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Tetrahedron

Tetrahedron Vol. 60, No. 18, 2004

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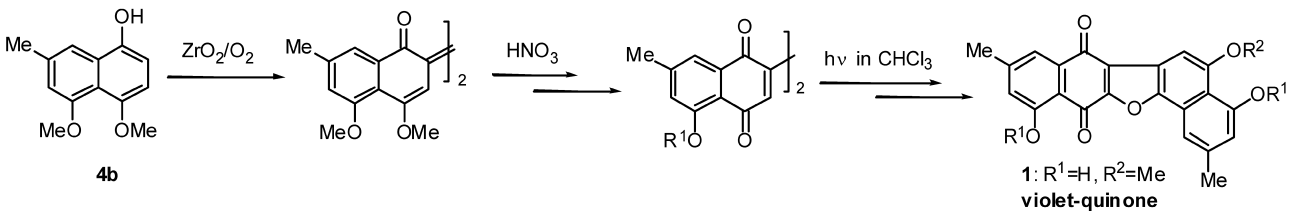
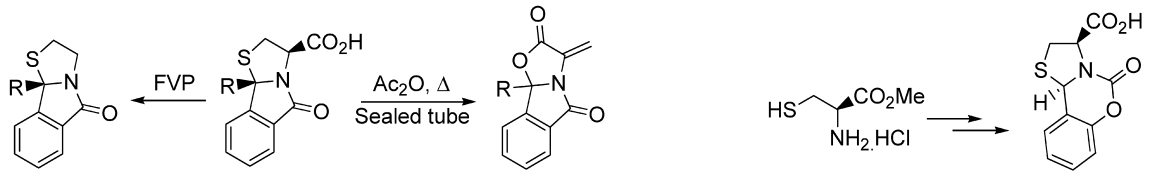
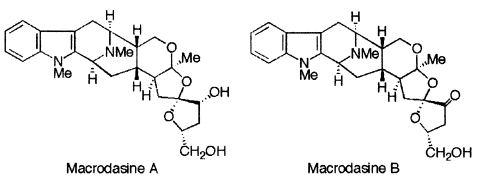
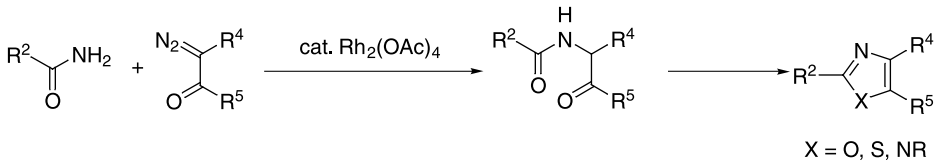
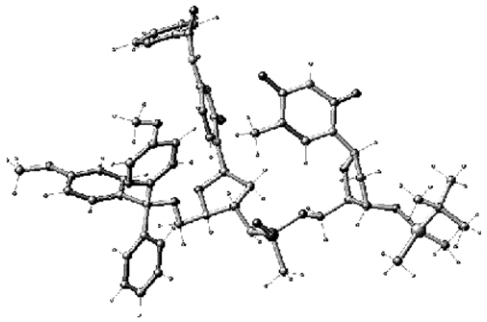


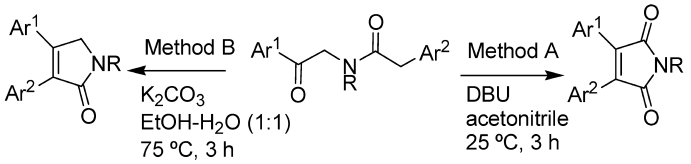


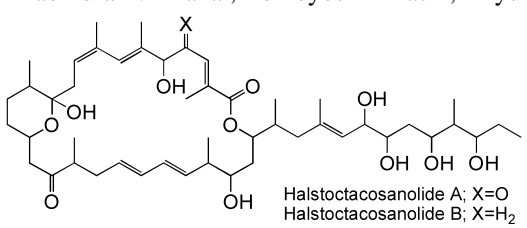


The bark extract of the Malayan *A. macrophylla* provided several indoles with unprecedented carbon skeletons, an unusual nitrogenous compound, a cytotoxic bisindole, several new macroline alkaloids, in addition to other known alkaloids. The structures of the new compounds were established by spectroscopic analysis.

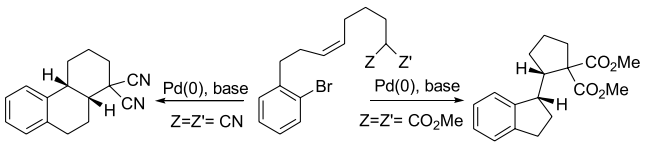




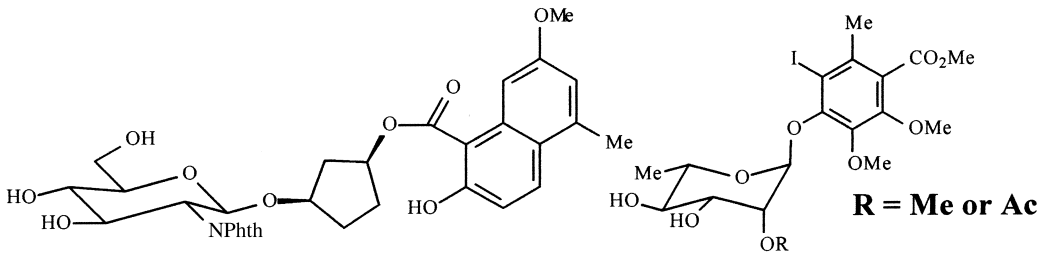


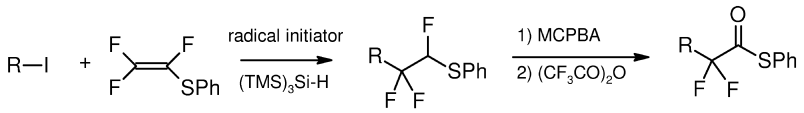


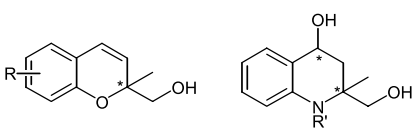
Genetically predicted polyketides were materialized by isolation of two new compounds, halstoctacosanolides A and B, from the fermentation broth of *Streptomyces halstedii* HC34, and the structures were determined to have a novel 28-membered macrolactone.

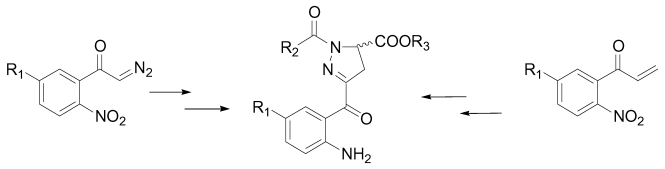


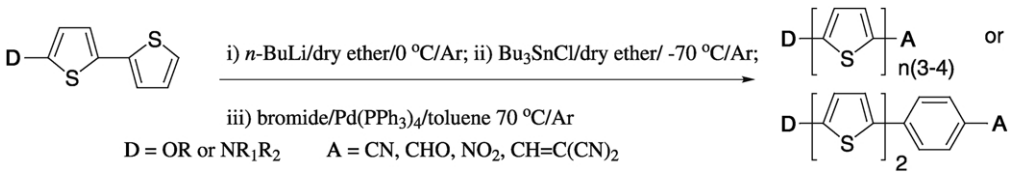
Regioselectivity and stereoselectivity of the biscyclization are depending on the stereochemistry of the initial alkene and on the nature of the nucleophile.

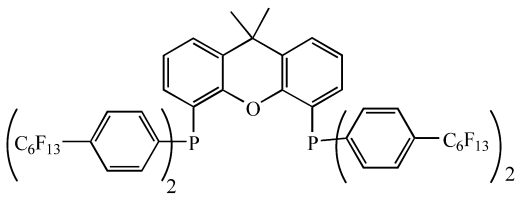


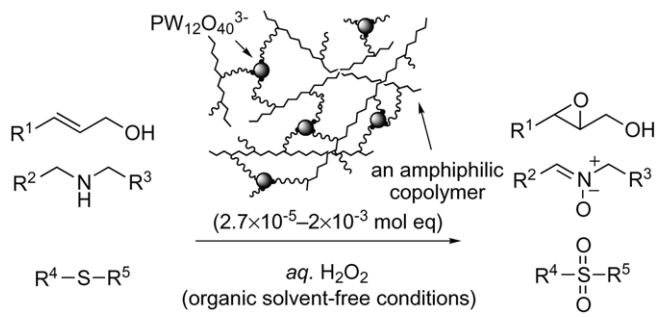


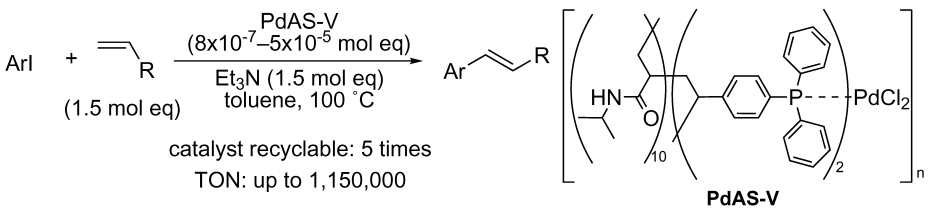


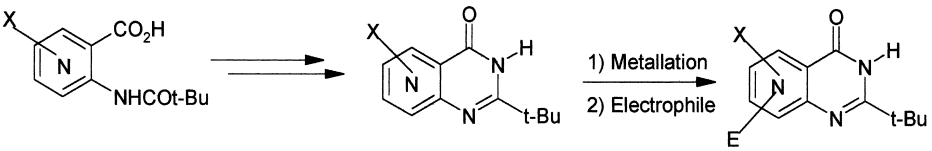












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ISSN 0040-4020

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Tetrahedron 60 (2004) 3941–3948
Tetrahedron

Biomimetic synthesis of the dinaphthofuranquinone violet-quinone, utilizing oxidative dimerization with the ZrO_2/O_2 system

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Received 10 February 2004; revised 11 March 2004; accepted 11 March 2004

Abstract—The first total and biomimetic synthesis of violet-quinone (**1**), which has a dinaphthofuranquinone (DNFQ) framework, is described. This synthesis features the oxidative dimerization of 1-naphthol **4** and the construction of the DNFQ framework by photochemical ring closure of 2,2′-binaphthoquinone **7** as a key intermediate. Compound **7** was prepared by the novel oxidative dimerization of **4** with a semiconductor (such as ZrO_2) in the presence of dioxygen, followed by oxidation of the resulting 2,2′-binaphthyl-1,1′-quinone **6** with HNO_3.

1. Introduction

Among natural biarylquinones, dinaphthofuranquinones (DNFQ) such as violet-quinone (**1**)[1] and balsaminone A (**2**),[2] and dibenzofuranquinones (DBFQ) such as popolohuanone E (**3**),[3] having the dibenzofuran-1,4-dione moiety as a key structural element, have been isolated from several plants and marine products. Compounds **2** and **3** show antipruritic activity[2] and selective cytotoxicity against A549 non-small cell lung cancer cells, respectively (Fig. 1).[3]

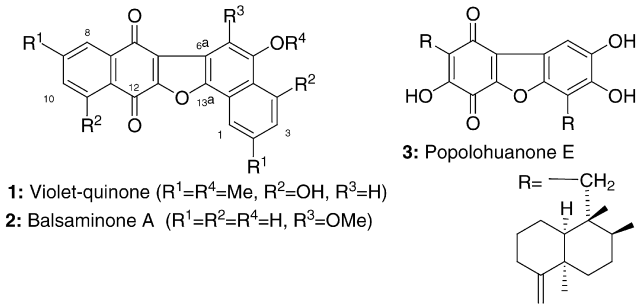

Figure 1.

A possible biogenetic pathway to biarylfuranquinones **E** (DNFQs and DBFQs) would involve (i) the oxidative biaryl coupling of the corresponding two aryls **A** (1-naphthols or phenols), (ii) selective oxidation of biaryls **B**, and (iii) subsequent intramolecular ring closure of biarylquinones **C** or **D** to form the corresponding furan rings, as shown in Figure 2.[3] There is some support for such a biogenetic pathway. Thus, violet-quinone (**1**), along with diosindigo B (**6c**) and biramentaceone (**7c**), which are related to the intermediates **C** and **D**, have been isolated from the heartwood of *Diospyros melanoxyloin*,[1] and its congener *Diospyros celebica*[4] also contains dihydrodiosindigo B (**5c**), corresponding to **B**, together with **6c** and **7c** (refer to Fig. 2, Schemes 2 and 3).

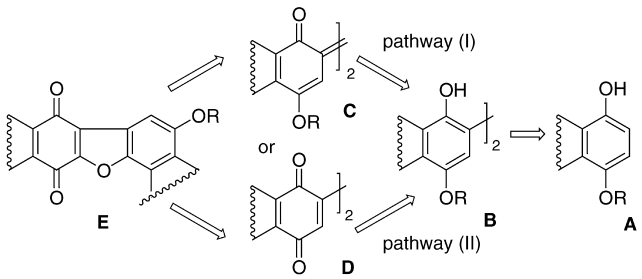

Figure 2.

Although the synthesis of **3** has been explored[5] owing to the biological activity of **3**, as described above, a total synthesis has not yet been achieved. Violet-quinone (**1**) has an analogous framework (the diarylfuran-1,4-dione moiety) to popolohuanone E (**3**). From the viewpoint of the structure–cytotoxic activity relationship of **3**, compound **1** is of great interest as a synthetic target. As yet, its synthesis has not yet been accomplished.

Several methods have been developed for the preparation of

Keywords: Violet-quinone; Dinaphthofuranquinone; First synthesis; Oxidation; Zirconium dioxide.
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0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.03.038

2,2′-binaphthyl derivatives such as **B**, **C** and **D** by the oxidative dimerization of 1-naphthols. These involve chemical,[6] electrolytic,[7] thermal disproportionation[8] and air oxidation[9] reactions. However, the reactions are difficult to control, mostly showing poor selectivity and low yield of the desired products, accompanied with side reactions.

Recently, much attention has been focused on the use of various semiconductor catalysts,[10] particularly TiO_2, to achieve a variety of organic reactions and syntheses on the basis of the concept of green chemistry.[11] More recently, we developed a new and efficient method for the direct synthesis of 2,2′-binaphthyls, utilizing an oxidative dimerization of 1-naphthols (NPOH), with semiconductors such as ZrO_2 and activated charcoal (Act-C) in the presence of dioxygen (O_2).[12]

This method has stimulated further studies with the aim of applying it to biomimetic synthesis of natural products. Here, we present the first total synthesis of violet-quinone (**1**), utilizing the oxidative dimerization of NPOH **4b** with the ZrO_2/O_2 reagent system.

2. Results and discussion

2.1. Synthetic plan

For our feasibility study, we envisioned the biomimetic synthesis of DNFQ **1** through pathway (I) or (II), based on our biogenetic hypotheses mentioned above (Fig. 2).

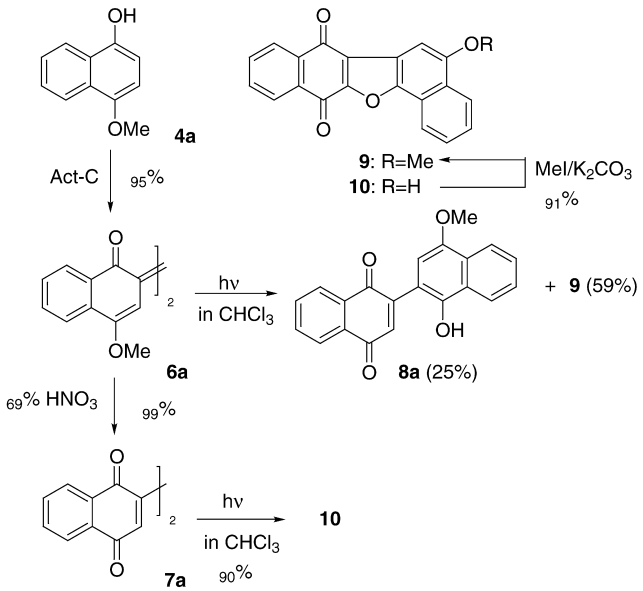
Pathway (I) consists of the formation of 2,2′-binaphthyl-1,1′-quinone (**6**; BNPTQ), which is related to intermediate **C**, by the oxidative dimerization of NPOH **4** and the construction of DNFQ framework by intramolecular ring closure of **6**. In contrast, pathway (II) involves the formation of 2,2′-binaphthoquinone (**7**; BNPQ), which is related to intermediate **D**, and the construction of DNFQ framework by ring closure of **7**. These approaches are attractive because of the simplicity of the reaction and its possible involvement in biosynthesis of naturally occurring DNFQs and DBFQs as described above.

2.2. Preliminary experiments for synthesis of violet-quinone

As a prelude to the synthesis of violet-quinone (**1**), preliminary experiments using 4-methoxy-1-naphthol **4a** as a model substrate were examined. These were based on pathways (I) and (II) described above (Scheme 1).

First, BNPTQ **6a**,[13a] the required model intermediate in pathway (I), was prepared in 95% yield by the oxidative dimerization of **4a** with the Act-C/O_2 system[12] in MeCN. The reaction with the ZrO_2/O_2 system under similar conditions gave **6a** (75%) along with 4-methoxy-1,2-naphthoquinone (17%). Subsequently, **6a** could be easily converted to BNPQ **7a**[13d] as the model intermediate in pathway (II), in 99% yield, by oxidation with 69% HNO_3.

Several methods have been reported for the construction of the DNFQ framework by ring closure of BNPTQs or



Scheme 1.

BNPQs. These include photochemical,[13a,d] thermal,[13e] and chemical (with acid[13d] or base[13f–h]) reactions. We examined the photolysis of **6a** and **7a** under various conditions.

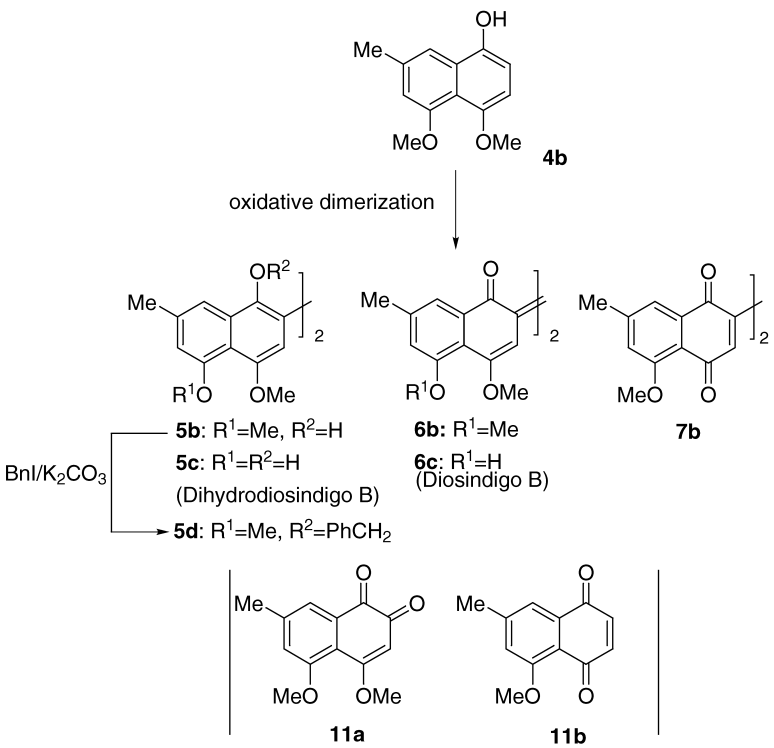
The photolysis of **6a** using a 60 W Hg lamp in $CHCl_3$ with a Pyrex vessel for a long time (80 h) gave **8a**[13a] in 88% yield. When we used a 450 W Hg lamp under similar conditions but for a short time (1 h), DNFQ **9** (59%) and **8a** (25%) were obtained. In the photolysis of **7a**, the best result was obtained with a 450 W Hg lamp in $CHCl_3$ for 2 h, affording DNFQ **10** in 90% yield, and methylation of **10** with CH_3I gave **9** in 91% yield. This mechanism for the photochemical conversion of **7a** into **10** was proposed to proceed via ring closure with rearrangement.[13d,i] The synthetic compounds **9**[13a] and **10**[13d,e] were identical with the corresponding compounds reported previously. In addition, the structures **9** and **10** were confirmed by analyses of the IR, ^{1}H-, ^{13}C NMR spectra with the aid of 2D NMR analyses.

2.3. First total synthesis of violet-quinone

On the basis of the results and information obtained from the above model experiments, the synthesis of **1** utilizing **7b** as the key intermediate was investigated based on pathway (II). First, NPOH **4b** was synthesized according to the protocol reported previously.[14a,b,15a]

In order to obtain BNPOH **5b** or BNPTQ **6b** as a precursor for obtaining **7b**, oxidative dimerization of **4b** using several reagents was examined (Table 1 and Scheme 2). The reaction with Ag_2O gave a mixture of **5b**, **6b** and the *ortho*-naphthoquinone **11a** (entry 1). However, **5b** could not be isolated because it proved very susceptible to air oxidation and decomposition. The structure **5b** was thus confirmed by converting this compound into the benzylated derivative **5d**.

In the case of the well-known AgO/HNO_3 system,[6m] the desired compound **7b** was obtained in one step, but the yield was not satisfactory (entry 2). Laatsch[15a] reported that the



Scheme 2.

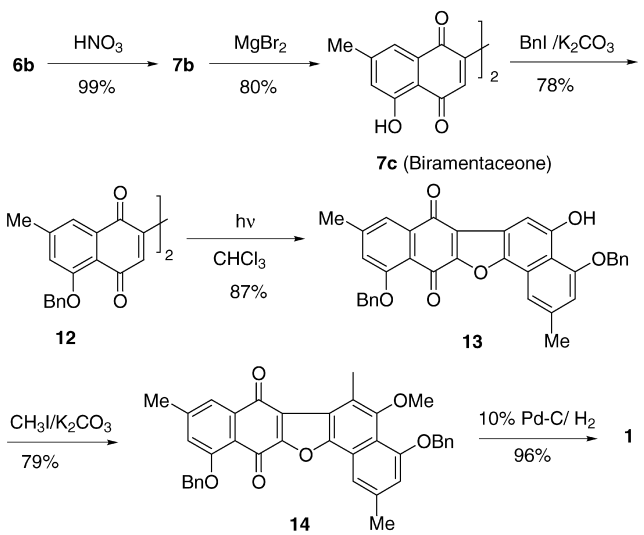
oxidative dimerization of **4b** with the Ag_2O/NEt_3 system[6n] gave only **6b** without any by-product. Re-examination of the reaction by us afforded **6b** (92%) together with the *para*-naphthoquinone **11b** (4%) (entry 3). The best result was obtained by employing the novel oxidative dimerization with the ZrO_2/O_2 reagent system, which we recently developed, affording BNPTQ **6b**[15a] selectively in excellent yield (entry 5). Subsequent oxidation of **6b** with 69% HNO_3 produced **7b**[15a] in 99% yield. When **6b** prepared by means of the above oxidation was used without purification, **7b** was obtained in a similar yield.

Furthermore, the synthesis of **1** from the resulting **7b** was investigated, as shown in Scheme 3. Magnesium bromide ($MgBr_2$) effected demethylation of the methoxyl group of **7b** to produce **7c** as a natural product, the so-called biramentaceone,[15a] in 80% yield. The naphtholic hydroxyl group of **7c** was protected with a benzyl group using benzyl iodide[16] in the presence of K_2CO_3 to yield BNPQ **12**. Subsequently, the ring closure of **12** was achieved by means of photolysis[13a,b] using a 450 W Hg lamp in $CHCl_3$ with a Pyrex vessel for 1 h to give DNFQ **13** in 87% yield. Next, compound **14** was obtained by methylation of **13** with methyl iodide. Finally, the reductive deprotection of the benzyl group of **14** with 10% Pd/C–H_2 gave violet-quinone (**1**) in 96% yield, as a violet solid. All physical data, such as the melting point, MS (Mass), IR (infrared) and ^1H NMR spectra of the synthetic product **1** were identical with those of the natural product.[1]

Table 1. Oxidative dimerization of **4b** with various reagents

Entry	Reagent	Time (h)	Product (isolated yield, %)				
			5d	**6b**	**7b**	**11a**	**11b**
1[a,b]	Ag_2O	0.5	30[c]	34	—	8	—
2[b]	AgO/HNO_3	0.5	—	—	50	—	17
3[b]	Ag_2O/NEt_3	0.5	—	92	—	—	4
4[a]	Act-C[d]/O_2	24	20[c]	44	—	11	9
5[e]	ZrO_2/O_2	19	—	96	—	—	—

[a] This reaction with Ag_2O in $CHCl_3$ gave a complex mixture containing **5b**. In order to isolate **5b**, we performed column chromatography of the reaction mixture under various conditions. However, all the attempts were unsuccessful, producing mainly solid mixtures of **5b** and **6b**. Furthermore, the mixture of **5b** and **6b** was treated with benzyl iodide/K_2CO_3 to give **5d** together with non-reacted **6b**.
[b] Under air at 23 °C.
[c] Yield from **4b**.
[d] Activated charcoal.
[e] Using a dioxygen (O_2)-saturated solvent (MeCN) at 70 °C.



Scheme 3.

2.4. Structure of violet-quinone

There is, to our knowledge, only one article relating to violet-quinone (**1**). This was published by Shidhu et al. in 1981.[1] In this article, a structural analysis of naturally occurring **1** based on the analysis of ^{1}H NMR spectrum was described. However, 2D NMR methods and ^{13}C NMR spectroscopy were not employed. We therefore confirmed the structure of the synthetic compound **1** by means of detailed analyses of the ^{1}H- and ^{13}C NMR spectra with the aid of various 2D NMR experiments.[17]

All ^{1}H- and ^{13}C NMR signal assignments, except for those of the carbons C6a and C6b, were confirmed by means of H–H COSY, C–H COSY and HMBC spectral analyses and by comparison of the spectra with those of the reference compounds **9**, **10** (which were synthesized by us), and balsaminone A (**2**) described in a previous report [2] (refer to Table 2 and Fig. 3).

The ^{1}H NMR spectrum of **1** showed the following signals: (i) two singlets (δ 2.52 and δ 2.47) due to the C2- and C9-methyl protons, a singlet (δ 4.18) due to the C5-methoxyl protons, and a singlet (δ 7.35) assignable to the C6-proton; (ii) a singlet (δ 12.12) due to the hydrogen-bonded hydroxyl at C-11 was observed at lower field than a singlet (δ 9.41) due to the C4-hydroxyl group. The ^{13}C NMR spectrum of **1** displayed signals for all 23 carbons in the molecule: one methoxyl, two carbonyls, two aromatic methyls and 18 aromatic carbons, five of which were protonated, eight quaternary, and five bearing oxygen (Table 2). Accordingly, the above data proved that violet-quinone has the structure **1**.

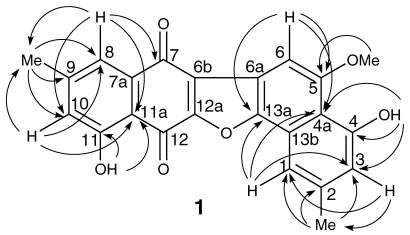


Figure 3. Long-range correlation in the HMBC spectrum of violet-quinone (**1**).

3. Conclusion

The first and biomimetic synthesis of the natural product violet-quinone (**1**) using BNTQ **7b** as a key intermediate was accomplished, based on pathway (II), in 11 steps from methyl 3-methyl-2-butenoate[14a] as a starting material with an overall yield of ca. 13%. In this first synthesis, a key intermediate **7b** was selectively synthesized in excellent yield by utilizing a novel oxidative dimerization of **4b** with the ZrO_2/O_2 system. Furthermore, the construction of the DNFQ framework from BNPQ **12** was achieved by photolysis using a 450 W Hg lamp. The structure of violet-quinone has been established as **1** by this synthesis. Investigations of the biological activity of the synthetic compounds **1**, **9** and **10** are in progress.

Table 2. ^{13}C and ^{1}H NMR spectral data (δ, ppm) for compounds **1**,[a] **2**,[a] **9**[a] and **10**[b]

Carbon no.	Violet-quinone (**1**)		Balsaminone A (**2**)[2]		**9**[c]		**10**[c]	
	^{13}C	^{1}H[d]	^{13}C	^{1}H	^{13}C	^{1}H[d]	^{13}C	^{1}H[d]
1	112.2	7.75 br s	121.2	8.48 m	121.0	8.43 d (8.2)	120.0	8.35 m
2	140.8		127.8	7.67 m	128.0	7.70 dt (8.2, 1.2)	127.9	7.79 br t (7.9)
3	115.9	6.92 d (0.9)	127.1	7.67 m	127.4	7.63 dt (8.2, 1.2)	126.8	7.71 br t (7.9)
4	155.3		123.1	8.34 m	123.7	8.38 d (8.2)	123.4	8.35 m
4a	113.1		125.6		127.1		125.7	
5	156.1		142.8		154.3		146.9	
6	95.0	7.35 s	134.7		96.5	7.46 s	98.6	7.55 s
6a	123.2[e]		114.6		125.3		124.1	
6b	125.4[e]		118.9		119.4		119.0	
7	181.3		174.6		174.3		173.4	
7a	133.3		133.7		133.4[f]		132.7	
8	121.3	7.57 d (0.9)	127.4	8.30 m	126.9[f]	8.25 m	125.9[f]	8.18 m
9	148.3		134.2	7.80 m	133.9[f]	7.78 m	133.78[f]	7.91 m
10	124.7	7.09 br s	133.8	7.80 m	133.8[f]	7.78 m	133.82[f]	7.91 m
11	162.9		126.7	8.30 m	126.7[f]	8.25 m	126.0[f]	8.18 m
11a	113.2		132.3		132.9[f]		132.3	
12	178.8		180.2		182.1		181.2	
12a	152.2		153.1		152.5		152.2	
13a	149.8		148.8		149.0		152.4	
13b	118.9		125.0		121.5		120.7	
2-Me	21.7	2.52 s						
4-OH		9.41 s						
5-OMe	55.8	4.18 s			56.2	4.12 s		
5-OH				6.33 s				10.54 s
6-OMe			63.4	4.09 s				
9-Me	22.2	2.47 s[f]						
11-OH		12.12 s						

[a] Data recorded in $CDCl_3$ at 300 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR).
[b] Data recorded in CD_3SOCD_3 at 300 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR).
[c] Assignment based on ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and HMBC spectra.
[d] Coupling constants (J in Hz) are in parentheses.
[e] Only the chemical schift of a methyl proton signal (δ 2.47) at the C9 position was different from that (δ 2.74) in the previous report.[1]
[f] Interchangeable.

4. Experimental

4.1. General

All melting points are uncorrected Infrared (IR) spectra were recorded with a JASCO IR-700 spectrometer, and ^{1}H- and ^{13}C NMR spectra with JEOL JNM-AL300 and JNM-alpha 500 spectrometers, with tetramethylsilane as an internal standard ($CDCl_3$, CD_3COCD_3 or CD_3SOCD_3 solution). Mass spectra were recorded on a JEOL JMS-D300 or Shimadzu QP-5000 spectrometer. Elemental analyses were done using a Yanaco CHN-MT-3 apparatus. Merck Kieselgel 60 (230–400 mesh), Wako silica gel C-200 (200 mesh) and Merck Kieselgel 60 F_{254} were used for flash column chromatography, column chromatography and thin-layer chromatography (TLC), respectively. Each organic extract was dried over $MgSO_4$ or Na_2SO_4. Photolyses were conducted with a Eikohsha 60 W low-pressure or 450 W high-pressure mercury lamp and irradiation was performed through a Pyrex vessel. The semiconductors, such as ZrO_2 and activated charcoal powders, are commercially available (Wako Pure Chemical Industries, Ltd, Japan).

4.1.1. Oxidative dimerization of 4a. *Method A* (*with the Act-C/O_2 system*). A slurry of activated charcoal powder (1 g) and **4a** (100 mg, 0.58 mmol) in a dioxygen-saturated MeCN (15 ml) was vigorously stirred at 70 °C for 16 h under normal laboratory light. A similar result was obtained in the dark. The insoluble reagent was filtered off and washed with MeCN, and then the filtrate was evaporated. The residue was subjected to flash column chromatography on silica gel. The eluate with CH_2Cl_2/hexane (1:1, v/v) gave 95 mg (95%) of 4,4′-dimethoxy-[2,2′]binaphthalenylidene-1,1′-dione (**6a**), as deep blue needles, mp 257–258 °C (lit.[13a] 256–258 °C). IR (KBr) cm^{-1}: 1606, 1584, 1561. ^{1}H NMR ($CDCl_3$) δ: 4.08 (6H, s, 4 and 4′-OMe), 7.48 (2H, broad t, J=7.7 Hz, 7 and 7′-H), 7.61 (2H, broad t, J=7.7 Hz, 6 and 6′-H), 7.79 (2H, broad d, J=7.7 Hz, 8 and 8′-H), 8.17 (2H, broad d, J=7.7 Hz, 5 and 5′-H), 8.42 (2H, s, 3 and 3′-H). LR-MS m/z: 344 (M^+). HR-MS calcd for $C_{22}H_{16}O_4$: 344.1044. Found: 344.1029.

Method B (*with the ZrO_2/O_2 system*). A slurry of ZrO_2 powder (5 g) and **4a** (100 mg, 0.58 mmol) in dioxygen-saturated MeCN (15 ml) was vigorously stirred at 70 °C for 1.5 h under normal laboratory light. The insoluble reagent was filtered off and washed with MeCN, and then the filtrate was evaporated. The residue was purified by the same method described above to give **6a** (75%) and 19 mg (17%) of 4-methoxy-1,2-naphthoquinone, as yellow needles (hexane–AcOEt), mp 192–193 °C (lit.[13j] 188–189 °C). IR (KBr) cm^{-1}: 1700, 1627, 1607, 1588. ^{1}H NMR ($CDCl_3$) δ: 4.03 (3H, s, 4-OMe), 5.99 (1H, s, 3-H), 7.59 (1H, dt, J=7.9, 1.3 Hz, 6 or 7-H), 7.70 (1H, dt, J=7.9, 1.3 Hz, 6 or 7-H), 7.87 (1H, dd, J=7.9, 1.3 Hz, 5-H), 8.13 (1H, dd, J=7.7, 1.5 Hz, 8-H). ^{13}C NMR ($CDCl_3$) δ: 56.81 (C4–OMe), 103.04 (C3), 124.73 (Ar–C), 129.05 (Ar–C), 130.33 (C4a or C8a), 131.52 (Ar–C), 131.96 (C4a or C8a), 134.96 (Ar–C), 168.68 (C4), 179.39 (C1 or C2), 179.49 (C1 or C2). LR-MS m/z: 188 (M^+). HR-MS calcd for $C_{11}H_8O_3$: 188.0479. Found: 188.0475.

4.1.2. Oxidation of 6a with 69% HNO_3. A mixture of 69% HNO_3 (2 ml) and **6a** (46 mg, 0.27 mmol) was stirred at 0 °C for 15 min. The reaction mixture was poured into a large volume of ice–water. The precipitated product was recrystallized from $CHCl_3$ to yield 42 mg (99%) of 2,2′-binaphthalenyl-1,4,1′,4′-tetraone (**7a**), as yellow needles, mp 288 °C (decomp.) (lit.[13b] 270–280 °C). IR (KBr) cm^{-1}: 1664, 1613, 1587. ^{1}H NMR ($CDCl_3$) δ: 7.07 (2H, s, 3 and 3′-H), 7.75–7.80 (4H, m, Ar–H), 8.12–8.16 (4H, m, Ar–H). LR-MS m/z: 314 (M^+). HR-MS calcd for $C_{20}H_{10}O_4$: 314.0576. Found: 314.0561.

4.1.3. Photolysis of 6a. *Method A* (*with a* 450 W *mercury lamp*). A solution of **6a** (25 mg, 0.15 mmol) in an argon-saturated $CHCl_3$ (10 ml) in a Pyrex vessel was irradiated using a 450 W high-pressure Hg lamp for 1 h, and then evaporated. The residue was subjected to flash column chromatography on silica gel. The eluate with CH_2Cl_2/hexane (2:1, v/v) gave 6 mg (25%) of 1′-hydroxy-4′-methoxy-[2,2′]binaphthalenyl-1,4-dione (**8a**) and 14 mg (59%) of 5-methoxy-dinaphtho[1,2-*b*;2′,3′-*d*]furan-7,12-dione (**9**).

Compound **8a**. Deep violet needles (benzene), mp 189 °C (lit.[13c] 185–186 °C). IR (KBr) cm^{-1}: 3328, 1655, 1590. ^{1}H NMR ($CDCl_3$) δ: 3.99 (3H, s, 4′-OMe), 6.56 (1H, s, 3′-H), 7.15 (1H, s, 3-H), 7.57–7.60 (2H, m, Ar–H), 7.81–7.85 (2H, m, Ar–H), 8.14–8.28 (3H, m, Ar–H), 8.39–8.42 (1H, m, Ar–H), 8.51 (1H, s, 1′-OH). ^{13}C NMR ($CDCl_3$) δ: 55.81 (C4′-OMe), 104.65 (C3′), 114.43 (C2′), 121.73 (C8′), 123.77 (C5′), 126.30 (C5 or C8), 126.72 (C6′ or C7′), 127.32 (C8a′), 127.55 (C6′ or C7′), 127.76 (C5 or C8), 127.99 (C4a′), 131.75 (C4a or C8a), 132.57 (C4a or C8a), 134.05 (C6 or C7), 134.82 (C6 or C7), 138.89 (C3), 145.48 (C1′), 149.71 (C4′), 150.00 (C2), 184.49 (C1 or C4), 188.49 (C1 or C4). LR-MS m/z: 330 (M^+). HR-MS calcd for $C_{21}H_{14}O_4$: 330.0888. Found: 330.0922.

Compound **9**. Orange needles ($CHCl_3$–hexane), mp 291.5–292.5 °C (lit.[13a] 293–295 °C). IR (KBr) cm^{-1}: 1665, 1590. LR-MS m/z: 328 (M^+).

Method B (*with a* 60 W *mercury lamp*). Photolysis of **6a** (25 mg, 0.15 mmol) was carried out under a 60 W low-pressure Hg lamp at 23 °C for 80 h by the same procedure under the conditions described above (method A) for the photolysis of **6a**. The crude product was purified by flash column chromatography on silica gel. The eluate with CH_2Cl_2/hexane (2:1, v/v) gave 21 mg (88%) of **8a**.

4.1.4. Photolysis of 7a. Photolysis of **7a** (20 mg, 0.06 mmol) was carried out at 23 °C for 2 h by the same procedure under the conditions described above (method A) for the photolysis of **6a**. The crude product was purified by recrystallization from $CHCl_3$–MeOH to yield 18 mg (90%) of 5-hydroxy-dinaphtho[1,2-*b*;2′,3′-*d*]furan-7,12-dione (**10**) as red needles, mp 305–308 °C (lit.[13e] 360 °C). IR (KBr) cm^{-1}: 3310, 1654, 1592. LR-MS m/z: 314 (M^+). HR-MS calcd for $C_{20}H_{10}O_4$: 314.0576. Found: 314.0560.

4.1.5. Methylation of 10. CH_3I (24 μl, 0.4 mmol) was added to a solution of **10** (30 mg, 0.10 mmol) and anhydrous K_2CO_3 (132 mg) in dry DMF (12 ml), and the solution was

stirred vigorously at 23 °C for 4 h. The reaction mixture was poured into ice–water, neutralized with 10% HCl, and extracted with $CHCl_3$. The organic layer was washed with H_2O, dried and concentrated. The residue was purified by recrystallization from $CHCl_3$–MeOH to yield 28 mg (91%) of **9**.

4.1.6. Oxidative dimerization of 1-naphthol 4b with various reagents. *Method A* (*entry* 1) (*with* Ag_2O). A mixture of **4b** (50 mg, 0.23 mmol) in $CHCl_3$ (10 ml) containing 1.5 equiv. of Ag_2O (80 mg, 0.344 mmol) was stirred at 23 °C in an air atmosphere for 1 h. The solvent was removed and the residue was subjected to flash column chromatography on silica gel using CH_2Cl_2/hexane (1:2, v/v) as an eluent to give a mixture of **5b** and **6b**, and 4,5-dimethoxy-7-methyl-1,2-naphthoquinone (**11a**). Benzyl iodide (268 μl, 2.26 mmol) was added to a solution of the mixture of **5b** and **6b**, and anhydrous K_2CO_3 (312 mg) in dry DMF (5 ml), and the solution was stirred vigorously at 23 °C for 40 min. The reaction mixture was poured into ice–water, neutralized with 10% HCl, and extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried and concentrated. The residue was subjected to flash column chromatography on silica gel. The eluate with hexane/AcOEt (10:1, v/v) gave 1,1′-dibenzyloxy-4,5, 4′,5′-tetramethoxy-[2,2′]binaphthalenyl-1,1′-diol (**5d**) and 4,5,4′,5′-tetramethoxy-7,7′-dimethyl-[2,2′]binaphthalenyli-dene-1,1′-dione (**6b**). Yields are listed in Table 1.

Compound **5d**. Pale yellow powder (AcOEt), mp 203.5–204.0 °C. IR (KBr) cm^{-1}: 2922, 1604. ^1H NMR ($CDCl_3$) δ: 2.50 (6H, s, 7 and 7′-Me), 3.91 (6H, s, 4 and 4′-OMe), 4.02 (6H, s, 5 and 5′-OMe), 4.71 (4H, s, 2×–CH_2–Ar), 6.78 (2H, d, J=1.29 Hz, 6 and 6′-H), 7.17 (2H, s, 3 and 3′-H), 7.20–7.30 (10H, m, Ar–H), 7.67 (2H, d, J=1.29 Hz, 8 and 8′–H). ^{13}C NMR ($CDCl_3$) δ: 22.23 (7- and 7′–Me), 56.55 (5-OMe), 56.62 (4-OMe), 75.14 (–CH$_2$–Ar), 108.27 (C3), 109.07 (C6), 114.49 (C8), 116.21 (C2), 127.46 (C4a), 127.87 (Ar–C), 128.10 (Ar–C), 128.35 (Ar–C), 132.104 (C8a), 136.65 (C7), 137.32 (Ar–C), 145.16 (C1), 152.82 (C4), 157.13 (C5). LR-MS m/z: 614 (M^+). HR-MS: calcd for $C_{40}H_{38}O_6$: 614.2658. Found: 614.2642. Anal. Calcd for $C_{40}H_{38}O_6$: C, 78.15; H, 6.23. Found: C, 78.13; H, 6.20.

Compound **6b**. Deep violet needles, mp 236.5–237 °C (lit.[15a] 228 °C). IR (KBr) cm^{-1}: 1589. ^1H NMR ($CDCl_3$) δ: 2.43 (6H, s, 7 and 7′-Me), 3.92 (6H, s, 4 and 4′-OMe), 4.05 (6H, s, 5 and 5′-OMe), 6.98 (2H, broad t, J=0.9 Hz, 6 and 6′-H), 7.70 (2H, broad t, J=0.9 Hz, 8 and 8′-H), 8.37 (2H, s, 3 and 3′-H). ^{13}C NMR ($CDCl_3$) δ: 21.76 (7 and 7′-Me), 56.08 (Ar–OMe), 56.91 (Ar–OMe), 103.21 (C3 and C3′), 117.99, 118.18 (C6 and C6′,or C8 and C8′), 121.74 (C6 and C6′,or C8 and C8′), 130.32 (Ar–C), 133.60 (Ar–C), 140.27 (Ar–C), 156.14 (Ar–C), 159.46 (Ar–C), 188.93 (C1 and C1′). HR-MS calcd for $C_{26}H_{24}O_6$: 432.1566. Found: 432.1563. Anal. Calcd for $C_{26}H_{24}O_6$: C, 72.21; H, 5.59. Found: C, 72.35; H, 5.56.

Compound **11a**. Orange powder ($CHCl_3$–hexane), mp 175.0–175.5 °C. IR (KBr) cm^{-1}: 1642, 1603, 1580. ^1H NMR ($CDCl_3$) δ: 2.42 (3H, s, 7 and 7-Me), 3.92, 3.97 (6H, each s, 4 and 5-OMe), 5.89 (1H, s, 3-H), 7.07 (1H, br s, 6-H), 7.63 (1H, d, J=0.74 Hz, 8-H). ^{13}C NMR ($CDCl_3$) δ: 21.6 (7-Me), 56.75, 56.81 (4 and 5-OMe), 102.1 (C3), 116.2 (C4a), 120.8, 123.8 (C6 and C8), 132.2 (C8a), 143.8 (C7), 158.2 (C5), 172.7 (C4), 179.1, 180.6 (C1 and C2). LR-MS m/z: 232 (M^+). Anal. Calcd for $C_{13}H_{12}O_4$: C, 67.23; H, 5.21. Found: C, 67.20; H, 5.19.

Method B (*entry 2*) (*with AgO*/40% *HNO*$_3$). To a mixture of **4b** (50 mg, 0.229 mmol) and AgO (284 mg, 2.29 mmol) in acetone (5 ml) was added 40% HNO_3 (1.5 ml) over 5 min. The reaction mixture was stirred at room temperature for 30 min, diluted with water and extracted with $CHCl_3$. The extracts were washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography on silica gel. The eluate with $CHCl_3$/AcOEt (20:1, v/v) gave 23 mg (50%) of 5,5′-dimethoxy-7,7′-dimethyl-[2,2′]binaphthalenyl-1,4,1′,4′-tetraone (**7b**) and 8 mg (17%) of 5-methoxy-8-methyl-1,4-naphthoquinone (**11b**).

Compound **7b**. Yellow amorphous powder ($CHCl_3$–MeOH), mp 279–281 °C (lit.[15a] 310 °C). IR (KBr) cm^{-1}: 1649, 1601, 1587. ^1H NMR ($CDCl_3$) δ: 2.50 (6H, s, 7 and 7′-Me), 4.02 (6H, s, 5 and 5′-OMe), 6.94 (2H, s, 3 and 3′-H), 7.13 (2H, broad s, 6 and 6′-H), 7.59 (2H, broad s, 8 and 8′-H). HR-MS calcd for $C_{24}H_{18}O_6$: 402.1098. Found: 402.1133. Anal. Calcd for $C_{24}H_{18}O_6$: C, 72.64; H, 4.51. Found: C, 72.60; H, 4.50.

Compound **11b**. Yellow needles (benzene), mp 169.5–170 °C (lit.[14b] 164–166 °C). IR (KBr) cm^{-1}: 1651, 1559. ^1H NMR ($CDCl_3$) δ: 2.48 (3H, s, 7-Me), 4.00 (3H, s, 5-OMe), 6.84 (2H, s, 2 and 3-H), 7.11 (1H, s, 6-H), 7.55 (1H, s, 8-H). HR-MS calcd for $C_{12}H_{10}O_3$: 202.0627. Found: 202.0614.

Method C (*entry* 3) (*with* Ag_2O/NEt_3). A mixture of **4b** (100 mg, 0.58 mmol) in $CHCl_3$ (20 ml) containing 0.2% NEt_3 and 20 equiv. of Ag_2O (2.66 g) was stirred at 23 °C in an air atmosphere for 1 h. The solvent was removed and the residue was subjected to flash column chromatography on silica gel. The eluate with CH_2Cl_2/hexane (1:2, v/v) gave **7b** and **11b**. Yields are listed in Table 1.

Method D (*entry* 4) (*with the Act-Cl*/O_2 *system*). Oxidation of **4b** (100 mg, 0.58 mmol) was carried out at 70 °C for 24 h by the same procedure under the conditions described above (method A) for the oxidative dimerization of **4a**. The crude product was purified by flash column chromatography on silica gel. The eluate with CH_2Cl_2/hexane (1:2, v/v) gave **5d**, **6b**, **11a** and **11b**. Yields are listed in Table 1.

Method E (*entry* 5) (*with the* ZrO_2/O_2 *system*). Oxidation of **4b** (100 mg, 0.58 mmol) was carried out at 70 °C for 19 h by the same procedure under the conditions described above (method B) for the oxidative dimerization of **4a**. The crude product was purified by recrystallization from benzene to yield **6b** (96%).

4.1.7. Oxidation of 6b with 69% HNO_3. A mixture of 69% HNO_3 (3 ml) and **6b** (80 mg, 0.17 mmol) was stirred at 0 °C for 15 min. The reaction mixture was poured into a large volume of ice–water. The precipitated product was

recrystallized from $CHCl_3$/MeOH to yield 73 mg (99%) of **7b**.

4.1.8. 5,5′-Dihydroxy-7,7′-dimethyl-[2,2′]binaphthalenyl-1,4,1′,4′-tetraone (biramentaceone) (7c). Magnesium bromide (2.2 g, 12 mmol) was added to a solution of **7b** (200 mg, 0.5 mmol) dissolved in anhydrous toluene (30 ml) and the whole was refluxed for 12 h. The reaction was quenched with cooled water and saturated NH_4Cl solution, and the whole stirred for 30 min. The mixture was extracted with $CHCl_3$, and the $CHCl_3$ layer was washed with H_2O, dried, concentrated, and then the residue was subjected to flash column chromatography on silica gel. The eluate with hexane/AcOEt (5:1, v/v) gave 150 mg (80%) of **7c** as an orange amorphous powder ($CHCl_3$–MeOH), mp 264–265 °C (decomp.) (lit.[15a] 260 °C). IR (KBr) cm^{-1}: 3426, 1665, 1641, 1574. ^1H NMR ($CDCl_3$) δ: 2.45 (6H, s, 7 and 7′-Me), 7.01 (2H, s, 3 and 3′-H), 7.12 (2H, dd, J=0.9, 1.7 Hz, 6 and 6′-H), 7.49 (2H, dd, J=0.9, 1.7 Hz, 8 and 8′-H), 11.79 (2H, s, 5 and 5′-OH). HR-MS calcd for $C_{22}H_{14}O_6$: 374.0786. Found: 374.0787. Anal. Calcd for $C_{22}H_{14}O_6$: C, 70.58; H, 3.77. Found: C, 70.47; H, 3.75.

4.1.9. 5,5′-Bis-benzyloxy-7,7′-dimethyl-[2,2′]binaphthalenyl-1,4,1′,4′-tetraone (12). Benzyl iodide (72 μl, 0.54 mmol) was added to a solution of **7c** (20 mg, 0.054 mmol) and anhydrous K_2CO_3 (76 mg) in dry DMF (10 ml), and the solution was stirred vigorously at 23 °C for 1 h. The reaction mixture was poured into ice–water, neutralized with 10% HCl, and extracted with $CHCl_3$. The organic layer was washed with H_2O, dried and concentrated. The residue was subjected to flash column chromatography on silica gel. The eluate with benzene/acetone (40:1, v/v) gave 24 mg (78%) of **12** as yellow amorphous powder ($CHCl_3$–MeOH), mp 192–193 °C. IR (KBr) cm^{-1}: 1654, 1598. ^1H NMR ($CDCl_3$) δ: 2.46 (6H, s, 7 and 7′-Me), 5.31 (4H, s, $-OCH_2$), 6.95 (2H, s, 3 and 3′-H), 7.17 (2H, broad d, J=0.7 Hz, 6 and 6′-H), 7.33–7.60 (10H, m, Ar–H), 7.58 (2H, broad d, J=0.7 Hz, 8 and 8′-H). HR-MS calcd for $C_{36}H_{26}O_6$: 554.1722. Found: 554.1740. Anal. Calcd for $C_{36}H_{26}O_6$: C, 77.96; H, 4.73. Found: C, 77.92; H, 4.70.

4.1.10. 4,11-Bis-benzyloxy-5-hydroxy-2,9-dimethyl-dinaphtho[1,2-*b*;2′,3′-*d*]furan-7,12-dione (13). A solution of **17** (20 mg, 0.036 mmol) in argon-saturated $CHCl_3$ (10 ml) in a Pyrex vessel was irradiated using a 450 W high-pressure Hg lamp for 1 h, and then evaporated. The residue was subjected to flash column chromatography on silica gel. The eluate with CH_2Cl_2/hexane (2:1, v/v) gave 18 mg (87%) of **13** as red needles ($CHCl_3$–MeOH), mp 258–258.5 °C. IR (KBr) cm^{-1}: 3406, 1662, 1598, 1505. ^1H NMR ($CDCl_3$) δ: 2.49 (3H, s, 9-Me), 2.54 (3H, s, 2-Me), 5.29 (2H, s, C4–OCH_2), 5.30 (2H, s, C11–OCH_2), 6.90 (1H, broad s, 3-H), 7.18 (1H, broad s, 10-H), 7.43 (1H, s, 6-H), 7.31–7.73 (10H, m, Ar–H), 7.73 (1H, broad d, J=0.9 Hz, 8-H), 7.93 (1H, broad d, J=0.9 Hz, 1-H), 9.39 (1H, s, 5-OH). ^{13}C NMR ($CDCl_3$) δ: 22.01 (C2–*Me*), 22.31 (C9–*Me*), 70.98 (C11–O*CH*$_2$), 72.09 (C4–O*CH*$_2$), 101.10 (C6), 110.08 (C3), 113.58 (C4a), 114.65 (C1), 118.37 (C11a), 120.21 (C10), 120.55 (C13b), 121.17 (C8), 122.70 (C6a or C6b), 123.27 (C6a or C6b), 126.67 (Ar–C), 127.83 (Ar–C), 128.14 (Ar–C), 128.66 (Ar–C), 129.10 (Ar–C), 129.17 (Ar–C), 134.80 (Ar–C), 135.86 (C7a), 136.25 (Ar–C), 138.40 (C2), 146.60 (C9), 147.08 (C13a), 153.56 (C5), 154.12 (C12a), 155.67 (C4), 159.89 (C11), 174.12 (C12), 181.49 (C7). HR-MS calcd for $C_{36}H_{26}O_6$: 554.1722. Found: 554.1867. Anal. Calcd for $C_{36}H_{26}O_6$: C, 77.96; H, 4.73. Found: C, 77.99; H, 4.75.

4.1.11. 4,11-Bis-benzyloxy-5-methoxy-2,9-dimethyl-dinaphtho[1,2-*b*;2′3′-*d*]furan-7,12-dione (14). Methyl iodide (0.14 μl, 2.29 mmol) was added to a solution of **13** (100 mg, 0.19 mmol) and anhydrous K_2CO_3 (260 mg) in dry DMF (10 ml), and the solution was stirred at 23 °C for 2 h with vigorous stirring. The reaction mixture was poured into ice–water, and extracted with $CHCl_3$. The organic layer was washed with H_2O, dried and concentrated. The residue was subjected to flash column chromatography on silica gel. The eluate with AcOEt/hexane (1:1, v/v) gave 85 mg (79%) of **14** as red needles ($CHCl_3$–MeOH), mp 322–324 °C. IR (KBr) cm^{-1}: 1662, 1598, 1567. ^1H NMR ($CDCl_3$) δ: 2.50 (3H, s, 2-Me), 2.55 (3H, s, 9-Me), 4.05 (3H, s, 5-OMe), 5.21 (2H, s, 4-OCH_2), 5.31 (2H, s, 11-OCH_2), 6.95 (1H, broad s, 3-H), 7.18 (1H, broad s, 10-H), 7.43 (1H, s, 6-H), 7.33–7.73 (10H, m, Ar-H), 7.73 (1H, s, 8-H), 7.93 (1H, s, 1-H). HR-MS calcd for $C_{37}H_{28}O_6$: 568.1878. Found: 568.1926. Anal. Calcd for $C_{37}H_{28}O_6$: C, 78.15; H, 4.96. Found: C, 78.13; H, 4.99.

4.1.12. 4,11-Dihydroxy-5-methoxy-2,9-dimethyl-dinaphtho[1,2-*b*;2′,3′-*d*]furan-7,12-dione (violet-quinone) (1). Compound **14** (46 mg, 0.08 mmol) was hydrogenated in the presence of 10% Pd/C (20 mg) in ethyl acetate (8 ml). The catalyst was removed, and the filtrate was concentrated. The residue was subjected to flash column chromatography on silica gel. The eluate with CH_2Cl_2 gave 30 mg (96%) of **1** as violet solid ($CHCl_3$–MeOH), mp 332–335 °C (lit.[1] 335–338 °C). IR (KBr) cm^{-1}: 3370, 1664, 1640, 1607. HR-MS calcd for $C_{23}H_{16}O_6$: 388.0942. Found: 388.0957. Anal. Calcd for $C_{23}H_{16}O_6$: C, 71.13; H, 4.15. Found: C, 71.23; H, 4.18.

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Tetrahedron 60 (2004) 3949–3955
Tetrahedron

Synthesis of tricyclic isoindoles and thiazolo[3,2-*c*][1,3]benzoxazines

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Received 21 January 2004; revised 21 January 2004; accepted 11 March 2004

Abstract—The thermolysis of (3*R*,9b*S*)-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylic acids in Ac_2O led to novel 3-methylene-2,5-dioxo-3*H*,9b*H*-oxazolo[2,3-*a*]isoindoles and chiral (9b*S*)-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindoles were obtained on FVP. Starting from L-cysteine methyl ester (3*R*,10b*R*)-5-oxo-2,3-dihydro-10b*H*-[1.3]thiazolo[3,2-*c*][1,3]benzoxazines were obtained as single stereoisomers. The thermolysis of (3*R*,10b*R*)-5-oxo-2,3-dihydro-10b*H*-[1.3]thiazolo[3,2-*c*][1,3]benzoxazine-3-carboxylic acid in Ac_2O gave 5-acetyl-2-phenyl-2,3-dihydrothiazole. The structures of methyl (3*R*,9b*S*)-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylate **1a** and methyl (2*R*,4*R*)-*N*-chlorocarbonyl-2-(2-hydroxyphenyl)thiazolidine-4-carboxylate **9** were determined by X-ray crystallography.

1. Introduction

The study of 1,3-dipolar cycloaddition of münchnones as an approach to chiral pyrrolo[1,2-*c*]thiazoles is an area of our current research interests.[1] In this context we became interested in exploiting the possibility of preparing 1,3-thiazolidine-4-carboxylic acids fused to five- and six-membered ring systems which could be used as potential münchnone precursors.

In a preliminary communication, we described the thermolysis (3*R*,9b*S*)-5-oxo-2,3,5,9b-tetrahydrothiazolo-[2,3-*a*]isoindole-3-carboxylic acids in acetic anhydride where no evidence for the generation of mesoionic species was observed. However, this study led to the development of a synthetic methodology to 3-methylene-2,5-dioxo-3*H*,9b*H*-oxazolo[2,3-*a*]isoindoles.[2] In this paper we report full details of the work on the synthesis and reactivity of 5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylic acids as well as of 5-oxo-2,3-dihydro-10b*H*-[1.3]thiazolo[3,2-*c*][1,3]benzoxazine derivatives.

2. Results and discussion

The reaction of L-cysteine methyl ester with 2-carboxybenzaldehyde was carried out following the general procedure reported earlier for the synthesis of thiazolidines.[3] The product was purified simply by recrystallisation. This resulted in the direct diastereoselective synthesis of methyl (3*R*,9b*S*)-5-oxo-2,3,5,9b-tetrahydro-thiazolo[2,3-*a*]isoindole-3-carboxylate **1a** in 71% yield (Scheme 1).

The structure of **1a** was confirmed by X-ray crystallography (Fig. 1). The absolute structure was determined by a Flack analysis (898 Friedel pairs, η=0.01(3)) that unambiguously assigns the *R*,*S* configuration to the chiral centers C3 and C9b.

Compound **1a** was converted into the corresponding acid **2a** in 91% yield ($[\alpha]_D^{25}=-343$, c=0.1, EtOH) by the reaction with lithium iodide in ethyl acetate and treatment with aqueous HCl (Scheme 1).

Compound **2a** can also be prepared as described by Oliver et al. directly from the reaction of 2-carboxybenzaldehyde with cysteine hydrochloride in the presence of pyridine.[4] This procedure gave 5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylic acid **2a** in 58% yield.

The reactivity of compound **2a** as a münchnone precursor was studied. Attempts were made to promote cyclodehydration by heating at reflux a solution of compound **2a** in acetic anhydride in the presence of dimethyl acetylenedicarboxylate. However, the expected 1*H*,3*H*-pyrrolo[1,2-*c*]thiazole was not obtained even when prolonged heating was used.

Keywords: Diastereoselectivity; Thiazolo[2,3-*a*]isoindoles; 3-Methylene-2,5-oxazolo[2,3-*a*]isoindoles; [1.3]Thiazolo[3,2-*c*][1,3]benzoxazines.
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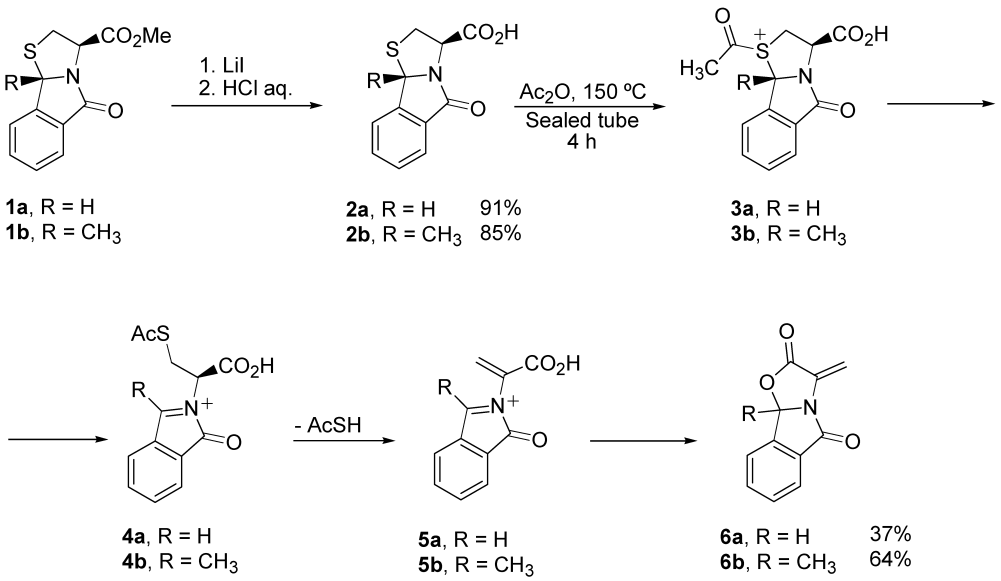
doi:10.1016/j.tet.2004.03.030



Scheme 1.

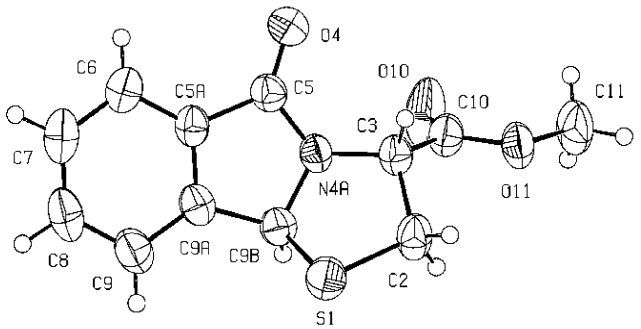


Figure 1. X-ray structure of compound **1a**.

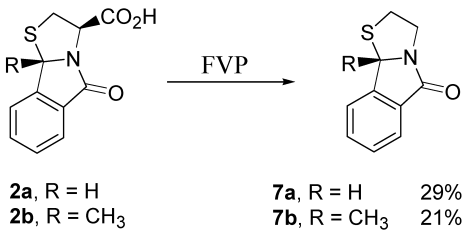
Based on the structure of methyl (3*R*,9b*S*)-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylate **1a**, determined by X-ray crystallography, we can explain this unsuccessful result. This tricyclic compound has a rigid structure and is characterized by having a value of 122.42° for the C5–N4A–C-3 bond angle (Fig. 1 and Table 1). A similar bond angle is expected for (3*R*,9b*S*)-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylic acid **2a**. The C-5, N-4A and C-3 atoms would be part of the mesoionic ring and a significant structure distortion had to occur in order to allow its formation. Thus, the generation of a münchnone from compound **2a** is not a favourable process.

Table 1. Bond angles (°) for compound **1a**

C2–S1–C9B	88.93(12)	C5–N4A–C9B	111.93(19)
C9B–N4A–C3	115.55(18)	N4A–C9B–C9A	103.77(19)
N4A–C9B–S1	104.45(16)	N4A–C5–C5A	106.3(2)
C3–C2–S1	106.91(17)	C9A–C5A–C5	108.9(2)
N4A–C3–C2	107.12(19)	C5A–C9A–C9B	108.6(2)
C5–N4A–C3	122.42(19)	C9A–C9B–S1	115.57(18)

Nevertheless, we carried out the reaction of (3*R*,9b*S*)-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylic acid **2a** with acetic anhydride and dimethyl acetylenedicarboxylate in a sealed tube. The solution was heated at 150 °C for 4 h. Although no 1,3-dipolar cycloadduct was obtained, 3-methylene-2,5-dioxo-3*H*,9b*H*-oxazolo[2,3-*a*]isoindole **6a** was isolated in 37% yield which (Scheme 1). The structure of **6a** was determined by X-ray crystallography.[2]

The mechanism proposed for the formation of compound **6a** is outlined in Scheme 2. The process can be regarded as involving the formal elimination of the elements of SH from (3*R*,9b*S*)-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylic acid **2a** leading to *N*-acyliminium ion **5a** followed by a 5-*endo*-trig cyclization. It represents the synthesis of an isoindole derivative (**6a**), a new member to a class of compounds having a significant number of applications.[5]



Scheme 2.

In order to determine the scope of this route to 3-methylene-2,5-dioxo-3*H*,9b*H*-oxazolo[2,3-*a*]isoindoles we prepared (3*R*,9b*S*)-9b-methyl-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylic acid **2b** (Scheme 1). The reaction of L-cysteine methyl ester with 2-acetylbenzoic acid was carried out in presence of sodium acetate in refluxing toluene for 5 h giving (3*R*,9b*S*)-9b-methyl-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylate **1b** in 75% yield with the value of $[\alpha]_D^{25}=-328.7$ (c=1.75, CH_2Cl_2). Compound **2b** was obtained from **1b** in 85% yield.

We carried out the reaction of (3*R*,9b*S*)-9b-methyl-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylic acid **2b** with acetic anhydride in a sealed tube (Scheme 1). The solution was heated at 150 °C for 4 h. In a process

analogous to that described for the synthesis of oxazolo[2,3-*a*]isoindole derivative **6a**, compound **2b** was converted into the tricyclic isoindole derivative 9b-methyl-3-methylene-2,5-dioxo-3*H*,9b*H*-oxazolo[2,3-*a*]isoindole **6b** in 64% yield. When the reaction of **2b** with acetic anhydride was performed in the presence of dimethyl acetylenedicarboxylate (sealed tube, 150 °C, 4 h) compound **6b** was isolated in lower yield (40%) but no 1,3-dipolar cycloadduct was formed.

The flash vacuum pyrolysis of (3*R*,9b*S*)-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylic acids **2a** and **2b** was also studied (Scheme 2). We found that on FVP (600 °C/3×10^{-2}–4×10^{-2} mbar) these compounds undergo decarboxylation to the corresponding chiral (9b*S*)-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindoles (**7a** and **7b**) in moderate yields.

Compounds **7a** and **7b** have been prepared before from the reaction of carboxybenzaldehyde or 2-acetylbenzoic acid with 2-aminoethanethiol. However, they were obtained as racemic mixtures.[4,6,7] Some 5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindoles substituted at C-9 with aryl and heteroaromatic groups have also been prepared as racemic mixtures although the separation of both enantiomers can be achieved by chromatography on cellulose triacetate.[5b]

Our synthetic procedure is particularly interesting since it allows the synthesis of (9b*S*)-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindoles directly as single enantiomers.

We then went on to investigate the possibility of preparing a tricyclic compound having a thiazolidine ring fused to a six-membered ring which should be a better münchnone precursor in terms of structural requirements than 5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindoles **2a** and **2b**. We defined 5-oxo-2,3-dihydro-10b*H*-[1,3]thiazolo[3,2-*c*][1,3]benzoxazine-3-carboxylic acid **11** as our target molecule (Scheme 3). The chemistry of [1,3]thiazolo[3,2-*c*][1,3]benzoxazines is an area of considerable interest since some derivatives show biological activity namely immunoacitvating action which makes this synthesis more appealing.[8]

The synthetic strategy is outlined in Scheme 3. Thiazolidine **8** was prepared by condensing L-cysteine methyl ester hydrochloride with salicylaldehyde in presence of potassium hydrogen carbonate. Our approach to construct the six-membered ring was to react methyl 2-(2-hydroxyphenyl)thiazolidine-4-carboxylate **8** with phosgene. The reaction was carried out at room temperature and after 6 h a product was isolated in 67% yield. Although it was expected to obtain directly the cyclization product, the characterisation data allow us to conclude that we were in the presence of methyl (2*R*,4*R*)-*N*-chlorocarbonyl-2-(2-hydroxyphenyl)thiazolidine-4-carboxylate **9**. This showed $[\alpha]_D^{25}$=+197 (*c*=0.1, EtOH). Thus, the reaction conditions used led to a diastereoselective N-acylation of thiazolidine **8**.

It is known that NMR spectra of *N*-acylthiazolidines at ambient temperature are usually complicated by the existence of rotamers.[1,9] In agreement with this we found that the ^{1}H and ^{13}C NMR spectra of thiazolidine **9** recorded at room temperature, showed two sets of signals.

The structure of methyl (2*R*,4*R*)-*N*-chlorocarbonyl-2-(2-hydroxyphenyl)thiazolidine-4-carboxylate **9** was confirmed by X-ray crystallography (Fig. 2). The absolute structure was determined by a Flack analysis (1358 Friedel pairs, η=−0.16(9)) that unambiguously assigns the *R*,*R* configuration to the chiral centers C2 and C4.

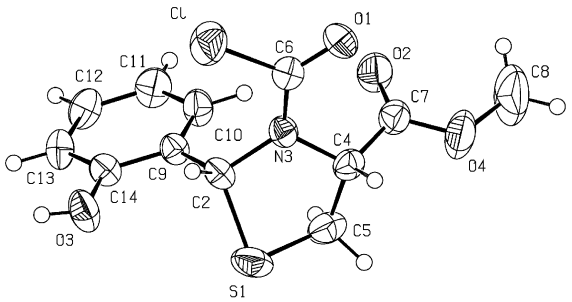
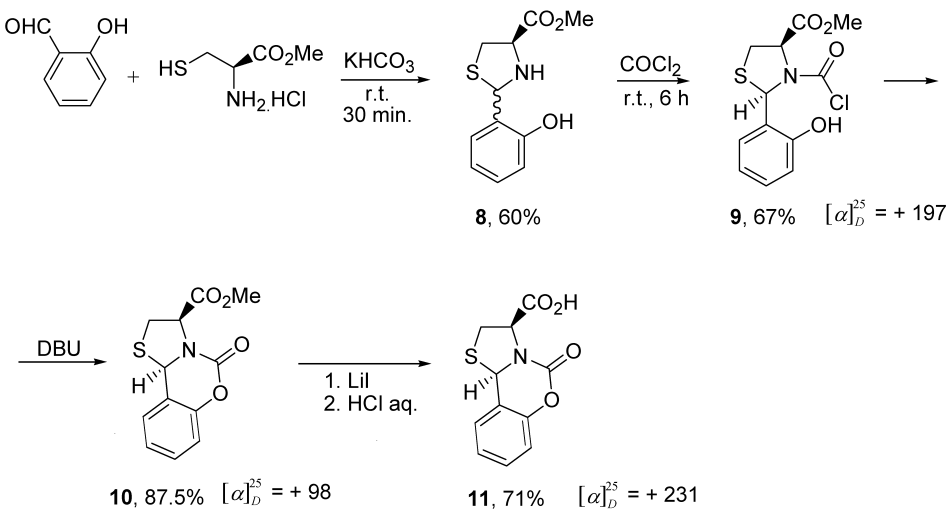


Figure 2. X-ray structure of compound **9**.

The thiazolidine ring adopts a twisted conformation around atom N3. The puckering parameters as defined by Cremer and Pople[10] are q_2=0.503(3) Å, ϕ_2=346.0(4)°, the φ_2 value



Scheme 3.

for the pure twisted conformation being 342°. There is an approximate C_2 axis running through N3 and the middle of the S1–C5 bond, the C_2 asymmetry parameter being 4.2(3)°. The exocyclic angles around the N3 atom show a large asymmetry; the sum of the valence angles around this atom is 358.9° indicating an insignificant degree of pyramidalization.

The least-squares planes of the hydroxyphenyl group and thiazolidine ring make an angle of 48.6(1)°. The methyl carboxylate substituent is in bissectional position with respect to the ring plane. The torsion angle O2–C7–C4–C5 is 86.0(4)°. The chlorocarbonyl group is planar but slightly titled with respect to the least squares plane defined by atoms N3, C2, C4 and C6 (the torsion angle C2–N3–C6–Cl is −4.5(4)°).

We studied the thermolysis of (2*R*,4*R*)-*N*-chlorocarbonyl-2-(2-hydroxyphenyl)thiazolidine-4-carboxylate **9** as a way to the corresponding cyclization product. However, even when a solution of **9** in sulpholane was heated at reflux did not lead to the desired product. The synthesis of methyl (3*R*,10b*R*)-5-oxo-2,3-dihydro-10b*H*-[1.3]thiazolo[3,2-*c*][1,3]benzoxazine-3-carboxylate **10** (87.5%) was achieved when thiazolidine **9** was treated with DBU (with DBN **10** was obtained in 76% yield). This new tricyclic compound **10** was obtained as single stereoisomer with $[\alpha]_D^{25}=+98$ (Scheme 3).

The (3*R*,10b*R*)-5-oxo-2,3-dihydro-10b*H*-[1.3]thiazolo[3,2-*c*][1,3]benzoxazine-3-carboxylic acid **11** was obtained in 71% yield by reacting compound **10** with lithium iodide in ethyl acetate followed by treatment with aqueous HCl (Scheme 3).

Attempts to generate the corresponding münchnone from 5-oxo-2,3-dihydro-10b*H*-[1.3]thiazolo[3,2-*c*][1,3]benzoxazine derivative **11** in presence of DMAD did not lead to positive results. However, the thermolysis of **11** in acetic anhydride, carried out in a sealed tube, led to the synthesis of 5-acetyl-2-phenyl-2,3-dihydrothiazole **14** in low yield (Scheme 4). The formation of this product can be rationalised as involving a double decarboxylation giving **12** which is converted into 2,3-dihydrothiazole **13** through prototropy. Acylation of this intermediate gives compound **14**.

3. Conclusion

In conclusion, we report a synthetic methodology to new tricyclic isoindole derivatives, 3-methylene-2,5-dioxo-3*H*,9b*H*-oxazolo[2,3-*a*]isoindoles through the thermolysis of (3*R*,9b*S*)-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylic acids in acetic anhydride.

Chiral (9b*S*)-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindoles (**7a** and **7b**) were also obtained from the flash vacuum pyrolysis of (3*R*,9b*S*)-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylic acids.

The diastereoselective synthesis of (3*R*,10b*R*)-5-oxo-2,3-dihydro-10b*H*-[1,3]thiazolo[3,2-*c*][1,3]benzoxazines (**10** and **11**) was accomplished and the thermolysis of **11** in acetic anhydride gave 5-acetyl-2-phenyl-2,3-dihydrothiazole **14**.

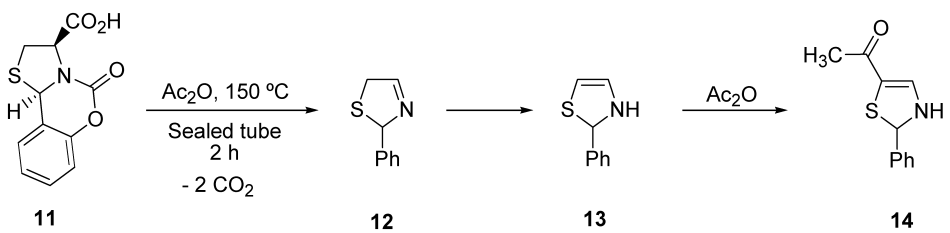
The work provided a range of isoindoles and thiazolobenzoxazines, compounds with potential biological activity.[5,8]

4. Experimental

4.1. General

^{1}H NMR spectra were recorded on a Bruker AMX300 instrument operating at 300 MHz. ^{13}C spectra were recorded on a Bruker AMX300 instrument operating at 75.5 MHz. The solvent is deuteriochloroform except where indicated otherwise. IR spectra were recorded on a Perkin–Elmer 1720X FTIR spectrometer. Mass spectra were recorded on a HP GC 6890/MSD5973 instrument under electron impact (EI) except where indicated otherwise. Optical rotations were measured on an Optical Activity AA-5 electrical polarimeter. Microanalyses were performed in the University of Coimbra using a EA 1108-CHNS-O Fisons instrument or in the University of Liverpool using a Carlo-Erba elemental analyser. Mp were recorded on a Reichert hot stage and are uncorrected. Flash column chromatography was performed with Merck 9385 silica as the stationary phase.

4.1.1. Methyl (3*R*,9b*S*)-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylate (1a). L-Cysteine methyl ester hydrochloride (3.45 g, 20 mmol) was dissolved in water (15 mL) and potassium hydrogen carbonate (2.0 g, 20 mmol) was added following the addition of a solution of the 2-carboxybenzaldeyde (3.3 g, 22 mmol) in ethanol (15 mL). The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was diluted with water and extracted with dichloromethane. The organic phase was dried and the solvent was evaporated off giving the methyl (3*R*,9b*S*)-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylate **1a** as a white solid (3.48 g, 71%). Mp 83.2–85.8 °C (from ethyl ether), lit.[6] 83–86 °C. ν_{max}



11 12 13 14

Scheme 4.

(KBr) 1745 and 1710 cm^{-1}; δ_H 3.59–3.70 (2H, m), 3.83 (3H, s), 5.25 (1H, dd, *J*=4.9, 7.1 Hz), 6.08 (1H, s), 7.48–7.63 (3H, m, Ar-H), 7.81–7.83 (1H, m, Ar-H); *m/z* 249 (M^+, 100%), 221 (8), 190 (83), 162 (44) and 146 (12). Anal. Calcd for $C_{12}H_{11}NO_3S$: C, 57.82; H, 4.45; N, 5.62; S, 12.86. Found: C, 57.72; H, 4.44; N, 5.64; S, 12.48%. $[\alpha]_D^{25}=-400.5$ (*c*=2.3, CH_2Cl_2).

4.1.2. Methyl (3*R*,9b*S*)-9b-methyl-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylate (1b). L-Cysteine methyl ester hydrochloride (0.865 g, 5 mmol) and sodium acetate (1.23 g, 15 mmol) were dissolved in toluene (50 mL) and a solution of 2-acetylbenzoic acid (0.825 g, 5 mmol) in toluene (50 mL) was added. The reaction mixture was at reflux for 5 h. The solution was washed with water, dried and the solvent was evaporated off. The crude product was purified by flash chromatography [hexane–ethyl acetate (1:1)]. Compound **1b** was obtained as a white solid (0.98 g, 74.5%). Mp 128.8–132.1 °C (from ethyl ether), lit.[6] 113–116 °C. ν_{max} (KBr) 1753 and 1695 cm^{-1}; δ_H 1.96 (3H, s, CO_2Me), 3.83 (1H, dd, *J*=8.7 Hz), 3.85 (3H, s), 3.95 (1H, dd, *J*=6.5 Hz), 5,15 (1H, dd, *J*=6.5, 8.7 Hz), 7.48–7.54 (2H, m, ArH), 7.60–7.63 (1H, m, ArH), 7.80–7.82 (1H, m, ArH). $[\alpha]_D^{25}=-328.7$ (*c*=1.75, CH_2Cl_2).

4.2. General procedure for the synthesis of 5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylic acids 2a and 2b

The (3*R*,9b*S*)-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-a]isoindole-3-carboxylate (**1a** or **1b**) (1 mmol) and LiI (4 mmol) were dissolved in ethyl acetate (1.3 mL). The reaction mixture was protected from light and heated at reflux for 6 h. Water was added (5 mL) and the solution was acidified with HCl 1 M and extracted with ethyl acetate. The organic phase was washed with water and with saturated aqueous solution of NaCl. The organic solvent was evaporated off. To the residue a saturated aqueous solution of $NaHCO_3$ was added and the solution was washed with dichloromethane. The aqueous solution was acidified with concentrated HCl and extracted with ethyl acetate. The organic phase was dried and the solvent was evaporated off giving the desired product.

4.2.1. (3*R*,9b*S*)-5-Oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylic acid 2a. The title compound was obtained as a white solid (91%). Mp 156.1–157.9 °C (from ethyl ether), lit.[4] 161–162 °C. δ_H ($CDCl_3$/DMSO-d_6) 3.71 (1H, dd, *J*=7.4, 12.0 Hz), 3.81 (1H, dd, *J*=6.6, 12.0 Hz), 5.03 (1H, approx. t, *J*=7.0 Hz), 6.06 (1H, s), 7.51–7.58 (3H, m, Ar-H), 7.85–7.88 (1H, m, Ar-H); *m/z* [compound **2a** treated with CH_2N_2] 249 [(M^+−H), 100%], 221 (5) and 190 (95). Anal. Calcd for $C_{11}H_9NO_3S$: C, 56.16; H, 3.86; N, 5.95; S, 13.63. Found: C, 55.87; H, 3.92; N, 5.81; S, 13.92%. $[\alpha]_D^{25}=-343$ (*c*=0.1, EtOH).

4.2.2. (3*R*,9b*S*)-9b-Methyl-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylic acid 2b. The title compound was obtained as a white solid (85%). Mp 162.3–164.9 °C (from ethyl ether). δ_H 1.97 (3H, s), 3.88 (1H, dd, *J*=8.5, 12.3 Hz), 4.05 (1H, dd, *J*=7.8, 12.3 Hz), 5.01 (1H, approx. t, *J*=8.2 Hz), 7.50–7.56 (2H, m, ArH), 7.63–7.69 (1H, m, ArH), 7.81–7.84 (1H, m, ArH). Anal. Calcd for $C_{12}H_{11}NO_3S$: C, 57.82; H, 4.45; N, 5.62; S, 12.86. Found: C, 57.69; H, 4.42; N, 5.33; S, 12.33%. $[\alpha]_D^{25}=-363$ (*c*=0.1, MeOH).

4.3. General procedure for the synthesis of 3-methylene-2,5-dioxo-3*H*,9b*H*-oxazolo[2,3-*a*]isoindoles 6a and 6b

A solution of 5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylic acid (**2a** or **2b**) (3 mmol) in Ac_2O (5 mL) was heated, in a sealed tube, at 150 °C for 4 h. The reaction was cooled to room temperature and was diluted with dichloromethane (50 mL). The organic phase was washed with saturated aqueous solution of $NaHCO_3$ and with water, dried ($MgSO_4$) and evaporated off. The crude product was purified by flash chromatography [ethyl acetate–hexane (1:2)].

4.3.1. 3-Methylene-2,5-dioxo-3*H*,9b*H*-oxazolo[2,3-*a*]isoindole 6a. The title compound was obtained as a white solid (37%). Mp 174.2–176.0 °C (from ethyl ether). δ_H 5.93 (1H, d, *J*=1.4 Hz), 5.96 (1H, d, *J*=1.4 Hz), 6.51 (1H, s), 7.68–7.79 (3H, m, Ar-H), 7.95–7.97 (1H, Ar-H); δ_C 88.0, 107.9, 125.1, 125.5, 130.1, 131.7, 131.9, 134.4, 141.1, 165.2, 169.3; *m/z* 201 (M^+, 33%), 172 (4), 157 (41) and 133 (100).

4.3.2. 9b-Methyl-3-methylene-2,5-dioxo-3*H*,9b*H*-oxazolo[2,3-*a*]isoindole 6b. The title compound was obtained as a light yellow solid (64%). Mp 145.4–147.6 °C (from ethyl ether). δ_H 2.00 (3H, s), 5.88 (1H, d, *J*=0.7 Hz), 6.03 (1H, d, *J*=0.7 Hz), 7.60–7.65 (2H, m, Ar-H), 7.71–7.76 (1H, m, Ar-H), 7.95–8.00 (1H, Ar-H); *m/z* 216 (MH^+, 3%), 198 (2), 188 (19) and 171 (100); δ_C 32.2, 73.8, 108.0, 122.6, 125.3, 129.0, 130.2, 134.0, 135.8, 148.0, 167.0, 192.3.

4.4. General procedure for the flash vacuum pyrolysis of 5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylic acids 2a and 2b

Pyrolysis of 5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylic acids **2a**or **2b** (1.5 mmol) at 600 °C/3×10^{-2}–4×10^{-2} mbar onto a surface cooled at −196 °C over a period of 2 h gave a yellowish pyrolysate [The rate of volatilisation of the starting material was controlled by the use of a Kugelrohr oven which heated the sample at 200 °C]. After cooling to room temperature the pyrolysate was removed from the cold finger with dichloromethane. The solvent was removed in vacuo and the residue purified by flash chromatography [SiO_2, ethyl-acetate–hexane (1:2)] for **7a** and [SiO_2, ethyl-acetate–hexane (1:3)] for **7b**.

4.4.1. (9b*S*)-5-Oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole 7a. The title compound was obtained as a white solid (29%). Mp 99.6–100.3 °C (from ethyl ether/hexane), lit.[9] 97–100 °C. δ_H 3.35–3.44 (3H, m), 4.44–4.45 (1H, m), 5.88 (1H, s), 7.49–7.56 (2H, m, Ar-H), 7.57–7.60 (1H, m, Ar-H), 7.80–7.82 (1H, m, Ar-H); δ_C 36.5, 44.5, 66.0, 123.2, 124.3, 129.2, 131.1, 132.6, 145.1, 170.8; *m/z* 191 (M^+, 84%), 163 (12), 145 (100), 117 (39), 90 (28) and 76 (14). $[\alpha]_D^{25}=-341$ (*c*=0.1, CH_2Cl_2).

4.4.2. (9b*S*)-9b-Methyl-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole 7b. The title compound was obtained

as an yellow oil (21%). δ_H 1.92 (3H, s), 3.35–3.50 (3H, m), 4.54–4.61 (1H, m), 7.45–7.51 (2H, m, Ar-H), 7.57–7.63 (1H, m, Ar-H), 7.76–7.79 (1H, m, Ar-H); *m/z* 205 (M^+, 100%), 190 (21), 158 (68) and 146 (66). $[\alpha]_D^{25}=-69$ (c=0.15, CH_2Cl_2).

4.4.3. Methyl 2-(2-hydroxyphenyl)thiazolidine-4-carboxylate 8. L-Cysteine methyl ester hydrochloride (3.45 g, 20 mmol) was dissolved in water (15 mL) and potassium hydrogen carbonate (2.0 g, 20 mmol) was added following the addition of a solution of the salicylaldehyde (2.68 g, 22 mmol) in ethanol (15 mL). The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was diluted with water and extracted with dichloromethane. The organic phase was dried and the solvent was evaporated off giving the methyl 2-(2-hydroxyphenyl)thiazolidine-4-carboxylate **8** (60%). Mp 66.7–68.0 °C (from ethyl ether). ν (KBr) 3277 and 1736 cm^{-1}; δ_H (two diastereoisomers, ratio 73:27) 3.20–3.25 (1H, m), 3.40–3.47 (1H, m), 3.78 and 3.83 (3H, 2xs), 4.07–4.19 (1H, m), 5.62 and 5.92 (1H, 2xd, J=5.7, 4.3 Hz respectively), 6.79–6.94 (2H, m, Ar-H), 7.16–7.26 (2H, m, Ar-H); *m/z* 239 (M^+, 19%), 224 (10), 193 (21), 180 (36), 163 (71), 146 (13) and 132 (100). Anal. Calcd for $C_{11}H_{13}NO_3S$: C, 55.21; H, 5.48; N, 5.85; S, 13.40. Found: C, 55.42; H, 5.72; N, 5.81; S, 13.02%.

4.4.4. Methyl (2*R*,4*R*)-*N*-chlorocarbonyl-2-(2-hydroxyphenyl)thiazolidine-4-carboxylate 9. The methyl 2-(2-hydroxyphenyl)thiazolidine-4-carboxylate **8** (3.75 g, 15.7 mmol) was dissolved in dichloromethane (20 mL) and potassium hydrogen carbonate (1.57 g, 15.7 mmol) and a solution of the phosgene in toluene (10 mL, 18.84 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 6 h. The reaction mixture was diluted with water and extracted with ethyl acetate. The organic phase was dried and the solvent was evaporated off giving the methyl (2*R*,4*R*)-*N*-chlorocarbonyl-2-(2-hydroxyphenyl)thiazolidine-4-carboxylate **9** as a white solid (67%). Mp 145.2–146.9 °C. ν (KBr) 3285, 1746 and 1698 cm^{-1}. δ_H (two rotamers) ($CDCl_3$/DMSO-d_6) 3.22–3.41 (2H, m), 3.86 and 3.89 (3H, 2xs), 4.83 and 5.07 (1H, dd, J=6.4, 9.4 Hz and approx. t, J=6.4 Hz, respectively), 6.49 and 6.56 (1H, 2xs), 6.83–6.90 (2H, m, Ar-H), 7.10–7.18 (1H, m, Ar-H), 7.79–7.86 (1H, m, Ar-H); δ_C (two rotamers): major: ($CDCl_3$/DMSO-d_6) 31.7, 52.7, 63.8, 66.1, 115.0, 119.1, 125.6, 126.4, 128.7, 147.7, 153.3, 168.8; minor: 32.4, 52.9, 64.2, 66.8, 115.3, 119.2, 124.2, 126.0, 128.9, 147.7, 153.7, 169.5. *m/z* 265 [(M^+−HCl), 4%], 264 (6), 206 (15) and 179 (100). Anal. Calcd for $C_{12}H_{12}NO_4SCl$: C, 47.77; H, 4.01; N, 4.64; S, 10.62. Found: C, 47.89; H, 4.23; N, 4.57; S, 10.93%. $[\alpha]_D^{25}=+197$ (c=0.1, CH_3COCH_3).

4.4.5. Methyl (3*R*,10b*R*)-5-oxo-2,3-dihydro-10b*H*-[1,3]thiazolo[3,2-*c*][1,3]benzoxazine-3-carboxylate 10. The methyl (2*R*,4*R*)-*N*-chlorocarbonyl-2-(2-hydroxyphenyl)thiazolidine-4-carboxylate **9** (0.84 g, 2.8 mmol) was dissolved in ethyl acetate (15 mL). DBU (2 mmol) was added and the reaction mixture was heated at 50 °C for 2 h. Water was added (15 mL) and the solution was extracted with ethyl acetate. The organic phase was washed with water and dried. The residue obtained upon removal of the solvent was purified by column chromatography [ethyl acetate–hexane (1:1)] giving compound **10** as a white solid (87.5%). Mp 127.4–129.1 °C (from ethyl acetate–hexane). δ_H 3.44 (1H, dd, J=0.94, 12.7 Hz), 3.63 (1H, dd, J=7.5, 12.7 Hz), 3.74 (3H, s), 4.88 (1H, dd, J=1.1, 7.5 Hz), 6.05 (1H, s), 7.12–7.22 (3H, m, Ar-H), 7.31–7.44 (1H, m, Ar-H); δ_C 34.0, 53.0, 62.1, 63.0, 116.5, 119.2, 125.1, 125.7, 130.2, 148.2, 149.0, 169.0; *m/z* 265 (M^+, 5%), 206 (17) and 179 (100). Anal. Calcd for $C_{12}H_{11}NO_4S$: C, 54.33; H, 4.18; N, 5.28. Found: C, 53.98; H, 4.43; N, 5.14%. $[\alpha]_D^{25}=+98$ (c=0.1, CH_2Cl_2).

4.4.6. (3*R*,10b*R*)-5-Oxo-2,3-dihydro-10b*H*-[1,3]thiazolo[3,2-*c*][1,3]benzoxazine-3-carboxylic acid 11. The methyl (3*R*,10b*R*)-5-oxo-2,3-dihydro-10b*H*-[1.3]thiazolo-[3,2-*c*][1,3]benzoxazine-3-carboxylate **10** (0.235 g, 1 mmol) and LiI (4 mmol) were dissolved in ethyl acetate (1.3 mL). The reaction mixture was protected from light and heated at reflux for 6 h. Water was added (5 mL) and the solution was acidified with HCl 1 M and extracted with ethyl acetate. The organic phase was washed with water and with saturated aqueous solution of NaCl. The organic solvent was evaporated off. To the residue a saturated aqueous solution of $NaHCO_3$ was added and the solution was washed with DCM. The aqueous solution was acidified with concentrated HCl and extracted with ethyl acetate. The residue obtained upon removal of the solvent was purified by column chromatography [ethyl acetate–hexane (1:1)] giving compound **11** light yellow solid (71%). Mp 173.2–175.3 °C (from ethyl ether–hexane). δ_H ($CDCl_3$/DMSO-d_6) 3.46–3.51 (1H, m), 3.64 (1H, dd, J=7.7, 12.7 Hz), 4.81 (1H, dd, J=1.2, 7.6 Hz), 6.07 (1H, s), 7.09–7.21 (3H, m, ArH), 7.33–7.38 (1H, m, ArH); δ_C ($CDCl_3$/DMSO-d_6) 36.6, 64.6, 65.6, 119.0, 122.0, 127.5, 128.4, 132.6, 148.5, 151.7, 172.7; *m/z* [compound **11** treated with CH_2N_2] 264 [(M^+−H), 5%], 206 (14) and 179 (100). Anal. Calcd for $C_{11}H_9NO_4S$: C, 52.58; H, 3.61; N, 5.57; S, 12.76. Found: C, 52.41; H, 3.38; N, 5.58; S, 12.83%. $[\alpha]_D^{25}=+231$ (c=0.1, MeOH).

4.4.7. 5-Acetyl-2-phenyl-2,3-dihydrothiazole 14. A solution of (3*R*,10b*R*)-5-oxo-2,3-dihydro-10b*H*-[1,3]thiazolo[3,2-*c*][1,3]benzoxazine-3-carboxylic acid **11** (0.75 g, 3 mmol) in Ac_2O (5 mL) was heated, in a sealed tube, at 150 °C for 2 h. The reaction was cooled to room temperature and was diluted with dichloromethane (50 mL). The organic phase was washed with saturated aqueous solution of $NaHCO_3$ and with water, dried ($MgSO_4$) and evaporated off. The crude product was purified by flash chromatography [ethyl acetate–hexane (1:3)] giving compound **14** as a white solid (4%). Mp 73.5–74.5 °C (from dichloromethane–hexane). ν (KBr) 1745, 1690 and 1639 cm^{-1}. δ_H 2.40 (1H, s), 4.70 (1H, s), 4.75 (1H, m, NH), 6.73 (1H, s), 7.10–7.22 (3H, m, Ar-H), 7.32–7.39 (2H, m, Ar-H); δ_C 30.6, 56.9, 97.4, 116.3, 120.7, 125.3, 125.4, 130.2, 130.3, 148.5, 194.3; *m/z* (CI–CH_4) 206 [(MH^+), 28%], 137 (5) and 75 (100).

4.5. X-ray structure determination of methyl (3*R*,9b*S*)-5-oxo-2,3,5,9b-tetrahydrothiazolo[2,3-*a*]isoindole-3-carboxylate 1a

Crystal data. $C_{12}H_{11}NO_3S$, M=249.28, tetragonal, space group $P4_12_12$ (#92), $a=b$=9.424(8), c=26.209(12) Å,

V=2390.1(11) Å^3, Z=8, D_c=1.386 g cm^{-3}, F_{000}=1040, μ=2.454 mm^{-1}, T=296 K. Number of independent intensities 2294 from transparent, colourless prism, 0.39×0.20×0.15 mm^3. Ψ-scan absorption correction applied, T_{min}=0.888, T_{max}=0.986. No significant crystal decay detected.

Data collection. X-ray measurements were performed on a Enraf-Nonius MACH3 diffractometer using ω–2θ scans up to θ_{max}=71.51°.

Structure solution and refinement. The structure was solved using methods using SHELXS97. R=0.0344 for 2116 reflections with $I>2\sigma$, R_w=0.0888 for 2294 reflections used in the refinement and 156 variable parameters. H-atoms were placed at calculated positions except those of the methyl group which were determined from a Fourier difference synthesis and refined as riding on their parent atoms using SHELXL97 defaults.

4.6. X-ray structure determination of methyl (2*R*,4*R*)-*N*-chlorocarbonyl-2-(2-hydroxyphenyl)thiazolidine-4-carboxylate 9

Crystal data. $C_{12}H_{12}ClNO_4S$, M=301.74, orthorhombic, space group $P2_12_12_1$ (#19), a=8.7424(16) Å, b=10.1480(7), c=15.857(3) Å, V=1406.8(4) Å^3, Z=4, D_c=1.425 g cm^3, F_{000}=624, μ=0.428 mm^{-1}, T=296 K. Number of independent intensities: 3210 from transparent, colourless prism, 0.37×0.20×0.15 mm^3. ψ-scan absorption correction applied, T_{min}=0.980, T_{max}=0.961. No significant crystal decay detected.

Data collection. X-ray measurements were performed on a Enraf-Nonius CAD-4 diffractometer using ω–2θ scans up to θ_{max}=27.44°.

Structure solution and refinement. The structure was solved using direct methods using SHELXS97. R=0.0409 for 2182 reflections with $I>2$, R_w=0.0952 for 3210 reflections used in the refinement and 175 variable parameters. H-atoms were placed at calculated positions except those of the methyl group which were determined from a Fourier difference synthesis and refined as riding on their parent atoms using SHELXL97 defaults.

Acknowledgements

Financial support from Chymiotechnon and Fundação para a Ciência e a Tecnologia (POCTI/36137/QUI/2000) is gratefully acknowledged.

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Tetrahedron 60 (2004) 3957–3966
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Unusual spirocyclic macroline alkaloids, nitrogenous derivatives, and a cytotoxic bisindole from *Alstonia*

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Received 21 January 2004; revised 17 February 2004; accepted 11 March 2004

Abstract—The bark extract of the Malayan *A. macrophylla* provided several novel indoles with unprecedented carbon skeletons, an unusual nitrogenous compound, a cytotoxic bisindole, several new macroline alkaloids, in addition to other known alkaloids. The structures of the new compounds were established by spectroscopic analysis.

1. Introduction

The genus *Alstonia* is characterised by a preponderance of the macroline-type indole and oxindole alkaloids.[1–9] We have previously reported the presence of new macroline indoles as well as oxindoles from the Malayan species, *A. angustifolia* var. *latifolia*, including isoalstonisine and macrogentine, which represent the first macroline oxindoles possessing the *S* configuration at the spirocyclic carbon.[3,4] In continuation of our studies of Malaysian *Alstonia*, we would like to report the structures of new alkaloids from *A. macrophylla*, including novel macroline alkaloids incorporating an unprecedented spiroketal unit,[9] unusual nitrogenous derivatives, and a cytotoxic bisindole.

2. Results and discussion

Macrodasine A **1** was obtained from the bark extract of *A. macrophylla* as a colourless oil, with $[\alpha]_D$=+36 (*c* 0.36, $CHCl_3$). The UV spectrum was characteristic of an indole chromophore with absorption maxima at 230 and 287 nm, while the IR spectrum (3411 cm^{-1}, broad) indicated the presence of hydroxyl functions. The EIMS of **1** showed a molecular ion at *m/z* 454, which analyzed for $C_{26}H_{34}N_2O_5$, requiring 11 degrees of unsaturation while the mass fragments which were observed at *m/z* 197, 182, 181, 170, and 144 are typical of macroline derivatives[10] and provided early indication that **1** contained a macroline core. The ^{13}C NMR spectrum (Table 2) gave a total of 26 separate carbon resonances (three methyls, six methylenes, 11 methines, and six quaternary carbons) in agreement with the molecular formula. In addition to the eight signals associated with the indole moiety, the ^{13}C NMR spectrum is notable for the presence of two oxymethylenes (δ 63.9, 64.3), two oxymethines (δ 77.7, 79.2), and two quaternary carbons each of which are flanked by two oxygen atoms (δ 105.5, 114.8), consistent with a highly oxygenated molecule as indicated by the molecular formula. The ^1H NMR spectrum of **1** (Table 1) showed the presence of an unsubstituted indole chromophore, from the signals due to four aromatic hydrogens, the presence of three methyl groups corresponding to the *N*(1)–Me (δ 3.63), *N*(4)–Me (δ 2.33), and Me(18) (δ 1.59), and a hydroxymethyl group from the presence of a pair of doublet of doublets at δ 3.43 and 3.77 (corresponding to the carbon resonance at δ 63.9).

The COSY spectrum disclosed some partial structures which are characteristic of a macroline skeleton, such as $NCHCH_2$ and $NCHCH_2CHCHCH_2O$, corresponding to the C(5)–C(6) and C(3)–C(14)–C(15)–C(16)–C(17) fragments.[3,4] This is further supported by the observed hydrogen chemical shifts and coupling behaviour for H(3), H(5), H(16), H(17), as well as the three characteristic methyl groups which are typical of a macroline compound (e.g., alstonerine).[3] At this stage, further analysis of the COSY spectrum was complicated by overlap of some key signals. Thus, two sets of partial structures can be proposed for the remaining fragments, viz., $CHCH_2$ and $OCHCH_2CHCH_2O$, versus $CHCH_2CHO$ and CH_2CHCH_2O, which with the aid of the HMBC data led to two possible structures, **1** and **2**, respectively. Structure **1** is distinguished by the incorporation of a 1,6-dioxaspiro[4.4]nonane substructure fused onto a macroline residue, while structure **2** on the other hand, is distinguished by the incorporation of contiguously fused tetrahydropyran and tetrahydrofuran rings onto the

Keywords: Alkaloids; NMR; Plants.
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doi:10.1016/j.tet.2004.03.031

Table 1. ^{1}H NMR spectral data of **1**, **3**, **4**, **5**, **11**, **12**, **13**, **14**, **15**, and **18**[a]

Position	**1**	**3**	**4**	**5**	**11**	**12**	**13**	**14**	**15**	**18**	Position	**18**
3	3.95 t (3)	4.79 brs	3.94 t (3)	4.78 br s	3.96 m	3.98 t (3)	3.97 t (4)	3.82 br s	3.82 br s	4.09 dd (4, 2)	3′	3.79 t (3)
5	2.98 d (7)	3.56 d (7)	2.99 d (7)	3.57 d (7)	2.87 d (7)	2.91 d (7)	2.91 d (7)	3.10 d (6)	3.10 d (6)	3.46 d (7)	5′	2.99 d (7)
6	2.39 m	3.04 d (18)	2.41 d (17)	3.02 d (17)	2.47 d (17)	2.43 d (17)	2.45 d (17)	2.48 d (16)	2.48 d (16)	2.54 m	6′	2.28 m
	3.27 dd (17, 7)	3.45 dd (18, 7)	3.28 dd (17, 7)	3.46 dd (17, 7)	3.25 dd (17, 7)	3.26 dd (17, 7)	3.27 dd (17, 7)	3.30 dd (16, 6)	3.30 dd (16, 6)	3.08 m		3.32 m
9	7.50 br d (8)	7.56 br d (8)	7.50 br d (8)	7.55 br d (8)	7.49 br d (8)	7.49 br d (8)	7.49 dd (8, 1)	7.33 d (8)	7.33 d (8)	7.52 br d (8)	9′	6.90 s
10	7.12 br t (8)	7.23 td (8, 1)	7.12 td (8, 1)	7.23 td (8, 1)	7.10 td (8, 1)	7.10 td (8, 1)	7.09 td (8, 1)	6.76 dd (8, 2)	6.76 dd (8, 2)	7.13 td (8, 1)	10′	—
11	7.21 td (8, 1)	7.35 td (8, 1)	7.21 td (8, 1)	7.35 td (8, 1)	7.19 td (8, 1)	7.18 td (8, 1)	7.17 td (8, 1)	—	—	7.22 td (8, 1)	11′	—
12	7.31 br d (8)	7.40 br d (8)	7.31 br d (8)	7.40 br d (8)	7.29 br d (8)	7.29 br d (8)	7.27 dd (8, 1)	6.84 d (2)	6.84 d (2)	7.32 br d (8)	12′	6.69 s
14	1.55 ddd (13, 5, 3)	1.78 br d (14)	1.56 m	1.79 dt (14, 5)	1.42 ddd (13, 5, 2)	1.54 ddd (12, 4, 3)	1.39 dt (13, 4)	1.81 m	1.81 m	1.98 m	14′	1.75 td (12, 3)
												2.04 m
	2.39 m	3.33 td (14, 4)	2.42 m	3.35 td (14, 3)	2.50 td (13, 4)	2.29 m	2.26 td (13, 4)	2.13 m	2.13 m	2.41 m	15′	2.54 m
15	1.85 m	1.92 dt (14, 5)	1.84 dt (12, 5)	1.96 dt (14, 5)	2.06 dt (13, 5)	1.97 dt (13, 4)	1.86 m	2.64 dt (11, 5)	2.64 dt (11, 5)	2.14 m	16′	1.84 dt (11, 4)
16	2.03 dt (12, 5)	2.33 dt (12, 5)	2.14 m	2.49 dt (13, 5)	2.15 dt (11, 5)	1.86 dt (11, 4)	1.86 m	1.92 m	1.92 m	1.57 m	17′	4.13 ddd
17	3.70 dd (12, 5)	3.75 dd (12, 5)	3.85 dd (12, 5)	3.88 dd (13, 5)	3.79 dd (11, 5)	3.73 dd (11, 4)	3.74 dd (11, 4)	4.17 ddd (11, 4, 2)	4.19 ddd (11, 4, 2)	3.95 dd (11, 3)		(11, 4, 1)
												4.37 t (11)
	4.04 t (12)	4.82 t (12)	4.08 t (12)	4.92 t (13)	4.07 t (11)	4.06 t (11)	4.07 t (11)	4.45 t (11)	4.50 t (11)	4.01 dd (11, 2)	18′	2.05 s
18	1.59 s	1.72 s	1.54 s	1.71 s	1.24 d (7)	1.15 d (6)	1.13 d (6)	2.09 s	2.17 s	1.72 s	21′	7.51 s
19	—	—	—	—	3.96 m	3.49 m	3.51 dq (10, 6)	—	—	—	*N*(1)-Me′	3.65 s
20	2.01 dd (12, 8)	2.07 m	2.02 m	2.10 m	1.07 m	1.46 m	1.69 m	—	—	3.32 m	*N*(4)-Me′	2.25 s
21	1.85 m	1.85 dd (13, 8)	2.02 m	2.10 m	3.69 dd (11, 4)	3.31 dd (11, 8)	3.83 d (7)	7.54 s	9.66 s	2.41 m	11′-OMe	3.87 s
	2.39 m	2.17 t (13)	2.15 dd (13, 11)	2.10 m	3.81 dd (11, 6)	3.49 m	3.83 d (7)	—	—	3.08 m		
23	4.13 d (5)	5.21 d (4)	—	—	—	—	1.68 s	—	—	—		
24	1.85 m	2.03 m	2.50 dd (17, 7)	2.28 dd (19, 7)	—	—	—	—	—	—		
	2.39 m	2.14 td (9, 4)	2.52 dd (17, 8)	2.69 dd (19, 7)	—	—	—	—	—	—		
25	4.42 m	4.37 dtd (9, 7, 4)	4.56 m	4.69 tt (7, 4)	—	—	—	—	—	—		
26	3.43 dd (12, 3)	3.98 dd (12, 7)	3.61 dd (12, 4)	4.15 dd (12, 4)	—	—	—	—	—	—		
	3.77 dd (12, 2)	4.21 dd (12, 4)	3.96 dd (12, 3)	4.30 dd (12, 3)	—	—	—	—	—	—		
N(1)–Me	3.63 s	3.69 s	3.63 s	3.69 s	3.62 s	3.62 s	3.60 s	—	—	3.55 s		
N(4)–Me	2.33 s	2.88 s	2.34 s	2.88 s	2.31 s	2.30 s	2.34 s	2.36 s	2.36 s	2.34 s		
23-OAc	—	2.02 s	—	—	—	—	—	—	—	—		
26-OAc	—	2.06 s	—	2.02 s	—	—	—	—	—	—		
11-OMe	—	—	—	—		—	—	3.84 s	3.84 s	—		

[a] $CDCl_3$, 400 MHz; assignments based on COSY and HMQC.

Table 2. ^{13}C NMR spectral data of **1**, **3**, **4**, **5**, **7**, **9**, **10**, **11**, **12**, **13**, **14**, **15** and **18**[a]

Position	**1**	**3**	**4**	**5**	**7**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**18**	Position	**18**
2	132.8	[b]	131.5	125.9	60.2	132.8	132.8	133.2	133.2	133.0	129.2	129.2	131.3	2′	132.9
3	53.3	55.1	53.3	55.7	36.9	53.5	53.1	53.7	53.6	53.5	54.8	54.8	53.1	3′	53.7
4	—	—	—	—	220.0	—	—	—	—	—	—	—	—	5′	54.7
5	54.8	56.2	54.7	56.4	69.1	54.5	54.9	54.6	55.1	54.9	55.1	55.1	59.2	6′	22.0
6	22.5	23.4	22.6	23.8	30.7	22.5	22.4	22.6	22.5	22.4	22.9	22.5	22.6	7′	105.4
7	106.4	105.4	106.5	105.9	26.7	106.6	106.6	106.6	106.7	106.7	106.4	106.4	105.9	8′	120.1
8	126.4	[b]	126.2	124.9	36.5	126.2	126.2	126.4	126.4	126.3	121.6	121.6	126.3	9′	118.7
9	118.0	118.4	118.0	118.6	68.4	118.1	117.9	118.1	117.9	117.9	118.2	118.2	118.2	10′	119.1
10	118.9	120.0	118.9	120.4	18.9	118.9	118.9	118.8	118.8	118.8	108.8	108.8	119.0	11′	153.6
11	121.0	[b]	121.0	123.2	69.4	121.0	121.0	120.8	120.7	120.8	156.2	156.2	120.9	12′	91.3
12	108.0	109.5	108.8	109.7	54.2	108.7	108.9	108.7	108.8	108.5	95.4	95.4	108.7	13′	136.5
13	136.9	137.5	137.1	137.6	203.4	137.2	137.0	136.9	137.0	136.9	136.8	136.8	137.0	14′	32.4
14	31.9	[b]	31.9	[b]	—	30.1	26.7	30.7	25.3	25.0	32.9	32.9	32.3	15′	22.8
15	26.5	25.9	27.1	25.9	—	27.0	26.1	28.6	26.7	27.1	23.0	23.0	31.5	16′	38.3
16	36.9	36.8	36.6	36.8	—	39.4	42.5	39.3	43.5	43.4	38.6	38.6	43.1	17′	67.7
17	64.3	[b]	68.8	62.9	—	68.8	67.1	68.9	67.6	67.5	67.7	68.1	66.5	18′	24.9
18	24.2	[b]	23.5	22.7	—	19.2	20.2	18.8	20.2	20.1	25.0	16.5	31.1	19′	195.4
19	105.5	105.4	106.1	104.9	—	69.4	67.8	71.2	70.5	70.3	195.6	170.0	213.2	20′	120.8
20	44.3	43.6	45.8	45.3	—	54.6	57.7	43.6	46.8	43.2	121.6	121.6	54.5	21′	157.4
21	34.7	35.2	35.9	35.6	—	204.7	203.0	63.1	61.6	62.9	157.6	188.8	32.0	*N*(1)-Me′	29.0
22	114.8	113.7	106.4	105.6	—	—	—	—	—	170.8	—	—	—	*N*(4)-Me′	41.2
23	77.7	78.8	209.4	208.6	—	—	—	—	—	20.3	—	—	—	11′-OMe	55.5
24	33.0	33.0	34.7	35.3	—	—	—	—	—	—	—	—	—		
25	79.2	76.4	75.0	72.4	—	—	—	—	—	—	—	—	—		
26	63.9	67.2	63.1	64.9	—	—	—	—	—	—	—	—	—		
N(1)–Me	29.0	29.4	29.0	29.5	42.2	29.1	29.0	29.0	29.0	28.9	—	—	28.9		
N(4)–Me	41.6	40.8	41.7	40.4	—	41.8	41.6	41.7	41.7	41.6	41.5	41.5	41.7		
23-OAc	—	21.1 170.0	—	—	—	—	—	—	—	—	—	—	—		
26-OAc	—	20.9 170.8	—	20.7 170.6	—	—	—	—	—	—	—	—	—		
11-OMe	—	—	—	—	—	—	—	—	—	—	55.8	55.8	—		

[a] $CDCl_3$, 100 MHz; assignments based on HMQC and HMBC.
[b] Not detected.

same macroline unit. Both structures accommodate the observed NMR chemical shifts as well as the HMBC correlation data. To resolve the difficulty in distinguishing the two structures, acetylation (Ac_2O, pyridine) was carried out which yielded a single diacetylated derivative, providing cogent support for structure **1**. Furthermore, conversion to the acetylated derivative resulted in a better resolved ^1H NMR spectrum (Table 1), which removed the earlier ambiguity associated with some of the key signals. Specifically, the signals for H(21), H(23), H(24) and H(25) were now sufficiently clear and well resolved in the acetate derivative **3** {whereas H(24) and H(21) were overlapping multiplets in **1**}, and indicated the presence of the key $OCHCH_2CHCH_2O$ fragment, corresponding to the C(23)–C(24)–C(25)–C(26) partial structure in **1**. In addition, the observed carbon resonance of δ 114.8 for the spirocyclic centre was in good agreement with that previously noted for the spirocarbon in compounds containing a 1,6-dioxaspiro[4.4]nonane unit.[11–14]

The ring junction stereochemistry between rings C, D, and E, is assumed to follow that in the known macroline compounds (e.g., alstonerine)[3] from the similarity of the chemical shifts and coupling patterns observed for the ring junction hydrogens, a supposition which is also in agreement with the NOE and NOESY data. The observed NOE between 18-methyl and H(17α) as well as H(20), fixes the E/F ring junction stereochemistry as *cis* {18-Me and H(20) both α}. The resonance for H(20) was a doublet of doublets with J=12, 8 Hz. Decoupling experiments indicated that the splittings were due to coupling with the two H(21). Since the stereochemistry of H(20) has been fixed as α, the 12 Hz coupling must be due to coupling to H(21β). Irradiation of H(23) causes NOE enhancement of H(25) and vice versa, indicating that they are *syn* to each other. Aside from these, further assignment of the remaining stereochemistry, such as that of the spirocyclic centre at C(22), was precluded by the unresolved signals of H(21) in **1**, which were fortuitously well resolved in the diacetate derivative **3**. Thus observation of the key NOE interaction between H(23) and H(21β) in **3**, not only allowed assignment of the configuration at the spirocarbon as *R*, but also fixes the stereochemistry of C(23) and C(25), respectively, as *R*, *R*.

Macrodasine B **4** was also obtained from the bark extract of *A. macrophylla*, as a colourless oil, with $[\alpha]_D$=+149 (*c* 0.067, $CHCl_3$). The UV spectrum was very similar to that of **1** with absorption maxima at 230 and 287 nm (log ε 3.95 and 3.23, respectively), characteristic of an unsubstituted indole chromophore, while the IR spectrum showed in addition to a broad OH band at 3435 cm^{-1}, another band at 1765 cm^{-1}, indicative of a five-membered cyclic ketone. The EIMS of **4** showed a molecular ion at *m*/*z* 452, which analyzed for $C_{26}H_{32}N_2O_5$, two mass units less than that of **1**, and requiring 12 degrees of unsaturation. In common with **1**, the mass fragments which were observed at *m*/*z* 197, 182, 181, 170, and 144 are characteristic of macroline derivatives,[10] and indicated that **4** also contained a macroline-like residue. The ^{13}C NMR spectrum (Table 2) gave a total of 26 separate carbon resonances (three methyls, six methylenes,

10 methines, and seven quaternary carbons) in agreement with the molecular formula, but differing from that of **1** by the addition of a quaternary carbon at the expense of a methine.

The ^{1}H and ^{13}C NMR spectral data share a number of common features with that of a typical macroline (as well as with **1**), indicating that rings A–E are essentially unchanged, but that substantial changes have occurred affecting rings F and G. Thus the ^{1}H NMR spectrum (Table 1) showed the presence of three methyl groups corresponding to the *N*(1)–Me (δ 3.63), *N*(4)–Me (δ 2.34), and Me(18) (δ 1.54), and a hydroxymethyl group, from the presence of a pair of doublet of doublets at δ 3.61 and 3.96 (corresponding to the carbon resonance at δ 63.1), which are similar to **1**. The COSY spectrum of **4** showed in addition to the $NCHCH_2$, $CHCH_2$, and $NCHCH_2CHCHCH_2O$ fragments, which are common to **1**, a CH_2CHCH_2O fragment, in place of the $OCHCH_2CHCH_2O$ fragment observed in **1**. Comparison of the ^{13}C NMR spectra of 1 and 4, showed that the two oxymethylenes at δ 68.8 and 63.1, corresponding to C(17) and C(26), respectively, are intact, as is the oxymethine corresponding to C(25) {δ 75.0 c.f. 79.2 in **1**}, and the quaternary carbon resonance due to the spiroacetal C(22), which was observed at δ 106.4. However, the other oxymethine at δ 77.7 corresponding to C(23) in **1**, is absent in the spectrum of **4**. Instead a ketone carbonyl resonance at δ 209.4 was observed in its place.

At this stage the structure of macrodasine B can be assembled as shown in structure **4**, which reveals it to be the 23-oxo derivative of **1**. The structure is consistent with the HMBC data (Fig. 1), as well as the observed cyclic ketone absorption at 1765 cm^{-1} in the IR spectrum, which is in excellent agreement with that of 3-oxacyclopentanones (1764 cm^{-1}) versus that for 3-oxacyclohexanones (1725 cm^{-1}).[15] In addition, the chemical shift and geminal coupling constant for H(24) {δ 2.50 dd, J=17, 7 Hz; 2.52, dd, J=17, 8 Hz} are highly diagnostic of geminal hydrogens adjacent to a carbonyl carbon.[4,16] Reaction of **4** with Ac_2O/pyridine yielded the monoacetate derivative **5**, in agreement with the proposed structure. The NOESY and NOE data are similar to those observed for **1** and confirmed the stereochemistry of the E/F ring junction {18-Me and H(20) both α},[9] in addition to the characteristic ring junction stereochemistries for the C/D/E rings, which correspond to that of a typical macroline.[3,4] In the case of macrodasine A **1**, assignment of the configuration at the spirocyclic C(22) was facilitated by the well-resolved H(21) and H(23) signals in the diacetate derivative **3**, which permitted NOE experiments to be carried out {NOE between H(23) and H(21β)},[9] which is precluded in the case of macrodasine B **4**, where C(23) is now a ketone carbonyl. The configuration at the spirocyclic C(22) and at C(25) in **4** are therefore tentatively assigned as *R* and *R*, respectively, on the grounds of a presumed close biogenetic relationship with **1**.

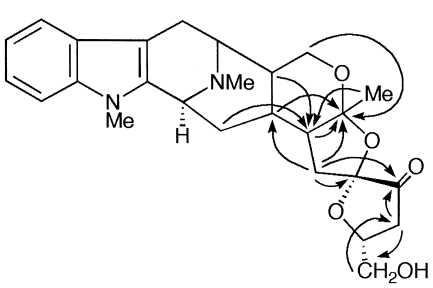


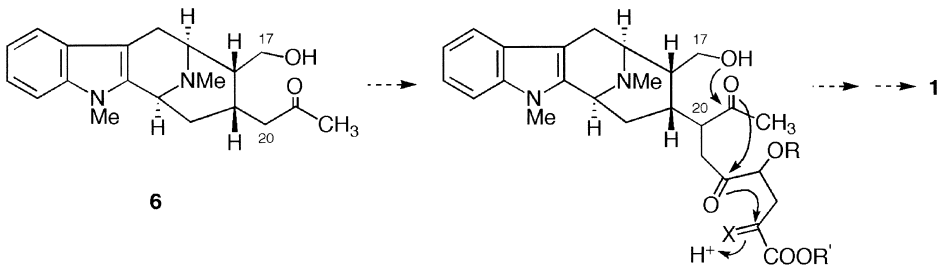
Figure 1. Selected HMBC (H to C) of **4**.

Macrodasines A **1** and B **4**, represent the first members of an unusual class of macroline compounds which have incorporated additional novel structural features, in the form of fused spirocyclic tetrahydrofuran rings, incorporating an unprecedented spiroacetal moiety. The spiroketal unit has been previously encountered in insect pheromones,[17–19] marine natural products,[11–14,20,21] microbial compounds,[17,22–26] plant steroidal derivatives[17] and various other plant secondary metabolites.[17] It has however not been found as a substructure in alkaloids. The macrodasines **1** and **4**, thus represent the first instances of the incorporation of a spiroketal unit in an indole alkaloid.

A tentative proposal for a possible pathway to these unusual compounds is from the ring-opened form of alstonerine **6**,[4,30] which on alkylation by a six-carbon fragment at C(20), followed by tandem intramolecular hemiketal formation (Scheme 1), yields the ring system of the macrodasines.[9]

An unusual nitrogenous compound, angustimalal **7** was also obtained from this study. It was isolated as a colourless oil, with $[\alpha]_D$=+78 (*c* 0.064, $CHCl_3$). The IR spectrum showed two carbonyl bands, one at 1717 cm^{-1}, corresponding to an aldehyde carbonyl, and another at 1741 cm^{-1}, indicative of a five-membered ring ketone. The characterstic Fermi doublets at 2767 and 2867 cm^{-1} were clear in this instance, and taken with the ^{1}H NMR signal at δ 10.0, confirmed the presence of the aldehyde function. The EIMS of **7** showed a molecular ion at *m/z* 237, the odd mass indicating the presence of a single nitrogen. HREIMS measurements gave the formula $C_{13}H_{19}NO_3$. The ^{13}C NMR spectrum (Table 2) showed a total of 13 peaks in agreement with the molecular formula (two methyls, three methylenes, seven methines and one quaternary carbon). Two methyl groups were indicated, a CH_3CH (δ 1.42) and an NCH_3 (δ 2.30). The quaternary carbon resonance at δ 220.0 is due to a ketone function while the methine at δ 203.4 corresponds to the aldehyde group. The COSY spectrum revealed the following partial structures, $NCHCH_2$, $NCHCH_2CHCHCH_2O$, and $CH_3CHCHCH{=}O$. The former two fragments are characteristic of macroline compounds and correspond to the C(5)–C(6) and C(3)–C(14)–C(15)–C(16)–C(17) fragments, respectively, of a macroline alkaloid, while the latter fragment, together with the two methine resonances at δ 26.7 and 36.5, corresponds to the ring E portion of a type-A macroline, such as talcarpine **9**. The molecule can therefore be assembled accordingly and requires only insertion of a ketone function to complete the structure of angustimalal as shown in **7**, which is in perfect agreement with the HMBC data (Fig. 2).

The ring junction stereochemistry was established from the NOESY spectrum and was in agreement with that in a typical macroline alkaloid (e.g., talcarpine **9**). The stereochemistries of the tetrahydropyran ring substituents were



Scheme 1. (X=O; or OH, H).

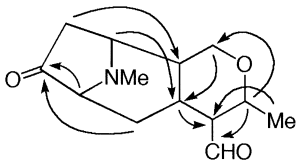


Figure 2. Selected HMBC (H to C) of **7**.

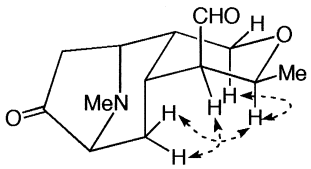


Figure 3. Selected NOE's of **7**.

also established on the basis of the NOESY spectrum (Fig. 3).

The stereochemistry of the two H(9) can be determined on the basis of their respective coupling constants (see Section 3). The α-oriented H(9) which is *trans*-diaxial with H(8) is seen as a triplet, with J=11 Hz. The observed NOE interaction of H(9α) with H(11) indicated that the methyl substituent is β. Similarly H(6α) can be distinguished from H(6β) on the basis of their coupling interactions. The NOE observed between H(6α)/H(11α), and between H(6β)/H(12) confirmed the β-stereochemistry of the C(12) aldehyde substituent (Fig. 3). This assignment is further vindicated by comparison of the chemical shifts of the aldehyde-H in angustimalal **7** (δ 10.0) with the shifts observed for the corresponding macroline alkaloids, talcarpine **9** (β-CHO, δ 9.95) and *N*(4)-methyl-*N*(4), 21-*seco*talpinine 10 (α-CHO, δ 9.41) (see Section 3).

The structure of angustimalal **7** shows that it retains all the features of the non-indole portion of a type-A macroline compound, except for the presence of an additional oxygenated carbon. A similar compound, angustimaline **8** (corresponding to the non-indole portion of a type-B macroline alkaloid in this case) has been encountered once recently, from the bark extract of another *Alstonia* species.[27] The origin of such compounds remains enigmatic, although a simple assumption (in the case of **7**) is that it is probably derived from fragmentation of a macroline-type precursor, possibly talcarpine **9** (which also occurs in the same plant), or its as yet unknown oxindole.

Three other new macroline indole derivatives were also obtained from the bark extract, macrocarpines A **11**, B **12**, and C **13**. A common feature of these three alkaloids is that they contain a saturated ring E, as exemplified by talcarpine **9**. Macrocarpine A **11** was obtained as a light yellowish oil, $[\alpha]_D$=+117 (c 0.11, $CHCl_3$). The IR spectrum showed the presence of a hydroxyl function (3400 cm^{-1}), while the UV spectrum indicated an indole chromophore. The EIMS of **11** showed a molecular ion at m/z 340, which analysed for $C_{21}H_{28}N_2O_2$. Examination of the ^1H and ^{13}C NMR spectral data (Tables 1 and 2, respectively) revealed a macroline compound resembling talcarpine **9** in all respects except for changes involving the substituents in the saturated E-ring, viz., the replacement of the 20β-CHO substituent by a 20β-hydroxymethyl substituent in **11**. This is clearly indicated by the presence of the hydroxymethyl signals (δ_H 3.69, 3.81; δ_C 63.1) in place of the aldehyde signals of talcarpine. The stereochemistry of the hydroxymethyl substituent at C(20) is readily confirmed from the observed NOE interaction between H(14β) and H(20) which is only possible if H(20) is α. The assignment is also confirmed by chemical correlation, by conversion of talcarpine **9** to **11** by $NaBH_4$ reduction, and oxidation of **11** to talcarpine **9** by PCC. Macrocarpine B **12** was obtained as a light yellowish oil, $[\alpha]_D$=−51 (c 0.34, $CHCl_3$). The IR (OH, 3400 cm^{-1}), UV (indole), and EIMS (M^+ m/z 340) spectral data were similar to that of **11**, as were the NMR spectral data (Tables 1 and 2), which were generally similar except for the noticeable difference in the shifts of C(14) and C(16). These similarities indicated that **12** is the C(20) epimer of **11** which is confirmed from the NOESY spectrum which showed NOE interaction between H(20)/H(16). The assignment was also confirmed by chemical correlation with *N*(4)-methyl-*N*(4), 21-*seco*talpinine **10**, via $NaBH_4$ reduction. Macrocarpine C **13** is readily shown to be the acetate derivative of macrocarpine B **12** from the spectral data. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) were similar to that of **12** except for the presence of a methyl resonance at δ 1.68 and the carbon signals at δ 20.3 and 170.8. The assignment was again supported by correlation with **12** via acetylation (Ac_2O/pyridine).

Two other new macroline derivatives **14** and **15** were obtained as an inseparable mixture of type-B and type-A forms (ratio 3:1, respectively), which co-eluted in column chromatography and proved resistant to further attempts at resolution by chromatography or fractional crystallization. The H(18) {methyl} and H(21) {aldehyde-H for **15**, vinylic-H for **14**} signals are clearly distinguishable in the ^1H NMR spectrum (Table 1), while the signals of H(17) are

partially overlapped. The rest of the hydrogen resonances of the two isomers are coincident. In the ^{13}C NMR spectrum (Table 2), the majority of the signals are coincident with the exception of C(6), C(17), C(18), C(19), and C(21). This behaviour has been observed previously in the case of the macroline indoles, alstonerine (type-B) and alstonerinal (type-A),[3] and in the case of the macroline oxindoles, *N*(1)-demethylalstonisine (type-B) and *N*(1)-demethylalstonal (type-A).[4] In the event, the spectral data (Tables 1 and 2) indicated that **14** and **15** are the *N*(1)-demethyl derivatives of alstophylline **16** and alstophyllal **17**, respectively. The latter two compounds also occur as a pair of unresolvable type-A and type-B isomers in both the stem and leaf extracts.

Two bisindole alkaloids were obtained, of which one was a new natural product. Perhentinine **18** was obtained as a light yellowish oil, $[\alpha]_D=-61$ (*c* 1.19, $CHCl_3$). The IR spectrum showed the presence of hydroxyl (3400 cm^{-1}), ketone (1701 cm^{-1}), and α,β-unsaturated ketone (1651, 1616 cm^{-1}) functions, while the UV spectrum indicated an indole chromophore, with charcteristic absorption maxima at 231 and 298 nm. The LSIMS spectrum of **18** showed the MH^+ ion at *m/z* 705, which analysed for $C_{43}H_{52}N_4O_5$. The ^1H NMR spectrum (Table 1) showed several clear features, *inter alia*, four aromatic hydrogen signals associated with an unsubstituted indole moiety, two aromatic singlets associated with another indole substituted at positions 10′ and 11′, a total of seven methyl singlets corresponding to two *N*(1)-methyls, two *N*(4)-methyls, two acetyls, and an aromatic methoxy group. Since only six aromatic hydrogens are observed and both indolic nitrogens are substituted, it is reasonable to conclude that the bisindole is branched from one of the aromatic carbon atoms of one monomer, with the adjacent position occupied by the methoxy substituent. The low field region also showed the presence of a vinylic singlet (δ 7.51), which with the associated 18′-methyl singlet at δ 2.05, indicated one monomer to be a type-B macroline. This is supported by the observation of the characteristic C(17′) hydrogen signals as a ddd and a triplet at δ 4.13 and 4.37, respectively.[3,4] The ^{13}C NMR spectrum (Table 2) showed a total of 43 carbon signals, comprising seven methyls, seven methylenes, 16 methines, and 13 quaternary carbon atoms.

Examination of the carbon spectrum revealed that one set of signals showed a correspondence to a 10′, 11′-disubstituted alstonerine.[3] Furthermore, the observed low-field resonances of both H(12′) and C(12′) at δ 6.69 and 91.3, respectively, are characteristic of oxygenation at the adjacent C(11′),[28,29] thus indicating position 11′ as the site of methoxy substitution and position 10′ as the site of branching of the bisindole from this monomeric unit.

The second moiety constituting the bisindole was deduced to be another macroline derivative from initial inspection of the NMR spectral data. The C(17) hydogens are observed as doublets of doublets at δ 3.95 and 4.01 and the acetyl hydrogens of C(18) are seen as a singlet at δ 1.72, features which are characteristic of ring E-opened, *seco*-macrolines.[4] Further examination of the NMR spectral data indicated that C(20), which is expected to be a methylene, is now substituted, appearing as a methine at δ 3.32 (δ_C 54.5). The NMR spectral data also revealed that this monomeric unit corresponds to the new *seco*-macroline, alstomicine, isolated from the leaf-extract of the same plant.[30] The point of branching in this second macroline unit must therefore be from C(20). This leaves the methylene C(21) unaccounted for, which is observed at δ 32.0 in the ^{13}C NMR spectrum (δ_H 2.41, 3.08). This methylene is directly attached to C(20) of the *seco*-macroline unit, from the COSY spectrum, as well as the HMBC spectrum {^3J from H(21) to C(19)}. In addition, the observation of another key three-bond correlation from H(9′) to this C(21) provided cogent support for the proposed structure, in which the two macroline units are connected by a methylene bridge, as shown in **18**, although the configuration at C(20) could not be established based on the present data. Perhentinine and the other known bisindole obtained, villalstonine, both showed moderate in vitro cytotoxicity towards the P388 murine leukemia cell line (IC_{50} 12.3 and 4.4 μg/ml, respectively) (the biological activity of *Alstonia* alkaloids will be reported separately).

In addition to the above new alkaloids, 12 other known alkaloids were also obtained from the bark extract of this plant, as detailed in Section 3. A notable feature of the alkaloidal composition, in addition to the novel structures discussed above, is the predominance of the macroline skeleton, which is a characteristic of *Alstonia*.

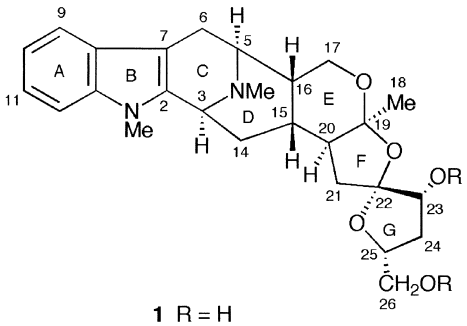
3. Experimental

3.1. General

UV spectra were recorded on a Shimadzu UV-3101PC spectrophotometer. IR spectra were recorded on a Perkin–Elmer RX1 FT-IR spectrophotometer. Optical rotations were measured on a JASCO DIP-370 digital polarimeter or an Atago Polax-D polarimeter. ESIMS was obtained on a Perkin–Elmer API 100 instrument. HREIMS and HRLSIMS measurements were carried out at Organic Mass Spectrometry, Central Science Laboratory, University of Tasmania, Tasmania, Australia. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on a JEOL JMN-LA400 spectrometer at 400 and 100 MHz, respectively. Assignments are confirmed by COSY, HMQC, HMBC, NOESY and NOE experiments. All solvents were of analytical grade and were distilled before use.

3.2. Collection, extraction and isolation

Plant material was collected in Terengganu, Malaysia (June, 2000) and was identified by Dr. K. M. Wong, Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia. Herbarium voucher specimens (K 659) are deposited at the Herbarium of the University of Malaya. Extraction of the ground bark material was carried out in the usual manner by partitioning the concentrated EtOH extract with dilute acid as has been described in detail elsewhere.[31] The alkaloids were isolated by initial column chromatography on silica gel using $CHCl_3$ with increasing proportions of MeOH, followed by rechromatography of appropriate partially resolved fractions using centrifugal TLC. Solvent systems used for centrifugal



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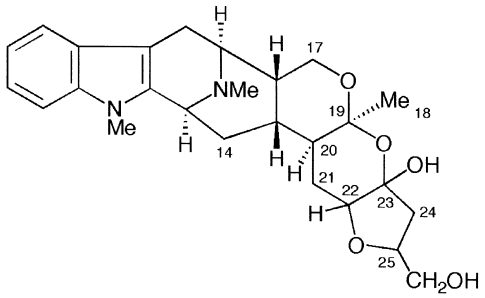

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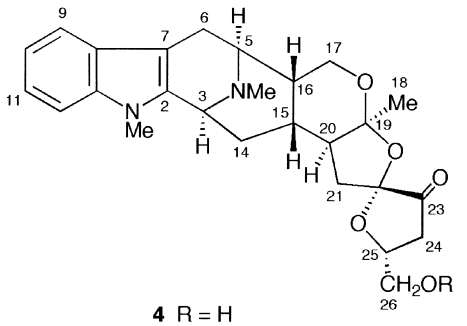

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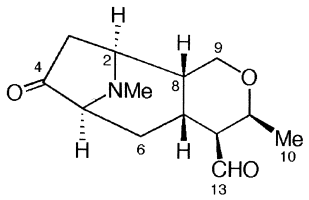

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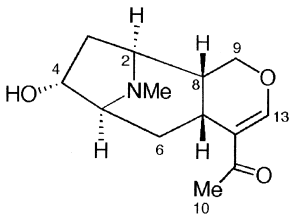

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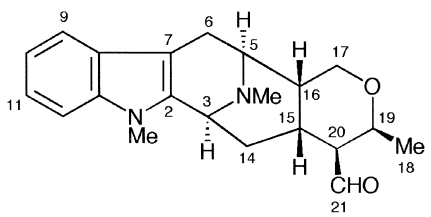
TLC were Et_2O–petroleum ether (1:1; 2:1), Et_2O, $CHCl_3$–MeOH (100:1), $CHCl_3$ (NH_3-saturated), and EtOAc (NH_3-saturated). The yields (g kg^{-1}) of the alkaloids were as follows: **1** (0.004), **4** (0.0012), **7** (0.0012), **9** (0.0005), **10** (0.010), **11** (0.002), **12** (0.031), **13** (0.028), **14** (0.0004), **15** (0.0002), **16** (0.027), **17** (0.016), **18** (0.0249), alstonisine (0.028), alstonal (0.0085), *N*(4)-demethylalstophylline oxindole (0.054), *N*(4)-demethylalstophyllal oxindole (0.035), villalstonine (0.393), pleiocarpamine (0.071), fluorocarpamine (0.029), 16*R*,19*E*-isositsirikine (0.004), and 11-methoxyakuammicine (0.0012).

3.2.1. Macrodasine A 1. $[\alpha]_D$=+36 ($CHCl_3$, *c* 0.36); IR (dry film) ν_{max} 3411 cm^{-1}; UV (EtOH), λ_{max} nm (log ε): 230 (3.88) and 287 (3.17). EIMS, *m/z* (rel. int.): 454 [M^+] (78), 439 (4), 424 (44), 367 (7), 197 (100), 182 (27), 181 (16), 170 (34), 144 (13), 70 (26), 57 (16) and 43 (36). HREIMS found, *m/z* 454.2462, Calcd for $C_{26}H_{34}N_2O_5$, 454.2468. ^1H and ^{13}C NMR: see Tables 1 and 2, respectively.

3.2.2. Macrodasine B 4. $[\alpha]_D$=+149 ($CHCl_3$, *c* 0.07); IR (film) ν_{max} 3435 and 1765 cm^{-1}; (EtOH), λ_{max} nm (log ε): 230 (3.95) and 287 (3.23). EIMS, *m/z* (rel. int.): 452 [M^+] (64), 437 (3), 421 (12), 366 (11), 322 (17), 293 (4), 237 (6), 197 (100), 182 (26), 181 (19), 170 (29), 144 (9), 85 (13), 70 (19), 57 (16) and 40 (35). HREIMS found, *m/z* 452.2326, Calcd for $C_{26}H_{32}N_2O_5$, 452.2311. ^1H and ^{13}C NMR spectral data, see Tables 1 and 2, respectively.

3.2.3. Acetylation of macrodasine A 1. Macrodasine A **1** (11 mg) was added to a mixture of acetic anhydride/pyridine (1:1; 2 ml) and the mixture stirred at room temperature for 2 h. The mixture was then poured into saturated Na_2CO_3 and extracted with CH_2Cl_2. Removal of the solvent followed by purification by centrifugal chromatography over SiO_2 (2% MeOH–$CHCl_3$) afforded 5 mg (38%) of the diacetate derivative **3** as a colourless oil; $[\alpha]_D$=+119 ($CHCl_3$, *c* 0.06); IR (film) ν_{max} 1739 and 1234 cm^{-1}; UV (EtOH), λ_{max} nm (log ε): 221 (4.17), 229 (4.22), 286 (3.56) and 293 (3.52). EIMS, *m/z* (rel. int.): 538 [M^+] (29), 465 (4), 281 (14), 253 (4), 207 (100), 197 (99), 182 (23), 167 (20), 144 (8), 96 (9), 70 (23) and 55 (18). ^1H and ^{13}C NMR spectral data, see Tables 1 and 2, respectively.

3.2.4. Acetylation of macrodasine B 4. Acetylation of macrodasine B **4** (4 mg) with Ac_2O/pyridine as described above gave the monoacetate derivative 5 as a colourless oil (2 mg, 46%); $[\alpha]_D$=+147 ($CHCl_3$, *c* 0.02); IR (film) ν_{max} 1767, 1739, and 1237 cm^{-1}; UV (EtOH), λ_{max} nm (log ε): 222 (4.41), 228 (4.48), 285 (3.79) and 293 (3.75). EIMS, *m/z* (rel. int.): 494 [M^+] (30), 366 (8), 322 (14), 197 (100), 182 (23), 170 (30), 158 (10), 144 (10) and 70 (33). ^1H and ^{13}C NMR spectral data, see Tables 1 and 2, respectively.



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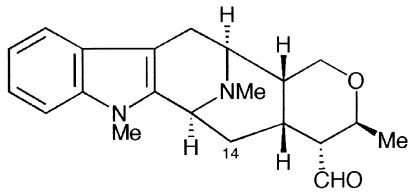

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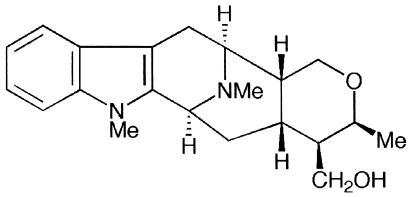

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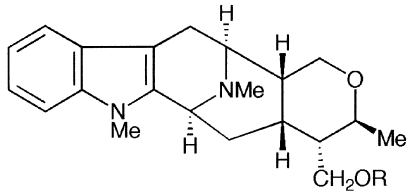

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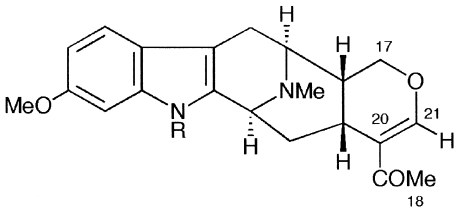
13 R = Ac



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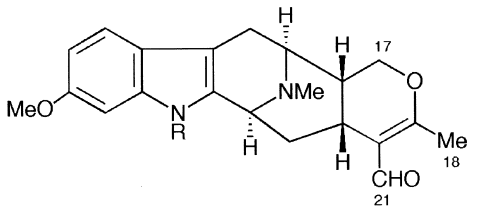
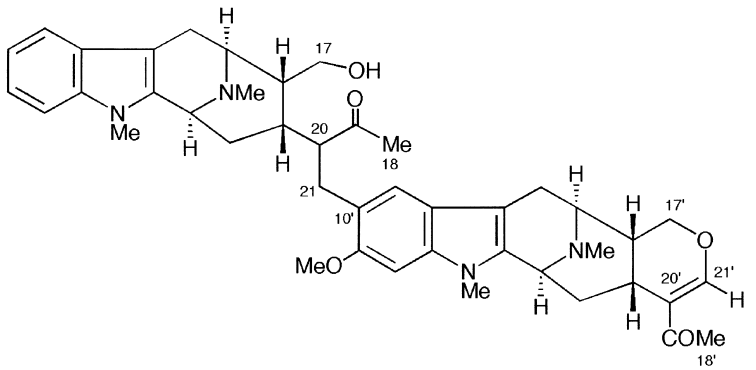
16 R = Me



15 R = H

17 R = Me



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3.2.5. Angustimalal 7. $[\alpha]_D$=+78 ($CHCl_3$, *c* 0.06); IR (film) ν_{max} 1741 and 1711 cm^{-1}; UV (EtOH), λ_{max} nm (log ε): 211 (3.09) and 256 (2.42). EIMS, *m/z* (rel. int.): 237 [M^+] (2), 209 (74), 194 (39), 180 (100), 166 (24), 150 (25), 138 (13), 124 (28), 110 (32), 94 (65), 84 (97), 70 (26), 57 (50) and 42 (74). HREIMS found, *m/z* 237.1375, Calcd for $C_{13}H_{19}NO_3$, 237.1365. ^1H (400 Hz; $CDCl_3$; Me_4Si) δ 1.42 (3H, d, *J*=7 Hz, 10-Me), 1.67 (1H, m, H-6), 1.95 (1H, s, H-12), 1.98 (1H, m, H-8), 2.06 (1H, d, *J*=19 Hz, H-3), 2.11 (1H, td, *J*=13, 3 Hz, H-6), 2.30 (3H, s, *N*-Me), 2.44 (1H, dt, *J*=13, 6 Hz, H-7), 2.68 (1H, dd, *J*=19, 7 Hz, H-3), 2.90 (1H, br s, H-5), 3.23 (1H, d, *J*=7 Hz, H-2), 3.90 (1H, dd, *J*=11, 5 Hz, H-9), 3.94 (1H, qd, *J*=7, 2 Hz, H-11), 4.15 (1H, t, *J*=11 Hz, H-9), 10.0 (1H, d, *J*=2 Hz, H-13); ^{13}C NMR spectral data, see Table 2.

3.2.6. Talcarpine 9. $[\alpha]_D$=−26 ($CHCl_3$, *c* 0.12); UV (EtOH), λ_{max} nm (log ε): 209 (3.86), 226 (4.01), 277 (2.65), 285 (2.91) and 294 (2.65). ESIMS, *m/z* (rel. int.): 339 [MH^+]. ^1H (400 Hz; $CDCl_3$; Me_4Si) δ 1.30 (3H, d, *J*=7 Hz, 18-Me), 1.45 (1H, ddd, *J*=12, 4, 3 Hz, H-14), 1.79 (1H, br s, H-20), 2.06 (1H, dt, *J*=11, 5 Hz, H-16), 2.20 (1H, m, H-15),

2.32 (3H, s, *N*(4)-Me), 2.45 (1H, d, *J*=16 Hz, H-6), 2.50 (1H, td, *J*=12, 4 Hz, H-14), 2.90 (1H, d, *J*=7 Hz, H-5), 3.27 (1H, dd, *J*=16, 7 Hz, H-6), 3.62 (3H, s, *N*(1)–Me), 3.89 (1H, dd, *J*=12, 5 Hz, H-17), 3.98 (2H, m, H-3 and H-19), 4.14 (1H, t, *J*=12 Hz, H-17), 7.10 (1H, td, *J*=8, 1 Hz, H-10), 7.19 (1H, td, *J*=8, 1 Hz, H-11), 7.29 (1H, br d, *J*=8 Hz, H-12), 7.49 (1H, br d, *J*=8 Hz, H-9), 9.95 (1H, d, *J*=3 Hz, H-21); ^{13}C NMR spectral data, see Table 2.

3.2.7. N(4)-Methyl-N(4), 21-*seco*talpinine 10. $[\alpha]_D$=+19 ($CHCl_3$, *c* 0.45); UV (EtOH), λ_{max} nm (log ε): 205 (3.95), 228 (4.21), 280 (3.00), 285 (3.42) and 300 (3.12). ESIMS, *m/z* (rel. int.): 339 [MH^+]. ^{1}H (400 Hz; $CDCl_3$; Me_4Si) δ 1.20 (3H, d, *J*=7 Hz, 18-Me), 1.28 (1H, m, H-14), 1.93 (1H, m, H-16), 2.31 (3H, s, *N*(4)-Me), 2.37 (3H, m, H-14, H-15, H-20), 2.49 (1H, d, *J*=16 Hz, H-6), 2.96 (1H, d, *J*=7 Hz, H-5), 3.30 (1H, dd, *J*=16, 7 Hz, H-6), 3.58 (3H, s, *N*(1)-Me), 3.75 (1H, dd, *J*=12, 5 Hz, H-17), 3.93 (2H, m, H-3, H-19), 4.06 (1H, t, *J*=12 Hz, H-17), 7.13 (1H, td, *J*=8, 1 Hz, H-10), 7.21 (1H, td, *J*=8, 1 Hz, H-11), 7.31 (1H, br d, *J*=8 Hz, H-12), 7.52 (1H, br d, *J*=8 Hz, H-9), 9.41 (1H, br s, H-21); ^{13}C NMR spectral data, see Table 2.

3.2.8. Macrocarpine A 11. $[\alpha]_D$=+117 ($CHCl_3$, *c* 0.11); IR (film) ν_{max} 3400 cm^{-1}; UV (EtOH), λ_{max} nm (log ε): 230 (4.15) and 286 (3.46). EIMS, *m/z* (rel. int.): 340 [M^+] (100), 309 (14), 226 (19), 197 (75), 182 (23), 170 (13) and 70 (18). HREIMS found, *m/z* 340.2142, Calcd for $C_{21}H_{28}N_2O_2$, 340.2151. ^{1}H and ^{13}C NMR spectral data, see Tables 1 and 2, respectively.

3.2.9. Reduction of 9 to 11. $NaBH_4$ (27 mg) was added to a solution of **9** (24 mg) in MeOH (2 ml) at room temperature and the mixture stirred for 5 h. Excess solvent was removed under reduced pressure and water (5 ml) was then added. The mixture was extracted with $CHCl_3$, dried (Na_2SO_4), and then chromatographed (SiO_2, centrifugal TLC, 1% MeOH–$CHCl_3$) to give macrocarpine A **11** (12 mg, 50%).

3.2.10. Oxidation of 11 to 9. PCC (9 mg) was added to solution of **11** (12 mg) in CH_2Cl_2 (2 ml) at room temperature and the mixture stirred for 4 h. Water (5 ml) was then added and the mixture was extracted with $CHCl_3$, dried (Na_2SO_4), and then chromatographed (SiO_2, centrifugal TLC, $CHCl_3$) to give **9** (3 mg, 25%).

3.2.11. Macrocarpine B 12. $[\alpha]_D$=−51 ($CHCl_3$, *c* 0.34); IR (film) ν_{max} 3400 cm^{-1}; UV (EtOH), λ_{max} nm (log ε): 230 (4.34) and 288 (3.64). EIMS, *m/z* (rel. int.): 340 [M^+] (75), 325 (10), 309 (13), 226 (15), 197 (100), 182 (43), 170 (19), 158 (16), 144 (13), 83 (11), 70 (31) and 40 (48). HREIMS found, *m/z* 340.2149, Calcd for $C_{21}H_{28}N_2O_2$, 340.2151. ^{1}H and ^{13}C NMR spectral data, see Tables 1 and 2, respectively.

3.2.12. Reduction of 10 to 12. Reduction of **10** (47 mg) with $NaBH_4$ (52 mg) as described above gave macrocarpine B **12** (27 mg, 57%).

3.2.13. Macrocarpine C 13. $[\alpha]_D$=−35 ($CHCl_3$, *c* 1.55); IR (film) ν_{max} 1737 cm^{-1}; UV (EtOH), λ_{max} nm (log ε): 230 (4.30) and 287 (3.62). EIMS, *m/z* (rel. int.): 382 [M^+] (85), 307 (11), 197 (100), 182 (33), 170 (26), 70 (32) and 43 (12). HREIMS found, *m/z* 382.2252, Calcd for $C_{23}H_{30}N_2O_3$, 382.2256. ^{1}H and ^{13}C NMR spectral data, see Tables 1 and 2, respectively.

3.2.14. Acetylation of 12 to 13. Acetylation of **12** (27 mg) with Ac_2O/pyridine as described above gave **13** (16 mg, 53%).

3.2.15. N(1)-Demethylalstophylline 14 and N(1)-demethylalstophyllal 15. EIMS, *m/z* (rel. int.): 352 [M^+] (74), 337 (6), 283 (12), 265 (4), 228 (12), 213 (79), 197 (28), 186 (100), 170 (19), 143 (12), 118 (5), 91 (6), 70 (40) and 40 (41). HREIMS found, *m/z* 352.1773, Calcd for $C_{21}H_{24}N_2O_3$, 352.1787. ^{1}H and ^{13}C NMR spectral data, see Tables 1 and 2, respectively.

3.2.16. Perhentinine 18. $[\alpha]_D$=−61 ($CHCl_3$, *c* 1.19); IR (film) ν_{max} 3400, 1701, 1651 and 1616 cm^{-1}; UV (EtOH), λ_{max} nm (log ε): 231 (4.25) and 298 (3.45). LSIMS, *m/z* (rel. int.): 705 [MH^+] (51), 661 (14), 379 (58), 239 (19) and 197 (100). HRLSIMS found, *m/z* 705.4019, Calcd for [$C_{43}H_{42}N_4O_5$+H], 705.4016. ^{1}H and ^{13}C NMR spectral data, see Tables 1 and 2, respectively.

Acknowledgements

We would like to thank the University of Malaya and IRPA for financial support of this work.

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N–H Insertion reactions of rhodium carbenoids. Part 5: A convenient route to 1,3-azoles☆

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Received 19 January 2004; revised 25 February 2004; accepted 11 March 2004

Abstract—Dirhodium(II) carboxylate catalysed reaction of diazocarbonyl compounds **2** in the presence of primary amides **1** results in the formation of α-acylaminoketones **3** (12 examples) by N–H insertion reaction of the intermediate rhodium carbene. The 1,4-dicarbonyl compounds **3** are readily converted into structurally diverse oxazoles **4** (11 examples) by cyclodehydration, thiazoles **5** (10 examples) by treatment with Lawesson's reagent, or imidazoles **6** (2 examples) by reaction with ammonia or methylamine.

1. Introduction

The 1,3-azoles—oxazoles, thiazoles, imidazoles—have attracted the attention of chemists for many years. The heteroaromatic imidazole ring plays a key role in the chemistry of the proteinogenic amino acid histidine, and oxazoles and thiazoles occur widely in a range of natural products, particularly the non-ribosomal peptides.[2,3] Recently there has been considerable interest in the use of 1,3-azoles as peptide mimetics.[4–6] The structural diversity of complex naturally occurring 1,3-azoles and the biological activity of synthetic analogues has ensured that new methods continue to be developed for their synthesis.[7]

Of the intermediates available for the synthesis of five-membered heteroaromatic rings, 1,4-dicarbonyl compounds are among the most versatile. In the field of 1,3-azole synthesis, the cyclodehydration of such a 1,4-dicarbonyl compound (an α-acylaminoketone) is the basis of the Robinson–Gabriel oxazole synthesis.[7] Although this reaction was discovered some time ago, it continues to undergo modification, for example, the preparation of the intermediate α-acylaminoketone by acylation of α-amino-β-ketoesters,[4,8] or by oxidation of β-hydroxyamides.[9] Recently we reported a new variation on the Robinson–Gabriel synthesis in which the key 1,4-dicarbonyl intermediate was obtained by a rhodium carbene N–H insertion reaction,[10] developed specifically for the synthesis of the amino acid derived oxazole building blocks of the natural products nostocyclamide and promothiocin A.[11,12] Subsequently, others used our protocol for the synthesis of oxazole-containing peptide mimetics,[5] whilst Janda and co-workers have developed a solid-phase variant of this reaction, and applied it to the synthesis of an array of oxazoles.[13,14] We now report further developments in the use of rhodium carbene N–H insertion reactions and their use in a general approach to the synthesis of 1,3-azoles.

2. Results and discussion

A range of primary amides was selected, comprising formamide **1a**, a simple alkanamide **1b**, aromatic and heteroaromatic amides **1c**-**1e**, the amide **1f** derived from piperidine-4-carboxylic acid (isonipecotic acid), and the oxazole amide **1g**. Amide **1d** was readily prepared from 5-methoxysalicylic acid, **1f** from piperidine-4-carboxamide, and **1g** from the corresponding ester **4c** (prepared by the method described herein); the other amides are commercially available. A range of six diazocarbonyl compounds **2** was also selected for study. The α-diazo-β-ketoesters **2a**-**2e** were prepared by diazo-transfer reaction[15] to the corresponding β-ketoesters using 4-acetamidobenzenesulfonyl azide as the reagent,[16] and azibenzil **2f** was obtained by the literature procedure by oxidation of benzil monohydrazone with manganese(IV) oxide.[17] The carbene N–H insertion reactions were generally carried out using dirhodium tetraacetate as catalyst and 1,2-dichloroethane as solvent, the diazocarbonyl compound being added by syringe pump over about 16 h. The resulting α-acylamino ketones **3** were formed in varying yield (13–82%) (Table 1) with no significant by-products being identified.

☆ See Ref. 1.

Keywords: Heterocyclic; Oxazole; Thiazole; Imidazole.
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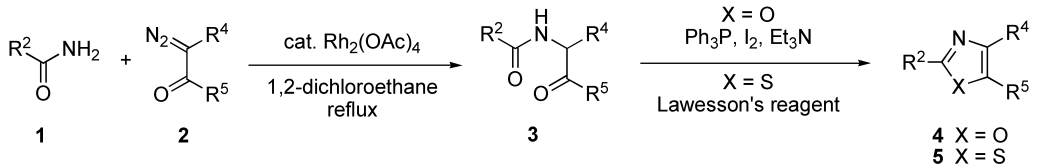
doi:10.1016/j.tet.2004.03.037

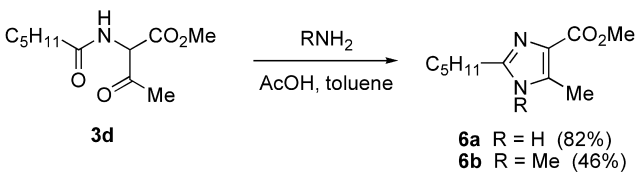
Table 1. Dirhodium(II) catalysed reactions of diazocarbonyl compounds **2** with amides **1**, and subsequent cyclisation of the ketoamides **3** to oxazoles **4** and thaizoles **5**



Amide **1**	R^2	Diazo **2**	R^4	R^5	**3**-**5**	**3** Yield (%)	**4** Yield (%)	**5** Yield (%)
1a	H	**2a**	CO_2Me	Me	**a**	43	45	60
1a	H	**2f**	Ph	Ph	**b**	54	78	94
1a	H	**2a**	CO_2Me	4-Cl–C_6H_4	**c**	55[a]	65	—
1b	C_5H_{11}	**2a**	CO_2Me	Me	**d**	82[b]	79	89
1c	Ph	**2b**	CO_2Et	Me	**e**	62	80	53
1d	2-BnO-5-MeO–C_6H_3	**2a**	CO_2Me	Me	**f**	26	23	55
1d	2-BnO-5-MeO–C_6H_3	**2c**	CO_2Et	Ph	**g**	13	67	—
1e	2-Thienyl	**2a**	CO_2Me	Me	**h**	80	80	69
1e	2-Thienyl	**2d**	CO_2Me	4-Cl–C_6H_4	**i**	36	72	89
1e	2-Thienyl	**2e**	CO_2Me	4-MeO_2C–C_6H_4	**j**	74	54	34
1f	*N*-Boc-piperidin-4-yl	**2a**	CO_2Me	Me	**k**	68	66	74
1g	5-(4-Cl–C_6H_4)oxazol-4-yl	**2a**	CO_2Me	Me	**l**	65	—	40

[a] Dirhodium tetraoctanoate as catalyst in dichloromethane solvent.
[b] Dichloromethane solvent.

With a range of 1,4-dicarbonyl compounds **3** in hand, their conversion into 1,3-azoles was investigated. First, cyclodehydration, using the triphenylphosphine-iodine-triethylamine protocol developed by Wipf and Miller,[9] gave the corresponding oxazoles **4** in 23–80% yield (Table 1), oxazole **4f** being the protected form of the terminal oxazolecarboxylate in the linear lipopeptide amamistatin A.[18] The thiazoles **5** were readily formed from the 1,4-dicarbonyl compounds by thionation with Lawesson's reagent.[19,20] Thus simply heating the α-acylaminoketones **3** with Lawesson's reagent in THF gave the thiazoles **5** in 34–94% yield (Table 1). Finally, two examples of imidazole formation were studied: simply treating 1,4-dicarbonyl compound **3d** with ammonium acetate and methylamine gave the imidazoles **6a** and **6b** in 82 and 46% yield, respectively (Scheme 1).[20]



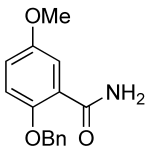
Scheme 1.

Thus by extending the scope of the rhodium carbene N–H insertion reaction, a number of α-acylaminoketones has been obtained. These 1,4-dicarbonyl compounds are useful precursors to a range of structurally diverse 1,3-azoles.

3. Experimental

3.1. General

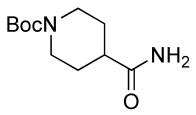
Commercially available reagents were used throughout without further purification unless otherwise stated; solvents were dried by standard procedures. Light petroleum refers to the fraction with bp 40–60 °C and ether refers to diethyl ether. Reactions were routinely carried out under a nitrogen atmosphere. Analytical thin layer chromatography was carried out using aluminium-backed plates coated with Merck Kieselgel 60 GF_{254}. Plates were visualised under UV light (at 254 and /or 360 nm). Flash chromatography was carried out using Merck Kieselgel 60 H silica or Matrex silica 60. Fully characterised compounds were chromatographically homogeneous. IR spectra were recorded in the range 4000–600 cm^{-1} using a Nicolet Magna FT-550 spectrometer. ^1H and ^{13}C NMR spectra were recorded using Bruker 300 and 400 MHz instruments (^1H frequencies, corresponding ^{13}C frequencies are 75 and 100 MHz); *J* values were recorded in Hz. In the ^{13}C NMR spectra, signals corresponding to CH, CH_2 or Me groups are noted; all others are C. High and low-resolution mass spectra were recorded on a Micromass GCT TOF High Resolution Mass Spectrometer, or at the EPSRC Mass Spectrometry Service (Swansea).



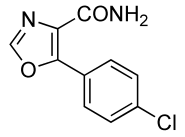
3.1.1. 2-Benzyloxy-5-methoxybenzamide 1d. (a) To a solution of 5-methoxysalicylic acid (2.00 g, 12 mmol) and potassium carbonate (8.20 g, 59 mmol) in DMF (40 ml) cooled to 0 °C was added benzyl bromide (4.24 ml, 36 mmol). The mixture was then stirred at 0 °C for 30 min and then overnight at ambient temperature. Ethyl acetate (100 ml) was added to the mixture and washed with aqueous potassium hydrogen sulfate (1 M; 100 ml), water (100 ml), saturated potassium hydrogen carbonate solution (100 ml) and saturated brine (100 ml). The organic layer was dried ($MgSO_4$) and concentrated to give a dark oil that was then dissolved in methanol (38 ml) and sodium hydroxide solution (40%, 12 ml) and stirred at ambient for 1 h. The reaction mixture was then acidified to pH 1 with dilute

hydrochloric acid solution (30 ml) and extracted with diethyl ether (3×15 ml). The organic extracts were dried ($MgSO_4$) and concentrated to yield the crude compound which was recrystallised from hexane–ethyl acetate (3:2) to yield 2-benzyloxy-5-methoxybenzoic acid as a colourless crystalline solid (1.70 g, 63%); mp 95–96 °C (lit.[18] mp 85–87 °C); ν_{max}(KBr)/cm^{-1} 3440, 3001, 2962, 2929, 2910, 2835, 1695, 1597, 1502, 1454, 1416, 1389, 1329, 1296, 1221, 1047, 1018, 914, 874, 854, 822, 744, 700; δ_H (300 MHz; $CDCl_3$) 9.70 (1H, s br, OH), 7.70 (1H, m, ArH), 7.43 (5H, m, ArH), 7.09 (2H, m, ArH), 5.26 (2H, s, OC*H*$_2$Ph), 3.83 (3H, s, OMe); δ_C (75 MHz; $CDCl_3$) 165.5, 155.0, 151.9, 134.8, 129.6 (CH), 129.5 (CH), 128.4 (CH), 122.5 (CH), 119.0, 116.6 (CH), 115.3 (CH), 73.4 (CH_2), 56.3 (Me).

(b) To a solution of the above acid (1.50 g, 58 mmol) and triethylamine (0.81 ml, 58 mmol) in THF (35 ml) cooled to 0 °C was added ethyl chloroformate (0.56 ml, 58 mmol) and stirred for 15 min. Ammonia solution (30%, 35 ml) in THF (15 ml) was then added to the reaction and stirred for 15 min and then concentrated. The solid residue was then partition with dichloromethane (30 ml) and water (30 ml). The aqueous was then washed again with dichloromethane (30 ml) and the combined organics were washed with saturated sodium hydrogen carbonate solution (30 ml), brine (30 ml), dried ($MgSO_4$) and concentrated to yield the title compound as a light brown crystalline solid (1.33g, 89%); mp 121–123 °C; (Found: M$^+$, 257.1064. $C_{15}H_{15}NO_3$ requires 257.1052); ν_{max}(KBr)/cm^{-1} 3452, 2426, 3314, 3252, 3160, 2924, 2827, 1660, 1598, 1578, 1491, 1429, 1368; δ_H (300 MHz; $CDCl_3$) 7.82 (2H, m, ArH), 7.40 (4H, m, ArH), 7.01 (2H, m, ArH), 5.84 (2H, s br, NH_2), 5.14 (2H, s, OC*H*$_2$Ph), 3.83 (3H, s, OMe); *m*/*z* (CI) 257 (M$^+$, 71%), 241 (22), 215 (10), 195 (7), 181 (3), 151 (16), 137 (7), 119 (3), 91 (54).



3.1.2. 1-*tert*-Butoxycarboxypiperidine-4-carboxamide 1f. To a stirred solution of the piperidine-4-carboxamide (1.00 g, 7.8 mmol) and di-*tert*-butyl dicarbonate (2.20 g, 10.1 mmol) in acetonitrile (15 ml) was added DMAP (95 mg, 0.78 mmol). The resulting solution was stirred overnight and then the solvent was removed under reduced pressure. The solid residue was then dissolved in dichloromethane (40 ml) and washed with saturated sodium hydrogen carbonate solution (40 ml). The aqueous was then extracted with dichloromethane (40 ml) and then the combined organic extracts were washed with saturated ammonium chloride solution (60 ml), water (60 ml) and brine (60 ml) and then dried ($MgSO_4$) and concentrated to yield the desired product as a colourless solid (1.70 g, 92%); mp 152–155 °C (lit.[21] mp 154–156 °C); ν_{max}(KBr)/cm^{-1} 3363, 3190, 2976, 2935, 2860, 1687, 1660, 1632, 1479, 1435, 1365, 1288, 1234, 1180, 1146, 1119, 1034, 926, 872, 769; δ_H (300 MHz; $CDCl_3$) 5.79 (1H, s, NH), 5.64 (1H, s, NH), 4.11 (2H, m, 2×CH), 2.72 (2H, m, 2×CH), 2.28 (1H, m, CH), 1.64 (2H, m, 2×CH), 1.57 (2H, m, 2×CH), 1.44 (9H, s, CMe_3); δ_C (75 MHz; $CDCl_3$) 177.4, 155.0, 80.1, 43.6 (CH), 29.0 (CH_2), 43.0 (CH_2), 28.8 (Me); *m*/*z* (CI) 229 (MH$^+$, 15%), 217 (4), 201 (14), 184 (2), 174 (6), 173 (100), 156 (7), 155 (76), 129 (6), 112 (7), 106 (2).



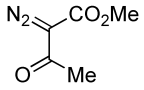
3.1.3. 5-(4-Chlorophenyl)-oxazole-4-carboxamide 1g. (a) A solution of oxazole ester **4c** (500 mg, 2 mmol) and sodium hydroxide (421 mg, 10 mmol) in THF (25 ml) and water (8 ml) was stirred overnight. The reaction mixture was concentrated and the residue was partitioned between dichloromethane (80 ml) and water (80 ml). The aqueous layer was then acidified to pH 1 with diluted hydrochloric acid and extracted with dichloromethane (150 ml). The organic layer was then washed with brine (75 ml), dried ($MgSO_4$) and the solvent removed under reduced pressure to 5-(4-chlorophenyl)oxazole-4-carboxylic acid as a colourless crystalline solid (411 mg, 87%); mp 178–179 °C; (Found: C, 53.5; H, 2.3; N, 6.1. $C_{10}H_6ClNO_3$ requires C, 53.7; H, 2.7; N, 6.3%); (Found: M$^+$, 224.0131. $C_{10}H_6^{35}ClNO_3$ requires 224.0114); ν_{max}(KBr)/cm^{-1} 3128, 3035, 2956, 2924, 2854, 1722, 1701, 1585, 1535, 1491, 1294, 1273, 1234, 1126, 1099, 1068, 1016, 989, 951, 883, 793, 762, 729, 640; δ_H (300 MHz; $CDCl_3$) 8.10 (2H, d, *J*=8.7 Hz, ArH), 7.93 (1H, s, H-2), 7.41 (2H, d, *J*=8.7 Hz, ArH), 5.66 (1H, br s, OH); δ_C (75 MHz; $CDCl_3$) 163.2, 153.1, 151.3 (CH), 135.3, 130.4 (CH), 129.0 (CH), 127.6, 126.0; *m*/*z* (CI) 226/224 (M$^+$, 7/13%), 209 (4), 208/206 (33/100), 182/180 (5/15), 179 (7), 154/152 (3/7), 139 (11), 125 (5).

(b) To a solution of the above acid (480 mg, 2 mmol) and triethylamine (0.30 ml, 2 mmol) in THF (30 ml) cooled to 0 °C was added ethyl chloroformate (0.21 ml, 2 mmol), and the mixture stirred for 15 min at 0 °C. Aqueous ammonia (30% w/w, 20 ml) and THF (15 ml) were then added, and the reaction mixture was stirred at ambient for 15 min. The reaction mixture was then concentrated under reduced pressure and the residue partitioned between water (100 ml) and dichloromethane (100 ml). The aqueous layer was extracted with further dichloromethane (100 ml) and the combined organics were washed with saturated sodium hydrogen carbonate solution (100 ml) and brine (100 ml), dried (Mg_2SO_4) and the solvent removed under reduced pressure to yield the title compound as a colourless crystalline solid (275 mg, 58%); mp 245–247 °C; (Found: M$^+$, 222.0194. $C_{10}H_7^{35}ClN_2O_2$ requires 222.0196); ν_{max}(KBr)/cm^{-1} 2404, 3361, 3290, 3201, 3128, 3080, 2924, 2852, 1699, 1608, 1597, 1533, 1491, 1400, 1329, 1257, 1194, 1119, 1093, 1061, 1016, 987, 953, 835, 793, 742, 708, 667; δ_H (300 MHz; $CDCl_3$) 8.51 (1H, s, H-2), 8.18 (2H, d, *J*=8.7 Hz, ArH), 7.72 (1H, s, NH), 7.60 (1H, s, NH), 7.51 (2H, d, *J*=8.7 Hz, ArH); δ_C (75 MHz; DMSO) 163.0, 150.6 (CH), 150.5, 150.4, 134.8, 129.9 (CH), 128.9 (CH), 126.2; *m*/*z* (CI) 224/222 (M$^+$, 11/17%), 221 (5), 208/206 (34/100), 187 (4), 186 (12).

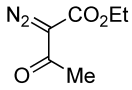
3.2. General method for diazo transfer

To a solution of the β-ketoester substrate (10 mmol) and

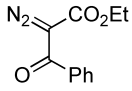
4-acetamidobenzenesulfonyl azide[16] (11 mmol) in acetonitrile (60 ml) at 0 °C was added triethylamine (30 mmol) dropwise. After stirring at room temperature for 16 h the reaction mixture was concentrated in vacuo and the resultant solid was triturated with ether–light petroleum. The filtrate was concentrated in vacuo and purified by flash chromatography on silica gel eluting with ethyl acetate–light petroleum (1:4) to yield the desired product.



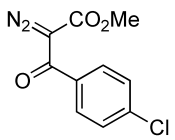
3.2.1. Methyl 2-diazo-3-oxobutanoate 2a. Obtained as a yellow oil according to the general procedure in 92% yield; data as previously described.[10]



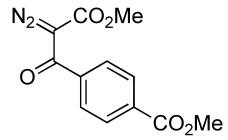
3.2.2. Ethyl 2-diazo-3-oxobutanoate 2b. According to the general procedure the title compound was obtained as a yellow oil (83%) (lit.[22] data not given); (Found: M^+, 156.0531. $C_6H_8N_2O_3$ requires 156.0535); ν_{max}(film)/cm^{-1} 2985, 2939, 2912, 2877, 2141, 1716, 1660, 1595, 1533, 1458, 1373, 1319, 1251, 1155, 1074, 1022, 966, 858, 744, 639; δ_H (300 MHz; $CDCl_3$) 4.24 (2H, q, J=7.1 Hz, OC*H*$_2$Me), 2.41 (3H, s, Me), 1.27 (3H, t, J=7.1 Hz, CH$_2$*Me*); δ_C (75 MHz; $CDCl_3$) 188.4, 159.5, 74.5, 59.6 (CH_2), 26.4 (Me), 12.4 (Me); *m*/*z*(CI) 156 (M^+, 26%), 129 (16), 111 (4), 102 (5), 101 (100), 87 (3), 85 (17), 83 (8).



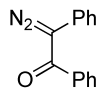
3.2.3. Ethyl 2-diazo-3-oxo-3-phenylpropanoate 2c. According to the general procedure, the title compound was obtained as a yellow oil (99%) (lit.[22] data not given); (Found: C, 60.8; H, 4.8; N, 13.0. $C_{11}H_{10}N_2O_3$ requires C, 60.6; H, 4.6; N, 12.9%); ν_{max}(film)/cm^{-1} 3058, 2976, 2940, 2904, 2868, 2136, 1721, 1685, 1629, 1598, 1578, 1450, 1368, 1301, 1260, 1178, 1112, 1015, 938, 917, 789, 748, 692, 671; δ_H (300 MHz; $CDCl_3$) 7.62 (2H, m, ArH), 7.52 (1H, m, ArH), 7.40 (2H, m, ArH), 4.24 (2H, q, J=7.3 Hz, OC*H*$_2$Me), 1.25 (3H, t, J=7.3 Hz, CH$_2$*Me*); δ_C (75 MHz; $CDCl_3$) 186.9, 160.9, 137.0, 132.2 (CH), 128.4 (CH), 128.0 (CH), 76.1, 61.4 (CH_2), 14.0 (Me); *m*/*z* (CI) 218 (M^+, 8%), 193 (65), 175 (21), 163 (100), 145 (85), 105 (53), 91 (4).



3.2.4. Methyl 3-(4-chlorophenyl)-2-diazo-3-oxopropanoate 2d. According to the general procedure the title compound was obtained as a yellow solid (89%); mp 104 °C (chloroform) (lit.[23] mp 105.5–107.5 °C); (Found: C, 50.1; H, 2.7; N, 11.7. $C_{10}H_7ClN_2O_3$ requires C, 50.3; H, 2.9; N, 11.7%); ν_{max}(KBr)/cm^{-1} 2955, 2919, 2848, 2141, 1716, 1624, 1583, 1434, 1342, 1265, 1127, 1086, 1015, 968, 902, 840, 753, 733, 687; δ_H (300 MHz; $CDCl_3$) 7.51 (2H, d, J=8.7 Hz, ArH), 7.32 (2H, d, J=8.7 Hz, ArH), 3.73 (3H, s, OMe); δ_C (75 MHz; $CDCl_3$) 183.7, 159.3, 136.7, 133.2, 128.0 (CH), 126.3 (CH), 74.6, 50.5 (Me); *m*/*z* (EI) 240/238 (M^+, 10/39%), 212 (7), 210 (15), 154 (4), 152 (8), 141 (67), 139 (100), 123 (48), 111 (78), 75 (47).



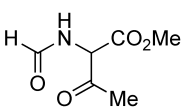
3.2.5. Methyl 2-diazo-3-(4-methoxycarbonylphenyl)-3-oxopropanoate 2e. According to the general procedure the title compound was obtained as a yellow solid (99%); mp 70–72 °C (ethyl acetate–light petroleum); (Found: C, 55.1; H, 3.6; N, 10.8. $C_{12}H_{10}N_2O_5$ requires C, 55.0; H, 3.8; N, 10.7%) (Found: M^+, 262.0588. $C_{12}H_{10}N_2O_5$ requires 262.0590); ν_{max}(KBr)/cm^{-1} 3027, 2950, 2996, 2919, 2853, 2131, 1721, 1624, 1434, 1409, 1281, 1189, 1132, 1107, 1020, 974, 963, 902, 866, 820, 784, 743, 707, 677; δ_H (300 MHz; $CDCl_3$) 8.02 (2H, d, J=8.6 Hz, ArH), 7.59 (2H, d, J=8.6 Hz, ArH), 3.87 (3H, s, OMe), 3.72 (3H, s, OMe); δ_C (75 MHz; $CDCl_3$) 186.8, 166.6, 161.5, 141.2, 133.5, 129.5 (CH), 128.6 (CH), 52.9 (Me), 52.8 (Me), diazo carbon not observed; *m*/*z*(CI) 262 (M^+, 11%), 235 (20), 205 (6), 204 (10), 203 (100), 191 (16), 179 (4), 163 (28), 159 (11), 131 (4).



3.2.6. Azibenzil 2f. Prepared in 87% yield by oxidation of benzil monohydrazone (2.50 g, 11 mmol) in chloroform (38 ml) using activated manganese dioxide (3.90 g, 44 mmol) according to the literature procedure,[17] mp 78–80 °C (lit.[24] mp 79–80 °C).

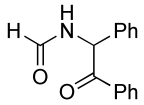
3.3. General procedure for N–H insertion reactions; preparation of α-acylamino ketones 3

To a solution of the amide **1** (5 mmol) and dirhodium tetraacetate (2.5 mol%) in 1,2-dichloroethane (10 ml), heated to reflux, was added a solution of the diazo compound **2** (7 mmol) in 1,2-dichloroethane dropwise over 16 h. The reaction mixture was then heated for a further 2–4 h until TLC analysis showed that the reaction was complete. The mixture was evaporated in vacuo and purified on silica gel eluting with ethyl acetate–light petroleum (1:4) to yield the product.

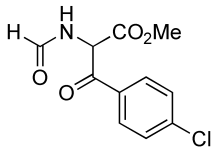


3.3.1. *N*-(1-Methoxycarbonyl-2-oxopropyl)formamide 3a. According to the general procedure, using formamide **1a**, diazo compound **2a** and 1,2-dichloromethane as solvent, the title compound was obtained as a colourless crystalline solid (43%); mp 68–70 °C (ethyl acetate–light petroleum); (Found: C, 45.2; H, 5.7; N, 8.7. $C_6H_9NO_4$ requires C, 45.3; H, 5.7; N, 8.8%); (Found: M^+, 160.0623. $C_6H_9NO_4$ requires 160.0610); ν_{max}(KBr)/cm^{-1} 3319, 3017, 2950, 2925, 2894,

2853, 1747, 1721, 1639, 1521, 1434, 1388, 1373, 1347, 1250, 1209, 1163, 1107, 1035, 968, 933, 886, 758, 661, 600; δ_H (300 MHz; $CDCl_3$) 8.28 (1H, s, CHO), 7.85 (1H, s br, NH), 5.34 (1H, d, *J*=7.0 Hz, C*H*NH), 3.86 (3H, s, OMe), 2.43 (3H, s, Me); δ_C (75 MHz; $CDCl_3$) 198.0, 166.5, 160.8 (CH), 62.0 (CH), 53.9 (Me), 28.4 (Me); *m*/*z* (CI) 160 (M^+, 36%), 156 (6), 132 (100), 128 (53), 117 (11), 85 (6), 83 (16).



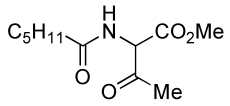
3.3.2. *N*-(2-Oxo-1,2-diphenylethyl)formamide 3b. To a suspension of formamide **1a** (234 mg, 5 mmol) and dirhodium tetraacetate (62 mg, 0.12 mmol) in dichloromethane (20 ml) was added a solution of azibenzil **2f** (1.5 g, 6.7 mmol) in dichloromethane (30 ml) over 15 min and stirred at room temperature overnight. The reaction was the heated to reflux for 4 h and then evaporated in vacuo and purified on silica gel eluting with ethyl acetate–light petroleum (2:3) to yield the title product as a beige solid (670 mg, 54%); mp 119–120 °C (lit.[25] mp 122 °C); (Found: C, 74.9; H, 5.5; N, 5.7. $C_{15}H_{13}NO_2$ requires C, 75.3; H, 5.5; N, 5.9%); ν_{max}(KBr)/cm^{-1} 3370, 3063, 3032, 2919, 2858, 2751, 1690, 1660, 1593, 1578, 1491, 1445, 1383, 1322, 1296, 1255, 1219, 1189, 1066, 984, 933, 881, 851, 779, 758, 738, 692, 677, 656; δ_H (300 MHz; $CDCl_3$) 8.28 (1H, s, CHO), 7.99 (2H, m, ArH), 7.54 (1H, m, NH), 7.42 (4H, m, ArH), 7.32 (4H, m, ArH), 6.66 (1H, d, *J*=7.4 Hz, C*H*NH); δ_C (75 MHz; $CDCl_3$) 195.6, 160.7 (CH), 137.3, 134.5, 134.4 (CH), 129.8 (CH), 129.7 (CH), 129.3 (CH), 129.0 (CH), 128.7 (CH), 57.6 (CH); *m*/*z* (CI) 239 (M^+, 3%), 212 (51), 196 (12), 195 (94), 167 (7), 149 (4), 134 (8), 105 (4).



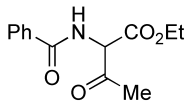
3.3.3. *N*-[2-(4-Chlorophenyl)-1-methoxycarbonyl-2-oxoethyl]formamide 3c. (a) According to the general procedure, using formamide **1a**, diazo compound **2d** and 1,2-dichloroethane as solvent, the title compound was obtained as a colourless solid (21%); mp 116–119 °C (lit.[26] mp 116–118 °C); (Found: C, 51.8; H, 3.7; N, 5.2. $C_{11}H_{10}ClNO_4$ requires C, 51.7; H, 3.9; N, 5.5%); ν_{max}(KBr)/cm^{-1} 3365, 3091, 3076, 3045, 3012, 2958, 2941, 2912, 2877, 2848, 1747, 1699, 1666, 1585, 1570, 1498, 1435, 1406, 1354, 1273, 1252, 1211, 1190, 1167, 1093, 999, 964, 849, 762, 660, 611, 538, 472; δ_H (300 MHz; $CDCl_3$) 8.23 (1H, s, CHO), 8.00 (2H, d, *J*=8.7 Hz, ArH), 7.43 (2H, d, *J*=8.7 Hz, ArH), 6.98 (1H, d, *J*=7.5 Hz, NH), 6.17 (1H, d, *J*=7.5 Hz, C*H*NH), 3.68 (3H, s, OMe); δ_C (75 MHz; $CDCl_3$) 190.2, 166.9, 160.9 (CH), 141.8, 132.5, 131.4 (CH), 129.7 (CH), 56.8 (CH), 54.0 (Me); *m*/*z* (EI) 255/257 (M^+, 7/1%), 227 (4), 224 (16), 223 (3), 196 (31), 168 (7), 140 (100), 139 (64), 133 (47), 113 (84), 112 (99), 111 (96), 104 (28), 85 (23), 77 (45), 75 (69), 51 (59).

(b) According to the general procedure, using dichloromethane as solvent, the title compound was obtained as a colourless solid (41%). See above for data.

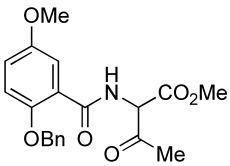
(c) According to the general procedure, using dichloromethane as solvent and dirhodium tetraoctanoate as catalyst, the title compound was obtained as a colourless solid (55%). See above for data.



3.3.4. *N*-(1-Methoxycarbonyl-2-oxopropyl)hexanamide 3d. According to the general procedure, using hexanamide **1b**, diazo compound **2a** and dichloromethane as solvent the title compound was isolated as an oily solid (82%); mp 44–46 °C; (Found: MH^+, 230.1387. $C_{11}H_{20}NO_4$ requires 230.1392); ν_{max}(film)/cm^{-1} 3375, 3298, 2960, 2925, 2868, 1752, 1726, 1665, 1511, 1434, 1358, 1271, 1214, 1158, 1025, 968; δ_H (300 MHz; $CDCl_3$) 6.70 (1H, app s, NH), 5.23 (1H, d, *J*=6.5 Hz, C*H*NH), 3.77 (3H, s, CO_2Me), 2.33 (3H, s, Me), 2.23 (2H, t, *J*=7.4 Hz, CH_2CO), 1.58 (2H, m, CH_2), 1.23 (4H, m, 2×CH_2), 0.83 (3H, m, Me); δ_C (75 MHz; $CDCl_3$) 199.1, 173.4, 167.1, 63.2 (Me), 53.7 (CH), 36.3 (CH_2), 31.7 (CH_2), 28.5 (Me), 25.4 (CH_2), 22.7 (CH_2), 14.3 (Me); *m*/*z* (CI) 230 (MH^+, 76%), 228 (14), 215 (13), 187 (25), 155 (4), 133 (7), 132 (100), 131 (4), 117 (4).

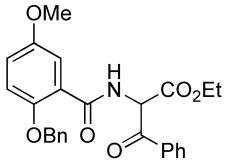


3.3.5. *N*-(1-Ethoxycarbonyl-2-oxopropyl)benzamide 3e. According to the general procedure using benzamide **1c** and diazo compound **2b**, the title compound was obtained as a colourless oil (62%) (lit.[27] data not given); (Found: M^+, 250.1080. $C_{13}H_{15}NO_4$ requires 250.1079); ν_{max}(film)/cm^{-1} 3401, 3058, 3027, 2981, 2940, 2868, 1752, 1726, 1659, 1603, 1578, 1516, 1481, 1445, 1373, 1337, 1265, 1209, 1178, 1102, 1071, 1015, 861, 799, 712, 692; δ_H (300 MHz; $CDCl_3$) 7.83 (2H, m, ArH), 7.46 (3H, m, ArH), 7.41 (1H, d, *J*=6.4 Hz, NH), 5.43 (1H, d, *J*=6.4 Hz, C*H*NH), 4.29 (2H, q, *J*=7.1 Hz, OCH_2Me), 2.46 (3H, s, Me), 1.29 (3H, t, *J*=7.1 Hz, CH_2Me); δ_C (75 MHz; $CDCl_3$) 199.1, 167.2, 166.5, 133.4, 132.5 (CH), 129.0 (CH), 127.6 (CH), 63.9 (CH), 63.1 (CH_2), 20.5 (Me), 14.4 (Me); *m*/*z* (CI) 250 (M^+, 88%), 233 (4), 232 (23), 208 (6), 207 (50), 204 (71), 188 (3), 172 (3), 161 (13), 160 (3), 133 (5), 122 (7), 105 (100).

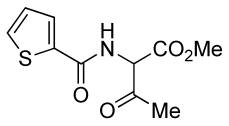


3.3.6. 2-Benzyloxy-5-methoxy-*N*-(1-methoxycarbonyl-2-oxopropyl)benzamide 3f. According to the general procedure using amide **1d** and diazo compound **2a** the title compound was obtained as a colourless solid (26%); mp 65–67 °C (ethyl acetate–light petroleum) (lit.[18] mp not given); (Found: M^+, 371.1362. $C_{20}H_{21}NO_6$ requires 371.1369); ν_{max}(KBr)/cm^{-1} 3437, 3365, 2924, 2356, 2336, 1752, 1721, 1644, 1603, 1496, 1455, 1440, 1388, 1352, 1306, 1281, 1214, 1173, 1143, 1040, 999, 892, 856, 815, 769, 743, 697, 666, 615; δ_H (300 MHz; $CDCl_3$) 9.23

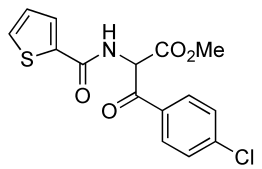
(1H, d, *J*=6.0 Hz, NH), 7.70 (1H, m, ArH), 7.40 (5H, m, ArH), 6.98 (2H, m, ArH), 5.43 (1H, d, *J*=6.0 Hz, C*H*NH), 5.26 (2H, s, OCH_2Ph), 3.80 (3H, s, OMe), 3.74 (3H, s, OMe), 2.36 (3H, s, Me); δ_C (100 MHz; $CDCl_3$) 198.6, 166.6, 164.7, 154.0, 151.4, 135.7, 128.7 (CH), 128.4 (CH), 128.0 (CH), 121.2, 120.2 (CH), 115.3 (CH), 115.0 (CH), 72.1 (CH_2), 63.9 (CH), 55.8 (Me), 53.1 (Me), 27.9 (Me); *m*/*z*(EI) 371 (M^+, 3%), 353 (4), 281 (3), 258 (6), 257 (47), 255 (6), 231 (3), 178 (6), 151 (45), 150 (65), 102 (59), 91 (100), 59 (7).



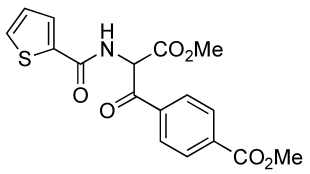
3.3.7. 2-Benzyloxy-*N*-(1-ethoxycarbonyl-2-oxo-2-phenylethyl)-5-methoxybenzamide 3g. According to the general procedure, using amide **1d** and diazo compound **2c**, the title compound was obtained as a colourless solid (13%); mp 85–88 °C (diethyl ether); (Found: C, 69.8; H, 5.5; N, 3.0. $C_{26}H_{25}NO_6$ requires C, 69.8; H, 5.6; N, 3.1%); ν_{max}(KBr)/cm^{-1} 3334, 3088, 3073, 2996, 2976, 2950, 2930, 2899, 2832, 1737, 1690, 1655, 1614, 1598, 1578, 1506, 1486, 1445, 1281, 1224, 1204, 1184, 1158, 1086, 1045, 1025, 979, 948, 902, 815, 769, 743, 687, 645; δ_H (300 MHz; $CDCl_3$) 9.66 (1H, d, *J*=7.1 Hz, NH), 8.34 (2H, d, *J*=7.7 Hz, ArH), 7.94 (1H, s, H-6), 7.82 (1H, m, ArH), 7.71 (3H, m, ArH), 7.62–7.48 (4H, m, ArH), 7.19 (2H, m, ArH), 6.59 (1H, d, *J*=7.1 Hz, C*H*NH), 5.50 (2H, s, OCH_2Ph), 4.36 (2H, q, *J*=7.0 Hz, CH_2Me), 4.01 (3H, s, OMe), 1.34 (3H, t, *J*=7.0 Hz, CH_2*Me*); δ_C (75 MHz; $CDCl_3$) 191.2, 166.4, 164.4, 153.5, 151.0, 135.4, 134.1 (CH), 133.8, 129.1 (CH), 128.4 (CH), 128.3 (CH), 127.9 (CH), 127.4 (CH), 121.1, 119.8 (CH), 114.9 (CH), 114.7 (CH), 71.6 (CH_2), 61.9 (CH_2), 58.8 (CH), 55.4 (Me), 13.5 (Me); *m*/*z* (EI) 447 (M^+, 22%), 429 (53), 401 (16), 357 (20), 340 (26), 339 (44), 297 (20), 258 (28), 257 (100), 239 (57), 212 (28), 151 (50), 150 (85), 91 (94).



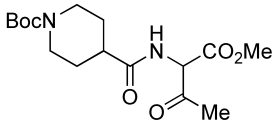
3.3.8. *N*-(1-Methoxycarbonyl-2-oxopropyl)thiophene-2-carboxamide 3h. According to the general procedure, using thiophene-2-carboxamide **1e**, diazo compound **2a** and 1,2-dichloromethane as solvent, the title compound was obtained as a colourless oil (80%); (Found: C, 49.8; H, 4.5; N, 5.4. $C_{10}H_{11}NO_4S$ requires C, 49.8; H, 4.6; N, 5.8%); ν_{max}(film)/cm^{-1} 3334, 3088, 3073, 2996, 2976, 2950, 2930, 2899, 2832, 1737, 1690, 1655, 1614, 1598, 1578, 1506, 1486, 1445, 1281, 1224, 1204, 1184, 1158, 1086, 1045, 1025, 979, 948, 902, 815, 769, 743, 687, 645; δ_H (300 MHz; $CDCl_3$) 7.61 (1H, dd, *J*=1.1, 3.8 Hz, ArH), 7.51 (1H, dd, *J*=1.1, 4.9 Hz, ArH), 7.19 (1H, d, *J*=6.4 Hz, NH), 7.08 (1H, dd, *J*=3.8, 4.9 Hz, ArH), 5.40 (1H, d, *J*=6.4 Hz, C*H*NH), 3.82 (3H, s, OMe), 2.42 (3H, s, OMe); δ_C (75 MHz; $CDCl_3$) 198.7, 166.9, 161.7, 137.6, 131.5 (CH), 129.5 (CH), 128.2 (CH), 63.6 (CH), 53.9 (Me), 28.5 (Me); *m*/*z* (EI) 241 (M^+, 20%), 199 (85), 167 (63), 112 (18), 110 (100), 83 (14).



3.3.9. *N*-[2-(4-Chlorophenyl)-1-methoxycarbonyl-2-oxoethyl)thiophene-2-carboxamide 3i. According to the general procedure, using thiophene-2-carboxamide **1e**, diazo compound **2d** and 1,2-dichloromethane as solvent, the title compound was obtained as a colourless crystalline solid (36%); mp 100 °C (diethyl ether); (Found: C, 53.2; H, 3.4; N, 4.0. $C_{15}H_{12}ClNO_4S$ requires C, 53.3; H, 3.6; N, 4.2%); ν_{max}(KBr)/cm^{-1} 3421, 3314, 3109, 3088, 3073, 3037, 2991, 2950, 2843, 1737, 1685, 1644, 1619, 1578, 1532, 1501, 1432, 1358, 1312, 1271, 1224, 1199, 1158, 1086, 1004, 958, 927, 861, 840, 764, 712, 605; δ_H (300 MHz; $CDCl_3$) 8.12 (2H, d, *J*=8.7 Hz, ArH), 7.64 (1H, dd, *J*=1.1, 3.9 Hz, ArH), 7.54 (1H, dd, *J*=1.1, 5.0 Hz, ArH), 7.50 (2H, d, *J*=8.7 Hz, ArH), 7.32 (1H, d, *J*=7.1 Hz, NH), 7.11 (1H, dd, 3.9, 5.0, ArH), 6.31 (1H, d, *J*=7.1 Hz, C*H*NH), 3.75 (3H, s, OMe); δ_C (75 MHz; $CDCl_3$) 190.7, 167.3, 161.8, 141.7, 137.6, 132.8, 131.6 (CH), 131.4 (CH), 129.7 (CH), 129.6 (CH), 128.2 (CH), 58.5 (CH), 53.5 (Me); *m*/*z* (CI) 340/338 (M^+, 32/100%), 322/320 (3/7), 308/306 (13/39), 278 (3), 254 (6), 226 (4), 140 (3), 138 (6).

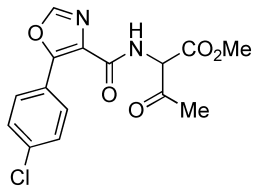


3.3.10. *N*-[1-Methoxycarbonyl-2-(4-methoxycarbonylphenyl)-2-oxoethyl)thiophene-2-carboxamide 3j. According to the general procedure, using thiophene-2-carboxamide **1e**, diazo compound **2e** and 1,2-dichloromethane as solvent the title compound was obtained as a colourless crystalline solid (74%); mp 188–190 °C (diethyl ether); (Found: C, 56.3; H, 4.1; N, 3.7. $C_{17}H_{15}NO_6S$ requires C, 56.5; H, 4.2; N, 3.9%); ν_{max}(KBr)/cm^{-1} 3309, 3249, 3113, 3097, 3067, 3041, 3017, 2957, 2848, 1710, 1690, 1634, 1530, 1502, 1453, 1433, 1421, 1405, 1361, 1317, 1285, 1261, 1237, 1205, 1169, 1113, 1041, 1005, 964, 928, 872; δ_H (300 MHz; $CDCl_3$) 8.22 (2H, d, *J*=8.7 Hz, ArH), 8.17 (2H, d, *J*=8.7 Hz, ArH), 7.65 (1H, dd, *J*=1.1, 3.8 Hz, ArH), 7.54 (1H, dd, *J*=1.1, 4.9 Hz, ArH), 7.33 (1H, d, *J*=7.3 Hz, NH), 7.11 (1H, dd, *J*=3.8, 4.9 Hz, ArH), 6.37 (1H, d, *J*=7.3 Hz, C*H*NH), 3.96 (3H, s, OMe), 3.75 (3H, s, OMe); δ_C (75 MHz; $CDCl_3$) 191.7, 167.1, 166.3, 161.8, 137.7, 137.5, 135.4, 131.6 (CH), 130.4 (CH), 129.9 (CH), 129.7 (CH), 128.2 (CH), 58.8 (CH), 53.9 (Me), 53.0 (Me); *m*/*z* (CI) 362 (M^+, 100%), 344 (7), 330 (25), 278 (3), 176 (3), 163 (8).



3.3.11. 1-*tert*-Butyloxycarbonyl-*N*-(1-methoxycarbonyl-2-oxopropyl)piperidine-4-carboxamide 3k. According to

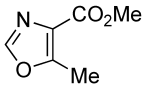
the general procedure the title product was isolated from reaction of amide **1f** and diazo compound **2a** in dichloromethane as an oily solid (68%); mp 76–79 °C; (Found: MH^+, 343.1871. $C_{16}H_{27}N_2O_6$ requires 343.1869); ν_{max}(KBr)/cm^{-1} 3280, 3057, 2978, 2960, 2937, 2860, 1757, 1695, 1639, 1541, 1435, 1365, 1342, 1279, 1250, 1214, 1173, 1107, 968, 764, 661; δ_H (300 MHz; $CDCl_3$) 6.68 (1H, d, *J*=6.3 Hz, NH), 5.24 (1H, d, *J*=6.3 Hz, C*H*NH), 4.14–4.07 (2H, m, 2×CH), 3.81 (3H, s, OMe), 2.81–2.73 (2H, m, 2×CH), 2.39 (4H, m, Me+CH), 1.85–1.81 (2H, m, 2×CH), 1.70–1.56 (2H, m, 2×CH), 1.44 (9H, s, CMe_3); δ_C (75 MHz; $CDCl_3$) 198.7, 174.5, 166.9, 155.0, 80.1, 63.2 (CH), 53.8 (Me), 43.5 (CH_2), 43.0 (CH), 28.8 (Me), 28.7 (CH_2), 28.5 (Me); *m*/*z* (CI) 343 (MH^+, 5%), 327 (2), 315 (6), 299 (2), 288 (7), 287 (73), 269 (40), 241 (10), 218 (12), 186 (7), 174 (22), 173 (100), 155 (21), 133 (6), 131 (83), 115 (7), 102 (7), 75 (17).



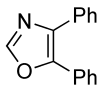
3.3.12. 5-Chlorophenyl-*N*-(1-methoxycarbonyl-2-oxo-propyl)oxazole-4-carboxamide 3l. To a solution of amide **1g** (385 mg, 1.73 mmol) and dirhodium tetraoctanoate (34 mg, 0.04 mmol) in dichloromethane (20 ml) heated to reflux was added a solution of diazo compound **2a** (320 mg, 2.25 mmol) in dichloromethane (10 ml) dropwise over 4 h. The reaction mixture was then concentrated under reduced pressure and the residue was triturated with diethyl ether to yield the title compound as a beige solid (280 mg, 48%). The trituration liquors were then reduced in vacuo and purified by flash column chromatography to yield further title compound (101 mg, 17%, total 381 mg, 65%); mp 122–125 °C; (Found: M^+, 337.0599. $C_{15}H_{13}^{35}ClN_2O_5$ requires 337.0591); ν_{max}(KBr)/cm^{-1} 3401, 3334, 3135, 3083, 3032, 2960, 2930, 2853, 1747, 1660, 1588, 1527, 1496, 1440, 1363, 1332, 1260, 1224, 1163, 1091, 1055, 1009, 979, 943, 927, 840, 784, 743 697, 641; δ_H (300 MHz; $CDCl_3$) 8.31 (1H, m, NH), 8.23 (2H, d, *J*=8.9 Hz, ArH), 7.89 (1H, s, H-2), 7.42 (2H, d, *J*=8.9 Hz, ArH), 5.40 (1H, d, *J*=6.8 Hz, C*H*NH), 3.86 (3H, s, OMe), 2.44 (3H, s, Me); δ_C (75 MHz; $CDCl_3$) 199.5, 168.0, 162.2, 154.0, 149.8 (CH), 137.9, 131.1 (CH), 130.3 (CH), 129.7, 126.7, 64.6 (CH), 55.1 (Me), 29.6 (Me); *m*/*z* (CI) 339/337 (M^+, 23/100%), 333 (9), 319 (5), 307/305 (15/51), 294 (14), 277 (5), 262 (3), 234 (2), 208/206 (3/8).

3.4. General procedure for oxazole formation

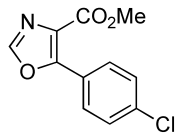
To a solution of triphenylphosphine (0.2 mmol) and iodine (0.2 mmol) in dry dichloromethane (10 ml) was added triethylamine (0.41 mmol) and then a solution of the keto amide substrate **3** in dry dichloromethane (3 ml). The reaction mixture was then stirred for 16 h and then evaporated in vacuo and purified on silica gel eluting with ethyl acetate–light petroleum (3:7) to yield the product.



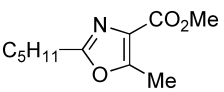
3.4.1. Methyl 5-methyloxazole-4-carboxylate 4a. According to the general procedure the title compound was obtained from **3a** as a colourless crystalline solid (45%); mp 45–47 °C (lit.[28] mp 46–48 °C); ν_{max}(KBr)/cm^{-1} 3114, 3017, 2955, 2925, 2848, 1701, 1603, 1516, 1440, 1393, 1347, 1327, 1235, 1199, 1168, 1096, 1071, 968, 943, 871, 810, 779, 656; δ_H (300 MHz; $CDCl_3$) 7.68 (1H, s, H-2), 3.85 (3H, s, OMe), 2.58 (3H, s, Me); δ_C (75 MHz; $CDCl_3$) 162.9, 157.0, 149.2 (CH), 127.5, 52.4 (Me), 12.2 (Me); *m*/*z* (FI) 141 (M^+, 100%).



3.4.2. 4,5-Diphenyloxazole 4b. According to the general procedure the title compound was obtained from **3b** as a colourless crystalline solid (78%); mp 42–43 °C (lit.[29] mp 44 °C); ν_{max}(KBr)/cm^{-1} 3122, 3076, 3026, 2926, 2852, 1674, 1603, 1510, 1477, 1443, 1363, 1217, 1124, 1107, 1068, 1053, 1026, 955, 912, 839, 762, 692, 644; δ_H (300 MHz; $CDCl_3$) 7.90 (1H, s, H-2), 7.57 (4H, m, ArH), 7.30 (6H, m, ArH); δ_C (75 MHz; $CDCl_3$) 149.5 (CH), 145.5, 134.4, 131.8 (2×C), 128.5 (CH), 128.4 (CH), 128.3 (CH), 127.9 (CH), 127.6 (CH), 126.5 (CH); *m*/*z* (EI) 237 (M^+, 22%), 195 (4), 165 (3).

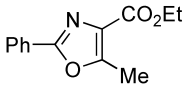


3.4.3. Methyl 5-(4-chlorophenyl)oxazole-4-carboxylate 4c. According to the general procedure the title compound was obtained from **3c** as a colourless crystalline solid (65%); mp 113–115 °C (lit.[30] mp 111–112 °C); (Found: C, 55.5; H, 3.1; N, 5.8. $C_{11}H_8ClNO_3$ requires C, 55.6; H, 3.4; N, 5.9%); ν_{max}(KBr)/cm^{-1} 3126, 3014, 2960, 2924, 2854, 1705, 1618, 1524, 1489, 1441, 1373, 1325, 1250, 1209, 1097, 1072, 1005, 823, 791, 642; δ_H (300 MHz; $CDCl_3$) 7.99 (2H, d, *J*=8.9 Hz, ArH) 7.84 (1H, s, H-2), 7.38 (2H, d, *J*=8.9 Hz, ArH), 3.87 (3H, s, OMe); δ_C (100 MHz; $CDCl_3$) 162.3, 154.7, 149.0 (CH), 136.7,129.7 (CH), 128.9 (CH), 126.6, 125.0, 52.5 (Me); *m*/*z* (CI) 237/239 (M^+, 23/13%), 234 (16), 209 (5), 208/206 (34/100), 186 (7), 139 (3).

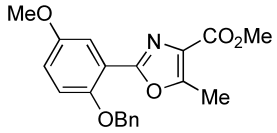


3.4.4. Methyl 5-methyl-2-pentyloxazole-4-carboxylate 4d. According to the general procedure the title compound was isolated from **3d** as a colourless oil (79%); (Found: MH^+, 212.1287. $C_{11}H_{18}NO_3$ requires 212.1287); ν_{max}(film)/cm^{-1} 2950, 2930, 2862, 1716, 1622, 1591, 1441, 1387, 1352, 1203, 1178, 1097, 980, 825, 789, 723, 641; δ_H (300 MHz; $CDCl_3$) 3.82 (3H, s, CO_2Me), 2.66 (2H, m, CH_2), 2.52 (3H, s, Me), 1.69 (2H, m, CH_2), 1.27 (4H, m, 2×CH_2), 0.83 (3H, m, Me); δ_C (75 MHz; $CDCl_3$) 163.9,

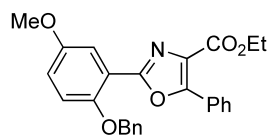
163.7, 156.8, 127.8, 52.6 (Me), 32.1 (CH_2), 28.8 (CH_2), 27.5 (CH_2), 23.1 (CH_2), 14.7 (Me), 12.7 (Me); *m/z* (CI) 212 (MH^+, 100%), 210 (8), 196 (3), 181 (5), 180 (41), 168 (6), 155 (8), 136 (2), 123 (4), 109 (1), 85 (1).



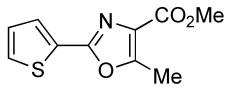
3.4.5. Ethyl 5-methyl-2-phenyloxazole-4-carboxylate 4e. According to the general procedure the title product was obtained from **3e** as a colourless solid (80%); mp 51–52 °C (ethyl acetate–light petroleum) (lit.[31] oil); (Found: C, 67.4; H, 5.7; N, 5.9. $C_{13}H_{13}NO_3$ requires C, 67.5; H, 5.7; N, 6.1%); ν_{max}(KBr)/cm^{-1} 3066, 2999, 2981, 2960, 2924, 2906, 2852, 1732, 1564, 1468, 1450, 1404, 1375, 1347, 1325, 1304, 1225, 1190, 1124, 1105, 1059, 1022, 841, 787, 710, 690; δ_H (300 MHz; $CDCl_3$) 8.07 (2H, m, ArH), 7.45 (3H, m, ArH), 4.45 (2H, q, *J*=7.1 Hz, OCH_2Me), 2.71 (3H, s, Me), 1.42 (3H, t, *J*=7.1 Hz, OCH_2*Me*); δ_C (75 MHz; $CDCl_3$) 162.9, 160.0, 156.6, 131.1 (CH), 129.2, 129.1 (CH), 127.0 (CH), 61.4 (CH_2), 14.8 (Me), 12.6 (Me), 1 Ar C unobserved; *m/z*(CI) 231 (M^+, 15%), 214 (6), 187 (4), 186 (28), 185 (4).



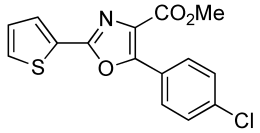
3.4.6. Methyl 2-(2-benzyloxy-5-methoxyphenyl)-5-methyloxazole-4-carboxylate 4f. According to the general procedure the title compound was obtained from **3f** as a white crystalline solid (23%); mp 96–99 °C (ethyl acetate–light petroleum) (lit.[18] mp 98–100 °C); ν_{max}(KBr)/cm^{-1} 3062, 3030, 3003, 2953, 2920, 2854, 2839, 1711, 1616, 1541, 1491, 1448, 1383, 1348, 1267, 1234, 1209, 1107, 1043, 868, 808, 781, 733, 692; δ_H (400 MHz; $CDCl_3$) 7.54 (3H, m, ArH), 7.42 (3H, m, ArH), 6.98 (2H, m, ArH), 5.14 (2H, s, OCH_2Ph), 3.95 (3H, s, CO_2Me), 3.83 (3H, s, OMe), 2.68 (3H, s, Me); δ_C (100 MHz; $CDCl_3$) 175.5, 163.0, 156.4, 153.8, 151.1, 137.0, 128.4 (CH), 128.1, 127.8 (CH), 127.0 (CH), 118.7 (CH), 117.1, 115.9 (CH), 114.3 (CH), 71.7 (CH_2), 56.0 (Me), 52.0 (Me), 12.1 (Me); *m/z*(EI) 353 (M^+, 67%), 336 (19), 321 (10), 310 (16), 279 (11), 278 (25), 277 (57), 262 (100), 231 (30), 224 (53), 216 (34), 202 (22), 174 (14), 167 (22) 150 (28), 149 (65), 125 (14), 111 (23), 97 (35), 91 (83), 71 (39), 57 (52).



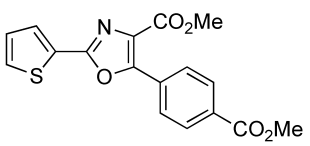
3.4.7. Ethyl 2-(2-benzyloxy-5-methoxyphenyl)-5-phenyloxazole-4-carboxylate 4g. According to the general procedure the title compound was obtained from **3g** as colourless crystalline solid (67%); mp 115–116 °C (ethyl acetate–light petroleum); (Found: C, 72.9; H, 5.4; N, 3.1. $C_{26}H_{23}NO_5$ requires C, 72.7; H, 5.4; N, 3.3%); ν_{max}(KBr)/cm^{-1} 3053, 3027, 2996, 2971, 2925, 2863, 1711, 1588, 1537, 1491, 1419, 1373, 1224, 1107, 1035, 1015, 866, 815, 743, 687; δ_H (300 MHz; $CDCl_3$) 7.71 (2H, m, ArH), 7.45 (1H, m, ArH), 7.31 (2H, m, ArH), 7.14 (6H, m, ArH), 6.83 (2H, m, ArH), 4.93 (2H, s, OCH_2Ph), 4.24 (2H, q, *J*=7.1 Hz, OCH_2Me), 3.66 (3H, s, OMe), 1.21 (3H, t, *J*=7.1 Hz, OCH_2*Me*); δ_C (75 MHz; $CDCl_3$) 162.9, 159.2, 155.6, 154.1, 151.8, 137.1, 130.4 (CH), 128.9 (2×CH), 128.7 (CH), 128.4 (CH), 128.2 (CH), 128.1, 127.6, 119.3 (CH), 117.0, 115.6 (CH), 115.1 (CH), 72.0 (CH_2), 61.8 (CH_2), 56.4 (Me), 14.7 (Me); *m/z*(EI) 429 (M^+, 34%), 412 (7), 383 (4), 355 (7), 338 (45), 324 (8), 293 (4), 279 (10), 277 (6), 224 (15), 167 (26), 151 (45), 149 (100), 139 (9), 113 (13), 105 (28), 91 (66), 71 (38), 57 (55).



3.4.8. Methyl 5-methyl-2-(thien-2-yl)oxazole-4-carboxylate 4h. According to the general procedure the title compound was obtained from **3h** as a colourless crystalline solid (80%), 158–159 °C (diethyl ether); (Found: C, 53.8; H, 3.9; N, 6.2. $C_{10}H_9NO_3S$ requires C, 53.8; H, 4.1; N, 6.3%); ν_{max}(KBr)/cm^{-1} 3078, 3063, 2991, 2945, 2914, 2852, 1726, 1639, 1603, 1588, 1496, 1440, 1414, 1368, 1317, 1260, 1219, 1178, 1107, 1055, 1015, 984, 856, 810, 779, 769, 712, 646, 630; δ_H(300 MHz; $CDCl_3$) 7.72 (1H, dd, *J*=1.1, 3.8 Hz, ArH), 7.45 (1H, dd, *J*=1.1, 5.1 Hz, ArH), 7.10 (1H, dd, *J*=3.8, 5.1 Hz, ArH), 3.93 (3H, s, OMe), 2.69 (3H, s, Me); δ_C (75 MHz; $CDCl_3$) 164.9, 158.1, 131.1 (CH), 131.0, 130.7 (CH), 130.5, 130.4, 130.1 (CH), 54.3 (Me), 14.3 (Me); *m/z* (EI) 223 (M^+, 58%), 192 (23), 163 (46), 130 (100), 110 (60), 95 (77), 60 (25).

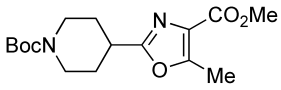


3.4.9. Methyl 5-(4-chlorophenyl)-2-(thien-2-yl)oxazole-4-carboxylate 4i. According to the general procedure the title compound was obtained from **3i** as a colourless crystalline solid (72%); mp 138–139 °C (methanol); (Found: C, 56.1; H, 2.9; N, 4.2. $C_{15}H_{10}ClNO_3S$ requires C, 56.3; H, 3.2; N, 4.4%); ν_{max}(KBr)/cm^{-1} 3117, 3093, 3065, 3001, 2953, 2917, 2844, 1718, 1606, 1578, 1554, 1486, 1441, 1421, 1353, 1309, 1221, 1185, 1093, 1037, 1017, 1005, 948, 924; δ_H (300 MHz; $CDCl_3$) 8.10 (2H, d, *J*=8.6 Hz, ArH), 7.82 (1H, dd, *J*=1.1, 3.8 Hz, ArH), 7.51 (1H, dd, *J*=1.1, 5.0 Hz, ArH), 7.47 (2H, d, *J*=8.6 Hz, ArH), 7.15 (1H, dd, *J*=3.8, 5.0 Hz, ArH), 3.97 (3H, s, OMe); δ_C (75 MHz; $CDCl_3$) 162.9, 156.6, 154.1, 136.9, 130.1 (2×CH), 129.7 (CH), 129.2 (CH), 128.7, 128.5 (CH), 128.4, 125.5, 53.0 (Me); *m/z* (EI) 321/319 (M^+, 30/43%), 316 (12), 291 (3), 290/288 (17/45), 284 (27).



3.4.10. Methyl 5-(4-methoxycarbonylphenyl)-2-(thien-2-yl)oxazole-4-carboxylate 4j. According to the general procedure the title compound was obtained from **3j** as a colourless crystalline solid (54%); mp 168 °C (methanol);

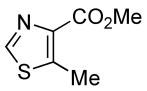
(Found: C, 59.2; H, 3.6; N, 3.9. $C_{17}H_{13}NO_5S$ requires C, 59.5; H, 3.8; N, 4.1%); ν_{max}(KBr)/cm^{-1} 3109, 3088, 3032, 2996, 2950, 2843, 1716, 1609, 1578, 1501, 1434, 1404, 1347, 1281, 1224, 1189, 1107, 1091, 1020, 1015, 943, 861, 810, 774, 723, 692; δ_H (300 MHz; DMSO) 8.26 (2H, d, J=8.7 Hz, ArH), 8.13 (2H, d, J=8.7 Hz, ArH), 7.96 (2H, m, ArH), 7.32 (1H, app t, J=4.1 Hz, ArH), 3.93 (3H, s, OMe), 3.90 (3H, s, OMe); δ_C (75 MHz; DMSO) 165.9, 162.0, 156.3, 152.8, 131.7 (CH), 131.1, 130.8, 130.3 (CH), 129.6 (CH), 129.2, 129.1 (CH), 128.7 (CH), 128.0, 52.8 (Me), 52.6 (Me); m/z (CI) 343 (M$^+$, 33%), 340 (5), 314 (3), 313 (6), 312 (29).



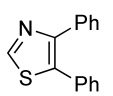
3.4.11. Methyl 2-(1-*tert*-butoxycarbonylpiperidin-4-yl)-5-methyloxazole-4-carboxylate 4k. According to the general procedure the title compound was isolated from **3k** as a colourless crystalline solid (66%); mp 101–103 °C; (Found: C, 59.1; H, 7.6; N, 8.5. $C_{16}H_{24}N_2O_5$ requires C, 59.2; H, 7.5; N, 8.6%); ν_{max}(KBr)/cm^{-1} 2981, 2962, 2937, 2850, 1718, 1682, 1622, 1425, 1346, 1252, 1234, 1211, 1167, 1107, 1026, 937, 783; δ_H (300 MHz; $CDCl_3$) 4.09 (2H, m, 2×CH), 3.87 (3H, s, CO_2Me), 2.90 (3H, m, 3×CH), 2.58 (3H, s, Me), 1.97 (2H, m, 2×CH), 1.75 (2H, m, 2×CH), 1.43 (9H, s, CMe_3); δ_C (75 MHz; $CDCl_3$) 164.8, 163.2, 156.5, 155.0, 127.5, 80.0, 52.3 (Me), 43.5 (CH), 36.0 (CH), 29.7 (CH_2), 28.8 (Me), 12.3 (Me); m/z (CI) 324 (M$^+$, 2%), 298 (3), 297 (11), 271 (3), 270 (15), 269 (100), 251 (55), 226 (4), 225 (25), 223 (32), 193 (3), 168 (14), 155 (2), 136 (5), 83 (5).

3.5. General procedure for thiazole formation

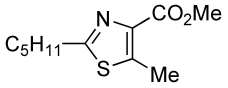
A solution of the keto amide substrate **3** (1.5 mmol) and Lawesson's reagent (3.0 mmol) in dry THF (10 ml) was heated to reflux for 4–6 h. The reaction mixture was then evaporated in vacuo and purified on silica gel eluting with ethyl acetate–light petroleum (3:7) to yield the product.



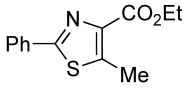
3.5.1. Methyl 5-methylthiazole-4-carboxylate 5a. According to the general procedure the title compound was obtained from **3a** as a colourless crystalline solid (60%); mp 62–65 °C (diethyl ether) (lit.[32] mp not given); (Found: M$^+$, 157.0200. $C_6H_7NO_2S$ requires 157.0198); ν_{max}(KBr)/cm^{-1} 3035, 2958, 2924, 2850, 1722, 1597, 1518, 1433, 1372, 1333, 1288, 1203, 1124, 1066, 955, 881, 831, 785, 762, 627; δ_H (300 MHz; $CDCl_3$) 8.51 (1H, s, H-2), 3.87 (3H, s, OMe), 2.73 (3H, s, Me); δ_C (75 MHz; $CDCl_3$) 163.2, 149.6 (CH), 145.4, 142.1, 52.6 (Me), 13.4 (Me); m/z (EI) 157 (M$^+$, 17%), 127 (6), 126 (65), 125 (100), 98 (12), 97 (23), 72 (4), 71 (11), 59 (8), 54 (3).



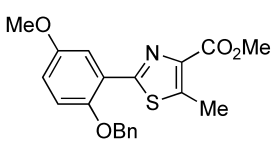
3.5.2. 4,5-Diphenylthiazole 5b. According to the general procedure the title compound was obtained from **3b** as a colourless crystalline solid (94%); mp 59–61 °C (lit.[33] mp 60–61 °C); (Found: C, 75.7; H, 4.6; N, 5.8. $C_{15}H_{11}NS$ requires C, 75.9; H, 4.7; N, 5.9%); ν_{max}(KBr)/cm^{-1} 3053, 2926, 2854, 2808, 1497, 1475, 1441, 1414, 1338, 1279, 1070, 1026, 999, 966, 899, 825, 766, 694; δ_H (300 MHz; $CDCl_3$) 8.84 (1H, s, H-2), 7.54 (2H, m, ArH), 7.34 (8H, m, ArH); δ_C (75 MHz; $CDCl_3$) 151.5 (CH), 151.0, 135.0, 133.4, 132.2, 130.1 (CH), 129.4 (CH), 129.2 (CH), 128.73, 128.71, 128.3 (CH); m/z (EI) 237 (M$^+$, 24%), 236 (6).



3.5.3. Methyl 5-methyl-2-pentylthiazole-4-carboxylate 5d. According to the general procedure the title compound was isolated from **3d** as a colourless oil (89%); (Found: MH$^+$, 228.1050. $C_{11}H_{18}NO_2S$ requires 228.1058); ν_{max}(film)/cm^{-1} 2954, 2929, 2858, 1716, 1504, 1437, 1221, 1068, 962, 866, 789, 768; δ_H (300 MHz; $CDCl_3$) 3.88 (3H, s, OMe), 2.91 (2H, t, J=7.8 Hz, CH_2), 2.69 (3H, s, Me), 1.70 (2H, m, CH_2), 1.34 (4H, m, 2×CH_2), 0.85 (3H, t, J=7.0 Hz, Me); δ_C (75 MHz; $CDCl_3$) 168.2, 163.3, 144.9, 140.5, 52.4 (Me), 33.8 (CH_2), 31.6 (CH_2), 30.2 (CH_2), 22.7 (CH_2), 14.3 (Me), 13.4 (Me); m/z (CI) 228 (MH$^+$, 100%), 226 (8), 212 (3), 197 (4), 196 (31), 184 (3), 171 (11), 139 (3).

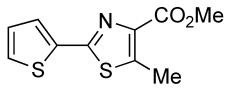


3.5.4. Ethyl 5-methyl-2-phenylthiazole-4-carboxylate 5e. According to the general procedure the title product was obtained from **3e** as a colourless solid (53%); mp 80–81 °C (ethyl acetate–light petroleum) (lit.[34] mp 59–61 °C); (Found: C, 63.1; H, 5.2; N, 5.6. $C_{13}H_{13}NO_2S$ requires C, 63.1; H, 5.3; N, 5.7%); ν_{max}(KBr)/cm^{-1} 2980, 2927, 2902, 2868, 2852, 1707, 1517, 1466, 1443, 1367, 1327, 1242, 1219, 1165, 1065, 1018, 974, 777, 698, 638; δ_H (300 MHz; $CDCl_3$) 8.15 (2H, m, ArH), 7.65 (3H, m, ArH), 4.66 (2H, q, J=7.1 Hz, OCH_2Me), 3.03 (3H, s, Me), 1.67 (3H, t, J=7.1 Hz, OCH$_2$*Me*); δ_C (75 MHz; $CDCl_3$) 164.1, 163.1, 144.9, 142.7, 133.3, 130.7 (CH), 129.3 (CH), 127.1 (CH), 61.6 (CH_2), 14.8 (Me), 13.8 (Me); m/z(CI) 247 (M$^+$, 13%), 230 (5), 203 (4), 202 (30), 201 (4).

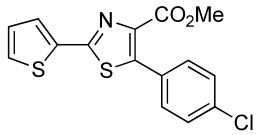


3.5.5. Methyl 2-(2-benzyloxy-5-methoxyphenyl)-5-methylthiazole-4-carboxylate 5f. According to the general procedure the title product was obtained from **3f** as a colourless crystalline solid (55%); mp 115–117 °C (methanol); (Found: C, 64.9; H, 5.0; N, 3.6. $C_{20}H_{19}NO_4S$ requires C, 65.0; H, 5.2; N, 3.8%); ν_{max}(KBr)/cm^{-1} 3058, 3032, 2996, 2946, 2914, 2868, 2838, 1701, 1650, 1609, 1506, 1455, 1440, 1414, 1388, 1312, 1276, 1235, 1173, 1117, 1071, 1035, 999, 871, 805, 779, 733, 692, 666; δ_H (400 MHz; $CDCl_3$) 7.94 (1H, d, J=3.1 Hz, ArH), 7.41 (5H, m, ArH), 6.95 (2H, m, ArH), 5.23 (2H, s, OCH_2Ph), 3.96 (3H, s, OMe), 3.87 (3H, s, OMe), 2.75 (3H, s, Me); δ_C

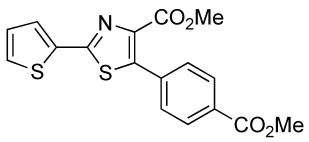
(100 MHz; $CDCl_3$) 163.4, 158.1, 154.0, 149.9, 145.6, 139.8, 136.3, 128.6 (CH), 128.2 (CH), 127.8 (CH), 122.7, 117.9 (CH), 114.4 (CH), 112.0 (CH), 71.6 (CH_2), 56.0 (Me), 52.0 (Me), 12.9 (Me); *m/z*(EI) 369 (M^+, 8%), 352 (7), 279 (5), 278 (16), 246 (5), 218 (8), 205 (7), 177 (7), 149 (100), 125 (5), 111 (8), 97 (12), 91 (23), 83 (12), 55 (16).



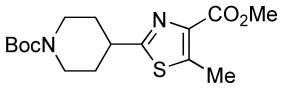
3.5.6. Methyl 5-methyl-2-(thien-2-yl)thiazole-4-carboxylate 5h. According to the general procedure the title product was obtained from **3h** as a colourless crystalline solid (69%); mp 113–115 °C (methanol); (Found: C, 49.9; H, 3.6; N, 5.7. $C_{10}H_9NO_2S_2$ requires C, 50.2; H, 3.8; N, 5.9%); ν_{max}(KBr)/cm^{-1} 3109, 2991, 2950, 2919, 2843, 1711, 1521, 1470, 1440, 1419, 1378, 1317, 1240, 1219, 1163, 1071, 912, 856, 840, 825, 781, 764, 702, 630; δ_H (300 MHz; $CDCl_3$) 7.47 (1H, d, *J*=3.7 Hz, ArH), 7.40 (1H, d, *J*=5.0 Hz, ArH), 7.06 (1H, dd, *J*=3.7, 5.0 Hz, ArH), 3.94 (3H, s, OMe), 2.78 (3H, s, Me); δ_C (75 MHz; $CDCl_3$) 163.2, 158.1, 144.8, 141.7, 136.8, 128.5 (CH), 128.2 (CH), 127.6 (CH), 52.6 (Me), 13.6 (Me); *m/z*(EI) 239 (M^+, 64%), 209 (8), 208 (21), 207 (65), 181 (8), 179 (47), 136 (4), 129 (9), 127 (100), 111 (5), 99 (4), 71 (8), 59 (10).



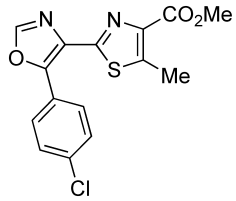
3.5.7. Methyl 5-(4-chlorophenyl)-2-(thien-2-yl)thiazole-4-carboxylate 5i. According to the general procedure the title compound was obtained from **3i** as a pale crystalline solid (89%); mp 146–149 °C (methanol); (Found: C, 53.2; H, 2.8; N, 4.0. $C_{15}H_{10}ClNO_2S_2$ requires C, 53.6; H, 3.0; N, 4.2%); ν_{max}(KBr)/cm^{-1} 3109, 3088, 3068, 3049, 3032, 2996, 2951, 2924, 1716, 1647, 1541, 1466, 1431, 1417, 1400, 1335, 1201, 1169, 1088, 1016, 999; δ_H (300 MHz; $CDCl_3$) 7.55 (1H, dd, *J*=1.1, 3.7 Hz, ArH), 7.46 (3H, m, ArH), 7.41 (2H, d, *J*=8.7 Hz, ArH), 7.10 (1H, dd, *J*=3.7, 5.0 Hz, ArH), 3.86 (3H, s, CO_2Me); δ_C (75 MHz; $CDCl_3$) 163.0, 160.8, 145.1, 141.2, 136.7, 136.3, 132.0 (CH), 129.6, 129.3 (CH), 129.1 (CH), 128.8 (CH), 128.5 (CH), 52.9 (Me); *m/z* (CI) 335/337 (M^+, 41/34%), 332 (12), 307 (5), 306/304 (27/60), 300 (7).



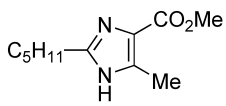
3.5.8. Methyl 5-(4-methoxycarbonylphenyl)-2-(thien-2-yl)thiazole-4-carboxylate 5j. According to the general procedure the title compound was obtained from **3j** as a pale crystalline solid (34%); mp 165–167 °C (methanol); (Found: C, 56.6; H, 3.5; N, 3.7. $C_{17}H_{13}NO_4S_2$ requires C, 56.8; H, 3.7; N, 3.9%); ν_{max}(KBr)/cm^{-1} 3119, 3117, 2955, 2914, 2848, 1721, 1654, 1603, 1537, 1470, 1424, 1327, 1281, 1260, 1214, 1178, 1112, 1081, 963, 917, 851, 764, 702; δ_H (300 MHz; DMSO) 8.06 (2H, d, *J*=8.5 Hz, ArH), 7.87 (1H, dd, *J*=1.1, 4.9 Hz, ArH), 7.82 (1H, dd, *J*=1.1, 3.8 Hz, ArH), 7.75 (2H, d, *J*=8.5 Hz, ArH), 7.25 (1H, dd, *J*=3.8, 4.9 Hz, ArH), 3.92 (3H, s, OMe), 3.78 (3H, s, OMe); δ_C (75 MHz; DMSO) 166.1, 162.0, 160.0, 143.3, 141.0, 135.5, 134.6, 130.7 (CH), 130.5 (CH), 130.4, 129.5 (CH), 129.3 (CH), 129.1 (CH), 52.7 (Me), 52.5 (Me); *m/z* (CI) 359 (M^+, 30%), 356 (3), 329 (7), 328 (32), 285 (3).



3.5.9. Methyl 2-(1-*tert*-butoxycarbonylpiperidin-4-yl)-5-methylthiazole-4-carboxylate 5k. According to the general procedure the title compound was isolated from **3k** as a colourless crystalline solid (74%); mp 75–77 °C; (Found: M^+, 340.1439. $C_{16}H_{24}N_2O_4S$ requires 340.1457); ν_{max}(KBr)/cm^{-1} 3174, 2980, 2958, 2933, 2872, 1741, 1709, 1597, 1454, 1377, 1338, 1306, 1265, 1238, 1205, 1155, 1111, 1088, 1053, 933, 856, 746; δ_H (300 MHz; $CDCl_3$) 4.20 (2H, m, 2×CH), 3.90 (3H, s, OMe), 3.16 (1H, m, CH), 2.79 (2H, m, 2×CH), 2.73 (3H, s, Me), 2.04 (2H, m, 2×CH), 1.63 (2H, m, 2×CH), 1.44 (9H, s, CMe_3); δ_C (75 MHz; $CDCl_3$) 168.9, 161.0, 152.7, 142.4, 138.3, 77.8, 50.2 (Me), 41.6 (CH_2), 39.0 (CH), 30.5 (CH_2), 26.5 (Me), 11.3 (Me); *m/z* (CI) 340 (M^+, 3%), 314 (3), 313 (14), 287 (5), 286 (9), 285 (100), 267 (40), 253 (4), 242 (9), 241 (56), 239 (33), 210 (2), 209 (7), 197 (3), 184 (24), 171 (3), 152 (9), 83 (7).

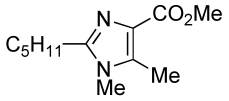


3.5.10. Methyl 2-[5-(4-chlorophenyl)oxazol-4-yl]-5-methylthiazole-4-carboxylate 5l. According to the general procedure **3l** was treated with Lawesson's reagent to yield the title compound as a colourless crystalline solid (40%); mp 210–212 °C; (Found: C, 53.6; H, 3.0; N, 8.2. $C_{15}H_{11}ClN_2O_3S$ requires C, 53.8; H, 3.3; N, 8.4%); ν_{max}(KBr)/cm^{-1} 3129, 3058, 3037, 2955, 2909, 2843, 1701, 1511, 1470, 1434, 1327, 1230, 1112, 1091, 1066, 1030, 1009, 912, 876, 839, 769, 744, 625; δ_H (300 MHz; $CDCl_3$) 8.47 (2H, d, *J*=8.7 Hz, ArH), 7.88 (1H, s, H-2), 7.42 (2H, d, *J*=8.7 Hz, ArH), 3.93 (3H, s, OMe), 2.79 (3H, s, Me); δ_C (75 MHz; $CDCl_3$) 160.9, 154.6, 147.2 (CH), 145.0, 144.2, 139.6, 133.7, 127.2, 126.8 (CH), 126.7 (CH), 123.8, 50.2 (Me), 11.1 (Me); *m/z* (FI) 334 (M^+, 100%).



3.5.11. Methyl 5-methyl-2-pentyl-1*H*-imidazole-4-carboxylate 6a. Ketoamide **3a** (1.0 g, 4.4 mmol), ammonium acetate (510 mg, 6.6 mmol) and glacial acetic acid (2 ml) were dissolved in toluene (50 ml) and heated under reflux for 2 h. The reaction was cooled, washed with brine, dried ($MgSO_4$), reduced in vacuo and purified by column chromatography to yield the title product as a colourless oil (756 mg, 82%); (Found: MH^+, 211.1454. $C_{11}H_{19}N_2O_2$ requires 211.1447); ν_{max}(film)/cm^{-1} 3399, 3308, 2956,

2928, 2866, 1739, 1602, 1539, 1434, 1373, 1292, 1264, 1187, 1106; δ_H (300 MHz; $CDCl_3$) 6.30 (1H, s, NH), 3.63 (3H, s, OMe), 2.24 (2H, t, J=7.4 Hz, CH_2), 1.91 (3H, s, Me), 1.71–1.61 (2H, m, CH_2), 1.35–1.29 (4H, m, 2×CH_2), 0.88 (3H, t, J=6.8 Hz, Me); δ_C (75 MHz; $CDCl_3$) 174.5, 171.6, 168.4, 93.3, 51.0 (Me), 36.8 (CH_2), 31.7 (CH_2), 25.9 (CH_2), 22.8 (CH_2), 19.5 (Me), 14.2 (Me); *m/z* (CI) 211 (MH^+, 28%), 198 (12), 197 (100), 195 (8), 179 (5), 157 (5), 154 (3), 131 (6), 130 (46), 129 (13), 113 (3), 99 (5), 98 (2), 70 (3).



3.5.12. Methyl 1,5-dimethyl-2-pentyl-1*H*-imidazole-4-carboxylate 6b. Ketoamide **3a** (250 mg, 1.1 mmol) and glacial acetic acid (2 ml) were dissolved in toluene (50 ml) to which was then added a solution of methylamine (2 M in THF, 0.82 ml, 1.7 mmol) and heated under reflux for 2 h. The reaction was cooled, washed with brine, dried ($MgSO_4$), reduced in vacuo and purified by column chromatography to yield the title product as an orange oil (114 mg, 46%); (Found: MH^+, 225.1603. $C_{12}H_{21}N_2O_6$ requires 225.1603); ν_{max}(film)/cm^{-1} 2958, 2928, 2858, 1701, 1577, 1530, 1439, 1373, 1216, 1068; δ_H (300 MHz; $CDCl_3$) 3.81 (3H, s, OMe), 3.42 (3H, s, NMe), 2.63 (2H, t, J=7.7 Hz, CH_2), 2.46 (3H, s, Me), 1.65–1.62 (2H, m, CH_2), 1.30–1.28 (4H, m, 2×CH_2), 0.84 (3H, t, J=6.6 Hz, Me); δ_C (75 MHz; $CDCl_3$) 164.7, 148.7, 136.5, 127.1, 51.5 (Me), 31.9 (CH_2), 30.5 (Me), 27.9 (CH_2), 27.6 (CH_2), 22.6 (CH_2), 14.2 (Me), 10.4 (Me); *m/z* (CI) 225 (MH^+, 100%), 224 (8), 209 (3), 195 (3), 194 (6), 193 (56), 181 (6), 169 (2), 168 (14), 150 (2), 136 (5), 116 (2), 99 (3).

Acknowledgements

We thank the EPSRC and Tripos Receptor Research for an Industrial CASE Award (to J.R.D.), and the EPSRC Mass Spectrometry Service at Swansea for mass spectra.

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Assignment of absolute configuration at phosphorus of P-chiral diastereomers of deoxyribonucleoside methanephosphonamidates by means of NMR spectroscopy☆,☆☆

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Received 16 December 2003; revised 19 February 2004; accepted 11 March 2004

Abstract—Recently, we have prepared a novel class of DNA analogues containing the [3′-NH–P(CH_3)(O)–O-5′] methanephosphonamidate linkage. Synthesis of such analogues requires preparation of the dinucleoside methanephosphonamidates N×N, where N is a 2′-deoxyribonucleoside moiety and × is the methanephosphonamidate linkage. Dimers T×T and C×T were obtained in a non-stereospecific manner giving rise to a pair of P-chiral diastereomers. Such diastereomers were effectively separated into fast and slow migrating ones by means of chromatographic methods (TLC). As described in our previous work (Nawrot et al. *Nucleic Acids Res.* **1998**, *26*, 2650), the stereochemistry of the phosphorus chiral center of T×T fast migrating diastereomer is R_P and of T×T slow migrating diastereomer is S_P, as established by means of 2D ROESY experiments. Here we describe assignment of the absolute configuration at the phosphorus center of fast and slow migrating diastereomers of C×T dimer. The 2D ROESY sequence with phosphorus decoupling during acquisition used in these measurements allowed observation of the P–Me group as a singlet instead of a $^1H-^{31}P$-coupled doublet. The apparent advantage of this approach was a much better signal to noise ratio and improved resolution in the F1 dimension. For the fast migrating C×T diastereomer an R_P and for slow migrating C×T diastereomer an S_P configuration was assigned. Conformational analysis of both pairs of diastereomers T×T and C×T indicates significant differences in sugar ring puckering, which strongly depend on the nature of the nucleobase at the 5′-terminus of the dimer. The ribose rings of the 3′-amino-2′,3′-dideoxycytidine moiety of both diastereomers of C×T adopt predominantly a C3′-*endo* (North) conformation, while thymine-substituted ribofuranoses originating either from C×T or T×T dimers prefer a C2′-*endo* (South) conformation.

1. Introduction

Among the many DNA analogues considered for therapeutic applications as antisense[1] and/or antigene[2] agents, much attention has been focused on oligo(deoxyribonucleoside methanephosphonate)s with the R_P configuration.[3] Such constructs are of great interest due to their improved affinity toward double-stranded DNA, resistance to *exo*-nucleolytic degradation, enhanced cellular uptake and low affinity for proteins.[4] Oligo(nucleotide 3′-NH–P(O)O^--5′-phosphoroamidate)s, introduced by Gryaznov,[5] are second generation antisense constructs. Besides stability to phosphorodiesterases, such DNA analogues possess excellent hybridisation properties toward RNA and single- or double-stranded DNA.[6] In view of the advantageous properties of both of the above DNA analogues, we designed oligomers with the combined structural features of both classes. Thus, we synthesized thymidine dimers linked by a novel P-chiral methanephosphonamidate [3′-NH–P(O)(CH_3)O-5′] moiety and introduced them into a DNA chain in alternate positions.[7–9] Such constructs exhibit resistance to nucleolytic degradation and, for oligomers originating from fast-migrating dimers (see below), enhanced affinity toward double-stranded DNA. These features make them useful molecular tools for the inhibition of gene expression by the antigene approach (as triplex forming oligonucleotides, TFOs). In our approach to the synthesis of such oligomers we prepared dinucleoside methanephosphonamidates N×N,

☆ CDRI Communication No. 6414.

☆☆ Supplementary data associated with this article can be found in the online version, at doi: 10.1016/j.tet.2004.03.035

Keywords: Dinucleoside methanephosphonamidates; NMR structure; Conformational analysis; Molecular modelling.

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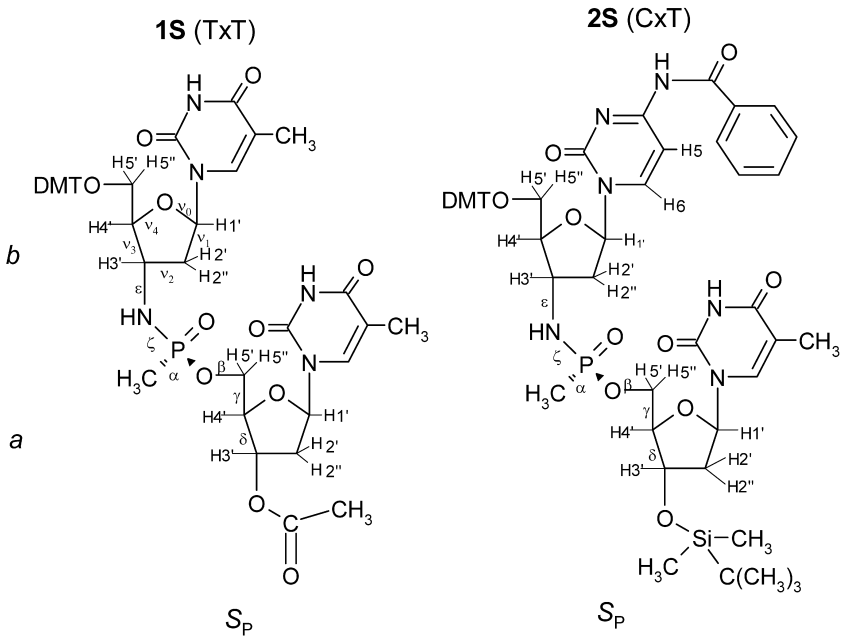

Figure 1. Schematic structures of Sp diastereomers of dimers T×T (**1S**) and C×T (**2S**) with the atom numbering and respective torsion angles. DMT is a 4,4′-dimethoxytrityl group.

Table 1. Spectral characteristics of diastereomers **F** and **S** of dimer C×T[a]

	2F		**2S**	
	b	*a*	*b*	*a*
(a) ^{1}H chemical shifts (ppm) of the ribofuranose rings of diastereomers **2** at 23 °C (*a* and *b* are as described in Figure 1, Thy is thymine and Cyt is cytosine residue)				
H1′	6.23	6.03	6.23	6.07
H2′	2.50	2.20	2.48	2.20
H2″	2.56	2.18	2.53	2.20
H3′	4.07	4.33	3.92	4.40
H4′	4.00	3.94	3.97	3.92
H5′	3.42	3.90	3.41	3.94
H5″	3.63	4.06	3.70	4.06
P–CH_3	1.33		1.30	
N–H	3.48		3.40	
$H6_{Thy}$		7.25		7.24
CH_{3Thy}		1.84		1.86
$H6_{Cyt}$	8.40		8.44	
$H5_{Cyt}$	7.20		7.28	
(b) ^{13}C chemical shifts (ppm) of diastereomers **2** from HMQC at 23 °C				
C1′	86.57	87.21	86.40	86.61
C2′	42.12	40.23	42.39	40.51
C3′	49.75	72.34	49.94	72.1
C4′	85.56	85.56	86.24	86.24
C5′	61.47	63.42	61.72	63.14
C6	145.26	137.07	144.94	113.61
C5	96.9		97.11	
CH_{3Thy}		12.61		12.82
P–CH_3	14.12		13.94	
(c) $^3J_{HH}$ coupling constants (Hz) of the ribofuranose rings of diastereomers **2F** and **2S** at 23 °C				
H1′–H2′	3.4	6.8	3.0	6.8
H1′–H2″	6.7	6.8	7.0	6.8
H2′–H3′	7.4	6.4	7.5	6.3
H2″–H3′	8.4	3.1	8.3	3.6
H2′–H2″	13.7	13.6	13.8	12.3
H3′–H4′	7.5	3.2	7.7	3.5
H4′–H5′	3.8	5.0	3.2	4.1
H4′–H5″	2.5	3.2	2.5	4.1
H5′–H5″	10.8	11.0	10.9	11.4

[a] Measurement error is ca. +/−0.2 Hz.

where N is T or dC, and × represents the methanephosphonamidate linkage.[10] As with the methanephosphonates, the non-stereospecific synthesis of the methanephosphonamidate dimers leads to a mixture of two diastereomers at phosphorus, namely S_P and R_P. Diastereomers are labelled by their silica gel mobility as fast (**F**) or slow (**S**) migrating ones. For a pair of dithymidine diastereomers T×T (**1**) an assignment of the absolute configuration at phosphorus as *R* for the fast migrating dimer (**1F**) and *S* for the slow migrating dimer (**1S**) has already been proposed on the basis of 2-D NMR ROESY experiments.[7] Here we present our study on the conformational analysis and the determination of the absolute configuration at phosphorus of the C×T diastereomers **2F** and **2S** by means of NMR spectroscopy and molecular modelling.

2. Results and discussion

2.1. Absolute configuration of methanephosphonamidate 2

The subjects of our study are the dinucleoside dimers T×T (**1**) and C×T (**2**) possessing a P-chiral methanephosphonamidate [3′-NH–P(O)(CH_3)O-5′] linkage. The structures of the S_P diastereomers of dimer T×T (**1S**) and C×T (**2S**) with the atom numbering and respective torsion angles are shown in Figure 1. The sugar ring of the 3′-terminal nucleoside is described as '*a*' and of the 5′-terminal nucleoside as '*b*'.

The full assignment of the structure of the **2F** (TLC fast migrating) and **2S** (TLC slow migrating) diastereomers in the liquid phase (chloroform solution) was carried out using 1D and 2D NMR techniques. In some experiments we have taken advantage of the Pulse Field Gradient (PFG) system in order to reduce the time of measurement and improve the quality of the spectra (e.g., reduction of T_1 noise).[11–13] The ^1H and ^{13}C chemical shifts as well as proton–proton ^3J coupling constants for **2F** and **2S** were assigned by means of $^1H-^1H$ PFG COSY, $^1H-^{13}C$ PFG HMQC and $^1H-^{13}C$ PFG HMBC experiments and are given in Table 1. The accuracy of $^3J_{HH}$ scalar coupling constants was verified by comparison of experimental and calculated ^1H NMR spectra employing the WINDAISY program (Fig. 2).[14] Selected regions of experimental spectra of diastereomers **2F** and **2S** are given in black and the respective calculated spectra are given in red. In order to simplify the analysis and simulation procedure, ^1H NMR spectra were recorded with ^{31}P decoupling.

The stereochemistry of the phosphorus chiral centers of **2F** and **2S** was established by means of 2D ROESY experiments. This approach was used previously for the assignment of absolute configuration of the dinucleoside methanephosphonates[15–18] and dithymidine methanephosphonamidates T×T (**1F** and **1S**).[7] Nuclear Overhauser effects (NOEs) between H-3′, H-4′ and H-5′ of the 2′-deoxyribose and protons of the P-methyl group were used as criteria for distinguishing between diastereomers with S_P and R_P configurations. The NOE between the P–Me and H-4′ of the 5′-terminal nucleoside was used for the determination of the R_P absolute configuration in the P–Me dimers (dinucleoside methanephosphonates).[15] The presence of a significant cross-peak between P–Me and H-3′ of the 5′-terminal nucleoside was reported to serve as a criterion for assignment of the S_P phosphorus configuration in dimeric methanephosphonates,[16] however further investigations of a variety of P–Me dimers only partially supported this assumption.[17] In some cases both diastereomers exhibit NOE cross-peaks from the P–Me to H-3′ of the 5′-terminal nucleoside.[15] For the R_P dinucleoside

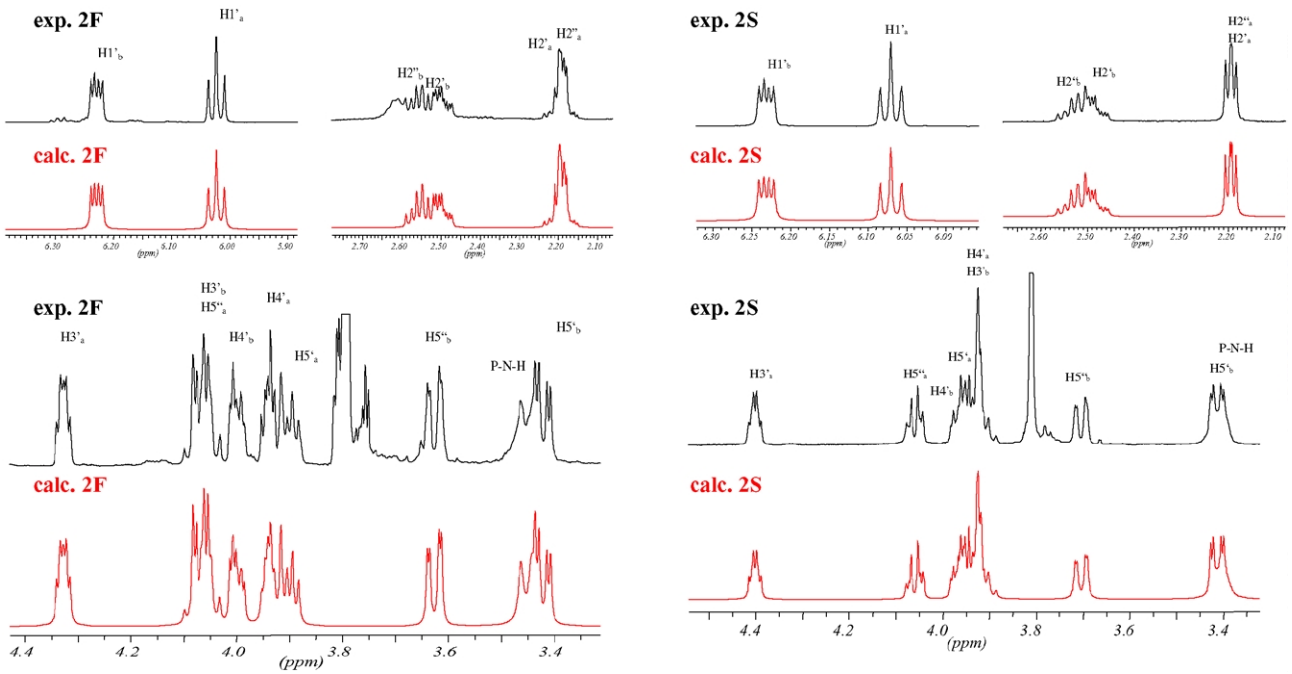

Figure 2. Comparison of experimental (500.13 MHz ^1H NMR) and calculated ^1H NMR spectra of diastereomers **2F** and **2S** employing the WINDAISY program version 940108, Bruker-Franzen Analytik. Selected regions of experimental spectra are shown in black and the respective calculated spectra are shown in red.

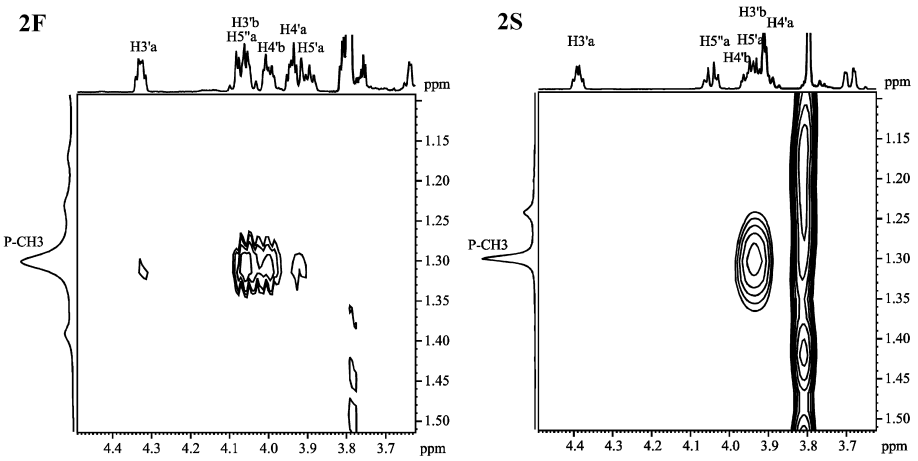

Figure 3. Selected regions of the 500.13 MHz ^{1}H NMR ROESY spectra of diastereomers **2F** and **2S**. Diagnostic cross-peaks between the *a* or *b* ribose rings protons and the protons of the P–Me group are present.

methanephosphonate NOE cross-peaks were also detected between the P–Me and H-5′ and H-5″ of the 3′-terminal nucleoside.[15] An assignment of absolute configuration at the phosphorus center in **1F** as R_P was based on the interaction between the P–Me and H-5′ of an a sugar ring.[7] Cross-peaks from the P–Me to H-4′ of the deoxyribose rings *a* and *b* were also present. A ROESY spectrum of dimer **1S** with an S_P configuration showed significant cross-peaks from the P–Me protons to H-3′ and H-4′ of the *b* deoxyribose moiety. Analogously, in the present work the most important information was obtained from inspection of cross-peaks between the P-methyl group located at the chiral centre and protons of the deoxyribose *a* and *b* rings of diastereomers **2F** and **2S**.

We used the ROESY sequence with phosphorus decoupling during acquisition. With this approach, the phosphorus-attached methyl group, which for the investigated compounds is the most diagnostic probe of stereochemistry at the phosphorus center, is observed as a singlet instead of a ^{1}H–^{31}P-coupled doublet. The apparent advantage of this approach is a much better signal to noise ratio and improved resolution in the F1 dimension. For the TLC fast migrating diastereomer C×T (**2F**) the NOE cross-peaks between the P-methyl group and protons 4′, 5′ and 5″ of the deoxyribose *a* ring as well as between protons 3′ and 4′ of the deoxyribose *b* ring are clearly separated (Fig. 3 **2F**). For the TLC slow migrating diastereomer **2S** only cross-peaks between the CH_3 and protons 3′ and 4′ of ribofuranose *b* ring are seen (Fig. 3 **2S**). Such a correlation pattern suggests an *R* configuration at the phosphorus atom of the TLC fast migrating diastereomer **2F** and an *S* configuration at P-chiral centre of the TLC slow migrating one **2S**.

Although the diagnostic NOEs of dinucleoside methanephosphonamidates are similar to those of dinucleoside methanephosphonates, in the spectra of both pairs of diastereomers, **1F** and **1S**, as well as **2F** and **2S**, there is a strong interaction between the P–Me and H-4′ of ribose *b*. This interaction may be due to the fact that the P–N bond (1.73 Å) in methanephosphonamidates is shorter than the P–O bond in the parent methanephosphonates (1.79 Å), causing decrease of the P–Me↔H-4′ of *b* interatomic distance, and thus, enhancing the NOE interaction. Molecular modelling performed previously[7] for dithymidine methanephosphonamidates **1F** and **1S** (with the help of HyperChem program, MM+ method) showed that the closest P–CH_3↔H-5′ of ribose *a* ring contact of 2.37 Å was in the structure of the R_P diastereomer. In contrast, the model structure of diastereomer S_P revealed the P–CH_3↔H-3′ of ribose *b* ring distance of 2.38 Å as the closest one. These data support our assignment of absolute configuration at the phosphorus atom as R_P for TLC fast migrating dimers **1F** and **2F** as well as S_P for TLC slow migrating dimers **1S** and **2S**.

2.2. Conformational analysis and molecular modelling of P-chiral diastereomers of dinucleoside methanephosphonamidates 1 and 2

In our previous work[7] the simple equation based on vicinal coupling constants between protons 1′ and 2′/2″[19] was used to establish population of conformers of each deoxyribose ring of **1F** and **1S**. In the present work we employed more elaborate methodology which allowed us to obtain the pseudorotation parameters. The phase angle *P*, and maximum puckering amplitude Ψ_m[20,21] for major and minor conformers were determined for the *a* and *b* sugar rings of the pairs of diastereomers **1** and **2**. In addition, these parameters were further used as constraints in molecular modelling of the most likely structure of the respective derivatives.

Conformational analysis of the sugar moiety was performed with the computer program PSEUROT[20–25] with the use of λ electronegativities[26] for the substituents along H–C–C–H fragments in the six-parameter generalized Karplus–Altona equation.[27] The vicinal coupling constants used as input were taken from the WINDAISY simulation. The following λ electronegativity values were used: 0.00 for H,

Table 2. Pseudorotational parameters of diastereomers **F** and **S** of dimers **1** and **2**. Parameters P, Ψ and $\%S$ characterize the North⇄South pseudorotational equilibrium of furanose rings *a* and *b* in each of the dinucleoside methanephosphonamidates. $^3J_{HH}$ coupling constants measurements were carried out at 23, 35 and 45 °C

Compound	Sugar	Pseudorotation parameters				% South			RMS
		P_N	$^N\Psi_m$	P_S	$^S\Psi_m$	296 K	308 K	318 K	
1F	*a*	7	30	156	30	77	78	78	0.205
	b	31	33	156	33	56	52	50	0.363
1S	*a*	37	36	196	36	81	76	76	0.065
	b	14	34	156	34	61	58	53	0.228
2F	*a*	7	29	171	29	69	68	71	0.079
	b	20	35	156	35	18	18	19	0.558
2S	*a*	16	31	171	34	65	65	64	0.051
	b	24	34	156	34	14	16	17	0.524

Pseudorotation parameters are in degrees, RMS error is in Hz.

0.58 for the heterocyclic base, 1.19 for the methanephosphonamidate [3′-NH–P(O)(CH_3)O-5′] moiety, 1.17 for OAc, 0.62 for C1′ and C4′, 0.67 for C2′, 1.40 for O4′ and 0.68 for C5′. In the optimization procedure the geometries and populations of both *N*-(C3′-*endo*) and S-type (C2′-*endo*) pseudorotamers were varied to obtain the best fit between the experimental and the calculated coupling constants. Our optimization procedure started with the following values: P_N=19°, Ψ_m^N=36°, P_S=156° and Ψ_m^S=36°. The puckering amplitude and phase angle of the minor conformer were kept frozen during individual iterative least-squares optimization, whereas parameters for the major conformer were freely optimized. The optimization resulted in the P and Ψ_m for the N- and S-type geometries of the sugar moieties which best agreed with the experimental $^3J_{HH}$ coupling constant data (Table 2).

The influence of the change of nucleobase on the conformation of the deoxyribose rings in the dinucleoside units is apparent when comparing puckering parameters for dimers **1** and **2**. The most significant distinction between dimers T×T and C×T is the conformation of the sugar rings *b* (Table 2). Conformational analysis of sugar rings in T×T (**1**) showed a preference for S-type conformers as is usual in deoxyribonucleosides and their 5′-phosphates. The comparison between *a* and *b* sugars in both slow and fast fractions of compound T×T (**1**) shows that populations of the S-type conformers in sugar ring *b* are ca. 20 unit percent lower that in the case of sugar ring *a*. It has been shown[28] that replacement of the OH group on C3′ of thymidine with an NH_2 substituent decreases the S-type population to ca. 40 percent. Thus it is reasonable to expect a lower S-type sugar population upon introduction of a methanephosphonamidate moiety as in the case of the sugar ring *b* in T×T (**1**).

In the case of compound C×T (**2**) the N⇄S pseudorotational equilibrium was biased towards S-type conformers for sugar *a* for both slow and fast fractions. For sugar *b* the N⇄S pseudorotational equilibrium was surprisingly strongly biased towards N-type conformers. Namely, the populations of S-type conformers at 296 K were 14 and 18% in **2S** and **2F**, respectively. This can be explained by two effects. First, there is a preference of the methanephosphonamidate moiety for N-type conformers, as already shown in the case of **1**. Second, there are stacking interactions of the modified cytosine bases in $CDCl_3$ that additionally drive the pseudorotational equilibrium towards the N-type conformers. Since the conformational preferences observed for 3′-substituted 3′-deoxythymidine derivatives strongly depend on the electronegativity of the 3′-substituent[28] and, as it is well known,[29] nucleoside phosphoramidates adopt predominantly a C3′-*endo* sugar ring conformation, it is reasonable to expect the methanephosphonamidate DNA analogues to adopt a North conformation for sugar rings substituted with an amino functionality that is opposite to a typical B-DNA helix ring puckering.[30] Interestingly, the chirality at phosphorus has little influence on the puckering parameters. The ratio of South to North conformers is only slightly dependent on the temperature of the experiment, resulting in subtle changes of the %S value. The biggest changes in population of the conformers as a function of the temperature are observed for sugar ring *b* of dimer **1S** [Δ(%S)=8].

Molecular modelling is an approach which allows rationalisation of constraints obtained by means of spectroscopic techniques and visualisation of the most reliable set or family of conformers. The PM3 semi-empirical method was used for calculations.[31,32] The 3D structures of **1** and **2**, calculated with partially frozen geometry, as obtained from NMR measurements, are shown in Figure 4. The calculated torsion angles which characterize chain geometry[30] and the data showing differences between energy of fully optimized and partially frozen structures of **1** and **2** are attached as Supplementary Material.

3. Conclusions

The absolute configuration at the phosphorus center of both T×T and C×T methanephosphonamidate dimers is assigned as R_P for fast migrating dimers, and as S_P for slow migrating dimers. Conformational analysis of both pairs of diastereomers indicates significant conformational differences in sugar ring puckering, which strongly depend on the nature of the nucleobase at the 5′-terminus of the dimer. The ribose rings of the 3′-amino-2′,3′-dideoxycytidine moiety of both diastereomers of C×T adopt predominantly C3′-*endo* (North) conformation, while thymine-substituted ribofuranoses in C×T or T×T dimers exist predominantly in a C2′-*endo* (South) conformation.

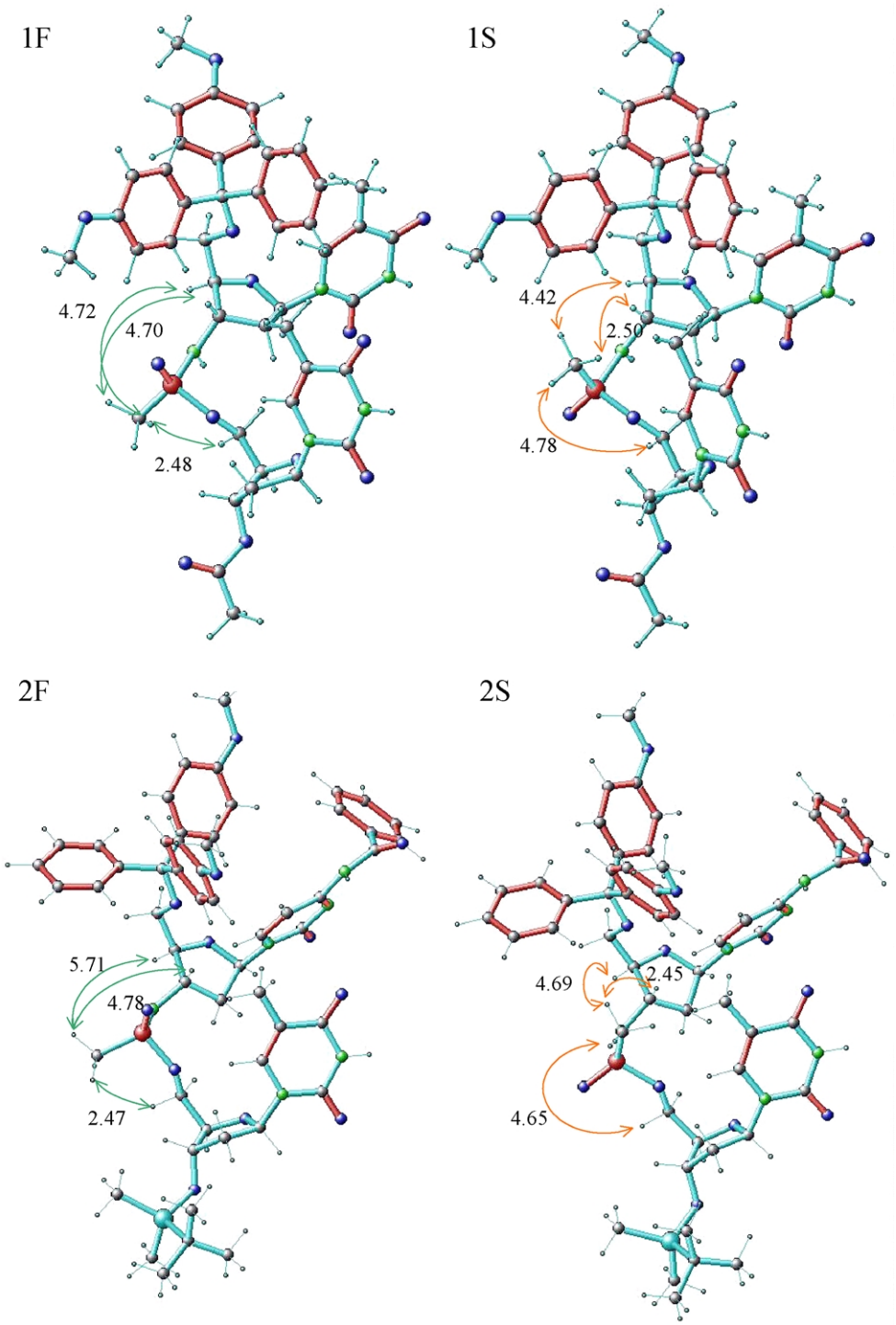

Figure 4. The 3D structures of diastereomers T×T (**1F** and **1S**) and C×T (**2F** and **2S**) obtained by means of the PM3 semi-empirical method. NOE-cross-peaks are indicated.

4. Experimental

4.1. Synthesis and purification of 2

Dinucleotide methanephosphonamidates T×T (**1**) and C×T (**2**) were synthesized and separated chromatographically into fast (**1F** and **2S**) and slow migrating diastereomers (**1F** and **2S**) as described previously.[7,10] If necessary, further purification was achieved by silica gel column chromatography in a gradient of methanol in chloroform (up to 5%). Compounds were dried in vacuo and then used for NMR measurements.

4.2. NMR measurements in the solution

The 5 mg samples of **1** or **2** were dissolved in 0.5 mL of $CDCl_3$. All spectra were recorded on Bruker Avance DRX 500 spectrometer, operating at 500.13 MHz for ^1H,

125.2578 MHz for ^{13}C and 202.46 MHz for ^{31}P. For all experiments original Bruker pulse programs were used. The chemical shift of $CDCl_3$ signal was used as a reference (δ=7.24 ppm for ^{1}H and δ=77.0 ppm for ^{13}C). 85% phosphoric acid was used as an external standard for ^{31}P spectra. The spectrometer was equipped with a Pulse Field Gradient Unit (50 G/cm). The inverse broadband probehead was used.

The COSY90 spectra were obtained from 1024 experiments with 4 scans of each. Relaxation delay was 1.5 s. The spectral width was 10 ppm (5000 Hz) in both dimensions. The data size in F2 was 4 K. Digital quadrature detection (DQD) was applied. Two 10 μs length *z*-gradient pulses, strength of about 5 G/cm each, were applied with 1 ms delay for gradient recovery. The FIDs were apodized with a sine-bell function in both dimensions. Final data were zero filled twice in both dimensions and symmetrized about the diagonal.

The ROESY spectra were recorded in a 2 K×1 K (F2×F1) data matrix. Digital quadrature detection was applied and 32 scans were accumulated in each experiment. The experiment was run in a phase sensitive mode with a 3650 ms cw pulse for the ROESY spin lock. The spectral width was 4500 Hz (9 ppm) in both dimensions. Data were processed with a sine-bell shape apodization function in both directions and TPPI in F1. No zero filling was applied.

The PFG-HMQC spectra were acquired in 1 K×4 K [F1(^{13}C)×F2(^{1}H)] data matrix. Three 1ms length z-gradient pulses, strength of about 25, 15 and 20 G/cm, in sequence were applied with 1 ms delay for gradient recovery. Spectral width was 4000 Hz (8 ppm) in F2 (^{1}H) and 25 kHz (200 ppm) in F1 (^{13}C). A Garp decoupling sequence was employed. Final data were processed with a sine function in F1 and qsine in F2 dimension.

The PFG-HMBC experiment was acquired in a 0.5 K×4 K (F1×F2) data matrix and 8 scans for each experiment. Three 1 ms length *z*-gradient pulses, strength of about 25, 15 and 20 G/cm, in sequence were applied with 50 μs delay for gradient recovery. Final data were processed with sine-bell and qsine-bell functions in F1 and F2 dimensions respectively.

4.3. Molecular modelling

All structures discussed were calculated by means of PM3 routine employing Gaussian 98 program running on the Silicon Graphic Power Challenger computer. The calculations were run until the minimum, defined by the following parameters: maximum force <0.000450; RMS force <0.000300; maximum displacement <0.001800, and RMS displacement <0.001200 was reached. Geometrical constraints were calculated for structures of compound **1** and **2** with fully optimised geometry as well with 'frozen' conformation of sugar rings as shown in Figure 4. Moreover, Figure 4 displays distances given in Å between protons for which NOE effects were observed (see Fig. 1) The data for fully optimised structures with distances between protons corresponding to those presented in Figure 4 are attached as Supplementary Material.

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A rapid and direct access to symmetrical/unsymmetrical 3,4-diarylmaleimides and pyrrolin-2-ones☆

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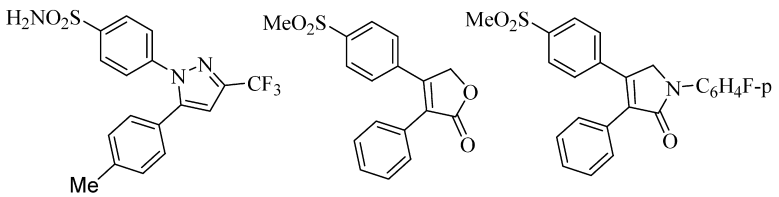
Received 3 December 2003; revised 19 February 2004; accepted 11 March 2004

Abstract—1,8-Diazabicyclo[5.4.0]undec-7ene (DBU) facilitated the oxidative cyclization of phenacyl amide in the presence of atmospheric oxygen under environmentally friendly conditions. The reaction has been studied under various conditions and a plausible mechanism is proposed. This 'green' reaction proceeds via intramolecular ring closure of the amide followed by subsequent reaction with molecular oxygen where DBU played a crucial role. A variety of phenacyl amides were treated with DBU in acetonitrile under an oxygen atmosphere to give the symmetrical/unsymmetrical 3,4-diarylsubstituted maleimides in good yields. Corresponding pyrrolin-2-ones however, were obtained in good to excellent yields when K_2CO_3 was used in place of DBU affording a practical synthesis of these compounds of potential biological interest.

1. Introduction

The tricyclic class of compound having two aryl groups attached to the vicinal positions of the central ring is the focus of many recent reports due to their importance for the development of selective cyclooxygenase-2 (COX-2) inhibitors.[1] This is exemplified by the development of several COX-2 inhibitors (Fig. 1) such as celecoxib[2a] (Celebrex) (**1**), rofecoxib[2b] (Vioxx) (**2**) or the pyrrolin-2-one derivative[3a] (**3**). These compounds are known to be useful for the treatment of inflammation and other related diseases with reduced gastrointestinal side effects when compared to traditional NSAIDs (non-steroidal anti-inflammatory drugs).[3b] Many of these compounds possess a common structural feature i.e. a central ring having a diaryl stilbene-like moiety with a methanesulfonyl or aminosulfonyl group at the C-4 position of one of the aryl rings. These groups usually confer optimal COX-2 inhibitory potency when one of them is present at the C-4 position of an appropriate aryl ring.[3c] In connection with our studies on the synthesis of novel diaryl heterocycles as COX inhibitors[4] we decided to explore the biological as well as pharmacological properties of **II**, having a maleimide or pyrrolin-2-one moiety as the central ring (Fig. 2).



1 (Celecoxib) **2** (Rofecoxib) **3**

Figure 1. Examples of tricyclic compounds as selective COX-2 inhibitors.

☆ DRF Publication No. 339; 3,4-diarylsubstituted maleimides are commonly known as 3,4-diarylpyrrole-2,5-dione or 3,4-diaryl-2,5-dihydro-1*H*-2,5-azoledione according to the IUPAC nomenclature.

Keywords: 3,4-Diarylmaleimide and pyrrolin-2-one; Oxidative cyclization; Phenacyl amide; Oxygen.
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doi:10.1016/j.tet.2004.03.036

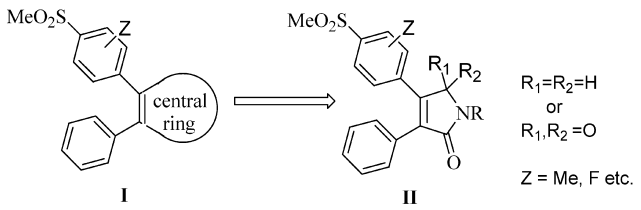
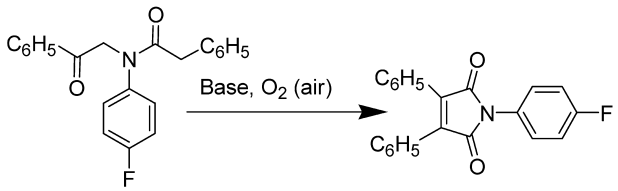


Figure 2. Design of new COX-2 inhibitor.

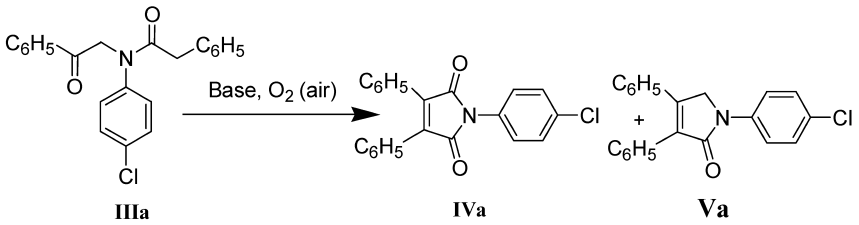
3,4-Disubstituted maleimides i.e. 3,4-disubstituted pyrrole-2,5-diones or 3,4-disubstituted-2,5-dihydro-1*H*-2,5-azole-diones are known to be useful for electrophotographic photoreceptors[5] as well as for maleimide-based fluorophores that are thiol-reactive probes for protein labeling[6a–c] or micromorphological probes[6d–e] for monitoring bulk polymerization. Maleimides, on the other hand, have been reported as rapid and time-dependent inhibitors of PGHS (prostaglandin endoperoxide synthase)[7a] and selective inhibitors of PKC (protein kinase C).[7b] They are also known to be useful as potent and selective inhibitors of glycogen synthase kinase-3 (GSK-3)[7c] as well as cyclin D1/CDK4 inhibitors.[7d]

A number of methods are available in the literature for the synthesis of symmetrical and unsymmetrical 3,4-disubstituted maleimides.[8–9] Among them, the most convenient involves the synthesis[7a,8d,10] from the corresponding maleic anhydrides (and appropriate amine in the presence of acid or base catalyst), which in turn are prepared via a number of methods[11] including the reaction of glyoxylic acids with acetic acids,[8f] or condensation of glyoxalate esters with acetamides.[9a] In both cases however, the required glyoxylic acids or esters are either not readily available or require complicated synthetic procedure. Use of diphenylfumaronitrile[9b] for the synthesis of diphenylmaleimide was also assessed recently and was found to be inappropriate due to the unsatisfactory yields of products, tedious purification procedure and difficulties in the preparation of starting materials.[12] Therefore an alternative single-step method has been developed employing arylacetonitrile and elemental iodine under strongly basic conditions.[12] While this method was found to be operative for the synthesis of symmetrical 3,4-disubstituted maleimides in low to reasonably good yields, its application in the preparation of unsymmetrical derivatives appeared to be unsuitable. Unlike maleimides, only few methods have been reported for the synthesis of 3,4-diarylpyrrolin-2-ones.[13] In our effort for the synthesis of 3,4-diaryl-substituted maleimides, we have developed a mild and environmentally friendly method for the preparation of such compounds via unusual oxidative cyclization of phenacyl amide [i.e. *N*1-(2-oxo-2-arylethyl)-*N*1,2-diaryl-acetamide][4b] (Scheme 1). However, only one example was investigated previously and the methodology was not established as a general protocol for the synthesis of these compounds. In this article we now describe this newly found single-step procedure as a general method for the synthesis of symmetrical/unsymmetrical 3,4-diaryl-substituted maleimides. We also describe a practical and general method for the synthesis of pyrrolin-2-one derivatives where generation of the corresponding maleimide as a side product was not observed.



Scheme 1. Base promoted cyclization of phenacyl amide.

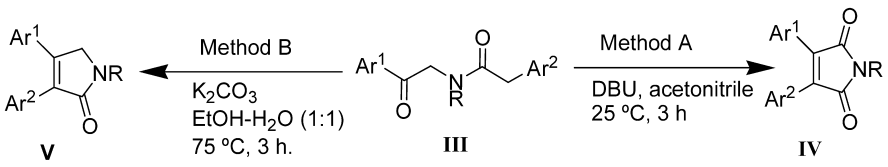
Table 1. Cyclization of *N*1-(2-oxo-2-phenylethyl)-*N*1,2-diphenylacetamide (**IIIb**) in the presence of different bases[a]



IIIa **IVa** **Va**

Entry	Base	Time (h)	Temperature (°C)	Yield (%)[b]	
				IVa	**Va**
1	DBU	3	25	67	11
2	Triethylamine	24	25	n.d.	21
3	Triethylamine	7	75	13	55
4	Diisopropylamine	7	75	11	43
5	Prolinol	7	75	10	40
6	NMP	24	75	n.d.	n.d.
7	NMM	24	75	n.d.	n.d.
8	K_2CO_3	3	75	n.d.	50
9	K_2CO_3	3	75	n.d.	87[c]

[a] Reactions were carried out by using **IIIa** (1.0 equiv.) and base (3.0 equiv.) in acetonitrile.
[b] Isolated yields.
[c] 1.5 equiv. of base was used and EtOH–H_2O (1:1) was used as a solvent. n.d.=not detected.



Scheme 2. Base promoted cyclization of phenacylamide in the presence of oxygen.

2. Results and discussion

2.1. Oxidative cyclization of phenacyl amide in the presence of various bases

Our earlier synthesis of 3,4-diaryl-substituted maleimide was carried out in acetonitrile using three equivalent of DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) as a base in the presence of atmospheric oxygen. However, DBU mediated cyclization of phenacyl amide led to the formation of pyrrolin-2-ones as major products in the absence of oxygen. While exclusion or inclusion of oxygen, and equivalents of base used were established as crucial factors for determining the nature of the products formed, the effect of basicity of the base used on product distribution was not studied extensively. We therefore, examined the cyclization reaction of *N*1-(2-oxo-2-phenylethyl)-*N*1,2-diphenylacetamide (**IIIa**) in the presence of a variety of bases as well as atmospheric oxygen and results are summarized in Table 1. The conversion time of **IIIa** to **IVa** in the presence of DBU was found to be much shorter than other amine bases such as triethylamine, diisopropylamine or prolinol. After 3 h, **IVa** was isolated in 67% yield using DBU (entry 1, Table 1) when 10–13% yield was observed using other bases (entries 3–5, Table 1). *N*-Methylmorpholine (NMM) and *N*-methylpyrrolidone (NMP) gave no product after 24 h. Interestingly, the inorganic base K_2CO_3 afforded the corresponding pyrrolin-2-one **Va** in 87% yield when the reaction was carried out at 75 °C in aqueous ethanol even in the presence of atmospheric oxygen (entry 9, Table 1). These results therefore suggest that intramolecular ring closure of **IIIa** could be carried out successfully by using a variety of bases when the conversion of **Va** to **IVa** occurred effectively in the presence of DBU only.

2.2. Synthesis of symmetrical/unsymmetrical 3,4-diaryl-substituted maleimides and pyrrolin-2-one derivatives

It is evident from Table 1 that intramolecular ring closure of phenacyl amide i.e. *N*1-(2-oxo-2-arylethyl)-*N*1,2-diarylacetamide can be utilized for the synthesis of 3,4-diaryl maleimides or corresponding pyrrolin-2-ones depending on the nature of the base used. To investigate the synthetic utility of this reaction a number of phenacyl amides **III** were treated with DBU in acetonitrile (Method A) or K_2CO_3 in aqueous ethanol (Method B) in the presence of atmospheric oxygen at 25 °C (Scheme 2). Results of this study are summarized in Tables 2 and 3.

As can be seen from Table 2, the oxidative cyclization reaction (Method A) proceeds well in the presence of various R groups in the starting amide **III**. Both symmetrical and unsymmetrical 3,4-diaryl-substituted maleimides were prepared efficiently via the one step procedures in good yields. Halogens (Cl and Br) are well tolerated during the course of the reaction irrespective of their presence in Ar^1 or R (entries 1–6, Table 1). Better yields of products **4** and **11** (entries 1 and 8) were obtained using the present methodology compared to the earlier method where these compounds were prepared in low yields from appropriately substituted pyridine-2-one in the presence of *m*-chloroperbenzoic acid (32 and 14%).[9c] Moreover, Method A has advantages over reported procedures involving the successive treatment of 2,3-dibromo-*N*-methylmaleimide with the moisture sensitive organo-magnesium bromide[8e,g] or the condensation of appropriate glyoxylate chloride (which usually decomposes in the presence of moisture) with aryl acetic acids[7b] for the synthesis of unsymmetrical maleimides. To asses the merit of the present methodology synthesis of 1-(4-chlorophenyl)-3,4-diphenyl-2,5-dihydro-1*H*-2,5-azoledione (**VIa**) was carried out in a bigger scale and 10 g of **VIc** was prepared efficiently in 60% yield.

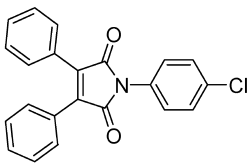
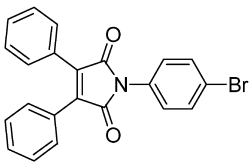
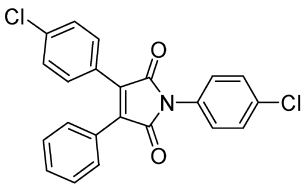
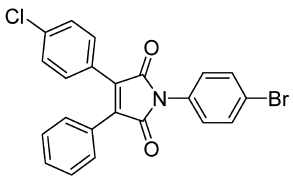
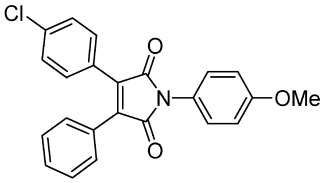
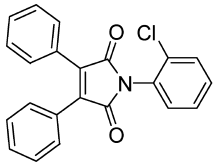
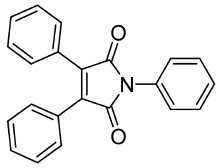
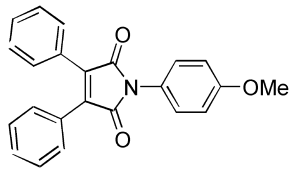
Good to excellent yields of 3,4-diaryl-substituted pyrrolin-2-ones (**V**) were also obtained when the reaction was carried out according to Method B (Table 3). The use of 1:1 ethanol–water in this method was found to be optimum as precipitation of reactants occurred in the presence of excess water whereas use of pure ethanol was found to be less effective (entry 8, Table 1). Products (**V**) isolated from the reaction mixture after dilution with water were often analytically pure. The observed high yield and purity of the isolated products as well as our continuing interest in the parallel synthesis strategy[14] prompted us to investigate the synthesis of **V** using parallel synthesis technique. Yields of products isolated after usual work-up is shown in Table 3.

We have described a direct and practical synthesis of 3,4-diarylmaleimides or pyrrolin-2-ones starting from a common amide. All phenacyl amides **III** used for the synthesis of maleimidies **IV** or pyrrolin-2-ones **V** were prepared from the appropriate *N*-phenacylaniline (**VI**) and arylacetyl chloride (**VII**) according to a similar procedure reported earlier (Scheme 3).[3a] *N*-Phenacylanilines (**VI**) were prepared from phenacyl bromide and corresponding anilines according to the known procedure.[15]

2.3. Application of the methodology

Having demonstrated the present methodology as an efficient tool for the preparation of a variety of diaryl-substituted maleimide as well as pyrrolin-2-one, synthesis of compounds of potential biological interest (Scheme 4) was undertaken. Because of our continuing interest in the development of COX-2 inhibitors for the treatment of inflammatory diseases with reduced ulcerogenic side effects we synthesized some methansulfone derivatives of **IV** as potential COX-2 inhibitors.[4a] Thus, 2-(4-fluoroanilino)-1-(3-methyl-4-methylsulfonylphenyl)-1-ethanone **4** was treated with phenylacetyl chloride to give the desired phenacyl

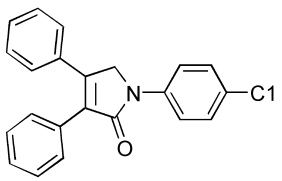
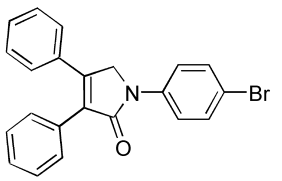
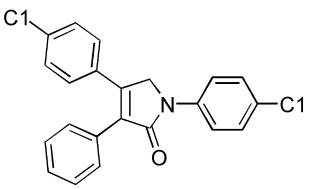
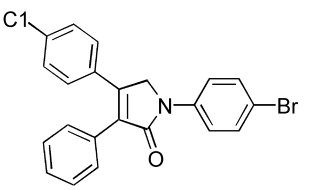
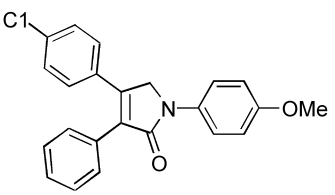
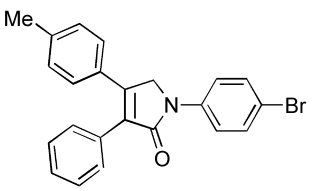
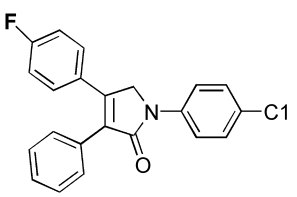
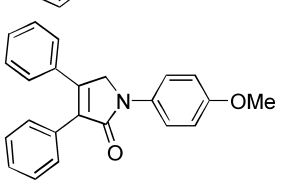
Table 2. Synthesis of 3,4-diaryl substituted maleimides[a]

Entry No.	Ar^1	Ar^2	R	Product (**IV**)[b]		Yield of **IV** (%)[c]
1	Phenyl	Phenyl	4-Chloro phenyl		**IVa**	67
2	Phenyl	Phenyl	4-Bromo phenyl		**IVb**	65
3	4-Chloro phenyl	Phenyl	4-Chloro phenyl		**IVc**	62
4	4-Chloro phenyl	Phenyl	4-Bromo phenyl		**IVd**	55
5	4-Chloro phenyl	Phenyl	4-Methoxy phenyl		**IVe**	71
6	Phenyl	Phenyl	2-Chloro phenyl		**IVf**	59
7	Phenyl	Phenyl	Phenyl		**IVg**	67
8	Phenyl	Phenyl	4-Methoxy phenyl		**IVh**	65

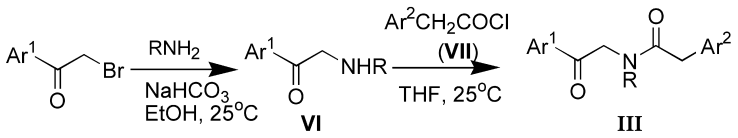
[a] Method A: reactions were carried out by using **III** (1.0 equiv.) and DBU (3 equiv.) in acetonitrile 25 °C for 3 h.
[b] Identified by ^{1}H NMR, IR, Mass.
[c] Isolated yields.

amide **5**, which on treatment with DBU in acetonitrile in the presence of atmospheric oxygen (Method A), yielded 4-methansulfonylphenyl substituted maleimide **6**. Phenacyl amide **5** on treatment with K_2CO_3 in aqueous ethanol (Method B) afforded pyrrolin-2-one **7**. Compound **7** showed 70 and 27% inhibition when tested against recombinant human COX-2 (expressed in sf9 insect cells using baculovirus) and COX-1 (Ram Seminal vesicles) enzyme in vitro[16] (% inhibition was recorded at10 μM concentration of the compound), respectively.

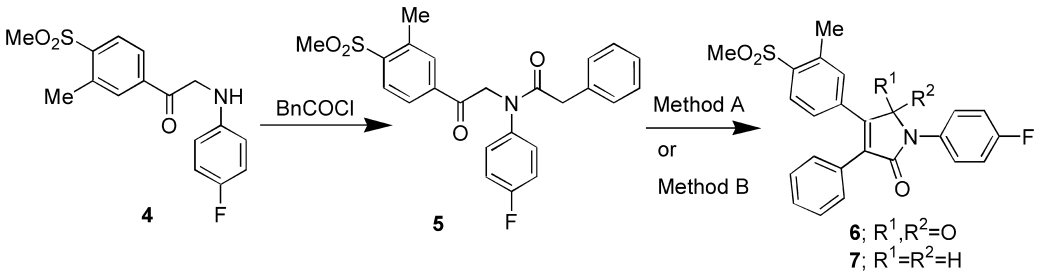
Table 3. Synthesis of 3,4-diaryl substituted pyrrolin-2-ones[a]

Entry No.	Ar^1	Ar^2	R	Product (**V**)[b]		Yield of **V** (%)[c]	
						Normal synthesis	Parallel synthesis
1	Phenyl	Phenyl	4-Chloro phenyl		**Va**	82	85
2	Phenyl	Phenyl	4-Bromo phenyl		**Vb**	87	83
3	4-Chloro phenyl	Phenyl	4-Chloro phenyl		**Vc**	85	85
4	4-Chloro phenyl	Phenyl	4-Bromo phenyl		**Vd**	85	86
5	4-Chloro phenyl	Phenyl	4-Methoxy phenyl		**Ve**	89	90
6	4-Methyl phenyl	Phenyl	4-Bromo phenyl		**Vf**	97	93
7	4-Fluoro phenyl	Phenyl	4-Chloro phenyl		**Vg**	94	93
8	Phenyl	Phenyl	4-Methoxy phenyl		**Vh**	85	87

[a] Method B: reactions were carried out by using **III** (1.0 equiv.) and K_2CO_3 (1.5 equiv.) in 1:1 ethanol–water at 75 °C for 3 h.
[b] Identified by ^1H NMR, IR, Mass.
[c] Isolated yields.



Scheme 3. Preparation of phenacyl amides **III**.[3a,15]



Scheme 4. Synthesis of COX-2 inhibitor.

3. Conclusions

To summarize, the present study demonstrates phenacyl amides as useful precursors for the synthesis of symmetrical and unsymmetrical 3,4-diarylsubstituted maleimides via an oxidative cyclization reaction. The cyclization could be carried out effectively in the presence of DBU and atmospheric oxygen. K_2CO_3 however, facilitated the cyclization of the same amide in aqueous ethanol affording the corresponding pyrrolin-2-one even in the presence of air. Since both reactions (Method A and B) were carried out in an open vessel, i.e. in the presence of atmospheric oxygen, no extra precautions (e.g. inert atmosphere, anhydrous condition) are needed for effective cyclization. They are amenable to scale-up synthesis and the methodology has been utilized for the synthesis of compounds of potential biological interest. Current efforts are now directed to the extension of this methodology to more complex molecules.

4. Experimental

4.1. General methods

Unless stated otherwise, reactions were performed in dried glassware under a nitrogen atmosphere. All the solvents used were commercially available and distilled before use. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254; Merck), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (SRL 230–400 mesh) using distilled petroleum ether, ethyl acetate, dichloromethane, chloroform and methanol. ^1H and ^{13}C NMR spectra were determined in $CDCl_3$, DMSO-d_6 or MeOH-d_4 solutions on Varian Gemini 200 MHz spectrometers. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ=0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constants (J) are given in Hertz. Infrared spectra were recorded on a Perkin–Elmer 1650 FT-IR spectrometer. UV spectra were recorded on Shimadzu UV 2100S UV–vis recording spectrophotometer. Melting points were determined using a Buchi melting point B-540 apparatus and are uncorrected. Thermal analysis data was generated with the help of Shimadzu DSC-50 detector. MS spectra were obtained on a HP-5989A mass spectrometer. Purity was determined by HPLC (AGIL-AUTO) using the condition specified in each case: column, mobile phase (range used), flow rate (range used), detection wavelength, retention times. Microanalyses were performed using Perkin–Elmer 2400 C H N S/O analyzer. All the arenes/heteroarenes used are commercially available.

4.2. General procedure for the preparation of phenacyl amide [*N*1-(2-oxo-2-arylethyl)-*N*1,2-diarylacetamide; III]

Step 1. To a mixture of arylamine (10.3 mmol) and $NaHCO_3$ (10.3 mmol) in ethanol was added the appropriately substituted α-bromoacetophenone (10.3 mmol) at 25 °C under a nitrogen atmosphere. The mixture was stirred vigorously for 6 h at the same temperature and then diluted with water (10 mL). The mixture was extracted with EtOAc (3×20 mL), the organic layers were combined, washed with water (2×15 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum to give the 2-arylamino-1-arylethanone (**VI**).

Step 2. To a solution of 2-arylamino-1-arylethanone (**VI**, 4.08 mmol) in dry THF (15 mL) was added arylacetyl chloride (**VII**, 4.08 mmol) at 25 °C under a nitrogen atmosphere. The mixture was stirred for 2 h then poured into water (50 mL) and extracted with EtOAc (3×30 mL). Combined organic layers were washed with water (2×20 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum to give the desired product.

4.2.1. 2-(4-Chlorophenylamino)-1-phenylethanone (VIa). Light brown solid; yield 83%; mp 177–178 °C (lit.[17a] 177–179 °C); δ_H (200 MHz, $CDCl_3$) 8.0 (d, J=7.3 Hz, 2H), 7.67–7.48 (m, 3H), 7.16 (d, J=8.6 Hz, 2H), 6.62 (d, J=8.8 Hz, 2H), 4.95 (bs, D_2O exchangeable, 1H, N–H), 4.58 (s, 2H, CH_2).

4.2.2. *N*1-(4-Chlorophenyl)-*N*1-(2-oxo-2-phenylethyl)-2-phenylacetamide (IIIa). Light orange solid; yield 85%; mp 88–90 °C; δ_H (200 MHz, $CDCl_3$) 7.93 (d, J=7.2 Hz, 2H), 7.58–7.08 (m, 12H), 5.07 (s, 2H, CH_2), 3.59 (s, 2H, CH_2); ν_{max} (KBr) 1738 (w), 1701, 1668, 1596 cm^{-1}; m/z (CI,

i-Butane) 364 (100, MH^+); found C, 72.37; H, 5.09; N, 3.95; $C_{22}H_{18}ClNO_2$ requires C, 72.63; H, 4.99; N, 3.85%.

4.2.3. 2-(4-Bromophenylamino)-1-phenylethanone (VIb). Brown solid; yield 60%; mp 165–166 °C (lit.[17b] 162 °C); δ_H (200 MHz, $CDCl_3$) 8.0 (d, J=7.3 Hz, 2H), 7.64 (d, J=7.3 Hz, 1H), 7.56 (m, 2H), 7.28 (d, J=8.8 Hz, 2H), 6.60 (d, J=8.6 Hz, 2H), 4.99 (bs, D_2O exchangeable, 1H, N–H), 4.59 (s, 2H, CH_2).

4.2.4. *N*1-(4-Bromophenyl)-*N*1-(2-oxo-2-phenylethyl)-2-phenylacetamide (IIIb). Off white solid; yield 71%; mp 90–92 °C; δ_H (200 MHz, $CDCl_3$) 7.92 (d, J=7.3 Hz, 2H), 7.57–7.09 (m, 12H), 5.07 (s, 2H, CH_2), 3.58 (s, 2H, CH_2); ν_{max} (KBr) 1738 (w), 1701, 1668, 1597 cm^{-1}; *m/z* (CI, *i*-Butane) 410 (100, M+2), 408 (80, M^+); found C, 64.55; H, 4.19; N, 3.62; $C_{22}H_{18}NO_2Br$ requires C, 64.72; H, 4.44; N, 3.43%.

4.2.5. 1-(4-Chlorophenyl)-2-(4-chlorophenylamino)ethanone (VIc). White solid; yield 77%; mp 155–156 °C (lit.[17c] 155–157 °C); δ_H (200 MHz, $CDCl_3$) 7.96 (d, J=7.8 Hz, 2H), 7.50 (d, J=7.8 Hz, 2H), 7.16 (d, J=8.3 Hz, 2H), 6.63 (d, J=8.3 Hz, 2H), 4.91 (D_2O exchangeable, 1H, N–H), 4.55 (s, 2H, CH_2).

4.2.6. *N*1-(4-Chlorophenyl)-*N*1-[2-(4-chlorophenyl)-2-oxoethyl]-2-phenylacetamide (IIIc). Light brown solid; yield 64%; mp 105–107 °C; δ_H (200 MHz, $CDCl_3$) 7.87 (d, J=8.6 Hz, 2H), 7.45–7.08 (m, 11H), 5.03 (s, 2H, CH_2), 3.57 (s, 2H, CH_2); ν_{max} (KBr) 1696, 1663, 1590 cm^{-1}; *m/z* (CI, *i*-Butane) 398 (100, MH^+); found C, 66.20; H, 4.29; N, 3.82; $C_{22}H_{17}NO_2Cl_2$ requires C, 66.34; H, 4.30; N, 3.52%.

4.2.7. 2-(4-Bromophenylamino)-1-(4-chlorophenyl)ethanone (VId). Brown solid; yield 75%; mp 165–166 °C (lit.[17c] 165–168 °C); δ_H (200 MHz, $CDCl_3$) 7.96 (d, J=8.3 Hz, 2H), 7.50 (d, J=8.3 Hz, 2H), 7.30 (d, J=8.8 Hz, 2H), 6.58 (d, J=8.8 Hz, 2H), 4.93 (bs, D_2O exchangeable, 1H, N–H), 4.55 (s, 2H, CH_2).

4.2.8. *N*1-(4-Bromophenyl)-*N*1-[2-(4-chlorophenyl)-2-oxoethyl]-2-phenylacetamide (IIId). Yellow solid; yield 73%; mp 104–108 °C; δ_H (200 MHz, $CDCl_3$) 7.87 (d, J=8.3 Hz, 2H), 7.52–7.08 (m, 11H), 5.02 (s, 2H, CH_2), 3.57 (s, 2H, CH_2); ν_{max} (KBr) 1698, 1665, 1590 cm^{-1}; *m/z* (CI, *i*-Butane) 444 (100, M+2), 442 (80, M^+); found C, 59.49; H, 3.88; N, 3.32; $C_{22}H_{17}BrNO_2Cl$ requires C, 59.68; H, 3.87; N, 3.16%.

4.2.9. 1-(4-Chlorophenyl)-2-(4-methoxyphenylamino)-ethanone (VIe). Off white solid; yield 91%; mp 116–118 °C (lit.[17d] 118–120 °C); δ_H (200 MHz, $CDCl_3$) 7.96 (d, J=8.3 Hz, 2H), 7.50 (d, J=8.8 Hz, 2H), 6.83 (d, J=8.8 Hz, 2H), 6.67 (d, J=8.8 Hz, 2H), 4.95 (D_2O exchangeable, 1H, N–H), 4.55 (s, 2H, CH_2), 3.76 (s, 3H, OCH_3).

4.2.10. *N*1-[2-(4-Chlorophenyl)-2-oxoethyl]-*N*1-(4-methoxyphenyl)-2-phenylacetamide (IIIe). Light brown solid; yield 86%; mp 67–69 °C; δ_H (200 MHz, $CDCl_3$) 7.86 (d, J=8.3 Hz, 2H), 7.40 (d, J=8.3 Hz, 2H), 7.28–7.09 (m, 7H), 6.88 (d, J=8.6 Hz, 2H), 5.02 (s, 2H, CH_2), 3.81 (s, 3H, OCH_3), 3.56 (s, 2H, CH_2); ν_{max} (KBr) 1701, 1655, 1590 cm^{-1}; *m/z* (CI, *i*-Butane) 394 (100, MH^+); found C, 70.05; H, 5.19; N, 3.72; $C_{23}H_{20}NO_3Cl$ requires C, 70.14; H, 5.12; N, 3.56%.

4.2.11. 2-(2-Chlorophenylamino)-1-phenylethanone (VIf). White solid; yield 50%; mp 104–105 °C (lit.[17e] 105 °C); δ_H (200 MHz, $CDCl_3$) 8.0 (d, J=7.3 Hz, 2H), 7.97–7.2 (m, 5H), 6.66 (d, J=7.8 Hz, 2H), 4.95 (D_2O exchangeable, 1H, N–H), 4.65 (s, 2H, CH_2).

4.2.12. *N*1-(2-Chlorophenyl)-*N*1-(2-oxo-2-phenylethyl)-2-phenylacetamide (IIIf). Off white solid; yield 50%; mp 116–118 °C; δ_H (200 MHz, $CDCl_3$) 7.95 (d, J=7.3 Hz, 2H), 7.69–7.21 (m, 10H), 7.10 (d, J=7.6 Hz, 2H), 5.88 (d, J=17.6 Hz, 1H), 4.24 (d, J=17.6 Hz, 1H), 3.50 (s, 2H, CH_2); ν_{max} (KBr) 1742, 1691, 1662 cm^{-1}; *m/z* (CI, *i*-Butane) 364 (100, MH^+); found C, 73.02; H, 4.89; N, 3.99; $C_{22}H_{18}NO_2Cl$ requires C, 72.63; H, 4.99; N, 3.85%.

4.2.13. 1-Phenyl-2-phenylaminoethanone (VIg). Yellow solid; yield 57%; mp 110–112 °C (lit.[17c] 113–115 °C); δ_H (200 MHz, $CDCl_3$) 8.03 (d, J=7.3 Hz, 2H), 7.67–7.48 (m, 3H), 7.27–7.19 (m, 2H), 6.79–6.70 (m, 3H), 4.95 (bs, D_2O exchagable, 1H, NH), 4.63 (s, 2H, CH_2); ν_{max} (KBr) 3370, 1693, 1603, 1512 cm^{-1}; *m/z* (CI, *i*-Butane) 212 (100, MH^+).

4.2.14. *N*1-(2-Oxo-2-phenylethyl)-*N*1,2-diphenylacetamide (IIIg). Brown solid; yield 80%; mp 108–110 °C; δ_H (200 MHz, $CDCl_3$) 7.93 (d, J=7.1 Hz, 2H), 7.59–7.09 (m, 13H), 5.1 (s, 2H, CH_2), 3.58 (s, 2H, CH_2); ν_{max} (KBr) 1698, 1659, 1594 cm^{-1}; *m/z* (CI, *i*-Butane) 330 (100, MH^+); found C, 80.05; H, 5.89; N, 4.54; $C_{22}H_{19}NO_2$ requires C, 80.22; H, 5.81; N, 4.25%.

4.2.15. 2-(4-Methoxyphenylamino)-1-phenylethanone (VIh). Off white solid; yield 50%; mp 95–96 °C (lit.[17f] 94–96 °C); δ_H (200 MHz, $CDCl_3$) 8.02 (d, J=8.1 Hz, 2H), 7.62–7.49 (m, 2H), 7.44 (d, J=8.1 Hz, 2H), 6.86–6.71 (m, 3H), 4.92 (bs, D_2O exchangeable, 1H), 4.60 (s, 2H, CH_2), 3.76 (s, 3H, OCH_3); ν_{max} (KBr) 3390, 1682, 1595, 1514 cm^{-1}; *m/z* (CI, *i*-Butane) 242 (100, MH^+).

4.2.16. *N*1-(4-Methoxyphenyl)-*N*1-(2-oxo-2-phenylethyl)-2-phenylacetamide (IIIh). White solid; yield 31%; mp 68–70 °C; δ_H (200 MHz, $CDCl_3$) 8.0 (d, J=7.3 Hz, 2H), 7.60 (d, J=6.9 Hz, 1H), 7.50 (m, 5H), 7.29 (d, J=6.9 Hz, 2H), 7.17 (d, J=7.3 Hz, 2H), 6.93 (d, J=8.9 Hz, 2H), 5.14 (s, 2H, CH_2), 3.88 (s, 3H, OMe), 3.64 (s, 2H, CH_2); ν_{max} (KBr) 1701, 1659 cm^{-1}; *m/z* (CI, *i*-Butane) 360 (100, MH^+); found C, 76.65; H, 6.01; N, 3.93; $C_{23}H_{21}NO_3$ requires C, 76.86; H, 5.89; N, 3.90%.

4.3. Method A. General procedure for the preparation of IV

To a solution of phenacyl amide **III** (1.3 mmol) in acetonitrile (40 mL) was added DBU (0.61 mL, 4.1 mmol) dropwise at 0–5 °C. The mixture was stirred at 25 °C for 3 h under air. After completion of the reaction the mixture was poured into ice-cold 3 M HCl solution (100 mL) with stirring. The solid precipitate was collected by filtration and then washed with water (2×8 mL) and petroleum ether

(2×5 mL). When a solid precipitate did not form the mixture was extracted with ethyl acetate (3×20 mL) and the combined organic layers were washed with water (2×20 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product thus obtained was purified by column chromatography using petroleum ether–EtOAc as eluant.

4.3.1. 1-(4-Chlorophenyl)-3,4-diphenyl-2,5-dihydro-1*H*-2,5-azoledione (IVa). Yellow solid; yield 67%; mp 186–188 °C; δ_H (200 MHz, $CDCl_3$) 7.52 (d, J=7.3 Hz, 2H), 7.46–7.38 (m, 12H); ν_{max} (KBr) 1760 (w), 1710, 1497 cm^{-1}; m/z (CI, i-Butane) 360 (100, MH^+); UV (MeOH, nm) 363.5, 289.0, 235.0; HPLC: 99.8%, INERTSIL ODS 3V (250×4.6 mm), 0.01 M KH_2PO_4)/CH_3CN 30/70, 1.0 mL/min, 235 nm, retention time 18.73 min; ^{13}C NMR (50 MHz, $CDCl_3$): 168.0 (C=O, 2C), 136.35 (2C), 133.37, 130.31 (2C), 130.10 (2C), 129.97 (4C), 129.22 (2C), 128.63 (4C), 128.28, 127.19 (2C); found C, 73.05; H, 4.09; N, 3.92; $C_{22}H_{14}NO_2Cl$ requires C, 73.44; H, 3.92; N, 3.89%.

4.3.2. 1-(4-Bromophenyl)-3,4-diphenyl-2,5-dihydro-1*H*-2,5-azoledione (IVb). Yellow solid; yield 65%; mp 198–200 °C; δ_H (200 MHz, $CDCl_3$) 7.63 (d, J=8.7 Hz, 2H), 7.53–7.37 (m, 12H); ν_{max} (KBr) 1766 (w), 1712, 1594 (w), 1491 cm^{-1}; m/z (CI, i-Butane) 406 (100, M+2), 404 (100, M^+); UV (MeOH, nm) 237; HPLC: 99.8%, INERTSIL ODS 3V (250×4.6 mm), 0.01 M KH_2PO_4/CH_3CN 30/70, 1.0 mL/min, 237 nm, retention time 23.46 min; ^{13}C NMR (50 MHz, $CDCl_3$): 165.0 (C=O, 2C), 131.80 (C), 126.99 (2C), 124.91 (4C), 124.77 (4C), 123.43 (4C), 123.05 (2C), 122.26 (2C), 116.0 (C); found C, 65.48; H, 3.69; N, 3.37; $C_{22}H_{14}NO_2Br$ requires C, 65.36; H, 3.49; N, 3.46%.

4.3.3. 3-(4-Chlorophenyl)-1-(4-chlorophenyl)-4-phenyl-2,5-dihydro-1*H*-2,5-azoledione (IVc). Yellow solid; yield 62%; mp 140–142 °C; δ_H (200 MHz, $CDCl_3$) 7.49–7.33 (m, 13H); ν_{max} (KBr): 1768 (w), 1710, 1594 (w), 1496 cm^{-1}; m/z (CI, i-Butane) 394 (100, MH^+); UV (MeOH, nm) 368.5, 302.5, 237.0; HPLC: 99.62%, INERTSIL ODS 3V (250×4.6 mm), 0.01 M KH_2PO_4/CH_3CN 30/70, 1.0 mL/min, 238 nm, retention time 28.89 min; ^{13}C NMR (50 MHz, $CDCl_3$): 168.94 (C=O, 2C), 136.38, 136.37, 135.03, 133.45, 131.32 (2C), 130.31, 130.16, 129.87 (2C), 129.23 (2C), 128.99 (2C), 128.76 (2C), 128.01, 127.13 (2C), 126.69; found C, 67.08; H, 3.38; N, 3.50; $C_{22}H_{13}NO_2Cl_2$ requires C, 67.02; H, 3.32; N, 3.55%.

4.3.4. 3-(4-Chlorophenyl)-1-(4-bromophenyl)-4-phenyl-2,5-dihydro-1*H*-2,5-azoledione (IVd). Pale yellow solid; yield 55%; mp 136–138 °C; δ_H (200 MHz, $CDCl_3$) 7.62 (d, J=8.6 Hz, 2H), 7.49–7.33 (m, 11H); ν_{max} (KBr): 1767, 1711, 1591 (w), 1490 cm^{-1}; m/z (CI, i-Butane) 440 (100, M+2), 438 (80, M^+); UV (MeOH, nm) 363.0, 300.5, 238.5; HPLC: 97.60%. INERTSIL ODS 3V (250×4.6 mm), 0.01 M KH_2PO_4/CH_3CN 30/70, 1.0 mL/min, 238 nm, retention time 32.38 min; ^{13}C NMR (50 MHz, $CDCl_3$): 168.91 (C=O, 2C), 136.38, 135.05, 132.20 (2C), 131.32 (2C), 131.01, 130.70, 130.33, 129.87 (2C), 129.57, 129.30, 129.00 (2C), 128.77 (2C), 127.40 (2C), 121.45; found C, 60.41; H, 2.98; N, 3.24; $C_{22}H_{13}NO_2ClBr$ requires C, 60.23; H, 2.99; N, 3.19%.

4.3.5. 3-(4-Chlorophenyl)-1-(4-methoxyphenyl)-4-phenyl-2,5-dihydro-1*H*-2,5-azoledione (IVe). Yellow solid; yield 71%; mp 146–148 °C; δ_H (200 MHz, $CDCl_3$) 7.52–7.32 (m, 11H), 7.0 (d, J=8.9 Hz, 2H), 3.84 (s, 3H, OMe); ν_{max} (KBr) 1763 (w), 1706, 1588 (w), 1511 cm^{-1}; m/z (CI, i-Butane) 390 (100, M^+); UV (MeOH, nm) 308.5, 235.0; HPLC: 99.18%, INERTSIL ODS 3V (250×4.6 mm), 0.01 M KH_2PO_4/CH_3CN 30/70, 1.0 mL/min, 235 nm, retention time 18.87 min; ^{13}C NMR (50 MHz, $CDCl_3$): 169.54 (C=O, 2C), 159.04, 136.49, 136.16, 134.87, 131.34 (2C), 130.14, 129.90 (2C), 128.93 (2C), 128.70 (2C), 128.26, 127.56 (2C), 126.95, 124.16, 114.40 (2C), 55.43; found C, 70.95; H, 4.14; N, 3.54; $C_{23}H_{16}ClNO_3$ requires C, 70.86; H, 4.14; N, 3.59%.

4.3.6. 1-(2-Chlorophenyl)-3,4-diphenyl-2,5-dihydro-1*H*-2,5-azoledione (IVf). Yellow solid; yield 59%; mp 195–197 °C; δ_H (200 MHz, $CDCl_3$) 7.56–7.39 (m, 14H); ν_{max} (KBr) 1766 (w), 1712, 1483 cm^{-1}; m/z (CI, i-Butane) 360 (100, MH^+); ^{13}C NMR (50 MHz, $CDCl_3$): 168.83 (C=O, 2C), 136.49, 133.20, 130.67, 130.46, 130.36, 130.02 (4C), 129.99 (4C), 129.63, 128.56 (4C), 128.36, 127.64; HPLC: 95%, INERTSIL ODS 3V (250×4.6 mm), 0.01 M KH_2PO_4/CH_3CN 30/70, 1.0 mL/min, 210 nm, retention time 15.24 min; found C, 73.50; H, 3.99; N, 3.81; $C_{22}H_{14}NO_2Cl$ requires C, 73.44; H, 3.92; N, 3.89%.

4.3.7. 1,3,4-Triphenyl-2,5-dihydro-1*H*-2,5-azoledione (IVg). White solid; yield 67%; mp 179–180 °C (lit.[18] 180–181 °C); δ_H (200 MHz, $CDCl_3$) 7.68–6.84 (m, 15H); m/z (CI, i-Butane) 326 (100, MH^+).

4.3.8. 1-(4-Methoxyphenyl)-3,4-diphenyl-2,5-dihydro-1*H*-2,5-azoledione (IVh). Yellow solid; yield 65%; mp 191–192 °C (lit.[9c] 193–194 °C); δ_H (200 MHz, $CDCl_3$) 7.67–6.88 (m, 14H), 3.80 (s, 3H, OCH_3); m/z (CI, i-Butane) 355 (100, MH^+).

4.3.9. Scale-up synthesis for IVc. To a solution of phenacyl amide **IIIc** (16 g, 0.04 mol) in acetonitrile (0.48 L) was added DBU (20 mL, 0.12 mol) dropwise at 0–5 °C. The mixture was stirred at 25 °C for 3 h in the open air and was then poured into cold 3 M HCl (0.84 L) with stirring. The mixture was extracted with ethyl acetate (3×0.20 L) and the combined organic layers were washed with water (2×0.20 L), dried over anhydrous Na_2SO_4 and concentrated under vacuum to afford 10.5 g of the crude product. The crude material obtained was re-crystallized from isopropanol to give 9.6 g (60%, 24.2 mmol) of the desired compound.

4.4. Method B. General procedure for the preparation of V

To a solution of phenacyl amide **III** (1.6 mmol) in 1:1 EtOH–H_2O (40 mL) was added powdered K_2CO_3 (2.4 mmol) and the mixture was stirred at 25 °C for 10 min. The mixture was then heated to 75 °C for 2.5 h. After completion of the reaction the mixture was cooled and poured into water (50 mL) with stirring. The solid precipitate was collected by filtration, washed with water (2×8 mL) and dried under vacuum to afford analytically pure product.

4.4.1. Parallel synthesis of V. Parallel synthesis was carried out using eight reaction flasks simultaneously each containing the appropriate amide **III** and powdered K_2CO_3. To a solution of phenacyl amide **III** (0.8 mmol) in 1:1 EtOH–H_2O (20 mL) was added powder K_2CO_3 (1.2 mmol) and the mixture was stirred at 25 °C for 10–15 min. The mixture was then heated to 75–80 °C for 2.5 h. After completion of the reaction each mixture was cooled and poured into water (25 mL) with stirring. In all cases products appeared as solid, and the filtered solid, after washing with cold hexane (2×5 mL), was analytically pure.

4.4.2. 1-(4-Chlorophenyl)-3,4-diphenyl-2,5-dihydro-1*H*-2-azolone (Va). Off white solid; yield 82%; DSC 178.27 °C; δ_H (200 MHz, $CDCl_3$) 7.80 (d, J=8.8 Hz, 2H), 7.40–7.35 (m, 12H), 4.74 (s, 2H, CH_2); ν_{max} (KBr) 1677, 1595 cm^{-1}; *m/z* (CI, *i*-Butane) 346 (100, MH^+); ^{13}C NMR (50 MHz, DMSO-d_6): 168.76 (C=O), 148.59, 138.14, 132.20, 131.72, 129.55, 129.36 (2C), 128.74 (2C), 128.61 (2C), 128.34 (2C), 128.10 (2C), 127.71 (2C), 127.36, 120.03 (2C), 52.25 (CH_2); HPLC: 95%, HICHROM RPB (250×4.6 mm), 0.01 M KH_2PO_4/CH_3CN 0/40, 5/40, 20/80, 30/80, 35/40, 40/40, 1.0 mL/min, 242 nm, retention time 23.91 min; found C, 76.22; H, 4.69; N, 3.87; $C_{22}H_{16}NOCl$ requires C, 76.41; H, 4.66; N, 4.05%.

4.4.3. 1-(4-Bromophenyl)-3,4-diphenyl-2,5-dihydro-1*H*-2-azolone (Vb). Light yellow solid; yield 87%; DSC 173.31 °C; δ_H (200 MHz, $CDCl_3$) 7.76 (d, J=9.1 Hz, 2H), 7.50 (d, J=9.1 Hz, 2H), 7.41–7.34 (m, 10H), 4.73 (s, 2H, CH_2); ν_{max} (KBr) 1678, 1588 cm^{-1}; *m/z* (CI, *i*-Butane) 392 (100, M+2), 390 (100, M^+); ^{13}C NMR (50 MHz, DMSO-d_6): 168.78 (C=O), 148.60, 138.56, 132.20, 131.72, 131.65, 129.56 (2C), 129.37 (2C), 128.62 (2C), 128.35 (2C), 128.11 (2C), 127.72 (2C), 120.38 (2C), 115.42, 52.20 (CH_2); HPLC: 99%, INERTSIL ODS 3V (150×4.6 mm), 0.01 M KH_2PO_4/CH_3CN 0/40, 10/40, 30/85, 40/85, 45/40, 50/40, 1.0 mL/min, 240 nm, retention time 30.06 min; found C, 67.50; H, 4.34; N, 3.71; $C_{22}H_{16}NOBr$ requires C, 67.71; H, 4.13; N, 3.59%.

4.4.4. 1,4-Di(4-chlorophenyl)-3-phenyl-2,5-dihydro-1*H*-2-azolone (Vc). Yellow solid; yield 85%; DSC 190.90 °C; δ_H (200 MHz, $CDCl_3$) 7.79 (d, J=9.0 Hz, 2H), 7.38–7.25 (m, 11H), 4.70 (s, 2H, CH_2); ν_{max} (KBr) 1668, 1594 cm^{-1}; *m/z* (CI, *i*-Butane) 380 (100, MH^+); ^{13}C NMR (50 MHz, DMSO-d_6): 168.58 (C=O), 147.37, 138.10, 134.25, 132.27, 131.43, 131.11, 129.54 (2C), 129.35 (2C), 128.78 (4C), 128.49 (2C), 128.30, 127.48, 120.09 (2C), 52.21 (CH_2); HPLC: 99%, INERTSIL ODS 3V (150×4.6 mm), 0.01 M KH_2PO_4/CH_3CN 0/70, 5/70, 15/85, 25/85, 30/70, 35/70, 1.0 mL/min, 240 nm, retention time 13.79 min; found C, 69.81; H, 3.99; N, 3.41; $C_{22}H_{15}NOCl_2$ requires C, 69.49; H, 3.98; N, 3.68%.

4.4.5. 1-(4-Bromophenyl)-4-(4-chlorophenyl)-3-phenyl-2,5-dihydro-1*H*-2-azolone (Vd). Off white solid; yield 85%; DSC 170.45 °C; δ_H (200 MHz, $CDCl_3$) 7.75 (d, J=7.3 Hz, 2H), 7.50 (d, J=7.1 Hz, 2H), 7.48–7.25 (m, 9H), 4.70 (s, 2H, CH_2); ν_{max} (KBr) 1669, 1590 cm^{-1}; *m/z* (CI, *i*-Butane) 426 (100, M+2), 424 (80, M^+); ^{13}C NMR (50 MHz, DMSO-d_6): 168.59 (C=O), 147.21, 138.49, 134.49, 134.30, 132.28, 131.66, 131.40, 131.04, 129.50 (2C), 129.36 (2C), 128.75 (2C), 128.45 (2C), 128.29, 120.32 (2C), 115.54, 52.11 (CH_2); HPLC: 99%, INERTSIL ODS 3V (150×4.6 mm), 0.01 M KH_2PO_4/CH_3CN 0/40, 10/40, 30/85, 40/85, 45/40, 50/40, 1.0 mL/min, 240 nm, retention time 32.73 min; found C, 62.47; H, 3.59; N, 3.01; $C_{22}H_{15}BrNOCl$ requires C, 62.21; H, 3.56; N, 3.30%.

4.4.6. 4-(4-Chlorophenyl)-1-(4-methoxyphenyl)-3-phenyl-2,5-dihydro-1*H*-2-azolone (Ve). White solid; yield 89%; DSC 154.86 °C; δ_H (200 MHz, $CDCl_3$) 7.70 (d, J=8.9 Hz, 2H), 7.43–7.28 (m, 9H), 6.94 (d, J=8.9 Hz, 2H), 4.69 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3); ν_{max} (KBr) 1681, 1594 cm^{-1}; *m/z* (CI, *i*-Butane) 376 (100, MH^+); ^{13}C NMR (50 MHz, DMSO-d_6): 168.06 (C=O), 155.72, 146.64, 134.04, 132.43, 131.69, 131.35, 129.47 (2C), 129.37 (2C), 128.73 (2C), 128.40 (2C), 128.17 (2C), 120.53 (2C), 114.05 (2C), 55.18 (OCH_3), 52.51 (CH_2); HPLC: 98%, INERTSIL ODS 3V (250×4.6 mm), 0.01 M KH_2PO_4/CH_3CN 0/40. 10/40, 30/85, 40/85, 45/40, 50/40, 1.0 mL/min, 240 nm, retention time 28.38 min; found C, 73.54; H, 4.99; N, 3.61; $C_{23}H_{18}NO_2Cl$ requires C, 73.50; H, 4.83; N, 3.73%.

4.4.7. 1-(4-Bromophenyl)-4-(4-methylphenyl)-3-phenyl-2,5-dihydro-1*H*-2-azolone (Vf). Yellow solid; yield 97%; DSC 167.72 °C; δ_H (200 MHz, $CDCl_3$) 7.76 (d, J=8.8 Hz, 2H), 7.50 (d, J=8.8 Hz, 2H), 7.40–7.22 (m, 7H), 7.12 (d, J=7.8 Hz, 2H), 4.71 (s, 2H, CH_2), 2.35 (s, 3H, CH_3); ν_{max} (KBr) 1680, 1598 cm^{-1}; *m/z* (CI, *i*-Butane) 404 (100, MH^+), 406 (100, M+2); ^{13}C NMR (50 MHz, DMSO-d_6): 168.91 (C=O), 148.56, 139.49, 138.62, 131.93, 131.66 (2C), 131.03, 129.40 (2C), 129.26, 129.22 (2C), 128.39 (2C), 128.06, 127.64 (2C), 120.34 (2C), 115.35, 52.10 (CH_2), 20.86 (CH_3); HPLC: 99%, INERTSIL ODS 3V (150×4.6 mm), 0.01 M KH_2PO_4/CH_3CN 0/75, 10/75, 15/80, 25/80, 30/75, 35/75, 1.0 mL/min, 210 nm, retention time 13.30 min; found C, 68.54; H, 4.39; N, 3.31; $C_{23}H_{18}NOBr$ requires C, 68.33; H, 4.49; N, 3.46%.

4.4.8. 1-(4-Chlorophenyl)-4-(4-fluorophenyl)-3-phenyl-2,5-dihydro-1*H*-2-azolone (Vg). Light yellow solid; yield 94%; DSC 163.04 °C; δ_H (200 MHz, $CDCl_3$) 7.80 (d, J=9.0 Hz, 2H), 7.39–7.30 (m, 9H), 7.01 (t, J=8.5 Hz, 2H), 4.71 (s, 2H, CH_2); ν_{max} (KBr) 1668, 1603 cm^{-1}; *m/z* (CI, *i*-Butane) 364 (100, MH^+); ^{13}C NMR (50 MHz, DMSO-d_6): 168.64 (C=O), 165.01, 160.08, 147.34, 138.07, 131.62, 131.54, 130.09, 129.92, 129.34, 129.02, 128.70, 128.60, 128.39 (2C), 128.15, 127.37, 119.86 (2C), 115.88, 115.45, 52.21 (CH_2); HPLC: 99%, INERTSIL ODS 3V (250×4.6 mm), 0.01 M KH_2PO_4/CH_3CN 0/75, 10/75, 15/80, 25/80, 30/75, 35/75, 1.0 mL/min, 210 nm, retention time 9.04 min; found C, 72.84; H, 3.98; N, 3.79; $C_{22}H_{15}NOClF$ requires C, 72.63; H, 4.16; N, 3.85%.

4.4.9. 1-(4-Methoxyphenyl)-3,4-diphenyl-2,5-dihydro-1*H*-2-azolone (Vh). White solid; yield 85%; DSC 139.94 °C; δ_H (200 MHz, $CDCl_3$) 7.73 (d, J=8.9 Hz, 2H), 7.42–7.33 (m, 10H), 6.94 (d, J=8.9 Hz, 2H), 4.73 (s, 2H, CH_2), 3.82 (s, 3H, OMe); ν_{max} (KBr) 1689 cm^{-1}; *m/z* (CI, *i*-Butane) 342 (80, MH^+), 341 (100); ^{13}C NMR (50 MHz, DMSO-d_6): 168.0 (C=O), 156.28, 146.57, 132.77, 132.61, 131.63, 129.59 (2C), 129.34 (2C), 128.72 (2C), 128.38 (2C), 128.18 (2C), 127.62 (2C), 120.64 (2C), 114.28, 55.44 (OCH_3), 53.0 (CH_2); HPLC: 97%, INERTSIL ODS 3V

(250×4.6 mm), 0.01 M KH_2PO_4/CH_3CN 0/60, 5/60, 15/80, 25/80, 30/60, 35/60, 1.0 mL/min, 234 nm, retention time 16.54 min; found C, 80.81; H, 5.68; N, 4.29; $C_{23}H_{19}NO_2$ requires C, 80.92; H, 5.61; N, 4.10%.

4.4.10. Preparation of 2-(4-fluoroanilino)-1-(3-methyl-4-methylsulfonylphenyl)-1-ethanone (4). To a mixture of *p*-fluoroaniline (1.14 g, 10.3 mmol) and $NaHCO_3$ (0.87 g, 10.3 mmol) in ethanol (25 mL) was added 2′-bromo-3-methyl-4-methylsulfonyl acetophenone (3 g, 10.3 mmol) under a nitrogen atmosphere at 25 °C. The mixture was stirred vigorously for 3.5 h then diluted with water (100 mL). The solid precipitated was filtered, washed with water (2×25 mL) and petroleum ether (2×10 mL) then dried under vacuum to give the title compound (2.9 g, 88%). Brown solid; mp 156–158 °C; δ_H (200 MHz, $CDCl_3$) 8.19 (d, *J*=8.7 Hz, 1H), 7.98–7.95 (m, 2H), 6.98–6.89 (m, 2H), 6.68–6.62 (m, 2H), 4.60 (s, 2H, CH_2), 3.12 (s, 3H, SO_2CH_3), 2.81 (s, 3H, CH_3); MS (CI, *i*-Butane) *m/z* 322 (M+1, 100); ^{13}C NMR (50 MHz, DMSO-d_6): 196.57 (C═O), 156.96, 144.73, 142.73, 138.84, 138.05, 131.97, 129.02, 125.95, 115.42, 114.97, 113.40, 113.26, 50.91 (CH_2), 43.14 (CH_3SO_2), 19.67(CH_3); HPLC: 98%, INERTSIL ODS 3V (250×4.6 mm), 0.01 M KH_2PO_4/CH_3CN 0/30, 5/30, 20/80, 30/80, 35/30, 40/30, 1.0 mL/min, 244 nm, retention time 16.3 min; found C, 59.85; H, 5.00; N, 4.30; $C_{16}H_{16}FNO_3S$ requires C, 59.80; H, 5.02; N, 4.36%.

4.4.11. Preparation of N*1*-(4-fluorophenyl)-N*1*-[2-(3-methyl-4-methylsulfonylphenyl)-2-oxoethyl]-2-phenylacetamide (5). To a solution of 2-(4-fluoroanilino)-1-(3-methyl-4-methylsulfonylphenyl)-1-ethanone (1.5 g, 4.67 mmol) in anhydrous THF (15 mL) was added phenacylchloride (0.72 g, 0.62 mmol) very slowly under nitrogen atmosphere at 25 °C. The mixture was stirred for 2 h and diluted with water (25 mL). The solid separated was filtered, washed with water (2×15 mL) followed by petroleum ether (2×5 mL) and dried under vacuum to give 1.6 g of the title compound in 78% yield, Low melting yellow solid; δ_H (200 MHz, $CDCl_3$) 8.12 (d, *J*=8.7 Hz, 1H), 7.89–7.86 (m, 2H), 7.32–7.23 (m, 5H), 7.10–7.01 (m, 4H), 5.03 (s, 2H, CH_2), 3.56 (s, 2H, CH_2), 3.08 (s, 3H, SO_2CH_3), 2.75 (s, 3H, CH_3); ν_{max} (KBr) 1698, 1660 cm^{-1}; MS (CI, *i*-Butane) *m/z* 440 (M+1, 100); ^{13}C NMR (50 MHz, DMSO-d_6): 193.03 (C═O), 171.32 (C═O), 164.51, 159.56, 146.66, 142.62, 138.90, 138.52, 138.29, 134.61, 131.96, 130.41, 130.24, 129.67, 128.87, 128.30, 126.67, 125.84, 116.70, 116.25, 56.60 (CH_2), 43.38 (CH_3SO_2), 40.56 (CH_2), 20.18 (CH_3); HPLC: 98%, Hichrom RPB (250×4.6 mm), 0.01 M KH_2PO_4/CH_3CN 0/40, 5/40, 20/80, 30/80, 35/40, 40/40, 1.0 mL/min, 243 nm, retention time 17.6 min; found C, 65.50; H, 5.01; N, 3.49; $C_{24}H_{22}$ FNO_4S requires C, 65.59; H, 5.05; N, 3.19%.

4.4.12. Preparation of 1-(4-fluorophenyl)-3-(3-methyl-4-methylsulfonylphenyl)-4-phenyl-2,5-dihydro-1*H*-2,5-azoledione (6). The title compound was prepared in 63% yield from *N*1-(4-fluorophenyl)-*N*1-[2-(3-methyl-4-methylsulfonylphenyl)-2-oxoethyl]-2-phenylacetamide (0.79 g, 1.58 mmol) using DBU (0.48 g, 1.58 mmol) according to the procedure described above (Method A). Light orange solid; mp>200 °C; δ_H (200 MHz, $CDCl_3$) 7.8–7.6 (m, 3H), 7.38–7.01 (m, 9H), 3.1 (s, 3H, SO_2CH_3), 2.58 (s, 3H, CH_3); IR (KBr, cm^{-1}) 1713, 1600, 1511; MS (CI, *i*-Butane) *m/z* 436 (M^+, 100); ^{13}C NMR (50 MHz, $CDCl_3$): 168.0 (C═O, 2C), 139.75, 138.11, 133.99, 133.85, 130.82, 130.82, 130.01 (2C), 129.54, 128.90 (2C), 128.05 (2C), 127.86, 124.46, 123.96, 119.09, 116.42, 116.12, 115.96, 43.61 (CH_3SO_2), 20.68 (CH_3); found C, 66.09; H, 4.18; N, 3.39; $C_{24}H_{18}FNO_4S$ requires C, 66.19; H, 4.17; N, 3.22%.

4.4.13. Preparation of 1-(4-fluorophenyl)-4-(3-methyl-4-methylsulfonylphenyl)-3-phenyl-2,5-dihydro-1*H*-2-azolone (7). The title compound was prepared in 69% yield from *N*1-(4-fluorophenyl)-*N*1-[2-(3-methyl-4-methylsulfonylphenyl)-2-oxoethyl]-2-phenylacetamide (1 g, 2.27 mmol) using K_2CO_3 (7.72 g, 3.40 mmol) in 1:1 EtOH–H_2O according to the procedure described above (Method A). White powder; mp 207–209 °C; δ_H (200 MHz, $CDCl_3$) 7.96 (d, *J*=8.4 Hz, 1H), 7.83–7.76 (m, 2H), 7.39–7.07 (m, 9H), 4.74 (s, 2H, CH_2), 3.09 (s, 3H, SO_2CH_3), 2.63 (s, 3H, CH_3); IR (KBr, cm^{-1}) 1680; MS (CI, *i*-Butane) *m/z* 421 (M^+, 100); ^{13}C NMR (50 MHz, DMSO-d_6): 168.64 (C═O), 161.9, 157.06, 144.33, 139.26, 138.21, 137.92, 135.94, 135.10, 131.63, 129.80, 129.44 (2C), 128.96, 128.70 (2C), 125.92, 120.75, 120.59, 116.13, 115.69, 52.70 (CH_2), 43.63 (CH_3SO_2), 20.29 (CH_3); HPLC: 98%, INERTSIL ODS 3V (250×4.6 mm), 0.01 M KH_2PO_4/CH_3CN 0/50, 5/50, 20/80, 30/80, 35/50, 40/50, 1.0 mL/min, 233 nm, retention time 17.6 min; found C, 68.29; H, 4.69; N, 3.49; $C_{24}H_{20}FNO_3S$ requires C, 68.39; H, 4.78; N, 3.32%.

Acknowledgements

The authors gratefully acknowledge Dr. A. Venkateswarlu, Dr. R. Rajagopalan and Professor J. Iqbal for their constant encouragement and the Analytical Department especially Dr. J. Moses Babu and Mrs. Sivalakshmi Devi for spectral support.

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Genome-inspired search for new antibiotics. Isolation and structure determination of new 28-membered polyketide macrolactones, halstoctacosanolides A and B, from *Streptomyces halstedii* HC34

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Received 8 January 2004; revised 20 February 2004; accepted 8 March 2004

Abstract—During the search for polyketide synthase (PKS) in the genome of *Streptomyces halstedii* HC34, we found clustered new genes which appeared to encode typical Type 1 PKSs beyond the cluster harboring the genes for the biosynthesis of antitumor antibiotic vicenistatin. The deduced domain configuration of these putative PKS genes allowed to predict a corresponding partial structure of polyketide, which was in turn materialized by isolation of new polyketide macrolactone halstoctacosanolides A and B from the fermentation broth of *S. halstedii* HC34. The structures of these metabolites were determined by spectroscopic means to have a novel 28-membered macrolactone structure. The partial structure deduced from the genetic data was completely compatible to the structures of halstoctacosanolides A and B. This success clearly demonstrates the present new approach of genome-inspired search for new antibiotics promising. Halstoctacosanolides A and B showed moderate antimicrobial activity against several microorganisms.

1. Introduction

Over the last several decades, a lot of antibiotics were isolated from various *Streptomecetes* and other microorganisms, and importance of antibiotics is well-recognized throughout the medicinal and agricultural fields.[1] Particularly, serious issue of resistance of pathogenic bacteria against commonly used antibiotics urged to develop more effective drugs of natural and synthetic origin. In addition to the conventional bioassay-guided approach including high-throughput screening, various methodologies particularly employing microbial genetic technology have emerged. Among those genome-driven approaches, combinatorial biosynthesis has recently been gaining relevance for generation of new structures. This technology utilizes combination of pertinent genes involved in the already known but different biosynthetic pathways to produce novel or modified metabolites.[2] Another approach to access to interesting natural products concerns to uncultured microbes, which involves initial isolation of DNA directly from soil (environmental DNA, eDNA) using PCR and its subsequent expression in the established expression systems. Although a successful achievement has recently been reported by this approach,[3] there are still some difficulties particularly in dealing with functionally unknown DNA of unknown origin. The third approach relies on the genetic information compiled by extensive genome projects.

Streptomyces bacteria and closely related genera are still extraordinary rich sources to be searched for a wide variety of secondary metabolites as lead to new drug candidates. Completion of the genome projects of *Streptomyces coelicolor*[4] and *Streptomyces avermitilis*[5] revealed the presence of a large number of gene clusters harboring the biosynthetic enzymes of secondary metabolites in the genome of these strains. Among these clusters are the biosynthetic genes for the previously identified secondary metabolites as well as those for the unidentified products, which have yet to be discovered from nature. It should be pointed out that, according to a mathematical modeling, only 3% of all biologically active metabolites produced by *Streptomycetes* have been identified so far.[6] A large number of antibiotics from this genus still await to be discovered.

In the last decade, general methods and appropriate probes for cloning of antibiotic biosynthetic gene clusters in

Keywords: 28-Membered macrolactone; Isolation; Structure determination; Polyketide synthase.
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doi:10.1016/j.tet.2004.03.027

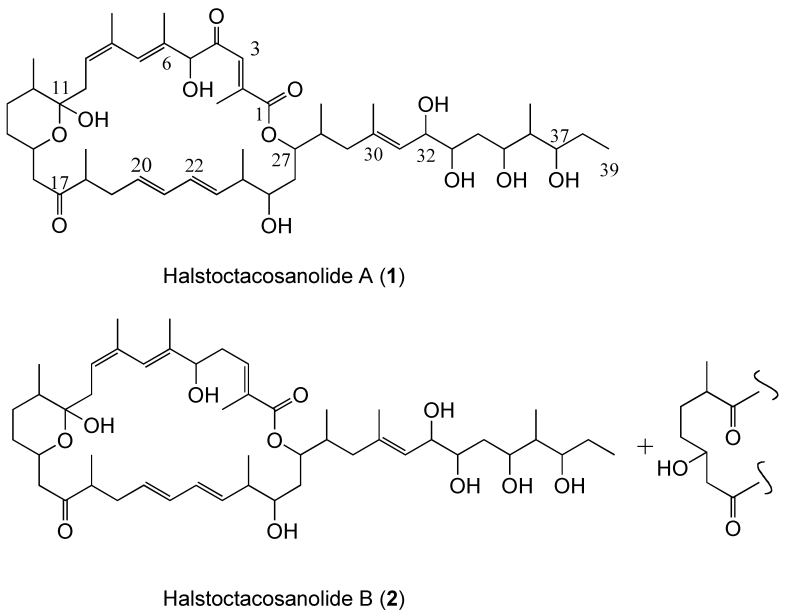

Figure 1. Structures of halstoctacosanolides A (**1**) and B (**2**).

Streptomycetes and related microorganisms have been well developed.[7] Recent advances in these area are extremely helpful us to understand whole biosynthetic scenarios of various biologically important natural products, especially of polyketides, and large knowledge bases successfully compiled to date are apparently useful for correlating the DNA base sequences to the polyketide products.[8] It is thus possible nowadays to estimate chemical natures of a starter- and extender units of a polyketide directly from the DNA sequence data of the corresponding biosynthetic gene cluster without knowing the actual product.[8,9] Therefore, an emerging rational approach to search for new antibiotics, particularly in *Streptomycetes*, may rely apparently on the accumulated DNA sequence information.

Streptomyces halstedii HC34 produces an antitumor antibiotic vicenistatin, the structure of which is comprised of a macrocyclic 20-membered lactam aglycon and an amino-sugar vicenisamine.[10,11] We reported recently that the extender units of the aglycon are derived from acetate and propionate in a standard polyketide biosynthetic pathway, whereas the aglycon precursor is primed by an unusual

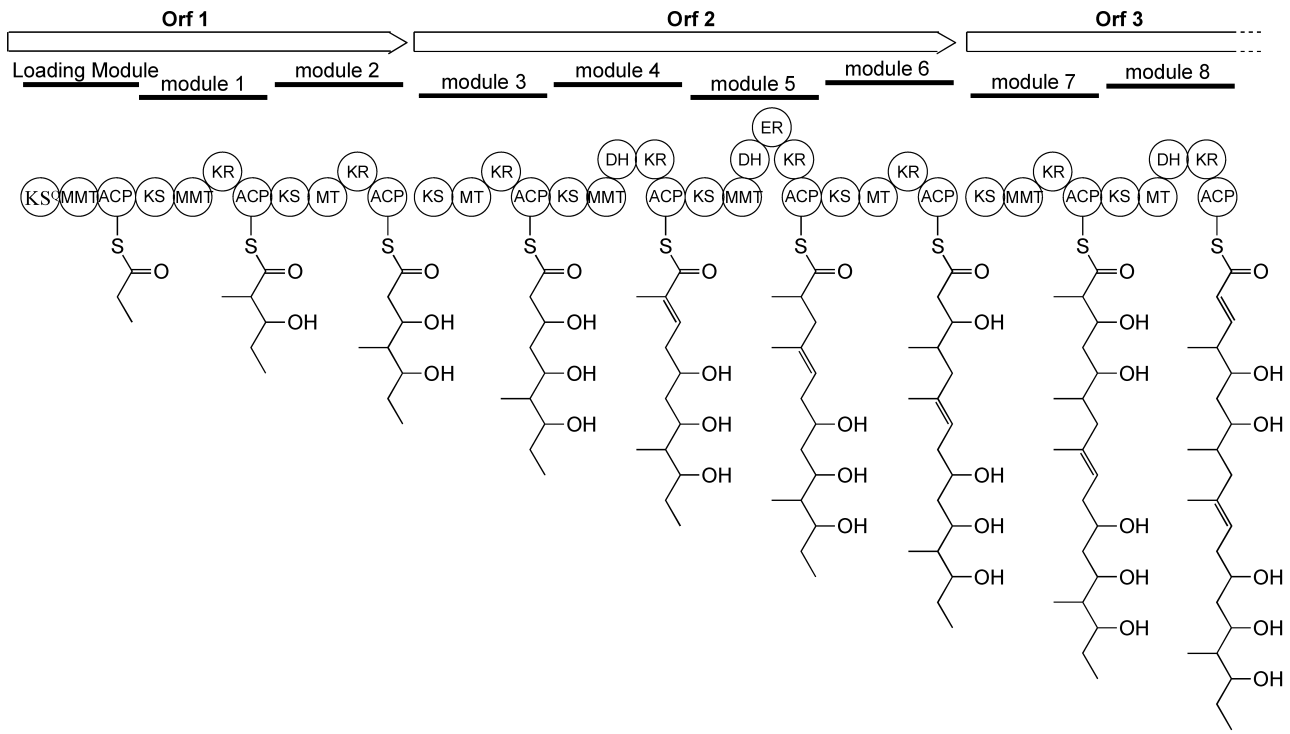

Figure 2. The PKS genes from *S. halstedii* HC34. Each circle represents an enzymatic domain in the PKS multifunctional proteins. MM, malonyl transferase; MMT, methylmalonyl transferase; ACP, acyl carrier protein; KS, ketoacyl-ACP synthase; KS^{Q}, inactive KS; KR, ketoacyl ACP reductase; DH, hydroxylthioester dehydratase; ER, enoyl reductase.

starter unit derived from 2-methylaspartate.[12–14] More recently, we have described cloning, sequencing and functional analysis of the biosynthetic gene cluster for vicenistatin.[15]

During the search for the polyketide synthase (PKS) genes in *S. halstedii* HC34, we found that this microorganism possesses a series of typical Type 1 PKS genes which are different from those responsible for the vicenistatin biosynthesis. These findings and the deduced sequence information strongly indicated possible production of so-far unidentified polyketide metabolite(s), which prompted us to start isolation and chemical studies. In this paper, we describe the isolation and structure determination of two new 28-membered macrolactones, which we named halstoctacosanolides A and B (Fig. 1).

2. Results and discussion

Based on the extensive analysis of the cosmid library of *S. halstedii* HC34,[15] we were able to identify a portion of a biosynthesis gene cluster of new polyketide (total ca. 45 kbp so far). These genes are composed of three open reading frames which appear to encode typical Type 1 PKS containing nine modules. From the homology search for the deduced amino acid sequences of these genes (DDBJ; accession No. AB158460), the starter unit and the extender units as well as the oxidation states of each unit could be clearly predicted. It was thus anticipated that these PKSs catalyze the biosynthesis of a polyketide chain containing a partial structure as shown in Figures 2 and 3. The most important point at this stage was that an anticipated molecule having this partial structure was shown to be unprecedented. Thus, we started its isolation and chemical studies.

To obtain new polyketide(s) from the fermentation broth of *S. halstedii* HC34, the same culture conditions as those for vicenistatin were appropriate.[10] After culture for 3 days, both supernatant and mycelium cake were separately extracted with ethyl acetate. After combining and concentrating the extracts, two new compounds were isolated and purified to homogeneity through repeated chromatography. We named these compounds as halstoctacosanolides A (**1**) and B (**2**), the structures of which were determined as follows.

The physico-chemical properties of halstoctacosanolide A (**1**) are summarized in Table 1. The molecular formula of **1** was established as $C_{48}H_{76}O_{12}$ on the basis of HRFAB-MS data. In the IR spectrum, **1** showed strong bands at 3410 and 1710 cm^{-1}, which revealed the presence of hydroxyl and carbonyl groups. The ^1H NMR spectrum in $CDCl_3$ (Fig. 4) indicated the presence of 10 methyl and 8 olefinic protons. Further, the ^{13}C NMR spectrum showed 48 carbon signals including two ketonic (δ 198.9, 215.4), an ester (δ 165.8), an

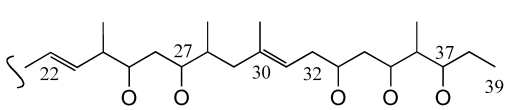

Figure 3. Partial structure deduced from genetic analysis.

Table 1. Physico-chemical properties of halstoctacosanolides A (**1**) and B (**2**)

	1	**2**
Appearance	Colorless powder	Colorless powder
Mp	79–80 °C	79–80 °C
$[\alpha]_D^{22}$	+43.2 (*c* 1.0, $CHCl_3$)	−22.3 (*c* 1.0, $CHCl_3$)
Molecular formula	$C_{48}H_{76}O_{12}$	$C_{48}H_{78}O_{11}$
HRFAB-MS		
Calcd: (*m/z*)	867.5234 $(M+Na)^+$	853.5442 $(M+Na)^+$
Found: (*m/z*)	867.5247	853.5393
UV λ_{max} (ε) (in MeOH)	233 nm (64,000)	226 nm (48,000)
IR ν (KBr); cm^{-1}	3410 (br), 2960, 2930, 1710	3420 (br), 2960, 2930, 1700
Elemental Anal.		
Calcd	C: 68.22; H: 9.06	C: 69.37; H: 9.46
Found	C: 68.17; H: 8.99	C: 69.12; H: 9.76

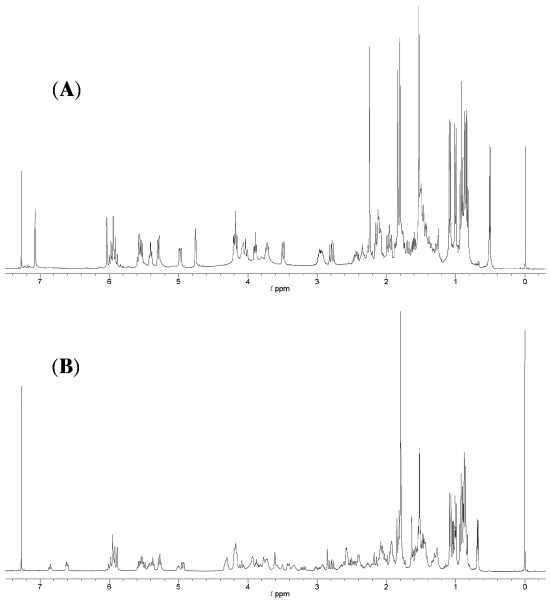

Figure 4. ^1H NMR spectra (400 MHz, $CDCl_3$) of (A); halstoctacosanolide A (**1**) and (B) halstoctacosanolide B (**2**).

acetal (δ 98.9), 12 olefinic, and 8 hydroxylated carbons. All carbon signals were divided into 10 methyl, 9 methylene, 21 methine, and 8 quaternary carbons by DEPT experiments as indicated in Table 2.

By ^1H–^1H COSY and HMBC experiments, one polyketide chain composed of nonadeca-ketides was established as shown in Figure 5. Since the long range coupling between 27-H and C-1 ester carbon was clearly detected in a HMBC experiment, **1** was found to have a 28-membered macrolactone structure. The geometries of the double-bonds at C-20 and C-22 were determined to be *E* by their spin–spin coupling constants ($J_{20,21}$=14.6 Hz and $J_{22,23}$=14.9 Hz). The other double-bond geometries were confirmed by NOE experiments. While essentially no NOE was observed between 3-H and 2-CH_3, the NOE between 2-CH_3 and 5-H was clearly detected, therefore the double bond at C-2 was determined to be *E*. Furthermore, the NOEs between 6-CH_3 and 8-CH_3, and 8-CH_3 and 9-H were observed, thus the double-bond geometries at C-6 is to be *E* and at C-8 to be *Z*. The double bond at C-30 was proved to be *E* by observation of the NOE between 32-H and 30-CH_3.

Table 2. NMR data of **1**, **3** and **4** in $CDCl_3$

No.	Halstoctaisaconolide A (**1**)		Compound **3**		Compound **4**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	165.79 (s)		166.51 (s)		167.74 (s)	
2	145.50 (s)		144.94 (s)		129.38 (s)	
3	126.70 (d)	7.07 (d, 1.6)	126.68 (d)	7.14 (d, 1.4)	136.93 (d)	6.69 (t, 6.5)
4	198.87 (s)		199.82 (s)		34.80 (t)	2.62/2.54 (m)
5	82.38 (d)	4.75 (d, 4.0)	83.16 (d)	4.67 (s)	74.81 (d)	4.30 (t, 5.9)
6	133.71 (s)		133.27 (s)		138.49 (s)	
7	129.40 (d)	6.04 (brs)	130.13 (d)	6.12 (s)	125.23 (d)	5.94 (s)
8	134.92 (s)		132.42 (s)		133.25 (s)	
9	122.56 (d)	5.41 (dd, 6.5, 7.4)	124.50 (d)	5.37 (m)	122.66 (d)	5.29 (t, 6.6)
10	39.91 (t)	1.89/1.97 (m)	34.05 (t)	1.98/2.23 (m)	33.58 (t)	2.03/2.28 (m)
11	98.92 (s)		101.01 (s)		101.21 (s)	
12	35.71 (d)	1.35 (m)	36.34 (d)	1.51 (m)	35.93 (d)	1.64 (m)
13	27.42 (t)	1.38/1.51 (m)	27.16 (t)	1.38/1.60 (m)	27.16 (t)	1.41/1.68 (m)
14	38.78 (t)	1.27/1.52 (m)	31.77 (t)	1.39/1.57 (m)	31.74 (t)	1.29/1.58 (m)
15	67.51 (d)	4.04 (brt, 10.7)	67.63 (d)	4.05 (brt, 11.0)	67.69 (d)	4.03 (t, 11.1)
16	48.15 (t)	2.13 (m)	47.82 (t)	2.23 (m)	47.80 (t)	2.25 (m)
	2.89 (dd, 10.5, 13.5)		2.78 (dd, 10.8, 14.4)		2.76 (dd, 10.4, 14.3)	
17	215.38 (s)		212.98 (s)		213.19 (s)	
18	48.05 (d)	2.97 (ddq, 10.0, 13.8, 7.2)	47.77 (d)	2.72 (dq, 13.4, 6.8)	47.80 (d)	2.65 (m)
19	34.75 (t)	2.15 (m)	34.92 (t)	2.18 (m)	34.98 (t)	1.97/2.57 (m)
	2.44 (ddd, 7.8, 10.0, 13.8)		2.53 (quintet, 7.1)			
20	130.68 (d)	5.57 (m)	129.79 (d)	5.56 (quintet, 7.1)	129.68 (d)	5.54 (dt, 14.2, 7.3)
21	131.47 (d)	5.97 (dd, 10.2, 14.6)	132.12 (d)	5.98 (m)	132.55 (d)	6.02 (m)
22	129.36 (d)	5.92 (ddd, 0.7, 10.2, 14.9)	130.39 (d)	5.95 (m)	130.10 (d)	5.98 (m)
23	136.22 (d)	5.55 (m)	135.20 (d)	5.34 (m)	135.63 (d)	5.37 (dd, 8.2, 14.7)
24	41.14 (d)	2.13 (m)	43.41 (d)	2.03 (m)	43.60 (d)	2.08 (m)
25	68.72 (d)	3.50 (d, 11.1)	71.88 (d)	3.19 (dd, 2.2, 9.8)	72.31 (d)	3.14 (t, 8.4)
26	31.98 (t)	1.46 (m)	33.62 (t)	1.49/1.63 (m)	33.74 (t)	1.43/1.64 (m)
	1.71 (dd, 11.2, 13.7)					
27	75.73 (d)	4.99 (dd, 2.5, 10.6)	75.72 (d)	5.12 (dd, 2.2, 9.8)	73.94 (d)	5.01 (dd, 2.4, 10.5)
28	31.68 (d)	2.35 (m)	32.92 (d)	2.16 (m)	33.00 (d)	2.16 (m)
29	44.93 (t)	1.95/2.11 (m)	44.69 (t)	1.98/2.07 (m)	44.66 (t)	2.00/2.06 (m)
30	136.15 (s)		137.30 (s)		137.69 (s)	
31	128.13 (d)	5.29 (d, 8.8)	127.47 (d)	5.27 (d, 9.0)	126.99 (d)	5.24 (d, 8.5)
32	72.25 (d)	4.18 (dd, 6.0, 8.8)	72.20 (d)	4.18 (m)	72.31 (d)	4.17 (m)
33	71.44 (d)	3.73 (ddd, 2.2, 6.0, 9.6)	71.50 (d)	3.72 (dt, 1.8, 8.1)	71.61 (d)	3.70 (t, 8.2)
34	32.34 (t)	1.48/1.79 (m)	38.19 (t)	1.41/1.86 (m)	38.13 (t)	1.43/1.83 (m)
35	73.77 (d)	4.19 (m)	73.52 (d)	4.17 (m)	73.29 (d)	4.13 (m)
36	40.63 (d)	1.51 (m)	41.10 (d)	1.49 (m)	41.19 (d)	1.50 (m)
37	79.08 (d)	3.90 (dt, 1.3, 6.9)	78.41 (d)	3.81 (dt 1.5, 6.1)	77.83 (d)	3.78 (t, 6.6)
38	28.08 (t)	1.47/1.59 (m)	28.13 (t)	1.43/1.53 (m)	27.88 (t)	1.40/1.55 (m)
39	10.44 (q)	0.92 (t, 7.4)	10.40 (q)	0.91 (t, 7.4)	10.48 (q)	0.90 (t, 7.4)
2-CH_3	14.95 (q)	2.25 (d, 1.4)	15.03 (q)	2.26 (d, 1.4)	12.75 (q)	1.82 (s)
6-CH_3	14.74 (q)	1.54 (d, 1.1)	14.98 (q)	1.55 (s)	14.60 (q)	1.54 (s)
8-CH_3	24.31 (q)	1.84 (s)	23.86 (q)	1.79 (s)	24.22 (q)	1.73 (s)
12-CH_3	16.13 (q)	0.52 (d, 6.7)	16.02 (q)	0.67 (d, 6.3)	16.26 (q)	0.80 (d, 6.1)
18-CH_3	16.46 (q)	1.09 (d, 7.2)	15.01 (q)	1.06 (d, 6.8)	14.62 (q)	1.04 (d, 4.9)
24-CH_3	11.20 (q)	1.01 (d, 7.0)	15.24 (q)	1.01 (d, 6.9)	16.15 (q)	1.02 (d, 5.1)
28-CH_3	14.23 (q)	0.87 (d, 6.9)	14.62 (q)	0.89 (d, 6.9)	14.62 (q)	0.85 (d, 6.8)
30-CH_3	16.28 (q)	1.81 (s)	16.41 (q)	1.79 (s)	16.57 (q)	1.77 (s)
36-CH_3	4.23 (q)	0.84 (d, 7.1)	4.63 (q)	0.82 (d, 7.1)	5.00 (q)	0.82 (d, 7.3)
11-OCH_3			47.63(q)	3.02 (s)	47.42 (q)	3.06 (s)

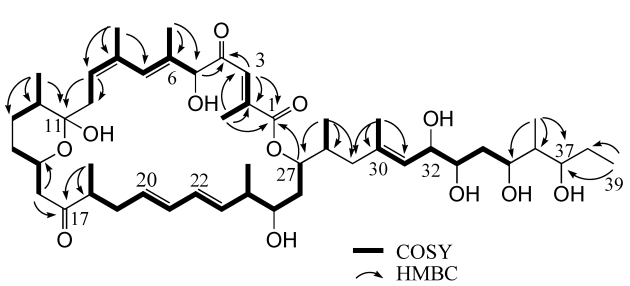

Figure 5. $^1H-^1H$ COSY and HMBC correlations of halstoctacosanolide A (**1**).

The remaining structure to be determined was the position of the oxygen functionalities forming a hemi-acetal ring. However, no direct information was available from the NMR spectra of **1** so far analyzed. To circumvent this difficulty, **1** was derivatized into its methyl acetal **3**. Thus, treatment of **1** with pyridinium *p*-toluenesulfonate in methanol gave a methyl acetal derivative **3** as a single product (Fig. 6). In **3**, an NOE between the introduced methoxy protons (δ 3.02) and 15-H was clearly observed, and the presence of a 6-membered cyclic acetal moiety in **3** was thus established as shown in Figure 6. All the data discussed above allowed us to deduce the planar structure of **1** as shown in Figure 1. The resulting structure of **1** strongly suggested that **1** is biosynthesized through rather regular

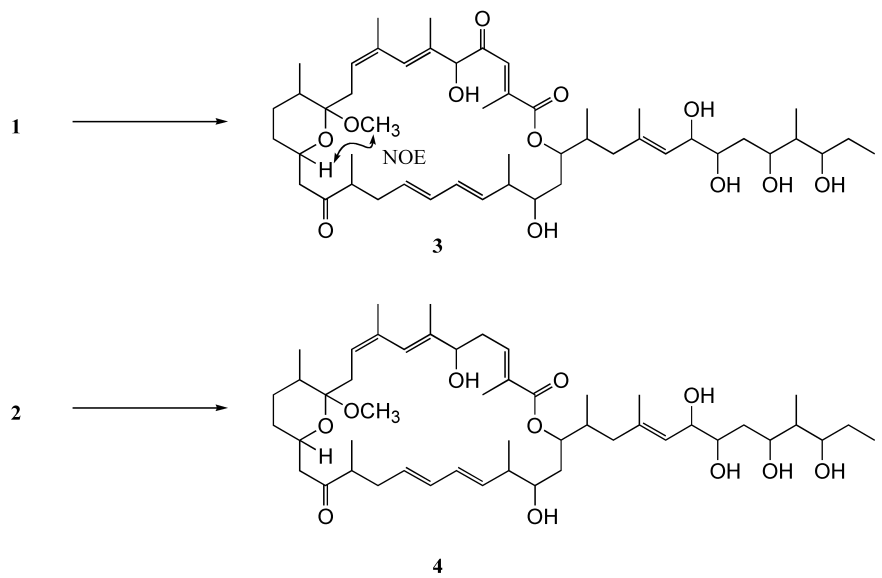

Figure 6. Derivatization of **1** and **2** into methyl acetal derivatives **3** and **4**, respectively.

polyketide pathway catalyzed by Type 1 PKSs, except for two features. The oxygen functionalities at the C-4 and C-32 positions of **1** appear to be irrelevant to the simple PKS pathway and may probably be introduced by post-PKS modifications. The partial structure (Figs. 2 and 3) deduced from the genetic data of new Type 1 PKSs is apparently incorporated into the structure of **1** (C-22 to C-38).

The physico-chemical data of **2** are also shown in Table 1. The molecular formula of **2** was established as $C_{48}H_{78}O_{11}$ on the basis of HRFAB-MS. The molecular formula suggested that the structure of **2** was a deoxygenated derivative of **1**. However, the structure determination of **2** was not straightforward because **2** was obtained as an inseparable mixture of two components. Although these two compounds could be separated by HPLC, each fraction spontaneously turned out to become similar mixture again. Thus, **2** was suggested to exist as an equilibrium mixture of two isomers. As was anticipated, the ^{1}H and ^{13}C NMR spectra were extremely complex. In the ^{1}H NMR spectrum (Fig. 4), the paired signals were observed and the ratio of each signal changed depending on the solvent (1:2 in $CDCl_3$ and 1:5 in CD_3OD), which well supported the state of this compound as an equilibrium mixture of two isomers in solution. In the ^{13}C NMR spectrum, more than 90 signals were observed. However, a key clue to solve this problem came from the observation that three ketonic carbon signals (δ 213.4, 214.7, 215.2) and one acetal signal (δ 99.1) were observed in the ^{13}C NMR spectrum. Thus, **2** appeared to exist in equilibrium of keto-hemiacetal isomerization. Since further structural analysis of natural **2** seemed to be difficult, methyl acetalization was performed again as in the case of **1**. The resulting methyl acetal derivative **4** (Fig. 6) showed rather simple spectra in various NMR experiments, and the spectra turned out to be quite similar to those of **3** as summarized in Table 2. Straightforward analysis of the NMR spectra of **4** including HMBC and NOE experiments (data not shown) indicated that **4** was a deoxo-derivative of **3** at C-4 as shown in Figure 6. Therefore, the natural **2** is a mixture of the ketonic and hemi-acetal forms as shown in Figure 1.

The planar structures of **1** and **2** were determined to be nonadeca-ketides of same biosynthetic origin having a 28-membered macrocyclic lactone ring as shown in Figure 1. A few examples containing a 28-membered lactone ring structure are known in nature as classical polyene antibiotics.[16–27] Halstoctacosanolides are the first examples containing such a 28-membered lactone of non-polyene antibiotics. Stereochemical analyses of **1** and **2** are now in progress.

Antibacterial activities of **1** and **2** were preliminarily tested, and the MIC (μg/ml) values are shown in Table 3. These compounds were moderately active against *Moraxella catarrhalis*.

As described above, halstoctacosanolides A and B appear to belong to the standard polyketides biosynthesized mainly by Type 1 PKSs with some post-PKS modifications. Particularly interesting is that the relevant partial structure of halstoctacosanolides A and B was first predicted from the

Table 3. MIC value (μg/mL) of **1** and **2**

Test organism	**1**	**2**	Midecamycin	Azithromycin
Staphylococcus aureus 209P JC-1	>64	64	0.5	0.25
Micrococcus luteus ATCC9341	32	32	0.06	0.03
Haemophilus influenzae Rd/*acrB*::*Km*	64	32	0.5	0.5
Streptococcus pneumoniae 1913	64	32	0.25	0.06
Streptococcus pyogenes Cook	32	32	0.13	0.06
Moraxella catarrhalis W-0506	0.5	<0.25	2	0.03

genomic data and it was in fact found in the natural products. Certain proof by means, for example, of gene disruption and phenotype analysis should be necessary to conclude the relationship between the genetic information and the resulting metabolites, and the present case is by no means an exception. Genetic studies will be described elsewhere. However, the present observation of complete agreement between the predicted partial constitution and the actual structure may convince of direct relationship between the deduced DNA sequence and halstoctacosanolides A and B. Most significant in the present study are to show that genomic analysis of microorganisms, particularly of *Streptomycetes* and related species, is extremely beneficial to directed search for new microbial metabolites and to chemical studies thereof, and to stimulate an emerging field of natural product chemistry.

More than 20,000 secondary metabolites have been described so far from *Streptomycetes* and other *Actinomycetes*. Particularly, the genus *Streptomyces* and closely related genera are still an extraordinary rich source of a wide variety of secondary metabolites as lead compounds for the development of new successful drugs. For example in *S. avermitilis*, 30 different secondary metabolite gene clusters were assigned[28] and several unidentified secondary metabolites were predicted from the genetic data,[5] although isolation of the predicted products has not necessarily been described fully. The findings provided by us and others apparently demonstrate strongly the potential of genetic information in *Streptomycetes* to unveil yet unknown substances.

In conclusion, we successfully demonstrated the isolation of new bioactive compounds, halstoctacosanolides A and B, by genome-inspired search. These compounds in fact escaped from attention with conventional activity-based screening. This approach appears to be promising to search for new antibiotics and useful in natural product chemistry.

3. Experimental

3.1. General procedures

Optical rotations were measured with a JASCO DIP-360 spectrometer. Mass spectra were obtained on a JEOL JMS-700 in FAB mode using 3-nitrobenzyl alcohol as matrix. UV and IR spectra were recorded on a Shimadzu UV-160A spectrophotometer and Horiba FT-710 spectrophotometer, respectively. NMR spectra were measured by using JEOL LA-400 and Bruker DRX-500 spectrometers. Chemical shifts are reported in δ values relative to internal tetramethylsilane (δ 0.00). Column chromatography was carried out with a Silica gel 60 (70–230 or 230–400 mesh, Merck) and preparative TLC was performed on PLC plate (Silica gel 60 F_{254}, Merck, 0.5 mm thickness).

3.2. Fermentation

The vicenistatin production medium was used as described,[10] containing potato starch 3%, soya flake 1.5%, yeast extract 0.2%, corn steep liquor 0.5%, NaCl 0.3%, $MgSO_4{\cdot}7H_2O$ 0.05%, $CoCl_2{\cdot}6H_2O$ 0.0005% and $CaCO_3$ 0.3%, the pH being adjusted to 7.1 before sterilization with 2 M NaOH. A 10 μL of spore suspension of *S. halstedii* HC 34 was added to an autoclaved 100 mL of this medium in a 500 mL baffled flask equipped with cotton plug. The culture was grown at 27 °C for 2 days with shaking at 200 rpm on a rotary shaker. Vegetative cultures (125 mL×12) of the production medium having the same composition were inoculated with 1 mL of the pre-culture, and were grown under the same conditions for 3 days.

3.3. Isolation and purification

The fermentation broth (1.5 L) was centrifuged to obtain a mycelium cake and supernatant. The mycelium cake was extracted with acetone (500 mL). The extract was filtered through a pad of Celite and the filtrate was evaporated to an aqueous suspension. The suspension, after being adjusted to pH10, was extracted three times with ethyl acetate. The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The supernatant was also extracted three times with ethyl acetate after being adjusted to pH10. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Then, the crude extracts thus obtained were combined and subjected to flash chromatography with silica gel, which was eluted with 10% methanol in ethyl acetate to afford a mixture of halstoctacosanolides. The mixture was further purified by preparative TLC (90% ethyl acetate in hexane) to give halstoctacosanolide A (**1**) (33 mg) and halstoctacosanolide B (**2**) (110 mg).

3.3.1. Preparation of halstoctacosanolide A methyl acetal (3). A solution of **1** (19.1 mg, 0.022 mmol) in methanol (3.0 mL) was treated with pyridinium *p*-toluenesulfonate (3 mg) for 3 h at room temperature. The reaction mixture was diluted with Tris–HCl buffer (1 mol/L, pH 8), and the resulting mixture was evaporated to remove methanol, whereupon the rest was extracted three times with ethyl acetate. The combined organic extracts was washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by preparative TLC (10% methanol in $CHCl_3$) to afford **3** (13.6 mg, 70%) as a pale yellow powder. Mp 84.8–85.5 °C; $[\alpha]_D^{29}=+34.9$ (*c* 1.23, $CHCl_3$); IR (KBr): 3400 (br), 2960, 2930, 1710 cm^{-1}; NMR data of **3** are shown in Table 3; HRFAB-MS calcd for $C_{49}H_{78}O_{12}Na$: *m/z*; 881.5391 ($M+Na^+$). Found: *m/z*; 881.5434.

3.3.2. Preparation of halstoctacosanolide B methyl acetal (4). The compound **2** was treated as in the same manner as described in the preparation of **3**, and a methyl acetal derivative **4** was obtained as colorless powder (27.7 mg, 78%). Mp 80.9–82.5 °C; $[\alpha]_D^{27}=-36.8$ (*c* 1.0, $CHCl_3$); IR (KBr): 3420(br), 2960, 2930, 1700 cm^{-1}; NMR data of **4** are shown in Table 3; HRFAB-MS calcd for $C_{49}H_{80}O_{11}Na$: *m/z*; 867.5598 ($M+Na^+$). Found: *m/z*; 867.5566.

Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research and the COE21 program from the Ministry of Education, Culture, Sports, Science and

Technology, Japan. The authors are grateful to Meiji Seika Kaisha Co Ltd. for the assay of antimicrobial activity.

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Tetrahedron 60 (2004) 4007–4017

A study on the regio- and stereoselectivity in palladium-catalyzed cyclizations of alkenes and alkynes bearing bromoaryl and nucleophilic groups

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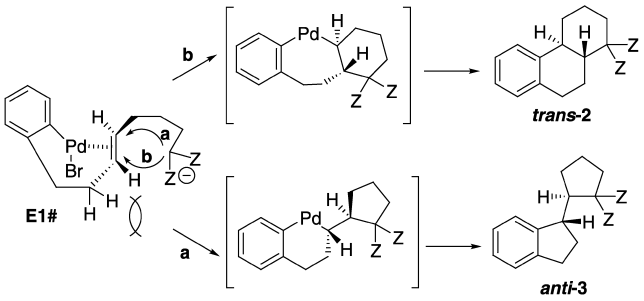
Received 6 October 2003; revised 30 January 2004; accepted 5 March 2004

Abstract—We have studied the remarkable dependence of the stereochemistry of the cyclization on the double bond geometry and of the effect of the bulkiness of the nucleophile on the regiochemistry of the palladium mediated cyclization of alkenes bearing aryl bromides and nucleophiles. In contrast, the cyclization of the acetylenic homologous substrates is not dependent on the nature of the nucleophile.

1. Introduction

Control of regio- and stereochemistry during the simultaneous creation of consecutive stereogenic centers continue to offer considerable challenge to organic chemists.[1] Transition metal-mediated tandem or cascade reactions have recently emerged as new and powerful methods, which are aimed at achieving this goal. The scope and limitations of such reactions have been the subject of recent reviews.[2] Following this trend, we have developed a new palladium-mediated cyclization reaction of unsaturated substrates bearing a nucleophilic substituent.[3] By using the intramolecular version of this strategy, we have already achieved the stereocontrolled synthesis of fused tricyclopentanoid[4] and linearly condensed hexahydro-1*H*-benz[*f*]indenes.[5] It is noteworthy that these cyclizations proceed in a completely *trans*-stereoselective manner since they involve attack of the carbon nucleophile onto the double bond which is electrophilically activated by the organopalladium species.

It was envisioned that application of the same concept to linear substrates having an internal *trans* double bond such as **E1** would either proceed via a 5-*exo* or a 6-*endo*-trig process leading to tricyclic compounds **2** and **3**, respectively (Scheme 1). We thought that the syntheses of these two tricyclic compounds would occur with concomitant stereocontrol of the two newly formed adjacent carbon centers since the nucleophile and the organopalladium species add in a *trans* fashion across the unsaturated linkage. This means



Scheme 1.

that, due to the stereochemistry of the initial double bond substrate, the ring fusion in compound **2** must be *trans*. The relative configuration of **3** would be fixed for the same reason. Moreover, examination of molecular models led us to believe that the bulkiness of the nucleophile would be a determining factor controlling the selectivity (5-*exo*- versus 6-*endo-trig*) of the reaction. In general, *exo*-cyclization is kinetically more favorable than the *endo* mode of attack. In the particular case of a substrate of type **1**, the geometric requirement for the intramolecular palladium-mediated cyclization in the 6-*endo-trig* process seems to induce less strain in the transition state relative to attack according to 5-*exo-trig* process. However, severe steric interactions between a bulky nucleophile and one of the allylic hydrogens of the linear substrate could be anticipated in the *endo*-cyclization mode. We were therefore interested to see if these steric interactions could be used as stereocontrolling elements during the cyclization. Indeed, a thin nucleophile would favor 5-*exo* pathway, while the 6-*endo-trig* would be preferred in the presence of a bulky nucleophile (Scheme 1). We also wanted to examine the

Keywords: Stereoselectivity; Biscyclization; Palladium.
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doi:10.1016/j.tet.2004.03.023

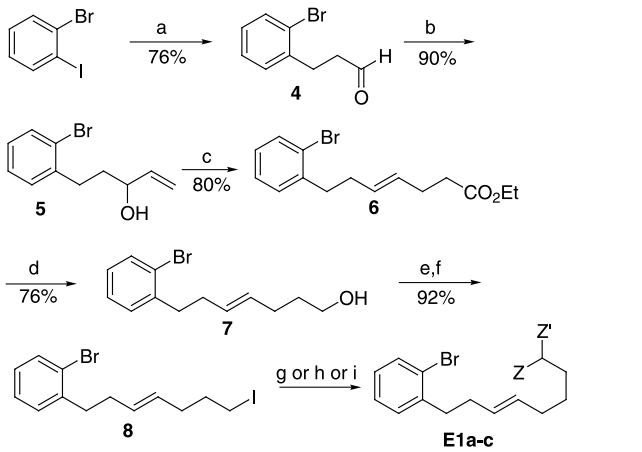
importance of olefin geometry on the stereochemical course of the cyclization.

In this paper, we report details of our work[6] and show that the cyclization proceeds with virtually complete regio- and stereocontrol. Moreover, the reaction is shown to be stereospecific with the stereochemical outcome depending on the geometry of the internal alkene.

2. Results and discussion

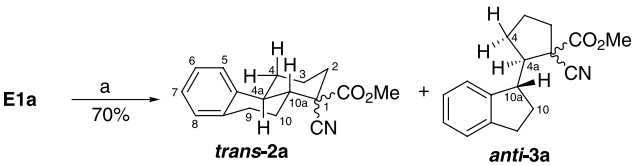
2.1. Cyclization of linear *trans*-alkenes E1a–c

In order to validate the feasibility of our strategy, the palladium catalyzed cyclization reactions of linear *trans* alkenes of type **E1a–c** differing in the bulkiness of the nucleophilic moiety were investigated. The cyclization substrates **E1a–c** were prepared via the route outlined in Scheme 2, in a seven-step sequence from commercially available 1-bromo-2-iodobenzene. Thus treatment of this dihalide with allylic alcohol, in DMF, at 50 °C, according to the procedure published by Jeffery[7] [$Pd(OAc)_2$, benzyltriethylammonium chloride, $NaHCO_3$] afforded the single aldehyde **4** in 92% yield. Treatment of **4** with vinylmagnesium bromide led to allylic alcohol **5** in 90% yield. The orthoester Claisen rearrangement of **5** proceeded in triethylorthoacetate at reflux to give 80% of the ester **6**. Reduction of **6** with lithium aluminium hydride in diethyl ether cleanly provided the alcohol **7** which was transformed to the iodide **8** via the corresponding mesylate. Substitution of the iodide group by the sodium salts of methyl cyanoacetate, dimethyl malonate and malononitrile, respectively, furnished the corresponding precursors **E1a–c**.



Scheme 2. Reagents and conditions: (a) allyl alcohol, $Pd(OAc)_2$ 5%, $NaHCO_3$, TEBA, DMF, 50 °C; (b) vinylmagnesium bromide, THF, −30–25 °C, 2 h; (c) $CH_3-C(OEt)_3$, propionic acid, reflux, 12 h; (d) $LiAlH_4$, ether, 25 °C, 30 min; (e) MsCl, TEA, CH_2Cl_2, 0 °C, 2 h;(f) NaI, acetone, reflux, 12 h; ((g) NaH, dimethylmalonate, DMF/THF 1/1, reflux, 18 h; (h) NaH, malononitrile, THF, reflux, 12 h; (i) NaH, methylcyanoacetate, DMF/THF 1/1, reflux, 12 h.

Initial experiments to cyclize the *trans* alkene **1a** using previously developed methodology in our group failed.[4] Therefore, when a solution of **1a** in THF was treated with 1.1 equiv. of *t*BuOK, followed by addition of 5 mol% of Pd(dppe),[8] no reaction was observed and starting material was recovered even after prolonged reflux times. Optimum conditions of our tandem biscyclization reaction performed on the substrate **E1a** involved formation of the enolate with *t*BuOK, in presence of 5 mol% of Pd(dppe), in dry 1-methyl-2-pyrrolidinone (NMP) at 50 °C. After 24 h, the starting material was consumed and a 1:1 mixture of two tricyclic compounds was obtained in 70% combined yield. The ^1H NMR of the crude reaction product revealed no traces of bicyclic products resulting from the competing Heck reaction (Scheme 3).



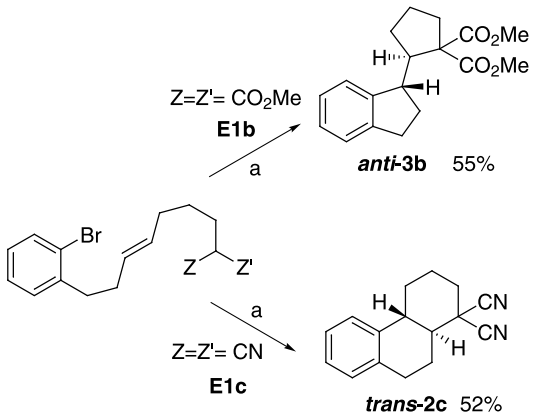
Scheme 3. Reaction conditions: (a) $Pd(OAc)_2$ 5%, dppe 10%, 1-heptene 10%, 18-C-6 crown ether 20%, *t*BuOK, NMP, 50 °C.

These two tricyclic compounds were separated by careful medium pressure liquid chromatography and their structures were confirmed by ^1H and ^{13}C NMR data. The solid less polar product was identified as one epimer of *trans*-**2a** by a 400 MHz two dimensional DQF COSY spectrum and a $^1H-^{13}C$ HMQC experiment (heteronuclear multiple quantum coherence) recorded in the phase-sensitive mode that permit identification of most of the hydrogens and carbons.[9] Thus, H_{4a} resonates at δ=2.12 ppm as doublet of a doublet of a doublet. The J_{4a-10a}, $J_{4a-4a\ ax}$ and $J_{4a-4a\ eq}$ constants were 12.1, 11.3, 3.9 Hz, respectively, and were consistent with the expected two large coupling constants J_{ax-ax} and one J_{ax-eq} of a *trans*-octahydrophenanthrene.[10]

The structure assigned to the liquid more polar product was one epimer of *anti*-**3a** by arguments analogous to those made for the assignment of *trans*-**2a**. In the ^1H NMR, the double doublet of doublets at 3.4 ppm is assigned to the H_{10a} angular proton. It is coupled to the adjacent H_{10} and $H_{10'}$ protons by coupling constants J_{10a-10}=8.3 Hz, $J_{10a-10'}$=7.1 Hz typical of a five-membered ring. The splitting of the H_{10a} signal is due to its coupling with the adjacent angular proton H_{4a} (J_{10a-4a}=11.5 Hz) and this confirms the expected *anti* relationship between them.

As expected, the palladium induced cyclization of **E1a** bearing a medium size nucleophile proceeded via both *exo* and *endo*-pathway, but surprisingly, only one diastereomer of *trans*-**2a** and *anti*-**3a** were formed at the carbon bearing the nitrile and the ester. The configuration of the quaternary center was not determined. Next, we attempted the cyclization of substrate **E1b** bearing a bulky nucleophile. This was carried out under the usual reaction conditions used for **E1a**. After 5 h at 60 °C, the reaction provided exclusively compound *anti*-**3b** which was isolated in 55% yield after chromatographic purification. No traces of the other regioisomer or of classical Heck reaction product were observed within the limits of ^1H NMR and capillary GC

sensitivities. The stereostructural assignments for the tricyclic compound were verified by comparison of its characteristic ^{1}H NMR data with those of *anti*-**3a**. In particular, the *trans* relationship between H_{10a} and H_{4a} was readily deduced from the coupling constant J_{10a-4a}=11 Hz (Scheme 4).



Scheme 4. Reaction conditions: (a) $Pd(OAc)_2$ 5%, dppe 10%, 1-heptene 10%, 18-C-6 crown ether 20%, *t*BuOK, NMP, 50 °C.

This result clearly indicates that the 5-*exo*-cyclization can be controlled by judicious choice of the nucleophile substituent. Finally, we turned our attention to the cyclization of the substrate **E1c** bearing a thin nucleophile. In contrast to the facile cyclization of substrates **E1a** and **E1b**, **E1c** appeared to be more resistant since using the procedure mentioned above, all the starting material was only consumed after 65 h at 60 °C.[11] A colorless solid was isolated in 52% yield after flash-chromatography and characterized as the regioisomer *trans*-**2c**, resulting from the 6-*endo-trig* cyclization process on the basis of spectroscopic correlation with *trans*-**2a** (Scheme 4). The *trans* junction of the ring was confirmed by its ^{1}H NMR spectrum in C_6D_6 in which the H_{10a} proton resonates at δ=1.50 ppm and its coupling pattern as a doublet (J=4.3 Hz) of triplet (J=12 Hz) consistent with a small J_{ax-eq} and two large J_{ax-ax} couplings. In addition, a single crystal X-ray diffraction analysis confirmed the stereochemical assignment of this diastereomer (Fig. 1).

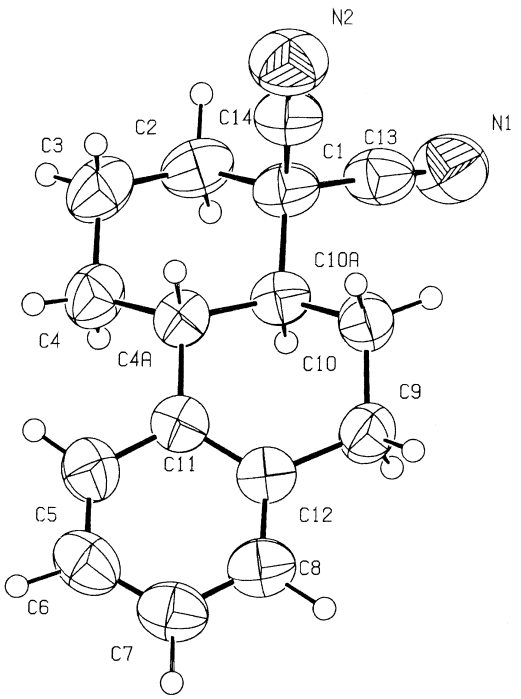
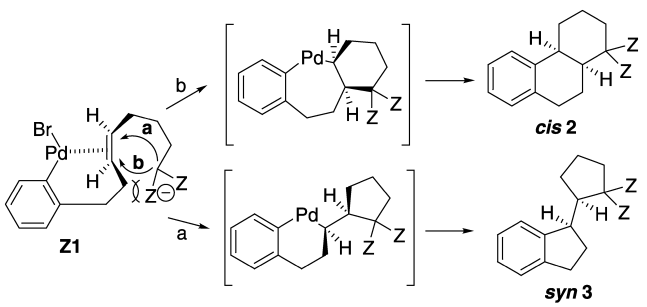


Figure 1. X-ray crystallographic structure of compound *trans*-**2c**: ORTEP view.

This last result shows that, in this case, the tandem carbopalladation–cyclization sequence proceeds with complete regio- and stereoselectivity leading to the *trans* perhydrophenanthrene ring. This system is very common in natural products, particularly in the carbon framework of steroids and many triterpenoids.[12]

2.2. Cyclization of linear *cis*-alkenes Z1a–c

Since the ring junction stereochemistry is governed by alkene geometry, a question which is raised by these successful preliminary results is the stereospecificity of this tandem carbopalladation–cyclization sequence. In order to gain further insight into this problem, we investigated the cyclization of *Z* linear substrates of type **1** (Scheme 5).

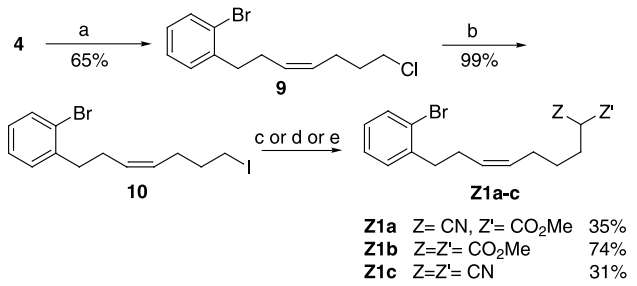


Scheme 5.

Using molecular model, we speculated as previously described for the cyclization of *trans* isomers, that the regioselectivity would depend again upon steric factors. Indeed, in the approach of the nucleophile to the internal double bond, a strong interaction was observed between one of the allylic hydrogens and the nucleophile. Bulky ones would therefore favor the 5-*exo* cyclization while smaller ones would shift the reaction to the expected 6-*endo* pathway. The success of such a reaction would lead to the synthesis of the *cis*-octahydrophenanthrene skeleton. It is noteworthy that a few synthetic methodologies have been developed to construct this core structure which is of current interest.[13]

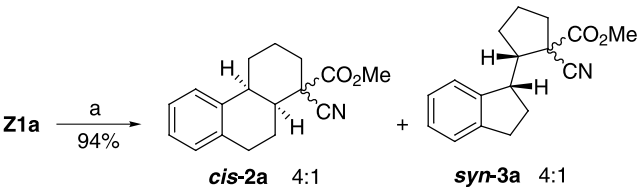
Access to the required starting material **Z1a–c** proved to be straightforward with the key step being a Wittig condensation of the known phosphonium salt[14] with the aldehyde **4** giving the *Z*-alkene **9** with complete stereoselectivity. Conversion of the chloride to the iodide **10** followed by reaction with the sodium salts of methyl cyanoacetate, dimethyl malonate and malononitrile, respectively, produced the corresponding precursors **Z1a**, **Z1b**, **Z1c** (Scheme 6).

Treatment of **Z1a** with Pd(dppe) (5 mol%) and *t*BuOK (1.1 equiv.) in NMP at 50 °C afforded an inseparable



Scheme 6. Reagents and conditions: (a) (4-chlorobutyl)-triphenylphosphonium bromide, KHMDS, THF, 0 °C; (b) NaI, acetone, reflux, 12 h; (c) NaH, methylcyanoacetate, DMF/THF 1/1, reflux, 12 h; (d) NAH, dimethylmalonate, DMF/THF 1/1 reflux, 18 h; (e) NaH, malononitrile, THF, reflux, 12 h.

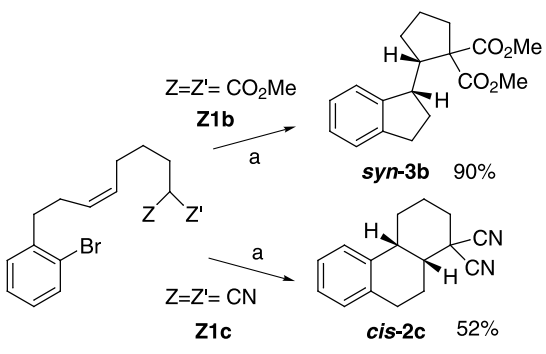
mixture of four isomeric tricyclic compounds in a ratio of about 4:4:1:1 after only 1.5 h (according to GC) and in 94% yield. We suspected that these four substrates were two couples of diastereomers for each of the regioisomers, *cis*-**2a** and *syn*-**3a**. The ^{1}H and ^{13}C NMR spectra of the mixture were significantly different from those reported for *trans*-**2a** and *anti*-**3a** and no traces of compounds resulting from a Heck reaction were observed. Because of the low regio- and diastereoselectivity (referred to C_1 carbon) exerted in this cyclization, we decided not to investigate which of the four isomers were predominant (Scheme 7).



Scheme 7. Reaction conditions: (a) $Pd(OAc)_2$ 5%, dppe 10%, 1-heptene 10%, 18-C-6 crown ether 20%, *t*BuOK, NMP, 50 °C.

Interestingly, treatment of **Z1b** under the same conditions gave *syn*-**3b** as a single diastereomer in 90% yield after 12 h at 50 °C (Scheme 8). Spectral data clearly indicated the five-membered ring: in particular, the C_{10} axial proton at δ 2–2.5 ppm has the expected coupling pattern (ddd, J=7.1, 8.8, 13.8 Hz). The *cis* relationship between H_{4a} and H_{10a} was established by the coupling constant (J=3 Hz) in the homonuclear decoupling spectrum (Scheme 8).

This result indicates that the pallado-catalyzed cyclization of linear compounds **1b** is stereospecific and that the



Scheme 8. Reaction conditions: (a) $Pd(OAc)_2$ 5%, dppe 10%, 1-heptene 10%, 18-C-6 crown ether 20%, *t*BuOK, NMP, 50 °C.

dimethylmalonate group again exerts a profound influence upon the regiochemistry of cyclization process by virtue of its steric bulk.

Finally, we have investigated the cyclization of **Z1c** bearing a smaller nucleophile. This reaction was performed at 50 °C for 48 h to afford the single crystalline *cis* octahydrophenanthrene **2c** in 52% yield (Scheme 8). The small ^{1}H NMR coupling (J=3.7 Hz) observed between the angular hydrogens confirmed the expected *cis* ring fusion of **2c**. The ^{13}C NMR spectrum displays two methine carbons (36.8 and 40 ppm) showing that the carbons of the ring junction in *cis*-**2c** are more shielded than those of *trans*-**2c** (38.8 and 44.5 ppm). This is in accordance with the fact that the ^{13}C NMR shift values for a *cis* ring junction of perhydrophenanthrenes are smaller than those for a *trans* junction.[15] Single X-ray diffraction analysis unambiguously established the expected stereochemistry as shown in Figure 2.

The remarkable influence of the double bond geometry of the starting material on the stereochemistry of the product was here also demonstrated. Furthermore, the regiochemistry of the cyclization could be controlled by the size of the nucleophile.

2.3. Cyclization of the acetylenic substrates 14a–b

It was of interest to examine the behavior of the corresponding acetylenic substrates under our standard conditions of cyclization. We wanted to know if the bulkiness of the nucleophile could also exert a beneficial directing effect on the regioselectivity during the palladium mediated cyclization leading either to cyclopentylidenindane **15** or to hexahydrophenanthrene **16** (Scheme 9).

To this end, syntheses of the two required acetylenic substrates were each accomplished in a four step sequence starting from the commercially available 2-bromobenzyl bromide as illustrated in Scheme 10. The required Grignard reagent was generated in situ in diethyl ether, from propargyl bromide and magnesium turnings and then added to 2-bromobenzyl bromide to provide **11** in 75% yield. Deprotonation of the resulting acetylenic product by lithium diisopropylamide (LDA) followed by addition of an excess of 1-bromo-3-chloropropane afforded chloride **12** in 60% yield. Halide exchange (NaI, acetone) gave the desired iodide **13** in excellent yield. This iodide was treated with the sodium salts of dimethylmalonate and malonitrile to respectively produce the corresponding acetylenic precursors **14a** and **14b**.

The cyclization of substrate **14a** under the conditions previously used for alkenyl compounds gave, after 24 h at 60 °C, an inseparable mixture of two products in a 3:2 ratio (as determined by ^{1}H NMR). In the ^{1}H NMR spectrum of the product mixture, the minor compound appeared to be **17** resulting from the competing Heck reaction with a proton triplet centered at 3.38 ppm characteristic of proton at the α position of a malonate function, and a vinylic proton at 4.6 ppm. For the major product, the absence of these two protons strongly suggested the biscyclization had taken place but the regiochemistry of the cyclization (6-*endo* versus 5-*exo*) could not be ascertained. In order to improve

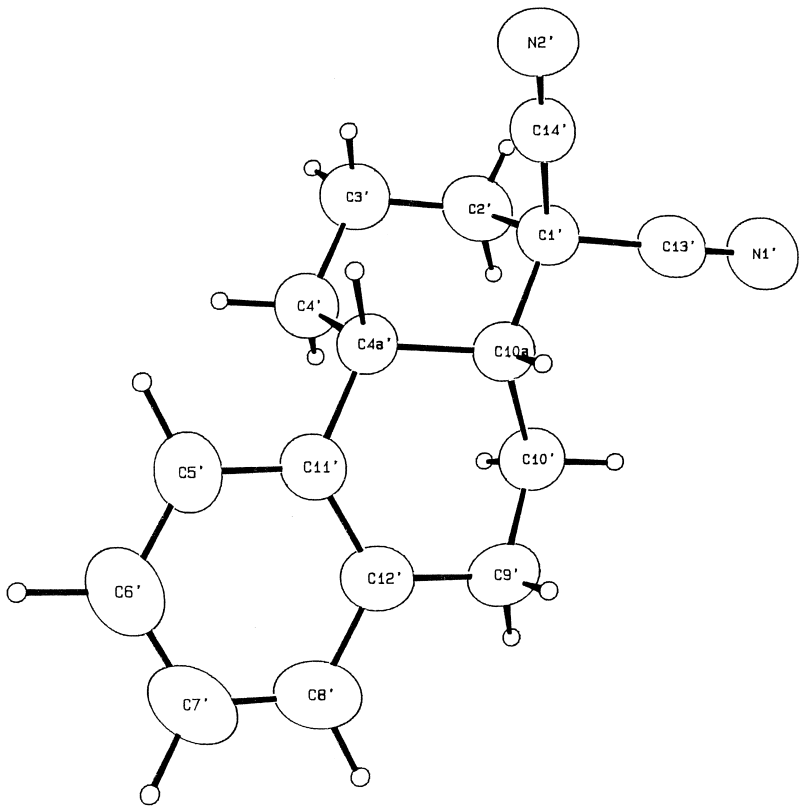
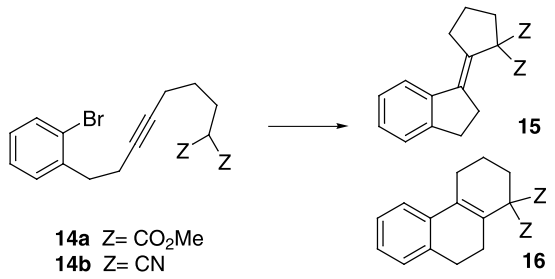
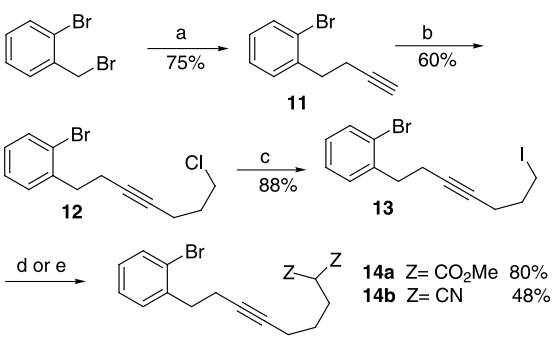


Figure 2. X-ray crystallographic structure of compound *cis*-**2c**: ORTEP view.



Scheme 9.



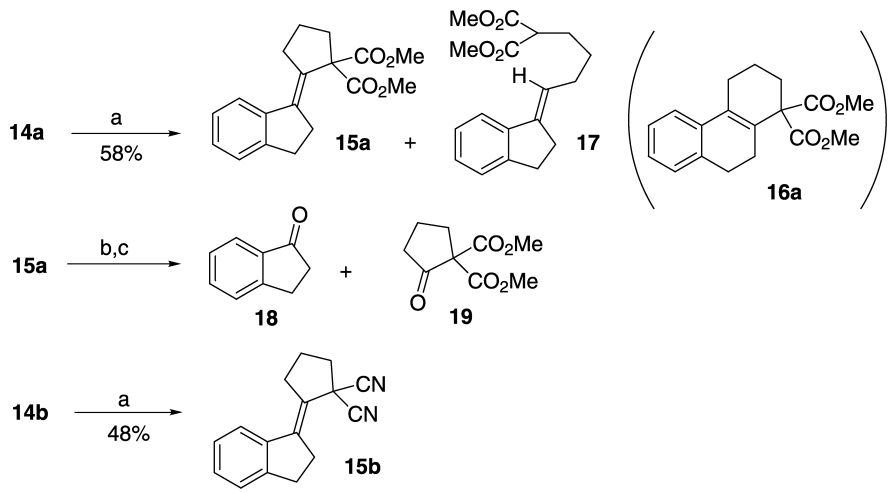
Scheme 10. Reagents and conditions: (a) propargyl bromide, Mg, $HgCl_2$ cat., ether THF, 0 °C; (b) LDA, −78 °C, 1 h then 1-bromo-3-chioro-propane, −60 °C to reflux, 12 h; (c) Nal, acetone, reflux, 12 h; (d) NaH, dimethylmalonate, DMF/THF 1:1, reflux, 18 h; (e) NaH, malononitrile, THF, reflux, 12 h.

the selectivity in favor of the biscyclized product, we decided to test the reaction in DMSO. In this solvent, the reaction was complete after 2 h at 90 °C leading to the previously obtained tricyclic compound (**15a** or **16a**) in 58% yield as the only isolable product. To determine the structure of this unsaturated substrate, the alkene was oxidatively cleaved by treatment with ozone[16] followed by addition of dimethylsulfide leading to two products. The mixture was analyzed by GC–MS proving the presence of 1-indanone **18** by comparison with an authentic sample. The second product shown an ion peak at M^+=200 according to the structure of **19**. This result clearly demonstrated the cyclopentylindanylidene structure **15a** and not **16a** for the biscyclization product (Scheme 11).

Same conditions were applied to **14b** leading after 2 h to a unique crystalline product in 48% yield. The regiochemistry of this compound was derived from a single X-ray diffraction analysis and revealed the cyclopentylindanylidene structure **15b** (Fig. 3). Contrary to the ethylenic substrates, the regioselectivity of the biscyclization process of the acetylenic homologs is independent of the bulkiness of the nucleophile.

3. Conclusion

In summary, we have demonstrated that the simple tandem palladium-catalyzed cyclization of linear compound of type *Z* or *E* proceeds with complete retention of the stereochemistry in a stereocontroled mode. Moreover, it was possible to effect either 5-*exo* or 6-*endo*-cyclization



Scheme 11. Reagents and conditions: (a) $Pd(OAc)_2$ 5%, dppe 10%, 1-heptene 10%, 18-C-6 crown ether 20%, *t*BuOK, DMSO, 90 °C, 2 h; (b) O_3, CH_2Cl_2, −78 °C; (c) DMS, −78 °C to 25 °C.

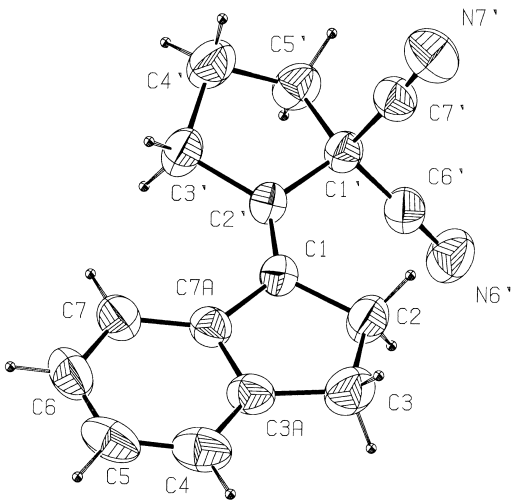


Figure 3. X-ray crystallographic structure of compound **15b**: ORTEP view.

selectively by appropriate choice of the electron withdrawing substituents of the nucleophile. *exo*-Cyclizations are observed when a sterically hindered nucleophile is employed. *endo*-Cyclizations leading to octahydrophenanthrene is the only reaction observed with a less sterically demanding nucleophile. Notably, these cyclizations proceed in a completely stereoselective *trans* manner. The reaction is then stereospecific, the stereochemistry is defined by that of the double bond in the initial substrate, the relative configuration of the indane substrates are hereby controlled. The biscyclization of acetylenic homologs could also be performed leading exclusively to cyclopentylidenindane structure, in that case the bulkiness of the nucleophile has no effect on the course of the reaction.

4. Experimental

4.1. General

All reactions were carried out under a nitrogen atmosphere using standard syringe, cannula and septa techniques. All reactions were monitored by thin layer chromatography carried out on 0.2 mm silica gel plates (60 F-254, Merck) or by gas chromatography on a DB 1 capillary column 30 m. Column chromatographies were performed on a silica gel Si 60 (40–63 mesh, Merck). Melting points are uncorrected. IR spectra were recorded on a Perkin–Elmer 337 instrument. Nuclear magnetic resonance spectra were obtained on a Brucker AC 200 spectrometer (^{1}H: 200 MHz or ^{13}C: 50 MHz) or on a Brucker AC 300 spectrometer (^{1}H: 300 MHz or ^{13}C: 75 MHz) using TMS as an internal standard. Chemical shifts were expressed in ppm downfield from TMS and coupling constants (*J*) in Hertz. Microanalysis were performed by Service Central d'Analyse du CNRS, Solaize, France. THF was distilled from Na/benzophenone, *N*-methyl pyrrolidone (NMP) and DMSO (dimethyl sulfoxide) were distilled under N_2 from CaH_2, DMF was distilled from P_2O_5 and Et_2O was distilled from LAH prior to use.

4.1.1. 3-(*o*-Bromophenyl)propan-1-al (4). To a solution of $Pd(OAc)_2$ (60 mg, 0.27 mmol), allylic alcohol (1.2 mL, 17.7 mmol), triethylbenzylammonium chloride (1.6 g, 7.1 mmol) and $NaHCO_3$ (1.48 g, 17.7 mmol) in 50 mL of DMF was added 1-bromo-2-iodobenzene (2.2 g, 7.9 mmol). The black solution was heated at 50 °C for 24 h. The mixture was quenched with saturated aqueous NH_4Cl solution (50 mL). The organic phase was extracted with Et_2O (3×100 mL), washed with brine (2×100 mL) and dried over Na_2SO_4. The solvent was evaporated and the residue purified by flash chromatography (PE/Et_2O=95:5) to give **4** as a yellow oil (1.15 g, 76%). ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.8 (2H, m), 3.1 (2H, m), 7.1 (1H, m), 7.25 (2H, m), 7.55 (1H, d, J=7.9 Hz), 9.85 (1H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.7, 43.6, 124.2, 127.6, 128.0, 130.5, 132.9, 139.7, 201.1. IR (neat): 3060, 2960, 2850, 2720, 1720, 1590, 1470, 1440, 1180, 1020, 750 cm^{-1}.

4.1.2. 5-(*o*-Bromophenyl)pent-1-en-3-ol (5). A solution of vinylmagnesium bromide 1 M in THF (11.1 mL) was added dropwise to a stirred solution of **4** (1.57 g, 7.37 mmol) in THF (20 mL) maintained at −30 °C. The solution was allowed to warm to room temperature. After stirring for 2 h,

the reaction was quenched with saturated aqueous NH_4Cl solution. The alcohol was extracted with Et_2O (3×50 mL), washed with brine (50 mL) and dried over Na_2SO_4. The solvent was evaporated and the residue purified by flash chromatography (PE/Et_2O=70:30) to give **5** as a yellow liquid (1.71 g, 90%). ^1H NMR (200 MHz, $CDCl_3$) δ 1.65 (1H, s), 1.75 (2H, m), 2.85 (2H, m), 4.15 (1H, m), 5.2 (2H, m), 5.95 (1H, ddd, J=17.2, 10.4, 6 Hz), 7.1 (1H, m), 7.25 (2H, m), 7.55 (1H, d, J=7.7 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 32.2, 37.1, 72.6, 115.7, 124.6, 127.6, 127.8, 130.6, 133.0, 141.0, 141.3. IR (neat): 3400, 3060, 2920, 2860, 1640, 1570, 1470, 1020, 990, 920, 900 cm^{-1}. Anal. calcd for $C_{11}H_{13}OBr$: C, 54.79; H, 5.43. Found: C, 55.20; H, 5.31.

4.1.3. Ethyl (*E*)-7-(*o*-bromophenyl)hept-4-enoate (6). The allylic alcohol **5** (1.71 g, 7.10 mmol) was refluxed with freshly distilled triethyl orthoacetate (39 mL, 214 mmol) and propionic acid (47 μl, 0.63 mmol) for 15 h. After removal of triethyl orthoacetate under vacuum, the residual oil was purified by flash chromatography (PE/Et_2O=70:30) to give ester **6** as a yellow oil (1.76 g, 80%). ^1H NMR (200 MHz, $CDCl_3$) δ 1.27 (3H, t, J=7.2 Hz), 2.34 (6H, m), 2.78 (2H, m), 4.15 (2H, q, J=7.2 Hz), 5.44 (1H, dt, J=15.4, 6.3 Hz), 5.54 (1H, dt, J=15.4, 6.3 Hz), 7.08 (1H, m), 7.25 (2H, m), 7.55 (1H, d, J=7.7 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.3, 27.9, 32.6, 34.2, 36.1, 60.2, 124.4, 128.3, 127.5, 129.1, 130.2, 130.4, 132.7, 141.1, 173.1. IR (neat): 3060, 2980, 2860, 1740, 1570, 1470, 1440, 1370, 1180, 1020, 970, 750, 660 cm^{-1}. Anal. calcd for $C_{15}H_{19}O_2Br$: C, 57.81; H, 6.15; O, 10.28. Found: C, 57.96; H, 6.02; O, 10.41.

4.1.4. (*E*)-7-(*o*-Bromophenyl)hept-4-en-1-ol (7). A solution of ester **6** (1.88 g, 6.04 mmol) in dry Et_2O (20 mL) was added dropwise to a cold (0 °C) stirred suspension of LAH (230 mg, 6.04 mmol) in dry Et_2O (50 mL). The mixture was stirred at room temperature for 1 h. Water (0.230 mL), 1 N NaOH (0.230 mL) then 3 mL of water were successively added until a precipitate appeared. The slurry was filtered through a pad of celite and the filtrate was dried over Na_2SO_4 and concentrated. The residual oil was purified by flash chromatography using (PE/AcOEt=90:10) to give alcohol **7** as a yellow oil (1.24 g, 76%). ^1H NMR (200 MHz, $CDCl_3$) δ 1.38 (1H, s), 1.58 (2H, qn, J=6.9 Hz), 2.06 (2H, m), 2.3 (2H, m), 2.79 (2H, m), 3.61 (2H, t, J=6.4 Hz), 5.44 (1H, dt, J=15.4, 5.5 Hz), 5.53 (1H, dt, J=15.4, 5.4 Hz), 7.08 (1H, m), 7.25 (2H, m), 7.55 (1H, d, J=7.6 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 29.0, 32.4, 32.9, 36.4, 62.6, 124.6, 127.4, 127.7, 129.8, 130.60, 130.7, 132.9, 141.3. IR (neat): 3320, 3060, 2920, 2860, 1590, 1570, 1470, 1440, 1020, 970, 750, 660 cm^{-1}. Anal. calcd for $C_{13}H_{17}OBr$: C, 58.01; H, 6.37; O, 5.94. Found: C, 57.79; H, 6.26; O, 5.53.

4.1.5. (*E*)-2-[7-(2-Bromophenyl)-hept-4-enyl] malonic acid dimethyl ester (E1b). Methane sulfonyl chloride (0.76 mL, 9.82 mmol) was added dropwise to a stirred solution of alcohol **7** (2.00 g, 7.43 mmol) in a mixture of CH_2Cl_2 (60 mL) and triethylamine (1.41 mL, 9.66 mmol). After stirring for 2 h at 0 °C and 3 h at room temperature, the reaction mixture was diluted with diethyl ether (150 mL) and the mixture was washed with a saturated aqueous NH_4Cl solution (70 mL), dried and concentrated in vacuo. The residue was dissolved in acetone and sodium iodide (2.20 g, 14.7 mmol) was added. The mixture was refluxed for 12 h and cooled to room temperature. Et_2O (200 mL) was added and the mixture was washed with a saturated aqueous $Na_2S_2O_3$ solution (2×100 mL), brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography using pure petroleum ether as eluent to give the iodide **8** as a yellow oil (2.6 g, 92%).

A dispersion of 60% NaH in mineral oil (90.0 mg, 2.24 mmol) was suspended in a mixture of THF (5 mL) and DMF (5 mL), and dimethylmalonate (282 μL, 2.47 mmol) was added dropwise. The resulting solution of sodium malonate was added dropwise to a stirred solution of the iodide derivative **8** (447 mg, 1.18 mmol) in THF (7.5 mL) and DMF (7.5 mL) and the mixture was heated overnight at 70 °C. The reaction was quenched with 5% HCl. The malonate was extracted with Et_2O (3×50 mL) and the organic phase washed with brine (50 mL), dried and concentrated in vacuo. The residue was purified by flash chromatography (PE/Et_2O=80:20) to afford **E1b** as a colorless oil (370 mg, 80%). ^1H NMR (200 MHz, $CDCl_3$) δ 1.4 (2H, m), 1.95 (2H, m), 2.03 (2H, m), 2.3 (2H, m), 2.75 (2H, m), 3.36 (1H, t, J=7 Hz), 3.75 (6H, s), 5.3–5.4 (2H, dt, J=15.1, 7 Hz), 7.19 (1H, m), 7.22 (2H, m), 7.53 (1H, d, J=7.5 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 28, 32.2, 32.9, 36.4, 52.0, 52.6, 124.6, 127.4, 127.6, 129.9, 130.4, 130.6, 132.9, 141.0, 170.0. IR (neat): 3060, 2960, 2920, 2840, 1735 (broad), 1570, 1470, 1440, 1150, 1020, 970, 750 cm^{-1}. Anal. calcd for $C_{18}H_{23}O_4Br$: C, 56.41; H, 6.05; O, 16.7. Found: C, 56.62; H, 5.99; O, 16.9.

4.1.6. (*E*)-9-(2-Bromophenyl)-2-cyano-non-6-enoic acid methyl ester (E1a). Prepared as above for compound **E1b**. Colorless oil (42%). ^1H NMR (200 MHz, $CDCl_3$) δ 1.31 (2H, m), 1.59 (2H, m), 1.88 (2H, m), 2.12 (2H, m), 2.79 (2H, t, J=22 Hz), 3.49 (1H, m), 3.82 (3H, s), 5.45 (2H, m), 7.09 (1H, m), 7.20 (2H, m), 7.51 (1H, d, J=8.4 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 26.5, 29.2, 31.5, 32.7, 36.1, 38.8, 53.4, 116.4, 124.5, 127.3, 127.6, 129.5, 130.43, 130.45, 132.7, 141.1, 166.0. IR (neat): 3060, 2960, 2860, 2240, 1700, 1565, 1470, 1440, 1260, 1120, 1020, 970, 750, 650.

4.1.7. (*E*)-2-[7-(2-Bromophenyl)-hept-4-enyl] malononitrile (E1c). A dispersion of 60% NaH in mineral oil (74.0 mg, 1.84 mmol) was suspended in THF (10 mL) and cooled at 0 °C. Malononitrile (130 mg, 1.98 mmol) in THF (10 mL) was added dropwise. The resulting solution of sodium malononitrile was added at room temperature to a solution of the iodide **8** (400 mg, 1.06 mmol) in THF (10 mL). The resulting mixture was refluxed overnight in THF. The reaction was quenched with a saturated aqueous NH_4Cl solution (20 mL). The dinitrile was extracted with Et_2O (3×50 mL) and the organic phase was washed with brine (50 mL), dried and concentrated in vacuo. The residue was purified by flash chromatography (PE/Et_2O=80:20) to afford **E1c** as a colorless oil (172 mg, 50%). ^1H NMR (300 MHz, $CDCl_3$) δ 1.64 (2H, m), 1.89 (2H, m), 2.08 (2H, m), 2.42 (2H, m), 2.8 (2H, m), 3.66 (1H, t, J=7 Hz), 5.35 (1H, dt, J=15.1, 7 Hz), 5.54 (1H, dt, J=15.1, 7 Hz), 7–7.4 (3H, m), 7.52 (1H, d, J=7.5 Hz). ^{13}C (75 MHz, $CDCl_3$) δ 22.5, 26.1, 30.0, 31.0, 32.6, 35.9, 112.6, 124.4, 127.4, 127.6, 128.9, 130.5, 131.1, 132.8, 140.9. IR (neat): 3060, 2920, 2870, 2260, 1620, 1590, 1570, 1470, 1440, 1140, 1020, 970,

750 cm^{-1}. Anal. calcd for $C_{16}H_{17}N_2Br$: C, 60.58; H, 5.40. Found: C, 60.80; H, 5.51.

4.2. General procedure for the preformation of the palladium (0) complex and formation of 2-indan-1-ylcyclopentane-1,1-dicarboxylic acid dimethyl ester (*anti*-3b)

The Pd (0) complex was preformed using the same experimental procedure for all cyclizations. Under N_2, a mixture of 5 mol% of $Pd(OAc)_2$, 10 mol% of dppe (1,2-bis(diphenylphosphino)ethane) and 10 mol% of 1-heptene in NMP (*N*-methyl pyrrolidone) was stirred and heated with a hairdrier until the mixture turned brick-red. On the one hand, a solution of *t*BuOK (49 mg, 0.43 mmol) and 18-crown-6 (21 mg, 0.08 mmol) in NMP (1 mL) was added to a solution of malonate **E1b** (150 mg, 0.39 mmol) in NMP (2 mL). The mixture was stirred at room temperature for 30 min. On the other hand, the palladium (0) complex was preformed in NMP (2 mL) by reaction of 1-heptene (0.56 mL, 0.04 mmol) with $Pd(OAc)_2$ (4.50 mg, 0.02 mmol) and dppe (15.9 mg, 0.04 mmol). The addition of the brick-red Pd(0) solution was made via a cannula to the malonate solution prepared above. The mixture was stirred at 50 °C for 3 h. The solution was directly purified by flash chromatography (PE/Et_2O=80:20) to afford *anti*-**3b** as a pure white solid (65 mg, 55%). ^1H NMR (300 MHz, $CDCl_3$) δ 1.45 (1H, ddd, J=3.6, 8.7, 13.5 Hz), 1.65 (1H, ddd, J=7.9, 11.2, 14.2 Hz), 1.85 (2H, m), 2.10 (3H, m), 2.56 (1H, dt, J=8.1, 13.6 Hz), 2.75 (2H, m), 3.0 (1H, dt, J=2.8, 10 Hz), 3.10 (1H, ddd, J=7.1, 9.7, 13.8 Hz), 3.75 (6H, s), 7.10–7,53 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.5, 30.9, 31.6, 31.9, 37.3, 47.6, 49.9, 52.2, 52.6, 63.0, 124.6, 125.5, 125.6, 126.6, 144.7, 146.6, 172.3, 174.0. IR (KBr): 3060, 2940, 2840, 1740. Anal. calcd for $C_{18}H_{22}O_4$: C, 71.50; H, 7.33. Found: C, 71.29; H, 7.68. Mp 50–52 °C.

4.2.1. *trans*-2-Cyano-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2-carboxylic acid methyl ester (*trans*-2a) and 1-cyano-2-indan-1-yl-cyclopentanecarboxylic acid methyl ester (*anti*-3a).

Same experimental procedure as for **E1b**. The mixture was stirred at 50 °C for 24 h. The solution was directly purified by flash chromatography (PE/Et_2O=80:20) to give *trans*-**2a** and *anti*-**3a** (combined yield: 70%).

trans-**2a**: ^1H NMR (400 MHz, $CDCl_3$) δ 1.15–1.30 (1H, m), 1.65–1.82 (2H, m), 1.83–1.98 (4H, m), 2.12 (1H, ddd, J=12.1, 11.3, 3.9 Hz), 2.5 (1H, m), 2.81 (3H, m), 3.8 (3H, s), 7–7.25 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.6, 26.0, 29.3, 30.1, 34.4, 38.8, 44.5, 52.1, 53.5, 117.8, 125.7, 126.1, 126.2, 129.0, 135.9, 138.2, 170.0.

anti-**3a**: ^1H NMR (400 MHz, $CDCl_3$) δ 1.72–2.10 (3H, m), 2.17–2.38 (4H, m), 2.50 (1H, m), 2.70 (1H, m), 2.79–3.01 (2H, m), 3.4 (1H, ddd, J=11.5, 8.3, 7.1 Hz), 3.90 (3H, s), 7.14–7.35 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.5, 31.0, 31.1, 31.3, 39.7, 48.3, 52.6, 53.0, 53.6, 118.8, 124.7, 124.8, 125.9, 126.9, 144.1, 145.3, 171.1.

4.2.2. *trans*-3,4,4a,9,10,10a-Hexahydro-2*H*-phenanthrene-1,1-dicarbonitrile (*trans*-2c).

Same experimental procedure as for **E1b**. Scale: 234 mg, 0.74 mmol of **E1c**.The mixture was stirred at 50 °C for 65 h. The solution was directly purified by flash chromatography (PE/Et_2O=90:10) to give *trans*-**2c** as a pure white solid (90 mg, 52%). IR (KBr): 3060, 2940, 2215, 1600. ^1H NMR (300 MHz, C_6D_6) δ 0.6 (1H, tdt, J=12.5, 12.5, 3.9 Hz), 1.1–1.4 (4H, m), 1.50 (1H, dt, J=4.3, 12 Hz), 1.70 (1H, dt, J=12.7, 3.4 Hz), 1.8 (1H, dd, J=13.3, 3 Hz), 1.95 (1H, ddt, J=12.6, 5.4 Hz), 2.35 (1H, m), 2.35 (2H, m), 6.8–7.2 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.9, 26.3, 28.9, 29.3, 34.8, 38.6, 39.3, 45.7, 116.0, 126.3, 126.6, 126.8, 129.3, 135.6, 137.2. Anal. calcd for $C_{16}H_{16}N_2$: C, 81.35; H, 6.82. Found: C, 81.14; H, 6.72. Mp 174–176 °C.

4.2.3. X-ray crystal structure analysis.

Crystal data for *trans*-**2c** at 295 K collected on a Nonius CAD 4. $C_{16}H_{16}N_2$, M=236.31, monoclinic, $C2/c$, a=26.332(4), b=7.4772(7), c=17.345(3) Å, α=90, β=130.648(12), γ=90°, V=2591.2(6) $Å^3$, Z=8, λ(Cu Kα)=1.54056 Å, D_c=1.212 g cm^{-3}, 2585 reflections, 211 parameters, R=0.0615 and Rw=0.1800 for 2264 reflections with $I>2\sigma(I)$. CCDC registration number 221276.

4.2.4. (*Z*)-2-[7-(2-Bromophenyl)-hept-4-enyl] malonic acid dimethyl ester (Z1b).

8.16 g (18.8 mmol) of phosphonium salt of 1-bromo-4-chlorobutane and 3.83 g (19.2 mmol) of potassium hexamethyldisilazane (KHMDS) were placed in a round bottomed flask flushed with N_2. At 0 °C, 77 mL of dry THF were added dropwise, the mixture turning to orange solution. After 15 min at 0 °C, aldehyde **4** (2.0 g, 9.4 mmol) in THF (17 mL) was added dropwise to the ylide solution and the resulting betaine-ylide solution was stirred at 0 °C for 3 h. The solvent was partially removed in vacuo and 20 mL of pentane were added in order to precipitate triphenylphosphine oxide. Filtration through a pad of silica gel and removal of solvent under reduced pressure gave a crude oil. Chromatography on silica gel using PE as eluent gave the compound **9** (1.76 g, 65%). ^1H NMR (300 MHz, $CDCl_3$) δ 1.73 (2H, qn, J=6.7 Hz), 2.14 (2H, m), 2.40 (2H, m); 2.70 (2H, t, J=7.5 Hz); 3.47 (2H, t, J=7 Hz), 5.38 (1H, dt, J=11, 7 Hz), 5.49 (1H, dt, J=11, 7 Hz), 7.1 (1H, m), 7.2 (2H, m), 7.55 (1H, d, J=7 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 24.4, 27.5, 32.4, 36.2, 44.4, 124.4, 127.2, 127.6, 128.9, 129.8, 130.5, 132.8, 141.0. IR (neat): 3050, 2960, 2870, 1570, 1470, 1440, 1200, 1120, 1050, 1025, 920, 750, 700 cm^{-1}.

Following the same experimental procedure as for **E1b**, using iodide **10** as intermediate, the residue was purified by flash chromatography (PE/Et_2O=80:20) to afford **Z1b** as an oil (292 mg, 74%). ^1H NMR (300 MHz, $CDCl_3$) δ 1.4 ppm (2H, m), 1.87 (2H, q, J=7.7 Hz), 2.00 (2H, q, J=7.1 Hz); 2.30 (2H, q, J=7.7 Hz), 2.76 (2H, t, J=7.7 Hz), 3.33 (1H, t, J=7.7 Hz), 3.75 (6H, s); 5.3–5.6 (2H, dt, J=10.7, 7 Hz), 7.05 (1H, m), 7.25 (2H, m), 7.5 (1H, d, J=8 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 26.7, 27.3, 27.6, 28.2, 36.2, 51.2, 52.4, 124.4, 127.3, 127.6, 129.0, 129.9, 130.6, 132.7, 141.0, 169.8. IR (neat): 3010, 2960, 2930, 2860, 1750 (broad), 1570, 1470, 1440, 1350, 1150, 1020, 750, 660 cm^{-1}. Anal. calcd for $C_{18}H_{23}O_4Br$: C, 56.41; H, 6.05. Found: C, 56.81; H, 6.25.

4.2.5. (*Z*)-9-(2-Bromophenyl)-2-cyano-non-6-enoic acid methyl ester (Z1a).

Same experimental procedure as for **E1b**. Colorless oil (35%). ^1H NMR (200 MHz, $CDCl_3$) δ

1.40–1.60 (2H, m), 1.80–1.92 (2H, m), 1.98–2.08 (2H, m), 2.29–2.40 (2H, m), 2.76 (2H, t, J=7 Hz), 3.46 (1H, t, J=7 Hz), 3.81 (3H, s), 5.28–5.55 (2H, m), 7.0–7.09 (1H, m), 7.19–7.25 (2H, m), 7.51 (1H, d, J=7.6 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 26.2, 26.6, 27.6, 29.3, 36.1, 37.3, 53.4, 116.4, 124.4, 127.3, 127.6, 129.2, 129.5, 130.7, 132.7, 140.9, 166.6. IR (neat): 3060, 3010, 2940, 2870, 2250, 1760, 1570, 1470, 1260, 1210, 1020, 970 cm^{-1}.

4.2.6. (*Z*)-2-[7-(2-Bromophenyl)-hept-4-enyl] malononitrile (Z1c). Same experimental procedure as for **E1c**. Scale: 500 mg, 1.32 mmol of iodide **10**. The residue was purified by flash chromatography (PE/Et_2O=70:30) to afford **Z1c** as an oil (180 mg, 31%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.5 (2H, m), 1.8 (2H, m), 2.2 (2H, m), 2.35 (2H, m), 2.8 (2H, m), 3.66 (1H, t, J=7 Hz), 5.36 (1H, dt, J=10.7, 7 Hz), 5.52 (1H, dt, J=10.7, 7 Hz), 7.1 (1H, m), 7.2 (2H, m), 7.5 (1H, d, J=7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.6, 25.8, 26.4, 27.7, 30.2, 36.1, 112.6, 125, 127.5, 127.9, 128.6, 130.4, 130.9, 132.4, 141. IR (neat): 3060, 3010, 2980, 2925, 2860, 2260, 1570, 1470, 1440, 1030, 970, 750, 660 cm^{-1}. Anal. calcd for $C_{16}H_{17}N_2Br$: C, 60.58; H, 5.40. Found: C, 60.81; H, 5.53.

4.2.7. 2-Indan-1-ylcyclopentane-1,1-dicarboxylic acid dimethylester (*syn*-3b). Same experimental procedure as for **E1b**. Scale: 120 mg, 0.3 mmol of **Z1b**. The mixture was stirred at 50 °C for 15 h. The solution was directly purified by flash chromatography (PE/Et_2O=80:20) to give (82 mg, 90%) of *syn*-**3b** as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.5 (3H, m), 1.75 (2H, m), 2.0 (2H, m), 2.5 (1H, ddd, J=7.1, 8.5, 13.6 Hz), 2.85 (2H, m), 3.2 (1H, dt, J=2.9, 7.2 Hz), 3.6 (1H, dt, J=2.9, 10 Hz), 3.75 (6H, s), 7.1–7.34 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.9, 26.4, 29.9, 32.2, 34.8, 44.7, 50.1, 52.6, 62.9, 123.9, 124.3, 126.4, 126.6, 143.7, 146.9, 173.0. IR (neat): 3060, 2940, 2840, 1740. Anal. calcd for $C_{18}H_{22}O_4$: C, 71.50; H, 7.33. Found: C, 71.45; H, 7.54.

4.2.8. *cis*-3,4,4a,9,10,10a-Hexahydro-2*H*-phenanthrene-1,1-dicarbonitrile (*cis*-2c). For dicarbonitrile compound, the experimental procedure was identical as previously. Scale: 210 mg, 0.66 mmol of **Z1c**. The mixture was stirred at 50 °C for 48 h. The solution was directly purified by flash chromatography (PE/Et_2O=90:10) to give *cis*-**2c** in 58% yield (90 mg) as a pure white solid. ^{1}H NMR (300 MHz, C_6D_6) δ 0.7–1.5 (7H, M), 1.6 (1H, ddt, J=13, 3, 1.5 Hz), 1.8 (1H, dt, J=13.2, 3.7 Hz), 2.25 (1H, ddd, J=17, 12, 6 Hz), 2.45 (1H, dd, J=17, 6 Hz), 2.8 (1H, dt, J=12.6, 3.7 Hz), 7.2 (4H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.5, 21.8, 28.0, 29.0, 29.5, 36.8, 37.0, 40.0, 116.0, 126.3, 126.7, 129.0, 129.2, 134.4, 139.0. IR (KBr): 3060, 2940, 2215. Anal. calcd for $C_{16}H_{16}N_2$: C, 81.30; H, 6.82. Found: C, 81.03; H, 6.80. Mp 113–115 °C.

4.2.9. X-ray crystal structure analysis. Crystal data for *cis*-**2c** at 293 K collected on a Nonius CAD 4. $C_{16}H_{16}N_2$, M=236.32, triclinic, $P-1$, a=9.218(1), b=9.596(1), c=16.064(2) Å, α=75.50(1), β=74.89(1), γ=75.01(1)°, V=1299.3(3) Å^3, Z=4, λ(CuKα)=1.54056 Å, D_c=1.209 g cm^{-3}, 5202 reflections, 421 parameters, R=0.072 and Rw=0.127 for 4496 reflections with $I>3\sigma(I)$. CCDC registration number 221277.

4.3. Alkyne series

4.3.1. 4-(*o*-Bromophenyl)-but-1-yne (11). In a round bottom flask flushed with N_2 were placed magnesium turnings (1.42 g, 58.3 mmol) and $HgCl_2$ (48 mg, 0.18 mmol) in diethyl ether (10 mL). At room temperature, few drops of pure propargyl bromide were added. After some minutes an exothermic reaction started and the mixture was cooled to 0 °C. When the exothermic reaction has subsided, the remainder of the propargyl bromide (3.6 mL, 48 mmol) in diethyl ether (20 mL) was added dropwise over a period of 1 h, while the temperature was maintained at 0 °C. After completion of the addition, the mixture was stirred for 1 h at 0 °C. Then the Grignard solution was allowed to warm to room temperature and was added via a cannula to a solution of 2-bromobenzyl bromide (10 g, 40 mmol) in THF (50 mL). After stirring at room temperature for 3 h the mixture was quenched with water (100 mL) and extracted with diethyl ether (3×100 mL). The organic layer was washed with brine (100 mL), dried and concentrated. To eliminate the excess of 2-bromobenzyl bromide the crude oil was dissolved in DMSO and sodium cyanide (6 g) was added (3 g/100 mL DMSO). After stirring for 12 h at room temperature the formation of polar 2-bromobenzyl cyanide was occurred. The mixture was quenched with water (200 mL), extracted with Et_2O (2×150 mL) and washed with brine (150 mL). The organic layer was dried, concentrated and purified by chromatography (PE/Et_2O=90:10) to afford **11** as a colorless oil (6.23 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.0 (1H, t, J=2.5 Hz), 2.54 (2H, td, J=2.5, 7.5 Hz), 3.0 (2H, t, J=7.5 Hz); 7.1 (1H, m), 7.2 (2H, m), 7.56 (1H, d, J=7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.8, 35.1, 69.1, 83.3, 124.0, 127.4, 128.2, 130.5, 130.8, 132.8.

4.3.2. 2-[7-(2-Bromophenyl)-hept-4-ynyl] malonic acid dimethyl ester (14a). At −20 °C, *n*BuLi (2.5 M in hexane) (2.11 mL, 5.28 mmol) was added dropwise to a solution of diisopropylamine (0.88 mL, 6.22 mmol) in THF (2 mL). The LDA solution was stirred for 1 h at −20 °C and was cooled to −78 °C. A solution of **11** (1.0 g, 4.8 mmol) in THF (2 mL) was added via a canula to the LDA solution. The mixture was stirred for 1 h at −78 °C. Finally, 1-bromo-3-chloropropane (0.71 mL, 7.2 mmol) was added via a syringe. The solution was allowed to warm at RT and was refluxed overnight. The mixture was cooled to RT, quenched with saturated aqueous NH_4Cl solution, extracted with Et_2O (3×50 mL). The organic layer was washed with brine (50 mL), dried and concentrated. The excess of 1-bromo-3-chloropropane was eliminated by distillation under atmospheric pressure. The starting material **11** was trapped in Et_2O (12 h, RT) by an aqueous solution of silver nitrate (14% in weight). The formation of water insoluble silver acetylide occurred. The mixture was diluted with Et_2O (20 mL), washed with water (10 mL) and brine (10 mL). The organic layer was dried, concentrated and purified by chromatography using PE as eluent to afford **12** as an yellow oil (1.48 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.9 ppm (2H, t, J=7.3 Hz), 2.33 (2H, m), 2.48 (2H, m); 2.93 (2H, t, J=7.4 Hz), 3.58 (2H, t, J=6.6 Hz), 7.10 (1H, m), 7.25 (2H, m), 7.5 (1H, d, J=7.9 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.1, 19.6, 32.2, 35.6, 44.4, 71.0, 80.0, 124.0, 127.2, 128.1, 130.7, 132.8, 141.0. IR (neat):

3060, 2960, 2920, 2860, 1570, 1470, 1440, 1290, 1120, 1020, 750 cm^{-1}.

12 (433 mg, 1.52 mmol) and sodium iodide (0.5 g, 3.3 mmol) were dissolved in acetone (10 mL) The solution was refluxed overnight and cooled to room temperature. The mixture was diluted in Et_2O (50 mL) and washed with saturated aqueous $Na_2S_2O_3$ solution (10 mL). The organic layer was dried, concentrated and purified by chromatography using PE to afford **13** as a pale yellow oil (507 mg, 88%). ^1H NMR (300 MHz, $CDCl_3$) δ 1.93 (2H, m), 2.27 (2H, m), 2.48 (2H, m), 2.93 (2H, t, J=7.4 Hz), 3.23 (2H, t, J=6.6 Hz), 7.1 (1H, m), 7.25 (2H, m), 7.52 (1H, d, J=7.9 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 6.3, 19.1, 19.7, 32.3, 35.5, 70, 80, 124, 127.3, 128, 130.7, 132.7, 141. IR (neat): 3060, 2960, 2920, 2860, 1570, 1470, 1440, 1220, 1020, 750, cm^{-1}.

At room temperature, NaH (90.5 mg, 3.77 mmol) washed in pentane was suspended in THF (25 mL) and dimethylmalonate (0.48 mL, 4.17 mmol) was added dropwise. The sodium enolate solution was stirred for 30 min at RT and was added dropwise to a solution of **13** (750 mg, 1.99 mmol) in DMF (15 mL). The resulting mixture was refluxed overnight and cooled to RT. The mixture was quenched with saturated aqueous NH_4Cl solution, extracted with Et_2O (3×50 mL) and washed with brine (50 mL). The organic layer was dried, concentrated and purified by chromatography (PE/Et_2O=90:10) to afford **14a** as a colorless oil (593 mg, 80%). ^1H NMR (200 MHz, $CDCl_3$) δ 1.48 (2H, m), 1.98 (2H, td, J=7.5 Hz), 2.19 (2H, tt, J=2.2, 7.5 Hz), 2.48 (2H, tt, J=2.2, 7.5 Hz), 2.91 (2H, t, J=7.5 Hz), 3.38 (1H, t, J=7.5 Hz), 3.75 (6H, s), 7.1–7.3 (3H, m), 7.51 (1H, d, J=8.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$) δ 18.5, 19.2, 26.6, 28.0, 35.7, 51.3, 52.5, 79.7, 80.1, 124.4, 127.3, 128.0, 130.8, 132.7, 140.0, 169.7. IR (neat): 3000, 2950, 1750 (br), 1440, 1390, 1200, 1150, 1030, 850, 755 cm^{-1}. Anal. calcd for $C_{18}H_{21}O_4Br$: C, 56.70; H, 5.56. Found: C, 56.54; H, 5.65.

4.3.3. 2-[7-(2-Bromophenyl)-hept-4-ynyl] malononitrile (14b). Under N_2 at 0 °C malononitrile (131 mg, 1.98 mmol) in THF (1 mL) was added dropwise to a solution of NaH washed in pentane (44 mg, 1.84 mmol) in THF (3.5 mL). After stirring at RT for 30 min, the sodium enolate solution was added dropwise to a solution of **13** (500 mg, 1.32 mmol) in THF (1 mL). The resulting mixture was refluxed overnight and cooled to RT. The solution was quenched with saturated aqueous NH_4Cl solution, extracted with Et_2O (3×50 mL) and washed with brine (50 mL). The organic layer was dried, concentrated and purified by chromatography (PE/Et_2O=85:15) to afford **14b** as an yellow oil (186 mg, 45%). ^1H NMR (200 MHz, $CDCl_3$) δ 1.78 (2H, m), 2.07 (2H, m), 2.28 (2H, m), 2.51 (2H, m), 2.94 (2H, t, J=7.3 Hz), 3.74 (1H, t, J=7 Hz), 7.05 (1H, m), 7.2 (2H,m), 7.51 (1H, d, J=8.1 Hz). ^{13}C NMR (50 MHz,$CDCl_3$): δ 17.7, 19.1, 22.3, 25.4. 29.9, 35.4, 78.7, 81.2, 112.5, 124.5, 127.4, 128.2, 130.7, 132.1, 139.8. IR (neat): 3060, 2950, 2860, 2260, 1440, 1030, 750 cm^{-1}. Anal. calcd for $C_{16}H_{15}N_2Br$: C, 60.96; H, 4.80. Found: C, 61.37; H, 5.1.

4.3.4. 2-Indan-1-ylidenecyclopentane-1,1-dicarboxylic acid dimethylester (15a). The palladium zero complex was preformed using the same procedure as the olefinic compound (concentration 0.05 M in DMSO). *t*BuOK (41 mg, 0.36 mmol) and 18-C-6 crown ether in DMSO (0.33 mL) were added to a solution of **14a** (125 mg, 0.33 mmol) in DMSO (0.66 mL). The sodium enolate solution was stirred for 30 min at RT. The palladium zero solution was added via a cannula to the sodium enolate solution and the mixture was stirred at 90 °C for 2 h. The mixture was cooled to RT, quenched with water, extracted with Et_2O (2×10 mL) and the organic layer was washed with brine (10 mL), dried, concentrated and purified by chromatography (PE/Et_2O=90:10) to afford **15a** as an yellow oil (57 mg, 58%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.87 (2H, t, J=7.2 Hz), 2.47 (2H, t, J=7.2 Hz), 2.72 (2H, m), 2.82 (2H, t, J=6.3 Hz), 2.97 (2H, t, J=6.3 Hz), 3.70 (6H, s), 7.22 (3H, m), 7.53 (1H, d, J=6.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.3, 30.68, 30.73, 33.2, 38.8, 52.7, 60.7, 124.9, 125.1, 126.2, 127.4, 132.7, 140.2, 142.3, 147.6, 171.9. MS *m*/*z*: 300.05 (16), 236 (12), 210.15 (16), 209.05 (100), 208.05 (54), 207.05 (12), 181.15 (14), 165.05 (13). IR (neat): 3060, 2940, 1750, 1430, 1250.

4.3.5. 1-Cyano-2-indan-1-ylidene cyclopentanecarboxylic acid methyl ester (15b). For dicarbonitrile compound **14b** (93 mg, 0.32 mmol) the experimental procedure was identical as previously, the mixture was stirred at 90 °C for 2 h and cooled to RT. The mixture was filtered through silica gel (PE/Et_2O=90:10) and was concentrated to afford **15b** as a transparent crystalline solid (34 mg, 50%). ^1H NMR (300 MHz, $CDCl_3$): δ 2.12 (2H, q, J=6.9 Hz), 2.64 (2H, t, J=7 Hz), 2.89 (2H, t, J=7 Hz), 3.15 (4H, s), 7.1–7.4 (3H, m), 7.5 (1H, d, J=7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.6, 30.6, 30.7, 31.9, 37.8, 41.0, 115.0, 124.6, 125.3, 125.6, 126.7, 129.2, 140.1, 144.5, 148.8. MS *m*/*z*: 234 (74), 206 (35), 205 (20), 178 (10), 156 (100), 155 (27), 116 (21), 115 (32). IR (neat): 3080, 2940, 2860, 2220. Mp 137–139 °C.

4.3.6. X-ray crystal structure analysis. Crystal data for **15b** at 295 K collected on a Nonius Kappa CCD. $C_{16}H_{14}N_2$, M=234.3, triclinic, $P-1$, a=7.546(2), b=10.267(2), c=17.602(4) Å, α=100.67(3), β=96.03(3), γ=108.85(3)°, V=1248.3(4) $Å^3$, Z=4, λ (Mo Kα)=0.71073 Å, D_c= 1.247 g cm^{-3}, 5664 reflections, 325 parameters, R=0.0502 and Rw=0.1072 for 1776 reflections with $I>4\sigma(I)$. CCDC registration number 221278.

4.4. X-ray diffraction studies

Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre (see registration numbers in experimental). Copies of the data can be obtained on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (e-mail: deposit@ccdc.cam.ac.uk).

Acknowledgements

D. Bruyère wishes to thank the Ministère de l'Education Nationale, de la Recherche et de la Technologie for a fellowship. The authors thank Prof. René Faure for X-ray diffraction analysis.

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Tetrahedron

Synthesis of analogues of calicheamicin and neocarzinostatin chromophore

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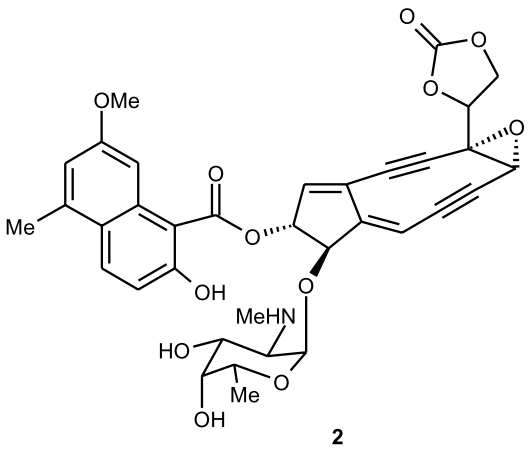
Received 3 December 2003; revised 6 February 2004; accepted 4 March 2004

Abstract—The work presents a synthetic route to the CD ring of calicheamicin and in the case of neocarzinostatin an approach to a functionalised cyclopentane-1,3-diol containing the naturally occurring naphthoate and a glucosamine motif. In the case of the NCS derivative some biological activity (cytotoxicity) was observed.

1. Introduction

Calicheamicin γ_1^I **1** from the soil microorganism *Micromonospora echinospora* has been the subject of numerous synthetic and biological studies.[1] Much of its biological activity can be ascribed to the way in which it binds specifically to 5′-TCCT and 5′-ACCT sequences in the minor groove of DNA,[2] and a limited number of analogues have also been prepared with a view to establish structure activity relationships. In particular, both Nicolaou[3] and Danishefsky[4] have prepared glycones with modifications to the A, B and E rings. Moutel and Prandi have prepared AB rings with acyclic E ring analogues, and a DCB analogue where an ester oxygen replaces the thioester linkage.[5] But there has been no investigation of the effects of changing the D-ring. We have devised some novel and flexible chemistry for the production of a range of CD-analogues.

Neocarzinostatin **2** from *Streptomyces carzinostaticus* was in fact the first member of the family of enediynes to be isolated,[6] and also has a range of biological activities including anti-proliferative activity.[7] Its central naphthoate is known to bind duplex DNA intercalatively,[8] but the role of the glycosyl unit has not been established. This natural product has also been the target of numerous synthetic studies[9] and one successful synthesis by Myers.[10] We have prepared a core structure that includes a homochiral polysubstituted cyclopentane with sugar and naphthoate units attached, in order to explore the essential features required for selective DNA duplex binding.



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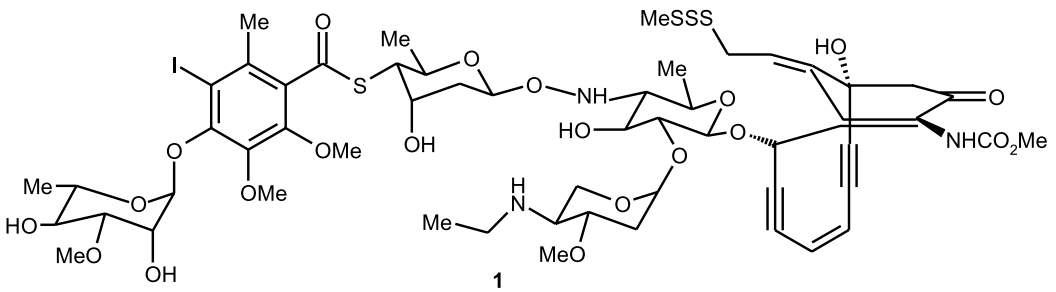

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Keywords: Neocarzinostatin; Calicheamicin; Cyclopentane-1,3-diol.
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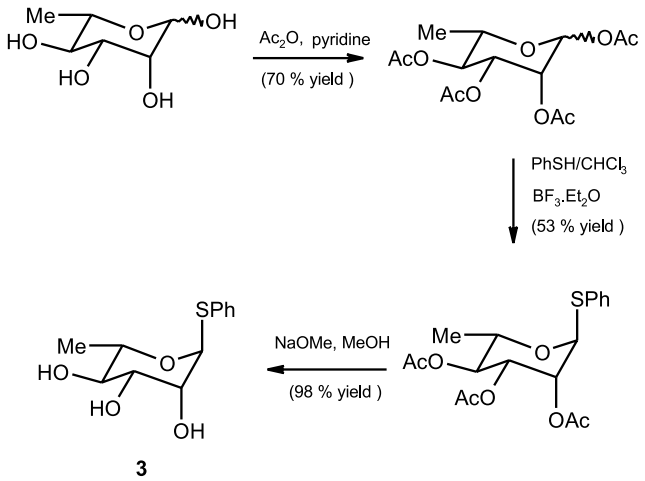
doi:10.1016/j.tet.2004.03.021

2. Results and discussion

Our key intermediate for the production of the CD ring system of calicheamicin was 1-thiophenyl-α-L-rhamnopyranoside **3** prepared from L-rhamnose via the sequence shown in Scheme 1 (overall yield for the three steps 35%).



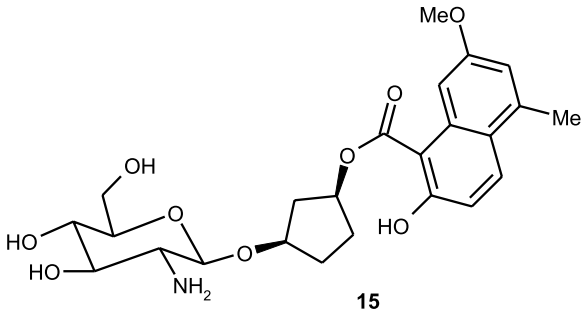
3

Scheme 1.

Compound **3** was then converted into the bis-acetal **4** using Ley's technology[11] (butan-2,3-dione in MeOH containing camphor sulphonic acid, 58%), and thence into the 2-methyl derivative **5** (MeI/NaH, 67%), and the 2-acetate **6** (acetic anhydride/pyridine, 76%). The thiophenyl group was now removed using aqueous NBS to provide an inseparable mixture of α:β anomers of the 2-methyl-derivative **7** (Scheme 2) (ratio around 1:1, anomeric ^{1}H singlet and doublet J=1.2 Hz); and a 6:1 ratio of anomers (major anomer ^{1}H singlet and minor anomer ^{1}H doublet J=1.2 Hz) of the 2-acetate **11** (again inseparable by flash chromatography), in yields of around 60–80% in each case. Presumably formation of an intermediate acetoxonium species improves the stereoselectivity for the formation of the α-anomer of **11**.

A Mitsunobu reaction with the phenol **8** which had been previously[12] synthesised by us was carried out on the free anomeric alcohols **7** (DEAD, Ph_3P, THF), and a 3:1 ratio of anomers of the protected CD-ring analogue **9** was obtained (in 83% yield). Removal of the bis-acetal using brief exposure to aqueous TFA provided a 3:1 anomeric mixture of the desired CD-ring analogue **10** (63%). These anomers were separated and NMR analysis suggested that the minor product was the desired α-anomer, since the relative δ-values for H-5 were 4.2 ppm (minor compound) and 3.1 ppm (major compound) reflecting the anisotropic effect of the aryl group upon H-5. In contrast, a Mitsunobu reaction on anomeric alcohols **11** provided none of the desired product, but reaction of the trichloroimidates **12** with the phenol **8** produced the protected CD-ring analogue **13**. Removal of the bis-acetal (as before) provided the other CD-ring analogue **14** as one pure anomer in an overall yield of 19% for the three steps. Since H-5 resonated at 4.2 ppm, we believe this to be the α-anomer, and this would be consistent with participation of an acetoxonium intermediate. While the yields of these reactions clearly require optimisation, this approach allows access to novel CD-ring analogues of calicheamicin which possess (in principle) a range of substituents at C-2, 3 and 4 of the rhamnose ring. Despite the efforts of Nicolaou,[3] Danishefski,[4] and Prandi,[5] this possibility has not been available before our work.

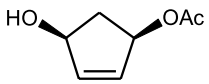
Our initial target in the neocarzinostatin series was the aminoglucoside of hydroxycyclopentyl naphthoate **15** in order to assess its biological activity.



15

The ultimate intention was the preparation of a library of core structures which carry conventional cytotoxic drugs and various carbohydrate moieties, and an investigation of the effects of these substituents on the intercalative binding of the naphthoate unit to duplex DNA. The naphthoic acid component **16** was prepared essentially according to the route described by Myers[13] (Scheme 3), though the initial Heck reaction was improved markedly through the use of DMF as co-solvent (time of reaction reduced from 12 to 2 h).

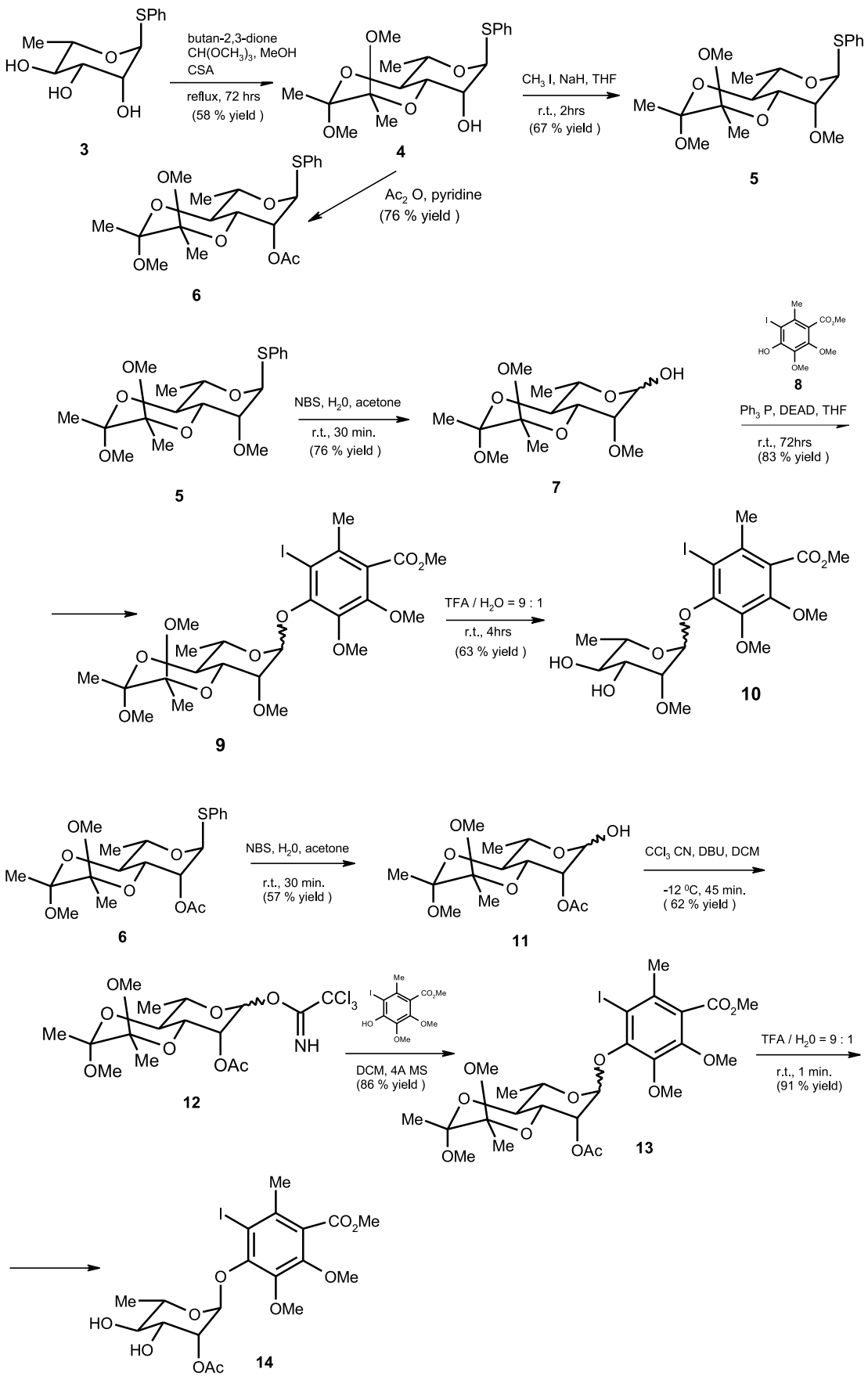
The homochiral hydroxycyclopent-2-enylacetate derivative **17** was prepared according to our optimisation[14] of an earlier preparation.[15]



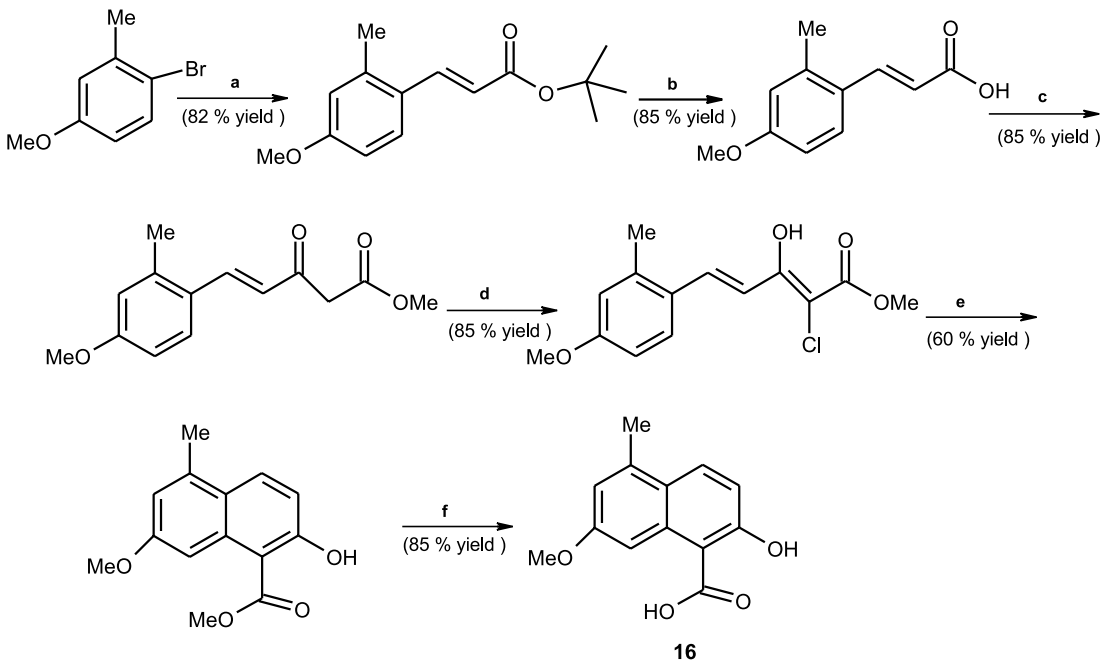
17

With these key substrates in hand, we employed the fully protected thioacetal of *N*-phthalimidoglucosamine **18** (prepared according to Scheme 4) as a model carbohydrate for investigation of the coupling methodology.

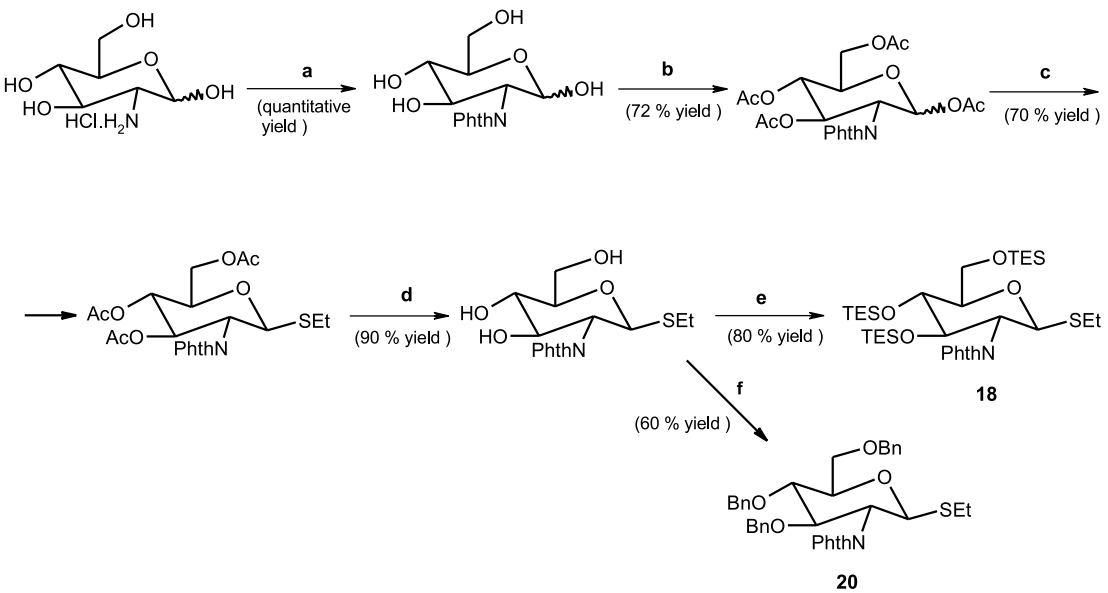
Reaction of this thioacetal with (1*R*,3*S*)-(+)-1-acetoxycyclopent-2-en-3-ol **17** in the presence of *N*-iodosuccinimide and catalytic BF_3 etherate[16] yielded the desired glycoside **19** (R=triethylsilyl) exclusively as the β-anomer. Unfortunately, during the work-up, one or more of the triethylsilyl protecting groups were lost and a complex mixture of products was obtained. In consequence, the fully benzylated thioglycoside **20** (R=benzyl) was prepared and this could be converted into the glycoside **21** (R=benzyl) (60%). Removal of the acetate with methanolic potassium carbonate to yield **22** was followed by coupling with **16** using DCC and DMAP in dichloromethane and thence conversion into the desired naphthoate ester **23** (60%) (Scheme 5). This was treated with Pearlman's catalyst


butan-2,3-dione
CH(OCH3)3, MeOH
CSA
reflux, 72 hrs
(58 % yield)
CH3 I, NaH, THF
r.t., 2hrs
(67 % yield)
Ac2 O, pyridine
(76 % yield)
NBS, H20, acetone
r.t., 30 min.
(76 % yield)
Ph3 P, DEAD, THF
r.t., 72hrs
(83 % yield)
TFA / H2O = 9 : 1
r.t., 4hrs
(63 % yield)
NBS, H20, acetone
r.t., 30 min.
(57 % yield)
CCl3 CN, DBU, DCM
-12 0C, 45 min.
(62 % yield)
DCM, 4A MS
(86 % yield)
TFA / H20 = 9 : 1
r.t., 1 min.
(91 % yield)
3
4
5
6
7
8
9
10
11
12
13
14

Scheme 2.



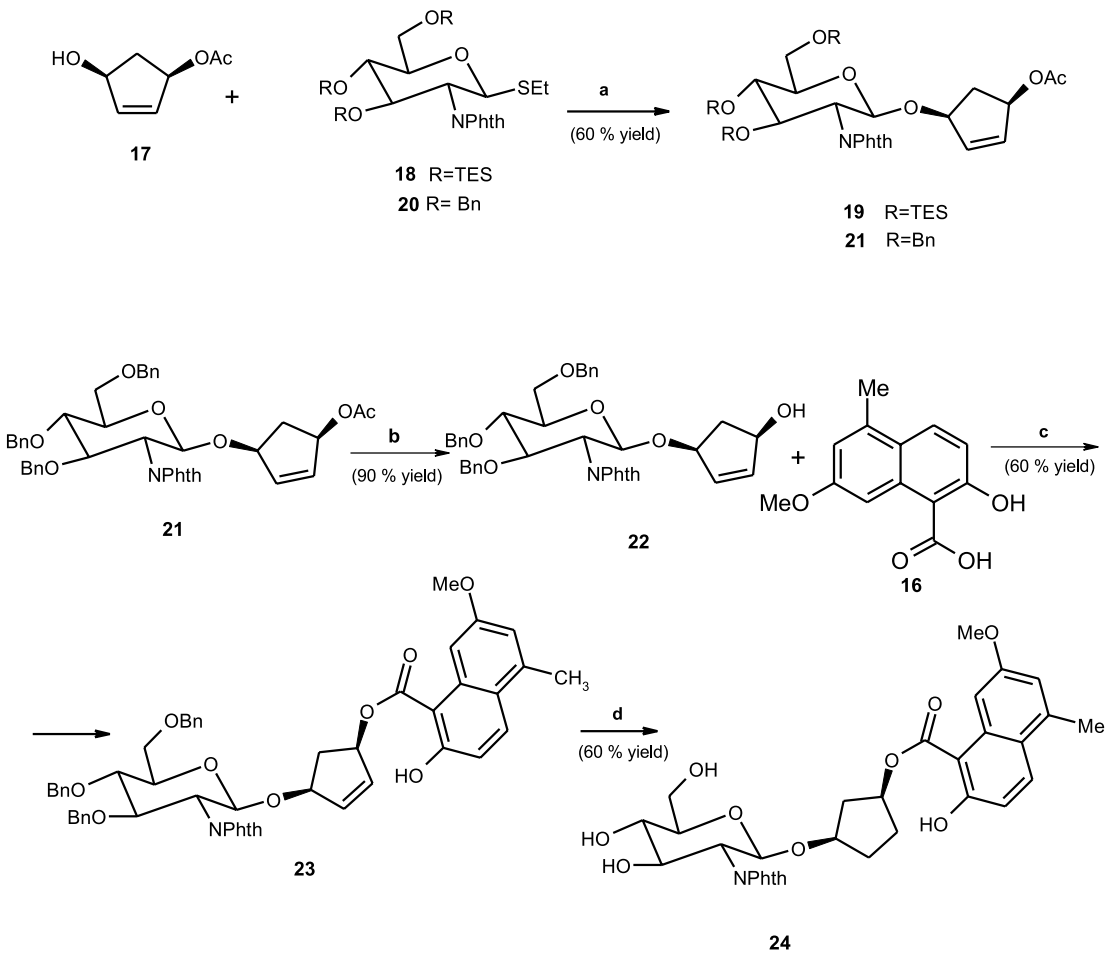
Scheme 3. Reagents and conditions: (a) *tert*-butyl acrylate, Et_3N, P(*o*-tol)$_3$, Pd(OAc)$_2$, 110 °C, 82%; (b) TFA, DCM, rt, quantitative; (c) CDI, magnesium methyl malonate, THF, rt, 85%; (d) SO_2Cl_2, C_6H_6, 70 °C (85%); (e) *hν*, Et_3N, MeOH, rt, 60%; (f) NaOH, 3:1 MeOH/H_2O, 80 °C, 85%.



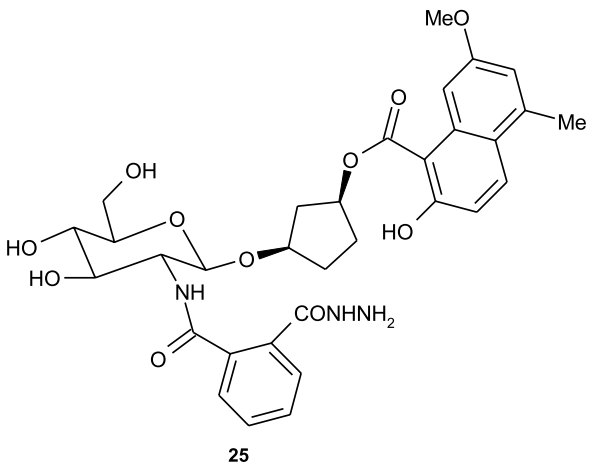
Scheme 4. (a) NaOH, phthalic anhydride, H_2O, overnight, rt, quantitative; (b) Ac_2O, pyridine, DMAP, overnight, 0 °C→rt, 72%; (c) EtSH, $CHCl_3$, $BF_3{\cdot}Et_2O$, 0 °C→rt, reflux 3 h, 70%; (d) NaOMe 25% (w/v) pH 8, MeOH, 90%; (e) triethylsilyltrifluoromethansulfonate, 2,6-lutidine, DMF, 0 °C, 4 h, 80%; (f) BnBr, Bu_4NI, NaH, DMF, 0 °C→rt, overnight, 60%.

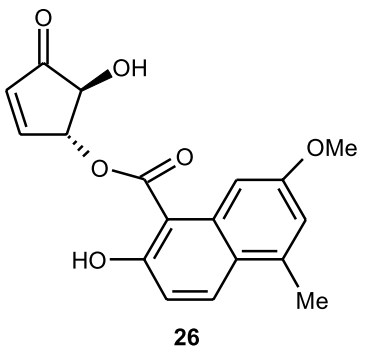
(palladium hydroxide) in methanol and in atmosphere of H_2 to provide the fully debenzylated adduct **24**.

Finally, removal of the phthalimide group was attempted using methanolic hydrazine. However, although partial hydrolysis was easily effected to provide the analogue **25** (ES^+: 640.2), further reaction led to production of only trace amounts of the desired analogue **15** (ES^+: 478.2). Clearly further work will be required to optimise this chemistry. Nonetheless, this work has established a viable route for the synthesis of our core structure, and future work will seek to produce a library of neocarzinostatin analogues in order to establish the optimum structure required to maximise binding to DNA.



Scheme 5. (a) NIS, $BF_3{\cdot}Et_2O$, 4 Å MS, DCM, 20 min, 60%; (b) 1 M K_2CO_3, MeOH, 90%; (c) DCC, DMAP, 0 °C→rt, 60%; (d) $Pd(OH)_2$, H_2, EtOH, 60%.





2.1. Biological evaluation

The calicheamicin CD ring analogues **10** and **14** and the neocarzinostatin core analogue **25** were evaluated for cytotoxic activity against a range of cancer cell lines in vitro. Cells were plated in RPMI1640 medium supplemented with foetal calf serum and 1% penicillin/streptomycin (1×10^3 cells/well in 24 well plates). Following a 24 h attachment period at 37°, the medium was removed from the wells and replaced with 1 ml of medium containing the appropriate compound at a range of concentrations. Cell counts were carried out using a Coulter counter and cell growth curves were plotted for a 7-day period. While compounds **10** and **14** exhibited no significant activity, compound **24** did exhibit modest activity at the level of 50 μM.

This compares with the results of Caddick and co-workers who reported[17] very recently that the non-glycosylated analogue **26** exhibited activity against a range of cancer cell lines with IC_{50}s typically in the range 2.5–5.0 μM.

3. Experimental

3.1. General

IR were recorded using a Perkin–Elmer 881 series double

beam spectrophotometer, and samples were run as thin films or in solution using NaCl plates. Low resolution and accurate mass data were recorded on a VG Autospec spectrometer and elemental analysis was carried out using a Perkin–Elmer 2400 CHN Microanalyser by ASEP, Queen's University Belfast. All compounds for which accurate mass data are provided were homogeneous by two-dimensional TLC and exhibited no spurious signals in the ^{1}H NMR spectra at 300 MHz. NMR spectra were recorded using Bruker DPX 300 and DRX 500 instruments. $[\alpha]_D$ Values are given in units of 10^{-1}deg cm^2 g^{-1}. Solvents were dried by distillation from calcium hydride (DCM, dichloromethane) or from sodium-benzophenone (THF, diethyl ether). Petrol refers to petroleum ether boiling range 40–60 °C. Compound **3** was prepared according to Ref. 18.

3.1.1. (2′*S*), (3′*S*)-Phenyl-3,4-*O*-2′,3′-dimethoxybutane-2′,3′-diyl-1-thio-α-L-rhamnopyranoside 4. Phenyl 1-thio-α-L-rhamnopyranoside **3** (4.1 g, 16 mmol) was dissolved in analar methanol (110 ml), and under a flow of argon, trimethyl orthoformate (6.9 ml, 45.2 mmol), butan-2,3-dione (1.76 ml, 20.1 mmol), and camphor sulphonic acid (240 mg-catalytic) were added sequentially. The reaction mixture was then refluxed for 72 h, after which time the *trans* diol had been protected. Upon cooling, the reaction was quenched by the addition of triethylamine to pH=7, and the solution was immediately concentrated onto flash silica for purification. The title compound (**4**) was isolated as a yellow foam (3.4 g, 58%). IR ($CHCl_3$) *ν*: 3450, 2949, 2833. ^{1}H NMR ($CDCl_3$, 500 MHz): *δ* 1.26 (3H, d, *J*=6.2 Hz, C-5–CH_3), 1.31, 1.32 (2s, 6H, C-2′–CH_3, C-3′–CH_3), 3.24, 3.30 (2s, 6H, C-2′–OCH_3, C-3′–OCH_3), 3.77 (at, 1H, *J*=10.2 Hz, H-4), 3.97 (dd, 1H, *J*=3.0, 10.2 Hz, H-3), 4.18 (dd, 1H, *J*=1.2, 3.0 Hz, H-2) 4.25 (m, 1H, H-5), 5.49 (as, 1H, H-1) 7.24 (3H, m, S–C_6H_5), 7.44 (2H, m, S–C_6H_5. ^{13}C NMR ($CDCl_3$, 500 MHz): *δ* 16.4, 17.6, 17.7, 47.6, 48.1, 67.7, 68.5, 68.7, 71.4, 87.8, 99.8, 100.3, 127.3, 134.3. HRMS (CI): calcd for $C_{18}H_{26}O_6S$ [M$^+$] 370.1450. Found: 370.1439. $[\alpha]_D^{20}$=−300.0 (*c*=1.31, $CHCl_3$).

3.1.2. 2-*O*-Methyl-(2′*S*), (3′*S*)-phenyl-3,4-*O*-2′,3′-dimethoxybutane-2′,3′-diyl-1-thio-α-L-rhamnopyranoside 5. The *trans* protected thioglycoside **4** (2.41 g, 65 mmol) in dry THF (50 ml) was added to sodium hydride (4.62 g, 97.5 mmol) under a flow of argon. Iodomethane (1.6 ml, 260 mmol) was then added dropwise, and the reaction mixture stirred at ambient temperature for 2 h. The reaction was then quenched by cooling the solution to 0 °C followed by a slow dropwise addition of methanol (20 ml). After evaporation to dryness, the residue was dissolved in DCM (100 ml), and the organic layer sequentially washed with H_2O (2×100 ml) and brine (1×100 ml). It was then dried ($MgSO_4$), filtered and concentrated onto flash silica for purification to yield the title compound as a pale yellow crystalline solid (1.7 g, 67%). IR ($CHCl_3$) *ν*: 2949, 2832, 1259. ^{1}H NMR ($CDCl_3$, 500 MHz): *δ* 1.28 (d, 3H, *J*=6.2 Hz, C-5–CH_3), 1.31, 1.34 (2s, 6H, C-2′–CH_3, C-3′–CH_3), 3.25, 3.31 (2s, 6H, C-2′–OCH_3, C-3′–OCH_3), 3.48 (s, 3H, C-2–OCH_3) 3.73 (dd, 1H, *J*=1.4, 3.0 Hz, H-2), 3.76 (at, 1H, *J*=10.3 Hz, H-4), 3.95 (dd, 1H, *J*=3.0, 10.3 Hz, H-3), 4.21 (m, 1H, H-5) 4.25 (d, 1H, *J*=1.4 Hz, H-1), 7.24 (3H, m, S–C_6H_5), 7.45 (2H, m, S–C_6H_5). ^{13}C NMR ($CDCl_3$, 125 MHz): *δ* 16.6, 17.8, 17.9, 47.7, 47.9, 58.5, 67.7, 68.6, 68.8, 80.5, 85.6, 99.5, 99.9, 127.2, 134.9. HRMS (CI): calcd for $C_{19}H_{28}O_6S$ [M$^+$] 384.1607. Found: 384.1595. $[\alpha]_D^{20}$=−270.4 (*c*=0.71, $CHCl_3$). Mp=124.4 °C.

3.1.3. 2-*O*-Acetyl-(2′*S*) (3′*S*)-phenyl-3,4-*O*-2′,3′-dimethoxybutane-2′,3′-diyl-1-thio-α-L-rhamnopyranoside 6. (2′*S*), (3′*S*)-Phenyl-3,4-*O*- 2′,3′-dimethoxybutane-2′,3′-diyl-1-thio-α-L-rhamnopyranoside **4** (1.91 g. 5.16 mmol) was dissolved in pyridine (5.4 ml), and the reaction mixture cooled to 0 °C. Acetic anhydride (1.47 ml) was added dropwise, and the reaction mixture allowed to warm to room temperature. After stirring at ambient temperature overnight, TLC showed complete reaction. The reaction mixture was then cooled to 4 °C, and quenched by the dropwise addition of methanol (6 ml). The pyridine was then removed under high vacuum and the residue was re-dissolved in CH_2Cl_2 (60 ml). The organic layer was washed sequentially with 1 M HCl (2×60 ml), sat. aq. $NaHCO_3$ (1×60 ml), H_2O (1×60 ml) and brine (1×60 ml), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography yielded the desired compound **6** as a white foam (1.61 g, 76%). IR ($CHCl_3$) *ν*: 2952, 1748, 1236. ^{1}H NMR ($CDCl_3$, 500 MHz): *δ* 1.24 (d, 3H, *J*=6.2 Hz, C-5–CH_3), 1.28, 1.31 (2s, 6H, C-2′–CH_3, C-3′–CH_3), 2.13 (s, 3H, C-2–OCOCH_3), 3.27, 3.29 (2s, 6H, C-2′–OCH_3, C-3′–OCH_3), 3.71 (at, 1H, *J*=10.2 Hz, H-4), 4.04 (dd, 1H, *J*=3.2, 10.2 Hz, H-3), 4.25 (m, 1H, H-5) 5.29 (dd, 1H, *J*=1.3, 3.2 Hz, H-2), 5.41 (as, 1H, H-1), 7.25 (3H, m, S–C_6H_5), 7.44 (2H, m, S–C_6H_5). ^{13}C NMR ($CDCl_3$, 125 MHz): *δ* 16.9, 17.9, 18.2, 21.6, 48.1, 48.5, 67.1, 68.3, 69.3, 72.8, 86.9, 100.2, 100.6, 128.0, 134.5, 170.9. HRMS (CI): calcd for $C_{19}H_{25}O_6S_1$ (M$^+$−OCH$_3$) 381.1371. Found: 381.1359. $[\alpha]_D^{20}$=−215.8 (*c*=0.76, $CHCl_3$).

3.1.4. 2-*O*-Methyl-(2′*S*), (3′*S*)-3,4-*O*-2′,3′-dimethoxybutane-2′,3′-diyl-L-rhamnopyranoside 7. Under a flow of argon, the protected thioglycoside **5** (300 mg, 0.78 mmol) was dissolved in analar acetone (10 ml), and cooled to 0 °C. *N*-Bromosuccinimide (0.278 g, 1.56 mmol) was added followed almost immediately by the addition of H_2O (0.5 ml). An immediate colour change from an orange solution to a yellow solution was apparent. Stirring was continued for a further 30 min, after which time a clear solution showed the end of the reaction. The acetone was removed under reduced pressure, and the residue re-dissolved in EtOAc (50 ml). The organic layer was washed with sat. aq. $NaHCO_3$ solution (2×50 ml) and brine (1×50 ml), dried ($MgSO_4$), filtered, and immediately concentrated onto flash silica for purification.

An intractable mixture of anomers of unknown stereochemistry was obtained, in a ratio of 1:1.2 (apparent from NMR studies) (178 mg, 76%). It has been found that the sugar proton signals for the two anomers, α, β, are impossible to distinguish. However the major anomer signals for other protons are highlighted in bold, and no integration values are noted. IR ($CHCl_3$) *ν*: 3416, 2834, 1453. ^{1}H NMR ($CDCl_3$, 500 MHz): *δ* 1.14 (d, *J*=6.6 Hz, C-5–CH_3), **1.18** (d, *J*=6.1 Hz, C-5–CH_3), **1.21**, **1.21** (2s, C-2′–CH_3, C-3′–CH_3), 1.23, 1.25 (2s, C-2′–CH_3, C-3′–CH_3), **3.17**, **3.18** (2 s, C-2′–OCH_3, C-3′–OCH_3), 3.19, 3.20 (2s, C-2′–OCH_3, C-3′–OCH_3), 3.32–4.00 (m, C-2, C-3,

C-4, C-5), **3.44** (s, C-2–OCH_3) 3.58 (s, C-2–OCH_3), 4.66 (as, 1H, C-1), **5.17** (d, 1.2H, J=1.2 Hz, C-1). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 15.6, 15.7, 16.3, 16.4, 16.6, 16.7, 46.6, 46.7, 46.9, 58.3, 60.3, 66.0, 66.9, 67.0, 67.7, 69.6, 70.6, 77.8, 78.1, 91.9, 92.7, 98.5, 98.5, 98.7, 98.8. HRMS (CI): calcd for $C_{13}H_{21}O_7$ [M^+-CH_3] 277.1287. Found: 277.1274.

3.1.5. 2-*O*-Acetyl-(2′*S*) (3′*S*)-3,4-*O*-2′,3′-dimethoxybutane-2′,3′-diyl-L-rhamnopyranoside 11. The thioglycoside **6** (1.61 g; 3.90 mmol) was dissolved in acetone (50 ml), and cooled to 0 °C. *N*-Bromosuccinimide (1.42 g; 7.97 mmol) was added followed immediately by the addition of H_2O (1.42 ml). A colour change from an orange solution to a yellow solution was apparent. Stirring was continued for a further 30 min, after which time a clear solution was visible. TLC showed formation of the desired alcohol as well as the presence of unreacted starting material. Stirring was continued for a further 30 min at room temperature, however, no further change was noted. The reaction mixture was immediately concentrated onto silica for purification by flash column chromatography. Yield 711 mg, (57%). The anomeric ratio was 6:1, α/β. The ^{1}H NMR signals are those for the α-anomer. The only two discernible 'β' signals were those of the anomeric proton and H-2, which are noted separately below. IR ($CHCl_3$) ν: 3440, 2932, 1732, 1373. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.26 (d, 3H, J=6.2 Hz, C-5–CH_3), 1.27, 1.29 (2s, 6H, C-2′–CH_3, C-3′–CH_3), 2.15 (s, 3H, C-2–OCOCH_3), 3.25, 3.26 (2s, 6H, C-2′–OCH_3, C-3′–OCH_3), 3.63 (at, 1H, J=10.0 Hz, H-4), 4.00 (m, 1H, H-5), 4.14 (dd, 1H, J=3.3, 10.0 Hz, H-3), 5.05 (dd, 1H, J=1.5, 3.3 Hz, H-2), 5.15 (as, 1H, H-1). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 15.6, 16.7, 16.8, 20.2, 46.7, 47.0, 64.5, 65.6, 67.8, 70.2, 91.7, 98.7, 99.1, 169.8. β-*anomer*. 4.90 (d, 1H, J=1.2 Hz, H-1), 5.29 (dd, 1H, J=1.2, 3.1 Hz, H-2). HRMS (CI): calcd for $C_{13}H_{21}O_7$ (M^+-OCH_3) 289.1287. Found: 289.1285.

3.1.6. Methyl 4-[2-*O*-methyl-(2′*S*), (3′*S*)-3,4-*O*- 2′,3′-dimethoxybutane-2′,3′-diyl-L-rhamnopyranosyl]-oxy-5-iodo-2,3-dimethoxy-6-methyl benzoate 9. Under a flow of argon, the phenol **8** (80 mg, 0.23 mmol) and the alcohol **7** (100 mg, 0.34 mmol) were dissolved in dry THF (2 ml) and triphenylphosphine (89 mg, 0.34 mmol) was added. The reaction mixture was then cooled to 0 °C, and DEAD (53.8 μl, 0.34 mmol) was added dropwise. The reaction mixture was then allowed to warm to room temperature and stirred at ambient temperature for 72 h. TLC showed major conversion to a new product, running between the alcohol and the phenol. This was then followed by the removal of solvents under reduced pressure and concentration onto flash silica for purification, yielding the title compound as a yellow oil (119 mg, 83%).

The following data shows an intractable mixture of anomers. It can be clearly seen from the ^{1}H NMR spectrum that there is a mixture in a ratio of 3:1, and investigation of the coupling constants reveals a probable ratio of 3:1, β/α. The signals for the major β-anomer in the proton spectrum are highlighted in bold type. IR ($CHCl_3$) ν: 2946, 1734, 1457. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.26–1.36 (m, C-5–CH_3, C-2′–CH_3, C-3′–CH_3), 2.29, **2.30** (2s, Ar–CH_3), **3.17**, 3.20, **3.21**, 3.26 (4s, C-2′–OCH_3, C-3′–OCH_3), 3.17–4.30 (m, H-2, H-3, H-4, H-5), 3.48 (s, Ar–CO_2CH_3), **3.70** (s, Ar–CO_2CH_3), 3.77 (s, Ar–OCH_3), **3.80**, **3.81** (2s, Ar–OCH_3), 3.84 (s, Ar–OCH_3), 3.85 (s, C-2–OCH_3), 3.86 (s, C-2–OCH_3), **4.99** (d, 0.75H, J=0.68 Hz, H-1), 5.50 (d, 0.25H, J=1.34 Hz, H-1). ^{13}C NMR ($CDCl_3$, 62 MHz): δ 15.5, 15.6, 16.7, 16.8, 16.9, 24.9, 46.7, 46.9, 47.1, 51.5, 59.9, 60.1, 60.3, 60.5, 66.8, 67.1, 68.7, 70.1, 70.5, 77.6, 77.8, 93.3, 98.5, 98.8, 98.9, 100.6, 102.3, 124.7, 132.7, 133.1, 142.4, 149.7, 150.7, 151.0, 166.7. HRMS (CI): calcd for $C_{24}H_{35}O_{11}I$ 626.1224. Found: 626.1198.

3.1.7. Methyl 4-[2-*O*-methyl-L-rhamnopyranosyl]-oxy-5-iodo-2,3-dimethoxy-6-methyl benzoate 10. Compound **9** (78 mg, 0.121 mmol) was cooled to −20 °C by placing the flask in an acetone/dry ice bath. A 9:1 mixture of trifluoroacetic acid/water (0.87 ml) was added dropwise and the reaction mixture stirred at this temperature for 4 h. After this time, TLC showed the formation of two anomers and thus the reaction was quenched by allowing the reaction mixture to warm to ambient temperature, and then immediately removing the TFA under high vacuum. The residue was then concentrated under reduced pressure onto flash silica for purification by flash chromatography and eluted with EtOAc to provide two anomers. R_f=0.56 'α' and R_f=0.41 β. Overall yield 40 mg, (63%); β −30 mg, (47%), α −10 mg, (16%). β-*Anomer*. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.29 (d, 3H, J=6.2 Hz, C-5–CH_3), 2.35 (s, 3H, Ar–CH_3), 3.11 (m, 1H, H-5), 3.38 (at, 3H, J=9.1 Hz, H-4), 3.47 (dd, 1H, J=3.6, 9.1 Hz, H-3), 3.84 (s, 3H, Ar–CO_2CH_3), 3.89, 3.89 (2s, 6H, Ar–OCH_3), 3.93 (s, 3H, C-2–OCH_3), 4.00 (dd, 1H, J=0.63, 3.6 Hz, H-2), 5.10 (as, 1H, H-1). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 17.4, 25.9, 52.6, 61.1, 61.5, 62.4, 72.4, 73.9, 74.1, 80.1, 94.7, 103.6, 126.1, 133.9, 143.6, 150.7, 151.6, 167.7. $[\alpha]_D^{20}$=−19 (c=1.0, MeOH). α-*Anomer*. IR ($CHCl_3$) ν: 3475, 2939, 1750, 1452. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.25 (d, 3H, J=6.9 Hz, C-5–CH_3), 2.37 (s, 3H, Ar–CH_3), 3.49 (at, 3H, J=8.0 Hz, H-4), 3.56 (s, 3H, Ar–CO_2CH_3), 3.84, 3.89 (2s, 6H, Ar–OCH_3), 3.93 (s, 3H, C-2–OCH_3), 3.56–3.93 (m, 1H, H-2), 4.07 (dd, 1H, J=3.3, 9.8 Hz, H-3), 4.20 (m, 1H, H-5), 5.66 (as, 1H, H-1). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 17.4, 25.9, 52.7, 59.0, 61.1, 61.6, 71.1, 72.8, 80.6, 93.4, 100.5, 125.6, 134.5, 143.2, 151.3, 151.9, 168.0. HRMS (CI): calcd for $C_{18}H_{25}O_9I$ [M^+] 512.0543. Found 513.0616. $[\alpha]_D$=−11.4 (c=0.66, MeOH).

3.1.8. 2-*O*-Acetyl-(2′*S*) (3′*S*)-3,4-*O*-2′,3′-dimethoxybutane-2′,3′-diyl-1-*O*-trichloroimidate-L-rhamnopyranoside 12. Under a flow of argon, the alcohol **11** (200 mg, 0.625 mmol) was dissolved in dry CH_2Cl_2 (4 ml), and trichloroacetonitrile (0.62 ml, 6.25 mmol) was added. After cooling to −12 °C, a catalytic amount of DBU (4.6 mg) in dry CH_2Cl_2 (1 ml) was added dropwise. Stirring was then continued at this temperature for 45 min. TLC showed complete disappearance of starting material, and so the reaction mixture was immediately concentrated onto flash silica for purification to yield 181 mg, (62%) of the trichloroimidates.

Due to the instability of the trichloroimidate the ^{1}H NMR spectrum was the only characterisation carried out on this compound. The spectrum only showed the presence of one anomer which was probably the β-anomer. ^{1}H NMR

($CDCl_3$, 300 MHz) δ: 1.27, 1.30 (2s, 6H, C-2′–CH_3, C-3′–CH_3), 1.33 (d, 3H, J=6.2 Hz, C-5–CH_3), 2.17 (s, 3H, C-2–OCOCH_3), 3.26, 3.27 (2s, 6H, C-2′–OCH_3, C-3′–OCH_3), 3.72 (at, 1H, J=10.1 Hz, H-4), 3.99 (m, 1H, H-5), 4.13 (dd, 1H, J=3.4, 10.1 Hz, H-3), 5.25 (dd, 1H, J=1.5, 3.4 Hz, H-2), 6.19 (d, 1H, J=1.5 Hz, H-1), 8.62 (bs, 1H, N–H).

3.1.9. Methyl 4-[2-*O*-acetyl-(2′*S*), (3′*S*)-3,4-*O*-2′,3′-dimethoxy butane-2′,3′ diyl-α-L-rhamnopyranosyl]-oxy-5-iodo-2,3-dimethoxy-6-methyl benzoate 13. Under an argon atmosphere, the imidate **12** (90 mg, 0.20 mmol), the phenol (52 mg, 0.15 mmol), and 4 Å molecular sieves (300 mg), were stirred in dry CH_2Cl_2 (2 ml) for 1 h at ambient temperature. The reaction mixture was then cooled to −70 °C, and boron trifluoride diethyl etherate (28 μl, 0.22 mmol) was added dropwise. With constant monitoring by TLC, the reaction mixture was allowed to warm to −50 °C over a period of 1 h. With the formation of the desired glycosylated compound apparent by TLC, the reaction was quenched by the addition of solid $NaHCO_3$ (10 mg) at −50 °C, followed by further warming to −20 °C and final addition of H_2O (1 ml). The reaction mixture was then diluted with CH_2Cl_2, the organic layer washed with sat. aq. $NaHCO_3$ (2×20 ml), dried over $MgSO_4$, filtered and concentrated onto flash silica for purification to yield 85 mg, (86%) of **13**. IR ($CHCl_3$) ν: 3054, 2987, 1734, 1653, 1457. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.26 (d, 3H, J=6.4 Hz, C-5–CH_3), 1.30, 1.32 (2s, 6H, C-2′–CH_3, C-3′–CH_3), 2.17 (s, 3H, C-2–OCOCH_3), 2.36 (s, 3H, Ar–CH_3), 3.27, 3.32 (2s, 6H, C-2′–OCH_3, C-3′–OCH_3), 3.75 (at, 1H, J=10.1 Hz, H-4), 3.83 (s, 3H, Ar–CO_2CH_3), 3.87 (s, 3H, Ar–OCH_3), 3.91 (s, 3H, Ar–OCH_3), 4.33 (m, 1H, H-5), 4.48 (dd, 1H, J=3.3, 10.1 Hz, H-3), 5.50 (dd, 1H, J=1.6, 3.3 Hz, H-2), 5.59 (d, 1H, J=1.6 Hz, H-1). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 16.7, 17.7, 17.8, 21.1, 25.3, 47.7, 48.2, 61.0, 61.6, 61.7, 65.8, 68.3, 69.6, 70.6, 93.1, 100.0, 100.9, 101.0, 125.5, 134.1, 142.7, 150.5, 151.2, 167.7, 170.3. HRMS (CI): calcd for $C_{24}H_{32}O_{11}I$ (M^+-OCH_3) 623.0989. Found: 623.1012. $[\alpha]_D^{20}=-56.1$ (c=0.66, $CHCl_3$).

3.1.10. Methyl 4-[2-*O*-acetyl-α-L-rhamnopyranosyl]-oxy-5-iodo-2, 3-dimethoxy-6-methyl benzoate 14. A dropwise addition of a 9:1 mixture of trifluoroacetic acid/H_2O (0.42 ml) to the acetal **14** (40 mg, 0.0611 mmol) was carried out at ambient temperature. After stirring for 1 min, the reaction was quenched by removing the TFA under high vacuum. The residue was then concentrated onto flash silica and purified using column chromatography (100% EtOAc), and the title compound was isolated as a white foam. Yield=30 mg, (91%). IR ($CHCl_3$) ν: 3441, 2937, 1773, 1460. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.30 (d, 3H, J=6.2 Hz, C-5–CH_3), 2.17 (s, 3H, C-2–OCOCH_3), 2.36 (s, 3H, Ar–CH_3), 3.59 (at, 1H, J=9.6 Hz, H-4), 3.84 (s, 3H, Ar–OCH_3), 3.87 (s, 3H, Ar–OCH_3), 3.92 (s, 3H, Ar–CO_2-CH_3), 4.20 (m, 1H, H-5), 4.40 (dd, 1H, J=3.6, 9.6 Hz, H-3), 5.53 (dd, 1H, J=1.7, 3.6 Hz, H-2), 5.72 (d, 1H, J=1.7 Hz, H-1). ^{1}H NMR ($CDCl_3$, 100 MHz) δ: 17.5, 21.0, 25.9, 52.6, 61.0, 61.6, 70.1, 70.7, 72.0, 73.0, 92.9, 100.1, 125.5, 134.3, 142.7, 150.5, 151.2, 167.7, 170.8. HRMS (CI): calcd for $C_{19}H_{25}O_{10}I$ [M^++NH_4^+] 558.0835. Found: 558.0825. $[\alpha]_D^{20}=-20.7$(c 0.58, MeO).

3.1.11. 3-(4′-Methoxy-2′-methyl-phenyl)-acrylic acid *tert*-butyl ester. A mixture of 4-bromo-3-methylanisole (5 ml, 3.48 mmol), tri-*o*-tolylphosphine (423 mg, 1.39 mmol), *tert*-butylacrylate (7.6 ml, 0.05 mol), Et_3N (5.8 ml, 4.20 mmol) and $Pd(OAc)_2$ all dissolved in anhydrous DMF (14 ml), was heated at 110 °C. After 30 min a precipitate had formed and the heating was continued for 2 h, then the reaction mixture was cooled and EtOAc was added. The reaction was washed with water, sat. $NaHCO_3$ solution, brine and the organic extract was dried ($MgSO_4$) and the solvent removed under reduced pressure. The residue was purified by column chromatography (eluent: petrol/ether, 95:5) to give the unsaturated ester as a white solid. Yield: 7.1 g (82%). R_f: 0.62 (petrol/ethyl acetate, 9:1). IR ($CHCl_3$): ν 2976, 1704, 1604, 1256, 1147, 863. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.53 (9H, s, *tert*-butyl), 2.41 (3H, s, CH_3), 3.80 (3H, s, OMe), 6.20 (1H, d, J=15.8 Hz, H-2), 6.75 (2H, m, H-5′/3′), 7.50 (1H, d, J=8.5 Hz, H-6′), 7.83 (1H, d, J=15.8 Hz, H-3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 20.3, 55.8, 112.0, 115.5, 118.4, 126.2 127.9 139.53, 140.8, 160.8, 166.8. m/z (EI): 248 ([M]$^+$, 17%), 192 (55), 175 (58), 147 (60), 132 (95), 104 (55), 77 (95), 56 (100). HRMS (EI): calcd for $C_{15}H_{20}O_3$ [M]$^+$: 248.1412. Found: 248.1418. Mp: 44–46 °C (lit.[13] 39 °C).

3.1.12. 2-Deoxy-2-phthalimido-D-glucopyranose. D-Glucosamine hydrochloride (1.0 g, 4.64 mmol) was dissolved in a 1 M solution of NaOH (240 mg, 5.8 mmol) in water (6.0 ml) and then phthalic anhydride (756 mg, 5.10 mmol) was added. The reaction mixture was left overnight at room temperature. The following day the reaction mixture was washed with diethyl ether to eliminate the excess of phthalic anhydride and then the water was concentrated under reduced pressure to give a white foam. The product was a mixture of α and β anomers in the ratio (α/β=1:1). Yield: quantitative. IR (KBr): ν 3420, 1636, 1586, 1559, 870, 839, 753, 696.

NMR data. The integration values below are not related to the α/β ratio. The integration values given treat the two anomers as separate compounds in an equal amount. ^{1}H NMR (D_2O, 500 MHz): δ 3.50–3.90 (11H, β H-2, α H-3, β H-3, α H-4, β H-4, α H-5, β H-5, α H-6, β H-6, α H-6′, β H-6′), 4.05 (1H, dd, J=10.6, 3.5 Hz, α H-2), 4.83 (1H, d, J=8.4 Hz, β H-1), 5.32 (1H, d, J=3.5 Hz, α H-1), 7.49–7.54 (6H, m, Phth.–CH), 7.65 (2H, m, H–Ar). ^{13}C NMR (D_2O, 125 MHz): δ 57.6 (α C-2), 60.7, 63.4, 63.6, 72.6, 73.0, 74.0, 74.6, 75.0, 77.2, 79.0, 93.8, 97.6, 130.2, 130.2, 131.3, 131.36, 132.77, 133.10, 133.39, 133.46, 137.39, 139.51, 176.30, 176.38, 178.22, 178.51. HRMS (EI): calcd for $C_{14}H_{15}NO_7$ [M]$^+$: 309.0848. Found: 309.0841.

3.1.13. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranoside. 2-Deoxy-2-phthalimido-D-glucopyranose (2.8 g, 9.38 mmol) was dissolved in pyridine (30 ml) and the solution was cooled to 0 °C. Acetic anhydride (48.80 mmol, 4.5 ml) was added dropwise with a catalytic amount of DMAP. The reaction mixture was then left at room temperature overnight. The following day the starting material had disappeared and the reaction was quenched by addition of MeOH (5 ml) at 0 °C. After evaporation of the solvents under reduced pressure, the pyridine was removed under high vacuum. The resultant oil was diluted with DCM and the organic phase was washed sequentially with 1 M

HCl (3×80 ml), sat. aq. $NaHCO_3$ (2×80 ml), H_2O (1×80 ml) and brine. After drying with $MgSO_4$ the extract was filtered and concentrated under pressure. Purification was carried out by flash chromatography (petrol/EtOAc, 6:4) to give a white solid as a mixture of anomers in the ratio (α/β, 1.5:1). Yield: 3.2 g (72%). IR (DCM): *ν*: 2940, 1755, 1721.

NMR data. The integration values below are not related to the α/β ratio. The integration values given treat the two anomers as separate compounds in an equal amount. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.87–2.12 (24H, α, β-CH_3), 4.04 (1H, m, β-H-5), 4.13–4.15 (2H, m, α, β-H-6), 4.20 (1H, m, α-H-5), 4.35–4.38 (2H, dd, *J*=12.4, 4.2 Hz, α, β-H-6′), 4.47–4.50 (1H, at, *J*=8.9 Hz, β-H-2), 4.71–4.74 (1H, dd, *J*=11.57, 3.4 Hz, α-H-2), 5.15–5.21 (1H, α, β-H-4), 5.87–5.89 (1H, at, *J*=9.1 Hz, β-H-3), 6.28 (1H, d, *J*=3.4 Hz, α-H-1), 6.52 (1H, d, *J*=8.9 Hz, β-H-1), 6.54–6.58 (1H, at, *J*=9.1 Hz, α-H-3), 7.74–7.76 (4H, m, α, β-Phth.–CH), 7.84–7.88 (4H, m, α, β-Phth.–CH). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 20.4, 21.0, 52.9, 53.6, 61.6, 67.0, 68.4, 69.4, 70.2, 70.6, 72.7, 89.8, 90.6, 123.7, 123.8, 131.2, 131.3, 134.47 (α, 167.39–170.65 (α, β-C=O). *m*/*z* (ES^+): 500.3 ([M^++Na).

3.1.14. Ethyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-glucopyranoside. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranoside (6.5 g, 13.70 mmol) was dissolved in $CHCl_3$ (80 ml) and $BF_3{\cdot}Et_2O$ (5.50 ml, 44.0 mmol) was added dropwise at 0 °C. The reaction mixture was stirred for 30 min at room temperature. Then EtSH (1.5 ml, 20.5 mmol) was added dropwise. The reaction was left for 2 h at room temperature and then refluxed for 4 h until disappearance of the starting material. The reaction was quenched with sat. sol. $NaHCO_3$ at 0 °C, extracted with DCM and washed with $NaHCO_3$ and water. The organic layers were dried over $MgSO_4$, the solvent was evaporated and the crude product was purified by flash column (petrol/EtOAc, 8:2) to give the β-anomer as a white solid. Yield: 4.6 g (70%). IR (DCM): *ν* 1750, 1718, 1636, 1387, 914, 722. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.21–1.24 (3H, t, *J*=13.5 Hz, CH_2CH_3), 1.87–2.04–2.11 (3H, 3×s, $COCH_3$), 2.63–2.74 (2H, m, CH_2CH_3), 3.90–3.91 (1H, m, C-5), 4.17–4.19 (1H, brd, *J*=11.9 Hz, H-6), 4.30–4.33 (1H, dd, *J*=4.4, 11.9 Hz, H-6′), 4.38–4.42 (1H, t, *J*=20.6 Hz, H-2), 5.17–5.20 (1H, t, *J*=18.8 Hz, H-4), 5.48–5.50 (1H, d, *J*=10.5 Hz, H-1), 5.82–5.85 (1H, t, *J*=19.2 Hz, H-3), 7.73–7.76 (2H, m, Phth.–CH), 7.84–7.86 (2H, m, Phth.–CH). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 15.3, 20.8, 21.0, 21.1, 24.7, 54.0, 62.7, 69.3, 71.9, 76.3, 81.6, 124.1, 131.5, 132.0, 134.7, 167.5, 168.2, 169.9, 170.5, 171.1. HRMS (EI): calcd for $C_{20}H_{20}NO_9$ [M^+−SEt], 418.1138. Found 418.1134. Mp: 115–116 °C. CHN; calcd for $C_{22}H_{25}NO_9S$: C, 55.10%; H, 5.25%; N, 2.92%. Found: C, 55.19%; H, 5.24%, N, 2.92%. $[\alpha]_D^{24}$=+39.5 (*c* 0.6, DCM). HRMS (EI): calcd for $C_{14}H_{20}O_9$ [M^++NH_4^+]: 350.1451. Found: 350.1464.

3.1.15. Ethyl 2,3,4-hydroxyl-2-deoxy-2-phthtalimido-1-thio-β-glucopyranoside. Ethyl-3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-glucopyranoside (14.0 g, 0.03 mol) was dissolved in MeOH and a few drops of NaOMe 25% sol. in MeOH was added until pH 8 was obtained and left to stir for 4 h at room temperature until disappearence of the starting material. The reaction mixture was neutralised with acid ion exchange resins, the solvent was evaporated and the crude product was purified by flash chromatography (EtOAc=100%) to give the target compound. Yield: 9.5 g (90%). R_f: 0.42 (EtOAc=100%). IR ($CHCl_3$): *ν* 3420, 1711, 1388. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.13–1.16 (3H, t, *J*=7.4 Hz, CH_2CH_3), 2.58–2.69 (2H, m, CH_2CH_3), 3.45–3.48 (1H, m, H-5), 3.67 (1H, at, *J*=5.5 Hz, H-4), 3.86–4.15 (2H, m, H-6 and H-6′), 4.10–4.15 (1H, m, H-2), 4.29–4.33 (1H, at, *J*=9.6 Hz, H-3), 5.31 (1H, d, *J*=10.4 Hz, H-1), 7.68–7.72 (2H, m, Phth.–CH), 7.72–7.81 (2H, m, Phth.–CH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 14.9, 21.0, 55.8, 61.9, 71.2, 72.6, 79.6, 81.3, 123.4, 123.8, 131.7, 134.1, 168.3, 168.5. *m*/*z* (ES^+): 376.4 ([M^++Na). CHN: C, 54.50; H, 5.57; N, 3.81. $C_{16}H_{19}NO_6S$ requires C, 54.38; H, 5.42; N, 3.96%. $[\alpha]_D$=+8.73 (*c* 0.91, DCM).

3.1.16. Ethyl-2,3,4-tri-*O*-triethylsilyl-2-deoxy-2-phthalimido-1-thio-β-glucopyranoside 18. Ethyl-2-deoxy-2-phthtalimido-1-thio-β-glucopyranoside (530 mg, 1.50 mmol) was dissolved in DMF (10.0 ml) and the solution was cooled to −78 °C. 2,6-Lutidine (1.6 ml, 13.41 mmol) and TESOTf (2.0 ml, 8.94 mmol) were then added. The reaction mixture was left for 3 h at 0 °C, it was then diluted with a sat. $NaHCO_3$ sol. and extracted with EtOAc. The organic phase was washed with water, the solvent was concentrated in vacuo and the crude was purified by flash column (petrol/EtOAc, 93:7) to afford a white foam. Yield: 830 mg (80%). R_f: 0.45 (petrol/EtOAc, 95:5). IR ($CHCl_3$) *ν*: 2955, 2877, 1778, 1716, 1386, 1111, 1009, 974. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.36–0.43 (6H, m, CH_2), 0.62–0.66 (9H, m, CH_3), 0.72–0.78 (12H, m, CH_2), 0.97–1.00 (18H, t, *J*=8.00 Hz, CH_3), 1.14 (3H, t, *J*=7.4 Hz, S–CH_3), 2.58–2.67 (2H, m, S–CH_2), 3.36 (1H, m, H-5), 3.67–3.70 (1H, t, *J*=9.6 Hz, H-4), 3.82–3.88 (2H, m, H-6 and H-6′), 4.13–4.17 (1H, t, *J*=10.4 Hz, H-2), 4.43–4.47 (1H, t, *J*=9.6 Hz, H-3), 5.18 (1H, d, *J*=10.4 Hz, H-1), 7.73–7.87 (4H, 2×m, Phth.–CH). ^{13}C NMR ($CDCl_3$, 500 MHz): δ 4.62, 7.1, 14.7, 23.5, 56.9, 62.2, 73.1, 75.0, 80.5, 81.2, 123.4, 132.0, 134.1, 168.1, 168.8. *m*/*z* (ES^+): 718.3 ([M^++Na). CHN: C, 58.55; H, 8.71; N, 2.05. $C_{34}H_{61}NO_6SSi_3$ requires C, 58.67; H, 8.84; N, 2.01%. $[\alpha]_D^{24}$=+20.0 (*c* 0.25, $CHCl_3$).

3.1.17. 1-*O*-[(1′*R*,4′*S*)-4′-Acetoxy]-cyclopent-2′-enyl-β-2-phthalimido-3,4,6-tri-*O*-triethylsilyl-β-glucopyranoside 19. Ethyl 2,3,4-tri-*O*-ethylsilyl-2-deoxy-2-phthalimido-1-thio-β-glucopyranoside (100 mg, 0.14 mmol) and (1*R*,4*S*)-(+)-4-hydrocyclopent-2-enylacetate (20 mg, 0.14 mmol) were stirred in DCM (4 ml) in the presence of chopped MS 4 Å (100 mg) for 20 min. The reaction mixture was cooled to −30 °C and NIS (97 mg, 0.43 mmol) was added and after 5 min. $BF_3{\cdot}Et_2O$ (5 μl, 0.04 mmol) was added. After 30 min the reaction was quenched with a 10% sol. of $Na_2S_2O_3$, washed with sat. $NaHCO_3$ sol. and extracted with EtOAc. The solvent was evaporated and the crude mixture was purified by flash chromatography (petrol/EtOAc, 98:2→9:1) to give a colourless oil. Yield: 30 mg (25%). R_f: 0.23 (petrol/EtOAc, 9:1). IR ($CHCl_3$) *ν*: 2955, 2878, 1734, 1717, 1386, 1240, 1065, 828. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.36–0.43 (6H, m, TES–CH_2), 0.63–0.66 (9H, m, TES–CH_3), 0.72–0.78 (12H, m, TES–CH_2), 0.97–1.00 (18H, m, TES–CH_3), 1.61–1.66 (1H, m, H-5′), 1.95 (3H, s, OAc), 2.63–2.66 (1H, m, H-5′), 3.24 (1H, m, H-5),

3.66–3.69 (1H, at, J=8.2 Hz, H-4), 3.82–3.85 (2H, m, H-6 and H-6′), 4.02–4.07 (1H, at, J=8.5 Hz, H-2), 4.36–4.40 (1H, at, J=8.2 Hz, H-3), 4.45 (1H, m, CH–O–sug.), 5.18 (1H, d, J=8.5 Hz, H-1), 5.35 (1H, m, CH–O–OAc), 5.74–5.78 (2H, m, =CH), 7.73–7.87 (4H, 2×m, Phth.–CH). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 4.61–7.1 (3×TES-C), 21.0, 29.7, 38.3, 57.8, 62.1, 73.3, 73.9, 76.6, 76.8, 81.0, 97.0, 123.2, 132.0, 132.9, 134.1, 135.7, 170.7. m/z (ES^+): 798.5 ([M^++Na, 100%), 634.6 (40), 502.6 (65), 301.5 (18). $[\alpha]_D^{24}$=−6.4 (c 0.46, $CHCl_3$).

3.1.18. Ethyl 2,3,4-tri-*O*-benzyl-2-deoxy-2-phthtalimido-1-thio-β-glucopyranoside 20. Ethyl 2-deoxy-2-phthtalimido-1-thio-β-glucopyranoside (2.70 g, 7.64 mmol), was dissolved in DMF (30 ml). Tetrabutylammonium iodide (TBAI) (300 mg, 0.76 mmol) and benzylbromine (5.71 ml, 45.88 mmol) were added. The solution was cooled down to 0 °C and then NaH (1.9 g, 45.88 mmol) was added slowly. The reaction mixture was left for 1 hr. at 0 °C and stirred at rt overnight. The following day the reaction was quenched with NH_4Cl sat. sol. and extracted with DCM. The organic phase was washed with NH_4Cl sat. sol. and brine. The solvent was evaporated and the crude product was purified by flash chromatography (petrol/EtOAc, 9:1) to give a white foam. Yield: 2.9 g (60%). R_f: 0.85 (petrol/EtOAc, 75:25). IR ($CHCl_3$) ν: 3005, 2926, 1773, 1715, 1387, 720. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.16–1.20 (3H, t, J=7.4 Hz, CH_2CH_3), 2.58–2.71 (2H, m, CH_2CH_3), 3.68 (1H, m, H-5), 3.76–3.80 (3H, m, H-4 and Bn–CH_2), 4.26 (1H, at, J=8.5 Hz, H-2), 4.37–4.84 (7H, 2×Bn–CH_2, H-3, H-6, H-6′), 5.25 (1H, d, J=10.4 Hz, H-1), 6.88–7.78 (19H, 3×Bn–CH_2, 1×Phth.–CH). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 14.9, 23.9, 54.9, 68.9, 73.4, 74.9, 75.01, 79.4, 79.5, 80.3, 81.0, 123.3–138.2 (24×C), 167.5, 168.0. HRMS (FAB): calcd for $C_{37}H_{37}NSO_6$ [$M]^+$: 623.2342. Found: 623.2360. $[\alpha]_D^{24}$=+8.0 (c 0.75, DCM).

3.1.19. 1-*O*-[(1′*R*,4′*S*)-4′-Acetoxy]-cyclopent-2′-enyl-β-2,3,4-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-glucopyranoside 21. Ethyl 2,3,4-tri-*O*-benzyl-2-deoxy-2-phthtalimido-1-thio-β-glucopyranoside (570.0 mg, 0.91 mmol) and (1*R*,4*S*)-(+)-4-hydroxycyclopent-2-enylacetate (130.0 mg, 0.91 mmol) were dissolved in DCM (8.0 ml) and stirred for 10 min at rt. The reaction mixture was cooled to −30 °C and chopped 4 Å molecular sieves (570 mg) and NIS (615 mg, 2.73 mmol) were added. After 10′ at −30 °C $BF_3{\cdot}Et_2O$ (23 μl, 0.18 mmol) was added and the reaction mixture was left for 20′ and then quenched with sodium thiosulfate 10% sol. The solution was filtered, diluted with DCM and washed with sodium sulfate, $NaHCO_3$ sat. sol. and water. The solvent was evaporated and the residue was purified by flash chromatography (petrol/EtOAc, 8:2→7:3). Yield: 460 mg (70%). R_f: 0.44 (petrol/EtOAc, 75:25). IR ($CHCl_3$) ν: 3030, 2868, 1776, 1732, 1714, 1389. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.67–1.72 (1H, dt, J=4.4 Hz, 14.6, H-5′), 1.94 (3H, s, CH_3), 2.67–2.72 (1H, m, H-5′), 3.65 (1H, m, H-5), 3.75–3.78 (3H, m, H-3 and Bn–CH_2), 4.18 (1H, at, J=8.5 Hz, H-2), 4.30 (1H, at, J=8.5 Hz, H-3), 4.42–4.85 (7H, H-6, H-6′, 2×Bn–CH_2 and H-1′), 5.27 (1H, d, J=8.5 Hz, H-1), 5.84 (1H, m, H-4′), 5.77 (1H, brd, J=5.6 Hz, CH=), 5.84 (1H, brd, J=5.6 Hz, CH=), 6.84–7.77 (19H, Bn–CH and Phth.–CH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.1, 38.2, 55.9, 68.7, 73.5, 74.8, 75.0, 79.2, 79.6, 81.1, 97.2, 123.2–138.2 (25×C), 133.1, 133.7, 170.8 (3×C). HRMS (FAB): calcd for $C_{42}H_{41}NO_9$ [$M]^+$: 703.2781. Found: 703.2794. $[\alpha]_D^{24}$=+6.1 (c 0.66, DCM).

3.1.20. 1-*O*-[(1′*R*,4′*S*)-4′-Hydroxy]-cyclopent-2′-enyl-β-2,3,4-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-glucopyranoside 22. Compound **21** (450 mg, 0.64 mmol) was dissolved in MeOH and few drops of a 1 M sol. of K_2CO_3 were added until pH 8. After 3 h the solvent was evaporated, EtOAc and water were added. The organic extracts were dried over $MgSO_4$, the solvent was evaporated in vacuum and the crude product was purified by flash chromatography (petrol/EtOAc, 6:4) to give the target compound. Yield: 380 mg (90%). R_f: 0.19 (petrol/EtOAc, 6:4). IR ($CHCl_3$) ν: 3470, 3030, 2870, 1774, 1713, 1389. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.57–1.61 (1H, dt, J=14.4 Hz, 4.1, H-5′), 2.58–2.64 (1H, m, H-5′), 3.70 (1H, m, H-5), 3.73–3.77 (3H, H-4 and CH_2), 4.15–4.18 (1H, at, J=8.5 Hz, H-2), 4.42 (1H, at, J=8.5 Hz, H-3), 4.42–4.85 (8H, 2×Bn–CH_2, H-6, H-6′, H-1′ and H-4′), 5.27 (1H, d, J=8.5 Hz, H-1), 5.74 (1H, d, J=8.6 Hz, CH=), 5.81 (1H, d, J=8.6 Hz, CH=), 6.86–7.77 (19H, Bn–CH_2 and Phth.–CH). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 41.7, 55.9, 68.8, 73.5, 74.8, 75.0, 75.1, 79.2, 79.7, 81.4, 97.1, 123.2–138.14 (25×C), 133.37, 137.60, 168.00. m/z (ES^+): 684.3 ([$M]^+$+Na). $[\alpha]_D^{24}$=+186.6 (c 0.75, DCM).

3.1.21. (4′*R*,1′*S*)-[4′-(2″-Hydroxy, 7″-methoxy-5″-methyl-naphthoate)]-[1′-*O*-1-3,4,6-tri-*O*-benzyl-2-deoxy-2-phthalimido-β-glucosyl]-1′,4′-dihydroxy-cyclopentene 23. The previous product (1.62 g, 2.45 mmol), naphthoic acid **16** (682 mg, 2.88 mmol) and DMAP (60 mg, 0.48 mmol) were dissolved in DCM (12.0 ml). The reaction mixture was cooled to 0 °C and then DCC (740 mg, 3.60 mmol) was added. The reaction was left at 0 °C for 1 h and then at rt overnight. The following day the reaction mixture was filtered, the solvent was concentrated in vacuo and the crude product was purified by flash chromatography (petrol/ EtOAc, 75:25) to give a white foam. Yield: 1.3 g (60%). R_f: 0.48 (petrol/EtOAc, 7:3). IR ($CHCl_3$) ν: 3450, 2922, 1773, 1713, 1615, 1388. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 2.05–2.10 (1H, dt, J=14.8 4.1 Hz,), 2.60 (3H, s), 2.91–2.95 (1H, m), 3.67 (1H, m), 3.71 (3H, s), 3.77 (3H), 4.13 (1H, at, J=9.6 Hz), 4.41 (1H, at, J=9.6 Hz), 4.55- 4.85 (7H, m), 5.34 (1H, d, J=8.5 Hz), 5.75 (1H, m), 5.97 (1H, d, J=5.7 Hz), 6.04 (1H, d, J=5.7 Hz), 6.84–7.54 (21H, m), 7.98 (2H, m). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 19.9, 38.4, 55.1, 55.9, 68.8, 73.5, 74.8, 75.0, 76.8, 78.4, 79.3, 79.7, 81.2, 97.7, 104.5, 117.0, 122.9, 127.3, 127.6, 127.7, 127.8, 127.9, 128.0, 128.0, 128.4, 128.5, 132.4, 132.9, 133.6, 134.2, 136.4, 136.5, 137.9, 138.0, 138.1, 159.5, 164.5, 172.1. HRMS (FAB): calcd for $C_{53}H_{49}NO_{11}$ [$M]^+$: 875.3305. Found: 875.3297. CHN: C, 72.15; H, 5.94; N, 1.91. $C_{53}H_{49}NO_{11}$ requires C, 72.55; H, 5.64; N, 1.60%. $[\alpha]_D^{24}$=+17.63 (c 0.7, DCM).

3.1.22. (4′*R*,1′*S*)-[4-(2″-Hydroxy-7″-methoxy-5″-methyl-naphthoate]-1′-*O*-1-2-deoxy-2-phthalimido-β-glucosyl-1′,4′-dihydroxy-cyclopentane 24. $Pd(OH)_2$ 20% (140 mg) was dissolved in EtOH (8.0 ml). The previous product (70 mg, 0.08 mmol) was added and then it was left to react with H_2 at rt under 1 atm pressure overnight. The following day the solution was filtered through celite the solvent was

evaporated and the crude product was purified by flash chromatography (DCM/MeOH, 9:1). Yield: 30 mg (60%). R_f: 0.47 (DCM/ MeOH, 9:1). IR (DCM) ν: 3414, 2926, 1775, 1713, 1615, 1388. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.68–1.78 (2H, m), 2.06 (1H, m), 2.24–2.31 (2H, 2×m), 2.59 (3H, s, CH_3), 2.82 (1H, m), 3.44–3.58 (3H, 2×m), 3.78 (3H, s, OCH_3), 3.80 (1H, m), 4.00–4.04 (1H, at, J=8.3 Hz), 4.13 (1H, m), 4.33 (1H, m), 5.32–5.36 (2H, m), 6.83 (1H, s), 6.93 (1H, d, J=9.2 Hz), 7.47–7.60 (4H, m), 7.94 (1H, d, J=9.2 Hz), 7.99 (1H, s). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 19.9, 30.4, 30.8, 39.5, 55.3, 55.7, 62.0, 71.7, 72.2, 75.7, 79.2, 79.2, 97.2, 103.3, 104.7, 115.9, 116.9, 122.9, 124.0, 131.3, 132.1, 133.9, 134.3, 136.5, 159.4, 163.3, 168.3, 171.4. HRMS (FAB): calcd for $C_{32}H_{33}NO_{11}$ [M^+]: 607.2054. Found: 607.2049. $[\alpha]_D^{24}=-64.8$ (c 1.05, DCM).

Acknowledgements

A. Ciral thanks the McClay Trust for a PhD studentship, A. McHale thanks the EPSCR and Dextra Laboratories, and we thank Dr. Hendrik van den Berg for the in vitro anti-cancer screening.

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Tetrahedron 60 (2004) 4031–4035
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Phenyl trifluorovinyl sulfide: a radical acceptor for preparation of *gem*-difluoromethylene compounds

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Received 9 February 2004; accepted 3 March 2004

Abstract—Phenyl trifluorovinyl sulfide was prepared from the reaction of trifluorovinyllithium and *S*-phenyl benzenethiosulfonate. The fluorinated olefin showed reactivity with alkyl radicals generated from halogen-abstraction from alkyl halides. Reactions with alkyl halides required tris(trimethylsilyl)silane as a chain transfer reagent to improve selectivity of the products. Initiation of radical reaction was effected by thermal decomposition of AIBN. Oxidation of the thioether products gave the corresponding sulfoxides, which were successively converted into α,α-difluoroalkanecarboxylic acid thiol esters by Pummerer reaction.

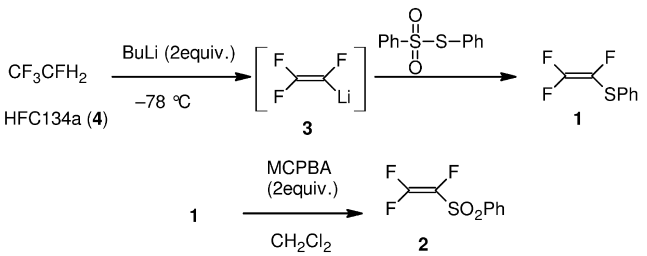
1. Introduction

Difluoromethylene compounds[1–3] have attracted increasing interest in recent years due to the wide variety of their biological activities.[4–6] α,α-Difluoroalkanecarboxlic acid derivatives are potentially versatile starting materials and synthetic building blocks for such biologically active difluoromethylene compounds. The regioselective addition of alkyl radicals[7,8] to the electron-deficient 1,1-dichloro-2,2-difluoroethene provided us with a promising route to α,α-difluoroalkanecarboxlic acids.[1,9–14] However, in order to avoid the use of the ozone-depleting chlorofluorocarbons for the environmental reason, currently another good radical acceptor is required to obtain difluoromethylene products regioselectively, which can be readily converted into synthetically useful functional groups with a difluoromethylene group. In this paper, we report new radical acceptors, phenyl trifluorovinyl sulfide (**1**) and phenyl trifluorovinyl sulfone (**2**), and the functional group conversion of the radical adducts to synthetically useful difluoroalkanecarboxylic acid thiol esters.

2. Results and discussion

2.1. Preparation of trifluorovinyl phenyl sulfide 1 and sulfone 2

Phenyl trifluorovinyl sulfide (**1**) and the corresponding sulfone (**2**) were previously prepared from phenylsulfenyl chloride and also a chlorofluorocarbon CF_2=CFCl in low yields.[15–17] Recently, new preparation methods of trifluorovinyllithium (**3**) from non-ozone-depleting 1,1,1,2-tetrafluoroethane (HFC-134a) (**4**) at −78 °C were reported.[18,19] According to these preparation methods, sulfenylation of anion **3** in Et_2O of with *S*-phenyl benzenethiosulfonate[20] was carried out to give sulfide **1** in 64% yield. The corresponding sulfone **2** was obtained by oxidation of **1** with 2 equiv. of *m*-chloroperbenzoic acid (MCPBA) in 80% yield (Scheme 1).[15]



Scheme 1.

2.2. Borane-assisted radical reaction of trifluorovinyl sulfide 1 and sulfone 2

In the presence of a trace amount of oxygen, alkylboranes

Keywords: Difluoromethylene; Radical reaction; Fluoro olefin; Pummerer reaction; Thiol ester.
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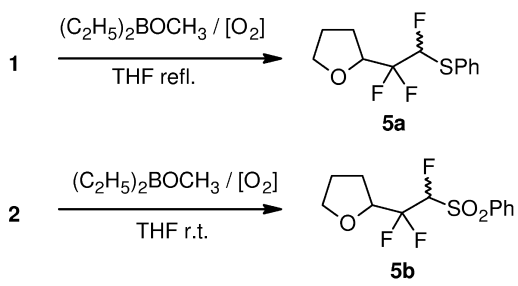
doi:10.1016/j.tet.2004.03.020

generate alkyl radicals, which abstract hydrogen from activated C–H bonds in ethers.[21] Reactivities of both trifluorovinyl compounds **1** and **2** with carbon radicals were compared by the reaction with methyl diethylboronate in THF (Scheme 2). Generated 2-oxolanyl radical reacted with **1** under refluxing to give a 1:1 diastereomer mixture of 2-substituted oxolane derivative **5a** in 76%. Similarly, sulfone **2** gave a 1:1 mixture of **5b** at room temperature in 77% yield. As expected from the reactions of geminal difluoroolefins with alkyl radicals, the addition reactions of radical to **1** and **2** were regioselective to form the new carbon–carbon bonds at the difluorinated carbons, and no regioisomers were detected. The regioselectivity was confirmed by their ^{19}F NMR spectra, in which two sets of diastereotopic fluorine signals of difluoromethylene groups were observed ($^{2}J^{19}F-^{19}F=250-270$ Hz).

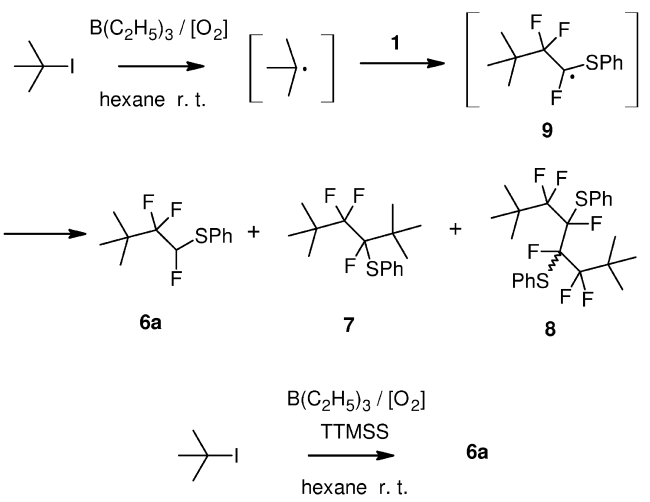


Scheme 2.

Although a competition experiment using an equimolar mixture of **1** and **2** gave a mixture of **5a** and **5b** in a 1:4 ratio and thus **2** is four times more reactive than **1**, the following radical reactions were conducted with sulfide **1** because of the comparable product yields and the shorter preparation steps.

2.3. Generation of alkyl radicals from organic halides and the reaction with thioether 1

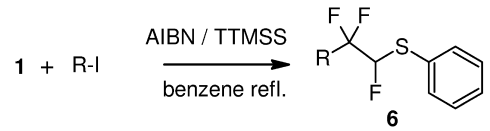
Alkyl radicals are generated from the reaction of alkyl halides and alkylborane/O_2.[22] A mixture of thioether **1**, *t*-butyl iodide, and triethylborane in hexane was stirred at room temperature in the presence of air, bearing in mind that atom-transfer reaction via alkyl radical from organic halides proceed to give olefin insertion products. However, a complex mixture of products containing several radical products such as **6a**, **7**, and **8** detected by GC–MS analysis was afforded (Scheme 3). Although these products prove that ethyl radical was generated from triethylborane and reacted with fluoroolefin **1** to give radical intermediate **9**, the radical **9** is too unreactive to abstract iodine from *t*-butyl iodide for chain propagation. When tris(trimethylsilyl)-silane (TTMSS) was added as a radical chain transfer reagent, the radical reaction proceeded smoothly to give mainly hydrogenated adduct **6a** in 65% yield.



Scheme 3.

Nevertheless, the radical reactions using $B(C_2H_5)_3$/air as the radical initiator had some problems in reproducibility particularly in the cases of primary halides. Thus, we adopted another conventional alkyl radical generation system using thermal decomposition of azobisisobutyronitrile (AIBN) under benzene reflux. The reaction was carried out by continual addition of small amounts of TTMSS and solid AIBN in every 3 min into the refluxing benzene solution of sulfide **1** and 3–5-fold excess amounts of the corresponding alkyl halide until the starting material **1** was completely consumed. In this manner, as well as *tert*-butyl iodide (entry 1) which also gave **6a** in somewhat lower yield than the reaction with $B(C_2H_5)_3$/air, primary (entries 2–4) and secondary (entries 5 and 6) halides gave adducts **6b–f** as summarized in Table 1. When TTMSS was used as the radical chain transfer reagent, a byproduct tris(trimethylsilyl)silyl halide had been often troublesome to remove from the reaction mixture. However, TTMSS was able to be readily removed by elution of the resulting benzene solution through a short column of basic alumina. While a small amount of the hydrolyzed silanol was eluted with adducts, it was readily removed by the successive chromatography on a SiO_2 column.

Table 1. Radical reaction of **1** with alkyl halides in the presence of AIBN and TTMSS



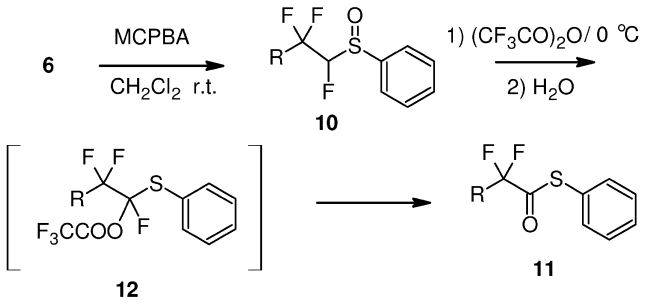
Entry	R	Product	Yield (%)
1	$(CH_3)_3C-$	**6a**	36
2	$CH_3(CH_2)_2-$	**6b**	68
3	$CH_3(CH_2)_5-$	**6c**	49
4	$CH_3(CH_2)_8-$	**6d**	67
5	$(CH_3)_2CH-$	**6e**	70
6	Cyclohexyl	**6f**	46

2.4. Conversion of radical adducts by Pummerer reaction to α,α-difluoroalkanecarboxylic acid thiol esters

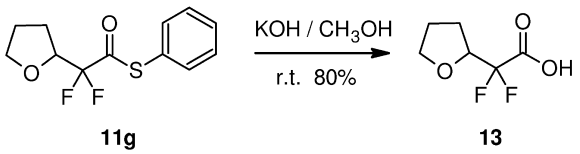
Versatility of this radical reaction depends on whether the fluorinated products can be smoothly converted into difluoroalkanecarboxylic acid derivatives, which are utilized as starting materials in various organic preparations.[23–26] The obtained α-fluorothioethers were oxidized with an equimolar amount of MCPBA at room temperature in CH_2Cl_2 to give the diastereomer mixtures of corresponding

sulfoxides **10a**–**g** as summarized in Table 2. Following Pummerer reaction with trifluoroacetic anhydride at 0 °C gave the desired α,α-difluoroalkanecalboxylic acid thiol esters **11a**–**g** after hydrolysis of the labile intermediate **12**. 2-Oxolanyl derivative **11g** was converted into the corresponding difluorocarboxylic acid **13**[10] in 80% yield on alkaline hydrolysis with KOH (Scheme 4).

Table 2. MCPBA oxidation of radical adducts followed by Pummerer reaction to thiol esters



Entry	Substrate	R	Sulfone	Thiol ester	Yield (%)
1	**6a**	$(CH_3)_3C-$	**10a**	**11a**	67
2	**6b**	$CH_3(CH_2)_2-$	**10b**	**11b**	64
3	**6c**	$CH_3(CH_2)_5-$	**10c**	**11c**	58
4	**6d**	$CH_3(CH_2)_8-$	**10d**	**11d**	41
5	**6e**	$(CH_3)_2CH-$	**10e**	**11e**	63
6	**6f**	Cyclohexyl	**10f**	**11f**	51
7	**5a**	2-Oxolanyl	**10g**	**11g**	63



Scheme 4.

In conclusion, phenyl trifluorovinyl sulfide prepared from sulfenylation of trifluorovinyllithium reacted with alkyl halides under radical reaction conditions to give difluoromethylene-containing thioether products regioselectively. Oxidation of the products followed by Pummerer reaction afforded synthetically useful α,α-difluoroalkanecarboxylic acid thiol esters.

3. Experimental

3.1. General

^{1}H NMR spectra were collected in $CDCl_3$ in the presence of TMS as an internal standard at 300 MHz. ^{19}F NMR spectra (282 MHz) were recorded in $CDCl_3$, and referenced based on internal $CF_3COOC_2H_5$ whose chemical shift was set at −75.75 ppm downfield (δ) from internal $CFCl_3$ in $CDCl_3$.

3.1.1. Phenyl trifluorovinyl sulfide (1). HFC134a (**4**) (ca. 6.0 g, 59 mmol) was liquefied at −78 °C and diluted in Et_2O (100 mL). To the Et_2O solution, BuLi (1.6 M in hexane; 62.5 mL, 100 mmol) was slowly added dropwise in 4 h at −78 °C. The resulted solution was then added dropwise into a solution of *S*-phenyl *p*-toluenethiosulfonate (12.5 g, 50 mmol) in Et_2O (300 mL) in 2 h below −45 °C. After warming up to room temperature, the resulted suspension was filtered through a bed of Celite®. The solvents of the filtrate were removed by distillation, and the residue was distilled with a glass tube oven (45 °C, 5 mm Hg) to give pure olefin **1** as colorless oil: 5.98 g (63% based on thiosulfonate); ^{1}H NMR δ 7.27–7.42 (m); ^{19}F NMR δ −88.4 (dd, J=46±2, 34±2 Hz), −106.5 (dd, J=122±2, 46±2 Hz), −149.4 (dd, J=122±2, 34±2 Hz). The NMR spectra were compatible with previously reported data.[15–17]

3.1.2. Phenyl trifluorovinyl sulfone (2). Sulfide **1** (190 mg, 1 mmol) and *m*-chloroperbenzoic acid (MCPBA) (430 mg, 2.5 mmol) was dissolved in CH_2Cl_2 (10 mL) and the mixture was stirred for 12 h at room temperature. The resulting solution was successively washed with aq. $Na_2S_2O_3$ and aq. $NaHCO_3$ solutions. Organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The residue was chromatographed on a SiO_2 column (1:1 hexane–EtOAc) to give sulfone **2** as colorless oil: 176 mg (80%); ^{1}H NMR δ 7.58–7.66 (m, 3H), 7.71–7.78 (m, 2H); ^{19}F NMR δ −85.2 (dd, J=40±2, 31±2 Hz), −95.0 (dd, J=122±2, 31±2 Hz), −175.2 (dd, J=122±2, 40±2 Hz).

3.1.3. 1,1,2-Trifluoro-1-oxolan-2-yl-2-phenylsulfanylethane (5a). A mixture of **1** (380 mg, 2 mmol) and $(C_2H_5)_2BOCH_3$ THF solution (1.0 M; 10 mL, 10 mmol) was refluxed for 12 h. The solvent was removed under reduced pressure and the residue was chromatographed on a short SiO_2 column (Et_2O) to give a 1:1 diastereomer mixture of **5a** as colorless oil: 400 mg (76%); ^{1}H NMR δ 1.91–2.18 (m, 4H), 3.84–3.93 (m, 2H), 4.28–4.59 (m, 1H), 6.01 (ddd, J=52.8, 16.8, 5.4 Hz, 0.5H), 6.01 (ddd, J=51.0, 17.4, 5.4 Hz, 0.5H), 7.33–7.39 (m, 3H), 7.55–7.59 (m, 2H); ^{19}F NMR δ −118.3 (dt, J=256±2, 18±2 Hz, 0.5F), −121.5 (dtd, J=262±2, 17±2, 6±2 Hz, 0.5F), −122.7 (dtd, J=262±2, 20±2, 6±2 Hz, 0.5F), −123.5 (dtd, J=256±2, 20±2, 6±2 Hz, 0.5F), −161.8 (dt, J=55±2, 18±2 Hz, 0.5F), −168.8 (dt, J=52±2, 18±2 Hz, 0.5F); EI-MS m/z (rel. %) 262 (M^+, 67), 141 (34), 77 (31), 71 (100). Anal. Calcd for $C_{12}H_{13}F_3OS$: C; 54.95, H; 5.00. Found: C; 55.03, H; 5.04.

3.1.4. 1,1,2-Trifluoro-1-oxolan-2-yl-2-phenylsulfonylethane (5b). A mixture of sulfone **2** (220 mg, 1 mmol) and 1.0 M $(C_2H_5)_2BOCH_3$ THF solution (5 mL) **5b** was stirred at room temperature for 12 h. The resulting mixture was worked up as above to give sulfone **5b** as colorless oil: 100 mg, (34%; 77% based on the consumed sulfone **2**); ^{1}H NMR δ 1.80–2.22 (m, 4H), 3.79–3.95 (m, 2H), 4.23 (ddddd, J=24.3, 9.0, 4.8, 3.6, 1.5 Hz, 0.5H), 4.68 (ddt, J=21.6, 7.8, 5.1 Hz, 0.5H), 5.50 (ddd, J=43.5, 20.1, 0.9 Hz, 0.5H), 5.56 (ddd, J=45.0, 16.8, 6.0 Hz, 0.5H), 7.57–7.65 (m, 2H), 7.71–7.78 (m, 1H), 7.98–8.02 (m, 2H); ^{19}F NMR δ −119.3 (dtd, J=267±2, 15±2, 6±2 Hz, 0.5F), −120.9 (dddd, J=267±2, 21±2, 12±2, 6±2 Hz, 0.5F), −122.3 (ddd, J=268±2, 21±2, 15±2 Hz, 0.5F), −127.2 (ddd, J=268±2, 24±2, 9±2 Hz, 0.5F), −189.4 (dddd, J=43±2, 15±2, 8±2, 5±2 Hz, 0.5F), −196.9 (dt, J=45±2,

13±2 Hz, 0.5F); EI-MS *m/z* (rel. %) 212 (7), 141 (10), 133 (36), 78 (70), 71 (100). Anal. Calcd for $C_{12}H_{13}F_3O_3S$: C; 48.98, H; 4.45. Found: C; 48.85, H; 4.58.

3.1.5. Triethylborane-initiated radical reaction of sulfide 1 with *tert*-butyl iodide. To a mixture of **1** (95 mg, 0.50 mmol), *tert*-butyl iodide (460 mg, 2.5 mmol), and TTMSS (250 mg, 1.00 mmol), $B(C_2H_5)_3$ hexane solution (1.0 M, 0.25 mL: 0.25 mmol) was added. Then the mixture was exposed with air and stirred at room temperature. $B(C_2H_5)_3$ hexane solution (1.0 M, 0.25 mL: 0.25 mmol) was added twice into the reaction mixture at 1 and 2 h later. After 3 h stirring, the mixture was diluted with hexane and washed with saturated aq. NaCl solution. The hexane solution was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue was chromatographed on a SiO_2 column to give 3,3,4-trifluoro-2,2-dimethyl-4-phenylsulfanylbutane (**6a**) as colorless oil: 80 mg (65%); ^{1}H NMR δ 1.15 (s, 9H), 5.90 (ddd, *J*=52.5, 19.8, 3.0 Hz, 1H), 7.35–7.38 (m, 3H), 7.56–7.59 (m, 2H); ^{19}F NMR δ −108.8 (dd, *J*=259±2, 21±2 Hz, 1F), −121.1 (dt, *J*=259±2, 18±2 Hz, 1F), −156.9 (dt, *J*=52±2, 18±2 Hz, 1F); EI-MS *m/z* (rel. %) 248 (31, M^+), 141 (100), 109 (13), 57 (35). Anal. Calcd for $C_{12}H_{15}F_3S$: C; 58.04, H; 6.09. Found: C; 58.20, H; 6.06.

3.1.6. AIBN initiated radical reaction of sulfide 1 with *tert*-butyl iodide. To a refluxing mixture of **1** (95 mg, 0.50 mmol) and *tert*-butyl iodide (0.32 mL, 2.5 mmol) in benzene (2 mL) under stirring, TTMSS (50 μL, ca. 0.16 mmol) and solid AIBN (2 mg, ca. 12 μmol) was continually added in every 3 min. The reaction was continued for 1 h. The reaction mixture was filtered through a short alumina column, and then the column was washed with hexane. After evaporation of the solvent, the residue was chromatographed on a SiO_2 column eluting with hexane to give pure **6a**: 98 mg (36%).

3.1.7. 1,2,2-Trifluoro-1-phenylsulfanylpentane (6b). The title compound was obtained as above from **1** (380 mg, 2.0 mmol) and 1-iodopropane (1.36 g, 4.0 mmol) as colorless oil: 316 mg (68%); ^{1}H NMR δ 1.00 (t, *J*=7.4 Hz, 3H), 1.59 (sex, *J*=7.4 Hz, 2H), 1.75–2.39 (m, 2H), 5.74 (ddd, *J*=53.0, 10.7, 8.0 Hz, 1H), 7.34–7.38 (m, 3H), 7.53–7.57 (m, 2H); ^{19}F NMR δ −161.6 (dt, *J*=52±2, 18±2 Hz, 1F), −108.5 (dqd, *J*=253±2, 18±2, 12±2 Hz, 1F), −106.5 (dqd, *J*=253±2, 15±2, 6±2 Hz, 1F); EI-MS *m/z* (rel. %) 234 (19, M^+), 141 (100), 109 (22), 77 (26), 51 (27). Anal. Calcd for $C_{11}H_{13}F_3S$: C; 56.39, H; 5.59. Found: C; 56.41, H; 5.77.

3.1.8. 1,2,2-Trifluoro-1-phenylsulfanyloctane (6c). The title compound was obtained as above from **1** (95 mg, 0.50 mmol) and 1-iodohexane (530 mg, 2.5 mmol) as colorless oil: 68 mg (49%); ^{1}H NMR δ 0.90 (t, *J*=6.9 Hz, 3H), 1.31–1.44 (m, 6H), 1.496–1.598 (m, 2H), 1.95–2.18 (m, 2H), 5.74 (ddd, *J*=52.8, 10.7, 8.0 Hz, 1H), 7.34–7.39 (m, 3H), 7.52–7.59 (m, 2H); ^{19}F NMR δ −161.6 (dt, *J*=54±2, 16±2 Hz, 1F), −108.5 (dqd, *J*=253±2, 21±2, 12±2 Hz, 1F), −106.5 (dqd, *J*=253±2, 18±2, 9±2 Hz, 1F); EI-MS *m/z* (rel. %) 276 (33, M^+), 141 (100), 109 (18), 77 (20), 51 (17). Anal. Calcd for $C_{14}H_{19}F_3S$: C; 60.84, H; 6.93. Found: C; 60.66, H; 7.12.

3.1.9. 1,2,2-Trifluoro-1-phenylsulfanylundecane (6d). The title compound was obtained as above from **1** (95 mg, 0.50 mmol) and 1-iodononane (640 mg, 2.5 mmol) as colorless oil: 108 mg (68%); ^{1}H NMR δ 0.88 (t, *J*=7.0 Hz, 3H), 1.13–1.42 (m, 12H), 1.54 (quint., *J*=7.4 Hz, 2H), 1.88–2.13 (m, 2H), 5.74 (ddd, *J*=52.7, 10.7, 8.0 Hz, 1H), 7.34–7.39 (m, 3H), 7.53–7.57 (m, 2H); ^{19}F NMR δ −161.6 (dt, *J*=54±2, 18±2 Hz, 1F), −108.5 (dqd, *J*=253±2, 18±2, 12±2 Hz, 1F), −106.5 (dqd, *J*=253±2, 18±2, 9±2 Hz, 1F); EI-MS *m/z* (rel. %) 319 (8, M^++1), 318 (42, M^+), 141 (100), 110 (17), 109 (19), 77 (11), 65 (11), 55 (16). Anal. Calcd for $C_{17}H_{25}F_3S$: C; 64.12, H; 7.91. Found: C; 63.90, H; 8.20.

3.1.10. 1,2,2-Trifluoro-3-methyl-1-phenylsulfanylbutane (6e). The title compound was obtained as above from **1** (95 mg, 0.50 mmol) and 2-iodopropane (430 mg, 2.5 mmol) as colorless oil: 82 mg (70%); ^{1}H NMR δ 1.09 (d, *J*=6.6 Hz, 3H), 1.10 (d, *J*=6.9 Hz, 3H), 2.31–2.52 (m, 1H), 5.82 (ddd, *J*=52.5, 14.4, 6.9 Hz, 1H), 7.36–7.38 (m, 3H), 7.55–7.59 (m, 2H); ^{19}F NMR δ −162.7 (dt, *J*=52±2, 17±2 Hz, 1F), −117.3 (dtd, *J*=253±2, 18±2, 6±2 Hz, 1F), −115.3 (dtd, *J*=253±2, 15±2, 8±2 Hz, 1F); EI-MS *m/z* (rel. %) 235 (3, M^++1), 234 (24, M^+), 141 (100), 110 (21), 109 (20), 77 (16), 65 (29), 51 (24). Anal. Calcd for $C_{11}H_{13}F_3S$: C; 56.39, H; 5.59. Found: C; 56.32, H; 5.79.

3.1.11. 1-Cyclohexyl-1,1,2-trifluoro-2-phenylsulfanylethane (6f). The title compound was obtained as above from **1** (380 mg, 2.0 mmol) and iodocyclohexane (2.10 g, 10 mmol) as colorless oil: 253 mg (46%); ^{1}H NMR δ 1.14–1.36 (m, 5H), 1.68–1.72 (m, 1H), 1.80–1.95 (m, 4H), 2.12 (m, 1H), 5.82 (ddd, *J*=52.8, 12.6, 9.3 Hz, 1H), 7.31–7.38 (m, 3H), 7.55–7.58 (m, 2H); ^{19}F NMR δ −162.9 (dt, *J*=52±2, 18±2 Hz, 1F), −116.1–114.0 (m, 2F); EI-MS *m/z* (rel. %) 274 (39, M^+), 141 (100), 110 (48), 77 (28), 55 (26). Anal. Calcd for $C_{14}H_{17}F_3S$: C; 61.29, H; 6.25. Found: C; 61.19, H; 5.94.

3.1.12. *S*-Phenyl 2,2-difluoro-3,3-dimethylbutanethioate (11a). Radical adduct **6a** (90 mg, 0.36 mmol) was dissolved in CH_2Cl_2 (2 mL). To the solution, MCPBA (74 mg, 0.43 mmol) was added in several portions. The resulting mixture was stirred for 12 h at room temperature. Then, the mixture was successively washed with 5% NaS_2O_3 solution and sat. $NaHCO_3$ solution. The CH_2Cl_2 solution was dried over $MgSO_4$, and the solvent was removed under reduced pressure to give crude sulfoxide **10a** (76 mg, 80%). Trifluoroacetic anhydride (0.5 mL) was added to the ice-cooled **10a** under stirring, and resulting mixture was stirred for 10 h at room temperature. Then, crashed ice (ca. 1 g) was added to the mixture and the mixture was stirred for 2 h. After neutralization with $NaHCO_3$, the mixture was extracted with Et_2O. The combined extracts were dried over Na_2SO_4, and the solvent was removed under reduced pressure. The residue was chromatographed on a silica gel column eluting with 5% EtOAc–hexane to give thioate **11a** as colorless oil: 59 mg (67%); ^{1}H NMR δ 1.13 (s, 9H), 7.40–7.48 (m, 5H); ^{19}F NMR δ −112.2 (s); EI-MS *m/z* (rel. %) 244 (19, M^+), 216 (11), 110 (100), 109 (50),107 (29), 87 (33), 65 (76), 59 (12), 51 (16). Anal. Calcd for $C_{12}H_{14}F_2OS$: C; 59.00, H; 5.78. Found: C; 58.89, H; 5.73.

3.1.13. *S*-Phenyl 2,2-difluoropentanethioate (11b). The title compound was obtained from radical adduct **6b** (289 mg, 1.23 mmol) as above: 180 mg (64%); ^{1}H NMR δ 0.98 (t, J=7.3 Hz, 3H), 1.55 (sex, J=7.5 Hz, 2H), 1.97–2.15 (m, 2H), 7.41–7.46 (m, 5H); ^{19}F NMR δ −103.6 (t, J=17±2 Hz); EI-MS m/z (rel. %) 230 (13, M^+),110 (100), 109 (45), 73 (16), 65 (28), 51 (12). Anal. Calcd for $C_{11}H_{12}F_2OS$: C; 57.37, H; 5.25. Found: C; 57.28, H; 5.56.

3.1.14. *S*-Phenyl 2,2-difluorooctanethioate (11c). The title compound was obtained from radical adduct **6c** (332 mg, 1.20 mmol) as above: 191 mg (58%); ^{1}H NMR δ 0.872 (t, J=6.7 Hz, 3H), 1.20–1.39 (m, 6H), 1.53 (quint, J=6 Hz, 2H), 1.98–2.15 (m, 2H), 7.33–7.44 (m, 5H); ^{19}F NMR δ −103.6 (t, J=17±2 Hz); EI-MS m/z (rel. %) 272 (7, M^+), 111 (10), 110 (100), 109 (29), 65 (10), 55 (11). Anal. Calcd for $C_{14}H_{18}F_2OS$: C; 61.74, H; 6.66. Found: C; 61.96, H; 6.99.

3.1.15. *S*-Phenyl 2,2-difluoroundecanethioate (11d). The title compound was obtained from radical adduct **6d** (114 mg, 0.36 mmol) as above: 46 mg (41%); ^{1}H NMR δ 0.86 (t, J=6.7 Hz, 3H), 1.17–1.36 (m, 10H), 1.50 (quint, J=7 Hz, 2H), 1.98–2.15 (m, 2H), 7.39–7.47 (m, 5H); ^{19}F NMR δ −103.6 (t, J=17±2 Hz); EI-MS m/z (rel. %) 314 (5, M^+), 110 (100), 109 (22), 69 (11), 57 (26), 55 (20). Anal. Calcd for $C_{17}H_{24}F_2OS$: C; 64.94, H; 7.69. Found: C; 64.85, H; 8.01.

3.1.16. *S*-Phenyl 2,2-difluoro-3-methylbutanethioate (11e). The title compound was obtained from radical adduct **6e** (100 mg, 0.43 mmol) as above: 61 mg (63%); ^{1}H NMR δ 1.08 (d, J=6.9 Hz, 6H), 2.42 (t-sep, J=15.3, 6.9 Hz, 1H), 7.38–7.58 (m, 5H); ^{19}F NMR δ −111.7 (d, J=15±2 Hz); EI-MS m/z (rel. %) 230 (18, M^+), 111 (10), 110 (100), 109 (44), 93 (17), 65 (85), 51 (14). Anal. Calcd for $C_{11}H_{12}F_2OS$: C; 57.37, H; 5.25. Found: C; 57.15, H; 5.41.

3.1.17. *S*-Phenyl 2-cyclohexyl-2,2-difluoroethanethioate (11f). The title compound was obtained from radical adduct **6f** (220 mg, 0.80 mmol) as above: 110 mg (51%); ^{1}H NMR δ 0.81–0.90 (m, 1H), 1.10–1.34 (m, 6H), 1.62–1.72 (m, 1H), 1.76–1.88 (m, 2H), 2.00–2.18 (m, 1H), 7.35–7.50 (m, 5H); ^{19}F NMR δ −110.9 (d, J=15±2 Hz); EI-MS m/z (rel. %) 270 (7, M^+), 113 (46), 110 (100), 109 (31), 93 (14), 77 (14), 73 (10), 65 (16), 51 (16). Anal. Calcd for $C_{14}H_{16}F_2OS$: C; 62.20, H; 5.97. Found: C; 62.02, H; 6.28.

3.1.18. *S*-Phenyl 2,2-difluoro-2-oxolan-2-ylethanethioate (11g). The title compound was obtained from radical adduct **5a** (210 mg, 0.80 mmol) as above: 131 mg (63%); ^{1}H NMR δ 1.86–2.28 (m, 4H), 3.86–4.00 (m, 2H), 4.46 (dtd, J=17.7, 7.2, 6.0 Hz, 1H), 7.457 (s, 5H); ^{19}F NMR δ −120.7 (dd, J=259±2, 15±2 Hz, 1F), −110.0 (dd, J=259±2, 6±2 Hz, 1F); EI-MS m/z (rel. %) 258 (17, M^+), 109 (46), 71 (100), 51 (40). Anal. Calcd for $C_{12}H_{12}F_2O_2S$: C; 55.80, H; 4.68. Found: C; 56.02, H; 4.77.

3.1.19. 2,2-Difluoro-2-oxolan-2-ylacetic acid (13). Thioate **11g** (140 mg, 0.54 mmol) and KOH (30 mg, 0.54 mmol) were dissolved in methanol (10 mL) and the mixture was stirred at room temperature for 5 h. Under reduced pressure, methanol was removed, and then after addition of water, the resulting mixture was washed with Et_2O. The aqueous layer was pored into 10% hydrochloric acid. The mixture was extracted with Et_2O and dried over $MgSO_4$. The solvent was removed under reduced pressure to give carboxylic acid **13** which was identical with an authentic sample:[10] 70 mg (78%).

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Tetrahedron 60 (2004) 4037–4049
Tetrahedron

A new approach to 2,2-disubstituted chromenes and tetrahydroquinolines through intramolecular cyclization of chiral 3,4-epoxy alcohols

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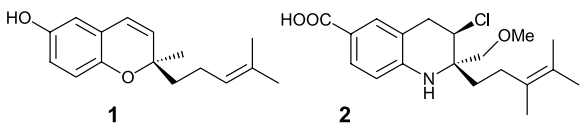
Received 29 October 2003; revised 9 February 2004; accepted 3 March 2004

Abstract—An efficient route to chiral chromene and tetrahydroquinoline ring models **3** and **4** was developed by means of the vanadium epoxidation of chiral homoallylic alcohols **12** and **19** followed by an intramolecular epoxide opening of 3,4-epoxy alcohols **14** and **20**. The configuration of all compounds was confirmed using NMR analysis.

1. Introduction

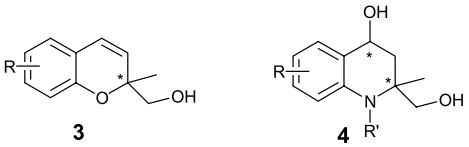
O and *N*-Heterocyclic compounds have attracted considerable attention because of their functionality in pharmaceutical chemistry. In particular, synthesis of benzo-fused *O* and *N*-heterocyclic compounds such as chromenes[1] and tetrahydroquinolines[2] is very important because they are found in a variety of natural products, which exert a broad range of bioactivities (e.g., antioxydants,[3] enzyme inhibitors,[4] antitumor agents,[5] antibiotic agents[6]). Numerous publications[7] described the preparation of these important classes of heterocycles. However, it remains a great challenge to establish stereogenic quaternary carbon on both chromene and tetrahydroquinoline ring systems. Only a few publications[8] have described an access to these types of compounds and have been directed to specific molecules such as, for example, cordiachromene[9] **1** or virantmycin[6f,g] **2** (Fig. 1).



1 2

Figure 1.

In a previous article, we described an efficient three-step procedure for the synthesis of substituted 2-hydroxymethyl-2-methyl-2*H*-chromenes.[10] Herein, we wish to report the extension of our versatile procedure to optically active 2-hydroxymethyl-2-methyl-2*H*-chromenes **3** and to another class of heterocycles, 2-hydroxymethyl-2-methyl-1,2,3,4-tetrahydroquinolin-4-ols **4** (Fig. 2).



3 4

Figure 2.

2. Results and discussion

2.1. Retrosynthetic analysis

A retrosynthetic analysis of target molecule **3** is delineated in Scheme 1. The formation of 2*H*-chromene moiety can be achieved by a ring closure of key chiral epoxide **6** under acidic conditions. This ring closure through path a (6-*exo-tet*) is a more favored process than path b (7-*endo-tet*) following Baldwin's rules.[11] Key intermediate **6** is based on a 3,4-epoxy-3-methyl-1-(2-subtituted phenyl)butan-1-ol subunit. The desired oxirane function can be introduced by a stereoselective epoxidation of protected homoallylic alcohol **7** with vanadium (IV)/*tert*-butylhydroperoxide as oxidants. Required homoallylic alcohol **7** can be synthesized by asymmetric allylation of either various substituted 2-hydroxybenzaldehyde **8** with (+) or (−)-β-methallyldiisopinocampheylborane **9**. Homoallylic alcohols can also be obtained by asymmetric reduction of ketone **10**

Keywords: Chromene; Tetrahydroquinoline; 2*H*-1-Benzopyrane.
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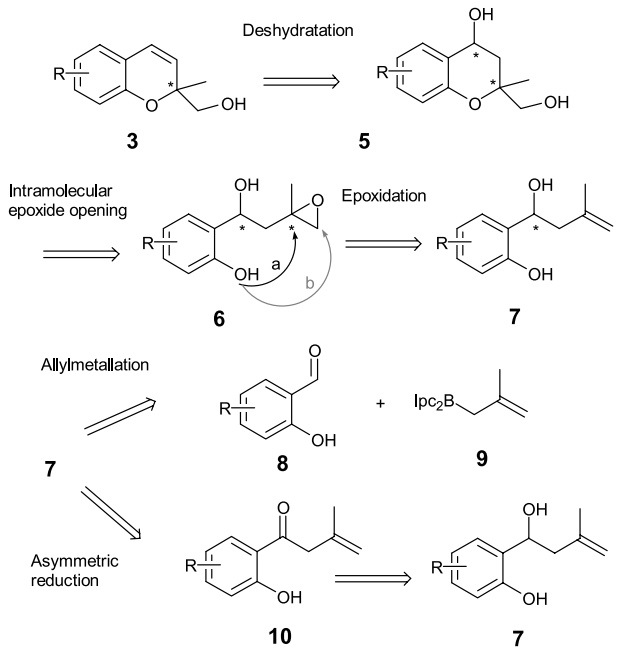
0040–4020/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2004.03.010

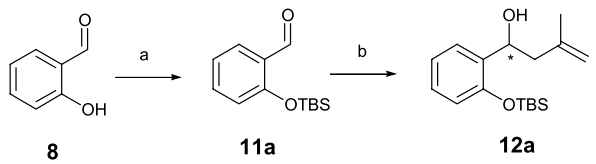
using CBS-oxazaborolidine. This strategy will be applied to nitrogen analogues to form target tetrahydroquinoline **4**.



Scheme 1. Retrosynthetic analysis of 2,2-disubstituted chromenes **3**.

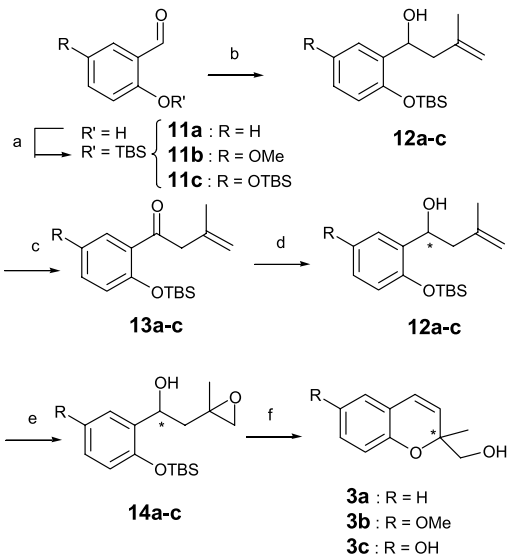
2.2. Asymmetric synthesis of 2,2-disubstituted chromenes 3

As depicted in Scheme 2, the reaction of salicylaldehyde with *tert*-butyldimethylsilylchloride (TBDMS-Cl) and imidazole in DMF afforded the corresponding 2-OTBS benzaldehyde **11a** in excellent yield. Brown's asymmetric allylation[12] (method A) of compounds **11a** with β-methallyldiisopinocampheylborane **9**, prepared from (+) or (−)-β-chlorodiisopinocampheylborane ((+) or (−)-Ipc_2BCl) and 2-methylpropenylmagnesium chloride, afforded corresponding chiral homoallylic alcohols (+) or (−)-**12a** in good yields and 80% ee (best value, Table 1).



Scheme 2. Reagents and conditions: (a) TBDMSCl, imidazole, DMF, rt; (b) (+) or (−)-methallyldiisopinocampheylborane **9**, THF, −78 °C, 2 h.

Accordingly, an improvement in ee values was necessary, so we turned our attention to a synthesis of enantiopure compounds **12a-c**, based on asymmetric reduction of corresponding ketones **13a-c** using (*R*) or (*S*)-CBS-oxazaborolidines[13] as chiral reducing agents (method B in Table 1, Scheme 3). β,γ-Unsaturated ketones **13a-c** were obtained by treatment of compounds **11a-c** with an excess of 2-methylpropenylmagnesium chloride followed by oxidation with Dess–Martin periodinane (DMP). Enantioselective reduction of β,γ-unsaturated ketones **13a-c** with (*R*) or (*S*)-CBS-oxazaborolidine reagent and catecholborane at −60 °C led to homoallylic alcohols **12a-c** with quantitative yields and ee values ranging from 85 to 95% (Table 1).



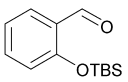
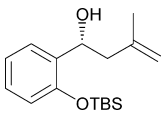
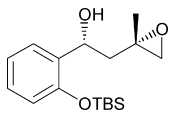
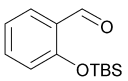
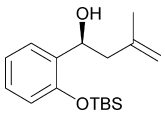
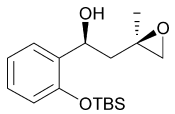
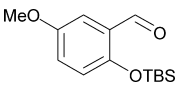
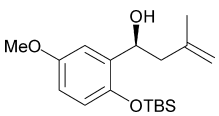
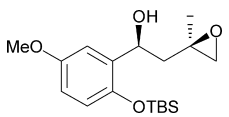
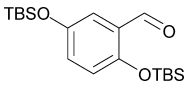
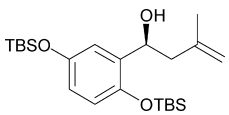
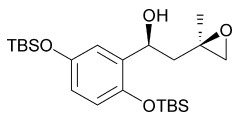
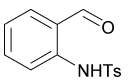
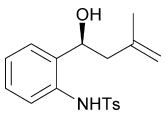
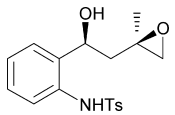
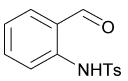
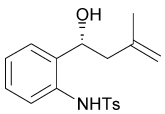
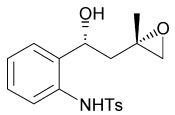
Scheme 3. Reagents and conditions: (a) TBDMSCl, imidazole, DMF, rt; (b) 2-methylpropenylmagnesium chloride, THF, 50 °C, 3 h; (c) DMP, DCM, 0 °C, 1 h; (d) (*R*) or (*S*)-CBS oxazaborolidine, catecholborane, toluene, −60 °C; (e) TBHP, $VO(acac)_2$, DCM, −10 °C, 5 h; (f) (i) TBAF, THF, 0 °C; (ii) CSA (4 mol%), toluene, reflux, 16 h.

The next step was the stereoselective epoxidation of chiral homoallylic alcohols **12a-c** by the usual and cheap procedure using *tert*-butylhydroperoxide (TBHP) and a catalytic amount of vanadylacetylacetonate ($VO(acac)_2$).[14] In each case, epoxyalcohols **14a-c** were obtained in both good yields and diastereoselectivities (Table 1, Scheme 3). Subsequent *O*-silyldeprotection of **14a-c** with TBAF followed by the ring closure in refluxing toluene with a catalytic amount of camphor sulfonic acid (CSA) (4 mol%) gave directly chromenes **3a-c**, as reported in a recent paper.[10] It should be noted that the overall process permitted synthesis of chromenes with good yields, but in all cases with a loss of optical purity (based on the ee value of starting epoxyalcohols **14a-c** (Table 2, entries 1–4). This result could be interpreted by a lack of selectivity during the cyclization step under acidic conditions or by a possible racemization via a retro-Claisen rearrangement, which has already been observed on such compounds upon exposure to light irradiation.[15]

In order to rationalize this result, we have first studied nucleophile centres which should be involved into the intramolecular cyclization reaction. According to Baldwin's rules, two pathways are favored. Scheme 4 presents the structures of the possible intermediates corresponding to this intramolecular cyclization reaction. For 6-*exo-tet* and 4-*exo-tet* favored cyclization reactions, which, respectively, involve one or two Walden inversion, two stereoisomers related to (+) or (−)-**3a** can be formed. These two competitive ring closures could explain the observed loss of optical purity for chromenes **3**.

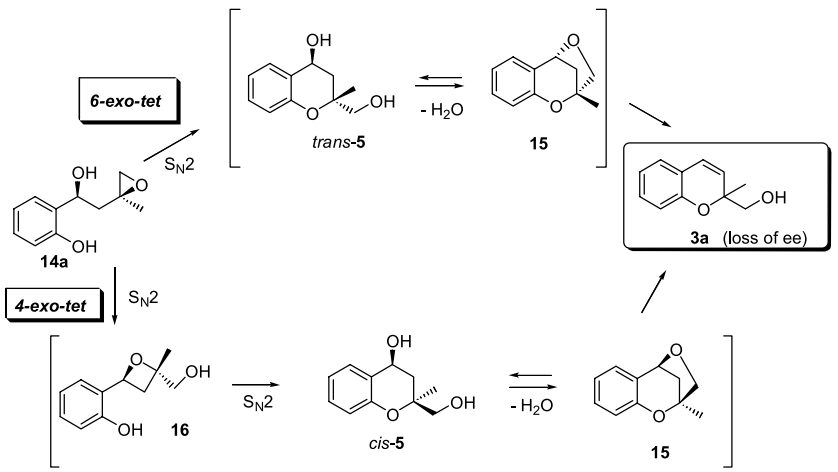
Alternatively, the hypothesis of a racemization reaction has

Table 1. Allylation and epoxidation reactions

Entry	Aldehydes	Method	Alcohols	Yields (%)[a] (ee)[b]	Epoxides	Yields (%)[a] (de)[c]
1		A[d]		79 (80%)		72 (84%)[e]
	11a	B[f]	(+)-(*R*)-**12a**	75 (93%)	(+)-(1*R*,3*S*)-**14a**	
2		A[g]		74 (80%)		72 (86%)[e]
	11a	B[h]	(−)-(*S*)-**12a**	72 (95%)	(−)-(1*S*,3*R*)-**14a**	
3		B[h]		70 (90%)		73 (84%)[e]
	11b		(−)-(*S*)-**12b**		(−)-(1*S*,3*R*)-**14b**	
4		B[h]		75 (85%)		70 (82%)[e]
	11c		(−)-(*S*)-**12c**		(−)-(1*S*,3*R*)-**14c**	
5		A[g]		84 (82%)		89 (82%)
	18		(+)-(*S*)-**19**		(+)-(1*S*,3*R*)-**20**	
6		A[d]		80 (84%)		96 (84%)
	18		(−)-(*R*)-**19**		(−)-(1*R*,3*S*)-**20**	

[a] Isolated yields.
[b] Enantiomeric excess were determined by HPLC.
[c] Diastereomeric excess determined by ^{1}H NMR spectroscopy.
[d] Reaction carried out with methallyldiisopinocampheylborane **9** prepared from (+)-DIPCl.
[e] Diastereomeric excess of epoxidation reaction with compounds **12** prepared by method B.
[f] Reaction carried out with (*S*)-CBS oxazaborolidine.
[g] Reaction carried out with methallyldiisopinocampheylborane **9** prepared from (−)-DIPCl.
[h] Reaction carried out with (*R*)-CBS oxazaborolidine.

also been taken in account. Exposure of chiral chromene **17**[9b] to the same experimental conditions used for the cyclization of **14** (refluxed toluene, CSA 4 mol%) led to a decrease in the optical activity of **17** (Scheme 5). Half-life for racemization of **17** is around 96 h. This result gave an important information about the rate of racemization of 2,2-dialkylchromene via a thermally induced retro-Claisen rearrangement.

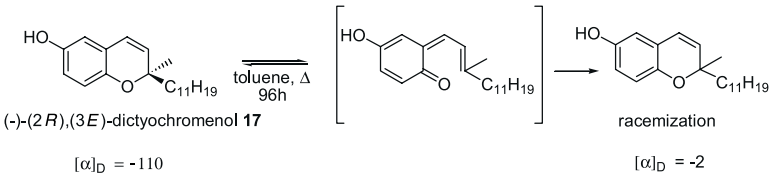


Scheme 4. Hypothetical mechanism for the formation of chromenes **3**.

From all these results, it seems that both the thermally induced racemization and the two possible ring closure pathways might limit stereoselectivity of this intramolecular cyclization reaction accounting for poor optical purity of chromenes **3**.

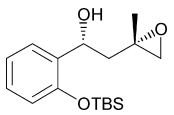
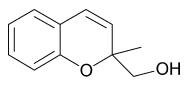
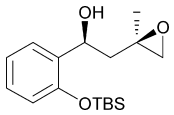
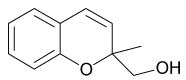
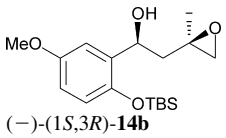
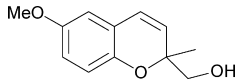
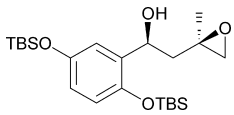
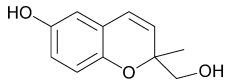
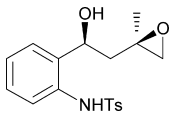
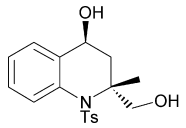
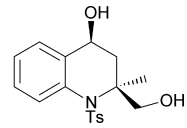
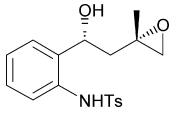
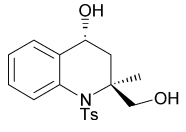
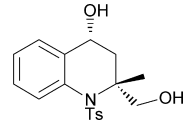
2.3. Asymmetric synthesis of 2,2-disubstituted-1,2,3,4-tetrahydroquinolin-4-ols 4

Tos-protected aminobenzaldehyde **18** was readily available in high yield by a two-step sequence on multigram scale from commercially available 2-aminobenzyl alcohol. After oxidation with manganese dioxide in dichloromethane to the corresponding aldehyde,[16] the amino group was protected as a sulfonamide. Compound **18** was easily transformed to homoallylic alcohols **19** (80<ee<86%) which were then converted to corresponding epoxyalcohols **20** using the same experimental conditions as above for the preparation of **14a-c** (Scheme 6, Table 1). It is noteworthy that in order to increase the ee of compounds **19**, we attempted, without any success, asymmetric reduction of the corresponding ketone.[17] At this stage in our synthetic strategy, behavior of **20** in the intramolecular epoxide opening sequence was investigated using conditions described by Morimoto et al.[6f,g] Treatment of **20** with trifluoroacetic acid in toluene at room temperature for 16 h expectedly provided desired 1,2,3,4-tetrahydroquinolin-4-ol **4** as the exclusive product of cyclization reaction. Moreover,

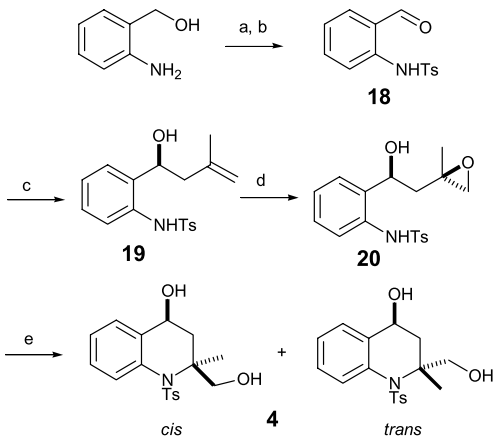


Scheme 5. Racemization of **17**.

Table 2. Synthesis of chromene **3a-c** and tetrahydroquinoline **4**

Entry	Epoxides	Alcohols		Yields (%)[a] (ee)
1	 (+)-(1*R*,3*S*)-**14a**	 (+)-**3a**		80 (46%)[b]
2	 (−)-(1*S*,3*R*)-**14a**	 (−)-**3a**		80 (44%)[b]
3	 (−)-(1*S*,3*R*)-**14b**	 (−)-**3b**		75 (43%)[b]
4	 (−)-(1*S*,3*R*)-**14c**	 (−)-**3c**		68 (35%)[b]
5	 (+)-(1*S*,3*R*)-**20**	 (+)-*trans*-(2*R*,4*S*)-**4**	 (+)-*cis*-(2*S*,4*S*)-**4**	45 (+)-*trans*-**4**: (88%)[c] (+)-*cis*-**4**: (40%)[c]
6	 (−)-(1*R*,3*S*)-**20**	 (−)-*trans*-(2*S*,4*R*)-**4**	 (−)-*cis*-(2*R*,4*R*)-**4**	58 (−)-*trans*-**4**: (94%)[c] (−)-*cis*-**4**: (63%)[c]

[a] Isolated overall yields.
[b] Enantiomeric excesses determined by HPLC on a chiral column.
[c] Enantiomeric excesses determined by HPLC on a chiral column after isolation of each diastereomer by flash chromatography.



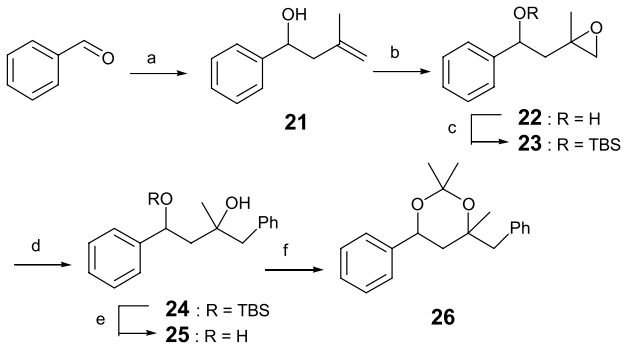
Scheme 6. Reagents and conditions: (a) MnO_2, DCM, rt; (b) acid *p*-toluene sulfonyl chloride, DCM, pyridine, rt; (c) (+) or (−)-methallyldiisopinocampheylborane **9**, THF, −78 °C, 2 h; (d) TBHP, $VO(acac)_2$, DCM, −5 °C, 5 h; (e) 2 equiv. of TFA, toluene, rt, 16 h.

it should be pointed out that compound **4** was obtained as a mixture of diastereomers (50:50) which were easily separated by column chromatography to furnish diastereomerically pure tetrahydroquinolines (+)-*cis*-**4** and (+)-*trans*-**4** from (+)-**20** and (−)-*cis*-**4** and (−)-*trans*-**4** from (−)-**20** in good overall yields (Table 2, entries 5 and 6). As for chromene's series, the loss of stereoselectivity in the last step might be explained by assuming that the two favored mechanisms are competitive.

In addition, the enantiomeric excess in which these heterocycles **4** were obtained, was determined by chiral HPLC showing that little or no racemization occurred at C1 during the process from **20** to tetrahydroquinoline **4** (Table 2).

2.4. Determination of relative and absolute configuration of the products

2.4.1. Determination of absolute configuration of 3,4-epoxy alcohols 14. While the oxidative reaction of homoallylic alcohols using vanadium (IV)[14] as a catalyst often gave epoxy alcohols with both good yields and diastereoselectivities, our major intention was to establish the relative configuration between C1 and new C3 stereogenic centre created during epoxidation and consequently, the absolute configuration at C3. With this idea in mind, we envisaged the conversion of epoxy alcohols to their corresponding six-membered acetonides in order to study their conformational properties by NMR spectroscopy. In order to simplify this study, the synthetic sequence described in Scheme 7 was carried out with epoxy alcohol **22** instead of more complex epoxy alcohols **14**. Thus, epoxy alcohol **22** was prepared in two steps starting from benzaldehyde and 2-methylpropenylmagnesium chloride followed by oxidation of the resultant product with *tert*-butylhydroperoxide (TBHP) and a catalytic amount of vanadylacetylacetonate ($VO(acac)_2$). According to these experimental conditions, **22** was obtained in good yield as a 80:20 mixture of diastereomers. Then, **22** was converted to acetonide **26** in a four-step sequence involving protection of the secondary alcohol, regioselective addition of phenyllithium, *O*-silyl deprotection and acetalization.



Scheme 7. Reagents and conditions: (a) 2-methylpropenylmagnesium chloride, THF, 50 °C, 3 h; (b) TBHP, $VO(acac)_2$, DCM, 0 °C, 8 h; (c) TBDMSCl, imidazole, DMF, rt; (d) PhLi, $BF_3{\cdot}Et_2O$, THF, −78 °C; (e) TBAF, THF, rt; (f) CSA, DMP, rt.

The relative configuration of **26** was determined by ^{13}C NMR analysis as shown in Figure 3.

The chemical shifts of the methyl of the acetal for major compound **26** were in accord with a 1,3-*syn* stereochemistry.[18] These results confirmed the *syn* stereoselectivity of the epoxidation with vanadium (IV)[14] as a catalyst.

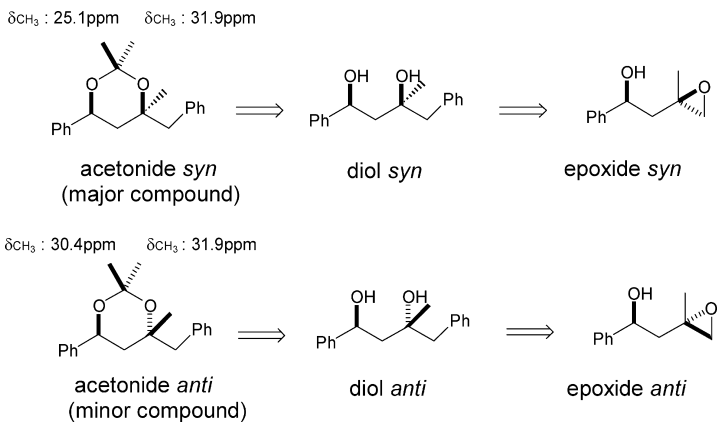


Figure 3. ^{13}C NMR analysis of epoxides.

2.4.2. Determination of absolute configuration of tetrahydroquinolines 4. The configurational assignment of compounds **4** was effected by NOESY experiments. As summarized in Figure 4, NOESY spectra clearly showed a significant NOE corresponding to the dipolar interactions between H4 and H2′ for compounds (−)-*trans*-**4** and (+)-*trans*-**4** when either H4 or H2′ were irradiated. This suggests a *cis* configuration between them for *trans*-**4**. On the other hand, the absence of a NOE enhancement on H4 for *cis*-**4** when H2′ was irradiated suggests a *trans* configuration of the latters. This result was confirmed by a NOE enhancement of H4 by irradiation of CH_3 even if it is difficult to evaluate because of interactions between H4 and H3 in the same time (same chemical shifts for H3 and CH_3). Therefore, the determined *cis* configuration between H4 and H2′ of *trans*-**4** confirms (2*R*,4*S*) and (2*S*,4*R*) absolute configuration of the stereogenic centres of (+)-*trans*-**4** and (−)-*trans*-**4**. The *cis* configuration between H4 and CH_3 of *cis*-**4** allows us to assign (2*S*,4*S*) and (2*R*,4*R*) absolute configuration of stereogenic centres of (+)-*cis*-**4** and (−)-*cis*-**4** compounds.

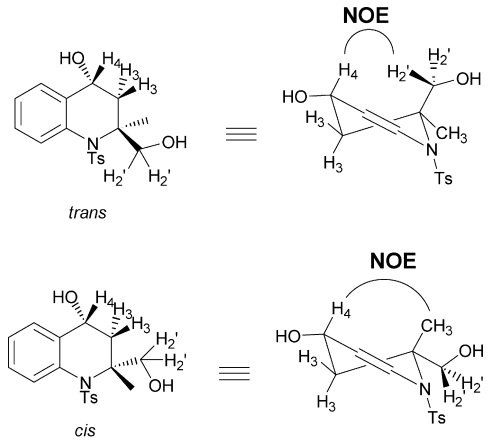


Figure 4. NOE correlation observed in *cis*- and *trans*-**4**.

3. Conclusion

In summary, we successfully extended our method for the stereoselective synthesis of chromene and tetrahydroquinoline rings. If the synthetic utility of the method was limited because of racemization of chromenes, the diastereomeric mixtures of tetrahydroquinolines were easily separated by column chromatography and the absolute configuration of diastereomers was thus assigned.

4. Experimental

4.1. Apparatus

^1H and ^{13}C NMR spectra were recorded on Bruker AC 200 and Bruker AMX 400 spectrometers in $CDCl_3$ as the solvent and TMS as the internal standard; chemical shifts (δ) are expressed in ppm and coupling constants (J) in Hertz. IR spectra were recorded on a Bruker vector 22 spectrometer. Mass spectra (*m*/*e* (% base peak)) were recorded on HP 5889A spectrometer in EI mode (70 eV) or in CI mode (with CH_4 or NH_3 as reacting gas). For high performance liquid chromatography (HPLC) analysis, a Hewlett–Packard model (HP 1050) equipped with a UV detector (254 nm) and a CHIRALCEL OD-H column were employed. Optical rotations were measured on a Perkin–Elmer 341 polarimeter. Melting points were determined on a C. REICHERT microscope apparatus and were uncorrected. Elemental analysis were carried out by CNRS Analysis Laboratory, Vernaison, France, on a Perkin–Elmer 2400 C, H, N elemental analyser.

4.2. Chemicals

Every starting material was obtained from commercial suppliers and used without further purification. Dichloromethane, ethylacetate were dried by distillation over P_2O_5. Diethylether, THF, benzene and toluene were distilled from sodium.

4.3. General procedure for formation of silylether 11a-c

To a solution of phenol derivative (3.5 mmol), imidazole (1 g, 14.8 mmol) in DMF (10 mL) was added *tert*-butyldimethylsilyl chloride (810 mg, 5.4 mmol). The reaction was heated 20 h at 80 °C, then 50 mL of water and 50 mL of EtOAc were added. The aqueous layer was extracted with EtOAc (3×40 mL), the combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (9:1, petroleum ether/EtOAc) to give the silylether **11a-c**.

4.3.1. 2-*tert*-Butyldimethylsilyloxybenzaldehyde 11a. Compound **11a** was obtained from salicyladehyde as a colorless oil (740 mg, 90% yield); ^1H NMR (200 MHz, $CDCl_3$): δ_H 0.26 (s, 6H, $2SiCH_3$), 1.01 (s, 9H, $3CH_3$), 6.85–7.82 (m, 4H, H_{ar}), 10.45 (s, 1H, CHO); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ_C −4.3, −2.9, 18.3, 25.6 (3C), 120.1, 121.4, 127.1, 128.2, 135.6, 158.3, 190.1; IR (film) ν 2956, 2931, 2859, 1689, 1599, 1479, 1254 cm^{-1}; MS-CI *m*/*z* (relative intensity) 237 (M+1, 100), 221 (30), 179 (28).

4.3.2. 4-Methoxy-2-*tert*-butyldimethylsilyloxybenzaldehyde 11b. Compound **11b** was obtained from 2-hydroxy-5-methoxybenzaldehyde (456 mg, 3.0 mmol) as a colorless oil (718 mg, 90% yield); ^1H NMR (200 MHz, $CDCl_3$): δ_H 0.24 (s, 6H, $2SiCH_3$), 1.01 (s, 9H, $3CH_3$), 3.80 (s, 3H, OMe), 6.80–7.28 (m, 3H, H_{ar}), 10.41 (s, 1H, CHO); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ_C −4.4 (2C), 18.3, 25.6 (3C), 55.7, 109.5, 121.6, 123.9, 127.1, 153.4, 154.0, 190.0; IR (film) ν 2956, 2931, 2886, 2858, 1683, 1489 cm^{-1}; MS-EI *m*/*z* (relative intensity) 266 (M^+, 0), 251 (1), 209 (100), 191 (12), 166 (19).

4.3.3. 2,5-Di-*tert*-butyldimethylsilyloxybenzaldehyde 11c. Compound **11c** was obtained from 2,5-dihydroxybenzaldehyde (420 mg, 3.0 mmol) as a colorless oil (660 mg, 90% yield); ^1H NMR (200 MHz, $CDCl_3$): δ_H 0.16 (s, 6H, $2CH_3$), 0.23 (s, 6H, $2CH_3$), 0.96 (s, 9H, $3CH_3$), 1.00 (s, 9H, $3CH_3$), 6.72–7.25 (m, 3H, H_{ar}), 10.37 (s, 1H, CHO); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ_C −4.5 (2C), −4.4 (2C), 18.1, 18.3, 25.6 (6C), 117.6, 121.1, 127.4, 127.9, 149.8, 153.4, 189.9; IR (film) ν 2956, 2931, 2859, 1689, 1488 cm^{-1}; MS-CI *m*/*z* (relative intensity) 367 (M+1, 100), 308 (11).

4.4. Preparation of methallylborane reagents 9

To a solution of diisopinocampheylborane chloride (4 equiv.) in anhydrous diethyl ether (20 mL) at 0 °C under argon was added dropwise a solution of 2-methylpropenyl magnesium chloride (1.5 equiv.) in THF. The reaction mixture was stirred 1 h at 0 °C and 1 h at room temperature. The formation of methallyldiisopinocampheylborane **9** is indicated by precipitation of the magnesium salts. The reagent can be readily isolated as a clear solution, free of magnesium salts, by passing the reaction mixture through a filtration chamber.

4.5. Typical procedure for preparation of homoallylic alcohols 12a

To the precedent clear filtrate **9** (free of magnesium salts) was added, dropwise, at −78 °C and under argon, a solution of compound **11a** (1 equiv.) in anhydrous THF (10 mL). The mixture was stirred for 3 h until TLC showed complete disappearance of starting material. The reaction mixture was then hydrolyzed with 1 N HCl (12 mL) and the organic layer was washed with brine (20 mL), dried over $MgSO_4$ and concentrated in vacuo. The crude product was heated 1 h in a Kugelrohr at 100 °C under 5 mbar in order to remove most of isopinocampheol formed during the reaction. The residue was purified by column chromatography on silica gel (8:2, petroleum ether/EtOAc).

4.5.1. (+)-(1*R*)-3-Methyl-1-(2-*tert*-butyldimethylsilyloxyphenyl)but-3-en-1-ol 12a. Compound (+)-(*R*)-**12a** was obtained from **11a** and **9** (prepared with (+)-DIP-Cl) as a pale yellow solid in 79% yield; $[\alpha]_D^{20}=+38.8$ ($c=1.2$, acetone); ee=80%; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.27 (s, 3H, $SiCH_3$), 0.28 (s, 3H, $SiCH_3$), 1.02 (s, 9H, $3CH_3$), 1.80 (s, 3H, CH_3), 2.27 (d, 1H, $J=3.2$ Hz, OH), 2.33 (dd, 1H, $J=14$, 9.6 Hz, H^2), 2.52 (dd, 1H, $J=14$, 3.2 Hz, H^2), 4.86 (s, 1H, H^4), 4.92 (s, 1H, H^4), 5.15 (dt, 1H, $J=3.2$, 3.2, 9.6 Hz, H^1), 6.79 (d, 1H, $J=8.0$ Hz, H_{ar}), 6.96 (dd, 1H, $J=7.2$, 7.6 Hz, H_{ar}), 7.13 (ddd, 1H, $J=7.2$, 8.0, 1.6 Hz, H_{ar}), 7.44 (dd, 1H, $J=7.6$, 1.6 Hz, H_{ar}); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ_C −4.1 (2C), 18.2, 22.4, 25.8 (3C), 46.3, 66.3, 113.7, 118.0, 121.2, 126.7, 127.8, 134.2, 142.8, 152.2; IR (film) ν 3424, 2956, 2931, 2859, 1488, 1254 cm^{-1}; MS-EI *m*/*z* (relative intensity) 292 (M^+, 1), 277 (1), 237 (62), 165 (38), 73 (100).

4.5.2. (−)-(1*S*)-3-Methyl-1-(2-*tert*-butyldimethylsilyloxyphenyl)but-3-en-1-ol 12a. Compound (−)-(*S*)-**12a** was obtained from **11a** and **9** (prepared with (−)-DIP-Cl) as a pale yellow solid in 74% yield; $[\alpha]_D^{20}=-35.9$ ($c=1.3$, acetone); ee=80%.

4.6. General procedure for the formation of racemic homoallylic alcohol 12a-c from aldehyde 11a-c

To a solution of 2-methylpropene magnesium chloride (0.6 M in THF) at −30 °C was added dropwise a solution of aldehyde **11a-c** (2.1 mmol) in THF (10 mL). The reaction was stirred 1 h at room temperature and then quenched with a saturated aqueous solution of NH_4Cl (20 mL). The aqueous layer was extracted with EtOAc (3×20 mL), the combined organic layers were dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (9:1, petroleum ether/EtOAc).

4.6.1. 3-Methyl-1-(2-*tert*-butyldimethylsilyloxyphenyl)but-3-en-1-ol 12a. Compound **12a** was obtained from **11a** as a colorless oil in 98% yield.

4.6.2. 3-Methyl-1-(5-methoxy-2-*tert*-butyldimethylsilyloxyphenyl)but-3-en-1-ol 12b. Compound **12b** was obtained from **11b** as a colorless oil in 97% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.24 (s, 3H, $SiCH_3$), 0.25 (s, 3H, $SiCH_3$), 1.01 (s, 9H, $3CH_3$), 1.81 (s, 3H, CH_3), 2.25 (d, 1H, $J=2.8$ Hz, OH), 2.31 (dd, 1H, $J=9.6$, 14.0 Hz, H^2), 2.51 (dd, 1H, $J=3.2$, 14.0 Hz, H^2), 3.77 (s, 3H, OMe), 4.87 (bs, 1H, H^4), 4.92 (bs, 1H, H^4), 5.11 (dt, 1H, $J=3.2$, 9.6, 2.8 Hz, H^1), 6.66 (dd, 1H, $J=8.8$, 2.8 Hz, H_{ar}), 6.71 (d, 1H, $J=8.8$ Hz, H_{ar}), 7.02 (d, 1H, $J=2.8$ Hz, H_{ar}); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ_C −4.2, −4.0, 18.1, 22.3, 25.8 (3C), 46.2, 55.6, 66.2, 111.7, 113.0, 113.7, 118.7, 135.0, 142.7, 145.9, 153.9; IR (film) ν 3472, 3074, 2956, 2931, 2858, 1495 cm^{-1}; MS-EI *m*/*z* (relative intensity) 322 (M^+, 1), 267 (24), 209 (39), 195 (36), 75 (44), 73 (100).

4.6.3. 3-Methyl-1-(2,5-di-*tert*-butyldimethylsilyloxyphenyl)but-3-en-1-ol 12c. Compound **12c** was obtained from **11c** as a colorless oil in 98% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.15 (s, 6H, $2SiCH_3$), 0.22 (s, 3H, $SiCH_3$), 0.23 (s, 3H, $SiCH_3$), 0.96 (s, 9H, $3CH_3$), 0.99 (s, 9H, $3CH_3$), 1.78 (s, 3H, CH_3), 2.24 (bs, 1H, OH), 2.29 (dd, 1H, $J=9.6$, 14.0 Hz, H^2), 2.47 (dd, 1H, $J=3.2$, 14.0 Hz, H^2), 4.84 (s, 1H, H^4), 4.89 (s, 1H, H^4), 5.05 (dd, 1H, $J=3.2$, 9.6 Hz, H^1), 6.57 (dd, 1H, $J=12.0$, 4.0 Hz, H_{ar}), 6.62 (d, 1H, $J=12.0$ Hz, H_{ar}), 6.89 (d, 1H, $J=4.0$ Hz, H_{ar}); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ_C −4.5 (2C), −4.1, −3.9, 18.1, 18.2, 22.4, 25.7 (6C), 46.2, 66.4, 113.7, 118.1, 118.5, 118.7, 134.9, 142.7, 146.4, 149.6; IR (film) ν 3489, 3075, 2956, 2930, 2858, 1488 cm^{-1}; MS-CI *m*/*z* (relative intensity) 422 (M^+, 21), 404 (100), 366 (81).

4.7. General procedure for oxidation of homoallylic alcohol 12a-c with Dess–Martin periodinane (DMP)

To a solution of alcohol **12a-c** (1.7 mmol) in DCM (10 mL) was added at 0 °C Dess–Martin Periodinane (1.06 g, 2.5 mmol). After 1 h, an aqueous solution of $NaHCO_3$ (10%) and $Na_2S_2O_3$ (10%) was added, the aqueous layer was extracted with DCM (3×20 mL). The combined organic layers were dried over $MgSO_4$, and concentrated in vacuo to give the unstable ketone **13a-c** used without further purification.

4.7.1. 3-Methyl-1-(2-*tert*-butyldimethylsilyloxyphenyl)but-3-en-1-one 13a. Compound **13a** was obtained from **12a** as a colorless oil in 98% yield; ^{1}H NMR (200 MHz, $CDCl_3$): δ_H 0.24 (s, 6H, $2SiCH_3$), 0.99 (s, 9H, $3CH_3$), 1.76 (s, 3H, CH_3), 3.70 (s, 2H, H^2), 4.78 (s, 1H, H^4), 4.90 (s, 1H, H^4), 6.82–7.51 (m, 4H, H_{ar}); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ_C −4.0 (2C), 18.3, 22.8, 25.8 (3C), 51.9, 114.7, 120.1, 121.1, 129.8, 131.5, 132.4, 139.5, 153.9, 201.6; IR (film) ν 2956, 2931, 2887, 2859, 1690, 1479, 1255, 910 cm^{-1}.

4.7.2. 3-Methyl-1-(5-methoxy-2-*tert*-butyldimethylsilyloxyphenyl)but-3-en-1-one 13b. Compound **13b** was obtained from **12b** as a colorless oil in 97% yield; ^{1}H NMR (200 MHz, $CDCl_3$): δ_H 0.22 (s, 6H, $2SiCH_3$), 0.99 (s, 9H, $3CH_3$), 1.77 (s, 3H, CH_3), 3.72 (s, 2H, H^2), 3.77 (s, 3H, OMe), 4.79 (bs, 1H, H^4), 4.91 (bs, 1H, H^4), 6.76–7.03 (m, 3H, H_{ar}); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ_C −4.1 (2C), 18.3, 22.8, 25.8 (3C), 51.8, 55.6, 113.2, 114.7, 119.1, 121.2, 131.5, 139.6, 147.8, 153.6, 201.2; IR (film) ν 2956, 2931, 2859, 1684, 1490 cm^{-1}.

4.7.3. 3-Methyl-1-(2,5-di-*tert*-butyldimethylsilyloxyphenyl)but-3-en-1-one 13c. Compound **13c** was obtained from **12c** as a colorless oil in 96% yield; ^{1}H NMR (200 MHz, $CDCl_3$): δ_H 0.15 (s, 6H, $2SiCH_3$), 0.20 (s, 6H, $2SiCH_3$), 0.96 (s, 9H, $3CH_3$), 0.97 (s, 9H, $3CH_3$), 1.75 (s, 3H, CH_3), 3.67 (s, 2H, H^2), 4.77 (s, 1H, H^4), 4.89 (s, 1H, H^4), 6.78–6.96 (m, 3H, H_{ar}); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ_C −4.5 (2C), −4.1 (2C), 18.1, 18.7, 22.8, 25.6 (3C), 25.8 (3C), 51.9, 114.7, 120.3, 120.7, 123.9, 131.8, 139.7, 148.1, 149.5, 202.5; IR (film) ν 2956, 2930, 2886, 2859, 1692, 1485, 1257, 909 cm^{-1}.

4.8. General procedure for enantioselective reduction of ketone 13a-c with CBS-oxazaborolidine

To a solution of ketone **13a-c** (1.7 mmol) and CBS-oxazaborolidine (0.17 mmol, 1 M in toluene) in anhydrous toluene (10 mL) at −60 °C under argon was added catecholborane (3.7 mmol, 1 M in THF). The reaction was stirred 16 h at this temperature and quenched with water (10 mL). The aqueous layer was extracted with EtOAc (3×10 mL), the combined organic layers were washed successively with an aqueous solution of $NaHCO_3$ (10%, 10 mL), an aqueous solution of HCl (1 N, 10 mL) and brine (10 mL). The organic layer was dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography.

4.8.1. (−)-(1*S*)-3-Methyl-1-(2-*tert*-butyldimethylsilyloxyphenyl)but-3-en-1-ol 12a. Compound (−)-**12a** was obtained by reduction of **13a** using (*R*)-CBS-oxazaborolidine as a colorless oil in 72% yield; $[\alpha]_D^{20}$=−44.3 (*c*=1, acetone); ee=95%.

4.8.2. (+)-(1*R*)-3-Methyl-1-(2-*tert*-butyldimethylsilyloxyphenyl)but-3-en-1-ol 12a. Compound (+)-**12a** was obtained by reduction of **13a** using (*S*)-CBS-oxazaborolidine as a colorless oil in 75% yield; $[\alpha]_D^{20}$=+43.8 (*c*=1.1, acetone); ee=93%.

4.8.3. (−)-(1*S*)-3-Methyl-1-(5-methoxy-2-*tert*-butyldimethylsilyloxyphenyl)but-3-en-1-ol 12b. Compound (−)-**12b** was obtained by reduction of **13b** using (*R*)-CBS-oxazaborolidine as a colorless oil in 70% yield; $[\alpha]_D^{20}$=−40.7 (*c*=0.8, acetone); ee=90%.

4.8.4. (−)-(1*S*)-3-Methyl-1-(2,5-di-*tert*-butyldimethylsilyloxyphenyl)but-3-en-1-ol 12c. Compound (−)-**12c** was obtained by reduction of **13c** using (*R*)-CBS-oxazaborolidine as a colorless oil in 75% yield; $[\alpha]_D^{20}$=−38.7 (*c*=0.8, acetone); ee=84%.

4.9. Representative procedure for epoxidation of chiral homoallylic alcohols 12a-c and 19 with vanadium (IV) and *tert*-butylhydroperoxide

To a blue solution of homoallylic alcohol and vanadyl acetylacetonate (0.05 equiv.) in anhydrous dichloromethane, was added, at −5 °C under argon, *tert*-butyl hydroperoxide (5.5 M in nonane, 2 equiv.). The resulting red-brown solution was stirred at −5 °C for 3–5 h. Then, the reaction mixture was poured into 10% aqueous $Na_2S_2O_3$ and extracted with dichloromethane. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (7:3, petroleum ether/EtOAc) to provide **14** and **20** as a mixture of diastereomers.

4.9.1. (−)-(1*S*,3*R*)-3,4-Epoxy-3-methyl-1-(2-*tert*-butyldimethylsilyloxyphenyl)butan-1-ol 14a. Compound (−)-**14a** was obtained from (−)-**12a** as a colorless oil in 72% yield; $[\alpha]_D^{20}$=−46.2 (*c*=0.75, acetone); de=86%; major isomer ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.28 (s, 6H, $2SiCH_3$), 1.02 (s, 9H, $3CH_3$), 1.46 (s, 3H, CH_3), 1.74 (dd, 1H, *J*=14.4, 10.0 Hz, H^2), 2.16 (dd, 1H, *J*=14.4, 3.2 Hz, H^2), 2.59–2.65 (2d, 2H, *J*=4.8 Hz, H^4), 2.98 (d, 1H, *J*=3.6 Hz, OH), 5.31 (dt, 1H, *J*=3.2, 3.6, 10.0 Hz, H^1), 6.65–6.71 (m, 2H, H_{ar}), 7.01–7.05 (m, 2H, H_{ar}); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ_C −4.0 (2C), 18.3, 21.2 25.8 (3C), 43.7, 53.7, 56.3, 66.6, 118.1, 121.3, 126.8, 127.9, 134.3, 151.9; minor isomer ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.25 (s, 6H, $2SiCH_3$), 1.01 (s, 9H, $3CH_3$), 1.38 (s, 3H, CH_3), 1.90 (dd, 1H, *J*=14.8, 10.4 Hz, H^2), 2.23 (dd, 1H, *J*=14.8, 2.4 Hz, H^2), 2.70 (d, 1H, *J*=4.0 Hz, H^4), 3.05 (d, 1H, *J*=4.0 Hz, H^4), 3.25 (d, 1H, *J*=2.4 Hz, OH), 5.31 (dt, 1H, *J*=2.4, 2.4, 10.4 Hz, H^1), 6.65–7.05 (m, 4H, H_{ar}); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ_C −4.3 (2C), 18.3, 22.6 25.7 (3C), 42.0, 52.9, 57.2, 65.9, 117.9, 121.3, 126.6, 127.9, 134.0, 151.9; IR (film) ν 3457, 2956, 2930, 2859, 1488, 1254 cm^{-1}; MS-CI *m/z* (relative intensity) 308 (M^+,80), 291 (100).

4.9.2. (+)-(1*R*,3*S*)-3,4-Epoxy-3-methyl-1-(2-*tert*-butyldimethylsilyloxyphenyl)butan-1-ol 14a. Compound (+)-**14a** was obtained from (+)-**12a** as a colorless oil in 72% yield; $[\alpha]_D^{20}$=+44.2 (*c*=1.1, acetone); de=84%.

4.9.3. (−)-(1*S*,3*R*)-3,4-Epoxy-3-methyl-1-(5-methoxy-2-*tert*-butyldimethylsilyloxyphenyl)butan-1-ol 14b. Compound (−)-**14b** was obtained from (−)-**12b** as a colorless oil in 73% yield; $[\alpha]_D^{20}$=−42.7 (*c*=1.1, acetone); de=84%; major isomer ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.25 (s, 6H, $2SiCH_3$), 1.01 (s, 9H, $3CH_3$), 1.47 (s, 3H, CH_3), 1.68 (dd, 1H, *J*=14.8, 10.0 Hz, H^2), 2.17 (dd, 1H, *J*=14.8, 2.8 Hz, H^2), 2.60–2.66 (2d, 2H, *J*=4.8 Hz, H^4), 3.01 (d, 1H, *J*=3.2 Hz, OH), 3.76 (s, 3H, OMe), 5.28 (dt, 1H, *J*=3.2, 2.8, 10.0 Hz, H^1), 6.65–6.71 (m, 2H, $2H_{ar}$), 7.01–7.05 (m, 1H, H_{ar}); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ_C −4.0 (2C), 18.3, 21.2, 25.6 (3C), 43.8, 53.7, 55.6, 56.3, 66.7, 111.7, 113.3, 118.8, 135.2, 145.6, 154.1; minor isomer ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.22 (s, 6H, $2SiCH_3$), 0.97 (s, 9H, $3CH_3$), 1.38 (s, 3H, CH_3), 1.90 (dd, 1H, *J*=14.8, 10.4 Hz, H^2), 2.23 (dd, 1H, *J*=14.8, 2.4 Hz, H^2), 2.71 (d, 1H, *J*=4.0 Hz, H^4), 2.07 (d, 1H, *J*=4.0 Hz, H^4), 3.31 (d, 1H, *J*=2.4 Hz, OH), 3.77 (s, 3H, OMe), 5.02 (dt, 1H, *J*=2.4, 2.4,

10.4 Hz, H^1), 6.65–7.05 (m, 3H, H_{ar}); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ_C −4.0 (2C), 18.3, 22.7, 25.9 (3C), 41.8, 52.8, 55.6, 57.3, 66.0, 111.7, 113.3, 118.6, 134.8, 145.5, 154.1; IR (film) ν 3473, 2930, 2858, 1495 cm^{-1}; MS-CI *m/z* (relative intensity) 338 (M^+, 62), 321 (M−17, 100).

4.9.4. (−)-(1*S*,3*R*)-3,4-Epoxy-3-methyl-1-(2,5-di-*tert*-butyldimethylsilyloxyphenyl)butan-1-ol 14c. Compound (−)-**14c** was obtained from (−)-**12c** as a colorless oil in 70% yield; $[\alpha]_D^{20}$=−39.1 (*c*=1.0, acetone); de=82%; major isomer ^1H NMR (400 MHz, $CDCl_3$): δ_H 0.16 (s, 6H, $2SiCH_3$), 0.24 (s, 6H, $2SiCH_3$), 0.96 (s, 9H, $3CH_3$), 1.01 (s, 9H, $3CH_3$), 1.45 (s, 3H, CH_3), 1.72 (dd, 1H, *J*=14.4, 10.0 Hz, H^2), 2.13 (dd, 1H, *J*=14.4, 3.2 Hz, H^2), 2.58–2.65 (2d, 2H, *J*=4.8 Hz, H^4), 2.91 (d, 1H, *J*=3.2 Hz, OH), 5.21 (dt, 1H, *J*=3.2, 3.2, 10.0 Hz, H^1), 6.58 (dd, 1H, *J*=8.8, 2.8 Hz, H_{ar}), 6.63 (d, 1H, *J*=8.8 Hz, H_{ar}), 6.90 (d, 1H, *J*=2.8 Hz, H_{ar}); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ_C −4.5 (2C), −4.0 (2C), 18.1, 18.3, 21.3, 25.7 (3C), 25.9 (3C), 43.8, 53.7, 56.3, 66.7, 118.2, 118.8, 118.9, 135.1, 146.1, 151.9; minor isomer ^1H NMR (400 MHz, $CDCl_3$): δ_H 0.16 (s, 6H, $2SiCH_3$), 0.23 (s, 6H, $2SiCH_3$), 0.96 (s, 9H, $3CH_3$), 0.98 (s, 9H, $3CH_3$), 1.38 (s, 3H, CH_3), 1.85 (dd, 1H, *J*=14.8, 10.0 Hz, H^2), 2.21 (dd, 1H, *J*=14.8, 2.4 Hz, H^2), 2.69 (d, 1H, *J*=4.0 Hz, H^4), 3.03 (d, 1H, *J*=4.0 Hz, H^4), 3.20 (d, 1H, *J*=2.4 Hz, OH), 4.99 (dt, 1H, *J*=2.4, 2.4, 10.0 Hz, H^1), 6.57–6.94 (m, 3H, H_{ar}); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ_C −4.3 (2C), −4.1 (2C), 18.1, 18.3, 22.7, 25.7 (3C), 25.9 (3C), 41.9, 52.9, 57.2, 66.0, 118.2, 118.8, 118.9, 134.8, 146.1, 151.8; IR (film) 3470, 2930, 2858, 1488 cm^{-1}; MS-CI *m/z* (relative intensity) 438 (M^+, 54), 421 (100).

4.10. General procedure for formation of 2*H*-1-benzopyran 3a-c

To a solution of epoxide **14a-c** (0.26 mmol) in THF (2 mL) was added at 0 °C TBAF (1 M in THF, 0.31 mmol). The reaction was stirred 1 h at room temperature and then hydrolyzed with a saturated aqueous solution of NH_4Cl (2 mL). The aqueous layer was extracted with EtOAc (3×10 mL), the organic layers were washed with brine (30 mL) and dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in toluene (2 mL) and a catalytic amount of CSA (4 mol%) was added and the reaction was heated at 80 °C for 16 h. After cooling, a saturated aqueous solution of $NaHCO_3$ (1 mL) was added, the combined organic layer was dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (8:2, petroleum ether/EtOAc) to give the 2*H*-1-benzopyran **3a-c**.

4.10.1. (−)-2-Hydroxymethyl-2-methyl-2*H*-1-benzopyran 3a. Compound (−)-**3a** was obtained from (−)-**14a** as a colorless oil in 80% yield; $[\alpha]_D^{20}$=−13.4 (*c*=0.4, acetone); ee=44%; ^1H NMR (200 MHz, $CDCl_3$): δ 1.36 (s, 3H, CH_3), 2.03 (bs, 1H, OH), 3.59 (d, 1H, *J*=11.6 Hz, $H^{1'}$), 3.68 (d, 1H, *J*=11.6 Hz, $H^{1'}$), 5.56 (d, 1H, *J*=9.9 Hz, H^3), 6.45 (d, 1H, *J*=9.9 Hz, H^4), 6.76–7.15 (m, 4H, H_{ar}); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 22.6, 68.7, 79.2, 116.1, 120.8, 121.1, 124.7, 126.6, 126.7, 129.3, 153.9; IR (film) ν 3396, 2972, 2927, 1486, 1240, 1053, 773 cm^{-1}; MS-EI *m/z* (relative intensity) 176 (M^+, 3), 145 (100), 115 (13), 91 (5). Anal. Calcd for $C_{11}H_{12}O_2$: C, 74.98; H, 6.86. Found C, 74.82; H, 6.91.

4.10.2. (+)-2-Hydroxymethyl-2-methyl-2*H*-1-benzopyran 3a. Compound (+)-**3a** was obtained from (+)-**14a** as a colorless oil in 80% yield; $[\alpha]_D^{20}$=+13.0 (*c*=0.5, acetone); ee=46%.

4.10.3. (−)-2-Hydroxymethyl-6-methoxy-2-methyl-2*H*-1-benzopyran 3b. Compound (−)-**3b** was obtained from (−)-**14b** as a colorless oil in 75% yield; $[\alpha]_D^{20}$=−10.2 (*c*=0.4, acetone); ee=43%; ^1H NMR (200 MHz, $CDCl_3$): δ 1.35 (s, 3H, CH_3), 2.03 (bs, 1H, OH), 3.58 (d, 1H, *J*=11.8 Hz, $H^{1'}$), 3.67 (d, 1H, *J*=11.8 Hz, $H^{1'}$), 3.75 (s, 3H, OCH_3), 5.62 (d, 1H, *J*=10.0 Hz, H^3), 6.42 (d, 1H, *J*=10.0 Hz, H^4), 6.56 (d, 1H, *J*=2.8 Hz, H_{ar}), 6.67 (dd, 1H, *J*=2.8, 8.8 Hz, H_{ar}), 6.73 (d, 1H, *J*=8.8 Hz, H_{ar}); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 22.3, 55.7, 68.5, 79.0, 111.8, 114.5, 116.7, 121.5, 124.9, 127.9, 146.2, 154.0; IR (film) ν 3432, 2932, 2834, 1492, 1040 cm^{-1}; MS-EI *m/z* (relative intensity) 206 (M^+, 5), 175 (100), 132 (18); anal.calcd for $C_{12}H_{14}O_3$: C, 69.88; H, 6.86. Found C, 69.95; H, 6.80.

4.10.4. (−)-2-Hydroxymethyl-6-hydroxy-2-methyl-2*H*-1-benzopyran 3c. Compound (−)-**3c** was obtained from (−)-**14c** as a colorless oil in 68% yield; $[\alpha]_D^{20}$=−7.6 (*c*=0.3, acetone); ee=35%; ^1H NMR (200 MHz, $CDCl_3$): δ 1.35 (s, 3H, CH_3), 3.58 (d, 1H, *J*=11.6 Hz, $H^{1'}$), 3.68 (d, 1H, *J*=11.6 Hz, $H^{1'}$), 5.61 (d, 1H, *J*=9.6 Hz, H^3), 6.38 (d, 1H, *J*=9.6 Hz, H^4), 6.50 (d, 1H, *J*=2.8 Hz, H_{ar}), 6.59 (dd, 1H, *J*=2.8, 8.4 Hz, H_{ar}), 6.68 (d, 1H, *J*=8.4 Hz, H_{ar}); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 22.3, 68.4, 79.3, 111.8, 114.8, 116.4, 121.5, 124.2, 127.8, 146.6, 154.2; IR (film) ν 3432, 2932, 2834, 1492, 1040 cm^{-1}; MS-CI *m/z* (relative intensity) 210 (M+18, 100), 192 (M^+, 51). Anal. Calcd for $C_{11}H_{12}O_3$: C, 68.74; H, 6.29. Found C, 68.82; H, 6.36.

4.11. 2-Tosylaminobenzaldehyde 18

To a solution of 2-aminobenzaldehyde (2.8 g, 23.3 mmol) and pyridine (4.1 mL, 51.3 mmol) in anhydrous dichloromethane (30 mL) was added, dropwise at room temperature under argon, a solution of *para*-toluenesulfonyl chloride (4.8 g, 25.6 mmol) in dry dichloromethane (20 mL). The resulting mixture was stirred for 20 h, poured into water (80 mL) and extracted with dichloromethane (3×50 mL). The combined organic layers were washed with saturated aqueous $CuSO_4$ (100 mL), brine (100 mL) and dried over $MgSO_4$. After concentration in vacuo the crude residue was triturated with a mixture of 8:2 petroleum ether/ethyl acetate (20 mL), filtered over celite, concentrated under vacuum and purified by column chromatography on silica gel (8:2, petroleum ether/EtOAc) to provide **18** (4.48 g, 70%) as a pale yellow solid; mp 128 °C; ^1H NMR (200 MHz, $CDCl_3$) δ_H 2.36 (s, 3H, CH_3), 7.12–7.26 (m, 4H, H_{ar}), 7.51–7.79 (m, 4H, H_{ar}), 9.82 (s, 1H, CHO), 10.80 (s, 1H, NHTs); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ_C 21.4, 117.7, 121.7, 122.9, 127.1 (2C), 129.7 (2C), 135.7, 136.0, 136.2, 139.1, 144.1, 195.0; IR (KBr) ν 1662, 1602, 1493 cm^{-1}; MS-EI *m/z* (relative intensity) 275 (M^+, 10), 120 (100), 91 (50), 65 (33), 39 (12).

4.12. Preparation of chiral homoallylic alcohols 19

Homoallylic alcohols **19** were prepared according to Section 4.5.

4.12.1. (−)-(*R*)-Methyl-1-(2-tosylaminophenyl)but-3-en-1-ol (−)-19. Compound (−)-(*R*)-**19** was obtained from **18** and **9** (prepared with (+)-DIP-Cl) as a pale yellow solid in 80% yield: mp 79 °C; $[\alpha]_D^{20}=-16.7$ (c=1.0, acetone); ee=84%; ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 1.69 (s, 3H, CH_3), 2.19 (dd, 1H, J=4, 14 Hz, H^2), 2.28 (dd, 1H, J=10, 14 Hz, H^2), 2.37 (s, 3H, CH_3), 2.56 (bs, 1H, OH), 4.68 (dd, 1H, J=10, 4 Hz, H^1), 4.75 (s, 1H, H^4), 4.92 (s, 1H, H^4), 7.02–7.72 (m, 8H, H_{ar}), 8.57 (s, 1H, NH); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ_C 21.4, 22.0, 45.1, 71.3, 114.6, 121.7, 124.4, 127.0 (2C), 127.5, 128.4, 129.5 (2C), 132.0, 135.8, 136.9, 141.3, 143.6; IR (KBr) ν 3491, 3239, 2922, 1159 cm^{-1}; MS-EI m/z (relative intensity) 276 (35), 91 (100), 65 (35).

4.12.2. (+)-(*S*)-Methyl-1-(2-tosylaminophenyl)but-3-en-1-ol (+)-19. Compound (+)-(*S*)-**19** was obtained from **18** and **9** (prepared with (−)-DIP-Cl) as a pale yellow solid in 84% yield: mp 79 °C; $[\alpha]_D^{20}=+17.0$ (c=1.0, acetone); ee=82%.

4.13. Preparation of epoxide 20

Epoxides **20** were prepared according to Section 4.9.

4.13.1. (−)-(1*R*,3*S*)-3,4-Epoxy-3-methyl-1-(2-tosylamino-phenyl)butan-1-ol (−)-20. Compound (−)-(1*R*,3*S*)-**20** was obtained from (−)-(*R*)-**19** (250 mg, 754 μmol) as a colorless oil (252 mg, 96%); $[\alpha]_D^{20}=-13.9$ (c=0.6, acetone); de=84%; ^{1}H NMR (200 MHz, $CDCl_3$); major isomer δ_H 1.33 (s, 3H, CH_3), 1.72 (dd, 1H, J=9.4, 14.6 Hz, H^2), 1.86 (dd, 1H, J=3.5, 14.6 Hz, H^2), 2.38 (s, 3H, CH_3), 2.57 (d, 1H, J=4.6 Hz, H^4), 2.61 (d, 1H, J=4.6 Hz, H^4), 3.70 (bs, 1H, OH), 4.99 (dd, 1H, J=3.5, 9.4 Hz, H^1), 6.99–7.72 (m, 8H, H_{ar}), 8.67 (s, 1H, NH); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ_C 21.1, 21.4, 42.2, 54.3, 56.2, 71.6, 122.0, 124.6, 127.1 (2C), 127.3, 128.5, 129.6 (2C), 132.7, 135.8, 137.1, 143.6; minor isomer (meaningful signals) δ_H 2.73 (d, 1H, J=3.7 Hz, H^4), 3.04 (d, 1H, J=3.7 Hz, H^4), 4.67 (dd, 1H, J=3.5, 9.4 Hz, H^1); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ_C 40.3, 52.2, 57.4, 71.3; IR (film) ν 3474, 3239, 2925, 1161 cm^{-1}; MS-EI m/z (relative intensity) 248 (8), 192 (25), 174 (20), 132 (100), 91 (24); MS-CI m/z (relative intensity) 364 (100), 347 (95).

4.13.2. (+)-(1*S*,3*R*)-3,4-Epoxy-3-methyl-1-(2-tosylaminophenyl)butan-1ol (+)-20. Compound (+)-(1*S*,3*R*)-**20** was obtained from (+)-(*S*)-**19** (600 mg, 1.81 mmol) as a colorless oil (560 mg, 89%); $[\alpha]_D^{20}=+10.8$ (c=1.0, acetone); de=82%.

4.14. Typical procedure for formation of compounds 4

To a solution of epoxide **20** in anhydrous toluene (25 mL) at room temperature under argon was added slowly trifluoroacetic acid (2 equiv.) and the solution was stirred for 16 h. The reaction mixture was then quenched with saturated aqueous $NaHCO_3$ (4 mL). The resulting mixture was poured into water (20 mL) and extracted with diethyl ether (3×20 mL). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (7:3, petroleum ether/EtOAc) to provide two diastereomers.

4.14.1. (−)-*trans*-(2*S*,4*R*)-2-(Hydroxymethyl)-2-methyl-1-tosyl-1,2,3,4-tetrahydroquinolin-4-ol (−)-4. Compound (−)-*trans*-(2*S*,4*R*)-**4** was obtained from (−)-(1*R*,3*S*)-**20** (220 mg, 0.63 mmol) as a colorless oil (64 mg, 29%); $[\alpha]_D^{20}=-3.0$ (c=0.5, acetone); ee=94%; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.43 (s, 3H, CH_3), 1.83 (dd, 1H, J=13, 11 Hz, H^3), 2.14 (dd, 1H, J=13, 5 Hz, H^3), 2.35 (s, 3H, CH_3), 3.90 (s, 2H, CH_2), 5.02 (dd, 1H, J=11, 5 Hz, H^4), 6.98–7.70 (m, 8H, H_{ar}); ^{13}C NMR (100.62 MHz, $CDCl_3$) δ_C 21.5, 25.0, 47.3, 78.2, 80.1, 80.3, 121.6, 124.4, 127.0 (2C), 127.1, 128.5, 129.6 (2C), 130.4, 136.0, 137.2, 143.6; IR (film) ν 3492, 3254, 2967, 2928, 2880, 1592, 1499, 1453, 1334, 1159 cm^{-1}; MS-EI m/z (relative intensity) 347 (M$^+$, 3), 274 (17), 192 (25), 174 (80), 144 (42), 118 (100), 117 (43), 91 (72), 65 (36), 39 (16); MS-CI m/z (relative intensity) 365 (M+NH_3, 18), 348 (MH$^+$, 100), 330 (61), 291 (37), 274 (69), 144 (21). Anal. Calcd for $C_{18}H_{21}O_4NS$: C, 62.18; H, 6.04; S, 9.21; N, 4.03. Found: C, 62.90; H, 6.34; S, 9.25; N, 4.05.

4.14.2. (−)-*cis*-(2*R*,4*R*)-2-(Hydroxymethyl)-2-methyl-1-tosyl-1,2,3,4-tetrahydroquinolin-4-ol (−)-4. Compound (−)-*cis*-(2*R*,4*R*)-**4** was obtained from (−)-(1*R*,3*S*)-**20** (220 mg, 0.63 mmol) as a white solid (64 mg, 29%); $[\alpha]_D^{20}=-4.0$ (c=0.2, acetone); ee=63%; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.52 (s, 3H, CH_3), 1.5–1.6 (m, 4H, H^3, CH_3), 1.89 (dd, 1H, J=14, 6 Hz, H^3), 2.01 (d, 1H, J=6 Hz, OH), 2.40 (s, 3H, CH_3), 2.85 (bt, 1H, J=6 Hz, OH), 3.52 (dd, 1H, J=12, 6 Hz, H$^{2'}$), 3.63 (dd, 1H, J=12, 6 Hz, H$^{2'}$), 4.54 (dd, 1H, J=12, 6 Hz, H^4), 7.21–7.34 (m, 8H, H_{ar}); IR (KBr) ν 3300, 3024, 2957, 2925, 2852, 1599, 1481, 1454, 1350, 1159, 1090 cm^{-1}; MS-EI m/z (relative intensity) 347 (1), 316 (20), 155 (12), 144 (100), 91 (41), 77 (13), 65 (19). Anal. Calcd for $C_{18}H_{21}O_4NS$: C, 62.18; H, 6.04; S, 9.21; N, 4.03. Found: C, 63.20; H, 6.38; S, 9.15; N, 4.05.

4.14.3. (+)-*trans*-(2*R*,4*S*)-2-(Hydroxymethyl)-2-methyl-1-tosyl-1,2,3,4-tetrahydroquinolin-4-ol (+)-4. Compound (+)-*trans*-(2*R*,4*S*)-**4** was obtained from (+)-(1*S*,3*R*)-**20** (300 mg, 0.86 mmol) as a colorless oil (68 mg, 23%); $[\alpha]_D^{20}=+2.8$ (c=0.3, acetone); ee=88%. Anal. Calcd for $C_{18}H_{21}O_4NS$: C, 62.18; H, 6.04; S, 9.21; N, 4.03. Found: C, 62.19; H, 6.05; S, 9.21; N, 4.03.

4.14.4. (+)-*cis*-(2*S*,4*S*)-2-(Hydroxymethyl)-2-methyl-1-tosyl-1,2,3,4-tetrahydroquinolin-4-ol (+)-4. Compound (+)-*cis*-(2*S*,4*S*)-**4** was obtained from (+)-(1*S*,3*R*)-**20** (300 mg, 0.86 mmol) as a white solid (66 mg, 22%); $[\alpha]_D^{20}=+2.0$ (c=0.2, acetone); ee=40%. Anal. Calcd for $C_{18}H_{21}O_4NS$: C, 62.18; H, 6.04; S, 9.21; N, 4.03. Found: C, 62.20; H, 6.04; S, 9.21; N, 4.08.

4.15. Formation of the acetonide

4.15.1. 3-Methyl-1-phenylbut-3-en-1-ol 21. To a solution

of 2-methylpropene magnesium chloride (0.6 M in THF, 6 mmol) at −30 °C was added dropwise a solution of benzaldehyde (530 mg, 0.5 mmol) in THF (10 mL). The reaction was stirred 1 h at room temperature and then quenched with a saturated aqueous solution of NH_4Cl (20 mL). The aqueous layer was extracted with EtOAc (2×20 mL), the organic layers were dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography (9:1, petroleum ether/EtOAc) to give a colorless oil (794 mg, 98% yield); ^{1}H NMR (200 MHz, $CDCl_3$): δ_H 1.78 (s, 3H, CH_3), 2.25 (bs, 1H, OH), 2.41 (d, 2H, J=6.4 Hz, H^2), 4.78 (t, 1H, J=6.4 Hz, H^1), 4.81–4.91 (m, 2H, H^4), 7.23–7.36 (m, 5H, H_{ar}); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ_C 22.3, 48.2, 71.3, 114.0, 125.7 (2C), 127.4, 128.3 (2C), 142.3, 144.0; IR (film) ν 3396, 3073, 3030, 2969, 2936, 1454, 700 cm^{-1}; MS-CI *m/z* (relative intensity) 180 (M+18, 26), 162 (M^+, 100), 145 (37).

4.15.2. 3,4-Epoxy-3-methyl-1-phenylbutan-1-ol 22. To a blue solution of homoallylic alcohol **21** (1.3 mmol, 620 mg) and vanadyl acetylacetonate (131 μmol, 35 mg) in anhydrous dichloromethane, was added, at −5 °C under argon, *tert*-butyl hydroperoxide (5.5 M in nonane, 0.47 mL, 2.6 mmol). The resulting red-brown solution was stirred at 0 °C for 5 h. Then, the reaction mixture was poured into 10% aqueous $Na_2S_2O_3$ and extracted with dichloromethane. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (7:3, petroleum ether/EtOAc) to provide **22** (180 mg, 78% yield) as a mixture of diastereomers (ratio 8:2); major isomer ^{1}H NMR (200 MHz, $CDCl_3$): δ_H 1.44 (s, 3H, CH_3), 2.00 (m, 2H, H^2), 2.60 (m, 2H, H^4), 3.05 (bs, 1H, OH), 4.95 (m, 1H, H^1), 7.25–7.35 (m, 5H, H_{ar}); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ_C 21.2, 45.3, 53.9, 56.2, 71.5, 125.6 (2C), 127.5, 128.4 (2C), 144.0; minor isomer ^{1}H NMR (200 MHz, $CDCl_3$): δ_H 1.39 (s, 3H, CH_3), 2.03 (m, 2H, H^2), 2.87 (m, 2H, H^4), 3.32 (bs, 1H, OH), 4.74 (m, 1H, H^1), 7.25–7.35 (m, 5H, H_{ar}); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ_C 22.6, 43.8, 52.9, 57.1, 71.1, 125.6 (2C), 127.5, 128.4 (2C), 143.6; IR (film) ν 3438, 2927, 1071, 760, 701 cm^{-1}; MS-CI *m/z* (relative intensity) 196 (M+18, 75), 178 (M^+, 48), 161 (M−17).

4.15.3. 3,4-Epoxy-3-methyl-1-phenyl-1-*tert*-butyldimethylsilyloxybutane 23. To a solution of **22** (620 mg, 3.5 mmol), imidazole (473 mg, 7.0 mmol) in DMF (4 mL) was added *tert*-butyldimethylsilyl chloride (780 mg, 5.2 mmol). The reaction was heated 18 h at room temperature, then 50 mL of water and 50 mL of EtOAc were added. The aqueous layer was extracted with ethyl acetate (3×40 mL), the organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (9:1, petroleum ether/EtOAc) to give the compound **23** (880 mg, 86% yield) as a mixture of diastereomers (ratio 8:2); major isomer ^{1}H NMR (200 MHz, $CDCl_3$): δ_H 0.17 (s, 3H, $SiCH_3$), 0.41 (s, 3H, $SiCH_3$), 1.25 (s, 9H, $3CH_3$), 1.68 (s, 3H, CH_3), 2.06 (m, 1H, H^2), 2.66 (m, 1H, H^2), 2.79 (m, 2H, H^4), 5.14 (m, 1H, H^1), 7.63–7.69 (m, 5H, H_{ar}); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ_C −4.5 (2C), 18.0, 22.2, 25.8 (3C), 47.9, 53.5, 55.1, 72.9, 125.9 (2C), 127.3, 128.1 (2C), 144.9; minor isomer ^{1}H NMR (200 MHz, $CDCl_3$): δ_H 0.13 (s, 3H, $SiCH_3$), 0.37 (s, 3H, $SiCH_3$), 1.25 (s, 9H, $3CH_3$), 1.79 (s, 3H, CH_3), 2.04 (m, 1H, H^2), 2.40 (m, 1H, H^2), 2.97 (m, 2H, H^4), 5.21 (m, 1H, H^1), 7.63–7.69 (m, 5H, H_{ar}); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ_C −5.0 (2C), 18.0, 21.3, 25.8 (3C), 48.5, 54.9, 55.3, 72.9, 125.8 (2C), 127.3, 128.1 (2C), 144.9; IR (film) ν 2956, 2929, 2857, 1092, 837 cm^{-1}; MS-CI *m/z* (relative intensity) 293 (M+1, 5), 221 (100), 161 (19), 132 (30).

4.15.4. 2-Methyl-1,4-diphenyl-4-*tert*-butyldimethylsilyloxybutan-2-ol 24. To a solution of $BF_3{\cdot}Et_2O$ (4 mmol), in THF (4 mL) was added at −78 °C phenyllithium (1.4 M, 2.8 mL, 4.0 mmol). Then, a solution of epoxide **23** (292 mg, 1 mmol) in THF (2 mL) was added quickly and the reaction was stirred 2 h at −60 °C. The reaction was quenched by addition of a saturated aqueous solution of NH_4Cl (25 mL), the organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (9:1, petroleum ether/EtOAc) to give the compound **24** (274 mg, 74% yield) as a mixture of diastereoisomers (ratio 8:2); major isomer ^{1}H NMR (200 MHz, $CDCl_3$): δ_H −0.19 (s, 3H, $SiCH_3$), 0.26 (s, 3H, $SiCH_3$), 1.07 (s, 9H, $3CH_3$), 1.54 (s, 3H, CH_3), 1.90 (m, 1H, H^3), 2.23 (m, 1H, H^3), 2.97 (bs, 2H, H^1), 4.60 (bs, 1H, OH), 5.19 (m, 1H, H^4), 7.40–7.51 (m, 10H, H_{ar}); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ_C −4.1 (2C), 17.8, 25.8 (3C), 28.2, 46.9, 50.2, 72.7, 74.7, 126.1, 127.1 (2C), 127.8 (2C), 128.1 (2C), 128.7, 130.7 (2C), 137.7, 144.6; minor isomer ^{1}H NMR (200 MHz, $CDCl_3$): δ_H −0.13 (s, 3H, $SiCH_3$), 0.33 (s, 3H, $SiCH_3$), 1.12 (s, 9H, $3CH_3$), 1.26 (s, 3H, CH_3), 1.84 (m, 1H, H^3), 2.27 (m, 1H, H^3), 3.05 (d, 1H, J=13.2 Hz, H^1), 3.26 (d, 1H, J=13.2 Hz, H^1), 4.53 (bs, 1H, OH), 5.39 (m, 1H, H^4), 7.40–7.51 (m, 10H, H_{ar}); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ_C −4.9 (2C), 17.8, 25.8 (3C), 28.2, 46.9, 49.1, 72.7, 74.7, 126.4, 127.1 (2C), 127.8 (2C), 128.1 (2C), 128.7, 130.4 (2C), 138.2, 144.6; MS-CI *m/z* (relative intensity) 388 (M+18, 5), 370 (M^+, 30), 238 (100).

4.15.5. 3-Methyl-1,4-diphenylbutan-1,3-diol 25. To a solution of **24** (290 mg, 0.78 mmol) in THF (2 mL) was added at 0 °C TBAF (1 M in THF, 0.86 mmol). The reaction was stirred 1 h at room temperature and then hydrolysed with a saturated aqueous solution of NH_4Cl (2 mL). The aqueous layer was extracted with EtOAc (3×10 mL), the organic layers were washed with brine (30 mL) and dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (7:3, petroleum ether/EtOAc) to give the compound **25** as a white solid (189 mg, 95% yield) as a mixture of diastereomers (ratio 8:2); mp 117 °C; major isomer ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.35 (s, 3H, CH_3), 1.67 (d, 1H, J=14.2 Hz, H^2), 1.93 (dd, 1H, J=14.2, 11.0 Hz, H^2), 2.73 (d, 1H, J=13.6 Hz, H^4), 2.78 (d, 1H, J=13.6 Hz, H^4), 3.06 (bs, 1H, OH), 4.05 (bs, 1H, OH), 5.00 (d, 1H, J=11.0 Hz, H^1), 7.16–7.35 (m, 10H, H_{ar}); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ_C 25.3, 48.5, 50.3, 71.8, 73.5, 125.6 (2C), 126.5 (2C), 127.3, 128.1 (2C), 128.3 (2C), 130.5, 136.6, 144.5; minor isomer (meaningful signals) ^{1}H NMR (200 MHz, $CDCl_3$): δ_H 1.12 (s, 3H, CH_3), 1.85 (m, 2H, H^2), 2.84 (d, 1H, J=13.2 Hz, H^4), 3.09 (d, 1H, J=13.2 Hz, H^4), 3.06 (bs, 1H, OH), 4.05 (bs, 1H, OH), 5.18 (m, 1H, H^1), 7.16–7.35 (m, 10H, H_{ar}); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ_C 28.7, 46.2, 49.1, 71.6, 73.6, 125.5 (2C), 126.4 (2C), 127.3, 128.2 (2C), 128.4 (2C), 130.4, 137.3, 144.6; IR (film) ν 3334, 3028, 2971, 2913,

700 cm^{-1}; MS-CI *m/z* (relative intensity) 274 (M+18, 62), 256 (M^+, 6), 238 (100), 221 (74).

4.15.6. 4-Benzyl-2,2,4-trimethyl-6-phenyl-1,3-dioxacyclohexane 26. To a solution of diol **25** (100 mg, 0.39 mmol) in 2,2-dimethoxypropane (3.5 mL) was added at rt CSA (2 mol%). After 1 h the reaction was quenched by addition of a saturated aqueous solution of $NaHCO_3$ (4 mL). The aqueous layer was extracted with EtOAc (3×10 mL), the combined organic layers were washed with brine (30 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (9:1, petroleum ether/EtOAc) to give compound **26** as a white solid (106 mg, 92% yield) as a mixture of diastereomers (ratio 8:2); mp 56 °C; major isomer ^1H NMR (400 MHz, $CDCl_3$): δ_H 1.41 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 1.54 (dd, 1H, *J*=2.0, 12.0 Hz, H^5), 1.56 (s, 3H, CH_3), 1.76 (dd, 1H, *J*=11.6, 12.0 Hz, H^5), 2.76 (d, 1H, *J*=12.0 Hz, PhC*H*), 2.81 (d, 1H, *J*=12.0 Hz, PhC*H*), 4.90 (dd, 1H, *J*=2.0, 11.6 Hz, H^6), 7.21–7.33 (m, 10H, H_{ar}); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ_C 25.1, 25.7, 31.9, 41.7, 51.6, 68.0, 73.5, 99.0, 125.9 (2C), 126.2, 127.4, 127.7 (2C), 128.4 (2C), 130.9 (2C), 137.3, 142.5; minor isomer ^1H NMR (200 MHz, $CDCl_3$): δ_H 1.24 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 1.58 (s, 3H, CH_3), 1.62–2.10 (m, 2H, H^5), 2.90 (d, 1H, *J*=13.6 Hz, PhC*H*), 3.16 (d, 1H, *J*=13.6 Hz, PhC*H*), 4.84 (m, 1H, H^6), 7.21–7.33 (m, 10H, H_{ar}); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ_C 26.8, 30.4, 31.7, 41.3, 47.5, 68.4, 74.1, 99.0, 125.5 (2C), 126.3, 127.4, 127.9 (2C), 128.4 (2C), 130.6 (2C), 138.0, 142.5; IR (film) ν 2990, 2938, 699 cm^{-1}; MS-CI *m/z* (relative intensity) 314 (M+18, 4), 296 (M^+, 1), 238 (86), 152 (100).

Acknowledgements

We are indebted to French Ministry of Education and CNRS for financial support. We thank Professor Jean-Paul Quintard for fruitful discussion.

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Tetrahedron 60 (2004) 4051–4069

Synthesis and iNOS/nNOS inhibitory activities of new benzoylpyrazoline derivatives

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Received 19 December 2003; revised 12 February 2004; accepted 2 March 2004

Abstract—A series of new Δ^2-pyrazoline derivatives has been synthesized by means of a 1,3-dipolar-cycloaddition reaction. Ethyl 3-(5-methoxy-2-nitrobenzoyl)-Δ^2-pyrazoline-5-carboxylate (**5a**) has been designed for the formation of the benzoylpyrazoline system present in these derivatives. Two synthetic routes have been utilized changing the starting products in the cycloaddition reaction. In both routes, the majority product obtained was only a Δ^2-pyrazoline. The intermediate ethyl 1-acyl-3-(2-nitrobenzoyl-5-substituted)-Δ^2-pyrazoline-5-carboxylate derivatives have been transformed into the final compounds by means of several chemical treatments. The compounds have been biologically evaluated as inhibitors of nitric oxide synthase (NOS), showing better affinity towards the inducible NOS isoform than versus neuronal NOS.

1. Introduction

Nitric oxide (NO) is a biologically active compound. The synthesis of NO is catalyzed by a family of enzymes called NO synthases (NOS). Three NOS isoforms have been well identified and named according to the cell type or conditions in which they were first described: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS).[1] Each one of the isoforms converts L-arginine to L-citrulline and nitric oxide utilizing NADPH and O_2 as cofactors, as well as the flavin-adenine dinucleotide (FAD), the flavin mononucleotide (FMN), tetrahydrobiopterin, heme and calcium-calmoduline.[2] Nitric oxide has important physiological functions including neurotransmission, blood pressure homeostasis, platelet aggregation, and immunological defense mechanisms.[3] The magnitude and duration of NO synthesis make its action physiological or pathological. Thus, although NO participates in the synaptic transmission in a normal way, the excessive levels which are produced by nNOS can become neurotoxic, and can be involved in different neurological disorders such as Alzheimer's disease,[4] the amyotrophic lateral sclerosis[5] or Hungtinton's disease.[6] On the other hand, the high production of NO by the inducible isoform of the nitric oxide synthase is related to disorders like the septic shock,[7] inflammatory arthritis,[8] and inflammatory bowel disease.[9] Up to now, the current research is orientated (a) to establish the adequate inhibition level of NOS so that its beneficial effects are kept, and (b) to identify more selective inhibitor compounds of each NOS isoenzyme, since the control of certain pathological states could be achieved. Although many inhibitors of NOS are known, very few of them show selectivity for only one isoform.[10–12] The studies carried out until this moment indicate that the goal of attaining selectivity for iNOS over nNOS is more difficult than achieving selectivity for iNOS over eNOS.[13]

Melatonin (*N*-acetyl-5-methoxytryptamine) **1** is a hormone that is synthesized and secreted into the general circulation by the pineal gland.[14] Inhibitory actions of melatonin in the rat[15,16] and human[17] central nervous system (CNS) have been reported. These inhibitory actions may be the cause of the anticonvulsant, hypnotic, antitumoral, antioxidant and neuroprotective properties.[18] Diverse experiments, have suggested that melatonin attenuates glutamate-mediated responses in the rat striatum.[19] The inhibitory effects of melatonin in the striatum may be mediated through inhibition of NOS, as has been reported in rat cerebellum and hypothalamus.[20–22] This inhibition is dose-dependent and calmodulin-dependent.[23]

Recently, the Δ^2-pyrazoline compounds have raised a great interest because of their multiple pharmacological applications such as antibacterials, antifungicals, anticonvulsants,[24] hypotensives,[25] antidepressants,[26] analgesics, antiinflammatories[27] and neuroprotectives.[28] In this paper,

Keywords: Addition reactions; Pyrazolines; Benzisoxazoles; Anti-inflammatory compounds.
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doi:10.1016/j.tet.2004.03.013

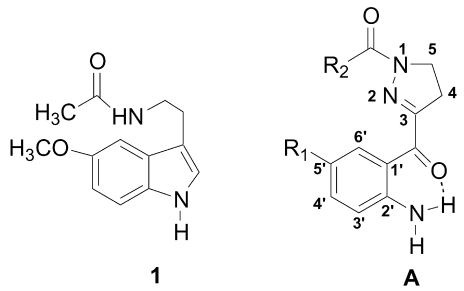
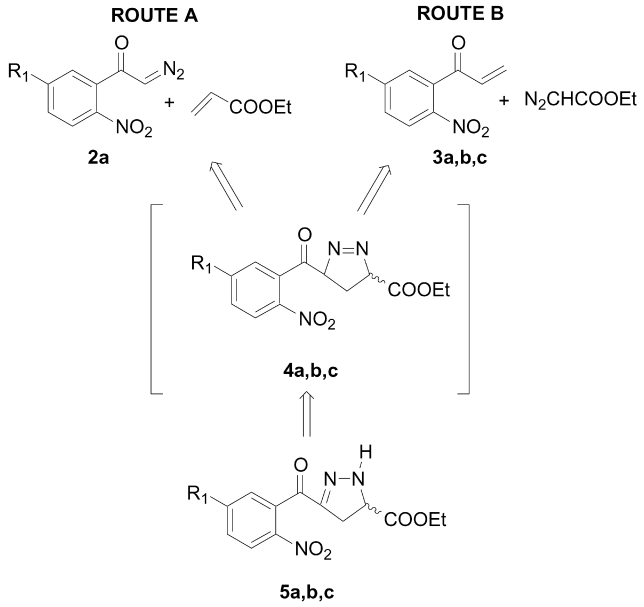


Figure 1.

taking as a prototype melatonin, we have carried out the synthesis of a new series of Δ^2-pyrazoline derivatives (represented by the general formula **A**) with the aim of searching for new selective inhibitors of NOS (Fig. 1).



Scheme 1.

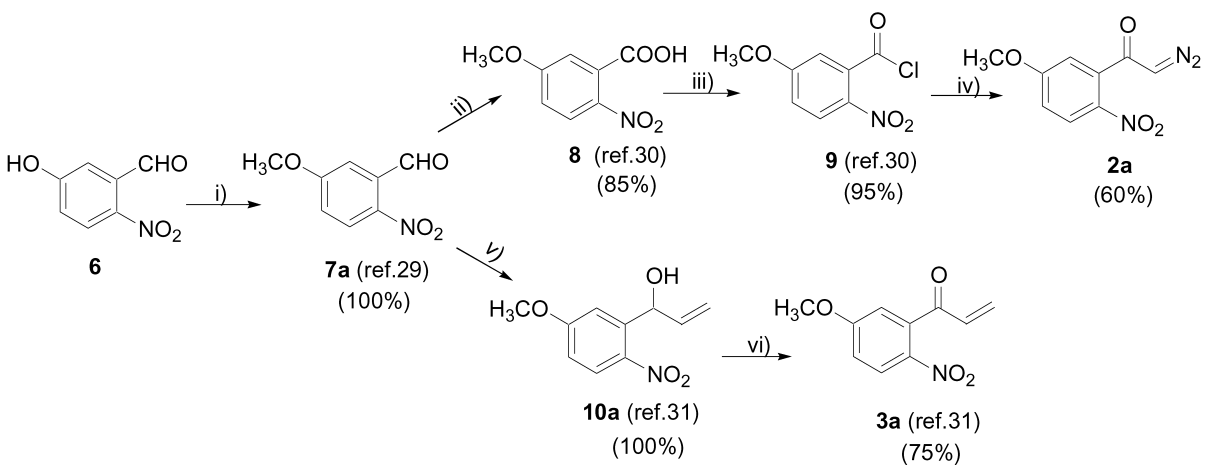
2. Results and discussion

2.1. Chemistry

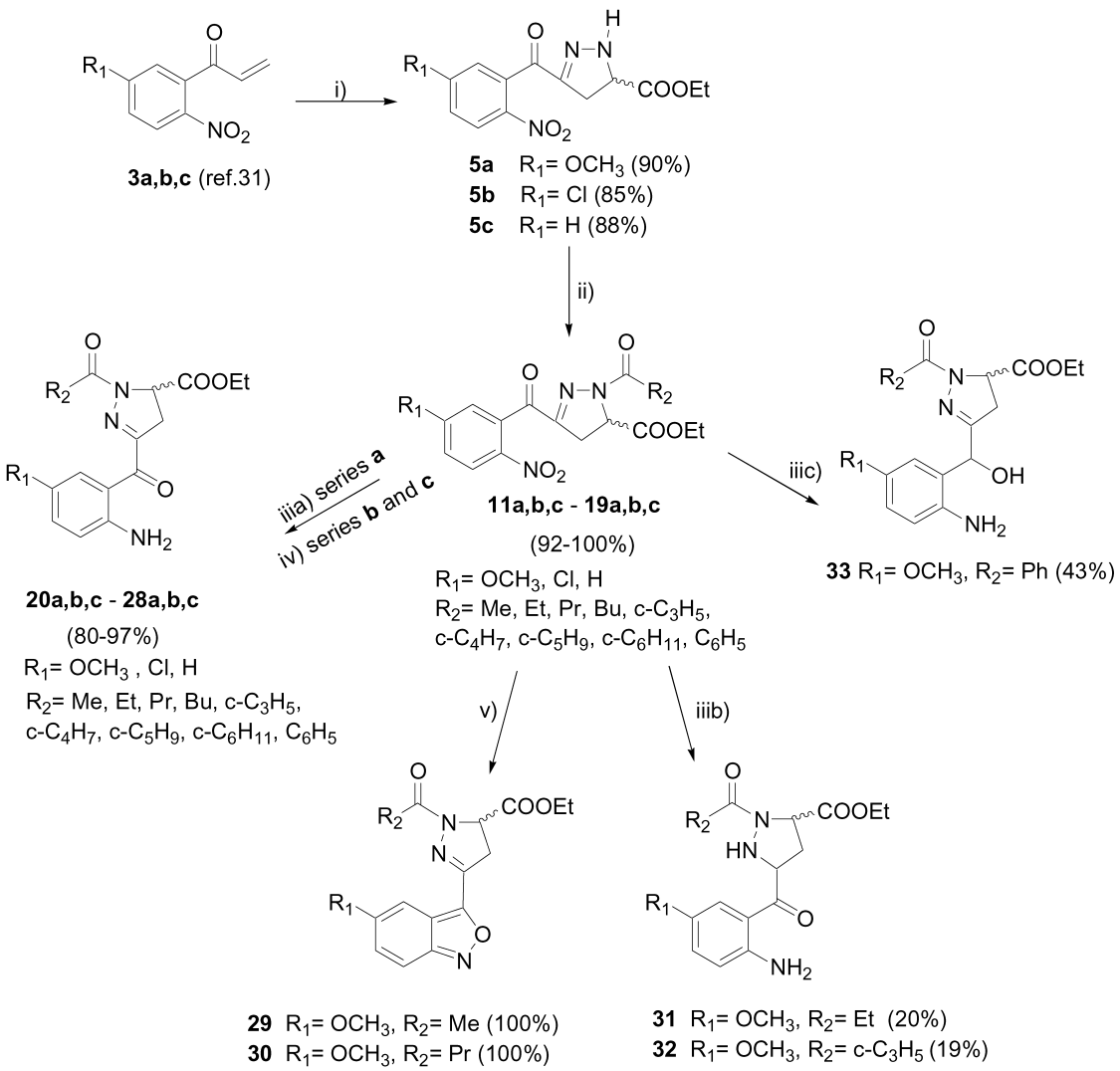
Scheme 1 shows the synthetic pathway used. The method employed for the formation of the ethyl 3-(2-nitro-5-substitutedbenzoyl)-Δ^2-pyrazoline-5-carboxylate derivatives is a 1,3-dipolar cycloaddition reaction. For the construction of the benzoylpyrazoline system by means of the procedure before mentioned, two options are possible, which seem to be of equal interest. The synthesis of pyrazoline **5a** has been taken as a model with the aim of analyzing which is the most suitable route to prepare these compounds. In the route A the 5-methoxy-2-nitrodiazoacetophenone **2a** acting as a 1,3-dipole, reacts with an active dipolarophile (ethyl acrylate) and in the route B, 5-methoxy-2-nitrophenyl vinyl ketone **3a**, acting as dipolarophile, reacts with ethyl diazoacetate. Both routes lead to the same intermediate ethyl 5-(5-methoxy-2-nitrobenzoyl)-Δ^1-pyrazoline-3-carboxylate **4a** which is not isolated, but it tautomerizes quickly to the racemate ethyl 3-(5-methoxy-2-nitrobenzoyl)-Δ^2-pyrazoline-5-carboxylate **5a**.

The two synthetic routes which lead to the intermediate **5a** are equally viable, and in both cases the yield of the 1,3-dipolar cycloaddition reaction was 80%. The choice of the route B as the method of synthesis for the construction of the benzoylpyrazolinic system was based on the easiness with which the starting compounds **2a** and **3a** were synthesized: for the preparation of the compound 5-methoxy-2-nitrodiazoacetophenone **2a**, 4 steps of synthesis with a global yield 48% were needed, whereas for the preparation of the compound 5-methoxy-2-nitrophenyl vinyl ketone **3a** only 3 steps of synthesis with a global yield 75% were necessary (Scheme 2).

Once the second strategy was chosen as the synthetic method, a modification of the conditions of reaction was carried out, using a base (pyridine) and a polar solvent (acetonitrile), so that the yield increased up to 90% in the case of Δ^2-pyrazoline **5a** and the time of reaction diminished (from 16 to 10 h). Accordingly, we took this



Scheme 2.



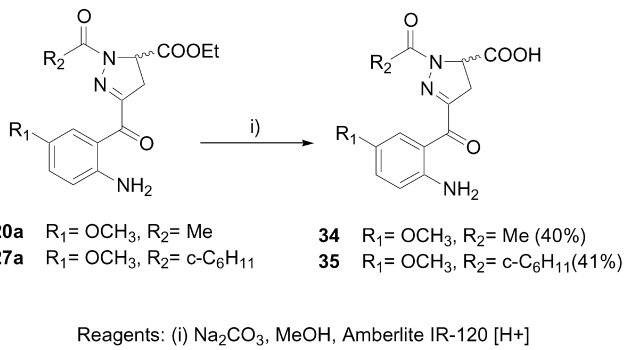
Reagents: (i) ethyl diazoacetate, Pyr, CH_3CN; (ii) Ac_2O o RCOCl, Et_3N, Cl_2CH_2; (iiia) H_2 Pd/C 10%, MeOH, 2,5 h (iv) Fe, $FeSO_4$, H_2O; (v) $SnCl_2$, EtOH; (iiib) H_2, Pd/C 10%, MeOH, 5 h.; (iiic) H_2, Pd/C 10%, MeOH, 7,5h.

Scheme 3.

procedure as the general method for the preparation of the benzoylpyrazolinic system in the different series a (R_1=OCH_3), b (R_1=Cl) and c (R_1=H). The use of pyridine could allow the change of 1-pyrazoline to 2-pyrazoline since its basic character facilitates the prototropy. Once the benzoylpyrazoline derivatives **5a**,**b**,**c** have been synthesized, they have been transformed into the corresponding acyl derivatives **11a**,**b**,**c**–**19a**,**b**,**c** by treating them with triethylamine and acetic anhydride or the corresponding acyl chloride. Twenty-seven intermediates are obtained, nine of each series, where the radical R_2 can be a lineal chain in the case of Me, Et, Pr and Bu, or a cyclic chain, in the case of *c*-C_3H_5, *c*-C_4H_7, *c*-C_5H_9, *c*-C_6H_{11} and Ph. The yields are similar in the three series and ranges between 92 and 100%. These acyl derivatives underwent diverse chemical treatments to perform the right modifications in the aromatic and pyrazolinic rings, with the aim of getting the different final compounds (Scheme 3).

The reduction of the nitro group belonging to the aromatic ring of the 27 acyl derivatives leads to compounds **20a**,**b**,**c**–**28a**,**b**,**c**. In series a, the reduction is accomplished by catalytic hydrogenation with Pd/C and the yield oscillates between 80 and 84%. In series b, the reduction was carried out with Fe and $FeSO_4$ in water, in order to avoid dechlorination, in this series the yield oscillates between 95 and 97%. In series c, the reduction was carried out whith $Fe/FeSO_4$ due to the better yield obtained with this method (95–96%).

The compounds **29** and **30** were obtained starting from the acyl derivatives **11a** and **13a** by treating them with $SnCl_2$ in ethanol with quantitative yield. The formation of these derivatives is justified by the attack of the hydroxyl group of the intermediate hydroxyamino reduction to the carbonyl group, the benzo[*c*]isoxazole ring being formed by posterior loss of a water molecule. Compounds **31**–**33** are formed by catalytic hydrogenation with Pd/C starting from the corresponding nitroarene (**12a**, **15a** and **19a**). Both, the quantity of catalyst and the time of reaction were higher



20a R_1= OCH_3, R_2= Me
27a R_1= OCH_3, R_2= c-C_6H_{11}

34 R_1= OCH_3, R_2= Me (40%)
35 R_1= OCH_3, R_2= c-C_6H_{11}(41%)

Reagents: (i) Na_2CO_3, MeOH, Amberlite IR-120 [H+]

Scheme 4.

than the ones used for the reduction of the aromatic nitro group, in this case the yield ranged between 19 and 43%. The derivatives **34** and **35** were obtained by hydrolysis starting from their esters **20a** and **27a** with Na_2CO_3, and posterior neutralization with Amberlite IR-120 $[H]^+$ resin, with 40–41% yield (Scheme 4).

2.2. Striatal nNOS and cerebral iNOS inhibitory activity

The effect of the final compounds on nNOS activity has been studied in striatum and rat brain (Table 1), with the object of evaluating its possible inhibition and selectivity versus the two isoforms of the nitric oxide synthase. The concentration of the compounds essayed has been 1 mM.

The nNOS activity was measured monitoring the conversion of L-[^{3}H]-arginine into L-[^{3}H]-citrulline, according to the method described by Bredt and coll.[32] For the measurement of the iNOS activity, the induction of the enzyme was achieved by means of the intravenous injection of lipopolysaccharide (LPS).

In general, the majority of compounds show better values of inhibition towards the iNOS isoform than towards the nNOS isoform. Next, a comparative analysis of the cerebral iNOS/striatal nNOS activities is carried out.

Among the benzoylpyrazoline derivatives **20a**,**b**,**c**–**28a**,**b**,**c**, compounds **28b** and **28c** with a phenyl group in R_2 showed higher affinity against iNOS than for nNOS. In **29** and **30** derivatives, there are not significant values of inhibition of both isoforms, and, accordingly, the elimination of the amino group in position 2 of the aromatic ring by formation of the benzo[*c*]isoxazole does not lead to positive results with regard to the NOS inhibition. This corroborates previous results obtained by our Group, which demonstrate the need of a hydrogen bond donor group in this position (an amino or amino monosubstituted group), for the formation of a hydrogen bond with the biologic target which is important for the NOS inhibitory activity.[12]

Compounds in which the rigidity has been diminished by reduction of the pyrazoline ring (compounds **31** and **32**) or

Table 1.

Compounds	Series	R_1	R_2	% nNOS inhibition	% iNOS inhibition
20	a	OCH_3	Me	21.74±4.20	7.07±1.67
	b	Cl		2.37±3.68	30.38±2.77
	c	H		—	—
21	a	OCH_3	Et	15.15±3.61	24.03±6.91
	b	Cl		3.90±5.29	25.39±1.61
	c	H		5.41±2.75	13.25±4.36
22	a	OCH_3	Pr	11.49±4.08	14.63±2.26
	b	Cl		3.71±3.53	20.34±3.78
	c	H		—	26.42±2.71
23	a	OCH_3	Bu	11.34±1.75	12.98±5.27
	b	Cl		4.57±3.21	8.86±7.39
	c	H		1.11±0.55	0.14±3.46
24	a	OCH_3	*c*-C_3H_5	4.94±2.38	8.25±5.25
	b	Cl		4.71±1.33	22.33±2.25
	c	H		5.49±0.34	14.50±6.48
25	a	OCH_3	*c*-C_4H_7	5.24±1.20	19.96±8.45
	b	Cl		—	4.43±9.62
	c	H		—	14.13±4.04
26	a	OCH_3	*c*-C_5H_9	11.59±0.57	3.53±5.92
	b	Cl		—	20.15±2.72
	c	H		—	23.28±3.14
27	a	OCH_3	*c*-C_6H_{11}	19.79±3.56	11.08±5.73
	b	Cl		—	4.94±1.37
	c	H		3.99±4.50	25.47±1.23
28	a	OCH_3	Ph	5.73±2.29	3.77±4.43
	b	Cl		—	35.62±3.23
	c	H		—	32.44±0.52
29	a	OCH_3	Me	—	—
30	a	OCH_3	Pr	—	—
31	a	OCH_3	Et	—	23.93±4.21
32	a	OCH_3	*c*-C_3H_5	—	22.05 ± 4.19
33	a	OCH_3	Ph	—	37.58±1.90
34	a	OCH_3	Me	—	22.66±6.07
35	a	OCH_3	*c*-C_6H_{11}	—	36.34±2.99

Values of inhibition striatal nNOS and cerebral iNOS. Each value is the mean of three experiments performed by triplicate in striatum (nNOS) and brain (iNOS) homogenates of rats.

by reduction of the carbonyl group (compound **33**), only present iNOS inhibition. Moreover, they present more significant inhibition values regarding the more similar rigid compounds **21a**, **24a** and **28a**. Again, the **33** derivative with a phenyl group in R_2 presents the highest value of iNOS inhibition.

The **34** and **35** acids obtained by hydrolysis of the ester function present affinity toward the iNOS isoform. The reduction of the size of the substituent leads to better inhibition values regarding their analogues **20a** and **27a**.

The comparative analysis between iNOS/nNOS activities shows that the compounds present better selectivity by the iNOS isoform versus nNOS. The inhibitory potency iNOS is improved with structures which present a decrease of the conformational rigidity or an acid group in position 5.

The results obtained with these compounds prompted us the attainment of selective inhibitors of an only NOS isoform, what could mean the control of certain pathologies. Besides, they would help us to know the structure and action mechanism of each isoform.

3. Conclusions

The synthesis and biologic evaluation of a new class of Δ^2-pyrazoline derivatives have been carried out. The benzoylpyrazoline moiety has been made by means of the 1,3-dipolar cycloaddition reaction using different starting compounds which lead to an only type of Δ^2-pyrazoline (**5a**). The compounds presented in this report show more affinity towards iNOS isoform, the values of inhibition produced by **28b**, **33** and **35** derivatives are remarkable. For the inhibition of the NOS activity, the presence of a free amino group in position 2 of the aromatic ring is important, since **29** and **30** compounds with a benzoisozaxole ring do not inhibit the nNOS and iNOS isoforms. This corroborates previous results obtained by our research group, where a hydrogen bond donor group in this position is needed.

4. Experimental

4.1. Chemistry

Reactions were performed under an inert atmosphere of argon. Solvents were dried according to standard methods. Melting points (mp) were taken in open capillaries on a Electrothermal melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a 400.1 MHz ^{1}H and 100.3 MHz ^{13}C NMR Bruker ARX-400 or 300.13 MHz ^{1}H and 75.58 MHz ^{13}C NMR Bruker AMX-300 spectrometers, and chemical shifts (ppm) are reported relative to the solvent peak ($CHCl_3$ in $CDCl_3$ at δ 7.24 and 77.1 ppm; CH_3OH in CD_3OD at 3.34 and 49.9 ppm). Signal are designated as follows: s, singlet; bs, broad singlet; d, doublet; dd, doublet of doublet; ddd, double doublet of doublet; t, triplet; pt, pseudotriplet; dt, double of triplet; tt, triplet of triplet; q, quadruplet; pc, pseudo-quadruplet; pq, pseudoquintuplet; m, multiplet. Coupling constants (J) are expressed in hertz. High-resolution mass spectroscopy (HRMS) was carried out on a VG AutoSpec Q high-resolution mass spectrometer (Fison Instrument). Elemental analyses were performed on a Perkin–Elmer 240 C and agreed with theoretical values within $\pm$0.4%. Flash-chromatography was carried out using silica gel 60, 230–240 mesh (Merck), and the solvent mixture reported within parentheses was used as eluent. Evaporations were carried out in vacuo with a rotary evaporator.

4.1.1. Starting materials

4.1.1.1. Synthesis of 5-methoxy-2-nitrodiazoacetophenone 2a. A 0.54 M solution of CH_2N_2 was added dropwise with stirring under argon at -10 °C to a solution of 5-methoxy-2-nitrobenzoyl chloride[30] (2 g, 9.27 mmol) in dry diethyl ether (10 mL). The reaction mixture was stirred for 3 h. Evaporation of the solvent rendered a residue that was purified by flash chromatography (ethyl acetate/hexane 1:2) to give **2**: 1.2 g (65% yield); thick oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, 1H, H-3′, $J_{3'-4'}$=9.0 Hz); 6.98 (dd, 1H, H-4′, $J_{4'-3'}$=9.0 Hz, $J_{4'-6'}$=2.8 Hz); 6.89 (d, 1H, H-6′, $J_{6'-4'}$=2.8 Hz); 5.4 (s, 1H, $-CH-N_2$); 3.9 (s, 3H, $-OCH_3$).^{13}C NMR (100 MHz, $CDCl_3$) δ 184.43 (C-1); 165.14 (C-5′); 140.45 (C-2′); 127.36 (C-3′, C-1′); 115.30 (C-4′); 113.44 (C-6′); 56.32 (C-2, $-OCH_3$). HR LSIMS calcd for $C_9H_7N_3O_4Na$ $(M+Na)^+$ 244.0334, found: 244.0333.

4.1.1.2. Synthesis of 2-nitrophenyl-5-substituted vinyl ketone 3a,b,c. 5-Methoxy-2-nitrobenzaldehyde **7a** (synthesized from 5-hydroxy-2-nitrobenzaldehide **6** with MeI and K_2CO_3 in THF),[29] commercial 5-chloro-2-nitrobenzaldehyde **7b** and commercial 2-nitrobenzaldehyde **7c**, were transformed into the corresponding allylic alcohols **10a**,**b**,**c** by quantitative addition of vinylmagnesium bromide.[31] Oxidation with CrO_3 leads to the 2-nitrophenyl-5-substituted vinylketone **3a**,**b**,**c**.[31]

4.1.1.3. General procedures for the preparation of compounds 5a,b,c. *Procedure 1*. Ethyl acrylate (0.04 mL, 0.39 mmol) was slowly added to stirred 5-methoxy-2-nitrodiazoacetophenone **2** (0.085 g, 0.39 mmol) at 65 °C. The reaction mixture was stirred for 16 h, CH_2Cl_2 was added, and washed with H_2O (2×20 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated. The residue was recrystallized from CH_2Cl_2/hexane.

Procedure 2. Ethyl diazoacetate (0.16 mL, 1.38 mmol) was slowly added to stirred 5-methoxy-2-nitrophenyl vinyl ketone **3a** (0.285 g, 1.38 mmol) at 65 °C. The reaction mixture was stirred for 16 h, CH_2Cl_2 was added, and washed with H_2O (2×20 mL). The organic phase was dried (Na_2SO_4), filtered and evaporated. The residue was recrystallized from CH_2Cl_2/hexane.

Procedure 3. Pyridine (0.013 mL, 0.172 mmol) was added to a solution of the corresponding 5-methoxy-2-nitrophenyl vinyl ketone **3a**,**b**,**c** (1.38 mmol) in 4 mL of dry acetonitrile. Ethyl diazoacetate was slowly added (0.16 mL, 1.38 mmol) to the stirred solution. The reaction mixture was stirred for 10 h and washed with 5% HCl (2×10 mL). The organic phase was dried (Na_2SO_4), filtered and evaporated to dryness. The residue was recrystallized from CH_2Cl_2/hexane.

*4.1.1.3.1. Ethyl 3-(5-methoxy-2-nitrobenzoyl)-Δ²-pyrazoline-5-carboxylate **5a***. Compound **5a** was obtained as a yellow solid (98.7 mg, 80% yield), as described in procedure 1. Compound **5a** was obtained as a yellow solid (98.7 mg, 80% yield), as described in the procedure 2. Compound **5a** was obtained as a yellow solid (111 mg, 90% yield) starting from **3a**,[31] as described in procedure 3; mp 144–146 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (d, 1H, H-3′, $J_{3'-4'}$=9.1 Hz); 7.00 (dd, 1H, H-4′, $J_{4'-3'}$=9.1 Hz, $J_{4'-6'}$=2.9 Hz); 6.87 (d, 1H, H-6′, $J_{6'-4'}$=2.9 Hz); 6.74 (bs, 1H, –NH); 4.49 (dd, 1H, H-5, $J_{5,4b}$=12.7 Hz, $J_{5,4a}$=5.6 Hz); 4.22 (c, 2H, –COOCH_2–CH_3, J=7.1 Hz); 3.89 (s, 3H, –OCH_3); 3.45 (H-4 a, J_{4a-4b}=17.5 Hz, J_{4a-5}=5.6 Hz); 3.29 (1H, H-4b, J_{4b-4a}=17.5 Hz, $J_{4b,5}$=12.7 Hz); 1.29 (t, 3H, –COO–CH_2–CH_3 J=7.1 Hz).^{13}C NMR (75 MHz, $CDCl_3$) δ187.41 (Ph–*C*O–); 171.27 (–*C*OO–CH_2–CH_3); 163.95 (C-5′); 150.07 (C-3); 140.29 (C-2′); 137.85 (C-1′); 126.59 (C-3′); 115.52 (C-4′); 113.58 (C-6′); 62.30 (–COO–*C*H_2–CH_3); 61.85 (C-5); 56.25 (–OCH_3); 33.44 (C-4); 14.17 (–COO–CH_2–*C*H_3). HR LSIMS calcd for $C_{14}H_{15}N_3O_6Na$ (M+Na)$^+$ 344.0858, found 344.0867.

*4.1.1.3.2. Ethyl 3-(5-chloro-2-nitrobenzoyl)-Δ²-pyrazoline-5-carboxylate **5b***. Compound **5b** was obtained as a yellow solid (382 mg, 85% yield) starting from **3b**,[31] as described in procedure 3; mp 143–146 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (d, 1H, H-3′, $J_{3'-4'}$=8.7 Hz); 7.55 (dd, 1H, H-4′, $J_{4'-3'}$=8.7 Hz, $J_{4'-6'}$=2.3 Hz); 7.44 (d, 1H, H-6′, $J_{6'-4'}$=2.3 Hz); 6.82 (bs, 1H, –NH); 4.52 (dd, 1H, H-5, J_{5-4b}=12.8 Hz, J_{5-4a}=5.7 Hz); 4.23 (c, 2H, –COO–CH_2–CH_3, J=7.1 Hz) 3.43 (dd, 1H, H-4a, J_{4a-4b}=17.6 Hz, J_{4a-5}=5.7 Hz); 3.28 (dd, 1H, H-4b, J_{4b-4a}=17.6 Hz, J_{4b-5}=12.8 Hz); 1.30 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.72 (Ph–*C*O–), 171.04 (–*C*OO–CH_2–CH_3); 149.49 (C-3); 145.81 (C-2′); 140.73 (C-5′); 136.68 (C-1′); 130.65 (C-4′); 129.27 (C-6′); 125.53 (C-3′); 62.39 (–COO–*C*H_2–CH_3); 61.97 (C-5); 35.17 (C-4); 14.17 (–COO–CH_2–*C*H_3). HR LSIMS calcd for $C_{13}H_{12}ClN_3O_5Na$ (M+Na)$^+$ 348.0363, found 348.0361.

*4.1.1.3.3. Ethyl 3-(2-nitrobenzoyl)-Δ²-pyrazoline-5-carboxylate **5c***. Compound **5c** was obtained as a yellow solid (353 mg, 88% yield) starting from **3c**,[31] as described in procedure 3; mp 107–109 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (d, 1H, H-3′, $J_{3'-4'}$=8.1 Hz); 7.71 (dt, 1H, H-5′, $J_{5'-4'}$=$J_{5'-6'}$=7.5 Hz, $J_{5'-3'}$=1.1 Hz); 7.60 (dt, 1H, H-4′, $J_{4'-3'}$=8.1 Hz, $J_{4'-5'}$=7.5 Hz, $J_{4'-6'}$=1.5 Hz); 7.49 (dd, 1H, H-6′, $J_{6'-5'}$=7.5 Hz, $J_{6'-4'}$=1.5 Hz); 6.75 (bs, 1H, –NH); 4.50 (dd, 1H, H-5, J_{5-4b}=12.6 Hz, J_{5-4a}=5.6 Hz); 4.22 (c, 2H, –COO–CH_2–CH_3, J=7.1 Hz); 3.45 (dd, 1H, H-4a, J_{4a-4b}=17.6 Hz, J_{4a-5}=5.6 Hz); 3.29 (dd, 1H, H-4b, J_{4b-4a}=17.6 Hz, J_{4b-5}=12.6 Hz); 1.29 (t, 3H, –COO–CH_2–CH_3 J=7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 187.38 (Ph–*C*O–); 171.20 (–*C*OO–CH_2–CH_3); 150.00 (C-3); 147.67 (C-2′); 135.15 (C-1′); 133.90 (C-5′); 130.78 (C-4′); 129.17 (C-6′); 124.03 (C-3′); 62.30 (–COO–*C*H_2–CH_3); 61.87 (C-5); 33.37 (C-4); 14.15 (–COO–CH_2–*C*H_3). HR LSIMS calcd for $C_{13}H_{13}N_3O_5Na$ (M+Na)$^+$ 314.0752, found 314.0753.

4.1.1.4. General procedure for the preparation of compounds 11a,b,c–19a,b,c. Triethylamine (a small excess molar) and acetic anhydride or the corresponding acyl chloride (a molar equivalent) was added to a solution of the corresponding pyrazoline **5a**, **5b** or **5c** (0.85 mmol) in dry CH_2Cl_2 (10 mL) at room temperature. The reaction mixture was stirred for 3 h, filtered and washed with H_2O, 10% HCl, 2 M NaOH, H_2O and brine. The organic phase was dried (Na_2SO_4), and filtered. Evaporation of the solvent rendered a residue that was purified by flash chromatography (ethyl acetate–hexane 1:4).

*4.1.1.4.1. Ethyl 1-acetyl-3-(5-methoxy-2-nitrobenzoyl)-Δ²-pyrazoline-5-carboxylate **11a***. White solid; yield 100%; mp 102–104 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (d, 1H, H-3′, $J_{3'-4'}$=9.1 Hz); 7.06 (dd, 1H, H-4′, $J_{4'-3'}$=9.1 Hz, $J_{4'-6'}$=2.7 Hz); 6.92 (d, 1H, H-6′, $J_{6'-4'}$=2.7 Hz); 4.94 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.2 Hz); 4.21 (c, 2H, –COO–CH_2–CH_3, J=7.1 Hz); 3.93 (s, 3H, –OCH_3); 3.52 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.28 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.2 Hz); 2.11 (s, 3H, –CO–CH_3); 1.27 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.06 (Ph–*C*O–); 169.84, 168.97 (–*C*OOCH_2CH_3, –N–*C*O–); 164.05 (C-5′); 152.51 (C-3); 140.75 (C-2′); 136.33 (C-1′); 126.57 (C-3′); 116.06 (C-4′); 114. 09 (C-6′); 62.33 (–COO*C*H_2CH_3); 59.89 (C-5); 56.36 (–OCH_3); 35.65 (C-4); 21.07 (–CO–*C*H_3); 14.04 (–COOCH_2*C*H_3). HR LSIMS calcd for $C_{16}H_{18}N_3O_7$ (M^++1) 364.1144, found 364.1144.

*4.1.1.4.2. Ethyl 3-(5-methoxy-2-nitrobenzoyl)-1-propionyl-Δ²-pyrazoline-5-carboxylate **12a***. White solid; yield 98%; mp 116–118 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (d, 1H, H-3′, $J_{3'-4'}$=9.1 Hz); 7.07 (dd, 1H, H-4′, $J_{4'-3'}$=9.1 Hz, $J_{4'-6'}$=2.8 Hz); 6.93 (d, 1H, H-6′, $J_{6'-4'}$=2.8 Hz); 4.94 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.2 Hz); 4.21 (c, 2H, –COO–CH_2–CH_3, J=7.1 Hz); 3.93 (s, 3H, –OCH_3); 3.51 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.27 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.2 Hz); 2.42 (m, 2H, –CO–CH_2–CH_3); 1.28 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz); 1.04 (t, 3H, –CO–CH_2–CH_3 J=7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.12 (Ph–*C*O–); 173.31 (–N–CO–); 169.10 (–*C*OO–CH_2–CH_3); 164.07 (C-5′); 152.34 (C-3); 140.77 (C-2′); 136.47 (C-1′); 126.59 (C-3′); 116.08 (C-4′); 114. 06 (C-6′); 62.18 (–COO–*C*H_2–CH_3); 59.04 (C-5); 56.36 (–OCH_3); 35.39 (C-4); 26.85 (–CO–*C*H_2–CH_3); 14.06 (–COO–CH_2–*C*H_3); 8.37 (–CO–CH_2–*C*H_3). HR LSIMS calcd for $C_{17}H_{19}N_3O_7Na$ (M+Na)$^+$ 400.1120, found 400.1120.

*4.1.1.4.3. Ethyl 1-butyryl-3-(5-methoxy-2-nitrobenzoyl)-Δ²-pyrazoline-5-carboxylate **13a***. White solid; yield 98%; mp 106–108 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (d, 1H, H-3′, $J_{3'-4'}$=9.1 Hz); 7.07 (dd, 1H, H-4′, $J_{4'-3'}$=9.1 Hz, $J_{4'-6'}$=2.8 Hz); 6.93 (d, 1H, H-6′, $J_{6'-4'}$=2.8 Hz); 4.95 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.2 Hz); 4.21 (C, 2H, –COOCH_2CH_3, J=7.1 Hz); 3.94 (s, 3H, –OCH_3); 3.51 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.27 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.2 Hz); 2.39 (m, 2H, –CO–CH_2–CH_2–CH_3); 1.55 (m, 2H, –CO–CH_2–CH_2–CH_3); 1.28 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz); 0.84 (t, 3H, –CO–CH_2–CH_2–CH_3, J=7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.16 (Ph–*C*O–); 172.61 (–N–CO–); 169.09 (–*C*OO–CH_2–CH_3); 164.08 (C-5′); 152.34 (C-3); 140.77 (C-2′); 136.50 (C-1′); 126.58 (C-3′); 116.11 (C-4′); 114. 06

(C-6′); 62.21 (–COO–CH_2–CH_3); 59.01 (C-5); 56.36 (–OCH_3); 35.42, 35.26 (–CO–CH_2–CH_2–CH_3, C-4); 18.03 (–CO–CH_2–CH_2–CH_3); 14.07 (–COO–CH_2–CH_3); 13.68 (–CO–CH_2–CH_2–CH_3). HR LSIMS calcd for $C_{18}H_{21}N_3O_7Na$ $(M+Na)^+$ 414.1277, found 414.1272.

4.1.1.4.4. Ethyl 3-(5-methoxy-2-nitrobenzoyl)-1-pentanoyl-Δ²-pyrazoline-5-carboxylate ***14a***. White solid; yield 96%; mp 73–75 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (d, 1H, H-3′, $J_{3'-4'}$=9.1 Hz); 7.07 (dd, 1H, H-4′, $J_{4'-3'}$=9.1 Hz, $J_{4'-6'}$=2.8 Hz); 6.93 (d, 1H, H-6′, $J_{6'-4'}$=2.8 Hz); 4.94 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.2 Hz); 4.21 (C, 2H, –COOCH_2CH$_3$, J=7.1 Hz); 3.93 (s, 3H, –OCH_3); 3.51 (dd, 1H, H-4a, J_{4a-4b}=18.6 Hz, J_{4a-5}=12.9 Hz); 3.26 (dd, 1H, H-4b, J_{4b-4a}=18.6 Hz, J_{4b-5}=6.2 Hz); 2.40 (m, 2H, –CO–CH_2–CH_2–CH_2–CH_3); 1.48 (m, 2H, –CO–CH_2–CH_2–CH_2–CH_3); 1.27 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz); 1.23 (m, 2H, –CO–CH_2–CH_2–CH_2–CH_3); 0.80 (t, 3H, –CO–CH_2–CH_2–CH_2–CH_3, J=7.3 Hz).^{13}C NMR (75 MHz, $CDCl_3$) δ 187.13 (Ph–*C*O–); 172.79 (–N–CO–); 169.05 (–*C*OO–CH_2–CH_3); 164.08 (C-5′); 152.33 (C-3); 140.77 (C-2′); 136.54 (C-1′); 126.55 (C-3′); 116.06 (C-4′); 114. 03 (C-6′); 62.19 (–COO–CH_2–CH_3); 59.03 (C-5); 56.37 (–OCH_3); 35.42 (C-4); 33.19 (–CO–CH_2–CH_2–CH_2–CH_3); 26.66 (–CO–CH_2–CH_2–CH_2–CH_3); 22.40 (–CO–CH_2–CH_2–CH_2–CH_3); 14.06 (–COO–CH_2–CH_3); 13.64 (–CO–CH_2–CH_2–CH_2–CH_3). HR LSIMS calcd for $C_{19}H_{23}N_3O_7Na$ $(M+Na)^+$ 428.1433, found 428.1434.

4.1.1.4.5. Ethyl 1-cyclopropanecarbonyl-3-(5-methoxy-2-nitrobenzoyl)-Δ²-pyrazoline-5-carboxylate ***15a***. White solid; yield 99%; mp 137–139 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (d, 1H, H-3′, $J_{3'-4'}$=9.1 Hz); 7.06 (dd, 1H, H-4′, $J_{4'-3'}$=9.1 Hz, $J_{4'-6'}$=2.8 Hz); 6.94 (d, 1H, H-6′, $J_{6'-4'}$=2.8 Hz); 4.94 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.4 Hz); 4.21 (c, 2H, –COO–CH_2–CH$_3$, J=7.1 Hz); 3.93 (s, 3H, –OCH_3); 3.53 (dd, 1H, H-4a, J_{4a-4b}=18.6 Hz, J_{4a-5}=12.9 Hz); 3.29 (dd, 1H, H-4b, J_{4b-4a}=18.6 Hz, J_{4b-5}=6.4 Hz); 2.12 (m, 1H, H-1$_{cycloprop.}$); 1.26 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz); 1.06–0.98, 0.85–0.78 (2m, 4H, H-2, H-3$_{cycloprop.}$).^{13}C NMR (75 MHz, $CDCl_3$) δ 187.29 (Ph–*C*O–); 173.12 (–N–CO–); 169.11 (–*C*OO–CH_2–CH_3); 164.09 (C-5′); 152.57 (C-3); 140.61 (C-2′); 136.63 (C-1′); 126.65 (C-3′); 116.09 (C-4′); 114. 01 (C-6′); 62.14 (–COO–CH_2–CH_3); 59.34 (C-5); 56.38 (–OCH_3); 35.37 (C-4); 14.08 (–COO–CH_2–CH_3); 11.45 (C-1$_{cycloprop.}$); 9.44, 9.39 (C-2, C-3$_{cycloprop.}$). HR LSIMS calcd $C_{18}H_{19}N_3O_7Na$ $(M+Na)^+$ 412.1120, found: 412.1122.

4.1.1.4.6. Ethyl 1-cyclobutanecarbonyl-3-(5-methoxy-2-nitrobenzoyl)-Δ²-pyrazoline-5-carboxylate ***16a***. White solid; yield 99%; mp 137–139 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (d, 1H, H-3′, $J_{3'-4'}$=9.1 Hz); 7.08 (dd, 1H, H-4′, $J_{4'-3'}$=9.1 Hz, $J_{4'-6'}$=2.8 Hz); 6.91 (d, 1H, H-6′, $J_{6'-4'}$=2.8 Hz); 4.93 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.1 Hz); 4.22 (c, 2H, –COO–CH_2–CH$_3$, J=7.1 Hz); 3.94 (s, 3H, –OCH_3); 3.48 (dd, 1H, H-4a, J_{4a-4b}=18.6 Hz, J_{4a-5}=12.9 Hz); 3.39 (m, 1-H, H-1$_{cyclobut.}$); 3.24 (dd, 1H, H-4b, J_{4b-4a}=18.6 Hz, J_{4b-5}=6.1 Hz); 2.25–2.15, 1.98–1.72 (2m, 6H, H-2, H-3, H-4$_{cyclobut.}$); 1.28 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.18 (Ph–*C*O–); 174.11 (–N–CO–); 169.09 (–*C*OO–CH_2–CH_3); 164.09 (C-5′); 152.36 (C-3); 140.67 (C-2′); 136.67 (C-1′); 126.56 (C-3′); 116.11 (C-4′); 113.94 (C-6′); 62.19 (–COO–CH_2–CH_3); 59.11 (C-5); 56.38 (–OCH_3); 37.36 (C-1$_{cyclobut.}$); 35.19 (C-4); 24.58, 24.30 (C-2, C-4$_{cyclobut.}$); 18.15 (C-3$_{cyclobut.}$); 14.08 (–COO–CH_2–CH_3). HR LSIMS calcd for $C_{19}H_{21}N_3O_7Na$ $(M+Na)^+$ 426.1277, found: 426.1281.

4.1.1.4.7. Ethyl 1-cyclopentanecarbonyl-3-(5-methoxy-2-nitrobenzoyl)-Δ²-pyrazoline-5-carboxylate ***17a***. White solid; yield 98%; mp 129–131 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (d, 1H, H-3′, $J_{3'-4'}$=9.1 Hz); 7.07 (dd, 1H, H-4′, $J_{4'-3'}$=9.1 Hz, $J_{4'-6'}$=2.8 Hz); 6.93 (d, 1H, H-6′, $J_{6'-4'}$=2.8 Hz); 4.94 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.2 Hz); 4.21 (m, 2H, –COOCH_2CH$_3$); 3.93 (s, 3H, –OCH_3); 3.50 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.26 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.2 Hz); 3.06 (m, 1H, H-1$_{cyclopent.}$); 1.65–1.40 (m, 8H, H-2, H-3, H-4, H-5$_{cyclopent.}$); (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz).^{13}C NMR (75 MHz, $CDCl_3$) δ 187.26 (Ph–*C*O–); 175.69 (–N–CO–); 169.14 (–*C*OO–CH_2–CH_3); 164.09 (C-5′); 152.18 (C-3); 140.71 (C-2′); 136.67 (C-1′); 126.58 (C-3′); 116.12 (C-4′); 113.96 (C-6′); 62.14 (–COO–CH_2–CH_3); 59.18 (C-5); 56.37 (–OCH_3); 41.89 (C-1$_{cyclopent.}$); 35.24 (C-4); 29.59, 29.20 (C-2, C-5$_{cyclopent.}$); 26.24, 26.12 (C-3, C-4$_{cyclopent.}$); 14.06 (–COO–CH_2–CH_3). HR LSIMS calcd for $C_{20}H_{23}N_3O_7Na$ $(M+Na)^+$ 440.1433, found: 440.1437.

4.1.1.4.8. Ethyl 1-cyclohexanecarbonyl-3-(5-methoxy-2-nitrobenzoyl)-Δ²-pyrazoline-5-carboxylate ***18a***. White solid; yield 98%; mp 116–118 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (d, 1H, H-3′, $J_{3'-4'}$=9.1 Hz); 7.07 (dd, 1H, H-4′, $J_{4'-3'}$=9.1 Hz, $J_{4'-6'}$=2.8 Hz); 6.93 (d, 1H, H-6′, $J_{6'-4'}$=2.8 Hz); 4.92 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.0 Hz); 4.19 (m, 2H, –COOCH_2CH$_3$); 3.93 (s, 3H, –OCH_3); 3.49 (dd, 1H, H-4a, J_{4a-4b}=18.6 Hz, J_{4a-5}=12.9 Hz); 3.24 (dd, 1H, H-4b, J_{4b-4a}=18.6 Hz, J_{4b-5}=6.0 Hz); 2.65 (tt, 1H, H-1$_{cyclohex.}$, $J_{transdiaxial}$=11.5 Hz, J_{cis}=3.2 Hz); 1.75–1.56 (m, 5H, H$_{ec.\ cyclohex.}$); 1.26 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz); 1.40–1.05 (m, 5H, H$_{ax.\ cyclohex.}$).^{13}C NMR (75 MHz, $CDCl_3$) δ 187.11 (Ph–*C*O–); 175.41 (–N–CO–); 169.05 (–*C*OO–CH_2–CH_3); 164.08 (C-5′); 152.06 (C-3); 140.88 (C-2′); 136.58 (C-1′); 126.43 (C-3′); 116.09 (C-4′); 114.06 (C-6′); 62.13 (–COO–CH_2–CH_3); 59.10 (C-5); 56.36 (–OCH_3); 41.55 (C-1$_{cyclohex.}$); 35.18 (C-4); 28.49, 28.45 (C-2, C-6$_{cyclohex.}$); 25.80, 25.54, 25.50 (C-3, C-4, C-5$_{cyclohex.}$); 14.06 (–COO–CH_2–CH_3). HR LSIMS calcd for $C_{21}H_{25}N_3O_7Na$ $(M+Na)^+$ 454.1590, found 454.1583.

4.1.1.4.9. Ethyl 1-benzoyl-3-(5-methoxy-2-nitrobenzoyl)-Δ²-pyrazoline-5-carboxylate ***19a***. White solid; yield 94%; mp 134–136 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (d, 1H, H-3′, $J_{3'-4'}$=9.1 Hz); 7.60 (dd, 2H, H-2, H-6$_{benz.}$, $J_{2\text{-}3benz.}$=7.1 Hz, $J_{2\text{-}4benz.}$=1.3 Hz); 7.40 (tt, 1H, H-4$_{benz.}$, $J_{4\text{-}3benz.}$=7.4 Hz, $J_{4\text{-}2benz.}$=1.3 Hz); 7.26 (pt, 2H, H-3, H-5$_{benz.}$, $J_{3\text{-}4benz.}$=7.4 Hz, $J_{3\text{-}2benz.}$=7.1 Hz); 7.01 (dd, 1H, H-4′, $J_{4'-3'}$=9.1 Hz, $J_{4'-6'}$=2.8 Hz); 6.87 (d, 1H, H-6′, $J_{6'-4'}$=2.8 Hz); 5.17 (dd, 1H, H-5, J_{5-4a}=12.7 Hz, J_{5-4b}=6.1 Hz); 4.26 (c, 2H, –COOCH_2CH$_3$, J=7.1 Hz); 3.89 (s, 3H, –OCH_3); 3.57 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.7 Hz); 3.31 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz,

J_{4b-5}=6.1 Hz); 1.30 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz).^{13}C NMR (75 MHz, $CDCl_3$) δ 187.18 (Ph–*C*O–); 168.96, 167.32 (–*C*OO–CH_2–CH_3, –N–CO–); 164.20 (C-5′); 153.30 (C-3); 140.49 (C-2′); 136.64 (C-1′); 132.04 (C-$1_{benz.}$); 131.89 (C-$4_{benz.}$); 129.97 (C-2, C-$6_{benz.}$); 127.72 (C-3, C-$5_{benz.}$); 126.52 (C-3′); 116.12 (C-4′); 113.95 (C-6′); 62.92 (–COO–*C*H_2–CH_3); 60.36 (C-5); 56.34 (–OCH_3); 34.90 (C-4); 14.13 (–COO–CH_2–*C*H_3). HR LSIMS calcd for $C_{21}H_{19}N_3O_7Na$ $(M+Na)^+$ 448.1120, found: 448.1118.

4.1.1.4.10. Ethyl 1-acetyl-3-(5-chloro-2-nitrobenzoyl)-Δ^2-pyrazoline-5-carboxylate ***11b***. White solid; yield 94%; mp 134–136 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (d, 1H, H-3′, $J_{3'-4'}$=8.7 Hz); 7.63 (dd, 1H, H-4′, $J_{4'-3'}$=8.7 Hz, $J_{4'-6'}$=2.3 Hz); 7.51 (d, 1H, H-6′, $J_{6'-4'}$=2.3 Hz); 4.96 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.1 Hz); 4.22 (c, 2H, –COO–CH_2–CH_3, J=7.1 Hz); 3.51 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.26 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.1 Hz); 2.12 (s, 3H, –CO–CH_3); 1.28 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 185.52 (Ph–*C*O–); 169.82, 168.79 (–*C*OO–CH_2–CH_3, –N–CO–); 152.10 (C-3); 146.33 (C-2′); 141.04 (C-5′); 135.11 (C-1′); 131.52 (C-4′); 129.61 (C-6′); 125.45 (C-3′); 62.33 (–COO–*C*H_2–CH_3); 59.11 (C-5); 35.48 (C-4); 21.08 (–CO–*C*H_3); 14.06 (–COO–CH_2–*C*H_3). HR LSIMS calcd for $C_{15}H_{14}ClN_3O_6Na$ $(M+Na)^+$ 390.0468, found 390.0462.

4.1.1.4.11. Ethyl 3-(5-chloro-2-nitrobenzoyl)-1-propionyl-Δ^2-pyrazoline-5-carboxylate ***12b***. White solid; yield 98%; mp 87–90 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, 1H, H-3′, $J_{3'-4'}$=8.7 Hz); 7.63 (dd, 1H, H-4′, $J_{4'-3'}$= 8.7 Hz, $J_{4'-6'}$=2.3 Hz); 7.51 (d, 1H, H-6′, $J_{6'-4'}$=2.3 Hz); 4.95 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.2 Hz); 4.21 (c, 2H, –COO–CH_2–CH_3, J=7.1 Hz); 3.49 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.25 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.2 Hz); 2.44 (m, 2H, –CO–CH_2–CH_3); 1.27 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz), 1.05 (t, 3H, –CO–CH_2–*C*H_3, J=7.5 Hz).^{13}C NMR (75 MHz, $CDCl_3$) δ 185.58 (Ph–*C*O–); 173.28 (–N–CO–); 168.91 (–*C*OO–CH_2–CH_3); 151.92 (C-3); 146.27 (C-2′); 141.03 (C-5′); 135.21 (C-1′); 131.51 (C-4′); 129.58 (C-6′); 125.45 (C-3′); 62.28 (–COO–CH_2–CH_3); 59.20 (C-5); 35.19 (C-4); 26.83 (–CO–*C*H_2–CH_3); 14.06 (–COO–CH_2–*C*H_3); 8.34 (–CO–CH_2–*C*H_3). HR LSIMS calcd for $C_{16}H_{16}ClN_3O_6Na$ $(M+Na)^+$ 404.0625, found 404.0630.

4.1.1.4.12. Ethyl 1-butyryl-3-(5-chloro-2-nitrobenzoyl)-Δ^2-pyrazoline-5-carboxylate ***13b***. White solid; yield 98%; mp 82–84 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, 1H, H-3′, $J_{3'-4'}$=8.7 Hz); 7.64 (dd, 1H, H-4′, $J_{4'-3'}$=8.7 Hz, $J_{4'-6'}$= 2.1 Hz); 7.52 (d, 1H, H-6′, $J_{6'-4'}$=2.1 Hz); 4.97 (dd, 1H, H-5, J_{5-4a}=13.0 Hz, J_{5-4b}=6.1 Hz); 4.22 (c, 2H, –COO–CH_2–CH_3, J=7.1 Hz); 3.50 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=13.0 Hz); 3.26 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.1 Hz); 2.46 (1 pq, –CO–CH_2–CH_2–CH_3, Ha, J_{gem}=15.3 Hz, J_{Ha-CH2}=7.4 Hz); 2.37 (1 pq, –CO–CH_2–CH_2–CH_3, Hb, J_{gem}=15.3 Hz, J_{Hb-CH_2}= 7.4 Hz); 1.57 (m, 2H, –CO–CH_2–CH_2–CH_3); 1.29 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz); 0.87 (t, 3H, –CO–CH_2–CH_2–CH_3, J=7.4 Hz).^{13}C NMR (100 MHz, $CDCl_3$) δ 185.60 (Ph–*C*O–); 172.56 (–N–CO–); 168.89 (–*C*OO–CH_2–CH_3); 151.92 (C-3); 146.28 (C-2′); 141.04 (C-5′); 135.24 (C-1′); 131.48 (C-4′); 129.60 (C-6′); 125.44 (C-3′); 62.28 (–COO–*C*H_2–CH_3); 59.18 (C-5); 35.21 (–CO–*C*H_2–CH_2–CH_3, C-4); 17.98 (–CO–CH_2–*C*H_2–CH_3); 14.07 (–COO–CH_2–*C*H_3); 13.68 (–CO–CH_2–CH_2–*C*H_3). HR LSIMS: calcd for $C_{17}H_{18}ClN_3O_6Na$ $(M+Na)^+$ 418.0781, found 418.0783.

4.1.1.4.13. Ethyl 3-(5-chloro-2-nitrobenzoyl)-1-pentanoyl-Δ^2-pyrazoline-5-carboxylate ***14b***. White solid; yield 95%; mp 97–99 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, 1H, H-3′, $J_{3'-4'}$=8.7 Hz); 7.63 (dd, 1H, H-4′, $J_{4'-3'}$= 8.7 Hz, $J_{4'-6'}$=2.3 Hz); 7.51 (d, 1H, H-6′, $J_{6'-4'}$=2.3 Hz); 4.96 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.1 Hz); 4.21 (c, 2H, –COO–CH_2–CH_3, J=7.1 Hz); 3.49 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.25 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.1 Hz); 2.42 (m, 2H, –CO–CH_2–CH_2–CH_2–CH_3); 1.51 (m, 2H, –CO–CH_2–CH_2–CH_2–CH_3); 1.27 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz); 1.25 (m, 2H, –CO–CH_2–CH_2–CH_2–CH_3) 0.82 (t, 3H, –CO–CH_2–CH_2–CH_2–CH_3, J=7.3 Hz).^{13}C NMR (75 MHz, $CDCl_3$) δ 185.60 (Ph–*C*O–); 172.76 (–N–CO–); 168.88 (–*C*OO–CH_2–CH_3); 151.94 (C-3); 146.26 (C-2′); 141.05 (C-5′); 135.30 (C-1′); 131.44 (C-4′); 129.58 (C-6′); 125.44 (C-3′); 62.28 (–COO–CH_2–CH_3); 59.19 (C-5); 35.23 (C-4); 33.14 (–CO–*C*H_2–CH_2–CH_2–CH_3); 26.60 (–CO–CH_2–*C*H_2–CH_2–CH_3); 22.25 (–CO–CH_2–CH_2–*C*H_2–CH_3); 14.06 (–COO–CH_2–*C*H_3); 13.65 (–CO–CH_2–CH_2–CH_2–*C*H_3).HR LSIMS calcd for $C_{18}H_{21}ClN_3O_6$ (M^++1) 410.1118, found 410.1118.

4.1.1.4.14. Ethyl 3-(5-chloro-2-nitrobenzoyl)-1-cyclopropanecarbonyl-Δ^2-pyrazoline-5-carboxylate ***15b***. White solid; yield 98%; mp 129–131 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (d, 1H, H-3′, $J_{3'-4'}$=8.7 Hz); 7.61 (dd, 1H, H-4′, $J_{4'-3'}$=8.7 Hz, $J_{4'-6'}$=2.3 Hz); 7.52 (d, 1H, H-6′, $J_{6'-4'}$=2.3 Hz); 4.95 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.1 Hz); 4.20 (m, 2H, –COO–CH_2–CH_3); 3.51 (dd, 1H, H-4a, J_{4a-4b}=18.6 Hz, J_{4a-5}=12.9 Hz); 3.27 (dd, 1H, H-4b, J_{4b-4a}=18.6 Hz, J_{4b-5}=6.3 Hz); 2.10 (m, 1H, H-$1_{cycloprop.}$); 1.26 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz); 1.02, 0.83 (2m, 4H, H-2, H-$3_{cycloprop.}$) ^{13}C NMR (75 MHz, $CDCl_3$) δ 185.73 (Ph–*C*O–); 173.11 (–N–CO–); 168.90 (–*C*OO–CH_2–CH_3); 152.13 (C-3); 146.12 (C-2′); 141.04 (C-5′); 135.35 (C-1′); 131.40 (C-4′); 129.59 (C-6′); 125.56 (C-3′); 62.21 (–COO–*C*H_2–CH_3); 59.51 (C-5); 35.16 (C-4); 14.10 (–COO–CH_2–*C*H_3); 11.50 (C-$1_{cycloprop.}$); 9.54, 9.47 (C-2, C-$3_{cycloprop.}$). HR LSIMS calcd for $C_{17}H_{16}ClN_3O_6Na$ $(M+Na)^+$ 416.0625, found: 416.0621.

4.1.1.4.15. Ethyl 3-(5-chloro-2-nitrobenzoyl)-1-cyclobutanecarbonyl-Δ^2-pyrazoline-5-carboxylate ***16b***. White solid; yield 98%; mp 97–99 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (d, 1H, H-3′, $J_{3'-4'}$=8.7 Hz); 7.64 (dd, 1H, H-4′, $J_{4'-3'}$=8.7 Hz, $J_{4'-6'}$=2.3 Hz); 7.50 (d, 1H, H-6′, $J_{6'-4'}$=2.3 Hz); 4.95 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}= 6.1 Hz); 4.22 (c, 2H, –COO–CH_2–CH_3, J=7.1 Hz); 3.47 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.41 (m, 1H, H-$1_{cyclobut.}$); 3.23 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.1 Hz); 2.25–2.15, 1.98–1.72 (2m, 6H, H-2, H-3, H-$4_{cyclobut.}$); 1.28 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 185.64 (Ph–*C*O–); 174.10

(–N–CO–); 168.90 (–*C*OO–CH_2–CH_3); 152.00 (C-3); 146.12 (C-2′); 141.07 (C-5′); 135.44 (C-1′); 131.42 (C-4′); 129.55 (C-6′); 125.45 (C-3′); 62.27 (–COO–*C*H_2–CH_3); 59.28 (C-5); 37.32 (C-$1_{cyclobut.}$); 35.01 (C-4); 24.71, 24.22 (C-2, C-$4_{cyclobut.}$); 18.18 (C-$3_{cyclobut.}$); 14.08 (–COO–CH_2–*C*H_3). HR LSIMS calcd for $C_{18}H_{18}ClN_3O_6Na$ $(M+Na)^+$ 430.0783, found: 430.0781.

4.1.1.4.16. Ethyl 3-(5-chloro-2-nitrobenzoyl)-1-cyclopentanecarbonyl-Δ²-pyrazoline-5-carboxylate ***17b***. White solid; yield 97%; mp 74–76 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (d, 1H, H-3′, $J_{3'-4'}$=8.7 Hz); 7.63 (dd, 1H, H-4′, $J_{4'-3'}$=8.7 Hz, $J_{4'-6'}$=2.3 Hz); 7.51 (d, 1H, H-6′, $J_{6'-4'}$=2.3 Hz); 4.96 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.1 Hz); 4.21 (m, 2H, –COO–C*H*$_2$–CH_3); 3.49 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.24 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.1 Hz); 3.06 (m, 1H, H-$1_{cyclopent.}$); 1.81–1.42 (m, 8H, H-2, H-3, H-4, H-$5_{cyclopent.}$); 1.27 (t, 3H, –COO–CH_2–C*H*$_3$, *J*=7.1 Hz).^{13}C NMR (75 MHz, $CDCl_3$) δ 185.69 (Ph–*C*O–); 175.68 (–N–CO–); 168.93 (–*C*OO–CH_2–CH_3); 151.80 (C-3); 146.15 (C-2′); 141.04 (C-5′); 135.41 (C-1′); 131.42 (C-4′); 129.57 (C-6′); 125.46 (C-3′); 62.21 (–COO–*C*H_2–CH_3); 59.33 (C-5); 41.85 (C-$1_{cyclopent.}$); 35.05 (C-4); 29.71, 29.20 (C-2, C-$5_{cyclopent.}$); 26.24, 26.11 (C-3, C-$4_{cyclopent.}$); 14.06 (–COO–CH_2–*C*H_3). HR LSIMS calcd for $C_{19}H_{20}ClN_3O_6$-Na $(M+Na)^+$ 444.0938, found 444.0940.

4.1.1.4.17. Ethyl 3-(5-chloro-2-nitrobenzoyl)-1-cyclohexanecarbonyl-Δ²-pyrazoline-5-carboxylate ***18b***. White syrup; yield 98%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (d, 1H, H-3′, $J_{3'-4'}$=8.7 Hz); 7.63 (dd, 1H, H-4′, $J_{4'-3'}$=8.7 Hz, $J_{4'-6'}$= 2.3 Hz); 7.51 (d, 1H, H-6′, $J_{6'-4'}$=2.3 Hz); 4.94 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.0 Hz); 4.20 (m, 2H, –COO–C*H*$_2$–CH_3); 3.47 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}= 12.9 Hz); 3.23 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.0 Hz); 2.66 (tt, H-$1_{cyclohex.}$, $J_{transdiaxial}$=11.5 Hz, J_{cis}=3.3 Hz); 1.72–1.04 (m, 10H, H-2, H-3, H-4, H-5, H-$6_{cyclohex.}$); 1.26 (t, 3H, –COO–CH_2–C*H*$_3$, *J*=7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 185.54 (Ph–*C*O–); 175.39 (–N–CO–); 168.84 (–*C*OO–CH_2–CH_3); 151.67 (C-3); 146.27 (C-2′); 140.97 (C-5′); 135.28 (C-1′); 131.39 (C-4′); 129.64 (C-6′); 125.31 (C-3′); 62.19 (–COO–*C*H_2–CH_3); 59.21 (C-5); 41.51 (C-$1_{cyclohex.}$); 34.97 (C-4); 28.56, 28.40 (C-2, C-$6_{cyclohex.}$); 25.53, 25.45, 25.38 (C-3, C-4, C-$5_{cyclohex.}$); 14.03 (–COO–CH_2–*C*H_3). HR LSIMS calcd for $C_{20}H_{22}ClN_3O_6Na$ $(M+Na)^+$ 458.1094, found 458.1097.

4.1.1.4.18. Ethyl 1-benzoyl-3-(5-chloro-2-nitrobenzoyl)-Δ²-pyrazoline-5-carboxylate ***19b***. White syrup; yield 98%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (d, 1H, H-3′, $J_{3'-4'}$= 8.7 Hz); 7.56 (m, 3H, H-4′, H-2, H-$6_{benz.}$); 7.45 (d, 1H, H-6′, $J_{6'-4'}$=2.2 Hz); 7.41 (tt, 1H, H-$4_{benz.}$, J_{4-3}=6.8 Hz, J_{4-2}=1.2 Hz); 7.27 (pt, 2H, H-3, H-$5_{benz.}$, $J_{3\text{-}2benz.}$=7.4 Hz, $J_{3\text{-}4benz.}$=6.8 Hz); 5.18 (dd, 1H, H-5, J_{5-4a}=12.8 Hz, J_{5-4b}=6.2 Hz); 4.25 (c, 2H, –COO–C*H*$_2$–CH_3, *J*= 7.1 Hz); 3.56 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}= 12.8 Hz); 3.31 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}= 6.2 Hz); 1.29 (t, 3H, –COO–CH_2–C*H*$_3$, *J*=7.1 Hz).^{13}C NMR (75 MHz, $CDCl_3$) δ185.62 (Ph–*C*O–); 168.75, 167.39 (–*C*OO–CH_2–CH_3, –N–CO–); 152.87 (C-3); 146.15 (C-2′); 141.06 (C-5′); 135.35 (C-1′); 133.20 (C-$1_{benz.}$); 131.94 (C-$4_{benz.}$); 131.26 (C-4′); 129.76 (C-2, C-$6_{benz.}$); 129.42 (C-6′); 127.72 (C-3, C-$5_{benz.}$); 125.37 (C-3′); 62.30 (–COO–*C*H_2–CH_3); 60.40 (C-5); 34.69 (C-4); 14.05 (–COO–CH_2–*C*H_3). HR LSIMS calcd for $C_{20}H_{16}ClN_3O_6Na$ $(M+Na)^+$ 452.0625, found 452.0623.

4.1.1.4.19. Ethyl 1-acetyl-3-(2-nitrobenzoyl)-Δ²-pyrazoline-5-carboxylate ***11c***. White solid; yield 99%; mp 112–114 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (d, 1H, H-3′, $J_{3'-4'}$=8.1 Hz); 7.78 (t, 1H, H-5′, $J_{5'-6'}$=$J_{5'-4'}$=7.5 Hz); 7.69 (dt, 1H, H-4′, $J_{4'-3'}$=8.1 Hz, $J_{4'-5'}$=7.5 Hz, $J_{4'-6'}$= 1.1 Hz); 7.57 (dd, 1H, H-6′, $J_{6'-5'}$=7.5 Hz, $J_{6'-4'}$=1.1 Hz); 4.96 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.1 Hz); 4.23 (c, 2H, –COO–C*H*$_2$–CH_3, *J*=7.1 Hz); 3.53 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.29 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.1 Hz); 2.12 (s, 3H, –CO–CH_3); 1.29 (t, 3H, –COO–CH_2–C*H*$_3$, *J*=7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.06 (Ph–*C*O–); 169.88, 168.92 (–*C*OO–CH_2–CH_3, –N–CO–); 152.44 (C-3); 148.26 (C-2′); 134.15 (C-5′); 133.67 (C-1′); 131.66 (C-4′); 129.59 (C-6′); 123.95 (C-3′); 62.27 (–COO–*C*H_2–CH_3); 58.99 (C-5); 35.62 (C-4); 21.05 (–CO–CH_3); 14.06 (–COO–CH_2–*C*H_3). HR LSIMS calcd for $C_{15}H_{15}N_3O_6Na$ $(M+Na)^+$ 356.0858, found 356.0858.

4.1.1.4.20. Ethyl 3-(2-nitrobenzoyl)-1-propionyl-Δ²-pyrazoline-5-carboxylate ***12c***. White solid; yield 98%; mp 106–108 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (d, 1H, H-3′, $J_{3'-4'}$=8.1 Hz); 7.77 (dt, 1H, H-5′, $J_{5'-6'}$=$J_{5'-4'}$=7.5 Hz, $J_{5'-3'}$=1.2 Hz); 7.68 (dt, 1H, H-4′, $J_{4'-3'}$=8.1 Hz, $J_{4'-5'}$=7.5 Hz, $J_{4'-6'}$=1.6 Hz); 7.55 (dd, 1H, H-6′, $J_{6'-5'}$= 7.5 Hz, $J_{6'-4'}$=1.6 Hz); 4.95 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.2 Hz); 4.21 (c, 2H, –COO–C*H*$_2$–CH_3, *J*=7.1 Hz); 3.50 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.26 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.2 Hz); 2.43 (m, 2H, –CO–C*H*$_2$–CH_3); 1.27 (t, 3H, –COO–CH_2–C*H*$_3$, *J*=7.1 Hz); 1.04 (t, 3H, –CO–CH_2–C*H*$_3$, *J*=7.5 Hz).^{13}C NMR (75 MHz, $CDCl_3$) δ 187.13 (Ph–*C*O–); 173.33 (–N–CO–); 169.04 (–*C*OO–CH_2–CH_3); 152.27 (C-3); 148.19 (C-2′); 134.15 (C-5′); 133.79 (C-1′); 131.60 (C-4′); 129.56 (C-6′); 123.97 (C-3′); 62.20 (–COO–*C*H_2–CH_3); 59.11 (C-5); 35.34 (C-4); 26.81 (–CO–*C*H_2–CH_3); 14.06 (–COO–CH_2–*C*H_3); 8.35 (–CO–CH_2–*C*H_3). HR LSIMS calcd for $C_{16}H_{17}N_3O_6Na$ $(M+Na)^+$ 370.1015, found 370.1014.

4.1.1.4.21. Ethyl 1-butyryl-3-(2-nitrobenzoyl)-Δ²-pyrazoline-5-carboxylate ***13c***. White syrup; yield 98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, 1H, H-3′, $J_{3'-4'}$= 8.1 Hz); 7.76 (t, 1H, H-5′, $J_{5'-6'}$=$J_{5'-4'}$=7.5 Hz); 7.67 (dt, 1H, H-4′, $J_{4'-3'}$=8.1 Hz, $J_{4'-5'}$=7.5 Hz, $J_{4'-6'}$=1.2 Hz); 7.54 (dd, 1H, H-6′, $J_{6'-5'}$=7.5 Hz, $J_{6'-4'}$=1.2 Hz); 4.95 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.1 Hz); 4.20 (c, 2H, –COO–C*H*$_2$–CH_3, *J*=7.1 Hz); 3.49 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.25 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.1 Hz); 2.42 (pq, 1H, –CO–C*H*$_2$–CH_2–CH_3, Ha, J_{gem}=15.0 Hz, J_{Ha-CH2}=7.4 Hz); 2.34 (pq, 1H, –CO–C*H*$_2$–CH_2–CH_3, Hb, J_{gem}=15.0 Hz, J_{Hb-CH2}=7.4 Hz); 1.54 (m, 2H, –CO–CH_2–C*H*$_2$–CH_3); 1.26 (t, 3H, –COO–CH_2–C*H*$_3$, *J*=7.1 Hz); 0.83 (t, 3H, –CO–CH_2–CH_2–C*H*$_3$, *J*=7.4 Hz).^{13}C NMR (100 MHz, $CDCl_3$) δ 187.10 (Ph–*C*O–); 172.53 (–N–CO–); 168.94 (–*C*OO–CH_2–CH_3); 152.23 (C-3); 148.07 (C-2′); 134.12 (C-5′); 133.73 (C-1′); 131.56 (C-4′); 129.47 (C-6′); 123.89 (C-3′);

62.12 (–COO–CH_2–CH_3); 59.01 (C-5); 35.29, 35.13 (–CO–CH_2–CH_2–CH_3, C-4); 17.91 (–CO–CH_2–CH_2–CH_3); 13.99, 13.58 (–COO–CH_2–CH_3, –CO–CH_2–CH_2–CH_3). HR LSIMS calcd for $C_{18}H_{21}N_3O_6Na$ $(M+Na)^+$ 398.1328, found 398.1325.

4.1.1.4.22. Ethyl 3-(2-nitrobenzoyl)-1-pentanoyl-Δ^2-pyrazoline-5-carboxylate ***14c***. White syrup; yield 96%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, 1H, H-3′, $J_{3'-4'}$= 8.1 Hz); 7.74 (dt, 1H, H-5′, $J_{5'-6'}=J_{5'-4'}$=7.5 Hz, $J_{5'-3'}$= 0.7 Hz); 7.65 (dt, 1H, H-4′, $J_{4'-3'}$=8.1 Hz, $J_{4'-5'}$=7.5 Hz, $J_{4'-6'}$=1.3 Hz); 7.52 (dd, 1H, H-6′, $J_{6'-5'}$=7.5 Hz, $J_{6'-4'}$= 1.1 Hz); 4.92 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.1 Hz); 4.17 (c, 2H, –COO–CH_2–CH_3, J=7.1 Hz); 3.48 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.23 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.1 Hz); 2.42 (pq, 1H, –CO–CH_2–CH_2–CH_2–CH_3, Ha, J_{gem}=15.1 Hz, J_{Ha-CH2}= 7.6 Hz); 2.33 (pq, 1H, –CO–CH_2–CH_2–CH_2–CH_3, Hb, J_{gem}=15.1 Hz, J_{Hb-CH2}=7.6 Hz); 1.45 (m, 2H, –CO–CH_2–CH_2–CH_2–CH_3); 1.23 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz); 1.17 (m, 2H, –CO–CH_2–CH_2–CH_2–CH_3) 0.75 (t, 3H, –CO–CH_2–CH_2–CH_2–CH_3, J=7.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ187.03 (Ph–CO–); 172.66 (–N–CO–); 168.86(–COO–CH_2–CH_3); 152.21 (C-3); 147.98 (C-2′); 134.08 (C-5′); 133.68 (C-1′); 131.49 (C-4′); 129.38 (C-6′); 123.82 (C-3′); 62.03 (–COO–CH_2–CH_3); 58.97 (C-5); 35.23 (C-4); 32.98 (–CO–CH_2–CH_2–CH_2–CH_3); 26.46 (–CO–CH_2–CH_2–CH_2–CH_3); 22.07 (–CO–CH_2–CH_2–CH_2–CH_3); 13.90, 13.48 (–COO–CH_2–CH_3, –CO–CH_2–CH_2–CH_2–CH_3). HR LSIMS calcd for $C_{18}H_{21}N_3O_6Na$ $(M+Na)^+$ 398.1328, found 398.13253.

4.1.1.4.23. Ethyl 1-cyclopropanecarbonyl-3-(2-nitrobenzoyl)-Δ^2-pyrazoline-5-carboxylate ***15c***. White solid; yield 98%; mp 116–118 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (dd, 1H, H-3′, $J_{3'-4'}$=8.1 Hz, $J_{3'-5'}$=1.2 Hz); 7.77 (dt, 1H, H-5′, $J_{5'-6'}=J_{5'-4'}$=7.5 Hz, $J_{5'-3'}$=1.2 Hz); 7.67 (dt, 1H, H-4′, $J_{4'-3'}$=8.1 Hz, $J_{4'-5'}$=7.5 Hz, $J_{4'-6'}$=1.6 Hz); 7.57 (dd, 1H, H-6′, $J_{6'-5'}$=7.5 Hz, $J_{6'-4'}$=1.6 Hz); 4.95 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.3 Hz); 4.21 (m, 2H, –COO–CH_2–CH_3); 3.52 (dd, 1H, H-4a, J_{4a-4b}=18.6 Hz, J_{4a-5}=12.9 Hz); 3.28 (dd, 1H, H-4b, J_{4b-4a}=18.6 Hz, J_{4b-5}=6.3 Hz); 2.11 (m, 1H, H-$1_{cycloprop.}$); 1.26 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz); 1.01–0.98, 0.84–0.79 (2m, 4H, H-2, H-$3_{cycloprop.}$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.27 (Ph–CO–); 173.14 (–N–CO–); 169.03 (–COO–CH_2–CH_3); 152.47 (C-3); 148.09 (C-2′); 134.14 (C-5′); 133.91 (C-1′); 131.52 (C-4′); 129.61 (C-6′); 124.03 (C-3′); 62.15 (–COO–CH_2–CH_3); 59.41 (C-5); 35.32 (C-4); 14.07 (–COO–CH_2–CH_3); 11.44 (C-$1_{cycloprop.}$); 9.39, 9.35 (C-2, C-$3_{cycloprop.}$). HR LSIMS calcd for $C_{17}H_{17}N_3O_6Na$ $(M+Na)^+$ 382.1015, found 382.1014.

4.1.1.4.24. Ethyl 1-cyclobutanecarbonyl-3-(2-nitrobenzoyl)-Δ^2-pyrazoline-5-carboxylate ***16c***. White syrup; yield 99%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (dd, 1H, H-3′, $J_{3'-4'}$=8.0 Hz, $J_{3'-5'}$=1.1 Hz); 7.77 (dt, 1H, H-5′, $J_{5'-6'}=J_{5'-4'}$=7.5 Hz, $J_{5'-3'}$=1.1 Hz); 7.68 (dt, 1H, H-4′, $J_{4'-3'}$= 8.0 Hz, $J_{4'-5'}$=7.5 Hz, $J_{4'-6'}$=1.6 Hz); 7.52 (dd, 1H, H-6′, $J_{6'-5'}$=7.5 Hz, $J_{6'-4'}$=1.6 Hz); 4.93 (dd, 1H, H-5, J_{5-4a}=12.8 Hz, J_{5-4b}=6.1 Hz); 4.21 (c, 2H, –COO–CH_2–CH_3, J=7.1 Hz); 3.47 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}= 12.9 Hz); 3.38 (m, 1H, H-$1_{cyclobut.}$); 3.24 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.1 Hz); 2.27–2.13, 1.98–1.73 (2m, 6H, H-2, H-3, H-$4_{cyclobut.}$); 1.27 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz).^{13}C NMR (75 MHz, $CDCl_3$) δ 187.23 (Ph–CO–); 174.11 (–N–CO–); 169.01 (–COO–CH_2–CH_3); 152.35 (C-3); 147.94 (C-2′); 134.20 (C-5′); 134.00 (C-1′); 131.53 (C-4′); 129.43 (C-6′); 123.95 (C-3′); 62.18 (–COO–CH_2–CH_3); 59.16 (C-5); 37.31 (C-$1_{cyclobut.}$); 35.13 (C-4); 24.68, 24.18 (C-2, C-$4_{cyclobut.}$); 18.13 (C-$3_{cyclobut.}$); 14.05 (–COO–CH_2–CH_3). HR LSIMS calcd for $C_{18}H_{19}N_3O_6Na$ $(M+Na)^+$ 396.1171, found 396.1169.

4.1.1.4.25. Ethyl 1-cyclopentanecarbonyl-3-(2-nitrobenzoyl)-Δ^2-pyrazoline-5-carboxylate ***17c***. White syrup; yield 97%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (dd, 1H, H-3′, $J_{3'-4'}$=8.1 Hz, $J_{3'-5'}$=1.1 Hz); 7.77 (dt, 1H, H-5′, $J_{5'-6'}=J_{5'-4'}$=7.5 Hz, $J_{5'-3'}$=1.1 Hz); 7.67 (dt, 1H, H-4′, $J_{4'-3'}$=8.1 Hz, $J_{4'-5'}$=7.5 Hz, $J_{4'-6'}$=1.6 Hz); 7.54 (dd, 1H, H-6′, $J_{6'-5'}$=7.5 Hz, $J_{6'-4'}$=1.6 Hz); 4.94 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.1 Hz); 4.20 (m, 2H, –COO–CH_2–CH_3); 3.49 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}= 12.9 Hz); 3.25 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}= 6.1 Hz); 3.06 (m, 1H, H-$1_{cyclopent.}$); 1.91–1.06 (m, 8H, H-2, H-3, H-4, H-$5_{cyclopent}$); 1.26 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.29 (Ph–CO–); 175.70 (–N–CO–); 169.05 (–COO–CH_2–CH_3); 152.17 (C-3); 147.96 (C-2′); 134.18 (C-5′); 133.99 (C-1′); 131.53 (C-4′); 129.45 (C-6′); 123.97 (C-3′); 62.12 (–COO–CH_2–CH_3); 59.22 (C-5); 41.84 (C-$1_{cyclopent.}$); 35.18 (C-4); 29.66, 29.14 (C-2, C-$5_{cyclopent.}$); 26.20, 26.07 (C-3, C-$4_{cyclopent.}$), 14.03 (–COO–CH_2–CH_3). HR LSIMS calcd for $C_{19}H_{21}N_3O_6Na$ $(M+Na)^+$ 410.1328, found 410.1326.

4.1.1.4.26. Ethyl 1-cyclohexanecarbonyl-3-(2-nitrobenzoyl)-Δ^2-pyrazoline-5-carboxylate ***18c***. White syrup; yield 98%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (dd, 1H, H-3′, $J_{3'-4'}$=8.1 Hz, $J_{3'-5'}$=1.1 Hz); 7.77 (dt, 1H, H-5′, $J_{5'-4'}$=7.6 Hz, $J_{5'-6'}$=7.5 Hz, $J_{5'-3'}$=1.1 Hz); 7.68 (dt, 1H, H-4′, $J_{4'-3'}$=8.1 Hz, $J_{4'-5'}$=7.6 Hz, $J_{4'-6'}$=1.6 Hz); 7.54 (dd, 1H, H-6′, $J_{6'-5'}$=7.5 Hz, $J_{6'-4'}$=1.6 Hz); 4.92 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.0 Hz); 4.19 (m, 2H, –COO–CH_2–CH_3); 3.48 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}= 12.9 Hz); 3.24 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}= 6.0 Hz); 2.65 (tt, H-$1_{cyclohex.}$, $J_{transdiaxial}$=11.5 Hz, J_{cis}=3.2 Hz); 1.72–1.04 (m, 10H, H-2, H-3, H-4, H-5, H-$6_{cyclohex.}$); 1.25 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.17 (Ph–CO–); 175.43 (–N–CO–); 168.97 (–COO–CH_2–CH_3); 152.07 (C-3); 148.10 (C-2′); 134.17 (C-5′); 133.91 (C-1′); 131.53 (C-4′); 129.52 (C-6′); 123.83 (C-3′); 62.13 (–COO–CH_2–CH_3); 59.12 (C-5); 41.50 (C-$1_{cyclohex.}$); 35.12 (C-4); 28.54, 28.38 (C-2, C-$6_{cyclohex.}$); 25.77, 25.53, 25.46 (C-3, C-4, C-$5_{cyclohex.}$), 14.03 (–COO–CH_2–CH_3). HR LSIMS calcd for $C_{20}H_{23}N_3O_6Na$ $(M+Na)^+$ 424.1484, found 424.1485.

4.1.1.4.27. Ethyl 1-benzoyl-3-(2-nitrobenzoyl)-Δ^2-pyrazoline-5-carboxylate ***19c***. White solid; yield 92%; mp 135–137 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (dd, 1H, H-3′, $J_{3'-4'}$=8.1 Hz, $J_{3'-5'}$=1.1 Hz); 7.70 (dt, 1H, H-5′, $J_{5'-4'}$= 7.7 Hz, $J_{5'-6'}$=7.4 Hz, $J_{5'-3'}$=1.1 Hz); 7.61 (m, 3H, H-4′, H-2, H-$6_{benz.}$); 7.49 (dd, 1H, H-6′, $J_{6'-5'}$=7.4 Hz, $J_{6'-4'}$= 1.5 Hz); 7.39 (tt, 1H, H-$4_{benz.}$, $J_{4\text{-}3benz.}$=6.7 Hz, $J_{4\text{-}2benz.}$= 1.2 Hz); 7.25 (pt, 2H, H-3, H-$5_{benz.}$, $J_{3\text{-}2benz.}$=7.1 Hz,

$J_{3\text{-}4benz.}$=6.7 Hz); 5.17 (dd, 1H, H-5, J_{5-4a}=12.7 Hz, J_{5-4b}=6.2 Hz); 4.26 (c, 2H, –COO–C*H*$_2$–CH$_3$, *J*=7.1 Hz); 3.57 (dd, 1H, H-4a, J_{4a-4b}=18.6 Hz, J_{4a-5}=12.7 Hz); 3.33 (dd, 1H, H-4b, J_{4b-4a}=18.6 Hz, J_{4b-5}=6.2 Hz); 0.97 (t, 3H, –COO–CH$_2$–C*H*$_3$, *J*=7.1 Hz). ^{13}C NMR (75 MHz, CDCl$_3$) δ 187.29 (Ph–*C*O–); 168.89, 167.33 (–*C*OO–CH$_2$–CH$_3$, –N–CO–); 153.34 (C-3); 148.26 (C-2′); 134.30 (C-5′); 134.02 (C-1′); 131.97 (C-1$_{benz.}$); 131.84, 131.42 (C-4$_{benz.}$, C-4′); 129.86 (C-2, C-6$_{benz.}$); 129.36 (C-6′); 127.67 (C-3, C-5$_{benz.}$); 123.90 (C-3′); 62.26 (–COO–*C*H$_2$–CH$_3$); 60.36 (C-5); 34.83 (C-4); 14.09 (–COO–CH$_2$–*C*H$_3$). HR LSIMS calcd for $C_{20}H_{17}N_3O_6Na$ (M+Na)$^+$ 418.1015, found: 418.1014.

4.1.2. Final products

4.1.2.1. General procedure for the preparation of compounds 20a–28a. A mixture of nitroarenes **11a–19a** (0.512 mmol) and 10% Pd/C (20 mg) was dissolved in methanol (30 mL) and stirred under a hydrogen atmosphere (70 psi) for 1.5 h. The mixture was filtered through celite and evaporated. The resultant residue was dissolved in CH_2Cl_2, and this solution was washed with water, dried (Na_2SO_4), and concentrated. The resultant solid was dissolved and purified by flash chromatography (ether–hexane 1:2).

4.1.2.1.1. Ethyl 1-acetyl-3-(2-amino-5-methoxybenzoyl)-Δ^2-pyrazoline-5-carboxylate ***20a***. Orange solid; yield 80%; mp 210–212 °C. ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.04 (d, 1H, H-6′, $J_{6'-4'}$=2.9 Hz); 7.02 (dd, 1H, H-4′, $J_{4'-3'}$=9.0 Hz, $J_{4'-6'}$=2.9 Hz); 6.65 (d, 1H, H-3′, $J_{3'-4'}$=9.0 Hz); 6.04 (bs, 2H, –NH$_2$); 4.90 (dd, 1H, H-5, J_{5-4a}=12.8 Hz, J_{5-4b}=6.2 Hz); 4.23 (c, 2H, –COO–C*H*$_2$–CH$_3$, *J*=7.1 Hz); 3.76 (s, 3H, –OCH$_3$); 3.61 (dd, 1H, H-4a, J_{4a-4b}=18.8 Hz, J_{4a-5}=12.8 Hz); 3.31 (dd, 1H, H-4b, J_{4b-4a}=18.8 Hz, $J_{4b,5}$=6.2 Hz); 2.41 (s, 3H, –CO–CH$_3$); 1.29 (t, 3H, –COO–CH$_2$–C*H*$_3$, *J*=7.1 Hz). ^{13}C NMR (75 MHz, CDCl$_3$) δ 186.37 (Ph–*C*O–); 169.93, 169.51 (–N–CO–, –*C*OO–CH$_2$–CH$_3$); 153.89 (C-3); 150.13 (C-5′); 146.90 (C-2′); 125.47 (C-4′); 118.62 (C-3′); 116.10 (C-1′); 114.31 (C-6′); 62.13 (–COO–*C*H$_2$–CH$_3$); 57.44 (C-5); 55.74 (–OCH$_3$); 38.05 (C-4); 21.52 (–CO–CH$_3$); 14.14 (–COO–CH$_2$–*C*H$_3$). HR LSIMS calcd for $C_{16}H_{19}N_3O_5Na$ (M+Na)$^+$ 356.1222, found 356.1223. Anal. for $C_{16}H_{19}N_3O_5$: calcd: C, 57.65; H, 5.75; N, 12.61. Found: C, 57.46; H, 5.70; N, 12.21.

4.1.2.1.2. Ethyl 3-(2-amino-5-methoxybenzoyl)- 1-propionyl-Δ^2-pyrazoline-5-carboxylate ***21a***. Orange solid; yield 80%; mp 140–141 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.03 (d, 1H, H-6′, $J_{6'-4'}$=3.0 Hz); 7.02 (dd, 1H, H-4′, $J_{4'-3'}$=9.0 Hz, $J_{4'-6'}$=3.0 Hz); 6.64 (d, 1H, H-3′, $J_{3'-4'}$=9.0 Hz); 6.06 (bs, 2H, –NH$_2$); 4.90 (dd, 1H, H-5, J_{5-4a}=12.8 Hz, J_{5-4b}=6.3 Hz); 4.23 (c, 2H, –COO–C*H*$_2$–CH$_3$, *J*=7.1 Hz); 3.76 (s, 3H, –OCH$_3$); 3.59 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.8 Hz); 3.28 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, $J_{4b,5}$=6.3 Hz); 2.78 (m, 2H, –CO–C*H*$_2$–CH$_3$); 1.28 (t, 3H, –COO–CH$_2$–C*H*$_3$, *J*=7.1 Hz); 1.21 (t, 3H, –CO–CH$_2$–C*H*$_3$, *J*=7.5 Hz). ^{13}C NMR (75 MHz, CDCl$_3$) δ 186.45 (Ph–*C*O–); 173.42 (–N–CO–); 169.62 (–*C*OO–CH$_2$–CH$_3$); 153.70 (C-3); 150.12 (C-5′); 146.88 (C-2′); 125.42 (C-4′); 118.61 (C-3′); 116.48 (C-1′); 114.30 (C-6′); 62.06 (–COO–*C*H$_2$–CH$_3$); 57.52 (C-5); 55.71 (–OCH$_3$); 37.76 (C-4); 27.33 (–CO–*C*H$_2$–CH$_3$); 14.13 (–COO–CH$_2$–*C*H$_3$); 8.91 (–CO–CH$_2$–*C*H$_3$). HR LSIMS calcd for $C_{17}H_{21}N_3O_5Na$ (M+Na)$^+$ 370.1378, found 370.1377. Anal. for $C_{17}H_{21}N_3O_5$: calcd: C, 58.78; H, 6.09; N, 12.10. Found: C, 59.15; H, 6.27; N, 11.81.

4.1.2.1.3. Ethyl 3-(2-amino-5-methoxybenzoyl)-1-butyryl-Δ^2-pyrazoline-5-carboxylate ***22a***. Orange solid; yield 80%; mp 153–155 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.04 (d, 1H, H-6′, $J_{6'-4'}$=2.9 Hz); 7.02 (dd, 1H, H-4′, $J_{4'-3'}$=9.0 Hz, $J_{4'-6'}$=2.9 Hz); 6.65 (d, 1H, H-3′, $J_{3'-4'}$=9.0 Hz); 6.07 (bs, 2H, –NH$_2$); 4.90 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.3 Hz); 4.20 (c, 2H, –COO–C*H*$_2$–CH$_3$, *J*=7.1 Hz); 3.77 (s, 3H, –OCH$_3$); 3.59 (dd, 1H, H-4a, J_{4a-4b}=18.8 Hz, J_{4a-5}=12.9 Hz); 3.29 (dd, 1H, H-4b, J_{4b-4a}=18.8 Hz, $J_{4b,5}$=6.3 Hz); 2.80 (pq, 1H, –CO–C*H*$_2$–CH$_2$–CH$_3$, Ha, J_{gem}=15.1 Hz, J_{Ha-CH2}=7.5 Hz); 2.68 (pq, 1H, –CO–C*H*$_2$–CH$_2$–CH$_3$, Hb, J_{gem}=15.1 Hz, J_{Hb-CH2}=7.5 Hz); 1.74 (m, 2H, –CO–CH$_2$–C*H*$_2$–CH$_3$); 1.28 (t, 3H, –COO–CH$_2$–C*H*$_3$, *J*=7.1 Hz); 0.97 (t, 3H, –CO–CH$_2$–CH$_2$–C*H*$_3$, *J*=7.4 Hz).^{13}C NMR (75 MHz, CDCl$_3$) δ 186.43 (Ph–*C*O–); 172.64 (–N–CO–); 169.61 (–*C*OO–CH$_2$–CH$_3$); 153.70 (C-3); 150.14 (C-5′); 146.93 (C-2′); 125.52 (C-4′); 118.63 (C-3′); 116.12 (C-1′); 114.23 (C-6′); 62.06 (–COO–*C*H$_2$–CH$_3$); 57.47 (C-5); 55.71 (–OCH$_3$); 37.79 (C-4); 35.83 (–CO–*C*H$_2$–CH$_2$–CH$_3$); 18.30, (–CO–CH$_2$–*C*H$_2$–CH$_3$); 14.14, 13.85 (–COO–CH$_2$–*C*H$_3$, –CO–CH$_2$–CH$_2$–*C*H$_3$). HR LSIMS calcd for $C_{18}H_{23}N_3O_5Na$ (M+Na)$^+$ 384.1535, found: 384.1537. Anal. for $C_{18}H_{23}N_3O_5$: calcd: C, 59.82; H, 6.41; N, 11.63. Found: C, 59.45; H, 6.46; N, 11.53.

4.1.2.1.4. Ethyl 3-(2-amino-5-methoxybenzoyl)-1-pentanoyl-Δ^2-pyrazoline-5-carboxylate ***23a***. Orange solid; yield 80%; mp 108–110 °C. ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.03 (d, 1H, H-6′, $J_{6'-4'}$=2.9 Hz); 7.01 (dd, 1H, H-4′, $J_{4'-3'}$=9.0 Hz, $J_{4'-6'}$=2.9 Hz); 6.64 (d, 1H, H-3′, $J_{3'-4'}$=9.0 Hz); 6.07 (bs, 2H, –NH$_2$); 4.89 (dd, 1H, H-5, J_{5-4a}=12.8 Hz, J_{5-4b}=6.3 Hz); 4.22 (c, 2H, –COO–C*H*$_2$–CH$_3$, *J*=7.1 Hz); 3.76 (s, 3H, –OCH$_3$); 3.58 (dd, 1H, H-4a, J_{4a-4b}=18.8 Hz, J_{4a-5}=12.8 Hz); 3.28 (dd, 1H, H-4b, J_{4b-4a}=18.8 Hz, $J_{4b,5}$=6.3 Hz); 2.81 (pq, 1H, –CO–C*H*$_2$–CH$_2$–CH$_2$–CH$_3$, Ha, J_{gem}=15.3 Hz, J_{Ha-CH2}=7.6 Hz); 2.70 (pq, 1H, –CO–C*H*$_2$–CH$_2$–CH$_2$–CH$_3$, Hb, J_{gem}=15.3 Hz, J_{Hb-CH2}=7.6 Hz); 1.69 (m, 2H, –CO–CH$_2$–C*H*$_2$–CH$_2$–CH$_3$); 1.40 (m, 2H, –CO–CH$_2$–CH$_2$–C*H*$_2$–CH$_3$) 1.27 (t, 3H, –COO–CH$_2$–C*H*$_3$, *J*=7.1 Hz); 0.91 (t, 3H, –CO–CH$_2$–CH$_2$–CH$_2$–C*H*$_3$, *J*=7.3 Hz).^{13}C NMR (75 MHz, CDCl$_3$) δ 186.44 (Ph–*C*O–); 172.77 (–N–CO–); 169.60 (–*C*OO–CH$_2$–CH$_3$); 153.67 (C-3); 150.13 (C-5′); 146.92 (C-2′); 125.45 (C-4′); 118.61 (C-3′); 116.14 (C-1′); 114.33 (C-6′); 62.04 (–COO–*C*H$_2$–CH$_3$); 57.49 (C-5); 55.71 (–OCH$_3$); 37.78 (C-4); 33.67 (–CO–*C*H$_2$–CH$_2$–CH$_2$–CH$_3$); 26.84 (–CO–CH$_2$–*C*H$_2$–CH$_2$–CH$_3$); 22.46 (–CO–CH$_2$–CH$_2$–*C*H$_2$–CH$_3$); 14.12, 13.85 (–COO–CH$_2$–*C*H$_3$, –CO–CH$_2$–CH$_2$–CH$_2$–*C*H$_3$). HR LSIMS calcd for $C_{19}H_{25}N_3O_5Na$ (M+Na)$^+$ 398.1691, found 398.1692. Anal. for $C_{19}H_{25}N_3O_5$: calcd: C, 60.79; H, 6.71; N, 11.90. Found: C, 60.47; H, 6.86; N, 11.55.

4.1.2.1.5. Ethyl 3-(2-amino-5-methoxybenzoyl)-1-cyclopropanecarbonyl-Δ^2-pyrazoline-5-carboxylate ***24a***. Orange solid; yield 84%; mp 118–120 °C. ^{1}H NMR (300 MHz,

$CDCl_3$) δ 8.02 (d, 1H, H-6′, $J_{6'-4'}$=2.9 Hz); 7.01 (dd, 1H, H-4′, $J_{4'-3'}$=9.0 Hz, $J_{4'-6'}$=2.9 Hz); 6.65 (d, 1H, H-3′, $J_{3'-4'}$=9.0 Hz); 6.03 (bs, 2H, $-NH_2$); 4.90 (dd, 1H, H-5, J_{5-4a}=12.8 Hz, J_{5-4b}=6.5 Hz); 4.22 (m, 2H, $-COO-CH_2-CH_3$); 3.73 (s, 3H, $-OCH_3$); 3.60 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.8 Hz); 3.30 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, $J_{4b,5}$=6.5 Hz); 2.57 (m, 1H, H-$1_{cycloprop.}$); 1.28 (t, 3H, $-COO-CH_2-CH_3$, J=7.1 Hz); 1.16–1.06, 0.92–0.87 (2m, 4H, H-2, H-$3_{cycloprop.}$).^{13}C NMR (75 MHz, $CDCl_3$) δ186.77 (Ph–CO–); 173.20 (–N–CO–); 169.60 ($-COO-CH_2-CH_3$); 153.62 (C-3); 150.07 (C-5′); 146.73 (C-2′); 125.25 (C-4′); 118.56 (C-3′); 116.26 (C-1′); 114.61 (C-6′); 62.01 ($-COO-CH_2-CH_3$); 57.93 (C-5); 55.66 ($-OCH_3$); 37.63 (C-4); 14.13 ($-COO-CH_2-CH_3$); 11.84 (C-$1_{cycloprop.}$); 9.10, 9.01 (C-2, C-$3_{cycloprop.}$). HR LSIMS calcd for $C_{18}H_{21}N_3O_5Na$ $(M+Na)^+$ 382.1378, found 382.1376. Anal. for $C_{18}H_{21}N_3O_5$: calcd: C, 60.16; H, 5.89; N, 11.69. Found: C, 60.51; H, 5.66; N, 12.02.

4.1.2.1.6. Ethyl 3-(2-amino-5-methoxybenzoyl)-1-cyclobutanecarbonyl-Δ²-pyrazoline-5-carboxylate ***25a***. Orange solid; yield 82%; mp 112–114 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97 (d, 1H, H-6′, $J_{6'-4'}$=2.9 Hz); 7.02 (dd, 1H, H-4′, $J_{4'-3'}$=9.0 Hz, $J_{4'-6'}$=2.9 Hz); 6.65 (d, 1H, H-3′, $J_{3'-4'}$=9.0 Hz); 6.03 (bs, 2H, $-NH_2$); 4.89 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.2 Hz); 4.23 (c, 2H, $-COO-CH_2-CH_3$, J=7.1 Hz); 3.82 (m, 1H, H-$1_{cyclobut.}$); 3.80 (s, 3H, $-OCH_3$); 3.56 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}= 12.9 Hz); 3.26 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, $J_{4b,5}$= 6.2 Hz); 2.44–2.13, 2.05–1.88 (2m, 6H, H-2, H-3, H-$4_{cyclobut.}$); 1.29 (t, 3H, $-COO-CH_2-CH_3$, J=7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 186.72 (Ph–CO–); 174.17 (–N–CO–); 169.62 ($-COO-CH_2-CH_3$); 153.55 (C-3); 150.17 (C-5′); 146.93 (C-2′); 125.52 (C-4′); 118.54 (C-3′); 116.34 (C-1′); 114.89 (C-6′); 62.06 ($-COO-CH_2-CH_3$); 57.57 (C-5); 55.85 ($-OCH_3$); 37.64 (C-$1_{cyclobut.}$); 37.56 (C-4); 25.22, 24.61 (C-2, C-$4_{cyclobut.}$); 18.49 (C-$3_{cyclobut.}$); 14.14 ($-COO-CH_2-CH_3$). HR LSIMS calcd for $C_{19}H_{23}N_3O_5Na$ $(M+Na)^+$ 396.1535, found 396.1533. Anal. for $C_{19}H_{23}N_3O_5$: calcd: C, 61.11; H, 6.21; N, 11.25. Found: C, 60.79; H, 6.43; N, 11.30.

4.1.2.1.7. Ethyl 3-(2-amino-5-methoxybenzoyl)-1-cyclopentanecarbonyl-Δ²-pyrazoline-5-carboxylate ***26a***. Orange solid; yield 84%; mp 125–127 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.03 (d, 1H, H-6′, $J_{6'-4'}$=2.9 Hz); 7.02 (dd, 1H, H-4′, $J_{4'-3'}$=9.0 Hz, $J_{4'-6'}$=2.9 Hz); 6.64 (d, 1H, H-3′, $J_{3'-4'}$=9.0 Hz); 6.03 (sa, 2H, $-NH_2$); 4.90 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.2 Hz); 4.21 (m, 2H, $-COO-CH_2-CH_3$); 3.76 (s, 3H, $-OCH_3$); 3.58 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.51 (m, 1H, H-$1_{cyclopent.}$); 3.27 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, $J_{4b,5}$=6.2 Hz); 1.91–1.54 (m, 8H, H-2, H-3, H-4, H-$5_{cyclopent.}$); 1.27 (t, 3H, $-COO-CH_2-CH_3$, J=7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 186.63 (Ph–CO–); 175.69 (–N–CO–); 169.65 ($-COO-CH_2-CH_3$); 153.51 (C-3); 150.14 (C-5′); 146.88 (C-2′); 125.45 (C-4′); 118.62 (C-3′); 116.23 (C-1′); 114.38 (C-6′); 61.99 ($-COO-CH_2-CH_3$); 57.60 (C-5); 55.69 ($-OCH_3$); 42.37 (C-$1_{cyclopent.}$); 37.60 (C-4); 30.26, 29.47 (C-2, C-$5_{cyclopent.}$); 26.17, 26.06 (C-3, C-$4_{cyclopent.}$); 14.13 ($-COO-CH_2-CH_3$). HR LSIMS calcd for: $C_{20}H_{25}N_3O_5Na$ $(M+Na)^+$ 410.1691, found 410.1695. Anal. for $C_{20}H_{25}N_3O_5$: calcd: C, 62.00; H, 6.50; N, 10.85. Found: C, 61.71; H, 6.70; N, 10.86.

4.1.2.1.8. Ethyl 3-(2-amino-5-methoxybenzoyl)-1-cyclohexanecarbonyl-Δ²-pyrazoline-5-carboxylate ***27a***. Orange solid; yield 82%; mp 127–129 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, 1H, H-6′, $J_{6'-4'}$=2.9 Hz); 7.02 (dd, 1H, H-4′, $J_{4'-3'}$=9.0 Hz, $J_{4'-6'}$=2.9 Hz); 6.65 (d, 1H, H-3′, $J_{3'-4'}$=9.0 Hz); 6.07 (bs, 2H, $-NH_2$); 4.89 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.2 Hz); 4.21 (m, 2H, $-COO-CH_2-CH_3$); 3.78 (s, 3H, $-OCH_3$); 3.57 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.26 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, $J_{4b,5}$=6.2 Hz); 3.15 (tt, 1H, H-$1_{cyclohex.}$, $J_{transdiaxial}$=11.6 Hz, J_{cis}=3.5 Hz); 2.02–1.46 (m, 10H, H-2, H-3, H-4, H-5, H-$6_{cyclohex.}$); 1.26 (t, 3H, $-COO-CH_2-CH_3$, J=7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 186.62 (Ph–CO–); 175.61 (–N–CO–); 169.62 ($-COO-CH_2-CH_3$); 153.63 (C-3); 150.18 (C-5′); 146.91 (C-2′); 125.50 (C-4′); 118.60 (C-3′); 116.18 (C-1′); 114.47 (C-6′); 61.97 ($-COO-CH_2-CH_3$); 57.48 (C-5); 55.89 ($-OCH_3$); 41.54 (C-$1_{cyclohex.}$); 37.53 (C-4); 28.98, 28.50 (C-2, C-$6_{cyclohex.}$); 25.83, 25.76, 25.59 (C-3, C-4, C-$5_{cyclohex.}$); 14.13 ($-COO-CH_2-CH_3$). HR LSIMS calcd for $C_{21}H_{27}N_3O_5Na$ $(M+Na)^+$ 424.1848, found 424.1845. Anal. for $C_{21}H_{27}N_3O_5$: calcd: C, 62.83; H, 6.78; N, 10.47. Found: C, 62.46; H, 6.85; N, 10.41.

*4.1.2.1.9. Ethyl 3-(2-amino-5-methoxybenzoyl)-1-benzoyl-Δ²-pyrazoline-5-carboxylate (****28a****).* Orange solid; yield 82%; mp 118–120 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (m, 3H, H-6′, H-$2_{benz.}$, H-$6_{benz.}$); 7.42 (m, 3H, H-$3_{benz.}$, H-$4_{benz.}$, H-$5_{benz.}$); 6.94 (dd, 1H, H-4′, $J_{4'-3'}$= 9.0 Hz, $J_{4'-6'}$=2.9 Hz); 6.61 (d, 1H, H-3′, $J_{3'-4'}$=9.0 Hz); 5.12 (dd, 1H, H-5, J_{5-4a}=12.7 Hz, J_{5-4b}=6.5 Hz); 4.28 (m, 2H, $-COO-CH_2-CH_3$); 3.64 (dd, 1H, H-4a, J_{4a-4b}= 18.7 Hz, J_{4b-5}=12.7 Hz); 3.36 (dd, 1H, H-4b, J_{4b-4a}= 18.7 Hz, J_{4b-5}=6.5 Hz); 3.26 (s, 3H, $-OCH_3$); 1.31 (t, 3H, $-COO-CH_2-CH_3$, J=7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 186.17 (Ph–CO–); 169.54, 168.19 ($-COO-CH_2-CH_3$, –N–CO–); 154.66 (C-3); 150.17 (C-5′); 146.86 (C-2′); 133.20 (C-$1_{benz.}$); 131.55 (C-$4_{benz.}$); 129.72 (C-$2_{benz.}$, C-$6_{benz.}$); 128.01 (C-$3_{benz.}$, C-$5_{benz.}$); 125.73 (C-4′); 118.56 (C-3′); 116.02 (C-1′); 114.04 (C-6′); 62.15 ($-COO-CH_2-CH_3$); 58.53 (C-5); 55.44 ($-OCH_3$); 37.38 (C-4); 14.17 ($-COO-CH_2-CH_3$). HR LSIMS calcd for $C_{21}H_{21}N_3O_5Na$ $(M+Na)^+$ 418.1378, found 418.1377. Anal. for $C_{21}H_{21}N_3O_5$: calcd: C, 63.79; H, 5.35; N, 10.63. Found: C, 63.51; H, 5.49; N, 10.70.

4.1.2.2. General procedure for the preparation of compounds 20b,c–28b,c. To a suspension of the corresponding nitroarene **11b**,**c**–**19b**,**c** (0.524 mmol) in refluxing water was added Fe (0.29 g, 5.24 mmol) and $FeSO_4$ (0.15 g, 0.524 mmol). The reaction mixture was refluxed for 3 h, filtered through Celite, and washed thoroughly with CH_2Cl_2. The aqueous phase was extracted with CH_2Cl_2 (3×15 mL) and EtOAc (3×15 mL). The organic phase was washed with brine, dried (Na_2SO_4), and evaporated. The residue was recrystallized from CH_2Cl_2/hexane.

4.1.2.2.1. Ethyl 1-acetyl-3-(2-amino-5-chlorobenzoyl)-Δ²-pyrazoline-5-carboxylate ***20b***. Orange solid; yield 95%; mp 193–195 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (d, 1H, H-6′, $J_{6'-4'}$=2.4 Hz); 7.25 (dd, 1H, H-4′,

$J_{4'-3'}$=8.8 Hz, $J_{4'-6'}$=2.4 Hz); 6.64 (d, 1H, H-3′, $J_{3'-4'}$=8.8 Hz); 6.28 (bs, 2H, $-NH_2$); 4.91 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}= 6.3 Hz); 4.24 (c, 2H, $-COO-CH_2-CH_3$, J=7.1 Hz); 3.61 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.29 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.3 Hz); 2.40 (s, 3H, $-CO-CH_3$); 1.29 (t, 3H, $-COO-CH_2-CH_3$, J= 7.1 Hz).^{13}C NMR (100 MHz, $CDCl_3$) δ 186.15 (Ph–*C*O–); 170.21, 169.41 (–N–CO–, –*C*OO–CH_2-CH_3); 153.06 (C-3); 150.07 (C-2′); 135.13 (C-4′); 132.64 (C-6′); 120.48 (C-5′); 118.52 (C-3′); 116.91 (C-1′); 62.18 ($-COO-CH_2-CH_3$); 57.59 (C-5); 37.69 (C-4); 21.46 ($-CO-CH_3$); 14.11 ($-COO-CH_2-CH_3$). HR LSIMS calcd for $C_{15}H_{17}ClN_3O_4$ (M^++1) 338.0907, found 338.0906. Anal. for $C_{15}H_{16}ClN_3O_4$: calcd: C, 53.34; H, 4.77; N, 12.44. Found: C, 52.99; H, 4.59; N, 12.15.

4.1.2.2.2. Ethyl 3-(2-amino-5-chlorobenzoyl)-1-propionyl-Δ²-pyrazoline-5-carboxylate ***21b***. Orange solid; yield 95%; mp 148–150 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.46 (d, 1H, H-6′, $J_{6'-4'}$=2.5 Hz); 7.24 (dd, 1H, H-4′, $J_{4'-3'}$=8.9 Hz, $J_{4'-6'}$=2.5 Hz); 6.63 (d, 1H, H-3′, $J_{3'-4'}$= 8.9 Hz); 6.28 (bs, 2H, $-NH_2$); 4.90 (dd, 1H, H-5, J_{5-4a}=12.8 Hz, J_{5-4b}= 6.3 Hz); 4.23 (c, 2H, $-COO-CH_2-CH_3$, J=7.1 Hz); 3.55 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}= 12.8 Hz); 3.25 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}= 6.3 Hz); 2.79 (m, 2H, $-CO-CH_2-CH_3$); 1.28 (t, 3H, $-COO-CH_2-CH_3$, J=7.1 Hz); 1.24 (t, 3H, $-CO-CH_2-CH_3$, J=7.5 Hz).^{13}C NMR (75 MHz, $CDCl_3$) δ 186.27 (Ph–*C*O–); 173.73 (–N–CO–); 169.52 (–*C*OO–CH_2-CH_3); 152.84 (C-3); 150.05 (C-2′); 135.08 (C-4′); 132.69 (C-6′); 120.47 (C-5′); 118.52 (C-3′); 116.98 (C-1′); 62.12 ($-COO-CH_2-CH_3$); 57.72 (C-5); 37.42 (C-4); 27.41 ($-CO-CH_2-CH_3$); 14.12 ($-COO-CH_2-CH_3$); 8.88 ($-CO-CH_2-CH_3$). HR LSIMS calcd for $C_{16}H_{18}ClN_3O_4Na$ $(M+Na)^+$ 374.0883, found 374.0882. Anal. for $C_{16}H_{18}ClN_3O_4$: calcd: C, 54.63; H, 5.16; N, 11.94 Found: C, 54.26; H, 4.76; N, 11.66.

4.1.2.2.3. Ethyl 3-(2-amino-5-chlorobenzoyl)-1-butyryl-Δ²-pyrazoline-5-carboxylate ***22b***. Orange solid; yield 95%; mp 144–146 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (d, 1H, H-6′, $J_{6'-4'}$=2.4 Hz); 7.26 (dd, 1H, H-4′, $J_{4'-3'}$=8.8 Hz, $J_{4'-6'}$=2.4 Hz); 6.65 (d, 1H, H-3′, $J_{3'-4'}$=8.8 Hz); 6.27 (bs, 2H, $-NH_2$); 4.92 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}= 6.2 Hz); 4.24 (c, 2H, $-COO-CH_2-CH_3$, J=7.1 Hz); 3.57 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.27 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.2 Hz); 2.82 (pq, 1H, $-CO-CH_2-CH_2-CH_3$, Ha, J_{gem}=14.9 Hz, J_{Ha-CH2}= 7.3 Hz); 2.69 (pq, 1H, $-CO-CH_2-CH_2-CH_3$, Hb, J_{gem}=14.9 Hz, J_{Hb-CH2}=7.3 Hz); 1.79 (m, 2H, $-CO-CH_2-CH_2-CH_3$); 1.29 (t, 3H, $-COO-CH_2-CH_3$, J= 7.1 Hz); 1.04 (t, 3H, $-CO-CH_2-CH_2-CH_3$, J=7.4 Hz). ^{13}C NMR (75.57 MHz, $CDCl_3$) δ 186.26 (Ph–*C*O–); 173.03 (–N–CO–); 169.48 (–*C*OO–CH_2-CH_3); 152.82 (C-3); 150.04 (C-2′); 135.08 (C-4′); 132.72 (C-6′); 120.50 (C-5′); 118.51 (C-3′); 116.98 (C-1′); 62.11 ($-COO-CH_2-CH_3$); 57.66 (C-5); 37.42 (C-4); 36.02 ($-CO-CH_2-CH_2-CH_3$); 18.66 ($-CO-CH_2-CH_2-CH_3$); 14.11, 13.94 ($-COO-CH_2-CH_3$, $-CO-CH_2-CH_2-CH_3$). HR LSIMS calcd for $C_{17}H_{20}ClN_3O_4Na$ $(M+Na)^+$ 388.1040, found 388.1041. Anal. for $C_{17}H_{20}ClN_3O_4$: calcd: C, 55.82; H, 5.51; N, 11.49 Found: C, 55.47; H, 5.44; N, 11.27.

4.1.2.2.4. Ethyl 3-(2-amino-5-chlorobenzoyl)-1-pentanoyl-Δ²-pyrazoline-5-carboxylate ***23b***. Orange solid; yield 95%; mp 108–110 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (d, 1H, H-6′, $J_{6'-4'}$=2.4 Hz); 7.24 (dd, 1H, H-4′, $J_{4'-3'}$=8.8 Hz, $J_{4'-6'}$=2.4 Hz); 6.64 (d, 1H, H-3′, $J_{3'-4'}$= 8.8 Hz); 6.28 (bs, 2H, $-NH_2$); 4.90 (dd, 1H, H-5, J_{5-4a}= 12.9 Hz, J_{5-4b}=6.3 Hz); 4.23 (c, 2H, $-COO-CH_2-CH_3$, J=7.1 Hz); 3.56 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}= 12.9 Hz); 3.26 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}= 6.3 Hz); 2.83 (pq, 1H, $-CO-CH_2-CH_2-CH_2-CH_3$, Ha, J_{gem}=14.9 Hz, J_{Ha-CH2}=7.7 Hz); 2.70 (pq, 1H, $-CO-CH_2-CH_2-CH_2-CH_3$, Hb, J_{gem}=14.9 Hz, J_{Hb-CH2}= 7.7 Hz); 1.71 (m, 2H, $-CO-CH_2-CH_2-CH_2-CH_3$); 1.45 (m, 2H, $-CO-CH_2-CH_2-CH_2-CH_3$); 1.28 (t, 3H, $-COO-CH_2-CH_3$, J=7.1 Hz); 0.94 (t, 3H, $-CO-CH_2-CH_2-CH_2-CH_3$, J=7.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 186.25 (Ph–*C*O–); 173.18 (–N–CO–); 169.48 (–*C*OO–CH_2-CH_3); 152.83 (C-3); 150.05 (C-2′); 135.08 (C-4′); 132.70 (C-6′); 120.50 (C-5′); 118.52 (C-3′); 116.99 (C-1′); 62.11 ($-COO-CH_2-CH_3$); 57.67 (C-5); 37.44 (C-4); 33.86 ($-CO-CH_2-CH_2-CH_2-CH_3$); 27.17 ($-CO-CH_2-CH_2-CH_2-CH_3$); 22.54 ($-CO-CH_2-CH_2-CH_2-CH_3$); 14.12, 13.84 ($-COO-CH_2-CH_3$, $-CO-CH_2-CH_2-CH_2-CH_3$). HR LSIMS calcd for $C_{18}H_{22}ClN_3O_4Na$ $(M+Na)^+$ 402.1196, found 402.1199. Anal. for $C_{18}H_{22}ClN_3O_4$: calcd: C, 56.92; H, 5.84; N, 11.06. Found: C, 56.52; H, 5.95; N, 10.92.

4.1.2.2.5. Ethyl 3-(2-amino-5-chlorobenzoyl)-1-cyclopropanecarbonyl-Δ²-pyrazoline-5-carboxylate ***24b***. Orange solid; yield 97%; mp 155–157 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50 (d, 1H, H-6′, $J_{6'-4'}$=2.4 Hz); 7.24 (dd, 1H, H-4′, $J_{4'-3'}$=8.8 Hz, $J_{4'-6'}$=2.3 Hz); 6.63 (d, 1H, H-3′, $J_{3'-4'}$=8.8 Hz); 6.28 (bs, 2H, $-NH_2$); 4.91 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.6 Hz); 4.22 (m, 2H, $-COO-CH_2-CH_3$); 3.57 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}= 12.9 Hz); 3.28 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}= 6.6 Hz); 2.57 (m, 1H, H-$1_{cycloprop.}$); 1.28 (t, 3H, $-COO-CH_2-CH_3$, J=7.1 Hz); 1.08, 0.98 (2m, H-2, H-$3_{cycloprop.}$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 186.41 (Ph–*C*O–); 173.43 (–N–CO–); 169.52 (–*C*OO–CH_2-CH_3); 152.96 (C-3); 150.01 (C-2′); 135.03 (C-4′); 132.75 (C-6′); 120.46 (C-5′); 118.50 (C-3′); 117.07 (C-1′); 62.06 ($-COO-CH_2-CH_3$); 58.08 (C-5); 37.32 (C-4); 14.13 ($-COO-CH_2-CH_3$); 11.92 (C-$1_{cycloprop.}$); 9.32, 9.24 (C-2, C-$3_{cycloprop.}$). HR LSIMS: calcd for $C_{17}H_{18}ClN_3O_4Na$ $(M+Na)^+$ 386.0883; found: 386.0885. Anal. for $C_{17}H_{18}ClN_3O_4$: calcd: C, 56.13; H, 4.99; N, 11.55. Found: C, 55.96; H, 4.85; N, 11.44.

4.1.2.2.6. Ethyl 3-(2-amino-5-chlorobenzoyl)-1-cyclobutanecarbonyl-Δ²-pyrazoline-5-carboxylate ***25b***. Orange solid; yield 96%; mp 134–136 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (d, 1H, H-6′, $J_{6'-4'}$=2.5 Hz); 7.26 (dd, 1H, H-4′, $J_{4'-3'}$=8.8 Hz, $J_{4'-6'}$=2.5 Hz); 6.64 (d, 1H, H-3′, $J_{3'-4'}$=8.8 Hz); 6.25 (bs, 2H, $-NH_2$); 4.90 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.2 Hz); 4.24 (m, 2H, $-COO-CH_2-CH_3$); 3.79 (m, 1H, H-$1_{cyclobut.}$); 3.54 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.24 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.2 Hz); 2.47–1.89 (m, 6H, H-2, H-3, H-$4_{cyclobut.}$); 1.30 (t, 3H, $-COO-CH_2-CH_3$, J= 7.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.30 (Ph–*C*O–); 174.43 (–N–CO–); 169.51 (–*C*OO–CH_2-CH_3); 152.69 (C-3); 149.98 (C-2′); 135.01 (C-4′); 132.77 (C-6′); 120.46 (C-5′); 118.48 (C-3′); 117.00 (C-1′); 62.10 ($-COO-CH_2-CH_3$);

57.75 (C-5); 37.87 (C-$1_{cyclobut.}$); 37.16 (C-4); 25.17, 24.41 (C-2, C-$4_{cyclobut.}$); 18.37 (C-$3_{cyclobut.}$); 14.13 (–COO–CH_2–CH_3). HR LSIMS calcd for $C_{18}H_{20}ClN_3O_4Na$ $(M+Na)^+$ 400.1040, found 400.1040. Anal. for $C_{18}H_{20}ClN_3O_4$: calcd: C, 55.52; H, 5.34; N, 11.12. Found: C, 57.11; H, 5.62; N, 10.87.

4.1.2.2.7. Ethyl 3-(2-amino-5-chlorobenzoyl)-1-cyclopentanecarbonyl-Δ^2-pyrazoline-5-carboxylate ***26b***. Orange solid; yield 96%; mp 135–137 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50 (d, 1H, H-6′, $J_{6'-4'}$=2.5 Hz); 7.24 (dd, 1H, H-4′, $J_{4'-3'}$=8.9 Hz, $J_{4'-6'}$=2.5 Hz); 6.63 (d, 1H, H-3′, $J_{3'-4'}$=8.9 Hz); 4.90 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.2 Hz); 4.22 (m, 2H, –COO–CH_2–CH_3); 3.55 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.47 (m, 1H, H-$1_{cyclopent.}$); 3.24 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.2 Hz); 2.11–1.58 (m, 8H, H-2, H-3, H-4, H-$5_{cyclopent.}$); 1.28 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 186.46 (Ph–*C*O–); 175.86 (–N–CO–); 169.54 (–*C*OO–CH_2–CH_3); 152.44 (C-3); 150.00 (C-2′); 135.00 (C-4′); 132.87 (C-6′); 120.57 (C-5′); 118.50 (C-3′); 117.16 (C-1′); 62.05 (–COO–*C*H_2–CH_3); 57.89 (C-5); 42.62 (C-$1_{cyclopent.}$); 37.21 (C-4); 30.11, 29.30 (C-2, C-$5_{cyclopent.}$); 26.26, 26.14 (C-3, C-$4_{cyclopent.}$); 14.13 (–COO–CH_2–*C*H_3). HR LSIMS calcd for $C_{19}H_{22}ClN_3O_4Na$ $(M+Na)^+$ 414.1196, found 414.1194. Anal. for $C_{19}H_{22}ClN_3O_4$: calcd: C, 58.24; H, 5.66; N, 10.72. Found: C, 58.08; H, 5.92; N, 10.70.

4.1.2.2.8. Ethyl 3-(2-amino-5-chlorobenzoyl)-1-cyclohexanecarbonyl-Δ^2-pyrazoline-5-carboxylate ***27b***. Orange solid; yield 95%; mp 135–137 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (d, 1H, H-6′, $J_{6'-4'}$=2.4 Hz); 7.25 (dd, 1H, H-4′, $J_{4'-3'}$=8.8 Hz, $J_{4'-6'}$=2.4 Hz); 6.64 (d, 1H, H-3′, $J_{3'-4'}$=8.8 Hz); 6.26 (bs, 2H, $-NH_2$); 4.88 (dd, 1H, H-5, J_{5-4a}=12.8 Hz, J_{5-4b}=6.1 Hz); 4.21 (m, 2H, –COO–CH_2–CH_3); 3.53 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.8 Hz); 3.24 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.1 Hz); 3.11 (tt, 1H, H-$1_{cyclohex.}$, $J_{transdiaxial}$=11.5 Hz, J_{cis}=3.4 Hz); 2.05–1.19 (m, 10H, H-2, H-3, H-4, H-5, H-$6_{cyclohex.}$); 1.27 (t, 3H, –COO–CH_2–*C*H_3, J=7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 186.20 (Ph–*C*O–); 175.79 (–N–CO–); 169.49 (–*C*OO–CH_2–CH_3); 152.67 (C-3); 150.07 (C-2′); 135.01 (C-4′); 132.79 (C-6′); 120.58 (C-5′); 118.50 (C-3′); 117.14 (C-1′); 62.02 (–COO–*C*H_2–CH_3); 57.75 (C-5); 42.11 (C-$1_{cyclohex.}$); 37.19 (C-4); 28.60, 28.58 (C-2, C-$6_{cyclohex.}$); 25.95, 25.89, 25.68 (C-3, C-4, C-$5_{cyclohex.}$); 14.12 (–COO–CH_2–*C*H_3). HR LSIMS calcd for $C_{20}H_{24}ClN_3O_4Na$ $(M+Na)^+$ 428.1355, found 428.1353. Anal. for $C_{20}H_{24}ClN_3O_4$: calcd: C, 59.18; H, 5.96; N, 10.35. Found: C, 58.80; H, 5.62; N, 10.05.

4.1.2.2.9. Ethyl 3-(2-amino-5-chlorobenzoyl)-1-benzoyl-Δ^2-pyrazoline-5-carboxylate ***28b***. Orange solid; yield 95%; mp 181–183 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.47 (d, 1H, H-6′, $J_{6'-4'}$=2.4 Hz); 7.96 (dd, 2H, H-$2_{benz.}$, H-$6_{benz.}$, $J_{2\text{-}3benz.}$=7.4 Hz, $J_{2\text{-}4benz.}$=1.6 Hz); 7.50 (m, 3H, H-3, H-$4_{benz.}$, H-$5_{benz.}$); 7.20 (dd, 1H, H-4′, $J_{4'-3'}$=8.9 Hz, $J_{4'-6'}$=2.4 Hz); 6.59 (d, 1H, H-3′, $J_{3'-4'}$=8.9 Hz); 6.25 (bs, 2H, $-NH_2$); 5.14 (dd, 1H, H-5, J_{5-4a}=12.8 Hz, J_{5-4b}=6.4 Hz); 4.28 (m, 2H, –COO–CH_2–CH_3); 3.61 (dd, 1H, H-4a, J_{4a-4b}=18.8 Hz, J_{4a-5}=12.8 Hz); 3.32 (dd, 1H, H-4b, J_{4b-4a}=18.8 Hz, J_{4b-5}=6.4 Hz); 1.31 (t, 3H, –COO–CH_2–*C*H_3, J=7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.90 (Ph–*C*O–); 169.48 (–*C*OO–CH_2–CH_3); 167.87 (–N–CO–); 153.95 (C-3); 150.09 (C-2′); 135.11 (C-4′); 133.48 (C-$1_{benz.}$); 132.53 (C-6′); 131.93 (C-$4_{benz.}$); 129.89 (C-2, C-$6_{benz.}$); 128.26 (C-3, C-$5_{benz.}$); 120.54 (C-5′); 118.52 (C-3′); 116.88 (C-1′); 62.22 (–COO–*C*H_2–CH_3); 58.83 (C-5); 36.84 (C-4); 14.18 (–COO–CH_2–*C*H_3). HR LSIMS calcd for $C_{20}H_{18}ClN_3O_4Na$ $(M+Na)^+$ 422.0883, found 422.0881. Anal. for $C_{20}H_{18}ClN_3O_4$: calcd: C, 60.08; H, 4.54; N, 10.51. Found: C, 59.80; H, 4.17; N, 10.46.

4.1.2.2.10. Ethyl 1-acetyl-3-(2-aminobenzoyl)-Δ^2-pyrazoline-5-carboxylate ***20c***. Yellow solid; yield 95%; mp100–102 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (dd, 1H, H-6′, $J_{6'-5'}$=8.2 Hz, $J_{6'-4'}$=1.5 Hz); 7.28 (ddd, 1H, H-4′, $J_{4'-3'}$=8.4 Hz, $J_{4'-5'}$=7.0 Hz, $J_{4'-6'}$=1.5 Hz); 6.71 (d, 1H, H-3′, $J_{3'-4'}$=8.4 Hz); 6.69 (dd, 1H, H-5′, $J_{5'-6'}$=8.2 Hz, $J_{5'-4'}$=7.0 Hz); 4.90 (dd, 1H, H-5, J_{5-4a}=12.8 Hz, J_{5-4b}=6.1 Hz); 4.23 (c, 2H, –COO–*C*H_2–CH_3, J=7.1 Hz); 3.60 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.8 Hz); 3.27 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.1 Hz); 2.40 (s, 3H, –CO–CH_3); 1.28 (t, 3H, –COO–CH_2–*C*H_3, J=7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.53 (Ph–*C*O–); 170.12, 169.51 (–N–CO–, –*C*OO–CH_2–CH_3); 153.38 (C-3); 151.20 (C-2′); 135.25 (C-4′); 133.62 (C-6′); 117.31 (C-3′); 116.92 (C-1′); 116.31 (C-5′); 62.08 (–COO–*C*H_2–CH_3); 57.52 (C-5); 37.90 (C-4); 21.46 (–CO–*C*H_3); 14.12 (–COO–CH_2–*C*H_3). HR LSIMS calcd for $C_{15}H_{17}N_3O_4Na$ $(M+Na)^+$ 326.1116, found 326.1116). Anal. for $C_{15}H_{17}N_3O_4$: calcd: C, 59.40; H, 5.65; N, 13.85. Found: C, 59.77; H, 5.60; N, 14.25.

4.1.2.2.11. Ethyl 3-(2-aminobenzoyl)-1-propionyl-Δ^2-pyrazoline-5-carboxylate ***21c***. Yellow solid; yield 95%; mp132–133 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (dd, 1H, H-6′, $J_{6'-5'}$=8.2 Hz, $J_{6'-4'}$=1.5 Hz); 7.32 (ddd, 1H, H-4′, $J_{4'-3'}$=8.5 Hz, $J_{4'-5'}$=7.0 Hz, $J_{4'-6'}$=1.5 Hz); 6.72 (d, 1H, H-3′, $J_{3'-4'}$=8.5 Hz); 6.70 (ddd, 1H, H-5′, $J_{5'-6'}$=8.2 Hz, $J_{5'-4'}$=7.0 Hz, $J_{5'-3'}$=1.1 Hz); 4.90 (dd, 1H, H-5, J_{5-4a}=12.8 Hz, J_{5-4b}=6.2 Hz); 4.23 (c, 2H, –COO–*C*H_2–CH_3, J=7.1 Hz); 3.58 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.8 Hz); 3.25 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.2 Hz); 2.77 (m, 2H, –CO–*C*H_2–CH_3); 1.28 (t, 3H, –COO–CH_2–*C*H_3, J=7.1 Hz); 1.20 (t, 3H, –CO–CH_2–*C*H_3, J=7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.62 (Ph–*C*O–); 173.60 (–N–CO–), 169.61 (–*C*OO–CH_2–CH_3); 153.14 (C-3); 150.92 (C-2′); 135.19 (C-4′); 133.62 (C-6′); 117.41 (C-3′); 117.13 (C-1′); 116.45 (C-5′); 62.02 (–COO–*C*H_2–CH_3); 57.64 (C-5); 37.59 (C-4); 27.27 (–CO–*C*H_2–CH_3); 14.11 (–COO–CH_2–*C*H_3); 8.75 (–CO–CH_2–*C*H_3). HR LSIMS calcd for $C_{16}H_{19}N_3O_4Na$ $(M+Na)^+$ 340.1273, found 340.1273. Anal. for $C_{16}H_{19}N_3O_4$: calcd: C, 60.56; H, 6.03; N, 13.24. Found: C, 60.26; H, 6.32; N, 13.13.

4.1.2.2.12. Ethyl 3-(2-aminobenzoyl)-1-butyryl-Δ^2-pyrazoline-5-carboxylate ***22c***. Yellow solid; yield 95%; mp 90–92 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (d, 1H, H-6′, $J_{6'-5'}$=8.2 Hz); 7.28 (m, H-4′); 6.67 (d, 1H, H-3′, $J_{3'-4'}$=8.6 Hz); 6.66 (m, H-5′); 6.26 (bs, 2H, $-NH_2$); 4.90 (dd, 1H, H-5, J_{5-4a}=12.8 Hz, J_{5-4b}=6.1 Hz); 4.22 (c, 2H, –COO–*C*H_2–CH_3, J=7.1 Hz); 3.57 (dd, 1H, H-4a, J_{4a-4b}=18.6 Hz, J_{4a-5}=12.8 Hz); 3.25 (dd, 1H, H-4b,

J_{4b-4a}=18.6 Hz, J_{4b-5}=6.1 Hz); 2.78 (pq, 1H, Ha, –CO–CH_2–CH_2–CH_3, J_{gem}=15.0 Hz, J_{Ha-CH2}=7.4 Hz); 2.66 (pq, 1H, Hb, –CO–CH_2–CH_2–CH_3, J_{gem}=15.0 Hz, J_{Hb-CH2}=7.4 Hz); 1.74 (m, 2H, –CO–CH_2–CH_2–CH_3); 1.27 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz); 0.99 (t, 3H, –CO–CH_2–CH_2–CH_3, J=7.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.59 (Ph–*C*O–); 172.85 (–N–CO–), 169.60 (–*C*OO–CH_2–CH_3); 153.15 (C-3); 151.64 (C-2′); 135.20 (C-4′); 133.62 (C-6′); 117.31 (C-3′); 116.65 (C-1′); 115.95 (C-5′); 61.99 (–COO–*C*H_2–CH_3); 57.54 (C-5); 37.63 (C-4); 35.68 (–CO–*C*H_2–CH_2–CH_3); 18.36 (–CO–CH_2–*C*H_2–CH_3) 14.09, 13.83 (–COO–CH_2–*C*H_3, –CO–CH_2–CH_2–*C*H_3). HR LSIMS calcd for $C_{17}H_{21}N_3O_4Na$ $(M+Na)^+$ 354.1429, found 354.1433. Anal. for $C_{17}H_{21}N_3O_4$: calcd: C, 61.62; H, 6.39; N, 12.68. Found: C, 61.24; H, 6.45; N, 12.63.

4.1.2.2.13. Ethyl 3-(2-aminobenzoyl)-1-pentanoyl-Δ²-pyrazoline-5-carboxylate ***23c***. Yellow solid; yield 95%; mp 88–90 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, 1H, H-6′, $J_{6'-5'}$=8.2 Hz); 7.30 (m, H-4′); 6.68 (d, 1H, H-3′, $J_{3'-4'}$=8.4 Hz); 6.66 (pt, 1H, H-5′, $J_{5'-6'}$=8.2 Hz, $J_{5'-4'}$=7.1 Hz); 6.24 (bs, 2H, $-NH_2$); 4.90 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.1 Hz); 4.22 (c, 2H, –COO–CH_2–CH_3, J=7.1 Hz); 3.58 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.25 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.1 Hz); 2.80 (pq, 1H, Ha, –CO–CH_2–CH_2–CH_2–CH_3, J_{gem}=15.0 Hz, J_{Ha-CH2}=7.6 Hz); 2.68 (pq, 1H, Hb, –CO–CH_2–CH_2–CH_2–CH_3, J_{gem}=15.0 Hz, J_{Hb-CH2}=7.6 Hz); 1.70 (m, 2H, –CO–CH_2–CH_2–CH_2–CH_3); 1.40 (m, 2H, –CO–CH_2–CH_2–CH_2–CH_3); 1.28 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz); 0.92 (t, 3H, –CO–CH_2–CH_2–CH_2–*C*H_3, J=7.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.61 (Ph–*C*O–); 173.05 (–N–CO–), 169.60 (–*C*OO–CH_2–CH_3); 153.15 (C-3); 151.63 (C-2′); 135.22 (C-4′); 133.66 (C-6′); 117.11 (C-3′); 116.70 (C-1′); 115.97 (C-5′); 62.01 (–COO–*C*H_2–CH_3); 57.56 (C-5); 37.65 (C-4); 33.62 (–CO–*C*H_2–CH_2–CH_2–CH_3); 27.03 (–CO–CH_2–*C*H_2–CH_2–CH_3) 22.44 (–CO–CH_2–CH_2–*C*H_2–CH_3); 14.11, 13.87 (–COO–CH_2–*C*H_3, –CO–CH_2–CH_2–CH_2–*C*H_3). HR LSIMS calcd for $C_{18}H_{23}N_3O_4Na$ $(M+Na)^+$ 368.1586, found 368.1589. Anal. for $C_{18}H_{23}N_3O_4$: calcd: C, 62.59; H, 6.71; N, 12.17. Found: C, 62.24; H, 6.90; N, 12.13.

4.1.2.2.14. Ethyl 3-(2-aminobenzoyl)-1-cyclopropane-carbonyl-Δ²-pyrazoline-5-carboxylate ***24c***. Yellow solid; yield 95%; mp 88–90 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (dd, 1H, H-6′, $J_{6'-5'}$=8.8 Hz, $J_{6'-4'}$=1.6 Hz); 7.30 (ddd, 1H, H-4′, $J_{4'-3'}$=8.3 Hz, $J_{4'-5'}$=7.0 Hz, $J_{4'-6'}$=1.6 Hz); 6.67 (m, H-5′); 6.24 (bs, 2H, $-NH_2$); 4.90 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.4 Hz); 4.22 (m, 2H, –COO–CH_2–CH_3); 3.60 (dd, 1H, H-4a, J_{4a-4b}=18.6 Hz, J_{4a-5}=12.8 Hz); 3.27 (dd, 1H, H-4b, J_{4b-4a}=18.6 Hz, J_{4b-5}=6.4 Hz); 2.55 (m, 1H, H-$1_{cycloprop.}$); 1.27 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz); 1.15–1.05, 0.96–0.89 (2m, 4H, H-2, H-$3_{cycloprop.}$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.74 (Ph–*C*O–); 173.34 (–N–CO–), 169.63 (–*C*OO–CH_2–CH_3); 153.36 (C-3); 151.58 (C-2′); 135.17 (C-4′); 133.73 (C-6′); 117.08 (C-3′); 116.78 (C-1′); 116.02 (C-5′); 61.97 (–COO–*C*H_2–CH_3); 57.94 (C-5); 37.55 (C-4); 14.12 (–COO–CH_2–*C*H_3); 11.80 (C-$1_{cycloprop.}$); 9.27, 9.21 (C-2, C-$3_{cycloprop.}$). HR LSIMS calcd for $C_{17}H_{19}N_3O_4Na$ $(M+Na)^+$ 352.1269, found 352.1273. Anal. for $C_{17}H_{19}N_3O_4$: calcd: C, 62.00; H, 5.81; N, 12.76. Found: C, 61.81; H, 6.20; N, 12.62.

4.1.2.2.15. Ethyl 3-(2-aminobenzoyl)-1-cyclobutane-carbonyl-Δ²-pyrazoline-5-carboxylate ***25c***. Yellow solid; yield 96%; mp 104–106 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, 1H, H-6′, $J_{6'-5'}$=7.6 Hz); 7.31 (m, 1H, H-4′); 6.68 (d, 1H, H-3′, $J_{3'-4'}$=7.9 Hz); 6.67 (m, 1H, H-5′); 6.22 (bs, 2H, $-NH_2$); 4.89 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.1 Hz); 4.23 (c, 2H, –COO–CH_2–CH_3, J=7.1 Hz); 3.77 (m, 1H, H-$1_{cyclobut.}$); 3.55 (dd, 1H, H-4a, J_{4a-4b}=18.6 Hz, J_{4a-5}=12.9 Hz); 3.23 (dd, 1H, H-4b, J_{4b-4a}=18.6 Hz, J_{4b-5}=6.1 Hz); 2.46–2.16, 2.04–1.85 (m, 6H, H-2, H-3, H-$4_{cyclobut.}$); 1.29 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.72 (Ph–*C*O–); 174.17 (–N–CO–), 169.62 (–*C*OO–CH_2–CH_3); 153.03 (C-3); 151.57 (C-2′); 135.19 (C-4′); 133.75 (C-6′); 117.08 (C-3′); 116.78 (C-1′); 115.93 (C-5′); 62.01 (–COO–*C*H_2–CH_3); 57.66 (C-5); 37.69 (C-$1_{cyclobut.}$); 37.40 (C-4); 25.15, 24.48 (C-2, C-$4_{cyclobut.}$); 18.33 (C-$3_{cyclobut.}$); 14.13 (–COO–CH_2–*C*H_3). HR LSIMS calcd for $C_{18}H_{21}N_3O_4Na$ $(M+Na)^+$ 366.1429, found 366.1430. Anal. for $C_{18}H_{21}N_3O_4$: calcd: C, 62.96; H, 6.16; N, 12.24. Found: C, 62.59; H, 6.19; N, 12.14.

4.1.2.2.16. Ethyl 3-(2-aminobenzoyl)-1-cyclopentane-carbonyl-Δ²-pyrazoline-5-carboxylate ***26c***. Yellow solid; yield 96%; mp 118–120 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (d, 1H, H-6′, $J_{6'-5'}$=8.1 Hz); 7.30 (m, 1H, H-4′); 6.68 (d, 1H, H-3′, $J_{3'4'}$=8.3 Hz); 6.66 (m, 1H, H-5′); 6.22 (bs, 2H, $-NH_2$); 4.90 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.1 Hz); 4.21 (m, 2H, –COO–CH_2–CH_3); 3.57 (dd, 1H, H-4a, J_{4a-4b}=18.6 Hz, J_{4a-5}=12.9 Hz); 3.47 (m, 1H, H-$1_{cyclopent.}$); 3.24 (dd, 1H, H-4b, J_{4b-4a}=18.6 Hz, J_{4b-5}=6.1 Hz); 2.04–1.55 (m, 8H, H-2, H-3, H-4, H-$5_{cyclopent.}$); 1.27 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.72 (Ph–*C*O–); 175.69 (–N–CO–), 169.65 (–*C*OO–CH_2–CH_3); 152.85 (C-3); 151.58 (C-2′); 135.15 (C-4′); 133.69 (C-6′); 117.09 (C-3′); 116.78 (C-1′); 115.93 (C-5′); 61.93 (–COO–*C*H_2–CH_3); 57.71 (C-5); 42.23 (C-$1_{cyclopent.}$); 37.42 (C-4); 30.06, 29.35 (C-2, C-$5_{cyclopent.}$); 26.33, 26.20 (C-3, C-$4_{cyclopent.}$); 14.10 (–COO–CH_2–*C*H_3). HR LSIMS calcd for $C_{19}H_{23}N_3O_4Na$ $(M+Na)^+$ 380.1586, found 380.1585. Anal. for $C_{19}H_{23}N_3O_4$: calcd: C, 63.85; H, 6.49; N, 11.76. Found: C, 63.63; H, 6.58; N, 11.76.

4.1.2.2.17. Ethyl 3-(2-aminobenzoyl)-1-cyclohexane-carbonyl-Δ²-pyrazoline-5-carboxylate ***27c***. Yellow solid; yield 96%; mp 117–119 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (dd, 1H, H-6′, $J_{6'-5'}$=8.2 Hz, $J_{6'-4'}$=1.2 Hz); 7.31 (m, 1H, H-4′); 6.68 (d, 1H, H-3′, $J_{3'4'}$=8.3 Hz); 6.66 (m, 1H, H-5′); 6.24 (bs, 2H, $-NH_2$); 4.89 (dd, 1H, H-5, J_{5-4a}=12.9 Hz, J_{5-4b}=6.1 Hz); 4.21 (m, 2H, –COO–CH_2–CH_3); 3.56 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}=12.9 Hz); 3.24 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}=6.1 Hz); 3.08 (tt, H-$1_{cyclohex.}$, $J_{transdiaxial}$=11.5 Hz, J_{cis}=3.2 Hz); 2.02–1.19 (m, 10H, H-2, H-3, H-4, H-5, H-$6_{cyclohex.}$); 1.26 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.62 (Ph–*C*O–); 175.61 (–N–CO–), 169.72 (–*C*OO–CH_2–CH_3); 152.94 (C-3); 151.60 (C-2′); 135.16 (C-4′); 133.65 (C-6′); 117.12 (C-3′); 116.78 (C-1′); 115.89 (C-5′); 61.94

(–COO–CH_2–CH_3); 57.58 (C-5); 41.86 (C-$1_{cyclohex.}$); 37.40 (C-4); 28.99, 28.52 (C-2, C-$6_{cyclohex.}$); 25.91, 25.76, 25.60 (C-3, C-4, C-$5_{cyclohex.}$); 14.10 (–COO–CH_2–CH_3). HR LSIMS calcd for $C_{20}H_{25}N_3O_4Na$ $(M+Na)^+$ 394.1742, found 394.1739. Anal. for $C_{20}H_{25}N_3O_4$: calcd: C, 64.67; H, 6.78; N, 11.31. Found: C, 64.44; H, 7.06; N, 11.26.

4.1.2.2.18. Ethyl 3-(2-aminobenzoyl)-1-benzoyl-Δ^2-pyrazoline-5-carboxylate ***28c***. Yellow solid; yield 95%; mp 125–127 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 (dd, 1H, H-6′, $J_{6'-5'}$=8.3 Hz, $J_{6'-4'}$=1.2 Hz); 7.95 (dd, 2H, H-2, H-$6_{benz.}$, $J_{2\text{-}3benz.}$=7.1 Hz, $J_{2\text{-}4benz.}$=1.5 Hz); 7.46 (m, 3H, H-4′, H-3, H-$5_{benz.}$); 7.27 (m, 1H, H-$4_{benz.}$); 6.65 (d, 1H, H-3′, $J_{3'4'}$=8.3 Hz); 6.56 (pt, 1H, H-5′, $J_{5'-6'}$=8.3 Hz, $J_{5'-4'}$= 7.0 Hz); 6.24 (sa, 2H, $-NH_2$); 5.13 (dd, 1H, H-5, J_{5-4a}= 12.8 Hz, J_{5-4b}=6.4 Hz); 4.27 (c, 2H, –COO–CH_2–CH_3, J=7.1 Hz); 3.64 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}= 12.8 Hz); 3.32 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}= 6.4 Hz); 1.31 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.34 (Ph–*C*O–); 169.56 (–*C*OO–CH_2–CH_3); 167.67 (–N–CO–); 154.18 (C-3); 151.68 (C-2′); 135.23 (C-4′); 133.70 (C-6′); 132.83 (C-$1_{benz.}$); 131.84 (C-$4_{benz.}$); 130.13 (C-2, C-$6_{benz.}$); 127.89 (C-3, C-$5_{benz.}$); 117.08 (C-3′);116.70 (C-1′); 115.99 (C-5′); 62.11 (–COO–*C*H_2–CH_3); 58.52 (C-5); 37.12 (C-4); 14.17 (–COO–CH_2–*C*H_3). HR LSIMS calcd for $C_{20}H_{19}N_3O_4Na$ $(M+Na)^+$ 388.1273, found 388.1273. Anal. for $C_{20}H_{25}N_3O_4$: calcd: C, 65.74; H, 5.24; N, 11.50. Found: C, 65.41; H, 4.97; N, 11.42.

4.1.2.3. General procedure for the preparation of compounds 29 and 30. A mixture of nitroarene **20a** or **22a** (0.511 mmol) and $SnCl_2$ (2.55 mmol) was dissolved in ethanol and was stirred under reflux for 1 h. The solution was neutralized to pH=7 with $NaHCO_3$, extracted with ethyl acetate (2×15 mL), and dried (Na_2SO_4). Evaporation of the solvent gave a residue which was recrystallized from CH_2Cl_2–hexane.

4.1.2.3.1. Ethyl 1-acetyl-3-(5-methoxybenzo[c]isoxazol-3-yl)-Δ^2-pyrazoline-5-carboxylate ***29***. Orange solid; yield 100%; mp 156–158 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, 1H, H-7′, $J_{7'-6'}$=9.5 Hz); 7.09 (dd, 1H, H-6′, $J_{6'-7'}$= 9.5 Hz, $J_{6'-4'}$=2.2 Hz); 6.98 (d, 1H, H-4′, $J_{4'-6'}$=2.2 Hz); 5.03 (dd, 1H, H-5, J_{5-4a}=12.6 Hz, J_{5-4b}=6.0 Hz); 4.25 (c, 2H, –COO–CH_2–CH_3, J=7.1 Hz); 3.87 (s, 3H, $-OCH_3$); 3.79 (dd, 1H, H-4a, J_{4a-4b}=18.2 Hz, J_{4a-5}=12.6 Hz); 3.51 (dd, 1H, H-4b, J_{4b-4a}=18.2 Hz, J_{4b-5}=6.0 Hz); 2.47 (s, 3H, –CO–CH_3); 1.30 (t, 3H, –COO–CH_2–CH_3, J= 7.1 Hz).^{13}C NMR (75 MHz, $CDCl_3$) δ 169.43, 169.36 (–*C*OO–CH_2–CH_3, –N–CO–); 158.15 (C-5′); 155.57, 154.97 (C-3, C-7′a); 144.35 (C-3′); 128.63 (C-6′); 117.46 (C-3′a); 117.09 (C-7′); 94.81 (C-4′); 62.25 (–COO–*C*H_2–CH_3); 57.35 (C-5); 55.52 ($-OCH_3$); 37.28 (C-4); 21.41 (–CO–*C*H_3); 14.15 (–COO–CH_2–*C*H_3). HR LSIMS calcd for $C_{16}H_{17}N_3O_5Na$ $(M+Na)^+$ 354.1069, found 354.1065. Anal. for $C_{16}H_{17}N_3O_5$: calcd: C, 58.00; H, 5.17; N, 12.68. Found: C, 57.68; H, 5.13; N, 12.49.

4.1.2.3.2. Ethyl 1-butyryl-3-(5-methoxybenzo[c]isoxazol-3-yl)-Δ^2-pyrazoline-5-carboxylate ***30***. Orange solid; yield 100%; mp 144–146 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, 1H, H-7′, $J_{7'-6'}$=9.6 Hz); 7.09 (dd, 1H, H-6′, $J_{6'-7'}$= 9.6 Hz, $J_{6'-4'}$=2.3 Hz); 6.99 (d, 1H, H-4′, $J_{4'-6'}$=2.3 Hz); 5.03 (dd, 1H, H-5, J_{5-4a}=12.6 Hz, J_{5-4b}= 6.0 Hz); 4.24 (c, 2H, –COO–CH_2–CH_3, J= 7.1 Hz); 3.87 (s, 3H, $-OCH_3$); 3.78 (dd, 1H, H-4a, J_{4a-4b}=18.2 Hz, J_{4a-5}=12.6 Hz); 3.50 (dd, 1H, H-4b, J_{4b-4a}=18.2 Hz, J_{4b-5}=6.0 Hz); 2.86 (pq, 1H, –CO–CH_2–CH_2–CH_3, H-a, J_{gem}=14.7 Hz, J_{Ha-CH2}= 7.5 Hz); 2.74 (pq, 1H, –CO–CH_2–CH_2–CH_3, H-b, J_{gem}= 14.7 Hz, J_{Hb-CH2}= 7.5 Hz); 1.79 (m, 2H, –CO–CH_2–CH_2–CH_3); 1.29 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz); 1.03 (t, 3H, –CO–CH_2–CH_2–CH_3, J=7.4 Hz).^{13}C NMR (75 MHz, $CDCl_3$) δ 172.09 (–N–CO–); 169.50 (–*C*OO–CH_2–CH_3); 158.12 (C-5′); 155.55, 154.14 (C-3, C-7′a); 144.11 (C-3′); 128.59 (C-6′); 117.43 (C-3′a); 117.10 (C-7′); 94.85 (C-4′); 62.18 (–COO–*C*H_2–CH_3); 57.44 (C-5); 55.46 ($-OCH_3$); 37.01 (–CO–*C*H_2–CH_2–CH_3); 36.03 (C-4); 18.49 (–CO–CH_2–*C*H_2–CH_3); 14.14, 14.11 (–COO–CH_2–*C*H_3, –CO–CH_2–CH_2–*C*H_3). HR LSIMS calcd for $C_{18}H_{21}N_3O_5Na$ $(M+Na)^+$ 382.1378, found 382.1379. Anal. for $C_{18}H_{21}N_3O_5$: calcd: C, 60.16; H, 5.89; N, 11.69. Found: C, 60.38; H, 6.19; N, 11.35.

4.1.2.4. General procedure for the preparation of compounds 31 and 32. A mixture of nitroarene **21a** or **24a** (0.512 mmol) and 10% Pd/C (60 mg) was dissolved in methanol (30 mL) and stirred under a hydrogen atmosphere (70 psi) for 5 h. The mixture was filtered through celite and evaporated. The resultant residue was dissolved in CH_2Cl_2, and this solution was washed with water, dried (Na_2SO_4), and concentrated. The resultant solid was dissolved and purified by flash chromatography (ethyl acetate–hexane 1:3).

4.1.2.4.1. Ethyl 5-(2-amino-5-methoxybenzoyl)-2-propionylpyrazolidine-3-carboxylate4 ***31***. Orange solid; yield 20%; mp 81–83 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00 (s, 1H, H-6′); 7.02 (dd, 1H, H-4′, $J_{4'-3'}$=8.9 Hz, $J_{4'-6'}$= 2.8 Hz); 6.66 (d, 1H, H-3′, $J_{3'-4'}$=8.9 Hz); 6.06 (bs, 2H, $-NH_2$); 5.33 (d, 1H, H-1, J_{1-5}=11.7 Hz); 4.96 (dd, 1H, H-3, J_{3-4a}=9.2 Hz, J_{3-4b}=6.5 Hz); 4.59 (m, 1H, H-5); 4.17 (m, 2H, –COO–CH_2–CH_3); 3.76 (s, 3H, $-OCH_3$); 2.93 (ddd, 1H, H-4a, J_{4a-4b}=13.0 Hz, J_{4a-3}=9.2 Hz, J_{4a-5}= 8.0 Hz); 2.61 (m, 2H, –CO–CH_2–CH_3); 2.12 (ddd, 1H, H-4b, J_{4b-4a}=13.0 Hz, J_{4b-5}=9.4 Hz, J_{4b-3}=6.5 Hz); 1.24 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz); 1.14 (t, 3H, –CO–CH_2–CH_3, J=7.5 Hz).^{13}C NMR (75 MHz, $CDCl_3$) δ 196.09 (Ph–*C*O–); 175.13 (–N–CO–); 171.33 (–*C*OO–CH_2–CH_3); 150.29 (C-5′); 146.50 (C-2′); 124.91 (C-4′); 119.14 (C-3′); 114.99 (C-1′); 112.45 (C-6′); 62.69 (C-5); 61.69 (–COO–*C*H_2–CH_3); 58.06 (C-3); 56.15 ($-OCH_3$); 37.97 (C-4); 26.95 (–CO–*C*H_2–CH_3); 14.17 (–COO–CH_2–*C*H_3); 9.15 (–CO–CH_2–*C*H_3). HR LSIMS calcd for $C_{17}H_{23}N_3O_5Na$ $(M+Na)^+$ 372.1535, found 372.1534. Anal. for $C_{17}H_{23}N_3O_5$: calcd: C, 58.44; H, 6.64; N, 12.03. Found: C, 58.06; H, 6.35; N, 12.27.

4.1.2.4.2. Ethyl 5-(2-amino-5-methoxybenzoyl)-2-cyclopropanecarbonylpyrazolidine-3-carboxylate ***32***. Orange solid; yield 19%; mp 78–80 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (d, 1H, H-6′, $J_{6'-4'}$=2.8 Hz); 7.02 (dd, 1H, H-4′, $J_{4'-3'}$=9.0 Hz, $J_{4'-6'}$=2.8 Hz); 6.68 (d, 1H, H-3′, $J_{3'-4'}$=9.0 Hz); 6.03 (bs, 2H, $-NH_2$); 5.13 (d, 1H, H-1, J_{1-5}=8.7 Hz); 4.98 (m, 1H, H-5); 4.74 (dd, 1H, H-3, J_{3-4a}= 9.4 Hz, J_{3-4b}=3.6 Hz); 4.23 (c, 2H, –COO–CH_2–CH_3,

J=7.1 Hz); 3.77 (s, 3H, –OCH_3); 2.64 (m, 1H, H-4a); 2.40 (m, 2H, H-4b, H-$1_{cycloprop.}$); 1.29 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz); 1.00–0.66 (m, 4H, H-2, H-$3_{cycloprop.}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.50 (Ph–*C*O–); 174.44 (–N–CO–); 171.96 (–*C*OO–CH_2–CH_3); 150.08 (C-5′); 146.40 (C-2′); 124.36 (C-4′); 118.95 (C-3′); 115.44 (C-1′); 113.40 (C-6′); 61.79 (C-5); 61.53 (–COO–*C*H_2–CH_3); 59.00 (C-3); 56.16 (–OCH_3); 36.89 (C-4); 14.20 (–COO–CH_2–*C*H_3); 11.54 (C-$1_{cycloprop.}$); 8.19, 8.06 (C-2, C-$3_{cycloprop.}$). HR LSIMS: calcd for $C_{18}H_{23}N_3O_5Na$ $(M+Na)^+$ 384.1535, found 384.1539. Anal. for $C_{18}H_{23}N_3O_5$: calcd: C, 59.82; H, 6.41; N, 11.63. Found: C, 59.43; H, 6.05; N, 11.29.

4.1.2.5. Synthesis of ethyl 3-(2-amino-5-methoxy-α-hydroxybencyl)-1-benzoyl-Δ^2-pyrazoline-5-carboxylate 33. A mixture of nitroarene **28a** (0.512 mmol) and 10% Pd/C (80 mg) was dissolved in methanol (30 mL) and stirred under a hydrogen atmosphere (70 psi) for 7.5 h. The mixture was filtered through celite and evaporated. The resultant residue was dissolved in CH_2Cl_2, and this solution was washed with water, dried (Na_2SO_4), and concentrated. The resultant solid was dissolved and purified by flash chromatography (ethyl acetate–hexane 1:2) to give **33**: 87 mg (43% yield); white solid; mp 74–76 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (dd, 2H, H-2, H-$6_{benz.}$, $J_{2\text{-}3benz.}$= 7.1 Hz, $J_{2\text{-}4benz.}$=1.4 Hz); 7.47 (tt, 1H, H-$4_{benz.}$, $J_{4\text{-}3benz.}$= 7.4, $J_{4\text{-}2benz.}$=1.4 Hz); 7.43 (pt, 2H, H-3, H-$5_{benz.}$, $J_{3\text{-}4benz.}$=7.4 Hz, $J_{3\text{-}2benz.}$=7.1 Hz); 6.78 (d, 1H, H-6′, $J_{6'-4'}$=2.9 Hz); 6.73 (dd, 1H, H-4′, $J_{4'-3'}$=8.6 Hz, $J_{4'-6'}$=2.9 Hz); 6.62 (d, 1H, H-3′, $J_{3'-4'}$=8.6 Hz); 5.46 (s, 1H, –C*H*–OH); 5.02 (dd, 1H, H-5, J_{5-4a}=12.2 Hz, J_{5-4b}=6.0 Hz); 4.18 (m, 1H, –COO–CH_2–CH_3); 3.74 (s, 3H, –OCH_3); 3.19 (dd, 1H, H-4a, J_{4a-4b}=18.4 Hz, J_{4a-5}= 12.2 Hz); 2.81 (dd, 1H, H-4b, J_{4b-4a}=18.4 Hz, J_{4b-5}= 6.0 Hz); 1.22 (t, 3H, –COO–CH_2–CH_3, J=7.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.84 (–*C*OO–CH_2–CH_3); 166.99 (–N–CO–); 159.59 (C-3); 153.11 (C-5′); 138.09 (C-2′); 133.48 (C-$1_{benz.}$); 131.33 (C-$4_{benz.}$); 129.76 (C-2, C-$6_{benz.}$); 127.84 (C-3, C-$5_{benz.}$); 124.65 (C-1′); 118.83 (C-3′); 115.02 (C-4′); 113.43 (C-6′); 70.49 (–CH–OH); 61.88 (–COO–*C*H_2–CH_3); 59.10 (C-5); 55.84 (–OCH_3); 36.11 (C-4); 14.05 (–COO–CH_2–*C*H_3). HR LSIMS: calcd for $C_{21}H_{23}N_3O_5Na$ $(M+Na)^+$ 420.1535, found 420.1529. Anal. for $C_{21}H_{23}N_3O_5$: calcd: C, 63.46; H, 5.83; N, 10.57. Found: C, 63.42; H, 6.17; N, 10.35.

4.1.2.6. General procedure for the preparation of compounds 34 and 35. An aqueous solution of Na_2CO_3 (2 M, 1.5 mL) was added with stirring to a suspension of the corresponding ester **20a** or **27a** (0.548 mmol) at 65 °C for 6 h. The reaction mixture was then cooled at room temperature and neutralized with resin Amberlite IR-120 [H^+], stirred carefully for 20 min, next a solution of NH_4OH (18%, 10 mL) was added and stirred for 20 min, the mixture was filtered and the filtrate was concentrated to dryness. The resulting residue was purified by flash chromatography (acetone–methanol 10:1).

*4.1.2.6.1. 1-Acetyl-3-(2-amino-5-methoxybenzoyl)-Δ^2-pyrazoline-5-carboxylic acid **34**.* Orange solid; yield 40%; mp 260–262 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 8.06 (d, 1H, H-6′, $J_{6'-4'}$=3.0 Hz); 6.98 (dd, 1H, H-4′, $J_{4'-3'}$=9.1 Hz, $J_{4'-6'}$=3.0 Hz); 6.74 (d, 1H, H-3′, $J_{3'-4'}$=9.1 Hz); 4.73 (dd, 1H, H-5, J_{5-4a}=12.6 Hz, J_{5-4b}=5.8 Hz); 3.75 (s, 3H, –OCH_3); 3.58 (dd, 1H, H-4a, J_{4a-4b}=18.7 Hz, J_{4a-5}= 12.6 Hz); 3.23 (dd, 1H, H-4b, J_{4b-4a}=18.7 Hz, J_{4b-5}= 5.8 Hz); 2.38 (s, 3H, –CO–CH_3). ^{13}C NMR (75 MHz, CD_3OD) δ 188.39 (Ph–*C*O–); 176.99 (–COOH); 172.11 (–N–CO–); 156.57 (C-3); 150.98 (C-5′); 148.90 (C-2′); 126.16 (C-4′); 119.48 (C-3′); 117.05 (C-1′); 115.46 (C-6′); 61.40 (C-5); 56.23 (–OCH_3); 39.86 (C-4); 21.78 (–CO–*C*H_3). HR LSIMS calcd for $C_{14}H_{14}DN_3O_5Na$ $(M+Na)^+$ 329.0970, found 329.0972. Anal. for $C_{14}H_{15}N_3O_5$: calcd: C, 55.08; H, 4.95; N, 13.76. Found: C, 54.72; H, 4.55; N, 13.36.

*4.1.2.6.2. 3-(2-Amino-5-methoxybenzoyl)-1-cyclohexanecarbonyl-Δ^2-pyrazoline-5-carboxylic acid **35**.* Orange solid; yield 41%; mp 276–278 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 8.03 (d, 1H, H-6′, $J_{6'-4'}$=2.9 Hz); 7.00 Hz (dd, 1H, H-4′, $J_{4'-3'}$=9.0 Hz, $J_{4'-6'}$=2.9 Hz); 6.74 (d, 1H, H-3′, $J_{3'-4'}$=9.0 Hz); 4.71 (dd, 1H, H-5, J_{5-4a}= 12.6 Hz, J_{5-4b}= 5.9 Hz); 3.78 (s, 3H, –OCH_3); 3.57 (dd, 1H, H-4a, J_{4a-4b}= 18.6 Hz, J_{4a-5}=12.6 Hz); 3.30 (m, 1H, H-$1_{cyclohex.}$); 3.21 (dd, 1H, H-4b, J_{4b-4a}=18.6 Hz, J_{4b-5}= 5.9 Hz); 2.05–1.73 (m, 5H, $H_{ec.\ cyclohex.}$); 1.54–1.27 (m, 5H, $H_{ax.\ cyclohex.}$). ^{13}C NMR (75 MHz, CD_3OD) δ 188.72 (Ph–*C*O–); 177.36 (–N–CO–); 176.99 (–COOH); 156.24 (C-3); 151.04 (C-5′); 148.78 (C-2′); 126.01 (C-4′); 119.45 (C-3′); 117.28 (C-1′); 115.46 (C-6′); 61.56 (C-5); 56.49 (–OCH_3); 42.98 (C-$1_{cyclohex.}$); 39.33 (C-4); 30.22, 29.63 (C-2, C-$6_{cyclohex.}$); 27.06, 26.92, 26.82 (C-3, C-4, C-$5_{cyclohex.}$). HR LSIMS: calcd for $C_{19}H_{23}N_3O_5Na$ $(M+Na)^+$ 396.1529, found 396.1535. Anal. for $C_{19}H_{23}N_3O_5$: calcd: C, 61.11; H, 6.21; N, 11.25. Found: C, 60.81; H, 5.95; N, 10.87.

4.2. Biological activity

4.2.1. Striatal nNOS activity determination. L-Arginine, L-citrulline, *N*-(2-hydroxymethyl)piperazine-*N*-(2-hydroxypropanesulfonic acid) (HEPES), DL-dithiothreitol (DTT), leupeptin, aprotinin, pepstatin, phenylmethylsulfonylfluoride (PMSF), hypoxantine-9-β-D-ribofuranosid (inosine), ethylene-glycol-bis-(β-aminoethyl ether)-*N*,*N*,*N*,*N*,-tetraacetic acid (EGTA), bovine serum albumin (BSA), Dowex-50 W (50x8-200), FAD, NADPH and 5,6,7,8-tetrahydro-L-biopterin dihydrocloride (H_4-biopterin) were obtained from Sigma Química (Spain). L-[^{3}H]-arginine (58 Ci/mmol) was obtained from Amersham (Amersham, Bucks, UK). Tris (hydroxymethyl)-aminomethane (Tris–HCl) and calcium chloride were obtained from Merck (Spain).

The rats were killed by cervical dislocation, and the striata were quickly collected and immediately used to measure NOS activity. Upon removal, the tissues were cooled in ice-cold homogenizing buffer (25 mM Tris, 0.5 mM DTT, 10 μg/mL leupeptin, 10 μg/mL pepstatine, 10 μg/mL aprotinine, 1 mM PMSF, pH 7.6). Two striata were placed in 1.25 mL of the same buffer and homogenized in a Polytron (10 s×6). The crude homogenate was centrifuged for 5 min at 1000*g*, and aliquots of the supernatant were either stored at −20 °C for total protein determination[33] or used immediately to measure NOS activity. The nNOS activity was measured by the Bredt and Snyder[32] method, monitoring the conversion of L-[^{3}H]-arginine to

L-[^{3}H]-citrulline. The final incubation volume was 100 μL and consisted of 10 μL crude homogenate added to a buffer to give a final concentration of 25 mM Tris, 1 mM DTT, 30 μM H_4-biopterin, 10 μM FAD, 0.5 mM inosine, 0.5 mg/mL BSA, 0.1 mM $CaCl_2$, 10 μM L-arginine, and 50 nM L-[^{3}H]-arginine, at pH 7.6. The reaction was started by the addition of 10 μL of NADPH (0.75 mM final) and continued for 30 min at 37 °C. Control incubations were performed by the omision of NADPH. The reaction was halted by the addition of 400 μL of cold 0.1 M Hepes, 10 mM EGTA, and 0.175 mg/mL L-citrulline, pH 5.5. The reaction mixture was decanted into a 2 mL column packet with Dowex-50 W ion-exchange resin (Na^+ form) and eluted with 1.2 mL of water. L-[^{3}H]-Citrulline was quantified by liquid scintillation spectroscopy. The retention of L-[^{3}H]-arginine in this process was greater than 98%. Specific enzyme activity was determined by subtracting the control value, which usually amounted to less than 1% of the radioactivity added. The nNOS activity was expressed as picomoles of L-[^{3}H]-citruline produced (mg of protein)$^{-1}$ min^{-1}.

4.2.2. Cerebral iNOS activity determination. The induction of the enzyme was achieved by intravenous inyection of lipopolysacharide (LPS) 10 mg/kg. Six hours after the injection, the rat brains were removed and homogenized in homogenizing buffer (25 mM Tris–HCl, 0.5 mM DTT, 10 μg/mL leupeptin, 10 μg/mL pepstatin, 10 μg/mL aprotinin, 1 mM PMSF, pH 7.6) cold (4 °C) for 0.05 mg tissue/mL buffer.

Once the homogenizing of the tissue has been obtained, it was incubated in the presence of EGTA 10 mM to eliminate the existing nNOS activity. The rest of the process followed to measure the iNOS activity is the same as the one described to measure the striatal nNOS activity.

Acknowledgements

We thank the Spanish Comisión Interministerial de Ciencia y Tecnología (SAF2002-01688) and from the Fondo de Investigación Sanitaria (PI021181) for financial support of this work. The award of a grant from the Junta de Andalucía to M.D.C.P. is gratefully acknowledged.

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Tetrahedron 60 (2004) 4071–4078

Synthesis of donor–acceptor substituted oligothiophenes by Stille coupling

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Received 13 June 2003; revised 4 November 2003; accepted 2 March 2004

Abstract—A synthesis of donor–acceptor-substituted oligothiophenes by Stille coupling is described. The 5′-estanyl derivatives, readily prepared from 5-alkoxy- and 5-amino-2,2′-bithiophenes **7** were coupled with the appropriate aryl or heteroaryl bromides to give the title compounds.

1. Introduction

The interest in future photonic devices such as frequency converters, light modulators and optical switches has led to the development of a variety of organic non-linear optical (NLO) chromophores.[1–3]

In the last few years, thiophene containing donor–acceptor substituted π systems have been extensively investigated.[4–19]

These novel push–pull systems exibit enhanced second-order polarizabilities β compared to biphenyls or stilbenes.[14,16] Donor–acceptor substituted oligothiophenes represent promising candidates for NLO applications.[1–4,13,17,20]

The synthesis of donor–acceptor oligothiophenes may be achieved by several methods such as cross-coupling reactions; Stille,[14–17,21–24] Suzuki,[25] or others[4,6,8,26–28] and by procedures involving thiophene ring formations.[19,29,30]

Recently we have developed an efficient method for the synthesis of 5-amino- and 5-alkoxy-2,2′-bithiophenes.[31] These compounds have proved to be versatile substrates in formylation, dicyanovinylation and tricyanovinylation reactions, permitting the preparation of several new donor–acceptor substituted bithiophenes.[32]

As part of our continuing interest in non-linear optical material[32–36] we report here the use of the readily available 5-amino- and 5-alkoxy-2,2′-bithiophenes in the Stille cross-coupling reaction with phenyl, thienyl and bithienyl bromides to obtain new donor–acceptor substituted oligothiophenes.

The Stille coupling was chosen because it is one of the most versatile methods for C–C bond formation for several reasons: (i) the organostannanes are readily prepared, purified and stored; (ii) the Stille conditions tolerate a wide variety of functional groups (e.g. CO_2R, CN, OH, CHO, NO_2); (iii) the reaction can be performed under mild conditions and (iv) in contrast to the Suzuki reaction, the Stille coupling can be run under neutral conditions.[37,38]

2. Results and discussion

2.1. Synthesis

A series of chromophores was synthesized with either alcoxy- or *N*,*N*-dialkylamino- donors and formyl, nitro and dicyanovinyl acceptors across a conjugated π-bridge containing a bithiophene-benzene, terthiophene or tetrathiophene moiety.

The bithiophenes **9d**, **10a–d**, **11d**, the terthiophenes **12b**, **13–15d** and the quaterthiophene **16d** were synthesized by $Pd(PPh_3)_4$ catalyzed cross coupling reactions of (tributylstannyl)bithiophenes **8a–d** with the acceptor-substituted bromo-aryl or heteroaryl compounds **1a–b**,**d**, **2a–c** and **6**.

The aryl, thienyl and the bithienyl bromides used were activated by electron withdrawing substituents such as

Keywords: 5-Alkoxy- and 5-amino-2,2′-bithiophenes; Stille coupling; Donor–acceptor oligothiophenes; UV–visible spectroscopy; Chromophores; Solvatochromism; Non-linear optical (NLO) material; NLO applications.
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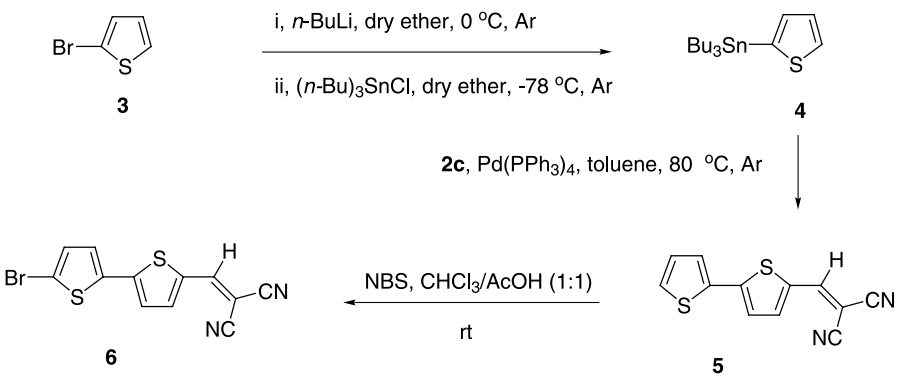
doi:10.1016/j.tet.2004.03.022

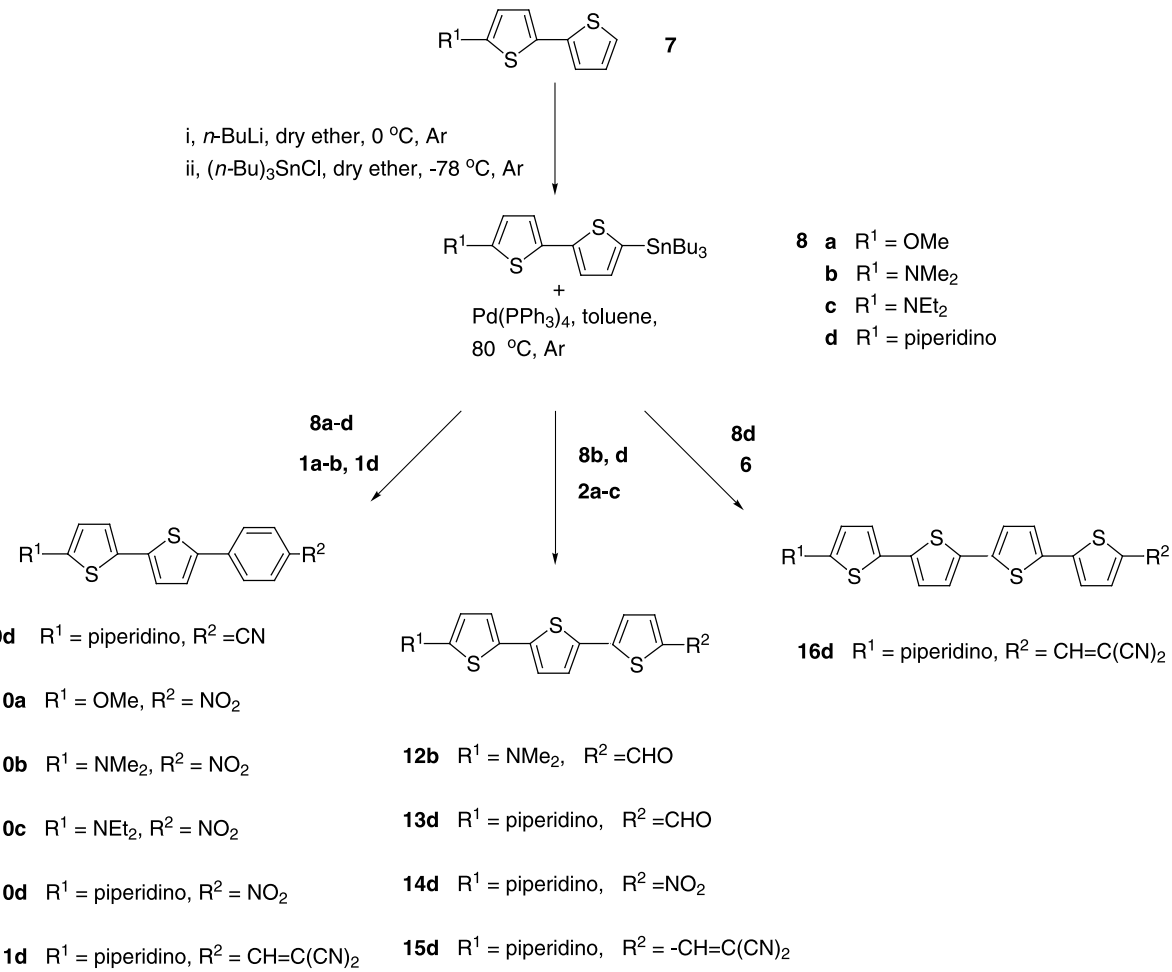


Scheme 1.

formyl, nitro and dicyanovinyl. The bromo derivatives 1-bromo-4-cyanobenzene **1a**, 1-bromo-4-nitrobenzene **1b**, 4-bromo-1-formylbenzene **1c**, 5-bromo-2-formylthiophene **2a** and 5-bromo-2-nitrothiophene **2b** were commercially available. The synthesis of the other bromo derivatives was achieved by several methods. Knoevenagel condensation[39] of the commercial available 4-bromo-1-formylbenzene **1c** and 5-bromo-2-formylthiophene **2a** with malononitrile in refluxing ethanol gave the corresponding dicyanovinyl derivatives 4-bromo-1-dicyanovinylbenzene **1d** and 5-bromo-2-dicyanovinylthiophene **2c** in 87 and 91% yield, respectively.

5′-Bromo-5-dicyanovinyl-2,2′-bithiophene **6** was obtained from 5-dicyanovinyl-2,2′-bithiophene **5** by bromination with NBS in a solution of chloroform–acetic acid (1:1) in 85% yield. Compound **5** was obtained in 55% yield, by Stille coupling of (tributhylstannyl)thiophene **4**[40] under $Pd(PPh_3)_4$ catalysis at 80 °C in toluene. Compound **4** was synthesized from the commercially available 2-bromothiophene **3** in quantitative yield, by lithiation, using *n*-BuLi at 0 °C, followed by transmetalation with tributyltin chloride at −78 °C (Scheme 1).

The bromo derivatives **1d**, **2c** and **6** described earlier were



Scheme 2.

synthesized in order to be coupled under Stille conditions with the stannane bithiophenes **8a–d**.

The synthesis of bithienylstannanes **8a–d** was achieved by metalation of 5-alkoxy- and 5-*N*,*N*-dialkylamino-2,2′-bithiophenes **7a–d**, using *n*-BuLi at 0 °C followed by transmetalation with tributyltin chloride at −78 °C (Scheme 2). The organotin compounds **8a–d** were obtained in good yields (81–90%) and were used in the Stille coupling reactions without further purification.

The Stille reactions were performed in toluene under an argon atmosphere and $Pd(PPh_3)_4$ (2 mol%) was used as palladium catalyst at 80 °C for 8–33.5 h (Scheme 2).

The donor–acceptor oligothiophenes were obtained in moderate to good yields 42–65% (Table 1). Better yields were obtained when more activated aryl or thienyl bromides were used in the Stille couplings. Therefore, bithiophene **10a** was synthesized in 65% yield (Table 1, entry 2) and terthiophene **15d** was obtained in 55% yield (Table 1, entry 10).

The influence of the activation of the aryl or heteroaryl bromides on the yield of the Stille coupling is demonstrated by comparison of the yield of **9d** (43%) (Table 1, entry 1) with the yield of **11d** (56%) (Table 1, entry 6). A better yield was obtained for compound **11d** due to the activation of the bromide **1d** by the dicyanovinyl group.

Waite[41] et al. reported the study of the polarizability and hyperpolarizability of terthiophene **12b** but no analytical data was described for this compound.

2.2. UV–visible study of oligothiophenes

Electronic absorption spectra of all the push–pull compounds **9–16** show an intense lowest energy charge-transfer absorption band in the UV–vis region. The position of this band is strongly influenced by the structure of the compounds, for example by the type of π bridge and the substitution pattern in the donor and acceptor moieties[19] (Table 1).

The influence of the strength of the acceptor group is demonstrated by comparison of the absorption maxima of compounds **13d** and **15d** as the longest wavelength transition is shifted from 456.0 nm in piperidino-T_3-CHO **13d** (Table 1, entry 8) to 545.5 nm in piperidino-T_3-[CH=C(CN)$_2$] **15d** (Table 1, entry 10). The influence of the strength of the donor group is demonstrated by comparison of the absorption maxima of compounds **10a** and **10c** as the longest wavelength transition is shifted from 413.0 nm in methoxy-T_2-4-NO_2-Ph **10a** (Table 1, entry 2) to 474.5 nm in *N*,*N*-diethyl-T_2-4-NO_2-Ph **10c** (Table 1, entry 4).

In general, the stronger the donor and/or acceptor group, the smaller the energy difference between ground and excited states, and the longer the wavelength of absorption.[14] According to Zyss[1] the increase of the β values characteristic of the NLO effects is accompanied by an increase of the λ_{max} in the UV–vis spectra.

Comparison of the electronic absorption spectra of piperidino-T_2-4-NO_2-Ph **10d** (Table 1, entry 5) (λ_{max}=453.0 nm) with piperidino-T_3-NO_2 **14d** (Table 1, entry 9) (λ_{max}=504.0 nm) reveals that the replacement of a benzene ring with a thiophene ring causes a dramatic red shift of the charge-transfer band. This observation clearly indicates that the incorporation of thiophene moieties in push–pull compounds enhances their charge-transfer properties.[4,8,13,20]

2.3. Solvatochromic behavior of oligothiophenes

Solvatochromism is easily quantified by UV–vis spectroscopy and is particularly suitable for the empirical determination of the polarity of a solvent[42,43] on a molecular-microscopic level. To evaluate the intermolecular forces between the solvents and the solute molecules we have measured absorption spectra of six oligothiophenes in 14 solvents of different solvatation character.

The maxima of the wavenumbers ν_{max} for compounds **10d**, **11d**, **12b**, **13d**, **15d** and **16d**, as well as the corresponding wavelength λ are listed in Table 2 and compared with the π^* determined by Kamlet and Taft.

The highest energy transitions are found with non-polar solvents such as hexane and cyclohexane. More polar solvents such as DMF resulted in lower energy transitions. This behavior has been defined as a positive solvatochromic response (between $\Delta\nu$=1333 cm^{-1} for **16d** and $\Delta\nu$= 3758 cm^{-1} for **11d**) that is related to a greater stabilization of the excited state relative to the ground state with increasing polarity of the solvent.

Table 1. Yields and UV–vis absorption spectra of the coupled donor–acceptor oligothiophenes **9–16**

Entry	Bromide	Bithienyl stannane	Product	Yield (%)	Reaction time [h]	λ_{max}[a] [nm] (ε)
1	**1a**	**8d**	Piperidino-T_2-4-CN-Ph **9d**	43	19	420.0 (18,660)
2	**1b**	**8a**	Methoxy-T_2-4-NO_2-Ph **10a**	65	10.5	413.0 (25,750)
3	**1b**	**8b**	*N*,*N*-Dimethyl-T_2-4-NO_2-Ph **10b**	42	19	461.0 (10,050)
4	**1b**	**8c**	*N*,*N*-Diethyl-T_2-4-NO_2-Ph **10c**	44	24.5	474.5 (16,800)
5	**1b**	**8d**	Piperidino-T_2-4-NO_2-Ph **10d**	53	8	453.0 (10,000)
6	**1d**	**8d**	Piperidino-T_2-4-[CH=C(CN)$_2$]-Ph **11d**	56	30	468.0 (21,400)
7	**2a**	**8b**	*N*,*N*-Dimethyl-T_3-CHO **12b**	46	20	465.5 (22,690)
8	**2a**	**8d**	Piperidino-T_3-CHO **13d**	51	17	456.0 (15,260)
9	**2b**	**8d**	Piperidino-T_3-NO_2 **14d**	53	33	504.0 (10,100)
10	**2c**	**8d**	Piperidino-T_3-[CH=C(CN)$_2$] **15d**	55	33.5	545.5 (23,770)
11	**6**	**8d**	Piperidino-T_4-[CH=C(CN)$_2$] **16d**	45	30	510.5 (12,000)

[a] All the UV/vis spectra were run in ethanol.

Table 2. UV–vis absorption maxima of bithiophenes **10d**, **11d**, terthiophenes **12b**, **13d**, **15d** and quaterthiophene **16d** in various solvents in comparison with π^* values by Kamlet and Taft[44]

Solvents	π^*	**10d**		**11d**		**12b**		**13d**		**15d**		**16d**	
		λ_{max} [nm]	ν_{max} [cm^{-1}]	λ_{max} [nm]	ν_{max} [cm^{-1}]	λ_{max} [nm]	ν_{max} [cm^{-1}]	λ_{max} [nm]	ν_{max} [cm^{-1}]	λ_{max} [nm]	ν_{max} [cm^{-1}]	λ_{max} [nm]	ν_{max} [cm^{-1}]
n-Hexane	−0.08	441.5	22,650	437.5	22,857	443.0	22,573	437.5	22,857	532.5	18,779	—	
Cyclohexane	0.00	446.5	22,396	443.0	22,573	448.0	22,321	443.0	22,573	539.0	18,552	—	
Diethyl ether	0.27	448.5	22,296	474.0	21,097	452.0	22,123	444.0	22,522	538.0	18,587	502.0	19,920
Dioxane	0.55	455.0	22,471	495.5	20,181	458.0	21,834	453.0	22,075	539.5	18,535	520.0	19,230
Ethyl acetate	0.55	454.0	22,026	491.5	20,345	457.0	21,881	450.0	22,222	538.5	18,570	513.0	19,493
Tetrahydrofuran	0.58	459.5	21,762	499.0	20,040	461.5	21,668	454.5	22,002	548.0	18,248	521.0	19,193
Acetone	0.71	458.5	21,810	493.5	20,263	461.5	21,668	454.5	22,002	544.5	18,365	515.0	19,417
Acetonitrile	0.75	457.0	21,881	488.5	20,470	462.5	21,621	453.0	22,075	542.0	18,450	503.0	19,880
Dimethylformamide	0.88	470.5	21,253	499.5	20,020	471.0	21,231	463.0	21,598	555.5	18,001	526.5	18,993
Ethanol	0.54	453.0	22,075	468.0	21,367	465.5	21,482	456.0	21,929	545.5	18,331	510.5	19,588
Methanol	0.60	450.0	22,222	468.5	21,344	464.5	21,528	454.5	22,002	539.0	18,552	504.5	19,821
Chloroform	0.58/0.76[45]	457.0	21,881	523.5	19,102	470.5	21,253	468.0	21,367	568.5	17,590	538.0	18,587
Dichloromethane	0.82	467.5	21,390	515.0	19,417	469.0	21,321	462.5	21,621	562.5	17,777	528.0	18,939
Toluene	0.54	459.5	21,762	511.0	19,569	462.0	21,645	454.0	22,026	552.5	18,099	533.0	18,761

Table 3. Correlation of UV–vis absorption maxima of bithiophenes **10d**, **11d**, terthiophenes **12b**, **13d**, **15d** and quaterthiophene **16d** and solvent parameter π^*[a]

Compounds	ν_0 [cm^{-1}]	Regression analysis s[b] [cm^{-1}]	r[b]
10d	22,588	−1120	−0.8084
11d	22,376	−3297	−0.8495
12d	22,415	−1294	−0.9410
13d	22,730	−1252	−0.9037
15d	18,698	−1003	−0.8869
16d[c]	20,019	−1002	−0.9150

[a] Applied solvents (π^* value): *n*-hexane (−0.08), cyclohexane (0.00), diethyl ether (0.27), dioxane (0.55), ethyl acetate (0.55), tetrahydrofuran (0.58), acetone (0.71), acetonitrile (0.75), dimethylformamide (0.88), ethanol (0.54), methanol (0.60), chloroform (0.76), dichloromethane (0.82), toluene (0.54).

[b] Intercept, slope, and correlation coefficient r of the linear solvatation energy relationship.

[c] Without *n*-hexane and cyclohexane.

Because of the pronounced solvatochromism, the good correlation with π^* values for the 14 solvents investigated (r=0.8495) and the long wavelength absorption in the visible range, **11d** seemed to be a very appropriate solvent polarity indicating dye (Table 3). The change in dipole moment on electronic excitation was shown to be oriented parallel to the transition dipole and is moreover constant over the whole charge transfer band.

The great number of aliphatic and dipolar aprotic solvents was chosen to determine the correlation behavior of ν_{max} (**11d**) and π^* because specific interactions were not expected. In fact a good correlation between absorption

Figure 1. Correlation between absorption wavenumbers ν_{max} (**11d**) and the π^* scale according to Kamlet and Taft. Aliphatic and dipolar aprotic solvents (◆), protic solvents (□), chlorinated solvents (△) and aromatic solvents (○).

wavenumbers of **11d** and π^* values (r=0.9431) of the corresponding solvents was obtained (Table 2).

However, as shown in Figure 1, the alcohols, aromatic and chlorinated solvents slightly deviate from this regression line. The behavior in chlorinated and aromatic solvents, which display the lowest energy transitions is noteworthy. Similar behavior has been observed for donor–acceptor molecules of oligothiophenes where the trend was rationalized as a consequence of an intramolecular charge transfer.[14]

The oligothiophene derivatives **9–16** were completely characterized by HRMS, ^{1}H spectroscopy and by IR and UV–vis spectroscopy.

The study of the thermal stability, the electrochemical and the non-linear optical properties of the new oligothiophenes are under way.

3. Experimental

3.1. General

^{1}H NMR spectra were obtained on a Varian Unity Plus Spectrometer at 300 MHz using the solvent peak as internal reference. The solvents are indicated in parenthesis before the chemical shift values (δ relative to TMS). Mp were determined on a Gallenkamp apparatus and are uncorrected. Infrared spectra were recorded on a Perkin–Elmer 1600 FTIR spectrophotometer. UV–vis spectra were recorded with a Shimadzu UV/2501/PC spectrophotometer using several solvents analytically pure (Merck). EI mass spectra EI (70 eV) and HRMS were run on a Unicam GC–MS 120. Elemental analysis was carried out on a Leco CHNS-932. Column chromatography was performed on Merck silica gel 60 (Art 9385). Light petroleum refers to solvent boiling in the range 40–60 °C.

All reactions were carried out under an argon atmosphere in dry glassware.

The phenyl and thienyl bromides **1a–c**, **2a–b** and **3** were purchased from Aldrich and used as received.

The synthesis of bithiophenes **7a–d** has been described elsewhere.[31]

3.2. General procedure for the synthesis of dicyanovinyl derivatives 1d and 2c from the corresponding formyl compounds 1c and 2a by Knoevenagel condensation

To a solution of malononitrile (1.2 g, 18 mmol) and the formyl derivatives **1c** and **2a** (15 mmol) in ethanol (50 ml) was added piperidine (1 drop). The solution was heated at reflux for 1 h, then cooled and the solvent was removed under reduced pressure to give the crude dicyanovinyl compounds. The resulting solids were recrystallized to give the title compounds **1d** and **2c**.

3.2.1. 4-Bromo-1-dicyanovinylbenzene 1d. Beige solid (87%). Mp: 160.5–161.6 °C. (ether/n-hexane). IR (nujol) ν 2224 (CN) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.70 (d, 2H, J=8.4 Hz, 2×Ar-H), 7.73 (s, 1H, CH=C(CN)$_2$), 7.78 (d, 2H, J=8.4 Hz, 2×Ar-H). Anal. calcd for $C_{10}H_5BrN_2$: C, 51.52; H; 2.15; N, 12.02. Found C, 51.34; H, 2.46; N, 11.84%.

3.2.2. 5-Bromo-2-dicyanovinylthiophene 2c. Pale orange solid (91%). Mp: 157–158 °C. (ether/n-hexane). UV (acetonitrile): λ_{max} nm (ε, /M^{-1} cm^{-1}) 317.5, (17,000). IR (nujol) ν 3310, 2224 (CN) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.25 (d, 1H, J=4.0 Hz, 4-H), 7.51 (d, 1H, J=4.0 Hz, 3-H), 7.75 (s, 1H, CH=C(CN)$_2$). MS (EI) m/z (%): 240 (M^{+81}Br, 98), 238 (M^{+79}Br, 100), 189 (10), 187 (10), 159 (51). HRMS: m/z (EI) for $C_8H_3{}^{81}BrN_2S$; calcd 239.9180; found: 239.9180. Anal. calcd for $C_8H_3BrN_2S$: C, 40.17; H; 1.26; N, 11.72; S, 13.39. Found C, 40.23; H, 1.49; N, 11.44%).

3.3. Synthesis of 5′-bromo-5-dicyanovinyl-2,2′-bithiophene 6

3.3.1. Synthesis of 2-(tri-*n*-butylstannyl)thiophene 4.[40] Under argon a solution of n-BuLi in hexanes (2.5 ml, 6.14 mmol, 2.5 M) was dropped into a stirred solution of **3** (3.07 mmol) in dry ether at 0 °C. After 1 h the mixture was cooled to −78 °C and a solution of tributyltin chloride (1 g/0.83 ml, 3.07 mmol) in dry ether was slowly added and the mixture was stirred overnight. The mixture was then added to water (50 ml). The aqueous layer was extracted with ether (3×30 ml). The combined organic layers were dried with magnesium sulfate, and the solvent was removed in vacuo to give the title product **4** as a pale brown oil in quantitative yield. ^{1}H NMR ($CDCl_3$) δ 0.80–1.00 (m, 15H, 3×(CH$_2$)$_2$CH_2CH_3)), 1.10–1.50 (m, 12H, 3×(CH_2)$_2$CH$_2$-CH_3), 7.20 (dd, 1H, J=3.3, 1.0 Hz, 3-H), 7.25–7.29 (m, 1H, 4-H), 7.66 (dd, 1H, J=4.7, 1.0 Hz, 5-H). Product **4** was used in the Stille coupling without further purification.

3.3.2. Synthesis of 5-dicyanovinyl-2,2′-bithiophene 5. A degassed solution of the 5-bromo-2-dicyanovinylthiophene **2c** (0.66 g, 2.8 mmol), the thienylstananne **4** (3.07 mmol) and Pd(PPh$_3$)$_4$ (0.056 mmol) in toluene (5 ml) was heated at 80 °C under argon. After 24 h the reaction mixture was cooled to room temperature, filtered and washed with a cold mixture of ether/petrol to give the pure 5-dicyanovinyl-2,2′-bithiophene **5** as a pale orange solid. The organic solution obtained from the filtration was washed with a saturated solution of KF (3×50 ml), water (3×50 ml) and a saturated solution of NaCl (100 ml). The resulting organic layer were dried with magnesium sulfate, and the solvent was removed in vacuo to give a brown oil. Overall yield: 55%. Recrystallization from n-hexane gave the pure 5-dicyanovinyl-2,2″-bithiophene **5** as a pale orange solid. Mp: 166.5–168.5 °C. IR (nujol) ν 2218 (CN) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.18–7.22 (m, 1H, 4′-H), 7.62 (d, 1H, J=4.5 Hz, 3-H), 7.67 (dd, 1H, J=3.8, 1.0 Hz, 3′-H), 7.78 (dd, 1H, J=5.0, 1.0 Hz, 5′-H), 7.89 (d, 1H, J=4.5 Hz, 3-H), 8.64 (s, 1H, CH=C(CN)$_2$).

3.3.3. Synthesis of 5′-bromo-5-dicyanovinyl-2,2′-bithiophene 6. To a stirred solution of 5-dicyanovinyl-2,2′-bithiophene **5** (0.1 g, 0.41 mmol) in a 1:1 (v/v) solution of chloroform–acetic acid (12 ml) was added NBS (0.073 g, 0.41 mmol) at rt. After 24 h the reaction mixture was washed with water (30 ml). The organic layer was dried with magnesium sulfate, and the solvent was removed in

vacuo to give the pure bithiophene **6** as a orange brownish solid (85%). Recrystallization from *n*-hexane gave the title compound as a pale orange solid. Mp: 193–195 °C. UV (acetonitrile): λ_{max} nm (ε, /M^{-1} cm^{-1}) 421.0 (21,290), 308.0 (240). IR (nujol) ν 2222 (CN) cm^{-1}. ^{1}H NMR (DMSO) δ 7.35 (d, 1H, J=4.2 Hz, 3′-H), 7.53 (d, 1H, J=4.2 Hz, 4′-H), 7.61 (d, 1H, J=4.5 Hz, 3-H), 7.88 (d, 1H, J=4.5 Hz, 4-H), 8.65 (s, 1H, C*H*=C(CN)$_2$). MS (EI) *m*/*z* (%): 322 (M^{+81}Br, 99), 320 (M^{+79}Br, 100). HRMS: *m*/*z* (EI) for $C_{12}H_5{}^{81}BrN_2S_2$; calcd 321.9057; found: 321.9058.

3.4. General procedure for the synthesis of 2-alkoxy- and 2-amino-substituted 5-(tri-*n*-butylstannyl)-2,2′-bithiophenes 8a–d

Under Ar a solution of *n*-BuLi in hexanes (1.3 ml, 3.21 mmol, 2.5 M) was dropped into a stirred solution of **7** (2.7 mmol) in dry ether at 0 °C. After 1 h the mixture was cooled to −78 °C and a solution of tri-*n*-butylchlorostannane (2.7 mmol) in dry ether was slowly added and the mixture was stirred overnight. The mixture was then added to water (50 ml). The aqueous layer was extracted with ether (3×30 ml). The combined organic layers were dried with magnesium sulfate, and the solvent was removed in vacuo to give product **8**. Derivatives **8a–d** were used in the Stille couplings without further purification.

3.4.1. 5-Methoxy-5′-(tri-*n*-butylstannyl)-2,2′-bithiophene 8a. Green oil (85%). ^{1}H NMR ($CDCl_3$) δ 0.80–1.00 (m, 15H, 3×$(CH_2)_2CH_2CH_3$), 1.20–1.40 (m, 12H, 3×$(CH_2)_2CH_2CH_3$), 3.90 (s, 3H, OCH_3), 5.80 (d, 1H, J=3.9 Hz, 4-H), 6.87 (d, 1H, J=3.9 Hz, 3-H), 7.00 (d, 1H, J=3.6 Hz, 3′-H), 7.07 (d, 1H, J=3.6 Hz, 4′-H).

3.4.2. 5-*N*,*N*-Dimethylamino-5′-(tri-*n*-butylstannyl)-2,2′-bithiophene 8b. Orange oil (90%). ^{1}H NMR ($CDCl_3$) δ 0.80–1.00 (m, 15H, 3×$(CH_2)_2CH_2CH_3$), 1.10–1.45 (m, 12H, 3×$(CH_2)_2CH_2CH_3$), 2.93 (6H, s, $N(CH_3)_2$), 5.80 (d, 1H, J=3.7 Hz, 4-H), 6.87 (d, 1H, J=3.7 Hz, 3-H), 7.00 (d, 1H, J=3.5 Hz, 4′-H) 7.07 (d, 1H, J=3.5 Hz, 3′-H).

3.4.3. 5-*N*,*N*-Diethylamino-5′-(tri-*n*-butylstannyl)-2,2′-bithiophene 8c. Pale brown oil (90%). ^{1}H NMR ($CDCl_3$) δ 0.80–1.00 (m, 15H, 3×$(CH_2)_2CH_2CH_3$), 1.10–1.45 (m, 12H, 3×$(CH_2)_2CH_2CH_3$), 1.20–1.30 (overlapped t, 6H, 2×CH_2CH_3), 3.25–3.35 (q, 4H, J=6.0 Hz, 2×CH_2CH_3), 5.78 (d, 1H, J=3.9 Hz, 4-H), 6.86 (d, 1H, J=3.9 Hz, 3-H), 7.00 (d, 1H, J=3.6 Hz, 4′-H) 7.05 (d, 1H, J=3.6 Hz, 3′-H).

3.4.4. 5-Piperidino-5′-(tri-*n*-butylstannyl)-2,2′-bithiophene 8d. Pale brown oil (81%). ^{1}H NMR ($CDCl_3$) δ 0.80–1.00 (m, 15H, 3×$(CH_2)_2CH_2CH_3$), 1.20–1.40 (m, 12H, 3×$(CH_2)_2CH_2CH_3$), 1.50–1.80 (m, 6H, 3×CH_2), 3.10–3.20 (m, 4H, 2×NCH_2), 5.98 (d, 1H, J=3.9 Hz, 4-H), 6.86 (d, 1H, J=3.9 Hz, 3-H), 7.00 (d, 1H, J=3.6 Hz, 4′-H) 7.08 (d, 1H, J=3.6 Hz, 3′-H).

3.5. General procedure for palladium-catalyzed cross-couplings of aryl 1a–b, 1d and heteroaryl bromides 2a–c and 6 with stannylbithiophene derivatives 8a–d

To a degassed solution of aryl **1a–b** and **1d**, thienyl **2a–c** or bithienyl **6** bromides (0.5 mmol), and bithienylstanannes **8a–d** (0.55 mmol) in toluene (5 ml) was added $Pd(PPh_3)_4$ (0.01 mmol). The mixture was heated at 80 °C under argon. After the given reactions times (TLC control, Table 1) the reaction mixture was cooled to room temperature and then filtered and washed with cold toluene to give the pure oligothiophenes **9d**, **10a–d**, **11d**, **12b** and **13d–16d**. The isolated solids were recrystallized. The organic solution obtained from the filtration was washed with a saturated solution of KF (3×30 ml), water (3×30 ml) and a saturated solution of NaCl (50 ml). The resulting organic layers were dried with magnesium sulfate, and the solvent was removed in vacuo to give oils which by ^{1}NMR reveal to be the stannanes derivatives used in excess.

3.5.1. 5′-(4″-Cyanophenyl)-5-piperidino-2,2′-bithiophene 9d. Orange solid (43%). Mp: 227–229 °C (ether). UV (EtOH): λ_{max} nm (ε, /M^{-1} cm^{-1}) 420.0 (18,660), 297.0 (7450), 255.0 s (8430), 239.0 (11,130), 215.0 s (13,340). IR (nujol) ν 2219 (CN) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.50–1.80 (m, 6H, 3×CH_2), 3.10–3.20 (m, 4H, 2×NCH_2), 6.00 (d, 1H, J=3.9 Hz, 4-H), 6.92–6.98 (m, 2H, 3 and 3′-H), 7.30 (d, 1H, J=4.2 Hz, 4′-H), 7.63 (br s, 4H, 4×Ar-*H*). MS (EI) *m*/*z* (%): 350 (M^+, 100). HRMS: *m*/*z* (EI) for $C_{20}H_{18}N_2S_2$; calcd 350.0911; found: 350.0916.

3.5.2. 5-Methoxy-5′-(4″-nitrophenyl)-2,2′-bithiophene 10a. Orange solid (65%). Mp: 169–171 °C (ether). UV (EtOH): λ_{max} nm (ε, /M^{-1} cm^{-1}) 413.0 (25,750), 289.0 (8680), 252.0 (1450), 213.0 s (1810). IR (nujol) ν 1593, 1531, 1505, 1351, 1200, 1158, 1111, 1048, 846, 800, 772, 749, 721, 666 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 3.94 (s, 3H, OCH_3), 6.18 (d, 1H, J=3.9 Hz, 4-H), 6.91 (d, 1H, J=3.9 Hz, 3-H), 7.03 (d, 1H, J=3.9 Hz, 3′-H), 7.37 (d, 1H, J=3.9 Hz, 4′-H), 7.70 (d, 2H, J=9.0 Hz, 2″ and 6″-H), 8.23 (d, 2H, J=9.0 Hz, 3″ and 5″-H). MS (EI) *m*/*z* (%): 317 (M^+, 44). Anal. calcd for $C_{15}H_{11}NO_3S_2$: C, 56.76; H, 3.47; N, 4.41; S, 20.20. Found: C, 56.51; H, 3.52; N, 4.44; S, 19.80. HRMS: *m*/*z* (EI) for $C_{15}H_{11}NO_3S_2$; calcd 317.0180; found: 317.0174.

3.5.3. 5-*N*,*N*-Dimethyl-5′-(4″-nitrophenyl)-2,2′-bithiophene 10b. Orange solid (42%). Mp: 243–245 °C (ethanol). UV (EtOH): λ_{max} nm (ε, /M^{-1} cm^{-1}) 461.0 (10,050), 322.0 (4650), 264.0 (5140), 211.0 s (8540). IR (nujol) ν 1592, 1563, 1534, 1504, 1450, 1425, 1331, 1278, 110, 1056, 919, 848, 795, 748, 688, 666 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.98 (s, 6H, 2×CH_3), 5.81 (d, 1H, J=3.9 Hz, 4-H), 6.94 (d, 1H, J=3.9 Hz, 3-H), 6.96 (d, 1H, J=3.9 Hz, 3′-H), 7.35 (d, 1H, J=3.9 Hz, 4′-H), 7.65 (d, 2H, J=8.9 Hz, 2″ and 6″-H), 8.20 (d, 2H, J=8.9 Hz, 3″ and 5″-H). MS (EI) *m*/*z* (%): 330 (M^+, 100). HRMS: *m*/*z* (EI) for $C_{16}H_{14}N_2O_2S_2$; calcd 330.0497; found: 330.0505.

3.5.4. 5-*N*,*N*-Diethyl-5′-(4″-nitrophenyl)-2,2′-bithiophene 10c. Dark red solid (44%). Mp: 181–183 °C (ethanol). UV (EtOH): λ_{max} nm (ε, /M^{-1} cm^{-1}) 474.5 (16,800), 360.0 (9630), 265.0 (7140). IR (nujol) ν 1591, 1504, 1332, 1280, 1183, 1108, 1057, 847, 791, 750, 722, 666 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 6H, J=7.0 Hz, 2×CH_2CH_3), 3.34 (q, 4H, J=7.0 Hz, 2×CH_2CH_3), 5.79 (d, 1H, J=3.9 Hz, 4-H), 6.92 (d, 1H, J=3.9 Hz, 3-H), 6.98 (d, 1H, J=3.9 Hz, 3′-H), 7.35 (d, 1H, J=3.9 Hz, 4′-H), 7.66 (d, 2H, J=9.0 Hz, 2″ and

6″-H), 8.21 (d, 2H, *J*=9.0 Hz, 3″ and 5″-H). MS (EI) *m/z* (%): 358 (M^+, 100). HRMS: *m/z* (EI) for $C_{18}H_{18}N_2O_2S_2$; calcd 358.0810; found: 358.0810.

3.5.5. 5-Piperidino-5′-(4″-nitrophenyl)-2,2′-bithiophene 10d. Brown solid (53%). Mp: 238–240 °C (ethanol). UV (EtOH): λ_{max} nm (ε, /M^{-1} cm^{-1}) 453.0 (10,000), 322.0 (4720), 316.0 (4710), 266.0 (4700), 213.0 s (8611). IR (nujol) ν 1592, 1504, 1493, 1329, 1275, 1247, 1117, 1066, 843, 796, 686, 666 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.50–1.80 (m, 6H, 3×CH_2), 3.16–3.20 (m, 4H, 2×NCH_2), 5.99 (d, 1H, *J*=3.9 Hz, 4-H), 6.95–6.98 (m, 2H, 3 and 3′-H), 7.35 (d, 1H, *J*=3.9 Hz, 4′-H), 7.66 (d, 2H, *J*=8.9 Hz, 2″ and 6″-H), 8.21 (d, 2H, *J*=8.9 Hz, 3″ and 5″-H). MS (EI) *m/z* (%): 370 (M^+, 100). HRMS: *m/z* (EI) for $C_{19}H_{18}N_2O_2S_2$; calcd 370.0810; found: 370.0814.

3.5.6. 5′-(4″-Dicyanovinylphenyl)-5-piperidino-2,2′-bithiophene 11d. Green solid with metal luster (56%). Mp: 232–233 °C. UV (ethanol): λ_{max} nm (ε, /M^{-1} cm^{-1}) 468.0 (21,400), 360.5 (13,386). IR (nujol) ν 2223 (CN). cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.45–1.70 (m, 6H, 3×CH_2), 3.10–3.20 (m, 4H, 2×NCH_2), 6.11 (d, 1H, *J*=4.5 Hz, 4-H), 7.08–7.14 (m, 2H, 3 and 3′-H), 7.69 (d, 1H, *J*=3.9 Hz, 4′-H), 7.90 (d, 2H, *J*=8.4 Hz, 2″ and 6″-H), 7.96 (d, 2H, *J*=8.4 Hz, 3″ and 5″-H), 8.44 (s, 1H, *CH*=C(CN)$_2$). MS (EI) *m/z* (%): 401 (M^+, 100). HRMS: *m/z* (EI) for $C_{23}H_{19}N_3S_2$; calcd 401.1020; found: 401.1022.

3.5.7. 5″-Formyl-5-*N*,*N*-dimethyl-2,2′:5′2″-terthiophene 12b. Brown solid (46%). Mp: 186–188 °C (ethanol) [lit.[41] (mp not quoted)]. UV (EtOH): λ_{max} nm (ε, /M^{-1} cm^{-1}) 465.5 (22,690), 342.0 (9300), 258.0 (12,010), 213.0 s (14,100). IR (nujol) ν 1650 (CHO) cm^{-1}. ^{1}H NMR δ 2.98 (s, 6H, 2×CH_3), 5.80 (d, 1H, *J*=4.4 Hz, 4-H), 6.87 (d, 1H, *J*=4.4 Hz, 4′ or 3′-H), 6.96 (d, 1H, *J*=3.9 Hz, 3-H), 7.17 (d, 1H, *J*=4.4 Hz, 3″-H), 7.22 (d, 1H, *J*=4.4 Hz, 3′ or 4′-H), 7.65 (d, 1H, *J*=4.4 Hz, 4″-H), 9.84 (s, 1H, C*H*O). MS (EI) *m/z* (%): 319 (M^+, 100). HRMS: *m/z* (EI) for $C_{15}H_{13}NOS_3$; calcd 319.0159; found: 319.0156.

3.5.8. 5″-Formyl-5-piperidino-2,2′:5′2″-terthiophene 13d. Brown solid (51%). Mp: 178–180 °C (ether). UV (EtOH): λ_{max} nm (ε, /M^{-1} cm^{-1}) 456.0 (15,260), 332.0 (6000), 257.0 (7570). IR (nujol) ν 1645 (CHO) cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.50–1.70 (m, 6H, 3×CH_2), 3.05–3.15 (m, 4H, 2×NCH_2) 6.10 (d, 1H, *J*=4.2 Hz, 4-H), 7.05 (d, 1H, *J*=3.9 Hz, 4′-H), 7.09 (d, 1H, *J*=4.2 Hz, 3-H), 7.45 (d, 1H, *J*=4.2 Hz, 3″-H), 7.48 (d, 1H, *J*=3.9 Hz, 3′-H), 7.96 (d, 1H, *J*=4.2 Hz, 4″-H), 9.84 (s, 1H, C*H*O). MS (EI) *m/z* (%): 359 (M^+, 100). HRMS: *m/z* (EI) for $C_{18}H_{17}NOS_3$; calcd 359.0472; found: 359.0482.

3.5.9. 5″-Nitro-5-piperidino-2,2′:5′2″-terthiophene 14d. Dark red solid (53%). Mp: 215–217 °C (ether). UV (EtOH): λ_{max} nm (ε, /M^{-1} cm^{-1}) 504.0 (10,100), 355.0 (2510), 219.0 (4350). IR (nujol) ν 1559, 1509, 1482, 1325, 1274, 1244, 1120, 1074, 1035, 852, 793, 759, 730, 666 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.50–1.80 (m, 6H, 3×CH_2), 3.17–3.22 (m, 4H, 2×NCH_2), 5.99 (d, 1H, *J*=3.9 Hz, 4-H), 6.89 (d, 1H, *J*=3.6 Hz, 4′ or 3′-H), 6.97 (d, 1H, *J*=3.9 Hz, 3-H), 7.00 (d, 1H, *J*=4.5 Hz, 3″-H), 7.24 (d, 1H, *J*=3.6 Hz, 3′ or 4′-H), 7.83 (d, 1H, *J*=4.5 Hz, 4″). MS (EI) *m/z* (%): 376 (M^+, 100). HRMS: *m/z* (EI) for $C_{17}H_{16}N_2O_2S_3$; calcd 376.0374; found: 376.0363.

3.5.10. 5″-Dicyanovinyl-5-piperidino-2,2′:5′,2″-terthiophene 15d. Dark purple solid (55%). Mp: 185–187 °C. UV (EtOH): λ_{max} nm (ε, /M^{-1} cm^{-1}) (Ethanol) 545.5 (23,770), 377.0 (10,992). IR (nujol) ν 2218 (CN) cm^{-1}. ^{1}H NMR (DMSO-d_6) 1.50–1.70 (m, 6H, 3×CH_2), 3.10–3.20 (m, 4H, 2×NCH_2), 6.13 (d, 1H, *J*=4.2 Hz, 4-H), 7.09 (d, 1H, *J*=3.9 Hz, 4′ or 3′-H), 7.17 (d, 1H, *J*=4.2 Hz, 3-H), 7.55 (d, 1H, *J*=4.2 Hz, 3″-H), 7.59 (d, 1H, *J*=3.9 Hz, 3′ or 4′-H), 7.86 (d, 1H, *J*=4.2 Hz, 4″-H), 8.57 (s, 1H, *CH*=C(CN)$_2$). MS (EI) *m/z* (%): 407 (M^+, 100). HRMS: *m/z* (EI) for $C_{21}H_{17}N_3S_3$; calcd 407.0585; found: 407.0594.

3.5.11. 5‴-Dicyanovinyl-5-piperidino-2,2′:5′,2″:5″,2‴-tetrathiophene 16d. Dark blue solid (45%). Mp: >230.0 °C (with decomposition). λ_{max} nm (ε, /M^{-1} cm^{-1}) (Ethanol) 510.5 (12,000). IR (nujol) ν 2218 (CN) cm^{-1}. ^{1}H NMR (DMSO-d_6) 1.42–1.70 (m, 6H, 3×CH_2), 3.10–3.20 (m, 4H, 2×NCH_2), 6.10 (d, 1H, *J*=4.2 Hz, 4-H), 7.05 (d, 1H, *J*=4.2 Hz, 4′ or 3′-H), 7.16 (d, 1H, *J*=4.2 Hz, 3-H), 7.34–7.38 (m, 2H, 3′ or 4′-H and 3″-H or 4″-H), 7.64 (d, 1H, *J*=4.2 Hz, 3‴-H), 7.67 (d, 1H, *J*=4.2 Hz, 4″or 3″-H), 7.89 (d, 1H, *J*=4.2 Hz, 4‴-H), 8.62 (s, 1H, *CH*=C(CN)$_2$). MS (EI) *m/z* (%): 489 (M^+, 100). HRMS: *m/z* (EI) for $C_{25}H_{19}N_3S_4$; calcd 489.0462; found: 489.0465.

Acknowledgements

Thanks are due to Foundation for Science and Technology (Portugal) for financial support through IBQF (UM) and through FEDER, POCTI (Ref. POCTI/QUI/37816/2001).

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Tetrahedron
Tetrahedron 60 (2004) 4079–4085

Towards the synthesis of perfluoroalkylated derivatives of Xantphos

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Received 18 December 2003; revised 30 January 2004; accepted 25 February 2004

Abstract—An analogue of Xantphos incorporating four perfluoroalkyl groups has been prepared and successfully used as a ligand in the rhodium-catalysed hydroformylation of 1-octene in toluene. A number of perfluoroalkylated xanthene backbones have also been synthesised, but their conversion into preferentially perfluorocarbon solvent soluble Xantphos-type ligands, suitable for catalysis in fluorocarbon solvents, has not been successful.

1. Introduction

The fluorous biphasic approach has been shown to be a useful tool for the efficient separation of catalyst from products in the rhodium-catalysed hydroformylation of long chain alkenes,[1] amongst other catalytic reactions.[2] We have recently shown that $P(C_6H_4\text{-}4\text{-}C_6F_{13})_3$ can be used in the rhodium-catalysed hydroformylation of 1-octene in the fluorous phase resulting in good selectivity to the required linear aldehyde with minimal rhodium leaching (0.05%) into the non-fluorous product phase on phase separation post reaction.[3] With a view to increasing the selectivity further, we investigated the perfluoroalkyl derivatives of analogues of established bidentate phosphines and phosphites that have been shown to offer excellent selectivity in this process.[4] Surprisingly, although a large number of perfluoroalkyl derivatised monodentate phosphorus(III) ligands, prepared by a variety of routes,[4] have been described in the literature, the number of perfluoroalkyl derivatised bidentate phosphorus(III) ligands is still relatively small with only those incorporating the ethyl backbone being perfluorocarbon soluble.[5] Xantphos has been shown to be a remarkable ligand for the rhodium-catalysed hydroformylation of long chain alkenes, giving exceptionally high selectivity to the industrially useful linear aldehyde (linear/branched ratio=50:1).[6] Here, we report our investigations directed towards the synthesis of perfluoroalkylated derivatives of Xantphos.

2. Results and discussion

The approach followed in this work draws upon precedents set in earlier Xantphos-[6] and fluorous-based[3] hydroformylation studies. The catalytic data available for Xantphos clearly shows that the bite angle of the bisphosphine is critical for the high selectivities achieved in the hydroformylation of long chain alkenes.[6] Therefore, in order to minimise the steric effects of the perfluoroalkyl groups, linear perfluoroalkyl groups directly attached to the aromatic rings in the *para* positions on the pendant arms (R_1 in Fig. 1) or the *meta* positions (for ease of synthesis) on the backbone (R_2) are likely to be the most successful. Indeed, water-soluble versions of Xantphos have incorporated sulfonate or dialkylamino groups in these positions.[7] Furthermore, although a wide range of perfluoroalkyl groups have been incorporated into phosphines, many include a so-called spacer group to ameliorate their electron-withdrawing effect. However, in the hydroformylation of alkenes, electron-withdrawing groups are known to lead to catalysts which give greater rates of reaction and higher ratios of linear/branched aldehydes in the final hydroformylation product mixtures than those with

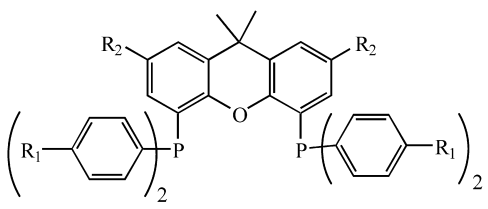

Figure 1. Xantphos skeleton, showing the sites where fluorinated side chains may be readily attached (R_1 and R_2).

Keywords: Fluorine; Fluorinated ligands; Phosphines; Homogeneous catalysis; Hydroformylation.
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doi:10.1016/j.tet.2004.02.070

greater electron density.[3] We, therefore, decided to focus solely on ligands with linear perfluoroalkyl groups directly attached to the aromatic rings thereby maximising the electronic advantages of these fluorinated substituents.

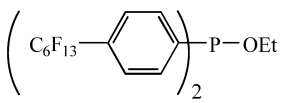


Figure 2. Bis(4-tridecafluoro-*n*-hexylphenyl) ethoxy phosphinite.

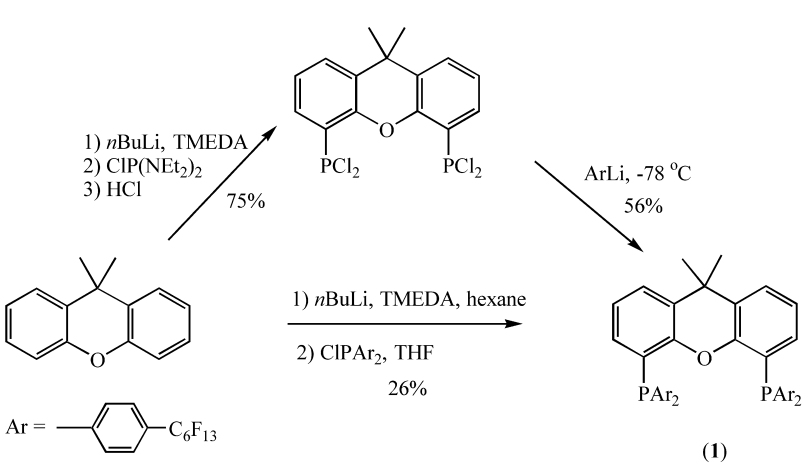
Although quenching the dilithiate of 9,9-dimethylxanthene with Ph_2PCl gives reproducibly high yields of Xantphos, the analogous reaction with $ClP(C_6H_4\text{-}4\text{-}C_6F_{13})_2$[8] in diethyl ether resulted in the isolation of only trace amounts of the desired perfluoroalkylated Xantphos. Instead, bis(4-tridecafluoro-*n*-hexylphenyl) ethoxy phosphinite was isolated, Figure 2. Decomposition of ethereal solvents by organolithiates is a well-known process, giving, in the case of diethyl ether, lithium ethoxide and ethene.[9] Here, therefore, the isolated phosphinite results from the reaction between chlorophosphine and lithium ethoxide. We have detected, by ^{31}P NMR spectroscopy, an analogous reaction during the synthesis of Xantphos itself although, in this case, ethoxydiphenyl phosphinite is only formed in trace amounts. This difference in reactivity between the two chlorophosphines is undoubtedly due to the presence of the highly electron-withdrawing perfluoroalkyl groups in $ClP(C_6H_4\text{-}4\text{-}C_6F_{13})_2$. A 17% yield of the perfluoroalkylated Xantphos (**1**) could be achieved by carrying out the reaction in hexane, but the low solubility of the chlorophosphine in this solvent required its addition as a refluxing suspension. By adding the chlorophosphine in THF to the lithiate in hexane, a similar yield could be obtained, which could be increased to 26% by refluxing the solution for 30 min prior to the addition of the chlorophosphine, Scheme 1.

The yield was improved further by following an alternative method. 4,5-Bis[bis(diethylamino)phosphine]-9,9,-dimethylxanthene, prepared via the method of van Leeuwen et al.[10] was converted to 4,5-bis(dichlorophosphino)-9,9-dimethylxanthene by reaction with dry HCl in ether. Reaction of this chlorophosphine with the aryl lithiate derived from 4-(tridecafluoro-*n*-hexyl)bromobenzene gave the perfluoroalkylated Xantphos (**1**) in a 56% yield, Scheme 1.

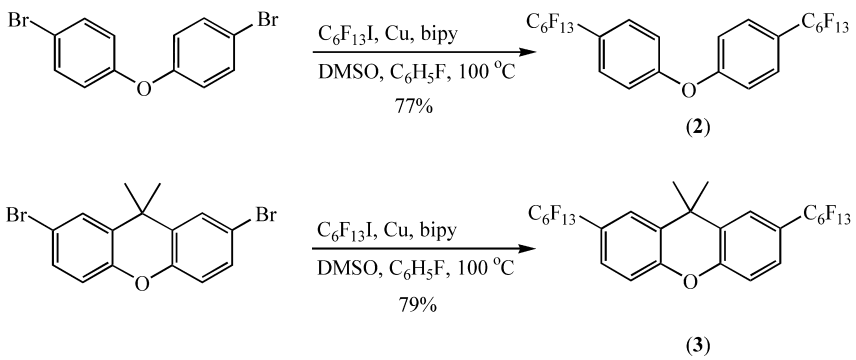
High fluorous phase solubility is required for the successful application of a ligand in a fluorous phase reaction. Previous work has suggested that 60% fluorine (w/w) is generally required for preferential solubility in a perfluorocarbon solvent over an organic solvent.[11] Although a single perfluoroalkyl group per aromatic ring is capable of generating a compound that is preferentially soluble, **1** has only 4 tails for 6 aromatic rings. With only 53.4% fluorine content it is, therefore, unsurprising that the perfluoroalkylated Xantphos (**1**) has a low solubility in perfluoro-1,3-dimethylcyclohexane (PP3). Partition coefficient determinations in a PP3/toluene biphase were complicated further by the low solubility of (**1**) in both phases. This low solubility has been previously noted for other high molecular weight fluorinated compounds. To increase the partition coefficient, the number of perfluoroalkyl groups on the molecule would need to be increased, which could be achieved by increasing the number of tails on the pendant arms. 3,5-Bis-substitution has been used in other ligand systems, but this has a dramatic influence on their steric properties, which would seriously compromise the high selectivity of Xantphos-based hydroformylation catalysts. Alternatively, using a silicon spacer unit, up to three tails per phenyl ring could be introduced. However, as illustrated by van Koten et al.[12] with a molecular weight in excess of 4500 Da, the absolute solubility of such a ligand in any solvent would be so low as to obviate its use in catalysis.

Therefore, to increase the percentage fluorine further, derivatising the xanthene backbone at position R_2 (Fig. 1) is likely to be the only viable approach. Perfluoroalkyl groups can be incorporated at position R_2 most simply by converting bis(4-bromophenyl)ether to bis(4-tridecafluoro-*n*-hexylphenyl)ether (**2**) by a copper mediated coupling reaction with $C_6F_{13}I$ which affords the desired product in a 77% yield. Similarly, 2,7-dibromo-9,9-dimethylxanthene (prepared by direct bromination of 9,9-dimethylxanthene) was converted to 2,7-bis(tridecafluoro-*n*-hexyl)-9,9-dimethylxanthene (**3**) in a 79% yield, Scheme 2.

Direct lithiation of (**2**) or (**3**) at room temperature (CAUTION! Lithiations of aromatic rings substituted with perfluoroalkyl groups have been reported to lead to explosion)[13] followed by quenching with $ClPPh_2$ in ether



Scheme 1.



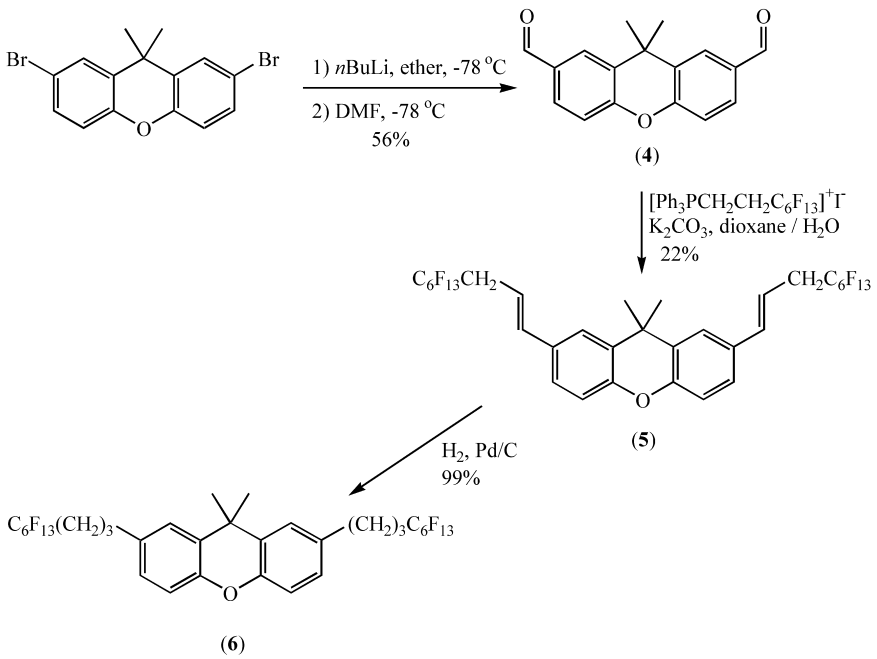
Scheme 2.

led to an inseparable mixture of phosphines being formed. The desired bisphosphine could be detected by ^{31}P NMR spectroscopy in each case, but could only be isolated in the case of (**2**). Even then, the bisphosphine could only be isolated with a purity of 90%. In each case, in addition to lithiating *ortho* to the oxygen, as occurs with 9,9-dimethylxanthene, lithiation *ortho* to the perfluoroalkyl group also occurs as evidenced by the strong P–F coupling in the ^{31}P NMR spectra of the quenched product.[8] It is apparent that the strongly electron-withdrawing perfluoroalkyl groups are having a profound effect on the regioselectivity of the lithiation. The most obvious way to solve this problem is to introduce a spacer between the perfluoroalkyl chain and the aromatic ring and there are numerous suitable groups now available.[12,14] We introduced an alkyl spacer group by conversion of 2,7-dibromo-9,9-dimethylxanthene to 9,9-dimethylxanthene-2,7-dicarbaldehyde (**4**), followed by a Wittig reaction with $[Ph_3PCH_2CH_2C_6F_{13}]^+I^-$ following the known methodology.[15] After hydrogenation with Pd/C in DCM, 2,7-bis(*1H*,*1H*,*2H*,*2H*,*3H*,*3H*-perfluorononanyl)-9,9-dimethylxanthene (**6**) could be isolated in an overall yield of 12% from the dibromoxanthene, Scheme 3.

Unfortunately, repeated attempts at lithiating **6**, followed by quenching with Ph_2PCl, failed to yield any of the desired product. Despite a strong colour change upon the addition of *n*-BuLi, the starting xanthene was recovered unchanged after reaction, along with a small amount of a product, the ^{19}F NMR spectrum of which suggested attack at or near the perfluoroalkyl groups had occurred. This infers that, rather than deprotonating the aromatic ring, lithiation of the alkyl spacer group had occurred.

2.1. Catalysis

The effect of the perfluoroalkyl groups on the donor ability of the phosphorus atoms has been probed previously by examination of the change in $^1J_{PPt}$ of the *cis*-[PtCl-$_2$-L] (L=bidentate or two monodentate phosphines).[5b] As can be seen from Table 1, perfluoroalkylated Xantphos (**1**) shows a similar trend to $(C_6H_4\text{-}4\text{-}C_6F_{13})_2PCH_2CH_2P(C_6H_4\text{-}4\text{-}C_6F_{13})_2$ with a decrease in the magnitude of $^1J_{PPt}$ as expected following the introduction of strongly electron-withdrawing groups.



Scheme 3.

Table 1. Pt–P coupling constants for platinum complexes containing bidentate ditertiary phosphines

Complex	$^1J_{PtP}$ (Hz)
cis-[$PtCl_2$(dppe)][a]	3594
cis-[$PtCl_2${($CH_2P(C_6H_4$-4-$C_6F_{13})_2$}$_2$][a]	3568
[$PtCl_2$(Xantphos)][b]	3694
[$PtCl_2$(**1**)][b]	3662

[a] CD_3COCD_3, data from Ref. 5b.
[b] $CDCl_3$.

Although **1** does not have sufficiently high perfluorocarbon solubility for use in a fluorous biphasic reaction, it can be successfully used as a ligand in the rhodium catalysed hydroformylation of 1-octene in toluene. We rationalised that the fluoroalkyl groups should have a beneficial effect in this reaction, since electron-withdrawing groups have been shown by others to increase rates of reaction and selectivity in such reactions.[16] Hydroformylation of 1-octene was carried out at 80 °C and 20 bar of 1:1 CO/H_2 using 2.2 equiv. of **1** with respect to rhodium. The results are shown in Table 2 and compared to the same reaction carried out with Xantphos. As with Xantphos, the selectivity for the required linear aldehyde is relatively high (linear aldehyde/branched aldehyde=22.9:1 compared to 4.7:1 for P(4-$C_6F_{13}C_6H_4)_3$). However, the isomerisation is greater when **1** is used compared with Xantphos. These findings mirror those found elsewhere for derivatives of thixantphos, where isomerisation to the 2-octene increased as phosphine basicity decreased.[16c] This has been attributed to an increased tendency of the branched alkyl rhodium species to form 2-octene instead of the branched aldehyde. A possible alternative explanation is that the poorer donor ability and larger size of **1** compared with Xantphos may reduce its coordinating power and leave some rhodium uncoordinated to the phosphine. This type of complex is a known alkene isomerisation catalyst, but is poorly selective and rather sluggish in hydroformylation reactions under these conditions. Support for this suggestion comes from the observation that lower loadings of ligand (**1**:Rh=1.7) give much lower linear/branched ratios and significant amounts of aldehydes derived from isomerised alkenes. The rhodium–Xantphos complexes are essentially inactive for the hydroformylation of internal alkenes. This last experiment was carried out in perfluoromethylcyclohexane. This is unlikely to be a medium effect, since our previous work has shown that triphenylphosphine is more selective when the reaction is carried out in a perfluorocarbon solvent as compared to toluene.[3b] At the end of the reaction, both organic and fluorous phases were yellow in colour, confirming that **1** is insufficiently fluorinated to immobilise the catalyst completely within the fluorous phase.

3. Conclusion

This work has shown the difficulties in attempting to generate a perfluorocarbon soluble analogue of a known bisphosphine, although a number of new perfluoroalkylated intermediates have been prepared and fully characterised. A derivative of Xantphos has been prepared incorporating four perfluoroalkyl groups. Unfortunately, this is not sufficient to render the bisphosphine preferentially soluble in a perfluorocarbon solvent and attempts at further derivatising this compound have failed. However, the derivatised Xantphos is still an effective ligand for the rhodium catalysed hydroformylation of 1-octene in toluene, offering good selectivity to the desired linear aldehydes but the fluorous groups appear to effect the amount of isomerisation.

4. Experimental

4.1. General Remarks

^1H, ^{19}F and ^{31}P NMR spectroscopies were carried out on a Bruker ARX250 spectrometer at 250.13, 235.34 and 101.26 MHz or a Bruker DPX300 spectrometer at 300.14, 282.41 and 121.50 MHz respectively and were referenced to external $SiMe_4$ (^1H), external $CFCl_3$ (^{19}F) and to external H_3PO_4 (^{31}P) using the high frequency positive convention. Due to the complicated spectra arising from the extensive coupling to the fluorine atoms, all ^{13}C NMR are quoted without the values for the perfluoroalkyl groups (105–120 ppm). Abbreviations for NMR spectral multiplicities are as follows: s=singlet, d=doublet etc., m=multiplet. Elemental analyses were performed by the Elemental Analysis Service at the University of North London. Mass spectra were recorded on a Kratos Concept 1H mass spectrometer. *cis*-[$PtCl_2(MeCN)_2$][17] and 4-(tridecafluoro-*n*-hexyl)bromobenzene[18] were prepared by the literature routes. 4,5-Bis(diethylaminophosphino)-9,9-dimethylxanthene was prepared via the method of Goertz et al.[11] [$Ph_3PCH_2CH_2C_6F_{13}$]$^+I^-$ was prepared via the method of Rocaboy et al.[15]

4.1.1. 4,5-Bis(dichlorophosphino)-9,9-dimethylxanthene. 4,5-Bis(diethylaminophosphino)-9,9-dimethylxanthene (0.98 g, 1.76 mmol) was dissolved in ether (30 mL). Concentrated HCl was bubbled through the solution for 15 min. The solution was then flushed with nitrogen for 2 h, filtered through celite and the solvent removed in vacuo to give a white solid (0.54 g, 75%). Anal. calcd for $C_{15}H_{12}OP_2Cl_4$: C, 43.90; H, 2.93. Found: C, 44.02; H,

Table 2. Products from the hydroformylation of 1-octene catalysed by rhodium complexes of Xantphos based ligands in toluene at 80 °C and 20 bar CO/H_2 (1:1)

Ligand	Ligand/Rh	1-Nonanal (%)	2-Methyloctanal (%)	% Isomer[a]	l:b[b]	k (s^{-1})[c]
Xantphos	2.2	82.1	1.9	3.4	43.5	1.2×10^{-4}
1	2.2	81.0	3.5	11.5	22.9	1.2×10^{-4}
1[d]	1.7	65.6	17.7	15.5	3.7	5.0×10^{-4}

[a] Percentage isomerisation to 2-octene.
[b] Linear over branched ratio.
[c] First order rate constant calculated from gas up take plots at constant pressure.
[d] In perfluoromethylcyclohexane.

2.92. *m/z* (EI): 412 (MH^+). ^1H NMR (C_6D_6) 7.80 (2H, dd, $^3J_{HH}$=7.6 Hz, $^4J_{HH}$=1.8 Hz, H3), 6.96 (2H, dd, $^3J_{HH}$=7.6 Hz, $^4J_{HH}$=1.8 Hz, H1), 6.79 (2H, t, $^3J_{HH}$=7.6 Hz, H2) 1.07 (6H, s, CH_3). $^{31}P\{^1H\}$ NMR (C_6D_6) 158.9 (s). ^{13}C NMR (C_6D_6) 150.8 (t, J_{PC}=13.5 Hz), 130.7, 130.4, 129.2, 128.3, 123.4, 34.4, 31.4.

4.1.2. 4,5-Bis-(bis(4-tridecafluoro-*n*-hexyl-phenyl)phosphino)-9,9-dimethylxanthene (1). 4-(Tridecafluoro-*n*-hexyl)bromobenzene (1.44 g, 3.03 mmol) was dissolved in diethyl ether (20 mL) under nitrogen and cooled to −78 °C. *n*-BuLi (1.9 mL of a 1.6 M solution in hexane, 3.04 mmol) was added over 1 h and the solution stirred for 4 h at −78 °C. 4,5-Bis(dichlorophosphino)-9,9-dimethylxanthene (0.31 g, 0.76 mmol) in diethyl ether (20 mL) was then added dropwise and the solution allowed to warm to room temperature overnight. Water was then added (30 mL), the organic solution separated and dried. The solvent was removed in vacuo to give a yellow solid. This was washed with perfluoro-1,3-dimethylcyclohexane (3×10 mL) to give a white solid (0.372 g, 27%). By repeatedly cooling the perfluoro-1,3-dimethylcyclohexane solution to −40 °C, a further 0.294 g of 4,6-bis-{bis(4-tridecafluoro-*n*-hexyl-phenyl}phosphino)-9,9-dimethylxanthene was collected (21%). *m/z* (FAB) 1851 (MH^+). Anal. calcd for $C_{63}H_{28}OP_2F_{52}$: C, 40.86; H, 1.51. Found: C, 40.76; H, 1.54. ^1H NMR (C_6D_6) 7.51–7.21 (18H, m, ArH), 6.97 (2H, t, $^3J_{HH}$=7.6 Hz, H2), 6.76 (2H, dd, $^3J_{HH}$=7.6 Hz, $^4J_{HH}$=1.6 Hz, H3), 1.62 (6H, s, CH_3). $^{19}F\{^1H\}$ NMR (C_6D_6) −81.45 (12F, t, $^4J_{FF}$=10.6 Hz, CF_3), −110.63 (8F, t, $^4J_{FF}$=14.6 Hz, α-CF_2), −121.78 (16F, m, CF_2), −123.14 (8F, m, CF_2), −126.41 (8F, m, CF_2). $^{31}P\{^1H\}$ NMR (C_6D_6) −18.2.

4.1.3. 4,4′-Bis(tridecafluoro-*n*-hexyl)phenyl ether (2). Perfluoro-*n*-hexyl iodide (55.31 g, 124.0 mmol) was added to a stirred solution of *para*-dibromodiphenyl ether (10.03 g, 30.6 mmol), copper bronze (15.75 g, 247.8 mmol) and 2,2′-bipyridine (1.34 g, 9.0 mmol) in DMSO (200 mL) and fluorobenzene (120 mL) under nitrogen at 100 °C. The solution was stirred for 3 days, cooled and poured on to an diethyl ether (300 mL)/water (300 mL) mixture and filtered. The organic layer was separated and washed three times with water, dried and the solvent removed in vacuo. The resulting yellow oil crystallised on standing and was washed with methanol to give a white powder (19.00 g, 77%). Mp 59–61 °C. Anal. calcd for $C_{24}H_8OF_{26}$ C, 35.73; H, 0.99. Found: C, 35.80; H, 0.96. *m/z* (FAB): 806 (M^+). ^1H NMR ($CDCl_3$) 7.59 (4H, d, $^3J_{HH}$=8.7 Hz, H3), 7.15 (4H, d, $^3J_{HH}$=8.7 Hz, H2); $^{19}F\{^1H\}$ NMR ($CDCl_3$) −81.29 (6F, t, $^4J_{FF}$=9.3 Hz, CF_3), −110.52 (4F, t, $^4J_{FF}$=14.6 Hz, CF_2), −121.89 (4F, m, CF_2), −122.27 (4F, m, CF_2), −123.25 (4F, m, CF_2), −126.59 (4F, m, CF_2).

4.1.4. Bis[(2-diphenylphosphino-4-tridecafluoro-*n*-hexyl)-phenyl]ether. 4,4′-Bis(tridecafluorohexyl)phenyl ether (0.81 g, 1.0 mmol) was dissolved in diethyl ether (30 mL). TMEDA (0.24 g, 2.1 mmol) was added, followed by *n*-BuLi (1.25 mL of a 1.6 M solution in hexane, 2.0 mmol) dropwise (CAUTION! Lithiations of aromatic rings substituted with perfluoroalkyl groups have been reported to lead to explosions[13]). The resulting red solution was stirred for 3 h and then quenched with chlorodiphenyl phosphine (0.44 g, 2.0 mmol). After stirring overnight, the solution was quenched with water, the organic layer removed, dried with sodium sulfate and the solvent removed in vacuo. The resulting semi-solid was passed down a short silica column to give a yellow solid (87% pure by NMR, 0.36 g, 31%). *m/z* (FAB): 1175 (MH^+). ^1H NMR ($CDCl_3$) 7.25 (2H, s, H5), 7.17 (8H, m, ArH), 7.05 (2H, d, $^3J_{HH}$=9.0 Hz, H2), 6.91 (12H, m, ArH), 6.29 (2H, dd, $^3J_{HH}$=9.4 Hz, $^4J_{HH}$=3.5 Hz, H3). $^{19}F\{^1H\}$ NMR ($CDCl_3$) −81.39 (6F, t, $^4J_{FF}$=8.5 Hz, CF_3), −110.52 (4F, t, $^4J_{FF}$=14.1 Hz, α-CF_2), −121.82 (4F, m, CF_2), −122.54 (4F, m, CF_2), −123.60 (4F, m, CF_2), −126.69 (4F, m, CF_2). $^{31}P\{^1H\}$ NMR ($CDCl_3$) −15.5 (s).

4.1.5. 2,7-Dibromo-9,9-dimethylxanthene. Bromine (0.51 mL, 10.0 mmol) in glacial acetic acid (1 mL) was added slowly to a stirred solution of 9,9-dimethylxanthene (0.843 g, 4.00 mmol) in acetic anhydride (10 mL) at 0 °C. The solution was then allowed to warm to room temperature and stirred for 2 h. The solution was then poured onto an excess of ice-cold water and the precipitate collected by filtration. The white solid was washed with sodium bisulfate (10% aqueous solution) and water and dried in vacuo (1.33 g, 90%). Anal. calcd for $C_{15}H_{12}OBr_2$: C, 48.95; H, 3.29. Found: C, 49.03; H, 3.19. Mp 113–115 °C. *m/z* (FAB): 368 ($[M]^+$). ^1H NMR ($CDCl_3$) 7.59 (2H, d, $^4J_{HH}$=2.3 Hz, H1), 7.40 (2H, dd, $^3J_{HH}$=8.7 Hz, $^4J_{HH}$=2.3 Hz, H3), 7.03 (2H, d, $^3J_{HH}$=8.7 Hz, H4), 1.70 (6H, s, CH_3). ^{13}C NMR ($CDCl_3$) 149.6, 132.0, 130.9, 129.4, 118.7, 116.1, 34.9, 32.6.

4.1.6. 2,7-Bis(tridecafluoro-*n*-hexyl)-9,9-dimethylxanthene (3). Tridecafluoro-*n*-hexyl iodide (4.82 g, 10.8 mmol), 2,7-dibromo-9,9-dimethylxanthene (1.00 g, 2.7 mmol), 2,2′-bipyridine (0.12 g, 0.8 mmol) and copper bronze (1.37 g, 21.6 mmol) were heated to 100 °C in DMSO (40 mL) and fluorobenzene (40 mL) for 4 days. The cooled solution was poured onto a mixture of diethyl ether (100 mL) and water (100 mL), filtered and the organic layer washed three times with water. After drying, the solvent was removed in vacuo and the resulting yellow solid triturated with methanol, giving a white solid, which was recovered by filtration (1.80 g, 79%). Anal. calcd for $C_{27}H_{12}OF_{26}$: C, 38.30; H. 1.42. Found: C, 38.29; H, 1.42. Mp 84–85 °C. *m/z* (FAB): 846 (M^+). ^1H NMR ($CDCl_3$) 7.55 (2H, bs, H1), 7.38 (2H, dd, $^3J_{HH}$=8.5 Hz, $^4J_{HH}$=1.6 Hz, H3), 7.11 (2H, d, $^3J_{HH}$=8.5 Hz, H4), 1.60 (6H, s, CH_3); $^{19}F\{^1H\}$ NMR ($CDCl_3$) −81.32 (6F, t, $^4J_{FF}$=10.6 Hz, CF_3), −110.58 (4F, t, $^4J_{FF}$=14.6 Hz, CF_2), −121.89 (4F, m, CF_2), −122.38 (4F, m, CF_2), −123.26 (4F, m, CF_2), −126.60 (4F, m, CF_2). ^{13}C NMR ($CDCl_3$) 152.8, 130.4, 127.0, 125.9, 125.0, 124.6, 124.2, 117.4, 34.6, 32.9.

4.1.7. 9,9-Dimethylxanthene-2,7-dicarbaldehyde (4). 2,7-Dibromo-9,9-dimethylxanthene (1.40 g, 3.80 mmol) was dissolved in diethyl ether (100 mL) and cooled to −78 °C. *n*-BuLi (5.10 mL of a 1.6 M solution in hexane, 8.16 mmol) was added over 30 min and the solution allowed to warm to room temperature over 90 min. After stirring for 4 h at room temperature, the solution was cooled to −78 °C and *N*,*N*-dimethylformamide (3.00 mL, 38.79 mmol) added. The solution was allowed to warm to room temperature overnight to give a yellow solution with a white precipitate. This was hydrolysed (100 mL), the organic layer separated, dried with sodium sulfate and the solvent removed in vacuo.

The yellow solid was recrystallised from ethanol to give a yellow solid (0.57 g, 56%). Mp 109–110 °C. *m/z* (FAB): 267 (MH^+). HRMS (FAB) 267.1022. Calcd for $C_{17}H_{14}O_3$ 267.1021. ^{1}H NMR ($CDCl_3$) 9.88 (2H, s, CHO), 7.93 (2H, d, $^4J_{HH}$=1.8 Hz, H1), 7.70 (2H, dd, $^3J_{HH}$=8.3 Hz, $^4J_{HH}$= 1.8 Hz, H3), 7.14 (2H, d, $^3J_{HH}$=8.3 Hz, H4), 1.65 (6H, s, CH_3). ^{13}C NMR ($CDCl_3$) 191.1, 154.6, 133.2, 130.8, 128.9, 117.9, 34.6, 31.4.

4.1.8. 2,7-Bis(*1H,2H,3H,3H*-perfluorononon-1-enyl)-9,9-dimethylxanthene (5). 9,9-Dimethylxanthene-2,7-dicarbaldehyde (3.30 g, 12.41 mmol), $[Ph_3PCH_2CH_2C_6F_{13}]^+I^-$ (20.13 g, 27.35 mmol) and potassium carbonate (4.46 g, 32.09 mmol) were heated to 110 °C for 3 days in dioxane (90 mL) and water (3 mL). After cooling, the solvent was removed in vacuo, the solid redissolved in dichloromethane (100 mL) and washed well with water. After drying with magnesium sulphate, the solvent was removed in vacuo and passed through a silica plug with hexane. The solvent was removed in vacuo to give a white glass (2.57 g, 22%). b.p. 202 °C at 0.05 mm Hg. Anal. calcd for $C_{33}H_{20}OF_{26}$: C, 42.77; H, 2.16. Found: C, 42.81; H, 2.17. *m/z* (FAB): 925 (M−H). ^{1}H NMR ($CDCl_3$) 7.23 (2H, m, H1), 6.99 (4H, m, H2, H3), 6.75 (2H, d, $^3J_{HH}$=11.4 Hz, C*H*Ar), 5.63 (2H, dt, $^3J_{HH}$=11.4 Hz, $^3J_{HH}$=7.3 Hz, C*H*CH_2), 3.02 (td, $^3J_{HF}$= 18.4 Hz, $^3J_{HH}$=7.3 Hz, CH_2), 1.55 (6H, s, CH_3); ^{19}F{^{1}H} NMR ($CDCl_3$) −81.27 (6F, t, $^4J_{FF}$=8.5 Hz, CF_3), −113.54 (4F, t, $^4J_{FF}$=14.2 Hz, α-CF_2), −122.37 (4F, m, CF_2), −122.39 (4F, m, CF_2), −123.68 (4F, m, CF_2), −126.66 (4F, m, CF_2). ^{13}C NMR ($CDCl_3$) 150.0, 135.7, 131.3, 130.2, 128.8, 126.8, 119.5, 118.0, 34.3, 32.6, 30.9 (t, $^2J_{CF}$= 22.2 Hz).

4.1.9. 2,7-Bis(*1H,1H,2H,2H,3H,3H*-perfluorononanyl)-9,9-dimethylxanthene (6). 2,7-Bis(*1H,2H,3H,3H*-perfluorononon-1-enyl)-9,9-dimethylxanthene (2.50 g, 2.70 mmol) was dissolved in dichloromethane (75 mL) and palladium on charcoal (0.25 g) added. The mixture was stirred overnight under an atmosphere of hydrogen and then filtered through a celite plug. The solvent was removed to give a white solid (2.49 g, 99%). Mp 101–102 °C. Anal. calcd for $C_{33}H_{24}OF_{26}$: C, 42.58; H, 2.58. Found: C, 42.63; H, 2.51. *m/z* (FAB): 929 (M−H). ^{1}H NMR ($CDCl_3$) 7.11 (2H, m, H1), 6.90 (4H, m, H2, H3), 2.61 (2H, t, $^3J_{HH}$= 7.1 Hz, CH_2), 1.88 (4H, m, CH_2CH_2), 1.54 (6H, s, CH_3). ^{19}F{^{1}H} NMR ($CDCl_3$) −81.40 (6F, t, $^4J_{FF}$=10.0 Hz, CF_3), −114.57 (4F, t, $^4J_{FF}$=13.9 Hz, α-CF_2), −122.41 (4F, m, CF_2), −122.38 (4F, m, CF_2), −124.02 (4F, m, CF_2), −126.65 (4F, m, CF_2). ^{13}C NMR ($CDCl_3$) 148.1, 134.1, 129.0, 128.1, 126.3, 115.5, 33.6, 33.1, 31.2, 29.2 (t, $^2J_{CF}$= 22.3 Hz), 21.1.

4.1.10. [{4,5-Bis-(bis(4-tridecafluoro-*n*-hexyl-phenyl)-phosphino)-9,9-dimethylxanthene}$PtCl_2$]. A slurry of *cis*-[$PtCl_2(MeCN)_2$] (0.009 g, 0.027 mmol) and 4,5-bis-(bis(4-tridecafluoro-*n*-hexyl-phenyl)phosphino)-9,9-dimethylxanthene (0.050 g, 0.027 mmol) in dichloromethane (20 mL) was heated to reflux for 15 h in a sealed tube under nitrogen to give a clear solution. After cooling, the solvent was removed in vacuo and the resulting solid was washed well with petroleum ether and dried in vacuo to give a white solid (0.024 g, 42%). Anal. calcd for $C_{63}H_{28}OP_2Cl_2F_{52}Pt$: C, 35.74; H, 1.32. Found: C, 35.69; H, 1.26. *m/z* (FAB): 2081 (M−Cl). ^{1}H NMR ($CDCl_3$) 7.66 (2H, d, $^3J_{HH}$=7.1 Hz, H3), 7.49 (8H, m), 7.29 (12H, m), 1.49 (6H, s, CH_3). ^{19}F{^{1}H} NMR ($CDCl_3$) −81.38 (12F, t, $^4J_{FF}$=8.5 Hz, CF_3), −112.50 (4F, m, CF_2), −112.68 (4F, m, CF_2), −122.11 (8F, m, CF_2), −122.99 (8F, m, CF_2), −123.49 (8F, m, CF_2), −126.85 (8F, m, CF_2). ^{31}P{^{1}H} NMR ($CDCl_3$) 7.5 (s, $^1J_{PtP}$=3674 Hz).

4.1.11. [{Xantphos}$PtCl_2$]. Prepared as above from Xantphos (0.139 g, 0.24 mmol) and *cis*-[$PtCl_2(MeCN)_2$] (0.083 g, 0.24 mmol) giving the product as a white powder (0.183 g, 90%). Anal. calcd for $C_{39}H_{32}OP_2Cl_2Pt$: C, 55.45; H, 3.79. Found: C, 55.39; H, 3.61. *m/z* (FAB): 844 (M^+). ^{1}H NMR ($CDCl_3$) 7.80 (2H, d, $^3J_{HH}$=7.3 Hz, H3), 7.70–7.10 (24H, m), 2.04 (6H, s, CH_3). ^{31}P{^{1}H} NMR ($CDCl_3$) 5.90 (s, $^1J_{PtP}$=3694 Hz).

4.2. Catalysis

An autoclave, fitted with a substrate injector containing 1-octene (1.0 mL, 6.37 mmol), a mechanical stirrer, a gas delivery system, an injection port and a thermocouple was flushed with CO/H_2 (1:1) to remove air. Degassed toluene (4.0 mL) containing dicarbonyl(2,4-pentanedionato)-rhodium(I) ([Rh(acac)$(CO)_2$], 0.01 mmol) and ligand (2 or Xantphos) (0.022 mmol) was added through the injection port against a stream of CO/H_2 using a syringe. The autoclave was pressurised with CO/H_2 (1:1) to 20 bar and the pressure released. This flushing procedure was repeated twice more. It was repressurised to 16 bar, the stirrer was started (600 rpm) and the autoclave was heated to 80 °C for 45 min. The 1-octene was then added to the autoclave by forcing it in through the substrate injector using a CO/H_2 pressure of 20 bar. The data recorder was started and the temperature, pressure in the autoclave and pressure in a ballast vessel, from which gas was fed into the autoclave through a mass flow controller to keep the pressure within the autoclave constant at 20 bar, were monitored and recorded every 5 s. After gas uptake had become very slow (5–8 h), the stirrer was stopped and the autoclave was allowed to cool. The gases were vented and the mixture was syringed into a sample vial for analysis by GC. Kinetic data were obtained from an analysis of the pressure drop in the ballast vessel. A similar reaction was carried out but using 2 (0.017 mmol) in perfluoromethylcyclohexane (4.0 mL). The resulting product consisted of two phases, both of them yellow. GC analysis was carried out on the upper phase.

Acknowledgements

We thank the Royal Society (EGH, AMS) and the EPSRC (DJA) for financial support.

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Tetrahedron 60 (2004) 4087–4096

Oxidation of allylic alcohols, amines, and sulfides mediated by assembled triphase catalyst of phosphotungstate and non-cross-linked amphiphilic copolymer

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Received 26 January 2004; accepted 19 February 2004

Abstract—A novel catalyst PWAA, an assembled complex of phosphotungstic acid ($H_3PW_{12}O_{40}$) and a non-cross-linked copolymer of *N*-isopropylacrylamide with an ammonium, was developed. It is an amphiphilic, cross-linked, and supramolecular insoluble complex and showed catalytic activity on oxidation with aqueous hydrogen peroxide. PWAA, used in 2.7×10^{-5}–2.0×10^{-3} mol equiv., catalyzed oxidation of allylic alcohols, amines, and sulfides efficiently. The turnover number (TON) of PWAA reached up to 35,000. PWAA showed a good stability in organic/aqueous media and was reused three to five times.

1. Introduction

A development of phase-transfer solid-phase catalyst that mediates aqueous–organic biphase reactions is one of the most important issues for recent synthetic organic chemistry and industrial engineering.[1,2] Using such triphase catalysts enables effective reactions between organic substrates and water-soluble reagents.[2] In addition to this, these solid-phase catalysts are easily separated by filtration or decantation from the system and are reused without any treatments. Hence, this catalytic system can enhance economy of the reaction and decrease environmental pollution under ideal conditions.

In order to realize this triphase catalytic oxidation system, one may say that using water-soluble oxidants such as hydrogen peroxide is suitable.[1] Although many toxic reagents such as Cr(VI), Mn(VII), Os(VIII) and Pb(IV) have been utilized for oxidation, such heavy metal species are stoichiometric or substoichimetric oxidants, and thus many toxic wastes must be disposed. On the contrary, hydrogen peroxide is sustainable and economical; it is a cheap and clean oxidant transformed into harmless water.[1c] Thus, the oxidation system promoted by triphase catalysts with hydrogen peroxide can fulfill all the requirements of economy, efficiency, and safety.

Over the past few decades, a considerable number of studies have been made on triphase catalysts which were immobilized with cross-linked polystyrene resins, silica gels or metals. These catalytic systems, however, generally resulted in lower catalytic activity compared with their soluble counterparts, and were often obliged to use hazardous chlorohydrocarbon solvents.[3] Besides, reuse of these catalysts was often difficult owing to the gradual decline of the catalytic activity. These problems made them less practical. Therefore, we decided to concentrate on developing triphase catalysts that were highly active and reusable.

In traditional triphase catalysts, as we have mentioned before, a catalytic species was anchored to a linker that was immobilized to a polymer resin or silica gel (Scheme 1, above). In our approach, however, the insoluble catalysts were constructed from self-assembly process of non-cross-linked amphiphilic copolymer ligands and inorganic species.[4] This process would promote the cross-linking of the copolymer by the inorganic species to provide networked, supramolecular, and insoluble complexes (Scheme 1, below). They might possess many mesopores where the inorganic species would be tightly supported by many ligands not to be dissociated. We expected such complexes should act as highly active catalysts based on the following points: (1) the complexes have a characteristic high-to-volume ratio to react with a substrate and a reagent, (2) they can capture these reagents effectively by their mesopores, and (3) the amphiphilic copolymers and the inorganic species might construct effective catalytic sites

Keywords: Catalysis; Oxidation; Polymer support; Self-assembly; Tungsten and compounds.
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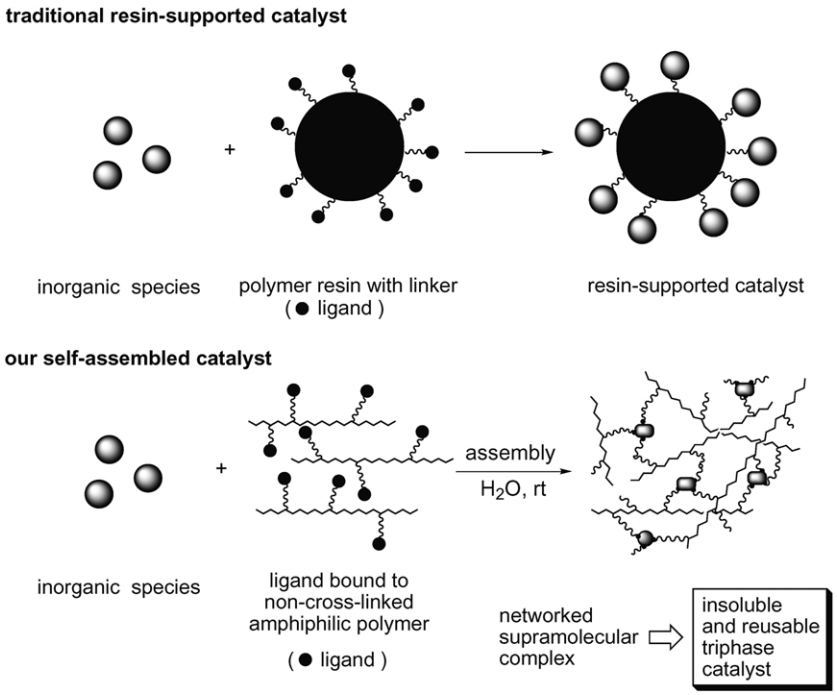
doi:10.1016/j.tet.2004.02.072



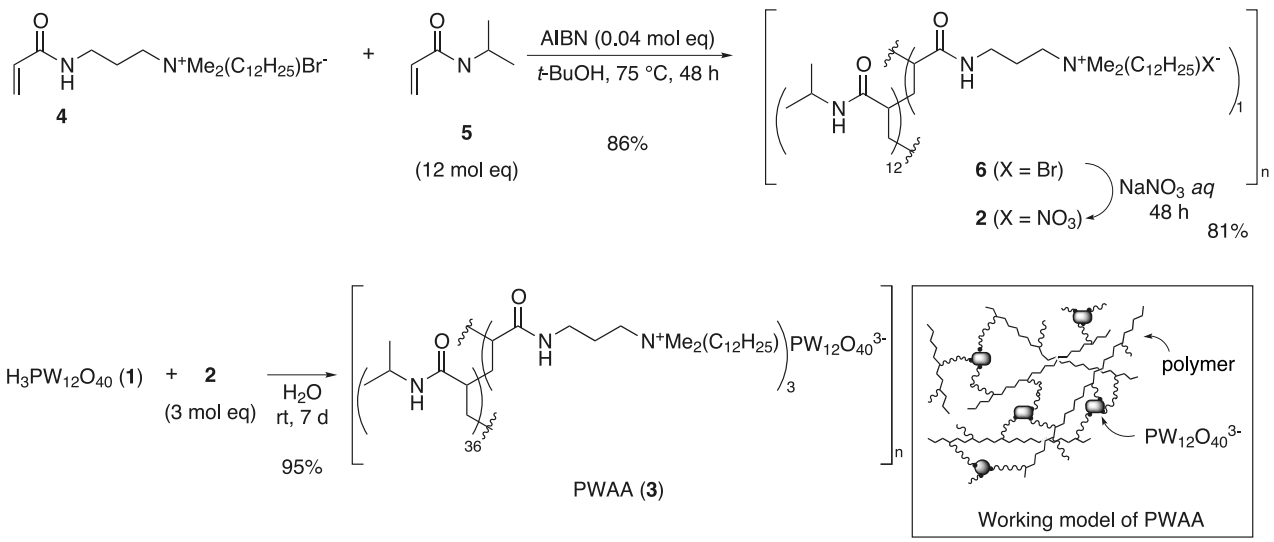
Scheme 1. Hypothesis of formation of a self-assembled complex for an insoluble oxidation catalyst.

with high affinity to both hydrophobic and hydrophilic reagents.

We herein report the full detail of our approach: a development of a highly active triphase catalyst PWAA (**3**), formed from phosphotungstic acid ($H_3PW_{12}O_{40}$) (**1**) and poly{[3-(*a*cryloylamino)propyl]dodecyldimethylammonium nitrate}-*co*-(*N*-isopropyl*a*crylamide)$_{12}$} (**2**) and its application to oxidation of allylic alcohols, amines and sulfides.[5] It was found that PWAA has a good catalytic activity on oxidation in both aqueous and organic solvent. It should be noted that the turnover number (TON=mol of a product/mol of a catalyst) of PWAA reached up to 35,000.

2. Preparation of a triphase catalyst PWAA

PWAA was prepared as shown in Scheme 2. Ammonium salt **4** was synthesized from commercially available *N*-[3-(dimethylamino)propyl]acrylamide with 1-bromododecane in 92% yield. Random copolymerizations of **4** with 12 mol equiv. of **5** were performed in the presence of 0.04 mol equiv. of AIBN in *t*-BuOH at 75 °C for 48 h, resulting in that non-cross-linked polymer **6** was formed. It was an amphiphilic polymer that was soluble in water, *t*-BuOH, and CH_2Cl_2. The ratio of the *N*-isopropylacryl amide unit to the ammonium unit to be 12/1 was determined by ^{1}H NMR. The bromide **6** was ion-exchanged to the



Scheme 2. Preparation of a novel triphase catalyst PWAA with the structure **3**.

nitrate **2**.[6] The molecular weight of **2** was wide-ranging (thousands to tens of thousands) as a result of gel-permeation chromatography relative to polystyrene standards. Thus, complexation to form PWAA was carried out according to the procedure for the preparation of $[\pi\text{-}C_5H_5N(CH_2)_{15}CH_3]_3PW_{12}O_{40}$.[7] A self-assembly process of **1** with **2** (3 mol equiv. as an ammonium unit) in water at room temperature resulted in the formation of white insoluble precipitates simultaneously. After stirring for 7 days at the same temperature, the resulted precipitate was washed and dried to give PWAA as white lumps.[8] PWAA were insoluble in H_2O and organic solvents such as MeOH, EtOH, *i*-PrOH, AcOEt, Me_2CO, CH_2Cl_2, toluene, Et_2O and hexane.

To elucidate the structure of PWAA, several spectroscopic measurements were examined. The elementary analysis showed that one complex unit of $\mathbf{3}\cdot 22H_2O$. The infrared spectrum of PWAA exhibited strong vibrations at 1080 (P═O), 978 (W═O), 897 and 818 cm^{-1}, while that of **1** exhibited them at 1080, 982, 893 and 808 cm^{-1}. Viewed in this light, the structure of the phosphotungstic acid unit of PWAA can be regarded as that of **1**.

Furthermore, we analyzed PWAA by gel-phase ^{31}P NMR; a broad singlet peak was detected at −13 ppm (Fig. 1). Since it was reported that the signals of $H_3PW_{12}O_{40}$ and $[\pi\text{-}C_5H_5N(CH_2)_{15}CH_3]_3PW_{12}O_{40}$ were observed at the similar frequency (−14.7 ppm[9] and −14.5 ppm,[10] respectively), it would be supported that phosphotungstate in PWAA maintained the heteropolyacidic structure such as the Keggin type ($PW_{12}O_{40}^{3-}$).[11]

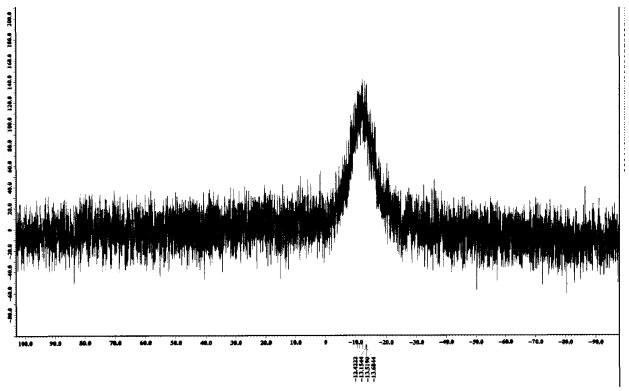


Figure 1. A gel-phase ^{31}P NMR chart of PWAA.

A scanning electron microscope (SEM) of PWAA was also investigated (Fig. 2). PWAA was treated with gold vapor by the sputter-coating method. It was observed that PWAA possessed many pores, whose diameter was about 1–10 μm (left) and hundreds nanometer or less (center). Further magnification (×50,000) showed many projections those lengths were less than 100 nm (right); all these things make it clear that PWAA has a high surface-to-volume ratio and many reactive sites. The further structural investigation of the catalyst is now under the way.

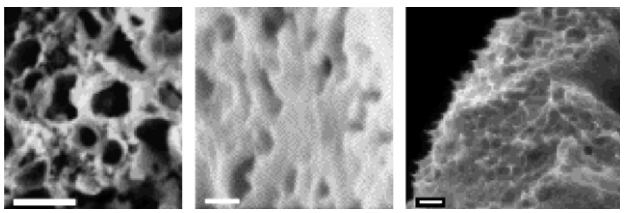
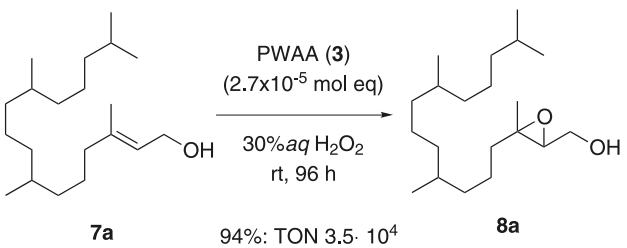


Figure 2. Scanning electron micrographs (SEM) of PWAA; (left): scale bar: 10 μm; (center): scale bar: 500 nm; (right): scale bar: 100 nm.

3. Epoxidation of allylic alcohols catalyzed by PWAA

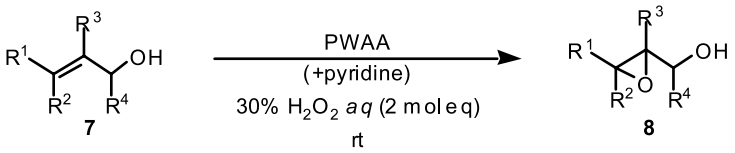
With the insoluble complex PWAA in hand, the epoxidation of allylic alcohols with aqueous H_2O_2 under the organic solvent-free conditions was examined.[12,13] We were pleased to find that PWAA showed a very high catalytic activity on epoxidation. In the presence of 2.7×10^{-5} mol equiv. of PWAA, the reaction of phytol (**7a**) with 2 mol equiv. of 30% aqueous H_2O_2 resulted in the corresponding epoxy alcohol **8a** in 94% yield (Scheme 3). TON of PWAA was approximately 35,000. This result showed that PWAA has a excellent catalytic activity among the precedent.

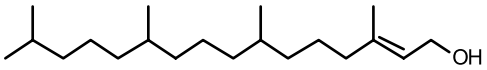
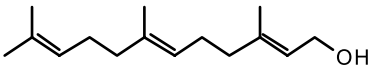
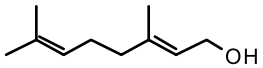
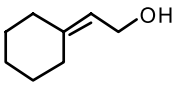
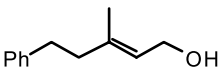
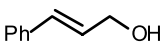
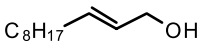
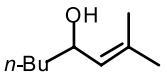
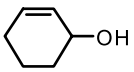
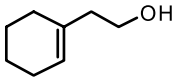
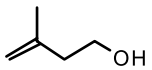


Scheme 3. Epoxidation of phytol (**7a**) promoted by PWAA.

Since the catalytic activity of PWAA was evaluated, a series of epoxidation of several allylic alcohols was performed in the presence of 5.0×10^{-4} mol equiv. of PWAA (Table 1). Hydrophobic substrates of **7a** and farnesol (**7b**) were converted to the corresponding epoxides in high yields. Epoxidation of **7a** proceeded in 7 h at room temperature to give **8a** in 96% yield with TON reaching approximately 2000 (entry 1). In the reaction of **7b**, 2,3-epoxy alcohol **8b** was obtained in 84% yield (entry 2). In this case, other trialkylsubstituted alkene moieties were intact. The epoxidation of less hydrophobic geraniol (**7c**) was messy to afford **8c** in 12% yield owing to acidic hydrolysis of the epoxide (entry 3). We found that the addition of a trace amount of pyridine was effective to reduce an acidity in this system and thus to prevent the epoxide-opening reactions.[14] The reaction of **7c** in the presence of PWAA and 6.0×10^{-3} mol equiv. of pyridine for 15 h resulted in the formation of **8c** in 80% yield (entry 4), where the C(6)–(7) double bond was not affected. Under identical conditions, trisubstituted allylic alcohols such as an exocyclic allylic alcohol **7d** and a linear one **7e** provided the corresponding epoxides in high yields (entries 5 and 6). Although disubstituted allylic alcohols were less reactive, they were converted to the corresponding epoxides in quantitative yields by using 2.0×10^{-3} mol equiv. of PWAA (entries 7 and 8). Besides, the diasteroselective epoxidation of 2-methyl-2-octen-4-ol (**7h**) furnished the *threo*-selective

Table 1. Epoxidation of allylic alcohols promoted by PWAA



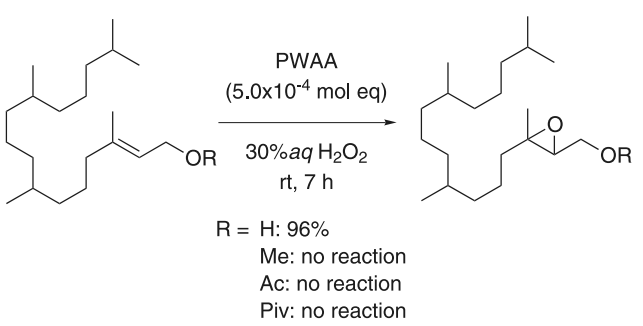
Entry	Substrate		**3b** (mol equiv.)	Pyridine (mol equiv.)	Time (h)	Yield (%)[a]
1		**7a**	5.0×10^{-4}	—	7	**8a**:96
2		**7b**	5.0×10^{-4}	—	13	**8b**:84
3		**7c**	5.0×10^{-4}	—	37	**8c**:12
4		**7c**	5.0×10^{-4}	6.0×10^{-3}	15	**8c**:80
5		**7d**	5.0×10^{-4}	6.0×10^{-3}	12	**8d**:83
6		**7e**	5.0×10^{-4}	6.0×10^{-3}	13	**8e**:96
7		**7f**	2.0×10^{-3}	9.6×10^{-2}	30	**8f**:quant
8		**7g**	2.0×10^{-3}	2.4×10^{-2}	33	**8g**:quant
9		**7h**	2.0×10^{-3}	9.6×10^{-2}	85	**8h**:73 (threo/erythro=91:9)
10		**7i**	2.0×10^{-3}	2.4×10^{-2}	—	[b]
11		**7j**	2.0×10^{-3}	2.4×10^{-2}	24	[c]
12		**7k**	2.0×10^{-3}	2.4×10^{-2}	—	[b]
13		**7l**	2.0×10^{-3}	2.4×10^{-2}	—	[b]

[a] Isolated yields.
[b] The product was not isolated because the reaction was messy.
[c] No reaction.

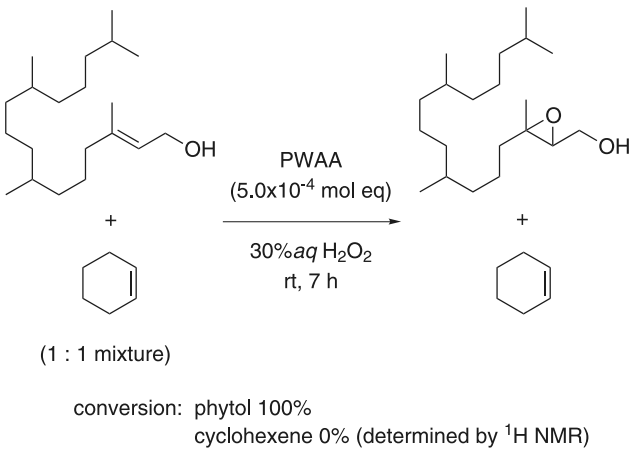
epoxy alcohol **8h** in 73% yield (*threo*/*erythro* (91:9)) (entry 9).

We further investigated the oxidation of cyclic allylic alcohol. In the case of 2-cyclohexen-1-ol (**7i**), decomposition of the product was so fast that the reaction was messy. This result might suggest that PWAA was not able to activate a *s-trans* allylic alcohol efficiently. In respect of chemoselectivity, cyclohexene (**7j**), an unmodified alkene, was not converted to cyclohexene oxide at all. Moreover, the epoxidation of homoallylic alcohols **7k** and **7l** was also slow and did not provide the corresponding products. These results indicated that hydroxyl group at allylic position was essential to proceed the epoxidation efficiently.

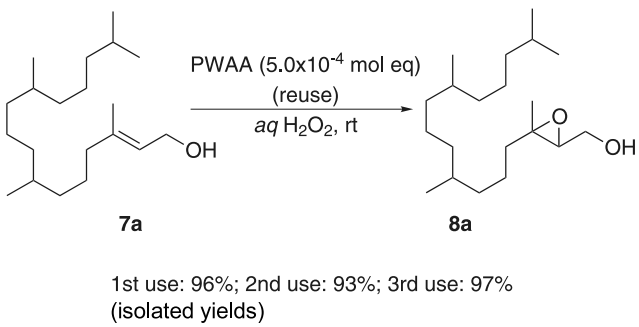
For reasons mentioned above, we investigated the proximity effect of allylic alcohol (Scheme 4). Epoxidation of a phytol-methyl ether, a phytol-acetyl ester and a phytol-pivaloyl ester under identical conditions did not proceed at all. Besides, the mixture of phytol (**7a**) and cyclohexene (**7j**) resulted in the quantitative conversion of phytol and the no reaction of cyclohexene under the identical conditions



Scheme 4. The epoxidation of phytol derivatives.



Scheme 5. Epoxidation of the mixture of phytol and cyclohexene promoted by PWAA.



Scheme 6. Epoxidation of phytol (**7a**) catalyzed by recycled PWAA.

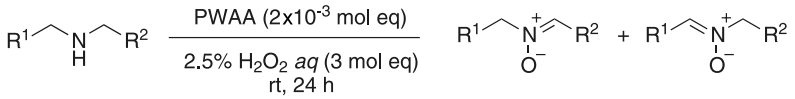
(Scheme 5). Considered these results, epoxidation was promoted by the interaction of hydroxyl group of allylic alcohol with PWAA which is similar to early transition metal catalyses.[15]

Moreover, recycling of PWAA in case of **7a** was examined as shown in Scheme 6. It was found that PWAA was reused three times; in the first to third cycle runs, the product **8a** was given in 96, 93, and 97% yields, respectively. The activity of PWAA was unchanged under the oxidation conditions through the consecutive runs, although PWAA was pulverized through runs.

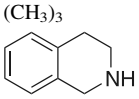
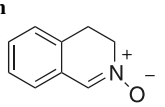
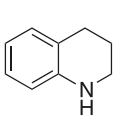
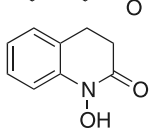
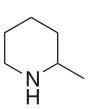
4. Oxidation of amines catalyzed by PWAA

Since PWAA efficiently promoted the epoxidation of allylic alcohols, we applied this oxidation to heteroatoms: secondary amines and sulfides.[16,17] Oxidation of secondary amines is the most straightforward method and the direct route to prepare nitrones, which are important substrates for the synthesis of nitrogen-containing bioactive compounds. It was beforehand confirmed that no oxidation of dibenzylamine (**9a**) with hydrogen peroxide was observed at room temperature. On the contrary, addition of 2×10^{-3} mol equiv. of PWAA to this reaction system proceeded oxidation to give the corresponding nitrone (**10a**) in 86% yield (Table 2, entry 1).[18] Bis(*p*-substituted benzyl)amines were also converted to the corresponding oximes under similar conditions. The reaction of bis[(4-trifluoromethyl)benzyl]amine (**9b**) proceeded smoothly to afford **10b** in 90% yield. TON of PWAA in this oxidation reached 450. However, bis(4-chlorobenzyl)amine (**9c**) and

Table 2. Oxidation of secondary amines by PWAA



9 **10** **10'**

Entry	Amines	R^1	R^2	Temperature (°C)	Time (h)	Nitrone	Yield (%)[a]
1	**9a**	Ph	Ph	rt	24	**10a**	89
2	**9b**	*p*-CF_3–C_6H_4	*p*-CF_3–C_6H_4	rt	24	**10b**	90
3	**9c**	*p*-Cl–C_6H_4	*p*-Cl–C_6H_4	rt	24	**10c**	56
4	**9d**	*p*-MeO–C_6H_4	*p*-MeO–C_6H_4	rt	48	**10d**	62
5	**9e**	*p*-MeO–C_6H_4	*p*-CF_3–C_6H_4	rt	24	**10e**+**10'**	94 (**10e**+**10e'**=1.7/1)
6	**9f**	Ph	*p*-CF_3–C_6H_4	rt	24	**10f**+**10f'**	80 (**10f**+**10f'**=1.3/1)
7	**9g**	Ph	*p*-CN–C_6H_4	40	24	**10g**+**10g'**	71 (**10g**+**10g'**=1.5/1)
8	**9h**	$(CH_3)_3$	Ph	rt	24	**10h**	34
9	**9i**			rt	12	**10i** 	70
10	**9j**			rt	12	**10j** 	30
11	**9k**			rt	12	—	[b]

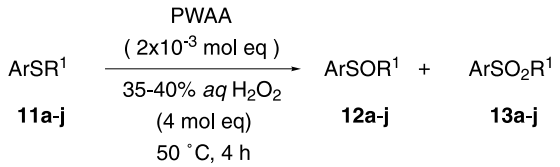
[a] Isolated yields.
[b] The product was not isolated because the reaction was messy.

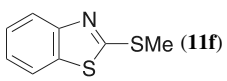
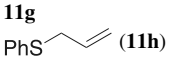
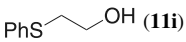
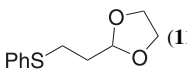
bis(4-methoxybenzyl)amine (**9d**) were converted to **10c** and **10d** in moderate yields. We further examined the regioselective oxidations of bis(*p*-substituted benzyl)amines. Although it seemed reasonable that the deprotonation took place at more acidic benzylic position selectively, the reactions gave nitrones in 71–94% yield albeit with low regioselectivity (1.3/1–1.7/1) (entries 5–7).[19] Turning to cyclic secondary amines, tetrahydroisoquinoline (**9i**) was converted to **10i**, which is the useful substrate for the synthesis of isoquinoline alkaloids, in 70% yield (entry 9).[16c] Oxidation of tetrahydroquinoline (**9j**) provided **10j** instead of the corresponding nitrone.[16d] (entry 10). The reaction of cyclic aliphatic amine **9k** was so messy because of the side reactions such as oxidative dimerization so that the corresponding product was not isolated (entry 11).

5. Oxidation of sulfides catalyzed by PWAA

Next, we turned our attention to oxidation of sulfides to sulfones (Table 3).[20,21] Sulfones have been utilized as the syntons for total synthesis of bioactive natural compounds. As depicted in parenthesis in Table 3, oxidation of **11a–k** with hydrogen peroxide in the absence of PWAA proceeded sluggishly to give mainly the corresponding sulfoxides **12a–k** in low to moderate yields[22] rather than sulfones **13a–k**. Meeting our expectations, PWAA efficiently proceeded the oxidation of sulfides to give sulfones under similar conditions. In the presence of 2×10^{-3} mol equiv. of PWAA, **11a** was converted into **13a** in 97% yield (entry 1). TON of PWAA reached approximately 500. The substituted aryl methyl sulfides were also converted to the corresponding sulfones in high yields (84–90%) (entries 3, 5 and 7). Similarly, oxidation of alkyl thiophenols also provided the corresponding sulfones in high yields (entries 9, 15, 17 and 19). It is notable that the catalytic system tolerates a wide variety of functional group. For example, methylthio benzothiazole (**11f**) that is a useful nucleophile for the Julia olefination[23] was converted to the sulfone **13f** in 78% yield (entry 11). In this reaction, benzothiazole ring was unaffected through the reaction. The chemoselective oxidation of methylthiobenzaldehyde (**11g**) proceeded to give **13g** in 86% yield with intactness of formyl group (entry 13). Besides, the oxidation of **11h** and **11i** proceeded efficiently to give **13h** and **13i** in high yields, where the olefin and alcohol were tolerated and β-elimination of the alcohol was not observed (entries 15 and 17). Sulfide **11j** with a cyclic acetal in the structure was converted to **13j**, which was the substrate for the preparation of the prostaglandin analogue, in 71% yield (entry 19).[24] On the other hand, the reaction of diphenyl sulfide **11k** hardly proceeded (entry 21).

Table 3. Oxidation of sulfides to sulfones with and without PWAA

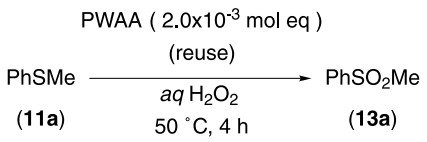


Entry	11	Catalyst	12 (%)[a]	13 (%)[a]
1[b]	PhSMe (**11a**)	PWAA	3	97
2[b]	**11a**	—	(74)	(26)
3	*p*-Me–C_6H_4SMe (**11b**)	PWAA	9	90
4	**11b**	—	(71)	(22)
5	*p*-Br–C_6H_4SMe (**11c**)	PWAA	12	87
6	**11c**	—	(70)	(15)
7	*p*-MeO–C_6H_4SMe (**11d**)	PWAA	6	84
8	**11d**	—	(76)	(24)
9	PhSEt (**11e**)	PWAA	3	91
10	**11e**	—	(75)	(17)
11[c]	 (**11f**)	PWAA	17	78
12[c]	**11f**	—	(9)	(0)
13	*p*-CHO–C_6H_4SMe (**11g**)	PWAA	—	86
14	**11g**	—	(53)	(33)
15[c]	 (**11h**)	PWAA	3	81
16[c]	**11h**	—	(80)	(10)
17	 (**11i**)	PWAA	—	Quant
18	**11i**	—	(80)	(13)
19	 (**11j**)	PWAA	11	71
20	**11j**	—	(54)	(trace)
21	PhSPh (**11k**)	PWAA	10	6
22	**11k**	—	4	(0)

The yields of the oxidations without PWAA were in parentheses.
[a] Isolated yields.
[b] 3 mol equiv. of H_2O_2 was used.
[c] The reaction was performed for 7 h.

The recycled activity of PWAA in the oxidation of sulfide **11a** was evaluated (Scheme 7). The oxidation of **11a** was performed under identical conditions, affording **13a** in 97% yield. In the repeated use of the recovered catalyst, PWAA mediated the second to fifth cycled runs to give **13a** in 82–88% yields. The reason to reduce yields in the second cycled run was unclear. One explanation for this may be that PWAA was pulverized and adsorbed onto the reaction vessel so that the efficiency of the reaction was reduced physically. In this respect, It was confirmed that the pulverization did not affect the intrinsic activity of PWAA: the recovered PWAA was analyzed by gel-phase ^{31}P NMR to show a broad peak at −13 ppm as well as the PWAA before use.[25]

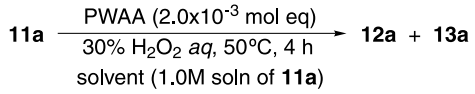
As stated above, all the reactions were performed under organic solvent-free conditions. PWAA was insoluble and stable in both aqueous and organic solvents. We expected that PWAA should efficiently catalyze the oxidation in any media. Hence, the activity of PWAA in an organic solvent was investigated. The reactions of **11a** to **13a** were carried out with various organic solvents as depicted in Table 4. Similar to the oxidation without organic solvent completed in 4 h to give **13a** in 97% yield (entry 1), the reaction with



1st use: 97%; 2nd use: 86%; 3rd use: 83%
4th use: 88%; 5th use: 82%
(isolated yields)

Scheme 7. Oxidation of **11a** catalyzed by recycled PWAA.

Table 4. Solvent effect on the oxidation catalyzed by PWAA



Entry	Solvent	**12a**[a]	**13a**[a]
1	(Neat)	3	97
2	Toluene	3	75
3	CH_2Cl_2	8	90
4	THF	8	91
5	Et_2O	—	96
6	DMF	—	100
7	EtOH	—	99

[a] Isolated yields.

aprotic hydrophobic solvents (toluene, CH_2Cl_2, THF, and Et_2O (entries 2–5)), hydrophilic solvent (DMF (entry 6)), and protic solvent (EtOH (entry 7)) progressed smoothly to furnish **13a** in high yields. On the contrary, the reaction in toluene proceeded slower to give **13a** in 75%. It seems reasonable that both organic solvent-free and -containing systems were effective for this oxidation.

6. Summary

In conclusion, we developed a highly active and reusable solid-phase catalyst, PWAA based on our strategy: the self-assembly of phosphotungstate and an ammonium salt with a non-cross-linked amphiphilic polymer. PWAA efficiently catalyzed the oxidations of allylic alcohols, amines, and sulfides with hydrogen peroxide in both aqueous and organic media. PWAA was reused three to five times and the turnover number of PWAA reached up to 35,000. While the reusable activity and stability of PWAA should be improved, we believe our concept will be useful for creating other solid-phase catalysts.[4]

7. Experimental

7.1. General

35–40% Hydrogen peroxide and $H_3PW_{12}O_{40}$ were used without any treatment Infrared (IR) spectra were recorded on a JASCO FT/IR-8000 Fourier transform infrared spectrophotometer. NMR spectra were recorded on a JEOL AL-400 spectrometer, opening at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR. Chemical shifts in $CDCl_3$ were reported in the δ scale relative to $CHCl_3$ (7.26 ppm for ^1H NMR and 77.00 ppm for ^{13}C NMR) as an internal reference. Gel-phase ^{31}P NMR spectra were recorded with a 600 MHz (^1H NMR) pulse Fourier transform NMR spectrometers in $CDCl_3$ suspension with 85% H_3PO_4 aqueous solution as an external standard. EIMS spectra were measured on JEOL SX-102A. Column chromatography was performed with silica gel Merck 60 (230–400 mesh ASTM).

7.1.1. [3-(Acryloylamino)propyl]dodecyldimethylammonium bromide (4). To the suspension of *N*-[3-(dimethylamino)propyl]acrylamide (1.0 mL; 6.07 mmol), Na_2CO_3 (0.161 g; 1.52 mmol) in MeOH and MeCN (12 mL each) was added $C_{12}H_{25}Br$ (2.92 mL; 12.1 mmol), and it was stirred at 60 °C for 48 h before it was filtered. The filtrate was evaporated, and purified by column chromatography (neutral silica gel; eluent: CH_2Cl_2/MeOH (gradient: 10/1–2/1 via 5/1) to afford **4** in 92% yield (2.27 g). Mp 57–70 °C; IR (KBr, cm^{-1}): 1628, 1670, 3443; ^1H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, J=7.1 Hz, 3H), 1.22–1.35 (m, 18H), 1.74 (m, 2H), 2.16 (m, 2H), 3.27 (s, 6H), 3.35–3.40 (m, 2H), 3.47–3.49 (m, 2H), 3.87 (m, 2H), 5.63 (d, J=11.5 Hz, 1H), 6.33 (d, J=17.1 Hz, 1H), 6.54 (dd, J=11.5, 17.1 Hz, 1H), 8.55 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.2, 22.7, 22.7, 22.9, 26.4, 29.2, 29.3, 29.4, 29.5, 29.6, 31.9, 36.4, 51.0, 62.9, 64.9. 126.5, 130.7, 166.40; MS (FAB): m/z 326 (M^++H, base peak); HRMS (FAB): calcd for $C_{20}H_{42}ON_2$ 326.3297, found 326.3300.

7.1.2. Poly{[3-(acryloylamino)propyl]dodecyldimethylammonium bromide}-*co*-(*N*-isopropylacrylamide)$_{12}$} (6). The solution of **4** (1.46 g; 3.60 mmol), *N*-isopropylacrylamide (4.89 g; 43.2 mmol) in *t*-BuOH (70 mL) was degassed by ultrasonication for 20 min under an argon atmosphere. After AIBN (23.7 mg; 0.144 mmol) was added and again degassed for 20 min, the solution was heated at 75 °C for 48 h under an argon atmosphere, and *t*-BuOH was evaporated. The residue was purified by sedimentation from CH_2Cl_2 and Et_2O to give **6** in 86% (5.44 g). IR (KBr, cm^{-1}): 1651, 3069, 3308; ^1H NMR (600 MHz, $CDCl_3$): δ 0.88 (t, J=9.6 Hz, 3H), 0.90–2.60 (m, 133H), 3.19–4.10 (m, 24H), 6.64 (br s, 13H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 13.8, 22.4, 26.1, 29.0, 29.2, 29.3, 31.6, 41.1, 42.1, 50.5, 174.2. Elementary anal. calcd for $C_{92n}H_{187n}N_{14n}O_{20n}Br_n$ as **6**·7$n$$H_2O$: C 58.5, H 10.0, N 10.4, found: C 58.8, H 10.3, N 10.3.

7.1.3. Poly{[3-(acryloylamino)propyl]dodecyldimethylammonium nitrate}-*co*-(*N*-isopropylacrylamide)$_{12}$} (2). The mixture of **6** (1.72 g) and 0.2 M aqueous $NaNO_3$ was vigorously stirred for 41 h, followed by heated at 60 °C to precipitate **2**, and supernatant was decanted. The residue was washed with H_2O, and dried in vacuo (~0.08 mmHg) to give **2b** in 81% yield (1.38 g). IR (KBr, cm^{-1}): 1651, 3065, 3298; ^1H NMR (600 NMR, $CDCl_3$): δ 0.88 (t, J=9.6 Hz, 3H), 0.90–2.60 (m, 133H), 3.20–4.10 (m, 24H), 6.61 (br s, 13H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.0, 22.5, 22.6, 26.4, 29.2, 29.4, 29.5, 31.8, 41.2, 42.2, 50.4, 174.3. Elementary anal. calcd for $C_{92n}H_{187n}N_{15n}O_{23n}$ as **2**·7$n$$H_2O$: C 59.0, H 10.1, N 11.2, found: C 59.3, H 10.3, N 11.4.

7.1.4. Preparation of an assembled catalyst PWAA (3) from 1 with 2. When an aqueous solution of **1** (334 mg in 33 mL of H_2O; **1** was dissolved in H_2O by ultrasonication.) was added to an aqueous solution of **2** (608 mg in 116 mL H_2O; 3 mol equiv. as an ammonium unit) at room temperature, white insoluble precipitates were simultaneously yielded. After being stirred for 7 days at the same temperature, the precipitate was filtered on a glass filter, washed thoroughly with water, and dried in vacuo (~0.08 mmHg) to give **3** in 95% yield (870 mg) as white lumps: IR (KBr, cm^{-1}) 1080, 982, 893, 808; gel-phase ^{31}P NMR δ −13 (br s). Elementary anal. calcd for $C_{276n}H_{563n}N_{42n}O_{101n}P_nW_{12n}$ as PWAA·22$n$$H_2O$: C 39.8, H 6.3, N 7.1, found: C39.6, H 6.6, N 7.2.

7.2. General procedure for assembled catalyst-promoted epoxidation of allylic alcohols with hydrogen peroxide

A 25-mL flask equipped with a magnetic stirring bar was charged with 2.52 mmol of **7a**, 5.05 mmol of 30% aqueous. H_2O_2 and 1.26 μmol of PWAA. After the mixture was stirred at room temperature for 7 h, toluene (or Et_2O, AcOEt could be used.) was added, and PWAA was filtered. The organic layer was separated, washed with saturated aqueous. $Na_2S_2O_3$, dried in vacuo, and purified by flash column chromatography (SiO_2; EtOAc/hexane=1:4 to 1:2) to give the epoxy alcohol **8a** in 96% isolated yield.

7.2.1. 2,3-Epoxy-3-methyl-5-phenyl-1-pentanol (8e). Colorless oil; IR (neat, cm^{-1}) 3406, 2932, 1454, 1032, 752, 702; ^1H NMR (400 MHz, $CDCl_3$): δ 1.34 (s, 3H), 1.72–1.80 (m, 1H), 1.94–2.01 (m, 1H), 2.32 (br s, 1H), 2.64–2.79 (m, 2H), 2.88 (dd, 1H, J=4.2, 6.6 Hz), 3.63 (br dd, 1H, J=6.6, 11.6 Hz), 3.73 (br dd, 1H, J=4.2, 11.6 Hz), 7.15–7.29 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.9, 31.3, 40.3, 60.9, 61.3, 63.0, 125.9, 128.1, 128.3, 128.4, 141.0; MS (EI): m/z 192 (M^+), 174, 143, 131, 117, 105, 91; HRMS (EI): calcd for $C_{12}H_{16}O_2$ 192.1150, found 192.1159.

7.3. General procedure for the oxidation of amines catalyzed by PWAA

To a suspension of PWAA (40 mg; 5×10^{-3} mmol) and **9a** (2.52 mmol) was added 2.5% H_2O_2 aqueous solution (7.56 mmol) dropwise for 50 min at 0 °C. The mixture was stirred at room temperature for 24 h, before it was diluted with AcOEt and filtered through a glass filter. Brine was added to the filtrate, and it was extracted with AcOEt (×3). The extract was washed with brine, dried over Na_2SO_4, filtered, dried in vacuo, and purified by column chromatography (SiO_2; EtOAc/hexane=1:5) to give **10a** in 86% yield.

7.3.1. *N*-((*Z*)-4-Methoxybenzylidene-4′-methoxybenzyl) *N*-oxide (10d). Mp 122–125 °C; IR (KBr, cm^{-1}): 1246, 2920 cm; ^1H NMR (400 MHz, $CDCl_3$): δ 3.81 (s, 3H), 3.82, (s, 3H), 4.95 (s, 2H), 6.88–6.94 (m, 4H), 7.26 (s, 1H), 7.37–7.41 (m, 2H), 8.17–8.21 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 55.3, 55.3, 70.1, 113.6, 114.2, 123.3, 125.3, 130.4, 130.6, 133.2, 159.8, 160.8; MS (EI): m/z 271 (M^+), 121 ($M^+-N(O)$=CHArOMe, base peak); HRMS (EI): calcd for $C_{16}H_{17}O_3N$ 271.1208, found 271.1205.

7.3.2. *N*-4-Methoxybenzyl-*N*-4-trifluoromethylbenzylamine (9e). To a solution of 5% Pd/C (80 mg) in methanol (27 mL) under H_2 atmosphere was added 4-trifluoromethylbenzaldehyde (1.23 mL; 9 mmol) and 4-methoxybenzaldehyde (1.08 mL; 9.9 mmol), and the resulting mixture was stirred at rt for 5 h. After the substrate was consumed (checked by TLC), the suspension was filtered. The filtrate was evaporated, purified by column chromatography (SiO_2; MeOH/CH_2Cl_2=1:100) to give **9e** in 72% yield (1.73 g; 6.50 mmol). Mp 29–30 °C; IR (neat, cm^{-1}): 1327, 3337; ^1H NMR (400 MHz, $CDCl_3$): δ 1.73 (br s, 1H), 3.73 (s, 2H), 3.79 (s, 3H), 3.84 (s, 2H), 6.87 (d, J=8.5 Hz, 2H), 7.25 (d, J=8.5 Hz, 2H), 7.45 (d, J=8.1 Hz, 2H), 7.57 (d, J=8.1 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.8, 52.9, 55.6, 114.1, 123.1, 125.4, 125.4, 125.4, 125.5, 125.8, 128.5, 129.1, 129.5, 132.2, 144.6, 158.9; MS (EI): m/z 295 (M^+), 159 ($M^+-NHCH_2ArOCH_3$), 121 ($M^+-NHCH_2ArOCF_3$, base peak); HRMS (EI): calcd for $C_{16}H_{16}ONF_3$ 295.1184, found 295.1180.

7.3.3. *N*-(*Z*)-4-Methoxybenzyl-*N*-4-(trifluoromethyl)-benzylidene *N*-oxide (10e). Mp 124–129 °C; IR (KBr, cm^{-1}): 1327, 3072 cm; ^1H NMR (400 MHz, $CDCl_3$): δ 3.82 (s, 3H), 5.02 (s, $CH_3OArCH_2N(O)$=CHArCF$_3$, 2H), 6.93–6.96 (m, 2H), 7.39–7.41 (m, 3H), 7.63 (d, J=8.3 Hz, 2H), 8.30 (d, J=8.3 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 55.4, 71.1, 114.4, 122.3, 124.6, 125.0, 125.1, 125.2, 128.4, 130.8, 131.1, 131.5, 132.2, 133.4, 160.1; MS (EI): m/z 309 (M^+), 121 ($M^+-N(O)$ =CHArCF$_3$, base peak); HRMS (EI): calcd for $C_{16}H_{14}O_2NF_3$ 309.0977, found 309.0971.

7.3.4. *N*-(*Z*)-4-Methoxybenzylidene-*N*-4-(trifluoromethyl)benzyl *N*-oxide (10e′). Mp 124–128 °C; IR (KBr, cm^{-1}): 1327, 3082; ^1H NMR (400 MHz, $CDCl_3$): δ 3.84 (s, 3H), 5.07 (s, CH_3OArCH=N(O)CH_2ArCF_3, 2H), 6.93 (d, J=8.8 Hz, 2H), 7.42 (s, CH_3OArCH=N(O)CH_2ArCF_3, 1H), 7.61 (d, J=8.3 Hz, 2H), 7.66 (d, J=8.3 Hz, 2H), 8.22 (d, J=8.8 Hz, 2H); ^{13}C NMR (100 MHz NMR, $CDCl_3$): δ 55.4, 70.0, 113.8, 123.0, 125.6, 125.7, 129.1, 130.5, 131.0, 134.2, 137.2, 161.1; MS (EI): m/z 309 (M^+), 159 ($M^+-N(O)$=CHArOCH$_3$, base peak); HRMS (EI): calcd for $C_{16}H_{14}O_2NF_3$ 309.0977, found 309.0978.

7.3.5. *N*-(*Z*)-4-Benzyl-*N*-4-nitrilebenzylidene *N*-oxide (10g). Mp 143–150 °C; IR (KBr, cm^{-1}): 2224, 3034; ^1H NMR (400 MHz, $CDCl_3$): δ 5.09 (s, ArCH_2-N(O)=CHArCN, 2H), 7.40–7.49 (m, *Ar*CH2N(O)= C*H*ArCN, 6H), 7.66 (d, J=8.6 Hz, 2H), 8.29 (d, J=8.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 71.9, 112.9, 118.4, 128.3, 129.0, 129.2, 132.0, 132.3, 132.4, 134.0; MS (EI): m/z 236 (M^+), 91 ($M^+-N(O)$=CHArCN, base peak); HRMS (EI): calcd for $C_{15}H_{12}ON_2$ 236.0950, found 236.0947.

7.3.6. *N*-(*Z*)-4-Benzylidene-*N*-4-nitrilebenzyl *N*-oxide (10g′). Mp 140–144 °C; IR (KBr, cm^{-1}): 2224, 3034; ^1H NMR (400 MHz, $CDCl_3$): δ 5.11 (s, ArCH=N(O)CH_2-ArCN, 2H), 7.39–7.44 (m, 3H), 7.51 (s, ArC*H*=N(O)-CH2ArCN, 1H), 8.29 (d, J=8.6 Hz, 2H), 8.21–8.24 (m, 2H); ^{13}C NMR (100 MHz, CDCl3): δ 70.1, 112.4, 117.9, 128.1, 128.2, 129.0, 129.6, 130.4, 132.1, 134.5, 137.9; MS (EI): m/z 236 (M^+), 116 ($M^+-N(O)$=CHAr, base peak); HRMS (EI): calcd for $C_{15}H_{12}ON_2$ 236.0950, found 236.0944.

7.4. General procedure for the oxidation of sulfides to sulfones catalyzed by PWAA

The mixture of PWAA, **11a** and 35–40% H_2O_2 aqueous solution was shaken by PetiSyther® (Shimadzu Scientific Research Inc. Japan) at 700 rpm at 50 °C for 4 h, it was diluted with AcOEt and filtered. To the filtrate was added saturated $Na_2S_2O_3$ and brine, and it was extracted with AcOEt (×3), dried over Na_2SO_4, filtered, dried in vacuo, and purified by column chromatography (SiO_2; MeOH/CH_2Cl_2=1:100) to give **13a** in 97% yield. While the shaker (PetiSyther®) for solid-phase syntheses was used in these

reactions, the glassware vessel equipped with a magnetic stirrer enabled to be also used.

Acknowledgements

We thank Ms. Junko Shimode and Ms. Maroka Kitsukawa (Teikyo University) for spectroscopic measurement, and Mr. Kiyoshi Abe (Teikyo University) and Mr. Shin-ichirou Kawabata (JEOL Ltd) for SEM measurement. This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Technology. Y.M.A.Y. thanks the Inoue Foundation for Science (IFS) for Inoue Research Award for Young Scientists, and Dainippon Ink and Chemicals, Inc. Award in Synthetic Organic Chemistry, Japan.

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18. The reaction oxidized by more concentrated H_2O_2 aqueous solution was less successful because dimerization of the substrates occurred. Besides, recycling of PWAA in case of **9a** was not efficient to afford **10a** in lower yield.

19. These oxidations were controlled kinetically, and the regioselectivity for the formation of nitrones was determined at the oxidation stage of dibenzyl hydroxyamines to nitrones. It was assured by the following results: no isomerization between **10e** and **10e′** was observed in the reaction of **10e** or **10e′** in the presence of PWAA and 2.5% H_2O_2; the reaction of **9e** in CH_2Cl_2 at room temperature provided **10e** and **10e′** in 95% yield with the same regioselectivity (**10e/10e′**=1.76/1); even in the reaction of **10e** or **10e′** with CH_2Cl_2 under identical conditions, isomerizations were hardly observed.
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Tetrahedron 60 (2004) 4097–4105

Assembled catalyst of palladium and non-cross-linked amphiphilic polymer ligand for the efficient heterogeneous Heck reaction

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Received 26 January 2004; accepted 19 February 2004

Abstract—The efficient heterogeneous Heck reaction was achieved by a new networked and supramolecular catalyst PdAS-V (**1b**). Employing of PdAS-V in 5.0×10^{-5} mol equiv. efficiently progressed the heterogeneous Heck reaction of a series of aryl iodides with acrylates, styrenes and acrylic acid. PdAS-V was successfully recycled five times without any decrease in its activity, and showed good stability in toluene and water, and hence the Heck reaction was efficiently performed in both reaction media. The use of 8.0×10^{-7} mol equiv. of PdAS-V resulted in the coupling product in 92% yield with the turnover number (TON) and the turnover frequency (TOF) of PdAS-V reached up to 1,150,000 and 12,000, respectively. The efficient synthesis of resveratrol was achieved via the PdAS-V-promoted Heck reaction.

1. Introduction

Development of reusable and solid-phase palladium catalysts is a very important theme in recent organic chemistry and industrial process.[1,2] Although homogeneous palladium catalysts are widely used and essential in organic synthesis, they have several drawbacks to be resolved. For example, palladium is an expensive and precious metal so that disposable palladium catalysts are wasteful, and perfect removal of palladium from a reaction mixture is bothersome and difficult, resulting in contamination of the products and the waste fluid by palladium. By contrast, reusable and solid-phase palladium catalysts, in an ideal system, will resolve these problems: such palladium catalysts are reused infinitely; a work-up of the reaction is simple and easy; they are recovered from the reaction mixture by simple filtration. Therefore, many immobilized and insoluble palladium catalysts have been reported, which were supported mainly onto insoluble resins, silica gels and metal oxides. Their catalytic system, however, has not been established in reality. Their catalytic activity is generally lower than that of homogeneous counterparts. Besides, they have a tendency to decrease the catalytic activity of themselves in repeated use owing to leaching of metal species from their supports.[2]

We conceived that new structural design and methodology to develop highly active, reusable and solid-phase palladium catalysts should be needed. The traditional resin or silica gel-supported palladium catalysts are prepared by the linking of palladium species onto insoluble supports (Scheme 1, above). On the other hand, we focused on a different strategy: self-assembled process between non-cross-linked amphiphilic polymer ligands and palladium to prepare the solid-phase catalysts (Scheme 1, below).[3] This process was expected to produce networked and supramolecular complexes where the polymers were cross-linked by palladium. Based on our strategy, PdAS (**1a**), a supramolecular complex of $(NH_4)_2PdCl_4$ (**2**) and poly[(*N*-isopropylacrylamide)$_{10}$-*co*-(4-diphenylstyrylphosphine)] (**3a**)), was developed as a solid-phase catalyst for the heterogeneous Suzuki–Miyaura reaction.[3c] PdAS, used in 8×10^{-7}–5×10^{-4} mol equiv., catalyzed efficiently the coupling, and was recycled 10 times without declining the catalytic activity.

Since PdAS was a highly active and reusable catalyst, we focused on its application to the efficiently recycled system of the Heck reaction. The Heck reaction, the coupling of sp^2-halides with alkenes promoted by palladium catalysts, is an important reaction for the synthesis of natural products and bioactive compounds as well as for the industrial process chemistry.[4] Although many efforts to prepare solid-phase catalysts for the Heck reaction have been made, homogeneous catalytic systems have advantages on catalytic activity.[5] In fact, it was known that designing recyclable system for the Heck reaction was more

Keywords: *N*-Isopropylacrylamide; Heck reaction; Palladium and compounds; Polymer support; Self-assembly.
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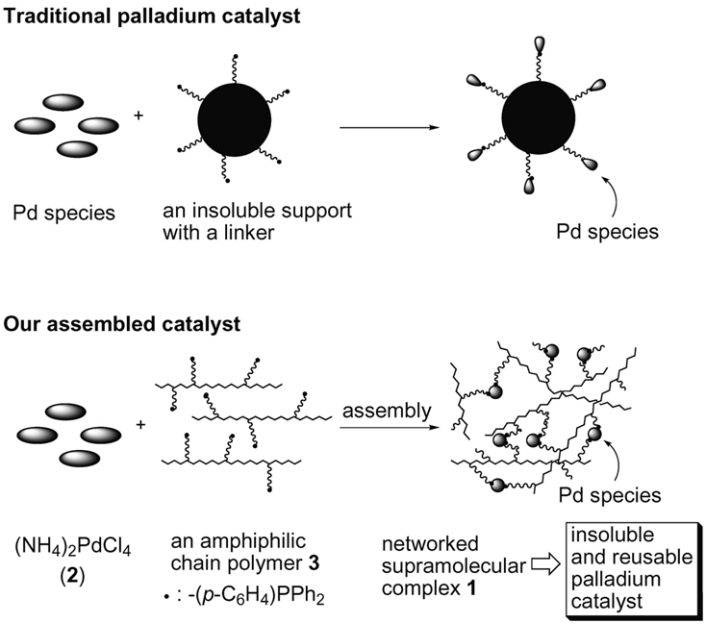
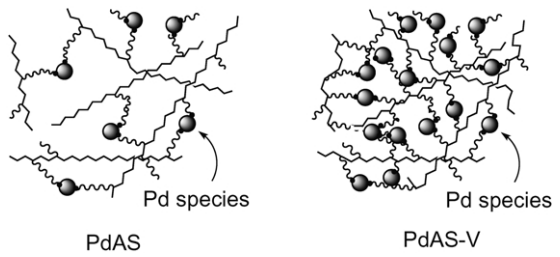
doi:10.1016/j.tet.2004.02.068



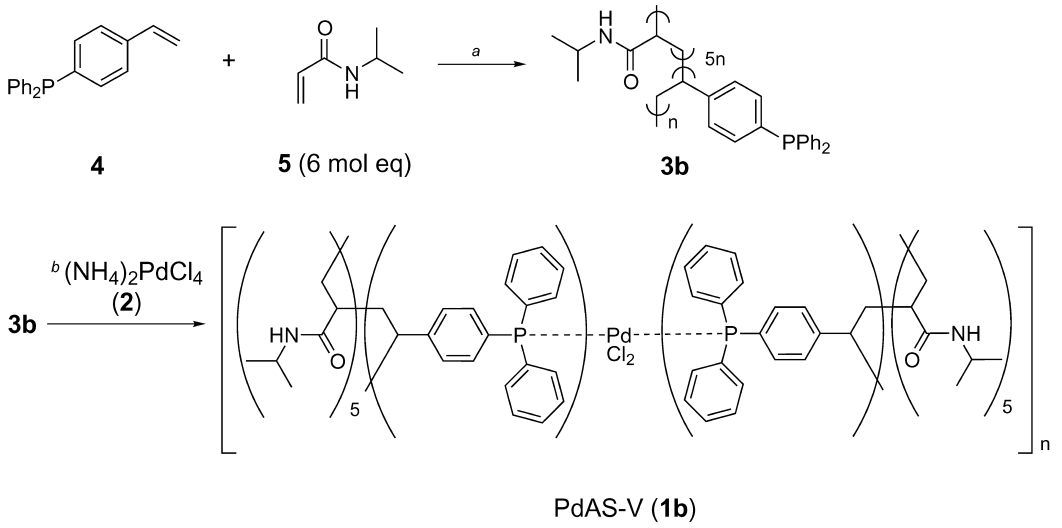
Scheme 1. Concept for the preparation of an assembled catalyst of palladium and non-cross-linked amphiphilic polymer.

challenging than that for the Suzuki–Miyaura reaction. These catalysts were less stable under the Heck reaction condition, and thus often decompose physically or chemically.[4a,b] For example, resulting salts accumulated in the reaction lead to degradation of the catalytic system and choke of catalysts under the Heck conditions. Besides, the reductive elimination of phosphonium cation causes depletion of phosphine-containing palladium catalysts.



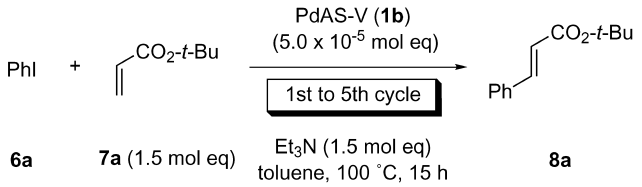
Scheme 2. Working model of PdAS and PdAS-V.

While a preliminary investigation by using PdAS was carried out, it was concluded that PdAS was not so effective owing to its pulverization under the recycled condition of the Heck reaction. We supposed that cross-linking in PdAS was not sufficient to preserve physical strength for the Heck reaction. This hypothesis struck us that a more cross-linked palladium catalyst should enhance the physical strength and the stability. Based on our working hypothesis, we have reported a reformed palladium solid-phase catalyst PdAS-V (**1b**) and partial results on the Heck reaction in toluene.[3e] In this article, we report here the full detail of the development of PdAS-V and a highly efficient and recyclable system for the heterogeneous Heck reaction.[6,7] This time, it is found that PdAS-V showed good stability not only in toluene but



Scheme 3. Preparation of assembled palladium catalyst PdAS-V. (a) AIBN (2.2 mol %), *t*-BuOH, 75 °C, 41 h, 82%; (b) (1) $(NH_4)_2PdCl_4$ (1 mol equiv.), **3b** (3 mol equiv. as PPh_2 unit), THF–H_2O, rt, 62 h, (2) added H_2O, (3) distilled with Dean–Stark equipment at 80 °C, (4) washed with H_2O, THF, and H_2O successively at 100 °C, 95%.

Table 1. Recycling of PdAS-V for the Heck reaction



Entry	Cycle	Yield (%)	TON	TOF (h^{-1})
1	1st cycle	92	18,400	1230
2	2nd cycle	93	18,600	1240
3	3rd cycle	95	19,000	1270
4	4th cycle	94	18,800	1250
5	5th cycle	95	19,000	1270
	A total TON	94,000	Av. TOF	1250

also in water, and thus both solvents were suitable for this reaction of versatile substrates such as alkylacrylates, styrenes, and acrylic acid with aryl iodides. It is noteworthy that employment of 8.0×10^{-7}–5.0×10^{-5} mol equiv. of PdAS-V facilitated the recycled system of the Heck reaction with the turnover number (TON (=mol of product/mol of catalyst)) up to 1,150,000 and the turnover frequency (TOF (h^{-1})=the turnover number per an hour) up to 12,000 h^{-1}. PdAS-V was reused five times without any decrease in its activity. Furthermore, the efficient synthesis of resveratrol, a promising COX-II inhibitor, was achieved via the PdAS-V-promoted Heck reaction.

2. Results and discussions

2.1. Preparation of PdAS-V

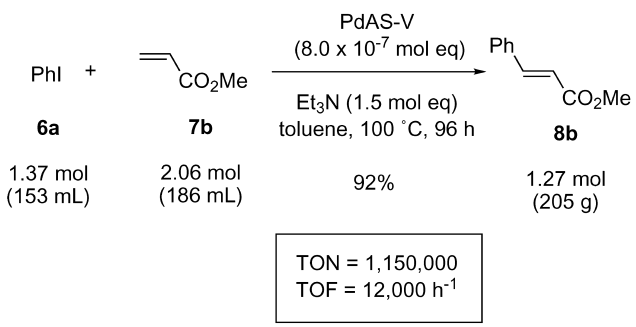
The difference of PdAS-V and PdAS was that the ratio of the *N*-isopropylacrylamide unit to the phosphine unit was 5/1 in PdAS-V while that in PdAS was 10/1.[7] Theoretically, the polymers in PdAS-V were cross-linked eight-fold more than those in PdAS per unit volume, and thus the amount of palladium in PdAS-V increased eight-fold over PdAS per unit content (Scheme 2). This implied that physical strength of PdAS-V was superior to that of PdAS, so that PdAS-V was expected to be prevented from pulverization under the Heck reaction conditions.

The reformed catalyst PdAS-V was prepared from **2** and **3b** using the method for the preparation of PdAS as shown in Scheme 3.[3c] Random copolymerization of 4-diphenylstyrylphosphine (**4**) with 6 mol equiv. of *N*-isopropylacrylamide (**5**) in the presence of 2.2 mol% of AIBN gave **3b** in 82% yield. The gel permeation chromatography showed that the molecular weight of **3b** was wide-ranging (approximately 5000–70,000). The ratio of the phosphine to the amide units in **3b** was determined by ^{1}H NMR measurements in $CDCl_3$ to be 1/5, and the phosphine unit was hardly oxidized in this polymerization as shown by ^{31}P NMR. This ratio of the phosphine to the amine unit as 1/5 was found to be reproducible in several lots. Thus, PdAS-V (**1b**) was prepared by self-assembly of **2** and **3b** (3 mol equiv. in phosphine) in THF and H_2O, resulting in the formation of precipitates. After the suspension was washed to remove a trace amount of unreacted palladium species and polymers, PdAS-V was obtained in 95% yield. It was a dark reddish solid that was insoluble in water, methanol, DMF, ethyl acetate, dichloromethane, THF and toluene as well as was PdAS, whereas polymer **3b** was soluble in organic solvents such as $CHCl_3$, CH_2Cl_2 and THF. Gel-phase ^{31}P NMR of PdAS-V showed the similar broad signals at 26.1 and 32.5 ppm as that of PdAS, which must be assigned as the peak of $PdCl_2(PPh_2Ar)_2$ and $ArPh_2P{=}O$, respectively. These results indicated that the structure of PdAS-V was analogous to that of PdAS, and thus the self-assembly process of **3b** and **2** to form the cross-linked and supramolecular complex was successful.

2.2. The catalytic activity of PdAS-V

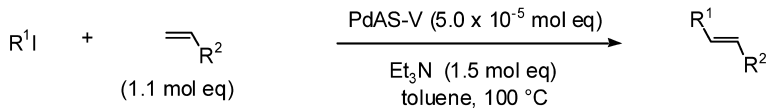
To check the potency of PdAS-V for the Heck reaction, PdAS-V was treated with the reaction of **6a** with 1.5 mol equiv. of **7a** in the presence of Et_3N in toluene at 100 °C (Table 1). The results agreed with our working hypothesis that PdAS-V was a highly active and reusable catalyst; the employment of 5.0×10^{-5} mol equiv. of PdAS-V in the 5th cycled run afforded **8a** in 95% yield with TON being 19,000 (entry 5).[8] PdAS-V was recycled five times without any loss of its activity. The average yield of five runs was 94%. A total turnover number of PdAS-V in the 1st to the 5th cycled runs was 94,000, and the average of TOF was 1250.

Since the recycled ability and high TON of PdAS-V was achieved in the Heck reaction, we further investigate the



Scheme 4. The heterogeneous Heck reaction catalyzed by 8×10^{-7} mol equiv. of PdAS-V. (a) The product **8b** was purified by crystallization.

Table 2. The Heck reaction of aryl iodides **6** with acrylates **7** and **9**



Entry	R^1I		$=R^2$		Time (h)	Product	Yield
1	**6a**		CO_2Me	**7b**	12	Ph, CO_2Me	**8b**:93%
2	**6a**		CO_2Bu	**7c**	20	Ph, CO_2Bu	**8c**:98%
3	**6a**		O, OPh, O	**7d**	20	Ph, O, OPh, O	**8d**:97%
4	**6a**		F_3C, CF_3, O, O	**7e**	5	Ph, F_3C, CF_3, O, O	**8e**:95%
5	EtO_2C, I	**6b**	**7b**		20	EtO_2C, CO_2Me	**8f**:95%
6	AcO, I	**6c**	**7b**		20	AcO, CO_2Me	**8g**:92%
7	Cl, I	**6d**	**7b**		20	Cl, CO_2Me	**8h**:95%
8	F, I	**6e**	CO_2Et	**7f**	20	F, CO_2Et	**8i**:93%
9	MeO, I	**6f**	**7b**		20	MeO, CO_2Me	**8j**:92%
10	I, OMe	**6g**	**7b**		40	CO_2Me, OMe	**8k**:90%
11	I, CF_3	**6h**	**7b**		60	CO_2Me, CF_3	**8l**:82%
12	**6a**		CO_2H	**9**	5	Ph, CO_2H	**10a**:93%[a]
13	O, I	**6i**	**9**		4	O, CO_2H	**10b**:90%[a]
14	**6f**		**9**		8	MeO, CO_2H	**10c**:87%[a]

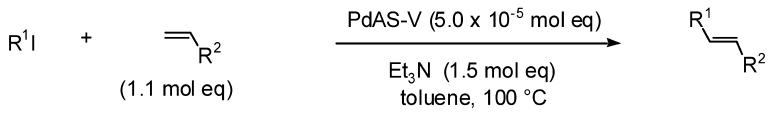
[a] The product was purified by recrystallization without column chromatography.

limitation of its catalytic activity. It was found that less than 1 ppm mol equiv. of PdAS-V catalyzed the coupling efficiently as shown in Scheme 4. The employment of 8×10^{-7} mol equiv. of PdAS-V in the coupling of **6a** (1.37 mol; 153 mL) with **7b** (2.06 mol; 186 mL) for 96 h provided **8b** (1.27 mol; 205 g) in 92% yield, isolated by crystallization. It is notable that PdAS-V promoted the reaction on a scale of more than 1 mol with TON and TOF in its reaction reaching 1,150,000 and 12,000 h^{-1}, respectively. That is, PdAS-V was the most active solid-phase catalyst for the Heck reaction. As far as we know, this is the highest TON value by the reusable catalysts for the Heck reaction.

2.3. The Heck reaction of aryl iodides with acrylates in toluene

In order to establish the scope of the sequence as depicted in Table 2, the coupling of various aryl halides with acrylates was investigated. All the reactions in Table 2 were performed under identical conditions as in Table 1: aryl iodide **6** (1 mol equiv.), alkene **7** (1.5 mol equiv.), PdAS-V

Table 3. The Heck reaction of aryl iodides **6** with styrenes **11**



Entry	R^1I		$=R^2$		Time (h)	Product	Yield
1	**6a**		Ph	**11a**	12	Ph, Ph	**12a**:90%
2	BzO, I	**6j**	**11a**		20	BzO, Ph	**12b**:86%[a]
3	AcO, I	**6c**	**11a**		20	AcO, Ph	**12c**:75%[a]
4	Cl, I	**6d**	**11a**		20	Cl, Ph	**12d**:87%[a]
5	MeO, I	**6f**	**11a**		20	MeO, Ph	**12e**:92%[a]
6	**6a**		OAc	**11b**	20	Ph, OAc	**12c**:95%[a]
7	**6a**		Cl	**11c**	20	Ph, Cl	**12d**:88%[a]
8	**6a**		OMe	**11d**	20	Ph, OMe	**12e**:93%[a]

[a] These products were purified by recrystallization without column chromatography.

(5.0×10^{-5} mol equiv.), Et_3N (1.5 mol equiv.) in toluene at 100 °C. Full conversions were achieved for these couplings in the presence of PdAS-V to afford cinnamic esters in high yields with TON and TOF of PdAS-V reached approximately 20,000 and 1000 h^{-1}, respectively. The reaction of **6a** with alkylacrylates **7b–e** proceeded in 5–20 h to give the corresponding couplings in 93–98% yields (entries 1–4). It is notable that the coupling of hexafluoroisopropyl acrylate (**7e**), an electron-deficient olefin, proceeded much faster and completed in 5 h to furnish **8e** in 95% yield (entry 4). Electron-deficient aryl iodides such as ethoxycarbonyl-, acetoxy-, chloro-, and fluoro-substituted iodobenzenes were also converted to **8f–i** in more than 90% yields (entries 5–8). The reaction system was applicable to the reaction of an electron-rich iodoarene (entry 9). Moreover, the coupling of *ortho*-substituted aryl iodides, sterically hindered substrate, proceeded to afford the corresponding products in high yields while it was slower (entries 10–11). Interestingly, the reactions of acrylic acid (**9**) in toluene were faster than that of alkyl acrylates to afford cinnamic acids **10a** and **10b** in 93 and 90% yields (Table 2).

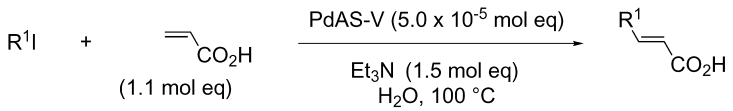
2.4. The Heck reaction of aryl iodides with styrenes in toluene

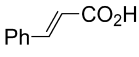
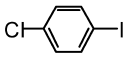
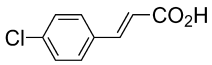
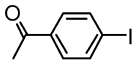
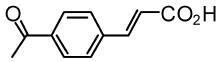
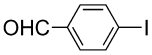
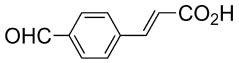
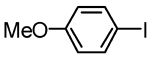
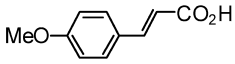
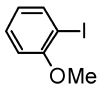
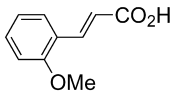
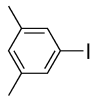
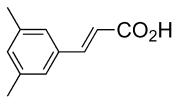
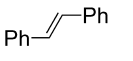
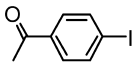
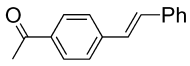
PdAS-V was applicable to the coupling of styrene derivatives **11**. The reaction conditions were identical with that in the reaction of acrylates. Aryl iodides with styrenes were also converted smoothly to the corresponding stilbenes in high yields with TON and TON being approximately 20,000 and 1000 h^{-1}. The reaction of iodobenzene (**6a**) with styrene (**11a**) was carried out, stilbene (**12a**) was obtained in 90% yield. Both electron-deficient (entries 2–4) and -donating (entry 5) aryl iodides were efficiently coupled with **11a** to provided the corresponding coupling products **12b–12e** in high yields. Besides, the electron-deficient and -donating styrenes **11b–d** were also useful reactants to give **12c–e** in approximately 90% yields (entries 6–8) (Table 3).

2.5. The Heck reaction in water

All the reactions above mentioned were performed in toluene. Since PdAS-V was composed of an amphiphilic polymer, it was expected that PdAS-V was also stable and works in water. Water is inexpensive, nontoxic, nonflammable, and easily available solvent. It nowadays receives much attention as a reaction solvent, although it has not been commonly used because palladium catalysts were generally unstable in water and hydrophobic substrates were insoluble in water.[8] Thus, the heterogeneous Heck reaction in water was investigated as shown in Table 4.[9] We were fueled by finding that PdAS-V has a good stability and activity even in water. The coupling of **6a** with acrylic acid (**9**) proceeded smoothly in 6 h to result in the formation of cinnamic acid (**10a**) in 94% yield (entry 1). Substituted aryl iodides including an *ortho*-substituted aryl iodide were also appropriate substrates in these couplings (entries 2–7). It was notable that styrene (**11a**) was also a useful reactant in water while both aryl iodides and styrene was not dissolved in water (entries 8 and 9). This result suggested that dispersion of reagents in water might be effective for promoting the reaction. Furthermore, it should be noted that

Table 4. The Heck reaction in water



Entry	R^1I		R^2		Time (h)	Product	Yield[a]
1		**6a**		**9**	6		**10a**:94%
2		**6d**	**9**		6		**10d**:91%
3		**6i**	**9**		4		**10b**:91%
4		**6k**	**9**		6		**10e**:94%
5		**6f**	**9**		24		**10c**:88%
6		**6g**	**9**		24		**10f**:95%
7		**6h**	**9**		8		**10g**:92%
8	**6a**			**11a**	36		**12a**:76%
9		**6i**	**11a**		30		**12f**:97%

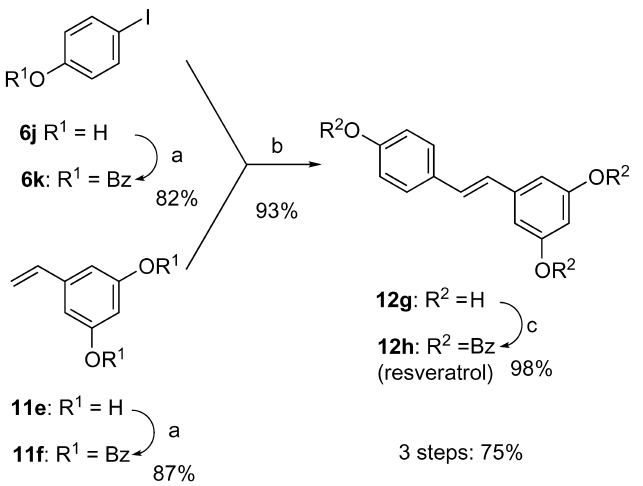
[a] These products were purified by recrystallization without column chromatography.

any catalytic activity in the reaction filtrate could not be observed, indicating obviously non-leaching of the metal catalyst from PdAS-V even in the reactions in water.

2.6. Efficient synthesis of resveratrol via the Heck reaction by PdAS-V

To demonstrate the utility of PdAS-V for the synthesis of bioactive compounds, resveratrol (**12h**) was synthesized via the heterogeneous Heck reaction. Resveratrol is a new type antitumor agent that can inhibit all three stages of cancer by inducing quinone reductase activity, inhibiting cyclooxygenase-2 (COX-2), and inducing the expression of nitroblue tetazolium reduction activity. Furthermore, it can inhibit the development of cardiovasacular disease through its ability as an antioxidant to inhibit platelet aggregation and eicosanoid synthesis and its ability to modulate lipoprotein metabolism.[10] However, it is isolated from natural sources in trace amounts,[10d] so that efficient chemical syntheses of **12h** are required.[11,12] The starting materials 4-iodophenol (**6j**) and 3,5-dihydroxystyrene (**11e**)[13] were protected by benzoyl group to afford **6k** and **11f** in 82 and 87% yield, respectively. The heterogeneous Heck reaction[12] of aryl iodide **6k** and alkene **11f** proceeded smoothly in the presence of PdAS-V to furnish the coupling **12g** in 93% yield. Deprotection of **12g** over NaOMe in THF and MeOH provided resveratrol (**12h**) in 98% yield. The total yield of resveratrol from commercially available **6j** was 75% in 3 steps (Scheme 5).

In conclusion, we have developed a new insoluble and reusable catalyst PdAS-V prepared from self-assembly of



Scheme 5. Efficient synthesis of resveratrol via the Heck reaction by PdAS-V. Reagents and conditions: (a) BzCl, pyridine, CH_2Cl_2, 0 °C; (b) **6k** (1 mol equiv.), **11f** (1.5 mol equiv.), PdAS-V (5×10^{-4} mol equiv.), Et_3N (1.5 mol equiv.), toluene, 100 °C, 12 h; (c) NaOMe, THF–MeOH, 50 °C, 5 h.

$(NH_4)_2PdCl_4$ (**2**) and non-cross-linked amphiphilic phosphine polymer **3b**. The heterogeneous Heck reaction using PdAS-V afforded the corresponding couplings in high yields with TON up to 1,150,000. Using only 5×10^{-5} mol equiv., PdAS-V was reused up to five times while still retaining its activity. PdAS-V was stable in toluene and water, so that it efficiently catalyzed the Heck reaction in these media. Resveratrol was synthesized via the Heck reaction by PdAS-V.

3. Experimental

3.1. General

All the products were isolated, and hence all the yields presented meant isolated yields. Melting points were uncorrected. ^1H and ^{13}C NMR spectra were recorded with 400 and 600 MHz (^1H NMR) pulse Fourier transform NMR spectrometers in $CDCl_3$ solution with tetramethylsilane as an internal standard. Gel-phase ^{31}P NMR spectra were recorded with a 600 MHz (^1H NMR) pulse Fourier transform NMR spectrometers in $CDCl_3$ suspension with 85% H_3PO_4 aqueous solution as an external standard. All the reactions were performed under an argon atmosphere unless cited.

3.2. Materials

Toluene was distilled from CaH_2 prior to use. Purchased aryl iodides, acrylates, styrenes, and triethyl amine were purified by distillation. *N*-isopropylacrylamide (purchased from Aldrich), AIBN, *t*-BuOH, and $(NH_4)_2PdCl_4$, were used without purification.

3.2.1. Poly[(*N*-isopropylacrylamide)$_5$-*co*-(4-diphenylphosphinostyrene)] (3b). To a solution of **4** (4.65 mmol) in *t*-BuOH (50 mL), after treatment of ultrasonication for 20 min at 60 °C to degass and dissolve **4** in *t*-BuOH, was added **5** (27.8 mmol) at rt, and the mixture was degassed by ultrasonication for 20 min. To the solution was added AIBN (0.10 mmol), and the resulting solution was again degassed by ultrasonication for 2×25 min, stirred at 75 °C for 41 h, and evaporated at 80 °C to give a crude polymer. It was purified by precipitation (×3) from CH_2Cl_2 (10 mL) and Et_2O (150 mL), dried in vacuo (ca. 0.08 mm Hg) to afford **3b** in 82% yield: IR (KBr, cm^{-1}): ν 3306, 2971, 2934, 1653, 1539, 1460, 747, 698; ^1H NMR (400 MHz, $CDCl_3$ with a trace of D_2O): δ 1.12 (br, 60H), 1.64–1.78 (br, 20H), 2.10 (br, 10H), 3.98 (br, 10H), 7.00–7.64 (br, 28H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.6, 41.3, 42.4, 128.3, 128.5, 133.4, 133.6, 174.2; ^{31}P NMR (243 MHz, $CDCl_3$): δ −3.0 (br, Ar_2PhP). Anal. Calcd for $C_{50n}H_{74n}O_{6n}N_{5n}P_n$ as **2**·1$n$$H_2O$: C 68.861%, H 8.550%, N 8.030%, found: C 68.207%, H 8.975%, N 8.395%.

3.2.2. Poly{dichlorobis[(*N*-isopropylacrylamide)$_5$-*co*-(4-diphenylstyrylphosphine)]palladium} (PdAS-V) (1b). All solvents were degassed by ultrasonication and argon substitution prior to use. To a well-stirred solution of **3b** (307 mg; 0.36 mmol in phosphine) in THF (72 mL) was added a solution of **2** (34.1 mg; 0.12 mmol) in H_2O (30 mL), and the mixture was again degassed. After the mixture stirred for 62 h at room temperature, a yellow precipitate was formed. Water (30 mL) was added to the suspension, and THF was removed at 80 °C for 4 h with Dean–Stark equipment to give a reddish precipitate. This precipitate was stirred at 100 °C successively in H_2O (100 mL) for 12 h, in THF (100 mL) for 3 h and in H_2O (100 mL) for 12 h to wash the unreacted palladium species and polymers. After drying in vacuo (ca. 0.08 mm Hg), a dark red solid **3** was obtained in almost quantitative yield: IR (KBr, cm^{-1}): ν 2971, 2934, 1651, 1537, 1460, 694; gel-phase ^1H NMR (600 MHz, $CDCl_3$): δ 1.06 (br, 60H), 1.54–2.10 (br, 30H), 3.68 (10H), 6.56–7.47 (br, 24H); gel-phase ^{13}C NMR (150 MHz, $CDCl_3$): δ 22.6, 41.3, 128.0, 174.1; gel-phase ^{31}P NMR (243 MHz, $CDCl_3$) 26.1 (br), 32.1 (br). Anal. Calcd for $C_{150n}H_{234n}O_{24n}N_{15n}P_3Pd_{1n}Cl_{2n}$ as PdAS·9$n$$H_2O$: C 62.090%; H 8.123%, N 7.240%, found: C 60.956%; H 8.445%, N 8.304%.

3.2.3. Recycle of PdAS-V for the Heck reaction (general procedure for the Heck reaction catalyzed by PdAS-V in toluene) (Table 1). The mixture of **6a** (4.1 mL; 36.5 mmol), **7a** (8.0 mL; 54.7 mmol), Et_3N (7.6 mL; 54.7 mmol) in toluene (18 mL) was degassed by untrasonication for 30 min. The solution was added to PdAS-V (5 mg; 1.82 μmol), and the resulting suspension was stirred at 100 °C for 15 h. After the reaction mixture was cooled to room temperature, methanol was added to the mixture, and the resulting solution with insoluble PdAS-V was filtered. At that time, PdAS-V was recovered on the filter. The filtrate was evaporated and it was diluted with EtOAc and water. The two-phase solution was extracted with EtOAc, washed with water and brine, dried over $MgSO_4$. The residue was purified by column chromatography or recrystallization (toluene–EtOH) to give **8a** in 82–95% yields. The recovered PdAS-V was dried in vacuo and reused.

3.2.4. Hexafluoropropyl cinnamate (8e). IR (KBr, cm^{-1}): ν 3088, 3034, 2971, 1748, 1636, 766, 691; ^1H NMR (400 MHz, $CDCl_3$): δ 5.83 (hept, J=2.9 Hz, 1H)), 6.38 (d, J=15.8 Hz, 1H), 7.25–7.34 (m, 3H), 7.42–7.45 (m, 2H), 7.75 (d, J=15.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): 66.6, 114.1–124.8 (m), 114.8, 128.5, 128.9, 131.3, 133.4, 149.1, 163.2; MS(EI): m/z 298 (M^+), 131, 103, 77; HR-MS (EI): calcd for $C_{12}H_8F_6O_2$ 298.0428, found 298.0423.

3.3. Synthesis of resveratrol

3.3.1. 3,5-Dibenzoyloxystyrene (11f). To a solution of 3,5-dihydroxystyrene (**11e**) (408 mg; 3.0 mmol) was added pyridine (1.21 mL; 15.0 mmol) and benzoyl chloride (1.04 mL; 9.0 mmol) at 0 °C. After the resulting solution was stirred for 1.5 h, water was added. The two-phase solution was extracted with CH_2Cl_2, washed with water and brine, dried over $MgSO_4$, and purified by column chromatography to give **11f** in 87% yield (898 mg; 2.61 mmol). IR (KBr, cm^{-1}): ν 3071, 2988, 1732, 1590; ^1H NMR (400 MHz, $CDCl_3$): δ 5.36 (d, J=11.0 Hz, 1H)), 5.80 (d, J=17.6 Hz, 1H), 6.72 (dd, J=11.0, 17.6 Hz, 1H), 7.10–7.11 (m, 1H), 7.21–7.22 (m, 2H), 7.54–7.55 (m, 4H), 7.63–7.67 (m, 2H), 8.20–8.22 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 114.9, 115.9, 116.9, 128.5, 129.1, 130.1, 133.6, 135.2,

139.9, 151.4, 164.5; MS(EI): *m/z* 344 (M^+), 105, 77; HR-MS (EI): Calcd for $C_{22}H_{16}O_4$ 344.1049, found 344.1055.

3.3.2. (*E*)-3,5,4′-Tribenzoyloxystyrene (12g) (the Heck reaction by PdAS-V). The mixture of **6k** (251 mg; 0.774 mmol), **11f** (400 mg; 1.16 mmol), Et_3N (0.162 mL; 1.16 mmol) in toluene (0.39 mL) was degassed by untrasonication for 30 min. The solution was added to PdAS-V (1.1 mg; 0.387 μmol), and the resulting suspension was stirred at 100 °C for 12 h. After the reaction mixture was cooled to room temperature and was filtered with EtOAc and water, the filtrate was washed with water and brine, dried over $MgSO_4$, and purified by column chromatography (eluent: hexane) to afford (*E*)-3,5,4′-tribenzoyloxystyrene (**12g**) in 93% yield (390 mg; 0.72 mmol). IR (KBr, cm^{-1}): ν 3061, 3034, 1738, 1599; ^1H NMR (400 MHz, $CDCl_3$): δ 7.06 (d, J=16.1 Hz, 1H)), 7.10–7.11 (m, 1H), 7.15 (d, J=16.1 Hz, 1H), 7.21–7.24 (m, 2H), 7.32–7.32 (m, 2H), 7.48–7.56 (m, 8H), 7.61–7.66 (m, 3H), 8.19–8.23 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 114.7, 117.1, 121.9, 127.1, 127.6, 128.4, 128.5, 129.1, 129.3, 129.6, 130.0, 130.1, 133.5, 133.6, 134.4, 139.6, 150.5, 151.5, 164.6, 164.8; MS(EI): *m/z* 540 (M^+), 105, 77; HR-MS (EI): Calcd for $C_{35}H_{24}O_6$ 540.1573, found 540.1570.

3.3.3. Resveratrol (12h). The solution of **12g** THF–MeOH was stirred at 50 °C for 5 h. After the mixture was cooled to rt, EtOAc and water was added. The two-phase solution was extracted with EtOAc, washed with water and brine, dried over $MgSO_4$, purified by column chromatography to afford resveratrol (**12h**) in 98% yield (22.4 mg; 0.098 mmol).

Acknowledgements

We thank Ms. Junko Shimode, and Ms. Maroka Kitsukawa for spectroscopic measurement. This work was partially supported by a Grant-in-Aid for Scientific Research from the ministry of Education, Science and Technology. Y. M. A. Y. thanks the Inoue Foundation for Science (IFS) for Inoue Research Award for Young Scientists, and Dainippon Ink and Chemicals, Inc. Award in Synthetic Organic Chemistry, Japan.

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Tetrahedron 60 (2004) 4107–4123
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First functionalization by metallation of the pyridine moiety of pyridopyrimidin-4(3*H*)-ones. Diazines. Part 36

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Received 14 November 2003; revised 9 February 2004; accepted 12 February 2004

Abstract—Starting from *o*-aminopyridine carboxylic acids, a general synthetic route leading to various pyridopyrimidin-4(3*H*)-ones is described. The first metallation and functionalization of the pyridine moiety has been studied and a regioselective metallation at the *peri*-position C_5 of the pyridine ring has been highlighted.

1. Introduction

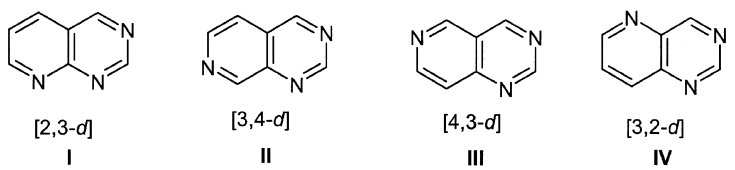
Among the pyridodiazines, the pyridopyrimidines have received much attention because of their potential biological activities as isosteres of quinazolines or pteridines. All four possible pyridopyrimidine systems, pyrido[2,3-*d*]pyrimidine **I**, pyrido[3,4-*d*]pyrimidine **II**, pyrido[4,3-*d*]pyrimidine **III** and pyrido[3,2-*d*]pyrimidine **IV** are known (Scheme 1). The pyrido[2,3-*d*]pyrimidine system has been more studied according to its medicinal applications such as inhibitor of the adenosine kinase[1] (AK) or dihydrofolate reductase[2] (DHFR) enzymes.

For the reason given above, the synthesis of pyridopyrimidine derivatives provides an interesting challenge. Construction of functionalized pyridopyrimidines involves cyclization of appropriately substituted pyrimidines or pyridines whose synthesis is not always easy. The functionalization via metallation of the pyridine moiety could provide a consistent strategy for the synthesis of new pyridopyrimidines.

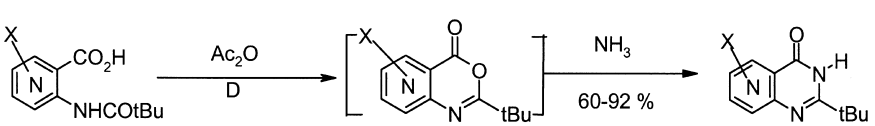
In previous papers, we have mentioned the lithiation of the benzene moiety of benzodiazines: cinnolines,[3] quinazolines,[4,5] quinoxalines and phtalazines.[5] As a continuation of our studies of metallation of *ortho*-condensed diazines, we report here the synthesis, the direct lithiation and functionalization of the pyridine moiety of pyridopyrimidin-4(3*H*)-ones.

Various syntheses of pyridopyrimidine systems have been previously described,[6] among them, a general synthetic route is the cyclization of *o*-acylaminopyridine carboxylic acids with acetic anhydride,[7] leading to pyrido[1,3]oxazin-4-ones intermediates which react with ammonia to give the expected pyridopyrimidin-4(3*H*)-ones (Scheme 2).

This synthetic route could be used with *o*-aminopyridine



Scheme 1.



Scheme 2.

Keywords: Metallation; Pyridopyrimidin-4(3*H*)-ones; Functionalization.
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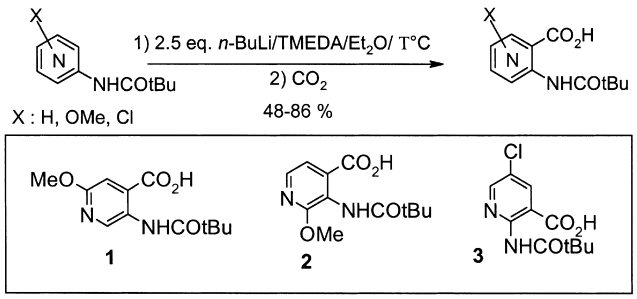
0040–4020/$ - see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.tet.2004.02.040

carboxylic acids as starting material which could be reacted with acid chlorides to give the expected *o*-acylaminopyridine carboxylic acids. The 2-aminonicotinic acid was the sole commercial material, the other *o*-acylaminopyridine carboxylic acids have been obtained from commercial aminopyridines which reacted with pivaloyl chloride to give the expected *N*-pivaloylaminopyridines. The pivaloylamino group, a very good *ortho*-directing group, allowed us to obtain *ortho* lithioderivatives which after reaction with carbon dioxide led to *o*-*N*-pivaloylaminopyridine carboxylic acids.[8] It could be noticed that, if most are known, three of them are new (**1**-**3**) ones (Scheme 3).



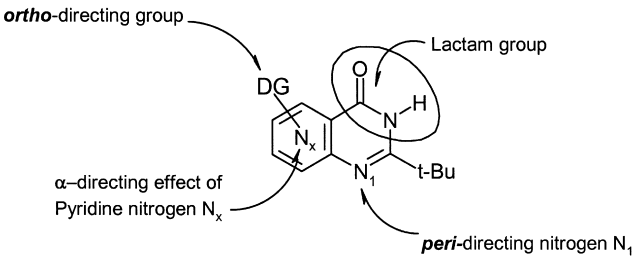
Scheme 3.

According to this general synthetic route, we have synthesized eight pyridopyrimidin-4(3*H*)-ones (**4**-**11**) among them compounds **6**-**11** are new ones. Compounds **4** and **5** were described as herbicides[9] (Scheme 4).

We have tested the direct lithiation and functionalization of the 2-*tert*-butylpyridopyrimidin-4(3*H*)-ones **4**-**11**.

During the lithiation of the benzene moiety of various benzodiazines,[3–5] it has been highlighted an exceptional regioselective metallation at the C_8 position, in *peri* to the ring nitrogen atom N_1. It has also been highlighted that the presence on the benzene moiety of a substituent inducing an *ortho*-directed metallation favored the lithiation reaction.

In the case of 2-*tert*-butylpyridopyrimidin-4(3*H*)-ones **4**-**11**, several parameters could be taken into account to direct the regioselectivity of the metallation (Scheme 5): the *peri* effect of the nitrogen atom N_1 of the pyrimidine moiety, the α effect of the nitrogen atom N_x of the pyridine moiety where the α position of the nitrogen atom was free and the *ortho*-effect of substituent such as a chlorine atom or a methoxy group for compounds **5**, **7**, **8**. For all these compounds except for **11**, it could be interesting to observe



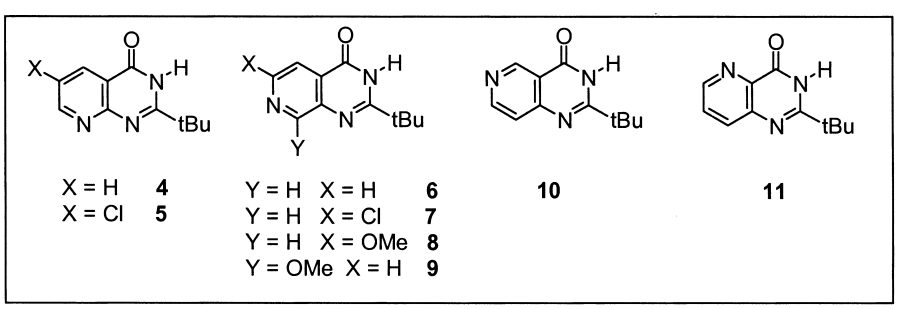
Scheme 5.

if the lactam group of the pyrimidinone moiety could have any influence on the *peri* position C_5 (Scheme 5).

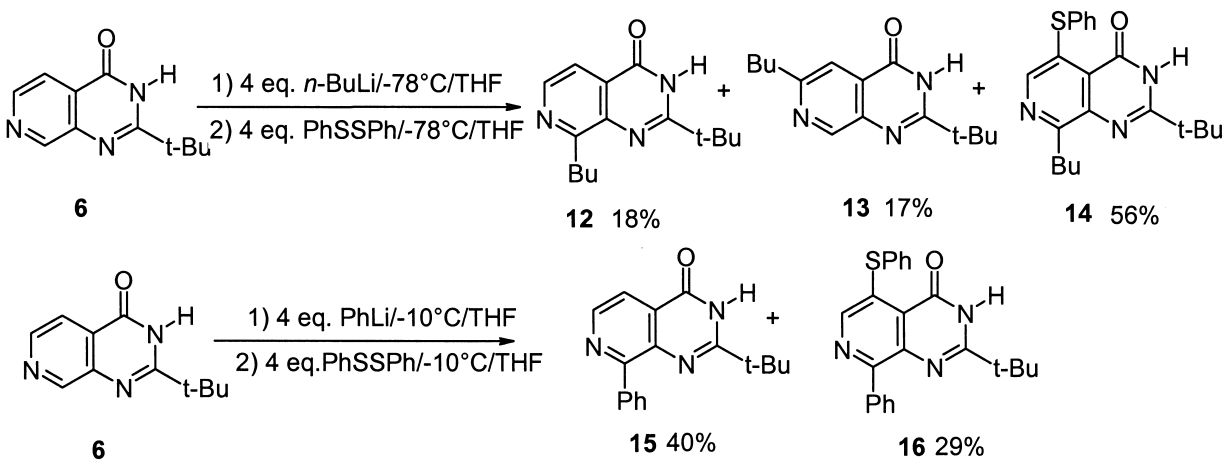
The lactam group has been previously used as *ortho*-directing group during the regioselective metallation at C_2 of the pyridine moiety of 5- or 6-methoxyquinolin-2(1*H*)-ones.[10] Otherwise, the lithiation of N_3-acylaminoquinazolin-4(3*H*)-ones has been described,[11] in this case, metallation occurred exclusively at C_2 which was influenced by the acylamino group on the ring nitrogen N_3. More recently, we have reported the metallation of quinazolones, these compounds underwent a regioselective metallation of the benzene moiety at the C_8 position, in *peri* to the ring nitrogen atom N_1, only when the benzene ring was substituted at C_7 position by a chlorine atom or a methoxy group.[5]

We have tested the metallation of 2-*tert*-butylpyridopyrimidin-4-(3*H*)-ones to appreciate if the presence of the pyridine nitrogen makes the deprotonation more easy than with quinazolones and could influence the regioselectivity. The presence of a *tert*-butyl group at the C_2 position has been chosen to avoid a nucleophilic attack of the metallating agent at this position[12,13] and to prevent the deprotonation on the carbon C_α of the lateral chain.[14–16]

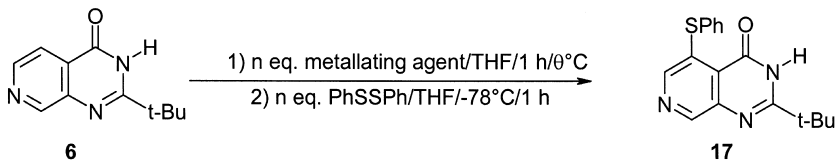
First, various attempts to metallate **6** with alkyllithiums have been performed. *n*-butyllithium and phenyllithium have been tested as metallating agent, followed by reaction with diphenyl disulfide as the electrophile. Treatment of **6** with 2 equiv. of *n*-butyllithium at −78 °C did not allow any reaction and 95% of starting material were recovered; use of 4 equiv. of butyllithium at this temperature afforded addition products (91%) besides a small amount of starting material (8%) (Scheme 6). Compounds **12** and **13** resulted from an addition at the α positions of the pyridine nitrogen (C_6 and C_8), whereas the main compound **14** resulted from an addition of *n*-butyllithium at C_8 followed by reaction



Scheme 4.



Scheme 6.



Scheme 7.

with electrophile at C_5, *peri* to the carbonyl of the lactam function.

When the reaction was performed with phenyllithium at −10 °C, two compounds **15** and **16** resulting from an addition reaction at C_8 were obtained in 69% total yield beside starting material (30%). For compound **14** as for compound **16**, we observed that the reaction with the electrophile has occurred at C_5 at the *peri* position of the lactam function.

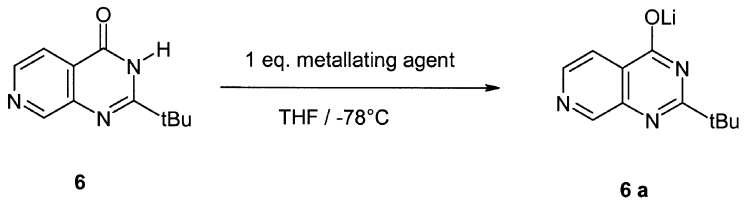
Reactions of addition observed with alkyllithiums as metallating agent urged us to use lithium alkylamides such as lithium 2,2,4,4-tetramethylpiperidide (LTMP) or lithium diisopropylamide (LDA) as metallating agent, which are known to be less nucleophilic than alkyllithiums. Later, the conditions of metallation have been established for compound **6**, with various amounts of lithium alkylamides as metallating agent, and diphenyl disulfide as the electrophile (Scheme 7, Table 1).

Table 1. Lithiation and functionalization of **6**

Entry	Metallating agent	*n* (equiv.)	Temperature *θ* (°C)	Compound **17** (%)
1	LTMP	2	−78	5
2	LTMP	4	−78	55
3	LTMP	4	0	45
4	LTMP	5	−78	75
5	LTMP	6	−78	90
6	LTMP	8	−78	97
7	LDA	8	−78	5

The results given in Table 1 revealed that a first attempt with 2 equiv. of LTMP at −78 °C afforded a small amount of **17** beside starting material (entry 1). With 4 equiv. of LTMP at −78 °C, the yield was improved to 55%, nevertheless, increasing the temperature to 0 °C gave a slightly lower yield (entries 2 and 3). A large excess of LTMP (6–8 equiv.) afforded **17** in very good yields (entries 5 and 6). When LDA, less basic than LTMP, was used even in large excess, only a few amount of **17** was obtained beside starting material (entry 7). The structure of compound **17** has been established thanks to its ^{1}H NMR spectrum which presented two singlets at 7.68 and 8.67 ppm assigned, to H_6 and H_8, highlighting a total regioselectivity at the C_5 position.

We have also tested if metallation could be performed by a mixture of bases. In the first step, the labile proton of the lactam function was trapped by 1 equiv. of base (*n*-BuLi or LTMP), leading to a lithium salt **6a** (Scheme 8) as this has been observed with its IR spectrum obtained with ReactIR™ spectrometer (Fig. 1). We could observe that the



Scheme 8.

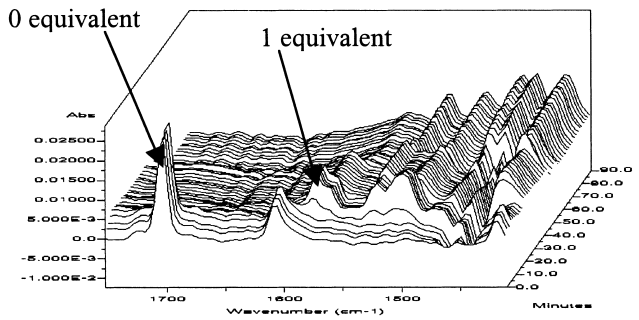


Figure 1. Spectroscopic analysis FTIR of **6** with *n* equivalent of *n*-butyllithium or LTMP.

introduction of 1 equiv. of metallating agent led to disappearance of peaks at 1700 and 1600 cm^{-1} assigned, respectively, to ν(C=O) and δ(N–H). The further equivalents of base could be used to induce either the metallation or additions.

Various attempts have been performed with a total of 5 equiv. of bases at −78 °C in THF for 1 h as total reaction time and with diphenyl disulfide as the electrophile. We have tested a mixture of lithium alkylamide–alkyllithium in various ratios. When a mixture of bases was used, the first base was reacted for 30 min, then the second base was introduced and reacted again for 30 min. The experimental conditions and results are given in Table 2.

Table 2. Metallation of **6** with a mixture of bases

Entry	Bases	Compound **6** (%)	Compound **17** (%)
1	5 equiv. LTMP	25	75
2	(1) 1 equiv. *n*-BuLi (2) 4 equiv. LTMP	35	65
3	(1) 4 equiv. LTMP (2) 1 equiv. *n*-BuLi	5	90
4	(1) 3 equiv. LTMP (2) 2 equiv. *n*-BuLi	25	75
5	(1) 2 equiv. LTMP (2) 3 equiv. *n*-BuLi	66	33
6	(1) 4 equiv. LDA (2) 1 equiv. *n*-BuLi	90	10

Treatment of **6** with 5 equiv. of LTMP as sole base provided the five-substituted compound **17** in 75% yield (entry 1). With 1 equiv. of *n*-butyllithium followed by reaction of 4 equiv. of LTMP (entry 2), we observed the formation of **17** in slightly lower yield (65%). For the entries 3–5, we have first used (*n*) equivalents of LTMP followed by reaction with (5−*n*) equivalents of *n*-butyllithium, the results revealed that a large excess of LTMP improved the yield of compound **17**. The best results have been obtained with the experimental conditions of entry 3. When LDA was used as alkylamide under the conditions of the entry 3, only small amount of **17** was obtained beside starting material (entry 6).

These results require some comments: when *n* equivalents of lithium alkylamides were first introduced followed by addition of (5−*n*) equivalents of *n*-butyllithium, the metallation reaction was observed without addition, even if *n*-butyllithium was in excess. In this case, it could be assumed that the first equivalent of lithium alkylamide trapped the labile proton of the lactam function, the other equivalents were used to give aggregates which then could favor metallation by LTMP or *n*-BuLi without occurrence of competitive addition reaction. We have nevertheless used the conditions of metallation given in Table 1 (entry 6) to functionalize the 2-*tert*-butylpyridopyrimidin-4(3*H*)-ones **4**-**11**, because the work up of the reaction mixture was easier.

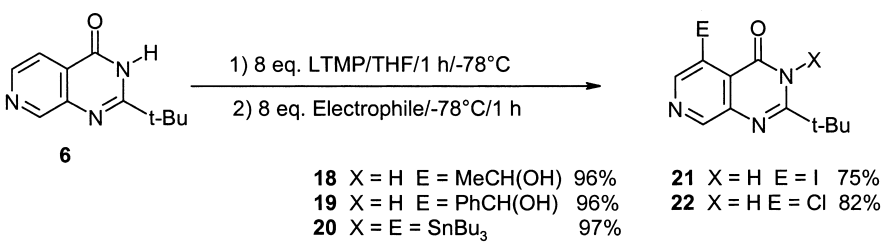
Treatment of **6** with 8 equiv. of LTMP at −78 °C followed by reaction with various electrophiles afforded five-substituted derivatives in very good yields (Scheme 9).

It must be noticed that when tributyltin chloride was used as the electrophile, a distannyl compound **20** was obtained, the presence of a lactam group was confirmed by its IR spectrum which exhibited a ν(CO) at 1688 cm^{-1}.

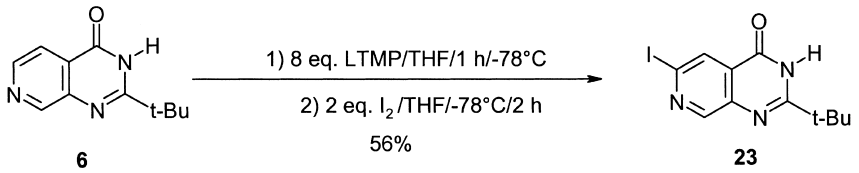
With iodine as the electrophile we have observed that the regioselectivity was dependent on the direct or inverse introduction of iodine. When iodine was introduced in the reaction mixture, two mono iodo derivatives at C_5 and C_6 were obtained, whereas compound **21** was the sole product isolated when the lithiated derivative was added to a solution of iodine in THF. The 6-iodo derivative **23** was obtained as sole product beside starting material (41%) by use of only 2 equiv. of iodine introduced in the reaction mixture after a 1 h reaction time (Scheme 10). Such a result could be explained by a 'halogen-dance' mechanism which has been previously described in the diazine series.[17]

It should be interesting to observe if the presence of a substituent such as a chlorine atom or a methoxy group could improve the reactivity towards lithiation and induce a particular regioselectivity. We have tested the metallation of substituted pyridopyrimidin-4(3*H*)-ones **7**-**9**.

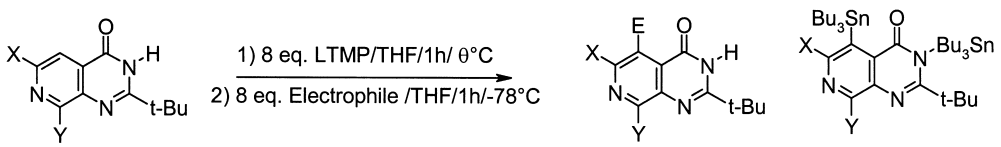
For all these compounds the C_5 position, *peri* to the



Scheme 9.



Scheme 10.



Scheme 11.

Table 3. Lithiation and functionalization of **7-9**

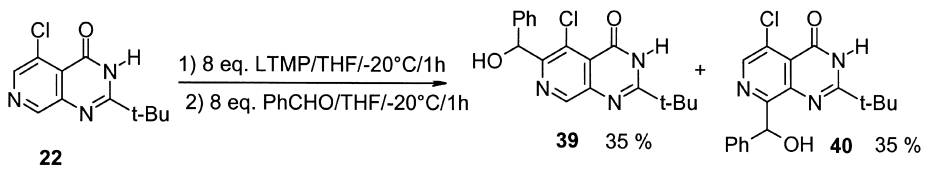
Starting material	X	Y	E	θ	Compounds (yield, %)
7	Cl	H	PhS	−78 °C	**24** (86)
			MeCH(OH)		**25** (97)
			PhCH(OH)		**26** (97)
			Bu_3Sn		**27** (84)
			I		**28** (87)
8	OMe	H	PhS	−78 to 0 °C	**29** (71)
			MeCH(OH)		**30** (92)
			PhCH(OH)		**31** (91)
			Bu_3Sn		**32** (75)
			I		**33** (88)
9	H	OMe	PhS	−78 to −20 °C	**34** (89)
			MeCH(OH)		**35** (90)
			PhCH(OH)		**36** (85)
			Bu_3Sn		**37** (75)
			I		**38** (85)

carbonyl group, was free as also one α position of the pyridine nitrogen atom. Various experimental conditions were tested and as before 8 equiv. of LTMP were necessary to obtain good yields (Scheme 11, Table 3).

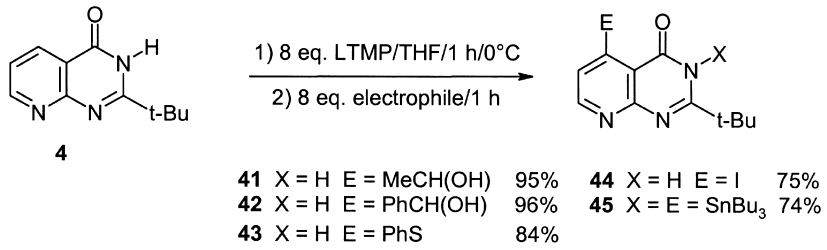
Under these experimental conditions, lithiation of **7-9** occurred exclusively at the C_5 position and reaction with various electrophiles led to 2-*tert*-butyl-5-substituted pyrido-[3,4-*d*]pyrimidin-4(3*H*)-ones **24-38** in good yields. As it has been previously mentioned, when tributyl tin chloride was used as the electrophile, the distannyl compounds **27**, **32** and **37** were obtained. It can be noticed that the presence of a methoxy group on the pyridine moiety required higher metallation temperature. For compounds **8** and **9** the metallating agent was introduced at −78 °C and the temperature was raised, respectively, to 0 °C and to −20 °C.

We have then tested the metallation of the 2-*tert*-butyl-5-chloropyrido [3,4-*d*]pyrimidin-4(3*H*)-one **22**, for this compound the C_5 position carried a chlorine atom and could not undergo lithiation, whereas positions C_6 and C_8 in α position to pyridine nitrogen atom were free. Treatment of **22** with 8 equiv. of LTMP at −20 °C for 1 h followed by reaction with benzaldehyde as electrophile afforded two compounds **39** and **40** in equal amounts with a global yield of 70% beside starting material (17%) (Scheme 12).

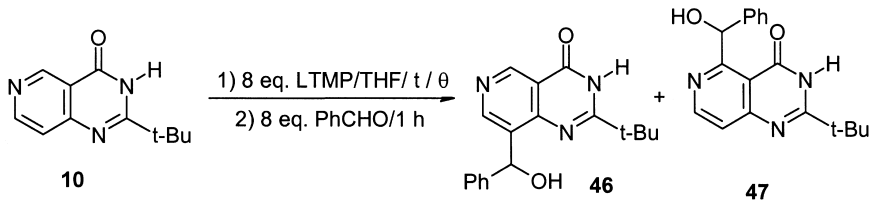
Despite the substitution of the C_5 position, this result indicated that metallation could occur at once at the C_6



Scheme 12.



Scheme 13.



Scheme 14.

Table 4. Metallation of compound **10**

Entry	Temperature , θ (°C)	Time, t (h)	Compound **46** (%)	Compound **47** (%)	Compound **10** (%)
1	−78	1	20	—	80
2	−20	1	92	3	5
3	0	1	75	18	7
4	20	1	62	29	9
5	20	2	9	74	16
6	20	3	7	76	17

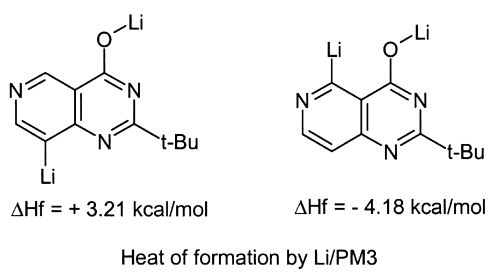
position influenced by the chlorine atom as *ortho*-directing group and the pyridine nitrogen atom, or at the C_8 position under influence of the two ring nitrogen atoms N_1 and N_7. It could be noticed that, if the single effect of the pyridine nitrogen N_7 was not sufficient to allow the metallation, the reaction became feasible when this effect was associated to an other effect such as an *ortho*-directing group or a *peri* ring nitrogen atom.

We have then tested the metallation of compounds **4** and **10** for which the C_5 position was kept free. Treatment of **4** with 8 equiv. of LTMP at 0 °C for 1 h, followed by reaction with various electrophiles led to five-substituted compounds **41**-**45** in good yields (Scheme 13).

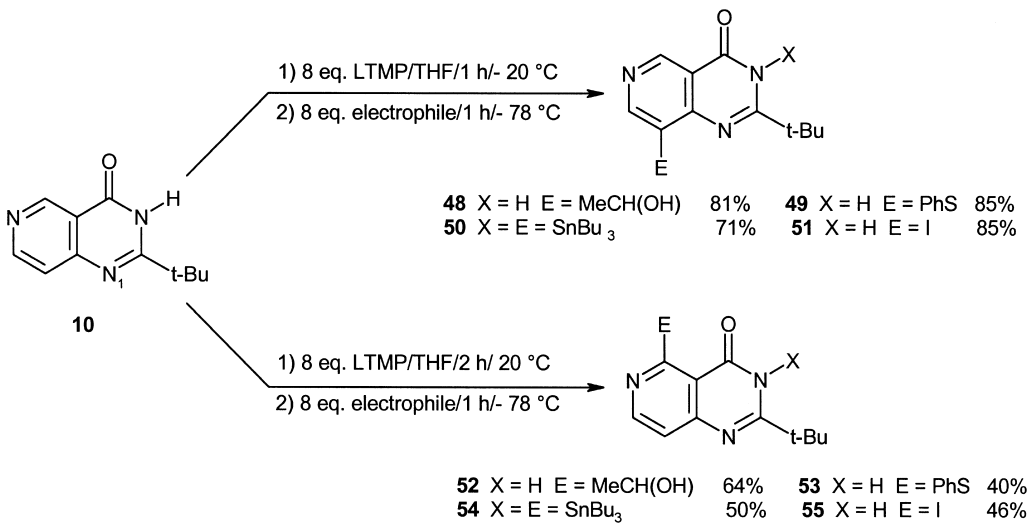
For compound **10**, the metallation reaction was performed with 8 equiv. of LTMP at various temperatures with benzaldehyde as the electrophile, under these conditions it has been observed that the regioselectivity was dependent on the temperature and the reaction time (Scheme 14, Table 4).

The results given in Table 4 revealed, that with a 1 h reaction time, only a few amount of **46** was obtained at −78 °C beside starting material (entry 1). When the temperature was raised to −20 °C, compound **46** was observed as the main product with a very good yield (entry 2). Then when temperature was increased from −20 °C to room temperature, we observed decreasing amounts of **46**, whereas the formation of **47** was growing up (entries 2–4). When the reaction was performed at 20 °C with rising reaction times, the compound **47** became the main product with 2 or 3 h for reaction time beside starting material (entries 4–6).

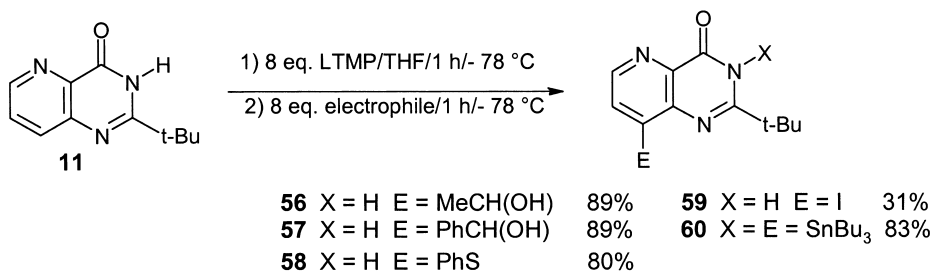
Regioselectivity of the lithiation could be discussed in terms of kinetic or thermodynamic control. Compound **47** which was obtained at higher temperatures and with a longer reaction time could be the thermodynamic compound while compound **46** obtained in softer conditions could be the kinetic compound. When deprotonation is thermodynamically controlled, heats of formation of the lithiated derivatives determined by semi-empirical Li/PM3 method could be examined as a simple approach to account for the regioselectivity. Heat of formation of the lithiated derivatives calculated by Li/PM3 method (Scheme 15) indicated that the C_5 lithiated intermediate is the more stable isomer, which is in agreement with the experimental results.



Scheme 15.



Scheme 16.



Scheme 17.

We have extended these results to other electrophiles and observed that at low temperature (−20 °C) with a reaction time of 1 h the eight-substituted compounds were obtained as main products in very good yields. When the reaction was performed at room temperature with a reaction time of 2 h, the C_5-substituted compounds were obtained in moderate yields (Scheme 16).

At last, we have tested the metallation of compound **11** for which the pyridine nitrogen atom is at the position 5, *peri* to the carbonyl group of the lactam function. It should be interesting to observe if the lithiation could be obtained and in the affirmative if a regioselectivity could de observed. Treatment of **11** with 8 equiv. of LTMP at −78 °C for 1 h followed by reaction with various electrophiles afforded the eight-substituted compounds **56-60** (Scheme 17).

2. Conclusion

Starting from *o*-aminopyridine carboxylic acids, a general synthetic route leading to various pyridopyrimidin-4(3*H*)-ones has been described. The first metallation and functionalization of the pyridine moiety has been studied. An original regioselective metallation at the *peri* position C_5 of the pyridine ring has been observed when an excess of metallating agent was used, allowing access to a wide range of new substituted pyridopyrimidin-4(3*H*)-ones. In some cases, control of the experimental conditions allowed the formation of *peri* compounds either at C_5 or C_8 positions. This general synthetic route associated to palladium cross-coupling reactions is promising to access to new compounds.

3. Experimental

Melting points were determined on a Kofler hot-stage. The ^1H and ^{13}C NMR spectra were recorded in deuteriochloroform or deuteriodymethylsulfoxide on Bruker instrument (Avance 300). Microanalyses were performed on a Carlo Erba CHNOS 1160 apparatus. The IR spectra were obtained as potassium bromide pellets with a Perkin Elmer FTIR 1650 spectrophotometer.

All reagents were of commercial quality and were purchased from Aldrich Chemical Co. or Acros. 2-*tert*-Butylpyrido [3,2-*d*]pyrimidine-4(3*H*)-one **4** was synthesized according to the procedure described in the literature.[9]

3.1. Procedure A for the metallation of various *N*-pivaloylaminopyridines

An oven-dried three-necked round bottom flask was cooled in a dessicator and then equipped with a thermometer and a magnetic stirrer, and flushed with a nitrogen inlet. The flask was charged with *N*-pivaloylaminopyridine and stoppered with a rubber septum and anhydrous THF or diethylether

was introduced. After cooling to −78 °C, metallating agent and TMEDA were added dropwise via syringe. The mixture was stirred for 15 min and heated to *T* (°C). After 3 h of stirring at *T* (°C), a precipitate appeared and the suspension was cooled again to −78 °C and poured onto an excess of dry ice. After 1 h, the reaction mixture was hydrolyzed with 50 mL of water and was allowed to warm to room temperature, and the organic layer was removed under reduced pressure. The residue was partitioned between water and ethyl ether, and the aqueous phase separated and again washed with ethyl ether. The aqueous layer was acidified with 50% hydrochloric acid to give an off-white solid that was filtered, thoroughly washed with water. The solid was triturated with acetone and filtered to leave a white solid.

3.2. Procedure B for preparation of 2-*tert*-butylpyrido-pyrimidin-4(3*H*)-ones via 2-*tert*-butylpyrido[1,3]oxazin-4-ones

Acetic anhydride (20–100 mL) and *o*-(*N*-pivaloylamino)-pyridinecarboxylic acids (2–8 g) were refluxed together for 2 h. The excess of anhydride was removed by distillation under reduced pressure to give crude pyrido-oxazinone, then ammonia 15 N (30–150 mL) was added, and the mixture was stirred at room temperature for 24 h. Evaporation of the solution or suspension under reduced pressure yielded the pyridopyrimidinone by precipitation and filtration. To complete the conversion of pyrido-oxazinone into pyridopyrimidinone, the mixture could be heated with 5% aqueous sodium hydroxide for 15 min.

3.3. Procedure C for direct metallation of 2-*tert*-butyl-pyrido[3,4-*d*]pyrimidin-4(3*H*)-one by *n*-butyl-lithium or phenyllithium

A solution of 2-*tert*-butylpyridopyrimidin-4(3*H*)-one (50 mg, 0.24 mmol) in anhydrous THF (20 mL) was cooled to −78 °C, at this temperature a solution of *n*-butyllithium or phenyllithium (*n* equivalents) in hexane was introduced dropwise under an atmosphere of dry argon. The mixture was stirred for 15 min and warmed to the temperature *T* (°C). After 1 h of stirring at *T* (°C), the temperature was decreased again to −78 °C and the diphenyl disulfide (4 equiv., 215 mg) was introduced in solution with THF (5 mL). After 1 h, hydrolysis was carried out using a mixture of water and ethanol (1:1), the organic layer was removed under reduced pressure. The aqueous phase was extracted with ethyl acetate (3×20 mL), the combined organic extracts were then dried over magnesium sulfate and evaporated. The crude product was purified by column chromatography on silica gel.

3.4. Procedure D for metallation of 2-*tert*-butylpyrido-pyrimidin-4(3*H*)-ones by lithium 2,2,6,6-tetramethyl-piperidide

A solution of *n*-butyllithium (1.6 or 2.5 M in hexane) was added to cold (−78 °C), stirred and anhydrous mixture of THF (15 mL) and 2,2,6,6-tetramethylpiperidine (TMPH) under an atmosphere of dry nitrogen. The mixture was warmed to 0 °C and after 30 min, the mixture temperature was cooled to −78 °C and added to a cold (−78 °C) solution of the 2-*tert*-butylpyridopyrimidin-4(3*H*)-one in THF (10 mL). Then, the mixture was stirred for 5 min and heated to *T* (°C). After 1 h of stirring at *T* (°C), the temperature was decreased to −78 °C and the electrophile introduced and stirring was continued for *t* hour(s) at this temperature. Hydrolysis was then carried out at −78 °C using a solution of water and ethanol (1:1). When the electrophile was iodine, the solution was decolorized with sodium thiosulfate. At room temperature, water (10 mL) was added to the mixture and THF was removed under reduced pressure. The aqueous layer was extracted with dichloromethane or ethyl acetate (3×20 mL), the combined organic extracts were then dried over magnesium sulfate and evaporated. The crude product was purified by column chromatography on silica gel.

3.5. Procedure E for metallation of 2-*tert*-butylpyrido-pyrimidin-4(3*H*)-ones by lithium 2,2,6,6- tetramethyl-piperidide

The workup of the procedures D and E is similar but differs only by the order of introduction of the electrophile. For procedure E, the lithiated product was introduced into the electrophile solution.

3.5.1. 3-(*tert*-Butylcarbonylamino)-6-methoxy-isonicotinic acid (1). Metallation of *N*-(6-methoxy-3-pyridyl)-2,2-dimethylpropanamide (10 g, 48 mmol) according to the procedure A with *n*-BuLi 2.5 M (2.5 equiv., 48 mL), TMEDA (2.5 equiv., 18.13 mL) in anhydrous ethyl ether (300 mL) at *T*=−10 °C gave 7.43 g (61%) of **1** as a colorless solid, mp 240–241 °C; ^{1}H NMR ($CDCl_3$): δ 1.15 (s, 9H, *tert*-butyl); 3.78 (s, 3H, OMe); 7.12 (s, 1H, H_5); 9.00 (s, 1H, H_2); 10.49 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 27.5 (3 $Me_{tert\text{-butyl}}$); 49.0 (CMe_3); 53.9 (OMe); 110.5 (CH); 129.8 (C_{py}); 130.7 (C_{py}); 140.6 (CH); 159.8 (C_{py}); 167.9 (CO); 176.5 (CO). Anal. Calcd for $C_{12}H_{16}N_2O_4$ (252.27): C, 57.13; H, 6.39; N, 11.10. Found: C, 57.39; H, 6.76; N, 10.88.

3.5.2. 3-(*tert*-Butylcarbonylamino)-2-methoxy-isonicotinic acid (2). Metallation of *N*-(2-methoxy-3-pyridyl)-2,2-dimethylpropanamide (10 g, 48 mmol) according to the procedure A with *n*-BuLi 2.5 M (2.5 equiv., 48 mL), TMEDA (2.5 equiv., 18.13 mL) in anhydrous diethyl ether (300 mL) at *T*=−10 °C gave 8.41 g (69%) of **2** as a colorless solid, mp 39–40 °C; ^{1}H NMR ($CDCl_3$): δ 1.25 (s, 9H, *tert*-butyl); 3.96 (s, 3H, OMe); 7.19 (d, J_{5-6}=5.6 Hz, 1H, H_5); 7.98 (d, J=5.6 Hz, 1H, H_6); 7.89 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 27.5 (3 $Me_{tert\text{-butyl}}$); 39.8 (CMe_3); 54.8 (OMe); 116.7 (CH); 119.8 (C_{py}); 133.4 (C_{py}); 142.9 (CH); 157.4 (C_{py}); 167.5 (CO); 177.9 (CO). Anal. Calcd for $C_{12}H_{16}N_2O_4$ (252.27): C, 57.13; H, 6.39; N, 11.10. Found: C, 56.98; H, 6.09; N, 11.51.

3.5.3. 2-(*tert*-Butylcarbonylamino)-5-chloronicotinic acid (3). Metallation of *N*-(5-chloro-2-pyridyl)-2,2-dimethylpropanamide (10 g, 47 mmol) according to the procedure A with *tert*-BuLi 1.5 M (2.25 equiv., 48 mL) in anhydrous THF (150 mL) at *T*=−78 °C gave 11.35 g (94%) of **3** as a white solid mp 238–239 °C; ^{1}H NMR (DMSO): δ 1.52 (s, 9H, *tert*-butyl); 8.14 (d, J=2.8 Hz, 1H, H_4); 8.35 (d, J=2.8 Hz, 1H, H_6); 12.41 (s, 1H, NH); ^{13}C NMR (DMSO): δ 27.3 ($Me_{tert\text{-butyl}}$); 39.7 (CMe_3); 67.3 (CCl); 120.6 (C_{py});

124.7 (C_{py}); 138.8 (CH); 148.1 (CH); 150.8 (C_{py}); 166.8 (CO); 175.7 (CO). Anal. Calcd for $C_{11}H_{13}N_2O_3Cl$ (256.69): C, 51.47; H, 5.10; N, 10.91. Found: C, 51.38; H, 5.07; N, 11.03.

3.5.4. 2-*tert*-Butyl-6-chloropyrido[2,3-*d*]pyrimidin-4(3*H*)-one (5). Reaction of 2-(*tert*-butyl-carbonylamino)-5-chloronicotinic acid (3 g) with acetic anhydride (30 mL) according to the procedure B, followed by reaction with 15 N ammonia (100 mL) gave 2.286 g (82%) of **5** as a colorless solid, mp>250 °C; ^{1}H NMR (DMSO): δ 1.39 (s, 9H, *tert*-butyl); 8.47 (d, J_{5-7}=2.6 Hz, 1H, H_5); 8.94 (d, J=2.6 Hz, 1H, H_7); 12.38 (s, 1H, NH); ^{13}C NMR (DMSO): δ 27.9 ($3Me_{tert\text{-butyl}}$); 38.0 (CMe_3); 116.7 (C_{py}); 128.4 (C_{py}); 134.2 (CH_{py}); 154.6 (CH_{py}); 156.9 (C_{py}); 162.5 (C_{py}); 167.0 (C_{py}). Anal. Calcd for $C_{11}H_{12}N_3OCl$ (237.69): C, 55.59; H, 5.09; N, 17.68. Found: C, 55.71; H, 5.04; N, 17.73.

3.5.5. 2-*tert*-Butylpyrido[3,4-*d*]pyrimidin-4(3*H*)-one (6). Reaction of 3-(*tert*-butylcarbonyl-amino)isonicotinic acid (2.8 g) with acetic anhydride (30 mL) according to the procedure B, followed by reaction with 15 N ammonia (30 mL) gave 1.61 g (63%) of **6** as a colorless solid, mp 208–209 °C; ^{1}H NMR ($CDCl_3$): δ 1.43 (s, 9H, *tert*-butyl); 7.96 (dd, J_{6-5}=5.3 Hz, J_{6-8}=0.76 Hz, 1H, H_6); 8.61 (d, J=5.3 Hz, 1H, H_5); 9.10 (d, J=0.76 Hz, 1H, H_8); 11.10 (s, 1H, NH); ^{13}C NMIR ($CDCl_3$): δ 28.5 ($3Me_{tert\text{-butyl}}$); 38.1 (CMe_3); 118.3 (CH_{py}); 125.8 (C_{py}); 144.0 (C_{py}); 146.3 (CH_{py}); 152.0 (CH_{py}); 163.1 (C_{py}); 164.5 (C_{py}). Anal. Calcd for $C_{11}H_{13}N_3O$ (203.24): C, 65.01; H, 6.45; N, 20.67. Found: C, 64.98; H, 6.44; N, 20.20.

3.5.6. 2-*tert*-Butyl-6-chloropyrido[3,4-*d*]pyrimidin-4(3*H*)-one (7). Reaction of 3-(*tert*-butyl-carbonylamino)-6-chloro-isonicotinic acid (8 g) with acetic anhydride (80 mL) according to the procedure B, followed by reaction with 15 N ammonia (150 mL) gave 6.72 g (90%) of **7** as a brown solid, mp 237–238 °C; ^{1}H NMR ($CDCl_3$): δ 1.43 (s, 9H, *tert*-butyl); 7.98 (s, 1H, H_5); 8.87 (s, 1H, H_8); 11.19 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.5 ($3Me_{tert\text{-butyl}}$); 38.2 (CMe_3); 118.9 (CH_{py}); 128.2 (C_{py}); 143.0 (C_{py}); 147.8 (C_{py}); 152.0 (CH_{py}); 162.2 (C_{py}); 164.7 (C_{py}). Anal. Calcd for $C_{11}H_{12}N_3O_2Cl$ (237.68): C, 55.59; H, 5.09; N, 17.68. Found: C, 55.53; H, 5.37; N, 17.72.

3.5.7. 2-*tert*-Butyl-6-methoxypyrido[3,4-*d*]pyrimidin-4(3*H*)-one (8). Reaction of 3-(*tert*-butyl-carbonylamino)-6-methoxy-isonicotinic acid (2.42 g) with acetic anhydride (30 mL) according to the procedure B, followed by reaction with 15 N ammonia (30 mL) gave 1.43 g (65%) **8** as a colorless solid, mp 248–249 °C; ^{1}H NMR ($CDCl_3$): δ 1.39 (s, 9H, *tert*-butyl); 3.96 (s, 3H, OMe); 7.37 (s, 1H, H_5); 8.70 (s, 1H, H_8); 10.49 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.5 ($3Me_{tert\text{-butyl}}$); 37.7 (CMe_3); 54.7 (OMe); 103.3 (CH_{py}); 129.3 (C_{py}); 138.8 (C_{py}); 149.2 (CH_{py}); 160.7 (C_{py}); 162.6 (C_{py}); 162.8 (C_{py}). Anal. Calcd for $C_{12}H_{15}N_3O_2$ (233.27): C, 61.79; H, 6.48; N, 18.01. Found: C, 61.67; H, 6.72; N, 17.79.

3.5.8. 2-*tert*-Butyl-8-methoxypyrido[3,4-*d*]pyrimidin-4(3*H*)-one (9). Reaction of 3-(*tert*-butyl-carbonylamino)-2-methoxy-isonicotinic acid (6.8 g) with acetic anhydride (80 mL) according to the procedure B, followed by reaction with 15 N ammonia (150 mL) and 5% aqueous sodium hydroxyde (100 mL) gave 5.93 g (93%) of **9** as a colorless solid, mp>250 °C; ^{1}H NMR ($CDCl_3$): δ 1.44 (s, 9H, *tert*-butyl), 4.07 (s, 3H, OMe); 7.54 (d, J_{5-6}=5.3 Hz, 1H, H_5); 8.08 (d, J=5.3 Hz, 1H, H_6); 11.15 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.6 ($3Me_{tert\text{-butyl}}$); 38.2 (CMe_3); 55.0 (OMe); 111.7 (CH_{py}); 127.5 (C_{py}); 134.8 (C_{py}); 142.7 (CH_{py}); 160.4 (C_{py}); 162.8 (C_{py}); 163.8 (C_{py}). Anal. Calcd for $C_{12}H_{15}N_3O_2$ (233.27): C, 61.79; H, 6.48; N, 18.01. Found: C, 61.82; H, 6.65; N, 18.31.

3.5.9. 2-*tert*-Butylpyrido[4,3-*d*]pyrimidin-4(3*H*)-one (10). Reaction of 4-(*tert*-butylcarbonyl-amino)nicotinic acid (6.44 g) with acetic anhydride (100 mL) according to the procedure B, followed by reaction with 15 N ammonia (100 mL) gave 5.155 g (87%) of **10** as a colorless solid, mp 248–249 °C; ^{1}H NMR (DMSO): δ 1.13 (s, 9H, *tert*-butyl); 7.30 (d, J_{7-8}=5.65 Hz, 1H, H_8); 8.56 (d, J=5.65 Hz, 1H, H_7); 9.02 (s, 1H, H_5); 12.03 (s, 1H, NH); ^{13}C NMR (DMSO): δ 27.9 ($3Me_{tert\text{-butyl}}$); 38.0 (CMe_3); 116.7 (C_{py}); 120.9 (CH_{py}); 149.6 (CH_{py}); 153.6 (CH_{py}); 161.9 (C_{py}); 168.2 (C_{py}). Anal. Calcd for $C_{11}H_{13}N_3O$ (203.24): C, 65.01; H, 6.45; N, 20.67. Found: C, 65.11; H, 6.43; N, 20.64.

3.5.10. 2-*tert*-Butylpyrido[3,2-*d*]pyrimidin-4(3*H*)-one (11). A solution of 3-aminopicolinic acid (1.50 g) and pivaloyl chloride (3.27 mL, 2.5 equiv.) in pyridine (10 mL) was refluxed for 30 min. The mixture was cooled to 10 °C, diluted with water and extracted with dichloromethane (3×25 mL). The combined organic extracts were then dried over magnesium sulfate and evaporated under reduced pressure to give crude pyrido-oxazinone. Then, 15 N ammonia (50 mL) was added and the mixture was stirred at room temperature for 24 h. Evaporation of the solution or suspension under reduced pressure yielded 1.71 g (78%) of **11** as a brown solid, mp 250–251 °C; ^{1}H NMR (DMSO): δ 1.42 (s, 9H, *tert*-butyl); 7.83 (dd, J_{6-7}=4.5 Hz, J_{7-8}=8.3 Hz, 1H, H_7); 8.09 (dd, J_{8-6}=1.5 Hz, J=8.3 Hz, 1H, H_8); 8.81 (dd, J=4.5, 1.5 Hz, 1H, H_6); 12.24 (s, 1H, NH); ^{13}C NMR (DMSO): δ 28.1 ($3Me_{tert\text{-butyl}}$); 37.6 (CMe_3); 129.0 (CH_{py}); 135.9 (CH_{py}); 137.9 (C_{py}); 145.3 (C_{py}); 149.1 (CH_{py}); 161.1 (C_{py}); 163.8 (C_{py}). Anal. Calcd for $C_{11}H_{13}N_3O$ (203.24): C, 65.01; H, 6.45; N, 20.67. Found: C, 64.87; H, 6.44; N, 20.82.

3.6. Metallation of 2-*tert*-butylpyrido[3,4-*d*]pyrimidin-4(3*H*)-one (6)

According to the procedure C with *n*-BuLi 1.6 M (4 equiv., 0.63 mL), T=−78 °C followed by reaction with diphenyldisulfide (4 equiv., 215 mg) gave after purification by column chromatography (silicagel, eluent: (1) dichloromethane, (2) diethyl ether) 11 mg (18%) of 8-*n*-butyl-2-*tert*-butylpyrido[3,4-*d*]pyrimidin-4(3*H*)-one (**12**), 10 mg (17%) of 6-*n*-butyl-2-*tert*-butylpyrido[3,4-*d*]pyrimidin-4(3*H*)-one (**13**) and 51 mg (56%) of 8-*n*-butyl-2-*tert*-butyl-5-phenylthiopyrido[3,4-*d*]pyrimidin-4(3*H*)-one (**14**).

3.6.1. 8-*n*-Butyl-2-*tert*-butylpyrido[3,4-*d*]pyrimidin-4(3*H*)-one (12). Light brown solid, mp 120–121 °C; ^{1}H NMR ($CDCl_3$): δ 0.90 (t, J=7.3 Hz, 3H, Me); 1.37 (m, 2H, CH_2); 1.40 (s, 9H, *tert*-butyl); 1.68 (m, 2H, CH_2); 3.23 (t, J=7.6 Hz, 2H, CH_2); 7.80 (d, J_{5-6}=5.5 Hz, 1H, H_5); 8.48 (d, J=5.5 Hz, 1H, H_6); 10.53 (s, 1H, NH); ^{13}C NMR

($CDCl_3$): δ 14.3 (Me); 23.1 (CH_2); 28.5 ($3Me_{tert\text{-}butyl}$); 31.9 (CH_2); 33.7 (CH_2); 38.2 (*C*Me_3); 116.3 (CH_{py}); 125.6 (C_{py}); 134.1 (C_{py}); 145.0 (CH_{py}); 162.7 (C_{py}); 163.6 (C_{py}); 163.9 (C_{py}). Anal. Calcd for $C_{15}H_{21}N_3O$ (259.35): C, 69.47; H, 8.16; N, 16.20. Found: C, 69.53; H, 8.11; N, 16.28.

3.6.2. 6-*n*-Butyl-2-*tert*-butylpyrido[3,4-*d*]pyrimidin-4(3*H*)-one (13). Light brown solid, mp 114–115 °C; ^1H NMR ($CDCl_3$): δ 0.88 (t, *J*=7.3 Hz, 3H, Me); 1.33 (m, 2H, CH_2); 1.39 (s, 9H, *tert*-butyl); 1.64 (m, 2H, CH_2); 3.08 (t, *J*=7.7 Hz, 2H, CH_2); 8.18 (s, 1H, H_5); 10.12 (s, 1H, NH); 10.22 (s, 1H, H_6); ^{13}C NMR ($CDCl_3$): δ 14.3 (Me); 23.0 (CH_2); 28.5 ($3Me_{tert\text{-}butyl}$); 30.0 (CH_2); 32.0 (CH_2); 32.9 (CH_2); 38.2 (*C*Me_3); 111.6 (C_{py}); 113.2 (C_{py}); 133.2 (CH_{py}); 136.3 (CH_{py}); 151.7 (CH_{py}); 152.9 (C_{py}); 162.2 (C_{py}); 167.4 (C_{py}). Anal. Calcd for $C_{15}H_{21}N_3O$ (259.35): C, 69.47; H, 8.16; N, 16.20. Found: C, 69.36; H, 8.09; N, 15.98.

3.6.3. 8-*n*-Butyl-2-*tert*-butyl-5-phenylthiopyrido[3,4-*d*]-pyrimidin-4(3*H*)-one (14). Colorless solid, mp 160–161 °C; ^1H NMR ($CDCl_3$): δ 0.86 (t, *J*=7.3 Hz, 3H, Me); 1.32 (m, 2H, CH_2); 1.45 (s, 9H, *tert*-butyl); 1.64 (m, 2H, CH_2); 3.08 (t, *J*=7.6 Hz, 2H, CH_2); 7.40 (m, 3H, Ph); 7.58 (m, 2H, Ph); 7.59 (s, 1H, H_6); 11.84 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 14.3 (Me); 23.0 (CH_2); 28.5 ($3Me_{tert\text{-}butyl}$); 31.6 (CH_2); 33.2 (CH_2); 38.3 (*C*Me_3); 121.3 (C_{py}); 130.0 (CH_{Ph}); 130.4 ($2CH_{Ph}$); 131.1 (C_{Ph}); 134.1 (C_{py}); 136.4 ($2CH_{Ph}$); 141.3 (CH_{py}); 142.6 (C_{py}); 158.2 (C_{py}); 163.3 (C_{py}); 164.5 (C_{py}). Anal. Calcd for $C_{21}H_{25}N_3OS$ (367.51): C, 68.63; H, 6.86; N, 11.43; S, 8.73. Found: C, 68.67; H, 6.79; N, 12.01; S, 8.82.

3.7. Metallation of 2-*tert*-butylpyrido[3,4-*d*]pyrimidin-4(3*H*)-one (6)

According to the procedure C with PhLi 1.8 M (4.2 equiv., 0.57 mL), *T*=0 °C, followed by reaction with diphenyl disulfide (4 equiv., 215 mg) gave after purification by column chromatography (silica, eluent: dichloromethane/diethylether, 7:3)) 27 mg (40%) of 2-*tert*-butyl-8-phenyl-pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (**15**) and 28 mg (29%) of 2-*tert*-butyl-8-phenyl-5-phenylthio-pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (**16**).

3.7.1. 2-*tert*-Butyl-8-phenylpyrido[3,4-*d*]pyrimidin-4(3*H*)-one (15). Light brown solid, mp 218–219 °C; ^1H NMR ($CDCl_3$): δ 1.39 (s, 9H, *tert*-butyl); 7.39 (m, 3H, Ph); 7.96 (d, J_{5-6}=4.9 Hz, 1H, H_5); 8.13 (m, 2H, Ph); 8.68 (d, *J*=4.9 Hz, 1H, H_6); 11.29 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.5 ($3Me_{tert\text{-}butyl}$); 38.5 (*C*Me_3); 117.6 (CH_{py}); 127.0 (C_{py}); 128.0 ($2CH_{Ph}$); 129.2 (CH_{Ph}); 131.4 ($2CH_{Ph}$); 138.0 (C_{Ph}); 141.6 (C_{py}); 145.7 (CH_{py}); 157.9 (C_{py}); 163.1 (C_{py}); 163.6 (C_{py}). Anal. Calcd for $C_{16}H_{16}N_3O$ (266.33): C, 72.16; H, 6.06; N, 15.78. Found: C, 72.19; H, 6.11; N, 15.81.

3.7.2. 2-*tert*-Butyl-8-phenyl-5-phenylthiopyrido[3,4-*d*]-pyrimidin-4(3*H*)-one (16). Colorless solid, mp>250 °C; ^1H NMR ($CDCl_3$): δ 1.44 (s, 9H, *tert*-butyl); 7.38 (m, 6H, Ph); 7.63 (m, 2H, Ph); 7.80 (s, 1H, H_6); 8.05 (m, 2H, Ph); 11.63 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.6 ($3Me_{tert\text{-}butyl}$); 38.6 (*C*Me_3); 122.4 (C_{py}); 127.9 ($2CH_{Ph}$); 128.8 (CH_{Ph}); 130.2 (CH_{Ph}); 130.6 ($2CH_{Ph}$); 130.8 (C_{Ph}); 131.0 ($2CH_{Ph}$); 136.2 (C_{py}); 136.4 ($2CH_{Ph}$); 138.0 (C_{Ph}); 142.0 (CH_{py}); 142.1 (C_{py}); 152.1 (C_{py}); 163.4 (C_{py}); 164.3 (C_{py}). Anal. Calcd for $C_{23}H_{21}N_3OS$ (387.51): C, 71.29; H, 5.46; N, 10.84; S, 8.27. Found: C, 71.34; H, 5.51; N, 10.90; S, 8.21.

3.7.3. 2-*tert*-Butyl-5-phenylthiopyrido[3,4-*d*]pyrimidin-4(3*H*)-one (17). Metallation of **6** (100 mg, 0.48 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 2.46 mL), TMPH (8 equiv., 0.67 mL), *T*=−78 °C, followed by reaction with diphenyl disulfide (8 equiv., 860 mg) in solution with anhydrous THF (5 mL), *t*=1 h, gave after purification by column chromatography (silicagel, eluent: petroleum ether/di ethyl ether , 1:1)) 159 mg (97%) of **12** as a yellow solid, mp>250 °C; ^1H NMR ($CDCl_3$): δ 1.46 (s, 9H, *tert*-butyl); 7.43 (m, 3H, Ph); 7.60 (m, 2H, Ph); 7.68 (s, 1H, H_6); 8.67 (s, 1H, H_8); 11.87 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.5 ($3Me_{tert\text{-}butyl}$); 38.2 (*C*Me_3); 121.4 (C_{py}); 130.3 (CH_{Ph}); 130.5 (C_{Ph}); 130.6 ($2CH_{Ph}$); 136.4 ($2CH_{Ph}$); 137.3 (C_{py}); 142.5 (CH_{py}); 144.7 (C_{py}); 146.5 (CH_{py}); 164.0 (C_{py}); 165.0 (C_{py}). Anal. Calcd for $C_{17}H_{17}N_3OS$ (311.40): C, 65.57; H, 5.50; N, 13.49; S, 10.30. Found: C, 65.33; H, 5.51; N, 13.26; S, 9.98.

3.7.4. 2-*tert*-Butyl-5-(1-hydroxyethyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (18). Metallation of **6** (100 mg, 0.48 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 2.46 mL), TMPH (8 equiv., 0.67 mL), *T*=−78 °C, followed by reaction with acetaldehyde (10 equiv., 0.27 mL), *t*=1 h, gave after purification by column chromatography (silicagel, eluent: petroleum ether/ethyl acetate, 1:1)) 116 mg (96%) of **18** as a white solid, mp 244–245 °C; ^1H NMR (DMSO): δ 1.24 (s, 9H, *tert*-butyl); 1.26 (d, *J*=6.4 Hz, 3H, Me); 5.32 (d, *J*=4.5 Hz, 1H, OH); 5.83 (dq, *J*=5.8, 4.5 Hz, 1H, C*H*OH); 8.70 (s, 1H, H_6); 8.74 (s, 1H, H_8); 12.02 (s, 1H, NH); ^{13}C NMR (DMSO): δ 26.3 (Me); 27.9 ($3Me_{tert\text{-}butyl}$); 37.5 (*C*Me_3); 64.3 (CHOH); 121.7 (C_{py}); 141.5 (C_{py}); 143.3 (CH_{py}); 143.6 (C_{py}); 149.5 (CH_{py}); 162.2 (C_{py}); 164.9 (C_{py}). Anal. Calcd for $C_{13}H_{17}N_3O$ (247.29): C, 63.14; H, 6.93; N, 16.96. Found: C, 62.98; H, 7.07; N, 16.53.

3.7.5. 2-*tert*-Butyl-5-(hydroxyphenylmethyl)pyrido[3,4-*d*]-pyrimidin-4(3*H*)-one (19). Metallation of **6** (50 mg, 0.24 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.33 mL), *T*=−78 °C, followed by reaction with benzaldehyde (8 equiv., 0.27 mL), *t*=1 h, gave after purification by column chromatography (silicagel, eluent: petroleum ether/ethyl acetate, 1:1)) 73 mg (96%) of **19** as a colorless solid, mp 194–195 °C; ^1H NMR ($CDCl_3$): δ 1.33 (s, 9H, *tert*-butyl); 5.49 (d, *J*=7.5 Hz, 1H, OH); 6.36 (d, *J*=7.5 Hz, 1H, C*H*OH); 7.22 (m, 5H, Ph); 8.42 (s, 1H, H_6); 9.05 (s, 1H, H_8); 10.70 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.5 ($3Me_{tert\text{-}butyl}$); 37.8 (*C*Me_3); 72.9 (CHOH); 123.2 (C_{py}); 127.0 ($2CH_{Ph}$); 127.8 (CH_{Ph}); 128.6 ($2CH_{Ph}$); 136.5 (C_{Ph}); 142.5 (C_{py}); 145.5 (C_{py}); 146.7 (CH_{py}); 152.5 (CH_{py}); 164.1 (C_{py}); 164.3 (C_{py}). Anal. Calcd for $C_{18}H_{19}N_3O_2$ (309.36): C, 69.88; H, 6.19; N, 13.58. Found: C, 69.79; H, 6.18; N, 13.68.

3.7.6. 2-*tert*-Butyl-5,N_3-bis(tributylstannyl)pyrido[3,4-*d*]-pyrimidin-4(3*H*)-one (20). Metallation of **6** (100 mg, 0.48 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 2.46 mL), TMPH (8 equiv., 0.67 mL), *T*=−78 °C, followed by reaction with tri-*n*-butylstannyl

chloride (8 equiv., 1.08 mL), t=1 h, gave after purification by column chromatography (silicagel, eluent: ethyl acetate) 372 mg (97%) of **20** as a colorless solid, mp 108–109 °C; ^{1}H NMR ($CDCl_3$): δ 0.80 (m, 18H, 6Me); 1.19 (m, 24H, 12CH_2); 1.37 (s, 9H, *tert*-butyl); 1.69 (m, 12H, 6CH_2); 8.62 (t, J_{H6-Sn}=10.7 Hz, 1H, H_6); 8.97 (t, J_{H8-Sn}=3.7 Hz, 1H, H_8); ^{13}C NMR ($CDCl_3$): δ 11.5 (CH_2); 13.9 (Me); 14.0 (Me); 17.3 (CH_2); 27.3 (CH_2); 27.7 (CH_2); 28.2 (CH_2); 28.6 (3Me$_{tert\text{-butyl}}$); 29.5 (CH_2); 37.8 (*C*Me$_3$); 131.0 (C_{py}); 135.1 (C_{py}); 144.1 (C_{py}); 151.3 (CH_{py}); 153.1 (CH_{py}); 162.9 (C_{py}); 163.6 (C_{py}). Anal. Calcd for $C_{35}H_{65}N_3OSn_2$ (781.34): C, 53.80; H, 8.39; N, 5.38. Found: C, 53.72; H, 8.31; N, 5.59.

3.7.7. 2-*tert*-Butyl-5-iodopyrido[34-*d*]pyrimidin-4(3*H*)-one (21). Metallation of **6** (50 mg, 0.24 mmol) according to the procedure E with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.33 mL), T=−78 °C, followed by reaction with iodine (8 equiv., 500 mg) in solution with anhydrous THF (5 mL), t=1 h, gave after purification by column chromatography (silicagel, eluent: dichloromethane/diethyl ether, 7:3)) 61 mg (75%) of **21** as a colorless solid, mp>250 °C; ^{1}H NMR (DMSO): δ 1.20 (s, 9H, *tert*-butyl), 8.71 (s, 1H, H_6), 8.78 (s, 1H, H_8), 12.12 (s, 1H, NH); ^{13}C NMR (DMSO): δ 27.8 (3Me$_{tert\text{-butyl}}$), 37.8 (*C*Me$_3$), 90.1 (C_{py}), 125.7 (C_{py}), 144.9 (C_{py}), 150.7 (CH_{py}), 155.2 (CH_{py}), 160.3 (C_{py}), 165.2 (C_{py}). Anal. Calcd for $C_{11}H_{12}N_3OI$ (329.14): C, 40.14; H, 3.67; N, 12.77. Found: C, 40.07; H, 3.59; N, 12.85.

3.7.8. 2-*tert*-Butyl-5-chloropyrido[3,4-*d*]pyrimidin-4(3*H*)-one (22). Metallation of **6** (500 mg, 2.46 mmol) according to the procedure E with *n*-BuLi 1.6 M (9 equiv., 13.85 mL), TMPH (9 equiv., 3.74 mL), T=−78 °C, followed by reaction with hexachloroethane (9 equiv., 5.25 g) in solution with anhydrous THF (15 mL), t=1 h, gave after filtration 476 mg (82%) of **22** as a colorless solid, mp>250 °C; ^{1}H NMR (DMSO): δ 1.45 (s, 9H, *tert*-butyl); 8.67 (s, 1H, H_6); 8.98 (s, 1H, H_8); 12.40 (s, 1H, NH); ^{13}C NMR (DMSO): δ 27.9 (3Me$_{tert\text{-butyl}}$); 37.7 (*C*Me$_3$); 122.8 (C_{py}); 128.0 (C_{py}); 145.3 (C_{py}); 145.9 (CH_{py}); 149.7 (CH_{py}); 160.0 (C_{py}); 166.3 (C_{py}). Anal. Calcd for $C_{11}H_{12}N_3OCl$ (237.68): C, 55.59; H, 5.09; N, 17.68. Found: C, 55.41; H, 5.18; N, 17.71.

3.7.9. 2-*tert*-Butyl-6-iodopyrido[3,4-*d*]pyrimidin-4(3*H*)-one (23). Metallation of **6** (50 mg, 0.24 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.33 mL), T=−78 °C, followed by reaction with iodine (2 equiv., 125 mg) in solution with anhydrous THF (5 mL), t=2 h, gave after purification by column chromatography (silicagel, eluent: dichloromethane/ethyl ether, 5:5)) 45 mg of **23** as a colorless solid (56%), mp 210–211 °C; ^{1}H NMR ($CDCl_3$): δ 1.42 (s, 9H, *tert*-butyl); 8.34 (s, 1H, H_6); 8.83 (s, 1H, H_8); 11.27 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.5 (3Me$_{tert\text{-butyl}}$); 38.3 (*C*Me$_3$); 111.6 (C_{py}); 127.4 (C_{py}); 129.4 (CH_{py}); 143.5 (C_{py}); 152.7 (CH_{py}); 161.8 (C_{py}); 165.2 (C_{py}). Anal. Calcd for $C_{11}H_{12}N_3OI$ (329.14): C, 40.14; H, 3.67; N, 12.77. Found: C, 40.26; H, 3.82; N, 12.13.

3.7.10. 2-*tert*-Buyl-6-chloro-5-phenylthiopyrido[3,4-*d*]-pyrimidin-4(3*H*)-one (24). Metallation of **7** (50 mg, 0.21 mmol) according to the procedure E with *n*-BuLi 1.6 M (8 equiv., 1.05 mL), TMPH (8 equiv., 0.29 mL), T=−78 °C, followed by reaction with diphenyl disulfide (8 equiv., 367 mg) in solution with anhydrous THF (5 mL), t=1 h, gave after purification by column chromatography (silicagel, eluent: dichloromethane/ethyl acetate, 9:1)) 62 mg (86%) of **24** as a yellow solid, mp 211–212 °C; ^{1}H NMR ($CDCl_3$): δ 1.34 (s, 9H, *tert*-butyl); 7.10 (m, 5H, Pb); 8.82 (s, 1H, H_8); 11.41 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.3 (3Me$_{tert\text{-butyl}}$); 38.1 (*C*Me$_3$); 126.6 (CH_{Ph}); 127.7 (C_{py}); 128.4 (2CH_{Ph}); 129.3 (C_{Ph}); 129.4 (2CH_{Ph}); 136.5 (C_{py}); 144.8 (C_{py}); 151.4 (CH_{py}); 153.5 (C_{py}); 161.6 (C_{py}); 165.6 (C_{py}). Anal. Calcd for $C_{17}H_{16}N_3OSCl$ (345.85): C, 59.04; H, 4.66; N, 12.15; S, 9.27. Found: C, 59.07; H, 4.67; N, 11.97; S, 9.22.

3.7.11. 2-*tert*-Butyl-6-chloro-5-(1-hydroxyethyl)pyrido-[3,4-*d*]pyrimidin-4(3*H*)-one (25). Metallation of **7** (50 mg, 0.21 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.05 mL), TMPH (8 equiv., 0.29 mL), T=−78 °C, followed by reaction with acetaldehyde (9 equiv., 0.11 mL), t=1 h, gave after purification by column chromatography (silicagel, eluent: dichloromethane/ethyl acetate, 8:2)) 51 mg (87%) of **25** as a colorless solid, mp 202–203 °C; ^{1}H NMR ($CDCl_3$): δ 1.42 (s, 9H, *tert*-butyl); 1.59 (d, J=6.8 Hz, 3H, Me); 5.58 (dq, J=6.2 Hz, 1H, C*H*OH); 6.13 (d, J=12.0 Hz, 1H, OH); 8.83 (s, 1H, H_8); 10.50 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 22.4 (Me); 28.5 (3Me$_{tert\text{-butyl}}$); 37.8 (*C*Me$_3$); 68.7 (CHOH); 125.4 (C_{py}); 137.8 (C_{py}); 145.5 (C_{py}); 146.9 (C_{py}); 151.2 (CH_{py}); 163.9 (C_{py}); 164.0 (C_{py}). Anal. Calcd for $C_{13}H_{16}N_3O_2Cl$ (281.74): C, 55.42; H, 5.72; N, 14.91. Found: C, 55.41; H, 5.95; N, 14.77.

3.7.12. 2-*tert*-Butyl-6-chloro-5-(hydroxyphenylmethyl)-pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (26). Metallation of **7** (50 mg, 0.21 mmol) according the to procedure D with *n*-BuLi 1.6 M (8 equiv., 1.05 mL), TMPH (8 equiv., 0.29 mL), T=−78 °C, followed by reaction with benzaldehyde (8 equiv., 0.17 mL), t=1 h, gave after purification by column chromatography (silicagel, eluent: petroleum ether/ethyl acetate, 5:5)) 70 mg (97%) of **26** as a colorless solid, mp 227–228 °C; ^{1}H NMR ($CDCl_3$): δ 1.28 (s, 9H, *tert*-butyl); 6.34 (d, J=12.0 Hz, 1H, C*H*OH); 6.64 (d, J=12.0 Hz, 1H, OH); 7.19 (m, 5H, Ph); 8.91 (s, 1H, H_8); 9.71 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.4 (3Me$_{tert\text{-butyl}}$); 37.7 (*C*Me$_3$); 72.9 (CHOH); 125.9 (C_{py}); 126.2 (2CH_{Ph}); 127.6 (CH_{Ph}); 128.6 (2CH_{Ph}); 135.5 (C_{py}); 142.1 (CH_{Ph}); 145.6 (C_{py}); 148.6 (C_{py}); 151.9 (CH_{py}); 162.8 (C_{py}); 164.1 (C_{py}). Anal. Calcd for $C_{18}H_{18}N_3O_2Cl$ (343.81): C, 62.88; H, 5.28; N, 12.22. Found: C, 62.68; H, 5.36; N, 11.92.

3.7.13. 2-*tert*-Butyl-5,N$_3$-bis(tributylstannyl)-6-chloro-pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (27). Metallation of **7** (50 mg, 0.21 mmol) according to the procedure E with *n*-BuLi 1.6 M (8 equiv., 1.05 mL), TMPH (8 equiv., 0.29 mL), T=−78 °C, followed by reaction with tri-*n*-butylstannyl chloride (8 equiv., 0.47 mL), t=1 h, gave after purification by column chromatography (silicagel, eluent: dichloromethane) 92 mg (84%) of **27** as an oil; ^{1}H NMR ($CDCl_3$): δ 0.79 (t, J=7.1 Hz, 9H, Me); 1.18 (m, 12H, CH_2); 1.35 (s, 9H, *tert*-butyl); 1.44 (m, 6H, CH_2); 8.72 (t, J_{H8-Sn}= 3.0 Hz, 1H, H_8); 8.89 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 14.1 (Me); 15.2 (CH_2); 27.7 (CH_2); 28.6 (3Me$_{tert\text{-butyl}}$); 29.5

(CH_2); 37.6 (CMe_3); 134.3 (C_{py}); 138.3 (C_{py}); 143.1 (C_{py}); 151.3 (CH_{py}); 156.6 (C_{py}); 162.3 (C_{py}); 162.6 (C_{py}). Anal. Calcd for $C_{23}H_{38}N_3OClSn$ (526.73): C, 52.45; H, 7.27; N, 7.98. Found: C, 52.41; H, 7.26; N, 7.79.

3.7.14. 2-*tert*-Butyl-6-chloro-5-iodopyrido[3,4-*d*]pyrimidin-4(3*H*)-one (28). Metallation of **7** (50 mg, 0.21 mmol) according to the procedure E with *n*-BuLi 1.6 M (8 equiv., 1.05 mL), TMPH (8 equiv., 0.29 mL), $T=-78$ °C, followed by reaction with iodine (8 equiv., 427 mg) in solution with anhydrous THF (5 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: ethyl acetate/dichloromethane, 1:9)) 66 mg (87%) of **28** as a yellow solid, mp 238–239 °C; ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 9H, *tert*-butyl); 8.74 (s, 1H, H_8); 11.52 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.5 ($3Me_{tert\text{-butyl}}$); 38.3 (CMe_3); 90.9 (C_{py}); 128.9 (C_{py}); 143.8 (C_{py}); 151.1 (C_{py}); 154.2 (C_{py}); 161.3 (C_{py}); 165.0 (C_{py}). Anal. Calcd for $C_{11}H_{11}N_3OClI$ (363.58): C, 36.34; H, 3.05; N, 11.56. Found: C, 36.42; H, 2.89; N, 11.05.

3.7.15. 2-*tert*-Butyl-6-methoxy-5-phenylthiopyrido[3,4-*d*]-pyrimidin-4(3*H*)-one (29). Metallation of **8** (50 mg, 0.21 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.07 mL), TMPH (8 equiv., 0.29 mL), $T=0$ °C, followed by reaction with dipheny disulfide (8 equiv., 380 mg) in solution with anhydrous THF (5 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: dichloromethane) 52 mg (71%) of **29** as a yellow solid, mp 210–211 °C; ^{1}H NMR ($CDCl_3$): δ 1.35 (s, 9H, *tert*-butyl); 3.72 (s, 3H, OMe); 7.10 (m, 5H, Ph); 8.61 (s, 1H, H_8); 10.50 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.4 ($3Me_{tert\text{-butyl}}$); 37.6 (CMe_3); 54.8 (OMe); 114.4 (C_{py}); 126.3 (CH_{Ph}); 128.8 (C_{Ph}); 128.9 ($2CH_{Ph}$); 128.9 ($2CH_{Ph}$); 137.5 (C_{py}); 140.4 (C_{py}); 148.0 (CH_{py}); 161.1 (C_{py}); 161.5 (C_{py}); 162.2 (C_{py}). Anal. Calcd for $C_{18}H_{19}N_3O_2S$ (341.43): C, 63.32; H, 5.61; N, 12.31; S, 9.39. Found: C, 63.21; H, 5.84; N, 12.35; S, 9.47.

3.7.16. 2-*tert*-Butyl-5-(1-hydroxyethyl)-6-methoxypyrido-[3,4-*d*]pyrimidin-4(3*H*)-one (30). Metallation of **8** (50 mg, 0.21 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.07 mL), TMPH (8 equiv., 0.29 mL), $T=0$ °C, followed by reaction with acetaldehyde (10 equiv., 0.10 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: petroleum ether/diethyl ether, 3:7)) 54 mg (92%) of **30** as a colorless solid, mp 227–228 °C; ^{1}H NMR ($CDCl_3$): δ 1.41 (s, 9H, *tert*-butyl); 1.53 (d, $J=6.4$ Hz, 3H, Me); 4.00 (s, 3H, OMe); 5.63 (d, $J=12.4$ Hz, 1H, OH); 5.77 (dq, $J=5.9$ Hz, 1H, *CH*OH); 8.66 (s, 1H, H_8); 11.14 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 23.4 (Me); 28.5 ($3Me_{tert\text{-butyl}}$); 37.5 (CMe_3); 54.8 (OMe); 64.5 (CHOH); 125.1 (C_{py}); 125.3 (C_{py}); 140.4 (C_{py}); 148.1 (CH_{py}); 159.0 (C_{py}); 160.3 (C_{py}); 165.3 (C_{py}). Anal. Calcd for $C_{14}H_{19}N_3O_3$ (277.32): C, 60.63; H, 6.91; N, 15.15. Found: C, 60.59; H, 7.12; N, 14.98.

3.7.17. 2-*tert*-Butyl-5-(hydroxyphenylmethyl)-6-methoxy-pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (31). Metallation of **8** (50 mg, 0.21 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.07 mL), TMPH (8 equiv., 0.29 mL), $T=0$ °C, followed by reaction with benzaldehyde (8 equiv., 0.18 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: dichloromethane/ethyl acetate, 7:3)) 67 mg (91%) of **31** as a colorless solid, mp>250 °C; ^{1}H NMR ($CDCl_3$): δ 1.41 (s, 9H, *tert*-butyl); 3.95 (s, 3H, OMe); 5.97 (d, $J=12.4$ Hz, 1H, *CH*OH); 6.84 (d, $J=12.4$ Hz, 1H, OH); 7.18 (m, 5H, Ph); 8.72 (s, 1H, H_8); 10.49 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.3 ($3Me_{tert\text{-butyl}}$); 37.3 (CMe_3); 55.0 (OMe); 68.8 (CHOH); 122.6 (C_{Ph}); 125.9 (C_{py}); 126.4 ($2CH_{Ph}$); 127.2 (CH_{Ph}); 128.3 ($2CH_{Ph}$); 140.5 (C_{py}); 143.6 (C_{py}); 148.9 (CH_{py}); 159.8 (C_{py}); 160.5 (C_{py}); 164.5 (C_{py}). Anal. Calcd for $C_{19}H_{21}N_3O_3$ (339.39): C, 67.24; H, 6.24; N, 12.38. Found: C, 67.28; H, 6.34; N, 12.16.

3.7.18. 2-*tert*-Butyl-5,N_3-bis(tributylstannyl)-6-methoxy-pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (32). Metallation of **8** (50 mg, 0.21 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.07 mL), TMPH (8 equiv., 0.29 mL), $T=0$ °C, followed by reaction with tri-*n*-butylstannyl chloride (8 equiv., 0.48 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: diethyl ether/petroleum ether, 5:5)) 130 mg (75%) of **32** as an oil; ^{1}H NMR ($CDCl_3$): δ 0.79 (m, 18H, Me); 1.13 (m, 24H, CH_2); 1.32 (s, 9H, *tert*-butyl); 1.42 (m, 12H, CH_2); 3.87 (s, 3H, OMe); 8.56 (t, $J_{H8-Sn}=3.03-3.39$ Hz, 1H, H_8); ^{13}C NMR ($CDCl_3$): δ 13.6 (CH_2); 14.2 (Me); 27.8 (CH_2); 28.7 ($3Me_{tert\text{-butyl}}$); 29.7 (CH_2); 37.3 (CMe_3); 54.2 (OMe); 121.4 (C_{py}); 135.0 (C_{py}); 139.4 (C_{py}); 148.9 (CH_{py}); 158.7 (C_{py}); 163.3 (C_{py}); 167.6 (C_{py}). Anal. Calcd for $C_{44}H_{67}N_3O_2Sn_2$ (811.36): C, 53.29; H, 8.32; N, 5.18. Found: C, 53.61; H, 8.04; N, 5.46.

3.7.19. 2-*tert*-Butyl-5-iodo-6-methoxypyrido[3,4-*d*]pyrimidin-4(3*H*)-one (33). Metallation of **8** (50 mg, 0.21 mmol) according to the procedure E with *n*-BuLi 1.6 M (8 equiv., 1.07 mL), TMPH (8 equiv., 0.29 mL), $T=0$ °C, followed by reaction with iodine (8 equiv., 432 mg) in solution with anhydrous THF (5 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: dichloromethane/diethyl ether, 9:1)) 67 mg (88%) of **33** as a yellow solid, mp>250 °C; ^{1}H NMR (DMSO): δ 1.39 (s, 9H, *tert*-butyl); 4.02 (s, 3H, OMe); 8.86 (s, 1H, H_8); 12.06 (s, 1H, NH); ^{13}C NMR (DMSO): δ 27.8 ($3Me_{tert\text{-butyl}}$); 37.4 (CMe_3); 55.7 (OMe); 75.6 (C_{py}); 129.1 (C_{py}); 139.6 (C_{py}); 147.5 (CH_{py}); 160.5 (C_{py}); 160.6 (C_{py}); 161.7 (C_{py}). Anal. Calcd for $C_{12}H_{14}N_3O_2I$ (359.16): C, 40.13; H, 3.93; N, 11.70. Found: C, 40.10; H, 4.02; N, 11.52.

3.7.20. 2-*tert*-Butyl-8-methoxy-5-phenylthiopyrido[3,4-*d*]-pyrimidin-4(3*H*)-one (34). Metallation of **9** (50 mg, 0.21 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.07 mL), TMPH (8 equiv., 0.29 mL), $T=-20$ °C, followed by reaction with diphenyl disulfide (8 equiv., 380 mg) in solution with anhydrous THF (5 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: ethyl acetate/petroleum ether, 5:5)) 65 mg (89%) of **34** as a yellow solid, mp 249–250 °C; ^{1}H NMR ($CDCl_3$): δ 1.47 (s, 9H, *tert*-butyl); 3.95 (s, 3H, OMe); 7.25 (s, 1H, H_6); 7.38 (m, 3H, Ph); 7.53 (m, 2H, Ph); 11.98 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.5 ($3Me_{tert\text{-butyl}}$); 38.3 (CMe_3); 54.9 (OMe); 123.8 (C_{py}); 127.1 (C_{Ph}); 129.5 (CH_{Ph}); 128.9 ($2CH_{Ph}$); 132.1 (C_{py}); 135.4 (C_{py}); 135.6 ($2CH_{Ph}$); 139.4 (CH_{py}); 157.8 (C_{py}); 163.7 (C_{py}); 164.7 (C_{py}). Anal. Calcd

for $C_{18}H_{19}N_3O_2S$ (341.43): C, 63.32; H, 5.61; N, 12.31; S, 9.39. Found: C, 62.99; H, 5.68; N, 12.61; S, 9.46.

3.7.21. 2-*tert*-Butyl-5-(1-hydroxyethyl)-8-methoxypyrido-[3,4-*d*]pyrimidin-4(3*H*)-one (35). Metallation of **9** (50 mg, 0.21 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.07 mL), TMPH (8 equiv., 0.29 mL), $T=-20$ °C, followed by reaction with acetaldehyde (10 equiv., 0.10 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: ethyl acetate) 53 mg (90%) of **35** as a colorless solid, mp>250 °C; ^{1}H NMR ($CDCl_3$): δ 1.44 (s, 9H, *tert*-butyl); 1.57 (d, $J=6.4$ Hz, 3H, Me); 4.06 (s, 3H, OMe); 4.91 (d, $J=7.9$ Hz, 1H, OH); 5.22 (dq, $J=6.9$ Hz, 1H, C*H*OH); 8.09 (s, 1H, H_6); 10.52 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 23.4 (Me); 28.6 ($3Me_{tert\text{-butyl}}$); 37.9 (CMe_3); 55.1 (OMe); 67.6 (CHOH); 124.8 (C_{py}); 130.9 (C_{py}); 136.3 (C_{py}); 141.1 (CH_{py}); 160.3 (C_{py}); 163.2 (C_{py}); 163.5 (C_{py}). Anal. Calcd for $C_{14}H_{19}N_3O_3$ (277.32): C, 60.63; H, 6.91; N, 15.15. Found: C, 60.32; H, 7.03; N, 14.96.

3.7.22. 2-*tert*-Butyl-5-(hydroxyphenylmethyl)-8-methoxy-pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (36). Metallation of **9** (50 mg, 0.21 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.07 mL), TMPH (8 equiv., 0.29 mL), $T=-20$ °C, followed by reaction with benzaldehyde (8 equiv., 0.18 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: ethyl acetate) 61 mg (85%) of **36** as a colorless solid, mp>250 °C; ^{1}H NMR ($CDCl_3$): δ 1.36 (s, 9H, *tert*-butyl); 4.05 (s, 3H, OMe); 5.44 (d, $J=8.7$ Hz, 1H, C*H*OH); 6.13 (d, $J=8.6$ Hz, 1H, OH); 7.26 (m, 5H, Ph); 7.84 (s, 1H, H_6); 9.82 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.6 ($3Me_{tert\text{-butyl}}$); 37.9 (CMe_3); 55.1 (OMe); 73.1 (CHOH); 122.6 (C_{py}); 125.9 (C_{py}); 126.8 ($2CH_{Ph}$); 127.5 (CH_{Ph}); 128.5 ($2CH_{Ph}$); 129.2 (C_{Ph}); 140.5 (C_{py}); 143.0 (C_{py}); 143.4 (CH_{py}); 163.4 (C_{py}); 167.7 (C_{py}). Anal. Calcd for $C_{19}H_{21}N_3O_3$ (339.39): C, 67.24; H, 6.24; N, 12.38. Found: C, 67.19; H, 6.39; N, 12.45.

3.7.23. 2-*tert*-Butyl-5,N$_3$-bis(tributylstannyl)-8-mehoxy-pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (37). Metallation of **9** (50 mg, 0.21 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.07 mL), TMPH (8 equiv., 0.29 mL), $T=-20$ °C, followed by reaction with tri-*n*-butylstannyl chloride (8 equiv., 0.48 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: diethyl ether) 130 mg (75%) of **37** as an oil; ^{1}H NMR ($CDCl_3$): δ 0.80 (m, 18H, Me); 1.07 (m, 24H, CH_2); 1.29 (s, 9H, *tert*-butyl); 1.48 (m, 12H, CH_2); 4.04 (s, 3H, OMe); 8.09 (t, $J_{H6-Sn}=11.7$ Hz, 1H, H_6); ^{13}C NMR ($CDCl_3$): δ 11.5 (CH_2); 13.9 (Me); 14.1 (Me); 17.8 (CH_2); 27.2 (CH2); 27.7 (CH2); 28.2 (CH2); 28.7 ($3Me_{tert\text{-butyl}}$); 29.6 (CH_2); 37.8 (CMe_3); 54.8 (OMe); 125.0 (C_{py}); 132.2 (C_{py}); 135.1 (C_{py}); 149.8 (CH_{py}); 160.8 (C_{py}); 161.8 (C_{py}); 163.1 (C_{py}). Anal. Calcd for $C_{44}H_{67}N_3O_2Sn_2$ (811.36): C, 53.29; H, 8.32; N, 5.18. Found: C, 53.55; H, 8.43; N, 5.20.

3.7.24. 2-*tert*-Butyl-5-iodo-8-methoxypyrido[3,4-*d*]pyri-midin-4(3*H*)-one (38). Metallation of **9** (50 mg, 0.21 mmol) according to the procedure E with *n*-BuLi 1.6 M (8 equiv., 1.07 mL), TMPH (8 equiv., 0.29 mL), $T=-20$ °C, followed by reaction with iodine (8 equiv., 432 mg) in solution with anhydrous THF (5 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: dichloromethane/ethyl acetate, 5:5)) 62 mg (80%) of **38** as a colorless solid, mp>250 °C; ^{1}H NMR (DMSO): δ 1.47 (s, 9H, *tert*-butyl); 4.03 (s, 3H, OMe); 8.44 (s, 1H, H_8); 11.78 (s, 1H, NH); ^{13}C NMR (DMSO): δ 28.6 ($3Me_{tert\text{-butyl}}$); 38.5 (CMe_3); 55.3 (OMe); 76.2 (C_{py}); 125.7 (C_{py}); 136.7 (C_{py}); 152.0 (CH_{py}); 161.1 (C_{py}); 161.8 (C_{py}); 164.3 (C_{py}). Anal. Calcd for $C_{12}H_{14}N_3O_2I$ (359.16): C, 40.13; H, 3.93; N, 11.70. Found: C, 40.09; H, 4.09; N, 11.64.

3.8. Metallation of 2-*tert*-Butyl-5-chloropyrido[3,4-*d*]-pyrimidin-4(3*H*)-one (22)

The titled compound (50 mg, 0.21 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.05 mL), TMPH (8 equiv., 0.29 mL), $T=-20$ °C, followed by reaction with benzaldehyde (8 equiv., 0.17 mL), $t=1$ h, gave after purification by preparative chromatography (C_{18} column (5 μm, 10×250 mm), eluent (4 mL/min): MeOH/water (55:45), UV detection (245 nm)), 25 mg (35%) of 2-*tert*-butyl-5-chloro-6-(hydroxyphenylmethyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **39** and 25 mg (35%) of 2-*tert*-butyl-5-chloro-8-(hydroxyphenylmethyl)pyrido[3,4-*d*]pyrimidin-4(3*H*)-one **40**.

3.8.1. 2-*tert*-Butyl-5-chloro-6-(hydroxyphenylmethyl)-pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (39). A colorless solid, mp 227–228 °C; ^{1}H NMR ($CDCl_3$): δ 1.39 (s, 9H, *tert*-butyl); 4.99 (d, $J=7.1$ Hz, 1H, OH); 6.15 (d, $J=7.1$ Hz, 1H, *CH*OH); 7.24 (m, 5H, Ph); 8.97 (s, 1H, H_8); 11.13 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.4 ($3Me_{tert\text{-butyl}}$); 38.1 (CMe_3); 72.2 (CHOH); 123.6 (C_{py}); 127.0 (C_{Ph}); 127.7 ($2CH_{Ph}$); 128.2 (CH_{Ph}); 128.8 ($2CH_{Ph}$); 142.0 (C_{py}); 145.5 (C_{py}); 148.5 (CH_{py}); 155.0 (C_{py}); 161.5 (C_{py}); 165.5 (C_{py}). Anal. Calcd for $C_{18}H_{18}N_3O_2Cl$ (343.81): C, 62.88; H, 5.28; N, 12.22. Found: C, 62.83; H, 5.44; N, 12.36.

3.8.2. 2-*tert*-Butyl-5-chloro-8-(hydroxyphenylmethyl)-pyrido[3,4-*d*]pyrimidin-4(3*H*)-one (40). A colorless solid, mp 250–251 °C; ^{1}H NMR (DMSO): δ 1.42 (s, 9H, *tert*-butyl); 6.17 (s, 1H, OH); 6.66 (d, $J=6.0$ Hz, 1H, *CH*OH); 7.37 (2m, 5H, Ph); 8.57 (s, 1H, H_6); 12.39 (s, 1H, NH); ^{13}C NMR (DMSO): δ 28.1 ($3Me_{tert\text{-butyl}}$); 38.0 (CMe_3); 70.9 (CHOH); 122.6 (C_{py}); 126.7 (C_{Ph}); 126.8 ($2CH_{Ph}$); 127.0 (CH_{py}); 127.1 (CH_{Ph}); 128.2 ($2CH_{Ph}$); 142.2 (C_{py}); 143.2 (C_{py}); 144.0 (C_{py}); 155.7 (C_{py}); 159.9 (C_{py}). Anal. Calcd for $C_{18}H_{18}N_3O_2Cl$ (343.81): C, 62.88; H, 5.28; N, 12.22. Found: C, 62.95; H, 5.43; N, 12.35.

3.8.3. 2-*tert*-Butyl-5-(1-hydroxyethyl)pyrido[2,3-*d*]pyri-midin-4(3*H*)-one (41). Metallation of **4** (50 mg, 0.24 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), $T=0$ °C, followed by reaction with acetaldehyde (8 equiv., 0.11 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: ethyl acetate) 57 mg (95%) of **41** as a colorless solid, mp>250 °C; ^{1}H NMR ($CDCl_3$): δ 1.46 (s, 9H, *tert*-butyl); 1.55 (d, $J=6.6$ Hz, 3H, Me); 4.39 (s, 1H, OH); 5.83 (dq, $J=6.6$ Hz, 1H, C*H*OH); 7.49 (d, $J_{6-7}=4.9$ Hz, 1H, H_6); 8.87 (d, $J=4.9$ Hz, 1H, H_7); 11.17 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 23.7 (Me), 28.5 ($3Me_{tert\text{-butyl}}$); 38.0 (CMe_3); 68.3 (CHOH); 113.0 (C_{py}); 120.1 (CH_{py}); 156.8 (CH_{py}); 158.6 (C_{py}); 161.0 (C_{py}); 165.4 (C_{py}), 165.8

(C_{py}). Anal. Calcd for $C_{13}H_{17}N_3O$ (247.29): C, 63.14; H, 6.93; N, 16.96. Found: C, 62.98; H, 7.07; N, 16.53.

3.8.4. 2-*tert*-Butyl-5-(hydroxyphenylmethyl)pyrido[2,3-*d*]-pyrimidin-4(3*H*)-one (42). Metallation of **4** (50 mg, 0.24 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), T=0 °C, followed by reaction with benzaldehyde (8 equiv., 0.27 mL), t=1 h, gave after purification by column chromatography (silicagel, eluent: diethyl ether/ethyl acetate, 1:1)) 73 mg (96%) of **42** as a colorless solid, mp 147–148 °C; ^{1}H NMR ($CDCl_3$): δ 1.38 (s, 9H, *tert*-butyl); 5.18 (d, J=6.8 Hz, 1H, OH); 6.50 (d, J=6.8 Hz, 1H, C*H*OH); 7.20 (d, J_{6-7}=4.9 Hz, 1H, H_6); 7.25 (m, 5H, Ph); 8.82 (d, J=4.9 Hz, 1H, H_7); 10.56 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.5 ($3Me_{tert\text{-butyl}}$); 37.9 (CMe_3); 73.6 (CHOH); 113.6 (C_{py}); 122.4 (CH_{py}); 127.3 ($2CH_{Ph}$); 128.2 (CH_{Ph}); 128.8 ($2CH_{Ph}$); 141.4 (C_{Ph}); 156.0 (C_{py}); 156.7 (CH_{py}); 161.1 (C_{py}); 165.1 (C_{py}); 165.7 (C_{py}). Anal. Calcd for $C_{18}H_{19}N_3O_2$ (309.36): C, 69.88; H, 6.19; N, 13.58. Found: C, 69.72; H, 6.26; N, 13.45.

3.8.5. 2-*tert*-Butyl-5-phenylthiopyridol[2,3-*d*]pyrimidin-4(3*H*)-one (43). Metallation of **4** (50 mg, 0.24 mmol) according to the procedure E with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), T=0 °C, followed by reaction with diphenyl disulfide (8 equiv., 430 mg) in solution with anhydrous THF (5 mL), t=1 h, gave after purification by column chromatography (silicagel, eluent: (1) dichloromethane, (2) diethyl ether/dichloromethane (3:7)) 64 mg (84%) of **43** as a yellow solid, mp 249–250 °C; ^{1}H NMR ($CDCl_3$): δ 1.50 (s, 9H, *tert*-butyl); 6.43 (d, J_{6-7}=5.65 Hz, 1H, H_6); 7.48 (m, 3H, Ph); 7.55 (m, 2H, Ph); 8.36 (d, J=5.65 Hz, 1H, H_7); 11.83 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.6 ($3Me_{tert\text{-butyl}}$); 38.3 (CMe_3); 112.2 (C_{py}); 118.3 (CH_{py}); 130.4 (C_{Ph}); 130.7 (CH_{Ph}); 130.7 ($2CH_{Ph}$); 136.4 ($2CH_{Ph}$); 154.1 (CH_{py}); 157.5 (C_{py}); 160.5 (C_{py}); 165.1 (C_{py}); 166.6 (C_{py}). Anal. Calcd for $C_{17}H_{17}N_3OS$ (311.40): C, 65.57; H, 5.50; N, 13.49; S, 10.30. Found: C, 65.48; H, 5.72; N, 13.21; S, 10.46.

3.8.6. 2-*tert*-Butyl-5-iodopyrido[2,3-*d*]pyrimidin-4(3*H*)-one (44). Metallation of **4** (50 mg, 0.24 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), T=0 °C, followed by reaction with iodine (8 equiv., 500 mg) in solution with anhydrous THF (5 mL), t=1 h, gave after purification by column chromatography (silicagel, eluent: (1) diethyl ether, (2) ethyl acetate/diethyl ether (1:1)) 60 mg (75%) of **44** as a colorless solid, mp 200–201 °C; ^{1}H NMR ($CDCl_3$): δ 1.49 (s, 9H, *tert*-butyl); 7.95 (d, J_{6-7}=4.9 Hz, 1H, H_6); 8.35 (d, J=4.9 Hz, 1H, H_7); 11.65 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 27.1 ($3Me_{tert\text{-butyl}}$); 37.1 (CMe_3); 104.4 (C_{py}); 114.9 (C_{py}); 134.9 (CH_{py});153.7 (CH_{py}); 158.2 (C_{py}); 161.8 (C_{py}); 165.1 (C_{py}). Anal. Calcd for $C_{11}H_{12}N_3OI$, (329.14): C, 40.14; H, 3.67; N, 12.77. Found: C, 40.23; H, 3.63; N, 12.26.

3.8.7. 2-*tert*-Buty1-5,N_3-bis(tributylstannyl)pyrido[2,3-*d*]-pyrimidin-4(3*H*)-one (45). Metallation of **4** (50 mg, 0.24 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), T=0 °C, followed by reaction with tri-*n*-butylstannyl chloride (8 equiv., 0.54 mL), t=1 h, gave after purification by column chromatography (silicagel, eluent: diethyl ether) 142 mg (74%) of **45** as an oil; ^{1}H NMR ($CDCl_3$): δ 0.82 (m, 18H, 6Me); 1.17 (m, 24H, $12CH_2$); 1.40 (s, 9H, *tert*-butyl); 1.54 (m, 12H, $6CH_2$); 7.51 (td, J_{6-7}=4.5 Hz, J_{H6-Sn}= 8.5 Hz, IH, H_6); 8.74 (q, J=4.1 Hz, J_{H7-Sn}=10.5 Hz, 1H, H_7); ^{13}C NMR ($CDCl_3$): δ 9.10 (CH_2); 14.0 (Me); 14.1 (Me); 17.9 (CH_2); 27.2 (CH_2); 27.7 (CH_2); 28.2 (CH_2); 28.6 ($3Me_{tert\text{-butyl}}$); 29.5 (CH_2); 37.9 (CMe_3); 121.2 (C_{py}); 131.3 (CH_{py}); 154.2 (CH_{py}); 158.7 (C_{py}); 159.3 (C_{py}); 164.6 (C_{py}); 164.6 (C_{py}). Anal. Calcd for $C_{35}H_{65}N_3OSn_2$ (781.34): C, 53.80; H, 8.39; N, 5.38. Found: C, 53.67; H, 8.24; N, 5.09.

3.8.8. 2-*tert*-Butyl-8-(hydroxyphenylmethyl)pyrido[4,3-*d*]-pyrimidin-4(3*H*)-one (46). Metallation of **10** (50 mg, 0.24 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), T= −20 °C, followed by reaction with benzaldehyde (8 equiv., 0.27 mL), t=1 h, gave after purification by column chromatography (silicagel, eluent: (1) dichioromethane/ethyl acetate (5:5), (2) ethyl acetate) 73 mg (96%) of **46** as a colorless solid, mp 186–187 °C; ^{1}H NMR ($CDCl_3$): δ 1.40 (s, 9H, *tert*-butyl); 5.65 (d, J=6.8 Hz, 1H, OH); 6.19 (d, J=6.8 Hz, 1H, C*H*OH); 7.21 (m, 3H, Ph); 7.37 (m, 2H, Ph); 8.67 (s, 1H, H_7); 9.31 (s, 1H, H_5); 11.81 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.4 ($3Me_{tert\text{-butyl}}$); 38.7 (CMe_3); 73.5 (CHOH); 116.3 (C_{py}); 126.7 ($2CH_{Ph}$); 127.9 (CH_{Ph}); 128.7 ($2CH_{Ph}$); 133.6 (C_{Ph}); 143.1 (C_{py}); 149.8 (CH_{py}); 151.9 (C_{py}); 152.1 (CH_{py}); 163.5 (C_{py}); 167.6 (C_{py}). Anal. Calcd for $C_{18}H_{19}N_3O_2$ (309.36): C, 69.88; H, 6.19; N, 13.58. Found: C, 69.48; H, 6.33; N, 13.49.

3.8.9. 2-*tert*-Butyl-5-(hydroxyphenylmethyl)pyrido[4,3-*d*]-pyrimidin-4(3*H*)-one (47). Metallation of **10** (50 mg, 0.24 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), T=20 °C, followed by reaction with benzaldehyde (8 equiv., 0.27 mL), t=1 h, gave after purification by column chromatography (silicagel, eluent: dichloromethane/ethyl acetate (1:1)) 51 mg (67%) of **47** as a colorless solid, mp 183–184 °C; ^{1}H NMR ($CDCl_3$): δ 1.32 (s, 9H, *tert*-butyl); 5.62 (d, J=8.7 Hz, 1H, OH); 6.84 (d, J=8.7 Hz, 1H, C*H*OH); 7.18 (m, 5H, Ph); 7.45 (d, J_{7-8}= 5.65 Hz, 1H, H_8); 8.70 (d, J=5.65 Hz, 1H, H_7); 10.86 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.4 ($3Me_{tert\text{-butyl}}$); 38.1 (CMe_3); 73.4 (CHOH); 113.8 (C_{py}); 121.6 (CH_{py}); 127.7 ($2CH_{Ph}$); 127.7 (CH_{Ph}); 128.5 ($2CH_{Ph}$); 143.5 (C_{Ph}); 151.4 (CH_{py}); 156.8 (C_{py}); 162.9 (C_{py}); 164.2 (C_{py}); 167.5 (C_{py}). Anal. Calcd for $C_{18}H_{19}N_3O_2$ (309.36): C, 69.88; H, 6.19; N, 13.58. Found: C, 69.67; H, 6.24; N, 13.34.

3.8.10. 2-*tert*-Butyl-8-(1-hydroxyethyl)pyrido[4,3-*d*]pyrimidin-4(3*H*)-one (48). Metallation of **10** (50 mg, 0.24 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), T= −20 °C, followed by reaction with acetaldehyde (8 equiv., 0.11 mL), t=1 h, gave after purification by preparative chromatography (C_{18} column (10 μm, 4.6×250 mm), eluent (1 mL/min): MeOH/water (7:3), UV detection (220 nm)), 49 mg (81%) of **48** as a colorless solid, mp 174–175 °C; ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 9H, *tert*-butyl); 1.62 (d, J=6.4 Hz, 3H, Me); 5.03 (d, J=7.53 Hz, 1H, OH); 5.20 (m, 1H, C*H*OH); 8.69 (s, 1H, H_7); 9.35 (s, 1H, H_5); 11.58 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 24.6 (Me); 28.4 ($3Me_{tert\text{-burtyl}}$),

38.6 (CMe_3); 68.3 (CHOH); 116.3 (C_{py}); 134.6 (C_{py}); 149.7 (CH_{py}); 151.1 (CH_{py}); 152.1 (C_{py}); 163.4 (C_{py}); 167.9 (C_{py}). Anal. Calcd for $C_{13}H_{17}N_3O$ (247.9): C, 63.14; H, 6.93; N, 16.96. Found: C, 63.02; H, 7.08; N, 16.78.

3.8.11. 2-*tert*-Butyl-8-phenylthiopyrido[4,3-*d*]pyrimidin-4(3*H*)-one (49). Metallation of **10** (50 mg, 0.24 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), $T=-20$ °C, followed by reaction with diphenyl disulfide (8 equiv., 430 mg) in solution with anhydrous THF (5 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: ethyl acetate/dichloromethane (1:1)) 65 mg (85%) of **49** as a colorless solid, mp 223–224 °C; ^{1}H NMR ($CDCl_3$): δ 1.42 (s, 9H, *tert*-butyl); 7.36 (m, 3H, Ph); 7.51 (m, 2H, Ph); 8.12 (s, 1H, H_7); 9.15 (s, 1H, H_5); 11.46 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.4 ($3Me_{tert\text{-butyl}}$); 38.7 (CMe_3); 115.8 (C_{py}); 129.5 (CH_{Ph}); 130.2 ($2CH_{Ph}$); 130.9 (C_{Ph}); 133.7 (C_{py}); 135.0 ($2CH_{Ph}$); 146.9 (CH_{py}); 150.5 (CH_{py}); 150.8 (C_{py}); 163.5 (C_{py}); 167.4 (C_{py}). Anal. Calcd for $C_{17}H_{17}N_3OS$ (311.40): C, 65.57; H, 5.50; N, 13.49; S, 10.30. Found: C, 65.38; H, 5.61; N, 13.12; S, 10.39.

3.8.12. 2-*tert*-Butyl-8,N$_3$-bis(tributylstannyl)pyrido[4,3-*d*]-pyrimidin-4(3*H*)-one (50). Metallation of **10** (50 mg, 0.24 mmol) according the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), $T=-20$ °C, followed by reaction with tri-*n*-butylstannyl chloride (8 equiv., 0.54 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: diethyl ether) 137 mg (71%) of **50** as a colorless solid, mp 125–126 °C; ^{1}H NMR ($CDCl_3$): δ 0.80 (m, 18H, 6Me); 1.19 (m, 24H, $12CH_2$); 1.43 (s, 9H, *tert*-butyl); 1.52 (m, 12H, $6CH_2$); 8.76 (t, $J_{H7-Sn}=11.4$ Hz, 1H, H_7); 9.35 (s, 1H, H_5); ^{13}C NMR ($CDCl_3$): δ 10.7 (CH_2), 14.0 (2Me); 18.1 (CH_2); 27.2 (CH_2); 27.9 (CH_2); 28.2 (CH_2); 28.6 ($3Me_{tert\text{-butyl}}$); 29.5 (CH_2); 38.4 (CMe_3); 115.9 (C_{py}); 136.4 (C_{py}); 150.3 (CH_{py}); 159.6 (C_{py}); 160.1 (CH_{py}); 164.5 (C_{py}); 166.7 (C_{py}). Anal. Calcd for $C_{35}H_{65}N_3OSn_2$ (781.34): C, 53.80; H, 8.39; N, 5.38. Found: C, 53.71; H, 8.13; N, 5.51.

3.8.13. 2-*tert*-Butyl-8-iodopyrido[4,3-*d*]pyrimidin-4(3*H*)-one (51). Metallation of **10** (50 mg, 0.24 mmol) according to the procedure E with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), $T=-20$ °C, followed by reaction with iodine (8 equiv., 500 mg) in solution with anhydrous THF (5 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: diethyl ether/dichloromethane (1:1)) 69 mg (85%) of **51** as a colorless solid, mp>250 °C; ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 9H, *tert*-butyl); 9.16 (s, 1H, H_7): 9.27 (s, lH, H_5); 11.19 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.5 ($3Me_{tert\text{-butyl}}$); 38.8 (CMe_3); 98.1 (C_{py}); 117.3 (C_{py}); 150.1 (CH_{py}); 154.3 (C_{py}); 161.4 (CH_{py}); 163.1 (C_{py}); 168.5 (C_{py}). Anal. Calcd for $C_{11}H_{12}N_3OI$ (329.14): C, 40.14; H, 3.67; N, 12.77. Found: C, 40.56; H, 3.83; N, 12.61.

3.8.14. 2-*tert*-Butyl-5-(1-hydroxyethyl)pyrido[4,3-*d*]pyrimidin-4(3*H*)-one (52). Metallation of **10** (50 mg, 0.24 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), $T=20$ °C, followed by reaction with acetaldehyde (8 equiv., 0.11 mL), $t=1$ h, gave after purification by preparative chromatography (C_{18} column (5 μm, 10×250 mm), eluent (4 mL/min): MeOH/water (45:55), UV detection (245 nm)), 39 mg (64%) of **52** as a colorless solid, mp 203–204 °C; ^{1}H NMR ($CDCl_3$): δ 1.44 (s, 9H, *tert*-butyl); 1.46 (d, $J=6.25$ Hz, 3H, Me); 5.11 (m, 1H, OH); 5.80 (m, 1H, C*H*OH); 7.44 (d, $J_{7-8}=5.7$ Hz, 1H, H_8); 8.64 (d, $J=5.7$ Hz, 1H, H_7); 11.76 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 25.1 (Me); 28.4 ($3Me_{tert\text{-butyl}}$); 38.2 (CMe_3); 68.5 (CHOH); 112.9 (C_{py}); 121.3 (CH_{py}); 151.3 (CH_{py}); 156.8 (C_{py}); 163.7 (C_{py}); 166.8 (C_{py}); 167.6 (C_{py}). Anal. Calcd for $C_{13}H_{17}N_3O$ (247.29): C, 63.14; H, 6.93; N, 16.96. Found: C, 62.95; H, 7.23; N, 16.94.

3.8.15. 2-*tert*-Butyl-5-phenylthiopyrido[4,3-*d*]pyrimidin-4(3*H*)-one (53). Metallation of **10** (50 mg, 0.24 mmol) according the procedure D **A** with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), $T=20$ °C, followed by reaction with diphenyl disulfide (8 equiv., 430 mg) in solution with anhydrous THF (5 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: ethyl acetate/dichloromethane (1:9)) 30 mg (40%) of **53** as a colorless solid, mp>250 °C; ^{1}H NMR ($CDCl_3$): δ 1.46 (s, 9H, *tert*-butyl); 7.10 (d, $J=5.65$ Hz, 1H, H_8); 7.25 (m, 3H, Ph); 7.53 (m, 2H, Ph); 8.29 (s, 1H, H_7); 11.88 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.5 ($3Me_{tert\text{-butyl}}$); 38.3 (CMe_3); 113.4 (C_{py}); 117.4 (CH_{py}); 129.4 ($2CH_{Ph}$); 129.4 (CH_{Ph}); 131.1 (C_{Ph}); 136.2 ($2CH_{Ph}$); 152.6 (CH_{py}); 156.6 (C_{py}); 163.6 (C_{py}); 164.0 (C_{py}); 167.5 (C_{py}). Anal. Calcd for $C_{17}H_{17}N_3OS$ (311.40): C, 65.57; H, 5.50; N, 13.49; S, 10.30. Found: C, 65.35; H, 5.74; N, 13.45; S, 10.37.

3.8.16. 2-*tert*-Butyl-5,N$_3$-bis(tributylstannyl)pyrido[4,3-*d*]-pyrimidin-4(3*H*)-one (54). Metallation of **10** (50 mg, 0.24 mmol) according to the procedure D **A** with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), $T=20$ °C, followed by reaction with tri-*n*-butylstannyl chloride (8 equiv., 0.54 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: ethyl acetate/petroleum ether (1:9)) 96 mg (50%) of **54** as a glassy solid, mp<50 °C; ^{1}H NMR ($CDCl_3$): δ 0.82 (m, 18H, 6Me); 1.18 (m, 24H, $12CH_2$); 1.34 (s, 9H, *tert*-butyl); 1.47 (m, 12H, $6CH_2$); 7.28 (d, $J_{7-8}=5.65$ Hz, lH, H_8); 8.87 (d, $J=5.65$ Hz, 1H, H_7); ^{13}C NMR ($CDCl_3$): δ 11.6 (CH_2); 14.0 (Me); 14.1 (Me); 17.9 (CH_2); 27.2 (CH_2); 27.8 (CH_2); 28.2 (CH_2); 28.6 ($3Me_{tert\text{-butyl}}$); 29.6 (CH_2); 37.9 (CMe_3); 119.3 (CH_{py}); 121.8 (C_{py}); 152.7 (C_{py}); 154.0 (CH_{py}); 163.4 (C_{py}); 165.4 (C_{py}); 181.0 (C_{py}). Anal. Calcd for $C_{33}H_{65}N_3OSn_2$ (781.34): C, 53.80; H, 8.39; N, 5.38. Found: C, 54.07; H, 8.46; N, 5.26.

3.8.17. 2-*tert*-Butyl-5-iodopyrido[4,3-*d*]pyrimidin-4(3*H*)-one (55). Metallation of **10** (50 mg, 0.24 mmol) according to the procedure E with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), $T=20$ °C, followed by reaction with iodine (8 equiv., 500 mg) in solution with anhydrous THF (5 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: diethyl ether/dichloromethane (2.5:7.5)) 37 mg (46%) of **55** as a brown solid, mp 213–214 °C; ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 9H, *tert*-butyl); 7.41 (d, $J_{7-8}=5.3$ Hz, 1H, H_8); 8.39 (d, $J=5.3$ Hz, 1H, H_7); 11.63 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.5 ($3Me_{tert\text{-butyl}}$); 38.6 (CMe_3); 117.4 (C_{py}); 117.9 (C_{py}); 122.0 (CH_{py}); 153.2 (CH_{py}); 155.9 (C_{py}); 162.0 (C_{py}); 167.7 (C_{py}). Anal. Calcd

for $C_{11}H_{12}N_3OI$ (329.14): C, 40.14; H, 3.67; N, 12.77. Found: C, 40.26; H, 3.87; N, 12.64.

3.8.18. 2-*tert*-Butyl-8-(1-hydroxyethyl)pyrido[3,2-*d*]pyrimidin-4(3*H*)-one (56). Metallation of **11** (50 mg, 0.24 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), $T=-78$ °C, followed by reaction with acetaldehyde (8 equiv., 0.11 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: ethyl acetate) 54 mg (89%) of **56** as a colorless solid, mp>250 °C; ^{1}H NMR ($CDCl_3$): δ 1.43 (s, 9H, *tert*-butyl); 1.58 (d, $J=6.4$ Hz, 3H, Me); 5.23 (q, $J=6.4$ Hz, 1H, C*H*OH); 7.50 (d, $J_{6-7}=4.15$ Hz, 1H, H_7); 8.73 (d, $J=4.15$ Hz, 1H, H_6); 10.82 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 24.0 (Me); 28.7 ($3Me_{tert\text{-butyl}}$); 38.1 (*C*Me_3); 69.1 (CHOH); 125.5 (CH_{py}); 137.7 (C_{py}); 143.9 (C_{py}); 150.0 (CH_{py}); 150.6 (C_{py}); 162.2 (C_{py}); 163.0 (C_{py}). Anal. Calcd for $C_{13}H_{17}N_3O$ (247.29): C, 63.14; H, 6.93; N, 16.96. Found: C, 63.06; H, 7.09; N, 16.52.

3.8.19. 2-*tert*-Butyl-8-(hydroxyphenylmethyl)pyrido-[3,2-*d*]pyrimidin-4(3*H*)-one (57). Metallation of **11** (50 mg, 0.24 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), $T=-78$ °C, followed by reaction with benzaldehyde (8 equiv., 0.27 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: ethyl acetate) 68 mg (89%) of **57** as a white solid, mp 216–217 °C; ^{1}H NMR (DMSO): δ 1.35 (s, 9H, *tert*-butyl); 6.31 (m, 1H, OH); 6.54 (m, 1H, C*H*OH); 7.22 (m, 3H, Ph); 7.49 (m, 2H, Ph); 7.94 (d, $J_{6-7}=4.9$ Hz, 1H, H_7); 8.72 (d, $J=4.9$ Hz, 1H, H_6); 11.07 (s, 1H, NH); ^{13}C NMR (DMSO): δ 28.6 ($3Me_{tert\text{-butyl}}$); 38.3 (*C*Me_3); 69.2 (CHOH); 124.2 (CH_{py}); 126.9 ($2CH_{Ph}$); 127.3 (CH_{Ph}); 128.3 ($2CH_{Ph}$); 137.1 (C_{Ph}); 142.2 (C_{py}); 144.4 (C_{py}); 148.3 (CH_{py}); 151.2 (C_{py}); 162.4 (C_{py}); 163.1 (C_{py}). Anal. Calcd for $C_{18}H_{19}N_3O_2$ (309.36): C, 69.88; H, 6.19; N, 13.58. Found: C, 69.92; H, 5.82; N, 12.96.

3.8.20. 2-*tert*-Butyl-8-phenylthiopyrido[3,2-*d*]pyrimidin-4(3*H*)-one (58). Metallation of **11** (50 mg, 0.24 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), $T=-78$ °C, followed by reaction with diphenyl disulfide (8 equiv., 430 mg) in solution with anhydrous THF (5 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: ethyl acetate/dichloromethane (1:1)) 60 mg (80%) of **58** as a colorless solid, mp>250 °C; ^{1}H NMR ($CDCl_3$): δ 1.47 (s, 9H, *tert*-butyl); 6.71 (d, $J=4.9$ Hz, 1H, H_7); 7.44 (m, 3H, Ph); 7.55 (m, 2H, Ph); 8.36 (d, $J_{6-7}=4.9$ Hz, 1H, H_6); 11.24 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.7 ($3Me_{tert\text{-butyl}}$); 38.3 (*C*Me_3); 123.1 (CH_{py}); 129.4 (C_{Ph}); 130.5 (CH_{Ph}); 130.6 ($2CH_{Ph}$); 136.3 ($2CH_{Ph}$); 142.3 (C_{py}); 144.6 (C_{py}); 148.9 (CH_{py}); 152.3 (C_{py}); 162.7 (C_{py}); 163.1 (C_{py}). Anal. Calcd for $C_{17}H_{17}N_3OS$ (311.40): C, 65.57; H, 5.50; N, 13.49; S, 10.30. Found: C, 65.28; H, 5.64; N, 12.94; S, 9.92.

3.8.21. 2-*tert*-Butyl-8-iodopyrido[3,2-*d*]pyrimidin-4(3*H*)-one (59). Metallation of **11** (50 mg, 0.24 mmol) according to the procedure E with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), T=−78 °C, followed by reaction with iodine (8 equiv., 500 mg) in solution with anhydrous THF (5 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: ethyl acetate) 25 mg (31%) of **59** as a colorless solid, mp 236–237 °C; ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 9H, *tert*-butyl); 8.15 (d, $J_{6-7}=4.9$ Hz, 1H, H_7); 8.31 (d, $J=4.9$ Hz, 1H, H_6); 10.93 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 28.7 ($3Me_{tert\text{-butyl}}$); 38.4 (*C*Me_3); 114.0 (C_{py}); 137.3 (C_{py}); 139.5 (CH_{py}); 146.6 (C_{py}); 149.4 (CH_{py}); 162.1 (C_{py}); 164.1 (C_{py}). Anal. Calcd for $C_{11}H_{12}N_3OI$ (329.14): C, 40.14; H, 3.67; N, 12.77. Found: C, 40.45; H, 4.12; N, 12.57.

3.8.22. 2-*tert*-Butyl-8,N$_3$-bis(tributylstannyl)pyrido[3,2-*d*]-pyrimidin-4(3*H*)-one (60). Metallation of **11** (50 mg, 0.24 mmol) according to the procedure D with *n*-BuLi 1.6 M (8 equiv., 1.23 mL), TMPH (8 equiv., 0.34 mL), T=−78 °C, followed by reaction with tri-*n*-butylstannyl chloride (8 equiv., 0.54 mL), $t=1$ h, gave after purification by column chromatography (silicagel, eluent: ethyl acetate/dichloromethane, 1:1) 159 mg (83%) of **60** as a glassy solid, mp<50 °C; ^{1}H NMR ($CDCl_3$): δ 0.82 (m, 18H, 6Me); 1.18 (m, 24H, $12CH_2$); 1.41 (s, 9H, *tert*-butyl); 1.50 (m, 12H, $6CH_2$); 7.51 (td, $J_{6-7}=4.15$ Hz, $J_{H6-Sn}=18.84$ Hz, 1H, H_7); 8.65 (d, $J=4.15$ Hz, 1H, H_6); ^{13}C NMR ($CDCl_3$): δ 10.8 (CH_2); 13.9 (Me); 14.0 (Me); 17.9 (CH_2); 27.2 (CH_2); 27.7 (CH_2); 28.2 (CH_2); 28.9 ($3Me_{tert\text{-butyl}}$); 29.4 (CH_2); 37.9 (*C*Me_3); 136.1 (C_{py}); 137.2 (CH_{py}); 148.4 (CH_{py}); 151.3 (C_{py}); 157.0 (C_{py}); 162.0 (C_{py}); 163.2 (C_{py}). Anal. Calcd for $C_{35}H_{65}N_3OSn_2$ (781.34): C, 53.80; H, 8.39; N, 5.38. Found: C, 53.62; H, 8.76; N, 5.23.

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