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Tetrahedron

Tetrahedron Vol. 62, No. 20, 2006

Contents

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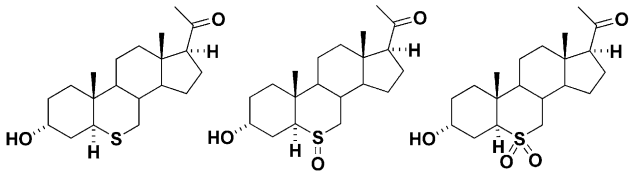
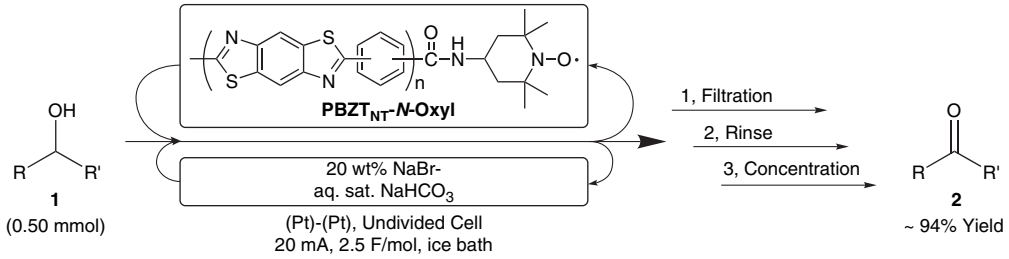
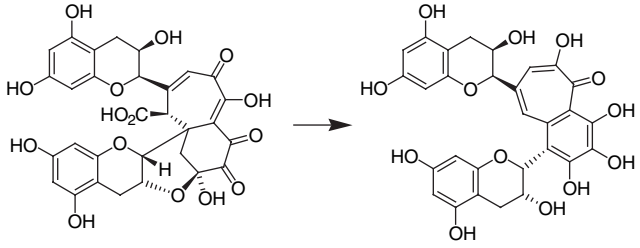
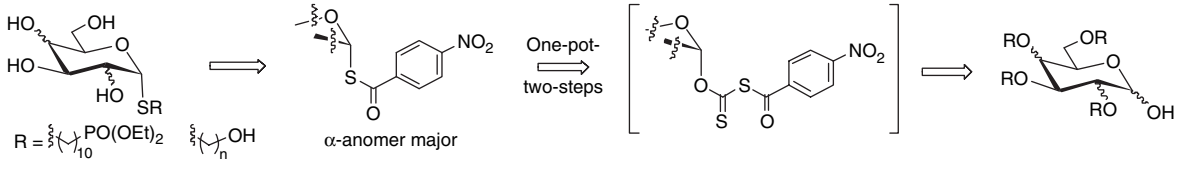
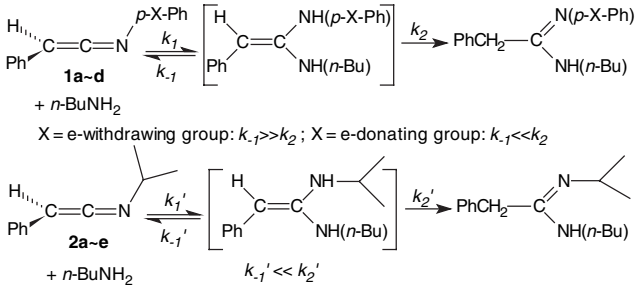
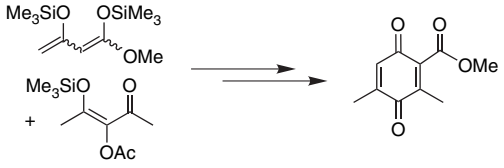


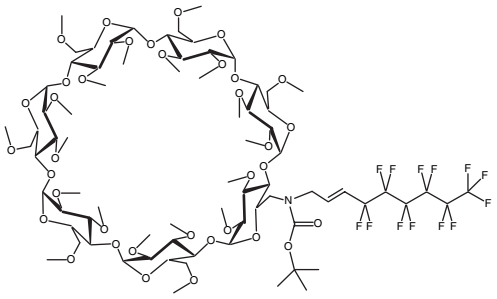


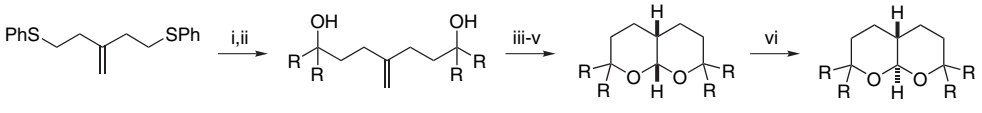




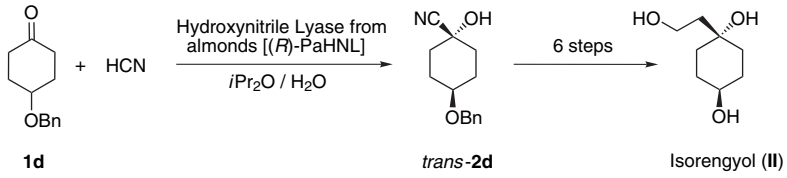


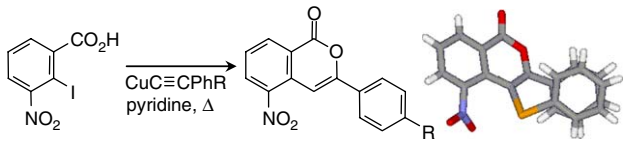


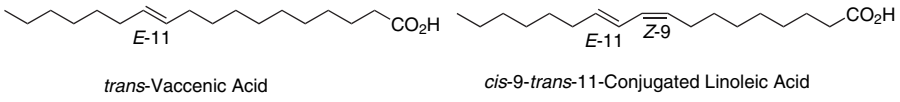


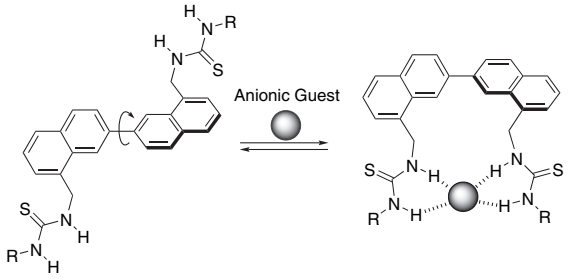


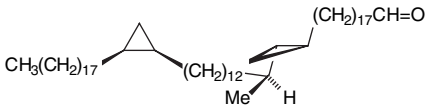
i, Li, DTBB (cat.), R_2CO, THF; ii, H_2O; iii, BH_3·THF; iv, H_2O_2, 3M NaOH; v, PCC, CH_2Cl_2; vi, *p*-TsOH (cat.), THF.





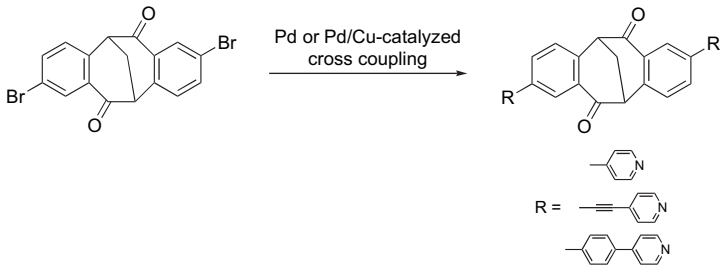


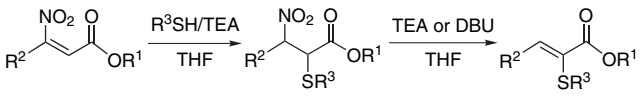


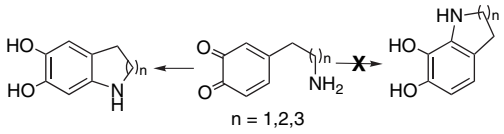


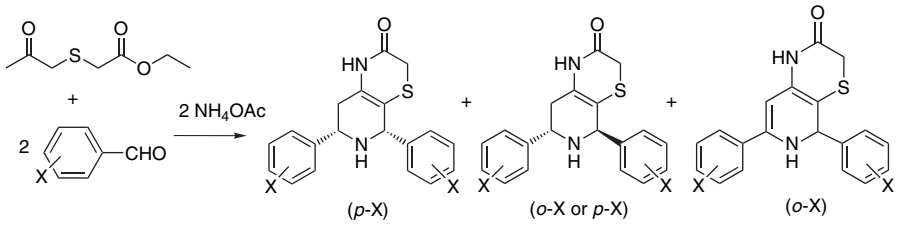
We report the synthesis of a single enantiomer of a meromycolate that contains one *cis*-1,2-dialkylcyclopropane and one alpha-methyl-*trans*-1,2-dialkylcyclopropane.

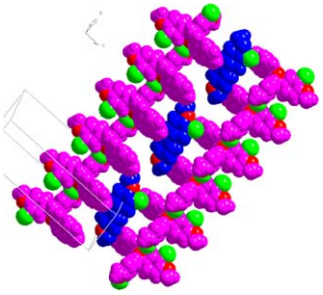
N represents a pyridine or a secondary alicyclic amine. Both reactions are stepwise, through a zwitterionic tetrahedral intermediate.

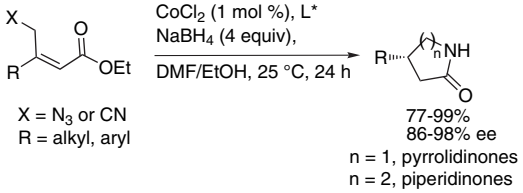


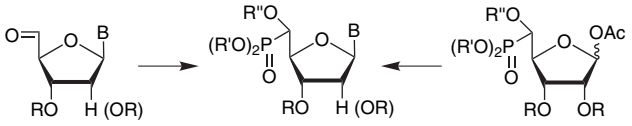


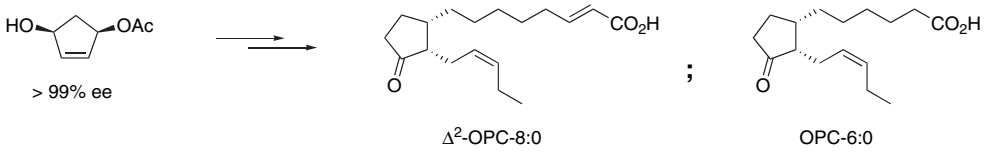




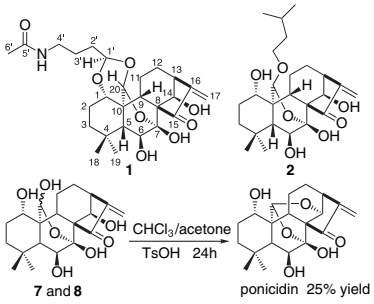


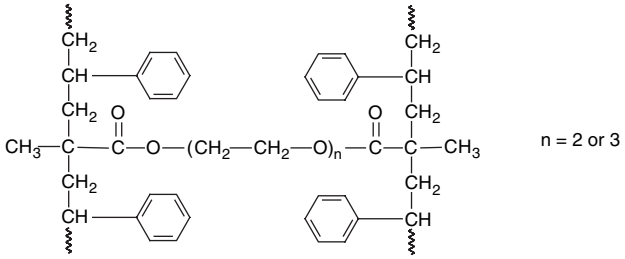


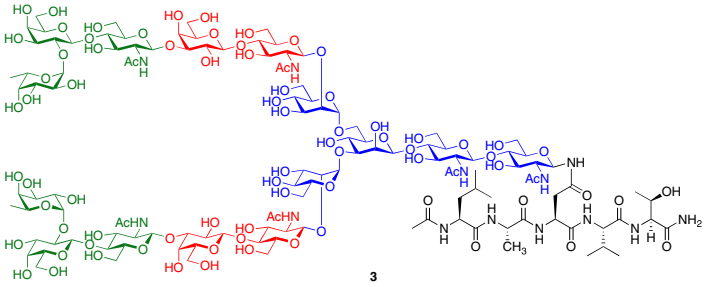




An extensive study of the diterpenoids produced by the species of *Isodon rubescens*, has led to the isolation of 12 new *ent*-kaurane diterpenoids, hebeirubescensins A–L (**1**–**12**), and 19 known analogues. Their structures were determined on the basis of spectroscopic analysis. Selected compounds were assayed for their inhibitory ability against human A549, HT-29, and K562 cells. Among them, hebeirubescensins B and C exhibited significant cytotoxicity with IC_{50} values of <2.0 μM. The structure–activity relationships were discussed.







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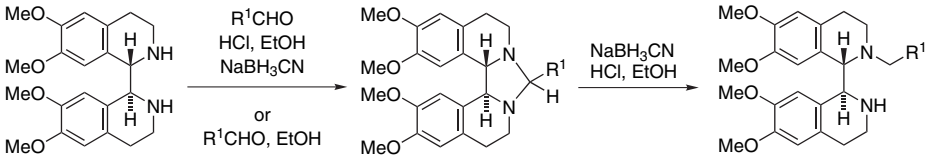

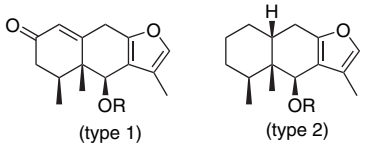

Ligularia virgaurea var. *virgaurea* of the title area was found to be divided into two types based on the furanoeremophilane composition and the ITS sequences.

*Corresponding author
ⓘ+ Supplementary data available via ScienceDirect

COVER

The total synthesis of an H-type blood group determinant in a model biological setting is described. The construct is comprised of a high mannose core structure with projecting lactose spacers, culminating in a two-copy presentation of the H-type blood group determinant itself. The pentadecasaccharide was assembled via a '5+2+3' coupling strategy and then further elaborated to generate the shown glycopeptide. *Tetrahedron* **2006**, *62*, 4954–4978.

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Tetrahedron

Daphtenidines A–D, new *Daphniphyllum* alkaloids from *Daphniphyllum teijsmannii*

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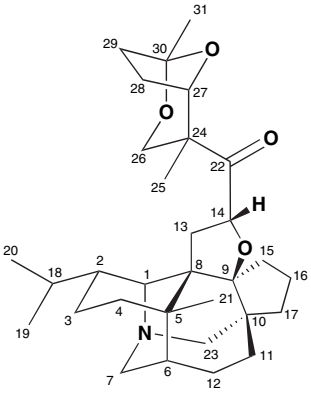
Abstract—Daphtenidines A–D (**1**–**4**), four new alkaloids were isolated from the leaves of *Daphniphyllum teijsmannii*, and the structures including relative stereochemistry were elucidated on the basis of spectroscopic data. Daphtenidines A (**1**) and B (**2**) were possessing daphnilactone A-type skeleton. This is the second isolation of daphnilactone A-type alkaloids from natural sources. Daphtenidine C (**3**) was 4-acetoxy form of daphmanidin A, while daphtenidine D (**4**) was 14-dehydro form of yuzurimine.

1. Introduction

Daphniphyllum alkaloids are a family of fused-heterocyclic natural products elaborated by trees of the genus *Daphniphyllum* (Daphniphyllaceae).[1,2] These ring systems have attracted great interest as challenging targets for total synthesis[3] as well as biosynthetic studies.[4] In our search for structurally unique and biogenetically interesting *Daphniphyllum* alkaloids,[5] four new *Daphniphyllum* alkaloids, daphtenidines A–D (**1**–**4**), were isolated from the leaves of *Daphniphyllum teijsmannii*. In this paper, we describe the isolation and structural elucidation of **1**–**4**.

2. Results and discussion

The leaves of *D. teijsmannii* were extracted with MeOH, and the MeOH extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted to pH 10 with saturated Na_2CO_3, were extracted with $CHCl_3$. $CHCl_3$-soluble materials were subjected to an LH-20 column (MeOH) followed by an amino silica gel column (hexane/EtOAc 1:0→0:1 and then $CHCl_3$/MeOH 1:0→0:1). The fractions eluted with hexane/EtOAc (4:1) were further purified by a silica gel column ($CHCl_3$/MeOH 1:0→0:1) to afford daphtenidines A (**1**, 0.00002% yield), B (**2**, 0.00002%), C (**3**, 0.0006%), and D (**4**, 0.0006%).



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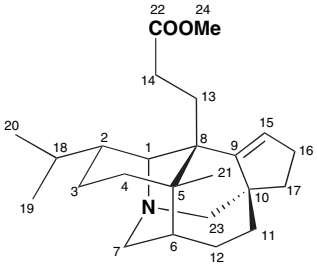

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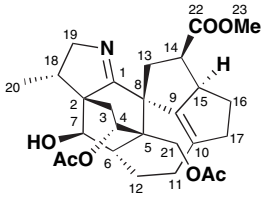

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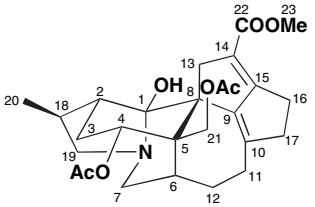

4

Daphtenidine A (**1**) showed the pseudomolecular ion peak at *m/z* 498 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{31}H_{47}N_1O_4$, was established by HRESIMS [*m/z* 498.3591, $(M+H)^+$ Δ +0.8 mmu]. The IR absorption implied the presence of carbonyl group (1730 cm^{-1}). The ^{13}C NMR (Table 1) spectrum of **1** gave signals including one ketone carbonyl,

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Table 1. ^{1}H and ^{13}C NMR data of daphtenidine A (**1**) in CD_3OD

Position	δ_H		δ_C	
1	3.29	(1H, m)	62.18	d
2	1.32	(1H, m)	46.30	d
3a	1.75	(1H, m)	29.16	t
3b	1.87	(1H, m)		
4a	1.43	(1H, m)	42.32	t
4b	1.99	(1H, m)		
5			41.09	s
6	1.67	(1H, m)	47.86	d
7a	3.83	(1H, d, 14.9)	61.61	t
7b	2.48	(1H, d, 14.9)		
8			48.69	s
9			101.52	s
10			53.99	s
11a	2.17	(1H, m)	29.55	t
11b	1.60	(1H, m)		
12a	2.12	(1H, m)	32.95	t
12b	1.57	(1H, m)		
13a	2.19	(1H, m)	33.76	t
13b	2.02	(1H, m)		
14	4.96	(1H, t, 8.2)	82.37	d
15a	2.27	(1H, m)	36.73	t
15b	1.29	(1H, m)		
16a	1.71	(1H, m)	19.94	t
16b	1.69	(1H, m)		
17a	1.73	(1H, m)	39.89	t
17b	1.47	(1H, m)		
18	1.70	(1H, m)	32.39	d
19	1.00	(3H, d, 6.10)	22.69	q
20	0.95	(3H, d, 6.87)	22.23	q
21	1.20	(3H, s)	28.75	q
22			215.80	s
23a	2.82	(1H, d, 14.5)	65.11	t
23b	2.59	(1H, d, 14.5)		
24			51.57	s
25	0.91	(3H, s)	19.63	q
26a	4.69	(1H, d, 12.6)	66.98	t
26b	3.69	(1H, d, 12.6)		
27	4.71	(1H, m)	84.43	d
28	2.03	(2H, m)	26.00	t
29a	1.89	(1H, m)	35.61	t
29b	2.10	(1H, m)		
30			107.33	s
31	1.37	(3H, s)	24.86	q

six sp^3 quaternary carbons, six sp^3 methines, thirteen sp^3 methylenes, and five methyls. Among them, two methylenes (δ_C 61.61 and 65.11) and one methine (δ_C 62.18) were ascribed to those bearing a nitrogen atom.

The ^{1}H–^{1}H COSY and HOHAHA spectra of **1** revealed connectivities of five partial structures **a** (C-1 to C-4, C-2 to C-18, and C-18 to C-19 and C-20), **b** (C-6 to C-7 and C-12, and C-11 to C-12), **c** (C-13 to C-14), **d** (C-15 to C-17), and **e** (C-27 to C-29) as shown in Figure 1. HMBC correlations of H_2-7 to C-1 (δ_C 62.18) and C-23 (δ_C 65.11), and H-23a to C-1 suggested that C-1, C-7, and C-23 were connected to each other through a nitrogen atom. Connections between C-4, C-6, and C-21 via C-5 were implied by HMBC cross-peaks of H_2-4 and H_3-21 to C-5. On the other hand, connections among C-11, C-17, and C-23 via C-10 were indicated by HMBC cross-peaks of H-23a to C-11 and C-17, and H-23b to C-10 and C-11. The connection between C-9 and C-10 was implied by HMBC cross-peaks of H-11b to C-9 and H-23b to C-9. HMBC cross-peaks of H-1 to C-5 and C-8, H_2-13 to C-1, C-8, and C-9, H-14 to C-9, and H_3-21 to C-8 suggested connectivities among units **a**, **b**, **c**, and **d**, to form a nitrogen-containing hexacyclic

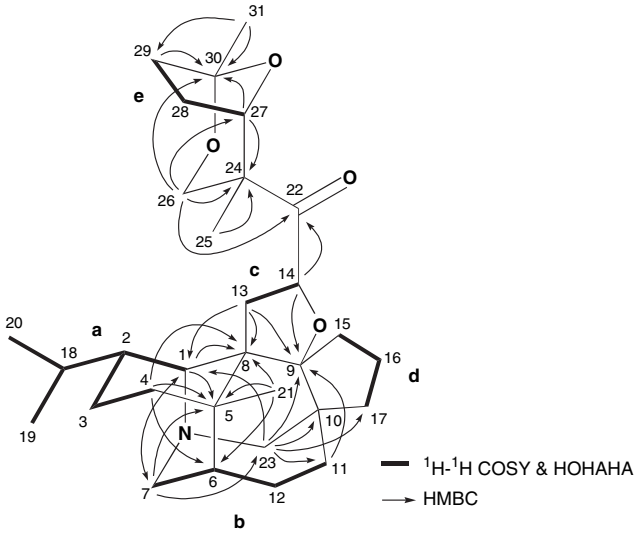


Figure 1. Selected 2D NMR correlations of daphtenidine A (**1**).

skeleton like daphnilactone A.[6] The presence of a 2,8-dioxabicyclo[3.2.1]octane moiety including unit **e** was deduced from HMBC cross-peaks of H_3-25 to C-24, H_2-26 to C-24, C-27, and C-30, H-27 to C-24 and C-30, H_2-29 to C-30, and H_3-31 to C-29 and C-30. HMBC cross-peaks of H-14 and H_2-26 to C-22 provided the connection between C-14 and C-24 via C-22. Thus, the gross structure of daphtenidine A was elucidated to be **1**.

The partial relative stereochemistry of **1** was deduced from ROESY correlations as shown in computer-generated 3D drawing (Fig. 2). These ROESY correlations indicated the relative configurations at C-2, C-9, and C-14, and chair forms of a cyclohexane (C-1 to C-5 and C-8) and a piperidine (N, C-1, C-8, and C-5 to C-7) ring in the 2-azabicyclo[3.3.1]-nonane moiety. The relative configuration at C-24 and a chair

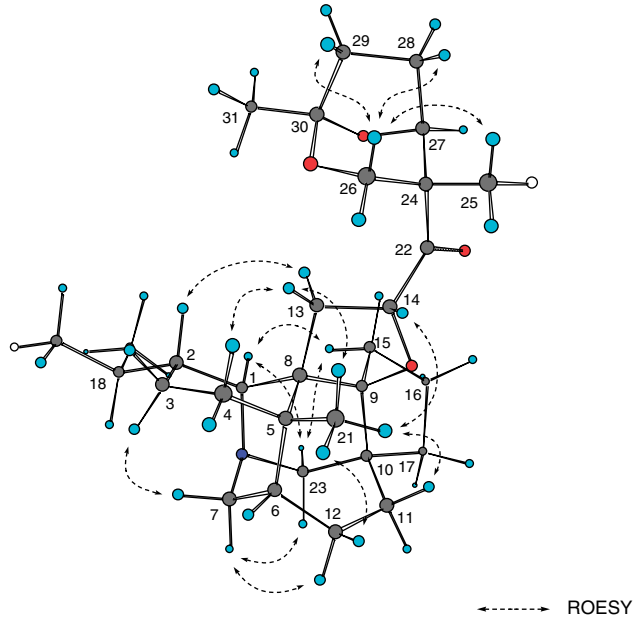


Figure 2. Selected ROESY correlations and relative stereochemistry of daphtenidine A (**1**).

form of the six-membered ring in the 2,8-dioxabicyclo [3.2.1]octane moiety was verified by ROESY correlations of H-26b to H-28 and H-29a.

Daphtenidine B (**2**) showed the pseudomolecular ion peak at *m/z* 372 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{24}H_{37}N_1O_2$, was established by HRESIMS [*m/z* 372.2899, $(M+H)^+$ Δ−0.3 mmu]. The IR absorption implied the presence of carbonyl group (1740 cm^{-1}). The ^{13}C NMR (Table 2) spectrum of **2** gave signals including one ester carbonyl, one sp^2 quaternary carbon, one sp^2 methine, three sp^3 quaternary carbons, four sp^3 methines, ten sp^3 methylenes, one methoxy, and three methyls. Among them, two methylenes (δ_C 55.34 and 73.65) and one methine (δ_C 60.23) were ascribed to those bearing a nitrogen atom.

Table 2. ^1H and ^{13}C NMR data of daphtenidine B (**2**) in CD_3OD

Position	δ_H		δ_C	
1	2.58	(1H, d, 4.4)	60.23	d
2	1.29	(1H, m)	41.45	d
3a	1.96	(1H, m)	28.82	t
3b	1.70	(1H, m)		
4a	1.96	(1H, m)	38.77	t
4b	1.51	(1H, m)		
5			38.16	s
6	1.64	(1H, m)	48.00	d
7a	3.73	(1H, dd, 15.0, 6.2)	55.34	t
7b	2.65	(1H, d, 15.0)		
8			43.72	s
9			151.16	s
10			51.40	s
11a	1.90	(1H, m)	35.58	t
11b	1.57	(1H, m)		
12a	1.79	(1H, m)	29.11	t
12b	1.57	(1H, m)		
13a	2.08	(1H, m)	26.46	t
13b	2.04	(1H, m)		
14a	2.40	(1H, m)	30.08	t
14b	2.04	(1H, m)		
15	5.44	(1H, s)	128.02	d
16a	2.48	(1H, m)	30.70	t
16b	2.24	(1H, ddd, 16.1, 8.48, 3.09)		
17a	1.69	(1H, m)	42.89	t
17b	1.57	(1H, m)		
18	1.62	(1H, m)	33.06	d
19	0.93	(3H, d, 6.50)	22.42	q
20	2.17	(3H, d, 6.22)	22.30	q
21	0.80	(3H, s)	25.32	q
22			177.42	s
23a	2.96	(1H, d, 13.3)	73.65	t
23b	2.77	(1H, d, 13.3)		
24	3.68	(3H, s)	53.09	q

The ^1H–^1H COSY and HOHAHA spectra of **2** revealed connectivities of four partial structures **a** (C-1 to C-4, C-2 to C-18, and C-18 to C-19 and C-20), **b** (C-6 to C-7 and C-12, and C-11 to C-12), **c** (C-13 to C-14), and **d** (C-15 to C-17) as shown in Figure 3. HMBC correlations were observed of H_2-7 to C-1 (δ_C 60.23) and C-23 (δ_C 73.65), and H-23a to C-1, suggesting that C-1, C-7, and C-23 were connected to each other through a nitrogen atom. The connection of a methoxycarbonyl group to C-14 was revealed from HMBC correlations of H-14 and H_3-24 to C-22. Connections among C-4, C-6, and C-21 via C-5 were indicated by HMBC cross-peaks of H_2-4, H-6, and H_3-21 to C-5. On the other hand, connections among C-11, C-17, and C-23 via C-10 were suggested by HMBC cross-peaks of H-11a, H-17a, and H-23a to C-10. Connections among C-10 and C-15 via C-9 were implied by HMBC cross-peaks of H-15 to C-10, H_2-16 to C-9, H-17a to C-9, and H-23a to C-9. HMBC cross-peaks for H-1 to C-5, H_2-13 to C-8 and C-9, H_3-21 to C-8 provided connectivities among all the units **a**–**d**, indicating the presence of a nitrogen-containing hexacyclic skeleton like daphnilactone A.[6] Thus, the gross structure of daphtenidine B was elucidated to be **2**.

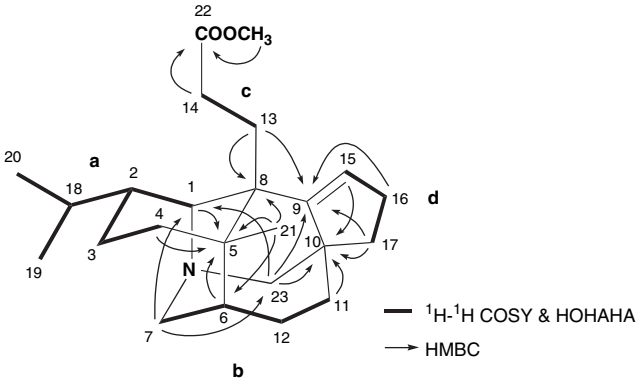


Figure 3. Selected 2D NMR correlations of daphtenidine B (**2**).

The relative stereochemistry of **2** was deduced from ROESY correlations as shown in computer-generated 3D drawing (Fig. 4). These ROESY correlations indicated the relative configuration at C-2 and chair forms of a cyclohexane (C-1 to C-5 and C-8) and a piperidine (N, C-1, C-8, and C-5 to C-7) ring in the 2-azabicyclo[3.3.1]nonane moiety.

Daphtenidine C (**3**) showed the pseudomolecular ion peak at *m/z* 486 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{27}H_{36}N_1O_7$, was established by HRESIMS [*m/z* 486.2473, $(M+H)^+$ Δ−1.9 mmu]. The IR absorption implied the presence of hydroxy group (3500 cm^{-1}) and carbonyl group (1730 cm^{-1}). The ^{13}C NMR (Table 3) spectrum of **3** gave signals including three ester carbonyls, three sp^2 quaternary

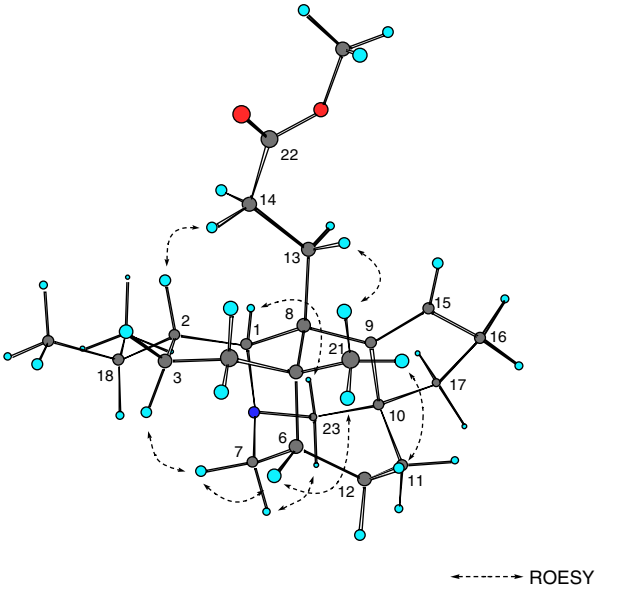


Figure 4. Selected ROESY correlations and relative stereochemistry of daphtenidine B (**2**).

Table 3. ^{1}H and ^{13}C NMR data of daphtenidine C (**3**) in CD_3OD

Position	δ_H		δ_C	
1			186.89	s
2			59.12	s
3	1.98	(2H, m)	33.50	t
4	4.89	(1H, dd, 9.6, 6.1)	73.17	d
5			53.14	s
6	2.39	(1H, m)	45.34	d
7	4.02	(1H, m)	67.95	d
8			47.42	s
9			136.17	s
10			142.43	s
11	2.27	(2H, m)	26.99	t
12a	2.05	(1H, m)	23.38	t
12b	1.90	(1H, m)		
13a	3.03	(1H, dd, 13.8, 4.3)	40.40	t
13b	2.42	(1H, m)		
14	3.15	(1H, dt, 10.0, 4.3)	44.53	d
15	3.54	(1H, m)	56.21	d
16a	1.89	(1H, m)	29.02	t
16b	1.27	(1H, m)		
17a	2.56	(1H, m)	44.77	t
17b	2.36	(1H, m)		
18	2.11	(1H, m)	38.73	d
19a	4.01	(1H, dd, 15.3, 6.9)	69.39	t
19b	3.46	(1H, d, 15.3)		
20	1.01	(3H, d, 7.1)	17.38	q
21a	4.38	(1H, d, 11.6)	66.85	t
21b	4.30	(1H, d, 11.6)		
22			177.95	s
23	3.62	(3H, s)	52.64	q
24			173.01	s
25	1.97	(3H, s)	21.83	q
26			172.64	s
27	2.00	(3H, s)	21.83	q

carbon, three sp^3 quaternary carbons, six sp^3 methines, eight sp^3 methylenes, one methoxy, and three methyls, implying that the structure of **3** was similar to that of daphmanidin A.[7] The ^{1}H–^{1}H COSY and HOHAHA spectra of **3** revealed connectivities of three partial structures **a** (C-6 to C-7 and C-12, and C-11 to C-12), **b** (C-13 to C-17), and **c** (C-18 to C-19 and C-20), which were connected to each other on the basis of HMBC correlations as shown in Figure 5. HMBC cross-peaks of H-4 and H_2-21 to acetyl carbonyl carbons (δ_C 173.01 and 172.64, respectively) revealed that the acetoxy groups were attached to C-4 and C-21.

The relative stereochemistry of **3** was deduced from NOESY correlations as shown in computer-generated 3D drawing (Fig. 6). These NOESY correlations indicated the relative configurations at C-4, C-7, C-14, and C-15 and the conformation of bicyclo[2.2.2]octane moiety (C-1 to C-8). Thus, daphtenidine C (**3**) was assigned to be 4-acetoxy form of daphmanidin A.

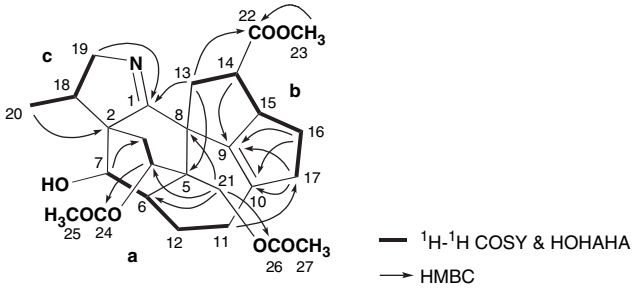


Figure 5. Selected 2D NMR correlations of daphtenidine C (**3**).

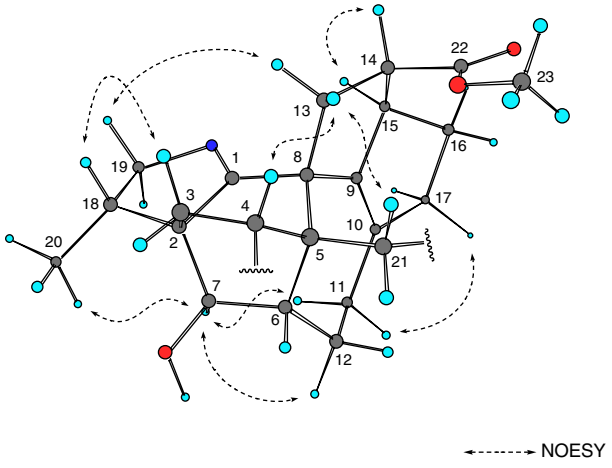


Figure 6. Selected NOESY correlations and relative stereochemistry of daphtenidine C (**3**). Two acetoxy groups were not shown.

Daphtenidine D (**4**) showed the pseudomolecular ion peak at *m/z* 486 $(M+H)^+$ in the ESIMS, and the molecular formula, $C_{27}H_{36}N_1O_7$, was established by HRESIMS [*m/z* 486.2490, $(M+H)^+$ $\Delta-0.2$ mmu]. The IR absorption implied the presence of hydroxy group (3480 cm^{-1}) and carbonyl group (1730 cm^{-1}). The ^{13}C NMR (Table 4) spectrum of **4** gave signals including three ester carbonyls, four sp^2 quaternary carbons, three sp^3 quaternary carbons, four sp^3 methines, nine sp^3 methylenes, one methoxy, and three methyls, implying that the structure of **4** was similar to that of

Table 4. ^{1}H and ^{13}C NMR data of daphtenidine D (**4**) in CD_3OD

Position	δ_H		δ_C	
1			98.35	s
2	2.39	(1H, m)	44.83	d
3a	2.00	(1H, m)	30.51	t
3b	1.74	(1H, m)		
4	5.42	(1H, dd, 11.2, 8.1)	75.14	d
5			46.56	s
6	2.60	(1H, m)	36.73	d
7a	3.40	(1H, d, 13.1)	60.34	t
7b	3.26	(1H, m,)		
8			53.71	s
9			152.45	s
10			154.28	s
11a	2.92	(1H, m)	27.56	t
11b	2.09	(1H, d, 16.8)		
12a	1.75	(1H, m)	30.34	t
12b	1.61	(1H, m)		
13a	3.37	(1H, m)	43.83	t
13b	3.08	(1H, d, 16.8)		
14			119.37	s
15			173.39	s
16	2.94.	(2H, m)	44.24	t
17a	2.72	(1H, m)	27.12	t
17b	2.63	(1H, m)		
18	2.75	(1H, m)	36.78	d
19a	3.58	(1H, dd, 11.8, 10.0)	65.58	t
19b	2.30	(1H, dd, 6.2, 11.8)		
20	1.12	(3H, d, 7.5)	16.51	q
21a	4.22	(1H, d, 11.2)	69.42	t
21b	4.17	(1H, d, 11.2)		
22			169.41	s
23	3.70	(3H, s)	52.36	q
24			173.17	s
25	1.97	(3H, s)	21.79	q
26			172.98	s
27	2.02	(3H, s)	22.09	q

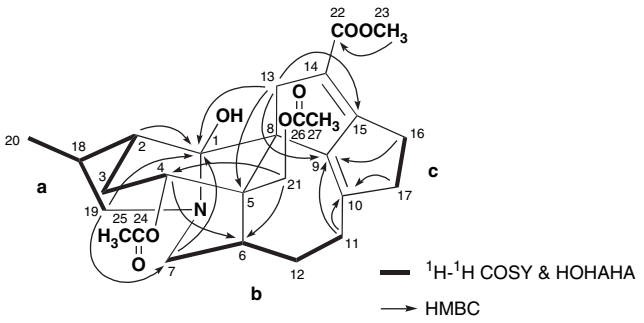


Figure 7. Selected 2D NMR correlations of daphtenidine D (**4**).

yuzurimine.[8] The ^{1}H–^{1}H COSY and HOHAHA spectra of **4** revealed connectivities of three partial structures **a** (C-2 to C-18 and C-18 to C-19 and C-20), **b** (C-6 to C-7 and C-12, and C-11 to C-12), and **c** (C-16 to C-17), which were connected to each other on the basis of HMBC correlations as shown in Figure 7. HMBC cross-peaks of H-4 and H_2-21 to acetyl carbonyl carbons (δ_C 173.17 and 172.98, respectively) revealed that the acetoxy groups were attached to C-4 and C-21.

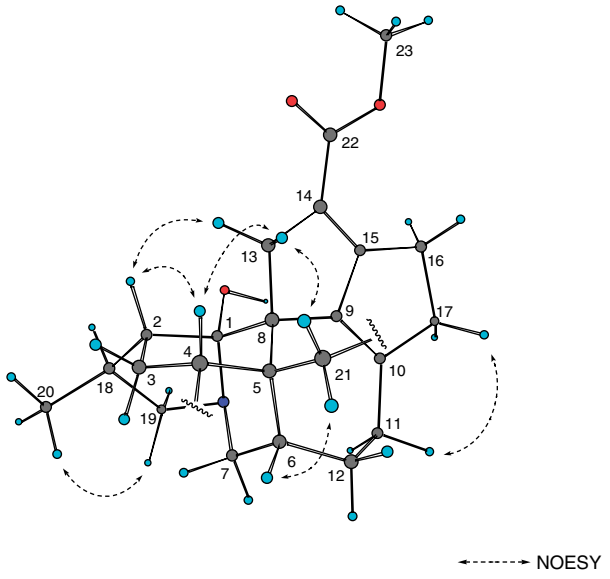
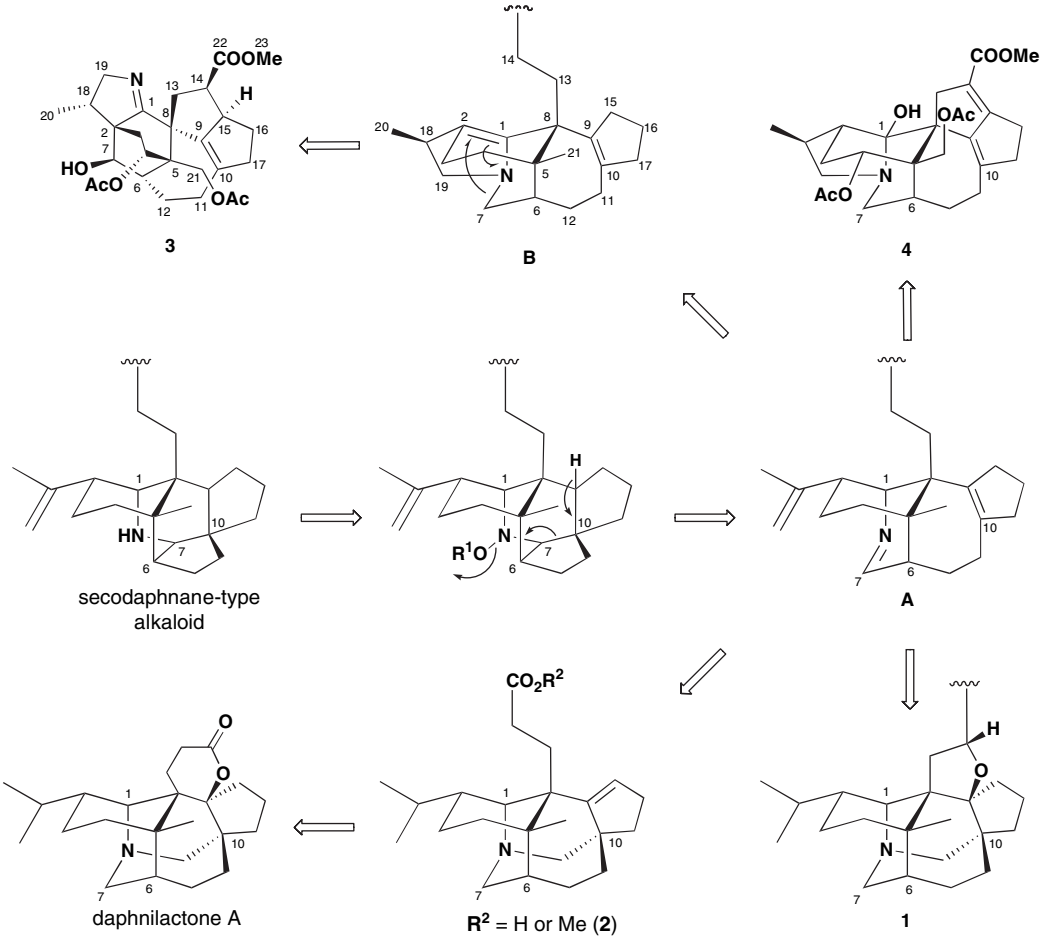


Figure 8. Selected NOESY correlations and relative stereochemistry of daphtenidine D (**4**). Two acetoxy groups were not shown.

The relative stereochemistry of **4** was deduced from NOESY correlations as shown in computer-generated 3D drawing (Fig. 8). These NOESY correlations indicated the relative configurations at C-4 and C-18, and the chair forms of a cyclohexane (C-1 to C-5 and C-8) and a piperidine (N, C-1, C-8, and C-5 to C-7) ring in the 2-azabicyclo[3.3.1]nonane moiety. Thus, daphtenidine D (**4**) was assigned to be 14-dehydro form of yuzurimine.



Scheme 1. Plausible biogenetic path of daphtenidines A–D (**1**–**4**).

Daphtenidines A (**1**) and B (**2**) were the second isolation of daphnilactone A-type alkaloids from natural sources. Daphtenidine A (**1**) was the first daphnilactone A-type alkaloid possessing a 2,8-dioxabicyclo[3.2.1]octane moiety. Daphtenidines C (**3**) and D (**4**) were the 4-acetoxy form of daphmanidin A and 14-dehydro form of yuzurimine, respectively. Biogenetically, daphtenidines A (**1**) and B (**2**) might be generated through an intermediate **A** from secodaphnane-type alkaloid,[9] followed by the formation of daphnilactone A (Scheme 1). Daphtenidine C (**3**) might be generated from intermediate **B** with cleavage of the N–C-7 bond followed by formation of the C-7–C-2 bond, while daphtenidine D (**4**) might be also generated from intermediate **A**.

Daphtenidines A–D (**1**–**4**) showed moderate cytotoxicity against murine lymphoma L1210 cells (IC_{50} 7, 3, 8, and 5 μg/mL, respectively) in vitro, while **1**–**4** did not show cytotoxicity against human epidermoid carcinoma KB cells (IC_{50}>10 μg/mL).

3. Experimental

3.1. General experimental procedures

The IR spectrum was recorded on a JASCO FTIR-230 and a Shimadzu UV-1600PC spectropolarimeter. ^1H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 and a Varian Unity INOVA 600 spectrometer. The 3.31 and 49.5 ppm resonances of residual CD_3OD were used as internal references for ^1H and ^{13}C NMR spectra, respectively. ESI mass spectra were obtained on a JEOL JMS-700TZ spectrometer.

3.2. Material

The leaves of *D. teijsmannii* were collected at Okinawa in 2002. The botanical identification was made by Prof. Takakazu Shinzato, University of the Ryukyus. A voucher specimen has been deposited in the herbarium of Hokkaido University.

3.3. Isolation

The leaves of *D. teijsmannii* (2.0 kg) were crushed and extracted with MeOH. The MeOH extract was treated with 3% tartaric acid (pH 2) and then partitioned with EtOAc. The aqueous layer was treated with saturated Na_2CO_3 (aq) to pH 10 and extracted with $CHCl_3$ to give a crude alkaloidal fraction. The alkaloidal fraction was purified by an LH-20 column (MeOH) followed by an amino silica gel column (hexane/EtOAc 1:0→0:1, and then $CHCl_3$/MeOH 1:0→0:1). The fractions eluted with hexane/EtOAc (4:1) were further purified by a silica gel column ($CHCl_3$/MeOH 1:0→0:1) to afford daphtenidines A (**1**, 0.4 mg, 0.00002% yield), B (**2**, 0.3 mg, 0.00002%), C (**3**, 11.8 mg, 0.0006%), and D (**4**, 11.7 mg, 0.0006%).

3.3.1. Daphtenidine A (1). Colorless amorphous solid; $[\alpha]_D^{23}$ −3 (*c* 0.1, MeOH); IR (neat) max 2920, 2860, and 1730 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS *m/z* 498 $(M+H)^+$; HRESIMS *m/z* 498.3591 (M+H; calcd for $C_{31}H_{48}NO_4$, 498.3583).

3.3.2. Daphtenidine B (2). Colorless amorphous solid; $[\alpha]_D^{23}$ +97 (*c* 0.1, MeOH); IR (neat) max 2920, 2850, and 1740 cm^{-1}; ^1H and ^{13}C NMR data (Table 2); ESIMS *m/z* 372 $(M+H)^+$; HRESIMS *m/z* 372.2899 (M+H; calcd for $C_{24}H_{38}N_1O_2$, 372.2902).

3.3.3. Daphtenidine C (3). Colorless amorphous solid; $[\alpha]_D^{23}$ −106 (*c* 1.0, MeOH); IR (neat) max 3500, 2960, and 1730 cm^{-1}; ^1H and ^{13}C NMR data (Table 3); ESIMS *m/z* 486 $(M+H)^+$; HRESIMS *m/z* 486.2473 (M+H; calcd for $C_{27}H_{36}N_1O_7$, 486.2492).

3.3.4. Daphtenidine D (4). Colorless amorphous solid; $[\alpha]_D^{23}$ +36 (*c* 1.0, MeOH); IR (neat) max 3480, 3010, 2940, and 1730 cm^{-1}; UV (MeOH) λ_{max} 301 nm (ε 11060); ^1H and ^{13}C NMR data (Table 4); ESIMS *m/z* 486 $(M+H)^+$; HRESIMS *m/z* 486.2490 (M+H; calcd for $C_{27}H_{36}N_1O_7$, 486.2492).

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Synthesis of end-functionalized π-conjugated porphyrin oligomers

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Abstract—4-(*S*-Acetylthiomethyl)phenyl- and pyrenyl-functionalized π-conjugated porphyrin oligomers were synthesized. The distribution of the length of the oligomers could be controlled by changing the ratio of the starting porphyrin to the capping molecules. Oligomers from dimers to heptamers were isolated using size exclusion chromatography. The spectroscopic properties of these oligomers were measured to determine the influences of the number of porphyrin units and capping molecules on the absorption and emission spectra.

1. Introduction

Molecular electronics is a fascinating area of fundamental research with the potential for many future applications.[1–4] In recent years, studies on the conductance of single or small numbers of molecules were reported with intriguing results, exhibiting such interesting properties as switching,[5,6] the Coulomb-blockade phenomenon,[7–9] field-effect transistor,[7–10] and the Kondo effect.[9,11] As an extension of these studies, the fabrication of larger nanostructures constructed from organic molecules as the functional moieties and conductive nanomaterials as the electron transport portion has been recognized as an important issue for future nanodevices (Fig. 1). Metal nanoparticles,[12,13] metal nanorods,[14] and carbon nanotubes[15,16] are promising candidates for conductive nanomaterials. In order to construct such nanocomposites made from conductive materials and organic molecules, functional groups are required to couple these moieties together. Mercapto or *S*-acetylthio groups are known to be good coupling groups to metals,[17] and aromatic hydrocarbons such as pyrene are known to adsorb to the surface of carbon nanotubes efficiently through π–π interactions.[18] For the purpose of constructing such nanocomposites[19–22] using a series of porphyrin oligomers as the organic portion,

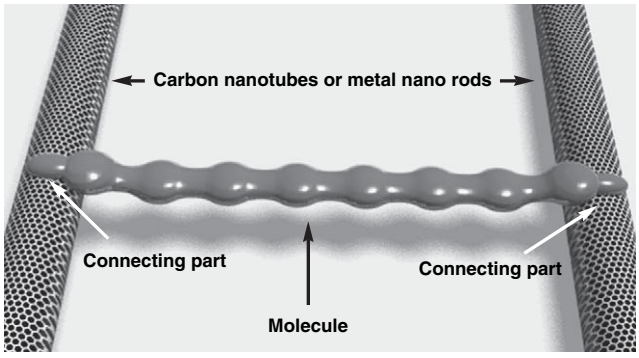

Figure 1. Schematic diagram of nanostructures consisting end-functionalized oligomers and conductive materials.

we synthesized a series of end-functionalized π-conjugated porphyrin oligomers. One series (**1a**–**g**) contains 1-[4-(*S*-acetylthiomethyl)phenyl]ethynyl groups and another series (**2a**–**g**) bears 2-pyrenylethynyl groups at the end of the molecules. Systematic spectroscopic studies of this series of oligomers were performed to study their electronic properties, especially to clarify the effects of the end-functional groups on the main π-conjugated electronic systems.

2. Results and discussion

2.1. Synthesis

End-functionalized porphyrin oligomers **1a**–**g** and **2a**–**g** were synthesized by the synthetic route shown in Scheme 1.

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2006.03.031.
Keywords: Porphyrin oligomers; Spectroscopic properties; 4-(*S*-Acetylthiomethyl)phenyl; Pyrenyl.
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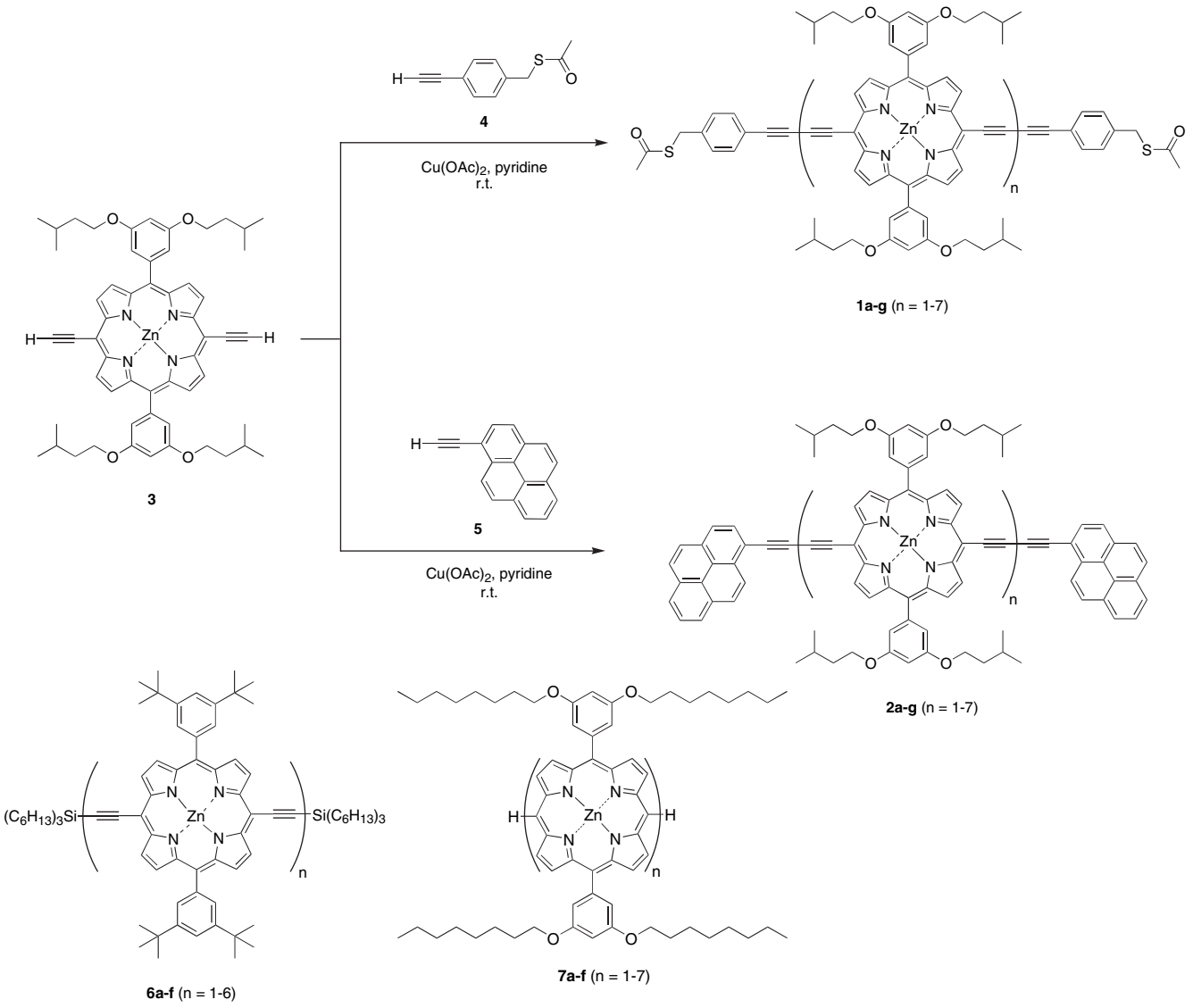

Scheme 1. Synthesis of end-functionalized porphyrin oligomers (**1a–g** and **2a–g**) and structures of silicon-functionalized (**6a–f**) and meso–meso linked (**7a–f**) porphyrin oligomers.

Functional alkynes **4** or **5** were used as the capping molecules. The oxidative coupling reaction of compound **3** and these capping molecules via copper catalysis gave a series of oligomers. The porphyrin-containing products were first purified by gel permeation chromatography (GPC) and further isolated by recycling high performance liquid chromatography GPC (HPLC–GPC). The details of the synthesis and isolation are presented in Section 4. The purities of these oligomers were confirmed by ^{1}H NMR, MALDI-TOF-MS, and analytical GPC (see Section 4 and Supporting information).

The distribution of oligomers could be controlled by changing the ratio of compound **3** to the capping molecules (**4** or **5**), as depicted in Figure 2. When the compound ratio of **3** to **4** was 1:2 (entry 1), the main product was monomer. When the ratio was 5:2 (entry 2), the major products ranged from tetramer to hexamer. When the ratio was 10:1 (entry 3), the distribution peak of oligomers centered on a decamer.

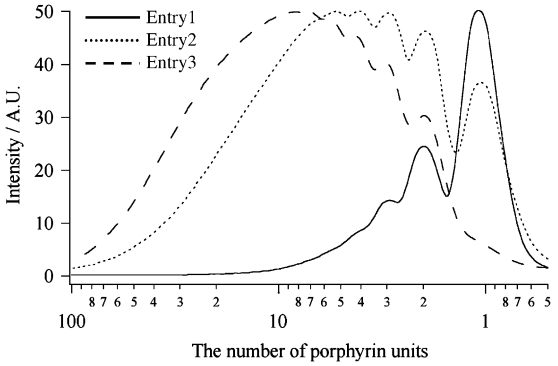

Figure 2. Analytical GPC data of porphyrin oligomers prepared by changing the compound ratio of **3** to **4**. The compound ratios of **3** to **4** were 1:2, 5:2, and 10:1 for entries 1, 2, and 3, respectively.

2.2. UV and fluorescence spectra of the oligomers

UV–vis absorption spectra of end-functionalized porphyrin oligomers **1a–g** and **2a–g** in THF are shown in Figure 3a

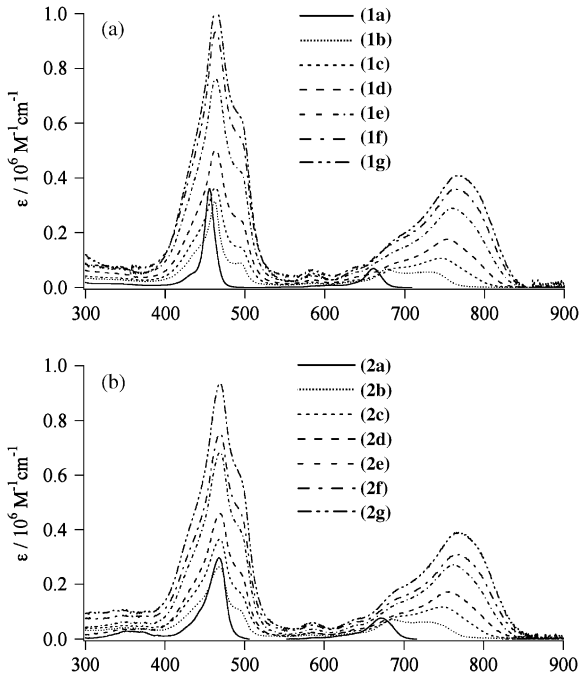

Figure 3. UV–vis spectra of: (a) acetylthiomethylbenzene-capped porphyrin oligomers **1a**–**g** and (b) pyrene-capped porphyrin oligomers **2a**–**g** measured in THF.

and b, respectively, and the peak wavelengths λ_{max} are tabulated in Table 1. The Soret peaks of the porphyrin oligomers were gradually red-shifted as the size of the oligomer increased. The Q bands were also red-shifted and intensified with increasing porphyrin units, indicating a high degree of conjugation. The Soret and Q band absorption maxima were dependent on the end-functional groups. Oligomers **2a**–**g** with pyrene end groups showed longer wavelength absorption compared to the corresponding oligomers **1a**–**g** with 4-(*S*-acetylthiomethyl)phenyl groups. The absorption coefficients of these oligomers became larger with the increasing number of porphyrin units. The absorption coefficients increased with a linear relationship to the number of porphyrin units with the exception of the monomer.

Table 1. The absorption λ_{max} (in THF), emission λ_{max} (in THF), and fluorescence quantum yield of porphyrin oligomers **1a**–**g** and **2a**–**g**

Porphyrin oligomer	Absorption λ_{max}/nm	Emission[a] λ_{max}/nm	Φ_F[b]
1a	455, 661	674	0.09
1b	462, 493, 583, 677, 725	750	0.10
1c	463, 583, 744	780	0.07
1d	463, 584, 752	792	0.08
1e	463, 585, 758	800	0.07
1f	463, 585, 764	804	0.07
1g	463, 585, 768	808	0.07
2a	467, 671	688	0.09
2b	468, 583, 685, 730	758	0.12
2c	468, 584, 747	782	0.09
2d	469, 584, 756	796	0.09
2e	469, 584, 762	802	0.07
2f	469, 584, 766	806	0.07
2g	469, 583, 769	808	0.07

[a] Emission spectra were taken for excitation at Soret band.
[b] Tetraphenylporphyrin in benzene (TPP, Φ_F=0.11) was used as a standard.[25]

Fluorescence data of these oligomers are also depicted in Table 1. These emission spectra show no dependency on excitation wavelength and the excitation spectra were coincident with the absorption spectra, which confirms that these emissions are not from any impurities. From the absorption and emission data, HOMO–LUMO energy gaps E_g were estimated. The values of E_g are plotted against the reciprocal of the number of porphyrin units ($1/N$) in Figure 4. These plots are linear, with no sign of saturation. The E_g value for **1a** obtained from absorption data (1.88 eV) is 0.03 eV larger than that for **2a** (1.85 eV), which is reasonably explained by the differences in the degree of resonance of the capping moieties. The E_g ($N=\infty$) values obtained from the intercepts for a series of **1** and **2** were coincident to be 1.57 eV from the absorption data and 1.48 eV from the emission data. This means that though the optical properties of the monomer were highly influenced by the capping molecules, the effect decreased with an increasing number of porphyrin units. Therefore, we can conclude that the optical properties of long oligomers were minimally influenced by the end-capping moieties. The reported E_g ($N=\infty$) obtained from the emission of a series of **6** was 1.34 eV, which is significantly lower than the values obtained for **1** and **2**.[23] This can be attributed to a difference in the measurement conditions. Because of the low solubility of **6**, a small amount of pyridine was added to the solution. Most likely, the pyridine molecules coordinated to the zinc metal of each porphyrin unit, lowering the E_g values by the extended resonance.[24]

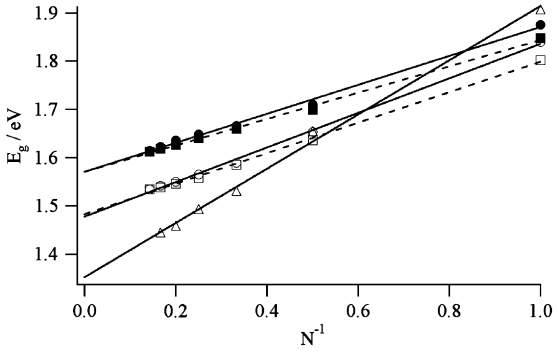

Figure 4. Plot of optical band gap energy E_g against the reciprocal of the number of porphyrin units $1/N$ for (●) **1a**–**g** (absorption), (■) **2a**–**g** (absorption), (○) **1a**–**g** (emission), (□) **2a**–**g** (emission), and (△) **6a**–**f** with pyridine (emission).[23]

The relative fluorescence quantum yields were determined using tetraphenylporphyrin (H_2TPP) as the standard (Φ_F=0.11).[25] The quantum yield of the monomers (**1a** and **2a**) was 0.09, and this decreased gradually with the increase in the number of porphyrin units (Table 1 and Fig. 5). The results shown are in sharp contrast with a report for meso–meso linked porphyrin oligomer **7**.[26–28] In the latter compounds, the quantum yield of the fluorescence for the monomer was 0.022 and increased as the number of porphyrin units increased in the arrays. This phenomenon is attributed to the increase of the rotational diffusion time by the anisotropic elongation of the molecules. As the rotational diffusion time increased, the natural radiative lifetime decreased, and consequently the fluorescence quantum yield increased. The reason for the difference between **7** and **1** or **2** is not clear at present, but the degree of conjugation between porphyrin moieties might be responsible.

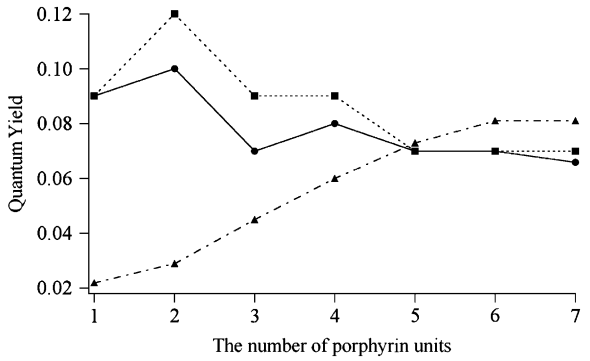

Figure 5. Plots of quantum yield versus the number of porphyrin units for (●) **1a–g**, (■) **2a–g**, and (▲) **7a–g**.[26]

3. Conclusion

We report the preparation and isolation of a series of end-functionalized porphyrin oligomers by a simple coupling reaction. The distribution of oligomers can be controlled by the ratio of porphyrin derivative to capping molecules. UV–vis absorption and fluorescence spectra of the monomers were affected by the capping groups; however, the effect decreased as the number of porphyrin units increased. The fluorescence quantum yield decreased as the number of porphyrin units increased, which showed a sharp contrast with a series of meso–meso coupled porphyrin oligomers. Construction of nanostructures consisting these end-functionalized molecules and metal particles or carbon nanotubes is now in progress.

4. Experimental

4.1. General

Compounds **3**,[29,30] **4**,[31] and **5**[32] were prepared according to published literature procedures. All solvents and reagents were of commercial reagent grade and were used without further purification except where noted. ^{1}H NMR spectra were recorded on a JEOL JNM-LA400 spectrometer and chemical shifts were reported in the delta scale relative to an internal standard of TMS (δ=0.00). Spectroscopic grade tetrahydrofuran (THF) and benzene were used as solvents for all spectroscopic measurements. UV–vis absorption spectra were recorded on a Shimadzu UV-3150 spectrophotometer. Fluorescence measurements were carried out with a JASCO FP-6600 spectrometer. Infrared spectra were obtained on a JASCO FT/IR-460plus. All MALDI-TOF-MS spectra were obtained using an Applied Biosystem Voyager DE-STR with 9-nitroanthracene and dithranol as the matrices. Analytical gel permeation chromatography (GPC) was carried out using Shimadzu LC-6A equipped with a diode array detector (MD-2015, JASCO) and two serially connected GPC KF-804L columns. THF was used as the eluent and the flow rate in all experiments was 1.0 mL/min. Recycling preparative GPC–HPLC was carried out on JAI LC-908 using serially connected preparative scale JAIGEL-2.5H, 3H, and 4H columns. Preliminary separation of the oligomers was performed by open column chromatography-GPC using Bio-Beads S-X1 (BioRad) and THF as the eluent.

4.2. General procedure

4.2.1. Preparation and isolation of 4-(*S*-acetylthiomethyl)phenyl functionalized porphyrin oligomers. In a round-bottomed flask, compounds **3** (52 mg, 57 μmol), **4** (11 mg, 57 μmol), and $Cu(OAc)_2$ (31 mg, 170 μmol) were dissolved in 5 mL of pyridine and the mixture was stirred at room temperature for 12 h. Water was added to the reaction and the resulting precipitate was filtered and washed with methanol. The precipitate was dissolved in THF and passed through an open column chromatograph using Bio-Beads S-X1 (BioRad) to obtain a mixture of the products containing porphyrin oligomers. These oligomers were further isolated by recycling GPC–HPLC to give the following: **1a** (5 mg, 6%), **1b** (19 mg, 31%), **1c** (8 mg, 14%), **1d** (6 mg, 10%), **1e** (5 mg, 8%), **1f** (3 mg, 5%), **1g** (1 mg, 2%), and larger oligomers (6 mg). The purity of each homologue was checked by the following analyses. The ^{1}H NMR spectrum of each sample showed consistent integrations for all the resonances, and no evidence of impurities was observed (Figs. 1S–7S). With the MALDI-TOF-MS spectra, no other homologues were detected for each isolated product (Fig. 15S). We conducted analytical GPC analyses of each product with simultaneous measurements of the absorption spectra using a diode array detector (Figs. 17S–20S). All the isolated oligomers showed a single peak clearly distinguishable from other oligomers by its retention time and absorption spectrum (Fig. 25S).

4.2.2. Control of product distribution by changing the ratio of 3 to 4 (entry 1). In a round-bottomed flask, **3** (52 mg, 57 μmol), **4** (225 mg, 118 μmol), and $Cu(OAc)_2$ (30 mg, 167 μmol) were dissolved in 5 mL of pyridine and the mixture was stirred at room temperature for 12 h. Water was added to the reaction and the resulting precipitate was filtered and washed with methanol. The precipitate was dissolved in THF and passed through an open column chromatograph using Bio-Beads S-X1 (BioRad) to obtain a mixture of the products containing porphyrin oligomers (55 mg). The distribution of the products was determined by analytical GPC of the mixture.

4.2.3. Control of product distribution by changing the ratio of 3 to 4 (entry 2). In a round-bottomed flask, **3** (51 mg, 55 μmol), **4** (4 mg, 20 μmol), and $Cu(OAc)_2$ (30 mg, 170 μmol) were dissolved in 5 mL of pyridine and the mixture was stirred at room temperature for 12 h. Water was added to the reaction and the resulting precipitate was filtered and washed with methanol. The precipitate was dissolved in THF and passed through an open column chromatograph using Bio-Beads S-X1 (BioRad) to obtain a mixture of the products containing porphyrin oligomers (42 mg). The distribution of the products was determined by analytical GPC of the mixture.

4.2.4. Control of product distribution by changing the ratio of 3 to 4 (entry 3). In a round-bottomed flask, **3** (51 mg, 56 μmol), a THF solution of **4** (52 μL solution of 10 mg/mL concentration, 2.8 μmol) and $Cu(OAc)_2$ (30 mg, 167 μmol) was dissolved in 5 mL of pyridine and the mixture was stirred at room temperature for 12 h. Water was added to the reaction and the resulting precipitate was filtered and washed with methanol. The precipitate was

dissolved in THF and passed through an open column chromatograph using Bio-Beads S-X1 (BioRad) to obtain a mixture of the products containing porphyrin oligomers (36 mg). The distribution of the products was determined by analytical GPC of the mixture.

4.2.5. Preparation and isolation of pyrenyl-functionalized porphyrin oligomers. In a round-bottomed flask, **3** (51 mg, 56 μmol), **5** (13 mg, 56 μmol), and $Cu(OAc)_2$ (30 mg, 167 μmol) were dissolved in 5 mL of pyridine and the mixture was stirred at room temperature for 12 h. Water was added to the reaction and the resulting precipitate was filtered, washed with methanol, and roughly purified by open column chromatography-GPC. The oligomers were isolated by a recycling GPC–HPLC to give the following: **2a** (13 mg, 18%), **2b** (14 mg, 23%), **2c** (10 mg, 16%), **2d** (7 mg, 12%), **2e** (4 mg, 6%), **2f** (3 mg, 6%), **2g** (2 mg, 4%), and larger oligomers (4 mg). The purity of each homologue was checked by the following analyses. The ^{1}H NMR spectra of each sample showed consistent integrations for all the resonances, and no evidence of impurities was observed (Figs. 8S–14S). With the MALDI-TOF-MS spectra, no other homologues were detected for each isolated product (Fig. 16S). We conducted analytical GPC analyses of each product with simultaneous measurements of the absorption spectra using a diode array detector (Figs. 21S–24S). All the isolated oligomers showed a single peak clearly distinguishable from other oligomers by its retention time and absorption spectrum (Fig. 26S).

4.2.5.1. Compound 1a. ^{1}H NMR (400 MHz, d_8-THF) δ 9.57 (d, J=5 Hz, 4H, β-pyrrole), 8.96 (d, J=5 Hz, 4H, β-pyrrole), 7.65 (d, J=8.3 Hz, 4H, Ar), 7.40 (d, J=8.3 Hz, 4H, Ar), 7.32 (d, J=2 Hz, 4H, Ar), 6.95 (t, J=2 Hz, 2H, Ar), 4.21 (t, J=7 Hz, 8H, OCH_2), 4.18 (s, 4H, SCH_2Ph), 2.34 (s, 6H, $C(O)CH_3$), 1.92 (sext, J=7 Hz, 8H, CH_2), 1.77 (m, J=7 Hz, 4H, CH), 1.00 (d, J=7 Hz, 24H, CH_3). HRMS (MALDI-TOF) calcd for $C_{78}H_{76}N_4O_6S_2Zn$ 1292.4492, found 1292.4489. UV–vis (THF) λ_{max} (log ε) 455 (5.56), 661 (4.84) nm. Fluorescence (THF, λ_{ex}=455 nm) λ_{em} 674 nm. IR (KBr) 2952, 2930, 2868, 2132, 2195, 1588, 1163 cm^{-1}.

4.2.5.2. Compound 1b. ^{1}H NMR (400 MHz, d_8-THF) δ 9.87 (d, J=5 Hz, 4H, β-pyrrole), 9.60 (d, J=5 Hz, 4H, β-pyrrole), 9.06 (d, J=5 Hz, 4H, β-pyrrole), 9.00 (d, J=5 Hz, 4H, β-pyrrole), 7.67 (d, J=8 Hz, 4H, Ar), 7.41 (d, J=8 Hz, 4H, Ar), 7.38 (d, J=2 Hz, 8H, Ar), 6.97 (t, J=2 Hz, 4H, Ar), 4.24 (t, J=6.6 Hz, 16H, OCH_2), 4.18 (s, 4H, SCH_2Ph), 2.34 (s, 6H, $C(O)CH_3$), 1.92 (sext, J=6.6 Hz, 16H, CH_2), 1.77 (m, J=6.6 Hz, 8H, CH), 1.01 (d, J=6.6 Hz, 48H, CH_3). HRMS (MALDI-TOF) calcd for $C_{134}H_{134}N_8O_{10}S_2Zn_2$ 2206.8241, found 2206.8234. UV–vis (THF) λ_{max} (log ε) 462 (5.50), 493 (4.95), 583 (4.19), 677 (4.82), 725 (4.75) nm. Fluorescence (THF, λ_{ex}=462 nm) λ_{em} 752 nm. IR (KBr) 2954, 2927, 2869, 2130, 2194, 1589, 1163 cm^{-1}.

4.2.5.3. Compound 1c. ^{1}H NMR (400 MHz, d_8-THF) δ 9.88 (m, 8H, β-pyrrole), 9.60 (d, J=5 Hz, 4H, β-pyrrole), 9.08 (m, 8H, β-pyrrole), 9.00 (d, J=5 Hz, 4H, β-pyrrole), 7.67 (d, J=8 Hz, 4H, Ar), 7.41 (d, 4H, Ar), 7.38 (m, 12H, Ar), 6.97 (m, 6H, Ar), 4.24 (m, 24H, OCH_2), 4.19 (s, 4H, SCH_2Ph), 2.35 (s, 6H, $C(O)CH_3$), 1.92 (m, 24H, CH_2), 1.77 (m, 12H, CH), 1.02 (m, 72H, CH_3). HRMS (MALDI-TOF) calcd for $C_{190}H_{192}N_{12}O_{14}S_2Zn_3$ 3121.1991, found 3121.2266. UV–vis (THF) λ_{max} (log ε) 463 (5.56), 583 (4.29), 744 (5.03) nm. Fluorescence (THF, λ_{ex}=463 nm) λ_{em} 786 nm. IR (KBr) 2954, 2927, 2868, 2129, 2162, 1590, 1156 cm^{-1}.

4.2.5.4. Compound 1d. ^{1}H NMR (400 MHz, d_8-THF) δ 9.88 (m, 12H, β-pyrrole), 9.60 (d, J=5 Hz, 4H, β-pyrrole), 9.08 (m, 12H, β-pyrrole), 9.00 (d, J=5 Hz, 4H, β-pyrrole), 7.67 (d, J=8 Hz, 4H, Ar), 7.41 (d, 4H, Ar), 7.38–7.44 (m, 16H, Ar), 6.97 (m, 8H, Ar), 4.24 (m, 32H, OCH_2), 4.19 (s, 4H, SCH_2Ph), 2.35 (s, 6H, $C(O)CH_3$), 1.92 (m, 32H, CH_2), 1.77 (m, 16H, CH), 1.02 (m, 96H, CH_3). MS (MALDI-TOF) calcd for $C_{246}H_{250}N_{16}O_{18}S_2Zn_4$ 4041.7, found 4041.5. UV–vis (THF) λ_{max} (log ε) 463 (5.70), 584 (4.43), 752 (5.24) nm. Fluorescence (THF, λ_{ex}=463 nm) λ_{em} 800 nm. IR (KBr) 2953, 2927, 2869, 2128, 2162, 1588, 1163 cm^{-1}.

4.2.5.5. Compound 1e. ^{1}H NMR (400 MHz, d_8-THF) δ 9.88 (m, 16H, β-pyrrole), 9.60 (d, J=5 Hz, 4H, β-pyrrole), 9.08 (m, 16H, β-pyrrole), 9.00 (d, J=5 Hz, 4H, β-pyrrole), 7.67 (d, J=8 Hz, 4H, Ar), 7.41 (d, 4H, Ar), 7.38–7.44 (m, 20H, Ar), 6.97 (m, 10H, Ar), 4.24 (m, 40H, OCH_2), 4.19 (s, 4H, SCH_2Ph), 2.35 (s, 6H, $C(O)CH_3$), 1.92 (m, 40H, CH_2), 1.77 (m, 20H, CH), 1.02 (m, 120H, CH_3). MS (MALDI-TOF) calcd for $C_{302}H_{308}N_{20}O_{22}S_2Zn_5$ 4964, found 4960. UV–vis (THF) λ_{max} (log ε) 463 (5.88), 585 (4.63), 758 (5.46) nm. Fluorescence (THF, λ_{ex}=463 nm) λ_{em} 814 nm. IR (KBr) 2953, 2926, 2868, 2128, 2162, 1588, 1162 cm^{-1}.

4.2.5.6. Compound 1f. ^{1}H NMR (400 MHz, d_8-THF) δ 9.88 (m, 20H, β-pyrrole), 9.60 (d, J=5 Hz, 4H, β-pyrrole), 9.08 (m, 20H, β-pyrrole), 9.00 (d, J=5 Hz, 4H, β-pyrrole), 7.67 (d, J=8 Hz, 4H, Ar), 7.41 (d, 4H, Ar), 7.38–7.44 (m, 24H, Ar), 6.97 (m, 12H, Ar), 4.24 (m, 48H, OCH_2), 4.19 (s, 4H, SCH_2Ph), 2.35 (s, 6H, $C(O)CH_3$), 1.92 (m, 48H, CH_2), 1.77 (m, 24H, CH), 1.02 (m, 144H, CH_3). MS (MALDI-TOF) calcd for $C_{358}H_{366}N_{24}O_{26}S_2Zn_6$ 5881, found 5876. UV–vis (THF) λ_{max} (log ε) 463 (5.97), 585 (4.81), 764 (5.56) nm. Fluorescence (THF, λ_{ex}=463 nm) λ_{em} 816 nm. IR (KBr) 2954, 2926, 2868, 2126, 2162, 1588, 1163 cm^{-1}.

4.2.5.7. Compound 1g. ^{1}H NMR (400 MHz, d_8-THF) δ 9.88 (m, 24H, β-pyrrole), 9.60 (d, J=5 Hz, 4H, β-pyrrole), 9.08 (m, 24H, β-pyrrole), 9.00 (d, J=5 Hz, 4H, β-pyrrole), 7.67 (d, J=8 Hz, 4H, Ar), 7.41 (d, 4H, Ar), 7.38–7.44 (m, 28H, Ar), 6.97 (m, 14H, Ar), 4.24 (m, 56H, OCH_2), 4.19 (s, 4H, SCH_2Ph), 2.35 (s, 6H, $C(O)CH_3$), 1.92 (m, 56H, CH_2), 1.77 (m, 28H, CH), 1.02 (m, 168H, CH_3). MS (MALDI-TOF) calcd for $C_{414}H_{424}N_{28}O_{30}S_2Zn_7$ 6799, found 6792. UV–vis (THF) λ_{max} (log ε) 463 (6.01), 585 (4.72), 768 (5.61) nm. Fluorescence (THF, λ_{ex}=463 nm) λ_{em} 816 nm. IR (KBr) 2953, 2927, 2869, 2121, 2166, 1588, 1162 cm^{-1}.

4.2.5.8. Compound 2a. ^{1}H NMR (400 MHz, d_8-THF) δ 9.57 (d, J=5 Hz, 4H, β-pyrrole), 9.02 (d, J=5 Hz, 4H, β-pyrrole), 8.83 (d, J=9 Hz, 2H, pyrene), 8.42–8.05 (16H, m, pyrene), 7.38 (d, J=2 Hz, 4H, Ar), 6.98 (t, J=2 Hz, 2H, Ar), 4.23 (t, J=7 Hz, 8H, OCH_2), 1.94 (sext, J=7 Hz, 8H, CH_2), 1.79 (m, J=7 Hz, 4H, CH), 1.01 (d, J=7 Hz, 24H,

CH_3). HRMS (MALDI-TOF) calcd for $C_{92}H_{76}N_4O_4Zn$ 1364.5152, found 1364.5150. UV–vis (THF) λ_{max} (log ε) 467 (5.47), 671 (4.88) nm. Fluorescence (THF, λ_{ex}=467 nm) λ_{em} 688 nm. IR (KBr) 2952, 2927, 2870, 2127, 2184, 1587, 1163 cm^{-1}.

4.2.5.9. Compound 2b. ^1H NMR (400 MHz, d_8-THF) δ 9.88 (d, J=5 Hz, 4H, β-pyrrole), 9.72 (d, J=5 Hz, 4H, β-pyrrole), 9.08 (d, J=5 Hz, 4H, β-pyrrole), 9.05 (d, J=5 Hz, 4H, β-pyrrole), 8.86 (d, J=9 Hz, 2H, pyrene), 8.44–8.06 (m, 16H, pyrene), 7.40 (d, J=2 Hz, 8H, Ar), 6.99 (t, J=2 Hz, 4H, Ar), 4.25 (t, J=7 Hz, 16H, OCH_2), 1.94 (sext, J=7 Hz, 16H, CH_2), 1.80 (m, J=7 Hz, 8H, CH), 1.02 (d, J=7 Hz, 48H, CH_3). HRMS (MALDI-TOF) calcd for $C_{148}H_{134}N_8O_8Zn_2$ 2278.8902, found 2278.8881. UV–vis (THF) λ_{max} (log ε) 468 (5.42), 580 (4.18), 685 (4.86), 730 (4.79) nm. Fluorescence (THF, λ_{ex}=468 nm) λ_{em} 758 nm. IR (KBr) 2953, 2927, 2869, 2127, 2184, 1588, 1164 cm^{-1}.

4.2.5.10. Compound 2c. ^1H NMR (400 MHz, d_8-THF) δ 9.91 (m, 8H, β-pyrrole), 9.73 (d, J=5 Hz, 4H, β-pyrrole), 9.10 (m, 8H, β-pyrrole), 9.05 (d, J=5 Hz, 4H, β-pyrrole), 8.86 (d, J=9 Hz, 2H, pyrene), 8.44–8.00 (m, 16H, pyrene), 7.42 (m, 12H, Ar), 6.99 (m, 6H, Ar), 4.25 (m, 24H, OCH_2), 1.96 (m, 24H, CH_2), 1.82 (m, 12H, CH), 1.03 (m, 72H, CH_3). HRMS (MALDI-TOF) calcd for $C_{204}H_{192}N_{12}O_{12}Zn_3$ 3193.2651, found 3193.2678. UV–vis (THF) λ_{max} (log ε) 468 (5.56), 584 (4.35), 747 (5.07) nm. Fluorescence (THF, λ_{ex}=468 nm) λ_{em} 782 nm. IR (KBr) 2954, 2928, 2868, 2125, 2167, 1587, 1162 cm^{-1}.

4.2.5.11. Compound 2d. ^1H NMR (400 MHz, d_8-THF) δ 9.91 (m, 12H, β-pyrrole), 9.73 (d, J=5 Hz, 4H, β-pyrrole), 9.10 (m, 12H, β-pyrrole), 9.05 (d, J=5 Hz, 4H, β-pyrrole), 8.86 (d, J=9 Hz, 2H, pyrene), 8.45–8.00 (m, 16H, pyrene), 7.42 (m, 16H, Ar), 6.99 (m, 8H, Ar), 4.26 (m, 32H, OCH_2), 1.96 (m, 32H, CH_2), 1.82 (m, 16H, CH), 1.03 (m, 96H, CH_3). MS (MALDI-TOF) calcd for $C_{260}H_{250}N_{16}O_{16}Zn_4$ 4117, found 4116. UV–vis (THF) λ_{max} (log ε) 469 (5.66), 584 (4.37), 756 (5.24) nm. Fluorescence (THF, λ_{ex}=469 nm) λ_{em} 796 nm. IR (KBr) 2954, 2925, 2869, 2126, 2167, 1588, 1163 cm^{-1}.

4.2.5.12. Compound 2e. ^1H NMR (400 MHz, d_8-THF) δ 9.91 (m, 16H, β-pyrrole), 9.73 (d, J=5 Hz, 4H, β-pyrrole), 9.10 (m, 16H, β-pyrrole), 9.05 (d, J=5 Hz, 4H, β-pyrrole), 8.86 (d, J=9 Hz, 2H, pyrene), 8.45–8.00 (m, 16H, pyrene), 7.42 (m, 20H, Ar), 6.99 (m, 10H, Ar), 4.26 (m, 40H, OCH_2), 1.96 (m, 40H, CH_2), 1.82 (m, 20H, CH), 1.03 (m, 120H, CH_3). MS (MALDI-TOF) calcd for $C_{316}H_{308}N_{20}O_{20}Zn_5$ 5034, found 5031. UV–vis (THF) λ_{max} (log ε) 469 (5.84), 584 (4.55), 762 (5.43) nm. Fluorescence (THF, λ_{ex}=469 nm) λ_{em} 802 nm. IR (KBr) 2951, 2927, 2870, 2126, 2167, 1588, 1162 cm^{-1}.

4.2.5.13. Compound 2f. ^1H NMR (400 MHz, d_8-THF) δ 9.91 (m, 20H, β-pyrrole), 9.73 (d, J=5 Hz, 4H, β-pyrrole), 9.10 (m, 20H, β-pyrrole), 9.05 (d, J=5 Hz, 4H, β-pyrrole), 8.86 (d, J=9 Hz, 2H, pyrene), 8.45–8.00 (m, 16H, pyrene), 7.42 (m, 24H, Ar), 6.99 (m, 12H, Ar), 4.26 (m, 48H, OCH_2), 1.96 (m, 48H, CH_2), 1.82 (m, 24H, CH), 1.03 (m, 144H, CH_3). MS (MALDI-TOF) calcd for $C_{372}H_{366}N_{24}O_{24}Zn_6$ 5953, found 5948. UV–vis (THF) λ_{max} (log ε) 469 (5.88), 584 (4.71), 766 (5.49) nm. Fluorescence (THF, λ_{ex}=469 nm) λ_{em} 806 nm. IR (KBr) 2956, 2927, 2869, 2126, 2162, 1588, 1163 cm^{-1}.

4.2.5.14. Compound 2g. ^1H NMR (400 MHz, d_8-THF) δ 9.91 (m, 24H, β-pyrrole), 9.73 (d, J=5 Hz, 4H, β-pyrrole), 9.10 (m, 24H, β-pyrrole), 9.05 (d, J=5 Hz, 4H, β-pyrrole), 8.86 (d, J=9 Hz, 2H, pyrene), 8.45–8.00 (m, 16H, pyrene), 7.42 (m, 28H, Ar), 6.99 (m, 14H, Ar), 4.26 (m, 56H, OCH_2), 1.96 (m, 56H, CH_2), 1.82 (m, 28H, CH), 1.03 (m, 168H, CH_3). MS (MALDI-TOF) calcd for $C_{428}H_{424}N_{28}O_{28}Zn_7$ 6868, found 6861. UV–vis (THF) λ_{max} (log ε) 469 (5.97), 583 (4.78), 769 (5.59) nm. Fluorescence (THF, λ_{ex}=469 nm) λ_{em} 808 nm. IR (KBr) 2954, 2927, 2869, 2126, 2162, 1588, 1163 cm^{-1}.

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CuI catalyzed C–N bond forming reactions between aryl/heteroaryl bromides and imidazoles in [Bmim]BF_4

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Abstract—By using CuI as the catalyst and L-Proline as the ligand, the Ullmann-type coupling reactions of aryl/heteroaryl bromides and imidazoles in [Bmim]BF_4 at 105–115 °C gave the corresponding *N*-arylimidazoles/*N*-heteroarylimidazoles in good yields. The system offers a convenient, recyclable, and environmentally benign method for these coupling reactions.

1. Introduction

N-Arylimidazoles and *N*-heteroarylimidazoles have been found useful for their biological effects and medicinal applications.[1] Recently, these arylimidazole derivatives show their important roles in the area of *N*-heterocyclic carbene chemistry.[2] Synthesis of these useful compounds becomes a meaningful work. One common strategy is the Ullmann-type C–N bond formations.[3] However, the reactions usually proceeds at high temperatures and their further applications would be limited. *N*-Arylimidazoles could be obtained by nucleophilic aromatic substitution,[4] but the method is suitable only for the substrates with electron withdrawing substituents. In 1998, Chan and Lam reported cupric acetate catalyzed coupling between aryl boronic acids and imidazoles.[5] The method could proceed at low temperatures, nevertheless the relatively expensive boronic reagents and the strict optimization of the condition limits its scope in synthetic areas.[6] Palladium catalyzed C–N bond forming coupling works effectively at mild temperatures, whereas the catalysts used is expensive and sensitive to air and moisture.[7]

Buchwald et al. firstly reported (CuOTf)·benzene/1,10-phenanthroline/*trans*,*trans*-dibenzylideneacetone catalyzed Ullmann-type reactions between aryl halides and imidazoles at low temperatures (110–125 °C),[8] and latterly found the diamines promoted CuI catalyzed coupling of aryl iodides and imidazoles.[9] However, Buchwald's method requires special catalysts or ligands. Recently, Ma and his co-workers developed the first amino acids promoted CuI catalyzed system in cross-coupling reactions, which were carried out in mild conditions.[10] Ma's protocol uses economical and readily available amino acids as promoters.

Room temperature ionic liquids (RTILs) are one class of non-molecular ionic solvents. They have special characteristics such as low vapor pressure, high polarity, and etc.[11] RTILs have been widely utilized in the area of organic chemistry as novel solvents.[11] In recent years, metal catalyzed coupling reactions have been studied in RTILs.[12] Ullmann-type reactions also have been tried in RTILs and many results revealed that they could proceed successfully in these special solvents.[13] Our laboratory reported a mild and efficient method for Ullmann-type coupling of vinyl bromides with imidazoles in ionic liquids, which was promoted by L-proline.[14]

On the other hand, although synthesis of *N*-arylimidazoles has been widely and intensively studied, researches on *N*-heteroarylation of imidazoles are still rare. Relating to this article, only two *N*-heteroarylimidazoles have been synthesized by Ullmann coupling methods.[15]

Herein we reported a copper (I) catalyzed cross-coupling reaction between aryl/heteroaryl bromides and imidazoles in RTIL, which would be a milder, economical, and recoverable way to obtain the *N*-arylimidazoles/*N*-heteroarylimidazoles. Considering the relatively rare reports on the latter and their important applications, our research mainly focused on the synthesis of *N*-heteroarylimidazoles.

2. Results and discussion

2.1. Synthesis of *N*-arylimidazoles/*N*-heteroarylimidazoles by Ullmann-type method in RTIL

Initially, CuI catalyzed coupling reactions between aromatic heterocyclic bromides and imidazoles in ([Bmim]BF_4) were studied. It was found that in the presence of some weak bases

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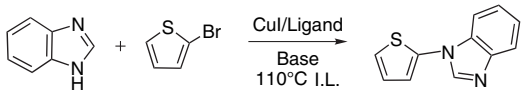
(such as K_2CO_3 and K_3PO_4), together with some α-amino acids as ligands, CuI could facilitate this type of coupling reactions in [Bmim]BF_4 under mild conditions. The coupling between aryl (including substituted phenyl and naphthyl) bromides and imidazoles under the similar conditions was also tested, as a supplement for the arylation of the imidazoles.

By using a moderate base (K_2CO_3), we firstly altered the ligand α-amino acids to search the most suitable promoter. It was found that the type of ligands affected this reaction significantly and L-proline seemed to be the best ligand (Table 1, entries 1–4).

Table 1. Effect of ligands and bases in one coupling reaction[a]



Entry	Ligand	Base	Yield (%)[b]
1	L-Alanine	K_2CO_3	73
2	L-Arginine	K_2CO_3	51
3	L-Serine	K_2CO_3	65
4	L-Proline	K_2CO_3	76
5	L-Proline	KOH	52
6	L-Proline	K_3PO_4	71
7	L-Proline	AcONa	26

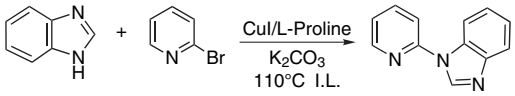
[a] Reaction conditions: CuI (0.9 mmol), base (5.4 mmol), amino acid (1.8 mmol), and benzimidazole (3 mmol), 2-bromothiophene (5.4 mmol), at 110 °C in [Bmim]BF_4 (3 ml), 24 h.
[b] Isolated yields.

After the favorable ligand has been sought out, several different bases were tried (Table 1, entries 4–7). By comparing the yields of these reactions, both K_2CO_3 and K_3PO_4 could facilitate this coupling reaction and K_2CO_3 was the best one. Although KOH was much stronger than the others, the yield was lower, and more complex mixture was obtained when the reaction was finished. In our opinion, when KOH was used as a base, the reagents or solvent might decompose and side reactions might exist. In contrast, AcONa was too weak to facilitate the metal exchange process and so the reactivity was lower.

The reactions also seem to be sensitive to the amount of catalyst and ligand. Different amount of the catalyst and ligand were added into the reaction systems. The yield was significantly improved when the amount of CuI was increased from 10 to 30 mol % gradually (Table 2). However, no obvious improvement was observed when the amount of CuI was raised from 30 to 40 mol %. This results show that the reaction might have been saturated when more than 40 mol % CuI was used. In our opinion, 30 mol % CuI is sufficient and optimum.

After optimizing the reaction conditions, we explored the scope of the coupling reactions between heteroaryl bromides and imidazoles. The results are listed in Table 3, entries 1–13. The coupling reactions between aryl (including substituted phenyl and naphthyl) bromides and imidazole were also carried out under the above-mentioned conditions. The reactions proceeded successfully and the yields were satisfactory (Table 3, entries 14–19).

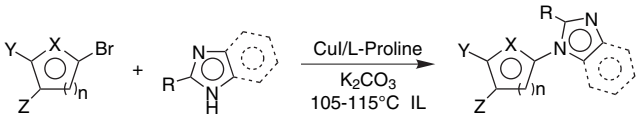
Table 2. Effect of the amount of CuI and ligand[a]



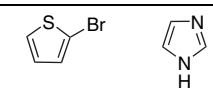
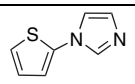
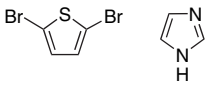
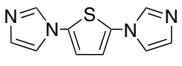
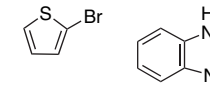
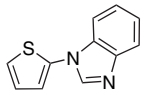
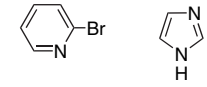
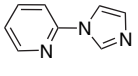
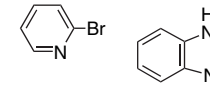
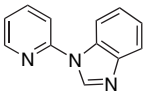
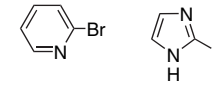
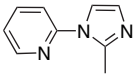
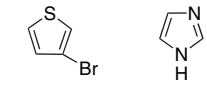
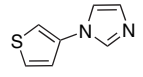
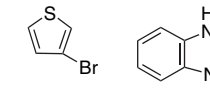
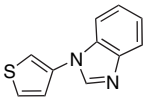
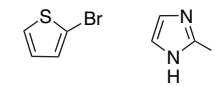
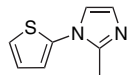
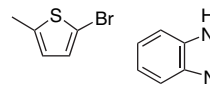
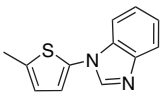
Entry	CuI (L-Proline) (mol %)	Yield (%)[b]
1	10 (20)	56
2	20 (40)	78
3	30 (60)	87
4	40 (80)	89

[a] Reaction conditions: CuI (0.3–1.2 mmol), base (5.4 mmol), amino acid (0.6–2.4 mmol), benzimidazole (3 mmol), 2-bromopyridine (5.4 mmol), at about 110 °C in [Bmim]BF_4 (3 ml), 10 h.
[b] Isolated yields.

Table 3. CuI/α-amino acid catalyzed coupling reactions of heteroaryl/aryl bromides with imidazoles in RTIL[a]

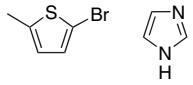
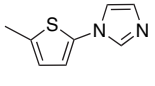
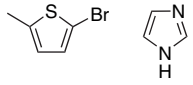
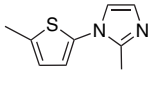
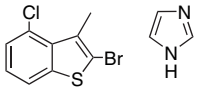
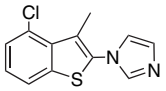
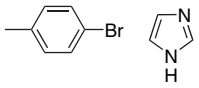
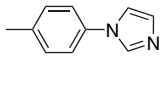
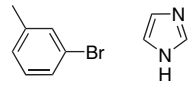
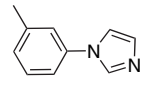
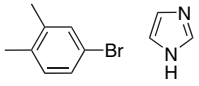
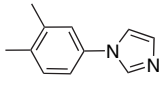
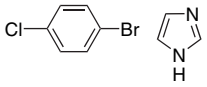
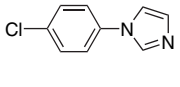
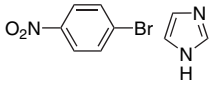
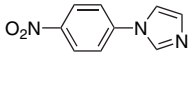
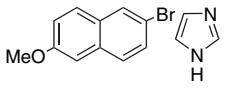
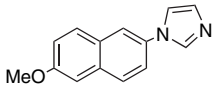


X=CH, S, N; n=1, 2; Y=H, CH_3, Br; Z=H, CH_3, Cl, NO_2; R=H, CH_3

Entry	Substrates	Product	Time (h)	Yield[b] (%)
1			26	74
2			50	77[c]
3			24	76
4			18	81
5			10	87
6			48	71
7			36	72
8			36	75
9			48	62
10			36	73

(continued)

Table 3. (*continued*)

Entry	Substrates	Product	Time (h)	Yield[b] (%)
11			48	68
12			48	46
13			48	19
14			24	82
15			24	75
16			24	70
17			24	80
18			24	62
19			24	85

[a] Reaction conditions: CuI (0.9 mmol), base (5.4 mmol), amino acid (1.8 mmol), imidazole (3 mmol), heteroaryl/aryl bromide (5.4 mmol), at 110 °C in [Bmim]BF_4 (3 ml).
[b] Isolated yields.
[c] CuI (1.8 mmol), base (10.8 mmol), amino acid (3.6 mmol), imidazole (6 mmol), 2,5-dibromothiophene (2 mmol), at 115 °C in [Bmim]BF_4 (5 ml).

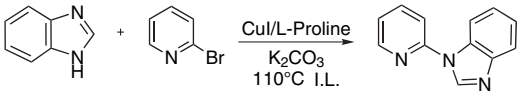
2-Bromopyridine is found to be the most reactive heteroaryl bromide in our experiment and benzimidazole seems to be a little more reactive than imidazole and alkyl-substituted imidazoles (Table 3, entries 4–6 and entries 7–9). We think both electrophilicity of heteroaryl bromides and the nucleophilicity of imidazoles might affect these reactions.

As shown in Table 3, the reaction is hypersensitive to the steric environment of the heteroaryl/aryl bromides and imidazoles, i.e., steric hindrance of the substrates obviously affects these coupling reactions. As indicated in Table 3 (entries 1, 4, 11 and entries 6, 9, 12), the conversion declined when imidazole is replaced by 2-methylimidazole. The effect of the 2-position steric hindrance on the bromides seems to be much stronger than on the imidazoles. As a result, the *o*-substituted heterocycle bromides such as 2-bromo-4-chloro-3-methylbenzo[*b*]thiophene are much more difficult to react with imidazole (Table 3, entry 13) probably because the methyl group on 3-position hinder the co-planarity of the imidazole and the thiophene moieties, which gives a negative effect on the product formation. Prolonging the reaction time from 48 to 72 h did not substantially improve the yield. The reactions between aryl bromides and imidazole could proceed successfully in the similar catalytic system (Table 3, entries 14–17) in relatively short reaction time (24 h). It is observed that these reactions tolerate both electron withdrawing and electron donating substituents on the bromides (Table 3, entries 14–19, yields 62–85%). The electron donating groups on the bromides are found to be more favorable for these reactions than the electron withdrawing ones (Table 3, entries 14 and 18). 2-Bromo-6-methoxy-naphthalene is found more reactive than phenyl bromides and the yield of the corresponding product obtained is also better.

2.2. Reusability and solvent effects of the coupling reactions in RTIL

One of the most attractive properties of ionic liquids is that the catalyst system could be recycled. The recovery possibility of the reaction has also been investigated. To our satisfaction, the results are rather good. After fourth runs, the system still gave good yield (Table 4). The catalyst system, including the solvent ionic liquids, the catalyst CuI and the ligand could be favorably recycled. Because of the high polarity of ionic liquid, the reaction mixture can be extracted directly by lower polar solvents. Therefore the workup of these reactions is simple. The recovered ionic liquid can be recycled after proper treatment.

Table 4. Reuse of CuI/L-proline/IL reaction system[a]

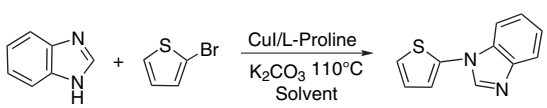


Cycle	Yield (%)	Cycle	Yield (%)[b]
1	87	3	80
2	82	4	76

[a] Reaction conditions: CuI (0.9 mmol), K_2CO_3 (5.4 mmol), L-proline (1.8 mmol), benzimidazole (3 mmol), 2-bromopyridine (5.4 mmol), at 110 °C in [Bmim]BF_4 (3 ml), 10 h. After reaction, the product was extracted with ether/ethyl acetate (v/v=4/1, 3×5 ml). The remaining ionic liquid was concentrated in vacuo (6.0 torr for 1 h at 110 °C), further amounts of base (5.4 mmol) and reactants were added and next turn began.
[b] Isolated yields.

Lastly, we have tried the reactions in traditional organic solvents such as DMF and DMSO. The yields obtained in these two solvents were also good but not as good as in the ionic liquid (Table 5).

Table 5. Effect of different solvents[a]



Entry	Solvent	Yield (%)[b]
1	[Bmim]BF_4	76
2	DMF	66
3	DMSO	71

[a] Reaction conditions: CuI (0.9 mmol), K_2CO_3 (5.4 mmol), L-proline (1.8 mmol), benzimidazole (3 mmol), 2-bromothiophene (5.4 mmol), at 110 °C, Solvent (3 ml), 24 h.
[b] Isolated yields.

3. Conclusions

In summary, we have developed an efficient and mild method to synthesize *N*-arylimidazoles/*N*-heteroarylimidazoles by Ullmann coupling strategy, in which ionic liquid was used as a favorable solvent. The procedure is simple and can be carried out under mild conditions, using comparatively cheap CuI as the catalyst and natural amino acid as the ligand. The conveniently recoverable and reusable catalytic system makes the methodology environmentally benign and economical.

4. Experimental

4.1. General information

All reagents and solvents used were analytical grade materials purchased from commercial sources and were used without further purification, if not stated otherwise. All the NMR spectra were recorded in $CDCl_3$ on a Bruker AMX-400 (400 MHz) instrument with TMS as internal standard. IR spectra were taken as liquid film or KBr plates. TLC was carried out with 0.2 mm silica gel plates (GF254). Visualization was accomplished by UV light or I_2 staining. The columns were hand packed with silica gel 60 (200–300 mesh). All reactions were carried out under atmosphere, if not stated otherwise. The ionic liquid, $[Bmim]BF_4$, was dehumidified in vacuo by heating at about 110 °C for 30 min prior to use. All products were confirmed by ^1H NMR, ^{13}C NMR, IR, and elemental/HRMS analysis (excepting the known compounds and the *N*-arylimidazoles).

4.2. General procedure for *N*-heteroarylation/*N*-arylation of imidazoles under the catalysis of CuI and α-amino acid in RTIL

4.2.1. Synthesis of 1-(thiophen-2-yl)-1*H*-imidazole in $[Bmim]BF_4$. Table 3, entry 1. CuI (1.71 g, 0.9 mmol), L-proline (0.207 g, 1.8 mmol), K_2CO_3 (0.745 g, 5.4 mmol) and dry $[Bmim]BF_4$ (3.00 ml) were added to a 10 ml flask. Stirred and humidified in vacuo by heating in an oil bath at 110 °C for 0.5 h. Imidazole (0.204 g, 3 mmol) was add to the stirred mixture, then bromothiophene (0.880 g, 5.4 mmol, 1.8 equiv) was added by a syringe into the flask (sealed). The reaction was monitored by TLC. After the reaction was completed, the resulting mixture was cooled to room temperature and was extracted by Et_2O/EtOAc (v/v=4/1, 3×5 ml). The extracts were combined and washed by water (2×10 ml), brine (15 ml), and dried ($MgSO_4$). Evaporating the solvent under reduced pressure gave a dark brown oil, which was further purified by column chromatography (v/v=4/1, Et_2O/EtOAc) to afford an viscous oil (0.316 g, 74%). The ^1H NMR is in accordance with literature.[15,16] ^1H NMR (400 MHz, $CDCl_3$) δ 6.92–6.95 (m, 2H), 7.07–7.10 (m, 1H), 7.11 (s, 1H), 7.14 (s, 1H), 7.72 (s, 1H); IR: 3079, 2920, 2858, 1611, 1565, 1502, 1450, 1331, 1228, 1197, 1046, 800, 743 cm^{-1}.

4.2.2. 2,5-Di(1*H*-imidazol-1-yl)thiophene. Table 3, entry 2, yield 77%. Mp 71–72 °C (lit.[16] mp 71–72 °C). ^1H NMR (400 MHz, $CDCl_3$) δ 6.92 (s, 2H), 7.18–7.19 (m, 4H), 7.75 (s, 2H); IR (KBr): 3103, 2925, 2870, 1649, 1575, 1481, 1310, 1251, 1047, 844, 808, 749 cm^{-1}.

4.2.3. 1-(Thiophen-2-yl)-1*H*-benz[*d*]imidazole. Table 3, entry 3, yield 76%. Viscous oil. ^1H NMR (400 MHz, $CDCl_3$) δ 7.07–7.09 (dd, J=3.9, 5.5 Hz, 1H), 7.12–7.14 (dd, J=1.4, 3.9 Hz, 1H), 7.26–7.28 (dd, J=1.4, 5.5 Hz, 1H), 7.32–7.36 (m, 2H), 7.53–7.56 (m, 1H), 7.83–7.87 (m, 1H), 8.06 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 110.75, 120.76, 122.06, 123.40, 123.61, 124.33, 126.63, 143.87, 137.23, 143.36, 143.75; IR: 3077, 2925, 2860, 1611, 1550, 1488, 1327, 1231, 1196, 743, 699 cm^{-1}; HRMS (ESI) calcd for $C_{11}H_8N_2SNa$ $(M+Na)^+$, 223.0306; found: $(M+Na)^+$, 223.0305.

4.2.4. 2-(1*H*-Imidazol-1-yl) pyridine.[15c,17] Table 3, entry 4, yield 81%. Mp 38–40 °C (lit.[17] mp 38–40 °C). ^1H NMR (400 MHz, $CDCl_3$) δ 7.12 (s, 1H), 7.29–7.32 (dd, J=5.0, 7.4 Hz, 1H), 7.76–7.78 (d, J=8.0 Hz, 1H), 7.92–7.96 (m, 2H), 8.44–8.46 (m, 1H), 8.54 (s, 1H); IR (KBr): 3078, 2926, 2865, 1611, 1601, 1488, 1328, 1231, 1196, 770, 650 cm^{-1}.

4.2.5. 1-(Pyridin-2-yl)-1*H*-benz[*d*]imidazole.[15a,b] Table 3, entry 5, yield 87%. Mp 59–60 °C (lit.[18] mp 59–60 °C). ^1H NMR (400 MHz, $CDCl_3$) δ 7.20–7.24 (m, 1H), 7.31–7.37 (m, 2H), 7.46–7.51 (m, 1H), 7.77–7.86 (m, 2H), 8.02–8.04 (m, 1H), 8.55 (s, 2H); IR (KBr): 3060, 3028, 2927, 2850, 1588, 1478, 1462, 1327, 1235, 1204, 773, 742 cm^{-1}.

4.2.6. 2-(2-Methyl-1*H*-imidazol-1-yl) pyridine. Table 3, entry 6, yield 71%. Viscous oil. ^1H NMR (400 MHz, $CDCl_3$) δ 2.55 (s, 3H), 6.98 (d, J=1.0 Hz, 1H), 7.24 (d, J=1.0 Hz, 1H), 7.25–7.27 (m, 2H), 7.78–7.83 (td, J=2.0, 8.0 Hz, 1H), 8.50–8.52 (d, J=4.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.16, 117.13, 118.86, 122.26, 127.74, 138.58, 144.83, 149.06, 150.58; IR: 3101, 2931, 2860, 1588, 1499, 1475, 1442, 1312, 1283, 787, 741, 677 cm^{-1}; HRMS (ESI) calcd for $C_9H_{10}N_3$ $(M+H)^+$, 160.0875; found: $(M+H)^+$, 160.0869.

4.2.7. 1-(Thiophen-3-yl)-1*H*-imidazole. Table 3, entry 7, yield 72%. Mp 82–83 °C. ^1H NMR (400 MHz, $CDCl_3$) δ 7.14 (d, J=1.2 Hz, 1H), 7.16 (d, J=1.6 Hz, 1H), 7.18–7.19 (dd, J=1.2, 2.8 Hz, 1H), 7.21 (s, 1H), 7.39–7.41 (dd, J=2.8, 5.4 Hz, 1H), 7.79 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 113.19, 116.50, 121.37, 127.12, 129.87, 135.81, 136.14; IR (KBr): 3110, 2928, 2858, 1555, 1494, 1337, 1247, 856, 777, 736, 658 cm^{-1}; Anal. Calcd for $C_7H_6N_2S$: C 55.97, H 4.03, N 18.65; found: C 56.08, H 4.03, N 18.68.

4.2.8. 1-(Thiophen-3-yl)-1*H*-benz[*d*]imidazole.[19] Table 3, entry 8, yield 75%. Mp 90–91 °C. ^1H NMR (400 MHz, $CDCl_3$) δ 7.23–7.25 (dd, J=1.6, 5.2 Hz, 1H), 7.28–7.32 (m, 2H), 7.33–7.35 (dd, J=1.6, 2.8 Hz, 1H), 7.46–7.48 (dd, J=3.2, 5.2 Hz, 1H), 7.49–7.53 (m, 1H), 8.07 (s, 1H), 7.83–7.88 (m, 1H); IR (KBr): 3106, 2963, 2858, 1548, 1491, 1226, 1194, 855, 789, 744, 642 cm^{-1}.

4.2.9. 2-Methyl-1-(thiophen-2-yl)-1*H*-imidazole. Table 3, entry 9, yield 62%. Viscous oil. ^1H NMR (400 MHz, $CDCl_3$) δ 2.37 (s, 3H), 6.95–6.96 (dd, J=1.6, 4.0 Hz, 1H), 6.98–6.99 (d, J=1.6 Hz, 1H), 7.00–7.02 (m, 2H), 7.23–7.26 (dd, J=1.2, 5.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.32, 121.92, 123.16, 123.76, 125.84, 127.68, 138.60, 145.94; IR: 3107, 2927, 2850, 1553, 1496, 1451,

1289, 984, 845, 703 cm^{-1}; HRMS (ESI) calcd for $C_8H_9N_2S$ $(M+H)^+$ 165.0486; found: $(M+H)^+$, 165.0481.

4.2.10. 1-(5-Methylthiophen-2-yl)-1*H*-benz[*d*]imidazole. Table 3, entry 10, yield 73%. Viscous oil. ^1H NMR (400 MHz, $CDCl_3$) δ 2.52 (s, 3H), 6.72–6.73 (d, J=3.6 Hz, 1H), 6.91–6.92 (d, J=3.6 Hz, 1H), 7.31–7.36 (m, 2H), 7.51–7.55 (m, 1H), 7.82–7.87 (m, 1H), 8.02 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.40, 110.37, 120.27, 121.96, 122.84, 123.78, 123.94, 133.80, 134.62, 138.06, 143.05, 143.27; IR: 3079, 2921, 2857, 1651, 1612, 1503, 1451, 1283, 919, 882, 800, 743, 676 cm^{-1}; HRMS (ESI) calcd for $C_{12}H_{11}N_2S$ $(M+H)^+$ 215.0643; found: $(M+H)^+$, 215.0637.

4.2.11. 1-(5-Methylthiophen-2-yl)-1*H*-imidazole. Table 3, entry 11, yield 68%. Viscous oil. ^1H NMR (400 MHz, $CDCl_3$) δ 2.50 (s, 3H), 6.64–6.65 (m, 1H), 6.79–6.80 (d, J=3.6 Hz, 1H), 7.16 (s, 2H), 7.72 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.30, 119.00, 120.21, 123.87, 129.80, 136.00, 136.54, 136.97; IR: 3112, 2921, 2859, 1644, 1568, 1510, 1474, 1302, 1215, 1038, 909, 801, 733, 657 cm^{-1}; HRMS (ESI) calcd for $C_8H_9N_2S$ $(M+H)^+$ 165.0486; found: $(M+H)^+$, 165.0481.

4.2.12. 2-Methyl-1-(5-methylthiophen-2-yl)-1*H*-imidazole. Table 3, entry 12, yield 46%. Mp 40–41 °C. ^1H NMR (400 MHz, $CDCl_3$) δ 2.33 (s, 3H), 2.45 (s, 3H), 6.61–6.62 (d, J=3.6 Hz, 1H), 6.70–6.71 (d, J=4.0 Hz, 1H), 6.94 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.33, 15.46, 122.08, 123.44, 123.72, 127.48, 135.72, 138.62, 146.25; IR: 3110, 2923, 2858, 1566, 1531, 1502, 1446, 1408, 1291, 1221, 1168, 1134, 985, 931, 802, 731, 671 cm^{-1}; Anal. Calcd for $C_9H_{10}N_2S$: C 60.64, H 5.65, N 15.72; found: C 60.74, H 5.80, N 15.70.

4.2.13. 1-(4-Chloro-3-methylbenzo[*b*]thiophen-2-yl)-1*H*-imidazole. Table 3, entry 13, yield 19%. Yellow needles. Mp 111–112 °C. ^1H NMR (400 MHz, $CDCl_3$) δ 2.29 (s, 3H), 7.19 (s, 1H), 7.25 (s, 1H), 7.37–7.40 (dd, J=2.0, 8.4 Hz, 1H), 7.68–7.71 (m, 2H), 7.74 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.11, 121.26, 122.26, 123.43, 125.75, 126.06, 130.12, 131.37, 133.64, 134.23, 138.30, 139.64; IR (KBr): 3088, 3044, 2920, 2861, 1649, 1581, 1541, 1476, 1443, 1283, 1234, 1151, 1103, 910, 853, 809, 734, 657 cm^{-1}; Anal. Calcd for $C_{12}H_9ClN_2S$: C 57.95, H 3.65, N 11.26; found: C 57.71, H 3.82, N 11.19.

4.2.14. 1-*p*-Tolyl-1*H*-imidazole.[20,21] Table 3, entry 14, yield 82%. Mp 45–47 °C (lit.[20] mp 45–48 °C). ^1H NMR (400 MHz, $CDCl_3$) δ 2.37 (s, 3H), 7.16–7.22 (m, 1H), 7.24 (s, 5H), 7.80 (s, 1H); IR (KBr): 3112, 2922, 2855, 1651, 1522, 815 cm^{-1}.

4.2.15. 1-*m*-Tolyl-1*H*-imidazole.[22] Table 3, entry 15, yield 75%. ^1H NMR (400 MHz, $CDCl_3$) δ 2.40 (s, 3H), 7.15–7.18 (m, 4H), 7.25 (s, 1H), 7.31–7.35 (m, 1H), 7.82 (s, 1H); IR: 3113, 2920, 2860, 1611, 1504, 1365, 785.13, 734.6, 691.2 cm^{-1}.

4.2.16. 1-(3,4-Dimethylphenyl)-1*H*-imidazole. Table 3, entry 16, yield 70%. ^1H NMR (400 MHz, $CDCl_3$) δ 2.29 (s, 3H), 2.31 (s, 3H), 7.09–7.11 (d, J=8.0 Hz, 1H), 7.15 (m, 1H), 7.18 (s, 1H), 7.19–7.21 (d, J=8.0 Hz, 1H), 7.23 (s, 1H), 7.82 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.25, 19.84, 118.33, 118.75, 122.61, 129.71, 130.66, 135.02, 135.48, 136.11, 138.33; IR: 3113, 2922, 2850, 2363, 1623, 1513, 1310, 814, 734 cm^{-1}.

4.2.17. 1-(4-Chlorophenyl)-1*H*-imidazole.[23] Table 3, entry 17, yield 80%. Mp 84–86 °C (lit.[23a] mp 85–87 °C). ^1H NMR (400 MHz, d_6-DMSO) δ 7.13 (s, 1H), 7.58–7.60 (td, J=2.0, 8.0 Hz, 2H), 7.70–7.72 (td, J=2.0, 8.0 Hz, 2H), 7.78 (s, 1H), 8.30 (s, 1H). IR (KBr): 3110, 2926, 2854, 1635, 1508, 1303, 830 cm^{-1}.

4.2.18. 1-(4-Nitrophenyl)-1*H*-imidazole.[15c,20] Table 3, entry 18, yield 62%. Mp 203–205 °C (lit.[20] mp 204.4–205.2 °C). ^1H NMR (400 MHz, $CDCl_3$) δ 7.31–7.32 (m, 1H), 7.43 (s, 1H), 7.73–7.78 (t, J=8.0 Hz, 1H), 7.81–7.83 (d, J=8.0 Hz, 1H), 8.04 (s, 1H), 8.28–8.30 (d, J=8.0 Hz, 1H), 8.33 (s, 1H); IR (KBr): 3100, 2924, 2853, 2363, 1621, 1529, 1358, 812 cm^{-1}.

4.2.19. 1-(6-Methoxynaphthalen-2-yl)-1*H*-imidazole.[24] Table 3, entry 19, yield 85%. Mp 84–85 °C (lit.[24] mp 85 °C). ^1H NMR (400 MHz, $CDCl_3$) δ 3.94 (s, 3H), 7.17 (d, J=2.0 Hz, 1H), 7.22–7.25 (m, 2H), 7.36 (s, 1H), 7.46–7.48 (dd, J=2.0, 8.5 Hz, 1H), 7.73–7.74 (m, 1H), 7.75–7.77 (m, 1H), 7.82–7.84 (d, J=8.5 Hz, 1H), 7.93 (s, 1H); IR (KBr): 3110, 2931, 2849, 2361, 1606, 1513, 1246, 852 cm^{-1}.

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Synthesis of 6-thia analogs of the natural neurosteroid allopregnanolone

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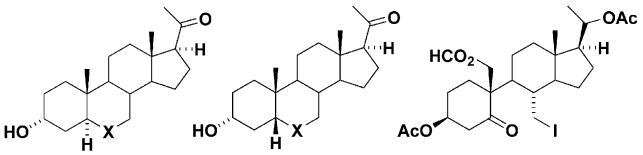
Abstract—A procedure is described for the preparation of 6-thiapregnanes in five steps from pregnenolone via a 5-oxo-7-iodo-secopregnane intermediate. The 6-thiasteroid obtained was converted into 6-thia-allopregnanolone and its sulfoxide and sulfone derivatives. The trans stereochemistry at the A/B ring junction was accomplished by stereoselective reduction of an intermediate hemithioketal with triethylsilane/$BF_3 \cdot Et_2O$. The compounds synthesized are analogs of natural neurosteroids, and exhibited $GABA_A$ receptor activity comparable to allopregnanolone.

1. Introduction

Neuroactive steroids are positive allosteric modulators of the $GABA_A$ receptor that interact with a specific steroid recognition site on the receptor–ion channel complex. They have potential therapeutic interest as anticonvulsants, anesthetics and for the treatment of several neurological and psychiatric disorders.[1] Endogenous neuroactive steroids (neurosteroids) such as 3α-hydroxy-5α-pregnan-20-one (allopregnanolone, **1**) and its 5β isomer (pregnanolone, **2**), are rapidly biotransformed when administered exogenously and several synthetic analogs of these compounds with improved bioavailability have been developed.[2] An important advance in this search was the finding that substituents conferring water-solubility to these lipophilic steroids could be incorporated at a number of different positions of the steroid nucleus, without loss of anesthetic activity.[3] Analogs obtained by isosteric replacement of carbon atoms of the steroid nucleus by heteroatoms (e.g., O) exhibit localized changes in hydrophobicity and hydrogen bonding acceptor capacity; however, at variance with the presence of additional polar substituents, these modifications do not produce major changes in the overall conformation of the steroid nucleus or increased steric bulk.

We have described a synthetic approach to 5α-H and 5β-H 6-oxapregnanes (**3** and **4**),[4] analogs of the endogenous neuroactive steroids **1** and **2**, via stereospecific cyclizations of secosteroid **5**.[5,6] These compounds were 100-fold less active than **1** in their in vitro activity on the $GABA_A$ receptor, suggesting a significant involvement of the lipophilic character of ring B in modulating ligand binding to neighboring sites. A recent report on the activity of 6-aza-allopregnanolone points to the same conclusion.[7] The replacement of oxygen by sulfur (other characteristics being equal) often generates marked changes in the biological activity of a compound.[8] These biological and physicochemical properties are mostly attributed to the different electronic properties (electronegativity) of the sulfur and oxygen atoms. The larger size of the sulfur atom, and its more disperse electron density, result in a higher lipophilicity and diminished hydrogen bond acceptor capacity. Also, the C–S bond is longer (ca. 1.8 Å) and the C–S–C angle (ca. 95–100°) is smaller than the corresponding oxygen counterparts, thus some changes in flexibility and conformation may be expected. Additionally, the soft sulfur atom can be selectively oxidized to the sulfoxide or sulfone moieties, giving rise to new derivatives with different steric bulk and dipolar moments stereospecifically directed towards the α- and/or β-faces. With these guidelines in mind, we decided to synthesize the 6-thia analog **6**. To our knowledge, only a few procedures for the synthesis of 6-thiasteroids have been reported,[9] none of which correspond to analogs of neuroactive steroids.



Keywords: 6-Thiasteroids; *S*-Oxo-thiasteroids; *S*,*S*-Dioxo-thiasteroids; Neurosteroid analogs; Allopregnanolone; $GABA_A$ receptor.
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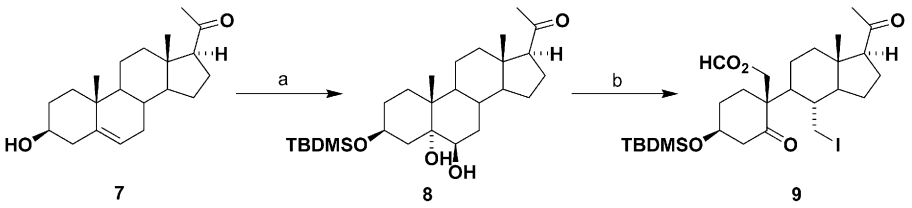
2. Results and discussion

2.1. Chemistry

By analogy with the approach used in the synthesis of the oxa-analogs **3** and **4**,[5,6] we focused our attention on the iodo substituent present in secosteroid **5**. This could be replaced by sulfur nucleophiles, giving rise to thioketals that may be transformed into the desired cyclic thioether. In this case however, the acetate group at C-3 in the 5-oxo secosteroid **5** proved to be too labile, giving elimination by-products throughout the synthesis; thus a silylated derivative was used.[10] Secosteroid **9** was synthesized from commercially available pregnenolone (**7**) in four steps (Scheme 1). Thus, pregnenolone was transformed into the 3β-*tert*-butyldimethylsilyloxy-5α,6β-diol **8** by epoxidation and cleavage (hydrogen peroxide, formic acid) followed by deformylation (methanolic base) to the 3β,5α,6β-trihydroxypregnane (one-pot)[11] and subsequent regioselective silylation of the less hindered equatorial 3β-hydroxy moiety. Treatment of **8** with HgO/I_2 (CCl_4, *hν*) gave the precursor secosteroid **9** in 42% yield. The structure of **9** was confirmed by comparison of its ^1H and ^{13}C NMR spectra with those of the acetylated analog **5** previously synthesized by us.[5]

Stereospecific reduction of the hemithioketal moiety in **11** to the 5α-H-cyclic thioether was accomplished in two steps by protection of the hydroxyl group at C-19 as a formate, followed by selective reduction of the hemithioketal moiety with Et_3SiH and $BF_3\cdot Et_2O$ in Cl_2CH_2. The attack of the hydride on the thiocarbenium intermediate occurred, as expected, from the less hindered α-face with stereoselective formation of the 5α-H-6-thiasteroid **12**. The pyranosic thiocarbenium ion preferently accepts nucleophiles from the α (axial) side due to the anomeric effect of the sulfur atom; this preference is magnified, relative to the tetrahydropyran system, because 1,3-diaxial repulsions are smaller as a result of the longer C–S bond.[12] The silyl protecting group was also cleaved in this step. The coupling pattern of the 3α-H resonance that appeared as a broad multiplet ($W_{1/2}$=15.3 Hz) typical of an axial hydrogen, was consistent with the α orientation of 5-H. Confirmation of that stereochemistry came from the observation of a strong NOE correlation between H-5 and H-3 in the NOESY spectrum of **12**, thus indicating the α orientation for H-5.

Inversion of the configuration at C-3, a requisite for agonistic $GABA_A$ receptor activity,[13] was accomplished in a straightforward way using the Mitsunobu reaction. Adequate choice



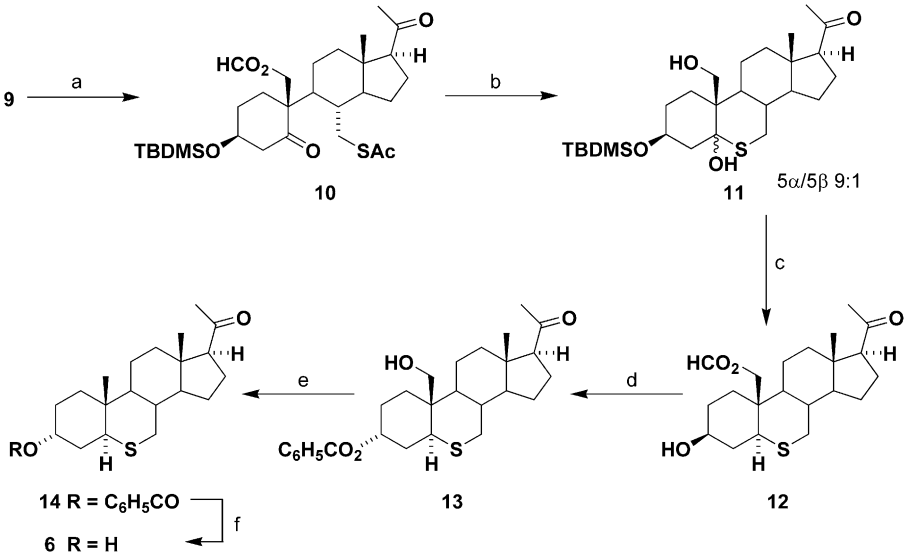
Scheme 1. Reagents and conditions: (a) i. HCO_2H, then 30% H_2O_2; ii. 40% NaOH, MeOH; iii. TBDMSCl, imidazole, DMF; (b) HgO, I_2, Cl_2CH_2–Cl_4C, *hν*, 25 °C, 4.5 h.

Reaction of **9** with potasium thioacetate gave the sulfur substituted secosteroid **10** in 70% yield (Scheme 2). The presence of the sulfur moiety was evident in the NMR and mass spectra. Particularly diagnostic was the shift of the 7-CH_2 hydrogen and carbon resonances (3.14/3.45 and 17.4 ppm for **9**; 2.95/3.05 and 31.5 ppm for **10**), consistent with the change of iodine by thioacetyl at that position. Treatment of **10** with base, simultaneously cleaved the C-19 formate and the C-7 thioacetate, with concomitant cyclization of the intermediate 7-sulfanyl anion to give the hemithioketal **11** as a 9:1 5α/5β mixture (78% yield).[†] Absence of the characteristic signal of a carbonyl group at C-5 in the ^{13}C NMR spectrum of **11** and the presence of a resonance at 81.9 ppm assigned to the hemithioketalic carbon of the 5-α isomer, indicated the success of this transformation. Attempts to purify the diastereomeric mixture on silica gel resulted in partial decomposition and isomerization of the α-epimer into the β-epimer giving rise to a new diastereomeric ratio of ca. 1:1.

[†] Determined in the ^1H NMR spectrum of the mixture, from the intensity ratio of the 13-CH_3 resonances for the α (0.68 ppm) and β (0.62 ppm) isomers. The coupling pattern of the 3α-H resonance of the major isomer, that appeared as a broad multiplet ($W_{1/2}$=22.3 Hz) typical of an axial hydrogen, was consistent with the α orientation of the 5-hydroxyl.

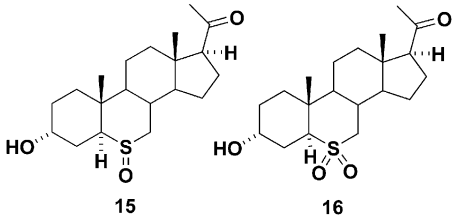
of the acid for this reaction was mandatory, taking into account that the following step involved the regioselective deprotection of the formate group at C-19. The 3α-benzoate in **13** proved to be adequate for this purpose. The success of the Mitsunobu reaction was evident in the ^1H NMR spectrum of the reaction product that showed the equatorial orientation of H-3 ($W_{1/2}$=7.5 Hz, at variance with the same hydrogen in **12**, vide supra). Aromatic hydrogens, belonging to the incorporated benzoyl moiety, were also observed. The 19-hydroxy derivative **13** was obtained by regioselective deprotection of the 19-ester moiety with hydrochloric acid in methanol; under these mild and controlled conditions, the 3α-benzoate was not affected.

Deoxygenation of the primary hydroxyl group attached at C-19 was then carried out using the Barton–Mc Combie procedure, by formation of the 19-imidazoylthionocarbonate derivative and reduction with diphenylsilane to give **14**; attempts to obtain this compound free of unreacted diphenylsilane were unsuccessful and so it was used directly in the next step. The low reactivity of the axial benzoate at C-3 required the use of strong base for its removal, which led to partial isomerization of the side chain at C-17. Smooth debenzoylation was achieved under nucleophilic conditions with sodium methylthiolate in DMF, to give 6-thia-allopregnanolone **6** as single product (61% yield from **13**).



Scheme 2. Reagents and conditions: (a) KSAc, acetone, 3 h, 25 °C; (b) NaOH, MeOH, 3 h, 25 °C; (c) i. formic acetic anhydride, py, 2 h, 25 °C; ii. Et_3SiH, $BF_3{\cdot}Et_2O$, Cl_2CH_2, 1 h, −15 °C; (d) i. benzoic acid, Ph_3P, DEAD, THF, 18 h, 25 °C; ii. 6 N HCl, MeOH, 1 h, 25 °C; (e) i. thiocarbonyldiimidazole, DMAP, Cl_2CH_2, 5 h, reflux; ii. Ph_2SiH_2, AIBN, toluene, 4.5 h, 115 °C; (f) $NaSCH_3$, DMF, 2 h, 100 °C.

Selective oxidation of **6** with potassium peroxymonosulfate (Oxone®) gave either sulfoxide **15** or sulfone **16**, depending on the product–reagent ratio and temperature.[14] Thus, treatment with Oxone® (1.3 equiv) in methanol at 0 °C gave sulfoxide derivative **15** as single product (87% yield) whereas excess of the same reagent (3 equiv) at room temperature gave sulfone **16** in 90% yield. Configuration of the sulfur atom in the sulfoxide **15** was inferred from the ^1H NMR spectrum, considering the shift in the 10-CH_3 resonance in sulfone **16** compared to **6** (+0.2 ppm) due to the axial oxygen on the sulfur. A negligible shift was observed in the transformation of **6** to the sulfoxide **15** (ca. +0.05 ppm) indicating that the oxygen was equatorially oriented. This is the expected stereochemistry for **15** considering attack of the reagent from the sterically less demanding α-face.



2.2. $GABA_A$ receptor activity

Biological activity of the three synthetic steroids was assayed by in vitro tests using [^{35}S]-*tert*-butylbicyclo[2,2,2]-phosporothionate (TBPS), [^{3}H]-flunitrazepam, and [^{3}H]-muscimol as radiolabelled ligands to γ-aminobutyric acid receptors ($GABA_A$). Crude membrane receptors from male rats' cerebellum were used to test the capacity of the steroids to displace the specific binding of the radioactive ligands.[13] Allopregnanolone was used as a positive control to check the viability of the methods. Preliminary results revealed that the synthetic steroids show a displacement pattern similar to that of allopregnanolone with an IC_{50} for [^{35}S]-TBPS binding in the 10^{-7} M range (Fig. 1). All three steroids

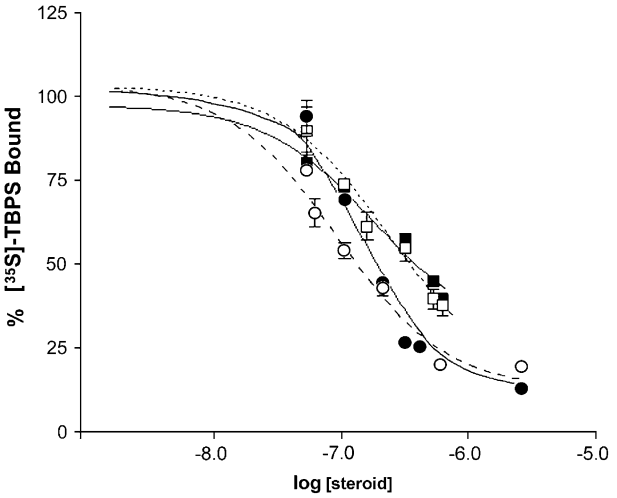


Figure 1. Inhibition of binding of [^{35}S]-*tert*-butylbicyclo-phosporothionate ([^{35}S]-TBPS) to membranes from rat cerebellum by allopregnanolone (**1**, ○), 3α-hydroxy-6-thia-5α-pregnan-20-one (6-thia-allopregnanolone, **6**, ●), *S*-oxo-3α-hydroxy-6-thia-5α-pregnan-20-one (**15**, □), and *S*,*S*-dioxo-3α-hydroxy-6-thia-5α-pregnan-20-one (**16**, ■). Cerebellum membrane preparations were incubated with 10 nM [^{35}S]-TBPS in the absence (100% binding) or presence of increasing concentrations of the steroids (50–600 nM). Picrotoxin (2 mM) was used to determine non-specific binding. Assays were carried out at 22 °C for 2 h in the presence of 5 μM GABA. Calculated IC_{50} values are: **1**, 92.8±14.1 nM; **6**, 171.2±39.2 nM; **15**, 241.1±97.0 nM; **16**, 200.3±37.1 nM.

stimulated in a similar way the binding of [^{3}H]-flunitrazepam and affected [^{3}H]-muscimol binding as well, rendering comparable results to allopregnanolone. Complete biological results will be published in a separate paper.

3. Conclusions

The first 6-thia analog of a neurosteroid and its oxidized derivatives 6-sulfoxide and 6-sulfone, were synthesized from

pregnenolone in 8.1, 7.0, and 7.3% yield, respectively. The key reduction of the intermediate hemithioketal to give the trans-fused A/B rings in the steroidal framework was accomplished stereospecifically, its stereochemical outcome being consistent with the intermediacy of a pyranosic thiocarbenium ion. Furthermore compound **13** is a synthetic precursor of C-19 substituted analogs. $GABA_A$ receptor activity for 6-thia-allopregnanolone (**6**) was similar to that of allopregnanolone (**1**) giving further support to our assumption that the decrease in activity observed for the 6-oxa and 6-aza analogs is related to their hydrogen bonding acceptor and/or donor properties at position 6. The small change in activity observed upon oxidation to the sulfoxide and sulfone analogs (**15** and **16**) indicates that lipophylicity and electrostatic potential changes in the vicinity of position 6 are not critical for $GABA_A$ receptor activity.

4. Experimental

4.1. General experimental procedures

Melting points were determined on a Fisher Johns apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions in a Bruker AC-200 NMR spectrometer at 200.13 (^1H) and 50.32 (^{13}C) MHz or a Bruker AM-500 at 500.13 (^1H) and 125.77 (^{13}C) MHz. NMR assignments are based on multiplicity determinations (DEPT) and 2D spectra (COSY, HETCOR, HMBC, HSQC, and NOESY) are obtained using standard Bruker software. Chemical shifts are given in parts per million (δ) downfield from TMS internal standard. IR spectra were measured as thin films (from dichloromethane solution) on KBr disks, using a FT-IR Nicolet Magna 550 spectrophotometer, wave-numbers are given in cm^{-1}. The electron impact mass spectra were obtained at 70 eV by direct inlet in a Shimadzu QP 5000 mass spectrometer. Thin layer chromatography was performed on silica gel 60 plates with fluorescent indicator. The plates were visualized with a 3.5% solution of phosphomolybdic acid in ethanol. Column chromatography was conducted on silica gel (230–400 mesh) or octadecyl-functionalized silica gel. All solvents were distilled and stored over 4 Å molecular sieves before use. Solvents were evaporated at 45 °C under vacuum. High-resolution mass spectra were measured at the Mass Spectrometry Facility of the Chemistry Department, University of California at Riverside. Pregnenolone was purchased from Steraloids Inc. (USA). All new compounds were determined to be >95% pure by ^1H NMR spectroscopy.

4.1.1. 3β-*tert*-Butyldimethylsilyloxy-5α,6β-dihydroxypregnan-20-one (8). A suspension of pregnenolone (**7**, 3.0 g, 9.48 mmol) in 88% formic acid (33 mL) was heated to 70–80 °C with stirring for 5 min and then cooled to 25 °C. The resulting thick slurry containing the 3-formate, was treated with 30% hydrogen peroxide (3.4 mL) keeping the temperature at 40 °C by cooling. After 20 min a pink solution resulted and stirring was continued at ca. 20 °C for 20 h. The reaction mixture was treated with boiling water (50 mL) with stirring, allowed to cool and the white solid was collected and dried. The solid was dissolved in methanol (100 mL) and the solution was treated with 40% sodium hydroxide (5.5 mL) at 0 °C and allowed to reach 25 °C. After 45 min the solution was neutralized with 2 N HCl (27 mL) and diluted with cold water (50 mL). 3β,5α,6β-Trihydroxypregnan-20-one precipitated as a white solid after cooling; it was collected, washed with cold water and dried under vacuum (3.34 g). The triol was dissolved in anhydrous DMF (45 mL) under nitrogen, imidazole (1.94 g, 28.50 mmol) and *tert*-butyldimethylsilyl chloride (2.87 g, 19.03 mmol) were added at 0 °C and the mixture was allowed to reach 25 °C. After 2 h the solution was poured into saturated aqueous NaCl (60 mL), extracted with diethyl ether (3×20 mL), dried with sodium sulfate and evaporated under vacuum. The white solid obtained was purified by flash chromatography on silica gel using hexane/ethyl acetate (90:10), to give the title compound **8** (3.17 g, 72% from **7**). Mp 202–203 °C (hexane/ethyl acetate) [found: C, 69.9, H, 10.5. $C_{27}H_{48}O_4Si$ requires: C, 69.78, H, 10.41]; ν_{max} 3479, 2937, 2860, 1697, 1466, 1359, 1251, 1077, 871, 834, 776; δ_H (500 MHz) 0.05 (6H, s, TBDMS–H), 0.63 (3H, s, 18-H), 0.87 (9H, s, TBDMS–H), 1.18 (3H, s, 19-H), 2.11 (3H, s, 21-H), 2.53 (1H, t, J=8.9 Hz, 17-H), 3.52 (1H, br s, 6-H), 4.07 (1H, m, 3-H); δ_C (50 MHz) −4.6 (CH_3Si), −4.5 (CH_3Si), 13.5 (C-18), 16.8 (C-19), 18.1 ($SiC(CH_3)_3$), 21.1 (C-11), 22.8 (C-16), 24.2 (C-15), 25.9 ($C(CH_3)_3$), 30.3 (C-8), 31.1 (C-2), 31.5 (C-21), 32.4 (C-1), 34.3 (C-7), 38.3 (C-10), 39.0 (C-12), 41.3 (C-4), 44.3 (C-13), 45.7 (C-9), 56.1 (C-14), 63.7 (C-17), 68.1 (C-3), 75.9 (C-6), 76.1 (C-5), 209.6 (C-20); *m/z* (EI) 465 (M+H, 0.1), 447 (M+H−H_2O, 0.2), 389 (2), 315 (24), 297 (30), 279 (8).

4.1.2. 3β-*tert*-Butyldimethylsilyloxy-19-formyloxy-7-iodo-6-nor-5,7-secopregnane-5,20-dione (9). To a solution of diol **8** (1.00 g, 2.15 mmol) in dry dichloromethane (85.0 mL) in a water-jacketed vessel, were added recently distilled carbon tetrachloride (85.0 mL), mercury(II) oxide (1.22 g, 4.73 mmol), and iodine (1.77 g, 7.00 mmol) under nitrogen. The solution was vigorously stirred and irradiated with two 300 W tungsten lamps (5000 lm each) at 25 °C. Three additional portions of mercury(II) oxide (1.22 g, 4.73 mmol) and iodine (1.77 g, 7.00 mmol) were added at intervals of 1 h. After 4.5 h the solution was diluted with dichloromethane (85.0 mL) and filtered. The organic layer was washed with a saturated solution of sodium thiosulfate (60 mL) and water (60 mL), dried, and evaporated under reduced pressure. The oily residue was purified by flash chromatography on octadecyl-functionalized silica gel using methanol/water (70:30) to give the title compound as a foamy yellow solid (0.85 g, 65%). ν_{max} 3488, 2950, 2887, 2858, 1726, 1700, 1467, 1360, 1179, 1052, 837, 778, 738, 700; δ_H (500 MHz) 0.03 (3H, s, TBDMS–H), 0.06 (3H, s, TBDMS–H), 0.69 (3H, s, 18-H), 0.86 (9H, s, TBDMS–H), 0.90 (1H, m, 8-H), 1.13 (1H, m, 15β-H), 1.39 (1H, m, 14-H), 1.48 (1H, m, 12α-H), 1.69 (1H, m, 2β-H), 1.75 (2H, m, 15α-H and 16α-H), 1.81–1.88 (4H, m, 11β-H, 2α-H, 9-H and 11α-H), 2.04 (1H, m, 1α-H), 2.09 (1H, m, 12β-H), 2.11 (1H, m, 1β-H), 2.12 (3H, s, 21-H), 2.15 (1H, m, 16β-H), 2.41 (1H, dt, J=13.9, 2.8 Hz, 4α-H), 2.57 (1H, t, J=9.2 Hz, 17-H), 3.14 (1H, dd, J=10.8, 2.8 Hz, 7a-H), 3.45 (1H, dd, J=10.8, 1.8 Hz, 7b-H), 3.49 (1H, dd, J=13.9, 3.5 Hz, 4β-H), 4.32 (1H, d, J=11.8 Hz, 19a-H), 4.45 (1H, m, 3α-H), 4.45 (1H, d, J=11.8 Hz, 19b-H), 8.09 (1H, s, formate); ^{13}C NMR (125 MHz) δ: −5.0 (C–CH_3Si), −4.9 (C–CH_3Si), 14.0 (C-18), 17.4 (C-7), 17.9 (C–CSi), 22.4 (C-16), 23.3 (C-11), 23.6 (C-15), 25.6 (C–CH_3), 27.4 (C-1), 28.8 (C-2), 31.3 (C-21), 37.6 (C-8), 38.6

(C-12), 39.6 (C-9), 43.1 (C-13), 49.1 (C-4), 53.7 (C-10), 55.4 (C-14), 63.2 (C-17), 65.2 (C-19), 70.4 (C-3), 160.8 (C-formate), 208.8 (C-20), 212.9 (C-5); *m/z* (FAB) 627 (M+Na$^+$, 11), 477 (9), 461 (100), 329 (40); HRMS (FAB) found 627.1980, $C_{27}H_{45}IO_5SiNa$ requires 627.1979.

4.1.3. 3β-*tert*-Butyldimethylsilyloxy-19-formyloxy-7-acetylsulfanyl-6-nor-5,7-seco-pregnane-5,20-dione (10). A mixture of secosteroid **7** (0.65 g, 1.08 mmol) and potassium thioacetate (0.37 g, 3.23 mmol) in dry acetone (33 mL) was stirred at room temperature for 3 h under nitrogen. The reaction mixture was diluted with dichloromethane (70 mL), filtered and the solvent was evaporated. The residue was purified by flash chromatography on silica gel with an hexane/ethyl acetate gradient to give thioacetate **10** (0.42 g, 70%) as a vitreous solid; ν_{max} 2955, 2932, 2894, 2857, 1725, 1700, 1464, 1357, 1174, 1128, 1041, 835; δ_H (500 MHz) 0.03 (3H, s, TBDMS–H), 0.05 (3H, s, TBDMS–H), 0.65 (3H, s, 18-H), 0.85 (9H, s, TBDMS–H), 1.23 (1H, m, 15β-H), 1.41-1.43 (2H, m, 14-H and 12α-H), 1.62 (1H, m, 15α-H), 1.67–1.69 (3H, m, 2β-H, 16α-H and 11β-H), 1.81 (1H, m, 11α-H), 1.88–1.91 (2H, m, 9-H and 2α-H), 1.98–1.99 (2H, m, 8-H and 1α-H), 2.08 (2H, m, 1β-H and 12β-H), 2.11 (3H, s, 21-H), 2.12 (1H, m, 16β-H), 2.30 (3H, s, CH_3COS), 2.36 (1H, dt, *J*=13.8, 2.8 Hz, 4α-H), 2.50 (1H, t, *J*=9.2 Hz, 17-H), 2.95 (1H, dd, *J*=13.9, 2.8 Hz, 7a-H), 3.05 (1H, dd, *J*=13.9, 3.0 Hz, 7b-H), 3.14 (1H, dd, *J*=13.8, 3.5 Hz, 4β-H), 4.36 (1H, d, *J*=11.8 Hz, 19a-H), 4.43 (1H, m, 3α-H), 4.48 (1H, d, *J*=11.8 Hz, 19b-H), 8.08 (1H, s, formate). δ_C (125 MHz) −5.0 (CH_3Si), −4.9 (CH_3Si), 13.1 (C-18), 17.9 (Si*C*$(CH_3)_3$), 22.6 (C-16), 23.7 (C-11), 24.4 (C-15), 25.6 (C(*C*$H_3)_3$), 27.5 (C-1), 28.8 (C-2), 30.9 (*C*H_3COS), 31.2 (C-21), 31.5 (C-7), 36.7 (C-8), 38.8 (C-12), 41.1 (C-9), 43.5 (C-13), 48.0 (C-4), 53.5 (C-14), 54.0 (C-10), 63.4 (C-17), 65.6 (C-19), 70.4 (C-3), 160.8 (formate), 193.8 (CH_3*C*OS), 208.8 (C-20), 211.9 (C-5); *m/z* (FAB) 575 (M+Na$^+$, 100), 494 (30), 493 (83), 441 (9), 419 (35), 361 (26); HRMS (FAB) found 575.2810, $C_{29}H_{48}O_6SSiNa$ requires 575.2839.

4.1.4. 3β-Hydroxy-19-formyloxy-6-thia-5α-pregnan-20-one (12). To a solution of thioacetate **10** (0.40 g, 0.72 mmol) in methanol (40.0 mL) was added 10% aqueous sodium hydroxide (10 mL) at 0 °C under nitrogen. The reaction mixture was warmed to 25 °C; after 3 h the solution was neutralized with 1 N hydrochloric acid (20 mL) and evaporated under vacuum to a fifth of its original volume. The remaining solution was diluted with dichloromethane (50 mL), washed with brine (20 mL), and evaporated to dryness. Purification on a silica gel column using a gradient of hexane/ethyl acetate gave hemithioketal **11** (5α/5β 9:1; 0.318 g, 91%). Data for the 5α-hydroxy isomer: δ_H (200 MHz) 0.05 (6H, s, TBDMS–H), 0.68 (3H, s, 18-H), 0.87 (9H, s, TBDMS–H), 2.11 (3H, s, 21-H), 2.35 (1H, dd, *J*=12.8, 4.0 Hz, 7α-H), 2.53 (1H, t, *J*=8.8 Hz, 17-H), 2.84 (1H, t, *J*=12.8 Hz, 7β-H), 3.71 (1H, d, *J*=12.6 Hz, 19a-H), 4.10 (1H, m, 3α-H), 4.25 (1H, d, *J*=12.6 Hz, 19b-H); δ_C (50 MHz) −4.7 and −4.6 (C–CH_3Si), 13.8 (C-18), 18.1 (Si*C*$(CH_3)_3$), 22.1 (C-16), 22.1 (C-11), 22.4 (C-15), 25.8 (C(*C*$H_3)_3$), 28.1 (C-2), 29.1 (C-1), 31.4 (C-21), 31.6 (C-7), 37.1 (C-8), 39.4 (C-12), 43.5 (C-13), 44.5 (C-10), 45.4 (C-9), 45.8 (C-4), 56.2 (C-14), 63.6 (C-17), 64.1 (C-19), 67.0 (C-3), 81.9 (C-5), 209.2 (C-20).

To a solution of the hemithioketal **11** obtained above (0.318 g, 0.659 mmol) in dry pyridine (14.2 mL) was added recently prepared formic acetic anhydride[15] (8.25 mL) at 25 °C under nitrogen. After 2 h the solution was poured into cold 2 N HCl (50 mL), extracted with dichloromethane (3×15 mL), dried with sodium sulfate, and the solvent was evaporated. The residue was dissolved in dry dichloromethane (50 mL) and triethylsilane (1.05 mL, 6.6 mmol) and $BF_3\cdot Et_2O$ (0.85 mL, 6.6 mmol) were added at −15 °C under nitrogen. After 1 h, cold water (20 mL) was added followed by solid sodium bicarbonate until neutral. The solution was washed with saturated sodium bicarbonate (20 mL) and brine (20 mL), dried with sodium sulfate, and the solvent was evaporated. Purification by column chromatography on silica gel with a gradient of hexane/ethyl acetate gave the 6-thiapregnane **12** (0.157 g, 64% from **10**). Mp 161–162 °C (hexane/ethyl acetate); ν_{max} 3407, 2937, 2869, 1715, 1360, 1174, 1060; δ_H (500 MHz) 0.64 (3H, s, 18-H), 0.89 (1H, td, *J*=11.4, 3.7 Hz, 9-H), 0.98 (1H, td, *J*=13.9, 3.6 Hz, 1α-H), 1.18 (1H, m, 14-H), 1.26–1.33 (2H, m, 15β-H and 12α-H), 1.38 (1H, m, 2β-H), 1.50 (1H, m, 11β-H), 1.55 (1H, m, 4α-H), 1.67–1.70 (2H, m, 16α-H and 15α-H), 1.77 (1H, ddd, *J*=13.9, 7.0, 3.7 Hz, 11α-H), 1.86–1.88 (1H, m, 8-H and 2α-H), 2.00–2.02 (1H, m, 4β-H and 12β-H), 2.10 (3H, s, 21-H), 2.19 (1H, m, 16β-H), 2.33 (1H, dt, *J*=14.0, 3.5 Hz, 1β-H), 2.41 (1H, dd, *J*=13.2, 11.3 Hz, 7α-H), 2.49 (1H, t, *J*=9.0 Hz, 17-H), 2.55 (1H, dd, *J*=13.2, 3.6 Hz, 7β-H), 2.72 (1H, dd, *J*=13.4, 3.6 Hz, 5α-H), 3.69 (1H, m, 3α-H), 4.48 (1H, d, *J*=12.8 Hz, 19a-H), 4.75 (1H, d, *J*=12.8 Hz, 19b-H), 8.12 (1H, s, formate); δ_C (125 MHz) 13.4 (C-18), 22.3 (C-11), 22.7 (C-16), 24.3 (C-15), 31.2 (C-1), 31.3 (C-2), 31.3 (C-21), 34.5 (C-7), 37.3 (C-4), 37.3 (C-8), 38.5 (C-10), 39.1 (C-12), 44.0 (C-13), 48.8 (C-5), 54.5 (C-9), 55.8 (C-14), 62.4 (C-19), 63.5 (C-17), 69.9 (C-3), 160.9 (formate), 208.9 (C-20); *m/z* (EI) 380 (M$^+$, 33), 335 (M−HCOOH, 24), 321 (4), 251 (8), 93 (55), 79 (82); HRMS (EI) found 380.2035, $C_{21}H_{32}O_4S$ requires 380.2021.

4.1.5. 3α-Benzoyloxy-19-hydroxy-6-thia-5α-pregnan-20-one (13). To a solution of 6-thiapregnane **12** (0.125 g, 0.328 mmol) in dry THF (5.0 mL), were added triphenylphosphine (0.258 g, 0.984 mmol), benzoic acid (0.094 g, 0.770 mmol), and DEAD (0.090 mL, 0.659 mmol) at 25 °C under nitrogen. The mixture was stirred for 18 h and the THF was evaporated under vacuum. The residue was purified by column chromatography on silica gel (cyclohexane/ethyl acetate), dissolved in methanol (26 mL) and 6 N HCl (5.9 mL, 35.8 mmol) added at 0 °C. The solution was allowed to reach 25 °C under nitrogen, after 1 h a saturated solution of potassium bicarbonate was added until neutral, the methanol was evaporated to a fifth of the original volume and the mixture was poured into dichloromethane (30 mL), washed with brine (1 mL), dried with sodium sulfate, and the solvent was evaporated. The light yellow solid was purified by column chromatography on silica gel using a gradient of hexane/ethyl acetate, to give the title compound **13** (0.140 g, 93%) as a white solid. Mp 86–88 °C (hexane/ethyl acetate) [found: C, 70.8, H, 8.2. $C_{27}H_{36}O_4S$ requires: C, 71.02, H, 7.95]; ν_{max} 3496, 2945, 2873, 1707, 1449, 1353, 1112, 715; δ_H (500 MHz) 0.72 (3H, s, 18-H), 0.99 (1H, td, *J*=11.4, 3.7 Hz, 9-H), 1.21 (1H, m, 14-H), 1.28–1.35 (2H, m, 15β-H and 1α-H), 1.43 (1H, td, *J*=12.8, 4.1 Hz,

12α-H), 1.67–1.72 (3H, m, 16α-H, 15α-H and 11β-H), 1.79–1.82 (2H, m, 2β-H and 11α-H), 1.93 (2H, m, 1β-H and 2α-H), 2.06–2.07 (2H, m, 4β-H and 12β-H), 2.11 (1H, m, 4α-H), 2.12 (3H, s, 21-H), 2.20 (1H, m, 16β-H), 2.28 (1H, qd, J=10.9, 3.8 Hz, 8-H), 2.50 (1H, dd, J=13.1, 11.4 Hz, 7α-H), 2.52 (1H, t, J=9.2 Hz, 17-H), 2.61 (1H, dd, J=13.1, 3.8 Hz, 7β-H), 3.16 (1H, dd, J=13.1, 3.8 Hz, 5α-H), 3.83 (1H, dd, J=12.2, 7.0 Hz, 19a-H), 4.34 (1H, d, J=12.2 Hz, 19b-H), 5.35 (1H, m, 3β-H), 7.46 (2H, m, *meta*-ArH), 7.59 (1H, m, *para*-ArH), 8.06 (2H, m, *ortho*-ArH); δ_C (50 MHz) 13.6 (C-18), 21.6 (C-11), 22.5 (C-16), 24.0 (C-15), 26.3 (C-2), 29.3 (C-1), 31.3 (C-21), 32.1 (C-4), 34.2 (C-7), 38.1 (C-8), 39.0 (C-12), 39.5 (C-10), 44.2 (C-13), 45.8 (C-5), 54.7 (C-9), 56.3 (C-14), 62.5 (C-19), 63.4 (C-17), 69.5 (C-3), 128.3 (*meta*-phenyl), 129.4 (*ortho*-phenyl), 130.5 (*ipso*-phenyl), 132.9 (*para*-phenyl), 165.5 (Ph*C*OO), 209.2 (C-20); *m*/*z* (EI) 456 (M^+, 10), 334 (61), 316 (5), 316 (5), 304 (25), 303 (24), 105 (41).

4.1.6. 3α-Hydroxy-6-thia-5α-pregnan-20-one (6). To a solution of 3α-benzoate **13** (0.127 g, 0.279 mmol) in dry dichloromethane (6.4 mL), were added thiocarbonyldiimidazole (0.253 g, 1.40 mmol) and 4-dimethylaminopyridine (0.002 g, 0.015 mmol) and the solution was refluxed under nitrogen for 5 h. The reaction mixture was evaporated to dryness and purified by column chromatography on silica gel with hexane/ethyl acetate (8:2) to give the intermediate 19-imidazoylthionocarbonate as a yellow solid (0.136 g). The solid was dissolved in anhydrous toluene (6.6 mL) and heated to 115 °C under nitrogen, diphenylsilane (0.265 mL, 1.450 mmol) was added followed by 18 aliquots (0.05 mL each) of a solution of AIBN in anhydrous toluene (0.158 g/mL, 1.8 equiv) at 15 min intervals. The solvent was evaporated and the residue was purified by column chromatography on silica gel with hexane/ethyl acetate as eluent to give an oily fraction of **14** containing residual diphenylsilane that could not be separated; δ_H (500 MHz) 0.65 (3H, s, 18-H), 1.09 (3H, s, 19-H), 2.11 (3H, s, 21-H), 2.44 (1H, dd, J=13.2, 12.2 Hz, 7α-H), 2.51 (1H, t, J=9.3 Hz, 17-H), 2.53 (1H, dd, J=13.2, 3.8 Hz, 7β-H), 3.09 (1H, dd, J=13.4, 3.5 Hz, 5α-H), 5.29 (1H, m, 3β-H), 7.47 (2H, m, *meta*-ArH), 7.58 (1H, m, *para*-ArH), 8.06 (2H, m, *ortho*-ArH); δ_C (125 MHz) 11.5 (C-19), 13.2 (C-18), 21.0 (C-11), 22.7 (C-16), 24.2 (C-15), 25.9 (C-2), 31.3 (C-21), 31.9 (C-1), 31.9 (C-4), 34.3 (C-7), 36.7 (C-8), 36.9 (C-10), 38.8 (C-12), 43.9 (C-13), 46.6 (C-5), 54.1 (C-9), 55.5 (C-14), 63.5 (C-17), 69.7 (C-3), 128.3 (*meta*-phenyl), 129.5 (*ortho*-phenyl), 130.7 (*ipso*-phenyl), 132.8 (*para*-phenyl), 165.5 (Ph*C*OO), 209.0 (C-20).

To a solution of the crude fraction obtained above containing **14**, in dry DMF (5.0 mL), was added sodium methanethiolate (0.254 g, 3.63 mmol) and the mixture was heated for 2 h at 100 °C under nitrogen. The resulting solution was cooled, poured into brine (10 mL), and extracted with ethyl ether (30 mL). The organic layer was washed with brine (2×10 mL), dried with sodium sulfate, and the solvent was evaporated. The yellowish solid was purified by column chromatography on silica gel using hexane/ethyl acetate (8:2 to 1:1) to give the title compound **6** as a white solid (0.057 g, 61% from **13**). Mp 174–175 °C (hexane/ethyl acetate); ν_{max} 3430, 2939, 2875, 1696, 1426, 1359, 1010, 752; δ_H (500 MHz) 0.63 (3H, s, 18-H), 0.90 (1H, m, 9-H), 1.03 (3H, s, 19-H), 1.17 (1H, m, 14-H), 1.25 (1H, m, 15β-H), 1.33 (1H, m, 11β-H), 1.40 (2H, m, 1α-H and 12α-H), 1.64–1.65 (3H, m, 1β-H, 4α-H and 4β-H), 1.66–1.67 (1H, m, 16α-H and 2α-H), 1.70–1.74 (4H, m, 15α-H, 2β-H, 11α-H and 8-H), 2.06 (1H, br d, J=11.6 Hz, 12β-H), 2.11 (3H, s, 21-H), 2.17 (1H, m, 16β-H), 2.45 (1H, dd, J=13.1, 11.2 Hz, 7α-H), 2.55 (1H, dd, J=13.1, 3.8 Hz, 7β-H), 2.56 (1H, t, J=9.3 Hz, 17-H), 3.12 (1H, dd, J=10.4, 6.7 Hz, 5α-H), 4.08 (1H, m, 3β-H); δ_C (125 MHz) 11.4 (C-19), 13.2 (C-18), 21.0 (C-11), 22.7 (C-16), 24.3 (C-15), 28.5 (C-2), 31.0 (C-1), 31.4 (C-21), 34.3 (C-7), 34.9 (C-4), 36.9 (C-8), 37.1 (C-10), 38.8 (C-12), 44.0 (C-13), 45.5 (C-5), 54.1 (C-9), 56.7 (C-14), 63.6 (C-17), 65.8 (C-3), 209.2 (C-20); *m*/*z* (EI) 336 (M^+, 5), 318 ($M-H_2O$, 10), 303 (2), 251 (2), 207 (1); HRMS (EI) found 336.2131, $C_{20}H_{32}O_2S$ requires 336.2123.

4.1.7. *S*-Oxo-3α-hydroxy-6-thia-5α-pregnan-20-one (15). To a solution of 6-thiapregnane **6** (0.009 g, 0.027 mmol) in methanol (1.0 mL) cooled to 0 °C, was added a suspension of Oxone® (0.011 g, 0.018 mmol) in water (0.8 mL). After 5 min, a saturated solution of sodium bisulfite (1.0 mL) was added, the methanol was evaporated and the resulting mixture was extracted with ethyl ether (10 mL). The residue obtained after evaporation of the solvent was purified by preparative TLC (dichloromethane/methanol 20:1) to give sulfoxide **15** (0.0082 g, 87%). Mp 182–183 °C (hexane/ethyl acetate); ν_{max} 3382, 2940, 2863, 1701, 1429, 1359, 1014, 756; δ_H (500 MHz) 0.64 (3H, s, 18-H), 0.95 (3H, s, 19-H), 1.15 (1H, td, J=11.4, 4.1 Hz, 9-H), 1.30 (1H, m, 11β-H), 1.31 (1H, m, 15β-H), 1.39 (1H, m, 14-H), 1.42 (1H, m, 12α-H), 1.50 (1H, m, 1α-H), 1.57 (1H, dd, J=13.1, 4.0 Hz, 1β-H), 1.64 (1H, m, 2β-H), 1.72–1.74 (4H, m, 2α-H, 11α-H, 16α-H and 15α-H), 1.78–1.79 (1H, m, 4β-H and 8-H), 2.05 (1H, br d, J=12.2 Hz, 12β-H), 2.12 (3H, s, 21-H), 2.19 (1H, m, 16β-H), 2.35 (1H, t, J=12.2 Hz, 7α-H), 2.36 (1H, m, 4α-H), 2.55 (1H, t, J=8.9 Hz, 17-H), 2.89 (1H, dd, J=13.2, 3.7 Hz, 5α-H), 3.41 (1H, dd, J=11.6, 2.8 Hz, 7β-H), 4.23 (1H, m, 3β-H); δ_C (125 MHz) 13.1 (C-19), 13.2 (C-18), 20.7 (C-11), 22.7 (C-16), 24.3 (C-15), 27.6 (C-2), 29.1 (C-4), 31.4 (C-21), 32.0 (C-1), 33.5 (C-8), 38.3 (C-12), 38.9 (C-10), 43.9 (C-13), 53.4 (C-9), 55.4 (C-14), 56.4 (C-7), 63.3 (C-17), 64.3 (C-3), 64.7 (C-5), 208.7 (C-20); MS (EI) *m*/*z* (%): 352 (M^+, 0.5), 318 (1), 298 (1), 173 (4), 121 (13); HRMS (EI) found 352.2070, $C_{20}H_{32}O_3S$ requires 352.2072.

4.1.8. *S,S*-Dioxo-3α-hydroxy-6-thia-5α-pregnan-20-one (16). To a solution of 6-thiapregnane **6** (0.0116 g, 0.0416 mmol) in methanol (1.3 mL) cooled to 0 °C, was added a suspension of Oxone® (0.038 g, 0.062 mmol) in water (1.0 mL). The reaction mixture was allowed to reach 25 °C and after 5 h a saturated solution of sodium bisulfite (1.3 mL) was added, the methanol was evaporated, and the residue was extracted with ethyl ether (10 mL). The residue obtained after evaporation of the solvent was purified by preparative TLC (dichloromethane/methanol 20:1) to give the sulfone **16** (0.0114 g, 90%). Mp 187–188 °C (hexane/ethyl acetate); ν_{max} 3491, 2947, 2865, 1699, 1289, 1131, 1012, 903; δ_H (500 MHz) 0.67 (3H, s, 18-H), 1.15 (1H, m, 9-H), 1.16 (3H, s, 19-H), 1.31–1.32 (2H, m, 14-H and 15β-H), 1.43 (2H, m, 11β-H and 12α-H), 1.53 (1H, m, 1α-H), 1.59 (1H, m, 1β-H), 1.67 (1H, m, 15β-H), 1.70 (2H, m, 2α-H and 2β-H), 1.73 (1H, m, 16α-H), 1.77 (1H, m, 11α-H),

1.93 (1H, m, 4β-H), 2.07 (1H, m, 12β-H), 2.12 (3H, s, 21-H), 2.20 (2H, m, 8-H and 16β-H), 2.24 (1H, m, 4α-H), 2.54 (1H, t, J=9.2 Hz, 17-H), 2.66 (1H, t, J=13.9 Hz, 7α-H), 3.08 (1H, dd, J=13.9, 3.4 Hz, 7β-H), 3.24 (1H, dd, J=12.9, 2.6 Hz, 5α-H), 4.28 (1H, m, 3β-H); δ_C (125 MHz) 12.2 (C-19), 13.1 (C-18), 20.8 (C-11), 22.6 (C-16), 23.9 (C-4), 24.1 (C-15), 27.7 (C-2), 31.4 (C-21), 32.9 (C-1), 34.2 (C-8), 38.2 (C-12), 39.4 (C-10), 43.8 (C-13), 52.5 (C-9), 54.8 (C-14), 56.2 (C-7), 61.2 (C-5), 63.2 (C-17), 64.2 (C-3), 208.5 (C-20); *m/z* (EI) 368 (M^+, 0.3), 350 ($M-H_2O$, 0.3), 298 (1), 207 (8), 121 (4), 44 (100); HRMS (EI) found 368.2016, $C_{20}H_{32}O_4S$ requires 368.2021.

4.2. Biological activity assays

4.2.1. Membrane preparation. Whole cerebellum from male Sprague–Dawley rats (200–250 g) was rapidly removed after sacrifice and stored at −80 °C. The material was thawed and homogenized in 5 vol (v/w) of ice-cold 0.32 M sucrose, using a Teflon-glass homogenizer at 1200 rpm. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C. The supernatant was carefully decanted and centrifuged for 20 min at 15,000*g* at 4 °C. The pellet was washed twice with 50 mM Tris–HCl buffer (pH 7.4) followed by centrifugation for 20 min at 15,000*g* at 4 °C. The final pellet was suspended in 1.2 mL of the same buffer and frozen at −20 °C. On the days of the assays, membranes were thawed, centrifuged for 20 min at 15,000*g* at 4 °C, and the pellet was washed twice with 100 vol of the corresponding ice-cold buffer by centrifugation (15,000*g*, 20 min). The final pellet was suspended in the incubation buffer to a protein concentration of approximately 8 mg/mL.[16]

4.2.2. [^{35}S]-*tert*-Butylbicyclo-phosporothionate ([^{35}S]-TBPS) binding. Binding assays were carried out using a previously described protocol with some modifications.[13] Aliquots (100 μL) of cerebellum membrane preparation were incubated with 10 nM [^{35}S]-TBPS (65.13 Ci/mmol, Perking Elmer Life Science Inc., Boston, MA) in the absence or presence of increasing concentration of the steroids (50–600 nM). The synthetic steroids and allopregnanolone, used as control, were dissolved in DMSO and diluted with the incubation buffer (1:1000; 50 mM Tris–HCl, 200 mM NaCl, pH 7.4) immediately before use; 2 mM picrotoxin was used to determine non-specific binding. Assays were carried out at 22 °C for 2 h in the presence of 5 μM GABA (Sigma–Aldrich Corp.) and terminated by rapid filtration through a glass fiber filter (Number 30, Schleicher & Schuell Inc., Keene, NH). Filter bound radioactivity was quantified by liquid scintillation spectrophotometry. IC_{50} (concentration at which half-maximal inhibition of control binding occurs) values were determined by linear computerized regression analysis after logit/log transformation.[17]

4.2.3. [^{3}H]-Flunitrazepam ([^{3}H]-FLU) binding. Aliquots (100 μL) of cerebellum membrane preparation were incubated with 3 nM [^{3}H]-FLU (85.2 Ci/mmol, Perking Elmer Life Science Inc., Boston, MA) in the absence or presence of increasing concentration of the steroids (50–600 nM). Allopregnanolone, was used as a standard and 1 mM diazepam (Roemmers Lab, Buenos Aires) was used as non-specific binding.[18] Incubations were carried out at 4 °C for 90 min in 50 mM Tris–HCl buffer (pH 7.4) in the absence of GABA and terminated by rapid filtration through a glass fiber filter as above.

4.2.4. [^{3}H]-Muscimol ([^{3}H]-Mus) binding. Aliquots (100 μL) of washed cerebellum membrane preparation in Tris–acetate 50 mM buffer pH 6.1 were incubated with 10 nM [^{3}H]-Mus (18.0 Ci/mmol, Perkin Elmer Life Science Inc., Boston, MA) in the absence or presence of increasing concentrations of the steroids (50–600 nM); 1 mM GABA (Sigma–Aldrich Corp.) was used to determine non-specific binding. Incubations were carried out at 4 °C for 60 min in the absence of GABA and terminated by rapid filtration through glass fiber filter as above.

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Electrooxidation of alcohols in an *N*-oxyl-immobilized rigid network polymer particles/water disperse system

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Abstract—The electrooxidation of alcohols in an aqueous disperse system with *N*-oxyl-immobilized poly(*p*-phenylene benzobisthiazole) network polymer particles (*PBZT$_{NT}$-N-Oxyl*) as a disperse phase was performed successfully in a simple beaker-type undivided cell under a constant current condition to afford the corresponding ketones, aldehydes, and/or carboxylic acid in moderate to good yields. Recycle use of both the *PBZT$_{NT}$-N-Oxyl* particles and the aqueous media could be achieved successfully by immobilization of additional *N*-oxyl moiety on the polymer particles in an appropriate interval. Notably, the shape and the particle size of *PBZT$_{NT}$-N-Oxyl* were not appreciably changed even after 60 times recycle use.

1. Introduction

Electrooxidation of alcohols mediated with *N*-oxyl compounds has been intensively investigated as a prominent tool for organic synthesis. The *N*-oxyl-mediated electrooxidation has been mainly carried out in polar organic solvents, such as acetonitrile or dichloromethane, containing rather high concentration of supporting electrolytes under a regulated potential condition in a divided cell.[1] The procedure is not necessarily satisfactory in terms of operational simplicity, manufacturing cost, and environmental stress. Torii and co-workers have developed an organic/aqueous two-phase system,[2] e.g., CH_2Cl_2/H_2O, in which the electrooxidation of alcohols is performed successfully by use of a simple beaker-type undivided cell under a constant current condition; hence, the operations are remarkably simple. However, there still remain serious problems in terms of environmental stress arising from the use of the harmful organic solvents, e.g., CH_2Cl_2.

In the last decade, various kinds of solid-supported reagents and catalysts have been developed for the oxidation of alcohols.[3] For example, *N*-oxyl-immobilized polymer particles[4] and silica gel[5] were prepared and employed as solid-supported catalysts. In this concern, we developed the electrooxidation of alcohols in a disperse system with *N*-oxyl-immobilized polymer particles, e.g., polyethylene[6] and poly(ethylene-*co*-acrylic acid),[7] or silica gel[8] as a disperse phase and an aqueous 20 wt % NaBr/satd $NaHCO_3$ solution as a disperse medium, in which most of alcohols would be adsorbed on the disperse phase and oxidize on the water/solid interface. Recycle use of the disperse phase and the aqueous disperse media was also investigated to offer a formally closed oxidation system. The disperse phases, e.g., polyethylene and poly(ethylene-*co*-acrylic acid) particles, and silica gel, however, suffered gradual degradation during the course of the recycle use. After repeating the use of the *N*-oxyl-immobilized polymer particles for several times, significant degradation into fine pieces brought about difficulty in the separation of the solid particles and the aqueous disperse media. In a practical sense, therefore, mechanically more tough solid particles (disperse phase) are desirable. In our continuing studies, we investigated the use of poly(*p*-phenylene benzobisthiazole) network polymer (PBZT$_{NT}$,[9] Fig. 1) as the disperse phase. This network polymer is constituted with

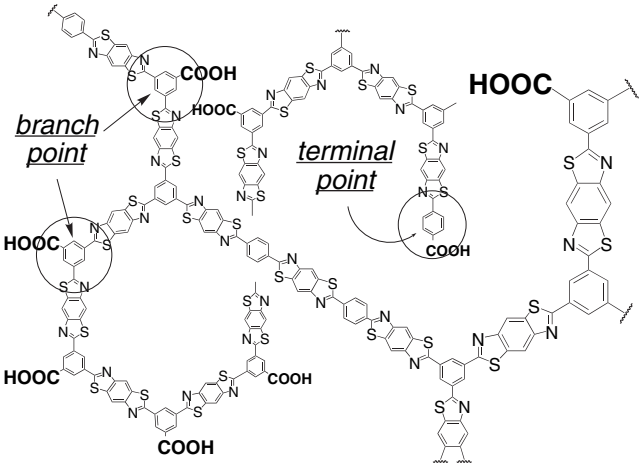

Figure 1. Structure of PBZT$_{NT}$.

Keywords: Electrooxidation; Alcohols; Polymer-supported *N*-oxyl; Disperse system; Aqueous solution.
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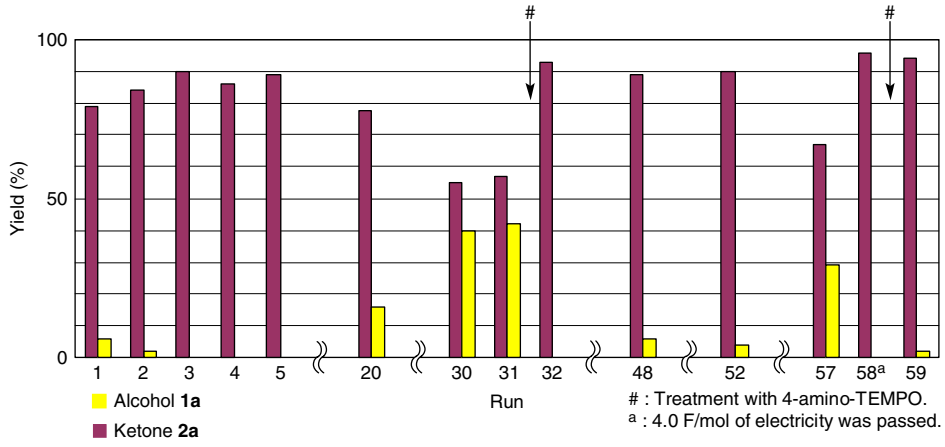
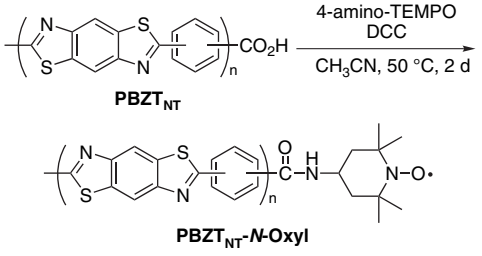


Figure 2. Recycle use of the *PBZT$_{NT}$-N-Oxyl*.

both straight chain segments and the branch point. The straight chain segments consist of benzobisthiazole and phenyl rings and is called PBZT. The PBZT is a rigid-rod polymer and the PBZT fiber is well known for its exceptional mechanical properties as well as its high thermal and chemical stabilities.[10] The PBZT$_{NT}$ also shows high mechanical, thermal, and chemical stability. In addition, many voids exist in the network and there are carboxyl groups at the branch and/or terminal point of the polymer chain, which can be used for immobilization of the *N*-oxyl moieties on the polymer particles. Herein, we describe that the PBZT$_{NT}$ particles were strong enough to survive without appreciable change in their shape and size even after more than 60 times recycle use for the electrooxidation of alcohols (Fig. 2).

2. Results and discussion

The *N*-oxyl-immobilized poly(*p*-phenylene benzobisthiazole) network polymer particles (*PBZT$_{NT}$-N-Oxyl*) were prepared by treatment of the PBZT$_{NT}$ particles with 4-amino-2,2,6,6-tetramethylpiperidine-*N*-oxyl (4-amino-TEMPO) in acetonitrile in the presence of dicyclohexylcarbodiimide (DCC) at 50 °C for 2 d (Scheme 1). The polymer particles were separated by filtration, washed successively with acetonitrile, water, ethanol, and ether, and dried under reduced pressure to afford the *PBZT$_{NT}$-N-Oxyl*. The weight of polymer particles increased from 400 to 450 mg, suggesting that ca. 0.6 mmol/g of the *N*-oxyl moiety was immobilized on the PBZT$_{NT}$.



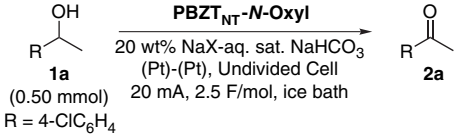
Scheme 1.

Electrolysis was carried out in a simple beaker-type undivided cell fitted with two platinum electrodes (1×1 cm^2 each). A typical procedure is as follows: a mixture of *PBZT$_{NT}$-N-Oxyl* (450 mg), and alcohol **1a** (R=4-Cl–C$_6$H$_4$; 0.5 mmol) in an aqueous satd NaHCO$_3$ containing 20 wt % NaBr was electrolyzed at 20 mA/cm^2 under vigorous stirring at 0 °C. After passage of 2.5 F/mol (1.67 h) of electricity, the *PBZT$_{NT}$-N-Oxyl* was separated by filtration and washed with EtOAc. The filtrates were extracted three times with EtOAc and the extracts and the washings were combined. Evaporation of the solvent afforded the corresponding ketone **2a** in 94% yield (Table 1, entry 1). The presence of both NaBr and NaHCO$_3$ is indispensable, since in the absence of each of them, the yields of **2a** decreased to 16–43% (entries 2 and 3). Neither NaCl nor NaI was effective since only 14% yield or no appreciable amount of the ketone **2a** was obtained by similar electrolysis with NaCl or NaI (entries 4 and 5).

Next, the required amount of *PBZT$_{NT}$-N-Oxyl* was examined (Table 2). When the electrooxidation of **1a** (0.5 mmol) was carried out with 400–200 mg of *PBZT$_{NT}$-N-Oxyl*, the corresponding ketone **2a** was obtained in good yield (entries 1–3). With less than 100 mg of the *PBZT$_{NT}$-N-Oxyl*, the yield of **2a** significantly decreased to 55–35%, suggesting that more than 200 mg of *PBZT$_{NT}$-N-Oxyl* would be required for the adsorption and the oxidation of 0.5 mmol of alcohol **1a** on the polymer surface.

The aqueous solution recovered after extractive workup process could also be used repeatedly. As shown in Figure 3, no significant change in the current efficiency and in the

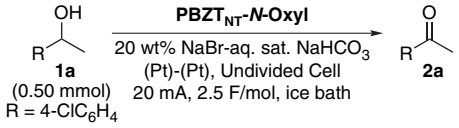
Table 1. Effects of halide salts and NaHCO$_3$[a]



Entry	NaX	NaHCO$_3$	Yield **2a** (%)[b]	Recovered **1a** (%)[b]
1	NaBr	Satd	94	4
2	None	Satd	16	75
3	NaBr	None	43	51
4	NaCl	Satd	14	74
5	NaI	Satd	—	94

[a] Electrooxidation of **1a** (0.50 mmol) using *PBZT$_{NT}$-N-Oxyl* (450 mg) in aqueous solution was carried out under a constant current (20 mA, 2.5 F/mol, 1.67 h) in an undivided cell under ice bath.
[b] Yields were determined by GC analysis using acetophenone as an internal standard.

Table 2. Effects of the amount of *PBZT$_{NT}$-N-Oxyl* [a]



Entry	*PBZT$_{NT}$-N-Oxyl* (mg)	*N*-Oxyl (mmol)	Yield **2a** (%)[b]	Recovered **1a** (%)[b]
1	450	0.27	94	4
2	400	0.24	94	4
3	200	0.12	94	4
4	100	0.06	55	41
5	50	0.03	35	63

[a] Electrooxidation of **1a** (0.50 mmol) using *PBZT$_{NT}$-N-Oxyl* in aqueous solution was carried out under a constant current (20 mA, 2.5 F/mol, 1.67 h) in an undivided cell under ice bath.
[b] Yields were determined by GC analysis using acetophenone as an internal standard.

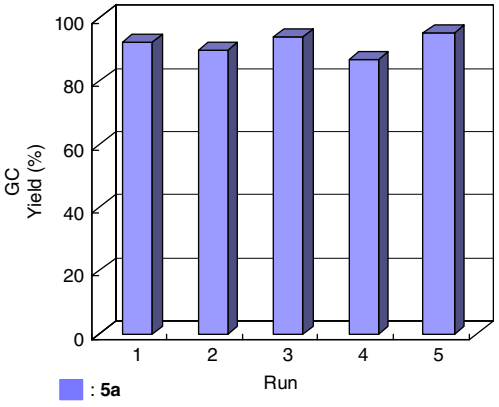
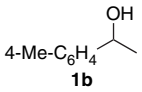
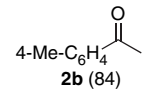
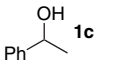
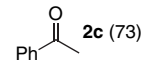
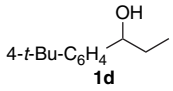
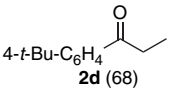
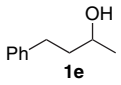
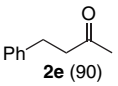
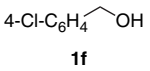
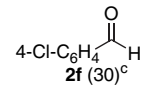
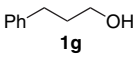
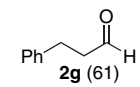
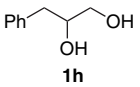
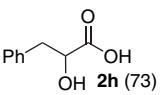
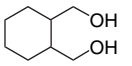
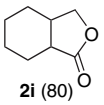


Figure 3. Recycle use of both the *PBZT$_{NT}$-N-Oxyl* and the aqueous media.

conversion yields was observed when both the aqueous solution and *PBZT$_{NT}$-N-Oxyl* were recovered and reused for the electrooxidation of **1a**. The recycling system, formally offers a totally closed system as illustrated in Scheme 2.

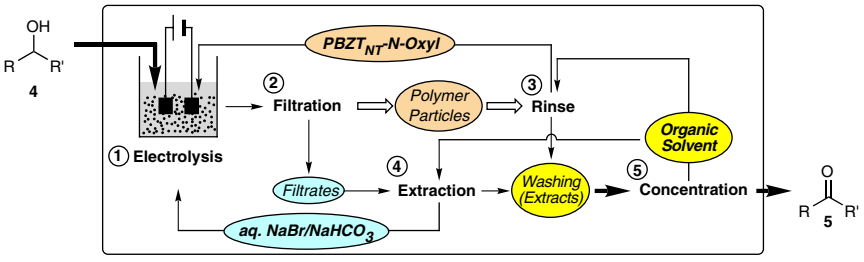
It is of interest to note that *PBZT$_{NT}$-N-Oxyl* could be easily recovered and used repeatedly. Thus, *PBZT$_{NT}$-N-Oxyl* was recovered by filtration after the electrolysis and used for the subsequent electrolysis. The results of the recycle use of *PBZT$_{NT}$-N-Oxyl* are shown in Figure 2. The yields of the ketone **2a** varied in the range of 79–90% through runs 1–20. After 30 times recycle use (run 30), however, the yield of **2a** decreased to 55% and 40% of **1a** was recovered. It is likely that some of the *N*-oxyl moieties would be peeled off from the *PBZT$_{NT}$-N-Oxyl* during the repetition of the electrolysis. Indeed, when the recovered *PBZT$_{NT}$-N-Oxyl* was treated again with 4-amino-TEMPO to immobilize the

Table 3. Electrooxidation of alcohols in the *PBZT$_{NT}$-N-Oxyl* dispersed water system[a]

Entry	Substrate	F/mol	Products (Yield, %)[b]
1	 **1b**	2.5	 **2b** (84)
2	 **1c**	2.5	 **2c** (73)
3	 **1d**	4.0	 **2d** (68)
4	 **1e**	4.0	 **2e** (90)
5	 **1f**	2.5	 **2f** (30)[c]
6[d]	**1f**		**2f** (86)
7[e,f]	 **1g**	2.5	 **2g** (61)
8[f]	 **1h**	4.5	 **2h** (73)
9[d]	 **1i**	4.5	 **2i** (80)

[a] Electrooxidation of **1a** (0.50 mmol) using *PBZT$_{NT}$-N-Oxyl* (450 mg) in aqueous solution was carried out under a constant current (20 mA) in an undivided cell under ice bath.
[b] Isolated yields.
[c] 4-Chlorobenzoic acid (7%) was obtained.
[d] Methyl ethyl ketone (1.5 mL) was used as a co-solvent.
[e] 3-Phenylpropanoic acid (28%) was obtained.
[f] Acetonitrile (1.5 mL) was used as a co-solvent.

N-Oxyl moiety, the efficiency of the *N*-oxyl-mediated oxidation of **1a** was almost completely recovered, affording **2a** in 93% yield (run 32). After additional 25 times recycle use (run 57), the yield of **2a** decreased to 67%. It is of interest to note that the yield of **2a** increased to over 90% by only passage of excess amount (3.0 F/mol) of electricity (run 58). Above all, it is likely that the immobilization process of the *N*-oxyl moiety on the PBZT$_{NT}$ particles in an appropriate interval enables the recycle use of the *PBZT$_{NT}$-N-Oxyl* for almost semi-permanent times (run >60).



Scheme 2. A totally closed electrolysis system.

The present electrooxidation in the *PBZT$_{NT}$-N-Oxyl*/Water disperse system could be successfully applied to various alcohols **1b–i**. The representative results are shown in Table 3. The electrooxidation of benzylic as well as aliphatic *sec*-alcohols **1b–e** proceeded smoothly to afford the corresponding ketones **2b–e** in good to excellent yields (entries 1–4). In contrast, the electrooxidation of *p*-chlorobenzyl alcohol (**1f**) under the standard condition afforded only 30% yield of the corresponding aldehyde **2f** together with the corresponding carboxylic acid (7%) (entry 5). When this electrooxidation was carried out in the presence of methyl ethyl ketone as a co-solvent, the yield of **2f** increased to 86% and no appreciable amount of the carboxylic acid was obtained (entry 6). The electrooxidation of aliphatic *prim*-alcohol **1g** and diols **1h** and **1i** was also performed by the use of methyl ethyl ketone or acetonitrile as a co-solvent to afford the corresponding aldehyde **2g**, carboxylic acid **2h** and lactone **2i** (entries 7–9).

3. Conclusion

In conclusion, the electrooxidation of alcohols **1** was successfully achieved in the disperse system with *N*-oxyl-immobilized poly(*p*-phenylene benzobisthiazole) network polymer (*PBZT$_{NT}$-N-Oxyl*) as a disperse phase and an aqueous 20 wt % NaBr/saturared $NaHCO_3$ as a disperse media. The disperse phase and the aqueous disperse media could be recovered and used repeatedly for the electrooxidation of alcohols, thereby offering a totally closed electrolysis system (Scheme 2).

4. Experimental

4.1. Preparation of poly(*p*-phenylene benzobisthiazole) network ($PBZT_{NT}$)

To a 500-mL reaction flask, equipped with a mechanical stirrer and an argon inlet/outlet adapters, were placed 2,5-diamino-1,4-benzenedithiol·dihydrochloride (6.17 g, 25.2 mmol) and PPA (115%, 125 g). The mixture was stirred under reduced pressure (<3 mmHg) and gradually heated up to 100 °C. On completion of degassing of hydrogen chloride, a stoichiometric amount of terephthalic acid (2.08 g, 12.6 mmol) and trimesic acid (1.75 g, 8.39 mmol) were added. The mixture was then heated to 140 °C under an argon atmosphere. The polymerization was carried out, while the solution viscosity was measured by a torque meter, and the gel point of the system was evaluated by the beginning of the precipitous climb of the viscosity. By stopping the reaction before the gelation, PBZT network particle was prepared. After the polymerization, the product was washed with sulfuric acid and water, and dried under reduced pressure at 100 °C to afford PBZT network as dark-brown powder: IR (KBr) 1708, 1100, 960, 690 cm^{-1}.

4.2. Preparation of *N*-oxyl-immobilized $PBZT_{NT}$: a typical procedure

A mixture of $PBZT_{NT}$ (400 mg), 4-amino-2,2,6,6-tetramethylpiperidine-*N*-oxyl (105 mg, 0.61 mmol) and DCC (119 mg, 0.58 mmol) in acetonitrile (10 mL) was heated at 50 °C for 2 d under an Ar atmosphere. The solid particles were separated by filtration and washed successively with acetonitrile, H_2O, MeOH, and Et_2O (20 mL each). The solids were dried under reduced pressure to afford *N*-oxyl-immobilized $PBZT_{NT}$ (451 mg, 0.6 mmol/g of the *N*-oxyl moiety was immobilized): black solids.

4.3. Electrooxidation of alcohols. A typical procedure

A mixture of *N*-oxyl-immobilized $PBZT_{NT}$ (450 mg) and 1-(4-chlorophenyl)ethanol **1a** (78.6 mg, 0.50 mmol) in an aqueous satd $NaHCO_3$ containing 20 wt % NaBr (5.0 mL) was placed in a beaker-type undivided cell. After stirring for 15 min, two platinum electrodes (1×1 cm^2) were immersed into the reaction mixture, and a constant current (20 mA, 1.67 h, 2.5 F/mol) was supplied at 0 °C under vigorous stirring. After electrolysis, the $PBZT_{NT}$ particles were separated by filtration and washed with EtOAc. The aqueous layer was extracted three times with EtOAc. The extracts and the washings were combined and dried over Na_2SO_4. Most of the solvents were evaporated and the residue was chromatographed on a silica gel column (hexane/EtOAc: 5/1) to afford 4-chloroacetophenone (**2a**, 70.0 mg, 0.45 mmol, 90%): a colorless liquid; R_f=0.46 (hexane/EtOAc: 5/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.60 (s, 3H, CH_3), 7.44 (d, J=8.6 Hz, 2H, Ar), 7.89 (d, J=8.6 Hz, 2H, Ar); IR (neat) 3006, 2970, 2904, 1687, 1590, 1572, 1176 cm^{-1}.

4.3.1. 4-Methylacetophenone (2b). A colorless liquid; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.42 (s, 3H, CH_3–Ar), 2.58 (s, 3H, CH_3), 7.26 (m, 2H, Ar), 7.86 (d, J=8.2 Hz, 2H, Ar); IR (neat) 3004, 2923, 1683, 1607, 1183 cm^{-1}.

4.3.2. Acetophenone (2c). A colorless liquid; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.61 (s, 3H, CH_3), 7.43–7.60 (m, 3H, Ar), 7.96 (d, J=7.6 Hz, 2H, Ar); IR (neat) 3004, 2923, 1686, 1599, 1266, 761 cm^{-1}.

4.3.3. 1-(4-*tert*-Butylphenyl)-1-propanone (2d). A colorless liquid; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.22 (t, J=7.2 Hz, 3H, CH_3), 1.34 (s, 9H, $(CH_3)_3C$), 2.98 (q, J=7.2 Hz, 2H, CH_2), 7.47 (d, J=8.6 Hz, 2H, Ar), 7.91 (d, J=8.6 Hz, 2H, Ar); IR (neat) 2967, 1687, 1607, 1228, 1192, 801 cm^{-1}.

4.3.4. 4-Phenyl-2-butanone (2e). A colorless liquid; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.14 (s, 3H, CH_3), 2.71–2.79 (m, 2H, CH_2–Ar), 2.86–2.95 (m, 2H, CH_2CO), 7.16–7.31 (m, 5H, Ar); IR (neat) 3027, 2923, 1717, 1497, 1454, 1162, 750 cm^{-1}.

4.3.5. 4-Chlorobenzaldehyde (2f). White solids; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.52 (d, J=8.5 Hz, 2H, Ar), 7.82 (d, J=8.5 Hz, 2H, Ar), 9.98 (s, 1H, CHO); IR (KBr) 3019, 2985, 2862, 1693, 1590, 1576, 1209 cm^{-1}.

4.3.6. 3-Phenylpropionaldehyde (2g). A colorless liquid; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.74–2.82 (m, 2H, CH_2–Ar), 2.90–3.02 (m, 2H, CH_2CO), 7.16–7.34 (m, 5H, Ar), 9.82 (s, 1H, CHO); IR (neat) 3029, 2928, 1725, 1604, 1455, 1180, 747 cm^{-1}.

4.3.7. 3-Phenylpropanoic acid-2-ol (2h). White solids; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.94–3.26 (m, 2H, CH_2),

4.49–4.55 (m, 1H, CH), 7.23–7.38 (m, 5H, Ar); IR (KBr) 3006, 2966, 1687, 1590, 1488, 1262, 1176 cm^{-1}.

4.3.8. *cis*-8-Oxabicyclo[4.3.0]nonan-7-one (2i). A colorless liquid; ^1H NMR (200 MHz, $CDCl_3$): δ 1.13–1.36 (m, 3H), 1.52–1.70 (m, 3H), 1.76–1.84 (m, 1H), 2.06–2.15 (m, 1H), 2.39–2.52 (m, 1H, CH), 2.59–2.69 (m, 1H, CH–C(O)–), 3.94 (d, J=9 Hz, 1H, –C(O)OCH_2–), 4.18 (dd, J=9 Hz, J=5 Hz, 1H, –C(O)OCH_2–); IR (neat) 2935, 1857, 1774, 1376, 1160, 1129 cm^{-1}.

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A new mechanism for oxidation of epigallocatechin and production of benzotropolone pigments

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Abstract—Enzymatic oxidation of (−)-epigallocatechin gave two new quinone dimers, dehydrotheasinensin C and proepitheaflagallin. Dehydrotheasinensin C has a hydrated cyclohexenetrione structure and its oxidation–reduction dismutation reaction yielded black tea polyphenols, theasinensins C and E, and desgalloyl oolongtheanin. The structure of proepitheaflagallin was determined based on spectroscopic data of its quinoxaline derivatives prepared by condensation with *o*-phenylenediamine. Proepitheaflagallin was decomposed on heating to give epitheaflagallin and hydroxytheaflavin. The former is a known black tea pigment and the latter is a new pigment with 1′,2′,3′-trihydroxy-3,4-benzotropolone moiety. The results revealed a new mechanism for the production of these pigments from epigallocatechin.

1. Introduction

Tea plant (*Camellia sinensis*) was originally used as a medicine and beverage in East Asia thousands of years ago and is currently of agricultural and commercial importance worldwide.[1] In addition to caffeine, the presence of four catechin monomers (epigallocatechin (**1**), epicatechin, and their 3-*O*-galloyl esters) in a high concentration (10–25% dry weight) is what makes tea plant distinctive from other polyphenol-rich plants.[1,2] The catechin composition of green tea, which is commonly consumed in China and Japan, is similar to that of fresh tea leaves, because the polyphenoloxidase is inactivated by steaming or roasting immediately after harvesting of fresh leaves. On the other hand, black tea contains a complex mixture of oxidation products produced by enzymatic oxidation of the original catechins, because the fresh leaves are rolled and crushed during black tea manufacturing, and thus, the catechins are mixed with active enzymes and oxygen molecules. The oxidation reaction yields numerous products, only some of which have so far been chemically clarified.[3,4]

Theaflavins are well known reddish-yellow pigments characteristic of black tea and with a unique 1′,2′-dihydroxy-3,4-benzotropolone moiety.[5] The pigments are formed by oxidative coupling between catechol-type catechins (epicatechin and its gallate) and pyrogallol-type catechins (**1** and its gallate). The presence of minor pigments with related benzotropolone structures has also been reported in black tea,[6–14] and these pigments were shown to be produced by a mechanism similar to that of theaflavin synthesis. Epitheaflagallin (**14**) and its 3-*O*-gallate, on the other hand, have 1′,2′,3′-trihydroxy-3,4-benzotropolone moiety, the hydroxylation pattern of which is different from those of theaflavins. These compounds were originally synthesized by in vitro experiments,[15] and their presence in commercial black tea was later disclosed by Nonaka et al.[16] It was presumed that **14** is produced by oxidative coupling between the B-ring of **1** and 1,2,3-trihydroxybenzene (pyrogallol) or gallic acid based on the results of in vitro enzymatic and chemical synthesis.[2]

Since the contents of **1** and its 3-*O*-galloyl ester account for over 70% of total tea catechins in tea leaves, oxidation of these pyrogallol-type catechins is most important in production of black tea polyphenols. Previously, we disclosed that dimers of the B-ring quinones of **1** and its gallate accumulate at the initial stage of tea fermentation.[17] Furthermore, a subsequent study showed that dehydrotheasinensin A (**2a**), one of the quinone dimers produced from (−)-epigallocatechin-3-*O*-gallate, is easily converted to stable products such as theasinensins[18] and oolongtheanins[19] by oxidation–reduction dismutation.[20] Since theasinensins are major black tea constituents,[2] this oxidation pathway is important in the production of black tea polyphenols. The results also indicated that pyrogallol-type catechin B-rings are much more easily oxidized compared to the galloyl groups. However, participation of the galloyl groups in catechin oxidation has also been reported recently,[7,11–14,21] and it was suggested that oxidation of galloyl groups generates minor products, resulting in a complex reaction mixture. Therefore, to further understand the oxidation of tea catechins with pyrogallol type B-rings, **1** should be used as the substrate rather than its

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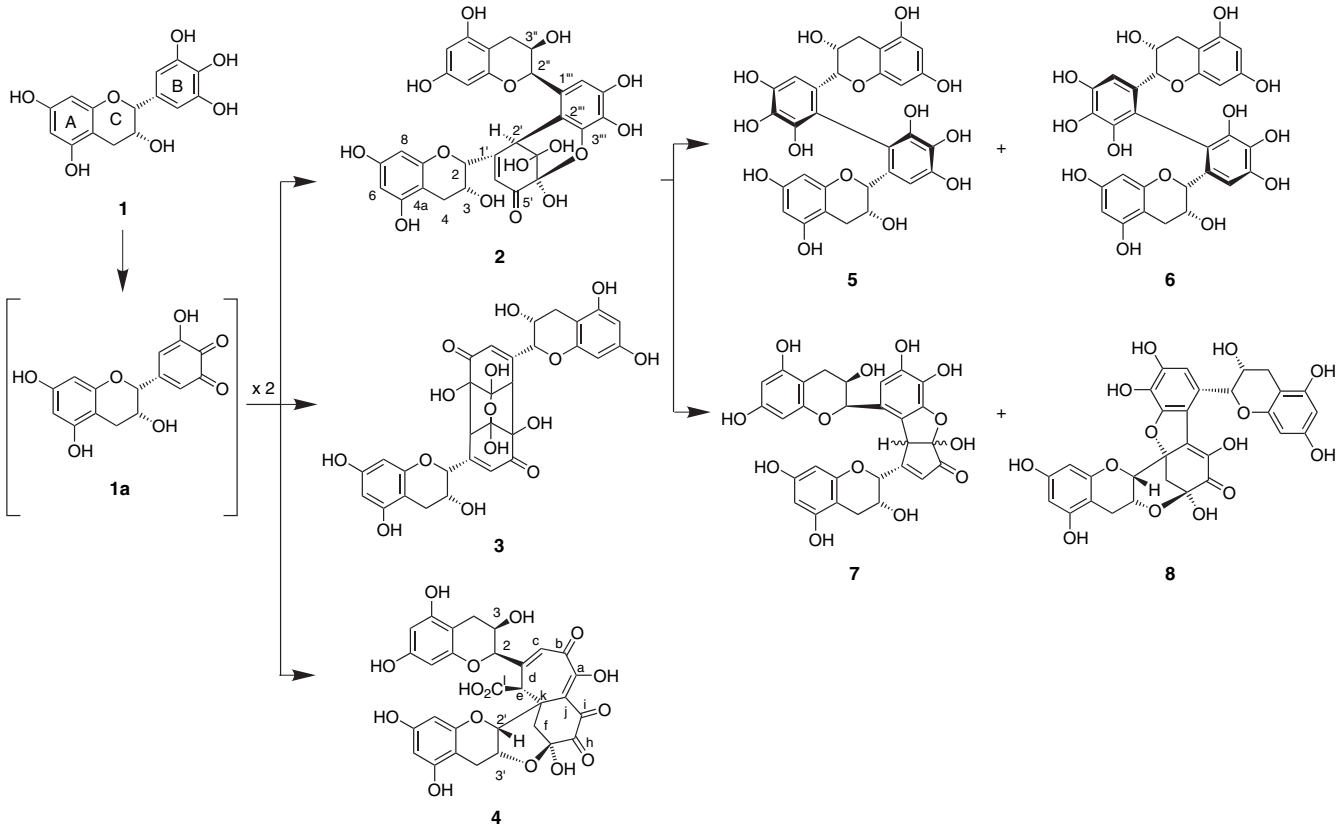
3-*O*-gallate. In the present study, we examined enzymatic oxidation of **1** and found a novel oxidation pathway producing **14** and a related new pigment.

2. Results and discussion

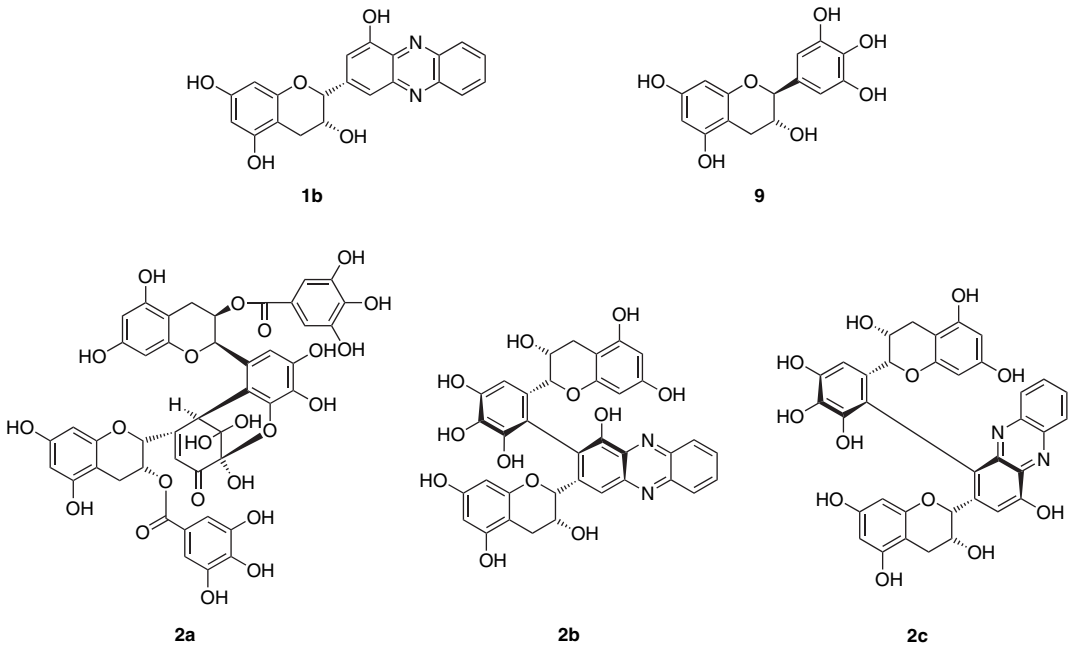
2.1. Enzymatic oxidation of epigallocatechin and isolation of quinone metabolites

Substrate **1**, which was prepared from commercial green tea,[18] was oxidized by mixing with a Japanese pear homogenate until the substrate disappeared. Japanese pear was used because it has strong catechin oxidation activity and does not result in interfering side products derived from compounds originally contained in the pear fruits.[22] The reaction mixture was cooled and acidified prior to separation, because quinone dimers are relatively stable in weakly acidic conditions.[20] After filtration, the filtrate was separated by MCI-gel CHP20P column chromatography to give compound **2**, and further chromatography of the remaining fractions yielded compounds **3** and **4** (Scheme 1). Among these products, compound **3** was identified as a symmetrical quinone dimer formerly synthesized as the radical oxidation product of **1** in an aprotic solvent.[23]

Major product **2** was obtained as a white amorphous powder and its NMR data were closely related to those of dehydrotheasinensin A (**2a**). In the ^{13}C NMR spectrum, signals of a conjugated ketone (δ 191.2), a trisubstituted double bond (δ 161.5 and 122.5), a benzyl methine (δ 45.3), and two hemiacetal carbons (δ 91.6 and 95.5) indicated the presence of a hydrated form of cyclohexenetrione moiety. In addition, the appearance of a $[M+H]^+$ ion peak at *m*/*z* 627 in the FABMS and the lack of signals of galloyl groups in the NMR spectra suggested that **2** is a desgalloyl analog of **2a**. On hydrogenation with dithiothreitol, **2** was converted to theasinensin C (**5**),[18] which has an *R*-biphenyl bond, showing that configuration of the benzyl methine carbon (C-2′) was *S*.[20] In addition, treatment of **2** with *o*-phenylenediamine yielded a phenazine derivative **2b**, which was previously obtained by similar treatment of crushed fresh tea leaves.[17] In neutral phosphate buffer, **2** was decomposed to give **5**, theasinensin E (**6**), which is an atropisomer of **5**,[19] desgalloyl oolongtheanin (**7**),[19] and dehydrotheasinensin E (**8**)[22] (Scheme 1). The former three products were produced by oxidation–reduction dismutation, which also occurs in the case of **2a**.[20] Product **8** was formed by isomerization at the C-2′ position of **2** and subsequent intramolecular acetal formation. These results allowed us to conclude the structure of **2**, which we named dehydrotheasinensin C. Products **2** and **3** were considered to be dimerization products of the B-ring *o*-quinone **1a**, which was initially generated by enzymatic hydrogenation. When *o*-phenylenediamine was directly added to the initial enzymatic oxidation mixture, phenazine derivatives **2b** and **2c**[17] were produced as the major products, and the phenazine derivative (**1b**)[24] derived from the monomer quinone (**1a**) was only obtained as one of the minor products. This result indicated that stereoselective dimerization of the quinone **1a** predominantly occurred and this reaction is the most important oxidation route of **1**.



Scheme 1. Production of **2**–**4** from **1** and decomposition of **2**.



Product **4** was obtained as brown amorphous powder and showed a $[M+H]^+$ peak at *m/z* 625 in FABMS, indicating that this compound is another oxidation product with a dimeric structure. This was supported by the observation of two sets of signals for the catechin A- and C-rings in the ^1H and ^{13}C NMR spectra. In the ^{13}C NMR spectrum, the remaining signals were attributed to an acetal (*δ* 94.0, C-g), a methylene (*δ* 37.4, C-f), a methine (*δ* 49.2, C-e), a quaternary carbon (*δ* 44.6, C-k), four olefinic carbons (*δ* 152.3, C-a; 128.2, C-c; 156.2, C-d; 120.5, C-j), two conjugated carbonyl carbons (*δ* 185.6, C-b; 191.6, C-h), and a carboxyl carbon (*δ* 166.0, C-l). The presence of a carboxyl group was supported by the appearance of a $[M-CO_2+H]^+$ peak at *m/z* 581 in FABMS. Since the molecular weight was calculated to be 624 from the results of FABMS, the molecular formula was suggested as $C_{30}H_{24}O_{15}$, and this was supported by elemental analysis. However, the ^{13}C NMR spectrum showed only 29 carbon signals, suggesting that one carbonyl carbon signal (C-i) was not detected in the spectrum, probably due to severe broadening caused by keto–enol tautomerization or hydration.

In the HMBC spectrum (Fig. 1), proton signals of the olefinic methine H-c and aliphatic methine H-e were correlated to the C-ring of C-2, and H-e and the aliphatic methylene H-f were correlated with C-2′. H-c was also coupled with C-d and the conjugated carboxyl carbon C-l. In addition, correlations of H-e with C-k, and C-j, H-f with C-g, C-h, C-k, and C-j, and other correlations illustrated in Figure 1 suggested connection between C-c–C-d (–C-2)–C-e (–C-l)–C-k (–C-2′, –C-j)–C-f–C-g–C-h. Observation of allyl ^1H–^1H coupling of H-c with H-2 and H-e in the ^1H–^1H COSY spectrum supported this structure. Formation of a hemiacetal ring between the acetal carbon C-g and C-3′ hydroxyl group was deduced from resonance of the C-ring of C-4′ at a higher field (*δ* 25. 5) compared to usual catechin C-4 carbons (*δ* 28), which was similarly observed in the case of **8** (*δ* 25.0).[22] C-b showed no correlation peaks with any protons; however, its chemical shift (*δ* 185.6) indicated that this carbonyl carbon is located between two double bonds. Furthermore, appearance of a ^4J correlation between H-e and C-a suggested that C-a is located between C-j and C-b. At this stage, a novel structure represented by formula **4** or its tautomer is proposed for this unstable oxidation product. However, we could not obtain further spectral evidence to conclude the complete structure of **4** because of tautomerization and gradual decomposition of the compound during NMR measurements.

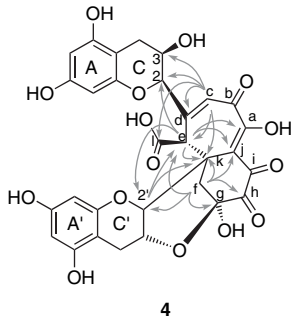
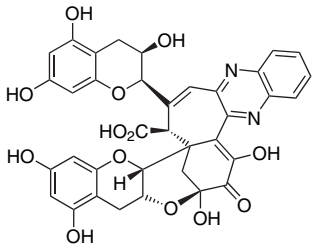


Figure 1. Selected HMBC correlations for **4**.

2.2. Phenazine derivatives of the quinone metabolites

To obtain further evidence on the structure of the new metabolite **4**, we next performed an experiment to trap the unstable oxidation products as quinoxaline derivatives by condensation with *o*-phenylenediamine, because compound **4** apparently has an α-diketone or equivalent structure.[17,20] After **1** was oxidized enzymatically, *o*-phenylenediamine was directly added to the reaction mixture, resulting in isolation of four new condensation products, **10** (yield 0.3%), **11** (0.6%), **12** (0.9%), and **13** (0.08%), along with **2b** (yield 33%), **2c** (6%), **1b** (3%), **3** (0.8%), **5** (0.5%), and **9** (0.3%), and recovery of **1** (7.7%). The results confirmed the predominant formation of **2** from **1** in the reaction mixture.



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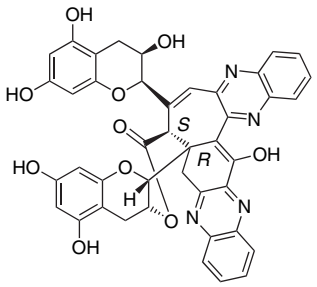

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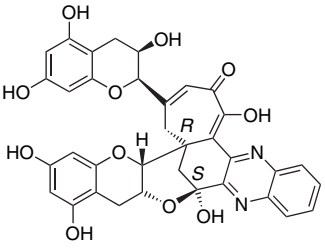
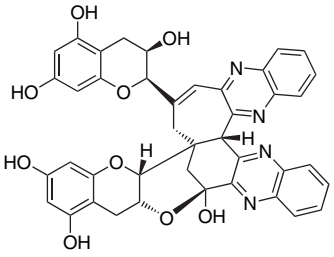


12



13

Product **10** was obtained as a brown amorphous powder and showed UV absorption at 354 nm. FABMS exhibited a $[M+H]^+$ peak at *m/z* 697 and $[M-CO_2+H]^+$ peak at 653. Since ^1H and ^{13}C NMR spectra (Table 1) indicated the presence of one quinoxaline ring,[17] the molecular weight of 696 coincided with that of the expected condensation product between **4** and *o*-phenylenediamine. The ^1H and ^{13}C NMR spectral data and HMBC correlations (Fig. 2) were also related to those of **4**, indicating a partially identical structure. The location of the quinoxaline ring at C-a (δ 152.7) and C-b (δ 152.4) was determined based on the HMBC correlation between these carbons and H-c. A chemical shift in C-h (δ 193.2) indicated that this carbonyl group is conjugated with a double bond. In addition, correlations of H-f with C-e, C-k, C-j, C-g, and C-h, and ^4J correlation of H-e to an oxygen-bearing olefinic carbon (C-i) allowed construction of a cyclohexenone ring. Furthermore, the upfield shift of C-4′ suggested formation of a hemiacetal ring between C-3′ and C-g. Thus, the plane structure of this quinoxaline derivative was concluded to be as shown in formula **10**. The NOESY correlations of H-e with H-f, H-2, H-3, and H-2′, and H-c with H-2 and H-3 supported this structure; however, the absolute configuration at C-e and C-k could not be determined at this stage, but was presumed to be the same as that of the quinoxaline derivatives **12** and **13** described below.

Product **11** was shown to have two quinoxaline units using FABMS (*m/z* 751 $[M+H]^+$) and according to the ^1H and ^{13}C NMR spectra (Table 1). Other NMR signals were related to those of **10**, except for the appearance of an sp^2 carbon signal at δ 152.6 (C-g) instead of an acetal carbon and a large low field shift of the C-ring of H-3′ (δ 5.60) compared to that of **10** (δ 4.83). The HMBC correlations (Fig. 2) revealed the presence of similar carbon strings from C-c to C-h through C-k. Correlations of H-c with C-a and C-b, and H-f with C-g and C-h confirmed the location of the quinoxaline units. The carboxyl group (δ 172.4, C-l), which was correlated with H-e, showed a small correlation peak with H-4′. Taking the large low field shift of H-3′ into account, the C-3′ hydroxyl group was believed to be esterified by this carboxyl group. Thus, the plane structure of **11** was deduced from these spectral data. As for the relative stereochemistry at C-e and C-k, H-e showed strong NOESY correlations with both methylene protons of C-f, indicating that H-e and H-f were located on the same side of the molecule.

Product **12** exhibited a $[M+H]^+$ peak at *m/z* 653 in FABMS, indicating a molecular weight 44 mass units smaller than that of **10**. In addition, the absence of the carboxyl carbon signal in the ^{13}C NMR spectrum suggested that **12** is a quinoxaline derivative of the decarboxylated form of **4**. The ^1H and ^{13}C NMR spectra revealed the presence of two methylenes (C-e and C-f), and HMBC correlations of these methylenes showed that the structure of **12** was partially similar to that of **10**, except for the absence of the carboxyl group at C-e (Fig. 2). The chemical shift of a carbonyl carbon (δ 186.1) indicated its location between two double bonds, and the HMBC correlations of this carbon with H-2 and H-e suggested that it is attributable to C-b. Correlations of H-c with C-a (δ 163.6), and H-e with C-j (δ 113.4) supported construction of a seven-membered ring structure. Although C-i (δ 151.7) showed no correlation peaks, correlation of C-h with H-f showed the location of the quinoxaline ring at C-h and C-i. The UV absorption of this product at 405 nm reflected the long conjugation of double bonds including the quinoxaline ring. The formation of an ether linkage between the C-3′ hydroxyl group and acetal carbon C-g was indicated by observation of an upfield shift of C-4′ and a ^4J HMBC correlation between H-4 and C-g. As for stereochemistry, strong NOEs between H-e and H-f in the NOESY spectrum indicated that these protons were located on the same side of the molecule. In addition, an NOE cross peak between H-2 and H-8′ was also observed (Fig. 3). Since the absolute configuration at C-2 and C-2′ was *R*, this observation indicated

Table 1. ^{1}H (500 MHz), ^{13}C (125 MHz) NMR data for phenazine derivatives **10–13** (δ in ppm, J in Hz)

Position	**10**		**11**		**12**		**13**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
2	4.65 (s)	81.4	4.94 (s)	80.7	4.88 (s)	80.5	4.79 (s)	80.1
3	4.40 (m)	67.3	4.57 (br s)	62.4	4.61 (br s)	62.8	4.63 (br s)	63.0
4	2.80–2.76 (2H, m)	28.8	2.94 (dd, 16.9, 2.1) 2.88 (dd, 16.9, 4.6)	27.2	2.97 (dd, 16.8, 4.1) 2.90 (dd, 16.8, 2.4)	29.7	2.93 (br d, 16.7) 2.85 (dd, 16.7, 4.6)	29.4
4a		99.7		99.4		99.5		99.3
5		157.2[c]		155.9[c]		157.7[b]		157.5[b]
6	5.99 (d, 2.5)[a]	96.2[d]	6.07 (d, 1.7)[a]	96.4[d]	6.05 (d, 2.2)[a]	96.6[c]	6.01 (br s)	96.5[c]
7		156.9[c]		157.5[c]		157.6[b]		157.5[b]
8	5.95 (d, 2.5)[a]	96.0[d]	6.05 (d, 1.7)[a]	95.3[d]	5.94 (d, 2.2)[a]	95.6[c]	6.01 (br s)	96.4[c]
8a		156.7[c]		157.4[c]		157.5[b]		157.0[b]
2′	5.04 (s)	70.9	4.33 (s)	73.1	4.13 (s)	72.4	4.24 (s)	73.1
3′	4.83 (m)	66.3	5.60 (br s)	72.4	4.03 (m)	66.0	3.66 (m)	66.2
4′	2.80–2.76 (2H, m)	25.6	2.80 (2H, m)	25.5	2.70 (dd, 17.4, 2.4) 2.64 (dd, 17.4, 4.6)	25.7	2.60 (dd, 17.6, 5.3) 2.50 (dd, 17.6, 1.6)	25.1
4a′		98.7		97.4		99.0		99.1
5′		156.6[c]		154.8[e]		157.2[b]		156.3[b]
6′	5.86 (d, 2.5)[b]	95.4[d]	5.97 (d, 2.1)[b]	95.4[f]	5.96 (d, 2.4)	96.5[c]	6.04 (d, 2.3)[a]	95.5[c]
7′		156.1[c]		157.8		155.8[b]		155.4[b]
8′	5.89 (d, 2.5)[b]	95.3[d]	5.98 (d, 2.1)[b]	96.9[f]	5.90 (d, 2.4)	95.2[c]	5.96 (d, 2.3)[a]	95.0[c]
8a′		56.0[c]		157.2[e]		155.6[b]		155.4[b]
a		152.7[e]		150.5		163.6		158.4
b		152.4[e]		152.6		186.1		155.0
c	6.91 (s)	129.7	7.46 (s)	130.4	6.77 (s)	128.3	7.42 (d, 1.6)	127.1
d		151.0		143.9		152.4		145.4
e	4.20 (s)	51.4	4.13 (s)	44.9	3.18 (d, 16.2) 2.96 (d, 16.2)	39.9[d]	3.29 (br d, 14.9) 1.91 (br d, 14.9)	34.3
f	2.63 (d, 13.5) 2.45 (d, 13.5)	40.7	4.04 (d, 16.0) 3.77 (d, 16.0)	38.0	2.52 (d, 13.2) 2.15 (d, 13.2)	39.9[d]	2.42 (d, 13.0) 2.36 (d, 13.0)	33.9
g		93.9		152.6		93.1		94.5
h		193.2		144.2		153.4		151.4
i		150.2		152.0		151.7		154.0
j		130.6[f]		115.2		113.4	4.91 (s)	53.4
k		55.1		54.3		41.2		53.3
l		174.9		172.4				
a-OH					16.60 (s)			
g-OH					6.30 (s)			
PHE	8.02–8.00 (1H, m) 7.95–7.93 (1H, m) 7.73–7.70 (2H, m)	141.2 140.4 130.6[f] 130.3 129.0 128.8	8.18–8.16 (1H, m) 8.09–8.04 (3H, m) 7.91–7.87 (2H, m) 7.83–7.77 (2H, m)	143.1, 142.2 141.8, 140.8 131.7, 131.4 131.3, 130.7 129.9, 129.7 129.7, 129.3	8.13 (1H, m) 8.10 (1H, m) 7.92 (1H, m) 7.85 (1H, m)	140.1 137.8 132.6 130.9 129.7 126.8	8.18 (1H, m) 8.00 (1H, m) 7.86–7.81 (2H, m) 7.78–7.74 (1H, m) 7.69 (1H, m) 7.54 (1H, m) 7.39 (1H, m)	143.5, 142.0 141.1, 140.2 131.2, 130.9 130.5, 130.1 129.8, 129.6 129.4, 129.3

[a–f] Assignments may be interchanged in each column.

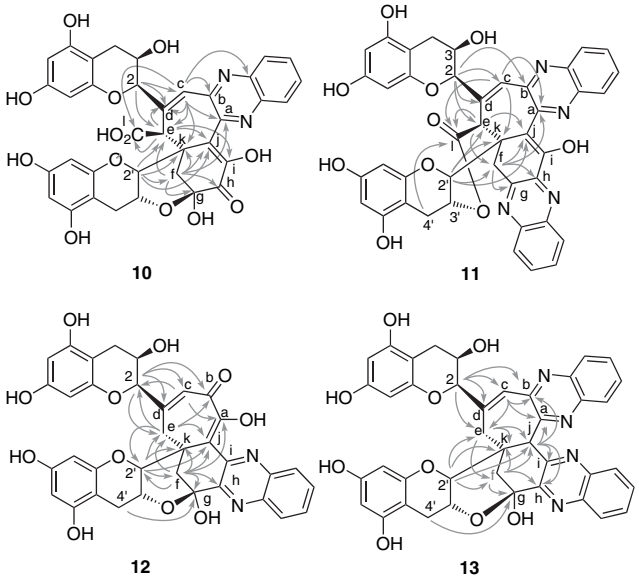


Figure 2. Selected HMBC correlations for **10–13**.

that the configurations at C-g and C-k were *S* and *R*, respectively.

The ^{1}H NMR spectra of product **13** were related to those of **12** (Table 1), showing signals of two sets of catechin A- and C-rings, two methylenes (H-e and H-f), and an olefinic methine (H-c). However, two sets of signals arising from quinoxaline moieties and a singlet aliphatic methine signal at δ 4.91 also appeared. In the ^{13}C NMR spectrum, a methine carbon (C-j) and two sp^{2} carbons bearing nitrogen atoms (C-a and C-b) were observed instead of the enone unit at C-b, C-a, and C-j in **12**. Remaining carbon signals and their HMBC correlations (Fig. 2) were similar to those of **12**, supporting close structural relationships between **12** and **13**. The C-a and C-b of **13** were correlated with both the H-c and newly appeared methine proton (H-j) in the HMBC spectrum, indicating that **13** was generated by condensation of additional *o*-phenylenediamine to the C-a and C-b positions of **12**. This was supported by the observation of a [M+Na]$^{+}$ peak at *m*/*z* 747 in the MALDI-TOF MS. In the NOESY

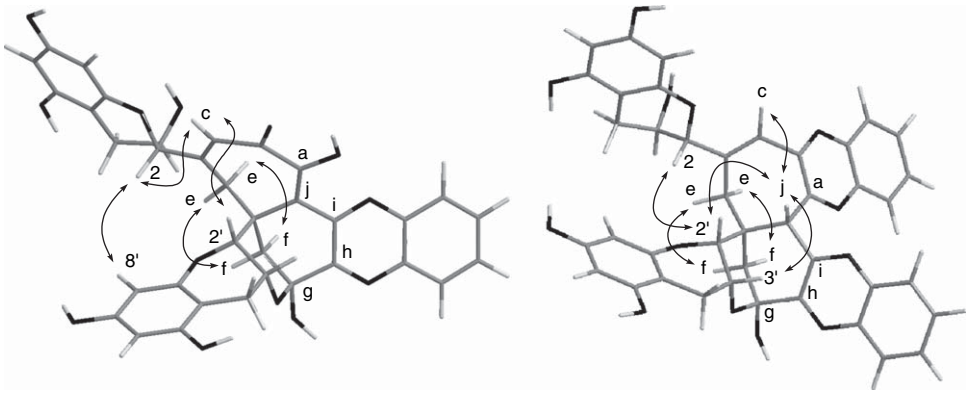


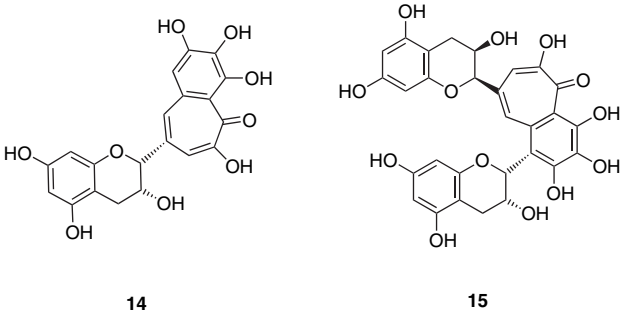
Figure 3. Selected NOESY correlations for **12** and **13**.

spectrum (Fig. 3), H-j showed NOE correlations with H-2′, H-3′, and H-c. In addition, NOEs between H-e and H-f, and between H-2 and H-2′ indicated that the configurations at C-g, C-j, and C-k were *S*, *R*, and *R*, respectively.

Production of the derivatives **10**–**13** not only supported the proposed structure of **4**, but also indicated the occurrence of decarboxylation at the C-e in the molecule of **4** in the following metabolism.

2.3. Degradation products of the quinone metabolites

The unstable product **4** was expected to be a precursor of some black tea polyphenols, as we have shown that **2** was a precursor of important black tea polyphenols **5**, **6**, and **7**. So, we examined decomposition of **4** on heating, which is expected to occur at the final stage of black tea manufacturing. An aqueous solution of **4** was heated at 80 °C for 10 min and then analyzed by HPLC. Photodiode array detection revealed production of two pigments, **14** and **15**; however, isolation of these pigments directly from the reaction mixture failed owing to the shortage of **4**. Isolation of **14** and **15** was achieved by performing a large-scale experiment in which the initial enzymatic oxidation products of **1** were heated and the unstable products including **2** and **4** were converted to more stable products (**5**, **6**, **7**, **8**, **14**, and **15**). Here, it might be of interest to note that MALDI-TOF MS analysis of the total mixture of phenolic products suggested production of a trimer and tetramer of **1**, though the peak intensity was much smaller than those of the dimeric products.



Pigment **14** was identified as epitheaflagallin, having a characteristic 1′,2′,3′-trihydroxy-3,4-benzotropolone unit, by comparisons of ^{1}H and ^{13}C NMR, UV and MS spectra.[16] At this stage, the unstable intermediate **4** was named proepitheaflagallin because it was found to be a precursor of the black tea pigment **14**.

Pigment **15** showed characteristic UV absorptions at 286, 309, 378, and 428 nm, which closely resembled to those of **14**. The ^{1}H and ^{13}C NMR spectra were also related to those of **14**, suggesting the presence of a trihydroxybenzotropolone unit. However, two sets of signals arising from catechin A- and C-rings were also observed. In addition, the absence of an aromatic singlet attributable to H-f of **14** in the ^{1}H NMR spectrum and low field shift of the C-f signal (δ 115.4, **14**: δ 111.8) in the ^{13}C NMR spectrum indicated that **15** has additional catechin A- and C-rings at the C-f position of compound **14**. This structure was confirmed by the HMBC correlations shown in Figure 4. Interestingly, the chemical shift of the C-i hydroxyl proton (δ 15.21) indicated hydrogen bonding of this hydroxyl group with the adjacent carbonyl group at C-a. This pigment was found to be a new compound and named hydroxytheaflavin.

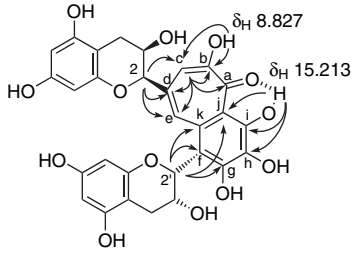
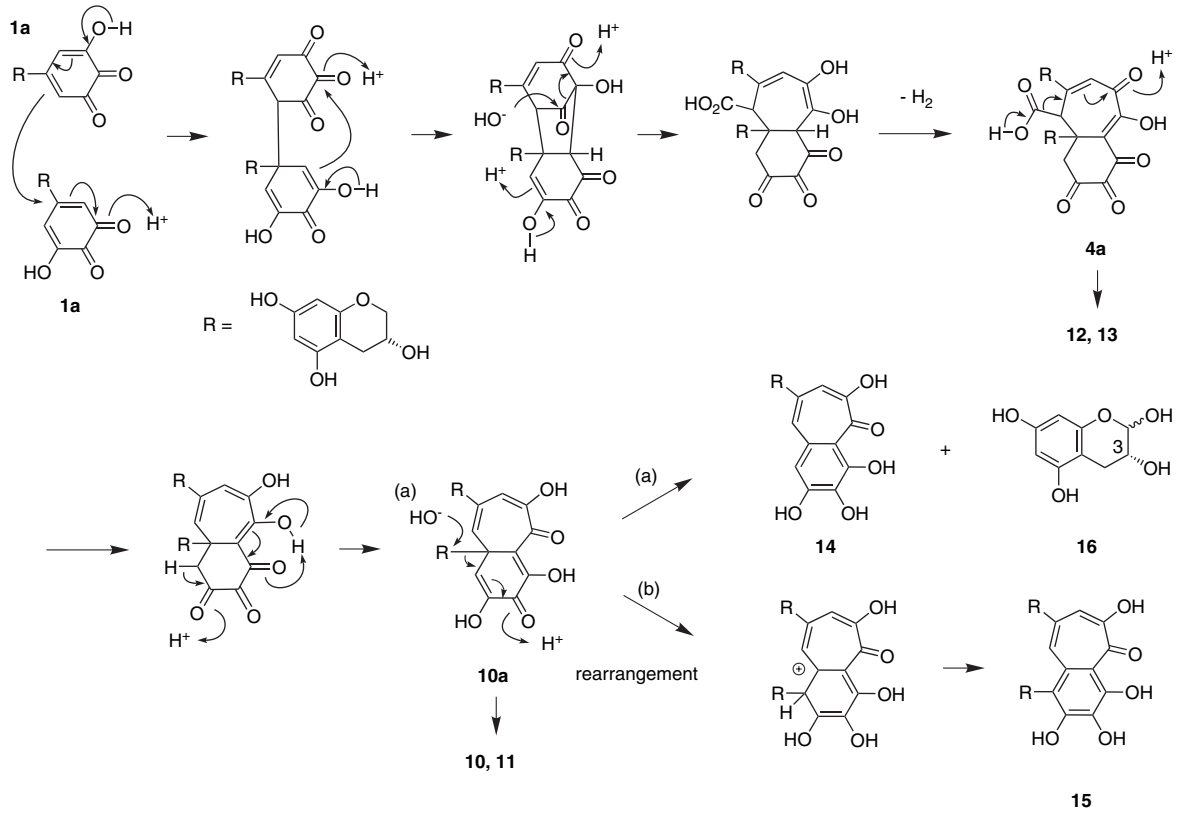


Figure 4. Selected HMBC correlations for **15**.

3. Conclusion

Many theaflavin related pigments, namely, theaflavins, isotheaflavins,[6,7] neotheaflavins,[7,8] theaflavic acids,[9,10] theaflavates,[7,11,12] theadibenzotropolones,[13,14] and theatribenzotropolone A,[14] have been isolated and synthesized. All were produced by oxidative condensation between a catechol ring and pyrogallol ring. Theaflagallins, including **14**, are exceptional because they were produced by condensation



Scheme 2. Proposed mechanisms for production of **14** and **15** from **1**.

between two pyrogallol rings.[15,16] Since pyrogallol itself does not occur in fresh tea leaf, **14** and its gallate found in black tea were considered to have formed by condensation with gallic acid via decarboxylation.[2,16] However, our results revealed that **14** was produced from **1** alone even in the absence of gallic acid, and we succeeded in confirming the key intermediate of this mechanism. Details of the proposed production of **14** and **15** are shown in Scheme 2. In the last step, migration or elimination of the flavan C-ring occurs; this was supported by the fact that compound **16**, which corresponds to the eliminated flavan A, C-rings were actually isolated from black tea.[20] Recently, we also demonstrated that the 3-*O*-galloyl ester of **14** is produced by enzymatic oxidation of the 3-*O*-gallate of **1**,[20] and the reaction probably proceeds in a similar manner.

4. Experimental

4.1. General

UV spectra were obtained with a JASCO V-560 UV/VIS spectrophotometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. ^{1}H, ^{13}C NMR, ^{1}H–^{1}H COSY, NOESY, HSQC and HMBC spectra were recorded in a mixture of acetone-d_6 and D_2O (19:1, v/v) at 27 °C with a Varian Unity plus 500 spectrometer operating at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR. Coupling constants are expressed in Hertz, and chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. MS were recorded on a JEOL JMS DX-303 spectrometer, and glycerol or *m*-nitrobenzyl alcohol was used as the matrix for the FABMS measurements. MALDI-TOF MS was measured on a Voyager-DE PRO (Applied Biosystems), and 2,5-dihydroxy benzoic acid (10 mg/mL) was used as the matrix.

Column chromatography was performed with Diaion HP20SS, MCI-gel CHP20P (75–150 μm, Mitsubishi Chemical Co.), Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemical Co. Ltd), TSK-gel Toyopearl HW-40 (TOSO Co. Ltd), and Chromatorex ODS (Fuji Silysia Chemical Ltd). TLC was performed on precoated Kieselgel 60 F_{254} plates (0.2 mm thick, Merck) with benzene–ethyl formate–formic acid (1:7:1, v/v) or chloroform–methanol–water (14:6:1, v/v) and spots were detected by ultraviolet (UV) illumination and by spraying with 2% ethanolic $FeCl_3$ or 10% sulfuric acid reagent followed by heating. Analytical HPLC was performed on a Cosmosil 5C_{18}-AR II (Nacalai Tesque Inc.) column (250×4.6 mm i.d.) with gradient elution from 10 to 30% (30 min) and from 30 to 75% (15 min) of CH_3CN in 50 mM H_3PO_4 (flow rate, 0.8 mL/min; detection: JASCO photodiode array detector MD-910). Epigallocatechin was isolated from commercial green tea and recrystallized from water.

4.2. Enzymatic oxidation of epigallocatechin and isolation of quinone metabolites

Japanese pear fruits (200 g) were homogenized with 200 mL of H_2O and filtered through four layers of gauze at 0 °C. The filtrate was mixed with an aqueous solution of **1** (1.5 g/75 mL) and vigorously stirred for 90 min at room temperature. The mixture was acidified with 2 mL of trifluoroacetic

acid (TFA) and directly applied to a column of MCI-gel CHP20P (3 cm i.d.×30 cm). After washing with 0.1% TFA, the column was eluted with 0–50% MeOH in 0.1% TFA (5% stepwise gradient elution, each 200 mL), and fractions (each 15 mL) were analyzed by HPLC. Fractions containing **2** were collected and lyophilized to give a white powder (422 mg). Fractions containing **3** and **4** were separately collected and purified by Chromatorex ODS (H_2O–MeOH) to give **3** (47 mg) and **4** (50 mg). Compound **3** was identified with a known oxidation product of **1** by comparison of the spectral data.[23]

4.2.1. Dehydrotheasinensin C (2). White amorphous powder, $[\alpha]_D^{20}$ −74.5 (*c* 0.1, MeOH); IR ν_{max} 3365, 2920, 1696, 1627, 1606 cm^{-1}; UV (MeOH) λ_{max} 269(ε 3640) nm; ^1H NMR (500 MHz, d_6-acetone) δ 2.70 (1H, dd, *J*=16.4, 4.4 Hz, H-4″), 2.73 (2H, br s, H-4), 2.95 (1H, dd, *J*=16.4, 4.4 Hz, H-4″), 4.17 (1H, m, H-3), 4.34 (1H, m, H-3″), 4.42 (1H, s, H-2′), 4.73 (1H, br s, H-2), 5.62 (1H, br s, H-2″), 5.94, 5.97, 5.97, 6.00 (each 1H, d, *J*=2.2 Hz, H-6, 8, 6″, 8″), 6.43 (1H, s, H-6′), 6.80 (1H, s, H-6‴); important long-range ^1H–^1H couplings observed in ^1H–^1H COSY, H-2/H-2′, H-2/H-6′; ^{13}C NMR (125 MHz, d_6-acetone) δ 27.5, 29.0 (C-4, C-4″), 45.3 (C-2′), 64.4 (C-3), 65.6 (C-3″), 75.5 (C-2″), 78.2 (C-2), 91.6 (C-3′), 95.0, 95.2, 95.5, 95.9, 96.1 (C-6, C-8, C-4′, C-6″, C-8″), 99.0, 99.1 (C-4a, C-4a′), 108.2 (C-6‴), 112.0 (C-2‴), 122.5 (C-6′), 127.3 (C-1‴), 132.4 (C-4‴), 142.3 (C-3‴), 145.1 (C-5‴), 155.4, 156.4, 156.7, 156.9 (2C), 156.9 (C-5, C-7, C-8a, C-5″, C-7″, C-8a″), 161.5 (C-1′), 191.2 (C-″); important HMBC correlations (H to C), H-2/C-1′, C-2′, C-6′, H-2′/C-1′, C-3′, C-4′, C-6′, C-1‴, C-2‴, C-3‴, H-2″/C-1‴, C-2‴, C-6‴, H-6‴/C-1‴, C-2‴, C-4‴, C-5‴; FABMS *m*/*z* 627 $[M+H]^+$, 609 $[M-H_2O+H]^+$; Anal. Calcd for $C_{30}H_{26}O_{15}\cdot 7H_2O$: C, 47.88; H, 5.36. Found: C, 47.89; H, 5.21.

4.2.1.1. Treatment of 2 with *o*-phenylenediamine. Compound **2** (2 mg) was treated with a solution of *o*-phenylenediamine (2 mg) in 5% AcOH in EtOH (1 mL) at room temperature for 1 h. HPLC analysis of the reaction mixture showed a product peak at t_R 30.3 min corresponding to the phenazine derivative **2b**.

4.2.1.2. Degradation of 2 under neutral conditions. Compound **2** (100 mg) was dissolved in 0.1 mM Na–K phosphate buffer (pH 7.1) (100 mL) and stirred for 3 h at room temperature. The mixture was acidified to pH 2 by addition of a few drops of diluted HCl and directly applied to a Sephadex LH-20 column (2 cm i.d.×20 cm) with water containing increasing proportions of MeOH (0–80%) to give **5** (24.0 mg), **6** (3.3 mg), **7** (22.3 mg), and **8** (6.8 mg).

4.2.2. Proepitheaflagallin (4). Brown amorphous powder, $[\alpha]_D^{20}$ 92.5 (*c* 0.1, MeOH); IR ν_{max} 3403, 1706, 1631, 1519, 1468 cm^{-1}; UV (MeOH) λ_{max} 271(ε 9490) nm; ^1H NMR (500 MHz, d_6-acetone) δ 2.09 (1H, d, *J*=13.0 Hz, H-f'), 2.58 (1H, d, *J*=13.0 Hz, H-f), 2.82 (2H, m, H-4′), 2.84 (1H, dd, *J*=16.8, 2.7 Hz, H-4), 2.91 (1H, dd, *J*=16.8, 4.3 Hz, H-4), 3.88 (1H, br s, H-2′), 4.47 (1H, br s, H-3), 4.52 (1H, m, H-3″), 4.81 (1H, s, H-2), 4.81 (1H, s, H-e), 5.94, 5.97, 5.95, 5.97, 6.03, 6.07 (each 1H, d, *J*=2.3 Hz, H-6, 8, 6″, 8″), 6.67 (1H, s, H-c); important long-range ^1H–^1H coupling observed in ^1H–^1H COSY, H-2/H-c, H-e/H-c, H-2′/H-f (δ 2.08); ^{13}C NMR (125 MHz, d_6-acetone) δ 25.5 (C-4′), 29.0 (C-4), 37.4 (C-f), 44.6 (C-k), 49.2 (C-e), 65.2 (C-3), 66.2 (C-3′), 94.0 (C-g), 95.2, 95.4, 96.6, 96.7 (C-6, C-8, C-6″, C-8″), 98.3, 99.1 (C-4a, C-4a′), 120.5 (C-j), 128.2 (C-c), 152.3 (C-a), 155.2, 155.3, 157.0, 157.5, 157.6 (2C) (C-5, C-7, C-8a, C-5′, C-7′, C-8a′), 156.2 (C-d), 116.0 (C-l), 185.6 (C-b), 191.6 (C-h) C–I was not detected; important HMBC correlations (H to C), H-c/C-2, C-3, C-d, C-e, C-k, C-l, H-e/C-2, C-2′, C-a, C-c, C-d, C-f, C-k, C-j, C-l, H-2/C-d, C-c, C-e, H-2′/C-e, C-f, C-k, H-f/C-e, C-k, C-j, C-g, C-h, C-2′; FABMS *m*/*z* 625 $[M+H]^+$, 581 $[M-CO_2+H]^+$; Anal. Calcd for $C_{30}H_{24}O_{15}\cdot 3.5H_2O$: C, 52.41; H, 4.54. Found: C, 52.14; H, 4.58.

4.3. Enzymatic oxidation of epigallocatechin and treatment with *o*-phenylenediamine

Japanese pear fruits (4 kg) were homogenized with 1.6 L of H_2O and filtered through four layers of gauze at 0 °C. The filtrate (4.0 L) was mixed with an aqueous solution of **1** (30 g/1.0 L) and vigorously stirred for 2.5 h at room temperature. The mixture was poured into EtOH (15 L) containing *o*-phenylenediamine (11.5 g) and AcOH (750 mL), and stirred gently for 30 min. After removal of insoluble precipitates by filtration, the filtrate was concentrated and applied to Diaion HP20SS (5.5 cm i.d.×55 cm) column chromatography. After washing the column with H_2O to remove sugars and AcOH, the products were eluted out with H_2O containing 0–100% MeOH (10% stepwise elution, each 1.0 L) yielding nine fractions: fr. 1 (4.0 g), fr. 2 (0.89 g), fr. 3 (16.5 g), fr. 4 (2.59 g), fr. 5 (1.81 g), fr. 6 (3.18 g), fr. 7 (1.07 g), fr. 8 (1.77 g), and fr. 9 (0.56 g). Fr. 1 was successively subjected to Sephadex LH-20 (0–60% MeOH, gradient elution), Diaion HP20SS (0–50% MeOH), and Chromatorex ODS (0–50% MeOH) chromatography to give **1** (2.3 g), **3** (265 mg), **5** (145 mg), and **9** (93 mg). Crystallization of fr. 2 from water yielded **1** (0.5 g). The recovery of starting material **1** was 9.3%. Separation of fr. 3 by successive chromatography over MCI-gel CHP20P (10–100% MeOH) and Sephadex LH-20 (20–100% MeOH) yielded **2b** (10.24 g) and **2c** (1.84 g), which were identified as isomers of phenazine derivatives prepared in our previous work.[17] Fr. 4 was applied to Sephadex LH-20 column chromatography (30–90% MeOH) and then further separated by Chromatorex ODS (20–80% MeOH) and MCI-gel CHP20P (25–75% MeOH) chromatography to yield a phenazine derivative **10** (97.2 mg). Fr. 6 was separated into two fractions, fr. 6-1 and fr. 6-2, by Sephadex LH-20 chromatography (50–90% MeOH). The concentration of aqueous MeOH solution of fr. 6-1 yielded reddish-brown powder (806 mg), which was identified as a phenazine derivative of epigallocatechin (**1b**). Fr. 6-2 gave microcrystalline powder of **12** (272 mg) from aqueous MeOH. Fr. 7 was separated by Chromatorex ODS (40–90% MeOH) and Sephadex LH-20 (30–90% MeOH) chromatography to give **1b** (200 mg). Fr. 8 was separated into two fractions, fr. 8-1 and fr. 8-2, by Sephadex LH-20 chromatography (30–100% MeOH). Fr. 8-1 was successively chromatographed over MCI-gel CHP20P (30–80% MeOH), Sephadex LH-20 (60–80% MeOH), Toyopearl HW-40 (40–80% MeOH), and Sephadex LH-20 (EtOH) to give **13** (23.6 mg). Chromatography of fr.

8-2 over Sephadex LH-20 (50–90% MeOH) yielded **11** (190.2 mg).

4.3.1. Phenazine derivative 10. Brown amorphous powder, $[\alpha]_D^{26}$ 104.5 (*c* 0.1, MeOH); IR (dry film) ν_{max} 3383, 1696, 1627, 1609, 1519, 1469 cm^{-1}; UV (MeOH) λ_{max} 354 (ε 7520) nm; ^1H and ^{13}C NMR data, see Table 1; important NOESY correlations: H-c/H-2 and H-3, H-e/H-2, H-3, H-2′, H-f (δ 2.63), and H-f (δ 2.452); FABMS *m/z* 697 $[M+H]^+$, 653 $[M-CO_2+H]^+$; Anal. Calcd for $C_{36}H_{28}N_2O_{13}\cdot 4.5H_2O$: C, 55.60; H, 4.80; N, 3.60. Found: C, 55.45; H, 4.82; N, 3.57.

4.3.2. Phenazine derivative 11. Brown amorphous powder, $[\alpha]_D^{26}$ 196.8 (*c* 0.1, MeOH); IR (dry film) ν_{max} 3366, 1715, 1626, 1609, 1519, 1467 cm^{-1}; UV (MeOH) λ_{max} 249 (ε 30,900), 382 (16,300) nm; ^1H and ^{13}C NMR data, see Table 1; important NOESY correlations: H-c/H-2 and H-3, H-e/H-2, H-3, H-f (δ 4.039), and H-f (δ 3.768), H-f (δ 4.039)/H-2′, H-e, and H-f (δ 3.768), H-f (δ 3.768)/H-2, H-e, and H-f (δ 4.039); FABMS *m/z* 751 $[M+H]^+$; Anal. Calcd for $C_{42}H_{30}N_4O_{10}\cdot 5.5H_2O$: C, 59.36; H, 4.86; N, 6.59. Found: C, 59.49; H, 4.78; N, 6.25.

4.3.3. Phenazine derivative 12. Yellow powder (from H_2O–MeOH, 4:1), $[\alpha]_D^{26}$ −104.5 (*c* 0.1, acetone); IR (dry film) ν_{max} 3394, 1697, 1631, 1521, 1469 cm^{-1}; UV (MeOH) λ_{max} 252 (ε 18,000), 405 (12,700) nm; ^1H and ^{13}C NMR data, see Table 1; important NOESY correlations: H-2/H-8′, H-c/H-2 and H-2′, H-e (δ 3.181)/H-f (δ 2.153) and H-e (δ 2.960), H-e (δ 2.960)/H-f (δ 2.524) and H-e (δ 3.181); FABMS *m/z* 653 $[M+H]^+$; Anal. Calcd for $C_{35}H_{28}N_2O_{11}\cdot 4.5H_2O$: C, 57.30; H, 5.08; N, 3.82. Found: C, 57.01; H, 5.39; N, 3.76.

4.3.4. Phenazine derivative 13. Brown amorphous powder, $[\alpha]_D^{26}$ 234.1 (*c* 0.1, MeOH); IR (dry film) ν_{max} 3369, 1715, 1626, 1489, 1467 cm^{-1}; UV (MeOH) λ_{max} 235 (ε 62,400), 324 (18,100) nm; ^1H and ^{13}C NMR data, see Table 1; important NOESY correlations: H-2/H-2′, H-j/H-2′, H-3′, and H-c, H-e (δ 3.287)/H-2, H-3 and H-e (δ 1.914), H-e (δ 1.914)/H-f (δ 2.361); TOF MS *m/z* 747 $[M+Na]^+$, 763 $[M+K]^+$; Anal. Calcd for $C_{41}H_{32}N_4O_9\cdot 6.5H_2O$: C, 58.50; H, 5.39; N, 6.66. Found: C, 58.59; H, 4.90; N, 6.54.

4.4. Degradation of 4

Aqueous solution of **4** (0.1 mg/0.1 mL) was heated at 80 °C for 10 min and then 5 μL of the solution was analyzed by HPLC. HPLC showed peaks arising from two pigments at 38.3 and 42.1 min, respectively. The retention times and UV absorptions of these peaks coincided with those of **14** and **15**, respectively.

4.5. Oxidation of epigallocatechin and heating of the reaction mixture

Japanese pear fruits (5.5 kg) were homogenized with 2.2 L of H_2O and filtered through four layers of gauze at 0 °C. The filtrate (6.7 L) was mixed with an aqueous solution of **1** (40 g/0.8 L) and vigorously stirred at room temperature, and then the mixture was analyzed by HPLC at intervals of 30 min. After 3 h, the starting material had almost disappeared and a large peak of **2** and moderate peak of **4** appeared. The mixture was poured into a stainless beaker (10 L) and heated until the temperature was elevated to 80 °C in order to inactivate the enzymes and degrade the unstable quinone dimers. The stainless beaker was then placed in ice water to cool down the reaction mixture. To the mixture, acetone (15 L) was added and stirred gently for 30 min, and then the precipitates were removed by filtration. A sample (50 mL) of the filtrate was concentrated and applied to a Diaion HP20SS (2 cm i.d.×10 cm). After washing the column with water, the polyphenols were eluted out with 70% MeOH and concentrated to dryness. The total mixture of products thus obtained was subjected to MALDI-TOF MS analysis. The spectrum showed large peaks at *m/z* 603 (relative intensity 37%), 633 (100%), and 649 (52%) corresponding to the $[M+Na]^+$ of **7**, $[M+Na]^+$ and $[M+K]^+$ of **5** or **6**, respectively. The spectrum also showed peaks at 936 (14%), 952 (12%), 972 (7%), 1212 (18%), 1228 (12%), and 1240 (10%). The remainder of filtrate was concentrated until acetone was removed and subjected to Diaion HP20SS (5.5 cm i.d.×55 cm), then the column was washed with H_2O. The products were eluted out with H_2O–MeOH (10% stepwise elution, each 1.0 L), yielding six fractions: fr. 1 (13.2 g), fr. 2 (5.7 g), fr. 3 (9.5 g), fr. 4 (4.5 g), fr. 5 (2.0 g), and fr. 6 (1.4 g). HPLC and TLC analyses indicated the presence of **5** and **6** in fr. 1, **1** in fr. 2, **1** and **7** in fr. 3, and **7** and **8** in fr. 4 as the major constituents of each fraction. HPLC analysis showed that fr. 6 contained the pigments produced from **4**. This fraction was separated by Sephadex LH-20 (40–100% MeOH) and Chromatorex ODS (20–80% MeOH) to give **14** (530.8 mg) and **15** (185.2 mg). Pigment **14** was identified as epitheaflagallin by comparison of spectral data and co-HPLC with authentic sample.[16]

4.5.1. Hydroxytheaflavin. Red amorphous powder, $[\alpha]_D^{20}$ −230.6 (*c* 0.1, MeOH); IR (dry film) ν_{max} 3366, 1627, 1604, 1517, 1467, 1430 cm^{-1}; UV (EtOH) λ_{max} 286 (ε 21,500), 309 (25,300), 378 (4990), 428 (2910) nm; ^1H NMR (500 MHz, d_6-acetone) δ 2.80 (1H, dd, *J*=16.8, 2.0 Hz, H-4), 2.93 (1H, dd, *J*=16.8, 4.3 Hz, H-4), 2.95 (1H, br d, *J*=17.0 Hz, H-4′), 3.06 (1H, dd, *J*=17.0, 4.3 Hz, H-4′), 4.36 (1H, br s, H-3), 4.72 (1H, br s, H-3′), 4.95 (1H, s, H-2), 5.94 (1H, s, H-2′), 5.96, 6.04 (each 1H, d, *J*=2.3 Hz, H-8 and H-6), 5.97, 6.09 (each 1H, d, *J*=2.3 Hz, H-8′ and H-6′), 7.51 (1H, d, *J*=0.9 Hz, H-c), 8.10 (1H, br s, H-e), 8.83 (1H, br s, OH at C-b), 15.21 (1H, s, OH at C-i); ^{13}C NMR (125 MHz, d_6-acetone) δ 29.4 (C-4), 29.9 (C-4′), 66.4 (C-3), 66.8 (C-3′), 78.6 (C-2′), 81.7 (C-2), 95.6, 96.9 (C-8 and C-6), 95.7, 96.5 (C-8′ and C-6′), 99.5 (C-4a), 99.6 (C-4a′), 115.4 (C-f), 117.3 (C-c), 117.4 (C-j), 127.8 (C-e), 132.0 (C-k), 135.8 (C-d), 136.0 (C-h), 151.8 (C-i), 152.2 (C-g), 154.9 (C-b), 156.6, 156.7, 157.6 (2C), 157.7, 157.9 (C-5, C-5′, C-7, C-7′, C-8a, and C-8a′), 183.1 (C-a); HR-FABMS *m/z* 581.1294 $[M+H]^+$ (Calcd for $C_{29}H_{25}O_{13}$: 581.1295).

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Unusual anomeric rearrangement of *para*-nitrobenzoylxanthate D-glycosides: a new direct stereoselective access to α-thioglycosides from pyranose sugars

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Abstract—A mild and general procedure for the synthesis of α-thioglycosides from glycopyranoses is described. The method involves the treatment of pyranose reductive sugar with sodium hydride, carbon disulfide, and *p*-nitrobenzoyl chloride, as a key step, to yield *p*-nitrobenzoyl-α-D-thioglycopyranose intermediates with high stereoselectivity, in a one-pot-two-step process. The interest of the strategy highlights a direct stereoselective access to ether-protected 1-thiol-α-D-glycopyranose derivatives (Gal, Glc, and Man) from pyranoses in the absence of anomeric 'Lewis acid' promoters.

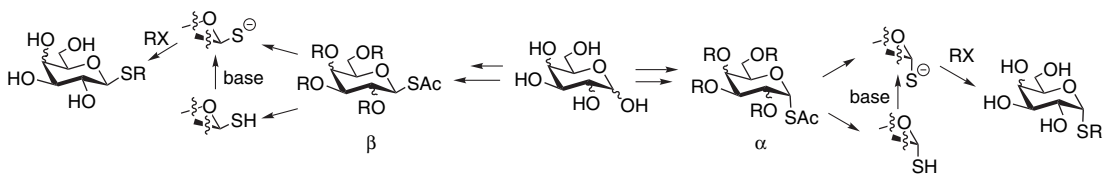
1. Introduction

The thiosaccharides, in which the oxygen of the glycosidic bond is replaced by a sulfur atom, are receiving considerable attention in glycobiology as potential enzyme substrates or inhibitors due to their resistance to acid/base or enzyme-mediated hydrolysis.[1–3] They have recently been highlighted as potent new therapeutic agents, for example, in anti-tumoral[4,5] or anti-HIV treatment.[6]

Within the range of general glycosylation methods, a number of glycosyl donors have been differentiated by the nature of the anomeric groups with a critical effect upon the activation step under acidic catalysis, neutral or basic conditons.[7–13] In the course of the stereoselective thioglycosylation process, halide, trichloroacetimidate, thio-alkyl or -aryl, and sulfoxide donors remain among efficient donors. The modulation of their reactivity with various promoters allows the anomeric substitution by nucleophilic partners including thiol-derivatives.[1,14]

However, base promoted S-alkylation of configurationally pure anomeric α and β-glycosyl thiol, xanthate or thiourea, by S_N2 displacement of alkylating agents has been found as one of the most elegant and powerful routes for the preparation of α and β-1-thioglycosides, respectively.[14,15] Indeed, it has been observed that the thioglycosyl anions do not mutarotate under basic conditions[1,16,17] and their anomerization is very slow[18] (Scheme 1).

The α and β-glycosyl thiolates are obtained from isolable α and β-anomeric thiols, respectively,[18–21] or by in situ



Scheme 1.

Keywords: Stereoselective α-thioglycosylation; α-Glycopyranoside thiols; α-Galactoside thiol; Thiolo-thiono rearrangement; α-Thiogalactolipids.
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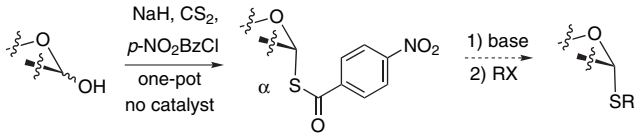
S-deacetylation of the corresponding anomeric thioacetates in the presence of bases (Et_2NH, MeONa,…).[1,17,22,23] The efficiency of the S-alkylation approach is overshadowed by the variable yields often associated with the generation of the anomeric thiolate and its subsequent reactivity with the electrophilic partner in solution. The use of costly hindered iminophosphorane bases has been recently proposed to minimize undesirable competing elimination and to avoid a trans-esterification side reaction when other sensitive *O*-ester protecting groups are present.[23] Furthermore, the access to configurationally pure α- and β-thioacetate (or xanthate and thiourea) anomers has been mainly performed by preliminary catalyzed S-glycosylation from suitable activated 2,3,4,6-ester-protected glycosyl halide, acetate and acetimidate donors in the presence of tetrabutylamonium salt of thioacetic acid and potassium thioacetate, respectively (or potassium ethyl xanthate and thiourea).[24–30] The attribute of the latter process, catalyzed by an ammonium salt or Lewis acid promoters, strongly depends on the nature of the activated donors and mostly affords high stereocontrol to produce 1,2-*trans*-thio-D-glycosides (β-gluco, β-galacto or α-manno) when a neighboring acyloxonium anomeric participation can be involved. Alternatively, Michael-addition of thionucleophiles to sugar enones has also been prone to achieve the access to thioglycosides.[5]

Due to the relative difficulty of conveniently achieving 1,2-*cis* glycosylation,[11] the introduction of a thioacetate group in a pure α-anomeric configuration in galacto or gluco series (or β-configuration for mannosides) and its subsequent in situ deacetylation for S-alkylation process has been promoted.[14] However, only a few syntheses of 1,2-*cis* thioglycosides have been reported.[14,24,31] Driguez and co-workers have used a fair combination of ester-protected α-1-thiolacetate glycosides,[31,32] derived from β-chloro and α-triphenylmetylthio-donors, to produce linear and branched α-thiooligosaccharides. In their convergent approaches, the authors performed the conversion of α-1-thiotrityl intermediates to α-1-thioacetate glycosides by subsequent $Hg(OAc)_2$, H_2S, and Ac_2O treatments. Alternatively, 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranose can be also produced almost exclusively by fair photochemical addition of thioacetic acid, catalyzed by *tert*-butyl or cumene hydroperoxide, from tetra-*O*-acetyl-1,5-anhydro-D-arabino-hex-1-enitol.[33–35]

We describe here a mild and general procedure for the direct access to novel *S*-*p*-nitrobenzoyl-α-D-thioglycopyranose from ether-protected D-glycopyranose precursors, which can be regarded as new potent intermediates in the preparation of α-glycoside thiolates. The method involves the treatment of the reductive sugar with sodium hydride, carbon disulfide, and *p*-nitrobenzoyl chloride, as a key step, to yield in a one-pot-two step process the corresponding *S*-*p*-nitrobenzoyl-α-D-thioglycopyranose with high stereoselectivity (Scheme 2).

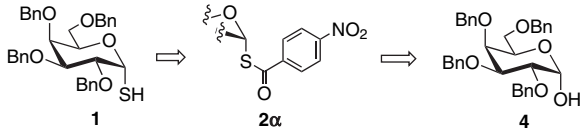


Scheme 2.

The results are discussed in terms of interpretation of an original mechanism and a first application to the preparation of α-thiogalactoconjugates following S-alkylation process.

2. Results and discussion

In our search for new thioglycosylation methods, we investigated a stereoselective access to 1-thiol-α-D-galactopyranose **1** (1,2-*cis*) derivatives from 2,3,4,6-tetra-*O*-benzyl-D-galactose **4**, via the 1-*S*-*p*-nitrobenzoyl-α-thiogalactopyranose **2α** (Scheme 3).

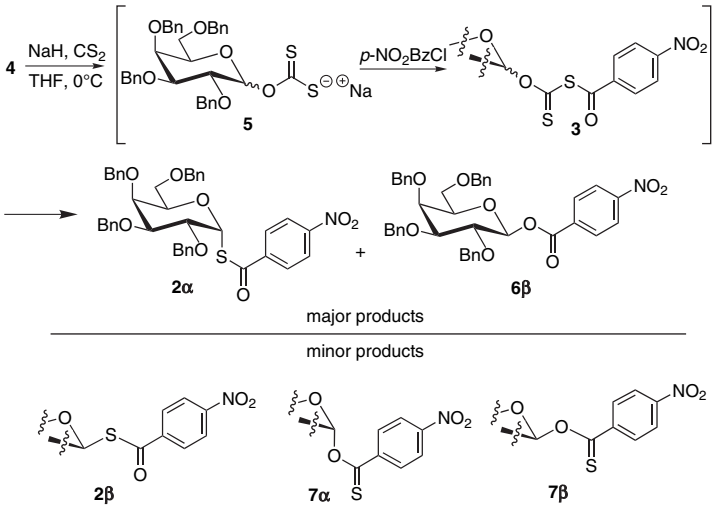


Scheme 3. Retrosynthesis of 1-thiol-α-D-galactopyranose **1**.

The 1-*S*-*p*-nitrobenzoyl-α-thiogalactopyranose **2α** resulted from an in situ rearrangement of the 1-*O*-(*S*-*p*-nitrobenzoyl)-dithiocarbonate-D-galactopyranose **3** formed by the treatment of tetrabenzyl-galactopyranose **4** with sodium hydride and carbon disulfide and subsequent addition of *p*-nitrobenzoyl chloride to the resulting xanthate salt (Scheme 4). When 1.1 equiv of each reagent were used, in THF, the 1-*S*-*p*-nitrobenzoyl-α-thiogalactopyranose **2α** and the 1-*O*-*p*-nitrobenzoyl-β-galactopyranose **6β** were identified as the major products formed in the reaction and were isolated in almost 50% yield. By-products, **2β**, **7α**, and **7β**, were also present in less than 10% overall yield and 25% of starting material **4** remained.

We attempted to improve the preparation of the thioester **2α** using the following optimized proportion of reagents: 1.2 equiv of NaH, 3 equiv of CS_2, and 1.2 equiv of *p*-NO_2BzCl in THF. The ratio between α-thioester **2α** and β-ester **6β** was evaluated and the most significant results are summarized in Table 1. When the reaction was run at 0 °C, the thioester **2α** and ester **6β** were formed in almost 60% yield (entry 1), with 20% of galactopyranose **4** remaining. After purification by chromatography on silica gel, compounds **2α** and **6β** were isolated in a 7/3 ratio. No decrease in the remaining initial pyranose **4** and no appearance of *O*-*p*-nitrobenzoyl anomer **6α** were observed when the reaction was continued for 6 h after the addition of *p*-NO_2BzCl, which indicates that sodium hydride and the acid chloride were consumed (entry 2). When the reaction was run entirely at rt, **2α** and **6β** were produced in the same proportion (5/5) in 67% yield (entry 3). The formation of the alkoxide at rt for 15 min prior to the addition of CS_2 and *p*-NO_2BzCl at 0 °C, increased the ratio in favor of **2α** (8/2, entry 4). Finally, the best selectivity leading to a **2α/6β** ratio of 9/1 (entry 5) was obtained when the alkoxide formation was carried out for 30 min. In these conditions, a **2α/6β** mixture was isolated in 78% yield and only 10% of galactopyranose **4** remained.

We then applied the latter procedure to manno- and gluco-pyranose rings. The results obtained in the manno series seemed to lend weight to this unusual anomeric phenomenon, which occurred in the absence of an activating catalyst.

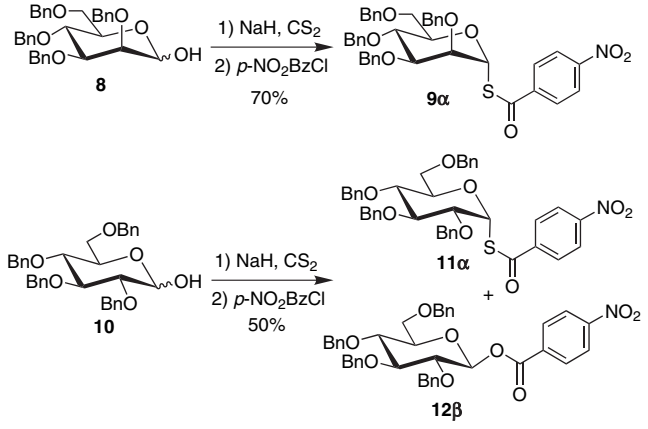


Scheme 4.

Table 1

Entry	NaH		CS_2	*p*-NO_2BzCl		Yield % of **2α+6β**	Ratio of **2α/6β**
	θ (°C)	*t* (min)	θ (°C)/60 min	θ (°C)	*t* (min)		
1	0	15	0	0	60	60	7/3
2	0	15	0	0	360	61	7/3
3	rt	15	rt	rt	60	67	5/5
4	rt	15	0	0	60	70	8/2
5	rt	30	0	0	60	78	9/1

Thus, by a similar one-pot rearrangement the targeted 1-*S*-*p*-nitrobenzoyl-α-thiomannopyranose ester **9α** was produced as the sole product of the reaction from the tetrabenzyl-mannopyranose **8** in 70% yield (Scheme 5).



Scheme 5.

When tetrabenzyl-glucopyranose **10** was subjected to the same reaction conditions, the results were confirmed but in a slightly lower yield and ratio (Scheme 5) as the mixture of α-thioester **11α** and β-ester **12β** was isolated in almost 50% yield, in a 6/4 ratio. The three homologue by-products, previously described in the galacto series, were also identified in the gluco series (<3% from ^{1}H NMR spectra of the crude reaction mixture) but were not isolated. The difference in the reactivity between the gluco and the galacto series is difficult to rationalize but can be correlated to results usually encountered in the literature for the preparation of α-glucoside derivatives, whereas galacto analogues are easier to prepare in good yields.

2.1. Mechanism interpretation

In a preliminary study on the galactose series, the formation of 1-*O*-(*S*-*p*-nitrobenzoyl)dithiocarbonate-D-galactopyranoses **3** was first suggested as key isolable intermediates in the transformation of tetrabenzyl-galactose **4** (1.1 equiv of NaH, 3 equiv of CS_2, and 1.1 equiv of *p*-NO_2BzCl).[36] However, recent advances in the elucidation of the mechanism have highlighted some misunderstanding of this hypothesis, mostly due to the spontaneous evolution of the reaction products even in mild storage conditions.[37] Therefore, the analysis of the crude reaction mixture and the characterization of the intermediates formed were not so straightforward.

Although the reaction should a priori take into account a rearrangement of intermediates **3α** and **3β** (Scheme 4), these latter appeared to be unstable and evolved differently in the medium to give the anomers **2**, **6**, and **7**, as shown by ^{1}H NMR (400 MHz) analysis (Fig. 1, Table 2). A reference experiment was initially recorded exposing galactose **4** to the conditions described in entry 5 (Table 1) but at a lower temperature (−20 °C) and concentrating the THF medium under vacuum only 20 min after the addition of the *p*-nitrobenzoyl chloride (instead of 60 min). Usual workup with dichloromethane/H_2O and the concentration of the dried organic layer were then achieved. The latter procedure revealed the appearance in the crude reaction mixture of all derivatives **2**, **3**, **6**, and **7**, which could be involved in the transformation of the pyranose **4** (Fig. 1A). The evolution of each derivative was then monitored using ^{1}H NMR analysis from the same sample kept for 60 min and 12 h, in $CDCl_3$ solution (Fig. 1B and C).

The presence of 1-*O*-(*S*-*p*-nitrobenzoyl)dithiocarbonate-α-D-galactopyranose **3α** (δH_1=7.00 ppm, d, $J_{1,2}$=3.3 Hz) and thus of 1-*S*-*p*-nitrobenzoyl-α-thiogalactopyranose **2α** (δH_1=6.50 ppm, d, $J_{1,2}$=5.3 Hz) was unambiguously

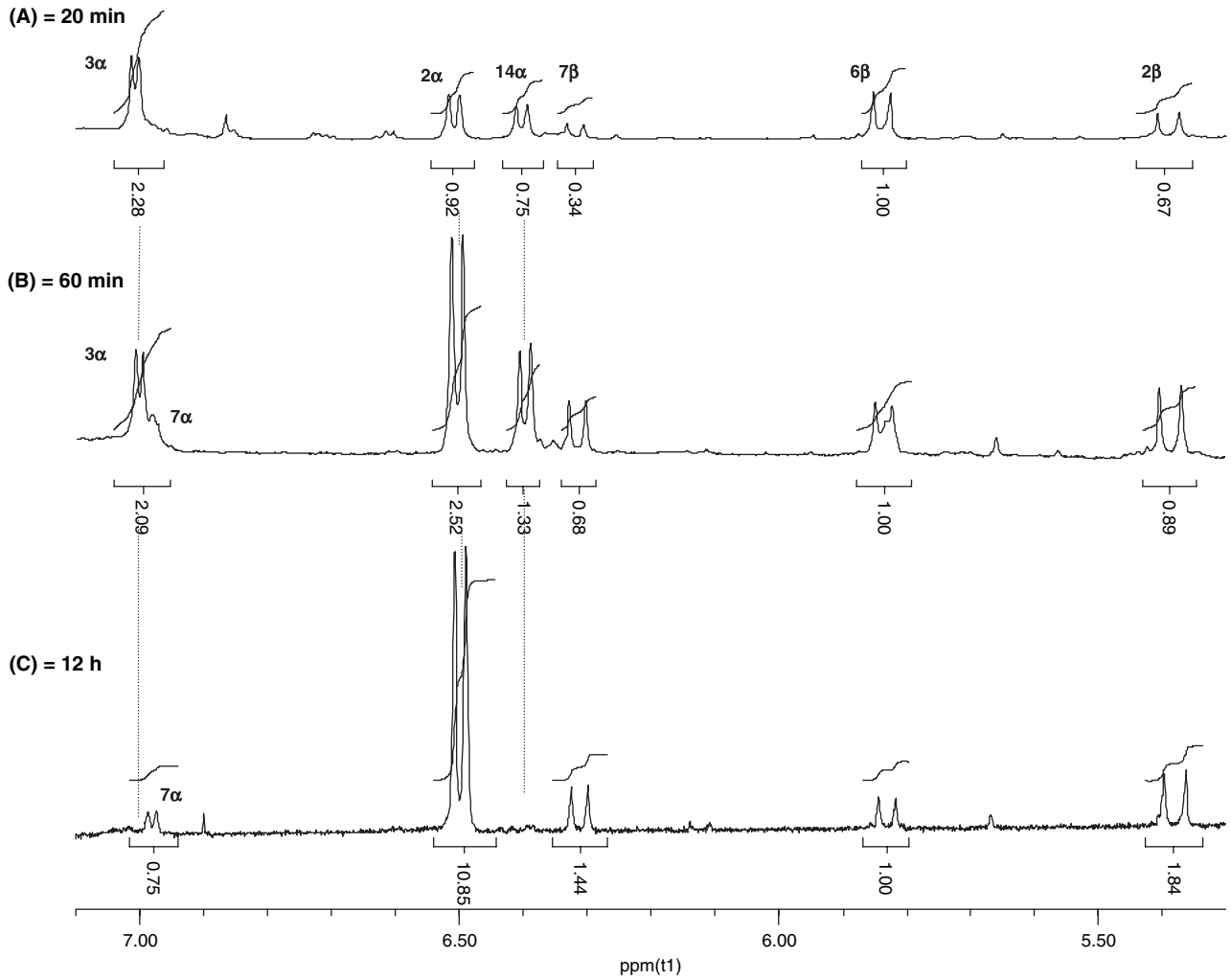
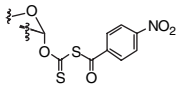
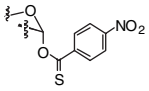
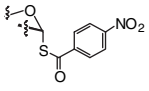
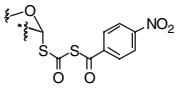
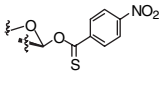
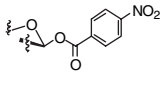
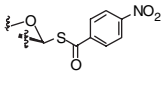


Figure 1. Zone of ^{1}H NMR spectra (5.3–7.1 ppm) showing the anomeric proton signals: evolution of the proportion of compounds **2**, **3**, **6**, **7**, and **14** with time: 20 min (A), 60 min (B), and 12 h (C).

Table 2

	 **3α**	 **7α**	 **2α**	 **14α**	 **7β**	 **6β**	 **2β**
H_1 (ppm)	7.00	6.91	6.50	6.38	6.30	5.84	5.37
J_{1-2} (Hz)	3.3	3.3	5.3	5.4	7.7	8.0	10.1

assigned (Fig. 1A–C). However, the appearance of a signal at 6.38 ppm (δH_1), which increased after 60 min (Fig. 1B) and disappeared after 12 h (Fig. 1C), revealed the existence of a novel intermediate **14α** (d, $J_{1,2}$=5.4 Hz) related to the rearrangement of **3α** into thioester **2α**. When repeating these NMR investigations in order to validate this observation, we have also observed, in some experiments, the total lack of the intermediate **3α** in favor of **14α** (Fig. 2A). Despite the difficulty in controlling the timing of the predominant formation of **14α**, this phenomenon was confirmed several times during ^{1}H NMR experiments, even when the same procedures leading to the data depicted in Fig. 1 were rigorously reproduced.[38] The identification of the 1-*S*-(*S*-*p*-nitrobenzoyl)dithiocarbonate-1-thio-α-D-galactopyranose **14α** (^{13}C NMR, δCO=183.8, 178.4 ppm for the two carbonyls), which is unstable on silica gel supports and in storage conditions, was confirmed by the analysis of its hydrolysis product, α-thiogalactopyranose **15α** (δH_1=5.82 ppm, t, $J_{1,2}$=$J_{1\text{-SH}}$=5.3 Hz). The formation of the latter was observed together with thioester **2α** (Fig. 2B) when silica gel was added to the crude mixture of a reaction in which **14α** was detected as the major α-anomer, whereas 1-*S*-*p*-nitrobenzoyl-α-thiogalactopyranose **2α** remained stable on silica gel.

This evidence led us to propose a revised mechanism for the stereoselective and concomitant formation of the α-thioester **2α** and β-ester **6β** from tetrabenzyl-galactopyranose **4** (Scheme 6). Our hypothesis is based on an original

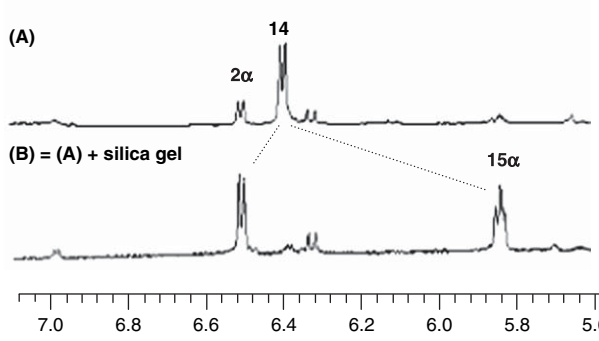
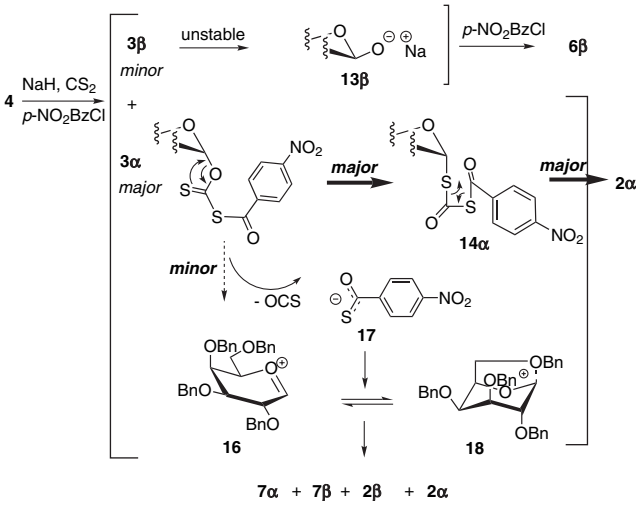


Figure 2. ^{1}H NMR spectra of crude reaction mixture after work-up: (A): reaction conditions: 1.2 equiv NaH, rt, 30 min, CS_2, 0 °C, 60 min, *p*-NO_2BzCl, 0 °C, 2 h; (B): (A)+silica gel.

and surprising one-pot-two-step stereospecific double rearrangement of 1-*O*-(*S*-*p*-nitrobenzoyl)dithiocarbonate-α-D-galactopyranose **3α** into 1-*S*-*p*-nitrobenzoyl-α-thiogalactopyranose **2α**, via the 1-*S*-(*S*-*p*-nitrobenzoyl)dithiocarbonate-1-thio-α-D-galactopyranose **14α**.



Scheme 6.

The anomer counterpart **3β** would not be able to achieve such transformations and the total absence of a signal related to the 1-*O*-(*S*-*p*-nitrobenzoyl)dithiocarbonate-β-D-galactopyranose anomer **3β** in ^{1}H NMR experiments (Figs. 1 and 2) prompted us to suspect its instability. In contrast, the remarkable conservation of the α-stereochemistry during a consecutive double rearrangement of **3α** can be highlighted. The mechanism could be envisaged as following a thiono/thiolo rearrangement of **3α** giving the intermediate **14α**, which could evolve through an anomeric pericyclic process to form **2α**.

Thiono/thiolo rearrangement, known as the Schrönberg rearrangement[39–41] in the case of thionocarbonates and as Newman–Karnes rearrangement[42–45] in the case of thiocarbamates, tends to usually occur at very high temperatures. Thus, to best of our knowledge, only two examples of such transformations have been previously mentioned on pyranose sugars, from a secondary C-4 carbamate[46] and an anomeric xanthate.[47] In both the cases, the process was initiated under acidic catalysis in refluxing solvent (THF or toluene) while, surprisingly, it occurred in situ between the glycosides **3α** and **14α** at 0 °C in the present case. Furthermore, it is interesting to note that the thionoester **7α** also spontaneously rearranged into the thioester **2α**, even when stored under argon at −10 °C.

Logically, the formation of by-products **2β**, **7α**, and **7β** should then involve the participation of an oxonium intermediate **16**, which could result from an inevitable partial degradation of dithiocarbonate **3α**. The addition of the released oxathiocarbonyl **17** to oxonium **16** would then also explain the presence of anomers **2** and **7**. This comment takes into account the significant increase in the H_1 signal of **2β** observed in the NMR experiments (Fig. 1A, B), while no trace of dithiocarbonate **3β** was detected. As the formation of the xanthate salt precursor (NaH, CS_2) cannot be considered as the determining step, if the dithiocarbonate **3β** is formed, it is too unstable to achieve its rearrangement into thioester **2β** and the appearance of the latter would then be understood as coming from the oxonium **16** in the presence of **17**.

In other end, according to Tsuboyama et al.,[48] who has described a one-step synthesis of *S*-1-(1′-phenyl-1*H*-tetrazolyl)-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose in the absence of catalytic activation by reacting *S*-*S*′-bis(1-phenyl-1*H*-tetrazol-5-yl)-dithiocarbonate with tetrabenzylglucopyranose, the predominant formation of **2α** from an oxonium ion **18** cannot be entirely ruled out.

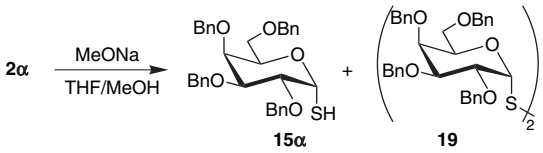
The formation of 1-*O*-*p*-nitrobenzoyl-β-D-galactopyranose **6β** should now be understood as a simple reaction of β-alcoholate anomer **13β** with the remaining *p*-nitrobenzoyl chloride reagent. Moreover, it should be noted that the protected 1-*O*-*p*-nitrobenzoyl anomer **6β** is also of interest as it has already been reported as an efficient glycoside donor in the usual stereoselective α-glycosylations.[49]

In conclusion to this mechanism study, it should be noted that the direct access to α-(*p*-nitrobenzoyl)-thioglycopyranose from pyranose sugars is quite remarkable and efficient particularly as it occurs in the absence of activating promoters with a high stereoselectivity.

2.2. Thioalkylation process

The study of the galacto series was then continued to evaluate the potential of the thioester **2α** for S-alkylation reactions. The de-esterification of **2α** was first attempted to obtain the corresponding α-thiol donor **15α**, anticipating that the corresponding thiolate anion could participate efficiently in the S_N2 displacement of electrophiles to generate a variety of α-thioglycoconjugates or α-thioglycosides. De-esterification of the thioester **2α** was first carried out at rt in THF with a catalytic amount of sodium methoxide (1 M solution in MeOH). In these conditions, the desired thiogalactopyranose **15α** was obtained with a concomitant formation of the disulfide **19** (Scheme 7).

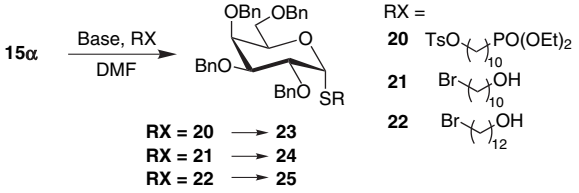
When the reaction was performed with an equimolar quantity of MeONa in a 1/1 THF/MeOH mixture at lower temperature[50] (−40, −15, and 0 °C), the thiol **15α** was formed quantitatively, as confirmed by ^{1}H NMR analysis of the



Scheme 7.

crude mixture of the reaction. Nevertheless, significant degradation occurred during flash chromatography on silica gel since the purified thiol compound was finally isolated with a maximum yield of 50%. This result suggested that thiol **15α** should be used in the next step without further purification. However, de-esterification of **2α** in the presence of NaH and imidazole (2 equiv) in acetonitrile, gave the disulfide **19** as the single product of the reaction (85% yield) and its subsequent reduction into the desired thiol **15α** was fully achieved by treatment with tributylphosphine (1 equiv) in wet THF.[51]

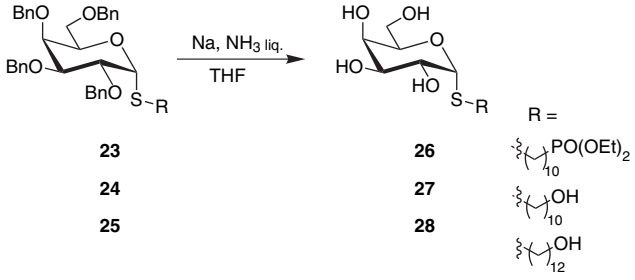
Alkylation of the thiogalactopyranose **15α** was then attempted in the presence of three electrophiles, 10-diethoxyphosphoryl-1-*O*-tosyldecanol **20**,[52] 1-bromodecanol **21**, and 1-bromododecanol **22** (Scheme 8). The results are summarized in Table 3.



Scheme 8.

The purified thiol **15α** treated with NaH (1 equiv), tosylate **20** (1.5 equiv), and crown-ether[53] (1 equiv) gave the desired alkylation product **23** as the major compound but the presence of the disulfide **19** and degradation products was also observed (entry 1). The thiol **15α**, generated in situ by reduction of the disulfide **19** (Fig. 3A) prior to the addition of Cs_2CO_3 (1 equiv) and tosyl **20** (1.2 equiv) in DMF,[54] gave quantitatively the 1-*S*-diethyldecanphosphonate-α-thiogalactoside **23** (entry 2). When electrophiles **21** and **22** were used, similar results were obtained to yield 1-(10-hydroxydecanyl)-α-thiogalactoside **24** and 1-(12-hydroxydodecanyl)-α-thiogalactoside **25**, respectively (entries 3 and 4). Although the ^{1}H NMR spectra of the crude products of the reactions showed a quantitative formation of the desired compounds (Fig. 3B), the thiogalactosides **23**, **24**, and **25** were recovered in moderate 25–30% yields after flash chromatography on silica gel. This inconvenience led us to attempt the following deprotection step without purification.

As predictable, attempts to achieve hydrogenolysis of the benzyl ether groups on thiogalactosides in the presence of Pd/C or $Pd(OH)_2$ failed while the debenzylation of **23**, **24**, and **25** by sodium in liquid ammonia[55,56] was successful, affording the targeted free α-thioglycosides **26**, **27**, and **28**, respectively (Scheme 9).



Scheme 9.

Thus, the reaction sequence: disulfide reduction, thiol-alkylation, and Birch reduction, was carried out without subsequent purification and, after final chromatography on silica gel, the free sugar analogues were obtained in almost 30% overall yields from 2,3,4,6-tetra-*O*-benzyl-α,β-D-galactopyranose.

3. Conclusion

We describe an original alternative for α-thioglycosylation, which compares well with results related in the literature following other methods starting from D-glycopyranose precursors.

The strategy involves, as a key step, the in situ double consecutive rearrangements of 2,3,4,6-tetra-*O*-benzyl-1-*O*-(*S*-*p*-nitrobenzoyl)dithiocarbonate-α-D-glycopyranoses into the corresponding 1-*S*-*p*-nitrobenzoyl-α-D-thioglycopyranoses. The latter rearrangement proceeds in the absence of anomeric activation with a high α-stereoselectivity from reductive sugars. As anticipated, the resulting α-glycoside 1-thioesters are expected to be involved as source of α-anomeric thiolate anions for *S*-alkylations with a total retention of the anomeric configuration. The study of the influence of the ether protecting groups on the efficiency of the methodology, in order to perform the access to branched thioglycosides by convergent approaches, is currently in progress by exchanging benzyloxy groups with *para*-methoxybenzyl, hydrolysis-sensitive substituents or mixed ester–ether groups.

Table 3

Entry	Starting material	Base	RX	Crown-ether	Product[a]	Isolated yield[b] (%)
1	**15α**	NaH	**20**	15-crown-5	**23** maj.[c]	25
2	**27** Reduction	Cs_2CO_3	**20**	None	**23** quant.	28
3	**27** Reduction	Cs_2CO_3	**21**	None	**24** quant.	25
4	**27** Reduction	Cs_2CO_3	**22**	None	**25** quant.	30

[a] Evaluated by ^{1}H NMR.
[b] After purification by flash chromotagraphy on silica gel.
[c] With traces of side products.

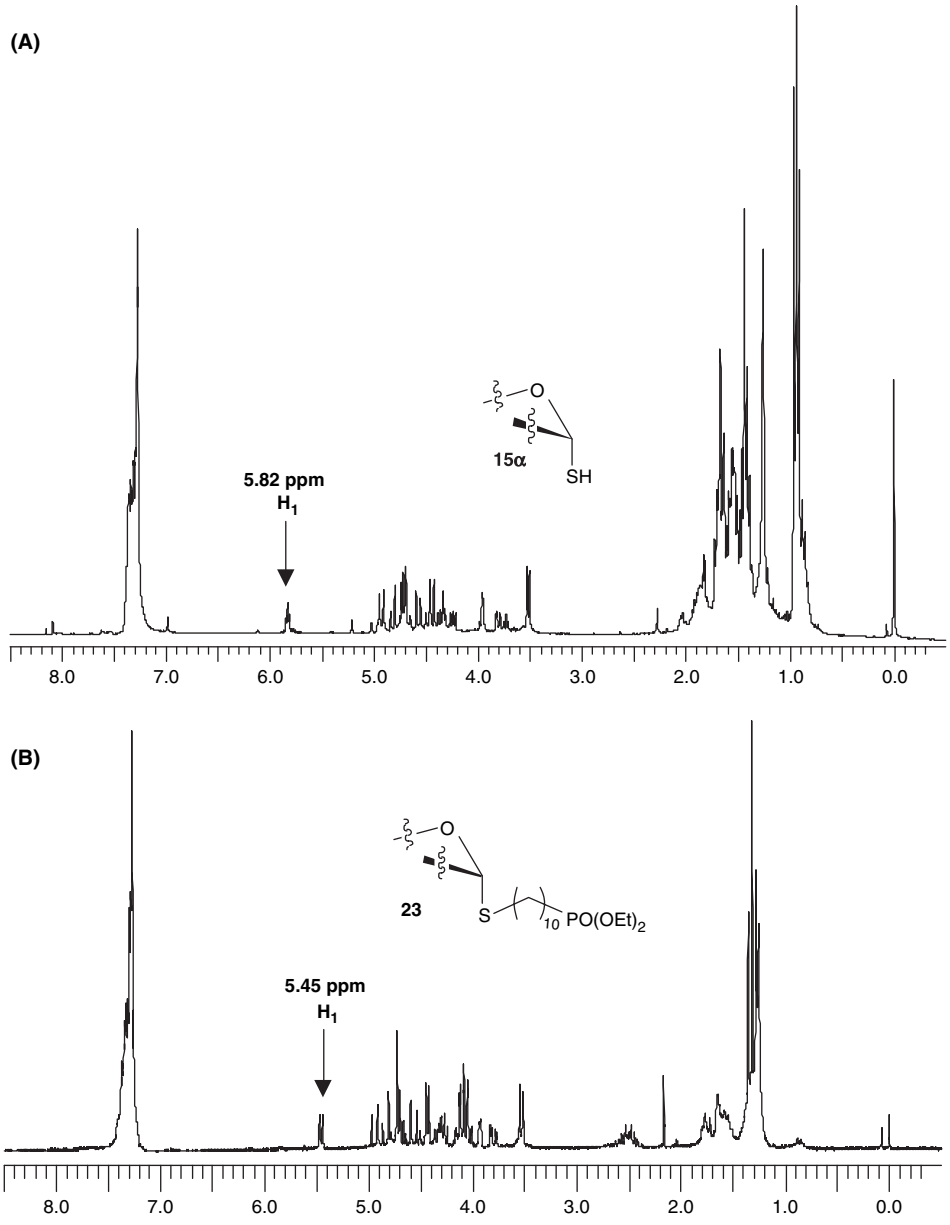


Figure 3. Crude NMR spectra of: (A) reduction of disulfide **19** into **15α** with tributylphosphine in wet THF; (B) alkylation of thiol **15α** into **23**.

The usefulness of this strategy has been illustrated by the first synthesis of three α-thiogalactoconjugates, which are of interest as potential glycomimics and an extension to the synthesis of thiofuranose analogues is underway. The thioglycosylation process is also now being exploited for the synthesis of thio-analogues of α-*O*-galactoceramides,[57] which express anti-cancer and anti-malaria potentials.

4. Experimental section

4.1. General methods

Solvents were purified and dried by standard methods prior to use.[58] All reactions were carried out under argon. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 400 at rt with TMS as internal standard for ^{1}H spectra. Coupling constants *J* are given in Hertz. All assignments were confirmed with the aid of two-dimensional ^{1}H, ^{1}H (COSYDFTP) or ^{1}H, ^{13}C (INVBTP) experiments using standard Bruker pulse programs. All reactions were monitored by TLC on commercially available pre-coated plates (Kieselgel 60 F_{254}) and the products were visualized with mostaïne solution (250 mL H_2O, 10.5 g of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 0.5 g of $Ce(SO_4)_2$, and 15 mL of H_2SO_4). Kieselgel 60, 230–400 mesh (Merck) was used for column chromatography. Optical rotations were measured at 20±1 °C on Perkin Elmer 341 in the indicated solutions whose concentrations are expressed in grams per 100 mL. Mass spectra were measured by CI with NH_3 on a quad. Hewlett Packard 5989A. HRMS were measured on an LCT spectrometer from Micromass (Lokspray, channel 2795 from Waters, flow: MeOH/H_2O 50/50: 0.2 mL/min) and were performed at the Institut de Chimie des Substances Naturelles, CNRS, Gif-sur-Yvette, France. FTIR spectra were obtained in the 500–4000 cm^{-1} range on a Bruker Vector 22 FT-IR spectrometer using NaCl pellets.

4.2. General procedure for the preparation of 1-*S*-*p*-nitrobenzoyl-2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-glycopyranose

To a stirred suspension of sodium hydride (0.14 g, 3.43 mmol) in 10 mL of THF was added a solution of 2,3,4,6-tetra-*O*-benzyl-α,β-D-glycopyranose (1.57 g, 2.86 mmol) in 10 mL of dry THF at rt under argon. After 30 min, carbon disulfide (0.52 mL, 8.60 mmol) was added dropwise to the reaction mixture at 0 °C and stirring was continued for 1 h at the same temperature. The reaction mixture was then treated with *p*-nitrobenzoyl chloride (0.64 g, 3.43 mmol) and was stirred for another 1 h at 0 °C. The solvent was removed under vacuum and the residue was dissolved in CH_2Cl_2. The resulted solution was washed with brine, dried over $MgSO_4$, concentrated, and purified by flash chromatography.

4.2.1. 1-*S*-*p*-Nitrobenzoyl-2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-galactopyranose (2α). Synthesized following the general procedure from 2,3,4,6-tetra-*O*-benzyl-α,β-D-galactopyranose **4** in 63% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.53 (m, 1H, H_{6a}), 3.61 (m, 1H, H_{6b}), 3.64 (dd, 1H, H_3, $^3J_{3-4}$=2.8, $^3J_{3-2}$=10.0), 3.97 (m, 1H, H_5), 4.03 (m, 1H, H_4), 4.37 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.7), 4.43 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.7), 4.48 (dd, 1H, H_2, $^3J_{2-3}$=10.0, $^3J_{2-1}$=5.3), 4.59 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.3), 4.71 (s, 2H, CH_2(OBn)), 4.74 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.7), 4.84 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.7), 4.96 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.3), 6.50 (d, 1H, H_1, $^3J_{1-2}$=5.3), 7.22–7.36 (m, 20H, CH(OBn)), 8.12 (d, 1H, CH_{ar}, ^3J=9.0), 8.28 (d, 1H, CH_{ar}, ^3J=9.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.3 (C_6), 73.3, 73.6 ($2CH_2$(OBn)), 74.0 (C_5), 74.5 (C_4), 75.1 ($2CH_2$(OBn)), 75.4 (C_2), 80.6 (C_3), 84.1 (C_1), 123.9 (CH_{ar}), 127.5–128.7 (CH(OBn)), 137.7–138.5 (Cq(OBn)), 141.7, 150.7 (Cq_{ar}), 188.3 (CO); IR ν (cm^{-1}) 1671 (C=O); $[\alpha]_D^{20}$ +141.1 (*c* 1.0, CH_2Cl_2); HRMS *m*/*z* calcd for $C_{41}H_{39}NO_8SNa$ 728.2294, found 728.2303.

4.2.2. 1-*S*-*p*-Nitrobenzoyl-2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranose (2β). Isolated in less than 3% yield during synthesis of thioester **2α**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.59 (m, 2H, $2H_6$), 3.72 (dd, 1H, H_3, $^3J_{3-2}$=9.1, $^3J_{3-4}$=2.4), 3.80 (m, 1H, H_5), 4.04 (m, 2H, H_2, H_4), 4.40 (d, 1H, CH_2(OBn), $^3J_{gem}$=11.7), 4.46 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.7), 4.63 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.5), 4.75 (m, 3H, CH_2(OBn)), 4.89 (d, 1H, CH_2(OBn), $^2J_{gem}$=10.8), 4.94 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.5), 5.37 (d, 1H, H_1, $^3J_{1-2}$=10.1), 7.24–7.35 (m, 20H, CH(OBn)), 8.04 (d, 2H, CH_{ar}, ^3J=8.8), 8.28 (d, 2H, CH_{ar}, ^3J=8.8); ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.2 (C_6), 72.9 (CH_2(OBn)), 73.7 (C_2 or C_4, CH_2(OBn)), 74.9, 75.9 ($2CH_2$(OBn)), 77.2 (C_2 or C_4), 78.0 (C_5), 82.4 (C_1), 84.4 (C_3), 124.1 (CH_{ar}), 127.8–128.6 (CH(OBn), CH_{ar}), 137.9, 138.0, 138.2, 138.6 (Cq(OBn)), 141.4, 150.8 (Cq_{ar}), 188.1 (CO); HRMS *m*/*z* calcd for $C_{41}H_{39}NO_8SNa$ 728.2294, found 728.2289.

4.2.3. 1-*O*-*p*-Nitrobenzoyl-2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranose (6β). Formed along with **2α** in 7% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.62 (m, 2H, $2H_6$), 3.71 (dd, 1H, H_3, $^3J_{3-4}$=2.7, $^3J_{3-2}$=9.6), 3.79 (m, 1H, H_5), 4.03 (d, 1H, H_4, $^3J_{4-3}$=2.7), 4.12 (dd, 1H, H_2, $^3J_{2-3}$=9.6, $^3J_{2-1}$=8.0), 4.41 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.6), 4.46 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.6), 4.65 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.6), 4.72–4.76 (m, 3H, CH_2(OBn)), 4.87 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.2), 4.96 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.6), 5.84 (d, 1H, H_1, $^3J_{1-2}$=8.0), 7.13–7.36 (m, 20H, CH(OBn)), 8.10 (d, 2H, CH_{ar}, ^3J=7.0), 8.21 (d, 2H, CH_{ar}, ^3J=7.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.1 (C_6), 73.0 (CH_2(OBn)), 73.2 (C_4), 73.7, 74.6 ($2CH_2$(OBn)), 75.0 (C_5), 75.4 (CH_2(OBn)), 77.8 (C_2), 82.7 (C_3), 95.5 (C_1), 123.6 (CH_{ar}), 127.8–128.6 (CH(OBn)), 131.3 (CH_{ar}), 134.8 (Cq_{ar}), 137.8–138.5 (Cq(OBn)), 150.9 (Cq_{ar}), 163.3 (CO); IR ν (cm^{-1}) 1732 (C=O), 1266 (O–CO); $[\alpha]_D^{20}$ –8.2 (*c* 1.1, CH_2Cl_2); HRMS *m*/*z* calcd for $C_{41}H_{39}NO_9Na$ 712.2523, found 712.2547.

4.2.4. 1-*O*-*p*-Nitrothiobenzoyl-2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranose (7α). Isolated in less than 3% yield during synthesis of thioester **2α**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.45 (m, 1H, H_{6a}), 3.55 (m, 1H, H_{6b}), 3.94 (m, 2H, H_3, H_5), 4.05 (m, 1H, H_4), 4.29 (m, 2H, H_2, CH_2(OBn)), 4.36 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.6), 4.55 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.6), 4.63 (d, 1H, CH_2(OBn), $^2J_{gem}$=12.7), 4.71 (d, 1H, CH_2(OBn), $^2J_{gem}$=12.7), 4.76 (s, 2H, CH_2(OBn)), 4.93 (d, 1H, CH_2(OBn), $^2J_{gem}$=12.1), 6.91 (d, 1H, H_1, $^3J_{1-2}$=3.3), 7.15–7.32 (m, 20H, CH(OBn)), 8.11 (m, 4H, CH_{ar}); ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.3 (C_6), 72.8 (CH_2(OBn)), 73.0 (C_5), 73.8 (CH_2(OBn)), 74.3 (C_4), 75.1, 75.8 ($2CH_2$(OBn)), 78.3 (C_3), 97.7 (C_1), 123.5 (CH_{ar}), 128.0–128.6 (CH(OBn)), 129.9 (CH_{ar}).

4.2.5. 1-*O*-*p*-Nitrothiobenzoyl-2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranose (7β). Isolated in less than 3% yield during synthesis of thioester **2α**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.62 (m, 2H, $2H_6$), 3.74 (dd, 1H, H_3, $^3J_{3-2}$=9.7, $^3J_{3-4}$=2.3), 3.81 (m, 1H, H_5), 4.05 (m, 1H, H_4), 4.29 (m, 1H, H_2), 4.40 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.5), 4.48 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.5), 4.60–4.79 (m, 4H, $2CH_2$(OBn)), 4.87 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.4), 4.96 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.3), 6.30 (d, 1H, H_1, $^3J_{1-2}$=7.7), 7.20–7.36 (m, 20H, CH(OBn)), 8.17 (m, 4H, CH_{ar}); ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.3 (C_6), 73.1 (CH_2(OBn)), 73.4 (C_4), 73.7 (CH_2(OBn)), 74.7 (C_5), 74.9, 75.4 ($2CH_2$(OBn)), 78.1 (C_2), 82.6 (C_3), 99.6 (C_1), 123.3 (CH_{ar}), 127.9–128.5 (CH(OBn)), 130.1 (CH_{ar}), 138.0, 138.4 (Cq(OBn)), 142.1, 150.2 (Cq_{ar}), 206.5 (CS).

4.2.6. 1-*S*-*p*-Nitrobenzoyl-2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-mannopyranose (9α). Obtained in 60% yield as the only product of the reaction following the general procedure from 2,3,4,6-tetra-*O*-benzyl-α,β-D-mannopyranose **8**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.72 (m, 2H, H_{6a}, H_5), 3.81 (m, 2H, H_{6b}, H_3), 3.87 (m, 1H, H_2), 4.12 (m, 1H, H_4), 4.53 (m, 4H, $2CH_2$(OBn)), 4.65 (d, 1H, CH_2(OBn), $^2J_{gem}$=12.1), 4.76 (d, 1H, CH_2(OBn), $^2J_{gem}$=12.4), 4.91 (d, 2H, CH_2(OBn), $^2J_{gem}$=11.0), 6.41 (d, 1H, H_1, $^3J_{1-2}$=1.7), 7.17–7.19, 7.25–7.34, 7.44–7.46 (m, 20H, CH(OBn)), 8.08 (d, 2H, CH_{ar}, ^3J=8.8), 8.31 (d, 2H, CH_{ar}, ^3J=8.8); ^{13}C NMR (100 MHz, $CDCl_3$) δ 69.0 (C_6), 71.9, 72.0, 73.6 ($3CH_2$(OBn)), 74.3 (C_4), 75.4 (CH_2(OBn)), 76.6 (C_2), 77.2 (C_5), 80.2 (C_3), 80.9 (C_1), 124.0 (CH_{ar}), 127.6–128.7 (CH(OBn)), 137.8–138.3 (Cq(OBn)), 141.2, 150.8 (Cq_{ar}), 187.5 (CO); IR ν (cm^{-1}) 1675 (C=O); MS (IC^+, NH_3) 723 (M+18); $[\alpha]_D^{20}$ +72.1 (*c* 1.0; CH_2Cl_2).

4.2.7. 1-*S*-*p*-Nitrobenzoyl-2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-glucopyranose (11α). Obtained following the general procedure from 2,3,4,6-tetra-*O*-benzyl-α,β-D-glucopyranose **10** in 36% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.62–3.83 (m, 5H, H_3, H_4, H_5, $2H_6$), 4.01 (dd, 1H, H_2, $^3J_{2-1}$=5.2, $^3J_{2-3}$=9.0), 4.44–4.99 (m, 8H, $4CH_2$(OBn)), 6.49 (d, 1H, H_1, $^3J_{1-2}$=5.2), 7.13–7.14 (m, 2H, CH(OBn)), 7.24–7.32 (m, 18H, CH(OBn)), 8.15 (d, 2H, CH_{ar}, ^3J=8.0), 8.31 (d, 2H, CH_{ar}, ^3J=8.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.1 (C_6), 73.2, 73.7 ($2CH_2$(OBn)), 75.2 (C_3 or C_4 or C_5), 75.4, 75.9 ($2CH_2$(OBn)), 77.2 (C_3 or C_4 or C_5), 78.7 (C_2), 83.1 (C_1), 83.7 (C_3 or C_4 or C_5), 123.8–124.0 (CH_{ar}), 127.9–128.8 (CH(OBn), CH_{ar}), 137.4, 137.9, 138.1, 138.6 (Cq(OBn)), 141.5, 150.8 (Cq_{ar}), 188.0 (CO); IR ν (cm^{-1}) 1672 (C=O); HRMS *m/z* calcd for $C_{41}H_{39}NO_8SNa$ 728.2294, found 728.2307.

4.2.8. 1-*O*-*p*-Nitrobenzoyl-2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranose (12β). Formed along with **11α** in 24% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68–3.84 (m, 6H, H_2, H_3, H_4, H_5, $2H_6$), 4.46–4.91 (m, 8H, $4CH_2$(OBn)), 5.87–5.91 (m, 1H, H_1), 7.16–7.32 (m, 20H, CH(OBn)), 8.12 (m, 2H, CH_{ar}), 8.25 (m, 2H, CH_{ar}); ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.1 (C_6), 73.6, 75.0 ($2CH_2$(OBn)), 75.7 (C_2 or C_3 or C_4 or C_5, $2CH_2$(OBn)), 77.2, 80.7, 85.0 (C_2 or C_3 or C_4 or C_5), 95.1 (C_1), 123.5 (CH_{ar}), 127.9–128.5 (CH(OBn)), 131.1 (CH_{ar}), 134.6 (Cq_{ar}), 137.8–138.3 (Cq(OBn)), 150.8 (Cq_{ar}), 163.1 (CO); IR ν (cm^{-1}) 1739 (C=O), 1268 (O–CO); MS (IC^-, NH_3) 689 [M]; $[\alpha]_D^{20}$ –34.2 (*c* 1.0, CH_2Cl_2); mp 74–76 °C; HRMS *m/z* calcd for $C_{41}H_{39}NO_9Na$ 712.2523, found 712.2531.

4.2.9. 2,3,4,6-Tetra-*O*-benzyl-1-*S*-(S-*p*-nitrobenzoyl)-dithiocarbonate-1-thio-α-D-galactopyranose (14α). ^{1}H NMR (400 MHz, $CDCl_3$) δ (analysis of the crude product) 6.38 (d, 1H, H_1, $^3J_{1-2}$=5.4); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.8, 178.4 (CO).

4.2.10. 2,3,4,6-Tetra-*O*-benzyl-1-thio-α-D-galactopyranose (15α). *Procedure A*: To a solution of thioester **2α** (0.845 g, 1.20 mmol) in 8 mL of a mixture of dry THF/MeOH (1/1) was added a solution of sodium methoxide (1 M in MeOH, 1.12 mL, 1.12 mmol). The reaction was stirred for 1 h and neutralized with Dowex acidic resin. The resin was filtered off and the filtrate was concentrated under reduced pressure to give the thiogalactopyranose **15α** (100% on NMR spectrum of the crude product). This was used immediately for the next step.

Procedure B: A solution of disulfide **19** (0.250 g, 0.22 mmol) in 3 mL of wet THF was treated with tributylphosphine (0.055 mL, 0.22 mmol). After 3 h at rt, the solvent was removed under vacuum and the residue was dissolved in EtOAc, washed with brine, dried over $MgSO_4$ then concentrated under reduced pressure to afford the thiogalactopyranose **15α** (100% on NMR spectrum of the crude product). This was used immediately for the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.81 (d, 1H, SH, $^3J_{SH-1}$=3.9), 3.52 (d, 2H, $2H_6$, $^3J_{6-5}$=6.4), 3.80 (dd, 1H, H_3, $^3J_{3-4}$=2.7, $^3J_{3-2}$=9.8), 3.95 (d, 1H, H_4, $^3J_{4-3}$=2.7), 4.24 (dd, 1H, H_2, $^3J_{2-3}$=9.8, $^3J_{2-1}$=5.3), 4.38 (m, 1H, H_5), 4.43 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.9), 4.47 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.9), 4.57 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.4), 4.66 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.5), 4.71 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.7), 4.72 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.5), 4.81 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.7), 4.92 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.4), 5.82 (dd, 1H, H_1, $^3J_{1-SH}$=3.9, $^3J_{1-2}$=5.3), 7.22–7.37 (m, 20H, CH(OBn)); ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.7 (C_6), 70.5 (C_5), 72.7–73.6 ($4CH_2$(OBn)), 74.9 (C_4), 76.0 (C_2), 78.8 (C_3), 79.8 (C_1), 127.6–128.5 (CH(OBn)), 138.0, 138.1, 138.6, 138.7 (Cq(OBn)); MS (IC^+, NH_3) 574 (M+18); $[\alpha]_D^{20}$ +125.3 (*c* 1.0, CH_2Cl_2); HRMS *m/z* calcd for $C_{34}H_{36}O_5SNa$ 579.2181, found 579.2194.

4.2.11. Bis[2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl]disulfide (19). To a solution of thioester **2α** (1.29 g, 1.83 mmol) in 20 mL of dry CH_3CN was added imidazole (0.25 g, 3.67 mmol) followed by sodium hydride (0.15 g, 3.67 mmol). The reaction mixture was stirred for 2 h and the solvent was removed. The crude product was diluted in EtOAc, washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. It was then purified by flash chromatography (15% EtOAc/petroleum ether) to afford 1.73 g of disulfide **19** as a white solid (85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.57 (dd, 1H, H_{6a}, $^2J_{6a-6b}$=9.0, $^3J_{6a-5}$=5.2), 3.67 (t, 1H, H_{6b}, $^2J_{6b-6a}$=$^3J_{6b-5}$=9.0), 3.79 (dd, 1H, H_3, $^3J_{3-2}$=9.9, $^3J_{3-4}$=2.3), 4.02 (d, 1H, H_4, $^3J_{4-3}$=2.3), 4.17 (dd, 1H, H_5, $^3J_{5-6a}$=5.2, $^3J_{5-6b}$=9.0), 4.29 (dd, 1H, H_2, $^3J_{2-1}$=5.3, $^3J_{2-3}$=9.9), 4.35 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.6), 4.46 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.6), 4.57 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.2), 4.60 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.6), 4.68 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.6), 4.79 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.6), 4.93 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.2), 5.40 (d, 1H, H_1, $^3J_{1-2}$=5.3), 7.24–7.35 (m, 20H, CH(OBn)); ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.2 (C_6), 70.6 (C_5), 72.6, 73.4, 73.6, 75.1 ($4CH_2$(OBn)), 74.9 (C_4), 76.7 (C_2), 79.4 (C_3), 87.1 (C_1), 127.6–128.4 (CH(OBn)), 138.1–138.8 (Cq(OBn)); MS (ES^+) 1133.5 (M+Na); $[\alpha]_D^{20}$ +270.5 (*c* 1.0, CH_2Cl_2).

4.3. General procedure for the preparation of thiogalactopyranosides (23), (24), and (25)

A solution of the thiogalactopyranose **15α** (0.70 g, 1.26 mmol), from the crude product of the disulfide reduction, in 10 mL of DMF was treated with cesium carbonate (0.41 g, 1.26 mmol). After 10 min at rt, the electrophile was added to the reaction mixture (10-diethoxyphosphoryl-1-*O*-tosyldecanol **20** (0.68 g, 1.5 mmol) for the preparation of **23**, 10-bromodecan-1-ol **21** (0.36 g, 1.5 mmol) for the preparation of **24**, and 10-bromododecan-1-ol **22** (0.40 g, 1.5 mmol) for the preparation of **25**). The solution was stirred at rt for 3 h then the reaction mixture was diluted with Et_2O and washed with brine. The organic layer was dried over $MgSO_4$ and then concentrated under vacuum. The crude product can be purified by flash chromatography at this step (15% EtOAc/petroleum ether) to afford 0.29 g of **23** (28%) or 0.22 g of **24** (25%). In the case of **25**, crude product was directly run in the next step.

4.3.1. 2,3,4,6-Tetra-*O*-benzyl-1-*S*-diethyldecanphosphonate-1-thio-α-D-galactopyranoside (23). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.17–1.75 (m, 24H, $9CH_2$, $2CH_3$(OEt)), 2.45–2.54 (m, 2H, CH_2S), 3.53 (m, 2H, $2H_6$), 3.81 (dd, 1H, H_3, $^3J_{3-4}$=2.4, $^3J_{3-2}$=9.9), 3.92 (d, 1H, H_4,

$^3J_{4-3}$=2.4), 4.06–4.11 (m, 4H, 2CH_2(OEt)), 4.26–4.31 (m, 2H, H_2, H_5), 4.40 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.8), 4.47 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.8), 4.57 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.5), 4.68 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.7), 4.70 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.8), 4.74 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.7), 4.83 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.8), 4.94 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.5), 5.45 (d, 1H, H_1, $^3J_{1-2}$= 5.5), 7.21–7.39 (m, 20H, CH(OBn)); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.7, 16.8 (2CH_3(OEt)), 22.5, 22.6, 25.2, 26.6, 29.1, 29.3, 29.4, 29.6, 30.7, 30.9 (10CH_2), 61.5, 61.6 (2CH_2(OEt)), 69.2 (C_6), 69.8 (C_5), 72.6, 73.5, 73.5, 74.9 (4CH_2(OBn)), 75.3 (C_4), 76.4 (C_2), 79.7 (C_3), 83.8 (C_1), 127.6–128.5 (CH(OBn)), 138.2, 138.4, 138.8, 139.0 (Cq(OBn)); ^{31}P NMR (80 MHz, $CDCl_3$) δ 32.61; $[\alpha]_D^{20}$ +79.3 (*c* 1.0, CH_2Cl_2).

4.3.2. 2,3,4,6-Tetra-*O*-benzyl-(10-hydroxydecanyl)-1-thio-α-D-galactopyranoside (24). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.16–1.60 (m, 16H, 8CH_2), 2.44–2.57 (m, 2H, CH_2S), 3.53 (m, 2H, 2H_6), 3.62 (t, 2H, CH_2OH, ^3J=6.6), 3.81 (dd, 1H, H_3, $^3J_{3-4}$=2.8, $^3J_{3-2}$=9.9), 3.92 (d, 1H, H_4, $^3J_{4-3}$=2.8), 4.28 (m, 2H, H_5, H_2), 4.40 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.8), 4.47 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.8), 4.57 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.5), 4.68 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.7), 4.70 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.8), 4.74 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.7), 4.83 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.8), 4.94 (d, 1H, CH_2(OBn), $^2J_{gem}$=11.5), 5.45 (d, 1H, H_1, $^3J_{1-2}$=5.5), 7.22–7.40 (m, 20H, CH(OBn)); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.8, 29.0–29.5, 23.9 (9CH_2), 63.1 (CH_2OH), 69.2 (C_6), 69.8 (C_5), 72.6, 73.5, 74.9 (4CH_2(OBn)), 75.3 (C_4), 76.4 (C_2), 79.7 (C_3), 83.7 (C_1), 127.7–128.4 (CH(OBn)), 138.2, 138.4, 138.7, 138.9 (Cq(OBn)); IR ν (cm−1) 3420 (OH), 2925 (CH_2), 2854 (CH_2); $[\alpha]_D^{20}$ +109.8 (*c* 1.0, CH_2Cl_2); HRMS *m/z* calcd for $C_{44}H_{56}O_6SNa$ 735.3695, found 735.3735.

4.4. General procedure for the debenzylation of thioglycosides

A solution of liquid ammonia was treated with sodium (0.057 mg, 2.40 mmol) at −78 °C. The thiogalactopyranoside **23**, **24** or **25** (crude product of the thioalkylation step) in 2 mL of THF was added to the reaction mixture. After 1 h 30 at −78 °C, the reaction mixture was neutralized with NH_4Cl (0.128 g, 2.40 mmol) before evaporation of the solvent. The crude product was purified by flash chromatography to afford the deprotected thioglycosides **26**, **27**, and **28** in 55% yield from disulfide **19** (i.e., over three steps).

4.4.1. 1-*S*-Diethyldecanphosphonate-1-thio-α-D-galactopyranoside (26). ^{1}H NMR (400 MHz, CD_3OD) δ 1.30–1.41, 1.52–1.63, 1.73–1.82 (m, 24H, 2CH_3(OEt), 9CH_2), 2.50–2.67 (m, 2H, CH_2S), 3.60 (dd, 1H, H_3, $^3J_{3-2}$=9.8, $^3J_{3-4}$=2.4), 3.71 (m, 2H, 2H_6), 3.89 (d, 1H, H_4, $^3J_{4-3}$= 2.4), 4.05–4.12 (m, 6H, H_2, 2CH_2(OEt)), 4.18 (m, 1H, H_5), 5.36 (d, 1H, H_1, $^3J_{1-2}$=5.3); ^{13}C NMR (100 MHz, CD_3OD) δ 16.7, 16.8 (2CH_3(OEt)), 30.0–31.5 (10CH_2), 62.6 (C_6), 63.0, 63.1 (2CH_2(OEt)), 69.8 (C_2), 70.9 (C_4), 72.2 (C_3), 72.6 (C_5), 87.4 (C_1); ^{31}P NMR (80 MHz, CD_3OD) δ 34.34; $[\alpha]_D^{20}$ +174,0 (*c* 0.3, MeOH).

4.4.2. (10-Hydroxydecanyl)-1-thio-α-D-galactopyranoside (27). ^{1}H NMR (300 MHz, CD_3OD) δ 1.39–1.66 (m, 14H, 7CH_2), 1.87 (m, 2H, CH_2), 2.51–2.68 (m, 2H, CH_2S), 3.54 (t, 2H, CH_2OH, ^3J=7.7), 3.61 (dd, 1H, H_3, $^3J_{3-2}$=10.1, $^3J_{3-4}$=3.3), 3.73 (m, 2H, H_6), 3.92 (m, 1H, H_4), 4.07 (dd, 1H, H_2, $^3J_{2-1}$=5.6, $^3J_{2-3}$=10.1), 4.18 (m, 1H, H_5), 5.37 (d, 1H, H_1, $^3J_{1-2}$=5.6); ^{13}C NMR (75 MHz, CD_3OD) δ 26.5, 26.9, 30.0, 30.3, 30.5, 30.6, 30.7, 30.8, 33.6 (9CH_2), 62.6 (C_6), 63.0 (CH_2OH), 69.7 (C_2), 71.0 (C_4), 72.2 (C_3), 72.5 (C_5), 87.5 (C_1).

4.4.3. (12-Hydroxydodecanyl)-1-thio-α-D-galactopyranoside (28). ^{1}H NMR (400 MHz, CD_3OD) δ 1.30–1.35 (m, 16H, 8CH_2), 1.41 (m, 2H, CH_2), 1.63 (m, 2H, CH_2), 2.51–2.65 (m, 2H, CH_2S), 3.54 (t, 2H, CH_2OH, ^3J=6.3), 3.60 (dd, 1H, H_3, $^3J_{3-2}$=9.9, $^3J_{3-4}$=3.5), 3.71 (m, 2H, H_6), 3.88 (m, 1H, H_4), 4.07 (dd, 1H, H_2, $^3J_{2-1}$=5.3, $^3J_{2-3}$=9.9), 4.18 (m, 1H, H_5), 5.37 (d, 1H, H_1, $^3J_{1-2}$=5.3).

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37. Thus, in our previous communication, some mistakes were made concerning the attribution of the NMR data of compound **3**, which should be identified in fact as compound **2**. This confusion has led to a wrong mechanism interpretation.
38. Attempt to run NMR experiment just after the addition of the reagents, without workup, failed.
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Kinetic studies for amination of ketenimines: change of rate-determining step by electron-withdrawing *N*-substituents through electronic effects

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Abstract—Kinetic studies for the amination of ketenimines **1a–d** and **2a–e** with *n*-BuNH$_2$ were carried out by means of UV spectrometry. Hammett equation was applied to the second-order rate constant (k_1) for the amination of **2a–e** and the Hammett plot demonstrated a linear free-energy relationship with a reaction constant (ρ_1) of 2.87, indicating that the second-order rate constant (k_1) corresponds to the first step of rate-determining C=N addition. In contrast, Hammett equation was applied to Kk_2 for the amination of **1a–d** and the Hammett plot was a convex curve with $\rho_D=\rho_1=7.08$ and $\rho_A=\rho_K+\rho_2=0.98$, indicating change of the rate-determining step. The electron-withdrawing *para*-substituents on the *N*-phenyl group of ketenimines significantly stabilize the first transition state of C=N addition, resulting in change of the rate-determining step to the second step of tautomerization. The *N*-substituent electronic effect has much more significant influence on the amination of ketenimines than the substituent electronic effect at C_β.

1. Introduction

Ketenimines and ketenes are important reactive intermediates, which occur as transients in many thermal and photochemical reactions.[1,2] There has been intense interest in their addition reactions, such as cycloadditions,[3] nucleophilic additions,[2b,2c,2g,4] electrophilic additions,[5] and radical additions.[6]

Recently, we have found that amination of ketenimines involved two steps including an initial addition to C=N, followed by tautomerization, and the metastable vinylidenediamine intermediate was caught and identified by proton NMR spectrometry.[7a] By means of proton NMR spectrometry and ab initio calculations, we also found that the rate-determining step for the amination of ketenimines was changed from the second step of tautomerization to the first step of C=N addition when *N*-substituent of the ketenimines is changed from phenyl group to *i*-propyl group[7b] (Fig. 1). We report herein kinetic studies for the amination of ketenimines by UV spectrometry to explore the substituent effect on the amination reactions.

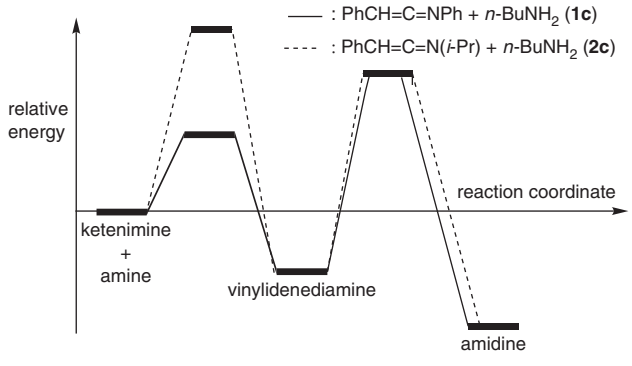

Figure 1. Cartoon picture of reaction profiles for the amination of **1c** and **2c**.

2. Results and discussion

Wavelength scan for the amination of *N*-phenylphenylketenimines **1c** with *n*-BuNH$_2$ in acetonitrile at both −10 and 20 °C by UV spectrophotometer showed a decaying absorption at λ_{max}=260 nm without any isosbestic point as shown in Figure 2, indicating the presence of appreciable amount of the corresponding reaction intermediate. This is consistent with our previous NMR and theoretical results that the rate-determining step is the second step of tautomerization and the reaction intermediate has been caught by low-temperature NMR spectrometry.[7] Therefore, accumulation of the reaction intermediate results in no isosbestic point in the wavelength scan of the amination reaction. On the other hand, wavelength scan for the amination of *N*-*i*-propyl-phenylketenimines **2c** with *n*-BuNH$_2$ in acetonitrile at 20 °C showed a decaying absorption at λ_{max}=270 nm with two isosbestic points as shown in Figure 3, indicating the absence of

Keywords: Amination; Ketenimine; Kinetic study; Hammett plot.
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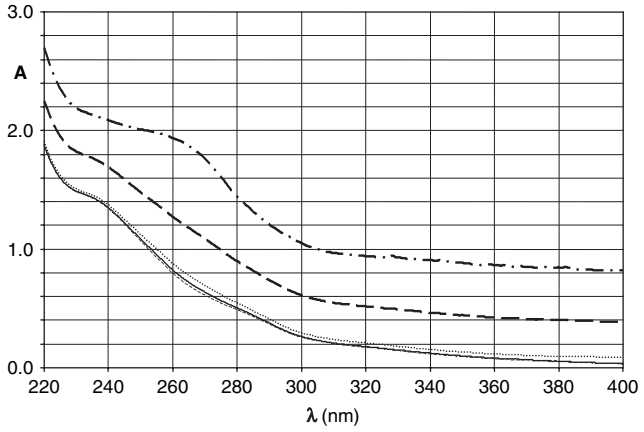


Figure 2. Wavelength scan for amination of **1c** with 0.37 mM *n*-$BuNH_2$ in CH_3CN at −10 °C at 0, 1, 3, 8, and 60 min with a decaying absorption at λ_{max}=260 nm.

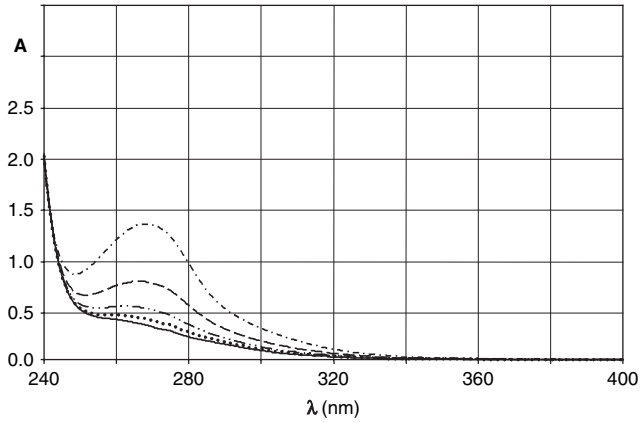


Figure 3. Wavelength scan for amination of **2c** with 0.4 M *n*-$BuNH_2$ in CH_3CN at 20 °C at 0, 1.5, 3, 4.5, and 17.5 h with a decaying absorption at λ_{max}=270 nm.

appreciable amount of the corresponding reaction intermediate. This is quite consistent with our previous NMR and theoretical results that the rate-determining step is the first step of C=N addition.[7b] The second step of tautomerization is much faster than the first step so that the corresponding reaction intermediate does not accumulate, resulting in appearance of isosbestic points in the wavelength scan of the amination reaction.

Time scans by UV spectrophotometer at λ=260 or 270 nm for the amination of **1a–d** or **2a–e** with excess of *n*-$BuNH_2$ in acetonitrile at 20 °C showed a first-order exponential decay for each of the reaction, and the observed rate constants (k_{obs}) are shown in Tables 1 and 2 (Schemes 1 and 2). The reactivity of **1a–d** to *n*-$BuNH_2$ is quite high, so kinetic studies of these reactions have been done in 0.2–0.6 mM of *n*-$BuNH_2$ in CH_3CN at 20 °C. On the other hand, the reactivity of **2a–e** to *n*-$BuNH_2$ is much lower, so kinetic studies of these reactions have been done at higher concentration (0.9, 0.8, 0.7, or 0.6 M for **2a–d**, and 0.05, 0.04, 0.03, or 0.02 M for **2e**) of *n*-$BuNH_2$ in CH_3CN at 20 °C.

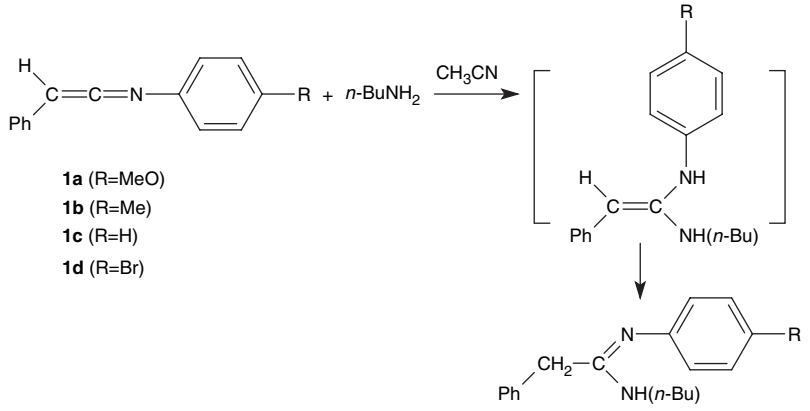
Based on our previous NMR and theoretical results,[7] a proposed mechanism for the amination of ketenimine is shown in Eq. 1. According to the steady-state approximation,[8] the rate law of the amination reactions was drawn in Eq. 2.

Table 1. Observed rate constants for the amination of **1a–d** in CH_3CN at 20 °C

[*n*-$BuNH_2$] (mM)	R	k_{obs} (s^{-1})	[*n*-$BuNH_2$] (mM)	R	k_{obs} (s^{-1})
0.6	MeO	9.64×10^{-3}	0.6	H	5.20×10^{-2}
0.5	MeO	7.67×10^{-3}	0.5	H	4.34×10^{-2}
0.4	MeO	5.92×10^{-3}	0.4	H	3.11×10^{-2}
0.3	MeO	4.22×10^{-3}	0.3	H	2.13×10^{-2}
0.2	MeO	2.60×10^{-3}	0.2	H	1.11×10^{-2}
0.6	Me	5.28×10^{-2}	0.6	Br	9.74×10^{-2}
0.5	Me	4.45×10^{-2}	0.5	Br	8.04×10^{-2}
0.4	Me	3.52×10^{-2}	0.4	Br	6.18×10^{-2}
0.3	Me	2.63×10^{-2}	0.3	Br	4.18×10^{-2}
0.2	Me	1.72×10^{-2}	0.2	Br	2.27×10^{-2}

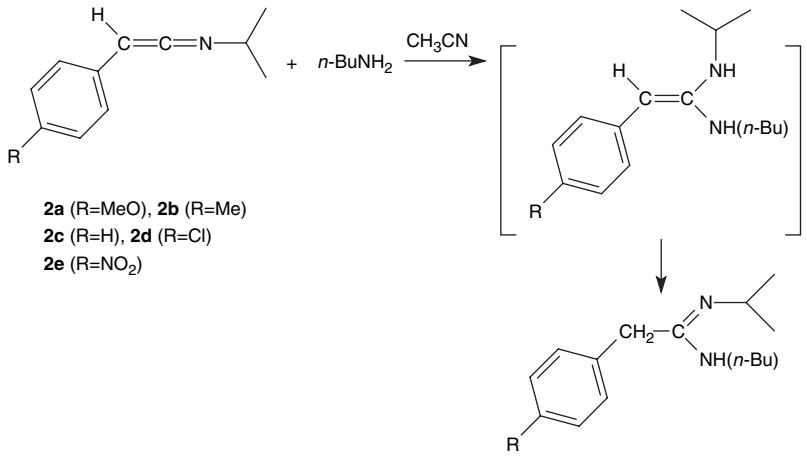
Table 2. Observed rate constants for the amination of **2a–e** in CH_3CN at 20 °C

[*n*-$BuNH_2$] (M)	R	k_{obs} (s^{-1})	[*n*-$BuNH_2$] (M)	R	k_{obs} (s^{-1})
0.9	MeO	2.51×10^{-4}	0.9	Me	3.60×10^{-4}
0.8	MeO	2.07×10^{-4}	0.8	Me	2.98×10^{-4}
0.7	MeO	1.57×10^{-4}	0.7	Me	2.28×10^{-4}
0.6	MeO	1.10×10^{-4}	0.6	Me	1.56×10^{-4}
0.9	H	7.25×10^{-4}	0.9	Cl	4.24×10^{-3}
0.8	H	5.95×10^{-4}	0.8	Cl	3.68×10^{-3}
0.7	H	4.77×10^{-4}	0.7	Cl	2.96×10^{-3}
0.6	H	3.85×10^{-4}	0.6	Cl	2.38×10^{-3}
0.05	NO_2	2.03×10^{-2}	0.04	NO_2	1.63×10^{-2}
0.03	NO_2	1.22×10^{-2}	0.02	NO_2	7.94×10^{-3}



1a (R=MeO)
1b (R=Me)
1c (R=H)
1d (R=Br)

Scheme 1.



2a (R=MeO), **2b** (R=Me)
2c (R=H), **2d** (R=Cl)
2e (R=NO_2)

Scheme 2.

Table 3. Second-order rate constants (k_1) for the amination of **2a–e** with *n*-$BuNH_2$ in CH_3CN at 20 °C[a]

R	k_1 ($M^{-1}\,s^{-1}$)	σ_p
MeO	4.73×10^{-4}	−0.27
Me	6.83×10^{-4}	−0.17
H	1.14×10^{-3}	0
Cl	6.30×10^{-3}	0.23
NO_2	4.13×10^{-1}	0.78

[a] ρ_1=2.87 (r^2=0.981).

When $k_2 \gg k_{-1}$, the first step of C=N addition becomes the rate-determining step and the rate law can be simplified into Eq. 3. According to the previous NMR and theoretical results,[7] the amination of **2a–e** is likely to follow this mechanism with the C=N addition as the rate-determining step. Then, a plot of k_{obs} for the amination of **2a–e** versus [*n*-$BuNH_2$] was drawn and it was a straight line for each of these amination reactions, indicating that the monitored process involves one molecule of *n*-$BuNH_2$. The slopes of these straight lines represent the corresponding second-order rate constants (k_1) of the first step of C=N addition, which are shown in Table 3. Hammett equation was applied to the second-order rate constants (k_1) of the first step of rate-determining C=N addition for the amination of **2a–e**, and is shown in Eq. 4. Based on this equation, a plot of $\log k_1(X)$ versus Hammett's substituent constant σ_p[9] was drawn, and it was a straight line with ρ_1=2.87 and a correlation coefficient r^2=0.981 (Table 3).

$$\text{Ketenimine(K)} + \text{Amine(A)} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Vinylidenediamine(V)} \xrightarrow{k_2} \text{Amidine} \quad (1)$$

According to the steady-state approximation,

$$\frac{d[V]}{dt} = k_1[A][K] - k_{-1}[V] - k_2[V] = 0;\ [V] = \frac{k_1}{k_{-1}+k_2}[A][K]$$

$$\text{rate} = \frac{-d[A]}{dt} = k_2[V] = \frac{k_1k_2}{k_{-1}+k_2}[A][K] \quad (2)$$

When $k_2 \gg k_{-1}$ and excess amine,

$$\text{rate} = \frac{-d[A]}{dt} = k_1[A][K] = k_{obs}[K] \quad (3)$$

$$\log\left(\frac{k_1(X)}{k_1(H)}\right) = \log k_1(X) - \log k_1(H) = \rho_1\sigma \quad (4)$$

When $k_2 \ll k_{-1}$ and excess amine, $K = k_1/k_{-1}$

$$\text{rate} = \frac{-d[A]}{dt} = \frac{k_1k_2}{k_{-1}}[A][K] = Kk_2[A][K] = k_{obs}[K] \quad (5)$$

$$\log Kk_2(X) - \log Kk_2(H) = \log\left(\frac{Kk_2(X)}{Kk_2(H)}\right) = \log\left(\frac{K(X)}{K(H)}\right) + \log\left(\frac{k_2(X)}{k_2(H)}\right) = \rho_K\sigma + \rho_2\sigma = (\rho_K + \rho_2)\sigma \quad (6)$$

The magnitude and sign of ρ of Hammett plot reflect the geometry of the transition state and indicate the electronic effects of the *para*-substituents to the remote reaction center.[8] Hammett plot for the amination of **2a–e** has a large and positive reaction constant ρ_1 (2.87), which indicates that the second-order rate constant (k_1) corresponds to the first step of rate-determining C=N addition for the amination of **2a–e**, the *para*-substituents on the phenyl group at C_β have direct resonance interaction with the reaction center at C_α, and the electron-withdrawing *para*-substituents on the phenyl group at C_β accelerate the reaction rate.

When $k_2 \ll k_{-1}$ in Eq. 2, the second step of tautomerization becomes the rate-determining step and the rate law can be simplified into Eq. 5. According to the previous NMR and theoretical results,[7] the amination of **1a–d** is assumed to follow this mechanism with the tautomerization as the rate-determining step. Then, a plot of k_{obs} for the amination of **1a–d** versus [*n*-$BuNH_2$] was drawn and it was a straight line

Table 4. Kk_2 or k_1 for the amination of **1a–d** with *n*-$BuNH_2$ in CH_3CN at 20 °C

R	Kk_2 (M^{-1} s^{-1})	σ_p
MeO	17.5[a]	−0.27
Me	89.4	−0.17
H	104	0
Br	470	0.23

[a] k_1 (s^{-1}) value.

for each of these amination reactions. The slopes of these straight lines represent the corresponding products (Kk_2) of the equilibrium constants of the first step by the second-order rate constants of the second step, which are shown in Table 4.

When Hammett equation was applied to the Kk_2 for the amination of **1a–d**, which corresponds to the reaction that includes one step prior to the rate-determining step, the ρ value should be a composite of the ρ_K and ρ_2 values,[10] which is shown in Eq. 6. Based on this equation, a plot of log Kk_2(X) versus Hammett's substituent constant σ_p[9] was drawn, and a convex curve was found as shown in Figure 4, indicating change of the rate-determining step. This is very similar to the substituent effect on the semicarbazone formation.[11]

As far as the amination of **1a–d** is concerned, for electron-withdrawing *para*-substituents on *N*-phenyl group, the first transition state of C═N addition is highly stabilized, resulting in the second step of tautomerization being the rate-determining step ($k_2 \ll k_{-1}$) and $\rho_A=\rho_K+\rho_2=0.98$ (Fig. 3). The ρ_K value is small because the *N*-substituent electronic effect on the relative stability between ketenimines and their corresponding vinylidenediamines is quite small based on the previous theoretical results.[7b] The ρ_2 value is also small since the *para*-substituents are far away from and not directly conjugated with the reaction center in the second step of tautomerization. Therefore, the small composite ρ_A value (0.98) is expected.

On the other hand, for electron-donating *para*-substituents on *N*-phenyl group in the amination of **1a–d**, the first transition state of C═N addition is much less stabilized, leading to the first C═N addition step being the rate-determining step ($k_2 \gg k_{-1}$) and $\rho_D=\rho_1=7.08$. Actually the rate law of Eq. 3 and the Hammett equation of Eq. 4 should be applied to this case, and the Kk_2 is supposed to be replaced by the k_1. The large and positive reaction constant ρ_1 (7.08) indicates that the first step of C═N addition is the rate-determining step, the *para*-substituents on *N*-phenyl group have a direct resonance interaction with the reaction center at C_α, and the electron-donating *para*-substituents decelerate the reaction rate. The reaction intermediate for the amination of **1a** with *N*-*p*-methoxyphenyl group couldn't be caught by low-temperature NMR spectrometry at −10 °C, and that confirms this mechanism. This ρ_1 value (7.08) is much higher than the ρ_1 value (2.87) for the amination of **2a–e**, clearly indicating that the first step of these amination reactions, which is the rate-determining step, involves C═N addition, instead of C═C addition.

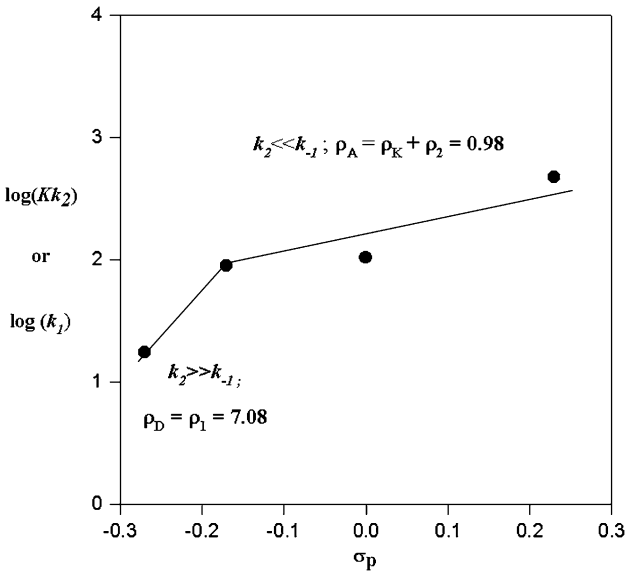


Figure 4. Hammett plot for the amination of **1a–d**.

In the amination of **2a–e**, electron-withdrawing *para*-substituents on the phenyl group at C_β stabilize the first transition state of C═N addition so that they accelerate the reaction rates. However, the extent to which they stabilize the first transition state is not significant enough to change the rate-determining step. On the other hand, in the amination of **1a–d**, electron-withdrawing *para*-substituents on *N*-phenyl group significantly stabilize the first transition state of C═N addition, and that does change the rate-determining step. It is known that steric effect of phenyl group is very close to that of *i*-propyl group,[12] so it is clear that the *N*-substituent electronic effect has much more significant influence on the amination of ketenimines than the substituent electronic effect at C_β.

In conclusion, the rate-determining step of the amination of **2a–e** is the first step of C═N addition, whose transition state is stabilized by electron-withdrawing substituents at C_β. On the other hand, electron-withdrawing *para*-substituents on *N*-phenyl group of ketenimines significantly stabilize the first transition state of C═N addition, resulting in change of the rate-determining step to the second step of tautomerization. Therefore, Hammett plot for the amination of **1a–d** is a convex curve, indicating change of the rate-determining step.

3. Experimental

3.1. General

Ketenimines **1a–c** and **2a–e** were known.[7] Ketenimine **1d** was prepared by the procedures given in the literature,[4h,4i,7] and its product analysis for the amination reaction is shown as follows. Product analyses for the kinetic studies of the amination of **1a–c** and **2a–e** have been done by NMR spectrometry and described in the previous publication.[7]

3.1.1. *N*-*p*-Tolylphenylketenimine (1d). Yield: 65%; ^1H NMR ($CDCl_3$) δ 2.39 (3H, s, CH_3), 5.26 (1H, s, CH), 7.18–7.40 (9H, m, PhH); ^{13}C NMR ($CDCl_3$) δ 21.1, 60.7, 123.6, 123.9, 124.6, 125.1, 125.4, 127.6, 128.2, 128.8, 129.3, 129.6, 132.7, 137.8, 190.5; IR (hexane) 2008 (C═C═N) cm^{-1}; HRMS (EI) *m/z* calcd for $C_{15}H_{13}N$ 207.1048, found 207.1050.

3.1.2. *N*-Butyl-*N*′-*p*-tolyl-2-phenylacetamidine. ^{1}H NMR ($CDCl_3$) δ 0.93 (3H, t, J=7.2 Hz, CH_3), 1.27–1.43 (4H, m, CH_2), 2.32 (3H, s, CH_3), 3.15 (2H, t, J=7.0 Hz, CH_2), 3.43 (2H, s, CH_2), 7.01–7.30 (9H, m, PhH); ^{13}C NMR ($CDCl_3$) δ 13.31, 20.07, 21.1, 31.17, 36.19, 41.02, 123.21, 124.52, 125.14, 128.28, 129.43, 129.82, 132.88, 136.01, 137.53; IR (thin film) 1653 (N–C═N) cm^{-1}; HRMS (EI) *m*/*z* calcd for $C_{19}H_{24}N_2$ 280.1939, found 296.1935.

3.1.3. Kinetic studies of amination of ketenimines 1a–d and 2a–e. Kinetics for the amination of **1a–d** and **2a–e** were carried out by injecting 2 μL of approximately 1 mM ketenimine solution in CH_3CN into 1 mL of *n*-$BuNH_2$ solution in CH_3CN, whose concentration was 0.6, 0.5, 0.4, 0.3, or 0.2 mM for **1a–d**, 0.9, 0.8, 0.7, or 0.6 M for **2a–d**, and 0.05, 0.04, 0.03, or 0.02 M for **2e**, in the thermostatic UV cell at 20 or −10 °C, monitoring the decrease in absorption at 260 or 270 nm with Perkin–Elmer Lambda 12 spectrometer. The SigmaPlot software was used to fit the exponential decays to get the rate constants. All rate constants were measured at least in duplicate with maximum deviations of ±5%.

Acknowledgment

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Synthesis of functionalized *p*-dihydrobenzoquinones and *p*-benzoquinones based on [3+3] cyclizations of 1,3-bis-silyl enol ethers

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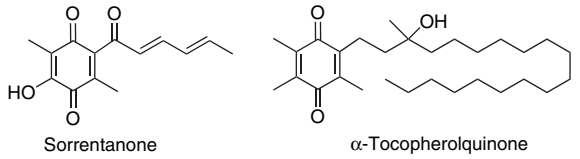
Abstract—Functionalized mono-protected *p*-dihydrobenzoquinones were prepared by [3+3] cyclization of 1,3-bis-silyl enol ethers with 2-acyloxy-3-(silyloxy)alk-2-en-1-ones. Deprotection and oxidation of the products afforded the corresponding *p*-benzoquinones.

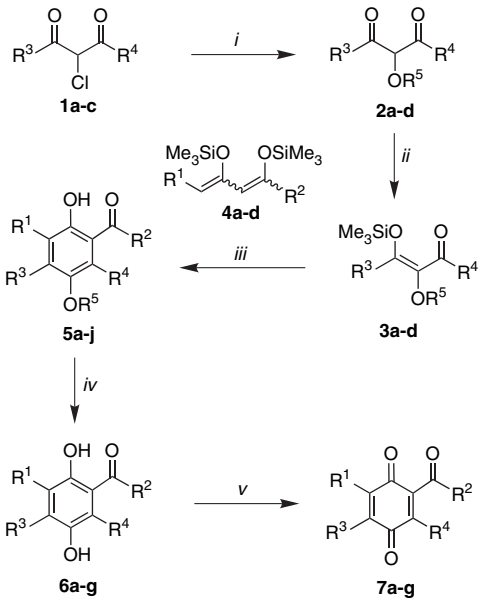
1. Introduction

p-Dihydrobenzoquinones[1,2] and *p*-benzoquinones[1,3,4] occur in a number of natural products, such as sorrentanone and α-tocopherolquinone (Scheme 1). They have found many technical and medicinal applications and represent important synthetic building blocks. Some years ago, Chan and co-workers reported an elegant approach to salicylates based on [3+3] cyclizations[5,6] of 1,3-bis-silyl enol ethers.[7] Recently, we reported the [3+3] cyclization of 1,3-bis-silyl enol ethers with 2-acyloxy-3-(silyloxy)alk-2-en-1-ones to give *p*-dihydrobenzoquinones.[8] Herein, we report full details of these studies. With regard to our preliminary communication,[8] we extended the methodology to the synthesis of *p*-benzoquinones. The transformations reported offer a convenient approach to functionalized mono-protected 1,4-dihydrobenzoquinones and to *p*-benzoquinones.



Scheme 1. *p*-Benzoquinones in natural products.

2-Chloro-1,3-diketones **1a–c** are readily available by reaction of 1,3-diketones with *N*-chlorosuccinimide (NCS).[9] The reaction of **1a–c** with sodium acetate and sodium benzoate afforded, according to a known procedure,[10] the 2-(acyloxy)alk-2-ene-1,3-diones **2a–d**. The reaction of **2a–d** with Me_3SiCl/NEt_3 gave the 2-acyloxy-3-(silyloxy)alk-2-en-1-ones **3a–d** (Scheme 2, Table 1). The $TiCl_4$ mediated [3+3] cyclization of **3a–d** with 1,3-bis-silyl enol ethers **4a–d** afforded the mono-protected *p*-dihydrobenzoquinones **5a–j**. Product **5f** contains one free and two orthogonally



Scheme 2. Synthesis of *p*-dihydrobenzoquinones **5a–j** and of *p*-benzoquinones **7a–g**; (i) NaOAc or NaOBz, DMSO, 3 h, 20 °C; (ii) Me_3SiCl, NEt_3, C_6H_6, 20 °C, 3 d; (iii) $TiCl_4$, CH_2Cl_2, −78→20 °C, 20 h; (iv) H_2SO_4 (5.0 M), THF, reflux, 36 h; (v) DDQ, C_6H_6, 3 h, 20 °C.

Keywords: Cyclization; Dihydroquinones; Oxidation; Quinones; Silyl enol ethers.
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Table 1. Products and yields

5,6,7	R^1	R^2	R^3	R^4	R^5	% (5)[a]	% (6)[a]	% (7)[a]
a	H	OMe	Me	Me	Ac	52	99[b]	90
b	H	OMe	Me	Ph	Ac	43	97[b]	84
c	Me	OEt	Me	Me	Ac	55	79	86
d	Et	OEt	Me	Me	Ac	54	91[b]	82
e	Et	OEt	Me	Ph	Ac	37	97[b]	82
f	OMe	OMe	Me	Me	Ac	50	83	86
g	H	OMe	Et	Et	Ac	51	98[b]	73
h	Me	OEt	Et	Et	Ac	39	—[c]	
i	H	OMe	Me	Me	Bz	55	—[c]	
j	Et	OEt	Me	Me	Bz	46	—[c]	

[a] Yields of isolated products.
[b] Used in crude form for the following step.
[c] Experiment not carried out.

protected hydroxyl groups. Treatment of a THF solution of **5a**–**g** with H_2SO_4 (5.0 M) resulted in clean deprotection to give **6a**–**g**; oxidation of the latter by DDQ afforded the *p*-benzoquinones **7a**–**g**.

The structure of all products was proved by spectroscopic methods. The structure of **5j** was independently confirmed by X-ray crystal structure analyses (Fig. 1).[11]

A great advantage of the methodology reported lies in the fact that mono-protected 1,4-dihydrobenzoquinones could be prepared without the need of regioselective protective group manipulations. The free hydroxyl group was successfully functionalized. For example, benzoate protected dihydroquinone **5i** was transformed into the corresponding enol triflate **8**. The Suzuki reaction of **8** with phenylboronic acid afforded the biaryl **9** (Scheme 3).

In conclusion, we have reported the synthesis of functionalized mono-protected *p*-dihydrobenzoquinones prepared by [3+3] cyclization of 1,3-bis-silyl enol ethers with 2-acyloxy-3-(silyloxy)alk-2-en-1-ones. Deprotection and oxidation of the products afforded the corresponding *p*-benzoquinones.

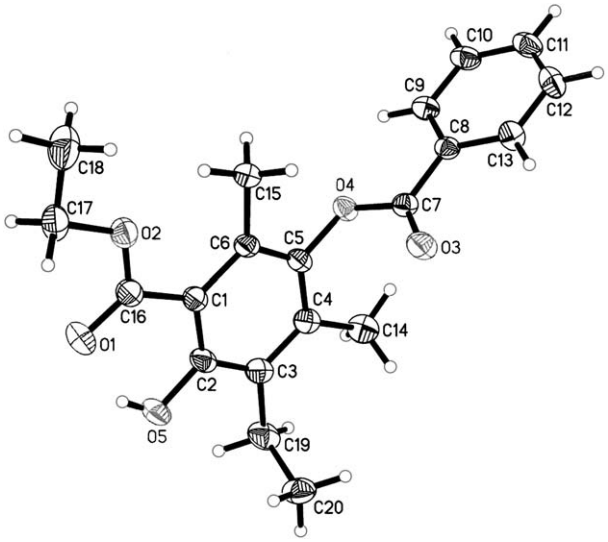
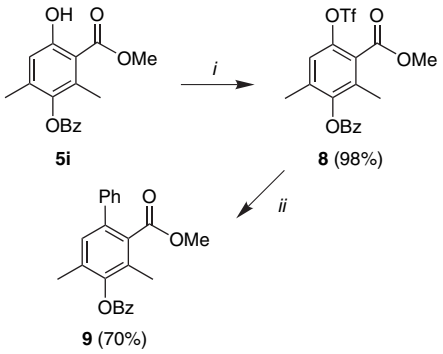


Figure 1. ORTEP plot of **5j** (non-hydrogen atoms are represented as thermal ellipsoids at the 30% probability level).



Scheme 3. (i) Tf_2O, pyridine, −78→−10 °C; (ii) phenylboronic acid, $Pd(PPh_3)_4$ (3 mol %), K_3PO_4 (1.5 equiv), dioxane, reflux.

2. Experimental

2.1. General comments

All solvents were dried by standard methods and all reactions were carried out under an inert atmosphere. For ^1H and ^{13}C NMR spectra the indicated deuterated solvents were used. Mass spectrometric data (MS) were obtained by electron ionization (EI, 70 eV), chemical ionization (CI, H_2O) or electrospray ionization (ESI). For preparative scale chromatography, silica gel (60–200 mesh) was used. Melting points are uncorrected.

2.2. General procedure for the synthesis of 2-acyloxy-1,3-diones (2b,g)

The 2-chloro-1,3-dione (1.0 mmol) was treated with sodium acetate (2.0 mmol) in anhydrous DMSO (2.60 mL/mmol) for 3 h at room temperature. After addition of water (50 mL), the organic layer was extracted with diethyl ether (3×). The combined organic layers were filtered, dried (Na_2SO_4) and concentrated. The residue was purified by chromatography (silica gel, 20% ethylacetate/heptane) to give the product.

2.2.1. Compound 2b. Starting with 2-chlorobenzoylacetone **1b** (3.00 g, 15.3 mmol), sodium acetate (2.50 g, 30.5 mmol) and DMSO (40 mL), **2b** was isolated as a colourless oil (2.40 g, 72%); ^1H NMR (300 MHz, $CDCl_3$): δ=2.22 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 6.25 (s, 1H, CH), 7.44–8.00 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=20.4, 26.8 (CH_3), 82.1 (CH), 128.0 (C), 128.7 (2C), 129.4 (2C), 134.2 (CH), 169.2, 190.8, 199.4 (C); IR (KBr): ν=3064 (w), 2934 (w), 1737 (s), 1693 (s), 1597 (m), 1449 (m), 1373 (m), 1228 (s), 1091 (m), 693 (m) cm^{-1}; MS (EI, 70 eV): *m/z* (%): 220.1 (M^+, 1), 178.1 (38), 136.0 (60), 105.0 (100), 77.0 (92); HRMS (EI): calcd for $C_{12}H_{12}O_4$ $[M]^+$: 220.0730; found: 220.0726.

2.2.2. Compound 2g. Starting with 4-chloro-3,5-heptanedione **1g** (3.0 g, 18.4 mmol), sodium acetate (3.00 g, 36.8 mmol) and DMSO (48 mL), **2g** was isolated as a colourless oil (2.50 g, 73%); ^1H NMR (300 MHz, $CDCl_3$): δ=1.01 (t, 6H, J=7.2 Hz, CH_3), 2.18 (s, 3H, CH_3), 2.50–2.74 (m, 4H, CH_2), 5.46 (s, 1H, CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=6.8 (2C), 20.4 (CH_3), 84.3 (CH), 169.3, 202.0 (2C, C);

IR (KBr): ν=2982 (w), 2943 (w), 1754 (s), 1737 (s), 1717 (s), 1374 (w), 1233 (s), 1091 (w) cm^{-1}; MS (EI, 70 eV): *m/z* (%): 186.0 (M^+, 1), 144.0 (20), 130.0 (20), 88.0 (94), 57.0 (100), 43.0 (95); elemental analysis: calcd (%) for $C_9H_{14}O_4$ (186.20): C 58.05, H 7.57; found: C 58.45, H 7.50.

2.3. General procedure for the synthesis of alkyl 3-acyloxy-6-hydroxy salicylates (5a–j)

To a stirred CH_2Cl_2 solution (2 mL/mmol) of 1,3-bis-silyl enol ether (1.0 mmol) and silyl enol ether (1.0 mmol) was added $TiCl_4$ (1.0 mmol) at −78 °C under argon atmosphere. The temperature of the reaction mixture was allowed to rise to 20 °C during 20 h and saturated aqueous solution of $NaHCO_3$ (10 mL) was added. The organic layer was separated and extracted with diethyl ether (3×30 mL). The combined organic layers were dried (Na_2SO_4), filtered and the filtrate was concentrated in vacuo. The residue was purified by column chromatography using 20% ethylacetate/heptane as eluent.

2.3.1. Compound 5a. Starting with 1,3-bis-silyl enol ether **4b** (1.00 g, 3.8 mmol), silyl enol ether **3a** (885 mg, 3.8 mmol) and $TiCl_4$ (0.42 ml, 3.8 mmol), **5a** was isolated as a colourless solid (470 mg, 52%), mp 79 °C; ^{1}H NMR (250 MHz, $CDCl_3$): δ=2.12 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 3.93 (s, 3H, OCH_3), 6.73 (s, 1H, ArH), 11.07 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=15.3, 17.1, 20.3, 52.1 (CH_3), 110.7 (C), 117.1 (CH), 132.1, 138.2, 141.0, 160.1, 169.1, 171.6 (C); IR (KBr): ν=3490 (w), 2990 (w), 2961 (m), 1759 (s), 1664 (s), 1621 (m), 1476 (m), 1439 (m), 1362 (m),1319 (m), 1194 (s), 1072 (m), 802 (m) cm^{-1}; MS (EI, 70 eV): *m/z* (%): 238 (M^+, 12), 196 (44), 164 (100); HRMS (EI): calcd for $C_{12}H_{14}O_5$ $[M]^+$: 238.0836; found: 238.0837.

2.3.2. Compound 5b. Starting with 1,3-bis-silyl enol ether **4b** (755 mg, 2.8 mmol), silyl enol ether **3b** (847 mg, 2.8 mmol) and $TiCl_4$ (0.31 ml, 2.8 mmol), **5b** was isolated as a colourless solid (366 mg, 43%), mp 97 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.80 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 3.39 (s, 3H, OCH_3), 6.91 (s, 1H, ArH), 7.08–7.33 (m, 5H, ArH), 10.89 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=17.0, 19.9, 51.7 (CH_3), 110.4 (C), 118.9, 126.8, 127.3 (2C), 128.2 (2C, CH), 136.4, 137.6, 138.8, 140.2, 159.5, 169.3, 170.7 (C); IR (KBr): ν=3526 (w), 3422 (w), 3055 (w), 3032 (w), 2954 (w), 1754 (s), 1670 (s), 1438 (m), 1358 (m), 1328 (m), 1237 (m), 1217 (s), 1205 (s), 1193 (s), 1072 (m), 705 (m) cm^{-1}; MS (EI, 70 eV): *m/z* (%): 300.1 (M^+, 3), 258.1 (35), 226.0 (100); elemental analysis: calcd (%) for $C_{17}H_{16}O_5$ (300.31): C 67.99, H 5.37; found: C 67.93, H 5.69.

2.3.3. Compound 5c. Starting with 1,3-bis-silyl enol ether **4c** (808 mg, 2.8 mmol), silyl enol ether **3a** (645 mg, 2.8 mmol) and $TiCl_4$ (0.30 ml, 2.8 mmol), **5c** was isolated as a colourless solid (410 mg, 55%), mp 96 °C; ^{1}H NMR (250 MHz, $CDCl_3$): δ=1.40 (t, 3H, J=8.4 Hz, CH_3), 2.09 (s, 3H, CH_3), 2.18 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 4.41 (q, 2H, J=7.0 Hz, OCH_2), 11.51 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=9.7, 11.9, 12.2, 13.4, 18.5 (CH_3), 59.7 (CH_2), 108.2, 121.8, 126.8, 134.3, 138.7, 156.4, 167.5, 169.8 (C); IR (KBr): ν=3413 (w), 2989 (w), 2970 (w), 2904 (w), 1753 (s), 1653 (s), 1404 (m), 1369 (m), 1312 (m), 1262 (m),1215 (s), 1197 (s), 1035 (w), 801 (m) cm^{-1}; GC–MS (EI, 70 eV): *m/z* (%): 266.1 (M^+, 4), 224.1 (18), 178.0 (100), 150.1 (15), 43.1 (72); HRMS (EI): calcd for $C_{14}H_{18}O_5$ $[M]^+$: 266.1149; found: 266.1147.

2.3.4. Compound 5d. Starting with 1,3-bis-silyl enol ether **4d** (615 mg, 2.0 mmol), silyl enol ether **3a** (472 mg, 2.0 mmol) and $TiCl_4$ (0.22 ml, 2.0 mmol), **5d** was isolated as a colourless solid (304 mg, 54%), mp 62 °C; ^{1}H NMR (250 MHz, $CDCl_3$): δ=1.10 (t, 3H, J=7.3 Hz, CH_3), 1.40 (t, 3H, J=7.0 Hz, CH_3), 2.11 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.65–2.75 (m, 2H, CH_2), 4.41 (q, 2H, J=7.0 Hz, OCH_2), 11.46 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=13.0, 13.1, 14.1, 15.3 (CH_3), 19.6 (CH_2), 20.3 (CH_3), 61.5 (CH_2), 110.2, 128.8, 129.6, 135.6, 140.7, 158.2, 169.3, 171.7 (C); IR (KBr): ν=3431 (w), 2979 (s), 2936 (m), 1757 (s), 1651 (s), 1465 (m), 1445 (m), 1395 (s), 1377 (s), 1367 (s), 1320 (s), 1278 (s), 1219 (s), 1042 (m), 806 (m) cm^{-1}; GC–MS (EI, 70 eV): *m/z* (%): 280.1 (M^+, 7), 238.1 (16), 192.1 (100), 164.0 (26), 43.0 (90); HRMS (EI): calcd for $C_{15}H_{20}O_5$ $[M]^+$: 280.1305; found: 280.1308.

2.3.5. Compound 5e. Starting with 1,3-bis-silyl enol ether **4d** (700 mg, 2.3 mmol), silyl enol ether **3b** (676 mg, 2.3 mmol) and $TiCl_4$ (0.25 ml, 2.3 mmol), **5e** was isolated as a colourless solid (290 mg, 37%), mp 79 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=0.65 (t, 3H, J=7.1 Hz, CH_3), 1.17 (t, 3H, J=7.5 Hz, CH_3), 1.79 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 2.78 (b, 2H, CH_2), 3.89 (q, 2H, J=7.1 Hz, OCH_2), 7.08–7.30 (m, 5H, ArH), 11.34 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=12.8, 13.0, 13.1 (CH_3), 19.9 (CH_2), 20.0 (CH_3), 60.8 (CH_2), 109.8 (C), 126.5, 127.3 (2C), 128.5 (2C, CH), 131.6, 133.3, 136.1, 138.3, 140.0, 157.7, 169.6, 170.9 (C); IR (KBr): ν=3444 (w), 2987 (m), 2937 (w), 1758 (s), 1655 (s), 1397 (m), 1376 (m), 1328 (m), 1279 (m), 1211 (s), 1117 (m), 1009 (w), 811 (w) cm^{-1}; GC–MS (EI, 70 eV): *m/z* (%): 342 (M^+, 4), 300 (38), 254 (95), 236 (28), 129 (57), 43 (100); elemental analysis: calcd (%) for $C_{20}H_{22}O_5$ (342.39): C 70.16, H 6.48; found: C 69.99, H 6.80.

2.3.6. Compound 5f. Starting with 1,3-bis-silyl enol ether **4f** (600 mg, 2.0 mmol), silyl enol ether **3a** (476 mg, 2.0 mmol) and $TiCl_4$ (0.22 ml, 2.0 mmol), **5f** was isolated as a colourless solid (263 mg, 50%), mp 68–72 °C; ^{1}H NMR (250 MHz, $CDCl_3$): δ=2.10 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 11.16 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=10.8, 15.4, 20.7, 52.7, 60.6 (CH_3), 111.6, 127.2, 131.5, 140.8, 145.3, 154.4, 169.5, 172.1 (C); IR (KBr): ν=3501 (w), 3009 (w), 2960 (w), 2937 (w), 1753 (s), 1660 (s), 1440 (s), 1352 (s), 1214 (s), 1071 (m), 1041 (m), 806 (m) cm^{-1}; GC–MS (EI, 70 eV): *m/z* (%): 268 (M^+, 10), 226 (17), 194 (100), 165 (38), 67 (46); HRMS (EI): calcd for $C_{13}H_{16}O_6$ $[M]^+$: 268.0941; found: 268.0942.

2.3.7. Compound 5g. Starting with 1,3-bis-silyl enol ether **4b** (500 mg, 1.9 mmol), silyl enol ether **3g** (496 mg, 1.9 mmol) and $TiCl_4$ (0.21 ml, 1.9 mmol), **5g** was isolated as a colourless oil (258 mg, 51%); ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.14 (t, 3H, J=7.4 Hz, CH_3), 1.25 (t, 3H,

J=7.5 Hz, CH_3), 2.32 (s, 3H, CH_3), 2.40–2.89 (m, 4H, CH_2), 3.95 (s, 3H, OCH_3), 6.78 (s, 1H, ArH), 11.16 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=12.9, 14.5, 20.5 (CH_3), 22.3, 23.6 (CH_2), 52.3 (CH_3), 110.2 (C), 115.6 (CH), 138.0, 140.3, 143.9, 160.4, 171.4 (C); IR (KBr): ν=2975 (m), 2879 (w), 1762 (s), 1665 (s), 1436 (m), 1369 (m), 1322 (m), 1232 (s), 1195 (s), 1079 (m), 808 (w) cm^{-1}; GC–MS (EI, 70 eV): *m/z* (%): 266 (M^+, 6), 235 (9), 224 (44), 192 (100), 149 (25); HRMS (EI): calcd for $C_{14}H_{18}O_5$ $[M]^+$: 266.1144; found: 266.1149.

2.3.8. Compound 5h. Starting with 1,3-bis-silyl enol ether **4c** (400 mg, 1.3 mmol), silyl enol ether **3g** (359 mg, 1.3 mmol) and $TiCl_4$ (0.15 ml, 1.3 mmol), **5h** was isolated as a colourless solid (155 mg, 39%), mp 40 °C; ^{1}H NMR (250 MHz, $CDCl_3$): δ=1.09 (t, 3H, J=7.6 Hz, CH_3), 1.13 (t, 3H, J=7.6 Hz, CH_3), 1.14 (t, 3H, J=7.3 Hz, CH_3), 2.21 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.40–2.98 (m, 4H, CH_2), 4.42 (q, 2H, J=7.0 Hz, OCH_2), 11.43 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=11.5, 13.1, 13.9, 14.7, 20.6 (CH_3), 21.7, 22.3, 61.7 (CH_2), 109.8, 123.6, 134.9, 140.1, 141.9, 158.7, 170.3, 171.5 (C); IR (KBr): ν=3430 (w), 2971 (m), 2937 (m), 1755 (s), 1651 (s), 1407 (m), 1370 (m), 1314 (m), 1274 (m), 1219 (s), 1197 (s), 1022 (m), 807 (m) cm^{-1}; MS (EI, 70 eV): *m/z* (%): 294.2 (M^+, 27), 252.2 (75), 206.1 (100), 163.1 (19); HRMS (EI): calcd for $C_{16}H_{22}O_5$ $[M]^+$: 294.1462; found: 294.1466.

2.3.9. Compound 5i. Starting with 1,3-bis-silyl enol ether **4b** (1.00 g, 3.8 mmol), silyl enol ether **3i** (1.12 g, 3.8 mmol) and $TiCl_4$ (0.42 ml, 3.8 mmol), **5i** was isolated as a colourless solid (625 mg, 55%), mp 80 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.17 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 3.95 (s, 3H, OCH_3), 6.78 (s, 1H, ArH), 7.51–8.26 (m, 5H, ArH), 11.16 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=15.5, 17.3, 52.2 (CH_3), 110.9 (C), 117.2, 128.7 (2C, CH), 128.9 (C), 130.2 (2C, CH), 132.5 (C), 133.8 (CH), 138.6, 141.1, 160.2, 164.7, 171.7 (C); IR (KBr): ν=3431 (w), 3034 (w), 2985 (w), 2958 (w), 1732 (s), 1661 (s), 1622 (m), 1443 (m), 1360 (m), 1323 (m), 1262 (s), 1236 (s), 1202 (s), 1153 (m), 1085 (s), 1062 (s), 1023 (m), 803 (m), 705 (s) cm^{-1}; MS (EI, 70 eV): *m/z* (%): 300.1 (M^+, 39), 269.1 (9), 105.0 (100), 77.0 (71); HRMS (EI): calcd for $C_{17}H_{16}O_5$ $[M]^+$: 300.0992; found: 300.0990.

2.3.10. Compound 5j. Starting with 1,3-bis-silyl enol ether **4d** (800 mg, 2.6 mmol), silyl enol ether **3i** (773 mg, 2.6 mmol) and $TiCl_4$ (0.29 ml, 2.6 mmol), **5j** was isolated as a colourless solid (410 mg, 46%), mp 89 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.13 (t, 3H, J=7.5 Hz, CH_3), 1.40 (t, 3H, J=7.2 Hz, CH_3), 2.16 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.74 (q, 2H, J=3.5 Hz, CH_2), 4.42 (q, 2H, J=7.0 Hz, OCH_2), 7.51–8.27 (m, 5H, ArH), 11.50 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ=13.2, 13.3, 14.2, 15.6 (CH_3), 19.8, 61.7 (CH_2), 128.7 (2C, CH), 129.1, 129.2, 129.8 (C), 130.2 (2C), 133.7 (CH), 135.9, 140.9, 158.4, 164.9, 171.9 (C); IR (KBr): ν=3437 (m), 2975 (m), 2933 (m), 2874 (w), 1731 (s), 1650 (s), 1611 (m), 1451 (m), 1394 (m), 1373 (m), 1321 (m), 1252 (s), 1205 (s), 1104 (s), 1066 (m), 1039 (m), 1026 (m), 807 (m), 715 (s) cm^{-1}; MS (EI, 70 eV): *m/z* (%): 342.2 (M^+, 67), 296.1 (50), 191.1 (23), 105.0 (100); elemental analysis: calcd (%) for $C_{20}H_{22}O_5$ (342.39): C 70.16, H 6.48; found: C 70.14, H 6.78.

2.4. General procedure for the synthesis of *p*-dihydrobenzoquinones (6a–g)

H_2SO_4 (5 M, 8 mL/mmol) was added to a solution of starting material (1.0 mmol) in THF (50 mL/mmol) and refluxed for 36 h. The reaction mixture was concentrated on rotary, taken up in dichloromethane (20 mL) and washed with water (50 mL). The organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by chromatography (silica gel, 20% ethylacetate/heptane) to give the product

2.4.1. Compound 6a. Starting with **5a** (265 mg, 1.1 mmol), 5 M H_2SO_4 (9 mL) and THF (50 mL), **6a** was isolated as a colourless solid (215 mg, 99%), mp 120 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.25 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 3.95 (s, 3H, OCH_3), 6.66 (s, 1H, ArH), 10.60 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=14.5, 16.9, 52.0 (CH_3), 110.8 (C), 116.6 (CH), 124.5, 132.5, 145.1, 156.0, 171.8 (C); IR (KBr): ν=3505 (s), 3086 (m), 2944 (m), 2862 (w), 1654 (s), 1621 (m), 1481 (s), 1442 (s), 1333 (s), 1237 (b), 1076 (m), 1050 (s), 967 (m), 796 (s), 719 (m) cm^{-1}; MS (EI, 70 eV): *m/z* (%): 196.0 (M^+, 63), 163.9 (100), 135.9 (40), 107.0 (18), 79.0 (16); HRMS (EI): calcd for $C_{10}H_{12}O_4$ $[M]^+$: 196.0725; found: 196.0730.

2.4.2. Compound 6b. Starting with **5b** (264 mg, 0.8 mmol), 5 M H_2SO_4 (8 mL) and THF (40 mL), **6b** was isolated as a colourless solid (220 mg, 97%), mp 138 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.28 (s, 3H, CH_3), 3.39 (s, 3H, OCH_3), 6.85 (s, 1H, ArH), 7.18–7.49 (m, 5H, ArH), 10.56 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=16.9, 51.5 (CH_3), 109.0 (C), 119.1 (CH), 126.5 (C), 127.9, 128.9 (2C), 129.2 (2C, CH), 133.7, 136.4, 144.2, 155.5, 170.9 (C); IR (KBr): ν=3530 (s), 3421 (b), 2945 (m), 2867 (w), 1670 (s), 1455 (s), 1434 (s), 1331 (s), 1184 (b), 1076 (m), 760 (m), 705 (m) cm^{-1}; MS (EI, 70 eV): *m/z* (%): 258.0 (M^+, 53), 226.0 (100), 197.9 (29), 141.0 (25), 115.0 (15); HRMS (EI): calcd for $C_{15}H_{14}O_4$ $[M]^+$: 258.0887; found: 258.0881.

2.4.3. Compound 6c. Starting with **5c** (70 mg, 0.2 mmol), 5 M H_2SO_4 (2 mL) and THF (14 mL), **6c** was isolated as a colourless solid (46 mg, 79%), mp 105 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.41 (t, 3H, J=5.3 Hz, CH_3), 2.17 (s, 3H, CH_3), 2.21 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 4.30 (s, 1H, OH), 4.41 (q, 2H, J=7.1 Hz, OCH_2), 10.97 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ=11.9, 13.2, 14.2, 14.6 (CH_3), 61.5 (CH_2), 110.1, 121.1, 123.0, 131.1, 144.6, 154.4, 171.9 (C); IR (KBr): ν=3382 (b), 2983 (m), 2940 (w), 1655 (s), 1616 (m), 1468 (m), 1439 (m), 1394 (m), 1376 (m), 1223 (m), 1268 (s), 1213 (s), 1049 (m), 1034 (m), 799 (m), 746 (m) cm^{-1}; MS (EI, 70 eV): *m/z* (%): 224.0 (M^+, 26), 177.9 (100), 15.0 (61); HRMS (EI): calcd for $C_{12}H_{16}O_4$ $[M]^+$: 224.1042; found: 224.1043.

2.4.4. Compound 6d. Starting with **5d** (84 mg, 0.3 mmol), 5 M H_2SO_4 (3 mL) and THF (15 mL), **6d** was isolated as a colourless solid (65 mg, 91%), mp 98 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.11 (t, 3H, J=7.5 Hz, CH_3), 1.43 (t, 3H, J=7.1 Hz, CH_3), 2.26 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 2.72 (q, 2H, J=7.5 Hz, CH_2), 4.43 (q, 2H, J=7.1 Hz, OCH_2), 10.92 (s, 1H, OH); ^{13}C NMR (75 MHz,

$CDCl_3$): δ=12.5, 13.5, 14.2, 14.6 (CH_3), 19.6, 61.4 (CH_2), 110.2, 121.2, 129.1, 130.5, 144.8, 154.2, 171.9 (C); IR (KBr): ν=3450 (s), 1979 (s), 2936 (m), 2873 (m), 1655 (s), 1614 (m), 1465 (m), 1393 (m), 1375 (s), 1323 (m), 1276 (s), 1212 (s), 1109 (m), 1096 (m), 1047 (m), 802 (m), 764 (m) cm^{-1}; MS (EI, 70 eV): *m/z* (%): 238.1 (M^+, 48), 192.1 (100), 177.1 (20), 164.1 (95), 149.1 (20), 121.1 (12), 91.1 (17); HRMS (EI): calcd for $C_{13}H_{18}O_4$ $[M]^+$: 238.1200; found: 238.1202.

2.4.5. Compound 6e. Starting with **5e** (166 mg, 0.4 mmol), 5 M H_2SO_4 (4 mL) and THF (25 mL), **6e** was isolated as a colourless solid (140 mg, 97%), mp 68 °C; ^1H NMR (300 MHz, $CDCl_3$): δ=0.65 (t, 3H, *J*=7.1 Hz, CH_3), 1.17 (t, 3H, *J*=7.5 Hz, CH_3), 2.34 (s, 3H, CH_3), 2.78 (q, 2H, *J*=7.5 Hz, CH_2), 3.89 (q, 2H, *J*=7.1 Hz, OCH_2), 7.18–7.47 (m, 5H, ArH), 11.01 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=12.4, 12.9, 13.3 (CH_3), 19.7, 60.6 (CH_2), 108.4 (C), 127.7 (CH), 128.5 (C), 128.9 (2C), 129.6 (2C, CH), 131.2, 131.9, 137.1, 143.8, 153.9, 171.1 (C); IR (KBr): ν=3531 (s), 2977 (m), 2934 (m), 2872 (w), 1653 (s), 1444 (m), 1398 (s), 1373 (s), 1330 (s), 1293 (m), 1249 (m), 1209 (s), 1080 (m), 1059 (m), 763 (m), 699 (m) cm^{-1}; MS (EI, 70 eV): *m/z* (%): 300.1 (M^+, 38), 254.1 (100), 139. (61), 221.0 (46), 183.1 (14), 129.0 (65); HRMS (EI): calcd for $C_{18}H_{20}O_4$ $[M]^+$: 300.1356; found: 300.1358.

2.4.6. Compound 6f. Starting with **5f** (106 mg, 0.3 mmol), 5 M H_2SO_4 (3 mL) and THF (20 mL), **6f** was isolated as a colourless solid (73 mg, 83%), mp 81 °C; ^1H NMR (300 MHz, $CDCl_3$): δ=2.22 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 4.44 (s, 1H, OH), 10.52 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=9.7, 14.1, 52.1, 60.3 (CH_3), 111.3, 119.2, 125.3, 144.6, 144.7, 149.6, 171.8 (C); IR (KBr): ν=3523 (s), 3152 (w), 3004 (w), 2964 (m), 2854 (w), 1642 (s), 1480 (m), 1441 (s), 1421 (s), 1371 (m), 1329 (s), 1274 (s), 1229 (s), 1122 (m), 1070 (m), 1048 (s), 1032 (m), 967 (m), 800 (s), 759 (m) cm^{-1}; MS (EI, 70 eV): *m/z* (%): 226.0 (M^+, 37), 194.0 (100), 165.0 (80), 151.0 (41), 95.1 (28), 83.0 (40); HRMS (EI): calcd for $C_{11}H_{14}O_4$ $[M]^+$: 226.0836; found: 226.0837.

2.4.7. Compound 6g. Starting with **5g** (170 mg, 0.4 mmol), 5 M H_2SO_4 (5 mL) and THF (30 mL), **6g** was isolated as a colourless oil (140 mg, 98%); ^1H NMR (400 MHz, $CDCl_3$): δ=1.19 (t, 3H, *J*=7.5 Hz, CH_3), 1.23 (t, 3H, *J*=7.5 Hz, CH_3), 2.61 (q, 2H, *J*=7.5 Hz, CH_2), 2.92 (q, 2H, *J*=7.5 Hz, CH_2), 3.95 (s, 3H, OCH_3), 6.69 (s, 1H, ArH), 10.58 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ=12.9, 14.5 (CH_3), 20.9, 22.3 (CH_2), 52.2 (CH_3), 110.1 (C), 115.6 (CH), 130.5, 138.4, 143.9, 156.1, 171.6 (C); IR (KBr): ν=3442 (m), 2966 (m), 2875 (m), 1662 (s), 1437 (s), 1325 (m), 1252 (m), 1213 (m), 1081 (m), 806 (w) cm^{-1}; GC–MS (EI, 70 eV): *m/z* (%): 224.1 (M^+, 18), 192.1 (100), 149.1 (38), 91.1 (10); HRMS (EI): calcd for $C_{12}H_{16}O_4$ $[M]^+$: 224.1043; found: 224.1041.

2.5. General procedure for the synthesis of *p*-benzoquinones (7a–g)

A solution of dihydroquinones (**6a–g**) (1.0 mmol) and DDQ (1.0 mmol) in benzene (20 mL/mmol) was stirred at room temperature for 2 h. The reaction mixture was filtered, dried (Na_2SO_4) and concentrated. The residue was purified by chromatography (silica gel, 30% ethylacetate/heptane) to give the product.

2.5.1. Compound 7a. Starting with **6a** (107 mg, 0.5 mmol), DDQ (124 mg, 0.5 mmol) and benzene (11 mL), **7a** was isolated as a yellow solid (95 mg, 90%), mp 48 °C; ^1H NMR (300 MHz, $CDCl_3$): δ=2.02 (s, 3H, CH_3), 2.05 (d, 3H, *J*=1.5 Hz, CH_3), 3.88 (s, 3H, OCH_3), 6.57 (d, 1H, *J*=1.5 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=13.5, 16.0, 52.7 (CH_3), 132.6 (CH), 137.0, 142.0, 146.0, 164.4, 183.5, 187.3 (C); IR (KBr): ν=3455 (w), 2955 (w), 1737 (s), 1658 (s), 1622 (m), 1440 (m), 1331 (s), 1238 (s), 1179 (m), 1043 (m), 898 (w) cm^{-1}; MS (EI, 70 eV): *m/z* (%): 222.0 (M^+, 82), 193.9 (83), 175.9 (100), 147.9 (93), 119.9 (36), 91.0 (61); HRMS (EI): calcd for $C_{10}H_{10}O_4$ $[M]^+$: 194.0573; found: 194.0574.

2.5.2. Compound 7b. Starting with **6b** (125 mg, 0.4 mmol), DDQ (110 mg, 0.4 mmol) and benzene (10 mL), **7b** was isolated as a yellow solid (105 mg, 84%), mp 128 °C; ^1H NMR (300 MHz, $CDCl_3$): δ=2.12 (s, 3H, CH_3), 3.64 (s, 3H, OCH_3), 6.71 (d, 1H, *J*=1.5 Hz, ArH), 7.25–7.29 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=16.2, 52.5 (CH_3), 128.1 (2C), 129.1 (2C), 129.8 (C, CH), 130.9 (C), 132.7 (CH), 136.8, 142.4, 146.1, 164.1, 183.8, 186.5 (C); IR (KBr): ν=3447 (w), 2950 (w), 1736 (s), 1653 (s), 1634 (m), 1432 (m), 1324 (m), 1237 (s), 1113 (m), 1036 (m), 755 (m), 698 (m) cm^{-1}; GC–MS (EI, 70 eV): *m/z* (%): 256.0 (M^+, 29), 224.0 (100), 196.0 (36), 168.0 (28), 139.0 (60); elemental analysis: calcd (%) for $C_{15}H_{12}O_4$ (256.25): C 70.31, H 4.72; found: C 70.08, H 4.85.

2.5.3. Compound 7c. Starting with **6c** (50 mg, 0.2 mmol), DDQ (51 mg, 0.2 mmol) and benzene (5 mL), **7c** was isolated as a yellow solid (42 mg, 86%), mp 46 °C; ^1H NMR (300 MHz, $CDCl_3$): δ=1.35 (t, 3H, *J*=7.1 Hz, CH_3), 2.02 (s, 9H, CH_3), 4.36 (q, 2H, *J*=7.1 Hz, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ=12.1, 12.4, 13.3, 14.1 (CH_3), 61.9 (CH_2), 137.0, 140.3, 141.11, 141.16, 164.4, 183.8, 186.9 (C); IR (KBr): ν=3452 (w), 2993 (w), 2930 (w), 1739 (s), 1648 (s), 1625 (m), 1374 (m), 1292 (s), 1216 (s), 1061 (m), 1023 (m), 713 (w) cm^{-1}; MS (EI, 70 eV): *m/z* (%): 222.0 (M^+, 82), 193.9 (83), 175.9 (100), 147.9 (93), 119.9 (36), 91.0 (61); HRMS (EI): calcd for $C_{12}H_{14}O_4$ $[M]^+$: 222.0882; found: 222.0887.

2.5.4. Compound 7d. Starting with **6d** (64 mg, 0.2 mmol), DDQ (61 mg, 0.2 mmol) and benzene (6 mL), **7d** was isolated as a yellow solid (50 mg, 82%), mp 43 °C; ^1H NMR (300 MHz, $CDCl_3$): δ=1.02 (t, 3H, *J*=7.5 Hz, CH_3), 1.34 (t, 3H, *J*=7.1 Hz, CH_3), 2.00 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.47 (q, 2H, *J*=7.5 Hz, CH_2), 4.36 (q, 2H, *J*=7.1 Hz, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ=11.8, 12.7, 13.2, 14.1 (CH_3), 19.6, 61.9 (CH_2), 137.1, 140.5, 140.9, 145.4, 164.4, 183.4, 187.3 (C); IR (KBr): ν=3531 (s), 2977 (m), 2934 (m), 2872 (w), 1653 (s), 1444 (m), 1398 (s), 1373 (s), 1330 (s), 1293 (m), 1249 (m), 1209 (s), 1080 (m), 1059 (m), 763 (m), 699 (m) cm^{-1}; MS (EI, 70 eV): *m/z* (%): 236.1 (M^+, 68), 190.1 (99), 162.1 (100), 134.1 (18), 105.1 (11), 91.1 (43), 67.1 (70); HRMS (EI): calcd for $C_{13}H_{16}O_4$ $[M]^+$: 236.1043; found: 236.1036.

2.5.5. Compound 7e. Starting with **6e** (92 mg, 0.3 mmol), DDQ (70 mg, 0.3 mmol) and benzene (7 mL), **7e** was isolated as a yellow solid (73 mg, 82%), mp 43 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.02 (t, 3H, J=7.1 Hz, CH_3), 1.06 (t, 3H, J=7.5 Hz, CH_3), 2.10 (s, 3H, CH_3), 2.56 (q, 2H, J=7.5 Hz, CH_2), 4.12 (q, 2H, J=7.1 Hz, OCH_2), 7.25–7.40 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=12.1, 12.8, 13.7 (CH_3), 19.8, 61.8 (CH_2), 128.0 (2C), 129.2 (2C), 129.6 (C, CH), 131.2, 136.9, 140.6, 141.8, 145.7, 164.0, 183.8, 186.6 (C); IR (KBr): ν=3442 (m), 2974 (m), 2937 (w), 2875 (w), 1736 (s), 1651 (s), 1615 (m), 1445 (m), 1373 (m), 1286 (s), 1221 (s), 1152 (m), 1021 (m), 699 (m) cm^{-1}; GC–MS (EI, 70 eV): m/z (%): 298.1 (M^+, 3), 252.0 (100), 237.0 (38), 224.1 (98), 129.0 (50); elemental analysis: calcd (%) for $C_{18}H_{18}O_4$ (298.33): C 72.47, H 6.08; found: C 72.28, H 5.83.

2.5.6. Compound 7f. Starting with **6f** (45 mg, 0.2 mmol), DDQ (46 mg, 0.2 mmol) and benzene (4 mL), **7f** was isolated as a yellow solid (38 mg, 86%), mp 40 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.95 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ=8.9, 13.5, 52.7, 61.0 (CH_3), 128.9, 135.4, 142.2, 154.7, 164.6, 180.0, 187.2 (C); IR (KBr): ν=3430 (s), 2956 (w), 1740 (m), 1655 (s), 1616 (m), 1289 (m), 1225 (m), 1152 (w), 1056 (w), 950 (w), 728 (w) cm^{-1}; GC–MS (EI, 70 eV): m/z (%): 224 (M^+, 22), 192 (65), 164 (64), 149 (19), 135 (16), 83 (66), 67 (100); HRMS (EI): calcd for $C_{11}H_{12}O_5$ $[M]^+$: 224.0679; found: 224.0678.

2.5.7. Compound 7g. Starting with **6g** (109 mg, 0.5 mmol), DDQ (110 mg, 0.5 mmol) and benzene (10 mL), **7g** was isolated as a yellow oil (80 mg, 73%); ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.10 (t, 3H, J=7.5 Hz, CH_3), 1.13 (t, 3H, J=7.5 Hz, CH_3), 2.42 (q, 2H, J=7.5 Hz, CH_2), 2.78 (dq, 2H, J=7.4 Hz, 1.7 Hz, CH_2), 3.89 (s, 3H, OCH_3), 6.52 (t, 1H, J=1.6 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ=11.5, 13.6 (CH_3), 21.6, 22.2 (CH_2), 52.6 (CH_3), 130.8 (CH), 136.3, 146.8, 151.1, 164.5, 184.2, 186.7 (C); IR (KBr): ν=2976 (m), 2941 (m), 1741 (s), 1653 (s), 1457 (w), 1338 (w), 1278 (m), 1223 (m), 1046 (m), 796 (w) cm^{-1}; GC–MS (EI, 70 eV): m/z (%): 222.1 (M^+, 2), 190.1 (100), 147.1 (13), 91.1 (28); HRMS (EI): calcd for $C_{12}H_{14}O_4$ $[M]^+$: 222.0887; found: 222.0886.

2.6. General procedure for the synthesis of triflate (8)

The reaction was carried out analogously by a known procedure. To a dichloromethane solution (10 mL/mmol) of **5i** (1.0 mmol) and triflic anhydride (1.2 mmol) was added pyridine (2.0 mmol) at −78 °C. The solution was allowed to warm to −10 °C within 4 h. The product was isolated by rapid chromatography (silica gel, dichloromethane) of the reaction mixture as a colourless solid.

2.6.1. Compound 8. Starting with **5i** (442 mg, 1.4 mmol), Tf_2O (0.29 mL, 1.7 mmol), pyridine (0.23 mL, 2.9 mmol) and dichloromethane (15 mL), **8** was isolated as a colourless solid (628 mg, 98%), mp 74 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.24 (s, 6H, CH_3), 3.95 (s, 3H, OCH_3), 7.10 (s, 1H, ArH), 7.52–8.24 (m, 5H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=14.1, 16.9, 52.7 (CH_3), 118.5 (q, J=318.2 Hz, CF_3), 121.3 (CH), 125.9, 128.2 (C), 128.8 (2C), 130.3 (2C, CH), 132.5 (C), 134.2 (CH), 135.3, 143.8, 147.9, 163.7, 164.7 (C); IR (KBr): ν=3435 (w), 3069 (w), 3006 (w), 2956 (w), 1745 (s), 1725 (s), 1454 (m), 1417 (s), 1282 (m), 1259 (s), 1245 (s), 1217 (s), 1190 (m), 1137 (m), 1079 (m), 1065 (m), 1015 (m), 827 (m), 707 (m) cm^{-1}; MS (EI, 70 eV): m/z (%): 432.1 (M^+, 1), 401.0 (6), 105.0 (100); HRMS (EI): calcd for $C_{18}H_{15}O_7SF_3$ $[M]^+$: 432.0485; found: 432.0480.

2.7. General procedure for Suzuki coupling (9)

A dioxane solution (5 mL per 1.0 mmol of triflate) of **8** (1.0 mmol), phenylboronic acid (1.3 mmol), K_3PO_4 (1.6 mmol) and $Pd(PPh_3)_4$ (0.03 mmol) was refluxed for 4 h. A saturated aqueous solution of ammonium chloride was added. The organic and the aqueous layer was separated and the latter was extracted (3×) with ether. The combined organic layers were dried (Na_2SO_4), filtered and the filtrate was concentrated in vacuo. The residue was purified by chromatography (silica gel, 20% ethylacetate/heptane) to give the product.

2.7.1. Compound 9. Starting with **8** (255 mg, 0.5 mmol), Phenylboronic acid (86 mg, 0.7 mmol), K_3PO_4 (188 mg, 0.8 mmol), Pd catalyst (21 mg, 0.017 mmol) and dioxane (4 mL), **9** was isolated as a colourless solid (145 mg, 70%), mp 62 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.21 (s, 3H, CH_3), 2.24 (s, 3H, CH_3), 3.55 (s, 3H, OCH_3), 7.14 (s, 1H, ArH), 7.24–8.27 (m, 10H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ=13.6, 16.6, 51.8 (CH_3), 127.3, 128.23 (2C), 128.26 (2C, CH), 128.3 (C), 128.7 (2C, CH), 128.9 (C), 129.9, 130.2 (2C, CH), 132.0, 132.4 (C), 133.8 (CH), 137.9, 140.5, 147.6, 164.2, 169.5 (C); IR (KBr): ν=3437 (m), 3061 (w), 3030 (w), 2949 (w), 1734 (s), 1451 (m), 1253 (s), 1235 (s), 1160 (s), 1082 (s), 1065 (s), 1024 (m), 709 (m) cm^{-1}; GC–MS (EI, 70 eV): m/z (%): 360.1 (M^+, 2), 329.1 (1), 223.0 (2), 165.1 (5), 152.0 (6), 105.0 (100); HRMS (EI): calcd for $C_{23}H_{20}O_4$ $[M]^+$: 360.1356; found: 360.1347.

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11. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-299670. Copies of this data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(1223)336033 or e-mail: deposit@ccdc.cam.ac.uk) or via http://www.ccdc.cam.ac.uk/conts/retrieving.html.

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Use of the olefin metathesis reaction for highly efficient β-cyclodextrin modification

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Abstract—A series of novel primary face mono-substituted β-cyclodextrin derivatives have been synthesised using the olefin metathesis reaction. Mono-6-allylamino-6-deoxy-β-cyclodextrin easily synthesised by nucleophilic substitution of mono-6-tosyl-β-cyclodextrin is the key synthon in the preparation of cyclodextrin derivatives mono-functionalised at the primary face by alkyl, aryl or perfluoroalkyl groups using Grubbs catalyst. In the cases of vinylbenzene and 1H,1H,2H-perfluoro-1-octene, the metathesis reactions yield with 95% stereoselectivity of the *E*-isomer.

1. Introduction

Olefin metathesis is one of the most powerful synthetic tools in organic chemistry, with a large scope of applications from natural product to polymer synthesis.[1–4] More specifically, this reaction has been applied with success to carbohydrate chemistry.[5,6] In the particular case of cyclodextrins (CDs) or cyclomaltoheptaoses, only a few examples have been reported so far. Thus, in 2000, Stoddart et al.[7] described the use of olefin metathesis to generate face-to-face 2-2′ and 3-3′ dimers of β-cyclodextrin. This was achieved by a homodimerisation reaction between mono-substituted cyclodextrins having ethylene oxy-alkene groups present at either the O-2 or the O-3 positions.

Following this, in 2002, Sinaÿ et al. synthesised 'head-to-head' dimers of α- and β-cyclodextrin using mono-substitution at the O-6 position with alkylidene chains.[8–10] More recently, the same authors described the synthesis of a heteroduplex system containing both α- and β-cyclodextrin disubstituted units using the metathesis reaction.[11]

Generally, the introduction of bioactive substituents onto the cyclodextrins has been treated in a case by case manner, with each coupling specific to the introduced recognition antenna. This has been achieved using esters,[12] amides[13] or thioalkyl[14] linkages with success. However, linkages using ether or amine functions are much more difficult. In order to make the introduction of antenna more efficient and allow rapid generation of libraries of such molecules, a 'base unit' consisting of a mono-substituted cyclodextrin capable of accepting a wide range of antennae under a standard coupling condition would seen to be highly attractive.

In this work, we describe an efficient synthesis by olefin metathesis reaction of new β-cyclodextrin derivatives mono-substituted at the primary face by different hydrophilic or hydrophobic chains linked by a nitrogen atom at the C-6 position. This reaction opens up the facile synthesis of a wide range of CDs derivatives using one simple building block.

2. Results and discussion

2.1. Synthesis and characterisation

The synthetic procedure for the synthesis of the new β-cyclodextrin derivatives **9**, **10**, **11**, **12** mono-functionalised at the primary face by different groups (oct-1-ene, vinylbenzene, 3-(2-methoxyethoxy)-prop-1-ene, 1H,1H,2H-perfluoro-1-octene) and the homodimer **13** was based on an olefin metathesis reaction in four steps from mono-6-allylamino-6-deoxy-β-cyclodextrin **1** (Scheme 1).[15]

The use of the Grubbs catalyst requires protection of the nitrogen atom and the lack of solubility of **1** in organic solvents

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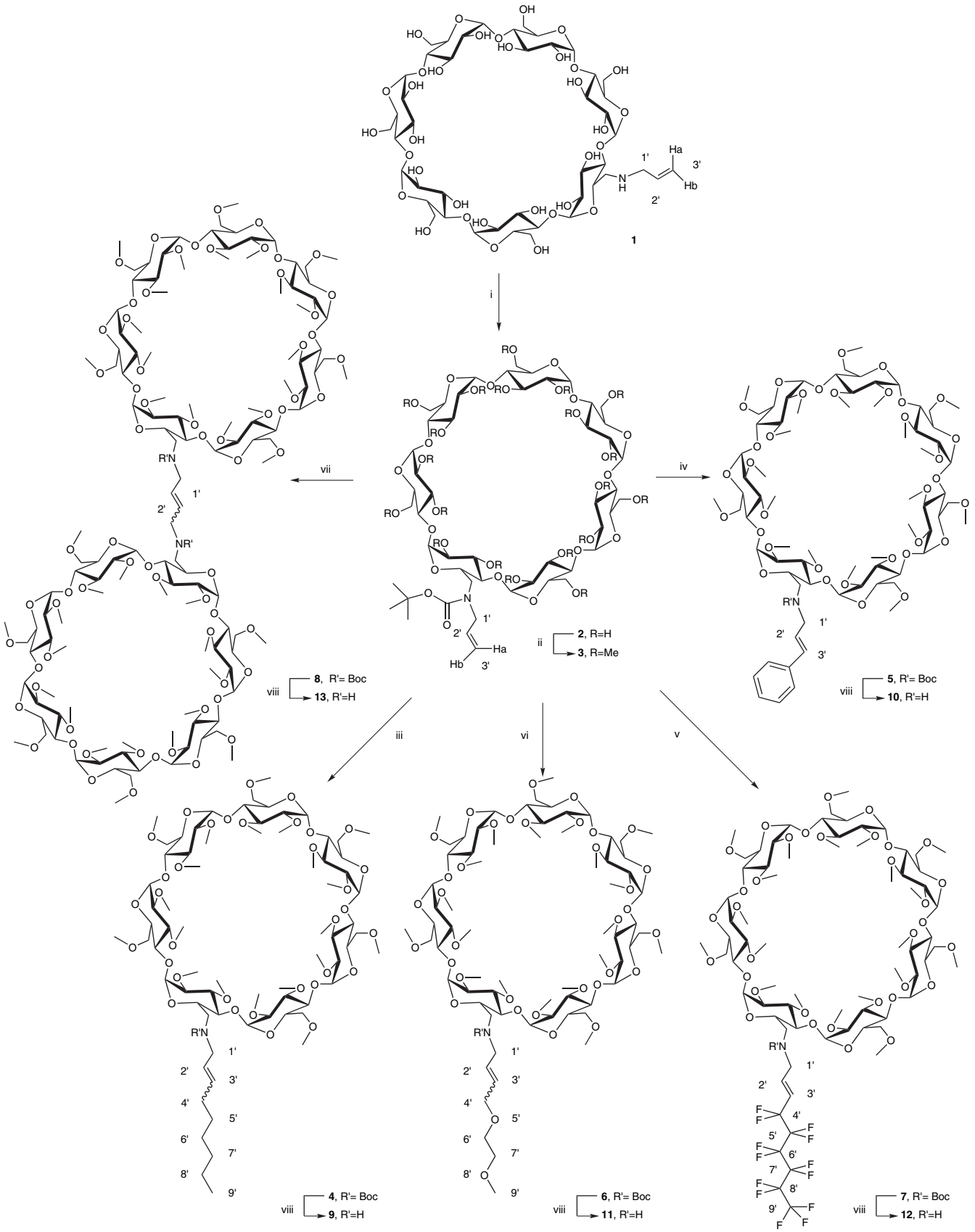

Scheme 1. Reagents and conditions: (i) Boc_2O, $NaHCO_3$, MeOH, ultrasonication; (ii) NaH, CH_3I, DMF, 20 °C; (iii) oct-1-ene, Grubbs catalyst [$Cl_2(PCy_3)2Ru{=}CHPh$], CH_2Cl_2, 55 °C; (iv) vinylbenzene, [$Cl_2(PCy_3)2Ru{=}CHPh$], CH_2Cl_2, 55 °C; (v) 3-(2-methoxyethoxy)-prop-1-ene, [$Cl_2(PCy_3)2Ru{=}CHPh$], CH_2Cl_2, 55 °C; (vi) 1H,1H,2H-perfluoro-1-octene, Grubbs–Hoveyda catalyst ($C_{31}H_{38}Cl_2N_2ORu$), CH_2Cl_2, 55 °C; (vii) [$Cl_2(PCy_3)2Ru{=}CHPh$], CH_2Cl_2, 55 °C; (viii) TFA, 20 °C.

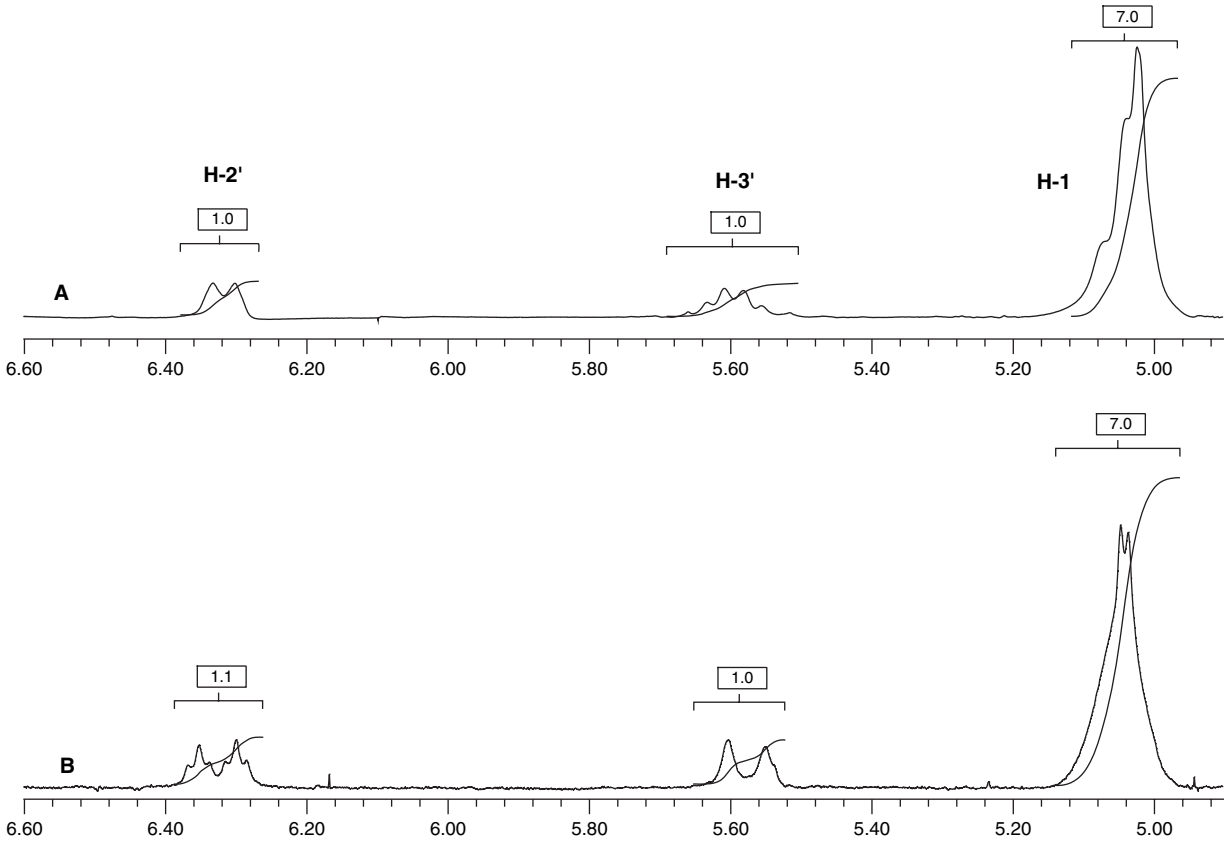

Figure 1. (A) ^{1}H NMR spectrum of **7**; (B) ^{1}H with ^{19}F decoupled NMR spectrum of **7**.

necessitates modification of the free hydroxyl groups at the O-2, O-3 and O-6 positions. N-protection was carried out using di-*tert*-butyl dicarbonate (Boc_2O) according to the conditions of peptide synthesis[16] to give **2** in 67% isolated yield. O-methylation at the six primary hydroxyl groups and the fourteen secondary hydroxyl groups of the β-CD **2** was realised using of 50 equiv of methyl iodide in dry DMF with 60 equiv of sodium hydride. The new cyclodextrin derivative **3** was purified by flash chromatography (eluent gradient $CHCl_3$/acetone, 100/0 to 60/40) in 67% isolated yield. The degree of the substitution was assessed by MALDI mass spectrometry (*m*/*z* 1576.7 $[M+Na]^+$ 1592.7 $[M+K]^+$) and the structure confirmed by ^{1}H, ^{13}C and HMBC NMR spectroscopy.

Mono(6^{A}-*N*-allyl-*N*-*tert*-butoxycarbonylamino,6^{A}-deoxy)-hexakis(6^{B},6^{C},6^{D},6^{E},6^{F},6^{G}-*O*-methyl)-heptakis(2,3-di-*O*-methyl)-β-cyclodextrin **3** (0.25 M in CH_2Cl_2) in the presence of the Grubbs catalyst $[Cl_2(PCy_3)_2Ru{=}CHPh]$ (15% mol/mol for **4** and **5**, 20% mol/mol for **6**) was reacted at 55 °C, under argon, with oct-1-ene, vinylbenzene or 3-(2-methoxyethoxy)-prop-1-ene[17] (2.5 equiv) during 18 h for **4** and **5**, 46 h for **6**. The cyclodextrins derivatives **4**, **5** and **6** were isolated by flash chromatography on silica gel with eluent gradient Et_2O/MeOH 100/0 to 96/4 for **4** and **5**, 100/0 to 95/5 for **6**, in 70, 73 and 53% yields, respectively. The coupling of the olefins was confirmed via ^{1}H NMR spectroscopy (500 MHz, solvent $CDCl_3$) by the changes in the chemical shifts of the vinylic protons situated at C-2′ and C-3′. The signal for the proton H-3′ shows the largest perturbation, being shifted downfield (5.46–6.38 ppm) in the new compounds **4**, **5** and **6** with respect to initial values (m, 4.95–5.07 ppm) in *N*-Boc-allyl-amino β-cyclodextrin **3**. Mono-substitution of β-cyclodextrin by a long chain, which may be hydrophobic or hydrophilic, is known to reduce the geometry of the cavity. This leads to a separation in the peaks for H-1 (m, 4.94–5.11 ppm) into three signals; a broad singlet at 4.94 ppm, integration 1, a multiplet around 5.03 ppm, integration 5 and a broad singlet at 5.11 ppm, integration 1. In the case of coupling with vinylbenzene there is less perturbation, one signal at 5.12 ppm, integration 1 and a second at 5.03 ppm, integration 6.

Determination of the *E*, *Z* selectivity of this reaction is not trivial as the signals of the vinylic protons of compounds **4** and **6** are present as multiplets. However for β-cyclodextrin derivative **5**, the coupling constant H-2′/H-3′ at 15.0 Hz confirms *E* geometry. The metathesis reaction yields stereoselectively (95%) the *E*-isomer,[†] in 73% yield, which is interesting when compared to the generally measured ratio *E*/*Z* isomer (7/3). It should be noted that in the case of the ether-linked 3-3′-β-CD dimer described by Stoddart, the authors concluded after much work, that the isomers were present in the 9:1 ratio.[7]

Given the facile nature of the above synthesis, we applied the reaction to the introduction of perfluoroalkyl chains, leading to the synthesis of the β-CD derivative **7**. Only few examples of metathesis have been published so far with fluorinated

[†] The actual ratio, as determined, is within the experimental limits of incertitude for ^{1}H NMR spectroscopy.

olefins with one or two fluorine atoms[18,19] or with longer fluorous chains.[20,21] In previous studies, we have synthesised mono-fluorinated amphiphilic cyclodextrin from mono-6-tosyl-β-cyclodextrin with 3-perfluorohexylpropanethiol by nucleophilic attack at the tosyl group.[22] Introduction of perfluoroalkyl chains in carbohydrates has been carried out using radical reactions from allylic compounds.[23] This method is not available for the cyclodextrins.[24] The novel derivative **7** was synthesised by a metathesis reaction between the olefinic cyclodextrin derivative **3** and 13 equiv of the 1H,1H,2H-perfluoro-1-octene in presence of the Grubbs–Hoveyda catalyst[25] ($C_{31}H_{38}Cl_2N_2ORu$) in CH_2Cl_2 at 55 °C for 9 days. Purification by flash chromatography yields **7** and the cyclodextrin dimer **8**. However, we have directly isolated **8** by the homodimerisation of the cyclodextrin derivative **3** in presence of the first generation Grubbs catalyst (14% mol/mol) at 50 °C for 21 h in 60% yield. Comparing the above results we propose that **8** is first synthesised as the kinetic product, which then reacts with the fluorous olefin to give **7**. Stereoselectivity of the metathesis reaction is again confirmed by NMR in $CDCl_3$ of **7**. In the ^1H decoupled ^{19}F spectrum (Fig. 1B) the appearance of a multiplet for H-2′ at 6.32 ppm and a doublet for H-3′ at 5.58 ppm with the coupling constant H-2′/H-3′=16 Hz is characteristic of the *E*-isomer. The anomeric signal H-1 was converted from a multiplet to a doublet ($^3J_{H\text{-}1/H\text{-}2}$=3.5 Hz). In the ^{13}C NMR C-2′ and C-3′ were observed at 139.4 and 117.5 ppm, respectively. Analysis by electrospray mass spectrometry (ESMS) confirms the structure of **7** (*m/z* 1894.9 $[M+Na]^+$ 959.0 $[M+2+Na]^{2+}$).

Selective removal of the *N*-Boc protecting group by trifluoroacetolysis (TFA, 20 °C) to gives the secondary amino cyclodextrin derivatives **9**, **10**, **11**, **12** and **13** in 94, 82, 94, 89 and 91% yields, respectively.

3. Conclusion

We have synthesised a variety of primary face mono-substituted β-cyclodextrin derivatives by the use of the metathesis reaction. This reaction gives direct access to β-cyclodextrin derivatives via a single synthon mono-6-allyl-6-deoxy-β-cyclodextrin **1** and various olefins presenting hydrophobic or hydrophilic nature. We also show the feasibility of this synthesis for the coupling of perfluoroalkyl-olefin. In the cases of vinylbenzene and 1H,1H,2H-perfluoro-1-octene, we obtained the *E*-isomer in 95% whereas the previous best stereoselectivities observed in the chemistry of cyclodextrins were 9:1. This metathesis reaction presents a highly promising route to novel mono-functionalised cyclodextrin derivatives.

4. Experimental

4.1. General

Ruthenium catalysts were purchased from Aldrich. β-Cyclodextrin was generously provided by Roquette (France). ^1H, ^{13}C, COSY, HSQC and HMBC NMR experiments were performed at 300 and 75 MHz, respectively, or 500 and 125 MHz, respectively, using a Bruker DRX 300 spectrometer or a Bruker AM 500 spectrometer. ^{19}F NMR experiments were obtained at 280 MHz with a Bruker ALS 300 spectrometer. It should be noted that mono-substitution of any cyclodextrin totally removes the axial symmetry of the molecule leading to considerable overlap in closely related chemical shifts of every proton or carbon and rendering spectral analysis extremely complex. ES mass spectra were measured using a Perkin–Elmer Sciex spectrometer and MALDI-TOF mass spectra were measured using Applied Biosystems Voyager-DE Pro. IR spectra were recorded on a Perkin–Elmer instrument. The optical rotations were tested on a Perkin–Elmer 241 Polarimeter.

4.1.1. Mono-(6^A-*N*-allylamino-6^A-deoxy)-cyclomaltoheptaose 1. The mono-(6^A-deoxy-6^A-(*p*-toluenesulfonyl))-cyclomaltoheptaose[26] was converted into **1** by the procedure described by Lai and Ng.[15] Yield: 81%; R_f (*n*-BuOH/EtOH/H_2O, 5/4/3)=0.06; mp: >250 °C; $[\alpha]_D$ +138 (*c* 1.000, MeOH); IR (cm^{-1}, KBr): 3395 (O–H), 2927 (C–H), 1657 (C=C), 1158 (C–O–C), 1033 (C–N); ^1H NMR (DMSO-d_6, 300 MHz, assignments by HSQC): δ (ppm): 2.69 (m, 1H, H-6aA), 2.92 (m, 1H, H-6bA), 3.18 (m, 2H, H-1′), 3.32–3.40 (m, 14H, H-4, H-5), 3.53–3.63 (m, 26H, H-2, H-3, H-6a, H-6b), 4.44 (br s, 6H, OH-6), 4.83 (br s, 7H, H-1), 5.03 (d, 1H, H-3′b, $^3J_{H\text{-}3'b/H\text{-}2'}$=10.1 Hz), 5.14 (d, 1H, H-3′a, $^3J_{H\text{-}3'a/H\text{-}2'}$=17.7 Hz), 5.67–5.81 (m, 15H, OH-2, OH-3, H-2′); ^{13}C NMR (DMSO-d_6, 75 MHz, assignments by HSQC): δ (ppm): 49.8 (C-6A), 52.5 (C-1′), 60.8 (C-6), 72.8 (C-2), 73.3 (C-5), 73.9 (C-3), 82.1–82.4 (C-4), 84.7 (C-4A), 102.8–103.1 (C-1), 116.1 (C-3′), 138.3 (C-2′); ESMS (+) *m/z*: $[M+Na]^+$ 1197.2; $C_{45}H_{75}NO_{34}$.

4.1.2. Mono-(6^A-*N*-allyl-*N*-*tert*-butoxycarbonylamino-6^A-deoxy)-cyclomaltoheptaose 2. To a solution of **1** (2.00 g, 1.70 mmol) in MeOH (50 mL) at room temperature were added Boc_2O (0.45 g, 2.05 mmol, 1.2 equiv) and $NaHCO_3$ (0.43 g, 5.11 mmol, 3 equiv). The reaction mixture was placed for 12 h in an ultrasonic bath and monitored by TLC. The solvent was evaporated and the white solid was purified by column chromatography (Silica gel 100 C_{18}-reversed phase, H_2O/MeOH, step gradient: 100/0 to 50/50) to afford **2** as a white powder. Yield: 67%; R_f (*n*-BuOH/EtOH/H_2O, 5/4/3)=0.44; mp: 206 °C; $[\alpha]_D$ +132 (*c* 1.015, MeOH); IR (cm^{-1}, KBr): 3395 (O–H), 2918 (C–H), 1676 (C=O), 1155 (C–N), 1028 (C–O–C); ^1H NMR (MeOD-d_4, 500 MHz, assignments by COSY and HSQC): δ (ppm): 1.51 (s, 9H, CH_3 Boc), 2.84 (dd, 1H, H-6aA, $^2J_{H\text{-}6aA/H\text{-}6bA}$= 14.5 Hz and $^3J_{H\text{-}6aA/H\text{-}5A}$=10.5 Hz), 3.18 (t, 1H, H-4A, $^3J_{H\text{-}4A/H\text{-}3A}$=$^3J_{H\text{-}4A/H\text{-}5A}$=9.5 Hz), 3.50–3.74 (m, 20H, H-2, H-4, H-5, H-1′b), 3.79–4.03 (m, 21H, H-3, H-6a, H-6b), 4.26 (t, 1H, H-5A, $^3J_{H\text{-}5A/H\text{-}4A}$=$^3J_{H\text{-}5A/H\text{-}6bA}$=10.3 Hz), 4.46 (d, 1H, H-1′a, $^2J_{H\text{-}1'a/H\text{-}1'b}$=14.5 Hz), 4.97–5.03 (m, 7H, H-1), 5.15 (m, 2H, H-3′), 5.80 (m, 1H, H-2′); ^{13}C NMR (MeOD-d_4 and 10% of Pyridine-d_5, 125 MHz, assignments by DEPT and HSQC): δ (ppm): 27.9, 28.6 (*C*H_3 Boc), 48.3 (C-6A), 51.8 (C-1′), 60.8 (C-6), 70.6 (C-5A), 72.4–74.1 (C-2, C-3, C-5), 80.1 (O–*C* Boc), 82.1–82.5 (C-4), 85.3 (C-4A), 101.7 (C-1A), 102.8–103.4 (C-1), 115.1 (C-3′), 134.1 (C-2′), 155.8 (–*C*=O Boc); ESMS (+) *m/z*: $[M+Na]^+$ 1296.4, $[M+H]^+$ 1275.4; $C_{50}H_{83}NO_{36}$.

4.1.3. Mono-(6^A-*N*-allyl-*N*-*tert*-butoxycarbonylamino-6^A-deoxy)-hexakis-6^B,6^C,6^D,6^E,6^F,6^G-*O*-methyl)-heptakis-(2,3-di-*O*-methyl)-cyclomaltoheptaose 3. NaH 60% (4.70 g, 117.6 mmol, 60 equiv, 3 equiv/OH) was added to

a solution of **2** (2.50 g, 1.9 mmol) in DMF (40 mL). The mixture was stirred for 3 h, then ICH_3 (6.10 mL, 98 mmol, 50 equiv, 2.5 equiv/OH) was added dropwise to the suspension (the temperature must be kept below 35 °C). After stirring for 18 h at room temperature, excess NaH was destroyed carefully by addition of methanol (10 mL) and the solution was diluted with water (50 mL). The organic layer was extracted with diethyl ether (3×100 mL). The combined organic layers were washed with water, dried over Na_2SO_4. The solvent was evaporated and the yellow solid was subjected to column chromatography (SiO_2, $CHCl_3$/acetone, step gradient: 100/0 to 60/40) to afford **3**. Yield: 65%; R_f (Et_2O/MeOH, 90/10)=0.26; mp: 80 °C; $[\alpha]_D$ +140 (*c* 1.025, $CHCl_3$); IR (cm^{-1}, KBr): 2930 (C–H), 1700 (C=O), 1164 (C–O–C), 1037 (C–N); ^1H NMR ($CDCl_3$, 300 MHz, assignments by COSY and HSQC): δ (ppm): 1.43 (s, 9H, CH_3 Boc), 2.86 (m, 1H, H-6aA), 3.10–3.13 (m, 8H, H-2, H-4A), 3.31–3.81 (m, 93H, H-3, H-4, H-5, H-6a, H-6b, 2-OC*H*$_3$, 3-OC*H*$_3$, 6-OC*H*$_3$, H-1′b), 4.01 (m, 1H, H-5A), 4.28 (m, 1H, H-1′a), 4.95–5.07 (m, 9H, H-1 and H-3′), 5.69 (m, 1H, H-2′); ^{13}C NMR ($CDCl_3$, 75 MHz, assignments by DEPT and HSQC): δ (ppm): 28.8–29.3 (CH_3 Boc), 49.2 (C-6A), 51.3 (C-1′), 58.5–62.1 (2-O*C*H$_3$, 3-O*C*H$_3$, 6-O*C*H$_3$), 71.0–71.8 (C-5, C-6), 79.7 (O–*C* Boc), 80.7–82.4 (C-2, C-3, C-4), 98.4 (C-1A), 99.4–100.5 (C-1), 116.5 (C-3′), 134.1 (C-2′), 155.3 (*C*=O Boc); MALDI-TOF-MS: $[M+Na]^+$ 1576.7, $[M+K]^+$ 1592.7; $C_{70}H_{123}NO_{36}$.

4.1.4. General procedure for metathesis reactions. To a solution of **3** (1.0 equiv) in freshly distilled CH_2Cl_2 (0.25 M) was added under argon the alkene (2.5 equiv) and Grubbs catalyst [$Cl_2(PCy_3)_2Ru$=CHPh] (15% mol/mol for **4**, **5** and 20% mol/mol for **6**). The solution was stirred at 50 °C and the reaction can be monitored by TLC (Et_2O/MeOH, 90/10). After stirring for 18 h (46 h for **6**), the solvent was evaporated and the black solid was subjected to column chromatography (SiO_2, Et_2O/MeOH, step gradient: 100/0 to 96/4 (100/0 to 95/5 for **6**) to afford the product.

4.1.4.1. Mono-(6^A-deoxy-6^A-(1-*N*-non-2-ene-*N*-*tert*-butoxycarbonylamino))-hexakis-(6^B,6^C,6^D,6^E,6^F,6^G-*O*-methyl)-heptakis-(2,3-di-*O*-methyl)-cyclomaltoheptaose 4. Yield: 70%; R_f (Et_2O/MeOH, 90/10)=0.63; mp (dec): 68 °C; $[\alpha]_D$ +133 (*c* 0.990, $CHCl_3$); IR (cm^{-1}, KBr): 2929 (C–H), 1697 (C=O), 1164 (C–O–C), 1037 (C–N); ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm): 0.81 (s, 3H, H-9′, $^3J_{H\text{-}9'/H\text{-}8'}$=6.5 Hz), 1.21–1.32 (m, 8H, H-5′, H-6′, H-7′, H-8′), 1.43 (s, 9H, C*H*$_3$ Boc), 1.98 (q, 2H, H-4′, $^3J_{H\text{-}4'/H\text{-}5'}$=$^3J_{H\text{-}4'/H\text{-}3'}$=7.0 Hz), 2.75 (m, 1H, H-6aA), 3.04 (t, 1H, H-4A, $^3J_{H\text{-}4A/H\text{-}5A}$=$^3J_{H\text{-}4A/H\text{-}3A}$=9.0 Hz), 3.09–3.13 (m, 7H, H-2), 3.30–3.82 (m, 93H, H-3, H-4, H-5, H-6a, H-6b, 2-OC*H*$_3$, 3-OC*H*$_3$, 6-OC*H*$_3$, H-1′b), 4.03 (m, 1H, H-5A), 4.35 (d, 1H, H-1′a, $^2J_{H\text{-}1'a/H\text{-}1'b}$=13.0 Hz), 4.94 (br s, 1H; H-1), 5.03 (m, 5H, H-1), 5.11 (br s, 1H, H-1), 5.30 (m, 1H, H-2′), 5.46 (m, 1H, H-3′); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm): 14.5 (C-9′), 23.0 (C-8′), 28.9–29.6 (C-5′, C-6′, CH_3 Boc), 32.1 (C-7′), 32.7 (C-4′), 48.9 (C-6A), 51.2 (C-1′), 58.8–62.1 (2-O*C*H$_3$, 3-O*C*H$_3$, 6-O*C*H$_3$), 71.0–72.0 (C-5, C-6), 79.5 (O–*C* Boc), 80.7–82.6 (C-2, C-3, C-4), 98.4 (C-1A), 99.8–100.5 (C-1), 133.4 (C-2′), 133.9 (C-3′), 155.3 (*C*=O Boc); MALDI-TOF-MS $[M+Na]^+$ 1662.7, $[M+K]^+$ 1676.7; $C_{76}H_{135}NO_{36}$.

4.1.4.2. Mono-(6^A-deoxy-6^A-(1-*N*-3-phenyl-prop-2-ene-*N*-*tert*-butoxycarbonylamino))-hexakis-(6^B,6^C,6^D,6^E,6^F,6^G-*O*-methyl)-heptakis-(2,3-di-*O*-methyl)-cyclomaltoheptaose 5. Yield: 73%; R_f (Et_2O/MeOH, 90/10)=0.55; mp (dec): 95 °C; $[\alpha]_D$ +135 (*c* 1.005, $CHCl_3$); IR (cm^{-1}, KBr): 2930 (C–H), 1700 (C=O), 1170 (C–O–C), 1036 (C–N); ^1H NMR ($CDCl_3$, 500 MHz): δ (ppm): 1.46 (s, 9H, C*H*$_3$ Boc), 2.80 (m, 1H, H-6aA), 3.07–3.13 (m, 8H, H-2, H-4A), 3.29–3.85 (m, 93H, H-3, H-4, H-5, H-6a, H-6b, 2-OC*H*$_3$, 3-OC*H*$_3$, 6-OC*H*$_3$, H-1′b), 4.06 (m, 1H, H-5A), 4.57 (d, 1H, H-1′a, $^2J_{H\text{-}1'a/H\text{-}1'b}$=14.0 Hz), 5.03 (m, 6H, H-1), 5.12 (br s, 1H, H-1A), 6.08 (m, 1H, H-2′), 6.38 (d, 1H, H-3′, $^3J_{H\text{-}3'/H\text{-}2'}$=15.0 Hz), 7.16 (t, 1H, H_{para}, $^3J_{H_{para}/H_{meta}}$=7.3 Hz), 7.24 (t, 2H, H_{meta}, $^3J_{H_{meta}/H_{para}}$=$^3J_{H_{meta}/H_{ortho}}$=7.3 Hz), 7.30 (d, 2H, H_{ortho}, $^3J_{H_{ortho}/H_{meta}}$=7.3 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm): 28.9–29.4 (CH_3 Boc), 49.5 (C-6A), 51.5 (C-1′), 58.0–62.3 (2-O*C*H$_3$, 3-O*C*H$_3$, 6-O*C*H$_3$), 71.1–71.5 (C-5, C-6), 79.7 (O–*C* Boc), 81.9–82.6 (C-2, C-3, C-4), 98.5 (C-1A), 99.6–100.6 (C-1), 125.6 (C-3′), 126.7 (C_{ortho}), 128.1 (C_{para}), 129.0 (C_{meta}), 132.1 (C-2′), 137.0 (C_{ipso}), 155.3 (*C*=O Boc); MALDI-TOF-MS $[M+Na]^+$ 1652.9, $[M+K]^+$ 1668.9; $C_{76}H_{127}NO_{36}$.

4.1.4.3. Mono-[6^A-deoxy-6^A-(1-*N*-5,8-dioxy-non-2-ene-*N*-*tert*-butoxycarbonylamino)]-hexakis-(6^B,6^C,6^D,6^E,6^F,6^G-*O*-methyl)-heptakis-(2,3-di-*O*-methyl)-cyclomaltoheptaose 6. Yield: 53%; R_f (Et_2O/MeOH: 90/10)=0.20; mp (dec): 76 °C; $[\alpha]_D$ +113 (*c* 1.005, $CHCl_3$); IR (cm^{-1}, KBr): 2928 (C–H), 1698 (C=O), 1164 (C–O–C), 1038 (C–N); ^1H NMR ($CDCl_3$, 500 MHz, assignments by COSY and HSQC): δ (ppm): 1.43 (s, 9H, CH_3 Boc), 2.71 (m, 1H, H-6aA), 3.03 (t, 1H, H-4A, $^3J_{H\text{-}4A/H\text{-}5A}$=$^3J_{H\text{-}4A/H\text{-}3A}$=9.0 Hz), 3.10–3.12 (m, 7H, H-2), 3.31–3.84 (m, 100H, H-3, H-4, H-5, H-6a, H-6b, 2-OC*H*$_3$, 3-OC*H*$_3$, 6-OC*H*$_3$, H-1′b, H-6′, H-7′, H-9′), 4.01 (m, 4H, H-4′, H-5A), 4.46 (d, 1H, H-1′a, $^2J_{H\text{-}1'a/H\text{-}1'b}$=13.5 Hz), 4.94 (br s, 1H, H-1A), 5.03 (m, 5H, H-1), 5.11 (br s, 1H, H-1), 5.60 (m, 2H, H-2′, H-3′); ^{13}C NMR ($CDCl_3$, 125 MHz, assignments by DEPT and HSQC): δ (ppm): 28.9–29.4 (*C*H$_3$ Boc), 49.2 (C-6A), 50.9 (C-1′), 58.5–62.2 (2-O*C*H$_3$, 3-O*C*H$_3$, 6-O*C*H$_3$, C-9′), 69.9 (C-6′), 70.2–72.0 (C-5, C-6, C-7′), 72.3 (C-4′), 79.6 (–O–*C*– Boc), 81.3–83.1 (C-2, C-3, C-4), 84.4 (C-4A), 98.5 (C-1A), 99.6–100.0 (C-1), 129.2 (C-2, C-3′), 155.2 (–*C*=O Boc); HR-MALDI-TOF-MS $[M+Na]^+$ 1664.8884, $[M+K]^+$ 1680.8875; $C_{74}H_{131}NO_{38}$.

4.1.5. Mono-[6^A-deoxy-6^A-(1-*N*-3-perfluorohexylprop-2-ene-*N*-*tert*-butoxy-carbonylamino)]-hexakis-(6^B,6^C,6^D,6^E,6^F,6^G-*O*-methyl)-heptakis-(2,3-di-*O*-methyl)-cyclomaltoheptaose 7. To a solution of **3** (0.25 M) in freshly distilled CH_2Cl_2 (0.390 g, 0.251 mmol) and 1H,1H,2H-perfluoro-1-octene (73 μL, 3.220 mmol, 13 equiv) was added under argon Hoveyda–Grubbs catalyst ($C_{31}H_{38}Cl_2N_2ORu$) (32 mg, 51 μmol, 20% mol/mol). The solution was stirred at 50 °C and the reaction can be monitored by TLC (Et_2O/MeOH, 90/10). After stirring for 9 days, the solvent was evaporated and the black solid was subjected to column chromatography (SiO_2, Et_2O/MeOH, step gradient: 100/0 to 95/5) to afford **7**. Yield: 27%; R_f (Et_2O/MeOH, 90/10)=0.80; mp (dec): 74 °C; $[\alpha]_D$ +111 (*c* 0.670, $CHCl_3$); IR (cm^{-1}, KBr): 2932 (C–H), 1701 (C=O), 1243–1180 (C–F), 1165 (C–O–C), 1039 (C–N); ^1H NMR ($CDCl_3$, 500 MHz, assignments by COSY and HSQC): δ (ppm):

1.43 (s, 9H, CH_3 Boc), 2.73 (m, 1H, H-6aA), 3.08–3.10 (m, 8H, H-2, H-4A), 3.27–3.85 (m, 93H, H-3, H-4, H-5, H-6a, H-6b, 2-OCH_3, 3-OCH_3, 6-OCH_3, H-1′b), 4.00 (m, 1H, H-5A), 4.58 (m, 1H, H-1′a), 5.02–5.04 (m, 7H, H-1), 5.58 (m, 1H, H-3′), 6.32 (d, 1H, H-2′, $^3J_{\text{H-2′/H-3′}}$=16.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz, assignments by DEPT and HSQC): δ (ppm): 28.6–29.2 (*C*H_3 Boc), 50.1 (C-6A, C-1′), 58.3–61.9 (2-O*C*H_3, 3-O*C*H_3, 6-O*C*H_3), 71.2–72.0 (C-5, C-6), 80.3 (–O–*C*– Boc), 81.1–83.8 (C-2, C-3, C-4), 98.4 (C-1A), 99.7–100.7 (C-1), 108.4–118.7 (C-4′, C-5′, C-6′, C-7′, C-8′, C-9′), 117.5 (C-3′), 139.4 (C-2′), 155.6 (–*C*=O Boc); ^{19}F NMR ($CDCl_3$, 280 MHz): δ (ppm): −81.3 (s, 3F, F-9′), −110.1 to −113.9 (m, 2F, F-4′), −122.1 (m, 2F, F-5′), −123.4 to −123.6 (m, 4F, F-6′, F-7′), −126.6 (m, 2F, F-8′); ES (+) *m*/*z*: $[M+Na]^+$ 1894.9, $[M+2+Na]^{2+}$ 959.0; $C_{76}H_{122}F_{13}NO_{36}$.

4.1.6. Boc-homodimer 8. To a solution (0.25 M) in freshly distilled CH_2Cl_2 of **3** (0.400 g, 0.257 mmol) was added under argon the Grubbs catalyst [$Cl_2(PCy_3)_2Ru$=CHPh] (30 mg, 36 μmol, 14% mol/mol). The solution was stirred at 50 °C and the reaction can be monitored by TLC (Et_2O/MeOH, 90/10). After stirring for 21 h, the solvent was evaporated and the black solid was subjected to column chromatography (SiO_2, Et_2O/MeOH, step gradient: 100/0 to 90/10) to afford **8**. Yield: 60%; R_f (Et_2O/MeOH, 75/25)=0.58; mp (dec): 107 °C; $[\alpha]_D$ +136 (*c* 0.825, $CHCl_3$); IR (cm^{-1}, KBr): 2930 (C–H), 1698 (C=O), 1165 (C–O–C), 1039 (C–N); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm): 1.41 (s, 18H, CH_3 Boc), 2.67 (m, 2H, H-6aA), 3.03 (t, 2H, H-4A, $^3J_{\text{H-4A/H-5A}}$=$^3J_{\text{H-4A/H-3A}}$=9.0 Hz), 3.11 (dd, 14H, H-2, $^3J_{\text{H-2/H-1}}$=3.0 Hz, $^3J_{\text{H-2/H-3}}$=9.5 Hz), 3.27–3.78 (m, 186H, H-3, H-4, H-5, H-6a, H-6b, 2-OCH_3, 3-OCH_3, 6-OCH_3, H-1′b), 4.01 (m, 2H, H-5A), 4.49 (m, 2H, H-1′a), 4.94 (br s, 2H, H-1A), 5.02 (m, 10H, H-1), 5.09 (m, 2H, H-1), 5.44 (m, 2H, H-2′); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm): 28.9–29.3 (*C*H_3 Boc), 49.2 (C-6A), 50.8 (C-1′), 58.5–62.2 (2-O*C*H_3, 3-O*C*H_3, 6-O*C*H_3), 70.2–72.1 (C-5, C-6), 79.7 (–O–*C*– Boc), 80.4–83.1 (C-2, C-3, C-4), 98.7 (C-1A), 99.5–100.8 (C-1), 128.4 (C-2′), 155.4 (–*C*=O Boc); MALDI-TOF-MS $[M+Na]^+$ 3103.4, $[M+K]^+$ 3119.3; $C_{138}H_{242}N_2O_{72}$.

4.1.7. Procedure for Boc deprotection of 4. A solution of compound **4** (0.150 g, 91.5 μmol) in trifluoroacetic acid (6 mL) was stirred overnight at room temperature. The solution was then diluted with water (30 mL). The organic layer was extracted with dichloromethane (3×20 mL). The combined organic layers were washed with a saturated solution of Na_2CO_3 (60 mL), dried over Na_2SO_4. The solvent was evaporated to afford **9**.

4.1.7.1. Mono-(6^A-deoxy-6^A-non-2-enamino)-hexakis-(6^B,6^C,6^D,6^E,6^F,6^G-*O*-methyl)-heptakis-(2,3-di-*O*-methyl)-cyclomaltoheptaose 9. Yield: 94%; R_f (Et_2O/MeOH, 85/15)=0.17; mp (dec): 80 °C; $[\alpha]_D$ +130 (*c* 1.000, $CHCl_3$); IR (cm^{-1}, KBr): 2928 (C–H), 1652 (C=C), 1155 (C–O–C), 1036 (C–N); ^{1}H NMR ($CDCl_3$, 500 MHz, assignments by COSY and HSQC): δ (ppm): 0.84 (t, 3H, H-9′, $^3J_{\text{H-9′/H-8′}}$=6.5 Hz), 1.13–1.29 (m, 8H, H-5′, H-6′, H-7′, H-8′), 1.98 (q, 2H, H-4′, $^3J_{\text{H-4′/H-5′}}$=$^3J_{\text{H-4′/H-3′}}$=7.0 Hz), 2.88 (dd, 1H, H-6aA, $^2J_{\text{H-6aA/H-6bA}}$=12.0 Hz, $^3J_{\text{H-6aA/H-5A}}$=6.5 Hz), 3.08–3.13 (m, 8H, H-2, H-6bA), 3.17–3.33 (m, 20H, H-1′, 6-OCH_3), 3.44–3.57 (m, 68H, H-3, H-4, H-6a, H-6b, 2-OCH_3, 3-OCH_3), 3.74–3.79 (m, 7H, H-5), 5.04–5.09 (m, 7H, H-1), 5.46 (m, 1H, H-2′), 5.58 (m, 1H, H-3′); ^{13}C NMR ($CDCl_3$, 125 MHz, assignments by DEPT and HSQC): δ (ppm): 14.4 (C-9′), 22.9 (C-8′), 28.9–29.6 (C-5′, C-6′), 32.0 (C-7′), 32.8 (C-4′), 49.6 (C-6A), 51.8 (C-1′), 58.6–61.8 (2-O*C*H_3, 3-O*C*H_3, 6-O*C*H_3), 69.5–71.7 (C-5, C-6), 80.4–83.3 (C-2, C-3, C-4), 99.1–99.6 (C-1), 128.5 (C-2′), 129.3 (C-3′); ES (+) *m*/*z*: $[M+H]^+$ 1538.6, $[M+Na]^+$ 1560.6; $C_{71}H_{127}NO_{34}$.

4.1.7.2. Mono-(6^A-deoxy-6^A-(3-phenyl-prop-2-enamino))-hexakis-(6^B,6^C,6^D,6^E,6^F,6^G-*O*-methyl)-heptakis-(2,3-di-*O*-methyl)-cyclomaltoheptaose 10. Yield: 82%; R_f (Et_2O/MeOH, 90/10)=0.11; mp (dec): 91 °C; $[\alpha]_D$ +137 (*c* 1.050, $CHCl_3$); IR (cm^{-1}, KBr): 2927 (C–H), 1652 (C=C), 1162 (C–O–C), 1034 (C–N); ^{1}H NMR ($CDCl_3$, 500 MHz, assignments by COSY and HSQC): δ (ppm): 3.05 (dd, 1H, H-6aA, $^2J_{\text{H-6aA/H-6bA}}$=11.9 Hz, $^3J_{\text{H-6aA/H-5A}}$=6.5 Hz), 3.15–3.20 (m, 8H, H-2, H-6bA), 3.23–3.39 (m, 18H, 6-OCH_3), 3.51–3.65 (m, 70H, H-3, H-4, H-6a, H-6b, 2-OCH_3, 3-OCH_3, H-1′), 3.79–3.90 (m, 7H, H-5), 5.11–5.15 (m, 7H, H-1), 6.29 (dt, 1H, H-2′, $^3J_{\text{H-2′/H-1′}}$=15.6 Hz, $^3J_{\text{H-2′/H-1′}}$=6.2 Hz), 6.55 (d, 1H, H-3′, $^3J_{\text{H-3′/H-2′}}$=15.6 Hz), 7.23–7.27 (m, 1H, H_{para}), 7.30 (t, 2H, H_{meta}, $^3J_{H_{meta}/H_{para}}$=$^3J_{H_{meta}/H_{ortho}}$=7.5 Hz), 7.36 (d, 2H, H_{ortho}, $^3J_{H_{ortho}/H_{meta}}$=7.5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz, assignments by DEPT and HSQC): δ (ppm): 50.2 (C-6A), 52.7 (C-1′), 59.2–62.3 (2-O*C*H_3, 3-O*C*H_3, 6-O*C*H_3), 70.5–72.3 (C-5, C-6), 80.6–83.8 (C-2, C-3, C-4), 99.6 (C-1A), 99.7–100.0 (C-1), 127.1 (C_{ortho}), 127.9 (C-3′), 128.4 (C_{para}), 129.4 (C_{meta}), 131.7 (C-2′), 137.6 (C_{ipso}); ES (+) *m*/*z*: $[M+H]^+$ 1530.9, $[M+H+Na]^{2+}$ 777.0; $C_{71}H_{119}NO_{34}$.

4.1.7.3. Mono-(6^A-deoxy-6^A-(5,8-dioxy-non-2-enamino))-hexakis-(6^B,6^C,6^D,6^E,6^F,6^G-*O*-methyl)-heptakis-(2,3-di-*O*-methyl)-cyclomaltoheptaose 11. Yield: 94%; R_f (Et_2O/MeOH, 80/20)=0.20; mp (dec): 65 °C; $[\alpha]_D$ +107 (*c* 0.500, $CHCl_3$); IR (cm^{-1}, KBr): 2926 (C–H), 1684 (C=C), 1162 (C–O–C), 1038 (C–N); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm): 2.94 (m, 1H, H-6aA), 3.07 (dd, 1H; H-6bA, $^2J_{\text{H-6bA/H-6aA}}$=9.8 Hz, $^3J_{\text{H-6bA/H-5A}}$=3.0 Hz), 3.12 (dd, 7H, H-2, $^3J_{\text{H-2/H-1}}$=3.3 Hz, $^3J_{\text{H-2/H-3}}$=9.3 Hz), 3.17–3.32 (m, 23H, H-1′, H-9′, 6-OCH_3), 3.43–3.57 (m, 74H, H-3, H-4, H-6a, H-6b, 2-OCH_3, 3-OCH_3, H-3′, H-4′), 3.74–3.80 (m, 7H, H-5), 3.93 (d, 2H, H-4′, $^3J_{\text{H-4′/H-3′}}$=3.0 Hz), 5.04–5.07 (m, 7H, H-1), 5.73 (m, 2H, H-2′, H-3′); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm): 49.3 (C-6A), 50.5–51.2 (C-1′), 58.8–61.9 (2-O*C*H_3, 3-O*C*H_3, 6-O*C*H_3, C-9′), 69.8 (C-6′), 71.3–71.8 (C-5, C-6, C-7′), 72.3 (C-4′), 80.5–83.2 (C-2, C-3, C-4), 99.2–99.5 (C-1), 127.8–128.5 (C-2′, C-3′); ES (+) *m*/*z*: $[M+H]^+$ 1542.9, $[M+H+Na]^{2+}$ 783.1; $C_{69}H_{123}NO_{36}$.

4.1.7.4. Mono-(6^A-deoxy-6^A-(3-perfluorohexylprop-2-enamino))-hexakis-(6^B,6^C,6^D,6^E,6^F,6^G-*O*-methyl)-heptakis-(2,3-di-*O*-methyl)-cyclomaltoheptaose 12. Yield: 89%; R_f (Et_2O/MeOH, 90/10)=0.40; mp (dec): 64 °C; $[\alpha]_D$ +99 (*c* 0.515, $CHCl_3$); IR (cm^{-1}, KBr): 2932 (C–H), 1653 (C=C), 1242–1191 (C–F), 1146 (C–O–C), 1040 (C–N); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm): 3.06 (m, 1H, H-6aA),

3.09–3.20 (m, 8H, H-2, H-6bA), 3.23–3.38 (m, 20H, H-1′, 6-OCH_3), 3.51–3.65 (m, 68H, H-3, H-4, H-6a, H-6b, 2-OCH_3, 3-OCH_3), 3.81–3.86 (m, 7H, H-5), 5.10 (d, 3H, H-1, $^3J_{H\text{-}1/H\text{-}2}$=2.6 Hz), 5.13 (br s, 4H, H-1), 5.84 (m, 1H, H-3′), 6.50 (d, 1H, H-2′, $^3J_{H\text{-}2'/H\text{-}3'}$=15.8 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm): 50.8 (C-6A), 51.5 (C-1′), 59.7–63.5 (2-OCH_3, 3-OCH_3, 6-OCH_3), 69.8–72.9 (C-5, C-6), 81.4–84.4 (C-2, C-3, C-4), 100.3–100.6 (C-1), 108.4–118.7 (C-4′, C-5′, C-6′, C-7′, C-8′, C-9′), 129.3 (C-3′), 143.4 (C-2′); ^{19}F NMR ($CDCl_3$, 280 MHz): δ (ppm): −81.4 (t, 3F, F-9′, $^3J_{F\text{-}9'/F\text{-}8'}$=9.6 Hz), −110.8 to −113.7 (m, 2F, F-4′), −122.3 (m, 2F, F-5′), −123.5 to −123.8 (m, 4F, F-6′, F-7′), −126.8 (m, 2F, F-8′); ES (+) *m/z*: $[M+Na]^+$ 1794.8, $[M+H]^+$ 1773.0, $[M+2Na]^{2+}$ 909.0, $[M+H+Na]^{2+}$ 898.0; $C_{71}H_{114}F_{13}NO_{34}$.

4.1.7.5. Homodimer 13. Yield: 91%; R_f (Et_2O/MeOH, 70/30)=0.07; mp (dec): 104 °C; $[\alpha]_D$ +140 (*c* 1.015, $CHCl_3$); IR (cm^{-1}, KBr): 2926 (C–H), 1654 (C=C), 1163 (C–O–C), 1037 (C–N); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm): 2.99 (m, 2H, H-6aA), 3.06 (dd, 2H; H-6bA, $^2J_{H\text{-}6bA/H\text{-}6aA}$=9.5 Hz, $^3J_{H\text{-}6bA/H\text{-}5A}$=3.0 Hz), 3.12 (dd, 14H, H-2, $^3J_{H\text{-}2/H\text{-}1}$=2.5 Hz, $^3J_{H\text{-}2/H\text{-}3}$=9.5 Hz), 3.17–3.32 (m, 40H, H-1′, 6-OCH_3), 3.43–3.58 (m, 136H, H-3, H-4, H-6a, H-6b, 2-OCH_3, 3-OCH_3), 3.73–3.78 (m, 14H, H-5), 5.04 (d, 8H, H-1, $^3J_{H\text{-}1/H\text{-}2}$=2.8 Hz), 5.06 (br s, 6H, H-1), 5.64 (m, 2H, H-2′); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm): 49.5–49.7 (C-6A), 51.8–52.2 (C-1′), 58.5–61.9 (2-OCH_3, 3-OCH_3, 6-OCH_3), 70.5–71.7 (C-5, C-6), 80.5–83.2 (C-2, C-3, C-4), 99.3–99.4 (C-1), 130.6 (C-2′); ES (+) *m/z*: $[M+H]^+$ 2880.7, $[M+2H]^{2+}$ 1441.4, $[M+3H]^{3+}$ 968.6; $C_{128}H_{226}N_2O_{68}$.

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Highly stereoselective synthesis of perhydropyrano[2,3-*b*]pyrans from the new 3-methylidenepentane-1,5-dianion synthon

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Dedicated to the memory of Professor Marcial Moreno Mañas

Abstract—4-Phenylsulfanyl-2-(2-phenylsulfanylethyl)but-1-ene (**2**) is a new 3-methylidenepentane-1,5-dianion synthon which on reaction with an excess of lithium powder and a catalytic amount of DTBB (2.5%) in the presence of a carbonyl compound in THF at 0 °C, leads, after hydrolysis, to the expected methylidenic diols **3**. These diols when subjected to successive hydroboration–oxidation and final oxidation, undergo spontaneous cyclisation to furnish a series of *cis*-perhydropyrano[2,3-*b*]pyrans (**4**) in a highly diastereoselective manner (>99% de). Acid-catalysed isomerisation of the *cis*-perhydropyrano[2,3-*b*]pyrans (**4**) leads, also stereoselectively, to the corresponding *trans*-perhydropyrano[2,3-*b*]pyrans (**5**). A discussion about the stability of **4** and **5** is also included.

1. Introduction

The perhydropyrano[2,3-*b*]pyran unit can be found in nature as a substructure of natural products, some of which exhibit interesting biological activities such as macralstonidine (**I**, from *Alstonia* species, with antimalarial activity)[1] or sapogenin triterpene **II** (from *Emmenospermum pancherianum*),[2] as well as in dipyranosides like **III** (key precursors for ansamycins)[3] (Chart 1). The perhydropyrano[2,3-*b*]pyran moiety also plays an important role in the synthesis and modification of carbohydrate scaffolds.[4] There is a variety of strategies that allow the construction of the perhydropyrano-[2,3-*b*]pyran skeleton, which normally involves intramolecular cyclisation over a preformed tetrahydropyran derivative

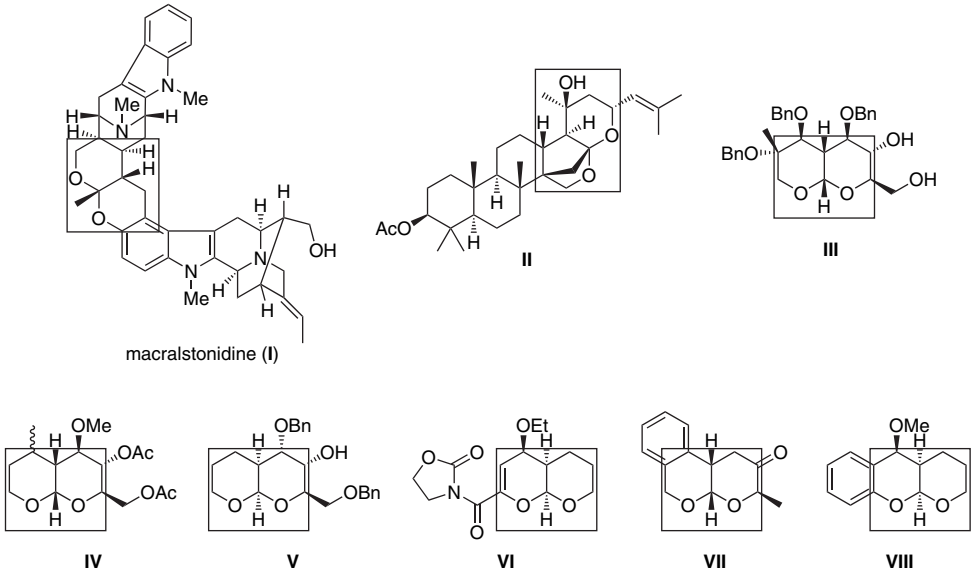

Chart 1.

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under radical,[4a,4c,5] acidic,[4b,6] Diels–Alder[4d,4e,7] or Heck[8] conditions. For instance, compound **IV** was obtained by 6-*exo* radical cyclisation of a but-3-enyl-2-deoxy-2-iodo-α-D-glycoside.[4a] Acid catalysis promoted the intramolecular cyclisation of a 2-deoxy-2-hydroxyalkyl-α-D-altropyranoside to give **V**.[4b] Compound **VI** is one example of intermolecular hetero Diels–Alder reaction of a substituted 1-oxabuta-1,3-diene with dihydro-2*H*-pyran,[7] whereas intramolecular Heck reaction of a hex-2-enepyranoside led to compound **VII**. More recently, different groups have focussed on the synthesis of pyranobenzopyrans of the type **VIII** by Lewis-acid catalysed intermolecular cyclisation of 3,4-dihydro-2*H*-pyran and salicylaldehyde derivatives.[9]

Due to our ongoing interest in the synthesis of fused bicyclic[10] and spirocyclic[11] polyether skeletons, we have preliminary reported about a new 3-methylidenepentane-1,5-dianion synthon, 4-phenylsulfanyl-2-(2-phenylsulfanylethyl)but-1-ene (**2**), that has found application in the straight synthesis of 1,7-dioxaspiro[4.5]decanes,[12a] perhydropyrano[2,3-*b*]pyrans[12a] and 1,6-dioxaspiro[4.4]nonanes.[12b] We want to report herein a more detailed study on the application of **2** to the stereoselective synthesis of *cis*-perhydropyrano [2,3-*b*]pyrans, including the synthesis of some enantiopure compounds, as well as to report about their acid-catalysed isomerisation to the corresponding trans derivatives. A discussion about the kinetically and thermodynamically controlled formation of the products is also included.

2. Results and discussion

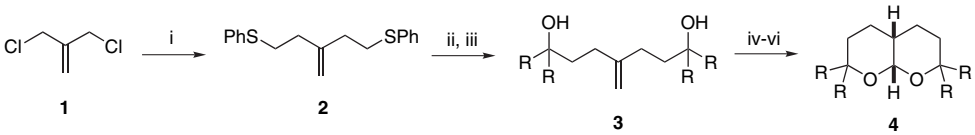
The general protocol followed for the obtention of perhydropyrano[2,3-*b*]pyrans is shown in Scheme 1. As already reported,[12] 4-phenylsulfanyl-2-(2-phenylsulfanylethyl)but-1-ene (**2**) was easily prepared from commercially available 3-chloro-2-(chloromethyl)prop-1-ene (**1**) with an organocuprate reagent derived from $PhSCH_2Li$ and CuCN. Reductive lithiation[13] of the carbon–sulfur bonds in **2** with an excess of lithium powder and a catalytic amount of DTBB (4,4′-di-*tert*-butylbiphenyl), in the presence of different ketones (Barbier conditions)[14] in THF, at 0 °C for 2 h, led after hydrolysis with water, to the corresponding methylidenic diols **3** (Table 1).[15] It is worthy to note that when the chiral ketones (−)-menthone and (−)-fenchone were used as electrophiles, the corresponding C_2-symmetric diols **3g** and **3h**, respectively, were obtained as single enantiomers.[15]

The transformation of diols **3** into the corresponding perhydropyrano[2,3-*b*]pyrans **4** was effected by successive hydroboration–oxidation with borane–hydrogen peroxide, and final oxidation with PCC (Scheme 1 and Table 1).[10] Under the reaction conditions shown in Scheme 1 (step vi), the spontaneous intramolecular ketalisation occurred in most of the cases with exclusive formation of the cis diastereoisomers and in high yields. Especially interesting from the structural point of view are the products derived from cyclic ketones (in particular polyether **4e**), which contain both spiro and fused bicyclic moieties. Compounds **4a** and **4c** showed to be in equilibrium with small amounts (~10%) of the corresponding precursor lactols (Table 1). The cis stereochemistry in **4** was initially assigned by comparison of the ^1H NMR chemical shift of H_{8a} (acetal proton) and the J H_{8a}, H_{4a} with the values appeared in the literature,[6b] as well as by NOE experiments, and unambiguously established by X-ray crystallography of compound **4f** (Fig. 1). The derivative of (−)-menthone (**4g**) was obtained as an enantiomerically pure compound, whereas **4h** (74%) was obtained together with the corresponding trans diastereoisomer (13%).

To the best of our knowledge this is the first procedure that allows the straight preparation of perhydropyrano[2,3-*b*]pyrans from a completely acyclic precursor and in a stereoselective manner. This methodology is, in fact, clearly advantageous by comparison with those based on the acidic treatment of 2-alkoxy-3-(3-hydroxypropyl)tetrahydropyran derivatives and reported independently by the groups of Deslongchamps[6a] and Duhamel.[6b] In these studies, perhydropyrano[2,3-*b*]pyrans were obtained in 10–90% de, as a result the acidic medium promoted both intramolecular cyclisation and cis–trans isomerisation. In contrast, the methodology described herein, due to the mild and inert reaction conditions utilised, allowed a complete kinetically controlled ketalisation, leading to *cis*-perhydropyrano[2,3-*b*]pyrans (**4**) in >99% de.

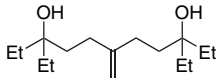
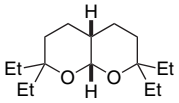
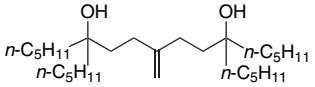
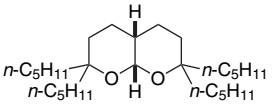
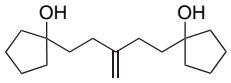
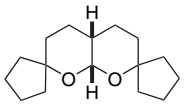
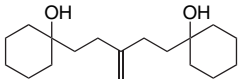
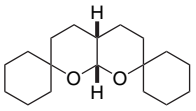
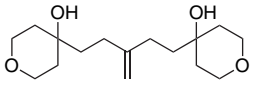
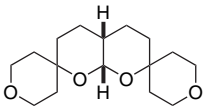
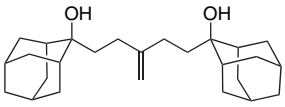
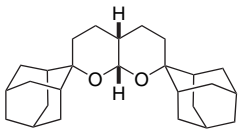
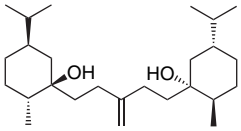
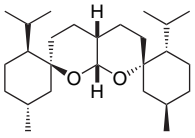
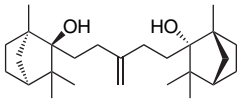
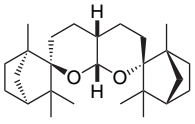
It must be noted that in contrast to the results obtained in Table 1, the cyclisation of the diol derived from diisopropylketone (**3i**) furnished a mixture of perhydropyrano[2,3-*b*]pyrans in a 1:3.5 cis/trans ratio, whereas the diol derived from di-*tert*-butylketone (**3j**) was exclusively obtained as the trans isomer (Scheme 2).[16]

We devised the possibility of obtaining stereoselectively *trans*-perhydropyrano[2,3-*b*]pyrans from the corresponding cis derivatives. Thus, by treating a variety of *cis*-perhydropyrano[2,3-*b*]pyrans **4** with *p*-toluenesulfonic acid in $CHCl_3$ at rt, a progressive isomerisation to the corresponding *trans*-perhydropyrano[2,3-*b*]pyrans **5** was observed (Scheme 3 and Table 2). Figure 2 shows the evolution of the cis–trans isomerisation vs time for compounds **4a**,**b**, **c**,**d**,**f**. A conversion of ≥80% was reached in all the cases after 35 h. The different substituents at the 2 and 7 positions seem to exert a little influence on the cis–trans isomerisation since most values for the trans isomers are near 85% after total equilibration. However, a high 8:92 cis/trans ratio after total equilibration was obtained for compound **4c** (Table 2,



Scheme 1. Reagents and conditions: (i) $PhSCH_2Li$, CuCN, LiCl, 0 °C, 2 h; (ii) Li, DTBB (2.5 mol %), R_2CO, THF, 0 °C, 2 h; (iii) H_2O; (iv) BH_3·THF, 0 °C, 6 h; (v) 33% H_2O_2, 3 M NaOH, 0 °C, 8 h; (vi) PCC, CH_2Cl_2, rt, 8 h.

Table 1. Preparation of the perhydropyrano[2,3-*b*]pyrans **4**

Product **3**[a]			Product **4**[a]		
No.	Structure	Yield (%)[b]	No.	Structure	Yield (%)[c]
3a		55	**4a**		82
3b		50	**4b**		91
3c		57	**4c**		84
3d		58	**4d**		87
3e		37[d]	**4e**		87
3f		33[e]	**4f**		76[f]
3g		48	**4g**		93[g]
3h		49	**4h**		74[h]

[a] All products were ≥95% pure (GLC and/or 300 MHz ^{1}H NMR) and were fully characterised by spectroscopic means (IR, ^{1}H and ^{13}C NMR, and MS).
[b] Isolated yield after column chromatography, unless otherwise stated (silica gel, hexane/EtOAc), based on the starting compound **2**.
[c] Yield of pure compounds **4** from the reaction crude (unless otherwise stated) based on the starting diol **3**.
[d] Purification by column chromatography was carried out with EtOAc/MeOH as eluant.
[e] Isolated yield after recrystallisation with hexane.
[f] Isolated yield after column chromatography (silica gel, hexane), based on the corresponding diol **3f**.
[g] As a single enantiomer.
[h] The corresponding trans diastereoisomer was obtained in 13% yield.

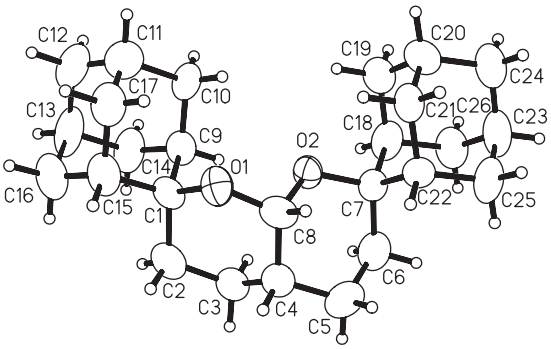
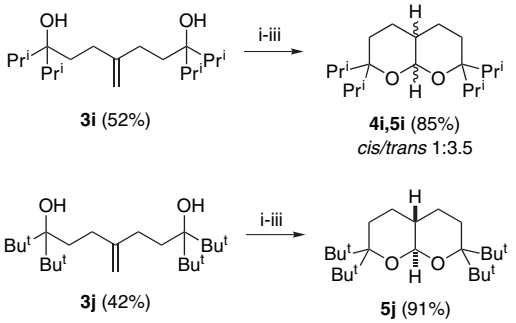
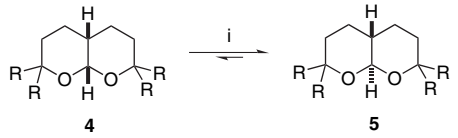


Figure 1. Plot showing the X-ray structure and atomic numbering of compound **4f**.



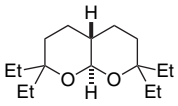
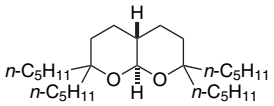
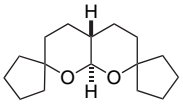
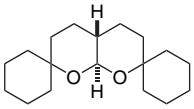
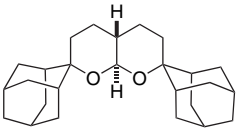
Scheme 2. (i) $BH_3 \cdot THF$, 0 °C, 6 h; (ii) 33% H_2O_2, 3 M NaOH, 0 °C, 8 h; (iii) PCC, CH_2Cl_2, rt, 8 h.

entry 3). This isomerisation is in favour of the trans products **5**, which showed to be more stereoselective than those reported previously.[6b] The trans stereochemistry in compounds **5** was assigned by comparing their δ H_{8a} (4.30–4.58 ppm) and J H_{8a}, H_{4a} (7.5–8.6 Hz) with those of the cis stereochemistry in compounds **4** (4.94–5.06 ppm, J=1.9–2.8 Hz). Nonetheless, this spectroscopic-structure correlation could be additionally confirmed by X-ray crystallography of compound **5f** (Fig. 3).



Scheme 3. (i) *p*-TsOH (cat.), $CHCl_3$ and rt.

Table 2. Isomerisation of the *cis*-perhydropyrano[2,3-*b*]pyrans **4**

Starting material	Product **5**		
	No.	Structure	Conversion (%)[a]
4a	**5a**		86
4b	**5b**		84
4c	**5c**		92
4d	**5d**		86
4f	**5f**		85

[a] Conversion determined by ^{1}H NMR.

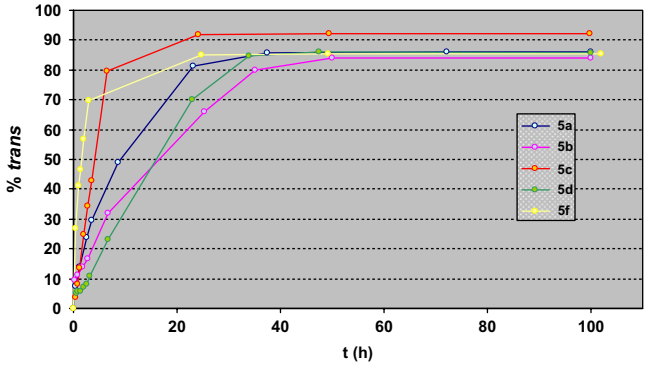


Figure 2. Graphic showing the cis–trans isomerisation of compounds **4** to **5** vs time, under the conditions depicted in Scheme 3.

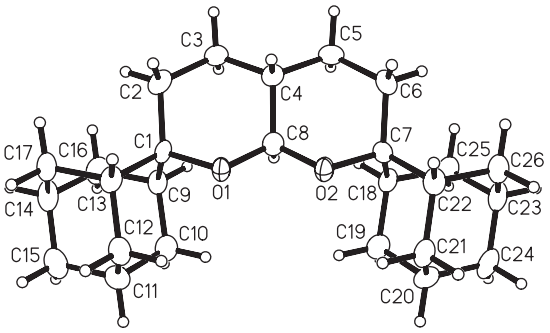
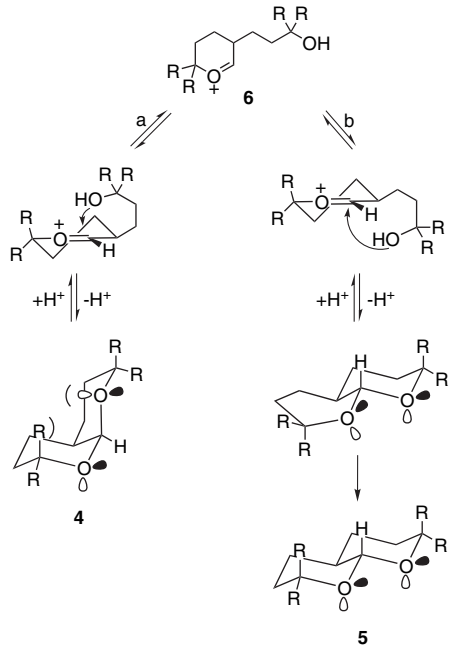


Figure 3. Plot showing the X-ray structure and atomic numbering of compound **5f**.

The high diastereo-control achieved in the cyclisation reaction to the *cis*-perhydropyrano[2,3-*b*]pyrans **4** can be explained if we accept that the hydroxyalkyl substituent adopts a pseudoequatorial position in the cation **6** and the nucleophilic attack occurs along a pseudoaxial trajectory to maximise the overlap of the HOMO of the nucleophile with the LUMO of the oxonium ion (Scheme 4, pathway a). This argument is equivalent to consider, as Deslongchamps et al. did,[6a] that the acetal formation will take place with minimum energy only when the intermediate oxonium ion **6** can develop an electron pair which becomes antiperiplanar to the newly formed carbon–oxygen bond in the final product (pathway a). Under these conditions, nucleophilic attack from the bottom face of the oxonium ion (α attack) cannot yield the *trans*-acetal directly in its more stable conformation (Scheme 4, pathway b), but must provide a disfavoured twist-boat conformation. The latter would then undergo a conformational change to the more stable chair–chair conformation of the *trans*-acetal **5**. On the other hand, the formation of the *cis*-perhydropyrano[2,3-*b*]pyrans **4** is expected to be favoured by a lower-energy, chair-like transition state (pathway a).[17]



Scheme 4. Kinetic vs thermodynamic ketalisation.

The specific conversion of the diols **3** into the *cis*-perhydropyrano[2,3-*b*]pyrans **4** can be considered as a result of a kinetically controlled reaction. The above described equilibration studies show that, at 25 °C, *cis*-acetals **4** are less stable than the trans isomers **5** by 0.99–1.47 kcal/mol (Table 3). Descotes et al. carried out the equilibration of *cis*- and *trans*-hexahydro-2*H*-pyrano[2,3-*b*]pyrans **7** and the resulting mixture contained 57% of cis and 43% of trans isomer at 80 °C (Chart 2).[18] Therefore, the cis isomer was more stable by 0.17 kcal/mol with an estimated value of 1.4 kcal/mol for the anomeric effect. Similar studies by Duhamel et al. on the dimethyl derivative **8** showed, however, that the *cis*-acetal (31%) was less stable than the trans isomer (69%) by 0.52 kcal/mol.[6b] The higher diastereoselectivity achieved in our equilibration studies [7.7–15.9% (cis), 84.1–92.3% (trans)], in comparison with the aforementioned examples, might be due to an extra and unfavourable 1,3-diaxial interaction, which is present in **4** (Chart 2). This 1,3-diaxial interaction could account for the major and exclusive formation of the trans diastereoisomers in the cyclisation reaction of the diols **3i** and **3j**, respectively, where the bulkier isopropyl and *tert*-butyl groups cannot be easily accommodated in a cis chair–chair conformation.

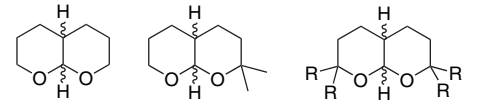


Chart 2. Equilibration studies of different 1,8-dioxadecalins.

The different results obtained in the equilibration studies of the *cis*- and *trans*-1,8-dioxadecalins shown in Chart 2, together with the anomalous behaviour observed in the cyclisation of the diols **3i**,**j**, encouraged us to carry out a short computational study about the geometry optimisation of some of the compounds of **4** and **5**, that allowed us to compare the calculated values with the experimental data.

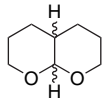
Table 3

Compound no.	ΔG° (kcal/mol)[a]	$\Delta(\Delta H_f)$ (kcal/mol)[b]
4a, **5a**	−1.079	2.858
4b, **5b**	−0.987	—
4c, **5c**	−1.469	0.459
4d, **5d**	−1.053	1.177
4e, **5e**	—	0.635
4f, **5f**	−1.039	1.221
4i, **5i**	—[c]	−1.311
4j, **5j**	—[d]	−1.819[e]

[a] Standard Gibbs energy was experimentally determined at 298 K for the isomerisation of the *cis*-perhydropyrano[2,3-*b*]pyrans **4** to the *trans*-perhydropyrano[2,3-*b*]pyrans **5** (see Scheme 3).
[b] Difference in heat of formation of compounds **4** and **5** [ΔH_f (**5**)−ΔH_f (**4**)] in a chair–chair conformation, unless otherwise stated, calculated by the PM3 semi-empirical method.
[c] Isomerisation of **4i** to **5i** was accompanied by decomposition.
[d] No isomerisation of **5j** (the starting material in this case) was observed after 20 min but only decomposition.
[e] Difference in heat of formation of compounds **4j** and **5j** in a chair–twist boat and chair–chair conformations, respectively.

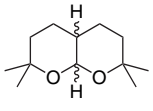
Theoretical studies dealing with the conformational analysis and relative stabilities of 1,8-dioxadecalins are very scarce.[19] In our case, PM3 semi-empirical calculations[20] were carried out for any of the derivatives **4a**,**c**,**d**,**e**,**f**,**i**,**j** and **5a**,**c**,**d**,**e**,**f**,**i**,**j**. In all the cases, the heat of formation was determined for a fused chair–chair conformation in the perhydropyrano[2,3-*b*]pyran core, as other conformations resulted to be less stable (Table 3). One exception was, however, compound **4j**, wherein a high steric hindrance of the *tert*-butyl substituents did not allow fixing of a chair–chair but a chair–twist boat conformation.

From Table 3 and as already mentioned above, the standard Gibbs energy for the isomerisation of the *cis*-perhydropyrano[2,3-*b*]pyrans **4** to the *trans*-perhydropyrano[2,3-*b*]pyrans **5**, clearly reveals that the latter (thermodynamic product) are more stable than the former (kinetic product), though the difference is in most cases around 1 kcal/mol. This result is, however, contradictory with the heats of formation calculated for compounds **4** and **5** (except for **4i**,**j** and **5i**,**j**), which indicate that compounds **4** are thermodynamically more stable than compounds **5**. The same trend was observed for the heats of formation of the simplest *cis*- and *trans*-hexahydro-2*H*-pyrano[2,3-*b*]pyrans **7** (Chart 3). In order to confirm the validity of the PM3 calculations, the simpler substituted *cis*- and *trans*-2,2,7,7-tetramethylperhydropyrano[2,3-*b*]pyrans **9** were subjected to both PM3 and DFT geometry optimisation at the B3LYP/6-31G* level.[21] Also in this case, the similar differences in energy point to the diastereoisomer *cis*-**9** as the more stable one (Chart 3). Therefore, we can conclude that, in general, the *cis*-perhydropyrano[2,3-*b*]pyrans **4** are more stable than the *trans*-perhydropyrano[2,3-*b*]pyrans **5**, under the calculation conditions (i.e., in the gas phase). It is not our aim to carry out a more detailed study about the effect of the solvent on the relative stability of *cis*- and *trans*-1,8-dioxadecalins. On the other hand, it is worthy to note that the only two cases for which the trans diastereoisomer has been calculated to be more stable than the cis one, namely **5i** and **5j**, are the only ones that have been experimentally obtained as the major and exclusive trans diastereoisomers, respectively. In these two cases, important repulsive interactions (1,3-diaxial and others) involving the isopropyl and *tert*-butyl substituents seem to dominate over the stabilising stereoelectronic effects (e.g., the anomeric effect), disfavouring the formation of the cis diastereoisomers.



7

E_{trans} - E_{cis} = 2.107 kcal/mol (PM3)



9

E_{trans} - E_{cis} = 0.951 kcal/mol (PM3)
E_{trans} - E_{cis} = 1.094 kcal/mol (DFT)

Chart 3.

3. Conclusion

A variety of symmetrically substituted *cis*-perhydropyrano[2,3-*b*]pyrans (kinetic products) have been synthesized stereoselectively from a new 3-methylidenepentane-1,5-dianion synthon and the acid-promoted isomerisation of the former to the corresponding trans isomers (thermodynamic products) also proceeds diastereoselectively.

4. Experimental

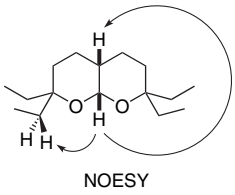
4.1. General

Melting points were obtained with a Reichert Thermovar apparatus. Optical rotations were measured with a Perkin–Elmer 341 polarimeter with a thermally jacketed 10 cm cell at approximately 20 °C. Concentrations (c) are given in g/100 mL and [α] values are given in units of 10^{-1} deg cm^2/g. NMR spectra were recorded on a Bruker Avance 300 and Bruker Avance 400 (300 and 400 MHz for ^{1}H NMR, and 75 and 100 MHz for ^{13}C NMR, respectively) using $CDCl_3$ as solvent and TMS as an internal standard; chemical shifts are given in (δ) parts per million and coupling constants (J) in hertz. Mass spectra (EI) were obtained at 70 eV on a Shimadzu QP-5000 and Agilent 5973 spectrometers, fragment ions in m/z with relative intensities (%) in parenthesis. HRMS analyses were carried out on a Finnigan MAT95S spectrometer. Elemental analyses were performed on a Carlo Erba CHNS-O EA1108 elemental analyser. The purity of volatile compounds and the chromatographic analyses (GLC) were determined with a Hewlett Packard HP-5890 instrument equipped with a flame ionisation detector and a 30 m capillary column (0.32 mm diameter, 0.25 μm film thickness), using nitrogen (2 mL/min) as carrier gas, $T_{injector}$=275 °C, T_{column}=60 °C (3 min) and 60–270 °C (15 °C/min); retention times (t_R) are given under these conditions. Column chromatography was performed using silica gel 60 of 40–60 microns. Thin-layer chromatography was carried out on TLC plastic sheets with silica gel 60 F_{254} (Merck). THF was directly used without any purification (Acros, 99.9%). Lithium powder was commercially available (MEDALCHEMY S. L.). PM3 calculations were carried out with the HyperChem7.5 molecular modelling package, whereas DFT calculations were carried out with the Gaussian03 package.[22]

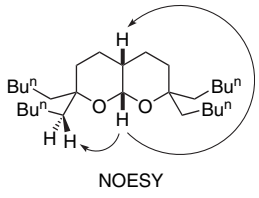
4.2. General procedure for the preparation of the *cis*-perhydropyrano[2,3-*b*]pyrans (4)

$BH_3\cdot$THF (1 M, 5 mL, 5 mmol) was added to a solution of the diol **3** (1 mmol) in THF (10 mL). After stirring for 6 h at rt, the reaction mixture was hydrolysed with water (5 mL) at 0 °C (ca. 5 min), followed by the consecutive addition of a 3 M NaOH (10 mL) and 33% vol of H_2O_2 (10 mL) solutions. The resulting mixture was stirred for 8 h followed by extraction with EtOAc (3×15 mL). The organic phases were dried over anhydrous Na_2SO_4 and the solvents were removed under reduced pressure (15 Torr), affording the corresponding triol crudes, which were subjected to oxidation as follows: pyridinium chlorochromate (2.4 mmol, 517 mg) was added to a solution of the corresponding triol in dichloromethane (10 mL) and the reaction mixture was stirred for 8 h. Then, it was filtered through a pad containing silica gel (bottom layer) and Celite (top layer), and washed with hexane, in order to remove the chromium salts. After removal of the solvents at reduced pressure (15 Torr), the expected *cis*-perhydropyrano[2,3-*b*]pyrans were obtained without any further purification, except in the case of compound **4f**, which was purified by column chromatography (silica gel, hexane).

4.2.1. 2,2,7,7-Tetraethyl-*cis*-perhydropyrano[2,3-*b*]pyran (4a). Colourless oil; t_R 13.32; R_f 0.67 (hexane/EtOAc 8:2); ν (film) 1087 cm^{-1} (CO); δ_H 0.83, 0.87 (12H, 2t, J=7.5, 4×CH_3), 1.20–1.70 (17H, m, 8×CH_2, C*H*CHO), 5.02 (1H, d, J=2.8, CHO); δ_C 7.6, 7.8 (4×CH_3), 21.5, 27.7, 29.1, 30.2 (8×CH_2), 34.2 (*C*HCHO), 76.6 (2×C), 93.3 (CHO); m/z 225 (M$^+$−29, 100%), 207 (30), 189 (57), 153 (15), 140 (23), 136 (10), 135 (86), 133 (50), 124 (12), 123 (19), 111 (21), 109 (19), 98 (12), 97 (17), 95 (35), 85 (13), 84 (14), 83 (25), 81 (12), 69 (39), 67 (11), 57 (42), 55 (45). HRMS calcd for $C_{16}H_{30}O_2$ 254.2246, found 254.2248.



4.2.2. 2,2,7,7-Tetrapentyl-*cis*-perhydropyrano[2,3-*b*]pyran (4b). Colourless oil; t_R 17.56; R_f 0.67 (hexane/EtOAc 8:2); ν (film) 1075 cm^{-1} (CO); δ_H 0.80–0.95 (12H, m, 4×CH_3), 1.15–2.00 (41H, m, 20×CH_2, C*H*CHO), 5.02 (1H, d, J=2.5, CHO); δ_C 14.1, 14.2 (4×CH_3), 21.6, 22.6, 22.7, 22.8, 22.9, 23.0, 23.2, 30.1, 32.6, 32.7, 38.6 (20×CH_2), 34.3 (*C*HCHO), 78.1 (2×C), 93.4 (CHO); m/z 407 (M$^+$−15, <1%), 338 (24), 337 (100). HRMS calcd for $C_{28}H_{54}O_2$ 422.4124, found 422.4117.



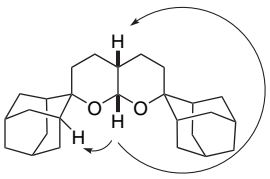
4.2.3. Dispiro[cyclopentane-1,2′-*cis*-tetrahydropyrano-[2,3-*b*]pyran-7′,1″-cyclopentane] (4c). Colourless oil; t_R 14.73; R_f 0.58 (hexane/EtOAc 8:2); ν (film) 1071 cm^{-1} (CO); δ_H 1.20–2.10 (25H, m, 12×CH_2, C*H*CHO), 4.94 (1H, d, J=2.5, CHO); δ_C 23.7, 23.9, 32.4, 36.8, 39.1, 39.5 (12×CH_2), 34.2 (*C*HCHO), 83.9 (2×C), 95.1 (CHO); m/z 250 (M$^+$, 47%), 169 (18), 151 (52), 150 (14), 147 (14), 139 (13), 138 (100), 137 (12), 136 (10), 135 (23), 134 (12), 133 (47), 132 (37), 123 (46), 122 (65), 121 (23), 120 (46), 119 (11), 109 (21), 108 (14), 107 (24), 105 (10), 97 (13), 96 (17), 95 (49), 94 (44), 93 (41), 91 (23), 85 (17), 83 (19), 82 (37), 81 (89), 80 (57), 79 (48), 77 (15), 69 (13), 68 (11), 67 (93), 57 (17), 55 (52), 54 (10), 53 (16). HRMS calcd for $C_{16}H_{26}O_2$ 250.1933, found 250.1940.

4.2.4. Dispiro[cyclohexane-1,2′-*cis*-tetrahydropyrano-[2,3-*b*]pyran-7′,1″-cyclohexane] (4d). Colourless oil; t_R

15.80; R_f 0.63 (hexane/EtOAc 8:2); ν (film) 1050 cm^{-1} (CO); δ_H 1.20–1.90 (29H, m, 14×CH_2, C*H*CHO), 5.03 (1H, d, J=2.5, CHO); δ_C 21.4, 21.8, 22.1, 23.8, 25.6, 26.3, 32.5 (14×CH_2), 34.7 (*C*HCHO), 73.4 (2×C), 93.1 (CHO); *m/z* 278 (M^+, 64%), 183 (20), 181 (10), 166 (15), 165 (100), 152 (36), 149 (11), 147 (43), 146 (36), 137 (15), 136 (31), 135 (11), 134 (14), 122 (14), 121 (39), 109 (22), 108 (17), 107 (15), 96 (19), 95 (49), 94 (26), 93 (13), 91 (12), 83 (12), 81 (64), 79 (25), 69 (12), 68 (10), 67 (42), 55 (43). HRMS calcd for $C_{18}H_{30}O_2$ 278.2246, found 278.2246.

4.2.5. Dispiro[oxacyclohexane-4,2′-*cis*-tetrahydropyrano-[2,3-*b*]pyran-7′,4″-oxacyclohexane] (4e). Colourless oil; t_R 17.34; R_f 0.55 (hexane/EtOAc 1:1); ν (film) 1101 cm^{-1} (CO); δ_H 1.20–2.05 (17H, m, 4×C$H_2$$CH_2$O, 2×C$H_2CH_2$CH, C*H*CHO), 3.62–3.72, 3.77–3.90 (8H, 2m, 4×CH_2O), 5.06 (1H, d, J=1.9, CHO); δ_C 20.8, 33.2, 35.3, 35.4 (4×*C*$H_2$$CH_2$O, 2×*C*$H_2$*C*$H_2$CH), 34.2 (*C*HCHO), 63.4, 63.9 (4×CH_2O), 70.9 (2×C), 93.2 (CHO); *m/z* 282 (M^+, 14%), 268 (86), 222 (18), 210 (14), 170 (14), 168 (17), 167 (78), 157 (17), 155 (37), 137 (12), 127 (19), 121 (14), 114 (35), 112 (18), 111 (22), 109 (15), 101 (28), 99 (40), 97 (21), 96 (100), 95 (14), 94 (11), 93 (10), 83 (21), 81 (19), 79 (30), 71 (12), 70 (10), 69 (10), 67 (29), 55 (39), 53 (12). HRMS calcd for $C_{16}H_{26}O_4$ 282.1831, found 282.1810.

4.2.6. Dispiro[adamantane-2,2′-*cis*-tetrahydropyrano-[2,3-*b*]pyran-7′,2″-adamantane] (4f). Colourless solid; R_f 0.72 (hexane/EtOAc 8:2); mp 180 °C (dec); ν (KBr) 1083 cm^{-1} (CO); δ_H 1.20–1.95, 2.00–2.10, 2.20–2.30, 2.40–2.50 (37H, 4m, 9×C*H*CH_2, 14×CH_2), 5.13 (1H, d, J=2.2, CHO); δ_C 21.3, 29.2, 29.7, 32.4, 33.1, 34.2, 34.4, 38.5 (14×CH_2), 27.7, 27.8 (4×*C*HCH_2*C*H), 37.4 (*C*HCHO), 77.2 (2×C), 92.7 (CHO); *m/z* 382 (M^+, 2%), 204 (48), 189 (18), 188 (23), 149 (19), 148 (100), 91 (10), 79 (11). Anal. calcd for $C_{26}H_{38}O_2$ C, 81.62; H, 10.01, found C, 81.60; H, 9.89.



NOESY

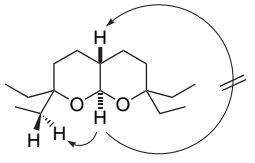
4.2.7. Dispiro[(1*S*,2*S*,4*R*)-1-isopropyl-4-methylcyclohexane-2,2′-*cis*-tetrahydropyrano[2,3-*b*]pyran-7′,2″-{(1*S*,2*S*,4*R*)-1-isopropyl-4-methylcyclohexane}] (4g). Colourless oil; t_R 18.43; R_f 0.59 (hexane/EtOAc 8:2); $[\alpha]_D$ −45.3 (*c* 2.3, $CHCl_3$); ν (film) 1040 cm^{-1} (CO); δ_H 0.75–1.00 (18H, m, 6×CH_3), 1.15–2.55 (27H, m, 4×C*H*CH_3, 3×C*H*CH_2, 10×CH_2), 4.94 (1H, d, J=1.9, CHO); δ_C 18.4, 20.1, 22.6, 22.7, 24.1, 25.5, 25.7, 26.1, 26.4, 27.2 (6×CH_3, 4×*C*HCH_3), 18.9, 20.7, 22.1, 24.9, 26.3, 32.7, 35.9, 36.0, 39.8, 46.3 (10×CH_2), 35.4 (*C*HCHO), 50.8, 52.2 (2×*C*HCHCH_3), 74.7, 77.7 (2×CO), 92.9 (CHO); *m/z* 391 (M^++1, 24%), 390 (81), 347 (32), 329 (30), 305 (16), 239 (21), 221 (23), 208 (20), 203 (50), 202 (100), 193 (10), 165 (12), 164 (39), 159 (12), 149 (17), 147 (14), 137 (19), 123 (11), 121 (12), 110 (10), 109 (27), 107 (12), 105 (11), 97 (11), 95 (32), 93 (14), 83 (14), 81 (31), 79 (11), 69 (32), 67 (17), 55 (30). HRMS calcd for $C_{26}H_{46}O_2$ 390.3498, found 390.3493.

4.2.8. Dispiro[(1*R*,2*S*,4*S*)-1,3,3-trimethylbicyclo[2.2.1]-heptane-2,2′-*cis*-tetrahydropyrano[2,3-*b*]pyran-7′,2″-{(1*R*,2*S*,4*S*)-1,3,3-trimethylbicyclo[2.2.1]heptane}] (4h). Colourless oil; t_R 19.19; R_f 0.60 (hexane/EtOAc 8:2); ν (film) 1036 cm^{-1} (CO); δ_H 0.85–2.35 (41H, m, 6×CH_3, 3×C*H*CH_2, 10×CH_2), 5.03 (1H, d, J=2.3, CHO); δ_C 18.2, 18.8, 22.6, 22.8, 23.1, 23.3 (6×CH_3), 21.8, 22.9, 24.7, 25.3, 25.8, 26.0, 29.7, 29.9, 41.3, 43.6 (10×CH_2), 30.4 (*C*HCHO), 45.2, 47.1 (4×*C*CH_3), 48.9, 50.3 (2×*C*HCH_2C), 82.6, 83.1 (2×CO), 94.3 (CHO); *m/z* 386 (M^+, 8%), 261 (24), 125 (16), 123 (45), 122 (17), 121 (14), 109 (13), 107 (32), 105 (11), 95 (12), 93 (13), 82 (10), 81 (100), 80 (14), 79 (23), 69 (30), 67 (17), 57 (12), 55 (36), 53 (10), 43 (44), 41 (61). HRMS calcd for $C_{26}H_{42}O_2$ 386.3185, found 386.3191.

4.3. General procedure for the preparation of the *trans*-perhydropyrano[2,3-*b*]pyrans (5)

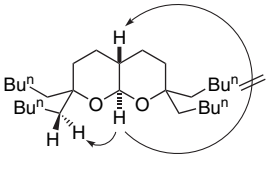
The *cis*-perhydropyrano[2,3-*b*]pyran **4** (30 mg) was dissolved in $CDCl_3$ (1 mL) and this solution was introduced into an NMR tube. *p*-Toluensulfonic acid (ca. 0.5 mg) was added to the NMR tube, the resulting mixture being monitored by ^1H NMR at different time intervals until no variation in the cis/trans rate was observed.

4.3.1. 2,2,7,7-Tetraethyl-*trans*-perhydropyrano[2,3-*b*]pyran (5a). Colourless oil; t_R 13.16; R_f 0.67 (hexane/EtOAc 8:2); ν (film) 1086 cm^{-1} (CO); δ_H 0.85, 0.88 (12H, 2t, J=7.5, 4×CH_3), 1.20–1.70 (17H, m, 8×CH_2, C*H*CHO), 4.33 (1H, d, J=8.4, CHO); δ_C 6.8, 7.7 (4×CH_3), 23.2, 25.0, 31.8, 32.6 (8×CH_2), 40.6 (*C*HCHO), 78.3 (2×C), 93.8 (CHO); *m/z* 225 (M^+−29, 100%), 207 (31), 189 (61), 153 (15), 140 (21), 135 (84), 133 (51), 124 (10), 123 (18), 111 (19), 109 (19), 97 (14), 95 (33), 85 (11), 84 (12), 83 (20), 81 (11), 69 (32), 57 (31), 55 (34). HRMS calcd for $C_{16}H_{30}O_2$ 254.2246, found 254.2242.



NOESY

4.3.2. 2,2,7,7-Tetrapentyl-*trans*-perhydropyrano[2,3-*b*]pyran (5b). Colourless oil; t_R 17.53; R_f 0.670 (hexane/EtOAc 8:2); ν (film) 1070 cm^{-1} (CO); δ_H 0.80–0.95 (12H, m, 4×CH_3), 1.15–2.00 (41H, m, 20×CH_2, C*H*CHO), 4.39 (1H, d, J=8.4, CHO); δ_C 14.1 (4×CH_3), 22.2, 23.9, 24.3, 25.1, 31.4, 33.5, 34.4, 36.0, 40.2, 41.6 (20×CH_2), 43.3 (*C*HCHO), 78.2 (2×C), 94.0 (CHO); *m/z* 407 (M^+−15, <1%), 338 (21), 337 (100). HRMS calcd for $C_{28}H_{54}O_2$ 422.4124, found 422.4119.

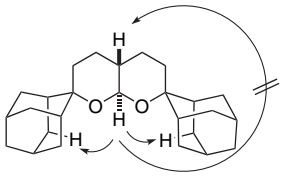


NOESY

4.3.3. Dispiro[cyclopentane-1,2′-*trans*-tetrahydropyrano[2,3-*b*]pyran-7′,1″-cyclopentane] (5c). Colourless oil; t_R 14.28; R_f 0.60 (hexane/EtOAc 8:2); ν (film) 1075 cm^{-1} (CO); δ_H 1.20–2.10 (25H, m, 12×CH_2, C*H*CHO), 4.30 (1H, d, J=7.8, CHO); δ_C 22.5, 24.3, 30.0, 35.6, 38.2, 38.9 (12×CH_2), 39.5 (*C*HCHO), 80.1 (2×C), 92.7 (CHO); *m/z* 251 (M^++1, 10%), 250 (52), 169 (19), 151 (54), 150 (14), 147 (13), 139 (13), 138 (100), 137 (12), 136 (10), 135 (23), 134 (11), 133 (46), 132 (37), 123 (44), 122 (67), 121 (22), 120 (47), 119 (12), 109 (21), 108 (14), 107 (24), 105 (10), 97 (12), 96 (16), 95 (49), 94 (43), 93 (42), 91 (23), 85 (17), 83 (18), 82 (34), 81 (86), 80 (56), 79 (46), 77 (13), 69 (12), 68 (11), 67 (90), 57 (16), 55 (50), 54 (10), 53 (15). HRMS calcd for $C_{16}H_{26}O_2$ 250.1933, found 250.1929.

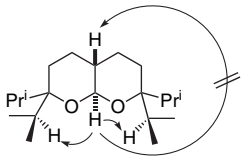
4.3.4. Dispiro[cyclohexane-1,2′-*trans*-tetrahydropyrano[2,3-*b*]pyran-7′,1″-cyclohexane] (5d). Colourless oil; t_R 15.76; R_f 0.65 (hexane/EtOAc 8:2); ν (film) 1070 cm^{-1} (CO); δ_H 1.20–2.00 (29H, m, 14×CH_2, C*H*CHO), 4.54 (1H, d, J=8.2, CHO); δ_C 21.6, 21.8, 24.9, 26.2, 30.7, 35.0, 40.1 (14×CH_2), 41.8 (*C*HCHO), 75.3 (2×C), 93.3 (CHO); *m/z* 278 (M^+, 60%), 183 (18), 166 (17), 165 (100), 152 (35), 149 (11), 147 (40), 146 (36), 137 (16), 136 (30), 134 (17), 122 (16), 121 (38), 109 (24), 108 (15), 107 (15), 96 (19), 95 (48), 94 (27), 93 (11), 91 (13), 83 (12), 81 (64), 79 (24), 69 (11), 67 (39), 55 (44). HRMS calcd for $C_{18}H_{30}O_2$ 278.2246, found 278.2252.

4.3.5. Dispiro[adamantane-2,2′-*trans*-tetrahydropyrano[2,3-*b*]pyran-7′,2″-adamantane] (5f). Colourless solid; R_f 0.71 (hexane/EtOAc 8:2); mp 165 °C (dec); ν (KBr) 1090 cm^{-1} (CO); δ_H 1.20–1.95, 2.00–2.35, 2.40–2.50 (37H, 3m, 9×C*H*CH_2, 14×CH_2), 4.55 (1H, d, J=7.8, CHO); δ_C 24.6, 27.5, 31.6, 31.9, 32.7, 33.8, 34.4, 38.3, 39.8 (14×CH_2), 27.8, 29.5 (4×*C*HCH_2*C*H), 41.5 (*C*HCHO), 79.0 (2×C), 91.5 (CHO); *m/z* 382 (M^+, <1%), 204 (50), 189 (17), 188 (24), 149 (23), 148 (100), 79 (10). Anal. calcd for $C_{26}H_{38}O_2$ C, 81.62; H, 10.01, found C, 81.69; H, 9.99.



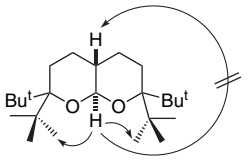
NOESY

4.3.6. 2,2,7,7-Tetraisopropyl-*trans*-perhydropyrano[2,3-*b*]pyran (5i). Colourless oil; t_R 15.16; R_f 0.68 (hexane/EtOAc 8:2); ν (film) 1367, 1384, 1074 cm^{-1} (CO); δ_H 0.85–1.05 (24H, m, 8×CH_3), 1.20–1.75 (9H, m, 4×CH_2, C*H*CHO), 2.00–2.10, 2.30–2.40 (4H, 2m, 4×C*H*CH_3), 4.58 (1H, d, J=8.6, CHO); δ_C 16.9, 18.3, 18.6, 19.0 (8×CH_3), 24.7, 25.0 (4×CH_2), 30.2, 32.9 (4×*C*HCH_3), 40.6 (*C*HCHO), 81.8 (2×C), 94.5 (CHO); *m/z* 310 (M^+, <1%), 268 (19), 267 (100), 249 (47), 231 (20), 181 (10), 179 (13), 165 (10), 163 (58), 161 (13), 137 (12), 125 (15), 123 (11), 121 (14), 111 (14), 109 (16), 107 (24), 99 (12), 97 (13), 95 (20), 93 (13), 83 (17), 81 (12), 71 (37), 69 (41), 67 (10), 57 (14), 55 (22). HRMS calcd for $C_{20}H_{38}O_2$ 310.2872, ($M^+-C_3H_7$) 267.2319, found 267.2296.



NOESY

4.3.7. 2,2,7,7-Tetra-*tert*-butyl-*trans*-perhydropyrano[2,3-*b*]pyran (5j). Colourless oil; t_R 16.75; R_f 0.74 (hexane/EtOAc 9:1); ν (film) 1041 cm^{-1} (CO); δ_H 1.06, 1.11 (36H, 2s, 12×CH_3), 1.20–1.85 (9H, m, 4×CH_2, C*H*CHO), 5.04 (1H, d, J=8.7, CHO); δ_C 25.5, 28.1 (4×CH_2), 30.6 (12×CH_3), 35.2 (*C*HCHO), 43.2, 43.6 (4×*C*CH_3), 83.8 (2×CO), 98.1 (CHO); *m/z* 366 (M^+, 2%), 309 (19), 291 (17), 235 (20), 153 (20), 151 (33), 135 (19), 123 (12), 109 (25), 107 (14), 97 (11), 95 (14), 83 (31), 57 (100), 55 (14). HRMS calcd for $C_{24}H_{46}O_2$ 366.3498, found 366.3501.



NOESY

4.4. X-ray crystallography

Compounds **4f** and **5f** were recrystallised from hexane. Data collection was performed on a Bruker Smart CCD diffractometer, based on three ω-scan runs (starting ω=−34°) at the values of ϕ=0, 120, 240° with the detector at 2θ=−32°. For each of these runs, 606 frames were collected at 0.3° intervals. An additional run at ϕ=0° of 100 frames was collected to improve redundancy. The diffraction frames were integrated using the SAINT[23] programme and the integrated intensities were corrected for Lorentz-polarisation effects with SADABS.[24]

X-ray data for **4f**: $C_{26}H_{38}O_2$, M=382.56; monoclinic, a=13.490(4) Å, b=11.681(3) Å, c=27.054(7) Å, β=102.798(5)°; V=4157.2(19) $Å^3$; space group $P21/c$; Z=8; D_c=1.222 Mg/m^{-3}; λ=0.71073 Å; μ=0.075 mm^{-1}; $F(000)$=1680; T=22±1 °C. The structure was solved by direct methods[25] and refined to all 6531 unique F_o^2 by full matrix least squares (SHELX97).[26] All the hydrogen atoms were placed at idealised positions and refined as rigid atoms. Final $wR2$=0.1806 for all data and 506 parameters; R_1=0.1045 for 2197 $F_o>4\sigma(F_o)$.

X-ray data for **5f**: $C_{26}H_{38}O_2$, M=382.56; monoclinic, a=13.1663(16) Å, b=6.6471(8) Å, c=23.712(3) Å, β=101.292(3)°; V=2035.1(4) $Å^3$; space group $P21/c$; Z=4; D_c=1.249 Mg/m^{-3}; λ=0.71073 Å; μ=0.076 mm^{-1}; $F(000)$=840; T=−100±1 °C. The structure was solved by direct methods[25] and refined to all 3590 unique F_o^2 by full matrix least squares (SHELX97).[26] All the hydrogen atoms were placed at idealised positions and refined as rigid atoms. Final $wR2$=0.1448 for all data and 253 parameters; R_1=0.1349 for 2010 $F_o>4\sigma(F_o)$.

Crystallographic data (excluding structure factors) for compounds **4f** and **5f** have been deposited in the Cambridge

Crystallographic Data Center as supplementary publication numbers CCDC 291410 and 294666. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 1223 336033; email: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk/data_request/cif).

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Chemo enzymatic synthesis of Rengyol and Isorengyol☆

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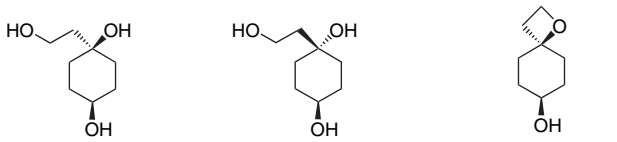
Abstract—Cyanohydrins **2** of *O*-protected 4-hydroxycyclohexanones **1** are excellent starting compounds for the synthesis of Isorengyol (**I**) and Rengyol (**II**). The cyano group of the *O*-benzyl derivative **2d** is first converted into the corresponding aldehyde **4**, which via Wittig olefination led to the vinyl compound **6**. Hydroboration of the trans-derivative (*trans*-**6**) leads, after debenzylation, to Isorengyol, whereas hydroboration and debenzylation of the cis-isomer (*cis*-**6**) gives Rengyol. With hydroxynitrile lyases (HNLs) as catalysts the stereoselective preparation of *cis*- as well as *trans*-cyanohydrin **2d** is possible, which enables the selective preparation of Isorengyol or Rengyol, respectively. The trans-configuration of Isorengyol and the cis-configuration of Rengyol were secured by X-ray crystal structure analysis.

1. Introduction

In 1984 Endo and Hikino have first isolated and characterized Rengyol (**I**) and Isorengyol (**II**) from the fruits of *Forsythia suspensa* Vahl (Scheme 1).[2] In traditional Chinese medicine these fruits, commonly called '*Rengyo*', are widely used because of their anti-inflammatory, antibactericidal, and antiemetic properties.[3] Isorengyol was not only found in *Forsythia* but also in the plants *Isoplexis chalcantha*,[4] *Millingtonia hortensis*,[5] and *Eurya tigang*.[6] Soon after its discovery chemical syntheses of Rengyol and Isorengyol have been developed.[7] The first stereoselective synthesis of Rengyol was performed by Hikino et al.[8] Starting from glucopyranosyl bromide, Rengyol was obtained in six steps and a total yield of 4%.[8] A completely different route was developed by the group of Ogasawara.[9] Starting from ethyl-2-(1-hydroxy-4-oxo-cyclohexa-2,5-dienyl)acetate Rengyol was obtained in 12 and Isorengyol in 16 steps.[9]



I: Rengyol **II**: Isorengyol **III**: Cleroindicin A

Scheme 1.

In 1996 Sun et al. isolated and characterized Cleroindicin A (**III**) from *Clerodendrum indicum*, which can be derived from Rengyol by intramolecular ether formation (Scheme 1).[10] Until today only one synthesis of Cleroindicin A is described in literature.[9]

The natural products **I**–**III**, contain a common 1,4-dihydroxy-cyclohexane substructure with an additional alkyl substituent in the 1-position (Scheme 1). In our methodically oriented investigations related to applications of hydroxynitrile lyases (HNL) in stereoselective organic syntheses, we have recently published the HNL-catalyzed addition of HCN to 4-substituted cyclohexanones.[11] Unexpectedly we observed trans-selectivity in the (*R*)-PaHNL-catalyzed additions and cis-selectivity with (*S*)-MeHNL as catalyst.[11] Since the transformation of a cyano group into a β-hydroxyethyl moeity can be achieved easily, cyanohydrins of 4-hydroxy-cyclohexanones should be ideal starting compounds for the syntheses of Rengyol, Isorengyol, and Cleroindicin A.

2. Results and discussion

In the publication mentioned above, the HNL-catalyzed reactions of *O*-protected 4-hydroxy-cyclohexanones **1** with hydrogen cyanide are described.[11b] With (*R*)-PaHNL from almonds (*Prunus amygdalus*) as catalyst the HCN addition preferentially gives the trans-products *trans*-**2**, with (*S*)-MeHNL from cassava (*Manihot esculenta*) as catalyst the cis-isomers *cis*-**2** are the major products (Scheme 2).[11b] In the nonenzymatic chemical addition of HCN to the cyclohexanones **1a–e** the isomeric cyanohydrins, *cis*-**2a–e** and *trans*-**2a–e** are obtained in almost equal amounts.[11b] Therefore, in the latter case a selective synthesis of either *cis*- or *trans*-1,4-substituted cyclohexanones is not possible.

☆ See Ref. 1.
Keywords: Rengyol; Isorengyol; Hydroxynitrile lyase; Cyanohydrins; cis/trans-Selectivity.
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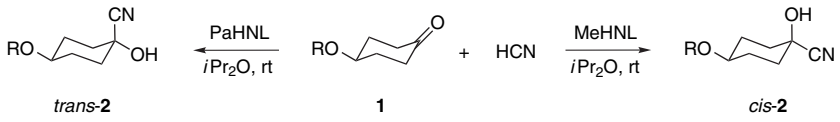
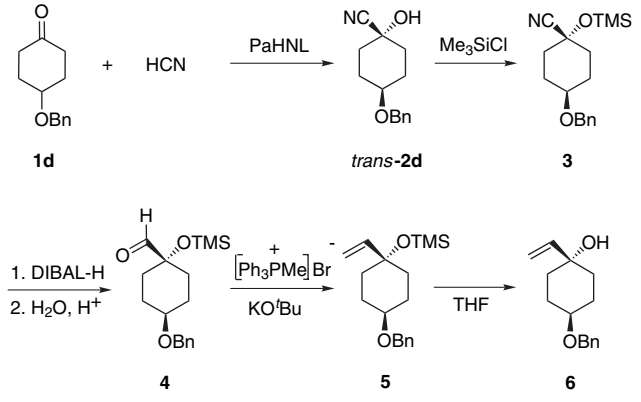


1, 2	a	b	c	d	e
R =	Me	*i*-Bu	allyl	benzyl	acetyl

Scheme 2.

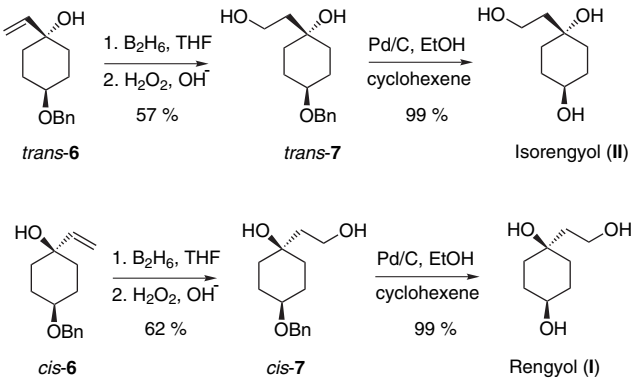
For the further transformations of the cyanohydrins **2** into Rengyol and Isorengyol, we have selected the benzyl protected cyanohydrin **2d** as educt for two reasons. The relatively high cis/trans-stereoselectivity of the HCN addition to **1d** with both the enzymes (cis/trans=2:98 with PaHNL and cis/trans=82:18 with MeHNL),[11b] and the mild reaction conditions for removal of the benzyl group by hydrogenolysis. For the synthesis of either Isorengyol or Rengyol the pure stereoisomers *trans*-**2d** and *cis*-**2d**, respectively, are required. However, since a separation of the cis/trans-isomers can be achieved without problems in a later stage in the synthesis, we first followed up reactions with the crude cyanohydrins obtained in the HNL-catalyzed reactions.

In Scheme 3 the synthesis of 4-benzyloxy-1-vinylcyclohexanol (**6**) starting from *trans*-**2d** is shown. Although the (*R*)-PaHNL-catalyzed HCN addition to **1d** using organic solvents and the enzyme supported on cellulose gives almost exclusively the *trans*-cyanohydrin, *trans*-**2d**,[11b] we have resorted to a two-phase reaction system,[12] which is easier to handle with defatted almond meal as enzyme source. This way **2d** was obtained as a cis/trans-mixture with a ratio of 25:75. Protection of the OH-function with a trimethylsilyl group[13] and hydrogenation with DIBAL-H[14] leads, after aqueous work up, to the corresponding (protected) aldehyde **4** (cis/trans=26:74). Wittig olefination of **4** results in the formation of the vinyl compound **5**.[15] The TMS-protecting group is removed by the treatment with *n*-Bu_4NF in THF leading to **6** (Scheme 3). At this stage the separation of the cis/trans-isomers was performed via chromatography on silica with PE/EE (7:1) to give pure *trans*-**6** and pure *cis*-**6**, respectively.



Scheme 3.

In Scheme 4 the straightforward transformations of *trans*-**6** to Isorengyol and *cis*-**6** to Rengyol are summarized. Hydroboration of the vinyl compounds *trans*-**6** and *cis*-**6**, with diborane in THF at 0 °C overnight and subsequent treatment of the reaction mixture with an alkaline solution of hydrogen peroxide (30%) at room temperature leads to the *O*-benzyl protected products *trans*-**6** and *cis*-**6**, respectively, which are isolated and purified.[16] The debenzylation to the natural products Isorengyol (**II**) and Rengyol (**I**) was performed via Pd/C catalyzed transfer hydrogenation using cyclohexene in abs EtOH (Scheme 4). Both Isorengyol and Rengyol are obtained quantitatively as colorless solids, which are recrystallized from acetone.



Scheme 4.

The cis/trans-isomer ratio of the starting cyanohydrin **2d** (Scheme 3) has been assigned by the chemical shift of the C-4 atom in ^{13}C NMR spectra. The absolute configuration of the 4-substituted cyclohexanone cyanohydrins has been determined earlier by X-ray crystallography of a corresponding 4-nitrobenzoyloxy derivative.[11b]

In none of the reactions performed (Schemes 3 and 4) isomerizations are to be expected. Thus structure assignments for the intermediates **3**–**6** are acceptable. The X-ray crystallographic structure of Isorengyol (**II**) (Fig. 1) confirms

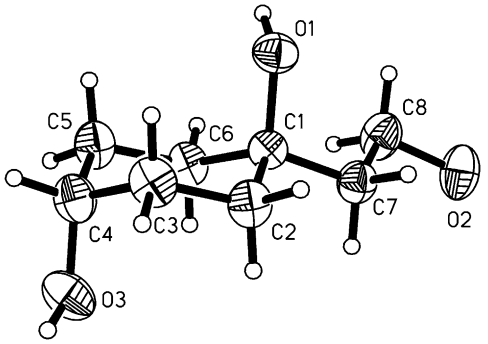


Figure 1. ORTEP plot of the trans-configurated Isorengyol (**II**).

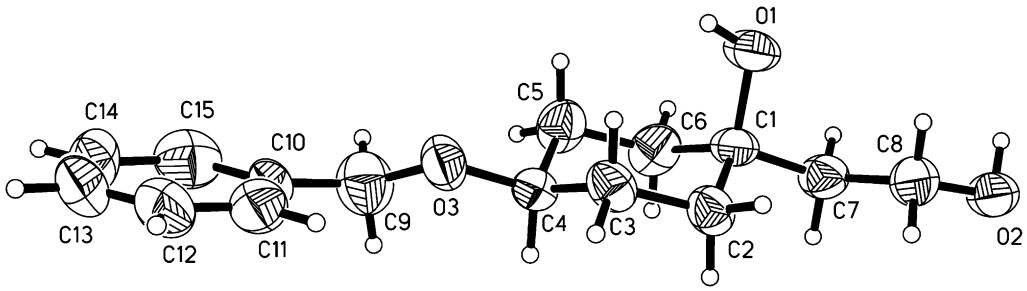


Figure 2. ORTEP plot of *cis*-4-benzyloxy-1-(2-hydroxyethyl)cyclohexane-1-ol (*cis*-**7**)—the *O*-benzyl derivative of Rengyol (**I**).

unambiguously the assigned trans-configuration.[17] The cis-configuration of Rengyol (**I**) is supported by the X-ray structure of the *O*-benzyl derivative *cis*-**7** (Fig. 2).[17]

3. Conclusions

Rengyol (**I**) and Isorengyol (**II**), isolated from *F. suspensa*, possess a variety of interesting biological activities, thus justifying that stereoselective chemical syntheses of these natural products are of great interest and importance. Cyanohydrins of 4-hydroxycyclohexanones are ideal starting materials for these naturally occurring 1,4-dihydroxycyclohexanones. *O*-Protected 4-hydroxycyclohexanones **1** are excellent substrates for hydroxynitrile lyase (HNL) catalyzed HCN additions leading to the corresponding cyanohydrins **2** for which an unexpected high cis- or trans-selectivity is observed.[11] With (*R*)-PaHNL from almonds the *trans*-1,4-dihydroxycyclohexanones are almost exclusively (≥98%) obtained, whereas with (*S*)-MeHNL from *cassava* high yields (≥82%) of the corresponding cis-compounds are obtained. The chemical transformations of the cyanohydrins **2** to the final products, i.e., Isorengyol (**II**) and Rengyol (**I**), are straightforward and can be performed without problems. By using almond meal as enzyme catalyst in a two-phase system, the cis/trans-ratio of the cyanohydrins **2d** is only 25:75. Nevertheless, the chemical transformations of this cis/trans-mixture of **2d** can be performed without difficulties. Separation of the cis/trans-isomers is best achieved via column chromatography of the vinyl compounds *trans*-**6** and *cis*-**6**, respectively. The last two steps to the final products Isorengyol (**II**) and Rengyol (**I**) are then performed separately with the pure isomers *trans*-**6** and *cis*-**6**, respectively.

A comparison of the syntheses of Rengyol and Isorengyol published already, with the procedures described in this paper, shows the following.

The synthesis of Rengyol by Hikino et al. was performed in six steps, starting from glucopyranosyl bromide, in 4% total yield. Ogasawara et al. synthesized Rengyol in 10 steps, starting from ethyl-2(1-hydroxy-4-oxo-cyclohexa-2.5-dienyl) acetate, in 14% total yield. Starting from 4-benzyloxy-cyclohexanone, we obtained Rengyol in seven steps and a total yield of 9.7%. The effort for the syntheses of the starting compounds in the three procedures cited is comparable.

For Isorengyol only one synthesis was published by Ogasawara et al. From the same starting compound as for Rengyol they obtained Isorengyol in 14 steps and a total yield of 3.64%. We obtained Isorengyol in seven steps and a total yield of 8.9%.

This comparison confirms the advantage of our approach if one combines the number of reaction steps and total yields, especially for the synthesis of Isorengyol.

4. Experimental

4.1. Materials and methods

Melting points were determined on a Büchi SMP-20 and are uncorrected. Unless otherwise stated, ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 250 F (250 MHz) and ARX 500 (500 MHz) in $CDCl_3$ with TMS as internal standard. ^{13}C NMR multiplicities were determined with DEPT experiments. Chromatography was performed using silica gel, grain size 0.040–0.063 mm (Fluka). cis/trans-Ratios: GC separations were conducted using capillary glass columns (20 m) with OV 1701, carrier gas 0.4–0.6 bar hydrogen. 4-Benzyloxycyclohexanone (**1d**) was prepared according to literature procedures.[18] All solvents were dried and distilled. Yields are not optimized.

4.1.1. 4-Benzyloxy-1-hydroxycyclohexanecarbonitrile (2d).[11b] (a) HNL-catalyzed reactions in organic solvents: the *O*-protected 4-hydroxycyclohexanone cyanohydrins **2a**–**e** were prepared according to Effenberger et al.[12] (b) HNL-catalyzed reactions in the two-phase system: to a vigorously stirred two-phase system of 24.51 g (120 mmol) **1d**[18] in 120 mL diisopropylether and 200 mL of an aqueous solution of crude (*R*)-PaHNL[12] (42,200 U), 300 mL of a solution of 6.48 g (240 mmol) HCN, prepared in situ,[12] in 300 mL diisopropylether was added. To determine the conversion rate and the cis/trans-ratio, 2 mL of the reaction mixture was diluted with 2 mL of diisopropylether and dried (Na_2SO_4). The organic phase was decanted and concentrated under reduced pressure. The residue was dissolved in 1 mL anhydrous CH_2Cl_2 and 100 μL acetic anhydride and 20 mg DMAP were added. After 48 h of stirring at room temperature the reaction mixture was filtered through a Büchner funnel and washed several times with diisopropylether. The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo to give 26.21 g (94% yield) **2d** as a pale yellow oil, cis/trans-ratio=25:75. *cis*-**2d**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.71–2.07 (m, 8H, 8CH), 3.39 (br s, 1H, OH), 3.73–3.78 (m, 1H, C^4H), 4.51 (s, 2H, CH_2Ph), 7.26–7.38 (m, 5H, H_{Ph}). *cis*-**2d**: ^{13}C NMR (63 MHz,

$CDCl_3$) δ 26.74 (C^3H_2, C^5H_2), 33.30 (C^2H_2, C^6H_2), 68.85 (C^1), 69.96 (CH_2Ph), 71.63 (C^4H), 121.76 (CN), 127.44, 127.56, 128.40, 138.47 (C_{Ph}). *trans*-**2d**: ^1H NMR (250 MHz, $CDCl_3$) δ 1.67–1.81 (m, 4H, 4CH), 1.95–2.03 (m, 2H, C^3H_{eq}, C^5H_{eq}), 2.19–2.29 (m, 2H, C^2H_{eq}, C^6H_{eq}), 2.93 (br s, 1H, OH), 3.45–3.54 (m, 1H, C^4H), 4.53 (s, 2H, CH_2Ph), 7.28–7.39 (m, 5H, H_{Ph}). *trans*-**2d**: ^{13}C NMR (63 MHz, $CDCl_3$) δ 26.54 (C^3H_2, C^5H_2), 33.99 (C^2H_2, C^6H_2), 68.34 (C^1), 70.17 (CH_2Ph), 73.27 (C^4H), 121.87 (CN), 127.46, 127.63, 128.45, 138.48 (C_{Ph}).

4.1.2. 4-Benzyloxy-trimethylsilyloxycyclohexanecarbonitrile (3).[13,14b] To a stirred solution of 15.74 g (224.82 mmol) imidazole in 280 mL dry DMF 14.25 g (131.15 mmol) chloro-trimethylsilane was added at 0 °C under an atmosphere of nitrogen. After stirring for 15 min, a solution of 26.00 g (112.41 mmol) **2d** in 50 mL dry DMF was added dropwise and the mixture was allowed to warm up slowly to room temperature with continued stirring for a further 1 h. Then 560 mL of water was added and the resulting mixture was extracted with diethyl ether (3×100 mL). The combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The crude cis/trans-mixture was purified by chromatography on SiO_2 using petroleum ether–EtOAc (7:1) as eluant (R_f=0.56) to afford 28.51 g (84% yield) **3** as a colorless oil, cis/trans-ratio=25:75. *cis*-**3**: ^1H NMR (500 MHz, $CDCl_3$) δ 0.25 (s, 9H, $Si(CH_3)_3$), 1.71–2.01 (m, 6H, 4CH, C^3H_{eq}, C^5H_{eq}), 2.02–2.16 (m, 2H, C^2H_{eq}, C^6H_{eq}), 3.53–3.55 (m, 1H, C^4H), 4.50 (s, 2H, CH_2Ph), 7.29–7.34 (m, 5H, H_{Ph}). *cis*-**3**: ^{13}C NMR (126 MHz, $CDCl_3$) δ 1.40 ($Si(CH_3)_3$), 26.90 (C^3H_2, C^5H_2), 34.82 (C^2H_2, C^6H_2), 69.87 (C^1), 69.98 (CH_2Ph), 71.90 (C^4H), 121.82 (CN), 127.43, 127.54, 128.39, 138.62 (C_{Ph}). *trans*-**3**: ^1H NMR (500 MHz, $CDCl_3$) δ 0.24 (s, 9H, $Si(CH_3)_3$), 1.64–2.11 (m, 6H, 4CH, C^3H_{eq}, C^5H_{eq}), 2.15–2.25 (m, 2H, C^2H_{eq}, C^6H_{eq}), 3.41–3.47 (m, 1H, C^4H), 4.52 (s, 2H, CH_2Ph), 7.29–7.34 (m, 5H, H_{Ph}). *trans*-**3**: ^{13}C NMR (126 MHz, $CDCl_3$) δ 1.30 ($Si(CH_3)_3$), 26.91 (C^3H_2, C^5H_2), 35.60 (C^2H_2, C^6H_2), 69.43 (C^1), 70.10 (CH_2Ph), 73.54 (C^4H), 121.80 (CN), 127.41, 127.54, 128.40, 138.64 (C_{Ph}). Anal. Calcd for $C_{17}H_{25}NO_2Si$ (303.48): C, 67.28; H, 8.30; N, 4.62. Found: C, 67.40; H, 8.30; N, 4.45.

4.1.3. 4-Benzyloxy-1-trimethylsilyloxycyclohexanecarbaldehyde (4).[14] To a vigorously stirred solution of 15.50 g (51.07 mmol) **3** in 125 mL dry *n*-hexane 86.8 mL of a DIBAL-H solution (1 M in *n*-hexane) was added dropwise at −45 °C under an inert gas atmosphere. Then the reaction mixture was allowed to warm up slowly to 15 °C and stirring at this temperature was continued for 5 h. The mixture was diluted with CH_2Cl_2 (100 mL) and quenched with a saturated aqueous solution of NH_4Cl (100 mL) and 0.5 M aqueous H_2SO_4 (250 mL). After vigorous stirring for 12 h at 15 °C, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3×100 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The crude cis/trans-mixture was purified by chromatography on SiO_2 using petroleum ether–EtOAc (10:1) as eluant (R_f (*cis*-**4**)=0.54, R_f (*trans*-**4**)=0.57) to afford 7.54 g (48% yield) of **4** as a colorless oil, cis/trans-ratio=26:74. ^1H NMR (500 MHz, $CDCl_3$)[14b] δ 0.14 (s, 6.9H, *trans*-$Si(CH_3)_3$), 0.15 (s, 2.1H, *cis*-$Si(CH_3)_3$), 1.41–2.04 (m, 8H, 8CH), 3.37–3.40 (m, 0.25H, *cis*-C^4H), 3.60–3.63 (m, 0.75H, *trans*-C^4H), 4.51 (s, 1.45H, *trans*-CH_2Ph), 4.56 (s, 0.55H, *cis*-CH_2Ph), 7.25–7.36 (m, 5H, H_{Ph}), 9.49 (s, 0.75H, *trans*-CHO), 9.54 (s, 0.25H, *cis*-CHO).^{13}C NMR (126 MHz, $CDCl_3$) δ 2.25 ($Si(CH_3)_3$), 25.13 (*trans*-C^3H_2, *trans*-C^5H_2), 26.53 (*cis*-C^3H_2, *cis*-C^5H_2), 27.77 (*trans*-C^2H_2, *trans*-C^6H_2), 30.41 (*cis*-C^2H_2, *cis*-C^6H_2), 69.87 (*trans*-CH_2Ph), 69.96 (*cis*-CH_2Ph), 72.67 (*trans*-C^4H), 75.38 (*cis*-C^4H), 79.31 (*cis*-C^1), 79.69 (*trans*-C^1), 127.36, 127.40, 127.49, 127.53, 128.34, 128.38, 138.82, 138.96 (C_{Ph}), 202.89 (*trans*-CHO), 203.73 (*cis*-CHO). Anal. Calcd for $C_{17}H_{26}O_3Si$ (306.48): C, 66.62; H, 8.55. Found: C, 66.34; H, 8.63.

4.1.4. (4-Benzyloxy-1-vinyl-cyclohexyloxy)trimethylsilane (5).[15] To a stirred solution of 4.79 g (42.68 mmol) potassium *tert*-butoxide in 100 mL dry THF 16.06 g (44.81 mmol) methyl-triphenylphosphonium bromide was added under nitrogen atmosphere at room temperature. The bright yellow reaction mixture was stirred for 0.5 h, and then a solution of 7.40 g (24.14 mmol) **4** in 100 mL dry THF was added dropwise. After stirring for 2 h, the precipitate was filtered off and washed with Et_2O. The combined filtrates were quenched with water and extracted with Et_2O. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude cis/trans-mixture was purified by chromatography on SiO_2 using a short column with petroleum ether–EtOAc (3:1) as eluant to give 2.25 g (31% yield) **5** as a colorless oil. ^1H NMR (500 MHz, $CDCl_3$) δ 0.09 (s, 6.4H, *trans*-$Si(CH_3)_3$), 0.10 (s, 2.6H, *cis*-$Si(CH_3)_3$), 1.26–1.49 (m, 8H, 8CH), 3.27–3.38 (m, 0.3H, *cis*-C^4H), 3.54–3.62 (m, 0.7H, *trans*-C^4H), 4.50 (s, 2H, *trans*-CH_2Ph), 4.56 (s, 0.6H, *cis*-CH_2Ph), 4.99–5.21 (m, 1.4H, CH=CH_2), 5.75–5.98 (m, 1H, CH=CH_2), 7.22–7.38 (5H, m, H_{Ph}). ^{13}C NMR (126 MHz, $CDCl_3$) δ 2.53 (*cis*-$Si(CH_3)_3$), 2.56 (*trans*-$Si(CH_3)_3$), 26.62 (*trans*-C^3H_2, *trans*-C^5H_2), 27.48 (*cis*-C^3H_2, *cis*-C^5H_2)^{cis}, 33.47 (*trans*-C^2H_2, *trans*-C^6H_2), 35.39 (*cis*-C^2H_2, *cis*-C^6H_2), 69.79 (*trans*-CH_2Ph), 69.83 (*cis*-CH_2Ph), 73.34 (*cis*-C^1), 73.96 (*trans*-C^4H), 74.24 (*trans*-C^1), 76.64 (*cis*-C^4H), 112.27 (*cis*-CH=CH_2), 113.00 (*trans*-CH=CH_2), 127.29, 127.38, 127.54, 128.30, 128.33, 139.15, 139.24 (C_{Ph}), 145.10 (*trans*-CH=CH_2), 145.56 (*cis*-CH=CH_2). HRMS (EI, 70 eV) calcd for $C_{18}H_{28}O_2Si^+$: 304.1859. Found: 304.1850.

4.1.5. 4-Benzyloxy-1-vinylcyclohexan-1-ol (6). To a solution of 2.19 g (7.19 mmol) **5** in 36 mL dry THF a solution of 2.50 g (7.92 mmol) *n*-Bu_4NF in 8 mL dry THF was added slowly at 0 °C. The mixture was stirred for 0.5 h. Then 42 mL H_2O and 56 mL CH_2Cl_2 were added, the organic layer separated, and the aqueous layer was extracted twice with CH_2Cl_2 (2×25 mL). The combined organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The crude cis/trans-mixture was purified and separated by chromatography on SiO_2 with petroleum ether–EtOAc (7:1) as eluant to give two main fractions as colorless oils: 0.55 g (33% yield) *cis*-**6** and 0.76 g (45% yield) *trans*-**6**. *cis*-**6**: IR (neat) 2928, 2855, 1453, 1363, 1252, 1229, 1068, 1028, 993, 959, 920, 841, 734, 695. ^1H NMR (500 MHz, $CDCl_3$) δ 1.36 (br s, 1H, OH), 1.48–1.54 (m, 2H, 2CH), 1.69–1.81 (m, 4H, 4CH), 1.86–1.91 (m, 2H, C^2H_{eq}, C^6H_{eq}), 3.34–3.40 (m, 1H, C^4H), 4.57 (s, 2H, CH_2Ph), 5.04 (dd, $^2J(H_C, H_B)$=1.3 Hz, *cis*-$^3J(H_C, H_A)$=10.8 Hz,

1H, $CH_A{=}CH_CH_B$), 5.26 (dd, 1H, $^2J(H_B, H_C)$=1.3 Hz, *trans*-$^3J(H_B, H_A)$=17.5 Hz, 1H, $CH_A{=}CH_CH_B$), 5.93 (dd, *cis*-$^3J(H_A, H_C)$=10.8 Hz, *trans*-$^3J(H_B, H_A)$=17.5 Hz, 1H, $CH_A{=}CH_CH_B$), 7.25–7.42 (m, 5H, H_{Ph}). ^{13}C NMR (126 MHz, $CDCl_3$) δ 27.40 (C^3H_2, C^5H_2), 35.24 (C^2H_2, C^6H_2), 69.77 (CH_2Ph), 70.93 (C^1), 76.14 (C^4H), 111.85 ($CH{=}CH_2$), 127.39, 127.49, 128.34, 139.05 (C_{Ph}), 145.46 ($CH{=}CH_2$). HRMS (MH^+) calcd for $C_{15}H_{21}O_2$: 233.1542. Found: 233.1538.

trans-**6**: IR (neat) 2931, 2860, 1453, 1360, 1089, 1063, 1027, 968, 920, 907, 847, 732, 696. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.30 (br s, 1H, OH), 1.41–1.49 (m, 2H, 2CH), 1.72–1.78 (m, 2H, 2CH), 1.85–1.92 (m, 4H, C^3H_{eq}, C^5H_{eq}, C^2H_{eq}, C^6H_{eq}), 3.61–3.63 (m, 1H, C^4H), 4.52 (s, 2H, CH_2Ph), 5.07 (dd, $^2J(H_C, H_B)$=1.3 Hz, *cis*-$^3J(H_C, H_A)$=10.8 Hz, 1H, $CH_A{=}CH_CH_B$), 5.27 (dd, 1H, $^2J(H_B, H_C)$=1.3 Hz, *trans*-$^3J(H_B, H_A)$=17.3 Hz, 1H, $CH_A{=}CH_CH_B$), 6.01 (dd, *cis*-$^3J(H_A, H_C)$=10.8 Hz, *trans*-$^3J(H_B, H_A)$=17.3 Hz, 1H, $CH_A{=}CH_CH_B$), 7.25–7.36 (m, 5H, H_{Ph}). ^{13}C NMR (126 MHz, $CDCl_3$) δ 26.14 (C^3H_2, C^5H_2), 32.84 (C^2H_2, C^6H_2), 69.85 (CH_2Ph), 71.64 (C^1), 73.35 (C^4H), 111.91 ($CH{=}CH_2$), 127.38, 128.33, 139.14 (C_{Ph}), 145.58 ($CH{=}CH_2$). HRMS (MH^+) calcd for $C_{15}H_{21}O_2$: 233.1542. Found: 233.1535. Anal. Calcd for $C_{15}H_{20}O_2$ (232.32): C, 77.55; H, 8.68. Found: C, 77.40; H, 8.83.

4.1.6. *cis*-4-Benzyloxy-1-(2-hydroxyethyl)cyclohexan-1-ol (*cis*-7).[16] To 480.9 mg (2.07 mmol) *cis*-**6** in 6 mL dry THF 4.5 mL of a 1 M solution of B_2H_6 in THF was added dropwise at 0 °C under nitrogen atmosphere and the mixture stirred overnight at 0 °C. The reaction mixture was oxidized by careful addition of 3.5 mL NaOH (3 M) and 0.78 mL 30% H_2O_2 and allowed to warm up slowly to room temperature. Stirring was continued for 30 min and then the mixture was extracted with CH_2Cl_2 (3×8 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by chromatography on SiO_2 with petroleum ether–EtOAc (1:3) as eluant to afford 320.0 mg (62% yield) *cis*-**7** as a white amorphous solid, recrystallized from iPr_2O (colorless prismatic crystals): mp 102 °C. IR (neat) 3331, 2929, 2973, 1453, 1362, 1165, 1102, 1070, 1050, 1019, 962, 937, 741, 697. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.33–1.38 (m, 2H, 2CH), 1.68–1.75 (m, 2H, 2CH), 1.69 (t, ^3J=5.7 Hz, CH_2CH_2OH), 1.83–1.85 (m, 4H, 4CH), 2.74 (br s, 1H, C^1OH), 2.96 (t, ^3J=4.6 Hz, 1H, CH_2CH_2OH), 3.33–3.39 (m, 1H, C^4H), 3.86 (dt, ^3J_d=4.6 Hz, ^3J_t=5.6 Hz, 2H, CH_2CH_2OH), 4.56 (s, 2H, CH_2Ph), 7.25–7.36 (m, 5H, H_{Ph}). ^{13}C NMR (126 MHz, $CDCl_3$) δ 27.28 (C^3H_2, C^5H_2), 35.09 (C^2H_2, C^6H_2), 42.21 (CH_2CH_2OH), 59.51 (CH_2CH_2OH), 69.79 (CH_2Ph), 71.55 (C^1), 76.20 (C^4H), 127.42, 127.51, 128.35, 138.98 (C_{Ph}). Anal. Calcd for $C_{15}H_{22}O_3$ (250.34): C, 71.97; H, 8.86. Found: C, 71.93; H, 8.86.

4.1.7. *trans*-4-Benzyloxy-1-(2-hydroxyethyl)cyclohexan-1-ol) (*trans*-7).[16] For reaction conditions and workup see above for *cis*-**7**. 598.0 mg (2.57 mmol) of *trans*-**6**, 7.4 mL dry THF, and 5.6 mL B_2H_6 (1 M in THF). Purification by column chromatography (SiO_2) with petroleum ether–EtOAc (1:3) as eluant affording 365.0 mg (57% yield) *trans*-**7** as a white amorphous solid: mp 38 °C. IR (neat) 3324, 3271, 2939, 2922, 1440, 1250, 1087, 1066, 1029, 1004, 973, 940, 731, 695, 648. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.49–1.54 (m, 2H, 2CH), 1.61–1.80 (m, 2H, 2CH), 1.74–1.80 (m, 2H, 2CH), 1.76 (t, ^3J=5.8 Hz, CH_2CH_2OH), 1.84–1.90 (m, 2H, 2CH), 3.14 (br s, 2H, C^1OH, CH_2CH_2OH), 3.56–3.60 (m, 1H, C^4H), 3.87 (t, ^3J=5.7 Hz, 2H, CH_2CH_2OH), 4.51 (s, 2H, CH_2Ph), 7.24–7.35 (m, 5H, H_{Ph}). ^{13}C NMR (126 MHz, $CDCl_3$) δ 26.47 (C^3H_2, C^5H_2), 33.19 (C^2H_2, C^6H_2), 41.18 (CH_2CH_2OH), 59.30 (CH_2CH_2OH), 69.91 (CH_2Ph), 72.35 (C^1), 74.08 (C^4H), 127.41, 127.52, 128.34, 139.03 (C_{Ph}). Anal. Calcd for $C_{15}H_{22}O_3$ (250.34): C, 71.97; H, 8.86. Found: C, 71.80; H, 8.93.

4.1.8. Rengyol (*cis*-1-(2-hydroxyethyl)cyclohexane-1,4-diol) (I).[8,9] To a solution of 86.8 mg (0.35 mmol) *cis*-**7** in 7 mL abs EtOH and 0.75 mL freshly distilled cyclohexene, 20 mg 10% Pd/C (Degussa Type E) was added. The suspension was stirred under reflux for 1 h. The mixture was allowed to cool down and was then filtered through Celite. The filtrate was concentrated under reduced pressure. The residue was recrystallized from acetone to afford 55.3 mg (99% yield) Rengyol (**I**) as a white amorphous solid: mp 120 °C. IR (neat) 2931, 2439, 2405, 1125, 1090, 1063, 1019, 959, 930, 907, 735, 666. ^{1}H NMR (500 MHz, methanol-d_4) δ 1.37–1.43 (m, 2H, C^2H_{ax}, C^6H_{ax}), 1.60–1.73 (m, 6H, C^3H_{ax}, C^5H_{ax}, C^3H_{eq}, C^5H_{eq}, C^2H_{eq}, C^6H_{eq}), 1.68 (t, ^3J=7.2 Hz, 2H, CH_2CH_2OH), 3.52 (tt, ^3J (C^4H_{ax}, C^3H_{ax})=^3J (C^4H_{ax}, C^5H_{ax})=9.9 Hz, ^3J (C^4H_{ax}, C^3H_{eq})=^3J (C^4H_{ax}, C^5H_{eq})=4.4 Hz, 1H, C^4H), 3.73 (t, ^3J=7.2 Hz, 2H, CH_2CH_2OH). ^{1}H NMR (300 MHz, pyridine-d_5)[5b,19] δ 1.51–1.61 (m, 2H, C^2H_{ax}, C^2H_{ax}), 2.01–2.12 (m, 4H, C^2H_{eq}, C^6H_{eq}, C^3H_{eq}, C^5H_{eq}), 2.04 (t, ^3J=6.6 Hz, 2H, CH_2CH_2OH), 2.24–2.38 (m, 2H, C^3H_{ax}, C^5H_{ax}), 3,94 (tt, ^3J (C^4H_{ax}, C^3H_{ax})=^3J (C^4H_{ax}, C^5H_{ax})=10.1 Hz, ^3J (C^4H_{ax}, C^3H_{eq})=^3J (C^4H_{ax}, C^5H_{eq})=4.0 Hz, 1H, C^4H), 4.22 (t, ^3J=6.6 Hz, 2H, CH_2CH_2OH). ^{13}C NMR (126 MHz, methanol-d_4) δ 31.28 (C^3H_2, C^5H_2), 36.04 (C^2H_2, C^6H_2), 45.57 (CH_2CH_2OH), 59.15 (CH_2CH_2OH), 70.76 (C^4H, C^1). ^{13}C NMR (126 MHz, pyridine-d_5)[5b,19] 31.86 (C^3H_2, C^5H_2), 36.26 (C^2H_2, C^6H_2), 45.21 (CH_2CH_2OH), 58.86 (CH_2CH_2OH), 69.80 (C^1), 70.03 (C^4H). Anal. Calcd for $C_8H_{16}O_3$ (160.21): C, 59.98; H, 10.07. Found: C, 59.92; H, 9.96.

4.1.9. Isorengyol (*trans*-1-(2-hydroxyethyl)cyclohexane-1,4-diol) (II).[9] To a solution of 242.2 mg (0.97 mmol) *trans*-**7** in 20 mL abs EtOH and 2.1 mL freshly distilled cyclohexene, 50 mg 10% Pd/C (Degussa Type E) was added. The suspension was stirred under reflux for 1 h. The mixture was allowed to cool down and was then filtered through Celite. The filtrate was concentrated under reduced pressure. Recrystallization from acetone afforded 153.8 mg (99% yield) Isorengyol (**II**) as colorless crystals: mp 109 °C. IR (neat) 3272, 2945, 2925, 2434, 1021, 979, 839, 730, 699, 672. ^{1}H NMR (500 MHz, methanol-d_4) δ 1.42–1.51 (m, 4H, 4CH), 1.74–1.87 (m, 4H, 4CH), 1.78 (t, ^3J=7.1 Hz, 2H, CH_2CH_2OH), 3.74–3.79 (m, 1H, C^4H), 3.75 (t, ^3J=7.1 Hz, 2H, CH_2CH_2OH). ^{1}H NMR (500 MHz, pyridine-d_5)[5b,19] δ 1.83–1.89 (m, 4H, 4CH), 2.15 (t, ^3J=6.5 Hz, 2H, CH_2CH_2OH), 2.18–2.29 (m, 4H, 4CH), 4.20–4.26 (m, 1H, C^4H), 4.25 (t, ^3J=6.5 Hz, 2H, CH_2CH_2OH). ^{13}C NMR (126 MHz, methanol-d_4) δ 30.69 (C^3H_2, C^5H_2), 34.22 (C^2H_2, C^6H_2), 43.03 (CH_2CH_2OH),

58.98 (CH_2CH_2OH), 68.52 (C^4H), 72.00 (C^1). ^{13}C NMR (126 MHz, pyridine-d_5)[5b,19] δ 31.24 (C^3H_2, C^5H_2), 34.59 (C^2H_2, C^6H_2), 43.26 (CH_2CH_2OH), 58.81 (CH_2CH_2OH), 67.41 (C^1), 71.29 (C^4H). Anal. Calcd for $C_8H_{16}O_3$ (160.21): C, 59.98; H, 10.07. Found: C, 60.11; H, 9.98.

Acknowledgments

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5-Nitroisocoumarins from tandem Castro–Stephens coupling—6-*endo*-dig cyclisation of 2-iodo-3-nitrobenzoic acid and arylethynes and ring-closure of methyl 2-alkynyl-3-nitrobenzoates with electrophiles

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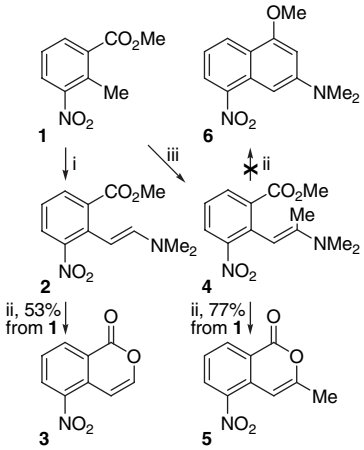
Abstract—Reaction of 2-iodo-3-nitrobenzoic acid with arylalkynyl copper(I) reagent gave 3-aryl-5-nitroisocoumarins. Castro–Stephens coupling was followed by in situ Cu-catalysed ring-closure. ^{1}H NMR and X-ray crystallography showed the cyclisations to be 6-*endo*, contrasting with reports of 5-*exo* cyclisation of analogous 2-iodobenzoate esters with alkynes. Sonogashira couplings of methyl 2-iodo-3-nitrobenzoate with phenylacetylene and with trimethylsilylacetylene gave the corresponding 2-alkynyl-3-nitrobenzoate esters. With $HgSO_4$, the phenylalkyne underwent 6-*endo* cyclisation to give 5-nitro-3-phenylisocoumarin. The disubstituted alkyne esters gave 4-phenylselenylisocoumarins with PhSeCl. 5-Nitro-3-phenyl-4-phenylselenylisocoumarin shows significant sterically-driven distortion of the isocoumarin ring. Reaction of methyl 3-nitro-2-phenylethynylbenzoate with ICl gave the 4-iodoisocoumarin. Thus the nitro group tends to direct these electrophile-driven cyclisations towards the 6-*endo* mode.

1. Introduction

As a part of our continuing research on the design and synthesis of heterocyclic compounds as enzyme inhibitors and potential drugs,[1–3] we required a series of 3-substituted-5-nitroisocoumarins. The synthesis of 5-nitroisocoumarin **3** (Scheme 1) is now well established,[4] through condensation of methyl 2-methyl-3-nitrobenzoate **1** with dimethylformamide dimethylacetal, followed by hydrolysis of the intermediate methyl *E*-2-(2-dimethylaminoethenyl)-3-nitrobenzoate **2** and cyclisation catalysed by wet silica. However, this synthesis cannot be extended to 3-methyl-5-nitroisocoumarin **5**, as the corresponding initial condensation with dimethylacetamide dimethylacetal takes an alternate course, giving a trisubstituted naphthalene **6**.[5] Compound **5** has been reported to be synthesised in moderate yield by an alternative route of Hurtley coupling of 2-bromo-3-nitrobenzoic acid with pentane-2,4-dione, followed by acyl cleavage and ring-closure with sodium chloride at very high temperature.[6] Although the corresponding condensations of **1** with dimethylacetals of Ar-substituted *N*,*N*-dimethylbenzamides would be expected to follow the isocoumarin-forming path, as they lack a reactive α-methylene or α-methyl, they are not easy to prepare and an alternative route was sought.



Scheme 1. Condensation of methyl 2-methyl-3-nitrobenzoate **1** with DMF–DMA (Ref. 4) and with DMA–DMA (Ref. 5), followed by different modes of cyclisation. Reagents: (i) $HC(OMe)_2NMe_2$, DMF, Δ; (ii) SiO_2, EtOAc and (iii) $MeC(OMe)_2NMe_2$, $AcNMe_2$, Δ.

Several groups have reported the cyclisation of 2-alkynylbenzoic acids and 2-alkynylbenzoate esters **7** under electrophilic conditions, although no examples have a substituent at

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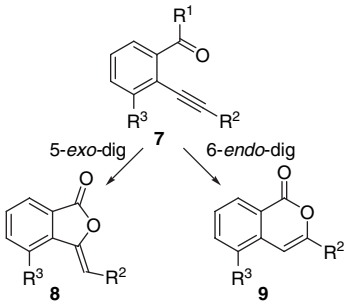
the 3-position of the benzoate (corresponding to the 5-position of the target isocoumarins). Moreover, there is debate about whether the reaction goes 5-*exo*-dig (giving ylidenephthalides **8**) or 6-*endo*-dig (giving isocoumarins **9**), as shown in Scheme 2 (R^3=H); both are favoured under Baldwin's Guidelines. For example, treatment of methyl 2-arylethynylbenzoates with $Hg^{(II)}$ under acidic conditions is reported to give intermediate mercurials from which 3-aryl-isocoumarins can be isolated by reduction with $NaBH_4$.[7,8] Similarly, reaction of the same starting materials with hydrogen iodide, electrophilic iodine reagents, bromine, sulfenyl chlorides and selenyl chlorides gave 3-arylisocoumarins and their 4-iodo, 4-bromo, 4-arylthio and 4-arylselenyl derivatives, respectively.[9–12] Dihydrofuroisocoumarins have also been synthesised by $Ag^{(I)}$-promoted 6-*endo*-dig cyclisation of the corresponding arylalkynylbenzoate esters.[13] In contrast, methyl 2-ethynylbenzoate undergoes 5-*exo*-dig cyclisation with iodine[12] and $Ag^{(I)}$-mediated cyclisation of 2-alkynylbenzoic acids affords the corresponding ylidenephthalides.[14] Under basic conditions (LiOH), methyl 2-(pent-1-ynyl)benzoate undergoes exclusive 6-*endo*-dig cyclisation whereas methyl 2-(3-hydroxypent-1-ynyl)benzoate gives a mixture of products from both cyclisation modes.[15] Regioisomeric mixtures are also formed during Pd-catalysed cyclisation of the former alkyne.[16] Cherry et al.[17] have shown very recently that treatment of 2-iodobenzoic acid with allenylstannanes under Pd-catalysed Stille conditions also gives isocoumarins through 6-*endo*-dig cyclisation of the intermediate 2-(3-substituted-allenyl)benzoic acids. 5-*exo*-Dig cyclisation is also evident at the lower (alcohol) oxidation level of the intramolecular nucleophilic oxygen under fluoride-ion catalysis.[18]



Scheme 2. Possible alternative cyclisation modes of 2-alkynylbenzoic acids **7** (R^1=OH) and 2-alkynylbenzoate esters (R^1=Oalkyl) via 5-*exo*-dig (giving **8**) and 6-*endo*-dig (giving **9**) routes. R^2=alkyl, aryl. R^3=H for previous examples; R^3=NO_2 in this paper.

While developing their synthesis of arylalkynes from iodoarenes and Cu^I-acetylides, Stephens and Castro[19] claimed that treatment of 2-iodobenzoic acid with Cu–C≡C–Ph gave 3-phenylisocoumarin **9** (R^2=Ph; R^3=H) by Cu-catalysed 6-*endo*-dig of the initial Castro–Stephens coupling product **7** (R^1=OH; R^2=Ph; R^3=H). However, this claim was later withdrawn[20] with correction of the characterisation of the product to be the benzylidine phthalide **8** (R^2=Ph; R^3=H), resulting from tandem Castro–Stephens coupling and 5-*exo*-dig cyclisation. Since then, an isocoumarin has been synthesised by this method[21] and mixtures of these isocoumarins and phthalides have been reported from analogous one-pot coupling–cyclisations of 2-iodobenzoic acid with terminal alkynes under Pd/Zn catalysis.[22]

2. Results and discussion

Given the dichotomy of reports for the outcome of the cyclisations, particularly in the Castro–Stephens tandem version, we initiated a short study on whether the mode of cyclisation would be influenced by the presence of the nitro group *ortho* to the alkyne. This strongly electron-withdrawing group should influence the electron distribution in the alkyne and was predicted to favour 6-*endo*-dig cyclisation by making the alkyne carbon further from the nitroarene more electrophilic (Fig. 1). Thus we investigated the tandem coupling–cyclisation of arylalkynes (as their Cu-acetylides) with 2-iodo-3-nitrobenzoic acid under Castro–Stephens conditions.

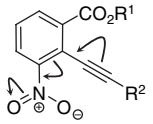
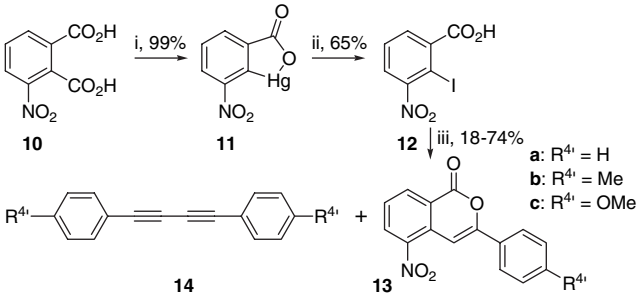


Figure 1. Proposed influence of the *ortho* nitro group on the electron-distribution in the alkyne in 2-alkynyl-3-nitrobenzoic acids and 2-alkynyl-3-nitrobenzoate esters. R^1=H or alkyl, R^2=H, silyl, alkyl and aryl.

The starting material, 2-iodo-3-nitrobenzoic acid **12**, was prepared in two steps from 3-nitrobenzene-1,2-dioic acid (**10**, 3-nitrophthalic acid). Decarboxylation/mercuration with mercury(II) acetate at high temperature in acetic acid gave the intermediate aryl-mercury **11**. Electrophilic replacement of the mercury with iodine, using crude **11**, gave **12** conveniently in excellent yield.

To test our hypothesis about the controlling effect of the nitro group, **12** was exposed to copper(I) phenylacetylide (prepared from phenylethyne and copper(I) iodide in aq ethanolic ammonia) under the classical conditions of boiling pyridine as solvent (Scheme 3). Conventional work-up gave a high yield of a single cyclisation product, along with a small amount of a highly non-polar by-product. The latter was readily identified as 1,4-diphenylbutadiyne **14a**,[23] derived from an unintended oxidative Glaser homo-coupling of the alkyne, despite carrying out the reaction under nitrogen. Careful examination of the ^{1}H NMR spectrum of the major (cyclisation) product suggested that it was the desired isocoumarin **13a**, rather than the alternative 5-*exo*-dig product **15a** (Fig. 2). Firstly, the chemical shift of the signal at δ 7.79 corresponds with the chemical shifts previously



Scheme 3. Synthesis of 2-iodo-3-nitrobenzoic acid **12** and tandem Castro–Stephens coupling and 6-*endo*-dig ring closure to give the 3-aryl-5-nitroisocoumarins **13**. Reagents: (i) $Hg(OAc)_2$, AcOH, reflux; (ii) I_2, KI, AcOH, H_2O, reflux and (iii) $R^{4'}$–C_6H_4C≡CCu, pyridine, reflux.

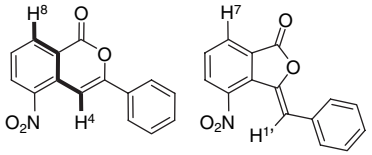


Figure 2. Structures of alternative 6-*endo*-dig (**13a**) and 5-*exo*-dig (**15a**) cyclisation products, showing the long-range ^{1}H–^{1}H NMR coupling path in **13a**.

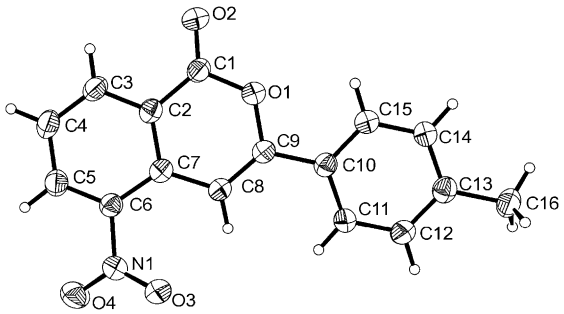
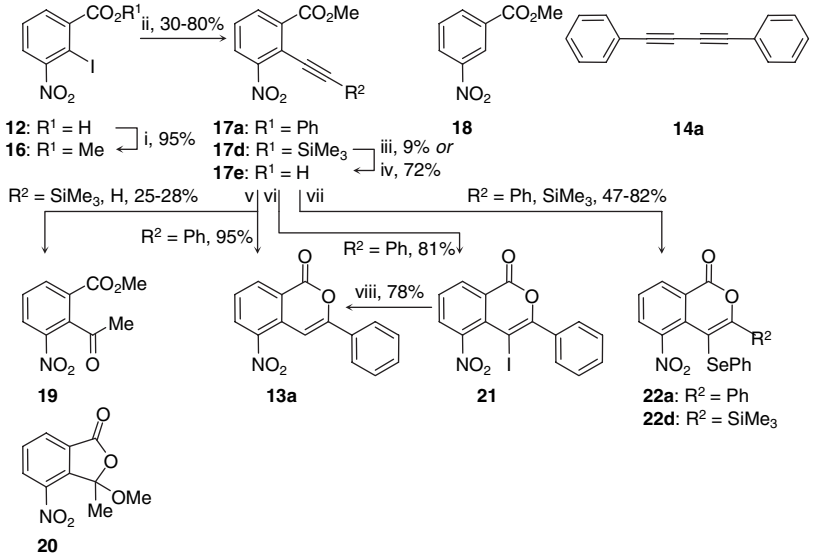


Figure 3. X-ray crystal structure of **13b**, with crystallographic numbering.

observed by us for the corresponding proton in 5-nitroisocoumarin (δ 7.39)[4] and for 3-phenylisocoumarin (δ 6.93)[24] better than it does with that observed for the alkene proton of 3-benzylidenephthalide (δ 6.40),[25] a close analogue of **15a**. Moreover, a small coupling (J 0.8 Hz) was observed for this proton signal. We have previously observed[4] a similar long-range coupling between H^4 and H^8 in 5-nitroisocoumarin and we propose that it arises from the extended-**W** ^{5}J H^4–H^8 coupling path shown for **13a** in Figure 2, rather than the alternative longer ^{6}J $H^{1'}$–H^7 path in **15a**. Moreover, the IR spectrum of **13a** showed an absorption band at 1739 cm^{-1}, which lies in the middle of the range 1730–1750 cm^{-1} for six-membered ring lactones but not in the corresponding range for five-membered ring lactones (1760–1780 cm^{-1}). These data point strongly towards the cyclisation having occurred in the 6-*endo*-dig mode. 2-Iodo-3-methylbenzoic acid was also treated with the Cu(I) acetylides of 4-methylphenylethyne and 4-methoxyphenylethyne under the same conditions. Unexpectedly, the yields of the cyclised products **13b**,**c** were lower, as were the yields of the Glaser coupling products **14b**,**c**. However, the NMR spectra of **13b**,**c** were similar to those of **13a**, with H^4 resonating at δ 7.80 and δ 7.66, respectively, indicating the same mode of cyclisation.

To confirm unequivocally that the products were indeed the isocoumarins, the structure of **13b** was established by X-ray crystallography. The structure and X-ray crystallographic numbering scheme are shown in Figure 3. In this structure, the isocoumarin bicycle is essentially planar. In particular, the protons and carbons of the extended-**W** $^{5}J_{H-H}$ NMR coupling path are shown to be coplanar, supporting the assignment of the observed long-range coupling. The 4-methylphenyl substituent in **13b** is only very slightly twisted out of the isocoumarin plane, with a dihedral angle of 4.8°; this closeness to coplanarity presumably reflects the presence of only one proton on the isocoumarin *ortho* to the 4-methylphenyl group. However, the nitro group is twisted some 22.4° out of the isocoumarin plane, owing to adverse steric interactions with the *peri*-hydrogen.

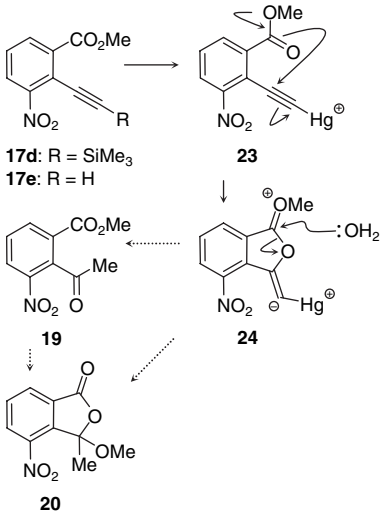
With the mode of Cu-catalysed cyclisation of the 2-(arylalkynyl)-3-nitrobenzoic acids firmly established as 6-*endo*-dig, attention was turned to study the mode(s) of cyclisation of representative methyl 2-(substituted)alkynyl-3-nitrobenzoates. To supply the starting material for this part of the study, 2-iodo-3-nitrobenzoic acid **12** was converted to its methyl ester **16** in the usual way (Scheme 4). Sonogashira coupling of this iodoarene with phenylethyne and with trimethylsilylethyne gave the disubstituted alkynes **17a**,**d**. The yield of **17a** was excellent but coupling of the more bulky alkyne with the sterically demanding *ortho*,*ortho*′-disubstituted iodoarene **16** gave a much lower yield (30%). In the latter case, the difficulty of the coupling was



Scheme 4. Sonogashira couplings of methyl 2-iodo-3-nitrobenzoate **16** and electrophile-driven cyclisations of methyl 2-alkynyl-3-nitrobenzoates **17a**,**d**,**e**. Reagents: (i) MeOH H_2SO_4; (ii) HC≡CPh or HC≡CSiMe$_3$, $(Ph_3P)_2PdCl_2$, Pr^i_2NH THF; (iii) Bu_4NF, THF; (iv) AgOTf, acetone, water, CH_2Cl_2; (v) $HgSO_4$, H_2SO_4, Me_2CO, Δ; (vi) ICl, CH_2Cl_2; (vii) PhSeCl, CH_2Cl_2 and (viii) HCO_2H, $Pd(OAc)_2$, Et_3N, DMF.

illustrated by the isolation of a 39% yield of the dehalogenated product **18**, arising from decomposition of the intermediate arylpalladium, which had failed to couple with the alkyne. Interestingly, despite the presence of copper(I) in the reaction mixture, no cyclised products were formed, although some runs with phenylethyne also gave 1,4-diphenylbutadiyne **14a**, the product of Glaser-like homodimerisation of the starting alkyne. Attempted desilylation of **17d** under a variety of basic conditions (e.g., potassium carbonate/methanol) caused extensive decomposition; indeed the classical fluoride ion-mediated desilylation gave only a maximum yield of 9% of **17e**. Removal of trimethylsilyl protection from alkynes with silver(I) nitrate is widely reported to be efficient if cyanide ion is added to break up the initially formed alkynylsilver(I).[26–28] However, Orsini et al.[29] have recently developed a selective desilylation of 1-trimethylsilyl-2-alkylalkynes with silver(I) triflate in a biphasic solvent system at room temperature, which obviates the use of large molar excesses of silver and of cyanide. Adapting this method to the current application, prolonged heating of **17d** with silver(I) triflate in a mixture of methanol, water and dichloromethane gave good yields (>70%) of the required alkyne **17e**. We attribute the need for the higher temperature and much longer reaction times (days, rather than hours) to the highly electron-deficient nature of the starting trimethylsilylalkyne.

Each of the three methyl 2-alkynyl-3-nitrobenzoates **17a**,**d**,**e** (carrying the diverse substituents Ph, $SiMe_3$ and H, respectively) was treated with three different electrophiles, to investigate whether or not cyclisation would occur and whether such cyclisation would be 5-*exo* or 6-*endo* (Scheme 4). Firstly, treatment of **17a** with mercury(II) sulfate under acidic conditions afforded an almost quantitative yield of a single product, the isocoumarin **13a**, which was identical to the material prepared by the Castro–Stephens one-pot method. 6-*endo* Cyclisation was relatively unsurprising in this case, in view of the exclusive formation of 3-phenylisocoumarin from methyl 2-phenylethynylbenzoate under the same conditions, as reported by Nagarajan and Balasubramanian.[7] Similar treatment of the analogous trimethylsilylalkyne **17d**, however, gave only methyl 2-acetyl-3-nitrobenzoate **19**, in modest yield after chromatography. The same ketone **19** was also isolated from the reaction of **17e** with mercury(II) sulfate and acid; the NMR spectrum also indicated the presence of a trace of the isomeric pseudoester **20**.[30] Formation of **19** from **17d** and **17e** clearly involves attack of an oxygen nucleophile on the alkyne carbon nearest to the benzene ring, in contrast to the formation of **13a** from **17a**, which must arise from 6-*endo* attack of the ester carbonyl oxygen on the carbon remote from the substituted ring. Thus the alkyne must be polarised in the opposite sense. Scheme 5 shows a mechanistic rationalisation for this change in reactivity. By analogy with the mechanism of the silver(I)-mediated desilylations,[29,31] we propose that transmetallation of **17d** occurs to form an alkynylmercury species, such as **23**. This intermediate could also be formed by direct metallation of **17e**. In intermediate **23**, it is likely that the polarisation of the alkyne caused by coordination to the mercury would over-ride the opposite polarisation induced by the two *ortho*-electron-withdrawing substituents (nitro, carbonyl) on the benzene ring. The ester carbonyl oxygen is then located oppositely for 5-*exo* nucleophilic attack, giving intermediate **24**. Hydrolysis would then afford the major product, the ketone **19**. It is not clear whether the minor side-product **20** is formed from **19** or directly from intermediate **24**.



Scheme 5. Proposed routes for the formation of **19** and **20** through 5-*exo* attack of the neighbouring ester carbonyl oxygen; the polarisation of the alkyne is driven by the mercury cation, rather than by the *ortho*-nitrophenyl group in this case.

The set of three alkynes **17a**,**d**,**e** was also treated with the electrophilic iodine reagent iodine monochloride at ambient temperature in dichloromethane. Only the phenylalkyne **17a** gave an identifiable product, affording 4-iodo-5-nitro-3-phenylisocoumarin **21** in high yield (Scheme 4). No isocoumarins or isobenzofuranones could be identified in the NMR spectra of the crude mixtures of products formed from the trimethylsilylalkyne **17d** or from the monosubstituted alkyne **17e**. The structure of **21** was confirmed as being the isocoumarin product of 6-*endo* cyclisation by two methods. Firstly, the IR spectrum showed an absorption at 1736 cm^{-1}, corresponding to a six-membered ring lactone of this type. Secondly, palladium-catalysed reductive deiodination of **21** with formic acid (by the general method of Rossi et al.[12]) gave an excellent yield of 5-nitro-3-phenylisocoumarin **13a**, identical to samples prepared by the one-pot Castro–Stephens method and the $Hg^{(II)}$-mediated cyclisation of **17a**.

Finally, the reactions of the electrophile phenylselenyl chloride with the alkynes **17a**,**d**,**e** were investigated (Scheme 4). As with the reaction with iodine monochloride, no isocoumarins or isobenzofuranones could be identified as products of the reaction with the monosubstituted alkyne **17e**. However, the disubstituted alkynes **17a**,**d** formed the corresponding 4-phenylselenylisocoumarins **22a**,**d** in moderate-to-good yields, through 6-*endo* cyclisation. Again, the IR spectra indicated 6-membered ring lactones, with bands at 1733 cm^{-1} for both isocoumarins. In view of the lack of suitable methods for reductive removal of the phenylselenyl group to provide material for comparison with **13a** synthesised previously by three independent routes, an X-ray crystal structure determination was carried out for 5-nitro-3-phenyl-4-phenylselenylisocoumarin **22a**. Large bright orange-red

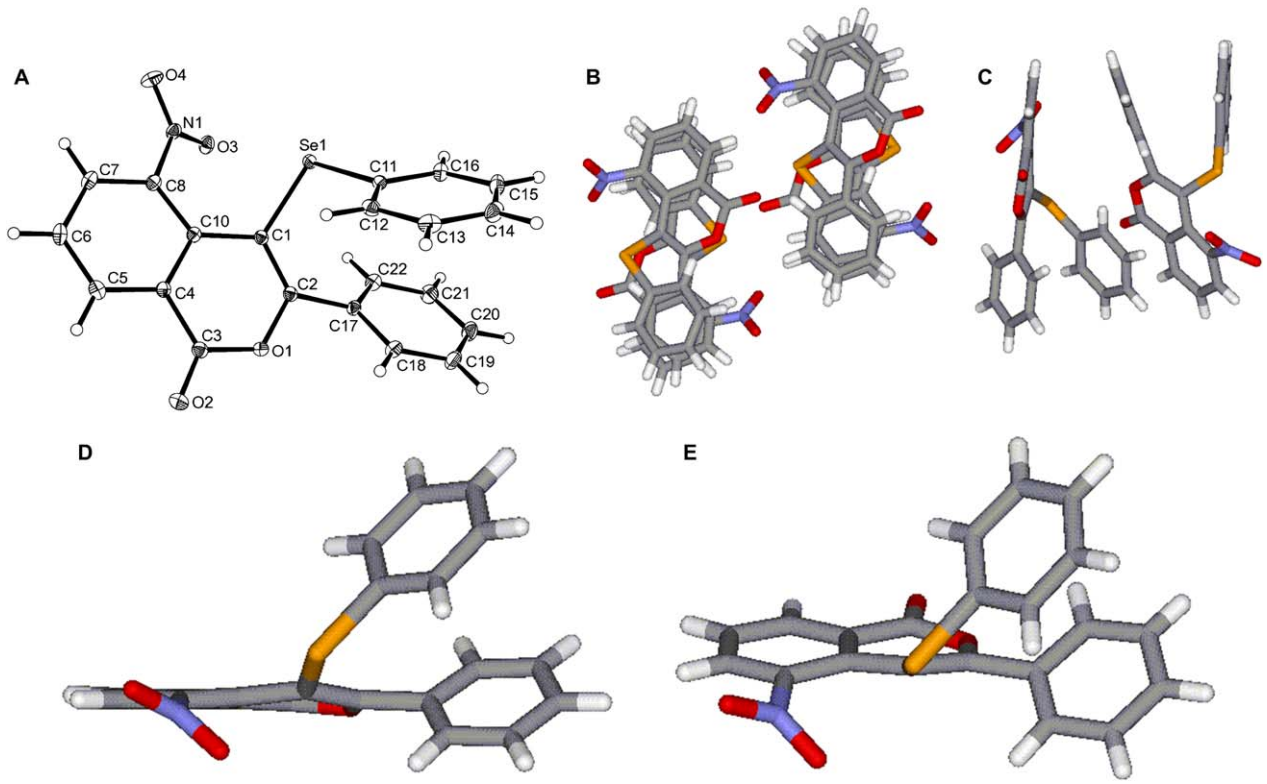


Figure 4. A. X-ray crystal structure of **22a**, with crystallographic numbering. B. Axial view of intermolecular and intramolecular π-stacking in the crystal of **22a**. C. Side view of intermolecular and intramolecular π-stacking of two molecules in the crystal of **22a**. D. View of single molecule of **22a** in the plane of the isocoumarin carbocyclic ring. E. View of single molecule of **22a** along the Se–C bond. Grey=C, white=H, blue=N, red=O and orange=Se.

crystals were formed from ethyl acetate. The structure and X-ray crystallographic numbering scheme are shown in Figure 4A. The most striking observation in this crystal structure is the intermolecular and intramolecular π-stacking of all three benzene rings in the molecule. Figure 4B shows four molecules in two parallel stacks, viewed from the axis of the stacking. The nature of the stacking is shown in the side view in Figure 4C, with the C–Ph and SePh rings stacked intramolecularly; these then stack intermolecularly with the carbocyclic ring of the isocoumarins. This isocoumarin also displays interesting conformational features within the molecule, as shown in Figure 4. The three adjacent substituents, phenyl, phenylselenyl and nitro, at the 3-, 4- and 5-positions, respectively, occupy very crowded regions of space. In particular, there is a severe *peri* interaction between the nitro and phenylselenyl groups. As for the structure of **13b**, the nitro group in **22a** is twisted out of the plane–plane subtended by atoms C2–C8 and C10 of the isocoumarin, by 36.9°. However, the severe steric crowding has a more profound effect, in that the pyranone ring of the isocoumarin of **22a** is forced out of the plane described; above, such that C-1 lies some 0.19 Å above same. This effect is demonstrated even more clearly by the position of the selenium atom at 0.93 Å above this mean plane, as depicted in Figure 4D (wherein the structure is viewed from the plane of the benzene ring) and in Figure 4E (in which the structure is viewed along the Se–C bond vector). Whereas the 3-aryl substituent was only twisted out of the isocoumarin plane in **13b** by <5°, the corresponding 3-phenyl in **22a** is twisted out of the isocoumarin mean plane by 36.5°. The presence of the adjacent bulky phenylselenyl is presumably responsible for this greater lack of coplanarity. The gross structure is dominated by interdigitating intra- and intermolecular stacking of the aromatic rings. The intermolecular centroid–centroid distance between the aromatic rings is 3.8 Å, while the comparable intermolecular aromatic–aromatic distances average 4.1 Å. This latter value reflects the fact that the π-stacking is offset in the intermolecular case.

3. Conclusions

In this paper, we have reported one-pot Castro–Stephens couplings of arylalkynes with 2-iodo-3-nitrobenzoic acid **12**, followed by Cu^{+}-catalysed cyclisation of the intermediate 2-arylalkynyl-3-nitrobenzoic acids in situ to give exclusively 3-aryl-5-nitroisocoumarins **13a–c**. These results contrast with the results reported by Stephens and Castro for the analogues lacking the nitro group, which cyclised in the 5-*exo* mode to give 5-benzylidene-isocoumarins.[20] We interpret this change in regiochemistry of cyclisation as being due to the nitro group inducing polarisation of the alkyne, making the remote sp-carbon more electrophilic as shown in Figure 1. Similarly, $Hg^{(II)}$-catalysed cyclisation of pre-formed methyl 2-phenylalkynyl-3-nitrobenzoate **17a** also proceeded in the 6-*endo* mode to give 2-nitro-3-phenylisocoumarin **13a**, again reflecting this polarisation of the alkyne. Cyclisations of methyl 3-nitro-2-phenylethynylbenzoate **17a** with iodine monochloride and with phenylselenyl chloride also followed the 6-*endo* route to give the isocoumarins **21** and **22a**, respectively, as did cyclisation of methyl 3-nitro-2-trimethylsilylethynylbenzoate **17d** with phenylselenyl chloride, affording the isocoumarins **22d**. Thus the regiochemistry of these cyclisations is also likely to be under

the control of the nitro group. In contrast, the formation of methyl 2-acetyl-3-nitrobenzoate **19** by treatment of **17d**,**e** with $Hg^{(II)}$ suggests that a 5-*exo* cyclisation may have been driven by the change in electron-distribution caused by the formation of an intermediate alkynylmercury complex. These studies extend the understanding of electrophile-driven cyclisations of 2-alkynylbenzoic acids and 2-alkynylbenzoate esters to the previously unreported cases where a powerful electron-withdrawing group is present, influencing the electron-distribution of the alkyne. This understanding will be useful in predicting modes of cyclisation in the synthesis of more complex isocoumarins.

4. Experimental

4.1. General

IR spectra were obtained as KBr discs and NMR spectra were obtained using solutions in $CDCl_3$, unless otherwise stated. Mass spectra were obtained using fast atom bombardment in the positive ion mode, unless otherwise stated. Solutions in organic solvents were dried with $MgSO_4$. Solvents were evaporated under reduced pressure. Mps were obtained using a Kofler–Galen hot stage microscope and are uncorrected.

4.1.1. 2-Hydroxymercuri-3-nitrobenzoic acid (11) and 2-iodo-3-nitrobenzoic acid (12). 3-Nitrobenzene-1,2-dicarboxylic acid **10** (21.1 g, 100 mmol) in hot aq NaOH (10%, 80 mL) was added to $Hg(OAc)_2$ (35.0 g, 110 mmol) in hot AcOH (5 mL) and water (70 mL). The mixture was boiled under reflux for 70 h, then filtered. The precipitate was washed (H_2O, then EtOH) and dried to give **11** (36.2 g, 99%) as a cream solid. Aq HCl (2 M, 12 mL) was added slowly to a boiling solution of **11** (36.2 g, 100 mmol) in aq NaOH (3.5%, 500 mL) and the mixture was allowed to cool to 25 °C. AcOH (3 mL) was then added, followed by KI (19.0 g, 114 mmol) and I_2 (29.0 g, 114 mmol) in water (30 mL). The mixture was boiled under reflux for 24 h, cooled and neutralised with aq NaOH, before being filtered and acidified with aq HCl (9 M). The precipitate was collected, dried and recrystallised (EtOH) to give **12** (19.1 g, 65%) as yellow crystals: mp 203–204 °C (lit.[32] mp 204–205.5 °C); IR ν_{max} 1375, 1542, 1712, 2800 cm^{-1}; ^1H NMR ($(CD_3)_2SO$) δ 7.66 (t, J=7.8 Hz, 1H, 5-H), 7.79 (dd, J=7.8, 1.7 Hz, 1H, 4-H), 7.92 (dd, J=7.8, 1.7 Hz, 1H, 6-H); MS m/z 293.9250 (M+H) ($C_7H_5INO_4$ requires 293.9263).

4.1.2. 5-Nitro-3-phenylisocoumarin (13a) and 1,4-diphenyl-1,3-butadiyne (14a). *Method A*. CuI (7.5 g, 39 mmol) in aq NH_3 (35%, 100 mL) was slowly added to phenylethyne (4.0 g, 39 mmol) in EtOH (200 mL). The mixture was stirred for 15 min. The precipitate was collected, washed with water (5×), EtOH (5×) and Et_2O (5×) and dried at 50 °C (20 torr) for 2 h to afford phenylethynylcopper(I) (4.7 g, 73%) as a bright canary-yellow solid. Compound **12** (3.0 g, 10 mmol) and phenylethynylcopper(I) (1.7 g, 10 mmol) were boiled under reflux in dry pyridine (100 mL) for 6 h under N_2. The mixture was poured into water (300 mL) and extracted with (Et_2O). The extract was washed (aq HCl (2 M), aq $NaHCO_3$ (5%), water). Evaporation and chromatography (hexane/EtOAc 12:1) yielded **14a** (105 mg, 5%) as a white solid: mp 85–86 °C (lit.[33] mp 88 °C); IR ν_{max} 2158 cm^{-1}; ^1H NMR δ 7.26–7.35 (6H, m, 2×3′,4′,5′-H_3), 7.45–7.50 (4H, m, 2×2′,6′-H_2); MS (EI) m/z 202.0784 (M) ($C_{16}H_{10}$ requires 202.0783). Further elution yielded **13a** (2.0 g, 74%) as yellow crystals: mp 142–143 °C; IR ν_{max} 1341, 1525, 1739 cm^{-1}; ^1H NMR δ 7.50–7.53 (3H, m, 3′,4′,5′-H_3), 7.62 (1H, t, J=7.8 Hz, 7-H), 7.79 (1H, d, J=0.8 Hz, 4-H), 7.93–7.97 (2H, m, 2′,6′-H_2), 8.51 (1H, dd, J=8.2, 1.2 Hz, 6-H), 8.65 (1H, ddd, J=8.2, 1.2, 0.8 Hz, 8-H); MS (EI) m/z 267.0532 (M) ($C_{15}H_9NO_4$ requires 267.0532), 237 (M−NO), 184, 150.

4.1.3. 5-Nitro-3-phenylisocoumarin (13a). *Method B*. Compound **17a** (2.5 g, 8.9 mmol) was boiled under reflux with $HgSO_4$ (3.0 g, 10 mmol) and concd H_2SO_4 (4 mL) in acetone (80 mL) for 48 h. The evaporation residue was extracted with $CHCl_3$. Evaporation and chromatography (hexane/Et_2O 9:1) gave **13a** (2.3 g, 95%) as yellow crystals, with data as above.

4.1.4. 5-Nitro-3-phenylisocoumarin (13a). *Method C*. HCO_2H (30 mg, 0.66 mmol) was added to a degassed mixture of **21** (130 mg, 0.33 mmol), Et_3N (100 mg, 1.0 mmol), $Pd(OAc)_2$ (6.6 mg, 7 μmol) and Ph_3P (13.2 mg, 13 μmol) in dry DMF (10 mL). The mixture was stirred at 60 °C for 4.5 h under Ar before being poured into water (50 mL). Extraction (EtOAc), drying, evaporation and chromatography (EtOAc/hexane 1:9) gave **13a** (69 mg, 78%) as pale yellow crystals, with data as above.

4.1.5. 3-(4-Methylphenyl)-5-nitroisocoumarin (13b) and 1,4-di(4-methylphenyl)-1,3-butadiyne (14b). Copper(I) 4-methylphenylacetylide was prepared and treated with **12**, as for the synthesis of **13a** (Method A), to give **14b** (0.4%) as pale yellow crystals: mp 180–181 °C (lit.[33] mp 183 °C); IR ν_{max} 2130 cm^{-1}; ^1H NMR δ 2.36 (6H, s, 2×Me), 7.14 (4H, d, J=8.1 Hz, 2×3′,5′-H_2), 7.41 (4H, d, J=8.1 Hz, 2×2′,6′-H_2); MS (EI) m/z 231.1141 (M+H) ($C_{18}H_{15}$ requires 231.1174). Further elution gave **13b** (18%) as yellow crystals: mp 161–162 °C; IR ν_{max} 1321, 1511, 1618, 1737 cm^{-1}; ^1H NMR δ 2.42 (3H, s, Me), 7.29 (2H, d, J=8.2 Hz, 3′,5′-H_2), 7.56 (1H, t, J=8.2 Hz, 7-H), 7.80 (1H, s, 4-H), 7.81 (2H, d, J=8.2 Hz, 2′,6′-H_2), 8.46 (1H, dd, J=8.2, 1.2 Hz, 6-H), 8.60 (1H, dt, J=8.2, 1.2 Hz, 8-H); MS (EI) m/z 282.0762 (M+H) ($C_{16}H_{11}NO_4$ requires 282.0766).

4.1.6. 3-(4-Methoxyphenyl)-5-nitroisocoumarin (13c) and 1,4-di(4-methoxyphenyl)-1,3-butadiyne (14c). Copper(I) 4-methoxyphenylacetylide was prepared and treated with **12**, for the synthesis of **13a** (Method A), to give **14c** (1%) as pale yellow crystals: mp 148–149 °C (lit.[34] mp 149 °C); IR ν_{max} 2145 cm^{-1}; ^1H NMR δ 3.82 (6H, s, 2×Me), 6.85 (4H, d, J=8.9 Hz, 2×3′,5′-H_2), 7.46 (4H, d, J=8.9 Hz, 2×2′,6′-H_2); MS (EI) m/z 262.0995 (M) ($C_{18}H_{14}O_2$ requires 262.0994), 247 (M−Me), 219 (M−Me−CO). Further elution gave **13c** (29%) as yellow crystals: mp 241–242 °C; IR ν_{max} 1346, 1511, 1620, 1738 cm^{-1}; ^1H NMR δ 3.88 (3H, s, Me), 6.99 (2H, d, J=9.0 Hz, 3′,5′-H_2), 7.54 (1H, t, J=8.2 Hz, 7-H), 7.76 (1H, s, 4-H), 7.88 (2H, d, J=9.0 Hz, 2′,6′-H_2), 8.46 (1H, dd, J=8.2, 1.2 Hz, 6-H), 8.59 (1H, dd, J=8.2, 1.2 Hz,

8-H); MS (EI) *m/z* 297.0639 (M) ($C_{16}H_{11}NO_5$ requires 297.0637), 277 (M−NO), 135.

4.1.7. Methyl 2-iodo-3-nitrobenzoate (16). Compound **12** (4.0 g, 14 mmol) was boiled under reflux with MeOH (120 mL) and concd H_2SO_4 (3 mL) for 48 h, then poured into ice-water. The precipitate was filtered, dried and recrystallised (MeOH) to give **16** (4.0 g, 95%) as yellow crystals: mp 65–66 °C (lit.[35] mp 62–64 °C); IR ν_{max} 1351, 1533, 1705 cm^{-1}; ^1H NMR δ 3.99 (3H, s, Me), 7.54 (1H, t, *J*=7.8 Hz, 5-H), 7.70 (1H, dd, *J*=7.8, 1.7 Hz, 4-H), 7.77 (1H, dd, *J*=7.8, 1.7 Hz, 6-H); MS (EI) *m/z* 306.9347 (M) ($C_8H_6INO_4$ requires 306.9342), 276 (M−OMe).

4.1.8. Methyl 3-nitro-2-(2-phenylethynyl)benzoate (17a) and methyl 3-nitrobenzoate (18). Compound **16** (3.0 g, 9.8 mmol) was stirred with $(Ph_3P)_2PdCl_2$ (300 mg, 0.4 mmol) and CuI (400 mg, 2.1 mmol) in dry THF (120 mL) and dry Pr^i_2NH (40 mL) at 45 °C for 30 min under Ar. Phenylethyne (1.5 g, 15 mmol) was added during 30 min and the mixture was stirred for 48 h. Filtration (Celite®), evaporation and chromatography (hexane/CH_2Cl_2 1:1) gave **17a** (2.2 g, 80%) as reddish-brown crystals: mp 59–60 °C; IR ν_{max} 1343, 1527, 1737, 2219 cm^{-1}; ^1H NMR δ 4.01 (3H, s, Me), 7.36–7.41 (3H, m, Ph 3,4,5-H_3), 7.49 (1H, t, *J*=7.8 Hz, 5-H), 7.57–7.62 (2H, m, Ph 2,6-H_2), 8.04 (1H, dd, *J*=7.8, 1.2 Hz, 4-H), 8.10 (1H, dd, *J*=7.8, 1.2 Hz, 6-H); ^{13}C NMR δ 52.81, 81.79, 102.61, 117.62, 122.06, 126.81, 127.63, 128.32, 129.44, 131.97, 133.56, 134.94, 151.87, 165.27; MS (EI) *m/z* 282.0757 (M+H) ($C_{16}H_{12}NO_4$ requires 282.0766), 266 (M−Me), 250 (M−OMe), 222 (M−CO_2Me). Further elution gave **18** (500 mg, 28%) as yellow crystals: mp 78–79 °C (lit.[36] mp 78 °C); ^1H NMR δ 3.99 (3H, s, Me), 7.66 (1H, t, *J*=7.8 Hz, 5-H), 8.37 (1H, ddd, *J*=7.8, 2.4, 1.2 Hz, 4-H), 8.42 (1H, ddd, *J*=7.8, 2.4, 1.2 Hz, 6-H), 8.87 (1H, t, *J*=2.4 Hz, 2-H). From some runs, 1,4-diphenylbutadiyne **14a** was also isolated.

4.1.9. Methyl 3-nitro-2-(2-trimethylsilylethynyl)benzoate (17d) and methyl 3-nitrobenzoate (18). Compound **16** (3.0 g, 9.8 mmol) in dry THF (120 mL) was added to $(Ph_3P)_2PdCl_2$ (300 mg, 0.4 mmol) and CuI (400 mg, 2.1 mmol) in dry Pr^i_2NH (40 mL) and the mixture was stirred at 45 °C for 30 min under Ar. Trimethylsilylethyne (1.1 g, 11 mmol) was added during 30 min. The mixture was stirred for 72 h at 45 °C. Filtration (Celite®), evaporation and chromatography (hexane/CH_2Cl_2 3:2) gave **17d** (810 mg, 30%) as a reddish-brown oil: IR (film) ν_{max} 1351, 1532, 1738, 2219 cm^{-1}; ^1H NMR δ 0.28 (9H, s, $SiMe_3$), 3.96 (3H, s, OMe), 7.49 (1H, t, *J*=7.8 Hz, 5-H), 7.96 (1H, dd, *J*=7.8, 1.6 Hz, 4-H), 8.01 (1H, dd, *J*=7.8, 1.6 Hz, 6-H); MS (EI) *m/z* 278.2361 (M+H) ($C_{13}H_{16}NO_4Si$ requires 278.2357). Further elution gave **18** (690 mg, 39%), with data as above.

4.1.10. Methyl 2-ethynyl-3-nitrobenzoate (17e). *Method A*. Bu_4NF (1.0 M in THF, 5.0 mL, 5.0 mmol) was added to **17d** (400 mg, 1.4 mmol) in THF (50 mL) and water (2.5 mL) at 0 °C and the mixture was stirred at 20 °C for 16 h. The mixture was diluted with Et_2O and washed with satd aq NH_4Cl and water. Evaporation and chromatography (hexane/EtOAc 7:3) gave **17e** (28 mg, 9%) as a pale yellow solid: mp 78–80 °C; IR (film) ν_{max} 1351, 1532, 1738, 2219 cm^{-1}; ^1H NMR δ 3.67 (1H, s, C≡CH), 3.90 (3H, s, Me), 7.48 (1H, t, *J*=7.8 Hz, 5-H), 7.91 (1 H, dd, *J*=7.8, 1.2 Hz, 4-H), 8.00 (1H, dd, *J*=7.8, 1.2 Hz, 6-H); MS (EI) *m/z* 205.0381 (M) ($C_{10}H_7NO_4$ requires 205.0375), 191 (M−CH_2), 175 (M−NO), 160 (M−NO−Me).

4.1.11. Methyl 2-ethynyl-3-nitrobenzoate (17e). *Method B*. Compound **17d** (250 mg, 0.9 mmol) was stirred with AgOTf (23 mg, 0.09 mmol) in a mixture of acetone (6.0 mL), water (1.5 mL) and CH_2Cl_2 (10.5 mL) for 7 d. Satd aq ammonium chloride (2 mL) was added and the mixture was extracted thrice with CH_2Cl_2. Evaporation and chromatography (EtOAc/hexane 1:6) gave **17e** (132 mg, 72%) with data as above.

4.1.12. Methyl 2-acetyl-3-nitrobenzoate (19). *Method A*. Compound **17d** (110 mg, 0.4 mmol) was boiled under reflux with $HgSO_4$ (148 mg) and concd H_2SO_4 (0.1 mL) in acetone (10 mL) for 48 h. The evaporation residue was extracted with $CHCl_3$. Evaporation and chromatography (toluene) gave **15** (25 mg, 28%) as a white solid: mp 81–82 °C (lit.[37] mp 81.5 °C); ^1H NMR δ 2.71 (3H, s, ArCOMe), 3.93 (3H, s, OMe), 7.65 (1H, t, *J*=8.2 Hz, 5-H), 8.33 (1H, dd, *J*=8.2, 1.2 Hz, 4-H or 6-H), 8.37 (1H, dd, *J*=8.2, 1.2 Hz, 6-H or 4-H); ^{13}C NMR δ 29.66, 53.09, 128.64, 129.47, 129.56, 136.20, 140.19, 146.04, 164.47, 200.03.

4.1.13. Methyl 2-acetyl-3-nitrobenzoate (19). *Method B*. Compound **17e** was treated with $HgSO_4$, as for Method A, to give **19** (25%) with properties as above. Also identified in trace amounts in the NMR spectrum was 3-methoxy-3-methyl-4-nitroisobenzofuran-1-one **20**:[30] ^1H NMR δ 2.05 (3H, s, CMe), 3.22 (3H, s, OMe), 7.43 (1H, m, 6-H), 8.22 (1H, dd, *J*=8.4, 0.9 Hz, 5-H or 7-H), 8.47 (1H, dd, *J*=8.8, 0.9 Hz, 7-H or 5-H).

4.1.14. 4-Iodo-5-nitro-3-phenylisocoumarin (21). Compound **17a** (114 mg, 0.37 mmol) was stirred with ICl (90 mg, 0.55 mmol) in CH_2Cl_2 (1.0 mL) for 2 h in the dark. The mixture was diluted with Et_2O (50 mL), washed with aq $Na_2S_2O_3$ and dried. Evaporation and chromatography (EtOAc/hexane 1:4) gave **21** (130 mg, 81%) as pale yellow crystals. A sample was recrystallised (EtOAc/hexane) to give a yellow powder: mp 154–156 °C; IR ν_{max} 1347, 1522, 1736 cm^{-1}; ^{13}H NMR δ 7.50 (3H, m, Ph 3,4,5-H_3), 7.66 (1H, t, *J*=7.8 Hz, 7-H), 7.75 (2H, m, Ph 2,6-H_2), 8.08 (1H, dd, *J*=7.8, 1.2 Hz, 6-H), 8.54 (1H, dd, *J*=7.8, 1.2 Hz, 8-H); ^{13}C NMR δ 61.58, 123.47, 128.35, 128.63, 130.46, 130.92, 131.21, 131.95, 133.25, 134.90, 150.06, 158.95, 159.55; MS (EI) *m/z* 392 (M−H), 265 (M−HI). Found: C, 46.1; H, 2.15; N, 3.69; $C_{15}H_8INO_4$ requires C, 45.83; H, 2.05; N, 3.56%.

4.1.15. 5-Nitro-3-phenyl-4-phenylselenylisocoumarin (22a). Compound **17a** (100 mg, 0.36 mmol) was stirred with PhSeCl (100 mg, 0.53 mmol) in CH_2Cl_2 (5.0 mL) under N_2 for 4 h. The mixture was washed (aq $NaHCO_3$) and dried. Evaporation and chromatography (EtOAc/hexane 1:7) gave **22a** (72 mg, 47%) as yellow crystals. A sample was recrystallised (EtOAc) to give orange-red crystals: mp 188–190 °C; IR ν_{max} 1354, 1533, 1733 cm^{-1}; ^1H NMR δ 6.77 (2H, ca. d, *J*=8.6 Hz, SePh 2,6-H_2), 6.95 (2H, ca. t, *J*=ca. 8 Hz, SePh 3,5-H_2), 7.07 (1H, tt, *J*=7.4, 1.2 Hz,

SePh 4-H), 7.32 (2H, ca. t, J=ca. 7.4 Hz, CPh 3,5-H_2), 7.39 (1H, tt, J=7.1, 1.5 Hz, CPh 4-H), 7.58 (2H, ca. d, J=ca. 7.5 Hz, CPh 2,6-H_2), 7.64 (1H, t, J=8.0 Hz, 7-H), 8.14 (1H, dd, J=8.0, 1.5 Hz, 6-H), 8.55 (1H, dd, J=8.0, 1.5 Hz, 8-H); ^{13}C NMR δ 102.24, 123.12, 127.61, 127.85, 128.04, 128.90, 130.34, 130.60, 131.05, 131.70, 132.18, 133.26, 133.68, 134.38, 137.27, 148.35, 159.90, 160.65; MS (EI) m/z 422 (M). Found: C, 59.8; H, 3.06; N, 3.32; $C_{21}H_{13}NO_4Se$ requires C, 59.73; H, 3.10; N, 3.32.

4.1.16. 5-Nitro-4-phenylselenyl-3-trimethylsilylisocoumarin (22d). Compound **17d** (110 mg, 0.4 mmol) was stirred with PhSeCl (114 mg, 0.6 mmol) in CH_2Cl_2 (3.0 mL) under N_2 for 24 h. The mixture was washed (aq $NaHCO_3$) and dried. Evaporation and chromatography (EtOAc/hexane 1:4) gave **22d** (136 mg, 82%) as a yellow solid. A sample was recrystallised (toluene) to give a yellow powder: mp 105–107 °C; IR ν_{max} 1733 cm^{-1}; ^{1}H NMR δ 0.34 (9H, s, $SiMe_3$), 7.00 (2H, m, Ph 2,6-H_2), 7.14 (3H, m, Ph 3,4,5-H_3), 7.38 (1H, t, J=7.8 Hz, 7-H), 7.80 (1H, dd, J=7.8, 1.4 Hz, 6-H), 8.50 (1H, dd, J=7.8, 1.4 Hz, 8-H); ^{13}C NMR δ 0.34, 110.26, 124.04, 126.67, 127.84, 128.30, 129.10, 129.36, 130.15, 130.33, 132.96, 133.73, 160.47, 175.22; MS (EI) m/z 419.0096 (M) ($C_{18}H_{17}NO^{80}Se^{28}Si$ requires 419.0092), 403 (M−CH_4). Found: C, 51.8; H, 4.15; N, 3.33; $C_{18}H_{17}NOSeSi$ requires C, 51.67; H, 4.10; N, 3.35.

4.1.17. X-ray crystallography. Compound 13b. *Crystal data*, $C_{16}H_{11}NO_4$, M=281.26, λ=0.71073 Å, monoclinic, space group $P2_1/n$, a=5.8870(2), b=12.0270(3), c=18.4880(7) Å, β=96.802(1)°, U=1299.79(7) Å^3, Z=4, D_c=1.437 mg m^{-3}, μ=0.105 mm^{-1}, F(000)=584, crystal size 0.40×0.13×0.08 mm, unique reflections=2964 [R_{int}=0.0686], observed $I>2\sigma(I)$=1523, data/restraints/parameters=2964/0/192, $R1$=0.0548 $wR2$=0.1228 (observed data), $R1$=0.1295 $wR2$=0.1617 (all data), max peak/hole 0.300 and −0.279 eÅ^{-3}, diffractometer=Nonius kappaCCD, software used, SHELXS,[38] SHELXL[39] and ORTEX.[40]

4.1.18. X-ray crystallography. Compound 22a. *Crystal data*, $C_{21}H_{13}NO_4Se$, M=422.28, λ=0.71073 Å, monoclinic, space group $P2_1/n$, a=11.8050(1), b=11.8550(1), c=13.3860(1) Å, β=113.969(1)°, U=1711.80(2) Å^3, Z=4, D_c=1.639 mg m^{-3}, μ=2.222 mm^{-1}, F(000)=848, crystal size 0.50×0.30×0.10 mm, unique reflections=3928 [R_{int}=0.0549], observed $I>2\sigma(I)$=3671, data/restraints/parameters=3928/0/245, $R1$=0.0248 $wR2$=0.0627 (observed data), $R1$=0.0275 $wR2$=0.0643 (all data), max peak/hole 0.380 and −0.568 eÅ^{-3}, software used, SHELXS,[38] SHELXL[39] and ORTEX.[40]

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CDCC-263103 and CDCC-288997. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

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Synthesis of *trans*-vaccenic acid and *cis*-9-*trans*-11-conjugated linoleic acid

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Abstract—The preparation of the monounsaturated fatty acid, *trans*-vaccenic acid **4** (TVA), using both Wittig and one-pot Julia-Kocieński olefination protocol, was achieved in good yield. Similarly a Wittig approach was employed for the stereoselective synthesis of *cis*-9-*trans*-11-conjugated linoleic acid **2** from *trans*-2-nonenal and (8-carboxyoctyl)triphenylphosphonium bromide **12**.

1. Introduction

Excessive dietary fatty acids, in particular saturated fatty acids, are associated with a number of chronic diet-related diseases including obesity, type 2 diabetes mellitus, cardiovascular disease and some cancers.[1] Together these conditions account for the majority of global mortality rates in the Western world. In contrast, unsaturated fatty acids, for example, linoleic acid (LA) **1**, are associated with reduced risk of these diet-related diseases.[1] Therefore, nutritional science is focussing on identifying 'healthy' fatty acids, which may be incorporated into functional foods or nutraceuticals. Recent evidence has demonstrated that a novel unsaturated fatty acid, conjugated linoleic acid (CLA) may have health promoting effects, inhibiting the development of cancer, atherosclerosis, diabetes and chronic inflammatory diseases.[2] Consequently CLA has become a popular health food supplement. CLA is a heterogeneous group of compounds, including a number of positional and geometrical isomers of linoleic acid (C18:2 *n*-6).[3] Significantly it has been demonstrated that the health effects of CLA are isomer specific, whereby the *cis*-9-*trans*-11 CLA (c9, t11-CLA) isomer **2** prevents disease processes that lead to atherosclerosis, diabetes, chronic inflammation and colon cancer.[4] However, the other main isomer, *trans*-10-*cis*-12 CLA **3** (t10, c12-CLA), appears to possess detrimental health effects including inducing a diabetic state, associated with hyperlipidaemia.[4] All commercially available supplements contain a mixture of these regioisomeric cis, trans-alkenes (**2** and **3**), since the current synthetic procedures used for their preparation are unselective. Therefore, any potential health benefit of *cis*-9-*trans*-11 CLA **2** is lost, due to the presence of the detrimental *trans*-10-*cis*-12 CLA **3** isomer.[5] A mixture of CLA (**2** and **3**) is obtained by following the base induced isomerisation of linoleic acid **1**.[3,6] However, this conversion leads to the mixture of regio- and stereoisomers that require lengthy and complicated separation (Fig. 1).

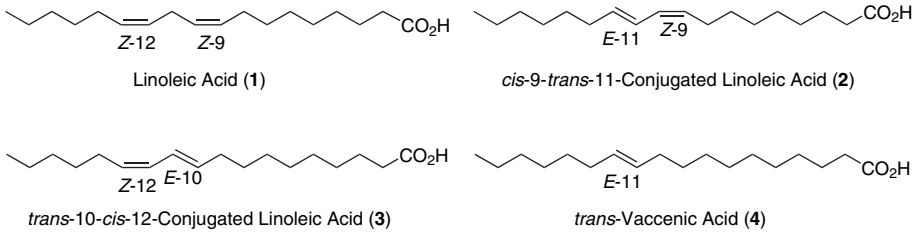

Figure 1. Structures of linoleic acid and related C-18 fatty acids.

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CLA is a natural food component found in the lipid fraction of meat, milk and dairy products, and *cis*-9-*trans*-11 CLA **2** is the principal dietary isomeric form of CLA.[7] It is now evident that the major trans-fatty acid found in ruminant meat and milk is *trans*-vaccenic acid **4** (TVA, *trans*-11 C18:1) which has been found to protect against chemically-induced mammary cancer in rats.[8] Biosynthetic evidence indicates that TVA **4** is readily converted by the bacterial enzyme Δ^9-desaturase into *cis*-9-*trans*-11-conjugated linoleic acid **2**. Initially it was proposed that this was the major source of CLA synthesis in the bovine mammary gland but it is now clear that several human tissues, in particular the intestine, can convert TVA **4** to *cis*-9-*trans*-11 CLA **2**.[9] Therefore, the biopotency of CLA food sources can be enhanced in vivo by the co-existence of TVA **4** in several food products (milk, yoghurt, cheese, butter and meats). Nevertheless, the presence of both fatty acids in foods impedes investigation of the individual effects of CLA and TVA. Additionally, currently there are no stereoisomerically pure forms of TVA **4** is commercially available for nutritional studies to determine whether the nature of the health effects ascribed to TVA are due to the fatty acid alone or attributable to metabolic conversion of TVA **4** to CLA **2**. This data are essential to define the health implication of CLA and TVA enriched functional foods and nutraceuticals for the development and application of enhanced human nutrition and health products. Consequently, we became interested in the stereoselective preparation of both *trans*-vaccenic acid **4** and *cis*-9-*trans*-11-conjugated linoleic acid **2**.

2. Results and discussion

Since it was known that *cis*-vaccenic acid undergoes a facile isomerisation to *trans*-vaccenic acid **4** with a mixture of $NaNO_2$–HNO_3,[10] the preparation of *trans*-vaccenic acid was achieved by using Wittig chemistry (see Fig. 2).[11] Thus, commercially available carboxylic acid **5** was converted into the phosphonium salt **6**, the purification of which was achieved by taking up the crude mixture and treatment with boiling ethyl acetate. This operation also facilitates the handling of **6** since on addition of ethyl acetate, **6** formed a solid.[12] Treatment of a slurry of **6**, in THF at −78 °C, with 2.5 equiv of LHMDS and then warming this mixture to 0 °C over 2 h resulted in the formation of the ylide. Subsequent re-cooling to −78 °C and addition of heptanal gave the Wittig product *cis*-vaccenic acid in good yield (70%) and as a ratio of alkenyl stereoisomers (*Z*/*E*: ca. 82:18—estimated by integration of the respective alkenyl signals in the ^{1}H NMR spectrum [*cis*-CVA: δ_H 5.34–5.41 ppm; *trans*-CVA: δ_H 5.38–5.47 ppm]).

Under the so-called 'salt-free' Wittig conditions using KHMDS as the base slightly better yields and stereoselectivities were observed for this process (89%; *Z*/*E*, ca. 88:12).[11,13] Isomerisation was then effected by following the conditions reported and *trans*-vaccenic acid **4** was obtained by iterative recrystallisation of the crude product from acetone (*E*/*Z*, >95:5).[10] This overall synthetic sequence proved a robust method for the preparation of *trans*-vaccenic acid **4** and was performed on scales of ca. 30 g (Wittig product).

Although the melting point of our synthetic material was consistent with that reported in the literature[10] and additional spectroscopic evidence supported the assigned structure we were not able to observe the trans-alkenyl coupling by proton NMR spectroscopy since the two alkenyl protons in both the *cis*- and *trans*-vaccenic acid samples were coincident. Therefore, in order to probe the stereochemistry of the alkene formed in this sequence we investigated a complementary Julia-Kocieński one-pot olefination protocol, since this method has been reported to generate high levels of trans-stereoselectivity during alkene formation (particularly when K^+ counter ions are employed).[14] Thus, the sulfonyl tetrazole **7** was synthesised by following the alkylation of 1-phenyl-1*H*-tetrazole-5-thiol **8** with 11-bromoundecanoic acid methyl ester and the oxidation of the sulfide adduct with *m*-CPBA. Subsequently, a solution of the sulfone **7** in THF was treated with KHMDS at approximately −55 °C[14] and stirring was continued for 40 min before addition of heptanal at the same temperature. The resultant alkene **9** was isolated in 50% yield and with reasonable trans-stereoselectivity (*E*/*Z*, ca. 85:15). Subsequent ester hydrolysis with LiOH

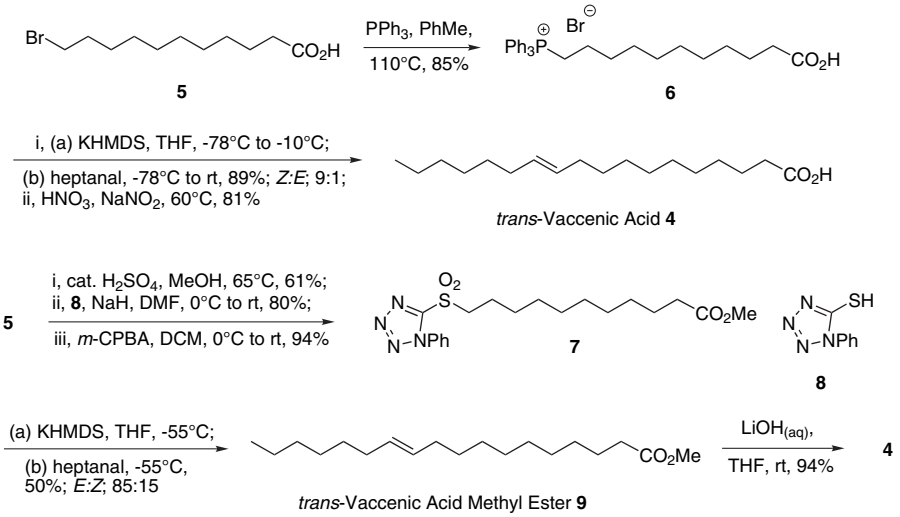

Figure 2. Synthesis of *trans*-vaccenic acid **4**.

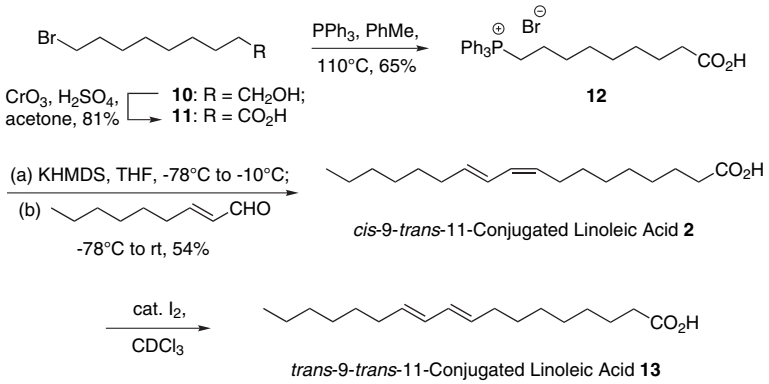

Figure 3. Synthesis of *cis*-9-*trans*-11-conjugated linoleic acid **4**.

gave the corresponding acid **2** and the spectroscopic data of the predominant stereoisomer were identical to that obtained from *trans*-vaccenic acid **2** accessed via the Wittig-isomerisation route.

Following these studies we then investigated the stereo- and regioselective preparation of *cis*-9-*trans*-11-conjugated linoleic acid **2**.[6] Arguably the most widely used method to synthesise this type of conjugated *cis*, *trans*-dienyl system present in CLA is based on the reduction of a *trans*-enyne.[6a,15] However, in their classical synthesis of the insect pheromone bombykol, Bestmann and co-workers demonstrated that the Wittig reaction between a non-stabilised ylide and a *trans*-α,β-unsaturated aldehyde affords the adduct in good levels with cis-stereoselectivity.[16] Therefore, we chose to employ a similar Wittig approach to construct the cis, trans-dienyl architecture present in *cis*-9-*trans*-11-CLA **2** (Fig. 3).

Jones oxidation of 9-bromononanol **10** gave the carboxylic acid **11**, which was converted into phosphonium bromide **12**.[17] As described for **6**, this material was purified by stirring in boiling ethyl acetate, removing the unreacted starting materials and also facilitating the formation of the phosphonium salt **12** as an amorphous solid. The subsequent Wittig reaction of **12** was performed using KHMDS as a base in an identical fashion to the previous synthesis of TVA **4**. Thus, *cis*-9-*trans*-11-conjugated linoleic acid **2** was isolated in 54% yield and the new double bond was formed with reasonable cis-selectivity (*Z*/*E*, ca. 85:15). The stereochemical integrity of the predominantly formed geometric isomer was confirmed by comparison with the commercially available material.[6e,18] In addition its isomerisation to *trans*-9-*trans*-11-conjugated linoleic acid **13** was achieved using approximately 5 mol % of I_2, as described[6a] and consequently we were able to confirm the identity of the minor impurity in the Wittig reaction.

In summary, we have achieved a straightforward stereoselective synthesis of *cis*-9-*trans*-11-conjugated linoleic acid **2**. The preparation of its bioprecursor, the monounsaturated fatty acid, *trans*-vaccenic acid **4**, was also performed using both Wittig and Julia-Kocieński olefination approaches. Significantly, these reaction sequences are scalable, enabling the preparation of multi-gram amounts of these biologically important fatty acids.

3. Experimental

3.1. General

Starting materials were purchased from commercial sources and were used without further purification. Anhydrous THF was distilled under nitrogen from the sodium-benzophenone ketyl radical. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded using a Bruker AMX400 spectrometer. Infrared spectroscopy was performed on a Perkin–Elmer Paragon 1000 FTIR spectrometer. Flash column chromatography, under moderate pressure was performed using silica gel—ICN 32–63, 60 Å. Melting points were recorded on an Electrothermal IA9000 digital melting point apparatus and were uncorrected.

3.1.1. (10-Carboxydecyl)triphenylphosphonium bromide 6. Under nitrogen a solution of 11-bromoundecanoic acid **5** (40.0 g, 0.15 mol, 1 equiv) and triphenylphosphine (43.42 g, 0.165 mol, 1.1 equiv) in toluene (250 mL) were heated to reflux for 24 h. On cooling the two phases were apparent and the upper phase was decanted and DCM (ca. 250 mL) was added to the viscous residue before the solvent was stripped in vacuo. The resultant solid was washed with boiling EtOAc (3×ca. 250 mL) and the white solid (67.4 g, 85%) was collected by filtration and dried in vacuo. Mp 103–105 °C; υ_{max} (Nujol/cm^{-1}) 3010, 2925, 2855, 1711, 1465, 1287; δ_H (400 MHz, $CDCl_3$) 1.19–1.35 (10H, m, CH_2), 1.57–1.69 (6H, m, CH_2), 2.46 (2H, t, *J* 7.0 Hz, CH_2), 3.70–3.81 (2H, m, CH_2), 7.66–7.78 (6H, m, ArH), 7.78–7.84 (9H, m, ArH); δ_C (100 MHz, $CDCl_3$) 22.3 (d, *J* 5.0 Hz), 22.8 (d, *J* 49.5 Hz), 24.4, 28.25, 28.3, 29.9 (d, *J* 15.5 Hz), 34.3, 118.4 (d, *J* 85.5 Hz), 130.5 (d, *J* 12.5 Hz), 133.6 (d, *J* 9.5 Hz), 135.0, 177.4; δ_P (162 MHz, $CDCl_3$) 25.65.

3.1.2. *cis*-Octadec-12-enoic acid (*cis*-vaccenic acid)

(a) Under nitrogen hexamethyldisilazane (28.8 mL, 0.138 mol, 3 equiv) in dry THF (250 mL) was cooled to −78 °C and treated with 1.6 M BuLi in hexane (72 mL, 0.115 mol, 2.5 equiv). Stirring was continued for 0.5 h before this solution of LHMDS was added to a slurry of the phosphonium salt **6** (24.25 g, 0.046 mol, 1 equiv) in THF (350 mL) at −78 °C via cannula. The resultant yellow–orange mixture was stirred between

−78 and 0 °C for 2 h before the solution was re-cooled to −78 °C and heptanal (8.4 mL, 0.06 mol, 1.3 equiv) was added dropwise. The mixture was stirred for 48 h during which period room temperature was gradually reached. EtOAc (250 mL) and 1 M HCl (250 mL) were added and the resultant aqueous phase was further extracted with EtOAc (2×250 mL). The combined organic phases were dried over $MgSO_4$ before filtration and solvent evaporation under reduced pressure gave the crude cis-alkene. Purification by flash column chromatography (Hex–EtOAc, 6:1) afforded the product (9.03 g, 70%) as a viscous yellow oil. R_f=0.3 (Hex–EtOAc, 6:1); υ_{max} (neat/cm^{-1}) 3005, 2925, 2855, 1713, 1586, 1464; δ_H (400 MHz, $CDCl_3$) 0.89 (3H, t, *J* 7.0 Hz, CH_3), 1.23–1.39 (20H, m, CH_2), 1.64 (2H, pent, *J* 7.5 Hz, CH_2), 1.98–2.07 (4H, m, CH_2), 2.36 (2H, t, *J* 7.5 Hz, CH_2), 5.34–5.41 (2H, m, CH), 11.20 (1H, br s, CO_2H); δ_C (100 MHz, $CDCl_3$) 14.1, 22.6, 24.7, 27.15, 27.2, 29.0, 29.05, 29.2, 29.4, 29.5, 29.7, 31.8, 34.1, 129.8, 129.9, 179.9.

(b) Under nitrogen, a stirred slurry of (10-carboxydecyl)triphenylphosphonium bromide **6** (10.0 g, 18.98 mmol, 1 equiv) in THF (120 mL) was cooled to −78 °C. Potassium bis(trimethylsilyl)amide (101 mL of 0.5 M solution in toluene, 50.49 mmol, 2.7 equiv) was added and the mixture was warmed to room temperature over 2 h. The deep red solution was then re-cooled to −78 °C before heptanal (3.66 mL, 26.22 mmol, 1.4 equiv) was added dropwise. The reaction was warmed to room temperature and stirred for 12 h. Work-up and purification was carried out as above and *cis*-vaccenic acid (4.76 g, 89%) was obtained whose data corresponded to the above data.

3.1.3. *trans*-Octadec-12-enoic acid (*trans*-vaccenic acid 4). With stirring *cis*-vaccenic acid (9.03 g, 32.02 mmol, 1 equiv) was treated with a solution of $NaNO_2$ (0.47 g, 6.81 mmol, 0.2 equiv) in water (1.8 mL), which was heated to 60 °C. After 0.5 h a solution of concd HNO_3 (2.3 mL) in water (2.3 mL) was added. The mixture was further stirred for 0.3 h before being removed from the oil bath. After reaching room temperature (ca. 1 h) Et_2O (30 mL) and water (30 mL) were added. The resultant aqueous phase was further extracted with Et_2O (4×30 mL) and the combined organic phases were dried over $MgSO_4$ before filtration and solvent evaporation under reduced pressure gave the crude trans-alkene. Purification by three repetitive recrystallisations from acetone afforded the product **4** (7.31 g, 81%) as a white solid. Mp 43.5–44 °C (acetone); R_f=0.25 (Hex–EtOAc, 6:1); υ_{max} (Nujol/cm^{-1}) 2954, 2923, 2852, 1712, 1462, 1414, 1377; δ_H (400 MHz, $CDCl_3$) 0.90 (3H, t, *J* 6.5 Hz, CH_3), 1.24–1.39 (20H, m, CH_2), 1.65 (2H, pent, *J* 7.5 Hz, CH_2), 1.95–2.04 (4H, m, CH_2), 2.36 (2H, t, *J* 7.5 Hz, CH_2), 5.38–5.47 (2H, m, CH_2), 10.05 (1H, br s, CO_2H); δ_C (100 MHz, $CDCl_3$) 14.1, 22.6, 24.6, 28.8, 29.0, 29.1, 29.2, 29.35, 29.4, 29.6, 31.7, 32.55, 32.6, 34.1, 130.3, 130.4, 180.3; *m/z* (CI) 300 (MNH_4^+, 100%); found 300.28983, $C_{18}H_{38}O_2N$ requires 300.29025 (−1.5 ppm); found C, 76.67; H, 11.85%; $C_{18}H_{34}O_2$ requires C, 76.54; H, 12.13%.

3.1.4. 11-Bromoundecanoic acid methyl ester.[19] A solution of 11-bromoundecanoic acid (5.0 g, 19.00 mmol, 1 equiv) and methanol (50 mL) was treated with concd H_2SO_4 (0.5 mL) and heated to reflux for 12 h. On cooling Et_2O (80 mL) and 1 M KOH (80 mL) were added. The resultant aqueous phase was further extracted with Et_2O (2×80 mL) and the combined organic phases were dried over $MgSO_4$. Filtration and solvent removal under reduced pressure gave the crude methyl ester which was purified by flash column chromatography (Hex–EtOAc, 19:1). Thus the methyl ester (2.734 g, 61%) was isolated as a colourless liquid. R_f=0.25 (Hex–EtOAc, 19:1); υ_{max} (neat/cm^{-1}) 2926, 2854, 1737, 1436, 1246, 1196, 1169; δ_H (400 MHz, $CDCl_3$) 1.22–1.31 (10H, m, CH_2), 1.42 (2H, pent, *J* 7.0 Hz, CH_2), 1.63 (2H, pent, *J* 7.0 Hz, CH_2), 1.84 (2H, pent, *J* 7.5 Hz, CH_2), 2.31 (2H, t, *J* 7.5 Hz, CH_2), 3.39 (2H, t, *J* 7.0 Hz, CH_2), 3.66 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 24.9, 28.1, 28.6, 29.0, 29.1, 29.2, 29.3, 32.7, 33.95, 34.0, 51.4, 174.2.

3.1.5. 11-(1-Phenyl-1*H*-tetrazole-5-sulfanyl)undecanoic acid methyl ester. Under nitrogen at 0 °C 60% w/w NaH (0.48 g, 12.0 mmol, 1.1 equiv) was added in one portion to a solution of 1-phenyl-1*H*-tetrazole-5-thiol **8** (1.96 g, 11.0 mmol, 1 equiv) in dry DMF (25 mL). After stirring for 0.5 h the methyl ester (2.56 g, 11.0 mmol, 1 equiv) was added. Stirring was maintained for 12 h. Et_2O (50 mL) and water (50 mL) were added and the resultant aqueous layer was further extracted with Et_2O (3×50 mL). The combined ethereal extracts were dried over $MgSO_4$. Filtration, solvent removal in vacuo and purification by flash column chromatography (Hex–EtOAc, 4:1) gave the sulfide (3.29 g, 80%) as a colourless waxy solid. Mp 34–36 °C; R_f=0.3 (Hex–EtOAc, 4:1); υ_{max} (neat/cm^{-1}) 2925, 1736, 1598, 1500, 1386, 1242, 1170; δ_H (400 MHz, $CDCl_3$) 1.25–1.38 (10H, m, CH_2), 1.44 (2H, pent, *J* 7.5 Hz, CH_2), 1.62 (2H, pent, *J* 7.5 Hz, CH_2), 1.83 (2H, pent, *J* 7.0 Hz, CH_2), 2.34 (2H, t, *J* 7.5 Hz, CH_2), 3.42 (2H, t, *J* 7.0 Hz, CH_2), 3.69 (3H, s, CH_3), 7.52–7.66 (5H, m, ArH); δ_C (100 MHz, $CDCl_3$) 24.9, 28.6, 29.0, 29.05, 29.1, 29.2, 29.3, 29.35, 33.3, 34.1, 51.5, 123.8, 129.8, 130.1, 133.7, 154.5, 174.3; *m/z* (ES^+) 399 (MNa^+, 100%), 377 (MH^+, 20%); found 377.1996, $C_{19}H_{29}N_4O_2S$ requires 377.2011 (−4.0 ppm).

3.1.6. 11-(1-Phenyl-1*H*-tetrazole-5-sulfonyl)undecanoic acid methyl ester 7. At 0 °C *m*-CPBA (1.38 g, 7.98 mmol, 3 equiv) was added in one portion to a solution of the sulfide (1.00 g, 2.66 mmol, 1 equiv) in DCM (40 mL). Stirring was maintained for two days. DCM (50 mL) and satd Na_2SO_3 (50 mL) were added and the mixture was partitioned for 1 h. The resultant aqueous layer was further extracted with DCM (50 mL) and the combined organic extracts were washed with satd $NaHCO_3$ (100 mL). The resultant aqueous layer was washed with DCM (3×50 mL) before drying over $MgSO_4$. Filtration and solvent removal in vacuo gave the sulfone **7** (1.02 g, 94%) as a colourless solid whose ^1H NMR spectrum indicated sufficient purity for further use. Mp 47–49 °C; R_f=0.2 (Hex–EtOAc, 4:1); υ_{max} (neat/cm^{-1}) 2918, 2848, 1725, 1595, 1437, 1341, 1155; δ_H (400 MHz, $CDCl_3$) 1.22–1.40 (10H, m, CH_2), 1.46–1.55 (2H, m, CH_2), 1.64 (2H, pent, *J* 7.5 Hz, CH_2), 1.96 (2H, pent, *J* 8.0 Hz, CH_2), 2.34 (2H, t, *J* 7.5 Hz, CH_2), 3.68 (3H, s, CH_3), 3.74 (2H, t, *J* 8.0 Hz, CH_2), 7.60–7.67 (3H, m, ArH), 7.72 (2H, d, *J* 7.5 Hz, ArH); δ_C (100 MHz, $CDCl_3$) 21.9, 24.9, 28.1, 28.8, 29.0, 29.05, 29.1, 29.2, 34.1, 51.5,

56.0, 125.0, 129.7, 131.4, 133.0, 153.4, 174.3; *m/z* (ES^+) 431 (MNa^+, 100%), 409 (MH^+, 15%); found 409.1913, $C_{19}H_{29}N_4O_4S$ requires 409.1910 (+0.8 ppm).

3.1.7. *trans*-Octadec-12-enoic acid methyl ester 9. At −55 °C a solution of the sulfone (0.644 g, 1.58 mmol, 1 equiv) in THF (10 mL) was treated with a 0.5 M solution of potassium bis(trimethylsilyl)amide in toluene (3.56 mL, 1.78 mmol, 1.1 equiv). Stirring was continued for 40 min at −55 °C before heptanal (0.29 mL, 2.054 mmol, 1.3 equiv) was added. The mixture was further stirred for 1 h at −55 °C before water (25 mL) and Et_2O (25 mL) were added. The mixture was extracted with Et_2O (3×25 mL) and the combined organic extracts were dried over $MgSO_4$. Filtration and solvent removal under reduced pressure and purification by flash column chromatography (Hex−EtOAc, 19:1) gave the alkene **9** (0.237 g, 50%) as a colourless oil. R_f=0.6 (Hex–EtOAc, 4:1); υ_{max} (neat/cm^{-1}) 2925, 2855, 1744, 1693, 1464, 1436; δ_H (400 MHz, $CDCl_3$) 0.91 (3H, t, *J* 6.5 Hz, CH_3), 1.22–1.39 (20H, m, CH_2), 1.63 (2H, pent, *J* 7.5 Hz, CH_2), 1.93–2.01 (4H, m, CH_2), 2.32 (2H, t, *J* 7.5 Hz, CH_2), 3.69 (3H, s, CH_3), 5.38–5.44 (2H, m, CH); δ_C (100 MHz, $CDCl_3$) 14.1, 22.7, 24.9, 28.8, 29.1, 29.2, 29.4, 29.45, 29.6, 31.8, 32.6, 34.1, 51.5, 130.3, 130.4, 174.4.

3.1.8. *trans*-Octadec-12-enoic acid (*trans*-vaccenic acid 4). A solution of the methyl ester (100 mg, 0.337 mmol, 1 equiv) in THF (3 mL) was treated with a solution of LiOH (24 mg, 1.01 mmol, 3 equiv) in water (3 mL). Stirring was continued for 48 h before Et_2O (20 mL) and 0.5 M HCl (20 mL) were added. The resultant aqueous layer was further extracted with Et_2O (3×20 mL) and the combined organic extracts were dried over $MgSO_4$. Filtration and solvent removal under reduced pressure gave the alkene (89 mg, 94%) as a white solid whose data was identical to that described above.

3.1.9. 9-Bromononanoic acid 11.[17] Chromium trioxide (1.34 g, 13.4 mmol, 1.5 equiv) and water (1.3 mL) was cooled to ca. 0 °C and concd H_2SO_4 (1.0 mL, 17.92 mmol, 2 equiv) was cautiously added followed by water (2.5 mL). After 5 min a solution of 9-bromo-1-nonanol **10** (2.0 g, 8.96 mmol, 1 equiv) in acetone (7 mL) was added dropwise. The reaction mixture was stirred for 2 h at 0 °C before warming to room temperature and stirring for 12 h. Et_2O (50 mL) and H_2O (50 mL) were added and the aqueous layer was further extracted with Et_2O (3×50 mL). The combined organic phases were then washed with a brine solution (100 mL) and the resultant organic phase was dried over $MgSO_4$, filtered and reduced in vacuo. The crude acid was purified by flash column chromatography (DCM; 0.5% AcOH) affording the product **11** (1.71 g, 81%) as a white solid. Mp 35–37 °C; R_f=0.25 (DCM); υ_{max} (Nujol/cm^{-1}) 2928, 2855, 1706, 1464, 1430, 1412, 1243, 1209, 936; δ_H (400 MHz, $CDCl_3$) 1.41 (8H, m, CH_2), 1.66 (2H, m, CH_2), 1.88 (2H, m, CH_2), 2.38 (2H, t, *J* 7.5 Hz, CH_2), 3.43 (2H, t, *J* 7.0 Hz, CH_2); δ_C (100 MHz, $CDCl_3$) 24.6, 28.0, 28.5, 28.9, 29.0, 32.7, 34.0, 180.1.

3.1.10. (8-Carboxyoctyl)triphenylphosphonium bromide 12.[17] 9-Bromononanoic acid **11** (2.00 g, 8.41 mmol, 1 equiv) and triphenylphosphine (2.21 g, 8.41 mmol, 1 equiv) were dissolved in toluene (15 mL). This mixture was heated to reflux for 24 h. On cooling, the upper layer was decanted and the lower layer was dissolved in DCM (3×20 mL) and reduced in vacuo. The resulting brown solid was washed in boiling EtOAc (3×50 mL), and a white solid (2.72 g, 65%) was collected by filtration and dried in vacuo. Mp 82–84 °C; υ_{max} (Nujol/cm^{-1}) 3058, 2930, 2857, 1718, 1644, 1439, 1113; δ_H (400 MHz, $CDCl_3$) 1.20 (6H, m, CH_2), 1.49 (6H, m, CH_2), 2.28 (2H, t, *J* 7.0 Hz, CH_2), 3.56 (2H, m, CH_2), 7.67–7.78 (15H, m, ArH); δ_C (100 MHz, $CDCl_3$) 22.2 (d, *J* 7.0 Hz), 22.4 (d, *J* 50.0 Hz), 24.4, 28.25, 28.3, 29.9 (d, *J* 15.5 Hz), 34.2, 117.9 (d, *J* 85.0 Hz), 130.4 (d, *J* 12.5 Hz), 133.4 (d, *J* 10.0 Hz), 134.9 (d, *J* 3.0 Hz), 176.8; δ_P (162 MHz, $CDCl_3$) 25.65.

3.1.11. *cis*-9-*trans*-11-Octadecdienoic acid (*cis*-9-*trans*-11-CLA) 2. Under nitrogen, a slurry of **12** (2.00 g, 4.00 mmol, 1 equiv) in THF (30 mL) was stirred at −78 °C and potassium bis(trimethylsilyl)amide (20 mL of a 0.5 M solution in toluene, 10.00 mmol, 2.5 equiv) was added dropwise. This mixture was warmed to room temperature over 2 h before re-cooling to −78 °C. *trans*-2-Nonenal (0.86 mL, 5.20 mmol, 1.3 equiv) was added dropwise and the mixture was stirred for 12 h during which room temperature was reached. EtOAc (50 mL) and 1 M HCl (50 mL) were added and the aqueous layer was further extracted with EtOAc (2×50 mL). The combined organic phases were dried over $MgSO_4$ before filtration and solvent evaporation under reduced pressure. The crude product was purified via flash column chromatography (Hex–EtOAc, 5:1) affording the title compound **2** (0.60 g, 54%) as a viscous yellow oil. R_f=0.25 (Hex–EtOAc, 5:1); υ_{max} (Nujol/cm^{-1}) 3019, 2955, 2926, 2855, 1711, 1465, 1465, 1411, 1259, 983; δ_H (400 MHz, $CDCl_3$) 0.90 (3H, t, *J* 7.0 Hz, CH_3), 1.23–1.45 (16H, m, CH_2), 1.65 (2H, pent, *J* 7.5 Hz, CH_2), 2.04–2.20 (4H, m, CH_2), 2.37 (2H, t, *J* 7.5 Hz, CH_2), 5.31 (1H, dt, *J* 7.5, 11.0 Hz, CH-9), 5.68 (1H, dt, *J* 7.0, 14.75 Hz, CH-12), 5.96 (1H, dd, app. t, *J* 11.0 Hz, CH-10), 6.31 (1H, dd, *J* 11.0, 14.75 Hz, CH-11); δ_C (100 MHz, $CDCl_3$) 14.1, 22.6, 24.6, 27.6, 28.9, 29.0, 29.1, 29.3, 29.4, 29.6, 31.7, 32.9, 125.52, 128.7, 129.9, 134.8, 179.8; *m/z* (ES^-) 279 ($M-H^-$, 100%); found 279.2314, $C_{18}H_{31}O_2$ requires 279.2324 (−3.6 ppm).

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UV–vis and fluorescence spectroscopic detection of anions by the conformational restriction of 2,2′-binaphthalene derivatives bearing thiourea groups through a methylene spacer

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Abstract—Novel fluorescence receptors, **2** and **3** based on 2,2′-binaphthalene possessing thiourea moieties via a methylene spacer have been synthesized. Hydrogen bonds of NH groups of thiourea moieties with acetate anion were confirmed by ^{1}H NMR study in MeCN-d_3. These receptors showed characteristic UV–vis spectral changes through isosbestic points on complexation with anions inspite of lacking conjugation between the chromophore and the binding sites in polar organic solvent such as acetonitrile. The UV–vis spectral changes arise from the conformational restriction of the 2,2′-binaphthyl skeleton on the complexation. The receptors exhibit high selectivities for AcO^- and F^-. The fluorescence intensity of the receptors decreases with the increasing amount of the AcO^-, however, addition of F^- induces a different change in its fluorescence spectrum, in which shorter emission of the receptors decreases with the increase in F^- concentration, while the longer emission of the receptors increases through an isoemissive point in MeCN. The results suggest that favorable dual-wavelength ratiometric fluorescence measurement can be conducted by the receptors for F^-.

1. Introduction

The chromo- and fluoroionophores for anion have been widely studied due to practical applications in environmental and biological utilities.[1] A new approach to signaling process of a fluoroionophore based on so-called 'molecular rigidification'[2] or 'conformational restriction'[3] for recognitions of sugars[2] and metal ions[3,4] has recently been developed. However, the anion receptor based on these ideas has been scarcely reported.[5] In many cases, conformational restriction of receptors cannot change UV–vis spectra of the receptors through complexation with guest molecules indicating that the electronic perturbation of the receptor molecules in ground state is not less effective on complexation. As a novel fluorophore, we have designed 2,2′-binaphthalene group[4,6] and the skeleton is expected to be advantageous for the construction of artificial receptors: (1) introduced binding sites at 8- and 8′-positions to 2,2′-binaphthalene form a convergent binding site upon complexation with target species; (2) two naphthyl groups showing fluorescence character and fluorescence intensity would change upon complexation; (3) cooperative complexation of two binding sites at 8- and 8′-positions restricts the motion of the rotation of two naphthyl groups connected with a single bond. As a result, changing overlap of larger π-surfaces of two naphthyl groups would induce a perturbation of UV–vis absorbance. Thiourea group has frequently been used as an anion recognition site to construct anion receptors.[7] We have developed a chromo- and fluoroionophore **1** based on 2,2′-binaphthalene skeleton bearing thiourea groups. Indeed, the receptor **1** shows remarkable UV–vis and fluorescence spectral changes upon the addition of anions such as AcO^- and F^- in MeCN.[6a] However, a contribution of an electronic effect of the conjugated thiourea groups with 2,2′-binaphthalene moiety plays an important role for the spectral changes on the complexations. We have recently reported that 2,2′-binaphthalene bearing aza-15-crown-5 ethers at 8- and 8′-positions via a methylene spacer forms an intramolecular sandwich complex with barium cation, which can be easily detectable by UV–vis spectroscopic changes.[4] As an extension of our previous works, we designed a novel chromo- and fluoroionophore based on 2,2′-binaphthalene bearing thiourea groups through a methylene spacer (receptors **2** and **3**) to break the electronic conjugation between the thiourea and the naphthyl moieties (Scheme 1). These receptors showed UV–vis and fluorescence spectral changes on complexation with anions inspite of lacking conjugation between the chromophore and the binding sites in polar organic solvent such as acetonitrile.

Keywords: Anion recognition; Fluorescence; UV–vis spectroscopy; 2,2′-Binaphthalene; Thiourea.
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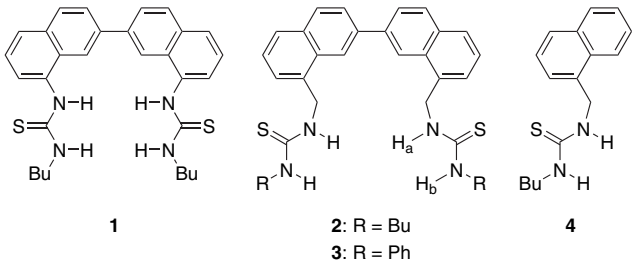
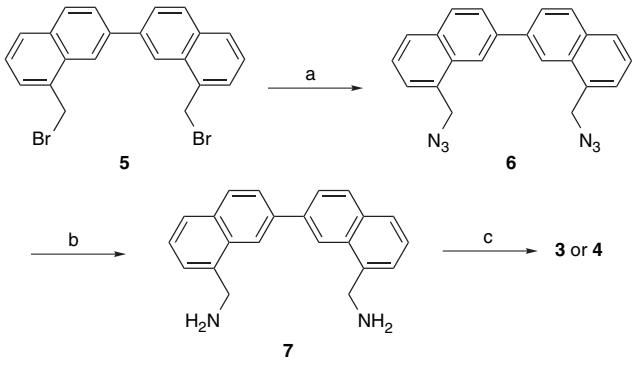


Scheme 1.

2. Results and discussion

Preparation of receptors **2** and **3** is depicted in Scheme 2. A synthetic intermediate, 8,8′-bis(bromomethyl)-2,2′-binaphthalene was prepared from bromobenzene in six steps as reported previously.[6b] Reaction of **5** with sodium azide in DMF gave diazide **6** in 80% yield. Hydrogenation of **6** catalyzed by 10% Pd/C in ethanol yielded diamine **7** quantitatively, which was immediately subjected to the reaction with the corresponding isothiocyanates in EtOH to afford the bisthiourea derivatives **2** and **3** in 36 and 71% yield, respectively. The products were characterized by ^{1}H NMR, electrospray ionization-mass spectroscopy (ESI-MS), and elemental analyses.



Scheme 2. Reagents and conditions: (a) NaN_3, DMF, 80 °C, 80%; (b) H_2, Pd/C, EtOH, quant.; (c) BuNCS, EtOH, 36%; PhNCS, EtOH, 71%.

Self-association of receptor **3** was evaluated by dilution experiments by ^{1}H NMR in MeCN-d_3 and was found to be negligible at least in the concentration range 6.25×10^{-4}–2.50×10^{-3} mol dm^{-3} from no chemical shift changes of any proton signals. Figure 1 shows the ^{1}H NMR spectrum of the receptor **3** in the absence and in the presence of tetrabutylammonium acetate and fluoride in MeCN-d_3, respectively. Significant large downfield shifts of both thiourea NH protons of **3** were observed upon the addition of AcO^- ($\Delta\delta$=2.99 ppm for H_a and 2.59 ppm for H_b) and F^- ($\Delta\delta$=3.62 ppm for H_a and 2.83 ppm for H_b). The result indicates strong hydrogen bonding formation between both thiourea NH's of **3** and guest anions and the equilibrium of the complexation is reached fast over the NMR timescale. ESI-MS (negative ion mode) of **2** and **3** in the presence of 1 equiv of AcO^-, $H_2PO_4^-$, and Cl^- (as tetrabutylammonium salts) showed peaks corresponding to 1:1 complex in good agreement with the isotope patterns. For instance, after the addition of AcO^- to the solution of **3**, ion peaks at *m/z* 580.9 and 641.2 were observed and these correspond to **3** (581.19 calcd for $[\mathbf{3}-H^+]^-$) and $\mathbf{3}\cdot AcO^-$ (641.21 calcd for $[\mathbf{3}+AcO^-]^-$), respectively.

The anion binding abilities of **2** and **3** were evaluated by UV–vis spectroscopic titration with anions such as AcO^-, $H_2PO_4^-$, HSO_4^-, NO_3^-, F^-, Cl^-, Br^-, and I^- (as their tetrabutylammonium salts) in MeCN. The absorbance of **3** at around 314 nm clearly decreased with increasing amount of F^- through isosbestic points at 301 and 336 nm indicating 1:1 complexation as shown in Figure 2b. A similar spectral change was observed upon the addition of AcO^- and a slightly smaller change was observed upon the addition of Cl^- (Fig. 3). In the case of $H_2PO_4^-$, spectra were altered without clear isosbestic points implying formation of

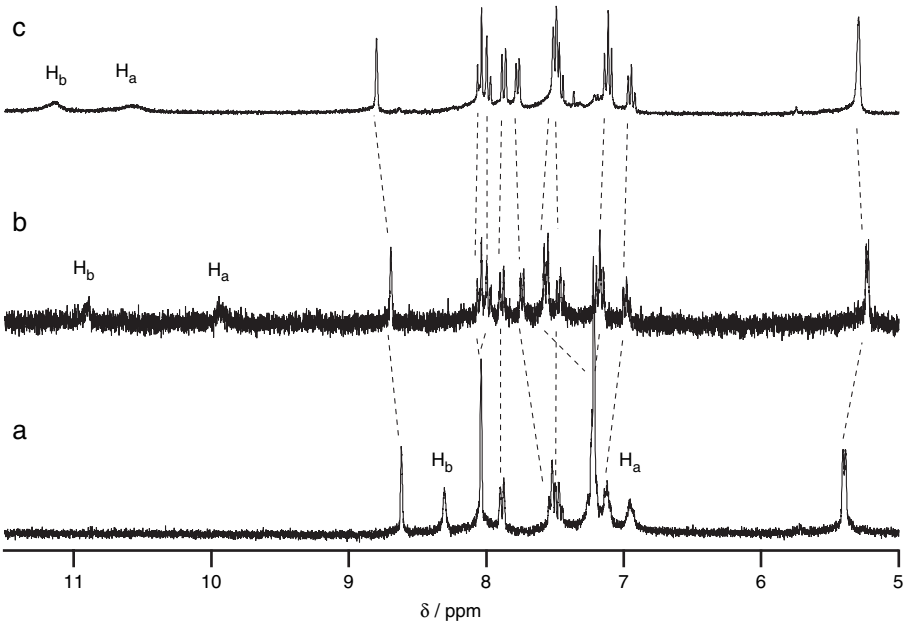


Figure 1. Partial ^{1}H NMR spectra of **3** in the absence (a) and presence of 1 equiv of tetrabutylammonium acetate (b) and tetrabutylammonium fluoride (c) in MeCN-d_3 at 298 K. [**3**]=2.5×10^{-3} mol dm^{-3}.

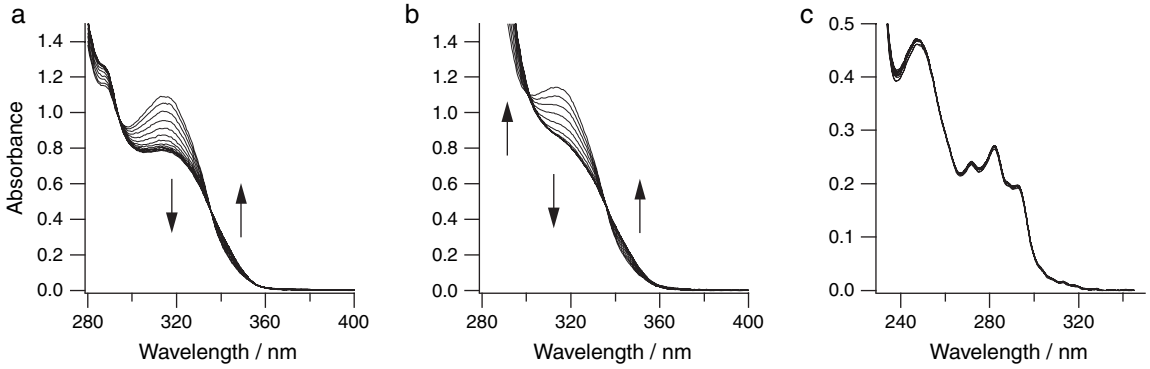


Figure 2. UV–vis spectral changes of **2** (a), **3** (b), and **4** (c) upon the addition of F^- in MeCN at 298 K. [receptor]=6.67×10^{-5} mol dm^{-3}.

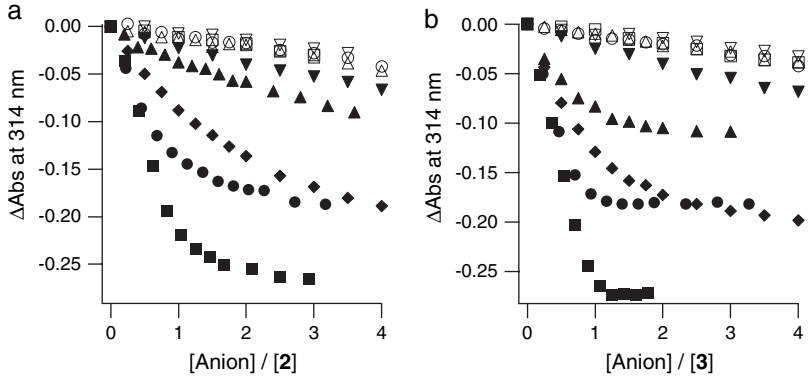
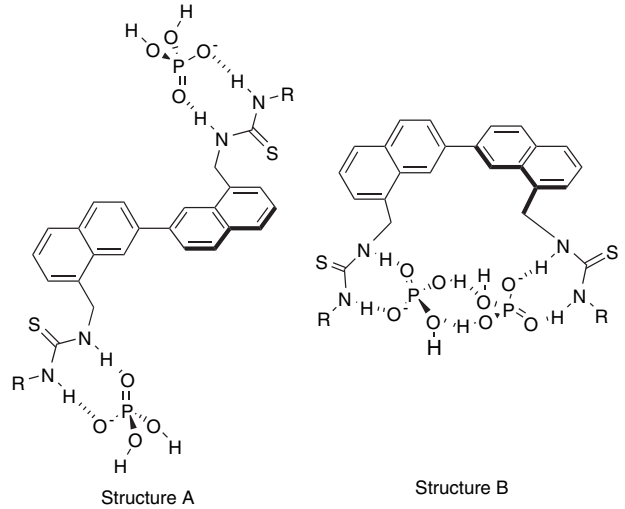


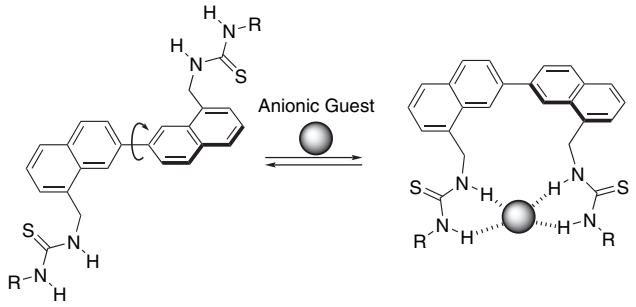
Figure 3. UV–vis spectral changes at 314 nm of **2** (a) and **3** (b) upon the addition of anions in MeCN at 298 K. [**3**]=6.67×10^{-5} mol dm^{-3}, AcO^- (●), $H_2PO_4^-$ (▲), $(EtO)_2PO_2^-$ (◆), HSO_4^- (△), NO_3^- (○), F^- (■), Cl^- (▼), Br^- (□), and I^- (▽).

a higher order complexation such as **3**/$H_2PO_4^-$=1:2. Two plausible structures of **3**/$H_2PO_4^-$=1:2 complex can be considered as shown in Scheme 3, i.e., two thiourea groups independently associate two $H_2PO_4^-$ (structure A) and two thiourea groups cooperatively associate $H_2PO_4^-$ dimer in which OH groups of each $H_2PO_4^-$ form intermolecular hydrogen bondings (structure B). To distinguish these two possible structures, UV–vis titrations of **2** and **3** with a phosphodiester and diethylphosphate was studied. The absorbance of **3** at around 314 nm decreased upon the addition of $(EtO)_2PO_2^-$ through isosbestic points at 301 and 341 nm as similar to the titration with F^-. This result indicates that the complex structure is consistent with structure B. Similar 1:2 complexes with $H_2PO_4^-$, in which two $H_2PO_4^-$ form a dimer by intermolecular hydrogen bonds, can be found in the literature.[8] However, the addition of Br^-, I^-, HSO_4^-, and NO_3^- virtually showed no spectral changes. The receptor **2** showed similar spectral changes of **3** through isosbestic points at 294 and 335 nm as shown in Figure 2a. Although the thiourea group of receptor **3** conjugates with phenyl group, the similar spectral changes of **2** strongly suggest that decrease at around 314 nm of **3** upon the addition of F^- cannot arise only from the electronic perturbation of thiourea groups on complexation. Gunnlaugsson and co-workers reported that 9,10-bisthioureidomethylanthracenes exhibit little UV–vis spectral changes upon the addition of anions in DMSO.[9] Hong and co-workers reported that 2,2′-bis(aminomethyl)biphenyl showed only small changes in the UV–vis spectrum during the titration with F^- in $CHCl_3$.[5] Titrations of monourea receptor **4** exhibited only minor changes upon the addition of anionic guests as shown in Figure 2c. These results clearly demonstrate that UV–vis spectral changes of **2** and **3** upon the addition of anionic species are characteristic and arise from the conformational restriction of the 2,2′-binaphthyl skeleton on the complexation (Scheme 4). There are two stable conformers of 2,2′-binaphthalene in solution and in the solid state. The dihedral angles of two naphthyl rings were reported to be 32–41° (*trans*-inclined conformer) and 136–148° (*cis*-inclined conformer)



Scheme 3.

in the ground state, respectively.[10] The UV–vis spectra of **2** and **3** in the absence of guest anions are thermodynamic average of these two predominant conformers. The addition of guest anions induced bathochromic shift around 314 nm suggesting that more planer (smaller dihedral angle of two naphthyl rings) *cis*-conformation of 2,2′-binaphthyl moiety would be formed by the cooperative complexation with two thiourea groups.



Scheme 4.

The stoichiometries of the complex between receptors and guest anions (F^-, AcO^-, and $H_2PO_4^-$) were established by Job's plots as shown in Figure 4. The minima at a mole fraction of 0.5 indicate 1:1 receptor–guest bindings for F^- and AcO^-. As shown in Figure 4, the minimum for complexation of **2** with $H_2PO_4^-$ at about 0.4 also suggests 1:2 complex formation. The association constants of **2** and **3** for anionic species except for $H_2PO_4^-$, which forms 1:2 complex with **2** and **3**, were calculated by nonlinear curve fitting of UV–vis titrations and the results are summarized in Table 1. The order of the association constants for anions was $F^->AcO^->(EtO)_2PO_2^->Cl^-$, which can be rationalized on the basis of the guest basicity in polar aprotic organic solvent such as DMSO.[11] The association constants of **3** are larger than those of **2** due to higher acidity of urea NH's of **3** which are in conjugation with the phenyl groups. The association constants of **4** could not be calculated due to small perturbation of UV–vis spectra as shown in Figure 2c.

The quantum yields of 8,8′-dimethyl-2,2′-binaphthalene, **2**, and **3** in MeCN were determined to be 0.36, 0.019, and 0.0041, respectively, suggesting that thiourea groups show significant quenching effect, which may be due to photoinduced electron transfer (PET) on the excited state.[9,12] Fluorescence titrations of **3** with anions excited at 301 nm, which is one of the isosbestic points during UV–vis titrations, were performed (Fig. 5). The fluorescence intensity of **3** decreases with the increase amount of the AcO^- and $H_2PO_4^-$ because of increasing the frequency of fluorescence quenching via PET by the complexation with anions. Interestingly, addition of F^- induces a different change in its fluorescence spectrum, in which shorter emission of **3** decreases with the increase in F^- concentration, while the longer emission of **3** increases through an isoemissive point at 492 nm. The fluorescence behavior of **2** also showed similar spectral changes of **3**. The results suggest that favorable dual-wavelength ratiometric fluorescence measurement[13] can be conducted by the receptors **2** and **3** for F^-. The fluorescence spectral change of **2** upon the addition of $(EtO)_2PO_2^-$ was similar to that of **2** upon the addition of AcO^-. Interestingly, the fluorescence change of **3** with $(EtO)_2PO_2^-$ was in same manner as observed in **3** with F^-, i.e., a ratiometric change through isoemissive point at around 470 nm. These results indicate the differences of these fluorescence changes arising from structural changes of 2,2′-binaphthyl backbone of the receptors rather than the character of anions. As shown in Figure 1, chemical shift changes of naphthyl CH's were in the same directions and almost the similar shifts upon the addition of AcO^- and F^- by ^1H NMR spectroscopy indicate that the differences of complex structures with anions

Table 1. The association constants for **2** and **3** with anions in MeCN determined by UV–vis spectroscopy

Anion	K_{11}/dm^3 mol^{-1}[a]	
	2	**3**
AcO^-	$2.21\pm0.34\times10^5$	$1.17\pm0.29\times10^6$
$H_2PO_4^-$	—[b]	—[b]
$(EtO)_2PO_2^-$	$1.34\pm0.01\times10^4$	$6.12\pm0.02\times10^4$
HSO_4^-	ND[c]	ND[c]
NO_3^-	ND[c]	ND[c]
F^-	$3.81\pm0.05\times10^5$	$1.42\pm0.18\times10^6$
Cl^-	$3.60\pm0.41\times10^3$	$2.31\pm0.26\times10^3$
Br^-	ND[c]	ND[c]
I^-	ND[c]	ND[c]

[a] [**2**]=[**3**]=6.67×10^{-5} mol dm^{-3}. Determined by UV–vis spectroscopy at 298 K.
[b] The data does not fit satisfactorily to a 1:1 binding model.
[c] The association constants could not be determined due to small spectral changes.

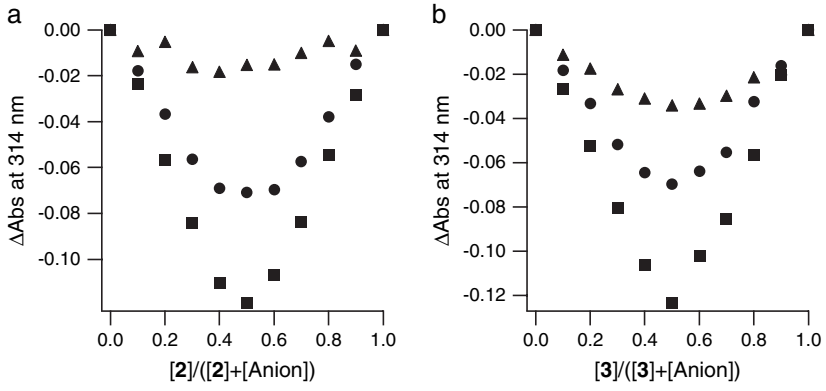


Figure 4. Job's plots for complexation of **2** (a) and **3** (b) with AcO^- (●), $H_2PO_4^-$ (▲), and F^- (■) determined by UV–vis spectroscopy in MeCN. [**3**]+[anion]=6.67×10^{-5} mol dm^{-3} at 298 K.

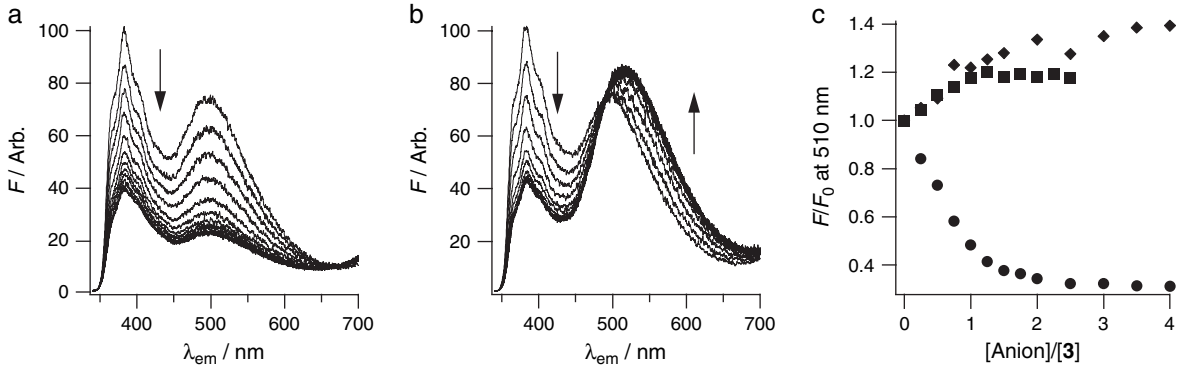


Figure 5. Fluorescence spectral changes of **3** (λ_{ex}=301 nm) upon the addition of AcO^- (a) and F^- (b) in MeCN at 298 K and fluorescence changes at 510 nm; (c) upon the addition of AcO^- (●), $(EtO)_2PO_2^-$ (◆), and F^- (■). [**3**]=3.33×10^{-5} mol dm^{-3}.

are small. The enhancement of the emission at the longer wavelength induced by the addition of F^- may be explained by more planar structure of 2,2′-binaphthyl moiety on complexation with AcO^-. The association constants of **2** and **3** for AcO^-, $(EtO)_2PO_2^-$, and F^- calculated from fluorescence titrations are listed in Table 2. The same trends were observed in the association constants derived from UV–vis titrations, however, somewhat different values were calculated because of weak fluorescence intensity (low-quantum yield), of the receptors during titrations.

Fluorescence titration of **4** with AcO^- excited at 260 nm was also conducted in MeCN. The fluorescence emission of **4** was quenched upon the addition of AcO^- and the association constant was calculated to be 1.5×10^3 dm^3 mol^{-1}. The association constant of **2** for AcO^- is almost 40 times higher than that of **4**. This observation is consistent with the cooperative binding by the two thiourea groups of **2**.

Table 2. The association constants for **2** and **3** with anions in MeCN determined by fluorescence spectroscopy

Anion	K_{11}/dm^3 mol^{-1}[a]	
	2[b]	**3**[c]
AcO^-	$6.36\pm1.87\times10^4$	$2.29\pm0.15\times10^5$
$(EtO)_2PO_2^-$	$2.20\pm0.10\times10^4$	$7.20\pm0.15\times10^4$
F^-	$1.03\pm0.27\times10^6$	$4.67\pm0.06\times10^5$

[a] [**2**]=[**3**]=3.33×10^{-5} mol dm^{-3}.
[b] λ_{ex}=294 nm.
[c] λ_{ex}=301 nm.

3. Conclusion

In conclusion, we have demonstrated preparation of 2,2′-binaphthalene derivatives bearing two thiourea groups through a methylene spacer and formation of 1:1 complex with various anions. These receptors show characteristic UV–vis and fluorescence spectral changes upon the addition of anions without conjugation between the chromophore and the binding sites. The receptors **2** and **3** exhibit high selectivities for AcO^- and F^-. As far as we know, this is the first example of the receptor that affects UV–vis spectra on anion recognition events based on the concept, 'conformational restriction' without conjugation between binding sites and chromophore.

4. Experimental

4.1. General

Most of the solvents and all reagents were obtained from commercial suppliers and used without further purification. DMF was dried over calcium hydride and distilled under reduced pressure. Dry acetonitrile was purchased from Kanto Kagaku Co., Ltd. Proton NMR spectra were recorded on JEOL AL-300 NMR spectrometer. Melting points were determined on a Yanagimoto Micro Melting Point Apparatus and are uncorrected. Electrospray ionization-mass spectra (ESI-MS) were recorded on an Applied Biosystems/MDS-Sciex API-100 spectrometer. UV–vis spectra were recorded on Shimadzu UV-2200A and UV-2500PC spectrometers with thermal regulator (±0.5 °C). Fluorescence spectra were recorded on a Hitachi F-4500 spectrofluorimeter. Column chromatography was performed by using Wakogel C-200 (silica gel, 70–250 mm, Wako Chemical Co., Ltd). Fluorescence quantum yields were measured with quinine sulfate in 0.5 mol dm^{-3} H_2SO_4 as a standard. Elemental analyses were performed at the Center of Instrumental Analysis of Gunma University.

4.2. Synthesis

4.2.1. 8,8′-Bis(azidomethyl)-2,2′-binaphthalene (6). To a solution of 8,8′-bis(bromomethyl)-2,2′-binaphthalene[6b] (250 mg, 0.568 mmol) in DMF (25 ml), was added sodium azide (74 mg, 1.14 mmol) and the mixture was stirred at 80 °C for 12 h under nitrogen atmosphere. The mixture was extracted with AcOEt/water and the organic layer was washed with water (100 ml×2) and brine. The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was chromatographed on silica gel ($CHCl_3$/hexane=1:1 as eluent) to give the product as white solids. Yield 167 mg, 80%. Mp 101–102 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 (s, 2H), 8.03 (d, 2H, J=8.6 Hz), 7.93 (dd, 2H, J_1=8.6, J_2=1.6 Hz), 7.90 (d, 2H, J=7.2 Hz), 7.52 (d, 2H, J=7.2 Hz), 7.48 (t, 2H, J=7.2 Hz), 4.85 (s, 4H).

4.2.2. 8,8′-Bis(aminomethyl)-2,2′-binaphthalene (7). A solution of 8,8′-bis(azidomethyl)-2,2′-binaphthalene (141 mg) in ethanol (30 ml) was hydrogenated at rt and atmospheric pressure in the presence of 10% Pd/C (10 mg) overnight.

The solution was filtered and the solvent was evaporated under reduced pressure to give 116 mg (96%) of 8,8′-bis(aminomethyl)-2,2′-binaphthalene as a viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (s, 2H), 8.00 (d, 2H, J=8.4 Hz), 7.89 (dd, 2H, J_1=8.4, J_2=1.7 Hz), 7.82 (d, 2H, J=7.9 Hz), 7.54 (d, 2H, J=6.2 Hz), 7.47 (dd, 2H, J_1=7.9, J_2=6.2 Hz), 4.43 (s, 4H), 1.71 (br s, 4H).

4.2.3. 8,8′-Bis(3-butylthioureidomethyl)-2,2′-binaphthalene (2). To a solution of 8,8′-bis(aminomethyl)-2,2′-binaphthalene (294 mg, 0.94 mmol) in 40 ml of ethanol, butyl isothiocyanate (217 mg, 1.88 mmol) was added at 0 °C. The mixture was stirred at rt for 40 h under nitrogen atmosphere in the dark. The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel (0.5% MeOH/$CHCl_3$). Recrystallization from toluene gave **2** as white solids. Yield 183 mg, 36%. Mp 185.9–188.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52 (s, 2H), 7.93 (d, 2H, J=8.6 Hz), 7.87 (d, 2H, J=8.6 Hz), 7.81 (d, 2H, J=8.3 Hz), 7.50 (d, 2H, J=6.6 Hz), 7.34 (dd, J_1=8.3, J_2=6.6 Hz), 6.37 (br s, 4H), 5.16 (br s, 4H), 3.26 (br s, 4H), 1.34 (br s, 4H), 1.13 (br s, 4H), 0.74 (t, 6H, J=7.2 Hz). Anal. Calcd for $C_{32}H_{38}N_4S_2$: C, 70.81; H, 7.06; N, 10.32. Found C, 71.04; H, 6.97; H, 10.32. ESI-MS (negative ion mode) calcd for $[C_{32}H_{38}N_4S_2-H]^-$: m/z 541.25; found: 541.2.

4.2.4. 8,8′-Bis(3-phenylthioureidomethyl)-2,2′-binaphthalene (3). To a solution of 8,8′-bis(aminomethyl)-2,2′-binaphthalene (447 mg, 1.43 mmol) in 40 ml of ethanol, phenyl isothiocyanate (387 mg, 2.86 mmol) was added at 0 °C. The mixture was stirred at rt for 1 d under nitrogen atmosphere in the dark. The precipitates were collected and washed with small amount of ethanol to give **3** as white solids. Yield 592 mg, 71%. Mp 194–197 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (s, 2H), 8.24 (br s, 2H), 8.01 (d, 2H, J=8.6 Hz), 7.96 (d, 2H, J=8.6 Hz), 7.85 (d, 2H, J=7.7 Hz), 7.49 (d, 2H, J=6.4 Hz), 7.42 (dd, 2H, J_1=6.4, J_2=7.7 Hz), 7.09–7.24 (m, 10H), 6.40 (br s, 2H), 5.52 (d, 4H, J=5.3 Hz). Anal. Calcd for $C_{36}H_{30}N_4S_2$: C, 74.19; H, 5.19; N, 9.61. Found C, 74.07; H, 5.43; H, 9.32. ESI-MS (negative ion mode) calcd for $[C_{36}H_{30}N_4S_2-H]^-$: m/z 581.18; found: 580.9.

4.2.5. 1-(3-Butylthioureidomethyl)naphthalene (4). To a solution of 1-aminomethylnaphthalene (2.04 g) in ethanol (60 ml), a solution of butyl isothiocyanate (1.50 g, 1.0 equiv) in ethanol (20 ml) was dropwise at 0 °C. The mixture was stirred at 0 °C to rt for 2 d under nitrogen atmosphere. The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel (1% MeOH/$CHCl_3$) to give 2.39 g (68%) of the product as colorless powder. Mp 105.0–105.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (d, 1H, J=7.7 Hz), 7.89 (dd, 1H, J_1=7.3, J_2=2.3 Hz), 7.85 (d, 1H, J=7.3 Hz), 7.46–7.60 (m, 4H), 5.84 (br s, 2H), 5.11 (d, 2H, J=4.0 Hz), 3.26 (br s, 2H), 1.51 (quint, 2H, J=7.4 Hz), 1.30 (six, 2H, J=7.4 Hz), 0.87 (t, 3H, J=7.4 Hz). Anal. Calcd for $C_{16}H_{20}N_2S$: C, 70.55; H, 7.40; N, 10.28. Found C, 70.55; H, 7.37; H, 10.41.

4.3. Spectral titration

In a typical experiment, a solution of receptor (6.67×10^{-5} mol dm^{-3} for UV–vis, 3.33×10^{-5} mol dm^{-3} for fluorescence spectra, respectively) in dry acetonitrile was titrated by increasing the amounts of tetrabutylammonium salt solutions of the anion of interest (5.0×10^{-3} mol dm^{-3}) at 298 K. After each addition of aliquots, the UV–vis and fluorescence spectra of the solution were recorded. The association constants were calculated from the titration data by a self-written nonlinear least-square-fitting program.

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The synthesis of one enantiomer of the α-methyl-*trans*-cyclopropane unit of mycolic acids

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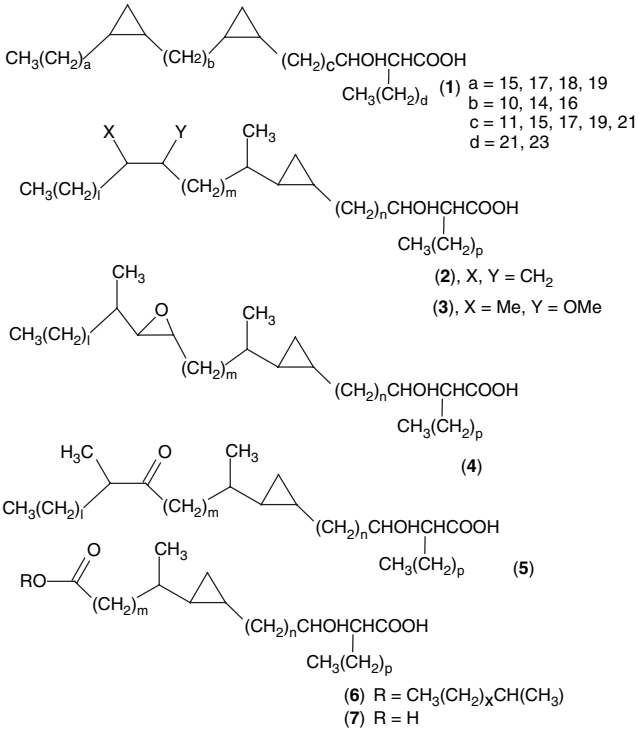
Abstract—We report the synthesis of a single enantiomer of an α-methyl-*trans*-cyclopropane unit present in a number of mycolic acids and its incorporation into a reported 1,2-dialkylcyclopropane meromycolate that contains one *cis*-1,2-dialkylcyclopropane and one α-methyl-*trans*-1,2-dialkylcyclopropane.

1. Introduction

Mycobacterial cell walls show unusually low permeability, a factor, which contributes to their resistance to therapeutic agents. This is believed to be due to an exceptionally thick mono-layer formed by the packing of C_{60}–C_{90} fatty acids (esters).[1] These 'mycolic acids', exemplified by structures such as **1**–**5**, show a variety of structural features including just *cis*-cyclopropanes **1** and α-methyl-*trans*-cyclopropanes **2** (α-mycolic acids),[2] various combinations of either above type of cyclopropane with α-methyl-β-methoxy groups (methoxymycolates), e.g., (**3**) and α-methyl-β-keto-groups (ketomycolates), e.g., (**5**), as well as molecules containing *cis*-alkene, α-methyl-*trans*-alkene[3–10] and α-methyl-*trans*-epoxy groups, e.g., **4** (l, m, n, p are all long alkyl chains).[7,11a–c] In each case there is a common β-hydroxyacid functionality, while the acids are generally present as mixtures of various chain lengths.[8,9] The balance of these structures, which is dependant on the mycobacterial species, changes membrane permeability and fluidity and hence resistance to a therapeutic agent.[11d] Although the hydroxyacid grouping is known to be of *R*,*R*-configuration for a number of bacteria,[12] little is known about the absolute stereochemistries of the other groups. There is some evidence that the 1-methyl-2-methoxy unit at the distal position from the hydroxyacid in mycolic acids **3** is *S*,*S* based on the additivity of optical rotations,[12c,7b] while other reports identify an *R*-stereochemistry for the three stereo-centres of the α-methyl-*trans*-epoxy unit in **4**.[13,7]

Mycolic acids containing *trans*-cyclopropanes at the position in the chain closest to the hydroxyacid have a particular effect on the cell wall and therefore on the sensitivity of mycobacterial species to hydrophobic antibiotics.[5] Although the ratio of *trans*- to *cis*-cyclopropanes in ketomycolic acids can in some cases exceed 6, and ratios of 0.2–0.6 are common in methoxymycolic acids, the proportion of *trans*-mycolates **2** to cis-isomers **1** in α-mycolic acids is generally either zero or below 10%.[9] In addition to those structures



Scheme 1.

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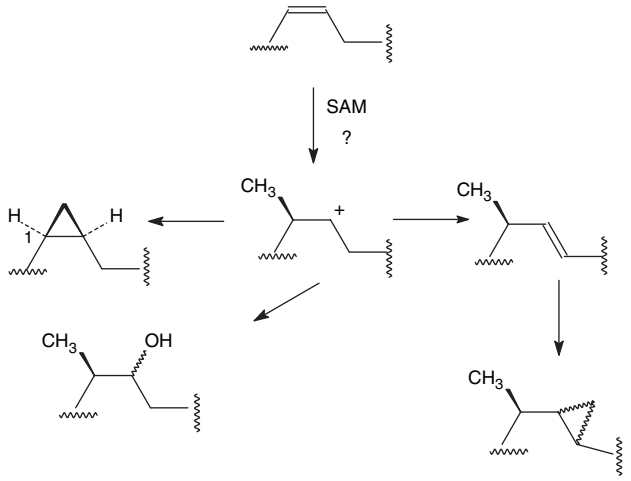
described above, two other types of α-methyl-*trans*-cyclopropane containing mycolic acid derivative, the wax esters **6**[10] and diacids **7** have been reported, while *Mycobacterium gordonae* contains derivatives of the dicarboxylate **7** (Scheme 1).[15]

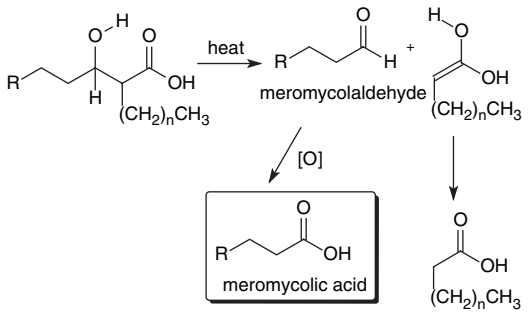
Much is now known about the enzymes that control the biosynthesis of mycolic acids,[16] and a number of proposals have been made to the relationship between routes to the different types, e.g., that the *cis*-cyclopropane unit, the α-methyl-*trans*-cyclopropane and the α-methyl-β-alkoxy unit are formed from a *Z*-alkene through a common cation (Scheme 2).[17] A consequence of this would be that the three sub-units should have a common absolute stereochemistry at the carbon bearing the methyl-group and C-1 of the *cis*-cyclopropane, e.g., Scheme 2.



Scheme 2.

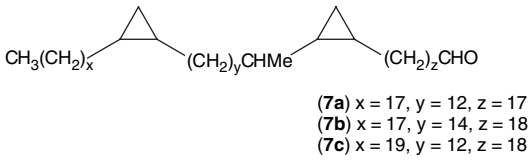
However, labelling studies show that the methyl branches in the α-methyl-*trans*-cyclopropane of mycolic acids from *Mycobacterium tuberculosis* are derived from the 2-position of acetate units, whereas those from *Mycobacterium smegmatis* are derived from C-1.[18] The *cma*A2 gene in virulent *M. tuberculosis* has been shown to be required for the synthesis of *trans*-cyclopropanes in both keto- and methoxymycolates.[19]

One of the standard methods for the characterisation of mycolic acids is thermolysis to fragment the hydroxyacid functionality and to give an aldehyde, or 'meromycolaldehyde' (Scheme 3).[1,2] This can be oxidised to the corresponding 'meromycolic acid'.



Scheme 3.

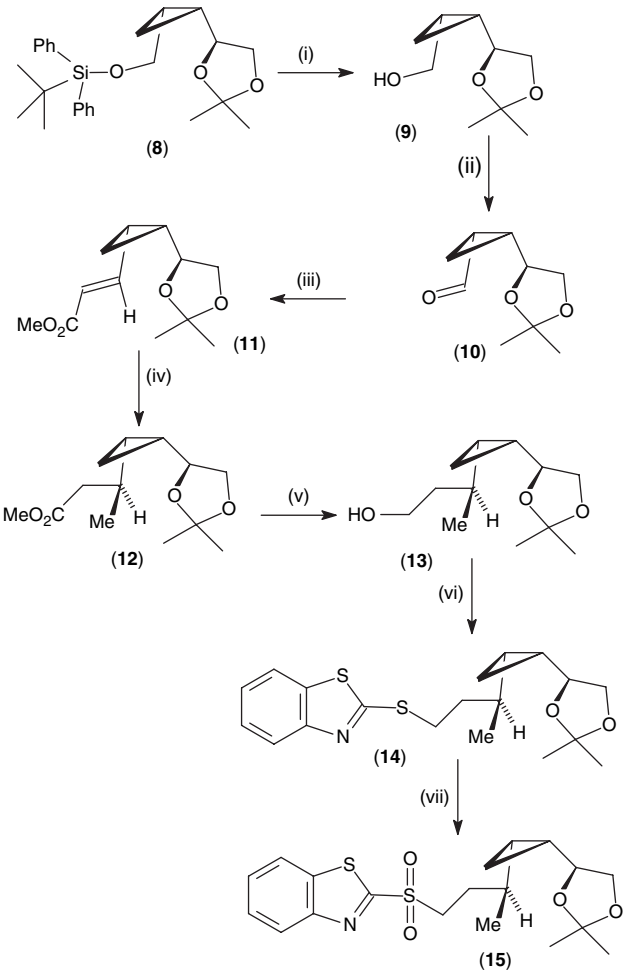
A pioneering paper reported the isolation of a meromycolaldehyde for which structure **7a** (Scheme 4) was proposed as the main component from the thermolysis of the mycolic acids from *Mycobacterium avium*, and proposed that the parent mycolic acid is (**2**, *a*=*c*=17, *b*=12, *d*=21), although it was noted that such a structure would have an odd number of carbons in the main chain.[2a] The assignment was based on MS data and on the presence of a methyl doublet in the NMR spectrum at δ 0.99. Both cyclopropanes were assigned cis-configurations in this paper. In all later examples, the cyclopropane α to the methyl-group is trans, in accord with the postulated biosynthetic pathways (Scheme 2).[1,17] For example, a similar assignment of a doublet at δ 0.99 to a methyl adjacent to a cyclopropane was made in ketomycolates, but now the cyclopropane was assigned as trans.[2b,9] The *M. avium* meromycolyl alcohol had a significantly larger $[\alpha]_D$ than that from *M. tuberculosis* (−1.33 compared to −0.13). Recent detailed studies,[8,9] have shown that the α-mycolic acids from *M. avium* have a principal di-*cis*-cyclopropane component (**1**, *a*=*c*=17, *b*=14, *d*=21) (together with two minor homologues), accompanied by a minor acid with an α-methyl-*trans*-cyclopropane **7b** and a related α-methyl-*trans*-alkene component. These findings may explain the original observation of a methyl branch signal in the low resolution NMR spectrum recorded in the earlier study.[2] These unusual α-mycolic acids have only been identified in members of the *M. avium* complex (**7b**) and *Mycobacterium kansasii* (**7b**,**c**).[8,9]



Scheme 4.

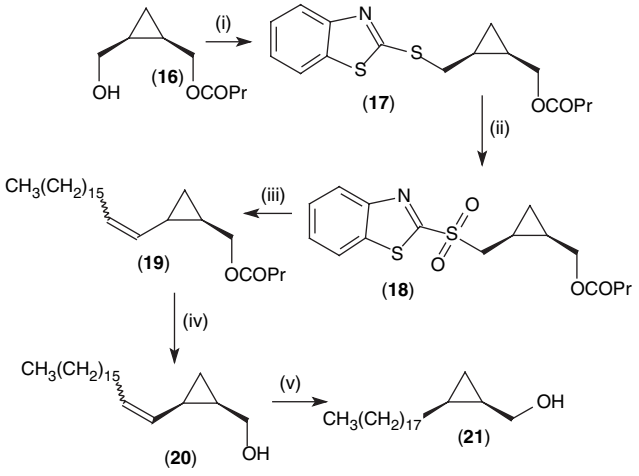
In no case has the absolute stereochemistry of the *trans*-cyclopropane or the relative stereochemistry of this and the adjacent methyl branch been determined. In recent studies, we have reported syntheses of single enantiomers of α-mycolates containing two *cis*-cyclopropanes,[14a,b] and of the corresponding meromycolates.[14c] We now report the preparation of a single α-methyl-*trans*-cyclopropane-containing building block that can be used in the synthesis of a range of mycolic acids and meromycolates containing this subunit; the method can be readily adapted to produce a number of absolute stereochemistries and any appropriate chain length. We also report its application in the preparation of a single enantiomer of **7a**. In separate papers we will report the synthesis of single enantiomers of compounds of type **2**, **3**, **5**, **6** and **7**.

Key to the route presented is the introduction of the α-methyl-cyclopropane fragment. This was achieved (Scheme 5) using the known acetal **8**[20] which was desilylated, oxidised to the corresponding aldehyde **10** and treated with methoxycarbonylmethylene tri-phenylphosphorane in toluene to give mainly **11** (Scheme 6). When toluene was replaced by methanol, a 2:1 mixture of *E*- and *Z*-isomers was obtained. Reaction of the *E*-isomer **11** with methyl magnesium bromide and copper bromide led to a single alkylated product **12**. A trace of a minor compound, probably the isomer with the opposite methyl-group stereochemistry, was also observed (1:15). Moreover, when the mixture of the *E*-alkene **11** with the *Z*-isomer was used, the same product was obtained.



(i) Bu_4NF, THF (85 %); (ii) PCC, CH_2Cl_2 (85 %); (iii) $Ph_3PCH{=}CHCO_2Me$, toluene, (81 %); (iv) MeMgBr, THF (72 %); (v) $LiAlH_4$, THF (88 %); (vi) 2-mercaptobenzthiazole, PPh_3, DEAD, thf (87 %); (vii) ammonium molybdate(VI) tetrahydrate, H_2O_2, methylated spirits (67%)

Scheme 5.



(i) Ph_3P, DEAD, THF, 2-mercaptobenzthiazole (73 %); (ii) $Mo_7O_{24}(NH_4)_6.4H_2O$, H_2O_2, IMS (92 %); (iii) heptadecanal, NaBSA, THF, -20 °C (68 %); (iv) $LiAlH_4$, THF (84 %); (v) H_2NNH_2, $CuSO_4$, $NaIO_4$, CH_3COOH, iPrOH (81 %)

Scheme 6.

Reduction of **12** with lithium aluminium hydride led the corresponding alcohol **13**; the absolute configuration of this was confirmed by X-ray crystallography of the corresponding 3,5-dinitrobenzoate, based on the known configuration of **8** (Fig. 1). The alcohol was converted into sulfide **14** which was oxidised to sulfone **15**; some deprotection of the acetal group occurred during this process, but the diol formed (20%) could be reprotected to give **15** in 95% yield.

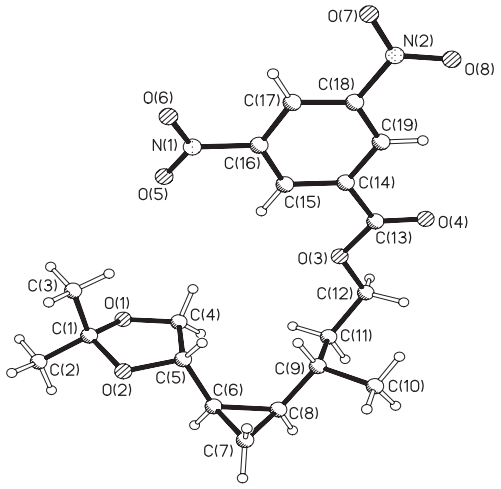


Figure 1. Molecular structure of the 3,5-dinitrobenzoate of **13**.

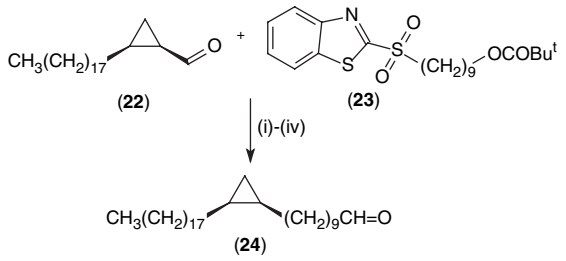
The next stage in the route to **7a** required the alcohol **21**. This was prepared from alcohol **16** (ee>95%, obtained by a modification of a route used by Grandjean),[21,22] which was converted into the sulfone **18**† and then coupled with heptadecanal (prepared by coupling heptylmagnesium bromide with 10-bromo-decanol in the presence of $LiCuCl_4$ to give heptadecanol, followed by PCC oxidation), in a Julia type reaction.[23a] Hydrolysis of the ester **19**, and saturation of the alkene using diimide gave alcohol **21**.[24]

Oxidation of **21** with PCC led to the aldehyde **22** in 85% yield. The sulfone **23** was prepared from nonan-1,9-diol. The diol was monoprotected with 2,2-dimethylpropanoyl (pivalyl) chloride, pyridine and DMAP (50%). The monopivalyl ester was converted into the sulfone by reaction with triphenylphosphine, DEAD and 2-mercaptobenz-thiazole, then oxidation with H_2O_2 and ammonium molybdate(VI) tetra-hydrate (72% overall). Homologation of the aldehyde **22** by reaction with sulfone **23** and sodium hexamethyldisilazide was followed by removal of the ester, saturation of the alkene and oxidation of the alcohol to give the aldehyde **24** (Scheme 7).

The sulfone **15** was condensed with **24** again in a modified Julia reaction,[23a] to form the dicyclopropane **25**. Saturation of the alkene and oxidative cleavage of the acetal group gave the aldehyde **26** which was epimerised using sodium methoxide in methanol to a 22:1 mixture of aldehydes **27** and **26** (Scheme 8).[22,25]

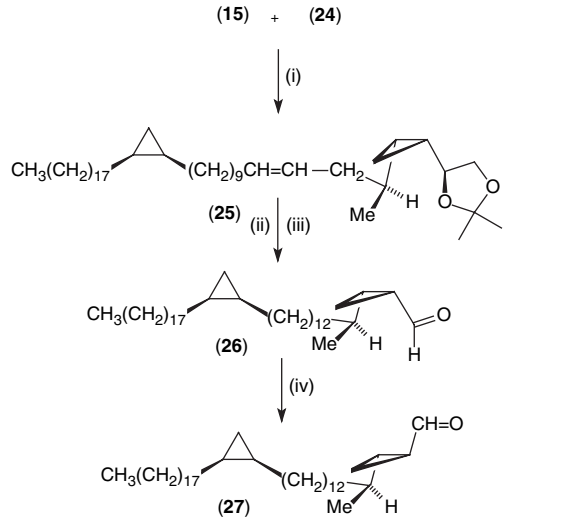
α-Methyl-*trans*-cyclopropanes have previously been obtained by cyclopropanation of 4-methylalk-3-en-1-ols,[26] by 1,3-elimination,[27] and by rearrangement of propargyl ethers.[28]

† The enantiomers of **17** and **18** have already been reported.[14]



(i) NaHMDS, THF, -20 °C (75 %); (ii) $LiAlH_4$, THF (91 %); (iii) H_2NNH_2, $CuSO_4$, $NaIO_4$, CH_3COOH, iPrOH (75 %); (iv) PCC, CH_2Cl_2 (95 %)

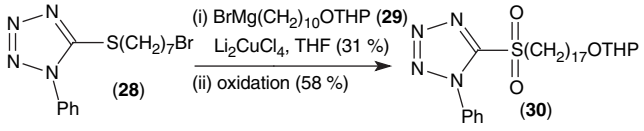
Scheme 7.



(i) LiHMDS, THF (78 %); (ii) $KO_2CN{=}NCO_2K$, AcOH, thf, MeOH (86 %); (iii) HIO_4 (97 %); (iv) NaOMe, MeOH, THF (70 %)

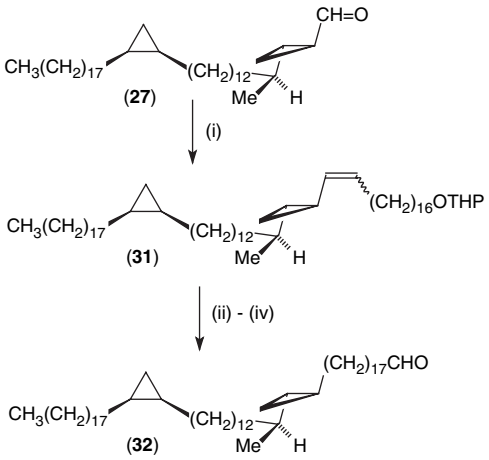
Scheme 8.

Finally, compound **27** was homologated at the aldehyde position to produce a C_{18}-chain. To achieve this, the difunctional species **30** was prepared by copper-catalysed coupling of the bromide **28** derived from 7-bromoheptanol, with the Grignard reagent **29**, followed by oxidation (Scheme 9).



Scheme 9.

The aldehyde **27** was coupled with the sulfone **30** in a Julia–Kocienski reaction.[23b] Deprotection of the THP ether **31** then saturation of the alkene using diimide, followed by oxidation gave the desired mycolaldehyde **32**, $[\alpha]_D^{22}$ +2.8 (*c* 1.02, $CHCl_3$). The overall ^1H NMR pattern for the cyclopropane signals of **32** was visually essentially identical to that reported for a mixture of mycolic acid esters containing both *cis*- and α-methyl-*trans*-cyclopropanes.[3,8,10] The *cis*-cyclopropane showed single protons at δ −0.32 (dt, *J* 4.3, 5.3 Hz) and 0.57 (dt, *J* 4.3, 8.0 Hz) and two protons within a multiplet at 0.62–0.71. The *trans*-cyclopropane unit showed three single-hydrogen multiplets at δ 0.1–0.2 and one at 0.45 (Scheme 10).



(i) (**30**), LiHMDS, THF (90 %); (ii) pTsOH, MeOH (95 %); (iii) H_2NNH_2, $CuSO_4$, Me_2CHOH, AcOH, $NaIO_4$ (85 %); (iv) PCC, CH_2Cl_2 (81 %)

Scheme 10.

Irradiation at δ 0.65 partly decoupled each of the signals at 0.57 and −0.32 (for the *cis*-cyclopropane unit), but also that at 0.2 and the methyl doublet at δ 0.9 (for the methyl adjacent to the *trans*-cyclopropane); it did not affect the signal at 0.45. The signal at δ 0.45 was coupled to each of those at 0.1–0.2 but not to the α-methyl-group signal at δ 0.9 (d, *J* 7.0 Hz). The signal for H_c is therefore part of the multiplet at 0.65 as previously identified on a natural sample, while those at δ 0.2 and 0.45, respectively, are assigned to H_b and H_a (Fig. 2) in contrast to an earlier assignment.[10] Moreover, the chemical shifts of the carbon signals for the two cyclopropanes and the adjacent CHMe group of the synthetic compound **32** were in very close agreement with those determined by Watanabe et al.;[10] thus the natural material gave signals at δ 18.6, 26.1 for the *trans*-cyclopropane CH-carbons, at 15.8 for the *cis*-cyclopropane CH-carbons and at 38.1 and 19.8 for the carbons of the CHMe fragment; the synthetic material showed corresponding signals at δ 18.62, 26.15, 15.79, 38.11 and 19.67. This close correspondence suggests that, even though the absolute stereochemistry of the natural material is still uncertain, the relative stereochemistry of the α-methyl-group and the cyclopropane is as in **32**.

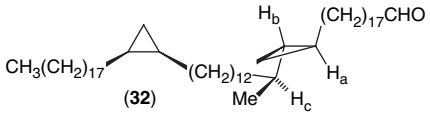


Figure 2.

Moreover, using **16** or its enantiomer,[14] and stereoisomers of intermediates related to **10**,[14,20] and the chemistry described above, it is expected that any absolute stereochemistry of mycolic acid containing an α-methyl-*trans*-cyclopropane unit may be synthesised.

2. Experimental

2.1. General

Unless stated, reagents were obtained from commercial suppliers. Solvents which had to be dry (e.g., ether, tetrahydrofuran) were dried over sodium wire. Boiling point of petroleum was 40–60 °C. Reactions under inert conditions were carried out under a slow stream of nitrogen from a balloon and septum. Those carried out at low temperatures were cooled using methylated spirit and liquid nitrogen. Silica gel (Merck 7736 silica gel) and silica plates used for thin layer and column chromatography were obtained from Aldrich. GLC was carried out on a Perkin–Elmer 8410 on a capillary column (15 m×0.53 mm). IR spectra were carried out on a Perkin–Elmer 1600 FTIR spectrometer from liquid films. ^{1}H NMR spectra were recorded on a Bruker AC250 or Advance 500 spectrometers. DEPT ^{13}C-spectra as reported are + for CH_2, − for CH or CH_3, dot for quaternary C. $[\alpha]_D$ values were recorded in $CHCl_3$ on a POLAAR 2001 Optical Activity polarimeter.

2.1.1. [(1*S*,2*R*)-2-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)cyclopropyl]methanol (9). Tetra-*n*-butylammonium fluoride (73 ml, 73 mmol) was added to a stirred solution of *tert*-butyl-[(1*S*,2*R*)-2-((*S*)-2,2-dimethyl[1,3]dioxolan-4-yl)cyclopropyl-methyl]diphenylsilane (**8**)[20] (23 g, 56 mmol) in dry tetrahydrofuran (100 ml), at 0 °C under nitrogen. The mixture was allowed to reach room temperature and stirred for 16 h, when TLC showed no starting material was left, then cooled to 5 °C and quenched with satd aq ammonium chloride (50 ml) and the product was extracted with ethyl acetate (3×150 ml). The combined organic layers were washed with brine (100 ml), water (100 ml), dried and evaporated to give an oil. Chromatography (1:1 petroleum/ethyl acetate) gave *[(1S,2R)-2-((S)-2,2-dimethyl[1,3]dioxolan-4-yl)cyclopropyl]methanol* (**9**) as a colourless oil (8.2 g, 85%) [Found M−H$^+$: 171.1023, $C_9H_{15}O$ requires: 171.1021], $[\alpha]_D^{22}$ −19.2 (*c* 1.24, $CHCl_3$), which showed δ_H (250 MHz, $CDCl_3$): 4.13 (1H, dd, *J* 5.5, 7.6 Hz), 3.85–3.77 (2H, m), 3.69 (1H, t, *J* 8 Hz), 3.43 (1H, dd, *J* 8.5, 11.3 Hz), 2.00 (1H, br s), 1.43 (3H, s), 1.34 (3H, s), 1.27–1.15 (1H, m), 1.03 (1H, br dq, *J* 5.5, 8.25 Hz), 0.89 (1H, br dt *J* 3.4, 6.1 Hz), 0.44 (1H, br q, *J* 5.5 Hz); δ_C (62.5 MHz, $CDCl_3$): 109.23, 77.47, 70.4, 63.24, 54.13, 27.6, 27.24, 18.5, 18.1, 8.8; ν_{max}: 3436, 2984, 2934 cm^{-1}.

2.1.2. (1*S*,2*R*)-2-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl) cyclopropanecarbaldehyde (10). Alcohol **9** (8.00 g, 46.5 mmol) in dichloromethane (25 ml) was added to a stirred suspension of pyridinium chlorochromate (20.05 g, 93 mmol) in dichloromethane (300 ml). The mixture was stirred vigorously for 2 h, when TLC showed no starting material, then poured into diethyl ether (500 ml), filtered through a pad of silica and washed well with ether. The filtrate was evaporated to give a yellow oil; chromatography (1:1 petroleum/ethyl acetate) gave an oil, *(1S,2R)-2-((S)-2,2-dimethyl[1,3]-dioxolan-4-yl)cyclopropanecarbaldehyde* (**10**) (6.9 g, 85%) [Found M−H$^+$: 169.0854; $C_9H_{13}O_3$ requires: 169.0854], $[\alpha]_D^{22}$ +36.1 (*c* 1.19, $CHCl_3$), which showed δ_H (250 MHz, $CDCl_3$): 9.41 (1H, d, *J* 5.2 Hz), 4.12 (1H, br t, *J* 6.1 Hz), 4.00 (1H, dd, *J* 6.1, 8 Hz), 3.66 (1H, br t, *J* 7.3 Hz), 2.02–1.95 (1H, m), 1.60 (1H, br t, *J* 7.3 Hz), 1.49–1.44 (1H, m), 1.42 (3H, s), 1.40–1.34 (1H, m), 1.33 (3H, s); δ_C (62.5 MHz, $CDCl_3$): 200.68, 109.55, 74.5, 69.4, 27.2, 26.7, 25.8, 25.6, 12.77; ν_{max}: 1702, 1370, 1063 cm^{-1}.

2.1.3. (*E*)-3-[(1*R*,2*R*)-2-((*S*)-2,2-Dimethyl[1,3]dioxolan-4-yl)cyclopropyl]acrylic acid methyl ester (11). Methyl (triphenylphosphoranylidene)acetate (14.23 g, 42 mmol) was added in portions to a stirred solution of aldehyde **10** (6.5 g, 38.2 mmol) in toluene (100 ml) at 10 °C. The mixture was allowed to reach room temperature and stirred for 24 h when GLC showed no starting material. The solvent was evaporated and the residue was refluxed with 1:1 petroleum/ether (50 ml) for 10 min. The precipitate was washed with petroleum/ether (30 ml). The solvent was evaporated and the residue was chromatographed (5:2 petroleum/ethyl acetate) to give *(E)-3-[(1R,2R)-2-((S)-2,2-dimethyl[1,3]-dioxolan-4-yl)cyclopropyl]acrylic acid methyl ester* (**11**) as a colourless oil (7.00 g, 81%) [Found M$^+$: 226.1200; $C_{12}H_{18}O_4$ requires: 226.1205], which showed δ_H (500 MHz, $CDCl_3$): 6.64 (1H, dd, *J* 10.4, 15.5 Hz), 5.95 (1H, d, *J* 15.5 Hz), 4.03 (1H, dd, *J* 6, 8.2 Hz), 3.83–3.78 (1H, m), 3.73 (3H, s), 3.64 (1H, br t, *J* 8.2 Hz), 1.79–1.73 (1H, m), 1.45 (3H, s), 1.41–1.37 (1H, m), 1.36 (3H, s), 1.30 (1H, dt, *J* 5.1, 8.2 Hz), 0.94 (1H, br q, *J* 5.4 Hz); δ_C (62.5 MHz, $CDCl_3$): 166.64, 148.88 (−), 120.97 (−), 109.17, 76.86 (−), 69.28 (+), 51.42 (−), 26.78 (−), 25.78 (−), 23.46 (−), 18.6(−), 13.79 (+); ν_{max}: 1701, 1643 cm^{-1}. There was less than 5% of the *Z*-isomer present. When methanol was used as a solvent, a 2:1 mixture of *E* and *Z*-isomers was obtained.

2.1.4. (*S*)-3-[(1*R*,2*R*)-2-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)cyclopropyl]butyric acid methyl ester (12). Methyl magnesium bromide (28.8 ml, 86.3 mmol) was added dropwise to a stirred suspension of copper bromide (6.2 g, 43 mmol) in dry tetrahydrofuran (100 ml) at −40 °C under nitrogen. The mixture was stirred for 30 min, then (*E*)-ester **11** (6.5 g, 28.7 mmol) in dry tetrahydrofuran (25 ml) was added dropwise at −30 °C. The mixture was allowed to reach −5 °C over 2 h when GLC showed no starting material, then quenched with satd aq ammonium chloride (30 ml) at −30 °C. The product was extracted with ethyl acetate (3×100 ml). The combined organic layers were washed with brine (50 ml), dried and evaporated to give a brown oil. Chromatography (5:2 petroleum/ethyl acetate) gave a colourless oil, *(S)-3-[(1R,2R)-2-((S)-2,2-dimethyl-[1,3]dioxolan-4-yl)-cyclopropyl]butyric acid methyl ester* (**12**) (5.00 g, 72%) [Found M$^+$: 242.1528; $C_{13}H_{22}O_4$ requires: 242.1518], $[\alpha]_D^{22}$ +11.65 (*c* 1.3, $CHCl_3$), which showed δ_H (500 MHz, $CDCl_3$): 4.09 (1H, dd, *J* 6, 7.6 Hz), 3.76 (1H, ddd, *J* 6.3, 7.85, 14 Hz), 3.68–3.65 (4H, including a singlet for the methoxy group), 2.33 (1H, dd, *J* 3.8, 14.8 Hz), 2.20 (1H, dd, *J* 9.8, 14.85 Hz), 1.52–1.59 (1H, m), 1.44 (3H, s), 1.35 (3H, s), 1.06 (3H, d, *J* 6.6 Hz), 0.94 (1H, dq, *J* 5.35, 8.2 Hz), 0.86 (1H, dt, *J* 4.7, 8.8 Hz), 0.77–0.71 (1H, m), 0.32 (1H, br q, *J* 5.32 Hz); δ_C (125 MHz, $CDCl_3$): 173.1, 108.74, 77.5, 70.2, 51.73, 42, 31.1, 27.1, 25.98, 23.3, 20.75, 19.6, 9.5; ν_{max}: 2984, 1737, 1061 cm^{-1}. There was less than ca. 6% of another isomer present. When a mixture of (2:1 *E*/*Z*) was used the same product was obtained.

2.1.5. (S)-3-[(1*R*,2*R*)-2-((*S*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)cyclopropyl]-butan-1-ol (13). Methyl ester **12** (4.2 g,

17.3 mmol) in dry tetrahydrofuran (15 ml) was added dropwise to a stirred suspension of lithium aluminium hydride (1.32 g, 34.7 mmol) in tetrahydrofuran (50 ml) at room temperature under nitrogen. The mixture was refluxed for 1 h, when TLC showed no starting material, then cooled to 0 °C and quenched with satd aq sodium sulfate decahydrate (20 ml) until a white solid formed. The precipitate was filtered off and washed with tetrahydrofuran (2×20 ml). The filtrate was evaporated to give a crude product; chromatography (1:1 petroleum/ethyl acetate) gave *(S)-3-[(1R,2R)-2-((S)-2,2-dimethyl[1,3]dioxolan-4-yl)cyclopropyl]-butan-1-ol* (**13**) as a colourless oil (3.26 g, 88%) [Found M^+-CH_3: 199.1344; $C_{11}H_{19}O_3$ requires: 199.1334], $[\alpha]_D^{22}$ −8.9 (*c* 1.53, $CHCl_3$), which showed δ_H (500 MHz, $CDCl_3$): 4.19 (1H, dd, *J* 5.65, 7.55 Hz), 3.88 (1H, dt, *J* 6, 7.86 Hz), 3.71 (2H, t, *J* 7.9 Hz), 3.67–3.62 (1H, br m), 1.75 (1H, br s), 1.67–1.59 (2H, m), 1.53–1.46 (1H, m), 1.45 (3H, s), 1.36 (3H, s), 1.04 (3H, d, *J* 6.6 Hz), 0.95 (1H, dq, *J* 5.65, 8.5 Hz), 0.85 (1H, dt, *J* 4.4, 8.5 Hz), 0.74–0.68 (1H, m), 0.28 (1H, br q, *J* 5.65 Hz); δ_C (125 MHz, $CDCl_3$): 108.5, 77.1, 70.1, 60.6, 40.3, 29.64, 26.78, 25.7, 23.87, 20.35, 19.1, 8.3; ν_{max}: 3432, 2983 cm^{-1}.

2.1.6. 3,5-Dinitrobenzoic acid (*S*)-3-[(1*R*,2*R*)-2-((*S*)-2,2-dimethyl[1,3]dioxolan-4-yl)cyclopropyl]butyl ester. 3,5-Dinitrobenzoyl chloride (0.42 g, 1.82 mmol), was added to a stirred solution of (*S*)-3-[(1*R*,2*R*)-2-((*S*)-2,2-dimethyl[1,3]dioxolan-4-yl)cyclopropyl]butan-1-ol (**13**) (0.3 g, 1.4 mmol) and pyridine (1.5 ml) in toluene (5 ml) at room temperature. The mixture was refluxed for 4 h then cooled to room temperature and the solvent was evaporated. The residue was treated with water (10 ml) and extracted with ether (3×20 ml). The combined organic layers were washed with brine, dried and evaporated to give a thick yellow oil; chromatography (1:1 petroleum/ether) gave a white solid, *3,5-dinitrobenzoic acid (S)-3-[(1R,2R)-2-((S)-2,2-dimethyl[1,3]dioxolan-4-yl)cyclopropyl]butyl ester* (0.32 g, 56%), mp 77–79 °C [Found C, 56.0, H, 5.8, N, 6.9; $C_{19}H_{24}O_8N_2$ requires: C, 55.88, H, 5.92, N, 6.85], $[\alpha]_D^{22}$ −26 (*c* 0.4, $CHCl_3$), which showed δ_H (250 MHz, $CDCl_3$): 9.23 (1H, br t, *J* 2.1 Hz), 9.14 (2H, br d, *J* 2.1 Hz), 4.56–4.42 (2H, m), 4.18–4.08 (1H, m), 3.81–3.68 (2H, m), 2.10–1.88 (1H, m), 1.81–1.67 (1H, m), 1.42 (3H, s), 1.38–1.29 (1H, m), 1.28 (3H, s), 1.13 (3H, d, *J* 6.4 Hz), 1.08–0.79 (3H, m), 0.33 (1H, br q, *J* 5 Hz); δ_C (62.5 MHz, $CDCl_3$): 162.5, 148.7, 133.8, 129.3, 122.38, 108.56, 70.1, 65.2, 33.8, 30.4, 28.75, 25.7, 23.5, 20.03, 19.1, 8.73; ν_{max}: 2923, 2853, 1732, 1544 cm^{-1}.

2.1.7. Crystal structure determination for the 3,5-dinitrobenzoate of (13). *Crystal data*: $C_{19}H_{24}N_2O_8$, M=408.4, orthorhombic, space group $P2_12_12_1$, a=5.6009(3), b=16.6472(8), c=21.3374(10) Å, V=1989.48(17) $Å^3$, Z=4, D_c=1.364 g cm^{-3}, μ=0.11 mm^{-1} (Mo Kα, λ=0.71073 Å), T=150 K. Of 14,574 reflections measured on a Bruker AXS SMART CCD diffractometer, 2057 were unique (θ<25°, R_{int}=0.038). The structure was solved by standard direct methods and refined on F^2 values; H atoms were constrained with a riding model. In the absence of significant anomalous scattering, Friedel pairs were merged, and the absolute configuration was assigned on the basis of the known configuration of compound **8**. R=0.033 (F values, $F^2>2s$), R_w=0.079 (F^2 values, all data), goodness-of-fit=1.125 for 266 refined parameters, final difference map within ±0.27 $eÅ^{-3}$. Software: Bruker SMART, SAINT and SHELXTL. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCD283460. Copies of the data can be obtained free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

2.1.8. 2-{(*S*)-3-[(1*R*,2*R*)-2-((*S*)-2,2-Dimethyl[1,3]-dioxolan-4-yl)cyclopropyl]butylsulfanyl}benzothiazole (14). Diethyl azodicarboxylate (2.56 g, 14.7 mmol) in dry tetrahydrofuran (5 ml) was added to a stirred solution of alcohol **13** (3 g, 14.0 mmol), triphenylphosphine (4.04 g, 15 mmol) and 2-mercaptobenzthiazole (2.46 g, 14.7 mmol) in tetrahydrofuran (20 ml) at 5 °C under nitrogen. The mixture was allowed to reach room temperature, stirred for 24 h, then the solvent was evaporated. The residue was dissolved in ethyl acetate (50 ml) and petroleum (50 ml), stirred for 15 min, filtered through Celite and evaporated to give a yellow oil. This was dissolved in ether, suspended on silica and then columned (1:1 petroleum/ether) to give *2-{(S)-3-[(1R,2R)-2-((S)-2,2-dimethyl[1,3]-dioxolan-4-yl)cyclopropyl]butylsulfanyl}benzothiazole* (**14**) as a pale yellow oil (4.5 g, 89%) [Found M^+: 363.1317; $C_{19}H_{25}O_2S_2N$ requires: 363.1327], $[\alpha]_D^{22}$ −45.05 (*c* 1.485, $CHCl_3$), which showed δ_H (250 MHz, $CDCl_3$): 7.88 (1H, br dd, *J* 0.9, 7.6 Hz), 7.76 (1H, br dd, *J* 0.9, 8 Hz), 7.42 (1H br dt, *J* 1.2, 7.3 Hz), 7.27 (1H, br dt, *J* 1.2, 8 Hz), 4.09 (1H, dd, *J* 5.8, 7.6 Hz), 3.76 (1H, br dt, *J* 5.8, 8.25 Hz), 3.63 (1H, br t, *J* 7.6 Hz), 3.52–3.24 (2H, m), 1.95–1.83 (1H, m), 1.81–1.65 (1H, m), 1.43 (3H, s), 1.33 (3H, s), 1.29–1.19 (1H, m), 1.10 (3H, d, *J* 6.4 Hz), 1.04–0.71 (4H, m), 0.31 (1H, br q, *J* 6.4 Hz); δ_C (62.5 MHz, $CDCl_3$): 166.6, 153.2, 135.1, 126.1, 124.2, 121.5, 120.9, 108.3, 77.2, 70, 36.7, 32.65, 31.1, 26.8, 25.6, 23.5, 19.6, 19.4, 8.9; ν_{max}: 3062, 2982, 1461, 1427, 1060 cm^{-1}.

2.1.9. 2-{(*S*)-3-[(1*R*,2*R*)-2-((*S*)-2,2-Dimethyl[1,3]dioxolan-4-yl)cyclopropyl]-butane-1-sulfonyl}benzothiazole (15). A solution of ammonium heptamolybdate(VI) tetrahydrate (1.34 g, 1.1 mmol), in 35% H_2O_2 (w/w) (5.3 ml, 54.4 mmol) was added dropwise at 5 °C to a stirred solution of compound **14** (4.2 g, 11.5 mmol) in methylated spirit (100 ml). The resulting yellow solution was stirred for 1 h at this temperature and then for 16 h at room temperature. The solvent was evaporated to give a yellow solid which was treated with water (50 ml) and extracted with dichloromethane (3×50 ml); the combined organic layers were washed with brine (50 ml), dried and evaporated to give the crude product. This was purified by chromatography (1:1 petroleum/ethyl acetate) to give *2-{(S)-3-[(1R,2R)-2-((S)-2,2-dimethyl[1,3]-dioxolan-4-yl)cyclopropyl]-butane-1-sulfonyl}benzothiazole* as a thick yellow oil (**15**) (3.08 g, 67.4%) [Found M^+: 395.123; $C_{19}H_{25}O_4S_2N$ requires: 395.1225], $[\alpha]_D^{22}$ −34.1 (*c* 1.36, $CHCl_3$), which showed δ_H (250 MHz, $CDCl_3$): 8.23 (1H, br d, *J* 8.2 Hz), 8.04 (1H, br d, *J* 8 Hz), 7.66 (1H, dt, *J* 1, 7.3 Hz), 7.61 (1H, dt, *J* 1, 8.2 Hz), 4.04 (1H, dd, *J* 6, 7.9 Hz), 3.76 (1H, br q, *J* 7.6 Hz), 3.63–3.56 (2H, m), 3.54–3.48 (1H, m), 2.03–1.96 (1H, m), 1.81–1.73 (1H, m), 1.41 (3H, s), 1.37–1.33 (1H, m), 1.31 (3H, s), 1.06 (3H, d, *J* 6.4 Hz), 0.99–0.92 (1H, dt,

J 5.4, 8.5 Hz), 0.84 (1H, br dt, *J* 4, 8.8 Hz), 0.72–0.65 (1H, m), 0.31 (1H, br q, *J* 5.4 Hz); δ_C (62.5 MHz, $CDCl_3$): 165.9, 152.6, 136.6, 128.1, 127.8, 125.4, 122.4, 76.5, 70, 52.5, 32.3, 29.2, 26.77, 25.65, 23.1, 19.6, 19.3, 8.46; ν_{max}: 2982, 1735, 1472, 1059 cm^{-1}. The second product was (*S*)-1-{(1*R*,2*R*)-2-[(*S*)-3-(benzothiazole-2-sulfonyl)-1-methylpropyl]cyclopropyl}ethane-1,2-diol (0.84 g, 20.45%) which was reprotected with 2,2-dimethoxypropane in dichloromethane in the presence of a catalytic amount of PTSA at room temperature in 95% yield.

2.1.10. Butyric acid (1*R*,2*S*)-2-(benzothiazole-2-sulfonylmethyl)cyclopropylmethyl ester (18). Diethyl azodicarboxylate (18.1 g, 103.7 mmol) in dry tetrahydrofuran (50 ml) was added to a stirred solution of *cis*-(1*R*,2*S*)-1-butyryloxymethyl-2-hydroxymethylcyclopropane (**16**) (17 g, 98.8 mmol),[22] triphenylphosphine (28.5 g, 108.72 mmol) and 2-mercaptobenzthiazole (17.35 g, 103.7 mmol) in dry tetrahydrofuran (200 ml) at 0 °C. The mixture was allowed to reach room temperature and stirred for 18 h. The solvent was evaporated and the residue was treated with petroleum/ether (5:2) (200 ml) and the precipitate was filtered off on a sinter. The filter cake was washed with petroleum/ether. The combined organic layers were evaporated to give a pale yellow oil, which was columned (5:2 petroleum/ethyl acetate) to give *butyric acid (1R,2S)-2-(benzothiazol-2-ylsulfanylmethyl)cyclopropylmethyl ester* (**17**) as a thick yellow oil (23.5 g, 73%); $[\alpha]_D^{22}$ −4.3 (*c* 1.48, $CHCl_3$) (lit. value (1*S*,2*R*)-isomer $[\alpha]_D^{22}$ +4.9 (*c* 2.4, $CHCl_3$);[14] [Found M^+: 321.083; $C_{16}H_{19}O_2S_2N$ requires: 321.086], which showed δ_H (250 MHz, $CDCl_3$): 7.83 (1H, br d, *J* 8.04 Hz), 7.72 (1H, br d, *J* 8.4 Hz), 7.46–7.33 (1H, m), 7.27–7.22 (1H, m), 4.32 (1H, dd, *J* 6.5, 12 Hz), 3.98 (1H, dd, *J* 8.5, 12 Hz), 3.52 (1H, dd, *J* 7.5, 13.27 Hz), 3.34 (1H, dd, *J* 7.4, 13.27 Hz), 2.34 (2H, t, *J* 7.3 Hz), 1.65 (2H, sext, *J* 5.3 Hz), 1.45–1.32 (2H, m), 0.93 (3H, t, *J* 7.3 Hz), 0.95–0.85 (1H, m), 0.37 (1H, br q, *J* 5.6 Hz); δ_C (62.5 MHz, $CDCl_3$): 173.7, 166.76, 153.2, 135.2, 126.00, 124.2, 121.4, 121, 64.1, 36.2, 33.9, 18.4, 16.13, 15.7, 13.7, 11.00; ν_{max}: 1733, 1458, 1427, 1180 cm^{-1}.

A solution of ammonium heptamolybdate(VI) tetra-hydrate (6.15 g, 4.9 mmol) in 35% H_2O_2 (w/w) (23.2 ml, 238 mmol) at 5 °C was added to a stirred solution of the above ester (17 g, 53 mmol) in methylated spirit (150 ml). The resulting yellow solution was stirred for 1 h at this temperature then for 16 h at room temperature. The solvent was evaporated to give a yellow solid, which was diluted with water (100 ml) and extracted with dichloromethane (3×100 ml). The combined organic layers were washed with brine (50 ml), dried and evaporated to give an oil. Chromatography (5:3 petroleum/ethyl acetate) gave butyric acid (1*R*,2*S*)-2-(benzothiazole-2-sulfonylmethyl)cyclopropylmethyl ester (**18**) as a thick yellow oil (17.3 g, 92%), which showed δ_H (250 MHz, $CDCl_3$): 8.21–8.15 (1H, m), 7.99–7.95 (1H, m), 7.62–7.45 (2H, m), 4.24 (1H, distorted dd, *J* 6, 12 Hz), 3.83–3.72 (2H, m), 3.35 (1H, distorted dd, *J* 9.5, 14.7 Hz), 2.22 (2H, t, *J* 7.4 Hz), 1.65 (2H, sext, *J* 7.4 Hz), 1.38–1.30 (2H, m), 0.95–0.80 (4H, m, including a triplet, *J* 7.36 Hz), 0.28 (1H, br q, *J* 5.7 Hz); δ_C (62.5 MHz, $CDCl_3$): 173.4, 165.8, 152.6, 136.8, 128, 127.6, 125.4, 122.3, 63.6, 55.1, 36, 18.35, 14.4, 13.6, 9.7, 9.1; ν_{max}: 1730, 1707, 1470, 762 cm^{-1}. $[\alpha]_D^{22}$ +56.1 (*c* 1.13, $CHCl_3$) (lit. value (1*S*,2*R*)-isomer $[\alpha]_D^{22}$ −58.3 (*c* 1.59, $CHCl_3$)[14]; *m/z*: M^+ (353), 266 ($M^+-C_4H_7O_2$), 155 ($M^+-C_7H_4S_2O_2N$).

2.1.11. Heptadecan-1-ol. 1-Bromoheptane (34 g, 189.8 mmol) in dry tetrahydrofuran (50 ml) was added dropwise to a suspension of magnesium turnings (5.96 g, 246.8 mmol) in dry tetrahydrofuran (80 ml) at a rate sufficient to maintain a steady reflux. Once the exothermic reaction had subsided, the mixture was refluxed for 1 h. The Grignard reagent was cooled to −10 °C, then 10-bromodecanol (15 g, 63.3 mmol) in tetrahydrofuran (50 ml) was added. The mixture was cooled to −40 °C followed by the addition of dilithium tetrachlorocuprate (5 ml) then stirred for 2 h at −40 °C and at room temperature for 12 h. Satd aq ammonium chloride (100 ml) was added together with ethyl acetate (100 ml). The organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (2×50 ml). The combined organic layers were washed with water, dried and evaporated to give a residue, which was columned (5:1 petroleum/ethyl acetate) to give heptadecan-1-ol as a white solid (14.5 g, 89.5%), mp 54–56 °C [Found M^+: 256.2777; $C_{17}H_{36}O$ requires: 256.2766], which showed δ_H (250 MHz, $CDCl_3$): 3.65 (2H, t, *J* 6.4 Hz), 1.62–1.27 (31H, m), 0.89 (3H, t, *J* 7 Hz); δ_C (62.5 MHz, $CDCl_3$): 62.9, 32.7, 31.92, 29.7 (v. broad), 29.45, 29.36, 25.7, 22.67, 14.1; ν_{max}: 3430 cm^{-1}.

2.1.12. Heptadecanal. Heptadecan-1-ol (15.0 g, 58 mmol) in dichloromethane (50 ml) was added at room temperature to a stirred suspension of pyridinium chlorochromate (25.2 g, 117 mmol) in dichloromethane (250 ml). A black colour appeared after 10 min. The mixture was stirred for 2 h, when TLC showed no starting material, then diluted with ether (500 ml) and filtered through a pad of silica. The solvent was evaporated to give a residue, which was purified by chromatography (5:1 petroleum/ether) to give heptadecanal as a white solid (13.6 g, 91%), mp 45–47 °C [Found M^+: 254.2614; $C_{17}H_{34}O$ requires: 254.2610], which showed δ_H (250 MHz, $CDCl_3$): 9.73 (1H, t, *J* 1.8 Hz), 2.43 (2H, dt, *J* 1.8, 7.3 Hz), 1.72–1.62 (2H, m), 1.43–1.21 (26H, m), 0.91 (3H, t, *J* 7 Hz); δ_C (62.5 MHz, $CDCl_3$): 202.89, 43.89, 33.98, 31.9, 29.68, 29.65, 29.63, 29.57, 29.42, 29.35, 29.23, 29.16, 29.06, 24.68, 22.67, 22.07, 14.07; ν_{max}: 1727 cm^{-1}.

2.1.13. ((1*R*,2*S*)-2-Octadecycyclopropyl)methanol (21). Sodium hexamethyldisilazide (62 ml, 62 mmol, 1 M) was added to a stirred solution of butyric acid (1*R*,2*S*)-2-(benzothiazole-2-sulfonylmethyl)cyclopropylmethyl ester (**18**) (16.8 g, 47.6 mmol) and heptadecanal (11 g, 43.3 mmol) in dry tetrahydrofuran (100 ml) under nitrogen at −20 °C. The mixture was stirred for 2 h at this temperature, then allowed to reach room temperature for 12 h. This was quenched with water (20 ml) at 0 °C, then diluted with ether (100 ml). The organic layer was separated and the aqueous layer was extracted with ether (2×50 ml). The combined organic layers were dried and evaporated to give a pale yellow oil, which was columned (20:1 petroleum/ether) to give 1.3:1 *(E/Z)-butyric acid (1R,2R)-2-octadec-1-enylcyclopropylmethyl ester* (**19**) (10.5 g, 68%). The (*E*/*Z*) mixture (8 g, 20.4 mmol) in tetrahydrofuran (50 ml) was added dropwise over 15 min to a suspension of lithium aluminium hydride (1.55 g, 40.8 mmol) in tetrahydrofuran (150 ml) at room temperature. The mixture was refluxed for 1 h, then cooled

to room temperature and quenched carefully with freshly prepared satd aq sodium sulfate decahydrate (40 ml) until a white precipitate formed, followed by the addition of magnesium sulfate (10 g). The mixture was stirred vigorously for 10 min, filtered through a pad of Celite and washed well with tetrahydrofuran (2×50 ml). The combined organic layers were dried and evaporated to give 1.3:1 *(E/Z)-((1R,2R)-2-octadec-1-enylcyclopropyl)methanol* (**20**) (5.4 g, 84.3%), which was used for the next step without purification.

Sodium metaperiodate (36.55 g, 170.8 mmol) in hot water (100 ml) was added over a 90 min at 70–80 °C to a stirred solution of alcohol **20** (5.5 g, 17 mmol) in isopropyl alcohol (100 ml), acetic acid (2 ml), satd aq copper sulfate (2 ml) and hydrazine hydrate (15 ml). The mixture was stirred for another 2 h to reach room temperature, then diluted with ether (150 ml). The organic layer was separated and the aqueous layer was extracted with ether (2×50 ml). The combined organic layers were washed with water (2×50 ml), dried and evaporated. The crude product was purified by chromatography (5:1 petroleum/ether) to give *((1R,2S)-2-octadecylcyclopropyl)methanol* (**21**) as a white solid (4.5 g, 81%), mp 54–56 °C [Found: M^+-H_2O: 306.3289, $C_{22}H_{42}$ requires 306.3287], $[\alpha]_D^{22}$ +11.98 (*c* 1.06, $CHCl_3$), which showed δ_H (500 MHz): 3.66 (1H, ddd, *J* 1.25, 7.25, 11.35 Hz), 3.61 (1H, br ddd, *J* 0.95, 8.2, 11 Hz), 1.58 (1H, br s), 1.50–1.41 (2H, m), 1.37–1.22 (32H, m), 1.15–1.08 (1H, m), 0.91 (3H, t, *J* 7 Hz), 0.85 (1H, m), 0.73 (1H, ddt, *J* 0.9, 4.7, 9.75 Hz), 0.03 to −0.028 (1H, br q, *J* 4.4 Hz); δ_C (62.5 MHz, $CDCl_3$): 63.3, 31.9, 30.15, 29.68, 29.64, 29.56, 29.34, 28.54, 22.66, 18.12, 16.14, 14.08, 9.45; ν_{max}: 3352 cm^{-1}.

2.1.14. ((1*R*,2*S*)-2-Octadecylcyclopropanecarbaldehyde (22). Alcohol **21** (2.5 g, 7.7 mmol) in dichloromethane (15, ml) was added to a stirred suspension of pyridinium chlorochromate (3.34 g, 15 mmol) in dichloromethane (50 ml) at room temperature. The mixture was stirred vigorously for 3 h, when TLC showed no starting material, poured into diethyl ether (200 ml) and filtered on a pad of silica then washed well with ether. The filtrate was evaporated to give a white solid; chromatography (5:2 petroleum/ether) gave *((1R,2S)-2-octadecylcyclopropanecarbaldehyde* (**22**) as a white solid (2.1 g, 84.5%), mp 39–41 °C [Found M^+: 322.3232; $C_{22}H_{42}O$ requires: 322.3236], $[\alpha]_D^{22}$ +7.62 (*c* 1.455, $CHCl_3$), which showed δ_H (250 MHz, $CDCl_3$): 9.35 (1H, d, *J* 5.5 Hz), 1.92–1.81 (1H, m), 1.65–1.23 (37H, m), 0.88 (3H, t, *J* 7 Hz); ν_{max} 1695 cm^{-1}.

2.1.15. 10-((1*R*,2*S*)-2-Octadecylcyclopropyl)decan-1-ol. Sodium hexamethyldisilazide (8.1 ml, 8.1 mmol) was added to a stirred solution of 2,2-dimethylpropionic acid 9-(benzothiazole-2-sulfonyl)nonyl ester (**23**) (2.85 g, 6.7 mmol) and aldehyde (**22**) (1.8 g, 5.6 mmol) in dry tetrahydrofuran (30 ml) under nitrogen at −10 °C. After 1 h at this temperature, it was allowed to reach room temperature for 12 h, then cooled to 0 °C, quenched with satd aq ammonium chloride (20 ml), then diluted with ether (100 ml). The organic layer was separated and the aqueous layer was extracted with ether (2×30 ml). The combined organic layers were dried and evaporated to give a pale yellow oil; chromatography (5:1 petroleum/ether) gave a yellow oil, *2,2-dimethylpropionic acid 10-((1R,2S)-2-octadecylcyclopropyl)dec-9-enyl ester* as a 1.3:1 (*E/Z*) mixture (2.24 g, 75%).

The above mixture (2.1 g, 3.9 mmol) in tetrahydrofuran (15 ml) was added dropwise over 5 min to a suspension of lithium aluminium hydride (0.3 g, 7.9 mmol) in tetrahydrofuran (20 ml) at room temperature. The mixture was refluxed for 1 h, then allowed to reach room temperature and quenched carefully with freshly prepared satd aq sodium sulfate decahydrate (10 ml) until a white precipitate was formed, followed by the addition of magnesium sulfate (10 g). The mixture was stirred vigorously for 10 min then filtered through a pad of Celite and washed well with tetrahydrofuran (2×25 ml). The combined organic layers were dried and evaporated to give *(E/Z)-10-((1R,2S)-2-octadecylcyclopropyl)dec-9-en-1-ol* as a colourless oil (1.6 g, 91%), which was used for the next step without purification.

Sodium metaperiodate (7.64 g, 35.7 mmol) in hot water (50 ml) was added over 1 h at 70–80 °C to a stirred solution of (*E/Z*)-10-((1*R*,2*S*)-2-octadecylcyclopropyl)dec-9-en-1-ol (1.6 g, 3.57 mmol) in isopropyl alcohol (50 ml), acetic acid (1 ml), satd aq copper sulfate (1 ml) and hydrazine hydrate (10 ml). The mixture was stirred for 2 h to reach room temperature, then diluted with ether (100 ml). The organic aqueous layer was extracted with ether (2×50 ml). The combined organic layers were washed with water (2×50 ml), dried and evaporated to give a solid; recrystallisation from petroleum gave *10-((1R,2S)-2-octadecylcyclopropyl)decan-1-ol* as a white solid (1.2 g, 75%), mp 58–60 °C [Found M^+: 450.4795, $C_{31}H_{62}O$ requires: 450.4801], $[\alpha]_D^{22}$ +0.54 (*c* 0.735, $CHCl_3$), which showed δ_H (500 MHz, $CDCl_3$): 3.66 (2H, t, *J* 6.65 Hz), 1.61 (2H, pen, *J* 6.65 Hz), 1.53 (1H, br s), 1.45–1.27 (48H, m), 1.18–1.12 (2H, m), 0.91 (3H, t, *J* 7 Hz), 0.68–0.63 (2H, m), 0.59 (1H, dt, *J* 4.1, 8.2 Hz), −0.31 (1H, br q, *J* 5.35 Hz); δ_C (62.5 MHz, $CDCl_3$): 63.11, 32.83, 31.92, 30.21, 29.7 (v. broad), 29.66, 29.61, 29.6, 29.44, 29.35, 28.72, 25.74, 22.68, 15.79, 14.1, 10.92; ν_{max}: 3352 cm^{-1}.

2.1.16. 10-((1*R*,2*S*)-2-Octadecyl-cyclopropyl)decanal (24). 10-((1*R*,2*S*)-2-Octadecylcyclopropyl)decan-1-ol (1.00 g, 2.2 mmol) in dichloromethane (10 ml) was added to a refluxing stirred suspension of pyridinium chlorochromate (1.2 g, 5.5 mmol) in dichloromethane (20 ml). The mixture was stirred vigorously for 2 h, when TLC showed no starting material. The mixture was cooled to room temperature and poured into ether (100 ml) and the precipitate filtered on a pad of silica and washed well with ether. The filtrate was evaporated to give a white solid; chromatography (5:1 petroleum/ether) gave *10-((1R,2S)-2-octadecyl-cyclopropyl) decanal* (**24**) as a white solid (0.95 g, 95%), mp 48–50 °C [Found M^+: 448.4647; $C_{31}H_{60}O$ requires: 448.4644], $[\alpha]_D^{24}$ +0.17 (*c* 1.18, $CHCl_3$), which showed δ_H (500 MHz, $CDCl_3$): 9.78 (1H, t, *J* 1.9 Hz), 2.43 (2H, dt, *J* 1.9, 7.6 Hz), 1.65 (2H, pen, *J* 7.5 Hz), 1.43–1.25 (46H, m), 1.19–1.12 (2H, m), 0.91 (3H, t, *J* 7 Hz), 0.69–0.65 (2H, m), 0.58 (1H, dt, *J* 4.1, 7.9 Hz), −0.31 (1H, br q, *J* 5.35 Hz); δ_C (125 MHz, $CDCl_3$): 202.77, 43.9, 31.9, 30.22, 30.2, 29.74, 29.7, 29.66, 29.62, 29.44, 29.36, 29.2, 28.72, 28.7, 22.68, 22.1, 15.78, 14.1, 10.9; ν_{max}: 1695 cm^{-1}.

2.1.17. 2, 2-Dimethylpropionic acid 9-hydroxynonyl ester. Trimethylacetyl chloride (22.57 g, 187 mmol) was added at room temperature to a stirred solution of 1,9-nonandiol (25 g, 155.9 mmol), pyridine (22.9 ml, 28 mmol) and

4-dimethylaminopyridine (0.5 g) in tetrahydrofuran (200 ml) at 10 °C. The reaction was allowed to reach room temperature and stirred for 16 h. A white precipitate was formed, and the mixture was diluted with ether (200 ml) and washed with dil hydrochloric acid (5%), then the organic layer was separated and the aqueous layer was re-extracted with ether (2×100 ml). The combined organic layers were washed with satd aq sodium bicarbonate (100 ml), water (100 ml), dried and evaporated to give a colourless oil. The crude product was columned, eluting with 5:1 petroleum/ethyl acetate, to give *2,2-dimethylpropionic acid 9-hydroxynonyl ester* as a colourless oil (18.83 g, 49%), [Found M^+: 244.2039; $C_{14}H_{28}O_3$ requires: 244.2038], which showed δ_H (250 MHz, $CDCl_3$): 4.4.03 (2H, t, *J* 6.73 Hz), 3.63 (2H, t, *J* 6.43 Hz), 2.04 (1H, br s), 1.62–1.57 (4H, m), 1.30 (10H, br s), 1.15 (9H, s), δ_C (62.5 MHz, $CDCl_3$): 178.7, 64.4, 62.3, 38.7, 32.7, 29.4, 29.3, 29.1, 28.5, 27.1, 25.8, 25.6; ν_{max}: 3440, 1729 cm^{-1}.

2.1.18. 2,2-Dimethylpropionic acid 9-(benzothiazol-2-ylsulfanyl)nonyl ester. Diethyl azodicarboxylate (11.24 g, 64.5 mmol) in dry tetrahydrofuran (25 ml) was added to a stirred solution of 2,2-dimethylpropionic acid 9-hydroxy-nonyl ester (15 g, 61.5 mmol), triphenylphosphine (17.73 g, 67.6 mmol) and 2-mercaptobenzthiazole (10.8 g, 64.5 mmol) in dry tetrahydrofuran (120 ml) at 0 °C, then allowed to reach room temperature and stirred for 18 h. The solvent was evaporated and the residue was treated with petroleum/ether (5:2) (200 ml) and the precipitate was filtered off on a sinter. The filter cake was washed with petroleum/ether. The combined organic layers were evaporated to give an oil; chromatography (5:1 petroleum/ether) gave *2,2-dimethylpropionic acid 9-(benzothiazol-2-ylsulfanyl)-nonyl ester* as a colourless oil (18 g, 75%) [Found M^+: 393.1790; $C_{21}H_{31}O_2S_2N$ requires: 393.1796], which showed δ_H (250 MHz, $CDCl_3$): 7.83 (1H, br d, *J* 8.04 Hz), 7.72 (1H, br d, *J* 8.4 Hz), 7.45–7.22 (2H, m), 4.00 (2H, t, *J* 6.4 Hz), 3.30 (2H, t, *J* 7 Hz), 1.82–1.74 (2H, pen, *J* 7 Hz), 1.58–1.54 (2H, m), 1.46–1.41 (2H, m), 1.28 (8H, br s), 1.15 (9H, s); δ_C (62.5 MHz, $CDCl_3$): 178.6, 167.35, 153.34, 135.1, 125.97, 124.1, 121.4, 120.9, 64.3, 33.6, 29.3, 29.1, 28.9, 28.7, 28.5, 27.2, 25.8, 15.2; ν_{max}: 1726 cm^{-1}.

2.1.19. 2,2-Dimethylpropionic acid 9-(benzothiazole-2-sulfonyl)nonyl ester (23). A solution of ammonium heptamolybdate(VI) tetra-hydrate (3.55 g, 2.8 mmol) in 35% H_2O_2 (w/w) (14 ml, 143.5 mmol) was added dropwise at 5 °C to a stirred solution of 2,2-dimethylpropionic acid 9-(benzothiazol-2-ylsulfanyl)nonyl ester (12 g, 30.5 mmol) in methylated spirit (200 ml). The resulting yellow solution was stirred for 1 h at this temperature then for 16 h at room temperature. The solvent was evaporated to give a yellow solid, which was diluted with water (100 ml) and the product was extracted with dichloromethane (3×100 ml). The combined organic layers were washed with brine (50 ml), dried and evaporated to give an oil. Chromatography (5:1 petroleum/ethyl acetate) gave *2,2-dimethylpropionic acid 9-(benzothiazole-2-sulfonyl)nonyl ester* (**23**) as a thick yellow oil (9.5 g, 73%) [Found M^+: 425.1711; $C_{21}H_{31}O_4S_2N$ requires: 425.1695], which showed δ_H (250 MHz, $CDCl_3$): 8.23 (1H, br d, *J* 8 Hz), 8.01 (1H, br d, *J* 8 Hz), 7.65–7.55 (2H, m), 4.02 (2H, t, *J* 6.7 Hz), 3.53–3.46 (2H, m), 1.92–1.82 (2H, m), 1.62–1.55 (2H, m), 1.45–1.35 (2H, m), 1.27 (8H, br s), 1.18 (9H, s); δ_C (62.5 MHz, $CDCl_3$): 178.62, 165.86, 152.7, 136.74, 127.99, 127.64, 125.4, 122.33, 64.3, 54.67, 38.7, 29, 28.8, 28.5, 28.16, 27.17, 25.78, 22.2; ν_{max}: 1723, 1148 cm^{-1}.

2.1.20. (*S*)-2,2-Dimethyl-4-{(1*R*,2*R*)-2-[(*S*)-1-methyl-13-((1*R*,2*S*)-2-octadecylcyclopropyl)tridecyl]cyclopropyl}-[1,3]dioxolane (25). Lithium hexamethyldisilazide (2.8 ml, 2.8 mmol) was added dropwise to a stirred solution of 2-{(*S*)-3-[(1*R*,2*R*)-2-((*S*)-2,2-dimethyl[1,3]dioxolan-4-yl) cyclo-propyl]butane-1-sulfonyl}benzothiazole (**18**) (0.91 g, 2.3 mmol) and 10-((1*R*,2*S*)-2-octadecylcyclopropyl)-decanal (**24**) (0.9 g, 2 mmol) in dry tetrahydrofuran (20 ml) under nitrogen at −5 °C. The reaction was exothermic and the temperature rose to 0 °C, resulting in a dark orange solution. The mixture was allowed to reach room temperature and stirred for 16 h, cooled to 0 °C and quenched with satd aq ammonium chloride (5 ml). The product was extracted with 1:1 petroleum/ether (2×50 ml); the combined organic layers were washed with brine, dried and evaporated to give a thick yellow oil. Chromatography (5:1 petroleum/ether) gave *(S)-2,2-dimethyl-4-{(1R,2R)-2-[(E/Z)-(S)-1-methyl-13-((1R,2S)-2-octadecylcyclopropyl)tridec-3-enyl]-cyclopropyl}-[1,3]dioxolane* (**25**) (0.98 g, 78%). Freshly distilled acetic acid (1.37 g, 22.9 mmol) in methanol (5 ml) was added slowly to a stirred solution of the above [1,3]-dioxo-lane (0.9 g, 1.43 mmol) and dipotassium azodicarboxylate (2.78 g, 14.3 mmol) in methanol/tetrahydrofuran (30 ml) (2:1) at room temperature. The mixture was stirred for 24 h then additional dipotassium azodicarboxylate (2.78 g) and acetic acid (1.4 g) were added and stirred for a further 24 h. The reaction was poured into water (15 ml) slowly and the product was extracted with petroleum/ether (3×30 ml). The combined organic layers were washed with satd aq sodium bicarbonate (15 ml), dried and evaporated, to give a residue; chromatography (10:1 petroleum/ether) gave *(S)-2,2-dimethyl-4-{(1R,2R)-2-[(S)-1-methyl-13-((1R,2S)-2-octadecylcyclopropyl)-tridecyl]cyclopropyl}[1,3]-dioxolane* as a colourless oil which solidified later (0.75 g, 86%) [Found M^+: 630.6300, $C_{43}H_{82}O_2$ requires: 630.6315], $[\alpha]_D^{24}$ −7.0 (*c* 1.1, $CHCl_3$); which showed δ_H (500 MHz, $CDCl_3$): 4.11–4.09 (1H, m), 3.72–3.68 (2H, m), 1.45 (3H, s), 1.42–1.13 (62H, br m, including a singlet integrating to 3H), 1.02 (3H, br s), 0.95–0.91 (1H, m), 0.88 (3H, t, *J* 7 Hz), 0.83 (1H, dt, *J* 4.5, 8 Hz), 0.71–0.64 (3H, m), 0.57 (1H, dt, *J* 4.5, 8.5 Hz), 0.24 (1H, br q, *J* 5.5 Hz), −0.33 (1H, br q, *J* 5 Hz); δ_C (125 MHz, $CDCl_3$): 108.27, 77.88, 70, 37.4, 33.3, 31.9, 30.2, 30, 29.7 (v. broad), 29.6, 29.36, 28.7, 27.1, 26.88, 25.74, 23.87, 22.68, 20, 19.2, 15.76, 14.1, 10.9, 9.04; ν_{max}: 2923, 2852, 1062 cm^{-1}.

2.1.21. *cis*-(1*R*,2*R*)-2-[(*S*)-1-Methyl-13-((1*R*,2*S*)-2-octadecylcyclopropyl)tridecyl]cyclopropanecarbaldehyde (26). Periodic acid (0.6 g, 2.3 mmol) was added to a stirred solution of (*S*)-2,2-dimethyl-4-{(1*R*,2*R*)-2-[(*S*)-1-methyl-13-((1*R*,2*S*)-2-octadecylcyclopropyl)tridecyl]-cyclopropyl} [1,3]dioxolane (0.7 g, 1.11 mmol) in dry ether (30 ml) under nitrogen at room temperature. The mixture was stirred for 16 h, when TLC showed no starting material. The precipitate was removed and the solvent was evaporated to give a residue; chromatography (10:1 petroleum/ether) gave *cis-(1R,2R)-2-[(S)-1-methyl-13-((1R,2S)-2-octadecylcyclopropyl) tridecyl]cyclopropanecarbaldehyde* (**26**) as a white solid

(0.6 g, 97%), mp 35–37 °C [Found M^+: 558.5751, $C_{39}H_{74}O$ requires: 558.5740], $[\alpha]_D^{24}$ −0.096 (*c* 1.04, $CHCl_3$); which showed δ_H (500 MHz, $CDCl_3$): 9.38 (1H, d, *J* 6 Hz), 1.97–1.91 (1H, m), 1.45–1.17 (61H, br m), 1.09 (3H, d, *J* 6.5 Hz), 0.93 (3H, t, *J* 6.5 Hz), 0.91–0.88 (1H, m), 0.72–0.65 (2H, m), 0.61 (1H, dt, *J* 3.5, 8 Hz), −0.29 (1H, br q, *J* 4.5 Hz); δ_C (125 MHz, $CDCl_3$): 201.76 (−), 37.41 (+), 32.3 (−), 32.03 (−), 31.9 (+), 30.2 (+), 29.86 (+), 29.73 (+), 29.7(+) (v. broad), 29.65 (+), 29.6 (+), 29.36 (+), 28.7 (+), 28.66 (−), 26.8 (+), 22.68 (+), 20.1 (−), 15.77 (−), 14.1 (−), 13.6 (+), 10.9 (+); ν_{max}: 2917, 2852, 1692 cm^{-1}.

2.1.22. *trans*-(1*S*,2*R*)-2-[(*S*)-1-Methyl-13-((1*R*,2*S*)-2-octadecylcyclopropyl)tridecyl]cyclopropanecarbaldehyde (27). Sodium methoxide (0.053 g, 0.985 mmol) was added to a stirred solution of *cis*-(1*R*,2*R*)-2-[(*S*)-1-methyl-13-((1*R*,2*S*)-2-octadecylcyclopropyl)tridecyl]cyclopropanecarbaldehyde (0.5 g, 0.896 mmol) in methanol (15 ml) and tetrahydrofuran (10 ml) and refluxed for 56 h. The mixture was cooled to room temperature, quenched with satd aq ammonium chloride (20 ml), and extracted with 1:1 petroleum/ether (3×30 ml). The combined organic layers were dried and evaporated to yield a thick oil which solidified later as a mixture of trans- and cis-isomers in the ratio 22:1. Chromatography (10:0.5 petroleum/ether) gave *trans-(1S,2R)-2-[(S)-1-methyl-13-((1R,2S)-2-octadecylcyclopropyl) tridecyl]cyclopropanecarbaldehyde* (**27**) as a jelly-like solid (0.35 g, 70%) [Found M^+: 558.5756, $C_{39}H_{74}O$ requires: 558.5740], $[\alpha]_D^{22}$ +9.6 (*c* 1.15, $CHCl_3$); which showed δ_H (500 MHz, $CDCl_3$): 9.00 (1H, d, *J* 5.65 Hz), 1.72–1.68 (1H, m), 1.44–1.22 (59H, br m), 1.19–1.13 (2H, br m), 0.99 (3H, br s), 0.98–0.92 (1H, m), 0.91 (3H, t, *J* 7 Hz), 0.69–0.64 (2H, br m), 0.58 (1H, dt, *J* 4.1, 8.2 Hz), −0.31 (1H, br q, *J* 5 Hz); δ_C (125 MHz, $CDCl_3$): 201, 36.8, 31.93, 30.42, 30.22, 29.88, 29.73, 29.7 (v. broad), 29.62, 29.36, 29.27, 28.72, 27.03, 22.7, 19.35, 15.77, 14.11, 13.27, 10.9; ν_{max}: 2921, 2848, 1690 cm^{-1}.

2.1.23. 5-(7-Bromo-heptylsulfanyl)-1-phenyl-1*H*-tetrazole. Diethyl azodicarboxylate (5.36 g, 30.76 mmol) in dry tetrahydrofuran (10 ml) was added to a stirred solution of 7-bromoheptan-1-ol (5 g, 25.6 mmol), triphenylphosphine (8.74 g, 33.3 mmol) and 1-phenyl-1H-tetrazol-5-thiol (5.48 g, 30.76 mmol) in tetrahydrofuran (100 ml) at 5 °C under nitrogen. The mixture was allowed to reach room temperature and stirred for 24 h, then the solvent was evaporated and the residue was dissolved in ether (150 ml) and petroleum (50 ml) and stirred for 15 min. The precipitate was removed through a pad of Celite and the filtrate was evaporated to give a pale yellow oil. This was dissolved in ether and suspended on silica and then columned (1:1 petroleum/ether) to give 5-(7-bromoheptylsulfanyl)-1-phenyl-1*H*-tetrazole as a pale yellow oil (5 g, 55%) [Found M^+: 356.0509; $C_{14}H_{19}N_4Br^{81}$ requires: 356.0493], which showed δ_H (250 MHz, $CDCl_3$): 7.55 (5H, br s), 3.38 (4H, br t, *J* 6.4 Hz), 1.82 (4H, br s), 1.47 (6H, br s); δ_C (62.5 MHz, $CDCl_3$): 154.4, 133.66, 130.1, 129.76, 123.8, 33.89, 33.21, 32.57, 28.94, 28.37, 28.1, 27.9; ν_{max}: 2931, 2855, 1499, 1411, 761, 694 cm^{-1}.

2.1.24. 1-Phenyl-5-[17-(tetrahydropyran-2-yloxy)heptadecylsulfanyl]-1*H*-tetrazole. 1-Bromo-10-tetrahydropyranyloxydecane (5.7 g, 17.7 mmol) in dry tetrahydrofuran (15 ml) was added dropwise to a suspension of magnesium turnings (0.56 g, 23 mmol) in dry tetrahydrofuran (20 ml) under nitrogen. Once the exothermic reaction had subsided, the mixture was refluxed for 1 h then cooled to room temperature. The Grignard reagent was added to a stirred solution of 5-(7-bromoheptylsulfanyl)-1-phenyl-1*H*-tetrazole (4.5 g, 12.67 mmol) in tetrahydrofuran (30 ml) at 5 °C. The mixture was cooled to −40 °C followed by the addition of dilithium tetrachlorocuprate (2.5 ml), stirred for 2 h at this temperature and at room temperature for 12 h, then satd aq ammonium chloride (100 ml) was added together with ether (100 ml). The organic layer was separated and the aqueous layer was re-extracted with ether (2×50 ml). The combined organic layers were washed with water, dried and evaporated to give a residue; chromatography (5:2 petroleum/ether) gave *1-phenyl-5-[17-(tetrahydropyran-2-yloxy)heptadecylsulfanyl]-1H-tetrazole* as a colourless oil (2 g, 31%), which showed δ_H (250 MHz, $CDCl_3$): 7.57 (5H, br s), 4.57 (1H, br t, *J* 3.9 Hz), 3.82–3.89 (1H, m), 3.74 (1H, dt, *J* 7, 9.4 Hz), 3.52–3.46 (1H, m), 3.42–3.32 (4H, including a triplet with coupling constant 7.5 Hz), 1.85–1.75 (4H, m), 1.58–1.22 (31H, br m); δ_C (62.5 MHz, $CDCl_3$): 154.5, 133.7, 130, 129.7, 123.8, 98.8, 67.67, 62.3, 33.8, 33.34, 33.2, 32.6, 30.76, 29.6 (v. broad), 29, 28.6, 28.4, 28.1, 27.9, 26.2, 25.5, 19.7; ν_{max}: 2916, 2852, 1499, 1033, 760 cm^{-1}.

2.1.25. 1-Phenyl-5-[17-(tetrahydro-pyran-2-yloxy)heptadecane-1-sulfonyl]-1*H*-tetrazole (30). To a stirred solution of 1-phenyl-5-[17-(tetrahydropyran-2-yloxy)heptadecylsulfanyl]-1*H*-tetrazole (1.5 g, 2.9 mmol) in methylated spirit (30 ml) was added dropwise a yellow solution of ammonium heptamolybdate(VI) tetra-hydrate (0.34 g, 0.27 mmol), in 35% H_2O_2 (w/w) (1.4 ml, 14.5 mmol) at 10 °C. The resulting yellow solution was stirred for 1 h at this temperature and then for 16 h at room temperature. The solvent was then evaporated to give a yellow solid, which was treated with water (50 ml) and satd aq sodium bicarbonate (25 ml) and then the product was extracted with dichloromethane (3×50 ml). The combined organic layers were washed with brine (50 ml), dried and evaporated to give the crude product. Chromatography (5:3 petroleum/ethyl acetate) gave a white solid, *17-(1-phenyl-1H-tetrazole-5-sulfonyl)-heptadecan-1-ol* (0.8 g, 59%), mp 65–67 °C [Found M^+: 464.2817; $C_{24}H_{40}O_3SN_4$ requires: 464.2821], which showed δ_H (250 MHz, $CDCl_3$): 7.71–7.55 (5H, m), 3.73 (2H, br t, *J* 7.9 Hz), 3.63 (2H, t, *J* 6.4 Hz), 1.97–1.88 (2H, br m), 1.81–1.25 (29H, br m); δ_C (62.5 MHz, $CDCl_3$): 153.45, 133, 131.4, 129.7, 125.1, 63.1, 55.97, 32.7, 29.6, 29.4, 29.1, 28.8, 28.1, 25.7, 21.9; ν_{max}: 3430, 1496, 1464, 1339, 1149, 909 cm^{-1}. Pyridinium *p*-toluenesulfonate (65 mg, 0.258 mmol) was added to a stirred solution of the alcohol (0.8 g, 1.7 mmol) and 3,4-dihydro-2*H*-pyran (0.3 g, 3.45 mmol) in dry dichloromethane (30 ml) under nitrogen at room temperature. The mixture was stirred for 2 h, when TLC showed no starting material, quenched with satd aq sodium bicarbonate (15 ml), water (10 ml) and extracted with dichloromethane (3×20 ml). The combined organic layer was dried and evaporated; chromatography of the residue, eluting with petroleum/ethyl acetate (5:1.5) gave a white solid, *1-phenyl-5-[17-(tetrahydropyran-2-yloxy) heptadecane-1-sulfonyl]-1H-tetrazole* (**30**) (0.93 g, 98%), mp 56–58 °C [Found $M-H^+$: 547.3327; $C_{29}H_{48}O_4SN_4$ requires:

547.3318], which showed δ_H (250 MHz, $CDCl_3$): 7.71–7.60 (5H, m), 4.58 (1H, t, *J* 3.9 Hz), 3.95–3.83 (1H, m), 3.77–3.71 (3H, m), 3.58–3.50 (1H, m), 3.43–3.34 (1H, m), 2.05–1.89 (2H, m), 1.85–1.22 (34H, br m); δ_C (62.5 MHz, $CDCl_3$): 153.5, 133.7, 131.4, 129.7, 125.1, 98.8, 67.7, 62.3, 56.00, 30.7, 29.64, 29.2, 28.9, 28.13, 26.2, 25.5, 21.9, 19.7; ν_{max}: 2924, 2852, 1497, 1465, 1342, 1152 cm^{-1}.

2.1.26. 18-{(1*S*,2*R*)-2-[(*S*)-1-Methyl-13-((1*R*,2*S*)-2-octadec-ylcyclopropyl)tridecyl]cyclopropyl}octadecan-1-ol. Lithium hexamethyldisilazide (0.81 ml, 0.81 mmol) was added dropwise to a stirred solution of tetrazole **30** (0.294 g, 0.537 mmol) and *trans*-(1*S*,2*R*)-2-[(*S*)-1-methyl-13-((1*R*,2*S*)-2-octadecylcyclopropyl)tridecyl]cyclopropanecarbaldehyde (0.25 g, 0.45 mmol) in dry tetrahydrofuran (15 ml) under nitrogen at 2–4 °C. The reaction was exothermic and the temperature rose to 6 °C, resulting in a yellow solution. This was allowed to reach room temperature and stirred for 2 h, when TLC showed no starting material. It was cooled to 0 °C and quenched with satd aq ammonium chloride (3 ml). The product was extracted with 1:1 petroleum/ether (3×30 ml); the combined organic layers were washed with brine, dried and evaporated to give a thick yellow oil; chromatography (10:0.5 petroleum/ether) gave *(E/Z)-2-(18-{(1R,2R)-2-[(S)-1-methyl-13-((1R,2S)-2-octadecylcyclopropyl)tridecyl]cyclopropyl}octadec-17-enyloxy) tetrahydropyran* (**31**) (0.36 g, 90%). *p*-Toluenesulfonic acid monohydrate (0.14 g, 0.738 mmol) was added to a stirred solution of (*E*/*Z*)-mixture **31** (0.325 g, 0.37 mmol) in tetrahydrofuran (15 ml) and methanol (4 ml) at room temperature. The mixture was stirred for 3 h, when TLC showed no starting material, then quenched with satd aq sodium bicarbonate (15 ml) and extracted with petroleum/ether (1:1) (3×30 ml). The combined organic layers were washed with brine (15 ml), water (15 ml), dried and evaporated, to give a white solid, *(E/Z)-18-{(1R,2R)-2-[(R)-1-methyl-13-((1R,2S)-2-octadecylcyclopropyl)tridecyl]cyclopropyl}octadec-17-en-1-ol* (0.28 g, 95%) which was used for the next step without purification. Sodium metaperiodate (1.34 g, 6.3 mmol) in hot water (25 ml) was added over 45 min to a stirred solution of the above (*E*/*Z*) mixture (0.25 g, 0.314 mmol) in isopropyl alcohol (30 ml), acetic acid (1 ml), satd aq copper sulfate (1 ml) and hydrazine hydrate (5 ml) at 60 °C. The temperature rose to 80 °C through the addition. The mixture was stirred for 2 h to reach room temperature then extracted with petroleum/ether (1:1) (2×50 ml), dried and evaporated to give a white solid. The reduction was repeated in order to complete the hydrogenation. Chromatography of the crude product (5:1 petroleum/ether) gave *18-{(1S,2R)-2-[(S)-1-methyl-13-((1R,2S)-2-octadecylcyclopropyl)tridecyl]cyclopropyl}octadecan-1-ol* (0.21 g, 85%), mp 60–62 °C [Found M^+: 798.8540, $C_{56}H_{110}O$ requires: 798.8557], $[\alpha]_D^{22}$ +2.44 (*c* 1.23, $CHCl_3$); which showed δ_H (500 MHz, $CDCl_3$): 3.65 (2H, t, *J* 6.6 Hz), 1.61–1.55 (2H, pent, *J* 6.6 Hz, and 1H, br s, for the hydroxyl group), 1.43–1.22 (86H, br m), 1.20–1.12 (4H, m), 0.904 (3H, d, *J* 6.65 Hz), 0.89 (3H, t, *J* 7 Hz), 0.69–0.63 (3H, m), 0.57 (1H, br dt, *J* 4.1, 8.2 Hz), 0.48–0.42 (1H, m), 0.22–0.18 (1H, m), 0.17–0.14 (1H, m), 0.13–0.09 (1H, m), −0.32 (1H, br q, *J* 4.75 Hz), δ_C (125 MHz, $CDCl_3$): 63.1 (+), 38.1 (−), 37.43 (+), 34.49 (+), 32.84 (+), 31.93 (+), 30.23 (+), 30.08 (+), 29.71 (+, v. broad), 29.66 (+), 29.62 (+), 29.44 (+), 29.36 (+), 28.73 (+), 27.26 (+), 26.15 (−), 25.75 (+), 22.69 (+), 19.69 (−), 18.63 (−), 15.79 (−), 14.1 (−), 10.92 (+), 10.5 (+); ν_{max}: 3406, 2910, 1215, 1056 cm^{-1}.

2.1.27. 18-{(1*S*,2*R*)-2-[(*S*)-1-Methyl-13-((1*R*,2*S*)-2-octadecylcyclopropyl)tridecyl]cyclopropyl}octadecanal (32). 18-{(1*S*,2*R*)-2-[(*S*)-1-Methyl-13-((1*R*,2*S*)-2-octadecylcyclopropyl)tridecyl]cyclopropyl}octadecan-1ol (0.15 g, 0.188 mmol) was dissolved in hot dichloromethane (5 ml) and added to a refluxing stirred suspension of pyridinium chlorochromate (0.1 g, 0.47 mmol) in dichloromethane (15 ml). The mixture was refluxed and stirred vigorously for 2 h, when TLC showed no starting material. The mixture was cooled to room temperature and poured into diethyl ether (50 ml), then the precipitate was filtered through a bed of silica and washed well with ether and the filtrate was evaporated to give a white solid. Chromatography (5:1 petroleum/ether) gave *18-{(1S,2R)-2-[(S)-1-methyl-13-((1R,2S)-2-octadecylcyclopropyltridecyl]cyclopropyl}octadecanal* as a white solid (**32**) (0.12 g, 80.5%), mp 48–50 °C [Found M^+: 796.8361, $C_{56}H_{108}O$ requires: 796.8400], $[\alpha]_D^{22}$ +2.8 (*c* 1.02, $CHCl_3$) which showed δ_H (500 MHz, $CDCl_3$): 9.77 (1H, t, *J* 1.8 Hz, C*H*O), 2.42 (2H, dt, *J* 1.6, 7.25 Hz, CH_2CHO),1.63 (2H, pent, *J* 7.22 Hz, CH_2CH_2CHO), 1.42–1.24 (82H, m, satd alkane), 1.23–1.12 (6H, m, satd alkane), 0.90 (3H, d, *J* 6.65 Hz, α-Me), 0.89 (3H, t, *J* 7 Hz, terminal CH_3), 0.71–0.62 (3H, m, 2×C*H*-*cis*-cyclopropane and C*H*CH_3), 0.57 (1H, dt, *J* 4.3, 8.0 Hz, CH_2-*cis*-cyclopropane), 0.48–0.42 (1H, m, CH-*trans*-cyclopropane), 0.22–0.178 (1H, m, CH-*trans*-cyclopropane), 0.169–0.138 (1H, m, CH_2-*trans*-cyclopropane), 0.128–0.093 (1H, m, CH_2-*trans*-cyclopropane), −0.32 (1H, dt, *J* 4.3, 5.3 Hz, CH_2-*cis*-cyclopropane); δ_C (125 MHz, $CDCl_3$): 202.84 (−), 43.92 (+), 38.11 (−), 37.43 (+), 34.49 (+), 31.93 (+), 30.23 (+), 30.08 (+), 29.7 (+, v. broad), 29.66 (+), 29.61 (+), 29.59 (+), 29.44 (+), 29.36 (+), 29.18 (+), 28.73 (+), 27.27 (+), 26. 15 (−), 22.68 (+), 22.11 (+), 19.67 (−), 18.62 (−), 15.79 (−), 14.1 (−), 10.92 (+), 10.49 (+); ν_{max}: 2918, 2847, 1720 cm^{-1}.

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Kinetics and mechanisms of the reactions of *S*-methyl chlorothioformate with pyridines and secondary alicyclic amines

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Abstract—The pyridinolysis of *S*-methyl chlorothioformate shows a biphasic Brønsted-type plot, in agreement with a stepwise mechanism and a change in the rate-limiting step, from formation of a zwitterionic tetrahedral intermediate ($T^{\pm}$) at high pK_a to its breakdown at low pK_a. The reaction of the same substrate with secondary alicyclic amines shows a linear Brønsted-type plot of slope 0.23, which is in accordance with a stepwise mechanism where formation of $T^{\pm}$ is the rate-determining step for the whole pK_a range examined.

1. Introduction

There have been many reports on the kinetics and mechanisms of the solvolysis[1,2] and aminolysis[3] of chloroformates (RO–CO–Cl). Nevertheless, the solvolysis of chlorothioformates (RS–CO–Cl) and chlorothionoformates (RO–CS–Cl),[4] as well as the aminolysis of the latter compounds[5] have received little attention. Furthermore, to our knowledge, there have been no investigations concerning the kinetics of the aminolysis of *S*-alkyl chlorothioformates.

The hydrolysis of *S*-methyl chlorothioformate (**1**) and the solvolysis of the *S*-ethyl analogue in several alcohol–water mixtures were found to be driven by an S_N1 mechanism.[4a,b] Nevertheless, the solvolysis of the latter substrate in pure methanol and ethanol, and in 90% ethanol–water is governed by an addition–elimination process.[4b]

The pyridinolysis of methyl chloroformate (**2**) in water shows a curved (biphasic) Brønsted-type plot, with slopes β=0.93 at low pK_a and β=0.15 at high pK_a.[3b] This was explained by a stepwise mechanism, through a zwitterionic tetrahedral intermediate ($T^{\pm}$), and a change in the rate-limiting step, from $T^{\pm}$ breakdown to $T^{\pm}$ formation as the amine becomes more basic.[3b] The reactions of piperidine and pyrrolidine, as well as a series of aliphatic amines, with isopropyl chloroformate are driven by an addition–elimination mechanism involving a $T^{\pm}$ intermediate.[3c] The pyridinolysis of phenyl and 4-nitrophenyl chloroformates in acetonitrile exhibits linear Brønsted-type plots with slopes (β) of ca. 0.3, which was attributed to a stepwise mechanism where the formation of the intermediate $T^{\pm}$ is the rate-limiting step.[3d]

The reactions of secondary alicyclic (SA) amines[5a] and pyridines[5b] with phenyl and 4-nitrophenyl chlorothionoformates (**3** and **4**, respectively) and those of the former amines with a series of aryl chloroformates,[3f,g] in water exhibit linear Brønsted-type plots consistent with a stepwise process, where the formation of the intermediate $T^{\pm}$ is the rate-limiting step.

On the other hand, the anilinolysis of a series of aryl chlorothionoformates in acetonitrile has been claimed to proceed by a concerted mechanism through a four-membered hydrogen-bonded cyclic transition state.[5c] Similarly, the phenolysis of **3** and **4** in an aqueous solution was found to be governed by a concerted process.[6]

Due to these apparent contradictory results and in order to clarify the mechanisms of the aminolysis of chlorothioformates and their oxy analogues, in the present work we undertake a kinetic and mechanistic study of the reactions of pyridines and SA amines with chlorothioformate **1** in aqueous solution. Specific aims are the comparison of these reactions between them, and with the pyridinolysis of the corresponding chloroformate (**2**) in water,[3b] and the pyridinolysis and SA aminolysis of *S*-methyl 4-nitrophenyl and *S*-methyl 2,4-dinitrophenyl thiocarbonates (**5** and **6**, respectively) in water.[7] Through these comparisons the effects of the amine nature and the S atom in the nonleaving group, as well as the influence of the leaving group on the kinetics and mechanism will be assessed.

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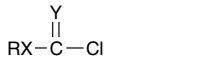
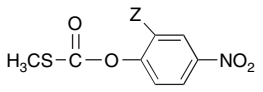


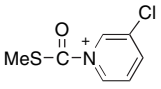
1 (R = methyl; X = S; Y = O)
2 (R = methyl; X = O; Y = O)
3 (R = phenyl, X = O; Y = S)
4 (R = 4-nitrophenyl, X = O; Y = S)



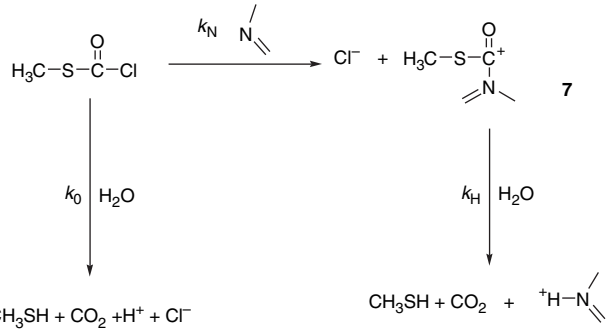
5 (Z = H)
6 (Z = NO_2)

2. Results and discussion

The spectrophotometric study of the reactions of **1** with pyridines in water, shows the appearance and disappearance of an intermediate at 270–320 nm. We assume this intermediate to be compound **7** (Scheme 1) because similar intermediates have been detected spectrophotometrically or isolated in the pyridinolysis of methyl chloroformate,[3b] aryl chlorothionoformates,[5b] and other compounds.[8] As an example, Figure 1 shows a plot of absorbance at 290 nm vs time obtained for the reaction of 3-chloropyridine with **1**. This intermediate is probably compound **7a**.



7a



Scheme 1.

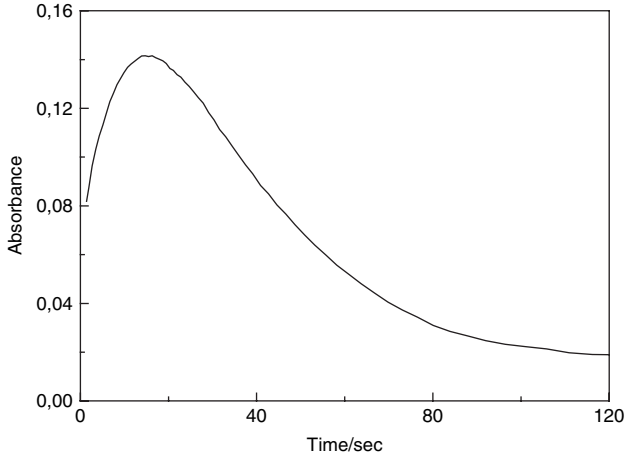
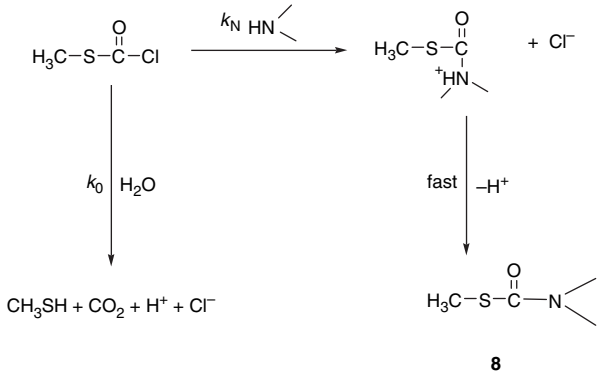


Figure 1. Plot of absorbance at 290 nm (1 cm cell) against time obtained for the reaction of **1** with 3-chloropyridine in an aqueous solution at 25.0 °C with an ionic strength of 0.2 M (KCl). Concentration of total amine 3.5×10^{-2} M, pH=2.97. The intermediate is presumably compound **7a**.

For the reactions involving SA amines, only the increase of a band in the 210–240 region is observed, probably because the corresponding cation intermediate rapidly deprotonates, leading to the stable carbamate-type product **8** (see Scheme 2).



8

Scheme 2.

Under amine excess over the substrate, pseudo-first-order rate coefficients (k_{obsd}) were obtained for all the reactions. The experimental conditions of the reactions and the values of k_{obsd} are shown in Tables 1and 2.

The rate law obtained for the reactions of **1** with the series of pyridines and SA amines is shown in Eqs. 1 and 2, where P represents either intermediate **7** (for pyridines, Scheme 1) or the corresponding thiocarbamate **8** (for SA amines, Scheme 2), S represents the substrate, and N the free amine. The rate constants k_0 and k_N are those for hydrolysis and aminolysis of the substrate, respectively.

Table 1. Experimental conditions and values of k_{obsd} for the pyridinolysis of **1**[a]

Pyridine substituent	pH	10^3 $[N]_{tot}$/M	10^2 k_{obsd}/s^{-1}	No. of runs
4-$N(CH_3)_2$	9.56	0.82–6.94	7.37–354	6
	9.87	0.82–8.17	12.0–568	7
4-NH_2	9.06	1.84–15.6	9.61–61.7	6
	9.37	0.85–7.22	16.4–59.0	6
	9.68	0.85–8.50	15.8–75.5	7
3,4-$(CH_3)_2$	6.46	1.77–7.09	6.58–17.9	6
	6.77	0.93–9.33	6.75–35.31	7
	7.08	0.54–5.35	5.54–24.4	7
H	5.06	1.01–10.1	3.63–15.9	7
	5.37	1.01–8.6	3.84–18.8	6
	5.68	1.01–10.1	7.45–23.7	7
3-$CONH_2$	3.12	14.5–58.1	10.9–23.4	6
	3.74	13.1–39.4	9.12–19.9	6
3-Cl	2.66	8.81–74.9	7.48–20.8	6
	2.97	8.81–88.1	5.55–32.6	7
4-CN	5.00[b]	8.32–83.2	1.98–6.03	7
	5.30[b]	8.32–83.2	1.89–6.57	7
	5.60[b]	8.22–82.2	1.76–5.92	7
3-CN	5.00[b]	8.28–82.8	1.29–2.72	7
	5.30[b]	8.32–83.2	1.11–2.83	7
	5.60[b]	8.32–83.2	1.08–2.78	7

[a] In aqueous solution, at 25.0 °C with an ionic strength of 0.2 M.
[b] Under the presence of an acetate buffer 0.005 M.

Table 2. Experimental conditions and values of k_{obsd} for the SA aminolysis of **1**[a]

Amine	pH	10^3 $[N]_{tot}$/M	10^3 k_{obsd}/s^{-1}	No. of runs
Piperidine[b]	8.0	0.5–5.0	8.66–15.7	6
	8.5	0.5–5.0	10.6–28.7	6
	9.0	0.5–5.0	18.3–58.6	6
Piperazine	9.64	0.05–0.50	35.6–277	6
	9.94	0.05–0.50	59.7–344	7
	10.24	0.05–0.50	67.8–365	7
1-(2-Hydroxyethyl)piperazine	9.08	0.05–0.50	50.3–104	7
	9.38	0.125–0.50	57.7–123	6
	9.68	0.05–0.50	62.0–162	7
Morpholine	8.48	0.5–5.0	123–846	7
	8.78	0.5–3.5	128–884	5
	9.08	0.10–1.25	70.2–577	8
Formylpiperazine	7.68	0.5–1.6	45.0–155	7
	7.98	0.25–2.50	38.5–340	7
	8.28	0.25–2.50	44.5–450	7
Piperazinium ion	5.51	0.5–5.0	15.9–132	7
	5.81	0.5–16.2	22.7–685	7
	6.11	0.5–5.0	24.4–225	7

[a] In aqueous solution, at 25.0 °C with an ionic strength of 0.2 M.
[b] Under the presence of borate buffer 0.01 M.

$$d[P]/dt = k_{obsd}[S] \quad (1)$$

$$k_{obsd} = k_0 + k_N[N] \quad (2)$$

The values of k_N were obtained as the slopes of plots of Eq. 2 at constant pH, and were pH independent. Tables 3 and 4 summarize the values of k_N found, together with those for the pK_a of the conjugate acids of the pyridines and SA amines, respectively.

Table 3. Values of pK_a of the conjugate acid of pyridines and of k_N for the pyridinolysis of **1**[a]

Pyridine substituent	pK_a	k_N[b]/s^{-1} M^{-1}
4-$N(CH_3)_2$	9.87	128±7
4-NH_2	9.42	117±8
3,4-$(CH_3)_2$	6.77	63±2
H	5.37	34±2
3-$CONH_2$	3.43	7.0±0.7
3-Cl	2.97	6.5±0.4
4-CN	2.20	0.57±0.02
3-CN	1.55	0.22±0.06

[a] In aqueous solution, at 25.0 °C, with an ionic strength of 0.2 M.
[b] Nucleophilic rate constant for the formation of intermediate **7** in Scheme 1.

Table 4. Values of pK_a for the conjugate acid of SA amines and of k_N for the SA aminolysis of **1**[a]

SA amine	pK_a	$10^{-2}k_N$[b]/s^{-1} M^{-1}
Piperidine	11.24	17.5±0.5
Piperazine	9.94	10.9±0.6
1(2-Hydroxyethyl)piperazine	9.38	3.44±0.2
Morpholine	8.78	4.88±0.2
Formylpiperazine	7.68	2.65±0.09
Piperazinium ion	5.81	0.82±0.02

[a] In aqueous solution, at 25.0 °C, and an ionic strength of 0.2 M.
[b] Nucleophilic rate constant for the formation of product **8** in Scheme 2.

The fact that the values of k_{obsd} increase as the concentration of the amine increases rules out an S_N1 mechanism for the aminolysis of the title substrate. This is in contrast to the ionization process found for the hydrolysis of this substrate.[4a]

The Brønsted-type plots of Figure 2 were obtained with the data in Table 3 for the pyridinolysis of the substrate and with the data of Table 4, after statistical correction of k_N and pK_a, for the reactions with SA amines. The latter were corrected with $p=2$ for all the conjugate acids of the SA amines, except piperazinium ion for which $p=4$, and with $q=2$ for piperazine ($q=1$ for the other SA amines).[9,10] The statistical parameter q is the number of equivalent basic sites in the amine and p is the number of equivalent protons in the conjugate acid of the amine.[10] As can be observed, the Brønsted-type plot is linear for the SA aminolysis and biphasic for the pyridinolysis.

The curved Brønsted line in Figure 2 for the pyridinolysis of **1** was calculated by means of a semiempirical equation (Eq. 3) based on the existence of a zwitterionic tetrahedral intermediate ($T^{\pm}$) on the reaction pathway (see Scheme 3).[3b,9,11]

This equation contains four parameters: β_1 and β_2, which are the Brønsted slopes at high and low pK_a, respectively, and $\log k_N^0$ and pK_a^0, which are the corresponding values at the center of the curvature.

$$\log\left(k_N/k_N^0\right) = \beta_2\left(pK_a - pK_a^0\right) - \log(1 + a/2) \quad (3)$$

$$\log a = (\beta_2 - \beta_1)\left(pK_a - pK_a^0\right)$$

The Brønsted curve was calculated by means of the following parameters: $\log k_N^0=1.0$, $pK_a^0=3.6$, $\beta_1=0.12$, and $\beta_2=1.0$ ($n=8$, $R=0.996$). The errors of the slopes are ±0.1, and those of pK_a^0 and $\log k_N^0$ are ±0.2 and ±0.1, respectively. The curved Brønsted plot can be explained by the existence of the intermediate $T^{\pm}$ and a change in the rate-limiting step

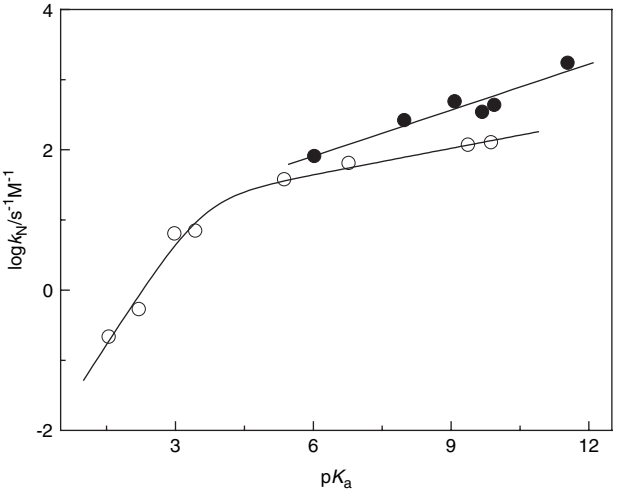
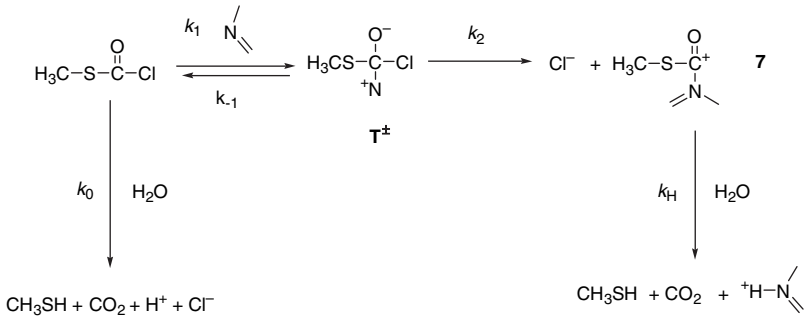


Figure 2. Brønsted plots for k_N, obtained in the reactions of **1** with pyridines (○) and SA amines (●), in an aqueous solution, 25.0 °C with an ionic strength of 0.2 M (KCl).



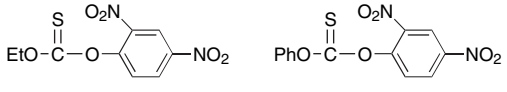
Scheme 3.

from that of k_2 in Scheme 3, to that of k_1, as the amine becomes more basic.[3b,11]

The values of β_1 and β_2 are in accordance with those reported for other aminolysis governed by the stepwise mechanisms: β_1=0.1–0.3 and β_2=0.8–1.1.[3b,9,11–14]

The slope of the linear Brønsted plot found for the SA aminolysis, β=0.23±0.05, is in agreement with the values of the Brønsted slopes found in stepwise mechanisms of similar reactions when the formation of the intermediate $T^{\pm}$ is the rate-limiting step.[3f,g,5a,11–14]

It is noteworthy that the rate constants of $T^{\pm}$ formation (k_1) for the reactions of **1** with SA amines (k_N=k_1) for the whole pK_a range are greater than those of isobasic pyridines (for amines with pK_a>3.6, where $k_2 \gg k_{-1}$), showing that SA amines are better nucleophiles than isobasic pyridines toward **1**. This result is surprising because for the reactions of these two amine series with chlorothionoformates **3** and **4** the k_1 values are similar,[5a,b] and for the reactions of these amines with ethyl and phenyl 2,4-dinitrophenyl thionocarbonates (**9** and **10**, respectively) the k_1 values for pyridines are 5–25 fold greater than those for isobasic SA amines.[12d,15] A possible explanation could be that both amine series show similar reactivities toward a thiocarbonyl carbon such as that in the chlorothionoformates, but SA amines exhibit steric hindrance toward the thiocarbonyl carbon of the dinitrophenyl derivatives when compared with the isobasic pyridines. The fact that SA amines show larger k_1 values than pyridines toward **1**, could be due to the harder character of the carbonyl carbon of this substrate, which makes it a better electrophile toward a harder base such as an SA amine relative to the softer isobasic pyridine.[16]



9 **10**

Figure 3 shows the Brønsted plots obtained for the pyridinolysis of **1** (this study) and methyl chloroformate (**2**).[3b] The pK_a value at the curvature center (pK_a^0) for the latter plot is 3.6,[3b] which is same as that found in this work. Therefore, the change of SMe to OMe, as the nonleaving group, in this case does not affect the pK_a^0 value significantly. This can be explained by taking into account that: (i) the inductive effects are more important than the resonance effects in a

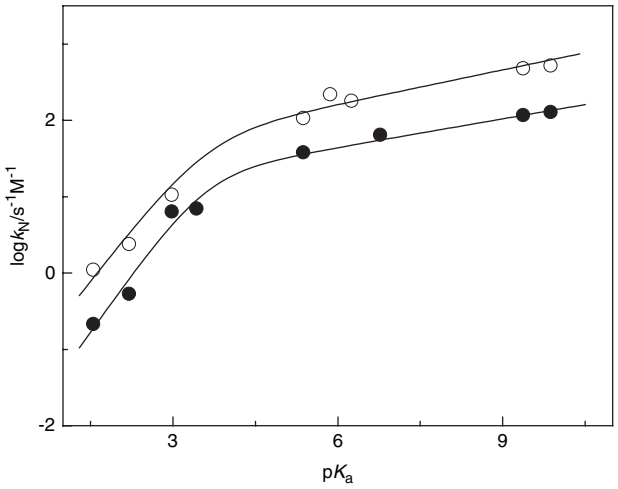


Figure 3. Brønsted plots for the pyridinolysis of **1** (this work; ●) and **2** (Ref. 3b; ○), in an aqueous solution, at 25.0 °C with an ionic strength of 0.2 M (KCl).

tetrahedral intermediate,[17] and (ii) these two groups have similar Hammett inductive values (σ_I=0.23 and 0.29 for SMe and OMe, respectively).[18] The similar electron-withdrawing abilities of these two groups should result in similar k_{-1}/k_2 ratios (see Scheme 3), which means similar pK_a^0 values.[19]

It can be seen in Figure 3 that the k_1 values for the pyridinolysis of **2** are greater than those for the same pyridinolysis of **1**. This result can be explained by the bulkier S atom relative to the O atom, which inhibits the attack by the pyridine. This behavior was also found for the stepwise pyridinolysis of methyl 2,4-dinitrophenyl carbonate and the corresponding *S*-methyl thiocarbonate.[7a,12a] Furthermore, the k_N values for rate-limiting expulsion of the nucleofuge from $T^{\pm}$ are also greater for methyl carbonates relative to their *S*-methyl thiol counterparts. This is the case for the pyridinolysis and SA aminolysis of methyl 4-nitrophenyl carbonate,[20,21] compared with the same aminolyses of the corresponding *S*-methyl derivative.[7a,b] Similarly, the k_N values obtained for the concerted SA aminolysis of the methyl 2,4-dinitrophenyl carbonate[21] are greater than those found for the same aminolysis of the corresponding *S*-methyl thiolcarbonate.[7b]

The fact that the shapes of the Brønsted-type plots in Figure 3 are the same indicates that both reactions are ruled by the same mechanism. An S_N1 process cannot be present

on the grounds that the k_N values increase with the basicity of the amine. This highlights the difference between the mechanism of the hydrolysis (and solvolysis in aqueous solvents) of *S*-alkyl chlorothioformates, which was found to be S_N1,[4a,b] with that of the aminolysis, which is an addition–elimination process.

Figure 4 shows the Brønsted-type plots for the pyridinolysis of **1** (this work) and those for the pyridinolysis of 4-nitrophenyl and 2,4-dinitrophenyl *S*-methyl carbonates (**5** and **6**, respectively).[7a] The curved plot for the reactions of **6** has been explained by a stepwise mechanism through a zwitterionic tetrahedral intermediate ($T^{\pm}$) and a change in the rate-limiting step,[7a] as for the pyridinolysis of **1**. The pK_a value at the curvature center is pK_a^0=7.3.[7a] The linear Brønsted plot found for the pyridinolysis of the mononitro derivative shows a slope of 1.1, which is consistent with a stepwise process where breakdown to products of the intermediate $T^{\pm}$ is the rate-limiting step.[7a] This means that for the latter reaction, $pK_a^0>10$.[7a] The fact that the pK_a^0 value increases as the leaving group becomes worse can be attributed to the decrease in the rate constant for expulsion of the nucleofuge from $T^{\pm}$ (k_2), which results in a greater k_{-1}/k_2 ratio for the mononitro derivative.[11,19]

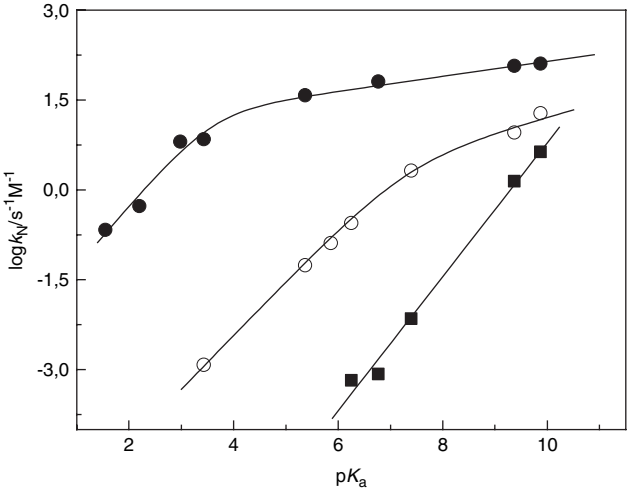


Figure 4. Brønsted-type plots for the pyridinolysis of **1** (this work; ●), **5** (Ref. 7a; ■) and **6** (Ref. 7a; ○), in an aqueous solution, 25.0 °C with an ionic strength of 0.2 M (KCl).

It can be seen in Figure 4 that the values of k_1 (for the two most basic pyridines) for the reactions of **1** are greater than those of **6**. For instance, for the reactions with 4-dimethylaminopyridine (pK_a=8.97) the k_1 values are 128 (this work) and 19 $s^{-1}\,M^{-1}$,[7a] respectively. This result can be explained by the greater steric hindrance towards the amine attack shown by 2,4-dinitrophenoxy group in **6** compared with Cl in **1**.

In the lower pK_a region of the Brønsted plots in Figure 4, where $k_N=k_1k_2/k_{-1}$, the k_N value of a given pyridine is about 10^4 times greater for its reaction with **1** relative to that with **6**. Considering that k_1 is about 10 times greater for **1**, the ratio k_2/k_{-1} must be about 10^3 times greater for **1** compared to **6**. This should be a consequence of the larger value of k_2 for the reactions of **1** relative to the dinitrophenyl derivative, since Cl should be a better nucleofuge than 2,4-dinitrophenoxy group.

The reactions of SA amines with **1** (this work) and **5**[7b] are stepwise. For the former, formation of the intermediate $T^{\pm}$ is the rate-limiting step (β=0.23), whereas for the latter the rate-limiting step is the breakdown of $T^{\pm}$ to the products (β=0.9).[7b] This is due to the much greater nucleofugality of Cl than 4-nitrophenoxy group from the corresponding $T^{\pm}$. In fact, for the aminolysis of **1** Cl is expelled faster than all the SA amines of the series from the tetrahedral intermediate, whereas for the reactions of **5** all the SA amines are expelled faster than the 4-nitrophenoxy group from the corresponding $T^{\pm}$ intermediate.

The reactions of **6** with SA amines are concerted,[7b] in contrast to the reactions of the same amines with **1**, which are stepwise (this work). This means that the 2,4-dinitrophenoxy group destabilizes the corresponding $T^{\pm}$ relative to Cl. An explanation to this behavior would be that the $T^{\pm}$ intermediate possessing the latter group would be much more crowded (and, therefore, more unstable) compared to that with Cl. This is supported by the fact that the reactions of these amines with 4-methylphenyl chloroformate are stepwise,[3g] in contrast to their reactions with 4-methylphenyl 2,4-dinitrophenyl carbonate, which are concerted.[22]

On the other hand, the pyridinolysis of **6** is stepwise,[7a] whereas the SA aminolysis of this substrate is concerted.[7b] This should be due to the fact that pyridines are worse nucleofuges from a $T^{\pm}$ intermediate than isobasic SA amines,[13] stabilizing, therefore, this intermediate, and making possible the change in mechanism, from concerted for the reactions with SA amines, to stepwise for those with pyridines.

3. Concluding remarks

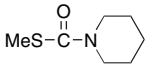
The following conclusions can be drawn from this and related works: (i) The pyridinolysis of *S*-methyl chlorothioformate (**1**) shows lower k_1 values relative to those of its oxy analog (**2**). (ii) Both reaction series show the same pK_a^0 value. (iii) The pK_a^0 values for the pyridinolysis of **1**, *S*-methyl 2,4-dinitrophenyl thiocarbonate (**6**), and *S*-methyl 4-nitrophenyl thiocarbonate (**5**) are 3.6, 7.3, and >10, respectively, showing that the value of pK_a^0 increases as the leaving group becomes worse. (iv) The value of k_1 for the pyridinolysis of **1** is 10 times greater than that for **6**. (v) The k_{-1}/k_2 ratio for the pyridinolysis of **1** is about 1000 times greater than that for **6**. (vi) The reactions of SA amines with **1** are stepwise in contrast to those of the same amines with **6**, which are concerted.

4. Experimental

4.1. Materials

The series of pyridines[3b] and secondary alicyclic (SA) amines[9] were purified either by distillation or recrystallization. *S*-Methyl chlorothioformate (**1**) was used as purchased. 1-(*S*-Methylcarbonyl)piperidine (**8a**), one of the products of the reaction of piperidine with **1**, was obtained by reaction of

the latter with piperidine in acetonitrile, and was identified by its H NMR spectrum.



8a

4.2. Kinetic measurements

These were carried out by means of a diode array spectrophotometer, in an aqueous solution, at 25.0±0.1 °C with an ionic strength of 0.2 M (KCl). The reactions were initiated by the addition (10 μL) of a stock solution of the substrate in acetonitrile into the aqueous solution of the amine (3 mL), at the appropriate pH, contained in a cell placed in the thermostated compartment of the spectrophotometer. The initial substrate concentration was $1–2\times10^{-5}$ M. Usually the pH was maintained by the corresponding amine–aminium pair, except in the reactions with 3-cyano and 4 cyano pyridines (where 0.005 M acetate buffer was used) and those with piperidine (where 0.01 M borate buffer was used). The reactions were followed at 220–500 nm and carried out under an excess (10-fold at least) of the amine over the substrate.

For the reactions with pyridines an intermediate was detected spectrophotometrically (270–320 nm), as shown by a fast absorbance increase followed by a slow decrease (see Section 2). In the case of the reactions with 3-chloropyridine we assume the intermediate to be 1-(*S*-methylcarbonyl)-3-chloropyridinium cation (**7a**). The kinetics for all the reactions were measured under conditions where decomposition of the intermediate was slower than its formation. Pseudo-first-order rate coefficients (k_{obsd}) were found for all the reactions by means of the kinetic software of the spectrophotometer.

4.3. Product studies

For the reaction with piperidine, compound **8a** was identified as one of the products. This was achieved by comparison of the UV–vis spectrum at the end of the reaction with that of an authentic sample at the same experimental conditions.

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Synthesis and X-ray crystallographic analysis of chiral pyridyl substituted carbocyclic molecular clefts

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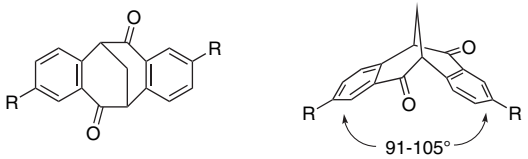
Abstract—Ditopic symmetrical bis(pyridyl) ligands incorporating the chiral dibenzobicyclo[*b*,*f*][3.3.1]nona-5a,6a-diene-6,12-dione cleft have been synthesised and characterised by NMR spectroscopy, mass spectrometry and X-ray crystallography. The ligands, which incorporate pyridyl groups directly connected to the carbocyclic cleft core or via alkyne or phenyl linkers were accessed from palladium-catalysed coupling reactions of 2,8-dibromodibenzobicyclo[*b*,*f*][3.3.1]nona-5a,6a-diene-6,12-dione. X-ray crystal analyses show the interplanar angles between the two cleft aromatic rings in these molecules, which range from 97.80(3) to 109.80(4)°.

1. Introduction

Oligopyridines are widely studied as ligands and building blocks in coordination and supramolecular chemistries today.[1] Most prevalent are those where the heteroaromatic units are directly connected to each other via a carbon–carbon single bond, examples include bipyridines, terpyridines and phenanthrolines. Oligopyridines of the types described are characterised by their rigid structure. In recent years, the incorporation of semi-rigid and flexible oligopyridines in supramolecular systems has also been the subject of intensive study. Typically, their structures contain a flexible alkyl bridging unit between the pyridyl groups.[2] The versatility of oligopyridines as ligands is reflected in many elegant syntheses of architecturally fascinating supramolecular structures, ranging from discrete species such as polygons,[3] catenanes,[4] rotaxanes,[5] single[6] and double-stranded helices[7] to extended systems, which include grids[8] and wires.[9]

Recently, the dibenzobicyclo[*b*,*f*][3.3.1]nona-5a,6a-diene-6,12-dione framework has been identified as a versatile supramolecular building block,[10–12] and the applications and potential of this system in supramolecular chemistry have been reviewed recently.[13] These molecular clefts can be considered as analogues of Tröger's base but contain a carbocyclic core that may be elaborated to introduce additional molecular recognition groups via the ketone functional groups. A key feature of the dibenzobicyclo[*b*,*f*][3.3.1]-nona-5a,6a-diene-6,12-dione **1**[14] is its angular or cleft-like framework that is imposed by the bicyclononane skeleton. Apart from its well-defined geometry, the cleft possesses an element of chirality as well as functional handles that allow access to new structural motifs with the potential for chiral molecular recognition processes. Thus, the carbonyl groups can be transformed to hydroxyls or oximes by reaction with lithium aluminium hydride and hydroxylamine, respectively.[10,12,14] The ready access to the bisarylbromide derivative **2** also allows entry into aryl and alkynyl-substituted clefts through metal catalysed cross-coupling reactions.[11]



1 R = H
2 R = Br

In the course of our studies towards the assembly of chiral metallomacrocycles of nanoscale dimensions, we required the efficient synthesis of bispyridyl derivatives of the clefts **L1**, **L2** and **L3** in which the distance between the pyridyl units was systematically varied. In addition, given that substituents may influence the size of the cleft angle,[11,12] X-ray crystallographic analyses were undertaken to provide measurements of the cleft angle in order to gauge the likely outcome of metal complexation and self-assembly studies.

Keywords: Molecular clefts; Supramolecular chemistry; Metal complexation; Suzuki cross-coupling; Sonogashira cross-coupling; X-ray analysis.
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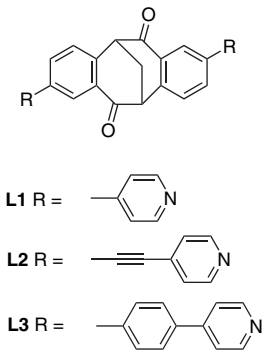


2. Results and discussion

2.1. Synthesis

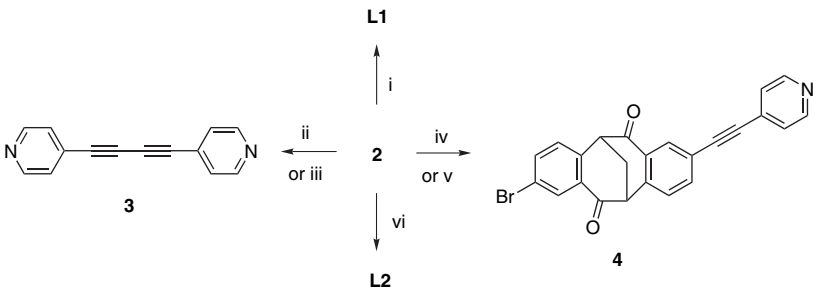
The target ligands **L1**, **L2** and **L3** are pyridyl, acetylenylpyridyl and *p*-(4′-pyridyl)phenyl derivatives of the parent molecular cleft **1**. While there have been numerous recent reports and reviews on catalysts and conditions to effect metal catalysed couplings,[15,16] the application of these methods to heterocycles is frequently limited and deactivation of the metal catalyst by heterocycles requires careful optimisation of reaction conditions.[17]

Ligand **L1** was prepared by the direct coupling of the dibromo cleft **2** with (4-pyridyl)boronic acid pinacol ester under the catalysis of $PdCl_2(PPh_3)_2$ (Scheme 1). The synthesis of the ligand **L2** from the dibromo cleft **2** and 4-ethynylpyridine was first attempted using the protocol developed for appending alkynyl units to the peripheral arms of the cleft (Scheme 1).[11] Thus, dibromo cleft **2** was treated with 4-ethynylpyridine hydrochloride, $PdCl_2(PPh_3)_2$ and CuI in neat triethylamine, that is, the standard Sonogashira conditions.[16] However, these conditions afforded only the homocoupled product, (4,4′-dipyridyl)butadiene **3**. Use of the more active $Pd(PPh_3)_4$ catalyst under similar conditions led to the same result. Further optimisation of the reaction by the use of $Pd(PPh_3)_4$–CuI as the catalytic system and the use of organic solvents showed that in contrast to most successful Sonogashira reactions that are performed using an amine base, the choice of solvent was critical to the success of the reaction. While the use of THF and MeCN resulted in formation of the monocoupled cleft **4** in 30% and 43% yield, respectively, the desired ligand **L2** was obtained in 83% yield with the use of DMF (Scheme 1).

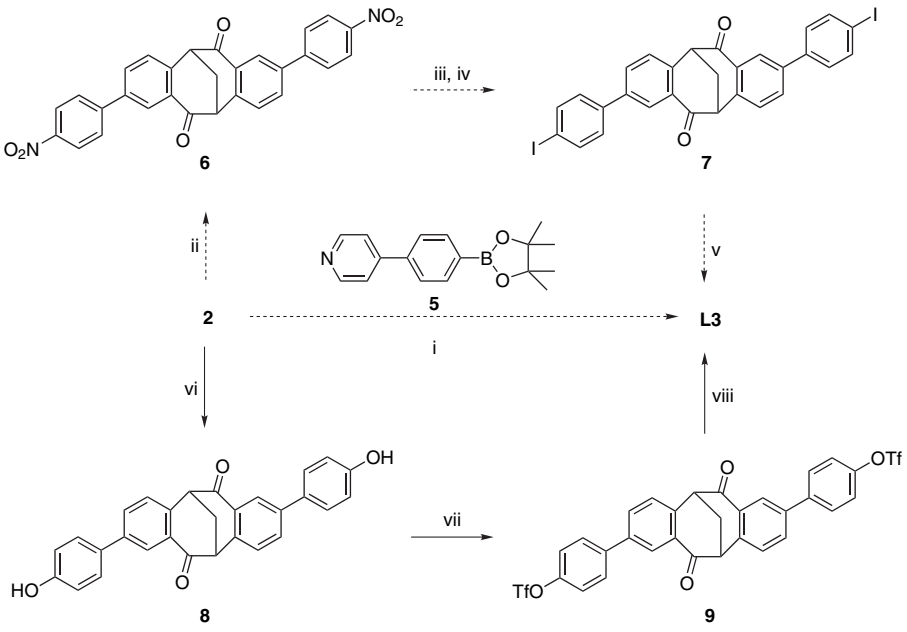
Ligand **L3** contains two new biaryl bonds and hence is accessible via a number of different routes (Scheme 2). The first route investigated used Suzuki coupling of the diaryl boronic ester **5** to the dibromo cleft **2**. The synthesis of the diaryl boronic ester **5** has not been reported previously, and required initial preparation of the corresponding aryliodide **11** (Scheme 3). Aniline **10**,[18] was converted to the iodide **11** according to the literature report.[19] However, in our hands, the literature yields could not be reproduced, and careful temperature control and the use of sodium nitrite/HBF_4 were required to furnish the diaryliodide **11** in only moderate yield of 44%. The diazotization step required slow addition of an aqueous solution of $NaNO_2$ (over 1.5 h) with the internal temperature of the reaction mixture being maintained at no higher than −40 °C. Iodination was effected with addition of KI also at −40 °C and allowing the reaction mixture to warm slowly to rt. Purification of the crude diaryliodide **11** according to the literature procedure by recrystallisation from hot toluene was unsuccessful due to the apparent insolubility of **11**. All attempts to convert the crude diaryliodide **11** to the boronic ester **5** were unsuccessful upon treatment with $PdCl_2(dppf)\cdot CH_2Cl_2$, bis(pinacolato)diborane and KOAc in dioxane or DMSO (Scheme 3).[20]

The alternative strategy to ligand **L3** involved initial attachment of a *para*-substituted boronic ester to the cleft followed by introduction of the pyridyl groups (Scheme 2). The first key step involved the coupling of the dibromo cleft **2** with 4-nitrophenylboronic acid. The nitro groups serve as functional handles enabling conversion of adduct **6** to the diiodide **7**, the latter being a suitable coupling partner for the subsequent Suzuki reaction with (4-pyridyl)boronic acid pinacol ester. This route failed as reaction of cleft **2** with 4-nitrophenylboronic acid under $PdCl_2(PPh_3)_2$ catalysis resulted predominantly in the monocoupled product **13** under forcing conditions and long reaction times. Hence the corresponding iodide **12** was prepared via the Sandmeyer reaction of the diamino cleft **15** (Scheme 4).[11] While the rate of the reaction improved by the use of the iodide **12** in place of the bromide **2**, the monocoupled product **14** was the major product due to its poor solubility, which resulted in precipitation during the reaction (Scheme 5). Changing the solvent and conditions was unsuccessful, and while minor amounts of the bisnitro product **6** were formed, the extremely low solubility of this product in all common organic solvents precluded efficient purification.

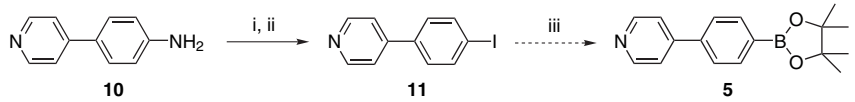
To avoid these problems, the alternative approach involved preparation of the more soluble bisphenol **8** via Suzuki



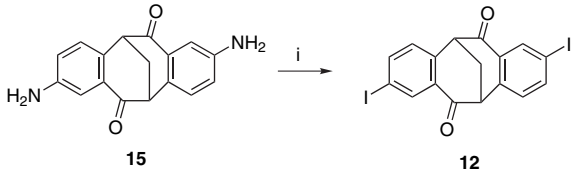
Scheme 1. *Reagents and conditions*: i. $PdCl_2(PPh_3)_2$, Na_2CO_3, (4-pyridyl)boronic acid pinacol ester, DME–H_2O; ii. $PdCl_2(PPh_3)_2$, CuI, Et_3N; iii. $Pd(PPh_3)_4$, CuI, Et_3N, THF; iv. $Pd(PPh_3)_4$, CuI, Et_3N, MeCN; v. $Pd(PPh_3)_4$, CuI, Et_3N, DMF.



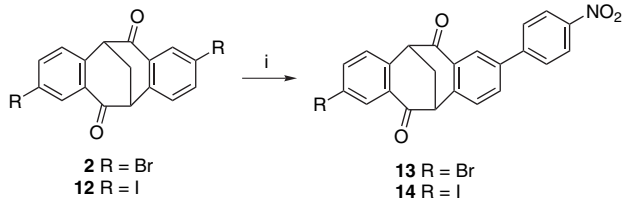
Scheme 2. *Reagents and conditions*: i. $PdCl_2(PPh_3)_2$, Na_2CO_3, DME–H_2O; ii. 4-nitrophenylboronic acid, $PdCl_2(PPh_3)_2$, Na_2CO_3, DME–H_2O; iii. Fe/CH_3CO_2H; iv. $NaNO_2$, H_2SO_4, KI; v. (4-pyridyl)boronic acid pinacol ester, $PdCl_2(PPh_3)_2$, Na_2CO_3, DME–H_2O; vi. 4-(hydroxy)boronic acid pinacol ester, $PdCl_2(PPh_3)_2$, Na_2CO_3, DME–H_2O; vii. Tf_2O, pyridine, CH_2Cl_2; viii. (4-pyridyl)boronic acid pinacol ester, $Pd(PPh_3)_4$, Na_2CO_3, DME–H_2O.



Scheme 3. *Reagents and conditions*: i. $NaNO_2$, 40% aq HBF_4, EtOH, −40 °C; ii. KI, −40 °C to rt; iii. bis(pinacolato)diborane, $PdCl_2(dppf)\cdot CH_2Cl_2$, KOAc, dioxane or DMSO.



Scheme 4. *Reagents and conditions*: i. H_2SO_4, $NaNO_2$, KI.



Scheme 5. *Reagents and conditions*: i. $PdCl_2(PPh_3)_2$ or $Pd(PPh_3)_4$, Na_2CO_3, 4-nitrophenylboromic acid, DME–H_2O.

reaction of the dibromo cleft **2** and (4-hydroxyphenyl)boronic acid pinacol ester, catalysed by $PdCl_2(PPh_3)_2$ (Scheme 2). In contrast to attempted preparation of **6**, the bisphenol **8** was formed in good yield and exhibited good solubility thus avoiding the problems encountered with the nitro compound. Bisphenol **8** was treated with triflic anhydride and pyridine to afford the corresponding bistriflate **9** as a suitable coupling partner for Suzuki reactions. However, under standard Suzuki reaction conditions,[21] treatment of (4-pyridyl)-boronic acid pinacol ester with bistriflate **9** in the presence of $PdCl_2(dppf)\cdot CH_2Cl_2$ (2.5 mol %), K_3PO_4 and KBr in dioxane gave no reaction. The use of more forcing conditions (increased catalyst loading, use of the more active $Pd(PPh_3)_4$ catalyst and prolonged reaction time) also resulted in no reaction. The failure of this reaction is most likely because of the precipitation of palladium black at the reaction onset, which results from the side reaction of the triflate with the triphenylphosphine ligand to afford phosphonium salts.[21,22] Accordingly, application of the methodology of Fu et al.[23] for coupling of aryl triflates and boronic acids was investigated. Following the reported optimal conditions, there was no reaction at rt using $Pd(OAc)_2$ and PCy_3 in DMF, and the successful synthesis of ligand **L3** required the use of $Pd(PPh_3)_4$ and sodium carbonate in DME–H_2O with heating. Purification of **L3** required chromatography, followed by recrystallisation from MeOH and trifluoroacetic acid.

2.2. X-ray crystallography

Single crystals of racemic **L1**, **L2** and **L3** suitable for X-ray diffraction analysis were obtained by recrystallisation from MeOH. Crystallographic details and pertinent metric details are summarised in Tables 1–3, and ORTEP depictions are provided in Figures 1 and 2. The crystal structure of ligand **L1** includes a water solvate molecule that forms a hydrogen bond with **L1**. The crystal structure of **L2** contains a disordered methanol solvate molecule, whilst the solid state

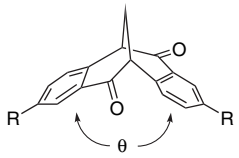
Table 1. Single crystal X-ray diffraction details for ligands **L1**, **L2** and **L3**

Compound	**L1**	**L2**	**L3**
Formula	$C_{27}H_{20}N_2O_3$	$C_{32}H_{22}N_2O_3$	$C_{39}H_{26}N_2O_2$
Crystal system	Orthorhombic	Monoclinic	Triclinic
Molecular weight	420.45	482.52	554.62
Size (mm^3)	0.31×0.27×0.26	0.33×0.17×0.15	0.31×0.26×0.10
Space group	*P*ccn(#56)	$P2_1/c$(#14)	$P\bar{1}$(#2)
a (Å)	17.3760(6)	7.499(6)	11.7599(10)
b (Å)	10.6974(4)	27.08(2)	15.3672(14)
c (Å)	10.8602(3)	12.224(10)	16.5565(14)
α (°)	—	—	95.6950(10)
β (°)	—	104.336(13)	107.2570(10)
γ (°)	—	—	97.1050(10)
V ($Å^3$)	2018.67(12)	2405(3)	2806.1(4)
D_{calcd} ($g\,cm^{-3}$)	1.383	1.333	1.313
Z	4	4	4
μ(Mo Kα) (mm^{-1})	0.091	0.086	0.081
$T(SADABS)_{min,max}$	0.891, 0.977	0.733, 1.000	—
$2\theta_{max}$ (°)	70.00	57	61
hkl range	−28 28, −17 17, −14 17	−10 9, −34 34, −16 16	−16 16, −22 21, −23 23
N	40,515	22,452	48,618
N_{ind} (R_{merge})	4405(0.025)	5603(0.078)	17,155(0.070)
N_{obs} [$I>2\sigma(I)$]	3505	3339	10,392
N_{var}	150	354	775
$R1(F)$,$wR2(F^2)$	0.0428, 0.1055	0.0560, 0.1287	0.0446, 0.0903
GoF (all)	1.270	1.401	1.148
ρ_{max} ($e\,Å^{-3}$)	−0.199, 0.463	−0.311, 0.306	−0.267, 0.385

Table 2. Selected bond lengths (Å) and angles (°) for ligands **L1**, **L2** and **L3**

Ligand **L1**			
O(1)–C(7)	1.2164(10)	C(5)–C(4)–C(10)–C(14)	25.88(12)
C(1)–C(8)–C(7)	108.78(7)	C(3)–C(4)–C(10)–C(11)	24.59(12)
Ligand **L2**			
O(1)–C(7)	1.228(2)	O(2)–C(15)	1.224(3)
C(7)–C(8)–C(9)	107.51(16)	C(1)–C(16)–C(15)	108.01(16)
Ligand **L3**			
O(1)–C(7)	1.2167(13)	O(3)–C(46)	1.2148(13)
O(2)–C(15)	1.2174(14)	O(4)–C(54)	1.2173(14)
C(7)–C(8)–C(9)	108.75(9)	C(46)–C(47)–C(48)	108.84(9)
C(1)–C(16)–C(15)	107.28(10)	C(40)–C(55)–C(54)	108.02(10)
C(5)–C(4)–C(18)–C(23)	−142.45(12)	C(44)–C(43)–C(57)–C(62)	−141.28(12)
C(11)–C(12)–C(29)–C(34)	34.17(17)	C(50)–C(51)–C(68)–C(69)	144.00(12)
C(22)–C(21)–C(24)–C(25)	46.43(17)	C(61)–C(60)–C(63)–C(64)	50.40(17)
C(33)–C(32)–C(35)–C(39)	38.33(18)	C(70)–C(71)–C(74)–C(78)	−34.18(17)

Table 3. Cleft angle θ and nitrogen–nitrogen separations for **L1**, **L2** and **L3**



Compound	Interplanar 'cleft' angle θ (°)	Distance between nitrogen atoms (Å)
L1	97.80(3)	13.57(1)
L2	102.47(8)	17.55(1)
L3[a]	103.30(4), 109.80(4)	21.35(1), 21.40(1)

[a] The asymmetric unit contains two independent molecules.

structure of ligand **L3** contains two crystallographically independent molecules.

The dihedral angle formed by the least square planes of the two opposing phenyl rings immediately attached to the bridgehead superstructure, provides a simple measure of the intramolecular cleft angle. Previous analyses of 2,8-disubstituted dibenzobicyclo[*b*,*f*][3.3.1]nona-5a,6a-diene-6,12-dione clefts have suggested substituent effects on cleft angles, with angles of 100–104° observed for clefts substituted with electron-donating aryl groups.[11] The cleft angles for **L1**, **L2** and **L3** (two independent molecules) are 97.80(3), 102.47(8), 103.30(4) and 109.80(4)°, respectively (Table 3). The single crystal structures indicate that the linker units of **L1**, **L2** and **L3** affect the overall shape of the oligopyridine clefts, though it is not clear if this reflects packing influences or electronic effects. The significantly different cleft angles found in the two independent molecules of **L3** suggest that packing influences are most likely to be the cause of the cleft angle variation, though we have not yet identified any particular causal contacts. The cleft angle of 109.80(4)° observed in the second molecule of **L3** is much like that which would be expected of an sp^3 carbon based geometry, suggesting perhaps that this molecule is actually least affected by packing influences. Paradoxically,

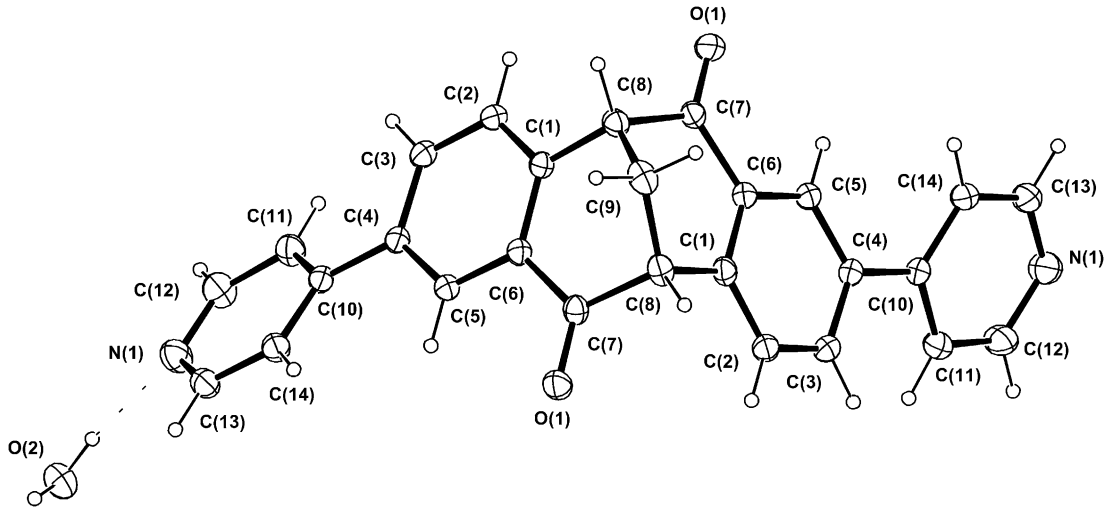


Figure 1. ORTEP depiction of structures of **L1** showing 50% displacement ellipsoids and the hydrogen bonding interaction with the solvate water molecule.

the **L3** intramolecular nitrogen–nitrogen distances are much the same at 21.35(1) and 21.40(1) (Å) (Table 3), and there is no obvious explanation for this contrast to the differing cleft angles found in the two molecules of **L3**.

The molecules of **L1** and **L3** pack with the apex of one molecule effectively nestling into the cleft of another, as suggested in Figure 2. In contrast, the stacking of molecules in the structure of **L2** is interleaved with the terminal residue of neighbouring molecules, as shown in Figure 3. The cleft angle of **L2** is between those of **L1** and **L3**, suggesting, in contrast to early observations that this packing difference does seem to influence the cleft angle. The solid state cleft angle of any particular molecule appears to be the outcome of subtle differences in packing and electronic influences, with no reliable systematic trend yet evident.

2.3. Metal complexation studies

Preliminary metal complexation studies of the racemic ligand **L1** with palladium(II) have been performed. Thus, a solution of $[Pd(ONO_2)_2(en)]$ in H_2O (16.7 mM) was treated with a solution of racemic ligand **L1** in MeOH (16.7 mM) at ambient temperature. ESI–MS analysis of the crude reaction mixture was consistent with the formation of the [2+2] metallomacrocycle and peaks at 631 $[M-2NO_3]^{2+}$, 508 $[M-4NO_3+7MeOH]^{4+}$, 380 $[M-4NO_3+3MeOH]^{4+}$ and 284 $[M-4NO_3]^{4+}$ were detected. Due to the chirality of

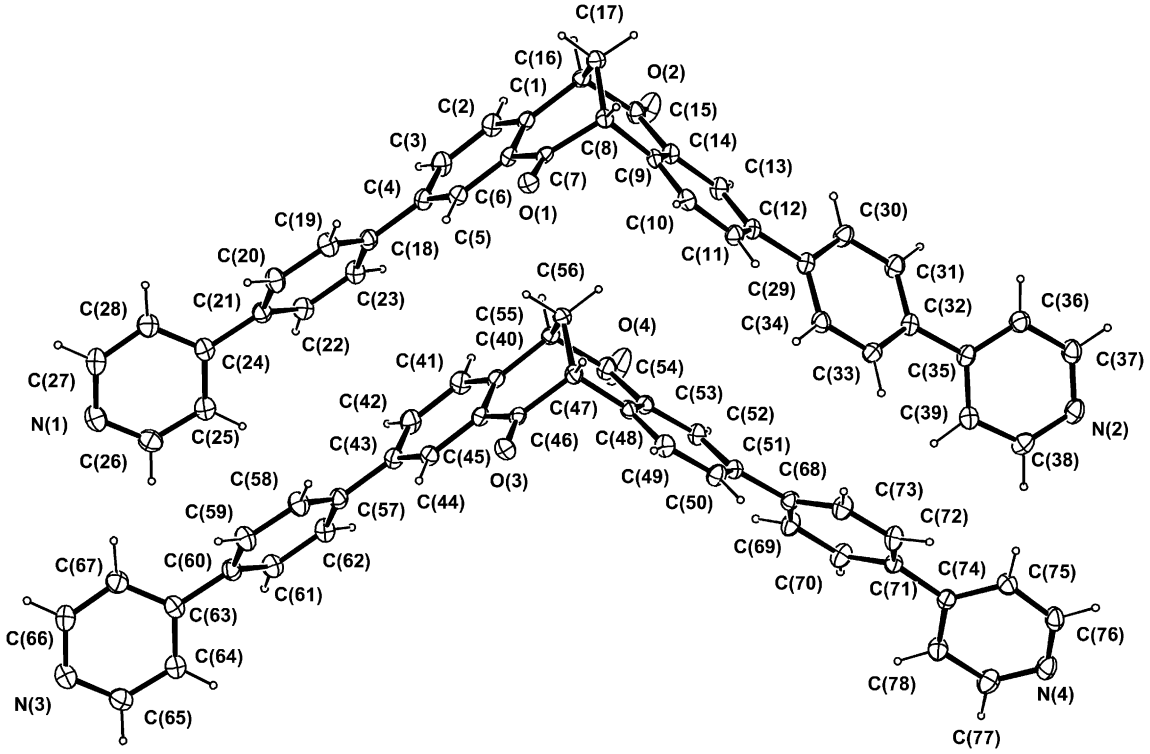


Figure 2. ORTEP depiction of structures of **L3** showing 50% displacement ellipsoids.

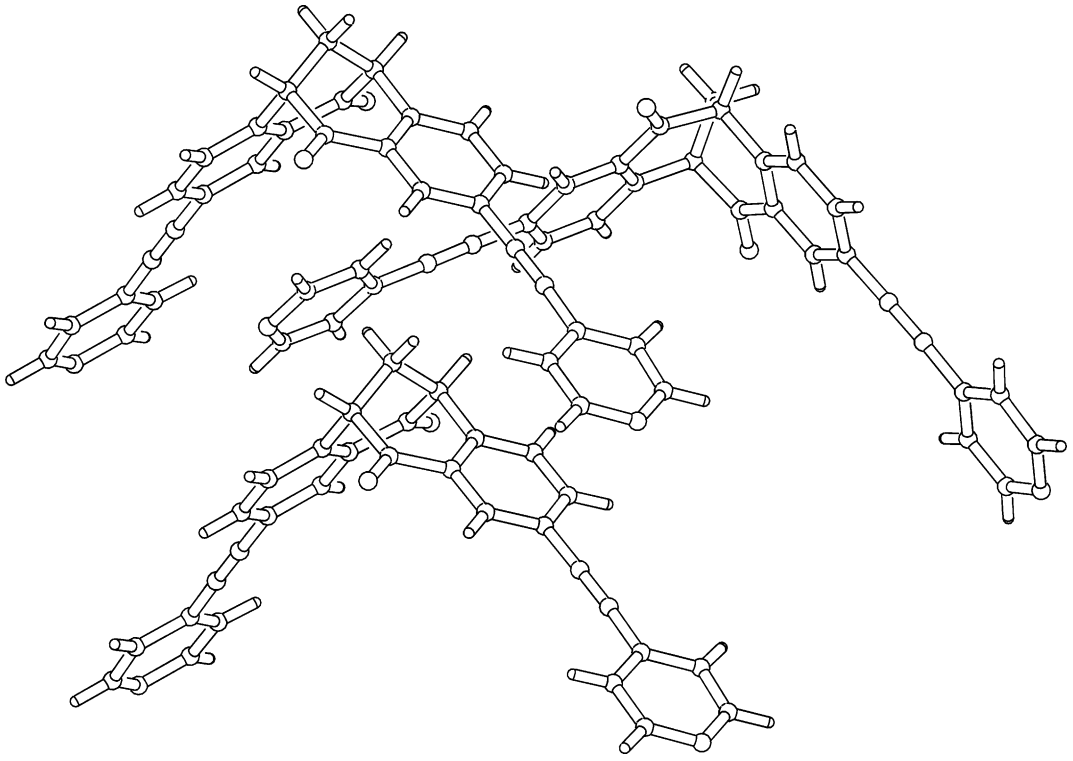


Figure 3. Ball and stick depiction of **L2** showing the interleaving of the molecules.

the ligand **L1**, the formation of stereoisomers is possible. In contrast to studies with bipyridyl clefts and zinc(II) [$Zn(OTf)_2$] where enantiomeric ligand–ligand recognition occurred,[24] the ^{1}H NMR spectrum of **L1** and Pd contained multiple signals consistent with formation of several complexes. Full characterisation of these complexes therefore requires studies with the pure enantiomers, which are accessible from optically pure **2**.[11]

Analogous results were obtained upon treatment of ligands **L2** and **L3** with [$Pd(ONO_2)_2$en]. These reactions were performed at more dilute concentrations due to the poor solubility of ligands **L2** and **L3** relative to **L1** in MeOH. ESI-MS analysis of the reaction mixtures was consistent with the formation of the corresponding [2+2] metallomacrocycles. In the case of **L2**, peaks were detected at *m*/*z* 616 $[M-4NO_3+4MeOH+10H_2O]^{4+}$ and 557 $[M-4NO_3+5MeOH+5H_2O]^{4+}$ while for **L3** peaks at *m*/*z* 833 $[M-2NO_3+MeOH+H_2O]^{2+}$, 720 $\{2[M-4NO_3]^{4+}\}$, 660 $[M-4NO_3+6MeOH+6H_2O]^{4+}$.

3. Conclusions

The new dibenzobicyclo[*b*,*f*][3.3.1]nona-5a,6a-diene-6,12-dione molecular clefts incorporating pyridyl, ethynylpyridyl and *p*-(4′-pyridyl)phenyl groups were prepared via Pd and Pd–CuI catalysed cross-coupling methodologies. The choice of solvent was critical in mediating the cross-coupling reactions, with polar solvents such as DME–H_2O and DMF being required to facilitate these reactions. X-ray crystallographic analyses of the clefts confirm previous studies, which have shown that the substituents on the aryl rings affect the dimensions, and packing motifs, of the cleft in the solid state. Preliminary metal complexation studies have confirmed that under appropriate conditions the ditopic symmetrical bis(pyridyl) ligands can be used in the assembly of chiral [2+2] metallomacrocycles. The incorporation of these ligands into the design of chiral metallomacrocycles of nanoscale dimensions is currently under investigation in these laboratories.

4. Experimental

4.1. General experimental procedures

Melting points were measured on a Reichardt hot-stage and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 300 K on Bruker DPX200 and Bruker DPX300 NMR spectrometers. Low and high resolution mass spectra (EI) were obtained on a Kratos MS902 spectrometer at 70 eV. Values of *m*/*z* are quoted with intensities expressed as percentages of the base peak in parentheses. Infrared spectra were recorded on a Shimadzu FTIR-8400S spectrometer. Single crystal X-ray analyses were performed on either an APEXII-FR591 diffractometer or a Bruker SMART 1000 CCD diffractometer. $PdCl_2(PPh_3)_2$ and 4-ethynylpyridine hydrochloride were purchased from Sigma-Aldrich. (4-Pyridine)boronic acid pinacol ester and (4-hydroxyphenyl)boronic acid pinacol ester were sourced from Boron Molecular Pty Ltd. 4-Pyridylboronic acid was purchased from Alfa Asar. $Pd(PPh_3)_4$[25] and (±)-2,8-dibromodibenzobicyclo[*b*,*f*][3.3.1]nona-5a,6a-diene-6,12-dione were synthesised according to literature procedures.[11]

4.1.1. (±)-2,8-Dipyridyldibenzobicyclo[*b*,*f*][3.3.1]nona-5a,6a-diene-6,12-dione L1. To a mixture of dibromo dione **2** (200 mg, 0.492 mmol), (4-pyridine)boronic acid pinacol ester (202 mg, 0.986 mmol), Na_2CO_3 (312 mg, 2.96 mmol) and $PdCl_2(PPh_3)_2$ (70 mg, 20 mol %) at rt were added

DME (10 mL) and H_2O (2 mL). The mixture was degassed and purged with nitrogen (six cycles), then heated at 85 °C under an atmosphere of nitrogen for three days. After cooling to rt, the solvents were removed in vacuo. The resulting residue was taken up in EtOAc (30 mL) and washed with H_2O (20 mL). The aqueous phase was separated and extracted with EtOAc (3×30 mL). The organic solutions were combined and washed with brine (30 mL), then dried (Na_2SO_4) and concentrated to dryness. Flash chromatography of the tan residue on silica, eluting with MeOH/hexane (1:99 to 1.5:98.5) afforded **L1** as a off-white solid [R_f 0.13; MeOH/hexane (1:99)] (154 mg, 78%); mp 114–116 °C; IR (KBr) 1688, 1682 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 3.08 (t, J=3.0 Hz, 2H), 4.15 (t, J=3.0 Hz, 2H), 7.47 (d, J=5.4 Hz, 2H), 7.63 (d, J=8.1 Hz, 2H), 7.82 (d, J=8.1, 2.0 Hz, 2H), 8.27 (d, J=2.0 Hz, 2H), 8.67 (br s, 2H); δ_C (300 MHz, $CDCl_3$) 31.9, 48.5, 121.5, 126.8, 129.5, 129.9, 132.8, 140.4, 149.4, 193.5; UV λ_{max} (MeOH) 374 (sh) (481), 356 (sh) (1307), 338 (1782), 324 (1883), 306 (2241), 274 (3123), 270 (3126); *m/z* (EI, %) 402 (M^+, 100), 385 (24), 374 (28), 357 (13), 332 (9), 331 (2), 296 (6), 280 (2), 266 (5), 239 (2), 207 (1), 201 (4), 167 (3), 152 (2), 132 (1), 89 (1), 79 (1), 52 (2). Found: $M^{\bullet+}$, 402.1369. $C_{27}H_{18}N_2O_2$ requires $M^{\bullet+}$, 402.1368. Crystals suitable for X-ray diffraction were obtained by recrystallisation from MeOH.

4.1.2. (±)-2,8-(4-Bromo-4′-ethynylpyridyl)dibenzobicyclo[*b*,*f*][3.3.1]nona-5a,6a-diene-6,12-dione 4. Triethylamine (1.0 mL, 7.25×10^{-3} mol) was added to a mixture of the dibromo cleft **2** (20.0 mg, 4.83×10^{-5} mol) and 4-ethynylpyridine hydrochloride (101 mg, 7.25×10^{-4} mol) in dry THF (4 mL) at rt. Under a steady stream of nitrogen, $Pd(PPh_3)_4$ (3 mg, 5 mol %) and CuI (0.5 mg, 5 mol %) were quickly added. The dark reaction mixture was evacuated and purged with nitrogen (3× freeze-pump-thaw cycles), then heated at 50 °C in the dark under an atmosphere of nitrogen for 50 h. After cooling to rt, the dark mixture was filtered, the solids washed with EtOAc (15 mL) and MeOH (15 mL). The filtrate was evaporated in vacuo and the resulting residue was taken up in EtOAc (30 mL). After washing with aq $NaHCO_3$ (30 mL), the layers were separated and the aqueous solution was extracted with EtOAc (3×30 mL). The organic solutions were combined and washed with aq $NaHCO_3$ (2×30 mL) and brine (30 mL), then dried (Na_2SO_4) and evaporated under reduced pressure. Flash column chromatography of the resultant brown residue on silica, eluting with MeOH/CH_2Cl_2 (1.5:98.5) afforded the cleft **4** (5.5 mg, 20%) as a white solid [R_f 0.31; MeOH/CH_2Cl_2 (1.5:98.5)]; mp>300 °C; IR (KBr) 1688, 1645, 1589, 1454, 1402 cm^{-1}; δ_H (300 MHz, $CDCl_3$) δ 3.01 (t, J= 2.9 Hz, 2H), 4.04 (br m, 1H), 4.09 (br m, 1H), 7.37 (d, J= 8.2 Hz, 1H), 7.57 (dd, J=8.0, 2.2 Hz, 1H), 7.67 (dd, J=7.9, 1.8 Hz, 1H), 7.73 (d, J=8.2 Hz, 1H), 7.88 (d, J=6.5 Hz, 2H), 8.10 (dd, J=1.8 Hz, 1H), 8.21 (dd, J=2.1 Hz 1H), 8.71 (d, J=6.4 Hz, 2H); *m/z* (EI, %) 429 ($M^{\bullet+}$, 100), 399 (33), 385 (26), 358 (45), 348 (41), 320 (62), 291 (36), 263 (28), 261 (9), 213 (12), 163 (11), 149 (29), 131 (18), 126 (14), 85 (18), 71 (28); Found: $M^{\bullet+}$ 427.0213. $C_{24}H_{14}BrN_1O_2$ requires $M^{\bullet+}$, 427.0208.

4.1.3. (±)-2,8-Bis(4-ethynylpyridyl)dibenzobicyclo[*b*,*f*][3.3.1]nona-5a,6a-diene-6,12-dione L2. Triethylamine (1.0 mL, 7.25×10^{-3} mol) was added to a mixture of the dibromo cleft **2** (20.0 mg, 4.83×10^{-5} mol) and 4-ethynylpyridine hydrochloride (40.0 mg, 2.90×10^{-4} mol) in dry DMF (5 mL) at rt. Under a steady stream of nitrogen, $Pd(PPh_3)_4$ (3 mg, 5 mol %) and CuI (0.5 mg, 5 mol %) were quickly added. The dark brown reaction mixture was evacuated and purged with nitrogen (3× freeze-pump-thaw cycles) then heated at 80 °C in the dark under an atmosphere of nitrogen for three days. After the cooling to rt, the reaction mixture was taken up in EtOAc (30 mL) and washed with H_2O (30 mL). The layers were separated and the aqueous solution was extracted with EtOAc (5×20 mL). The organic solutions were combined and washed with H_2O (4×30 mL) and brine (30 mL), then dried (Na_2SO_4) and evaporated under reduced pressure. Flash column chromatography of the resulting brown residue on silica, eluting with a gradient of MeOH/CH_2Cl_2 (1.5:98.5 to 4:96) afforded ligand **L2** (18 mg, 83%) as a white solid [R_f 0.31; MeOH/CH_2Cl_2 (4:96)]; mp>300 °C; IR (KBr) 2231, 2210, 1686, 1589 cm^{-1}; δ_H (300 MHz, $CDCl_3$) δ 3.04 (t, J=2.8 Hz, 2H), 4.10 (t, J=2.8 Hz, 2H), 7.45 (d, J=6.2 Hz, 4H), 7.52 (d, J=8.0 Hz, 2H), 7.79 (dd, J=8.0, 1.9 Hz, 2H), 8.17 (s, 2H), 8.62 (d, J= 6.2 Hz, 4H); δ_C (300 MHz, $CDCl_3$) 31.7, 48.6, 88.4, 92.1, 123.3, 125.6, 129.0, 129.3, 130.8, 131.9, 137.2, 140.2, 149.9, 192.8; *m/z* (EI, %) 450 ($M^{\bullet+}$, 100), 422 (29), 421 (25), 405 (19), 380 (10); Found: $M^{\bullet+}$ 450.1360. $C_{31}H_{18}N_2O_2$ requires $M^{\bullet+}$, 450.1368. Crystals suitable for X-ray diffraction were obtained by recrystallisation from MeOH.

4.1.4. (±)-2,8-Di(4-hydroxyphenyl)dibenzobicyclo[*b*,*f*][3.3.1]nona-5a,6a-diene-6,12-dione 8. To a mixture of dibromo cleft **2** (100 mg, 0.249 mmol), (4-hydroxyphenol) boronic acid pinacol ester (110 mg, 0.500 mmol), Na_2CO_3 (80.0 mg, 0.755 mmol) and $PdCl_2(PPh_3)_2$ (3.5 mg, 20 mol %) were added DME (5 mL) and H_2O (1 mL). The mixture was degassed and purged with nitrogen (six cycles), then heated at 85 °C under an atmosphere of nitrogen for 24 h. After cooling to rt and evaporation of solvent, the residue was taken up in EtOAc (30 mL) and washed with 3 M HCl (30 mL). The layers were separated and the aqueous phase was extracted with EtOAc (3×30 mL). The organic solutions were combined and washed with brine (30 mL), then dried (Na_2SO_4) and evaporated in vacuo. The resulting off-white solid was washed with cold Et_2O (30 mL) then dried under high vacuum to afford bisphenol **8** as an off-white solid (60 mg, 58%) [R_f 0.34; MeOH/hexane (1:9)]; mp>300 °C; IR (KBr) 3219 (br), 1670 cm^{-1}; δ_H (200 MHz, *d*-DMSO) 3.02 (br m, 2H), 4.07 (br m, 2H), 6.83 (d, J=8.6 Hz, 2H), 7.46 (d, J=8.6 Hz, 2H), 7.51 (d, J=8.1 Hz, 2H), 7.83 (dd, J=8.1, 2.0 Hz, 2H), 7.97 (d, J=2.0 Hz, 2H), 9.66 (br s, 2H); *m/z* (EI, %) 432 (M^+, 100), 419 (73), 404 (33), 388 (10), 362 (9), 327(8), 312 (10), 299 (14), 294 (15), 265 (22), 263 (11), 240 (11), 211 (43), 209 (34), 191 (9), 189 (12), 185 (22), 183 (15), 155 (21), 149 (41), 125 (18), 121 (23), 107 (23), 105 (95), 81 (48), 79 (64), 77 (69), 67 (48), 55 (54). Found: $M^{\bullet+}$, 432.1366. $C_{29}H_{20}O_4$ requires $M^{\bullet+}$, 432.1362.

4.1.5. (±)-2,8-Di(phenyl-4-trifluoromethanesulfonate) dibenzobicyclo[*b*,*f*][3.3.1]nona-5a,6a-diene-6,12-dione 9. Pyridine (0.10 mL, 1.28×10^{-3} mol) was added to bisphenol **8** (115 mg, 2.66×10^{-4} mol) suspended in dry CH_2Cl_2 (17 mL) at rt under an atmosphere of nitrogen. The resultant

solution was cooled to 0 °C (ice bath) followed by addition of triflic anhydride (0.15 mL, 8.81×10^{-4} mol). After stirring at rt for 2 h, the reaction was quenched with aq $NaHCO_3$ (1 mL) and the mixture extracted into Et_2O (3×15 mL). The organic solution was washed with satd aq $CuSO_4$ (3×15 mL), H_2O (1×15 mL), and brine (1×15 mL), dried (Na_2SO_4) and concentrated in vacuo to afford crude bistriflate **9** as a yellow oil (183 mg, 99%) [R_f 0.93; MeOH/$CHCl_3$ (1:9)], which was used in the next reaction without further purification; δ_H (200 MHz, $CDCl_3$) 3.07 (t, J=2.6 Hz, 2H), 4.13 (t, J=2.6 Hz, 2H), 7.33 (d, J=8.8 Hz, 2H), 7.59 (d, J=8.0 Hz, 2H), 7.60 (d, J=8.8 Hz, 2H), 7.74 (dd, J=8.0, 2.2 Hz, 2H), 8.18 (d, J=2.2 Hz, 2H).

4.1.6. (±)-2,8-Bis(4-phenylpyridyl)dibenzobicyclo[*b*,*f*][3.3.1]nona-5a,6a-diene-6,12-dione L3. To a mixture of the bistriflate **9** (183 mg, 2.63×10^{-4} mol), 4-pyridinylboronic acid (100 mg, 8.13×10^{-4} mol) and sodium carbonate (140 mg, 1.32×10^{-3} mol) were added DME (20 mL) and H_2O (6 mL). The mixture was evacuated and purged with nitrogen (3× freeze-pump-thaw cycles) and heated at 85 °C in the dark under an atmosphere of nitrogen for 46 h. After cooling to rt, the mixture was taken up in CH_2Cl_2 (250 mL) and washed with H_2O (120 mL). The layers were separated and the organic layer was washed with aq Na_2CO_3 (2×50 mL). The aqueous solution was extracted with CH_2Cl_2 (100 mL); the organic solutions were combined then dried (Na_2SO_4) and evaporated in vacuo. Flash column chromatography of the residue on silica, eluting with MeOH/CH_2Cl_2 (3:97), followed by recrystallisation of the resulting white solid from MeOH and trifluoroacetic acid afforded ligand **L3** as colourless crystals (100 mg, 68%) [R_f 0.27; MeOH/CH_2Cl_2 (4:96)]; mp>300 °C; IR (KBr) 1683, 1653, 1558 cm^{-1}; δ_H (200 MHz, $CDCl_3$) 3.10 (m, 2H), 4.15 (m, 2H), 7.55 (d, J=5.9 Hz, 4H), 7.63 (d, J=8.0 Hz, 2H), 7.72 (s, 8H), 7.83 (dd, J=8.0, 2.0 Hz, 2H), 8.29 (d, J=2.0 Hz, 2H), 8.69 (d, J=5.9 Hz, 4H); *m*/*z* (EI, %) 554 (M^+, 40), 553 (100), 526 (77), 277 (15), 242 (9), 228 (8), 165 (9), 156 (23), 149 (19), 105 (11), 95 (15), 91 (20), 77 (69), 79 (27), 67 (28), 55 (37). Found: $M^{\bullet+}$, 544.1991. $C_{39}H_{26}N_2O_2$ requires $M^{\bullet+}$, 554.1994. Crystals suitable for X-ray diffraction were obtained by recrystallisation from MeOH and trifluoroacetic acid.

4.1.7. Attempted preparation of (±)-2,8-bis(4-nitrophenyl)dibenzobicyclo[*b*,*f*][3.3.1]nona-5a,6a-diene-6,12-dione 6. Using the same protocol for the synthesis of cleft **L1**, the reaction of the dibromo cleft **2** (78 mg, 0.19 mmol), Na_2CO_3 (123 mg, 1.16 mmol), 4-nitrophenylboronic acid (74 mg, 0.44 mmol) and $PdCl_2(PPh_3)_2$ (30 mg, 43 μmol) followed by flash chromatography, eluting with EtOAc/hexane (3:1) afforded the crude monocoupled product **13** as a cream solid (55 mg, 64%) [R_f 0.65; EtOAc/hexane (2:1)]; δ_H (200 MHz) 3.05–3.02 (2H, m), 4.11–4.05 (2H, m), 7.81–7.58 (6H, m), 8.31–8.10 (4H, m); *m*/*z* (EI, %) 448.9 (MH^+, 97%), 446.9 (M^+, 100), 419.0 (33), 263.2 (32). Crude **13** was treated further with Na_2CO_3 (123 mg, 1.16 mmol), 4-nitrophenylboronic acid (74 mg, 0.44 mmol) and $PdCl_2(PPh_3)_2$ (30 mg, 43 μmol). ^1H NMR analysis of the resulting product indicated a complex mixture of products.

4.1.8. (±)-2,8-Diiododibenzobicyclo[*b*,*f*][3.3.1]nona-5a,6a-diene-6,12-dione 12. An aqueous solution (1 mL) of sodium nitrite (23 mg, 0.33 mmol) was added over 2 h to a stirred solution of cleft **15** (34 mg, 0.12 mmol) in water (1.5 mL) and concd H_2SO_4 (four drops) at 0 °C. After 30 min, an aqueous solution (1.5 mL) of KI (79.5 mg, 0.48 mmol) was slowly added. The reaction mixture was warmed to rt and left to stand for 40 min. The mixture was poured onto water (40 mL) and extracted with EtOAc (3×30 mL). The combined organic extracts were washed with 10% aq $Na_2S_2O_7$ (100 mL) and brine (100 mL), then dried (Na_2SO_4) and concentrated in vacuo. Flash chromatography of the orange residue on silica, eluting with EtOAc/hexane (1:5) afforded the diiodide **12** as an off-white coloured solid (42.5 mg, 70%) [R_f 0.81 (CH_2Cl_2)]; mp 243.5–245.5 °C; IR (NaCl) 1683 cm^{-1}; δ_H (200 MHz) 2.96 (t, J=3.0 Hz, 2H), 3.96 (t, J=3.0 Hz, 2H), 7.19 (d, J=8.1 Hz, 2H), 7.83 (dd, J=8.1, 2.0 Hz, 2H), 8.27 (d, J=2.0 Hz, 2H); *m*/*z* (ES^+, %) 500.8 ($M+H^+$, 18%), 499.8 (M^+, 100), 218.2 (41), 189.2 (36). Anal. Calcd for $C_{17}H_{10}I_2O_2$: (%) C 40.83, H 2.02. Found: C 41.07, H 2.12.

4.2. Metal complexation

A solution of ligand **L1** (10 mg, 2.49×10^{-5} mol) in MeOH (1.5 mL) was added dropwise to a solution of [$Pd(ONO_2)_2$(en)] (7.25 mg, 2.49×10^{-5} mol) in H_2O (1.5 mL) at 20 °C. The homogeneous solution was stirred at 20 °C for four days. The residue was redissolved in CD_3OD/D_2O (1:1) and analysed by ^1H NMR spectroscopy. The ^1H NMR spectrum contained multiple peaks consistent with the formation of a mixture of stereoisomers. Analysis by ESI mass spectrometry showed peaks consistent with the formation of the [2+2] macrocycle, *m*/*z* 631 $[M-2NO_3]^{2+}$, 508 $[M-4NO_3+7MeOH]^{4+}$, 380 $[M-4NO_3+3MeOH]^{4+}$ and 284 $[M-4NO_3]^{4+}$.

In an analogous fashion, methanolic solutions of ligand **L2** (4.49 mg, 1×10^{-5} mol; 1.2 mL) and ligand **L3** (5.54 mg, 1×10^{-5} mol; 3.3 mL) were independently treated with [$Pd(ONO_2)_2$(en)] (2.91 mg, 1×10^{-5} mol) in H_2O (1.2 mL). After stirring at 20 °C for 6 h, the crude reaction mixtures were analysed by ESI mass spectrometry. Peaks consistent with the formation of the [2+2] metallomacrocycles were observed as follows: for **L2**-containing macrocycle, *m*/*z* 833 $[M-2NO_3+MeOH+H_2O]^{2+}$, 720 $[M-4NO_3]^{4+}$, 661 $[M-4NO_3+6MeOH+6H_2O]^{4+}$; for **L3**-containing macrocycle, *m*/*z* 616 $[M-4(NO_3)]^{4+}$ and 557 $[M-4NO_3+5MeOH+5H_2O]^{4+}$.

4.3. X-ray diffraction

Data collections were undertaken at 150(2) K, graphite monochromated Mo Kα radiation (0.71073 Å). For **L1** and **L3**, an APEXII-Kappa-FR591 rotating anode diffractometer was used for the data collection, while for **L2**, a Bruker SMART 1000 sealed tube diffractometer was used. The diffraction data integration and reduction for **L1**, **L2** and **L3** were undertaken with SAINT and XPREP,[26] subsequent computations were carried out with the XTAL[27] and WinGX[28] graphical user interfaces. A multi-scan correction determined with SADABS[29] was applied to the data of **L1** and **L2**. There was no crystal decay during the data collections. The structures were solved by direct methods with SIR97[30] and extended and refined with SHELXL97.[31]

Anisotropic displacement parameters were refined for the non-hydrogen atoms. The asymmetric unit for **L1** contains half of the molecule located on a two fold axis passing through the bridgehead carbon site C(9), and there is also a water molecule residing on a two fold axis. In addition to the cleft molecule, the asymmetric unit for **L2** contains a methanol solvate disordered over two sites with occupancies refined and then fixed at 0.5. The asymmetric unit for **L3** contains two crystallographically independent molecules. ORTEP[32] depictions are provided in Figures 1 and 2.

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited to the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 293459, 29360 and 293461. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223 336033 or email: deposit@ccdc.cam.ac.uk].

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Nucleophilic α-addition to β-nitroacrylates: application to the synthesis of α-thioacrylates

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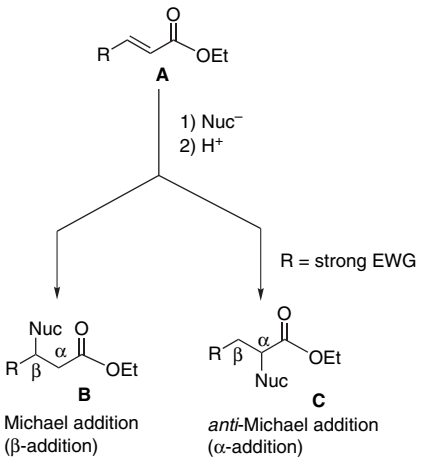
Abstract—The α-addition of alkyl or aryl thionucleophiles to β-nitro-α,β-unsaturated alkenoates in THF in the presence of TEA or DBU gave access to the α-thio-α,β-unsaturated alkenoates. The reaction occurred via formation of β-nitro-α-thioalkanoates and concomitant elimination of nitrous acid from the α-adducts.

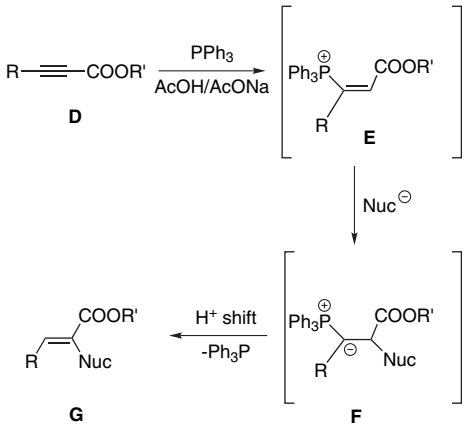
1. Introduction

The Michael reaction is one of the most versatile methods in organic synthesis for the construction of new carbon–carbon or carbon–heteroatom bonds.[1] The Michael addition between various nucleophiles and α,β-unsaturated alkenoates **A** (Scheme 1) in most cases occurs regioselectively at the β-position to give products of type **B** (β-adduct). The regioselectivity of the Michael reaction can be inverted by attaching groups with strongly electron-withdrawing properties at the β carbon, which leads to the formation of the α-substituted products[2–4] of type **C** (α-adduct).



Scheme 1. The α- vs β-addition of the nucleophiles to Michael acceptors.

Recently, Trost and Dake[5] showed that the regioselectivity of Michael additions can be redirected from the classical β-addition to the abnormal α-addition when triphenylphosphine is used as a catalyst. They have shown that PPh_3-catalyzed addition of amines to 2-alkynoates esters **D** occurs at the α-carbon to give 2-amino alkenoates **G**. The overall α-addition resulted from β-addition of PPh_3 to alkynoates **D**, which led to the formation of the vinyl phosphonium intermediate **E**. The latter serves as an α-Michael acceptor (relative to the carbonyl group) and reacts with the nucleophile to give intermediate **F**, which furnishes, after elimination of triphenylphosphine, product **G** with a net result being overall α-substitution (Scheme 2).



Scheme 2. The α-addition of nucleophiles to 2-alkynoates in the presence of PPh_3.

Our recent theoretical studies of the Michael reaction reveal that the reaction barriers to α- vs β-addition decrease as the strength of the electron-withdrawing group on the β carbon increases. For those with sufficiently strong electron-withdrawing groups, α-addition becomes favoured.[6] For instance, it was predicted that methyl 3-nitropropenoate should react with nucleophiles to give an α-substituted

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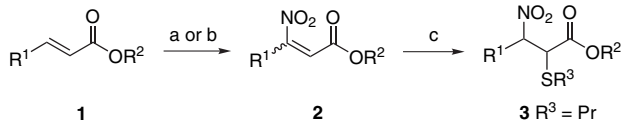
adduct.[6a] On the basis of the theoretical calculations we rationalized that 3-nitro-2-alkenoates would serve as a convenient substrate leading to the formation of *anti*-Michael adducts. After in situ elimination of nitrous acid, such an adduct would be expected to provide access to α-substituted α,β-unsaturated alkenoates, which can serve as precursors for further chemical transformations. Herein, we report application of β-nitroacrylates as α-Michael acceptors in a new approach for the synthesis of α-thioacrylates through an in situ elimination of nitrous acid from the α-adducts.

Very recently, Ballini and his group[7] utilized reactions of β-nitroacrylic esters with C-nucleophiles for the synthesis of polyfunctionalized α,β-unsaturated esters prepared via an α-substitution and elimination process. The α-addition of organozinc cuprates, dialkylzinc reagents or trimethylaluminium to β-nitroacrylate esters has been used in the synthesis of optically active β-amino acids.[8] Addition of indoles to the α-carbon of β-nitroacrylates has also been reported.[9]

The α-thioacrylates have been used as Michael acceptors[10] and as dienophiles in Diels–Alder reactions.[11] They have been synthesized by procedures that involve multistep reactions such as: (i) condensation of α-phenylthio acetate carbanions with aldehydes and ketones,[12] (ii) Pummerer-style dehydration of sulfoxides,[13] (iii) *ipso*-substitution of the bromine in α-bromo Michael acceptors,[14] or (iv) nucleophilic substitution—Wittig reaction of α-hypervalent iodine functionalized phosphonium ylide.[15] The above procedures often require harsh conditions and frequently give products with low selectivity.

2. Results and discussion

The starting 3-nitroacrylates **2a–b** were prepared in 70–75% yield by reactions of the corresponding acrylic esters **1a–b** with $NaNO_2$–ceric ammonium nitrate (CAN) followed by dehydration ($MsCl/Et_3N/CH_2Cl_2/-20\ ^\circ C$) as reported recently (Scheme 3).[16] The β-nitrocinnamate derivatives **2c–d** were obtained directly from the corresponding cinnamates **1c–d** with $NaNO_2$–CAN in 57–65% yields. Attempts to prepare the 4-nitrocinnamate analogue using this method were unsuccessful. The 3-nitroacrylic esters **2a** and **2b** were obtained as single *E* isomers while **2c** and **2d** were obtained as a mixture of *E* and *Z* isomers in ratios of 2:1 and 5:1, respectively.



Scheme 3. α-Addition of thiolate nucleophiles to β-nitroacrylates. Reagents and condition: (a) $CAN/NaNO_2/CH_3CN$; (b) (i) $CAN/NaNO_2/CH_3CN$, (ii) $MsCl/Et_3N/CH_2Cl_2/20\ ^\circ C$; (c) R^3SH/Et_3N (cat)/THF rt.

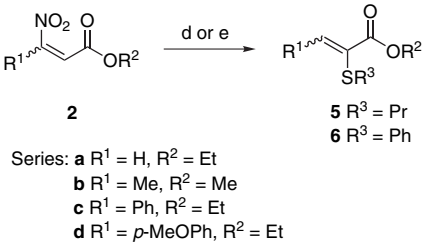
Treatment of 3-nitroacrylates **2a**(*E*) (Scheme 3) with propanethiol in the presence of a catalytic amount of triethylamine (TEA) in tetrahydrofuran (THF) at ambient temperature provided the α-substituted product **3a** in 78% yield (Table 1, entry 1). Analogous treatment of 3-nitrocrotonate **2b**(*E*) and 3-nitrocinnamate **2c–d**(*E*/*Z*) afforded α-adducts **3b–d** as a 1:1 mixture of diastereomers (Table 1, entries 2–4). In the case of **3b**, the diastereomers were separated by column chromatography. The ^1H NMR spectrum for **3b** verified that the adduct was derived from an α-addition since the presence of two sets of signals corresponding to the hydrogens at C2 (d, J=10.5 Hz) and C3 (dq, J=10.5, 7,0 Hz) instead of signals for two hydrogens at C2 was observed.

Table 1. Reaction parameters for the α-addition of thiolate nucleophiles to β-nitroacrylates

Entry	Product	Yield (%)[a]	Condition[b]
1	**3a**	78	c
2	**3b**	84	c
3	**3c**	79	c
4	**3d**	78	c
5	**4c**	86	c
6	**4d**	72	c

[a] Isolated yield.
[b] See Scheme 3.

Contrary to the reactions with propanethiol, treatment of β-nitroacrylates with thiophenol produced the stable α-adducts **4c** and **4d** only in the case of β-nitrocinnamic substrates **2c–d** (Table 1, entries 5 and 6). Treatment of aliphatic **2a** and **2b** with thiophenol initially produced the *anti*-Michael addition products **4a** and **4b** (TLC) as well, but these adducts underwent subsequent in situ elimination of HNO_2 to produce α-thioacrylates **6a** and **6b** even in the absence of TEA (Scheme 4).



Scheme 4. Synthesis of α-thioacrylates. Reagents and condition: (d) R^3SH/Et_3N/THF rt; (e) R^3SH/DBU/THF (60 °C oil bath).

In order to optimalize the formation of α-thio-α,β-unsaturated alkanoates **5** and **6** we tested different reaction conditions, such as various ratios of the substrates to nucleophiles and base, as well as different solvents and temperature. We found that for the reaction of aliphatic alkenoates **2a** and **2b** with propanethiol, the highest yields of **5a** and **5b** were obtained with a 1:1.2:1 ratio of **2a** or **2b**/propanethiol/TEA in THF at ambient temperature (Table 2, entries 1 and 2). Using the same reaction conditions the α-phenylthioalkenoates **6a** and **6b** were also obtained (Table 2, entries 5 and 6) in high yield. The α-thiocrotonates **5b** and **6b** were isolated as *Z* isomers. The *Z*-stereochemistry of **6b** was established by comparison of the chemical shift of β-vinyl proton signal, which occurs at a lower field (δ 7.48) than of the corresponding *E* isomer (δ 6.54) with the literature values.[13d,15a]

Table 2. Reaction parameters for the synthesis of α-thioacrylates

Entry	Product	Yield (%)[a]	Condition[b]	Ratio[c] of *E*/*Z*
1	**5a**	82	d	—
2	**5b**	88	d	0:1
3	**5c**	79	e	1:0
4	**5d**	40	e	1:0
5	**6a**	78	d	—
6	**6b**	84	d	0:1
7	**6c**	78	e	0:1
8	**6d**	44	e	0.6:1

[a] Isolated yield.
[b] See Scheme 4.
[c] Estimated by ^{1}H NMR.

In the reaction of cinnamates **2c–d**, the propylthio-**3c–d** as well as phenylthio α-adducts **4c–d** were stable, so stronger base (DBU) and longer reaction times and/or higher temperatures were necessary for the conversion to α-thioacrylates **5** and **6** (Scheme 4). Thus, treatment of **2c** (*E*/*Z*, 1.0:0.5) with propanethiol in the presence of 1.2 equiv DBU in THF at 60 °C for 24 h led to the formation of α-(propanethiol)cinnamate **5c** as a single *E* isomer in 79% yield. Analogous treatment of **2c** with thiophenol (4 h) afforded **6c** in 82% yield as a mixture of isomers (*E*/*Z*, 0.2:1.0).[13d,15a] Prolonged heating led to conversion of the isomers resulting in formation of the more stable *Z* isomer, which was isolated in 78% yield (Table 2, entries 3 and 7). In both the cases the intermediary α-addition products **3c** or **4c** were also isolated in low yields from the reaction mixture (<10%). Interestingly, even treatment of 3-nitro-*p*-methoxycinnamate **2d** with propanethiol as well as with thiophenol led to the formation of quite stable α-adducts **3d** and **4d**, which required heating to convert them to the α-thiocinnamates **5d**(*E*) or **6d** (*E*/*Z*; 0.6:1.0) isomers (Table 2, entries 4 and 8).

It was found that addition of thionucleophiles to β-nitroacrylic esters **2a–d** in THF with an equivalent amount of base affords α-thioacrylates **5** or **6** in a one pot process via *anti*-Michael addition with the formation of β-nitro-α-thio intermediates **3** or **4** and subsequent elimination of nitrous acid. These results indicate that the kind of substituents at the β-position (alkyl or aryl) in β-nitroacrylates and the pK_a of the thionucleophiles are two important factors contributing to the stabilization of α-addition products **3** and **4**.

3. Conclusion

In summary, we have developed an efficient method for the synthesis of α-alkyl(or aryl)thio-α,β-unsaturated alkenoates via α-addition of nucleophiles to β-nitroalkenoates, followed by in situ elimination of nitrous acid from the resulting α-adducts in basic conditions.

4. Experimental

4.1. General

THF was distilled from sodium benzophenone under nitrogen. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were determined in $CHCl_3$ on a Bruker Avance-400 instrument. When the spectra were recorded for the mixture of isomers the signals for the respective isomers were assigned based on the COSY and HETCOR experiments. Mass spectra (MS) and HRMS were obtained with electron impact (EI, 20 eV) technique. Merck kieselgel 60-F_{254} sheets were used for TLC and products were detected with 254 nm light. Merck kieselgel 60 (230–400 mesh) was used for column chromatography.

4.1.1. Ethyl 3-nitro-2-propylthiopropanoate (3a) (procedure A). Propanethiol (0.15 mL, 126 mg, 1.66 mmol) and TEA (24 μL, 17 mg, 0.17 mmol) were added to a stirred solution of **2a** (60 mg, 0.41 mmol) in THF (2 mL) at ambient temperature. After 10 min, the resulting mixture was evaporated to dryness under vacuum and the oily residue was column chromatographed (hexane→5% EtOAc/hexane) to give **3a** (71 mg, 78%) as an oil: IR ($CHCl_3$) 1735 (C=O), 1550 (NO_2) cm^{-1}; ^{1}H NMR δ 1.01 (t, *J*=7.3 Hz, 3H), 1.32 (t, *J*=7.1 Hz, 3H), 1.64 (dq, *J*=2.1, 7.3 Hz, 2H), 2.68 (dt, *J*=2.1, 7.3 Hz, 2H), 3.94 (dd, *J*=10.2, 5.1 Hz, 1H), 4.27 (q, *J*=7.1 Hz, 2H), 4.58 (dd, *J*=14.8, 5.1 Hz, 1H), 4.90 (dd, *J*=14.8, 10.2 Hz, 1H); ^{13}C NMR δ 13.2, 13.4, 22.6, 33.8, 42.1, 62.1, 74.4, 169.5; HRMS (EI) *m*/*z* calcd for $C_8H_{15}NO_4S$ (M^+) 221.0722, found 221.0711.

4.1.2. Methyl 3-nitro-2-propylthiobutanoate (3b). Treatment of **2b** (*E*; 76 mg, 0.52 mmol) with propanethiol (0.19 mL, 158 mg, 2.08 mmol) by procedure A gave **3b** (93 mg, 84%) as a separable mixture (1:1) of two diastereomers: IR ($CHCl_3$) 1736 (C=O), 1550 (NO_2) cm^{-1}. The less polar isomer had: ^{1}H NMR δ 0.97 (t, *J*=7.3 Hz, 3H), 1.61 (qd, *J*=7.3, 3.5 Hz, 2H), 1.78 (d, *J*=7.0 Hz, 3H), 2.64 (td, *J*=7.3, 2.1 Hz, 2H), 3.71 (d, *J*=10.5 Hz, 1H), 3.77 (s, 3H), 4.84 (dq, *J*=10.5, 7.0 Hz, 1H); ^{13}C NMR δ 13.2, 17.8, 22.4, 33.6, 48.5, 52.8, 82.1, 170.2. The more polar isomer had: ^{1}H NMR δ 0.91 (t, *J*=7.3 Hz, 3H), 1.51–1.58 (m collapsed with d, *J*=6.7 Hz, 5H), 1.78 (d, *J*=7.0 Hz, 3H), 2.54–2.61 (m, 2H), 3.65 (d, *J*=10.0 Hz, 1H), 3.72 (s, 3H), 4.79 (dq, *J*=10.0, 6.7 Hz, 1H); ^{13}C NMR δ 13.3, 18.1, 22.6, 34.4, 49.3, 52.8, 83.4, 169.6; HRMS (EI) *m*/*z* calcd for $C_8H_{15}NO_4S$ (M^+) 221.0722, found 221.0710.

4.1.3. Ethyl 3-nitro-3-phenyl-2-propylthiopropanoate (3c). Treatment of **2c** (*E*/*Z*, 1.0:0.5; 47 mg, 0.19 mmol) with propanethiol (0.07 mL, 58 mg, 0.76 mmol) by procedure A gave **3c** (50 mg, 79%) as a mixture (1:1) of two diastereomers as yellow oil: IR ($CHCl_3$) 1752 (C=O), 1568 (NO_2) cm^{-1}. One isomer had: ^{1}H NMR δ 0.80 (t, *J*=7.3 Hz, 3H), 0.90 (t, *J*=7.1 Hz, 3H), 1.62 (sextet, *J*=7.3, 2.8 Hz, 2H), 2.30–2.38 (m, 2H), 3.90 (q, *J*=7.1 Hz, 2H), 4.56 (d, *J*=11.5 Hz, 1H), 5.39 (d, *J*=11.5 Hz, 1H), 7.18–7.27 (m, 5H, Ar); ^{13}C NMR δ 13.2, 13.4, 22.3, 33.9, 48.5, 63.0, 91.3, 128.0, 128.5, 128.8, 136.3, 162.2. Second isomer had: ^{1}H NMR δ 0.80 (t, *J*=7.3 Hz, 3H), 1.29 (t, *J*=7.1 Hz, 3H), 1.62 (sextet, *J*=7.3, 2.8 Hz, 2H), 2.30–2.38 (m, 2H), 4.27 (qd, *J*=7.1, 2.7 Hz, 2H), 4.57 (d, *J*=10.7 Hz, 1H), 5.34 (d, *J*=10.7 Hz, 1H), 7.18–7.27 (m, 5H, Ar); ^{13}C NMR δ 13.2, 13.9, 22.4, 34.4, 49.7, 63.4, 92.0, 128.4, 128.5, 128.9, 136.8, 162.6; HRMS (EI) *m*/*z* calcd for $C_{14}H_{19}NO_4S$ (M^+) 297.1035, found 297.1032.

4.1.4. Ethyl 3-(4-methoxyphenyl)-3-nitro-2-propylthiopropanoate (3d). Treatment of **2d** (*E*/*Z*, 1.0:0.2; 52 mg, 0.21 mmol) with propanethiol (74 μL, 69 mg, 0.83 mmol)

by procedure A gave **3d** (53 mg, 78%) as a mixture (1:1) of two isomers as an oil: IR ($CHCl_3$) 1752 (C=O), 1569 (NO_2) cm^{-1}. One isomer had: ^1H NMR δ 0.91 (t, J=7.3 Hz, 3H), 1.05 (t, J=7.1 Hz, 3H), 1.52–1.55 (m, 2H), 2.36–2.44 (m, 2H), 3.81 (s, 3H), 4.03 (m, 2H), 4.62 (d, J=11.4 Hz, 1H), 5.42 (d, J=11.4 Hz, 1H), 6.86–6.89 (m, 2H, Ar), 7.27–7.30 (m, 2H, Ar); ^{13}C NMR δ 13.3, 13.6, 22.4, 33.8, 48.1, 55.2, 63.0, 91.6, 114.2, 127.9, 129.2, 159.6, 162.3. Second isomer had: ^1H NMR δ 0.91 (t, J=7.3 Hz, 3H), 1.36 (t, J=7.1 Hz, 3H), 1.52–1.55 (m, 2H), 2.36–2.44 (m, 2H), 3.82 (s, 3H), 4.37 (qd, J=7.1, 2.4 Hz, 2H), 4.64 (d, J=10.5 Hz, 1H), 5.39 (d, J=10.3 Hz, 1H), 6.86–6.89 (m, 2H, Ar), 7.27–7.30 (m, 2H, Ar); ^{13}C NMR δ 13.2, 13.9, 22.4, 34.2, 49.1, 55.4, 63.4, 92.2, 114.3, 128.5, 129.7, 159.6, 162.7; HRMS (EI) *m/z* calcd for $C_{15}H_{21}NO_5S$ (M^+) 327.1140, found 327.1138.

4.1.5. Ethyl 3-nitro-3-phenyl-2-phenylthiopropanoate (4c). Treatment of **2c** (*E*/*Z*, 1.0:0.5; 57 mg, 0.26 mmol) with thiophenol (32 μL, 34 mg, 0.31 mmol) by procedure A (without addition of TEA) gave **4c** (73 mg, 86%) as a mixture (1:1) of two isomers as an oil: IR ($CHCl_3$) 1752 (C=O), 1566 (NO_2) cm^{-1}. One isomer had: ^1H NMR δ 0.90 (t, J=7.1 Hz, 3H), 3.89–3.96 (m, 2H), 4.84 (d, J=10.6 Hz, 1H), 5.46 (d, J=10.6 Hz, 1H), 6.99–7.05 (m, 2H, Ar), 7.15–7.21 (m, 8H, Ar); ^{13}C NMR δ 13.4, 52.2, 63.1, 90.5, 127.8, 128.4, 128.6, 128.9, 129.1, 130.6, 134.8, 135.7, 162.1. Second isomer had: ^1H NMR δ 1.29 (t, J=7.1 Hz, 3H), 4.22–4.27 (m, 2H), 4.87 (d, J=11.5 Hz, 1H), 5.49 (d, J=11.5 Hz, 1H), 6.99–7.05 (m, 2H, Ar), 7.15–7.21 (m, 8H, Ar); ^{13}C NMR δ 13.9, 53.7, 63.5, 91.3, 128.4, 128.5, 128.7, 129.0, 129.2, 131.5, 135.1, 135.8, 162.6; HRMS (EI) *m/z* calcd for $C_{17}H_{17}O_4SN$ (M^+) 331.0878, found 331.0889.

4.1.6. Ethyl 3-(4-methoxyphenyl)-3-nitro-2-phenylthiopropanoate (4d). Treatment of **2d** (*E*/*Z*, 1.0:0.2; 50 mg, 0.2 mmol) with thiophenol (24 μL, 26 mg, 0.22 mmol) by procedure A gave **4d** (52 mg, 72%) as a mixture (1:1) of two isomers as an oil: IR ($CHCl_3$) 1752 (C=O), 1569 (NO_2) cm^{-1}. One isomer had: ^1H NMR δ 1.04 (t, J=7.1 Hz, 3H), 3.78 (s, 3H), 4.03–4.06 (m, 2H), 4.92 (d, J=10.5 Hz, 1H), 5.52 (d, J=10.5 Hz, 1H), 6.77–6.81 (m, 2H, Ar), 7.02–7.07 (m, 2H, Ar), 7.26–7.27 (m, 4H, Ar), 7.29–7.32 (m, 1H, Ar); ^{13}C NMR δ 13.5, 51.9, 55.3, 63.1, 90.7, 114.0, 127.5, 128.9, 129.0, 130.8, 132.2, 134.7, 159.6, 162.1. Second isomer had: ^1H NMR δ 1.38 (t, J=7.1 Hz, 3H), 3.80 (s, 3H), 4.26–4.35 (m, 2H), 4.94 (d, J=11.4 Hz, 1H), 5.53 (d, J=11.4 Hz, 1H), 6.77–6.81 (m, 2H, Ar), 7.02–7.07 (m, 2H, Ar), 7.26–7.27 (m, 4H, Ar), 7.29–7.32 (m, 1H, Ar); ^{13}C NMR δ 13.9, 53.1, 55.5, 63.5, 91.4, 114.1, 127.7, 129.1, 129.6, 131.6, 132.6, 135.1, 159.6, 162.6; HRMS (EI) *m/z* calcd for $C_{18}H_{19}O_5SN$ (M^+) 361.0984, found 361.1004.

4.1.7. Ethyl 2-propylthiopropenoate (5a) (procedure B). Propanethiol (37 μL, 31.4 mg, 0.41 mmol) and TEA (47 μL, 34.3 mg, 0.34 mmol) were added to a stirred solution of **2a** (50 mg, 0.34 mmol) in THF (2 mL) at ambient temperature and stirring was continued for 10 h. The resulting mixture was evaporated to dryness under vacuum and the residue was column chromatographed (hexane→1% EtOAc/hexane) to give **5a** (28 mg, 82%) as an oil: IR ($CHCl_3$) 1712 (C=O), 1614 (C=C) cm^{-1}; ^1H NMR δ 0.97 (t, J=7.3 Hz, 3H), 1.26 (t, J=7.1 Hz, 3H), 1.64 (sextet, J=7.3 Hz, 2H), 2.62 (t, J=7.3 Hz, 2H), 4.20 (q, J=7.1 Hz, 2H), 5.33 (s, 1H), 6.27 (s, 1H); ^{13}C NMR δ 13.6, 14.1, 21.2, 33.5, 61.7, 118.8, 137.9, 164.6; HRMS (EI) *m/z* calcd for $C_8H_{14}O_2S$ (M^+) 174.0714, found 174.0710.

4.1.8. Methyl 2-propylthio-2(*Z*)-butenoate (5b). Treatment of **2b** (*E*; 46 mg, 0.32 mmol) with propanethiol (34 μL, 29 mg, 0.38 mmol) and TEA (44 μL, 32.3 mg, 0.32 mmol) by procedure B gave **5b** (27 mg, 88%) as an oil: IR ($CHCl_3$) 1710 (C=O), 1610 (C=C) cm^{-1}; ^1H NMR δ 0.89 (t, J=7.3 Hz, 3H), 1.46 (sextet, J=7.3 Hz, 2H), 1.95 (d, J=7.0 Hz, 3H), 2.61 (t, J=7.3 Hz, 2H), 3.72 (s, 3H), 7.21 (q, J=7.0 Hz, 1H); ^{13}C NMR δ 13.2, 16.6, 23.0, 35.7, 52.7, 128.3, 146.5, 166.2; HRMS (EI) *m/z* calcd for $C_8H_{14}O_2S$ (M^+) 174.0714, found 174.0708.

4.1.9. Ethyl 3-phenyl-2-propylthio-2(*E*)-propenoate (5c). Treatment of **2c** (*E*/*Z*, 1.0:0.5; 47 mg, 0.21 mmol) with propanethiol (23 μL, 19 mg, 0.25 mmol) and DBU (31 μL, 32 mg, 0.21 mmol) by procedure B (stirring was continued for 24 h at 60 °C) gave separable mixture of **5c** (42 mg, 79%) and **4c** (5 mg, 8%; as a mixture of two isomers). Compound **5c** had: IR ($CHCl_3$) 1693 (C=O), 1602 (C=C) cm^{-1}; ^1H NMR δ 0.83 (t, J=7.2 Hz, 3H), 1.32 (t, J=7.1 Hz, 3H), 1.46 (sextet, J=7.2 Hz, 2H), 2.37 (t, J=7.2 Hz, 2H), 4.25 (q, J=7.1 Hz, 2H), 5.93 (s, 1H), 7.33–7.35 (m, 2H, Ar), 7.39–7.41 (m, 3H, Ar); ^{13}C NMR δ 13.2, 14.4, 23.0, 34.7, 60.0, 115.9, 128.0, 128.4, 128.7, 138.8, 160.6, 165.9; HRMS (EI) *m/z* calcd for $C_{14}H_{18}O_2S$ (M^+) 250.1027, found 250.1029.

4.1.10. Ethyl 3-(4-methoxyphenyl)-2-propylthio-2(*E*)-propenoate (5d). Treatment of **2d** (*E*/*Z*, 1.0:0.2; 70 mg, 0.21 mmol) with propanethiol (30 μL, 25 mg, 0.33 mmol) and DBU (50 μL, 51 mg, 0.33 mmol) by procedure B (stirring was continued for 24 h at 60 °C) gave separable mixture of **5d** (31 mg, 40%) and **4d** (28 mg, 30%; as a mixture of two isomers). Compound **5d** had: IR ($CHCl_3$) 1693 (C=O), 1607 (C=C) cm^{-1}; ^1H NMR δ 0.85 (t, J=7.2 Hz, 3H), 1.32 (t, J=7.1 Hz, 3H), 1.46 (sextet, J=7.2 Hz, 2H), 2.41 (t, J=7.2 Hz, 2H), 3.85 (s, 3H), 4.23 (q, J=7.1 Hz, 2H), 5.92 (s, 1H), 6.91–6.93 (m, 2H, Ar), 7.29–7.31 (m, 2H, Ar); ^{13}C NMR δ 13.3, 14.4, 23.0, 29.7, 34.8, 55.3, 59.9, 113.8, 115.7, 129.4, 131.2, 160.1, 165.9; HRMS (EI) *m/z* calcd for $C_{15}H_{20}O_3S$ (M^+) 280.1133, found 280.1128.

4.1.11. Ethyl 2-phenylthiopropenoate (6a) (procedure C). Thiophenol (25 μL, 27.3 mg, 0.25 mmol) and TEA (3 μL, 2.5 mg, 0.025 mmol) were added to a stirred solution of **2a** (30 mg, 0.21 mmol) in THF (2 mL) at ambient temperature and stirring was continued for 2 h. The resulting mixture was evaporated to dryness under vacuum and the residue was column chromatographed (hexane→1% EtOAc/hexane) to give **6a**[13b] (33 mg, 78%) as an oil: IR ($CHCl_3$) 1720 (C=O), 1610 (C=C) cm^{-1}; ^1H NMR δ 1.24 (t, J=7.1 Hz, 3H), 4.26 (q, J=7.1 Hz, 2H), 5.26 (s, 1H), 6.32 (s, 1H),

7.34–7.42 (m, 3H, Ar), 7.48–7.51 (m, 2H, Ar); ^{13}C NMR δ 14.1, 61.8, 122.6, 128.7, 129.5, 134.1, 139.1, 143.4, 164.7.

4.1.12. Methyl 2-phenylthio-2(*Z*)-butenoate (6b). Treatment of **2b** (*E*; 50 mg, 0.34 mmol) with thiophenol or benzenethiol (42 μL, 45.5 mg, 0.41 mmol) by procedure C gave **6b**[13d] (61 mg, 84%) as an oil: IR ($CHCl_3$) 1715 (C=O), 1607 (C=C) cm^{-1}; ^{1}H NMR δ 2.02 (d, J=7.0 Hz, 3H), 3.61 (s, 3H), 7.07–7.10 (m, 1H, Ar), 7.12–7.19 (m, 4H, Ar), 7.48 (q, J=7.0 Hz, 1H); ^{13}C NMR δ 16.7, 52.5, 125.9, 127.3, 127.9, 128.9, 135.7, 149.3, 165.9.

4.1.13. Ethyl 3-phenyl-2-phenylthio-2(*Z*)-propenoate (6c). DBU (33 μL, 33 mg, 0.22 mmol) was added to a stirred solution of **2c** (*E*/*Z*, 1.0:0.5; 48 mg, 0.22 mmol) in THF (2 mL) containing thiophenol (27 μL, 29 mg, 0.26 mmol), and stirring was continued for 24 h at 60 °C. After cooling to ambient temperature, CH_2Cl_2 (5 mL) and 5% HCl (4 mL) were added. The organic layer was separated and was washed (H_2O, brine), dried ($MgSO_4$) and column chromatographed (hexane→1% EtOAc/hexane) to give **6c**[15a] (48 mg, 78%) and **4c** (5 mg, 7%; as a mixture of two isomers). Compound **6c** had: IR ($CHCl_3$) 1706 (C=O), 1602 (C=C) cm^{-1}; ^{1}H NMR δ 0.97 (t, J=7.1 Hz, 3H), 4.03 (q, J=7.1 Hz, 2H), 7.07–7.12 (m, 1H, Ar), 7.14–7.23 (m, 4H, Ar), 7.28–7.35 (m, 3H, Ar), 7.73–7.77 (m, 2H, Ar), 8.00 (s, 1H); ^{13}C NMR δ 13.6, 61.8, 125.8, 126.4, 127.8, 128.3, 128.4, 128.7, 128.9, 129.9, 130.8, 134.4, 135.7, 145.1, 166.1.

4.1.14. Ethyl 3-(4-methoxyphenyl)-2-phenylthio-2(*E*/*Z*)-propenoate (6d). Treatment of **2d** (*E*/*Z*, 1.0:0.2; 40 mg, 0.16 mmol) with thiophenol (20 μL, 21 mg, 0.19 mmol) and DBU (31 μL, 22 mg, 0.22 mmol) as described for **6c** gave **6d** (*E*/*Z*, 0.6:1.0; 22 mg, 44%): IR ($CHCl_3$) 1706 (C=O), 1601 (C=C) cm^{-1}. *Z*-Isomer had: ^{1}H NMR δ 1.09 (t, J=7.1 Hz, 3H), 3.86 (s, 3H), 4.13 (q, J=7.1 Hz, 2H), 6.92–6.94 (m, 2H, Ar), 7.23–7.31 (m, 5H, Ar), 7.91–7.93 (m, 2H, Ar), 8.12 (s, 1H); ^{13}C NMR δ 13.9, 55.2, 61.7, 113.8, 116.0, 126.0, 128.1, 128.9, 130.2, 133.0, 145.9, 161.1, 166.5. *E* Isomer had: ^{1}H NMR δ 1.36 (t, J=7.1 Hz, 3H), 3.72 (s, 3H), 4.28 (q, J=7.1 Hz, 2H), 6.09 (s, 1H), 6.64–6.66 (m, 2H, Ar), 7.06–7.11 (m, 2H, Ar), 7.14–7.19 (m, 5H, Ar); ^{13}C NMR δ 14.3, 55.4, 60.2, 113.2, 122.0, 127.0, 127.5, 128.4, 130.7, 133.5, 136.0, 158.2, 159.8, 165.9; HRMS (EI) *m*/*z* calcd for $C_{18}H_{18}O_3S$ (M^+) 314.0977, found 314.0988.

Notes: The 4-methoxybenzaldehyde and **4d** were also detected in the reaction mixture.

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An MO study of regioselective amine addition to *ortho*-quinones relevant to melanogenesis

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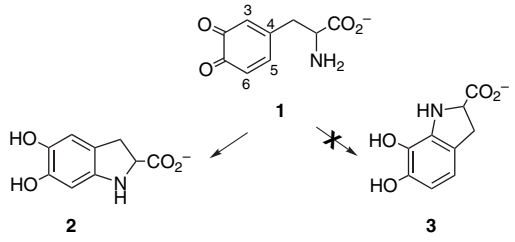
Abstract—Energy profiles for alternative intramolecular cyclisations of 4-(aminoalkyl)-*ortho*-quinones have been calculated using the AM1 method and ab initio energies of the transition states are determined. In all the cases cyclisation at position 5 occurs via a significantly lower energy transition state than cyclisation at position 3. This is consistent with experimental observations. Optimal trajectories for attack have been determined from a study of the reactions of methylamine with 4-methyl-*ortho*-quinone. For cyclisation of aminoalkyl derivatives deviation from the optimal direction is less for reaction at position 5 but constraint on angle of attack only partially accounts for the regioselectivity. Intrinsic differences in the electronic energies of the alternative transition states are the main contributor to regioselectivity. The relative energies of transition states can be modified by variation of the substituent at position 4. The calculations suggest that seven-membered ring formation may occur via a boat transition state and steric hindrance in the seven-membered transition states may account for the experimentally observed influence of *N*-substituents on the mode of reaction.

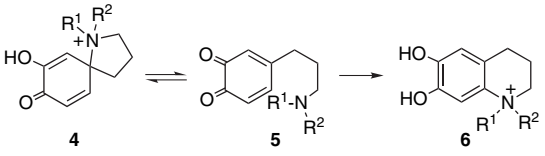
1. Introduction

An important early step in the biosynthesis of eumelanin (a black-brown pigment) is the intramolecular cyclisation of dopaquinone **1** to cyclodopa **2**.[1–3] No evidence of the alternative cyclisation mode (**1**→**3**) (Scheme 1) has been observed.

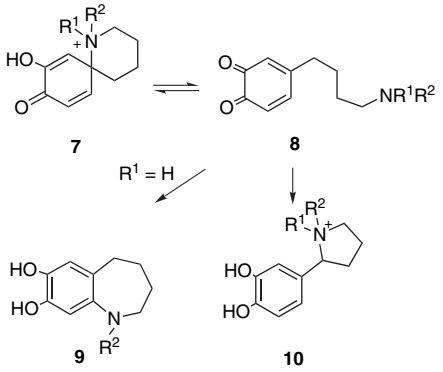


Scheme 1.

We have studied a number of analogues of dopaquinone **1**, including higher homologues, using NMR, tyrosinase oximetry and pulse radiolysis.[4–9] In none of our studies have we observed evidence of the alternative cyclisation at position 3. Increasing the chain length still results in exclusive cyclisation at position 5, although for propyl- and butylamines **5** and **8** (Schemes 2 and 3) we have observed that the spiro derivatives **4** and **7**, resulting from attack at position 4, are the kinetic products and these rapidly equilibrate to the thermodynamic products **6** and **9**. Recently we have isolated a stable spirocyclic product.[5] In the case of the *n*-butyl tertiary amines **8** ($R^1 \neq H$, $R^2 \neq H$) cyclisation is not observed and much slower formation of the isomeric *para*-quinomethane occurs leading to the product **10**. Other modifications of the side chain (Eqs. 1[5] and 2[9]) similarly give exclusively the product from cyclisation at position 5.



Scheme 2.



Scheme 3.

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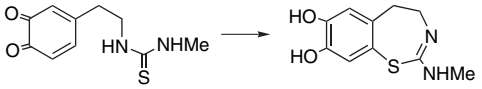
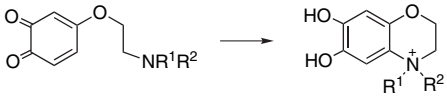
The regioselectivity of these intramolecular cyclisations is consistent with the intermolecular reactions of *ortho*-quinones with amines and other nucleophiles, which usually give Michael addition products at position 5.[10] This is in interesting contrast with the reactions of *ortho*-quinones with thiols (RSH), which even under basic conditions (RS^-), give exclusively or mainly the 6-addition products (Scheme 4).[11–13] This mode of reaction with cysteine is an early step in the formation of pheomelanin (a red-yellow pigment).[3]

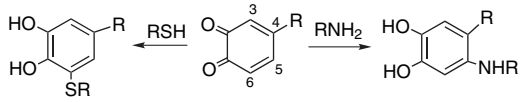


(1)



(2)

In addition to being relevant to the biosynthesis of eumelanin and pheomelanin, the formation of endogenous *ortho*-quinones may be associated with the initiation of some cancers by reaction with DNA-amines or glutathione.[14,15] The mechanisms of these reactions are also of fundamental chemical interest. To explore the origins of the selectivity in these quinone reactions, we have carried out molecular orbital studies. In this paper we report the results for inter- and intramolecular reactions of *ortho*-quinones with primary amines.

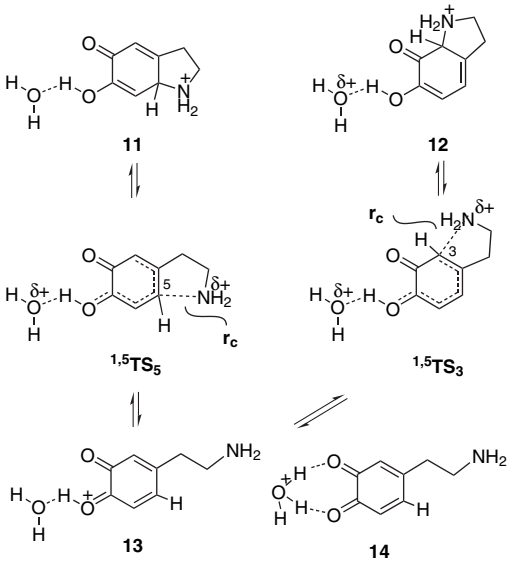


Scheme 4.

2. Results and discussion

2.1. 1,5-Intramolecular cyclisation and intermolecular reactions

The retro-1,5-cyclisations **11**→**13** and **12**→**13** (Scheme 5) were studied using the AM1 method by increasing the appropriate C–N interatomic distance (r_c) in increments of ca. 0.05–0.1 Å. For each value of the reaction coordinate (r_c) the energy was minimised with respect to all other variables. In the following discussion, for intramolecular cyclisations the size of the ring forming in the transition state (**TS**) is designated by a superscript and the ring position of the reaction by a subscript (e.g., ^{1,5}**TS$_5$**). To allow for the role of proton transfer during quinone amine cyclisation in vivo, leading to intermediate enols (e.g., **11** and **12**), a hydrogen bonded water molecule was included in the calculations. In fact the calculated retro-reactions (Scheme 5) led to a protonated *ortho*-quinone **13**, rather than a hydrogen bonded quinone. However, the hydrogen bonded quinone **14** (ΔH_f 70.54 kcal mol^{-1}) was calculated to be only 1.2 kcal mol^{-1} higher in energy than the protonated quinone **13** (ΔH_f 69.36 kcal mol^{-1}). Since this study is primarily concerned with the relative energies of similar alternative transition states formed from common precursors (e.g., **13** or **14**) the exact nature of the solvation of the precursor amines is not an essential requirement. It is beyond the scope of this study to investigate accurate absolute energies of the species involved.



Scheme 5.

The AM1 energy profiles for the alternative reaction pathways to positions 5 and 3 via the transitions states ^{1,5}**TS$_5$** and ^{1,5}**TS$_3$** are shown in Figure 1. In agreement with experimental observations it can be seen that cyclisation at the 5-position is favoured. For both the cyclisations the transition state is calculated to occur at a C–N separation of ca. 2.2 Å. To obtain a more accurate estimate of the relative transition state energies their electronic energies were determined by ab initio calculations at the RHF/6-31G** level using the AM1 geometries. The results of these calculations and the relative energies of transition states are summarised in

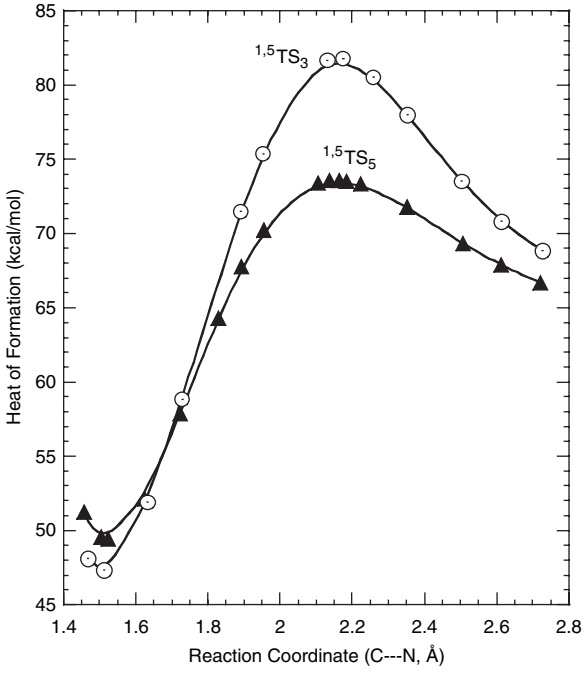


Figure 1. AM1 energy profiles for cyclisation of 4-(2-aminoethyl)-*ortho*-quinone at positions 3 (○) and 5 (▲) (Scheme 5).

Table 1. Ab initio electronic energies (au) and relative energies (kcal mol^{-1}) for transition states

Reaction mode	Transition state (**TS**)	RHF/6-31G** (a.u.)	Rel energy (kcal mol^{-1})[a]
Intermolecular	$\mathbf{TS_3}$	−589.58129	12.1 (6.3)
	$\mathbf{TS_5}$	−589.60063	0.0 (0.0)
	$\mathbf{TS_6}$	−589.59264	5.0 (2.6)
1,5-Intramolecular	$^{1,5}\mathbf{TS_3}$	−588.40625	17.7 (8.2)
	$^{1,5}\mathbf{TS_5}$	−588.43439	0.0 (0.0)
1,6-Intramolecular	$^{1,6}\mathbf{TS_3}$ (chair)	−627.43690	15.9 (7.4)
	$^{1,6}\mathbf{TS_3}$ (twist boat)	−627.43210	18.9 (8.3)
	$^{1,6}\mathbf{TS_5}$ (chair)	−627.46217	0.0 (0.0)
	$^{1,6}\mathbf{TS_5}$ (twist boat)	−627.45742	3.0 (1.2)
1,7-Intramolecular	$^{1,7}\mathbf{TS_3}$ (chair)	−666.44647	14.1 (5.9)
	$^{1,7}\mathbf{TS_3}$ (boat)	−666.45430	9.2 (6.0)
	$^{1,7}\mathbf{TS_5}$ (chair)	−666.46891	0.0 (0.0)
	$^{1,7}\mathbf{TS_5}$ (boat)	−666.47077	−1.2 (0.0_5)

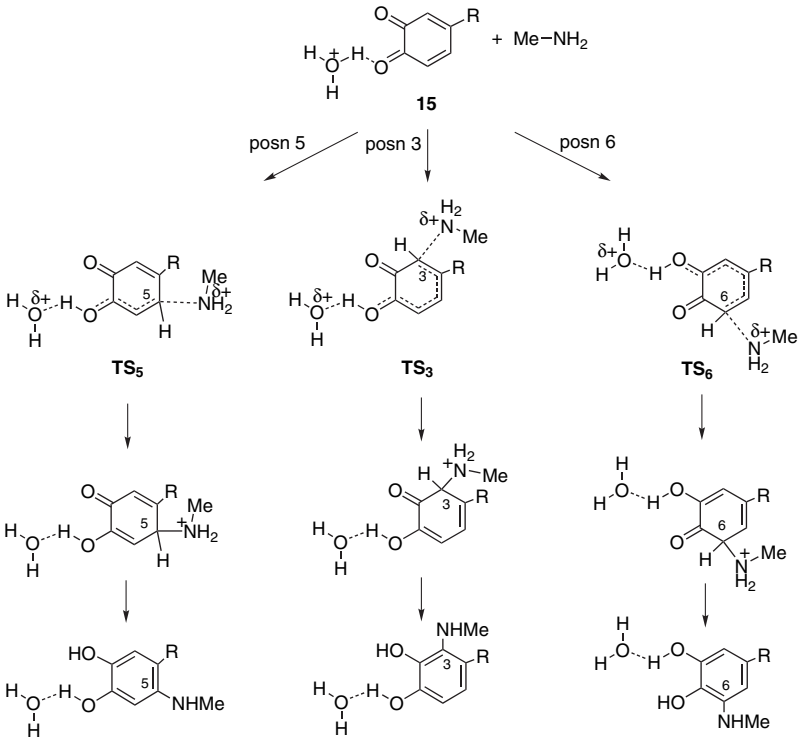
[a] Defined with respect to the lowest energy AM1 transition state for the reactant(s); values in brackets are for the AM1 calculations.

Table 1. It can be seen that the ab initio method suggests an even greater energy difference (17.7 kcal mol^{-1}) between transition states $^{1,5}\mathbf{TS_5}$ and $^{1,5}\mathbf{TS_3}$ than the AM1 method (8.2 kcal mol^{-1}) and the results are entirely consistent with the observed regioselectivity.

Based on these calculations there is a significant energetic preference for cyclisation at the 5-position of the ring. At this stage it was not clear whether this energy difference arises from constraints on the angle at which the amine can approach the sp^2 carbon atom or inherent electronic energy differences in the isomeric transition states. To determine the optimum angle of approach for each ring position and the relative energies of unrestricted acyclic transition states we calculated the energy profiles of the reactions of methylamine at positions 3, 5 and 6 of 4-methyl-*ortho*-quinone **15** (R=Me) (Scheme 6). Again the retro-reactions were studied using the appropriate C–N separation as reaction coordinate and increasing this parameter by increments of ca. 0.05–0.1 Å. For reaction at positions 3 and 6, after the transition state had been passed in the retro-reactions there was a tendency for the nitrogen to attack the neighbouring carbonyl group. This is consistent with experimental observation (see below). To prevent this, for r_c>2.25 Å the angle N–C3–C2 (or N–C6–C1) was restricted to that at the transition state (92°). Otherwise, all variables except the reaction coordinate were minimised.

The results of these AM1 calculations are shown in Figure 2. Single-point ab initio energies were calculated for the transition states (Table 1). As for the 1,5-intramolecular cyclisation reactions, reaction at the 5-position is calculated to be energetically preferred over reaction at the 3-position by 6.3 kcal mol^{-1} (AM1) and 12.1 kcal mol^{-1} (ab initio). The transition state for reaction at position 6 is intermediate in energy. We observed that there is a shallow energy minimum at C5–N 2.75 Å (Fig. 2), which is presumably due to a weak favourable interaction between the quinone and the amine. These calculations are in agreement with experimental observations in which reactions of *ortho*-quinones with amines are found to occur by conjugate addition at position 5.[16–18] If this mode of reaction is prevented by substituents or steric hindrance then nucleophilic attack at one of the carbonyl carbons occurs.[19]

We assume that the calculated angles of approach are optimal for the intermolecular methylamine reactions for which there are no constraints at the transition state. These optimal angles (see Fig. 3) are: (i) for attack at C5: N–C5–C6=99.7°,



Scheme 6.

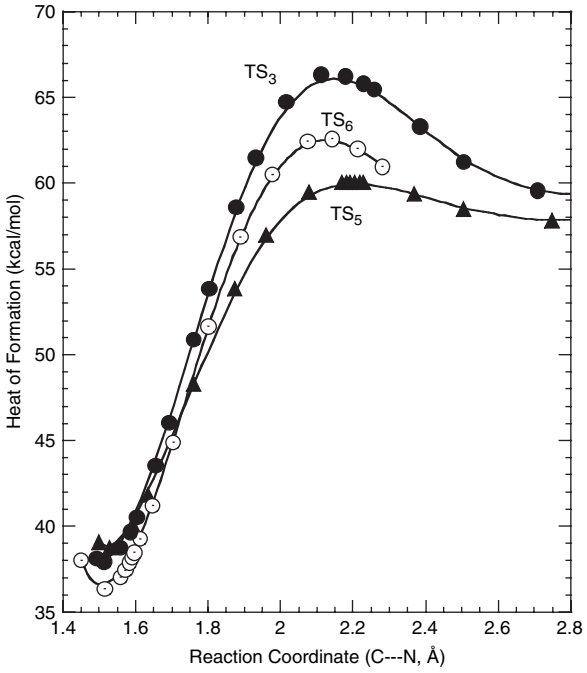


Figure 2. AM1 energy profiles for reaction of methylamine with 4-methyl-*ortho*-quinone at positions 3 (●), 5 (▲) and 6 (○) (Scheme 6).

N–C5–C4=97.3° and (ii) for attack at C3: N–C3–C4=102.8°, N–C3–C2=94.2°.

It is instructive to compare these with the corresponding angles of approach in the constrained intramolecular reactions (Scheme 5). The optimal directions of approach were defined relative to atoms C4,C5,C6 (attack at C5) or C2,C3,C4 (attack at C3) using the angles determined above for the intermolecular reactions of methylamine. The deviations from the optimal direction in transition state ^{1,n}**TS$_5$** can then be estimated in terms of the angular deviation (Φ) from the optimal direction and the orientation of this deviation (ϑ) relative to the C5–C6 bond as shown in Figure 3a. Similar parameters (Ψ and π) can be defined with respect to the C3–C4 bond for cyclisation at position 3 (Fig. 3b).

Using this approach we calculated the following deviations from optimal nucleophilic attack for the 2-aminoethyl side chain: (i) cyclisation at position 5 (i.e., ^{1,5}**TS$_5$**) [Φ=11.8° and υ=198°] and (ii) cyclisation at position 3 (i.e., ^{1,5}**TS$_3$**) [Ψ=17.9° and π=46°]. It can be seen that neither mode of 1,5-intramolecular cyclisation permits the optimum direction of nucleophilic attack but the observed cyclisation at position 5 is closest (Φ=12° vs Ψ=18°) (see also Table 2).

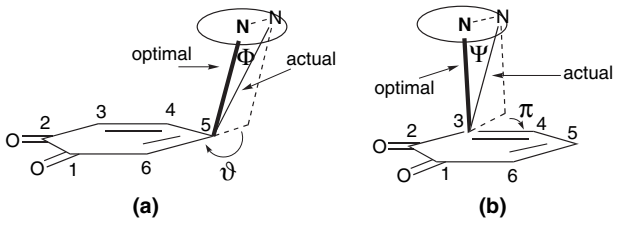


Figure 3. Definition of optimal and actual directions for intramolecular nucleophilic attack by amines at (a) position 5 and (b) position 3.

Table 2. Calculated angles of nucleophilic attack at transition states relative to optimal angle (Fig. 3)

Reaction mode	Transition state (**TS**)	Position 5		Position 3	
		Φ	ϑ	Ψ	π
Intermolecular	—	0°	0°	0°	0°
1,5-Intramolecular	—	12°	198°	18°	46°
1,6-Intramolecular	Chair	5°	209°	6°	14°
	Twist boat	4°	219°	5°	4°
1,7-Intramolecular	Chair	4°	265°	6°	306°
	Boat	2°	262°	1°	342°

Figure 4 shows the calculated structures of the ^{1,5}**TS$_5$** and ^{1,5}**TS$_3$** transition states together with the optimal direction of approach, estimated from the reaction with methylamine, indicated by the yellow dummy atom.

To determine the influence of distorting the direction of nucleophilic attack away from the optimal direction on transition state energy we investigated the effect of constraining the transition states for the intermolecular methylamine reactions to the corresponding 1,5-intramolecular trajectories. For attack at position 5 angles N–C5–C6 and N–C5–C4 were fixed at the values in ^{1,5}**TS$_5$** (i.e., N–C5–C6=110° and N–C5–C4=88°). All parameters other than C5–N (2.2 Å) were allowed to relax. This distortion resulted in an AM1 calculated heat of formation of the transition state of 71.3 kcal mol^{-1}, which is an increase of 11.2 kcal mol^{-1} over the optimal transition state energy. Clearly, deviation of 12° (Fig. 3) from the optimal trajectory causes a significant increase in energy. A similar AM1 calculation for attack at position 3 using the angles for ^{1,5}**TS$_3$** (N–C3–C4=90° and N–C3–C2=110°) resulted in an increase of transition state energy of 14.2 kcal mol^{-1}. The difference in the AM1 energies of the distorted transition states (9.2 kcal mol^{-1}) is comparable to the energy difference between the cyclic transition states ^{1,5}**TS$_5$** and ^{1,5}**TS$_3$** (8.2 kcal mol^{-1}). This suggests that the distortion from the ideal angles (for which the energy difference is 6.3 kcal mol^{-1}) contributes ca. 2–3 kcal mol^{-1} to the total AM1 energy difference between the transition states (8.2 kcal mol^{-1}). Ab initio calculations on the same distorted acyclic transition states suggest that distortion contributes ca. 6–9 kcal mol^{-1} to the calculated energy difference of 17.7 kcal mol^{-1} between ^{1,5}**TS$_5$** and ^{1,5}**TS$_3$**. Based on these estimates, there is clearly a positive correlation between an increase in Φ or Ψ and increase in transition

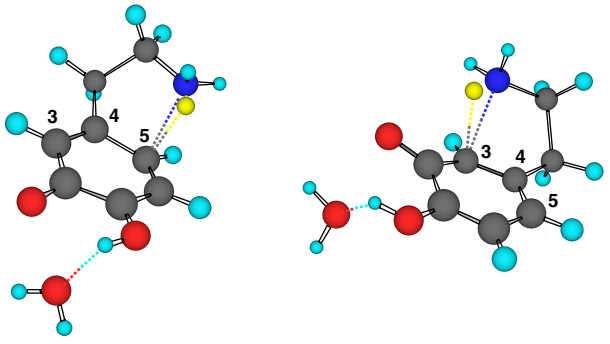


Figure 4. Calculated transition states ^{1,5}**TS$_5$** and ^{1,5}**TS$_3$** including optimal angles for nucleophilic attack (yellow atoms) estimated from intermolecular reaction of $MeNH_2$.

state energy, and this effect can be expected to favour intramolecular nucleophilic attack at position 5. This may partly explain why intramolecular attack at position 5 is preferred but clearly it is not the only effect favouring reaction at position 5.

Even when there is no constraint on angle of approach, as in the methylamine–4-methylquinone reaction (Scheme 6; R=Me), there is a clear preference for nucleophilic attack at position 5 (Fig. 2). We conclude, therefore, that the main source of the energetic preference for attack at position 5 is the intrinsic difference in the electronic energies of the two similar but different transition states (**TS$_5$** vs **TS$_3$**) (Scheme 6). In the preferred mode of reaction (**TS$_5$**) one α,β-unsaturated ketone function remains unperturbed during reaction whereas in the alternative mode (**TS$_3$**) both the formal C–C double bonds of the quinone are broken to form the transition state (Scheme 6).

It is of interest to investigate the influence of the substituent R on the relative energies of the transition states **TS$_5$** and **TS$_3$** (Scheme 6). Assuming that the transition states occur at the same C–N separation (2.2 Å), we have investigated the relative energies for nine substituents (R) for which there is a wide variation in electronic properties. Apart from the reaction coordinate all structural parameters were optimised for each transition state and the results are summarised in Table 3, together with the corresponding Swain and Lupton electronic parameters ($\mathcal{F}$, $\mathcal{R}$ and $\mathcal{R}^+$).[20]

$$\Delta H_f[\mathbf{TS_3}-\mathbf{TS_5}] = 4.000 - 5.646\,\mathcal{R}^+ \quad n=9,\ r=0.959 \quad (3)$$

$$\Delta H_f[\mathbf{TS_3}-\mathbf{TS_6}] = 0.611 - 11.376\,\mathcal{R}^+ \quad n=9,\ r=0.950 \quad (4)$$

From Table 3 it can be seen that even in the absence of a substituent (R=H) attack at position 5 is favoured over position 3 by ca. 5 kcal mol^{-1}, as measured by the calculated difference in transition state energies (ΔH_f [**TS$_3$**−**TS$_5$**]). In general, reaction at position 5 becomes increasingly favoured as the negative resonance effect of the substituent R increases (i.e., Swain and Lupton resonance constant $\mathcal{R}$ negative). This can be interpreted as a favourable resonance/hyperconjugation interaction between substituent R and the α,β-unsaturated ketone fragment that is retained throughout the reaction at position 5 but which is lost (or less favourable) when reaction occurs at position 3. In contrast, when the substituent R has a positive resonance effect (i.e., CF_3 and CN) resonance favours the alternative transition state (**TS$_3$**) but the effect is not large enough to reverse the preferred mode of reaction. In this context it is instructive to consider the transition state for reaction at position 3 in terms of partial formation of a dienolate anion (cf. **16** and **17**). In this mode of reaction (**TS$_3$**) negative charge can be expected to develop at position 4 (and 6) and this can be expected to be stabilised by substituents with a positive resonance effect (i.e., CF_3 and CN). In contrast, substituents with a negative resonance effect will not favour **TS$_3$**, while at the same time favouring **TS$_5$**. A good correlation between $\mathcal{R}$ and ΔH_f [**TS$_3$**−**TS$_5$**] was found and this was improved using the cationic constant $\mathcal{R}^+$ (Eq. 3) $\left(\sigma^+=\mathcal{F}+\mathcal{R}^+\right)$. Use of $\mathcal{R}^+$ does not seem unreasonable as *ortho*-quinones are electron deficient species.

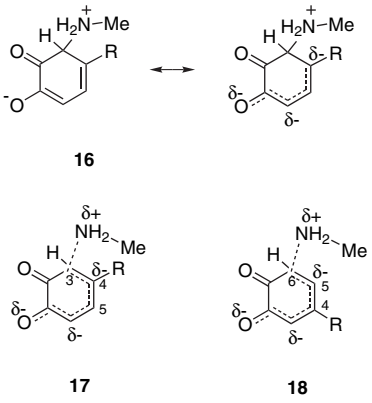
This interpretation of the substituent effect at position 4 is reinforced by considering the influence of substituents on competition between nucleophilic attack at positions 3 and 6 (i.e., **17** vs **18**). In this case the transition states (**TS$_3$** and **TS$_6$**) are identical when R=H. Any difference when R is varied is therefore entirely due to the nature of the substituent. Table 3 shows the calculated differences in transition state energies (ΔH_f [**TS$_3$**−**TS$_6$**]) for the same set of substituents. As might be expected by considering the structures **17** and **18**, substituents R with a negative resonance effect (e.g., Me, OMe) favour transition state **TS$_6$** and those with a positive resonance effect (e.g., CF_3, CN) favour transition state **TS$_3$**. However, in all cases **TS$_5$** is still the energetically favoured mode of reaction. There is a good correlation between the difference in calculated transition state energy ΔH_f [**TS$_3$**−**TS$_6$**] and $\mathcal{R}$, and this correlation is improved using $\mathcal{R}^+$ (Eq. 4).

We conclude that for 1,5-cyclisation of 4-(2-aminoethyl)-*ortho*-quinones the intrinsic difference in electronic energy of the alternative transition states favours cyclisation at position 5. The energy advantage of cyclisation at position 5 may be related to the fact that during this mode of reaction an α,β-unsaturated ketone function remains unperturbed. In addition, the alkyl chain at position 4 probably increases the preference for position 5 due to a negative resonance effect. This mode of reaction is also enhanced by the more favourable trajectory of approach to the ring carbon atom compared to reaction at position 3.

Table 3. AM1 calculated transition state energies for a series of 4-substituted *ortho*-quinones reacting with methylamine

R	ΔH_f [**TS$_5$**]	ΔH_f [**TS$_3$**]	ΔH_f [**TS$_6$**]	ΔH_f [**TS$_3$**−**TS$_5$**]	ΔH_f [**TS$_3$**−**TS$_6$**]	$\mathcal{F}$	$\mathcal{R}$	$\mathcal{R}^+$
H	69.2	74.1	74.1	4.9	0	0	0	0
Me	60.1	66.3	62.6	6.2	3.7	−0.04	−0.13	−0.27
Cl	65.7	71.1	68.6	5.4	2.5	0.41	−0.15	−0.30
OH	23.9	33.8	22.1	9.9	11.7	0.29	−0.64	−1.21
CF_3	−78.5	−76.4	−69.9	2.1	−6.5	0.38	0.19	0.23
SH	72.1	80.3	68.6	8.2	10.7	0.28	−0.11	(−1)[a]
OMe	27.9	38.2	25.9	10.3	12.3	0.26	−0.51	−1.04
SMe	64.4	73.7	60.7	9.3	13.0	0.20	−0.18	−0.74
CN	108.8	112.1	114.1	3.3	−0.2	0.51	0.19	0.15

[a] Estimated.



2.2. 1,6-Intramolecular cyclisation

Cyclisation of the 3-aminopropyl derivative **19** to positions 3 or 5 leading to the intermediates **20** or **21** (Scheme 7) was modelled as described for the 2-aminoethyl derivative. In this case two alternative transition states were found for each position of reaction. For both the pathways the lower energy transition state has a chair conformation and the higher energy transition state had a twist boat conformation. However, in each case the twist boat structure is only ca. 1 kcal mol^{-1} higher in energy than the chair structure and these alternative conformations are not relevant to the preferred mode of cyclisation. As for the 2-aminoethyl derivative, cyclisation at position 5 is calculated to be kinetically favoured. The calculated energy profiles are shown in Figure 5. The difference in heats of formation of the alternative chair transition states ^{1,6}**TS$_5$** and ^{1,6}**TS$_3$** are 7.4 kcal mol^{-1} (AM1) and 15.9 kcal mol^{-1} (ab initio) (Table 1). The calculated angles of approach relative to the optimum, as defined in Figure 3, for all four transition states are shown in Table 2. For these cyclisations the deviations from optimal (Φ,$\Psi \approx$ 4–6°) are much smaller than for the five-membered transition states and there is no significant difference between reaction at position 3 and position 5. This effect may marginally favour reaction at position 5 but it is small. The factors that favour reaction at position 5 are probably essentially those that favour similar attack in the intermolecular methylamine reaction discussed above.

2.3. 1,7-Intramolecular cyclisation

Finally we have calculated the cyclisation pathway for the 4-aminobutyl side chain **22**. As for the 3-aminopropyl cyclisations, each mode of ring-closure can occur via a chair or boat transition state (Fig. 6), with each pair being close in energy (Scheme 7). As previously, the cyclisation at position 5 is calculated to be energetically preferred with a difference in calculated heats of formation between ^{1,7}**TS$_5$** and ^{1,7}**TS$_3$** of 5.9 kcal mol^{-1} (AM1) and 10.4 kcal mol^{-1} (ab initio) (Table 1). For all four modes of reaction deviation from the optimal angle of approach is small (Table 2). It is significant to note that at the RHF/6-31G** level the boat transition states are calculated to be more stable than the chair transition states (Table 1) and for ^{1,7}**TS$_5$** (boat) the angle of approach deviates only 1° from the optimal angle (Table 2). It is therefore possible that these cyclisations occur, at least partially, via the boat transition states (Fig. 6b).

A feature of the transition states for reaction at position 5 is the close approach of the protons on C5 and C3′ for ^{1,7}**TS$_5$** (chair) (2.19 Å) and on C5 and C2′ for ^{1,7}**TS$_5$** (boat) (2.08 Å) (Fig. 6). Similar close interactions do not arise in the transition states for shorter chains, and these interactions in the seven-membered transition states may be the primary

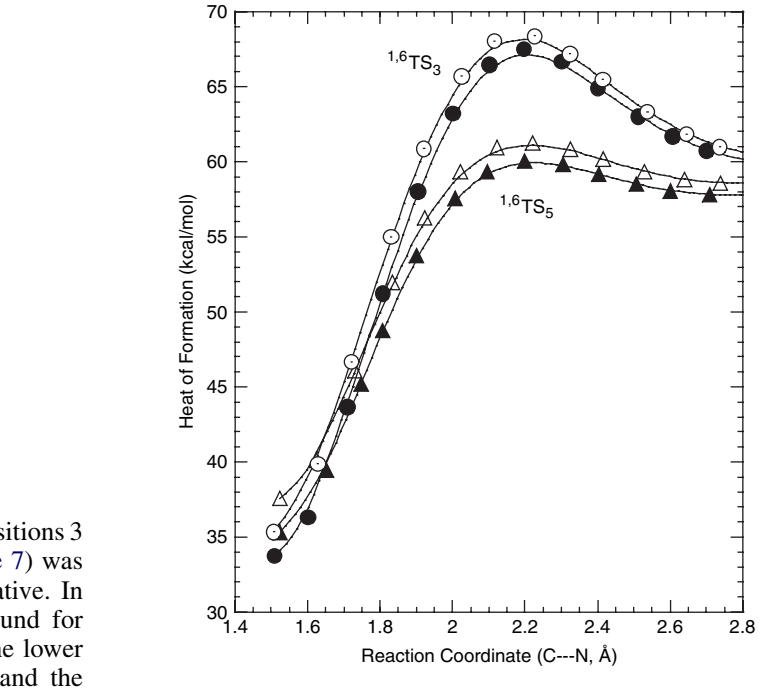
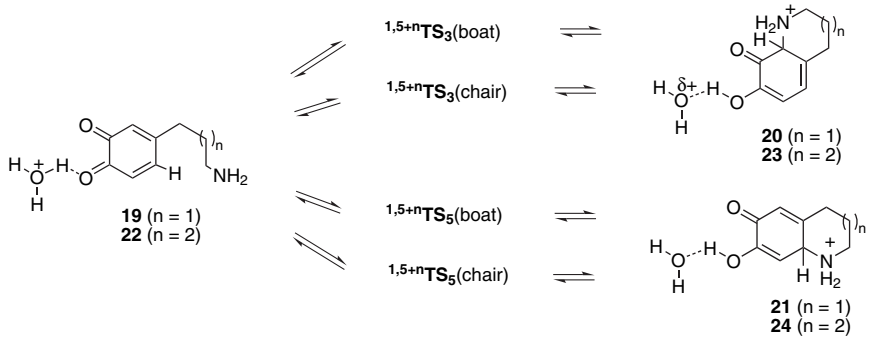


Figure 5. AM1 energy profiles for cyclisation of 4-(3-aminopropyl)-*ortho*-quinone at positions 3 (●, chair; ○, twist boat) and 5 (▲, chair; △, twist boat) (Scheme 7).



Scheme 7.

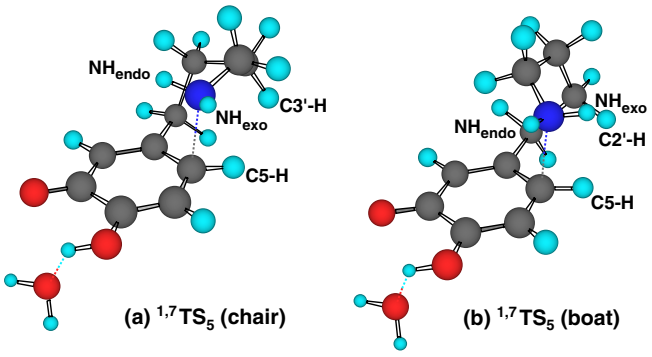


Figure 6. Calculated ^{1,7}**TS$_5$** chair and boat transition states.

source of substituent effects that we have observed experimentally.[6,21] In particular, we have observed that tertiary amine derivatives (–NR$_2$) do not cyclise at either position 5 or 3. Instead quinomethane formation occurs via a pathway that is normally much slower (Scheme 3; **8**→**10**). Significantly, calculations on the *N*,*N*-dimethyl, *N*,*N*-diethyl and *N*,*N*-di-*n*-propyl chair transition states showed that the C5-H, C3′-H separation decreased to 2.09 Å (and for the boat transition states the C5-H, C2′-H separation decreased to 2.00 Å). In contrast to tertiary amines, simple secondary amines (–NHR, R=alkyl) do cyclise via a ^{1,7}**TS$_5$** transition state and calculations on the NHMe (*exo*) and NHPr (*exo*) derivatives gave increased chair C5-H, C3′-H separations of 2.17 and 2.18 Å. Similarly, the NHMe (*endo*) and NHPr (*endo*) derivatives gave increased boat C5-H, C2′-H separations of 2.08 and 2.06 Å. We have observed that the NH^{t}Bu derivative also does not readily cyclise and behaves like a tertiary amine leading to quinomethane formation.[6,21] A calculation of this amine showed the C5-H, C3′-H separation to be 2.07 Å, together with other unfavourable H–H interactions (<2.1 Å) due to the methyl substituents. Significantly, the NH^{i}Pr derivative does cyclise[6,21] and a calculation showed that this amine can adopt a conformation in the ^{1,7}**TS$_5$** chair transition state, in which the ^{i}Pr C–H bond is *anti* to the N–C4′ bond, such that the C5–H, C3′–H separation is 2.18 Å.

Table 4. Calculated close H–H interactions for ^{1,7}**TS$_5$** transition states

Nucleophile NR$_2$	^{1,7}**TS$_5$** (chair) C5-H–C3′-H (Å)	^{1,7}**TS$_5$** (boat) C5-H–C2′-H (Å)
NH$_2$	2.19	2.08
NMe$_2$	2.09	2.00
NEt$_2$	2.09	2.01
N^{n}Pr$_2$[a]	2.09	2.01
NHMe (*exo*)	2.17	2.03
NHMe (*endo*)	2.12	2.08
NH^{n}Pr (*exo*)[b]	2.18	2.05
NH^{n}Pr (*endo*)[b]	2.11	2.06
NH^{i}Pr (*exo*)[b]	2.18	2.07
NH^{t}Bu (*exo*)[a]	2.07	2.03

[a] These groups do not undergo 1,7-cyclisation in experimental studies.[6,21]
[b] These groups have been shown experimentally to undergo 1,7-cyclisation.[6,21]

It therefore appears that an adverse C5–H, C2′– or C3′–H interaction in the transition state is a significant factor in inhibiting ^{1,7}**TS$_5$** cyclisation and that derivatives, which do not cyclise in experimental studies[6,21] have AM1 calculated separations that are less than those that do cyclise. The results are summarised in Table 4. We believe that these subtle steric interactions in the transition states inhibit 1,7-cyclisation of tertiary amines and *N*-*t*-butyl derivatives allowing slower alternative reactions to occur (Scheme 3).

3. Conclusions

Our calculations show that for both inter- and intramolecular additions of amines to *ortho*-quinones reaction at position 5 of the ring is favoured over reaction at position 3. The calculated heats of formation of all reactants, products and transition states are summarised in Table 5. Although we have previously noted that there is a shallow energy minimum at r_c~2.75 Å, to avoid ambiguity the heats of formation of the reactants shown in Table 5 are calculated for maximum separation of ring and amine or, for intermolecular reactions, a separation of 10 Å.

The calculations suggest that reaction at position 5 is favoured due to the intrinsically lower electronic energy of the '**TS$_5$**' transition states in which an α,β-unsaturated ketone function remains unperturbed throughout the reaction. The energy difference can be modified by the nature of the substituent at position 4 but no substituent effects are large enough to reverse the regioselectivity. A secondary factor is the trajectory of the incoming amine and for five-member ring formation, which is particularly relevant to melanogenesis (Scheme 1), this also favours reaction at position 5.

Table 5. AM1 stationary point energies for *ortho*-quinone–amine reactions

Reaction mode	Transition state (**TS**)	Reactant(s) ΔH_f [R]	Product ΔH_f [P]	**TS** ΔH_f [**TS**]	$\Delta\Delta H$ ΔH_f [**TS**]−ΔH_f [R]
Intermolecular	**TS$_3$**	62.16	37.99	66.34	4.18
	TS$_5$	62.16	38.75	60.06	−2.10
	TS$_6$	62.16	36.36	62.61	0.45
1,5-Intramolecular	^{1,5}**TS$_3$**	69.36	47.32	81.74	12.38
	^{1,5}**TS$_5$**	69.36	49.42	73.57	4.21
1,6-Intramolecular	^{1,6}**TS$_3$** (chair)	61.97	33.75	67.50	5.53
	^{1,6}**TS$_3$** (twist boat)	61.97	35.39	68.42	6.45
	^{1,6}**TS$_5$** (chair)	61.97	35.31	60.08	−1.89
	^{1,6}**TS$_5$** (twist boat)	61.97	37.56	61.26	−0.71
1,7-Intramolecular	^{1,7}**TS$_3$** (chair)	55.35	30.66	60.62	5.27
	^{1,7}**TS$_3$** (boat)	55.35	31.70	60.70	5.35
	^{1,7}**TS$_5$** (chair)	55.35	30.91	54.75	−0.60
	^{1,7}**TS$_5$** (boat)	55.35	32.79	54.81	−0.54

4. Calculations

The MOPAC program within *CS CHEM3D Pro*® (CambridgeSoft Corporation, Cambridge MA, USA) was used for AM1 calculations,[22] and the GaussView 3.0 program (Gaussian, Inc, Pittsburgh PA, USA) for ab initio calculations.[23] Transition states were stationary points. For improved energies single-point RHF/6-31G** calculations were performed on AM1 optimised geometries.

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A tandem multi-component synthesis of 5,7-diaryl-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-*b*][1,4]thiazin-2(3*H*)-ones

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Abstract—The five-component reaction of ethyl 2-[(2-oxopropyl)sulfanyl]acetate, aromatic aldehydes, and ammonium acetate affords two diastereomers of 5,7-diaryl-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-b][1,4]thiazin-2(3*H*)-ones via a novel tandem Mannich-enamine-substitution sequence. Presumably, they are generated from ethyl 2-[(4-oxo-2,6-diaryl-3-piperidinyl)sulfanyl]acetates. During the formation of the *trans*-5,7-diaryl-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-b][1,4]-thiazin-2(3*H*)-ones from ethyl 2-[(4-oxo-2,6-diaryl-3-piperidinyl)sulfanyl]acetates, the configuration at the carbon bearing an aryl group adjacent to the enamide C=C double bond is inverted via ring opening and closure. When *o*-substituted benzaldehydes were employed in this reaction, 5,7-diaryl-5,6-dihydro-1*H*-pyrido[3,4-b][1,4]thiazin-2(3*H*)-ones were obtained via air oxidation, along with *trans*-5,7-diaryl-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-b][1,4]-thiazin-2(3*H*)-ones.

1. Introduction

Heterocycles containing nitrogen and sulfur possess important biological activities. For instance, the piperidine sub-structure is ubiquitous in nature as it is found in many biologically active compounds such as alkaloids.[1] Compounds incorporating a piperidine ring exhibit biological activities like anti-parkinson,[2] antidepressant[3] and analgesic.[4] Thiazines display many important biological activities such as sedative,[5] neuroleptic,[6] antitussive,[7] anti-fungal, anti-inflammatory and anthelmintic,[8] antipsoriatic[9] and anti-HIV.[10] The derivatives of thiazine act as myocardial calcium channel modulators[11] and also as matrix metalloproteinase inhibitors.[12] Hence, it was considered of interest to synthesis heterocycles comprising these two rings in a one-pot synthesis employing a tandem sequence. Tandem reactions are very useful in constructing molecules of complex structures rapidly with elegance and economy in an eco-friendly manner as wastage is minimised in this protocol.

2. Results and discussion

The synthesis of 5,7-diaryl-5,6,7,8-tetrahydro-1*H*-pyrido [3,4-b][1,4]thiazin-2(3*H*)-ones **5** was planned via a multi-component reaction employing a Mannich-enamine-substitution tandem sequence as depicted in Scheme 1.

Accordingly, a series of 5,7-diaryl-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-b][1,4]thiazin-2(3*H*)-ones **5** (42–74%) were prepared in a one-pot reaction by gently warming the reactants, ethyl 2-[(2-oxopropyl)sulfanyl]acetate **1**, aromatic aldehyde and ammonium acetate in a 1:2:2 molar ratio in ethanol and stirring at ambient temperature for about 5–7 days. When 1 mol of ammonium acetate was used, the reaction was found to afford ethyl 2-[(4-oxo-2,6-diaryl-3-piperidinyl)sulfanyl]acetate **2**, showing that the product-selectivity of the reaction can be tuned statistically. Since, thiazinones were targeted as the final products, only one of the intermediate piperidones was synthesised and the *cis* relationship between the aryl rings in **2** was established.

Ethyl 2-[(2-oxopropyl)sulfanyl]acetate **1** required for the synthesis of the thiazinones was prepared by the reaction of chloroacetone and ethyl 2-sulfanylacetate in presence of potassium carbonate in chloroform medium in 95% yield. In contrast, the preparation of **1** was reported earlier in 62.5% yield from the reaction of chloroacetone and ethyl 2-sulfanylacetate in presence of sodium ethoxide in ethanol under reflux.[13] The ketoester **1** has one active methyl and two active methylene groups. From the structures of **2** and **5**, it follows that the reaction occurs regioselectively, wherein the methylene and methyl adjacent to the keto carbonyl are involved in the reaction, although the methylene adjacent to the sulfur and ester functions should be more acidic relative to the acetyl methyl (Scheme 1). This may probably be ascribed to the greater nucleophilicity of the carbanion formed at the terminal methyl carbon relative to the carbanion generated at the methylene carbon attached to the ester and sulfur functionalities.

Keywords: Piperidine; Thiazine; Ethyl 2-[(2-oxopropyl)sulfanyl]acetate; Tandem; Mannich; Enamine.
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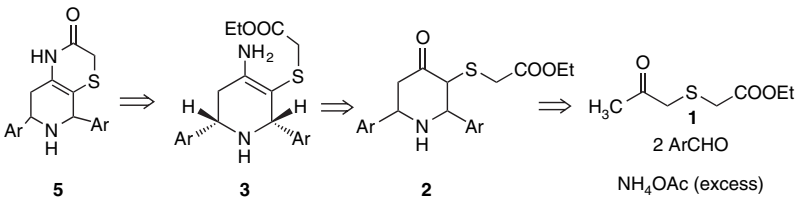


Scheme 1. Retrosynthesis of thiazinones **5**.

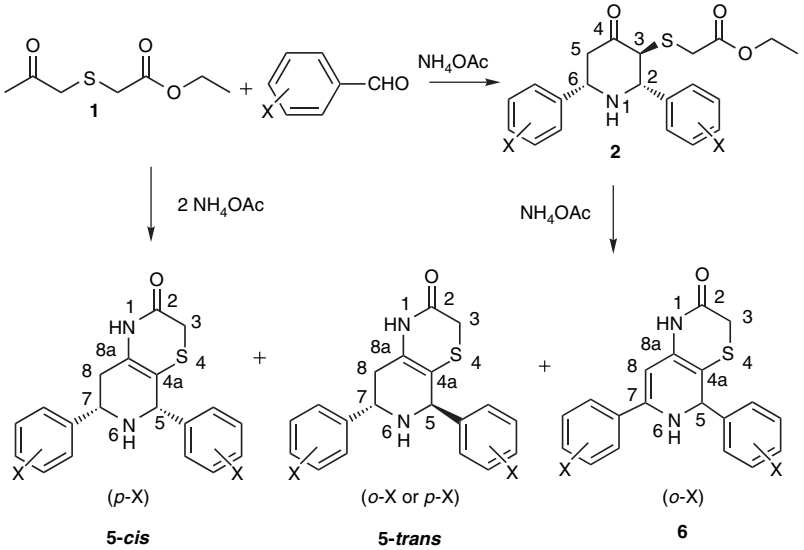
This five-component tandem reaction involving *para*-substituted benzaldehydes affords two diastereomers of thiazinones, **5**-*cis* and **5**-*trans*, which differ in the relative orientations of the aryl rings at C-5 and C-7. The *cis*- and *trans*-isomers of **5a**–**5d** were separated using flash column chromatography. From the ^{1}H NMR spectroscopic data of the reaction mixture, it is found that the ratio of the *trans* and *cis* isomers, **5a**–**5d** is ~0.70:1.00. In the case of *ortho*-substituted benzaldehydes, only the **5**-*trans* and the air-oxidised product, 5,7-diaryl-5,6-dihydro-1*H*-pyrido[3,4-b][1,4]thiazin-2(3*H*)-ones, **6** were obtained (Scheme 2). The yields of **5** and **6** are given in Table 1.

Table 1. Yields of thiazinones, **5a**–**6g**

Comp	X	Yield (%)	Total yield (%)
5a-*cis*	H	38	67
5a-*trans*		29	
5b-*cis*	*p*-Cl	37	63
5b-*trans*		26	
5c-*cis*	*p*-Me	33	54
5c-*trans*		21	
5d-*cis*	*p*-F	32	48
5d-*trans*		16	
5e-*trans*	*o*-Cl	39	49
5f-*trans*	*o*-Me	33	42
5g-*trans*	*o*-MeO	60	74
6e	*o*-Cl	10	
6f	*o*-Me	9	
6g	*o*-MeO	14	

The structure and stereochemistry of **2** was established from ^{1}H, ^{13}C and 2D NMR spectroscopic data. That the ring system in **2f** adopts a chair conformation is evident from the splitting pattern and *J* values of the signals of the ring protons. The proton H-6 gives a doublet of doublets at 4.65 ppm with *J* values of 12 and 3 Hz corresponding to the vicinal diaxial and axial–equatorial couplings, respectively. This points to the equatorial orientation of the *o*-chlorophenyl group at C-6. The axial orientation of the sulfanyl ester side chain at C-3 is inferred from the *J* value of 2 Hz for the protons, H-2 and H-3 giving signals at 4.93 and 3.79 ppm, respectively. The diastereotopic methylene protons, H-5 each appear as a doublet of doublets at 2.60 and 3.62 ppm, respectively, while the diastereotopic methylene protons adjacent to sulfur appear as doublets at 3.01 and 3.12 ppm with a *J* value of 15 Hz. The methyl and the methylenic protons of the ester functionality appear at 1.20 and 4.00 ppm, respectively. The results of X-ray studies are also in accord with the above structure **2f**[14] (Fig. 1).

The *cis*- and *trans*-isomers of **5a**–**5g** were characterised using ^{1}H, ^{13}C and 2D NMR spectroscopic data. The ^{1}H NMR spectrum of **5a**-*cis* gives a doublet of doublets with *J* values of 11 and 4 Hz at 4.20 ppm ascribable to the axial–pseudoaxial and axial–pseudoequatorial couplings of H-7 with the diastereotopic protons at C-8. This points to the equatorial orientation of the phenyl ring at C-7. The diastereotopic methylene protons, H-3 give two doublets at 3.12



Scheme 2. Formation of thiazinones **5** and **6** via tandem reactions.

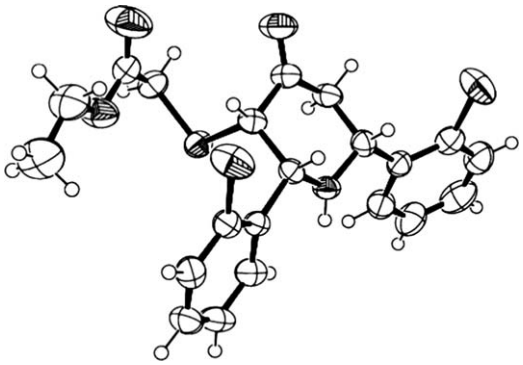


Figure 1. X-ray structure of ethyl 2-[2,6-bis(2-chlorophenyl)-4-oxo-3-piperidinyl]sulfanylacetate **2f**.

and 3.39 ppm with the *J* value of 15 Hz, this assignment being confirmed by a HMBC correlation of these protons with the carbonyl carbon at 164.2 ppm and the C-4a at 111.0 ppm. One of the two protons of 8-CH_2 appears as a doublet of doublets of doublets at 2.37 ppm with *J* values of 16, 4 and 2 Hz corresponding to the geminal coupling, pseudoequatorial–axial coupling with H-7, and a long range coupling with H-5. Another proton of 8-CH_2 group also appears as a doublet of doublets of doublets at 2.58 ppm with *J* values of 16, 11 and 4 Hz corresponding to the geminal coupling, pseudoaxial–axial coupling with H-7 and a long range coupling with H-5. The benzylic proton H-5 appears at 4.75 ppm as a doublet of doublets with *J* values of 4 and 2 Hz resulting from the long range coupling with the allylic protons at C-8, which is evident from H,H-COSY spectroscopic data. The aromatic protons appear in the region of 7.27–7.46 ppm, while the amine and amide protons appear at 1.65 and 7.65 ppm, respectively.

In the ^{1}H NMR spectrum of **5a**-*trans*, a doublet of doublets due to H-7 is obtained at 4.08 ppm with *J* values of 10 and 4 Hz, ascribable to pseudoaxial–axial and axial–pseudoequatorial coupling, respectively, with 8-CH_2 protons. This shows that the phenyl ring at C-7 is oriented equatorially. The diastereotopic protons at C-3 give two doublets at 3.31 and 3.44 ppm (J=15 Hz). The two allylic protons at C-8 appear as a multiplet at 2.53 ppm. Unlike in the case of **5a**-*cis*, the proton, H-5 of **5a**-*trans* appears as a singlet at 4.74 ppm, while the amine and amide protons give signals at 1.70 and 7.45 ppm, respectively.

An unambiguous assignment of the heterocyclic ring carbon chemical shifts for **5a**-*cis* and **5a**-*trans* was arrived at from the proton chemical shift values and C,H-COSY correlations. The ^{13}C chemical shifts of 5,7-diaryl-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-b][1,4]thiazin-2(*3H*)-ones show that the carbon signals of **5a**-*trans* isomers appear slightly more shielded than the corresponding carbons of the *cis* isomer (**5a**-*cis*). For instance, the quaternary carbon, C-4a and the homoallylic carbon, C-7 of **5a**-*trans* appear shielded by 3.7 and 6.7 ppm, respectively, compared to the corresponding carbons of the *cis* isomer. Presumably, this is ascribable to the *syn* axial interaction between the C-5 aryl ring and the axial proton H-7 of the **5a**-*trans* isomer. The ratio of the **5**-*trans* to **5**-*cis* isomers ~0.70:1.00 found from the ^{1}H NMR spectrum of the reaction mixture suggests that the **5**-*trans* is slightly less stable than the **5**-*cis* isomer in the case of aryl rings with *p*-substituent. The C-3 of **5a**-*cis* gives a signal at 30.6 ppm, while the quaternary carbon, C-4a appears at 111.0 ppm. The assignment of the chemical shift of C-4a is also further supported by the HMBC spectroscopic data, which shows correlation between C-4a and H-3 proton. The allylic carbons, C-5 and C-8 appear at 62.7 and 36.4 ppm, respectively, the assignments being aided by the C,H-COSY correlations. Similarly, the homoallylic carbon C-7 was assigned to the signal at 57.9 ppm. The signal of another quaternary carbon C-8a appears at 142.6 ppm. The homoallylic proton, H-7 appearing at 4.20 ppm shows a weak correlation with quaternary carbon, C-8a at 142.6 ppm in the HMBC spectrum. The aromatic carbons appear in the region of 126.6–139.7 ppm.

The stereochemistry of the *cis*- and *trans*-isomers of **5** arrived at from NMR studies is also in complete agreement with that determined by the X-ray crystallographic studies of crystals of **5b**-*cis* (Fig. 2), **5e**-*trans* (Fig. 3) and **5g**-*trans* (Fig. 4).

In the thiazinone ring of **5**-*cis* isomers, both six membered rings adopt a half chair conformation and the aryl rings at 5- and 7-positions are oriented equatorially and pseudoequatorially. For the **5**-*trans* isomers, the aryl rings at 5- and 7-positions are oriented equatorially and pseudoaxially in contrast to their *cis* relationship for the aryl rings found in

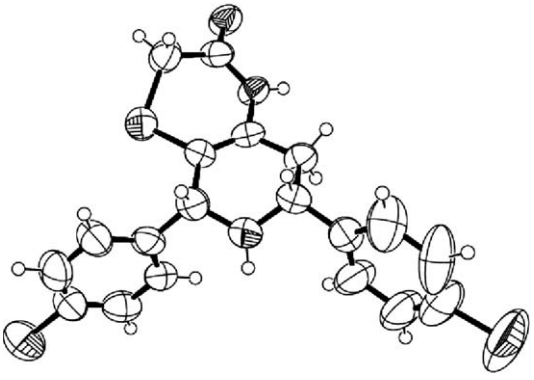


Figure 2. X-ray structure of *cis*-5,7-bis(4-chlorophenyl)-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-*b*][1,4]thiazin-2(3*H*)-one **5b**-*cis*.

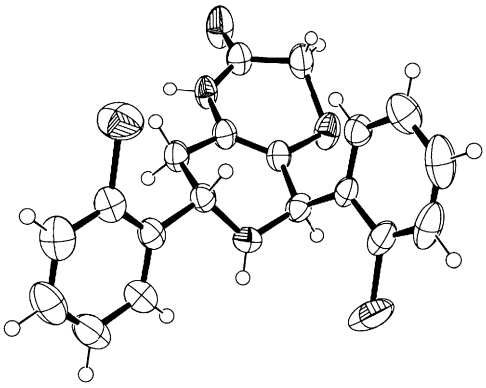


Figure 3. X-ray structure of *trans*-5,7-bis(2-chlorophenyl)-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-*b*][1,4]thiazin-2(3*H*)-one **5e**-*trans*.

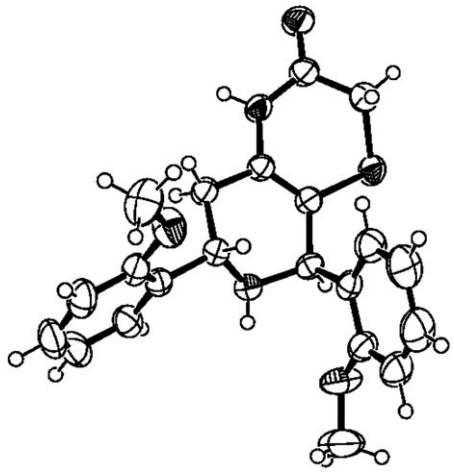
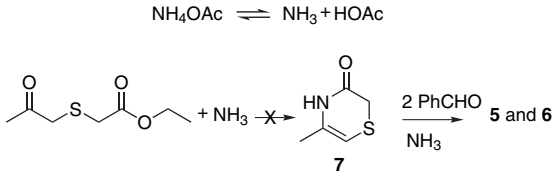


Figure 4. X-ray structure of *trans*-5,7-bis(2-methoxyphenyl)-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-*b*][1,4]thiazin-2(3*H*)-one **5g**-*trans*.

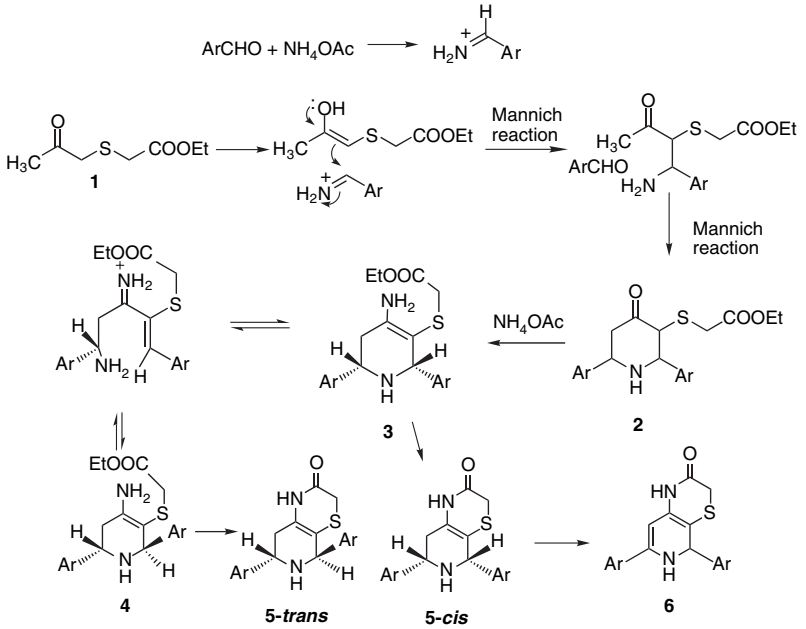
piperidone **2f**, although **2f** is the precursor for **5e**-*trans* as found from the conversion of **2f** into **5e**-*trans* upon treatment of the former with ammonium acetate under the reaction conditions in a separate reaction. This suggests that during the formation of **5e**-*trans* from **2f**, the configuration at C-5 is inverted, presumably via the opening and reformation of the ring with inversion at one of the benzylic positions of the piperidine ring as shown in Scheme 3. The probable mechanism of formation of the thiazinones is given in Scheme 3, wherein two Mannich reactions, enamine formation, ring opening and closure and condensation occur in a tandem sequence. In the case of *o*-substituted benzaldehydes, the *trans*-tetrahydrothiazinones undergo air oxidation to furnish the dihydrothiazinones **6** as minor product, whose structure was elucidated from NMR spectroscopic data. For instance, in the ^{1}H NMR spectrum of **6e**, the two diastereotopic protons at C-3 give two doublets at 3.33 and 3.44 ppm (J=14 Hz). The H-5 appears as a singlet at 4.77 ppm, while the alkenic proton, H-8 appears as a singlet at 5.49 ppm. The amino and the amide protons give broad singlets at 1.70 and 7.76 ppm, respectively. That the reaction proceeding through an alternative mechanistic pathway via an initially formed **7** depicted in Scheme 4 is not involved under the present reaction conditions is evident from the following. The reaction of ethyl 2-[(2-oxopropyl)sulfanyl]acetate **1** with ammonium acetate both under room temperature and at reflux for 8 days in the absence of benzaldehyde and the subsequent examination of the reaction mixture employing ^{1}H NMR, ^{13}C NMR and GC–MS techniques failed to show the formation of even a small amount of the thiazine **7**.



Scheme 4. Mechanism for the formation of thiazinones **5** and **6** through **7**.

3. Conclusion

The present study reports a concise, one-pot, multi-component tandem synthesis of thiazinones, which could possess important biological activities. The complete structure and stereochemistry of the products have been elucidated using NMR spectroscopic and X-ray crystallographic studies. In view of the pharmacophoric nature of the piperidine and thiazine ring systems, investigations are now currently in progress in our group with a view to using the thiazinones as synthons for the construction of more complex molecules and to prepare enantiomerically pure compounds belonging to this class.



Scheme 3. Mechanism of formation of thiazinones.

4. Experimental

4.1. General methods

All melting points reported in this work were measured in open capillaries. Flash chromatography was performed on silica gel (230–400 mesh). The ^{1}H and ^{13}C NMR spectra have been measured at 300 and 75 MHz, respectively, using Bruker 300 MHz (Avance) instrument in $CDCl_3$ solvent using tetramethylsilane as internal standard. Chemical shifts were reported as δ values (ppm). All one- and two-dimensional NMR spectra were obtained using standard Bruker software throughout. IR spectra were recorded on a JASCO FTIR instrument (KBr pellet in case of solids and CH_2Cl_2 in case of liquids). Elemental analysis were done using Vario EL III instrument. Crystals suitable for X-ray analysis were obtained by crystallisation from ethanol and ethyl acetate mixture.

4.2. Synthesis of thiazin-2(3*H*)-ones from benzaldehyde and *p*-substituted benzaldehydes—general procedure

Ammonium acetate (0.663 g, 8.4 mmol) was dissolved in ethanol (3 mL) by heating. Benzaldehyde (0.582 mL, 5.7 mmol) and ethyl 2-[(2-oxopropyl)sulfanyl]acetate (0.338 mL, 2.8 mmol) were added to this solution, the mixture heated until the colour of the solution turned yellow and left at room temperature for 5–7 days. After the completion of the reaction, the two diastereomers were separated using flash column chromatography performed on silica gel (230–400 mesh) and petroleum ether and ethyl acetate mixture. The products were further recrystallised from ethanol and ethyl acetate.

4.2.1. *cis*-5,7-Diphenyl-5,6,7,8-tetrahydro-1*H*-pyrido [3,4-*b*][1,4]thiazin-2(3*H*)-one 5a-*cis*. Isolated as colourless solid. (347 mg, 38%) mp=202 °C; υ_{max} (KBr) 3199, 1672 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.65 (1H, s), 2.37 (1H, ddd, *J*=16, 4, 2 Hz), 2.58 (1H, ddd, *J*=16, 11, 4 Hz), 3.12 (1H, d, *J*=15 Hz), 3.39 (1H, d, *J*=15 Hz), 4.20 (1H, dd, *J*=11, 4 Hz), 4.75 (1H, dd, *J*=4, 2 Hz), 7.27–7.46 (10H, m), 7.65 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 30.6, 36.4, 57.9, 62.7, 111.0, 126.6, 127.8, 128.4, 128.5, 128.6, 128.7, 128.8, 139.7, 142.6, 164.2. Anal. Calcd for $C_{19}H_{18}N_2OS$: C, 70.78; H, 5.63; N, 8.69. Found C, 70.73; H, 5.67; N, 8.70.

4.2.2. *trans*-5,7-Diphenyl-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-*b*][1,4]thiazin-2(3*H*)-one 5a-*trans*. Isolated as colourless solid. (265 mg, 29%) mp=199 °C; υ_{max} (KBr) 3430, 1648 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.70 (1H, s), 2.53 (2H, m), 3.31 (1H, d, *J*=15 Hz), 3.44 (1H, d, *J*=15 Hz), 4.08 (1H, dd, *J*=10, 4 Hz), 4.74 (1H, s), 7.22–7.39 (10H, m), 7.45 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 30.4, 36.0, 51.2, 60.1, 107.3, 126.6, 127.7, 128.0, 128.1, 128.6, 128.7, 130.0, 140.8, 142.3, 164.1. Anal. Calcd for $C_{19}H_{18}N_2OS$: C, 70.78; H, 5.63; N, 8.69. Found C, 70.76; H, 5.69; N, 8.60.

4.2.3. *cis*-5,7-Bis(4-chlorophenyl)-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-*b*][1,4]thiazin-2(3*H*)-one 5b-*cis*. Isolated as pale yellow solid. (410 mg, 37%) mp=195 °C; υ_{max} (KBr) 3285, 1671 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.78 (1H, s), 2.34 (1H, ddd, *J*=16, 4, 2 Hz), 2.52 (1H, ddd, *J*=16, 11, 4 Hz), 3.12 (1H, d, *J*=15 Hz), 3.37 (1H, d, *J*=15 Hz), 4.15 (1H, dd, *J*=11, 4 Hz), 4.72 (1H, dd, *J*=4, 2 Hz), 7.28–7.49 (10H, m), 8.18 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 30.5, 36.5, 57.2, 61.9, 110.4, 128.0, 128.6, 128.7, 128.8, 130.2, 133.6, 134.3, 138.2, 141.0, 164.0. Anal. Calcd for $C_{19}H_{16}Cl_2N_2OS$: C, 58.32; H, 4.12; N, 7.16. Found C, 58.29; H, 4.18; N, 7.14.

4.2.4. *trans*-5,7-Bis(4-chlorophenyl)-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-*b*][1,4]thiazin-2(3*H*)-one 5b-*trans*. Isolated as colourless solid. (288 mg, 26%) mp=180 °C; υ_{max} (KBr) 3350, 1681 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 2.33 (1H, m), 3.21 (1H, d, *J*=15 Hz), 3.35 (1H, d, *J*=15 Hz), 4.34 (1H, s), 4.63 (1H, s), 3.97 (1H, m), 7.15–7.27 (10H, m), 8.83 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 30.2, 35.2, 50.6, 59.1, 107.1, 128.1, 128.8, 128.9, 129.5, 130.0, 133.6, 134.1, 140.1. Anal. Calcd for $C_{19}H_{16}Cl_2N_2OS$: C, 58.32; H, 4.12; N, 7.16. Found C, 58.39; H, 4.08; N, 7.21.

4.2.5. *cis*-5,7-Bis(4-methylphenyl)-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-*b*][1,4]thiazin-2(3*H*)-one 5c-*cis*. Isolated as colourless solid. (328 mg, 33%) mp=180 °C; υ_{max} (KBr) 3402, 1675 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 2.1 (1H, s), 2.31 (3H, s), 2.32 (2H, m), 2.34 (3H, s), 2.54 (1H, ddd, *J*=14, 11, 4 Hz), 3.10 (1H, d, *J*=15 Hz), 3.37 (1H, d, *J*=15 Hz), 4.14 (1H, dd, *J*=11, 4 Hz), 4.70 (1H, dd, *J*=4, 2 Hz), 7.11–7.33 (8H, m), 8.9 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 21.1, 21.2, 30.6, 36.5, 57.6, 62.4, 111.1, 126.4, 128.6, 129.1, 129.2, 136.7, 137.4, 138.3, 139.7, 164.1. Anal. Calcd for $C_{21}H_{22}N_2OS$: C, 71.97; H, 6.33; N, 7.99. Found C, 71.90; H, 6.35; N, 8.07.

4.2.6. *trans*-5,7-Bis(4-methylphenyl)-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-*b*][1,4]thiazin-2(3*H*)-one 5c-*trans*. Isolated as paste. (208 mg, 21%); υ_{max} (CH_2Cl_2) 3328, 1675 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 2.53 (2H, m), 3.27 (3H, s), 3.25 (1H, d, *J*=15 Hz), 3.41 (1H, d, *J*=15 Hz), 3.39 (3H, s), 4.07 (1H, m), 4.75 (1H, s), 5.03 (1H, s), 7.08–7.26 (8H, m), 8.50 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 21.0, 21.1, 30.2, 35.4, 50.9, 59.4, 107.2, 126.7, 128.1, 129.3, 129.4, 130.0, 137.1, 137.6, 138.1, 138.6, 164.8. Anal. Calcd for $C_{21}H_{22}N_2OS$: C, 71.97; H, 6.33; N, 7.99. Found C, 71.94; H, 6.25; N, 7.91.

4.2.7. *cis*-5,7-Bis(4-fluorophenyl)-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-*b*][1,4]thiazin-2(3*H*)-one 5d-*cis*. Isolated as colourless solid. (325 mg, 32%) mp=205 °C; υ_{max} (KBr) 3212, 1671 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.83 (1H, s), 2.35 (1H, ddd, *J*=16, 4, 2 Hz), 2.54 (1H, ddd, *J*=16, 11, 4 Hz), 3.10 (1H, d, *J*=15 Hz), 3.37 (1H, d, *J*=15 Hz), 4.16 (1H, dd, *J*=11, 4 Hz), 4.73 (1H, dd, *J*=4, 2 Hz), 6.98–7.43 (8H, m), 8.50 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 30.9, 57.7, 62.3, 36.9, 111.1, 115.6, 115.7, 115.9, 116.0, 128.6, 128.7, 130.8, 130.9, 135.9, 138.8, 164.6. Anal. Calcd for $C_{19}H_{16}F_2N_2OS$: C, 63.67; H, 4.50; N, 7.82. Found C, 63.66; H, 4.57; N, 7.72.

4.2.8. *trans*-5,7-Bis(4-fluorophenyl)-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-*b*][1,4]thiazin-2(3*H*)-one 5d-*trans*. Isolated as colourless solid. (163 mg, 16%) mp=198 °C; υ_{max} (KBr) 3091, 1650 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 2.51 (m), 3.29 (1H, d, *J*=15 Hz), 3.44 (1H, d, *J*=15 Hz), 4.06 (1H, m),

4.70 (1H, s), 6.15 (1H, s), 6.99–7.50 (8H, m), 7.71 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 30.3, 35.7, 50.5, 59.2, 107.6, 115.3, 115.4, 115.6, 115.7, 115.8, 116.1, 128.4, 128.8, 129.7, 129.8, 130.0, 164.6. Anal. Calcd for $C_{19}H_{16}F_2N_2OS$: C, 63.67; H, 4.50; N, 7.82. Found C, 63.64; H, 4.49; N, 7.89.

4.3. Synthesis of thiazin-2(3*H*)-ones from *o*-substituted benzaldehydes—general procedure

The procedure described above for benzaldehyde and *p*-substituted benzaldehydes was used as such here. In these reactions, a mixture of **5**-*trans* and **6** were obtained, which were separated by flash column chromatography using silica gel (230–400 mesh) and petroleum ether and ethyl acetate mixture.

4.3.1. *trans*-5,7-Bis(2-chlorophenyl)-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-*b*][1,4]thiazin-2(3*H*)-one 5e-*trans*. Isolated as colourless solid. (432 mg, 39%) mp=181 °C; υ_{max} (KBr) 3295, 1658 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 2.02 (1H, s), 2.53 (2H, m), 3.33 (1H, d, *J*=15 Hz), 3.44 (1H, d, *J*=15 Hz), 4.38 (1H, dd, *J*=11, 4 Hz), 5.21 (1H, s), 7.16–7.57 (8H, m), 8.49 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 30.3, 34.3, 47.5, 56.9, 106.0, 126.5, 127.3, 127.5, 128.8, 129.3, 129.4, 129.6, 131.0, 130.2, 133.1, 134.2, 137.3, 139.3, 164.3. Anal. Calcd for $C_{19}H_{16}N_2Cl_2OS$: C, 58.32; H, 4.12; N, 7.16. Found C, 58.33; H, 4.10; N, 7.11.

4.3.2. *trans*-5,7-Bis(2-methylphenyl)-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-*b*][1,4]thiazin-2(3*H*)-one 5f-*trans*. Isolated as colourless solid. (328 mg, 33%) mp=141 °C; υ_{max} (KBr) 3420, 1678 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.96 (1H, s), 2.39 (3H, s), 2.42 (3H, s), 2.57 (2H, m), 3.27 (1H, d, *J*=15 Hz), 3.40 (1H, d, *J*=15 Hz), 4.24 (1H, dd, *J*=11, 4 Hz), 4.96 (1H, s), 7.03–7.42 (8H, m), 8.99 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 19.0, 19.4, 30.3, 33.9, 46.7, 56.7, 107.3, 125.4, 125.6, 126.3, 127.4, 127.5, 127.9, 130.5, 130.8, 131.2, 136.0, 136.6, 137.8, 139.7, 164.6. Anal. Calcd for $C_{21}H_{22}N_2OS$: C, 71.97; H, 6.33; N, 7.99. Found C, 71.90; H, 6.31; N, 8.06.

4.3.3. *trans*-5,7-Bis(2-methoxyphenyl)-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-*b*][1,4]thiazin-2(3*H*)-one 5g-*trans*. Isolated as colourless solid. (651 mg, 60%) mp=161 °C; υ_{max} (KBr) 3442, 1679 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.92 (1H, s), 2.46 (2H, m), 3.32 (1H, d, *J*=15 Hz), 3.43 (1H, d, *J*=15 Hz), 3.62 (3H, s), 3.83 (3H, s), 4.24 (1H, dd, *J*=11, 4 Hz), 5.16 (1H, s), 6.76–7.42 (8H, m), 7.93 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 30.4, 34.4, 45.2, 54.4, 55.1, 55.2, 106.5, 110.3, 110.4, 120.0, 120.8, 126.2, 128.3, 128.4, 128.9, 129.0, 130.8, 131.0, 156.7, 157.4, 164.2. Anal. Calcd for $C_{21}H_{22}N_2O_3S$: C, 65.95; H, 5.80; N, 7.32. Found C, 65.86; H, 5.75; N, 7.34.

4.3.4. 5,7-Bis(2-chlorophenyl)-5,6-dihydro-1*H*-pyrido[3,4-*b*][1,4]thiazin-2(3*H*)-one 6e. Isolated as colourless solid. (111 mg, 10%) mp=136 °C; υ_{max} (KBr) 3307, 1680 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.70 (1H, s), 3.33 (1H, d, *J*=14 Hz), 3.44 (1H, d, *J*=14 Hz), 4.77 (1H, s), 5.49 (1H, s), 6.75–7.53 (9H, m), 7.76 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 30.9, 48.5, 110.2, 118.9, 120.3, 126.5, 126.8, 127.3, 128.7, 129.0, 129.3, 129.6, 129.8, 130.2, 130.6, 133.4, 134.5, 136.5, 163.5. Anal. Calcd for $C_{19}H_{14}Cl_2N_2OS$: C, 58.62; H, 3.62; N, 7.20. Found C, 58.58; H, 3.60; N, 7.23.

4.3.5. 5,7-Bis(2-methylphenyl)-5,6-dihydro-1*H*-pyrido[3,4-*b*][1,4]thiazin-2(3*H*)-one 6f. Isolated as colourless solid. (90 mg, 9%) mp=155 °C; υ_{max} (KBr) 3400, 1670 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.7 (1H, s), 2.26 (3H, s), 2.29 (3H, s), 3.25 (1H, d, *J*=14 Hz), 3.45 (1H, d, *J*=14 Hz), 4.44 (1H, s), 5.20 (1H, s), 6.64–7.25 (9H, m), 8.60 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 18.7, 19.1, 30.7, 54.5, 119.8, 120.0, 125.5, 126.2, 127.1, 128.1, 128.3, 130.0, 130.4, 131.3, 134.8, 136.9, 137.2, 137.3, 137.7, 138.3, 164.6. Anal. Calcd for $C_{21}H_{20}N_2OS$: C, 72.38; H, 5.79; N, 8.04. Found C, 72.39; H, 5.75; N, 8.00.

4.3.6. 5,7-Bis(2-methoxyphenyl)-5,6-dihydro-1*H*-pyrido[3,4-*b*][1,4]thiazin-2(3*H*)-one 6g. Isolated as paste. (151 mg, 10%); υ_{max} (CH_2Cl_2) 3425, 1665 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.7 (1H, s), 3.10 (1H, d, *J*=15 Hz), 3.27 (1H, d, *J*=15 Hz), 3.80 (3H, s), 3.85 (3H, s), 4.81(1H, s), 5.60 (1H, s), 6.64–7.53 (9H, m), 8.00 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 33.0, 49.7, 55.1, 55.4, 109.8, 111.4, 111.1, 119.8, 120.1, 120.4, 124.0, 127.6, 127.9, 128.2, 128.8, 129.6, 129.8, 133.2, 133.6, 170.0. Anal. Calcd for $C_{21}H_{20}N_2O_3S$: C, 66.29; H, 5.30; N, 7.36. Found C, 66.27; H, 5.26; N, 7.38.

4.4. Synthesis of ethyl 2-[2,6-bis(2-chlorophenyl)-4-oxo-3-piperidinyl]sulfanylacetate 2f

Ammonium acetate (0.215 g, 2.8 mol) was dissolved in ethanol (3 mL) by heating. *ortho*-Chlorobenzaldehyde (0.630 mL, 5.7 mol) and ethyl 2-[(2-oxopropyl)sulfanyl] acetate (0.338 mL, 2.8 mmol) were added to this solution and the mixture was heated until the colour of the solution turned yellow. The solution was kept at room temperature for 4 days. The solid precipitated was filtered off, washed with ethanol, and recrystallised from ethanol and ethyl acetate as colourless solid. (597 mg, 49%) mp=178 °C; υ_{max} (CH_2Cl_2) 3307, 1731, 1706 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.20 (3H, t, *J*=7 Hz), 1.65 (1H, s), 2.60 (1H, dd, *J*=15, 3 Hz), 3.01 (1H, d, *J*=15 Hz), 3.12 (1H, d, *J*=15 Hz), 3.62 (1H, dd, *J*=15, 12 Hz), 3.79 (1H, d, *J*=2 Hz), 4.00 (2H, m), 4.65 (1H, dd, *J*=12, 3 Hz), 4.93 (1H, d, *J*=2 Hz), 7.23–7.90 (8H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 13.9, 33.5, 42.3, 55.2, 56.4, 59.3, 61.5, 126.5, 127.6, 127.8, 129.0, 129.1, 129.5, 129.6, 129.7, 132.2, 132.4, 136.0, 139.5, 169.4, 203.6. Anal. Calcd for $C_{21}H_{21}Cl_2NO_3S$: C, 57.54; H, 4.83; N, 3.20. Found C, 57.49; H, 4.88; N, 3.13.

4.5. Synthesis of ethyl 2-[(2-oxopropyl)sulfanyl]acetate 1

Ethyl 2-sulfanylacetate (0.912 mL, 5.6 mmol) was dissolved in chloroform (10 mL). To this solution, chloroacetone (0.451 mL, 5.6 mmol) and potassium carbonate (0.392 g, 2.8 mmol) were added. The mixture was stirred under cold condition for 4–5 h. After the completion of the reaction, the product was extracted with chloroform, washed with water, dried over anhydrous calcium chloride and the solvent evaporated in vacuo. The pure product was obtained as a pale yellow liquid (0.936 g, 95%) bp 97 °C, density

1.4760 mg m^{-3}. υ_{max} (CH_2Cl_2) 1725, 1649, 1203; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.29 (3H, t, J=7 Hz), 2.30 (2H, s), 3.25 (2H, s), 3.46 (1H, s), 4.17 (2H, q, J=7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 13.7, 27.8, 32.9, 41.7, 61.0, 169.3, 202.5. Anal. Calcd for $C_7H_{12}O_3S$: C, 47.71; H, 6.86. Found C, 47.68; H, 6.81.

4.6. X-ray crystallographic determination of compounds of 2f, 5b-*cis*, 5e-*trans* and 5g-*trans*

Data were collected at room temperature on an Enraf-Nonius MACH 3 four-circle diffractometer (Mo Kα radiation, λ=0.71073 Å) for compounds **2f**, **5b**-*cis*, **5e**-*trans* and **5g**-*trans*. The data collection, integration, and data reduction for **2f**, **5b**-*cis*, **5e**-*trans*, and **5g**-*trans* were performed using CAD-4 EXPRESS[15] and XCAD4[16] programmes and an empirical absorption correction was applied using ψ scan method.[17] The unit cell parameters were determined by a least square fitting of 25 randomly selected strong reflections and an empirical absorption correction was applied using the azimuthal scan method. The structures were solved by direct methods (SHELXS 97)[18] and subsequent Fourier synthesis and refined by full matrix least squares on SHELXL 97[19] for all non-hydrogen atoms for **2f**, **5b**-*cis*, **5e**-*trans* and **5g**-*trans*. All hydrogen atoms were placed in calculated positions.

4.6.1. Compound 2f. $C_{21}H_{21}Cl_2NO_3S$, M=438.35, monoclinic, space group $Pccn$, a=25.5182(15) Å, b=19.3676(11) Å, c=8.3266(5) Å, V=4115(3) Å^3, Z=8, F(000)=1824, μ=0.439 mm^{-1}, D_c=1.415 mg m^{-3}. The reflections collected were 3845 of which 3594 unique [$R_{(int)}$=0.0459]; 1664 reflections $I>2\sigma(I)$, R_1=0.0677 and wR_2=0.1772 for 1664 [$I>2\sigma(I)$] and R_1=0.1731 and wR_2=0.2397 for all (3594) intensity data. Goodness of fit=1.027, residual electron density in the final Fourier map was 0.372 and −0.637 eÅ^{-3}. CCDC number is 290756.

4.6.2. Compound 5b-*cis*. $C_{19}H_{16}C_{l2}N_2OS$, M=391.31, monoclinic, space group $P2_1/n$, a=17.891(4) Å, b=5.052(6) Å, c=24.603(5) Å, V=4115(3) Å^3, Z=4, F(000)=808, μ=0.402 mm^{-1}, D_c=1.195 mg m^{-3}. The reflections collected were 4179 of which 3792 unique [$R_{(int)}$=0.0334]; 1114 reflections $I>2\sigma(I)$, R_1=0.0661 and wR_2=0.1873 for 1664 [$I>2\sigma(I)$] and R_1=0.1882 and wR_2=0.2241 for all (3792) intensity data. Goodness of fit=0.752, residual electron density in the final Fourier map was 0.453 and −0.271 eÅ^{-3}. CCDC number is 290758.

4.6.3. Compound 5e-*trans*. $C_{19}H_{16}C_{l2}N_2OS$, M=391.31, monoclinic, space group $P2_1/c$, a=7.1010(4) Å, b=26.6410(14) Å, c=9.8301(8) Å, V=1789.7 Å^3, Z=4, F(000)=808, μ=0.489 mm^{-1}, D_c=1.452 mg m^{-3}. The reflections collected were 3893 of which 3157 unique [$R_{(int)}$=0.0118]; 2128 reflections $I>2\sigma(I)$, R_1=0.0355 and wR_2=0.0912 for 2128 [$I>2\sigma(I)$] and R_1=0.0729 and wR_2=0.1200 for all (3157) intensity data. Goodness of fit=1.053, residual electron density in the final Fourier map was 0.463 and −0.455 eÅ^{-3}. CCDC number is 290757.

4.6.4. Compound 5g-*trans*. $C_{21}H_{22}N_2O_3S$, M=382.47, monoclinic, space group P-1, a=9.764 Å, b=10.972 Å, c=11.061 Å, V=965.3 Å^3, Z=2, F(000)=404, μ=0.191 mm^{-1}, D_c=1.316 mg m^{-3}. The reflections collected were 4046 of which 3392 unique [$R_{(int)}$=0.0110]; 2694 reflections $I>2\sigma(I)$, R_1=0.0347 and wR_2=0.0970 for 2694 [$I>2\sigma(I)$] and R_1=0.0492 and wR_2=0.1062 for all (3392) intensity data. Goodness of fit=1.037, residual electron density in the final Fourier map was 0.261 and −0.215 eÅ^{-3}. CCDC number is 290759.

5. Supplementary material

Crystal data and structure refinement for **2f**, **5b**-*cis*, **5e**-*trans* and **5g**-*trans*. Atomic coordinates ($\times 10^4$) and equivalent isotopic displacement parameters (Å$^2\times 10^3$) for **2f**, **5b**-*cis*, **5e**-*trans* and **5g**-*trans*.

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14. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the

Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 290756–290759. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK, +44 1223 336408 or e-mail: deposit@ccdc.cam.ac.uk).

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Nanoarchitecture self-assembly and photochromic studies of 2,2-diarylnaphthopyrans

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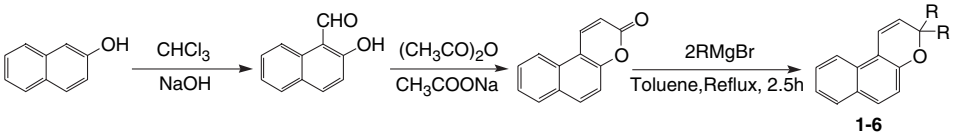
Abstract—The synthesis and photochromic properties of novel 2,2-diarylnaphthopyrans were described. Significantly, the nanostructured architecture through two-component self-assembly of a photochromic naphthopyran and an asymmetric biphenyl was determined by X-ray diffraction. The structure motifs of nanocavities were formed by Cl···O interactions and Ar–H···Cl hydrogen bonds among the photochromic naphthopyran molecules. It was further shown by TEM that the dimensions of cavity structures were up to nanometer level, which provides the potential to capture useful nanoscale entities and control photochromism in organic materials.

1. Introduction

The design and synthesis of photochromic molecules is an intense research area because of their potential applications in optical memory, photo-optical switching, and nonlinear optics.[1–6] Naphthopyrans are one of the most studied and important class of photochromic compounds.[7,8] Nowadays, it is possible to obtain any color using only naphthopyrans.[9] Owing to their good photochromic properties, associated with high fatigue resistance, naphthopyrans have been used in the manufacture of photochromic lenses that darken in sunlight.[10] This photochromic behavior is based on a photoinduced reversible opening of the pyran ring that converts the colorless form (closed form) into the isomers (opened form).[8] The observation of a distinct absorption spectrum is due to the extensively π-conjugated system present in the opened form.[11]

Quite few self-assembly of photochromic molecules were reported. In this paper, we intended to report the preparation of a new series of photochromic 2,2-diarylnaphthopyrans **1**–**6** (Scheme 1) and the study of their photochromic behaviors in various media including organic solvents and polymethyl methacrylate (PMMA). Significantly, the nanostructured architecture was realized through two-component self-assembly of a photochromic naphthopyran and an asymmetric biphenyl in the crystal. It was further shown by TEM that the dimensions of cavity structures were up to nanometer level, which provided the potential to capture useful nanoscale entities and control photochromism in organic



Scheme 1. Routine of synthesis of 2,2-diarylnaphotopyrans derivatives **1**–**6** where R=3-CF_3–C_6H_4, 4-$(CH_3)_3C$–C_6H_4, 4-CH_3S–C_6H_4, 2,4-dimethoxy-1-phenyl, 3-F-4-CH_3O–C_6H_3, and 3-Cl-4-CH_3O–C_6H_3.

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materials. We are presently exploring a method of replacing the guests by other biphenyls or dipyridines.

2. Results and discussion

2.1. X-ray crystal structural studies

The X-ray crystal structures of 2,2-diarylnaphthopyrans **3** (R=4-CH_3S–C_6H_4) were presented in Figure 1a. An asymmetric unit consisted of two molecules in the compound **3** (CCDC 258742). The packing of molecules (b) in compound **3** were given in Figure 1b. The crystals were monoclinic, in space group $P2_1$. Compound **3** exhibited 2-D supramolecular structures, as illustrated in Figure 1b. An extended zigzag tape was generated via C1–H1···S3 and C47–H47A···S4. Recrystallization of compound **4** (R=2,4-dimethoxy-phenyl) from ethanol yielded a clathrate with ethanol as guest. The packing structure of compound **4** (CCDC 258582), given in Figure 2, displayed a nanometer cavity structure.[12]

2.2. Nanoarchitecture self-assembly

As shown in Figure 3, compound **6** yielded the clathrate with an asymmetric biphenyl **I** from ethanol solution (CCDC 272162). The two phenyl rings themselves and naphtho moiety itself of molecule **6** in the crystal were approximately planar and dihedral angles were 75.0° between the naphtho ring and one phenyl (Ph) group (C14C15C16C17C18C19), and 61.3° between the naphtho ring and the other one (C21C22C23C2C25C26), respectively. Moreover, the dihedral angle between two phenyl rings themselves of compound **6** was 89.0°.

The substituent groups on the phenyl rings of molecule **6** had an insignificant effect on the length of the C_{sp^3}−O bond and on the nonplanarity of the pyran ring. It was shown in Figure 3 that the pyran ring was nonplanar, with folding along the O1···C11 and O1···C12 axes. The dihedral angle was 23.1° between the O1/C13/C12 and O1/C12/C11 planes, and 12.7° between the O1/C12/C11 and O1/C11/C10 planes, while the corresponding values in 3,3-(1-phenyl)-3*H*-naphtho[2,1-*b*]pyran were 22.9 (2) and 10.0 (2)°, respectively.[13]

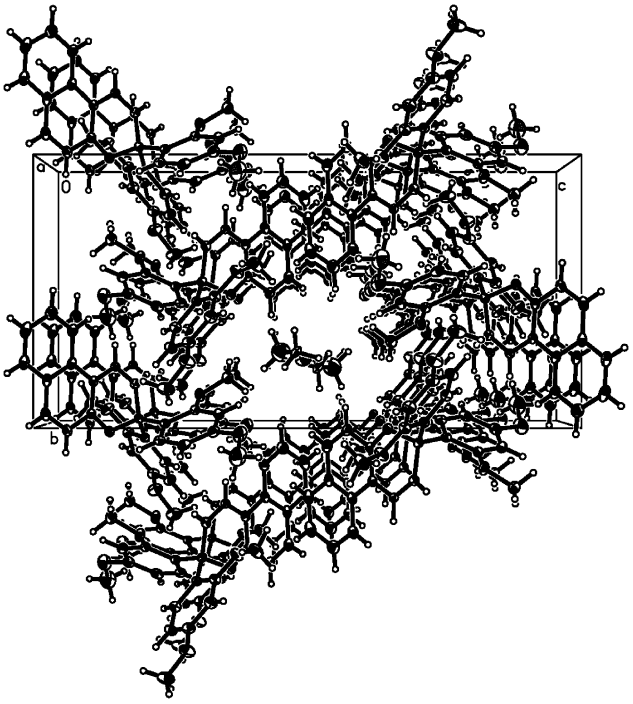

Figure 2. Packing of molecules of compound **4**.

A weak C13–O1 bond was confirmed by the X-ray diffraction data. The bond length was 1.465 (5) Å and was longer than the normal C–O bond (1.41–1.43 Å)[14] in six-member heterocycle, but this kind of change was coincident with other spiro compounds. The angles of the C_{sp^3}−O bond with the Ph ring planes (C14C15C16C17C18C19) and (C21C22C23C24C25C26) were 54.5 and 67.8°, respectively.

The crystal structure of the clathrate (Fig. 4) exhibited the molecules of **6** to form a centrosymmetric dimer in the cell with a space group of *C*2/*c*. Weak Cl···O (3.182 Å) interactions and Ar–H···Cl (2.906 Å) hydrogen bonds linked the

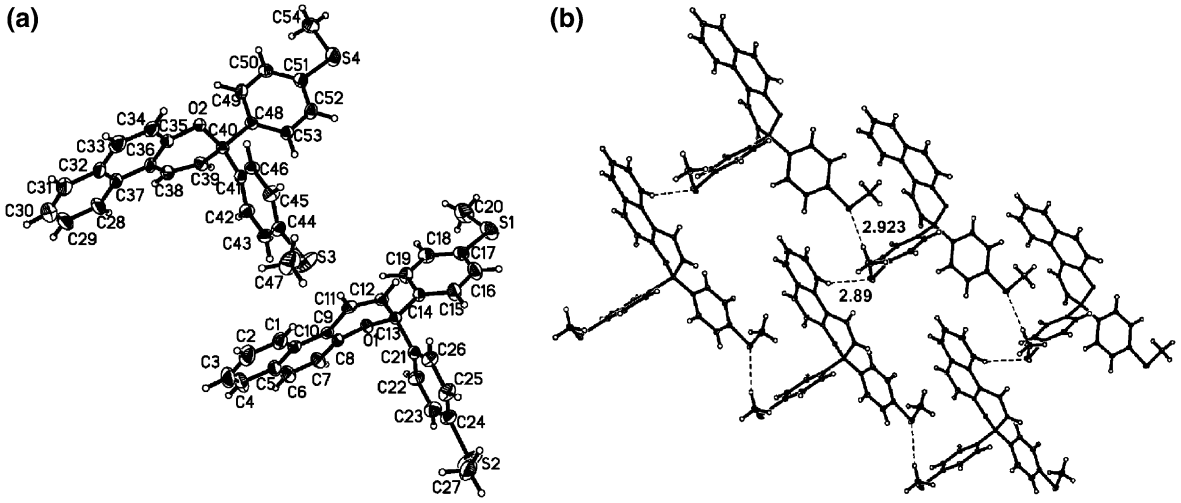

Figure 1. X-ray crystallographic structure (a) and packing of molecules (b) of compound **3**.

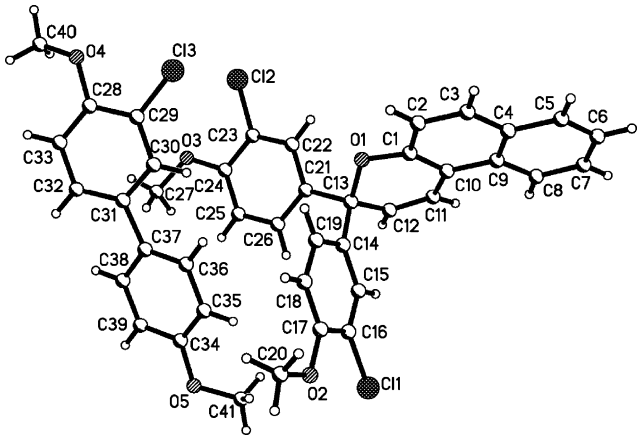

Figure 3. The self-assembled structure of molecules **6** and **I**.

two host molecules, and Ar–H···Cl (2.752 Å) hydrogen bond linked the host–guest molecule. As shown in Figure 4, the clathrate of **6** and **I** in their crystal from ethanol had host–guest stoichiometry of 4:1.

The crystal packing of molecule **6** showed nanostructured cavities along the *b* axis (Fig. 5) with the dimensions of 15.9×8.5 Å and 3.6×8.5 Å, respectively. The potential of these cavities to capture useful nanoscale entities and control photochromism in crystals are currently being explored.

Partial lattice voids were occupied by the protruding chloro groups of the helicene host molecules, so that an alternating arrangement of guest and substituents occurs. The molecular conformation of compound **I** in its crystal and the packing space filling representations of compound **I** and **6** were shown in Figure 6 and Figure 7, respectively.

2.3. TEM image of compound 6

Compound **6** was ground and dissolved in ethanol, then volatilized into a film for TEM image. Crystal morphology of compound **6** was studied by TEM on Philip FEI TECNAI-20. TEM image of photochromic compound **6** was shown in Figure 8, which verified that the whole arrangement of molecular self-assembly was of cavity structure in the crystal. To our surprise, the dimension of cavity structure determined by TEM was up to nanometer level, which is rarely found in organic compounds. It would be of great use for the molecular assembly suggesting the opportunities for inclusion complex formation.

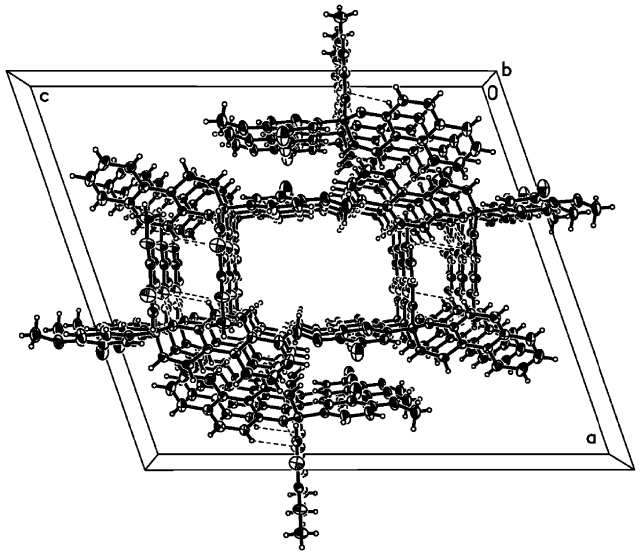

Figure 5. Crystal packing diagram of compound **6** along *b* axis.

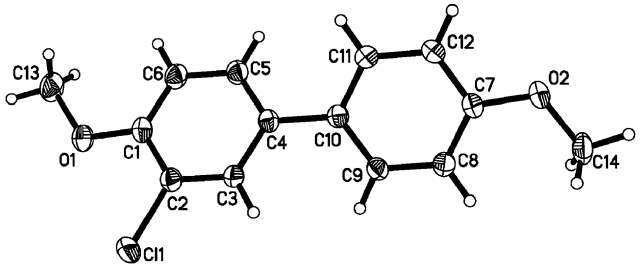

Figure 6. The molecular structure of compound **I**.

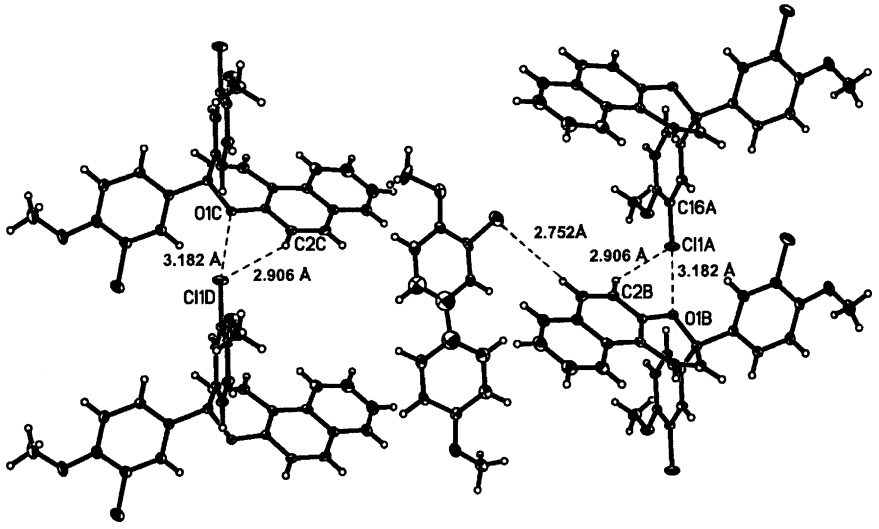

Figure 4. Packing excerpt of helicene compound **6** and **I** (4:1) with Cl···O and Ar–H···Cl contacts (dashed lines) along the *a* axis.

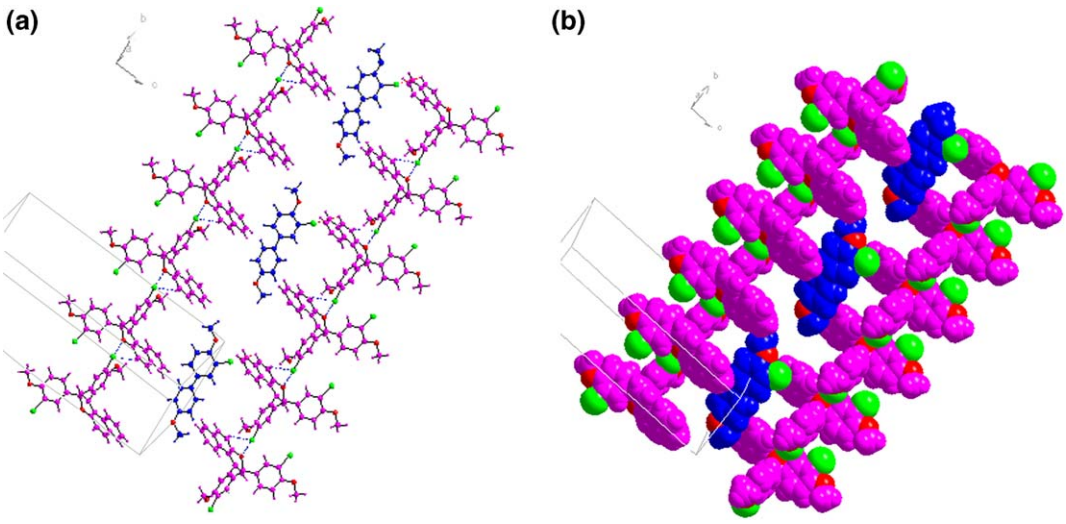

Figure 7. The packing stick (a) and space filling representations (b) of compound **6** and **I**.

2.4. Photochromic properties

The nanostructural assemblies had some influence on the photochromic properties of this series. Compound **6** yielded the clathrate with an asymmetric biphenyl **I** from ethanol solution. The nanostructural assembly is carried out in crystalline state. Although visible color change is observed with the naked eye in crystalline state, photochromism in crystalline state cannot be tested in the existing experimental circumstances.

The absorption spectra of compounds **1**–**6** were observed in solution. It was noteworthy that an obvious color change occurred on irradiation by UV-light or sunlight. Upon excitation at 365 nm, compounds **1**–**6** exhibit photochromic behaviors at room temperature in chloroform, with a visible absorption band at 409–498 nm, corresponding to the generation of the open ring form (Table 1). As shown in Figure 9, upon excitation at 365 nm, compound **4** in chloroform solution exhibited excellent photochromism, with a strong absorption band at 483 nm, resulting from the generation of the open ring form. The open ring form is thermally unstable and readily undergoes thermal bleaching, which follows first-order kinetics, to the ring-closed form.

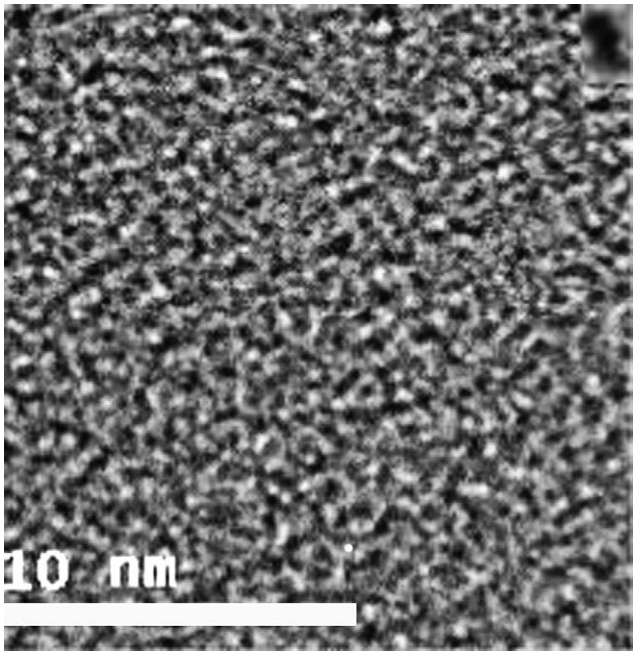

Figure 8. TEM image of the crystal of photochromic compound **6**.

Table 1. The maximum absorption wavelength (λ_{max}) of compounds **1**–**6** in chloroform solution (10^{-5} mol/L) after UV–vis irradiation

Compd	Irradiation time (s)	λ_{max} in solution (nm)	Absorption intensity
1	60	409	0.20
2	60	469	0.21
3	60	498	0.05
4	30	483	1.02
5	60	443	0.04
6	30	473	0.19

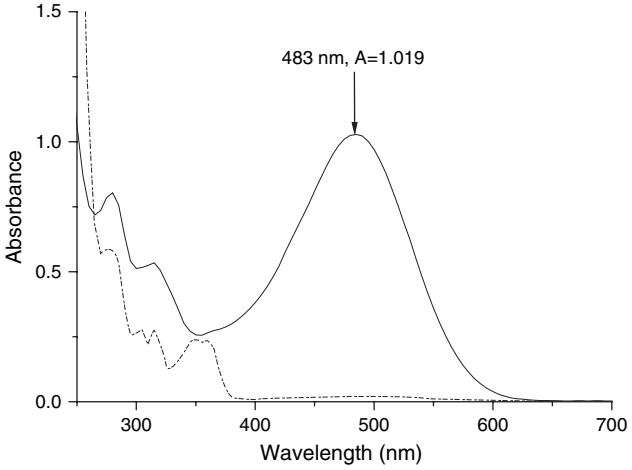

Figure 9. UV–vis absorption spectra of compound **4** before (·····) and after (——) in acetone.

2.4.1. Absorption spectra in polymer films. Films of all species were prepared (2 wt % loading) in PMMA with 1 μm thickness. The state of these compounds in PMMA

Table 2. The maximum absorption wavelength (λ_{max}) of compounds **1**–**6** in PMMA films (2 wt % loading) after UV–vis irradiation

Compd	Irradiation time	λ_{max} in PMMA film (nm)	Absorption intensity	$t_{A_0/2}$ (min)
1	60	409	0.10	170
2	60	450	0.09	160
3	60	479	0.06	140
4	30	486	1.25	670
5	60	465	0.07	150
6	30	463	0.10	170

was amorphous without self-assembly. The absorbance of each film at its λ_{max} was recorded immediately after irradiation for 30 s at 365 nm with a 12 W ultraviolet lamp. Upon excitation at 365 nm, compounds **1**–**6** exhibited photochromic behaviors at room temperature in PMMA, with a visible absorption band at 409–486 nm, corresponding to the generation of the open ring form (Table 2). The absorption intensities of compounds **1**, **2**, **3**, **5**, and **6** at the maximum wavelength in films after UV–vis irradiation were small, but that of **4** was large. In the discussion below, we only described the films of **4** and **6**. The coloration and bleaching of **4** (a) and **6** (b) following irradiation were shown in Figure 10.

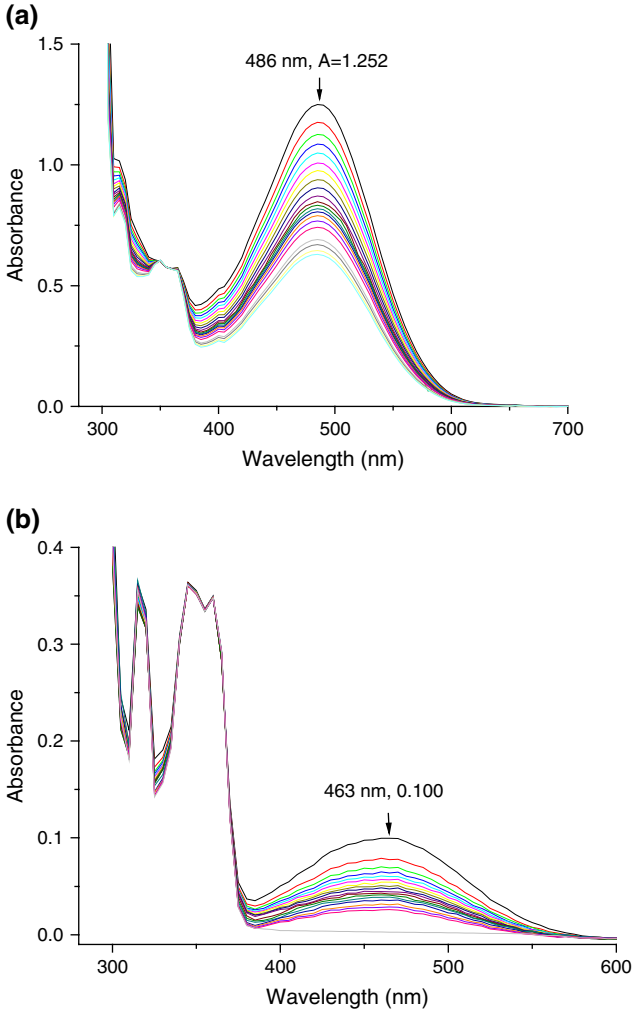

Figure 10. Absorption spectra of compounds **4** (a) and **6** (b) in PMMA films after irradiation at 365 nm with a 12 W ultraviolet lamp for 30 s at different decay times.

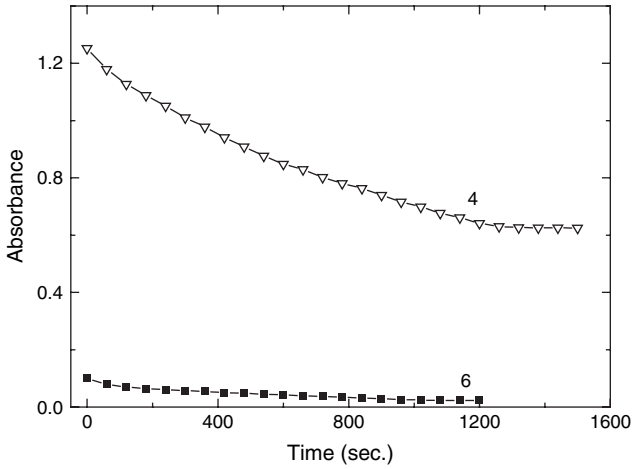

Figure 11. Absorption changes at the λ_{max} of **4** (▽) and **6** (■) in PMMA during the decoloration process at room temperature after the irradiation at 365 nm with a 12 W ultraviolet lamp.

Figure 2 showed that the representative UV–vis absorption spectra changed in the photomerocyanines with time. Following UV–vis irradiation for 30 s, a new absorption band appeared with a maximum absorption wavelength of **4** at 486 nm or **6** at 463 nm, which demonstrated that they exhibited excellent photochromism.

The two-methoxy substituents in the phenyl ring led to red shifts of the absorption band and the electron-donor groups stabilized the open-ring isomers.[15] The photochromic performance is based on the photoinduced reversible opening of the pyran ring as described in the literature.[16]

The thermally reversible processes of compounds **4** and **6** in PMMA films were studied by plotting absorbances at the same λ_{max} for a given compound at different time. A typical example of the plot made for compounds **4** and **6** was illustrated in Figure 11. As shown in Figure 3, the intensity of absorption band at its λ_{max} was decreased gradually during the decoloration process.

2.4.2. The parameter $t_{A_0/2}$ for the fatigue resistance in PMMA films. Sixteen slices of thin-films of **4** and **6** (2 wt % loading) in PMMA were prepared, respectively. All films were irradiated at the same time with a 400 W high-pressure mercury lamp. The absorbances at the λ_{max} of a given compound at different irradiation time were recorded on a spectrophotometer immediately after irradiation. A plot of the absorbance against the irradiation time was given in Figure 12. The parameter $t_{A_0/2}$ obtained from the plot is defined as the required time in minute decreasing the initial absorbance (A_0) at the λ_{max} of the merocyanine form to the half value ($A_0/2$). As shown in Table 2, the $t_{A_0/2}$ of **4** was calculated to be 670 min, and the $t_{A_0/2}$ of **6** was 170 min through a linear extrapolation of the data.[17] It seemed that electron donors increased the lifetime of the open ring form.

2.4.3. Evaluation of the fatigue resistance of 4 in PMMA films. PMMA film was irradiated with a 12 W ultraviolet lamp. The absorbances at λ_{max} of compound **4** were recorded on a spectrophotometer immediately before and after UV

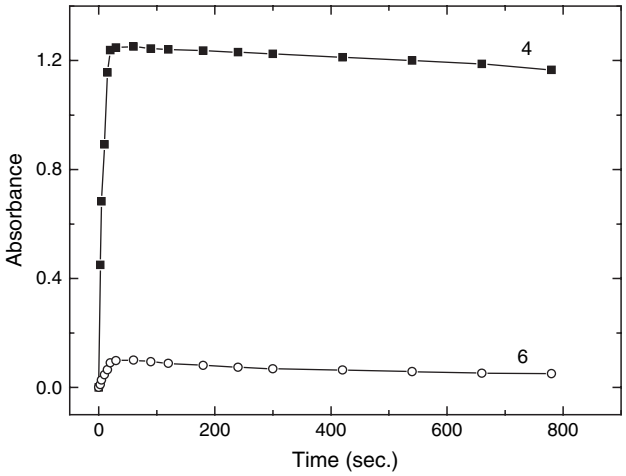

Figure 12. The absorption changes at the λ_{max} of **4** (λ_{max} =486 nm, 2 wt % loading) and **6** (λ_{max}=463 nm, 2 wt % loading) in PMMA films under continuous UV–vis irradiation with a 400 W high-pressure mercury lamp.

irradiation in a 40-time cycle. The absorbances at λ_{max} were zero before UV irradiation (ring-closed form), but they were different after UV irradiation (ring-opened form). On gradual increase of cycle times, the absorbances at λ_{max} were gradually decreased after UV irradiation (ring-opened form). A plot of the absorbance against the cycle times was made as shown in Figure 13. The fatigue resistance was examined after 280-cycle irradiation of UV–vis. As shown in Figure 13, the fatigue resistance of compound **4** was examined and recorded. After 280-cycle UV–vis irradiation, the absorbance at maximum wavelength was kept in 99.1% of that of first irradiation in open ring form. As it was extrapolated to 1000 cycles, the absorbance of maximum wavelength after UV–vis irradiation would be kept in 96.9% of that of first irradiation in open ring form ($A=A_0(1-X)^n$, where n: cycle times, A_0: the original absorption intensity; X: the variational absorption intensity). It was shown that 2,2-diarylnaphthopyrans exhibited excellent stability. The useful lifetime of the photochromic films is of utmost importance to its commercial success.

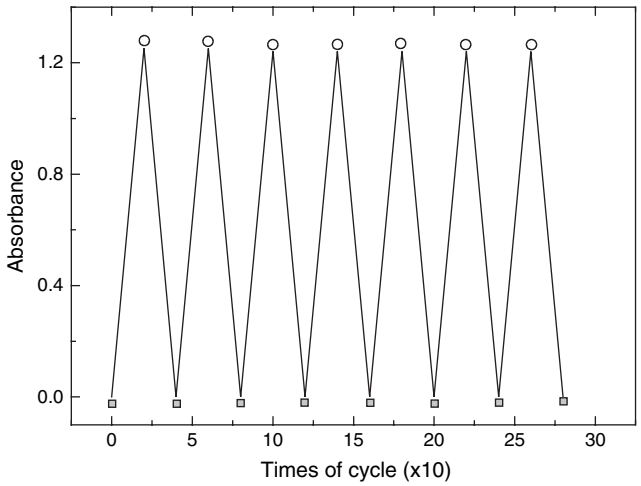

Figure 13. Photoinduced absorption changes for compound **4** in PMMA film at λ_{max}; photoirradiation started at each point of □ with visible light, finished at the point of ○ and started with UV light.

3. Experimental

3.1. General procedure for the synthesis of 2,2-diarylnaphthopyrans (entries 1–6)

The target compounds **1**–**6** were prepared in moderate yields through reactions of Grignard reagents with naphthopyran biones in dry tetrahydrofuran (THF) instead of toluene.[18–20]

Prepared Grignard reagent (10 mmol) in diethyl ether was added to naphthopyran bione (5 mmol, synthesized as described in the literature[18]) in dry THF under reflux.[19,20] The mixtures were stirred until no starting materials remained in TLC. The reaction mixture was then cooled to room temperature and ethyl ether was added to extract the organic layer. The organic phases were combined, and dried with anhydrous magnesium sulfate. Solvent evaporation gave a crude product, which was purified by a silica-gel column chromatography using petroleum ether/ethyl ether as eluent. Recrystallization from ethanol or acetone gave a crystalline material.

3.1.1. 2,2-Bis(3-trifluoro methylphenyl)-3*H*-naphtho[2,1-*b*]pyran (1). Yield: 46%. Mp: 103–104 °C. ^{1}H NMR: δ 6.21 (d, 1H, J=6.6 Hz), 7.14–7.43 (m, 14H), 7.75 (d, 1H, J=6.0 Hz). IR (KBr): 1622, 1510 (C=C) cm^{-1}, 1330, 1224 (C–F) cm^{-1}. EIMS: m/z 471 (M^+). Anal. Calcd for $C_{27}H_{16}F_6O$: C, 68.94; H, 3.40. Found: C, 68.70; H, 3.41.

3.1.2. 2,2-Bis(4-tertiary butylphenyl)-3*H*-naphtho[2,1-*b*]pyran (2). Yield: 36%. Mp: 194–195 °C. ^{1}H NMR: δ 1.27 (s, 18H), 6.28 (d, 1H, J=6.6 Hz), 7.20–7.72 (m, 14H), 7.93 (d, 1H, J=5.4 Hz). IR (KBr): 2962, 1463, 1361 (C=C) cm^{-1}. EIMS: m/z 447 (M^+). Anal. Calcd for $C_{33}H_{34}O$: C, 88.79; H, 7.62. Found: C, 88.51; H, 7.61.

3.1.3. 2,2-Bis(4-sulfur methylphenyl)-3*H*-naphtho[2,1-*b*]pyran (3). Yield: 36%. Mp: 142–143 °C. ^{1}H NMR: δ 2.43 (s, 6H), 6.17 (d, 1H, J=6.6 Hz), 7.14–7.71 (m, 14H), 7.93 (d, 1H, J=5.6 Hz). IR (KBr): 1618, 1510 (C=C) cm^{-1}. EIMS: m/z 427 (M^+). Anal. Calcd for $C_{27}H_{22}OS_2$: C, 76.06; H, 5.16. Found: C, 76.28; H, 5.14.

3.1.4. 2,2-Bis(2,4-dimethoxyphenyl)-3*H*-naphtho[2,1-*b*]pyran (4). Yield: 23%. Mp: 94–95 °C. ^{1}H NMR: δ 2.77 (s, 6H), 2.84 (s, 6H), 6.45 (d, 1H, J=6.8 Hz), 7.00–7.11 (m, 12H), 7.91 (d, 1H, J=5.8 Hz). IR (KBr): 1608, 1501 (C=C) cm^{-1}. EIMS: m/z 455 (M^+). Anal. Calcd for $C_{29}H_{26}O_5$: C, 76.65; H, 5.73. Found: C, 76.43; H, 5.72.

3.1.5. 2,2-Bis(3-fluoro-4-methoxyphenyl)-3*H*-naphtho[2,1-*b*]pyran (5). Yield: 41%. Mp: 132–134 °C. ^{1}H NMR: δ 3.85 (s, 6H), 6.12 (d, 1H, J=6.4 Hz), 6.96–7.71 (m, 12H), 7.95 (d, 1H, J=5.4 Hz). IR (KBr): 1621, 1514 (C=C) cm^{-1}. EIMS: m/z 431 (M^+). Anal. Calcd for $C_{27}H_{20}F_2O_3$: C, 75.34; H, 4.65. Found: C, 75.20; H, 4.64.

3.1.6. 2,2-Bis(3-chloro-4-methoxyphenyl)-3*H*-naphtho[2,1-*b*]pyran (6). Yield: 32%. Mp: 107–108 °C. ^{1}H NMR: δ 3.84 (s, 6H), 6.11 (d, 1H, J=9.8 Hz), 6.86–7.68 (m, 12H), 7.94 (d, 1H, J=8.4 Hz). IR (KBr): 1631, 1498 (C=C) cm^{-1}. EIMS: m/z 463 (M^+). Anal. Calcd for $C_{27}H_{20}O_3Cl_2$: C, 69.98; H, 4.32. Found: C, 69.96; H, 4.31.

3.2. Preparation of thin polymer films

To 60 mL of toluene, 10 g of the polymer was added and stirred until completely dissolved into transparent liquid by heating. Then a specified amount (weight percent loading) of **1**–**6** was added to the polymer solution and stirred well to mix. This solution was poured into a Petri dish and kept in a dark room. After complete evaporation of the solvent, the dish was baked in an oven at 60 °C for 20 min. The film was then peeled off from the dish. The resulting films were kept in a dark room.

Acknowledgments

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Co-catalyzed reductive cyclization of azido and cyano substituted α,β-unsaturated esters with $NaBH_4$: enantioselective synthesis of (*R*)-baclofen and (*R*)-rolipram

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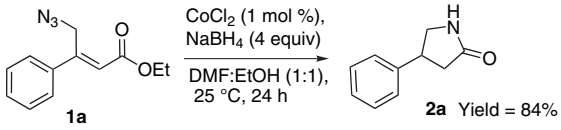
Abstract—Sodium borohydride in combination with a catalytic amount of $CoCl_2$ has been found to be an excellent catalytic system in reductive cyclizations of suitably substituted azido and cyano groups of α,β-unsaturated esters to afford γ and δ-lactams in high yields. The process has been demonstrated for the enantioselective synthesis of (*R*)-baclofen, (*R*)-rolipram, and (*R*)-4-fluorophenylpiperidinone, a key intermediate for (−)-paroxetine.

1. Introduction

Metal hydrides, particularly sodium borohydride and lithium aluminum hydride have emerged as prominent reducing agents capable of reducing most functional groups.[1] Moreover, by attaching organic ligands at the B or Al centers, or changing the metal counterion, one can modulate the scope, regio, and stereoselectivity of such reductions.[2] Quite recently, transition metal salts have been used as catalysts or additives in conjunction with $NaBH_4$ or $LiAlH_4$ in order to enhance the reducing power of these reagents.[3] In particular, the cobalt–sodium borohydride system[3d] is a well-known reducing system, capable of selectively reducing a variety of functional groups including C=C, N_3, CN, etc. when present alone. In this paper we disclose a single step, new synthetic procedure for the reductive cyclization of azido and cyano substituted α,β-unsaturated esters with $NaBH_4$ catalyzed by cobalt chloride, leading to synthesis of γ and δ-lactams in high yields.

2. Results and discussion

In continuation with our studies[4a] on simultaneous reduction of multifunctional moieties, we were interested in subjecting γ-azido olefinic ester **1a** to reduction with the $NaBH_4$–$CoCl_2$ system in order to obtain the corresponding γ-amino esters. To our surprise, γ-lactam **2a** was obtained in 84% yield, presumably due to the simultaneous reduction of both the azido group and C–C double bond followed by its cyclization, all occurring concurrently in a single step (Scheme 1). In the absence of $CoCl_2$, no reaction took place; also, other metal salts such as $PdCl_2$, $CuCl_2$, $NiCl_2$, etc. were not effective in achieving the transformation of γ-azido ester **1a** to the corresponding γ-lactam **2a**.



Scheme 1. Co-catalyzed reductive cyclization of γ-azido ester with $NaBH_4$.

For systematic evaluation of this study, the required starting γ-azido esters **1a**–**g** were prepared in three steps as presented in Table 1: (i) Pd-catalyzed arylation of ethyl crotonate with the respective aryl boronic acids **3a**–**g** producing α,β-unsaturated esters **4a**–**g**; (ii) allylic bromination of the methyl group in **4** with NBS yielding the bromo derivatives **5a**–**g**; and (iii) nucleophilic displacement of bromide with azide giving γ-azido esters **1a**–**g**. Alternatively, alkyl and aryl α,β-unsaturated esters **4** were also obtained in high yields, although in two steps, by the Reformatsky reaction of the corresponding ketones (see Scheme 4 in Section 4 for their preparation) with ethyl bromoacetate, followed by dehydration of the resulting alcohols with *p*-TSA.[4a]

It was of interest to develop the asymmetric version of this reductive cyclization process. As a model compound,

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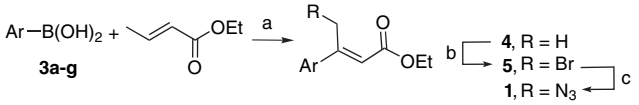
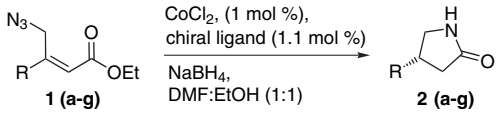
Table 1. Preparation of starting γ-azido olefinic esters **1a–g**: Pd-catalyzed arylation,[a] allylic bromination[b] followed by bromide displacement with azide[c]



Entry	Ar	Yield (%)[a]		
		4 (R=H)[b]	**5** (R=Br)[c]	**1** (R=N_3)[d]
a	Ph	86	72	86
b	4-ClPh	82	88	77
c	4-FPh	80	84	80
d	2-MeOPh	91	80	90
e	4-MeOPh	93	86	95
f	3-CpO-4-MeOPh[e]	92	90	99
g	*t*-C_4H_9	82[f]	76	91

[a] Isolated yield after chromatographic purification.
[b] Ethyl crotonate (2 mmol), boronic acid **3** (1 mmol), cat. $Pd(OAc)_2$ (1 mol %), Na_2CO_3 (1.5 mmol), O_2 (1 atm), DMF, 50 °C, 12 h, 80–93%.
[c] Olefinic ester **4** (1 mmol), NBS (1.2 mmol), AIBN (1 mol %), CCl_4, 80 °C, 24 h, 72–90%.
[d] Bromo ester **5** (1 mmol), NaN_3 (3 mmol), EtOH/H_2O (1:1), 50 °C, 12 h, 86–99%.
[e] Cp=cyclopentyl.
[f] Prepared by Reformatsky route.

Table 2. $CoCl_2$-catalyzed reductive cyclization of γ-azido-α,β-unsaturated esters with $NaBH_4$[a] in the presence of chiral ligands



Entry	R	Chiral ligand	Yield of **2** (%)[b]	% ee[c] (Configuration)
a	Ph	**6**	86	51 (*R*)
b	4-ClPh	**6**	82	89 (*R*)
	4-ClPh	**7**	86	05
	4-ClPh	**8**	80	12
	4-ClPh	**9**	73	05
c	4-FPh	**6**	80	[d]
d	2-MeOPh	**6**	91	[d]
e	4-MeOPh	**6**	93	98 (*R*)
f	3-CpO-4-MeOPh[e]	**6**	92	92 (*R*)
g	*t*-C_4H_9	**6**	77	[d]

[a] Conditions: azido ester (1 mmol), $CoCl_2$ (1 mol %), chiral ligands **6–9** (1.1 mol %), $NaBH_4$ (4 mmol), DMF/EtOH (1:1), 25 °C, 24 h, 77–93%.
[b] Isolated yield after chromatographic purification.
[c] Determined by comparison of $[\alpha]_D$ with the reported values as well as by chiral HPLC analysis.
[d] % ee not determined.
[e] Cp=cyclopentyl.

4-chlorophenyl-γ-azido olefinic ester **1b** was chosen and subjected to reduction with $CoCl_2$ (1 mol %), $NaBH_4$ (4 equiv) in the presence of ligands **6–9** (1.1 mol %) (Fig. 1), at 25 °C and obtained the corresponding chiral γ-lactam **2b** in high yields with good enantioselectivity (Table 2).

In order to systematically explore the utility of this catalytic system for the synthesis of various 4-substituted pyrrolidin-2-ones, a variety of γ-azido olefinic esters **1a–g** were successfully screened to afford the corresponding 4-substituted pyrrolidin-2-ones **2a–g** in excellent yields. Table 2 shows the results of several such azido esters, which underwent reductive cyclization smoothly under the reaction conditions. The methodology also works well in the case of an aliphatic system (entry *g*). As can be seen from Table 2, only (4*S*)-(+)-phenyl-α-[(4*S*)-phenyloxazolidin-2-ylidine]-2-oxazoline-2-acetonitrile **6** gave high optical induction in the product (up to 98% ee) (Fig. 1).

These γ-lactams **2(a–g)** are the precursors to γ-aminobutyric acid (GABA) analogues, which are of great interest due to their importance in various nervous system functions (Fig. 1).[5] For instance, the strongly lipophilic β-substituted analogue, 4-amino-3-(4-chlorophenyl)butyric acid (**10**, baclofen) is until now the only available selective agonist of the $GABA_B$ receptor.[6] Also the cyclic GABA analogue, 4-(3-cyclopentyloxy-4-methoxyphenyl)pyrrolidin-2-one (**2f**, rolipram) is known for its potent inhibitor activity of the cardiac cyclic AMP phosphodiesterase, found in brain tissue.[7] Research has shown that for both the compounds, the pharmacological activity resides in the (*R*)-enantiomers only.[8] The 4-arylpiperidine moiety (**11**) is also an important structural element in many biologically active compounds, possibly due to its similarity to the aryl alkylamine pharmacophore common to neurotransmitters such as serotonin or dopamine. Notably, (−)-paroxetine (**11**) possessing a 4-arylpiperidine moiety, is used in the treatment of depression, obsessive–compulsive disorder, and panic disorder.[9] Several stereoselective syntheses of (*R*)-baclofen (**10**),[4] (*R*)-rolipram (**2f**),[10] and (−)-paroxetine (**11**)[11] have already been reported (Fig. 2).

In view of its biological importance, chiral γ-lactam **2b** thus prepared by the present protocol, was subjected to

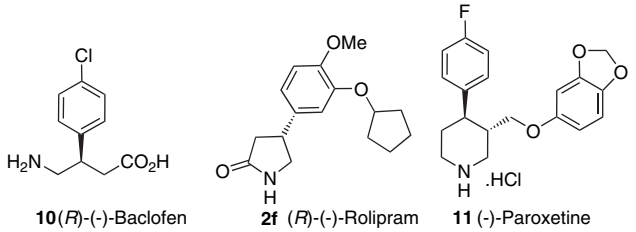


Figure 2.

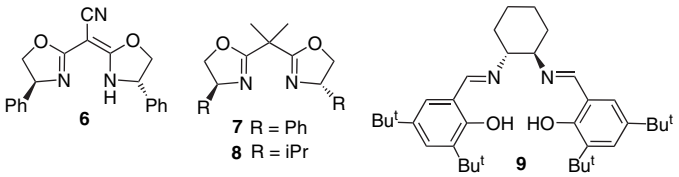
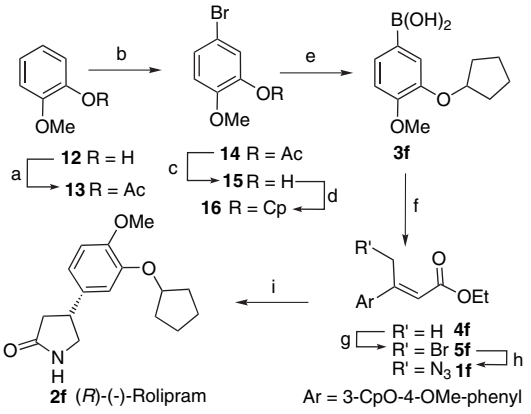


Figure 1.

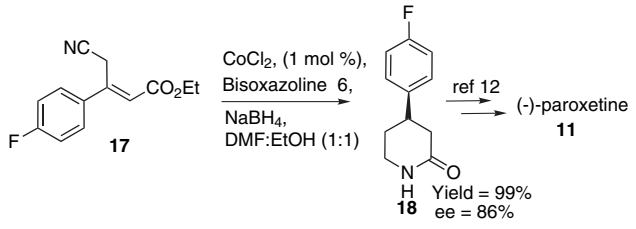
hydrolysis with 6 M HCl to give the optically active (*R*)-baclofen (**10**) as its hydrochloride salt in 73% yield and 88% ee.

Aryl boronic acid **3f** utilized in (*R*)-rolipram synthesis was prepared in five steps from guaiacol **14** as shown in Scheme 2. Acetyl protection of the guaiacolic hydroxy group has facilitated the selective bromination occurring at the *p*-position to the methoxy group. The boronic acid **3f** was then used for arylation of ethyl crotonate using Pd catalysis to obtain α,β-unsaturated esters **4f**, which were further transformed to azido intermediate **1f**. Finally, γ-azido ester **1f** when subjected to Co-catalyzed reduction with $NaBH_4$ yielded (*R*)-rolipram in 92% yield and 92% ee.



Scheme 2. Synthesis of (*R*)-rolipram from guaiacol. Conditions: (a) Ac_2O, concd H_2SO_4, 100 °C, 7 h, 97%; (b) NBS, CH_3CN, 50 °C, 4 h, 99%; (c) aq 10% $NaHCO_3$, MeOH, reflux, 6 h, 95%; (d) cyclopentyl bromide, K_2CO_3, DMF, 60 °C, 12 h, 89%; (e) BuLi, $B(OMe)_3$, −78 °C, 3 h; aq HCl (10%), reflux, 6 h, 15%; (f) ethyl crotonate, boronic acid **3f**, cat. $Pd(OAc)_2$, Na_2CO_3, O_2 (1 atm), DMF, 50 °C, 12 h, 92%; (g) olefinic ester **4f**, NBS, AIBN, CCl_4, 80 °C, 24 h, 90%; (h) bromoester **5f**, NaN_3, EtOH/H_2O (1:1), 50 °C, 12 h, 99%; (i) azido ester **1f** (1 mmol), $CoCl_2$ (1 mol %), ligand **6** (1.1 mol %), $NaBH_4$ (4 mmol), DMF/EtOH (1:1), 25 °C, 24 h, 92%, 92% ee.

Further, when cyano ester **17**, was subjected to Co-catalyzed asymmetric reduction with $NaBH_4$-bisoxazoline **6** at 25 °C, the corresponding chiral δ-lactam **18**, an important intermediate for (−)-paroxetine,[12] was obtained in 99% yield and 86% ee (Scheme 3). The cyano ester **17** was in turn prepared from the corresponding bromoester **5c** by displacement with cyanide using NaCN in dry DMF at 25 °C in 81% yield.



Scheme 3. Synthesis of intermediate **18** for (−)-paroxetine.

3. Conclusion

In conclusion, we have developed a new one-step procedure in which suitably substituted azido and cyano functions of α,β-unsaturated esters undergo reductive cyclization with $NaBH_4$ in the presence of a catalytic amount of $CoCl_2$ affording γ and δ-lactams in high yields. The methodology has been successfully applied to an efficient, enantioselective synthesis of (*R*)-baclofen, (*R*)-rolipram, and (*R*)-4-fluorophenylpiperidinone, a key intermediate for (−)-paroxetine.

4. Experimental

4.1. General information

Solvents were purified and dried by standard procedures before use; petroleum ether of boiling range 60–80 °C was used. Melting points are uncorrected. HPLC analyses were performed on a chiral column (Chiralpak®). Optical rotations were measured on a Jasco DIP 181 digital polarimeter. Infrared spectra were recorded on a Shimadzu FTIR-8400 spectrometer. ^1H NMR and ^{13}C NMR were recorded on Bruker AC-200 and MSL-300 NMR spectrometers, respectively. Mass spectra were obtained with a Finnigan MAT-1020 B-70 eV mass spectrometer. Elemental analysis was carried on a Carlo Erba CHNS-O analyzer.

4.2. General experimental procedure for the synthesis of α,β-unsaturated esters from aryl boronic acids (arylation route)

A 25 ml flask with reflux condenser attached was charged with boronic acid **3** (1 mmol), ethyl crotonate (0.228 g, 0.25 ml, 2 mmol), Na_2CO_3 (0.159 g, 1.5 mmol), catalytic amount of $Pd(OAc)_2$ (0.022 g, 0.1 mmol), and DMF (5 ml) under oxygen (1 atm). The flask was heated at 50 °C for 12 h. After completion of the reaction (monitored by TLC), the reaction mixture was allowed to cool to 25 °C. The organic layer was separated and the aqueous layer was extracted with EtOAc (2×10 ml). The combined organic extracts were washed with water (5 ml) followed by brine (10 ml) and concentrated under reduced pressure to give the crude product. This was then purified by column chromatography eluting with 10% ethyl acetate in petroleum ether to get the olefinic esters **4**.

4.3. General experimental procedure for the synthesis of α,β-unsaturated esters from ketones (Reformatsky route)

A 100 ml two-necked RB flask was charged with activated zinc (3.40 g, 44 mmol), and kept under an N_2 atmosphere. Dry benzene (30 ml) was introduced and the reaction mixture was heated to 80 °C (oil bath temp). A solution of ethyl bromoacetate (7.25 g, 44 mmol) and ketone (40 mmol) in dry benzene (20 ml) was added dropwise to the reaction mixture. After completion of the addition, the resulting reaction mixture was refluxed for 6 h, cooled to 25 °C, and quenched by adding ice-cold 4 M H_2SO_4 (30 ml). The crude hydroxyester was extracted with diethyl ether, evaporated under reduced pressure, and then was subjected to dehydration using Dean–Stark apparatus with *p*-toluenesulphonic acid (0.7 g, 3.68 mmol) in toluene at reflux. Water generated during the dehydration was separated azeotropically and

then toluene was distilled off. The crude olefinic ester **4** was purified by column chromatography packed with silica gel, eluting with EtOAc/petroleum ether (1:10) to give **4**.

4.3.1. (*E*)-Ethyl 3-phenylbut-2-enoate (4a). Yield: 80%; gum; IR ($CHCl_3$, cm^{-1}): 428, 694, 756, 871, 950, 1000, 1026, 1045, 1076, 1170, 1215, 1242, 1272, 1344, 1365, 1377, 1448, 1492, 1575, 1600, 1627, 1656, 1710, 1959, 2360, 2979, 3031, 3058, 3028, 3303, 3398; ^1H NMR (500 MHz, $CDCl_3$): δ 1.31 (t, J=7.4 Hz, 3H), 2.58 (s, 3H), 4.20 (q, J=7.4 Hz, 2H), 6.11 (s, 1H), 7.33–7.54 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.10, 18.47, 59.29, 116.99, 121.71, 125.96, 127.96, 128.18, 132.05, 154.92, 166.14; MS *m*/*z* (% rel intensity): 190 (70), 175 (5), 161 (50), 145 (100), 131 (5), 115 (90), 102 (10), 91 (40), 77 (20), 57 (20), 51 (30); Analysis: $C_{12}H_{14}O_2$ requires C, 75.76; H, 7.42; found C, 75.70; H, 7.36%.

4.3.2. (*E*)-Ethyl 3-(4-chlorophenyl)but-2-enoate (4b). Yield: 82%; viscous liquid; IR ($CHCl_3$, cm^{-1}): 840, 887, 1056, 1109, 1182, 1288, 1493, 1578, 1594, 1641, 1725, 1915, 2116, 3001; ^1H NMR (200 MHz, $CDCl_3$): δ 1.31 (t, J=7.0 Hz, 3H), 2.54 (s, 3H), 4.19 (q, J=8.0 Hz, 2H), 6.08 (s, 1H), 7.29 (d, J=8.7 Hz, 2H), 7.37 (d, J=9.0 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.29, 17.60, 59.58, 117.62, 127.47, 128.61, 134.93, 140.55, 153.57, 165.95; MS *m*/*z* (% rel intensity): 224 (M^+, 96), 209 (8), 195 (55), 179 (100), 152 (32), 115 (92); Analysis: $C_{12}H_{13}ClO_2$ requires C, 64.15; H, 5.83; found C, 64.12; H, 5.80%.

4.3.3. (*E*)-Ethyl 3-(4-fluorophenyl)but-2-enoate (4c). Yield: 90%; yellow viscous liquid; IR ($CHCl_3$, cm^{-1}): 696, 770, 878, 1045, 1175, 1280, 1345, 1366, 1445, 1577, 1620, 1710, 2988, 3062; ^1H NMR (200 MHz, $CDCl_3$): δ 1.33 (t, J=7.0 Hz, 3H), 2.56 (s, 3H), 4.22 (q, J=7.0 Hz, 2H), 6.09 (s, 1H), 7.05 (d, J=8.6 Hz, 2H), 7.45 (d, J=9.0 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.25, 17.56, 59.52, 116.21, 118.13, 127.73, 130.66, 138.52, 153.47, 162.43, 165.78; Analysis: $C_{12}H_{13}FO_2$ requires C, 69.22; H, 6.29; found C, 69.12; H, 6.21%.

4.3.4. (*E*)-Ethyl 3-(2-methoxyphenyl)but-2-enoate (4d). Yield: 80%; gum; IR ($CHCl_3$, cm^{-1}): 754, 806, 883, 1026, 1112, 1163, 1228, 1247, 1274, 1369, 1434, 1461, 1490, 1579, 1598, 1639, 1712, 1726, 2837, 2937, 2977, 3072, 3452; ^1H NMR (500 MHz, $CDCl_3$): δ 1.21 (t, J=7.7 Hz, 3H), 2.39 (s, 3H), 3.72 (s, 3H), 4.10 (q, J=7.7 Hz, 2H), 5.77 (s, 1H), 6.77 (d, J=8.3 Hz, 1H), 6.81 (t, J=7.3 Hz, 1H), 7.02 (d, J=7.8 Hz, 1H), 7.16 (d, J=7.8 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.90, 19.38, 54.90, 59.16, 110.73, 118.88, 120.19, 128.35, 129.09, 132.73, 156.02, 166.14; Analysis: $C_{13}H_{16}O_3$ requires C, 70.89; H, 7.32; found C, 70.80; H, 7.30%.

4.3.5. (*E*)-Ethyl 3-(4-methoxyphenyl)but-2-enoate (4e). Yield: 93%; gum; IR ($CHCl_3$, cm^{-1}): 589, 668, 701, 759, 840, 928, 978, 1018, 1040, 1083, 1123, 1184, 1215, 1272, 1411, 1509, 1572, 1606, 1672, 1695, 1736, 2400, 3019, 3617; ^1H NMR (500 MHz, $CDCl_3$): δ 1.22 (t, J=7.0 Hz, 3H), 2.47 (s, 3H), 3.72 (s, 3H), 4.11 (q, J=7.0 Hz, 2H), 6.02 (s, 1H), 6.79 (d, J=8.7 Hz, 2H), 7.35 (d, J=8.7 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.13, 17.34, 54.99, 59.40, 113.65, 115.13, 127.41, 134.08, 154.52, 160.31, 166.73; MS *m*/*z* (% rel intensity): 220 (90), 205 (3), 191 (10), 175 (100), 161 (5), 148 (90), 131 (15), 115 (20), 103 (15), 91 (25), 77 (20), 63 (10), 51 (12); Analysis: $C_{13}H_{16}O_3$ requires C, 70.89; H, 7.32; found C, 70.72; H, 7.26%.

4.3.6. (*E*)-Ethyl 3-(3-(cyclopentyloxy)-4-methoxyphenyl)but-2-enoate (4f). Yield: 88%; colorless liquid; ^1H NMR (200 MHz, $CDCl_3$): δ 1.32 (t, J=7.1 Hz, 3H), 1.61–1.64 (m, 2H), 1.85–1.92 (m, 6H), 2.55 (s, 3H), 3.84 (s, 3H), 4.18 (q, J=6.0 Hz, 2H), 4.79–4.80 (m, 1H), 6.02 (s, 1H), 6.78 (d, J=10.0 Hz, 1H), 6.96 (s, 1H), 7.00 (d, J=3.7 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.27, 17.67, 23.93, 32.74, 55.94, 59.60, 80.63, 111.51, 113.31, 115.37, 119.22, 134.59, 147.36, 151.13, 155.04, 166.93; Analysis: $C_{18}H_{24}O_4$ requires C, 71.03; H, 7.95; found C, 71.00; H, 7.90%.

4.3.7. (*E*)-Ethyl 3,4,4-trimethylpent-2-enoate (4g). Yield: 82%; colorless liquid; IR ($CHCl_3$, cm^{-1}): 740, 870, 965, 1035, 1163, 1200, 1272, 1340, 1465, 1635, 1732, 2907; ^1H NMR (200 MHz, $CDCl_3$): δ 1.10 (s, 9H), 1.26 (t, J=7.0 Hz, 3H), 2.14 (s, 3H), 4.10 (q, J=7.0 Hz, 2H), 5.68 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.92, 28.08, 37.34, 58.66, 115.66, 166.10; Analysis: $C_{10}H_{18}O_2$ requires C, 70.55; H, 10.66; found C, 70.52; H, 10.52%.

4.4. General experimental procedure for the synthesis of γ-bromo-α,β-unsaturated esters (5a–g) (allylic bromination)

A solution of α,β-unsaturated ester **4** (15.62 mmol), NBS (2.81 g, 17.2 mmol), and AIBN (0.102 g, 0.62 mmol) in dry CCl_4 (35 ml) was refluxed under nitrogen atmosphere for 10 h. The resulting reaction mixture was cooled to room temperature and then filtered through a sintered funnel to separate succinimide formed during the reaction. The filtrate was concentrated under reduced pressure to obtain bromoester **5**. It was then purified by column chromatography packed with silica gel to give pure bromoester **5**, as a pale yellow gum.

4.4.1. (*Z*)-Ethyl 4-bromo-3-phenylbut-2-enoate (5a). Yield: 72%; gum; IR ($CHCl_3$, cm^{-1}): 447, 460, 696, 767, 881, 960, 1020, 1047, 1095, 1176, 1218, 1286, 1344, 1367, 1448, 1492, 1577, 1596, 1623, 1710, 1766, 1890, 1955, 2345, 2935, 2979, 3058, 3537; ^1H NMR (500 MHz, $CDCl_3$): δ 1.33 (t, J=7.4 Hz, 3H), 4.25 (q, J=7.4 Hz, 2H), 4.95 (s, 2H), 6.18 (s, 1H), 7.38–7.53 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.04, 26.34, 60.24, 119.55, 126.36, 128.53, 129.47, 138.19, 152.90, 165.18; Analysis: $C_{12}H_{13}BrO_2$ requires C, 53.55; H, 4.87; found C, 53.63; H, 4.81%.

4.4.2. (*Z*)-Ethyl 4-bromo-3-(4-chlorophenyl)but-2-enoate (5b). Yield: 88%; yellow colored liquid; IR ($CHCl_3$, cm^{-1}): 649, 734, 908, 1012, 1095, 1182, 1288, 1340, 1369, 1490, 1625, 1710, 2982, 3060; ^1H NMR (200 MHz, $CDCl_3$): δ 1.34 (t, J=7.0 Hz, 3H), 4.26 (q, J=8.0 Hz, 2H), 4.94 (s, 2H), 6.19 (s, 1H), 7.36 (d, J=9.0 Hz, 2H), 7.47 (d, J=9.0 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.14,

26.13, 60.57, 120.01, 127.87, 128.94, 135.74, 136.77, 151.80, 165.22; MS *m/z* (% rel intensity): 304 (M^+, 5), 289 (5), 224 (8), 179 (10), 152 (100), 137 (55), 115 (92), 101 (48), 91 (22), 75 (15); Analysis: $C_{12}H_{12}BrClO_2$ requires C, 47.48; H, 3.98; found C, 47.50; H, 3.83%.

4.4.3. (*Z*)-Ethyl 4-bromo-3-(4-fluorophenyl)but-2-enoate (5c). Yield: 84%; gum; IR ($CHCl_3$, cm^{-1}): 742, 912, 1214, 1090, 1185, 1283, 1332, 1363, 1495, 1622, 1715, 2980, 3055; ^1H NMR (200 MHz, $CDCl_3$): δ 1.32 (t, J=7.0 Hz, 3H), 4.25 (q, J=7.0 Hz, 2H), 4.94 (s, 2H), 6.14 (s, 1H), 7.0 (d, J=8.6 Hz, 2H), 7.52 (d, J=9.0 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.86, 25.97, 60.03, 115.24, 119.36, 128.30, 130.41, 134.19, 151.56, 164.62; Analysis: $C_{12}H_{12}BrFO_2$ requires C, 50.20; H, 4.21; found C, 50.16; H, 4.20%.

4.4.4. (*Z*)-Ethyl 4-bromo-3-(2-methoxyphenyl)but-2-enoate (5d). Yield: 80%; gum; IR ($CHCl_3$, cm^{-1}): 682, 754, 806, 883, 933, 950, 1022, 1041, 1112, 1176, 1240, 1269, 1298, 1340, 1367, 1434, 1461, 1488, 1579, 1598, 1631, 1710, 2042, 2837, 2937, 2977, 3060; ^1H NMR (200 MHz, $CDCl_3$): δ 1.33 (t, J=7.4 Hz, 3H), 3.84 (s, 3H), 4.26 (q, J=7.0 Hz, 2H), 5.03 (s, 2H), 5.95 (s, 1H), 6.92 (d, J=8.2 Hz, 1H), 6.99 (t, J=7.4 Hz, 1H), 7.21–7.27 (m, 1H), 7.33–7.41 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.89, 28.26, 55.09, 59.98, 110.52, 120.34, 121.66, 128.09, 130.08, 130.30, 153.82, 156.03, 164.96; Analysis: $C_{13}H_{15}BrO_3$ requires C, 52.19; H, 5.05; found C, 52.20; H, 5.10%.

4.4.5. (*Z*)-Ethyl 4-bromo-3-(4-methoxyphenyl)but-2-enoate (5e). Yield: 86%; gum; IR ($CHCl_3$, cm^{-1}): 412, 428, 667, 757, 810, 833, 879, 950, 1024, 1031, 1047, 1095, 1174, 1217, 1253, 1288, 1342, 1369, 1438, 1461, 1514, 1573, 1604, 1708, 1890, 2057, 2430, 2549, 2839, 2904, 2937, 2981, 3014, 3398, 3568; ^1H NMR (500 MHz, $CDCl_3$): δ 1.23 (t, J=7.2 Hz, 3H), 3.73 (s, 3H), 4.14 (q, J=7.0 Hz, 2H), 4.86 (s, 2H), 6.05 (s, 1H), 6.80 (d, J=8.9 Hz, 2H), 7.40 (d, J=8.9 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.93, 26.07, 54.98, 59.97, 113.86, 117.25, 127.67, 152.16, 160.67, 165.28; MS *m/z* (% rel intensity): 300 (10), 298 (10), 254 (60), 252 (60), 239 (3), 228 (5), 219 (40), 191 (60), 174 (8), 163 (10), 145 (100), 131 (15), 115 (12), 103 (60), 91 (10), 77 (70), 63 (30), 40 (80); Analysis: $C_{13}H_{15}BrO_3$ requires C, 52.19; H, 5.05; found C, 52.13; H, 5.11%.

4.4.6. (*Z*)-Ethyl 4-bromo-3-(3-(cyclopentyloxy)-4-methoxyphenyl)but-2-enoate (5f). Yield: 90%; crystalline solid; mp: 167–169 °C (recrystallized from petroleum ether); IR ($CHCl_3$, cm^{-1}): 666, 756, 808, 858, 990, 1024, 1095, 1164, 1218, 1256, 1299, 1348, 1367, 1440, 1513, 1578, 1597, 1619, 1708, 2038, 2572, 2872, 2961, 3331; ^1H NMR (200 MHz, $CDCl_3$): δ 1.37 (t, J=8.0 Hz, 3H), 1.66 (m, 2H), 1.89–1.95 (m, 6H), 3.89 (s, 3H), 4.27 (q, J=6.0 Hz, 2H), 4.84 (s, 1H), 4.96 (s, 2H), 6.15 (s, 1H), 6.86 (d, J=3.0 Hz, 1H), 7.11 (s, 1H), 7.13 (d, J=4.0 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.34, 24.14, 26.33, 32.86, 55.86, 60.27, 80.53, 111.59, 113.27, 117.72, 119.48, 130.83, 147.68, 151.67, 152.86, 165.53; Analysis: $C_{18}H_{23}BrO_4$ requires C, 56.41; H, 6.05; found C, 56.33; H, 6.00%.

4.4.7. (*Z*)-Ethyl 3-(bromomethyl)-4,4-dimethylpent-2-enoate (5g). Yield: 76%; IR ($CHCl_3$, cm^{-1}): 412, 430, 460, 688, 707, 730, 745, 877, 962, 1033, 1095, 1155, 1193, 1267, 1338, 1371, 1469, 1631, 1720, 1755, 2358, 2873, 2908, 2968, 3417; ^1H NMR (200 MHz, $CDCl_3$): δ 1.19 (s, 9H), 1.28 (t, J=7.0 Hz, 3H), 4.15 (q, J=7.0 Hz, 2H), 4.53 (s, 2H), 5.87 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.96, 24.18, 28.96, 37.60, 59.83, 117.73, 163.56; MS (*m/z*, % rel intensity): 248 (5), 235 (3), 205 (10), 189 (3), 169 (5), 155 (50), 141 (10), 127 (30), 123 (20), 109 (15), 95 (45), 79 (20), 57 (50), 41 (100); Analysis: $C_{10}H_{17}BrO_2$ requires C, 48.21; H, 6.88; found C, 48.13; H, 6.73%.

4.5. General experimental procedure for the synthesis of γ-azido-α,β-unsaturated esters (1a–g) (azidation)

A solution of bromoester **5** (6.1 mmol) and sodium azide (0.594 g, 9.14 mmol) in ethanol/water (80:20, 15 ml) mixture was taken in 50 ml RB and refluxed for 8 h. The resulting yellow solution was concentrated under reduced pressure to yield crude azido ester **1**, which was purified by column chromatography packed with silica gel to give pure azido ester **1** as colorless viscous liquid.

4.5.1. (*Z*)-Ethyl 4-azido-3-phenylbut-2-enoate (1a). Yield: 86%; gum; IR ($CHCl_3$, cm^{-1}): 443, 669, 698, 757, 1035, 1182, 1215, 1371, 1448, 1710, 1845, 2104, 2343, 2360, 3018, 3421; ^1H NMR (500 MHz, $CDCl_3$): δ 1.25 (t, J=7.4 Hz, 3H), 4.17 (q, J=7.4 Hz, 2H), 4.69 (s, 2H), 6.25 (s, 1H), 7.33–7.43 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.73, 47.79, 60.12, 120.40, 126.29, 128.39, 129.28, 138.28, 150.16, 165.23; Analysis: $C_{12}H_{13}N_3O_2$ requires C, 62.33; H, 5.67; N, 18.17; found C, 62.28; H, 5.65; N, 18.09%.

4.5.2. (*Z*)-Ethyl 4-azido-3-(4-chlorophenyl)but-2-enoate (1b). Yield: 77%; colorless liquid; IR ($CHCl_3$, cm^{-1}): 415, 427, 435, 457, 478, 1179, 1350, 1591, 1713, 2101, 2982; ^1H NMR (200 MHz, $CDCl_3$): δ 1.32 (t, J=7.0 Hz, 3H), 4.24 (q, J=8.0 Hz, 2H), 4.74 (s, 2H), 6.30 (s, 1H), 7.34 (d, J=8.0 Hz, 2H), 7.43 (d, J=8.0 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.96, 48.03, 60.53, 121.04, 127.87, 128.87, 135.67, 136.95, 149.19, 165.33; Analysis: $C_{12}H_{12}ClN_3O_2$ requires C, 54.25; H, 4.55; N, 15.82; found C, 54.12; H, 4.48; N, 15.80%.

4.5.3. (*Z*)-Ethyl 4-azido-3-(4-fluorophenyl)but-2-enoate (1c). Yield: 80%; gum; IR ($CHCl_3$, cm^{-1}): 730, 830, 910, 1014, 1090, 1185, 1250, 1372, 1453, 1495, 1590, 1630, 1710, 2104, 2991; ^1H NMR (200 MHz, $CDCl_3$): δ 1.33 (t, J=7.0 Hz, 3H), 4.22 (q, J=7.0 Hz, 2H), 4.72 (s, 2H), 6.29 (s, 1H), 7.09 (d, J=8.8 Hz, 2H), 7.54 (d, J=8.8 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.13, 47.86, 61.13, 115.31, 119.42, 128.27, 131.63, 134.21, 151.54, 164.75; Analysis: $C_{12}H_{12}FN_3O_2$ requires C, 57.83; H, 4.85; N, 16.86; found C, 57.82; H, 4.88; N, 16.80%.

4.5.4. (*Z*)-Ethyl 4-azido-3-(2-methoxyphenyl)but-2-enoate (1d). Yield: 90%; gum; IR ($CHCl_3$, cm^{-1}): 650, 755, 821, 836, 887, 941, 1030, 1094, 1120, 1177, 1250, 1293, 1371, 1425, 1478, 1515, 1581, 1611, 1703, 1888, 2100, 2548, 2830, 2900, 2930, 2984, 3337; ^1H NMR (200 MHz, $CDCl_3$): δ 1.32 (t, J=7.0 Hz, 3H), 3.85 (s, 3H),

4.22 (q, J=7.0 Hz, 2H), 4.79 (s, 2H), 6.00 (s, 1H), 6.86–6.99 (m, 2H), 7.17–7.38 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.03, 49.98, 55.24, 60.28, 110.56, 120.74, 121.88, 128.53, 129.67, 130.34, 152.83, 156.40, 165.55; Analysis: $C_{13}H_{15}N_3O_3$ requires C, 59.76; H, 5.79; N, 16.08; found C, 59.75; H, 5.80; N, 16.10%.

4.5.5. (*Z*)-Ethyl 4-azido-3-(4-methoxyphenyl)but-2-enoate (1e). Yield: 95%; gum; IR ($CHCl_3$, cm^{-1}): 666, 758, 810, 834, 885, 937, 1029, 1096, 1115, 1174, 1254, 1291, 1369, 1421, 1462, 1513, 1574, 1604, 1712, 1891, 2101, 2550, 2831, 2906, 2937, 2981, 3332; ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.33 (t, J=7.2 Hz, 3H), 3.83 (s, 3H), 4.24 (q, J=7.2 Hz, 2H), 4.77 (s, 2H), 6.31 (s, 1H), 6.93 (d, J=8.6 Hz, 2H), 7.49 (d, J=8.6 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.96, 47.56, 55.06, 60.20, 113.98, 118.39, 127.87, 130.34, 149.63, 160.77, 165.77; MS *m*/*z* (% rel intensity): 261 (10), 253 (10), 225 (5), 219 (100), 204 (5), 191 (70), 173 (5), 163 (80), 145 (30), 131 (35), 115 (15), 103 (40), 83 (30), 77 (35), 55 (40), 40 (55); Analysis: $C_{13}H_{15}N_3O_3$ requires C, 59.76; H, 5.79; N, 16.08; found C, 59.63; H, 5.72; N, 16.10%.

4.5.6. (*Z*)-Ethyl 4-azido-3-(3-(cyclopentyloxy)-4-methoxyphenyl)but-2-enoate (1f). Yield: 99%; light yellow liquid; IR ($CHCl_3$, cm^{-1}): 667, 756, 1045, 1166, 1216, 1253, 1373, 1442, 1514, 1598, 1734, 2104, 2966, 3019, 3403; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.23 (t, J=6.0 Hz, 3H), 1.52 (m, 2H), 1.72–1.85 (m, 6H), 3.76 (s, 3H), 4.14 (q, J=6.0 Hz, 2H), 4.65 (s, 2H), 4.72 (s, 1H), 6.18 (s, 1H), 6.76 (d, J=3.0 Hz, 1H), 6.96 (s, 1H), 7.00 (d, J=4.0 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.72, 23.59, 32.34, 47.48, 55.40, 59.95, 80.25, 111.37, 113.07, 118.21, 119.36, 130.47, 147.30, 149.70, 151.42, 165.43; Analysis: $C_{18}H_{23}N_3O_4$ requires C, 62.59; H, 6.71; N, 12.17; found C, 62.63; H, 6.54; N, 12.10%.

4.5.7. (*Z*)-Ethyl 3-(azidomethyl)-4,4-dimethylpent-2-enoate (1g). Yield: 91%; colorless gum; IR ($CHCl_3$, cm^{-1}): 680, 710, 734, 875, 971, 1034, 1100, 1160, 1269, 1340, 1376, 1630, 1735, 2109, 2968; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.14 (s, 9H), 1.29 (t, J=7.0 Hz, 3H), 4.17 (q, J=7.0 Hz, 2H), 4.27 (s, 2H), 6.00 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.95, 24.12, 29.00, 36.55, 59.75, 117.52, 163.00; Analysis: $C_{10}H_{17}N_3O_2$ requires C, 56.85; H, 8.11; N, 19.89; found C, 56.73; H, 8.10; N, 19.90%.

4.5.8. Preparation of (*E*)-ethyl 4-cyano-3-(4-fluorophenyl)but-2-enoate (17) (cyanation). In a 25 ml flask were added γ-bromoester **5c** (2.87 g, 10 mmol), NaCN (0.735 g, 15 mmol), and dry DMF (20 ml) under an argon atmosphere. The reaction mixture was heated at 50 °C for 6 h (monitored by TLC). After completion of the reaction it was diluted with water (5 ml) and extracted with EtOAc (4×15 ml); the combined organic extracts were washed with brine (10 ml), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give the crude product. This was further purified by column chromatography on silica gel using EtOAc/petroleum ether (2:8) as eluent to afford cyano ester **17** (1.88 g, 81%).

Yield: 81%; yellow colored liquid; IR ($CHCl_3$, cm^{-1}): 827, 1027, 1103, 1164, 1240, 1317, 1369, 1427, 1512, 1602, 1735, 2216, 2360, 2983, 3066; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.20 (t, J=8.0 Hz, 3H), 3.88 (s, 2H), 4.14 (q, J=8.0 Hz, 2H), 5.77 (s, 1H), 7.41 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.14, 39.32, 61.38, 99.20, 116.48, 126.11, 128.98, 130.41, 137.04, 155.63, 168.27; MS *m*/*z* (% rel intensity): 233 (M^+, 25), 218 (5), 205 (8), 188 (10), 174 (7), 161 (100), 133 (55), 121 (8), 96 (40), 75 (20), 57 (15); Analysis: $C_{13}H_{12}FNO_2$ requires C, 66.94; H, 5.19; N, 6.01; found C, 67.00; H, 5.20; N, 6.00%.

4.6. General experimental procedure for enantioselective synthesis of 4-substituted pyrrolidin-2-ones (2a–g) (asymmetric reduction)

To azido ester **1** (10 mmol) in a 25 ml RB flask was added a solution of $CoCl_2\cdot 6H_2O$ (0.021 g, 0.1 mmol) in ethanol (2 ml), followed by a solution of (4*S*)-(+)-phenyl-α-[(4*S*)-phenyloxazolidin-2-ylidine]-2-oxazoline-2-acetonitrile **6** (0.039 g, 0.12 mmol) in EtOH (1 ml) under nitrogen atmosphere. After dilution with DMF (1 ml), the clear, dark blue solution was degassed by three freeze–thaw cycles. To this mixture, which was kept under nitrogen, was added sodium borohydride solution (1.514 g, 40 mmol) in DMF (1 ml), which resulted in an instantaneous color change to yellow. The slightly foaming solution was immediately degassed by three freeze–thaw cycles. The evacuated flask containing the yellow, slightly turbid solution was stirred at 25 °C. In the beginning, slow H_2-evolution was observed, which gradually ceased after 1 h. Toward the end of the reaction, solid precipitate along with a brown-yellow foam began to form. After completion of the reaction (monitored by TLC), the reaction mixture was transferred to a separatory funnel containing 50 ml of EtOAc and 75 ml of water, and extracted with EtOAc. The organic layer was washed three times with brine solution, dried over anhydrous Na_2SO_4, and concentrated in vacuum. Column chromatographic purification (EtOAc/petroleum ether 4:1) afforded pyrrolidin-2-one **2** as solid.

4.6.1. (*R*)-4-Phenylpyrrolidin-2-one (2a). Yield: 86%; colorless solid; mp: 112 °C; $[\alpha]_D^{25}$ −18.90 (*c* 0.91, $CHCl_3$); HPLC: 92% ee, Chiralpak®, λ=210 nm, 10% EtOH/hexane, 1 ml/min, retention time: (*R*) 9.5 min; IR ($CHCl_3$, cm^{-1}): 665, 703, 756, 1092, 1218, 1373, 1490, 1697, 2100, 3211, 3422; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.35–2.47 (dd, J=16.0, 8.0 Hz, 1H), 2.65–2.77 (dd, J=16.0, 8.0 Hz, 1H), 3.32–3.41 (m, 1H), 3.60–3.82 (m, 2H), 7.25–7.36 (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 38.22, 39.51, 49.43, 126.28, 128.40, 132.00, 136.65, 175.53; Analysis: $C_{10}H_{11}NO$ requires C, 64.60; H, 6.19; N, 10.76; found C, 64.53; H, 6.11; N, 10.69%.

4.6.2. (*R*)-4-(4-Chlorophenyl)pyrrolidin-2-one (2b). Yield: 84%; colorless solid; mp: 115–117 °C; $[\alpha]_D^{25}$: –34.82 (*c* 1.0, EtOH), 89% ee, {Lit.[4,12] $[\alpha]_D^{25}$: −39 (*c* 1.0, EtOH)}; HPLC: 92% ee, Chiralpak®, λ=210 nm, 10% EtOH/hexane, 1 ml/min, retention time: (*R*) 9.8 min; IR ($CHCl_3$, cm^{-1}): 486, 550, 625, 676, 829, 1015,1106, 1168, 1273, 1297, 1410, 1460, 1488, 1666, 1763, 1911, 2228, 2840, 2898, 2952, 3106, 3197, 3443; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.39–2.51 (dd, J=16.9, 8.4 Hz, 1H), 2.68–2.81 (dd, J=16.9, 8.7 Hz, 1H), 3.35–3.43 (m, 1H), 3.62–3.84

(m, 2H), 7.18 (d, J=9.1 Hz, 2H), 7.31 (d, J=9.1 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 38.22, 39.51, 49.43, 128.02, 128.83, 132.72, 140.59, 178.01; MS m/z (% rel intensity): 195 (M^+, 15), 140 (24), 138 (100), 75 (5); Analysis: $C_{10}H_{10}ClNO$, requires C, 61.39; H, 5.15; N, 7.16; found: C, 61.30; H, 5.10; N, 7.19%.

4.6.3. 4-(4-Fluorophenyl)pyrrolidin-2-one (2c). Yield: 80%; colorless solid; mp: 98–99 °C; $[\alpha]_D^{25}$: −27.54 (c 1.12, MeOH); IR ($CHCl_3$, cm^{-1}): 669, 704, 760, 1095, 1210, 1375, 1493, 1695, 2105, 3209, 3417; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.53–2.69 (m, 2H), 3.37–3.46 (m, 1H), 3.61–3.82 (m, 2H), 7.06 (d, J=8.6 Hz, 2H), 7.51 (d, J=9.0 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 38.12, 39.68, 49.51, 115.31, 128.74, 134.14, 151.22, 178.00; Analysis: $C_{10}H_{10}FNO$, requires C, 67.03; H, 5.62; N, 7.82; found: C, 67.00; H, 5.58; N, 7.79%.

4.6.4. 4-(2-Methoxyphenyl)pyrrolidin-2-one (2d). Yield: 91%; colorless solid; mp: 118 °C; $[\alpha]_D^{25}$: −27.62 (c 1.10, MeOH); IR ($CHCl_3$, cm^{-1}): 664, 673, 685, 731, 1046, 1250, 1441, 1542, 1694, 2405, 2977; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.56–2.60 (d, J=8.0 Hz, 2H), 3.40–3.46 (m, 1H), 3.69–3.77 (m, 2H), 6.84–6.95 (m, 2H), 7.17–7.25 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 35.17, 48.22, 55.17, 110.49, 117.84, 120.67, 127.36, 128.06, 129.89, 157.21, 175.00; Analysis: $C_{11}H_{13}NO_2$ requires C, 69.09; H, 6.85; N, 7.32; found C, 69.10; H, 6.80; N, 7.20%.

4.6.5. (*R*)-4-(4-Methoxyphenyl)pyrrolidin-2-one (2e). Yield: 93%; colorless solid; mp: 121–123 °C; $[\alpha]_D^{25}$: −21.00 (c 1.00, MeOH); HPLC: 92% ee, Chiralpak®, λ=210 nm, 10% EtOH/hexane, 1 ml/min; IR ($CHCl_3$, cm^{-1}): 495, 516, 546, 564, 604, 641, 685, 772, 829, 884, 926, 1030, 1059, 1080, 1112, 1161, 1184, 1247, 1298, 1316, 1352, 1413, 1458, 1516, 1558, 1611, 1680, 1889, 1957, 2021, 2051, 2430, 2515, 2840, 2905, 2959, 3090, 3200; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.85 (s, 1H), 2.41–2.54 (d, J=7.2 Hz, 1H), 2.67–2.79 (d, J=8.6 Hz, 1H), 3.37–3.45 (m, 1H), 3.63–3.80 (m, 2H), 3.83, (s, 3H), 6.88 (d, J=8.6 Hz, 2H), 7.19 (d, J=8.6 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 38.44, 39.43, 49.80, 54.95, 113.98, 127.54, 134.05, 158.38, 178.19; Analysis: $C_{11}H_{13}NO_2$ requires C, 69.09; H, 6.85; N, 7.32; found C, 69.00; H, 6.72; N, 7.30%.

4.6.6. (*R*)-4-(3-(Cyclopentyloxy)-4-methoxyphenyl)pyrrolidin-2-one: (*R*)-(−)-rolipram (2f). Yield: 92%; colorless solid; mp: 133–134 °C (recrystallized from $CHCl_3$); $[\alpha]_D^{25}$ –27.80 (c 1.01, MeOH), 92% ee, {Lit.[12] $[\alpha]_D^{25}$: −30.2 (c 1.0, MeOH)}; HPLC: 92% ee, Chiralpak®, λ=210 nm, 10% EtOH/hexane, 1 ml/min, Retention time: (*R*) 10.3 min; IR ($CHCl_3$, cm^{-1}): 508, 601, 619, 641, 686, 772, 816, 877, 973, 1002, 1025, 1060, 1145, 1164, 1236, 1250, 1272, 1310, 1438, 1513, 1592, 1686, 1701, 1868, 2042, 2575, 2942, 3098, 3200; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.62 (m, 2H), 1.82–1.93 (m, 6H), 2.50 (d, J=8.7 Hz, 1H), 2.74 (d, J=8.7 Hz, 1H), 3.40 (t, J=8.0 Hz, 1H), 3.62 (q, J=8.7 Hz, 1H), 3.77 (t, J=9.0 Hz, 1H), 3.83 (s, 3H), 4.77 (m, 1H), 6.76 (s, 2H), 6.82 (s, 1H), 7.05 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 20.95, 23.95, 32.74, 38.20, 39.77, 49.97, 56.07, 80.54, 112.18, 113.77, 118.73, 134.34, 147.84, 149.13, 175.42, 178.61; MS (m/z, % rel intensity): 275 (10), 208 (5), 207 (80), 196 (5), 168 (5), 151 (10), 150 (100), 135 (15), 125 (20), 93 (15), 77 (10), 65 (15); Analysis: $C_{16}H_{21}NO_3$ requires C, 69.79; H, 7.69; N, 5.09; found C, 69.63; H, 7.67; N, 5.03%.

4.6.7. 4-*tert*-Butylpyrrolidin-2-one (2g). Yield: 77%; colorless solid; mp: 81 °C; $[\alpha]_D^{25}$: −6.17 (c 0.74, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.01 (s, 9H), 1.98–2.32 (m, 1H), 2.22–2.59 (m, 2H), 3.31–3.56 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 23.82, 33.46, 35.20, 41.18, 41.63, 176.30; Analysis: $C_8H_{15}NO$ requires C, 68.04; H, 10.71; N, 9.92; found C, 68.00; H, 10.72; N, 9.78%.

4.6.8. (*R*)-(−)-Baclofen hydrochloride (10). Lactam **2b** (0.170 g, 0.9 mmol) in 6 M HCl (4 ml) was heated at 100 °C for 16 h. The excess of water in the reaction mixture was removed under reduced pressure to obtain solid residue, which was triturated in isopropanol affording (*R*)-baclofen hydrochloride (**10**) as a colorless solid (0.164 g).

Yield: 73%; colorless solid; mp: 196–197 °C; $[\alpha]_D^{25}$: –1.76 (c 0.5, H_2O) 88% ee {Lit.[4b] $[\alpha]_D^{25}$: −2.00 (c 0.6, H_2O)}; IR ($CHCl_3$, cm^{-1}): 698, 704, 758, 1090, 1490, 1550, 1620, 2955, 2092, 3200; ^{1}H NMR (200 MHz, DMSO-d_6+$CDCl_3$): 2.51–2.71 (m, 2H), 3.42–3.65 (m, 2H), 4.15–4.21 (m, 1H), 7.01–7.21 (m, 4H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 37.57, 38.88, 43.00, 128.27, 129.62, 131.57, 138.95, 171.95; MS m/z (% rel intensity): 195 (10), 140 (61), 138 (100), 125 (6), 115 (10), 103 (45), 89 (9), 77 (29); Analysis: $C_{10}H_{13}Cl_2NO_2$ requires C, 48.02; H, 5.24; N, 5.60; found C, 48.24; H, 5.15; N, 5.49%.

4.6.9. Preparation of (*R*)-4-(4-fluorophenyl)piperidin-2-one (18). To a 1.01 g (4.33 mmol) of cyano ester **17** in a 15 ml RB flask was added a solution of $CoCl_2\cdot 6H_2O$ (0.009 g, 0.04 mmol) in ethanol (4 ml), followed by a solution of (4*S*)-(+)-phenyl-α-[(4*S*)-phenyloxazolidin-2-ylidine]-2-oxazoline-2-acetonitrile (**6**) (0.016 g, 0.048 mmol) in ethanol (2 ml) under a nitrogen balloon. After dilution with DMF (2 ml), the clear, dark blue solution was degassed by three freeze–thaw cycles. The solution, which was kept under nitrogen, was then added to sodium borohydride solution (0.64, 17 mmol) in DMF (2 ml) resulted in an instantaneous color change to yellow. The slightly foaming solution was immediately degassed by three freeze–thaw cycles. The evacuated flask containing the yellow, slightly turbid solution was stirred at room temperature. In the beginning, slow H_2-evolution was observed, which gradually ceased after 1 h. Toward the end of the reaction, solid precipitated and brown-yellow foam began to form. After completion of reaction (as monitored by TLC), the reaction mixture was transferred to a separatory funnel with 50 ml of EtOAc and 50 ml of water, diluted with 25 ml of ice water, and extracted with EtOAc. The organic layer was washed three times with brine solution, dried over anhydrous Na_2SO_4, and concentrated in vacuum. Column chromatographic purification (EtOAc/petroleum ether 4:1) afforded 0.828 g (99%) of piperidin-2-one **18** in 86% ee as a colorless solid.

Yield: 99%; crystalline solid; mp: 158 °C (recrystallized from $CHCl_3$); $[\alpha]_D^{25}$ +16.63 (c 1.00, $CHCl_3$), 86% ee, {Lit.[11] $[\alpha]_D^{25}$: +19.0 (c 1.02, $CHCl_3$)}; IR ($CHCl_3$, cm^{-1}):

627, 675, 832, 1013, 1102, 1159, 1275, 1300, 1414, 1458, 1476, 1685, 1756, 1910, 2231, 2842, 2895, 2950, 3100, 3201, 3440; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.78–1.95 (m, 1H), 2.02–2.10 (m, 1H), 2.40 (dd, J=17.6, 10.7 Hz, 1H), 2.64 (dd, J=17.6, 5.3 Hz, 1H), 3.00–3.31 (m, 1H), 3.37–3.43 (m, 2H), 7.11 (d, J=8.5 Hz, 3H), 7.27 (d, J=8.5 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 29.48, 37.90, 38.70, 41.20, 127.88, 128.91, 132.64, 141.98, 171.76; Analysis: $C_{11}H_{12}FNO$ requires C, 68.38; H, 6.26; N, 7.25; found C, 68.33; H, 6.20; N, 7.23%.

4.6.10. Preparation of 2-methoxyphenyl acetate (13). To a mixture of guaiacol **12** (2.48 g, 20 mmol) and acetic anhydride (40 ml) was added three drops of concd H_2SO_4 with vigorous stirring. The reaction mixture was heated at 100 °C for 6 h and then allowed to cool to 25 °C and stirring continued for 3 h (monitored by TLC). The organic layer was separated and the aqueous layer was extracted with ether (2×15 ml). The combined ethereal extracts were washed with water (15 ml) followed by brine (15 ml) and concentrated under reduced pressure to give crude product, which was further purified by column chromatography on silica gel using petroleum ether/EtOAc (9:1) as eluent to afford acetate **13** (3.22 g).

Yield: 97%; colorless liquid; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.27 (s, 3H), 3.78 (s, 3H), 6.89–7.14 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 20.28, 55.57, 112.32, 120.52, 122.62, 126.62, 151.03, 168.64; MS (*m*/*z*, % rel intensity): (M^+ 166, 5), 124 (100), 109 (90), 95 (8), 91 (4), 81 (60), 77 (18), 64 (15); Analysis: $C_9H_{10}O_3$ requires C, 65.05; H, 6.07; found C, 64.93; H, 6.11%.

4.6.11. Preparation of 5-bromo-2-methoxyphenyl acetate (14). A solution of guiacolic ester **13** (2.59 g, 15.62 mmol) and NBS (2.81 g, 17.2 mmol) in dry CH_3CN (35 ml) was heated at 60 °C under a nitrogen atmosphere for 10 h. The resulting reaction mixture was cooled to room temperature. The organic layer was separated and the aqueous layer was extracted with EtOAc (2×15 ml). The combined organic extracts were washed with a saturated solution of sodium sulfite (15 ml), water (15 ml) followed by brine (15 ml), and concentrated under reduced pressure to give crude product, which was further purified by column chromatography on silica gel using petroleum ether/EtOAc (9:1) as eluent to afford pure 3.75 g of pale yellow color gum, bromoester **14**.

Yield: 99%; pale yellow color gum; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.27 (s, 3H), 3.75 (s, 3H), 6.77 (d, J=10.0 Hz, 1H), 7.18 (d, J=3.0 Hz, 1H), 7.24–7.29 (d, J=10.0 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 20.21, 55.79, 111.66, 113.54, 125.78, 129.34, 140.19, 150.41, 168.23; MS (*m*/*z*, % rel intensity): (M^+ 244, 10), 204 (100), 187 (60), 173 (3), 161 (10), 143 (4), 123 (5), 108 (5), 94 (7), 79 (20), 71 (3), 63 (8); Analysis: $C_9H_9BrO_3$ requires C, 44.11; H, 3.70; found C, 44.00; H, 3.65%.

4.6.12. Preparation of 5-bromo-2-methoxyphenol (15). A 25 ml flask was charged with ester **14** (3.66 g, 15 mmol), 10% $NaHCO_3$ (2.5 g, 22 mmol), and MeOH (15 ml) under nitrogen atmosphere. The solution was allowed to reflux for 3 h. Then EtOAc (25 ml) was added to reaction mixture. The organic layer was separated and the aqueous layer was extracted with EtOAc (2×15 ml). The combined organic extracts were washed with water (15 ml) followed by brine (15 ml) and concentrated under reduced pressure to give crude product, which was further purified by column chromatography on silica gel using petroleum ether/EtOAc (3:1) as eluent to phenol **15** (2.85 g).

Yield: 95%; yellow solid; mp: 102–105 °C (recrystallized from EtOAc); ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.77 (s, 3H), 6.58 (d, J=10.0 Hz, 1H), 6.83–6.89 (d, J=10.0 Hz, 1H), 6.97 (d, J=2.0 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 56.05, 111.88, 113.39, 117.95, 122.76, 145.88, 146.58, 159.74; MS (*m*/*z*, % rel intensity): (M^+ 204, 20), 202 (20), 189 (20), 160 (20), 131 (5), 116 (3), 107 (7), 91 (5), 79 (900), 62 (100); Analysis: $C_7H_7BrO_2$ requires C, 41.41; H, 3.48; found C, 41.38; H, 3.52%.

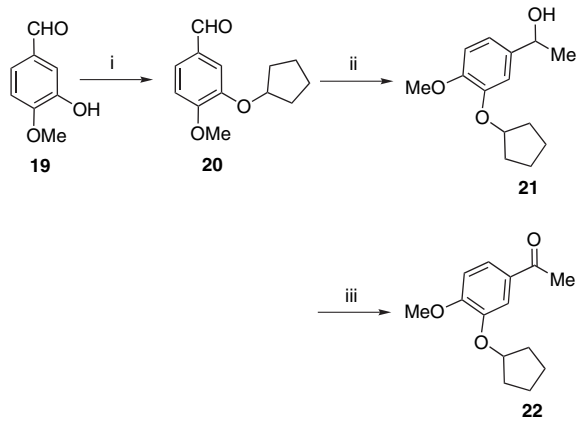
4.6.13. Preparation of 4-bromo-2-(cyclopentyloxy)-1-methoxybenzene (16). To a mixture of bromo phenol **15** (2.04 g, 10 mmol) and K_2CO_3 (1.2 g, 20 mmol) in DMF (40 ml) was added cyclopentyl bromide through a syringe at 50 °C with vigorous stirring. After completion of the reaction (monitored by TLC), the reaction mixture was allowed to cool to room temperature. The organic layer was separated and the aqueous layer was extracted with EtOAc (2×15 ml). The combined organic extracts were washed with water (15 ml) followed by brine (15 ml) and concentrated under reduced pressure to give crude product, which was further purified by column chromatography on silica gel using petroleum ether/EtOAc (9:1) as eluent to afford bromo ether **16** (2.42 g).

Yield: 89%; yellowish liquid; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.59–1.87 (m, 8H), 3.79 (s, 3H), 4.71 (m, 1H), 6.67 (d, J=10.0 Hz, 1H), 6.96–7.00 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 23.55, 32.27, 55.53, 80.20, 112.14, 112.91, 117.54, 122.80, 148.13, 148.97; MS (*m*/*z*, % rel intensity): (M^+ 272, 10), 270 (10), 202 (100), 187 (60), 173 (5), 159 (10), 142 (5), 123 (7), 108 (7), 94 (20), 79 (70), 63 (40); Analysis: $C_{12}H_{15}BrO_2$ requires C, 53.15; H, 5.58; found C, 53.10; H, 5.50%.

4.6.14. Preparation of 3-(cyclopentyloxy)-4-methoxyphenylboronic acid (3f). To a mixture of bromo ether **16** (4.56 g, 20 mmol) in diethyl ether (40 ml) was added dropwise through a syringe, 1 M *n*-BuLi solution in hexane (15 ml) at −78 °C with vigorous stirring. The reaction mixture was stirred for 1 h and then $B(OMe)_3$ (2 ml) was added. After stirring for 1 h, the reaction mixture was quenched with satd NH_4Cl solution (10 ml) and organic layer was separated and the aqueous layer was extracted with ether (2×15 ml). The combined ethereal extracts were washed with water (15 ml) followed by brine (15 ml) and concentrated under reduced pressure to give crude boronic acid **3f** (0.236 g), which was used, as it was for further reactions.

Yield: 15%; gray color solid; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.82 (m, 8H), 2.53 (br s, 2H), 3.73 (m, 1H), 3.95 (s, 3H), 7.02 (d, J=8.0 Hz, 1H), 7.71 (s, 1H), 7.81 (d, J=8.0 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 24.25, 33.01, 55.94, 80.59, 121.34, 129.66, 147.21, 153.90; Analysis: $C_{12}H_{17}BO_4$ requires C, 61.05; H, 7.26; found C, 61.10; H, 7.20%.

4.6.15. Synthetic strategy for preparation of 1-(3-(cyclopentyloxy)-4-methoxyphenyl)ethanone (22).



(i) Cyclopentyl bromide (1.5 equiv), K_2CO_3 (1.2 equiv), DMF, 60 °C, 12 h, 85%; (ii) MeMgBr (1.3 equiv), Et_2O, 0–25 °C, 1 h, 76%; (iii) $H_2Cr_2O_7$, Et_2O, 0–25 °C, 4 h, 75%.

4.6.16. Preparation of 3-(cyclopentyloxy)-4-methoxybenzaldehyde (20). To a mixture of isovaniline **19** (3.04 g, 20 mmol) and K_2CO_3 (1.76 g, 20 mmol) in DMF (40 ml) was added cyclopentyl bromide (3.73 g, 25 mmol) through a syringe at 50 °C with vigorous stirring. After completion of the reaction (monitored by TLC), the reaction mixture was allowed to cool to room temperature. The organic layer was separated and the aqueous layer was extracted with EtOAc (2×15 ml). The combined organic extracts were washed with water (15 ml) followed by brine (15 ml) and concentrated under reduced pressure to give crude product, which was further purified by column chromatography on silica gel using petroleum ether/EtOAc (9:1) as eluent to afford ether **20** (3.74 g).

Yield: 85%; yellow solid; mp: 107–110 °C (recrystallized from EtOAc); IR ($CHCl_3$, cm^{-1}): 426, 446, 452, 590, 742, 868, 902, 936, 968, 1022, 1238, 1362, 2038, 2360, 2606, 2720, 3078, 3354, 3624; ^1H NMR (200 MHz, $CDCl_3$): δ 1.63–2.00 (m, 8H), 3.93 (s, 3H), 4.86 (m, 1H), 6.65 (d, J=8.0 Hz, 1H), 7.41–7.45 (m, 2H), 9.84 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 23.66, 32.30, 55.64, 80.01, 110.45, 111.74, 125.85, 129.60, 147.80, 155.04, 190.47; MS (*m*/*z*, % rel intensity): (M^+ 220, 10), 177 (5), 151 (100), 137 (10), 122 (12), 108 (15), 95 (8), 79 (20), 65 (20); Analysis: $C_{13}H_{16}O_3$ requires C, 70.89; H, 7.32; found C, 70.63; H, 7.25%.

4.6.17. Preparation of 1-(3-(cyclopentyloxy)-4-methoxyphenyl)ethanol (21). A dry, argon flushed 100 ml round-bottom flask, equipped with a magnetic stirring (bar, reflux water condenser, and dropping funnel, was charged with 2–3 crystals of iodine, and magnesium turnings (0.675 g, 28 mmol) in dry diethyl ether (20 ml) at 25 °C. Then methyl iodide (3.8 g, 2.12 ml, 28 mmol) was added dropwise at 25 °C in diethyl ether. After stirring for 3–4 h, the dropping funnel was replaced by a rubber septum. The reaction flask was cooled in ice water, followed by slow addition of aldehyde **20** (5.0 g, 23 mol) in ether over a period of 10 min. Then it was allowed to stir over night. The reaction mixture was quenched with a saturated solution of NH_4Cl, poured into water (50 ml), and extracted with ether (3×50 ml). The combined organic fractions were washed with brine, then dried over Na_2SO_4, and concentrated under reduced pressure to afford crude alcohol **21**. The crude alcohol was purified by column chromatography packed with silica gel, eluting with petroleum ether/EtOAc (5:1) gave 4.12 g of **21**.

Yield: 76%; gray solid; mp: 123–125 °C (recrystallized from EtOAc+petroleum ether); ^1H NMR (200 MHz, $CDCl_3$): δ 1.43 (d, J=6.0 Hz, 3H), 1.86–1.99 (m, 8H), 3.81 (s, 3H), 4.75–4.84 (m, 2H), 6.76–6.92 (m, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.93, 23.85, 24.51, 24.91, 32.74, 56.23, 69.98, 74.10, 74.43, 80.49, 112.47, 113.46, 117.65, 118.57, 118.72, 137.28, 138.83, 147.98, 149.38; MS (*m*/*z*, % rel intensity): (M^+ 236, 20), 218 (5), 196 (5), 168 (40), 153 (100), 135 (10), 125 (40), 107 (5), 93 (17), 77 (10), 65 (12); Analysis: $C_{14}H_{20}O_3$ requires C, 71.16; H, 8.53; found C, 71.13; H, 8.51%.

4.6.18. Preparation of 1-(3-(cyclopentyloxy)-4-methoxyphenyl)ethanone (22). To a mixture of alcohol **21** (3.54 g, 15 mmol) in diethyl ether (40 ml) was added dropwise through an addition funnel freshly prepared chromic acid solution (15 ml) under ice-cold condition with vigorous stirring. The reaction mixture was allowed to warm to room temperature and stirring continued for 3 h (monitored by TLC). The organic layer was separated and the aqueous layer was extracted with ether (3×15 ml). The combined ethereal extracts were washed with water (15 ml) followed by brine (15 ml) and concentrated under reduced pressure to give the crude product, which was further purified by column chromatography on silica gel using petroleum ether/EtOAc (9:1) as eluent to afford cyclopentyl acetophenone **22** (2.63 g).

Yield: 75%; brown colored solid; mp: 127–129 °C (crystallized from EtOAc); IR ($CHCl_3$, cm^{-1}): 408, 430, 442, 456, 466, 472, 588, 642, 668, 776, 808, 878, 898, 1076, 1132, 1178, 1216, 1356, 1584, 1676, 2360, 2870, 2960; ^1H NMR (200 MHz, $CDCl_3$): δ 1.72–1.91 (m, 8H), 2.56 (s, 3H), 3.91 (s, 3H), 4.81–4.90 (m, 1H), 6.85 (d, J=10.0 Hz, 1H), 7.53–7.57 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 23.92, 26.05, 32.63, 55.94, 80.42, 110.38, 113.54, 122.84, 130.30, 147.54, 154.23, 196.68; Analysis: $C_{14}H_{18}O_3$ requires C, 71.77; H, 7.74; found C, 71.75; H, 7.68%.

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Nucleoside 5′-*C*-phosphonates: reactivity of the α-hydroxyphosphonate moiety

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Abstract—We found that various dialkyl phosphites, dialkyl trimethylsilyl phosphites, and tris-trimethylsilyl phosphite reacted smoothly with nucleoside 5′-aldehydes to afford epimeric nucleoside 5′-*C*-phosphonates in high yields. A number of these compounds in both the 2′-deoxyribo and *ribo* series were prepared. In the case of 2′-deoxythymidine-5′-aldehyde, a thorough study was made on the influence of the 3′-hydroxyl protecting group, type of phosphite, base, and solvent, on the yield and epimeric ratio of the resulting 5′-hydroxyphosphonates. Partial stereoselectivity in favour of either *R* or *S* epimers was observed. An attempt to transform the α-hydroxyl of the phosphonate moiety into a halo or azido moiety was not successful. Only intramolecular substitution reaction of the mesyloxy group for an alkoxy residue of the 2-hydroxyethyl ester took place in a low yield.

1. Introduction

Nucleoside phosphonic acids, the well-known structurally diverse analogues of natural nucleotides, exhibit virtually absolute stability against enzymes of nucleotide catabolism, such as phosphomonoesterases and nucleotidases.[1] Among them, several types exhibit, after in vivo phosphorylation, remarkable antiviral properties.[2] The potential biological effects of nucleoside phosphonic acids have been the driving force in the search for novel nucleotide analogues with the P–C linkage. Nucleoside α-hydroxyphosphonic acids bearing a phosphoryl moiety attached directly to one of the carbon atoms of the sugar ring could undoubtedly be interesting compounds in this respect. Wiemer[3] and Králíková[4] reported 3′- and 2′-α-hydroxyphosphonate derivatives of nucleosides (Fig. 1). These 2′- and 3′-nucleotide analogues, however, did not exhibit any antiviral properties. A short account on the synthesis of regioisomeric compounds bearing the 5′-hydroxyphosphonate moiety **4a** was also reported by Králíková.[5,6] Recently Wiemer[7] described the synthesis of arabinosylcytosine 5′-hydroxyphosphonate **4b** which showed interesting biochemical properties. The nucleoside 5′-hydroxyphosphonates are related to the known 5′-deoxynucleoside 5′-phosphonates[8] **5** that lack chirality on the C5′ atom (Fig. 1).

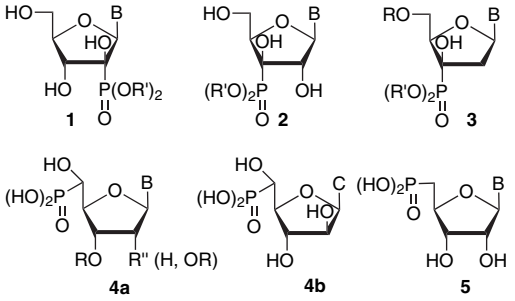

Figure 1. Examples of isopolar non-isosteric nucleoside phosphonic acids.

Herein, we present the synthesis of a number of nucleoside 5′-*C*-phosphonates (5′-hydroxyphosphonates) in 2′-deoxyribo **12–15** and *ribo* series **48–53** bearing an additional chiral centre on the C5′ atom (Schemes 1 and 4, Tables 1 and 3), and demonstrate the reactivity of the 5′-hydroxy group of the hydroxyphosphonate moiety. We prepared these compounds employing the general method of nucleophilic addition of phosphites to carbonyl compounds.[9–11] We found it interesting to subject these compounds to further transformation reactions to obtain a variety of new derivatives. In addition, the free nucleoside 5′-*C*-phosphonic acids **38–41** and **54–58** represent a pool of potential antimetabolites which could inhibit, for instance, different mammalian 5′-nucleotidases, as described earlier[12] for another type of nucleoside 5′-phosphonic acids prepared in our laboratory.[13,14] Also the inhibition of thymidine phosphorylase, the enzyme involved in angiogenesis,[15] by nucleoside 5′-*C*-phosphonic acids could be expected, similarly to recently reported case of acyclic nucleoside phosphonic acids.[16]

Keywords: Nucleoside 5′-aldehydes; Oxidation; Phosphonates; Addition reaction; Nucleophilic substitution.
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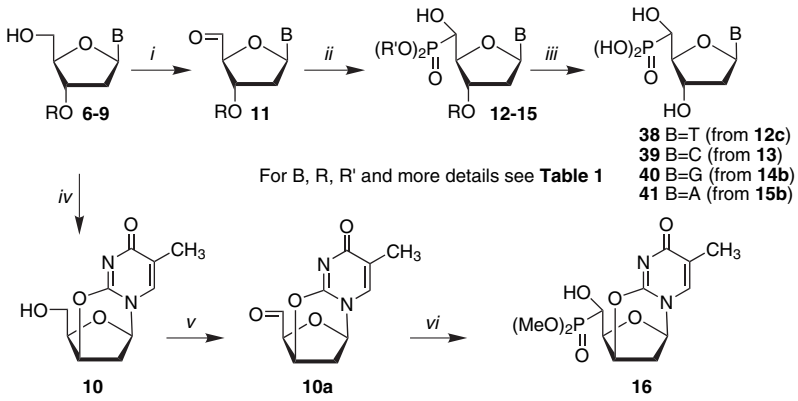


Scheme 1. (i) DMSO–$(COCl)_2$ or DMSO–DCC method; (ii) $(R'O)_2(O)PH$, Et_3N; (iii) (a) Me_3SiBr, CH_3CN, 24 h, rt, (b) satd ammonia in 50% aq ethanol, 48 h, rt, (c) 1 M TBAF in THF, 16 h, rt; (iv) Ph_3P, DEAD (from **10** R=H, B=T); (v) DMSO, DCC, pyridine, TFA; (vi) $(MeO)_2(O)PH$, Et_3N.

Table 1. Epimeric ratios and yields of prepared 2′-deoxynucleoside 5′-*C*-phosphonates (for general structures see Scheme 1)

Starting nucleoside	Oxidation method	Phosphite	Product	B	R	R′	*R*/*S* ratio[a]	Yield %[b]
6a	A[c]	$(Me_3SiO)_3P$	**12a**	T	TBDPS	H	37/63	85
		$(MeO)_2POH$	**12b**			Me	21/79	78
		$(EtO)_2POH$	**12c**			Et	19/81	77
		$(i\text{PrO})_2POH$	**12d**			*i*Pr	13/87	67
6b		$(MeO)_2POH$	**12e**		TBDMS	Me	20/80	71
6c	B[d]	$(MeO)_2POH$	**12f**		Bz	Me	42/58	52
		$(EtO)_2POH$	**12g**			Et	50/50	58
6d		$(EtO)_2POH$	**12h**		Piv	Et	33/67	54
6e		$(MeO)_2POH$	**12i**		DMTr	Me	19/81	60
7	A[c]	$(MeO)_2POH$	**13**	C^{Bz}	TBDPS	Me	15/85	84
8a			**14a**	G^{Bz}			34/66	82
8b			**14b**	G^{iBu}			40/60	76
9a			**15a**	A^{Bz}			25/75	69
9b	B[d]		**15b**	A^{Bz}	DMTr		22/78	80
10			**16**	T	—	—	37/63	34

[a] Epimeric ratio determined from ^1H NMR spectra.
[b] Isolated yield.
[c] DMSO–$(COCl)_2$.
[d] DMSO–DCC–pyridine–TFA.

2. Results and discussion

2.1. Preparation of 2′-deoxyribonucleoside 5′-*C*-phosphonates

The synthesis of protected nucleoside hydroxyphosphonates **12–16** in the 2′-deoxy series was accomplished by nucleophilic addition of various phosphites to nucleoside 5′-aldehydes **11** and **10a** (Scheme 1, Table 1) obtained by oxidation of protected nucleosides **6–9**, **10** using the Swern (DMSO–$(COCl)_2$)[17] or modified[18] Moffatt procedures (DMSO–DCC).[19,20] They were used in further reaction without purification and characterization. Whereas the 3′-*O*-silyl-protected 2′-deoxyribonucleosides **6a**, **6b**, **7**, **8a**, **8b** and **9a** were smoothly oxidized by the Swern procedure[17] (Method A), for the oxidation of 2′-deoxynucleosides bearing 3′-*O*-acyl protecting groups **6c**, **6d**, 3′-*O*-DMTr derivatives **6e** and **9b**, and 2,3′-anhydrothymidine (**10**) the DMSO–DCC method[18] was used (Method B) (Table 1). In contrast to literature data, [17] the 3′-*O*-acyl-protected 2′-deoxynucleosides **6c** and **6d** were not stable under conditions of the Swern oxidation procedure.[17]

We attempted to increase the stereoselectivity of the addition of dialkyl phosphites to nucleoside 5′-aldehydes (Table 1) by changing several factors, such as the solvent and the base used, and the type of phosphorus acid esters. We found that the solvent did not have any effect on the stereoselectivity, but the yields of phosphonates were different. Thus, DCM provided better yields of 5′-*C*-phosphonates than THF and acetonitrile. Increasing the amount of triethylamine (from 1 to 5 equiv) or the use of saturated ammonia in dioxane as a weak base did not influence the ratio of epimers. The use of DBU instead of triethylamine caused destruction of the starting nucleoside 5′-aldehyde. No change in the ratio of the epimeric phosphonates under the addition of diethyl phosphite in the presence of either lithium bis (trimethylsilyl)amide or *tert*-butylmagnesium chloride at −78 °C was found, but the yields of 5′-*C*-phosphonates were significantly reduced. On the other hand, only little changes in epimeric ratios were found if various dialkyl phosphites were used in the presence of triethylamine (Table 1); however, tris-trimethylsilyl phosphite exhibited significantly lower stereoselectivity in the addition reaction. The comparison of various types of 3′-*O*-protecting groups revealed (Table 1) that the use of 3′-*O*-acyl groups resulted in decrease of reaction preferences for the (*S*)-epimers.

The epimeric, protected nucleoside 5′-*C*-phosphonates **12–15** were separable by RP-HPLC, but on silica gel the

separation seldom took place. During our experiments, we succeeded in the separation of the epimers of dimethyl-(2-*N*-benzoyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxyguanosin-5′-*C*-yl-phosphonate) (**14a**) on C18 silica. In case of thymidine phosphonate **12**, we examined several ways in which to separate individual epimers. We found that removal of the 3′-*O*-protecting group of dimethyl phosphonates **12b** and **12f** resulted in better resolution of epimers on C18 silica. Unfortunately, hydrolysis of the silyl or benzoyl 3′-*O*-protecting group was accompanied by partial hydrolysis of one methyl ester group (epimeric monomethyl esters were very difficult to separate). In addition, the removal of 3′-*O*-benzoyl group from phosphonate **12f** led to the change of elution order of epimers on silica gel (not on C18 silica), and to significant increase of ΔR_f of epimers on TLC. In the case of dimethyl-(3′-*O*-*tert*-butyldimethylsilyl-2′-deoxythymidin-5′-*C*-yl-phosphonate) (**12e**), we succeeded in the separation of the substantial part of minor (*R*)-epimer by crystallization from ethyl acetate–ethanol mixture. Likewise, crystallization of the epimeric mixture of dimethyl-(3′-*O*-benzoyl-2′-deoxythymidinyl-5′-*C*-phosphonate) (**12f**) provided the major (*S*)-epimer.

2.2. ^{1}H and ^{13}C NMR study

Individual epimers **14a** were subjected to NMR analysis to determine the configuration at the 5′-carbon atom. The structural assignment was carried out using characteristic chemical shifts (protons and carbons of the nucleobase and substituents), homonuclear 2D-COSY and 2D-ROESY spectra (deoxyribose protons), and heteronuclear ^{1}H, ^{13}C-2D-HMQC spectra (deoxyribose carbon atoms). Vicinal couplings *J*(H,H) showed a high preference for the C2′-*endo* form of the deoxyribofuranse ring in both epimers (~85% in the major and ~95% in the minor epimer). The preferred syn-orientation of the nucleobase was determined from 2D-ROESY spectra. Determination of the configuration at C5′ is closely connected with the conformation around C4′–C5′ bond. The high value of *J*(P,C3′)=14.6 Hz and low value of *J*(P,H4′)=2.3 Hz in the major epimer indicates the trans-arrangement of P and C3′ which, together with a *gauche*-relationship of H4′/H5′ as indicated by *J*(H4′,H5′)=2.3 Hz, establishes the (*S*)-configuration at C5′ (Fig. 2). The configuration of minor epimer **14a** is therefore 5′(*R*) and the set of its vicinal couplings (*J*(P,C3′)=8.8 Hz, *J*(P,H4′)=5.4 Hz and *J*(H4′,H5′)=6.4 Hz) indicate comparable populations of *trans* and *gauche* conformers around C4′–C5′ bond (Fig. 2). Lower values of *J*(P,H4′) and *J*(H4′,H5′) were observed for major isomer in five-epimeric pairs **12–15**, **25–26** and **54–58**, indicating the 5′(*S*)-configuration for the major isomer, similar to the above discussed pair **14a**, **14b**. It should be noted that this relation between *J*(P,H4′) and *J*(H4′,H5′) in the 5′(*S*) and 5′(*R*) isomers does not hold in general for all nucleotides studied in this paper. For example, in deprotected epimeric pairs **38–41** and/or in 2,3-di-*O*-isopropylidene derivatives **48–53** prepared from precursors with known ratio of 5′(*S*) and 5′(*R*) isomer, the observed relative values of corresponding *J*(H,H) are opposite. A similar situation was also found in nucleosides **16** and **36**, obviously due to the different preferred conformation around the C4′–C5′ bond in the presence of additional sugar ring.

2.3. Attempt to convert α-hydroxyphosphonate moiety into α-halophosphonate (Scheme 2)

Reaction of the 5′-hydroxyl of **12b** with CBr_4 or CCl_4 and triphenylphosphine in dioxane to prepare a halo derivative **17** failed. Whereas at rt we observed no reaction, under heating total decomposition of **12b** took place. Also the attempt to obtain fluoro derivative **18** in the reaction of **12b** with DAST[21] in dichloromethane at −78 °C failed, and we isolated only a small amount of product of elimination, namely 1-(3-*O*-*tert*-butyldiphenylsilyl-2,5-dideoxy-β-D-*glycero*-pent-4-enofuranosyl)thymine. Therefore, on reaction with methansulfonyl chloride in pyridine, the phosphonate **12b** was first transformed into 5′-*O*-mesyl derivative **19**, which was used for the reaction with TBAF in dimethylformamide in the presence of powdered molecular sieves. The mesyl derivative **19** was completely stable at rt, but under heating provided a mixture of products. The reaction of **19** with tris(dimethylamino)sulfonium difluorotrimethyl silicate (TASF)[22] in various solvents (dichloromethane, acetonitrile and dimethylformamide) resulted in quantitative conversion of **19** into its 3-*N*-methyl derivative (structure not shown); no fluoro derivative **18** was formed.

2.4. Attempt to convert α-hydroxyphosphonate into α-azidophosphonate

An attempt to displace the mesyloxy group of adenine derivative **20** by an azide ion caused only quantitative cleavage of one methyl ester group giving monomethyl derivative **21** (Scheme 2). We also did not succeed in the preparation of a more reactive 5′-*O*-trifluoromethanesulfonyl derivative

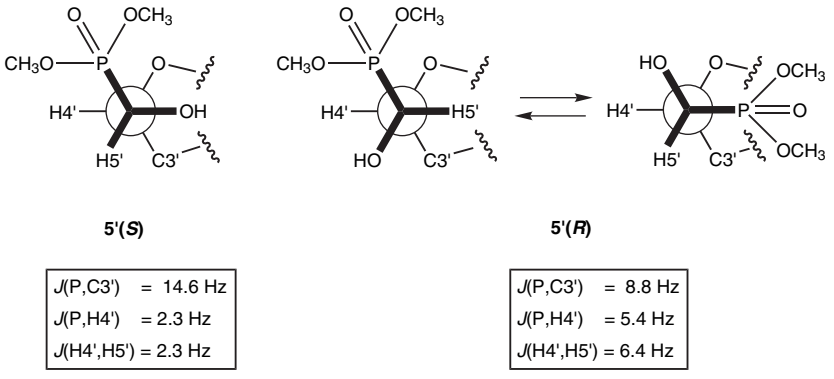
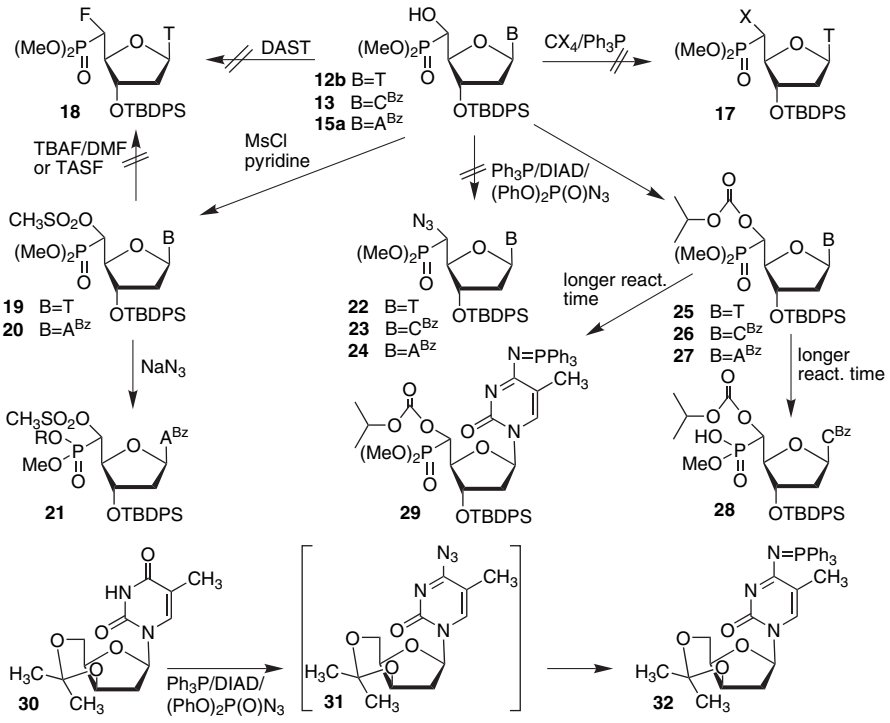


Figure 2. Epimers **14a**: preferred conformations around C4′–C5′ in both epimers and vicinal coupling constants used for determination of the configuration at carbon C5′.



Scheme 2. Reactions on the 5′-hydroxyphosphonate moiety.

intended for use in the reaction with sodium azide. At −15 °C, the reaction of phosphonate **12b** with triflic anhydride did not proceed, whereas at rt total decomposition of starting compound **12b** was observed.

Mitsunobu reaction[23] of phosphonates **12b**, **13** and **15a** with diphenylphosphoryl azide, diisopropyl azodicarboxylate, and triphenylphosphine did not provide the expected 5′-azido derivatives **22**–**24** but gave the 5′-*O*-isopropoxycarbonyl derivatives **25**–**27** in quantitative yield (Scheme 2). Reaction proceeded with partial inversion of configuration, as it was proved in the reaction with both the epimeric mixture and the individual epimers of compound **12b** (for results, see Table 2). The formation of carbonates under Mitsunobu conditions was observed by Battaglia[24] in case of branched hydroxy derivatives of dioxolanones. During prolonged reaction time we observed, in the case of cytosine compound **26**, the loss of one methyl ester group under formation of monoester **28**. The calculated molecular mass of **28** corresponded with that of found by HRMS. In addition, the derivative **25** underwent subsequent reaction on the thymine residue. We detected (by RP-HPLC) the formation of another pair of peaks with higher retention times than starting epimers **25**. Using 3′,5′-*O*-isopropylidene-*xylo*-thymidine (**30**) as a model compound, we proved that the reaction on the thymine residue led to triphenylphosphoranylidene derivative **32**, most probably via 4-azido-2-pyrimidone derivative **31**. Thus, we believe that the newly formed pair of peaks with higher retention times in the reaction of **12b**→**25** (Scheme 2) are the 5′-epimeric triphenylphosphoranylidene derivatives **29**.

Table 2. Changes of epimeric ratio in the reaction **12b**→**25** (Scheme 2)

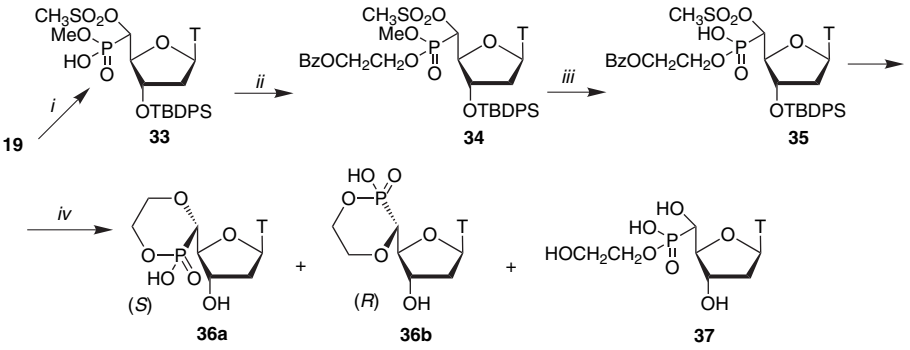
R/*S* epimeric ratio	
Reactant **12b**	Product **25**
22/78	43/57
0/100	64/36
100/0	17/83

2.5. Intramolecular substitution reaction of 5′-*O*-mesyl group

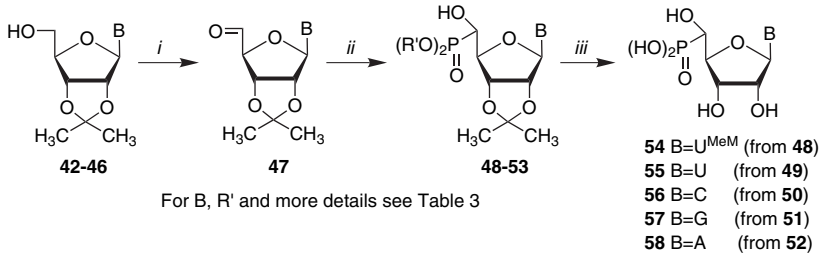
The failure of substituting a mesyloxy group for an azido moiety led us to check the possibility of an intramolecular nucleophilic substitution reaction. Thus, monomethyl ester **33** prepared by treatment of **19** with aqueous pyridine was condensed by a phosphotriester method[25] with 2-benzoyloxyethanol to give mixed diester **34**, which was demethylated in aqueous pyridine to give the 2-benzoyloxyethyl ester **35** (Scheme 3). This compound underwent a slow intramolecular nucleophilic substitution of the 5′-*O*-mesyloxy group with the hydroxy moiety of the 2-hydroxyethyl ester residue in refluxing 1 M sodium methoxide in methanol. After complete removal of the silyl protecting group, RP-HPLC isolation provided both epimers of cyclic monoester **36a** and **36b** as well as 2-hydroxyethyl ester **37** in low yields, along with thymine as the main product.

2.6. Preparation of ribonucleoside 5′-*C*-phosphonates

The synthesis of protected ribonucleoside hydroxyphosphonates **48**–**53** was accomplished by nucleophilic addition of phosphites to nucleoside 5′-aldehydes of general formula **47** obtained by oxidation of protected nucleosides **42**–**46** using the DMSO–DCC procedure[18] (Scheme 4, Table 3). Again in this case, we observed reaction preferences for the formation of (*S*)-epimers (Table 3). Separation of epimeric pairs of phosphonates **48**–**53** was more effective on C18 silica than on silica gel.



Scheme 3. (i) Aqueous pyridine (60%), 50 °C; (ii) $BzOCH_2CH_2OH$, MSNT, 4-methoxypyridine-*N*-oxide, pyridine; (iii) 60% aq pyridine, 50 °C; (iv) 1 M CH_3ONa in CH_3OH, reflux.



Scheme 4. (i) DCC–DMSO (Method B); (ii) $(R'O)_2(O)PH$, Et_3N; (iii) (a) Me_3SiBr, CH_3CN, 24 h, rt, (b) concd aq ammonia, 48 h, rt, (c) 0.025 M H_2SO_4 in 50% aq dioxane, 16 h, rt.

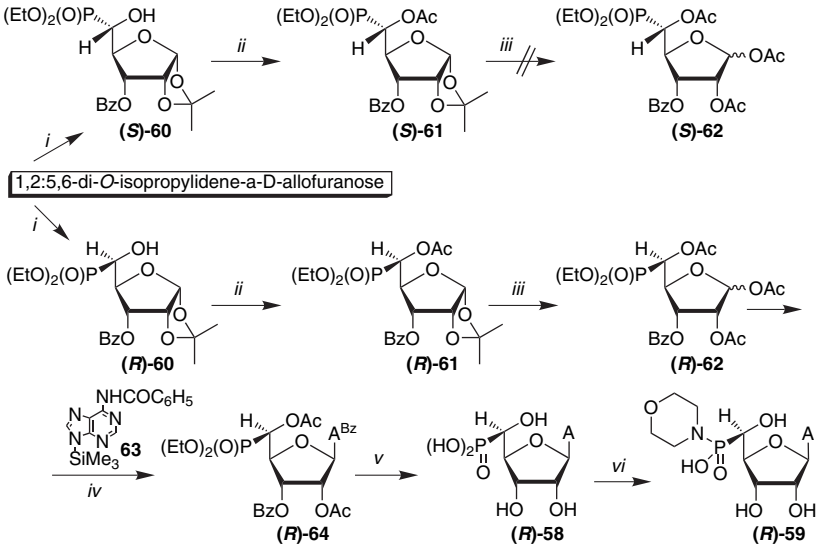
Table 3. Epimeric ratios and yields of prepared ribonucleoside 5′-*C*-phosphonates (for general structure see Scheme 4)

Starting nucleoside	Oxidation method	Phosphite	Product	B	R′	*R*/*S* ratio[a]	Yield %[b]
42	B[c]	$(EtO)_2POH$	**48**	U^{MeM}	Et	30/70	31
43			**49**	U		45/55	46
44			**50**	C^{Bz}		43/57	57
45		$(MeO)_2POH$	**51**	G^{Bz}	Me	40/60	66
46		$(EtO)_2POH$	**52**	A^{Bz}	Et	33/67	32
		$(MeO)_2POH$	**53**	A^{Bz}	Me	17/83	42

[a] Epimeric ratio determined from ^1H NMR spectra.
[b] Isolated yield.
[c] DMSO–DCC–pyridine–TFA.

Therefore, we examined the nucleosidation reaction as a potential synthetic route for the preparation of pure epimers of ribonucleoside 5′-*C*-phosphonates (Scheme 5). The starting sugar-phosphonate synthons (*S*)-**60** and (*R*)-**60** (Scheme 5) were easily available by addition of diethyl phosphite to 3-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-*ribo*-pentodialdo-1,4-furanose according to a modified Otmar procedure.[26] Both epimers were separated on silica gel. The 5′-hydroxyl of these compounds was acetylated in pyridine with acetic anhydride, and acetyl derivatives (*S*)-**61** and (*R*)-**61** were subjected to acetolysis to obtain sugar-phosphonate synthons (*S*)-**62** and (*R*)-**62**. Surprisingly, only (*R*)-**61** underwent clean acetolysis with formation of the expected product (*R*)-**62** which afforded, on a nucleosidation reaction with silylated 6-*N*-benzoyladenine **63**, the phosphonate (*R*)-**64**. On the other hand, acetolysis of compound (*S*)-**61** resulted unexpectedly in a mixture of products, so that the route following nucleosidation reaction with silylated 6-*N*-benzoyladenine **63** did not afford any desired nucleoside phosphonate. The reason for instability of (*S*)-**62** toward acetolysis is not clear. The observation that this synthetic route can provide only (*R*)-phosphonates led us back to the original synthetic route. We found that morpholidate derivatives of epimeric adenine compound **58** exhibited significant difference in retention times on C18 silica. To identify the (*R*)-epimer in an epimeric mixture, we prepared morpholidate (*R*)-**59** from the free phosphonate (*R*)-**58**, morpholine and DCC.

Deprotection of fully protected nucleoside phosphonates **12b**, **13**, **14b**, **15b** and **48**–**52** was performed as follows. First, the alkyl ester groups of the phosphorus moiety were removed by treatment with bromotrimethylsilane (DMTr group of **15b** was also cleaved off under these conditions), then *N*- and *O*-acyl protected groups were removed in concd aq ammonia–ethanol mixture and, finally, either silyl groups were removed by treatment with TBAF in THF or the isopropylidene groups were hydrolyzed in 0.025–0.05 M sulfuric acid. Free phosphonic acids **38**–**41** and **54**–**58** were purified on DEAE-Sephadex column followed by RP-HPLC. Obtained compounds were transformed into sodium salts on Dowex 50 (Na^+), and finally freeze-dried from water.



Scheme 5. (i) (a) Benzoyl cyanide, Et_3N, DCM–acetonitrile, (b) 60% aq acetic acid, 50 °C, (c) $NaIO_4$, 70% aq acetone; (ii) Ac_2O, pyridine; (iii) Ac_2O, AcOH, DCM, H_2SO_4; (iv) $SnCl_4$, acetonitrile; (v) (a) concd aq ammonia–ethanol mixture, (b) Me_3SiBr, acetonitrile; (vi) DCC, morpholine, *t*-BuOH–H_2O, reflux.

2.7. Biological properties

Cytostatic activity of protected phosphonates and free phosphonic acids was examined on L 1210, L 929 and HeLa S3 cell lines. Antiviral properties of the same compounds were examined on HIV-1, HIV-2 and Moloney sarcoma virus (MSV), and on the selected DNA viruses (HSV-1 (KOS), HSV-1 (B), HSV-1 (McIntyre), HSV-2 (G), HSV-2 (196), HSV-2 (Lyons), VV, VSV, HSV-1 TK^- (B2006) and HSV-1 TK^- (VMW1837)). Neither phosphonates **12**–**15** and **48**–**53** nor phosphonic acids **38**–**41** and **54**–**58** exhibited any significant cytostatic and/or antiviral activities.

3. Conclusion

We have established an efficient synthesis of nucleoside 5′-*C*-phosphonates in 2′-deoxyribo and *ribo* series bearing a new centre of chirality on the C5′ atom via base-catalyzed nucleophilic addition of various phosphites to nucleoside 5′-aldehydes, and thus enlarged the family of enzyme-stable nucleotide analogues. The reactivity of 5′-hydroxyl of the nucleoside 5′-hydroxyphosphonates was found to be very specific, and the conversion of the hydroxyl into a halo or azido moiety failed. Only acylation and sulfonylation reactions proceeded smoothly in quantitative yields. The prepared nucleoside phosphonic acids offer the possibility for their further evaluation as inhibitors of mammalian 5′-nucleotidases, bisubstrate inhibitors of thymidine and purine nucleoside phosphorylases, and, after transformation into nucleoside 5′-triphosphate analogues, as substrates/inhibitors of DNA and RNA polymerases.

4. Experimental

4.1. General

The solvents were evaporated at 40 °C and 2 kPa, and the products were dried over phosphorus pentoxide at 50–70 °C and 13 Pa. The course of the reactions was checked by TLC cards (Fluka, Merck) whereby the products were detected by UV monitoring and by spraying with 1% ethanolic solution of 4-(4-nitrobenzyl)pyridine followed by heating and treating with gaseous ammonia (blue colour of diesters of phosphonic acids). For flash column chromatography, silica gel 40–60 μm (Fluka) was used. The TLC and the preparative silica gel chromatography were carried out in the following solvent systems (v/v): chloroform–ethanol 9/1 (C1); ethyl acetate–acetone–ethanol–water 4/1/1/1 (H1); ethyl acetate–acetone–ethanol–water 6/1/1/1 (H3); 2-propanol–concd aq ammonia–water 7/1/2 (I); 50% EtOAc–toluene (T1), 20% EtOAc–toluene (T2). Analytical HPLC was performed on Nucleosil 100–5 C18 (4.6×150 mm; Macharey–Nagel) using a linear gradient of methanol in 0.1 M TEAA. Preparative reversed-phase chromatography was carried out on octadecyl silica column (25×250 mm, 20 μm, IOCB Prague); compounds were eluted with a linear gradient of methanol in water at 15 ml/min. UV spectra and thermal characteristics were taken on a Cary Bio 100 (Varian) spectrophotometer. High resolution FAB mass spectra were recorded on a ZAB-EQ (VG Analytical) instrument with glycerol and thioglycerol as matrices. NMR spectra were measured on a Varian UNITY-500 spectrometer (^1H at 500 MHz; ^{13}C at 125.7 MHz frequency) in DMSO-d_6 and/or D_2O at 20 °C. The chemical shifts were referenced either to solvent signal (converted to δ scale using relations δ_H(DMSO)=2.50 and δ_C(DMSO)=39.7 ppm) or to DSS (in D_2O). Proton 2D-COSY spectra were used for the structural assignment of coupled protons and 2D-ROESY spectra for detection of the NOE contacts. Carbon-13 chemical shifts and coupling constants *J*(C,P) were obtained from broad band proton-decoupled spectra using APT pulse sequence.

Method A: Swern oxidation procedure [DMSO–$(COCl)_2$].[17] Dimethylsulfoxide (0.21 ml, 3 mmol) was added dropwise to a stirred solution of oxalyl chloride (0.13 ml, 1.5 mmol) in dichloromethane (3.5 ml) at −78 °C under argon atmosphere. After 10 min, a solution of protected nucleoside (1 mmol) (see Table 1) in dichloromethane (7 ml) was added

dropwise and the reaction mixture was stirred at −78 °C for 30 min. The reaction was quenched by addition of triethylamine (0.7 ml, 5 mmol). The resulting suspension was stirred for additional 5 min at low temperature, allowed to warm to rt, and the appropriate phosphite (2 mmol) (see Table 1) was added. The reaction mixture was set aside overnight at rt (TLC in C-1), diluted with chloroform, extracted with water, and dried over anhydrous sodium sulfate. Chromatography of the crude product on a silica gel (elution with a linear gradient of 0–10% ethanol in chloroform) afforded the desired phosphonate (Table 1).

Method B: Modified Moffatt oxidation procedure [DMSO–DCC].[18] Pyridine (0.08 ml, 1 mmol) followed by TFA (0.04 ml, 0.5 mmol) was added at rt to a stirred solution of protected nucleoside (1 mmol) (Tables 1 and 3) and DCC (0.64 g, 3 mmol) in DMSO (4 ml). After 16 h, appropriate phosphite (2 mmol) (Tables 1 and 3) and triethylamine (0.7 ml, 5 mmol) were added and the mixture was stirred for further 16 h (TLC in C-1). The reaction mixture was diluted with chloroform, filtered through Celite, and the filtrate was extracted with water and organic layer was dried over anhydrous sodium sulfate. Chromatography of the crude product on silica gel (elution with a linear gradient of 0–10% ethanol in chloroform) afforded the expected phosphonate (Tables 1 and 3).

Method C: Removal of ester protecting groups of phosphonate moiety. 2,6-Lutidine (0.58 ml, 5 mmol) and bromotrimethylsilane (0.66 ml, 5 mmol) were sequentially added to a solution of phosphonate diester **12c**, **13**, **14b**, **15b**, **48–52** (1 mmol) in acetonitrile (5 ml), the mixture was set aside at rt for 48 h (TLC in C-1, H-1 and I), and then concentrated in vacuo. Aqueous 2 M TEAB (2 ml) was added, the solution was concentrated, and the residue was co-distilled several times with methanol to destroy TEAB and finally with ethanol. The obtained *N*- and/or *O*-protected nucleoside phosphonic acids **12–15** (R′=H) and **48–52** (R′=H) were subjected to further deprotection steps (Method D, E or F).

Method D: Removal of N-protecting groups. The solution or suspension of *N*-protected cytosine- (**13** and **50**, R′=H), guanine- (**14** and **51**, R′=H) and adenine-containing (**15** and **52**, R′=H) phosphonic acid, obtained by Method C, in methanol or ethanol (50 ml/mmol) was saturated with gaseous ammonia at 0 °C. The mixture was stirred in a tightly stoppered flask at rt overnight (TLC in C-1, H-1 and I). Deprotection of *N*-benzoylguanine derivatives **14** and **51** (R′=H) took place in an autoclave at 60 °C overnight. Alternatively, deacylations were also performed in a 1/1 mixture of concd aq ammonia–ethanol for 16 h with identical results. Solvent was evaporated, and the residue was dried by co-distillation with ethanol (3×20 ml). The crude deacylated compounds **13** and **14** (B=C, G; R′=H, R=TBDPS) were subjected to treatment according to Method E, derivatives **50–52** (B=C, G, A; R′=H) to treatment by Method F, and adenine phosphonic acid **41** (obtained from **15** ($B=A^{Bz}$; R=R′=H)) to the final purification step (Method G).

Method E: Removal of silyl protecting group. The silyl derivatives **12–14** (B=T, C, G; R′=H, R=TBDPS) obtained by Method D were co-distilled with dry toluene, dissolved in 0.5 M TBAF in THF (10 ml/mmol), and the reaction mixture was stirred for 24 h at rt under exclusion of moisture. In case of less soluble compounds, an equal volume of pyridine was added, and the solution was concentrated to half volume. Silyl group cleavage ability of TBAF in pyridine was at least as efficient as in THF alone. After removal of the silyl group (TLC in H-1 and I), water was added to the reaction mixture, the solution was concentrated in vacuo, and finally Dowex 50 (Et_3NH^+, 20 ml/mmol) in 50% aqueous ethanol (50 ml/mmol) was added to remove *tetra-n*-butylammonium cations. The suspension was filtered, the resin was washed with 50% aqueous ethanol, and the combined filtrates were evaporated. Crude phosphonic acids **38–40** were subjected to final purification step (Method G).

Method F: Removal of 2′,3′-O-isopropylidene protecting group. The products **50–52** (B=C, G, A; R′=H) obtained by Method D were dissolved in aqueous 0.05 M sulfuric acid (100 ml/mmol) and the solution was set aside overnight at rt (TLC in H-1 and I). Solution of crude cytidine **56**, guanosine **57**, and adenosine **58** phosphonic acids was applied onto a column of Dowex 50 (H^+) and, after washing with water, the product was eluted by 3% aqueous ammonia. In the case of uridine derivatives **48** and **49** ($B=U^{MeM}$, U; R′=H) obtained by Method C, the cleavage of isopropylidene group was achieved with aqueous 80% acetic acid at 80 °C for 3 h. The reaction mixture was concentrated, and the residue co-distilled several times with water. The obtained crude products **54–58** were subjected to final purification step (Method G).

Method G: Final purification of nucleoside phosphonic acids. Phosphonic acids **38–41** and **54–58** were purified by chromatography on DEAE-Sephadex A-25 (HCO_3^-). The compounds were eluted by a linear gradient of 0–0.2 M TEAB. Fractions were pooled and evaporated, the residue was co-distilled several times with methanol and then applied onto C18 column (25×300 mm) in a solution of aqueous 2 M TEAB (10 ml/mmol). The products were eluted by a linear gradient of methanol in water (0–10%), then transformed into sodium salts on a column of Dowex 50 (Na^+), and finally freeze-dried from water.

4.1.1. 3′-*O*-*tert*-Butyldiphenylsilyl-2′-deoxythymidin-5′-*C*-ylphosphonic acid (12a). Phosphonate **12a** was obtained from tris(trimethylsilyl) phosphite (1.0 ml, 3 mmol) and the aldehyde prepared from 3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxythymidine[27] (480 mg, 1 mmol) according to Method A. The reaction was quenched by addition of methanol (5 ml) and 2 M TEAB (5 ml), and the solution was heated at 80 °C for 2 h (TLC in H1). After evaporation of the solvent, the partially protected phosphonate was purified on RP C18 column (elution with a linear gradient of methanol in water). Yield, 562 mg (85%; white foam) of triethylammonium salt of **12a** (*R*/*S* 37/63). For $C_{26}H_{33}N_2NaO_8PSi$ $(M+Na)^+$ calcd: 583.1643, found: 583.1642. ^1H NMR—see Tables 4 and 5.

4.1.2. Dimethyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxythymidin-5′-*C*-ylphosphonate (12b). The title compound **12b** was obtained on reaction of dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxythymidine[27] (480 mg, 1 mmol) according to Method A. Yield, 459 mg (78%; white foam) of **12b** (*R*/*S* 21/79). For $C_{28}H_{37}N_2O_8PSi$ (588.66) calcd: 57.13%

Table 4. ^{1}H chemical shifts of compounds **12–16** in DMSO-d_6

Compound	H-1′	H-2′	H-2″	H-3′	H-4′	H-5′	5′-OH	Nucleobase
(*S*)-**12a**[a]	6.15	1.86	2.00	4.51	4.30	3.39	4.50	T: 11.20 (NH), 8.18 (H-6), 1.71 (5-Me)
(*R*)-**12a**[a]	6.20	1.85	1.75	4.97	4.42	3.57	4.50	T: 11.20 (NH), 8.02 (H-6), 1.70 (5-Me)
(*S*)-**12b**[a,b]	6.31	1.96	2.16	4.46	4.09	3.46	6.07	T: 11.32 (NH), 7.82 (H-6), 1.71 (5-Me)
(*R*)-**12b**[a,b]	6.36	1.94	1.87	4.64	4.24	3.98	6.28	T: 11.30 (NH), 7.57 (H-6), 1.74 (5-Me)
(*S*)-**12c**[a,c]	6.31	1.96	2.17	4.45	4.11	3.36	5.99	T: 11.31 (NH), 7.86 (H-6), 1.71 (5-Me)
(*R*)-**12c**[a,c]	6.36	1.95	1.86	4.69	4.26	3.92	6.26	T: 11.30 (NH), 7.65–7.40 (H-6), 1.74 (5-Me)
(*S*)-**12d**[a,d]	6.29	1.96	2.19	4.44	4.13	3.27	5.85	T: 11.30 (NH), 7.89 (H-6), 1.71 (5-Me)
(*R*)-**12d**[a,d]	6.35	1.95	1.85	4.73	4.27	3.83	6.22	T: 11.30 (NH), 7.50–7.40 (H-6), 1.74 (5-Me)
(*S*)-**12e**[b,e]	6.17	2.14	2.06	4.49	4.02	4.12	6.21	T: 11.30 (NH), 7.88 (H-6), 1.76 (5-Me)
(*R*)-**12e**[b,e]	6.20	2.14	1.96	4.65	3.96	4.04	6.35	T: 11.33 (NH), 7.64 (H-6), 1.78 (5-Me)
(*S*)-**12f**[b,f]	6.31	2.33	2.46	5.53	4.42	4.34	6.47	T: 11.36 (NH), 7.97 (H-6), 1.79 (5-Me)
(*R*)-**12f**[b,f]	6.27	2.42	2.35	5.73	4.37	4.26	6.45	T: 11.37 (NH), 7.68 (H-6), 1.81 (5-Me)
(*S*)-**12g**[c,f]	6.30	2.30–2.50		5.52	4.42	4.28	6.37	T: 11.36 (NH), 7.98 (H-6), 1.78 (5-Me)
(*R*)-**12g**[c,f]	6.26	2.30–2.50		5.74	4.40	4.18	6.44	T: 11.35 (NH), 7.68 (H-6), 1.80 (5-Me)
(*S*)-**12h**[c,g]	6.19	2.23	2.23	5.23	4.17	4.09	6.23	T: 11.36 (NH), 7.93 (H-6), 1.79 (5-Me)
(*R*)-**12h**[c,g]	6.15	2.28	2.08	5.48	4.15	4.20	6.40	T: 11.36 (NH), 7.64 (H-6), 1.77 (5-Me)
(*S*)-**12i**[b,h]	6.27	1.88	2.09	4.29	3.58	3.27	5.98	T: 11.30 (NH), 7.85 (H-6), 1.70 (5-Me)
(*R*)-**12i**[b,h]	6.14	1.61	0.95	4.37	4.37	3.89	6.10	T: 11.26 (NH), 7.52 (H-6), 1.71 (5-Me)
(*S*)-**13**[a,b,i]	6.31	1.94	2.46	4.49	4.20	3.48	6.12	C^{Bz}: 11.25 (NH), 8.55, 7.32 (H-5, H-6)
(*R*)-**13**[a,b,i]	6.36	2.12	1.93	4.71	4.37	4.06	6.23	C^{Bz}: 11.28 (NH), 8.35, 7.25 (H-5, H-6)
(*S*)-**14a**[a,b,i]	6.45	2.51	2.46	4.60	4.17	3.50	6.08	G^{Bz}: 12.36, 11.91 (2×NH), 8.27 (H-8)
(*R*)-**14a**[a,b,i]	6.45	2.77	2.19	4.77	4.30	3.97	6.18	G^{Bz}: 12.35, 11.91 (2×NH), 8.24 (H-8)
(*S*)-**14b**[a,b,i]	6.36	2.44	2.44	4.58	4.16	3.50	6.04	G^{iBu}: 12.29, 11.65 (2×NH), 8.22 (H-8)
(*R*)-**14b**[a,b,i]	6.35	2.70	2.17	4.75	4.29	3.92	6.16	G^{iBu}: 12.10, 11.65 (2×NH), 8.19 (H-8)
(*S*)-**15a**[a,b,i]	6.31	2.87	2.24	4.57	4.29	3.95	6.03	A^{Bz}: 11.22 (NH), 8.66, 8.54 (H-2, H-8)
(*R*)-**15a**[a,b,i]	6.39	2.44	2.14	4.75	4.35	4.07	6.17	A^{Bz}: 11.20 (NH), 8.53, 8.32 (H-2, H-8)
(*S*)-**15b**[b,h,i]	6.65	2.55	2.55	4.43	3.64	3.32	6.19	A^{Bz}: 11.23 (NH), 8.74, 8.71 (H-2, H-8)
(*R*)-**15b**[b,h,i]	6.45	2.35	1.27	4.59	4.46	4.03	6.13	A^{Bz}: 11.19 (NH), 8.72, 8.61 (H-2, H-8)
(*S*)-**16**[b]	5.78	2.58	2.50	5.26	4.29	3.74	6.35	T: 7.58 (H-6), 1.76 (Me)
(*R*)-**16**[b]	5.85	2.52	2.52	5.23	4.34	4.02	6.01	T: 7.56 (H-6), 1.75 (Me)

[a] 3′-OTBDPS: 7.55–7.65 (m, 4H) and 7.36–7.52 (m, 6H) (2×C_6H_5), 1.07–1.10 (s, 9H, *t*-Bu).
[b] P(OMe)$_2$: 3.32–3.68 (2×d, 2×3H).
[c] P(OEt)$_2$: 3.80–4.10 (m, 4H, 2×P–OCH$_2$), 1.10–1.23 (2×d, 2×CH$_3$).
[d] P(*i*Pr)$_2$: 4.49–4.59 (m, 2H, 2×P–OCH), 1.13, 1.14, 1.20 and 1.21 (4×d, 4×CH$_3$).
[e] 3′-TBDMS: 0.87 (s, 9H, *t*-Bu), 0.10 (s, 6H, 2×CH$_3$).
[f] 3′-OBz: 8.02 (m, 2H, *o*-ArH), 7.70 (m, 1H, *p*-ArH) and 7.56 (m, 2H, *m*-ArH).
[g] 3′-OPiv: 3.90–4.07 (m, 4H, 2×P–OCH$_2$), 1.17 (s, 9H, 3×CH$_3$).
[h] 3′-ODMTr: 7.43 (m, 2H), 7.35 (m, 2H) and 7.25 (m, 1H) (C_6H_5); 7.30 (m, 4H) and, 6.93 (m, 4H) (2×C_6H_4), 3.74 (s, 6H, 2×OCH$_3$).
[i] NBz: 8.05 (m, 2H, *o*-ArH), 7.65 (m, 1H, *p*-ArH) and 7.55 (m, 2H, *m*-ArH).

C, 6.34% H, 4.76% N; found: 56.78% C, 6.23% H, 4.51% N. MS (FAB): 589.2 (M+H)$^+$. ^{1}H NMR—see Tables 4 and 5.

4.1.3. Diethyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxythymidin-5′-*C*-ylphosphonate (12c). Phosphonate **12c** was obtained from diethyl phosphite (0.26 ml, 2 mmol) and the aldehyde prepared from 3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxythymidine[27] (480 mg, 1 mmol) according to Method A. Yield, 475 mg (77%; white foam) of **12c** (*R*/*S* 19/81). For $C_{30}H_{41}N_2O_8SiP$ (616.72) calcd: 58.43% C, 6.70% H, 4.54% N; found: 58.19% C, 6.67% H, 4.43% N. MS (FAB): 617.4 (M+H)$^+$. ^{1}H NMR—see Tables 4 and 5.

4.1.4. Diisopropyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxythymidin-5′-*C*-ylphosphonate (12d). Phosphonate **12d** was obtained from diisopropyl phosphite (0.33 ml, 2 mmol) and the aldehyde prepared from 3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxythymidine[27] (480 mg, 1 mmol) according to Method A. Yield, 432 mg (67%; white foam) of **12d** (*R*/*S* 13/87). For $C_{32}H_{45}N_2O_8SiP$ (644.27) calcd: 59.60% C, 7.04% H, 4.35% N; found: 59.97% C, 7.08% H, 4.02% N. MS (FAB): 645.3 (M+H)$^+$. ^{1}H NMR—see Tables 4 and 5.

4.1.5. Dimethyl-3′-*O*-*tert*-butyldimethylsilyl-2′-deoxythymidin-5′-*C*-ylphosphonate (12e). Phosphonate **12e** was obtained from dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 3′-*O*-*tert*-butyldimethylsilyl-2′-deoxythymidine[28] (356 mg, 1 mmol) according to Method A. Yield, 330 mg (71%; white solid) of **12e** (*R*/*S* 20/80). Crystallization of **12e** from ethanol–ethyl acetate afforded 55 mg of single epimer (*R*)-**12e** (white crystals), mp 235 °C. For $C_{18}H_{33}N_2NaO_8PSi$ (M+Na)$^+$ calcd: 487.1642, found: 487.1651. ^{1}H NMR—see Tables 4 and 5. ^{13}C NMR—see Table 10.

4.1.6. Dimethyl-3′-*O*-benzoyl-2′-deoxythymidin-5′-*C*-ylphosphonate ((*S*)-12f). Phosphonate **12f** was obtained from dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 3′-*O*-benzoyl-2′-deoxythymidine[29] (346 mg, 1 mmol) according to Method B. Yield, 236 mg (52%; white solid) of **12f** (*R*/*S* 42/58). Crystallization of **12f** from chloroform afforded 100 mg of single epimer (*S*)-**12f** (white crystals), mp 224 °C. For $C_{19}H_{24}N_2O_9P$ (M+H)$^+$ calcd: 455.1219, found: 455.1235. ^{1}H NMR—see Tables 4 and 5. ^{13}C NMR—see Table 10.

4.1.7. Diethyl-3′-*O*-benzoyl-2′-deoxythymidin-5′-*C*-ylphosphonate (12g). Phosphonate **12g** was obtained from diethyl phosphite (0.26 ml, 2 mmol) and the aldehyde prepared from 3′-*O*-benzoyl-2′-deoxythymidine[29] (346 mg, 1 mmol) according to Method B. Yield, 280 mg (58%; white foam) of **12g** (*R*/*S* 50/50). For $C_{21}H_{27}N_2O_9P$ (482.44)

Table 5. ^{1}H coupling constants in compounds **12–16** in DMSO-d_6

Compound	1′,2′	1′,2″	2′,2″	2′,3′	2″,3′	3′,4′	4′,5′	5′,OH	4′,P	5′,P	P,OH
(*S*)-**12a**[a]	8.8	5.4	12.9	5.1	1.0	1.5	2.2	n	4.2	12.7	n
(*R*)-**12a**[a]	9.5	5.1	12.9	4.4	1.0	1.0	3.4	n	4.9	11.0	n
(*S*)-**12b**[a,b]	9.1	5.3	13.1	5.1	1.0	1.7	2.0	6.9	1.5	12.6	9.0
(*R*)-**12b**[a,b]	9.7	5.1	13.2	4.4	1.0	1.0	4.8	6.8	4.8	9.8	5.1
(*S*)-**12c**[a,b]	9.0	5.4	13.2	5.1	1.0	1.5	1.7	7.1	0.5	12.7	9.0
(*R*)-**12c**[a,b]	9.8	5.1	12.9	5.9	1.0	1.0	4.6	6.8	4.6	10.5	5.1
(*S*)-**12d**[a,c]	9.0	5.4	13.2	5.4	1.0	1.0	1.5	7.1	0.5	13.4	9.8
(*R*)-**12d**[a,c]	10.0	5.1	13.2	4.4	1.0	1.0	4.6	6.8	4.6	10.3	5.5
(*S*)-**12e**[a,b]	8.3	5.9	13.2	5.4	2.4	2.4	2.4	7.0	2.7	12.3	8.8
(*R*)-**12e**[a,b]	9.8	5.1	12.9	4.6	1.0	1.0	4.4	6.8	3.9	10.0	5.0
(*S*)-**12f**[a,b]	8.9	5.8	14.0	6.1	1.5	1.6	2.2	6.9	2.5	12.1	8.8
(*R*)-**12f**[a,b]	8.9	6.0	14.4	6.2	1.9	2.2	3.8	6.7	4.7	10.4	6.7
(*S*)-**12g**[a,d]	9.0	5.9	n	6.1	1.5	1.5	2.4	7.1	0.5	12.2	9.0
(*R*)-**12g**[a,d]	9.0	5.9	n	6.1	2.0	2.0	3.4	6.8	4.0	10.5	7.1
(*S*)-**12h**[a,d]	7.8	7.1	n	5.0	1.4	2.0	2.0	7.1	n	n	7.8
(*R*)-**12h**[a,d]	9.3	5.6	13.2	6.1	1.2	1.2	3.4	6.8	n	10.5	7.1
(*S*)-**12i**[a,b]	9.6	5.4	13.2	5.7	0.5	0.5	0.5	7.0	0.5	13.5	10.6
(*R*)-**12i**[a,b]	10.1	5.0	13.7	5.4	1.2	n	6.0	7.0	n	9.2	7.0
(*S*)-**13**[b,e]	8.3	5.4	13.2	5.1	1.0	1.0	1.7	7.1	1.7	12.7	9.0
(*R*)-**13**[b,e]	8.0	5.4	13.4	4.5	1.0	1.0	4.9	6.8	4.6	9.5	6.5
(*S*)-**14a**[b]	8.6	6.0	13.3	5.0	1.8	1.4	2.3	7.2	2.3	12.4	7.2
(*R*)-**14a**[b]	10.0	5.5	13.4	4.6	<1	<1	6.4	7.2	5.4	8.8	7.2
(*S*)-**14b**[b]	7.3	7.3	n	3.4	3.4	2.0	2.0	7.0	1.0	8.5	8.7
(*R*)-**14b**[b]	8.0	5.4	13.2	5.0	1.0	1.0	6.5	6.5	5.0	8.5	6.5
(*S*)-**15a**[b]	8.6	5.6	13.6	4.9	1.0	1.0	5.0	7.3	5.5	9.5	8.0
(*R*)-**15a**[b]	8.9	5.0	13.2	5.6	1.0	1.0	5.6	6.6	5.9	9.5	7.5
(*S*)-**15b**[b]	8.8	6.2	n	4.9	0.5	1.0	1.5	8.3	1.0	6.6	8.3
(*R*)-**15b**[b]	9.8	5.4	12.9	5.0	1.0	1.0	7.3	8.6	7.3	7.6	7.1
(*S*)-**16**[b]	0.5	3.9	12.9	1.8	2.7	2.4	9.1	6.0	6.8	6.0	6.0
(*R*)-**16**[b]	2.6	2.6	n	2.3	2.3	2.4	6.2	7.6	5.5	10.6	7.6

n=Unresolved multiplet, *J* could not be determined.
[a] $J(6,CH_3)$=1.0–1.3 Hz.
[b] $J(P,OCH_3)$=10.0–10.5 Hz.
[c] $J(CH,CH_3)$=6.1–6.3 Hz.
[d] $J(CH_2,CH_3)$=7.1.
[e] $J(5,6)$=7.6 Hz.

calcd: 52.23% C, 5.60% H, 5.80% N; found: 51.83% C, 5.96% H, 5.69% N. MS (FAB): 483.4 $(M+H)^+$. ^{1}H NMR—see Tables 4 and 5.

4.1.8. Diethyl-3′-*O*-trimethylacetyl-2′-deoxythymidin-5′-*C*-ylphosphonate (12h). Phosphonate **12h** was obtained from diethyl phosphite (0.26 ml, 2 mmol) and the aldehyde prepared from 2′-deoxy-3′-*O*-trimethylacetylthymidine[30] (326 mg, 1 mmol) according to Method B. Yield, 250 mg (54%; white solid) of **12h** (*R*/*S* 33/67). For $C_{19}H_{31}N_2O_9P$ (462.43) calcd: 49.30% C, 6.70% H, 6.05% N; found: 48.92% C, 6.81% H, 5.85% N. MS (FAB): 463.4 $(M+H)^+$. ^{1}H NMR—see Tables 4 and 5.

4.1.9. Dimethyl-3′-*O*-dimethoxytrityl-2′-deoxythymidin-5′-*C*-ylphosphonate (12i). Phosphonate **12i** was obtained from dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 2′-deoxy-3′-*O*-dimethoxytritylthymidine[31] (545 mg, 1 mmol) according to Method B. Yield, 391 mg (60%; yellowish foam) of **12i** (*R*/*S* 19/81). For $C_{33}H_{37}N_2NaO_{10}P$ $(M+Na)^+$ calcd: 675.2084, found: 675.2078. ^{1}H NMR—see Tables 4 and 5.

4.1.10. Dimethyl-4-*N*-benzoyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxycytidin-5′-*C*-ylphosphonate (13). Phosphonate **13** was obtained from dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 4-*N*-benzoyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxycytidine[32] (569 mg, 1 mmol) according to Method A. Yield, 569 mg (84%; white foam) of **13** (*R*/*S* 15/85). For $C_{34}H_{40}N_3NaO_8PSi$ $(M+Na)^+$ calcd: 700.2220, found: 700.2228. ^{1}H NMR—see Tables 4 and 5.

4.1.11. Dimethyl-2-*N*-benzoyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxyguanosin-5′-*C*-ylphosphonate (14a). Phosphonate **14a** was obtained from dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 2-*N*-benzoyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxyguanosine[32] (610 mg, 1 mmol) according to Method A. Yield, 589 mg (82%; white foam) of **14a** (*R*/*S* 34/66). For $C_{35}H_{41}N_5O_8PSi$ $(M+H)^+$ calcd: 718.2462, found: 718.2461. ^{1}H NMR—see Tables 4 and 5. ^{13}C NMR—see Table 10.

4.1.12. Dimethyl-2-*N*-isobutyryl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxyguanosin-5′-*C*-ylphosphonate (14b). Phosphonate **14b** was obtained from dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 2-*N*-isobutyryl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxyguanosine[32] (562 mg, 1 mmol) according to Method A. Yield, 509 mg (76%; white foam) of **14b** (*R*/*S* 40/60). For $C_{32}H_{43}N_5O_8PSi$ $(M+H)^+$ calcd: 684.2619, found: 684.2614. ^{1}H NMR—see Tables 4 and 5.

4.1.13. Dimethyl-6-*N*-benzoyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxyadenosin-5′-*C*-ylphosphonate (15a). Phosphonate **15a** was obtained from dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 6-*N*-benzoyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxyadenosine[33] (594 mg, 1 mmol) according to Method A. Yield, 484 mg

(69%; white foam) of **15a** (*R*/*S* 25/75). For $C_{35}H_{41}N_5O_7PSi$ $(M+H)^+$ calcd: 702.2513, found: 702.2517. ^1H NMR—see Tables 4 and 5.

4.1.14. Dimethyl-6-*N*-benzoyl-2′-deoxyadenosin-3′-*O*-dimethoxytrityl-5′-*C*-ylphosphonate (15b). Phosphonate **15b** was obtained from dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 6-*N*-benzoyl-2′-deoxy-3′-*O*-dimethoxytrityladenosine[30] (658 mg, 1 mmol) according to Method B. Yield, 612 mg (80%; yellowish foam) of **15b** (*R*/*S* 22/78). For $C_{40}H_{40}N_5NaO_9P$ $(M+Na)^+$ calcd: 788.2461, found: 788.2460. ^1H NMR—see Tables 4 and 5.

4.1.15. Dimethyl-2,3′-anhydro-2′-deoxythymidin-5′-*C*-ylphosphonate (16). Phosphonate **16** was obtained from dimethyl phosphite (0.18 ml, 2 mmol) and the aldehyde prepared from 2,3′-anhydro-2′-deoxythymidine[34] (673 mg, 3 mmol) according to Method B. Yield, 337 mg (34%; white solid) of **16** (*R*/*S* 37/63). For $C_{12}H_{18}N_2O_7P$ $(M+H)^+$ calcd: 333.0852, found: 333.0844. ^1H NMR—see Tables 4 and 5.

4.1.16. Dimethyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxy-5′-*O*-isopropoxycarbonylthymidin-5′-*C*-ylphosphonate (25). Triphenylphosphine (525 mg, 2 mmol), diethyl-azodicarboxylate (0.52 ml, 2.4 mmol), and diphenylphosphoryl azide (0.47 ml, 2.4 mmol) were sequentially added to a solution of hydroxyphosphonate **12b** (*R*/*S* 18/82) (589 mg, 1 mmol) in THF (12 ml) at 0 °C. After 30 min (TLC in C-1), the reaction was stopped by addition of abs methanol (1 ml) and the solution was evaporated. Chromatography of the residue on silica gel (elution with a linear gradient of 0–10% ethanol in chloroform) afforded compound **25**. Yield, 453 mg (67%; yellowish foam) of **25** (*R*/*S* 43/57). HR FAB: For $C_{32}H_{44}N_2O_{10}PSi$ $(M+H)^+$ calcd: 675.2503, found: 675.2493. $\nu_{max}(CHCl_3)$ 3390 (w, NH), 3136 (vvw, =C–H), 3092 (w, Ph), 3073 (w, Ph), 3052 (w, Ph), 1710 (s, sh, C=O), 1690 (vs, C=O), 1590 (w, Ph), 1489 (w, Ph), 1428 (m, Ph), 1387 (w, CH_3), 1377 (m, CH_3), 1364 (m, *t*-Bu), 1255 (s, P=O), 1041 (s, COPOC), 998 (m, Ph), 703 (m, Si–O) cm^{-1}. ^1H NMR—see Tables 6 and 7. ^{13}C NMR—see Table 10.

4.1.17. Dimethyl-4-*N*-benzoyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxy-5′-*O*-isopropoxycarbonylcytidin-5′-*C*-ylphosphonate (26). Triphenylphosphine (908 mg, 3.46 mmol), diethyl-azodicarboxylate (0.9 ml, 4.2 mmol), and diphenylphosphoryl azide (0.81 ml, 4.2 mmol) were sequentially added to a solution of hydroxyphosphonate **13** (*R*/*S* 14/86) (1.17 g, 1.73 mmol) in THF (12 ml) at 0 °C. After 30 min (TLC in C-1), the reaction was stopped by addition of abs methanol (1 ml) and the solution was evaporated. Chromatography of the residue on silica gel (elution with a linear gradient of 0–10% ethanol in chloroform) afforded compound **26**. Yield, 453 mg (67%; yellowish foam) of **26** (*R*/*S* 80/20). For $C_{38}H_{45}N_3O_{10}PSi$ $(M-H)^-$ calcd: 762.2612, found: 762.2618. $\nu_{max}(CHCl_3)$ 3405 (w, NH_2), 3073 (m, Ph), 3055 (w, Ph), 2495 (vw, br, P–OH), 1377 (m, CH_3), 1744 (s, C=O, 1698 (s, C=O, 1651 (s, sh), 1642 (s), 1624 (s, sh), 1567 (s), 1487 (vs), 1392 (s), 1363

Table 6. ^1H chemical shifts in compounds **25–41**

Compound	Solvent	H-1′	H-2′	H-2″	H-3′	H-4′	H-5′	Nucleobase
(*S*)-**25**[a–c]	DMSO-d_6	6.35	1.99	2.16	4.44	4.25	4.88	T: 11.35 (NH), 7.95 (H-6), 1.72 (5-Me)
(*R*)-**25**[a–c]	DMSO-d_6	6.38	n	n	4.48	4.03	4.29	T: 11.39 (NH), 7.68 (H-6), 1.76 (5-Me)
(*S*)-**26**[a–d]	DMSO-d_6	6.18	1.67	2.35	4.54	4.50	4.73	C^{Bz}: 11.25 (NH), 8.72 and 7.29 (H-5, H-6)
(*R*)-**26**[a–d]	DMSO-d_6	6.24	2.00	2.30	4.70	n	4.32	C^{Bz}: 11.25 (NH), 8.88 and 7.30 (H-5, H-6)
(*S*)-**27**[a–d]	DMSO-d_6	6.39	2.60	2.35	4.72	4.32	5.05	A^{Bz}: 8.47 (H-2, H-8)
(*S*)-**33**[a,e,f]	DMSO-d_6	6.26	1.97	1.92	4.83	4.36	4.32	T: 11.24 (NH), 8.14 (H-6), 1.70 (5-Me)
(*R*)-**33**[a,e,f]	DMSO-d_6	6.30	1.82	1.53	4.79	4.47	4.49	T: 11.26 (NH), 8.02 (H-6), 1.68 (5-Me)
(*S*)-**35**[a,f,g]	DMSO-d_6	6.27	1.89	1.96	4.74	3.30–3.90		T: 11.25 (NH), 7.85 (H-6), 1.68 (5-Me)
(*R*)-**35**[a,f,g]	DMSO-d_6	6.31	1.50	1.80	4.80	4.30–3.90		T: 11.25 (NH), 7.83 (H-6), 1.65 (5-Me)
(*S*)-**36a**[h]	D_2O	6.27	2.48	2.36	4.71	4.26	3.80	T: 7.72 (H-6), 1.92 (5-Me)
(*R*)-**36b**[i]	D_2O	6.31	2.40	2.33	4.81	4.27	3.99	T: 7.83 (H-6), 1.90 (5-Me)
(*S*)-**38**	D_2O	6.24	2.41		4.56	4.13	3.88	T: 7.78 (H-6), 1.91 (5-Me)
(*R*)-**38**	D_2O	6.28	2.44	2.33	4.56	4.20	4.23	T: 7.68 (H-6), 1.88 (5-Me)
(*S*)-**39**	D_2O	6.20	2.47	2.34	4.49	4.14	3.84	C: 7.98 and 6.06 (H-5, H-6)
(*R*)-**39**	D_2O	6.25	2.44	2.35	4.69	4.27	4.05	C: 8.06 and 6.05 (H-5, H-6)
(*S*)-**40**	D_2O	6.20	2.72	2.49	4.70	4.25	3.89	G: 7.92
(*R*)-**40**	D_2O	6.23	2.75	2.53	4.92	4.36	4.00	G: 8.02
(*S*)-**41**	D_2O	6.43	2.82	2.54	4.73	4.37	3.97	A: 8.32, 8.19 (H-2, H-8)
(*R*)-**41**	D_2O	6.44	2.83	2.56	4.94	4.45	4.05	A: 8.34, 8.21 (H-2, H-8)

n=Chemical shift value was not determined.

[a] 3′-OTBDPS: 7.64 (m, 4H) and 7.55–7.43 (m, 6H) (2×C_6H_5), 1.07 (s, 9H, *t*-Bu).

[b] $P(OMe)_2$: 3.32–3.67 (2×d, 2×3H).

[c] 5′-O–CO–O–*i*Pr: 4.72 (m, 1H, CH), 1.21 and 1.14 (2×s, 2×CH_3).

[d] Bz: 8.02 (m, 2H, *o*-ArH), 7.70 (m, 1H, *p*-ArH), 7.56 (m, 2H, *m*-ArH).

[e] P(OMe): 3.22–3.30 (d, 3H, $J(CH_3,P)\sim10.5$ Hz).

[f] 5′-SO_2CH_3: 2.17–2.54 (s, 3H, CH_3).

[g] BOM: 5.34, 4.59 (2×m, O–CH_2–CH_2–O), 8.02 (m, 2H, *o*-ArH), 7.70 (m, 1H, *p*-ArH) and 7.56 (m, 2H, *m*-ArH) (C_6H_5).

[h] P–O–CH_2–CH_2–O: 4.43 (m, 1H, J(Ha,Hb)=12.2 Hz, J(Ha,Hc)=2.6 Hz, J(Ha,Hd)=11.2 Hz, J(Ha,P)=2.8 Hz, Ha); 4.18 (m, 1H, J(Hb,Ha)=12.2 Hz, J(Hb,Hc)=1.2 Hz, J(Hb,Hd)=2.6 Hz, J(Hb,P)=17.3 Hz, Hb); 3.96 (m, 1H, J(Hc,Ha)=2.6 Hz, J(Hc,Hb)=1.2 Hz, J(Hc,Hd)=12.6 Hz, J(Hc,P)=1.2 Hz, Hc); 3.77 (m, 1H, J(Hd,Ha)=11.2 Hz, J(Hd,Hb)=2.6 Hz, J(Hd,Hc)=12.6 Hz, Hd).

[i] P–O–CH_2–CH_2–O: 4.44 (1H, m, J(Ha,Hb)=2.3 Hz, J(Ha,Hc)=2.4 Hz, J(Ha,Hd)=11.5 Hz, J(Ha,P)=2.4 Hz, Ha); 4.17 (1H, m, J(Hb,Ha)=12.3 Hz, J(Hb,Hc)=0.8 Hz, J(Hb,Hd)=2.5 Hz, J(Hb,P)=17.8 Hz, Hb); 3.98 (1H, m, J(Hc,Ha)=2.4 Hz, J(Hc,Hb)=0.8 Hz, J(Hc,Hd)=12.7 Hz, Hc); 3.80 (1H, m, J(Hd,Ha)=11.5 Hz, J(Hd,Hb)=2.5 Hz, J(Hd,Hc)=12.7 Hz, Hd).

Table 7. Coupling constants in compounds **25**–**41**

Compound	Solvent	1′,2′	1′,2″	2′,2″	2′,3′	2″,3′	3′,4′	4′,5′	4′,P	5′,P
(*S*)-**25**[a–c]	DMSO-d_6	8.3	5.9	13.7	5.1	2.7	2.4	4.4	2.7	11.7
(*R*)-**25**[a–c]	DMSO-d_6	9.0	5.4	n	4.9	1.0	1.0	7.6	7.6	12.2
(*S*)-**26**[b–d]	DMSO-d_6	7.6	5.6	13.7	5.0	1.0	n	2.8	n	12.2
(*R*)-**26**[b–d]	DMSO-d_6	9.0	5.0	13.4	5.0	1.0	n	6.0	n	10.8
(*S*)-**27**[b,c]	DMSO-d_6	7.2	7.2	n	5.0	2.5	3.5	5.6	3.5	11.1
(*S*)-**33**[a,c]	DMSO-d_6	9.3	5.7	13.2	4.8	1.0	1.0	3.4	8.8	10.0
(*R*)-**33**[a,c]	DMSO-d_6	8.8	6.0	12.9	4.8	1.5	2.0	2.0	8.3	11.3
(*S*)-**35**[a]	DMSO-d_6	8.7	5.6	13.4	5.1	2.0	n	n	n	n
(*R*)-**35**[a]	DMSO-d_6	8.8	5.9	13.2	5.0	1.0	n	n	n	n
(*S*)-**36a**[a]	D_2O	6.2	7.9	14.3	2.5	5.9	2.3	8.6	4.5	4.7
(*R*)-**36b**[a]	D_2O	6.3	7.6	14.1	3.2	6.0	2.4	4.1	4.3	5.3
(*S*)-**38**[a]	D_2O	6.6	6.6	n	6.0	6.0	5.1	5.6	3.4	11.7
(*R*)-**38**[a]	D_2O	6.4	6.4	14.2	n	n	n	1.0	4.6	13.9
(*S*)-**39**	D_2O	6.3	5.9	13.9	6.1	6.1	5.8	5.8	4.0	11.2
(*R*)-**39**	D_2O	5.6	6.6	13.8	6.0	6.0	1.0	1.0	5.1	12.7
(*S*)-**40**	D_2O	6.3	6.8	13.7	6.6	5.4	4.6	4.6	2.9	12.0
(*R*)-**40**	D_2O	6.3	6.8	13.7	6.5	5.6	4.2	1.7	1.7	13.4
(*S*)-**41**	D_2O	6.3	6.6	14.4	6.3	3.9	4.7	3.7	3.0	12.7
(*R*)-**41**	D_2O	6.6	6.6	13.8	5.9	3.4	3.0	2.2	2.0	13.7

n=Unresolved multiplet, *J* could not be determined.
[a] $J(6,CH_3)$=1.0–1.3 Hz.
[b] $J(P,OCH_3)$=10.5 Hz.
[c] $J(CH,CH_3)$=6.3 Hz.
[d] $J(5,6)$=7.6 Hz.

(m, *t*-Bu), 1261 (vs), 1185 (s), 1083 (s), 1061 (s), 704 (s, Si–O) cm^{-1}. ^1H NMR—see Tables 6 and 7.

4.1.18. Dimethyl-6-*N*-benzoyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxy-5′-*O*-isopropoxycarbonyladenosin-5′-*C*-ylphosphonate (27). Derivative **27** was prepared from hydroxyphosphonate **15a** (*R*/*S* 24/76) (774 mg, 1.1 mmol) according to the procedure described for compound **25**. Yield, 340 mg (40%; yellowish foam) of **27** (*R*/*S* 84/16). For $C_{39}H_{45}N_5O_9PSi$ $(M-H)^-$ calcd: 786.2725, found: 786.2723. $\nu_{max}(CHCl_3)$ 3413 (vw, NH_2), 3072 (w, Ph), 3059 (w, Ph), 3030 (w, Ph), 1750 (m, C=O), 1645 (m, NH), 1612 (m, base), 1582 (m, base), 1591 (m, sh, Ph), 1561 (w, sh, Ph), 1489 (w, Ph), 1428 (m, Ph), 1390 (m, sh, *t*-Bu), 1363 (m, *t*-Bu),1258 (vs, P=O), 1063 (s, COPOC), 1041 (s, COPOC), 703 (s, Si–O) cm^{-1}. ^1H NMR—see Tables 6 and 7.

4.1.19. [1-(2-Deoxy-3,5-*O*-isopropyliden-β-D-*threo*-pentofuranosyl)-5-methylpyrimid-2-on-4-yl] iminotriphenylphosphorane (32). Phosphorane **32** was prepared from 3′,5′-*O*-isopropylidene derivative **30**[35] (140 mg, 0.5 mmol) according to the procedure described for compound **25**. Yield, 73 mg (27%; yellowish solid) of the derivative **32**. For $C_{31}H_{33}N_3O_4P$ $(M+H)^+$ calcd: 542.2208, found: 542.2203. $\nu_{max}(CHCl_3)$ 3102 (w, =C–H), 3080 (w, =C–H), 3062 (w, =C–H), 1738 (s, C=O), 1729 (s, C=O), 1708 (m, sh, C=O), 1632 (m, base), 1592 (w, base), 1562 (m, base), 1484 (w, base), 1438 (s, CH_3), 1083 (s), 1120 (vs), 1162 (s), 1175 (s), 1195 (s) cm^{-1}. ^1H NMR, δ_H (500 MHz, $CDCl_3$) 8.20 (1H, q, *J* 1.3 Hz, H-6); 7.65–7.48 (15H, m, 3×Ph), 6.32 (1H, dd, *J* 1.2, 7.6 Hz, H-1′), 4.55 (1H, bdd, *J* 4.5, 2.6, <1 Hz, H-3′), 4.30 (1H, dd, *J* 13.8, 1.3 Hz, H-5′a), 4.26 (1H, dd, *J* 13.8, 2.1 Hz, H-5′b), 4.01 (1H, m, *J* 2.6, 2.1, 1.3 Hz, H-4′), 2.75 (1H, ddd, *J* 15.3, 7.6, 4.5 Hz, H-2″), 2.46 (3H, d, *J* 1.3 Hz, 5-CH_3), 2.36 (1H, bdd, *J* 15.3, 1.2, <1 Hz, H-2′), 1.51 (3H, br s, CH_3), 1.35 (3H, br s, CH_3); δ_C (125.7 MHz, $CDCl_3$+CD_3OD 9/1) 151.84 (C-2), 142.33 (C-4), 132.43 (C-6), 131.12 (d, *J*(C,P) 3.0 Hz, 3×*p*-C, 3×Ph), 131.81 (d, *J*(C,P) 10.3 Hz, 6×*m*-C, 3×Ph), 131.0 (3×*i*-C, 3×Ph), 128.49 (d, *J*(C,P) 12.2 Hz, 6×*o*-C, 3×Ph), 101.91 (C-5), 97.97 (>C<), 86.88 (C-1′), 76.22 (C-4′), 68.19 (C-3′), 60.28 (C-5′), 40.91 (C-2′), 28.72 (CH_3), 18.25 (CH_3), 12.92 (5-CH_3).

4.1.20. Methyl-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxy-5′-*O*-methansulfonylthymidin-5′-*C*-ylphosphonate (33). Phosphonate **12b** (425 mg, 0.72 mmol) dried by co-distillation with pyridine (2×15 ml) was treated with mesyl chloride (0.28 ml, 3.6 mmol) in pyridine (7 ml) at 0 °C until the starting compound disappeared (TLC in C-1 and H-1). The reaction was quenched by addition of water (1 ml). Chloroform (50 ml) was added, the organic layer was washed by saturated solution of sodium hydrogencarbonate, dried over anhydrous Na_2SO_4 and evaporated. The residue was treated with 60% aqueous pyridine (20 ml) at 50 °C for 16 h (TLC in C1 and H1). After complete demethylation the solution was concentrated in vacuo, the residue was co-distilled with ethanol and treated with Dowex 50×2 (Et_3N^+) in 75% aqueous ethanol to remove *N*-methylpyridinium cations. Product **33** was purified on silica gel column using elution with a linear gradient of H-3 in ethyl acetate and followed by a linear gradient of H-1 in H-3. Yield, 433 mg (79%; yellowish foam) of triethylammonium salt of the derivative **33**. For $C_{28}H_{37}N_2NaO_{10}PSSi$ $(M+Na)^+$ calcd: 675.1574, found: 675.1572. ^1H NMR—see Tables 6 and 7.

4.1.21. (2-Hydroxyethyl)-3′-*O*-*tert*-butyldiphenylsilyl-2′-deoxy-5′-*O*-methansulfonylthymidin-5′-*C*-ylphosphonate (35). A mixture of the derivative **33** (433 mg, 0.57 mmol), 2-*O*-benzoyloxyethanol (246 mg, 1.48 mmol), and 4-methoxypyridine-*N*-oxide (MPNO) (1.32 g, 10.53 mmol) dried by co-distillation with toluene and dichloromethane was treated with MSNT (1.01 g, 3.42 mmol) in dichloromethane (7 ml) (TLC in C-1 and H-1). The reaction was quenched by addition of a saturated solution of sodium hydrogencarbonate.

Chloroform (50 ml) was added, organic layer was washed with saturated solution of sodium hydrogencarbonate, dried over anhydrous Na_2SO_4, and evaporated. Mixed diester **34** was heated with 60% aqueous pyridine (20 ml) at 50 °C for 16 h (TLC in C1 and H1). The solution was concentrated in vacuo, and the residue was co-distilled with ethanol and treated with Dowex 50×2 (Et_3N^+) in 75% aqueous ethanol to remove *N*-methylpyridinium cations. Product **35** was purified on silica gel column using elution with a linear gradient of H3 in ethyl acetate and followed by a linear gradient of H1 in H3. Yield, 493 mg (97%; yellowish foam) of **35** (Et_3NH^+ salt). For $C_{36}H_{43}N_2NaO_{12}PSSi$ $(M+Na)^+$ calcd: 809.1941, found: 809.1935. ^1H NMR—see Tables 6 and 7.

4.1.22. (5*S*)-[*P*→2″-*O*-*cyclo*]-2′-Deoxy-5′-*O*-(2″-hydroxyethyl)thymidin-5′-*C*-ylphosphonate (36a) and (5*R*)-[*P*→2″-*O*-*cyclo*]-2′-deoxy-5′-*O*-(2″-hydroxyethyl)thymidin-5′-*C*-ylphosphonate (36b). The solution of monoester **35** (493 mg, 0.55 mmol) in 1 M sodium methoxide was heated at 55 °C for 4 d (checked by RP-HPLC). The reaction mixture was treated with Dowex 50 (Et_3NH^+) and products were purified on RP-HPLC. Yield, 15 mg (8%; white solid) of (*S*)-epimer **36a** and 17 mg (9%; white solid) of (*R*)-epimer **36b**. For $C_{12}H_{17}N_2NaO_8P$ $(M+Na)^+$ calcd: 371.0620, found: 371.0618 for **36a** and 371.0629 for **36b**. ^1H NMR—see Tables 6 and 7. ^{13}C NMR—see Table 10.

4.1.23. 2′-Deoxythymidin-5′-*C*-ylphosphonic acid (38). Phosphonic acid **38** was prepared from phosphonate **12c** (*R*/*S* 19/81) (1.161 g, 1.88 mmol) by consecutive application of Methods C, E and G. Yield, 454 mg (75%) of **38** (Na^+ salt; white lyophilizate) (*R*/*S* 18/82). For $C_{10}H_{14}N_2O_8P$ $(M-H)^-$ calcd: 321.0488, found: 321.0494. ν_{max}(KBr) 3417 (m, vbr, NH_2), 3270 (m, vbr, sh, OH), 3067 (m, =C–H), 2818 (m, br), 1696 (vs, br, C=O), 1479 (m, base), 1448 (w, base), 1410 (w, sh, base), 1388 (w, sh, base), 1372 (w, base), 1278 (m, base), 1077 (s, br, C–OH), 1055 (s, br, C–OH), 962 (m, PO_3^{-2}), 902 (m, br, PO_3^{-2}) cm^{-1}. ^1H NMR—see Tables 6 and 7.

4.1.24. 2′-Deoxycytidin-5′-*C*-ylphosphonic acid (39). Phosphonic acid **39** was prepared from phosphonate **13** (*R*/*S* 15/85) (162 mg, 0.24 mmol) by consecutive application of Methods C, D, E and G. Yield, 55 mg (75%) of **39** (Na^+ salt; white lyophilizate) (*R*/*S* 15/85). For $C_9H_{13}N_3O_7P$ $(M-H)^-$ calcd: 306.0491, found: 306.0498. ν_{max}(KBr) 3429 (vs, vbr, NH_2 or OH), 3200 (m, br, sh, NH_2 or OH), 1646 (s, C=O), 1612 (m, sh, base), 1530 (w, base), 1494 (m, base), 1294 (w, C–NH_2), 1249 (w, base), 1086 (m, C–OH), 1048 (m, sh, C–OH), 968 (w, PO_3^{-2}), 897 (m, br, PO_3^{-2}) cm^{-1}. ^1H NMR—see Tables 6 and 7.

4.1.25. 2′-Deoxyguanosin-5′-*C*-ylphosphonic acid (40). Phosphonic acid **40** was prepared from phosphonate **14b** (*R*/*S* 24/76) (1.56 g, 2.17 mmol) by consecutive application of Methods C, D, E and G. Yield, 158 mg (21%) of **40** (Na^+ salt; white lyophilizate) (*R*/*S* 29/71). For $C_{10}H_{13}N_5O_7P$ $(M-H)^-$ calcd: 346.0553, found: 346.0553. ν_{max}(KBr) 3417 (s, br, NH_2 or OH), 3120 (m, br, NH_2 or OH), 2765 (m, br, NH_2 or OH), 1694 (vs, C=O), 1607 (m, base), 1580 (w, sh, base), 1535 (w, base), 1483 (w, base), 1410 (w, base), 1179 (w, base), 1074 (m, vbr, C–OH), 968 (w, PO_3^{-2}), 899 (w, br, PO_3^{-2}) cm^{-1}. ^1H NMR—see Tables 6 and 7.

4.1.26. 2′-Deoxyadenosin-5′-*C*-ylphosphonic acid (41). Phosphonic acid **41** was prepared from phosphonate **15b** (*R*/*S* 22/78) (234 mg, 0.33 mmol) by consecutive application of Methods C, D and G. Yield, 89 mg (81%) of **41** (Na^+ salt; white lyophilizate) (*R*/*S* 17/83). For $C_{10}H_{13}N_5O_6P$ $(M-H)^-$ calcd: 330.0604, found: 330.0602. ν_{max}(KBr) 3424 (s, vbr, NH_2 or OH), 3262 (v, br, NH_2 or OH), 3108 (s, br, NH_2 or OH), 3155 (m, br, sh NH or OH), 2675 (w, br, sh, NH_2 or OH), 1603 (m, sh, ring), 1581 (w, base), 1507 (w, sh, base), 1478 (m, base), 1429 (w, base), 1334 (w, base), 1306 (w, base), 1193 (C–OH), 1083 (vs, C–OH), 997 (m, PO_3^{-2}), 907 (w, PO_3^{-2}), 796 (w, base), 739 (w, br, base), 641 (w, base) cm^{-1}. ^1H NMR—see Tables 6 and 7.

4.1.27. Diethyl-2′,3′-*O*-isopropylidene-3-*N*-methoxymethyluridine-5′-*C*-ylphosphonate (48). Phosphonate **48** was obtained from diethyl phosphite (0.258 ml, 2 mmol) and the aldehyde prepared from 2′,3′-*O*-isopropyliden-3-*N*-methoxymethyluridine[36] (328 mg, 1 mmol) according to Method B. Yield, 144 mg (31%; yellowish foam) of **48** (*R*/*S* 30/70). For $C_{18}H_{29}N_2O_{10}P$ (464.40) calcd: 46.55% C, 6.29% H, 6.03% N; found: 46.31% C, 6.35% H, 5.90% N. MS (FAB): 465.1 $(M+H)^+$. ^1H NMR—see Tables 8 and 9.

4.1.28. Diethyl-4-*N*-benzoyl-2′,3′-*O*-isopropylidenecytidine-5′-*C*-ylphosphonate (50). Phosphonate **50** was obtained from diethyl phosphite (0.258 ml, 2 mmol) and the aldehyde prepared from 4-*N*-benzoyl-2′,3′-*O*-isopropylidenecytidine[37] (390 mg, 1 mmol) according to Method B. Yield, 298 mg (57%; yellowish foam) of **50** (*R*/*S* 43/57). For $C_{23}H_{30}N_3O_9P$ (523.48) calcd: 52.77% C, 5.78% H, 8.03% N; found: 52.44% C, 5.72% H, 7.89% N. MS (FAB): 524.1 $(M+H)^+$. ^1H NMR—see Tables 8 and 9.

4.1.29. Dimethyl-2-*N*-benzoyl-2′,3′-*O*-isopropylideneguanosin-5′-*C*-ylphosphonate (51). Phosphonate **51** was obtained from dimethyl phosphite (0.183 ml, 2 mmol) and the aldehyde prepared from 2-*N*-benzoyl-2′,3′-*O*-isopropylideneguanosine[38] (427 mg, 1 mmol) according to Method B. Yield, 353 mg (66%; white foam) of **51** (*R*/*S* 40/60). For $C_{22}H_{27}N_5O_9P$ $(M+H)^+$ calcd: 536.1546, found: 536.1554. ^1H NMR—see Tables 8 and 9.

4.1.30. Diethyl-6-*N*-benzoyl-2′,3′-*O*-isopropylideneadenosin-5′-*C*-ylphosphonate (52). Phosphonate **52** was obtained from diethyl phosphite (0.258 ml, 2 mmol) and the aldehyde prepared from 6-*N*-benzoyl-2′,3′-*O*-isopropylideneadenosine[39] (411 mg, 1 mmol) according to Method B. Yield, 175 mg (32%; white foam) of **52** (*R*/*S* 33/67). For $C_{24}H_{30}N_5O_8P$ (547.50) calcd: 52.65% C, 5.52% H, 12.79% N; found: 52.34% C, 5.57% H, 12.86% N. MS (FAB): 548.5 $(M+H)^+$. ^1H NMR—see Tables 8 and 9.

4.1.31. Dimethyl-6-*N*-benzoyl-2′,3′-*O*-isopropylideneadenosin-5′-*C*-ylphosphonate (53). Phosphonate **53** was obtained from dimethyl phosphite (0.183 ml, 2 mmol) and aldehyde prepared from 6-*N*-benzoyl-2′,3′-*O*-isopropylideneadenosine[39] (411 mg, 1 mmol) according to Method B. Yield 218 mg (42%; white foam) of **53** (*R*/*S* 17/83). For $C_{22}H_{27}N_5O_8P$ $(M+H)^+$ calcd: 520.0597, found: 520.0594. ^1H NMR—see Tables 8 and 9.

4.1.32. 3-*N*-Methoxymethyluridin-5′-*C*-ylphosphonic acid (54). Phosphonic acid **54** was prepared from phosphonate

Table 8. ^{1}H chemical shifts in compounds **48–62**

Compound	Solvent	H-1′	H-2′	H-3′	H-4′	H-5′	5′-OH	Nucleobase
(*S*)-**48**[a–c]	DMSO-d_6	5.87	4.97	4.99	4.22	4.07	6.23	U^{MeM}: 7.84 (H-6), 5.78 (H-5)
(*R*)-**48**[a–c]	DMSO-d_6	5.92	4.84	4.87	4.33	4.15	6.39	U^{MeM}: 8.07 (H-6), 5.79 (H-5)
(*S*)-**50**[b–d]	DMSO-d_6	5.88	4.97	5.02	4.33	4.21	6.20	C^{Bz}: 11.32 (NH), 8.28 (H-6), 7.35 (H-5)
(*R*)-**50**[b–d]	DMSO-d_6	5.91	4.85	4.89	4.43	4.18	6.22	C^{Bz}: 11.30 (NH), 8.59 (H-6), 7.33 (H-5)
(*S*)-**51**[b,d,e]	DMSO-d_6	6.13	5.39	5.27	4.30	3.98	6.30	G^{Bz}: 11.32 (NH), 8.24 (H-8)
(*R*)-**51**[b,d,e]	DMSO-d_6	6.16	5.23	5.16	4.40	4.26	6.29	G^{Bz}: 11.33 (NH), 8.36 (H-8)
(*S*)-**52**[b–d]	DMSO-d_6	6.29	5.46	5.23	4.41	4.03	n	A^{Bz}: 11.23 (NH), 8.77, 8.65 (H-2, H-8)
(*R*)-**52**[b–d]	DMSO-d_6	6.31	5.29	5.08	4.53	4.22	n	A^{Bz}: 11.22 (NH), 8.82, 8.76 (H-2, H-6)
(*S*)-**53**[b,d,e]	DMSO-d_6	6.29	5.46	5.23	4.41	4.03	n	A^{Bz}: 11.23 (NH), 8.77, 8.65 (H-2, H-8)
(*R*)-**53**[b,d,e]	DMSO-d_6	6.31	5.29	5.08	4.43	4.22	n	A^{Bz}: 11.23 (NH), 8.81, 8.75 (H-2, H-8)
(*S*)-**54**[a]	D_2O	5.98	4.41	4.50	4.47	4.14	—	U^{MeM}: 8.13 (H-6), 6.00 (H-5)
(*R*)-**54**[a]	D_2O	5.92	4.40	4.33	4.35	3.99	—	U^{MeM}: 8.11 (H-6), 5.98 (H-5)
(*S*)-**55**	D_2O	5.91	4.39	4.45	4.45	4.10	—	U: 8.15 (H-6)
(*R*)-**55**	D_2O	5.87	4.39	4.24	4.29	3.87	—	U: 7.98 (H-6)
(*S*)-**56**	D_2O	5.89	4.32	4.39	4.47	4.12	—	C: 8.16 (H-6), 6.04 (H-5)
(*R*)-**56**	D_2O	5.84	4.34	4.15	4.26	3.83	—	C: 7.89 (H-6), 6.04 (H-5)
(*S*)-**57**	D_2O	5.87	4.73	4.71	4.53	4.04	—	G: 8.02 (H-8)
(*R*)-**57**	D_2O	5.83	4.68	5.15	5.13	3.94	—	G: 7.91 (H-8)
(*S*)-**58**	D_2O	6.06	4.75	4.71	4.59	4.06	—	A: 8.42, 8.21 (H-2, H-8)
(*R*)-**58**	D_2O	6.08	4.74	4.46	4.41	3.90	—	A: 8.32, 8.19 (H-2, H-8)
(*S*)-**60**[b,c,f]	DMSO-d_6	5.90	4.90	4.96	4.43	3.99	5.85	—
(*R*)-**60**[b,c,f]	DMSO-d_6	5.91	4.88	5.20	4.53	4.16	6.10	—
(*R*)-**62(β)**[c,f,g]	DMSO-d_6	6.09	5.37	5.73	4.72	5.57	n	—
(*R*)-**62(α)**[c,f,g]	DMSO-d_6	6.39	5.32	5.82	4.74	5.52	n	—

n=Not detected.
[a] MeM: 5.16–5.37 (s or 2×d, 2H, N–CH_2), 3.26–3.44 (s, 3H, OCH_3).
[b] –O–C(CH_3)$_2$–O–: 1.29–1.58 (2×s, 2×3H).
[c] P(OEt)$_2$: 3.95–4.10 (2×m, 2×2H, 2×CH_2), 1.04–1.25 (2×t, 2×3H, 2×CH_3).
[d] NBz: 7.87–8.05 (m, 2H), 7.52–7.70 (m, 1H), 7.44–7.56 (m, 2H).
[e] P(OMe)$_2$: 3.60–3.62 and 3.51–3.52 (2×d, 2×3H).
[f] 3′-OBz: 8.02 (m, 2H), 7.70 (m, 1H), 7.56 (m, 2H).
[g] 1′,2′,3′-triOAc: 1.85–2.17 (3×s, 3×3H).

48 (*R*/*S* 30/70) (600 mg, 1.29 mmol) by consecutive application of Methods C, F and G. Yield, 461 mg (97%) of **54** (Na^+ salt; white lyophilizate) (*R*/*S* 45/55). For $C_{11}H_{16}N_2O_{10}P$ $(M-H)^-$ calcd: 367.0543, found: 367.0541. ν_{max}(KBr) 3417 (s, NH_2 or OH), 3272 (s, vbr, sh, NH_2 or OH), 3108 (m, NH_2 or OH), 2970 (m, CH_3), 2836 (m, CH_3), 1714 (s, C=O), 1660 (vs, br, C=O), 1629 (s, sh, base), 1463 (s, base), 1414 (m, base), 1115 (s, COC), 1083 (s, C–OH), 1056 (s, sh, C–OH), 972 PO_3^{-2}), 912 (m, PO_3^{-2}) cm^{-1}. ^{1}H NMR—see Tables 8 and 9.

Table 9. Coupling constants in compounds **48–62**

Compound	Solvent	1′,2′	2′,3′	3′,4′	4′,5′	4′,P	5′,P	5′,OH	P,OH
(*S*)-**48**[a–c]	DMSO-d_6	2.2	6.6	2.2	5.6	5.1	8.8	6.8	6.6
(*R*)-**48**[a–c]	DMSO-d_6	2.7	6.4	2.9	3.9	0.5	11.5	7.0	8.8
(*S*)-**50**[a–c]	DMSO-d_6	2.2	6.4	2.4	5.1	4.9	8.8	6.4	6.4
(*R*)-**50**[a–c]	DMSO-d_6	2.2	6.1	2.9	3.4	2.7	10.8	8.0	8.0
(*S*)-**51**[d]	DMSO-d_6	2.7	6.2	2.2	5.7	4.5	8.4	6.7	5.7
(*R*)-**51**[d]	DMSO-d_6	2.7	6.2	2.2	3.8	3.4	11.1	6.9	8.9
(*S*)-**52**[b]	DMSO-d_6	2.9	6.1	2.2	4.9	4.4	9.8	6.8	7.1
(*R*)-**52**[b]	DMSO-d_6	2.9	6.1	2.2	3.2	2.8	12.0	7.3	6.5
(*S*)-**53**[d]	DMSO-d_6	2.9	6.1	2.0	5.0	4.2	9.3	6.6	7.3
(*R*)-**53**[d]	DMSO-d_6	2.9	6.1	2.2	3.4	3.1	12.0	6.3	7.3
(*S*)-**54**[a]	D_2O	4.6	4.9	4.9	1.7	1.7	13.2	—	—
(*R*)-**54**[a]	D_2O	3.4	5.1	6.1	3.4	6.3	12.9	—	—
(*S*)-**55**[a]	D_2O	3.5	n	n	1.5	n	13.0	—	—
(*R*)-**55**[a]	D_2O	3.3	5.3	6.6	5.4	2.9	11.9	—	—
(*S*)-**56**[a]	D_2O	2.7	5.1	7.1	1.7	1.7	12.9	—	—
(*R*)-**56**[a]	D_2O	2.4	5.4	7.1	7.1	2.9	11.2	—	—
(*S*)-**57**	D_2O	5.0	5.5	3.4	2.0	1.2	13.9	—	—
(*R*)-**57**	D_2O	5.5	5.3	4.3	3.6	2.6	12.7	—	—
(*S*)-**58**	D_2O	4.6	5.1	4.6	1.5	1.5	13.9	—	—
(*R*)-**58**	D_2O	4.6	4.9	4.9	5.0	2.4	12.0	—	—
(*S*)-**60**[b]	DMSO-d_6	3.8	5.1	8.5	2.7	2.7	13.0	8.0	8.4
(*R*)-**60**[b]	DMSO-d_6	3.9	5.6	7.6	2.4	3.9	13.0	6.6	12.8
(*R*)-**62(β)**[b]	DMSO-d_6	1.0	5.0	6.6	3.4	2.6	13.1	—	—
(*R*)-**62(α)**[b]	DMSO-d_6	4.2	6.5	2.7	2.6	2.6	12.6	—	—

[a] J(5,6)=7.3–8.3 Hz.
[b] J(CH_2,CH_3)=7.1 Hz.
[c] J(P,OCH_2)=7.3 Hz.
[d] J(P,OCH_3)=10.5 Hz.

4.1.33. Uridin-5′-*C*-ylphosphonic acid (55). Diester **49** was obtained from diethyl phosphite (0.516 ml, 4 mmol) and the aldehyde prepared from 2′,3′-*O*-isopropylideneuridine[40] (568 mg, 2 mmol) according to Method B. This derivative was transformed into free phosphonic acid **55** by consecutive application of Methods C, F and G. Yield, 298 mg (46%) of **55** (Na^+ salt; white lyophilizate) (*R*/*S* 45/55). For $C_9H_{12}N_2O_9P$ $(M-H)^-$ calcd: 323.0280, found: 323.0290. ν_{max}(KBr) 3418 (s, vbr, NH_2 or OH), 3248 (s, vbr, sh, NH_2 or OH), 2806 (m, vbr, NH_2 or OH), 1782 (m, sh, base), 1699 (vs, vbr, C═O), 1628 (s, sh, base), 1470 (m, base), 1441 (m, base), 1398 (m, base), 1274 (m, base), 1105 (s, br, OH), 1058 (s, br, C–OH), 969 (m, PO_3^{-2}), 921 (w, PO_3^{-2}) cm^{-1}. ^1H NMR—see Tables 8 and 9. ^{13}C NMR—see Table 10.

4.1.34. Cytidin-5′-*C*-ylphosphonic acid (56). Phosphonic acid **56** was prepared from phosphonate **50** (*R*/*S* 43/57) (342 mg, 0.65 mmol) by consecutive application of Methods C, D, F and G. Yield, 105 mg (50%) of **56** (Na^+ salt; white lyophilizate) (*R*/*S* 44/56). For $C_9H_{13}N_3O_8P$ $(M-H)^-$ calcd: 322.0440, found: 322.0435. ν_{max}(KBr) 3427 (vs, br, NH_2 or OH), 3206 (m, br, sh, NH_2 or OH), 1652 (s, C═O), 1610 (m, base), 1521 (w, base), 1499 (m, base), 1287 (w, C–NH_2), 1253 (w, base), 1211 (w, base), 1185 (w, br, base), 1120 (m, base), 1089 (m, C–OH), 1043 (m, C–OH), 971 (w, PO_3^{-2}), 899 (w, br, PO_3^{-2}) cm^{-1}. ^1H NMR—see Tables 8 and 9.

4.1.35. Guanosin-5′-*C*-ylphosphonic acid (57). Phosphonic acid **57** was prepared from phosphonate **51** (*R*/*S* 40/60) (700 mg, 1.3 mmol) by consecutive application of Methods C, D, F and G. Yield 378 mg (80%) of **57** (Na^+ salt; white lyophilizate) (*R*/*S* 44/56). For $C_{10}H_{13}N_5O_8P$ $(M-H)^-$ calcd: 362.0503, found: 362.0504. ν_{max}(KBr) 3417 (m, vbr, NH_2 or OH), 3119 (m, vbr, NH_2 or OH), 2766 (w, br, NH_2 or OH)), 1698 (vs, C═O), 1550 (m, base), 1582 (w, sh, base), 1536 (w, base), 1485 (w, base), 1412 (w, base), 1102 (w, base), 1180 (w, base), 1070 (m, vbr, C–OH), 972 (w, PO_3^{-2}) cm^{-1}. ^1H NMR—see Tables 8 and 9.

4.1.36. Adenosin-5′-*C*-ylphosphonic acid (58). Phosphonic acid **58** was prepared from phosphonate **52** (*R*/*S* 33/67) (175 mg, 0.32 mmol) by consecutive application of Methods C, D, F and G. Yield, 85 mg (77%) of **58** (Na^+ salt; white lyophilizate) (*R*/*S* 40/60). For $C_{10}H_{13}N_5O_7P$ $(M-H)^-$ calcd: 346.0553, found: 346.0553. ν_{max}(KBr) 3419 (vs, br, NH_2 or OH), 3265 (s, br, sh, NH_2 or OH), 3217 (s, br, NH_2 or OH), 3120 (m, br, sh, NH_2 or OH), 2675 (w, br, sh, NH_2 or OH), 1606 (m, sh, base), 1579 (w, base), 1505 (w, sh, base), 1476 (m, base), 1419 (w, base), 1334 (w, base),

Table 10. ^{13}C NMR data of compounds **12e**, **12f**, **14a**, **25**, **36** and **55**

Compound	Solvent	C-1′	C-2′	C-3′ *J*(C,P)	C-4′ *J*(C,P)	C-5′ *J*(C,P)	Nucleobase
(*S*)-**12b**[a]	DMSO-d_6	85.03	~39.7	75.51 d (15.1)	86.86	66.68 (164.3)	T: 163.86 (C-4); 150.59 (C-2); 136.34 (C-6); 109.29 (C-5); 12.41 (5-CH_3)
(*S*)-**12e**[b]	DMSO-d_6	84.80	39.54	73.49 d (14.6)	86.72 (~0)	66.69 d (165.0)	T: 163.99 (C-4); 150.66 (C-2); 136.57 (C-6); 109.28 (C-5); 12.51 (5-CH_3)
(*R*)-**12e**[c]	DMSO-d_6	84.03	39.54	73.07 (4.9)	86.89 (10.8)	63.72 (159.2)	T: 163.86 (C-4); 150.75 (C-2); 136.09 (C-6); 109.86 (C-5); 12.32 (5-CH_3)
(*S*)-**12f**[d]	DMSO-d_6	84.82	37.34	76.86 d (14.6)	83.97 (~0)	67.12 d (165.0)	T: 163.89 (C-4); 150.62 (C-2); 136.35 (C-6); 109.56 (C-5); 12.48 (5-CH_3)
(*R*)-**12f**[e]	DMSO-d_6	84.60	38.03	73.96	83.66 (10.2)	68.13 (160.6)	T: 164.13 (C-4); 150.62 (C-2); 135.96 (C-6); 111.36 (C-5); 12.17 (5-CH_3)
(*S*)-**14a**[f]	DMSO-d_6	83.98	41.18	75.74 (14.6)	87.44 (~0)	66.74 (163.1)	G^{Bz}: 169.32, 132.53, 128.71(2), 128.73(2), 133.24 (NBz); 155.27 (C-6), 148.55 (C-4), 139.39 (C-2), 137.86 (C-8), 120.57 (C-5)
(*R*)-**14a**[g]	DMSO-d_6	83.27	38.61	74.97 (8.8)	87.54 (6.8)	67.11 (160.2)	G^{Bz}: 169.35, 132.53, 128.71(2), 128.76(2), 133.35 (NBz); 155.28 (C-6), 149.06 (C-4), 139.40 (C-2), 138.22 (C-8), 120.84 (C-5)
(*S*)-**25**[h]	DMSO-d_6	84.28*	38.75	73.60 d (9.2)	84.25*	70.17 d (163.5)	T: 163.68 (C-4), 150.56 (C-2), 135.44 (C-6), 109.82 (C-5), 12.23 (5-CH_3)
(*S*)-**36a**[i]	D_2O	89.14	41.54	74.38	88.38 d (5.9)	80.38 d (140.1)	T: 169.49 (C-4), 154.50 (C-2), 140.53 (C-6), 113.92 (C-5), 14.52 (5-CH_3)
(*R*)-**36b**[j]	D_2O	87.91	41.54	73.63 d (3.4)	88.11 d (6.8)	80.44 d (141.1)	T: 169.51 (C-4), 154.65 (C-2), 140.60 (C-6), 114.01 (C-5), 14.56 (5-CH_3)
(*S*)-**55**	D_2O	88.71	74.75	67.42	84.94 d (12.7)	69.48 d (142.6)	U: 166.36 (C-4), 151.69 (C-2), 141.87 (C-6), 101.92 (C-5)
(*R*)-**55**	D_2O	90.02	74.28	70.14	84.10 d (3.9)	69.71 d (148.4)	U: 166.41 (C-4), 151.61 (C-2), 142.23 (C-6), 101.83 (C-5)

[a] $P(OCH_3)_2$: 52.99 and 52.53 (2×d, *J*(C,P)=6.8 Hz); TBDPS: 135.44(4), 132.90, 132.78, 130.38, 130.35, 128.28(2) and 128.25(2) (2×C_6H_5), 26.87(3) and 18.76 (*t*-Bu).
[b] $P(OCH_3)_2$: 53.17 and 52.63 (2×d, *J*(C,P)=6.8 Hz); TBDMS: 25.86(3) and 17.83 (*t*-Bu), 4.65 and 4.52 (2×Me).
[c] $P(OCH_3)_2$: 53.32 and 52.62 (2×d, *J*(C,P)=6.8 Hz); TBDMS: 25.78(3) and 17.70 (*t*-Bu), 4.69 and 4.52 (2×Me).
[d] OBz: 165.48 (CO); 133.86(1), 129.60(2), 129.42 (1) and 129.00(2) (C_6H_5); 53.19 and 52.66 (2×d, *J*(C,P)=6.8 Hz, $P(OCH_3)_2$).
[e] $P(OCH_3)_2$: 53.87 and 53.24 (2×d, *J*(C,P)=7.3 Hz); OBz: 165.58 (CO); 133.44(1), 129.61(2), 129.24 (1) and 128.42(2) (C_6H_5).
[f] $P(OCH_3)_2$: 53.03 and 52.41 (2×d, *J*(C,P)=5.9 Hz); TBDPS: 135.51(2), 135.48(2), 132.90, 132.75, 130.46, 130.42, 128.34(2) and 128.32(2) (2×C_6H_5), 26.94(3) and 18.81 (*t*-Bu).
[g] $P(OCH_3)_2$: 52.88 and 52.34 (2×d, *J*(C,P)=6.8 Hz); TBDPS: 135.64(2), 135.56(2), 132.79, 132.25, 130.31, 130.21, 128.22(2) and 128.12(2) (2×C_6H_5), 26.96(3) and 18.90 (*t*-Bu).
[h] O–CO–O*i*Pr: 153.08 (d, *J*(C,P)=4.6 Hz, O–CO–O); 73.35, 21.40 and 21.25 (*i*Pr); TBDPS: 135.44(4), 132.60, 132.42, 130.38, 130.36, 128.20(2) and 128.18(2) (2×C_6H_5); 26.79(3) and 18.76 (*t*-Bu); $P(OCH_3)_2$: 53.58 (d, *J*(C,P)=6.4) and 53.24 (d, *J*(C,P)=6.0 Hz).
[i] P–O–CH_2–CH_2–O: 71.81 (d, *J*(C,P)=6.8 Hz); 70.15 (d, *J*(C,P)=3.4 Hz).
[j] P–O–CH_2–CH_2–O: 71.80 (d, *J*(C,P)=6.8 Hz); 70.51 (d, *J*(C,P)=2.9 Hz).
* The assignment of signals may be interchanged.

1297 (w, base) 1102 (vs, C–OH), 1069 (vs, C–OH), 989 (m, PO_3^{-2}), 905 (PO_3^{-2}) cm^{-1}. ^1H NMR—see Tables 8 and 9.

4.1.37. (*R*)-Adenosin-5′-*C*-ylphosphonic acid ((*R*)-58). 6-*N*-Benzoyladenine (1.6 g, 6.7 mmol) in hexamethyldisilazane (67 ml, 315 mmol) was refluxed in the presence of trimethylsilyl chloride (6.7 ml, 53.6 mmol) at 150 °C under stirring and exclusion of moisture for 10 h. Volatiles were removed under reduced pressure and the resulting oil was co-distilled with xylene (2×50 ml) and acetonitrile (2×50 ml) to remove traces of HMDS. A solution of diethyl phosphonate (*R*)-**62** (2.871 g, 5.56 mmol) (dried by co-distillation with acetonitrile) in acetonitrile (2×20 ml) was added under argon to the silylated base and then tin tetrachloride (11.12 mmol, 1.31 ml) was added. The mixture was kept at rt for 24 h (TLC in C1 and H1). The reaction mixture was then diluted with pyridine (2 ml) and toluene (40 ml) and stirred overnight. The thick suspension (precipitated complex of tin tetrachloride with pyridine) was filtered through Celite, washed with chloroform, and the solvents were removed under diminished pressure. All protecting groups of the product (*R*)-**64** were removed by consecutive application of Methods C, D and G. Yield, 669 mg (35%) of (*R*)-**58** (Na^+ salt; white lyophilizate) (*R*/*S* 100/0). For $C_{10}H_{13}N_5O_7P$ $(M+H)^+$ calcd: 346.0553, found: 346.0550. ^1H NMR—see Tables 8 and 9.

4.1.38. Adenosin-5′-*C*-ylphosphonyl morpholide ((*R*)-59). The sodium salt of adenosine 5′-*C*-phosphonate (*R*)-**58** (10 mg, 0.029 mmol) was converted to pyridinium salt on Dowex 50 (5 ml, Py). Morpholine (0.012 ml, 0.13 mmol) was added to the pyridinium salt, the mixture was evaporated, the residue was co-distilled with ethanol (5×15 ml), dissolved in a mixture of *tert*-butanole–water (2 ml, 1/1) and then morpholine (0.023 ml, 0.26 mmol) and DCC (40 mg, 0.19 mmol) were added to the solution. The reaction mixture was kept under reflux for 15 h. Then volatiles were removed under reduced pressure, the residue was suspended in water (10 ml), filtered through Celite, and the filtrate was extracted with ether (2×10 ml). The aqueous layer was concentrated and the residue was co-distilled with ethanol (2×20 ml) and suspended in 1/1 ethanol–ether mixture. Morpholide (*R*)-**59** was filtered and dried in vacuo. Yield, 22 mg; mixture of (*R*)-**59** and *N*-morpholino-dicyclohexylcarboxamidine (47%, apparent molecular weight calculated from UV spectrum (ε=14,000) was 1522). For $C_{14}H_{20}N_6O_7P$ $(M-H)^-$ calcd: 415.1216, found: 415.1210. δ_H (500 MHz, DMSO-d_6) 8.35 (1H, s, H-2), 8.13 (1H, s, H-8), 7.28 (2H, br s, NH_2), 5.84 (1H, d, *J* 3.2 Hz, H-1′), 5.16 (1H, dd, *J* 5.2, 3.2 Hz, H-2′), 4.17 (1H, dd, *J* 6.3, 5.2 Hz, H-3′), 4.03 (1H, ddd, *J* 6.8, 6.3, 3.4 Hz, H-4′), 3.56 (1H, dd, *J* 9.8, 6.8 Hz, H-5′), 3.69 (2H, m), 3.42 (2H, m), 2.97 (2H, m), 2.93 (2H, m).

4.1.39. (5*R*)-Diethyl-(3-*O*-benzoyl-1,2-*O*-isopropylidene-D-ribofuranos-5-*C*-yl)phosphonate ((*R*)-60) and (5*S*)-diethyl-(3-*O*-benzoyl-1,2-*O*-isopropylidene-D-ribofuranos-5-*C*-yl)phosphonate ((*S*)-60). Benzoyl cyanide (7.2 g, 55 mmol) followed by triethylamine (0.7 ml, 5 mmol) were added to the solution of 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose[41] (13.01 g, 50 mmol, co-distilled with toluene) in acetonitrile (60 ml) at 0 °C, and the reaction mixture was stirred at rt for 15 h (TLC in T1). After addition of abs methanol (2 ml) the solvent was evaporated in vacuo and the residue was partitioned between chloroform (300 ml) and water (3×300 ml). The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was dissolved in 60% acetic acid (600 ml) and the reaction mixture heated at 50 °C for 3 h (TLC in T1 and C1). Acetic acid was evaporated and the product was partitioned between chloroform and water. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The obtained 3-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-allofuranose was dissolved in an acetone–water mixture (7/3, 300 ml) and the solution of sodium periodate (12.84 g, 60 mmol) in the same solvent (250 ml) was added at 0 °C under vigorous stirring (TLC in T1 and C1). When the starting compound disappeared the mixture was diluted with acetone (200 ml), cooled to 0 °C, and filtered through Celite. After evaporation of the solvent the crude aldehyde was co-distilled with toluene and treated with diethyl phosphite (7.74 ml, 66 mmol) in dichlormethane (100 ml) in the presence of triethylamine (2.78 mmol, 20 mmol) at 80 °C for 15 h (TLC in T1 and C1). The reaction mixture was diluted with ethyl acetate, filtered through Celite, and after evaporation of solvent the product was isolated by chromatography on silica gel (elution with a linear gradient of 0–50% ethyl acetate in toluene). Yield, 2.54 g of product (*R*)-**60**, 3.65 g of product (*S*)-**60** and 11.11 g of a mixture of both products (total yield, 64%; all fractions as yellowish oil). For $C_{19}H_{28}O_9P$ $(M+H)^+$ calcd: 431.1471, found: 431.1466 for (*R*)-**60** and 431.1468 for (*S*)-**60**. ^1H NMR—see Tables 8 and 9.

4.1.40. (5*R*)-Diethyl-(1,5-di-*O*-acetyl-3-*O*-benzoyl-D-ribofuranos-5-*C*-yl)phosphonate ((*R*)-62). Sugar phosphonate (*R*)-**60** (3.0 g, 6.97 mmol) dried by co-distillation with pyridine (2×15 ml) was treated with acetic anhydride (3.29 ml, 34.9 mmol) in pyridine (50 ml) in the presence of DMAP (50 mg, 0.41 mmol) at rt under exclusion of moisture until the starting compound disappeared (15 h; TLC in C1 and H1). The reaction was quenched by addition of abs methanol (2 ml), the solvent was evaporated, the residue was diluted with ethyl acetate, and the organic layer was washed with 10% citric acid (3×150 ml) and dried over anhydrous Na_2SO_4. After evaporation of ethyl acetate, the residue (*R*)-**61** was co-distilled with toluene (2×20 ml) and treated with acetic anhydride (3.0 ml, 31.8 mmol), acetic acid (1.9 ml, 31.8 mmol) and sulfuric acid (0.3 ml, 4 mmol) in dichloromethane (20 ml) at 0 °C for 48 h (TLC in C1 and H1). Then anhydrous sodium acetate (470 mg, 5.7 mmol) was added to neutralize sulfuric acid, and the reaction mixture was stirred for 20 min. The solvents were evaporated under oil-pump vacuum at 40 °C in a bath, the residue was dissolved in ethyl acetate, and washed with a saturated solution of sodium chloride. The organic layer was dried over anhydrous Na_2SO_4. Chromatography of the crude product on silica gel (elution with a linear gradient of acetone in toluene) afforded the expected phosphonate. Yield, 2.871 g (80%; yellowish oil) of product (*R*)-**62** (α/β 1/4). For $C_{22}H_{30}O_{12}P$ $(M+H)^+$ calcd: 517.1475, found: 517.1474. ^1H NMR—see Tables 8 and 9.

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Synthesis of Δ²-OPC-8:0 and OPC-6:0

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Abstract—CuCN-catalyzed reaction of the (1*R*)-isomer of 4-cyclopentene-1,3-diol monoacetate with TBDPSO$(CH_2)_6$MgCl produced an S_N2-type product regioselectively in high yield. Mitsunobu inversion of the product and subsequent Claisen rearrangement furnished aldehyde with the two side chains, from which the title compounds were synthesized efficiently.

1. Introduction

While the biological properties of *epi*-jasmonic acid produced in plants via the linolenic acid cascade (Fig. 1) have been well studied,[1,2] only a little attention has been paid to the upper metabolic intermediates. Tendril coiling response is one such function for 12-oxo-PDA found in 1993.[3] Since then, induction of secondary metabolites,[4] up-regulation of several genes,[5] and expression of the specific genes[6] have been disclosed. Furthermore, the specific enzyme for the β-oxidation was identified.[7] However, the biological profile of OPC-*n*:0 (*n*=8, 6, 4) has not been studied, while biological activity of the trans isomer of 11-*epi*-OPC-6:0 methyl ester in racemic form has been studied to date.[8]

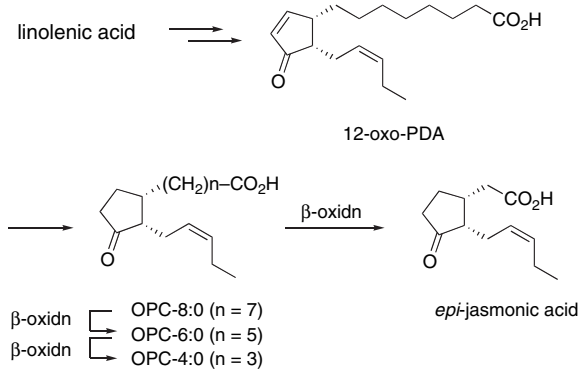

Figure 1. Metabolic sequence to *epi*-jasmonic acid.

Keywords: Allylation; Copper; Stereoselective synthesis; OPC-6:0; Linolenic acid metabolism.
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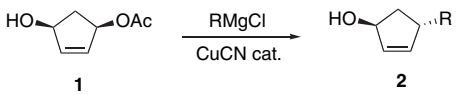
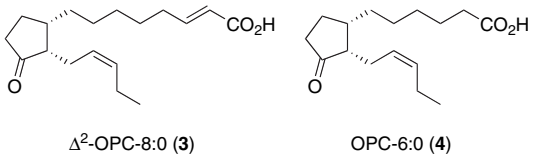
To elucidate the biological function of OPC-*n*:0 (*n*=8, 6, 4), these compounds in chemically pure form are definitely required. In these compounds, the two side chains on the cyclopentane ring are projected in the same direction, and the lower (2*Z*)-pentenyl chain is attached to the α position of the cyclopentanone core. Consequently, synthesis should be designed in a manner that it would not induce isomerization to the thermodynamically more stable trans isomer. However, the previous syntheses[9] of OPC-*n*:0 (*n*=8, 6, 4) have hardly been successful in controlling the stereocenters except for our approach[10] to OPC-8:0, which is briefly described next.

Several years ago, we reported installation of an alkyl group on the ring of 4-cyclopentene-1,3-diol monoacetate (**1**) through CuCN-catalyzed allylation with RMgCl to produce S_N2-type product **2** (Eq. 1).[11,12] The reaction was then applied to the synthesis of OPC-8:0 as well as 12-oxo-PDA with great success.[9] These compounds synthesized as chemically pure forms have been used for the biological studies mentioned above.[6,7]



(1)

Later we studied the synthesis of Δ²-OPC-8:0 (**3**) and OPC-6:0 (**4**) (Fig. 2).[13] The former compound possessing the double bond at the Δ² position would be resistant to the β-oxidation[14] on the analogy of Δ²-prostaglandins,[15] and hence be a useful tool for chemical biology to elucidate the pure property of OPC-8:0.[16] Herein, we present the synthesis in detail.

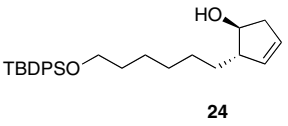


Δ^2-OPC-8:0 (**3**) OPC-6:0 (**4**)

Figure 2. Compounds synthesized through the procedures mentioned in this paper.

2. Results and discussion

According to our original method for the S_N2-type reaction of **1** (Eq. 1),[11] 3 equiv of RMgCl is required to complete the reaction. To reduce the quantity of the reagent **5**, monoacetate (1*R*)-**1** (>99% ee), prepared according to the literature procedure,[17] was treated first with 1 equiv of *t*-BuMgCl at 0 °C (Scheme 1). Subsequently, 2 equiv of TBDPSO$(CH_2)_6$MgCl (**5**) was added to the solution of the resulting magnesium alkoxide at −18 °C. The reaction proceeded with 92% regioselectivity giving the S_N2-type product **6**, which was isolated as a mixture with TBDPSO$(CH_2)_6$OH (calculated yield of **6** by ^{1}H NMR was 81%) after chromatographic separation of the minor regioisomer **24**. Mitsunobu inversion of the mixture using AcOH, DIAD, and PPh_3 in toluene at −78 °C afforded **7** and TBDPSO$(CH_2)_6$OAc, and the mixture was separated by chromatography to afford pure **7** in 77% yield from (1*R*)-**1**. Hydrolysis of the acetate **7** yielded alcohol **8** in high yield. Claisen rearrangement of alcohol **8** with CH_2=CHOEt proceeded at 180–190 °C to afford aldehyde **9**, which upon Jones oxidation produced acid **10** in 90% yield. Lactonization with KI_3 provided iodo-lactone **11**, which underwent reaction with Bu_3SnH to afford lactone **12** in high yield.



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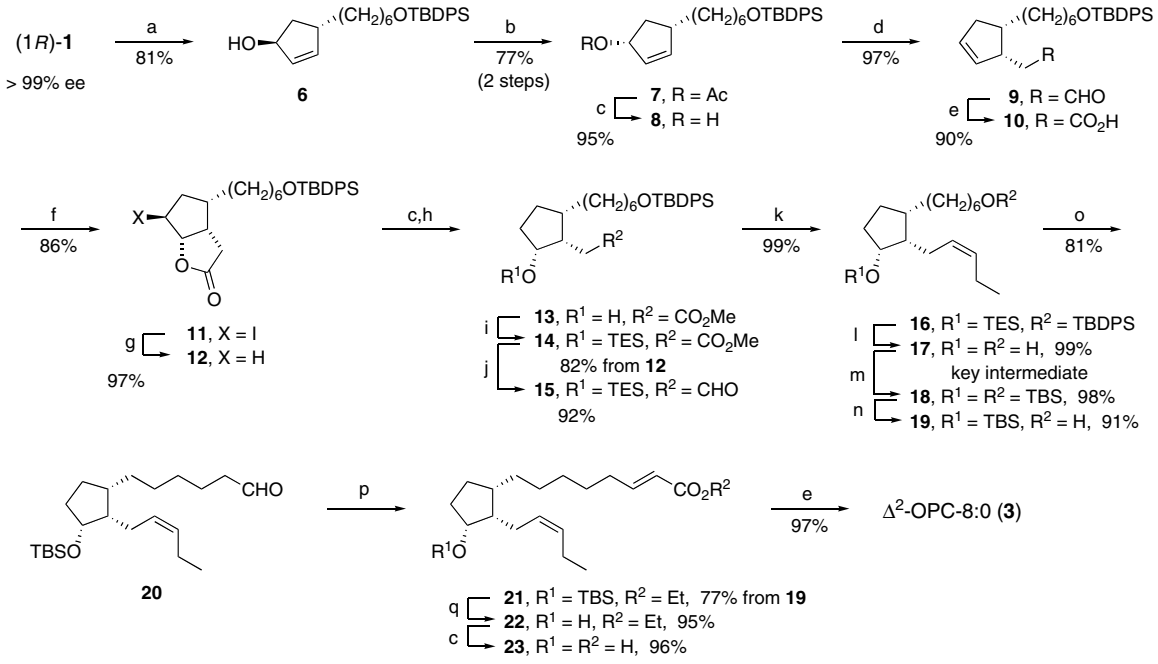
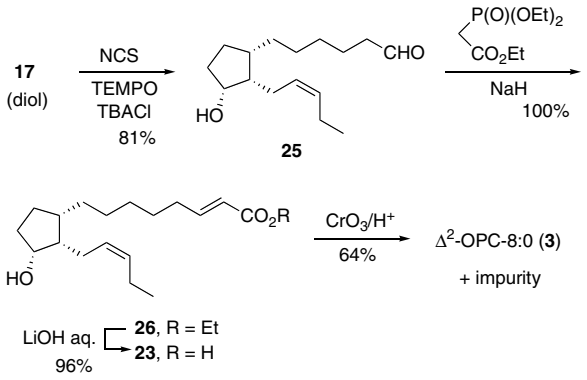
To construct the complete (2*Z*)-pentenyl side chain, lactone **12** was hydrolyzed into the hydroxyl acid, which upon esterification with CH_2N_2 afforded the hydroxyl methyl ester **13**. Subsequently, the silylation of **13** with TESCl immediately after filtration through a short silica gel column produced silyl ether **14** in 82% yield from **12**.[18,19] DIBAL-H reduction of ester **14** followed by Wittig reaction of the resulting aldehyde **15** with Ph_3P=CHEt furnished *cis*-olefin **16**, which underwent deprotection with Bu_4NF to afford the key diol **17** in quantitative yield.

In order to transform diol **17** into Δ^2-OPC-8:0 (**3**) in a short way through hydroxyl aldehyde **25** (Scheme 2), diol **17** was oxidized into hydroxyl aldehyde **25** with NCS in the presence of catalytic TEMPO and Bu_4NCl.[20] The reaction proceeded in good yield.[21] However, the ^{13}C NMR spectrum of **25** after chromatography revealed contamination with an olefinic impurity, though in less than 10%, which was probably derived from TEMPO-catalyzed olefin isomerization. Without further purification, **25** was subjected to the Horner–Emmons reaction to produce ester **26**, which was hydrolyzed to give hydroxyl acid **23**. Finally, Jones oxidation produced **3**. However, the impurity could not be eliminated even after these reactions and accompanying chromatographic purification.

After the unsuccessful results mentioned above, a sequence leading to the target compound **3** was established, which is

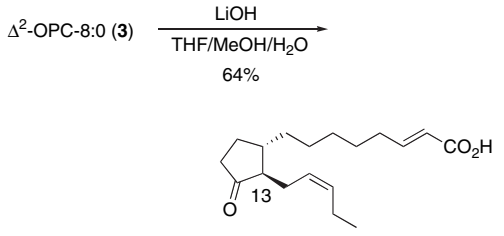


Scheme 1. Synthesis of Δ^2-OPC-8:0. (a) *t*-BuMgCl (1 equiv) then ClMg$(CH_2)_6$OTBDPS (**5**) (2 equiv), CuCN (0.3 equiv), −18 °C; (b) AcOH, DIAD, PPh_3, −78 °C; (c) LiOH aq; (d) CH_2=CHOEt, $Hg(OAc)_2$ (0.23 equiv), 180–190 °C; (e) CrO_3; (f) KI_3, $NaHCO_3$; (g) Bu_3SnH, AIBN; (h) CH_2N_2; (i) TESCl, imidazole; (j) DIBAL-H, −78 °C; (k) $Ph_3PC_3H_7Br$, $NaN(TMS)_2$; (l) Bu_4NF; (m) TBSCl, imidazole; (n) PPTS, EtOH/CH_2Cl_2; (o) PCC; (p) $(EtO)_2P$(=O)CH_2CO_2Et, LiCl, DBU; (q) AcOH, aq THF.



Scheme 2. An attempted synthesis of Δ^2-OPC-8:0.

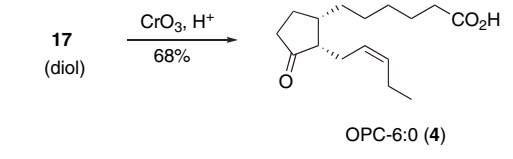
presented in Scheme 1. Silylation of diol **17** with TBSCl afforded **18**, which upon exposure to PPTS in EtOH and CH_2Cl_2 (1:1) at 5–10 °C afforded primary alcohol **19** in 91% yield.[22] PCC oxidation of **19** followed by Horner–Emmons reaction of the resulting aldehyde **20** under the Masamune conditions[23] afforded the α,β-unsaturated ester **21**. Desilylation of **21** followed by hydrolysis of the resulting alcohol **22** gave hydroxyl acid **23** in good yield. Finally, Jones oxidation furnished Δ^2-OPC-8:0 (**3**) in 97% yield.[24] Furthermore, **3** was subjected to epimerization under alkaline conditions to afford the 13-epimer of **3** (i.e., **27**) (Eq. 2). The ^{13}C NMR spectra (75 MHz) of **3** and **27** differed from each other (especially, 35.4, 38.7, and 53.7 ppm for **3**; 38.1, 41.2, 55.1 ppm for **27**), and established <5% contamination of **27** in **3**, while the ^1H NMR spectra and R_f values of **3** and **27** were superimposed on each other.



(2)

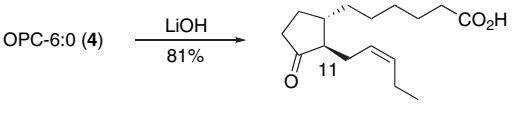
27 (13-epimer of **3**)

Next, Jones oxidation of the key diol **17** afforded OPC-6:0 (**4**) in 68% yield (Eq. 3).[24] In addition, **4** was exposed to aqueous LiOH for epimerization at C(11) (Eq. 4). The 11-epimer of **4** (i.e., **28**) thus synthesized in 81% yield was identical to the racemic 11-epimer reported in the literature[9c] by ^1H NMR, ^{13}C NMR, and IR spectroscopy. Purity of OPC-6:0 (**4**) was >95% by calculation of the specific signal heights at 38.7 and 53.7 ppm for **4** and 41.2 and 55.1 ppm for **28**, in the ^{13}C NMR spectrum of **4**.



(3)

OPC-6:0 (**4**)



(4)

28
(11-epimer of OPC-6:0)

3. Conclusion

We have secured the synthesis of Δ^2-OPC-8:0 (**3**) and OPC-6:0 (**4**). Consequently the specific function of OPC-8:0 will be elucidated by using Δ^2-OPC-8:0 (**3**). Moreover, a full metabolic study of linolenic acid will be carried out using these compounds synthesized previously,[10] here, and elsewhere.[25]

4. Experimental

4.1. General methods

Infrared (IR) spectra are reported in wave numbers (cm^{-1}). The ^1H NMR (300 and 500 MHz) and ^{13}C NMR (75 MHz) spectra were measured in $CDCl_3$ using $SiMe_4$ (δ=0 ppm) and the center line of $CDCl_3$ triplet (δ=77.1 ppm) as internal standards, respectively. Purity of the title compounds was confirmed by elemental analysis in most of cases or by the spectral method (^1H NMR and ^{13}C NMR) in case the satisfactory results were not recorded.

4.2. Synthesis of the diol key intermediate 17

4.2.1. (4*S*,1*S*)-4-[6-{(*tert*-Butyldiphenylsilyl)oxy}hexyl]-2-cyclopenten-1-ol (6). To an ice-cold solution of (1*R*)-**1** (505 mg, 3.55 mmol, >99% ee) in THF (21 mL) was added *t*-BuMgCl (4.60 mL, 0.76 M in THF, 3.50 mmol) and the solution was stirred at 0 °C for 10 min to prepare the alkoxide of (1*R*)-**1**. To this solution was added CuCN (95 mg, 1.06 mmol) and a solution of $ClMg(CH_2)_6OTBDPS$ (10.2 mL, 0.70 M in THF, 7.14 mmol) at −18 °C. The resulting mixture was stirred for 4 h at −18 °C and diluted with saturated NH_4Cl and EtOAc. After being stirred vigorously at room temperature, the layers were separated, and the aqueous layer was extracted with EtOAc twice. The combined extracts were dried ($MgSO_4$), and concentrated to afford an oil, which was a mixture of **6**, regioisomer **24**, and $TBDPSO(CH_2)_6OH$. Ratio of **6** and **24** was 92:8 by ^1H NMR spectroscopy. The mixture was subjected to chromatography to collect fractions (1.86 g) consisting of **6** (1.22 g and 81% yield) and $TBDPSO(CH_2)_6OH$ (0.64 g) by ^1H NMR spectroscopy. This mixture was used for the next reaction without further purification: ^1H NMR (300 MHz, $CDCl_3$) (selected signals) δ 1.05 (s, 9H), 1.75 (ddd, *J*=14, 7, 5 Hz, 1H), 1.90 (ddd, *J*=14, 8, 3 Hz, 1H), 2.78–2.90 (m, 1H), 4.80–4.89 (m, 1H), 5.81 (dt, *J*=5.5, 2 Hz), 5.94 (ddd, *J*=5.5, 2, 1 Hz, 1H). Regioisomer **24**: ^1H NMR (300 MHz, $CDCl_3$) δ 1.04 (s, 9H), 1.2–1.6 (m, 11H), 2.24 (d, *J*=17 Hz, 1H), 2.43–2.58 (m, 1H), 2.70 (ddq, *J*=17, 6, 2 Hz, 1H), 3.65 (t, *J*=6 Hz, 2H), 4.04–4.12 (br s, 1H), 5.62–5.72 (m, 2H), 7.33–7.46 (m, 6H), 7.63–7.70 (m, 4H).

4.2.2. (4*S*,1*R*)-4-[6-{(*tert*-Butyldiphenylsilyl)oxy}hexyl]-2-cyclopentenyl acetate (7). To a solution of the above mixture, Ph_3P (2.60 g, 9.91 mmol), and AcOH (0.71 mL, 12 mmol) in toluene (17 mL) was added DIAD (2.09 mL, 10.1 mmol) at −78 °C. The mixture was stirred for 5 h at −78 °C and poured into saturated $NaHCO_3$ and hexane with vigorous stirring. The layers were separated, and the aqueous layer was extracted with hexane twice. The combined extracts were dried ($MgSO_4$) and concentrated to

give a residue, which was purified by chromatography (hexane/EtOAc) to furnish *cis*-acetate **7** exclusively (1.26 g, 77% from (1*R*)-**1**): $[\alpha]_D^{24}$ −3 (*c* 1.19, $CHCl_3$); IR (neat) 1735, 1242, 1112 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.04 (s, 9H), 1.20–1.62 (m, 11H), 2.03 (s, 3H), 2.45–2.63 (m, 2H), 3.65 (t, *J*=6.5 Hz, 2H), 5.57–5.66 (m, 1H), 5.75 (dt, *J*=6, 2 Hz, 1H), 5.99 (d, *J*=5.5 Hz, 1H), 7.32–7.45 (m, 6H), 7.59–7.76 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.3, 21.5, 25.8, 27.0, 27.8, 29.5, 32.6, 36.4, 36.7, 44.4, 64.0, 80.0, 127.6, 128.8, 129.6, 134.2, 135.7, 141.2, 171.1. Anal. Calcd for $C_{29}H_{40}O_3Si$: C, 74.95; H, 8.68. Found: C, 74.85; H, 8.74.

4.2.3. (4*S*,1*R*)-4-[6-{(*tert*-Butyldiphenylsilyl)oxy}hexyl]-2-cyclopenten-1-ol (8). To an ice-cold solution of acetate **7** (1.81 g, 3.89 mmol) in THF (16 mL), MeOH (8 mL), and H_2O (4 mL) was added $LiOH\cdot H_2O$ (898 mg, 21.4 mmol). The mixture was stirred at room temperature overnight and diluted with saturated NH_4Cl and EtOAc with stirring at room temperature. The layers were separated, and the aqueous layer was extracted with EtOAc two times. The combined extracts were dried ($MgSO_4$) and concentrated to leave an oil, which was purified by chromatography (hexane/EtOAc) to give alcohol **8** (1.57 g, 95%): $[\alpha]_D^{28}$ –12 (*c* 1.11, $CHCl_3$); IR (neat) 3319, 1112, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.05 (s, 9H), 1.17–1.65 (m, 12H), 2.44–2.60 (m, 2H), 3.65 (t, *J*=6.5 Hz, 2H), 4.75–4.87 (m, 1H), 5.77 (dt, *J*=5.5, 2 Hz, 1H), 5.88 (d, *J*=5.5 Hz, 1H), 7.30–7.50 (m, 6H), 7.62–7.74 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.3, 25.8, 27.0, 27.9, 29.5, 32.6, 36.9, 40.6, 44.5, 64.0, 77.5, 127.7, 129.6, 133.0, 134.2, 135.7, 139.1. Anal. Calcd for $C_{27}H_{38}O_2Si$: C, 76.72; H, 9.06. Found: C, 76.68; H, 9.19.

4.2.4. (1*S*,2*S*)-2-([6-{(*tert*-Butyldiphenylsilyl)oxy}hexyl]-4-cyclopentenyl)ethanal (9). A sealed glass tube containing alcohol **8** (917 mg, 2.17 mmol), $Hg(OAc)_2$ (162 mg, 0.508 mmol), ethyl vinyl ether (2.4 mL, 25 mmol), and benzene (5 mL) was immersed in an oil bath set at 190 °C. After 70 h at 180–190 °C, the solution was cooled to room temperature and transferred with benzene to a flask containing K_2CO_3 (701 mg, 5.07 mmol). The mixture was stirred for 30 min and filtered through a pad of Celite with EtOAc. The filtrate was concentrated to give an oil, which was purified by chromatography (hexane/EtOAc) to give aldehyde **9** (944 mg, 97%): $[\alpha]_D^{25}$ –45 (*c* 1.39, $CHCl_3$); IR (neat) 1726, 1112, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.05 (s, 9H), 1.18–1.61 (m, 10H), 1.94 (dd, *J*=15, 9 Hz, 1H), 2.23 (ddd, *J*=16, 9, 2 Hz, 1H), 2.28–2.33 (m, 1H), 2.39 (dd, *J*=15, 8 Hz, 1H), 2.49 (ddd, *J*=16, 5, 2 Hz, 1H), 3.00–3.12 (m, 1H), 3.65 (t, *J*=6.5 Hz, 2H), 5.77 (s, 2H), 7.32–7.46 (m, 6H), 7.63–7.75 (m, 4H), 9.79 (t, *J*=2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.3, 25.8, 26.9, 28.7, 29.6, 30.7, 32.6, 37.2, 41.3, 41.6, 44.6, 64.0, 127.6, 129.6, 131.2, 134.1, 134.2, 135.6, 202.9. Anal. Calcd for $C_{29}H_{40}O_2Si$: C, 77.62; H, 8.99. Found: C, 77.54; H, 9.07.

4.2.5. (1*S*,2*S*)-2-([6-{(*tert*-Butyldiphenylsilyl)oxy}hexyl]-4-cyclopentenyl)acetic acid (10). To an ice-cold solution of aldehyde **9** (1.18 g, 2.63 mmol) in acetone (26 mL) was added Jones reagent (4 M solution) slowly until the color of the reagent persisted (ca. 0.7 mL). After 10 min of stirring at the same temperature, 2-propanol was added to destroy the excess reagent. The resulting mixture was filtered through a pad of Celite with Et_2O. The filtrate was washed with brine three times to make the aqueous solution slightly acidic (pH 4). The aqueous layer was extracted with EtOAc three times. The combined extracts were dried ($MgSO_4$) and concentrated. The residue was purified by chromatography (hexane/EtOAc) to afford acid **10** (1.09 g, 90%): $[\alpha]_D^{26}$ –43 (*c* 0.394, $CHCl_3$); IR (neat) 3100, 1707, 1112 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.05 (s, 9H), 1.20–1.62 (m, 10H), 1.80–1.93 (m, 1H), 2.02 (dd, *J*=15, 10 Hz, 1H), 2.12–2.32 (m, 2H), 2.37 (dd, *J*=15, 5.5 Hz, 1H), 2.86–3.09 (m, 1H), 3.58 (t, *J*=6.5 Hz, 2H), 5.66–5.88 (m, 2H), 7.19–7.44 (m, 6H), 7.52–7.70 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.4, 25.9, 27.0, 28.8, 29.7, 30.4, 32.7, 34.8, 37.2, 41.5, 43.3, 64.1, 127.6, 129.5, 131.2, 134.1, 134.3, 135.6, 179.5. Anal. Calcd for $C_{29}H_{40}O_3Si$: C, 74.95; H, 8.68. Found: C, 74.83; H, 8.90.

4.2.6. Iodo-lactone 11. To an ice-cold solution of acid **10** (1.74 g, 3.74 mmol) in Et_2O (13 mL) and THF (13 mL) was added $NaHCO_3$ (989 mg, 11.8 mmol) dissolved in H_2O (24 mL) and the mixture was stirred for 30 min. Then an aqueous solution of I_2 (1.99 g, 7.84 mmol) and KI (3.91 g, 23.6 mmol) in H_2O (12 mL) was added. The resulting dark brown mixture was stirred at room temperature for 16 h under the dark and poured into aqueous $Na_2S_2O_3$ with vigorous stirring. The mixture was extracted with EtOAc three times. The combined extracts were dried ($MgSO_4$) and concentrated to give an oil, which was purified by chromatography (hexane/EtOAc) to furnish **11** (1.90 g, 86%): $[\alpha]_D^{23}$ +2 (*c* 1.2, $CHCl_3$); IR (neat) 1787, 1166, 1111, 702 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.05 (s, 9H), 1.22–1.42 (m, 7H), 1.48–1.72 (m, 4H), 2.09 (dd, *J*=15, 6 Hz, 1H), 2.49 (dd, *J*=18, 4 Hz, 1H), 2.59 (dd, *J*=18, 10 Hz, 1H), 2.58–2.73 (m, 1H), 3.05–3.16 (m, 1H), 3.66 (t, *J*=6.5 Hz, 2H), 4.46 (d, *J*=5 Hz, 1H), 5.27 (d, *J*=6 Hz, 1H), 7.32–7.50 (m, 6H), 7.64–7.72 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.4, 25.8, 27.0, 28.4, 28.5, 28.9, 29.6, 30.1, 32.6, 39.0, 40.3, 40.5, 64.0, 92.8, 127.6, 129.5, 134.1, 135.5, 176.4. Anal. Calcd for $C_{29}H_{39}O_3Si$: C, 58.97; H, 6.66. Found: C, 58.98; H, 6.77.

4.2.7. Lactone 12. To a solution of iodo-lactone **11** (165 mg, 0.279 mmol) in benzene (0.9 mL) were added Bu_3SnH (0.23 mL, 0.86 mmol) and AIBN (5 mg, 0.03 mmol). After 1 h of reflux, the reaction was quenched by addition of NaF (59 mg, 1.41 mmol). The slurry was stirred at room temperature for 30 min, and filtered through a pad of Celite with EtOAc. The filtrate was concentrated, and a residue was purified by chromatography (hexane/EtOAc) to afford **12** (126 mg, 97%): $[\alpha]_D^{24}$ –5.7 (*c* 0.53, $CHCl_3$); IR (neat) 1771, 1111, 703 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.97 (s, 9H), 1.10–1.36 (m, 8H), 1.42–2.20 (m, 7H), 2.35 (dd, *J*=19, 6 Hz, 1H), 2.42 (dd, *J*=19, 10 Hz, 1H), 2.78–2.92 (m, 1H), 3.58 (t, *J*=6 Hz, 2H), 4.95 (t, *J*=6 Hz, 1H), 7.33–7.43 (m, 6H), 7.63–7.69 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.3, 25.7, 26.9, 28.5, 28.7, 28.9, 29.5, 30.6, 32.5, 33.1, 40.5, 42.8, 63.9, 86.2, 127.6, 129.6, 134.2, 135.6, 178.1. Anal. Calcd for $C_{29}H_{40}O_3Si$: C, 74.95; H, 8.68. Found: C, 75.03; H, 8.72.

4.2.8. Methyl ester 14. To an ice-cold solution of lactone **12** (52 mg, 0.11 mmol) in THF (0.72 mL), MeOH (0.24 mL),

and H_2O (0.24 mL) was added $LiOH \cdot H_2O$ (24 mg, 0.57 mmol). The mixture was stirred at room temperature for 3 h, cooled to −18 °C, and diluted with saturated NH_4Cl. The resulting mixture was acidified to ca. pH 4 with 1 N HCl, and extracted with Et_2O three times. The extracts were dried ($MgSO_4$) at −18 °C and $MgSO_4$ was filtered out. The filtrate was treated with excess CH_2N_2 in Et_2O at −18 °C for 5 min. The solution was concentrated and the residue was passed through a short column of silica gel with Et_2O as an eluent to give the hydroxyl methyl ester **13** after evaporation.

To an ice-cold solution of the above ester **13** and imidazole (19 mg, 0.28 mmol) in DMF (1 mL) was added TESCl (0.038 mL, 0.23 mmol). The solution was stirred at room temperature for 14 h and diluted with saturated $NaHCO_3$ and hexane. The layers were separated, and the aqueous layer was extracted with hexane twice. The combined extracts were dried ($MgSO_4$) and concentrated to give a residue, which was purified by chromatography (hexane/EtOAc) to afford silyl ester **14** (55 mg, 82% from lactone **12**) after chromatography (hexane/EtOAc): $[\alpha]_D^{23}$ +0.1 (*c* 1.98, $CHCl_3$); IR (neat) 1741, 1112 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.55 (q, *J*=8 Hz, 6H), 0.93 (t, *J*=8 Hz, 9H), 1.04 (s, 9H), 1.08–1.93 (m, 15H), 2.20 (dd, *J*=15, 5.5 Hz, 1H), 2.32–2.44 (m, 1H), 2.46 (dd, *J*=15, 7.5 Hz, 1H), 3.64 (t, *J*=6.5 Hz, 2H), 3.65 (s, 3H), 4.17–4.24 (m, 1H), 7.33–7.43 (m, 6H), 7.64–7.70 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 4.9, 6.9, 19.3, 25.9, 26.9, 28.1, 28.4, 29.5, 29.7, 31.7, 32.7, 32.8, 39.6, 43.8, 51.4, 64.1, 75.0, 127.6, 129.6, 134.3, 135.7, 174.9. Anal. Calcd for $C_{36}H_{58}O_4Si_2$: C, 70.77; H, 9.57. Found: C, 70.78; H, 9.53.

4.2.9. Aldehyde 15. To a solution of ester **14** (474 mg, 0.776 mmol) in CH_2Cl_2 (8 mL) was added DIBAL-H (1.0 mL, 0.94 M in hexane, 0.94 mmol) at −78 °C. The reaction was carried out at the same temperature for 1 h, and quenched with MeOH (0.40 mL, 9.9 mmol). After 10 min at −78 °C, a solution of H_2O (0.50 mL, 28 mmol) diluted with THF (0.50 mL) was added and the cooling bath was removed. The resulting mixture was stirred for 30 min and NaF (782 mg, 18.6 mmol) was added to it. After 30 min of vigorous stirring, the resulting mixture was filtered through a pad of Celite with EtOAc and the filtrate was concentrated to afford a residue, which was purified by chromatography (hexane/EtOAc) to give aldehyde **15** (415 mg, 92%): $[\alpha]_D^{26}$ −6 (*c* 0.53, $CHCl_3$); IR (neat) 1726, 1112 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.56 (q, *J*=8 Hz, 6H), 0.94 (t, *J*=8 Hz, 9H), 1.06 (s, 9H), 1.1–2.0 (m, 15H), 2.13–2.27 (m, 1H), 2.43–2.60 (m, 2H), 3.66 (t, *J*=6.5 Hz, 2H), 4.19–4.29 (m, 1H), 7.34–7.48 (m, 6H), 7.64–7.75 (m, 4H), 9.82 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 4.8, 6.9, 19.3, 25.8, 26.9, 27.7, 28.3, 29.6, 31.9, 32.1, 32.6, 38.9, 39.4, 43.0, 64.0, 75.2, 127.6, 129.5, 134.2, 135.6, 203.5. Anal. Calcd for $C_{35}H_{56}O_3Si_2$: C, 72.36; H, 9.72. Found: C, 72.21; H, 9.73.

4.2.10. Olefin 16. To an ice-cold suspension of *n*-propyltriphenylphosphonium bromide (861 mg, 2.23 mmol) in THF (1 mL) was added a solution of $NaN(TMS)_2$ (2.1 mL, 0.99 M in THF, 2.1 mmol). The mixture was stirred at room temperature for 40 min and cooled to 0 °C. A solution of aldehyde **15** (351 mg, 0.604 mmol) in THF (3 mL) was added. The mixture was stirred at room temperature overnight. Hexane and saturated NH_4Cl were added to the mixture at 0 °C with vigorous stirring. The layers were separated and aqueous layer was extracted with hexane twice. The combined extracts were dried ($MgSO_4$) and concentrated to leave a residue, which was purified by chromatography (hexane/EtOAc) to afford olefin **16** (363 mg, 99%): $[\alpha]_D^{25}$ +2.2 (*c* 0.368, $CHCl_3$); IR (neat) 1428, 1112, 740, 701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.56 (q, *J*=8 Hz, 6H), 0.95 (t, *J*=8 Hz, 9H), 0.96 (t, *J*=7.5 Hz, 3H), 1.04 (s, 9H), 1.15–1.88 (m, 16H), 1.98–2.24 (m, 4H), 3.65 (t, *J*=6.5 Hz, 2H), 4.13 (q, *J*=5 Hz, 1H), 5.24–5.48 (m, 2H), 7.34–7.43 (m, 6H), 7.64–7.70 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 5.0, 7.0, 14.4, 19.3, 20.8, 22.4, 25.9, 26.9, 28.5, 28.8, 29.8, 31.8, 32.7, 33.4, 39.9, 48.7, 64.1, 75.5, 127.6, 129.5, 129.9, 131.1, 134.3, 135.7. Anal. Calcd for $C_{38}H_{62}O_2Si_2$: C, 75.18; H, 10.29. Found: C, 75.38; H, 10.14.

4.2.11. Diol 17. To an ice-cold solution of olefin **16** (135 mg, 0.222 mmol) in THF (1 mL) was added Bu_4NF (1.1 mL, 1.0 M in THF, 1.1 mmol) slowly. The resulting solution was stirred at 65 °C for 1 h, cooled to 0 °C, and diluted with EtOAc and saturated NH_4Cl with vigorous stirring. The mixture was filtered through a pad of Celite with EtOAc. The layers were separated, and the aqueous layer was extracted with EtOAc twice. The combined extracts were dried ($MgSO_4$) and concentrated to leave a residue, which was purified by chromatography (hexane/EtOAc) to afford diol **17** (56 mg, 99%): $[\alpha]_D^{24}$ +12 (*c* 0.388, $CHCl_3$); IR (neat) 3350, 1056 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.98 (t, *J*=7.5 Hz, 3H), 1.19–1.95 (m, 17H), 2.20–2.50 (m, 5H), 3.64 (t, *J*=6.5 Hz, 2H), 4.14–4.26 (m, 1H), 5.34–5.49 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5, 20.9, 22.8, 25.9, 28.9, 29.1, 29.8, 31.8, 32.9, 33.2, 40.1, 47.9, 63.1, 75.5, 128.7, 132.1. Anal. Calcd for $C_{16}H_{30}O_2$: C, 75.54; H, 11.89. Found: C, 75.50; H, 11.80.

4.3. Synthesis of Δ^2-OPC-8:0

4.3.1. Olefin 18. To an ice-cold mixture of TBSCl (106 mg, 0.682 mmol) and imidazole (54 mg, 0.79 mmol) in DMF (1 mL) was added a solution of diol **17** (57 mg, 0.224 mmol) in DMF (1.3 mL). The resulting solution was stirred at room temperature overnight, and diluted with hexane and saturated NH_4Cl at 0 °C with vigorous stirring. The organic layer was separated, and the aqueous layer was extracted with hexane twice. The combined extracts were dried ($MgSO_4$) and concentrated to afford an oily residue, which was purified by chromatography (hexane/EtOAc) to furnish disilyl ether **18** (106 mg, 98%): $[\alpha]_D^{27}$ −0.3 (*c* 0.59, $CHCl_3$); IR (neat) 1255, 1102, 836, 774 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.02 (s, 6H), 0.05 (s, 6H), 0.88 (s, 9H), 0.90 (s, 9H), 0.97 (t, *J*=7.5 Hz, 3H), 1.06–1.76 (m, 15H), 1.77–1.90 (m, 1H), 2.00–2.17 (m, 4H), 3.59 (t, *J*=7 Hz, 2H), 4.07–4.14 (m, 1H), 5.26–5.48 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.2, −4.9, −4.4, 14.3, 18.1, 18.5, 20.8, 22.8, 25.9, 26.1, 28.8, 28.9, 29.8, 31.8, 33.0, 33.7, 39.8, 49.0, 63.5, 75.6, 129.6, 131.2.

4.3.2. Alcohol 19. A solution of olefin **18** (243 mg, 0.503 mmol) and PPTS (152 mg, 0.605 mmol) in EtOH (3 mL) and CH_2Cl_2 (3 mL) was stirred for 27 h at

5−10 °C, and diluted with saturated $NaHCO_3$ and EtOAc. The phases were separated and the aqueous layer was extracted with EtOAc twice. The combined organic portions were dried ($MgSO_4$) and concentrated under reduced pressure to obtain an oily residue, which was purified by chromatography (hexane/EtOAc) to afford alcohol **19** (168 mg, 91%): $[\alpha]_D^{23}$ 0 (*c* 0.52, $CHCl_3$); IR (neat) 3323, 1253, 1067 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.02 (s, 6H), 0.87 (s, 9H), 0.96 (t, *J*=7.5 Hz, 3H), 1.1–1.9 (m, 17H), 1.99–2.17 (m, 4H), 3.63 (t, *J*=7 Hz, 2H), 4.07–4.14 (m, 1H), 5.26–5.48 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.0, −4.4, 14.3, 18.1, 20.8, 22.8, 25.8, 25.9, 28.7, 28.8, 29.8, 31.7, 32.9, 33.7, 39.7, 49.0, 63.2, 75.5, 129.6, 131.3.

4.3.3. Ethyl ester 21. To an ice-cold solution of alcohol **19** (30 mg, 0.081 mmol) in CH_2Cl_2 (1 mL) was added PCC (26 mg, 0.12 mmol). The resulting mixture was stirred at room temperature for 1 h. The mixture was filtered through a pad of Celite with hexane, and the filtrate was washed with brine three times. The combined extracts were dried ($MgSO_4$) and concentrated to give an oil, which was semi-purified by chromatography (hexane/EtOAc) to give aldehyde **20**.

To an ice-cold mixture of LiCl (5 mg, 0.12 mmol) in CH_3CN (0.2 mL) were added diethylphosphonoacetic acid ethyl ester (0.049 mL, 0.24 mmol), DBU (0.048 mL, 0.32 mmol) and a solution of above aldehyde **20** in CH_3CN (0.8 mL). The resulting solution was stirred at room temperature for 6 h, and diluted with hexane and saturated $NaHCO_3$ at 0 °C with vigorous stirring. The organic layer was separated, and the aqueous layer was extracted with hexane twice. The combined extracts were dried ($MgSO_4$) and concentrated to afford an oily residue, which was purified by chromatography (hexane/EtOAc) to furnish ethyl ester **21** (27 mg, 77% from alcohol **19**): $[\alpha]_D^{21}$ 0 (*c* 0.38, $CHCl_3$); IR (neat) 1725, 1655, 1256 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.02 (s, 3H), 0.07 (s, 3H), 0.87 (s, 9H), 0.96 (t, *J*=7.5 Hz, 3H), 1.1–1.9 (m, 16H), 1.28 (t, *J*=7 Hz, 3H), 1.95–2.23 (m, 4H), 4.07–4.13 (m, 1H), 4.18 (q, *J*=7 Hz, 2H), 5.25–5.48 (m, 2H), 5.80 (dt, *J*=16, 1.5 Hz, 1H), 6.96 (dt, *J*=16, 7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.0, −4.4, 14.3, 14.6, 18.1, 20.8, 22.8, 25.9, 28.1, 28.5, 28.8, 29.5, 31.6, 32.3, 33.7, 39.7, 49.0, 60.2, 75.5, 121.2, 129.5, 131.3, 149.7, 166.9.

4.3.4. Alcohol 22. A mixture of ethyl ester **21** (43 mg, 0.098 mmol) in THF (0.2 mL), H_2O (0.2 mL), and AcOH (0.6 mL) was stirred at 30 °C for 60 h, and diluted with EtOAc and saturated $NaHCO_3$ at 0 °C with vigorous stirring. The organic layer was separated, and the aqueous layer was extracted with EtOAc twice. The combined extracts were dried ($MgSO_4$) and concentrated to afford an oily residue, which was purified by chromatography (hexane/EtOAc) to furnish alcohol **22** (30 mg, 95%): $[\alpha]_D^{26}$ +13 (*c* 0.52, $CHCl_3$); IR (neat) 3490, 1722, 1653 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.97 (t, *J*=7.5 Hz, 3H), 1.1–2.0 (m, 15H), 1.28 (t, *J*=7 Hz, 3H), 2.0–2.3 (m, 6H), 4.08–4.25 (m, 3H), 5.34–5.44 (m, 2H), 5.80 (d, *J*=16 Hz, 1H), 6.95 (dt, *J*=16, 7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.33, 14.34, 20.8, 22.7, 28.1, 28.6, 29.0, 29.4, 31.7, 32.3, 33.2, 40.0, 47.9, 60.2, 75.4, 121.3, 128.7, 132.2, 149.5, 166.9.

4.3.5. Hydroxyl acid 23. To an ice-cold solution of alcohol **22** (16 mg, 0.0496 mmol) in THF (0.6 mL), MeOH (0.2 mL), and H_2O (0.2 mL) was added $LiOH\cdot H_2O$ (10 mg, 0.24 mmol). The mixture was stirred at room temperature overnight and diluted with saturated NH_4Cl and EtOAc with stirring at room temperature. The layers were separated, and the aqueous layer was extracted with EtOAc twice. The combined extracts were dried ($MgSO_4$) and concentrated to leave an oil, which was purified by chromatography (CH_2Cl_2/EtOAc) to give hydroxyl acid **23** (14 mg, 96%): $[\alpha]_D^{23}$ +13 (*c* 0.33, $CHCl_3$); IR (neat) 3410, 1699, 1652 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, *J*=7.5 Hz, 3H), 1.13–2.44 (m, 21H), 4.15–4.25 (m, 1H), 5.22–5.51 (m, 2H), 5.82 (d, *J*=16 Hz, 1H), 7.06 (dt, *J*=16, 7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 20.8, 22.7, 28.0, 28.6, 29.0, 29.5, 31.7, 32.4, 33.1, 40.0, 47.8, 75.5, 120.8, 128.7, 132.3, 152.3, 171.8.

4.3.6. Δ^2-OPC-8:0 (3). To an ice-cold solution of the above hydroxyl acid **23** (28 mg, 0.095 mmol) in acetone (0.9 mL) was added Jones reagent (4 M solution) dropwise at 0 °C until the color of the reagent persisted (a few drops). After 10 min of stirring at 0 °C, *i*-PrOH was added to destroy the excess reagent. The mixture was filtered through a pad of Celite with EtOAc. The filtrate was washed with brine three times, dried ($MgSO_4$), and concentrated to give an oil, which was purified by chromatography (CH_2Cl_2/EtOAc) to give Δ^2-OPC-8:0 (**3**) (27 mg, 97%): $[\alpha]_D^{25}$ +51 (*c* 0.14, $CHCl_3$); IR (neat) 3196, 1738, 1699 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, *J*=7.5 Hz, 3H), 1.1–2.4 (m, 20H), 5.36–5.46 (m, 2H), 5.83 (d, *J*=16 Hz, 1H), 7.05 (dt, *J*=16, 7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 20.7, 22.6, 24.9, 27.5, 27.9, 28.1, 29.3, 32.3, 35.4, 38.7, 53.7, 120.8, 126.2, 133.1, 152.2, 171.8, 220.3.

4.3.7. 13-Epimer 27. To an ice-cold solution of Δ^2-OPC-8:0 (**3**) (22 mg, 0.0752 mmol) in THF (0.6 mL), MeOH (0.2 mL), and H_2O (0.2 mL) was added $LiOH\cdot H_2O$ (21 mg, 0.50 mmol). The mixture was stirred at room temperature for 2 h and diluted with saturated NH_4Cl and EtOAc with stirring. The layers were separated, and the aqueous layer was extracted with EtOAc twice. The combined extracts were dried ($MgSO_4$) and concentrated to leave an oil, which was purified by chromatography (CH_2Cl_2/EtOAc) to give 13-epimer **27** (14 mg, 64%): ^1H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, *J*=7.5 Hz, 3H), 1.2–2.4 (m, 20H), 5.19–5.48 (m, 2H), 5.83 (dt, *J*=16, 1.5 Hz, 1H), 7.07 (dt, *J*=16, 7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.3, 20.7, 25.5, 27.0, 27.1, 27.9, 29.4, 32.3, 34.7, 38.1, 41.2, 55.1, 120.6, 125.5, 133.6, 152.1, 170.8, 220.8.

4.4. Synthesis of OPC-6:0

4.4.1. OPC-6:0 (4). To an ice-cold solution of the above diol **17** (38 mg, 0.15 mmol) in acetone (1.5 mL) was added Jones reagent (4 M solution) dropwise at 0 °C until the color of the reagent persisted (a few drops). After 5 min of stirring at 0 °C, *i*-PrOH was added to destroy the excess reagent. The mixture was filtered through a pad of Celite with EtOAc. The filtrate was washed with H_2O three times, dried ($MgSO_4$), and concentrated to give an oil, which was purified by chromatography (hexane/EtOAc) to give **4** (27 mg, 68%): $[\alpha]_D^{23}$ +43 (*c* 0.274, $CHCl_3$); IR (neat) 3080, 1738,

1709 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J=7.5 Hz, 3H), 1.18–1.48 (m, 8H), 1.56–1.72 (m, 2H), 1.76–2.42 (m, 10H), 5.27–5.49 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 20.7, 22.6, 24.7, 24.8, 27.4, 28.0, 29.3, 33.9, 35.4, 38.7, 53.7, 126.2, 133.1, 179.2, 220.3.

4.4.2. 11-Epimer 28. To an ice-cold solution of OPC-6:0 (**4**) (27 mg, 0.10 mmol) in THF (0.6 mL), MeOH (0.2 mL), and H_2O (0.2 mL) was added $LiOH\cdot H_2O$ (21 mg, 0.50 mmol). The mixture was stirred at room temperature for 2.5 h and diluted with saturated NH_4Cl and EtOAc with stirring. The layers were separated, and the aqueous layer was extracted with EtOAc twice. The combined extracts were dried ($MgSO_4$) and concentrated to leave an oil, which was purified by chromatography (hexane/EtOAc) to give 11-epimer **28** (22 mg, 81%): ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 20.7, 24.8, 25.6, 26.9, 27.2, 29.4, 34.1, 34.6, 38.2, 41.2, 55.1, 125.4, 133.5, 179.6, 220.7. The ^1H NMR (300 MHz, $CDCl_3$) and the above ^{13}C NMR spectra of 11-epimer **28** were identical with the racemic 11-epimer reported in the literature.[9c]

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18. The filtration was inevitably necessary step to remove the moisture involved in crude **13** in order to achieve the successful silylation.
19. Hydroxyl acid obtained by hydrolysis of lactone **12** and the hydroxyl ester **13** are highly prone to revert to **12**. The isolation and handling should be executed as fast as possible at low temperatures (preferably 0 °C or below) (see Section 4).
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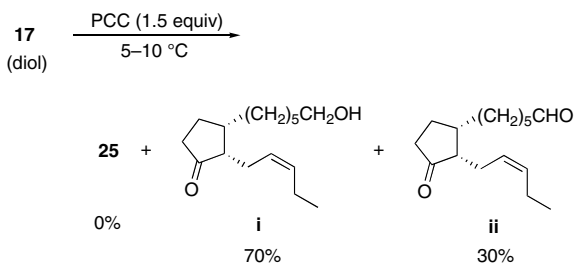
21. PCC oxidation of **17** at 5–10 °C afforded the mixture of **i** and **ii**, while little **25** was obtained.



22. The conditions used here for the selective desilylation are also successful in other cases.
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24. Although being thermodynamically less stable than the trans isomer, no epimerization took place during the Jones oxidation at the last stage and chromatography on silica gel. See Ref. 10 for stability of OPC-8:0.
25. We also accomplished synthesis of OPC-4:0.

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Cytotoxic *ent*-kauranoid derivatives from *Isodon rubescens*

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Abstract—An extensive study of the diterpenoids produced by the species of *Isodon rubescens*, has led to the isolation of 12 new *ent*-kaurane diterpenoids, hebeirubescensins A–L (**1**–**12**), and 19 known analogues. Their structures were determined on the basis of spectroscopic analysis. Selected compounds were assayed for their inhibitory ability against human A549, HT-29, and K562 cells. Among them, hebeirubescensins B and C exhibited significant cytotoxicity with IC_{50} values of <2.0 μM. The structure–activity relationships were discussed.

1. Introduction

Herbal drugs have been widely used for thousands of years in traditional Chinese medicine for the treatment of human disease. *Isodon* species are claimed to exhibit antitumor and anti-inflammatory activities, diterpenoids with a diversity of highly oxygenated structures are the major bioactive constituents of this genus.[1] Given the important bioactivities, structural complexity, and interesting chemical diversity of the composition of this genus, since 1976, more than 50 *Isodon* species in China have been investigated systematically by our group. About 500 new diterpenoids including kauranoids, abietanoids, labdanoids, pimaranoids, isopimaranoids, gibberellanoids, and clerodanoids have been isolated and characterized.[2,3] Among them, some *ent*-kauranoids have potent anti-tumor activities with very low toxicity, for instance: maoecrystal P,[4] eriocalyxin B,[5] oridonin, and ponicidin.[6]

Isodon rubescens belongs to the genus *Isodon* and is commonly used as an antitumor and anti-inflammatory herb in China. It has been stated that this herb is useful for the treatment of cancers of liver, pancreas, esophagus, breast, and rectum. Previous phytochemical studies showed that this species was rich in *ent*-kauranoids. Oridonin and ponicidin, two 7,20-epoxy-*ent*-kauranoids, are the major bioactive constituents of this plant.[7] Recently, pharmaceutical study showed that oridonin and ponicidin had significant antiangiogenic activity.[8] More recently, these two compounds were found to be potent inhibitors of NF-κB transcription activity and the expression of its downstream targets, COX-2 and inducible nitric-oxide synthase.[9] With an aim to isolate more potent and selective NF-κB inhibitors, we further systematically reinvestigated the chemical constituents of *I. rubescens*, and 31 7,20-epoxy-*ent*-kauranoids, including 12 new ones, hebeirubescensins A–L (**1**–**12**), were isolated. In this paper, the isolation, structure elucidation, and cytotoxic properties of those new *ent*-kauranoids are reported below.

2. Results and discussion

Hebeirubescensin A (**1**) was obtained as an amorphous powder. It exhibited an even pseudomolecular ion peak at *m/z* 514 $[M+Na]^+$ in the ESIMS spectrum, suggesting that it might be a N-containing compound. The HRESIMS data (*m/z* 514.2430 $[M+Na]^+$, calcd for 514.2416) further confirmed this assumption, giving rise to the molecular formula $C_{26}H_{37}NO_8$. Its strong IR absorptions at 3387 and 1711 cm^{-1} suggested the presence of hydroxyl and carbonyl groups. The ^{13}C NMR data (Table 1) in combination with analysis of the DEPT and HSQC spectra revealed 26 carbon

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Table 1. NMR data for hebeirubescensin A (**1**) in C_5D_5N, δ (ppm)

No.	^1H	^{13}C	HMBC	ROESY
1	3.78 (dd, *J*=12.5, 5.7 Hz)	76.7 d	C-2, 3, 9, 20, 1′	H-3, 5, 3′
2α	1.92–2.04 (m)	22.7 t	C-1, 4	H-20, 1′, and H_3-19
2β	1.50–1.57 (m)		C-1, 4	H-1, 3β
3α	1.26 (overlapped)	38.3 t	C-1, 2, 4	
3β	1.33–1.42 (m)		C-1, 2, 4	H-1
4		38.6 s		
5	1.44 (d, *J*=8.6 Hz)	57.5 d	C-1, 4, 6, 10, 18, 19	H-1, 9, H_3-18, and OH-6
6	4.23 (dd, *J*=11.4, 8.6 Hz)	75.5 d	C-5, 7, 8, 10	H-20 and H_3-19
7		100.5 s		
8		61.8 s		
9	1.89 (overlapped)	53.6 d	C-5, 11, 12, 14, 15, 20	H-5
10		33.2 s		
11α	2.78–2.87 (m)	23.6 t	C-9, 12	H-14, 12α
11β	1.87 (overlapped)		C-8, 9, 12, 13	
12α	2.27–2.36 (m)	31.4 t	C-9, 11, 13, 16	H-11α, 13
12β	1.43 (overlapped)		C-9, 11, 13, 14, 16	
13	3.16 (br d, *J*=9.8 Hz)	43.7 d	C-8, 11, 12, 15, 16, 17	H-12α, 14
14	5.49 (br s)	72.9 d	C-7, 8, 12, 13, 16	H-11α, 13
15		208.9 s		
16		153.2 s		
17	5.50 (br s), 6.26 (br s)	119.8 t	C-12, 13, 15, 16	
18	1.27 (s)	33.8 q	C-3, 4, 5, 19	H-5
19	1.00 (s)	23.3 q	C-3, 4, 5, 18	H-2α, 6, 20
20	5.75 (br s)	98.4 d	C-1, 7, 9, 10, 1′	H-2α, 6, 1′ and H_3-19
1′	5.09 (br s)	93.6 d	C-1, 20, 3′	H-2α, 20
2′	1.80–1.91 (overlapped)	32.5 t	C-1′, 4′	
3′	1.78–1.90 (overlapped)	25.0 t	C-4′	H-1
4′	3.50 (q, like, *J*=5.2 Hz)	39.5 t	C-2′, 3′, 5′	NH
5′		170.9 s		
6′	2.05 (s)	23.2 q	C-5′	
NH	8.50 (br s)		C-4′, 5′	H-4′
6-OH	6.55 (d, *J*=11.4 Hz)		C-6, 7	H-5

signals due to seven quaternary carbons, eight methines, eight methylenes, and three methyls, of which 20 were assigned to the diterpene skeleton, and the remaining six were ascribed to the other moiety. Careful analysis of the NMR data of the diterpene part indicated that it was a 7,20-epoxy-kauranoid due to the characteristic signal of a hemiketal carbon (C-7 at δ_C 100.5). Comparison of the ^1H and ^{13}C NMR data of the diterpene part with those of rabdoternins E (**14**) and F (**13**), two known 7,20-epoxy-kauranoids that had been isolated as well, suggested that the diterpene part in **1** was strongly resembling to that of rabdoternin F (**13**).[10] Further analysis of 2D NMR data allowed us to determine the gross structure of the diterpene part as shown in Figure 1. The other moiety contained one N- and six C-atoms, including one methyl (δ_C 23.2), three methylene (δ_C 32.5, 25.0, and 39.5, respectively), a carbonyl group (δ_C 170.9), and one acetal group (δ_C 93.6). The CH_2-4′ group resonating at δ_H 3.50 was linked with an acetamide NH (δ_H 8.50), as deduced from a ^1H–^1H COSY correlation of H_2-4′ with NH (Fig. 1), as well as from the HMBC correlations of NH with C-4′, and of H_2-4′ with C-2′ and C-5′ (Fig. 1). In the ^1H–^1H COSY spectrum, the overlapped H_2-2′ and H_2-3′ resonances (δ_H 1.78–1.91) exhibited correlations with H-1′ (δ_H 5.09) and H_2-4′, respectively, suggesting that C-1′ to C-4′ were anchored in a line. The connection of two parts was provided by the HMBC correlations of H-1′ with C-1 (δ_C 76.7, d) and C-20 (δ_C 98.4, d), and of H-20 (δ_H 5.75, br s) with C-1′ (δ_C 93.6, d), giving rise to the connectivities of C-1′ to C-1 and C-20 through an acetal group.

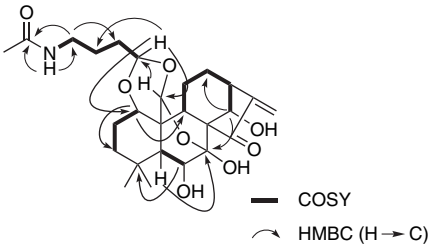


Figure 1. Key correlations of HMBC and ^1H–^1H COSY for **1**.

The relative stereochemistry of compound **1** was established using information from ROESY spectrum and by comparison of its spectroscopic data to those of rabdoternin F (**13**).[10] The same relative stereochemistry of diterpene part in compound **1** as in **13** was deduced from the similar carbon and proton chemical shifts and ROESY correlations found in **1** (Table 1). Considering that all the kauranoids isolated from the genus *Isodon* possessed an *ent*-configuration, **1** was also presumed to be an *ent*-kauranoid. The *S* configuration for C-20 was suggested from the strong ROESY correlations of H-20 with H_3-19 and H-6 as shown in computer-generated 3D drawing (Fig. 2). The configuration of C-1′ was inferred to be *R*, judging from ROESY cross-peaks of H-1′ with H-20 and H-2α. Thus, the structure of **1**, named as hebeirubescensin A, was unambiguously determined to be (1α,20*S*)-6β,7β,14β-trihydroxy-1,20-{[(1*R*)-4-(acetylamino)butane-1,1-diyl]dioxy}-7α,20-epoxy-*ent*-kaur-16-en-15-one.

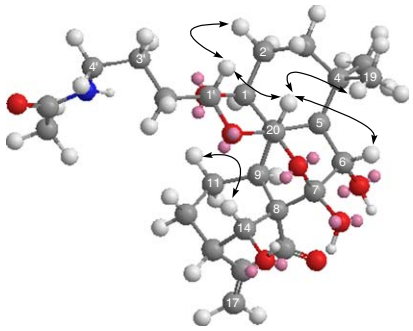


Figure 2. Key ROESY correlations for **1**.

Hebeirubescensins B–L (**2**–**12**) were analogues of hebeirubescensin A. Their stereostructure determination was thus aided by comparison of their spectroscopic data with those of **1** and some known *ent*-kauranoids. However, complete NMR studies on each new metabolite were performed in order to unambiguously determine the structures of the isolated compounds and to assign all the proton and carbon resonances. In particular, COSY and HSQC spectra, in combination with HMBC spectrum, acquired for all new compounds, showed that compounds **2**–**12** contained the same *ent*-kaurene core and similar oxygenation patterns. Interpretation of HMBC spectrum also allowed us to locate the substitution groups on methine carbons, while ROESY spectrum gave the relative stereochemistry information of chiral centers. Some key points for structure elucidations of compounds **2**–**12** were described below.

Hebeirubescensin B (**2**) was obtained as colorless prisms, possessing a molecular formula of $C_{25}H_{38}O_7$ as established by HRESIMS (calcd *m/z* 473.2515; found *m/z* 473.2511, $[M+Na]^+$). Its IR absorptions at 3395 and 1710 cm^{-1} suggested the presence of hydroxyl and carbonyl groups. Comparison of the ^1H and ^{13}C NMR data of **2** with those of rabdoternin F (**13**),[10] suggested that both compounds were closely similar and sharing the same oxygenation pattern. The only difference was in the signals due to the substitution group at C-20, including the absence of methoxyl carbon with the appearance of an isoamoxyl group on the basis of NMR data at δ_C 22.4 (q), 22.5 (q), 25.4 (d), 38.7 (t), and 67.6 (t) and δ_H 0.80 (6H), 1.68 (1H), 1.54 (2H), 4.09, and 3.55 (each 1H, q like, J=7.8 Hz) (Table 2 and Supplementary data) in **2**. Thus, the gross structure of **2** was determined to be a 7,20-epoxy-*ent*-kauranoid with the substitution of an isoamoxyl group at C-20, which was further confirmed by the HMBC correlations of H-20 with C-1′, and of H-1′ with C-20. The ROESY correlations of H-20 with H-6 and H_3-19 suggested that C-20 possessed an *S* configuration. Therefore, compound **2** was elucidated as (20*S*)-1α,6β,7β,14β-tetrahydroxy-20-isoamoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one.

Hebeirubescensins B and C (**2** and **3**) were obtained initially as a mixture by silica gel column chromatography and then separated by recrystallization and semipreparative HPLC. Both compounds had the same molecular formula, $C_{25}H_{38}O_7$, as determined by HRESIMS. Careful analysis of their ^1H and ^{13}C NMR data indicated that **2** and **3** might be C-20 epimers. Detailed comparison of NMR data of **3** with those of rabdoternin E (**14**),[10] showed that **3** and **14** were closely identical with each other, except for the substitution group at C-20. The stereochemistry at C-20 was further confirmed by the key ROESY correlation between H-20 and H-11α. Thus, hebeirubescensin C was elucidated

Table 2. ^{13}C NMR data for hebeirubescensins B–L (**2**–**12**) in C_5D_5N, δ (ppm)

No.	2	3	4	5	6	7	8	9	10	11	12[a]
1	75.4	74.2	75.3	75.1	31.1	75.2	75.0	75.5	30.4	73.8	31.8
2	31.0	30.9	31.0	29.5	18.9	30.5	31.1	30.9	18.7	28.5	15.5
3	39.5	40.5	39.5	39.7	41.6	39.4	40.9	39.3	41.6	39.9	41.5
4	34.1	33.2	34.1	34.3	34.2	34.1	33.3	33.3	34.2	34.3	33.6
5	60.1	59.0	60.0	60.3	58.6	59.9	59.6	57.8	57.5	57.9	53.7
6	74.7	74.7	74.6	74.5	73.8	74.8	74.7	73.6	73.8	74.6	75.2
7	99.7	100.1	99.7	97.0	101.4	99.3	99.9	101.8	101.6	97.1	96.3
8	62.2	63.0	62.1	59.8	53.8	62.4	63.2	52.8	52.6	53.4	53.3
9	53.7	56.6	53.7	58.8	52.1	54.1	56.7	45.1	44.3	50.0	47.5
10	43.7	45.2	43.7	44.0	40.3	43.3	45.6	43.6	39.4	42.7	35.9
11	23.3	21.8	23.3	66.2	63.5	23.5	21.8	21.6	17.7	63.3	18.9
12	31.3	31.3	31.3	39.1	45.1	31.5	31.4	34.0	33.1	42.7	32.0
13	44.4	43.4	44.4	35.0	47.3	44.4	43.4	47.0	46.8	37.3	43.4
14	73.7	72.9	73.7	27.8	76.6	73.9	73.0	76.1	76.1	27.5	76.9
15	210.2	208.5	210.2	212.0	73.2	210.6	208.8	73.2	73.0	75.3	73.6
16	153.3	153.5	153.3	154.3	160.4	153.7	153.6	161.4	161.5	161.9	157.5
17	118.7	119.6	118.8	115.4	109.3	118.6	119.4	108.8	109.0	106.8	111.5
18	33.5	35.9	33.5	33.3	33.9	33.6	35.9	33.3	33.9	33.1	33.1
19	22.3	23.2	22.2	22.7	22.9	22.2	23.6	22.2	22.6	22.8	22.5
20	102.3	99.6	102.4	101.7	102.4	96.1	93.2	103.7	103.7	64.3	66.8
1′	67.6	67.1	75.7	67.3	67.2						
2′	38.7	38.8	28.6	38.7	39.0						
3′	25.4	25.5	19.5	25.5	25.4						
4′	22.5	22.6	19.4	22.5	22.7						
5′	22.4	22.7		22.5	22.6						
OCH_3								55.3	56.1		

[a] Other signals: 12, 171.1, 171.0, 169.9 (C=O), 22.1, 21.6, 21.3 (CH_3) (OAc).

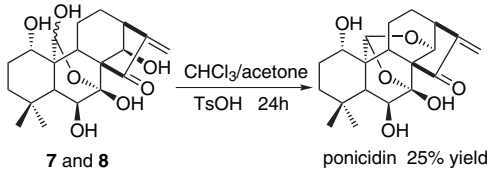
as (20*R*)-1α,6β,7β,14β-tetrahydroxy-20-isoamoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one.

Hebeirubescensin D (**4**) was isolated as colorless needles, and the molecular formula $C_{24}H_{36}O_7$ was deduced from pseudomolecular ion $[M+Na]^+$ at *m/z* 459 in ESIMS and NMR data, and further confirmed by the positive HRESIMS (*m/z* 459.2353 $[M+Na]^+$). The NMR spectroscopic data of **4** were closely identical to those of **2** (Table 2), except for the substitution group at C-20. Besides the signals for diterpene moiety, the ^{13}C NMR and DEPT spectra of **4** (Table 2) displayed four signals for two methyls (δ_C 19.5 and δ_C 19.4), one methine (δ_C 28.6), and one oxymethylene (δ_C 75.7) ascribed for an isobutoxyl residue. Therefore, compound **4** was concluded to be (20*S*)-1α,6β,7β,14β-tetrahydroxy-20-isobutoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one.

Hebeirubescensin E (**5**) was found by HRESIMS to possess the molecular formula, $C_{25}H_{38}O_7$, the same as those of **2** and **3**. Detailed analysis of the NMR spectra of **2** and **5** made it clear that these two compounds were similar except for the presence of a hydroxyl group at C-11 and the absence of a hydroxyl group at C-14 in **5**. The β-orientation for OH-11 was suggested from the intense ROESY correlation of H-11 with H-14α and the *trans* coupling constant between H-9 and H-11 (*J*=8.5 Hz). Thus, compound **5** was concluded to be (20*S*)-1α,6β,7β,11β-tetrahydroxy-20-isoamoxy-7α,20-epoxy-*ent*-kaur-16-en-15-one.

Hebeirubescensin F (**6**) was obtained as an amorphous powder. It exhibited a quasimolecular ion peak at *m/z* 475.2671 $[M+Na]^+$ in the HRESIMS spectrum, suggesting a molecular formula $C_{25}H_{40}O_7$. Its IR and NMR spectral data suggested **6** to be a 7,20-epoxy-*ent*-kauranoid, with an isoamoxyl group and six oxygenated carbons. A careful analysis of the 2D NMR spectral data and comparison with rubescensin C (**23**),[11] led to the conclusions that the C-6, C-7, C-11, C-14, and C-15 positions were each substituted by a hydroxyl group, and the isoamoxyl group was at C-20, on the basis of the HMBC correlations of H-20 (δ_H 5.55) with C-1′ (δ_C 67.2, t), and of H_2-1′ (δ_H 4.06 and 3.48, each 1H) with C-20. Moreover, because of the ROESY correlations of H-14 with H-11, both hydroxyl groups at C-11 and C-14 were deduced to be sharing the same β-orientation. Therefore, compound **6** was determined to be (20*S*)-6β,7β,11β,14β,15β-pentahydroxy-20-isoamoxy-7α,20-epoxy-*ent*-kaur-16-ene.

Hebeirubescensins G and H (**7** and **8**) were isolated as an inseparable mixture of two isomers. Their HRESIMS spectra gave a pseudomolecular ion peak at *m/z* 403.1738 $[M+Na]^+$, consistent with the molecular formula $C_{20}H_{26}O_7$. Besides the absence of signal for OCH_3 group, most NMR signals of compounds **7** and **8** were nearly identical to those of **13** and **14**, respectively. Thus, **7** and **8** were determined to be a C-20 epimers. Detailed 2D NMR analysis confirmed this structure to be 1α,6β,7β,14β,20-pentahydroxy-7α,20-epoxy-*ent*-kaur-16-en-15-one. Interestingly, we found that this epimer could be converted into ponicidin (**27**) during the separation on silica gel column eluted with cyclohexane–chloroform–acetone (5:5:2). So the conversion of this epimer into ponicidin under mild acid condition was investigated (Scheme 1). It was therefore assumed that **7** and **8** might be the biosynthetic precursor of ponicidin.



Scheme 1.

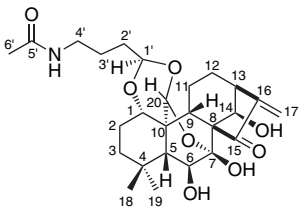
Hebeirubescensin I (**9**) was isolated as amorphous powder, and its molecular formula $C_{21}H_{32}O_7$ was established by HRESIMS. Comparison of the NMR data of **9** with those of rabdoternin F (**13**) led to the deductions that the only difference was the ketone group at C-15 in **13** being replaced by a hydroxyl group in **9**. The β-orientation of hydroxyl group at C-15 was suggested by the absence of any ROE of H-15 and the abnormal upfield shift of C-9 (δ_C 45.1, d) due to the γ-steric compression effect between OH-15 and H-9.[12,13] Thus, compound **9** was concluded to be (20*S*)-1α, 6β,7β,14β,15β-pentahydroxy-20-methoxy-7α,20-epoxy-*ent*-kaur-16-ene.

Hebeirubescensin J (**10**) was assigned to have the molecular formula $C_{21}H_{32}O_6$ from its HRESIMS and NMR data. Comparison of the spectral data of **10** with those of **9** (Table 2) showed similarities except for the substitution of a hydroxyl group at C-1 in **9** being replaced by a methylene group (δ_C 30.4, t) in **10**. The similar consideration allowed us to determine the relative stereochemistry of OH-15 with a β-orientation. Thus, the structure of **10** was established as (20*S*)-6β,7β,14β,15β-tetrahydroxy-20-methoxy-7α,20-epoxy-*ent*-kaur-16-ene.

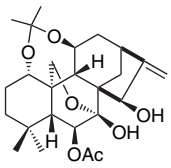
Hebeirubescensin K (**11**) was obtained as amorphous powder. The only differences between the ^1H NMR spectrum of maoyecrystal F and **11** were that of **11** lacked a methyl signal of acetyl group and the signal for the H-6α was shifted upfield from δ_H 5.27 in mayecrystal F to δ_H 4.26 in **11**.[26] This fact suggested **11** was 1α,6β,7β,11β,15β-pentahydroxy-7α,20-epoxy-*ent*-kaur-16-ene.

The molecular formula of hebeirubescensin L (**12**) was determined to be $C_{26}H_{36}O_8$ from the HRESIMS. The ^1H NMR spectrum almost superimposable with that of rabdoternin C (**15**),[14] the only exception due to the hydroxyl group at C-14 in **15** with an acetyl group in **12**. HMBC correlation of the carbonyl group of this additional acetate with H-14 unambiguously located this residue at C-14. Thus, compound **12** was determined to be 7β-hydroxy-6β,14β,15β-triacetoxy-7α,20-epoxy-*ent*-kaur-16-ene.

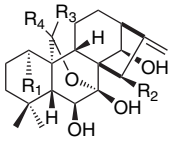
The structures of the known compounds **13–31** were established to be rabdoternin F (**13**),[10] rabdoternin E (**14**),[10] rabdoternin C (**15**),[14] oridonin (**16**),[15,16] rubescensin O (**17**),[13] rabdoternin B (**18**),[14] lasiokaurin (**19**),[17,18] rabdoternin A (**20**),[14] enmenol (**21**),[19] lasiodonin (**22**),[17,18] rubescensin C (**23**),[11] rabdoternin G (**24**),[10] rabdoternin D (**25**),[10] rubescensin Q (**26**),[20] ponicidin (**27**),[21] macrocalin B (**28**),[22] xerophilusin B (**29**),[23] acetonide of maoyecrystal F (**30**),[20,26] and trichokaurin (**31**),[24,25] by comparison of their spectral data with literature values.



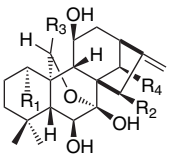
1



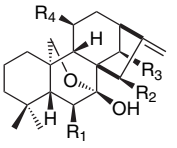
30



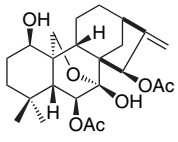
	R_1	R_2	R_3	R_4
2	OH	= O	OC_5H_{11}	H
3	OH	= O	H	OC_5H_{11}
4	OH	= O	OC_4H_9	H
7	OH	= O	OH	H
8	OH	= O	H	OH
9	OH	OH	OCH_3	H
10	H	OH	OCH_3	H
13	OH	= O	OCH_3	H
14	OH	= O	H	OCH_3
16	OH	= O	H	H
17	OH	OH	H	OCH_3
18	OH	OH	= O	
19	OAc	= O	H	H
20	H	OH	= O	
21	OH	OH	H	H



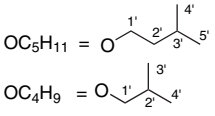
	R_1	R_2	R_3	R_4
5	OH	= O	OC_5H_{11}	H
6	H	OH	OC_5H_{11}	OH
22	OH	= O	H	H
11	OH	OH	H	H
23	H	OH	H	OH
24	OH	= O	OCH_3	H

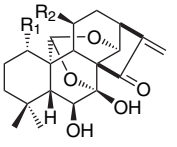


	R_1	R_2	R_3	R_4
12	OAc	OAc	OAc	H
15	OAc	OAc	OH	H
25	OAc	OH	OH	OH
26	OAc	OH	OH	H



31





	R_1	R_2
27	OH	H
28	H	OH
29	H	H

3. Biological activity

The cytotoxicities of compounds **1**–**14**, **16**, **19**, **22**, and **24** against A549, HT-29, and K562 cells were summarized in Table 3. Compounds **1**–**5**, **7**, **8**, **13**, **14**, **16**, **19**, **22**, and **24** showed inhibitory effects against those tumor cells, while compounds **6**, and **9**–**12** were completely inactive, which suggested that the cyclopentanone conjugated with an *exo*-methylene group was the active center for the inhibitory effect.[27] Moreover, careful examination of the results allowed us to determine some other structure–activity relationship. A better activity was observed when the carbon C-20 was an *S* configuration. Additionally, the presence of a hydroxyl group at C-11 would result in a marked decrease in cytotoxicity. Finally, compounds **2** and **3** were more potent than compounds **4**, **7**, **8**, **13**, and **14**, which indicated that the isoamoxyl group at C-20 could greatly improve the cytotoxicity. Further investigations of their cytotoxic

Table 3. Cytotoxicity data of compounds **1**–**14**, **16**, **19**, **22**, and **24** with IC_{50} values (μM)[a]

	1	**2**	**3**	**4**	**5**	**6**	**7** and **8**	**9**	**10**	**11**	**12**	**13**	**14**	**16**	**19**	**22**	**24**
A549	4.80	0.68	1.17	5.32	1.67	>100	8.99	>100	>100	>100	>100	2.16	2.83	9.54	6.04	46.47	53.21
HT–29	16.57	1.19	1.93	6.04	2.84	>100	18.42	>100	>100	>100	>100	5.81	6.30	12.31	9.61	31.62	15.88
K562	5.93	1.21	1.88	7.31	6.17	>100	8.32	>100	>100	>100	>100	6.84	7.82	7.86	3.08	13.52	42.71

[a] Amrubicin hydrochloride (positive control): IC_{50}=0.82 (A549), 4.36 (HT-29), and 1.26 (K562), respectively.

activities and structure–activities relationships are in progress and will be described later.

4. Conclusion

In conclusion, this was the first report of a N-containing *ent*-kauranoids, hebeirubescensin A (**1**), isolated from genus *Isodon*. Additionally, a series of new *ent*-kauranoids, named hebeirubescensins B–F (**2**–**6**), were the first examples of *ent*-kauranoids having an isoamoxyl or isobutoxyl group in the molecule. This discovery expanded considerably the library for this class of natural products. Most importantly, cytotoxicity assay showed that isoamoxyl group at C-20 could increase the lipophilicity of *ent*-kauranoids leading to apparent improvement of cytotoxicity, and this discovery could therefore serve as a scaffold for the synthesis of more potent modified diterpenoids.

5. Experimental

5.1. General

Melting points were obtained on an XRC-1 apparatus and were uncorrected. Optical rotations were carried out on a Perkin–Elmer model 241 polarimeter. IR spectra were measured in a Bio-Rad FTS-135 spectrometer with KBr pellets. MS were recorded on a VG Auto spec-3000 spectrometer or on a Finnigan MAT 90 instrument. 1D and 2D NMR spectra were taken on a Bruker AM-400 and a Bruker DRX-500 instrument with TMS as internal standard, respectively. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18}, 9.4 mm× 25 cm column. Column chromatography were performed either on silica gel (200–300 mesh. Qingdao Marine Chemical Inc., China), silica gel H (10–40 μm, Qingdao Marine Chemical Inc., China), or Lichroprep RP-18 gel (40–63 μm, Merck, Dramstadt, Germany).

5.2. Extraction and isolation

The leaves of *I. rubescens* were purchased in Hehuachi herbal market, Chengdu, Sichuan Province, People's Republic of China, in January 2004, and were identified by Prof. Xi-Wen Li. A voucher specimen was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences. The powdered air-dried leaves of *I. rubescens* (4.5 kg) were extracted with 70% aq acetone (3×20 L) at rt overnight. The extract was partitioned between H_2O and EtOAc. The EtOAc layer (165 g) was chromatographed on MCI-gel CHP 20P (90% CH_3OH–H_2O, 100% CH_3OH). The 90% CH_3OH fraction (145 g) was chromatographed over silica gel (200–300 mesh, 1.5 kg), eluted in a step gradient manner with $CHCl_3$–acetone (1:0 to 0:1) to afford fractions I–VIII. Fraction II was submitted to repeated chromatography over silica gel (petroleum–acetone, from 30:1 to 10:1; cyclohexane–2-propanol, 60:1) and RP-18, followed by semipreparative and preparative HPLC to yield compounds **2** (1.1 g), **3** (7 mg), **4** (11 mg), **5** (8 mg), and **12** (5 mg). Fraction III was first submitted to chromatography over RP-18 (CH_3OH–H_2O, from 0:1 to 1:0) and silica gel ($CHCl_3$–acetone, from 40:1 to 20:1), followed by semipreparative HPLC to yield compounds **6** (13 mg), **15** (25 mg), **29** (4 mg), **30** (24 mg), and **31** (46 mg). In the same way, fraction IV yielded compounds **10** (16 mg), **13** (1.21 g), **14** (0.42 g), **25** (7 mg), **26** (6 mg), **27** (305 mg), and **28** (3 mg). Compound **16** (12.3 g) was obtained from fraction V by recrystallization from CH_3OH. The remnant of fraction V was separated by silica gel chromatography and semipreparative HPLC to afford compounds **17** (13 mg), **18** (13 mg), **19** (6 mg), **20** (7 mg), **22** (562 mg), and **24** (11 mg). Compounds **7** and **8** (45 mg), **9** (26 mg), **11** (7 mg), **21** (3 mg), **23** (23 mg) were obtained from fraction VI. Compound **1** (9 mg) was obtained from fraction VII by repeated silica gel chromatography and semipreparative HPLC.

5.2.1. Hebeirubescensin A (1). Amorphous powder; $[\alpha]_D^{19}$ −23.2 (*c* 0.12, CH_3OH); IR (KBr) ν_{max} 3387, 2948, 2875, 1711, 1643, 1552, 1453, 1369, 1094 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) see Table 1; HRESIMS (positive ion) *m/z* 514.2430 (calcd for $C_{26}H_{37}NO_8Na$ $[M+Na]^+$, 514.2416).

5.2.2. Hebeirubescensin B (2). Colorless prisms; mp 194–195 °C; $[\alpha]_D^{19}$ −2.67 (*c* 0.26, CH_3OH); IR (KBr) ν_{max} 3395, 2956, 2871, 1710, 1643, 1458, 1092, 1063, 985 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) see Supplementary data; ^{13}C NMR (C_5D_5N, 100 MHz) see Table 2; HRESIMS (positive ion) *m/z* 473.2511 (calcd for $C_{25}H_{38}O_7Na$ $[M+Na]^+$, 473.2515).

5.2.3. Hebeirubescensin C (3). Amorphous powder; $[\alpha]_D^{19}$ −27.5 (*c* 0.32, CH_3OH); IR (KBr) ν_{max} 3417, 2954, 2870, 1711, 1644, 1463, 1142, 1095, 1060, 992 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) see Supplementary data; ^{13}C NMR (C_5D_5N, 100 MHz) see Table 2; HRESIMS (positive ion) *m/z* 473.2513 (calcd for $C_{25}H_{38}O_7Na$ $[M+Na]^+$, 473.2515).

5.2.4. Hebeirubescensin D (4). Amorphous powder; $[\alpha]_D^{19}$ −26.2 (*c* 0.32, CH_3OH); IR (KBr) ν_{max} 3375, 2957, 2933, 1711, 1645, 1459, 1370, 1218, 1091, 996 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) see Supplementary data; ^{13}C NMR (C_5D_5N, 100 MHz) see Table 2; HRESIMS (positive ion) *m/z* 459.2353 (calcd for $C_{24}H_{36}O_7Na$ $[M+Na]^+$, 459.2358).

5.2.5. Hebeirubescensin E (5). Amorphous powder; $[\alpha]_D^{19}$ −43.1 (*c* 0.38, CH_3OH); IR (KBr) ν_{max} 3374, 2956, 2934, 1710, 1642, 1458, 1370, 1083, 1052, 969 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) see Supplementary data; ^{13}C NMR (C_5D_5N, 100 MHz) see Table 2; HRESIMS (positive ion) *m/z* 473.2514 (calcd for $C_{25}H_{38}O_7Na$ $[M+Na]^+$, 473.2515).

5.2.6. Hebeirubescensin F (6). Amorphous powder; $[\alpha]_D^{19}$ −31.6 (*c* 0.15, CH_3OH); IR (KBr) ν_{max} 3315, 2955, 1628, 1463, 1366, 1104, 982 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) see Supplementary data; ^{13}C NMR (C_5D_5N, 100 MHz) see Table 2; HRESIMS (positive ion) *m/z* 475.2671 (calcd for $C_{25}H_{40}O_7Na$ $[M+Na]^+$, 475.2671).

5.2.7. Hebeirubescensin G (7). Amorphous powder; IR (KBr) ν_{max} 3332, 2945, 2871, 1709, 1645, 1460, 1364, 1094, 1055, 909 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) see Supplementary data; ^{13}C NMR (C_5D_5N, 100 MHz) see

Table 2; HRESIMS (positive ion) *m/z* 403.1738 (calcd for $C_{20}H_{28}O_7Na$ [M+Na]$^+$, 403.1733).

5.2.8. Hebeirubescensin H (8). Amorphous powder; IR (KBr) ν_{max} 3332, 2945, 2871, 1709, 1645, 1460, 1364, 1094, 1055, 909 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) see Supplementary data; ^{13}C NMR (C_5D_5N, 100 MHz) see Table 2; HRESIMS (positive ion) *m/z* 403.1738 (calcd for $C_{20}H_{28}O_7Na$ [M+Na]$^+$, 403.1733).

5.2.9. Hebeirubescensin I (9). Amorphous powder; $[\alpha]_D^{19}$ −12.5 (*c* 0.10, CH_3OH); IR (KBr) ν_{max} 3363, 2931, 2869, 1622, 1454, 1359, 1073, 1025, 983 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) see Supplementary data; ^{13}C NMR (C_5D_5N, 100 MHz) see Table 2; HRESIMS (positive ion) *m/z* 419.2040 (calcd for $C_{21}H_{32}O_7Na$ [M+Na]$^+$, 419.2046).

5.2.10. Hebeirubescensin J (10). Amorphous powder; $[\alpha]_D^{19}$ −19.1 (*c* 0.20, CH_3OH); IR (KBr) ν_{max} 3423, 2926, 1629, 1449, 1204, 1104, 984 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) see Supplementary data; ^{13}C NMR (C_5D_5N, 100 MHz) see Table 2; HRESIMS (positive ion) *m/z* 403.2090 (calcd for $C_{21}H_{32}O_6Na$ [M+Na]$^+$, 403.2096).

5.2.11. Hebeirubescensin K (11). Amorphous powder; $[\alpha]_D^{19}$ −10.8 (*c* 0.15, CH_3OH); IR (KBr) ν_{max} 3332, 2945, 2871, 1645, 1460, 1364, 1094, 1055, 909 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) see Supplementary data; ^{13}C NMR (C_5D_5N, 100 MHz) see Table 2; HRESIMS (positive ion) *m/z* 389.1943 (calcd for $C_{20}H_{30}O_6Na$ [M+Na]$^+$, 389.1940).

5.2.12. Hebeirubescensin L (12). Amorphous powder; $[\alpha]_D^{19}$ −57.2 (*c* 0.42, CH_3OH); IR (KBr) ν_{max} 3539, 2949, 1740, 1372, 1249, 1057, 899 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz) see Supplementary data; ^{13}C NMR (C_5D_5N, 100 MHz) see Table 2; HRESIMS (positive ion) *m/z* 499.2315 (calcd for $C_{26}H_{36}O_8Na$ [M+Na]$^+$, 499.2308).

5.3. Conversion of 7 and 8 into ponicidin

Hebeirubescensins G and H (**7** and **8**, 10 mg) were added to acetone (5 ml) and TsOH (2.0 mg), then stirred and kept at rt for 24 h, and then evaporated the solvent and subjected to RP-18 column eluted with CH_3OH–H_2O (40:60) to give ponicidin (**27**) (2.5 mg).

5.4. Cytotoxicity assay

Cytotoxicity of compounds against suspended tumor cells was determined by trypan blue exclusion method and against adherent cells was determined by sulforhodamine B (SRB) assay. Cells were plated in 96-well plate 24 h before treatment and continuously exposed to different concentrations of compounds for 72 h. After compound treatment, cells were counted (suspended cells) or fixed and stained with SRB (adherent cells) as described by Monks et al.[28]

Acknowledgments

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Polystyrene resins cross-linked with di- or tri(ethylene glycol) dimethacrylates as supports for solid-phase peptide synthesis

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Abstract—Polystyrene resins cross-linked with di(ethylene glycol) dimethacrylate (DEGDMA) and tri(ethylene glycol) dimethacrylate (TEGDMA), DEGDMA–PS and TEGDMA–PS, were synthesized by suspension copolymerization. Four functionalized resins, chloromethyl resin, 4-hydroxymethylphenoxymethyl resin (Wang resin), 4-methylbenzhydrylamine resin (MBHA resin) and 2-chlorotrityl chloride resin, were prepared from DEGDMA–PS and TEGDMA–PS. DEGDMA–PS and TEGDMA–PS showed high reactivity in the functionalization reactions in comparison with Merrifield resin (polystyrene cross-linked with divinylbenzene, DVB–PS). DEGDMA–PS–Wang resin and TEGDMA–PS–Wang resin were used as the solid-phase support for the synthesis of a difficult sequence, the fragment of acyl carrier protein 65–74. The yields of the crude peptide synthesized using DEGDMA–PS–Wang resin, TEGDMA–PS–Wang resin and DVB–PS–Wang resin were 92.3%, 91.6% and 78.8%, respectively. The purities of the crude peptides were 85.7%, 88.1% and 73.3%, respectively.

1. Introdution

Solid-phase synthesis method has been widely used in the synthesis of peptides, proteins and small organic molecules and in combinatorial chemistry.[1–4] In the process of the solid-phase synthesis, polymer supports play a critical role.[5–7] Polystyrene cross-linked with divinylbenzene (DVD–PS) with low cross-linkage developed by Merrifield in 1960s[8] is still the most commonly used polymer support for solid-phase synthesis because of the advantages such as good mechanical and chemical stabilities and the facility of derivations with a wide variety of functional groups for substrate attachment. The use of DVB–PS resin in many cases, however, is accompanied by difficulty during synthesis. This difficulty is primarily due to the high hydrophobic character of the matrix, the short and rigid cross-linker (DVB) connecting the PS backbone and the presence of local higher cross-link density regions because of higher reactivity of divinylbenzene relative to styrene in the free radical copolymerization.[9,10] Although DVB–PS resin with low cross-linkage is highly swellable in most of the solvents used in solid-phase peptide synthesis, swelling degree in the higher cross-link density regions should be much smaller. Thus the reactivity of the functional groups in these regions must be lower due to the steric hindering effect and lower diffusion rate of the reagents, eventually leading to a low yield of the target product in the solid-phase synthesis. In order to circumvent the inherent problems associated with DVB–PS resins, new cross-linkers have been designed both to increase the flexibility of the polymer backbone to allow for better diffusion through the matrix and also to impart a variety of solvent-like properties to the resins,[7] such as tetraethyleneglycol diacrylate,[11] 1,6-hexanediol diacrylate,[12,13] 1,4-butanediol dimethacrylate,[14,15] α,ω-bis(4-vinylbenzyl) ethers of mono-, di-, tetra-, or hexa(ethylene glycol),[16] α,ω-bis(4-vinylbenzyl) ethers of 1,4-butanediol or polytetrahydrofuran,[17] tri(propylene glycol) glycerolate diacrylate[18] and glycerol dimethacrylate[19] were copolymerized with styrene to prepare polystyrene resins as supports for solid-phase synthesis. Some of the resins cross-linked by these cross-linkers have been used for solid-phase peptide synthesis and exhibited high synthetic efficiency.[11–15,18–20] An alternative to improve the hydrophobic character of DVB–PS resin is grafting poly(ethylene glycol) (PEG) onto the resin, forming the so-called TentaGels.[21] The functional capacity of TentaGel resins is, however, fairly low. In this paper, two cheap and commercially available cross-linkers, di(ethylene glycol) dimethacrylate (DEGDMA) and tri(ethylene glycol) dimethacrylate (TEGDMA), were used to prepare novel polystyrene resins,[22] DEGDMA–PS and TEGDMA–PS, as shown in Figure 1. Dimethacrylates rather than diacrylates were chosen

Keywords: Solid-phase peptide synthesis; Di(ethylene glycol) dimethacrylate; Tri(ethylene glycol) dimethacrylate; Solid support.
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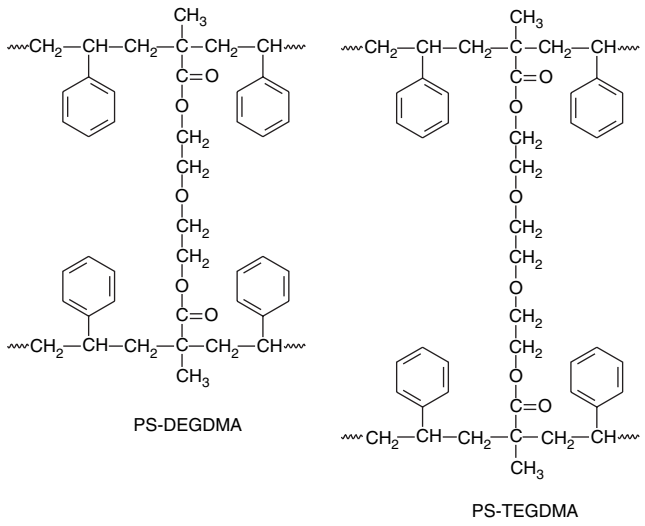


Figure 1. Structures of DEGDMA–PS resin and TEGDMA–PS resin.

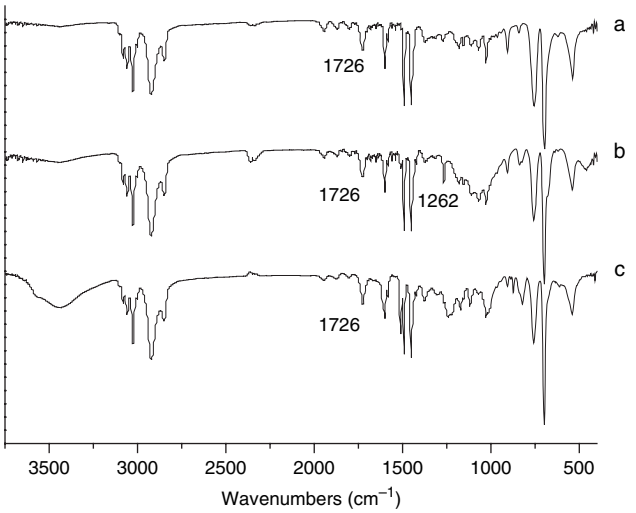


Figure 2. FTIR spectra of: (a) 3-wt % DEGDMA–PS resin, (b) clorometh-ylated 3-wt % DEGDMA–PS resin and (c) 3-wt % DEGDMA–PS–Wang resin.

for two reasons. The first reason is that polymethacrylates are much more chemically stable than polyacrylates. For example, saponification of cross-linked poly(methyl methacrylate) was much more difficult than that of cross-linked poly(methyl acrylate).[23] The second reason is that, in comparison with acrylates, methacrylates have more favourable copolymerization parameter with styrene[24] and thus the cross-linkage is more uniformly distributed in the resin made with the dimethacrylates as the cross-linker than diacrylates.

2. Results and discussion

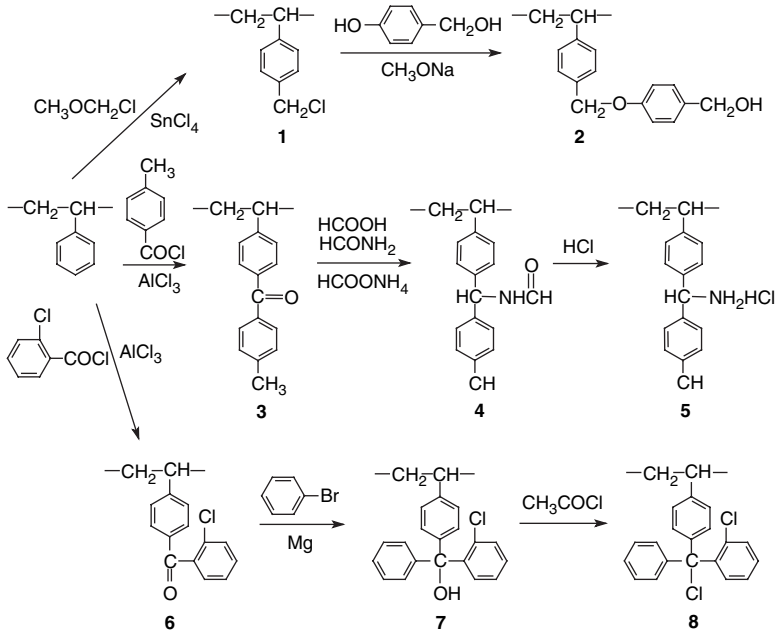
Beaded DEGDMA–PS and TEGDMA–PS resins were prepared by suspension radical copolymerization of styrene with DEGDMA and TEGDMA, respectively, with various cross-link degrees (1.6–5.3-wt % cross-linker). FTIR spectrum of 3-wt % DEGDMA–PS showed absorption at 1726 cm^{-1} (Fig. 2a), corresponding to ester carbonyl groups of the cross-liner. The swelling properties of DEGDMA–PS and TEGDMA–PS as well as 1-wt % DVB–PS gel in five solvents are listed in Table 1. It was shown that DEGDMA–PS and TEGDMA–PS resins swelled to a much greater extent than DVD–PS with a comparable amount of DVB. It was also showed that DEGDMA–PS and TEGDMA–PS resins had better swelling performance than DVB–PS resin in polar solvent compared with non-polar solvent, as indicated by ratio of swelling degree in DMF to that in CH_2Cl_2. Interestingly, DEGDMA–PS swelled to a greater extent than TEGDMA–PS with the same mole-% cross-linkage. Swelling of a gel-like resin was considered a prerequisite for facilitating reactions to occur within the solid support.[25–28] However, resins with over low cross-linkage have insufficient mechanical stability and swell excessively; excessive swelling increases the amount of solvent required for washing and the volumes of reagents needed to promote efficient synthesis. For the comparison with 1-wt % DVB–PS resin, a most commonly used support for solid-phase synthesis, 3-wt % DEGDMA–PS and 2.7-wt % TEGDMA–PS resins, which have similar or close to the swelling properties with 1-wt % DVB–PS, were used in all subsequent studies. Four functionalized resins with linkers commonly used in solid-phase peptide synthesis, chloromethyl resin (**1**), 4-hydroxymethylphenoxymethyl resin (Wang resin) (**2**), 4-methylbenzhydrylamine resin (MBHA resin) (**5**) and 2-chlorotrityl chloride resin (**8**), were prepared from 3-wt % DEGDMA–PS and 2.7-wt % TEGDMA–PS resins, as shown in Scheme 1.

The 3-wt % DEGDMA–PS and 2.7-wt % TEGDMA–PS resins were chloromethylated by Fridel–Craft alkylation

Table 1. Swelling degree (ml/g) of DEGDMA–PS and TEGDMA–PS compared to DVB–PS

Cross-linker	Cross-link degree		Dioxane	THF	DMF	Benzene	CH_2Cl_2	DMF/CH_2Cl_2[a]
	wt %	mol %						
DEGDMA	2.1	0.9	9.0	9.8	6.0	10.0	9.6	0.63
	2.4	1.0	8.3	8.6	5.4	8.8	8.4	0.64
	3.0	1.3	6.2	6.8	4.5	7.7	7.1	0.63
	4.1	1.8	5.5	5.8	4.2	6.4	6.4	0.66
	4.5	2.0	5.1	5.7	4.0	5.2	6.0	0.67
TEGDMA	1.6	0.6	7.7	9.0	5.9	9.4	9.3	0.63
	2.2	0.8	7.3	8.1	5.2	8.4	8.3	0.63
	2.7	1.0	7.0	7.4	5.0	7.7	7.4	0.68
	5.3	2.0	4.6	5.2	3.7	6.2	5.2	0.71
DVB	1.0	0.8	6.6	7.3	4.4	7.4	7.2	0.61

[a] Ratio of swelling degree in DMF to that in CH_2Cl_2.



Scheme 1. Synthetic scheme.

reaction using chloromethyl methyl ether catalyzed by anhydrous $SnCl_4$. FTIR spectrum of the resin showed absorption of chloromethyl group at 1262 cm^{-1} (see Fig. 2b) and the absorption at 1726 cm^{-1} of ester carbonyl groups of the cross-linkers remained unchanged (see Fig. 2a and b). Under the same chloromethylation conditions as shown in Section 3, the chloromethylation loadings of 3-wt % DEGDMA–PS, 2.7-wt % TEGDMA–PS and 1-wt % DVB–PS were 1.31, 1.27 and 0.85 mmol/g, respectively, indicating the higher reactivity of the resins with the two new cross-linkers as compared to DVB.

The 3-wt % DEGDMA–PS–Wang and 2.7-wt % TEGDMA–PS–Wang, were obtained by treating chloromethylated 3-wt % DEGDMA–PS (1.31 mmol Cl/g) and chloromethylated 2.7-wt % TEGDMA–PS (1.27 mmol Cl/g), respectively, with 4-hydroxybenzyl alcohol in the presence of CH_3ONa. The complete conversion was judged by no detectable residual chlorine content and disappearance of the FTIR absorption of chloromethyl group at 1262 cm^{-1} (as shown in Fig. 2b and c for DEGDMA–PS resin) of the resulting resins. The absorption at 1726 cm^{-1} of ester carbonyl groups of the cross-linkers remained unchanged, indicating that the cross-linkers were stable under the reaction condition. The loading capacities of 3-wt % DEGDMA–PS–Wang and 2.7-wt % TEGDMA–PS–Wang resins were determined by coupling Fmoc-amino acid to the resin and were found to be 0.86 and 0.80 mmol/g, respectively.

4-Methylbenzhydrylamine resin derived from 3-wt % DEGDMA–PS was prepared. The reactions were traced by FTIR spectra as shown in Figure 3. Adsorption peak of the ketone resin (**3**) at 1656 cm^{-1} (Fig. 3b) was caused by the ketone carbonyl group. When ketone resin (**3**) was transformed to formamide resin (**4**), adsorption peak at 1656 cm^{-1} disappeared and a new peak at 1695 cm^{-1} (amide carbonyl group) appeared. Complete hydrolysis of the formamide group of formamide resin (**4**) was confirmed by the disappearance of the absorption band of amide carbonyl group at 1695 cm^{-1} and 3-wt % DEGDMA–PS–MBHA (**4**) was obtained. In all the reactions, FTIR adsorption band of ester groups of the cross-linker remained unchanged, indicating that the reactions had no effect on the cross-linker introduced in the resin.

The 3-wt % DEGDMA–PS–2-chlorotrityl chloride resin (**8**) was prepared based on Scheme 1. The functionalization was confirmed by FTIR spectra, as shown in Figure 4. As in the case of the preparation of 3-wt % DEGDMA–PS–2-MBHA resin, cross-linker remained unchanged in the reactions. Under the same reaction conditions as shown in Section 3, the loadings of 2-chlorotrityl chloride resins prepared with 3-wt % DEGDMA–PS and 1-wt % DVB–PS resins were

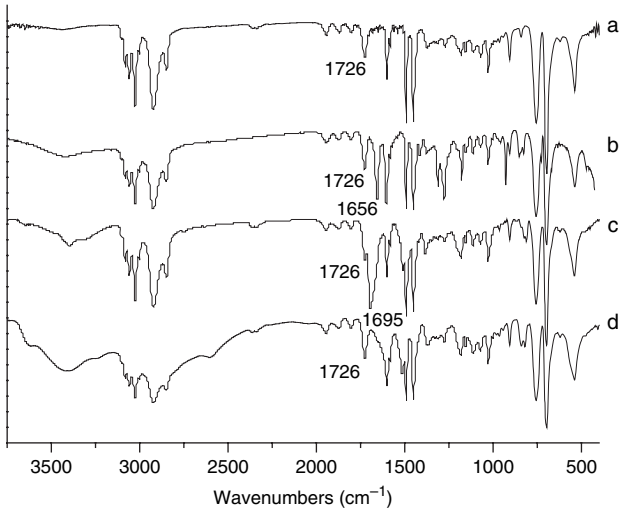


Figure 3. FTIR spectra of: (a) 3-wt % DEGDMA–PS resin, (b) 3-wt % DEGDMA–ketone resin, (c) 3-wt % DEGDMA–PS–formamide resin and (d) 3-wt % DEGDMA–PS–MBHA resin.

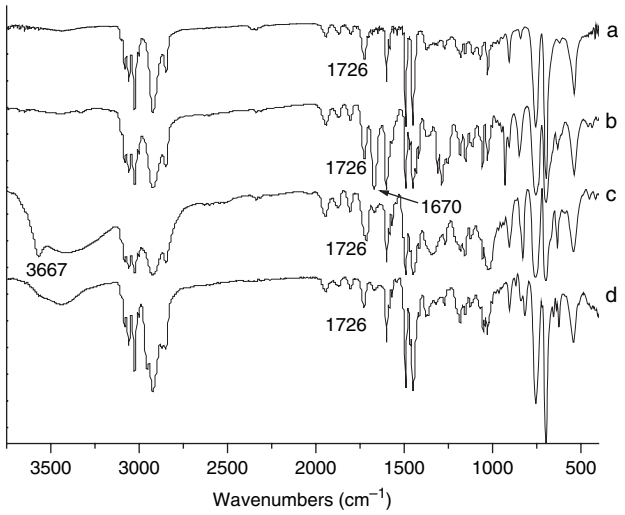


Figure 4. FTIR spectra of: (a) 3-wt % DEGDMA–PS resin, (b) 3-wt % DEGDMA–ketone resin, (c) 3-wt % DEGDMA–PS–alcohol resin and (d) 3-wt % DEGDMA–PS–2-chlorotrityl chloride resin.

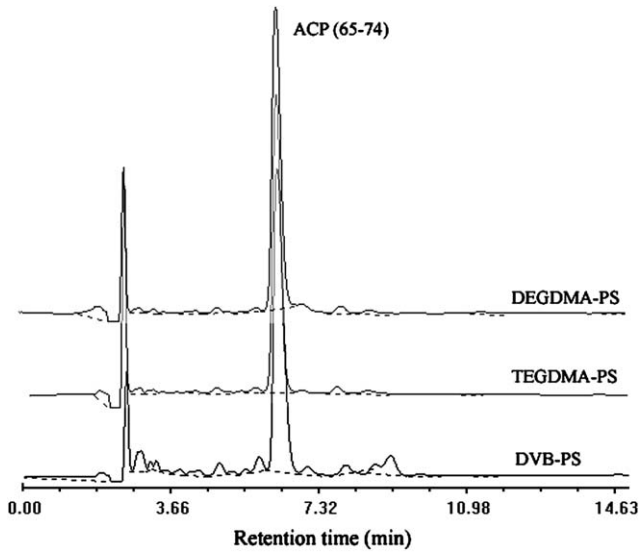


Figure 5. HPLC analysis of unpurified cleavage products. Conditions: ZORBAX SB-C18 (5 μm, 4.6×150 mm, Agilent), detected at 220 nm using 484 Tunable Absorbance Detector (Waters), 18% acetonitrile/water containing 0.05% TFA at 0.8 ml/min using 510 HPLC Pump (Waters).

determined by Fmoc-amino acid loading analysis to be 1.86 and 1.58 mmol/g, respectively.

To evaluate the application of the supports for solid-phase peptide synthesis, 3-wt % DEGDMA–PS–Wang (loading: 0.86 mmol/g) and 2.7-wt % TEGDMA–PS–Wang (loading: 0.80 mmol/g) resins were used for the synthesis of a classic difficult sequence, acyl carrier protein (65–74)[29] (ACP (65–74)) ($V^{65}Q^{66}A^{67}A^{68}I^{69}D^{70}L^{71}I^{72}N^{73}G^{74}$) by Fmoc-strategy. For comparison, 1-wt % DVB–PS–Wang resin (loading: 0.72 mmol/g) was used to synthesize the same peptide fragment in the same synthetic conditions. The yields of the crude peptide synthesized using DEGDMA–PS–Wang resin, TEGDMA–PS–Wang resin and DVB–PS–Wang resin were 92.3%, 91.6% and 78.8%, respectively, calculated on the basis of the first amino acid substitution. The purity of the acyl carrier protein fragment from each resin was tested by the HPLC on a C18 column, as shown in Figure 5. The purities of the crude peptide obtained from 1-wt % DVB–PS–Wang resin, 3-wt % DEGDMA–PS–Wang resin and 2.7-wt % TEGDMA–PS–Wang resin were 73.3%, 85.7% and 88.1%, respectively. The main peak of the HPLC chromatograms was confirmed to be ACP (65–74) by LC–MS, as shown in Figure 6. It was indicated that the purities of the peptide synthesized using 3-wt % DEGDMA–PS–Wang resin and 2.7-wt % TEGDMA–PS–Wang resin were higher than that by using 1-wt % DVB–PS–Wang resin. The 3-wt % DEGDMA–PS resin and 2.7-wt % TEGDMA–PS resin also showed high reactivity in the preparation of chloromethyl and 2-chlorotrityl chloride resins. These high reaction efficiencies may be caused by long, flexible, hydrophilic and uniformly distributed cross-linkers introduced in the resins. The 2.7-wt % TEGDMA–PS resin was slightly better than 3-wt % DEGDMA–PS resin based on the synthesis of ACP (65–74).

In summary, polystyrene resins cross-linked with DEGDMA or TEGDMA retained the advantages of DVB–PS resin, i.e., low cost, good mechanical and chemical stability and the facility of derivations with a wide variety of functional groups for substrate attachment. DEGDMA–PS and TEGDMA–PS also possessed higher reactivity in functionalization reactions. The purity of the peptide synthesized using the new supports was high when compared with the conventional DVB–PS resin.

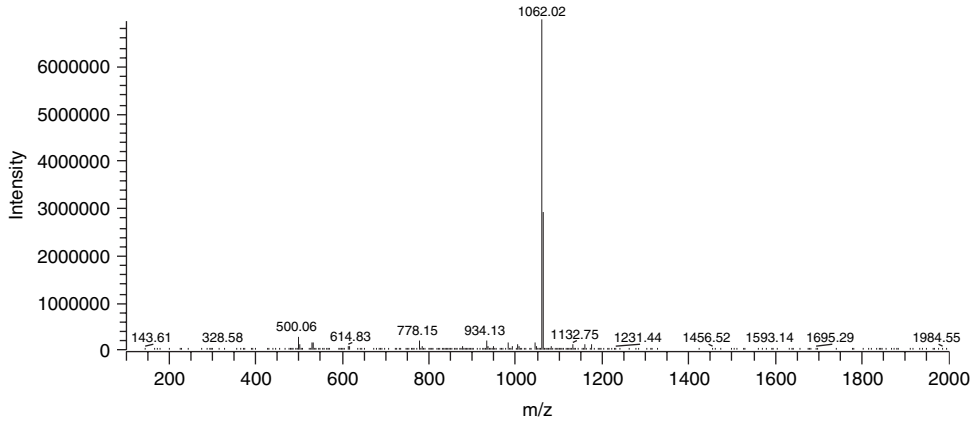


Figure 6. ESI–MS analysis of the unpurified peptide.

3. Experimental

3.1. Materials

Di(ethylene glycol) dimethacrylate (DEGDMA), tri(ethylene glycol) dimethacrylate (TEGDMA) and *N*,*N*-diisopropyl ethylamine were purchased from Aldrich. Styrene, divinylbenzene (55%), chloromethyl methyl ether, 1-wt % DVB–PS and 1-wt % DVB–PS–Wang resin (loading: 0.72 mmol/g) were from Hecheng Science & Technology Co. Ltd. 4-Hydroxybenzyl alcohol was from Lancaster. Sodium methoxide was from Fluka. Anhydrous tin tetrachloride, anhydrous aluminium chloride and magnesium were from Tianjin No. 2 Chemical Plant. 4-Toluoyl chloride, 2-chlorobenzoyl chloride, acetyl chloride and bromobenzene were from Tainyu Chemical Co. Formic acid (85%), formamide and ammonium formate were from Tianjin No. 1 Chemical Plant. Fmoc-amino acids were from Advanced ChemTech. Trifluoroacetic acid (TFA), piperidine, thioanisole, *N*,*N*′-dicyclohexylcarbodiimide (DCC), 4-dimethylamino pyridine and 1-hydroxybenzotiazole (HOBt) were from GL Biochem Ltd.

3.2. Synthesis of DEGDMA–PS and TEGDMA–PS resins

Cross-linked polystyrene resins were synthesized by suspension copolymerization. The following is a description of the synthesis of 3-wt % DEGDMA–PS as an example. A mixture of styrene (97 g), DEGDMA (3 g) and dibenzoyl peroxide (0.5 g) was suspended in 500 ml water containing 0.5% poly(vinyl alcohol) (average polymerization degree 1700, hydrolization degree 88%) with N_2 protection in a 1000 ml three-necked flask. The size of the monomer solution droplets in the aqueous phase was controlled by adjusting the stirring speed. The suspension was heated to 80 °C to initiate radical polymerization. The polymerization was carried out for 8 h at the same temperature and each droplet was converted into an individual resin bead. The polymer beads were collected by filtration and washed thoroughly with hot water to remove poly(vinyl alcohol), acetone and methanol. The resin was then extracted using 1,2-dichloroethane in a Soxhlet extractor to remove linear polymers. Beads of 100–200 mesh sizes were collected. The yield was 72%.

3.3. Swelling determination

Swelling degree was determined by the syringe method.[19] Briefly, a resin sample (500 mg) was taken in a 10-ml syringe fitted with a teflon filter at the bottom. The solvent was sucked into the syringe, and after 3 h, excess solvent was removed by applying force on the piston and the volume of the swollen resin was recorded.

3.4. Chloromethylation of polystyrene resin

Polystyrene resin (10 g) was suspended in dichloromethane (100 ml). The mixture was stirred at room temperature for 60 min and then cooled to 0 °C. To this mixture was added a solution of chloromethyl methyl ether (10 ml) and anhydrous $SnCl_4$ (1.2 ml) in dichloromethane (50 ml). The mixture was stirred at 4 °C for 50 min. The resulting resin was washed thoroughly with dichloromethane, methanol, water and methanol and then dried under vacuum to give chloromethylated polystyrene resin (**1**).

3.5. Synthesis of 4-hydroxymethylphenoxylmethyl (Wang) resin (2)

Chloromethylated polystyrene resin (5 g) was suspended in *N*,*N*-dimethylacetamide (25 ml) and the mixture was stirred for 60 min. To this mixture was added a solution of 4-hydroxybenzyl alcohol (2.1 equiv) in *N*,*N*-dimethylacetamide (15 ml) and CH_3ONa (1.4 equiv) powder. Under protection of N_2 the mixture was stirred at 50 °C for 8 h. The resulting resin was washed thoroughly with water, 1,4-dioxane and methanol and then dried under vacuum.

3.6. Synthesis of 4-methylbenzhydrylamine (MBHA) resin (5)

The 3-wt % DEGDMA–PS resin (10 g) was suspended in dichloromethane (140 ml). The mixture was stirred at room temperature for 60 min and was then cooled to −10 °C. To this mixture was added dropwise a solution of 4-toluoyl chloride (3.1 g) and anhydrous $AlCl_3$ (2.7 g) in dichloromethane (60 ml) and the temperature of the reaction mixture was maintained below 0 °C during the dropping. The reaction mixture was heated to 20 °C and stirred for 14 h. After washing with dichloromethane, methanol, water and methanol, ketone resin (**3**) was obtained. The ketone resin (**3**) (10 g) was treated with $HCOONH_4$ (24 g), HCOOH (16 ml) and $HCONH_2$ (25 ml) in nitrobenzene (100 ml) at 160–168 °C for 40 h to form formamide resin (**4**). The resulting resin (**4**) (10 g) was then treated with concentrated HCl (20 ml) in ethanol (10 ml) at refluxing for 6 h to form 3-wt % DEGDMA–PS–MBHA resin (**5**). The loading of 3-wt % DEGDMA–PS–MBHA resin (**5**) was 0.85 mmol/g determined by Fmoc-amino acid loading method.

3.7. Synthesis of 2-chlorotrityl chloride resin (8)

Polystyrene resin (10 g) was suspended in dichloromethane (100 ml). The mixture was stirred at room temperature for 60 min and then was cooled to −10 °C. To this mixture was added dropwise a solution of 2-chlorobenzoyl chloride (12 g) and anhydrous $AlCl_3$ (9 g) in dichloromethane (50 ml) and the temperature of the reaction mixture was maintained below 0 °C during the dropping. Then the reaction was carried out at refluxing for 4 h. After washing with dichloromethane, methanol, water and methanol, ketone resin (**6**) was obtained. The resulting ketone resin (**6**) (10 g) was treated with Mg (4.5 g) and bromobenzene (25 ml) in THF (160 ml) at 65–68 °C for 48 h. Then the reaction mixture was cooled to room temperature. To this mixture was added a solution of dioxane/water/concentrated HCl (5/1/1, v/v/v, 300 ml). The mixture was stirred at room temperature for 2 h. The resulting resin was washed with dioxane/water/concentrated HCl (5/1/1, v/v/v), acetone, water, methanol and dried under vacuum to give alcohol resin (**7**). The alcohol resin (**7**) (10 g) was suspended in toluene (120 ml). To this mixture was added acetyl chloride (20 ml) and then the mixture was heated to 80 °C and stirred for 4 h. The resulting resin was washed with toluene, dichloromethane and petroleum ether and then dried under vacuum to give 2-chlorotrityl chloride resin (**8**).

3.8. Determination of amino acid loading capacity of resin by Fmoc-amino acid loading analysis

For Wang resin and MBHA resins: Resin sample (1 g) was coupled with Fmoc-Gly (5 equiv) using diisopropylcarbodiimide (5 equiv), 4-dimethylamino pyridine (50 mg) and HOBt (50 mg) in DMF (10 ml) at room temperature, and the mixture was gently stirred overnight. The resin was filtered, washed with DMF and methanol and dried under vacuum. The Fmoc-amino acid attached resin (50 mg) was suspended in 20% piperidine in DMF (5 ml) and the mixture was stirred for 30 min. The filtrate of the reaction mixture together with the washing filtrate was collected and diluted to a volume of 100 ml. The resin loading capacity was obtained based on the UV absorption of the diluted solution at 290 nm. The calibration graph was made by the UV absorption of the deprotection solution of Fmoc-Gly using the similar procedure.

For 2-chlorotrityl chloride resin: Resin sample (1 g) was treated with Fmoc-Gly (3 mmol, 0.89 g) and *N*,*N*-diisopropyl ethylamine (9 mmol, 1.95 g) in dichloromethane (10 ml) at room temperature for 2 h. The resin was filtered, washed with dichloromethane, DMF and methanol and dried under vacuum. The following operation was the same as above.

3.9. Synthesis of fragment of acyl carrier protein 65–74

Incorporation of C-terminal Fmoc-Gly to the Wang resins: Wang resin (1 g) was treated with Fmoc-Gly (3 equiv), HOBt (3 equiv) and DCC (3 equiv) in DMF (15 ml) for 8 h. The resulting resin was washed thoroughly with DMF, ethanol and DMF and was then treated with acetic anhydride (1 ml) and pyridine (1 ml) in DMF (15 ml) for 4 h and then washed as above. The loading capacity of Fmoc-Gly on the resin was estimated by measuring the absorbance at 290 nm of an accurately weighed resin in 3 ml of 20% piperidine in DMF for 30 min.

General procedure for peptide synthesis: Fmoc-Gly attached resin (0.5 g) was swelled in DMF for 30 min. To this mixture was added 20% piperidine in DMF (7 ml) and the mixture was stirred for 30 min to remove Fmoc group. The resulting resin was washed thoroughly with DMF, ethanol and DMF. Then the resin was treated next with Fmoc-amino acid (3 equiv), HOBt (3 equiv) and DCC (3 equiv) in DMF (7 ml) for 2 h. The unreacted amino groups in the resin were masked by shaking acetic anhydride (0.5 ml) and pyridine (0.5 ml) in DMF (7 ml) for 30 min. This cycle of operation was repeated for the stepwise incorporation of remaining amino acids.

Cleavage of the peptide from the resin: The peptide attached resin was treated with TFA/thioanisole/water (95/2.5/2.5, v/v/v, 10 ml) at room temperature for 4 h. The resin was filtered off and washed with TFA (two times). The combined filtrate was concentrated under vacuum and the peptide was precipitated by ether.

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A highly convergent synthesis of an N-linked glycopeptide presenting the H-type 2 human blood group determinant

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We dedicate this paper to the magnificent accomplishments of Raymond Lemieux

Abstract—The total synthesis of an H-type blood group determinant in a model biological setting is described. The construct is comprised of a high mannose core structure with projecting lactose spacers, culminating in a two-copy presentation of the H-type blood group determinant itself. Key reactions that were used in this construction include sulfonamidohydroxylation (see **15**→**18**) and benzoate-directed glycosylation via an activated thiophenyl donor (see **34**→**36**). Another key strategic element involved the epimerization of an interior core glucoside to reach the β-mannoside (see **37**→**38**) required in the ring C sugar of the high mannose core.

1. Introduction

Among the great variety of functions assumed by carbohydrates in biological systems, the role of the carbohydrate domain of glycoproteins in molecular recognition has been a focus of particular attention for many scientific disciplines.[1] Indeed, the growing field of glycobiology is devoted to defining the effects of protein glycosylation on a range of phenomena, including fertilization,[2] tumor metastasis,[3] and immune response.[4] Specific glycosylation patterns also serve as markers for tumorigenesis and are used in blood profiling.[5] A major challenge facing the field of glycobiology is the limited natural accessibility of homogeneous glycopeptides, which can be attributed to difficulties in isolation, compounded by their often microheterogeneic nature. Certainly, the development of technologies to allow access to homogeneous glycopeptides would be of significant value in probing critical interactions at the interface of glycochemistry and glycobiology. Despite the daunting challenges associated with the preparation of glycopeptides in the laboratory, we envisioned that chemical synthesis could yet prove to be the most viable way to provide the field of glycobiology with 'precision tools' for the study of glycoproteins.[6,7]

An ongoing focus of our laboratory has been the development of methodologies that enable the preparation of complex, fully synthetic glycopeptides.[8] An early goal of this research program was the assembly of pentacyclic glycopeptide, **1**, in which the carbohydrate domain is N-linked to the peptide through an asparagine γ-carboxamide, not dissimilar to the way Nature combines such structures. We describe, herein, the design and execution of the synthesis of **1**.[9] We note that, for reasons discussed below, we also accomplished the synthesis of **2**, which differs from **1** only in the absolute stereochemistry of the peptide domain.[10]

As a further demonstration of the advances in the field of glycopeptide synthesis, we set for ourselves the rather ambitious ultimate goal of synthesizing an N-linked, 15-ring glycan construct (**3**), containing *a mature H-type 2 blood group determinant* at its non-reducing end. Nothing of this scope had previously been undertaken in synthesizing N-linked glycopeptides. The manner in which we accomplished the synthesis of this complex target compound is described herein.[11]

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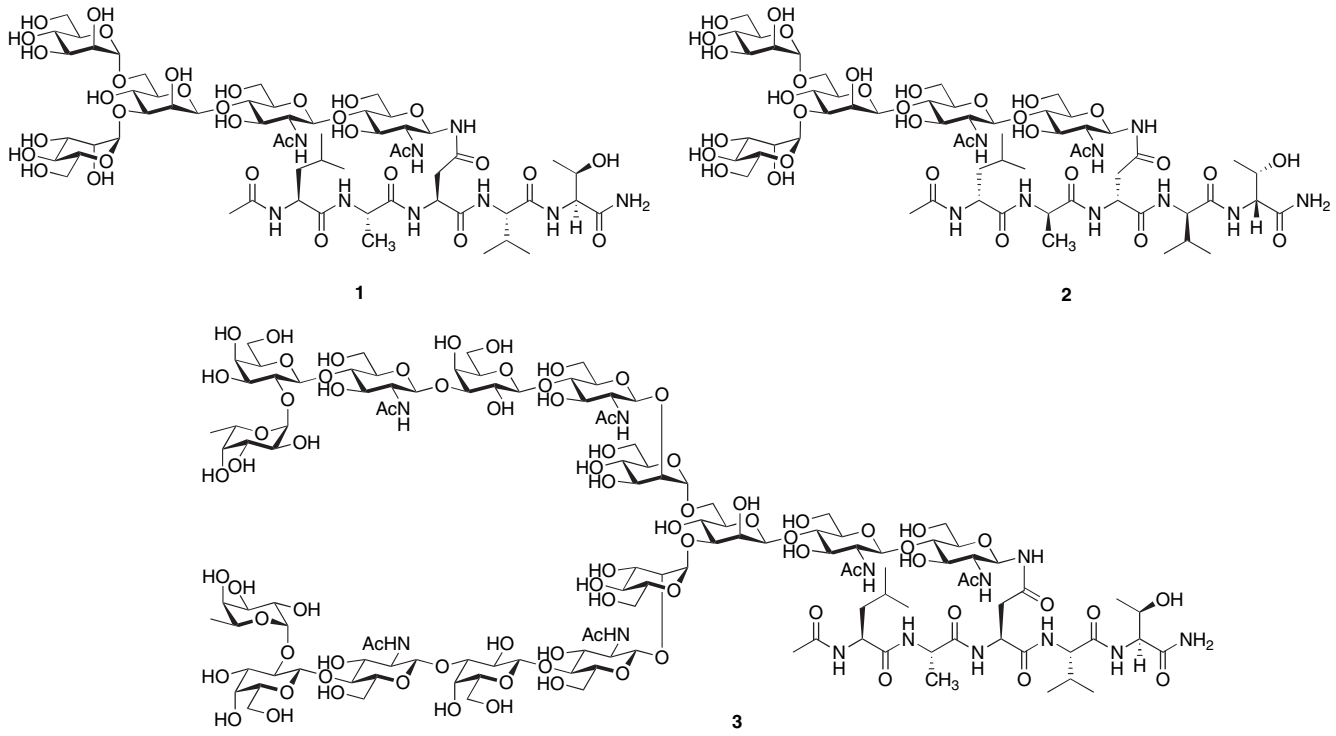


2. Results and discussion

In charting a program for glycopeptide synthesis, provisions must be made for assembling the glycan domain. To the extent that the ultimate goal is that of simulating the motifs of Nature, the glycan synthesis itself may well be quite complex. In addition, a polypeptide domain must be assembled. Depending on the situation, the polypeptide domain may be introduced as a single block, or in the form of segments to be subsequently ligated. In either case, the need for the glycan to be coupled to an appropriate peptide chain in the final stages of the synthesis requires careful selection of appropriate glycosylation and protection strategies, with due planning from the outset. Indeed, the implementation of an efficient global deprotection sequence is critical to the success of the enterprise.

In the case of N-linked glycopeptides, the anomeric amine required for eventual merger with a peptide-based acyl donor can be generated in several forms (Fig. 1). Coupling of the 'per protected' glycosylamine produces a glycopeptide, which is then subjected to global deprotection. While the coupling step can be high yielding, maintenance of anomeric configurational homogeneity of the N-linked peptide may be problematic.[12] Indeed, coupling of the anomeric amino group of a perbenzylated glycan (path *a*) or peracetylated glycan (path *b*) to the γ-carboxyl of an aspartyl residue on the peptide chain can produce a difficult

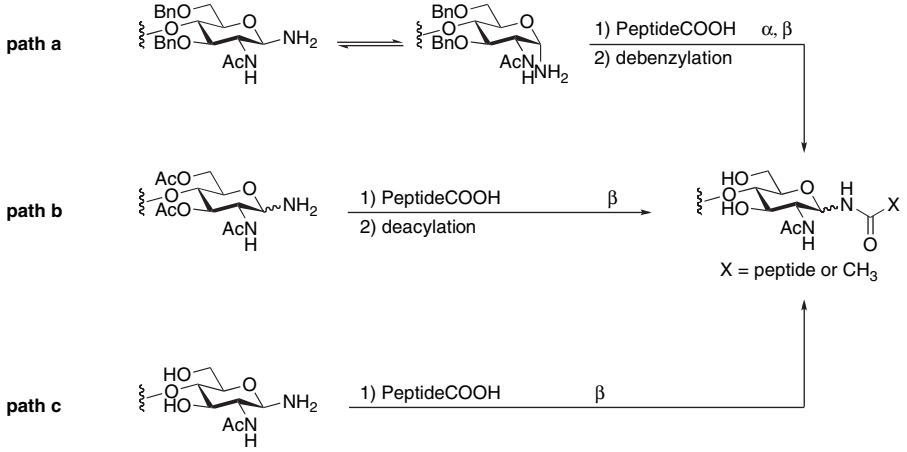
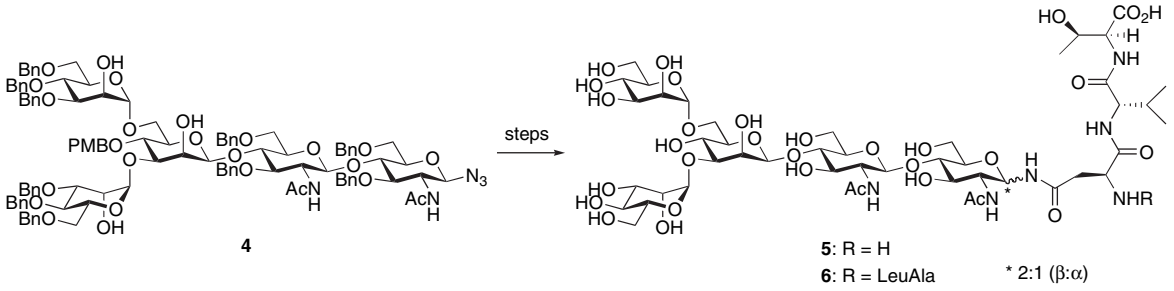


Figure 1. Comparison of glycopeptide assembly: (a) O-benzylated (electron-donating) glycosylamine, was coupled with peptide acid, followed by debenzylation. In a complex glycosylamine system, peptide conjugation led to α/β mixture of glycopeptide due to anomerization of benzylated glycosylamine; (b) O-acetylated glycosylamine (electron-deficient) was acylated with peptide acid. In this system, potential migration of acetyl from $O \rightarrow N$ led to the formation of glycosyl acetamide (X=CH_3) in addition to the desired β-glycopeptide; (c) selective N-acylation of a polyhydroxyl glycosylamine can be achieved with peptide acid utilizing minimum protection.

to separate mixture of *N*-aspartyl anomers or anomeric acetamide.

In this connection, we note that in an earlier initiative in our laboratory, we had accomplished the maximally convergent synthesis of an N-linked glycopeptide.[12] A pentacyclic glycan was prepared using methodology instituted under our paradigm of glycal assembly. Even in this complex setting, the glycal linkage was convertible to the required terminal glucosamine linkage. In the final stage of that effort, reduction of β-anomeric azide **4**, in the context of a substantially protected glycan, produced the glycosylamine. Following aspartylation with tripeptide and pentapeptide donors and then global deprotection, high mannose tripeptide **5** and pentapeptide **6** were each isolated as 2:1 (β:α) anomeric mixtures. The precise point at which anomeric configurational integrity was compromised (cf. inter alia, reduction of the azide, acylation or deprotection) is not clear.



Fortunately, as demonstrated by Lansbury[13] fully deprotected glycans bearing β-configured anomeric amino functions can be efficiently acylated, taking advantage of the significantly higher nucleophilicity of the amino group (Fig. 1, path *c*). The stereochemical integrity of the reducing terminus is preserved in the case of fully deprotected glycans. Furthermore, use of fully deprotected free glycans and glycosylamines obviates the need for late stage global deprotection after formation of the glycopeptide.

In planning the synthesis of the five-ring glycopeptide **1**, we anticipated that the required glycan might be assembled through various glycal-based methods.[14,15] Following global deprotection, the glycan would be aminated at its reducing end, using the procedure developed by Kochetkov,[16] and then subjected to conjugation with the peptide (vide infra) as reported by Lansbury.[13]

As in our previously reported syntheses of **5** and **6**,[12] we hoped that a terminal glycal (see structure type **10**) would prove suitable for the eventual presentation of the 1-β-amino function of the A ring (*N*-acetylglucosamine) to the peptide, as required under path *c* (Fig. 1). The pentasaccharide glycal would be obtained with high convergence by coupling of a donor derived from 'pre-trimannose glycal' **7** with prechitobiose glycal **8**. Both **7** and **8** can be accessed in short order from D-glucal.[12,15]

However, a significant issue presents itself in this otherwise attractive coupling strategy. The glycal assembly method, which we favored, was well suited to fashion the C-ring as a β-glucoside (cf. **9**) rather than as the requisite β-mannoside. The high convergence of our proposed synthesis seemed sufficiently attractive to justify, if necessary, an indirect route to reach the β-mannoside BC connection. The thought was to exploit the expected ease of first appending the C-ring as a β-glucoside. This would be followed by epimerization of the C2 center of the C-ring (see asterisk, structure **9**), thereby producing the mannoside (**10**). In essence, the question was whether the efficiency of the assembly phase, enabled by glycal logic, would vindicate the awkwardness of the need for a late stage gluco→manno epimerization sequence (Fig. 2).

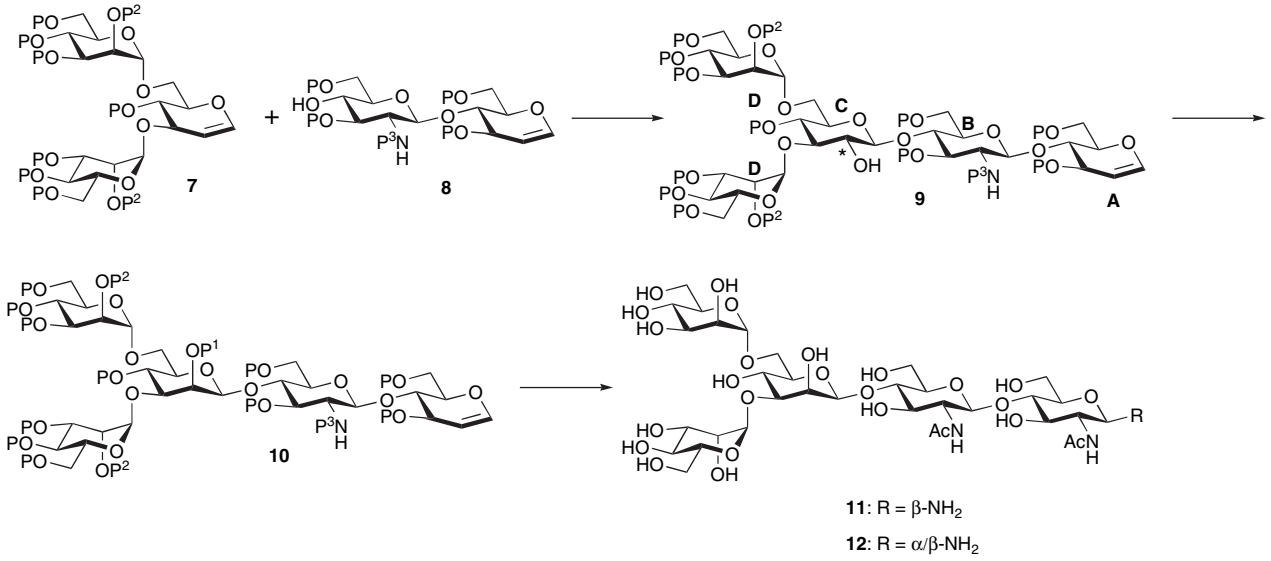


Figure 2. Synthetic plan for the core pentasaccharide glycosylamine. *P*, P^1, P^2, and P^3 represent protecting groups.

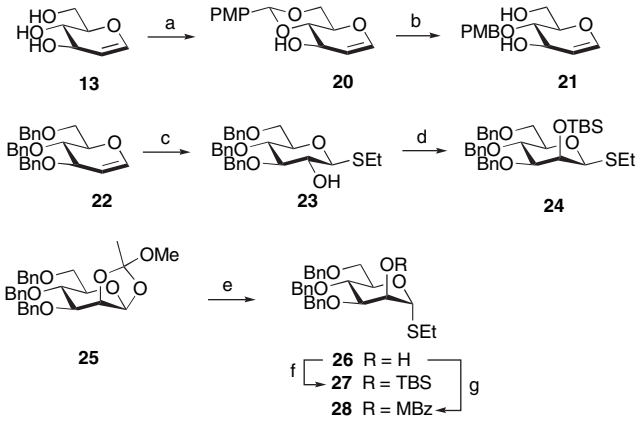
2.1. Discussion of results

Our venture commenced with the preparation of the building blocks from which the core pentasaccharide glycan (cf. **11**) could be synthesized. From the outset, we recognized that it would be important to develop a program that would allow for maximum diversification during the assembly of the mature glycan. Hopefully the strategy would not only encompass this endeavor, but would also establish a framework for future projects. As shown in Schemes 1 and 2, D-glucal (**13**) would serve as the common starting material for each building block. Additionally, the synthesis would make use of a tactic for azaglycosylation that had been developed in our laboratory some 15 years ago.[17]

The synthesis of the pre-chitobiose glycal (cf. **19**) commenced with 3,6-dibenzylglucal **14**, which had in turn been synthesized from D-glucal (**13**) as previously reported (Scheme 1).[17] Acetylation of the free alcohol in **14** provided the fully protected glucal **15**. Iodosulfonamidation followed by thiolysis of the intermediary aziridine at the anomeric center gave rise to thiodonor **17**. The original glucal **14** then served as an acceptor in the methyl triflate promoted glycosylation with **17**. Following methanolysis of the C4′ acetate, the pre-chitobiose glycal acceptor **19** was in hand.

Our most convergent route to the requisite pre-trimannose glycal of the type **7** would be through the selective introduction of α-mannosyl residues at C3 and C6 of glycal **21**. The synthesis of **21** was accomplished in a two-step sequence starting, once again, from D-glucal (**13**). Thus, engagement of the C4 and C6 alcohols of **13** as a *p*-methoxybenzylidene derivative gave rise to **20** (Scheme 2). Reductive cleavage of the acetal linkage afforded the appropriately protected glycal **21**.

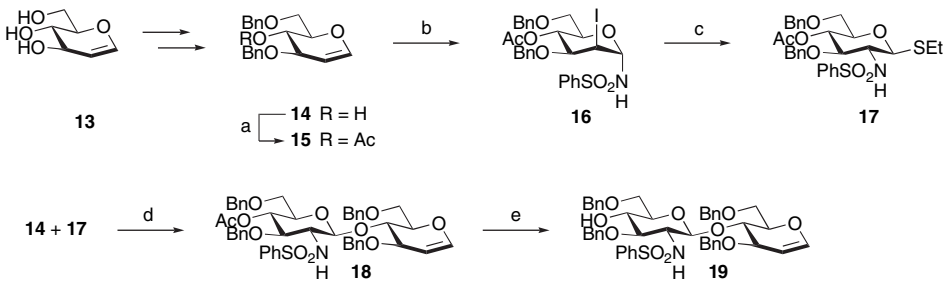
The required α-mannoside donor, **24**, was synthesized from commercially available tri-*O*-benzyl-D-glucal (**22**). Thus, stereoselective epoxidation with dimethyldioxirane (DMDO), followed by selective ring opening with ethanethiol, in the presence of trifluoroacetic anhydride, gave rise to thioglycoside **23**. Inversion of the alcohol at C2 was accomplished by Moffat-like oxidation[18] followed by stereoselective reduction of the resulting ketone with sodium borohydride. The now inverted alcohol was then protected to afford thiodonor **24**.



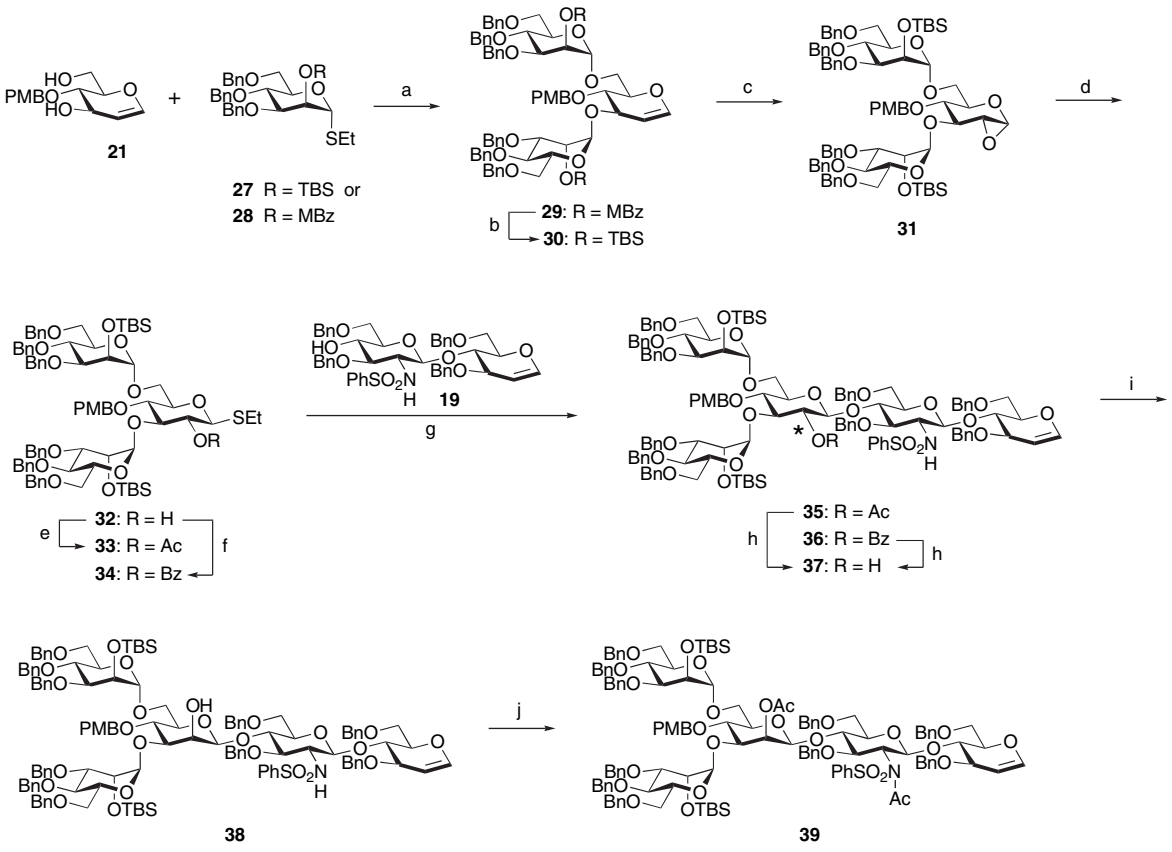
Scheme 2. Synthesis of the α-mannoside donor and acceptor from D-glucal: (a) *p*-anisaldehyde dimethyl acetal, PPTS, THF, 48%; (b) Dibal-H, DCM, 75%; (c) 1. DMDO, DCM, 2. EtSH, TFAA, 89% in two steps; (d) 1. DMSO, Ac_2O, 2. $NaBH_4$, DCM, MeOH, 76% in two steps, 3. TBSOTf, DCM, Et_3N, 93%; (e) 1. EtSH, $HgBr_2$, CH_3CN, 95%, 2. NaOMe, MeOH; (f) TBSOTf, DCM, Et_3N, 93%; (g) *p*-toluoyl chloride, DCM, Et_3N, 75%.

Alternatively, monosaccharides of this type may be prepared from the commercially available orthoester **25** in three steps, beginning with mercuric bromide-mediated ring opening with ethanethiol followed by methanolysis of the resulting acetate to afford **26**. The free alcohol of **26** can be reprotected with TBS triflate or *p*-toluoyl chloride (4-methylbenzoyl chloride) to afford **27** or **28**, respectively. It should be noted that this alternate route is more attractive due to its amenability to the production of large quantities of the α-mannoside donor.

With the mono- and disaccharide building blocks in hand, we began assembly of the pentasaccharide (cf. **11**), and ultimately construction of the target pentasaccharide–pentapeptides **1** and **2**. This exercise would serve to field-test and, indeed, validate our plan for synthesizing complex glycopolypeptides carrying biological information (cf. **3**). Thus, the construction of the α-trimannoside donor commenced with di-α-mannosylation of the glycal diol acceptor **21** (Scheme 3). Diglycosylation with 2-*O*-TBS protected thiomannoside **27**, using methyl triflate as the activating agent, afforded trisaccharide glycal **30** in 54% yield. The same transformation proceeded in somewhat higher yield (63%) to afford **29** when ester-protected thiomannoside **28** was used as the donor. However, because this ester group would later interfere with the epimerization sequence required at



Scheme 1. Synthesis of the pre-chitobiose glycal acceptor building block from D-glucal: (a) Ac_2O, pyridine, DMAP, 94%; (b) IDCP (Iodonium-di-*sym*-collidine perchlorate), $PhSO_2NH_2$, DCM, 0 °C, 94%; (c) EtSH, LHMDS, DMF, −40 °C–0 °C, 62%; (d) MeOTf, DTBP (2,6-di-*tert*-butylpyridine), DCM, 0 °C to rt, 70%, β/α 6:1; (e) NaOMe, MeOH, 90%.



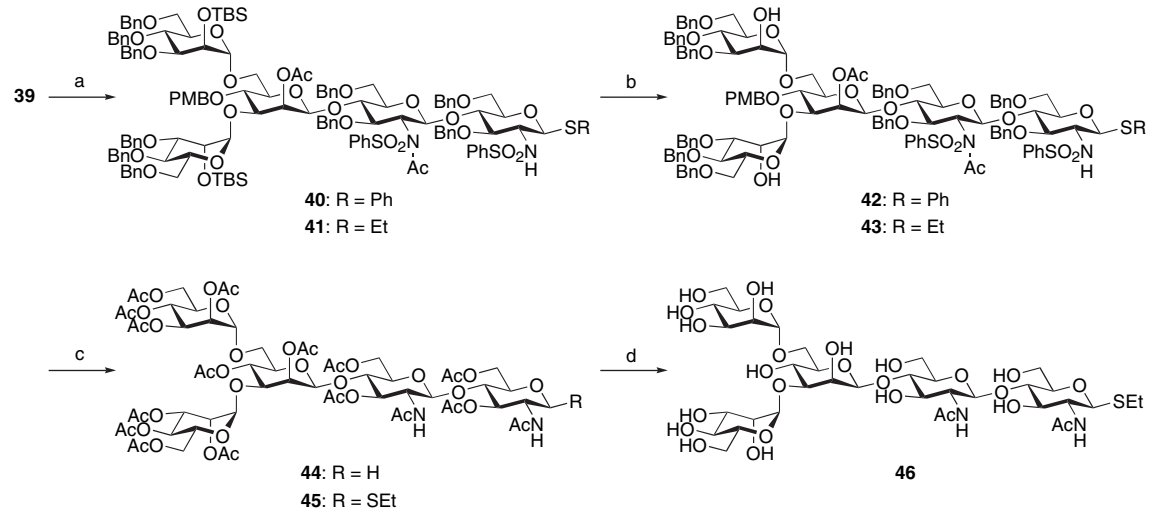
Scheme 3. Synthesis of the core pentasaccharide glycal: (a) **27** or **28**, MeOTf, DTBP, DCM, 54% for **30**, 63% for **29**; (b) 1. NaOMe, MeOH, 77%, 2. TBSOTf, Et_3N, DCM, 95%; (c) DMDO, DCM, crude yield >99%; (d) EtSH, TFAA, DCM, 76%; (e) Ac_2O, Et_3N, DCM, 91%; (f) BzCl, pyridine, DCM, 90%; (g) **19**, MeOTf, DTBP, DCM, 64% for **35**, 71% for **36**; (h) $LiAlH_4$, Et_2O; (i) 1. Dess–Martin periodinane, DCM, 2. L-Selectride, THF; (j) Ac_2O, pyridine, DMAP, DCM, 82% in four steps.

the pentasaccharide stage, the ester protecting groups in **29** had to be converted to silyl groups (**29**→**30**). While this two-step sequence could easily be achieved, the overall route was not highly efficient.

In either case, the double bond of the trisaccharide **30** was epoxidized with DMDO to afford **31**. Unfortunately, the direct coupling between this epoxide as a donor, and the pre-chitobiose acceptor **19** was never successful. Despite significant efforts to optimize this type of transformation, only trace amounts of pentasaccharide product could be detected by this seemingly straightforward approach. Fortunately we had earlier developed a modality to deal with such a contingency.[9,19] Applied to the case at hand, epoxide **31** was transformed into the thioglycoside alcohol **32**, which was subsequently protected as either an acetate (**33**) or benzoate (**34**). Both the β-glucoside bond donors were evaluated in coupling with the acceptor **19**. Coupling with trisaccharide acetate **33** provided corresponding pentasaccharide **35**, albeit in modest yield (64%). In practice, glycosylation was significantly complicated by orthoester formation. By contrast, upon using benzoate thioglucoside **34** as donor, the reaction was quite reproducible, providing pentasaccharide **36** in a more acceptable 71% yield. Not surprisingly, the benzoate proved to be a superior *trans*-glycosylation directing group.

The proposal had indeed been realized in a highly convergent fashion. However, it would now be necessary to convert the C-ring from gluco to the manno stereochemistry, by overall epimerization at C2 (compound **37**, see asterisk).[20] The overall inversion at this center would require gaining access to a C-ring uloside (i.e., 2-keto) functionality which could subsequently be reduced to provide, hopefully, a C2 axial alcohol (cf. **38**). In the event, deprotection of neither the acetate in **35** or the benzoate in **36** could be achieved under Zemplén conditions.[21] However, either acyl group could easily be cleaved reductively with LAH, yielding alcohol **37**. The equatorial alcohol in **37** was then oxidized with Dess–Martin periodinane,[22] giving rise to an unstable ketone that was immediately reduced using L-Selectride.[23] In this way, alcohol **38**, with the C2-axial hydroxyl group, was obtained in 82% yield over four steps. Subsequent acylation of the alcohol afforded diacetate **39**.

At this point, appropriate functionalization of the reducing end glycal and global deprotection had to be achieved prior to introduction of the peptide chain. Toward this end, glucal **39** was subjected to iodosulfonamidation and thiolysis, thereby giving rise to phenyl thioglycoside **40** in 85% yield (Scheme 4). The latter was subjected to TBAF-mediated desilylation, and the resultant crude product, **42**, was reacted with sodium in liquid ammonia, followed by peracetylation of the fully deprotected glycan. Unfortunately, as spectroscopic analysis of the product demonstrated, the erstwhile thiophenyl group was no longer present, having presumably been cleaved during the dissolving metal reduction. The observed product of this sequence, **44**, contained two anomeric protons.



Scheme 4. Upgrading of the pentasaccharide terminal glycal: (a) 1. IDCP, $PhSO_2NH_2$, DCM, 2. PhSH, LHMDS, DMF, 85% in two steps for **40**, or EtSH, LHMDS, DMF, 81% in two steps for **41**; (b) TBAF, THF, 80%; (c) 1. Na/NH_3, THF, 2. Ac_2O, pyridine, DMAP, 70% for **44**, 77% for **45**; (d) NaOMe, MeOH, 86%.

We reasoned that the phenyl ring of the anomeric thiophenyl function could well have facilitated the reduction of the C–S linkage. Accordingly, it was surmised that an alkyl thioglycoside might be more stable under the same reductive conditions. In the event, when ethylthioglycoside **43** (obtained from glucal **39** in a similar sequence) was subjected to the action of sodium in liquid ammonia followed by peracetylation and purification, the desired product **45**, containing an intact C–S bond, was isolated in 77% yield. The *O*-esters in **45** were then saponified to provide the fully deprotected pentasaccharide **46** in 86% yield.

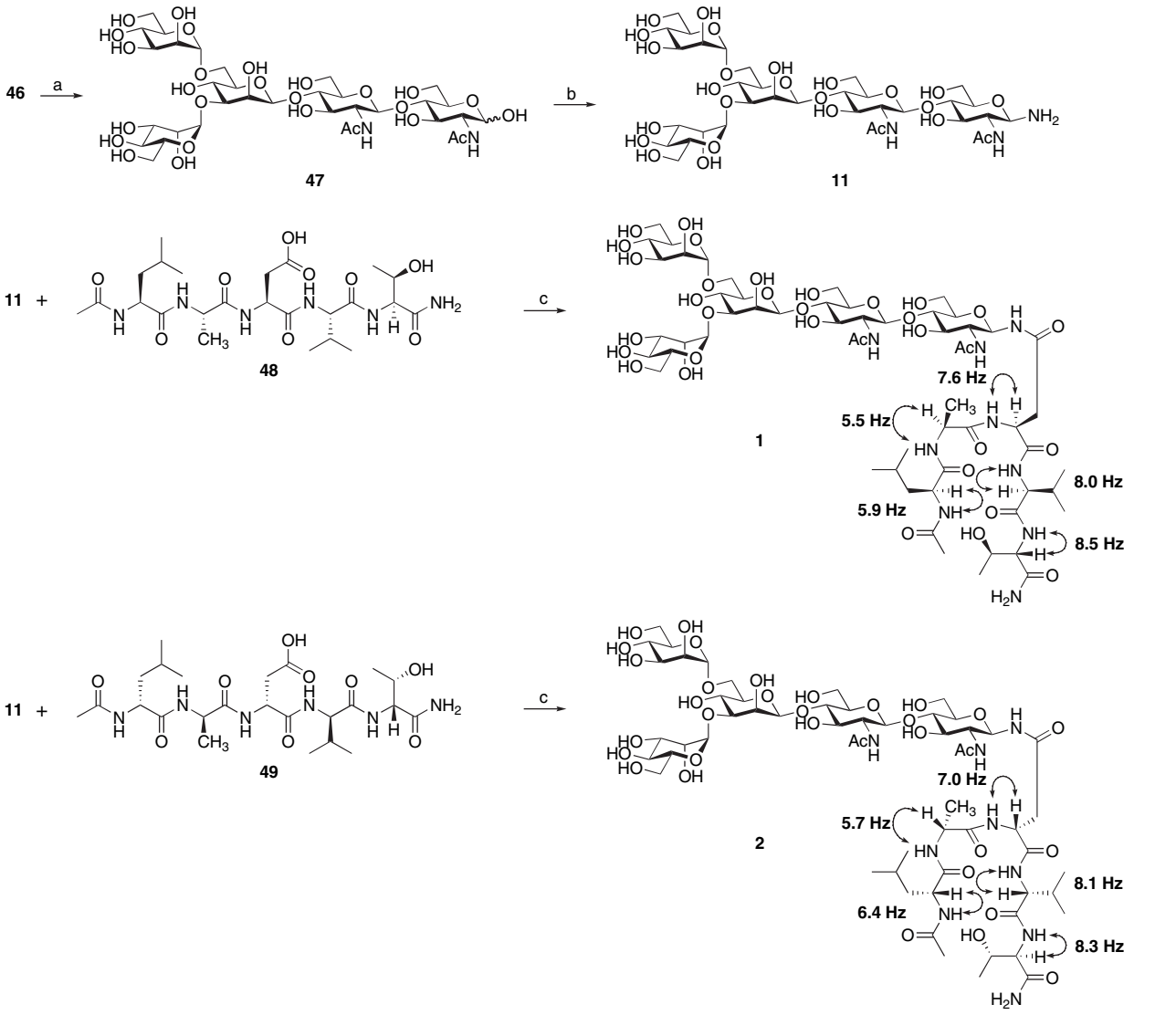
In order to implement a Kochetkov–Lansbury sequence for building the glycopeptide domain, it would be necessary to liberate, in some as yet unspecified fashion, an anomeric hydroxyl group. Thus, overall hydrolysis of the thioglycoside functionality in **46** would perhaps lead us to **11**. Though reaction of **46** with NBS in aqueous THF turned out to be difficult to reproduce, hydrolysis with $HgCl_2$ proceeded efficiently[24] to afford the reducing saccharide **47** in 95% yield, after gel filtration. Finally, this alcohol was converted into β-anomeric amine **11** using the Kochetkov protocol;[16] no α-anomer was detected in the ^1H NMR spectrum of the product.

Two pentapeptide enantiomers, one composed entirely of L-amino acids (**48**) and the other of D-amino acids (**49**), were prepared using an automatic Fmoc-synthesizer on a Rink amide MBHA resin. Each of the peptides was conjugated with the amine **11** using HOBt/HBTU to activate the aspartic acid. The diastereomeric β-N-linked glycopeptides **1** and **2** were isolated in 40% yield, with no observable formation of the α-anomer (Scheme 5).

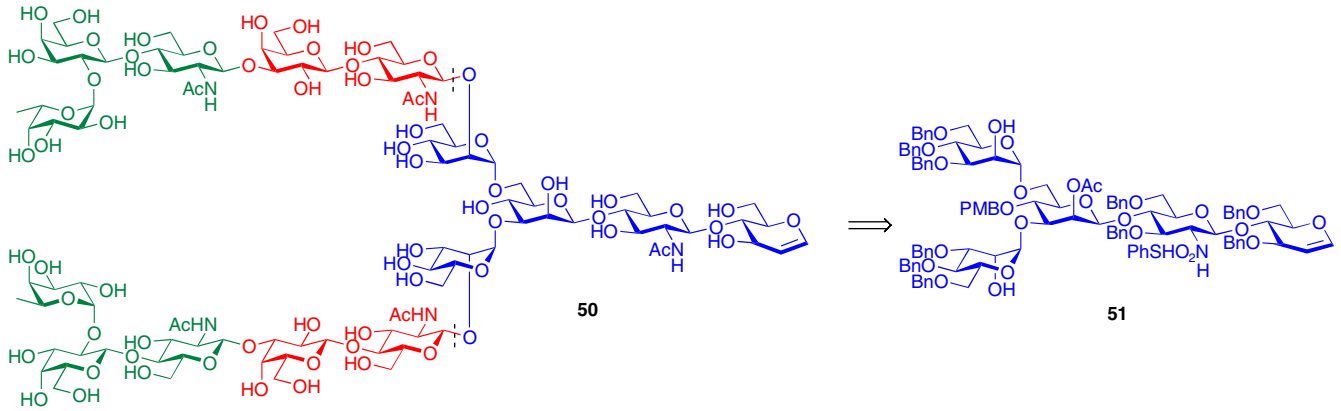
At this point, it is appropriate to discuss our reasons for preparing not only glycopeptide **1**, but also glycopeptide **2**, which differs only in the absolute stereochemistry of the peptide domain. An important area of study in the field of glycobiology is the evaluation of the structural configurations of glycopeptides. Early work in this field has focused on the evaluation of structural interactions within carbohydrate or peptide domains; however, little is known about communication across domains.[25] This gap can be attributed, in large part, to the very limited accessibility of homogeneous glycopeptide constructs. Given our interest in assembling such constructs through convergent chemical synthesis, we chose to prepare **1** and **2** in order to probe questions of stereochemical communication between the carbohydrate and peptide domains.[10]

Indeed, the results of high-field NMR studies performed on **1** and **2** strongly indicate the existence of stereochemical interactions between the peptide and carbohydrate domains of these N-linked glycopeptides. Although the NOESY patterns of compounds **1** and **2** were quite similar, as expected, distinct shift differences were observed for the protons at the central amino acid residues, as indicated in Figure 3. Thus, distinct shifts were observed for the amide NH protons of asparagine (to which the carbohydrate is linked) and the adjacent valine residue. Since amide proton chemical shifts are good indicators of peptide backbone conformations, these results clearly demonstrated that a global change in peptide stereochemistry impacts the structural conformation of the glycopeptide.[26] Importantly, the results of this exercise show that cross-domain communication cannot be solely attributed to the bulk of the carbohydrate moiety.[10]

Having field-tested the technologies required for glycal assembly and peptide conjugation in the syntheses of glycopeptides **1** and **2**, we were prepared to take on the challenge of synthesizing our ultimate target compound: a pentadecasaccharide glycan construct linked to a mature H-type 2 blood determinant (**3**). As will be seen, many new difficulties had to be overcome to meet this challenge. We turn first to our approach to the synthesis of the glycal portion of the molecule (**50**). In this system, the H-type 2 trisaccharides (shown in green) are appended to the common pentasaccharide domain (shown in blue) through two lactosamine spacer units (shown in red).



Scheme 5. Construction of glycopeptides: (a) $HgCl_2$, $CaCO_3$, H_2O, 95%; (b) NH_4HCO_3, H_2O, >99%; (c) **48** or **49**, HOBt, HBTU, $^{i}Pr_2NEt$, DMSO, 40%.



A logical disconnection, shown in structure **50** (see lines), would lead us to the common pentasaccharide domain as a key building block. The appropriately protected pentasaccharide intermediate (**51**) would be easily obtained through desilylation of the previously synthesized **39** (Scheme 3).

At this stage, two approaches for the preparation of the pentadecasaccharide **50** were considered. In the first, the di- and trisaccharide precursors **52** and **53** would be assembled to form the H-type 2-lactosamine donor pentasaccharide **54**. The latter would then be joined with **51** through

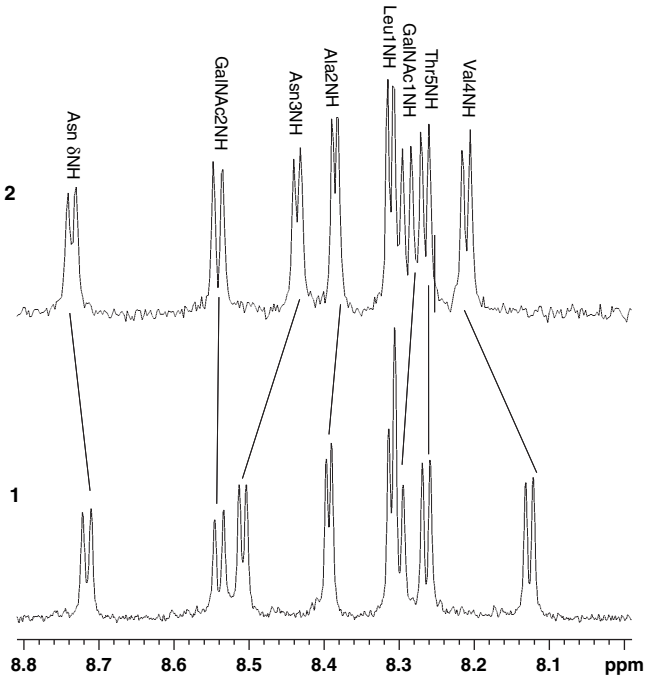


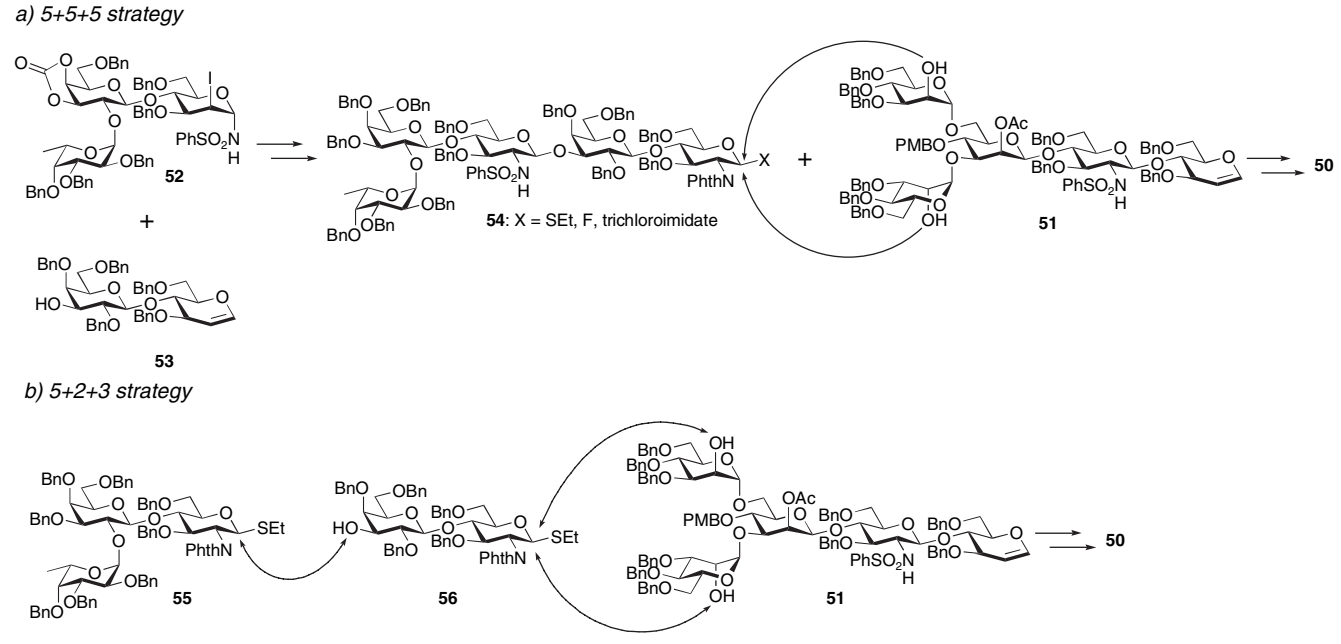
Figure 3. Amide proton spectra of **1** and **2** recorded at 800 MHz at 5 °C. Samples were dissolved in 90% H_2O/10% D_2O ([**1**]=5 mM, [**2**]=1 mM) and the pH was adjusted with a 10 mM phosphate buffer (**1**: pH 3.5, **2**: pH 4.2). The H_2O signal was suppressed using the Watergate method.

diglycosylation, as shown, in a highly convergent '5+5+5' coupling reaction (Scheme 6a). A second, more linear, approach would involve sequential coupling of the di- and trisaccharide precursors to the initial pentasaccharide unit (**51**). Thus, an appropriately protected lactosamine derivative **56** would be coupled to **51**. Then, following selective deprotection, diglycosylation with the H-type 2 trisaccharide would occur to afford the glycal **50** (Scheme 6b).

The more convergent '5+5+5' approach was investigated first. Unfortunately, the projected 5+5+5 coupling reaction did not occur cleanly upon activation of either the thioethyl-, fluoro- or trichloroacetimidate derivatives of donor **54**. However, on the basis of mass spectral analysis, it appeared that 5+5 coupling did in fact occur at each of the acceptor sites in **51**. The stereochemistry of these glycosylations could not be determined in the context of the inhomogeneous product. However, in no fraction could we claim mass spectral evidence that the 15-mer had arisen from an actual 5+5+5 coupling.

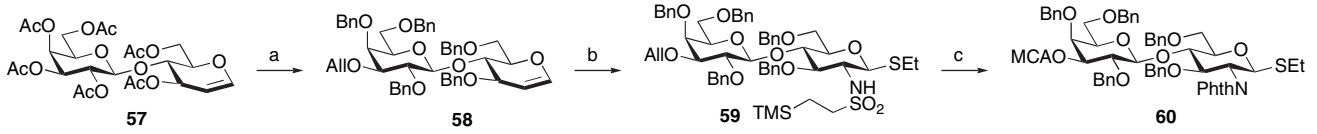
Since at least one glycosylation had occurred, we surmised that each of the glycosylation acceptor sites in **51** was, in principle, a competent acceptor. Perhaps with a more reactive glycosyl donor, two-fold glycosylation could be achieved. We reasoned that smaller, properly activated lactosamine donors, such as **56**, might allow for two-fold glycosylation of **51**. With proper selection of the lactosamine protecting groups, two new acceptor sites could be unveiled subsequent to coupling with **51**, at which point two activated H-type 2 donors, such as **55**, could be concurrently installed, producing the pentadecasaccharide via a '5+2+3' coupling strategy (Scheme 6b).

With these considerations in mind, we chose compound **60** as our lactosamine donor. This compound incorporates an orthogonally protected hydroxyl group at C3′ as well as a strongly β-directing phthalamide at C2. The synthesis of **60** commenced with the hexaacetyllactal **57** (Scheme 7). This compound was subjected to deacetylation with ammonia in methanol, and the resulting hexaol was selectively allylated at C3′ through the intermediacy of dibutyltin acetal formation.[27] Benzylation of the remaining hydroxyl groups afforded lactal **58** in 50% yield over four steps. The reducing end was then functionalized using the previously developed silylethylsulfonamide (SES) methodology.[28]



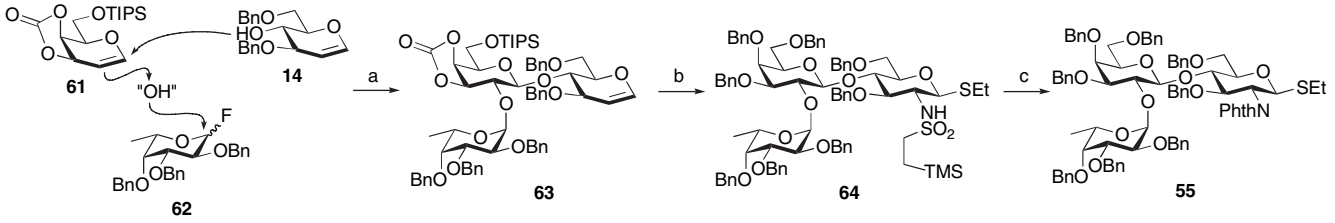
Scheme 6. Assembly of divalent H-type 2 antigen-encoded 15-mer glycal: comparison of the 5+5+5 and 5+2+3 strategies.

Thus, iodosulfonamidation with 2-trimethylsilylethylsulfonamide, followed by thiolysis, with concurrent migration of sulfonamide from C-1 to C-2 and ejection of iodide, provided thioglycoside **59** in 85% yield (two steps). Finally, the sulfonyl and allyl protecting groups were converted to phthaloyl[29] and chloroacetyl, respectively, to provide the desired lactosamine donor **60**.



Scheme 7. Synthesis of the lactosamine building block: (a) 1. NH_3, MeOH, 2. Bu_2SnO, PhH, 3. allyl bromide, Bu_4NBr, 4. BnBr, NaH, DMF, 50% in four steps; (b) 1. IDCP, $TMSCH_2CH_2SO_2NH_2$, 75%, 2. EtSH, LiHMDS, DMF, 85%; (c) 1. CsF, DMF, 90 °C, 94%, 2. phthalic anhydride, pyridine, then Piv_2O or Ac_2O, >99%, 3. $RhCl(PPh_3)_2$, DABCO, THF; 1 N HCl, 74%; 4. $(ClCH_2CO)_2O$, DTBP, cat. DMAP, DCM, 97%.

Glycal assembly was once again used to prepare the H-type 2 trisaccharide donor **55** (Scheme 8). Galactal **61** was selectively α-epoxidized with DMDO and the resulting epoxide was directly coupled with 3,6-dibenzylglucal **14** in the presence of $ZnCl_2$. The newly formed C2′ hydroxyl in the resulting disaccharide was fucosylated with **62**, to furnish trisaccharide glucal **63** in 50% yield (three steps). Perbenzylation and functionalization of the reducing end, following the procedure described for lactosamine **60** (vide supra), provided the 2-*N*-phthaloyl thioglycoside donor **55** required for the introduction of H-type 2 specificity.



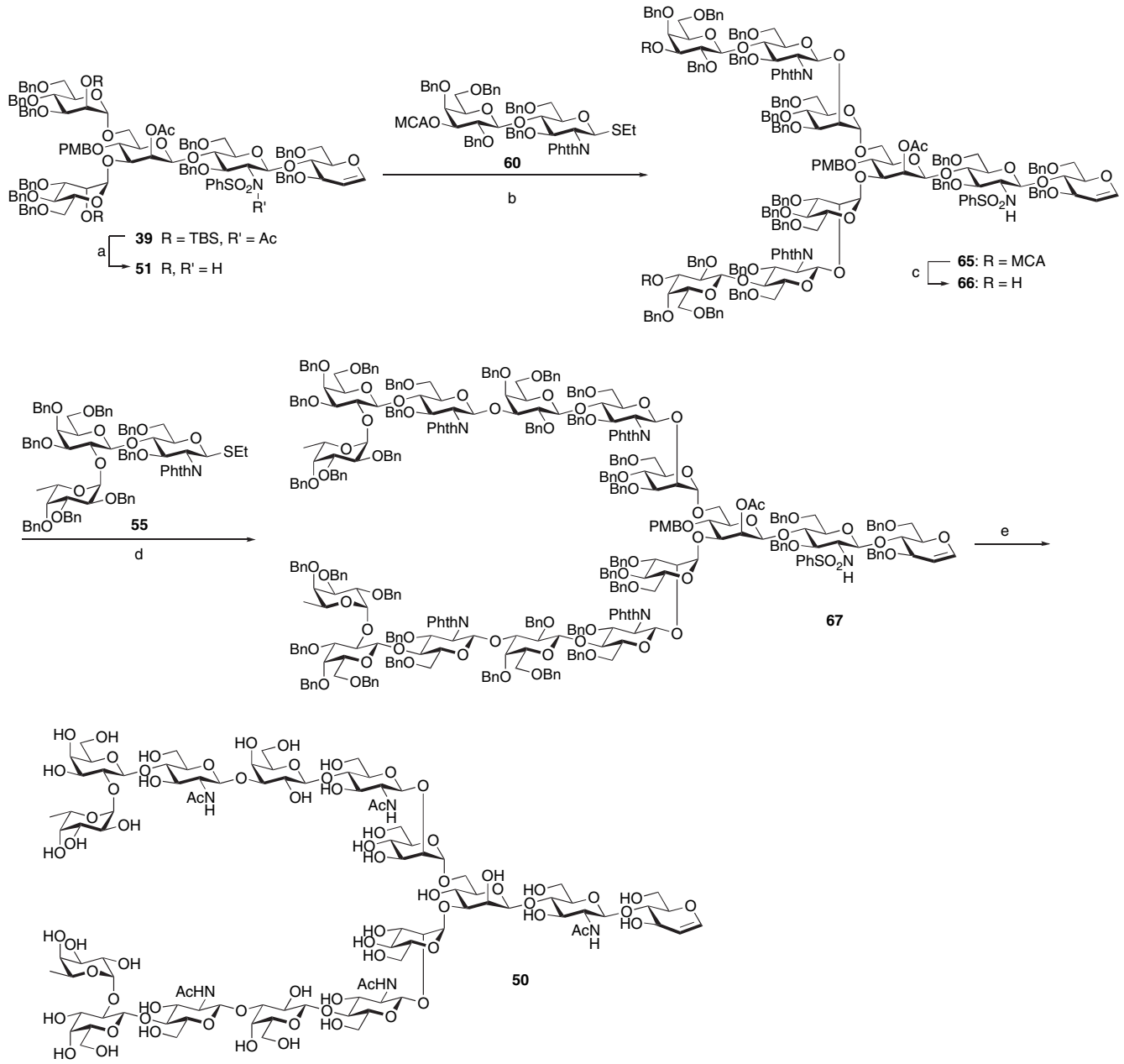
Scheme 8. Synthesis of the wing trisaccharide **55**: (a) 1. **61**, DMDO, DCM; 2. **14**, $ZnCl_2$, DCM; 3. **62**, $SnCl_2$, AgOTf, DTBP, 50% (three steps); (b) 1. TBAF, THF, 2 h then K_2CO_3, MeOH; 2. BnBr, NaH, DMF, 85% (two steps); 3. IDCP, $TMSCH_2CH_2SO_2NH_2$; 4. EtSH, LiHMDS, DMF −40 °C to 0 °C, 75% (two steps); (c) 1. CsF, DMF, 100 °C, 5 d, 65%; 2. phthalic anhydride, pyridine; 3. Ac_2O, 97% (two steps).

With the required subunits and technology in hand, we turned to the assembly of the pentadecasaccharide glycal. Thus, the C2 hydroxyl groups in **39**, previously protected with silyl groups, were now unveiled with TBAF to afford diol **51**. The latter was coupled with excess lactosamine donor **60**, using methyl triflate as a promoter (Scheme 9).[30] Importantly, the diglycosylation of **51**, which had failed with pentasaccharide donor **54**, could be easily achieved with disaccharide donor **60**, to provide the desired nonasaccharide **65** in 62% yield. Next, the chloroacetate groups at C3′ of the lactosamine units in **65** were removed under neutral conditions, while keeping the acetate on the central mannose unit and the terminal glycal intact. Finally, the H-type 2 trisaccharides (**55**) were introduced and, once again, diglycosylation proceeded smoothly to give rise to pentadecasaccharide **67** in 78% yield.

At this stage, the challenge was that of achieving global deprotection of the pentadecasaccharide system. Though our ultimate strategy was to deprotect following functionalization of the reducing end, we field-tested the deprotection sequence on the unfunctionalized glycal **67**. Thus, the phthaloyl and *O*-acetate groups were first removed using ethylenediamine. Next, the sulfonamide, *p*-methoxybenzyl, and benzyl linkages were reductively cleaved with sodium in liquid ammonia. The crude product was peracetylated, purified through normal phase chromatography, and then deacetylated under Zemplén conditions, to afford fully deprotected glycal **50**.

Having established the feasibility of the deprotection sequence with the glycal, **67**, we sought to apply the same logic to the deprotection of the functionalized glycan. Thus, iodosulfonamidation of glycal **67** gave the *trans*–diaxial adduct **68**, which was then subjected to thiolysis with LiSEt in the hope of obtaining the corresponding thioglycoside **69** (Scheme 10). However, this transformation failed to provide **69**; instead, the reducing hemiacetal **70** was isolated from the reaction mixture in 40% yield. In fact, we were never able to gain access to **69**, whereas the hydrolysis product **70** was isolated even under strictly anhydrous conditions.

As we were unable to convert the glycal into the reducing end thioglycoside in the context of the pentadecasaccharide, we decided to investigate the possibility of direct global deprotection of the oligosaccharide hemiacetals under dissolving metal reduction conditions. Such conditions, while clearly too harsh for the hemiacetal in the open aldehyde form, may leave the 'reducing end' intact provided that it is 'protected' via total dominance of the ring closed form in liquid ammonia. The results of our model studies (summarized in Table 1) demonstrate the truly remarkable stability and survivability of the reducing oligosaccharides under these conditions. A series of reducing glycans, including examples with amides and hydroxyls at C-2, were deprotected with yields of isolated peracetylated products typically above 70%.[31]



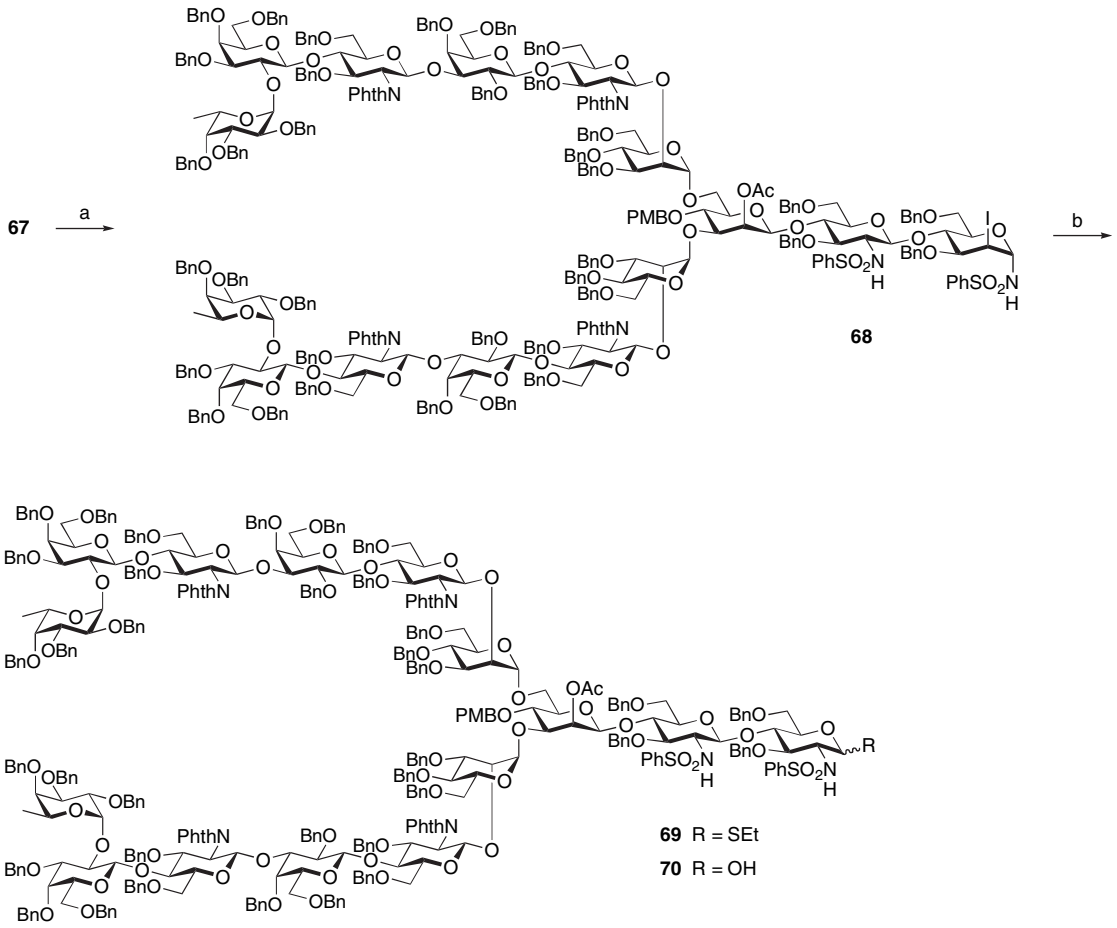
Scheme 9. Synthesis of the 15-mer glycal: (a) TBAF, THF, 77%; (b) **60**, MeOTf, DTBP, DCM, 0 °C to rt, 62%; (c) thiourea, $NaHCO_3$, EtOH, 99%; (d) **55**, MeOTf, DTBP, DCM, Et_2O, 78%; (e) 1. $NH_2CH_2CH_2NH_2$, EtOH, reflux; 2. Na/NH_3, THF; 3. Ac_2O, pyridine, DMAP; 4. NaOMe, MeOH (30%, four steps).

This pivotal finding paved the way for a much more efficient and straightforward approach to the preparation of N-linked glycans. Not only is the need for a moisture-sensitive and problematic thiolysis step obviated, but both isomeric sulfonamides can be utilized in the hydrolysis, greatly simplifying the transformation and allowing higher throughput of material.

We were eager to apply this novel approach to our pentadecasaccharide system (Scheme 11). Thus, the phthalimides in **67** were first converted into acetamides (cf. **71**). Then, the glycal was subjected to iodosulfonamidation, followed by basic hydrolysis of the resulting product mixture, to afford pentadecasaccharide **72**. At this point, the stage was set for the critical global deprotection.

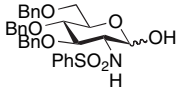
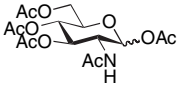
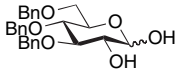
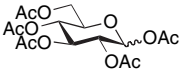
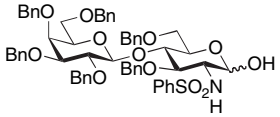
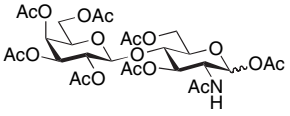
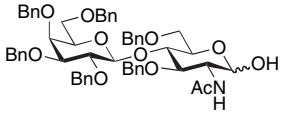
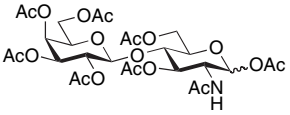
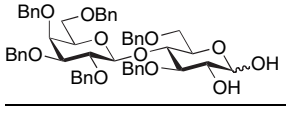
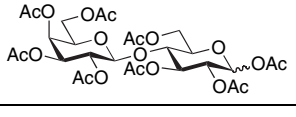
Hemiacetal **72** was subjected to reduction with sodium in liquid ammonia (Scheme 12),[32] and happily, the reducing end hemiacetal proved sufficiently robust; the fully deprotected material, **73**, was isolated in 57% yield over two steps, following partial reacetylation and reverse-phase column chromatography. The reducing alcohol was then converted into anomeric amine **74** under Kochetkov amination conditions,[16] and the latter was then coupled with pentapeptide **48** to furnish the N-linked glycopeptide **3**, presenting the H-type 2 blood group specificity.

The ability to gain access through chemical synthesis to such an advanced N-linked glycopeptide in a homogeneous state provided a unique opportunity to investigate not only its glycoarchitecture but also the biological implications of the



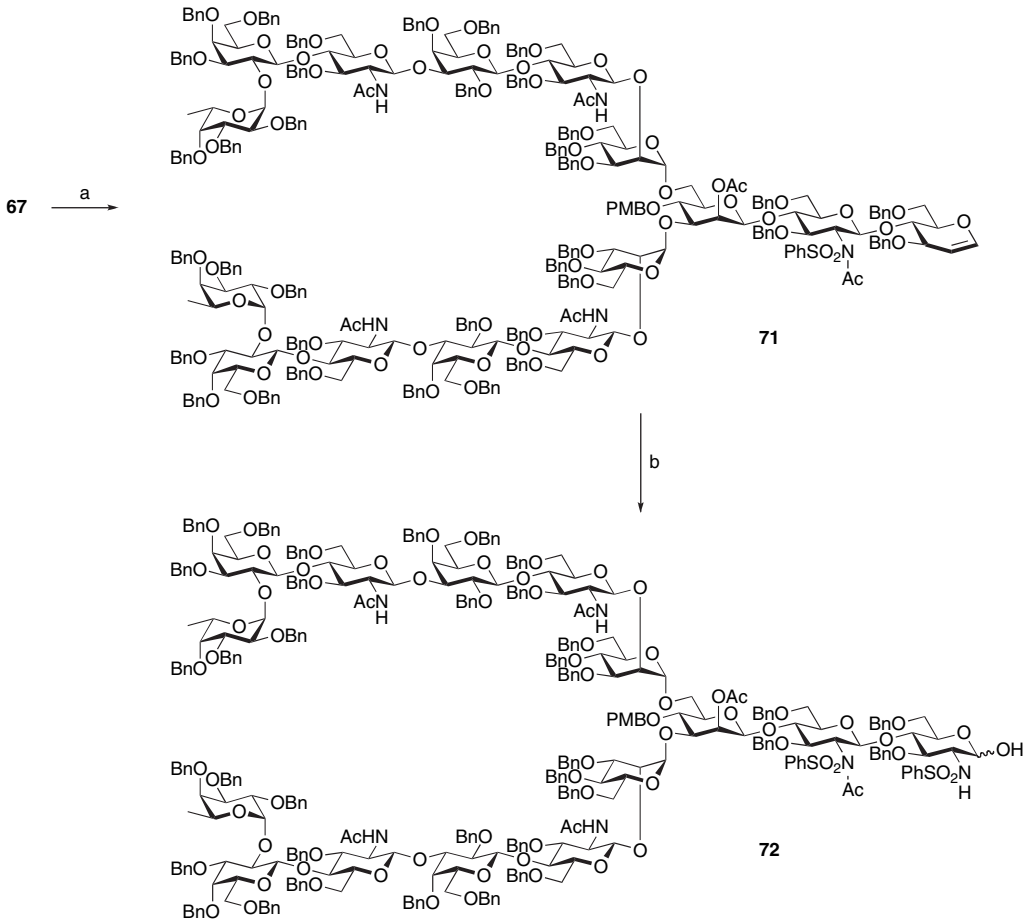
Scheme 10. Functionalization of the terminal glycal: (a) $PhSO_2NH_2$, IDCP; (b) EtSH, LiHMDS, DMF, **70** only, 40% (two steps).

Table 1. Dissolving metal reduction of glycal-derived 1-hydroxy sugars

Deprotection substrate	Peracetylated substrate	Time (min)	Yield[a] (%)
		60	64
		20	65
		60	76
		60	72
		20	79

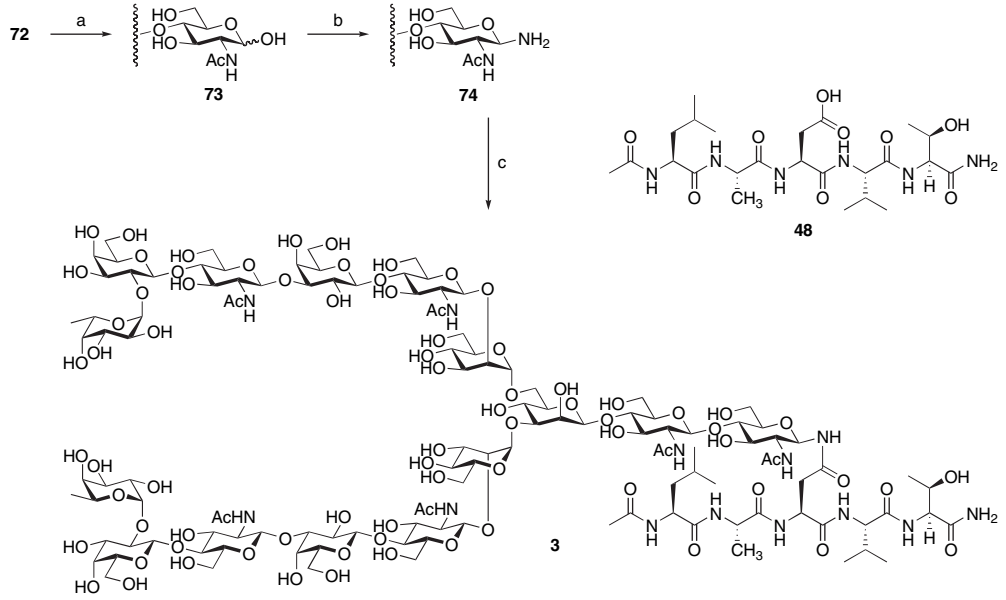
Reactions were performed by the addition of the saccharide in THF to a stirred solution of Na (6 equiv perbenzyl group) in NH_3 at −78 °C. After quenching and workup, the crude product was acetylated for analysis.

[a] Isolated yield following column chromatography.



Scheme 11. Upgrading of the terminal glycal to a hemiacetal: (a) 1. $NH_2CH_2CH_2NH_2$, EtOH, 2. Ac_2O, pyridine, DMAP, 85% (two steps); (b) 1. IDCP, $PhSO_2NH_2$, DCM, 74%; 2. LiHMDS, AgOTf, THF, H_2O, 63% or aq K_2CO_3, THF, 60%.

attached H-type 2 specificity. Indeed, the ^1H NMR spectrum of **3** (Fig. 4) is extremely well resolved, indicative of a high degree of structural order.[25] Moreover, the functionality of the H-type 2 blood group determinants was confirmed in an enzyme-linked immunosorbent assay (ELISA) where glycopeptide **3** reacted with an antibody against the H-type



Scheme 12. Stereocontrolled synthesis of a β-N-linked 15-mer glycopeptide containing the H-type 2 blood group determinants: (a) 1. Na/NH_3, THF; 2. Ac_2O, MeOH, 57% (two steps); (b) NH_4HCO_3, H_2O, >99%; (c) **48**, HOBt, HBTU, DIPEA, DMSO, 20%.

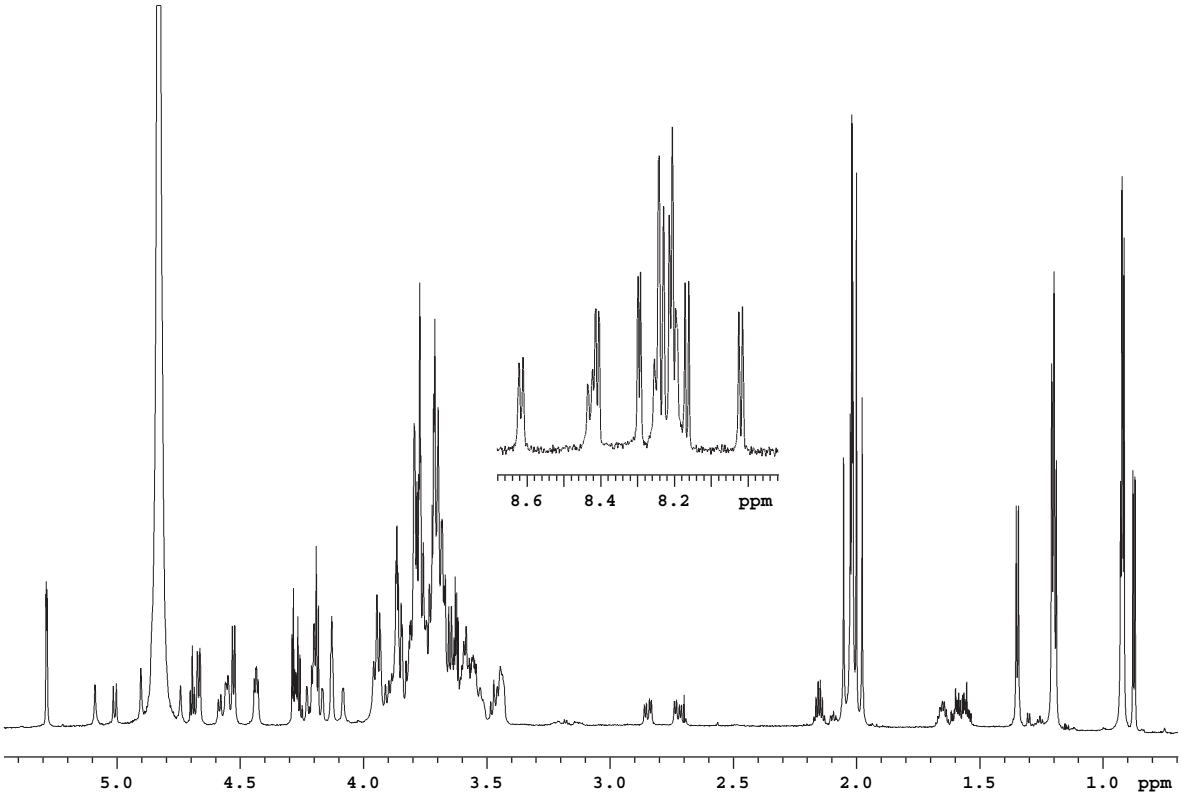


Figure 4. The ^{1}H NMR spectrum (800 MHz) of **3** in D_2O at 20 °C and pH 3.7 (phosphate buffer). The inset shows the secondary NH signals of amides from the peptide backbone, side chain, and GlcNAc sites when the spectrum is taken in H_2O at 5 °C and pH 3.7 (phosphate buffer).

2 determinant (Fig. 5). This antibody is highly specific for the H-type 2 blood group determinant, and does not react with related structures, such as H-type 1, Le^a, or Le^x.[33,34]

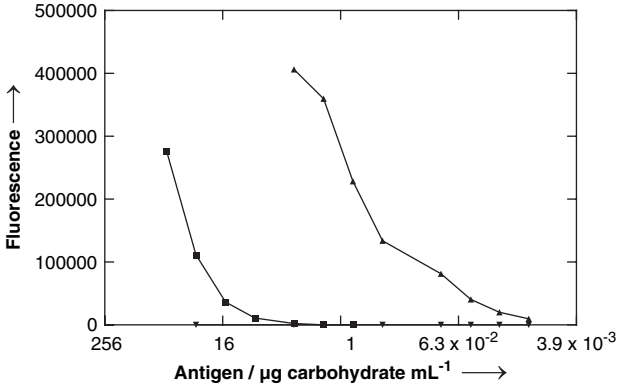


Figure 5. Reactivity of glycopeptide **3** (■), H-type 2 active mucin (▲) as a positive control, and Le^x/Le^a active mucin (▼) as negative control with the antibody against H-type 2 determinants (mAbSA) as determined with an ELISA. The mucin preparations have been previously reported.[33]

3. Conclusion

In conclusion, the goals of our investigation were substantially met. The logic of glycal assembly, honed over a decade, proved equal to the task of synthesizing the complex oligosaccharide **71**. The glycal olefin proved susceptible to our sulfonamidohydroxylation methodology. More importantly, the powerful Birch reduction methodology for the simultaneous cleavage of multiple benzyl groups was appropriate, even in this most difficult context (cf. **72**→**73**). Happily the Kotchetkov–Lansbury amination–aspartylation was also appropriate (cf. **73**→**3**).

With the ability to reach reasonable quantities of homogeneous structures containing this level of complexity in the laboratory come new questions. For instance, is it possible to develop methodology wherein complex glycopeptides can be ligated to create even more biorelevant structures? If so, can the chemistry be conducted in such a fashion that it would allow for the conservation of the fragile glycosidic linkages? One could then consider even more challenging systems as targets for chemical synthesis. One of the most exciting dimensions of synthetic chemistry, in our view, is its capacity to deal with still more biopertinent structures which might illuminate Nature's ways and even, occasionally serve to modulate these processes in constructive directions. Having reached the H-type 2 human blood group structural context by chemical synthesis, we were led to wonder if a system of the complexity of, say, erythropoietin[35] might be accessible via suitable advances in methodology. It is toward such goals that we have recently directed considerable attention.[36]

4. Experimental

4.1. General

All glassware was dried in an oven at 140 °C before use. All experiments were conducted under an atmosphere of dry

argon unless indicated otherwise. Solvents were dried as follows: methylene chloride, diethyl ether, tetrahydrofuran (THF), benzene, and toluene were obtained from a dry solvent system (alumina) and used without further drying. Pyridine and triethylamine were freshly distilled from CaH_2. All NMR spectra were recorded on a Bruker model AMX-400 (^{1}H: 400 MHz, ^{13}C: 100 MHz) or a Bruker model DRX-500 (^{1}H: 500 MHz, ^{13}C: 125 MHz) NMR spectrometer. Chemical shifts are reported in parts per million (ppm) from internal tetramethylsilane (^{1}H NMR spectra, δ 0.00 ppm) or the residual solvent signal of $CDCl_3$ (^{13}C NMR spectra, δ 77.23 ppm). Infrared spectra were taken on a Perkin Elmer 1600 Series FTIR spectrometer using thin film deposition on polished NaCl plates. Peaks are reported in wavenumbers (cm^{-1}). Low and high-resolution mass spectra were obtained from a PE Sciex API 100 instrument under EI mode, and are reported in units of *m*/*z*. Column chromatography (low-pressure chromatography) was performed with E. Merck silica gel 60 (40–63 mesh). Thin layer chromatography (TLC) was carried out on Merck silica gel 60 F-254 glass-backed plates. TLC visualization was done with a 254 nm UV lamp and potassium permanganate or phosphomolybdic acid staining solution. All chemicals were purchased from Aldrich Chemical Co. and used as received. While we cannot rule out the possibility of anomeric mixtures during glycosylation reactions, we were unable to observe such mixtures and the yields reported for these reactions are for the pure anomer listed.

4.1.1. Improved synthesis of 4-*p*-methoxybenzylglucal (21). To a solution of 4,6-*p*-methoxybenzylidine glucal[15] (**20**) (6.00 g, 23 mmol) in CH_2Cl_2 (20 mL) at −78 °C was added Dibal-H (1.5 M solution in toluene, 58 mL, 87 mmol). After removing the cooling bath, the resulting mixture was stirred at room temperature for 4 h and then diluted with CH_2Cl_2 (200 mL). The mixture was treated with saturated aq KNa-tartrate (100 mL) and was stirred for 15 h. The organic layer was separated, washed with brine, and dried over Na_2SO_4 and then concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=1:1) to afford **21** (4.5 g, 17 mmol, 74%) as a white waxy solid. $[\alpha]_D^{25}$ 15.3 (*c*=2.86; CH_2Cl_2); IR (thin film): 3285, 2955, 2933, 2836, 1649, 1613, 1514, 1232, 1173, 1086, 952, 814, 761 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.26 (d, *J*=8.6 Hz, 2H), 6.85 (d, *J*=8.6 Hz, 2H), 6.30 (dd, *J*=1.3, 6.0 Hz, 1H), 4.75–4.65 (m, 3H), 4.30 (br d, *J*=5.4 Hz, 1H), 3.88–3.77 (m, 3H), 3.75 (s, 3H), 3.57 (dd, *J*=6.7, 8.8 Hz, 1H), 2.79 (br s, 1H), 2.70 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=159.4, 144.1, 130.2, 129.7, 114.0, 103.3, 77.4, 76.6, 73.4, 71.6, 68.9, 61.7, 55.2.

4.1.2. Synthesis of 3,4,6-tribenzyl-1-thioethyl-β-mannoside. 3,4,6-Tribenzyl-1-thioethyl-β-glucopyranoside[12] (**23**) (9.29 g, 18.7 mmol) was treated with DMSO/Ac_2O (100 mL/50 mL) at room temperature for 3 d. The reaction mixture was then diluted with diethyl ether (1 L) and washed with H_2O (5×200 mL), saturated aq Na_2CO_3 (3×200 mL), and brine. The crude ketone was dried over $MgSO_4$ and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (60 mL) and MeOH (60 mL) then cooled to 0 °C. $NaBH_4$ (2.13 g, 37.8 mmol) was added in several portions and the reaction mixture was allowed to warm to room temperature then stirred for another 20 min. The reaction was quenched with H_2O (20 mL) and extracted with ether (3×300 mL). The combined extracts were washed with saturated aq $NaHCO_3$ (200 mL) and brine (300 mL) then dried ($MgSO_4$) and concentrated. The crude material was purified by silica gel chromatography (hexane/EtOAc=6.5:1) to afford the desired 3,4,6-tribenzyl thioethyl mannoside (7.05 g, 14.2 mmol, 76%) as a white foam.

4.1.3. Synthesis of 2-*tert*-butyldimethylsilyl-3,4,6-tribenzyl-1-thioethyl-β-mannoside (24). To a solution of 3,4,6-tribenzyl-1-thioethyl-β-mannoside (500 mg, 1.01 mmol) in CH_2Cl_2 (5 mL) was added triethylamine (2.81 mL, 20.2 mmol) and TBSOTf (464 μL, 2.02 mmol) drop wise at 0 °C. The reaction was slowly warmed to room temperature and stirred for 3 h. The reaction was diluted with EtOAc (100 mL), washed with saturated aq $NaHCO_3$ and brine then dried ($MgSO_4$), and concentrated in vacuo. Purification by silica column chromatography (hexane/EtOAc=10:1) yielded **24** (570 mg, 0.94 mmol, 93%). $[\alpha]_D^{25}$ −36.1 (*c*=4.09, CH_2Cl_2); IR (thin film): 3087, 2926, 2916, 1496, 1463, 1362, 1251, 1102, 1027, 966, 834 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.40–7.21 (m, 15H), 4.90 (d, *J*=8.8 Hz, 1H), 4.81 (d, *J*=11.8 Hz, 1H), 4.69 (d, *J*=4.5 Hz, 1H), 4.62 (d, *J*=4.7 Hz, 1H), 4.58 (d, *J*=5.3 Hz, 1H), 4.51 (d, *J*=4.0 Hz, 1H), 4.49 (s, 1H), 4.22 (d, *J*=2.4 Hz, 1H), 3.93 (t, *J*=9.5 Hz, 1H), 3.75–3.68 (m, 2H), 3.52–3.48 (m, 2H), 2.75–2.72 (m, 1H), 1.33 (t, *J*=7.3 Hz, 3H), 0.98–0.96 (m, 9H), 0.20–0.13 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=137.3, 136.9, 136.8, 127.0, 126.9, 126.8, 126.8, 126.4, 126.3, 126.2, 125.9, 83.9, 82.7, 78.8, 73.7, 73.1, 71.8, 71.3, 71.1, 68.1, 24.8, 24.3, 17.3, 13.8, −4.9, −5.4, −5.7; HRMS calcd for $C_{35}H_{48}O_5S_1Si_1Na$: 631.2889, found: 631.2883.

4.1.4. Synthesis of 2-*tert*-butyldimethylsilyl-3,4,6-tribenzyl-1-thioethyl-α-mannoside (27). 2-Acetyl-3,4,6-tribenzyl-1-thioethyl-α-mannoside (14.1 g, 26 mmol) was dissolved in MeOH (50 mL) then treated with NaOMe (25% solution in MeOH, 0.6 mL, 2.62 mmol). The reaction was stirred for 12 h and then was neutralized with Amberlyst 15 and filtered. The resin was washed with MeOH (3×50 mL). The combined filtrates were concentrated under reduced pressure and azeotroped with benzene (3×30 mL) then dried under high vacuum to afford the free alcohol (12.8 g). The crude alcohol was dissolved in CH_2Cl_2 (50 mL) and Et_3N (15 mL, 107.6 mmol) then cooled to 0 °C. TBSOTf (7.83 mL, 34 mmol) was added and the mixture was warmed to room temperature over 4 h then diluted with EtOAc (500 mL). The solution was washed with saturated aq $NaHCO_3$ and brine then dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=20:1) to afford **27** (14.7 g, 24.2 mmol, 93% over two steps) as a clear, colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.20–7.40 (m, 15H), 5.10 (d, *J*=1.2 Hz, H-2), 4.72 (d, *J*=10.8 Hz, 1H), 4.60 (d, *J*=11.7 Hz, 1H), 4.58 (d, *J*=12.3 Hz, 1H), 4.50 (d, *J*=11.7 Hz, 1H), 4.40 (d, 12.3 Hz, 1H), 4.38 (d, *J*=10.8 Hz, 1H), 4.10 (s, 1H), 4.07 (m, 1H), 3.95 (dd, *J*=9.4, 9.4 Hz, 1H), 3.60–3.80 (m, 3H), 2.52 (m, 2H), 1.25 (t, *J*=7.5 Hz, 3H), 0.85 (s, 9H), 0.09, 0.00, −0.02 (3s, 12H). HRMS calcd for $C_{35}H_{48}O_5S_1Si_1Na$: 631.2889, found: 631.2878.

4.1.5. Synthesis of 3,4,6-tribenzyl-2-*p*-toloyl-1-thioethyl-α-mannoside (28). To a solution of 3,4,6-tribenzyl-1-thioethyl-α-mannoside (8.0 g, 16 mmol) in CH_2Cl_2 (50 mL) was added triethylamine (25 mL, 18 mmol) followed by *p*-toluoyl chloride (8.5 mL, 63.7 mmol) at 0 °C. The resulting mixture was warmed to room temperature and stirred for 2 d. Saturated aq $NaHCO_3$ (50 mL) was added and the mixture was stirred vigorously for 15 h then diluted with EtOAc (500 mL). After separation, the organic extract was washed with saturated aq $NaHCO_3$ and brine then dried (Na_2SO_4) and concentrated in vacuo. Purification over silica gel (hexane/EtOAc=20:1, then 10:1) yielded **28** (7.4 g, 12.1 mmol, 75%) as yellowish syrup. ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.89–7.91 (m, 2H), 7.19–7.33 (m, 15H), 7.08–7.10 (m, 2H), 5.65 (dd, *J*=1.4, 3.0 Hz, 1H), 5.38 (d, *J*=1.4 Hz, 1H), 4.79, (d, *J*=10.5 Hz, 1H), 4.71, (d, *J*=11.2 Hz, 1H), 4.69 (d, *J*=11.9 Hz, 1H), 4.47 (d, *J*=11.2 Hz, 1H), 4.44 (d, *J*=11.9 Hz, 1H), 4.40 (d, *J*=10.5 Hz, 1H), 4.14 (ddd, *J*=1.6, 3.4, 9.7 Hz, 1H), 4.07 (dd, *J*=9.2, 9.7 Hz, 1H), 3.93 (dd, *J*=3.0, 9.2 Hz, 1H), 3.87 (dd, *J*=3.4, 10.6 Hz, 1H), 3.69 (dd, 1.6, 10.6 Hz, 1H), 2.50–2.64 (m, 2H), 2.34 (s, 3H), 1.21 (t, *J*=7.4 Hz, 3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ=166.1, 144.2, 138.7, 138.7, 138.1, 131.2, 130.3, 129.4, 128.6, 128.4, 128.3, 128.0, 127.9, 127.9, 127.8, 127.5, 126.5, 82.9, 79.0, 76.0, 74.8, 73.7, 72.3, 71.8, 70.9, 69.3, 25.9, 22.0, 15.3.

4.1.6. Synthesis of disilyl dimannosyl trisaccharide glycal 30. *Method A (p-toluoyl protecting group)*: Thiodonor **28** (7.4 g 12.1 mmol) and glucal acceptor **21** (1.0 g, 4 mmol) were mixed and azeotroped with toluene (3×40 mL). The residue was dissolved in CH_2Cl_2 (20 mL) and Et_2O (20 mL) followed by the addition of 2,6-di-*tert*-butylpyridine (13.45 mL, 60 mmol) and freshly dried 4 Å molecular sieves (10 g). The resulting slurry was stirred for 30 min at room temperature then cooled to 0 °C. MeOTf (5.42 mL, 48 mmol) was added and the mixture was slowly warmed to room temperature and stirred for 15 h at which point Et_3N (5 mL, 35.8 mmol) was added. After an additional 15 min of stirring, the solution was filtered through a pad of Celite and concentrated. The residue was purified over silica gel (hexane/EtOAc=5:1) to yield **29** (3.3 g, 2.41 mmol, 63%) as a thick, colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.05 (m, 4H), 7.20–7.50 (m, 23H), 6.80 (d, *J*=8.7 Hz, 2H), 6.30 (d, *J*=6.4 Hz, 1H), 5.70 (d, *J*=1.0 Hz, 1H), 5.60 (d, *J*=2.4 Hz, 1H), 5.22 (d, *J*=1.7 Hz, 1H), 5.05 (d, *J*=1.6 Hz, 1H), 4.50–5.00 (m, 10H), 4.50–4.70 (m, 10H), 3.70–4.30 (m, 7H), 3.58 (s, 3H), 2.45 (s, 3H), 2.43 (s, 3H).

The trisaccharide glycal **29** (3.3 g, 2.4 mmol) was dissolved in MeOH (20 mL) and toluene (5 mL) and NaOMe (25% solution in MeOH, 0.2 mL, 0.87 mmol) was added. The mixture was stirred at room temperature for 15 h then concentrated in vacuo. The residue was purified over silica gel (hexane/EtOAc=2:1, then 1:1) to afford the dihydroxylated glycal (2.2 g, 1.94 mmol, 77%) as a thick syrup.

The dihydroxy glycal (2.0 g, 1.76 mmol) was dissolved in CH_2Cl_2 (20 mL) and cooled to 0 °C. Triethylamine (1.22 mL, 8.75 mmol) and TBSOTf (1.2 mL, 5.27 mmol) were added and the resulting mixture was stirred at room temperature for 3 h then diluted with EtOAc (200 mL). The organic layer was washed with saturated aq $NaHCO_3$ and brine then dried (Na_2SO_4) and concentrated. The residue was purified over silica gel (hexane/EtOAc=20:1, then 10:1) to afford **30** (2.3 g, 1.69 mmol, 95%) as a thick syrup. $[\alpha]_D^{25}$ 24.4 (*c*=0.6, $CHCl_3$); IR (thin film) 3030, 2926, 2855, 1648, 1514, 1454, 1380, 1249, 1043, 835, 777 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.40–7.12 (m, 32H), 6.79 (dd, *J*=2.0, 6.4 Hz, 2H), 6.21 (dd, *J*=1.2, 6.4 Hz, 1H), 5.01 (dd, *J*=2.2, 6.1 Hz, 1H), 4.94 (d, *J*=1.9 Hz, 1H), 4.84 (d, *J*=10.8 Hz, 2H), 4.79 (d, *J*=2.0 Hz, 1H), 4.78 (d, *J*=11.7 Hz, 1H), 4.68–4.60 (m, 6H), 4.56–4.45 (dd, *J*=3.0, 11.9 Hz, 4H), 4.51 (dd, *J*=3.6, 10.8 Hz, 1H), 4.36 (dd, *J*=1.7, 7.1 Hz, 1H), 4.13 (t, *J*=2.4 Hz, 1H), 4.02–3.82 (m, 8H), 3.78–3.63 (m, 7H), 3.73 (s, 3H), 0.89 (b, 18H), 0.10 (s, 3H), 0.06 (s, 3H), 0.05 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=159.3, 144.2, 138.6, 138.5, 138.4, 130.1, 129.2, 128.3, 128.2, 128.2, 128.2, 128.2, 128.1, 127.6, 127.6, 127.4, 127.4, 127.3, 127.2, 113.9, 102.6, 102.3, 101.2, 80.0, 79.9, 79.2, 75.1, 75.0, 74.9, 74.7, 74.0, 73.1, 73.0, 72.6, 72.4, 72.1, 70.4, 69.6, 69.4, 69.2, 65.7, 55.2, 25.8, 25.7, 18.1, 0.0, −4.5, −4.8, −4.8; HRMS calcd for $C_{80}H_{102}O_{15}Si_2Na$: 1381.6650, found: 1381.6670.

Method B (TBS protecting group): Thiodonor **27** (8 g, 13 mmol) and glucal acceptor **21** (1.15 g, 4.38 mmol) were mixed and azeotroped with benzene (3×30 mL), and then further dried under high vacuum. To the residue was added CH_2Cl_2 (100 mL) followed by 2,6-di-*tert*-butylpyridine (14.7 mL, 65 mmol) and freshly dried 4 Å molecular sieves (10 g). The slurry was stirred at room temperature for 30 min then cooled to 0 °C and MeOTf (5.94 mL, 52.5 mmol) was added. The reaction was slowly warmed to room temperature over 4 h with vigorous stirring then quenched with saturated aq $NaHCO_3$ (10 mL) and diluted with EtOAc (500 mL). The mixture was filtered through a pad of Celite, and the filtrate was washed with saturated aq $NaHCO_3$ (50 mL) and brine (50 mL) then dried (Na_2SO_4) and concentrated in vacuo. The residue was purified over silica gel (hexane/EtOAc=1:0, 10:1, then 5:1) to yield **30** (3.2 g, 2.36 mmol, 53%) as a thick syrup.

4.1.7. Synthesis of dimannosyl trisaccharide alcohol thioglycoside 32. To a solution of **30** (1.0 g, 0.73 mmol) in CH_2Cl_2 (10 mL) was added 4 Å MS (flame-dried, 1 g). The resulting slurry was stirred at room temperature for 30 min then cooled to 0 °C. Dimethyldioxirane (0.072 M in acetone, 12.2 mL, 0.876 mmol) was added *very slowly* (*important*) under vigorous stirring over 1 h. After the addition was complete, the mixture was stirred for 10 min and the volatiles were removed under a stream of dry N_2. The crude epoxide (**31**) was azeotroped with benzene (×3) then dissolved in CH_2Cl_2 (5 mL) and cooled to −78 °C. Ethanethiol (2.5 mL, 33.75 mmol) and trifluoroacetic anhydride (10 μL, 0.07 mmol) were added and the reaction was stirred for 10 min then quenched with Et_3N (100 μL, 0.72 mmol). After removing the volatiles under a N_2 stream, the residue can be azeotroped with benzene (×2) for direct acylation or can be purified over silica gel (hexane/EtOAc=5:1) to afford the thioglycoside **32** (0.80 g, 0.58 mmol, 76%) as a thick syrup. $[\alpha]_D^{24}$ 12.8 (*c*=0.7, $CHCl_3$); IR (thin film): 3472, 2927, 2856, 1613, 1514, 1454, 1360, 1250, 1137, 1092, 1049, 978, 835, 777 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.40–7.10 (m, 32H), 6.80 (d, *J*=8.4 Hz, 1H), 4.90 (d, *J*=1.8 Hz, 1H), 4.84 (d, *J*=10.8 Hz, 2H), 4.79 (d, *J*=11.8 Hz, 1H), 4.65

(d, J=11.0 Hz, 2H), 4.58–4.45 (m, 9H), 4.39 (d, J=10.6 Hz, 1H), 4.34 (d, J=9.6 Hz, 1H), 4.08 (dd, J=2.2 Hz, 2H), 4.04–4.01 (m, 1H), 3.91 (dd, J=9.0, 18.0 Hz, 2H), 3.84 (d, J=2.4 Hz, 1H), 3.84–3.66 (m, 11H), 3.59 (t, J=8.1 Hz, 1H), 3.43–3.32 (m, 3H), 2.75–2.60 (m, 2H), 1.23 (t, J=7.6 Hz, 3H), 0.90 (s, 9H), 0.88 (s, 9H), 0.10 (s, 3H), 0.07 (s, 3H), 0.05 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=159.3, 138.6, 138.6, 138.2, 138.2, 138.1, 128.8, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 127.7, 127.6, 127.5, 127.5, 127.4, 127.4, 127.3, 127.2, 114.0, 102.8, 100.9, 90.8, 84.8, 78.1, 76.3, 75.1, 74.9, 74.9, 74.6, 73.2, 72.9, 72.7, 72.1, 72.0, 70.1, 69.4, 69.3, 69.1, 65.7, 55.2, 18.1, 15.0, −4.5, −4.5, −4.7, −4.9; FAB(+)MS: 1460, 1439, 1357, 963; HRMS calcd for $C_{82}H_{108}O_{16}SSi_2Na$: 1459.6790, found: 1459.6760.

4.1.8. Synthesis of dimannosyl trisaccharide acetate thioglycoside 33. To a room temperature solution of **32** (3.01 g, 2.09 mmol) in CH_2Cl_2 was added Ac_2O (1.07 g, 10.4 mmol), Et_3N (2.11 g, 20.9 mmol), and a catalytic amount of DMAP. After stirring for 2 h the reaction was concentrated in vacuo and the remaining residue was purified directly by silica gel chromatography to afford **33** (2.81 g, 1.90 mmol, 91%). $[\alpha]_D^{24}$ 24.6 (c=1.0, $CHCl_3$); IR (thin film): 2927, 2855, 1750, 1613, 1514, 1454, 1361, 1249, 1225, 1050, 835, 777 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.40–7.05 (m, 30H), 6.75 (d, J=8.4 Hz, 2H), 5.06 (br s, 1H), 4.98–4.72 (m, 6H), 4.70–4.40 (m, 8H), 4.29 (d, J=8.5 Hz, 1H), 4.08–4.06 (br m, 3H), 3.98–3.55 (m, 14H), 3.68 (s, 3H), 3.48–3.42 (m, 1H), 3.39–3.35 (m, 1H), 2.65–2.60 (m, 1H), 2.23 (s, 3H), 0.91 (s, 9H), 0.84 (s, 9H), 0.12 (s, 3H), 0.08 (s, 3H), 0.02 (s, 3H), −0.06 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=169.9, 166.3, 159.1, 138.9, 138.7, 138.6, 138.5, 138.4, 129.7, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 127.7, 127.5, 127.3, 127.3, 127.2, 127.2, 127.2, 113.9, 102.1, 101.2, 83.5, 79.9, 79.5, 78.4, 74.9, 74.7, 74.6, 74.0, 73.2, 73.0, 72.9, 72.3, 72.2, 72.0, 71.1, 69.4, 69.2, 65.5, 60.3, 55.2, 25.7, 25.7, 24.0, 22.1, 21.2, 18.1, 18.1, 14.8, −4.5, −4.5, −4.8, −5.1; HRMS calcd for $C_{84}H_{110}O_{17}SSi_2Na$: 1501.6900, found: 1501.6900.

4.1.9. Synthesis of trisaccharide benzoate thioglycoside 34. To a solution of **32** (3.28 g, 2.34 mmol) and pyridine (5 mL, 61.8 mmol) in CH_2Cl_2 (50 mL) was added benzoyl chloride (1.08 mL, 9.36 mmol) and the resulting mixture was stirred at room temperature for 4 d. At that point, propanol (1 mL) was added and stirring was continued for an additional hour. The mixture was diluted with EtOAc (200 mL) and washed with saturated aq $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and then concentrated in vacuo. The residue was purified over silica gel (hexane/EtOAc=3:1) to afford **34** (3.2 g, 2.11 mmol, 90%) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.80 (d, J=7.0 Hz, 2H), 7.36 (m, 2H), 6.90–7.30 (m, 18H), 6.70 (d, J=7.0 Hz, 2H), 5.20 (dd, J=8.0, 8.0 Hz, 1H), 5.00 (d, J=1.8 Hz, 1H), 4.76 (d, J=10.5 Hz, 1H), 4.70 (d, J=2.0 Hz, 1H), 4.65 (d, J=12.5 Hz, 1H), 4.63 (d, J=12.5 Hz, 1H), 4.54 (d, J=12.5 Hz, 1H), 3.98–4.05 (m, 2H), 3.85 (m, 2H), 3.75 (dd, J=2.0, 12.0 Hz, 1H), 3.63 (s, 3H), 3.57 (m, 1H), 3.40 (br d, 1H), 3.20 (d, J=12.0 Hz, 1H), 3.60 (m, 2H), 1.20 (t, J=7.0 Hz, 3H), 0.82 (s, 9H), 0.72 (s, 9H), 0.03 (s, 3H), 0.00 (s, 3H), −0.12 (s, 3H), −0.18 (s, 3H); LRMS calcd for $C_{89}H_{112}O_{17}SSi_2$: 1540.72, found: 1542 (M+H).

4.1.10. Synthesis of pentasaccharide glycal acetate 35. Trisaccharide donor **33** (1.03 g, 0.695 mmol) and disaccharide acceptor **19** (561 mg, 0.695 mmol) were combined and azeotroped with benzene (×2). Freshly activated 4 Å molecular sieves (4.0 g) were added, followed by CH_2Cl_2 (8 mL) and 2,6-di-*tert*-butylpyridine (1.09 mL, 4.9 mmol) and the mixture was stirred for 45 min at room temperature. The reaction was cooled to −10 °C and MeOTf (0.47 mL, 4.2 mmol) was slowly added. The reaction mixture was stirred at −8 °C for 10 h, at −5 °C for 6 h and finally at 5 °C for 6 h then quenched with Et_3N (2.0 mL, 14.3 mmol), filtered through a plug of silica, washed with $NaHCO_3$, and extracted with EtOAc. The combined organic extracts were washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. Purification by silica gel chromatography (hexane/EtOAc=3:1) yielded **35** (994 mg, 0.45 mmol, 64%) as a white foam. $[\alpha]_D^{24}$ −5.3 (c=1.31, CH_2Cl_2); IR (thin film) 2247, 1650, 1612, 1586, 1571, 1514, 1498, 1454, 1361, 1249, 910, 836 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.87 (d, J=7.7 Hz, 2H), 7.45–7.19 (m, 45H), 6.76 (d, J=8.8 Hz, 2H), 6.34 (d, J=6.0 Hz, 1H), 5.05 (br d, J=1.6 Hz, 1H), 4.95–4.77 (m, 6H), 4.75–4.35 (m, 24H), 4.23 (d, J=6.2 Hz, 1H), 4.16–3.95 (m, 7H), 3.90–3.70 (m, 14H), 3.75 (s, 3H), 3.70–3.35 (m, 14H), 3.24–3.19 (m, 1H), 1.98 (s, 3H), 0.94 (s, 9H), 0.88 (s, 9H), 0.10 (s, 3H), 0.06 (s, 6H), −0.03 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ=170.3, 159.0, 144.4, 141.8, 138.8, 138.7, 138.7, 138.6, 138.6, 138.5, 138.4, 137.9, 137.9, 137.8, 133.5, 128.6, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.1, 128.1, 128.0, 127.9, 127.7, 127.6, 127.4, 127.4, 127.3, 127.3, 127.2, 127.2, 127.2, 113.8, 101.6, 101.3, 100.0, 99.8, 99.4, 79.8, 79.5, 77.9, 75.4, 75.0, 74.7, 74.5, 74.3, 74.2, 74.0, 73.6, 73.4, 73.3, 73.3, 73.2, 73.0, 72.9, 72.8, 72.5, 72.5, 72.2, 72.1, 71.7, 70.2, 69.9, 69.1, 69.0, 67.7, 65.5, 56.2, 55.2, 25.7, 25.6, 21.1, 18.1, −4.4, −4.5, −4.6, −4.7, −5.1; HRMS calcd for $C_{128}H_{153}NO_{27}SSi_2Na$: 2246.9786, found: 2246.9840.

4.1.11. Synthesis of pentasaccharide glycal benzoate 36. Trisaccharide donor **34** (2.2 g, 1.45 mmol) and disaccharide acceptor **19** (1.5 g, 1.85 mmol, 1.3 equiv) were combined and azeotroped with benzene (3×20 mL). The residue was dissolved in dry CH_2Cl_2 (20 mL) then activated 4 Å molecular sieves (5 g) and 2,6-di-*tert*-butylpyridine (1.30 mL, 5.78 mmol) were added. The slurry was stirred at room temperature for 30 min then cooled to 0 °C. MeOTf (0.40 mL, 3.53 mmol) was added and the resulting mixture was stirred at a temperature between 0 °C and room temperature for 2 d then quenched by the addition of saturated aq $NaHCO_3$ (1 mL) and diluted with EtOAc (200 mL). After filtration through a short pad of Celite, the filtrate was washed with saturated aq $NaHCO_3$ (20 mL) and brine (20 mL) then dried (Na_2SO_4) and concentrated in vacuo. The residue was purified over silica gel (hexane/EtOAc=3:1) to afford **36** (2.36 g, 1.03 mmol, 71%) as a white foam. R_f 0.5 (hexane/EtOAc=3:1), $[\alpha]_D^{25}$ −5.0 (c=1.0, $CHCl_3$); IR (thin film): 3468, 3030, 2933, 2858, 1651, 1496, 1092 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.70 (d, J=8.0 Hz, 2H), 7.20–7.50 (m, 62H), 6.78 (d, J=8.0 Hz, 1H), 6.40 (d, J=6.2 Hz, 1H), 4.40–4.59 (m, 22H), 4.60 (d, J=8.0 Hz, 1H), 3.95–4.15 (m, 6H), 0.93 (s, 9H), 0.92 (s, 9H), 0.00 (s, 3H), 0.01 (s, 3H), 0.04 (s, 3H), 0.06 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=166.3, 159.5, 144.7, 142.5, 139.0, 139.0,

138.9, 130.3, 128.9, 128.8, 128.7, 128.6, 128.6, 128.5, 128.4, 128.1, 128.1, 127.9, 127.8, 127.8, 127.6, 127.6, 127.3, 114.7, 102.7, 101.7, 101.2, 100.2, 99.2, 81.9, 80.1, 80.0, 74.9, 74.4, 74.2, 74.1, 73.8, 73.7, 73.3, 73.3, 72.7, 72.6, 72.2, 70.4, 69.5, 68.3, 65.8, 57.5, 55.6, 26.2, 26.0, −4.4, −4.1, −4.2, −4.8; LRMS calcd for $C_{133}H_{155}NO_{27}SSi_2Na$: 2310, found: 2310 (M+Na).

4.1.12. Synthesis of pentasaccharide dimannosyl glycal alcohol (37). Pentasaccharide glucal **36** (330 mg, 0.144 mmol) was azeotropically dried with benzene (3×25 mL) then placed under high vacuum for 15 min. Et_2O (20 mL) was added and the solution was cooled to −40 °C. Lithium aluminum hydride (1 M solution in Et_2O, 0.58 mL, 0.58 mmol) was added and the solution was stirred at 0 °C for 1 h. After diluting the solution with saturated aq $NaHCO_3$ (10 mL) and extracting with EtOAc (3×15 mL), the combined organic extracts were washed with brine (10 mL), dried ($NaSO_4$), and concentrated in vacuo. The residue was placed on high vacuum to afford the crude product as a white foam (330 mg, >95%), which was used in the next reaction without any further purification. For analytical data, the crude product was purified over a short column of silica gel (R_f 0.50; EtOAc/hexane=1:4). $[\alpha]_D^{24}$ 0.9 (c=1.73, CH_2Cl_2); IR (thin film) 3468, 3351, 3063, 3030, 2927, 2856, 1650, 1612, 1586, 1514, 1498, 1453, 1360, 1249, 1208, 1093, 910, 835, 735, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=7.75 (d, J=7.5 Hz, 2H), 7.45–7.18 (m, 45H), 6.81 (d, J=8.6 Hz, 2H), 6.31 (d, J=6.1 Hz, 1H), 4.93–4.84 (m, 6H), 4.70–4.41 (m, 24H), 4.29 (d, J=7.6 Hz, 1H), 4.14–4.01 (m, 7H), 3.98–3.71 (m, 14H), 3.79 (s, 3H), 3.67–3.62 (m, 2H), 3.53–3.36 (m, 9H), 3.28–3.19 (m, 2H), 0.96 (s, 9H), 0.92 (s, 9H), 0.12 (s, 3H), 0.06 (s, 3H), 0.03 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=159.1, 144.3, 141.7, 138.9, 138.6, 138.6, 138.5, 138.3, 138.2, 138.1, 137.5, 132.1, 130.0, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4, 127.4, 127.4, 127.3, 127.3, 127.2, 127.1, 113.8, 103.0, 102.3, 101.1, 100.7, 100.3, 80.0, 79.1, 79.0, 76.8, 75.8, 75.0, 75.0, 74.8, 74.5, 74.5, 74.0, 73.8, 73.4, 73.1, 73.0, 72.9, 72.5, 72.0, 71.8, 70.4, 70.0, 69.2, 69.1, 69.0, 68.7, 67.5, 66.0, 58.7, 55.2, 25.7, 25.7, 18.0, −4.6, −4.7, −4.8, −5.0; FAB(+)MS: 2205, 2192, 2130, 2115, 2110, 1988; HRMS calcd for $C_{126}H_{151}NO_{26}SSi_2Na$: 2204.9681, found: 2204.9680.

4.1.13. Synthesis of pentasaccharide trimannosyl glycal alcohol 38. Glycal alcohol **37** (330 mg, 0.144 mmol) was azeotropically dried with toluene (3×5 mL) then placed under high vacuum for 15 h. The residue was dissolved in CH_2Cl_2 (9 mL) and pyridine (106 μL, 1.23 mmol) was added. In an argon-filled glove bag, Dess–Martin reagent (330 mg, 0.77 mmol) was added in one portion. The resulting mixture was stirred at room temperature until the starting material had been consumed (about 1 h). At that point, the reaction was quenched by adding saturated aq $Na_2S_2O_3$ (1 mL) and diluted with EtOAc (100 mL). The organic layer was washed with saturated aq $Na_2S_2O_3$ (10 mL) and brine (10 mL) then dried (Na_2SO_4) and concentrated to afford the intermediate keto-compound (330 mg) pure enough for further use. After azeotropically drying the residue with toluene (3×), it was dissolved in dry THF (8 mL) and cooled to −40 °C. L-Selectride (1 M solution in THF, 0.35 mL, 0.35 mmol) was added drop wise and the reaction was stirred at −40 °C, then warmed to room temperature and stirred for an additional 2 h. The reaction was quenched by adding saturated aq $NaHCO_3$ and H_2O_2 (33% in water, 2.4 mL). After stirring for 30 min, the mixture was diluted with EtOAc (100 mL) and the combined organic layers were washed with saturated aq $NaHCO_3$, saturated aq $Na_2S_2O_3$, and brine then dried (Na_2SO_4) and concentrated to afford a residue (320 mg) of suitable purity for the next reaction. For analytical data, the residue was purified over silica gel (hexane/EtOAc=4:1). (R_f 0.50; EtOAc/hexane=1:4); $[\alpha]_D^{24}$ −2.6 (c=2.05, CH_2Cl_2); IR (thin film) 3473, 3276, 1650, 1612, 1586, 1514, 1498, 1470, 1454, 1361, 1328, 1249, 1094, 910, 836, 778 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=7.79 (d, J=7.5 Hz, 2H), 7.38–7.18 (m, 54H), 6.84 (d, J=8.5 Hz, 2H), 6.36 (d, J=6.1 Hz, 1H), 4.96 (br s, 1H), 4.93 (br s, 1H), 4.88 (br s , 1H), 4.81 (br s, 1H), 4.90–4.39 (m, 24H), 4.33–4.30 (m, 2H), 4.28 (br s, 1H), 4.19 (br s, 1H), 4.10–3.50 (m, 30H), 3.80 (s, 3H), 3.49–3.45 (m, 3H), 0.99 (s, 18H), 0.16 (s, 3H), 0.12 (s, 6H), 0.11 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=159.1, 144.3, 141.7, 138.8, 138.7, 138.6, 138.5, 138.2, 138.1, 138.1, 138.0, 137.7, 137.6, 132.1, 130.1, 128.7, 128.5, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.6, 127.4, 127.4, 127.3, 127.3, 127.2, 127.1, 113.7, 102.2, 101.1, 100.6, 100.5, 100.1, 84.2, 80.1, 79.7, 75.9, 75.7, 75.3, 74.9, 74.5, 74.5, 73.4, 73.4, 73.1, 73.1, 73.0, 72.9, 72.8, 72.3, 70.3, 70.2, 69.8, 69.2, 69.0, 67.6, 66.5, 58.4, 55.1, 25.8, 18.1, −4.6, −4.7, −4.8, −5.0; FAB(+)MS: 2207, 2132, 2115, 1990; HRMS calcd for $C_{126}H_{151}NO_{26}SSi_2Na$: 2204.9680, found: 2204.9700.

4.1.14. Synthesis of acetylated pentasaccharide trimannosyl glycal alcohol 39. The crude alcohol (320 mg) was dissolved in CH_2Cl_2 (8 mL) then treated with Ac_2O (1.2 mL, 12.7 mmol), Et_3N (1.2 mL, 14.3 mmol), and DMAP (5 mg, 0.04 mmol) at room temperature for 15 h. The reaction mixture was diluted with EtOAc (100 mL), and the organic layer was washed with saturated aq $NaHCO_3$ (15 mL) and brine (15 mL) then dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified over silica gel to afford **39** (270 mg, 0.119 mmol, 82% in four steps) as a white foam. R_f 0.5 (hexane/EtOAc=1:1); ^1H NMR (500 MHz, $CDCl_3$): δ=7.98 (d, J 7.5 Hz, 2H), 7.20–7.50 (m, 57H), 6.74 (d, J=6.5 Hz, 2H), 6.35 (d, J=6.5 Hz, 1H), 5.44 (s, 1H), 5.40 (d, J=8.0 Hz, 1H), 4.40–5.00 (m, 36H), 3.60–3.80 (m, 22H), 3.58 (m, 4H), 2.08 (s, 3H), 1.97 (s, 3H), 0.89 (s, 9H), 0.86 (s, 9H), 0.02 (s, 3H), 0.01 (s, 3H), 0.00 (s, 3H), −0.02 (s, 3H); LRMS calcd for $C_{130}H_{155}NO_{28}SSi_2Na$: 2289, found: 2289 (M+Na).

4.1.15. Synthesis of ethylthio pentasaccharide 41. Pentasaccharide glycal **39** (353 mg, 0.155 mmol) and benzenesulfonamide (98 mg, 0.62 mmol) were combined and azeotropically dried with benzene (3×5 mL). The residue was dissolved in CH_2Cl_2 (15 mL), mixed with freshly dried 4 Å MS (1.5 g), and stirred at room temperature for 30 min. Iodonium-di-*sym*-collidine perchlorate (583 mg, 1.25 mmol) in CH_2Cl_2 (5 mL) was added via cannula to the mixture, and the resulting suspension was stirred at 0 °C for 30 min at which point it was warmed to room temperature and filtered through a short pad of Celite. The solids

were washed with EtOAc and the combined filtrates were washed with saturated $Na_2S_2O_3$ (×2), $CuSO_4$ (×4), brine, $Na_2S_2O_3$, and brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. Purification over silica gel (hexane/EtOAc=2:1) yielded the intermediate iodosulfonamide, which was taken on crude. The iodosulfonamide was subsequently dissolved in DMF (5 mL) and cooled to −40 °C. A solution of LiHMDS (1 M in THF, 467 μL, 0.47 mmol) and ethanethiol (80 μL, 1.08 mmol) was cooled to −40 °C and added via cannula to the solution containing the iodosulfonamide. The mixture was stirred at −40 °C for 3 h and subsequently diluted with ether (150 mL), washed with saturated aq $NaHCO_3$ and brine then dried (Na_2SO_4) and concentrated. The residue was purified over silica gel (hexane/EtOAc=2:1) to afford the desired thioglycoside **41** (293 mg, 0.12 mmol, 81%) as white foam. $[\alpha]_D^{24}$ 10 (c=1.3, $CHCl_3$); IR (thin film): 3275, 2925, 2855, 1747, 1453, 1361, 1328, 1247, 1157, 1092, 736 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=7.00–8.00 (m, 64H), 6.75 (d, J=6.4 Hz, 2H), 5.60 (d, J=9.0 Hz, 1H), 5.35 (d, J=2.8 Hz, 1H), 5.15 (s, 2H), 4.90 (s, 1H), 4.40–4.85 (m, 33H), 4.20 (d, J=7.1 Hz, 1H), 3.72 (s, 3H), 3.40–3.80 (m, 27H), 2.50 (m, 2H), 2.10 (s, 3H), 1.10 (t, J=7.6 Hz, 3H), 0.90 (s, 9H), 0.88 (s, 9H), 0.02 (s, 6H), 0.01 (s, 6H), 0.00 (s, 6H); HRMS calcd for $C_{138}H_{166}N_2O_{30}S_3Si_2$: 2483.0225, found: 2483.0264 (M+H).

4.1.16. Synthesis of the desilylated pentasaccharide thioglycoside 43. The thioglycoside **41** (170 mg, 0.07 mmol) in THF (5 mL) was stirred with TBAF (1 M solution in THF, 0.7 mL, 0.70 mmol) at room temperature for 36 h. At that point, the solution was concentrated and the residue was diluted with EtOAc. The organic layer was washed with saturated aq NH_4Cl and brine then dried (Na_2SO_4) and concentrated. The residue was purified over silica gel (hexane/EtOAc=1:1, then 2:3) to afford the desired diol **43** (120 mg, 0.054 mmol, 80%) as a white foam. R_f 0.25 (hexane/EtOAc=1:1); $[\alpha]_D^{24}$ −6 (c=1.5, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ=7.86 (d, J=7.4 Hz, 2H), 7.76 (d, J=7.4 Hz, 2H), 7.00–7.40 (m, 60H), 6.75 (d, J=8.5 Hz, 2H), 5.70 (d, J=6.4 Hz, 1H), 5.25 (s, 1H), 5.10 (s, 1H), 4.90 (s, 1H), 4.30–4.60 (m, 33H), 4.10–4.20 (m, 2H), 3.40–4.00 (m, 27H), 3.35 (m, 2H), 3.20 (m, 2H), 2.95 (d, J=7.0 Hz, 2H), 2.4 (m, 2H), 2.08 (s, 3H), 1.10 (t, J=6.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=169.2, 158.3, 140.5, 137.4, 137.2, 137.1, 136.9, 136.8, 128.2, 127.5, 127.4, 127.3, 127.3, 127.2, 127.1, 127.0, 126.9, 126.8, 126.8, 126.8, 126.7, 126.6, 126.3, 126.9, 100.7, 98.9, 98.5, 97.0, 93.0, 86.6, 81.7, 79.3, 78.6, 78.6, 78.0, 77.7, 72.4, 72.3, 72.3, 71.0, 70.6, 70.4, 67.8, 67.6, 67.0, 43.8, 37.8, 23.8, 18.8, 13.7; LRMS calcd for $C_{124}H_{136}N_2O_{29}S_3$: 2213, found: 2236 (M+Na) positive, 2248 (M+Cl) negative.

4.1.17. Synthesis of peracetylated thioglycoside pentasaccharide 45. To a deep blue solution of sodium metal (58 mg, 2.52 mmol) in NH_3 (~10 mL) at −78 °C was added a solution of thioglycoside **43** (58 mg, 0.03 mmol) in THF (2 mL). The solution was stirred at −78 °C for 45 min then quenched by the addition of solid NH_4Cl (96 mg, 1.79 mmol) and MeOH (1 mL). The volatiles were removed under a stream of N_2 and the residue was treated with Ac_2O (2 mL, 21.2 mmol), pyridine (2 mL, 24.7 mmol), and DMAP (10 mg, 0.08 mmol). The resulting mixture was stirred at room temperature for 15 h then concentrated at reduced pressure. The residue was dissolved in EtOAc (100 mL), washed with saturated aq $NaHCO_3$ and brine then dried (Na_2SO_4) and concentrated. Purification over silica gel (EtOAc/MeOH=95:5) yielded the peracetylated thioglycoside **45** (31 mg, 0.020 mmol, 77%) as a slightly yellow foam. R_f 0.5 (EtOAc/MeOH=95:5); $[\alpha]_D^{24}$ −15 (c=2.2, $CHCl_3$); IR (thin film): 2900, 1750, 1420, 1280, 1050 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=6.11 (d, J=9.3Hz, 1H), 5.59 (d, J=9.7 Hz, 1H), 5.58–5.20 (m, 6H), 5.20–4.90 (m, 8H), 4.76 (s, 1H), 4.57 (s, 1H), 4.42–3.90 (m, 19H), 2.62 (m, 2H), 2.59–1.76 (16s, 48H), 1.20 (t, J=7.3 Hz, 3H); ^{13}C NMR (500 MHz, $CDCl_3$): δ=171.0, 170.8, 170.7, 170.6, 170.5, 170.4, 170.3, 170.2, 170.1, 169.9, 169.8, 169.7, 113.7, 101.2, 98.6, 97.1, 96.7, 84.4, 77.3, 77.0, 76.8, 76.0, 74.1, 72.7, 72.5, 72.0, 69.7, 69.3, 69.2, 69.2, 68.8, 68.3, 68.2, 66.8, 65.6, 65.5, 62.4, 62.2, 62.1, 60.4, 54.2, 52.9, 24.3, 23.2, 23.0, 21.0, 20.9, 20.9, 20.8, 20.8, 20.7, 20.6, 20.6, 19.7, 14.7, 14.2; HRMS calcd for $C_{64}H_{90}N_2O_{39}S$: 1542.4840, found: 1542.4863.

4.1.18. Synthesis of deacetylated thioglycoside 46. To a solution of the peracetylated pentasaccharide **45** (30 mg, 0.02 mmol) in MeOH (2 mL) was added NaOMe (25% solution in MeOH, 30 μL, 0.13 mmol). The mixture was stirred at room temperature for 15 h then purified directly using lipophilic LH-20 Sephadex. The appropriate fractions were combined and concentrated in a lyophilizer to afford the thioglycoside **46** (16 mg, 16.7 μmol, 86%). R_f 0.80 (EtOAc/^{i}PrOH/H_2O=1:1:1); ^1H NMR (500 MHz, D_2O, 24 °C): δ=5.12 (s, 1H), 4.93 (s, 1H), 4.68 (d, J=10.7 Hz, 1H), 4.62 (d, J=8.9 Hz, 1H), 4.27 (s, 1H), 4.08 (s, 1H), 3.99 (s, 1H), 3.52–3.96 (m, 30H), 2.75 (m, 2H), 2.10 (s, 3H), 2.05 (s, 3H), 1.25 (t, J=7.4 Hz, 3H); ^{13}C NMR (125 MHz, D_2O, 24 °C): δ=174.8, 173.6, 104.4, 103.3, 102.2, 101.3, 85.9, 82.8, 81.3, 80.8, 76.8, 75.8, 75.4, 74.7, 73.9, 72.7, 72.2, 71.9, 69.4, 69.1, 68.1, 63.4, 63.2, 62.0, 61.6, 56.7, 55.8, 50.1, 25.2, 23.5, 23.1, 15.4; HRMS calcd for $C_{36}H_{62}N_2O_{25}S$: 954.3362, found: 954.3382.

4.1.19. Synthesis of free pentasaccharide 47. To a solution of ethylthioglycoside **46** (16 mg, 16.7 μmol) in H_2O (5 mL) was added $HgCl_2$ (30 mg, 83.3 μmol) and then $CaCO_3$ (30 mg, 167 μmol). The mixture was stirred for 15 h at room temperature and then purified via size-exclusion chromatography. The appropriate fractions were collected and lyophilized to afford **47** (16 mg, 17.5 μmol, 95%) as a α/β mixture of anomers (2:3 or 3:2). R_f 0.70 (EtOAc/^{i}PrOH/H_2O=1:1:1); ^1H NMR (500 MHz, D_2O, 24 °C): δ=5.20 (s, 1H), 5.11 (s, 1H), 4.99 (s, 1H), 4.26 (s, 2H), 4.08–3.43 (m, 30H), 2.08 (s, 3H), 2.04 (s, 3H); LRMS calcd for $C_{34}H_{58}N_2O_{26}$: 910, found: 933 (M+Na), 915 (M−H_2O+Na).

4.1.20. Synthesis of glycosylamine 11. To the solution of free sugar **47** (16 mg, 0.018 mmol) in H_2O (14 mL) was added solid NH_4HCO_3 (9.0 g, 114 mmol). The resulting mixture was stirred at room temperature until all the starting material was completely converted (monitored by ^1H NMR and TLC, EtOAc/^{i}PrOH/H_2O=1:1:1). During this period, NH_4HCO_3 needs to be continually added to keep the solution saturated. After completion of the reaction, the stirring bar was removed and the solution was lyophilized. The solid

material was several times redissolved in H_2O (5 mL) and lyophilized until the residue exhibited a constant weight. A white powder (17 mg, 18.7 μmol, >99%) was obtained. R_f 0.34 (EtOAc/^{i}PrOH/H_2O=1:1:1); ^{1}H NMR (500 MHz, D_2O, 24 °C): δ=5.12 (s, 1H), 4.93 (d, J=1.4 Hz, 1H), 4.62 (d, J=7.8 Hz, 1H), 4.27 (s, 1H), 4.16 (d, J=9.0 Hz, 1H), 4.08 (m, 1H), 3.98 (m, 3H), 3.48–3.94 (br m, 28H), 2.09 (s, 3H), 2.05 (s, 3H); HRMS calcd for $C_{34}H_{59}N_3O_{25}Na$: 932.3437, found: 932.3468 (M+Na).

4.1.21. Synthesis of the N-linked pentasaccharide–pentapeptide adduct 1. Pentasaccharide glycosylamine **11** (4 mg, 4.40 μmol) and L-pentapeptide **48**[37] (3.68 mg, 6.60 μmol) were mixed in anhydrous DMSO (0.5 mL) and HOBt (2.97 mg, 22 μmol), HBTU (9.48 mg, 22 μmol), and Hünig's base (15 μL, 8.8 μmol) were added. After stirring for 2 d at room temperature, the reaction mixture was lyophilized and purified on a reverse-phase Vydac C18-column (4.6×250 mm) to afford **1** (2.5 mg, 1.72 μmol, 40%). HPLC gradient: A: 0.1% TFA/H_2O, B: 0.09% TFA/70% CH_3CN/H_2O, gradient 5→50% B over 23 min; 50→100% B over 5 min; glycopeptide elutes at 21 min. ^{1}H NMR (800 MHz, H_2O, Watergate method, 24 °C): 8.52 (d, J=8.9 Hz, 1H), 8.33 (d, J=9.6 Hz, 1H), 8.33 (d, J=7.3 Hz, 1H), 8.22 (d, J=5.9 Hz, 1H), 8.12 (d, J=8.2 Hz, 2H), 8.08 (d, J=8.0 Hz, 1H), 7.92 (d, J=8.0 Hz, 1H), 7.42 (s, 1H), 7.04 (s, 1H); (500 MHz, D_2O, 30 °C) δ=5.24 (s, 1H, H-1c), 5.15 (d, J=9.7 Hz, 1H, H-1a), 5.04 (s, 1H, H-1d), 4.90 (s, 1H, H-1d), 4.84 (t, J=6.3 Hz, 1H), 4.76 (d, J=12.6 Hz, 1H, H-1b), 4.33–4.48 (m, 22H), 4.19 (s, 1H), 3.70–4.10 (m, 18H), 3.00 (dd, J=6.4, 16.4 Hz, 1H), 2.87 (dd, J=6.4, 16.7 Hz, 1H), 2.30 (m, 1H), 2.20 (s, 3H, Ac), 2.15 (s, 3H, Ac), 2.13 (s, 3H, Ac), 1.76 (m, 1H), 1.50 (d, J=7.2 Hz, 3H, CH_3), 1.34 (d, J=6.4 Hz, 3H, CH_3), 1.07 (d, J=6.5 Hz, 6H, $2CH_3$), 1.02 (d, J=6.4 Hz, 3H, CH_3); ^{13}C (200 MHz, H_2O, Watergate method, 24 °C): δ=105.2 (C-1), 104.2 (C-1), 103.3 (C-1), 102.5 (C-1), 81 (C-1a); HRMS (FAB) calcd for $C_{58}H_{99}N_9O_{33}Na$: 1472.6237, found: 1472.6282.

4.1.22. Synthesis of the N-linked pentasaccharide–pentapeptide adduct 2. Same procedure as in the synthesis of **1**. ^{1}H NMR (500 MHz, D_2O, 36 °C): 5.01 (s, 1H), 4.93 (d, J=9.5 Hz, 1H), 4.81 (s, 1H), 4.67 (s, 1H), 4.60 (t, J=7.0 Hz, 1H), 4.52 (d, J=7.5 Hz, 1H), 4.18–4.25 (m, 2H), 4.19 (m, 2H), 3.98 (s, 1H), 3.88 (s, 1H), 2.75 (dd, J=6.5, 15.0 Hz, 1H), 2.60 (m, 2H), 2.10 (m, 1H), 1.97 (s, 3H), 1.93 (s, 3H), 1.90 (s, 3H), 1.78 (br s, 1H), 1.58 (m, 1H), 1.50 (m, 2H), 1.28 (d, J=7.0 Hz, 3H), 1.12 (d, J=7.0 Hz, 3H), 0.87 (s, 6H), 0.81 (d, J=6.4 Hz, 3H).

4.1.23. Synthesis of allyl lactal 58. 3′-Allyl lactal[38] (6.50 g, 18.68 mmol) was suspended in DMF (160 mL) and cooled to 0 °C. NaH (60% in oil, 5.98 g, 149.44 mmol) was added in one portion and the reaction was maintained at 0 °C for 30 min. Benzyl bromide (22.2 mL, 186.8 mmol) was added drop wise and the reaction was stirred at room temperature for 24 h. The reaction was cooled to 0 °C and quenched by drop wise addition of glacial AcOH (2.24 g, 37 mmol). Most of the solvent (DMF) was removed by evaporation under high vacuum and the residue was diluted with saturated aq $NaHCO_3$ (100 mL) and washed with EtOAc (5×100 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=10:1) to afford **58** (13.58 g, 17 mmol, 91%) as a white foam. $[\alpha]_D^{25}$ −9.8 (c=3.9, $CHCl_3$); IR (thin film): 3028, 2865, 1650, 1453, 1097 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.71–7.24 (m, 25H), 6.45 (d, J=6.2 Hz, 1H), 5.93 (m, 1H), 5.28 (dd, J=1.6, 12.3 Hz, 1H), 5.18 (dd, J=1.5, 10.4 Hz, 1H), 4.97 (d, J=12.3 Hz, 1H), 4.87 (m, 1H), 4.74 (m, 2H), 4.66–4.44 (m, 7H), 4.38 (m, 2H), 4.26 (m, 1H), 4.17 (m, 4H), 3.85–3.31 (m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=144.4, 138.8, 138.7, 138.1, 137.9, 128.4, 128.3, 128.2, 128.2, 128.1, 127.9, 127.7, 127.6, 127.5, 127.4, 127.3, 82.0, 79.3, 75.9, 75.1, 73.6, 73.5, 73.3, 73.2, 72.3, 71.8, 70.3, 68.6, 68.0; HRMS (FAB) calcd for $C_{50}H_{54}O_9Na$: 821.3666, found: 821.3666.

4.1.24. Synthesis of iodosulfonamide. To a flask containing lactal **58** (6.0 g, 7.51 mmol), 2-(trimethylsilyl)ethanesulfonamide (5.44 g, 30.0 mmol), and powdered 4 Å molecular sieves (3.0 g, freshly flame-dried) was added CH_2Cl_2 (30 mL) and the mixture was stirred at room temperature for 30 min. The mixture was cooled to 0 °C and I(*sym*-collidine)$_2ClO_4$ (2.23 g, 4.76 mmol), prepared in situ from Ag(collidine)$_2ClO_4$ (10.13 g, 22.53 mmol) and 5.52 g I_2 (21.78 mmol) in the presence of powdered 4 Å molecular sieves (3.0 g, freshly flame-dried) in CH_2Cl_2 (130 mL) was added via cannula. The reaction was stirred for 1.5 h, then diluted with CH_2Cl_2 (150 mL) and filtered. The filtrate was washed with saturated aq $Na_2S_2O_3$ (3×75 mL), saturated aq $CuSO_4$ (5×75 mL), saturated aq $Na_2S_2O_3$ (1×75 mL), and brine (1×75 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=4:1 to 3:1) to afford the desired diaxial isomer (6.26 g, 5.65 mmol, 75%) as well as a small amount of the diequatorial isomer (0.589 g, 0.90 mmol, 12%). $[\alpha]_D^{25}$ −12.6 (c=0.9, $CHCl_3$); IR (thin film): 3258, 3029, 2867, 1455, 1337, 1107 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.46–7.22 (m, 25H), 5.93 (m, 1H), 5.34 (dd, J=1.6, 17.2 Hz, 1H), 5.20 (m, 2H), 4.93–4.37 (m, 12H), 4.19 (m, 4H), 4.08 (m, 1H), 3.85–3.34 (m, 10H), 3.04 (m, 1H), 1.06 (m, 2H), 0.07 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=138.5, 138.3, 137.9, 137.7, 137.1, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.4, 116.8, 103.7, 81.8, 79.6, 79.1, 79.0, 78.2, 75.3, 74.6, 74.4, 73.9, 73.6, 73.5, 73.4, 73.1, 72.4, 71.9, 68.9, 67.4, 51.0, 29.8, 10.4, −2.1; HRMS (FAB) calcd for $C_{55}H_{68}INO_{11}SSi$: 1105.3286, found: 1105.3290.

4.1.25. Synthesis of sulfonamidoethylthioglycoside 59. The iodosulfonamide (6.26 g, 5.65 mmol) was dissolved in DMF (20 mL) and added drop wise to a solution of ethanethiol (2.09 mL, 28.25 mmol, 5 equiv) and LiHMDS (1 M solution in THF, 17 mL, 17 mmol) in DMF (80 mL) and cooled to −40 °C. The reaction was allowed to slowly warm to room temperature and stirred for 12 h. Most of the solvent (DMF) was removed by evaporation under high vacuum and the residue was diluted with saturated aq $NaHCO_3$ (100 mL) and washed with EtOAc (3×100 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=3.5:1) to afford **59** (5.0 g,

4.80 mmol, 85%) as a white glass. $[\alpha]_D^{25}$ −10.2 (c=3.1, $CHCl_3$); IR (thin film): 3268, 3029, 2868, 1454, 1091 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=7.38–7.18 (m, 25H), 5.92 (m, 1H), 5.34 (d, J=17.5 Hz, 1H), 5.19 (d, J=10.5 Hz, 1H), 4.93 (d, J=11.8 Hz, 2H), 4.77–4.36 (m, 12H), 4.16 (m, 2H), 3.97–3.37 (m, 13H), 3.13 (m, 2H), 2.71 (m, 2H), 1.28 (t, J=7.4 Hz, 3H), 1.05 (m, 2H), −0.01 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=138.7, 138.6, 138.2, 137.9, 134.8, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.4, 127.4, 127.3, 116.3, 103.1, 83.8, 82.0, 81.2, 79.8, 79.7, 76.6, 75.2, 74.5, 73.8, 73.4, 73.2, 73.0, 71.6, 68.3, 67.9, 58.0, 24.0, 14.8, −2.0.

4.1.26. Synthesis of ethylthioglycoside lactosamine. To the solution of thiosulfonamide **59** (5.00 g, 4.80 mmol) in DMF (500 mL) was added powdered CsF (3.64 g, 24.0 mmol) and the mixture was stirred at 90 °C for 2 d. The solution was cooled to room temperature and most of the solvent (DMF) was removed by evaporation under high vacuum. The remaining residue was diluted with saturated aq $NaHCO_3$ (100 mL) and washed with Et_2O (3×100 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=1:1 to 1:2 with 2% of Et_3N v/v) to afford the title compound (3.96 g, 3.75 mmol, 94%) as a white glass. $[\alpha]_D^{25}$ −12.2 (c=1.0, $CHCl_3$); IR (thin film): 3029, 2921, 2867, 1453, 1090 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=7.48–7.22 (m, 25H), 5.92 (m, 1H), 5.34 (dd, J=1.7, 7.2 Hz, 1H), 5.19 (m, 2H), 4.97 (d, J=11.1 Hz, 1H), 4.81 (m, 2H), 4.57–4.28 (m, 8H), 4.18 (d, J=5.3 Hz, 2H), 3.99–3.17 (m, 5H), 3.53–3.33 (m, 6H), 2.95 (m, 1H), 2.72 (m, 2H), 1.29 (t, J=7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=139.0, 138.9, 138.8, 138.0, 134.9, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 119.4, 102.7, 86.5, 84.8, 82.3, 79.9, 79.8, 76.2, 75.3, 75.2, 74.6, 73.4, 73.0, 71.6, 68.4, 68.1, 55.7, 24.1, 15.4; HRMS (FAB) calcd for $C_{52}H_{61}NO_9S$: 876.4145, found: 876.4156.

4.1.27. Synthesis of phthalamidoethylthioglycoside. To a solution of the lactosamine (3.70 g, 4.22 mmol) in pyridine (60 mL) was added phthalic anhydride (0.94 g, 6.33 mmol) and the reaction was stirred at room temperature for 45 min. Pivalic anhydride (4.72 g, 25.32 mmol) was added and the reaction was stirred at 85 °C for 5 d. The solution was cooled to room temperature and the pyridine was removed by evaporation. The remaining residue was dissolved in EtOAc (150 mL) and washed with saturated aq $NaHCO_3$ (3×50 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=10:1) to afford the title compound (4.25 g, 4.22 mmol, >99%) as a white foam. $[\alpha]_D^{25}$ 22.2 (c=0.7, $CHCl_3$); IR (thin film): 3029, 3026, 2923, 2867, 1774, 1713, 1386, 1086 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=7.81 (m, 1H), 7.67 (m, 3H), 7.37–7.21 (m, 20H), 6.97 (m, 2H), 6.85 (m, 3H), 5.94 (m, 1H), 5.35 (dd, J=1.7, 7.2 Hz, 1H), 5.25 (d, J=10.0 Hz, 1H), 5.19 (dd, J=1.1, 10.5 Hz, 1H), 4.92–4.81 (m, 4H), 4.56–4.17 (m, 10H), 4.16 (m, 2H), 3.86 (m, 1H), 3.76 (m, 2H), 3.47–3.32 (m, 7H), 2.68 (m, 2H), 1.18 (t, J=8.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=168.1, 167.5, 139.1, 138.8, 138.5, 138.1, 134.9, 133.8, 133.7, 131.7, 128.4, 128.2, 128.2, 128.1, 128.0, 127.8, 127.7, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 126.8, 123.4, 123.2, 116.4, 102.9, 82.2, 80.9, 80.0, 79.7, 78.1, 77.8, 75.4, 74.5, 74.3, 73.6, 73.4, 73.0, 71.5, 68.3, 68.2, 54.8, 23.7, 14.9; HRMS (FAB) calcd for $C_{60}H_{63}NO_{11}SNa$: 1028.4019, found: 1028.4040.

4.1.28. Synthesis of 3′-hydroxy ethylthioglycoside. The phthalamidoethylthioglycoside (4.25 g, 4.22 mmol) was dissolved in EtOH/H_2O (80 mL, 1:1 v/v). $RhCl(PPh_3)_2$ (0.39 g, 0.42 mmol) and DABCO (0.947 g, 8.44 mmol) were added and the reaction was heated to 90 °C for 3 h. At that point, the reaction was cooled to room temperature and diluted with THF (50 mL) and 1 N HCl (40 mL). The mixture was stirred at room temperature for 48 h. The organic solvents and water were removed by evaporation and the residue was dissolved in EtOAc (150 mL) then washed with saturated aq $NaHCO_3$ (3×50 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=5:1) to afford the title compound (3.0 g, 3.1 mmol, 74%) as a white foam. $[\alpha]_D^{25}$ 22.1 (c=0.6, $CHCl_3$); IR (thin film): 3472, 3029, 2868, 1774, 1713, 1307, 1078 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=7.79 (m, 1H), 7.67 (m, 3H), 7.36–7.22 (m, 20H), 6.98 (m, 2H), 6.86 (m, 3H), 5.23 (d, J=10.1 Hz, 1H), 4.87 (m, 2H), 4.73 (m, 2H), 4.58 (m, 2H), 4.47–4.30 (m, 7H), 4.07 (m, 1H), 3.82 (m, 2H), 3.51 (m, 6H), 2.68 (m, 2H), 1.15 (t, J=7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=168.1, 167.5, 138.8, 138.7, 138.4, 138.3, 137.9, 131.7, 128.5, 128.4, 128.3, 128.2, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.4, 126.9, 123.5, 102.9, 80.9, 80.7, 79.6, 78.0, 77.9, 75.9, 74.9, 74.4, 74.0, 73.4, 73.3, 73.1, 68.2, 68.1, 54.7, 23.8, 14.9; HRMS (FAB) calcd for $C_{57}H_{59}NO_{11}SNa$: 988.3706, found: 988.3744.

Propyl phthalamidoethylthioglycoside (product of allylic double bond reduction) was isolated as a by-product (0.62 g, 15%). If DABCO is excluded, the yield of desired product is much higher (91%), but isomerization required 3 d to reach completion.

4.1.29. Synthesis of ethylthioglycoside donor 60. To a solution of 3′-hydroxy ethylthioglycoside (3.0 g, 3.1 mmol) in CH_2Cl_2 (10 mL) was added chloroacetic anhydride (2.65 g 15.5 mmol), DMAP (cat.), and di-*tert*-butylpyridine (DTBP, 1.78 g, 9.3 mmol) and the reaction was stirred at room temperature for 24 h. The solution was diluted with CH_2Cl_2 (100 mL) and washed with saturated aq $NaHCO_3$ (3×50 mL) and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=10:1) to afford **60** (3.142 g, 3.02 mmol, 97%) as a white foam. $[\alpha]_D^{25}$ 49.5 (c=0.2, $CHCl_3$); IR (thin film): 2922, 1771, 1713, 1386, 1076 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=7.83 (m, 1H), 7.71 (m, 3H), 7.37–7.22 (m, 20H), 6.98 (m, 2H), 6.87 (m, 3H), 5.23 (d, J=10.1 Hz, 1H), 4.84 (m, 3H), 4.65–4.26 (m, 12H), 4.10 (m, 1H), 3.93 (d, J=3.2 Hz, 1H), 3.87 (m, 1H), 3.77–3.42 (m, 7H), 2.69 (m, 2H), 1.19 (t, J=7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=168.1, 167.5, 166.6, 138.3, 138.2, 138.1, 137.9, 137.8, 133.8, 133.7, 131.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.7, 127.7, 127.6. 127.5, 127.4, 126.9, 123.5, 123.2, 102.7, 80.9, 79.5, 77.9, 77.8, 77.6,

75.1, 74.9, 74.7, 74.5, 73.3, 73.1, 72.6, 67.9, 67.4, 54.6, 40.5, 23.7, 14.9; HRMS (FAB) calcd for $C_{59}H_{60}ClNO_{12}SNa$: 1064.3422, found: 1064.3386.

4.1.30. Synthesis of perbenzylated H-type 2 trisaccharide. To a solution of glycal **63**[39] (9.9 g, 9.07 mmol) in THF (110 mL) was added TBAF (1 M solution in THF, 108 mL, 108 mmol) and the reaction was stirred at room temperature for 2 h. At that point MeOH (110 mL) and K_2CO_3 (15.0 g, 108 mmol) were added and the reaction was continued to stir at room temperature for an additional 12 h. The reaction was quenched by the addition of NH_4Cl, filtered through a pad of Celite, dried (Na_2SO_4), and concentrated. The crude triol was passed through a short plug of silica gel using EtOAc as eluent then concentrated. The resulting yellow oil was diluted with DMF (130 mL) then cooled to 0 °C and NaH (60% suspension in oil, 2.92 g, 72.9 mmol) was added in a few portions. The reaction was maintained at 0 °C for 30 min then benzyl bromide (12.8 mL, 108 mmol) was added drop wise and the reaction was stirred at room temperature for 14 h. The reaction was cooled to 0 °C and quenched by drop wise addition of MeOH. The solution was diluted with saturated aq $NaHCO_3$ (100 mL) and washed with EtOAc (3×300 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=10:1) to afford the H-type trisaccharide (9.05 g, 7.71 mmol, 85%) as a white foam. $[\alpha]_D^{25}$ −22.0 (c=2.7, $CHCl_3$); IR (thin film): 3029, 2869, 1453, 1098 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=7.42–7.01 (m, 40H), 6.28 (d, J=6.3 Hz, 1H), 5.69 (d, J=2.9 Hz, 1H), 4.94 (d, J=11.6 Hz, 1H), 4.87–4.80 (m, 5H), 4.60–4.38 (m, 14H), 4.23 (m, 1H), 4.14 (m, 1H), 4.04–3.95 (m, 4H), 3.80 (m, 1H), 3.72–3.57 (m, 7H), 1.19 (d, J=6.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=143.9, 139.0, 138.9, 138.5, 138.4, 138.3, 138.0, 137.9, 137.7, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.6, 127.4, 127.4, 127.3, 127.2, 126.3, 101.3, 99.2, 97.0, 84.5, 79.5, 78.1, 75.5, 75.3, 74.7, 74.4, 73.6, 73.5, 73.2, 72.8, 72.6, 72.0, 71.3, 71.1, 70.3, 70.1, 68.7, 67.7, 66.2, 16.6; HRMS (FAB) calcd for $C_{74}H_{78}O_{13}Na$: 1197.5340, found: 1197.5389.

4.1.31. Synthesis of iodosulfonamide. To a solution of the H-type 2 trisaccharide glycal (6.64 g, 5.65 mmol) in CH_2Cl_2 (160 mL) was added 2-(trimethylsilyl)ethanesulfonamide (1.53 g, 8.45 mmol) and powdered 4 Å molecular sieves (5 g, freshly flame-dried). The mixture was stirred at room temperature for 20 min and then cooled to 0 °C. Iodonium-di-*sym*-collidine perchlorate (5.3 g, 11.32 mmol) was added in one portion and the reaction was stirred for 1 h and subsequently diluted with CH_2Cl_2 (100 mL) and filtered. The filtrate was washed with saturated aq $Na_2S_2O_3$ (2×75 mL), saturated aq $CuSO_4$ (1×75 mL), saturated aq $Na_2S_2O_3$ (1×75 mL), and brine (1×75 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue (8.5 g) was used without further purification. $[\alpha]_D^{25}$ −20.2 (c=0.7, $CHCl_3$); IR (thin film): 3260, 3029, 2949, 1453, 1331, 1139, 1068 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=7.37–7.00 (m, 40H), 5.65 (d, J=3.8 Hz, 1H), 5.09 (t, J=10.4 Hz, 1H), 4.92–4.17 (m, 21H), 4.15 (m, 3H), 4.00 (m, 1H), 3.89 (m, 2H), 3.77–3.62 (m, 5H), 3.51 (m, 4H), 1.15 (d, J=6.4 Hz, 3H), 1.05 (m, 2H), −0.04 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=138.7, 138.1, 138.0, 137.5, 137.1, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.7, 127.4, 127.3, 126.4, 102.9, 97.3, 84.2, 80.0, 79.6, 78.2, 77.9, 77.9, 75.4, 75.0, 74.8, 74.6, 74.4, 74.0, 73.7, 73.6, 73.5, 73.0, 72.7, 72.1, 72.0, 71.6, 69.3, 67.5, 66.2, 51.0, 30.5, 17.2, 10.4, −1.9; HRMS (FAB) calcd for $C_{79}H_{92}INO_{15}SSi$: 1504.4899, found: 1504.4963.

4.1.32. Synthesis of sulfonamidoethylthioglycoside 64. The iodosulfonamide (8.5 g) was suspended in DMF (5 mL) and cooled to −40 °C. To this solution was added a mixture of ethanethiol (2.5 mL, 33.7 mmol) and LiHMDS (1 M solution in THF, 16.95 mL, 16.95 mmol) in DMF (30 mL). The reaction was warmed to room temperature then stirred for 12 h. At that point, the reaction was diluted with saturated aq $NaHCO_3$ (75 mL) and washed with EtOAc (3×500 mL). The organic layer was dried over Na_2SO_4 and concentrated under a stream of N_2. The residue was purified by silica gel chromatography (hexane/EtOAc=10:1) to afford **64** (6 g, 4.24 mmol, 75% over two steps) as a white glass. $[\alpha]_D^{25}$ −33.6 (c=0.5, $CHCl_3$); IR (thin film): 3261, 3029, 2869, 1453, 1326, 1098 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=7.38–7.05 (m, 40H), 5.70 (d, J=3.7 Hz, 1H), 4.95 (m, 2H), 4.92–4.33 (m, 21H), 3.96 (m, 3H), 3.73 (m, 1H), 3.70–3.42 (m, 9H), 3.02 (m, 2H), 2.71 (m, 2H), 1.30 (t, J=7.4 Hz, 3H), 1.06 (d, J=6.5 Hz, 3H), 1.06 (m, 2H), 0.02 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=138.8, 138.7, 138.6, 138.4, 138.1, 138.0, 137.9, 128.6, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5, 127.5, 127.3, 127.3, 127.2, 127.2, 126.1, 101.5, 97.4, 83.9, 83.6, 81.2, 80.2, 79.2, 77.8, 76.3, 75.7, 74.7, 74.5, 73.7, 73.4, 73.3, 73.2, 72.7, 72.4, 72.1, 70.9, 68.9, 68.1, 66.3, 57.8, 51.4, 23.9, 16.7, 14.9, 10.4, −1.9; HRMS (FAB) calcd for $C_{81}H_{97}NO_{15}S_2SiNa$: 1438.5966, found: 1438.5990.

4.1.33. Synthesis of ethylthioglycoside amine. The sulfonamidoethylthioglycoside **64** (2.80 g, 1.97 mmol) was suspended in DMF (15 mL) and CsF (1.5 g, 9.86 mmol) was added. The reaction was stirred at 100 °C for 5 d then cooled to room temperature, diluted with saturated aq $NaHCO_3$ (75 mL), and washed with EtOAc (3×75 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=5:1) to afford the free amine (1.63 g, 1.30 mmol, 65%) as a white glass. IR (thin film): 3382, 3029, 2868, 1453, 1099 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=7.32–7.07 (m, 40H), 5.71 (d, J=3.7 Hz, 1H), 5.22 (d, J=10.3 Hz, 1H), 4.95 (d, J=11.6 Hz, 1H), 4.85 (d, J=11.3 Hz, 1H), 4.77–4.20 (m, 18H), 4.05–3.25 (m, 14H), 2.90 (m, 1H), 2.72 (m, 2H), 1.32 (t, J=7.5 Hz, 3H), 1.21 (d, J=6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=138.8, 138.6, 138.5, 138.4, 138.3, 138.0, 137.9, 128.5, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1, 127.0, 126.1, 100.7, 97.3, 86.2, 84.5, 84.1, 79.1, 77.8, 75.6, 75.5, 75.2, 74.7, 74.6, 73.5, 73.3, 73.1, 72.6, 72.2, 72.1, 70.8, 68.3, 68.0, 66.3, 55.5, 23.9, 16.7, 15.4; HRMS (FAB) calcd for $C_{76}H_{85}NO_{13}SNa$: 1274.5639, found: 1274.5596.

4.1.34. Synthesis of phthalamidoethylthioglycoside 55. To a solution of the ethylthioglycoside amine (800 mg,

0.639 mmol) in pyridine (5 mL) was added phthalic anhydride (142 mg, 0.958 mmol) and the reaction was stirred at room temperature for 45 min. Acetic anhydride (1.2 mL, 12.7 mmol) was added and the reaction was stirred at 85 °C for 5 h. The solution was cooled to room temperature, diluted with 50 mL of saturated aq $NaHCO_3$, and washed with EtOAc (3×50 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/EtOAc=10:1) to afford **55** (860 mg, 0.626 mmol, 98%) as a white foam. $[\alpha]_D^{25}$ −37.5 (c=0.4, $CHCl_3$); IR (thin film): 3028, 2926, 2867, 1801, 1714, 1453, 1100 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ=8.00 (m, 1H), 7.67 (m, 3H), 7.33–7.04 (m, 35H), 6.88–6.75 (m, 5H), 5.70 (d, J=3.8 Hz, 1H), 5.19 (d, J=10.0 Hz, 1H), 4.95 (d, J=11.6 Hz, 1H), 4.86 (d, J=12.0 Hz, 1H), 4.75–4.62 (m, 14H), 4.04 (m, 2H), 3.92 (m, 1H), 3.86 (dd, J=2.5, 10.2 Hz, 1H), 3.77 (m, 2H), 3.58 (dd, J=2.7, 9.7 Hz, 1H), 3.47 (m, 4H), 2.69 (m, 2H), 1.33 (d, J=6.5 Hz, 3H), 1.19 (t, J=7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=167.9, 167.5, 138.9, 138.8, 138.7, 138.6, 138.5, 138.3, 138.1, 138.0, 133.9, 133.8, 131.7, 131.6, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.5, 127.4, 127.4, 127.3, 127.2, 127.1, 126.8, 126.1, 123.4, 123.2, 100.7, 97.5, 83.9, 81.1, 80.3, 79.2, 78.1, 77.7, 77.7, 76.4, 75.6, 74.8, 74.5, 74.4, 73.5, 73.4, 73.1, 72.5, 72.3, 72.2, 70.8, 68.3, 66.4, 54.7, 23.7, 16.7, 15.0; HRMS (FAB) calcd for $C_{84}H_{87}NO_{15}SNa$: 1404.5964, found: 1404.5696.

4.1.35. Synthesis of core mannose pentasaccharide acceptor 51. To a solution of glycal **39** (90 mg, 41 μmol) in THF (3 mL) was added TBAF (1 M solution in THF, 0.4 mL, 0.4 mmol) and the reaction was stirred at room temperature for 48 h. At that point, the reaction mixture was concentrated and purified over silica gel (hexane/EtOAc=1:1) to afford **51** (63 mg, 31.5 μmol, 77%) as a white foam. R_f 0.42 (EtOAc/hexane=2:1); ^1H NMR (500 MHz, $CDCl_3$): δ=7.68 (d, J=7.4 Hz, 2H), 7.08–7.32 (m, 57H), 6.72 (d, J=8.6 Hz, 2H), 6.21 (d, J=6.1 Hz, 1H), 5.19 (d, J=2.9 Hz, 1H), 5.03 (s, 1H), 4.87 (s, 1H), 4.20–4.72 (m, 29H), 3.30–3.95 (m, 32H), 3.22 (m, 1H), 2.98 (br d, J=6.0 Hz, 1H), 2.19 (s, 3H), ^{13}C NMR (125 MHz, $CDCl_3$): δ=159.9, 145.0, 142.0, 139.0, 138.8, 138.5, 138.4, 130.4, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 114.5, 102.3, 101.1, 100.9, 100.6, 98.1, 80.3, 80.2, 76.5, 75.6, 75.3, 75.2, 74.8, 73.9, 73.8, 73.6, 72.6, 72.1, 72.0, 71.0, 69.4, 68.5, 66.5, 60.9, 58.3, 55.8, 21.7; LRMS (FAB) calcd for $C_{116}H_{125}NO_{27}S$: 1996, found: 2019 (M+Na); 2031 (M+Cl) and 1995 (M−H).

4.1.36. Synthesis of nonasaccharide glycal 65. Lactosamine donor **60** (1.18 g, 1.12 mmol) and pentasaccharide acceptor **51** (0.37 g, 0.18 mmol) were combined and azeotropically dried with toluene (3×20 mL). The residue was dissolved in CH_2Cl_2 (10 mL) and 2,6-di-*tert*-butylpyridine (1 mL, 4.46 mmol) then freshly dried 4 Å MS (1.0 g) was added. The resulting slurry was stirred at room temperature for 30 min then cooled to 0 °C. MeOTf (0.41 mL, 3.63 mmol) was added drop wise, and the mixture was slowly warmed to room temperature then stirred for 2 d. The reaction was quenched with saturated aq $NaHCO_3$ (5 mL), diluted with EtOAc (100 mL), and filtered through Celite. The filtrate was washed with saturated aq $NaHCO_3$ (20 mL) and brine then dried (Na_2SO_4) and concentrated. The residue was purified over silica gel (EtOAc/hexane=1:4, then EtOAc/toluene=4:1) to afford the nonasaccharide glycal **65** (440 mg, 0.111 mmol, 62%) as a white foam. ^1H NMR (500 MHz, $CDCl_3$): δ=7.68 (d, J=7.4 Hz, 2H), 6.70–7.50 (m, 115H), 6.60 (d, J=8.6 Hz, 2H), 6.19 (d, J=6.1 Hz, 1H), 5.20 (d, J=8.0 Hz, 1H), 4.99 (d, J=8.0 Hz, 1H), 4.98 (s, 1H), 4.79 (s, 1H), 4.50–4.78 (m, 17H), 4.25–4.49 (m, 48H), 3.20–4.20 (m, 70H), 1.98 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=170.9, 166.9, 159.1, 144.7, 141.8, 139.3, 138.8, 138.7, 138.6, 138.5, 138.2, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 123.5, 114.0, 103.2, 100.7, 100.6, 98.2, 97.1, 80.3, 78.3, 75.3, 75.2, 74.6, 74.5, 73.7, 73.6, 73.5, 73.0, 72.7, 70.8, 67.8, 55.9, 55.3, 40.8, 40.7, 21.4; LRMS (FAB) calcd for $C_{230}H_{233}Cl_2N_3O_{51}S$: 3954, found: 2000 (M+2Na)/2e.

4.1.37. Synthesis of dechloroacetylated nonasaccharide 66. To a solution of the nonasaccharide glycal **65** (430 mg, 0.11 mmol) in toluene (2 mL) and ethanol (30 mL) were added thiourea (42 mg, 0.55 mmol) and solid $NaHCO_3$ (140 mg, 1.66 mmol). The mixture was heated at 75 °C for 15 h, cooled to room temperature, and concentrated. The residue was purified over silica gel (toluene/EtOAc=4:1) to afford diol **66** (410 mg, 0.11 mmol, >99%) as a white foam. ^1H NMR (500 MHz, $CDCl_3$): δ=7.80 (d, J=8.4 Hz, 2H), 6.80–7.60 (m, 115H), 6.70 (d, J=8.5 Hz, 2H), 6.30 (d, J=6.2 Hz, 1H), 5.35 (d, J=7.9 Hz, 1H), 5.25 (d, J=8.3 Hz, 1H), 5.15 (s, 1H), 4.95 (s, 1H), 4.00–4.93 (m, 71H), 3.20–3.95 (m, 52H), 2.95 (br d, 3H), 2.85 (br, 1H), 2.74 (d, J=6.0 Hz, 1H), 2.20 (s, 1H), 2.10 (s, 1H), 2.08 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=170.9, 159.2, 144.8, 141.9, 139.3, 139.2, 138.9, 138.8, 138.6, 138.4, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 125.7, 123.5, 114.2, 103.8, 103.4, 100.8, 100.6, 99.4, 97.2, 81.0, 80.8, 78.4, 76.3, 75.3, 75.2, 74.6, 74.5, 74.4, 73.7, 73.6, 73.5, 73.4, 73.3, 73.0, 72.8, 70.9, 69.8, 68.5, 55.9, 55.4, 21.4; LRMS (FAB) calcd for $C_{226}H_{231}N_3O_{49}S$: 3802.5, found: 1925 [M+2Na]/2, 1936 [M+2Cl]/2.

4.1.38. Synthesis of pentadecasaccharide glycal 67. The H-type 2 trisaccharide donor **55** (1.0 g, 0.72 mmol) and nonasaccharide diol acceptor **66** (410 mg, 0.108 mmol) were azeotropically dried with toluene (3×20 mL) and then dried under high vacuum for 15 h. The residue was dissolved in CH_2Cl_2 (10 mL), ether (20 mL), and 2,6-di-*tert*-butylpyridine (DTBP, 658 μL, 2.94 mmol, 27 equiv) and then freshly dried 4 Å molecular sieves (3 g) were added. After stirring for 30 min at room temperature, the mixture was cooled to 0 °C and treated with MeOTf (306 μL, 2.71 mmol). The reaction was slowly warmed to room temperature and stirred for 48 h. The reaction was quenched with saturated aq $NaHCO_3$ and then filtered through a pad of Celite. The filtrate was washed with saturated aq $NaHCO_3$ and brine then dried (Na_2SO_4) and concentrated. The residue was purified over silica gel (toluene/EtOAc=4:1) to afford glycal **67** (560 mg, 0.09 mmol, 78%) as a white foam. R_f 0.5 (toluene/EtOAc=4:1); ^1H NMR (500 MHz, $CDCl_3$): δ=7.70 (d, J=8.2 Hz, 2H), 6.60–7.60 (m, ~200H), 6.48 (d, J=8.5 Hz, 2H), 6.25 (d, J=6.2 Hz, 1H), 5.65 (s, 2H), 5.30 (d,

J=6.8 Hz, 2H), 4.75–5.05 (m, 14H), 4.50–4.70 (m, 20H), 4.05–4.50 (m, 92H), 3.60–4.00 (m, 50H), 3.00–3.60 (m, 70H), 1.88 (s, 3H), 1.38 (d, J=6.3 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=172.5, 169.5, 168.8, 159.5, 144.9, 142.8, 139.8, 139.7, 138.6, 138.5, 138.3, 136.5, 134.5, 124.8, 116.5, 114.5, 104.5, 103.7, 102.8, 101.5, 98.0, 97.5, 96.0, 84.0, 78.0, 77.4, 76.5, 76.4, 76.3, 76.2, 75.6, 75.4, 75.2, 74.8, 74.6, 74.5, 74.2, 74.1, 73.7, 72.5, 71.9, 71.7, 68.7, 67.2, 66.8, 57.5, 54.8, 37.0, 31.2, 22.0, 18.7; LRMS (FAB) calcd for $C_{390}H_{393}N_5O_{79}S$: 6442, found: 2171 (M+3Na)/3; 1634 (M+4Na)/4.

4.1.39. Synthesis of deprotected pentadecasaccharide 50. To a solution of **67** (100 mg, 0.02 mmol) in toluene (2 mL) and EtOH (15 mL) was added ethylenediamine (0.70 mL). The mixture was heated at 80 °C for 24 h then the volatiles were removed at reduced pressure. The residue was purified over silica gel ($MeCl_2$/MeOH=20:1) to yield the free amine intermediate (90 mg).

A solution of free amine (42 mg, 0.01 mmol) in THF (2 mL) was cooled to 78 °C. Gaseous NH_3 (~10 mL) was condensed into the reaction vessel and solid sodium (95 mg, 4.1 mmol) was added under vigorous stirring. The resulting blue solution was stirred at −78 °C for 1 h then quenched with solid NH_4Cl and MeOH (5 mL) until the solution turned clear. The resulting mixture was stirred for additional 60 min at −78 °C then warmed to room temperature. The NH_3 was removed under stream of nitrogen then further dried under high vacuum for 2 h. Acetic anhydride (1 mL), pyridine (1 mL), and DMAP (20 mg) were added to the dry glycan. The mixture was stirred at room temperature for 24 h then the volatiles were removed in vacuo. The residue was purified over silca gel ($MeCl_2$/MeOH=20:1). To the residue was added MeOH (0.2 mL) and NaOMe (10 μL, 0.5 M in MeOH). The reaction was stirred at room temperature overnight then concentrated in vacuo. The residue was purified by size-exclusion chromatography (LH-20 column) using MeOH/H_2O (1:1) as eluent to afford pentadecasaccharide **50** as a white powder (5.5 mg 30%). ^{1}H NMR (500 MHz, D_2O, 40 °C): 6.55 (d, J=6 Hz, 1H), 5.40 (s, 2H, 2Fuc-1), 5.25 (s, 1H), 5.05 (s, 1H), 4.50–4.95 (m, 17H), 4.45 (br s, 1H), 4.18–4.40 (m, 6H), 3.60–4.20 (m, 78H), 2.25 (s, 3H), 2.20 (m, 12H), 1.35 (m, 6H); LRMS (FAB) calcd for $C_{100}H_{165}N_5O_{72}$: 2589, found: 1317 (M+2Na)/2, 1329 (M+2Cl)/2.

4.1.40. Synthesis of acetylated 15-mer glycal 71. To a solution of glycal **67** (53 mg, 8.22 μmol) in ethanol (15 mL) and toluene (2 mL) was added ethylenediamine (0.26 mL, 3.88 mmol). The resulting mixture was heated at 85 °C for 2 d then cooled and concentrated. The residue was stirred at room temperature with acetic anhydride (1 mL, 10.59 mmol), pyridine (2 mL, 25 mmol), and DMAP (5 mg, 0.04 mmol) for 15 h and then concentrated. The residue was purified over silica gel ($CHCl_3$/MeOH=20:1, containing 1% Et_3N) to afford the *N*-acetylated product **71** (43 mg, 7.19 μmol, 85%) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.20 (d, J=8.0 Hz, 2H), 7.20–7.60 (m, ~180H), 6.83 (d, J=8.5 Hz, 2H), 6.54 (d, J=8.0 Hz, 1H), 5.90 (d, J=2.0 Hz, 2H), 5.65 (d, J=6.0 Hz, 1H), 5.50 (m, 3H), 3.50–5.30 (m, ~170H), 2.17 (s, 3H), 2.10 (s, 3H), 1.87 (s, 3H), 1.82 (s, 3H), 1.64 (s, 3H), 1.62 (s, 3H), 1.45 (d, J=5.5 Hz, 6H); ^{13}C NMR (500 MHz, $CDCl_3$, selected peaks): δ=168.9, 137.7, 137.4, 137.0, 136.8, 136.7, 127.9, 127.4, 127.3, 127.2, 127.1, 127.0, 126.9, 126.8, 126.6, 126.5, 126.4, 126.1, 124.4, 112.7, 101.6, 96.5, 82.9, 73.8, 73.5, 73.4, 72.4, 72.1, 72.0, 71.5, 71.3, 71.2, 67.4, 67.1, 65.3, 24.7, 22.0, 20.4, 15.8; LRMS calcd for $C_{368}H_{395}N_5O_{76}S$: 6136, found: 2046 (M+3H)/3e, 2068 (M+3Na)/3e.

4.1.41. 15-mer Iodosulfonamidation product. The pentadecasaccharide glycal **71** (130 mg, 21.2 μmol) and benzenesulfonamide (35 mg, 0.222 mmol) were mixed and azeotropically dried with toluene (3×5 mL). After addition of freshly dried 4 Å MS (500 mg) and CH_2Cl_2 (8 mL), the suspension was cooled to −15 °C. Under an atmosphere of argon, iodonium-di-*sym*-collidine perchlorate (80 mg, 0.17 mmol) in CH_2Cl_2 (2 mL) was added and the reaction was stirred at 0 °C for 30 min. After diluting with EtOAc (150 mL), the reaction mixture was filtered through a pad of Celite, which was thoroughly washed with EtOAc (20 mL). The combined filtrates were successively washed with saturated aq $Na_2S_2O_3$ (3×20 mL), saturated aq $CuSO_4$ (2×20 mL), saturated aq $Na_2S_2O_3$ (20 mL), and brine (20 mL) then dried (Na_2SO_4) and concentrated in vacuo. The residue was purified over silica gel (4% MeOH in CH_2Cl_2) to afford the intermediate iodosulfonamide (100 mg, 15.6 μmol, 74%) as a slightly yellow foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ=6.80–7.60 (m, ~190H), 6.60 (d, J=6.8 Hz, 2H), 5.60 (s, 2H), 5.20–5.40 (bm, 5H), 3.20–5.00 (m, ~100H), 1.85 (s, 3H), 1.83 (s, 3H), 1.62 (s, 3H), 1.61 (s, 3H), 1.58 (s, 3H), 1.38 (s, 3H), 1.20 (d, J=6.4 Hz, 6H), LRMS (FAB) calcd for $C_{374}H_{401}IN_6O_{78}S_2$: 6414, found: 2162 (M+3Na)/3e.

4.1.42. Hydrolysis of iodosulfonamide: synthesis of 2-sulfonamido-1-hydroxy pentadecasaccharide 72. *Procedure A*: To a solution of iodosulfonamide (100 mg, 15.6 μmol) in THF (2 mL) was added saturated aq K_2CO_3 (2 mL) and the reaction was stirred at room temperature for 15 h. The reaction mixture was diluted with EtOAc (100 mL) and then washed with saturated aq NH_4Cl (10 mL) and brine (10 mL), dried over Na_2SO_4 and concentrated. Chromatography over silica gel (hexane/EtOAc=1:1→1:5) afforded the desired hydrolysis product **72** (60 mg, 9.5 μmol, 60%) as a white foam.

Procedure B: A solution of the iodosulfonamide (60 mg, 9.35 μmol) in THF (2 mL) was cooled to −78 °C and then LiHMDS (1 M solution in THF, 60 μL, 60 μmol) and AgOTf (4 mg, 15.6 μmol) in H_2O (1 mL) were added. The resulting mixture was allowed to warm gradually to room temperature and stirred for 15 h. Upon complete consumption of starting material, saturated aq $NaHCO_3$ (5 mL) was added. The reaction was diluted with EtOAc (100 mL) and washed with brine (10 mL) then dried (Na_2SO_4) and concentrated. Chromatography over silica gel afforded **72** (38 mg, 6.0 μmol, 63%). ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.87 (d, J=2.0 Hz, 2H), 7.75 (d, J=2.0 Hz, 2H), 6.80–7.30 (m, ~190H), 6.61 (d, J=6.8 Hz, 2H), 6.60 (d, J=3.0 Hz, 2H), 3.20–5.00 (m, ~105H), 1.91 (s, 3H), 1.85 (s, 3H), 1.58 (s, 3H), 1.55 (s, 3H), 1.53 (s, 3H), 1.46 (s, 3H), 1.20 (d, J=6.0 Hz, 6H); LRMS calcd for $C_{374}H_{402}N_6O_{79}S_2$: 6306, found: 2125 (M+3Na)/3e.

4.1.43. Global Birch-type deprotection: synthesis of 1-hydroxy-pentadecasaccharide 73. A solution of **72** (62 mg, 9.83 μmol) in THF (1 mL) was cooled to −78 °C. Gaseous NH_3 (~10 mL) was condensed into the reaction vessel and solid sodium (55 mg, 2.39 mmol) was added, under vigorous stirring. The resulting blue solution was stirred at −78 °C for 1 h and then quenched with solid NH_4Cl and MeOH (5 mL). The, now colorless, solution was stirred for an additional 60 min at −78 °C then warmed to room temperature. The NH_3 was evaporated under a stream of nitrogen and then placed under high vacuum for 2 h. The resulting residue was dissolved in MeOH (1 mL) and stirred with acetic anhydride (0.2 mL, 211 μmol) at room temperature for 2 h. Upon completion of the reaction, the mixture was purified via size-exclusion chromatography. The volatiles were removed at reduced pressure until the residue exhibited constant weight to give the desired saccharide **73** (15 mg, 5.64 μmol, 57%). ^{1}H NMR (D_2O, 500 MHz, 24 °C): δ=5.32 (s, 2H, 2H-1Fuc), 5.20 (s, 1H, H-1 Man), 5.12 (s, 1H, H-1 Man), 4.96 (s, 1H, H-1 Man), 4.70 (d, J=8.2 Hz, 2H, 2H-1), 4.59 (d, J=7.6 Hz, 2H, 2H-1), 4.55 (d, J=7.8 Hz, 3H, 3H-1), 4.47 (d, J=8.0 Hz, 2H, 2H-1), 4.11–4.24 (m, 5H), 3.49–3.99 (m, br, ~85H), 2.09 (s, 9H, 3Ac), 2.05 (s, 9H, 3Ac), 1.24 (d, J=6.4 Hz, 6H, $2CH_3$). LRMS calcd for $C_{103}H_{170}N_6O_{74}$: 2664, found: 2688 (M+Na), 1355 (M+2Na)/2, 2698 (M+Cl).

4.1.44. Synthesis of pentadecasaccharide glycosylamine 74. To a room temperature solution of pentadecasaccharide **73** (15 mg, 5.64 μmol) in water (4 mL) was added a large excess of solid NH_4HCO_3. The saturated solution was stirred at room temperature for 3 d. It is important to keep the solution saturated by continually adding NH_4HCO_3. After complete consumption of the starting material, the reaction mixture was lyophilized. The white solid was redissolved in water and again lyophilized. This procedure was repeated until the weight remained constant. Pentadecasaccharide glycosylamine **74** (15 mg, 5.64 μmol, >99%) was obtained as a white powder. ^{1}H NMR (D_2O, 500 MHz, 24 °C): δ=5.32 (d, J=3.2 Hz, 2H, 2H-1Fuc), 5.13 (s, 1H, H-1 Man), 4.94 (s, 1H, H-1 Man), 4.72 (d, J=8.0 Hz, 2H, 2H-1), 4.60 (d, J=8.0 Hz, 2H, 2H-1), 4.56 (d, J=8.0 Hz, 3H, 3H-1), 4.48 (d, J=7.8 Hz, 2H, 2H-1), 4.12–4.28 (m), 3.48–4.00 (m, ~90H), 2.09, 2.05, 2.01 (s, 15H, 5Ac), 1.95 (s, 3H, Ac), 1.25 (d, J=6.5 Hz, 6H, $2CH_3$); LRMS calcd for $C_{102}H_{171}N_7O_{73}$: 2662, found: 2684 (M+Na).

4.1.45. Pentadecasaccharide pentapeptide conjugate (3). To a solution of glycosylamine **74** (10 mg, 3.75 μmol) in anhydrous DMSO (0.5 mL) at room temperature was added Hünig's base (100 μL DIEA/DMSO, prepared from 12.8 μL DIEA in 1 mL of DMSO, 7.34 μmol), pentapeptide **48**[37] (4 mg, 7.2 μmol), 1-hydroxybenzotriazole (100 μL HOBt/DMSO, prepared from 25.2 mg HOBt in 1 mL of DMSO, 18.8 μmol), and HBTU (100 μL HBTU/DMSO, prepared from 80.4 mg HBTU in 1 mL of DMSO, 18.8 μmol). The reaction was stirred at room temperature for 36 h (suspension turned into solution) and then lyophilized and purified on a reverse-phase Vydac C18-column (4.6×250 mm) to afford glycopeptide **3** (2.3 mg, 20%) as a white powder. HPLC gradient: A: 0.1% TFA/H_2O, B: 0.09% TFA/70% CH_3CN/H_2O, gradient 5→50% B over 23 min; 50→100% B over 5 min. ^{1}H NMR (***H_2O***, 800 MHz, 22 °C): δ=8.74 (d, J=8.8 Hz, 1H, NH), 8.54 (d, J=9.6 Hz, 1H, NH), 8.52 (d, J=7.2 Hz, 2H, 2NH), 8.42 (d, J=5.6 Hz, 2H, 2NH), 8.36 (d, J=5.6 Hz, 1H, NH), 8.35 (d, J=9.6 Hz, 1H, NH), 8.34 (d, J=12.8 Hz, 1H, NH), 8.32 (d, J=6.4 Hz, 1H, NH), 8.31 (d, J=8.8 Hz, 1H, NH), 8.29 (d, J=8.8 Hz, 2H, 2NH), 8.14 (d, J=8.8 Hz, 2H, 2NH), 7.60 (s, 1H, NH, C-terminal), 7.24 (s, 1H, NH, C-terminal); ^{1}H NMR (***D_2O***, 800 MHz, 22 °C): δ=5.18 (d, J=3.2 Hz, 2H, 2H-1Fuc), 4.99 (s, 1H, H-1 Man), 4.91 (d, J=9.6 Hz, 1H, NH), 4.80 (s, 1H, H-1 Man), 4.64 (s, 1H, H-1 Man), 4.60 (dd, J=6.4, 7.2 Hz, 1H), 4.57 (d, J=8.0 Hz, 2H, 2H-1), 4.49 (d, J=8.0 Hz, 1H, H-1b), 4.46 (d, J=8.0 Hz, 2H, 2H-1), 4.44 (d, J=8.0 Hz, 2H, 2H-1), 4.34 (d, J=7.2 Hz, 1H, H-1), 4.33 (d, J=7.2 Hz, H-1), 4.15–4.20 (m), 4.06–4.14 (m), 3.40–4.00 (m), 2.85 (dd, J=6.4, 16.1 Hz, 1H), 2.64 (dd, J=6.7, 16.1 Hz, 1H), 2.06 (m, 1H, $CH(CH_3)_2$, 1.95 (s, 3H, Ac), 1.93 (s, 3H, Ac), 1.92 (s, 6H, 2Ac), 1.91 (s, 3H, Ac), 1.90 (d, J=7.3 Hz, 6H, $2CH_3$Fuc), 1.09 (d, J=7.7 Hz, 3H, CH_3), 0.82 (d, J=5.9 Hz, 3H, CH_3), 0.81 (d, J=6.4 Hz, 6H, $2CH_3$), 0.77 (d, J=8.0 Hz, CH_3); LRMS calcd for $C_{126}H_{211}N_{13}O_{81}$: 3202, found: 3225 (M+Na), 1624 (M+2Na)/2e, 1637 (M+2Cl)/2 in FAB mode.

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An effective method for the preparation of mono *N*-alkyl derivatives of 1,1′-bis(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline)

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Abstract—The condensation of 1,1′-bis(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline) with alkyl, aralkyl and aryl aldehydes, but not ketones, in ethanol or chloroform provides useful cyclic aminal [8-substituted 5,6,10,11,15b,15c-hexahydro-2,3,13,14-tetramethoxy-8*H*-imidazo[5,1-*a*:4,3-*a*′]diisoquinoline] intermediates that when subsequently treated with sodium cyanoborohydride in ethanol, followed by the addition of 2 M hydrochloric acid, gave monosubstituted *N*-alkyl 1,1′-bis(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline) derivatives in very high yields. The rates of the initial condensation with four different aldehydes were measured, and the entire sequence was successfully applied in one example to a 'one-pot' process; this signals a versatile route to differentially *N*-substituted 1,1′-bis(1,2,3,4-tetrahydroisoquinoline) derivatives.

1. Introduction

Reductive alkylation[1–14] is a general method for the preparation of tertiary amines from secondary amines, and hydride reagents have been used for this purpose with particular success.[4,6] We envisaged that this method would provide a means of attaching substituents through the nitrogen atoms of *rac*-1,1′-bis(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline) **1** in an ongoing study of heterocyclic ligands by our group[15,16] and that of others.[17,18] However, it has been reported[19] that while the corresponding *meso* compound underwent two-fold reductive N,N′-dimethylation through treatment with formalin/sodium borohydride, the *racemic* compound **1** gave none of the expected product but a modest yield of an N-cyanomethylation product. As far as we can determine, this reaction has not been pursued. In our hands, two-fold reductive alkylation of *rac*-bisisoquinoline **1** with sodium cyanoborohydride, under neutral or acidic conditions, or with sodium (triacetoxy)borohydride, also proved impossible to achieve in one step. Instead, the heterocycle **1** condensed extremely efficiently under the reaction conditions with aldehydes, to yield the corresponding aminal derivatives **2**. Compounds **2** were stable to reduction under the reaction conditions. Further investigation revealed that the condensation reaction was general and proceeded with a range of aldehydes, but not ketones, under mild conditions that neither require acid catalysis nor the presence of the reducing agent (Scheme 1).

Apart from the example of reductive methylation, previous literature reactions between reduced bisisoquinolines like **1** and aldehydes have normally involved only the use of formaldehyde as partner.[20–22] Motivation for these studies has been as a structural tool because the products, through the equivalence or otherwise of the new aminal methylene protons, can be used to distinguish the original heterocycle from its *meso* diastereomer.

2. Results and discussion

2.1. Heterocycle with aldehyde/ketone condensation

In the current study, bis(tetrahydroisoquinoline) **1** was found to react completely with three, and in many cases fewer, equivalents of aliphatic, aromatic and heteroaromatic aldehydes in ethanol at room temperature within an hour. Treatment with butanone under similar conditions failed to yield any condensation product, although acetone was found to react in low yield under more forcing conditions.[23] The ability to use pi-excessive and pi-deficient carbocyclic and heterocyclic aldehydes is an illustration of the wide generality of the reaction. Workup was achieved simply by evaporation

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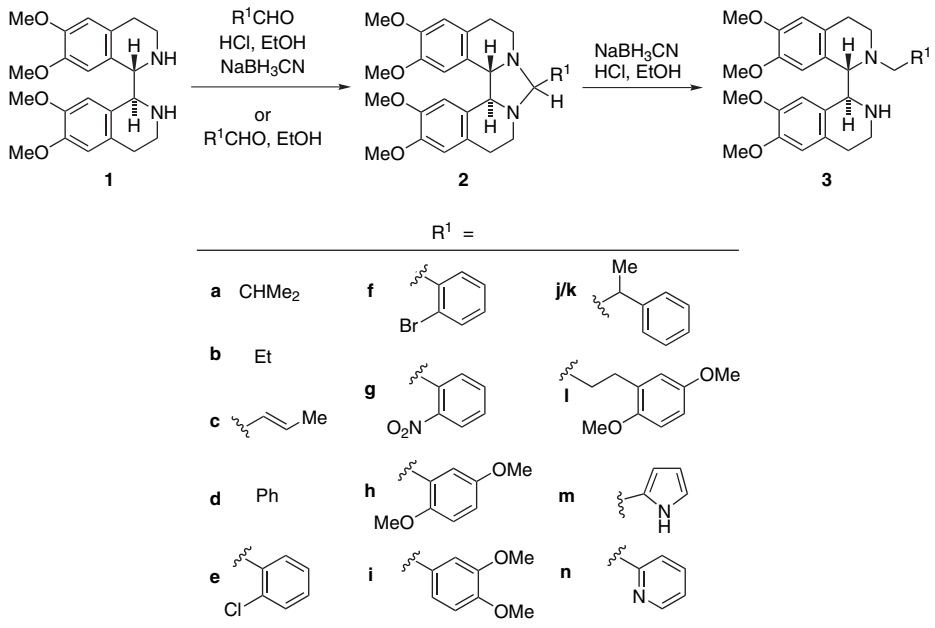


Scheme 1.

of solvent under reduced pressure (to remove any non-volatile unreacted aldehyde) and recrystallisation.

In the case of reaction with 2-phenylpropanal, leading to derivative **2j/k**, evaporation of solvent and analysis by ^{1}H NMR spectroscopy revealed formation of diastereomers, as shown by the appearance of duplicate sets of aromatic singlets for the H5, H5′, H8 and H8′ protons. Such detail was not important for the present study but was pursued for characterisation purposes. The product **2j/k** was recrystallised in order to attempt separation of the unequally distributed (62:38) diastereoisomers. The minor component **2j** crystallised in preference to the major isomer **2k**, and several recrystallisations gave a sample of the single diastereomer **2j** as a pure compound. It was not possible to determine the stereochemistry at the stereogenic centres in the bisisoquinoline portion of the molecule relative to the aldehyde-derived centre by spectroscopic methods and a sample could not be suitably crystallised for X-ray crystallography. Attempts to isolate a pure sample of the second diastereomer **2k** led only to production of more of the minor isomer **2j**. Eventually the crystallisation process gave very significant conversion of the material to what was originally the minor isomer **2j**. It was therefore concluded that the original mixture **2j/2k** resulted from a degree of kinetic control, so that the original major product **2k** was a kinetic rather than the thermodynamic product.

2.2. Rate comparisons

Qualitative differences were perceived in the rates of reactions with certain aldehydes. Attempts were made to compare rates of conversion at ice-bath temperatures in ethanol, but the results after evaporative workup were ambiguous. Attention then turned to deuteriochloroform as a solvent, so that progress in reactions could be monitored directly by ^{1}H NMR spectroscopy. Initially, the reaction of isobutyraldehyde with a slight sub-equimolar quantity of bis(tetrahydroisoquinoline) **1** in deuteriochloroform at 273 K was monitored in the probe of the NMR instrument using residual chloroform as an internal standard. The reaction was found to reach 86% conversion (percentage product over the sum of the bisisoquinoline **1** and product **2a**) within 48 min. A natural log plot of the aldehyde/bisisoquinoline **1** concentration ratio versus time subtended a straight line that revealed a rate of 8.809×10^{-5} mol/s with an R^2 factor of 0.98. Unfortunately other aldehydes of interest reacted too slowly at 273 K to yield useful data for comparisons. Reactions of isobutyraldehyde, 2-chlorobenzaldehyde, 2,5-dimethoxybenzaldehyde and 3,4-dimethoxybenzaldehyde, with slightly sub-equimolar quantities of bis(tetrahydroisoquinoline) **1**, were then carried out sequentially at 308 K. Conversions of 85% of the products **2a**, **2e**, **2h** and **2i** were attained after 6, 37, 108 and 260 min, respectively (Fig. 1). Further, calculation of rates, as above, yielded values of 38.56×10^{-5}, 7.259×10^{-5}, 0.2591×10^{-5} and 0.2452×10^{-5} mol/s, respectively, which translated into relative rates of 157: 29.6: 1.06: 1.00 (Table 1). It was concluded that the

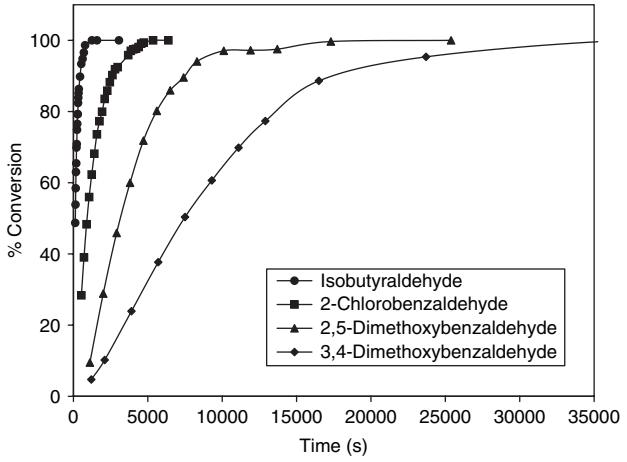
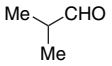
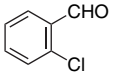
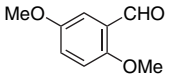
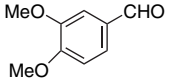


Figure 1. Plot of conversion versus time for reactions of 1,1′-bis(tetrahydroisoquinoline) **1** with isobutyraldehyde, 2-chlorobenzaldehyde, 2,5-dimethoxybenzaldehyde and 3,4-dimethoxybenzaldehyde.

Table 1. Reaction rates for the condensation of 1,1′-bis(tetrahydroisoquinoline) **1** with aldehydes as determined by ^{1}H NMR spectroscopy in $CDCl_3$ at 308 K

Aldehyde	Reaction rate ($\times 10^{-5}$ mol/s)	Relative rate
	38.56 (8.809)[a]	157
	7.259	29.6
	0.2591	1.06
	0.2452	1.00

[a] Reaction rate at 273 K.

condensation efficiency was directly related to the electrophilicity of the carbonyl partner, an observation that also explained the reluctance of ketones to participate within reasonable reaction times.

The reliability of the difference in rates between 2,5- and 3,4-dimethoxybenzaldehyde was tested through a competition reaction. An equimolar mixture of the two aldehydes was treated in deuteriochloroform with a slightly sub-equimolar (total 2 mol of aldehydes to 1 mol of bisisoquinoline) quantity of bis(tetrahydroisoquinoline) **1** at 308 K, and again the progress of the reaction monitored by ^{1}H NMR spectroscopy. On this occasion dimethylsulfone (DMS) was used as an internal standard. There was 53% conversion based on the amounts of new isoquinoline derivatives observed after 15 h, and the expected products, **2h** and **2i**, were formed in a ratio of 80:20, respectively. The latter selectivity was remarkable given the similarity of the aldehydes, and appeared from the linear trend in product formation for each of **2h** and **2i** not to arise through equilibration. The greater reactivity of the 2,5-dimethoxybenzaldehyde was attributed to the proximity of the 2-methoxy group to the aldehyde and its inductive effect on the carbonyl carbon. In continuation of the study, a competitive reaction at 273 K involving equimolar quantities of bis(tetrahydroisoquinoline) **1**, isobutyraldehyde and 2,5-dimethoxybenzaldehyde in deuteriochloroform gave 75% conversion to a 100:0 ratio of products **2a** and **2h**, respectively, within 48 min.

These results reinforced the qualitative differences in reactivity that had already been observed and were fully consistent with carbonyl electrophilicity as the major controlling factor. It is notable that there were sufficient differences in the rates to enable high selectivity to be achieved between reasonably similar aldehydes.

2.3. Reductive cleavage

In order to test the possible intermediacy of the condensation products **2** in the originally planned double reductive alkylation sequence, the isobutyraldehyde condensation product **2a** was subjected to treatment with excess sodium cyanoborohydride in ethanol in the presence of hydrochloric acid. The reaction failed when the reducing reagent was initially added to an acidified ethanol solution of the substrate **2a**. Treatment at room temperature or at reflux gave only the unreacted substrate **2a** in small but steadily decreasing amounts with time. Similarly, reduction using sodium (triacetoxy)-borohydride in dichloroethane, failed. Unfortunately, there was no distinct new product evident as a result of consumption of the substrate. In contrast, when 2 M aqueous hydrochloric acid was added slowly to a solution of the substrate **2a**, immediately after addition of sodium cyanoborohydride, complete conversion to a new product was observed to take place in the absence of side products within an hour at room temperature. Aqueous extractive workup afforded the anticipated *N*-isobutyryl product **3a** in virtually quantitative yield. Treatment of aminals **2h** and **2l** with sodium cyanoborohydride, and then hydrochloric acid, similarly afforded the mono-alkylated derivatives **3h** and **3l** in high yield, thereby suggesting that the process was general.

In retrospect, the demands of this late order of addition of acid would naturally preclude formation of the doubly alkylated product in a one-pot process, since one could not achieve the second aminal formation and the second cleavage until acid was added. However, it would leave open the possibility of a general 'one-pot' mono-alkylation procedure, from the parent bis(tetrahydroisoquinoline) **1**. Unsymmetrically *N*-alkylated derivatives of bis(tetrahydroisoquinoline) **1** should therefore be accessible by the relatively simple procedure of aldehyde condensation followed by reductive ring opening. This new method was demonstrated in one case, bis(tetrahydroisoquinoline) **1** to its *N*-isobutyl derivative in 86% yield, and future work will exploit this sequence in the synthesis of unsymmetrical ligands **4** for host–guest chemistry and catalysis.

2.4. Spectroscopic comparisons

The ^{1}H and ^{13}C NMR spectra of the condensation products **2** were useful from a structural standpoint. As expected, they showed clearly the unsymmetrical nature of the two isoquinoline ring systems through the appearance of separate signals for each of the chemically non-equivalent protons and carbons. More importantly, amongst all the resonances in the ^{1}H NMR spectra the signals for H15b/H15c and H1/H15 showed the most major differences in chemical shift in all derivatives, with those at H15b and H15 appearing at 0.7–0.8 and ca. 0.5 ppm, respectively, higher field than the signals for the corresponding protons from the second ring system (Fig. 2). Also notable, the signals for $H_\beta 5$ resonated at ca. 0.5 ppm higher field than the signals for their geminal partners, $H_\alpha 5$, in all cases, and those for $H_\alpha 10$ resonated at 0.4–0.5 ppm higher field than their geminal partners, $H_\beta 10$, but only in the cases where the group directly attached at C9 was aromatic, where R^1 was alkyl, aralkyl or alkenyl, the H10 signals were almost identical in chemical shift. The high field shift of the signals mentioned above was obviously related to the now unsymmetrical nature of the molecules and the concomitant presence of the aminal substituent that imparted asymmetry to the molecule; the extreme positions of these particular signals were more especially probably a reflection of pyramidalization through sp^3 hybridisation

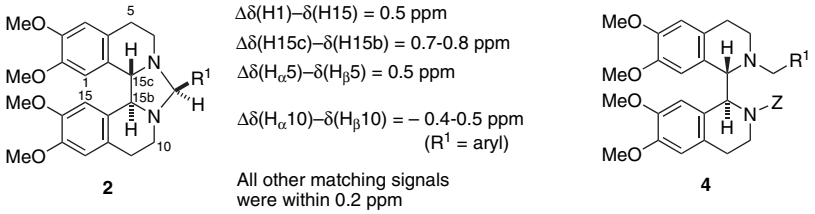


Figure 2.

at the nitrogens and comparatively fixed positions of the protons relative to the rest of the molecule due to rigidity in the imidazolidine rings. The major points of chemical shift difference in the ^{13}C NMR spectra were at C15b/C15c (3–6 ppm), C5/C11 (3–4 ppm) and C6/C10 (4–8 ppm). Consideration of these structural findings will assist in future ligand design since they reflect the more significant regions of the ligands and something of their conformational preferences in the ground state.

Molecular ions were not observed in the electron impact mass spectra of derivatives **2a**–**n**, but in all cases a large peak, often the base peak, was observed at 191 amu less than that expected for the molecular ion. This was consistent with fragmentation of the molecular ion through loss of one isoquinoline ring ($C_{11}H_{13}NO_2$) as a neutral species. The only exception to this behaviour was that of the nitrobenzaldehyde derivative **2g**, which gave a relatively minor fragment ion at M−191. Instead, the base peak had occurred at *m*/*z* 191. In the molecules derived from non-aromatic aldehydes there was also observed fragment ions through loss of the C-8 substituent, thus C_3H_7 and C_2H_5 from **2a** and **2b**, respectively.

3. Conclusion

In summary, a method has been developed that enables unsymmetrical, mono *N*-substituted 1,1′-bis(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline) derivatives to be prepared efficiently. The process involves a kinetically driven condensation reaction between the bis(tetrahydroisoquinoline) **1** and various aldehydic partners, which can be highly selective. The intermediates and products of the overall process show distinctive spectroscopic properties that are helpful in defining structural features for future ligand design.

4. Experimental

4.1. Condensation reactions of bis(tetrahydroisoquinoline) 1 with aldehydes

General procedure: Bis(tetrahydroisoquinoline) **1** (77 mg, 0.20 mmol) was dissolved in absolute EtOH (8 mL) and the solution stirred at ambient temperature as the aldehyde (0.60 mmol) was added dropwise or in solid portions. The mixture was then maintained at room temperature or warmed to reflux for the time specified, when TLC analysis indicated that the starting bisisoquinoline **1** had been consumed. Volatile material was removed by rotary evaporation and the residue was recrystallised.

4.1.1. Reaction with isobutyraldehyde. This reaction occurred at room temperature within 1 h to yield *5,6,10,11,15b,15c-hexahydro-8-(1-methylethyl)-2,3,13,14-tetramethoxy-8H-imidazo[5,1-a:4,3-a′]diisoquinoline* **2a** as white needles (78 mg, 90%) mp 172–174 °C (EtOAc), R_f 0.52 (MeOH) (Found: C, 70.97; H, 7.75; N, 6.14. $C_{26}H_{34}N_2O_4$ requires: C, 71.21; H, 7.81; N, 6.39%). IR (KBr): 1607, 1515, 1464, 1348, 1272, 1247, 1225, 1129, 851, 779 cm^{-1}. UV (MeOH): 242, 288 nm. ^{1}H NMR ($CDCl_3$) δ: 1.13 (3H, d, *J* 6.7 Hz, 8-CH(CH_3)$_a$(CH$_3$)$_b$), 1.14 (3H, d, *J* 6.8 Hz, 8-CH(CH$_3$)$_a$(CH_3)$_b$), 2.08 (1H, m, 8-C*H*(CH$_3$)$_a$(CH$_3$)$_b$), 2.49 (1H, m, H$_\beta$5), 2.85 (1H, m, H$_\beta$6), 2.89 (2H, m, (H11)$_2$), 2.90 (1H, m, H$_\alpha$5), 2.98 (2H, m, (H10)$_2$), 3.09 (1H, m, H$_\alpha$6), 3.48 (1H, d, *J* 9.3 Hz, H15b), 3.52 (3H, s, 14-OMe), 3.64 (1H, d, *J* 7.3 Hz, H8), 3.69 (3H, s, 2-OMe), 3.84 (3H, s, 13-OMe), 3.88 (3H, s, 3-OMe), 4.21 (1H, d, *J* 9.3 Hz, H15c), 5.93 (1H, s, H15), 6.45 (1H, s, H1), 6.63 (1H, s, H12), 6.73 (1H, s, H4); ^{13}C NMR ($CDCl_3$) δ: 19.6 (8-CH(CH$_3$)$_a$(*C*H$_3$)$_b$), 20.2 (8-CH(*C*H$_3$)$_a$(CH$_3$)$_b$), 26.5 (C5), 29.9 (C11), 30.2 (8-*C*H(CH$_3$)$_a$(CH$_3$)$_b$), 40.9 (C10), 49.8 (C6), 55.5 (14-OMe), 55.6 (13-OMe), 55.8 (3-OMe), 55.9 (2-OMe), 65.3 (C15c), 67.8 (C15b), 88.3 (C8), 110.8 (C12), 111.05 (C15), 111.11 (C4), 113.3 (C1), 125.6 (C15a), 127.0 (C15d), 127.1 (C11a), 130.2 (C4a), 145.9 (C14), 146.3 (C2), 147.4 (C3), 147.5 (C13); Mass spectrum (EI): *m*/*z* 395 (M−43, 21%), 247 (87), 232 (100).

4.1.2. Reaction with propionaldehyde. This reaction occurred at room temperature within 50 min to yield *8-ethyl-5,6,10,11,15b,15c-hexahydro-2,3,13,14-tetramethoxy-8H-imidazo[5,1-a:4,3-a′]diisoquinoline* **2b** as white needles (74 mg, 87%) mp 196–197 °C (EtOH), R_f 0.41 (MeOH) (Found: C, 70.51; H, 7.37; N, 6.40. $C_{25}H_{32}N_2O_4$ requires: C, 70.73; H, 7.60; N, 6.60%). IR (KBr): 1607, 1480, 1459, 1349, 1254, 1227, 1130, 850, 788 cm^{-1}. UV (MeOH): 242, 288 nm. ^{1}H NMR ($CDCl_3$) δ: 1.11 (3H, t, *J* 7.5 Hz, 8-CH$_2$CH_3), 1.83 (2H, dq, *J* 7.0, 7.5 Hz, 8-CH_2CH$_3$), 2.43 (1H, br d, *J* 14.7 Hz, H$_\beta$5), 2.82 (1H, m, H$_\alpha$11), 2.84 (1H, m, H$_\beta$6), 2.90 (1H, m, H$_\alpha$5), 2.98 (1H, m, H$_\alpha$10), 3.00 (1H, m, H$_\beta$11), 3.06 (1H, m, H$_\alpha$6), 3.10 (1H, m, H$_\beta$10), 3.50 (1H, d, *J* 9.0 Hz, H15b), 3.56 (3H, s, 14-OMe), 3.68 (3H, s, 2-OMe), 3.84 (3H, s, 13-OMe), 3.87 (3H, s, 3-OMe), 4.00 (1H, d, *J* 7.0 Hz, H8), 4.29 (1H, d, *J* 9.0 Hz, H15c), 5.97 (1H, s, H15), 6.37 (1H, s, H1), 6.64 (1H, s, H12), 6.72 (1H, s, H4); ^{13}C NMR ($CDCl_3$) δ: 10.2 (8-CH$_2$*C*H$_3$), 23.1 (8-*C*H$_2$CH$_3$), 25.3 (C5), 29.2 (C11), 38.9 (C10), 46.2 (C6), 55.7 (13-OMe), 55.7 (14-OMe), 55.8 (3-OMe), 55.9 (2-OMe), 64.7 (C15c), 69.7 (C15b), 83.5 (C8), 110.9 (C12), 111.5 (C4), 111.8 (C15), 112.9 (C1), 125.2 (C15a), 126.7 (C11a), 127.5 (C15d), 129.6 (C4a), 146.0 (C14), 146.39 (C2 or C3), 147.42 (C2 or C3), 147.9 (C13);

Mass spectrum (EI): *m/z* 395 (M−29, 4%), 247 (2), 233 (100), 218 (87).

4.1.3. Reaction with crotonaldehyde. This occurred at room temperature within 40 min to yield *5,6,10,11,15b,15c-hexahydro-8-(prop-1-enyl)-2,3,13,14-tetramethoxy-8H-imidazo[5,1-a:4,3-a′]diisoquinoline* **2c** as white needles (80 mg, 92%) mp 201–202 °C (EtOH), R_f 0.59 (MeOH) (Found: C, 71.51; H, 7.68; N, 6.32. $C_{26}H_{32}N_2O_4$ requires: C, 71.54; H, 7.39; N, 6.42%). IR (KBr): 1610, 1518, 1453, 1349, 1257, 1227, 1131, 965, 854, 777 cm^{-1}. UV (MeOH): 242, 287 nm. ^1H NMR ($CDCl_3$) δ: 1.83 (3H, dd, *J* 6.4, 1.5 Hz, 8-CHCHCH_3), 2.45 (1H, br d, *J* 12.5 Hz, $H_\beta5$), 2.82 (1H, m, $H_\beta6$), 2.83 (1H, m, $H_\alpha11$), 2.87 (1H, m, $H_\alpha5$), 2.96 (1H, m, $H_\beta11$), 2.98 (1H, m, $H_\alpha6$), 3.09 (1H, m, $H_\alpha10$), 3.09 (1H, m, $H_\beta10$), 3.54 (1H, d, *J* 9.0 Hz, H15b), 3.63 (3H, s, 14-OMe), 3.73 (3H, s, 2-OMe), 3.86 (3H, s, 13-OMe), 3.89 (3H, s, 3-OMe), 4.32 (1H, d, *J* 9.0 Hz, H15c), 4.36 (1H, d, *J* 7.5 Hz, H8), 5.66 (1H, dd, *J* 15.5, 1.5 Hz, 8-C*H*CHCH$_3$), 5.98 (1H, dq, *J* 15.5, 6.4 Hz, 8-CHC*H*CH$_3$), 6.17 (1H, s, H15), 6.48 (1H, s, H1), 6.66 (1H, s, H12), 6.74 (1H, s, H4); ^{13}C NMR ($CDCl_3$) δ: 17.9 (8-CHCH*C*H$_3$), 25.5 (C5), 29.2 (C11), 41.1 (C10), 45.4 (C6), 55.7 (13-OMe), 55.8 (14-OMe), 55.9 (3-OMe), 56.0 (2-OMe), 64.1 (C15c), 69.4 (C15b), 83.8 (C8), 111.2 (C12), 111.57 (C15), 111.60 (C4), 112.7 (C1), 125.8 (C15a), 126.9 (C11a), 128.0 (C15d), 129.0 (8-*C*HCHCH$_3$), 129.9 (C4a), 132.2 (8-CH*C*HCH$_3$), 146.2 (C14), 146.5 (C2), 147.5 (C3), 147.9 (C13); Mass spectrum (EI): *m/z* 246 (M−190, 17%), 245 (100), 244 (60), 230 (46).

4.1.4. Reaction with benzaldehyde. This reaction occurred at reflux within 16.5 h to yield *5,6,10,11,15b,15c-hexahydro-8-phenyl-2,3,13,14-tetramethoxy-8H-imidazo[5,1-a:4,3-a′]diisoquinoline* **2d** as white needles (85 mg, 91%) mp 215–217 °C (EtOAc), R_f 0.76 (MeOH) (Found: C, 73.61; H, 6.84; N, 5.71. $C_{29}H_{32}N_2O_4$ requires: C, 73.71; H, 6.82; N, 5.93%). IR (KBr): 1606, 1515, 1461, 1346, 1255, 1224, 1126, 850, 786 cm^{-1}. UV (MeOH): 242, 288 nm. ^1H NMR ($CDCl_3$) δ: 2.44 (1H, br d, *J* 12.9 Hz, $H_\beta5$), 2.46 (1H, m, $H_\alpha10$), 2.72 (1H, m, $H_\alpha11$), 2.86 (1H, m, $H_\beta11$), 2.88 (1H, m, $H_\alpha6$), 2.88 (1H, m, $H_\beta6$), 2.88 (1H, m, $H_\beta10$), 3.00 (1H, m, $H_\alpha5$), 3.65 (3H, s, 14-OMe), 3.75 (3H, s, 2-OMe), 3.76 (1H, d, *J* 8.7 Hz, H15b), 3.84 (3H, s, 13-OMe), 3.91 (3H, s, 3-OMe), 4.51 (1H, d, *J* 8.7 Hz, H15c), 5.18 (1H, s, H8), 6.23 (1H, s, H15), 6.54 (1H, s, H1), 6.64 (1H, s, H12), 6.76 (1H, s, H4), 7.35 (1H, t, *J* 7.5 Hz, H4″), 7.41 (2H, dd, *J* 7.5, 6.4 Hz, H3″ and H5″), 7.67 (2H, d, *J* 6.4 Hz, H2″ and H6″); ^{13}C NMR ($CDCl_3$) δ: 25.5 (C4), 29.2 (C11), 41.1 (C10), 46.0 (C6), 55.7 (13-OMe), 55.8 (14-OMe), 55.8 (3-OMe), 56.0 (2-OMe), 64.4 (C15c), 70.5 (C15b), 84.8 (C8), 111.2 (C12), 111.59 (C15), 111.64 (C4), 112.7 (C1), 125.8 (C15a), 127.0 (C11a), 128.15 (C4″), 128.2 (C1″), 128.20 (C15d), 128.22 (C3″ and C5″), 129.1 (C2″ and C6″), 130.0 (C4a), 138.1, 146.2 (C14), 146.5 (C2), 147.5 (C3), 148.0 (C13); Mass spectrum (EI): *m/z* 282 (M−190, 16%), 281 (100), 266 (20), 177 (18).

4.1.5. Reaction with 2-chlorobenzaldehyde. This reaction occurred at reflux within 8.5 h to yield *8-(2-chlorophenyl)-5,6,10,11,15b,15c-hexahydro-2,3,13,14-tetramethoxy-8H-imidazo[5,1-a:4,3-a′]diisoquinoline* **2e** as white needles (77 mg, 76%) mp 201–202 °C (EtOAc), R_f 0.78 (MeOH) (Found: C, 68.87; H, 6.09; N, 5.41. $C_{29}H_{31}N_2O_4Cl$ requires: C, 68.70; H, 6.16; N, 5.53%). IR (KBr): 1605, 1515, 1461, 1361, 1337, 1257, 1223, 1128, 1030, 808, 784, 776 cm^{-1}. UV (MeOH): 242, 288 nm. ^1H NMR ($CDCl_3$) δ: 2.38 (1H, m, $H_\alpha10$), 2.47 (1H, br d, *J* 15.1 Hz, $H_\beta5$), 2.70 (1H, m, $H_\alpha11$), 2.83 (1H, m, $H_\alpha6$), 2.83 (1H, m, $H_\beta6$), 2.87 (1H, m, $H_\beta10$), 2.88 (1H, m, $H_\beta11$), 3.02 (1H, ddd, *J* 15.1, 9.4, 6.0 Hz, $H_\alpha5$), 3.64 (3H, s, 14-OMe), 3.75 (3H, s, 2-OMe), 3.79 (1H, d, *J* 9.0 Hz, H15b), 3.84 (3H, s, 13-OMe), 3.91 (3H, s, 3-OMe), 4.49 (1H, d, *J* 9.0 Hz, H15c), 5.48 (1H, s, H8), 6.21 (1H, s, H15), 6.54 (1H, s, H1), 6.63 (1H, s, H12), 6.77 (1H, s, H4), 7.28 (1H, ddd, *J* 7.2, 7.1, 2.3 Hz, H4′), 7.32 (1H, ddd, *J* 7.2, 7.1, 1.9 Hz, H5′), 7.43 (1H, dd, *J* 7.2, 1.9 Hz, H3′), 7.98 (1H, dd, *J* 7.2, 2.3 Hz, H6′); ^{13}C NMR ($CDCl_3$) δ: 25.8 (C5), 29.3 (C11), 41.7 (C10), 46.1 (C6), 55.7 (13-OMe), 55.85 (14-OMe), 55.85 (3-OMe), 56.0 (2-OMe), 64.0 (C15c), 70.3 (C15b), 81.7 (C8), 111.1 (C12), 111.7 (C4), 111.7 (C15), 112.8 (C1), 125.9 (C15a), 126.5 (C5′), 127.1 (C11a), 128.0 (C15d), 129.2 (C4′), 129.5 (C3′), 130.0 (C4a), 130.9 (C6′), 135.4 (C1′), 135.8 (C2′), 146.2 (C14), 146.5 (C2), 147.5 (C3), 148.0 (C13); Mass spectrum (EI): *m/z* 317 (M−189, 32%), 315 (100), 300 (19), 280 (17), 177 (25).

4.1.6. Reaction with 2-bromobenzaldehyde. This was performed on exactly half the normal scale and occurred at reflux within 2.5 h. The crude product was chromatographed on alumina, eluting with a light petroleum–Et_2O–MeOH gradient to yield *8-(2-bromophenyl)-5,6,10,11,15b,15c-hexahydro-2,3,13,14-tetramethoxy-8H-imidazo[5,1-a:4,3-a′]diisoquinoline* **2f** as white needles (40 mg, 72%) mp 207.5–209 °C (EtOAc), R_f 0.80 (EtOAc) (Found: C, 63.24; H, 5.87; N, 5.09. $C_{29}H_{31}N_2O_4Br$ requires: C, 63.16; H, 5.67; N, 5.08%). IR (KBr): 1517, 1463, 1340, 1314, 1259, 1232, 1126, 1019, 857, 787, 749 cm^{-1}. UV (MeOH): 214, 287 nm. ^1H NMR ($CDCl_3$) δ: 2.37 (1H, m, $H_\alpha10$), 2.48 (1H, br d, *J* 15.1 Hz, $H_\beta5$), 2.71 (1H, m, $H_\alpha11$), 2.77–2.93 (4H, m, $H_\alpha6$, $H_\beta6$, $H_\beta10$ and $H_\beta11$), 3.03 (1H, ddd, *J* 15.1, 8.7, 5.7 Hz, $H_\alpha5$), 3.64 (3H, s, 14-OMe), 3.76 (3H, s, 2-OMe), 3.78 (1H, d, *J* 9.0 Hz, H15b), 3.85 (3H, s, 13-OMe), 3.91 (3H, s, 3-OMe), 4.50 (1H, d, *J* 9.0 Hz, H15c), 5.40 (1H, s, H8), 6.23 (1H, s, H15), 6.56 (1H, s, H1), 6.63 (1H, s, H12), 6.77 (1H, s, H4), 7.20 (1H, ddd, *J* 7.9, 7.5, 1.5 Hz, H4′), 7.38 (1H, ddd, *J* 7.5, 7.5, 1.1 Hz, H5′), 7.62 (1H, dd, *J* 7.9, 1.1 Hz, H3′), 7.97 (1H, dd, *J* 7.5, 1.5 Hz, H6′); ^{13}C NMR ($CDCl_3$) δ: 25.9 (C5), 29.4 (C11), 41.7 (C10), 46.1 (C6), 55.7 (13-OMe), 55.9 (14-OMe), 55.9 (3-OMe), 56.0 (2-OMe), 64.1 (C15c), 70.2 (C15b), 83.8 (C8), 111.2 (C12), 111.6 (C15), 111.7 (C4), 112.8 (C1), 125.6 (C1′), 126.0 (C15a), 127.1 (C11a), 127.1 (C5′), 128.1 (C15d), 129.6 (C4′), 130.0 (C4a), 131.4 (C6′), 132.8 (C3′), 137.4 (C2′), 146.2 (C14), 146.5 (C2), 147.5 (C3), 148.0 (C13); Mass spectrum (EI): *m/z* 361 (M(^{81}Br)−191, 88%), 359 (100), 280 (43), 191 (41), 176 (43).

4.1.7. Reaction with 2-nitrobenzaldehyde. This reaction occurred at reflux within 3.5 h to yield *5,6,10,11,15b,15c-hexahydro-8-(2-nitrophenyl)-2,3,13,14-tetramethoxy-8H-imidazo[5,1-a:4,3-a′]diisoquinoline* **2g** as pale yellow needles (58 mg, 56%) mp 192–194 °C (EtOAc), R_f 0.61 (MeOH) (Found: C, 67.05; H, 6.27; N, 7.90. $C_{29}H_{31}N_3O_6$ requires: C, 67.30; H, 6.04; N, 8.12%). IR (KBr): 1608, 1517, 1463, 1350, 1258, 1226, 1131, 1018, 857, 786 cm^{-1}. UV

(MeOH): 242, 288 nm. ^{1}H NMR ($CDCl_3$) δ: 2.32 (1H, m, H_α10), 2.50 (1H, m, H_β5), 2.68 (1H, m, H_α11), 2.80 (1H, m, H_β10), 2.80 (1H, m, H_β11), 2.87 (1H, m, H_α6), 2.87 (1H, m, H_β6), 3.01 (1H, m, H_α5), 3.63 (3H, s, 14-OMe), 3.70 (1H, d, *J* 9.0 Hz, H15b), 3.75 (3H, s, 2-OMe), 3.83 (3H, s, 13-OMe), 3.91 (3H, s, 3-OMe), 4.45 (1H, d, *J* 9.0 Hz, H15c), 5.59 (1H, s, H8), 6.21 (1H, s, H15), 6.55 (1H, s, H1), 6.61 (1H, s, H12), 6.77 (1H, s, H4), 7.48 (1H, ddd, *J* 7.9, 7.5, 1.5 Hz, H4′), 7.63 (1H, ddd, *J* 7.9, 7.5, 1.1 Hz, H5′), 7.78 (1H, dd, *J* 7.9, 1.5 Hz, H6′), 8.15 (1H, dd, *J* 7.9, 1.1 Hz, H3′); ^{13}C NMR ($CDCl_3$) δ: 26.0 (C5), 29.3 (C11), 42.0 (C10), 46.4 (C6), 55.7 (13-OMe), 55.9 (14-OMe), 55.9 (3-OMe), 56.0 (2-OMe), 64.2 (C15c), 70.0 (C15b), 79.7 (C8), 111.2 (C12), 111.6 (C4), 111.3 (C15), 112.8 (C1), 123.5 (C6′), 125.8 (C15a), 127.1 (C11a), 127.7 (C15d), 128.8 (C4′ or C5′), 130.0 (C4a), 130.7 (C3′), 132.0 (C4′ or C5′), 132.4 (C1′), 146.2 (C14), 146.6 (C2), 147.6 (C3), 148.0 (C13), 151.2 (C2′); Mass spectrum (EI): *m*/*z* 326 (M−191, 12%), 192 (27), 191 (100), 176 (35).

4.1.8. Reaction with 2,5-dimethoxybenzaldehyde. This reaction, on 20 times larger scale, occurred at room temperature within 1 h to yield *8-(2,5-dimethoxyphenyl)-5,6,10,11,15b,15c-hexahydro-2,3,13,14-tetramethoxy-8H-imidazo[5,1-a:4,3-a′]diisoquinoline* **2h** as colourless needles (1.56 g, 75%) mp 179–180 °C (EtOAc), R_f 0.59 (EtOAc) (Found: C, 69.93; H, 6.48; N, 5.07. $C_{31}H_{36}N_2O_6$ requires: C, 69.91; H, 6.81; N, 5.26%). IR (KBr): 3001, 2910, 2834, 1610, 1518, 1353, 1258, 1155, 1127, 1021, 856, 785, 712 cm^{-1}. UV (MeOH): 224, 291 nm. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.42 (1H, br d, *J* 14.9 Hz, H_β5), 2.46 (1H, m, H_α10), 2.70 (1H, m, H_α11), 2.86 (2H, m, H_α6 and H_β6), 2.90 (2H, m, H_β10 and H_β11), 3.01 (1H, ddd, *J* 14.9, 9.4, 5.6 Hz, H_α5), 3.63 (3H, s, 14-OMe), 3.74 (3H, s, 2-OMe), 3.78 (1H, d, *J* 9.1 Hz, H15b), 3.80 (3H, s, 5′-OMe), 3.84 (3H, s, 13-OMe), 3.84 (3H, s, 2′-OMe), 3.91 (3H, s, 3-OMe), 4.46 (1H, d, *J* 9.1 Hz, H15c), 5.53 (1H, s, H8), 6.15 (1H, s, H15), 6.51 (1H, s, H1), 6.61 (1H, s, H12), 6.75 (1H, s, H4), 6.86 (1H, dd, *J* 8.7, 2.3 Hz, H4′), 6.89 (1H, d, *J* 8.7 Hz, H3′), 7.55 (1H, d, *J* 2.3 Hz, H6′); ^{13}C NMR ($CDCl_3$, 75.6 MHz) δ: 25.7 (C5), 29.3 (C11), 41.6 (C10), 46.1 (C6), 55.6 (2-OMe), 55.7 (13-OMe), 55.78 (14-OMe), 55.85 (3-OMe), 56.0 (5′-OMe), 56.4 (2′-OMe), 63.9 (C15c), 70.6 (C15b), 78.7 (C8),111.0 (C12), 111.6 (C4), 111.7 (C15), 111.8 (C4′), 112.9 (C1), 113.6 (C3′), 115.9 (C6), 126.1 (C11a), 127.1 (C15a), 127.4 (C1′), 128.2 (C4a), 130.1 (C15d), 146.0 (C14), 146.4 (C2), 147.4 (C3), 147.8 (C13), 153.2 (C2′), 153.5 (C5′); Mass spectrum (EI): *m*/*z* 341 (M−191, 100%), 310 (22), 191 (58), 176 (70), 151 (28), 146 (32), 133 (25), 121 (37), 91 (39), 77 (41), 57 (26).

4.1.9. Reaction with 3,4-dimethoxybenzaldehyde. This reaction, on twice the scale and with only 2 equiv of aldehyde, occurred at room temperature within 1 h to yield *8-(3,4-dimethoxyphenyl)-5,6,10,11,15b,15c-hexahydro-2,3,13,14-tetramethoxy-8H-imidazo[5,1-a:4,3-a′]diisoquinoline* **2i** as colourless prisms (0.141 g, 68%) mp 202.5–204 °C (EtOAc), R_f 0.56 (EtOAc) (Found: C, 69.79; H, 7.20; N, 5.05. $C_{31}H_{36}N_2O_6$ requires: C, 69.91; H, 6.81; N, 5.26%). IR (KBr): 2934, 2835, 1609, 1515, 1464, 1259, 1227, 1128, 1019, 857, 780 cm^{-1}. UV (MeOH): 231, 283 nm. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.45 (1H, m, H_β5), 2.48 (1H, m, H_α10), 2.75 (1H, m, H_α11), 2.85 (1H, m, H_α6), 2.87 (1H, m, H_β10), 2.87 (1H, m, H_β11), 2.90 (1H, m, H_β6), 3.01 (1H, m, H_α5), 3.64 (3H, s, 2-OMe), 3.75 (3H, s, 14-OMe), 3.78 (1H, obscured d, H15b), 3.86 (3H, s, 13-OMe), 3.91 (3H, s, 3-OMe), 3.92 (6H, s, 3′-OMe and 4′-OMe), 4.50 (1H, d, *J* 9.0 Hz, H15c), 5.18 (1H, s, H8), 6.20 (1H, s, H15), 6.52 (1H, s, H1), 6.64 (1H, s, H12), 6.77 (1H, s, H4), 6.93 (1H, d, *J* 8.3 Hz, H5′), 7.22 (1H, br s, H2′), 7.24 (obscured d, H6′); ^{13}C NMR ($CDCl_3$, 75.6 MHz) δ: 25.5 (C5), 29.2 (C11), 40.8 (C10), 46.0 (C6), 55.65 (13-OMe), 55.80 (3′-OMe or 4′-OMe), 55.82 (3′-OMe or 4′-OMe), 55.82 (2-OMe), 55.88 (3-OMe), 56.0 (14-OMe), 64.3 (C15c), 70.3 (C15b), 84.5 (C8), 110.6 (C5′), 111.0 (C12), 111.48 (C15), 111.53 (C4), 111.7 (C2′), 112.6 (C1), 121.1 (C6′), 125.6 (C15a), 126.9 (C11a), 128.0 (C15d), 129.9 (C4a), 130.4 (C1′), 146.1 (C14), 146.4 (C2), 147.4 (C3), 147.9 (C13), 148.8 (C3′ or C4′), 148.9 (C3′ or C4′); Mass spectrum (EI): *m*/*z* 531 ((M−H)$^+$, 0.5%), 395 (0.75), 341 (100), 326 (26), 191 (13), 176 (20), 151 (15), 77 (9).

4.1.10. Reaction with 2-phenylpropanal. The reaction with 2-phenylpropanal on twice the scale occurred at room temperature within 0.5 h to yield a 62:38 diastereomeric mixture of products (218 mg, 84%) mp 145–151 °C. Fractional crystallisation using EtOAc afforded the minor diastereomer *(8R*,15bR*,15cS*,1′R*)-5,6,10,11,15b,15c-hexahydro-8-(1-phenylethyl)-2,3,13,14-tetramethoxy-8H-imidazo[5,1-a:4,3-a′]diisoquinoline* **2j** as colourless prisms mp 184–186 °C (EtOAc), R_f 0.65 (EtOAc) (Found: C, 74.27; H, 7.50; N, 5.41. $C_{31}H_{36}N_2O_4$ requires: C, 74.37; H, 7.25; N, 5.60%). IR (KBr): 2921, 1609, 1514, 1463, 1353, 1275, 1254, 1228, 1130, 1019, 854, 780, 699 cm^{-1}. UV (MeOH): 211, 286 nm. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.53 (3H, d, *J* 6.9 Hz, $(H2')_3$), 2.13 (1H, ddd, *J* 13.6, 3.2, 3.2 Hz, H_α10), 2.17 (1H, d, *J* 15.2 Hz, H_β11), 2.29 (1H, ddd, *J* 12.2, 11.9 Hz, H_β10), 2.72 (1H, ddd, *J* 11.8, 3.2, 3.2 Hz, H_α11), 2.89 (1H, ddd, *J* 16.1, 4.0, 3.9 Hz, H_β5), 2.98 (1H, ddd, *J* 16.1, 9.7, 5.7 Hz, H_α5), 3.12 (2H, m, H_α6 and H_β6), 3.19 (1H, m, H1′), 3.45 (1H, d, *J* 9.0 Hz, H15b), 3.55 (3H, s, 2-OMe), 3.68 (3H, s, 14-OMe), 3.86 (6H, s, 3-OMe and 13-OMe), 4.12 (1H, m, H8), 4.28 (1H, d, *J* 9.0 Hz, H15c), 5.93 (1H, s, H1), 6.37 (1H, s, H15), 6.64 (1H, s, H12), 6.66 (1H, s, H4), 7.25 (1H, t, *J* 7.3 Hz, H4″), 7.34 (2H, dd, *J* 7.5, 7.3 Hz, H3″ and H5″), 7.41 (2H, d, *J* 7.5 Hz, H2″ and H6″); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 19.1 (C2′), 20.1 (C11), 30.0 (C5), 40.8 (C6), 42.5 (C1′), 48.4 (C10), 56.1 (2-OMe), 56.2 (3-OMe), 56.3 (13-OMe), 56.4 (14-OMe), 66.0 (C15c), 69.2 (C15b), 88.0 (C8), 111.3 (C4), 111.7 (C12), 112.0 (C1), 113.5 (C15), 125.9 (C15d), 127.0 (C4″), 127.8 (C4a), 128.0 (C11a), 128.7 (C1″), 128.9 (C2″, C3″, C5″, C6″), 130.9 (C15a), 146.4 (C2), 146.7 (C14), 147.8 (C13), 148.2 (C3); Mass spectrum (EI): *m*/*z* 499 ((M−H)$^+$, 0.5%), 393 (42), 309 (53), 294 (27), 191 (100), 176 (25), 105 (22).

The initially major isomer could not be obtained pure but was identified as *(8R*,15bR*,15cS*,1′S*)-5,6,10,11,15b,15c-hexahydro-8-(1-phenylethyl)-2,3,13,14-tetramethoxy-8H-imidazo[5,1-a:4,3-a′]diisoquinoline* **2k** through analysis of the ^{1}H and ^{13}C NMR data of a highly enriched sample, colourless prisms, mp 184–185 °C (dec), R_f 0.75 (EtOAc). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.44 (3H, d, *J* 6.8 Hz, $(H2')_3$), 2.39 (1H, br d, *J* 15.1 Hz, H_β5), 2.46 (1H, m, H_α6), 2.58 (1H, m, H_β6), 2.67 (1H, m, H_β11), 2.69 (1H, m, H_α10), 2.77 (1H, m,

H_α5), 2.97 (1H, m, H_α11), 3.15 (1H, m, H_β10), 3.22 (1H, m, H1′), 3.43 (1H, d, *J* 9.0 Hz, H15b), 3.57 (3H, s, 14-OMe), 3.78 (3H, s, 2-OMe), 3.83 (3H, s, 13-OMe), 3.86 (1H, br s, H8), 3.87 (3H, s, 3-OMe), 4.20 (1H, d, *J* 9.0 Hz, H15c), 6.30 (1H, s, H15), 6.62 (1H, s, H12), 6.68 (1H, s, H4), 6.73 (1H, s, H1), 7.21 (1H, t, *J* 7.2 Hz, H4″), 7.32 (2H, dd, *J* 7.5, 7.2 Hz, H3″ and H5″), 7.37 (2H, d, *J* 7.5 Hz, H2″ and H6″); ^{13}C NMR ($CDCl_3$, 75.6 MHz) *δ*: 16.2 (C2′), 27.6 (C5), 30.4 (C11), 42.1 (C1′), 43.3 (C10), 50.6 (C6), 55.80 (12-OMe), 55.83 (13-OMe), 55.93 (3-OMe), 56.1 (2-OMe), 65.4 (C15c), 66.4 (C15b), 88.1 (C8), 110.5 (C15), 111.38 (C4), 111.43 (C12), 114.1 (C1), 126.1 (C4″), 127.1 (C15d), 128.0 (C15a), 128.1 (C3″, C5″), 128.3 (C2″, C6″), 128.5 (C11a), 131.0 (C4a), 144.7 (C1″), 146.3 (C14), 146.6 (C2), 147.6 (C13), 148.0 (C3).

4.1.11. Reaction with 3-(2,5-dimethoxyphenyl)propanal. This reaction was performed on four times the scale with only 1.5 mol equiv of aldehyde, at room temperature within 1 h to yield *8-[2-ethyl(2,5-dimethoxyphenyl)]-5,6,10,11,15b,15c-hexahydro-2,3,13,14-tetramethoxy-8H-imidazo[5,1-a:4,3-a′]diisoquinoline* **2l** as white rosettes (0.292 g, 58%) mp 181–183.5 °C (EtOAc), R_f 0.37 (EtOAc) (Found: C, 70.63; H, 7.10; N, 4.89. $C_{33}H_{40}N_2O_6$ requires: C, 70.69; H, 7.19; N, 5.00%). IR (KBr): 2927, 2830, 2360, 1609, 1504, 1464, 1228, 1126, 1050, 855 cm^{-1}. UV (MeOH): 240, 278 nm. ^{1}H NMR ($CDCl_3$, 300 MHz) *δ*: 2.09 (1H, m, H1′), 2.48 (1H, br d, *J* 13.6 Hz, H_β5), 2.72 (1H, m, H_a2), 2.86 (1H, m, H_β11), 2.86 (1H, m, H_β6), 2.91 (1H, m, H_α5), 2.92 (1H, m, H_b2), 3.05 (1H, m, H_α10), 3.07 (1H, m, H_α11), 3.08 (1H, m, H_α6), 3.33 (1H, m, H_β10), 3.55 (1H, d, *J* 9.0 Hz, H15b), 3.60 (3H, s, 13-OMe), 3.71 (3H, s, 3-OMe), 3.78 (3H, s, 2″-OMe), 3.82 (3H, s, 5″-OMe), 3.87 (3H, s, 14-OMe), 3.90 (3H, s, 2-OMe), 4.16 (1H, dd, *J* 9.0, 4.1 Hz, H8), 4.32 (1H, d, *J* 9.0 Hz, H15c), 6.04 (1H, s, H15), 6.41 (1H, s, H1), 6.68 (1H, s, H12), 6.72 (1H, dd, *J* 8.7, 3.0 Hz, H4″), 6.74 (1H, s, H4), 6.80 (1H, d, *J* 8.7 Hz, H3″), 6.83 (1H, d, *J* 3.0 Hz, H6″); ^{13}C NMR ($CDCl_3$, 75.6 MHz) *δ*: 25.4 (C5), 27.1 (C2′), 29.3 (C11), 30.3 (C1′), 39.1 (C10), 46.2 (C6), 55.6 (2-OMe), 55.7 (13-OMe), 55.7 (5″-OMe), 55.8 (2-OMe), 55.8 (5″-OMe), 55.9 (3-OMe), 64.7 (C15c), 69.8 (C15b), 82.1 (C8), 110.9 (C12), 111.0 (C4″), 111.2 (C3″), 111.5 (C4), 111.9 (C15), 112.9 (C1), 116.1 (C6″), 125.4 (C11a), 126.8 (C15a), 127.7 (C4a), 129.7 (C15d), 131.8 (C1″), 146.1 (C13), 146.4 (C3), 147.4 (C2), 148.0 (C14), 151.7 (C5″), 153.5 (C2″); Mass spectrum (EI): *m/z* 559 (M−1$^+$, 0.8%), 409 (1), 395 (7), 369 (15), 338 (6), 219 (10), 218 (100), 202 (8), 191 (12).

4.1.12. Reaction with pyrrole-2-carboxaldehyde. This reaction occurred at reflux within 13 h to yield *5,6,10,11,15b,15c-hexahydro-8-(pyrrol-2-yl)-2,3,13,14-tetramethoxy-8H-imidazo[5,1-a:4,3-a′]diisoquinoline* **2m** as a microcrystalline white solid (80 mg, 87%) mp 234–236 °C (EtOAc), R_f 0.75 (MeOH) (Found: C, 70.33; H, 6.67; N, 9.07. $C_{27}H_{31}N_3O_4$ requires: C, 70.26; H, 6.77; N, 9.10%). IR (KBr): 1605, 1514, 1455, 1345, 1262, 1226, 1123, 1017, 855, 716 cm^{-1}. UV (MeOH): 241, 283 nm. ^{1}H NMR ($CDCl_3$) *δ*: 2.45 (1H, m, H_β5), 2.48 (1H, m, H_α10), 2.73 (1H, m, H_α11), 2.83 (1H, m, H_β6), 2.88 (1H, m, H_β11), 2.89 (1H, m, H_β10), 2.95 (1H, m, H_α5), 2.96 (1H, m, H_α6), 3.63 (3H, s, 14-OMe), 3.69 (1H, d, *J* 9.0 Hz, H15b), 3.75 (3H, s, 2-OMe), 3.85 (3H, s, 13-OMe), 3.90 (3H, s, 3-OMe), 4.42 (1H, d, *J* 9.0 Hz, H15c), 5.22 (1H, s, H8), 6.19 (1H, s, H15), 6.25 (1H, m, H4′), 6.36 (1H, br s, H3′), 6.51 (1H, s, H1), 6.64 (1H, s, H12), 6.75 (1H, s, H4), 6.77 (1H, br s, H5′), 9.09 (1H, br s, NH); ^{13}C NMR ($CDCl_3$) *δ*: 25.5 (C5), 29.2 (C11), 41.3 (C10), 46.2 (C6), 55.7 (13-OMe), 55.8 (3-OMe), 55.9 (14-OMe), 56.0 (2-OMe), 63.8 (C15c), 69.9 (C15b), 79.0 (C8), 108.6 (C3′ or C4′), 108.7 (C3′ or C4′), 111.2 (C12), 111.5 (C15), 111.7 (C4), 112.7 (C1), 117.0 (C5′), 125.5 (C15a), 127.0 (C11a), 127.7 (C15d), 128.3 (C2′), 129.9 (C4a), 146.2 (C14), 146.6 (C2), 147.6 (C3), 148.0 (C13); Mass spectrum (EI): *m/z* 271 (M−190, 15%), 270 (100), 255 (21), 191 (12), 176 (18).

4.1.13. Reaction with pyridine-2-carboxaldehyde. This reaction occurred at reflux within 2 h to yield *5,6,10,11,15b,15c-hexahydro-8-(pyridin-2-yl)-2,3,13,14-tetramethoxy-8H-imidazo[5,1-a:4,3-a′]diisoquinoline* **2n** as tan needles (48 mg, 50%) mp 200–201 °C (acetone), R_f 0.58 (MeOH) (Found: C, 70.95; H, 6.47; N, 8.76. $C_{28}H_{31}N_3O_4$ requires: C, 71.02; H, 6.60; N, 8.87%). IR (KBr): 1607, 1513, 1460, 1336, 1255, 1224, 1126, 1017, 849, 788, 759 cm^{-1}. UV (MeOH): 242, 288 nm. ^{1}H NMR ($CDCl_3$) *δ*: 2.39 (1H, ddd, *J* 10.5, 5.3, 4.3 Hz, H_α10), 2.47 (1H, br d, *J* 14.7 Hz, H_β5), 2.73 (1H, ddd, *J* 15.8, 4.5, 3.8 Hz, H_α11), 2.83 (1H, m, H_β6), 2.84 (1H, m, H_β11), 2.92 (1H, m, H_α6), 2.93 (1H, m, H_β10), 3.00 (1H, m, H_α5), 3.65 (3H, s, 14-OMe), 3.74 (1H, d, *J* 9.0 Hz, H15b), 3.76 (3H, s, 2-OMe), 3.84 (3H, s, 13-OMe), 3.90 (3H, s, 3-OMe), 4.53 (1H, d, *J* 9.0 Hz, H15c), 5.13 (1H, s, H8), 6.29 (1H, s, H15), 6.59 (1H, m, H1), 6.62 (1H, s, H12), 6.76 (1H, s, H4), 7.26 (1H, ddd, *J* 7.5, 4.1, 1.1 Hz, H5′), 7.75 (1H, ddd, *J* 7.5, 7.5, 1.9 Hz, H4′), 7.82 (1H, d, *J* 7.5 Hz, H3′), 8.67 (1H, d, *J* 4.1 Hz, H6′); ^{13}C NMR ($CDCl_3$) *δ*: 25.8 (C5), 29.3 (C11), 41.9 (C10), 46.3 (C6), 55.8 (3-OMe), 55.7 (13-OMe), 55.9 (14-OMe), 56.1 (2-OMe), 64.7 (C15c), 70.1 (C15b), 86.1 (C8), 111.2 (C12), 111.3 (C15), 111.7 (C4), 112.9 (C1), 123.0 (C5′), 123.1 (C3′), 126.1 (C15a), 127.1 (C11a), 128.0 (C15d), 130.3 (C4a), 136.5 (C4′), 146.2 (C14), 146.6 (C2), 147.6 (C3), 147.9 (C13), 149.3 (C6′), 159.0 (C2′); Mass spectrum (EI): *m/z* 395 (M−78, 6%), 283 (18), 282 (100), 281 (68), 190 (20), 176 (12), 93 (18).

4.2. Kinetic studies of the condensation reactions of bis(tetrahydroisoquinoline) 1 with aldehydes

General procedure: A solution of aldehyde (0.0521 mmol) in $CDCl_3$ (0.2 mL) was added to an ice-cooled solution of bis(tetrahydroisoquinoline) **1** (20 mg, 0.0521 mmol) in $CDCl_3$ (0.5 mL) in a 5 mm diameter NMR tube. The NMR tube was immediately capped, shaken vigorously and inserted into the temperature-conditioned NMR probe. The composition of the mixture was analysed by ^{1}H NMR spectroscopy at regular intervals, and the integration of relevant signals and thus percentage composition or component abundance relative to residual chloroform as an internal standard was measured and calculated.

Relative reaction rates were initially assessed in a preliminary study and then measured under precisely defined conditions. For the fastest reaction, that with isobutyraldehyde, each datum point was collected using spectra acquired with one scan, an acquisition time of 2.6 s and a recycle

delay of 10 s. For the remaining slower reactions, each datum point was extracted from an experiment that was acquired using 16 scans with an acquisition time of 4.6 s and a recycle delay of 6 s.

Reaction at 273 K with:

(a) isobutyraldehyde gave unreacted bis(tetrahydroisoquinoline) **1** (δ 4.51, H1, H1′) and imidazolidine **2a** (δ 6.00, H15; 6.49, H1) in the integral ratio of 1.24:1.00 (85% conversion).
(b) 2-chlorobenzaldehyde gave unreacted bis(tetrahydroisoquinoline) **1** (δ 6.60, H5, H5′) and imidazolidine **2e** (δ 6.20, H15; 6.53, H1) in the integral ratio of 1.00:0.02 (2% conversion).
(c) 2,5-dimethoxybenzaldehyde gave unreacted bis(tetrahydroisoquinoline) **1** (δ 6.79, H8, H8′) but no imidazolidine **2h** (δ 6.15, H15; 6.51, H1) leaving the integral ratio of 1.00:0.00 (0% conversion).

4.3. Reductive cleavage of imidazolidines 2a, 2h and 2l

General procedure: Aqueous HCl (10 mL of 2 M/g solution of imidazolidine) was added dropwise to a suspension of imidazolidine and $NaBH_3CN$ (3 mol equiv) in absolute EtOH (100 mL/g of imidazolidine) at room temperature. The mixture immediately became clear and the solution was measured to have pH 1. The resultant mixture was stirred at ambient temperature for 1 h, then satd aq $NaHCO_3$ (20 mL/g of imidazolidine) was added dropwise followed by H_2O. The mixture was extracted with EtOAc, the extracts were dried over Na_2SO_4 and the solution was evaporated to dryness. Chromatography of the residue on alumina, eluting with a gradient of light petroleum, EtOAc and MeOH, afforded the major product.

4.3.1. Reductive cleavage of imidazolidine (2a). Imidazolidine **2a** (0.150 g, 0.342 mmol) afforded *2-isobutyl-6,6′,7,7′-tetramethoxy-1,1′-bis(1,2,3,4-tetrahydroisoquinoline)* **3a** as colourless needles (0.144 g, 95%) mp 130–131 °C (EtOAc), R_f 0.44 (EtOAc/Al_2O_3) (Found: C, 70.98; H, 8.33; N, 6.03. $C_{26}H_{36}N_2O_4$ requires: C, 70.88; H, 8.24; N, 6.36%). IR (KBr): 2954, 2835, 1608, 1518, 1466, 1360, 1262, 1226, 1116, 1032, 866, 776 cm^{-1}. UV (MeOH): 215, 285 nm. ^1H NMR ($CDCl_3$, 300 MHz) δ: 0.90 (3H, d, J 6.8 Hz, 2″-(CH_3)$_a$(CH_3)$_b$), 0.92 (3H, d, J 6.8 Hz, 2″-(CH_3)$_a$(CH_3)$_b$), 1.79 (1H, m, H2″), 2.21 (1H, dd, J 12.4, 7.5 Hz, H$_a$1″), 2.37 (1H, dd, J 12.4, 6.8 Hz, H$_b$1″), 2.56 (1H, m, H$_\beta$4), 2.73 (1H, ddd, J 15.8, 4.9, 4.5 Hz, H$_\alpha$4′), 2.83 (1H, m, H$_\beta$3), 2.88 (1H, m, H$_\alpha$4), 2.91 (1H, m, H$_\beta$4′), 3.02 (1H, ddd, J 12.0, 5.6, 4.5 Hz, H$_\alpha$3′), 3.27 (1H, ddd, J 12.0, 9.0, 4.9 Hz, H$_\beta$3′), 3.43 (1H, m, H$_\alpha$3), 3.45 (3H, s, 7-OMe), 3.47 (3H, s, 7′-OMe), 3.58 (1H, d, J 8.6 Hz, H1), 3.83 (3H, s, 6-OMe), 3.83 (3H, s, 6′-OMe), 3.95 (1H, d, J 8.6 Hz, H1′), 5.77 (1H, s, H8), 5.79 (1H, s, H8′), 6.58 (1H, s, H5), 6.59 (1H, s, H5′); ^{13}C NMR ($CDCl_3$, 75.6 MHz) δ: 20.3 (2″-(CH_3)$_a$(CH_3)$_b$), 20.9 (2″-(CH_3)$_a$(CH_3)$_b$), 23.1 (C4), 26.7 (C2″), 29.0 (C4′), 39.1 (C3′), 43.4 (C3), 55.2 (7-OMe), 55.3 (7′-OMe), 55.8 (6-OMe, 6′-OMe), 59.0 (C1′), 62.4 (C1″), 65.0 (C1), 111.2 (C5), 111.3 (C5′), 113.9 (C8), 114.3 (C8′), 126.4 (C8a), 126.9 (C8a′), 127.0 (C4a), 127.6 (C4a′), 145.4 (C7), 145.5 (C7′), 147.5 (C6), 147.6 (C6′); Mass spectrum (EI): m/z 441 (M^+, 0.1%), 365 (0.3), 249 (13), 248 (100), 206 (27), 191 (19), 176 (24), 41 (28).

4.3.2. Reductive cleavage of imidazolidine (2h). Imidazolidine **2h** (0.100 g, 0.188 mmol) afforded *2-(2,5-dimethoxybenzyl)-6,6′,7,7′-tetramethoxy-1,1′-bis(1,2,3,4-tetrahydroisoquinoline)* **3h** as colourless needles (0.96 g, 96%) mp 133.5–135 °C (EtOAc), R_f 0.38 (EtOAc/Al_2O_3) (Found: C, 69.73; H, 7.46; N, 4.93. $C_{31}H_{38}N_2O_6$ requires: C, 69.64; H, 7.16; N, 5.24%). IR (KBr): 2934, 2832, 2361, 1610, 1516, 1464, 1262, 1232, 1112, 1023, 862 cm^{-1}. UV (MeOH): 221, 289 nm. ^1H NMR ($CDCl_3$, 300 MHz) δ: 2.49 (1H, m, H$_\beta$4′), 2.61 (1H, m, H$_\alpha$4), 2.85 (3H, m, H$_\alpha$3′, H$_\beta$3′ and H$_\alpha$4′), 2.95 (1H, m, H$_\beta$3), 2.97 (1H, m, H$_\beta$4), 3.43 (3H, s, 7′-OMe), 3.45 (3H, s, 7-OMe), 3.46 (1H, m, H$_\alpha$3), 3.57 (1H, d, J 9.1 Hz, H1), 3.66 (1H, d, J 12.3 Hz, H$_a$1″), 3.73 (1H, d, J 12.3 Hz, H$_b$1″), 3.74 (3H, s, 2‴-OMe), 3.77 (3H, s, 5‴-OMe), 3.80 (3H, s, 6′-OMe), 3.85 (3H, s, 6-OMe), 4.09 (1H, d, J 9.1 Hz, H1′), 5.67 (1H, s, H8), 5.76 (1H, s, H8′), 6.54 (1H, s, H5′), 6.63 (1H, s, H5), 6.79 (1H, m, H6‴), 6.80 (1H, m, H3‴), 6.82 (1H, m, H4‴); ^{13}C NMR ($CDCl_3$, 75.6 MHz) δ: 23.0 (C4), 27.6 (C4′), 38.1 (C3′), 43.1 (C3), 52.7 (C1″), 55.2 (7′-OMe), 55.3 (7-OMe), 55.6 (2‴-OMe), 55.8 (6′-OMe, 5‴-OMe), 55.9 (6-OMe), 57.6 (C1′), 62.5 (C1), 111.1 (C5′), 111.3 (C5), 111.6 (C4‴), 112.7 (C3‴), 113.9 (C8′), 114.2 (C8), 117.4 (C6‴), 124.7 (C8a), 124.9 (C8a′), 126.7 (C4a′), 127.0 (C4a), 128.0 (C1‴), 145.6 (C7), 145.7 (C7′), 147.8 (C6′), 147.9 (C6), 152.3 (C2‴), 153.3 (C5‴); Mass spectrum (EI): m/z 535 (M^+, 0.3%), 343 (17), 342 (91), 192 (52), 176 (27), 164 (33), 151 (100), 121 (60), 91 (25), 77 (25).

4.3.3. Reductive cleavage of imidazolidine (2l). Imidazolidine **2l** (0.200 g, 0.357 mmol) afforded *2-[3-(2,5-dimethoxybenzyl)propyl]-6,6′,7,7′-tetramethoxy-1,1′-bis(1,2,3,4-tetrahydroisoquinoline)* **3l** as colourless needles (0.157 g, 78%) mp 83.5–85.5 °C (EtOAc), R_f 0.40 (EtOAc/Al_2O_3) (Found: C, 68.55; H, 7.56; N, 4.45. $C_{33}H_{42}N_2O_6\cdot$EtOAc requires: C, 68.28; H, 7.74; N, 4.30%). IR (KBr): 2934, 2831, 2324, 1610, 1515, 1464, 1261, 1223, 1115, 1026, 861 cm^{-1}. UV (MeOH): 216, 287 nm. ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.79 (2H, tt, J 7.5, 7.1 Hz, H2″), 2.56 (1H, m, H$_\beta$4), 2.58 (2H, m, H1″), 2.61 (2H, m, H3″), 2.74 (1H, m, H$_\alpha$4′), 2.86 (1H, m, H$_\beta$4′), 2.88 (1H, m, H$_\beta$3), 2.88 (1H, m, H$_\alpha$4), 3.01 (1H, ddd, J 12.2, 6.7, 5.3 Hz, H$_\alpha$3′), 3.29 (1H, ddd, J 12.2, 8.7, 4.9 Hz, H$_\beta$3′), 3.46 (1H, m, H$_\alpha$3), 3.46 (3H, s, 7′-OMe), 3.48 (3H, s, 7-OMe), 3.66 (1H, d, J 8.3 Hz, H1), 3.75 (3H, s, 5‴-OMe), 3.77 (3H, s, 2‴-OMe), 3.83 (6H, s, 6-OMe and 6′-OMe), 3.99 (1H, d, J 8.3 Hz, H1′), 5.78 (1H, s, H8), 5.80 (1H, s, H8′), 6.59 (1H, s, H5), 6.60 (1H, s, H5′), 6.68 (1H, dd, J 8.3, 3.0 Hz, H4‴), 6.70 (1H, d, J 3.0 Hz, H6‴), 6.76 (1H, d, J 8.3 Hz, H3‴); ^{13}C NMR ($CDCl_3$, 75.6 MHz) δ: 23.3 (C4), 27.9 (C3″), 28.2 (C2″), 28.7 (C4′), 39.1 (C3′), 43.6 (C3), 53.2 (C1″), 55.3 (7′-OMe), 55.4 (7-OMe), 55.6 (5‴-OMe), 55.82 (6′-OMe), 55.84 (2‴-OMe), 55.9 (6-OMe), 58.8 (C1′), 63.8 (C1), 110.7 (C4‴), 111.2 (C5), 111.3 (C5′, C3‴), 113.6 (C8′), 114.1 (C8), 116.2 (C6‴), 125.8 (C8a), 126.4 (C8a′), 126.9 (C4a′), 127.4 (C4a), 131.7 (C1‴), 145.6 (C7), 145.7 (C7′), 147.7 (C6, C6′), 151.7 (C5‴), 153.4 (C2‴); Mass spectrum (EI): m/z 563 (M^+, 0.25%), 371 (21), 370 (100), 206 (26), 192 (61), 176 (28), 121 (20), 91 (22), 77 (26), 43 (45).

4.4. 'One-pot' mono-alkylation of bis(tetrahydroisoquinoline) 1 with isobutyraldehyde

A solution of bis(tetrahydroisoquinoline) **1** (0.200 g, 0.521 mmol) and isobutyraldehyde (0.113 g, 1.562 mmol) in EtOH (20 mL) was stirred under argon at room temperature for 1 h. $NaBH_3CN$ (0.098 g, 1.562 mmol) and 2 M HCl (2.0 mL) were added sequentially, whereupon the white suspension immediately became clear. The resultant mixture was stirred at room temperature for 1 h, then satd aq $NaHCO_3$ (5 mL) was added dropwise followed by H_2O and EtOAc. The layers were separated and the aqueous layer was extracted with EtOAc (5×20 mL). The combined organic extracts were dried over Na_2SO_4 and the solution evaporated to dryness, to afford a white crystalline solid. Analysis of the crude material by ^1H NMR spectroscopy indicated 100% conversion to the mono-alkylated product. Flash chromatography on Al_2O_3, eluting with EtOAc, afforded *2-isobutyl-6,6′,7,7′-tetramethoxy-1,1′-bis(1,2,3,4-tetrahydroisoquinoline)* **3a** as a white crystalline solid (0.197 g, 86%), which was identical by ^1H NMR spectroscopy to the earlier product.

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Chemical constituents of *Ligularia virgaurea* and its diversity in southwestern Sichuan of China

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Abstract—Chemical constituents of root extract and the nucleotide sequences of the *atpB–rbcL* intergenic region and the internal transcribed spacers (ITSs) of the ribosomal RNA gene were studied for *Ligularia virgaurea* var. *virgaurea* collected in southwestern Sichuan Province of China. Eleven samples were collected. Four of them were found to contain four new furanoeremophilanes, virgaurenones A–D, as well as a new eremophilanolide, virgaurenolide A. The other samples contained different furanoeremophilanes and their derivatives including nor-type of compounds, two of which were new. Diversity was found to be present in the nucleotide sequences as well. The chemical composition was found to be correlated with the ITS variation but not with the geographic distribution of the samples.

1. Introduction

Ligularia Cass. (Compositae (or Asteraceae)) is a genus highly diversified in the eastern Qinghai–Tibet Plateau and adjacent areas, and more than 100 species are extant therein.[1] The genus is diversified particularly in the Hengduan Mountains and this area is considered to be the main center of the on-going evolution of the genus.[2] Therefore, *Ligularia* species in the Hengduan Mountains provide a good set of subjects for the study of plant evolution. We have been studying inter- and intra-specific diversity of *Ligularia* in the Hengduan Mountains by the combination of chemical and genetic approaches. As the chemical index, we have chosen furanoeremophilanes, since they have been found in *Ligularia*[3] as well as related genera such as *Farfugium*,[4] *Petasites*,[5] and *Syneilesis*,[6] and since the presence/absence of furanosesquiterpenes can be easily examined by Ehrlich's test on TLC.[7] As the genetic index, we have chosen the DNA sequences of the *atpB–rbcL* intergenic region in the plastid genome and the two internal transcribed spacers (ITSs) of the ribosomal RNA gene in the nuclear genome. The regions are non-coding and variations therein are thought to be neutral to evolution.[8] Hence, the regions are routinely analyzed in the studies of plant diversity and phylogeny.[8]

We have obtained the following findings so far. *Ligularia tongolensis* (Franch.) Hand.-Mazz., *Ligularia cymbulifera* (W.W. Smith) Hand.-Mazz., and *Ligularia atroviolacea* (Franch.) Hand.-Mazz., all belonging to the section Corymbosae, were close to one another with respect to the composition of furanoeremophilanes and the nucleotide sequence of the *atpB–rbcL* region.[9] However, while *L. tongolensis* was found to be diverse in both indices, *L. cymbulifera* was uniform in both. More recently, we have found that samples of *Ligularia pleurocaulis* (Franch.) Hand.-Mazz. of northwestern Yunnan and those in southwestern Sichuan were different.[10] 3β-Angeloyloxyfuranoeremophil-1(10)-en-6β-ol and furanoligularenone were the characteristic components, respectively, for the Yunnan and the Sichuan specimens. The *atpB–rbcL* sequence variation was not correlated with the chemical composition, but the ITS variation was.

Our present focus is on *Ligularia virgaurea* (Maxim.) Mattf. Both *L. virgaurea* and *L. pleurocaulis*, one of our previous subjects, belong to section Senecillis[11] and inhabit alpine meadows of more than 4000 m in altitude. While *L. pleurocaulis* is extant only in the Yunnan and the Sichuan Provinces, *L. virgaurea* is widely distributed in Yunnan, Sichuan, Qinghai, and Gansu in China, and in Nepal and

Keywords: *Ligularia virgaurea*; Sesquiterpene; Furanoeremophilane; *atpB–rbcL*; ITS; Diversity.
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Bhutan.[11] The morphological variation within the species is so large that three varieties, *virgaurea*, *pilosa*, and *oligocephala*, have been proposed.[11–13] Chemical constituents in the root of *L. virgaurea* collected in Qinghai and Gansu have been studied. Shi and co-workers obtained some eremophilanolides from var. *oligocephala* from Qinghai.[14] Jia and co-workers studied the chemical constituents of this species, presumably var. *virgaurea*, from Gansu,[15] and isolated several benzofuranes including cacalol, a rearranged carbon skeleton of eremophilane, and a methylated derivative of it. Here, we report that *L. virgaurea* var. *virgaurea* in southwestern Sichuan Province produces furanoeremophilanes and its derivatives, some of which are new. We also report that the plant can be grouped into two on the basis of the chemical composition and the ITS sequences.

2. Results

Eleven samples of *L. virgaurea* var. *virgaurea* were collected in southwestern Sichuan Province (Table 1 and Fig. 1). The roots of each sample were extracted with ethanol and the alcoholic solutions were subjected to Ehrlich's test on TLC plates. Two patterns of the Ehrlich-positive spots were observed. Four samples (samples 2, 5, 7, and 11; designated hereafter as type 1) showed two major components at R_f=0.45 and 0.53 (hexane–AcOEt=7/3) with yellow Ehrlich coloring, which implied the presence of furanoeremophilanes with some functional group. The other seven samples (samples 1, 3, 4, 6, 8, 9, and 10; type 2) showed several pink spots on TLC. The major spots appeared at R_f=0.54 and 0.59. The pattern was almost the same for all the seven samples, indicating that their composition of furanoeremophilanes was similar. The distribution of the two types did not coincide with the geographical distribution of the samples (Table 1 and Fig. 1).

Furanoeremophilanes and the related compounds were isolated and their structures were determined. The two components observed for the type-1 samples were purified and identified as new compounds, **1** and **2**. These two compounds were the major constituents in the type-1 samples (**1**: R_f=0.53, **2**: R_f=0.45). Related new enones **3** and **4** as well as a new lactone **5** and a known lactone **6**[14] were also

Table 1. Collection locality and *atpB–rbcL* genetic type of *L. virgaurea* samples

Sample	Locality	Elevation (m)	Ehrlich type[a]	*atpB–rbcL*	ITS clade[b]
1	Xiangcheng	4100, 4300[c]	2	G-A11	A
2	Wumingshan	4000	1	G-A10	B
3	Yading	4000	2	G-A9	A
4	Daocheng	4100	2	G-A11	A
5	Daocheng	4400	1	G-A10	B
6	Haizishan	4300	2	G-A11	A
7	Haizishan	4400	1	G-A10	B
8	Jiawa	3800	2	G-A11	A
9	Litang	4100	2	G-A12	A
10	Honglong	4300	2	G-A11	A
11	Gaoersishan	4200	1	A-A10, 344G	B

[a] Type 1=yellow color; type 2=pink color. See text.
[b] See Table 4.
[c] Collected at two locations close to each other. The chemical components were judged to be the same based on TLC. The DNA sequences were the same.

Figure 1. Locations where samples of *L. virgaurea* var. *virgaurea* (open squares) were collected. Filled triangles and double circles indicate major peaks and major cities, respectively.

isolated from the type-1 samples as minor constituents. The Ehrlich-positive components in the type-2 samples included four furanoeremophilanes: ligularol (=petasalbin, **7**),[3a,16] its methyl ether **8**,[5b] ethyl ether **9**,[7,17] and furanoeremophilane-6β,10β-diol (**10**).[3b] Compounds **7** (R_f=0.54) and **8/9** (R_f=0.59) were the major Ehrlich-positive components. Compound **9** is considered to be an artifact generated during the ethanol extraction process. Eight non-furano types of compounds **11–18** were also isolated from these samples, among which **11** and **14** were new. Compounds **12**[18] and **13**[19] had been isolated from *Petasites japonicus* subsp. *giganteus*. Although compound **15** has not been reported, this compound seems to be an artifact during ethanol extraction.[‖] Compounds **16**,[3g] **17**,[20] and **18**[21] had been known. The structures of the seven new compounds, **1–5**, **11**, and **14**, were determined as follows (Charts 1 and 2).

The molecular formula of compound **1**, named as virgaurenone A, was determined as $C_{20}H_{24}O_4$ by HRCIMS. The IR spectrum indicated the presence of two carbonyl moieties (1720, 1680 cm^{-1}). ^1H and ^{13}C NMR data are shown in Tables 2 and 3, respectively. The ^{13}C NMR spectrum gave 20 peaks including 10 sp^2 carbons, two of which were due to carbonyl groups (δ 196.8, 167.1). The resonance at δ 6.84 was assigned to the α proton of a tri-substituted furan ring, which was supported by four signals at δ 118.3 (C), 120.1 (C), 139.6 (CH), and 148.6 (C). The substitution of an angelate group was suggested by the signals of methyl groups at δ 1.98 (dq) and 1.82 (quint), and carbon signals at δ 167.1 (CO), 128.5 (C), 140.2 (CH), 20.7 (Me), and 15.9 (Me). The HMBC spectrum showed correlations from the methyl group at C-4 into C-3, C-4, and C-5, from the methyl group at C-5 into C-4, C-5, C-6, and C-10, and from the methyl group at C-11 into C-7, C-11, and C-12. With the results of a COSY experiment, this compound was established to possess the eremophilane skeleton and

[‖] Although compound **15** seems to be an artifact, the data were presented in Section 5, because a search for this compound with SciFinder made no hit and we considered the data to be worth inclusion for future comparison.

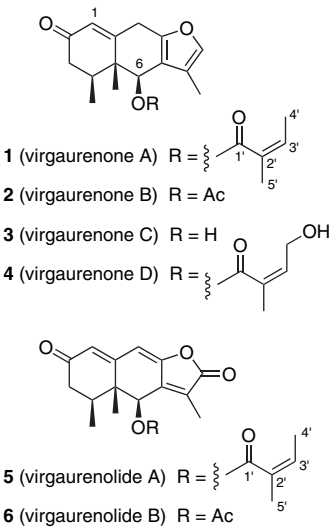

Chart 1. Chemical constituents of type-1 samples of *L. virgaurea* var. *virgaurea*.

the angelate moiety at C-6. The stereochemistry was established as shown in the formula, since NOE was detected between H-6 and H-4, and between H-9β and H-14.

The ^{1}H NMR spectrum of compound **2**, virgaurenone B, $C_{17}H_{20}O_4$, was very similar to that of compound **1** except for the presence of an acetyl group and the absence of an angeloyl group (Table 2). The IR spectrum indicated the presence of carbonyl groups (1730, 1680 cm^{-1}). Because a singlet proton at δ 6.18 had a correlation into C-1′, an acetoxy group (δ 1.70) was determined to be substituted at C-6. The enone moiety (1(10)-en-2-one) was present as in **1**, which was confirmed by the 2D experiment. The stereochemistry was determined by the NOESY spectrum. Therefore, the structure was established as depicted in the formula. The sample nicely crystallized from EtOAc and the structure was solved by X-ray crystallography to support the structure deduced as above (Fig. 2) (see Section 5).

Compound **3** (virgaurenone C) showed a quasi-molecular ion peak at *m*/*z* 247 (CIMS) and an absorption of a hydroxy group at 3440 cm^{-1} in its IR spectrum. The ^{13}C NMR spectrum displayed 15 peaks including a carbonyl group (δ 197.0, Table 3). These data implied that compound **3** should contain the basic terpene part of both virgaurenones A and B.

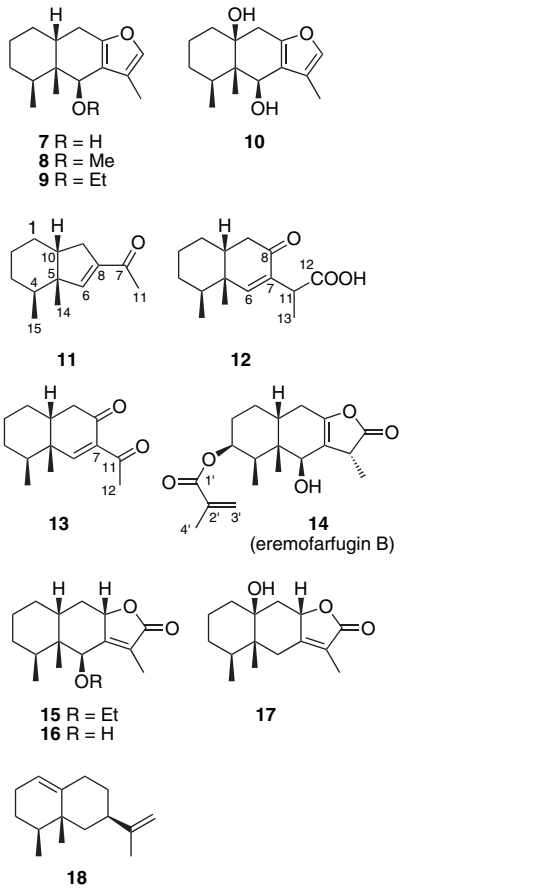

Chart 2. Chemical constituents of type-2 samples of *L. virgaurea* var. *virgaurea*.

Actually, 2D NMR, especially HMBC, supported this assumption. The NOESY spectrum confirmed the stereochemistry as depicted in the formula, since correlations between H-6 and H-4, and between H-9β and H-14 were observed.

All CD spectra of **1**–**3**, indicated positive absorptions around 240 nm, which were due to π–π* transitions in α,β-unsaturated enone systems.[22] Thus, the absolute configurations of these compounds should be as depicted in the formula, since the conformation of each molecule was indicated by the NOESY spectra and the helicity attributed to this system must be clockwise.

Table 2. ^{1}H NMR data of compounds **1**–**5** (600 MHz, C_6D_6)

No.	**1**		**2**		**3**		**4**		**5**	
1	5.69	d, 1.8	5.68	d, 1.2	5.69	d, 1.6	5.67	d, 1.9	5.735	s
3α	2.71	dd, 16.2, 4.5	2.69	dd, 16.2, 5.4	2.25	dd, 15.9, 4.4	2.66	dd, 16.0, 4.7	2.02	ddd, 17.4, 4.4, 1.1
3β	2.10	dd, 16.2, 4.5	2.12	dd, 16.2, 4.2	2.02	dd, 15.9, 6.6	2.09	dd, 16.0, 4.1	1.93	dd, 17.4, 13.4
4	1.94	m	1.91	m	1.94	m	1.87	m	1.78	dqd, 13.4, 6.6, 4.4
6	6.34	dq, 2.4, 1.2	6.18	dq, 2.4, 1.2	4.31	br d, 6.6	6.25	dq, 2.5, 1.4	5.86	q, 1.9
9α	2.73	d, 17.4	2.71	d, 17.6	2.71	d, 17.6	2.70	d, 17.3	5.30	s
9β	3.12	br d, 17.4	3.10	br d, 17.6	3.08	d, 17.6	3.08	br d, 17.3	—	
12	6.84	s	6.85	s	6.87	s	6.81	s	—	
13	1.78	d, 1.2	1.72	d, 1.2	1.93	d, 1.4	1.71	d, 1.4	1.56	d, 1.9
14	0.90	s	0.87	s	0.65	s	0.85	s	0.74	s
15	0.70	d, 6.6	0.72	d, 7.2	0.83	d, 7.1	0.68	d, 6.8	0.57	d, 6.6
2′	—		1.70	s	—		—		—	
3′	5.78	qq, 7.8, 1.8	—		—		6.05	tq, 5.2, 1.6	5.740	qq, 7.1, 1.4
4′	1.98	dq, 6.6, 1.2	—		—		4.49	m	1.89	dq, 7.1, 1.4
5′	1.82	quint, 1.2	—		—		1.74	q, 1.6	1.68	quint, 1.4

Table 3. ^{13}C NMR data of compounds **1–5** (150 MHz, C_6D_6)

No.	1	2	3	4	5
1	126.9	126.9	126.5	126.9	129.1
2	196.8	196.9	197.0	196.7	195.4
3	42.7	42.7	42.8	42.6	43.5
4	33.9	33.9	34.9	33.9	38.0
5	44.4	44.3	45.0	44.3	44.3
6	69.7	70.2	71.1	70.4	73.1
7	118.3	118.0	120.6	117.9	143.7
8	148.6	148.6	147.7	148.7	151.2
9	31.4	31.4	31.2	31.3	107.0
10	156.0	156.1	158.9	155.8	157.4
11	120.1	120.0	120.3	119.9	125.5
12	139.6	139.6	139.3	139.7	169.1
13	8.7	8.6	9.2	8.6	8.4
14	17.2	16.9	14.9	17.1	13.8
15	16.0	15.9	16.5	15.9	16.9
1′	167.1	170.2	—	167.0	166.0
2′	128.2	20.4	—	126.5	126.4
3′	140.2	—	—	147.4	142.8
4′	15.9	—	—	61.0	16.0
5′	20.7	—	—	19.8	20.3

The molecular formula of compound **4** (virgaurenone D), determined as $C_{20}H_{24}O_5$, indicated that a C_5 ester should be attached to the eremophilane skeleton, since the ^{1}H NMR spectrum of **4** was very similar to those of **1**, **2**, and **3** (see Table 2). From careful analysis of the HMBC spectrum, it was inferred that the terpene part was the same as that of **3**, but the ester part was not an angelate. The methyl group at δ 1.74 (H-5′) had correlations into C-1′, C-2′, and C-3′. The proton at δ 6.05 (H-3′) was coupled with the protons at δ 4.49 (2H, H-4′). Therefore, this ester should be 4-hydroxy-2-methyl-2-butenoate and the whole structure was established as depicted in the formula, including the stereochemistry.

The molecular formula of virgaurenolide A (**5**) was $C_{20}H_{22}O_5$ and the degree of unsaturation was 10. The ^{13}C NMR spectrum indicated 11 sp^2 carbons, three of which were due to carbonyl groups. The IR spectrum showed absorptions of a lactone (1780 cm^{-1}), an ester (1720 cm^{-1}), and a carbonyl (1660 cm^{-1}) group. The HMBC spectrum clearly indicated the presence of a 1(10)-en-2-one moiety, which was further conjugated to the lactone ring. Therefore, the HMBC spectrum indicated that the basic skeleton must be 2-oxoeremophil-1(10),7(11),8-triene-12,8-olide. This

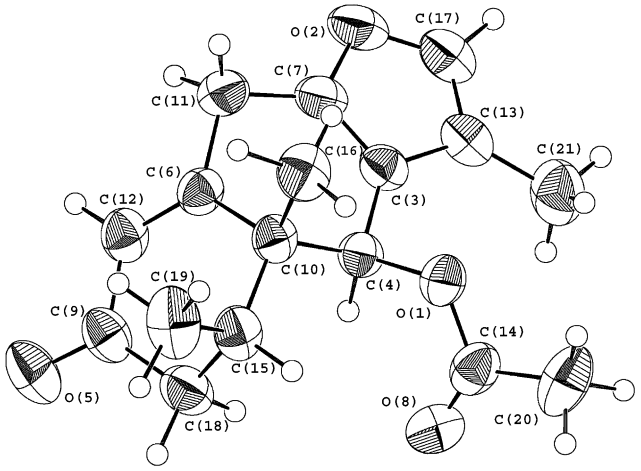

Figure 2. The X-ray crystal structure of **2**.

was presumably produced from the corresponding furanoeremophilane (**1**) by oxidation in the plant. The stereochemistry was determined as depicted in the formula, since NOE was observed between H-6 and H-4. The acetylated compound **6** (virgaurenolide B) had been isolated recently from *L. virgaurea* var. *oligocephala*.[14]

Compound **11** showed a molecular ion peak at *m*/*z* 192, whose molecular formula was calculated as $C_{13}H_{20}O$ by HRMS. The ^{13}C NMR spectrum indicated the presence of a carbonyl group (δ 195.6), which was supported by the absorption at 1670 cm^{-1} in the IR spectrum. An acetyl group was detected at δ 1.97 (3H, s) in the ^{1}H NMR spectrum. The ^{1}H NMR spectrum also showed the presence of a *tert*-methyl and a *sec*-methyl group as well as one proton absorption at δ 6.30 assignable to an olefinic proton. Since the total number of ^{13}C NMR signals was 13 and the degree of unsaturation was 4, this compound was judged to be a bicyclic dinor-sesquiterpene. The HMBC spectrum indicated correlations from the methyl group at C-5 into C-4, C-5, C-6, and C-10. The methyl group at C-4 had correlations into C-3, C-4, and C-5. These results indicated a dinor-eremophilane skeleton. The sequence of protons of H-4, H-3, H-2, H-1, and H-10 were indicated by the COSY spectrum. NOE was detected between H-10 and H-14 and indicated that two carbocycles were cis-fused. Thus, the 12,13-dinor-eremophilane skeleton was established. Similar compounds with an oxygen functionality at C-3 had been known.[5e]

Compound **14** indicated a characteristic absorption at 1800 cm^{-1} in its IR spectrum, suggesting the presence of an enol γ-lactone as precedented in eremofarfugin A.[5c,5d] The molecular formula was determined to be $C_{19}H_{26}O_5$ by HRMS. Both the ^{1}H and the ^{13}C NMR spectra obtained at room temperature showed very broad peaks and were not analyzable. Therefore, spectra were obtained at 50 °C in C_6D_6 and analyzed. The HMBC spectrum indicated correlations from the methyl group at C-5 to C-4, C-5, C-6, and C-10, from the methyl group at C-4 to C-3, C-4, and C-5, and from the methyl group at C-11 to C-7, C-11, and C-12. A hydroxy group (3500 cm^{-1}) must be at C-6 (δ 65.3), while an acyl group should be at C-3 (δ 72.3), because H-3 (δ 5.19) had a correlation into C-1′ (δ 166.5). The acyl group was an α-methylacrylate, because a methyl group at C-2′ had correlations into C-1′, C-2′, and C-3′ in the HMBC spectrum. The stereochemistry was determined as depicted in the formula by the NOESY spectrum. One of the authors previously isolated eremofarfugin A from *Farfugium japonicum*, which had an angelate group substituted at C-3 instead of α-methylacrylate.[3f] Compound **14** lacks one methyl group of eremofarfugin A and was named as eremofarfugin B.

Purification of DNA from the samples, the amplification of the *atpB–rbcL* region, and the determination of the base sequence of the region, consisting of about 740 base-pairs, were carried out as described previously.[9,10] Five variants, G-A9, G-A10, G-A11, G-A12, and A-A10, 344G, according to our designation,[††] were identified (Table 1). The base

[††] The 28th base was A or G and the number of an A stretch around the 510th base was 9, 10, 11, or 12. The variant type was designated by the combination of A/G and A9/A10/A11/A12, such as G-A12; 344G denotes the 344th position to be G in place of T in the other samples. See Ref. 9.

```
ITS1
TCGAAACCTGCATAGCAGAACGACCCGTGAACATGTAACAACAATCGGGTGTCCTTGGTA  60
TCGGGCTCTTGTTCGATTAATTGGATGCCTTGTCGATGTGCGTCTTTGGTCAGCCCTTTG 120
GGTCCTAAGGACGTCACATTGGCGCAACAACAAACCCCCGGCACGGCATGTGCCAAGGAA 180
AATTAAACTTAAGAAGGGCTTGTACCATGCTTCCCCGTTTGCGGGGTTTGCATGGGACGT 240
GGCTTCTTTATAATCA                                              256

ITS2
ATCGCGTCGCCCCCACCACGCCTCCTCGATGAGRATGCTTGGATGTGGGCGGAGATTGGT  60
CTCCCGTTCCTAYGGTGCGGTTGGCTAAAACAGGAGTCCCCTTCGACGGACGCACGATTA 120
GTGGTGGTTGACAAGACCCTCTTATCAAGTTGTGCGTTCTAAGGAGCAAGGAATGTCTCT 180
TCAATGACCCCAATGTGTCGTCCTGTGACGATGCTTCGACC                     221
```

Figure 3. The nucleotide sequences of ITS1 and ITS2 of sample 5. They have been deposited to DDBJ/EMBL/GenBank together with the sequence of the 5.8S rRNA region between the ITSs and sequences flanking the ITSs (accession number AB245093).

sequences of the ITSs of the ribosomal RNA gene were also determined as previously (Fig. 3).[10] Multiple bases were observed at some positions, as thousands of copies of the gene are present in a cell and there can be variations among the copies.[8] Sequence variation among the samples was observed at a number of base positions, as summarized in Table 4. Analysis of the variation using the PHYLIP program package[23] indicated that the samples can be grouped into two clades, tentatively designated A and B in Tables 1 and 4, with a bootstrap value of 95.5% at the branch of the clades.

3. Discussion

In the present study, *L. virgaurea* was examined with respect to the chemical composition and the nucleotide sequences of the *atpB–rbcL* region and the ITSs of the ribosomal RNA gene. Seven new compounds, virgaurenones A–D (**1**–**4**), virgaurenolide A (**5**), compound **11**, and eremofarfugin B (**14**), were obtained together with several known compounds. All the isolated compounds had the eremophilane or a related skeleton: Compounds **1**–**4** and **7**–**10** were furanoeremophilanes, and **5**, **6**, **12**, **14**–**17** were their oxidized compounds; compound **13** had only 14 carbons and was considered to be a decarboxylated product of **12**; compound **11** was presumably a decarbonylatively ring-contracted product of **13**. Since eremophilanolides and related benzofurans have been obtained from *L. virgaurea* samples collected in Qinghai[14] and in Gansu,[15] production of eremophilanes must be common in this species. The present results showed that furanoeremophilanes are also produced by this species.

The presence of intra-specific variation was apparent. Four of the 11 collected samples (type 1) contained virgaurenones A–D (**1**–**4**) and their oxidized compounds (**5** and **6**). In contrast, these compounds were absent in the other seven samples (type 2). Petasalbin methyl and ethyl ethers (**8** and **9**) and 8-oxoeremophil-6-en-12-oic acid (**12**) were common to these samples. Types 1 and 2 can be considered quite different from each other, since no common components were isolated from both the types. This contrasts with our previous observation with *L. pleurocaulis*, in which two types had several compounds in common.[10] Interestingly, all compounds isolated from the type-1 samples had a ketone group at C-2 position. Virgaurenones A–D are the first examples of furanoeremophilanes having the 1(10)-en-2-one system, in which the hydrogen at 9-position is highly acidic.

Intra-specific variation was also observed in nucleotide sequences, both in the *atpB–rbcL* region and in the ITSs. Although no clear correlation was observed between the chemical composition and the *atpB–rbcL* sequence, grouping based on the composition (types 1 and 2) and that based on the ITS sequences (clades A and B) agreed (Table 1). We have reported a similar finding for *L. pleurocaulis*, as mentioned in Section 1.[10] The present agreement further supports our previous interpretation[10] that the chemical–genetic correlation is stronger for the ITSs of the rRNA gene because the terpene-related genes and the rRNA gene are encoded in the nuclear genome. Thus the chemical diversity in *L. virgaurea* seems to result from a difference(s) of a genetic character, but not from an environmental difference(s).

Interestingly, classification of the *L. virgaurea* samples on the basis of the composition type and the ITS clade showed no geographic division, while the chemical–genetic grouping of *L. pleurocaulis* agreed with geographic division.[10] Within the Daocheng/Litang area where we collected samples of both species, *L. pleurocaulis* was found to be almost uniform,[10] whereas *L. virgaurea* was diverse. It is also noteworthy that *atpB–rbcL* sequence of sample 11 is rather different from the others. This fact and the difference of the

Table 4. Variations in ITS1 and ITS2 in *L. virgaurea* var. *virgaurea*

Sample	Base position																							Clade
	ITS1											ITS2												
	11	65	66	74	124	130	132	134	135	235	240	32	34	73	84	91	162	168	175	179	188	217	220	
1	C	A	C	C	C	R	Y	Y	Y	S	C	G	A	T	R	C	A	A	A	A	C	C	C	A
3	Y	R	C	Y	C	R	C	T	Y	G	Y	G	A	T	R	M	A	A	R	A	Y	C	C	A
4	Y	A	C	Y	C	R	C	T	Y	G	Y	G	A	T	R	C	A	A	A	A	C	C	C	A
6	Y	A	C	Y	C	R	Y	T	Y	G	Y	G	A	T	R	C	A	A	A	A	C	C	C	A
8	C	A	C	Y	C	R	C	T	Y	G	Y	G	A	T	R	C	A	A	A	A	C	C	C	A
9	C	A	C	Y	C	R	C	T	Y	G	Y	G	A	T	R	C	A	A	A	A	C	C	C	A
10	C	A	C	Y	C	R	C	T	Y	G	C	G	A	T	R	C	A	A	A	A	C	C	C	A
2	C	G	Y	C	C	G	C	T	C	G	T	A	R	Y	G	C	A	W	G	C	C	Y	Y	B
5	C	G	C	C	C	G	C	T	C	G	T	A	R	Y	G	C	A	A	G	C	C	C	C	B
7	C	G	C	C	Y	G	C	T	C	G	T	A	R	Y	G	C	A	A	G	C	C	Y	C	B
11	C	G	C	C	C	G	C	T	C	G	T	A	A	C	G	C	W	W	G	C	C	C	C	B

The base numbering is for each ITS region as in Figure 2. All the multiplicities are listed irrespective of the relative occurrences of the two bases. Y=C+T; R=A+G; M=A+C; S=C+G; W=A+T.

chemical composition between a previous[15] and the present studies suggest that *L. virgaurea* var. *virgaurea* may harbor further diversity outside the areas of the current survey.

4. Conclusion

Seven new compounds were isolated from *L. virgaurea* var. *virgaurea* collected in southwestern Sichuan Province of China, and their structures were established. Four of them, virgaurenones A–D were the first examples of the furanoeremophilane having a 1(10)-en-2-one moiety. The presence of diversity in the species was demonstrated. The samples could be classified into two groups on the bases of their composition of the extracted compounds. The base variations in the ITSs also showed that the samples can be grouped into two, and this grouping was in concord with the chemical one. These results suggest that the chemical difference has a genetic origin.

5. Experimental

5.1. General

Specific rotations and CD spectra were measured on a JASCO DIP-1000 and a JASCO J-725 auto recording polarimeter; IR spectra, on a JASCO FT/IR-5300 spectrophotometer; ^{1}H and ^{13}C NMR spectra, on a Varian Unity 600 (600 MHz and 150 MHz, respectively) and a JEOL ECP 400 (400 MHz and 100 MHz, respectively) spectrometer. Mass spectra, including high-resolution ones, were recorded on a JEOL JMS-700 MStation. X-ray crystallographic analysis was carried out on a Mac Science MXC 18 diffractometer using a DIP image plate. Chemcopak Nucleosil 50-5 (4.6×250 mm) with a solvent system of hexane–ethyl acetate was used for HPLC (a JASCO pump system). Silica gel 60 (70–230 mesh, Fuji Sylisia) was used for column chromatography. Silica gel 60 F_{254} plates (Merck) were used for TLC. Determination of the DNA sequences of the *atpB–rbcL* intergenic region and the ITSs was carried out as described previously.[9,10]

5.2. Plant materials

Samples of *L. virgaurea* var. *virgaurea* were collected in August 2003 and 2005 at 11 locations (Table 1 and Fig. 1). Samples 2, 3, 4, and 6 were collected in 2003, and samples 1, 5, and 7–11 were collected in 2005. Each plant was identified by Xun Gong, one of the authors.

5.3. Ehrlich's test

The root of each plant (about 10 g) was harvested and extraction with ethanol was started immediately without drying. Extraction was continued at room temperature for several days. After filtering and without concentrating, extracts were subjected to TLC (Kieselgel 60 F_{254}, layer thickness 0.2 mm) using hexane–ethyl acetate (7:3) as the solvent. The TLC plate was dipped in a 1% solution of *p*-dimethylaminobenzaldehyde in ethanol. The plate was dried and then dipped in a 1 M solution of hydrochloric acid in aqueous ethanol and the resultant coloring was photographed.

5.4. Extraction, purification, and structure determination

For the samples collected in 2003, the collected roots of *L. virgaurea* were cut into small pieces without drying, and immediately extracted with EtOH at room temperature. The extract was filtered and concentrated to afford an oily residue with an aqueous phase. AcOEt was added to this oil/aqueous mixture and the organic layer was recovered. Evaporation of the solvent afforded an oily residue, to which water soluble starch was added for handling purpose. For the samples collected in 2005, the roots were dried and extracted with AcOEt at room temperature. Oily extracts were obtained by the standard method.

Components of the extract of sample 2 (1.98 g with starch, extracted from 65 g of fresh root) were separated by silica gel (50 g) column chromatography (hexane–EtOAc, in gradient) to give **1** (46.1 mg) and **2** (37.5 mg). Compound **2** was recrystallized from EtOAc to give rectangular crystals.

Components of the extract of sample 6 (5.4 g with starch, extracted from 95 g of fresh root) were separated by silica gel column chromatography (hexane–EtOAc, in gradient) to give five fractions. The second fraction (29 mg) was further subjected to HPLC (Nucleosil 50-5, 4.6×250 mm, 1 mL/min, hexane–EtOAc (95:5)) to afford **9** (3.6 mg), **8** (4.7 mg), and **11** (6.6 mg). The fourth fraction (69.7 mg) was subjected to silica gel column chromatography (hexane–EtOAc, in gradient) followed by HPLC (Nucleosil 50-5, 4.6×250 mm, 3 mL/min, hexane–EtOAc (97:3)) to give **13** (2.1 mg)[19] and **15** (1.3 mg). The fifth fraction (831 mg) was further subjected to silica gel column chromatography ($CHCl_3$–EtOAc, in gradient) to give **12** (140.8 mg).[18]

Components of the extract of sample 3 (4.3 g with starch, extracted from 120 g of fresh root) were separated by silica gel column chromatography (hexane–EtOAc, in gradient) to afford 10 fractions. The first fraction (102.1 mg), further separated by HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (95:5)), contained petasalbin methyl ether (**8**) (45.5 mg) and ethyl ether (**9**) (41.4 mg). The seventh fraction (59 mg) was subjected to silica gel column chromatography (hexane–EtOAc, in gradient) followed by HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (80:20)) to give **10** (4.6 mg),[3b] **14** (1.1 mg), and **13** (1.0 mg).[19] The ninth fraction (54.6 mg) was subjected to HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (80:20)) to afford **14** (4.6 mg). The 10th fraction (326.4 mg) was subjected to silica gel column chromatography ($CHCl_3$–EtOAc, in gradient) to give **12** (39.5 mg).

The extract of sample 4 (1 g with starch, extracted from 45 g of fresh root) was subjected to silica gel column chromatography (hexane–EtOAc, in gradient) and the resulting 10 fractions were further subjected to a combination of column chromatography and HPLC. However, the compounds were very labile and no compound was isolated in pure. Eremophila-1(10),11-diene (**18**) alone was detected by GC–MS.

Components of the extract of sample 5 (818 mg, extracted from 11 g of fresh root) were separated by silica gel column

chromatography (hexane–EtOAc, in gradient) to afford six fractions. The second fraction was virgaurenone A (**1**) (190.9 mg) and the third fraction was virgaurenone B (**2**) (195.6 mg). The fourth fraction (19.3 mg) was subjected to HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (80:20)) to give virgaurenolide A (**5**) (4.3 mg). The fifth fraction (17.8 mg) was subjected to HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (80:20)) to afford **6** (5.0 mg).[14]

Components of the extract of sample 7 (464 mg, extracted from 5 g of fresh root) were separated by silica gel column chromatography (hexane–EtOAc, in gradient) to afford seven fractions. The second fraction was virgaurenone A (**1**) (50.4 mg) and the fourth fraction was virgaurenone B (**2**) (79.6 mg). The third fraction (11.3 mg) was further separated by HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (9:1)) to afford **1** (1.0 mg) and **2** (2.3 mg).

Components of the extract of sample 8 (699 mg, extracted from 5 g of fresh root) were separated by silica gel column chromatography (hexane–EtOAc, in gradient) to afford seven fractions. The third fraction was ligularol (**7**) (17.0 mg). The sixth fraction (62.2 mg) was purified by HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (80:20)) to afford **16** (1.3 mg). The seventh fraction (263 mg) was further separated by silica gel column chromatography ($CHCl_3$–EtOAc, in gradient) to give **17** (24.1 mg).[20]

Components of the extract of sample 10 (1.3 g, extracted from 13 g of fresh root) were separated by silica gel column chromatography (hexane–EtOAc, in gradient) to afford seven fractions. The second (39.3 mg) and third (21.8 mg) fractions were purified by HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (95:5)) to afford ligularol (**7**) (8.2 mg). The fifth fraction (95.7 mg) was further separated by HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (80:20)) to afford **16** (2.5 mg) and **17** (19.7 mg). The seventh fraction (158 mg) was separated by HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (80:20)) to give **12** (6.7 mg).

Components of the extract of sample 11 (1.3 g, extracted from 15 g of fresh root) were separated by silica gel column chromatography (hexane–EtOAc, in gradient) to afford nine fractions. The fourth fraction was virgaurenone A (**1**) (160.4 mg) and the fifth fraction was virgaurenone B (**2**) (155.4 mg). The eighth fraction (24.4 mg) was purified by HPLC (Nucleosil 50-5, 4.6×250 mm, 2 mL/min, hexane–EtOAc (70:30)) to afford virgaurenone C (**3**) (5.8 mg), **6** (0.4 mg), and virgaurenone D (**4**) (8.9 mg).

5.4.1. 6β-Angeloyloxyfuranoeremophil-1(10)-en-2-one (1). A pale yellow oil; $[\alpha]_D^{20}$ +19.0 (*c* 1.15, EtOH); MS (CI) *m/z* 329 $[M+H]^+$, 271, 269, 229 (base), 228, 186, 83; HRMS (CI) obsd 329.1749 $[M+H]^+$ Calcd for $C_{20}H_{25}O_4$ 329.1753; FTIR (KBr) 1720, 1680, 1620 cm^{-1}; CD $[\theta]_{243\ nm}$ +18,600; see Tables 2 and 3 for ^1H and ^{13}C NMR, respectively.

5.4.2. 6β-Acetoxyfuranoeremophil-1(10)-en-2-one (2). Pale yellow rectangular crystals; mp 142.5–143.5 (from EtOAc); Analysis obsd C: 70.62, H: 7.08%, Calcd for $C_{17}H_{20}O_4$ C: 70.81, H: 6.99%; $[\alpha]_D^{19}$ −7.79 (*c* 0.94, EtOH); MS (CI) *m/z* 289 $[M+H]^+$, 246, 229 (base), 213, 186, 175, 123; HRMS (CI) obsd 289.1434 $[M+H]^+$ Calcd for $C_{17}H_{21}O_4$ 289.1440; FTIR (KBr) 1740, 1680, 1620 cm^{-1}; CD (EtOH) $[\theta]_{214\ nm}$ −17,900, $[\theta]_{238\ nm}$ +25,700; see Tables 2 and 3 for ^1H and ^{13}C NMR, respectively. X-ray data: orthorhombic, $P2_12_12_1$, a=8.9140 (3) Å, b=12.4900 (5) Å, c=13.9270 (4)Å, α=90.00°, β=90.00°, γ=90.00°, V=1550.57 (9) $Å^3$, Z=4; Mo Kα radiation, λ=0.71073; 2849 measured reflections, 2840 independent reflections, 2764 observed reflections, θ_{max}=25.73°; refinement on F^2, full matrix least-squares refinement, R(all)=0.0459, wR(ref)=0.1162, S(ref)=1.161, 2840 reflections; extinction correction, *SHELXL*, extinction coefficient=0.113 (8). Crystallographic data for compound **2** have been deposited at the Cambridge Crystallographic Data Center as supplementary publication number CCDC 295246. Copies of the data can be obtained, free of charge, via www.ccdc.cam.ac.uk/data_request/cif, or by mailing to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223 336033 or e-mail: data_request@ccdc.cam.ac.uk].

5.4.3. 6β-Hydroxyfuranoeremophil-1(10)-en-2-one (3). A pale yellow oil; $[\alpha]_D^{21}$ −24.3 (*c* 0.58, EtOH); MS (CI) *m/z* 247 $[M+H]^+$, 229 (base); HRMS (CI) obsd 247.1328 $[M+H]^+$, Calcd for $C_{15}H_{19}O_3$ 247.1334; FTIR (KBr) 3440, 1670, 1620 cm^{-1}; CD (EtOH) $[\theta]_{222\ nm}$ −10,000, $[\theta]_{248\ nm}$ +11,500, $[\theta]_{320\ nm}$+3600; see Tables 2 and 3 for ^1H and ^{13}C NMR, respectively.

5.4.4. 6β-(*Z*-4′-Hydroxy-2-methyl-2-butenoyl)oxyfuranoeremophil-1(10)-en-2-one (4). A pale yellow oil; $[\alpha]_D^{20}$ −7.1 (*c* 0.89, EtOH); MS (FAB) *m/z* 345 $[M+H]^+$, 154 (base), 136; HRMS (FAB) obsd 345.1722 $[M+H]^+$, Calcd for $C_{20}H_{25}O_5$ 345.1702; FTIR (KBr) 3400, 1710, 1670, 1630 cm^{-1}; see Tables 2 and 3 for ^1H and ^{13}C NMR, respectively.

5.4.5. 6β-Angeloyloxy-2-oxoeremophil-1(10),7(11),8-triene-12,8-olide (5). A pale yellow oil; $[\alpha]_D^{20}$ −344 (*c* 0.43, EtOH); MS (CI) *m/z* 343 $[M+H]^+$ (base), 245; HRMS (CI) obsd 343.1547 $[M+H]^+$, Calcd for $C_{20}H_{23}O_5$ 343.1545; FTIR 1780, 1720, 1660, 1640 cm^{-1}; CD (EtOH) $[\theta]_{223\ nm}$ −1460, $[\theta]_{266\ nm}$+3400, $[\theta]_{308\ nm}$ −5900; see Tables 2 and 3 for ^1H and ^{13}C NMR, respectively.

5.4.6. 1-[(3a*R*,4*S*,7a*R*)-3a,4,5,6,7a-Hexahydro-3a,4-dimethyl-1*H*-inden-2-yl]ethanone (11). A colorless oil; $[\alpha]_D^{20}$ +5.6 (*c* 0.49, EtOH); MS *m/z* 192 $[M]^+$ 177, 149 (base), 136, 122, 107, 93, 79; HRMS obsd 192.1523 $[M]^+$, Calcd for $C_{13}H_{20}O$ 192.1515; FTIR (KBr) 1670 cm^{-1}; ^{13}C NMR (150 MHz, C_6D_6) δ 17.3 (C-14), 17.4 (C-15), 22.3 (C-2), 24.5 (C-1), 26.0 (C-11), 29.4 (C-3), 33.7 (C-9), 37.0 (C-4), 46.6 (C-10), 50.0 (C-5), 144.0 (C-8), 153.2 (C-6), 195.6 (C-7); ^1H NMR (600 MHz, C_6D_6) δ 0.67 (3H, d, J=6.6 Hz, H-15), 0.84 (3H, s, H-14), 0.86 (1H, m, H-3), 1.09 (1H, m, H-4), 1.11 (1H, m, H-3), 1.18 (1H, m, H-2), 1.31 (1H, m, H-2), 1.34 (1H, m, H-1), 1.42 (1H, m, H-1), 1.75 (1H, m, H-10), 1.97 (3H, s, H-11), 2.43 (1H, ddd, J=16.0, 11.3, 2.4 Hz, H-9α), 2.57 (1H, dd, J=16.0, 8.1 Hz, H-9β), 6.30 (1H, d, J=2.4 Hz, H-6).

5.4.7. 6β-Hydroxy-3β-(2′-methylacryloyl)oxy-11β*H*-eremophil-7-en-12,8-olide (14). A colorless oil; $[\alpha]_D^{18}$ −64.2 (*c* 0.11, EtOH); MS (CI) *m/z* 335 $[M+H]^+$, 317, 249 (base), 231, 195, 109; HRMS (CI) obsd 335.1860 $[M+H]^+$, Calcd for $C_{19}H_{27}O_5$ 335.1859; FTIR (KBr) 3400, 1800, 1710 cm^{-1}; CD $\Delta\varepsilon_{217\,nm}$ −19.2; ^{13}C NMR (150 MHz, C_6D_6) δ 7.8 (C-15), 14.3 (C-13), 18.3 (C-4′), 19.0 (C-14), 25.4 (C-9), 25.9 (C-2), 29.5 (C-1), 32.8 (C-10), 35.0 (C-4), 39.1 (C-11), 42.0 (C-5), 65.3 (C-6), 72.3 (C-3), 116.1 (C-7), 125.2 (C-3′), 137.1 (C-2′), 145.1 (C-8), 166.5 (C-1′), 179.0 (C-12); ^1H NMR (600 MHz, C_6D_6) δ 0.67 (3H, br s, H-14), 0.79 (3H, d, *J*=7.1 Hz, H-15), 1.10 (3H, d, *J*=7.7 Hz, H-13), 1.85 (3H, dd, *J*=1.3 Hz, 0.8, H-4′), 3.04 (1H, br quint, H-11), 4.33 (1H, br s, H-6), 5.19 (1H, m, H-3), 5.22 (1H, quint, *J*=1.3 Hz, H-3′), 6.16 (1H, quint, *J*=0.8 Hz, H-3′). Other ^1H NMR signals could not be assigned due to broadening, see Ref. 3f.

5.4.8. 6β-Ethoxyeremophil-7(11)-en-12,8α-olide (15). ‖ A colorless oil; $[\alpha]_D^{21}$ +112.0 (*c* 0.10, EtOH); MS (CI) *m/z* 279 $[M+H]^+$ (base), 233, 169, 154, 126; HRMS (CI) obsd 279.1964 $[M+H]^+$, Calcd for $C_{17}H_{27}O_3$ 279.1960; FTIR 1760 cm^{-1}; CD $\Delta\varepsilon_{226\,nm}$ +4.61 (EtOH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.6, 15.3, 16.3, 20.0, 20.5, 25.9, 29.1, 30.6, 34.6, 35.1, 42.8, 64.3, 77.4, 78.0, 124.2, 159.7, 170.0; ^1H NMR (600 MHz, $CDCl_3$) δ 0.77 (3H, d, *J*=6.3 Hz, H-15), 1.08 (3H, s, H-14), 1.15 (3H, t, *J*=6.9 Hz, H-2′), 1.25–1.44 (7H, m), 1.70 (1H, td, *J*=14.0, 11.5 Hz, H-9α), 1.74 (1H, m, H-10), 1.86 (3H, d, *J*=1.6 Hz, H-13), 2.06 (1H, m, H-9β), 2.97 (1H, dq, *J*=9.2, 7.0 Hz, H-1′), 3.39 (1H, dq, *J*=9.2, 7.0 Hz, H-1′), 4.15 (1H, br s, H-6α), 4.94 (1H, m, H-8β).

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