become the $i+1$ residue of the target APC system is introduced (Fig. 2).[16] To prove the concept of the strategy, the side chains of Ala (R^3=Me), Phe (R^3=Bn), Gly (R^3=H), Val (R^3=*iso*-propyl) and again the unnatural Phg (Phenylglycine, R^3=Ph) were introduced in the model systems (Scheme 2).

4.3. Diels–Alder reaction, introduction of the conformational restriction in the target compound

The key step in the synthesis of the APC systems is the Diels–Alder reaction of the functionalised pyrazinone systems with ethene (Scheme 3). This reaction is carried out in a steel bomb at 135 °C under 35 atm ethene pressure. Completion of the reaction takes 12 to 48 h depending on the nature of the substrate. As can be expected, it takes a longer time for the more hindered substrates to be fully converted into the adducts. In general, the reaction is left for 16 h, and if starting material is still observed on TLC after 16 h, the reaction is relaunched for another 12 h. The imidoyl chloride formed after addition is prone to hydrolysis; especially in the case where R^3=Ph this hydrolysis is very fast and almost immediately upon opening the steel bomb the bislactam is formed. In other cases, the (usually partially hydrolysed) mixture is evaporated to dryness, redissolved in chloroform and stirred in a flask open to air moisture to complete the hydrolysis.[17,18] Presumably, traces of HCl already formed accelerate autocatalytic hydrolysis.

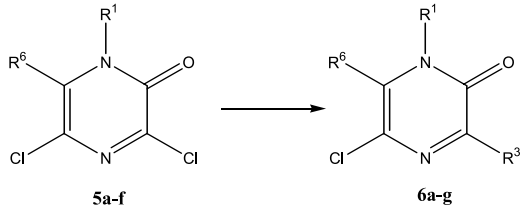
These cycloaddition reactions with ethene have also been investigated by us for similar pyrazinone compounds under somewhat milder conditions using microwave irradiation (reaction was performed in an ethene saturated solution instead of under 35 atm ethene pressure).[19] In this case, however, a specialised microwave reactor is needed. For the larger scale syntheses of these compounds we still rely on the more classical approach described above.

During this step of the reaction, two goals are achieved. First of all the conformational restriction of the target APC systems is introduced. Secondly the *cis*-relationship between the amine and the carboxylate, which we believe to be important for the β-turn inducing properties (see design hypothesis), is fixed in the precursor molecule due to the stereospecific *syn*-addition of the Diels–Alder reaction.

4.4. Conversion of bislactams into APC systems via selective methanolysis reaction: scope and limitations.[20]

The last step in the synthesis of the target compounds relies on the selectivity observed in the methanolysis reaction of a secondary lactam in the presence of a tertiary one.[21] This behaviour is in agreement with the general observation that the rate-determining step during acid catalysed hydrolysis of amides is the attack of water on an *O*-protonated amide to form a tetrahedral intermediate: indeed, steric retardation of the latter process appears to be the governing factor in many cases.[22] However, in the case of simple secondary and tertiary *N*-methyl amides, an anomalous behaviour is observed since the latter react slightly faster upon acidic hydrolysis. This result is probably due to non-steric factors, for example, σ-donation by the *N*-alkyl substituents and/or solvation effects.[22]

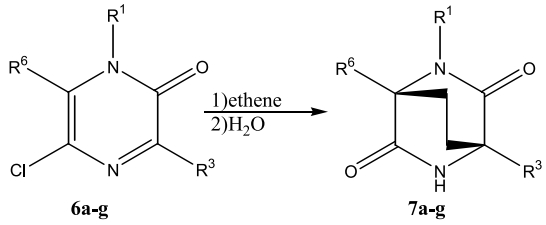
In order to check the generality of the approach, a profound study of the factors governing the selectivity and the



	R^1	R^3	R^6	Method	Yield (%)
6a	Bn	Me	H	Stille coupling	91
6b	Bn	Ph	Me	Stille coupling	92
6c	Bn	H	Ph	Pd/C	92
6d	PMB	Me	Isopropyl	Grignard reaction	45
6e	PMB	Bn	H	Grignard reaction	72
6f	PMB	Isopropyl	Me	Grignard reaction	68
6g	PMB	Me	Me	Grignard reaction/ Stille coupling	62 89

PMB=*p*-methoxybenzyl

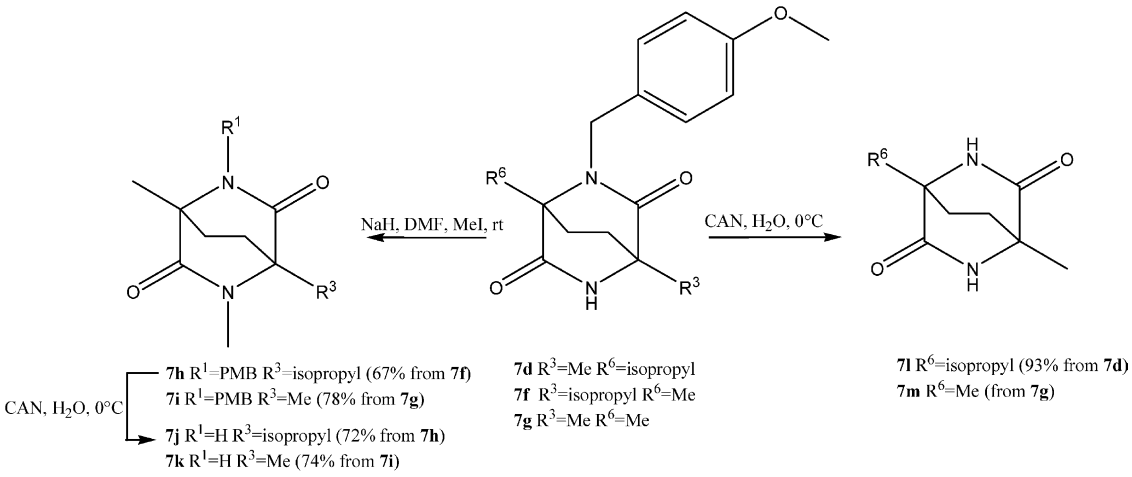
Scheme 2. Functionalisation of the 3-position of the pyrazinones.



	R^1	R^3	R^6	Yield (%)
7a	Bn	Me	H	79
7b	Bn	Ph	Me	88
7c	Bn	H	Ph	82
7d	PMB	Me	Isopropyl	56
7e	PMB	Bn	H	81
7f	PMB	Isopropyl	Me	52
7g	PMB	Me	Me	64

PMB=*p*-methoxybenzyl

Scheme 3. Diels–Alder reaction/hydrolysis of the pyrazinones.



Scheme 4. Preparation of test compounds for methanolysis study.

feasability of the methanolysis of 2,5-diazabicyclo-[2.2.2]octane-3,6-dione systems was performed.[20] The role of bulky substituents at the bridgehead positions 1 and 4 of the dione on the selectivity of the methanolysis as well as the effect of the secondary or tertiary nature of the lactam moiety was investigated.

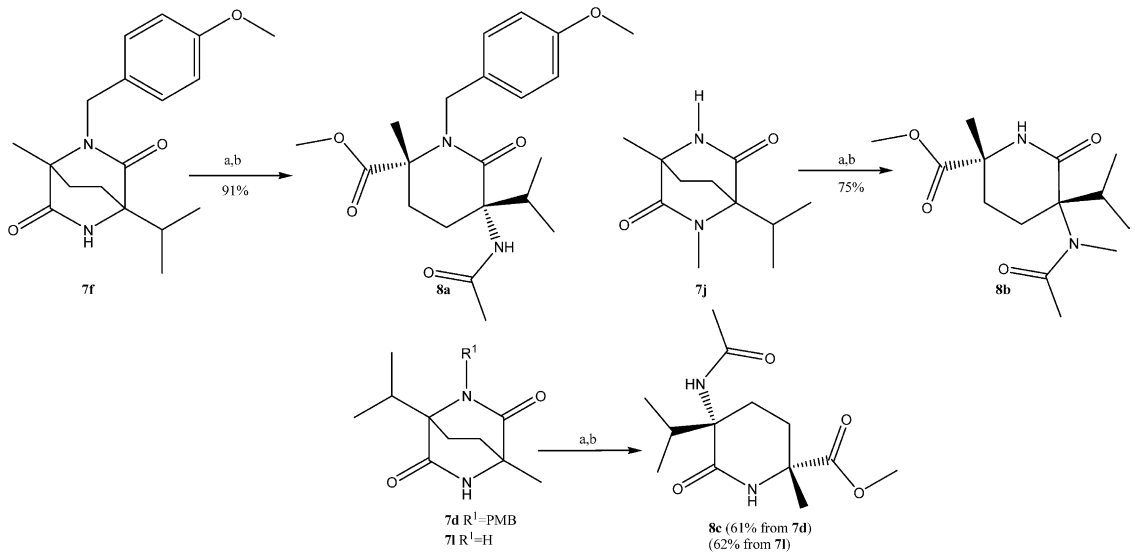
To be able to study the effects mentioned, it was necessary to synthesize a number of analogues of the bislactam systems. The conversion of **7f** and **7g** to **7j** and **7k**, respectively, was done by first methylating the secondary amide function with methyl iodide followed by removal of the PMB-group using CAN (Scheme 4). The 2-*N*-deprotected bislactam **7j** precipitated out of the reaction mixture. Direct removal of the PMB-group of bislactam **7d** and **7g** was also effected using CAN (Scheme 4). Compounds **7l** and **7m** were formed, respectively. Compound **7m** was directly used in the next methanolysis step after extraction.

4.4.1. Effect of bulky groups on the selectivity of the methanolysis process. First, we studied the effect of bulky substituents on the bridgehead positions 1 and 4 of the diones (Scheme 5). Thus treatment of 4-isopropyl-2,5-diazabicyclo[2.2.2]octane-3,6-dione **7f** with a HCl-saturated methanol solution for 16 h resulted in a selective cleavage of the secondary lactam moiety. In order to prevent recyclisation upon neutralisation of the reaction mixture, the newly formed primary amine was trapped as the *N*-substituted acetamide by addition of triethylamine and acetic anhydride yielding compound **8a**. When compound **7j** was subjected to the acid methanolysis/*N*-acetylation sequence, selective cleavage of the tertiary lactam afforded the APC system **8b**. The 4-isopropyl group appears to shield the 'top'(2,3) lactam function irrespective of its secondary or tertiary nature. Hence, relief of the bicyclic strain can be attained only by attack of methanol at the sterically more accessible lactam carbonyl group.

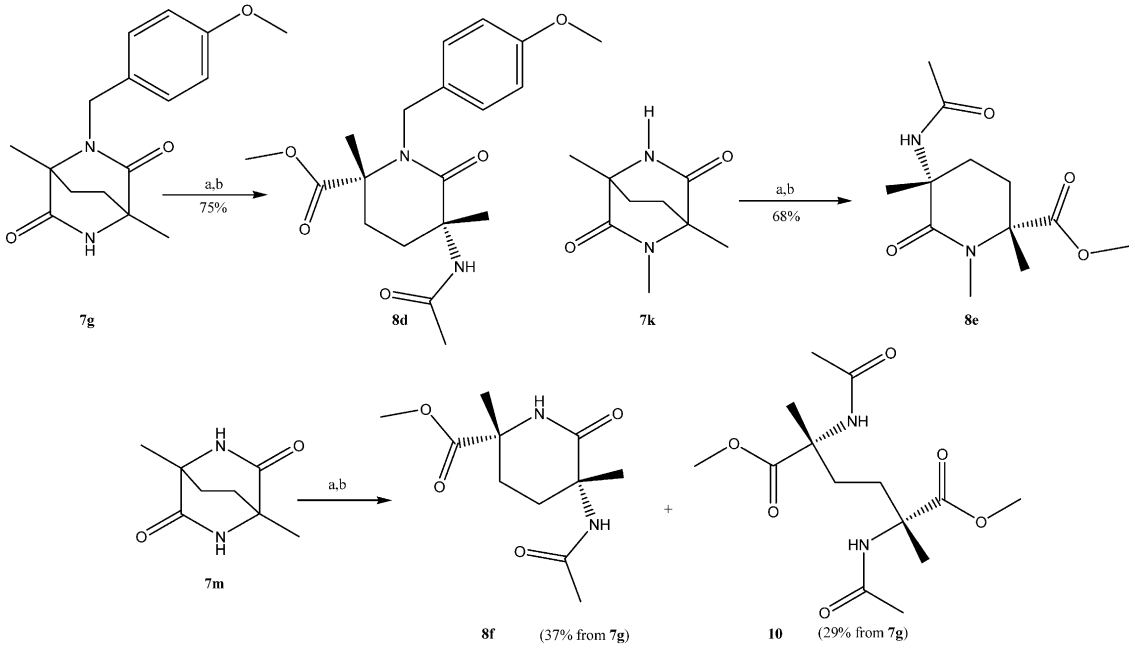
This statement also holds for the methanolysis of precursor **7l**. In this case, the 6-carbonyl function is shielded by the isopropyl group in position 1 resulting in selective cleavage of the 'upper' 2,3-amide linkage. When precursor **7d** is subjected to acid methanolysis compound **8c** is also readily formed. According to our experience the *p*-methoxybenzyl group is not easily removed from a lactam nitrogen; hence the less hindered tertiary lactam probably is cleaved first followed by removal of the benzylic group from the amine formed after cleavage.

4.4.2. Effect of secondary or tertiary lactam groups on the selectivity of the methanolysis process. In a second part, we studied the selectivity for methanolysis in the presence of two equal non bulky substituents in α-position, that is, two methyl groups (Scheme 6). Acidic methanolysis of the bicyclic system **7g** resulted in selective cleavage of the secondary amide to afford the monocyclic lactam **8d**. A comparable behaviour was observed for 2-benzyl-1,4-diphenyl-2,5-diazabicyclo[2.2.2]octane-3.6-dione.[21] When the secondary and tertiary amides were interchanged as in compound **7k** again the secondary amide was cleaved selectively to afford **8e**. Treatment of compound **7m** with an HCl-saturated methanol solution for 48 h and trapping of the intermediate amine as the *N*-acetyl derivative furnished the monocyclic secondary lactam **8f**. However, when the methanolysis was continued for one week, a mixture of singly cleaved product **8f** and doubly cleaved compound **10** was obtained. The absence of bicyclic strain accounts for the much slower second cleavage. These results indicate that a secondary lactam is cleaved preferentially without risk for a tertiary lactam cleavage if bulky substituents are absent. A second cleavage might occur if the reaction is left for a longer period of time if a secondary lactam function is present in the APC system formed.[6]

To unravel the reasons for the preferential acidic cleavage of the secondary lactam group in the bridged bislactam compounds, we performed a molecular mechanics model study of the two tetrahedral geminal diol intermediates corresponding to attack of water on either one of the two lactam functions of compound **7k** This study clearly revealed a much higher energy (difference ca. 6–7 kcal/mol) for the geminal diol formed next to the *N*-methyl group as compared to the one formed next to NH. Indeed, since the methyl group is now located on an sp^3 *N*-atom in a tight boat conformation it experiences a severe eclipsing interaction with one neighbouring OH group (Fig. 7).



Scheme 5. Effect of bulky substituents on the selectivity of the methanolysis. (a) MeOH, HCl, rt; (b) Ac_2O, Et_3N, rt.



Scheme 6. Effect of the secondary and tertiary nature of the lactam moiety on the selectivity of the methanolysis. (a) MeOH, HCl, rt; (b) Ac_2O, Et_3N, rt.

From our combined results it appears that the preferential cleavages observed for the bislactams studied here can be ascribed to two interrelated effects, that is, relief of the bicyclic strain which is modulated by the steric retardation caused by the *N*-alkyl and bridgehead substituents. While the first effect enhances the rate of acid hydrolysis of the bicyclic versus the monocyclic lactams, the latter apparently directs the attack of the nucleophile to the less sterically hindered lactam carbonyl group.

The site of cleavage in the APC systems was determined by 2D NMR spectroscopy using the long range ^{13}C–^{1}H couplings between the carbonyl groups and the neighbouring protons (HMBC). The principle is explained for compound **8b**: a coupling is noticed between the carbonyl of the methyl ester and the 2-methyl protons as well as a coupling between the *N*-methyl protons and the acetyl carbonyl group as seen in **8b**. These two couplings are not consistent with structure **11**, formed by cleavage of the other lactam group (Fig. 8).

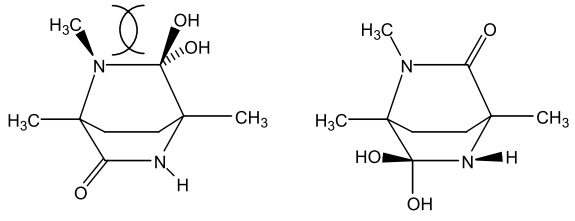


Figure 7. Eclipsing interactions in *gem*-diol intermediates corresponding to hydrolysis at CONMe and CONH groups of **7k**.

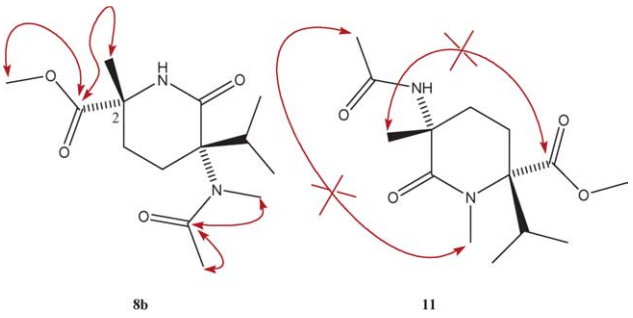


Figure 8. In theory compounds **8b** and **11** can be obtained upon methanolysis of compound **7j**. Structure **8b** is consistent with HMBC-data.

These observations can be summarised as follows: the main driving force of the reaction is alleviation of the bicyclic strain, which enhances the rate of acid hydrolysis of the bicyclic lactam relative to subsequent cleavage of the monocyclic lactam formed. The selectivity of the methanolysis reaction further depends on the steric factors involving the *N*-alkyl and bridgehead substituents. An isopropyl group at the α-position of the lactam carbonyl seems to completely prevent its sensitivity to methanolysis. In this case, the bicyclic strain will be relieved by cleaving the other lactam function, irrespective of its secondary or tertiary lactam nature. In the absence of bulky α-substituents, secondary lactam functions cleave more readily than tertiary ones. When after the first methanolysis a secondary lactam is still present, a second slow cleavage can occur but tertiary lactams seem to be stable.

The other bicyclic compounds **7a**–**7c** and **7e** also reacted in accordance with the observations made above (Scheme 7): because no bulky substituents are present, the secondary lactam function cleaves selectively during methanolysis. After trapping of the primary amine with acetic anhydride all compounds **8g**–**8j** are isolated in good yields.

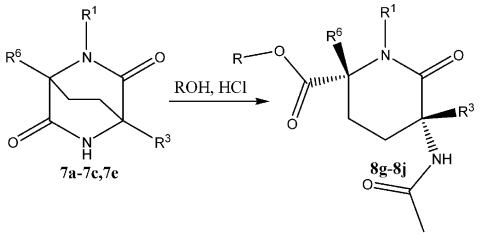
The insights gained in the reactivity of these systems in methanolysis slightly narrow the scope of the method: according to our results it will be impossible to get a secondary (or tertiary) side chain substituent at the *C*-terminal residue ($i+2$ position) of the dipeptide mimic because steric shielding prevents cleavage at the corresponding carbonyl position in the bislactam. In other cases, cleavage of the secondary lactam group is selective and our general synthesis strategy is applicable.

It has to be noted that the methanolysis reactions are somewhat difficult to monitor. TLC-monitoring is not possible in this case. Moreover, chemical ionisation mass spectral analysis of a neutralised sample extract suffers from the drawback that methanol can be lost thermally from the methyl ester products to form back the bridged bislactam starting material. However, the No-D NMR procedure described recently by Hoye et al. (using non-deuterated solvents) in this case appears to be the method of choice to get a quantitative view of what is happening in the mixture (Fig. 9).[23] The main advantage of this method is that aliquots can be taken directly from the reaction mixture to monitor the progress of the reaction.[24] The expansion of the spectrum clearly shows that the signal to noise ratio with these non deuterated solvent experiments is still sufficient to monitor the compound of interest. This is illustrated in Figure 9 where the acid catalysed methanolysis of compound **7g** is monitored by checking the disappearance of the characteristic methyl singlets in the starting material (indicated with ○ in Fig. 9). The signals of the corresponding methyl protons in the reaction product are already visible after 5 min (indicated with × in Fig. 9).

5. Analysis of β-turn properties of a model compound

In order to check the β-turn inducing properties of the APC systems synthesised, an NMR analysis was performed on compound **4** (Fig. 10). This was prepared by direct conversion of **8g** to the *N*-methyl amide by reaction with 33% $MeNH_2$ in ethanol, followed by evaporation of the reaction mixture and recrystallisation.

In the ^{1}H NMR spectrum (Fig. 10), the protons H^A and H^B on the ethylene moiety show up between 2.5 and 1.5 ppm. From the 2D COSY spectrum, the protons H^B neighbouring H^C can be identified as the signals at 2.05 and 1.19 ppm. The broader signals are assigned to the axial protons because these show mutual axial–axial couplings. The more complex signal pattern observed for H^{Bax} compared to H^{Aax} is due to coupling with H^C. In theory two conformations A and B are possible for this APC system: one with H^C pseudoaxial and one with H^C pseudoequatorial on the ring (Fig. 11). Proton H^C shows up as a broad doublet with one coupling of 7 Hz and a smaller coupling that was not resolved; this originally was interpreted as conformation B in which H^C has a big axial coupling with H^{Bax} and a small gauche coupling with H^{Beq}.[14] However, in a NOESY spectrum of this compound, a number of NOEs are observed which do not correspond with this initial proposal. For instance, the NOE found between N*H*CH_3 and the axial proton H^{Aax} is not consistent with conformer B. On the other hand this signal and the other NOEs observed fully agree with conformation A. Moreover this is also in accordance with the results of the molecular mechanics analysis (global minimum conformation) described before.



	R^1	R^3	R^6	R	Yield (%)
8g	Bn	Me	H	Me	91
8h*	Bn	Ph	Me	Et	82
8i	Bn	H	Ph	Me	84
8j	PMB	Bn	H	Me	75

* acid catalysed ethanolysis was used in this case
PMB=*p*-methoxybenzyl

Scheme 7. Methanolysis of the adducts and trapping with AcOAc.

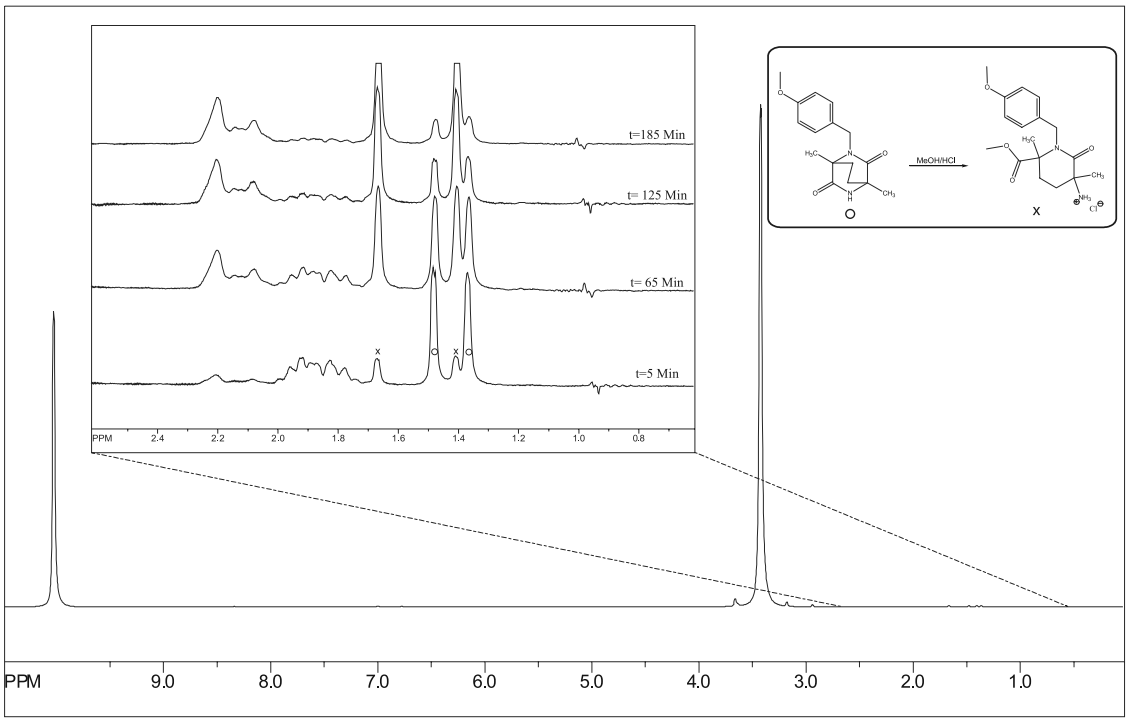


Figure 9. No-D NMR spectrum of the methanolysis mixture of **7g** at different time intervals (inset box).

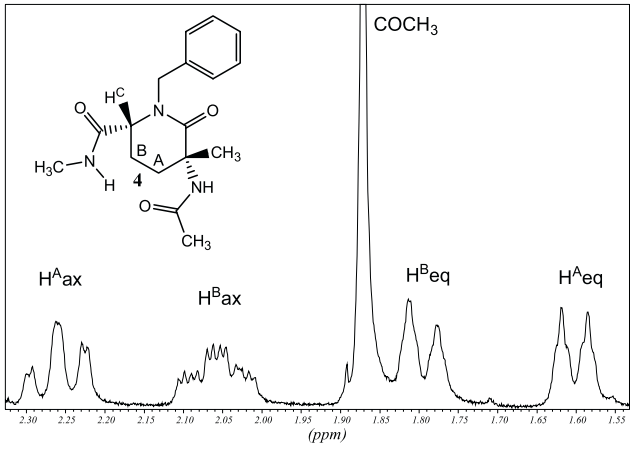


Figure 10. Ethylene bridge region in the ^{1}H NMR spectrum of the model compound.

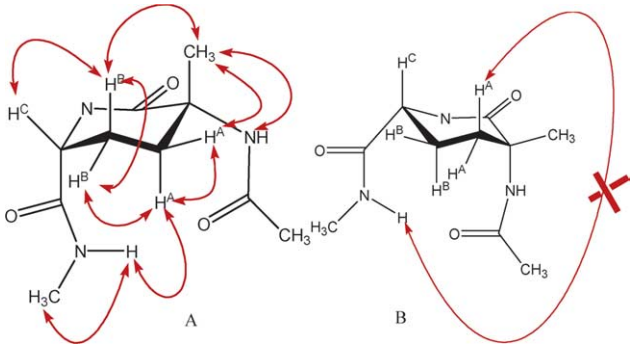


Figure 11. Structure determination based on observed NOE signals (*N*-Bn not shown in figures).

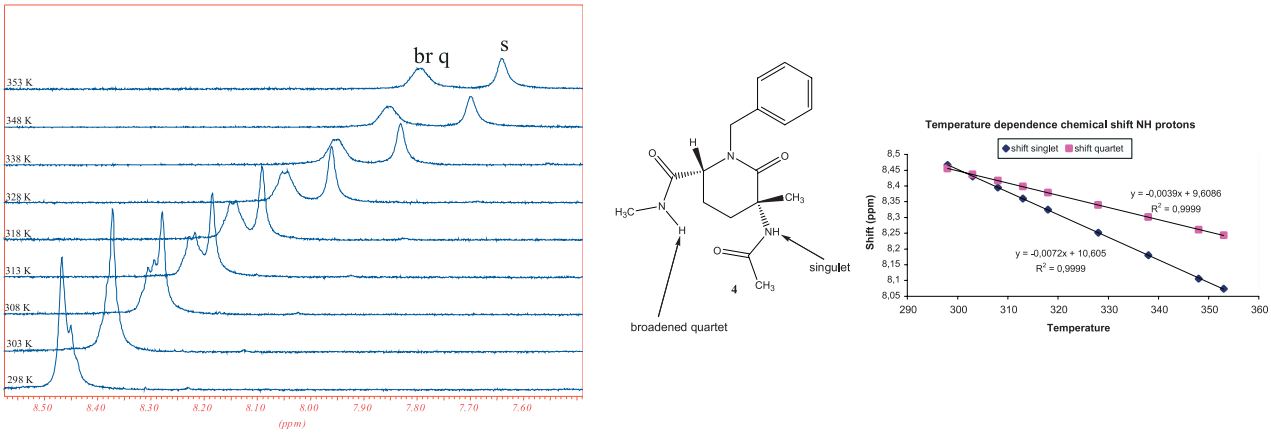


Figure 12. Hydrogen bond analysis of the model compound.

The presence of a hydrogen bond was also checked by ^{1}H NMR spectroscopy on compound **4**. The temperature dependence of the chemical shift of a hydrogen bonded amide proton is small (0 to −3 ppb/°C) when compared to the temperature dependence of a solvent exposed proton (< −7 ppb/°C).[7] The chemical shifts of the amide protons N*H*CO (singlet at $\delta = 8.47$ ppm) and N*H*Me (broadened quartet at $\delta = 8.46$ ppm) in DMSO-d_6 were recorded at different temperatures (Fig. 12). Linear regression on the collected data points provided us with the following results:the chemical shift dependence of −3.9 ppb for N*H*Me suggests this proton is shielded from the solvent and hydrogen bonding might be responsible for this shielding. The N*H*CO proton on the other hand is solvent exposed (shift dependence −7.2 ppb/°C). The value of −3.9 ppb just falls out of the region for hydrogen bonding; possibly this is due to a conformationial equilibrium with a non hydrogen bonded species or to the fact that the solvent shielding of a hydrogen bond in a model compound is less pronounced.[25] Further evidence for the presence of the hydrogen bond was obtained by checking the solvent dependency of the chemical shift of the amide protons upon changing the solvent from DMSO to $CDCl_3$. The results are summarised in Table 1. The small dependence of the chemical shift of the N*H*Me proton (0.14 ppm) also is in agreement with a hydrogen bond (or at least with shielding from the solvent). The exposed N*H*COMe proton is not shielded and therefore undergoes a large shift of 2.9 ppm upon switching the solvent.

6. Conclusion

A general non-Pro containing type VI-like β-turn mimetic was designed based on the structural resemblance between the systems of Kemp and Robinson/Germanas. In our hypothesis, we stated the turn inducing properties of these systems arise from the *cis*-relationship between an amine and a carboxylate function in an APC system. Based on this hypothesis, we developed a synthetic method for functionalised systems of this type. Key steps in the synthesis are the generation of a functionalised pyrazinone mimicking the side chains of the target compound, Diels–Alder reaction of this pyrazinone with ethene to impose the conformational restriction on the system and to fix the stereochemistry in the target compounds and finally a selective methanolysis reaction to form the APC systems. The scope and limitations of this sequence were further investigated.

Turn inducing properties were analysed by means of a combined NMR/molecular modelling analysis. Based on these results, it is concluded that our design hypothesis is correct and that these functionalised APC systems are good candidates for β-turn induction. These external turn mimics can be functionalised with the side chains of the dipeptide they are mimicking. The synthesis allows the introduction of both natural and non-natural amino acid side chains.

Introducing enantioselectivity in the system and scanning peptides with these type VI-like turn inducing systems is under current investigation.

7. Experimental

7.1. Analytical instruments

Melting points were taken using an Electrothermal IA 9000 digital melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin–Elmer 1600 Fourier transform spectrometer. Mass spectra were run using a Hewlett Packard MS-Engine 5989A apparatus for EI and CI

Table 1. Chemical shift dependence of amide protons of model compound **4**

δ_{NHMe} DMSO (ppm)	δ_{NHMe} $CDCl_3$ (ppm)	$\Delta\delta_{NHMe}$ (ppm)	δ_{NHCO} DMSO (ppm)	δ_{NHCO} $CDCl_3$ (ppm)	$\Delta\delta_{NHCO}$ (ppm)
8.46	8.32	0.14	8.47	5.88	2.59

Both the computational and the NMR analysis results indicate the design hypothesis is valid: hence this type of functionalised APC systems are promising candidates for β-turn induction.

spectra, and a Kratos MS50TC instrument for exact mass measurements performed in the EI mode at a resolution of 10,000. For the NMR spectra (δ, ppm) a Bruker AMX 400 and a Bruker Avance 300 spectrometer were used. Analytical and preparative thin layer chromatography was carried out using Merck silica gel 60 PF-224, for column chromatography 70–230 mesh silica gel 60 (E.M. Merck) was used as the stationary phase.

7.2. Synthesis

7.2.1. Synthesis of dichloropyrazinones. For the preparation of the pyrazinones, we refer to the corresponding references:**5a**,[13] **5b**,[15g] **5c**,[21] **5e**,[21a] **5f**.[9a]

7.2.2. Synthesis of pyrazinone 5d. The general procedure for the synthesis of pyrazinones 5 is used (0.1 mol scale) with the following modification: after the addition of oxalyl chloride, the mixture is stirred at room temperature for 4 h. Subsequently 1 mL of DMF is added to the mixture. The solution is stirred for another 48 h. After evaporation, the crude mixture is treated as described in the general procedure.

7.2.3. 3,5-Dichloro-6-isopropyl-1-(4-methoxybenzyl)-2(1*H*)-pyrazinone (5d). Yield: 85%; melting point: 107–108 °C (EtOH); IR (KBr) cm^{-1}: 2930 (s, $CH(CH_3)_2$), 2827 (s, OCH_3), 1680 (s, CO), 1615 (s, CN); ^1H NMR (400 MHz, $CDCl_3$, ppm, *303 K*): 7.07 (d, 2H, $J=8.2$ Hz, ArH), 6.87 (d, 2H, $J=8.2$ Hz, ArH), 5.38 (s, 2H, CH_2Ph), 3.79 (s, 3H, OCH_3), 3.31 (br s, 1H, $CH(CH_3)_2$), 1.27 (d, 6H, $J=7.1$ Hz, $CH(CH_3)_2$); (400 MHz, $CDCl_3$, ppm, *213 K*): major conformer ($\pm$90%) 7.02 (d, 2H, $J=8.6$ Hz, ArH), 6.84 (d, 2H, $J=8.7$ Hz, ArH), 6.04 (d, 1H, $J=15.7$ Hz, CH_2Ph), 4.65 (d, 1H, $J=15.7$ Hz, CH_2Ph), 3.74 (s, 3H, OCH_3), 3.15 (heptuplet, 1H, $J=7.0$ Hz, $CH(CH_3)_2$), 1.34 (d, 3H, $J=6.8$ Hz, $CH(CH_3)_2$), 0.96 (d, 3H, $J=6.8$ Hz, $CH(CH_3)_2$). minor conformer ($\pm$10%) 7.02 (d, 2H, ArH), 6.84 (d, 2H, ArH), 5.30 (d, 1H, CH_2Ph), 5.18 (d, 1H, CH_2Ph), 3.88 (heptuplet, 1H, $J=7.0$ Hz, $CH(CH_3)_2$), 3.74 (s, 3H, OCH_3), 1.34 (d, 3H, $J=7.0$ Hz, $CH(CH_3)_2$), 1.15 (d, 3H, $J=7.0$ Hz, $CH(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): 159.5 (C*para*), 153.2 (C2), 143.9 (C3), 143.5 (C*ipso*), 127.9 (C*meta*), 126.8 (C6), 123.7 (C5), 114.6 (C*ortho*), 55.3 (OCH_3), 49.3 (CH_2Ph), 29.9 ($CH(CH_3)_2$), 18.2 (CH_3); EIMS *m/z* (%): 326 ($M^{\circ+}$, 100); HRMS: calcd for $C_{15}H_{16}Cl_2N_2O_2$: 326.0589; Found: 326.0605.

7.2.4. Functionalisation of the 3-position of pyrazinones. For the preparation of the substituted pyrazinones, we refer to the corresponding references:**6a**,[15g] **6b**,[15h] **6c**,[21] **6g**.[9a]

7.3. General procedure for the Grignard reaction on pyrazinones

To a cooled solution (−78 °C) of 1 mmol of pyrazinone in dry THF is slowly added via canula a solution of the corresponding Grignard reagent (1.3 mmol). The solution is kept at low temperature until completion of the reaction (30 min–2 h). The mixture is worked up at low temperature by addition of saturated NH_4Cl solution and extracted with ether. The organic layers are dried over $MgSO_4$, filtered and evaporated. The crude compounds are purified by column chromatography (silicagel, $CH_2Cl_2 \rightarrow CH_2Cl_2$–EtOAc 70:30).

7.3.1. 5-Chloro-6-isopropyl-1-(4-methoxybenzyl)-3-methyl-2(1*H*)-pyrazinone (6d). Yield: 45%; melting point: 97 °C (CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$, ppm): 7.06 (d, 2H, $J=8.0$ Hz, ArH), 6.87 (d, 2H, $J=8.1$ Hz, ArH), 5.36 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3), 3.26 (br s, 1H, $CH(CH_3)_2$), 2.49 (s, 3H, CH_3), 1.27 (d, 6H, $J=7.3$ Hz, $CH(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): 159.5 (C*para*), 156.6 (CO), 154.4 (C3), 141.7 (C*ipso*), 128.1 (C*meta*), 128.0 (C6), 125.8 (C5), 114.8 (C*ortho*), 55.7 (OCH_3), 48.0 (CH_2Ph), 30.1 ($CH(CH_3)_2$), 21.2 ($CH(CH_3)_2$), 18.8 (CH_3); EIMS *m/z* (%): 306 ($M^{\circ+}$, 8), 121 ($C_8H_9O^+$, 100); HRMS: calcd for $C_{16}H_{19}ClN_2O_2$: 306.1131; Found 306.1147.

7.3.2. 3-Benzyl-5-chloro-1-(4-methoxybenzyl)-2(1*H*)-pyrazinone (6e). Yield: 72%; melting point: 102–103 °C; IR (KBr, cm^{-1}): 1648 (CO), 1586 (CN); ^1H NMR (300 MHz, $CDCl_3$, ppm): 7.44 (d, 2H, $J=7$ Hz, Ar), 7.35–7.30 (m, 5H, ArH), 7.01 (s, 1H, H6), 6.9 (d, 2H, $J=8.7$ Hz, *meta* PMB), 4.93 (s, 2H, N–CH_2 of PMB), 4.12 (s, 2H, CH_2Ph), 3.8 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): 159.8 (C*para*), 158.8 (C2), 154.4 (C3), 136.3 (C*ipso* Bn), 130.2 (CH-Ar), 129.3 (CH-Ar), 128.3 (CH-Ar), 126.5 (CH-Ar), 125.8 (C6), 125.7 (C*ipso* PMB), 124.3 (C5), 114.4 (*meta*PMB), 55.18 (OCH_3), 51.8 (CH_2 of PMB), 39.8 (CH_2 of Bn); EIMS *m/z* (%): 340 (8, $M^{\circ+}$), 121 (100, CH_3O–C_6H_4–$CH_2^+\cdot$); HRMS: calcd for $C_{19}H_{17}N_2O_2Cl$: 340.0978; Found: 340.0972.

7.3.3. 5-Chloro-3-isopropyl-1-(4-methoxybenzyl)-6-methyl-2(1*H*)-pyrazinone (6f). Yield: 68%; melting point: 89 °C (EtOH) IR (KBr) cm−1: (2862 (s, CH_3), 2871 (s, $CH(CH_3)_2$), 1648 (s, CONBn); ^1H NMR (300 MHz, $CDCl_3$, ppm): 7.10 (d, 2H, $J=8.7$ Hz, ArH), 6.83 (d, 2H, $J=8.4$ Hz, ArH), 5.23 (s, 2H, CH_2Ph), 3.75 (s, 3H, OCH_3), 3.45 (heptuplet, 1H, $J=6.9$ Hz, CH), 2.34 (s, 3H, CH_3), 1.23 (d, 6H, $J=6.9$ Hz, $CH(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): 161.6 (C*para*), 159.6 (C2), 155.8 (C3), 133.2 (C6), 128.7 (C*meta*), 127.4 (C5), 126.4 (C*ipso*), 114.8 (C*ortho*), 55.6 (OCH_3), 48.5 (CH_2Ph), 31.2 ($CH(CH_3)_2$), 20.4 ($CH(CH_3)_2$), 17.0 (CH_3); EIMS *m/z* (%): 306 ($M^{\circ+}$, 14); 121 ($C_8H_9O^+$, 100); HRMS: calcd for $C_{16}H_{19}ClN_2O_2$: 306.1131; Found: 306.1115.

7.4. Diels–Alder reaction. General procedure for the synthesis of compounds 7a–7g

The pyrazinone precursor (1 mmol) is dissolved in 30 mL of toluene and the solution is transferred to a steel bomb. The mixture is heated under ethene pressure (35 atm) at 135 °C for 1–2 days. Upon cooling and removal of ethene, the solvent is evaporated under reduced pressure. The imidoyl chloride intermediates are further hydrolised to the desired compounds **7a–7g** by stirring them in $CHCl_3$ open to air moisture.

These compounds are further purified by column chromatography (silica gel, CH_2Cl_2–EtOAc (95–5)). For the synthesis of compound **7c**, the imidoyl chloride intermediate is treated with 50 mL of moisturised EtOAc

containing a drop of HCl solution for 1 night. Upon addition of 10 mL of water, the solution is neutralised with K_2CO_3. The organic phase is separated and dried over $MgSO_4$ and evaporated under reduced pressure. The crude product is further purified by column chromatography (silica gel, CH_2Cl_2).

7.4.1. 5-Benzyl-1-methyl-2,5-diazabicyclo[2.2.2]octane-3,6-dione (7a). Yield: 79%; melting point: 170 °C (hexane/CH_2Cl_2); IR (KBr) cm^{-1}: 1707 (s, CO); ^1H NMR (400 MHz, $CDCl_3$, ppm): 7.34–7.24 (m, 5H, PhH), 6.44 (s, 1H, NH), 4.77 (d, 1H, $J=14.5$ Hz, CH_2Ph), 4.38 (d, 1H, $J=14.5$ Hz, CH_2Ph), 3.89 (br s, 1H, H4), 1.95–1.65 (m, 4H, CH_2CH_2), 1.54 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): 171.9 (CO, broad), 171.3 (CO), 136.2 (PhC*ipso*), 128.9;128.2;128.0 (ArC), 60.2 (C4, broad, visible in an HMQC spectrum), 58.2 (C1), 48.6, 32.5 (CH_2), 24.8 (CH_2), 18.3 (CH_3); EIMS *m/z* (%): 244 ($M^{\circ+}$, 84), 111 ($M^{\circ+}-CONCH_2Ph^{\circ}$, 43), 91 ($PhCH_2^+$, 100), 83 ($M^{\circ+}-CONCH_2$-$Ph^{\circ}$–CO, 60); HRMS: calcd for $C_{14}H_{16}N_2O_2$: 244.1212; Found: 244.1213.

7.4.2. 2-Benzyl-1-methyl-4-phenyl-2,5-diazabicyclo-[2.2.2]octane-3,6-dione (7b). Yield: 88%; melting point: 227 °C (CH_2Cl_2/hexane); IR (KBr) cm^{-1}: 1692 (s, CO); ^1H NMR (400 MHz, $CDCl_3$, ppm): 7.20–7.48 (m, 10H, PhH), 6.36 (s, 1H, NH), 4.84 (d, 1H, $J=16$ Hz, CH_2Ph), 4.56 (d, 1H, $J=16$ Hz, CH_2Ph), 2.32 (ddd, 1H, $J=14$ Hz, 10 Hz, 5 Hz, H8′), 2.16 (ddd, 1H, $J=14$ Hz, 10 Hz, 5 Hz, H8), 2.08 (ddd, 1H, $J=13.5$ Hz, 10 Hz, 5 Hz, H7), 1.96 (ddd, 1H, $J=13.5$ Hz, 10 Hz, 5 Hz, H7′), 1.55 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): 172.7 (CO), 171.1 (CO), 126.9–138.0 (ArC), 63.4 (C4), 61.8 (C1), 45.1 (CH_2Ph), 39.2 (CH_2), 29.3 (CH_2), 16.6 (CH_3); EIMS *m/z* (%): 320 ($M^{\circ+}$, 37), 187 ($M^{\circ+}-CONCH_2Ph^{\circ}$, 69), 159 ($M^{\circ+}-CONCH_2Ph^{\circ}$–CO, 100), 91 ($PhCH_2^+$, 68); HRMS: calcd for $C_{20}H_{20}N_2O_2$: 320.1525; Found: 320.1523.

For spectral data of compound **7c** we refer to Ref. 21.

7.4.3. 1-Isopropyl-2-(4-methoxybenzyl)-4-methyl-2,5-diazabicyclo[2.2.2]octane-3,6-dione (7d). Yield: 56%; melting point: 176 °C; IR (KBr) cm^{-1}: 3189 (NH), 1687 (CO); ^1H NMR (300 MHz, $CDCl_3$, ppm): 7.12 (d, 2H, $J=8.1$ Hz, ArH), 6.81 (d, 2H, $J=8.1$ Hz, ArH), 6.74 (s, 1H, NH), 5.03 (d, 1H, $J=16.1$ Hz, CH_2Ph), 4.37 (d, 1H, $J=16.1$ Hz, CH_2Ph), 3.78 (s, 3H, OCH_3), 2.37 (heptuplet, 1H, $J=6.6$ Hz, $CH(CH_3)_2$), 1.97–1.88 (m, 2H, CH_2), 1.82–1.64 (m, 2H, CH_2), 1.50 (s, 3H, CH_3), 1.35 (d, 3H, $J=6.6$ Hz, $CH(CH_3)_2$), 1.13 (d, 3H, $J=6.6$ Hz, $CH(CH_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): 174.7 (CO), 172.7 (CO), 159.1 (C*para*), 131.0 (C*ipso*), 128.7 (C*meta*), 114.4 (C*ortho*), 68.0 (C4), 57.1 (C1), 55.6 (OCH_3), 45.4 (CH_2Ph), 32.6 (CH_2), 29.1 (CH_2), 29.1 (CH_3), 19.9 ($CH(CH_3)_2$), 18.9 ($CH(CH_3)_2$); EIMS *m/z* (%): 316 ($M^{\circ+}$, 25), 152 ($M^{\circ+}\cdot-CONHC_8H_9O$, 36), 121 ($C_8H_9O^+$, 100); HRMS: calcd for $C_{18}H_{24}N_2O_3$: 316.1787; Found: 316.1779.

7.4.4. 1-Benzyl-5-(4-methoxybenzyl)-2,5-diazabicyclo-[2.2.2]octane-3,6-dione (7e). Yield: 81%; melting point: 184 °C; IR (KBr, cm^{-1}): 3209 (NH), 1704 (CO), 1689 (CO); ^1H NMR (300 MHz, $CDCl_3$, ppm): 7.41–7.24 (m, 5H, ArH), 7.19 (d, 2H, $J=8.5$ Hz, *ortho* PMB), 6.86 (d, 2H, $J=8.5$ Hz, *meta*PMB), 5.17 (s, 1H, NH), 4.76 (d, 1H, $J=14$ Hz, N–CH of PMB), 4.32 (d, 1H, $J=14$ Hz, N–CH of PMB), 3.86 (m, 1H, H4), 3.79 (s, 3H, OCH_3), 3.5 (d, 1H, $J=14$ Hz, Ph-CH), 3.07 (d, 1H, $J=14$ Hz, Ph-CH), 1.98 (m, 1H, bridge H), 1.88–1.64 (m, 3H, bridge H); ^{13}C NMR ($CDCl_3$, 100 MHz): 171.5 (CO), 170.7 (CO), 159.4 (C*para*), 134.7 (*ipso* benzyl), 130.4, 129.5, 129.1 (ArCH), 128.4 (C*ipso* PMB), 127.5 (ArCH), 114.3 (C*meta* PMB), 60.4 (C1), 59.0 (C4), 55.2 (OCH_3), 48.0 (CH_2 of PMB), 37.2 (CH_2-Ph), 30.8, 24.8 (C7, C8); EIMS *m/z* (%): 350 ($M^{\circ+}$, 61), 229 ($M^{\circ+}-CH_3O-C_6H_4-CH_2^{\cdot}$, 34), 186 ($C_{12}H_{12}NO^+$, 60), 121 ($CH_3O-C_6H_4-CH_2^+$, 100); HRMS: calcd for $C_{21}H_{22}N_2O_3$: 350.1630; Found: 350.1630.

7.4.5. 4-Isopropyl-2-(4-methoxybenzyl)-1-methyl-2,5-diazabicyclo[2.2.2]octane-3,6-dione (7f). Yield: 52%; melting point: 207 °C (EtOH); IR (KBr) cm^{-1}: 3347 (m, NH), 2832 (s, OCH3), 1696 (s, CO), 1668 (s, CO); ^1H NMR (400 MHz, $CDCl_3$, ppm): 7.09 (d, 2H, $J=8.6$ Hz, ArH), 6.82 (d, 2H, $J=8.4$ Hz, ArH), 5.95 (s, 1H, NH), 4.70 (d, 1H, $J=15.6$ Hz, CH_2Ph), 4.47 (d, 1H, $J=15.6$ Hz, CH_2Ph), 3.78 (s, 3H, OCH_3), 2.44 (q, 1H, $J=6.9$ Hz, $CH(CH_3)_2$), 2.04–1.97 (m, 1H, CH_2), 1.84–1.75 (m, 3H, CH_2+CH_2), 1.48 (s, 3H, CH_3), 1.14 (d, 3H, $J=6.9$ Hz, $CH(CH_3)_2$), 1.10 (d, 3H, $J=6.9$ Hz, $CH(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): 173.4 (CO), 172.0 (CO), 158.8 (C*para*), 130.6 (C*ipso*), 128.3 (C*meta*), 114.3 (C*ortho*), 62.7 (C4), 61.4 (C1), 55.2 (OCH_3), 43.9 (CH_2Ph), 33.2 (CH_2), 29.0 ($CH(CH_3)_2$), 28.0 (CH_2), 17.9 ($CH(CH_3)_2$), 17.1 ($CH(CH_3)_2$), 16.5 (CH_3); EIMS *m/z* (%): 316 ($M^{\circ+}$, 46), 121 ($C_8H_9O^+$, 100); HRMS: calcd for $C_{18}H_{24}N_2O_3$: 316, 1787; Found: 316, 1797.

7.4.6. 2-(4-Methoxybenzyl)-1,4-dimethyl-2,5-diazabicyclo[2.2.2]octane-3,6-dione (7g). Yield: 64%; melting point: 177 °C; IR (KBr) cm^{-1}: 3180 (NH), 1692 (CO); ^1H NMR (300 MHz, $CDCl_3$, ppm): 7.40 (s, 1H, NH), 7.08 (d, 2H, $J=8.8$ Hz, ArH), 6.81 (d, 2H, $J=8.8$ Hz, ArH), 4.71 (d, 1H, $J=16$ Hz, CH_2Ph), 4.45 (d, 1H, $J=16$ Hz, CH_2Ph), 3.77 (s, 3H, OCH_3), 1.91–1.71 (m, 4H, CH_2), 1.53 (s, 3H, CH_3), 1.46 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): 174.1 (CO), 173.2 (CO), 159.2 (C*para*), 130.8 (C*ipso*), 128.4 (ArC), 114.5 (ArC), 62.1 (C1/C4), 57.8 (C1/C4), 55.6 (OCH_3), 44.4 (CH_2Ph), 33.6 (CH_2), 32.5 (CH_2), 18.9 (CH_3), 16.9 (CH_3); EIMS *m/z* (%): 288 ($M^{\circ+}$, 43), 124 ($M^{\circ+}-CONHC_8H_9O^{\cdot}$, 87), 121 ($C_8H_9O^+$, 100); HRMS: calcd for $C_{16}H_{20}N_2O_3$: 288.1474; Found: 288.1484.

7.5. General procedure for the synthesis of 7h and 7i

To a stirred solution of 1 mmol of **7f** or **7g** in DMF was added 1.4 mmol of NaH. After stirring for 5 min at room temperature 1.2 mmol of MeI was added to this mixture. The reaction was kept at room temperature for 16 h. The crude product was obtained after workup with saturated NH_4Cl solution followed by extraction and evaporation of the solvent. The products were purified by column chromatography (Silicagel, CH_2Cl_2–EtOAc, 60:40 for **6i** and 80:20 for **6h**).

7.5.1. 1-Isopropyl-5-(4-methoxybenzyl)-2,4-dimethyl-2,5-diazabicyclo[2.2.2]octane-3,6-dione (7h). Yield: 67%; melting point: colorless oil; IR (NaCl) cm^{-1}: 1679

(CO); ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 7.04 (d, 2H, J=8.4 Hz, H*meta*), 6.79 (d, 2H, J=8.4 Hz, H*ortho*), 4.70 (d, 1H, J=15.6 Hz, CH_2Ph), 4.36 (d, 1H, J=15.6 Hz, CH_2Ph), 3.74 (s, 3H, OCH_3), 2.99 (s, 3H, NCH_3), 2.34 (heptuplet, 1H, J=6.8 Hz, *CH*$(CH_3)_2$), 1.98–1.92 (m, 1H, CH_2), 1.82–1.73 (m, 2H, CH_2+CH_2), 1.63–1.57 (m, 1H, CH_2), 1.46 (s, 3H, CH_3), 1.34 (d, 3H, J=6.8 Hz, CH$(CH_3)_2$), 1.26 (d, 3H, J=6.8 Hz, CH(*CH*$_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): 174.1 (*C*ONCH_3), 171.2 (CO), 158.7 (C*para*), 130.6 (C*ipso*), 128.0 (C*meta*), 114.0 (C*ortho*), 66.5 (C4), 60.5 (C1), 55.3 (OCH_3), 43.7 (CH_2Ph), 33.0 (CH_2), 29.8 (*C*H$(CH_3)_2$), 28.5 (NCH_3), 27.0 (CH_2), 19.1 (CH(*C*H$_3)_2$), 18.5 (CH(*C*H$_3)_2$), 17.2 (CH_3); EIMS *m/z* (%): 330 ($M^{\circ+}$, 88), 209 ($M^{\circ+}-C_8H_9O^{\cdot}$, 27), 167 ($M^{\circ+}-CONHC_8H_9O^{\cdot}$, 70), 121 ($C_8H_9O^+$, 100); HRMS: calcd for $C_{19}H_{26}N_2O_3$: 330.1943; Found: 330.1944.

7.5.2. 2-(4-Methoxybenzyl)-1,4,5-trimethyl-2,5-diazabicyclo[2.2.2]octane-3,6-dione (7i). Yield: 78%; melting point: 102 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 7.01 (d, 2H, J=8.8 Hz, ArH), 6.74 (d, 2H, J=8.8 Hz, ArH), 4.69 (d, 1H, J=16.0 Hz, CH_2Ph), 4.33 (d, 1H, J=16.1 Hz, CH_2Ph), 3.69 (s, 3H, OCH_3), 2.87 (s, 3H, NCH_3), 1.87–1.59 (m, 4H, 2×CH_2), 1.54 (s, 3H, CH_3), 1.43 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): 172.6 (CO), 172.4 (CO), 159.1 (C*para*), 130.8 (C*ipso*), 128.4 (ArC), 114.4 (ArC), 61.5 (C1/C4), 61.1 (C1/C4), 55.6 (OCH_3), 44.4 (CH_2Ph), 33.1 (CH_2), 32.0 (CH_2), 27.4 (NCH_3), 17.9 (CH_3), 17.5 (CH_3); EIMS *m/z* (%): 302 ($M^{\circ+}$, 56), 138 ($M^{\circ+}-CONHC_8H_9O^{\cdot}$, 100), 121 ($C_8H_9O^+$, 98); HRMS: calcd for $C_{17}H_{22}N_2O_3$: 302.1630; Found: 302.1639.

7.6. General procedure for the CAN deprotection

The *para*-methoxybenzyl protected bislactam-system is dissolved in acetonitrile and cooled in an ice bath. 3eq of CAN, dissolved in a minimum amount of H_2O, are added dropwise. After stirring for 3 h, the solution is extracted with CH_2Cl_2 and the combined organic layers are dried with $MgSO_4$. Upon removal of the solvent, the crude product is purified by chromatography (MeOH/CH_2Cl_2). The unprotected product precipitates out of the reaction solution in case of compound **7j** or **7l**.

7.6.1. 1-Isopropyl-2,4-dimethyl-2,5-diazabicyclo[2.2.2]octane-3,6-dione (7j). Yield: 72%; melting point: 113 °C; IR (KBr) cm^{-1}: 3184 (s, NH), 1690 (s, CO); ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 6.51 (s, 1H, NH), 2.99 (s, 3H, NCH_3), 2.31 (heptuplet, 1H, J=6.8 Hz, *CH*$(CH_3)_2$), 2.05–1.72 (m, 4H, 2×CH_2), 1.47 (s, 3H, CH_3), 1.30 (d, 3H, J=6.9 Hz, CH(*CH*$_3)_2$), 1.21 (d, 3H, J=6.9 Hz, CH(*CH*$_3)_2$); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): 174.6 (CO), 172.5 (CO), 56.6 (C4), 53.4 (C1), 32.3 (CH_2), 29.6 (NCH_3), 28.8 (*C*H$(CH_3)_2$), 27.1 (CH_2), 19.3 (CH_3), 18.9 (CH_3), 18.8 (CH_3); EIMS *m/z* (%): 210 ($M^{\circ+}$, 4), 165 ($M^{\circ+}-HCONH^{\cdot}$, 100), 153 ($M^{\circ+}-CONHMe^{\cdot}$, 44); HRMS: calcd for $C_{11}H_{18}N_2O_2$: 210.1368; Found: 210.1363.

7.6.2. 1,2,4-Trimethyl-2,5-diazabicyclo[2.2.2]octane-3,6-dione (7k). Yield: 74%; melting point: oil; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 6.72 (s, 1H, NH), 2.92 (s, 3H, NCH_3), 1.98–1.79 (m, 4H, CH_2), 1.54 (s, 3H, CH_3), 1.50 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): 173.8 (CO), 172.8 (CO), 61.5 (C1/C4), 57.5 (C1/C4), 32.7 (CH_2), 32.6 (CH_2), 27.3 (NCH_3), 18.9 (CH_3), 17.2 (CH_3).

7.6.3. 1-Isopropyl-4-methyl-2,5-diazabicyclo[2.2.2]octane-3,6-dione (7l). Yield: 93%; melting point: 201 °C; ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 8.33 (s, 1H, NH), 8.26 (s, 1H, NH), 2.07 (heptuplet, 1H, J=6.6 Hz, *CH*$(CH_3)_2$), 1.87–1.57 (m, 4H, CH_2), 1.22 (s, 3H, CH_3), 1.02 (d, 3H, J=6.6 Hz, CH(*CH*$_3)_2$), 1.00 (d, 3H, J=6.6 Hz, CH(*CH*$_3)_2$); ^{13}C NMR (75 MHz, DMSO-d_6, ppm): 174.3 (CO), 173.2 (CO), 62.7 (C1/C4), 56.6 (C1/C4), 32.7 (CH_2), 28.6 (CH_2), 18.0, 17.7, 17.1 (CH_3, CH); EIMS *m/z* (%): 196 ($M^{\circ+}$, 4), 151 ($M^{\circ+}-H_2NCOH$, 86), 121 ($C_8H_9O^+$, 100); HRMS: calcd for $C_{10}H_{16}N_2O_2$: 196.1212; Found: 196.1205.

7.7. Conversion of bislactams into APC systems via selective methanolysis reaction. General procedure for the methanolysis reaction

A solution of 1 mmol of bicyclic adduct **7** in 15 mL of MeOH is cooled to 0 °C. This solution is saturated with dry HCl gas for 5 min. Alternatively, 1 mL of $SOCl_2$ is slowly added to the methanol solution under cooling (CAUTION vigorous reaction). Upon completion of the reaction (check mass spectrum or NO-D NMR), the solution is evaporated under reduced pressure and the crude residue is dissolved in 8 mL of acetic anhydride. The mixture is cooled in an ice bath and Et_3N is added until precipitation of triethyl ammonium salts is observed. Upon removal of the ammonium salts, the solution is evaporated and the product **7** is purified by column chromatography (Silicagel, EtOAc).

7.7.1. Methyl 5-(acetylamino)-5-isopropyl-1-(4-methoxybenzyl)-2-methyl-6-oxo-2-piperidinecarboxylate (8a). Yield: 91%; melting point: 112 °C (CH_2Cl_2/Hex); IR (KBr) cm^{-1}: 3390 (m, NH), 2839 (w, OCH_3), 1740 (s, COOEt), 1682 (s, CO), 1648 (s, CO); ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 7.13 (d, 2H, J=8.7 Hz, H*ortho*), 6.76 (d, 2H, J=8.7 Hz, H*meta*), 6.60 (s, 1H, NH), 5.36 (d, 1H, J=15.4 Hz, CH_2Ph), 3.63 (d, 1H, J=15.4 Hz, CH_2Ph), 3.33 (s, 3H, OCH_3), 3.26 (s, 3H, OCH_3), 3.19 (dt, 1H, J=14.7 Hz, 3.9 Hz, $H4_{eq}$), 2.32 (td, 1H, J=14.3 Hz, 3.8 Hz, $H4_{ax}$), 2.10 (heptuplet, 1H, J=6.8 Hz, *CH*$(CH_3)_2$), 1.89 (dt, 1H, J=14.8 Hz, 4.2 Hz, $H3_{eq}$), 1.72 (td, 1H, J=14.3 Hz, 3.4 Hz, $H3_{ax}$), 1.55 (s, 3H, CH_3CO), 1.29 (s, 3H, CH_3), 1.10 (d, 3H, J=6.8 Hz, CH(*CH*$_3)_2$), 0.97 (d, 3H, J=6.8 Hz, CH(*CH*$_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): 173.9 (CON+COOCH_3), 169.5 (CH_3CONH), 158.5 (C*para*), 130.4 (C*ipso*), 128.3 (C*ortho*), 113.7 (C*meta*), 65.9 (C2), 61.8 (C5), 55.1 (OCH_3), 52.7 (OCH_3), 48.6 (CH_2Ph), 35.2 (*C*H$(CH_3)_2$), 31.7 (CH_2), 25.5 (CH_3), 24.8 (CH_2), 24.5 (CH_3), 17.7 (CH_3), 17.1 (CH_3); EIMS *m/z* (%): 390 ($M^{\circ+}$, 17), 303 ($C_{18}H_{25}NO_3^+$, 41), 121 ($C_8H_9O^+$, 100); HRMS: calcd for $C_{21}H_{30}N_2O_5$: 390, 2153; Found: 390, 2155.

7.7.2. Methyl 5-[acetyl(methyl)amino]-5-isopropyl-2-methyl-6-oxo-2-piperidinecarboxylate (8b). Yield: 75%; melting point: 93.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 5.87 (s, 1H, NH), 3.79 (s, 3H, OCH_3), 2.96 (s, 3H, NCH_3), 2.49–2.43 (m, 2H, *CH*$(CH_3)_2+CH_2$), 2.23 (ddd, 1H, J=14.1 Hz, 9.7 Hz, 3.7 Hz, CH_2), 2.07 (s, 3H, $COCH_3$), 1.92 (ddd, 1H, J=13.8 Hz, 9.3 Hz, 3.7 Hz, CH_2), 1.79 (ddd, 1H, J=13.8 Hz, 7.8 Hz, 3.7 Hz, CH_2), 1.42 (s, 3H, CH_3), 1.12

(d, 3H, $J=6.8$ Hz, CH(CH_3)$_2$), 1.06 (d, 3H, $J=6.8$ Hz, CH(CH_3)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): 174.2 ($COOCH_3$), 171.8 ($CONHCH_3$), 170.2 (CO), 66.5 (C5), 58.5 (C2), 52.8 (OCH_3), 34.8 (NCH_3), 33.8 (*C*H(CH_3)$_2$), 28.6 (CH_2), 27.5 (CH_3), 26.4 (CH_2), 24.3 ($COCH_3$), 19.3 (CH(*C*H_3)$_2$), 18.6 (CH(*C*H_3)$_2$); EIMS *m/z* (%): 241 ($M^{\circ+}-COCH_3^{\cdot}$, 81), 225 ($M^{\circ+}-COOCH_3^{\cdot}$, 29), 212 ($M^{\circ+}-CH_3NCOCH_3^{\cdot}$, 67), 199 (100).

7.7.3. Methyl 5-(acetylamino)-5-isopropyl-2-methyl-6-oxo-2-piperidinecarboxylate (8c). Yield: 62%; melting point: oil; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 6.00 (s, 1H, NH), 5.94 (s, 1H, NH), 3.79 (s, 3H, OCH_3), 2.46–2.43 (m, 1H, CH_2-4), 2.41–2.38 (m, 1H, CH_2-3), 2.27–2.22 (m, 1H, CH_2-4), 2.19 (heptuplet, 1H, CH(CH_3)$_2$), 1.95 (s, 3H, CH_3CO), 1.83 (dt, 1H, $J=12.6$, 10.5 Hz, CH_2-3), 1.43 (s, 3H, CH_3), 1.03 (d, 3H, CH(*C*H_3)$_2$), 1.00 (d, 3H, CH(*C*H_3)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): 174.0 ($COOCH_3$), 173.3 (CONH), 169.7 ($COCH_3$), 61.1 (C5), 59.7 (C2), 53.0 (OCH_3), 35.0 (*C*H(CH_3)$_2$), 29.2 (CH_2-5), 27.4 (CH_3), 24.9 (CH_2-4), 24.1 (CH_3CO), 18.2 (CH(*C*H_3)$_2$), 17.0 (CH(*C*H_3)$_2$).

7.7.4. Methyl 5-(acetylamino)-1-(4-methoxybenzyl)-2,5-dimethyl-6-oxo-2-piperidinecarboxylate (8d). Yield: 75%; melting point: oil; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 7.09 (d, 2H, $J=8.6$ Hz, H*ortho*), 6.82 (d, 2H, $J=8.6$ Hz, H*meta*), 6.74 (s, 1H, NH), 5.22 (d, 1H, $J=15.8$ Hz, CH_2Ph), 3.81 (d, 1H, $J=15.8$ Hz, CH_2Ph), 3.77 (s, 3H, OCH_3), 3.74 (s, 3H, $COOCH_3$), 2.74–2.72 (m, 1H, CH_2), 2.22–2.18 (m, 1H, CH_2), 2.05–2.01 (m, 2H, CH_2), 1.96 (s, 3H, $COCH_3$), 1.65 (s, 3H, CH_3-5), 1.48 (s, 3H, CH_3-2); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): 174.6 (CO), 173.9 ($COOCH_3$), 169.7 ($COCH_3$), 158.5 (C*para*), 130.7 (C*ipso*), 128.1 (C*ortho*), 114.1 (C*meta*), 65.7 (C5), 56.4 (C2), 55.2 (OCH_3), 52.9 ($COOCH_3$), 48.1 (CH_2Ph), 32.5 (CH_2), 29.4 (CH_2), 25.7 (CH_3-2), 24.7 (CH_3-5), 24.2 ($COCH_3$); EIMS *m/z* (%): 362 ($M^{\circ+}$, 15), 275 ($C_{16}H_{21}NO_3^+$, 22), 136 ($C_8H_{10}NO^+$, 100), 121 ($C_8H_9O^+$, 92); HRMS: calcd for $C_{19}H_{26}N_2O_5$: 362.1842; Found: 362.1854.

7.7.5. Methyl 5-(acetylamino)-1,2,5-trimethyl-6-oxo-2-piperidinecarboxylate (8e). Yield: 68%; melting point: oil; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 6.62 (s, 1H, NH), 3.76 (s, 3H, OCH_3), 2.88 (s, 3H, NCH_3), 2.70–2.68 (m, 1H, CH_2), 2.22–2.19 (m, 1H, CH_2), 1.99–1.96 (m, 2H, CH_2+CH_2), 1.95 (s, 3H, $COCH_3$), 1.57 (s, 6H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): 173.8 ($CONCH_3$), 173.6 ($COOCH_3$), 169.6 (CONH), 64.5 (C2), 56.4 (C5), 52.9 (OCH_3), 32.1 (CH_2), 30.9 (NCH_3), 29.2 (CH_2), 25.1 (CH_3), 24.3 (CH_3), 24.3 (CH_3CO); EIMS *m/z* (%): 197 ($M^{\circ+}-CH_3CONH_2$, 100), 138 (($M^{\circ+}-CH_3CONH_2$)–$COOCH_3^{\cdot}$, 22).

7.7.6. Methyl 5-(acetylamino)-2,5-dimethyl-6-oxo-2-piperidinecarboxylate (8f). Yield: 37%; melting point: 155 °C; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 6.27 (s, 1H, NH), 5.97 (s, 1H, NH), 3.80 (s, 3H, OCH_3), 2.48–2.43 (dm, 1H, $J=14$ Hz, CH_2eq), 2.30 (dt, 1H, $J=14.0$, 3.7 Hz, CH_2eq), 2.18 (td, 1H, $J=13.8$, 3.7 Hz, CH_2ax), 1.95 (s, 3H, $COCH_3$), 1.78 (td, 1H, $J=13.8$, 4.4 Hz, CH_2ax), 1.54 (s, 3H, CH_3), 1.45 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): 174.7 (CO), 174.3 (CO), 170.1 (CO), 60.4 (C2/C5), 55.7 (C2/C5), 53.4 (OCH_3), 30.8 (CH_2), 30.3 (CH_2), 27.9 ($COCH_3$), 24.8 (CH_3), 24.1 (CH_3); EIMS *m/z* (%): 242 ($M^{\circ+}$, 2), 183 ($M^{\circ+}-COOCH_3^{\cdot}$, 100), 141 ($C_7H_{13}N_2O^+$, 41), 124 ($C_7H_{10}NO^+$, 36); HRMS: calcd for $C_{11}H_{18}N_2O_4$: 242.1267; Found: 242.1278.

7.7.7. Methyl 5-(acetylamino)-1-benzyl-5-methyl-6-oxo-2-piperidinecarboxylate (8g). Yield: 91%; melting point: 137 °C (hexane/CH_2Cl_2); IR (KBr) cm^{-1}: 1738 (s, CO), 1666 (br s, CO); ^{1}H NMR ($CDCl_3$, 400 MHz): 7.36–7.26 (m, 3H, PhH), 7.19–7.15 (m, 2H, PhH), 6.58 (s, 1H, NH), 5.49 (d, 1H, $J=15$ Hz, CH_2Ph), 3.97 (dd, 1H, $J=4$ Hz, 3 Hz, H2), 3.76 (s, 3H, OCH_3), 3.70 (d, 1H, $J=15$ Hz, CH_2Ph), 2.72–2.60 (m, 1H, CH_2), 2.17–2.03 (m, 3H, CH_2CH_2), 1.97 (s, 3H, CH_3), 1.63 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): 173.4 (CO), 171.6 (CO), 169.7 (CO), 136.2 (ArC), 128.8; 128.1; 127.8 (ArCH), 58.4 (C2), 56.8 (C5), 52.6 (OCH_3), 50.1 (CH_2Ph), 29.9 (CH_2), 24.8 (CH_3), 24.1 (CH_3), 23.0 (CH_2); EIMS *m/z* (%): 318 ($M^{\circ+}$, 32), 259 ($M^{\circ+}-NH_2COCH_3$, 49), 231 ($M^{\circ+}-NH_2COCH_3$–CO, 100), 91 ($PhCH_2^+$, 98); HRMS: calcd for $C_{17}H_{22}N_2O_4$: 318.1580; Found: 318.1578.

7.7.8. Ethyl 5-(acetylamino)-1-benzyl-2-methyl-6-oxo-5-phenyl-2-piperidinecarboxylate (8h). Yield: 82%; melting point: 61 °C (CH_2Cl_2/hexane); IR (KBr) cm^{-1}: 1722 (s, CO), 1636 (s, CO); ^{1}H NMR ($CDCl_3$, 400 MHz): 7.48–7.25 (m, 10H, PhH), 7.23 (s, 1H, NH), 5.26 (d, 1H, $J=16$ Hz, CH_2Ph), 4.22 (q, 2H, $J=7$ Hz, OCH_2), 3.97 (d, 1H, $J=16$ Hz, CH_2Ph), 3.35 (ddd, 1H, $J=14$ Hz, 1.5 Hz, 1.5 Hz, $H3_{eq}$ or $H4_{eq}$), 2.48 (ddd, 1H, $J=14$ Hz, 14 Hz, 1.5 Hz, $H3_{ax}$ or $H4_{ax}$), 2.14 (ddd, 1H, $J=14$ Hz, 1.5 Hz, 1.5 Hz, $H3_{eq}$ or $H4_{eq}$), 1.90 (ddd, 1H, $J=14$ Hz, 14 Hz, 1.5 Hz, $H3_{ax}$ or $H4_{ax}$), 1.91 (s, 3H, $COCH_3$), 1.44 (s, 3H, CH_3), 1.29 (t, $J=7$ Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): 173.3 (CO), 172.0 (CO), 169.2 (CO), 140.6 and 138.1 (PhC*ipso*), 126.9–128.5 (ArC), 66.1 (C5), 62.3 (OCH_2), 62.1 (C2), 49.7 (CH_2Ph), 31.2 (C3 or C4), 27.8 (C3 or C4), 25.7 (CH_3), 24.4 ($COCH_3$), 14.2 (CH_3); EIMS *m/z* (%): 408 ($M^{\circ+}$, 14), 335 (86), 91 ($PhCH_2^+$, 100) HRMS: calcd for $C_{24}H_{28}N_2O_4$: 408.2049; Found: 408.2055.

For the spectral data of compound **8i** we refer to Ref. 21.

7.7.9. Methyl 5-(acetylamino)-5-benzyl-1-(4-methoxybenzyl)-6-oxo-2-piperidinecarboxylate (8j). Yield: 75%; melting point: oil; IR (NaCl, cm^{-1}): 3397 (NH), 1747 (CO), 1652 (CO); ^{1}H NMR ($CDCl_3$, 400 MHz): 7.22–7.18 (m 3H, ArH), 7.14 (d, 2H, $J=8.5$ Hz, *ortho* PMB), 7.05–6.98 (m, 2H, Ar H), 6.87 (d, 2H, $J=8.5$ Hz, *meta* PMB), 6.45 (s, 1H, NH), 5.29 (s, 1H, $J=14.5$ Hz, N–C*H* of PMB), 3.95 (br d, 1H, $J=5$ Hz, H2), 3.81 (s, 3H, Ar-OCH_3), 3.76–3.68 (m, 4H, CH_3–O–CO + N–C*H* of PMB), 3.41 (d, 1H, $J=13$ Hz, Ph-C*H*–C5), 3.14 (d, 1H, $J=13$ Hz, Ph-C*H*–C5), 2.83 (br d, 1H, $J=14$ Hz, $H4_{eq}$), 2.15 (ddd, 1H, $J=14$, 14, 4 Hz, H4ax), 2.01–1.89 (m, 4H, $NHCOCH_3+H3_{eq}$), 1.8 (dddd, 1H, $J=14$, 14, 6, 4 Hz, H3ax); ^{13}C NMR ($CDCl_3$, 100 MHz): 171.7, 171.6 (C6 and *C*$OOCH_3$), 169.7 (*C*ONH), 159.3 (C*para*-PMB), 135.9 (*ipso*-Bn), 130.3, 130.1, 128, (Ar CH), 127.9 (*ipso*-PMB), 126.9 (ArCH), 114.0 (*meta* PMB), 60.3 (C5), 58.5 (C2), 55.2 (Ar OCH_3), 52.5 ($COOCH_3$), 50.0 (N–CH_2 of PMB), 43.1 (C5–CH_2-Ph), 29.2 (C4), 24.1 (*C*H_3CONH), 22.8 (C3); EIMS *m/z* (%): 424 (6, $M^{\circ+}$), 333 (19, $M^{\circ+}-C_6H_5$–$CH_2^{\cdot}$), 121 (100,

CH_3O–C_6H_4–CH_2^+); HRMS: calcd for $C_{24}H_{28}N_2O_5$: 424.1998; Found: 424.2006.

7.7.10. Dimethyl 2,5-bis(acetylamino)-2,5-dimethylhexanedioate (10). Yield: 29%; melting point: oil; ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 6.33 (s, 2H, NH), 3.76 (s, 6H, OCH_3), 2.27–2.16 (m, 2H, CH_2), 1.99 (s, 6H, $COCH_3$), 1.96–1.94 (m, 2H, CH_2), 1.54 (s, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, ppm): 177.7 (CO), 174.9 (CO), 60.1 (C_{quat}), 53.2 (OCH_3), 30.7 (CH_2), 24.4 (CH_3), 24.0 (CH_3); EIMS *m/z* (%): 316 ($M^{\circ+}$, 1), 198 ($M^{\circ+}-(CH_3CONH_2)_2$, 45), 156 ($C_8H_{14}NO_2^+$, 100); HRMS: calcd for $C_{14}H_{24}N_2O_6$: 316.1634; Found: 316.1651.

7.8. Synthesis of model compound 4

The piperidinone derivative **8g** is dissolved in a 33% solution of $MeNH_2$ in ethanol and stirred for 12 h at room temperature. The reaction mixture is evaporated and the residue is recrystallised from hexane/dichloromethane.

7.8.1. 5-(Acetylamino)-1-benzyl-*N*,5-dimethyl-6-oxo-2-piperidinecarboxamide (4). Yield: 92%; melting point: 217 °C (hexane/CH_2Cl_2); IR (KBr) cm^{-1}: 3444 (m, NH), 3343 (s, NH), 1690 (s, CO), 1633 (br s, CO); ^{1}H NMR ($CDCl_3$, 400 MHz): 8.32 (s, 1H, N*H*Me), 7.33–7.21 (m, 5H, ArH), 5.88 (s, 1H, NH), 5.44 (d, 1H, H=15 Hz, BnH), 3.95 (br d, 1H, J=6 Hz, $H2_{eq}$), 3.67 (d, 1H, J=15 Hz, BnH), 2.84 (d, 3H, J=5 Hz, NCH_3), 2.51 (td (ddd), 1H, J=14 Hz, 4 Hz, H4ax), 2.08 (dm (dddd), 1H, J=14 Hz, H3eq), 2.03 (s, 3H, CH_3), 1.96 (tq (dddd), 1H, J=14 Hz, 4 Hz, H3ax), 1.62 (dt (ddd), 1H, J=14 Hz, 4 Hz, H4eq), 1.50 (s, 3H, CH_3); ^{1}H NMR (DMSO-d_6, 400 MHz): 8.47 (s, 1H, NH), 8.46 (q, 1H, J=5 Hz, N*H*Me), 7.36–7.23 (m, 3H, ArH), 7.18–7.14 (br d, 2H, J=7 Hz, ArH), 5.13 (d, 1H, J=15 Hz, BnH), 3.72 (d, 1H, J=15 Hz, BnH), 3.71 (br d, 1H, J=7 Hz, H2), 2.62 (d, 3H, J=5H, NCH_3), 2.26 (td (ddd), 1H, J=13 Hz, 4 Hz, H4ax), 2.06 (tq (dddd), J=13 Hz, 4 Hz, H3ax), 1.87 (s, 3H, CH_3), 1.80 (dtm (dddd), 1H, J=13 Hz, 4 Hz, H3eq), 1.60 (dt (ddd), 1H, J=13 Hz, 4 Hz, H4eq), 1.39 (s, 3H, CH_3); Coupling between H2 and H3 is also observed in the COSY spectrum both in $CDCl_3$ and in DMSO-d_6; ^{13}C NMR ($CDCl_3$, 100 MHz): 171.1 (CO), 170.8 (CO), 170.2 (CO), 136.6 (ArC), 128.7; 128.3; 127.7 (ArCH), 61.3 (C2), 55.5 (C5), 49.9 (CH_2Ph), 30.4 (CH_2), 26.3 (CH_3), 25.6 (CH_3), 23.2 (CH_3), 23.1 (CH_2); EIMS *m/z* (%): 317 ($M^{\circ+}$, 15), 259 ($M^{\circ+}-CONHCH_3$, 100), 91 ($C_7H_7^+$, 58); HRMS: calcd for $C_{17}H_{23}N_3O_3$: 317.1739; Found: 317.1738.

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16. This is dependent on the site of cleavage of a bicyclic lactam further on in the synthesis.
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18. Addition of a drop of water to the toluene mixture in which the Diels–Alder reaction is performed avoids the subsequent hydrolysis step.
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25. These values are derived from the analysis of small cyclic peptides. It is possible that for smaller model systems the shielding of the NH in a hydrogen bond is less efficient. So less stringent criteria might be adopted here.

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A general synthesis of water soluble upper rim calix[*n*]arene guanidinium derivatives which bind to plasmid DNA

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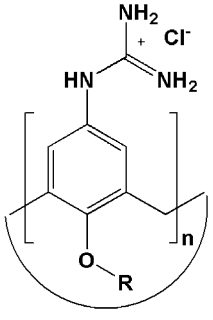
Abstract—The reaction of O-alkylated *p*-aminocalix[*n*]arenes ($n=4$, 6, 8) with *N*,*N*′-di(*tert*-butoxycarbonyl)thiourea in the presence of $HgCl_2$ and subsequent removal of the protective groups with hydrochloric acid led to the new water soluble calix[*n*]guanidinium derivatives (*p*-guanidiniumcalix[*n*]arenes) **20–23**. With the exception of tetraoctyl-*p*-guanidiniumcalix[4]arene **21**, which forms a macroscopic gelatinous aggregate even at very low concentration, all the synthesised guanidinium calixarenes show good solubility in water and sharp NMR signals. Moreover, these compounds are not cytotoxic and bind to plasmid DNA.

1. Introduction

Since the majority of biological processes take place in aqueous environment, the synthesis of water soluble receptors is of paramount interest in bioorganic chemistry. Artificial receptors can mimic natural processes such as the specific recognition of bioactive molecules (antigens, microbial/viral pathogens, nucleotides) or enzymatic transformations of substrates, helping to reveal their mechanisms.



20, n = 4, R = Pr
21, n = 4, R = Oct
22, n = 6, R = Me
23, n = 8, R = Me

Since calix[*n*]arenes[1,2] are cavity containing compounds that can be easily functionalised both at the upper and at the lower rim, they provide a suitable molecular scaffold for building up such receptors. In order to render the lipophilic calix[*n*]arenes soluble in water it is necessary to introduce polar, preferably charged, moieties.[3] The guanidinium group is also particularly attractive in this context because it is considerably effective for binding guests of anionic nature (carboxylates, phosphates, sulfates, nitrates etc.), through hydrogen bonding and charge-pairing interactions.[4] In collaboration with Reinhoudt's group we have recently shown[5,6] that guanidinium derivatives of calix[4]arenes, bearing adamantyl groups at the lower rim, can interact with β-cyclodextrin self-assembled monolayers (β-CD SAM) allowing patterns to be written on molecular printboards. This finding makes water soluble, guanidinium functionalised calixarenes also attractive in nanoscience. In this paper, we report the results of a systematic investigation on the introduction of guanidinium groups at the upper rim of calix[*n*]arenes ($n=4$, 6, 8) having alkyl groups of different length at the lower rim, together with some preliminary solubility features, binding properties to plasmid DNA and cytotoxicity data. En route to the guanidiniumcalix[*n*]arenes, several new calixarene derivatives, which may be useful for further transformations have been synthesised and characterised.

2. Results and discussion

2.1. Synthesis

The synthesis of *p*-guanidiniumcalix[*n*]arenes **20–23**

Keywords: Water soluble calixarenes; Macrocycles; DNA recognition; Cytotoxicity.
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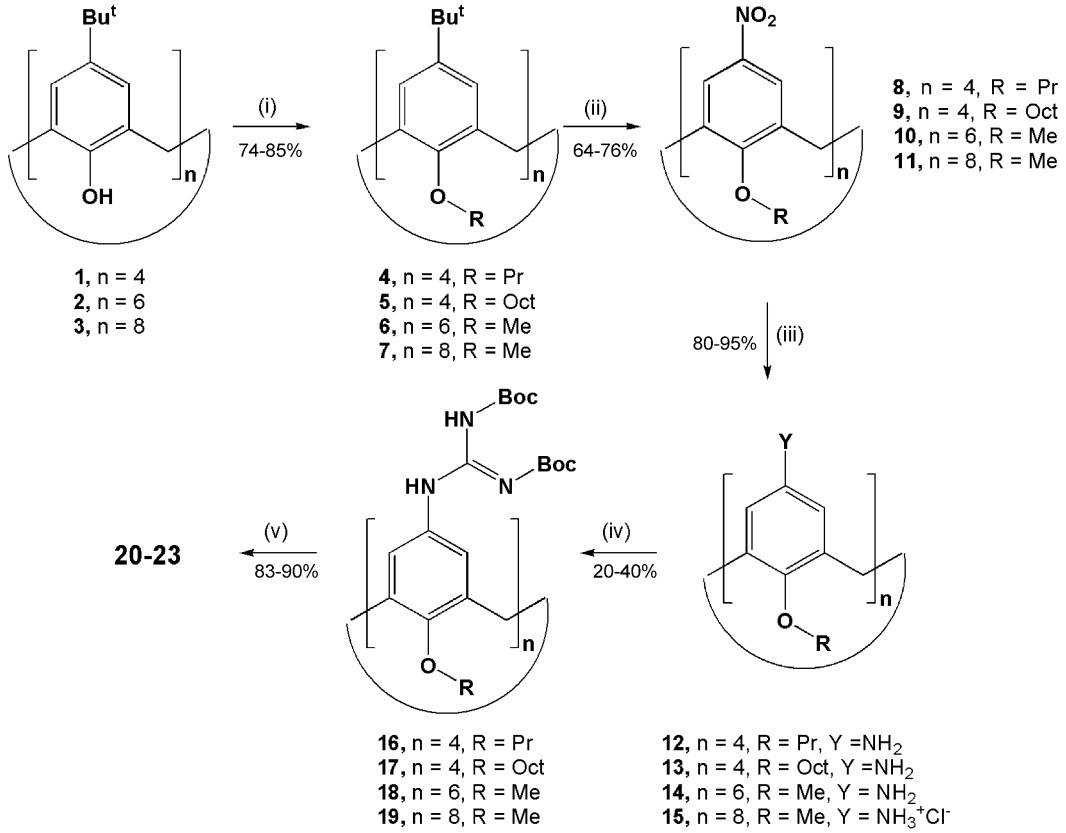

Scheme 1. Synthesis of the *p*-guanidiniumcalix[*n*]arenes. (i) RI/NaH/DMF (dry), rt; (ii) $NaNO_3/CF_3COOH$, rt; (iii) $NH_2NH_2\cdot H_2O$/Pd/C (10%) in EtOH (**8**, **9**) or in $CH_3OCH_2CH_2OH$ (**10**, **11**), reflux; (iv) $HgCl_2$/Boc-NH–C(S)–NH-Boc/DMF (dry), Et_3N (dry); (v) concd HCl/1,4-dioxane.

(Scheme 1) involves the transformation of calixarenes **1**, **2** and **3** into their corresponding peralkylated derivatives **4** (76% yield), **5** (74%), **6** (79%) and **7** (85%) by reaction with an excess of alkylating agent and NaH in dry DMF.[7,8] *Ipso* nitration by $NaNO_3$ in CF_3COOH gave nitro derivatives in acceptable yields: **8** (76%), **9** (71%), **10** (65%) and **11** (64%). Subsequent reduction using $NH_2NH_2\cdot H_2O$ and a catalytic amount of Pd/C (10%) either in refluxing ethanol (**8**, **9**) or in 2-methoxyethanol (**10**, **11**) led to the aminocalixarenes **12**[9] (93% yield), **13** (80%), **14** (86%) and **15** (88%). All the amino compounds, except calix[8]-arene **15**, were prepared in their free base form. In the case of **15**, we achieved a better yield when the ammonium salt was isolated. The final guanidinium products were formed by a $HgCl_2$ catalysed nucleophilic addition of the amino-calixarenes to *N*,*N*′-di(*tert*-butoxycarbonyl)thiourea[10] in dry DMF, yielding the protected guanidine calixarenes (**16**–**19**), followed by cleavage of the protective groups using hydrochloric acid (**20**–**23**).

2.2. Solubility in water, DNA recognition and cytotoxicity

In general, two properties are required for compounds to be applicable in biological systems. On the one hand is their good solubility in water and on the other is the low (or no) cytotoxicity. For the present cationic calix[*n*]arenes, we determined the molar absorption coefficient (ε) and their maximum solubility (c_{max}) in water by means of UV–vis spectroscopy (Table 1). The limiting solubility in water was found to be in the range 4×10^{-3}–1×10^{-2} mol dm^{-3}. We obtained a linear dependence of the absorbance on the concentration of the macrocycle which, together with the clear and sharp peaks observed in the ^{1}H NMR spectra of compounds **20**, **22** and **23** in water (see e.g., Fig. 1) rules out the formation of micellar aggregates for these compounds, in the investigated concentration range. The *p*-guanidinium-calix[4]arene **21** behaves quite differently since it forms a

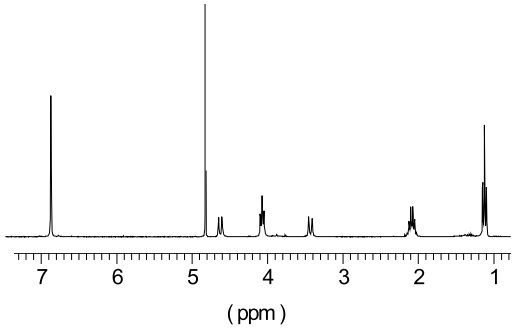

Figure 1. ^{1}H NMR of **20** in D_2O (300 MHz, 298 K, $c=9\times10^3$ mol dm^{-3}).

Table 1. The molar absorption coefficients (ε) and limiting solubility values (c_{max}) in water for selected guanidinium derivatives (UV–vis, $\lambda=280$ nm, 298 K)

Compound	ε (dm^3 mol^{-1} cm^{-1})	c_{max} (mol dm^{-3})
20	3300	1×10^{-2}
22	4640	8×10^{-3}
23	5120	4×10^{-3}

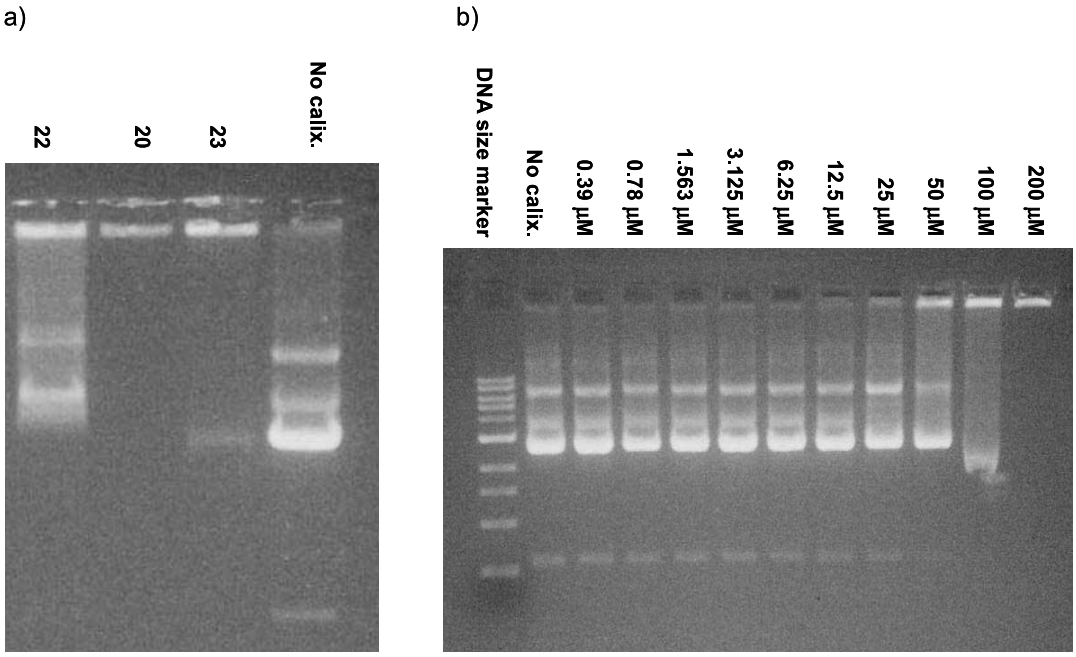

Figure 2. Agarose gel electrophoresis mobility shift assay (EMSA): (a) binding of plasmid pEGFP-Cl by **20**, **22** and **23** (200 μM). (b) Binding of plasmid pEGFP by **20** at different concentrations. The different bands correspond to different plasmid coiled conformations.

gelatinous macroscopic aggregate in water, even at very low concentration.

Preliminary DNA-binding studies of compounds **20**, **22** and **23** were performed on 4731 bp plasmid pEGFP-C1 (Clontech) and monitored by agarose gel Electrophoresis Mobility Shift Assay (EMSA). The best linear shift activity was observed for **20** (Fig. 2(a)), which starts to bind the plasmid at 12.5 μM (Fig. 2(b)) and showed complete plasmid complexation between 100 and 200 μM.

We also tested the cytoxicity of the molecules using an MTT assay.[11] RD-4 cells (3000 in 0.1 ml medium/well) were plated on 96-well plates and different amounts of **20**, **22** and **23** were added. At various times, the relative number of metabolically active cells was assessed by reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT). For calixarene **20** no cytotoxicity was found at a 12.5 μM concentration and **22** and **23** showed no toxicity even at 25 μM (see Supplementary material).

In conclusion, we prepared and characterised four new upper rim guanidinium functionalised calix[*n*]arenes ($n=4$, 6, 8). Preliminary studies on the cationic derivatives **20**, **22** and **23** show their good solubility in water and reasonably low cytotoxicity. These properties, together with the proven ability of the compounds to bind plasmid DNA, in the μM concentration range, suggest possible biological applications.

On the other hand, the *p*-guanidiniumcalix[4]arene (**21**), functionalised with octyl groups at the lower rim, forms a gelatinous macroscopic aggregate in water, even at very low concentration which suggests a peculiar aggregation behaviour, which is currently under study in more detail.

3. Experimental

3.1. General

All reactions were carried out under nitrogen atmosphere. Dry solvents were prepared according to standard procedures and stored over molecular sieves. Melting points were determined in under nitrogen sealed capillaries with an Electrothermal apparatus. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, on Bruker AV300 and AC300 spectrometers (partially deuterated solvents were used as internal standard). IR spectra were recorded on a Perkin–Elmer 298 apparatus. UV spectra were registered on a Perkin–Elmer UV–vis Lambda BIO 20 spectrophotometer. Mass spectra were registered in ESI and MALDI TOF mode on a Micromass ZMD and on a M@LDI-LR Mass spectrometer, respectively. Elemental analyses were performed using CHN 1106 Carlo Erba instrument and are reported as percentages. TLC was performed on silica gel Merck 60 F254 sheets and flash chromatography on 230–240 mesh Merck 60 silica gel.

The derivatives **4**[7], **6**[8], **7**[8], **12**[9] were prepared according to known literature procedures.

3.1.1. 5,11,17,23-Tetra-*tert*-butyl-25,26,27,28-tetraoctyloxycalix[4]arene 5. To a suspension of **1** (1 g, 1.54×10^{-3} mol) in DMF (15 ml) cooled at 0 °C, NaH (0.74 g, 1.85×10^{-2} mol) was added as a 60% oil suspension. After 30 min, 1-iodo-octane (3.3 ml, 1.85×10^{-2} mol) was put into the mixture. The cooling bath was removed and the reaction mixture was allowed to stir overnight. The rest of NaH was decomposed by water (20 ml) and the product was extracted with CH_2Cl_2 (2×30 ml). The organic layer was washed with 1 N HCl (3×30 ml) and dried over $MgSO_4$. The residue of evaporation was purified by flash column

chromatography on silica gel eluting with hexane and hexane/CH_2Cl_2 6:1 to obtain the pure product as a semisolid compound (1.2 g, 71%). Mp: 68–70 °C (acetone). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.88 (s, 8H, Ar*H*), 4.52 (d, J=12.4 Hz, 4H ArCH_2Ar), 3.95 (t, J=7.7 Hz, 8H, OCH_2(CH$_2$)$_6$CH$_3$), 3.21 (d, J=12.5 Hz, 4H, ArCH_2Ar), 2.13 (brt, 8H, OCH$_2$-CH_2(CH$_2$)$_5$CH$_3$), 1.36–1.52 (m, 40H, O(CH$_2$)$_2$(CH_2)$_5$CH$_3$, 1.19 (s, 36H, *Bu^t*) 1.01 (t, J=6.9 Hz, 12H, O(CH$_2$)$_7$CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.8, 144.1, 133.9, 124.9, 75.5, 33.8, 32.1, 31.5, 31.1, 30.5, 30.1, 29.8, 26.4, 22.8, 14.2. MS (MALDI TOF): calculated *m*/*z* for $[M+Na]^+$= 1120.8; found $[M+Na]^+$=1120.8. EA calculated for $C_{76}H_{120}O_4$: C, 83.15; H, 11.02. Found: C, 83.36; H 11.12.

3.2. General procedure for the *ipso* nitration of calix[*n*]arenes

Calixarene (1 mmol) and $NaNO_3$ (10 mmol for each *tert*-butyl group) were put into a round-bottom flask and then CF_3COOH (10 mmol for each *tert*-butyl group) was added dropwise. The mixture was allowed to stir at rt overnight.

3.2.1. 5,11,17,23-Tetranitro-25,26,27,28-tetrapropoxycalix[4]arene 8. The reaction was stopped by addition of water (200 ml) and extracted with CH_2Cl_2 (2×100 ml). The organic layer was washed with water (150 ml) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the pure product was obtain by trituration with MeOH (50 ml) as a pale yellow powder (76%). The analytical data corresponded to the literature.[12]

3.2.2. 5,11,17,23-Tetranitro-25,26,27,28-tetraoctyloxycalix[4]arene 9. The preparation was analogous to the one described for **8**. Yield 71% (pale yellow powder). Mp: 154–156 °C (CH_2Cl_2–MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (s, 8H, Ar*H*), 4.50 (d, J=13.9 Hz, 4H ArCH_2Ar), 3.97 (t, J=7.4 Hz, 8H, OCH_2(CH$_2$)$_6$CH$_3$), 3.38 (d, J=14.1 Hz, 4H, ArCH_2Ar), 1.87 (brt, J=6.5 Hz, 8H, OCH$_2$CH_2(CH$_2$)$_5$-CH$_3$), 1.66 (m, 40H, O(CH$_2$)$_2$(CH_2)$_5$CH$_3$, 0.88 (t, J= 6.9 Hz, 12H, O(CH$_2$)$_7$CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.1, 142.8, 135.4, 123.9, 76.2, 31.8, 31.1, 30.2, 29.7, 29.4, 26.3, 22.6, 14.0. MS (MALDI TOF): calculated *m*/*z* for $[M+Na]^+$=1075.6; found $[M+Na]^+$=1075.8. EA calculated for $C_{60}H_{84}N_4O_{12}$: C, 68.42; H, 8.04; N, 5.32. Found: C, 68.71; H 8.19; N, 5.39.

3.2.3. 5,11,17,23,29,35-Hexanitro-37,38,39,40,41,42-hexamethoxycalix[6]arene 10. The reaction was stopped by addition of water (200 ml) and the formed precipitate was filtrated off. This crude product was dissolved in the mixture CH_2Cl_2–toluene 1:1 (150 ml) and evaporated under reduced pressure (twice). The pure product was obtained by trituration with the mixture CH_2Cl_2–MeOH 10:1 as a pale yellow powder (65%). Mp: 250 °C dec (CH_2Cl_2–MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (s, 12H, Ar*H*), 4.12 (s, 12H, ArCH_2Ar), 3.78 (s, 18H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.9, 143.9, 134.0, 124.3, 60.0, 30.8. MS (ESI): calculated *m*/*z* for $[M+Na]^+$=1013.2; found $[M+Na]^+$= 1013.3. EA calculated for $C_{48}H_{42}N_6O_{18}$: C, 58.18; H, 4.27; N, 8.48. Found: C, 58.40; H 4.39; N, 8.62.

3.2.4. 5,11,17,23,29,35,41,47-Octanitro-37,38,39,40,41,42,43,44-octamethoxycalix[8]arene 11. The preparation was analogous to the one described for **10**. The pure product was obtained by crystallisation from CH_2Cl_2–hexane 20:1. Yield 64% (pale yellow powder). Mp: 250 °C dec (CH_2Cl_2–hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (s, 16H, Ar*H*), 4.12 (s, 16H, ArCH_2Ar), 3.78 (s, 24H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.9, 143.9, 134.0, 124.3, 60.8, 30.8. MS (ESI): calculated *m*/*z* for $[M+Na]^+$=1343.3; found $[M+Na]^+$=1343.6. EA calculated for $C_{64}H_{56}N_8O_{24}$: C, 58.18; H, 4.27; N, 8.48. Found: C, 58.44; H 4.42; N, 8.59.

3.3. General procedure for the reduction of nitrocalix[*n*]arenes

To a suspension of nitrocalix[*n*]arene (0.1 mmol) either in ethanol (for **8** and **9**) or in 2-methoxyethanol (for **10** and **11**), $NH_2NH_2\cdot H_20$ (0.5 mmol for each nitro group) and Pd/C (10%) (catalytic amount) were added. The reaction mixture was refluxed and stirred overnight. The catalyst was filtered off through a paper filter.

3.3.1. 5,11,17,23-Tetraamino-25,26,27,28-tetraoctyloxycalix[4]arene 13. The paper filter was washed with CH_2Cl_2 (20 ml) and all solvents were removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (30 ml) and dried over $MgSO_4$. The pure product was obtained after evaporation of the volatiles as a orange powder (80%). Mp: 187–189 °C (CH_2Cl_2–MeOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.94 (s, 8H, Ar*H*), 4.15 (d, J=12.5 Hz, 4H, ArCH_2Ar), 3.66 (t, J=7.4 Hz, 8H, OCH_2-(CH$_2$)$_6$CH$_3$), 3.30 (brs, 8H, ArNH_2), 2.78 (d, J=12.6 Hz, 4H ArCH_2Ar), 1.84 (brt, 8H, OCH$_2$CH_2(CH$_2$)$_5$CH$_3$), 1.28 (m, 40H, O(CH$_2$)$_2$(CH_2)$_5$CH$_3$, 0.86 (t, J=6.9 Hz, 12H, O(CH$_2$)$_7$CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 147.4, 141.9, 134.2, 114.0, 74.5, 31.4, 30.6, 29.7, 29.5, 29.1, 26.0, 22.0, 13.7. MS (MALDI TOF): calculated *m*/*z*for $[M+H]^+$=955.7; found $[M+H]^+$=955.6.

3.3.2. 5,11,17,23,29,35-Hexaamino-37,38,39,40,41,42-hexamethoxycalix[6]arene 14. The paper filter was washed with CH_2Cl_2 (20 ml) and all solvents were removed under reduced pressure. The residue was dissolved in a small amount of CH_2Cl_2 and the pure product was obtain after precipitation with hexane as a pale green powder (86%). Mp: 200 °C dec (CH_2Cl_2–hexane). ^{1}H NMR (300 MHz, CD_3OD) δ 6.44 (s, 12H, Ar*H*), 3.78 (s, 12H, ArCH_2Ar), 3.23 (s, 18H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.8, 142.1, 134.7, 115.5, 60.7, 30.2. MS (ESI): calculated *m*/*z* for $[M+Na]^+$=833.4; found $[M+Na]^+$=833.7.

3.3.3. 5,11,17,23,29,35,41,47-Octaamino-37,38,39,40,41,42,43,44-octamethoxycalix[8]arene·8HCl 15. The paper filter was washed with a mixture of 1 M HCl (6 ml) in ethanol (10 ml). All solvents were removed under reduced pressure. The residue was dissolved in a small amount of CH_3OH and the pure product was obtained after precipitation with toluene as a pale brown powder (88%). Mp: 200 °C dec (CH_3OH–toluene). ^{1}H NMR (300 MHz, D_2O) δ 6.81 (s, 16H, Ar*H*), 3.88 (s, 16H, ArCH_2Ar), 3.31 (s, 24H, OCH_3). ^{13}C NMR (75 MHz, D_20) δ 150.4, 138.8, 132.3, 105.2, 63.9, 32.6. MS (ESI): calculated *m*/*z* for $[M+H]^+$= 1082.6; found $[M+H]^+$=1082.7.

3.4. General procedure for the synthesis of the bis-Boc calix[*n*]arene guanidine derivatives

To a mixture of aminocalixarene (1 mmol), bis-Boc-thiourea (1.2 mmol for each amino group) and Et_3N (3 mmol for each amino group) in dry DMF (15 ml), mercury chloride (1.2 mmol for each amino group) was added. The reaction mixture was stirred overnight. The formed black suspension was diluted with CH_2Cl_2 (10 ml) and filtered through a paper filter. The filtrate was distilled off under reduced pressure. The residue was purified by flash column chromatography on silica gel eluting with hexane/Et_2O 4:1 and 7:3 to obtain the pure product as a white powder.

3.4.1. Compound 16. Yield 33%. Mp: 250 °C dec (CH_2Cl_2–MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.59 (s, 4H, BocN*H*), 9.80 (s, 4H, ArN*H*), 6.90 (s, 8H, Ar*H*), 4.41 (d, J=13.1 Hz, 4H, ArCH_2Ar), 3.81 (t, J=7.7 Hz, 8H, OCH_2-CH_2CH_3), 3.14 (d, J=13.2 Hz, 4H, ArCH_2Ar), 1.92 (m, 8H, $OCH_2CH_2CH_3$), 1.49 (s, 36H, *Bu*^{t}), 1.44 (s, 36H, *Bu*^{t}), 0.96 (t, J=7.7 Hz, 12H, $OCH_2CH_2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.5, 153.9, 153.5, 152.0, 134.6, 130.60, 122.0, 82.9, 79.0, 76.8, 31.3,28.1, 23.0, 10.2. MS (ESI): calculated m/z for $[M+Na]^+$=1644.9; found $[M+Na]^+$=1644.8. EA calculated for $C_{84}H_{124}N_{12}O_{20}$: C, 62.20; H, 7.71; N, 10.36. Found: C, 62.53; H 7.88; N, 10.51.

3.4.2. Compound 17. Yield 40%. Mp:166–168 °C (CH_2Cl_2–MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.59 (s, 4H, BocN*H*), 9.85 (s, 4H, ArN*H*), 6.88 (s, 8H, Ar*H*), 4.44 (d, J=13.2 Hz, 4H, ArCH_2Ar), 3.90 (t, J=7.6 Hz, 8H, OCH_2-$(CH_2)_6CH_3$), 3.27 (d, J=13.0 Hz, 4H, ArCH_2Ar), 1.93 (brs, 8H, $OCH_2CH_2(CH_2)_5CH_3$), 1.50 (s, 36H, *Bu*^{t}), 1.46 (s, 36H, *Bu*^{t}), 1.34 (m, 40H, $O(CH_2)_2(CH_2)_5CH_3$), 0.90 (t, J=7.0 Hz, 12H, $O(CH_2)_7CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.8, 154.6, 154.1, 152.9, 135.5, 129.7, 124.1, 83.2, 80.0, 75.5, 31.9, 31.2, 30.1, 29.8, 29.5, 28.1, 26.2, 22.6, 14.0. MS (ESI): calculated m/z for $[M+Na]^{2+}$=974.1; found for $[M+Na]^{2+}$=974.2. EA calculated for $C_{104}H_{164}N_{12}O_{20}$: C, 65.66; H, 8.69; N, 8.83. Found: C, 65.73; H 8.85; N, 8.93.

3.4.3. Compound 18. Yield 25%. Mp: 250 °C dec (CH_2Cl_2–MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.61 (s, 6H, BocN*H*), 10.01 (s, 6H, ArN*H*), 7.15 (s, 12H, Ar*H*), 3.90 (s, 12H, ArCH_2Ar), 3.29 (s, 18H, OCH_3), 1.50 (s, 54H, *Bu*^{t}), 1.45 (s, 54H, *Bu*^{t}). ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.1, 153.5, 134.8, 131.6, 123.3, 83.0, 80.0, 60.2, 30.5, 28.0. MS (ESI): calculated m/z for $[M+Na]^+$=2287.2; found $[M+Na]^+$=2286.9. EA calculated for $C_{114}H_{162}N_{18}O_{30}$: C, 60.46; H, 7.21; N, 11.13. Found: C, 60.75; H 7.35; N, 11.27.

3.4.4. Compound 19. Yield 20%. Mp: 250 °C dec (CH_2Cl_2–MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.58 (s, 8H, BocN*H*), 10.03 (s, 8H, ArN*H*), 7.12 (s, 16H, Ar*H*), 3.99 (s, 16H, ArCH_2Ar), 3.46(s, 24H, OCH_3), 1.48 (s, 72H, *Bu*^{t}), 1.43 (s, 72H, *Bu*^{t}). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.0, 153.9, 153.5, 153.0, 134.4, 131.9, 123.4, 83.2, 79.0, 60.6, 30.4, 27.9. MS (ESI): calculated m/z for $[M+Na]^+$= 3041.6; found $[M+Na]^+$=3041.4. EA calculated for $C_{152}H_{216}N_{24}O_{40}$: C, 60.46; H, 7.21; N, 11.13. Found: C, 60.81; H 7.32; N, 11.31.

3.5. General procedure for the synthesis of *p*-guanidiniumcalix[*n*]arenes

To a solution of the bis-Boc calix[*n*]arene guanidine derivative (0.1 mmol) in 1,4-dioxane (20 ml), concentrated HCl (1 mmol for each Boc group) was added dropwise. The reaction mixture was stirred for 24 h. The solvents were removed under reduced pressure. The residue of the evaporation was dissolved in MeOH (1 ml) and by addition of ethyl acetate the product was precipitated as a white powder.

3.5.1. Compound 20. Yield 86%. Mp: 250 °C dec (MeOH–ethyl acetate). ^{1}H NMR (300 MHz, CD_3OD) δ 6.67 (s, 8H, Ar*H*), 4.52 (d, J=13.2 Hz, 4H, ArCH_2Ar), 3.94 (t, J=7.2 Hz, 8H, OC$H_2CH_2CH_3$), 3.30 (d, J=13.2 Hz, 4H, ArCH_2Ar), 2.00 (m, 8H, $OCH_2CH_2CH_3$), 1.04 (t, J=7.2 Hz, 12H, $OCH_2CH_2CH_3$). ^{13}C NMR (75 MHz, CD_3OD) δ 158.1, 157.4, 137.9, 130.1, 126.9, 78.7, 32.0, 24.7, 11.0. MS (ESI): calculated m/z for [M−4HCl]=820.5; found m/e $[M+H–4HCl]^+$=821.8.

3.5.2. Compound 21. Yield 90%. Mp: 250 °C dec (MeOH–ethyl acetate). ^{1}H NMR (300 MHz, CD_3OD) δ 6.70 (s, 8H, Ar*H*), 4.50 (d, J=13.2 Hz, 4H, ArCH_2Ar), 3.96 (t, J=6.8 Hz, 8H, OC$H_2(CH_2)_6CH_3$), 3.30 (d, J=13.2 Hz, 4H, ArCH_2Ar), 2.00 (m, 8H, $OCH_2CH_2(CH_2)_5CH_3$), 1.44–1.34 (m, 40H, $O(CH_2)_2(CH_2)_5CH_3$), 0.92 (brt, J=6.8 Hz, 12H $O(CH_2)_7CH_3$). ^{13}C NMR (75 MHz, CD_3OD) δ 158.0, 157.3, 137.9, 130.2, 127.0, 77.2, 33.5, 31.9, 31.5, 31.2, 28.0, 24.1, 14.8. MS (ESI): calculated m/z for [M−4HCl]=1100.8; found m/e $[M+2H–4HCl]^{2+}$=551.4.

3.5.3. Compound 22. Yield 89%. Mp: 250 °C dec (MeOH–ethyl acetate). ^{1}H NMR (300 MHz, CD_3OD) δ 6.97 (s, 12H, Ar*H*), 4.01 (s, 12H, ArCH_2Ar), 3.48 (s, 18H, OCH_3). ^{13}C NMR (75 MHz, CD_3OD) δ 158.4, 157.1, 137.4, 131.6, 128.0, 61.7, 31.4. MS (ESI): calculated m/z for [M−6HCl]=1062.5; found $[M+H–6HCl]^+$=1063.8.

3.5.4. Compound 23. Yield 83%. Mp: 250 °C dec (MeOH–ethyl acetate). ^{1}H NMR (300 MHz, CD_3OD) δ 6.96 (s, 16H, Ar*H*), 4.04 (s, 16H, ArCH_2Ar), 3.56 (s, 24H, OCH_3). ^{13}C NMR (75 MHz, CD_3OD) δ 158.0, 157.1, 136.9, 131.5, 127.7, 61.7, 31.1. MS (ESI): calculated m/z for [M−8HCl]=1416.7; found $[M+H–8HCl]^+$=1418.1.

3.6. DNA binding reactions

Binding reactions were performed in a total volume of 14 μl containing 10 μl of 20 mM Tris–HCl (pH 8), 1 μl of plasmid (1 μg of pEGFP-C1) and 3 μl of **20**, **22** and **23** at different concentrations, ranging from 0.4 to 200 μM. The binding reaction was allowed to proceed at room temperature for 1 h, then 5 μl of a 1 g/ml solution of glycerol in H_2O were added to each reaction mixture and the mixtures were loaded on a TA (40 mM Tris–acetate) 1% agarose gel. The gel was run for 2.5 h in TA buffer at 10 V/cm, EDTA was omitted from the buffers because of competition with DNA in the reaction.

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Supplementary data

Supplementary data associated with this article (including cytotoxicity data for compounds **20**, **22** and **23**) can be found, in the online version, at doi:10.1016/j.tet.2004.09.047

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Isolation and total synthesis of gymnastatin N, a POLO-like kinase 1 active constituent from the fungus *Arachniotus punctatus*

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Abstract—A high throughput screen against POLO-like kinase 1 (Plk1), an anti-cancer target, identified an active extract from the fungus *Arachniotus punctatus*. Bioassay guided fractionation led to the isolation of the new natural product, gymnastatin N (**1**) and the known compound aranorosinol A (**2**) with IC_{50} values of 13 and 118 μM, respectively. A 12′-hydroxy analog of gymnastatin N, **3**, was also isolated as a minor component. Gymnastatin N (**1**) was found to be a 52:48 mixture of (1*S*,6′*R*) and (1*R*,6′*R*) diastereomers, by synthesis of the four possible diastereomers and comparison of the optical rotation and chiral HPLC profile of each diastereoisomer with the natural product. Analogues of **1** were synthesized and evaluated against the Plk1 assay and these SAR studies suggested that the diene and free carboxylic acid moieties might be responsible for its bioactivity.

1. Introduction

POLO-like kinases play a pivotal role in various stages of cell division. Recent studies on this novel family of enzymes have unraveled their functions in centrosome maturation and bipolar spindle formation at the onset of mitosis.[1] They are also involved in the activation of cyclin-dependent kinase (Cdk)1-cyclin B.[2] In addition, they have been implicated in the activation of anaphase-promoting complex (APC) and the inactivation of Cdk1 at the point of mitotic exit.[1] POLO-like kinase 1 (Plk1) is a highly conserved mitotic serine/threonine kinase which has been shown to be commonly overexpressed in cancer cell lines.[3] Its expression could reflect the degree of malignancy and proliferation in these cells. Due to its essential roles in cell-cycle regulation, Plk1 could serve as a suitable diagnostic and prognostic marker for tumour progression and as a target for anti-cancer therapy. We have initiated a screening programme of our natural product collection, directed towards the identification of potent Plk1 inhibitors as anti-tumour drugs.

A high throughput screen against Plk1 identified an active extract from the fungus *Arachniotus punctatus*. Bioassay guided fractionation led to the isolation of the new natural product, gymnastatin N (**1**) and the known compound aranorosinol A (**2**) (Fig. 1),[4] with IC_{50} values of 13 and 118 μM, respectively. A 12′-hydroxy analog of gymnastatin N, **3**, was also isolated as a minor component (Fig. 1). This class of fungal derived natural products contains a 4,6-dimethyl-dodecadien-2*E*,4*E*-oic acid unit connected to a tyrosine unit through an amide linkage. The tyrosine unit can have various degrees of oxygenation, halogenation, cyclisation and esterification, as found in aranorosin (**4**),[5] aranorosinol A (**2**), aranorosinol B,[4] aranochlor A and aranochlor B from *Pseudoarachniotus roseus*,[6] gymnastatin A (**5**) to E,[7] F to H (**6**)[8] and I (**7**) to M[9] from *Gymnastella dankaliensis* (Fig. 1). These compounds have been reported to have antibacterial and anti-tumour activity. The total synthesis of aranorosin (**4**),[10,11] gymnastatin A (**5**),[12] gymnastatin H (**6**)[8] and gymnastatin I (**7**),[9] along with various interconversions between compounds, have enabled the absolute stereochemistry of most compounds in this class to be determined. To date, all of these compounds and gymnasterone A from *G. dankaliensis*,[13] have a (6*R*) configuration at the 4,6-dimethyl-dodecadien-2*E*,4*E*-oic acid unit. The occurrence of the (6*S*) configuration is rare: it has only been reported in the isolation of (6*S*)-4,6-dimethyl-dodecadien-2*E*,4*E*-oic acid and its phomalactone ester derivative, from a *Phomopsis* sp.[14]

2. Results and discussion

Approximately 300 out of the 90,000 extracts screened showed more than 40% inhibition against the Plk1 assay at a

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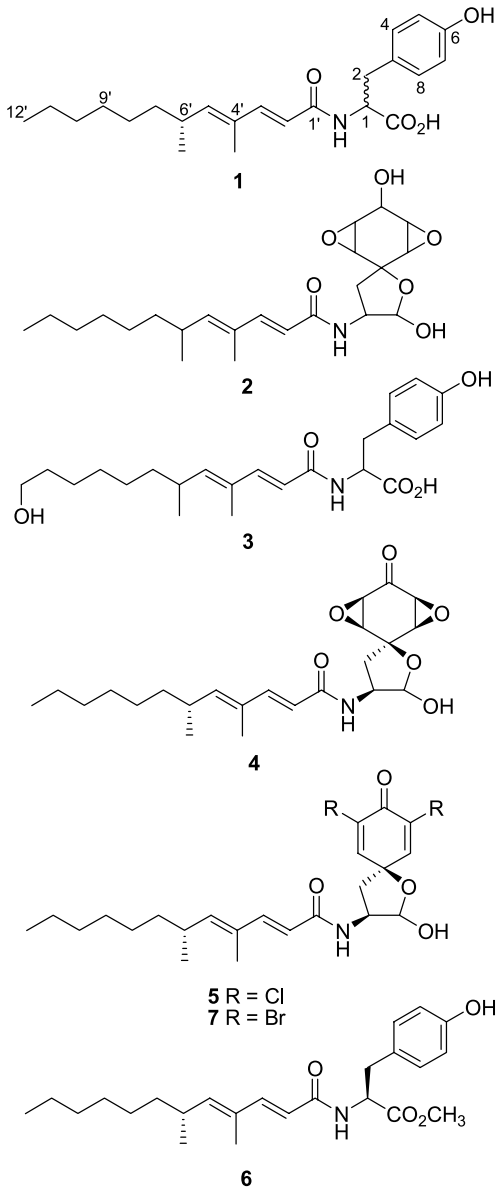


Figure 1. Gymnastatins and aranorosinol A.

concentration of 250 μg/mL. These extracts were tested against Cdk2, a serine/threonine kinase, to eliminate common serine/threonine kinase inhibitors and the 20 samples that had more than 5 fold potency against Plk1 versus Cdk2 were selected for further progression. One of these samples was the CH_3OH extract of the fungus *A. punctatus* and bioassay guided fractionation led to the isolation of gymnastatin N (**1**) and aranorosinol A (**2**) as the active components. Aranorosinol A (**2**) was identified by comparison of the spectroscopic data with that previously reported.[4] A 12′-hydroxy analogue of gymnastatin N, **3**, was isolated as a minor component.

Gymnastatin N (**1**) was isolated as a colourless oil that gave an $[M-H]^-$ ion peak at *m/z* 386.2335 and an $[M+H]^+$ ion peak at *m/z* 388.2470 in the (−)- and (+)-HR-ESIMS respectively, which were consistent with a molecular formula of $C_{23}H_{33}NO_4$. Analysis of the NMR data of **1** (^1H, ^{13}C, COSY, HSQCED and HMBC experiments) established the presence of a 4,6-dimethyl-dodecadien-

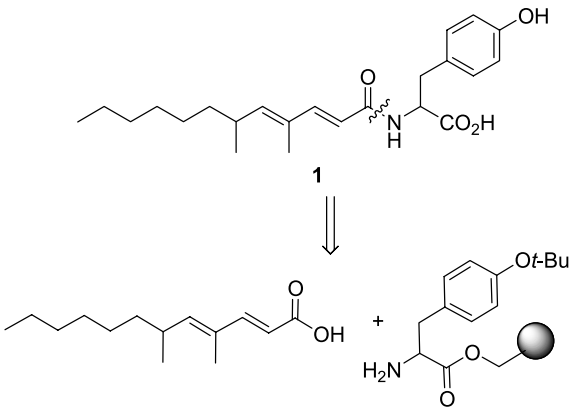


Figure 2. Retrosynthetic analysis of gymnastatin N.

2*E*,4*E*-oic acid derived unit, which was identical with that previously reported,[4–14] and a tyrosine unit. A HMBC correlation from the tyrosine NH (δ_H 8.14) to the carbonyl carbon (δ_C 165.3) of the 4,6-dimethyl-dodecadien-2*E*,4*E*-oic acid derived unit enabled the structure of **1** to be determined as the acid derivative of gymnastatin H (**6**).[8] Although gymnastatin H (**6**) has been synthesized, no optical rotation or NMR data has been reported.

Gymnastatin N (**1**) possesses two chiral centers, one residing at the tyrosine unit, the other at the aliphatic side chain and a total synthesis of each diastereoisomer was undertaken to establish its absolute stereochemistry. A retrosynthetic analysis (Fig. 2) shows that **1** can be disconnected at the amide bond to give the tyrosine unit and the long chain aliphatic acid. The acid component was synthesized using the protocols reported in literature with slight modifications.[10,11] For ease of purification, the assembly of the molecule was performed on solid phase at the final stage.

The preparations of both enantiomers of the acid side chain **8** were reported by Wipf et al.[10] The (*S*)-isomer was obtained by methylating the acylated (*S*)-4-benzyloxazolidinone while the (*R*)-isomer was obtained by alkylating the propionylated (*S*)-4-benzyloxazolidinone. However, the alkylation of the corresponding potassium, sodium, or lithium enolate with hexyl iodide failed to give the desired product and the problem was circumvented by employing the more reactive reagent hexyl triflate. The exact outcome of the earlier failures was not reported in detail.

In our study, alkylation of the (*S*)-propionylated oxazolidinone using sodium enolate and hexyl bromide gave no conversion at −78 °C. However, by raising the reaction temperature to 4 °C, self-acylation of the propionylated oxazolidinone was observed, giving the acylated isomers **11**

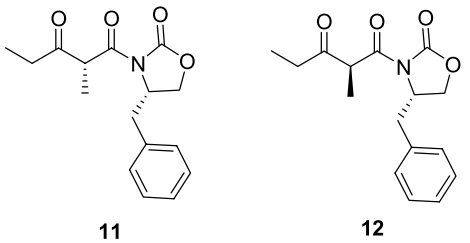
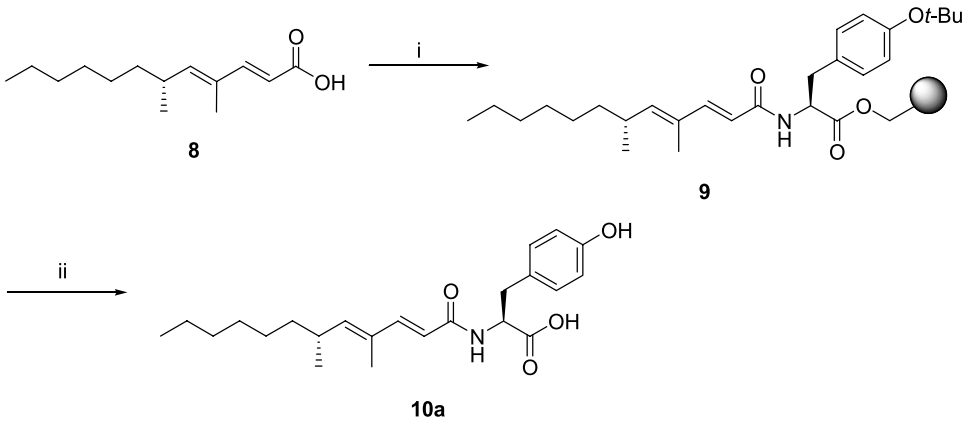


Figure 3. Self-acylated isomers.



Scheme 1. Synthesis of gymnastatin N. Reagents and conditions: (i) Wang resin-bound L-Tyr(*t*-Bu), PyBOP, HOBt, diisopropylethylamine, anhydrous DMA, rt, 18 h; (ii) TFA–CH_2Cl_2 1:1, rt, 2 h (93% from step i).

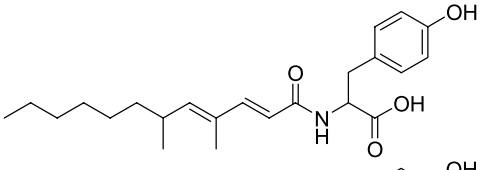
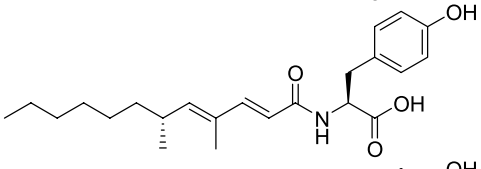
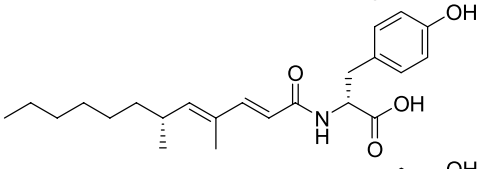
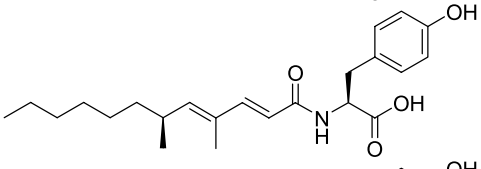
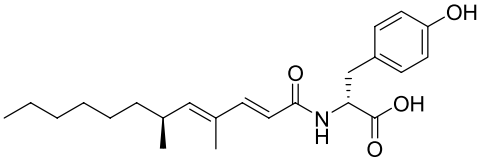
and **12** in a ratio of 61:39 (Fig. 3). The alkylation was also attempted using $TiCl_4$, but this was unsuccessful. There was no self-acylation and the starting material was recovered.

These initial failures led us to attempt the alternative synthetic route by first acylating the (*R*)-4-benzyloxazolidinone with octanoyl chloride following by methylation. This route gave a higher yield and was more appropriate for our purpose. Subsequent reductive cleavage of the methylated imide, oxidation, iterative Horner–Emmons–Wittig reactions and hydrolysis, afforded the (*R*)-acid component **8** in an overall 23% yield, starting from oxazolidinone. Adopting the same approach, the (*S*)-acid component was also obtained in an overall 20% yield from (*S*)-4-benzyloxazolidinone.

PyBOP-mediated coupling of the resin-bound L-tyrosine with the (*R*)-acid side chain **8** gave the amide **9** (Scheme 1). The resultant resin **9** had to be washed extensively with DMF to remove any impurities. Subsequent treatment with 50% TFA, to both remove the *t*-Bu protecting group and execute resin cleavage, gave the final product **10a** in a 93% yield over the two steps. The other three stereoisomers **10b–d** were also prepared by reacting the (*R*)-side chain with D-tyrosine, and (*S*)-side chain with L- or D-tyrosine respectively (Table 1).

The absolute stereochemistry of gymnasatin N (**1**) could not be ascertained by direct comparison with the ^1H or ^{13}C NMR spectra of the synthetic products as the four

Table 1. Structures, optical rotations, and HPLC retention times of gymnastatin N and isomers

Entry	Structure	$[\alpha]_D^{30}$ (*c* 0.2, EtOH)	R_t (min)
Gymnastatin N		−32.5	20.8, 21.3
10a		+27.5	21.4
10b		−94.0	20.8
10c		+87.5	21.3
10d		−20.0	20.7

stereoisomers **10a–d** had identical NMR data. This phenomenon was also encountered by Wipf and co-workers during their synthesis of aranorosin where they had to resort to comparison of optical rotations for each diastereomer to establish the absolute configuration of the natural product.[10] However, none of the optical rotations of the synthetic stereoisomers **10a–d** matched that of the gymnastatin N (Table 1).

Therefore, gymnastatin N was probably a mixture of enantiomers or diastereomers. To determine whether this was the case, the four synthetic stereoisomers and natural product were analyzed by HPLC using a ChiralCel®OD-R column (Fig. 4) and the retention times are shown in Table 1. The HPLC analysis of gymnastatin N showed that it was not a single compound, but instead two peaks that appeared at the retention times 21.3 and 20.8 min in a ratio

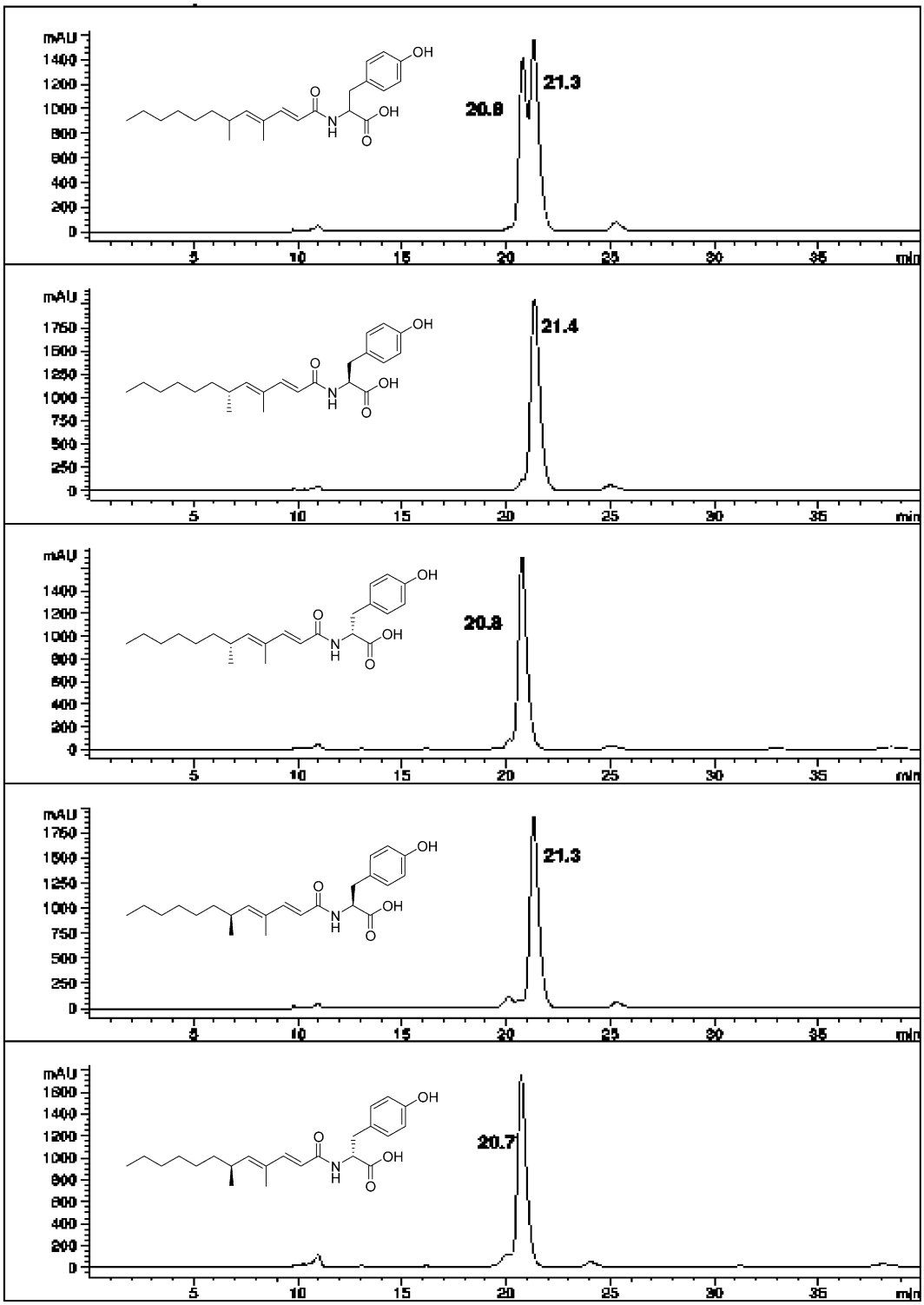


Figure 4. HPLC chromatograms of gymnastatin N and compounds **10a–d**.

of 58:42. Considering the optical rotation and HPLC profile, it was clear that gymnastatin N was a mixture of the diastereomers **10a** and **10b**. This was confirmed by obtaining the optical rotation of a mixture of isomers **10a** and **10b** at a ratio of 58:42 ($[\alpha]_D^{30}$ −35.5), which is similar to that obtained for gymnastatin N ($[\alpha]_D^{30}$ −32.5).

Compound **3** (Fig. 1) displayed an $[M-H]^-$ ion peak at *m/z* 402.2178 in the (−)-HR-ESIMS. These data were consistent with a molecular formula of $C_{23}H_{33}NO_5$ for **3**, which was 16 mass units more than **1**. The NMR data of **3** was almost identical to **1** except that the terminal methyl group on the aliphatic chain was replaced with a primary alcohol (δ_H 3.31; δ_C 60.2). Hence, the structure of **3** was established as the 12′-hydroxy derivative of gymnastatin N.

The IC_{50} values of gymnastatin N (**1**) and aranorosinol A (**2**) were 13 and 118 μM in the Plk1 assay, 45 μM and inactive in Cdk2 assay, respectively (Table 2). To improve the activity profile of **1**, a series of SAR studies was conducted. The importance of the carboxylic acid group and the conjugated diene was firstly assessed by preparing the methyl ester **13** and the fully saturated product **14** (Fig. 5). Both derivatives showed a decrease of activity against Plk1, indicating that the free acid and the conjugated diene functionalities might be essential for bioactivity.

A range of analogues with variations in amino acid moiety and aliphatic chain were also prepared. They were synthesized on solid support either through the reaction of free amino groups with acid chlorides, or PyBOP-mediated

Table 2. Yields, Plk1 and Cdk2 IC_{50} of gymnastatin N, its analogues, and aranorosinol A

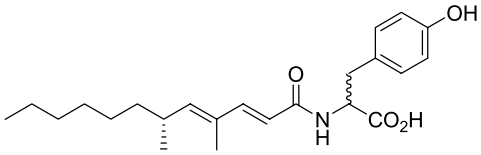
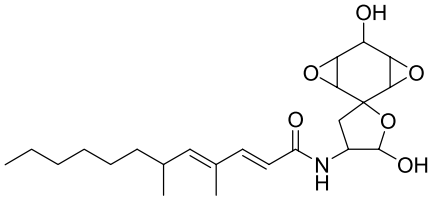
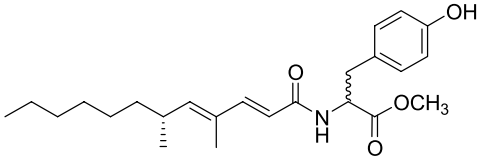
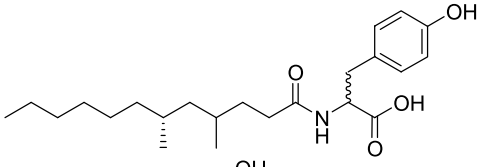
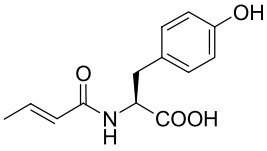
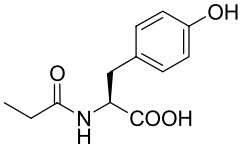
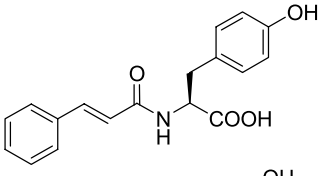
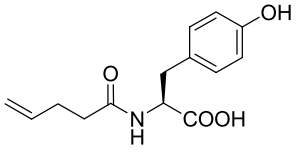
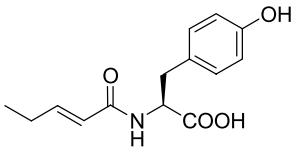
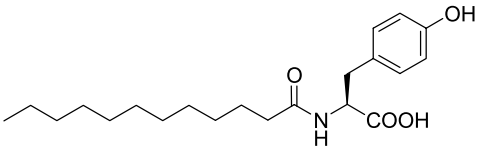
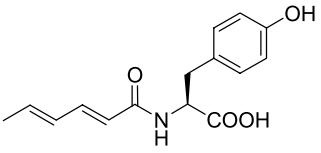
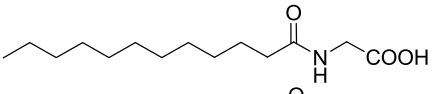
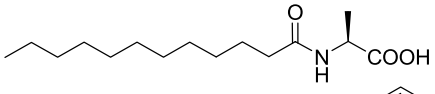
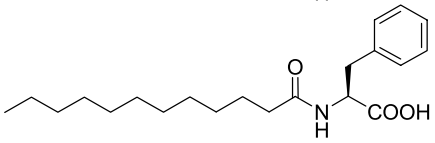
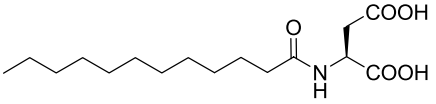
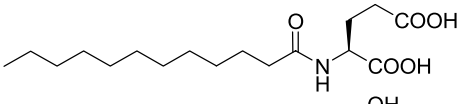
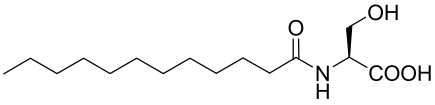
Entry	Structure	Yield (%)	Plk1[a] IC_{50} (μM)	Cdk2[b] IC_{50} (μM)
1		—	13	45
2		—	118	>208
13		92	>208	>208
14		89	82	254
15a		98	>334	>334
15b		96	>351	>351
15c		98	>268	>268
15d		98	>317	>317

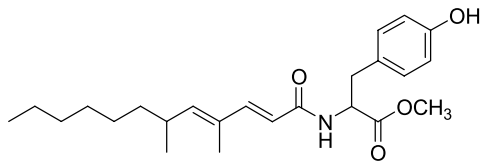
Table 2 (*continued*)

Entry	Structure	Yield (%)	Plk1[a] IC_{50} (μM)	Cdk2[b] IC_{50} (μM)
15e		98	73	>332
15f		99	63	>229
15g		99	2.2	17
15h		98	87	>324
15i		98	121	>307
15j		99	65	>240
15k		98	61	>264
15l		96	144	>253
15m		94	>290	>290

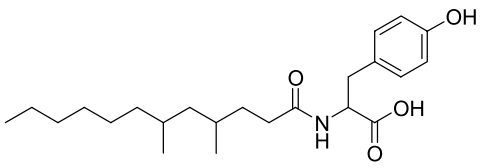
[a] The Plk1 assay was carried out in triplicates.
[b] The Cdk2 assay was carried out in duplicates.

coupling with acids (Scheme 2). The products **15a**–**m** were cleaved off resin in high yields (Table 2). The purities of the compounds are greater than 95% as assessed by ^{1}H NMR spectroscopy. Among all the analogues screened, the compound **15g**, which contained the tyrosine moiety and the conjugated diene, exhibited the most potent activity against Plk1.

In conclusion, the new natural product gymnastatin N (**1**) and the known compound aranorosinol A (**2**) were identified as Plk1 inhibitors. To our knowledge, this is the first report

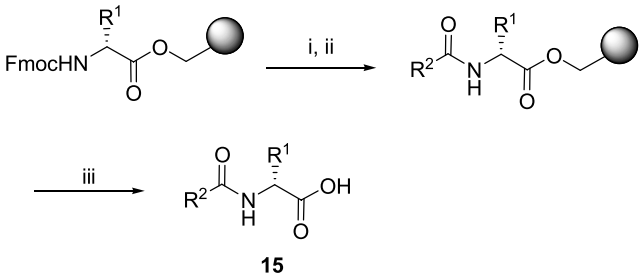


13



14

Figure 5. Derivatives of gymnastatin N.



15

Scheme 2. Solid phase synthesis of gymnastatin N analogues. Reagents and conditions: (i) 20% piperidine/DMF, rt, 20 min, 2 cycles; (ii) a cid chloride, diisopropylethylamine, anhydrous CH_2Cl_2, rt, 1 h or acid, PyBOP, HOBt, diisopropylethylamine, anhydrous DMA, rt, 18 h; (iii) TFA–CH_2Cl_2 1:1, rt, 18 h.

on the natural product inhibitors of Plk1. Total syntheses of all four stereoisomers of **1** showed that it is a mixture of two diastereomers **10a** and **10b**. We believe that the presence of both diastereomers is not an artifact as neither heating nor base was employed in the fermentation or the isolation process. Preliminary SAR studies indicated that the free acid and the conjugated diene groups might be responsible for the activity.

3. Experimental

3.1. General experimental procedures

Specific optical rotation was determined using a Jasco DIP-1000 Digital Polarimeter. ^{1}H and ^{13}C NMR spectra of the natural products were acquired on a Bruker Avance DRX-500 NMR spectrometer, using 5 mm inverse (^{1}H, G-COSY, multiplicity-edited G-HSQC, G-HMBC and G-ROESY spectra) or normal (^{13}C spectra) probeheads equipped with *z*-gradients, while NMR data on the synthetic compounds was acquired using a Bruker AVANCE-400 NMR. Spectra were calibrated to residual protonated solvent signals and all chemical shift values (δ) are given in ppm. Infrared spectra were taken on a Bio-Rad Excalibur Series FT-IR spectrometer. Melting points were determined by a Büchi 535 melting point apparatus. HPLC was performed on a Waters system equipped with a Waters 996 PDA detector, Waters 600 gradient controller and pump, Waters 717plus autosampler, Millennium software and a Waters fraction collector II. Preparative HPLC was done using a Waters Delta-Pak™ C_{18} (40 mm I.D.$\times$ 100 mm, 15 μ) column, while semi-preparative HPLC was done using either a Phenomenex Luna C18(2) (10 mm I.D.$\times$150 mm, 5 μ) or Phenomenex Phenyl-Hexyl (10 mm I.D.$\times$150 mm, 5 μ) columns for separation of the natural products. HR-ESIMS data was collected on an Applied Biosystems Mariner TOF mass spectrometer, using sodium trifluoroacetate as an internal standard for both positive and negative ionization modes. Chiral HPLC analyses were carried out on a ChiralCel®OD-R column (4.6 mm I.D.$\times$ 250 mm): isocratic elution with 55% (0.04% TFA/CH_3CN), 45% (0.04% TFA/H_2O); flow rate of 0.5 mL/min over 35 min. The SPA beads and ^{33}P-ATP were purchased from Amersham Biosciences, while the enzymes and peptide substrates were provided by GlaxoSmithKline. The chemical reagents and resins were purchased from Sigma-Aldrich, Novabiochem, and Bachem. Anhydrous CH_2Cl_2 was freshly distilled over calcium hydride. Fritted polyethylene filtration tubes and teflon taps were bought from Jones Chromatography.

3.2. Microorganism and Fermentation

The fungal strain *A. punctatus* is deposited in the MerLion Pharmaceuticals culture collection (F31134). The strain was sub-cultured on Malt Extract Agar (MEA) for 14 days. An aqueous seed medium (50 mL) containing 0.4% yeast extract, 1% malt extract and 0.4% glucose was placed in 250 mL Erlenmeyer flasks and was sterilized at 121 °C for 30 min. Five agar plugs were used for inoculation of seed culture and the inoculated flasks were shaken on a rotary shaker (200 rpm) at 24 °C for 5 days. Seed culture (5 mL) was transferred to each of the twenty 250 mL Erlenmeyer flasks with solid fermentation medium (containing 50 mL of 4% maltose, 12% Glucidex, 1% bacteriological peptone, 1.5% cotton seed flour, 1.5% cane molasses, 0.5% $MgSO_4\cdot 7H_2O$, 0.5% $CaCO_3$, 0.2% $FeSO_4\cdot 7H_2O$, 0.002% $ZnSO_4\cdot 7H_2O$, 0.2% L-tryptophan and 6 g of vermiculite). Fermentation was carried out at 24 °C for 14 days.

3.3. Extraction and isolation

The biomass from the 20 Erlenmeyer flasks were extracted with CH_3OH, and evaporated to dryness under vacuum. The crude CH_3OH extract (130 g) was partitioned between $CHCl_3$ and H_2O, and the $CHCl_3$ extract (3.5 g) was separated using a C18 column using a 20% stepwise gradient from H_2O to CH_3OH. The 80% CH_3OH active fraction was separated using C18 preparative HPLC (gradient elution from 0 to 100% [0.1% HCOOH/CH_3CN and 0.1% HCOOH/H_2O] in 25 min, 18 mL/min) and C18 semi-preparative HPLC (gradient elution from 60 to 75% [0.1% HCOOH/CH_3CN and 0.1% HCOOH/H_2O] in 25 min, 4 mL/min) to afford gymnastatin N (**1**) (5 mg). Adjacent fractions were also found to be active and was purified by HPLC using a phenylhexyl column (gradient elution from 43 to 50% [0.1% HCOOH/CH_3CN and 0.1% HCOOH/H_2O] in 20 min, 4 mL/min) gave aranorosinol A (**2**) (7 mg). During the process of isolation, another compound more polar than **1** that had a similar UV characteristics was observed, and was purified on a semi-preparative HPLC using phenylhexyl column (isocratic 40% [0.1% HCOOH/CH_3CN and 0.1% HCOOH/H_2O], 4 mL/min) and Sephadex LH-20 (eluent CH_3OH) to give 12′-hydroxy gymnastatin N (**3**) (0.5 mg).

3.3.1. Gymnastatin N (1). Colourless oil; $[\alpha]_D^{30}$ −32.5° (*c* 0.2, EtOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.83 (t, 3H, J=6.6 Hz, H-12′), 0.92 (dd, 3H, J=6.6, 1.9 Hz, 6′-CH_3), 1.21 (br m, 10H, H-7′ to H11′), 1.71 (s, 3H, 4′-CH_3), 2.49 (1H, under DMSO, H-6′), 2.74 (dd, 1H, J=13.9, 9.5 Hz, H-2a), 2.94 (dd, 1H, J=13.9, 4.7 Hz, H-2b), 4.39 (m, 1H, H-1) 5.58 (d, 1H, J=9.8 Hz, H-5′), 5.97 (d, 1H, J=15.4 Hz, H-2′), 6.63 (d, 2H, J=8.2 Hz, H-5 and H-7), 6.95 (d, 1H, J=15.4 Hz, H-3′), 6.99 (d, 2H, J=8.2 Hz, H-4 and H-8), 8.14 (d, 1H, J=8.2 Hz, NH), 9.19 (br. s, 1H, 1-COOH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 12.4 (4′-CH_3), 13.9 (C-12′), 20.5 (6′-CH_3), 22.0 (C-11′), 26.8 (C-9′*), 28.7 (C-8′*), 31.2 (C-10′), 32.4 (C-6′), 36.1 (C-2), 36.7 (C-7′), 54.1 (C-1), 114.9 (C-5, C-7), 119.3 (C-2′), 127.8 (C-3), 129.9 (C-4, C-8), 130.9 (C-4′), 144.1 (C-3′), 145.5 (C-5′), 155.8 (C-6), 165.3 (C-1′), 173.3 (1-COOH), * interchangeable; (−)-HR-ESIMS m/z 386.2335 $[M-H]^-$ (calcd for $C_{23}H_{32}NO_4$, 386.2331); (+)-HR-ESIMS m/z 388.2470 $[M+H]^+$ (calcd for $C_{23}H_{34}NO_4$, 388.2488).

3.3.2. 12′-Hydroxy gymnastatin N (3). Colourless oil; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.92 (dd, 3H, J=6.62, 1.9 Hz, 6′-CH_3), 1.22 (br. m, 10H, H-7′ to H11′), 1.70 (s, 3H, 4′-CH_3), 2.49 (1H, under DMSO, H-6′), 2.77 (dd, 1H, J=13.6, 7.2 Hz, H-2a), 2.95 (br d, 1H, H-2b), 3.31 (under H_2O, 1H, H-12′), 4.14 (m, 1H, H-1) 5.55 (d, 1H, J=9.8, H-5′), 5.98 (d, 1H, J=15.4, H-2′), 6.56 (d, 2H, J=8.2 Hz, H-5 and H-7), 6.91 (br d, 3H, H-3′, H-4 and H-8), 7.56 (br s, 1H, NH), 9.19 (br s, 1H, 1-COOH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 12.4 (4′-CH_3), 20.5 (6′-CH_3), 25.4 (C-11′), 26.9 (C-9′*), 29.0

(C-8′*), 32.2 (C-10′), 32.5 (C-6′), 36.5 (C-2), 36.7 (C-7′), 55.1 (C-1), 60.65 (C-12′), 114.6 (C-5, C-7), 120.3 (C-2′), 128.9 (C-3), 130.1 (C-4, C-8), 131.0 (C-4′), 143.2 (C-3′), 144.8 (C-5′), 155.4 (C-6), 164.5 (C-1′), 172.7 (1-COOH), * interchangeable; (−)-HR-ESIMS *m/z* 402.2278 $[M-H]^-$ (calcd for $C_{23}H_{32}NO_5$, 402.2280).

3.4. Biological assays

The Plk1 assay was performed in 384-well plates. Fractions and compounds were incubated with Plk1 in a reaction mixture containing 25 nM Plk1 enzyme, 1 μM peptide substrate, 0.15 mg SPA beads, 1 μM ATP, 0.3 mg/mL heparin, 0.25 mg/mL bovine serum albumin, 7.5 mM $MgCl_2$, and 12.5 mM HEPES pH 7.5. After 90 min of incubation at rt, the reaction was terminated by the addition of 50 μL stop reagent containing 2.5 mg/mL SPA beads and 100 mM EDTA in PBS buffer. Radioactivity was measured using a Microbeta Counter (PerkinElmer) after an overnight bead settling period. The assay procedure for Cdk2 assay is similar to that for Plk1 assay except that it involves a 30 min incubation at room temperature with the final reaction condition containing 0.64 μg/mL Cdk2 enzyme, 2.5 μM peptide substrate, 1.4 μM ATP, 0.1 mg/mL bovine serum albumin, 10 mM $MgCl_2$, and 100 mM HEPES pH 7.5.

3.5. Synthetic procedure for compounds 10

To the Wang resin-bound Fmoc-L- or D-Tyr(*t*-Bu) (0.63–0.74 mmol/g; 80 mg, 0.05–0.059 mmol) contained in a fritted polyethylene filtration tube was added 20% piperidine/DMF (2 mL). The resin suspension was stirred gently at rt for 20 min and then filtered. The Fmoc deprotection was carried out again. The resin was then washed with CH_2Cl_2 (3×5 mL), followed by drying under vacuum. To the dried resin was added acid **8** (0.1–0.18 mmol), PyBOP (0.1–0.18 mmol) and HOBt (0.1–0.18 mmol). This mixture was suspended in anhydrous DMA (1–1.2 mL), and distilled diisopropylethylamine (0.2–0.36 mmol) was added. The suspension was stirred gently at rt for 18 h, then filtered. The resin was washed successively with DMF (6×5 mL), THF–H_2O 3:2 (3×5 mL), THF (3×5 mL), CH_2Cl_2 (3×5 mL), then dried under vacuum. The derivatised resin **9** was transferred into a round bottom flask, and treated with TFA–CH_2Cl_2 (1:1, 2 mL) at rt for 2 h. Following that, the cleavage suspension was filtered and the resin was washed with CH_2Cl_2 (3×5 mL). The filtrate and collected washings were concentrated under reduced pressure to afford the product **10**.

3.5.1. (1*S*,6′*R*)-2-[(2*E*,4*E*)-4,6-Dimethyldodeca-2,4-dienamido]-3-(4-hydroxy-phenyl)propanoic acid (10a). Yield: 18.1 mg (93%). Colourless oil. ν_{max} (thin film) 3600–3100, 3313, 1748, 1640 cm^{-1}.

3.5.2. (1*R*,6′*R*)-2-[(2*E*,4*E*)-4,6-Dimethyldodeca-2,4-dienamido]-3-(4-hydroxy-phenyl)propanoic acid (10b). Yield: 17.3 mg (85%). White solid, mp 158–160 °C (decomposed). ν_{max} (Nujol) 3600–3100, 3323, 1754, 1639 cm^{-1}.

3.5.3. (1*S*,6′*S*)-2-[(2*E*,4*E*)-4,6-Dimethyldodeca-2,4-dienamido]-3-(4-hydroxy-phenyl)propanoic acid (10c). Yield: 19.0 mg (96%). White solid, mp 158–160 °C (decomposed). ν_{max} (Nujol) 3600–3100, 3328, 1754, 1639 cm^{-1}.

3.5.4. (1*R*,6′*S*)-2-[(2*E*,4*E*)-4,6-Dimethyldodeca-2,4-dienamido]-3-(4-hydroxy-phenyl)propanoic acid (10d). Yield: 20.0 mg (92%). Colourless oil. ν_{max} (thin film) 3600–3100, 3313, 1748, 1640 cm^{-1}.

3.5.5. Methyl 2-[(2*E*,4*E*)-4,6-dimethyldodeca-2,4-dienamido]-3-(4-hydroxyphenyl)-propanoate (13). The product was synthesized according to the literature procedure with modifications in the amounts of reagents used:[15] Gymnastatin N (2.2 mg, 5.68 μmol) was dissolved in diethyl ether (1 mL); Diazald® (100 mg, 0.47 mmol), Carbitol® (0.5 mL, 3.72 mmol) was dissolved in diethyl ether (1 mL); 37% KOH (1 mL). The reaction mixture was left at 0 °C for 3 h with occasional shaking. Excess diazomethane was quenched by adding acetic acid (2–3 drops). The solvent was then removed under reduced pressure to afford the product as colourless oil (2.0 mg, 92%). R_f (50% EtOAc/hexane) 0.71; ^1H NMR (400 MHz, $CDCl_3$) δ 0.86 (3H, td, J=7.0, 2.6 Hz), 0.96 (3H, d, J=6.6 Hz), 1.22–1.26 (10H, m), 1.74 (3H, d, J=1.0 Hz), 2.49 (1H, m), 3.06 (1H, dd, J=13.9, 5.4 Hz), 3.12 (1H, dd, J=13.9, 6.5 Hz), 3.73 (3H, s), 4.95 (1H, m), 5.63 (1H, br d, J=9.7 Hz), 5.72 (1H, d, J=15.5 Hz), 5.94 (1H, br dd, J=7.4, 2.5 Hz), 6.74 (2H, d, J=7.9 Hz), 6.95 (2H, d, J=7.9 Hz), 7.23 (1H, dd, J=15.5, 2.5 Hz); (+)-HR-ESIMS *m/z* 402.2641 $[M+H]^+$ (calcd for $C_{24}H_{35}NO_4$, 402.2644).

3.5.6. 2-(4,6-Dimethyldodecanamido)-3-(4-hydroxyphenyl)propanoic acid (14). To a solution of gymnastatin N (2.9 mg, 7.49 μmol) in ethanol (1 mL) was added 10% Pd/C (50 mg). The suspension was bubbled with hydrogen at room temperature for 10 cycles (each cycle took approximately 2 min), then filtered through a pad of Celite. The pad was washed further with ethanol (5×5 mL). The filtrate and washings were combined and concentrated under reduced pressure to give the product as colourless oil (2.6 mg, 89%). R_f (EtOAc) 0.29; ^1H NMR (400 MHz, CD_3OD) δ 0.80–0.97 (9H, m), 1.19–1.52 (16H, m), 2.15 (2H, br tt, J=7.1, 7.1 Hz), 2.86 (1H, dd, J=13.7, 7.6 Hz), 3.10 (1H, dd, J=13.7, 4.6 Hz), 4.45 (1H, m), 6.65 (2H, d, J=8.4 Hz), 7.02 (2H, d, J=8.4 Hz); (+)-HR-ESIMS *m/z* 414.2628 $[M+Na]^+$ (calcd for $C_{23}H_{37}NO_4Na$, 414.2620).

3.6. Synthetic procedure for compounds 15 using acid chloride coupling

To the Wang resin-bound Fmoc-protected amino acid (0.44–0.7 mmol/g; 100 mg, 0.044–0.07 mmol) in a fritted polyethylene filtration tube was added 20% piperidine/DMF (2 mL). The resin suspension was stirred gently at rt for 20 min and then filtered. The Fmoc deprotection was carried out again. The resin was then washed with CH_2Cl_2 (3×5 mL), followed by drying under vacuum. The dried resin was suspended in anhydrous CH_2Cl_2 (2 mL). Distilled diisopropylethylamine (0.44–0.7 mmol) was added. After 5 min of stirring, the acid chloride (0.44–0.7 mmol) was introduced dropwise. The suspension was stirred gently at rt for 1 h, then filtered. The resin was washed with CH_2Cl_2 (3×5 mL), then dried under vacuum. The derivatised resin was transferred into a round bottom flask, and treated with

TFA–CH_2Cl_2 (1:1, 2 mL) at rt for 18 h. Following that, the cleavage suspension was filtered and the resin was washed with CH_2Cl_2 (3×5 mL). The filtrate and collected washings were concentrated under reduced pressure to afford the product. This procedure was employed for the syntheses of compounds **15a–d**, **f**, **h–m**. Sasrin™ resin-bound Fmoc-L-Ser(*t*-Bu) was used for the preparation of compound **15m**.

3.7. Synthetic procedure of compounds 15 using PyBOP coupling

To the Wang resin-bound Fmoc-amino acid (0.45–0.63 mmol/g; 100 mg, 0.045–0.063 mmol) contained in a fritted polyethylene filtration tube was added 20% piperidine/DMF (2 mL). The resin suspension was stirred gently at rt for 20 min, then filtered. The Fmoc deprotection was carried out again. The resin was then washed with CH_2Cl_2 (3×5 mL), followed by drying under vacuum. To the dried resin was added acid (0.23–0.32 mmol), PyBOP (0.23–0.32 mmol) and HOBt (0.23–0.32 mmol). This mixture was suspended in anhydrous DMA (2 mL), and distilled diisopropylethylamine (0.45–0.63 mmol) was added. The suspension was stirred gently at rt for 18 h, then filtered. The resin was washed successively with DMF (3×5 mL), THF–H_2O 3:2 (3×5 mL), THF (3×5 mL), CH_2Cl_2 (3×5 mL), then dried under vacuum. The derivatised resin was transferred into a round bottom flask, and treated with TFA–CH_2Cl_2 (1:1, 2 mL) at rt for 18 h. Following that, the cleavage suspension was filtered and the resin was washed with CH_2Cl_2 (3×5 mL). The filtrate and collected washings were concentrated under reduced pressure to afford the product. This procedure was employed for the syntheses of compounds **15e** and **15g**.

3.7.1. *N*-(*trans*-Crotonyl)-L-tyrosine (15a). Yield: 11.1 mg (98%). Pale yellow gum. ν_{max} (Nujol) 3600–3100, 3286, 1720, 1670 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 1.83 (3H, dd, J=6.9, 1.7 Hz), 2.89 (1H, dd, J=14.0, 8.7 Hz), 3.11 (1H, dd, J=14.0, 5.2 Hz), 4.64 (1H, dd, J=8.7, 5.2 Hz), 5.96 (1H, dq, J=15.3, 1.7 Hz), 6.68 (2H, dt, J=8.5, 2.0 Hz), 6.75 (1H, dq, J=15.3, 6.9 Hz), 7.03 (2H, dt, J=8.5, 2.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 17.8, 37.8, 55.6, 116.1, 125.8, 129.2, 131.2, 141.3, 157.3, 168.3, 175.3; (+)-HR-ESIMS m/z 250.1081 $[M+H]^+$ (calcd for $C_{13}H_{16}NO_4$, 250.1079).

3.7.2. *N*-(1-Oxopropyl)-L-tyrosine (15b). Yield: 10.1 mg (96%). Pale yellow oil. ν_{max} (thin film) 3600–3100, 1727, 1646 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 1.04 (3H, t, J=7.6 Hz), 2.17 (2H, qd, J=7.6, 0.8 Hz), 2.84 (1H, dd, J=14.0, 9.1 Hz), 3.10 (1H, dd, J=14.0, 5.1 Hz), 4.58 (1H, dd, J=9.1, 5.1 Hz), 6.69 (2H, dt, J=8.6, 2.0 Hz), 7.03 (2H, dt, J=8.6, 2.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 10.3, 29.9, 37.7, 55.3, 116.1, 129.2, 131.3, 157.3, 175.3, 176.8; (+)-HR-ESIMS m/z 238.1072 $[M+H]^+$ (calcd for $C_{12}H_{16}NO_4$, 238.1079).

3.7.3. *N*-(*trans*-Cinnamoyl)-L-tyrosine (15c). Yield: 13.3 mg (98%). Colourless oil. ν_{max} (thin film) 3600–3100, 1729, 1650 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 2.94 (1H, dd, J=14.0, 8.5 Hz), 3.16 (1H, dd, J=14.0, 5.2 Hz), 4.73 (1H, dd, J=8.5, 5.2 Hz), 6.66 (1H, d, J=15.8 Hz), 6.70 (2H, dt, J=8.6, 2.1 Hz), 7.07 (2H, dt, J=8.6, 2.1 Hz), 7.35–7.40 (3H, m), 7.49 (1H, d, J=15.8 Hz), 7.54 (2H, br dd, J=7.6, 1.6 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 37.8, 55.7, 116.2, 121.4, 128.9, 129.1, 129.9, 130.9, 131.3, 136.2, 142.2, 157.3, 168.3, 175.0; (+)-HR-ESIMS m/z 312.1236 $[M+H]^+$ (calcd for $C_{18}H_{18}NO_4$, 312.1236).

3.7.4. *N*-(4-Pentenoyl)-L-tyrosine (15d). Yield: 11.7 mg (98%). Pale yellow oil. ν_{max} (thin film) 3600–3100, 1727, 1645 cm^{-1}; ^1H NMR (400 MHz, acetone-d_6) δ 2.25–2.27 (4H, m), 2.89 (1H, dd, J=13.9, 8.2 Hz), 3.08 (1H, dd, J=13.9, 5.1 Hz), 4.65 (1H, m), 4.89 (1H, dd, J=9.8, 1.6 Hz), 4.98 (1H, br d, J=17.1 Hz), 5.76 (1H, m), 6.74 (2H, d, J=8.4 Hz), 7.07 (2H, d, J=8.4 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 30.8, 36.1, 37.7, 55.3, 115.7, 116.1, 129.2, 131.3, 138.2, 157.3, 175.2; (+)-HR-ESIMS m/z 264.1231 $[M+H]^+$ (calcd for $C_{14}H_{18}NO_4$, 264.1236).

3.7.5. *N*-(*trans*−2-Pentenoyl)-L-tyrosine (15e). Yield: 11.5 mg (98%). Pale yellow oil. ν_{max} (thin film) 3600–3100, 1724, 1667 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 1.05 (3H, t, J=7.5 Hz), 2.20 (2H, qdd, J=7.5, 6.5, 1.7 Hz), 2.89 (1H, dd, J=14.0, 8.8 Hz), 3.11 (1H, dd, J=14.0, 5.2 Hz), 4.65 (1H, dd, J=8.8, 5.2 Hz), 5.95 (1H, dt, J=15.4, 1.7 Hz), 6.69 (2H, dt, J=8.6, 2.0 Hz), 6.79 (1H, dt, J=15.4, 6.5 Hz), 7.03 (2H, dt, J=8.6, 2.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 12.8, 26.1, 37.7, 55.5, 116.2, 123.3, 129.1, 131.2, 147.7, 157.3, 168.5, 175.0; (+)-HR-ESIMS m/z 264.1236 $[M+H]^+$ (calcd for $C_{14}H_{18}NO_4$, 264.1236).

3.7.6. *N*-Dodecanoyl-L-tyrosine (15f). Yield: 15.2 mg (99%). Pale yellow solid, mp 122–125 °C. ν_{max} (Nujol) 3600–3100, 3302, 1706, 1643 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 0.90 (3H, t, J=7.1 Hz), 1.20–1.37 (16H, m), 1.50 (2H, tt, J=7.4, 7.4 Hz), 2.15 (2H, t, J=7.4 Hz), 2.83 (1H, dd, J=14.0, 9.4 Hz), 3.11 (1H, dd, J=14.0, 4.9 Hz), 4.60 (1H, dd, J=9.4, 4.9 Hz), 6.69 (2H, dt, J=8.5, 2.0 Hz), 7.03 (2H, dt, J=8.5, 2.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 14.4, 23.7, 26.9, 30.1, 30.5, 30.6, 30.7, 33.1, 36.8, 37.7, 55.2, 116.2, 129.1, 131.2, 157.3, 175.1, 176.2; (+)-HR-ESIMS m/z 364.2485 $[M+H]^+$ (calcd for $C_{21}H_{34}NO_4$, 364.2488).

3.7.7. *N*-(*trans,trans*-2,4-Hexadienoyl)-L-tyrosine (15g). Yield: 17.0 mg (99%). Colourless oil. ν_{max} (thin film) 3600–3100, 1719, 1656 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 1.82 (3H, d, J=6.4 Hz), 2.90 (1H, dd, J=14.0, 8.6 Hz), 3.11 (1H, dd, J=14.0, 5.2 Hz), 4.66 (1H, dd, J=8.6, 5.2 Hz), 5.92 (1H, dd, J=15.2, 0.4 Hz), 6.06–6.23 (2H, m), 6.69 (2H, dt, J=8.6, 2.0 Hz), 7.03 (2H, dt, J=8.6, 2.0 Hz), 7.08 (1H, dd, J=15.5, 10.5 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 18.6, 37.8, 55.5, 116.2, 122.3, 129.1, 131.1, 131.2, 139.0, 142.7, 157.3, 168.8, 175.0; (+)-HR-ESIMS m/z 276.1237 $[M+H]^+$ (calcd for $C_{15}H_{18}NO_4$, 276.1236).

3.7.8. *N*-Dodecanoylglycine (15h). Yield: 12.5 mg (98%). White solid, mp 112–114 °C. ν_{max} (Nujol) 3600–3100, 3318, 1703, 1644 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 0.90 (3H, t, J=7.0 Hz), 1.24–1.32 (16H, m), 1.62 (2H, br tt, J=7.5, 7.5 Hz), 2.24 (2H, t, J=7.5 Hz), 3.89 (2H, s); ^{13}C NMR (100 MHz, CD_3OD) δ 14.4, 23.7, 26.8, 30.3, 30.5, 30.6, 30.7, 33.1, 36.8, 41.8, 173.2, 176.7; (+)-HR-ESIMS m/z 258.2067 $[M+H]^+$ (calcd for $C_{14}H_{28}NO_3$, 258.2069).

3.7.9. *N*-Dodecanoyl-L-alanine (15i). Yield: 17.1 mg (98%). White solid, mp 77–79 °C. ν_{max} (Nujol) 3600–3100, 3314, 1705, 1645 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 0.90 (3H, t, J=7.0 Hz), 1.24–1.32 (16H, m), 1.38 (3H, d, J=7.3 Hz), 1.61 (2H, br tt, J=7.3, 7.3 Hz), 2.22 (2H, br td, J=7.3, 1.3 Hz), 4.37 (1H, q, J=7.3 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 14.4, 17.6, 23.7, 26.9, 30.3, 30.5, 30.6, 30.7, 33.1, 36.7, 176.1, 176.2; (+)-HR-ESIMS m/z 272.2224 $[M+H]^+$ (calcd for $C_{15}H_{30}NO_3$, 272.2226).

3.7.10. *N*-Dodecanoyl-L-phenylalanine (15j). Yield: 18.2 mg (99%). White solid, mp 95–96 °C. ν_{max} (Nujol) 3600–3100, 3310, 1707, 1606 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 0.90 (3H, t, J=7.1 Hz), 1.16–1.36 (16H, m), 1.48 (2H, tt, J=7.4, 7.4 Hz), 2.14 (2H, t, J=7.4 Hz), 2.92 (1H, dd, J=13.9, 9.7 Hz), 3.22 (1H, dd, J=13.9, 4.8 Hz), 4.67 (1H, dd, J=9.7, 4.8 Hz), 7.17–7.29 (5H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 14.4, 23.7, 26.9, 30.1, 30.5, 30.6, 30.7, 33.1, 36.8, 38.4, 54.8, 127.7, 129.4, 130.2, 138.6, 174.8, 176.2; (+)-HR-ESIMS m/z 348.2534 $[M+H]^+$ (calcd for $C_{21}H_{34}NO_3$, 348.2539).

3.7.11. *N*-Dodecanoyl-L-aspartate (15k). Yield: 13.5 mg (98%). Pale yellow solid, mp 101–104 °C. ν_{max} (Nujol) 3600–3100, 3284, 1704, 1659 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 0.90 (3H, t, J=7.0 Hz), 1.24–1.32 (16H, m), 1.60 (2H, br tt, J=7.6, 7.6 Hz), 2.23 (2H, t, J=7.6 Hz), 2.77 (1H, dd, J=16.8, 7.1 Hz), 2.85 (1H, dd, J=16.8, 5.2 Hz), 4.73 (1H, dd, J=7.1, 5.2 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 14.4, 23.7, 26.9, 30.2, 30.5, 30.6, 30.8, 33.1, 36.8, 37.0, 50.3, 174.0, 174.2, 176.1; (+)-HR-ESIMS m/z 316.2123 $[M+H]^+$ (calcd for $C_{16}H_{30}NO_5$, 316.2124).

3.7.12. *N*-Dodecanoyl-L-glutamate (15l). Yield: 22.0 mg (96%). White solid, mp 103–105 °C. ν_{max} (Nujol) 3600–3100, 3329, 1728, 1676 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 0.90 (3H, t, J=7.1 Hz), 1.24–1.32 (16H, m), 1.62 (2H, br tt, J=7.3, 7.3 Hz), 1.92 (1H, m), 2.18 (1H, m), 2.24 (2H, t, J=7.3 Hz), 2.40 (2H, t, J=7.8 Hz), 4.43 (1H, dd, J=9.3, 5.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 14.4, 23.7, 26.9, 27.9, 30.3, 30.5, 30.6, 30.7, 31.3, 33.1, 36.8, 53.0, 175.1, 176.3, 176.5; (+)-HR-ESIMS m/z 330.2278 $[M+H]^+$ (calcd for $C_{17}H_{32}NO_5$, 330.2281).

3.7.13. *N*-Dodecanoyl-L-serine (15m). Yield: 15.0 mg (94%). White solid, mp 108–113 °C. ν_{max} (Nujol) 3600–3100, 3337, 1738, 1609 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 0.90 (3H, t, J=7.1 Hz), 1.24–1.36 (16H, m), 1.63 (2H, br tt, J=7.5, 7.5 Hz), 2.28 (2H, td, J=7.5, 1.6 Hz), 3.81 (1H, dd, J=11.2, 4.1 Hz), 3.89 (1H, dd, J=11.2, 4.9 Hz), 4.49 (1H, dd, J=4.9, 4.1 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 14.4, 23.7, 26.9, 30.3, 30.47, 30.49, 30.6, 30.7, 33.1, 36.9, 56.1, 63.0, 173.5, 176.4; (+)-HR-ESIMS m/z 288.2178 $[M+H]^+$ (calcd for $C_{15}H_{30}NO_4$, 288.2175).

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Pseudoasymmetry, stereogenicity, and the *RS*-nomenclature comprehended by the concepts of holantimers and stereoisograms

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Abstract—The concepts of holantimer and stereoisogram are applied to comprehensive discussions on the term 'pseudoasymmetry', where the concept of *RS*-stereogenicity is used as a more definite concept than usual stereogenicity. Thereby, three relationships contained in each stereoisogram can be definitely specified: an enantiomeric relationship is related to chiral/achiral, an *RS*-diastereomeric relationship is related to *RS*-stereogenic/*RS*-astereogenic, and a holantimeric relationship is related to scleral/ascleral, which is coined to keep the terminology in a balanced fashion. Such stereoisograms are classified into five types (Types I–V) by virtue of the three relationships. Among them, Type I, III, and V are selected as a set of *RS*-stereogenic units: chiral/ascleral *RS*-stereogenic unit (or Type I unit), chiral/scleral *RS*-stereogenic unit (or Type III unit), and achiral/scleral *RS*-stereogenic unit (or Type V unit). Thereby, the term 'pseudoasymmetric stereogenic units' should be replaced by the term 'achiral/scleral *RS*-stereogenic units' (or 'Type V units').

1. Introduction

The term 'pseudoasymmetric atom' was originally proposed to rationalize the fact that a tetrahedral molecule with different ligands ABpp̄ is achiral but a tetrahedral molecule with different ligands ABCD is chiral, where A, B, C, and D are atoms or achiral ligands (groups), while p and p̄ represent an enantiomeric pair of chiral ligands. This term has undergone significant change of connotation, as pointed out by Mislow.[1] Most textbooks on stereochemistry have adopted the original meaning of the term, as found in Eliel–Wilen's textbook.[2] However, the latest IUPAC 1996 rule[3] has defined the term as containing an atom of a chiral molecule in agreement with the revised *RS*-nomenclature.[4] The target of this paper is to settle the present inconsistency of the term by using the concepts of *holantimer* and *stereoisogram* which we have recently proposed for integrated discussions on chirality and stereogenicity.[5] Thereby, these concepts will be demonstrated to provide us with a versatile terminology for restructuring stereochemistry.

Keywords: Pseudoasymmetry; Stereogenicity; *RS*-Nomenclature; Holantimers; Stereoisograms.

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2. Problem setting

2.1. Connotation of 'pseudoasymmetry'

The short history of changes in the connotation of the term 'pseudoasymmetric atom', although it has once been discussed in Mislow's paper,[1] should be reviewed in detail from our viewpoint, because the present paper will be devoted to a settlement of the dispute concerning the term.

(1) Typical examples of pseudoasymmetry are the two achiral diastereomers (**1** and **2**) of 2,3,4-trihydroxyglutaric acid, where four substituents on the central carbon C(3), i.e. H, OH, *R*-CH(OH)COOH, and *S*-CH(OH)COOH, are different in isolation but the resulting molecules are achiral.
(2) However, the IUPAC 1968 tentative rule[6] has defined the term 'pseudoasymmetric atom' (E-5.8) as follows: 'An atom is termed pseudoasymmetric when bonded tetrahedrally to one pair of enantiomeric groups (+)-a and (−)-a and also to two atoms or groups b and c that are different from a, different from each other, and not enantiomeric with each other' (Def. 1). Then, it has selected a chiral **3** along with an achiral **1** as examples of containing pseudoasymmetric atoms and stated explicitly that molecules containing pseudoasymmetric atoms may be achiral or chiral (E-5.8 Note 2).
(3) Prelog and Helmchen have defined 'pseudoasymmetry' as 'the duality that results from the two ways in which two enantiomorphic ligands can be combined with two

enantiotopic half-spaces' (Def. 2) after their elaborate *geometrical* consideration.[7] Obviously, this definition (Def. 2) has presumed that a molecule characterized by pseudoasymmetry is achiral, as they have stated that 'chiral stereomodels and molecules cannot exhibit any pseudoasymmetry because enantiotopic half-spaces can exist only in achiral stereomodels and molecules (Fig. 1)'.

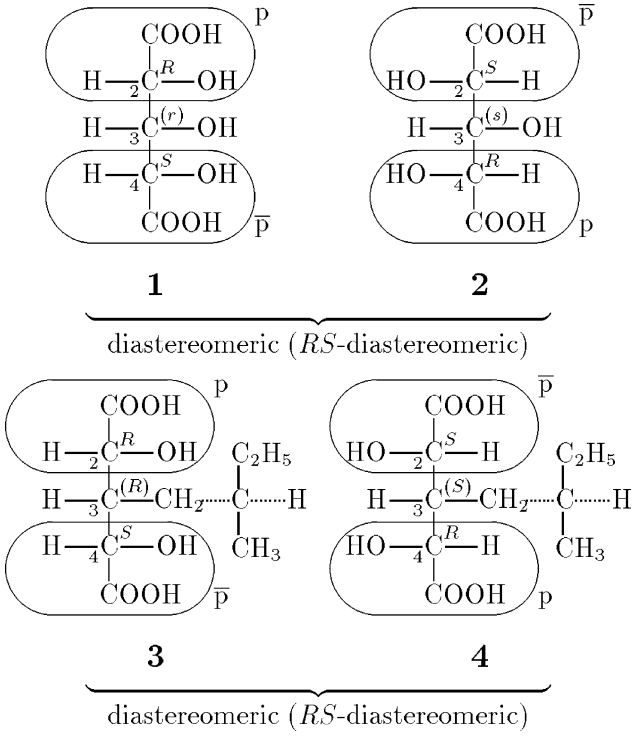

Figure 1. Diastereomeric achiral 2,3,4-trihydroxyglutaric acids (**1** and **2**) and related diastereomeric chiral molecules (**3** and **4**). The *R* for the central atom of **3** and the *S* for the central atom of **4** are designated by the lowercase letters (*r* and *s*) under the IUPAC 1996 rule.

(4) One response was a disputation to Prelog–Helmchen's paper,[7] where Hirschmann and Hanson have expanded their discussions to support Def. 1,[8] Hirschmann–Hanson's criticism[8] has been later characterized as being unjustified from the geometrical point of view.[4]

(5) The other response to Prelog–Helmchen's paper[7] was a revision of the IUPAC rule. Thus, Rule E-4.8 of IUPAC recommendations 1974[9] has defined the term 'pseudoasymmetric atom' as follows: 'An atom is termed pseudoasymmetric when bonded tetrahedrally to one pair of enantiomeric groups (+) -a and (−)-a and also to two atoms or achiral groups b and c that are different from each other'. Compare this definition with Def. 1 in which group b (or c) may be achiral or chiral. The IUPAC 1974 rule omitted the chiral example **3** but maintained the achiral example **1** as compared with Rule E-5.8 of the IUPAC 1968 tentative rules.[6] Moreover, Note 2 of Rule E-5.8 has been deleted. This means that the IUPAC 1974 rule has supported Def. 2.

(6) Prelog and Helmchen have revised the *RS*-nomenclature, where pseudoasymmetry is treated in the form of 'pseudoasymmetric stereogenic units'.[4] Thus, they have stated that, in a tetrahedral pseudoasymmetric stereogenic unit, 'ligands which differ only in their topography, i.e. which are enantiomorphic, are situated within heterotopic half-space.' Note that the term 'enantiotopic half-space' in Def. 2 has been changed into 'heterotopic half-space'. This means that Def. 2 is nullified so as to support Def. 1.

(7) The more recent IUPAC 1996 rule[3] has defined 'pseudoasymmetric carbon atom' as 'the traditional name for a tetrahedrally coordinated carbon atom bonded to four different entities, two and only two of which have the same constitution but opposite chirality sense. The *r*/*s* descriptors of pseudoasymmetric carbon atoms are invariant on reflection in a mirror (i.e. *r* remains *r*, and *s* remains *s*), but are reversed by the exchange of any two entities (i.e. *r* becomes *s*, and *s* becomes *r*).' Examples cited are **1** and **2** as achiral molecules along with hyoscyamine **5** shown in Fig. 2. This means the IUPAC 1996 rule supports Def. 1.

(8) Eliel–Wilen's textbook on stereochemistry[2] has adopted Def. 2, where it states that 'pseudoasymmetric pseudoasymmetric atom' (page 1204) is: 'A stereogenic [but achirotopic] atom of whose four distinct ligands two are enantiomorphic, as in Cabℓ$^{+}$ℓ$^{-}$. Molecules containing such atoms are achiral, but interchange of two ligands gives rise to diastereomers. The descriptors for pseudoasymmetric atoms are *r* and *s*'.

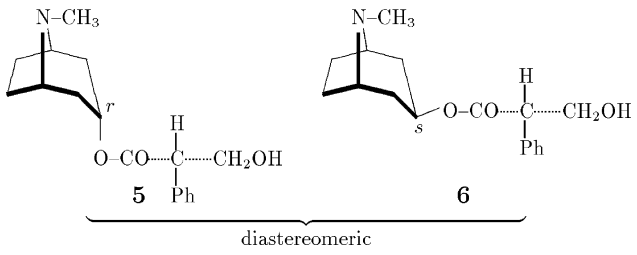

Figure 2. Diastereomeric chiral hyoscyamines (**5** and **6**). The *r* for the central atom of **5** and the *s* for the central atom of **6** are designated according to the IUPAC 1996 rule. However, they are designated by the uppercase letters (*R* and *S*) according to the present article.

Since the definitions enumerated above (though they are summed up into Def. 1 and Def. 2) are based on highly abstruse arguments by disputatious experts, the reader would yield to a sense of frustration and confusion, as remarked by Mislow when he closed his discussion on the term 'pseudoasymmetry' in his review entitled 'Stereo-Stereochemical Terminology and Its Discontents'.[1] For the sake of convenience, we call this situation 'the pseudoasymmetry problem' and we refer to the two mutually incompatible definitions (Def. 1 and Def. 2) as 'extended pseudoasymmetry' and '(proper) pseudoasymmetry', respectively.

2.2. Targets of the present paper

By the inspection of the short history concerning the term 'pseudoasymmetry', the confusing changes of connotation can be ascribed to a longstanding problem of what the *RS*-nomenclature specifies, 'chirality' or 'stereogenicity'. As shown by the term 'pseudoasymmetric stereogenic units' in Prelog–Helmchen's revision (Item 6),[4] the

RS-nomenclature is now concluded to be concerned with stereogenicity, but not with chirality. However, this point has not been fully clarified because of the lack of a logical framework for discriminating between chirality and stereogenicity. The following items bringing the confusing changes of connotation provide us with the targets of the present paper:

(9) The seemingly endless change in the connotation of the term stems from an accumulation of definition terms whose correct meanings are not always shared by all of the organic chemists. In particular, the term 'diastereomeric' has not been definitely specified insomuch as diastereomers are defined as stereoisomers that are not enantiomers. As a result, in contrast to the definite term 'enantiomer', the term 'diastereomer' connotes several kinds of stereoisomers (e.g. *RS*-diastereomers concerning tetrahedral molecules, polychiral diastereomers such as aldo/keto-hexoses, *endo*/*exo*-isomers such as hyoscyamine (**5**/**6**), and *cis*/*trans*- or *E*/*Z*-isomers concerning cycloalkanes and alkenes).

The concept of *holantimers*, which we have recently proposed to specify the meaning of diastereomers[5] will be applied to the solution of the pseudoasymmetry problem.

(10) There have frequently occurred unconscious misunderstandings because each definition has been described by altered sentences. For example, the term 'enantiomorphic' described in Item 3 has been correctly used to be related to an 'enantiotopic' relationship in an achiral molecule. However, Hirschmann and Hanson[8] have criticized this usage by stating that 'the enantiomorphic ligands of the pseudoasymmetric atom *must always* occupy enantiotopic positions [emphasis added]' as a citation from Prelog–Helmchen's paper (Item 3). The words *must always* were added by Hirschmann and Hanson[8] to reinforce their own standpoint; this addition has obviously altered the original meaning of Prelog–Helmchen's phrase containing the term 'enantiomorphic', because the connotation of the term 'pseudoasymmetric atom' was changed from Prelog–Helmchen's definition (Def. 2, Item 3)[7] into Hirschmann–Hanson's one (Def. 1, Item 4).[8,10] This misunderstanding is confirmed by the fact that the term 'enantiomorphic' described in Item 6 has been correctly used to be related to a 'heterotopic' relationship in a chiral molecule.

To avoid such arbitrary additions or alterations, we will use a *stereoisogram* as an illustrative and definite diagram for representing an enantiomeric relationship and a holantimeric relationship. This stereoisogram results in another definite relationship, i.e. *RS*-diastereomeric relationship, which is useful to discuss stereogenicity (strictly speaking, *RS*-stereogenicity) separately from chirality. The three definite relationships will be applied to the solution of the pseudoasymmetry problem.

(11) The revised *RS*-nomenclature by Prelog and Helmchen[4] has specified 'tetrahedral stereogenic units' (chirality centers, chirality planes, and chirality axes) and 'pseudoasymmetric stereogenic units' (pseudoasymmetric centers, pseudoasymmetric planes, and pseudoasymmetric axes). The 'pseudoasymmetric stereogenic units' may exist in achiral and chiral molecules. In contrast, Eliel–Wilen's textbook[2] has defined 'stereogenic element' as 'a focus of stereoisomerism (stereogenic center, axis, or plane) in a molecule such that interchange of two ligands attached to an atom in such a molecule (e.g. a and b in a tetrahedral atom Cabcd or in an alkene abC=Cab or in an allene species abC=C=Cab) leads to a stereoisomer. If the element is chirotopic, for example, if it occurs in a chiral molecule, one speaks of a chiral center, axis, or plane, but if the element is achirotopic as in abC=Cab these terms are appropriate, yet the center, axis, or plane is stereogenic.' Thus, the term corresponding to 'pseudoasymmetric stereogenic units' (as the revised *RS*-nomenclature) is not described in Eliel–Wilen's textbook, which only refers to the lowercase descriptor *r* or *s* for a pseudoasymmetric atom. Thus, the difference of attitudes toward the term 'pseudoasymmetry' tends to be hidden consciously or unconsciously without logical rationalization.

Hence, it is another target of the present paper to show what happens as a result of the difference between Prelog–Helmchen's methodology and Eliel–Wilen's methodology. This difference can be clarified to be a critical one by the concepts of holantimer and stereoisogram.

(12) A mathematical procedure for enumerating such isomers as **1** and **2** has been reported, where pseudoasymmetric cases have been taken into consideration.[11] Combinatorial enumerations of similar nonrigid isomers with rotatable ligands have been investigated by the USCI (unit-subduced-cycle-index) approach.[12,13] However, a non-mathematical approach is desirable in order to reach an intuitive comprehension of such pseudoasymmetric phenomena. This is a further target of the present paper.

3. Holantimers and stereoisograms

3.1. Holantimers, enantiomers, and *RS*-diastereomers

Let us consider the molecule **3**, where we place A=H, p=*R*-CH(OH)COOH, $\bar{p}$=*S*-CH(OH)COOH, q=*R*-CH_2-$CH(CH_3)C_2H_5$, and $\bar{q}$=*S*-$CH_2CH(CH_3)C_2H_5$.

Then we obtain **7** as a promolecule in which the symbol A represents an achiral proligand, the symbols p, $\bar{p}$, q, and $\bar{q}$ are chiral proligands, as shown in Fig. 3. Although we should use the term 'proligand' (or protoligand) that means a structureless ligand with chirality/achirality,[14] the term 'ligand' is used as long as it causes no confusion.

The corresponding *holantimer* **8** is obtained when each chiral ligand is replaced by its enantiomeric ligand, each achiral ligand is replaced by itself, and the skeleton is fixed. This operation is represented by the numbering of vertices of the tetrahedral skeleton. Thus, the original numbers 1 to 4

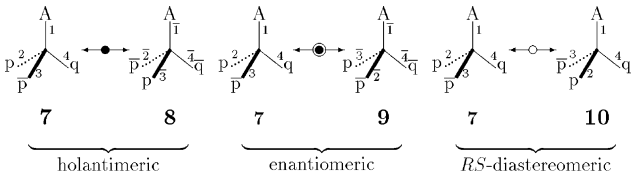

Figure 3. Holantimeric (⟷●⟷), enantiomeric (⟷◉⟷), and *RS*-diastereomeric (⟷○⟷) relationships for tetrahedral derivatives with Ap p̄q (and Ap$\overline{pq}$). The symbol A represents an achiral ligand in isolation, while the symbols p and p̄ (or q and q̄) represent a pair of enantiomeric ligands in isolation. Each number with an overbar shows that a ligand at this position has an opposite chirality. The diastereomeric (*RS*-diastereomeric) pair of **7** and **10** corresponds to the pair of **3** and **4**, where we place A=H, p=*R*-CH(OH)COOH, p̄=*S*-CH(OH)COOH, q=*R*-$CH_2CH(CH_3)C_2H_5$, and q̄=*S*-$CH_2CH(CH_3)C_2H_5$.

of the pair of molecules, which are linked with an underbrace in the left of Fig. 3, are maintained with respect to their locations but altered into $\bar{1}$ to $\bar{4}$. Note that each overbar represents the change of chirality of the ligand located and that achiral ligands are presumed to be changed into themselves under this operation.

On the other hand, the corresponding *enantiomer* **9** is obtained when each chiral ligand is replaced by its enantiomeric ligand, each achiral ligand is replaced by itself, and the skeleton is reflected. This operation is represented by the numbering for the vertices of the pair of molecules shown in the center of Fig. 3.

By constructing the holantimer of the enantiomer **9** or by constructing the enantiomer of the holantimer **8**, we obtain **10** shown as the rightmost pair in Fig. 3. The resulting molecule **10** is called *RS-diastereomer*. Note that the *RS*-diastereomer corresponds to **4** shown in Fig. 1. The *RS*-diastereomer defined in either of the operations, which result in the identical molecule, is a special case of usual diastereomers. The definition via a holantimeric relationship provides an *RS*-diastereomer as a distinct molecule, whereas such usual diastereomers connote several kinds of stereoisomers.

3.2. Stereoisograms of five types

The original molecule **7** and the three stereoisomers, i.e. the holantimer (**8**), the enantiomer (**9**), and the *RS*-diastereomer (**10**) are aligned as shown in Fig. 4, which is called *stereoisogram*. Since the four molecules contained in the stereoisogram shown in Fig. 4 are different from each other, they are linked by double-headed arrows modified by the symbols designating respective relationships. This type of stereoisograms is called *stereoisograms of Type III*.

As found easily, there exist three pairs of relationships in a stereoisogram. The horizontal double-headed arrows (⟷○⟷) represent *RS*-diastereomeric relationships (e.g. **7/10** and **9/8**), which are special cases of usual diastereomeric relationships. Hence, the horizontal axis, which is related to *RS*-stereogenicity as a special case of stereogenicity, is called *S*-axis (S: stereogenicity). The open circle on the double-headed arrow for each *RS*-diastereomeric relationship means that the skeleton is reflected.

The vertical double-headed arrows (⟷◉⟷) represent

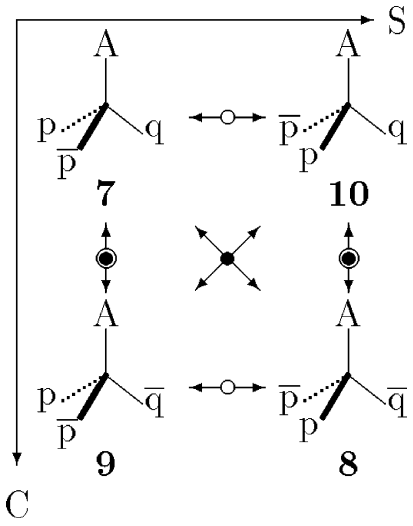

Figure 4. Stereoisogram of stereogenicity type III for a tetrahedral molecule. This stereoisogram corresponds to the diastereomeric pair of **3** and **4**, where we place A=H, p=*R*-CH(OH)COOH, p̄=*S*-CH(OH)COOH, q=*R*-$CH_2CH(CH_3)C_2H_5$, and q̄=*S*-$CH_2CH(CH_3)C_2H_5$.

enantiomeric relationships (e.g. **7/9** and **10/8**). Hence, the vertical axis is called *C*-axis (C: chirality). The encircled solid circle on the double-headed arrow for each enantiomeric relationship means that the skeleton is reflected and the chirality of each ligand is changed.

The diagonal double-headed arrows (⟷●⟷) represent holantimeric relationships (e.g. **7/8** and **9/10**). The solid circle on the double-headed arrow for each holantimeric relationship means that the chirality of each ligand is changed.

Similarly, we obtain Fig. 5 as a stereoisogram for characterizing **1** and **2**, where we place A=H, B=OH, p=*R*-CH(OH)COOH, and p̄=*S*−CH(OH)COOH. In this case, the enantiomer at the bottom left of Fig. 5 is identical with the original molecule **11** and the holantimer at the

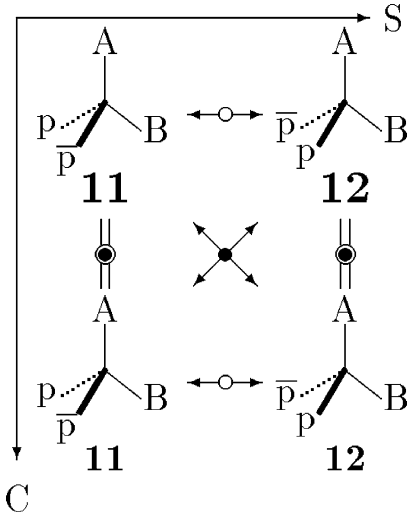

Figure 5. Stereoisogram of stereogenicity type V for a tetrahedral molecule. This stereoisogram corresponds to the diastereomeric pair of **1** and **2**, where we place A=H, B=OH, p=*R*-CH(OH)COOH, and p̄=*S*-CH(OH)COOH.

bottom right of Fig. 5 is identical with the *RS*-diastereomer **12** so that the vertical relationships are represented by equality symbols modified with the symbol (⊙). It follows that the molecules at issue are achiral, where an operation due to an enantiomeric relationship generate homomers. This type of stereoisograms is called *stereoisograms of Type V*. It should be noted that an achiral molecule is regarded as being *self-enantiomeric*, where the term 'homomer' is used to designate two or more identical molecules even if their numbering modes of vertices (cf. Fig. 3) are altered.

Tetrahedral molecules with various substitution patterns have been exhaustively enumerated with respect to their molecular formulas and to their point-group symmetries.[14,15] By means of the concepts of holantimers and stereoisograms, all of the enumerated tetrahedral molecules are examined so as to reveal that their stereoisograms can be classified into five types (Types I to V) shown in Fig. 6, which have been called *stereogenicity types* in a previous paper.[5] Although the reported figure has been concerned only with tetrahedral molcecules,[5] the existence of five types of stereoisograms can be proved generally in any left–right distinguishable skeletons. Hence, Fig. 6 is a modified diagram, which covers all molecules derived from any left–right distinguishable skeleton, where the symbols **A** and $\bar{\mathbf{A}}$ (or **B** and $\bar{\mathbf{B}}$) represent a pair of enantiomers, which are derived from the skeleton.

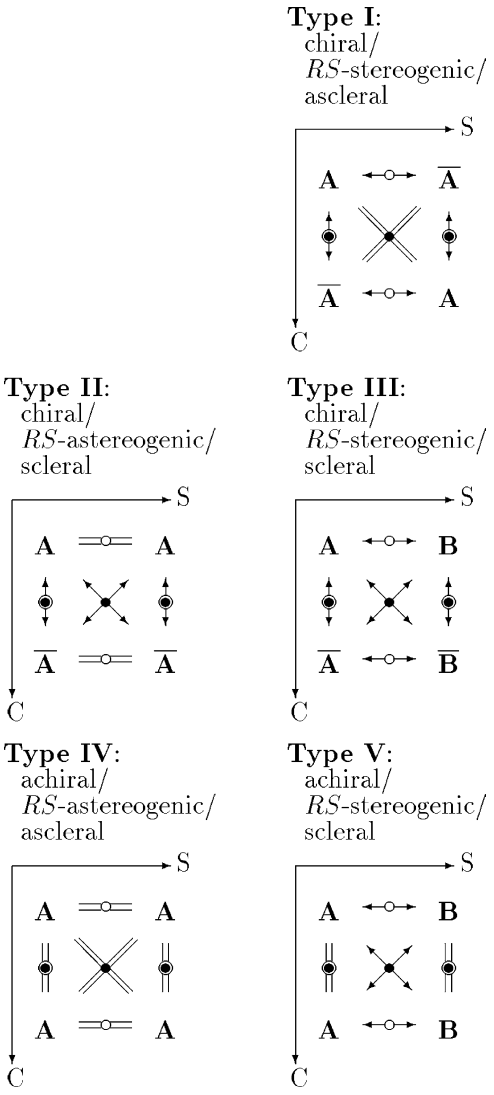

Figure 6. Stereoisograms of five types. The figure reported[5] has been modified to cover all molecules derived from a left–right distinguishable skeleton. The symbols **A** (or **B**) and $\bar{\mathbf{A}}$ (or $\bar{\mathbf{B}}$) represent a pair of enantiomers.

4. So-called 'pseudoasymmetry'

4.1. Stereogenicity type V

So-called pseudoasymmetric atoms are contained in molecules of stereogenicity type V, which represent achiral/*RS*-stereogenic cases, as shown in Fig. 5. The two achiral molecules (**11** and **12**) are *RS*-diastereomeric to each other. Thus, Type V corresponds to the pseudoasymmetry of Def. 2, which is described in Item 1 and in Item 5. If the priority of ligands is determined to be $A > B > p > \bar{p}$, **11** is concluded to have *s*-configuration, while **12** is concluded to have *r*-configuration. The lowercase letters are used because the atoms are concerned with the pseudoasymmetry (Def. 2).

The stereogenicity type V is determined by the modes of three relationships: vertical equality symbols modified with the symbol ⊙ (achiral, i.e. homomeric under the enantiomeric relationship), horizontal double-headed arrows modified with the symbol ○ (*RS*-stereogenic pair), and diagonal double-headed arrows modified with the symbol ● (holantimeric pair).

It should be emphasized that the determination of the type V is based on the modes of the three relationships appearing in the stereoisogram. In other words, the determination does not require any terms concerning properties in molecule (e.g. enantiotopic or achirotopic), which are in turn used in Items 3 and 8. On the contrary, such terms as concerning properties in molecule (e.g. enantiotopic or achirotopic) can be derived from the structure of **11** and **12**. According to the recent scheme reported by us,[16] the sphericity terms are more convenient to discuss such in-molecule properties. This will be discussed later in the present paper.

4.2. Stereogenicity type III

4.2.1. Extended pseudoasymmetric cases of type III. Let us next examine the stereoisogram shown in Fig. 4, which is an example of stereogenicity type III. According to Item 2, this case corresponds to the extended pseudoasymmetry of Def. 1. If the priority of ligands is determined to be $A > p > \bar{p} > q$, the molecule **7** is concluded to have *S*-configuration, while the molecule **10** is concluded to have *R*-configuration. On the other hand, the priority described above also brings the priority $A > p > \bar{p} > \bar{q}$, the molecule **9** is concluded to have *S*-configuration, while the molecule **8** is concluded to have *R*-configuration. Comparison between **7** and **9** (or between **10** and **8**) shows such invariance of the descriptors on reflection as described in Item 6 and Item 7, so that they exhibit extended pseudoasymmetry due to Def. 1. Here, we use the uppercase letters, although they should be replaced by lowercase letters if we obey Def. 1.

Thus, the determination of extended pseudoasymmetry relies on the fact that the enantiomers **7** and **9** (or **10** and **8**) provide the same *S*-configuration (or *R*-configuration). If the *RS*-nomenclature is concerned with chirality (along *C*-axis), such enantiomers should give opposite descriptors. This means that the *RS*-nomenclature is not concerned with chirality (along *C*-axis). In fact, the *RS*-nomenclature is based on the *RS*-stereogenicity described by *S*-axis (i.e. the comparison between **7** and **10**) shown in Fig. 4.

According to Def. 1, **13** shown in Fig. 7 has an extended pseudoasymmetric atom. By considering its holantimer **16**, we can obtain the corresponding stereoisogram (Fig. 7), which is another example of stereogenicity type III. According to Items 2, 6, and 7, this case corresponds to an extended pseudoasymmetry of Def. 1. If the priority of ligands is determined to be $p > \bar{p} > q > r$, **13** is concluded to have *R*-configuration, while **14** is concluded to have *S*-configuration. Because the priority described above also brings the priority $p > \bar{p} > \bar{q} > \bar{r}$, the molecule **15** is concluded to have *R*-configuration, while the molecule **16** is concluded to have *S*-configuration. Comparison between **13** and **15** (or between **14** and **16**) shows the invariance of the descriptors on reflection, which is described in Item 6 and Item 7. As a result, they exhibit extended pseudoasymmetry due to Def. 1. Here, we use the uppercase letters, although they should be replaced by lowercase letters if we obey Def. 1.

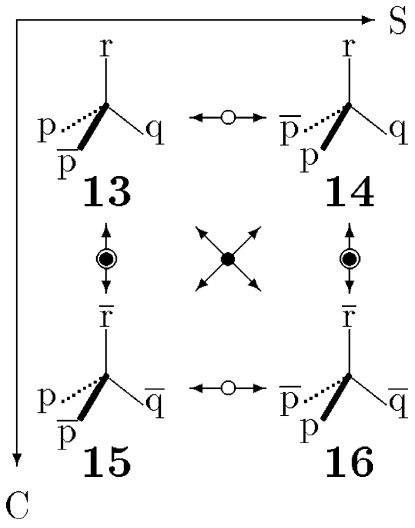

Figure 7. Stereoisogram of stereogenicity type III for a tetrahedral molecule. All of the ligands are chiral, where each pair of p/p̄, q/q̄, or r/r̄ represents a pair of enantiomeric ligands in isolation.

The stereogenicity types of Figs. 4 and 7 are determined to be Type III by means of the modes of three relationships: vertical double-headed symbols modified with the symbol ⊙ (enantiomeric pair), horizontal double-headed arrows modified with the symbol ○ (*RS*-stereogenic pair), and diagonal double-headed arrows modified with the symbol ● (holantimeric pair). It should be noted that the determination does not require any terms concerning properties in molecule (e.g. enantiotopic or achirotopic), which are used in Items 3 and 8.

4.2.2. General cases of type III. The stereoisogram of Type III shown in Fig. 8 illustrates the three relationships for a molecule (**17**) with ligands A, B, C, and p (or p̄): vertical double-headed arrows modified with the symbol ⊙ (enantiomeric pair), horizontal double-headed arrows modified with the symbol ○ (*RS*-stereogenic pair), and diagonal double-headed arrows modified with the symbol ● (holantimeric pair). If the priority of ligands is determined to be $A > B > C > p$, **17** is concluded to have *R*-configuration, while **18** is concluded to have *S*-configuration. The priority described above also brings the priority $A > B > C > \bar{p}$, so that **19** is concluded to have *S*-configuration, while **20** is concluded to have *R*-configuration.

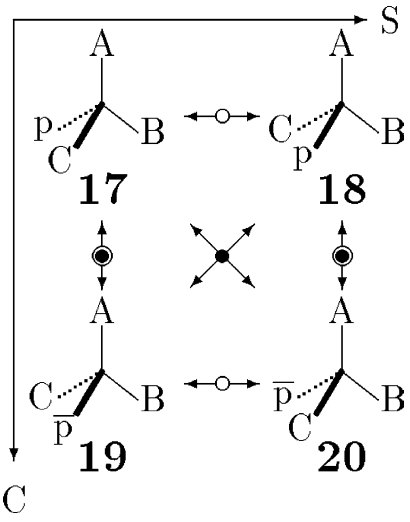

Figure 8. Stereoisogram of stereogenicity type III for a tetrahedral molecule with the ligand pattern ABCp (and ABCp̄).

It should be emphasized that the three relationships in extended pseudoasymmetric cases of Type III (e.g. Figs. 4 and 7) are equivalent to those in general cases of Type III (e.g. the stereoisogram of Type III shown in Fig. 6).

4.3. Sphericities of ligand orbits

Let us next consider in-molecule properties of each molecule, which has been described above as an example. The resulting properties will be combined with the results derived from stereoisograms so as to comprehend the pseudoasymmetry problem.

In **11** or **12** (Fig. 5) referred to as a 'pseudoasymmetric' case (Type V), the four ligands A, B, p, and p̄ are classified into three equivalence classes (orbits);[16–18] namely, a pair of enantiomeric ligands p and p̄ in **11** or **12** constructs a two-membered enantiospheric orbit, ligand A belongs to a one-membered homospheric orbit, and ligand B belongs to another one-membered homospheric orbit. Inasmuch as the ligands p and p̄ belong to the same orbit, they are equivalent to each other, whereas they are regarded as being different (nonequivalent) from the viewpoint of stereogenicity (i.e. the naming due to the *RS*-nomenclature).

In **7** or **10** (Fig. 4) referred to as an '(extended) pseudoasymmetric' case of Type III, on the other hand, the four ligands A, p, p̄, and q are classified into four

equivalence classes (orbits),[16–18] i.e. a one-membered hemispheric orbit containing A, a one-membered hemispheric orbit containing p, a one-membered hemispheric orbit containing $\bar{p}$, and a one-membered hemispheric orbit containing q. On the same line, the four ligands A, p, $\bar{p}$, and $\bar{q}$ contained in **9** or **8** (Fig. 4) respectively belong to one-membered hemispheric orbits. The ligands p and $\bar{p}$ are different (nonequivalent) from the viewpoint of chirality (or geometry) as well as from the viewpoint of stereogenicity, i.e. from the viewpoint of the naming due to the *RS*-nomenclature.

In **17** or **18** (Fig. 8) referred to as a general case of Type III, the four ligands A, B, C, and p are classified into four equivalence classes (orbits),[17,18,16] i.e. a one-membered hemispheric orbit containing A, a one-membered hemispheric orbit containing B, a one-membered hemispheric orbit containing C, and a one-membered hemispheric orbit containing p. On the same line, the four ligands A, B, C, and $\bar{p}$ contained in **19** or **20** (Fig. 8) respectively belong to one-membered hemispheric orbits.

The discussion described here has confirmed the counter-argument by Prelog and Helmchen,[4] where Hirschmann and Hanson's criticism (Items 4 and 10)[8] has been characterized as unjustified.

4.4. Proper pseudoasymmetry

Now, we have reached a starting point to discuss so-called 'pseudoasymmetry' on a logical basis. In this subsection, we will demonstrate that 'extended pseudoasymmetry' due to Def. 1 has no reason to be adopted and that 'proper pseudoasymmetry' due to Def. 2 is ascribed to Type V.

4.4.1. Extended pseudoasymmetry vs. proper pseudoasymmetry. Let us discuss whether there exist reasons or not in summing up extended pseudoasymmetric cases and proper pseudoasymmetric cases into one category by virtue of Def. 1.

The term '(proper) pseudoasymmetry' stems from the presumptions: (1) that A, B, p, and $\bar{p}$ are different from each other, though p and $\bar{p}$ are enantiomeric to each other in isolation and (2) that p and $\bar{p}$ occasionally become equivalent in an achiral molecule **11** having AB$p\bar{p}$. Because p and $\bar{p}$ are different in isolation but become equivalent in molecule, it is referred to by the term 'pseudoasymmetry' as an exceptional case.

In contrast, for extended pseudoasymmetric cases such as **7**, the four ligands A, p, $\bar{p}$, and q are different in molecule; and, at the same time, they are different in isolation. The different ligands (A, p, $\bar{p}$, and q) in isolation still remain different (nonequivalent) in molecule so that this case is by no means an exceptional case that is to be differentiated from general cases (e.g. **17**). In other words, the standpoint of Def. 1 implicitly regards the nonequivalent ligands p and $\bar{p}$ as being equivalent by using the term 'pseudoasymmetric'. Obviously, this standpoint of Def. 1 cannot be justified even by saying that 'the *r*/*s* descriptors of pseudoasymmetric carbon atoms are invariant on reflection in a mirror (i.e. *r* remains *r*, and *s* remains *s*)' (Item 7).

The conclusion described in the preceding paragraph can be confirmed by the examination of stereoisograms. Thus, the stereoisograms of extended pseudoasymmetric cases such as Figs. 4 and 7 belong to Type III: the three relationships (enantiomeric, holantimeric and *RS*-diastereomeric relationships) are characterized by double-headed arrows. On the other hand, the stereoisograms of proper pseudoasymmetric cases such as Fig. 5 belong to Type V: the enantiomeric relationships are represented by equality symbols and the other two relationships are characterized by double-headed arrows.

Chemically speaking, p and $\bar{p}$ contained in (proper) pseudoasymmetric cases such as **11** can be differentiated only by the stereoselective attack of chiral reagents, but not by the attack of achiral reagents. In contrast, p and $\bar{p}$ in extended pseudoasymmetric cases such as **7** can be differentiated by the attack of achiral (and chiral) reagents. This chemically important feature is concealed by summing up (proper) pseudoasymmetric cases (e.g. **11**) and extended pseudoasymmetric cases (e.g. **7**) into the same category 'pseudoasymmetry' by Def. 1.

4.4.2. Extended pseudoasymmetric cases of type III vs. general cases of type III. Let us next discuss whether there exist reasons or not in differentiating extended pseudoasymmetric cases of Type III from general cases of Type III.

As shown above, the different ligands of an extended pseudoasymmetric case (e.g. A, p, $\bar{p}$, and q in **7**) in isolation still remain different (nonequivalent) in molecule. This situation holds true for the different ligands of a general case (e.g. A, B, C, and p in **17**). Hence, such an exceptional picking-up of p and $\bar{p}$ by the term 'pseudoasymmetry' has no reason.

Chemically speaking, p and $\bar{p}$ in extended pseudoasymmetric cases (e.g. **7**) can be differentiated by the attack of achiral reagents as well as by the attack of chiral reagents. On the same line, the ligands A, B, C, and p in general cases of Type III (e.g. **17**) can be differentiated by the attack of achiral reagents as well as by the attack of chiral reagents. It follows that there is no reason to differentiate the extended pseudoasymmetric cases of Type III (e.g. **7**) from the general cases of Type III (e.g. **17**).

From the viewpoint based on the concept of stereoisogram, the question is whether the stereoisograms of Type III shown in Figs. 4 and 7 are permitted to be treated separately from such stereoisograms of Type III as shown in Fig. 8. As found easily, the stereoisograms of extended pseudoasymmetric cases such as Figs. 4 and 7 belong to Type III, which has the same features as those of general cases of Type III (e.g. Type III of Fig. 8): the three relationships (enantiomeric, holantimeric and *RS*-diastereomeric relationships) are characterized by double-headed arrows. According to the concept of stereoisogram, there is also no reason to differentiate the extended pseudoasymmetric cases of Type III (e.g. **7** and **13**) from the general cases of Type III (e.g. **17**).

5. Chirality, *RS*-stereogenicity, and sclerality

5.1. Stereogenicity type I

Let us examine the stereoisogram of Type I shown in Fig. 9 (a special case of the top diagram of Fig. 6), which is characterized by means of the modes of three relationships: vertical double-headed arrows modified with the symbol ⊙ (enantiomeric pair), horizontal double-headed arrows modified with the symbol ○ (*RS*-stereogenic pair), and diagonal equality symbols modified with the symbol ● (homomeric for the holantimeric relationship; i.e. self-holantimeric). The term 'self-holantimeric' represents the asclerality of the molecule **21**, as discussed in the next paragraph.

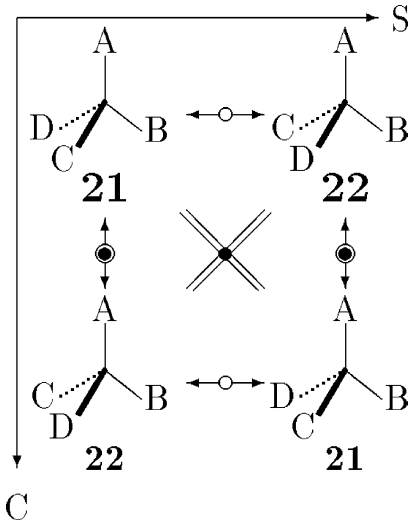

Figure 9. Stereoisogram of stereogenicity type I for a tetrahedral molecule with the ligand pattern ABCD.

When we describe the feature of the stereoisogram of Type I, we become aware of unbalance in the traditional terminology. A vertical double-headed arrow and a vertical equality symbol, both of which are modified with the symbol ⊙, correspond to the pair of terms chiral/achiral; a horizontal double-headed arrow and a horizontal equality symbol, both of which are modified with the symbol ○, correspond to the pair of terms *RS*-stereogenic/*RS*-astereogenic. However, there are no terms for designating a diagonal double-headed arrow and a diagonal equality symbol. To designate the property of giving a non-superimposable holantimer, we coin the term 'scleral', since the skeleton is fixed. Thereby, a pair of the terms scleral/ascleral can be derived. According to this terminology, a diagonal double-headed arrow and a diagonal equality symbol, both of which are modified with the symbol ●, correspond to the pair of terms scleral/ascleral. Thereby, the Type I of Fig. 6 (e.g. concretely Fig. 9) is referred to as chiral/*RS*-stereogenic/ascleral.

5.2. Stereogenicity types as stereogenic units

As described in Item 11, the revised *RS*-nomenclature by Prelog and Helmchen[4] supports Def. 1 and adopts the terms 'tetrahedral stereogenic units' and 'pseudoasymmetric stereogenic units'. Eliel–Wilen's textbook[2] supports Def. 2 but seems to adopt the *RS*-nomenclature for so-called 'pseudoasymmetric' cases of Def. 1. Probably because the adoption Def. 2 is incompatible to the partial adoption of Def. 1, Eliel–Wilen's textbook does not refer to 'pseudoasymmetric stereogenic units', where the terms 'stereogenic units (elements)' and 'chirality units (elements)' are described. The IUPAC 1996 rule[3] makes light of 'pseudoasymmetric carbon atom' as the traditional name, but substantially adopts the revised *RS*-nomenclature and Def. 1, whereas it seems not to adopt 'pseudoasymmetric units' as stereogenic units, as found Item 7. The subtle difference between their attitudes toward the term 'pseudoasymmetry' has arisen from the fact that Def. 1 is closely linked with the revised *RS*-nomenclature, which is regarded as a standard for stereochemical nomenclature.

The discussions described in the preceding sections enable us to recognize stereogenicity types I, III, and V (e.g. Fig. 6) as a set of *RS*-stereogenic units: chiral/ascleral *RS*-stereogenic unit (Type I unit), chiral/scleral *RS*-stereogenic unit (Type III unit), and achiral/scleral *RS*-stereogenic unit or simply achiral *RS*-stereogenic (Type V unit).

A chiral/ascleral *RS*-stereogenic unit (Type I) represents a case in which the enantiomeric relationship is occasionally superposed onto the *RS*-diastereomeric relationship because the holantimeric relationship produces homomers (i.e. ascleral).

A chiral/scleral *RS*-stereogenic unit (Type III) represents a case in which all of the three pairs of relationships produce different (nonequivalent) molecules. Although two modes of stereoisograms of Type III (e.g. Figs. 4 and 7) can be specified by Def. 1 as 'extended pseudoasymmetric' cases, they should not be separated from Type III, as discussed above in detail.

An achiral/scleral *RS*-stereogenic unit (Type V) represents a case in which the holantimeric relationship is occasionally superposed onto the *RS*-diastereomeric relationship because the enantiomeric relationship produces homomers (i.e. achiral). Although this case corresponds to the term 'pseudoasymmetry', the present definition of Type V is based on such a stereoisogram as illustrated in Fig. 5.

Concretely speaking, the lowercase descriptors *r* and *s* for 'extended pseudoasymmetry' (Def. 1) should be replaced by the uppercase descriptors *R* and *S*, which correspond to Type III described in the present paper. As a result, the use of *r* and *s* should be restricted to designate (proper) pseudoasymmetry (Def. 2), which corresponds to Type V described in the present paper.

5.3. Conceptual revolution

The concepts of holantimer and stereoisogram force us to encounter a conceptual revolution with respect to the dichotomy between enantiomers and diastereomers.

Diastereomers have been traditionally defined as stereoisomers that are not enantiomers. The dichotomy between enantiomers and diastereomers is oversimplified because

there exist several kinds of diastereomers. This over-simplification, however, is understandable and may be treated also within the traditional terminology of stereochemistry, even though the present approach can accomplish this task more comprehensively on the basis of the concepts of holantimer and stereoisogram.

In contrast, stereoisograms of Type I (e.g. Fig. 2 as a special case of the top diagram in Fig. 9) bring about a conceptual revolution which cannot be treated within the traditional terminology: *In a Type I stereoisogram, the diastereomeric relationships (strictly speaking RS-diastereomeric relationships) are superposed onto the enantiomeric relationships*. Obviously, this viewpoint is a counterargument to the traditional definition of diastereomers. In other words, the traditional stereochemistry has never recognized the asclerality appearing in the stereoisograms of Type I. The viewpoint becomes possible only when a holantimeric relationship is comprehended to be capable of generating homomers (e.g. the homomers of **A** (or $\bar{\mathbf{A}}$) represented by the equality symbol of the holantimeric relationship) after proposing the concepts of holantimer and stereoisogram.

The traditional way of stereochemistry has taken an unbalanced view of the three relationships appearing in a stereoisogram. For example, as for cases of the stereoisogram of Type I shown in Fig. 6, the traditional way has focused attention on the vertical relationship (*C*-axis) between **A** and $\bar{\mathbf{A}}$. But it does not refer to the horizontal relationship (*S*-axis), because of the lack of such logical frameworks as stereoisograms. On the other hand, as for cases of the stereoisogram of Type V shown in Fig. 6, it has focused attention on the horizontal relationship (*S*-axis) between **A** and **B** but not on the vertical relationship (*C*-axis).

The traditional way is more confusing for cases of the stereoisogram of Type III shown in Fig. 6 so that the attention to the horizontal relationship (*S*-axis) between **17** and **18** is unconsciously mixed up with the attention to the vertical relationship (*C*-axis) between **17** and **19**. In fact, the discussions on 'extended pseudoasymmetry' have pointed out this type of confusion. Obviously, a shift of viewpoint occurs between the *S*-axis and the *C*-axis, although they are conceptually distinct from each other.

Such shifts of viewpoint, which have not been ever revealed by the traditional terminology of stereochemistry, have caused confusion concerning the term 'pseudoasymmetry' and the *RS*-nomenclature. By the inspection of the stereoisograms of Types I, III, and V (Fig. 6), the *RS*-nomenclature is concluded to be based on the *RS*-stereogenicity (*S*-axis) contained in the Type I, III, or V stereoisogram. Even in the Type I stereoisogram, the *RS*-nomenclature specifies the *RS*-diastereomeric relationship that is superposed onto the enantiomeric relationship.

If one intends to maintain the traditional dichotomy between enantiomers and diastereomers, he/she would be forced to take an unbalanced or inconsistent way in which the *RS*-nomenclature for Type I molecules is based on the *C*-axis, while the *RS*-nomenclature for Type III or Type V molecules is based on the *S*-axis. Hence, the traditional dichotomy should be modified as a result of the conceptual revolution.

6. *RS*-stereogenicity and other stereogenicities

The concepts of holantimer and stereoisogram are useful to test the acceptability of the *RS*-nomenclature (cf. Item 9). Let us consider hyoscyamine (**5**) as an example (Fig. 2). This compound can be regarded as a special case of Type III (cf. Fig. 4), where the ring is conceptually opened to give a hypothetical pair of enantiomeric ligands (p and $\bar{\mathrm{p}}$). This conceptual ring opening (factorization) is a basis of the *RS*-nomenclature described in Fig. 2.

The present approach allows us to treat hyoscyamine (**5**) from an alternative viewpoint, where the molecule can be regarded as a bicyclic skeleton with a chiral ligand p. When we consider the bicyclic skeleton as a kind of stereogenic unit, we obtain a stereoisogram shown in Fig. 9. This stereoisogram is concluded to be Type II, which is chiral and *RS*-astereogenic. It follows that hyoscyamine is desirable not to be characterized in terms of the *RS*-nomenclature. Obviously, hyoscyamine (**5**) is determined to be an *endo*-isomer, even if the ligand p is replaced by a hydroxyl group. Thus, the relationship between the *endo*-isomer **5** and the corresponding *exo*-isomer **6** is diastereomeric, but not *RS*-diastereomeric.

The case shown in Fig. 10 gives us a problem of factorization depth. When the *RS*-nomenclature is applied to hyoscyamine (**23**), it requires a conceptual ring-opening to determine *R*-configuration, as described above. Thereby, the naming of **5** is ascribed to that of **7** or **10**. On the other hand, the alternative approach shown in Fig. 10 does not contain such a conceptual ring-opening.

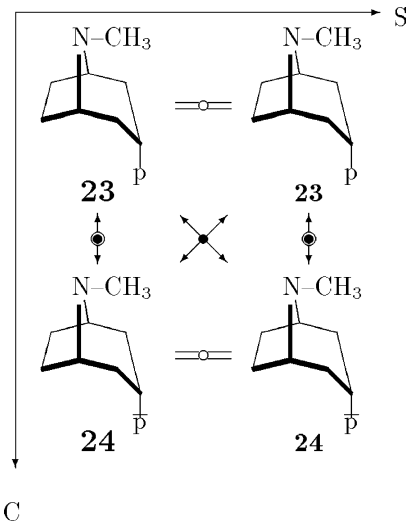

Figure 10. Stereoisogram of stereogenicity type II for hyoscyamines. The bicyclic skeleton is regarded as an *RS*-stereogenic unit.

The fact that such alternative viewpoints are possible is by no means the drawback of the present approach, because such alternative viewpoints may be generated by using any other approaches. It should be emphasized that the present

approach is capable of formulating the alternative viewpoints through a common framework based on the concepts of holantimer and stereoisogram.

It should be noted that the *RS*-astereogenicity derived from the alternative approach is different from the usual stereogenicity. The difference is open to further investigations in order especially to clarify *E*/*Z*-stereoisomerism about a cycloalkane ring as well as about a double bond.

7. Conclusion

The concepts of holantimer and stereoisogram are applied to determine the scope of so-called pseudoasymmetry, which has been specified by two mutually incompatible definitions. These concepts require the separation of *RS*-stereogenicity from stereogenicity in order to complete definite discussions. Each stereoisogram contains three relationships, the first two of which specify the well-known enantiomeric relationship related to chiral/achiral and the newly-specified *RS*-diastereomeric relationship related to *RS*-stereogenic/*RS*-astereogenic. The remaining third relationship is a holantimeric relationship, which is related to scleral/ascleral coined now to keep balanced terminology. In terms of the three relationships, stereoisograms are classified into five types (Types I–V), among which Type I, III, and V are selected as a set of *RS*-stereogenic units: chiral/ascleral *RS*-stereogenic unit (or Type I unit), chiral/scleral *RS*-stereogenic unit (or Type III unit), and achiral/scleral *RS*-stereogenic unit (or Type V unit). Thereby, the term 'pseudoasymmetric stereogenic units' should be replaced by the term 'achiral/scleral *RS*-stereogenic units' or 'Type V units'.

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10. Footnote i in Mislow's paper[1] has described that the two –CH(OH)COOH (in an achiral 2,3,4-trihydroxyglutaric acid) are not 'enantiomorphic' according to Prelog and Helmchen,[7] whereas they are 'enantiomorphic' according to Hirschmann and Hanson.[8] However, Prelog and Helmchen[7] have also regarded them as being 'enantiomorphic' if the term 'enantiomorphic ligands' means 'enantiomeric ligands in isolation'. Strictly speaking, the term 'the enantiomorphic ligands of the pseudoasymmetric atom' has different meanings because the term 'pseudoasymmetric atom' connotes different meanings in Def. 2[7] and in Def. 1.[8] Thus, 'the enantiomorphic ligands of the pseudoasymmetric atom' according to Def. 2[7] belong to a two-membered enantiospheric orbit, whereas 'the enantiomorphic ligands of the pseudoasymmetric atom' according to Def. 1[8] belong to distinct (independent) one-membered hemispheric orbits or to a two-membered enantiospheric orbit. This statement based on the sphericity terms[16–18] is simpler and more definite than the statements of Def. 2 (e.g. enantiotopic half-spaces in Item 3) and of Def. 1 (e.g. heterotopic half-spaces in Item 6). The term 'heterotopic half-space' described in Item 3 cannot be always characterized geometrically, although the term 'enantiotopic half-space' in Item 6 can be strictly defined geometrically. Note that a heterotopic space (other than an enantiotopic space) is independent in itself, i.e. it is not one of half-spaces, while enantiotopic spaces appear pairwise to construct enantiotopic half-spaces.
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A convenient synthesis of quinolines by reactions of *o*-isocyano-β-methoxystyrenes with nucleophiles

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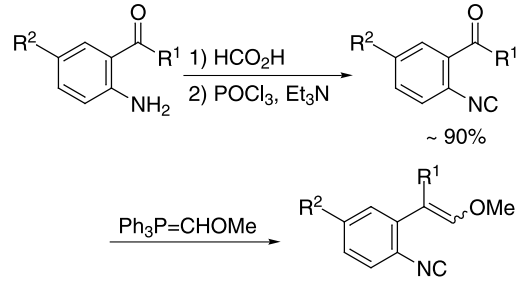
Abstract—2,4-Disubstituted quinolines have been synthesized by reactions of *o*-isocyano-β-methoxystyrenes, which can be easily prepared from commercially available *o*-aminophenyl ketones in three steps, with alkyl(or aryl)lithiums in generally good yields. Subsequently, *o*-isocyano-β-methoxystyrenes have also proved to react efficiently with lithium dialkylamides to afford the corresponding 4-substituted *N*,*N*-dialkylquinolin-2-amines in satisfactory yields.

There has been substantial interest in quinoline derivatives, because some of them are known to occur in nature,[1] and exhibit a wide variety of biological activities.[2] Moreover, they have been utilized as intermediates for the design of biologically active compounds.[3] Therefore, a large number of general methods for the preparation of substituted quinolines have recently been reported,[4] and any new general route to quinoline derivatives is of interest and value. In this paper we wish to report in full on the results of our investigation,[5,6] which offer a simple and general method for preparing 2,4-disubstituted quinolines and 4-substituted *N*,*N*-dialkylquinolin-2-amines by reactions of *o*-isocyano-β-methoxystyrenes with alkyl(or aryl)lithiums and lithium dialkylamides, respectively.[7] 2-Alkylated quinoline derivatives[8] and quinolin-2-amines[9] have been reported to exhibit notable biological activities.

1. Results and discussion

o-Isocyano-β-methoxystyrenes **1** were prepared in three steps from commercially available 2-aminophenylketones as shown in Scheme 1. Thus, formylation of 2-aminophenyl ketones with formic acid afforded the corresponding formamides, which were dehydrated by treatment with phosphoryl chloride/triethylamine to afford 2-isocyanophenyl ketones. Wittig reaction of these isocyano ketones with (methoxymethyl)triphenylphosphonium ylide gave *o*-isocyano-β-methoxystyrene derivatives **1**, as a mixture of stereoisomers in each case. The ratios of stereoisomers were determined by ^{1}H NMR spectral data (see Section 2).

The reactions of *o*-isocyano-β-methoxystyrenes **1** with aryl(or alkyl)lithiums **2** were conducted as shown in Scheme 2. Thus, after treatment of the isocyanides **1** with organolithiums **2** (1.5 equiv) at −78 °C in 1,2-dimethoxyethane (DME), the mixtures were allowed to warm to room temperature. Usual aqueous workup, followed by purification using preparative TLC on silica gel, gave 2,4-disubstituted quinolines **3**. The results summarized in Table 1 demonstrate that the good yields of the desired 2,4-disubstituted quinolines **3a**–**3i** were obtained in general (entries 1–9), though somewhat poorer yields of the desired products **3j** and **3k** were obtained by using *o*-isocyano-β-methoxy-α-methylstyrene (**1c**) (entries 10 and 11). When the reactions were conducted in THF, rather diminished



1a R^1 = Ph, R^2 = H 72% (*E*:*Z* = ~1:1)
1b R^1 = Ph, R^2 = Cl 67% (*E*:*Z* = ~1:1)
1c R^1 = Me, R^2 = H 70% (*E*:*Z* = ~7:3)

Scheme 1.

Keywords: Isocyanide; Lithium amide; Organolithium; Quinoline; Styrene.
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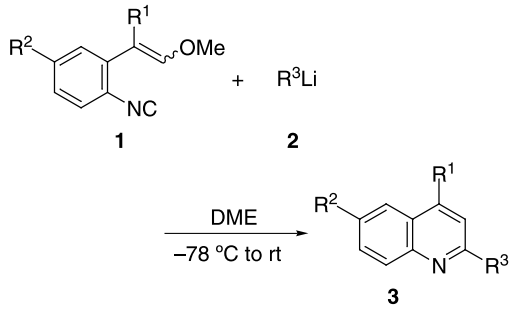


Scheme 2.

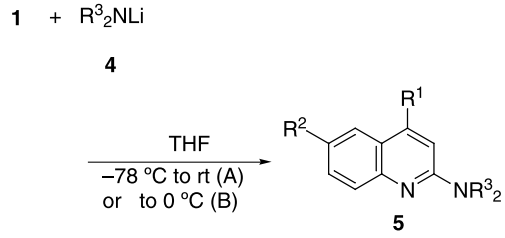
Table 1. Preparation of 2,4-disubstituted quinolines **3** according to Scheme 2

Entry	Isocyanide **1**	Organolithium **2**	**3** (Yield/%)[a]
1	**1a** (R^1=Ph, R^2=H)	**2a** (R^3=*n*-Bu)	**3a** (79)
2	**1a**	**2b** (R^3=*sec*-Bu)	**3b** (84)
3	**1a**	**2c** (R^3=*tert*-Bu)	**3c** (89)
4	**1a**	**2d** (R^3=Ph)	**3d** (91)
5	**1a**	**2e** (R^3=*p*-Tol)	**3e** (88)
6	**1a**	**2f** (R^3=2-thienyl)	**3f** (74)
7	**1a**	**2g** (R^3=2-furyl)	**3g** (75)
8	**1b** (R^1=Ph, R^2=Cl)	**2c**	**3h** (87)
9	**1b**	**2d**	**3i** (85)
10	**1c** (R^1=Me, R^2=H)	**2c**	**3j** (58)
11	**1c**	**2d**	**3k** (55)

[a] Isolated yields after purification by preparative TLC on silica gel.

yields of the desired products were obtained. For example, the reaction of **1a** with **2a** in THF under the same conditions as described above in DME led to the formation of rather complex reaction mixture and the desired product **3a** was obtained only in 41% yield. This is probably attributable to the lability of THF to organolithiums. It should be noted that the reaction of **1a** with PhMgBr gave an intractable mixture of products, from which no more than a trace amount of **3a** was obtained.

We anticipated that the use of lithium dialkylamide in place of alkyl(or aryl)lithiums would be expected to afford *N*,*N*-dialkylquinolin-2-amine derivatives, and the reactions of *o*-isocyano-β-methoxystyrenes **1** with lithium dialkylamides **4** were carried out as shown in Scheme 3. Thus, isocyanides **1** were treated with lithium amides **4** (1.2 equiv in general), generated by the treatment of secondary amines with butyllithium, in THF at −78 °C, and then the mixtures

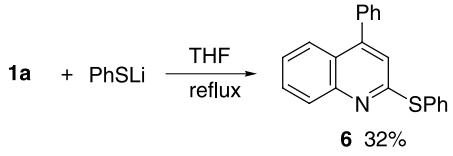


Scheme 3.

were allowed to warm to room temperature (Method A). Usual aqueous workup, followed by purification using preparative TLC on silica gel, gave 4-substituted *N*,*N*-dialkylquinolin-2-amines **5**. The results are summarized in Table 2. Two equivalents each of lithium dialkylamides generated from bulky secondary amines, such as diisopropylamine or dicyclohexylamine, were used for satisfactory production of the desired products (entries 2, 3 and 11). In order to obtain satisfactory yields of **5h** and **5i** (entries 9 and 10), the reaction temperature was raised to only 0 °C (Method B) considering the lability of chloride under the reaction conditions (entry 8). Although the reactions using *o*-isocyano-β-methoxy-α-phenylstyrenes **1a** and **1b** gave the desired quinoline-2-amines **5a**–**5i** in moderate to fair yields (entries 1–7, 9 and 10), the use of *o*-isocyano-β-methoxy-α-methylstyrene (**1c**) afforded the desired products **5j** and **5k** only in rather poor yields (entries 11 and 12). In these cases rather complex reaction mixtures including small quantities of the starting **1c** were obtained.

Subsequently, the possibility of the preparation of 2-sulfenylated quinoline derivatives was examined. Scheme 4 shows that benzenethiolate is usable as a nucleophile in the present method but under reflux conditions in THF to afford 2-phenylthio-4-phenylquinoline (**6**) in low yield. This reaction gave a rather complicated reaction mixture including a fair amount of the starting **1a**.

The production of quinoline derivatives **3**, **5**, and **6** may be

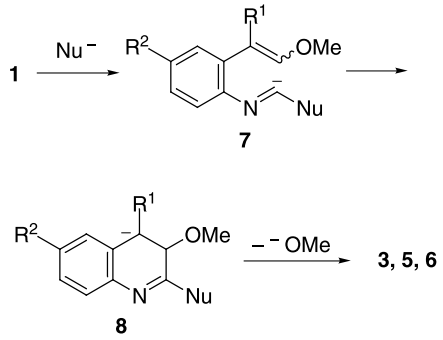


Scheme 4.

Table 2. Preparation of quinolin-2-amines **5** according to Scheme 3

Entry	Isocyanide **1**	Lithium amide **4**	Equiv	Method	**5** (Yield/%)[a]
1	**1a**	**4a** (NR^3_2=NEt_2)	1.2	A	**5a** (60)
2	**1a**	**4b** (NR^3_2=N*i*-Pr_2)	2.0	A	**5b** (61)
3	**1a**	**4c** (NR^3_2=N*c*-Hex_2)	2.0	A	**5c** (45)
4	**1a**	**4d** (NR^3_2=pyrrolidin-1-yl)	1.2	A	**5d** (64)
5	**1a**	**4e** (NR^3_2=piperidin-1-yl)	1.2	A	**5e** (72)
6	**1a**	**4f** (NR^3_2=morpholin-1-yl)	1.2	A	**5f** (55)
7	**1a**	**4g** (NR^3_2=4-methylpiperazin-1-yl)	1.2	A	**5g** (77)
8	**1b**	**4d**	1.2	A	**5h** (35)
9	**1b**	**4d**	1.2	B	**5h** (50)
10	**1b**	**4e**	1.2	B	**5i** (52)
11	**1c**	**4b**	2.0	A	**5j** (16)
12	**1c**	**4e**	1.2	A	**5k** (15)

[a] Isolated yields after purification by preparative TLC on silica gel.



Scheme 5.

interpreted as illustrated in Scheme 5. The α-addition of a nucleophile to the isocyano carbon of **1** resulted in formation of the imidoyl anion intermediate **7**. This anion attacks to the α-carbon atom of the methoxyvinyl moiety of **7** to afford the benzyl anion intermediate **8**, which, after a loss of methoxide, provides **3**, **5**, and **6**. The yields of the products are thought to depend upon the nucleophilicity of the nucleophiles. The poorer results of the reactions using **1c** is presumed to be ascribed to the lower stability of the corresponding intermediate benzyl anions compared to those from **1a** and **1b**.

In conclusion, we have demonstrated that the reactions of *o*-isocyano-β-methoxystyrene derivatives with nucleophiles, such as alkyl(or aryl)lithiums, lithium dialkylamides, or lithium benzenethiolate, provide a new method for the preparation of 2,4-disubstituted quinolines. The present method may find some value in organic synthesis because of its efficiency, the ready availability of the starting materials and the ease of operation.

2. Experimental

2.1. General

The melting points were determined on a Laboratory Devices MEL-TEMP II melting-point apparatus and are uncorrected. The IR spectra were recorded on a Perkin–Elmer 1600 Series FT IR spectrometer. The ^{1}H NMR spectra were determined using $SiMe_4$ as an internal reference with a JEOL JNM-GX270 FT NMR spectrometer operating at 270 MHz in $CDCl_3$. Low-resolution mass spectra were recorded on a JEOL AUTOMASS 20 spectrometer (Center for Joint Research and Development, this University). High-resolution mass spectra were performed on a JEOL JMS AX505 HA spectrometer (Faculty of Agriculture, this University). Thin-layer chromatography (TLC) was carried out on Merck Kieselgel 60 PF_{254}. All of the solvents used were dried over appropriate drying agents and distilled under argon prior to use.

2.2. Starting materials

2-Isocyanophenyl ketones were prepared from the corresponding commercially available 2-aminophenyl ketones by formylation with formic acid in toluene at reflux temperature, followed by dehydration with $POCl_3/Et_3N$ in THF at 0 °C. 2-Isocyanobenzophenone: 87% yield from 2-aminobenzophenone; a pale-yellow solid; mp 95–96 °C (hexane–CH_2Cl_2); IR (KBr disk) 2123, 1660 cm^{-1}; ^{1}H NMR δ 7.45–7.7 (7H, m), 7.81 (2H, dd, J=8.3, 1.6 Hz). Calcd for $C_{14}H_9NO$: C, 81.14; H, 4.38; N, 6.76. Found: C, 81.11; H, 4.33; N, 7.01. 5-Chloro-2-isocyanobenzophenone: 88% yield from 2-amino-5-chlorobenzophenone; a pale-yellow solid; mp 84–85 °C (hexane–CH_2Cl_2); IR (KBr disk) 2125, 1662 cm^{-1}; ^{1}H NMR δ 7.4–7.7 (6H, m), 7.82 (2H, dd, J=8.2, 1.3 Hz). Calcd for $C_{14}H_8ClNO$: C, 69.58; H, 3.34; N, 5.80. Found: C, 69.28; H, 3.51; N, 5.53. 2-Isocyanoacetophenone: 74% from 2′-aminoacetophenone; a yellow oil; IR (neat) 2124, 1696 cm^{-1}; ^{1}H NMR δ 2.72 (3H, s), 7.45–7.6 (3H, m), 7.78 (1H, dd, J=7.9, 1.6 Hz). The last isocyanide was rather unstable and used in the next step without any purification after workup. All other chemicals used in this study are commercially available.

2.3. Typical procedure for the preparation of isocyanostyrenes 1

2.3.1. 1-Isocyano-2-(2-methoxy-1-phenylethenyl)benzene (1a). To a stirred suspension of (methoxymethyl)triphenylphosphonium chloride (0.96 g, 2.9 mmol) in THF (15 mL) at 0 °C under argon was added butyllithium (2.9 mmol; 1.6 M solution in hexanes) dropwise; the mixture was stirred for 15 min. To this ylide solution 2-isocyanobenzophenone (0.50 g, 2.4 mmol) in THF (5 mL) was added. The mixture was allowed to warm to room temperature and stirring was continued for 30 min. The reaction was quenched by adding water (20 mL) and the organic materials were extracted with Et_2O three times (20 mL each). The combined extracts were washed with water three times and then brine, dried over anhydrous K_2CO_3, and evaporated. The crude product was purified by chromatography on silica gel to give **1a** (0.42 g, 72%) as a pale-yellow viscous oil; a mixture of stereoisomers (*E*/*Z*= ca. 1:1): R_f 0.66 (1:3 EtOAc–hexane); IR (neat) 2123, 1636 cm^{-1}; ^{1}H NMR δ 3.81 (1.5H, s), 3.83 (1.5H, s), 6.38 (0.5H, s), 6.69 (0.5 H, s), 7.05–7.5 (9H, m); MS *m*/*z* 235 (M^+, 97), 165 (100). Calcd for $C_{16}H_{13}NO$: M, 235.0997. Found: *m*/*z* 235.0994.

2.3.2. 4-Chloro-1-isocyano-2-(2-methoxy-1-phenylethenyl)benzene (1b). A pale-yellow viscous oil; a mixture of stereoisomers (*E*/*Z*=ca. 1:1): R_f 0.61 (1:3 EtOAc–hexane); IR (neat) 2123, 1636 cm^{-1}; ^{1}H NMR δ 3.82 (1.5H, s), 3.84 (1.5H, s), 6.40 (0.5 H, s), 6.69 (0.5H, s), 7.10 (1H, dd, J= 7.9, 1.6 Hz), 7.2–7.4 (7H, m); MS *m*/*z* 269 (M^+, 100). Calcd for $C_{16}H_{12}ClNO$: M, 269.0607. Found: *m*/*z* 269.0624.

2.3.3. 1-Isocyano-2-(2-methoxy-1-methylethenyl)benzene (1c). A pale-yellow oil; a mixture of stereoisomers (*E*/*Z*=*ca.* 7:3): R_f 0.31 (1:10 EtOAc–hexane); IR (neat) 2124, 1666 cm^{-1}; ^{1}H NMR δ 1.91 (2.1H, d, J=1.3 Hz), 2.00 (0.9H, d, J=1.3 Hz), 3.63 (2.1H, s), 3.73 (0.9 H, s), 6.13 (0.7H, q, J=1.3 Hz), 6.23 (0.3H, q, J=1.3 Hz), 7.15–7.4 (4H, m); MS *m*/*z* 173 (M^+, 60), 130 (100). Calcd for $C_{11}H_{11}NO$: M, 173.0841. Found: *m*/*z* 173.0861.

2.4. Typical procedure for the preparation of 2,4-disubstituted quinolines 3

2.4.1. 2-Butyl-4-phenylquinoline (3a). To a stirred solution of isocyanostyrene **1a** (0.12 g, 0.51 mmol) in DME (2.5 mL) at −78 °C under argon was added dropwise butyllithium (0.77 mmol; 1.57 M in hexane solution). After stirring for 30 min at this temperature, the mixture was allowed to warm to room temperature and stirring was continued for an additional 30 min. Saturated aqueous ammonium chloride (15 mL) was added and the mixture was extracted with Et_2O three times (15 mL each). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by preparative TLC on silica gel to give **3a** (0.11 g, 79%) as a pale-yellow viscous oil: R_f 0.67 (1:3 EtOAc–hexane); IR (neat) 3058, 2955, 1592, 1557, 1490, 765, 701 cm^{-1}; ^1H NMR δ 0.97 (3H, t, J=7.3 Hz), 1.47 (2H, sextet, J=7.3 Hz), 1.75–1.9 (2H, m), 3.01 (2H, t, J=7.9 Hz), 7.24 (1H, s), 7.43 (1H, ddd, J=8.2, 7.3, 1.3 Hz), 7.5–7.6 (5H, m), 7.68 (1H, ddd, J=8.2, 7.3, 1.3 Hz), 7.86 (1H, dd, J=8.2, 1.3 Hz), 8.11 (1H, dd, J=8.2, 1.3 Hz); MS m/z 261 (M^+, 1.1), 246 (6.9), 232 (19), 219 (100). Calcd for $C_{19}H_{19}N$: C, 87.31; H, 7.33; N, 5.36. Found: C, 87.09; H, 7.20; N, 5.36.

2.4.2. 2-(1-Methylpropyl)-4-phenylquinoline (3b). A pale-yellow viscous oil; R_f 0.74 (1:3 EtOAc–hexane); IR (neat) 3056, 2960, 1592, 1557, 1491, 772, 701 cm^{-1}; ^1H NMR δ 0.93 (3H, t, J=7.3 Hz), 1.40 (3H, d, J=6.9 Hz), 1.65–2.0 (2H, m), 2.95–3.15 (1H, m), 7.23 (1H, s), 7.43 (1H, ddd, J=8.2, 7.3, 1.3 Hz), 7.5–7.55 (5H, m), 7.68 (1H, ddd, J=8.2, 7.3, 1.3 Hz), 7.86 (1H, dd, J=8.2, 1.3 Hz), 8.12 (1H, dd, J=8.2, 1.3 Hz); MS m/z 261 (M^+, 3.3), 246 (45), 233 (100). Calcd for $C_{19}H_{19}N$: C, 87.31; H, 7.33; N, 5.36. Found: C, 87.07; H, 7.29; N, 5.20.

2.4.3. 2-(1,1-Dimethylethyl)-4-phenylquinoline (3c). A pale-yellow solid; mp 85–88 °C (hexane); IR (KBr disk) 3058, 1962, 1602, 1589, 1553, 1488, 778, 761, 706 cm^{-1}; ^1H NMR δ 1.50 (9H, s), 7.42 (1H, ddd, J=8.2, 7.3, 1.3 Hz), 7.44 (1H, s), 7.45–7.55 (5H, m), 7.66 (1H, ddd, J=8.2, 7.3, 1.3 Hz), 7.84 (1H, dd, J=8.2, 1.3 Hz), 8.12 (1H, dd, J=8.2, 1.3 Hz); MS m/z 261 (M^+, 43), 246 (100). Calcd for $C_{19}H_{19}N$: C, 87.31; H, 7.33; N, 5.36. Found: C, 87.48; H, 7.09; N, 5.27.

2.4.4. 2,4-Diphenylquinoline (3d). A pale-yellow solid; mp 120–122 °C (hexane) (lit.,[10] 114 °C); the spectral data for this product were identical to those reported previously.[11]

2.4.5. 2-(4-Methylphenyl)-4-phenylquinoline (3e). A pale-yellow solid; 116–117 °C (hexane–Et_2O) (lit.,[12] 106 °C); the spectral data for this product were identical to those reported previously.[11]

2.4.6. 4-Phenyl-2-(2-thienyl)quinoline (3f).[13a] A pale-yellow solid; mp 89–92 °C (hexane–Et_2O) (lit.,[13b] 83–85 °C); IR (KBr disk) 3065, 1590, 1548, 1489, 1428, 828, 772, 761, 712, 700 cm^{-1}; ^1H NMR δ 7.15 (1H, dd, J=5.0, 3.6 Hz), 7.35–7.6 (7H, m), 7.65–7.75 (3H, m), 7.83 (1H, dd, J=8.2, 1.3 Hz), 8.15 (1H, d, J=8.2 Hz); MS m/z 287 (M^+, 100).

2.4.7. 2-(2-Furyl)-4-phenylquinoline (3g).[13a] A pale-yellow solid; mp 109–111 °C (hexane–Et_2O) (lit.,[13b] 100–102 °C); IR (KBr disk) 3091, 1595, 1550, 1495, 1084, 1007, 776, 764, 742, 702 cm^{-1}; ^1H NMR δ 6.59 (1H, dd, J=3.3, 1.7 Hz), 7.23 (1H, dd, J=3.3, 1.0 Hz), 7.4–7.6 (6H, m), 7.63 (1H, dd, J=1.7, 1.0 Hz), 7.71 (1H, ddd, J=8.2, 7.3, 1.3 Hz), 7.77 (1H, s), 7.86 (1H, dd, J=8.2, 1.3 Hz), 8.19 (1H, dd, J=8.2, 1.3 Hz); MS m/z 271 (M^+, 100).

2.4.8. 6-Chloro-2-(1,1-dimethylethyl)-4-phenylquinoline (3h). A pale-yellow viscous oil; R_f 0.81 (1:3 hexane–AcOEt); IR (neat) 3060, 2961, 1590, 1550, 1484, 1137, 701 cm^{-1}; ^1H NMR δ 1.48 (9H, s), 7.4–7.6 (6H, m including s at δ 7.45), 7.60 (1H, dd, J=8.9, 2.3 Hz), 7.80 (1H, d, J=2.3 Hz), 8.05 (1H, d, J=8.9 Hz); MS m/z 295 (M^+, 37), 280 (100). Calcd for $C_{19}H_{18}ClN$: C, 77.15; H, 6.13; N, 4.74. Found: C, 76.96; H, 5.95; N, 4.55.

2.4.9. 6-Chloro-2,4-diphenylquinoline (3i).[14a] A pale yellow solid; mp 114–117 °C (hexane–Et_2O) (lit.,[14b] 127–129 °C); IR (KBr disk) 3026, 1590, 1544, 1483, 1357, 826, 776, 756, 704, 690 cm^{-1}; ^1H NMR δ 7.4–7.6 (8H, m), 7.66 (1H, dd, J=8.9, 2.3 Hz), 7.84 (1H, s), 7.86 (1H, d, J=2.3 Hz), 8.15–8.2 (3H, m); MS m/z 315 (M^+, 100).

2.4.10. 2-(1,1-Dimethylethyl)-4-methylquinoline (3j).[15] A pale-yellow viscous oil; R_f 0.84 (1:3 hexane–AcOEt); IR (neat) 3061. 2956, 1602, 1558, 1507, 1480, 1448, 758 cm^{-1}; ^1H NMR δ 1.46 (9H, s), 2.69 (3H, s), 7.35 (1H, s), 7.48 (1H, ddd, J=8.2, 6.9, 1.3 Hz), 7.65 (1H, ddd, J=8.2, 6.9, 1.3 Hz), 7.93 (1H, dd, J=8.2, 1.3 Hz), 8.05 (1H, dd, J=8.2, 1.3 Hz); MS m/z 199 (M^+, 54), 184 (100).

2.4.11. 4-Methyl-2-phenylquinoline (3k).[16] A pale-yellow viscous oil; R_f 0.65 (1:3 hexane–AcOEt); the spectral data for this compound were identical to those reported previously.[17]

2.5. Typical procedure for the preparation of quinolin-2-amine derivatives 5

2.5.1. 2-Diethylamino-4-phenylquinoline (5a). To a stirred solution of lithium diethylamide (0.51 mmol; generated by the standard method from diethylamine and butyllithium) at −78 °C under argon was added a solution of isocyanostyrene **1a** (0.10 g, 0.43 mmol) in THF (1.0 mL). After stirring for 30 min at this temperature, the mixture was allowed to warm to room temperature and stirring was continued for an additional 30 min. Similar workup as described for the above typical procedure followed by purification by preparative TLC on silica gel to give **5a** (71 mg, 60%) as a pale-yellow viscous oil; R_f 0.70 (1:3 EtOAc–hexane); IR (neat) 3060, 2969, 1610, 1597, 1548, 1450, 1426, 1360, 1246, 770, 701 cm^{-1}; ^1H NMR δ 1.26 (6H, t, J=6.9 Hz), 3.68 (4H, q, J=6.9 Hz), 6.74 (1H, s), 7.08 (1H, ddd, J=8.2, 6.9, 1.3 Hz), 7.4–7.55 (6H, m), 7.57 (1H, dd, J=8.2, 1.3 Hz), 7.72 (1H, dd, J=8.6, 1.3 Hz); MS m/z 276 (M^+, 29), 247 (100). Calcd for $C_{19}H_{20}N_2$: C, 82.57; H, 7.29; N, 10.14. Found: C, 82.55; H, 7.49; N, 10.13.

2-Aminoquinoline derivatives **5b–k** were prepared according to the procedure described above, excepting that 2.0 mol amounts each of lithium diisopropylamide (for **5b** and **5j**)

and lithium dicyclohexylamide (for **5c**) were used and that the preparation of **5h** and **5i** were conducted at −78–0 °C.

2.5.2. 2-Diisopropylamino-4-phenylquinoline (5b). A pale-yellow solid; mp 104–105 °C (hexane); IR (KBr disk) 3056, 2928, 1607, 1593, 1544, 1491, 1478, 1373, 1350, 1244, 771, 706 cm^{-1}; ^1H NMR δ 1.41 (12H, d, J=6.9 Hz), 4.42 (2H, septet, J=6.9 Hz), 6.81 (1H, s), 7.07 (1H, ddd, J=8.2, 6.9, 1.3 Hz), 7.4–7.6 (7H, m), 7.72 (1H, dd, J=8.6, 1.3 Hz); MS m/z 304 (M^+, 29), 261 (100). Calcd for $C_{21}H_{24}N_2$: C, 82.85; H, 7.95; N, 9.20. Found: C, 82.59; H, 8.12; N, 9.39.

2.5.3. 2-Dicyclohexylamino-4-phenylquinoline (5c). A pale-yellow solid; mp 167–169 °C (hexane–Et_2O); IR (KBr disk) 3062, 2927, 2846, 1608, 1597, 1545, 1493, 1473, 1376, 1216, 758, 704 cm^{-1}; ^1H NMR δ 1.15–1.50 (6H, m), 1.65–1.9 (10H, m), 2.0–2.2 (4H, m), 3.75–3.9 (2H, m), 6.84 (1H, m), 7.06 (1H, ddd, J=8.2, 6.9, 1.3 Hz), 7.4–7.6 (7H, m), 7.70 (1H, dd, J=8.2, 1.3 Hz); MS m/z 384 (M^+, 25), 301 (100). Calcd for $C_{27}H_{32}N_2$: C, 84.33; H, 8.39; N, 7.28. Found: C, 84.28; H, 8.60; N, 7.23.

2.5.4. 4-Phenyl-2-(pyrrolidin-1-yl)quinoline (5d). A pale-yellow solid; mp 145–148 °C (hexane–Et_2O) (lit.,[18] mp 139.5 °C); IR (KBr disk) 3051, 2967, 2861, 1604, 1593, 1547, 1500, 1427, 1342, 785, 759, 713 cm^{-1}; ^1H NMR δ 2.0–2.1 (4H, m), 3.65–3.7 (4H, m), 6.65 (1H, s), 7.10 (1H, ddd, J=8.2, 6.9, 1.3 Hz), 7.4–7.55 (6H, m), 7.59 (1H, dd, J=8.2, 1.0 Hz), 7.77 (1H, d, J=8.6 Hz); MS m/z 274 (M^+, 39), 245 (100).

2.5.5. 4-Phenyl-2-(piperidin-1-yl)quinoline (5e). A pale-yellow solid; mp 121–123 °C (hexane–Et_2O); IR (KBr disk) 3050, 2929, 2825, 1610, 1596, 1547, 1492, 1429, 1230, 778, 761, 710 cm^{-1}; ^1H NMR δ 1.69 (6H, br s), 3.7–3.8 (4H, m), 6.90 (1H, s), 7.13 (1H, ddd, J=8.2, 6.9, 1.3 Hz), 7.4–7.55 (6H, m), 7.59 (1H, dd, J=8.2, 1.3 Hz), 7.74 (1H, d, J=8.2 Hz); MS m/z 288 (M^+, 96), 259 (100). Calcd for $C_{20}H_{20}N_2$: C, 83.30; H, 6.99; N, 9.71. Found: C, 83.19; H, 7.08; N, 9.39.

2.5.6. 2-(Morpholin-1-yl)-4-phenylquinoline (5f). A pale-yellow solid; mp 124–126 °C (hexane–Et_2O); IR (KBr disk) 3060, 2965, 1605, 1594, 1548, 1491, 1425, 1229, 1125, 762 cm^{-1}; ^1H NMR δ 3.72–3.78 (4H, m), 3.82–3.88 (4H, m), 6.88 (1H, s), 7.19 (1H, ddd, J=8.2, 6.9, 1.3 Hz), 7.45–7.60 (6H, m), 7.64 (1H, dd, J=8.2, 1.3 Hz), 7.78 (1H, d, J=8.2 Hz); MS m/z 290 (M^+, 58), 259 (100). Calcd for $C_{19}H_{18}N_2O$: C, 78.59; H, 6.25; N, 9.65. Found: C, 78.48; H, 6.34; N, 9.66.

2.5.7. 2-(4-Methylpiperazin-1-yl)-4-phenylquinoline (5g). A pale-yellow solid; mp 124–126 °C (hexane–Et_2O) (lit.,[19] mp 123–129 °C); IR (KBr disk) 3057, 2832, 2847, 1609, 1595, 1547, 1492, 1424, 1424, 1231, 772, 699 cm^{-1}; ^1H NMR δ 2.36 (3H, s), 2.56 (4H, t, J=5.1 Hz), 3.80 (4H, t, J=5.1 Hz), 6.90 (1H, s), 7.16 (1H, ddd, J=8.2, 6.9, 1.3 Hz), 7.4–7.6 (6H. m), 7.61 (1H, dd, J=8.2, 1.3 Hz), 7.76 (1H, dd, J=8.2, 1.3 Hz); MS m/z 303 (M^+, 5.3), 233 (100).

2.5.8. 6-Chloro-4-phenyl-2-(pyrrolidin-1-yl)quinoline (5h). A pale-yellow solid; mp 158–160 °C (hexane–Et_2O); IR (KBr disk) 3068, 2852, 1605, 1543, 1471, 1438, 1413, 1347, 1072, 965, 858, 705 cm^{-1}; ^1H NMR δ 2.0–2.1 (4H, m), 3.6–3.65 (4H, m), 6.65 (1H, s), 7.4–7.55 (7H, m), 7.68 (1H, d, J=8.9 Hz); MS m/z 308 (M^+, 45), 279 (100). Calcd for $C_{19}H_{17}ClN_2$: C, 73.90; H, 5.55; N, 9.07. Found: C, 74.11; H, 5.53; N, 8.85.

2.5.9. 6-Chloro-4-phenyl-2-(piperidin-1-yl)quinoline (5i). A pale-yellow solid; mp 136–139 °C (hexane–Et_2O); IR (KBr disk) 3060, 2925, 2846, 1595, 1543, 1483, 1413, 1223, 953, 778, 708 cm^{-1}; ^1H NMR δ 1.65–1.7 (6H, m), 3.7–3.8 (4H, m), 6.91 (1H, s), 7.4–7.55 (7H, m), 7.66 (1H, d, J=8.9 Hz); MS m/z 322 (M^+, 100). Calcd for $C_{20}H_{19}ClN_2$: C, 74.41; H, 5.93; N, 8.68. Found: C, 74.40; H, 6.02; N, 8.66.

2.5.10. 2-Diisopropylamino-4-methylquinoline (5j). A pale-yellow solid; mp 60–62 °C (hexane); IR (KBr disk) 3059, 2966, 1606, 1552, 1493, 1469, 1428, 1353, 1238, 1150, 753 cm^{-1}; ^1H NMR δ 1.39 (12H, d, J=6.9 Hz), 2.57 (3H, s), 4.38 (2H, septet, J=6.9 Hz), 6.74 (1H, s), 7.15 (1H, ddd, J=8.2, 6.9, 1.3 Hz), 7.46 (1H, ddd, J=8.2, 6.9, 1.3 Hz), 7.63 (1H, dd, J=8.2, 1.3 Hz), 7.72 (1H, dd, J=8.2, 1.3 Hz); MS m/z 242 (M^+, 10), 199 (100). Calcd for $C_{16}H_{22}N_2$: C, 79.29; H, 9.15; N, 11.56. Found: C, 79.15; H, 9.16; N, 11.54.

2.5.11. 4-Methyl-2-(piperidin-1-yl)quinoline (5k).[20] A pale-yellow solid; mp 119–120 °C (hexane–AcOEt) (lit.,[21] mp 119–121 °C); the spectral data for this compound were identical to those reported previously.[21]

2.6. 4-Phenyl-2-(phenylthio)quinoline (6)

To a stirred solution of benzenethiol (86 mg, 0.55 mmol) in THF (1.5 mL) at −78 °C under argon was added butyllithium (0.55 mmol; 1.6 M in hexanes). After stirring for 15 min, isocyanostyrene **1a** (0.10 g, 0.43 mmol) was added and the mixture was allowed to warm to room temperature, then it was heated at reflux temperature for 7.5 h. After similar workup as described for the above typical procedure, followed by separation by preparative TLC on silica gel to give **6** (43 mg, 32%) as a pale-yellow solid; mp 99–101 °C (hexane–Et_2O); IR (KBr disk) 3062, 1609, 1578, 1540, 773, 745, 702, 690 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 6.96 (1H, s), 7.35–7.5 (9H, m), 7.6–7.7 (3H, m), 7.76 (1H, dd, J=8.4, 1.1 Hz), 8.00 (1H, dd, J=8.4, 0.7 Hz); MS m/z 313 (M^+, 100). Calcd for $C_{21}H_{15}NS$: C, 80.48; H, 4.82; N, 4.47; S, 10.23. Found: C, 80.50; H, 4.65; N, 4.37; S, 10.21.

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Four new dimeric triterpene glucosides from *Sanguisorba officinalis*

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Abstract—In search for bioactive compounds from the roots of *Sanguisorba officinalis* L. (Rosaceae), four new dimeric triterpene glucosides, namely sanguidioside A, B, C, and D (**1**–**4**) were isolated. Alkaline hydrolysis of **1**–**2** afforded the corresponding dimeric aglycones (**1a** and **2a**). Meanwhile, a ready intra-molecular transesterification was observed, providing dimeric triterpenes **1b** and **2b**. Alkaline hydrolysis of the crude dimmeric saponin also provided a new dimeric triterpene, sanguidiogenin (**9**). The structures of all these compounds are elucidated via spectroscopic and chemical methods, and are further confirmed by the X-ray diffraction analysis of the dimeric aglycone **2a**. Compound **3** represents the first dimeric saponin of an oleanolic acid and an ursonic acid derivative, while compound **4** is the first dimeric saponin of oleanolic acid derivatives.

1. Introduction

Sanguisorba officinalis (Rosaceae) distributes widely in China. Its roots have been used as a traditional Chinese medicine for treatment of internal hemorrhage and inflammation.[1,2] From the roots, triterpenoids, mainly 19α-hydroxyl ursolic acid (pomolic acid) derivatives have been isolated.[2–5] In continuous search for bioactive constituents from the abundant saponin components (~3% weight of the dry roots) of *S. officinalis*, we isolated four new dimmeric triterpene glycosides **1**–**4**, along with the known zi-yu glycoside I (**5**),[2] niga-ichigoside F1 (**6**),[3,8] rosamutin (**7**),[4] sauvissimoside R_1 (**8**)[6] and a new dimeric triterpene, sanguidiogenin E (**9**), isolated from the alkaline hydolyzate of the dimeric saponin fraction of the crude extract. Dimeric triterpene glycosides have been found from the genus *Rubus* of the Rosaceae family,[7,8] but not yet from the genus *Sanguisorba*. On mild alkaline hydrolysis of **1** and **2**, the dimeric triterpene aglycones **1a** and **2a**, and their intramolecular transesterification isomers **1b** and **2b** were obtained, respectively. The triterpene monomers **1c** and **1d** were obtained from **1** under forced alkaline conditions. All these compounds (**1**–**9**) were found inactive in MTT cytotoxicity assay against tumor cell lines. (i.e. HGC and MKN 28).[9]

Keywords: *Sanguisorba officinalis*; Rosaceae; Dimeric saponins; Intramolecular transesterification; X-ray diffraction.
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2. Results and discussion

Compound **1** was obtained as white amorphous powders. Positive results from both Libermann–Burchard and Molish reactions reveal it to be a saponin. The HRESIMS, ^{13}C NMR (Table 1), and DEPT data for compound **1** indicate a molecular formula of $C_{72}H_{110}O_{23}$. Considering the numbers of carbon and degrees of unsaturation, a dimeric triterpenoid saponin is implied.[8] Ten tertiary methyls and two secondary methyls are present in the ^1H NMR spectrum. Also present are two methine proton singlets (δ 2.98, 3.00, each 1H) characteristic of H-18 and H-18′ of pomolic acid derivatives, two olefinic proton singlets, two anomeric proton doublets (each $J=8.0$ Hz) at lower field indicating ester bond linkages of sugars with β configuration, as well as one unique singlet at the lowest field (δ 6.42, 1H) implying the ester bond between two units of the dimmer. ^{13}C NMR spectrum shows twelve methyl groups, a pair of methine carbons representing C-19 and C-19′ of pomolic acid derivatives, two pairs of olefinic carbons, and four carboxylic groups. These evidences indicate the two units of compound **1** are both pomolic acid derivatives. When comparing its ^{13}C NMR data with reference, we found two sets of signals both resembling the 2α,3β,19α-trihydroxy-urs-12-ene-23-carboxyl-28-*O*-β-D-glucopyranosyl ester (sauvissimoside R_1).[6] Differences exist mainly in the ring A (or ring A′) of each unit where they might connect to each other. 2D NMR methods were then applied to elucidate the structure and the linking pattern of the two rings.

Table 1. ^{13}C NMR assignments for compounds **1–4** and the hydrolysis products (pyridine-d_5)

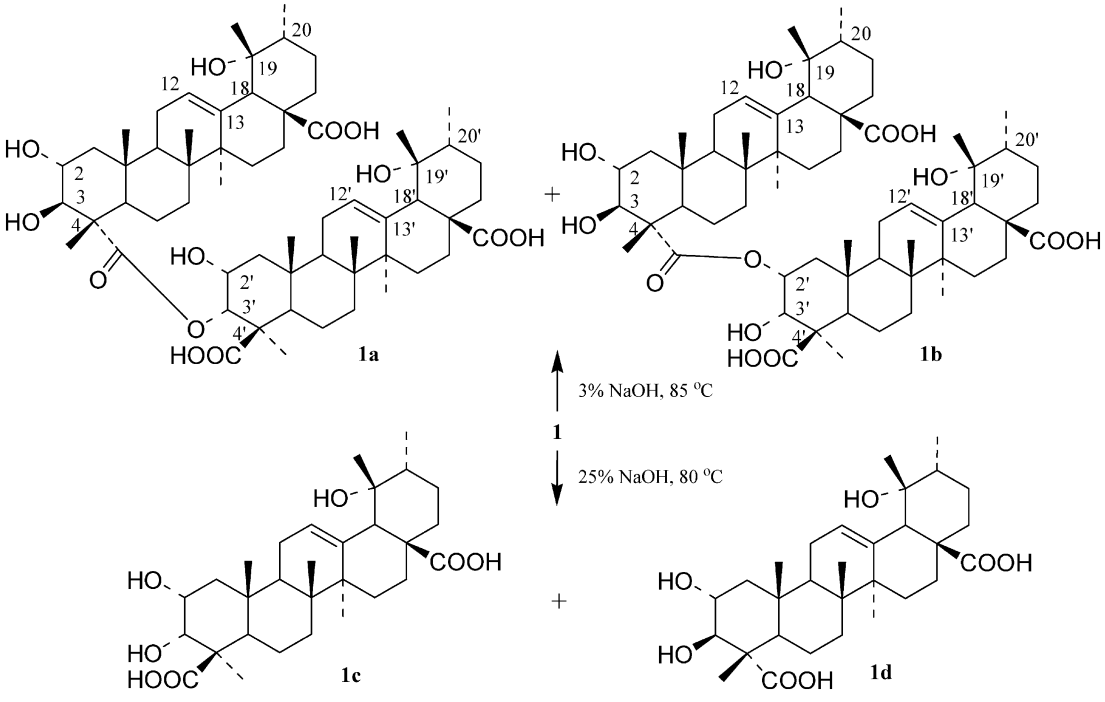
C	1	1a	1b	1c	2	2a	2b	3	3a	4	9
Unit a											
1	48.4	48.3	48.2		44.3	44.1	43.8	48.0	48.0	48.0	43.8
2	68.4	68.4	68.5		67.2	66.9	66.5	68.2	68.3	68.1	66.5
3	82.3	82.2	81.0		74.7	74.9	74.8	82.4	82.3	82.6	75.0
4	56.0	55.9	55.3		51.0	50.9	49.8	55.9	55.8	56.2	50.8
5	51.5	51.5	52.7		49.7	49.7	49.2	51.5	51.5	51.6	49.7
6	22.2	22.1	21.4		21.0	21.1	20.8	22.1	22.0	22.1	21.7
7	33.2	33.3	33.2		34.3	34.2	34.1	33.0	33.2	33.0	33.5
8	41.0	40.7	40.6		40.7	40.9	40.8	40.6	40.3	40.6	41.1
9	48.4	48.4	48.2		47.4	47.3	47.3	48.9	48.9	48.9	47.1
10	38.3	38.3	38.7		39.3	39.3	39.2	38.4	38.4	38.5	39.3
11	24.3	24.2	24.2		24.6	24.4	24.3	24.4	24.3	24.4	24.3
12	128.3	127.9	127.8		128.7	128.4	128.1	123.6	123.4	124.2	128.4
13	139.5	140.1	140.0		139.9	140.1	140.0	144.5	144.9	144.2	140.2
14	42.3	42.3	42.2		42.5	42.5	42.5	42.2	42.3	42.3	42.2
15	29.3	29.3	29.3		29.3	29.3	29.3	29.1	29.1	29.2	29.1
16	26.2	26.5	26.5		26.5	26.6	26.6	28.0	28.5	28.1	26.2
17	48.7	48.4	483		49.0	48.5	48.5	46.4	46.1	46.6	48.4
18	54.4	54.6	54.6		54.7	54.8	54.8	44.5	44.9	44.7	54.9
19	73.0	73.0	72.9		72.9	72.9	72.9	81.1	81.4	81.2	72.7
20	42.3	42.4	42.5		42.4	42.5	42.5	35.6	35.7	36.7	42.6
21	26.9	27.1	27.1		26.9	27.1	27.1	29.0	29.3	29.2	27.0
22	37.9	38.6	38.6		37.9	38.6	38.6	33.1	33.7	33.2	38.6
23	177.7	177.6	180.1		25.6	26.0	25.5	177.7	177.7	177.7	25.8
24	12.6	12.7	13.5		177.4	177.5	177.1	12.5	12.6	12.7	178.2
25	17.9	17.7	17.5		16.3	16.3	15.5	17.7	17.6	17.9	17.2
26	17.7	17.5	17.2		17.6	17.3	17.3	17.8	17.7	17.9	16.7
27	24.6	24.8	24.7		24.7	24.7	24.6	25.2	25.2	25.2	25.0
28	177.2	180.8	180.8		177.2	180.8	180.8	177.3	181.0	177.5	181.5
29	27.3	27.4	27.3		27.2	27.4	27.3	28.9	28.9	29.0	27.1
30	16.8	17.0	16.9		16.9	16.9	16.9	24.9	24.9	24.8	16.8
Unit b											
1′	45.1	45.0	39.9	44.0	44.7	44.6	39.6	45.0	45.0	44.4	44.1
2′	65.7	65.6	72.4	66.2	65.3	65.2	72.1	65.6	65.5	66.0	65.6
3′	78.3	78.2	72.4	74.8	78.1	78.2	72.2	78.2	78.3	74.3	79.2
4′	48.8	48.8	50.0	49.5	49.3	49.3	50.1	48.7	48.8	54.8	49.3
5′	51.0	51.0	49.1	49.2	51.4	51.2	49.1	51.1	51.1	47.6	51.3
6′	20.8	20.6	20.7	20.9	20.8	20.7	20.6	20.7	20.6	21.1	20.7
7′	34.3	34.3	34.1	34.2	34.3	34.4	34.1	34.3	34.2	33.7	34.1
8′	41.0	40.7	40.7	40.8	40.7	40.8	40.7	40.9	40.7	40.3	40.4
9′	47.7	47.6	47.3	47.4	47.6	47.6	47.3	47.5	47.5	48.2	48.2
10′	39.3	39.3	39.5	39.3	39.3	39.2	39.6	39.3	39.2	39.5	39.4
11′	24.4	24.4	24.4	24.4	24.6	24.5	24.4	24.4	24.4	24.5	24.4
12′	128.8	128.4	128.2	128.4	128.9	128.2	128.3	128.7	128.4	124.2	123.1
13′	139.5	140.1	140.1	140.1	139.4	140.4	140.2	139.4	140.1	144.7	145.4
14′	42.4	42.4	42.5	42.6	42.5	42.5	42.5	42.3	42.4	42.5	42.5
15′	29.5	29.5	29.4	29.4	29.5	29.5	29.4	29.4	29.6	29.3	29.2
16′	26.4	26.7	26.6	26.7	26.4	26.6	26.6	26.3	26.6	28.3	28.6
17′	48.9	48.5	48.5	48.5	48.9	48.5	48.5	48.8	48.5	46.8	46.2
18′	54.7	54.8	54.7	54.5	54.7	54.9	54.8	54.6	54.8	45.1	44.8
19′	73.0	73.0	72.8	72.9	73.0	73.0	72.9	72.9	73.0	81.4	81.4
20′	42.4	42.6	42.5	42.6	42.3	42.5	42.5	42.3	42.5	36.7	35.8
21′	26.9	27.1	27.1	27.1	26.9	27.1	27.1	26.8	27.1	29.3	29.5
22′	37.9	38.6	38.6	38.6	37.9	38.6	38.6	37.9	38.6	33.2	33.7
23′	24.9	24.9	25.2	25.4	25.1	25.4	25.2	24.7	24.9	67.3	25.7
24′	179.3	179.2	177.4	180.5	179.5	179.5	180.2	179.2	180.8	178.4	181.0
25′	15.2	15.1	15.0	15.1	15.4	15.4	15.0	15.0	15.0	15.0	13.9
26′	17.7	17.5	17.4	17.5	17.8	17.5	17.4	17.6	17.4	17.7	17.6
27′	24.7	24.8	24.6	24.7	24.8	25.0	24.6	24.5	24.4	24.7	26.1
28′	177.2	180.8	180.8	180.8	177.3	180.8	180.8	177.1	181.0	177.5	181.6
29′	27.4	27.5	27.3	27.3	27.5	27.3	27.3	27.4	27.5	29.0	28.6
30′	16.9	16.9	16.9	17.0	16.9	16.9	16.9	16.9	16.9	25.0	24.9
28-Glc											
1″	96.0				96.0			96.0		96.1	
2″	74.2				74.2			74. 3		74.4	
3″	79.1				79.0			79.1		79.1	
4″	71.4				71.5			71.1		71.2	
5″	79.4				79.2			79.5		79.6	
6″	62.5				62.6			62.2		62.3	

Table 1 (*continued*)

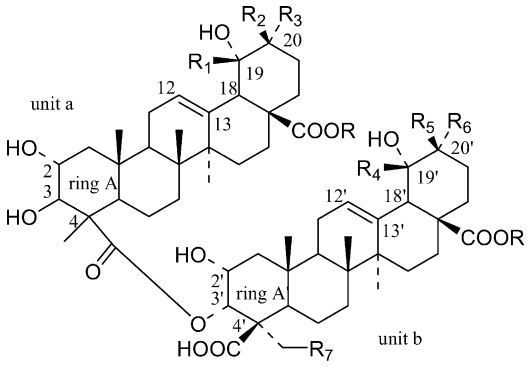
C	1	1a	1b	1c	2	2a	2b	3	3a	4	9
28′-Glc											
1‴	96.0				96.1			96.0		96.1	
2‴	74.2				74.4			74. 2		74.4	
3‴	79.1				79.2			79.1		79.1	
4‴	71.4				71.5			71.3		71.2	
5‴	79.4				79.5			79.5		79.6	
6‴	62.6				62.7			62.3		62.3	

Two pairs of diagnostic protons belonging to different spin system (δ 5.05, 6.42, H-2′, 3′; δ 4.28, 4.34, H-2, 3) are indicated by the ^{1}H–^{1}H COSY correlations, indicating ring A of unit a and ring A′ of unit b are both dihydroxylated. The lowest field proton signal (δ 6.42, s) shows HMBC correlations with seven carbons (including one methylene, one methine, one hydroxymethine, one liphatic quaternary carbon, one methyl, and two carboxyl carbons). Because HMBC correlations are observed from their protons to the 25′-Me, the methylene and the methine of the seven signals are assigned as C-1′ and C-5′, respectively. Accordingly, the proton at δ 6.42 could be assigned as H-3′. NOESY correlations observed between H-2′, H-3′ and 25′-Me support the β configurations of H-2′, and H-3′. The supposed configurations of the two protons (H-2′, H-3′) are confirmed by their multiplet pattern, coupling constants, and chemical shifts. Consequently, the carboxylic carbon having HMBC correlation with both H-3′ and H-5′ can only be substituted at the tertiary C-4′. No NOESY correlation observed between 24′-Me and 25′-Me suggest the β-configuration of 24′-COOH. Based on the evidences stated above this unit (unit b) is determined to be 2α,3α,19α-trihydroxyurs-12-ene-24,28-dioic acid (2α-hydroxyl-24-carboxyl-3-epi-pomolic acid), a new triterpene. In similar fashion, the structure of unit a is elucidated as 2α,3β,19α-trihydroxyurs-12-ene-23,28-dioic acid (2α-hydroxyl-23-carboxypomolic acid).[8] HMBC correlation from H-3′ to 24-COOH, along with the downfield shift of H-3′ indicate that units a and b are brought together through an ester bond between C-24 and C-3′.

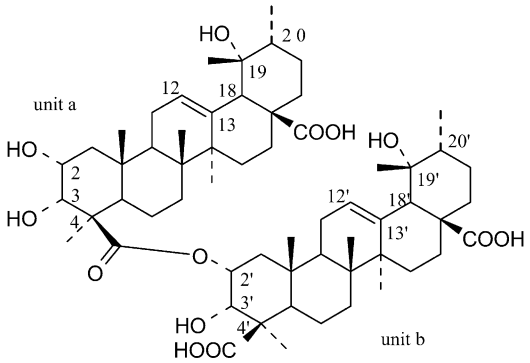
Alkaline hydrolysis of **1** with 3% NaOH at 85 °C for 24 h provided D-glucose and two dimeric triterpenes **1a** and **1b** (Scheme 1) in a ratio of 2:1, with no monomer being obtained. This is quite unlike the structurally similar coreanoside F1, a dimeric triterpenoid saponin from the genus *Rubus* bearing an additional 23-OH group, which was hydrolyzed easily to monomers (2% K_2CO_3–EtOH, reflux, 4 h).[8] When compound **1** was treated with 15% NaOH at 45 °C, aglycone **1a** was obtained as the major product. Differences between the ^{13}C NMR data of **1a** and **1b** lie in the downfield shift of C-2′ from δ 65.6 (**1a**) to 72.4 (**1b**), and downfield shift of C-2′ from δ 78.2 (**1a**) to 72.4 (**1b**). Correspondingly in ^{1}H NMR data, downfield shift from δ 5.05 (**1a**) to 6.36 (**1b**) is observed with H-2′, and highfield shift from δ 6.43 (**1a**) to 4.94 (**1b**) is observed with H-3′. These results indicate that compound **1b** is produced from **1a** via an intra-molecular transesterification reaction. The ready intra-molecular transesterification supports the α-configurations of the C-2′ and C-3′ hydroxyl groups. It also explains the resistance of dimeric **1a** (or **1b**) to hydrolysis into monomers. When compound **1** was treated



Scheme 1. Alkaline hydrolysis of compound **1**.



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	3-OH	C-4	R
1	Me	H	Me	Me	H	Me	H	β	*S*	glc
2	Me	H	Me	Me	H	Me	H	α	*R*	glc
2a	Me	H	Me	Me	H	Me	H	α	*R*	H
3	H	Me	Me	Me	H	Me	H	β	*S*	glc
3a	H	Me	Me	Me	H	Me	H	β	*S*	H
4	H	Me	Me	H	Me	Me	OH	β	*S*	glc
9	Me	H	Me	H	Me	Me	H	α	*R*	H



2b

Figure 1. Structures of compounds **1**–**4** and the hydrolysis products (**2a**, **2b**, **3a**, and **9**).

under forced alkaline conditions (25% NaOH at 80 °C), the expected monomers **1c** and **1d** were obtained (Scheme 1).[8] These evidences confirmed the structure of compound **1** (Fig. 1), which is given a trivial name sanguidioside A. The new triterpene **1c** is named as sanguic acid.

Compound **2** was obtained as white amorphous powders. Its HRESIMS, ^{13}C NMR (Table 1), and DEPT data indicate a molecular formula of $C_{72}H_{110}O_{23}$, suggesting an isomer of **1**. Its ^{1}H NMR data are similar to that of **1**, with the same numbers of and similar chemical shiftings of tertiary and secondary methyl groups, characteristic H-18 and 18′ methine proton singlets of pomolic acid derivatives, olefinic proton singlets, anomeric proton doublets (each J=8.0 Hz) indicating sugars of β configuration, and the unique H-3′ singlet in the lowest field implying the ester bond between two units of the dimmer. Additionally, its ^{13}C NMR spectrum resembles well with **1**. These data indicate the two units of compound **2** are also both pomolic acid derivatives. The diagnostic H-2′ and H-3′ of unit b are observed at δ 5.09 (1H, d, J=10.5 Hz) and 6.41 (1H, s) just as in the case of **1**, suggesting an identical structure and linking position of the ring A′ of unit b. Obvious differences emerge at δ 4.83 (1H, d, J=11.0 Hz) and 4.94 (1H, s) in **2**. Analysis of 2D NMR suggests they are H-2 and H-3 of unit a possessing the same configuration as their unit b counterpart. By analysis of 2D NMR data, the rest of each of the two units is also revealed to be identical. Consequently the two units are proved to be both 2α,3α,19α-trihydroxyurs-12-ene-24,28-dioic acid (sanguic acid, **1c**), and the two parts are brought together through an ester bond between C-24 of unit a and C-3′ of unit b. Consequently, the structure of **2** is determined as shown in Figure 1, and is named as sanguidioside B.

Alkaline hydrolysis of **2** with 3% NaOH at 85 °C for 24 h yielded D-glucose and the dimeric triterpene aglycones **2a** and **2b** (Fig. 1) in a ratio of 2:1, with no monomers being obtained. The structure and stereochemistry of **2a** are confirmed by X-ray crystallography (Fig. 2).[10] It can be explained from the crystal data that the downfield shifting of Me-25 (δ 1.43) and Me-26 (δ 1.36) compared to Me-25′ (δ 1.29) and Me-26′ (δ 1.28) is due to staying in the deshelding plane of unit b. And the relative milder shift of Me-26 is due to its longer distance to unit b than that of Me-25. Similarly, Me-27′ (δ 1.97) of unit b lies in the deshielding region of unit a and experiences large downfield shifting. The reason of the largest highfield shifting of Me-23′ (δ 1.73) is attributed to the proximity of the shielding region of two carbonyl groups, C-24 and C-24′. Similar shiftings are also observed in the case of compound **2**.

Compound **3** was obtained as white amorphous powders. The HRESIMS, ^{13}C NMR (Table 1), and DEPT data for compound **3** indicate a molecular formula of $C_{72}H_{110}O_{23}$, implying another isomer of the dimeric saponin series. Unlike compounds **1** and **2**, its ^{1}H NMR spectrum shows one more tertiary methyl and one less secondary methyl. With one of the two methine proton singlet (δ 3.00, H-18 of compound **1**) of pomolic acid derivative being absent, a broad single peak integrating for two protons emerges at δ 3.57. Additionally, in the ^{13}C NMR data, with the disappearing of one characteristic tertiary carbon (δ 72.9, C-19 of compound **1**), a methine appears at δ 81.1. By comparing with reference, compound **3** is proved to be a dimeric triterpenoid saponin made up of an oleanolic acid derivative and a pomolic acid derivative.[5]

Two pairs of diagnostic protons belonging to different spin system (δ 5.05, 6.46, H-2′, 3′; δ 4.28, 4.32, H-2, 3) are indicated by the ^{1}H–^{1}H COSY correlations, indicating ring A of unit a and ring A′ of unit b have the same structures and linking pattern like **1**. Because of both having HMBC correlations with C-18, one olefinic proton (H-12) is associated with the Me-30 of oleanolic acid type moiety. NOESY correlation observed between Me-26 of unit a and this olefinic proton (H-12) helps to determine unit a is oleanolic acid type. Thus, through interpretation of 2D NMR spectra, the two units are found respectively to be 2α,3β,19α-trihydroxyl-23-carboxyl-oleanolic acid (unit a) and 2α,3α,19α-trihydroxyurs-12-ene-24,28-dioic acid (sanguic acid, **1c**, unit b).

Alkaline hydrolysis of **3** with 3% NaOH at 85 °C for 24 h

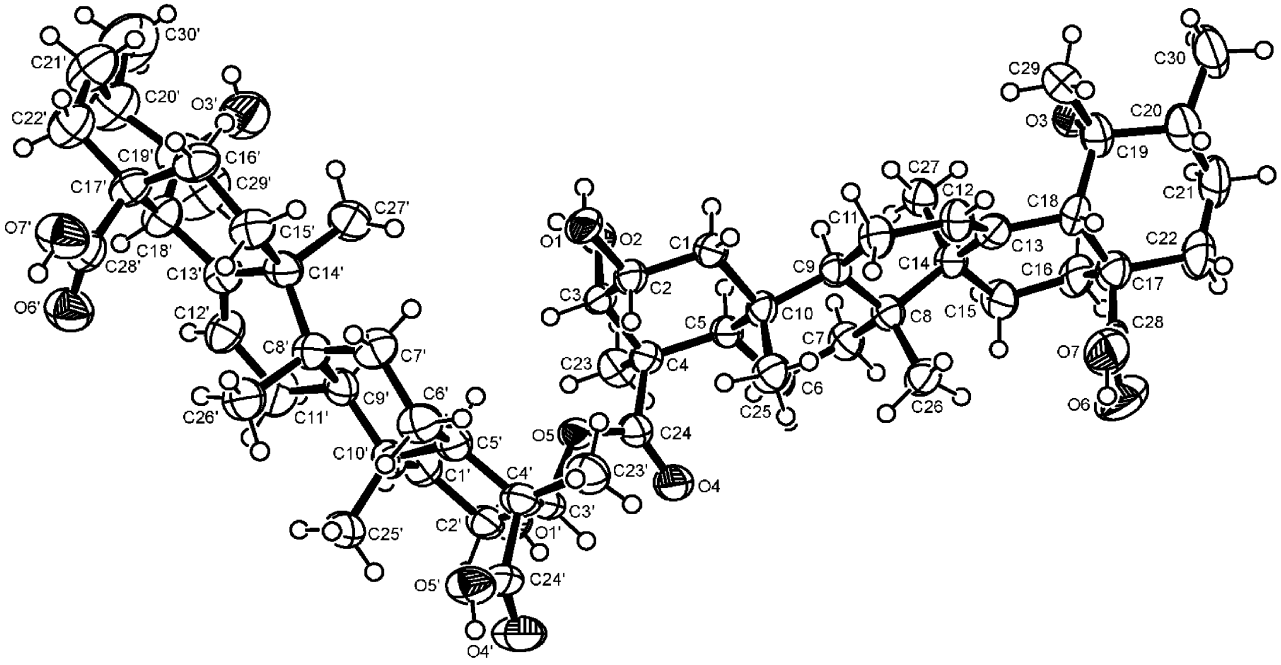


Figure 2. ORTEP drawing of compound **2a**.

yielded D-glucose and the dimeric triterpene aglycone **3a**. Consequently, the structure of **3** is determined as shown in Figure 1, and is named as sanguidioside C, which represents the first case of a dimric saponin made up of two different types of triterpene skeletons.

Compound **4** was obtained as white amorphous powders. The HRESIMS, ^{13}C NMR (Table 1), and DEPT data for compound **3** indicate a molecular formula of $C_{72}H_{110}O_{24}$, having one more hydroxyl group than compounds **1**–**3**. But unlike the previous compounds, no characteristic singlets for H-18 (or 18′) of pomolic acid derivative was observed in the ^{1}H NMR spectrum, and the twelve methyl groups are all tertiary. By comparing with reference, compound **4** is proved to be a dimeric triterpenoid saponin made up of two oleanolic acid derivatives.[5] Through analysis of the 2D NMR data, the two units are respectively elucidated as 2α,3β,19α-trihydroxyl-23-carboxyl-oleanolic acid (unit a) and 2α,3α,19α,23-tetrahydroxyl-24-carboxyl-oleanolic acid (unit b). The linking pattern between the ring A and A′ of both units are found to be the same as previous compounds. Consequently, the structure of **4** is determined as shown in Figure 1, and is named as sanguidioside D. This is the first case of a dimeric saponin made up of two oleanolic acid derivatives.

When the crude dimer complex (see Section 3.3) was treated with 3% NaOH at 85 °C for 24 h, a new dimeric triterpene (**9**), namely sanguidiogenin E, was isolated. The HRESIMS, ^{13}C NMR (Table 1), and DEPT data for compound **9** indicate a molecular formula of $C_{60}H_{90}O_{13}$. Analysis of its 2D NMR data reveal that its unit a and the linking pattern of the two units are identical to **2a**. But unlike **1a**, **2a** and **3a** which all have sanguic acid (**1c**) as their unit b, its unit b is determined as an oleanolic acid derivative (Fig. 1).

3. Experimental

3.1. General experimental procedures

Melting points were determined with an X-4 melting point apparatus (Taike Instruments Co.). Optical rotations were taken with a Perkin–Elmer 241 polarimeter. IR data were obtained using a Perkin–Elmer 16 PC FT-IR spectrometer. ^{1}H, ^{13}C, and 2D NMR spectra were recorded in pyridine-d_5 on AVANCE-500 (Bruker) NMR spectrometer. Chemical shifts are expressed in δ (ppm) with reference to pyridine-d_5. HRESIMS were measured on a FTMS-7 instrument (Bruker Daltonics). GC experiments were carried out on a HP-1 TCD instrument (Hewlett–Packard), using HP-Chiral column (30×0.25×1.0, 20% permethylated β-cyclodextrin). The conditions selected for analysis were: front inlet 250 °C, column 80 °C→230 °C, 5 °C/min. Open column chromatography was carried out using silica gel (200–300 mesh, Qingdao Marine Chemical Co., Qingdao, People's Republic of China) or octadecyl silica gel (ODS, 25–40 μm, Fuji) as stationary phase. TLC was conducted on Si gel 60 F_{254} S plates (Merck). All chemical reagents (AR grade) were purchased from Shanghai Reagent Co. Ltd.

3.2. Plant material

The roots of *S. officinalis* were purchased from Nanjing Chinese Medicine Co. (China). A voucher specimen (No. SIOC-Bio-20030320) has been deposited in the State Key Laboratory of Bio-organic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, People's Republic of China.

3.3. Extraction and isolation

Dried roots of *S. officinalis* (10 kg) were extracted with EtOH (95%). The extract was concentrated, defatted with

Table 2. ^{1}H NMR assignments for compounds **1–4** (pyridine-d_5)[a]

No	1	2	3	4
Unit a				
2	4.28 (1H)	4.83 (1H, br d, J=11.0 Hz)	4.28 (1H)	4.27 (1H)
3	4.34 (1H)	4.94 (1H, s)	4.32 (1H)	4.35 (1H)
12	5.56 (1H, br s)	5.61 (1H, br s)	5.50 (1H, br s)	5.52 (1H, br s)
15α	2.46–2.56 (1H, m)	2.47 (1H)	2.40 (1H)	2.32 (1H)
16β	3.07–3.17 (1H, m)	3.11 (1H)	2.79–2.89 (1H, m)	2.80 (1H)
18	2.98 (1H, s)	3.00 (1H, s)	3.57 (1H)	3.55 (1H, br s)
19			3.57 (1H)	3.58 (1H, s)
23		2.02 (3H, s)		
24	1.67 (3H, s)		1.67 (3H, s)	1.67 (3H, s)
25	1.19 (3H, s)	1.42 (3H, s)	1.19 (3H, s)	1.16 (3H, s)
26	1.28 (3H, s)	1.40 (3H, s)	1.25 (3H, s)	1.20 (3H, s)
27	1.70 (3H, s)	1.72 (3H, s)	1.64 (3H, s)	1.58 (3H, s)
28				
29	1.44 (3H, s)	1.45 (3H, s)	1.22 (3H, s)	1.19 (3H, s)
30	1.14 (1H, d, J=6.4 Hz)	1.13 (3H, d, J=6.8 Hz)	1.04 (3H, s)	1.02 (3H, s)
Unit b				
2′	5.05 (1H)	5.09 (1H, br d, J=10.5 Hz)	5.05 (1H, br d, J=10.6 Hz)	5.21 (1H, br d, J=10.1 Hz)
3′	6.42 (1H, s)	6.41 (1H, s)	6.46 (1H, s)	6.78 (1H, s)
12′	5.61 (1H, br s)	5.65 (1H, br s)	5.65 (1H, br s)	5.59 (1H, br s)
15′α	2.54–2.64 (1H, m)	2.58 (1H)	2.54–2.64 (1H, m)	2.44 (1H)
16′β	3.15–3.26 (1H, m)	3.17 (1H)	3.11–3.21 (1H, m)	2.84 (1H)
18′	3.00 (1H, s)	3.02 (1H, s)	3.04 (1H, s)	3.61 (1H, s)
19′				3.70 (1H, br s)
23′	1.73 (3H, s)	1.74 (3H, s)	1.74 (3H, s)	4.11 (1H) 4.87 (1H, d, J=9.9 Hz)
24′				
25′	1.32 (3H, s)	1.33 (3H, s)	1.33 (3H, s)	1.34 (3H, s)
26′	1.37 (3H, s)	1.39 (3H, s)	1.38 (3H, s)	1.29 (3H, s)
27′	1.92 (3H, s)	1.95 (3H, s)	1.85 (3H, s)	1.75 (3H, s)
28′				
29′	1.52 (3H, s)	1.50 (3H, s)	1.55 (3H, s)	1.25 (3H, s)
30′	1.18 (1H, d, J=6.4 Hz)	1.15 (3H, d, J=6.8 Hz)	1.18 (3H)	1.07 (3H, s)
28-Glc				
1″	6.35 (1H, d, J=8.0 Hz)	6.33 (1H, d, J=7.6 Hz)	6.44 (1H, d, J=8.2 Hz)	6.39 (1H, d, J=8.22 Hz)
2″	4.29 (1H)	4.26 (1H)	4.28 (1H)	4.26 (1H)
3″	4.37 (1H)	4.35 (1H)	4.35 (1H)	4.34 (1H)
4″	4.42 (1H)	4.41 (1H)	4.42 (1H)	4.42 (1H)
5″	4.09–4.14 (1H, m)	4.04–4.10 (1H, m)	4.19 (1H)	4.04–4.10 (1H, m)
6″	4.47 (1H, br d, J=12.3 Hz), 4.52–4.58 (1H, m)	4.45 (1H), 4.52 (1H)	4.47 (1H), 4.53 (1H)	4.44 (1H), 4.49 (1H)
28′-Glc				
1‴	6.35 (1H, d, J=8.0 Hz)	6.34 (1H, d, J=7.6 Hz)	6.36 (1H, d, J=7.9 Hz)	6.40 (1H, d, J=8.22 Hz)
2‴	4.29 (1H)	4.29 (1H)	4.29 (1H)	4.26 (1H)
3‴	4.37 (1H)	4.37 (1H)	4.38 (1H)	4.34 (1H)
4‴	4.42 (1H)	4.41 (1H)	4.44 (1H)	4.42 (1H)
5‴	4.09–4.14 (1H, m)	4.08–4.14 (1H, m)	4.22 (1H)	4.04–4.10 (1H, m)
6‴	4.47 (1H, br d, J=12.3 Hz), 4.52–4.58 (1H, m)	4.46 (1H), 4.54 (1H)	4.48 (1H), 4.55 (1H)	4.44 (1H), 4.49 (1H)

[a] Signals overlapped are not labeled with multiplicity.

cyclohexane, and partitioned sequentially with $CHCl_3$ and *n*-BuOH. The *n*-BuOH layer was dried in vacuo to yield 460 g crude total saponins. 400 g of it was then separated by silica gel column chromatography using $CHCl_3$–CH_3OH as solvent to yield seven fractions. Fraction 6 was then subjected to ODS silica gel column eluted with MeOH–H_2O (30:70–70:30) to get a complex of dimmers (3.4 g). 1.9 g of this complex was then subjected to ODS silica gel column to afford **1** (590 mg), **2** (193 mg), **3** (122 mg), and **4** (15 mg). Fractions 2 and 3 were further separated by silica gel column chromatography using $CHCl_3$–CH_3OH as solvent to yield **5** (35 g), **6** (17 mg), **7** (15 mg), and **8** (22 g).

3.3.1. Sanguidioside A (1). White amorphous powder, mp 224–226 °C, $[\alpha]_D^{18}$=+26.5 (*c* 4.6, MeOH); IR ν_{max} (KBr) 3429, 2934, 1722, 1656, 1458, 1381, 1231, 1075, 1032, 931, 773, 680 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 1343 $[M+H]^+$; HRESIMS *m/z* found 1365.7364 (Calcd for $C_{72}H_{110}O_{23}Na$, 1365.7336).

3.3.2. Sanguidioside B (2). White amorphous powder, $[\alpha]_D^{20}$=+25.6 (*c* 3.5, MeOH); IR ν_{max} (KBr) 3431, 2932, 1726, 1551, 1456, 1379, 1228, 1074, 971, 534 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 1342 $[M]^+$; HRESIMS *m/z* found 1365.7348 (Calcd for $C_{72}H_{110}O_{23}Na$, 1365.7336).

3.3.3. Sanguidioside C (3). White amorphous powder, $[\alpha]_D^{19}$=+21.8 (*c* 0.9, MeOH); IR ν_{max} (KBr) 3436, 2933, 1725, 1656, 1507, 1457, 1392, 1305, 1233, 1072, 988; ^{1}H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 1342 $[M]^+$;

HRESIMS *m/z* found 1365.7314 (Calcd for $C_{72}H_{110}O_{23}Na$, 1365.7336).

3.3.4. Sanguidioside D (4). White amorphous powder, $[\alpha]_D^{20}=+18.0$ (*c* 1.0, MeOH); IR ν_{max} (KBr) 3416, 2931, 1717, 1677, 1456, 1349, 1204, 1140, 1071, 839, 800, 723, 532 cm^{-1}; ^1H and ^{13}C NMR see Tables 1 and 2; ESIMS *m/z* 1358 $[M]^+$; HRESIMS *m/z* 1381.7237 (Calcd for $C_{72}H_{110}O_{24}Na$, 1381.7285).

3.4. Alkaline hydrolysis

A 3% NaOH solution (10 mL) of compound **1** (200 mg) was heated at 85 °C for 24 h, and was then neutralized with 3 N HCl. The solution was extracted with water–saturated *n*-BuOH. The butanol layer was concentrated and then subjected to Si gel chromatography for separation, affording compounds **1a** (40 mg) and **1b** (19 mg). When **1** (40 mg) was treated with 15% NaOH at 80 °C for 24 h, only **1a** (10 mg) was obtained as the major product. Treatment of **1** (100 mg) with 25% NaOH at 80 °C for 24 h afforded **1c** (11 mg) and **1d** (8 mg).

Compound **2** (100 mg) was treated with 3% NaOH at 85 °C for 24 h, affording **2a** (23 mg) and **2b** (9 mg) after Si gel chromatography. Under similar conditions hydrolysis of compound **3** (40 mg) afforded **3a** (7 mg).

The dimer complex (1.0 g) was treated with 3% NaOH at 85 °C for 24 h affording **1a**, **2a**, and a new dimeric triterpene, sanguidiogenin E (**9**, 8 mg) (Table 3).

3.4.1. Sanguidiogenin A (1a). White amorphous powder, mp 256–258 °C, $[\alpha]_D^{20}=+43.6$ (*c* 2.0, MeOH); IR ν_{max} (KBr) 3398, 2927, 1701, 1451, 1368, 1232, 1045, 762, 668 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; MALDIMS *m/z* 1041 $[M+Na]^+$; HRESIMS *m/z* found 1041.6250 (Calcd for $C_{60}H_{90}O_{13}Na$, 1041.6279).

3.4.2. Pseudosanguidiogenin A (1b). White amorphous powder, mp 249–251 °C, $[\alpha]_D^{20}=+27.0$ (*c* 1.0, MeOH); IR ν_{max} (KBr) 3449, 2934, 1707, 1459, 1380, 1234, 1048, 764, 651 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 1041 $[M+Na]^+$; HRESIMS *m/z* found 1041.6244 (Calcd for $C_{60}H_{90}O_{13}Na$, 1041.6279).

3.4.3. Sanguic acid (1c). White amorphous powder, mp 253–255 °C, $[\alpha]_D^{19}=+16.0$ (*c* 0.15, MeOH); IR ν_{max} (KBr) 3423, 2933, 1694, 1459, 1381, 1233, 1033, 766, 691 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 541 $[M+Na]^+$; HRESIMS *m/z* found 541.3125 (Calcd for $C_{30}H_{46}O_7Na$, 541.3141).

3.4.4. Sanguidiogenin B (2a). Colorless cuboid, $[\alpha]_D^{22}=+33.7$ (*c* 1.3, MeOH); IR ν_{max} (KBr) 3413, 2934, 1710,

Table 3. ^1H NMR assignments for the hydrolysis products (pyridine-d_5)[a]

No	1a	1b	1c	2a	2b	3a	9
Unit a							
2	4.25–4.34 (1H, m)	4.22–4.32 (1H, m)		4.90 (1H)	4.93 (1H)	4.25–4.35 (1H, m)	4.89 (1H)
3	4.37 (1H, d, *J*=9.1 Hz)	4.65 (1H, d, *J*=6.5 Hz)		4.91 (1H, s)	4.86 (1H, s)	4.35 (1H, d, *J*=9.4 Hz)	4.91 (1H, s)
12	5.59 (1H, br s)	5.62 (1H, br s)		5.66 (1H, br s)	5.66 (1H, br s)	5.54 (1H, br s)	5.61 (1H, br s)
15α	2.36 (1H)	2.30 (1H)		2.38 (1H)	2.33 (1H)	2.17 (1H)	2.46 (1H)
16β	3.13 (1H)	3.11 (1H)		3.13 (1H)	3.13 (1H)	2.77–2.87 (1H, m)	3.19 (1H)
18	3.10 (1H, s)	3.09 (1H, s)		3.11 (1H, s)	3.11 (1H)	3.63–3.71 (1H, m)	3.66 (1H)
19						3.61 (1H)	3.66 (1H)
23				2.04 (3H, s)	1.85 (3H, s)		2.01 (3H, s)
24	1.68 (3H, s)	1.72 (3H, s)				1.69 (3H, s)	
25	1.15 (3H, s)	1.12 (3H, s)		1.43 (3H, s)	1.21 (3H, s)	1.17 (3H, s)	1.59 (3H, s)
26	1.18 (3H, s)	1.12 (3H, s)		1.36 (3H, s)	1.20 (3H, s)	1.15 (3H, s)	1.41 (3H, s)
27	1.80 (3H, s)	1.65 (3H, s)		1.76 (3H, s)	1.72 (3H, s)	1.70 (3H, s)	1.77 (3H, s)
29	1.48 (3H, s)	1.46 (3H, s)		1.50 (3H, s)	1.44 (3H, s)	1.27 (3H, s)	1.49 (3H, s)
30	1.20 (1H, d, *J*=6.7 Hz)	1.17 (1H, d, *J*=6.7 Hz)		1.19 (1H, d, *J*=6.3 Hz)	1.17 (3H)	1.17 (3H, s)	1.20 (1H, d, *J*=6.6 Hz)
Unit b							
2′	5.05 (1H, br d, *J*=9.7 Hz)	6.36 (1H, br d, *J*=6.6 Hz)	5.02 (1H)	5.08 (1H, br d, *J*=11.1 Hz)	6.28 (1H, br d, *J*=10.4 Hz)	5.07 (1H, br d, *J*=10.7 Hz)	5.16 (1H, br d, *J*=10.1 Hz)
3′	6.43 (1H, s)	4.94 (1H, s)	4.86 (1H, s)	6.43 (1H, s)	4.96 (1H, s)	6.47 (1H, s)	6.42 (1H, s)
12′	5.66 (1H, br s)	5.63 (1H, br s)	5.68 (1H, br s)	5.64 (1H, br s)	5.66 (1H, br s)	5.69 (1H, br s)	5.54 (1H, br s)
15α′	2.42 (1H)	2.39 (1H)	2.37–2.47 (1H, m)	2.42 (1H)	2.43 (1H)	2.42 (1H)	2.24 (1H)
16β′	3.26–3.16 (1H, m)	3.17 (1H)	3.13–3.24 (1H, m)	3.19 (1H)	3.17 (1H)	3.16 (1H)	2.89 (1H)
18′	3.12 (1H, s)	3.11 (1H, s)	3.13 (1H, s)	3.12 (1H, s)	3.11 (1H)	3.15 (1H, s)	
23′	1.80 (3H, s)	1.83 (3H, s)	1.90 (3H, s)	1.73 (3H, s)	1.86 (3H, s)	1.75 (3H, s)	1.71 (3H, s)
25′	1.29 (3H, s)	1.38 (3H, s)	1.34 (3H, s)	1.29 (3H, s)	1.44 (3H, s)	1.29 (3H, s)	1.32 (3H, s)
26′	1.26 (3H, s)	1.25 (3H, s)	1.29 (3H, s)	1.28 (3H, s)	1.29 (3H, s)	1.26 (3H, s)	1.25 (3H, s)
27′	1.95 (3H, s)	1.74 (3H, s)	1.78 (3H, s)	1.97 (3H, s)	1.77 (3H, s)	1.87 (3H, s)	1.92 (3H, s)
29′	1.55 (3H, s)	1.52 (3H, s)	1.51 (3H, s)	1.50 (3H, s)	1.49 (3H, s)	1.59 (3H, s)	1.25 (3H, s)
30′	1.23 (1H, d, *J*=6.7 Hz)	1.20 (1H, d, *J*=6.7 Hz)	1.19 (1H, d, *J*=6.4 Hz)	1.21 (1H, d, *J*=6.3 Hz)	1.19 (3H)	1.23 (3H, d, *J*=6.6 Hz)	1.10 (3H, s)

[a] Signals overlapped are not labeled with multiplicity.

1551, 1458, 1380, 1230, 1174, 1055, 971, 533 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 1017 $[M-H]^+$; HRESIMS *m/z* found 1041.6285 (calcd for $C_{60}H_{90}O_{13}Na$, 1041.6279).

3.4.5. Pseudosanguidiogenin B (2b). White amorphous powder, $[\alpha]_D^{20}=+32.1$ (*c* 0.88, MeOH); IR ν_{max} (KBr) 3450, 2934, 1707, 1554, 1458, 1379, 1233, 1173, 1057, 970, 828, 533 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 1017 $[M-H]^+$; HRESIMS *m/z* found 1041.6275 (Calcd for $C_{60}H_{90}O_{13}Na$, 1041.6279).

3.4.6. Sanguidiogenin C (3a). White amorphous powder, $[\alpha]_D^{20}=+33.3$ (*c* 0.43, MeOH); IR ν_{max} (KBr) 3416, 2930, 1701, 1656, 1459, 1378, 1237, 1167, 1074, 975 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 1041 $[M+Na]^+$; HRESIMS *m/z* found 1041.6265 (Calcd for $C_{60}H_{90}O_{13}Na$, 1041.6279).

3.4.7. Sanguidiogenin E (9). White amorphous powder, $[\alpha]_D^{20}=+15.5$ (*c* 0.3, MeOH); IR ν_{max} (KBr) 3422, 2928, 1686, 1458, 1380, 1207, 1145, 1053, 842, 802, 724 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2; ESIMS *m/z* 1017 $[M-H]^+$; HRESIMS *m/z* found 1041.6245 (Calcd for $C_{60}H_{90}O_{13}Na$, 1041.6279).

3.5. Analysis of the sugar residue

Compound **1** (20 mg) was heated under reflux in 20 mL of 1 M HCl (MeOH–H_2O, 1:1) for 3 h. After removal of the solvent, the residue was partitioned between *n*-BuOH and H_2O. The aq. layer was neutralized with Dowex (HCO_3^-), then filtered. The filtrate was evaporated down to 2 mL, then treated with $NaBH_4$ (40 mg) at room temperature for 3 h. Excessive $NaBH_4$ was removed with 30% AcOH. After evaporation at 60 °C and washing with 0.1% hydrochloride acid (in MeOH) repeatedly until the BO_3^{3-} was removed, the reaction mixture was heated to dryness at 105 °C for 15 min, followed by the addition of pyridine (dry, 0.5 mL) and Ac_2O (0.5 mL). The mixture was incubated in a water bath at 100 °C for 1 h, and partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was concentrated for GC analysis. Monosaccharide was identified as D-glucose. By the same method, the monosaccharide was identified as D-glucose for compounds **2**, **3**, and **4**.

3.6. X-ray analysis of 2a

Crystal data: $C_{60}H_{90}O_{13}\cdot CH_3OH\cdot 5H_2O$; $M_r=1441.44$, dimensions $0.516\times0.345\times0.317$ mm^3, orthorhombic, $P2_1$, $a=12.8400(16)$ Å, $b=20.176(3)$ Å, $c=28.305(4)$ Å, $\alpha=90°$, $\beta=90°$, $\gamma=90°$, $V=7332.6(16)$ $Å^3$, $Z=4$, $D_{calc}=1.034$ g/cm^3, $F_{000}=2488.00$, $\mu(Mo\ K\alpha)=0.075$ mm^{-1}. Data collection was performed on a SMART CCD using graphite monochromated radiation ($\lambda=0.71073$ Å); 13581 unique reflections were collected to $\theta_{max}=25.50°$, in which 7856 reflections were observed [$F^2>4\sigma(F^2)$]. The structure was solved by direct methods (SHELXTL version 97) and refined by full-matrix least-squares on F^2. In the structure refinements, non-hydrogen atoms were refined anisotropically. Hydrogen atoms bonded to carbons were placed on the geometrically ideal positions by the 'ride on' method. Hydrogen atoms bounded to oxygen were located by the difference Fourier method and were included in the calculation of structure factors with isotropic temperature factors. The final indices were $R=0.0971$, $Rw=0.2652$ and $S=0.988$.

Acknowledgements

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10. Crystallographic data for compound **2a** reported in this paper have been deposited with the Cambridge Crystallographic Data Center and allocated the deposition number CCDC 249311. 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].

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Intramolecular Heck cyclization to the galanthamine-type alkaloids: total synthesis of (±)-lycoramine

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Abstract—A novel approach towards the construction of the galanthamine skeleton was demonstrated by the total synthesis of (±)-lycoramine. The key steps include a Pd-catalyzed intramolecular cyclization to form the seven-membered azepane ring and a spontaneous intramolecular Michael addition to afford the five-membered furan ring. This synthetic route has also been demonstrated to be useful for the preparation of novel derivatives with simplified galanthamine skeletons.

1. Introduction

Galanthamine ((−)-**1**),[1] an alkaloid isolated from the *Amaryllidaceae* species, has been shown to be a potent acetylcholinesterase (AChE) inhibitor and significantly enhances cognitive functions in patients who suffered from Alzheimer's disease (AD).[2–4] Galanthamine was first approved in Austria and most recently in the rest of Europe and in the United States for the treatment of AD.

Galanthamine is produced by isolation from botanical sources (e.g., *Galanthus nivalis*, *G. narcissus*, *G. leucojum*, or *G. crinium*) and these sources are limited.[5] Thus, synthetic approaches to the large-scale production of galanthamine have been sought and a number of total syntheses of galanthamine have appeared in the literatures.[6–26] Most of them utilized a biomimetic oxidative bisphenol coupling to create the critical spiroquarternary carbon of galanthamine.[6–19] Lycoramine ((−)-**2**, 1,2-dihydrogalanthamine), another galanthamine-type alkaloid, has been claimed to have significant activities in inhibiting the formation of peptide bond in protein synthesis.[20] In a formal synthesis of **2**, the intramolecular Heck reaction was used to construct the quaternary carbon center via a 6-*exo* cyclization.[21] From then on, several groups have utilized palladium-catalyzed cyclization to build the quaternary center of galanthamine-type alkaloids.[22–26]

Galanthamine is structurally related to morphine in topology (Chart 1). Morphine and its simplified analogs morphinan, benzomorphan, and 4-phenylpiperidine derivatives, remain the most widely used analgesics for the treatment of severe pain. Therefore, derivatives containing simplified galanthamine skeleton may be potential candidates for the development of novel AChE inhibitors. Prior research in this laboratory has focused on the development of novel synthetic strategies for the efficient preparation of compounds

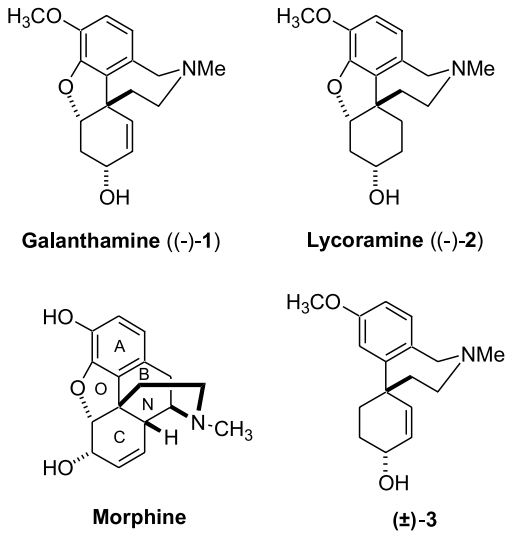

Chart 1.

Keywords: Galanthamine; Lycoramine; Intramolecular cyclization; Heck reaction.

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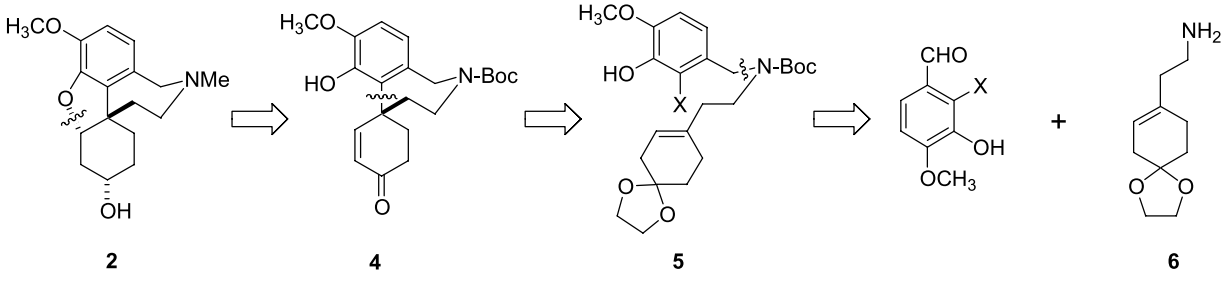
possessing morphine partial structures.[27–32] The Heck reaction was one of the key reactions used for construction of the O ring in the ANO and ACNO ring systems of morphine.[28] This experience has prompted us to extend our methodology to the synthesis of structurally simplified galanthamine analogs, including the 12-aza-benzo[*h*]spiro [5.6]dodecane (±)-**3**, which retains most of the rigidity of galanthamine and demonstrated the desired, albeit weak AChE inhibition.[33] To further explore the utilities of our methodology, we have achieved the total synthesis of the galanthamine-type alkaloid lycoramine ((±)-**2**). Here, we report in detail the novel synthetic strategy for the preparation of (±)-**2**.

2. Results and discussion

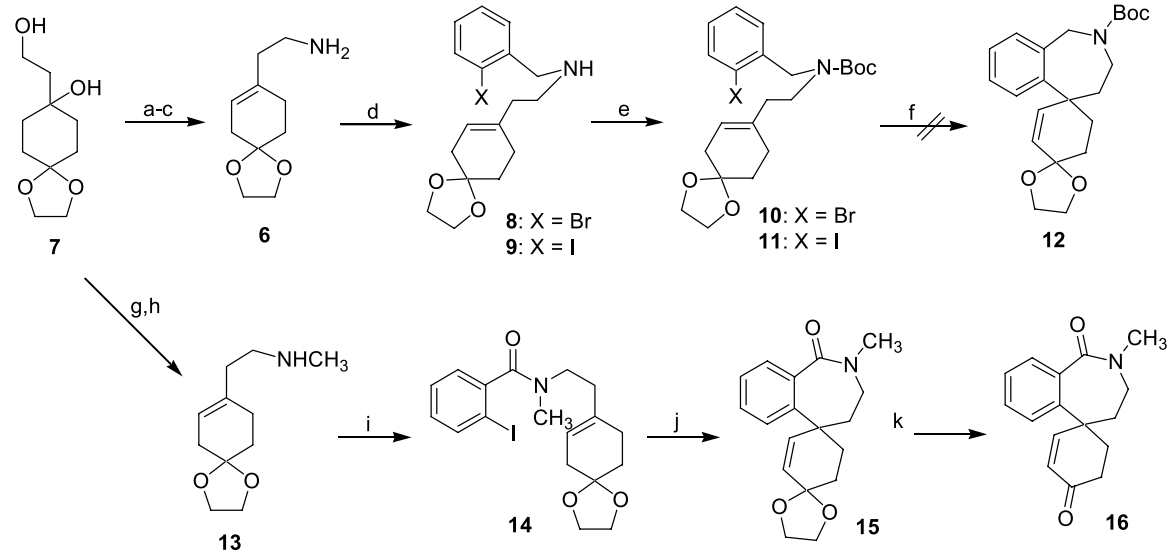
Retrosynthetic consideration led us to propose the route as shown in Scheme 1. The dihydrofuran ring in **2** could be generated by an intramolecular Michael addition of the phenol group to the enone functionality of **4**. The seven-membered tetrahydroazepine ring in compound **4** could be formed via a Pd-catalyzed 7-*exo-trig* cyclization of the key intermediate **5**. Compound **5** can be synthesized from the coupling of a 2-haloisovanillin and 2-(1,4-dioxa-spiro[4,5] dec-7-en-8-yl)ethylamine (**6**) via a reductive amination reaction.

The starting amine **6** was prepared from diol **7** via a reaction sequence of Mitsunobu reaction with phthalimide, dehydration, and deprotection. Diol **7** was obtained from cyclohexane-1,4-dione monoethylene ketal, as previously described.[33] Reductive amination of 2-halobenzaldehydes with amine **6**, followed by protection of the secondary amine intermediates with $(Boc)_2O$ afforded compounds **10** and **11**, respectively (Scheme 2). However, all attempts to effect the intramolecular Heck reaction to prepare the desired cyclized product **12** failed, resulting primarily in dehalogenated products. Earlier reports suggested that compounds with an *endo*-amide group may favor the intramolecular Heck reaction due to the added conformational restriction.[34,35] Therefore, we turned our attention to the use of *endo*-amide intermediates, such as compound **14**.

Treatment of compound **7** with MsCl, followed by methylamine, resulted in nucleophilic substitution and dehydration to afford the secondary amine **13**. The advantage of this alternative process for the preparation of the said amines was to avoid the use of very toxic reagents, DEAD and hydrazine, for the Mitsunobu and the subsequent deprotection steps, respectively. Compound **14** was prepared by the coupling of 2-iodobenzoic acid with amine **13**. To our delight, compound **14** underwent the desired intramolecular Pd-catalyzed cyclization smoothly to provide spiro-amide **15** in 67% yield. However, the desired cyclization was not observed when the bromo-analog of



Scheme 1. Retrosynthetic analysis of galanthamine-type alkaloids (X=Br or I).



Scheme 2. Reagents and conditions: (a) phthalimide, DEAD, PPh_3, THF, rt; (b) *p*-TsOH, toluene, reflux; (c) $N_2H_4 \cdot H_2O$, ethanol, reflux; (d) 2-bromobenzaldehyde or 2-iodobenzaldehyde, CH_2Cl_2, rt; then $NaBH_4$, MeOH, rt; (e) $(Boc)_2O$, $NaHCO_3$, MeOH, rt; (f) Heck reaction conditions; (g) MsCl, CH_2Cl_2, −23 °C to rt; (h) 40% CH_3NH_2, reflux; (i) 2-iodobenzoic acid, $SOCl_2$, THF, −23 °C to rt; (j) $Pd(OAc)_2$, PPh_3, K_2CO_3, CH_3CN, 130 °C; (k) 1 N HCl, rt.

compound **14** was subjected to the same Heck reaction condition. Hydrolysis of the ketal function in **15** with HCl afforded enone **16** in 63% yield.

With the success in the synthesis of compound **16**, we then turned our efforts to the construction of the complete tetracyclic skeleton of galanthamine. Thus, 2-iodoisovanillin (**18**) was prepared from isovanillin in four steps according to the literature.[36] The aldehyde and phenol groups of isovanillin were protected by reacting with trimethyl orthoformate and chloromethyl methyl ether, respectively, to afford compound **17**. Treatment of **17** with *n*-butyl lithium and I_2, followed by acidic work-up furnished compound **18**. Direct oxidation of **18** to the corresponding acid failed due to the sensitivity of its phenol group. Therefore, the phenol group of **18** was first protected as a benzyl ether, and then the protected aldehyde was oxidized with $KMnO_4$ to give acid **19**. Coupling of compound **19** with amine **13** afforded amide **20** in 73% yield (Scheme 3).

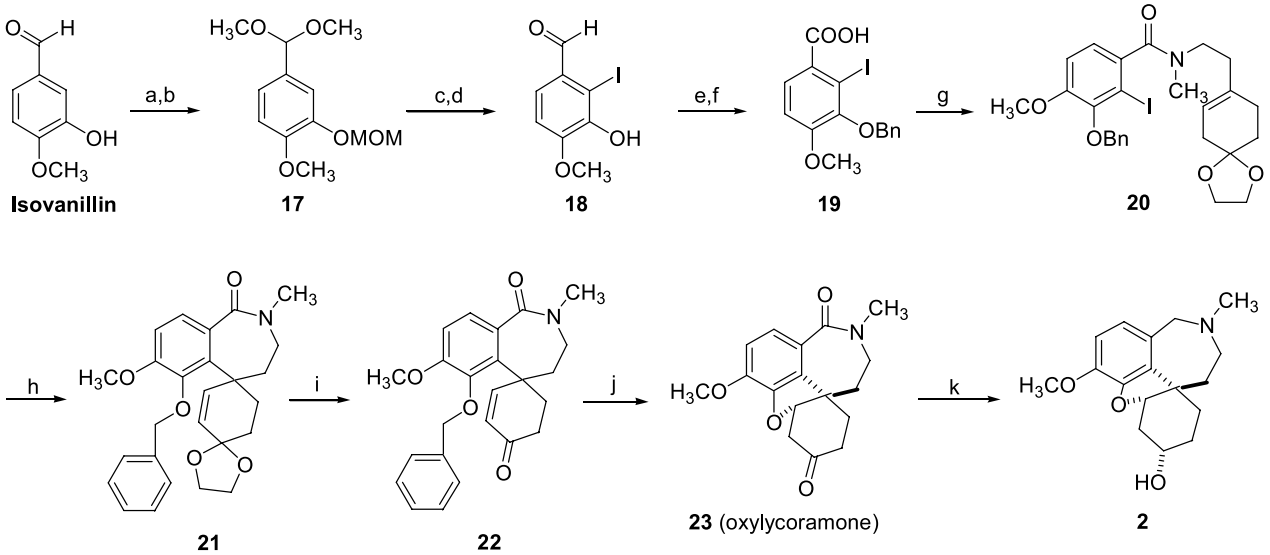
When compound **20** was subjected to Heck reaction condition using $Pd(OAc)_2$, Ph_3P, and K_2CO_3 in CH_3CN, the desired intramolecular cyclization was achieved to provide dispiro-compound **21**. Other reaction conditions, with different phosphine ligands (Ph_3As, BINAP), bases (NEt_3, Ag_2CO_3, proton sponge), and solvents (THF, DMF), resulted in the formation of **21** from **20** in lower yields. Longer reaction time or higher reaction temperature resulted in significant decomposition of the starting compound **20**. The ethylene ketal group of **21** was removed easily when put in contact with silical gel in methanol to give ketone **22** in 95% yield. Subsequent removal of the benzyl group in **22** with $SnCl_4$ was accompanied by a spontaneous intramolecular Michael addition to afford the tetracyclic oxolycoraminone (**23**)[37] in 75% yield. Simultaneous reduction of both the ketone and amide groups of **23** with $LiAlH_4$ afforded (±)-**2**[37,38] with excellent diastereoselectivity (de >95%). The hydroxyl group of (±)-**2** was assigned to be *cis* to the oxide ring based on analysis of its ^1H NMR spectrum, which is identical to that of an authentic sample of lycoramine.

In summary, we have demonstrated a novel synthetic route to the galanthamine skeleton and its simplified analogs, such as **16**. To our knowledge, the direct construction of the seven-membered ring of galanthamine-type alkaloids via an intramolecular Heck reaction as exemplified by the total synthesis of (±)-**2** has not been documented yet. The total synthesis starts from simple cyclohexane-1,4-dione monoethylene ketal and isovanillin, and completes in eight steps with an overall yield of 3%. Notable features of this synthetic route include a Pd-catalyzed intramolecular cyclization and a spontaneous intramolecular Michael addition to form the seven-membered azepane ring and the five-membered furan ring, respectively. This synthetic strategy may be developed into an alternative process for galanthamine production, and provide derivatives possessing simplified galanthamine skeleton as potential drug candidates. Further work towards the synthesis of novel galanthamine-related compounds and their pharmacological studies are in progress.

3. Experimental

3.1. General procedures

Melting points were taken in capillary tubes on a MEL-TEMP II apparatus by Laboratory Devices and are uncorrected. NMR spectra were recorded on Bruker DPX-200 and AMX-400 FT-NMR spectrometers; chemical shifts were recorded in parts per million downfield from Me_4Si. Mass spectra were recorded on a Jeol JMS-D300 mass spectrometer; HRMS was obtained with a Jeol-HX110 mass spectrometer. Elemental analyses were performed with a Perkin–Elmer 2400-CHN instrument. TLC was performed on Merck (art. 5715) silica gel plates and visualized under UV light (254 nm), upon treatment with iodine vapor or upon heating after treatment with 5%



Scheme 3. Reagents and conditions: (a) trimethy orthoformate, CH_2Cl_2, rt; (b) LDA, MOMCl, THF, −78 °C; (c) *n*-butyllithium, I_2, −78 °C; (d) 3 N HCl, rt; (e) benzyl bromide, K_2CO_3, acetone, rt; (f) $KMnO_4$, acetone–H_2O, rt; (g) $SOCl_2$, THF, −23 °C; then **13**, NEt_3, THF, −23 °C to rt; (h) $Pd(OAc)_2$, PPh_3, K_2CO_3, CH_3CN, 130 °C; (i) silical gel, MeOH, rt; (j) $SnCl_4$, CH_2Cl_2, rt; (k) LAH, THF, reflux.

phosphomolybdic acid in ethanol. Flash column chromatography was performed with Merck (art. 9385) 40–63 μm silical gel 60. Anhydrous tetrahydrofuran was distilled from sodium-benzophenone prior to use.

3.1.1. (2-Bromobenzyl)-[2-(1,4-dioxa-spiro[4.5]dec-7-en-8-yl)ethyl]carbamic acid *tert*-butyl ester (10). A mixture of **6**[33] (355 mg, 1.94 mmol) and 2-bromobenzaldehyde (390 mg, 2.13 mmol) in CH_2Cl_2 (30 mL) was stirred at rt for 30 min. The mixture was evaporated and was added MeOH (20 mL) and $NaBH_4$ (232 mg, 5.82 mmol). The resulting mixture was stirred at rt for 1 h. The solution was evaporated and the residue was partitioned with EtOAc (3×15 mL) and H_2O (10 mL). The combined extract was dried over $MgSO_4$, filtered, and evaporated. The residue was chromatographed (silical gel; EtOAc) to afford **8** (442 mg, 65%) as a yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.24–1.78 (m, 4H), 1.92–2.02 (m, 4H), 2.67 (t, J=6.5 Hz, 2H), 3.83 (s, 2H), 3.94 (s, 4H), 5.04 (s, 1H), 7.08 (t, J=7.0 Hz, 1H), 7.24 (t, J=7.0 Hz, 1H), 7.35 (d, J=6.5 Hz, 1H), 7.50 (d, J=7.8 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 27.7, 31.5, 36.0, 37.7, 47.0, 54.1, 64.7, 108.4, 109.9, 120.4, 124.3, 127.8, 128.9, 130.7, 133.1, 135.4. To a solution of **8** (442 mg, 1.26 mmol) in MeOH (30 mL) was added $(Boc)_2O$ (330 mg, 1.51 mmol) and $NaHCO_3$ (260 mg, 3.05 mmol) and stirred at rt for 2 h. The mixture was evaporated and the residue was partitioned with EtOAc (2×20 mL) and H_2O (10 mL). The combined extract was dried over $MgSO_4$, filtered, and evaporated. The residue was chromatographed (silical gel; EtOAc/*n*-hexane 2:3) to afford **10** (368 mg, 64%) as a yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.36–1.47 (m, 9H), 1.75 (t, J=6.4 Hz, 2H), 2.16–2.22 (m, 6H), 3.23–3.27 (m, 2H), 3.93 (s, 4H), 4.45–4.49 (m, 2H), 5.30 (s, 1H), 7.04–7.29 (m, 3H) 7.49 (d, J=7.8 Hz, 1H); MS (EI, 70 eV) *m*/*z* 451 (M^+), 298, 168; HRMS calcd for $C_{22}H_{30}BrNO_4^+$: 451.1358, found 451.1356.

3.1.2. [2-(1,4-Dioxa-spiro[4.5]dec-7-en-8-yl)ethyl]-(2-iodobenzyl)carbamic acid *tert*-butyl ester (11). [2-(1,4-Dioxa-spiro[4.5]dec-7-en-8-yl)ethyl]-(2-iodobenzyl)amine (**9**) was synthesized using compound **6** and 2-iodobenzaldehyde as described above to afford **9** in 55% yield as a yellow oil. Compound **11** was then synthesized from compound **9** as described above to afford **11** in 53% yield as a yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.36–1.49 (m, 9H), 1.90–1.94 (m, 2H), 2.10–2.16 (m, 6H), 3.20–3.21 (m, 2H), 3.93 (s, 4H), 4.35–4.44 (m, 2H), 5.30 (s, 1H), 6.91 (t, J=7.4 Hz, 1H), 7.11 (d, J=7.4 Hz, 1H), 7.27 (t, J=7.4 Hz, 1H), 7.78 (d, J=7.5 Hz, 1H); MS (EI, 70 eV) *m*/*z* 499 (M^+), 398, 216; HRMS calcd for $C_{22}H_{30}INO_4^+$: 499.1220, found 499.1233.

3.1.3. [2-(1,4-Dioxa-spiro[4,5]dec-7-en-8-yl)ethyl]-methylamine (13). To a stirred solution of diol **7**[33] (3.44 g, 17 mmol) and Et_3N (12 mL) in CH_2Cl_2 (125 mL) was added MsCl (2.64 mL, 34 mmol). The resulting mixture was stirred at rt under N_2 for 1 h. The solvent was evaporated and then water (100 mL) was added to the residue. The solution was extracted with CH_2Cl_2 (100 mL ×2) and the combined extract was dried over $MgSO_4$, filtered, and evaporated to afford methanesulfonic acid 2-(1,4-dioxa-spiro[4,5]dec-7-en-8-yl)ethyl ester (2.37 g, 53%) as a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.71–1.77 (t, J=8.6 Hz, 2H), 2.15–2.22 (m, 4H), 2.37–2.43 (t, J=8.6 Hz, 2H), 2.97 (s, 3H), 3.91–3.96 (m, 4H), 4.23–4.30 (m, 2H), 5.40–5.41 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 27.6, 30.9, 35.6, 36.4, 37.4, 64.3, 68.3, 107.6, 121.8, 132.1. To a stirred mixture of the mesylate (1.29 g, 4.92 mmol) and Et_3N (1 mL) in THF (10 mL) was added methylamine (40% in water, 2.55 mL). The reaction mixture was heated at reflux overnight and the solvent was evaporated. The residue was treated with water (15 mL) and then extracted with a solution of IPA/$CHCl_3$ (1:4; 3×15 mL). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to afford **13** (870 mg, 90%) as a yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.70–1.75 (m, 3H), 2.15–2.20 (m, 5H), 2.40 (s, 3H), 2.63 (t, J=7.4 Hz, 2H), 3.94 (s, 4H), 5.33 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 27.8, 31.0, 33.5, 35.6, 41.4, 55.5, 64.3, 107.8, 119.8, 134.7; MS (EI, 70 eV) *m*/*z* 198 (M^++1), 157 (base); HRMS calcd for $C_{11}H_{19}NO_2^+$: 197.1416, found 197.1432.

3.1.4. *N*-[2-(1,4-Dioxa-spiro[4.5]dec-7-en-8-yl)-ethyl]-2-iodo-*N*-methyl-benzamide (14). To a solution of 2-iodobenzoic acid (1.50 g, 6.04 mmol) in THF (50 mL) was added $SOCl_2$ (0.7 mL, 6.64 mmol) at −78 °C and stirred for 30 min. The temperature was allowed to raise to −20 °C, and then a solution of **13** (1.20 g, 6.56 mmol) and Et_3N (2.3 mL, 18.1 mmol) in THF (15 mL) was added. The mixture was stirred for 6 h and the reaction temperature was slowly raised to rt. The solvent was evaporated and the residue was partitioned with CH_2Cl_2 (50 mL) and H_2O (20 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was chromatographed (silica gel; EtOAc/*n*-hexane 3:2) to afford **14** (1.62 g, 63%) as a yellow oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.60 (t, J=6.0 Hz, 1H), 1.72 (t, J=6.0 Hz, 1H), 1.84–1.88 (m, 1H), 2.0–2.3 (m, 5H), 2.72 and 3.04 (s, 3H), 3.06–3.11 (m 1H), 3.51–3.65 (m, 1H), 3.87 and 3.90 (s, 4H), 5.16 and 5.38 (s, 1H), 6.91–7.05 (m, 1H), 7.12–7.17 (m, 1H), 7.27–7.31 (m, 1H), 7.71–7.77 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 27.3, (30.9, 32.4), 34.0, (35.6, 36.3), 45.3, 49.4, 64.2, (92.1, 92.6), (107.4, 107.7), (120.2, 120.8), (126.8, 127.2), (128.0, 128.2), (129.8, 129.9), (133.3, 134.4), 138.9, (142.4, 142.8), (170. 170.6); IR (neat) cm^{-1} 3450, 2926, 1636, 1488; MS (EI, 70 eV) *m*/*z* 427 (M^+), 166; HRMS calcd for $C_{18}H_{22}INO_3^+$: 427.0644, found 427.0621. (Since the amide group in **14** could adopt either a *cis* or *trans* configuration, some of the ^{1}H and ^{13}C signals appear as pairs.)

3.1.5. 12-Methyl-12-aza-benzo[*h*]spiro[5.6]dodec-1-en-3,11-dione (16). A solution of **14** (950 mg, 2.22 mmol), $Pd(OAc)_2$ (54 mg, 0.22 mmol), triphenylphosphine (233 mg, 0.89 mmol), and K_2CO_3 (618 mg, 4.44 mmol) in acetonitrile (150 mL) was heated in a sealed round bottle at 130 °C for 36 h. The mixture was cooled, filtered, and evaporated to afford crude 14-methyl-1,4-dioxa-14-aza-benzo[*j*]dispiro[4.2.6.2]hexadec-6-en-13-one (**15**). The crude **15** was treated with 1 N HCl for 30 min. The resulting mixture was evaporated and partitioned with CH_2Cl_2 (50 mL) and H_2O (20 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by chromatography (silica gel; EtOAc/*n*-hexane 3:2) to afford **16** (349 mg, 63%). Compound **16** was recrystallized from EtOAc as colorless crystals: ^{1}H NMR

(200 MHz, $CDCl_3$) δ 2.11–2.39 (m, 6H), 3.15 (s, 3H), 3.18–3.38 (m, 1H), 3.38–3.50 (m, 1H), 6.06 (d, $J=10.6$ Hz, 1H), 6.91 (dd, $J=10.4$, 1.0 Hz, 1H), 7.21–7.25 (m, 1H), 7.35–7.40 (m, 2H), 7.71–7.75 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 34.3, 34.4, 38.0, 42.4, 43.7, 48.2, 126.5, 127.9, 129.0, 130.4, 130.7, 135.6, 138.9, 156.5, 170.6, 198.8; IR (KBr) cm^{-1} 3478, 1680, 1639; MS (EI, 70 eV) *m/z* 255 (M^+, base). Anal. Calcd for $C_{16}H_{17}NO_2$: C, 75.27; H, 6.71; N, 5.49. Found: C, 75.21; H, 6.65; N, 5.26.

3.1.6. 3-Benzyloxy-2-iodo-4-methoxybenoic acid (19). A solution of 2-iodo-isovanillin (**18**)[36] (1.50 g, 5.39 mmol), benzyl bromide (2.0 mL, 7.54 mmol), and K_2CO_3 (3.75 g, 27.1 mmol) in acetone (60 mL) was heated at reflux for 2 h. The solvent was evaporated and the crude was purified by recrystallization from EtOAc to give 3-benzyloxy-2-iodo-4-methoxy-benaldehyde (1.97 g, 99%) as a light yellow solid. To a solution of 3-benzyloxy-2-iodo-4-methoxy-benaldehyde (1.30 g, 3.53 mmol) in acetone (60 mL) was added $KMnO_4$ (19.5 g, 12.3 mmol) in water (30 mL) and stirred for 24 h. The mixture was filtered to remove MnO_2 and the filtrate was partitioned with 1 N NaOH (20 mL) and CH_2Cl_2 (100 mL). The organic layer was collected to recover the starting material. The aqueous layer was separated, acidified with 3 N HCl (20 mL), and extracted with a solution of IPA/$CHCl_3$ (1:4; 80 mL $\times$2). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to afford **19** (1.55 g, 75%) as a yellow solid. This solid was recrystallized from CH_2Cl_2 to give colorless crystals: mp 176–178 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 3.92 (s, 3H), 5.0 (s, 3H), 6.93 (d, $J=8.7$ Hz, 1H), 7.33–7.41 (m, 3H), 7.58–7.60 (m, 2H), 7.87 (d, $J=8.7$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.1, 74.2, 95.8, 111.2, 125.9, 128.1, 128.3, 128.5, 129.5, 136.8, 148.6, 156.1, 171.0; IR (neat) cm^{-1} 1687, 1577, 1274; MS (EI, 70 eV) *m/z* 384 (M^+), 91 (base); HRMS calcd for $C_{15}H_{13}IO_4^+$: 383.9859, found 383.9847.

3.1.7. 3-Benzyloxy-*N*-[2-(1,4-dioxa-spiro[4.5]dec-7-en-8-yl)-ethyl]-2-iodo-4-methoxy-*N*-methyl-benzamide (20). A mixture of **19** (355 mg, 0.93 mmol), $SOCl_2$ (0.11 mL, 1.02 mmol), and DMF (three drops) in THF (30 mL) was stirred at −78 °C for 30 min. The reaction temperature was raised to −40 °C and then a solution of **13** (240 mg, 1.21 mmol) and Et_3N (0.36 mL, 2.77 mmol) in THF (10 mL) was added. The resulting mixture was stirred and the temperature was slowly raised to rt. The solution was evaporated and the residue was partitioned with EtOAc (3$\times$15 mL) and H_2O (10 mL). The combined extract was dried over $MgSO_4$, filtered, and evaporated to afford **20** (382 mg, 73%) as a yellow oil: ^1H NMR (400 MHz, $CDCl_3$) δ 1.60–1.68 (m, 1H), 1.73–1.77 (m, 1H), 1.80–1.93 (m, 1H), 2.16–2.34 (m, 5H), 2.72 and 3.05 (s, 3H), 3.12–3.20 (m, 1H), 3.60–3.62 (m, 1H), 3.83 (s, 3H), 3.90–3.93 (m, 4H), 4.91–4.97 (m, 2H), 5.19 and 5.40 (s, 1H), 6.90–6.92 (m, 2H), 7.30–7.35 (m, 3H), 7.49–7.55 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.4, 31.0, (32.6, 32.4), (35.5, 36.5), 45.6, 49.7, 64.3, 74.4, (92.1, 92.5), (107.6, 107.9), (112.7, 112.9), (120.2, 120.8), (122.8, 123.2), (128.1, 128.3), (128.5, 128.6), 131.9, (132.1, 132.6), (135.9, 136.3), 136.9, 147.8, 152.4, (170.2, 170.6); IR (neat) cm^{-1} 2927, 1633, 1261; MS (EI, 70 eV) *m/z* 563 (M^+), 367 (base); HRMS calcd for $C_{26}H_{30}INO_5^+$: 563.1169, found 563.1143. (Since the amide group in **20** could adopt either a *cis* or *trans* configuration, some of the ^1H and ^{13}C signals appear as pairs.)

3.1.8. 9-Benzyloxy-10-methoxy-14-methyl-1,4-dioxa-14-aza-benzo[*j*]dispiro[4.2.6.2]hexadec-6-en-13-one (21). A mixture of **20** (250 mg, 0.44 mmol), $Pd(OAc)_2$ (9 mg, 0.04 mmol), triphenylphosphine (23 mg, 0.088 mmol), and K_2CO_3 (123 mg, 0.86 mmol) in CH_3CN (40 mL) was heated in a sealed round bottle at 130 °C for 5 days. The resulting mixture was filtered, evaporated, and partitioned with CH_2Cl_2 (50 mL) and H_2O (20 mL). The organic extract was dried over $MgSO_4$, filtered, and evaporated. The residue was purified with prepared TLC (silica gel; EtOAc/*n*-hexane/$NH_{3(conc)}$ 3:2:0.005) to afford compound **21** (53 mg, 28%) as an oil with recovered starting material **20** (21%) and de-iodinated by-product (15%). Compound **21**: ^1H NMR (200 MHz, $CDCl_3$) δ 1.58–1.95 (m, 4H), 2.12–2.29 (m, 2H), 2.96–3.03 (m, 1H), 3.09 (s, 3H), 3.29–3.43 (m, 1H), 3.87 (s, 3H), 3.92–4.00 (m, 4H), 4.68 (d, $J=10.3$ Hz, 1H), 4.87 (d, $J=10.3$ Hz, 1H), 5.32 (d, $J=10.6$ Hz, 1H), 6.30 (d, $J=10.1$ Hz, 1H), 6.88 (d, $J=8.4$ Hz, 1H), 7.24–7.38 (m, 3H), 7.42–7.50 (m, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 30.9, 34.2, 38.5, 43.3, 44.0, 48.1, 55.6, 64.2, 64.4, 74.0, 105.7, 110.1, 121.9, 126.3, 127.9, 128.1, 132.0, 135.3, 138.1, 141.6, 146.2, 155.3, 171.1; MS (EI, 70 eV) *m/z* 435 (M^+), 91 (base); HRMS calcd for $C_{26}H_{29}NO_5^+$: 435.2046, found 435.2030.

3.1.9. 7-Benzyloxy-8-methoxy-12-methyl-12-aza-benzo[*h*]spiro[5.6]dodec-1-en-3,11-dione (22). To a solution of **21** (53 mg, 0.12 mmol) in methanol (10 mL) was added silical gel (20 mg) and stirred at rt for 1 h. The mixture was filtered and evaporated. The residue was partitioned with CH_2Cl_2 (50 mL) and H_2O (20 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated to afford compound **22** (45 mg, 95%) as a light yellow oil: ^1H NMR (400 MHz, $CDCl_3$) δ 1.89 (dd, $J=14.4$, 1.4 Hz, 1H), 2.09 (ddd, $J=12.0$, 9.6, 2.8 Hz, 1H), 2.19–2.23 (m, 1H), 2.24–2.32 (m, 2H), 2.41–2.44 (m, 1H), 3.05–3.11 (m, 1H), 3.11 (s, 3H), 3.39–3.48 (m, 1H), 3.90 (s, 3H), 4.40 (d, $J=10.8$ Hz, 1H), 4.98 (d, $J=10.8$ Hz, 1H), 5.69 (d, $J=10.0$ Hz, 1H), 6.94 (d, $J=10.0$ Hz, 1H), 7.23 (d, $J=8.4$ Hz, 1H), 7.25–7.34 (m, 5H), 7.50 (d, $J=8.4$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.3, 34.7, 39.7, 42.2, 44.0, 48.0, 55.7, 74.1, 110.9, 123.4, 126.8, 127.7, 127.9, 128.5, 129.2, 133.4, 136.8, 145.5, 155.0, 158.5, 170.5, 200.0; IR (neat) cm^{-1} 3428, 2938, 1672, 1635; MS (EI, 70 eV) *m/z* 391 (M^+), 91 (base); HRMS calcd for $C_{24}H_{25}NO_4^+$: 391.1784, found 391.1773.

3.1.10. Oxylycoramone (23).[37] To s solution of compound **22** (29 mg, 0.074 mmol) was added $SnCl_4$ (72 mg, 0.27 mmol) in CH_2Cl_2 (5 mL). After 30 min, the mixture was evaporated and partitioned with CH_2Cl_2 (20 mL) and $NH_{3(conc)}$ (10 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated to afford compound **23** (16.7 mg, 75%) as a light yellow oil: ^1H NMR (400 MHz, $CDCl_3$) δ 1.80–1.88 (m, 1H), 1.92–1.99 (m, 1H), 2.01–2.08 (m, 2H), 2.17–2.22 (m, 1H), 2.45 (td, $J=7.0$, 2.2 Hz, 1H), 2.76 (dd, $J=8.6$, 1.4 Hz, 1H), 2.99 (dd, $J=8.6$, 1.4 Hz, 1H), 3.13 (s, 3H), 3.15–3.20 (m, 1H), 3.67–3.78 (m, 1H), 3.88 (s, 3H), 4.91 (s, 1H), 6.87 (d, $J=8.4$ Hz, 1H), 7.39 (d, $J=$

8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.8, 35.8, 36.7, 40.7, 43.0, 48.0, 49.5, 56.0, 91.0, 112.3, 123.8, 123.9, 130.7, 146.1, 146.6, 168.9, 207.8; IR (neat) cm^{-1} 2928, 1719, 1636; MS (EI, 70 eV) *m/z* 301 (M^+, base); HRMS calcd for $C_{17}H_{19}NO_4^+$: 301.1314, found 301.1301.

3.1.11. Lycoramine (2).[37,38] To a solution of **23** (20 mg, 0.066 mmol) in THF (15 mL) was added LAH (5 mg, 0.13 mmol) and heated at reflux for 24 h. The mixture was evaporated and the residue was chromatographed (silica gel; CH_2Cl_2/MeOH 8:1) to afford **2** (10 mg, 53%) as a light yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51–1.56 (m, 1H), 1.63–1.71 (m, 1H), 1.73–1.80 (m, 2H), 1.83–1.89 (m, 1H), 1.91–1.98 (m, 1H), 2.36 (s, 3H), 2.34–2.49 (m, 2H), 3.04 (d, J=14.4 Hz, 1H), 3.21 (t, J=12.8 Hz, 1H), 3.62 (d, J=14.8 Hz, 1H), 3.81 (s, 3H), 4.01 (d, J=14.8 Hz, 1H), 3.99–4.05 (m, 1H), 4.34 (s, 1H), 6.57 (d, J=8.4 Hz, 1H), 6.63 (d, J=8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.8, 27.7, 31.0, 31.6, 41.5, 46.7, 54.0, 55.9, 60.3, 65.4, 90.0, 110.8, 122.0, 128.1, 136.2, 144.3, 146.0; IR (neat) cm^{-1} 3365, 2932, 1506; MS (EI, 70 eV) *m/z* 289 (M^+, base); HRMS calcd for $C_{17}H_{23}NO_3^+$: 289.1678, found 289.1678.

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