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Tetrahedron

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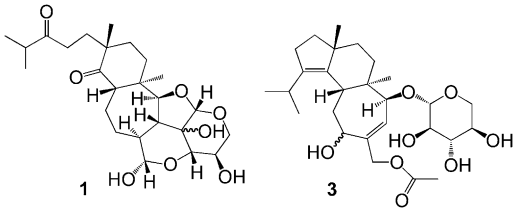
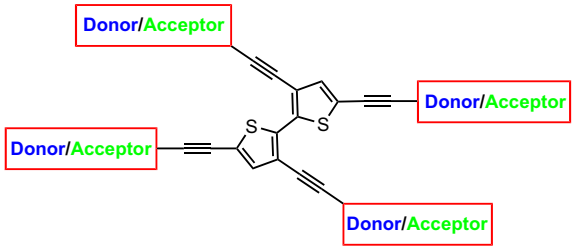
Contents

ARTICLES



Two novel compounds, erinacines J (**1**) and K (**3**) were isolated from the cultured mycelia of *Hericium erinaceum*. Erinacine K showed anti-MRSA activity.

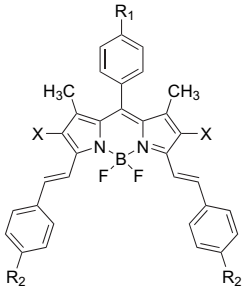


Two-photon absorption (TPA) chromophores were synthesized by sequential reactions of 3,5,3′,5′-tetrabromo-[2,2′]bithiophene with different terminal alkynes possessing electron donor and/or acceptor. Their TPA cross-section can be fine-tuned by the substitutents.

α-L-Rha-(1→3)-α-L-Rha-(1→2)-α-L-Rha(1→2)-α-L-Rha-OAll

α-L-Rha-(1→2)-α-L-Rha-(1→3)-α-L-Rha-(1→3)-α-L-Rha-(1→2)-α-L-Rha(1→2)-α-L-Rha-OAll

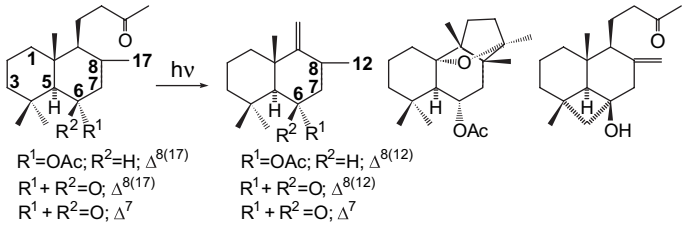
α-L-Rha-(1→3)-α-L-Rha-(1→2)-α-L-Rha-(1→2)-α-L-Rha-(1→3)-α-L-Rha-(1→3)-α-L-Rha-(1→2)-α-L-Rha(1→2)-α-L-Rha-OPr

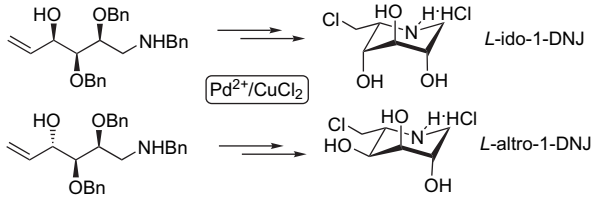


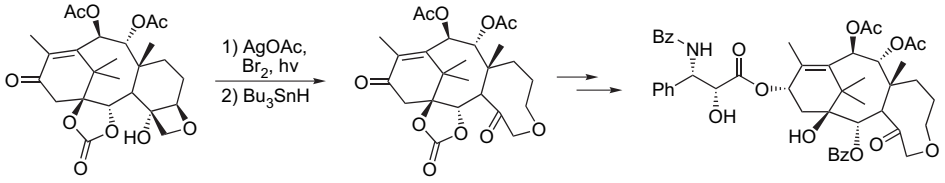
11= R_1=H, R_2=$OCH_2COOtBu$, X=Br

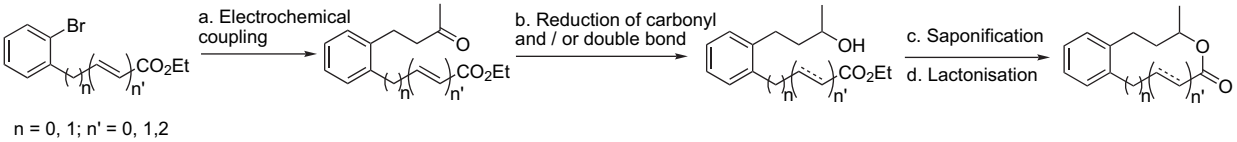
15= R_1=$OCH_2COOtBu$, R_2=$OCH_2COOtBu$, X=Et

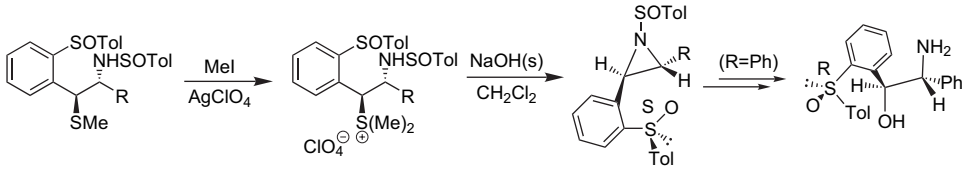
13= R_1=$OCH_2COOtBu$, R_2=$OCH_2COOtBu$, X=Br

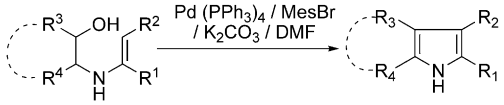


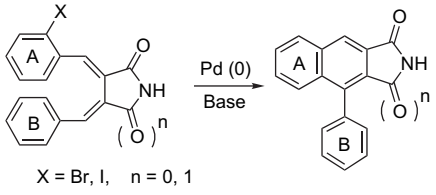




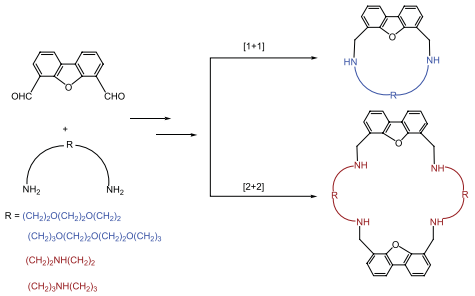


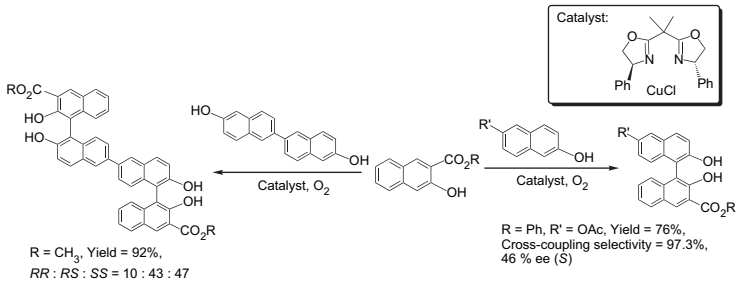


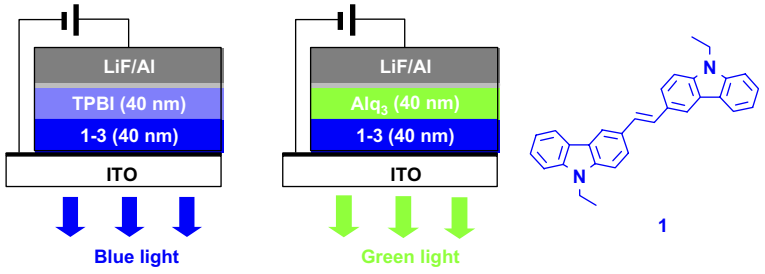




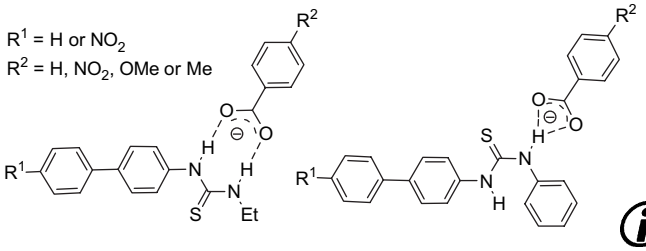
The discovery of a new Pd-catalyzed benzannulation reaction of bisbenzylidenesuccinic acid derivatives during process research on arylnaphthalene lignan aza-analogues is described.

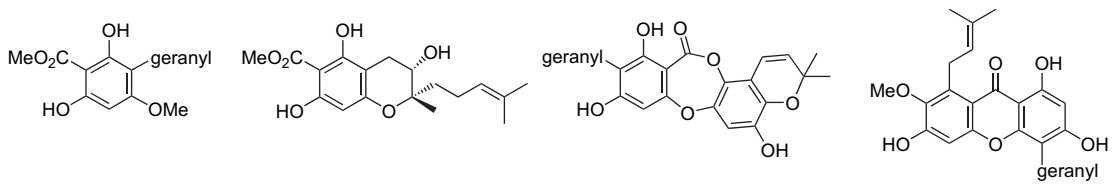




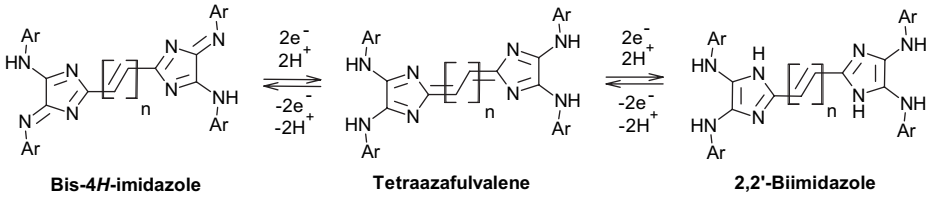


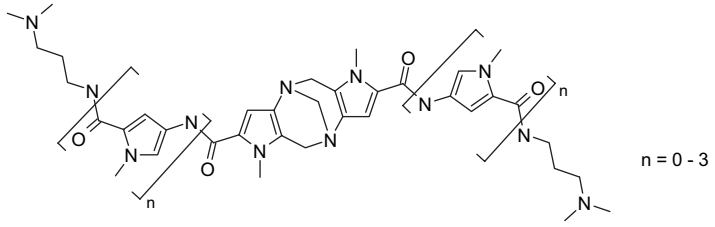
Six new biphenyl thiourea derivatives have been prepared to be used in carboxylate sensing. Experiments carried out with these ligands have demonstrated that the type of interaction with TBA carboxylates is strongly dependent on the substituents in the thiourea moiety. These interactions go from the formation of 1:1 hydrogen-bonded complexes to acid–base reactions.

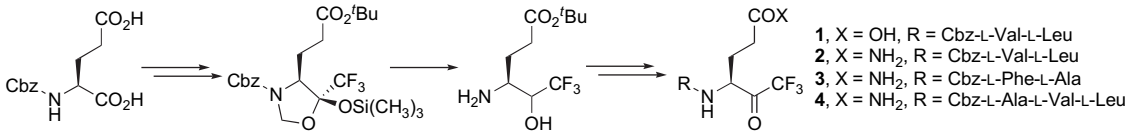


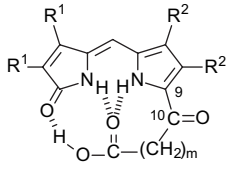


Seven phloroglucinols, two depsidones, and three xanthones were isolated form the twigs of *Garcinia parvifolia*. Their antibacterial and antioxidant activities were evaluated.

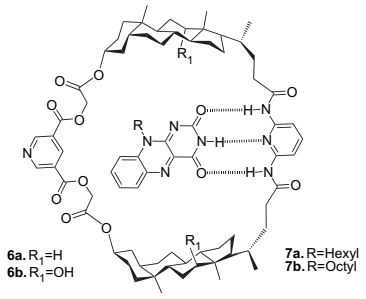


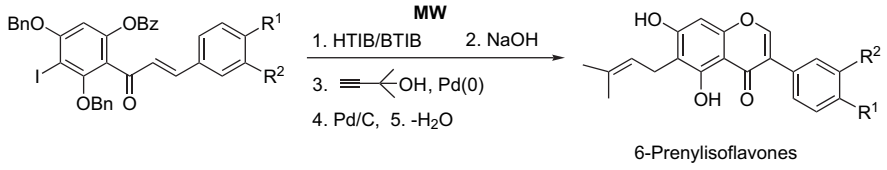


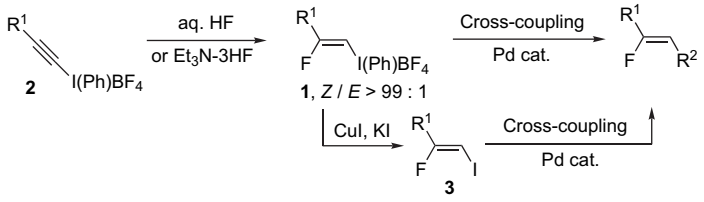




A series of dipyrrinones (**1**–**6**) with varying lengths of ω-oxo-alkanoic acid chains attached to C(9) display hydrogen bonding between the CO_2H and dipyrrinone lactam and pyrrole.







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Supplementary data available via ScienceDirect

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Five new isocoumarins from Sonoran desert plant-associated fungal strains *Paraphaeosphaeria quadriseptata* and *Chaetomium chiversii*☆

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Abstract—Five new isocoumarins, paraphaeosphaerins A–C and chaetochiversins A and B, biogenetically related to monocillin I and radicicol, have been isolated from solid agar cultures of *Paraphaeosphaeria quadriseptata* and *Chaetomium chiversii*, two fungal strains living in association with the Sonoran desert plants, *Opuntia leptocaulis* and *Ephedra fasciculata*, respectively. A new chroman-4-one, aposphaerin C, was also isolated from *P. quadriseptata*. Their structures and stereochemistry were elucidated using a combination of ^{1}H and ^{13}C homo- and hetero-nuclear 2D NMR techniques, ^{1}H NMR analysis of Mosher's esters, and chemical correlations.

1. Introduction

Recent studies have demonstrated that plant-associated fungi are rich sources of structurally diverse natural products, some with interesting biological activities.[2] In our continuing search for bioactive and/or novel metabolites of endophytic and rhizosphere fungi of the Sonoran desert plants, we have investigated EtOAc extracts of *Paraphaeosphaeria quadriseptata* occurring in the rhizosphere of the Christmas cactus (*Opuntia leptocaulis* DC.; Cactaceae) and *Chaetomium chiversii* endophytic in Mormon tea (*Ephedra fasciculata* A. Nels.; Ephedraceae). Here we report the isolation and characterization of five new isocoumarins, paraphaeosphaerins A–C (**1**–**3**) and chaetochiversins A and B (**4** and **5**) biogenetically related to monocillin I (**6**) and radicicol (**7**), a new chroman-4-one, aposphaerin C (**8**), and three known chromones, eugenetin (**9**), 6-methoxy-methyleugenin (**10**), and 6-hydroxy-methyleugenin (**11**). Previous studies of *P. quadriseptata* and *C. chiversii* have resulted in the isolation of cytotoxic and heat shock protein-90 (Hsp90) inhibitory β-resorcylic acid lactone macrolides, monocillin I (**6**) and radicicol (**7**), respectively.[1] Isolation of two 10-membered macrolides, modiolides A and B, from the marine-derived *Paraphaeosphaeria* sp. N-119 has recently been reported.[3]

2. Results and discussion

Liquid–liquid partitioning[4] of the EtOAc extract of a solid culture of *P. quadriseptata* followed by size exclusion chromatography of the $CHCl_3$ soluble fraction on Sephadex LH-20 and chromatographic separation of the fraction eluted with hexane/CH_2Cl_2 (1:4) and CH_2Cl_2/acetone (3:2) over a column of silica gel and preparative TLC furnished compounds **1**–**3** and **8**, in addition to the previously isolated monocillin I (**6**).[5]

Paraphaeosphaerin A (**1**) was obtained as a white amorphous solid that was analyzed for $C_{18}H_{18}O_6$ by a combination of HRFABMS and ^{13}C NMR spectroscopy and indicated ten degrees of unsaturation. Its UV spectrum with absorption maxima at 378, 360.5, 345.5, 330, and 270 nm was indicative of a conjugated chromophore and its IR spectrum with absorption bands at 3380, 1664, 1620, and 1570 cm^{-1} suggested the presence of OH/NH, α,β-unsaturated lactone carbonyl and olefinic groups. In the ^{1}H NMR spectrum of **1** (Table 1), in addition to other signals, a chelated OH (δ 11.12), a set of *meta*-coupled one-proton doublets [δ 6.24 and 6.32 (J=2.0 Hz)], and five olefinic/aromatic protons [δ 7.28 (dd, J=15.2, 11.4 Hz), 6.22 (s), 6.15 (dd, J=11.4, 10.2 Hz), 6.05 (d, J=15.2 Hz), and 5.56 (dd, J=10.2, 8.3 Hz)] were observed. The ^{13}C NMR spectrum of **1** (Table 2) indicated the presence of an α,β-unsaturated lactone/ester carbonyl, three oxygenated and nine non-oxygenated olefinic/aromatic carbons. In the HMBC spectrum, the proton at δ 6.22 (H-4) showed a correlation with a quaternary carbon at δ 99.9 (C-8a) and an aromatic carbon at δ 104.4 (C-5) bearing one of the *meta*-coupled protons [δ 6.32 (H-5)].

☆ See Ref. 1.

Keywords: *Paraphaeosphaeria quadriseptata*; *Chaetomium chiversii*; Endophytic and rhizosphere fungi; Paraphaeosphaerins; Chaetochiversins; Aposphaerin C; Structure elucidation.

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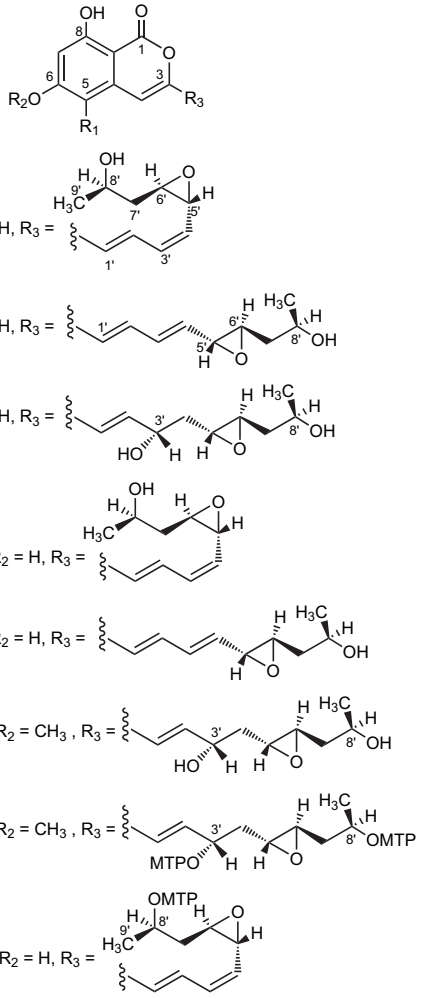


The proton at δ 6.32 (H-5) showed HMBC correlations with a quaternary carbon at δ 99.9 (C-8a), an aromatic carbon at δ 102.8 (C-7) bearing the remaining *meta*-coupled proton [δ 6.24 (H-7)], and an aromatic carbon at δ 107.6 (C-4) to which the proton at δ 6.22 is attached. The proton at δ 6.24 (H-7) showed HMBC correlations with the quaternary carbon at δ 99.9 (C-8a) and the aromatic carbon at δ 104.4 (C-5) (Fig. 1). These data suggested paraphaeosphaerin A to be a 6,8-dihydroxy-isocoumarin with a substituent at C-3 extending the conjugation of the C-3(4) double bond. The MS of **1** had a prominent ion at *m/z* 177 due to the dihydroxy-isocoumarin fragment further confirming this partial structure. In addition to the above low-field signals, the ^{1}H NMR spectrum of **1** indicated the presence of a tertiary methyl group at δ 1.25 (d, J=6.1 Hz), three methine protons at δ 4.69 (dd, J=8.3, 3.3 Hz), 4.42 (m), and 4.07 (dd, J=6.4, 3.3 Hz) attached to oxygenated carbons, and two methylene protons at δ 1.98 (ddd, J=13.0, 5.6, 2.4 Hz) and 1.68 (ddd, J=13.0, 9.4, 6.4 Hz). The ^{13}C NMR spectrum of **1** (Table 2) when analyzed with the help of edited HSQC spectra[6] showed the presence of one methyl, one methylene, and seven methine carbons in addition to the carbons encountered for the isocoumarin moiety. Of the seven methines, three were in the oxygenated region and four were in the olefinic region. These ^{1}H and ^{13}C NMR signals were assigned to the spin system –CH=CH–CH=CH–CH(O)–CH(O)–CH_2–CH(O)CH_3 (partial formula $C_9H_{12}O_3$) with the help of COSY, HSQC, and HMBC spectra. Since the dihydroxy-isocoumarin

Table 2. ^{13}C NMR data of compounds **1**–**5**

Position	**1**[a]	**2**[a]	**3**[a]	**4**[b]	**5**[b]
1	166.0 s	165.2 s	165.4 s	165.6 s	165.7 s
3	152.9 s	151.7 s	151.1 s	153.9 s	153.9 s
4	107.6 d	106.5 d	106.4 d	103.8 d	103.6 d
4a	137.4 s	139.4 s	139.3 s	138.1 s	137.0 s
5	104.4 d	103.5 d	102.3 d	107.1 s	107.9 s
6	166.3 s	165.6 s	165.7 s	161.7 s	162.9 s
7	102.8 d	102.2 d	103.6 d	103.2 d	103.3 d
8	164.5 s	164.5 s	163.2 s	162.7 s	163.2 s
8a	99.9 s	99.1 s	99.1 s	100.4 s	100.1 s
1′	125.2 d	122.8 d	120.8 d	125.1 d	123.3 d
2′	129.1 d	129.8 d	136.5 d	130.0 d	130.7 d
3′	129.6 d	132.4 d	68.9 d	129.4 d	134.3 d
4′	136.5 d	136.7 d	38.9 t	137.0 d	137.9 d
5′	84.1 d	86.7 d	55.3 d	84.0 d	83.6 d
6′	78.5 d	77.2 d	56.3 d	78.4 d	72.5 d
7′	43.8 t	41.9 t	40.3 t	43.7 t	44.3 t
8′	75.0 d	74.7 d	65.6 d	75.0 d	74.4 d
9′	21.5 q	20.8 q	22.8 q	21.4 q	21.8 q

[a] At 125 MHz ($CDCl_3$+CD_3OD).
[b] At 125 MHz ([$^2H_6$]-acetone), assignments were based on HSQC and HMBC experiments.

Table 1. ^{1}H NMR data of compounds **1**–**5**

Position	**1**[a]	**2**[a]	**3**[a]	**4**[b]	**5**[b]
4	6.22 s	6.20 s	6.21 s	6.84 s	6.82 s
5	6.32 d (2.0)	6.31 d (2.2)	6.30 d (2.2)		
7	6.24 d (2.0)	6.25 d (2.2)	6.23 d (2.2)	6.57 s	6.57 s
1′	6.05 d (15.2)	6.06 d (15.2)	6.22 d (15.5)	6.47 d (15.1)	6.43 d (15.3)
2′	7.28 dd (15.2, 11.4)	6.99 dd (15.2, 11.1)	6.52 dd (15.5, 5.0)	7.47 dd (15.1, 11.7)	7.01 dd (15.3, 10.9)
3′	6.15 dd (11.4, 10.2)	6.43 dd (15.2, 11.1)	4.45 m	6.28 dd (11.7, 10.6)	6.47 dd (15.3, 10.9)
4′	5.56 dd (10.2, 8.3)	5.99 dd (15.2, 6.3)	1.87 dt (14.1, 6.1)	5.69 dd (10.6, 8.5)	6.18 dd (15.3, 6.3)
4′			1.70 dt (14.1, 5.9)		
5′	4.69 dd (8.3, 3.3)	4.48 dd (6.3, 2.5)	2.80 dt (5.9, 2.2)	4.66 dd (8.5, 3.4)	4.49 dd (6.3, 3.1)
6′	4.07 dd (6.4, 3.3)	4.28 br t (6.3)	2.87 dt (5.9, 2.2)	4.12 dt (6.9, 3.4)	4.39 m
7′a	1.98 ddd (13.0, 5.6, 2.4)	2.11 ddd (13.2, 5.7, 1.1)	1.69 dt (14.0, 6.9)	1.99 ddd (12.8, 5.7, 2.7)	2.07 m
7′b	1.68 ddd (13.0, 9.4, 6.4)	1.67 ddd (13.2, 9.6, 6.3)	1.52 dt (14.0, 4.7)	1.71 ddd (12.8, 9.1, 6.9)	1.69 ddd (12.8, 9.5, 4.6)
8′	4.42 m	4.40 m	3.93 m	4.24 m	4.37 m
9′	1.25 d (6.1)	1.22 d (6.1)	1.60 d (5.3)	1.23 d (6.0)	1.19 d (6.1)
6-OH					
8-OH	11.12 s	11.20 s	10.95 s	11.13 s	11.19 s

[a] At 600 MHz ($CDCl_3$+CD_3OD), J values in hertz (in parenthesis).
[b] At 600 MHz (acetone-d_6), J values in hertz (in parenthesis).

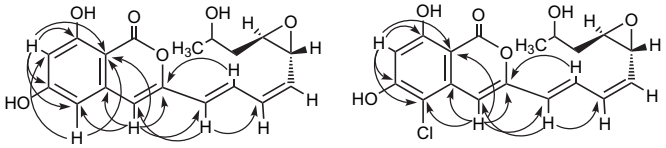
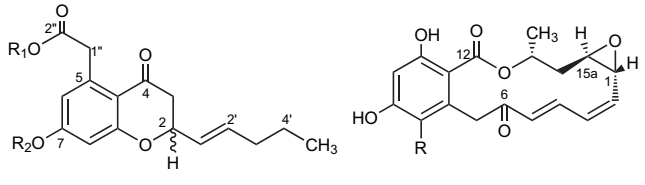


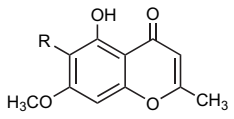
Figure 1. Selected HMBC correlations for paraphaeosphaerin A (**1**) and chaetochiversin A (**4**).

moiety accounts for $C_9H_5O_4$ with the molecular formula $C_{18}H_{18}O_6$ of **1**, the partial formula for the side-chain at C-3 should be $C_9H_{13}O_2$. In order to fulfill this requirement and the unsaturation number of 10 for paraphaeosphaerin A, an oxirane ring at C-5′(6′) and an OH substituent at C-8′ have been proposed. The cross peaks between δ_C 107.6 (C-4) and δ_H 6.32 (H-5) and 6.05 (H-1′), δ_C 125.2 (C-1′) and δ_H 6.22 (H-4) and 6.15 (H-3′) in the HMBC spectrum established the connectivity between the isocoumarin moiety and the side-chain. The relative configurations of the vicinal protons H-1′–H-6′ of the side-chain of paraphaeosphaerin A follow their characteristic coupling constants suggesting a trans relationship of the olefinic protons, H-1′ and H-2′ (J=15.2 Hz), and a cis relationship for H-3′ and H-4′ (J=10.2 Hz). The coupling constant of 3.3 Hz indicated a trans relationship of the protons (H-5′ and H-6′) on the oxirane ring.[7] The foregoing evidence suggested an isochromen-1-one structure **1** for paraphaeosphaerin A and this was confirmed by chemical transformation of monocillin I (**6**) to paraphaeosphaerin A (**1**). Treatment of **6** with potassium *tert*-butoxide in *tert*-butanol/DMF[8] yielded a product identical (TLC, MS, ^{1}H NMR, and ^{13}C NMR) with **1**. In addition to confirming the gross structure, formation of **1** from **6** also indicated that the absolute configurations of the three asymmetric centers of **1** to be identical with those of monocillin I (**6**). Since all three asymmetric centers of **6** have been determined by X-ray crystallographic analysis to have the *R* configuration,[9] the asymmetric carbons (C-5′, C-6′, and C-8′) of **1** must have the same *R* configuration. On the basis of the above data, the structure of paraphaeosphaerin A was elucidated as 3-(8′*R*-hydroxy-5′*R*,6′*R*-oxireno-1′*E*,3′*Z*-diennonyl)-6,8-dihydroxy-isochromen-1-one [6,8-dihydroxy-3-(8′*R*-hydroxy-5′*R*,6′*R*-oxirenonona-1′*E*,3′*Z*-dienyl)-1*H*-2-benzopyran-1-one] (**1**).



8 $R_1 = R_2 = H$
15 $R_1 = CH_2CH_3$, $R_2 = H$
16 $R_1 = R_2 = CH_3$

6 R = H
7 R = Cl

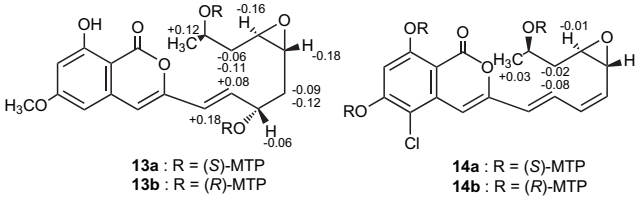


9 $R = CH_3$
10 $R = CH_2OCH_3$
11 $R = CH_2OH$

Paraphaeosphaerin B was obtained as an amorphous white solid. Its molecular formula was established as $C_{18}H_{18}O_6$ from HRFABMS and indicated ten degrees of unsaturation. The ^{1}H NMR spectral data of **2** (Table 1) were similar to those of **1**, except for the H-3′/H-4′ coupling constant (Table 1). The $J_{3',4'}$ of **2** was found to be 15.2 Hz (compared with 10.2 Hz observed for **1**) suggesting the *E* stereochemistry for the C-3′(4′) double bond in **2**. The stereochemical relationship between **1** and **2** was further confirmed by the treatment of **1** with iodine converting it into its more stable *E* isomer **2**. Paraphaeosphaerin B was thus identified as 3-(8′*R*-hydroxy-5′*R*,6′*R*-oxirenonona-1′*E*,3′*E*-dienyl)-6,8-dihydroxy-isochromen-1-one (**2**).

Paraphaeosphaerin C (**3**), isolated as a white amorphous solid, was determined to have the molecular formula $C_{18}H_{20}O_7$ by a combination of HRFABMS and ^{13}C NMR spectral data and indicated nine degrees of unsaturation. ^{1}H and ^{13}C NMR data (Tables 1 and 2, respectively) showed very close resemblance to those of **1** and **2** suggesting that **3** had the same carbon skeleton. However, ^{1}H and ^{13}C NMR spectra of **3** lacked signals due to two olefinic hydrogens/carbons but showed the presence of a methylene carbon (δ_H 1.87; δ_C 38.9) and an oxygenated methine carbon (δ_H 4.45; δ_C 68.9). In the ^{1}H–^{1}H COSY spectrum, the H-1′ at δ 6.22 (d, J=15.5 Hz) showed a correlation with H-2′ at δ 6.52 (dd, J=15.5, 5.0 Hz), which also was found to have a cross peak with 3′-H (δ 4.45); 3′-H also showed correlations with 4′-H at δ 1.70 (m) and 1.87 (dt, J=14.1 and 6.1 Hz) suggesting that C-3′ is oxygenated. Detailed analysis of 1D and 2D NMR spectra permitted the assignment of all proton and carbon signals of paraphaeosphaerin C (Tables 1 and 2). Since there was an additional asymmetric carbon atom in **3** compared with **1** and **2**, the absolute stereochemistry of this was determined by the application of a modified Mosher's method.[10] Reaction of the monomethyl paraphaeosphaerin C (**12**) with (*S*)- and (*R*)-α-methoxy-α-trifluoromethylphenylacetic (MTP) acids afforded (*R*)- and (*S*)-MTPA esters (**13a** and **13b**; Fig. 2), respectively. Analysis of $\Delta\delta$ values (Fig. 2) confirmed the *S* absolute stereochemistry for C-4′ and *R* absolute stereochemistry for C-8′. The structure of paraphaeosphaerin C was thus established as 3-(4′*S*,8′*R*-dihydroxy-5′*R*,6′*R*-oxirenonona-1′*E*-enyl)-6,8-dihydroxy-isochromen-1-one (**3**).

Aposphaerin C (**8**) was determined to have the molecular formula $C_{16}H_{18}O_5$ by HRMS, which was consistent with its ^{13}C and HSQC NMR data and indicated eight degrees of unsaturation. It had IR absorption bands at 3525, 1704, and 1651 cm^{-1} indicating the presence of hydroxyl, carboxylic acid, carbonyl, and conjugated carbonyl functionalities.



13a : R = (*S*)-MTP
13b : R = (*R*)-MTP
14a : R = (*S*)-MTP
14b : R = (*R*)-MTP

Figure 2. $\Delta\delta$ value [($\Delta\delta$ in ppm)=$\delta_S-\delta_R$] obtained for (*S*)- and (*R*)-MTP esters (**13a** and **13b**, respectively) of monomethylparaphaeosphaerin A (**12**) and (**14a** and **14b**, respectively) of chaetochiversin A (**4**).

Its UV spectrum with absorption maxima at 311.5, 277.5, 237.0, and 220.5 nm was characteristic of a 7-hydroxychroman-4-one structure.[11] Comparison of its ^{1}H and ^{13}C NMR data with those reported for aposphaerin B (**15**) suggested that **15** may be the ethyl ester of aposphaerin C (**8**) and this was further confirmed by the application of 2D NMR techniques including ^{1}H–^{1}H COSY and HMBC. Methylation of **8** with CH_3I/K_2CO_3 in acetone yielded its dimethyl derivative **16**. Isolation of aposphaerin B and the related octaketide, cavoxinone[11] as racemic mixtures has led to the suggestion that they have been formed by non-enzymatic cyclizations of appropriate olefinic open chain precursors.[11] The absence of any optical rotation associated with aposphaerin C (**8**) suggested that it may also have been formed by a similar process. The structure of aposphaerin C was thus elucidated as 3,4-dihydro-7-hydroxy-4-oxo-2-(1*E*-pentenyl)-2*H*-1-benzo-pyran-5-acetic acid (**8**).

Initial liquid–liquid partitioning of the EtOAc extract of the endophytic fungus, *C. chiversii*, indicated that the cytotoxicity was concentrated in the 80% aqueous MeOH soluble fraction. Size exclusion chromatography of this fraction on Sephadex LH-20 followed by column chromatography over silica gel and repeated thin layer chromatography furnished radicicol (**7**),[1] two new isocoumarins, chaetochiversins A (**4**) and B (**5**), and the three known chromones **9**–**11**. Chaetochiversin A (**4**) was obtained as a pale yellow solid that was analyzed for $C_{18}H_{17}ClO_6$ by a combination of HRFABMS and ^{13}C NMR spectroscopy. Its IR, UV, and ^{1}H NMR spectra were similar to those of paraphaeosphaerin A (**1**) indicating that it is an isocoumarin with 8′-hydroxy-5′,6′-oxirenonona-1′,3′-dienyl side-chain as a substituent. The ^{1}H NMR spectrum of **4** (Table 1) showed the absence of one of the *meta*-coupled protons suggesting that this may be substituted by a Cl atom. Detailed analysis of the HMBC spectra of **4** as for **1** (Fig. 1) suggested that the side-chain is at C-3 position of the isocoumarin moiety. By analogy with the structure of radicicol (**7**), the Cl atom in **4** was placed at C-5. Treatment of radicicol (**7**) with potassium *tert*-butoxide in DMF[8] as for monocillin I above, yielded **4** confirming its structure and the absolute configuration *R* of the three asymmetric centers. The *R* configuration of the C-8′ of chaetochiversin A was further confirmed by ^{1}H NMR analysis[10,12] of its (*S*)- and (*R*)-MTPA esters (**14a** and **14b**; Fig. 2), respectively. On the basis of the above data, the structure of chaetochiversin A was elucidated as 3-(8′*R*-hydroxy-5′*R*,6′*R*-oxirenonona-1′*E*,3′*Z*-dienyl)-5-chloro-6,8-dihydroxy-isochromen-1-one (**4**). Chaetochiversin B (**5**) was obtained as a pale yellow solid. Its molecular formula was established as $C_{18}H_{17}ClO_6$ by a combination of HRFABMS and ^{13}C NMR spectroscopy. ^{1}H and ^{13}C NMR spectral data of **5** were found to be very similar to those of **4**, except the coupling constant between H-3′ and H-4′ (J=15.3 Hz) suggesting the trans relationship of these protons. Treatment of **4** with iodine yielded **5** confirming its structure and configuration of the three asymmetric centers. Chaetochiversin B was therefore identified as 3-(8′*R*-hydroxy-5′*R*,6′*R*-oxirenonona-1′*E*,3′*E*-dienyl)-5-chloro-6,8-dihydroxy-isochromen-1-one (**5**). Comparison of spectral data of the remaining metabolites of *C. chiversii* with those reported in the literature allowed these to be identified as eugenetin (**9**),[13] 6-methoxymethyleugenin (**10**),[14] and 6-hydroxymethyleugenin (**11**).[14,15] Of the compounds encountered in this study only monocillin I (**6**) and radicicol (**7**) were found to exhibit cytotoxic activity.[1]

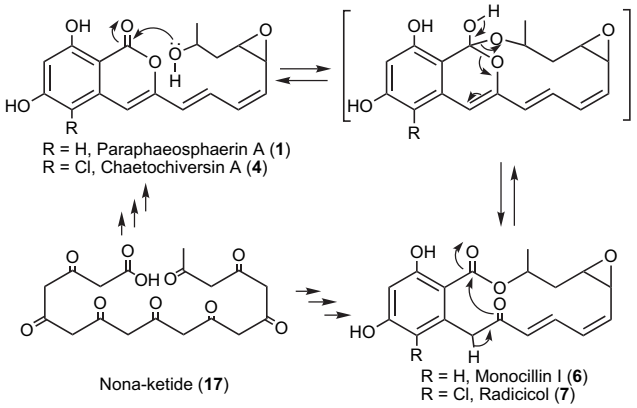


Figure 3. Possible biosynthetic relationship between paraphaeosphaerin A (**1**), monocillin I (**6**), chaetochiversin A (**4**), and radicicol (**7**).

Co-occurrence of the isocoumarins, paraphaeosphaerins A–C (**1**–**3**) and chaetochiversins A and B (**4** and **5**), and the β-resorcylic acid lactone macrolides, monocillin I (**6**) and radicicol (**7**), in these fungal strains suggests that their biosynthesis may involve the common nonaketide precursor (**17**). As depicted in Fig. 3, it may also be possible that the biosyntheses of these macrocyclic lactones may involve the intermediacy of isocoumarins; it is noteworthy that isocoumarins structurally related to paraphaeosphaerins have served as intermediates in recent chemical syntheses of monocillin I.[16] The possibility that these isocoumarins are artifacts arising as a result of the isolation process was ruled out as TLC analysis indicated the presence of these compounds in the original EtOAc extracts and prolonged treatment of monocillin I (**6**) and radicicol (**7**) with MeOH, the solvent used for the extraction of the fungal cultures, failed to yield even trace amounts of paraphaeosphaerin A (**1**) or chaetochiversin A (**4**). The absence of the macrolactone corresponding to paraphaeosphaerin C (**3**) in the EtOAc extract of *P. quadriseptata* further suggests that the isocoumarins encountered in this study are genuine natural products.

3. Experimental

3.1. General experimental procedures

Melting points were determined with an electrothermal melting point apparatus and were uncorrected. Optical rotations were measured with a Jasco Dip-370 digital polarimeter using $CHCl_3$ or MeOH as solvent. UV spectra were recorded on a Shimadzu UV-1601 UV–VIS spectrophotometer. IR spectra for KBr discs were recorded on a Shimadzu FTIR-8300 spectrometer. ^{1}H and 2D NMR spectra were recorded in $CDCl_3$+CD_3OD or acetone-d_6 with a Bruker DRX-600 instrument at 600 MHz for ^{1}H NMR using residual $CHCl_3$ or acetone as internal standard. ^{13}C spectra were recorded with a Bruker DRX-500 instrument at 125 MHz. The chemical shift values (δ) are given in parts per million (ppm), and the coupling constants are in hertz. Low resolution and high resolution MS were recorded, respectively, on Shimadzu LCMS-8000 QPα and JEOL HX110A spectrometers.

3.2. Culturing, extraction, and isolation of metabolites of *P. quadriseptata*

The fungal strain isolated from the rhizosphere of the Christmas cactus (*O. leptocaulis* DC.) growing in Tucson, Arizona was identified by Ms. Donna Bigelow (Division of Plant Pathology, Department of Plant Sciences, University of Arizona) as *P. quadriseptata* by analysis of the ITS regions of the ribosomal DNA as described previously.[5] The strain is deposited in the Department of Plant Pathology and Southwest Center for Natural Products Research and Commercialization of the University of Arizona microbial culture collection under the accession number Opl-1-F20 (AH-45-00-F20). For isolation of bioactive compounds, the fungus was cultured and processed as reported previously[5] to afford the cytotoxic EtOAc extract (1.85 g). A portion (1.80 g) of this extract was partitioned between hexane and 80% aqueous MeOH, and the cytotoxic aqueous MeOH fraction was diluted to 60% aqueous MeOH by the addition of water and extracted with $CHCl_3$. Evaporation of $CHCl_3$ under reduced pressure yielded a pale brown semisolid (1.31 g) that was found to be cytotoxic. A portion (1.30 g) of this was subjected to gel permeation chromatography on a column of Sephadex LH-20 (40.0 g) made up in hexane/CH_2Cl_2 (1:4) and eluted with hexane/CH_2Cl_2 (1:4) (700 mL), CH_2Cl_2/acetone (3:2) (250 mL), CH_2Cl_2/acetone (1:4) (250 mL), and finally with MeOH (250 mL). Twenty-four fractions (50 mL each) were collected (F_1–F_{24}) of which fractions F_7–F_{17} were found to be cytotoxic. These fractions were combined and further fractionated on silica gel (13.0 g) by elution with CH_2Cl_2 followed by increasing amounts of MeOH in CH_2Cl_2. Fractions eluted with 0.5% MeOH in CH_2Cl_2 were found to be cytotoxic and these fractions were combined and evaporated to yield monocillin I (**6**) (521 mg). Chromatography of the fraction F_{17} (114.0 mg) on silica gel (3.0 g) by elution with CH_2Cl_2 followed by increasing amounts of MeOH in CH_2Cl_2 afforded 43 fractions. Of these, the fraction (15.9 mg) eluted with 2% MeOH in CH_2Cl_2 afforded **1** (5.7 mg). Column chromatography of the fraction F_{18} (86.0 mg) on silica gel (3.0 g) by elution with Et_2O followed by Et_2O containing increasing amounts of MeOH afforded several fractions. Of these, the early fractions (4.1 mg) eluted with Et_2O yielded **8** (3.1 mg). Fraction F_{19} (97.0 mg) was further fractionated on silica gel (3.5 g) by elution with increasing amounts of MeOH in CH_2Cl_2 followed by MeOH to give 42 fractions. Of these, the fraction (25.6 mg) eluted with 5% MeOH in CH_2Cl_2 was further separated on preparative TLC on silica gel (CH_2Cl_2/MeOH, 98:2) to give **1** (1.2 mg) and **2** (4.9 mg). Later fractions eluted with 5% MeOH in CH_2Cl_2 were combined and further purified by repeated preparative TLC (silica gel) to give **3** (5.2 mg).

3.2.1. Paraphaeosphaerin A (1). Off-white amorphous solid; mp 178–180 °C; $[\alpha]_D^{25}$ +4.2 (*c* 2.0, MeOH); UV (MeOH) λ_{max} (log ε) 378 (4.23), 360.5 (4.41), 345.5 (4.35), 330 (4.29), 270 (4.66), 208.5 (4.11) nm; IR (KBr) ν_{max} 3380, 1664, 1620, 1569, 1504, 1466, 1366, 1242, 1161, and 1080 cm^{-1}; HRFABMS *m/z* 331.1182 $[M+H]^+$ (calcd for $C_{18}H_{19}O_6$, 331.1182); ^1H and ^{13}C NMR data, see Tables 1 and 2.

3.2.2. Paraphaeosphaerin B (2). Off-white amorphous solid; mp 174–176 °C; $[\alpha]_D^{25}$ +7.2 (*c* 2.0, MeOH); UV (MeOH) λ_{max} (log ε) 378.0 (4.93), 360.5 (5.12), 345.5 (5.05), 329.5 (4.10), 270.0 (5.36), 208.5 (4.51) nm; IR (KBr) ν_{max} 3390, 1663, 1620, 1570, 1508, 1462, 1362, 1242, 1165, 1080, 1038, and 972 cm^{-1}; HRFABMS *m/z* 331.1182 $[M+H]^+$ (calcd for $C_{18}H_{19}O_6$, 331.1182); ^1H and ^{13}C NMR data, see Tables 1 and 2.

3.2.3. Paraphaeosphaerin C (3). Off-white amorphous solid; mp 132–134 °C; $[\alpha]_D^{25}$ −5.8 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 354.5 (4.75), 339 (4.32), 312 (4.75), 301 (4.75), 259 (5.47), 203 (4.88) nm; IR (KBr) ν_{max} 3395, 1678, 1628, 1578, 1508, 1462, 1366, 1238, 1169, and 1072 cm^{-1}; HRFABMS *m/z* 349.1287 $[M+H]^+$ (calcd for $C_{18}H_{21}O_7$, 349.1287); ^1H and ^{13}C NMR data, see Tables 1 and 2.

3.2.4. Aposphaerin C (8). White solid; mp 154–156 °C; UV (MeOH) λ_{max} (log ε) 311.5 (2.75), 277.5 (3.09), 237 (3.06), 220.5 (3.24) nm; IR (KBr) ν_{max} 3525, 1651, 1589, 1504, 1454, 1362, 1288, 1157, and 1053 cm^{-1}; ^1H NMR [$^2H_6$]-acetone δ: 6.41 (1H, d, *J*=2.4 Hz, H-6), 6.35 (1H, d, *J*=2.4 Hz, H-8), 5.90 (1H, ddt, *J*=15.6, 6.6, 0.8 Hz, H-2′), 5.69 (1H, ddt, *J*=15.6, 6.6, 1.3 Hz, H-1′), 4.89 (1H, m, H-2), 3.96 (1H, d, *J*=16.6 Hz, H-1″a), 3.89 (1H, d, *J*=16.6 Hz, H-1″b), 2.69 (1H, dd, *J*=16.5, 11.8 Hz, H-3a), 2.55 (1H, dd, *J*=16.5, 3.3 Hz, H-3b), 2.07 (2H, br t, *J*=7.2 Hz, H_2-3′), 1.42 (2H, dq, *J*=7.2, 7.2 Hz, H_2-4′), 0.91 (3H, t, *J*=7.2 Hz, CH_3-5′); ^{13}C NMR [$^2H_6$]-acetone δ: 192.4 (C-2″), 172.5 (C-4), 165.8/164.2 (C-7/C-8a), 141.1 (C-5), 135.6 (C-2′), 129.5 (C-1′), 115.5 (C-6), 114.3 (C-4a), 103.6 (C-8), 79.1 (C-2), 45.1 (C-3), 41.6 (C-1″), 35.4 (C-3′), 23.3 (C-4′), 14.4 (C-5′); HRFABMS *m/z* 291.1232 $[M+H]^+$ (calcd for $C_{16}H_{19}O_5$, 291.1232).

3.2.5. Conversion of monocillin I (6) to 1. To a solution of **6** (20 mg) in DMF (500 μL) was added a solution of *t*-BuOK (33 mg) in *t*-BuOH (200 μL) and warmed at 50 °C for 10 min and allowed to stand at 25 °C for 40 min. Water (5 mL) was added to the reaction mixture, acidified with 2 N HCl, and extracted with EtOAc (3×15 mL). Purification by preparative TLC (silica gel) using 10% MeOH in CH_2Cl_2 as eluant gave **1** (1.2 mg).

3.2.6. Isomerization of paraphaeosphaerin A (1) to paraphaeosphaerin B (2). A solution of iodine (0.2 mg) in EtOAc (100 μL) was added to a solution of **1** (1.0 mg) in EtOAc (100 μL) and stirred at 25 °C. After 5 min MeOH (100 μL) was added to the reaction mixture, solvents removed under reduced pressure, and the crude product was purified by preparative TLC (silica gel) using 10% MeOH in CH_2Cl_2 as eluant to give **2** (0.8 mg).

3.2.7. Monomethyl paraphaeosphaerin C (12). K_2CO_3 (20 mg) and CH_3I (200 μL) were added to a solution of **3** (2.0 mg) in acetone (400 μL) and stirred at 25 °C for 4 h after which the reaction mixture was filtered and the filtrate evaporated under reduced pressure. The crude product was purified by preparative TLC (silica gel) using 8% MeOH in CH_2Cl_2 as eluant to give **12** as a colorless semisolid (2.1 mg); ^1H NMR acetone-d_6 δ: 11.09 (1H, s, OH), 6.63 (1H, dd, *J*=15.5, 5.0 Hz, H-2′), 6.62 (1H, s, H-4), 6.60 (1H, d, *J*=2.0 Hz, H-5), 6.49 (1H, d, *J*=2.0 Hz, H-7), 6.43 (1H, dd, *J*=15.5, 1.5 Hz, H-1′), 4.55 (1H, dd, *J*=6.0,

5.0 Hz, H-3′), 3.96 (1H, m, H-8′), 3.92 (3H, s, OMe), 2.88 (1H, dt, J=6.0, 2.0 Hz, H-6′), 2.85 (1H, dt, J=6.0, 2.0 Hz, H-5′), 1.91 (1H, dt, J=13.5, 6.0 Hz, H-4′a), 1.77 (1H, dt, J=13.5, 6.0 Hz, H-4′b), 1.73 (1H, dt, J=13.5, 6.0 Hz, H-7′a), 1.52 (1H, dt, J=13.5, 6.0 Hz, H-7′b), 1.18 (3H, d, J=6.3 Hz, CH_3).

3.2.8. Preparation of the (*R*)- and (*S*)-MTPA esters of 12. Monomethyl paraphaeosphaerin A (**12**) (1.0 mg, 2.76 μmol) in anhydrous EtOAc (100 μL) was added to a stirred solution of (*R*)-α-methoxy-α-trifluoromethylphenyl acetic acid (3.0 mg, 12.82 μmol), DCC (3.3 mg, 16.02 μmol), and 4-PP (catalytic amount) in anhydrous EtOAc (150 μL) and stirred at 25 °C. After 4 h (TLC control) the reaction mixture was filtered through a cotton plug and EtOAc was removed. Resulting residue was purified by preparative TLC (silica gel) using 1% methanol in CH_2Cl_2 as eluant to give (*R*)-MTPA ester (**13a**) (1.6 mg). The process was repeated as above, but using (*S*)-α-methoxy-α-trifluoromethylphenyl acetic acid, to afford the (*S*)-MTPA ester (**13a**) (1.5 mg).

*(R)-MTPA ester of **12*** (**13a**): White semisolid; selected ^{1}H NMR signals ($CDCl_3$, 600 MHz) δ: 11.0 (1H, s, OH), 6.48 (1H, d, J=2.1 Hz, H-5), 6.41 (1H, dd, J=15.5, 6.7 Hz, H-2′), 6.36 (1H, d, J=2.1 Hz, H-7), 6.17 (1H, s, H-4), 6.09 (1H, d, J=15.5 Hz, H-1′), 5.75 (1H, dd, J=13.1, 6.7 Hz, H-3′), 5.27 (1H, m, H-8′), 2.72 (1H, dd, J=6.1, 3.8 Hz, H-5′), 2.69 (1H, m, H-6′), 2.02 (1H, m, H-4′a), 1.85 (1H, m, H-7′a), 1.82 (1H, m, H-4′b), 1.81 (1H, m, H-7′b), 1.30 (3H, d, J=6.4 Hz, CH_3).

*(S)-MTPA ester of **12*** (**13b**): White semisolid; selected ^{1}H NMR signals ($CDCl_3$, 600 MHz) δ: 11.03 (1H, s, OH), 6.49 (1H, d, J=2.1 Hz, H-5), 6.49 (1H, dd, J=13.2, 7.4 Hz, H-2′), 6.37 (1H, d, J=2.1 Hz, H-7), 6.29 (1H, s, H-4), 6.27 (1H, d, J=13.2 Hz, H-1′), 5.69 (1H, dd, J=12.2, 6.2 Hz, H-3′), 5.23 (1H, m, H-8′), 2.54 (1H, m, H-5′), 2.53 (1H, m, H-6′), 1.90 (1H, m, H-4′a), 1.80 (1H, m, H-7′a), 1.75 (1H, m, H-7′b), 1.73 (1H, m, H-4′b), 1.37 (3H, d, J=6.4 Hz, CH_3).

3.2.9. Methyl aposphaerin C (16). K_2CO_3 (10 mg) and CH_3I (100 μL) were added to a solution of aposphaerin C (**8**) (1.0 mg) in acetone (200 μL) and stirred at 25 °C for 14 h. Reaction mixture was then filtered and the filtrate evaporated under reduced pressure to give **16** as a white semisolid (1.0 mg); ^{1}H NMR ($CDCl_3$) δ: 6.41 (1H, d, J=2.1 Hz, H-6), 6.34 (1H, d, J=2.1 Hz, H-8), 5.84 (1H, dt, J=15.5, 6.7 Hz, H-2′), 5.63 (1H, dd, J=15.5, 6.8 Hz, H-1′), 4.85 (1H, m, H-2), 3.96 (1H, d, J=16.6 Hz, H-1″a), 3.89 (1H, d, J=16.6 Hz, H-1″b), 3.79 (3H, s, OMe), 3.69 (3H, s, OMe), 2.73 (1H, dd, J=16.5, 12.4 Hz, H-3a), 2.59 (1H, dd, J=16.5, 3.1 Hz, H-3b), 2.05 (2H, br t, J=7.2 Hz, H_2-3′), 1.42 (2H, dq, J=7.3, 7.3 Hz, H_2-4′), 0.90 (3H, t, J=7.3 Hz, CH_3-5′); MS m/z 319 ($[M+H]^+$, in +APCI mode), 317 ($[M-H]^-$, in −APCI mode).

3.3. Culturing, extraction, and isolation of metabolites of *C. chiversii*

The fungal strain was isolated from the stem of *E. fasciculata* growing in South mountain park in Phoenix, Arizona, and was identified by Microbial ID Inc, Newark, DE as *C. chiversii*. The strain is deposited in the School of Life Sciences, Arizona State University, and the Southwest Center for Natural Products Research and Commercialization of the University of Arizona microbial culture collection under the accession numbers 7-EPH-2S and CS-36-62, respectively. For isolation of secondary metabolites, the fungus was cultured and processed as described previously[1] to obtain the EtOAc extract (2.01 g). A portion (1.75 g) of this extract was partitioned between hexane and 80% aqueous MeOH. Evaporation of solvents under reduced pressure yielded hexane (0.535 g) and 80% aqueous MeOH (1.031 g) fractions. A portion (1.0 g) of the 80% aqueous MeOH fraction was subjected to gel permeation chromatography on a column of Sephadex LH-20 (30 g) in hexane/CH_2Cl_2 (1:4) and eluted with hexane/CH_2Cl_2 (1:4) (800 mL), CH_2Cl_2/acetone (3:2) (500 mL), CH_2Cl_2/acetone (1:4) (300 mL), CH_2Cl_2/MeOH (1:1) (300 mL), and finally with MeOH (500 mL). Seventy fractions (20 mL each) were collected and pooled based on their TLC patterns to yield 20 combined fractions (F_1–F_{20}). Fraction 3 (F_3) was chromatographed over a column of silica gel (3.0 g) made up in CH_2Cl_2/hexane (3:2) and eluted with CH_2Cl_2/hexane (3:2 and 3:1), CH_2Cl_2 followed by CH_2Cl_2 containing increasing amounts of MeOH. Sixty fractions were collected and fractions having similar TLC patterns were combined to give six sub-fractions (A–F). Sub-fraction A was separated on preparative TLC (silica gel) using 1% MeOH in CH_2Cl_2 as eluant to give **9** (2.8 mg). Sub-fraction E was separated on preparative TLC (silica gel) using 3% MeOH in CH_2Cl_2 to give **10** (1.2 mg). Fraction F_5 was purified by preparative TLC (silica gel) using 6% MeOH in CH_2Cl_2 as eluant to give **11** (2.8 mg). Fraction F_{12} (250 mg) was chromatographed over a column of silica gel (Fluka G 60, 6.0 g) made up in CH_2Cl_2 and eluted with CH_2Cl_2 containing increasing amounts of MeOH. Eighty fractions (4 mL each) were collected and fractions having similar TLC patterns were combined to give eight sub-fractions. The sub-fraction eluted with 0.5% MeOH in CH_2Cl_2 was washed with hexane/CH_2Cl_2 (2:3) to give **7** (102.0 mg). Fractions F_{14} and F_{15} were combined and chromatographed over a column of silica gel (2.5 g) made up in CH_2Cl_2 and eluted with CH_2Cl_2 followed by CH_2Cl_2 containing increasing amounts of MeOH. Sixty-two fractions (7.5 mL each) were collected and fractions having similar TLC behavior were combined to give 16 sub-fractions. Sub-fraction eluted with 2% MeOH in CH_2Cl_2 was further separated by preparative RP-TLC (RP-18) using 25% H_2O in MeOH as eluant to give **4** (1.3 mg). Preparative TLC (RP-18, 25% H_2O in MeOH) purification of another sub-fraction eluted with 2% MeOH in CH_2Cl_2 gave **5** (1.1 mg).

3.3.1. Chaetochiversin A (4). Pale yellow amorphous solid; mp 186–188 °C; $[\alpha]_D^{23}$ +5.3 (*c* 1.0, MeOH); UV (EtOH) λ_{max} (log ε) 392 (4.85), 374 (5.10), 357 (5.10), 340 (5.07), 326 (5.08), 312 (5.02), 292 (5.35), 273 (5.39); IR (KBr) ν_{max} 3390, 1681, 1616, 1566, 1461, 1392, 1357, 1238, 1184, 1076, and 988 cm^{-1}; HRFABMS m/z $[M+H]^+$ 365.7908 (calcd for $C_{18}H_{18}ClO_6$, 365.7903); ^{1}H and ^{13}C NMR data, see Tables 1 and 2.

3.3.2. Chaetochiversin B (5). Pale yellow amorphous solid; mp 179–181 °C; $[\alpha]_D^{23}$ +8.6 (*c* 1.0, MeOH); UV (EtOH) λ_{max}

(log ε) 392 (4.75), 373 (5.00), 357 (5.01), 340 (4.99), 326 (4.99), 292 (5.29), 274 (5.30); IR (KBr) ν_{max} 3387, 1679, 1618, 1569, 1463, 1390, 1360, 1240, 1184, and 1076 cm^{-1}; HRFABMS *m/z* $[M+H]^+$ 365.7908 (calcd for $C_{18}H_{18}ClO_6$, 365.7903); ^1H and ^{13}C NMR data, see Tables 1 and 2.

3.3.3. Eugenetin (9). White crystalline solid; mp 160–162 °C (lit.[13] 159–160 °C); ^1H NMR data were consistent with literature values;[13] APCIMS (+)ve mode *m/z* 221 $[M+H]^+$.

3.3.4. 6-Methoxymethyleugenin (10). White crystalline solid; mp 192–193 °C; ^1H NMR data were consistent with literature values;[14] APCIMS (+)ve mode *m/z* 219 $[M+H-MeOH]^+$.

3.3.5. 6-Hydroxymethyleugenin (11). White crystalline solid; mp 197–198 °C (lit.[15] 198–199 °C); ^1H NMR data were consistent with literature values;[13] APCIMS (+)ve mode *m/z* 219 $[M+H-H_2O]^+$.

3.3.6. Conversion of radicicol (7) to chaetochiversin A (4). To a solution of **7** (10.0 mg) in DMF (0.5 mL) was added *t*-BuOK (11.3 mg). The reaction mixture was warmed to 50 °C and stirred for 3 h, after which it was poured into brine and extracted with EtOAc (3×30 mL). EtOAc extracts were combined, washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure and the crude product separated on preparative TLC (silica gel) using 6% MeOH in CH_2Cl_2 as eluant to give **4** (5.0 mg) and unreacted radicicol (**7**) (1.2 mg).

3.3.7. Preparation of the (*R*)- and (*S*)-MTPA ester derivatives of chaetochiversin A. Chaetochiversin A (**4**, 0.5 mg) was transferred into a clean NMR tube and was dried completely under the vacuum of an oil pump. Deuterated pyridine (0.6 mL) and (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) chloride (5.0 μL) were added into the NMR tube immediately under a stream of N_2, and then the NMR tube was shaken carefully to mix the sample and MTPA chloride evenly. The reaction NMR tube was permitted to stand at room temperature for 8 h. ^1H NMR data of the (*R*)-MTPA ester derivative (**14a**) of **7** (500 MHz, pyridine-d_5) δ: 6.690 (1H, dd, *J*=15.2, 10.1 Hz, H-2′), 6.832 (1H, s, H-4), 6.660 (1H, s, H-7), 6.357 (1H, d, *J*=15.2 Hz, H-1′), 6.262 (1H, dd, *J*=11.7, 10.6 Hz, H-3′), 6.115 (1H, dd, *J*=15.2, 6.3 Hz, H-4′), 5.813 (1H, m, H-8′), 4.864 (1H, m, H-5′), 4.436 (1H, m, H-6′), 2.298 (1H, dd, *J*=13.1, 5.2 Hz, H-7′a), 1.897 (1H, ddd, *J*=13.1, 9.4, 4.7 Hz, H-7′b), 1.189 (1H, d, *J*=6.1 Hz, CH_3-8′); APCIMS (+)ve mode *m/z* 625 $[M+H]^+$. In the manner described for **14a** another portion of **4** (0.5 mg) was reacted in a second NMR tube with (*S*)-(−)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) chloride (5.0 μL) at room temperature for 8 h using pyridine-d_5 (0.6 mL) as solvent, to afford the (*S*)-MTPA ester (**14b**). ^1H NMR data of **14b** (500 MHz, pyridine-d_5) δ: 6.952 (1H, dd, *J*=15.2, 11.8 Hz, H-2′), 6.821 (1H, s, H-4), 6.675 (1H, s, H-7), 6.562 (1H, dd, *J*=15.2, 10.8 Hz, H-3′), 6.265 (1H, d, *J*=15.2 Hz, H-1′), 5.840 (1H, m, H-8′), 5.765 (1H, dd, *J*=15.2, 6.4 Hz, H-4′), 4.808 (1H, dd, *J*=6.4, 3.4 Hz, H-5′), 4.425 (1H, m, H-6′), 2.215 (1H, dd, *J*=13.1, 5.2 Hz, H-7′a), 1.877 (1H, ddd, *J*=13.1, 9.4, 4.7 Hz, H-7′b), 1.216 (1H, d, *J*=6.1 Hz, CH_3-8′); APCIMS (+)ve mode *m/z* 625 $[M+H]^+$.

3.3.8. Conversion of chaetochiversin A (4) to chaetochiversin B (5). A solution of iodine (0.2 mg) in EtOAc (100 μL) was added to a solution of **4** (1.0 mg) in EtOAc (100 μL) and stirred at 25 °C. After 5 min (TLC control), MeOH (100 μL) was added to the reaction mixture, solvents removed under reduced pressure and the crude product was purified by preparative TLC (silica gel) using 8% MeOH in CH_2Cl_2 as eluant to afford **5** as a pale yellow solid (0.9 mg).

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Generation and electrophilic substitution reactions of 3-lithio-2-methyleneaziridines

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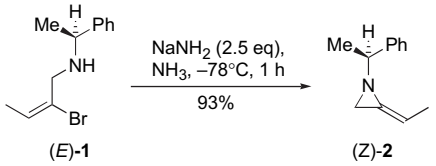
Abstract—2-Methyleneaziridinyl anions can be produced by selective deprotonation of the parent aziridine at C-3 using *sec*-BuLi/TMEDA. Subsequent reaction with a wide variety of electrophiles including MeI, $ICH_2CH_2CH_2CH_2Cl$, $PhCH_2Br$, allyl bromide, Me_3SiCl, Bu_3SnCl, PhCHO and Ph_2CO provides the corresponding C-3 substituted derivatives in moderate to good yields (43–91%). In the case of homochiral methyleneaziridines bearing an (*S*)-α-methylbenzyl group on nitrogen, high levels of diastereocontrol (up to 90%de) can be achieved in this lithiation/alkylation sequence.

1. Introduction

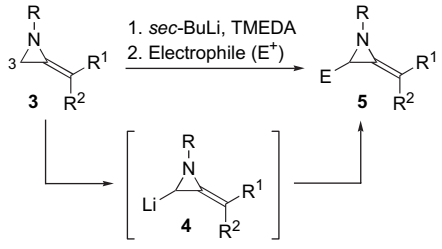
2-Methyleneaziridines are emerging as powerful vehicles for chemical synthesis. Recently, these highly strained heterocycles have been shown to participate in a number of useful transformations including [3+4] cycloadditions,[1] multi-component reactions,[2] radical cascades,[3] and several palladium-catalysed processes.[4–6] Of course, for these reactions to be of broad scope and utility, concise methods for the synthesis of these N-heterocycles are required. Simple 2-methyleneaziridines can easily be made in high yield by reaction of the corresponding 2-bromoallylamines with sodium amide in liquid ammonia.[7] This remarkable cyclisation tolerates considerable structural variation with respect to the substituents on nitrogen and the double bond, and proceeds with net stereochemical inversion at the trigonal carbon atom undergoing substitution.[8] For example, cyclisation of (*E*)-**1** yields (*Z*)-**2** in excellent yield (Scheme 1). Other routes to the parent heterocycles are also known.[9]

To assemble substrates for Lewis acid catalysed intramolecular [3+4] cycloadditions,[1] efficient routes to methyleneaziridines bearing C-3 substituents were required. The synthesis of such derivatives has little literature precedent.[10] Whilst they might conceivably be prepared by ring closure of the appropriately substituted vinyl bromide in an analogous



Scheme 1. Facile synthesis of C-3 unsubstituted methyleneaziridines by ring closure.

manner to that illustrated in Scheme 1, an alternative approach based upon C-3 functionalisation of a preformed 2-methyleneaziridine appeared more flexible and direct (Scheme 2). Early work by Quast and Weise Vélez had established the potential suitability of this approach by showing that 1-*tert*-butyl-2-methyleneaziridine **3** (R=^{t}Bu; R^1, R^2=H) can be deprotonated with *sec*-butyllithium at −78 °C to give organolithium **4** (R=^{t}Bu; R^1, R^2=H) and further alkylated with a limited range of electrophiles



Scheme 2. Strategy for the functionalisation of C-3 substituted methyleneaziridines.

Keywords: Aziridines; Organolithiums; Strained compounds.
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(MeOD, MeI or Me_3SiCl) to yield **5** (R=^{t}Bu; R^1, R^2=H; E=D, Me_3Si or Me).[10a] In the present article, the scope and limitations of this approach to C-3 substituted methyleneaziridines are examined in detail. Using a range of different methyleneaziridines and electrophiles, it is determined that this method provides a highly practical approach to many C-3 substituted methyleneaziridines.[11] Moreover, using simple chiral, non-racemic methyleneaziridines (R=(*S*)-CHMePh), useful levels of asymmetric induction can be achieved in this process.

2. Results and discussion

2.1. Precursor synthesis

The lithiation/alkylation reactions of wide range of methyleneaziridines have been studied. Eleven different methyleneaziridines **2**, **6–13** were prepared for use in these investigations (Fig. 1). In most instances, the compounds were made according to published procedures by sodium amide induced cyclisation of the corresponding 2-bromoallylamine [(*Z*)-**7**,[8] (*E*)-**7**,[8] **10**,[12] **11**,[13] **12**,[13] (*Z*)-**2**,[8] (*E*)-**2**[8] and **13**[2d]]. The examples incorporating the α-methylbenzyl substituent were produced as the (*S*)-enantiomer.

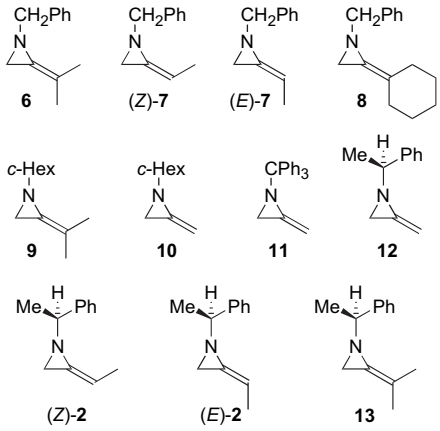
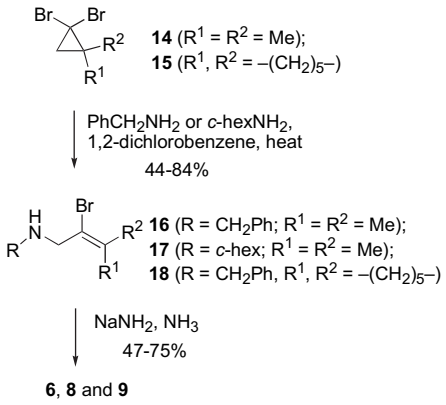


Figure 1. Substrates for lithiation/alkylation studies.

Three new methyleneaziridines, namely **6**, **8** and **9**, were made by a simple two-step sequence. Ring opening[14] of 1,1-dibromocyclopropane **14**[15] with benzylamine and cyclohexylamine yielded vinyl bromides **16** and **17**, respectively (Scheme 3). In a similar manner, opening of **15**[16] with benzylamine provided **18**. For **16** and **18**, the ring opening was performed at 170 °C and high yields (68–84%) were obtained. Using cyclohexylamine (bp 134 °C), the opening was conducted at 120 °C, which may account for the reduced yield of **17** (44%). Ring closure of bromides **16–18** with sodium amide[7,8] proceeded uneventfully to provide methyleneaziridines **6**, **8** and **9** in moderate to good yields.

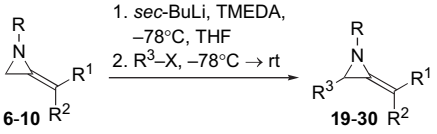
2.2. Lithiation/electrophilic substitution reactions using simple methyleneaziridines

In the original study by Quast and Weise Vélez, it was established that complete lithiation of 1-*tert*-butyl-2-methyleneaziridine at C-3 could be accomplished by treatment



Scheme 3. Synthesis of new methyleneaziridines **6**, **8** and **9**.

with 1.5–2.0 equiv of *sec*-BuLi/tetramethylethylenediamine (TMEDA) in diethyl ether at −78 °C for 7.5 h.[10a] Herein, the lithiation of **6–10** was achieved under very similar conditions and was conducted in THF at −78 °C using a small excess of *sec*-BuLi (1.1–1.5 equiv) and TMEDA (1.1–1.2 equiv) as cosolvent. Complete lithiation at C−3 was achieved in ≤6 h under these conditions. Quenching the lithiated methyleneaziridines with electrophiles and warming to room temperature provided the C-3 substituted products **19–30** in good to excellent yields (Scheme 4 and Table 1). In most cases, a small excess of the electrophile was used (1.2–1.5 equiv). However, for the synthesis of aziridines **29** and **30**, the electrophile was used as the limiting reagent (0.9 equiv). This was necessary because aziridines **28–30** could not be purified by chromatography due to their instability, and excess 1-chloro-4-iodobutane and tributyltin chloride were difficult to remove by distillation. The range of carbon-based electrophiles that can be used in this chemistry is quite broad. Successful alkylations were realised using a variety of alkyl iodides (Table 1, entries 1, 2, 8 and 12), benzyl bromide (Table 1, entry 11), benzophenone (Table 1, entry 4) and benzaldehyde (Table 1, entry 3). In the case of 1-chloro-4-iodobutane, selective displacement of the iodide was observed (Table 1, entry 12). No diastereoselectivity was witnessed in the reaction with benzaldehyde and **21** was produced as ca. 1:1 mixture of diastereomers. Heteroatom-based electrophiles can also be used (Table 1, entries 5 and 13). This chemistry accommodates considerable changes in the methyleneaziridine structure. Variation in the extent of substitution of the exocyclic double bond is well tolerated (Table 1, entries 1, 6–8 and 11). Furthermore, changes in the nature of the N-substituent are possible (Table 1, entry 1 and 9) although no reaction is witnessed with *N*-trityl-2-methyleneaziridine (Table 1, entry 14). The selective lithiation of *N*-benzyl substituted derivatives **6–9** at C-3 is especially notable, with no competitive benzylic deprotonation being observed. Unfortunately, initial attempts to extend this chemistry to the synthesis of 3,3′-disubstituted



Scheme 4. Functionalisation of simple methyleneaziridines via deprotonation/alkylation.

Table 1. Alkylation of simple methyleneaziridines

Entry	Aziridine	R	R^1	R^2	Electrophile	Product	R^3	%Yield[a]
1	**6**	CH_2Ph	Me	Me	MeI	**19**	Me	79
2	**6**	CH_2Ph	Me	Me	(*E*)-PhCH=CH$CH_2CH_2CH_2I$	**20**	$CH_2CH_2CH_2CH$=CHPh	73
3	**6**	CH_2Ph	Me	Me	PhCHO	**21**	CH(OH)Ph	74[b]
4	**6**	CH_2Ph	Me	Me	Ph_2CO	**22**	$C(OH)Ph_2$	73
5	**6**	CH_2Ph	Me	Me	Me_3SiCl	**23**	$SiMe_3$	63
6	(*Z*)-**7**	CH_2Ph	Me	H	Ph_2CO	(*Z*)-**24**	$C(OH)Ph_2$	62
7	(*E*)-**7**	CH_2Ph	H	Me	Ph_2CO	(*E*)-**24**	$C(OH)Ph_2$	73
8	**8**	CH_2Ph	$-(CH_2)_5-$		(2-furanyl)$CH_2CH_2CH_2I$	**25**	$CH_2CH_2CH_2$(2-furanyl)	61
9	**9**	c-Hex	Me	Me	MeI	**26**	Me	64
10	**9**	c-Hex	Me	Me	Me_3SiCl	**27**	$SiMe_3$	64
11	**10**	c-Hex	H	H	$PhCH_2Br$	**28**	CH_2Ph	86
12	**10**	c-Hex	H	H	$ClCH_2CH_2CH_2CH_2I$	**29**	$CH_2CH_2CH_2CH_2Cl$	70[c]
13	**10**	c-Hex	H	H	Bu_3SnCl	**30**	$SnBu_3$	91[c]
14	**11**	CPh_3	H	H	$PhCH_2Br$	n/a	n/a	0

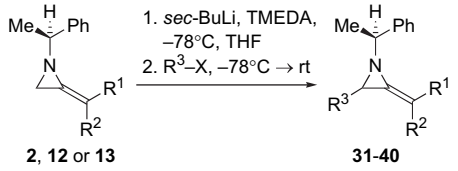
[a] Isolated yield after purification by silica gel chromatography or distillation.
[b] Produced as a separable mixture of two diastereomers (**21a**: 35%; **21b**: 39%).
[c] Yield based upon electrophile which was used as limiting reagent (0.9 equiv).

methyleneaziridines by repeating the lithiation/alkylation sequence on the alkylated products have not been fruitful.

2.3. Diastereocontrolled lithiation/alkylation reactions

Since a new asymmetric centre is produced at C-3 during the lithiation/alkylation sequence (Scheme 2), it was of interest to establish if any stereochemical control could be achieved in this transformation. Indeed, Quast and Weise Vélez had attempted this in the lithiation/alkylation reactions of 1-methyl-2-methyleneaziridine by using an external chiral ligand [(*S*,*S*)-(+)-1,4-bis(dimethylamino)-2,3-dimethoxybutane] as cosolvent. Unfortunately, the levels of enantioselectivity achieved were very modest (12.4% ee).[10b,17] We reasoned that much better levels of asymmetric induction might be achieved by incorporating a chiral, nonracemic element within the nitrogen substituent of the aziridine such that it might exert influence and control on the lithiation/alkylation process. For this study, the α-methylbenzyl group was chosen as the chiral control element because of its simplicity, low cost and widespread use in asymmetric synthesis.[18]

The diastereocontrolled alkylation reactions of four chiral methyleneaziridines, namely (*E*)-**2**, (*Z*)-**2**, **12** and **13** were studied (Scheme 5 and Table 2). Treatment of homochiral (*S*)-**12** with *sec*-BuLi and TMEDA in THF for 7 h, then benzyl bromide, provided **31** in good yield but as an inseparable 53:47 mixture of diastereomers (14% de before purification) (Table 2, entry 1). Using benzophenone as electrophile, an increase in diastereoselectivity was witnessed (Table 2, entry 2 cf. entry 1). Interestingly, a similar trend was seen for (*E*)-**2** (Table 2, entry 4 cf. entry 3) and (*Z*)-**2** (Table 2, entry 6 cf. entry 5). Moreover, the introduction of methyl groups on



Scheme 5. Diastereocontrolled deprotonation/alkylation of homochiral methyleneaziridines.

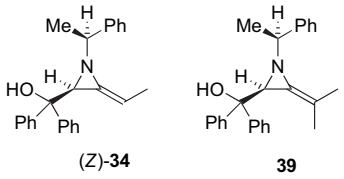
the exocyclic double bond leads to a significant improvement in the level of diastereoselectivity observed (Table 2, entries 1, 3, 5 and 9), with substitution cis to the aziridine nitrogen giving the greatest improvement. Thus, high levels of diastereocontrol (80–90% de) were achieved using 2-isopropylidineaziridine (*S*)-**13** with a range of electrophiles, producing **35**–**40** in moderate to good yields (Table 2, entries 7–12). With the exception of **36**, the alkylated products were readily isolated as single diastereomers after silica gel chromatography. For (*Z*)-**2**, high selectivities (up to 86%) were obtained in some instances (Table 2, entry 6). The relative stereochemistry within (*Z*)-**34** and **39** have been unambiguously established by X-ray crystallography (Fig. 2).[19] All the other alkylated aziridines **31**–**40** are tentatively assigned as having the same configuration at C-3 in the major diastereomer.

The origin of the large differences in the levels of diastereoselectivity observed in the lithiation/alkylation of aziridines (*E*)-**2** and (*Z*)-**2**, **12** and **13** remains unclear. As aziridinyl anions are normally configurationally stable, we suggest that the selectivity in these reactions most likely arises from diastereocontrol in the initial lithiation of the aziridine ring. At first glance, the methyl groups on the alkene terminus appear too remote to exert any increased bias in the stereoselectivity of this process. However, the stereochemical analysis is complicated by the fact that these enantiomerically pure methyleneaziridines exist as mixtures of diastereomers as a result of N-inversion. In an attempt to ascertain if the dynamics of nitrogen inversion play a role in the selectivity of the lithiation process, we have quantified the effect of alkene substitution on the rate of inversion. To simplify the analysis, *N*-benzyl substituted derivatives **6**, (*Z*)-**7**, (*E*)-**7** and *N*-benzylmethyleneaziridine (**41**)[12] were used as the populations of the N-invertomers were equal. The ^1H NMR spectra of the methyleneaziridines were recorded over a range of temperatures at 500 MHz and rate constants obtained for the exchanging signals ascertained by line shape matching with simulated spectra produced using WINDNMR.[20] From the rate constants, the Gibbs free energy of activation ($\Delta G^{\ddagger}$) for the inversion process was determined for each aziridine by use of Eyring plots. The following data were obtained: **41**, $\Delta G^{\ddagger}$=43.5 kJ mol^{-1};

Table 2. Diastereoselective alkylations with homochiral methyleneaziridines

Entry	Aziridine	R^1	R^2	Electrophile	Product	R^3	%Yield[a]	Crude %de[b]
1	**12**	H	H	$PhCH_2Br$	**31**	CH_2Ph	70[c]	14
2	**12**	H	H	Ph_2CO	**32**	$C(OH)Ph_2$	70[d,e]	56
3	(*E*)-**2**	H	Me	$PhCH_2Br$	(*E*)-**33**	CH_2Ph	51[f]	26
4	(*E*)-**2**	H	Me	Ph_2CO	(*E*)-**34**	$C(OH)Ph_2$	68[d]	56
5	(*Z*)-**2**	Me	H	$PhCH_2Br$	(*Z*)-**33**	CH_2Ph	59[f]	48
6	(*Z*)-**2**	Me	H	Ph_2CO	(*Z*)-**34**	$C(OH)Ph_2$	83[d]	86
7	**13**	Me	Me	MeI	**35**	Me	47	80
8	**13**	Me	Me	BuI	**36**	Bu	53[g]	n.d.
9	**13**	Me	Me	$PhCH_2Br$	**37**	CH_2Ph	68	88
10	**13**	Me	Me	$CH_2{=}CHCH_2Br$	**38**	$CH_2CH{=}CH_2$	63	84
11	**13**	Me	Me	Ph_2CO	**39**	$C(OH)Ph_2$	43[d]	88
12	**13**	Me	Me	Me_3SiCl	**40**	$SiMe_3$	80	90

[a] Isolated yield of major diastereomer after purification by silica gel chromatography unless otherwise stated.
[b] Ratio determined by ^1H NMR analysis prior to purification.
[c] Isolated as a 53:47 mixture of diastereomers.
[d] To remove excess Ph_2CO, treated with $NaBH_4$ in EtOH prior to purification.
[e] Isolated as a 80:20 mixture of diastereomers.
[f] Combined yield of separated diastereomers.
[g] Isolated as a 93:7 mixture of diastereomers.



(*Z*)-**34** **39**

Figure 2. Determination of relative stereochemistry in (*Z*)-**34** and **39** by X-ray crystallography.

(*Z*)-**7**, $\Delta G^{\ddagger}$=52.0 kJ mol^{-1}; (*E*)-**7**, $\Delta G^{\ddagger}$=54.2 kJ mol^{-1} and **6**, $\Delta G^{\ddagger}$=57.2 kJ mol^{-1} (at 298 K). From these measurements, it is apparent that the N-inversion barrier is raised by alkene substitution. However, it remains to be established if this is an important factor in the observed changes in diastereoselectivity witnessed in the lithiation/alkylation reactions of (*E*)- and (*Z*)-**2**, **12** and **13** and work focused on uncovering the origin of the selectivity in these reactions is ongoing.

3. Conclusions

It has been established that lithiation/alkylation of methyleneaziridines provides a convenient method for the synthesis of a wide range of C-3 substituted derivatives. Good yields are obtained in many cases and the reaction tolerates considerable variation in the structure of both the methyleneaziridine and the electrophile. Highly diastereoselective alkylations can be achieved with α-methylbenzyl substituted methyleneaziridines (up to 90% de). The best levels of selectivity arise when the alkene substituent cis to the aziridine nitrogen is larger than a hydrogen. This chemistry provides a general approach to C-3 substituted methyleneaziridines and as such will assist in the development of new applications of these N-heterocycles in synthesis.

4. Experimental

4.1. General

Anhydrous solvents were purchased in Sure/Seal™ bottles from Sigma–Aldrich Co., or dried prior to use by distillation. All other solvents and reagents were used as received or purified by standard protocols. All experiments were performed under an inert atmosphere in oven-dried glassware. Column chromatography was carried out using Matrex silica 60. Optical rotations were determined using an Optical Activity Ltd AA1000 Polarimeter. Melting points were measured using Gallenkamp MPD350 apparatus and are reported uncorrected. Infrared spectra were recorded on a Nicolet MAGNA 550 or Perkin–Elmer 'Spectrum One' FT-IR spectrometer with internal calibration. ^1H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, on a Bruker ACF-300 or AM-300; or at 400 and 100 MHz, respectively, on a Bruker DRX-400, DPX-400 or AV-400 spectrometer. Low-resolution mass spectra were recorded on a Kratos Profile HV3 or Micromass Quattro II mass spectrometer fitted with an electron ionisation source, or an Esquire 2000 platform with electrospray ionisation. High-resolution mass spectra were obtained using a Finnigan MAT 95XP, Finnigan MAT 900XLT, Micromass 70-VSEQ or VG-7070E instrument. Elemental analyses were carried out on a Perkin–Elmer 2400 CHN or Carlo Erba 1160 elemental analyser.

4.2. General method A: synthesis of amines 16–18

To a stirred solution of dibromocyclopropane **14** or **15** (1.0 M equiv) in 1,2-dichlorobenzene was added the amine (2.2–3.0 M equiv) and potassium carbonate (1.00–1.05 M equiv). The mixture was then heated at 120 or 170 °C for 65–72 h. On cooling to room temperature, sodium hydroxide (2 M aqueous solution) was added. The mixture was extracted with diethyl ether, the combined organic extracts washed with brine then dried over $MgSO_4$. Diethyl ether was removed under reduced pressure and 1,2-dichlorobenzene by distillation (60 °C/15 mmHg). Further purification by column chromatography gave the title amines.

4.2.1. *N*-(2-Bromo-3-methyl-2-butenyl)-1-benzylamine (16). Compound **14** (5.00 g, 21.9 mmol), benzylamine (5.17 g, 48.2 mmol) and potassium carbonate (3.18 g, 23.0 mmol) in 1,2-dichlorobenzene (40 mL) were reacted according to general method A for 72 h at 170 °C. After

work-up, column chromatography (10% EtOAc in petroleum ether) gave **16** (4.68 g, 84%) as a pale yellow oil. IR (film) 3448, 2978, 2932, 1735, 1644 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.40–7.26 (m, 5H), 3.74 (s, 2H), 3.59 (s, 2H), 1.96 (s, 3H), 1.78 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 140.4, 134.1, 128.8, 128.7, 127.4, 121.9, 53.0, 51.9, 25.9, 21.0; MS (CI) *m/z* 254 (MH^+: ^{81}Br), 252 (MH^+: ^{79}Br); HRMS (CI) calcd for $C_{12}H_{17}BrN$ 254.0544, found 254.0537.

4.2.2. *N*-(2-Bromo-3-methyl-2-butenyl)-1-cyclohexylamine (17). Compound **14** (10.0 g, 43.9 mmol), cyclohexylamine (9.58 g, 96.6 mmol) and potassium carbonate (6.08 g, 44.0 mmol) in 1,2-dichlorobenzene (40 mL) were reacted according to general method A for 72 h at 120 °C. After work-up, column chromatography pretreated with triethylamine (10% EtOAc and 0.5% Et_3N in petroleum ether) gave **17** (4.76 g, 44%) as a pale orange oil. IR (film) 3329, 2929, 2852, 1444, 1111, 1009 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 3.54 (s, 2H), 2.36 (m, 1H), 1.88 (s, 3H), 1.81 (s, 3H), 1.81–1.79 (m, 2H), 1.77–1.68 (m, 2H), 1.65–1.50 (m, 2H), 1.25–1.08 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) 132.8, 122.1, 54.5, 50.5, 33.5, 26.1, 25.5, 24.9, 20.5; MS (EI) *m/z* 247 (M^+: ^{81}Br), 245 (M^+: ^{79}Br); HRMS (EI) calcd for $C_{11}H_{20}NBr$ 245.0779, found 245.0770; Anal. Calcd for $C_{11}H_{20}NBr$: C, 53.67; H, 8.19; N, 5.69%. Found: C, 53.41; H, 8.46; N, 5.61%.

4.2.3. *N*-(2-Bromo-2-cyclohexylideneethyl)-1-benzylamine (18). Compound **15** (5.00 g, 18.7 mmol), benzylamine (5.99 g, 55.9 mmol) and potassium carbonate (2.70 g, 19.5 mmol) in 1,2-dichlorobenzene (40 mL) were reacted according to general method A for 65 h at 170 °C. After work-up, column chromatography pretreated with triethylamine (5–10% EtOAc in petroleum ether) gave **18** (3.73 g, 68%) as a pale yellow oil. IR (film) 3336, 3062, 3026, 2923, 2851, 1640, 1604, 1447, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 7.22–7.36 (m, 5H), 3.70 (s, 2H), 3.60 (s, 2H), 2.45 (br t, J=5.8 Hz, 2H), 2.22 (br t, J=5.9 Hz, 2H), 1.92 (s, 1H), 1.54–1.62 (m, 4H), 1.49–1.54 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 141.2, 140.1, 128.4, 128.2, 127.0, 119.1, 52.1, 51.4, 35.8, 31.4, 28.0, 27.3, 26.4; MS (EI) *m/z* 293 (M^+: ^{81}Br), 291 (M^+: ^{79}Br); HRMS (EI) calcd for $C_{15}H_{20}BrN$ 293.0779, found 293.0779.

4.2.4. 1-Benzyl-2-(1-methylethylidene)aziridine (6). A three-necked flask was fitted with a cold-finger condenser and a gas inlet. Iron(III) nitrate nonahydrate (12.0 mg, 0.03 mmol) was added and the system was flushed with $CaCl_2$ dried ammonia. A dry ice/acetone mixture was added to the condenser and ammonia (50 mL) was condensed into the flask. Sodium (0.600 g, 26.1 mmol) was added in small portions, each time waiting for the disappearance of the blue colouration prior to the next addition. After cooling to −78 °C, a solution of **16** (2.65 g, 10.4 mmol) in a small volume of diethyl ether was added slowly to the grey suspension. After 1 h, the mixture was diluted with diethyl ether and quenched by the dropwise addition of water (*Caution*). After the ammonia had evaporated, diethyl ether was added and the mixture was stirred for 2 min. The organic phase was separated and washed successively with 0.1 M acetic acid, 10% NaOH and brine, dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure to give the crude product, which was purified by bulb-to-bulb distillation to afford **6** (1.31 g, 73%) as a pale yellow oil. IR (film) 1799, 1496, 1452 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6 at 90 °C) 7.36–7.25 (m, 5H), 3.62 (s, 2H), 2.06 (s, 2H), 1.72 (s, 3H), 1.66 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 138.9, 128.4, 128.2, 127.1, 124.2, 104.1, 62.4, 31.7, 20.4, 19.1; MS (CI) *m/z* 173 (M^+); HRMS (CI) calcd for $C_{12}H_{16}N$ (MH^+) 174.1282, found 174.1276; Anal. Calcd for $C_{12}H_{16}N$: C, 83.40; H, 8.80; N, 8.15%. Found: C, 83.20; H, 8.75; N, 8.10%.

4.2.5. *N*-Benzyl-2-cyclohexylideneaziridine (8). A three-necked flask was fitted with a cold-finger condenser and a gas inlet. Iron(III) nitrate nonahydrate (3.4 mg, 8.42 μmol) was added and the system was flushed with $CaCl_2$ dried ammonia. A dry ice/acetone mixture was added to the condenser and ammonia (40 mL) was condensed into the flask. Sodium (0.195 g, 8.48 mmol) was added in small portions, each time waiting for the disappearance of the blue colouration prior to the next addition. A solution of **18** (1.00 g, 3.40 mmol) in Et_2O (2 mL) was added slowly to the grey suspension, which was subsequently stirred for 1.5 h. The mixture was diluted with diethyl ether (20 mL) and quenched by the dropwise addition of water (10 mL) (*Caution*). After the ammonia had evaporated, diethyl ether (20 mL) was added and the mixture was stirred for 2 min. The organic phase was separated and washed successively with 0.1 M acetic acid (20 mL), 10% NaOH (20 mL) and brine (20 mL), dried over $MgSO_4$ and filtered. Removal of the solvent under reduced pressure followed by column chromatography (5–10% EtOAc in petroleum ether) gave **8** (0.340 g, 47%) as a pale yellow oil. IR (film) 3063, 3028, 2921, 2850, 1792, 1599, 1446, 1276 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.42–7.30 (m, 5H), 3.89 (br s, 1H), 3.51 (br s, 1H), 2.74–1.85 (m, 6H), 1.63–1.54 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) 138.9, 128.4, 128.3, 127.2, 121.4, 112.1, 62.8, 31.3, 30.2, 28.0, 27.8, 26.6; MS (EI) *m/z* 213 (M^+); HRMS (EI) calcd for $C_{15}H_{19}N$ 213.1518, found 213.1519.

4.2.6. 2-Isopropylidene-1-(cyclohexyl)aziridine (9). To a three-necked flask fitted with a cold-finger condenser and a gas inlet was added sodium amide (9.93 g, 255 mmol). The system was flushed with $CaCl_2$ dried ammonia then a dry ice/acetone mixture was added to the condenser and ammonia (90 mL) was condensed into the flask. Compound **17** (2.10 g, 8.53 mmol) was added, then the mixture stirred for 3 h. Diethyl ether was added followed by the dropwise addition of water (*Caution*). After the ammonia had evaporated, water and diethyl ether were added and the mixture stirred for 2 min. The organic phase was separated and the aqueous phase extracted with diethyl ether (2×). The combined organic extracts were washed successively with 10% NaOH and brine, dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure to give the crude product, which was purified by bulb-to-bulb distillation (90 °C/1 mmHg) to afford **9** (1.05 g, 75%) as a colourless oil. IR (film) 3027, 2929, 2852, 1792, 1444, 1367, 1229, 1127, 1019 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 2.05–1.84 (m, 3H), 1.79 (s, 3H), 1.78–1.71 (m, 3H), 1.72 (s, 3H), 1.70–1.61 (m, 1H), 1.60–1.49 (m, 1H), 1.46–1.28 (m, 2H), 1.25–1.07 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 124.0, 103.0, 66.8, 32.9, 29.1, 25.8,

24.8, 20.9, 19.9; MS (EI) *m/z* 165 (M^+); HRMS (EI) calcd for $C_{11}H_{19}N$ 165.1517, found 165.1522.

4.3. General method B: lithiation/electrophile trapping of methyleneaziridines (2, 6–10, 12 and 13)

To a stirred solution of the aziridine (1.0 M equiv) in THF at −78 °C, was added TMEDA (1.1–1.2 M equiv) and *sec*-BuLi (1.1–1.5 M equiv) dropwise. The reaction was stirred at −78 °C for 3.5–7 h, then quenched with the electrophile (0.9–1.5 equiv) and allowed to warm to room temperature overnight. Water was added, the layers separated, and the aqueous phase extracted with diethyl ether (2×). The combined organic extracts were dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. Purification by column chromatography or bulb-to-bulb distillation provided the title compounds (**19–40**). For some reactions using benzophenone, an additional reductive step was included in the work-up to facilitate removal of excess electrophile (vide infra).

4.3.1. 1-Benzyl-2-isopropylidene-3-methylaziridine (19). Compound **6** (0.173 g, 1 mmol) was reacted with *sec*-BuLi (1.3 M in hexanes, 1.15 mL, 1.5 mmol) and TMEDA (0.140 g, 1.2 mmol) in THF (10 mL) for 6 h in accordance with general method B, then iodomethane (0.170 g, 1.2 mmol) was added. After work-up, purification by column chromatography (5% EtOAc and 0.5% Et_3N in petroleum ether) gave **19** (0.148 g, 79%) as a colourless oil. IR (film) 2965, 2923, 1806, 1495, 1452, 1147, 1021, 730, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 7.37–7.30 (m, 4H), 7.28–7.22 (m, 1H), 4.11 (d, *J*=13.9 Hz, 1H), 3.28 (d, *J*=13.9 Hz, 1H), 2.04 (br s, 1H), 1.75 (s, 3H), 1.71 (s, 3H), 1.29 (d, *J*=5.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 139.2, 130.4, 128.2, 127.9, 126.8, 103.8, 61.5, 39.4, 20.4, 18.9, 17.4; MS (ES) *m/z* 188 (MH^+); HRMS (ES) calcd for $C_{13}H_{18}N$ 188.1434, found 188.1432.

4.3.2. (*E*)-1-Benzyl-2-isopropylidene-3-(5-phenylpent-4-enyl)aziridine (20). Compound **6** (0.519 g, 3 mmol) was reacted with *sec*-BuLi (1.2 M in hexanes, 3.75 mL, 4.5 mmol) and TMEDA (0.420 g, 3.6 mmol) in THF (20 mL) for 6 h in accordance with general method B, then [(*E*)-5-iodo-pent-1-enyl]benzene (0.979 g, 3.6 mmol) in THF (7 mL) was added. After work-up, purification by column chromatography (5% EtOAc and 0.5% Et_3N in petroleum ether) gave **20** (0.697 g, 73%) as a slightly yellow oil. IR (film) 3027, 2947, 1792, 1486, 1449, 702 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 7.32–7.28 (m, 9H), 7.24–7.20 (m, 1H), 6.31 (d, *J*=15.8 Hz, 1H), 6.14 (dt, *J*=15.8, 6.8 Hz, 1H), 4.20 (d, *J*=13.3 Hz, 1H), 3.28 (d, *J*=13.3 Hz, 1H), 2.14 (q, *J*=6.8 Hz, 2H), 2.05 (t, *J*=5.7 Hz, 1H), 1.79 (s, 3H), 1.76 (s, 3H), 1.66–1.55 (m, 2H), 1.49–1.39 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 139.1, 137.8, 130.6, 130.2, 129.9, 128.5, 128.4, 128.3, 127.1, 126.8, 125.9, 104.0, 62.0, 44.0, 32.6, 31.8, 27.1, 20.6, 19.0; MS (ES) *m/z* 318 (MH^+); HRMS (EI^+) calcd for $C_{23}H_{27}N$ 317.2138, found 317.2142.

4.3.3. (1-Benzyl-2-isopropylideneaziridin-3-yl)phenylmethanol (21). Compound **6** (0.173 g, 1 mmol) was reacted with *sec*-BuLi (1.3 M in hexanes, 1.15 mL, 1.5 mmol) and TMEDA (0.140 g, 1.2 mmol) in THF (10 mL) for 6 h in accordance with general method B, then benzaldehyde (0.127 g, 1.2 mmol) was added. After work-up, purification by column chromatography (5% EtOAc and 0.5% Et_3N in petroleum ether) gave **21a** (after washing with *n*-pentane) (0.098 g, 35%) and **21b** (0.109 g, 39%) as white solids. Compound **21a**: mp 101–102 °C; IR (film) 3157, 2858, 1800, 1490, 1452, 1054, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 7.33–7.23 (m, 10H), 4.42 (dd, *J*=6.0, 4.5 Hz, 1H) 4.13 (d, *J*=12.9 Hz, 1H), 3.27 (d, *J*=12.9 Hz, 1H), 2.41–2.38 (m, 1H), 2.36 (d, *J*=6.0 Hz, 1H), 1.74 (s, 3H), 1.59 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 141.7, 138.5, 128.7, 128.6, 128.3, 127.59, 127.57, 126.03, 125.98, 106.0, 74.4, 61.6, 50.2, 20.5, 19.2; MS (ES) *m/z* 280 (MH^+); HRMS (ES) calcd for $C_{19}H_{22}NO$ 280.1696, found 280.1695. Compound **21b**: mp 102–103 °C; IR (film) 3131, 2851, 1494, 1447, 1252, 1036, 1024, 701 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 7.34–7.24 (m, 10H), 4.81 (dd, *J*=3.3, 1.5 Hz, 1H), 4.21 (d, *J*=13.5 Hz, 1H), 3.37 (d, *J*=13.5 Hz, 1H), 3.06 (d, *J*=1.5 Hz, 1H), 2.43 (d, *J*=3.3 Hz, 1H), 1.73 (s, 3H), 1.50 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 141.7, 138.3, 128.5, 128.2, 128.1, 127.6, 127.3, 126.0, 124.4, 106.5, 70.5, 60.7, 48.7, 20.7, 19.2; MS (ES) *m/z* 280 (MH^+); HRMS (ES) calcd for $C_{19}H_{22}NO$ 280.1696, found 280.1695.

4.3.4. (1-Benzyl-2-isopropylideneaziridin-3-yl)diphenylmethanol (22). Compound **6** (0.173 g, 1 mmol) was reacted with *sec*-BuLi (1.3 M in hexanes, 1.15 mL, 1.5 mmol) and TMEDA (0.140 g, 1.2 mmol) in THF (10 mL) for 6 h in accordance with general method B, then benzophenone (0.219 g, 1.2 mmol) in THF (1 mL) was added. After work-up, purification by recrystallisation (EtOAc/*n*-pentane) gave **22** (0.258 g, 73%) as a white crystalline solid. Mp 128–129 °C; IR (film) 3373, 3017, 2891, 1793, 1491, 1444, 1319, 1123, 1017, 742 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 7.41 (d, *J*=7.0 Hz, 2H), 7.31–7.13 (m, 13H), 4.04 (d, *J*=13.0 Hz, 1H), 3.79 (s, 1H), 3.65 (d, *J*=13.0 Hz, 1H), 3.08 (s, 1H), 1.64 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 146.6, 145.2, 137.6, 128.8, 128.3, 127.93, 127.86, 127.4, 126.9, 126.8, 126.5, 126.2, 123.9, 106.7, 74.5, 60.7, 51.0, 20.1, 19.5; MS (ES) *m/z* 356 (MH^+); HRMS (ES) calcd for $C_{25}H_{26}NO$ 356.2009, found 356.2010.

4.3.5. 1-Benzyl-2-isopropylidene-3-trimethylsilylaziridine (23). Compound **6** (0.173 g, 1 mmol) was reacted with *sec*-BuLi (1.3 M in hexanes, 1.15 mL, 1.5 mmol) and TMEDA (0.140 g, 1.2 mmol) in THF (10 mL) for 6 h in accordance with general method B, then chlorotrimethylsilane (0.130 g, 1.2 mmol) was added. After work-up, purification by column chromatography (5% EtOAc and 0.5% Et_3N in petroleum ether) gave **23** (0.155 g, 63%) as a colourless oil. IR (film) 2962, 2914, 1789, 1453, 1247, 836, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 7.35–7.28 (m, 4H), 7.27–7.22 (m, 1H), 4.39 (d, *J*=13.1 Hz, 1H), 2.90 (d, *J*=13.1 Hz, 1H), 1.78 (s, 3H), 1.73 (s, 3H), 1.24 (s, 1H), −0.10 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 139.3, 129.4, 128.4, 128.1, 127.0, 101.2, 64.2, 35.7, 20.3, 18.9, −2.8; MS (ES) *m/z* 246 (MH^+); HRMS (ES) calcd for $C_{15}H_{24}NSi$ 246.1673, found 246.1674.

4.3.6. (*Z*)-(1-Benzyl-2-ethylideneaziridin-3-yl)diphenylmethanol (24). Compound (*Z*)-**7** (0.200 g, 1.26 mmol) was reacted with *sec*-BuLi (1.3 M in hexanes, 1.25 mL, 1.63 mmol) and TMEDA (0.174 g, 1.50 mmol) for 5 h in

THF (10 mL) according to general method B, then benzophenone (0.297 g, 1.63 mmol) in THF (1 mL) was added. To remove unreacted benzophenone, the crude material was dissolved in EtOH (20 mL) and cooled to 0 °C. Sodium borohydride (0.108 g, 2.85 mmol) was added and the mixture allowed to warm to room temperature. After stirring for 4 h, the reaction was quenched by the dropwise addition of NH_4Cl solution, then basified using $NaHCO_3$ solution. The resulting mixture was extracted with Et_2O (3×25 mL) and the combined organic phases washed with brine (75 mL), dried ($MgSO_4$) and evaporated. Column chromatography (2% EtOAc in petroleum ether) gave (*Z*)-**24** (0.267 g, 62%) as a colourless oil, which crystallised on standing. Mp 87–89 °C; IR (film) 3444, 3058, 3028, 1785, 1600, 1493, 1447 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 7.36–7.08 (m, 15H), 5.13 (q, *J*=6.8 Hz, 1H), 4.03 (d, *J*=13.1 Hz, 1H), 3.66 (s, 1H), 3.63 (d, *J*=13.1 Hz, 1H), 2.93 (s, 1H), 1.69 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 146.4, 144.7, 137.0, 129.8, 128.9, 128.3, 128.1, 128.0, 127.5, 127.3, 126.8, 126.3, 97.0, 74.8, 60.3, 50.2, 13.4; MS (ES) *m/z* 342 (MH^+); HRMS (ES) calcd for $C_{24}H_{24}NO$ 342.1852, found 342.1855; Anal. Calcd for $C_{24}H_{23}NO$: C, 84.42; H, 6.79; N, 4.10%. Found: C, 84.34; H, 6.80; N, 4.00%.

4.3.7. (*E*)-(1-Benzyl-2-ethylideneaziridin-3-yl)diphenylmethanol (24). Compound (*E*)-**7** (0.200 g, 1.26 mmol) was reacted with *sec*-BuLi (1.3 M in hexanes, 1.25 mL, 1.63 mmol) and TMEDA (0.174 g, 1.50 mmol) in THF (10 mL) for 5 h according to general method B, then benzophenone (0.297 g, 1.63 mmol) in THF (1 mL) was added. To remove unreacted benzophenone, the crude material was dissolved in EtOH (20 mL) and cooled to 0 °C. Sodium borohydride (0.108 g, 2.85 mmol) was added and the mixture allowed to warm to room temperature. After stirring for 4 h, the reaction was quenched by the dropwise addition of NH_4Cl solution, then basified using $NaHCO_3$ solution. The resulting mixture was extracted with Et_2O (3×25 mL) and the combined organic phases washed with brine (75 mL), dried ($MgSO_4$) and evaporated. The product was purified (2% EtOAc in petroleum ether) to give a colourless oil, which crystallised on standing. Recrystallisation from acetone/water gave (*E*)-**24** (0.316 g, 73%) as clear colourless crystals. Mp 83–86 °C; IR (film) 3446 (br), 3060, 3028, 1782, 1600, 1494, 1449 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.37–7.14 (m, 15H), 5.09 (q, *J*=6.8 Hz, 1H), 3.82 (d, *J*=12.9 Hz, 1H), 3.68 (d, *J*=12.9 Hz, 1H), 3.64 (s, 1H), 3.03 (s, 1H), 1.26, (d, *J*=6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 146.9, 145.4, 138.1, 130.3, 129.0, 128.8, 128.5, 128.4, 127.9, 127.5, 127.1, 126.7, 97.7, 75.0, 62.0, 50.9, 14.4; MS (CI) *m/z* 342 (MH^+); HRMS (CI) calcd for $C_{24}H_{24}NO$ 342.1852, found 342.1861.

4.3.8. 1-Benzyl-2-cyclohexylidene-3-[3-(furan-2-yl)propyl]aziridine (25). Compound **8** (0.460 g, 2.16 mmol) was reacted with *sec*-BuLi (1.3 M in hexanes, 2.5 mL, 3.24 mmol) and TMEDA (0.300 g, 2.58 mmol) in THF (20 mL) for 5 h in accordance with general method B, then 1-iodo-3-(furan-2-yl)propane (560 mg, 2.37 mmol) was added. After work-up, purification by column chromatography (1.5% EtOAc and 0.5% Et_3N in petroleum ether) gave **25** (0.423 g, 61%) as a pale yellow oil. IR (film) 3086, 3062, 3028, 2923, 2850, 1794, 1592, 1446 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 7.40–7.29 (m, 6H), 6.28 (dd, *J*=3.1, 1.9 Hz, 1H), 5.92 (dd, *J*=3.1, 0.7 Hz, 1H), 4.16 (d, *J*=13.3 Hz, 1H), 3.22 (d, *J*=13.3 Hz, 1H), 2.57 (m, 2H), 2.21 (m, 4H), 2.05 (br s, 1H), 1.66–1.54 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) 156.1, 140.7, 139.1, 128.5, 128.3, 127.1, 127.0, 112.3, 110.0, 104.7, 62.5, 43.4, 31.8, 31.6, 30.1, 28.2, 27.9, 27.6, 26.6, 25.8; MS (EI) *m/z* 322 (MH^+); HRMS (EI) calcd for $C_{22}H_{26}NO$ 320.2014, found 320.2014.

4.3.9. 1-Cyclohexyl-2-isopropylidene-3-methylaziridine (26). Compound **9** (0.200 g, 1.21 mmol) was reacted with *sec*-BuLi (1.0 M in cyclohexane/hexane (98/2), 1.58 mL, 1.58 mmol) and TMEDA (0.169 g, 1.45 mmol) in THF (10 mL) for 5 h in accordance with general method B, then iodomethane (0.258 g, 1.82 mmol) was added. After work-up, purification by column chromatography (0.5% Et_3N in petroleum ether) gave **26** (0.138 g, 64%) as a yellow oil. IR (film) 2924, 2852, 1798, 1444, 1173, 1147, 891 cm^{-1}; ^1H NMR (400 MHz, C_6D_6) 2.30–2.14 (m, 1H), 1.91 (s, 3H), 1.87 (s, 3H), 1.88–1.72 (m, 5H), 1.68–1.46 (m, 3H), 1.36 (d, *J*=5.6 Hz, 3H), 1.30–1.13 (m, 3H); ^{13}C NMR (100 MHz, C_6D_6) 131.6, 100.9, 66.1, 36.3, 33.6, 32.8, 26.1, 24.7 (2C), 21.0, 19.7, 18.3; MS (CI) *m/z* 180 (MH^+); HRMS (CI) calcd for $C_{12}H_{22}N$ 180.1752, found 180.1749.

4.3.10. 1-Cyclohexyl-2-isopropylidene-3-trimethylsilylaziridine (27). Compound **9** (0.200 g, 1.21 mmol) was reacted with *sec*-BuLi (1.0 M in cyclohexane/hexane (98/2), 1.58 mL, 1.58 mmol) and TMEDA (0.169 g, 1.45 mmol) in THF (10 mL) for 5 h in accordance with general method B, then chlorotrimethylsilane (0.199 g, 1.83 mmol) was added. After work-up, purification by column chromatography (0.5% Et_3N in petroleum ether) gave **27** (0.183 g, 64%) as a colourless oil. IR (film) 2929, 2847, 1777, 1444, 1239, 1106, 866, 840 cm^{-1}; ^1H NMR (400 MHz, C_6D_6) 2.23–2.12 (m, 1H), 1.94 (s, 3H), 1.87 (s, 3H), 1.86–1.75 (m, 3H), 1.74–1.66 (m, 2H), 1.61–1.53 (m, 1H), 1.51–1.42 (m, 1H), 1.31–1.15 (m, 3H), 1.14–1.07 (m, 1H), 0.21 (s, 9H); ^{13}C NMR (100 MHz, C_6D_6) 130.0, 99.3, 68.5, 33.9, 33.2, 32.5, 26.1, 24.82, 24.81, 21.1, 19.6, −2.7; MS (EI) *m/z* 237 (M^+); HRMS (EI) calcd for $C_{14}H_{27}NSi$ 237.1913, found 237.1922.

4.3.11. 3-Benzyl-1-cyclohexyl-2-methyleneaziridine (28). Compound **10** (0.535 g, 3.90 mmol) was reacted with *sec*-BuLi (1.4 M in hexanes, 3.06 mL, 4.28 mmol) and TMEDA (0.498 g, 4.29 mmol) in THF (5 mL) for 3.5 h in accordance with general method B, then benzyl bromide (0.733 g, 4.29 mmol) was added. After work-up, purification by filtration through a short plug of basic alumina (petroleum ether) followed by bulb-to-bulb distillation of the unreacted starting materials gave **28** (0.764 g, 86%) as a clear, yellow oil. IR (film) 3028, 2828, 1770, 1496, 1451, 1165, 698 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.34–7.27 (m, 5H), 4.74 (s, 1H), 4.68 (s, 1H), 2.93–2.80 (m, 2H), 2.12 (t, *J*=6.3 Hz, 1H), 1.98–1.88 (m, 1H), 1.85–1.62 (m, 3H), 1.60–1.38 (m, 3H), 1.32–1.07 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) 141.9, 139.3, 128.9, 128.4, 126.4, 82.7, 66.9, 42.6, 39.4, 32.9, 32.4, 25.7, 24.57, 24.51; MS (EI) *m/z* 227 (M^+); HRMS (ES) calcd for $C_{16}H_{22}N$ (MH^+) 228.1752, found 228.1756; Anal. Calcd for $C_{16}H_{21}N$: C, 84.52; H, 9.32; N, 6.16%. Found: C, 84.51; H, 9.64; N, 5.93%.

4.3.12. 3-(4-Chlorobutyl)-1-cyclohexyl-2-methyleneaziridine (29). Compound **10** (0.190 g, 1.39 mmol) was reacted with *sec*-BuLi (1.3 M in hexanes, 1.16 mL, 1.51 mmol) and TMEDA (0.175 g, 1.51 mmol) in THF (5 mL) for 3.5 h in accordance with general method B, then 1-chloro-4-iodobutane (0.275 g, 1.26 mmol) was added. After work-up, purification by filtration through a short plug of basic alumina (petroleum ether) followed by bulb-to-bulb distillation of the unreacted starting materials gave **29** (0.200 g, 70% based on electrophile) as a clear, light yellow oil. IR (film) 2931, 2855, 1771, 1449, 1265, 1173, 739 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 4.67 (s, 1H), (s, 1H), 3.55 (t, J=6.6 Hz, 2H), 1.87–1.17 (m, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) 142.1, 82.3, 66.8, 44.9, 41.2, 32.9, 32.5, 32.3, 31.6, 25.7, 24.8, 24.59, 24.57; MS (CI) m/z 230 (MH^+: ^{37}Cl), 228 (MH^+: ^{35}Cl); HRMS (ES) calcd for $C_{13}H_{23}NCl$ 228.1519, found 228.1518.

4.3.13. 1-Cyclohexyl-2-methylene-3-(tri-*n*-butylstannyl)-aziridine (30). Compound **10** (0.311 g, 2.27 mmol) was reacted with *sec*-BuLi (1.3 M in hexanes, 1.92 mL, 2.50 mmol) and TMEDA (0.290 g, 2.49 mmol) in THF (5 mL) for 3.5 h in accordance with general method B, then tri-*n*-butyltin chloride (0.701 g, 2.16 mmol) was added. After work-up, purification by bulb-to-bulb distillation of the unreacted starting materials followed by filtration through a short plug of basic alumina (petroleum ether) gave **30** (0.833 g, 91% based on electrophile) as a clear light yellow oil. IR (film) 2928, 2854, 1761, 1456, 1362, 1210, 1128, 806 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 4.66 (s, 1H), 4.41 (s, 1H), 1.89–1.19 (m, 24H), 0.95–0.86 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) 143.5, 80.1, 69.8, 33.3, 32.7, 31.7, 29.1, 29.0, 27.3, 25.8, 24.66, 24.62, 13.7, 9.5; MS (CI) m/z 428 (MH^+); HRMS (ES) calcd for $C_{21}H_{42}NSn$ 428.2339, found 428.2342.

4.3.14. (1′*S*,3*R*)- and (1′*S*,3*S*)-3-Benzyl-2-methylene-1-(1-phenylethyl)aziridine (31). Compound (*S*)-**12** (0.200 g, 1.26 mmol) was reacted with *sec*-BuLi (1.0 M in cyclohexane/hexane (98/2), 1.51 mL, 1.51 mmol) and TMEDA (0.175 g, 1.51 mmol) in THF (10 mL) for 7 h in accordance with general method B, then benzyl bromide (0.320 g, 1.87 mmol) was added. After work-up, purification by distillation (170 °C/1 mmHg) gave **31** (0.220 g, 70%) as a 47:53 mixture of diastereomers and as a colourless oil. IR (film) 3062, 3032, 2965, 2919, 2832, 1767, 1495, 1449, 1152, 830, 748, 702 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 7.37–7.20 (m, 7H), 7.19–7.11 (m, 2H), 7.06–7.01 (m, 1H), 4.87 (s, 0.47H), 4.75 (s, 0.47H), 4.56 (s, 0.53H), 4.26 (s, 0.53H), 3.03 (q, J=6.7 Hz, 0.47H), 2.99 (q, J=6.7 Hz, 0.53H), 2.94–2.86 (m, 1.53H), 2.71 (dd, J=14.3, 6.4 Hz, 0.47H), 2.18 (t, J=6.4 Hz, 0.53H), 2.12 (t, J=6.4 Hz, 0.47H), 1.51 (d, J=6.7 Hz, 1.41H), 1.26 (d, J=6.7 Hz, 1.59H); ^{13}C NMR (100 MHz, $CDCl_3$) 143.94, 143.90, 142.3, 141.6, 139.2, 138.7, 129.0, 128.6, 128.49, 128.47, 128.4, 128.3, 127.2, 127.1, 126.8, 126.4, 126.2, 125.9, 83.5, 83.4, 68.2, 67.6, 43.3, 42.6, 39.1, 38.9, 23.9, 23.1; MS (EI) m/z 249 (M^+); HRMS (CI) calcd for $C_{18}H_{20}N$ 250.1595, found 250.1593.

4.3.15. [(1′*S*,3*R*)- and (1′*S*,3*S*)-2-Methylene-1-(1-phenylethyl)aziridin-3-yl]diphenylmethanol (32). Compound (*S*)-**12** (0.200 g, 1.26 mmol) was reacted with *sec*-BuLi (1.4 M in cyclohexane, 1.08 mL, 1.51 mmol) and TMEDA (0.174 g, 1.50 mmol) in THF (10 mL) for 5 h in accordance with general method B, then benzophenone (0.297 g, 1.63 mmol) in THF (1 mL) was added. After work-up, the residue was redissolved in ethanol (20 mL) and cooled to 0 °C. Sodium borohydride (0.108 g, 2.85 mmol) was added and the solution allowed to warm to room temperature and stirred for 4 h. The reaction was quenched with saturated NH_4Cl solution, then basified using saturated $NaHCO_3$ solution. The resulting mixture was extracted with diethyl ether (3×25 mL) and the combined organic extracts dried ($MgSO_4$), filtered and the solvent removed in vacuo. Impurities were removed by distillation (140 °C/0.2 mmHg) to give **32** (0.301 g, 70%) as a pale yellow oil. IR (film) 3451, 3087, 3060, 3028, 1771, 1658, 1599, 1493, 1448 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 7.80 (d, J=8.3 Hz, 0.4H), 7.62–6.86 (m, 14.6H), 4.93 (s, 0.2H), 4.73 (s, 0.2H), 4.67 (s, 0.8H), 4.41 (d, J=1.4 Hz, 0.8H), 3.74 (d, J=1.4 Hz, 0.8H), 3.49 (d, J=1.4 Hz, 0.2H), 3.31 (q, J=6.6 Hz, 0.2H), 3.22 (q, J=6.6 Hz, 0.8H), 2.94 (s, 0.8H), 2.90 (s, 0.2H), 1.48 (d, J=6.6 Hz, 0.6H), 0.94 (d, J=6.6 Hz, 2.4H); ^{13}C NMR (100 MHz, $CDCl_3$) 146.4, 145.6, 144.7, 144.4, 143.0, 142.3, 137.3, 136.5, 132.5, 130.1, 128.38, 128.36, 128.2, 128.1, 128.0, 127.7, 127.57, 127.54, 127.48, 127.24, 127.17, 127.1, 127.04, 126.99, 126.7, 126.6, 126.4, 126.2, 84.8, 74.9, 50.4, 49.1, 23.1, 22.7; MS (ES) m/z 342 (MH^+); HRMS (ES) calcd for $C_{24}H_{24}NO$ 342.1852, found 342.1849.

4.3.16. (1′*S*,3*R*)- and (1′*S*,3*S*)-(*E*)-3-Benzyl-2-ethylidene-1-(1-phenylethyl)aziridine (33). Compound (*E*)-**2** (0.218 g, 1.26 mmol) was reacted with *sec*-BuLi (1.3 M in hexanes, 1.25 mL, 1.63 mmol) and TMEDA (0.174 g, 1.50 mmol) in THF (10 mL) for 5 h in accordance with general method B, then benzyl bromide (0.279 g, 1.63 mmol) was added. After work-up, purification by column chromatography (10% Et_2O in petroleum ether) gave successively (*E*)-**33a** (0.059 g, 18%) and (*E*)-**33b** (0.108 g, 33%) as colourless oils. Compound (*E*)-**33a**: IR (film) 3062, 3028, 2954, 2921, 2858, 1780, 1686, 1493, 1453 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.40–7.20 (m, 10H), 4.71 (q, J=6.6 Hz, 1H), 2.93–2.85 (m, 3H), 2.20 (t, J=6.3 Hz, 1H), 1.57 (d, J=6.6 Hz, 3H), 1.47 (d, J=6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 144.7, 140.0, 135.1, 129.5, 128.8, 128.6, 127.8, 127.4, 126.8, 95.1, 68.4, 44.4, 39.9, 23.6, 14.9; MS (ES) 264 (MH^+); HRMS (EI) calcd for $C_{19}H_{20}N$ 262.1596, found 262.1583. Compound (*E*)-**33b**: IR (film) 3060, 3027, 2968, 2923, 2854, 1776, 1602, 1493 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.40–7.01 (m, 10H), 5.33 (q, J=6.6 Hz, 1H), 2.93 (q, J=6.8 Hz, 1H), 2.93 (dd, J=14.0, 5.9 Hz, 1H), 2.69 (dd, J=14.0, 6.7 Hz, 1H), 2.16 (dd, J=6.7, 5.9 Hz, 1H), 1.65 (d, J=6.7 Hz, 3H), 1.47 (d, J=6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 144.7, 139.4, 135.8, 129.5, 128.7, 128.6, 127.4, 127.1, 126.5, 95.5, 68.9, 43.7, 39.6, 24.4, 15.1; MS (EI) m/z 263 (M^+); HRMS (EI) calcd for $C_{19}H_{21}N$ 262.1596, found 262.1612.

4.3.17. [(1′*S*,3*S*)-(*E*)-2-Ethylidene-1-(1-phenylethyl)aziridin-3-yl]diphenylmethanol (34). Compound (*E*)-**2** (0.218 g, 1.26 mmol) was reacted with *sec*-BuLi (1.3 M in hexanes, 1.25 mL, 1.63 mmol) and TMEDA (0.174 g, 1.50 mmol) in THF (10 mL) for 5 h in accordance with general method B, then benzophenone (0.297 g, 1.63 mmol) in

THF (1 mL) was added. After work-up, the residue was redissolved in ethanol (20 mL) and cooled to 0 °C. Sodium borohydride (0.108 g, 2.85 mmol) was added and the solution allowed to warm to room temperature and stirred for 4 h. The reaction was quenched with saturated NH_4Cl solution, then basified using saturated $NaHCO_3$ solution. The resulting mixture was extracted with diethyl ether (3× 25 mL) and the combined organic extracts dried ($MgSO_4$), filtered and the solvent removed in vacuo. Purification by column chromatography (10% Et_2O in petroleum ether) gave (*E*)-**34** (0.302 g, 68%) as a colourless oil. IR (film) 3441 (br), 3057, 2966, 1778, 1598, 1491, 1447 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.39–6.92 (m, 15H), 4.78 (q, *J*=6.8 Hz, 1H), 3.97 (s, 1H), 3.16 (q, *J*=6.6 Hz, 1H), 3.09 (s, 1H), 1.37, (d, *J*=6.8 Hz, 3H), 0.98 (d, *J*=6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 147.0, 145.4, 144.0, 129.9, 128.7, 128.5, 128.4, 127.64, 127.57, 127.4, 126.9, 126.7, 97.4, 74.7, 67.1, 51.2, 23.5, 14.3; MS (EI) *m/z* 355 (M^+); HRMS (EI) calcd for $C_{25}H_{25}NO$ 355.1936, found 355.1952.

4.3.18. (1′*S*,3*R*)- and (1′*S*,3*S*)-(*Z*)-3-Benzyl-2-ethylidene-1-(1-phenylethyl)aziridine (33). Compound (*Z*)-**2** (0.218 g, 1.26 mmol) was reacted with *sec*-BuLi (1.3 M in hexanes, 1.25 mL, 1.63 mmol) and TMEDA (0.174 g, 1.50 mmol) in THF (10 mL) for 5 h in accordance with general method B, then benzyl bromide (0.280 g, 1.64 mmol) was added. After work-up, purification by column chromatography (10% Et_2O in petroleum ether) gave successively (*Z*)-**33a** (0.138 g, 42%) and (*Z*)-**33b** (0.057 g, 17%) as colourless oils. Compound (*Z*)-**33a**: IR (film) 3060, 3027, 2968, 2920, 1779, 1601, 1493, 1448, 1306, 1132, 1028, 745, 695 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.40–7.18 (m, 10H), 5.02 (q, *J*=6.8 Hz, 1H), 2.95 (q, *J*=6.6 Hz, 1H), 2.86 (d, *J*=6.4 Hz, 2H), 2.17 (t, *J*=6.4 Hz, 1H), 1.26 (d, *J*=6.6 Hz, 3H), 1.06 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 145.1, 139.4, 135.1, 129.3, 128.78, 128.76, 127.8, 127.6, 126.7, 95.6, 68.4, 44.0, 39.9, 23.7, 13.6; MS (EI) *m/z* 263 (M^+); HRMS (EI) calcd for $C_{19}H_{20}N$ 262.1596, found 262.1590. Compound (*Z*)-**33b**: IR (film) 3063, 3028, 2973, 2928, 1779, 1604, 1494, 1453 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.40–7.00 (m, 10H), 5.18 (q, *J*=6.8 Hz, 1H), 3.18 (q, *J*=6.6 Hz, 1H), 2.84 (dd, *J*=14.0, 5.3 Hz, 1H), 2.62 (dd, *J*=14.0, 7.3 Hz, 1H), 2.17 (dd, *J*=7.3, 5.3 Hz, 1H), 1.90 (d, *J*=6.6 Hz, 3H), 1.57 (d, *J*=6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 144.4, 139.5, 135.8, 129.0, 128.7, 128.6, 127.5, 127.3, 126.5, 95.6, 67.7, 42.4, 39.6, 24.4, 14.4; MS (EI) *m/z* 263 (M^+); HRMS (EI) calcd for $C_{19}H_{20}N$ 262.1596, found 262.1603.

4.3.19. (1′*S*,3*S*)-(*Z*)-2-Ethylidene-1-(1-phenylethyl)aziridin-3-yl)diphenylmethanol (34). Compound (*Z*)-**2** (0.218 g, 1.26 mmol) was reacted with *sec*-BuLi (1.3 M in hexanes, 1.25 mL, 1.63 mmol) and TMEDA (0.174 g, 1.50 mmol) in THF (10 mL) for 5 h in accordance with general method B, then benzophenone (0.297 g, 1.63 mmol) in THF (1 mL) was added. After work-up, the residue was redissolved in ethanol (20 mL) and cooled to 0 °C. Sodium borohydride (0.108 g, 2.85 mmol) was added and the solution allowed to warm to room temperature and stirred for 4 h. The reaction was quenched with saturated NH_4Cl solution, then basified using saturated $NaHCO_3$ solution. The resulting mixture was extracted with diethyl ether (3×25 mL) and the combined organic extracts dried ($MgSO_4$), filtered and the solvent removed in vacuo. Column chromatography (10% Et_2O in petroleum ether) gave (*Z*)-**34** (0.371 g, 83%) as a colourless oil, which crystallised on standing. $[\alpha]_D^{21}$ −11.4 (*c* 2.2, $CHCl_3$); mp 102–105 °C; IR (film) 3422, 3053, 3028, 1782, 1595, 1490, 1445 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.55–7.20 (m, 15H), 5.09 (q, *J*=6.6 Hz, 1H), 3.88 (s, 1H), 3.16 (q, *J*=6.8 Hz, 1H), 2.93 (s, 1H), 1.08 (d, *J*=6.8 Hz, 3H), 0.85 (d, *J*=6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 146.7, 144.5, 143.8, 129.4, 128.2, 128.0, 127.8 (2C), 127.23, 127.18, 127.0, 126.9, 126.7, 126.0, 97.2, 74.6, 66.3, 50.0, 22.9, 12.9; MS (EI) *m/z* 355 (M^+); HRMS (EI) calcd for $C_{25}H_{25}NO$ 355.1936, found 355.1918; Anal. Calcd for $C_{25}H_{25}NO$: C, 84.45; H, 7.09; N, 3.94%. Found: C, 84.40; H, 7.07; N, 3.92%.

4.3.20. (1′*S*,3*R*)-2-Isopropylidene-3-methyl-1-(1-phenylethyl)aziridine (35). Compound **13** (0.200 g, 1.07 mmol) was reacted with *sec*-BuLi (1.0 M in cyclohexane/hexane (98/2), 1.39 mL, 1.39 mmol) and TMEDA (0.149 g, 1.28 mmol) in THF (8 mL) for 5 h in accordance with general method B, then iodomethane (0.227 g, 1.60 mmol) was added. After work-up, purification by column chromatography (0.5% Et_3N in petroleum ether) gave **35** as a single diastereomer (0.100 g, 47%) and as a yellow oil. $[\alpha]_D^{21}$ −235 (*c* 1.01, $CHCl_3$); IR (film) 3032, 2970, 2919, 2847, 1798, 1449, 1147, 702 cm^{-1}; ^1H NMR (400 MHz, C_6D_6) 7.57–7.53 (m, 2H), 7.31–7.24 (m, 2H), 7.21–7.15 (m, 1H), 2.93 (q, *J*=6.5 Hz, 1H), 1.90 (q, *J*=5.6 Hz, 1H), 1.77 (s, 3H), 1.46 (d, *J*=6.5 Hz, 3H), 1.42 (d, *J*=5.6 Hz, 3H), 1.31 (s, 3H); ^{13}C NMR (100 MHz, C_6D_6) 146.0, 131.2, 128.3, 127.5, 127.1, 102.3, 68.2, 37.6, 24.0, 20.8, 19.0, 18.1; MS (CI) *m/z* 202 (MH^+); HRMS (CI) calcd for $C_{14}H_{20}N$ 202.1595, found 202.1605.

4.3.21. (1′*S*,3*R*)- and (1′*S*,3*S*)-3-Butyl-2-isopropylidene-1-(1-phenylethyl)aziridine (36). Compound **13** (0.200 g, 1.07 mmol) was reacted with *sec*-BuLi (1.0 M in cyclohexane/hexane (98/2), 1.39 mL, 1.39 mmol) and TMEDA (0.149 g, 1.28 mmol) in THF (8 mL) for 5 h in accordance with general method B, then 1-iodobutane (0.294 g, 1.60 mmol) was added. After work-up, purification by column chromatography (0.5% Et_3N in petroleum ether) gave **36** as a 93:7 mixture of diastereomers (0.137 g, 53%) and as a yellow oil. IR (film) 3027, 2965, 2929, 2852, 1792, 1449, 1229, 758, 691 cm^{-1}; ^1H NMR (400 MHz, C_6D_6) *Major diastereomer*: 7.59–7.55 (m, 2H), 7.31–7.24 (m, 2H), 7.20–7.13 (m, 1H), 2.93 (q, *J*=6.6 Hz, 1H), 1.94–1.90 (m, 1H), 1.81 (s, 3H), 1.79–1.50 (m, 4H), 1.49 (d, *J*=6.6 Hz, 3H), 1.45–1.36 (m, 2H), 1.32 (s, 3H), 0.99 (t, *J*=7.3 Hz, 3H); ^{13}C NMR (100 MHz, C_6D_6) *Major diastereomer*: 146.1, 130.7, 128.3, 127.1 (2C), 102.3, 68.2, 42.6, 32.6, 30.0, 24.2, 22.9, 21.1, 19.1, 14.0; MS (CI) *m/z* 244 (MH^+); HRMS (CI) calcd for $C_{17}H_{26}N$ 244.2065, found 244.2074.

4.3.22. (1′*S*,3*R*)-3-Benzyl-2-isopropylidene-1-(1-phenylethyl)aziridine (37). Compound **13** (0.200 g, 1.07 mmol) was reacted with *sec*-BuLi (1.0 M in cyclohexane/hexane (98/2), 1.39 mL, 1.39 mmol) and TMEDA (0.149 g, 1.28 mmol) in THF (8 mL) for 5 h in accordance with general method B, then benzyl bromide (0.274 g, 1.60 mmol) was added. After work-up, purification by column chromatography (0.5% Et_3N in petroleum ether) gave **37** (0.201 g,

68%) as a single diastereomer and as a colourless oil. $[\alpha]_D^{22}$ −152 (*c* 1.0, $CHCl_3$); IR (film) 3062, 3027, 2960, 2919, 2847, 1787, 1598, 1495, 1444, 1127, 758, 697 cm^{-1}; ^1H NMR (400 MHz, C_6D_6) 7.53–7.51 (m, 2H), 7.39–7.33 (m, 2H), 7.30–7.22 (m, 4H), 7.21–7.15 (m, 2H), 2.97 (dd, *J*=13.8, 5.6 Hz, 1H), 2.92 (dd, *J*=13.8, 6.9 Hz, 1H), 2.86 (q, *J*=6.6 Hz, 1H), 2.15 (dd, *J*=6.9, 5.6 Hz, 1H), 1.72 (s, 3H), 1.30 (s, 3H), 1.25 (d, *J*=6.6 Hz, 3H); ^{13}C NMR (100 MHz, C_6D_6) 145.9, 140.0, 130.2, 129.3, 128.36, 128.32, 127.5, 127.1, 126.3, 103.0, 68.2, 44.1, 39.8, 24.1, 21.1, 19.1; MS (EI) *m/z* 277 (M^+); HRMS (EI) calcd for $C_{20}H_{23}N$ 277.1830, found 277.1843.

4.3.23. (1′*S*,3*R*)-3-Allyl-2-isopropylidene-1-(1-phenylethyl)aziridine (38). Compound **13** (0.200 g, 1.07 mmol) was reacted with *sec*-BuLi (1.0 M in cyclohexane/hexane (98/2), 1.39 mL, 1.39 mmol) and TMEDA (0.149 g, 1.28 mmol) in THF (8 mL) for 5 h in accordance with general method B, then allyl bromide (0.189 g, 1.56 mmol) was added. After work-up, purification by column chromatography (0.5% Et_3N in petroleum ether) gave **38** (0.153 g, 63%) as a single diastereomer and as a colourless oil. $[\alpha]_D^{22}$ −210 (*c* 1.03, $CHCl_3$); IR (film) 3073, 3027, 2970, 2919, 2852, 1798, 1644, 1490, 1454, 1372, 1219, 1137, 988, 912, 758, 702 cm^{-1}; ^1H NMR (400 MHz, C_6D_6) 7.55–7.52 (m, 2H), 7.30–7.25 (m, 2H), 7.21–7.16 (m, 1H), 6.10–6.00 (m, 1H), 5.24–5.12 (m, 2H), 2.90 (q, *J*=6.6 Hz, 1H), 2.46 (dd, *J*=6.8, 5.9 Hz, 2H), 1.94 (br t, *J*=5.9 Hz, 1H), 1.78 (s, 3H), 1.46 (d, *J*=6.6 Hz, 3H), 1.29 (s, 3H); ^{13}C NMR (100 MHz, C_6D_6) 145.9, 136.0, 129.9, 128.3, 127.5, 127.1, 116.1, 102.9, 68.0, 42.0, 37.6, 24.2, 21.1, 19.1; MS (CI) *m/z* 228 (MH^+); HRMS (CI) calcd for $C_{16}H_{22}N$ 228.1752, found 228.1756.

4.3.24. [(1′*S*,3*S*)-2-Isopropylidene-1-(1-phenylethyl)aziridin-3-yl]diphenylmethanol (39). Compound **13** (0.200 g, 1.07 mmol) was reacted with *sec*-BuLi (1.0 M in cyclohexane/hexane (98/2), 1.39 mL, 1.39 mmol) and TMEDA (0.149 g, 1.28 mmol) in THF (8 mL) for 5 h in accordance with general method B, then benzophenone (0.292 g, 1.60 mmol) in THF (1 mL) was added. After work-up, the residue was redissolved in ethanol (15 mL) and cooled to 0 °C. Sodium borohydride (0.090 g, 2.38 mmol) in ethanol (3 mL) was added and the solution allowed to warm to room temperature and stirred for a further 4 h. The reaction was quenched with NH_4Cl solution. The aqueous phase was basified using $NaHCO_3$ then extracted with diethyl ether. The combined organic phases were dried ($MgSO_4$), and the solvent was removed under reduced pressure. Purification by column chromatography (0.5% Et_3N in petroleum ether) gave **39** as a single diastereomer (0.168 g, 43%) and as a white solid. $[\alpha]_D^{22}$ −111 (*c* 1.01, $CHCl_3$); IR (KBr) 3293, 2975, 2916, 2853, 1444, 1135, 754, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 7.67–7.56 (m, 4H), 7.40–7.26 (m, 7H), 7.25–7.21 (m, 4H), 4.08 (s, 1H), 3.18 (t, *J*=6.6 Hz, 1H), 3.15 (s, 1H), 1.36 (s, 3H), 1.08 (s, 3H), 0.91 (d, *J*=6.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 147.0, 145.3, 144.5, 128.3, 128.1, 128.0, 127.3, 127.0, 126.8, 126.3, 126.1, 123.9, 107.0, 74.2, 66.6, 50.7, 23.4, 20.6, 19.5; MS (CI) *m/z* 370 (MH^+); HRMS (CI) calcd for $C_{26}H_{28}NO$ 370.2171, found 370.2185; Anal. Calcd for $C_{26}H_{27}NO$: C, 84.51; H, 7.37; N, 3.79%. Found: C, 84.63; H, 7.55; N, 3.46%.

4.3.25. (1′*S*,3*R*)-2-Isopropylidene-1-(1-phenylethyl)-3-trimethylsilylaziridine (40). Compound **13** (0.200 g, 1.07 mmol) was reacted with *sec*-BuLi (1.0 M in cyclohexane/hexane (98/2), 1.39 mL, 1.39 mmol) and TMEDA (0.149 g, 1.28 mmol) in THF (8 mL) for 5 h in accordance with general method B, then chlorotrimethylsilane (0.175 g, 1.61 mmol) was added. After work-up, purification by column chromatography (0.5% Et_3N in petroleum ether) gave **40** as a single diastereomer (0.222 g, 80%) and as a colourless oil. $[\alpha]_D^{21}$ −133 (*c* 1.07, $CHCl_3$); IR (film) 3062, 3021, 2960, 2919, 2847, 1782, 1490, 1444, 1265, 1091, 1004, 866, 830, 753, 691 cm^{-1}; ^1H NMR (400 MHz, C_6D_6) 7.55–7.52 (m, 2H), 7.30–7.23 (m, 2H), 7.21–7.18 (m, 1H), 2.82 (q, *J*=6.5 Hz, 1H), 1.77 (s, 3H), 1.43 (d, *J*=6.5 Hz, 3H), 1.32 (s, 3H), 1.20 (s, 1H), 0.24 (s, 9H); ^{13}C NMR (100 MHz, C_6D_6) 146.0, 129.5, 128.3, 127.5, 127.1, 100.4, 70.3, 33.8, 24.4, 20.8, 18.9, −2.7; MS (CI) *m/z* 260 (MH^+); HRMS (CI) calcd for $C_{16}H_{26}NSi$ 260.1835, found 260.1824.

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Four different types of hydrogen bonds observed in 1,2-bis(*N*-benzenesulfonylamino)benzenes due to conformational properties of the sulfonamide moiety

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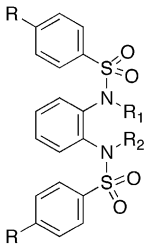
Abstract—The crystal structures of 1,2-bis(*N*-benzenesulfonylamino)benzenes with secondary and/or tertiary sulfonamide groups were determined by X-ray crystallographic analysis. Every Ar-sulfonamide group existed in synclinal conformation in the crystals even though it was secondary or tertiary. Each compound showed different types of hydrogen bonds in the crystal structure. 1,2-Bis(*N*-benzenesulfonylamino)-benzene (**1**) formed two double hydrogen bonds connected to the next molecules, 1-(*N*-benzenesulfonylamino)-2-(*N*-benzenesulfonyl-*N*-methylamino)benzene (**2**) contained double hydrogen bond involved by both the sulfonamide moieties, 1,2-bis(*N*-4-toluenesulfonylamino)-benzene (**3**) had both intra- and intermolecular hydrogen bonds, and 1-(*N*-methyl-*N*-4-toluenesulfonylamino)-2-(*N*-4-toluenesulfonylamino)-benzene (**4**) had one double hydrogen bond involved by only one sulfonamide moiety. Sulfonamides **1** and **3** formed infinite arrays of the molecules, and sulfonamides **2** and **4** formed racemic dimer of their conformational enantiomers via the hydrogen bonds.

1. Introduction

An aromatic sulfonamide moiety is one of the important structural fragments that is commonly seen in biologically active compounds.[1] Recently, several groups have used the aromatic sulfonamide structure to construct molecular recognition molecules,[2] or nanoporous networks in crystalline states.[3] Although Adsmond and Grant[4] discussed hydrogen bonding topology and Näther et al. reported on effects of the crystal solvent on the conformation of *p*-phenylenediamine derivatives with secondary sulfonamides,[5] the conformational properties of aromatic sulfonamides have not been well studied compared to the stereochemistry of the aromatic amides.[6,7] We investigated *o*-phenylenediamine derivatives showing interesting optical properties in the crystalline state,[8] and confirmed that the sulfonamide moiety of *o*-phenylenediamine derivatives existed in synclinal conformation[8d] even though the sulfonamide was secondary or tertiary. Due to this synclinal conformation, that is, by directing the amide hydrogen and the sulfonyl oxygen to the same side, secondary sulfonamides of *o*-phenylenediamine can form double hydrogen bonds intermolecularly.



	R	R_1	R_2
1	H	H	H
2	H	CH_3	H
3	CH_3	H	H
4	CH_3	CH_3	H

In this study, we demonstrated that four different types of hydrogen bonds resulting from a combination of inter- and/or intramolecular and single or self-complementary double hydrogen bonds formed in the crystals of aromatic sulfonamides of *o*-phenylenediamine.

2. Results and discussion

The crystal structure of 1,2-bis(*N*-benzenesulfonylamino)-benzene (**1**) was very recently reported by Bryan et al.[9]

Keywords: Aromatic sulfonamide; Synclinal conformation; Hydrogen bond; X-ray structure.
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Table 1. Crystal data for compounds **2**–**4**

Crystal	**2**	**3**	**4**
Formula	$C_{19}H_{18}N_2O_4S_2$	$C_{20}H_{20}N_2O_4S_2$	$C_{21}H_{22}N_2O_4S_2$
Mol wt	402.47	416.09	430.53
Crystal system	Monoclinic	Orthorhombic	Triclinic
Space group	$P2_1/n$	$Pccn$	P-1
a (Å), α (°)	12.9598 (7)	37.065 (8)	10.0135 (7), 90.9480 (10)
b (Å), β (°)	11.2294 (6), 93.0170 (10)	8.4075 (18)	10.2644 (8), 92.9720 (10)
c (Å), γ (°)	13.4081 (7)	13.006 (3)	10.7915 (8), 108.3800 (10)
V (Å^3)	1948.59 (18)	4053.0 (15)	1050.55 (13)
D_c (Mg m^{-3})	1.372	1.365	1.361
Z	4	8	2
R_1 [$I>2\sigma(I)$]	0.0378	0.0560	0.0420
wR_2 [$I>2\sigma(I)$]	0.0920	0.1383	0.1026
CCDC no.	607420	607418	607419

The other 1,2-bis(*N*-benzenesulfonylamino)benzenes (**2**–**4**) were prepared from *o*-phenylenediamine or *N*-methyl-1,2-phenylenediamine and corresponding sulfonyl chlorides. For each compound, recrystallization from ethyl acetate produced colorless prisms, which were suitable for X-ray crystallographic analysis. Crystal data are summarized in Table 1.

The crystal of 1,2-bis(*N*-benzenesulfonylamino)benzene (**1**) belonged to the space group $P2_1/c$.[9] This compound has two secondary sulfonamide bonds that form S═O···H–N hydrogen bonds. In the crystal, a complementary double hydrogen bond between adjacent molecules was observed, and both enantiomeric conformers were alternately arranged by double hydrogen bonds, forming an infinite chain of molecules (Table 2, Fig. 1a) as reported in Ref. 9. The crystal of 1-(*N*-benzenesulfonylamino)-2-(*N*-benzenesulfonyl-*N*-methylamino)benzene (**2**) belonged to the space group $P2_1/n$. In this compound, one of the two sulfonamide bonds is secondary, and the other is tertiary. In the crystal, both enantiomers formed dimer through a self-complementary double hydrogen bond between the amide proton of the secondary sulfonamide group and the oxygen of the tertiary sulfonamide group, but not the oxygen of the secondary sulfonamide group (Table 2, Fig. 1b). The crystal of 1,2-bis(*N*-4-toluenesulfonylamino)-benzene (**3**), which has two same secondary sulfonamide groups as compound **1**, belonged to the space group *Pccn*. In the crystal, two sulfonamide bonds were involved in both intra- and intermolecular hydrogen bonds (Table 2, Fig. 1c). Both enantiomers were alternately arranged, and formed an infinite chain structure through single intermolecular hydrogen bond. The crystal of 1-(*N*-methyl-*N*-4-toluenesulfonylamino)-2-(*N*-4-toluenesulfonylamino)-benzene (**4**), which contains secondary and tertiary sulfonamide bonds, as well as compound **2**, does belong to the space group *P*-1. In the crystal, both enantiomers formed dimer through a self-complementary double hydrogen bond between the two secondary sulfonamide moieties (Table 2, Fig. 1d). Although this double hydrogen bond was intrinsically the same as that of compound **1**, the infinite hydrogen bonding array was not observed because the tertiary sulfonamide group terminated the intermolecular hydrogen bonds.

Each crystal contained pairs of both conformational enantiomers in the unit cell in contrast to the crystal of 1,2-bis(*N*-benzenesulfonyl-*N*-methylamino)benzene, which had single enantiomers in the unit cell.[8d] In addition, each sulfonamide moiety of all compounds existed in the synclinal conformation in crystals even though the sulfonamide group was secondary or tertiary (Fig. 2, Table 3). Therefore, the S═O and the N–H bonds of the secondary sulfonamide groups were located in the same directions on the sulfonamide bond, the sulfur atom of which had a tetrahedral geometry, and the nitrogen atom of which had a trigonal geometry, providing an intermolecular hydrogen bonding network.

Table 2. Hydrogen bond geometry (Å, °) in compounds **1**–**4**

	D–H···A	d(D–H)	d(H···A)	d(D···A)	D–H···A
1[a]	N1–H1···O2[b]	0.79(2)	2.15(2)	2.9310(19)	169(2)
	N2–H2···O3[c]	0.814(19)	2.275(19)	3.0318(18)	154.8(19)
2	N1–H1···O4[d]	0.73(2)	2.38(2)	3.068(2)	157(2)
3	N1–H1···O3[e]	0.75(4)	2.24(4)	2.992(4)	176(4)
	N2–H2···O1	0.75(3)	2.31(3)	2.885(4)	135(3)
4	N1–H1···O1[f]	0.82(2)	2.34(2)	3.084(2)	151(2)

[a] Ref. 9.
[b] Symmetry code: $-x+1, -y+1, -z$.
[c] Symmetry code: $-x+2, -y+1, -z$.
[d] Symmetry code: $-x+1, -y+2, -z$.
[e] Symmetry code: $x, -y+3/2, z+1/2$.
[f] Symmetry code: $-x+2, -y+1, -z+1$.

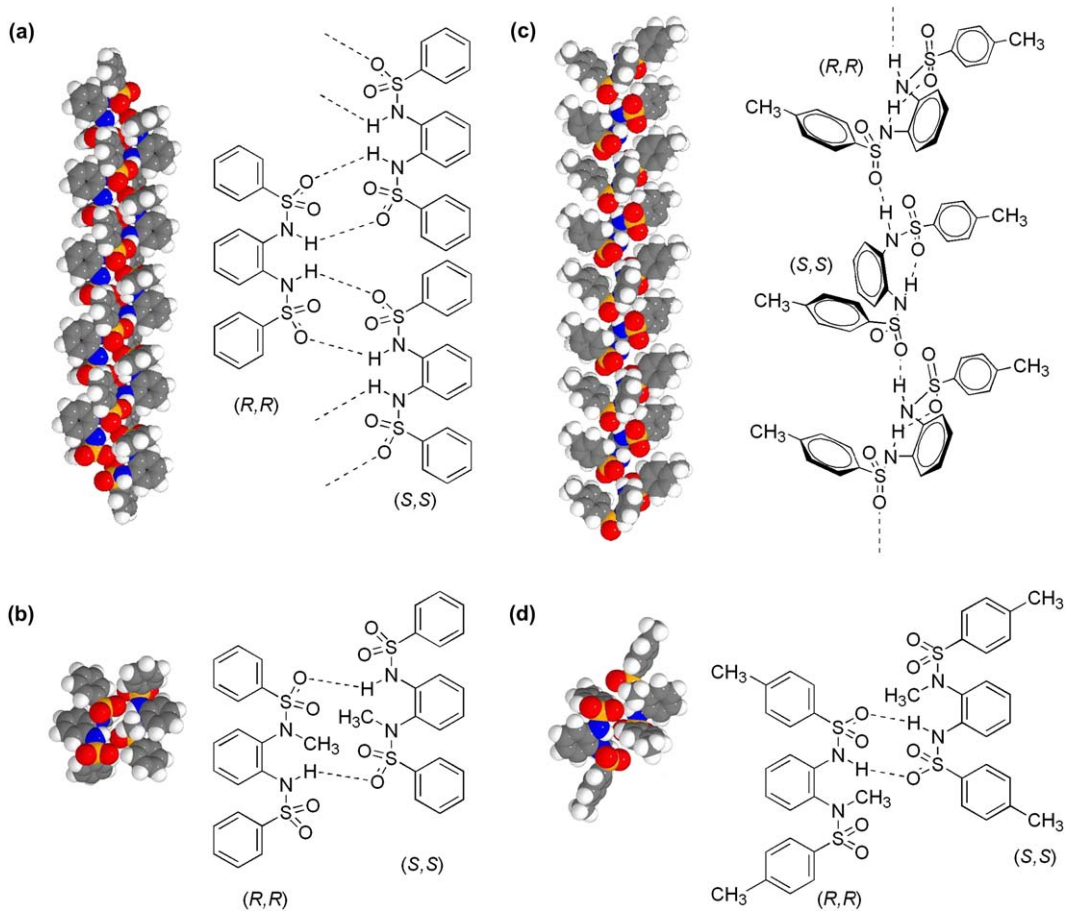

Figure 1. Spacefilling models of molecules, which formed hydrogen bonds in the crystal, their schematic representations of compounds (a) **1**,[9] (b) **2**, (c) **3**, and (d) **4**.

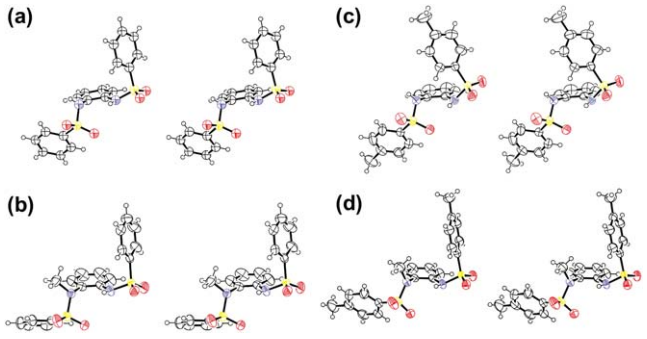

Figure 2. ORTEP stereoviews of compounds (a) **1**,[9] (b) **2**, (c) **3**, and (d) **4**. The thermal ellipsoids are drawn at the 50% probability level.

Table 3. Torsion angle (°) of sulfonamides in compounds **1–4**

	1[a]	**2**	**3**	**4**
Ar–N(R^1)–S–Ar	−65.23	−68.20	−63.98	−72.88
Ar–N(R^2)–S–Ar	61.36	59.87	60.31	60.51

[a] Ref. 9.

3. Conclusion

In conclusion, we demonstrated the existence of four different types of hydrogen bonds resulting from a combination of inter- and/or intramolecular and single or complementary double hydrogen bonds in crystals of aromatic sulfonamides of *o*-phenylenediamine. The sulfonamide moieties existed in synclinal conformations even though the sulfonamide was secondary or tertiary, producing various types of hydrogen bonds. A sulfonamide has the potential to construct 2-D or 3-D networks through intermolecular hydrogen bonds especially because of the synclinal conformation of secondary sulfonamides, which allows the arrangement of the amide hydrogen and sulfonyl oxygen in the same directions. We are currently exploring the supramolecular chemistry of macrocycles containing aromatic sulfonamide moieties, and the unique molecular array of aromatic sulfonamides resulting from intra- or intermolecular interactions such as hydrogen bonds, CH–π interactions, and π–π interactions in crystals.

4. Experimental

4.1. 1-(*N*-Benzenesulfonylamino)-2-(*N*-benzenesulfonyl-*N*-methylamino)benzene (2)

Benzenesulfonyl chloride (0.64 mL, 5.0 mmol) was added dropwise to a solution of *N*-methyl-1,2-phenylenediamine (0.23 mL, 2.0 mmol) in dry CH_2Cl_2 (20 mL) and pyridine (0.81 mL) at 0 °C, with stirring under Ar atmosphere. After stirring at room temperature for 2 h, benzenesulfonyl chloride (0.26 mL, 2.0 mmol) was added once again to the

reaction mixture. After 2.5 h, the reaction mixture was poured into ice, and was extracted with CH_2Cl_2 (50 mL). The organic layer was successively washed with 2 M HCl (10 mL), brine (15 mL), and dried over Na_2SO_4. The solvent was evaporated. The crude product was recrystallized from CH_2Cl_2 and methanol to produce pure 1-(*N*-benzenesulfonylamino)-2-(*N*-benzenesulfonyl-*N*-methylamino)benzene (0.78 g, 96%). Mp 156 °C. IR (KBr) ν 1180, 1350, 3280 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 2.50 (s, 3H), 6.17 (dd, J=8.0, 1.6 Hz, 1H), 6.89–6.95 (m, 1H), 7.24–7.31 (m, 1H), 7.40–7.56 (m, 7H), 7.58–7.64 (m, 1H), 7.71 (dd, J=8.0, 2.0 Hz, 1H), 7.79–7.84 (m, 2H), 7.98 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.9, 125.1, 125.8, 126.1, 127.4, 128.2, 128.9, 129.2, 132.8, 133.5, 133.6, 134.7, 135.2, 139.9. MS (FAB): m/z=403 $[M+H]^+$. HRMS Calcd for $C_{19}H_{18}N_2O_4S_2Na$ $[M+Na]^+$: 425.0605. Found 425.0616. Anal. Calcd for $C_{19}H_{18}N_2O_4S_2$: C, 56.70; H, 4.51; N, 6.96. Found: C, 56.70; H, 4.52; N, 6.90.

4.2. 1,2-Bis(*N*-4-toluenesulfonylamino)benzene (3)

4-Toluenesulfonyl chloride (0.95 g, 5.0 mmol) was added to a solution of *o*-phenylenediamine (0.22 g, 2.0 mmol) in pyridine (20 mL) at 0 °C, with stirring under Ar atmosphere. After stirring at room temperature for 3 h, 4-toluenesulfonyl chloride (0.19 g, 1.0 mmol) was added once again to the reaction mixture. After 5.5 h, the reaction mixture was poured into ice, and was extracted with AcOEt (50 mL). The organic layer was washed with 2 M HCl (10 mL), brine (10 mL), saturated $NaHCO_3$ aq (10 mL), brine (10 mL×2), and dried over Na_2SO_4. The solvent was evaporated. The crude product was recrystallized from CH_2Cl_2 and *n*-hexane to give pure 1,2-bis(*N*-4-toluenesulfonylamino)benzene (0.67 g, 81%). Mp 206 °C (lit.[10] 204–205 °C). IR (KBr) ν 1150, 1320, 3210 cm^{-1}. ^1H NMR (400 MHz, DMSO): δ 2.33 (s, 6H), 6.87–6.93 (m, 2H), 6.93–6.99 (m, 2H), 7.30 (d, J=8.0 Hz, 4H), 7.56 (d, J=8.0 Hz, 4H), 9.23 (br s, 2H). ^{13}C NMR (100 MHz, DMSO): δ 21.0, 122.6, 125.1, 126.9, 129.6, 130.2, 136.5, 143.3. MS (FAB): m/z=417 $[M+H]^+$. HRMS Calcd for $C_{20}H_{20}N_2O_4S_2Na$ $[M+Na]^+$: 439.0762. Found 439.0775. Anal. Calcd for $C_{20}H_{20}N_2O_4S_2$: C, 57.67; H, 4.84; N, 6.73. Found: C, 57.60; H, 4.90; N, 6.72.

4.3. 1-(*N*-Methyl-*N*-4-toluenesulfonylamino)-2-(*N*-4-toluenesulfonylamino)benzene (4)

4-Toluenesulfonyl chloride (0.95 g, 5.0 mmol) was added dropwise to a solution of *N*-methyl-1,2-phenylenediamine (0.23 mL, 2.0 mmol) in dry CH_2Cl_2 (20 mL) and pyridine (0.81 mL) at 0 °C, with stirring under Ar atmosphere. After stirring at room temperature for 3.3 h, 4-toluenesulfonyl chloride (0.38 g, 2.0 mmol) was added once again to the reaction mixture. After 2 h, the reaction mixture was poured into ice, and was extracted with CH_2Cl_2 (50 mL). The organic layer was successively washed with 2 M HCl (10 mL), brine (10 mL), and dried over Na_2SO_4. The solvent was evaporated. The crude product was recrystallized from CH_2Cl_2 and methanol to give pure 1-(*N*-methyl-*N*-4-toluenesulfonylamino)-2-(*N*-4-toluenesulfonylamino)benzene (0.66 g, 77%). Mp 138–140 °C. IR (KBr) ν 1173, 1347, 3247 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 2.36 (s, 3H), 2.43 (s, 3H), 2.56 (s, 3H), 6.91 (ddd, J=7.8, 7.8, 1.4 Hz, 1H), 7.21–7.29 (m, 5H), 7.39 (ddd, J=8.3, 1.8, 1.8 Hz, 2H), 7.65–7.73 (m, 3H), 7.93 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.5, 21.6, 38.9, 124.5, 125.4, 126.2, 127.5, 128.3, 129.1, 129.5, 129.6, 132.0, 133.5, 135.5, 137.0, 143.6, 144.5. MS (FAB): m/z=431.2 $[M+H]^+$. Anal. Calcd for $C_{21}H_{22}N_2O_4S_2$: C, 58.58; H, 5.15; N, 6.51. Found: C, 58.84; H, 4.97; N, 6.55.

4.4. X-ray crystallographic study

X-ray data were collected on Bruker ApexII CCD detector (for compounds **2** and **4**) and Bruker Smart1000 CCD detector (for compound **3**) with graphite monochromated Mo K_α (λ=0.71073 Å) radiation at room temperature. The crystal structures were solved by direct methods SHELXS-97 (Sheldrick, 1997) and refined by full-matrix least-squares SHELXL-97 (Sheldrick, 1997). All non-hydrogen atoms were refined anisotropically. The sulfonamide hydrogen atoms were located in a difference map and their coordinates were refined. Other hydrogen atoms were included as their calculated positions.

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Erinacines J and K from the mycelia of *Hericium erinaceum*

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Abstract—Two novel compounds, erinacines J (**1**) and K (**3**), were isolated from the cultured mycelia of *Hericium erinaceum*. Their structures were determined by spectral analyses. Erinacine K showed anti-MRSA activity.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has developed resistance to most antibiotics and is one of the most prevalent pathogen in nosocomial infections. Therefore, anti-MRSA substances are urgently required. We screened extracts of various mushrooms and their mycelia for anti-MRSA activity. Since the extract from the cultured mycelia of *Hericium erinaceum* showed potent activity, we tried to isolate the active principles from the fungus. We wish to report here the isolation, the structure determination of two novel compounds named erinacine J (**1**) and K (**2**), and the inhibitory activity of the two compounds and the other erinacines.

2. Results and discussion

The extract of the lyophilized mycelia of *H. erinaceum* was successively extracted with $CHCl_3$, EtOAc, acetone, and MeOH. Since the $CHCl_3$-soluble fraction showed significant activity, this fraction was repeatedly chromatographed on the basis of the result of the bioassay. As a consequence, two new compounds were purified and named as erinacine J (**1**) and K (**3**).

Erinacine J (**1**) was isolated as colorless crystals.[1] FABMS of **1** showed ion peaks at *m/z* 467 ($[M+H]^+$) and *m/z* 489 ($[M+Na]^+$). Its molecular formula was determined as $C_{25}H_{38}O_8$ by HRESIMS, indicating the presence of seven degrees of unsaturation in the molecule. The ^1H NMR (Table 1) and ^{13}C NMR (Table 2) spectra of **1** along with DEPT and HMQC showed two methyls attached at quaternary carbons, an isopropyl attached at an sp^2 carbon [δ_H 1.07 (6H, d, J=7.0), δ_C 18.7; 2.68 (1H, heptet, J=7.0), 41.8], seven sp^3 methylenes, eight sp^3 methines, three sp^3 quaternary carbons (δ_C 43.6, 47.4, 81.0), two carbonyls (δ_C 216.7, 217.4) (Tables 1 and 2). These data suggested that this compound is an analog of erinacines.[2–6] The HMBC correlations (H19,20/C3, H18/C3, H1/C3, H2/C3) indicated a partial structure, $(CH_3)_2CHCOCH_2CH_2$–, (Fig. 1). Cross peaks (H1/C9, H1/C4, H17/C1, H8/C1) between the terminal methylene (C1) in the sequence and the other protons or carbons suggested the further linkage, C1–$C(CH_3)$(CO–)CH_2–. The other part of the structure except for the sugar part was determined by the following HMBC correlations; H17/C8, H17/C9, H17/C4, H8/C17, H8/C4, H8/C7, H10/C4, H5/C4, H5/C7, H5/C6, H5/C10, H5/C11, H7/C5, H10/C5, H11/C5, H16/C5, H16/C7, H16/C6, H16/C14, H7/C16, H14/C16, H14/C7, H14/C6, H14/C13, H14/C12, H10/C12, H11/C12, H11/C13, H13/C11, H13/C6, and H15/C11. The structure of the highly modified sugar part and the bonds between the sugar and the aglycon was also confirmed by the HMBC correlations; cross peaks between H1′/C2′, H3′/C2′, H3′/C4′, H5′/C1′, H5′/C3′, H5′/C4′, H1′/C14, H1′/C13, H3′/C13, H3′/C15, H13/C2′, H14/C1′, H14/C2′, and H15/C3′ were observed in the spectrum. The COSY data of **1** also supported this structure (data not shown). The chemical shifts of the ^1H and ^{13}C NMR signals of the compound were similar to those of **2** obtained in this study, and especially those of the sugar part were almost same as each other. Compound **2**, CJ-14,258, which has been reported as a κ opioid receptor agonist, and its stereochemistry except for C-2′ has been determined.[2] Formation of **1** in the mycelia can be envisioned as an oxidative cleavage of C3–C4 bond of **2**.

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Table 1. ^{1}H NMR data [δ_H (the number of protons, multiplicity, *J* in Hertz)] for erinacine J (**1**), CJ-14,258 (**2**), and erinacine K (**3**) (in CD_3OD)[a]

Position	**1**	**2**	**3**
1	1.65 (2H, dd, 7.9, 7.9)	1.56 (1H, m); 1.61 (1H, m)	1.50 (1H, m); 1.58 (1H, m)
2	2.52 (1H, m); 2.58 (1H, m)	2.29 (2H, m)	2.30 (2H, m)
5	2.72 (1H, m)	2.30 (1H, m)	2.81 (1H, m)
7	1.72 (1H, m); 1.94 (1H, m)	1.36 (1H, m); 1.51 (1H, m)	1.12 (1H, m); 1.99 (1H, m)
8	1.72 (1H, m); 1.84 (1H, ddd, 13.3, 13.1, 5.4)	1.38 (2H, m)	1.40 (1H, m); 1.51 (1H, m)
10	1.21 (1H, m); 2.05 (1H, m)	1.70 (1H, m); 1.82 (1H, m)	2.10 (2H, m)
11	0.93 (1H, m); 2.17 (1H, m)	1.01 (1H, m); 2.21 (1H, m)	4.66 (1H, m)
12	1.68 (1H, m)	1.68 (1H, m)	—
13	1.98 (1H, dd, 12.2, 8.9)	1.97 (1H, dd, 12.2, 8.5)	5.78 (1H, d, 5.8)
14	3.89 (1H, d, 8.9)	3.99 (1H, d, 8.5)	3.81 (1H, m)
15	4.82 (1H, d, 8.5)	4.82 (1H, d, 8.5)	4.60 (1H, d, 13.0); 4.67 (1H, d, 13.0)
16	0.88 (3H, s)	0.96 (3H, s)	0.83 (3H, s)
17	1.20 (3H, s)	1.08 (3H, s)	1.07 (3H, s)
18	2.68 (1H, heptet, 7.0)	2.79 (1H, heptet, 7.0)	3.02 (1H, heptet, 6.7)
19,20	1.07 (6H, d, 7.0)	0.96 (3H, d, 7.0); 0.97 (3H, d, 7.0)	0.98 (6H, d, 6.7)
1′	4.95 (1H, s)	4.91 (1H, s)	4.23 (1H, d, 7.2)
2′	—	—	3.22 (1H, dd, 7.2, 8.9)
3′	3.82 (1H, d, 8.9)	3.81 (1H, d, 8.5)	3.30 (1H, dd, 8.9, 8.5)
4′	4.14 (1H, m)	4.16 (1H, m)	3.45 (1H, ddd, 8.5, 9.9, 5.2)
5′	3.13 (1H, dd, 11.0, 11.3); 3.79 (1H, dd, 11.0, 5.2)	3.12 (1H, dd, 11.3, 11.0); 3.79 (1H, m)	3.13 (1H, dd, 11.6, 9.9); 3.79 (1H, dd, 11.6, 5.2)
CH_3CO–			2.05 (3H, s)

[a] These assignments were established by decoupling, COSY, DEPT, HMQC, and HMBC experiments.

Erinacine K (**3**) was purified as pale yellow oil, and its molecular formula, $C_{27}H_{42}O_8$, was determined by HRESIMS. The NMR data (Tables 1 and 2) and HMBC experiment (Fig. 1) indicated that this compound was also an erinacine analog having a simple sugar.[3–7] The plane structure of the compound was determined by HMBC correlations (Fig. 1); H1/C2, H1/C3, H1/C4, H1/C9, H1/C17, H1/C8, H2/C3, H2/C4, H2/C9, H18/C2, H18/C3, H18/C4, H19,20/C3, H19/C20, H20/C19, H5/C3, H5/C4, H5/C10, H17/C1, H17/C9, H17/C4, H17/C8, H8/C9, H8/C4, H8/C6, H7/C6, H16/C6, H16/C7, H16/C5, H16/C14, H11/C5, H11/C10, H13/C11, H13/C12, H13/C6, H13/C14, H13/C15, H15/C11, and H15/C13. The structure of the compound was also supported by its COSY experiments (data not shown). This compound had an acetoxy group at C15; cross peaks between H15/CH_3CO and CH_3CO/CH_3CO were detected in its HMBC spectrum. The sugar was identified as D-xylose by hydrolysis of **3** with β-glucosidase and ^{1}H NMR data of the product. The position of the glycosidic linkage between the aglycon and the sugar was determined by H14/C1′ and C1′/H14 cross peaks in the HMBC spectrum (Fig. 1). The relative stereochemistry of **3** was deduced by NOESY and ROESY experiments; cross peaks between H5/H17 and H14/H16 were observed. The stereochemistry at C11 remains undetermined since its NOESY and ROESY experiments did not give any valuable information.

Compounds **1**, **3**, and the other erinacine analogs obtained in this study [**2**, erinacine A (**4**), C (**5**), and E (**6**)] were evaluated in anti-MRSA assays.[6,7] MIC of **2**, **3**, **4**, and **5** were 62.5, 500, 500, and 62.5 μM, respectively. Although **6** gave halos by the direct drop method, this compound was inert even at 1 mM by the micro-plate method. Compound **1** did not show any activity by the both method. This result indicated that the three-ring skeleton of the aglycon in the active compounds was indispensable to the anti-MRSA activity.

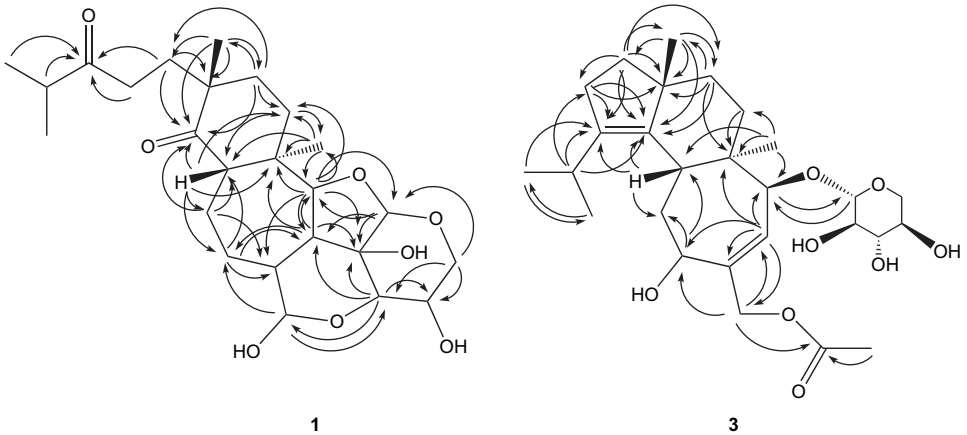

Figure 1. HMBC correlations in **1** and **3**.

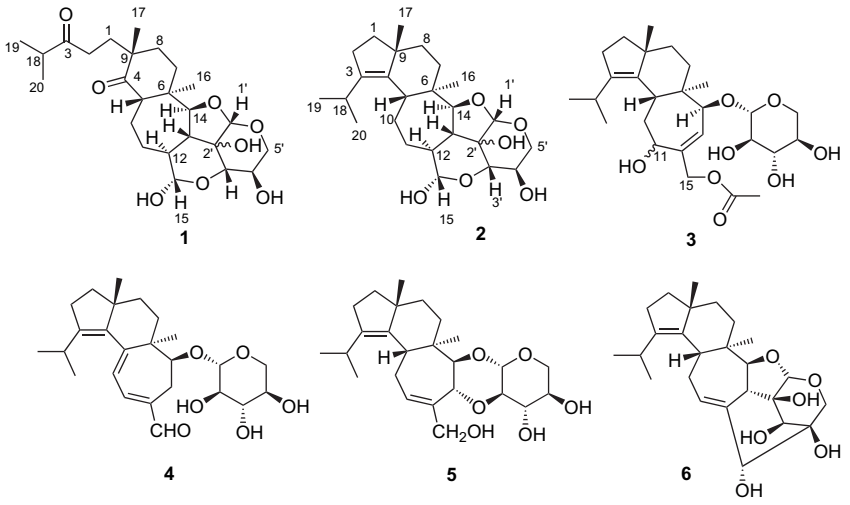

Table 2. ^{13}C NMR data (δ_C) for erinacine J (**1**), CJ-14,258 (**2**), and erinacine K (**3**) (in CD_3OD)[a]

Position	1	2	3
1	33.3	39.3	39.2
2	36.3	28.9	29.4
3	217.4	139.3	140.4
4	216.7	140.2	140.2
5	56.2	48.3	41.3
6	43.6	41.6	44.1
7	27.6	39.2	33.0
8	35.2	37.7	38.1
9	47.4	50.6	50.5
10	19.8	26.4	37.2
11	34.8	36.9	71.6
12	45.8	45.6	132.4
13	47.9	47.1	129.9
14	93.3	94.6	86.4
15	97.6	97.9	66.7
16	19.7	19.6	17.0
17	25.2	25.5	24.9
18	41.8	28.2	28.1
19, 20	18.7	21.7	21.0
		22.3	22.0
1′	108.6	108.4	107.2
2′	81.0	81.3	75.2
3′	85.8	86.0	77.8
4′	69.4	69.4	71.2
5′	65.5	65.5	66.7
CH$_3$CO–			21.0
CH$_3$*CO*			172.7

[a] These assignments were established by DEPT, HMQC, and HMBC experiments.

3. Experimental

3.1. General

^{1}H NMR spectra (one- and two-dimensional) were recorded on a JEOL lambda-500 spectrometer at 500 MHz, while ^{13}C NMR spectra were recorded on the same instrument at 125 MHz. The FABMS spectra were recorded on a JEOL DX-303HF and the HRESIMS spectra were measured on a JMS-T100LC mass spectrometer. A JASCO grating infrared spectrophotometer was used to record the IR spectra. The $[\alpha]_D$ spectra were measured by using a JASCO DIP-1000 spectropolarimeter. HPLC separations were performed with a JASCO Gulliver system using an ODS column (Grandpack ODS-A S-5 YC, 20×300 mm, Masis, Japan). Silica gel plate (Merck F_{254}) and silica gel 60 N (Merck 100–200 mesh) were used for analytical TLC and for flash column chromatography, respectively.

3.2. Fungus materials and cultivation

The strain, HE-01003, of *H. erinaceum* was collected in Fukushima Prefecture, Japan, in October, 2001. The isolate is deposited in the culture collection of IBI Corporation. The components of the mycelia culture were as follows; glucose 4%, polypeptone 0.3%, yeast extract 0.3%, KH_2PO_4 0.05%, and Na_2HPO_4 0.05% in the distilled water. The culture medium was adjusted to pH 5.5. The aerated liquid culture was carried out in a 1.5 ton tank containing 1000 L of the medium, and incubated at 25 °C for 30 days.

3.3. Extraction and isolation

After the cultivation, the wet mycelia of *H. erinaceum* were obtained by centrifugation (7000×g, 30 min) and lyophilized. The dried mycelia (about 200 g) were successively extracted with $CHCl_3$ (4 L, twice), EtOAc (4 L, twice), acetone (6 L, three times), and then MeOH (8 L). The residue (4.97 g) obtained after removing $CHCl_3$ was fractionated by silica gel flash column chromatography (100%, 80%, 60% $CHCl_3$/acetone, 90%, 70% $CHCl_3$/MeOH, MeOH, each 1 L) to obtain 13 fractions. Fraction 12 (464.8 mg) was further separated by silica gel flash column chromatography (90% $CHCl_3$/MeOH) and six fractions were obtained. Fraction 12–3 (122.0 mg) was separated by reversed-phase HPLC (80% MeOH) to afford compound **1** (12.7 mg). On the other hand, fraction 13 (142.9 mg) was subjected to reversed-phase HPLC (80% MeOH) to provide compounds **2** (0.9 mg) and **3** (3.1 mg).

3.3.1. Erinacine J (1). Colorless crystals, mp 125–128 °C. $[\alpha]_D$ −16.1 (*c* 1.00, MeOH). IR ν_{max} (KBr) cm^{-1}: 3418, 2934, 1706. HRESIMS *m/z* 489.2461 [M+Na]$^+$ (calcd for $C_{25}H_{38}NaO_8$, 489.2464).

3.3.2. Erinacine K (3). Pale yellow oil. $[\alpha]_D$ −18.4 (*c* 0.290, MeOH). IR ν_{max} (neat) cm^{-1}: 3398, 2926, 1725. HRESIMS *m/z* 517.2782 [M+Na]$^+$ (calcd for $C_{27}H_{42}NaO_8$, 517.2777).

3.4. Bioassay

3.4.1. Direct drop method. Pre-incubated MRSA suspension (100 μl) is spread on the surface of agar medium in Petri dishes. Solution of each sample (2 μl) was dropped on the agar. After incubation at 37 °C overnight, the activity (formation of halo) was observed.

3.4.2. Micro-plate method. Each sample at various concentrations was added to each well in a 96-well micro-plate. Pre-incubated MRSA suspension is added to the wells. After incubation at 37 °C for 16 h, inhibition was evaluated on the basis of turbidity of the culture media in the wells. Minimum inhibitory concentration (MIC) of each sample was defined as the minimum concentration that gave no turbidity of the culture media.

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Two-photon absorption chromophores with a tunable [2,2′]bithiophene core

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Abstract—Sonogashira coupling between 3,5,3′,5′-tetrabromo-[2,2′]bithiophene and various terminal alkynes provides two-photon absorption (TPA) chromophores **1**–**6**, which possess electron donor (D) and/or acceptor (A) alkynyl substituents at 3(3′) and 5(5′) sites of the bithiophene core. The up-converted fluorescence emission excited at 800 nm (Ti:sapphire femtosecond laser, ~100 fs pulses) was used to determine the two-photon absorption cross-sections (σ) of these compounds. The corresponding TPA cross-section (σ) values ranging from 132 to 1120 GM (10^{-50} cm^4 s photon^{-1}) can be fine-tuned by the substitutents. The quadrupolar-type (A-π-D-π-A) chromophore **5** exhibits the largest σ value (1120 GM) in CH_2Cl_2 upon 800 nm excitation.

1. Introduction

The two-photon process involving simultaneous absorption (nonresonance–resonance) of two photons was first predicted by Goppert-Mayer in 1931,[1] and experimentally observed in the 1960s.[2] Two-photon absorption (TPA) offers the advantage of high transmission at low incident intensity for fundamental frequencies well below the band gap. Furthermore, due to the quadratic dependence of the two-photon absorption probability on intensity, the absorption can theoretically be confined to a volume of order λ^3 (where λ is the laser wavelength) under tight-focusing conditions. Therefore, one is able to initiate two-photon polymerization for three-dimensional optical data storage and microfabrication using near infrared (NIR) laser source.[3] Another important application of TPA is confocal microscopy, which is an important tool for obtaining three-dimensional (3D) images of biological specimens such as tissues and cells.[4] Two-photon confocal laser scanning microscopy has several advantages over its single-photon counterpart. Except for the depth resolution due to the quadratic dependence of the two-photon induced fluorescence intensity on the excitation power, the NIR excitation light provides much better penetration ability compared to UV and even visible light in many organic materials where the linear attenuation and scattering are high.[5] Other important applications of TPA chromophores include two-photon optical power-limiting,[6] two-photon up-converted lasing,[7] and photodynamic therapy.[8]

In light of above fundamental applications, significant progress has been made on the development of organic conjugated molecules with large TPA cross-sections.[9] Among these, quadrupolar and octupolar-type molecules have received considerable interests because of the significant enhancement of the TPA cross-section in these molecules compared to their dipolar-type analogues.[10] Further increment of TPA cross-section has been achieved by incorporating TPA chromophores in a dendrimer to increase the density of chromophores.[11] In their seminal report, Marder and Perry illustrated that molecules with a D-π-D, A-π-A, D-π-A-π-D or A-π-D-π-A motif (D = electron donor; A = electron acceptor; π = conjugated bridge) were potential quadrupolar-type TPA chromophores, which could exhibit exceptional large TPA cross-sections.[12] Based on previous studies,[12] it was concluded that TPA cross-sections were larger in molecules with more effective conjugation length and higher polarizability. Accordingly, coplanarity of the conjugation bridge is beneficial to the increase of the TPA cross-sections. Practically, for the biological application, it is advantageous to have the two-photon peak occurring at or near 800 nm where most of organic and biological

Keywords: Two-photon absorption; Bithiophene; Sonogashira coupling reaction.
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materials have large optical transparency. Also, there is a greater penetration depth in tissue with reduced photodamage upon ~800 nm excitation.

It is thus important to gain detailed insights into the relationship between molecular structure and TPA cross-section σ. The results may provide a guideline for future design and synthesis of organic molecules with highly efficient TPA. To achieve this goal, we have been interested in the systematic development of organic quadrupolar- or octupolar-type TPA chromophores.[13] Herein, we report the investigations on the TPA properties of a series of novel quadrupolar chromophores based on 3,3′,5,5′-tetrasubstituted-[2,2′]-bithiophene core. In this approach, 3,5,3′,5′-tetrabromo-[2,2′]bithiophene[14] was subjected to Sonogashira coupling reaction[15] with terminal alkynes based on the following considerations: (1) the substituents introduced at the 3 and 3′ sites can differ in the electronic property from those introduced at the 5 and 5′ sites due to different reactivity of the 3(3′) and 5(5′) bromos and (2) incorporation of ethynyl entity in the conjugation chain normally results in less effective conjugation length.[16] This strategic design may avoid shifting the one-photon absorption towards 800 nm, while the coplanarity of aromatic rings, especially the central [2,2′]-bithiophene core, can be retained upon electronic tuning.

2. Results and discussion

2.1. Synthesis and characterization

Figure 1 depicts the structures of the new series of compounds **1**–**6**, and their corresponding synthetic routes are illustrated in Scheme 1. These compounds were synthesized via Sonogashira coupling[15] of 3,5,3′,5′-tetrabromo-[2,2′]bithiophene and appropriate terminal alkynes catalyzed by 3 mol % of $PdCl_2(PPh_3)_2$ and CuI in diisopropylamine. Compounds **1**–**3**, in which all four bromine atoms in 3,5,3′,5′-tetrabromo-[2,2′]bithiophene are replaced by the same substituents, were isolated in good yields (**1**, 62%; **3**, 80%) except for **2** (20%) if slight excess of terminal alkynes were used. Conversely, if only ~2 equiv of terminal alkynes were used and the reaction was allowed to proceed at lower temperature (40 °C), the Sonogashira coupling selectively occurred at the 5 and 5′ sites to afford the dibromo intermediates (**Ia** and **Ib**) in moderate to good yields. This is consistent with the previous reports[17] that the halo atom at the 2- or 5-site of halothiophene moieties was more reactive to undergo Sonogashira coupling reaction. These dibromo intermediates can subsequently undergo further Sonogashira coupling reactions with different terminal alkynes to afford **4**–**6** in 30–40% yields. Compounds **1**–**6** are moderately soluble in common solvents and have been characterized by ^1H and ^{13}C NMR and mass spectroscopic studies.

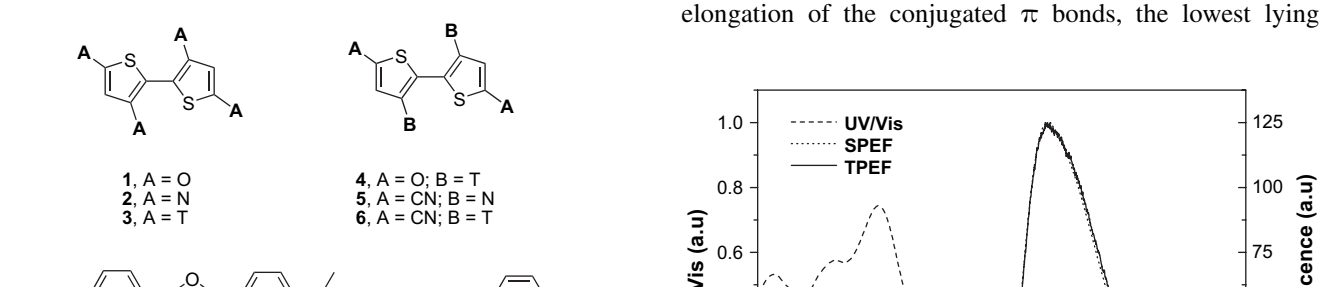
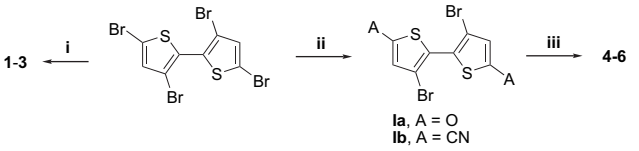


Figure 1. Molecular structures of compounds **1**–**6** and various alkynes moieties.



i. 1.5 mol% $PdCl_2(PPh_3)_2$, 0.5 mol% CuI, 1.5 mol% PPh_3, 4.4 equiv. terminal alkynes, *i*-Pr_2NH, rt 30 min., then reflux 2 days
ii. 1.5 mol% $PdCl_2(PPh_3)_2$, 0.5 mol% CuI, 1.5 mol% PPh_3, 2.2 equiv. terminal alkynes, *i*-Pr_2NH, rt 5 h, 40 °C 5 h, then reflux 1 day
iii. 3 mol% $PdCl_2(PPh_3)_2$, 1 mol% CuI, 3 mol% PPh_3, 2.2 equiv. terminal alkynes, *i*-Pr_2NH, rt 30 min., then reflux 2 days

Scheme 1.

2.2. Linear absorption and single-photon-excited fluorescence (SPEF)

Steady state absorption and emission spectra of the representative compound **5** are depicted in Figure 2, and the associated photophysical properties for **1**–**6** are listed in Table 1. The lower lying energy band ($\lambda_{abs}^{(1)}$>430 nm) in compounds **1**–**3** is tentatively attributed to the π–π* extending network through the substituents (A=O, N or T, see Fig. 1) and the [2,2′]bithiophene core. Support of this viewpoint is given by the disappearance of this band upon replacing the 3- and 3′-substituents by the bromine atom. Furthermore, the substituent (O, N or T) alone exhibits the lowest absorption band of<400 nm. For compounds **4**–**6**, in addition to the elongation of the conjugated π bonds, the lowest lying

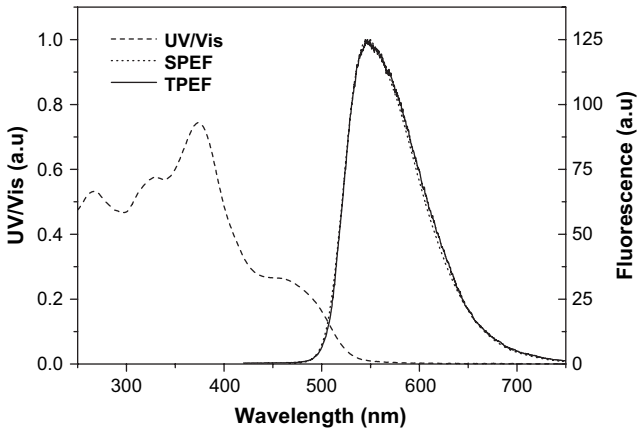


Figure 2. One-photon absorption (dashed line), single-photon-excited fluorescence (SPEF, λ_{ex}=400 nm), and two-photon excitation fluorescence (TPEF, λ_{ex}=800 nm) spectra of compound **5** in CH_2Cl_2. (SPEF and TPEF are in dotted and solid lines, respectively).

Table 1. One- and two-photon properties of compounds **1**–**6** and references

Compounds	$\lambda_{abs}^{(1)}$[a] (nm)	$\lambda_{em}^{(1)}$[b] (nm)	Φ_f[c]	$\lambda_{abs}^{(2)}$[d] (nm)	σ[e,f,g] (GM)
1	444,350,313,292	517	0.42	800	295 (0.22)
2	463,357,318,268	543	0.37	800	236 (0.16)
3	431,324,315,248	487	0.41	800	132 (0.21)
4	443,336,296	498	0.22	800	615 (0.62)
5	458,374,329	547	0.21	800	1120 (1.07)
6	440,333,253	492	0.10	800	665 (0.98)
C-480			0.87	800	168.2
R-6G			0.98	800	38.67

[a] $\lambda_{abs}^{(1)}$ of the one-photon absorption spectra in nanometers.
[b] $\lambda_{em}^{(1)}$ of the one-photon emission spectra in nanometers.
[c] Fluorescence quantum yield.
[d] $\lambda_{abs}^{(2)}$ of the two-photon absorption spectra in nanometers.
[e] TPA cross-section in 10^{-50} cm^4 s/photon (GM).
[f] Numbers in the parenthesis are relative σ/MW.
[g] Samples measured in CH_2Cl_2 at a concentration of 10^{-4} M, Coumarin 480 (**C-480**) and Rhodamine 6G (**R-6G**) measured in MeOH at a concentration of 10^{-4} M. Compounds **1**, **3**, and **4** compared to Coumarin 480; **2**, **5**, and **6** compared to Rhodamine 6G.

Table 2. The S_0–S_1 excitation energies and oscillation strengths of the twisted and planar **4** and **5** calculated with ZINDO//HF/3-21G* method

State	E_{cal} (eV)	λ_{cal} (nm)	f
4			
Twisted[a]			
S_1	2.95	420	1.1298
Planar			
S_1	2.51	493	1.0196
5			
Twisted[b]			
S_1	2.95	420	0.7889
Planar			
S_1	2.48	499	0.8650

[a] The dihedral angles of the twisted and planar forms are 56.28° and −179.9°, respectively.
[b] The dihedral angles of the twisted and planar forms are −56.49° and 177.3°, respectively.

absorption band with $\lambda_{abs}^{(1)}$ above 440 nm, to a certain extent, may also possess a charge transfer character from the 3- (or 3′-) donor to the 5- (or 5′-) acceptor site bridged by [2,2′]-bithiophene core. These assignments are supported by the theoretical approach on two prototypical compounds, **4** and **5** (see Table 2 and Fig. 3), in which, upon HOMO→LUMO transition in **5**, the electron density significantly decreases in electron donating group *N* (see Fig. 1 for definition), accompanied by the increase of the electron density in the electron accepting group *CN*.

2.3. TPA cross-sections

The up-converted fluorescence emission technique[18] was used to determine the two-photon absorption cross-sections (σ) of the studied compounds. In order to eliminate contribution from the excited-state absorption, the femtosecond (~100 fs) pulsed laser was used for the measurement. The two well characterized TPA chromophores, Coumarin 480 (with a TPA cross-section value of 168.2 GM)[19a] and Rhodamine 6G (with a TPA cross-section value of 38.67 GM),[19b] were used as the references. TPA cross-sections of compounds **1**–**6** obtained from the two-photon excitation fluorescence (TPEF) are compiled in Table 1. For a clear comparison, the TPA cross-section per unit mass was also calculated and listed in Table 1. A prototypical TPEF spectrum of **5** is displayed in Figure 2, while the representative two-photon excitation (TPE) spectra (750–840 nm) of **5** and **6** in CH_2Cl_2 are displayed in Figure 4. Comparing TPE and single-photon absorption spectra for **5** and **6**, the TPE peak appears at an energy slightly higher than twice that of the corresponding linear absorption maximum, indicating a different selectivity of transition, in part, between one and two-photon absorption. Nevertheless, compounds **1**–**6** have nearly superimposable single-photon-excited fluorescence and TPEF spectra, supporting that both emissions originate from the same lowest lying transition in the singlet manifold.

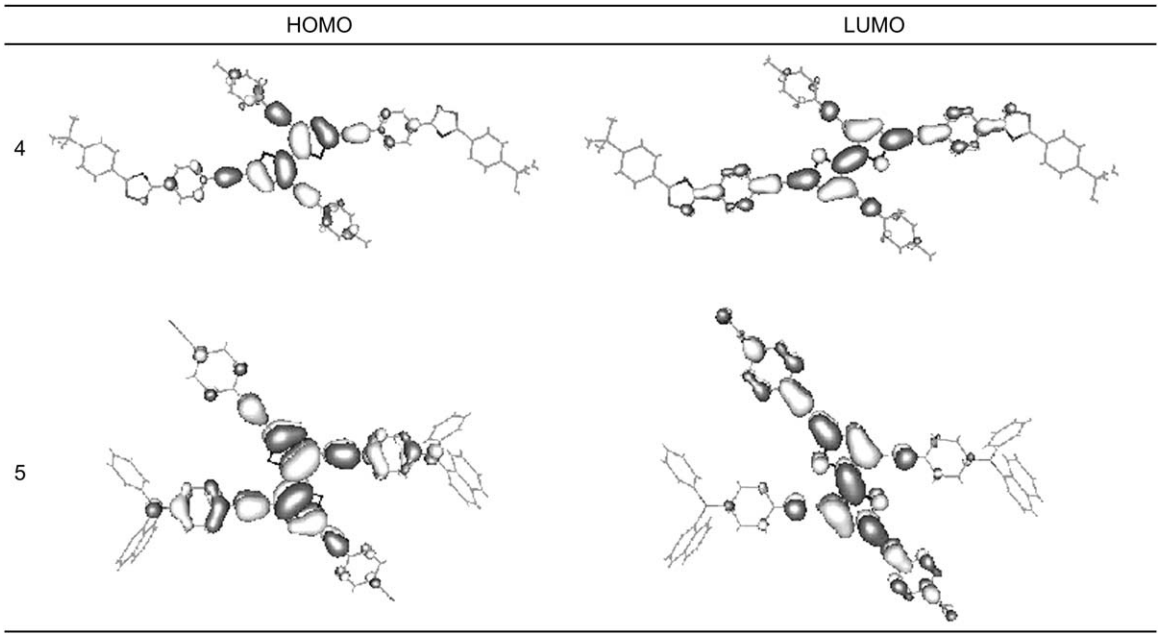


Figure 3. The HOMO and LUMO of planar compounds **4** and **5**. Note that the first singlet excited state is dominated by the HOMO→LUMO transition.

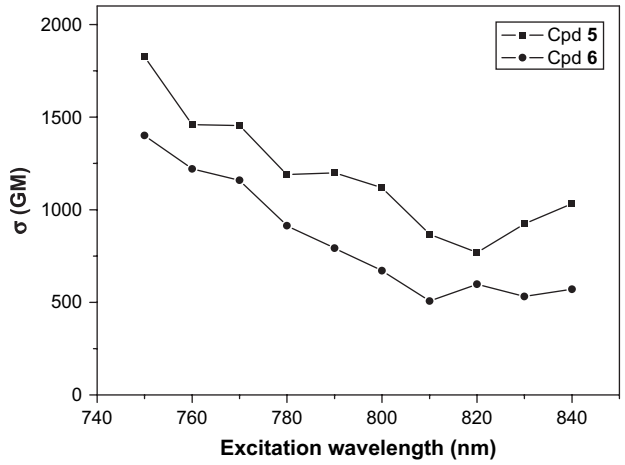


Figure 4. The two-photon excitation (TPE) spectra (750–840 nm) of **5** and **6** in CH_2Cl_2.

According to the data, compound **4** has significantly higher TPA cross-section than that of **1**, though the latter seems to have a larger conjugation length. Qualitatively, if one considers thiophene and oxadizaole moieties to be π-excessive and π-deficient, respectively, the two peripheral oxadiazole-containing segments at the 5 and 5′ positions together with the [2,2′]bithiophene core constitute an A-π-D-π-A motif for TPA chromophore **4**. This consideration requires the coplanarity of the molecular structure. Though the PM3 method indicates that each compound (**1**–**6**) possesses a dominant twisted form, the thermodynamic properties of the twisted and planar forms in the ground state are nearly degenerate based on the ab initio (HF/3-21G*) calculation, i.e., energy difference ($E_{planar}-E_{twisted}$) are 0.3 and −0.05 kcal/mol for **4** and **5**, respectively. In addition, due to the negligible C(2)-C(2′) rotation barrier there should exist a very fast equilibrium between these rotational isomers at room temperature. Upon excitation, the conversion from twisted to planar form should be fast and highly exothermic (see Table 2), such that the consequence is suited for a quadrupolar mechanism.

As such, the two tolyl-containing segments in **4** will enhance the electron donor character of the [2,2′]bithiophene core, while the two oxadiazole-containing segments at the 3 and 3′ positions (compound **1**) have an opposite effect. Likewise, both **5** and **6** also possess an A-π-D-π-A motif and the TPA cross-section of **5** is nearly 1.7 times as large as that of **6**. This trend can be attributed to a stronger π-donor for diarylamine (**5**) than that of the methyl group (**6**), resulting in an increase of σ value in **5**. Compound **6** has somewhat larger σ value than **4**, implying that the cyanophenyl moiety can lead to greater polarizability than the oxadiazole segment.

3. Conclusion

In summary, we have designed and synthesized a new series of [2,2′]bithiophene-based quadrupolar-type chromophores exhibiting large TPA cross-sections. We also demonstrate that the incorporation of push and pull segments at the 3/3′ and 5/5′ sites with different arrangements significantly alter the corresponding TPA property. We thus believe that an increment of the electron donor/acceptor strength in TPA chromophores with an A-π-D-π-A motif should enlarge the quadrupole moment and hence the TPA cross-section. On this basis, further fine-tuning of the TPA properties on [2,2′]bithiophene-based chromophores should be feasible via the variation of the donor, acceptor as well as the conjugation chain. In view of applications, on the basis of the [2,2′]bithiophene core, conversion of these compounds into polymers for the device fabrication is also feasible. This should greatly extend the usefulness of [2,2′]bithiophene-based chromophores in the field of information storage, microfabrication, etc. Works focused on these issues are currently in progress.

4. Experimental

4.1. General procedures and spectroscopic measurements

Unless otherwise specified, all the reactions were carried out under nitrogen atmosphere using standard Schlenk techniques. Solvents were dried by standard procedures. All column chromatography was performed with the use of silica gel (230–400 mesh, Macherey–Nagel GmbH & Co.) as the stationary phase. The ^{1}H NMR spectra were recorded on a Bruker AMX400 spectrometer. Electronic absorption spectra were measured in various solvents using a Cary 50 Probe UV–visible spectrophotometer. Emission quantum yields were measured with reference to Coumarin 1 or 6 in CH_3CN.[20] Mass spectra (FAB) were recorded on a JMS-700 double focusing mass spectrometer (JEOL, Tokyo, Japan). Elementary analyses were performed on a Perkin–Elmer 2400 CHN analyzer. 2-Bromothiophene, sodium azide, bromobenzonitrile, 4-*tert*-butylbenzyl chloride, trimethylsilylacetylene,*N*-phenyl-1-naphthylamine, 4-ethynyltoluene,*N*,*N*-diphenylamine, and hydrobromic acid were purchased from Acros. 1-Bromo-4-iodobenzene and Br_2 were purchased from Aldrich. Potassium hydroxide and magnesium sulfate were purchased from SOWA. The starting materials 3,5,3′,5′-tetrabromo-[2,2′]bithiophene,[21] 2-(4-*tert*-butyl-phenyl)-5-(4-ethynyl-phenyl)-[1,3,4]oxadiazole,[22] and (4-ethynyl-phenyl)-naphthalen-1-yl-phenyl-amine[23] were prepared according to the literature procedures with slight modifications.

4.2. General procedures for the synthesis of identical tetra-substituted compounds

Compounds 3,5,3′,5′-tetrakis-2-(4-*tert*-butyl-phenyl)-5-(4-ethynyl-phenyl)-[1,3,4]oxadiazole-[2,2′]bithiophene (**1**), 3,5,3′,5′-tetrakis(4-ethynyl-phenyl)-naphthalenyl-phenyl-amine-[2,2′]bithiophene (**2**), and 3,5,3′,5′-tetrakis-*p*-tolyl-ethynyl-[2,2′]bithiophene (**3**) were synthesized by a similar procedure as the following description. To a flask containing 3,5,3′,5′-tetrabromo-[2,2′]bithiophene (1 equiv), $PdCl_2(PPh_3)_2$ (3 mmol % per bromo atom), CuI (1 mmol % per bromo atom), PPh_3 (3 mmol % per bromo atom), and aromatic acetylene (4.4 equiv per halogen atom) was added *i*-Pr_2NH (50 mL). The resulting mixture was stirred at room temperature for 30 min, allowed to reflux for two days. The solvent was removed under vacuum, and the residue was extracted with CH_2Cl_2 and brine. Removal of CH_2Cl_2 provided

a yellow residue, which was purified by column chromatography using THF/*n*-hexane as eluent and followed by recrystallization from CH_2Cl_2 and MeOH.

4.2.1. Compound (1). Orange solid, yield 62% (360 mg). Mp: 333–335 °C. IR (KBr): ν_{max} 3055 w, 2930 w, 2130 w cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 8.17–8.13 (m, 8H), 8.04 (d, J=7.8 Hz, 8H), 7.77 (d, J=8.0 Hz, 4H), 7.67 (d, J=7.8 Hz, 4H), 7.53 (d, J=8.0 Hz, 8H), 7.41 (s, 2H), 1.35 (s, 36H). ^{13}C NMR ($CDCl_3$): δ 31.11, 35.11, 84.49, 87.74, 95.09, 96.05, 119.61, 120.87, 121.96, 123.90, 125.56, 126.10, 126.82, 126.90, 131.96, 132.03, 135.48, 139.06, 155.52, 155.57, 163.73, 164.90. FAB MS (*m*/*z*): 1367 (M^++H). HRMS Calcd for $C_{88}H_{71}N_8O_4S_2$: 1367.5040. Found: 1367.5033. Anal. Calcd for $C_{88}H_{70}N_8O_4S_2$: C, 77.28; H, 5.16; N, 8.19. Found: C, 76.90; H, 5.22; N, 8.01.

4.2.2. Compound (2). Orange solid, yield 20% (350 mg). Mp: 246 °C (decomp.). IR (KBr): ν_{max} 3058 w, 2130 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.88 (t, J=9.6 Hz, 4H), 7.79 (t, J=9.0 Hz, 4H), 7.68–7.63 (m, 4H), 7.52–7.44 (m, 8H), 7.42–7.33 (m, 12H), 7.21–7.17 (m, 12H), 7.15 (s, 2H), 7.10 (t, J=6.7 Hz, 8H), 7.01 (t, J=7.6 Hz, 4H), 6.91–6.83 (m, 8H). ^{13}C NMR ($CDCl_3$): δ 84.24, 86.67, 95.12, 96.49, 115.85, 117.36, 120.47, 121.78, 122.96, 125.65, 125.99, 126.08, 127.71, 128.28, 128.76, 129.32, 132.10, 132.96, 134.66, 138.72, 144.72. FABMS (*m*/*z*): 1434 (M^+). HRMS Calcd for $C_{104}H_{66}N_4S_2$: 1434.4729. Found: 1434.4740. Anal. Calcd for $C_{104}H_{66}N_4S_2$: C, 87.00; H, 4.63; N, 3.90. Found: C, 87.11; H, 4.90; N, 3.82.

4.2.3. Compound (3). Yellow solid, yield 80% (1.02 g). Mp: 225–227 °C. IR (KBr): ν_{max} 3055 w, 2930 w, 2130 w cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.50 (s, J=8.1 Hz, 4H), 7.4 (s, J=8.0 Hz, 4H), 7.27 (s, 2H), 7.19 (d, J=7.9 Hz, 4H), 7.15 (d, J=8.0 Hz, 4H), 2.37 (s, 6H), 2.35 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 21.58, 81.50, 84.72, 92.62, 95.37, 96.61, 119.56, 119.97, 122.05, 129.19, 129.28, 131.42, 134.63, 138.08, 138.96. FABMS (*m*/*z*): 622 (M^+). HRMS Calcd for $C_{44}H_{30}S_2$: 622.1789. Found: 622.1790. Anal. Calcd for $C_{44}H_{30}S_2$: C, 84.85; H, 4.85. Found: C, 84.47; H, 5.02.

4.3. General procedures for the synthesis of dibromo-substituted compounds

Compounds 3,3′-dibromo-5,5′-2-(4-*tert*-butyl-phenyl)-5-(4-ethynyl-phenyl)-[1,3,4]oxadiazole-[2,2′]bithiophene (**Ia**) and 4,4′-(3,3′-dibromo-2,2′-bithiophene-5,5′-diyl)bis(ethyne-2,1-diyl)-dibenzonitrile (**Ib**) were synthesized by a similar procedure as the following description. To a flask containing 3,5,3′,5′-tetrabromo-[2,2′]bithiophene (1 equiv), $PdCl_2(PPh_3)_2$ (1.5 mmol % per bromo atom), CuI (0.5 mmol % per bromo atom), PPh_3 (1.5 mmol % per bromo atom), and aromatic acetylene (2.2 equiv per halogen atom) was added *i*-Pr_2NH (70 mL). The resulting mixture was stirred at room temperature for 5 h, then heated to 40 °C for 5 h and allowed to reflux for one day. The solvent was removed under vacuum, and the residue was extracted with CH_2Cl_2 and brine. Removal of CH_2Cl_2 provided an orange residue, which was purified by column chromatography using THF/*n*-hexane as eluent and followed by recrystallization from CH_2Cl_2 and MeOH.

4.3.1. Compound (Ia). Yellow solid, yield 25% (250 mg). Mp: 285 °C (decomp.). IR (KBr): ν_{max} 3045 m, 2910 w, 2115 w cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 8.13 (d, J=8.3 Hz, 4H), 8.05 (d, J=8.0 Hz, 4H), 7.66 (d, J=8.3 Hz, 4H), 7.54 (d, J=8.0 Hz, 4H), 7.27 (s, 2H), 1.36 (s, 18H). ^{13}C NMR ($CDCl_3$): δ 31.10, 35.12, 86.52, 95.02, 97.10, 112.10, 112.85, 114.80, 125.45, 126.10, 126.29, 126.82, 128.29, 132.41, 132.98, 155.57. FABMS (*m*/*z*): 922 (M^+). HRMS Calcd for $C_{48}H_{36}Br_2N_4O_2S_2$: 922.0646. Found: 922.0626. Anal. Calcd for $C_{48}H_{36}Br_2N_4O_2S_2$: C, 62.34; H, 3.92; N, 6.06. Found: C, 62.21; H, 3.87; N, 6.12.

4.3.2. Compound (Ib). Yellow solid, yield 60% (840 mg). Mp: 253 °C (decomp.). IR (KBr): ν_{max} 3046 m, 2200 w, 2120 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.61 (d, J=7.8 Hz, 4H), 7.51 (d, J=7.8 Hz, 4H), 7.02 (s, 2H). ^{13}C NMR ($CDCl_3$): δ 90.57, 90.79, 111.20, 112.04, 114.76, 117.99, 127.96, 132.59, 132.93, 133.35. FABMS (*m*/*z*): 571 (M^+). HRMS Calcd for $C_{26}H_{10}Br_2N_2S_2$: 571.8652. Found: 571.8632. Anal. Calcd for $C_{26}H_{10}Br_2N_2S_2$: C, 54.37; H, 1.76; N, 4.88. Found: C, 54.86; H, 1.72; N, 4.64.

4.4. General procedures for the synthesis of compounds 4–6

Compounds 3,3′-tetrakis-*p*-tolylethynyl-5,5′-2-(4-*tert*-butyl-phenyl)-5-(4-ethynyl-phenyl)-[1,3,4]oxadiazole-[2,2′]-bithiophene (**4**), 4,4′-(3,3′-bis((4-(naphthalen-1-yl(phenyl)-amino)phenyl)ethynyl)-2,2′-bithiophene-5,5′-diyl)bis(ethyne-2,1-diyl)dibenzonitrile (**5**), and 4,4′-(3,3′-bis(*p*-tolyl-ethynyl)-2,2′-bithiophene-5,5′-diyl)bis(ethyne-2,1-diyl)di-benzonitrile (**6**) were synthesized by a similar procedure as the following description. To a flask containing dibromo-compounds (**Ia** or **Ib**) (1 equiv), $PdCl_2(PPh_3)_2$ (3 mmol % per bromo atom), CuI (1 mmol % per bromo atom), PPh_3 (3 mmol % per bromo atom), and aromatic acetylene (2.2 equiv per halogen atom) was added *i*-Pr_2NH (100 mL). The resulting mixture was stirred at room temperature for 30 min, allowed to reflux for two days. The solvent was removed under vacuum, and the residue was extracted with CH_2Cl_2 and brine. Removal of CH_2Cl_2 provided an orange-red residue, which was purified by column chromatography using THF/*n*-hexane as eluent and followed by recrystallization from CH_2Cl_2 and MeOH.

4.4.1. Compound (4). Orange solid, yield 30% (113 mg). Mp: 325 °C (decomp.). IR (KBr): ν_{max} 3055 w, 2930 w, 2130 w cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 8.02 (d, J=8.0 Hz, 4H), 8.01 (d, J=7.8 Hz, 4H), 7.68 (d, J=7.6 Hz, 4H), 7.64 (d, J=8.0 Hz, 4H), 7.52 (d, J=7.8 Hz, 4H), 7.24 (d, J=7.6 Hz, 4H), 7.00 (s, 2H), 2.41 (s, 6H), 1.35 (s, 18H). ^{13}C NMR ($CDCl_3$): δ 21.42, 31.08, 35.08, 85.76, 89.14, 95.01, 97.41, 112.06, 114.78, 120.85, 125.27, 126.08, 126.27, 126.80, 128.19, 128.26, 129.00, 132.96, 137.81, 155.53. FABMS (*m*/*z*): 995 (M^++H). HRMS Calcd for $C_{66}H_{51}N_4O_2S_2$: 995.3453. Found: 995.3455. Anal. Calcd for $C_{66}H_{50}N_4O_2S_2$: C, 79.65; H, 5.06; N, 5.63. Found: C, 79.90; H, 5.11; N, 5.81.

4.4.2. Compound (5). Orange solid, yield 40% (226 mg). Mp: 242 °C (decomp.). IR (KBr): ν_{max} 3055 w, 2200 w, 2150 w cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.89 (dd, J=3.1, 8.2 Hz, 4H), 7.80 (d, J=8.2 Hz, 2H), 7.58 (d, J=8.0 Hz,

4H), 7.51 (d, J=8.0 Hz, 4H), 7.49–7.44 (m, 4H), 7.41–7.33 (m, 8H), 7.31 (s, 2H), 7.25–7.21 (m, 4H), 7.13 (d, J=7.2 Hz, 4H), 7.02 (t, J=7.2 Hz, 2H), 6.91 (d, J=8.4 Hz, 4H). ^{13}C NMR ($CDCl_3$): δ 87.51, 92.24, 94.08, 94.10, 111.41, 112.24, 114.91, 115.50, 118.18, 122.82, 123.39, 123.90, 124.30, 124.55, 124.75, 125.25, 125.54, 128.16, 129.35, 129.53, 129.70, 132.29, 132.49, 132.79, 133.14, 133.56, 147.15, 147.50. FABMS (m/z): 1050 (M^+). HRMS Calcd for $C_{74}H_{42}N_4S_2$: 1050.2851. Found: 1050.2858. Anal. Calcd for $C_{74}H_{42}N_4S_2$: C, 84.54; H, 4.03; N, 5.33. Found: C, 84.64; H, 4.31; N, 5.34.

4.4.3. Compound (6). Orange solid, yield 26% (80 mg). Mp: 282 °C (decomp.). IR (KBr): ν_{max} 3055 w, 2920 w, 2230 w, 2150 w cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.63 (d, J=8.8 Hz, 4H), 7.57 (d, J=8.8 Hz, 4H), 7.50 (d, J=8.0 Hz, 4H), 7.36 (s, 2H), 7.21 (d, J=8.0 Hz, 4H), 2.39 (s, 6H). ^{13}C NMR ($CDCl_3$): δ 21.39, 81.52, 81.80, 112.24, 114.74, 118.18, 125.23, 126.90, 128.16, 128.94, 128.96, 131.95, 132.59, 132.91, 137.78. FABMS (m/z): 644 (M^+). HRMS Calcd for $C_{44}H_{24}N_2S_2$: 644.1381. Found: 644.1374. Anal. Calcd for $C_{44}H_{24}N_2S_2$: C, 81.96; H, 3.75; N, 4.34. Found: C, 82.10; H, 3.51; N, 4.61.

4.5. Measurement of two-photon cross-section by the two-photon-induced fluorescence method

The setup for TPEF excitation spectra and TPEF excitation cross-section measurement has been described in our previous report.[13a] In brief, a femtosecond mode-locked Ti:sapphire laser (Spectra Physics) generates ~100 fs pulses at repetition rate of 82 MHz with an average power of 300–400 mW. The laser beam was focused on a sample cell (1 cm) by a lens with the focal length 6 cm. To minimize the effects of re-absorption, the excitation beam was focused as close as possible to the wall of the quartz cell, which faced the slit of the imaging spectrograph. TPEF was detected at a direction perpendicular to the pump beam. The TPEF was focused by a lens with the focal length 8 cm, and was coupled via an optical fiber (Acton, ILG-45-20-3) into an optical spectrum analyzer. Our optical spectrum analyzer consists of a CCD with detector control (ICCD-576G, Princeton Instruments. Inc.) in conjunction with a monochromator (SpectraPro-275, Acton Research Corporation) was used as a recorder.

The TPA and TPEF cross-sections (σ and σ_e, respectively) are basic parameters to evaluate a material's TPA and TPEF properties. From TPEF intensity data, σ_e and σ can be evaluated by using Eqs. 1 and 2,[19a,24] expressed as, where r stands for the reference compound, n for the refractive index of solvents applied, and F for the integrated fluorescence intensity; the concentration of the molecules in solution was denoted as C.

$$\sigma_e = \sigma_{e,r}\frac{F n_r C_r}{F_r n C} \quad (1)$$

$$\sigma\Phi = \sigma_e \quad (2)$$

The TPEF cross-section σ_e is supposed to be linearly dependent on the TPA cross-section (σ) with the TPEF quantum yield Φ' as the coefficient.[19a] In most reports, the SPEF quantum yield Φ was adopted instead of Φ', because Φ' is difficult to be measured. By referencing the TPEF cross-section of Coumarin 480 to be 146.3 GM (1 GM = 10^{-50} cm^4 s/photon)[19a] and Rhodamine 6G to be 37.90 GM (1 GM = 10^{-50} cm^4 s/photon).[19b] The TPA cross-sections of these compounds were obtained (Table 1). The TPEF spectra of all these compounds were taken when they were excited at 800 nm in CH_2Cl_2. The TPEF of Coumarin 480 or Rhodamine 6G was measured as a standard under the same experimental conditions. We obtained the relative TPEF cross-sections σ_e of these compounds by comparing their TPEF to that of Coumarin 480 (compounds **1**, **3**, and **4**) or Rhodamine 6G (compounds **2**, **5**, and **6**) under exactly the same experimental conditions.

4.6. Theoretical calculation

All calculations are done with the Gaussian 03 program.[25] Hartree Fock is used with the basis set 3-21G* (hereafter designated as B3LYP).[26] The calculated minima have been carefully checked by frequency analyses to examine whether the number of the imaginary frequency is zero. Note that molecules considered here are large enough to preclude the use of ab initio methods with large basis sets. Alternatively, the electronic properties and UV–visible spectra (single-point calculation) were calculated with the ZINDO/S-CI method on the optimized structures.[27]

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Synthetic oligorhamnans related to the most common O-chain backbone from phytopathogenic bacteria

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Abstract—The synthesis of the tetrasaccharide rhamnanic motif α-L-Rha-(1→3)-α-L-Rha-(1→2)-α-L-Rha-(1→2)-α-L-Rha and its dimerization to octasaccharide have been developed. Three different pathways toward the dimerization have been investigated; the best one was based on a [4+2]+2 stepwise condensation of a rhamnose tetrasaccharide with two rhamnosyl *N*-phenyl trifluoroacetimidates as glycosyl donors and on an orthogonal set of protecting groups consisting of benzoyl, levulinoyl, and allyl groups.

1. Introduction

The mechanism of pathogenic agent recognition by plants is still unknown, even if many efforts toward understanding are currently underway.[1] It has been recently suggested that the recognition is analogous to the innate immunity system of animals,[2] which is based on the perception of pathogen-associated molecular patterns (PAMPs), characteristic structures of the pathogen indispensable for its growth within the host.[3] Since lipopolysaccharides (LPS) cover almost 80% of the cell surface, they are one of a group of general elicitors that can be recognized by plants to trigger a defense response; this role is induced especially by lipid-A and core[4] that are the most highly conserved regions of LPS in different Gram negative bacteria.[5] A recent study showed that some synthetic oligorhamnans are also able to trigger defense responses in plants and therefore they are PAMP.[6] The oligosaccharides used in that work were the rhamnose trisaccharide **A** and its dimer and trimer; they were chosen as first compounds for phytopathogenic tests, since **A** represents the motif of the most general backbone of the LPS *O*-antigenic region (O-chain) from phytopathogenic bacteria. Nevertheless, only a few bacterial strains present **A** as a repeating unit of their O-chain backbone; the most common O-chain backbones are characterized by motifs such as **B** or **C** that differ from **A** by the addition of a 3-linked- or 2-linked-rhamnose unit (Fig. 1).[7] So far, tetrasaccharide

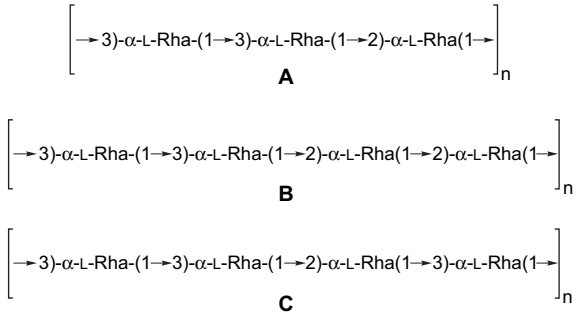

Figure 1. Common rhamnanic backbones of *O*-antigen polysaccharides from phytopathogenic bacteria.

motif **B** has been most frequently found in the O-chains of LPS from phytopathogenic bacteria. In this paper, the synthesis of **B** and its oligomerization is described; the synthetic oligorhamnans obtained will be the object of molecular mechanics calculations and phytopathogenic tests, in order to compare their 3D-structures and their eventual biological activities with the oligorhamnans related to motif **A**.

2. Results and discussion

The synthesis of α-linked oligorhamnans was the target of several reports in the last two decades;[8] recently, a methyl and an octyl glycoside bearing a tetrasaccharide corresponding to motif **B** were also synthesized,[9] nevertheless their oligomerization was not attempted. The synthetic approach described in this paper aimed at the synthesis

Keywords: Rhamnose; Glycosylation; Oligosaccharide; Lipopolysaccharide; Phytopathogenic bacteria.
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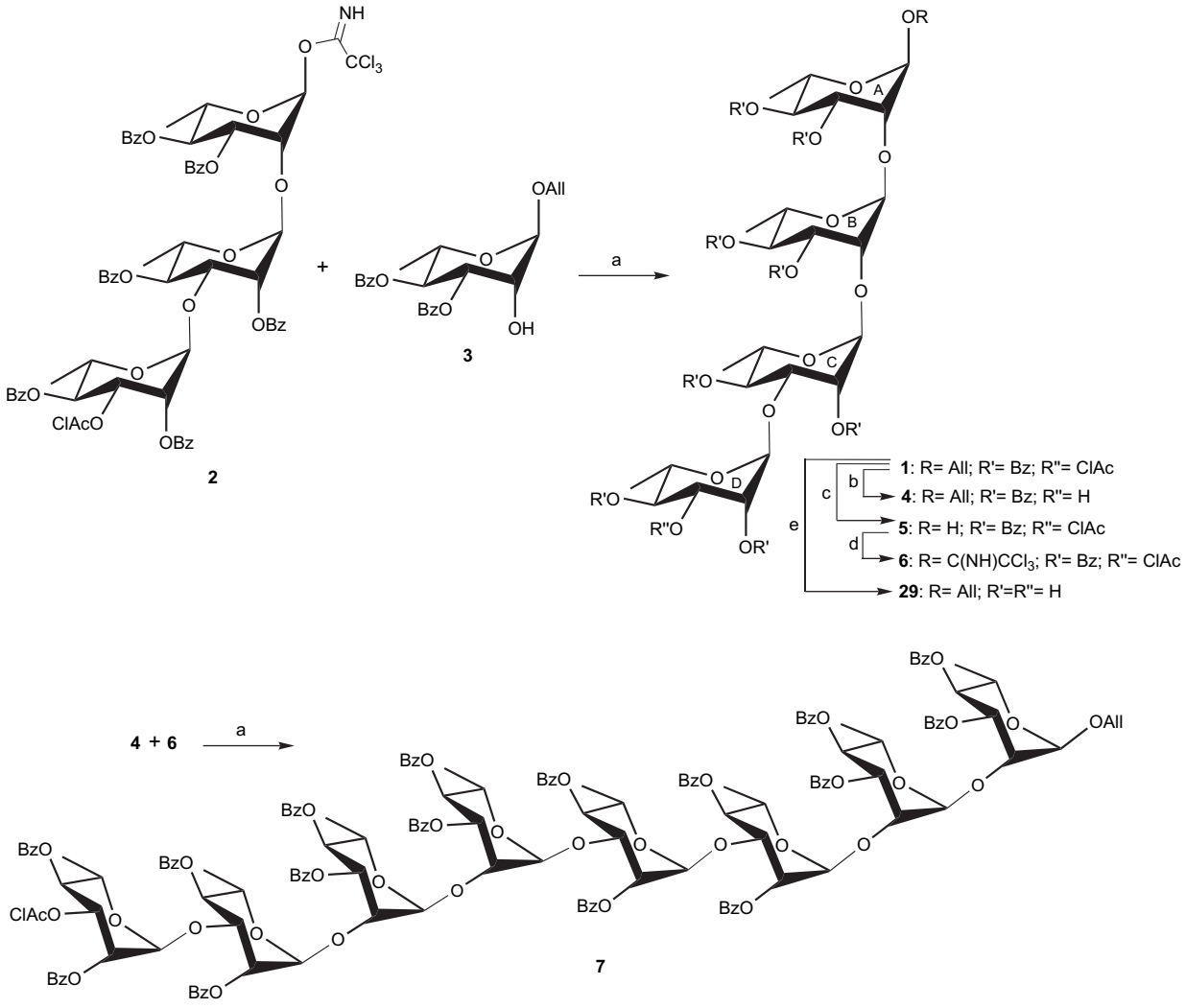
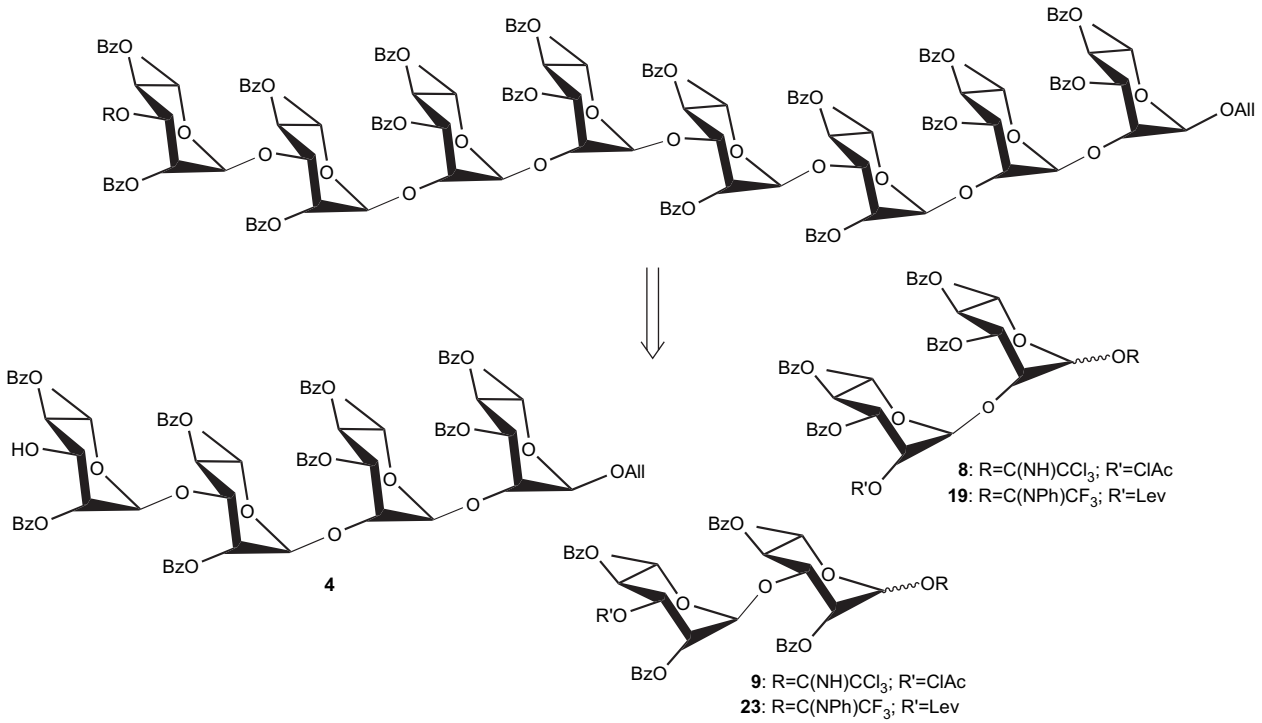
of a tetrasaccharide building-block that would be easily functionalized both as a glycosyl donor and a glycosyl acceptor, thus permitting stepwise condensation to higher oligosaccharides. Therefore, the protection pattern of this tetrasaccharide should have two orthogonal temporary protecting groups at positions O-1_A and O-3_D and a permanent protecting group at the other positions; an allyl group was chosen for the anomeric position, a chloroacetyl for position O-3_D, and benzoyls for the other positions. Tetrasaccharide **1** with this protection pattern was obtained by condensing trisaccharide donor **2**[10] with acceptor **3**[11] in CH_2Cl_2 at −50 °C with $BF_3\cdot OEt_2$ as activator (72% yield) (Scheme 1). The α-configuration of the newly formed glycosidic linkage was ascertained by the heteronuclear C_1–H_1 coupling constant of 172 Hz measured in a *J*-coupled HSQC experiment. Selective removal of the chloroacetyl moiety was easily achieved by treating an aliquot of **1** with thiourea; the tetrasaccharide acceptor **4** was obtained in 71% yield. Another aliquot of **1** was de-O-allylated with $PdCl_2$ in 1:1 CH_2Cl_2/MeOH to obtain a tetrasaccharide glycosyl donor, the resulting hemiacetal **5** (80%) was subsequently activated by treatment with Cl_3CCN and DBU to give the trichloroacetimidate **6** in 61% yield. Unfortunately, the glycosylation of **6** with **4** was unsuccessful; octasaccharide **7** was obtained in very low yield (<15%) by activating **6** with $BF_3\cdot OEt_2$ at −50 °C. No better yield was observed even by changing several reaction conditions. Interestingly, the glycosylation of trisaccharide trichloroacetimidate **2**, that is related to **6**, with rhamnose oligosaccharides had been already successfully accomplished.[10] Thus, we hypothesized that the upper limit for such couplings between rhamnose oligosaccharides was the use of a trisaccharide donor and a new strategy for the dimerization of **1** was planned. This new approach was to dimerize **1** by stepwise condensation of tetrasaccharide acceptor **4** with two different disaccharide donors that would have a temporary protecting group at positions O-2_B and O-3_B, respectively. In analogy to the [4+4] strategy, a chloroacetyl was chosen as temporary protecting group; compounds **8** and **9** was therefore, designed as suitable disaccharide donors (Scheme 2).

The synthesis of compound **8** was started by treating the known diol **10**[12] with BzCl in 2:1 CH_2Cl_2/Py at −30 °C to give selectively 3,4-di-*O*-benzoylated alcohol **11** in 88%



Scheme 1. Reagents and conditions: (a) $BF_3\cdot OEt_2$, AW-300 4 Å MS, CH_2Cl_2, −50 °C, to **1**: 75 min, 72%; to **7**: 2 h, <15%; (b) NH_2CSNH_2, 1:1 EtOH/DMF, rt, 2 days, 71%; (c) $PdCl_2$, 1:1 CH_2Cl_2/MeOH, rt, 2 days, 80%; (d) Cl_3CCN, DBU, 0 °C, 3 h, 61%; (e) NaOMe, MeOH, 40 °C, overnight, 78%.

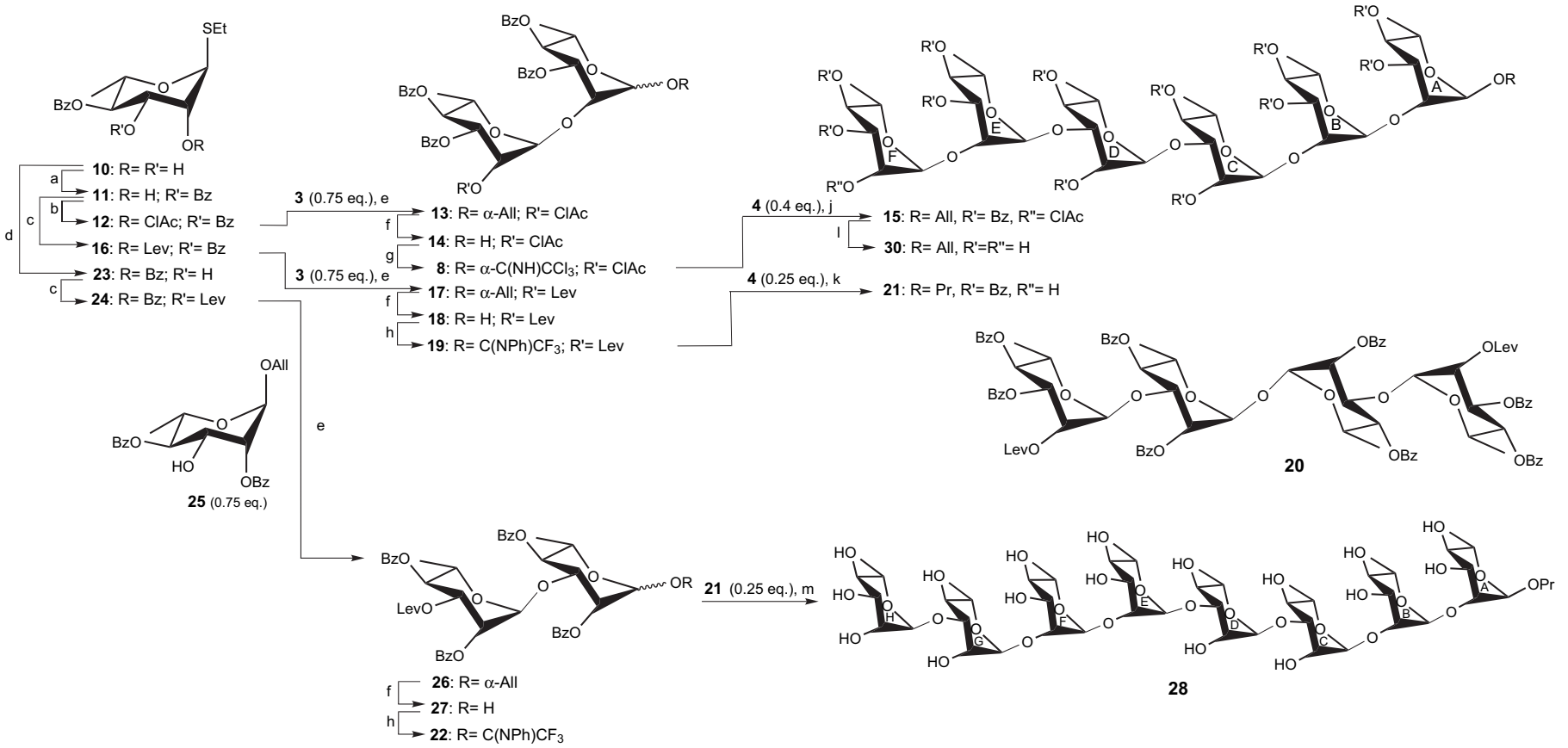


Scheme 2. [4+2]+2 Strategy for the dimerization of the rhamnanic motif **B**.

yield (Scheme 3). Compound **11** was chloroacetylated (56%) and the resulting fully-protected thioglycoside **12** was coupled with **3** by activation with NIS/TfOH at −30 °C. Disaccharide **13** was obtained in unsatisfactory yield (45%); this result was consistent with a recent report on the poor outcome of a condensation reaction involving a 2-chloroacetylated trichloroacetimidate as rhamnosyl donor.[13] In spite of the limited yield of the coupling, compound **13** was de-O-allylated with $PdCl_2$ to give hemi-acetal **14** (53%) that was converted in turn to trichloroacetimidate **8** (80%). Condensation of **8** with tetrasaccharide acceptor **4** at −50 °C with $BF_3 \cdot OEt_2$ as activator gave hexasaccharide **15** in moderate yield (48%).

The low global yield of the synthetic path from diol **10** to hexasaccharide **15** (5%) necessitated the re-designing of this synthetic path. The chloroacetyl temporary protecting group was replaced with a levulinoyl group; in addition the trichloroacetimidate leaving group on the disaccharide donor was replaced by a *N*-phenyltrifluoroacetimidate,[14] because the latter was recently shown to be very effective in glycosylation reactions involving deoxysugars (Scheme 2).[15] Thus, alcohol **11** was treated with levulinic acid (LevOH) in the presence of *N*,*N*′-diisopropylcarbodiimide (DIPC) and DMAP to give the 2-*O*-levulinoylated thioglycoside **16** (91%) that was coupled with **3** by activation with NIS/TfOH at −30 °C to afford disaccharide **17** in 83% yield. Hemi-acetal **18** was obtained from **17** with $PdCl_2$ (83%) and then converted into *N*-phenyltrifluoroacetimidate **19** (72%) by treatment with $CF_3C(NPh)Cl$ and NaH (Scheme 3).[16] Coupling of disaccharide donor **19** with tetrasaccharide acceptor **4** by activation with TMSOTf in CH_2Cl_2 at 0 °C proceeded very satisfyingly. Actually, an exact yield of this condensation was not obtained, since, after column chromatography, the resulting hexasaccharide was contaminated by traces of tetrasaccharide **20**, a side product due to self-condensation of **19**. Treatment with hydrazinium acetate in 7:1 CH_2Cl_2/MeOH cleaved the Lev group and reduced the allyl aglycon to propyl by the diimide generated in situ,[17] affording pure hexasaccharide acceptor **21** in 77% yield (calculated from **3**). A *J*-coupled HSQC experiment on **21** confirmed the α-configuration of the newly formed glycosidic linkage ($^1J_{C,H}$=172 Hz). The global yield of the synthetic path from **10** to **21** was much better with Lev than ClAc as O-2 temporary protecting group (30% vs 5%).

This result prompted us to re-design also the second disaccharide donor with a Lev temporary group. Thus, the synthesis of donor **22** (to replace **9**) was undertaken. Diol **10** was regioselectively 2-O-benzoylated via *ortho*-ester as already reported[13] and the resulting alcohol **23** was converted to 3-*O*-levulinoylated thioglycoside **24** (89%). Coupling of **24** with acceptor **25**[18] proceeded in high yield (87%) by activation of **24** with NIS/TfOH at −30 °C. Disaccharide **26** was de-O-allylated (60%) and the resulting hemi-acetal **27** was converted into *N*-phenyltrifluoroacetimidate **22** (58%). Elongation of hexasaccharide **21** by condensation with disaccharide donor **22** gave, after column chromatography, an octasaccharide contaminated by tetrasaccharide **20**. Benzoyl and levulinoyl deprotection by Zemplén transesterification of the mixture afforded pure propyl octasaccharide **28** (49% calculated from **21**) after size exclusion chromatography, the ^1H NMR spectrum of which is reported in Figure 2. Similarly, ester deprotection of **1** and **15** gave tetrasaccharide **29** (78%) and hexasaccharide **30** (90%) (Schemes 1 and 3). Compounds **28**, **29**, and **30** are currently the object of molecular mechanics calculations and phytopathogenic tests.



Scheme 3. Reagents and conditions: (a) BzCl, 2:1 CH_2Cl_2/Py, −30 °C, 2.5 h, 88%; (b) $ClCH_2COCl$, 1:1 Py/DMF, rt, 4 h, 56%; (c) LevOH, DIPC, DMAP, rt, to **16**: 1 h, 91%; to **24**: 30 min, 89%; (d) see Ref. 12; (e) NIS, TfOH, AW-300 4 Å MS, CH_2Cl_2, −30 °C, to **13**: 90 min, 45%; to **17**: 90 min, 83%; to **26**: 3 h, 87%; (f) $PdCl_2$, 3:1 CH_2Cl_2/MeOH, rt, overnight, to **14**: 53%; to **18**: 83%; to **27**: 60%; (g) Cl_3CCN, DBU, CH_2Cl_2, 0 °C, 2 h, 80% (α/β=6:1); (h) $CF_3C(NPh)Cl$, NaH, 4 Å MS, CH_2Cl_2, 0 °C to rt, 4 h, to **19**: 72% (α/β=1:1); to **22**: 58% (α/β=1:1); (j) $BF_3 \cdot OEt_2$, AW-300 4 Å MS, CH_2Cl_2, −50 °C, 4 h, 48%; (k) (i) TMSOTf, AW-300 4 Å MS, CH_2Cl_2, 0 °C, 3 h; (ii) N_2H_4, AcOH, 7:1 CH_2Cl_2/MeOH, rt, 2 h, 77% over two steps; (l) NaOMe, 3:1 MeOH/CH_2Cl_2, 40 °C, overnight, 77%; (m) (i) TMSOTf, AW-300 4 Å MS, CH_2Cl_2, 0 °C, overnight, (ii) NaOMe, 3:1 MeOH/CH_2Cl_2, 40 °C, 2 days, 49% over two steps.

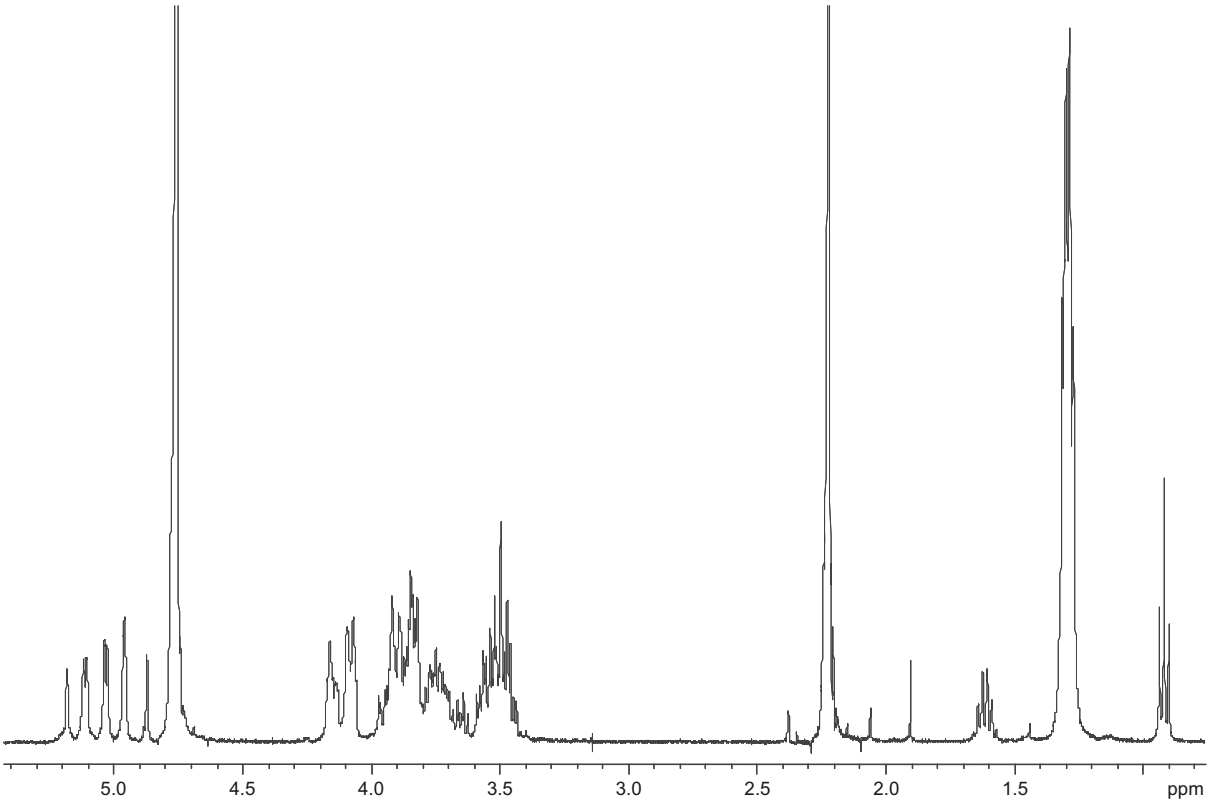


Figure 2. ^{1}H NMR spectrum (D_2O, 400 MHz; acetone as internal standard) of the octasaccharide **28**.

3. Experimental

3.1. General methods

^{1}H and ^{13}C NMR spectra were recorded on Varian XL-200 (^{1}H: 200 MHz; ^{13}C: 50 MHz), Varian Gemini-300 (^{1}H: 300 MHz; ^{13}C: 75 MHz) or Bruker DRX-400 (^{1}H: 400 MHz; ^{13}C: 100 MHz) instruments in $CDCl_3$ ($CHCl_3$ as internal standard, ^{1}H: C*H*Cl_3 at δ 7.26; ^{13}C: *C*DCl_3 at δ 77.0) and in D_2O (acetone as internal standard, ^{1}H: (CH_3)$_2$CO at δ 2.22; ^{13}C: (*C*H_3)$_2$CO at δ 31.5). Assignment of proton and carbon chemical shifts of the deprotected oligosaccharides was based on 2D NMR experiments such as COSY, TOCSY, NOESY, and HSQC. Heteronuclear C_1–H_1 coupling constants were measured with *J*-coupled HSQC experiments. Positive ESI-MS spectra were recorded on a Finnigan LCQ-DECA ion trap mass spectrometer. Positive MALDI-MS spectra were recorded on an Applied Biosystem Voyager DE-PRO MALDI-TOF mass spectrometer in the positive mode; compounds were dissolved in the appropriate solvent at a concentration of 1 mg/mL and 1 μL of these solutions were mixed with 1 μL of a 20 mg/mL solution of 2,5-dihydroxybenzoic acid in 7:3 CH_3CN/0.1 M aqueous TFA. IR spectra were recorded on a JASCO-FTIR-430 spectrometer. Optical rotations were measured on a JASCO P-1010 polarimeter. Analytical thin layer chromatography (TLC) was performed on aluminum plates precoated with Merck silica gel 60 F_{254} as the adsorbent. The plates were developed with 5% H_2SO_4 ethanolic solution and then heated to 130 °C. Column chromatography was performed on Merck Kieselgel 60 (63–200 mesh), except where differently specified. Gel-filtration chromatographies were performed on a Sephadex G-10 column (2.0×90 cm) with water as eluant.

3.1.1. Allyl (2,4-di-*O*-benzoyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzoyl-α-L-rhamnopyranoside (1). A mixture of **3** (179 mg, 0.43 mmol) and **2** (734 mg, 0.56 mmol) was coevaporated three times with toluene, the residue was dried, and then mixed with freshly activated AW-300 4 Å molecular sieves, suspended under argon in CH_2Cl_2 (15 mL), and stirred at −50 °C. $BF_3{\cdot}OEt_2$ (35 μL, 0.28 mmol) was then added. After 75 min the reaction mixture was quenched with some drops of Et_3N. After filtration over a Celite pad, the mixture was concentrated to give a residue that after column chromatography (5:1 to 3:1 petroleum ether/ethyl acetate) afforded **1** (480 mg, 72%) as a white foam. $[\alpha]_D$ +95.8 (*c* 1.0, CH_2Cl_2). IR (thin film, NaCl) 3030, 2913, 1720, 1458, 1275 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.15–7.09 (m, 40H), 5.98 (m, 1H), 5.83 (dd, *J*=9.9, 3.1 Hz, 1H), 5.68–5.57 (m, 3H), 5.54 (br s, 1H), 5.43–5.27 (m, 5H), 5.18 (br s, 1H), 5.17 (br s, 1H), 5.12 (br s, 1H), 5.01 (br s, 1H), 4.80 (br s, 1H), 4.50 (dd, *J*=10.0, 3.4 Hz, 1H), 4.40 (br s, 1H), 4.36 (br s, 1H), 4.30 (dd, *J*=9.7, 3.6 Hz, 1H), 4.23 (dq, *J*=9.6, 6.1 Hz, 1H), 4.13 (m, 3H), 4.00 (dq, *J*=10.0, 6.1 Hz, 1H), 3.72 (AB d, *J*=14.9 Hz, 1H), 3.68 (AB d, *J*=14.9 Hz, 1H), 1.41 (d, *J*=6.1 Hz, 3H), 1.35 (d, *J*=6.1 Hz, 3H), 1.20 (d, *J*=6.1 Hz, 3H), 1.01 (d, *J*=6.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 165.8–165.3 (CO), 133.8–133.3 (C_{ipso}, $OCH_2CH{=}CH_2$),

129.8–128.3 (C-Ar), 118.1 ($OCH_2CH{=}\mathit{C}H_2$), 100.8, 99.3, 99.0, 97.9 (C_1^A, C_1^B, C_1^C, C_1^D), 78.0, 77.5, 75.1 (C_2^A, C_2^B, C_3^C), 72.8, 71.8, 71.7, 71.6, 71.5, 71.3, 70.6, 70.5, 70.3, 68.1, 67.5, 67.4, 67.3, 66.9 (C_2^C, C_2^D, C_3^A, C_3^B, C_3^D, C_4^A, C_4^B, C_4^C, C_4^D, C_5^A, C_5^B, C_5^C, C_5^D, $OCH_2CH{=}CH_2$), 40.3 ($\mathit{C}H_2Cl$), 17.6–17.2 (C_6^A, C_6^B, C_6^C, C_6^D). MALDI-MS for $C_{85}H_{79}ClO_{26}$ (*m*/*z*): M_r (calcd) 1550.45; M_r (found) 1573.27 $(M+Na)^+$. Anal. Calcd: C 65.78, H 5.13. Found: C 65.97, H 5.10.

3.1.2. Allyl (2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzoyl-α-L-rhamnopyranoside (4). A solution of **1** (189 mg, 0.12 mmol) was dissolved in 1:1 EtOH/DMF (12 mL) and then thiourea was added (93 mg, 1.29 mmol). After 2 days stirring at rt, the solution was diluted with CH_2Cl_2, washed with 1 M HCl, 1 M $NaHCO_3$, and water. The organic layer was collected, dried, and concentrated to give a residue that, after column chromatography (4:1 petroleum ether/ethyl acetate) afforded **4** (128 mg, 71%) as a white foam. $[\alpha]_D$ +116.9 (*c* 1.7, CH_2Cl_2). IR (thin film, NaCl) 3053, 3031, 2925, 1723, 1460 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.17–7.07 (m, 40H), 5.98 (m, 1H), 5.85 (dd, *J*=9.9, 3.1 Hz, 1H), 5.64 (m, 2H), 5.58 (t, *J*=9.8 Hz, 1H), 5.53 (br s, 1H), 5.39 (m, 2H), 5.29 (d, *J*=10.8 Hz, 1H), 5.21 (br s, 1H), 5.13–5.05 (m, 4H), 5.02 (br s, 1H), 4.80 (br s, 1H), 4.48 (dd, *J*=9.6, 3.1 Hz 1H), 4.41 (br s, 1H), 4.37 (br s, 1H), 4.30 (dd, *J*=9.7, 3.6 Hz, 1H), 4.23 (dq, *J*=9.6, 6.1 Hz, 1H), 4.13 (m, 2H), 4.03 (m, 2H), 2.17 (br s, 1H), 1.39 (d, *J*=6.1 Hz, 3H), 1.34 (d, *J*=6.1 Hz, 3H), 1.16 (d, *J*=6.1 Hz, 3H), 1.01 (d, *J*=6.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.8–165.3 (CO), 133.9–133.3 (C_{ipso}, $OCH_2\mathit{C}H{=}CH_2$), 130.0–128.2 (C-Ar), 118.1 ($OCH_2CH{=}\mathit{C}H_2$), 100.8, 99.2, 99.0, 98.3 (C_1^A, C_1^B, C_1^C, C_1^D), 77.9, 77.0, 75.3, 75.1, 73.3–71.7, 70.6, 68.4, 68.3, 68.0, 67.4, 66.9 (C_2^A, C_2^B, C_2^C, C_2^D, C_3^A, C_3^B, C_3^C, C_3^D, C_4^A, C_4^B, C_4^C, C_4^D, C_5^A, C_5^B, C_5^C, C_5^D, $OCH_2CH{=}CH_2$), 17.6–17.3 (C_6^A, C_6^B, C_6^C, C_6^D). MALDI-MS for $C_{63}H_{78}O_{25}$ (*m*/*z*): M_r (calcd) 1474.48; M_r (found) 1497.42 $(M+Na)^+$. Anal. Calcd: C 67.56, H 5.33. Found: C 67.67, H 5.30.

3.1.3. (2,4-Di-*O*-benzoyl-3-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (6). Compound **1** (299 mg, 0.19 mmol) was dissolved in 1:1 MeOH/CH_2Cl_2 (8.0 mL), $PdCl_2$ (8.6 mg, 95 μmol) was then added, and the mixture was vigorously stirred at rt for 2 days, after that it was filtered over a Celite pad, diluted with CH_2Cl_2, and washed with 5 M NaCl. The organic layer was collected, dried, and concentrated to give **5** (227 mg, 80%) that was then dissolved in CH_2Cl_2 (15 mL) under Ar atmosphere. The solution was cooled to 0 °C and then treated with Cl_3CCN (76 μL, 0.76 mmol) and DBU (6.7 μL, 45 μmol). After 3 h the solution was concentrated at 30 °C. The residue was subjected to neutral alumina (Brockman grade 1) column chromatography (9:2 petroleum ether/ethyl acetate) to give **6** (152 mg, 61%) as a white foam. $[\alpha]_D$ +101.5 (*c* 1.0, CH_2Cl_2). IR (thin film, NaCl) 3023, 2970, 1741, 1650 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.39 (s, 1H), 8.15–7.11 (m, 40H), 6.47 (br s, 1H), 5.84 (dd, *J*=9.8, 3.2 Hz, 1H), 5.72 (t, *J*=9.8 Hz, 1H), 5.68 (dd, *J*=9.6, 3.2 Hz, 1H), 5.60 (t, *J*=9.8 Hz, 1H), 5.55 (br s, 1H), 5.42 (m, 2H), 5.31 (t, *J*=9.5 Hz, 1H), 5.19 (br s, 2H), 5.17 (br s, 1H), 4.88 (br s, 1H), 4.63 (br s, 1H), 4.51 (dd, *J*=9.5, 3.3 Hz, 1H), 4.40 (br s, 1H), 4.33 (m, 2H), 4.13 (dq, *J*=9.5, 6.1 Hz, 1H), 4.01 (dq, *J*=9.5, 6.1 Hz, 1H), 3.72 (AB d, *J*=14.9 Hz, 1H), 3.68 (AB d, *J*=14.9 Hz, 1H), 1.43 (d, *J*=6.1 Hz, 3H), 1.39 (d, *J*=6.1 Hz, 3H), 1.30 (d, *J*=6.1 Hz, 3H), 0.93 (d, *J*=6.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.0–165.4 (CO), 161.4 ($Cl_3C\mathit{C}{=}NH$), 133.8–133.4 (C_{ipso}), 130.0–128.3 (C-Ar), 100.9, 100.2, 100.1, 97.2 (C_1^A, C_1^B, C_1^C, C_1^D), 78.1, 75.1, 74.9, 72.9–71.1, 70.5, 70.2, 69.8, 69.7, 67.7–67.5 (C_2^A, C_2^B, C_2^C, C_2^D, C_3^A, C_3^B, C_3^C, C_3^D, C_4^A, C_4^B, C_4^C, C_4^D, C_5^A, C_5^B, C_5^C, C_5^D), 40.3 (CH_2Cl), 17.7–17.3 (C_6^A, C_6^B, C_6^C, C_6^D). Anal. Calcd: C 60.91, H 4.56, N 0.85. Found: C 61.09, H 4.45, N 0.88.

3.1.4. Ethyl 3,4-di-*O*-benzoyl-1-thio-α-L-rhamnopyranoside (11). A solution of **10** (2.935 g, 9.40 mmol) in 2:1 CH_2Cl_2/Py (12 mL) was cooled to −30 °C and then treated with a 1.1 M solution (2.25 mL) of BzCl in 2:1 CH_2Cl_2/Py. After stirring for 2.5 h, the mixture was treated with some drops of water, heated to rt, and then diluted with CH_2Cl_2. The mixture was washed with water, 1 M HCl, and water again. The organic layer was collected, dried, and concentrated to give a residue that, after column chromatography (7:1 petroleum ether/ethyl acetate) afforded **11** (3.446 g, 88%) as a white foam. $[\alpha]_D$ +8 (*c* 0.5, CH_2Cl_2). IR (thin film, NaCl) 3063, 3025, 2926, 1707, 1605 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 7.99–7.30 (m, 10H), 5.62 (t, *J*=9.8 Hz, 1H), 5.53 (dd, *J*=9.8, 2.6 Hz, 1H), 5.37 (br s, 1H), 4.48 (dq, *J*=9.8, 6.2 Hz, 1H), 4.39 (br s, 1H), 2.71 (app oct, *J*=8.4 Hz, 2H), 2.04 (br s, 1H), 1.35 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.6 (CO), 132.9, 132.8 (C_{ipso}), 129.4–128.0 (C-Ar), 84.1 (C_1), 72.7, 71.6, 70.5, 66.7 (C_2, C_3, C_4, C_5), 24.8 ($S\mathit{C}H_2CH_3$), 17.1 (C_6), 14.5 ($SCH_2\mathit{C}H_3$). ESIMS for $C_{22}H_{24}O_6S$ (*m*/*z*): M_r (calcd) 416.13; M_r (found) 439.33 $(M+Na)^+$. Anal. Calcd: C 63.44, H 5.81. Found: C 63.66, H 5.88.

3.1.5. Ethyl 3,4-di-*O*-benzoyl-2-*O*-chloroacetyl-1-thio-α-L-rhamnopyranoside (12). A solution of **11** (100 mg, 0.24 mmol) 1:1 pyridine/DMF (2.0 mL) was treated with $ClCH_2COCl$ (86 μL, 1.08 mmol) and then stirred at rt for 4 h. The mixture was coevaporated several times with toluene, then diluted with CH_2Cl_2, and washed with water. The organic layer was dried and concentrated to give a residue that was subjected to column chromatography (7:1 petroleum ether/ethyl acetate) to afford **12** (66 mg, 56%) as a yellowish oil. $[\alpha]_D$ −17.4 (*c* 2.1, CH_2Cl_2). IR (thin film, NaCl) 3060, 3025, 2959, 1721, 1596 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 7.99–7.30 (m, 10H), 5.68–5.52 (m, 3H), 5.36 (br s, 1H), 4.50 (dq, *J*=9.8, 6.2 Hz, 1H), 4.18 (s, 2H), 2.70 (m, 2H), 1.34 (m, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 166.5, 165.6, 165.3 (CO), 133.3 ($2C_{ipso}$), 129.7–128.3 (C-Ar), 81.7 (C_1), 73.7, 71.5, 70.0, 67.3 (C_2, C_3, C_4, C_5), 40.6 (CH_2Cl), 25.6 ($S\mathit{C}H_2CH_3$), 17.5 (C_6), 14.9 ($SCH_2\mathit{C}H_3$). ESIMS for $C_{24}H_{25}ClO_7S$ (*m*/*z*): M_r (calcd) 492.10; M_r (found) 513.39 $(M+Na)^+$. Anal. Calcd: C 58.47, H 5.11. Found: C 58.77, H 5.02.

3.1.6. Allyl (3,4-di-*O*-benzoyl-2-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzoyl-α-L-rhamnopyranoside (13). A mixture of **3** (50 mg, 0.12 mmol) and **12** (79 mg, 0.16 mmol) was coevaporated three times with

toluene, the residue was dried, and then mixed with freshly activated AW-300 4 Å molecular sieves and NIS (45 mg, 0.20 mmol). The mixture was suspended in CH_2Cl_2 (4.0 mL) under an Ar atmosphere and rapidly cooled to −30 °C. A 115 mg/mL solution of TfOH in CH_2Cl_2 (54 μL, 40 μmol) was then added. After 90 min the reaction mixture was rapidly filtered over a Celite pad, diluted with CH_2Cl_2, and washed with 10% $Na_2S_2O_3$ and 1 M $NaHCO_3$. The organic layer was collected, dried and concentrated to give a foamy residue. After column chromatography (7:1 petroleum ether/ethyl acetate), **13** (46 mg, 45%) was recovered as a white foam. $[\alpha]_D$ +45.1 (*c* 1.0, CH_2Cl_2). IR (thin film, NaCl) 3055, 3026, 2948, 1726, 1600, 1255 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 8.02–7.30 (m, 20H, H-Ar), 5.98 (m, 1H), 5.87 (dd, *J*=10.0, 3.2 Hz, 1H), 5.80 (dd, *J*=10.0, 3.2 Hz, 1H), 5.70 (dd, *J*=3.2, 1.8 Hz, 1H), 5.62 (t, *J*=10.0 Hz, 1H), 5.50 (t, *J*=10.0 Hz, 1H), 5.39 (d, *J*=17.0 Hz, 1H), 5.28 (d, *J*=10.4 Hz, 1H), 5.00 (br s, 2H), 4.34–4.12 (m, 5H), 4.08 (AB d, *J*=14.9 Hz, 1H), 3.98 (AB d, *J*=14.9 Hz, 1H), 1.39 (d, *J*=6.1 Hz, 3H), 1.30 (d, *J*=6.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 166.0, 165.7, 165.6, 165.4, 165.1 (CO), 133.4–133.1 (C_{ipso}, $OCH_2CH{=}CH_2$), 129.8–128.4 (C-Ar), 117.9 ($OCH_2CH{=}CH_2$), 99.0, 97.6 (C_1^A, C_1^B), 76.7, 71.7, 71.6, 71.4, 71.1, 69.3, 68.2, 67.5, 67.0 (C_2^A, C_2^B, C_3^A, C_3^B, C_4^A, C_4^B, C_5^A, C_5^B, $OCH_2CH{=}CH_2$), 40.5 (CH_2Cl), 17.6 (C_6^A, C_6^B). ESIMS for $C_{45}H_{43}ClO_{14}$ (*m/z*): M_r (calcd) 842.23; M_r (found) 865.49 $(M+Na)^+$. Anal. Calcd: C 64.09, H 5.14. Found: C 64.18, H 5.07.

3.1.7. (3,4-Di-*O*-benzoyl-2-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzoyl-L-rhamnopyranosyl trichloroacetimidate (8). Compound **13** (130 mg, 0.15 mmol) was dissolved in 3:1 CH_2Cl_2/MeOH (4.0 mL), $PdCl_2$ (5.4 mg, 60 μmol) was then added, and the mixture was vigorously stirred overnight. It was then filtered over a Celite pad, diluted with CH_2Cl_2, and washed with 5 M NaCl. The organic layer was dried and concentrated to give **14** (65 mg, 53%) that was then dissolved in CH_2Cl_2 (3.0 mL) under Ar atmosphere. The solution was cooled to 0 °C and then treated with Cl_3CCN (33 μL, 0.33 mmol) and DBU (4.3 μL, 29 μmol). After 2 h the solution was concentrated at 30 °C. The residue was subjected to neutral alumina (Brockman grade 1) column chromatography (6:1 petroleum ether/ethyl acetate) to give **8** (61 mg, 80%; α/β= 6:1) as a white foam. $[\alpha]_D$ +34.5 (*c* 1.0, CH_2Cl_2). IR (thin film, NaCl) 3048, 3020, 2970, 1738, 1655 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) (α-anomer) δ 8.76 (s, 1H), 8.02–7.33 (m, 20H, H-Ar), 6.46 (d, *J*=1.8 Hz, 1H), 5.86 (dd, *J*=10.0, 3.3 Hz, 1H), 5.81 (dd, *J*=10.0, 3.3 Hz, 1H), 5.73 (t, *J*=10.0 Hz, 1H), 5.70 (dd, *J*=3.3, 1.5 Hz, 1H), 5.53 (t, *J*=10.0 Hz, 1H), 5.07 (d, *J*=1.5 Hz, 1H), 4.55 (dd, *J*=3.3, 1.8 Hz 1H), 4.35 (m, 2H), 4.09 (AB d, *J*=15.0 Hz, 1H), 4.00 (AB d, *J*=15.0 Hz, 1H), 1.45 (d, *J*=6.2 Hz, 3H), 1.37 (d, *J*=6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) (α-anomer) δ 165.9, 165.7, 165.4, 165.3 (CO), 161.4 ($Cl_3CC{=}NH$), 133.4–133.1 (C_{ipso}), 129.9–128.4 (C-Ar), 99.9, 95.8 (C_1^A, C_1^B), 77.2, 74.3, 72.5, 71.8, 71.6, 71.5, 71.0, 70.6, 69.3, 68.6 (C_2^A, C_2^B, C_3^A, C_3^B, C_4^A, C_4^B, C_5^A, C_5^B), 40.3 (CH_2Cl), 17.7, 17.3 (C_6^A, C_6^B). Anal. Calcd: C 55.77, H 4.15, N 1.48. Found: C 56.01, H 4.11, N 1.45.

3.1.8. Allyl (3,4-di-*O*-benzoyl-2-*O*-chloroacetyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzoyl-α-L-rhamnopyranoside (15). A mixture of **4** (30 mg, 20 μmol) and **8** (57 mg, 60 μmol) was coevaporated three times with toluene, the residue was dried, and then mixed with freshly activated AW-300 4 Å molecular sieves, suspended under argon in CH_2Cl_2 (2.0 mL) and stirred at −50 °C. An 85 mg/mL solution of $BF_3{\cdot}OEt_2$ in CH_2Cl_2 (50 μL, 30 μmol) was then added. After 3 h the reaction mixture was quenched with some drops of Et_3N. After filtration over a Celite pad, the mixture was concentrated. The residue was subjected to column chromatography (11:1 to 6:1 toluene/ethyl acetate) to give **15** (22 mg, 48%) as a white foam. $[\alpha]_D$ +92 (*c* 0.5, CH_2Cl_2). IR (thin film, NaCl) 3024, 2927, 2857, 1722, 1604, 1449, 1272 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.15–7.24 (m, 60H, H-Ar), 6.00 (m, 1H), 5.85 (dd, *J*=9.9, 3.1 Hz, 1H), 5.74 (dd, *J*=9.9, 3.4 Hz, 1H), 5.65–5.51 (m, 4H), 5.44–5.30 (m, 7H), 5.23 (br s, 1H), 5.19 (br s, 1H), 5.14 (br s, 1H), 5.03 (br s, 1H), 4.94 (br s, 1H), 4.81 (br s, 1H), 4.71 (br s, 1H), 4.59 (br s, 1H), 4.49 (dd, *J*=9.6, 3.2 Hz, 1H), 4.38–4.32 (m, 3H), 4.22–4.14 (m, 3H), 4.03–3.91 (m, 3H), 3.82 (dq, *J*=9.8, 6.2 Hz, 1H), 3.73 (dq, *J*=9.9, 6.2 Hz, 1H), 3.70 (AB d, *J*=14.9 Hz, 1H), 3.65 (AB d, *J*=14.9 Hz, 1H), 3.61 (dq, *J*=9.9, 6.2 Hz, 1H), 1.42 (d, *J*=6.2 Hz, 3H), 1.37 (d, *J*=6.2 Hz, 3H), 1.15 (d, *J*=6.2 Hz, 3H), 1.00 (d, *J*=6.2 Hz, 3H), 0.85 (d, *J*=6.2 Hz, 3H), 0.79 (d, *J*=6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.8–165.0 (Ph*C*O), 133.8–133.3 (C_{ipso}, $OCH_2CH{=}CH_2$), 130.3–128.2 (C-Ar), 117.9 ($OCH_2CH{=}CH_2$), 100.9, 100.8, 100.4, 99.6, 99.5, 98.9 (C_1^A, C_1^B, C_1^C, C_1^D, C_1^E, C_1^F), 77.8, 76.9, 76.2, 75.4, 73.2, 73.1, 73.0, 72.2–71.5, 70.5, 70.0, 69.9, 68.2–67.0 (C_2^A, C_2^B, C_2^C, C_2^D, C_2^E, C_2^F, C_3^A, C_3^B, C_3^C, C_3^D, C_3^E, C_3^F, C_4^A, C_4^B, C_4^C, C_4^D, C_4^E, C_4^F, C_5^A, C_5^B, C_5^C C_5^D, C_5^E, C_5^F, $OCH_2CH_2{=}CH_2$), 40.3 (CH_2Cl), 17.5–17.0 (C_6^A, C_6^B, C_6^C, C_6^D, C_6^E, C_6^F). MALDI-MS for $C_{125}H_{115}ClO_{38}$ (*m/z*): M_r (calcd) 2258.68; M_r (found) 2281.40 $(M+Na)^+$. Anal. Calcd: C 66.41, H 5.13. Found: C 66.66, H 5.00.

3.1.9. Ethyl 3,4-di-*O*-benzoyl-2-*O*-levulinoyl-1-thio-α-L-rhamnopyranoside (16). Compound **11** (1.541 g, 3.70 mmol) was dissolved in CH_2Cl_2 (15 mL) and levulinic acid (1.89 mL, 18.5 mmol), DMAP (271 mg, 2.20 mmol), and DIPC (2.90 mL, 18.5 mmol) were added in succession. The mixture was stirred for 1 h at rt, then filtered over a Celite pad, diluted with CH_2Cl_2, and washed with water. The organic layer was dried and concentrated to afford a residue that after column chromatography (4:1 petroleum ether/ethyl acetate) gave **12** (1.735 g, 91%) as a yellowish oil. $[\alpha]_D$ −7 (*c* 0.5, CH_2Cl_2). IR (thin film, NaCl) 3060, 3020, 2985, 2930, 1726, 1601, 1452 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 8.00–7.30 (m, 10H), 5.61–5.51 (m, 3H), 5.30 (br s, 1H), 4.46 (dq, *J*=8.8, 6.4 Hz, 1H), 2.71–2.63 (m, 6H), 2.03 (s, 3H), 1.26 (t, *J*=6.2 Hz, 3H), 1.21 (d, *J*=6.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 205.8 (CH_2COCH_3), 171.3 ($OCOCH_2CH_2$), 165.9–165.6 (Ph*C*O), 133.6, 133.4 (C_{ipso}), 130.0–128.4 (C-Ar), 81.6 (C_1), 71.5, 71.4, 69.9, 66.6 (C_2, C_3, C_4, C_5), 37.2, 29.1, 27.5, 25.1 ($COCH_2CH_2COCH_3$), 17.0 (C_6), 14.4 (SCH_2CH_3). ESIMS for $C_{27}H_{30}O_8S$ (*m/z*): M_r (calcd) 514.17; M_r (found) 537.41 $(M+Na)^+$. Anal. Calcd: C 63.02, H 5.88. Found: C 63.26, H 5.65.

3.1.10. Allyl (3,4-di-*O*-benzoyl-2-*O*-levulinoyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzoyl-α-L-rhamnopyranoside (17). A mixture of **3** (544 mg, 1.32 mmol) and **16** (1.018 g, 1.98 mmol) was coevaporated three times with toluene, the residue was dried, and then mixed with freshly activated AW-300 4 Å molecular sieves and NIS (282 mg, 2.43 mmol). The mixture was suspended in CH_2Cl_2 (4.0 mL) under an Ar atmosphere, rapidly cooled to −30 °C, and treated with TfOH (43 μL, 0.49 mmol). After 90 min the reaction mixture was rapidly filtered over a Celite pad, diluted with CH_2Cl_2, and washed with 10% $Na_2S_2O_3$ and 1 M $NaHCO_3$. The organic layer was collected, dried, and concentrated to give a residue that after column chromatography (7:2 petroleum ether/ethyl acetate) afforded **17** (946 mg, 83%) as a white foam. $[\alpha]_D$ +60.9 (*c* 1.0, CH_2Cl_2). IR (thin film, NaCl) 3050, 3026, 1731, 1604, 1263 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 8.03–7.28 (m, 20H), 5.97 (m, 1H), 5.82 (dd, *J*=10.0, 3.4 Hz, 1H), 5.78 (dd, *J*=10.0, 3.0 Hz, 1H), 5.63 (m, 2H), 5.50 (t, *J*=10.0 Hz, 1H), 5.38 (d, *J*=17.2 Hz, 1H), 5.27 (d, *J*=10.6 Hz, 1H), 5.02 (d, *J*=1.6 Hz, 1H), 4.96 (d, *J*=1.4 Hz, 1H), 4.34–4.06 (m, 5H), 2.60 (s, 4H), 2.08 (s, 3H), 1.38 (d, *J*=6.2 Hz, 3H), 1.30 (d, *J*=6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 205.8 (CH_2COCH_3), 171.3 ($OCOCH_2$), 165.9, 165.6, 165.5, 165.3 (Ph*C*O), 133.6–133.2 (C_{ipso}, $OCH_2CH{=}CH_2$), 130.1–128.4 (C-Ar), 117.9 ($OCH_2CH{=}CH_2$), 99.6, 97.8 (C_1^A, C_1^B), 76.9, 72.1, 71.8, 71.2, 70.3, 69.7, 68.4, 67.6, 67.1 (C_2^A, C_2^B, C_3^A, C_3^B, C_4^A, C_4^B, C_5^A, C_5^B, $OCH_2CH{=}CH_2$), 37.9, 29.8, 28.0 ($COCH_2CH_2COCH_3$), 17.7, 17.6 (C_6^A, C_6^B). ESIMS for $C_{48}H_{48}O_{15}$ (*m*/*z*): M_r (calcd) 864.30; M_r (found) 887.41 $(M+Na)^+$. Anal. Calcd: C 66.66, H 5.59. Found: C 66.86, H 5.50.

3.1.11. (3,4-Di-*O*-benzoyl-2-*O*-levulinoyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzoyl-L-rhamnopyranosyl *N*-phenyl-trifluoroacetimidate (19). Compound **17** (838 mg, 0.97 mmol) was dissolved in 3:1 CH_2Cl_2/MeOH (4.0 mL), $PdCl_2$ (103 mg, 0.58 mmol) was then added, and the mixture was vigorously stirred overnight. It was then filtered over a Celite pad, diluted with CH_2Cl_2, and washed with 5 M NaCl. The organic layer was dried and concentrated to give **14** (664 mg, 83%) that was then mixed with freshly powdered 4 Å MS and suspended in CH_2Cl_2 (12 mL) under Ar atmosphere. The mixture was cooled to 0 °C and then treated with $CF_3C(NPh)Cl$ (148 μL, 1.26 mmol) and NaH (60% dispersion in mineral oil; 58 mg, 1.45 mmol). After 4 h the solution was concentrated at 30 °C. The residue was subjected to neutral alumina (Brockman grade 1) column chromatography (6:1 petroleum ether/ethyl acetate) to give **19** (577 mg, 72%; α/β=1:1) as a white foam. IR (thin film, NaCl) 3026, 3012, 2917, 1732, 1600, 1452 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 8.08–6.84 (m, 50H), 6.40 (br s, 1H), 5.99 (br s, 1H), 5.88–5.43 (m, 11H), 5.13 (br s, 1H), 5.07 (br s, 1H), 5.04 (br s, 1H), 4.60–4.52 (m, 2H), 4.22–4.13 (m, 2H), 2.57 (s, 4H), 2.56 (s, 4H), 2.01 (s, 6H), 1.45 (d, *J*=6.0 Hz, 3H), 1.22 (d, *J*=6.0 Hz, 3H), 1.18 (d, *J*=6.0 Hz, 3H), 1.15 (d, *J*=6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 205.8 (CH_2COCH_3), 171.2 ($OCOCH_2$), 165.8, 165.7, 165.4, 165.3 (Ph*C*O), 143.2 (C=N), 133.4–133.1 (C_{ipso}), 130.0–115.3 (C-Ar), 99.3, 98.3, 95.5, 93.9 ($2C_1^A$, $2C_1^B$), 76.9, 76.8, 74.1, 73.3, 72.5, 72.0, 71.5, 71.4, 71.0, 70.5, 69.9, 69.8, 69.7, 69.5, 67.8, 67.1 ($2C_2^A$, $2C_2^B$, $2C_3^A$, $2C_3^B$, $2C_4^A$, $2C_4^B$, $2C_5^A$, $2C_5^B$), 37.8, 29.7, 27.9 ($COCH_2CH_2COCH_3$), 17.7, 17.3 ($2C_6^A$, $2C_6^B$). ESIMS for $C_{53}H_{48}F_3NO_{15}$ (*m*/*z*): M_r (calcd) 995.30; M_r (found) 1018.40 $(M+Na)^+$. Anal. Calcd: C 63.92, H 4.86, N 1.41. Found: C 63.67, H 4.72, N 1.38.

3.1.12. Propyl (3,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→3)-(2,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-(3,4-di-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-*O*-benzoyl-α-L-rhamnopyranoside (21). A mixture of **4** (73 mg, 49 μmol) and **2** (195 mg, 0.20 mmol) was coevaporated three times with toluene, the residue was dried, and then mixed with freshly activated AW-300 4 Å molecular sieves, suspended under argon in CH_2Cl_2 (6.0 mL), and stirred at 0 °C. A 10 mg/mL solution of TMSOTf in CH_2Cl_2 (50 μL, 2.2 μmol) was then added. After 3 h the reaction mixture was quenched with some drops of Et_3N. After filtration over a Celite pad, the mixture was concentrated. The residue was subjected to column chromatography (5:1 to 3:2 petroleum ether/ethyl acetate) to give a foamy residue that was dissolved in CH_2Cl_2 (7.0 mL) and then treated with a 26 mg/mL solution of hydrazinium acetate in MeOH (1.0 mL, 0.28 mmol). After 2 h stirring at rt, the mixture was concentrated; a column chromatography (7:1 toluene/ethyl acetate) on the residue afforded **21** (82 mg, 77%) as a white foam. $[\alpha]_D$ +100.8 (*c* 2.0, CH_2Cl_2). IR (thin film, NaCl) 3019, 2924, 1729, 1600, 1269 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.11–7.25 (m, 60H, H-Ar), 5.82 (dd, *J*=10.0, 3.2 Hz, 1H), 5.61–5.52 (m, 5H), 5.41–5.34 (m, 4H), 5.29 (t, *J*=9.6 Hz, 1H), 5.26 (br s, 1H), 5.15 (br s, 1H), 5.10 (br s, 1H), 5.00 (br s, 1H), 4.93 (br s, 1H), 4.91 (br s, 1H), 4.76 (br s, 1H), 4.57 (br s, 1H), 4.48 (dd, *J*=9.6, 3.2 Hz, 1H), 4.36 (m, 2H), 4.22 (m, 3H), 4.11 (m, 1H), 3.99–3.88 (m, 3H), 3.73 (m, 2H), 3.48 (dq, *J*=9.4, 6.2 Hz, 1H), 1.70 (app sextet, *J*=7.4 Hz, 2H), 1.37 (d, *J*=6.2 Hz, 3H), 1.33 (d, *J*=6.0 Hz, 3H), 1.11 (d, *J*=6.1 Hz, 3H), 0.99 (t, *J*=7.4 Hz, 3H), 0.96 (d, *J*=6.1 Hz, 3H), 0.84 (d, *J*=6.2 Hz, 3H), 0.75 (d, *J*=6.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.8–165.0 (Ph*C*O), 133.9–133.3 (C_{ipso}), 130.0–128.0 (C-Ar), 100.8, 100.7, 100.4, 99.3, 99.2, 98.9 (C_1^A, C_1^B, C_1^C, C_1^D, C_1^E, C_1^F), 77.6, 76.9, 76.3, 75.4, 74.8, 73.3, 73.0, 72.0–71.4, 70.6, 70.0, 69.8, 68.4–67.3 (C_2^A, C_2^B, C_2^C, C_2^D, C_2^E, C_2^F, C_3^A, C_3^B, C_3^C, C_3^D, C_3^E, C_3^F, C_4^A, C_4^B, C_4^C, C_4^D, C_4^E, C_4^F, C_5^A, C_5^B, C_5^C, C_5^D, C_5^E, C_5^F, $OCH_2CH_2CH_3$), 22.7 ($OCH_2CH_2CH_3$), 17.4–16.9 (C_6^A, C_6^B, C_6^C, C_6^D, C_6^E, C_6^F), 10.5 ($OCH_2CH_2CH_3$). MALDI-MS for $C_{123}H_{116}O_{37}$ (*m*/*z*): M_r (calcd) 2184.72; M_r (found) 2207.49 $(M+Na)^+$. Anal. Calcd: C 67.57, H 5.35. Found: C 67.80, H 5.22.

3.1.13. Ethyl 2,4-di-*O*-benzoyl-3-*O*-levulinoyl-1-thio-α-L-rhamnopyranoside (24). Compound **23** (86 mg, 0.21 mmol) was dissolved in CH_2Cl_2 (1.0 mL) and levulinic acid (172 μL, 2.07 mmol), DMAP (13 mg, 0.10 mmol), and DIPC (326 μL, 2.07 mmol) were added in succession. The mixture was stirred for 30 min at rt, then diluted with CH_2Cl_2, and washed with water. The organic layer was dried and concentrated to afford a residue that after column chromatography (4:1 petroleum ether/ethyl acetate) gave **24** (96 mg, 89%) as a colorless oil. $[\alpha]_D$ +11.5 (*c* 1.0, CH_2Cl_2). IR (thin film, NaCl) 3058, 3020, 2928, 1726, 1599, 1452 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz) δ 8.12–7.41

(m, 10H), 5.62 (br s, 1H), 5.51 (m, 2H), 5.39 (br s, 1H), 4.43 (dq, J=9.0, 6.4 Hz, 1H), 2.71 (dt, J=7.6, 3.8 Hz, 2H), 2.63 (dt, J=7.6, 3.8 Hz, 2H), 2.48 (dq, J=7.2, 3.2 Hz, 1H), 2.33 (dq, J=7.2, 3.2 Hz, 1H), 2.00 (s, 3H), 1.30 (m, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 205.7 (CH_2COCH_3), 171.5 ($OCOCH_2CH_2$), 165.6, 165.4 (Ph*C*O), 133.4, 133.3 (C_{ipso}), 129.8–128.4 (C-Ar), 82.0 (C_1), 72.1, 71.8, 69.7, 67.1 (C_2, C_3, C_4, C_5), 37.5, 29.2, 27.7, 25.4 ($COCH_2CH_2COCH_3$, SCH_2CH_3), 17.4 (C_6), 14.7 (SCH_2CH_3). ESIMS for $C_{27}H_{30}O_8S$ (*m/z*): M_r (calcd) 514.17; M_r (found) 537.29 $(M+Na)^+$. Anal. Calcd: C 63.02, H 5.88. Found: C 62.95, H 5.96.

3.1.14. Allyl (2,4-di-*O*-benzoyl-3-*O*-levulinoyl-α-L-rhamnopyranosyl)-(1→3)-2,4-di-*O*-benzoyl-α-L-rhamnopyranoside (26). A mixture of **25** (53 mg, 0.13 mmol) and **24** (86 mg, 0.17 mmol) was coevaporated three times with toluene, the residue was dried, and then mixed with freshly activated AW-300 4 Å molecular sieves and NIS (47 mg, 0.21 mmol). The mixture was suspended in CH_2Cl_2 (4.0 mL) under an Ar atmosphere, rapidly cooled to −30 °C, and treated with TfOH (5.5 μL, 63 μmol). After 3 h the reaction mixture was rapidly filtered over a Celite pad, diluted with CH_2Cl_2, and washed with 10% $Na_2S_2O_3$ and 1 M $NaHCO_3$. The organic layer was collected, dried, and concentrated to give a residue that after column chromatography (10:1 to 8:1 toluene/ethyl acetate) afforded **26** (97 mg, 87%) as a white foam. $[\alpha]_D$ +84.4 (*c* 1.0, CH_2Cl_2). IR (thin film, NaCl) 3052, 3026, 1733, 1604, 1263 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.23–7.32 (m, 20H), 5.92 (m, 1H), 5.57 (t, J=9.8 Hz, 1H), 5.52 (m, 2H), 5.42–5.21 (m, 3H), 5.16 (br s, 2H), 5.05 (br s, 1H), 4.48 (dd, J=10.0, 3.4 Hz, 1H), 4.24 (ddt, J=12.8, 5.0, 1.2 Hz, 1H), 4.14–4.00 (m, 3H), 2.37 (dt, J=7.4, 3.8 Hz, 2H), 2.23 (dt, J=7.4, 3.8 Hz, 2H), 1.86 (s, 3H), 1.35 (d, J=6.2 Hz, 3H), 1.16 (d, J=6.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 205.6 (CH_2COCH_3), 170.6 ($OCOCH_2CH_2$), 166.0, 165.6, 165.4, 164.8 (Ph*C*O), 133.4, 133.1 (C_{ipso}, $OCH_2CH{=}CH_2$), 129.8–128.3 (C-Ar), 117.8 ($OCH_2CH{=}CH_2$), 99.1, 96.3 (C_1^A, C_1^B), 75.9, 73.0, 72.2, 71.3, 70.2, 68.7, 68.4, 67.2, 66.7 (C_2^A, C_2^B, C_3^A, C_3^B, C_4^A, C_4^B, C_5^A, C_5^B, $OCH_2CH{=}CH_2$), 37.5, 29.2, 27.6 ($COCH_2CH_2COCH_3$), 17.5, 17.2 (C_6^A, C_6^B). ESIMS for $C_{48}H_{48}O_{15}$ (*m/z*): M_r (calcd) 864.30; M_r (found) 887.48 $(M+Na)^+$. Anal. Calcd: C 66.66, H 5.59. Found: C 66.95, H 5.65.

3.1.15. (2,4-Di-*O*-benzoyl-3-*O*-levulinoyl-α-L-rhamnopyranosyl)-(1→2)-2,4-di-*O*-benzoyl-L-rhamnopyranosyl *N*-phenyl-trifluoroacetimidate (22). Compound **26** (178 mg, 0.21 mmol) was dissolved in 3:1 CH_2Cl_2/MeOH (4.0 mL), $PdCl_2$ (19 mg, 0.11 mmol) was then added, and the mixture was vigorously stirred overnight. It was then filtered over a Celite pad, diluted with CH_2Cl_2, and washed with 5 M NaCl. The organic layer was dried and concentrated to give **27** (104 mg, 60%) that was then mixed with freshly powdered 4 Å MS and suspended in CH_2Cl_2 (6.0 mL) under Ar atmosphere. The mixture was cooled to 0 °C and then treated with $CF_3C(NPh)Cl$ (19 μL, 0.16 mmol) and NaH (60% dispersion in mineral oil; 7.6 mg, 0.19 mmol). After 4 h the solution was concentrated at 30 °C. The residue was subjected to neutral alumina (Brockman grade 1) column chromatography (10:1 to 5:1 petroleum ether/ethyl acetate) to give **22** (73 mg, 58%; α/β=1:1) as a white foam. IR (thin film, NaCl) 3028, 3012, 2925, 1728, 1595, 1452 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.25–6.80 (m, 50H), 6.43 (br s, 1H), 6.08 (br s, 1H), 5.70–5.57 (m, 3H), 5.40–5.12 (m, 11H), 4.52 (dd, J=10.0, 3.4 Hz, 1H), 4.16–4.07 (m, 3H), 2.38 (m, 4H), 2.25 (m, 4H), 1.88 (s, 3H), 1.40 (d, J=6.0 Hz, 3H), 1.27 (d, J=6.0 Hz, 3H), 1.20 (d, J=6.0 Hz, 3H), 1.18 (d, J=6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 206.1 (CH_2COCH_3), 170.9 ($OCOCH_2$), 165.8, 165.6, 165.5, 164.8 (Ph*C*O), 143.0 (C=N), 133.7–133.3 (C_{ipso}), 130.0–115.3 (C-Ar), 99.3, 94.1, 93.6 ($2C_1^A$, $2C_1^B$), 75.1, 72.4, 72.0, 71.3, 70.6, 70.1, 69.4, 68.9, 68.7, 67.6 ($2C_2^A$, $2C_2^B$, $2C_3^A$, $2C_3^B$, $2C_4^A$, $2C_4^B$, $2C_5^A$, $2C_5^B$), 37.6, 29.3, 27.7 ($COCH_2CH_2COCH_3$), 17.7, 17.3 ($2C_6^A$, $2C_6^B$). ESIMS for $C_{53}H_{48}F_3NO_{15}$ (*m/z*): M_r (calcd) 995.30; M_r (found) 1018.29 $(M+Na)^+$. Anal. Calcd: C 63.92, H 4.86, N 1.41. Found: C 63.59, H 4.74, N 1.38.

3.1.16. Propyl α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranoside (28). A mixture of **21** (40 mg, 18 μmol) and **22** (72 mg, 72 μmol) was coevaporated three times with toluene, the residue was dried, and then mixed with freshly activated AW-300 4 Å molecular sieves, suspended under argon in CH_2Cl_2 (3.0 mL), and stirred at 0 °C. An 8.2 mg/mL solution of TMSOTf in CH_2Cl_2 (50 μL, 1.8 μmol) was then added. After stirring the reaction mixture at 0 °C overnight, some drops of Et_3N were added. The mixture was filtered over a Celite pad and concentrated. The residue was subjected to column chromatography (10:1 to 8:1 toluene/ethyl acetate) to give a foamy residue that was dissolved in 3:1 MeOH/CH_2Cl_2 (2.0 mL) and then treated with a 3.5 M methanolic solution of NaOMe (90 μL, 0.31 mmol). The solution was heated to 40 °C and stirred at this temperature over 2 days; it was then neutralized with Amberlist-15 H^+, filtered, and concentrated. The residue was subjected to a gel-filtration chromatography to give **28** (10.8 mg, 49%) as a white wax. $[\alpha]_D$ +81 (*c* 0.6, H_2O). ^{1}H NMR (D_2O, 400 MHz) δ 5.19 (br s, 1H, H_1^E), 5.12 (br s, 1H, H_1^F), 5.11 (br s, 1H, H_1^B), 5.04 (br s, 1H, H_1^H), 5.02 (br s, 1H, H_1^D), 4.96 (br s, 2H, H_1^C, H_1^G), 4.87 (br s, 1H, H_1^A), 4.15 (m, 3H, H_2^C, H_2^D, H_2^G), 4.08 (m, 4H, H_2^B, H_2^E, H_2^F, H_2^H), 3.96 (dd, $J_{3,4}$=9.8 Hz, $J_{3,2}$=3.2 Hz, 1H, H_3^E), 3.92–3.82 (m, 9H, H_2^A, H_3^A, H_3^B, H_3^C, H_3^D, H_3^F, H_3^G, H_3^H, H_5^E), 3.77–3.72 (m, 8H, H_5^A, H_5^B, H_5^C, H_5^D, H_5^F, H_5^G, H_5^H), 3.65 (dt, J_{gem}=13.9 Hz, J_{vic}=6.5 Hz, 1H, $OCHHCH_2CH_3$), 3.56–3.46 (m, 9H, H_4^A, H_4^B, H_4^C, H_4^D, H_4^E, H_4^F, H_4^G, H_4^H, $OCHHCH_2CH_3$), 1.61 (app sextet, J=7.0 Hz, 2H, $OCH_2CH_2CH_3$), 1.29 (m, 24H, H_6^A, H_6^B, H_6^C, H_6^D, H_6^E, H_6^F, H_6^G, H_6^H), 0.91 (t, J=7.0 Hz, 3H, $OCH_2CH_2CH_3$); ^{13}C NMR (D_2O, 100 MHz) δ 103.0 (C_1^D, C_1^H), 102.7 (C_1^C, C_1^G), 101.6 (C_1^E), 101.5 (C_1^B, C_1^F), 98.9 (C_1^A), 79.0 (C_2^A), 78.9 (C_2^B, C_2^E, C_2^F), 78.8 (C_3^C, C_3^G), 78.3 (C_3^D), 72.9 (C_4^A, C_4^B, C_4^E, C_4^F, C_4^H), 72.0 (C_4^C, C_4^D, C_4^G), 70.6 (C_3^E), 70.4 (C_3^A, C_3^H, C_5^E), 70.3 (C_3^B, C_3^H, $OCH_2CH_2CH_3$), 70.1 (C_5^B, C_5^C, C_5^D, C_5^F, C_5^G, C_5^H), 69.8 (C_5^A), 22.5 ($OCH_2CH_2CH_3$), 17.4 (C_6^A, C_6^B, C_6^C, C_6^D, C_6^E, C_6^F, C_6^G, C_6^H), 10.4 ($OCH_2CH_2CH_3$). MALDI-MS for $C_{51}H_{88}O_{33}$ (*m/z*): M_r (calcd) 1228.52; M_r (found) 1251.15 $(M+Na)^+$. Anal. Calcd: C 49.83, H 7.22. Found: C 49.59, H 7.44.

3.1.17. Allyl α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranoside (29). A solution of **1** (24 mg, 15.5 μmol)

in MeOH (1.0 mL) was treated with a 0.7 M methanolic solution of NaOMe (500 μL, 0.35 mmol). The solution was heated to 40 °C and stirred at this temperature overnight; it was then neutralized with Amberlist-15 H^+, filtered, and concentrated. The residue was subjected to a gel-filtration chromatography to give **29** (7.8 mg, 78%) as a white wax. $[\alpha]_D$ +25 (*c* 0.5, H_2O). ^1H NMR (D_2O, 400 MHz) δ 6.12 (m, 1H, $OCH_2CH{=}CH_2$), 5.52 (d, J=17.2 Hz, 1H, *trans* $OCH_2CH{=}CHH$), 5.47 (d, J=10.4, 1H, *cis* $OCH_2CH{=}CHH$), 5.25 (br s, 1H, H-1_B), 5.20 (br s, 1H, H-1_D), 5.12 (br s, 1H, H-1_C), 5.08 (br s, 1H, H-1_A), 4.40 (br d, J=5.1 Hz, 1H, $OCHHCH{=}CH_2$), 4.37 (br d, J=5.1 Hz, 1H, $OCHHCH{=}CH_2$), 4.31 (br s, 1H, H_2^C), 4.25 (br s, 1H, H_2^B), 4.22 (br s, 1H, H_2^D), 4.09 (br s, 1H, H_2^A), 4.07–3.96 (m, 5H, H_3^A, H_3^B, H_3^C, H_3^D, H_5^D), 3.93–3.86 (m, 3H, H_5^A, H_5^B, H_5^C), 3.70 (t, J=9.8 Hz, 1H, H_4^C), 3.63 (m, 3H, H_4^A, H_4^B, H_4^D), 1.45 (m, 12H, H_6^A, H_6^B, H_6^C, H_6^D); ^{13}C NMR (D_2O, 100 MHz) δ 133.5 ($OCH_2CH{=}CH_2$), 119.1 ($OCH_2CH{=}CH_2$), 102.8 (C_1^D), 102.4 (C_1^C), 101.4 (C_1^B), 97.7 (C_1^A), 78.9 (C_2^A), 78.5 (C_2^B), 78.4 (C_2^C), 72.5–72.4 (C_4^A, C_4^B, C_4^D), 71.6 (C_4^C), 70.5–70.1 (C_2^C, C_2^D, C_3^A, C_3^B, C_3^D, C_5^D), 69.7 (C_5^C), 69.6 (C_5^A), 69.5 (C_5^B), 68.6 ($OCH_2CH{=}CH_2$), 17.0–16.9 (C_6^A, C_6^B, C_6^C, C_6^D). MALDI-MS for $C_{27}H_{46}O_{17}$ (*m/z*): M_r (calcd) 642.27; M_r (found) 643.49 $(M+Na)^+$. Anal. Calcd: C 50.46, H 7.21. Found: C 50.20, H 7.36.

3.1.18. Allyl α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranoside (30). A solution of **15** (22 mg, 9.7 μmol) in 3:1 $MeOH/CH_2Cl_2$ (2.0 mL) was treated with a 3.5 M methanolic solution of NaOMe (90 μL, 0.31 mmol). The solution was heated to 40 °C and stirred at this temperature overnight; it was then neutralized with Amberlist-15 H^+, filtered, and concentrated. The residue was subjected to a gel-filtration chromatography to give **30** (7.0 mg, 77%) as a white wax. $[\alpha]_D$ +47 (*c* 0.5, H_2O). ^1H NMR (D_2O, 400 MHz) δ 5.96 (m, 1H, $OCH_2CH{=}CH_2$), 5.36 (d, J=17.2, 1H, *trans* $OCH_2CH{=}CHH$), 5.31 (d, J=10.4 Hz, 1H, *cis* $OCH_2CH{=}CHH$), 5.22 (br s, 1H, H_1^E), 5.10 (br s, 1H, H_1^B), 5.02 (br s, 1H, H_1^D), 4.96 (br s, 2H, H_1^C, H_1^F), 4.92 (br s, 1H, H_1^A), 4.24 (br d, J=5.1 Hz, 1H, $OCHHCH{=}CH_2$), 4.21 (br d, J=5.1 Hz, 1H, $OCHHCH{=}CH_2$), 4.17 (br s, 1H, H_2^C), 4.14 (br s, 1H, H_2^D), 4.11 (br s, 1H, H_2^B), 4.08 (br s, 2H, H_2^E, H_2^F), 4.01–3.77 (m, 13H, H_2^A, H_3^A, H_3^B, H_3^C, H_3^D, H_3^E, H_3^F, H_5^A, H_5^B, H_5^C, H_5^D, H_5^E, H_5^F), 3.58 (m, 2H, H_4^C, H_4^D), 3.51–3.44 (m, 4H, H_4^A, H_4^B, H_4^E, H_4^F), 1.29 (m, 18H, H_6^A, H_6^B, H_6^C, H_6^D, H_6^E, H_6^F); ^{13}C NMR (D_2O, 100 MHz) δ 133.9 ($OCH_2CH{=}CH_2$), 119.5 ($OCH_2CH{=}CH_2$), 103.0 (C_1^D), 102.9 (C_1^C, C_1^F), 101.7 (C_1^B, C_1^E), 98.0 (C_1^A), 79.3 (C_2^A), 79.0 (C_2^E), 78.9 (C_2^B), 78.8 (C_2^C), 78.5 (C_3^D), 72.8–72.2 (C_4^A, C_4^B, C_4^C, C_4^D, C_4^E, C_4^F), 70.7–70.5 (C_2^C, C_2^D, C_2^F, C_3^A, C_3^B, C_3^E C_3^F, C_5^C), 70.0–69.8 (C_5^A, C_5^B, C_5^D, C_5^E, C_5^F), 68.9 ($OCH_2CH{=}CH_2$), 17.4–17.3 (C_6^A, C_6^B, C_6^C, C_6^D, C_6^E, C_6^F). MALDI-MS for $C_{39}H_{66}O_{25}$ (*m/z*): M_r (calcd) 934.39; M_r (found) 935.41 $(M+H)^+$. Anal. Calcd: C 50.10, H 7.12. Found: C 49.91, H 7.29.

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Distyryl-boradiazaindacenes: facile synthesis of novel near IR emitting fluorophores

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Abstract—Boradiazaindacenes with methyl substituents at 3 and 5 positions were for the first time shown to undergo efficient double condensation reactions with an aromatic aldehyde yielding a series of extended conjugation dyes. These new fluorophores have absorption maxima in the range of 650–660 nm. The dyes reported here have large quantum yields with 20 nm Stokes' shifted emission peaks. The straightforward synthesis of such red shifted BODIPY derivatives is important in relation to the synthesis of novel and useful fluorescent chemosensors. In addition, this facile transformation may make these new fluorophores' building blocks in the construction of large functional supramolecular systems.

1. Introduction

Boradiazaindacenes (a.k.a., BODIPY dyes, BDPs, difluorobora-dipyrromethenes, etc.) are well known[1] fluorescent dyes with many applications, such as fluorescent labeling of biomolecules,[2] ion sensing, and signaling,[3] energy transfer cassettes,[4] light harvesting systems,[5] and fluorescent stains.[6] The parent dye absorbs near 480 nm and emits around 490 nm. While this is satisfactory for many applications, longer wavelength excitability and emission would be highly valuable considering Rayleigh scattering and pigmentation problems in many biological samples.[7] Thus, there have been many attempts[4b,8] to move the peak absorption wavelength to the red end of the visible spectrum, with varying degrees of success. The use of benzo- or naphthopyrroles leading to fused BODIPY's may hamper the solubility to a significant extent. In recent years we and others have shown[3g,9] that the absorption and emission characteristics of boradiazaindacenes can be altered to a great extent by simple condensation reactions of 3,5-dimethylboradiazaindacenes with *p*-dialkylamino substituted aromatic aldehydes. However, only one of the slightly acidic methyl groups was reported to condense to yield monostyryl derivatives. The dyes obtained showed strong charge transfer characteristics with reduced emission quantum yields in polar solvents. Here, we are reporting the first synthesis of doubly styryl substituted boradiazaindacenes (DS-BODIPY's), starting from 3,5-dimethylboradiazaindacene derivatives. The condensation reactions seem not to be limited to dialkylamino substituted aromatic aldehydes, which is an important finding for the broader applicability of the derivatization reaction. The novel alkoxystyryl derivatives have large quantum yields in polar solvents, thus partly demonstrating their potential as fluorescent labels.

2. Results and discussion

The synthesis of the dyes **11**, **13**, and **15** starts with the preparation of the standard BODIPY dyes **5**, **8**, and **9** (Scheme 1). 8-Phenyl- and 8-*tert*-butyloxycarbonylmethoxyphenyl derivatives were synthesized using appropriate aldehydes and purified by a standard work-up. These dyes were then treated with aldehyde **2** under reflux with azeotropic removal of water. In the other compounds, additional bromine substituents were placed as auxochromic groups. The reaction with the aldehyde produced both single and double condensation products, which can be separated by silica gel column chromatography. The presence of *tert*-butyl groups improved organic solubility to a great extent as expected. The absorption spectra of the dyes **10**–**15** were obtained in a polar protic solvent, isopropanol. The spectra are shown in Figure 1. The second styryl group causes a bathochromic shift of about 100 nm. The bromine substitution at the pyrrolic positions results in an additional 11 nm of shift toward the red end of the visible spectrum. These dyes show remarkable red fluorescence even under ambient light. The fluorescence spectra were also obtained in isopropanol. The novel fluorophores had relatively small Stokes' shifts of 15 nm, with

Keywords: DS-BODIPY; BODIPY derivatives; Near IR emitting dyes; Fluorophores; Chromophores.
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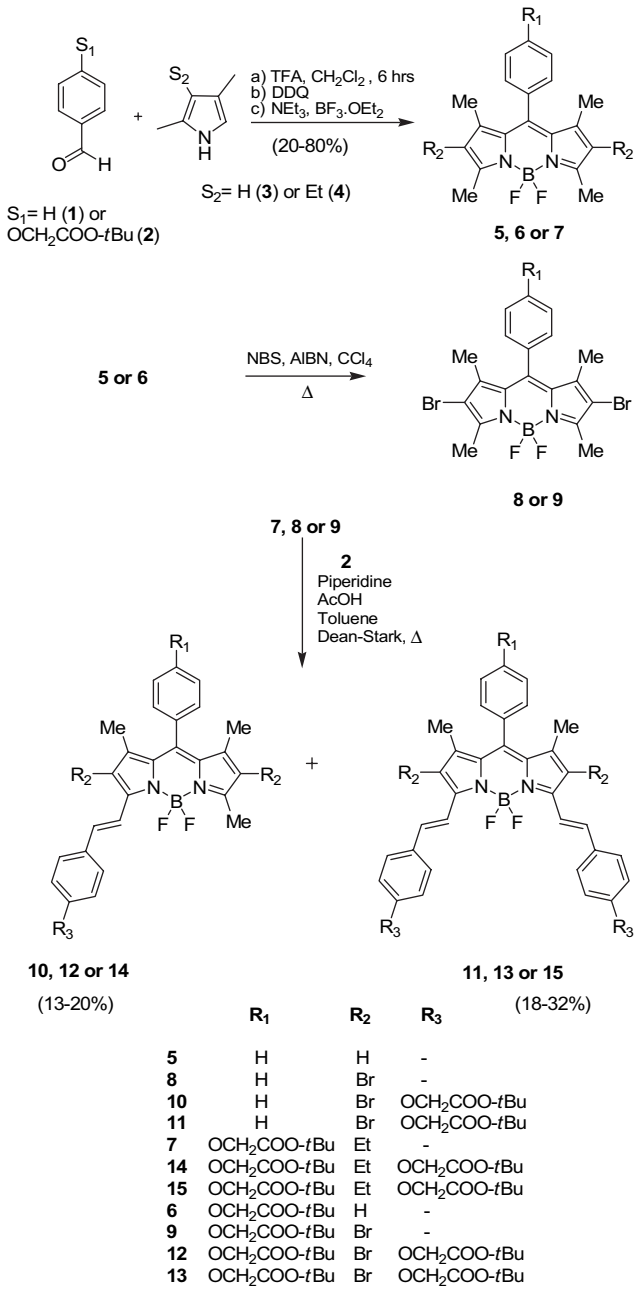

	R_1	R_2	R_3
5	H	H	-
8	H	Br	-
10	H	Br	OCH_2COO-*t*Bu
11	H	Br	OCH_2COO-*t*Bu
7	OCH_2COO-*t*Bu	Et	-
14	OCH_2COO-*t*Bu	Et	OCH_2COO-*t*Bu
15	OCH_2COO-*t*Bu	Et	OCH_2COO-*t*Bu
6	OCH_2COO-*t*Bu	H	-
9	OCH_2COO-*t*Bu	Br	-
12	OCH_2COO-*t*Bu	Br	OCH_2COO-*t*Bu
13	OCH_2COO-*t*Bu	Br	OCH_2COO-*t*Bu

Scheme 1. Synthesis of monostyryl- and distyryl-boradiazaindacene dyes.

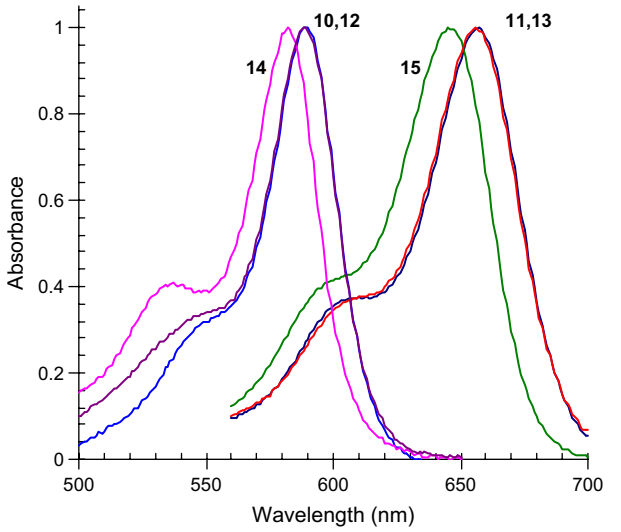

Figure 1. Normalized absorption spectra of extended conjugation BODIPY dyes in isopropanol. Compounds **10**, **12**, and **14** are monostyryl derivatives, whereas, **11**, **13**, and **15** are distyryl compounds.

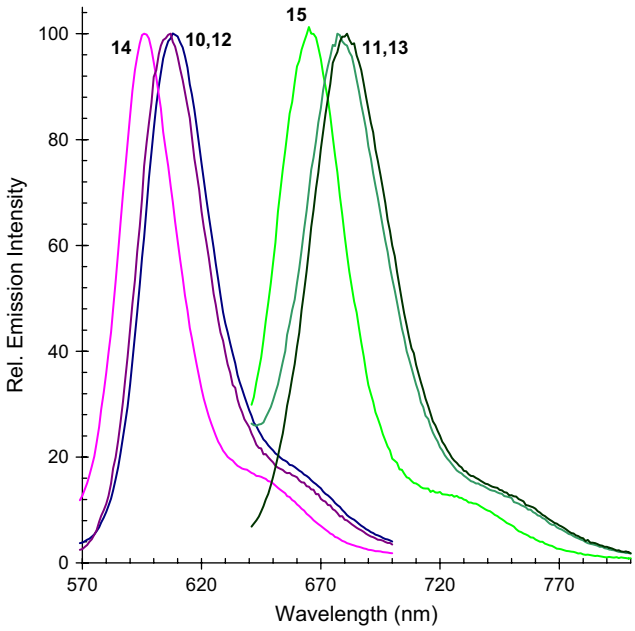

Figure 2. Normalized emission spectra of extended conjugation BODIPY dyes in isopropanol. Compounds **10**, **12**, and **14** are monostyryl derivatives, whereas, **11**, **13**, and **15** are distyryl compounds. The excitation wavelength was 530 nm for the monostyryl dyes and 610 nm for the distyryl dyes. Slit widths were 5 nm.

sharp emission peaks (Fig. 2). Compound **11** had the most red shifted emission peak at 679 nm. More importantly however, the fluorophores had only very little internal charge transfer characteristics; the emission peak position was only slightly moved bathochromically on changing the solvent from toluene to DMSO (Fig. 3). The quantum yield of emission for the dyes **11**, **13**, and **15** was determined using bis[4-(dimethylamino)-2-hydroxyphenyl]squaraine as a reference[10] (Table 1). The extinction coefficients were also very large ($\log \varepsilon$ 4.94–5.00), thus the brightness factor ($\Phi_f \times \varepsilon$) for these novel fluorophores is in fact larger than fluorescein. This straightforward derivatization of parent boradiazaindacene structures to yield near IR emitting dyes is not only important for the development of new biologically relevant fluorescent labels, but also may very well transform these dyes into building blocks in functional supramolecular systems. We are at present investigating such paths for further development.

3. Conclusion

We have synthesized and characterized near IR emitting boradiazaindacene dyes in a very straightforward reaction. This is the first report of the double condensation reaction with 3,5-dimethylboradiazaindacenes. The use of Dean–Stark apparatus seems to be critical in removing any water

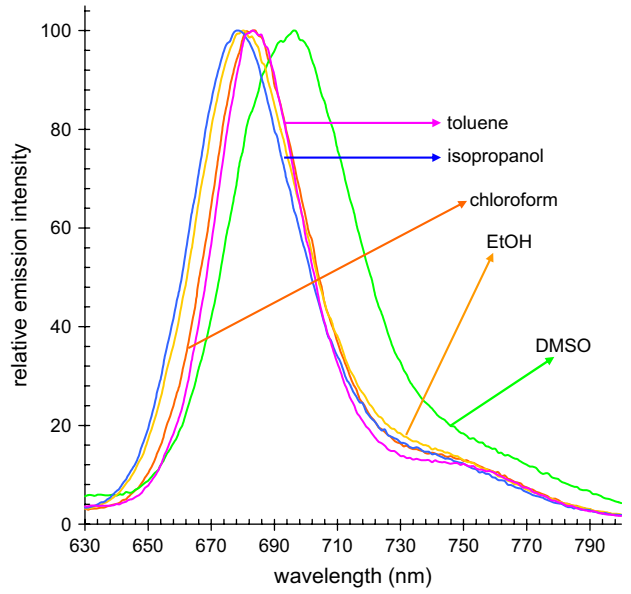

Figure 3. Normalized emission spectra of distyryl-BODIPY dye **13** in solvents of varying polarities. Excitation was at 610 nm with 5 nm slit widths.

Table 1. Selected spectral data of distyryl-BODIPY compounds **11**, **13**, and **15**

Distyryl-BODIPY	**11**	**13**	**15**
λ_{max} (abs, nm)	657	646	656
λ_{max} (em, nm)	679	668	678
fwhm (nm)	41	36	43
ε (M^{-1} cm^{-1})	1.01×10^5	8.85×10^4	8.79×10^4
Φ_{em}	0.42	0.44	0.40

formed in the reaction and thus shifting the equilibrium to the double condensation products, which are the distyrl-BODIPY dyes. The simplicity of the modification would allow facile synthesis of many other BODIPY dyes with desired functional groups. The synthesis has a modular character. The dyes display only a small degree of solvatochromism, this is most likely due to alkoxy group being a weakly electron donor substituent compared to dialkylamino group found in many strong ICT-character chromophores. With the well known advantages of working with red or near IR emitting fluorophores, we have no doubt that this series of boradiazaindacenes will be attractive candidates for practical applications.

4. Experimental

4.1. General

The compounds were characterized and analyzed by Nuclear Magnetic Resonance spectroscopy (NMR), UV–vis spectroscopy, and fluorescence spectroscopy. ^{1}H and ^{13}C Nuclear Magnetic Resonance spectra of all compounds were recorded in $CDCl_3$ with Bruker Gmbh DPX-400, 400 MHz High Performance Digital FTNMR Spectrometer. UV–vis spectra were recorded by Varian Bio 100 UV–vis Spectrophotometer. Fluorescence spectra were recorded using Varian Cary Eclipse Fluorescence Spectrophotometer. All solvents were distilled over $CaCl_2$ before use. *tert*-Butyl 2-(4-formylphenoxy)acetate was synthesized according to literature.[11] All chemicals were obtained from Aldrich, unless noted otherwise. Merck Silica Gel 60 F_{254} TLC Aluminum Sheets were used in monitoring reactions by thin layer chromatography. Merck Silica Gel 60 (particle size 0.040–0.0963 mm, 230–400 mesh ASTM) was used in column chromatography.

4.2. Synthesis

4.2.1. 2,6-Dibromo-1,3,5,7-tetramethyl-8-phenyl-4,4′-difluoroboradiazaindacene (8). 8-Phenyl-BODIPY (**5**, 0.33 g, 1.02 mmol), AIBN (0.335 g, 2.04 mmol), and NBS (0.363 g, 2.04 mmol) were refluxed for 30 min in CCl_4 (15 mL). Crude product was then concentrated under vacuum, and purified by silica gel column chromatography (hexane–EtOAc, 3:1). The red colored fraction was collected and the solvent was removed under reduced pressure to yield the desired compound (**5**) (393.3 mg, 80%).

^{1}H NMR (400 MHz, $CDCl_3$): 1.37 (s, 6H, CH_3), 2.56 (s, 6H, CH_3), 7.15–7.2 (m, 2H, Ar–H), 7.42–7.48 (m, 3H, Ar–H).

^{13}C NMR (100 MHz, $CDCl_3$): 153.9, 142.1, 140.6, 134.4, 130.5, 129.5, 129.4, 129.2, 127.8, 28.0, 13.6. Elemental analysis: Found: C, 47.45; H, 3.62; N, 5.99. $C_{19}H_{17}BBr_2F_2N_2$ requires: C, 47.35; H, 3.56; N, 5.81. ESI-MS (*m*/*z*): 482 [M^+].

4.2.2. Monostyryl- and distyryl-BODIPY dyes (10 and 11). Compound **8** (500 mg, 1.037 mmol) and *tert*-butyl 2-(4-formylphenoxy)acetate (**2**, 0.245 g, 1.037 mmol) were refluxed in a mixture of toluene (50 mL), glacial acetic acid (0.77 mL), and piperidine (0.94 mL). Any water formed during the reaction was removed azeotropically by heating overnight in a Dean–Stark apparatus. Crude product was then concentrated under vacuum, and purified by silica gel column chromatography (EtOAc–hexane, 1:4). The blue colored fraction was collected and the solvent was removed under reduced pressure to yield the bright red fluorescent compound **10** (145 mg, 20%).

R_f 0.65. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.29 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.42 (s, 9H, $C(CH_3)_3$), 2.56 (s, 3H, CH_3), 4.5 (s, 2H, OCH_2), 6.84 (d, J=8.7 Hz, 2H, C=CH), 7.16–7.22 (m, 2H, Ar–H), 7.42–7.54 (m, 6H, Ar–H), 8.00 (d, J=16.6 Hz, 1H, C=CH).

^{13}C NMR (100 MHz, $CDCl_3$): 167.1, 159.0, 154.0, 148.5, 141.5, 140.6, 140.1, 138.5, 134.6, 131.3, 131.0, 130.5, 129.5, 129.4, 129.1, 128.0, 118.2, 116.3, 115.0, 110.1, 109.7, 82.5, 65.7, 28.0, 13.8, 13.6, 10.6. Elemental analysis: Found: C, 54.71; H, 4.58; N, 3.93. $C_{32}H_{31}BBr_2F_2N_2O_3$ requires: C, 54.89; H, 4.46; N, 4.00. ESI-MS (*m*/*z*): 707 [M^+].

The green colored fraction was collected and the solvent was removed under reduced pressure to yield the distyryl dye **11** (300 mg, 32%).

R_f 0.47. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.37 (s, 6H, CH_3), 1.42 (s, 18H, $C(CH_3)_3$), 4.5 (s, 4H, OCH_2), 6.88

(d, J=8.8 Hz, 4H, C=CH), 7.23 (dd, J=2.1 Hz, J=5.8 Hz, 2H, Ar–H), 7.42–7.47 (m, 3H, Ar–H), 7.51–7.58 (m, 6H, Ar–H), 8.01 (d, J=16.6 Hz, 2H, C=CH).

^{13}C NMR (100 MHz, $CDCl_3$): 168.6, 159.8, 149.3, 141.9, 139.9, 139.5, 135.7, 132.9, 131.4, 130.4, 130.3, 130.2, 129.9, 129.2, 117.3, 115.8, 83.5, 66.5, 29.0, 14.6. Elemental analysis: Found: C, 58.82; H, 5.01; N, 2.98. $C_{45}H_{45}BBr_2F_2N_2O_6$ requires: C, 58.85; H, 4.94; N, 3.05. ESI-MS (m/z): 918 [M^+].

4.2.3. 2,6-Diethyl-1,3,5,7-tetramethyl-8-(4-*tert*-butoxycarbonyl-methyloxyphenyl)-4,4′-difluoroboradiazaindacene (7). 2,4-Dimethyl-3-ethylpyrrole (**4**, 0.81 g, 6.55 mmol) and *tert*-butyl 2-(4-formylphenoxy)acetate (**2**, 0.75 g, 3.18 mmol) were dissolved in absolute CH_2Cl_2 (200 mL) under N_2 atmosphere, one drop of TFA was added and the solution was stirred at rt until TLC analysis showed complete consumption of the aldehyde. At this point, a solution of tetrachlorobenzoquinone (0.81 g, 3.18 mmol) in CH_2Cl_2 (150 mL) was added, stirring was continued for 15 min. Then, Et_3N (10.0 mL) and $BF_3\cdot OEt_2$ (10.0 mL) were added. After stirring for another 12 h, crude product was washed three times with water, dried over Na_2SO_4, and evaporated to dryness. The residue was chromatographed on silica gel ($CHCl_3$–MeOH, 96:4) to afford 324 mg (yield: 20%) of **7** in the form of orange needles.

^{1}H NMR (400 MHz, $CDCl_3$): 0.90 (t, J=7.5 Hz, 6H, CH_3), 1.25 (s, 6H, CH_3), 1.42 (s, 9H, $C(CH_3)_3$), 2.13–2.26 (q, J=7.5 Hz, 4H, CH_2), 4.50 (s, 2H, OCH_2), 6.94 (d, J=8.5 Hz, 2H, Ar–H), 7.1 (d, J=8.5 Hz, 2H, Ar–H).

^{13}C NMR (100 MHz, $CDCl_3$): 165.9, 156.6, 151.8, 138.2, 136.6, 130.9, 129.3, 127.87, 127.0, 113.4, 80.7, 64.1, 26.2, 15.2, 12.8, 10.7, 10.0. Elemental analysis: Found: C, 68.35; H, 7.39; N, 5.40. $C_{29}H_{37}BF_2N_2O_3$ requires: C, 68.24; H, 7.31; N, 5.49. ESI-MS (m/z): 510 [M^+].

4.2.4. Monostyryl- and distyryl-BODIPY dyes (14 and 15). A similar procedure was followed in the synthesis of these monostyryl and distyryl dyes; thus, the BODIPY dye **7** (0.2662 g, 0.5215 mmol) and the aldehyde **2** (0.246 g, 1.043 mmol), piperidine (0.47 mL) and acetic acid (0.39 mL) were used in this reaction. The desired compounds were purified by silica gel column chromatography ($CHCl_3$–hexane, 5:1). The blue colored fraction was collected and the solvent was removed under reduced pressure to yield the monostyryl compound **14** (50 mg, 13%).

R_f 0.35. ^{1}H NMR (400 MHz, $CDCl_3$): 0.92 (t, J=7.3 Hz, 3H, CH_3), 1.04–1.09 (t, J=7.3 Hz, 3H, CH_3), 2.26 (q, 7.5 Hz, 2H, CH_3), 2.50 (s+q, 5H, CH_3+CH_2), 4.46 (s, 2H, OCH_2), 4.52 (s, 2H, OCH_2), 6.84 (d, J=8.5 Hz, 1H, C=CH), 6.94 (d, J=9.6 Hz, 2H, Ar–H), 7.03–7.22 (m, 3H, Ar–H), 7.34 (d, J=6.7 Hz, 1H, Ar–H), 7.48 (d, J=9.6 Hz, 2H, Ar–H).

^{13}C NMR (100 MHz, $CDCl_3$): 167.8, 167.7, 158.5, 158.3, 155.0, 149.2, 139.1, 138.7, 138.5, 134.5, 133.5, 132.8, 132.1, 131.2, 129.7, 128.9, 128.5, 118.4, 115.2, 114.9, 82.5, 82.4, 65.9, 65.8, 28.1, 28.0, 18.3, 17.1, 14.5, 14.1, 12.7, 11.9, 11.5. Elemental analysis: Found: C, 69.34; H, 7.01; N, 3.95. $C_{42}H_{51}BF_2N_2O_6$ requires: C, 69.23; H, 7.05; N, 3.84. ESI-MS (m/z): 728 [M^+].

The green colored fraction was collected and then the solvent was removed under reduced pressure to yield the distyryl compound **15** (90 mg, 18%).

R_f 0.29. ^{1}H NMR (400 MHz, $CDCl_3$): 1.08 (t, J=7.3 Hz, 6H, CH_3), 1.30 (s, 6H), 1.42 (s, 27H, $C(CH_3)_3$), 2.48–2.58 (q, J=7.3 Hz, 4H, CH_2), 4.49 (s, 4H, OCH_2), 4.52 (s, 2H, OCH_2), 6.86 (d, J=8.6 Hz, 2H, Ar–H), 6.95 (d, J=8.5 Hz, 2H, Ar–H), 7.09–7.2 (m, 4H, Ar–H), 7.49 (d, J=8.6 Hz, 4H, Ar–H), 7.59 (d, J=16.7 Hz, 2H, C=CH).

^{13}C NMR (100 MHz, $CDCl_3$): 166.8, 166.7, 157.5, 157.4, 149.4, 137.8, 134.1, 132.6, 132.3, 130.1, 128.9, 128.1, 127.7, 117.6, 114.3, 113.9, 111.3, 81.6, 81.5, 64.9, 64.8, 27.1, 27.0, 17.4, 13.1, 10.7. Elemental analysis: Found: C, 69.88; H, 6.98; N, 2.99. $C_{55}H_{65}BF_2N_2O_9$ requires: C, 69.76; H, 6.92; N, 2.96. ESI-MS (m/z): 946 [M^+].

4.2.5. 1,3,5,7-Tetramethyl-8-(4-*tert*-butoxycarbonyl-methyloxyphenyl)-4,4′-difluoroboradiazaindacene (6). A procedure very similar to the synthesis of compound **7** was applied in the synthesis of this BODIPY dye. Thus, *tert*-butyl 2-(4-formylphenoxy)acetate (**2**, 1.5 g, 6.35 mmol), 2,4-dimethylpyrrole (**3**, 1.25 g, 13.1 mmol), Et_3N (10.0 mL), $BF_3\cdot OEt_2$ (10.0 mL), and 1.62 g of DDQ were used in this reaction. The residue was chromatographed on silica gel ($CHCl_3$–MeOH, 95:5) to afford 0.784 g of compound **6** in the form of orange needles. Yield: 27%.

^{1}H NMR (400 MHz, $CDCl_3$): 1.35 (s, 6H, CH_3), 1.42 (s, 9H, $C(CH_3)_3$), 2.48 (s, 6H, CH_3), 4.55 (s, 2H, OCH_2), 5.88 (s, 2H, Pyr–H), 6.94 (d, J=8.5 Hz, 2H, Ar–H), 7.10 (d, J=8.5 Hz, 2H, Ar–H).

^{13}C NMR (100 MHz, $CDCl_3$): 167.6, 158.5, 155.3, 143.1, 141.5, 131.8, 129.3, 127.9, 121.2, 115.3, 82.6, 65.8, 61.7, 28.0, 14.5. Elemental analysis: Found: C, 66.17; H, 6.55; N, 6.11. $C_{25}H_{29}BF_2N_2O_3$ requires: C, 66.09; H, 6.43; N, 6.17. FABMS (m/z): 454 [M^+].

4.2.6. 2,6-Dibromo-1,3,5,7-tetramethyl-8-(4-*tert*-butoxycarbonyl-methyloxyphenyl)-4,4′-difluoroboradiazaindacene (9). Compound **6** (0.784 g, 1.73 mmol), AIBN (0.57 g, 3.46 mmol), and NBS (0.616 g, 3.46 mmol) were refluxed for 40 min in CCl_4 (40 mL). Crude product was concentrated under reduced pressure and purified by silica gel column chromatography ($CHCl_3$). The red colored fraction was collected and the solvent was removed under reduced pressure to yield the desired compound (**9**) (761 mg, 72%).

^{1}H NMR (400 MHz, $CDCl_3$): 1.34 (s, 6H, CH_3), 1.42 (s, 9H, $C(CH_3)_3$), 2.52 (s, 6H, CH_3), 4.52 (s, 2H, OCH_2), 6.96 (d, J=8.5 Hz, 2H, Ar–H), 7.08 (d, J=8.5 Hz, 2H, Ar–H).

^{13}C NMR (100 MHz, $CDCl_3$): 167.5, 158.9, 153.9, 143.0, 140.6, 130.7, 129.2, 127.2, 115.6, 111.7, 82.7, 82.7, 82.6, 68.0, 65.8, 28.0, 13.8. Elemental analysis: Found: C, 49.02; H, 4.49; N, 4.49. $C_{25}H_{27}BBr_2F_2N_2O_3$ requires: C, 49.05; H, 4.45; N, 4.58; O, 7.84. FABMS (m/z): 612 [M^+].

4.2.7. Monostyryl- and distyryl-BODIPY dyes (12 and 13). The applied procedure was very similar to the synthesis of other styryl dyes in this study. The dibromo compound **9** (0.761 g, 1.24 mmol), **2** (0.587 g, 2.49 mmol), piperidine (11.2 mL), and acetic acid (0.93 mL) were used in this reaction. Following the usual work-up, the reaction mixture was purified by silica gel column chromatography ($CHCl_3$–hexane, 5:1). The blue colored fraction was collected and the solvent was removed under reduced pressure to yield the desired compound **12** (0.154 g, 15%).

R_f 0.39. ^{1}H NMR (400 MHz, $CDCl_3$): 1.35 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.47 (s, 18H, $C(CH_3)_3$), 2.58 (s, 3H, CH_3), 4.47 (s, 2H, OCH_2), 4.53 (s, 2H, OCH_2), 6.83 (d, J=8.7 Hz, 2H, Ar–H), 6.98 (d, J=8.5 Hz, 2H, Ar–H), 7.12 (d, J=8.5 Hz, 2H, Ar–H), 7.54–7.45 (m, 3H), 8.1 (d, J=15.5 Hz, 1H, C═CH).

^{13}C NMR (100 MHz, $CDCl_3$): 167.6, 166.5, 159.1, 158.3, 156.2, 148.6, 143.0, 141.5, 138.3, 132.9, 129.5, 128.3, 126.9, 121.0, 115.5, 114.9, 82.7, 80.3, 65.8, 61.5, 39.3, 28.0, 23.2, 13.3. Elemental analysis: Found: C, 54.86; H, 4.88; N, 3.43. $C_{38}H_{41}BBr_2F_2N_2O_6$ requires: C, 54.97; H, 4.98; N, 3.37. ESI-MS (m/z): 830 [M^+].

The green colored fraction was collected and then the solvent was removed under reduced pressure to yield the desired compound **13** (0.248 g, 20%).

R_f 0.29. ^{1}H NMR (400 MHz, $CDCl_3$): 1.39 (s, 6H, CH_3), 1.43 (s, 18H, $OC(CH_3)_3$), 1.50 (s, 9H, $OC(CH_3)_3$), 4.49 (s, 4H, OCH_2), 4.54 (s, 2H, OCH_2), 6.85 (d, J=8.6 Hz, 4H, Ar–H), 6.96 (d, J=8.5 Hz, 2H, Ar–H), 7.11 (d, J=8.6 Hz, 2H, Ar–H), 7.5 (m, 6H), 8.0 (d, J=16.6 Hz, 2H, C═CH).

^{13}C NMR (100 MHz, $CDCl_3$): 166.7, 166.5, 157.9, 157.8, 147.3, 139.9, 137.6, 131.3, 129.5, 128.6, 128.3, 126.7, 115.4, 115.4, 114.5, 113.9, 109.1, 81.7, 81.6, 64.7, 64.6, 27.0, 27.0, 12.9. Elemental analysis: Found: C, 58.46; H, 5.35; N, 2.68. $C_{51}H_{55}BBr_2F_2N_2O_9$ requires: C, 58.42; H, 5.29; N, 2.67. ESI-MS (m/z): 1048 [M^+].

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Photodegradation of some 14,15-bisnorlabdene-13-ones, derived from larixol. Synthesis of drimanic dienes with functional groups at C-6

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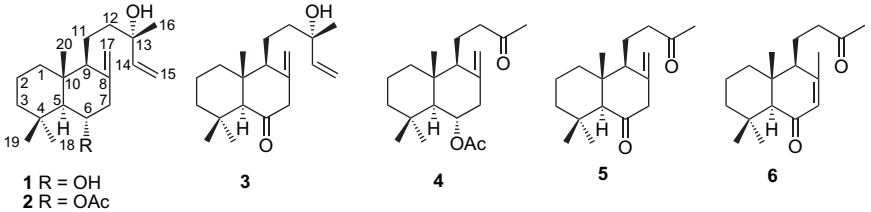
Abstract—Valuable chiral drimanic dienic synthons have been prepared by a photolytic Norrish type II degradation of the corresponding 14,15-bisnorlabdene-13-ones. Minor by-products with unexpected bi- and tricyclic structures were formed and some of them were isolated and identified.

1. Introduction

Drimane sesquiterpenes continue to attract attention due to their wide range of biological activities.[1,2] One way for the preparation of natural optically active drimanes is to start from the related and easily accessible labdane diterpenes.[3] For the synthesis of drimanes with functional groups at C-6, the labdane diol larixol **1** is a suitable starting material because it is easily available from the oleoresin of larch (*Larix decidua*, *Larix europea*).[4] For the conversion of larixol **1** into drimanes it is necessary to shorten its side chain with one isoprenic unit, which necessitates a multistep sequence. Recently, an efficient method for the oxidative degradation of the side chains of larixyl acetate **2** and keto-alcohol **3** to methylketones **4** and **5**, respectively, has been developed using oxidation with $KMnO_4$ in CH_2Cl_2.[5,6] Methylketone **5** can be isomerized with sodium methoxide in good yield to 14,15-bisnorlabd-7-ene-6,13-dione **6**[5,7] (Scheme 1). These 14,15-bisnorlabdene-13-ones **4**–**6** would be suitable starting chirons for the preparation of drimanes when a Norrish II type photochemical degradation could be realized, through which another three carbons would be eliminated. In this way a multifunctionalized drimane skeleton should be obtained as one of the major reaction products.[8,9] In addition, information would be obtained about the influence of structural variation in the starting



Scheme 1.

Keywords: Larixol; 14,15-Bisnorlabdene-13-ones; Chiral drimane synthons; Photolysis; Norrish type II reactions.
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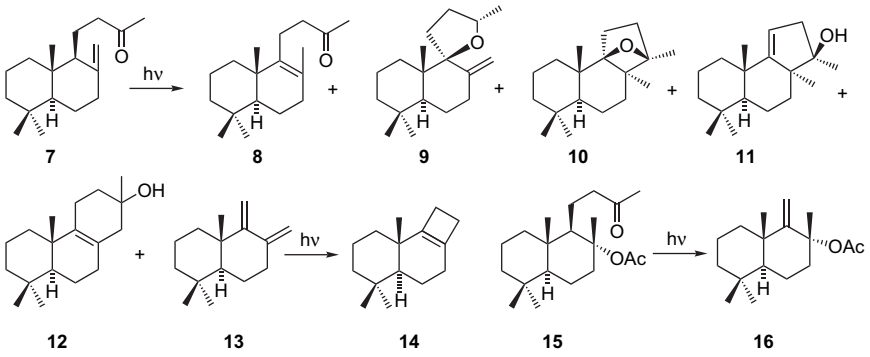


Scheme 2.

14,15-bisnorlabdenes on the yields and product formation of the photolytic reactions.

In 1976 Jeger and co-workers[8] established that exhaustive photolysis of labdanic methylketone **7** led to a complex mixture of more than 12 products, from which compounds **8**–**13** were isolated and identified, one of these being the drimanic diene **13** (yield 18%) (Scheme 2). Upon further irradiation this compound was transformed into the unstable tricyclic hydrocarbon **14**. This group[9] also investigated the photolysis of the enantiomer of the ketone **7** and demonstrated that the product composition depends on the duration of the irradiation and on the reaction temperature. Thus, the yield of the enantiomer of the diene **13** was quantitative at −72 °C, at 0 °C it constituted 72% (conversion 64%) and at 35 °C only 31% (conversion 81%).

Nakano and Mailo[10] have elaborated a method for the transformation of ketone **7** into diene **13** in a high yield (~78%), but with a low conversion (~7%). The unreacted ketone **7** could be recovered nearly quantitatively and can be used again. Some other photolytic degradations have been published[11] giving comparable results, with the 8α-acetoxy-14,15-bisnorlabdan-13-one **15**, giving 90% yield of alkene **16** with 50% conversion, as a favourable exception.[12,13]

2. Results and discussion

The above mentioned experiments show that the photolysis of bisnorlabdenic methylketones **4**–**6** should preferably be performed in a solution of low-molecular weight hydrocarbons (*n*-pentane, *n*-hexane and light petroleum ether), at low temperature and using a short period of irradiation. Although under these conditions the conversion of the ketones is low, the formation of secondary reaction products usually can be avoided and the unreacted starting material can be recycled.

The Norrish II type photochemical degradation of the 14,15-bisnorlabdenes **4**–**6** was carried out in hexane solution at 5 °C for 3 h and the main reaction product and several predominating side products were isolated by chromatography and their structures elucidated.

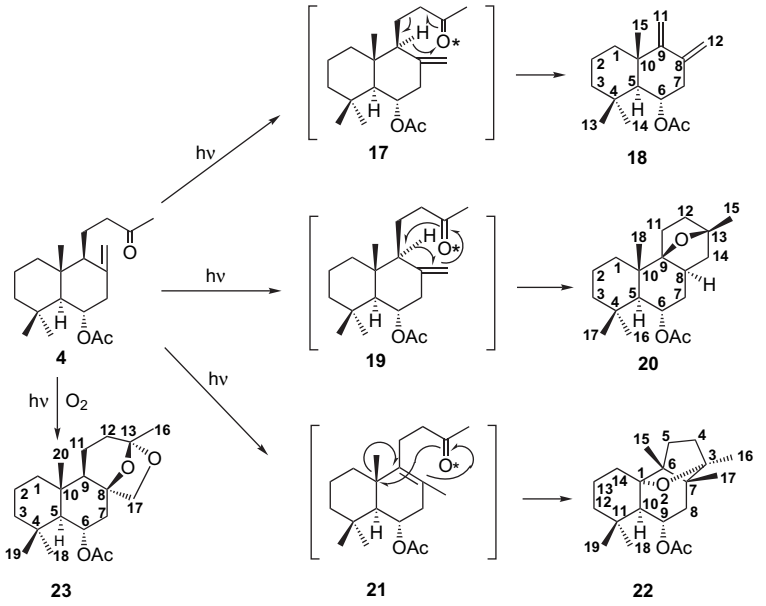
The 6α-acetoxy-14,15-bisnorlabd-8(17)-ene-13-one **4** was prepared from larixyl acetate **2** as described by Bolster et al.[5] The photolytic cleavage of this compound leads to a complex mixture of more than 10 products (TLC data). Column chromatography of the crude reaction product on SiO_2 permits the recovery of 88% of the starting ketone **4**, the conversion being 12%. The conversion of ketone **4** increases at longer irradiation times, but simultaneously the complexity of the reaction mixture also increases.

Among the reaction products the expected drimanic acetoxydiene **18** could be isolated as the main product in 76% yield, calculated on converted ketone **4** (Scheme 3). Its structure follows from the spectral data, which indicate an acetoxy group, three methyl groups at quaternary carbon atoms and a conjugated system consisting of two exocyclic double bonds.

The next compound, eluted in 3.5% yield, calculated on converted ketone **4**, turns out to be 6α-acetoxy-9,13β-epoxy-13β-methylpodocarpane **20**. The structure of this compound was confirmed by analytical and spectral data,[14] and by X-ray analysis (Fig. 1).[15] A probable way of formation of **20** is indicated in formula **19** in Scheme 3.

The next product **22**, eluted in 2.4% yield, calculated on converted ketone **4**, is an oxygen containing compound with an elemental composition identical to that of the starting compound **4**. From the ^{13}C NMR data it can be concluded that this substance contains six methyl groups (one of them belongs to the acetate group), four quaternary, two tertiary and seven secondary carbon atoms, and an oxygen atom connected to two quaternary carbon atoms. Finally the structure of compound **22** was determined by X-ray analysis (Fig. 2),[15] which showed its tricyclic nature with the *cis*-decalin skeleton condensed with a cyclopentane ring. Its formation can be explained by isomerization of the double bond in ketone **4** to the endocyclic position as in ketone **21**[8] followed by the photochemical rearrangement and cyclization that is indicated in Scheme 3.

The next compound, eluted from the chromatographic column in 17% yield, calculated on converted ketone **4**, was the photodegradation product **23**. The NMR evidence shows that this compound contains five methyl groups (including the acetoxy group), four completely substituted, three tertiary and seven secondary carbon atoms, and also two oxygen containing rings. In one of them, the oxygen atom is connected to two completely substituted carbon atoms and in the second ring the oxygen atom is connected to one



Scheme 3.

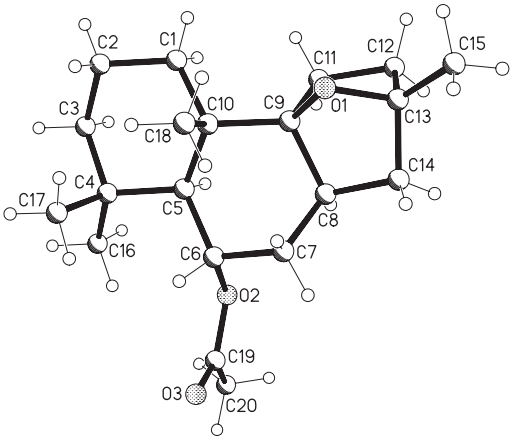


Figure 1. X-ray structure of compound **20**.

completely substituted and one secondary carbon atoms. These data led to the conclusion that this compound contains an intramolecular ketal group. This structural moiety is confirmed by the presence of the signals of an AB-system at 3.31 ppm and 3.76 ppm, and a singlet of a methyl group at 1.42 ppm in the NMR spectrum. From comparison of its spectral data with those of similar known diastereomeric ketoketals from the literature[16,17] the 6α-acetoxy-8β,13; 13α,17-diepoxy-14,15-bisnorlabdane structure **23** was attributed to this compound. This structure was proven by X-ray analysis of its monocrystal (Fig. 3).[15] Finally the starting acetoxyketone **4** was eluted from the column (88%).

Compound **23** is formed as the result of a reaction between acetoxyketone **4** (probably, in the photoexcited state) and oxygen from the reaction medium. Although the reaction was carried out in an inert atmosphere, the solution was not degassed before the photolysis and apparently enough oxygen was dissolved in the reaction medium to allow the formation of this product. This supposition is supported by two additional reactions, one carried out in an atmosphere of argon, the other under oxygen. In the reaction under argon 78% of the acetoxyketone **4** was recovered, the yield of acetoxydiene **18** was 72%, and the yield of acetoxyketal **23** was

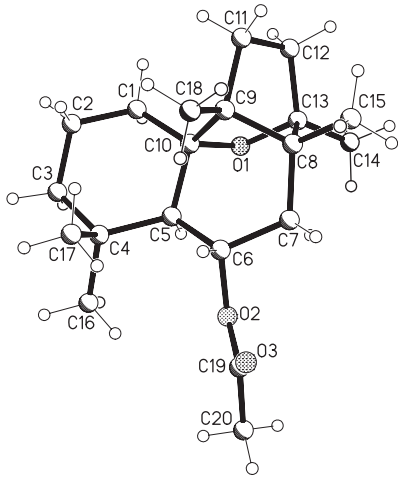


Figure 2. X-ray structure of compound **22**.

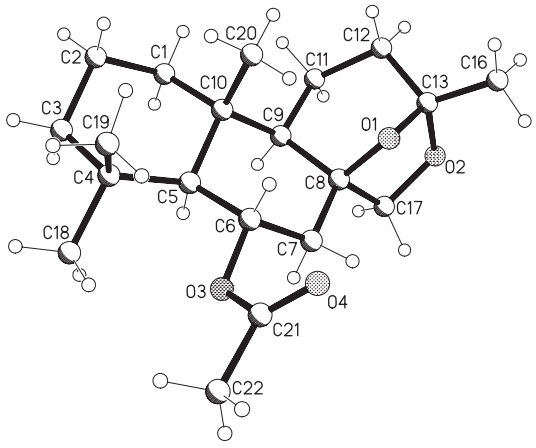
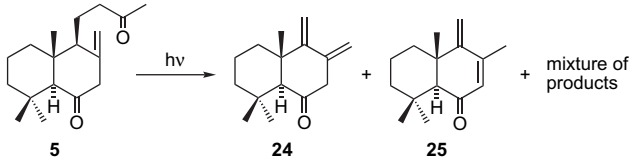


Figure 3. X-ray structure of compound **23**.

26%. In the reaction under oxygen only 46% of **4** was recovered and 14% of **18** and 40% of **23** was obtained. This 40% yield of **23** obtained under oxygen is 1.5 times higher than the yield of **23** under argon and 2.3 times higher than the 17% yield of **23** under nitrogen. Formation of **23** via epoxidation of the double bond in **4** cannot be excluded, but seems to be less probable because the reaction medium was neutral.

The photochemical degradation of the diketone **5** also affords a mixture of products, but in this case the conversion was rather high (~63%). The yield of the major Norrish type II reaction product, drim-8(12),9(11)-diene-6-one **24**, was 36% based on converted starting diketone **5** (Scheme 4). The reason for this moderate yield is again the formation of a large number of by-products, with the isomerized ketone **25** as the major one. The structure of ketodiene **24** is confirmed by elemental and spectral analyses. The spectra showed two exocyclic double bonds and a carbonyl group. Besides it contains five completely substituted, one tertiary and six secondary carbon atoms, and three methyl groups bonded to completely substituted carbon atoms.



Scheme 4.

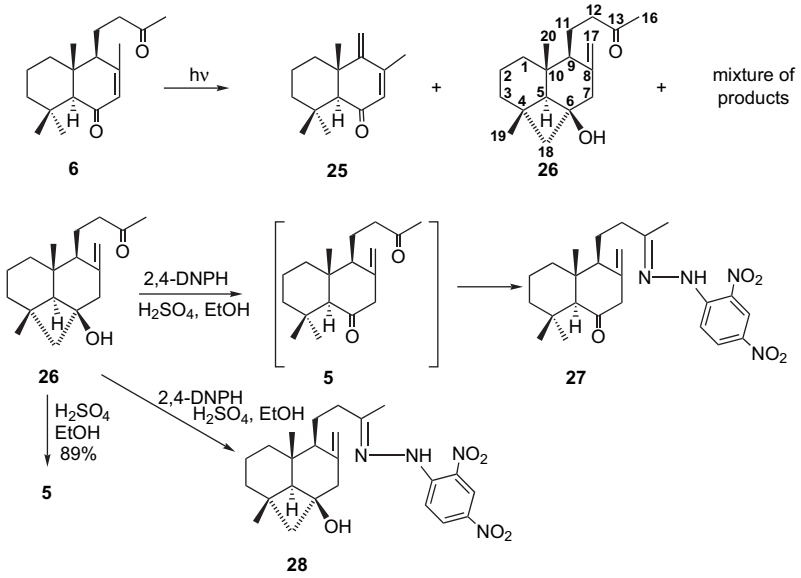
The second product, ketodiene **25**, was isolated in 19% yield based on converted diketone **5**. Its formation can be explained either by isomerization of the starting diketone **5**, before its photofragmentation, or by isomerization of ketodiene **24**. Both **24** and **25** are useful starting materials for drimane synthesis and their combined yields of 55% together with accessibility and the relatively high conversion of the starting ketone **5** makes this to be one of the shortest routes to highly functionalized drimanes.

The photolysis of diketone **6** gave a very complex mixture of at least 12 products, with one main component. Also in this case the reaction was not carried out with full transformation of the starting compound. The conversion was 23%, and the starting material was recovered by chromatography. According to spectral data, the major reaction product was the expected result from the Norrish type II reaction of diketone **6**, namely, the known drim-7,9(11)-diene-6-one **25**, which could be isolated in 67.5% yield based on converted ketone **6** (Scheme 5). The spectral data of this compound were identical to those in the literature.[4,18]

Besides ketodiene **25** a second liquid compound **26** could be isolated from the photolysis mixture. According to analytical and spectral data this compound has the same composition as the starting diketone **6** and contains a tertiary hydroxylic and methylketonic groups, an exocyclic double bond and, unlike the starting compound **6**, only three methyl groups. However, the spectral data of this compound did not allow a full characterization of the structure. For an unambiguous structure determination a 2,4-DNPH derivative of **26** was prepared for X-ray analysis.

Upon chromatographic purification of the reaction product, two 2,4-DNPH fractions with identical R_f value were collected, which had different melting points and crystal forms. X-ray studies[15] revealed that both these fractions consist of mixed crystals of 2,4-DNPH's **27** and **28** with different ratios of them. The 13-mono-2,4-DNPH **27** derived from the known 14,15-bisnorlabd-8(17)-ene-6,13-dione **5**, in which compound **26** is isomerized on its treatment with a solution of 2,4-dinitrophenylhydrazine in EtOH containing H_2SO_4 (Scheme 5). Indeed, in a separate experiment it was shown that on interaction with an ethanolic solution of H_2SO_4 the hydroxyketone **26** isomerized to diketone **5** (Fig. 4).

The second compound represented the 2,4-DNPH **28**, which contains an exocyclic double bond in C-8-C-17 position and a cyclobutane ring, obtained as the result of bond formation between C-18 and C-6. We didn't investigate in detail the



Scheme 5.

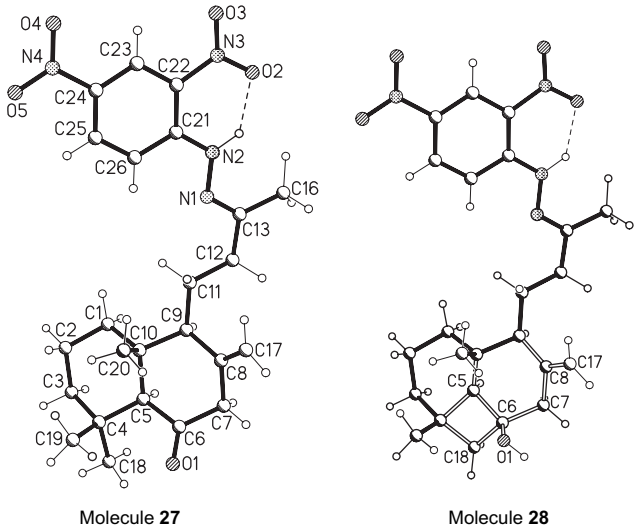


Figure 4. X-ray structures of the major **27** (74.7%) and the minor **28** fractions (25.3%) of the molecules in the crystal of compound **27**·0.5H_2O. The open lines and numbered atoms of **28** in Figure 4 correspond to the fragment whose position does not coincide in the structure with the corresponding fragment of compound **27**.

process of formation of compound **26** from the diketone **6**. However it should be mentioned that analogical transformations with the formation of compounds containing strained cyclobutane rings have been earlier observed on photolysis of some triterpene derivatives.[19–22]

Comparison of the influence of the structural variation in ketones **4**–**6** on the yield of the desired Norrish type II products in the photolysis reactions does not allow firm conclusions. The relatively good results in the photolysis of ketone **5**, a good yield in combination with a high conversion, cannot be explained easily. Although the end products are stabilized by conjugation, this is also the case in the products of the photolysis of ketones **4** and **6**, but their reactions follow the usual high yield low conversion pattern.

It should be noted that compounds **27** and **28** form mixed crystals in different ratios. In the crystal of compound **27** 25.3% of the molecules are isomorphously substituted by molecules of compound **28**. The composition of the crystal is {0.747(**27**)·0.253(**28**)·0.5H_2O}. Two different molecules reside statistically in the same position in the crystal. The positions of the majority of atoms for two different molecules overlap in the crystal structure within the resolution of data (Fig. 5). In each molecule, N-2-H···O-2 intramolecular hydrogen bonds facilitates the planarity of the hydrazone and 2,4-dinitrophenyl moieties.

The asymmetric part of the unit cell contains two molecules of **28a** and **28b** as crystallographically independent units (Fig. 6), which differ by their conformation. The torsion angle around the C-12-C-13 bond is *syn-clynal* in molecule **a** and *syn-periplanar* in molecule **b**. Two six-membered rings have chair conformation and four-membered ring is folded in both molecules. The OH groups are axial with respect to the four- and six-membered rings and trans to the H atom on C-5. The intramolecular N-2-H···O-2 H-bonds are presented in both molecules **a** and **b**.

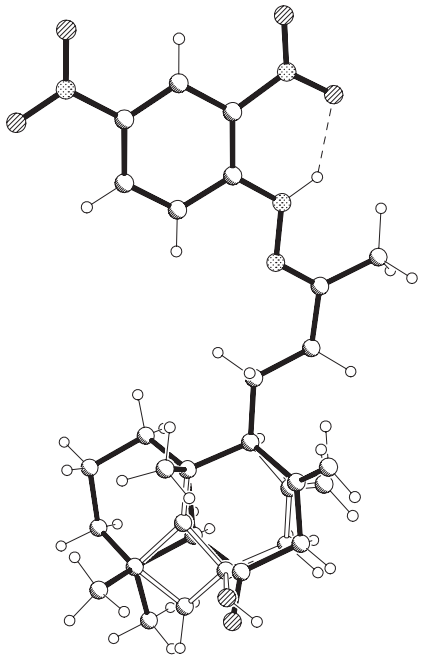


Figure 5. The overlap of positions of two different molecules in the crystal structure of **27**.

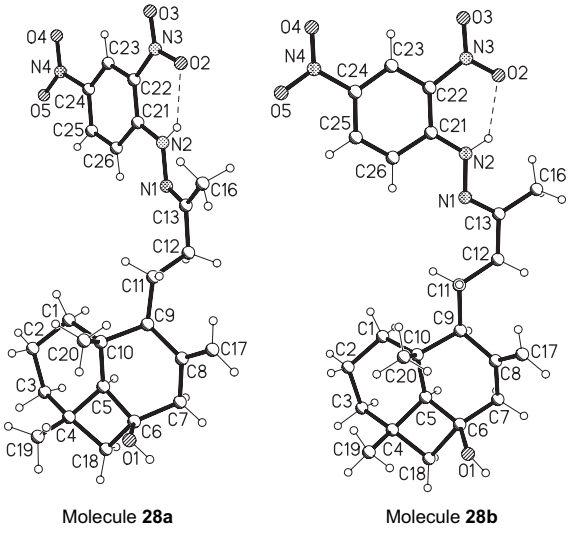


Figure 6. X-ray structure of two different molecules (**a** and **b**) in the crystal of compound **28** with a numbering scheme.

In the crystal of **28** molecules **a** partially (33.2%) are isomorphically substituted by molecules of compound **27** (Fig. 7). The overall composition of crystal **28** is {0.834(**28**)/0.166(**27**)}.

3. Conclusions

It was shown that the drimanic dienic syntons **18**, **24** and **25** can be prepared by a photolytic Norrish type II reaction of the corresponding 14,15-bisnorlabdene-13-ones **4**, **5** and **6**, respectively. Besides the major reaction products, complex mixtures of minor by-products with unexpected bi- and tricyclic structures were formed, part of which were isolated

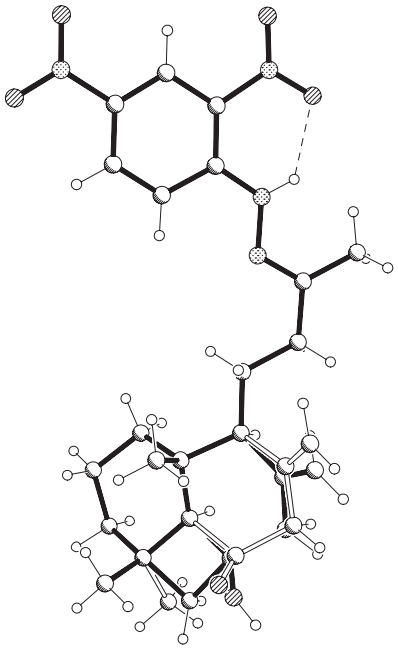


Figure 7. The overlap of positions of molecule **28a** and **27** in the crystal structure of **28**.

and identified. The good conversion (63%) of ketone **5**, combined with the good total yield of dienes **24** and **25** (55%) makes the photolysis of this easily accessible ketone as the best starting material for the synthesis of highly functionalized drimanes.

4. Experimental

4.1. General

Melting points (mp) were determined in capillary tubes and on a Boetius hot stage. IR spectra were obtained on Bio-Rad-Win-IR and Perkin–Elmer Models spectrometer in CCl_4. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AC-E 200 (200.13 and 50.32 MHz, respectively) and on a Bruker Avance DRX 400 (400.13 and 100.61 MHz) spectrometers. Chemical shifts are given in parts per million values in δ scale with $CHCl_3$ as reference (set δ_H at 7.24 ppm and δ_C 77.00 ppm) and coupling constants in Hertz. Carbon substitution degrees were established by DEPT pulse sequence. Mass spectra (MS) were run on an AEI MS 902 spectrometer (EI, 70 eV). Optical rotations were determined on a Perkin–Elmer 241 polarimeter with a 1 dm microcell, using $CHCl_3$ as solvent. Photolytic cleavage was effectuated by *n*-hexane in quartz flask using UV high-pressure lamps Heraeus TQ-718 Hg vapour, 700 W and LP Hg vapour, 450 W. For analytical TLC Merck silica gel 60 G in 0.25 mm layers was used. Chromatographic column separations were carried out on Merck silica gel 60 (70–230 mesh) using petroleum ether (bp 40–60 °C) and mixtures of petroleum ether with EtOAc of increasing polarity. All solvents were purified and dried by standard techniques just before use. Usual work-up means that water was added to the reaction mixture, which was then extracted with ether, the combined organic layers were washed with brine, dried over Na_2SO_4 or $MgSO_4$ and solvent evaporated under reduced pressure.

4.1.1. Photolysis of 6α-acetoxy-14,15-bisnorlabd-8(17)-ene-13-one (4). A solution of compound **4** (5.345 g, 16.7 mmol) in dry hexane (800 mL) was poured into the quartz flask of the photoreactor and a stream of dry nitrogen was bubbled through the solution before and during the irradiation. The solution was cooled to 5 °C and irradiated for 3 h with a UV lamp (750 W). Evaporation of the solvent afforded a yellow oil, which was purified by column chromatography on silica gel (200 g, eluent: PE/EtOAc 9:1), to give *6α-acetoxy-drim-8(12),9(11)-diene* **18** (410 mg, yield 76%, conversion 13%) as an colourles oil; [Found: C, 77.6; H, 10.4. $C_{17}H_{26}O_2$ requires C, 77.82; H, 9.99%]; $[\alpha]_D^{20}$ +27.34 (*c* 1.22); ν_{max} 1742, 1639, 1241, 896 cm^{-1}; δ_H (400.13 MHz) 5.22 (1H, m, *H*-6), 4.95 (1H, m), 4.91 (1H, d, *J* 1.6 Hz) (C*H*$_2$=), 4.73 (1H, m), 4.64 (1H, d, *J* 1.6 Hz) (C*H*$_2$=), 2.82 (1H, dd, *J* 13.4, 5.2 Hz, *H*-7α), 2.06–2.22 (1H, m, *H*-1), 2.05 (3H, s, OCO*Me*), 1.20–1.70 (7H, m), 1.03 (3H, s, *Me*), 1.00 (3H, s, *Me*), 0.94 (3H, s, *Me*); δ_C (100.61 MHz) 170.3 (C), 159.0 (C), 145.4 (C), 110.9 (CH_2), 104.3 (CH_2), 72.0 (CH), 54.1 (CH), 43.7 (CH_2), 41.0 (CH_2), 39.8 (C), 38.0 (CH_2), 35.8 (CH_3), 33.8 (C), 22.6 (CH_3), 22.3 (CH_3), 21.9 (CH_3), 18.8 (CH_2); *m/z* 262 (M^+, 18), 260 (100), 189 (24), 149 (20), 125 (44), 115 (42), 69 (32), 42 (46%); HRMS (EI): M^+, found 262.2132. $C_{17}H_{26}O_2$ requires 262.1933.

Further elution with the same eluent yielded 23 mg (3.5%) of *6α-acetoxy-9,13β-epoxy-13β-methylpodocarpane* **20** as white crystals, mp 156–157 °C (from hexane); [Found: C, 75.4; H, 10.2. $C_{20}H_{32}O_3$ requires C, 74.96, H, 10.21%]; $[\alpha]_D^{20}$ +7.67 (*c* 0.01); ν_{max} (Nujol) 1733, 1248, 1090, 1016 cm^{-1}; δ_H (400.13 MHz) 5.19 (1H, dd, *J* 8.0, 5.2 Hz, *H*-6), 2.00 (3H, s, OCO*Me*), 0.90–1.98 (16H, m), 1.42 (3H, s, *Me*), 1.16 (3H, s, *Me*), 1.05 (3H, s, *Me*), 0.83 (3H, s, *Me*); δ_C (100.61 MHz) 170.7 (C), 91.3 (C), 81.3 (C), 72.5 (CH), 50.7 (CH), 45.2 (CH_2), 42.9 (CH_2), 39.4 (CH), 39.0 (CH_2), 37.1 (C), 36.2 (CH_2), 34.9 (CH_2), 34.3 (C), 34.2 (CH_3), 33.1 (CH_2), 23.8 (CH_3), 21.9 (CH_3), 21.2 (CH_3), 19.4 (CH_3), 18.7 (CH_2).

The next compound eluted with the same eluent was *(1S,3S,6R,7R,9S,10R)-3,6,7,11,11-pentamethyl-2-oxatetracyclo[8.4.0.0^{1,6}.0^{3,7}]tetradec-9-yl acetate* **22** (16 mg, yield 2.4%) as crystals, mp 102–103 °C (from *n*-hexane); [Found: C, 75.1; H, 10.2. $C_{20}H_{32}O_3$ requires C, 74.95; H, 10.07%]; $[\alpha]_D^{20}$ +1.53 (*c* 0.01); ν_{max} (Nujol) 1724, 1235, 1006, 954, 908 cm^{-1}; δ_H (400.13 MHz) 5.24 (1H, dd, *J* 15.6, 7.6 Hz, *H*-6), 2.01 (3H, s, OCO*Me*), 1.23–2.18 (13H, m), 1.09 (3H, s, *Me*), 1.06 (3H, s, *Me*), 0.90 (6H, s, *Me*$_2$), 0.76 (3H, s, *Me*); δ_C (100.61 MHz) 170.7 (C), 85.1 (C), 84.9 (C), 68.8 (CH), 58.1 (CH), 49.6 (C), 49.1 (C), 41.4 (CH_2), 35.0 (CH_2), 34.5 (CH_2), 34.1 (CH_3), 33.9 (C), 33.0 (CH_2), 30.1 (CH_2), 26.0 (CH_3), 21.7 (CH_3), 19.7 (CH_2), 17.8 (CH_3), 17.4 (CH_3), 15.7 (CH_3).

The next compound, eluted from the chromatographic column with the same solvent mixture was *6α-acetoxy-8β,13;13,17-diepoxy-14,15-bisnorlabdane* **23** (118 mg, 17%), mp 121–122 °C (from *n*-hexane); [Found: C, 71.7; H, 9.6. $C_{20}H_{32}O_4$ requires C, 71.39; H, 9.59%]; $[\alpha]_D^{20}$ +25.3 (*c* 0.04); ν_{max} (Nujol) 1730, 1243, 1155, 1015 cm^{-1}; δ_H (400.13 MHz) 5.33 (1H, dt, *J* 11.08, 4.48 Hz, *H*-6), 3.76 (1H, d, *J* 6.75 Hz), 3.31 (1H, d, *J* 6.75 Hz, *H*$_2$-17), 2.03

(3H, s, OCOMe), 1.42 (3H, s, *Me*), 1.18 (3H, s, *Me*), 1.16 (3H, s, Me), 0.90–2.20 (14H, m), 0.91 (3H, s, *Me*); δ_C (100.61 MHz) 169.9 (C), 108.8 (C), 81.7 (C), 75.7 (CH_2), 70.5 (CH), 57.4 (CH), 49.7 (CH), 43.7 (CH_2), 41.2 (CH_2), 40.3 (C), 40.2 (CH_2), 36.7 (CH_3), 33.5 (CH_2), 33.2 (C), 24.9 (CH_3), 22.5 (CH_3), 21.9 (CH_3), 18.2 (CH_2), 17.7 (CH_3), 16.9 (CH_2).

The last compound, eluted from the chromatographic column with the same solvent mixture, was the *starting ketone* **4** (4.687 mg, 88%).

4.1.2. Photolysis of acetoxyketone (4) in argon atmosphere. A solution of acetoxyketone **4** (100 mg, 0.313 mmol) in dry *n*-hexane (320 mL) was poured into the quartz flask of the photoreactor and a stream of dry argon was bubbled through the solution before and during the irradiation. The solution was cooled to 5 °C and irradiated for 2 h with the 450-W UV lamp. The removal of the solvent afforded an yellow oil (119 mg), which was purified by column chromatography on silica gel (12 g, eluent PE/EtOAc 85:15), yielding *acetoxydiene* **18** (15 mg, 78.5%) as a colourless oil, unreacted *starting material* **4** (78 mg, 78%) and *acetoxyketal* **23** (6 mg, 26%).

4.1.3. Photolysis of acetoxyketone (4) in oxygen atmosphere. A solution of **4** (100 mg, 0.313 mmol) in dry *n*-hexane (320 mL) was poured into the quartz flask of the photoreactor and a stream of dry oxygen was bubbled through the solution before and during the irradiation. The solution was cooled to 5 °C and irradiated for 2 h with the 450-W UV lamp. The removal of the solvent afforded a yellow oil (114 mg), which was purified by column chromatography on silica gel (12 g, eluent: PE/EtOAc 85:15), yielding *acetoxydiene* **18** (7 mg, 14%) as an oil, unreacted *starting material* **4** (46 mg, 46%) and *acetoxyketal* **23** (23 mg, 40%).

4.1.4. Photolysis of 14,15-bisnorlabd-8(17)-ene-6,13-dione (5). A solution of the diketone **5** (260 mg, 0.94 mmol) in dry hexane (800 mL) was poured into quartz flask of the photoreactor and a stream of dry nitrogen was bubbled through the solution before and during the irradiation. The solution was cooled to 5 °C and irradiated for 4 h with the 700-W UV lamp. The removal of the solvent afforded the crude product (224 mg), which was purified by column chromatography on silica gel (22 g, eluent: PE/EtOAc, 98:2), to yield *ketodienes* **24** (46 mg, 36%), and **25** (25 mg, 19.4%) as yellow oils and unreacted *starting material* **5** (97 mg, 37%).

Drim-8(12),9(11)-diene-6-one (**24**), oil; [Found: C, 82.2; H, 10.1. $C_{15}H_{22}O$ requires C, 82.51; H, 10.16%]; $[\alpha]_D^{22}$ +15.3 (*c* 0.085); ν_{max} (liquid film) 1715, 1673, 1628, 901 cm^{-1}; δ_H (200.13 MHz) 5.08 (1H, s), 5.02 (1H, m) (CH_2=), 4.80 (1H, s), 4.69 (1H, m) (CH_2=), 3.14 (1H, d, *J* 16.6 Hz), 3.04 (1H, d, *J* 16.6 Hz, H_2-7), 2.18 (1H, s, *H*-5), 1.00–1.80 (6H, m), 1.20 (3H, s, *Me*), 0.97 (6H, s, Me_2); δ_C (50.32 MHz) 208.1 (C), 158.4 (C), 144.1 (C), 111.8 (CH_2), 106.6 (CH_2), 62.5 (CH), 50.7 (CH_2), 42.9 (CH_2), 40.6 (C), 37.8 (CH_2), 32.9 (CH_3), 32.8 (C), 23.6 (CH_3), 21.8 (CH_3), 18.6 (CH_2).

Drim-7,9(11)-diene-6-one (**25**), oil; $[\alpha]_D^{25}$ −101.3 (*c* 0.075); ν_{max} (liquid film) 3092, 1710, 1667, 893, 853 cm^{-1}; δ_H (200.13 MHz) 5.76 (1H, s, *H*-7), 5.25 (1H, s), 5.19 (1H, d, *J* 1.66 Hz, H_2-11), 2.19 (1H, s, *H*-5), 1.97 (3H, s, *Me*), 1.20–1.90 (6H, m), 1.12 (3H, s, *Me*), 1.09 (3H, s, *Me*), 1.08 (3H, s, Me); δ_C (50.32 MHz) 200.3 (C), 156.1 (C), 149.6 (C), 128.0 (CH), 111.8 (CH_2), 61.1 (CH), 43.1 (CH_2), 42.9 (C), 37.8 (CH_2), 33.3 (CH_3), 32.7 (C), 23.2 (CH_3), 21.7 (CH_3), 20.3 (CH_3), 18.4 (CH_2). Lit.[18] $[\alpha]_D^{20}$ −160.8; the spectral data are identical with those described in the literature.[4,18]

4.1.5. Photolysis of 14,15-bisnorlabd-7-ene-6,13-dione (6). A solution of diketone **6** (420 mg, 1.51 mmol) in dry *n*-hexane (800 mL) was poured into the quartz flask of the photoreactor and a stream of dry nitrogen was bubbled through the solution before and during the irradiation. The solution was cooled to 5 °C and irradiated for 4 h with the 700-W UV lamp. The removal of the solvent afforded a yellow oil (384 mg), which was purified by column chromatography on silica gel (35 g, eluent: PE/EtOAc 9:1), to give successively *diene* **25** (73 mg, 67.5%), *ketoalcohol* **26** as an oil (24 mg, 17.5%) and unreacted *starting material* **6** (283 mg, 67%).

The spectral data of ketodiene **25** are identical with those mentioned above.

Cyclobuto(18→6)-14,15-bisnorlabd-8(17)-ene-6-ol-13-one (**26**), oil; [Found: C, 78.1; H, 10.4. $C_{18}H_{28}O_2$ requires C, 78.21; H, 10.21%]; $[\alpha]_D^{20}$ +45.5 (*c* 0.02); ν_{max} 3577, 3090, 1705, 1635, 1145, 885 cm^{-1}; δ_H (400.13 MHz) 5.03 (1H, s), 4.79 (1H, s) (CH_2=), 2.12 (3H, s, CO*Me*), 2.05–2.62 (6H, m), 1.49 (3H, s, *Me*), 0.86–1.90 (11H, m), 0.85 (3H, s, *Me*); δ_C (100.61 MHz) 209.0 (C), 147.5 (C), 112.7 (CH_2), 78.6 (C), 59.9 (CH), 58.2 (CH), 54.5 (CH_2), 53.2 (CH_2), 42.3 (CH_2), 42.0 (CH_2), 41.9 (CH_2), 41.0 (C), 37.9 (C), 30.0 (CH_3), 21.4 (CH_2), 20.2 (CH_3), 19.6 (CH_2), 12.6 (CH_3).

4.1.6. Preparation of 2,4-DNPH of ketoalcohol (26). To the solution of ketoalcohol **26** (20 mg) in ethanol (1 mL) was added freshly prepared solution of 2,4-dinitrophenylhydrazine (30 mg) in EtOH (0.8 mL) containing 0.05 mL of concd H_2SO_4. The mixture was stirred at room temperature for 3 h (the course of the reaction was controlled by TLC). Then the reaction mixture was diluted with water (4 mL) and extracted with diethyl ether (3×10 mL). The organic layer was washed with water and dried. After removal of the solvent under reduced pressure the crude product (53 mg) was purified by column chromatography on silica gel (5 g, eluent: PE/EtOAc 95:5), to give two fractions (18 mg and 14 mg), and both were rechromatographied on SiO_2 (3 g). Two products were obtained and recrystallized from a *n*-hexane–EtOAc mixture to give **27** mp 133–133.5 °C and **28** mp 155–157.5 °C. Their structures were established by X-ray analysis.

4.1.7. Isomerization of hydroxyketone (26). To a solution of hydroxiketone **26** (3.5 mg, 0.013 mmol) in 0.5 mL EtOH were added two drops of concd H_2SO_4 and the mixture was stirred at room temperature for 48 h (the course of the reaction was controlled by TLC). Then reaction mixture was diluted with water (5 mL) and extracted with diethyl ether (2×5 mL). The organic layer was washed with

water (2×5 mL) and dried. The removal of the solvent under reduced pressure afforded *ketone* **5** (3.1 mg, 88.6%), mp 73–74 °C (from petroleum ether); $[\alpha]_D^{20}$ +79.0 (*c* 1.0); ν_{max} (KBr) 1714, 1644, 894 cm^{-1}; δ_H (200.13 MHz) 4.87 (1H, s), 4.59 (1H, br s) (H_2-17), 3.06 (1H, d, *J* 13.5 Hz), 2.95 (1H, d, *J* 13.5 Hz) (H_2-7), 2.55–2.75 (1H, m), 2.30–2.54 (1H, m) (H_2-12), 2.12 (3H, s, *Me*), 1.17 (3H, s, *Me*), 1.00–2.20 (11H, m), 0.96 (3H, s, *Me*), 0.64 (3H, s, Me); δ_C (50.32 MHz) 208.8 (C), 207.9 (C), 143.1 (C), 109.7 (CH_2), 66.2 (CH), 56.0 (CH), 55.7 (CH_2), 42.3 (CH_2), 41.2 (C), 38.6 (CH_2), 32.7 (C), 32.5 (CH_3), 30.1 (CH_3), 21.5 (CH_3), 18.8 (CH_2), 17.6 (CH_2), 15.7 (CH_3); *m/z* (M^+, 54), 258 (20), 151 (57), 124 (20), 123 (94), 109 (100), 107 (26), 95 (40), 81 (45), 43 (57%). Lit.[6] mp 72–74 °C; $[\alpha]_D^{20}$ +80.4.

4.2. Crystal structure determination

X-ray diffraction measurements for **20** and **22** were performed at room temperature on a KUMA Diffraction KM4 diffractometer using graphite monochromatized Mo Kα radiation for compound **20** and Cu Kα for compound **22**. Lattice parameters were obtained from least-squares refinement of 29 reflections with $7.5\leq 0\geq 18°$ for **20** and 30 reflections with $17.5\leq 0\geq 65°$ for **22**. Intensities were measured using ω/20 and ω scan technique at variable speed 1.2–12° min^{-1}. The data were corrected for Lorentz and polarization effects, but not for absorption. X-ray diffraction measurements for **23** were performed at 120 K temperature on a Nonius Kappa CCD diffractometer using graphite monochromatized Mo Kα radiation and at 100 K on Xcalibur Px kappa-geometry diffractometer, using graphite-monochromated Cu Kα radiation for **27** and **28**. Analytical absorption correction was applied for **27** and **28**. The structure were solved by direct methods and refined by full-matrix least-square techniques based on F^2. The non-H atoms were refined with anisotropic displacement parameters. Hydrogen atoms in all structures and non-H atoms of minor fraction molecules in disordered fragments in the structures **27** and **28** were refined using isotropic thermal factors. Calculations were performed using SHELX-97 crystallographic software package.[22]

4.2.1. Crystal data for 20.[23] $C_{20}H_{32}O_3$, Mr=320.46 g mol^{-1}, size 0.30×0.10×0.10 mm^3, orthorhombic, space group $P2_12_12_1$, a=7.946(2) Å, b=13.503(3) Å, c=16.798(3) Å, V=1802.3(7) $Å^3$, Z=4, ρ_{calcd}=1.181 g cm^{-3}, μ(Mo Kα)=0.77 cm^{-1}, F(000)=704, 2217 reflections in h(−10/1), k(0/17), l(0/21), measured in the range $1.94\leq\Theta\leq 26.82$, completeness Θ_{max}=94.5%, 2091 independent reflections, R_{int}=0.0340, 209 parameters, 0 restraints, $R1_{obs}$=0.0466, $wR2_{obs}$=0.1273, $R1_{all}$=0.0971, $wR2_{all}$=0.1500, GOOF=1.023, largest difference peak and hole: 0.211/−0.192 e $Å^{-3}$.

4.2.2. Crystal data for 22.[23] $C_{20}H_{32}O_3$, Mr=320.46 g mol^{-1}, size 0.25×0.10×010 mm^3, orthorhombic, space group $P2_12_12_1$, a=9.420(2) Å, b=11.300(2) Å, c=17.302(3) Å, V=1841.7(6) $Å^3$, Z=4, ρ_{calcd}=1.156 g cm^{-3}, μ(Cu Kα)=5.93 cm^{-1}, F(000)=704, 2122 reflections in h(−11/1), k(−14/0), l(−21/0), measured in the range $4.67\leq\Theta\leq 73.49$, completeness Θ_{max}=94.1%, 2004 independent reflections, R_{int}=0.0513, 209 parameters, 0 restraints, $R1_{obs}$=0.0510, $wR2_{obs}$=0.1382, $R1_{all}$=0.1572, $wR2_{all}$=0.1802, GOOF=1.002, largest difference peak and hole: 0.366/−0.252 e $Å^{-3}$.

4.2.3. Crystal data for 23.[23] $C_{20}H_{32}O_4$, Mr=336.46 g mol^{-1}, size 0.25×0.10×0.10 mm^3, monoclinic, space group $P2_1$, a=6.099(1) Å, b=28.941(6) Å, c=21.090(4) Å, β=90.08(3)°, V=3722.6(12) $Å^3$, Z=8, ρ_{calcd}=1.201 g cm^{-3}, μ(Mo Kα)=0.82 cm^{-1}, F(000)=1472, 21171 reflections in h(−7/7), k(−34/34), l(−24/24), measured in the range $1.19\leq\Theta\leq 25.00$, completeness Θ_{max}=94.5%, 11801 independent reflections, R_{int}=0.0740, 886 parameters, 7 restraints, $R1_{obs}$=0.0774, $wR2_{obs}$=0.1237, $R1_{all}$=0.1228, $wR2_{all}$=0.1369, GOOF=1.030, largest difference peak and hole: 0.249/−0.272 e $Å^{-3}$.

4.2.4. Crystal data for 26.[23] $C_{24}H_{32}N_4O_5\cdot 0.5(H_2O)$, Mr=465.54 g mol^{-1}, size 0.45×0.11×0.04 mm^3, monoclinic, space group $C2$, a=16.717(5) Å, b=6.565(3) Å, c=22.362(6) Å, β=106.78(3)°, V=2349.7(14) $Å^3$, Z=4, ρ_{calcd}=1.316 g cm^{-3}, μ(Cu Kα)=7.73cm^{-1}, F(000)=988, 9122 reflections in h(−20/18), k(−8/6), l(−19/27), measured in the range $4.13\leq\Theta\leq 76.09$, completeness Θ_{max}=90.1%, 3774 independent reflections, R_{int}=0.0425, 416 parameters, 1 restraints, $R1_{obs}$=0.0558, $wR2_{obs}$=0.1391, $R1_{all}$=0.0655, $wR2_{all}$=0.1493, GOOF=1.008, largest difference peak and hole: 0.411/−0.271 e $Å^{-3}$.

4.2.5. Crystal data for 27.[23] $C_{24}H_{32}N_4O_5$, Mr=456.54 g mol^{-1}, size 0.15×0.10×0.03 mm^3, orthorhombic, space group $P2_12_12_1$, a=7.157(3) Å, b=15.012(5) Å, c=43.51(2) Å, V=4675(3) $Å^3$, Z=8, ρ_{calcd}=1.297 g cm^{-3}, μ(Cu Kα)=7.51 cm^{-1}, F(000)=1952, 41120 reflections in h(−7/8), k(−18/14), l(−46/53), measured in the range $3.58\leq\Theta\leq 72.50$, completeness Θ_{max}=99.4%, 8882 independent reflections, R_{int}=0.1533, 623 parameters, 0 restraints, $R1_{obs}$=0.0837, $wR2_{obs}$=0.1784, $R1_{all}$=0.1527, $wR2_{all}$=0.2181, GOOF=0.994, largest difference peak and hole: 0.261/−0.237 e $Å^{-3}$.

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$PdCl_2/CuCl_2$-catalysed chlorocyclisation of sugar-derived aminoalkenitols in the synthesis of new iminohexitols

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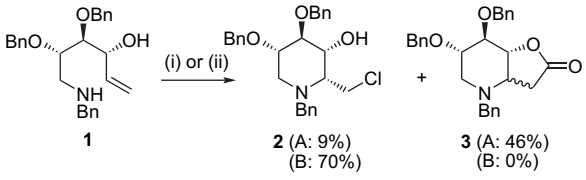
Abstract—The total synthesis of two novel L-*ido* and L-*altro* configured 6-chloro-1,5,6-trideoxyiminohexitols featuring a highly diastereoselective Pd(II)/$CuCl_2$-catalysed chlorocyclisation of sugar-derived aminoalkenitols has been accomplished. The requisite substrates were, in turn, prepared from chiral pool materials starting from the cheap and commercially available methyl-α-D-gluco- and methyl-α-D-galactopyranoside.

1. Introduction

Polyhydroxylated piperidines (azasugars and iminohexitols) are naturally occurring alkaloids with a wide range of unique biological properties,[1] which have led to synthetic and medicinal interest in their research producing an impressive array of functional analogues.[2] Thus, D-*galacto*,[3] L-*galacto*,[4] D-*gluco*[5] and L-*gulo*[6] configured 6-chloro-1,5,6-trideoxyiminohexitols are known. Recently, we described[7] the unexpected formation of partially protected L-*ido*-azasugar **2** along with desired lactones **3** during a Pd(II)-catalysed cyclocarbonylation of methyl-α-D-glucopyranoside derived aminoalkenitol **1** (conditions (i), Scheme 1). Clearly, $CuCl_2$ (used in excess as reoxidant for $Pd^0 \rightarrow Pd^{II}$) promoted the competitive chlorocyclisation of **1** at a comparable rate, even in the presence of carbon monoxide. This was proven by the 'blind' experiment with exclusion of CO from the reaction mixture with **2** being formed exclusively (conditions (ii), Scheme 1). To the best of our knowledge, there is only one more report[8] describing such a transformation, while the analogous iodocyclisation[9] on similar substrates is well documented. Thus, we decided to explore the Pd(II)/$CuCl_2$-catalysed chlorocyclisation of sugar-derived aminoalkenitols in more detail and herein we present its utility in the synthesis of two new C-6 chlorinated 1,5,6-trideoxyazasugars **12** and **13**.

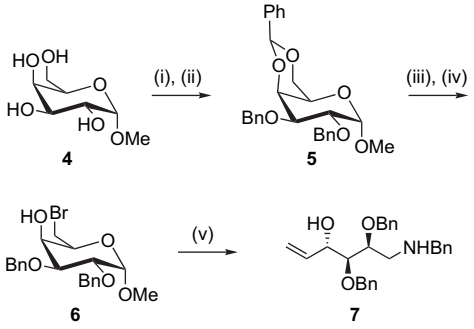


Scheme 1. Reagents and conditions: (i) CO, cat. Pd(II), $CuCl_2$, AcONa, AcOH; (ii) cat. Pd(II), $CuCl_2$, AcONa, AcOH.

2. Results and discussion

2.1. Preparation of substrates

In addition to the aminoalkenitol **1**,[7] we have chosen its diastereoisomer **7**[10] as a second substrate, the latter being prepared via a five-step sequence starting from commercially available methyl-α-D-galactopyranoside **4** (Scheme 2). Thus, 4,6-*O*-benzylidenation,[11] followed by 2,3-di-*O*-benzylation[12] afforded fully protected sugar **5**,[13] which was



Scheme 2. Reagents and conditions: (i) $PhCH(OEt)_2$, CSA, $CHCl_3$, reflux, 2 h, 88%; (ii) BnBr, NaH, DMF, rt, 3 h, 59%; (iii) 2% H_2SO_4, MeOH, rt, 4 h, 71%; (iv) Ph_3P, CBr_4, pyridine, 0–60 °C, 3 h, 61%; (v) Zn dust, $BnNH_2$, $NaBH_3CN$, ^{n}PrOH/H_2O, reflux, 3 h, 88%.

Keywords: Palladium catalysis; Copper(II) chloride; Aminoalkenitols; Azasugars; Chlorocyclisation; Total synthesis.
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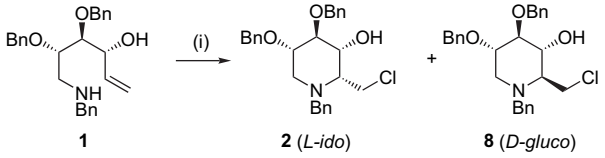
hydrolysed and subsequently halogenated to furnish primary bromide **6**.[14] The last transformation involved a one-pot three-step sequence (reductive elimination, ring opening and reductive amination) providing the desired aminoalkenitol **7** in 23% overall yield (Scheme 2).

2.2. $PdCl_2/CuCl_2$-catalysed chlorocyclisations

With both substrates **1** and **7** on hand, we subjected them to the $PdCl_2/CuCl_2$-catalysed chlorocyclisation under various reaction conditions. First, the influence of solvent on the chemoselectivity and/or diastereoselectivity (noticed[7] during aminocarbonylation of **1**) of the transformation was evaluated with aminoalkenitol **1** (Scheme 3).



1 **2** (*L-ido*) **8** (*D-gluco*)

Scheme 3. Reagents and conditions: (i) 0.1 equiv $PdCl_2$, 3 equiv $CuCl_2$, 3 equiv AcONa, see Table 1.

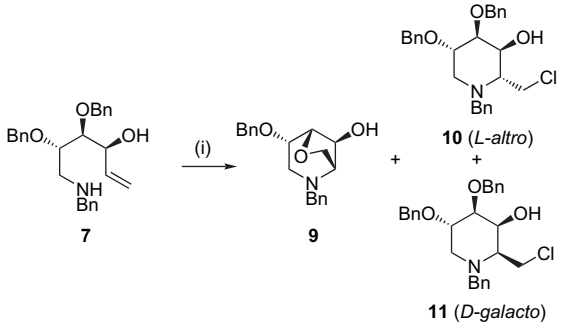
In all cases, L-*ido* configured C-6 chlorinated azasugar **2** was obtained (with full conversion of **1**) as a major product (resulting from the intramolecular Si-attack of nucleophilic amine to the Pd^{II}-activated double bond) along with its minor D-*gluco* diastereomer **8** (Table 1). The solvent of choice turned out to be glacial AcOH (Entry 1), which gave results superior to other solvents in terms of both yield (70%) and diastereoselectivity (90% de). Only slightly lower combined yields of products were obtained in CH_2Cl_2 (65%), THF (60%) and MeOH (59%), however, with considerably diminished des (71% and/or 67%, Entries 3–5). Chlorocyclisation of **1** in DMF (Entry 2) furnished a substantial amount of unidentified side products resulting in an unacceptably low yield (21%) of desired azasugars **2** and **8**. It is important to note that our best conditions (Entry 1) are far superior in terms of diastereoselectivity to those already reported[9b] for the iodocyclisation (5–26% de) of the perbenzylated analogue of **1**.

Table 1. $PdCl_2/CuCl_2$-catalysed chlorocyclisation of aminoalkenitol **1**

Entry	Solvent	Conditions	Yield (%)[a]	Ratio of **2/8** (de, %)[b]
1	AcOH	23 °C, 48 h	70	19/1 (90)
2	DMF	30 °C, 24 h	21	8/1 (82)
3	DCM	30 °C, 24 h	65	6/1 (71)
4	THF	30 °C, 24 h	60	6/1 (71)
5	MeOH	23 °C, 24 h	59	5/1 (67)
6	Toluene	30 °C, 48 h	56	3/1 (33)

[a] Isolated combined yield of **2**+**8** after FLC.
[b] Diastereomeric ratio determined by HPLC analysis of crude reaction mixture.

The same catalytic conditions were applied to the aminoalkenitol **7** and in this case we observed a significant solvent effect on the chemoselectivity and diastereoselectivity of the transformation (Table 2). In all of the solvents tested (Entries 1–6), we noticed the unexpected and rather surprising formation of bicyclic derivative **9** resulting in a lower combined yields of desired C-6 chlorinated azasugars L-*altro* **10** and D-*galacto* **11** in comparison to combined yields of **2**+**8** obtained from substrate **1** (Scheme 4).



7 **9** **10** (*L-altro*) **11** (*D-galacto*)

Scheme 4. Reagents and conditions: (i) see Table 2.

Considering the products distribution first, the best combined yields of **10** and **11** (53% and 54%) were obtained in DMF (Entry 2) and THF (Entry 4). On the other hand, the reaction was more selective towards the formation of **9** when conducted in glacial AcOH (48–52%, Entries 1, 7 and 8). Evaluating the diastereoselectivity second, the highest ratio of **10**/**11** (90% de) was obtained again in glacial AcOH (Entry 1), regardless of the amount of $CuCl_2$ used (Entries 7–9). DMF as a solvent performed comparably well (88% de). Due to its much higher chemoselectivity the latter is a solvent of choice for the preparation of L-*altro* **10** (Entry 2), which is again formed via Si-attack analogous

Table 2. Pd(II)/$CuCl_2$-catalysed chlorocyclisation and bicyclisation of aminoalkenitol **7**

Entry	Solvent	Catalyst and additive(s)	Conditions	Yield of **10**+**11**/**9** (%)[a]	Ratio of **10**/**11** (de, %)[b]
1	AcOH	0.1 equiv $PdCl_2$, 3 equiv $CuCl_2$, 3 equiv AcONa	25 °C, 24 h	28/49	19/1 (90)
2	DMF	0.1 equiv $PdCl_2$, 3 equiv $CuCl_2$, 3 equiv AcONa	25 °C, 19 h	53/11	15/1 (88)
3	DCM	0.1 equiv $PdCl_2$, 3 equiv $CuCl_2$, 3 equiv AcONa	29 °C, 24 h	36/8	5/1 (67)
4	THF	0.1 equiv $PdCl_2$, 3 equiv $CuCl_2$, 3 equiv AcONa	25 °C, 24 h	54/10	2/1 (33)
5	Toluene	0.1 equiv $PdCl_2$, 3 equiv $CuCl_2$, 3 equiv AcONa	29 °C, 24 h	43/9	1/2 (33)
6	MeOH	0.1 equiv $PdCl_2$, 3 equiv $CuCl_2$, 3 equiv AcONa	25 °C, 24 h	32/7	1/3 (50)
7	AcOH	0.1 equiv $PdCl_2$, 2 equiv $CuCl_2$, 2 equiv AcONa	30 °C, 48 h	35/52	19/1 (90)
8	AcOH	0.1 equiv $PdCl_2$, 1 equiv $CuCl_2$, 1 equiv AcONa	28 °C, 48 h	25/48	19/1 (90)
9	AcOH	0.1 equiv $PdCl_2$, 3 equiv $CuCl_2$	30 °C, 24 h	27/50	19/1 (90)
10[c]	AcOH	0.1 equiv $PdCl_2$, 3 equiv AcONa	28 °C, 96 h	0/0	—
11[c]	AcOH	0.1 equiv $Pd(OAc)_2$, 2 equiv benzoquinone, 3 equiv AcONa	30 °C, 24 h	—/0	—

[a] Isolated combined yields of **10**+**11** and **9** after FLC.
[b] Diastereomeric ratio determined by HPLC analysis of crude reaction mixture.
[c] Full conversion of **7** with concomitant formation of complex mixture of unidentified products.

to **1**. Interestingly, the use of either toluene (Entry 5) or methanol (Entry 6) as solvents caused reversal of the diastereoselectivity in favour of D-*galacto* **11**, albeit with poor des (33% and 50%). In addition, AcONa was not an essential component (originally used as a basic trap to quench HCl eliminated during the catalytic cycle) of the reaction mixture (Entry 9). More importantly, $CuCl_2$ turned out to be an indispensable reagent for this particular transformation, as either its exclusion (Entry 10) or its replacement for benzoquinone provides neither the bicycle **9** nor the chlorinated azasugars **10/11**. Instead, complex reaction mixtures of unidentified products were formed while the substrate **7** was fully consumed (Table 2).

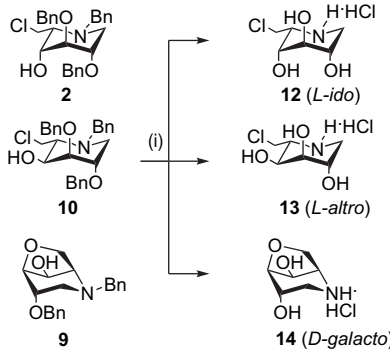
The unexpected formation of bicycle **9** from aminoalkenitol **7** under the applied reaction conditions might be explained as follows: the initial bis-coordination of $PdCl_2$ with both (C-3)OBn group and C=C moiety of **7** followed by the intramolecular Re-attack of *N*-nucleophile promotes the formation of a σ-Pd-complex I in a geometrically favourable $^1C_4$ chair conformation. As the presence of $CuCl_2$ is essential for the course of the transformation (Entries 1–9), we envisaged the subsequent formation of a heterobimetallic[15] σ-Pd/Cu-complex I. Its final reductive elimination releases both the bicycle **9** and the catalyst, which consequently re-enters the catalytic cycle (Scheme 5). Such $PdCl_2$/$CuCl_2$-catalysed *N*,*O*-bicyclisation of unsaturated 1,3-aminoalcohol constitutes a novel synthetic route for the preparation of 6-oxa-2-aza-bicyclo[3.2.1]octane skeleton.[16] On the other hand, the formation of chloroderivative **10** can be reasoned in a following way: Pd^{2+}-promoted activation of double bond of **7** followed by nucleophilic addition from its Si-face leads to σ-Pd-complex II with concomitant elimination of HCl. Subsequent formation of σ-Pd/Cu-complex II in the presence of $CuCl_2$ and the final reductive elimination furnishes desired **10** and regenerates $PdCl_2$ at the same time (Scheme 5).

The global deprotection of advanced intermediates **2** and **10** (isolated by careful flash chromatography of corresponding pure diastereomeric mixtures) using catalytic hydrogenolysis completed the total synthesis of two new azasugars, namely hydrochlorides of 6-chloro-1,5,6-trideoxy-1,5-imino-L-iditol **12** and 6-chloro-1,5,6-trideoxy-1,5-imino-L-altritol **13**. It is noteworthy that compounds **2**, **10**, **12** and **13** all exist in a $^1C_4$ conformation in $CDCl_3$ solution as evidenced by vicinal coupling constants in the ^1H NMR spectra (Table 3).

Table 3. Determination of $^1C_4$ conformation of **2**, **10**, **12** and **13**

Compound	Vicinal coupling (*J*) constants in ^1H NMR (Hz)
2	$J_{4,5}$=3, $J_{2,3}$=$J_{3,4}$=4.8
10	$J_{3,4}$=2.3, $J_{4,5}$=6.7
12	$J_{4,5}$=1.5, $J_{2,3}$=$J_{3,4}$=3.5
13	$J_{2,3}$=$J_{3,4}$=3.5, $J_{4,5}$=10.4

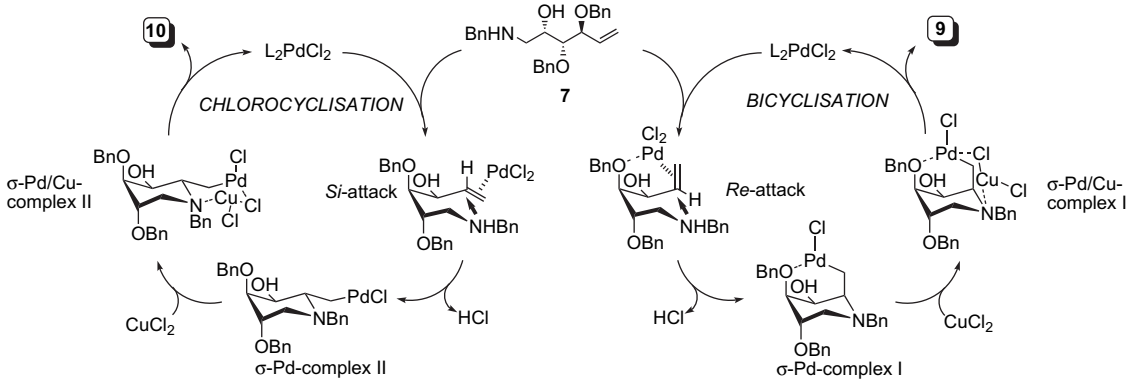
In addition, final total debenzylation of bicycle **9** afforded the hydrochloride of known[17] conformationally locked analogue of a strong glycosidase inhibitor 1-deoxy-D-galactonojirimycin, namely 1,5-dideoxy-3,6-anhydro-1,5-imino-D-galactitol **14** (Scheme 6).



Scheme 6. Reagents and conditions: (i) H_2/Pd–C, HCl, EtOH, rt, 24 h, 100%.

3. Conclusion

In conclusion, we have performed the total synthesis of two new C-6 chlorinated iminohexitols L-*ido* **12** and L-*altro* **13** featuring a $PdCl_2$/$CuCl_2$-catalysed chlorocyclisation of sugar-derived aminoalkenitols **2** and **7**. In addition, the $PdCl_2$/$CuCl_2$-catalysed *N*,*O*-bicyclisation of the aminoalkenitol **7** afforded a known bicyclic compound D-*galacto* **14**. This compound represents a conformationally locked analogue of the strong glycosidase inhibitor 1-deoxy-D-galactonojirimycin.



Scheme 5. Tentative mechanism of Pd(II)/$CuCl_2$-catalysed chlorocyclisation and bicyclisation of aminoalkenitol **7**.

4. Experimental

4.1. General

All reagents were used as received without further purification unless otherwise specified. All solvents were distilled before use: THF and toluene from Na, MeCN from P_2O_5, DMF from KOH, MeOH from MeONa, CH_2Cl_2 from activated 4 Å molecular sieves. Flash column liquid chromatography (FLC) was performed on Kieselgel 60 (40–63 μm). HPLC was performed on Separon SGX column (4×125 mm, 5 μm) using MeOH/H_2O as an eluent (80/20 v/v) and UV (254 nm) detection (flow rate 0.5 ml min^{-1}, injection volume 20 μl, column temperature 20 °C). Optical rotations were measured with a PolarL-μP polarimeter with a 10,000 cm cell at λ=589 nm. Elemental analyses were performed by the Microanalytical Service of Slovak University of Technology. Infrared (IR) spectra were recorded on a Nicolet Magna 750 FTIR spectrometer. NMR spectra were recorded on Varian VXR-300 (300 MHz) and Inova 600 (600 MHz) spectrometers. Chemical shifts (δ) are quoted in parts per million and the residual protic solvent was used as internal reference. The following abbreviations were used to characterise signal multiplicities: singlet (s), doublet (d), triplet (t), multiplet (m), broad (b). The COSY and NOESY techniques were used in assignment of ^1H–^1H relationships and the determination of relative configuration. The multiplicities of carbons were assigned from a broad-band decoupled analysis used in conjunction with APT. The HETCOR and HMQC techniques were used throughout for the assignment of the ^1H–^{13}C relationships.

4.1.1. (2*S*,3*S*,4*S*)-1-Benzylamino-2,3-di-*O*-benzyl-hex-5-ene-2,3,4-triol (7). Acid treated zinc dust [(17.95 g, 274.54 mmol, 60 equiv) prepared by gradual washing with 3% HCl (100 ml), H_2O (3×80 ml), EtOH (30 ml), acetone (30 ml), dry Et_2O (30 ml) and finally dried in vacuo] was added to the solution of bromoalcohol **6** (2.0 g, 4.576 mmol) in ^{n}PrOH/H_2O (19/1, 80 ml) mixture with vigorous stirring. Then $BnNH_2$ (7.38 g, 7.5 ml, 68.63 mmol, 15 equiv) and $NaBH_3CN$ (575 mg, 9.151 mmol, 2 equiv) were added and the resulting mixture was stirred at reflux for 3 h. After cooling to room temperature the suspension was filtered through Celite pad and washed with EtOH (20 ml). The filtrate was evaporated in vacuo and the residue was dissolved in Et_2O (200 ml) and treated with 20% aq HCl solution (20 ml). After 30 min the mixture was basified with 15% aq NaOH solution (pH 9), the organic phase was separated and the water layer was extracted with $CHCl_3$ (2×200 ml). The combined organic extracts were dried over $MgSO_4$ and evaporated in vacuo giving the crude product, which was purified by FLC (silicagel, hexanes/AcOEt/NH_4OH=6/4/0.03) to afford pure aminoalkenitol **7** (1.68 g, 88%) as a pale-yellow oil. R_f=0.18 (hexanes/AcOEt=1/1); $[\alpha]_D^{20}$ −13.1 (*c* 1.3, $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 2.90 (m, 2H, H-1), 3.22–3.50 (br s, 2H, exchange with D_2O, OH, NH), 3.59 (dd, 1H, $J_{3,2}$=5.1, $J_{3,4}$=5.9 Hz, H-3); 3.69 (d, 1H, J=12.9 Hz, NCH_2Ph), 3.73 (m, 1H, H-2), 3.78 (d, 1H, J=12.9 Hz, NCH_2Ph), 4.29 (ddt, 1H, $J_{4,6E}$=$J_{4,6Z}$=1.6, $J_{4,3}$=$J_{4,5}$=5.9 Hz, H-4), 4.48 (d, 1H, J=11.4 Hz, OCH_2Ph), 4.58 (d, 1H, J=11.4 Hz, OCH_2Ph), 4.59 (d, 1H, J=11.4 Hz, OCH_2Ph), 4.66 (d, 1H, J=11.4 Hz, OCH_2Ph), 5.15 (dt, 1H, $J_{6E,4}$=$J_{6E,6Z}$=1.6, $J_{6E,5}$=10.5 Hz, H-6*E*), 5.33 (dt, 1H, $J_{6Z,4}$=$J_{6Z,6E}$=1.6, $J_{6Z,5}$=17.2 Hz, H-6*Z*), 6.00 (ddd, 1H, $J_{5,4}$=5.9, $J_{5,6E}$=10.5, $J_{5,6Z}$=17.2 Hz, H-5); δ_C (75 MHz, $CDCl_3$) 47.5 (t, C-1), 53.8 (t, N*C*H_2Ph), 72.2 (d, C-4), 72.4 (t, O*C*H_2Ph), 73.2 (t, O*C*H_2Ph), 79.6 (d, C-2), 81.4 (d, C-3), 115.3 (t, C-6), 127.3, 127.8, 127.8, 127.9, 128.0, 128.3, 128.4, 128.5 (all d, all *C*H-Ph), 138.0, 138.2 (2×s, 2×*C*q-Ph), 138.5 (d, C-5), 139.2 (s, *C*q-Ph); ν_{max} (film on KBr)/cm^{-1} 698, 737, 1028, 1071, 1090, 1454, 1496, 2864, 3030, 3031, 3063, 3310; *m*/*z* (APCI$^+$) 418 $[M+1]^+$, 440 $[M+Na]^+$; found C 77.65, H 7.61, N 3.53%; $C_{27}H_{31}NO_3$ requires C 77.67, H 7.48, N 3.35%.

4.1.2. Typical procedure for $PdCl_2$/$CuCl_2$-catalysed chloroaminocyclisation of 7. Aminoalkenitol **7** (670 mg, 1.61 mmol), $PdCl_2$ (28 mg, 0.161 mmol, 0.1 equiv), $CuCl_2$ (648 mg, 4.82 mmol, 3 equiv) and AcONa (395 mg, 4.82 mmol, 3 equiv) were suspended in a glacial AcOH (16 ml) and the resulting deep-green heterogeneous mixture was stirred under Ar at 25 °C over 24 h. The light-green suspension was filtered over Celite pad (3×2 cm), solids were washed with AcOH (20 ml) and the filtrate was co-evaporated with toluene (20 ml) in vacuo. The resulting yellow-green-brown oil (932 mg) was taken up to AcOEt (100 ml), washed with 10% aq $NaHCO_3$ solution (2× 50 ml) and the combined water layers were extracted with AcOEt (50 ml). The combined organic extracts were washed with brine (50 ml), dried over anhydrous Na_2SO_4 and evaporated in vacuo to yield a brown oil (622 mg), which was purified by FLC (20 g of silicagel, column 2.5× 19.5 cm, gradient elution: hexanes/AcOEt/Et_3N=460/40/3→250/250/3) to afford two fractions: first eluted the pure diastereomeric mixture of **10**/**11** (203 mg, 28%, 19/1) as a pale-yellow oil, second eluted the pure bicycle **9** (256 mg, 49%) as a pale-yellow waxy solid.

Data for *N*-benzyl-2,3-di-*O*-benzyl-6-chloro-1,5,6-trideoxy-1,5-imino-L-altritol **10**: R_f=0.58 (hexanes/AcOEt=3/1); $[\alpha]_D^{24}$ +38 (*c* 1.38, CH_2Cl_2); δ_H (300 MHz, $CDCl_3$) 2.45 (br s, 1H, exchange with D_2O, OH), 2.58 (dd, 1H, $J_{1,2}$=2.6, $J_{1,1'}$=13.5 Hz, H-1), 2.71 (dd, 1H, $J_{1',2}$=6.1, $J_{1',1}$=12.9 Hz, H-1′), 2.93 (m, 1H, $J_{5,6}$=5.6, $J_{5,4}$=6.3 Hz, H-5), 3.42 (d, 1H, J=12.3 Hz, NCH_2Ph), 3.71 (m, 2H, H-2, H-3), 3.80 (dd, 1H, $J_{6,5}$=5.6, $J_{6,6'}$=12.0 Hz, H-6), 3.95 (dd, 1H, $J_{6',5}$=3.4, $J_{6',6}$=12.0 Hz, H-6′), 4.08 (d, 1H, J=12.6 Hz, NCH_2Ph), 4.18 (dd, 1H, $J_{4,3}$=2.3, $J_{4,5}$=6.7 Hz, H-4), 4.38 (d, 1H, J=12.0 Hz, OCH_2Ph), 4.47 (d, 1H, J=12.0 Hz, OCH_2Ph), 4.58 (d, 1H, J=11.5 Hz, OCH_2Ph), 4.66 (d, 1H, J=11.5 Hz, OCH_2Ph), 7.24–7.37 (m, 15H, 3×Ph); δ_C (75 MHz, $CDCl_3$) 41.2 (t, C-6), 48.4 (t, C-1), 57.3 (t, N*C*H_2Ph), 63.6 (d, C-5), 66.8 (d, C-4), 71.2 (t, O*C*H_2Ph), 72.4 (t, O*C*H_2Ph), 72.8 (d, C-2), 78.2 (d, C-3), 127.1, 127.5, 127.8, 127.9, 128.3, 128.5, 128.7 (all d, all *C*H-Ph), 137.8, 138.3, 138.7 (all s, all *C*q-Ph); ν_{max} (film on KBr)/cm^{-1} 699, 738, 1028, 1076, 1103, 1454, 1495, 2808, 2872, 3029, 3062, 3448, 3547; *m*/*z* (MALDI) 513 $[M+Na+K]^+$; found C 71.63, H 6.52, Cl 7.92, N 3.15%; $C_{27}H_{30}ClNO_3$ requires C 71.75, H 6.69, Cl 7.84, N 3.10%.

Data for *N*-benzyl-2-*O*-benzyl-3,6-anhydro-1,5-dideoxy-1,5-imino-D-galactitol **9**: R_f=0.08 (hexanes/AcOEt=3/1); $[\alpha]_D^{24}$ +18 (*c* 0.9, CH_2Cl_2); δ_H (300 MHz, $CDCl_3$) 2.28 (br s, 1H, exchange with D_2O, OH), 2.43 (dd, 1H, $J_{1,2}$=3.5, $J_{1,1'}$=13.0 Hz, H-1), 2.79 (d, 1H, $J_{1',1}$=13.0 Hz, H-1′),

3.25 (br d, 1H, $J_{5,6}$=3.0 Hz, H-5), 3.55 (d, 1H, J=13.5 Hz, NCH_2Ph), 3.62 (d, 1H, J=13.5 Hz, NCH_2Ph), 3.65 (t, 1H, $J_{2,3}$=5.1 Hz, H-2), 3.78 (dd, 1H, $J_{6,5}$=3.6, $J_{6,6'}$=9.3 Hz, H-6), 4.02 (d, 1H, $J_{6',6}$=9.0 Hz, H-6′), 4.18 (d, 1H, $J_{3,2}$= 5.1 Hz, H-3), 4.44 (d, 1H, J=12.3 Hz, OCH_2Ph), 4.51 (d, 1H, $J_{4,5}$=1.2 Hz, H-4), 4.55 (d, 1H, J=12.3 Hz, OCH_2Ph), 7.25–7.36 (m, 10H, 2×Ph); δ_C (75 MHz, $CDCl_3$) 48.3 (t, C-1), 59.3 (t, NCH_2Ph), 63.9 (t, C-6), 64.0 (d, C-5), 71.5 (t, OCH_2Ph), 73.1 (d, C-4), 76.4 (d, C-2), 80.3 (d, C-3), 127.3, 127.6, 127.7, 128.0, 128.4, 128.9 (all d, all *C*H-Ph), 137.9, 138.2 (2×s, 2×*C*q-Ph); IR: ν_{max} (KBr)/cm^{-1} 697, 739, 746, 891, 1029, 1049, 1057, 1114, 1133, 1454, 2804, 2883, 2951, 3395; *m/z* (MALDI) 323 [M−H-1]$^+$; found C 73.79, H 7.14, N 4.32%; $C_{20}H_{23}NO_3$ requires C 73.62, H 7.22, N 4.30%.

4.1.3. Typical procedure for the catalytic debenzylation of 2 or 10. Protected piperidine **2** or **10** (87 mg, 0.193 mmol) was dissolved in EtOH (8 ml), 10% Pd–C (55 mg) was added followed by 35% aq HCl (10 drops) and the resulting black suspension was stirred under hydrogen atmosphere (balloon) at 23 °C over 12 h. The reaction mixture was filtered, solids were washed with EtOH (2×5 ml) and the clear filtrate was co-evaporated with AcOEt (5 ml) in vacuo yielding **12** or **13** (42 mg, 100%) as a pale-yellow hygroscopic foam.

Data for 6-chloro-1,5,6-trideoxy-1,5-imino-L-iditol hydrochloride **12**: $[\alpha]_D^{24}$ +18 (*c* 0.26, EtOH); δ_H (600 MHz, CD_3OD) 3.31 (ddd, 1H, $J_{1,3}$=1.1, $J_{1,2}$=2.2, $J_{1,1'}$=13.2 Hz, H-1), 3.48 (dd, 1H, $J_{1',2}$=1.9, $J_{1',1}$=13.2 Hz, H-1′), 3.70 (dtd, 1H, $J_{5,4}$=1.5, $J_{5,6'}$=5.8, $J_{5,6}$=8.5 Hz, H-5), 3.90 (ddd, 1H, $J_{6,4}$=0.6, $J_{6,5}$=8.7, $J_{6,6'}$=12.0 Hz, H-6), 3.97 (dd, 1H, $J_{6',5}$=5.7, $J_{6',6}$=12.0 Hz, H-6′), 3.99 (m, 1H, $J_{2,1'}$=1.9, $J_{2,1}$= 2.1, $J_{2,3}$=3.1 Hz, H-2), 4.00 (dd, 1H, $J_{4,5}$=1.1, $J_{4,3}$=3.5 Hz, H-4), 4.01 (m, 1H, $J_{3,2}$=3.2, $J_{3,4}$=3.7 Hz, H-3); δ_C (125 MHz, CD_3OD) 42.3 (t, C-6), 47.5 (t, C-1), 58.1 (d, C-5), 67.7 (d, C-4), 67.9 (d, C-2), 69.5 (d, C-3); ν_{max} (film on KBr)/cm^{-1} 756, 1002, 1054, 1131, 1560, 2437, 2534, 3014, 3273, 3421; found C 33.21, H 5.88, Cl 32.45, N 6.33%; $C_6H_{13}Cl_2NO_3$ requires C 33.05, H 6.01, Cl 32.51, N 6.42%.

Data for 6-chloro-1,5,6-trideoxy-1,5-imino-L-altritol hydrochloride **13**: $[\alpha]_D^{24}$ −19 (*c* 0.27, EtOH); δ_H (600 MHz, CD_3OD) 3.18 (dd, 1H, $J_{1,2}$=2.5, $J_{1,1'}$=12.9 Hz, H-1), 3.42 (dd, 1H, $J_{1',2}$=1.4, $J_{1',1}$=13.0 Hz, H-1′), 3.57 (dtd, 1H, $J_{5,6'}$= 2.9, $J_{5,6}$=7.6, $J_{5,4}$=10.4 Hz, H-5), 3.95 (dd, 1H, $J_{6,5}$=7.6, $J_{6,6'}$=12.6 Hz, H-6), 3.96 (t, 1H, $J_{3,4}$=3.2, $J_{3,2}$=3.8 Hz, H-3), 4.00 (dd, 1H, $J_{4,3}$=2.9, $J_{4,5}$=10.4 Hz, H-4), 4.05 (m, 1H, $J_{2,1}$=2.2, $J_{2,3}$=4.2 Hz, H-2), 4.12 (dd, 1H, $J_{6',5}$=2.9, $J_{6',6}$=12.6 Hz, H-6′); δ_C (125 MHz, CD_3OD) 43.4 (t, C-6), 46.4 (t, C-1), 57.5 (d, C-5), 66.1 (d, C-4), 67.7 (d, C-2), 70.4 (d, C-3); ν_{max} (film on KBr)/cm^{-1} 756, 881, 1071, 1434, 2517, 3346; found C 32.88, H 6.05, Cl 32.60, N 6.40%; $C_6H_{13}Cl_2NO_3$ requires C 33.05, H 6.01, Cl 32.51, N 6.42%.

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Synthesis, biology, and modeling of a C-4 carbonyl C,D-*seco*-taxoid

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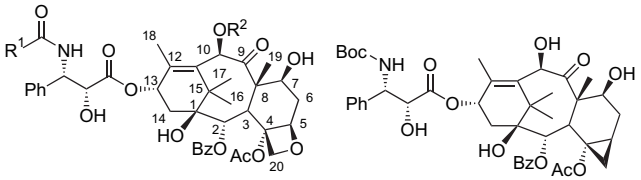
Dedicated with profound respect and affection to the memory of Professor Pierre Potier

Abstract—A C,D-*seco*-paclitaxel derivative **26** was prepared from taxine and tested for biological activity. Chemical reactivity of the *seco*-compounds proved to be substantially modified, with respects to taxoids. The corresponding C,D-*seco*-taxoid does not show tubulin stabilizing activity or cytotoxicity. Explanation of these observations based on molecular modeling is provided.

1. Introduction

Taxol **1**[1,2] and Taxotere **2**[2,3] are currently considered as the most efficient therapeutic agents for the treatment of several types of cancer. In order to understand better their unique mechanism of action, which would allow for design of the analogs of reduced structural complexity, yet with improved properties (i.e., higher activity, better solubility, activity against multidrug-resistant (MDR) tumors, etc.), extensive structure–activity relationship (SAR) studies have been performed. These studies indicated that the oxetane ring may be one of the crucial structural units of biologically active taxoids; its exact role, however, remained a matter of debate.[4] According to one explanation, the four-membered ring may serve purely as a rigidifying element, that imposes a proper conformational bias to the taxane core, thus forcing the functional groups at C-2, C-4, and C-13 to assume the appropriate positions for productive interactions with tubulin receptor. Alternatively, electronic effects may be important, with the heteroatom being involved in stabilizing dipolar, or hydrogen bonding, interactions with tubulin protein. For both hypotheses—'conformational' and 'electronic'—experimental support exists, as well as some contradictory data. Thus, substitution of oxygen in ring D by nitrogen,[5]



sulfur,[6] or selenium[6b] results in the loss of activity, although the geometries of the corresponding azetidine, thietane, and selenetane derivatives do not differ very much, as compared to the parent oxetane. These findings infer the prevalence of the electronic factors. On the other hand, the lack of bioactivity of most of D-*seco*-taxoids with various degree of oxygenation at positions C-4, C-5, and C-20, suggests that the role of the oxetane ring may be 'the conformational lock' of the diterpene scaffold.[7,4b] In line with this hypothesis, a taxoid **3**, containing cyclopropane ring in place of oxetane, showed the tubulin polymerizing activity comparable to that of docetaxel.[8] Recently, it was shown that, contrary to long

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lasting belief, no ring D is required for tubulin polymerizing activity of taxoids: on the basis of molecular modeling it was predicted that **4** would have a strong microtubule stabilizing activity, which was subsequently experimentally confirmed.[9] However, it should be noted that both **3** and **4** are at least by two orders of magnitude less cytotoxic with respect to paclitaxel or docetaxel; thus, while not necessary for the tubulin stabilizing activity, the oxetane ring still might be essential for cytotoxicity.

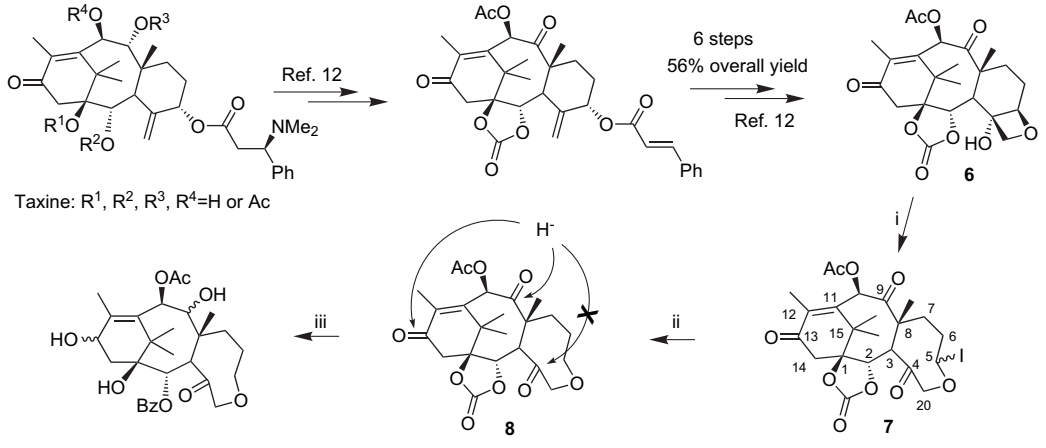
2. Results and discussion

In order to discern between the conformational and electronic effects of the oxetane ring, we endeavored to synthesize a C,D-*seco*-taxoid of type **5** and to evaluate its biological activity. While conformationally more flexible, the *seco*-derivative maintains the functionalization pattern of paclitaxel. The lack of biological activity of the compound would point to a conformational constraint as the principal (if not the sole) effect of the oxetane ring; on the contrary, eventual cytotoxicity of the *seco*-derivative would indicate the importance of the oxygenation pattern (i.e., the electronic effect of the oxetane oxygen atom). We decided to perform our study on 7-deoxy-taxoids: given that a 7-hydroxyl group is not required for cytotoxicity,[10] synthetic transformations could be performed on derivatives that require less protective group manipulation. In addition, others[11] and we[12] have shown that taxine B[13]—a pseudo-alkaloid readily isolated from the renewable needles of the European yew (*Taxus baccata*)—constitutes a suitable starting material, amenable to structural patterns relevant for this study.[14]

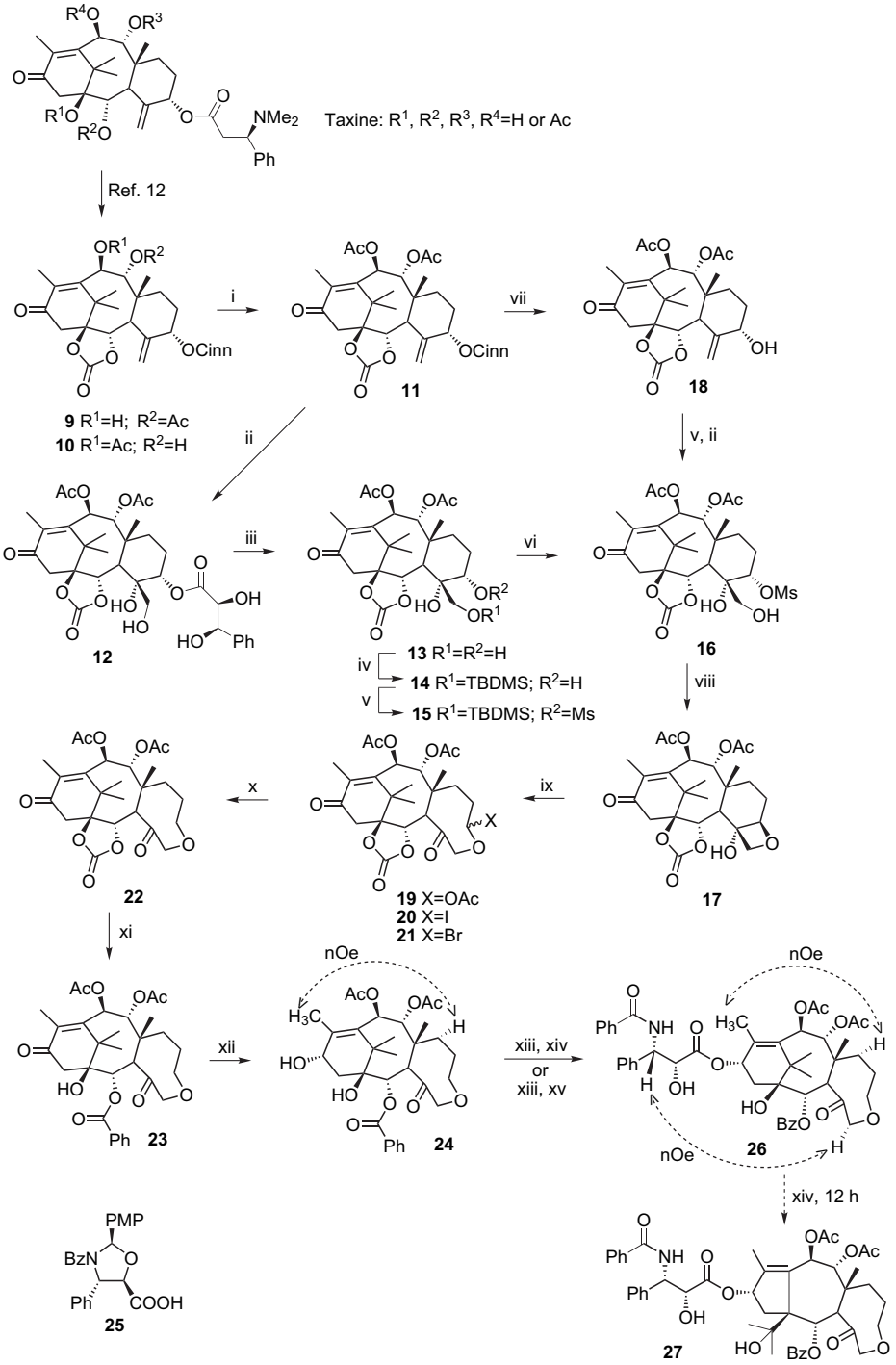
Our initial plan called for the scission of the central C-4–C-5 bond in previously described alcohol **6**,[12] via a fragmentation of the corresponding alkoxyl radical (Scheme 1). After some experimentation, the iodine-transfer fragmentation was cleanly effected using diacetoxyiodobenzene (DIB)/iodine reagent under photolytic conditions.[15] Subsequent reduction with tributyltin hydride (TBTH) afforded the desired intermediate **8** in good yield. For the reaction to proceed efficiently, a high concentration and an excess of TBTH are needed (under the conventional conditions for reductions with TBTH, rearrangements occur and unidentified products are formed). This skeletal transformation reversed the order of reactivity of carbonyl groups, with respect to the parent taxane: while C-9 carbonyl group in paclitaxel is resistant even to $LiAlH_4$,[16] in 4,5-*seco*-derivative it underwent rapid, but stereorandom reduction with $NaBH_4$, as well as C-13 carbonyl. On the contrary, the newly formed ketone at C-4 proved resistant toward $NaBH_4$, $NaBH_4/CeCl_3$, or $NaBH_3CN$, while a large excess of $NaBH_4$ resulted in decomposition of material.

In order to overcome this difficulty, the target structure was modified by substituting the C-9 α-acetate for the C-9 carbonyl group: a change that should simplify the synthetic procedure without affecting the biological activity of the final product (Scheme 2).[17] After the acetylation of a mixture of cinnamoyltaxicines **9** and **10**, selective removal of the cinnamoyl side chain in **10**, in the presence of two acetate units and a cyclic carbonate, was accomplished using our previously developed procedure:[12] dihydroxylation of **11** with OsO_4 resulted in stereoselective introduction of two requisite hydroxyl groups into the taxane core, while simultaneously activating (by intramolecular hydrogen bonding in **12**) the intermediate 2,3-dihydroxy-3-phenylpropanoate side chain toward alcoholysis in a buffered methanolic solution. The resulting triol **13** was converted into oxetane **17** by a slightly modified four-step protocol developed by the CNRS group[11a] (the cyclization step had to be performed with DIPEA in refluxing toluene; the reagents described in the literature proved unsuccessful). Alternatively, the conversion of **11** into the advanced cyclization precursor **16** could be effected via a three-step procedure based on hydroxylamine promoted selective cinnamate cleavage.[6a,18] The latter route is shorter and avoids the use of protective groups; however, the overall yield of **17** is lower (calculated yields for the transformation **11**→**17**, via tetraol **12**: 38%; via allylic alcohol **18**: 23%).

With compound **17** in hand, several reagents for the generation of tertiary alkoxyl radicals were tried for the fragmentation step. Surprisingly, the major product in the reaction with DIB/iodine was acetate **19** (29%), accompanied by the desired iodide **20** (17%); this is in sharp contrast to the same reaction with **6**, which proceeded in 73% yield without



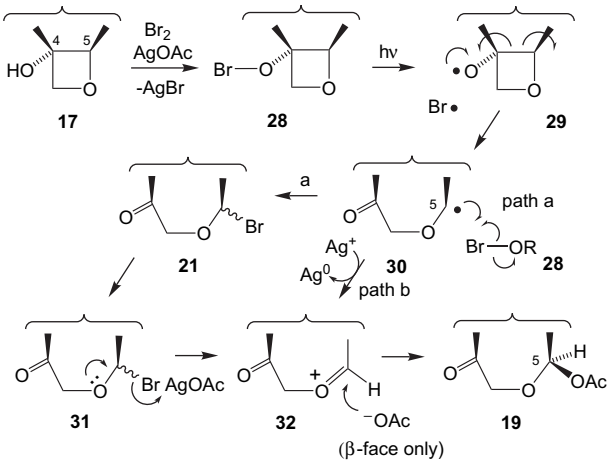
Scheme 1. Reagents and conditions: (i) $PhI(OAc)_2$ (1.5 equiv), I_2 (1.5 equiv), benzene, 15 °C, 250 W Xenophot lamp, 73%; (ii) Bu_3SnH (10 equiv, 0.4 M), benzene, 15 °C, 250 W Xenophot lamp, 80%; (iii) $NaBH_4$, $CeCl_3$, EtOH, rt.



Scheme 2. Reagents and conditions: (i) Ac_2O, pyridine, CH_2Cl_2, DMAP, 93%; (ii) OsO_4, NMO, THF, H_2O, *t*-BuOH; (iii) KOAc, MeOH, 73% over two steps; (iv) TBDMSCl, DMAP, Et_3N, CH_2Cl_2; (v) MsCl, pyridine; (vi) TBAF, THF, 58% from **13**; (vii) NH_2OH, HCl, Et_3N, THF, EtOH, H_2O, 50%; (viii) DIPEA, PhMe, Δ, 24 h, 90%; (ix) AgOAc, Br_2, PhH, h*ν*, see text for conditions, **19** (60%) or **21** (61%); (x) Bu_3SnH, AIBN, PhH, h*ν*, 61% over two steps; (xi) (a) PhLi, THF, −80 °C; (b) Ac_2O, DMAP, CH_2Cl_2, 62%; (xii) $NaBH_4$, MeOH, 72%; (xiii) **25**, DCC, DMAP, PhMe, 75 °C; (xiv) 5% *p*-TsOH, MeOH, 30 min, 70% from **24**; (xv) CAN, H_2O, CH_3CN, 40 °C, 2.5 h, 61% from **24**.

complication. Reagents such as mercuric oxide/iodine,[19] or silver oxide/bromine,[20] were also unsuccessful, afforded **20**, or **21**, in very low yield (<10%), along with some unidentified products. Finally, we found that the fragmentation step could be accomplished with silver acetate/bromine reagent,[19a,b] under carefully controlled conditions. As a result both bromide **21** and acetate **19** could be obtained chemoselectively, depending on the stoichiometry of reactants. When the reaction was performed with 1.4 equiv of AgOAc and 2.5 equiv of Br_2, the required bromide **21** was the predominant product isolated as a 1:1 mixture of stereoisomers. With 4 equiv of AgOAc and 2 equiv of Br_2 the chemoselectivity of the reaction is reversed and only acetate **19** is obtained. Interestingly, the acetate was formed stereoselectively, with strong predominance of the β-isomer (β:α=10.5:1), as determined by NOESY experiment.

The formation of bromide mixture **21** and acetate **19** can be understood as the consequence of different mechanistic pathways. In the first case, the AgOAc/Br_2 reagent produces a hypobromite at C-4 (C_4OBr), which is cleaved to an alkoxyl radical **29** under the influence of light (Scheme 3). As part of the chain reaction process, hypobromite **28** delivers bromine to both faces of the ring-opened radical **30** (path a) accountable for the product mixture. An MM3* Monte Carlo conformational search[21,22] of the flexible eight-membered ring radical corresponding to **30** with the C-5 carbon center modeled as a carbon free radical shows that both faces of the ring are free to react with **28** to give **21** (Fig. 1).[23]



Scheme 3. Proposed hypobromite reaction of **17** to give **21** and subsequent formation of oxonium cation **32** in the presence of excess AgOAc. Intermediate **32** is proposed to lead chemoselectively to β-acetate **19**.

The stereospecific formation of acetate **19** proceeds with a two-fold excess of AgOAc over Br_2. We speculate that under these conditions the 50:50 mixture of bromides **21** (Schemes 2 and 3) is likewise formed. However, the Lewis acid silver ion or silver acetate then coordinates with bromine to give complex **31** (Scheme 3, path a). Facile elimination can then provide the stabilized oxonium cation **32**. Alternatively, in the presence of excess AgOAc electron-transfer may predominate over the bromine transfer reaction, which results in direct oxidation of radical intermediate **30** to carbocation **32** (Scheme 3, path b).[24] The C–C=O^+–CH moiety introduces an element of rigidity in the *seco*-C,D eight-membered ring as suggested by a Monte Carlo conformational search of the analog of **32** modeled as the corresponding alkene. Unlike in the radical, the ring adopts a low energy conformation that presents only the face corresponding to the β-isomer as available for capturing the acetate nucleophile (**32**, Scheme 3 and Fig. 2). Bromide does not reassociate with the cation as it is removed as insoluble AgBr.

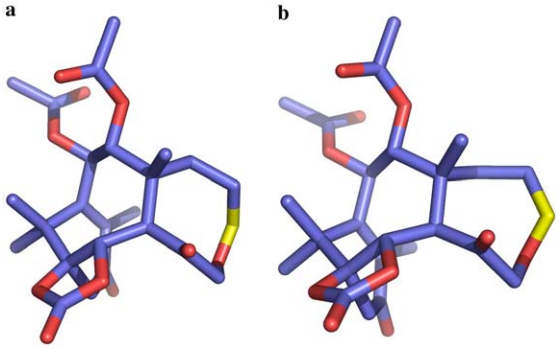


Figure 1. The first (a) and second (b) low energy MM3* conformers of the radical intermediate **30** (Scheme 3). The C-5 free radical carbon is highlighted in yellow.

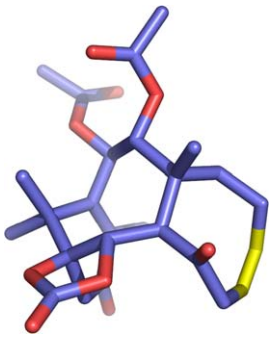


Figure 2. Low energy conformer of oxonium cation **32** (Scheme 3) modeled as the corresponding alkene-**21**. Only the β-face is exposed to nucleophilic attack. The O=C^+ bond modeled by C=C is highlighted in yellow.

Due to its instability on silica-gel, the crude bromide **21** was not purified, but directly reduced with TBTH to afford ketone **22** in 60% yield (from **17**). The installation of benzoate ester into C-2 position was effected by treatment with phenyllithium,[25] followed by reacetylation and the reduction of the C-13 carbonyl group in **23** with $NaBH_4$. At this stage we planned the reduction of the C-4 carbonyl group in **24** followed by acetylation of the so formed secondary alcohol. To our surprise, **24** proved unreactive toward a variety of reducing agents, such as $NaBH_4$ (20 equiv, rt), $NaBH_4/CeCl_3$, $BH_3 \cdot Me_2S$ (rt), L-Selectride (−70→0 °C), Red-Al (−60→+50 °C), or Noyori transfer-hydrogenation (performed separately with both (*R*,*R*)- and (*S*,*S*)-enantiomers of TsDPEN ligand).[26] Under the conditions of reduction with Et_3SiH/BF_3 only the formation of products of the Lewis acid-catalyzed rearrangement were observed. With $LiAlH_4$ a complex mixture of products was obtained. At low temperatures, DIBALH in excess induced only the reductive hydrolysis of the acetate groups, while a very complex mixture of products was formed at rt. The attempted Noyori reduction of **22** was very slow and resulted only in reductive deprotection of the cyclic carbonate unit. With other reducing reagents occasional reduction of the C-13 carbonyl group was observed, but the C-4 carbonyl remained intact. Thus, similar to compound **8**, fragmentation of the central bond in the condensed C,D-system of **17** brought about a major conformational change in the taxane core and rendered the newly formed carbonyl group inaccessible to reducing agents.

To understand the remarkable lack of reactivity at the C-4 carbonyls of **22** and **24**, we examined the conformational profile of the molecules to learn what structural features might contribute to sequester the C=O groups at C-4. In a preliminary communication,[14] we performed a 10,000-step

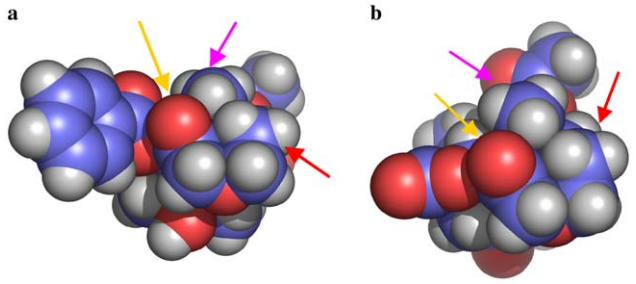


Figure 3. The lowest energy conformations for taxanes **24** and **22** and the disposition of the C-4 carbonyl. (a) For **24**, the C_4=O (yellow arrow) is surrounded by the C-2 phenyl (left), the C-8 methylene (red arrow) and the C-19 methyl (top, magenta arrow); (b) for **22**, the cyclic carbonate effectively replaces the C-2 phenyl in **24** as a shielding unit for C_4=O.

MMFF/GBSA/H_2O conformational search for ketone **24** and located a boat-chair (BC) conformation as the global minimum with a predicted population of 99.5% at 298 K. This form is identical to the lowest energy torsional isomer of cyclooctanone.[27] The BC conformer, embedded in the taxane architecture, occludes approach to either face of the C_4=O group (center, yellow arrow) by reducing agents (Fig. 3a). The plane of the carbonyl is shielded on three sides by the C-2 phenyl group (left), the C-8 CH_2 group (right, red arrow), and the C-19 Me group (top, magenta arrow) causing the C-4 carbon to be buried within the folds of the taxane. This observation is substantiated by calculation of zero solvent accessible surface for C-4.

In Figure 3a the C-2 phenyl of **24** is shown as a bulky impediment to the approach of a reducing agent to the α-face of the C-4 carbonyl. By comparison, the cyclic carbonate in **22** is both smaller and, to some extent, twisted away from the C-4 position by virtue of attachment at C-1 (Fig. 3b). Yet, it too prevents reduction. To understand this, we performed an MMFF conformational search of the C,D-ring of **22** similar to that of **24**. The lowest energy conformer likewise sustains the BC shape. Surprisingly, the cyclic carbonate oxygen, though much smaller than a phenyl ring, blocks approach to C-4 equally effectively. The distance between the C-4 carbon and the C-2 carbonate oxygen atom is 2.7 Å, indicating that the two atoms are most likely crowded (van der Waals sum=3.5 Å).[28] The steric repulsion might, nonetheless, be mediated by a LP→π* interaction from O to C(=O). Similar to the benzoyl ester **24**, the solvent accessible surface area for C-4 in **22** is estimated to be a diminutive 0.35 $Å^2$. One implication of the C_4=O shielding from C-2 is that reduction of the C-4 carbonyl in the absence of either the carbonate or the C-2 phenyl ring, namely the bare C-2 OH, is likewise predicted to fail.

Thus, the reluctance of **24** toward reduction hampered the introduction of acetoxy substituent into C-4 position. Although this structural feature is known to be important for the biological activity of taxoids, we proceeded further with the appendage of the side chain and the conversion of **24** into a derivative suitable for biological evaluation. Esterification of the C-13 hydroxyl group was achieved through the coupling of **24** with the protected side chain **25**, using a previously described procedure.[29,12] Acidic hydrolysis of *p*-methoxybenzylidene protective group afforded the required C,D-*seco*-derivative **26** in good yield (70% over two steps). However, prolonged reaction times (12 h instead of 30 min) lead to the rearrangement of the A-ring in **26** and formation of 11(15-1)-abeotaxane **27**. We found that, alternatively, the deprotection step could be performed oxidatively, with CAN in aqueous acetonitrile, which obviates the need for close monitoring of the progress of the reaction.

For the evaluation of biological activity, compound **26** was submitted to the tubulin test,[30] as well as to cytotoxicity assays against rat glioma C6 cell lines,[31] and these results were compared to those obtained with paclitaxel. The result of the tubulin test showed **26** to be devoid of microtubule stabilizing activity. At 11.9 mM concentration, **26** effected only 7% inhibition of the microtubule disassembly process (for comparison, paclitaxel showed 50% inhibition at 1 μM concentration). The staining with crystal violet showed that paclitaxel reduced the number of C6 glioma cells in a dose-dependent manner, with an LC_{50} value of approx. 5 nmol (Fig. 4a). In contrast, compound **26** failed to display significant cytotoxicity in the same concentration range (Fig. 4a). The absence of cytotoxic activity of **26** was also confirmed in the MTT assay for mitochondrial respiration (Fig. 4b). Therefore, the observed lack of microtubule stabilizing

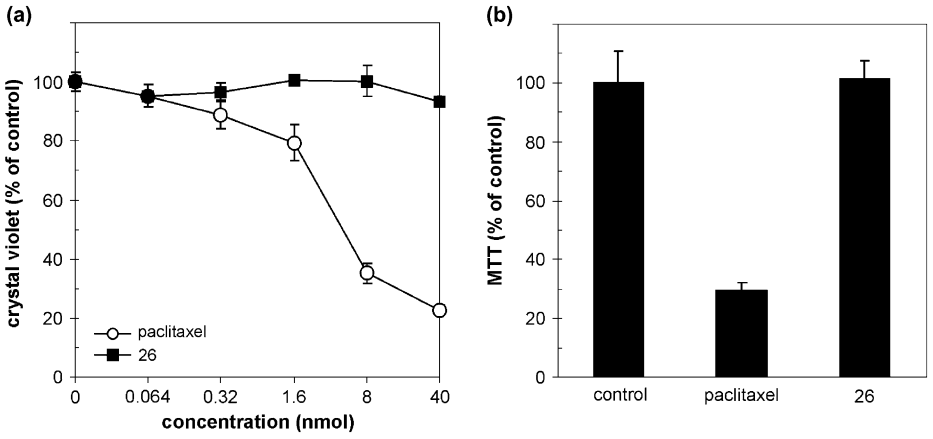


Figure 4. Cytotoxicity of paclitaxel and compound **26**. (a) C6 cells were incubated alone (control) or with various doses of paclitaxel or **26**; (b) C6 cells were incubated in the absence (control) or presence of 40 nmol of paclitaxel or **26**. Cell viability was assessed after 48 h by crystal violet (a) or MTT assay (b). The results from the representative of three separate experiments are presented as mean±SD of triplicate observations.

activity of **26** can be associated with the complete loss of cytotoxicity at pharmacologically relevant concentrations.

These findings confirmed the importance of the precise conformation of the C,D-ring system in taxoids for the biological activity. However, the fact that compound **26** lacks the C-4 acetate group makes interpretations of the biological tests somewhat ambiguous, as it is difficult to estimate, which of the two structural features in **26** (the conformational change, or the absence of the C-4 acetate) contributes more to the loss of biological activity.

Another factor concerns the relocation of the oxetane oxygen and the adjacent carbons. A conformation of compound **26** was constructed to incorporate the global minimum BC conformation of the *seco*-C,D unit as depicted in Figure 3a. In addition, the C-13 side chain was oriented in the T-Taxol conformation.[32,33] The corresponding structure was rigidly docked with the Glide protocol[34,22] into the taxane pocket of β-tubulin. The pose that resembles most closely the electron crystallographic structure of paclitaxel is depicted in Figure 5.

A significant difference between the structures of T-Taxol and **26** is the conformational reorganization of atoms in the *seco*-C,D rings. In paclitaxel (PTX), the C-4 acetate is found on the α-face of the molecule and directed into the concave region of the baccatin core. Simultaneously, the oxygen of the oxetane ring is oriented upward toward the β-face and outward forming weak contact with Thr274 of β-tubulin.[4a,32] In striking contrast, compound **26**, to a first approximation, inverts the roles of these two functionalities. The C═O replacement for the C-4 acetate is β oriented, while the oxetane oxygen along with its adjacent methylene groups is folded α and found deep within the concave baccatin cavity. The relative docking poses for PTX and **26** (Fig. 5) reflect the inside-out relationship operating within the C,D region of the molecules. One critical difference is that the C-4 acetate of PTX enjoying close contact with the C-3′ phenyl ring in the hydrophobic basin of the tubulin binding site[32] is lost in **26**. Another stems from the latter's α orientation of the bulky *seco*-C,D moiety. As a result of steric clashes with binding pocket residues Leu371, Pro274, and Phe272, the molecule is obligated to sit much higher in the pocket than PTX as depicted in Figure 5. Unfortunately, this results in the loss of pivotal hydrophobic interactions between ligand and protein. For example, instead of surrounding His229 in a stacked arrangement, the C-3′ benzamido and C-2 benzoyl moieties of **26** are moved much further up in the pocket and well outside of van der Waals contact. The situation is reminiscent of bridged taxanes proposed to be similarly dislodged from deep residence in the tubulin binding cleft as a result of ligand/protein steric encounters. The compounds are 10 to 30-fold less active than PTX.[35]

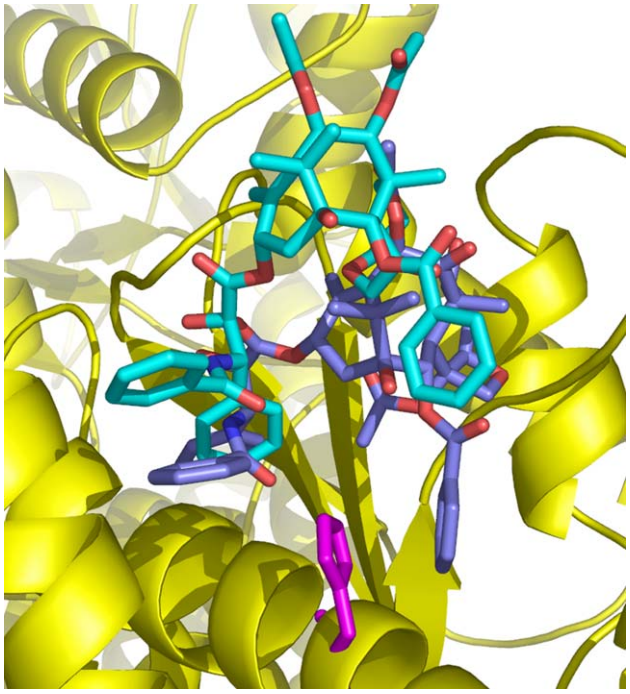


Figure 5. Docking poses of Taxol (blue) and **26** (cyan) within the taxane/tubulin binding pocket.

The proposed conformation of **26** (Fig. 5) is corroborated with the results of NOESY experiments (Scheme 2). A cross peak between H-20α and H-3′ is in agreement with the modeled conformation with C-20 curled into the concave region of the baccatin core and with a H-20α to H-3′ distance of 2.8 Å in the docked conformation. In addition, NOESY cross peaks between H-7α and H-18 are observed for both **26** and **24**, in accord with the concave conformation of the molecules. Taken together these observations support the proposal that the molecule adopts the pose pictured in Figure 5 and consequently experiences the steric clash suggested.

To summarize, a method is developed for the efficient preparation of C,D-*seco*-taxoids. The conformational change, induced by the scission of the C-4–C-5 bond in the taxane core, brings about important modifications in chemical reactivity of the 4,20-*seco*-derivatives and results in the loss of biological activity of the corresponding C,D-*seco*-taxoid.

3. Experimental

3.1. General

All chromatographic separations were performed on silica, 10–18 μm, 60A, ICN Biomedicals. Standard techniques were used for the purification of reagents and solvents. NMR spectra were recorded on a Varian Gemini 200 spectrometer, ^{1}H NMR at 200 MHz, ^{13}C NMR at 50 MHz, for samples in deuterated chloroform. Chemical shifts are expressed in parts per million using tetramethylsilane as internal standard, coupling constants (*J*) are in hertz. The NOESY spectra were acquired on a Varian Inova Unity 600 MHz spectrometer in deuterated chloroform at 25 °C, with mixing times of 400 ms and 500 ms for compounds **26** and **24**, respectively. IR spectra were recorded on a Perkin–Elmer 457 grating FT instrument, and are expressed in cm^{-1}. Mass spectrometric analysis was provided by the Emory University Mass Spectrometry Center using an ACQ Advantage mass spectrometer. Microanalyses were

performed at the Vario EL III instrument CHNOS Elementar Analyzer, Elementar Analysensysteme GmbH, Hanau, Germany. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected.

3.1.1. Compound 8. In a Pyrex, external water-cooled reactor, a deaerated suspension of **6** (30 mg, 0.067 mmol), diacetoxyiodobenzene (32 mg, 0.1 mmol), and iodine (25.4 mg, 0.1 mmol) in benzene (3 mL) was irradiated for 20 min with a 250 W Xenophot sun-lamp focalized light, with stirring under an argon atmosphere. The reaction mixture was diluted with CH_2Cl_2, washed successively with 5% aq $Na_2S_2O_3$ and water, and dried over anhyd $MgSO_4$. After the solvent was removed under reduced pressure, the crude **7** (60 mg) was used in the next step without further purification.

In a Pyrex, external water-cooled reactor, a deaerated solution of crude iodide **7** (60 mg), TBTH (101 mg, 0.348 mmol), AIBN (2 mg), and benzene (0.9 mL) was irradiated for 10 min with a 250 W Xenophot sun-lamp focalized light, with stirring under an argon atmosphere. After removal of the solvent under reduced pressure, the crude product was purified by dry flash chromatography (eluent: benzene/ethyl acetate=8/2) to give compound **8** (17.8 mg, 59%) as a colorless film. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.72 (1H, s, H-10), 4.42 (2H, br s, H-2 superimposed with H-3), 3.94 (1H, d, J=17.2 Hz, H-20), 3.86–3.72 (1H, m, H-5), 3.64–3.62 (1H, m, H-5), 3.52 (1H, d, J=17.2 Hz, H-20), 2.99 (1H, d, J=19.6 Hz, H-14), 2.76 (1H, d, J=19.4 Hz, H-14), 2.29 (3H, s, H-18), 2.25 (3H, s, Ac), 1.97–1.30 (4H, m, H-6 and H-7), 1.35 (3H, s, H-17 or H-16), 1.28 (3H, s, H-16 or H-17), 1.25 (3H, s, H-19). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 205.24 (C, C-4), 202.15 (C, C-9), 195.31 (C, C-13), 169.30 (C, Ac), 151.55 (C, CO), 147.02 (C, C-11), 141.99 (C, C-12), 88.41 (C, C-1), 78.77 (CH, C-2), 76.18 (CH_2, C-20), 75.99 (CH, C-10), 72.55 (CH_2, C-5), 57.28 (C, C-8), 48.49 (CH, C-3), 41.41 (CH_2, C-15), 40.68 (C, C-14), 36.00 (CH_2, C-7), 31.26 (CH_3, C-17 or C-16), 21.93 (CH_2, C-6), 20.74 (CH_3, Ac), 18.34 (CH_3, C-16 or C-17), 16.96 (CH_3, C-19), 15.28 (CH_3, C-18). IR (film) ν_{max}: 2960, 1820, 1749, 1694, 1270, 1236, 1028. HRMS (MALDI-FTMS) calcd for $C_{23}H_{27}O_9Na$ (M+Na$^+$) 471.1631, found 471.1641.

3.1.2. Compound 11. A mixture of 9-*O*-acetyl-5-*O*-cinnamoyltaxicine 1,2-carbonate **9** and 10-*O*-acetyl-5-*O*-cinnamoyltaxicine 1,2-carbonate **10** (200 mg, 0.37 mmol) was dissolved in CH_2Cl_2 (6 mL) and treated with acetic anhydride (160 mg, 1.57 mmol), pyridine (80 mg, 1.01 mmol), and DMAP (5 mg, 0.04 mmol). The reaction mixture was stirred at rt for 2.5 h, diluted with CH_2Cl_2, washed with 1.5 N HCl and water, dried over anhyd $MgSO_4$, and evaporated under reduced pressure. Purification of the residue by dry flash chromatography (eluent: petroleum ether/ethyl acetate=7/3) afforded 9-*O*,10-*O*-diacetyl-5-*O*-cinnamoyltaxicine 1,2-carbonate **11** (210 mg, 93%) as a white amorphous solid (mp 233–235 °C). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.76–7.72 (2H, m), 7.67 (1H, d, J=16.0 Hz), 7.46–7.42 (3H, m), 6.31 (1H, d, J=15.9 Hz), 6.07 (1H, d, J=10.2 Hz), 5.77 (1H, d, J=10.2 Hz), 5.55 (1H, br s), 5.39 (1H, s), 5.37 (1H, s), 5.02 (1H, d, J=5.8 Hz), 3.40 (1H, d, J=5.5 Hz), 2.96 (2H, br s), 2.32 (3H, s), 2.11 (3H, s), 2.07 (3H, s), 2.10–1.60 (4H, m), 1.70 (3H, s), 1.35 (3H, s), 1.01 (3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 196.38 (C), 169.87 (C), 169.43 (C), 165.76 (C), 152.25 (C), 150.14 (C), 146.00 (CH), 141.96 (C), 139.49 (C), 134.22 (C), 130.42 (CH), 128.85 (CH), 128.31 (CH), 118.60 (CH_2), 117.16 (CH), 88.60 (C), 79.06 (CH), 76.93 (CH), 75.29 (CH), 72.74 (CH), 44.46 (C), 42.99 (CH), 40.93 (CH_2), 40.86 (C), 32.54 (CH_3), 27.26 (CH_2), 27.02 (CH_2), 20.60 (CH_3), 20.41 (CH_3), 19.94 (CH_3), 17.19 (CH_3), 14.24 (CH_3). IR (KBr) ν_{max}: 2998, 2958, 1818, 1750, 1714, 1686, 1638, 1374, 1310, 1270, 1202, 1163, 1032. HRMS (FAB) calcd for $C_{34}H_{39}O_{10}$ (MH$^+$) 607.2543, found 607.2579. $[\alpha]_D^{25}$ +251 (*c* 1.0, CH_2Cl_2).

3.1.3. Compound 13. To a solution of **11** (800 mg, 1.32 mmol) in a mixture of THF (19 mL) and water (9.5 mL) was added NMO (910 mg, 6.74 mmol), followed by osmium tetraoxide (1.27 mL, 2.5% solution in *t*-BuOH). The reaction mixture was stirred for 6 h at rt, then Florisil (900 mg), sodium dithionite (2.5 g), and water (10 mL) were added, and stirring was continued for 30 min. The reaction mixture was filtered and the filtrate was extracted with CH_2Cl_2. The organic extract was dried over anhyd $MgSO_4$ and concentrated under reduced pressure. Crude **12** (800 mg, 90%) was used in the next step without further purification.

To a solution of **12** (800 mg) in methanol (104 mL) was added methanolic KOAc (4.93 mL, 0.1 M solution) and the reaction mixture was stirred and heated to reflux for 15 min. The solvent was evaporated under reduced pressure and the residue was diluted with water, extracted with CH_2Cl_2, the extract was dried over anhyd $MgSO_4$ and concentrated under reduced pressure. Purification by dry flash chromatography (eluent: benzene/ethyl acetate=1/1) afforded compound **13** (490 mg, 81%) as a colorless film. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.04 (1H, d, J=10.4 Hz), 5.62 (1H, d, J=10.2 Hz), 4.86 (1H, d, J=4.6 Hz), 4.05 (1H, br s), 3.98 (1H, d, J=19.3 Hz), 3.98 (1H, d, J=10.6 Hz), 3.80–3.60 (1H, m), 3.65 (1H, br s), 3.54 (1H, d, J=10.6 Hz), 3.01 (1H, br s), 2.93 (1H, d, J=4.6 Hz), 2.81 (1H, d, J=19.2 Hz), 2.21 (3H, s), 2.10 (3H, s), 2.04 (3H, s), 1.97–1.48 (4H, m), 1.67 (3H, s), 1.32 (3H, s), 0.89 (3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 198.07 (C), 170.33 (C), 169.36 (C), 153.38 (C), 148.73 (C), 143.25 (C), 89.09 (C), 80.20 (CH), 75.36 (CH), 74.85 (C), 72.58 (CH), 70.21 (CH), 62.34 (CH_2), 43.50 (C), 41.59 (CH), 41.39 (C), 40.93 (CH_2), 32.50 (CH_3), 24.40 (CH_2), 23.58 (CH_2), 20.69 (CH_3), 20.56 (CH_3), 20.03 (CH_3), 18.63 (CH_3), 13.80 (CH_3). IR (film) ν_{max}: 3611, 3477, 2995, 2960, 1808, 1748, 1688, 1474, 1401, 1375, 1272, 1238, 1025. HRMS (FAB) calcd for $C_{25}H_{35}O_{11}$ (MH$^+$) 511.2179, found 511.2170. $[\alpha]_D^{25}$ +178 (*c* 1.0, CH_2Cl_2).

3.1.4. Compound 14. A solution of **13** (470 mg, 0.92 mmol), triethylamine (540 mg, 5.35 mmol), DMAP (15 mg, 0.12 mmol), and TBDMSCl (660 mg, 4.41 mmol) in CH_2Cl_2 (13 mL) was stirred for 5 days at rt under an argon atmosphere. The reaction mixture was diluted with CH_2Cl_2, washed with ice-cold satd aq NH_4Cl and water, and dried over anhyd $MgSO_4$. The solvent removal under reduced pressure followed by purification by dry flash chromatography (eluent: benzene/ethyl acetate=8/2) afforded silyl

ether **14** (523 mg, 91%) as a white foam. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.05 (1H, d, J=10.3 Hz), 5.62 (1H, d, J=10.3 Hz), 4.81 (1H, d, J=4.7 Hz), 4.01 (1H, d, J=19.4 Hz), 3.98 (1H, d, J=9.4 Hz), 3.94 (1H, br s), 3.56 (1H, br s), 3.49 (1H, d, J=9.2 Hz), 2.95 (1H, d, J=4.5 Hz), 2.79 (1H, d, J=19.2 Hz), 2.70 (1H, br s), 2.22 (3H, s), 2.09 (3H, s), 2.04 (3H, s), 2.00–1.50 (4H, m), 1.65 (3H, s), 1.31 (3H, s), 0.90 (9H, s), 0.86 (3H, s), 0.14 (3H, s), 0.10 (3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 197.96 (C), 170.24 (C), 169.29 (C), 152.70 (C), 148.57 (C), 143.27 (C), 88.45 (C), 79.80 (CH), 75.38 (CH), 74.76 (C), 72.56 (CH), 70.01 (CH), 63.29 (CH_2), 43.48 (C), 41.41 (C), 41.11 (CH), 40.99 (CH_2), 32.48 (CH_3), 25.69 (CH_3), 24.44 (CH_2), 23.49 (CH_2), 20.69 (CH_3), 20.54 (CH_3), 20.01 (CH_3), 18.59 (CH_3), 18.12 (C), 13.82 (CH_3), −5.58 (CH_3). IR (film) ν_{max}: 3479, 2956, 2934, 1813, 1750, 1690, 1470, 1373, 1237, 1091, 1023. HRMS (FAB) calcd for $C_{31}H_{49}O_{11}Si$ (MH^+) 625.3044, found 625.3028.

3.1.5. Compound 15. To a cold (0 °C) solution of **14** (40 mg, 0.064 mmol) in pyridine (2 mL) was added mesyl chloride (110 mg, 0.96 mmol). The reaction mixture was stirred at 0 °C for 15 min and then for 24 h at rt. The reaction mixture was diluted with CH_2Cl_2, washed successively with ice-cold 2.5% HCl, aq $NaHCO_3$, and water, and dried over anhyd $MgSO_4$. After removal of the solvent under reduced pressure, the residue was purified by dry flash chromatography (eluent: benzene/ethyl acetate=8/2) to give mesylate **15** (34 mg, 77%) as a colorless film. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.04 (1H, d, J=10.4 Hz), 5.64 (1H, d, J=10.2 Hz), 4.79 (1H, d, J=4.6 Hz), 4.75 (1H, br s), 4.01 (1H, d, J=19.1 Hz), 3.99 (1H, d, J=9.8 Hz), 3.92 (1H, s), 3.54 (1H, d, J=9.7 Hz), 2.96 (3H, s), 2.89 (1H, d, J=4.9 Hz), 2.83 (1H, d, J=19.9 Hz), 2.27 (3H, s), 2.11 (3H, s), 2.10–1.50 (4H, m), 2.05 (3H, s), 1.65 (3H, s), 1.33 (3H, s), 0.92 (9H, s), 0.90 (3H, s), 0.16 (3H, s), 0.12 (3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 197.56 (C), 170.20 (C), 169.22 (C), 152.43 (C), 149.52 (C), 143.44 (C), 88.36 (C), 81.22 (CH), 79.24 (CH), 75.20 (CH), 73.87 (C), 72.63 (CH), 63.18 (CH_2), 43.46 (C), 42.61 (CH), 41.48 (C), 41.01 (CH_2), 38.66 (CH_3), 32.67 (CH_3), 25.68 (CH_3), 25.13 (CH_2), 25.06 (CH_2), 20.65 (CH_3), 20.54 (CH_3), 19.99 (CH_3), 18.99 (CH_3), 18.06 (C), 13.77 (CH_3), −5.51 (CH_3), −5.57 (CH_3). IR (film) ν_{max}: 3481, 2957, 2935, 1815, 1750, 1688, 1472, 1370, 1265, 1235, 1174, 1094, 1025. HRMS (FAB) calcd for $C_{32}H_{51}O_{13}SSi$ (MH^+) 703.2820, found 703.2814.

3.1.6. Compound 16. To a solution of **15** (320 mg, 0.45 mmol) in THF (27 mL) was added a solution of tetrabutylammonium fluoride trihydrate (156 mg, 0.49 mmol) in THF (3 mL). The reaction mixture was stirred at rt for 5 min and ethyl acetate was added. The resulting solution was washed with aq $NaHCO_3$ and water and dried over anhyd $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by dry flash chromatography (eluent: benzene/ethyl acetate=6/4) to give **16** (216 mg, 83%) as a colorless film. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.03 (1H, d, J=10.2 Hz), 5.63 (1H, d, J=10.4 Hz), 4.87 (1H, d, J=4.4 Hz), 4.79 (1H, br s), 4.11 (1H, br s), 3.99 (1H, d, J=19.3 Hz), 3.99 (1H, d, J=11.3 Hz), 3.85 (1H, br s), 3.62 (1H, d, J=10.9 Hz), 3.00 (3H, s), 2.85 (1H, d, J=4.4 Hz), 2.83 (1H, d, J=19.3 Hz), 2.25 (3H, s), 2.11 (3H, s), 2.05 (3H, s), 2.10–1.65 (4H, m), 1.66 (3H, s), 1.33 (3H, s), 0.92 (3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 197.64 (C), 170.29 (C), 169.31 (C), 153.12 (C), 149.68 (C), 143.33 (C), 88.94 (C), 81.75 (CH), 79.57 (CH), 75.20 (CH), 73.85 (C), 72.63 (CH), 62.16 (CH_2), 43.44 (C), 43.01 (CH), 41.44 (C), 40.91 (CH_2), 38.60 (CH_3), 32.66 (CH_3), 24.93 (2×CH_2), 20.63 (CH_3), 20.54 (CH_3), 19.96 (CH_3), 18.90 (CH_3), 13.75 (CH_3). IR (film) ν_{max}: 3563, 3455, 2967, 2934, 1806, 1750, 1685, 1414, 1375, 1346, 1273, 1238, 1033. HRMS (FAB) calcd for $C_{26}H_{37}O_{13}S$ (MH^+) 589.1955, found 589.1998. $[\alpha]_D^{25}$ +152 (*c* 1.0, ethyl acetate).

3.1.7. Compound 17. A solution of **16** (62 mg, 0.1 mmol) and DIPEA (95 mg, 0.74 mmol) in anhyd toluene (15.2 mL) was heated to reflux with stirring for 24 h. The solvent was removed under reduced pressure and the residue was purified by dry flash chromatography (eluent: benzene/ethyl acetate=6/4) to give compound **17** (47 mg, 90%) as a colorless film. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.96 (1H, d, J=10.4 Hz), 5.73 (1H, d, J=10.6 Hz), 4.94 (1H, d, J=5.1 Hz), 4.81–4.76 (1H, m), 4.64 (1H, d, J=9.0 Hz), 4.47 (1H, d, J=9.2 Hz), 3.73 (1H, d, J=19.1 Hz), 3.12 (1H, s), 2.80 (1H, d, J=19.3 Hz), 2.19 (1H, d, J=5.3 Hz), 2.14 (3H, s), 2.11 (3H, s), 2.40–1.60 (4H, m), 2.06 (3H, s), 1.70 (3H, s), 1.43 (3H, s), 1.32 (3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 197.47 (C), 170.22 (C), 169.42 (C), 152.92 (C), 149.53 (C), 142.52 (C), 88.42 (C), 87.83 (CH), 80.00 (CH_2), 79.42 (CH), 75.25 (CH), 74.52 (C), 72.67 (CH), 46.56 (CH), 42.68 (C), 41.35 (C), 40.71 (CH_2), 32.41 (CH_3), 27.82 (CH_2), 26.40 (CH_2), 20.70 (CH_3), 20.60 (CH_3), 20.05 (CH_3), 16.32 (CH_3),13.93 (CH_3). IR (film) ν_{max}: 3479, 2993, 2965, 1812, 1750, 1690, 1375, 1271, 1238, 1022. HRMS (FAB) calcd for $C_{25}H_{33}O_{10}$ (MH^+) 493.2074, found 493.2317. $[\alpha]_D^{25}$ +147 (*c* 1.0, ethyl acetate).

3.1.8. Compound 18. To a solution of **11** (300 mg, 0.49 mmol) in a mixture of THF, EtOH, and water (1:1:1, 54 mL) were added hydroxylamine hydrochloride (170 mg, 2.47 mmol) and triethylamine (250 mg, 2.47 mmol). The solution was stirred and heated at 80 °C for 30 h. The reaction mixture was diluted with CH_2Cl_2 and water, the organic layer was separated and dried over anhyd $MgSO_4$. After evaporation of the solvent under reduced pressure, the residue was purified by dry flash chromatography (eluent: petroleum ether/ethyl acetate=6/4) to give compound **18** (120 mg, 50%) as a colorless film. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.11 (1H, d, J=10.2 Hz), 5.71 (1H, d, J=10.2 Hz), 5.39 (1H, br s), 5.18 (1H, br s), 4.95 (1H, d, J=5.7 Hz), 4.20 (1H, br s), 3.61 (1H, d, J=5.5 Hz), 2.90 (2H, br s), 2.25 (3H, s), 2.10 (3H, s), 2.07 (3H, s), 1.85–1.60 (4H, m), 1.68 (3H, s), 1.32 (3H, s), 0.96 (3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 196.86 (C), 170.09 (C), 169.62 (C), 152.74 (C), 148.74 (C), 144.79 (C), 142.56 (C), 114.87 (CH_2), 88.78 (C), 79.71 (CH), 75.52 (CH), 74.49 (CH), 72.63 (CH), 44.72 (2×C), 40.80 (CH and CH_2), 32.41 (CH_3), 29.42 (CH_2), 26.16 (CH_2), 20.63 (CH_3), 20.45 (CH_3), 19.96 (CH_3), 17.04 (CH_3), 14.28 (CH_3). IR (KBr) ν_{max}: 2998, 2958, 1818, 1750, 1714, 1686, 1638, 1374, 1310, 1270, 1202, 1163, 1032. HRMS (FAB) calcd for $C_{25}H_{32}O_9Na$ (MNa^+) 499.1944, found 499.1930. $[\alpha]_D^{25}$ +179 (*c* 1.1, CH_2Cl_2).

3.1.9. Compound 19. In a Pyrex, external water-cooled reactor, to a deaerated suspension of **17** (96 mg, 0.195 mmol), silver acetate (130 mg, 0.778 mmol), and benzene (8.8 mL) was added a solution of bromine (62 mg, 0.387 mmol) in benzene (0.7 mL). The reaction mixture was irradiated for 5 min with a 250 W Xenophot sun-lamp focalized light, with stirring under an argon atmosphere. The reaction mixture was filtered and diluted with CH_2Cl_2, washed successively with 5% $Na_2S_2O_3$, aq $NaHCO_3$, and water, and dried over anhyd $MgSO_4$. The solvent was removed under reduced pressure and the crude product was purified by dry flash chromatography (eluent: benzene/ethyl acetate=7/3) to give acetate **19** (β/α=10.5/1) (65 mg, 60%) as a colorless film. Spectroscopic data for major isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.08 (1H, d, J=10.5 Hz, H-10), 5.96–5.94 (1H, m, H-5), 5.75 (1H, d, J=10.5 Hz, H-9), 4.94 (1H, d, J=7.1 Hz, H-2), 4.16 (1H, d, J=17.6 Hz, H-20), 3.95 (1H, d, J=7.1 Hz, H-3), 3.74 (1H, d, J=17.6 Hz, H-20), 2.98 (1H, d, J=19.4 Hz, H-14), 2.73 (1H, d, J=19.4 Hz, H-14), 2.29 (3H, s, H-18), 2.15–2.05 (1H, m, H-7), 2.13 (3H, s, Ac), 2.09 (3H, s, Ac), 2.08 (3H, s, Ac), 1.85–1.72 (1H, m, H-6), 1.70 (3H, s, H-16 or H-17), 1.60–1.50 (1H, m, H-7), 1.50–1.40 (1H, m, H-6), 1.34 (3H, s, H-17 or H-16), 1.02 (3H, s, H-19). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 204.94 (C, C-4), 195.06 (C, C-13), 169.65 (C, Ac), 169.33 (C, Ac), 168.82 (C, Ac), 151.60 (C, CO), 148.75 (C, C-11), 142.09 (C, C-12), 95.71 (CH, C-5), 88.49 (C, C-1), 77.37 (CH, C-2), 74.54 (CH, C-9), 71.99 (CH, C-10), 69.12 (CH_2, C-20), 47.52 (CH, C-3), 45.76 (C, C-8), 40.99 (CH_2, C-14), 40.82 (C, C-15), 32.30 (CH_3, C-16 or C-17), 28.73 (CH_2, C-7), 24.98 (CH_2, C-6), 20.91 (CH_3, Ac), 20.71 (CH_3, Ac), 20.52 (CH_3, Ac), 20.09 (CH_3, C-17 or C-16), 18.25 (CH_3, C-19), 14.94 (CH_3, C-18). IR (film) ν_{max}: 2998, 1821, 1750, 1693, 1375, 1235, 1025. HRMS: $C_{27}H_{34}O_{12}Na$ 573.1948, found 573.1959.

3.1.10. Compound 22. In a Pyrex, external water-cooled reactor, to a deaerated suspension of **17** (220 mg, 0.447 mmol), silver acetate (112 mg, 0.670 mmol), and benzene (21 mL) was added a solution of bromine (143 mg, 0.894 mmol) in benzene (1.9 mL). The reaction mixture was irradiated for 5 min with a 250 W Xenophot sun-lamp focalized light, with stirring under an argon atmosphere. The reaction mixture was filtered and diluted with CH_2Cl_2, washed successively with 5% $Na_2S_2O_3$, aq $NaHCO_3$, and water, and dried over anhyd $MgSO_4$. After concentration under reduced pressure, the crude **21** (260 mg, equimolar mixture of diastereoisomers) was used in the next step without further purification.

In a Pyrex, external water-cooled reactor, a deaerated solution of bromides **21** (260 mg, 0.447 mmol), TBTH (1.3 g, 4.47 mmol), AIBN, and benzene (8 mL) was irradiated for 15 min with a 250 W Xenophot sun-lamp focalized light, with stirring under an argon atmosphere. After removal of the solvent under reduced pressure, the crude product was purified by dry flash chromatography (eluent: benzene/ethyl acetate=8/2) to give compound **22** (135 mg, 61%) as a colorless film. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.10 (1H, d, J=10.5 Hz, H-10), 5.74 (1H, d, J=10.5 Hz, H-9), 4.94 (1H, d, J=7.0 Hz, H-2), 4.06 (1H, d, J=7.0 Hz, H-3), 3.91 (1H, d, J=17.2 Hz, H-20), 3.78–3.64 (1H, m, H-5), 3.59–3.52 (1H, m, H-5), 3.48 (1H, d, J=17.2 Hz, H-20), 2.98 (1H, d, J=19.3 Hz, H-14), 2.73 (1H, d, J=19.4 Hz, H-14), 2.26 (3H, s, H-18), 2.12 (3H, s, Ac), 2.25–2.10 (1H, m, H-7), 2.07 (3H, s, Ac), 1.69 (3H, s, H-16 or H-17), 1.72–1.30 (3H, m, H-6 and H-7), 1.33 (3H, s, H-17 or H-16), 0.99 (3H, s, H-19). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 207.14 (C, C-4), 195.49 (C, C-13), 169.80 (C, Ac), 169.38 (C, Ac), 151.81 (C, CO), 148.93 (C, C-11), 141.91 (C, C-12), 88.45 (C, C-1), 77.38 (CH, C-2), 75.91 (CH_2, C-20), 74.80 (CH, C-9), 72.47 (CH_2, C-5), 71.97 (CH, C-10), 46.90 (CH, C-3), 44.97 (C, C-8), 40.82 (CH_2, C-14), 40.70 (C, C-15), 32.19 (CH_3, C-16 or C-17), 31.63 (CH_2, C-7), 21.20 (CH_2, C-6), 20.56 (CH_3, Ac), 20.32 (CH_3, Ac), 19.90 (CH_3, C-17 or C-16), 17.83 (CH_3, C-19), 14.66 (CH_3, C-18). IR (film) ν_{max}: 2958, 1818, 1747, 1692, 1373, 1268, 1234, 1026. HRMS (MALDI-FTMS) calcd for $C_{25}H_{32}O_{10}Na$ (M+Na$^+$) 515.1888, found 515.1885. $[\alpha]_D^{25}$ +180 (*c* 1.0, ethyl acetate).

3.1.11. Compound 23. A cold (−80 °C) solution of **22** (70 mg, 0.142 mmol) in THF (15 mL) was treated with PhLi (240 mg, 1.5 mL of a 1.9 M solution in toluene) and stirred at −80 °C for 6 h under an argon atmosphere. The reaction mixture was quenched with satd aq NH_4Cl (10 mL) and then allowed to warm to rt. After dilution with CH_2Cl_2, the organic layer was separated, dried over anhyd $MgSO_4$, and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (6 mL) and acetic anhydride (147 mg, 1.42 mmol) and DMAP (87 mg, 0.71 mmol) was added. The mixture was stirred for 30 min at rt and diluted with CH_2Cl_2. The resulting solution was washed with satd aq $NaHCO_3$, 1.5 M HCl and water, and dried over anhyd $MgSO_4$. After removal of the solvent under reduced pressure the residue was purified by column chromatography (eluent: petroleum ether/ethyl acetate=7/4) to give benzoate **23** (47 mg, 58%, 62% based on recovered carbonate **22**) as a colorless film, followed by the starting compound **22** (4 mg). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.86–7.82 (2H, m), 7.61–7.53 (1H, m), 7.47–7.39 (2H, m), 6.20 (1H, d, J=10.5 Hz), 6.01 (1H, d, J=10.6 Hz), 5.88 (1H, d, J=7.7 Hz), 4.32 (1H, d, J=7.7 Hz), 3.87 (1H, d, J=16.0 Hz), 3.78–3.64 (1H, m), 3.53–3.45 (1H, m), 3.37 (1H, d, J=16.1 Hz), 2.94 (1H, d, J=19.6 Hz), 2.80 (1H, d, J=19.4 Hz), 2.26 (3H, s), 2.11 (3H, s), 2.09 (3H, s), 2.05–1.45 (4H, m), 1.76 (3H, s), 1.27 (3H, s), 1.00 (3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 211.00 (C), 198.55 (C), 169.73 (C), 169.62 (C), 165.14 (C), 151.45 (C), 140.03 (C), 133.28 (CH), 129.53 (C), 129.47 (CH), 128.45 (CH), 77.98 (C), 77.36 (CH_2), 74.71 (CH), 72.72 (CH_2), 72.21 (CH), 72.17 (CH), 50.84 (CH), 44.17 (CH_2 and C), 42.50 (C), 33.83 (CH_3), 31.45 (CH_2), 21.54 (CH_2), 20.78 (CH_3), 20.54 (CH_3), 20.01 (CH_3), 17.88 (CH_3), 13.99 (CH_3). IR (film) ν_{max}: 3497, 2986, 1746, 1680, 1451, 1374, 1269, 1231, 1096, 1026. HRMS (TOF MS ES+) calcd for $C_{31}H_{39}O_{10}$ (MH$^+$) 571.2543, found 571.2548. $[\alpha]_D^{25}$ +112 (*c* 1.0, ethyl acetate).

3.1.12. Compound 24. A solution of **23** (40 mg, 0.070 mmol) in methanol (5.7 mL) was treated with excess of $NaBH_4$ (53 mg, 1.40 mmol) for 1 h at rt. The reaction was quenched with satd aq NH_4Cl and the resulting mixture was stirred for 10 min. After dilution with water, the reaction mixture was extracted twice with CH_2Cl_2 and dried over anhyd $MgSO_4$. Concentration under reduced pressure, followed by purification of the residue by column chromatography (eluent: benzene/ethyl acetate=6/4) gave alcohol

24 (29 mg, 72%) as a white amorphous solid (mp 139–141 °C). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.87–7.83 (2H, m), 7.59–7.51 (1H, m), 7.45–7.37 (2H, m), 6.11 (1H, d, J=10.4 Hz), 5.89 (1H, d, J=10.4 Hz), 5.76 (1H, d, J=7.1 Hz), 4.73–4.61 (1H, m), 4.40 (1H, d, J=6.9 Hz), 4.07 (1H, d, J=16.0 Hz), 3.95–3.77 (1H, m), 3.69–3.55 (1H, m), 3.47 (1H, d, J=16.2 Hz), 3.14 (1H, d, J=10.9 Hz), 2.63 (1H, dd, J_1=15.8 Hz, J_2=10.4 Hz), 2.29 (3H, s), 2.23–2.16 (1H, m), 2.10–1.28 (4H, m), 2.07 (3H, s), 2.05 (3H, s), 1.66 (3H, s), 1.07 (3H, s), 0.99 (3H, s). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 210.69 (C), 169.96 (C), 169.76 (C), 165.19 (C), 142.73 (C), 133.99 (C), 133.17 (CH), 129.85 (C), 129.51 (CH), 128.45 (CH), 77.09 (C), 76.49 (CH_2), 75.85 (CH), 73.56 (CH_2), 72.88 (CH), 72.41 (CH), 69.35 (CH), 51.11 (CH), 43.95 (C), 41.92 (CH_2), 41.53 (C), 31.87 (CH_2), 28.98 (CH_3), 21.62 (CH_2), 20.96 (CH_3), 20.61 (CH_3), 20.38 (CH_3), 17.94 (CH_3), 16.74 (CH_3). IR (film) ν_{max}: 3474, 2932, 2875, 1737, 1630, 1452, 1374, 1244, 1094, 1024. HRMS (TOF MS ES+) calcd for $C_{31}H_{40}O_{10}Na$ ($M+Na^+$) 595.2519, found 595.2493. $[\alpha]_D^{25}$ +41 (*c* 1.0, ethyl acetate).

3.1.13. Compound 26. To a solution of carboxylic acid **25** (73.0 mg, 0.181 mmol), alcohol **24** (26.0 mg, 0.045 mmol) and DMAP (22.2 mg, 0.181 mmol) in toluene (11.0 mL) was added DCC (37.4 mg, 0.181 mmol) in toluene (1.4 mL), at rt, under an argon atmosphere. The reaction mixture was stirred at 75 °C for 30 min. After filtration and removal of the solvent under reduced pressure, the product was roughly purified by short column chromatography (eluent: benzene/ethyl acetate=7/3) to give the protected ester (41.3 mg, 93%).

Acidic deprotection: the product from the previous step was treated with 5% methanolic *p*-TsOH (7.0 mL) at rt for 30 min. The reaction mixture was diluted with ethyl acetate, washed successively with aq $NaHCO_3$ and brine, dried over anhyd $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography (eluent: hexane/ethyl acetate=1/1) to give ester **26** (26.9 mg, 75%) as a colorless film.

Deprotection with CAN: to a stirred solution of protected compound (15 mg, 0.015 mmol) in acetonitrile (0.9 mL) was added a solution of CAN (25.2 mg, 0.046 mmol) in water (0.9 mL). The resulting yellow mixture was stirred at 40 °C for 2.5 h, diluted with CH_2Cl_2, organic layer was separated and dried over anhyd $MgSO_4$. The residue was purified by column chromatography (eluent: hexane/ethyl acetate=1/1) to give compound **26** (8.7 mg, 66%) as a colorless film. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.03 (2H, dd, J_1=8.0 Hz, J_2=1.0 Hz, Ar), 7.81 (2H, dd, J_1=8.5 Hz, J_2=1.5 Hz, Ar), 7.54–7.48 (4H, m, Ar), 7.43 (2H, t, J=7.5 Hz, Ar), 7.40–7.30 (5H, m, Ar), 7.25 (1H, d superimposed with $CDCl_3$, J=9.5 Hz, N–H), 6.07 (1H, d, J=10.5 Hz, H-10), 6.06–6.03 (1H, m, H-13), 5.93 (1H, d, J=9.5 Hz, H-3′), 5.93 (1H, d, J=10.5 Hz, H-9), 5.79 (1H, d, J=7.5 Hz, H-2), 4.73 (1H, br s, H-2′), 4.65 (1H, d, J=16.0 Hz, H-20), 4.41 (1H, d, J=7.5 Hz, H-3), 3.88 (1H, br s, OH), 3.86–3.78 (1H, m, H-5), 3.68–3.65 (1H, m, H-5), 3.48 (1H, d, J=16.0 Hz, H-20), 2.46 (1H, dd, J_1=16.0 Hz, J_2=9.5 Hz, H-14), 2.39 (1H, dd, J_1=16.0 Hz, J_2=5.0 Hz, H-14), 2.15–2.00 (1H, m, H-7), 2.08 (3H, s, Ac), 2.05 (3H, s, Ac), 1.96 (3H, s, H-18), 1.70 (3H, s, H-17 or H-16), 1.65–1.50 (1H, m, H-7), 1.40–1.20 (2H, m, H-6), 1.13 (3H, s, H-16 or H-17), 0.99 (3H, s, H-19). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 211.21 (C, C-4), 171.79 (C-1′), 169.93 (C, Ac), 169.75 (C, Ac), 166.51 (C, Ar), 165.51 (C, Ar), 138.70 (C, Ar), 137.99 (C, C-12), 135.39 (C, C-11), 134.12 (C, Ar), 133.10 (CH, Ar), 131.79 (CH, Ar), 129.95 (CH, Ar), 129.67 (C, Ar), 128.69 (CH, Ar), 128.65 (CH, Ar), 128.40 (CH, Ar), 127.94 (CH, Ar), 127.00 (CH, Ar), 126.92 (CH, Ar), 77.31 (CH_2, C-20), 75.47 (CH, C-9), 74.09 (CH, C-2′), 73.07 (CH, C-2), 72.87 (CH_2, C-5), 72.32 (CH, C-13), 71.68 (CH, C-10), 54.20 (CH, C-3′), 51.55 (CH, C-3), 44.15 (C, C-8), 41.53 (C, C-15), 36.72 (CH_2, C-14), 31.24 (CH_2, C-7), 28.91 (CH_3, C-16 or C-17), 21.61 (CH_2, C-6), 20.94 (CH_3, Ac), 20.63 (2×CH_3, Ac and C-17 or C-16), 17.94 (CH_3, C-19), 16.04 (CH_3, C-18). The signal corresponding to C-1 could not be detected under the recording conditions. IR (film) ν_{max}: 3443, 2931, 1737, 1662, 1517, 1487, 1452, 1373, 1243, 1096, 1025. HRMS (ESI-TOF high acc) calcd for $C_{47}H_{54}NO_{13}$ (MH^+) 840.3589, found 840.3575. $[\alpha]_D^{25}$ +95 (*c* 1.6, ethyl acetate).

3.1.14. Spectroscopic data for 27. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.01 (2H, dd, J_1=7.0 Hz, J_2=1.5 Hz, Ar), 7.78–7.75 (2H, m, Ar), 7.54–7.50 (3H, m, Ar), 7.46–7.36 (4H, m, Ar), 7.34–7.29 (2H, m, Ar), 7.20–7.17 (2H, m, Ar), 6.95 (1H, d, J=9.5 Hz, N–H), 6.41 (1H, d, J=9.5 Hz, H-2), 6.19 (1H, d, J=10.0 Hz, H-10), 5.89–5.87 (1H, m, H-3′), 5.88 (1H, d, J=10.0 Hz, H-9), 5.79 (1H, br t, J=6.5 Hz, H-13), 4.69 (1H, d, J=2.0 Hz, H-2′), 4.38 (1H, d, J=17.0 Hz, H-20), 4.34 (1H, d, J=9.0 Hz, H-3), 3.83–3.77 (1H, m, H-5), 3.60–3.57 (1H, m, H-5), 3.37 (1H, d, J=17.5 Hz, H-20), 2.52 (1H, dd, J_1=15.5 Hz, J_2=6.0 Hz, H-14), 2.44 (1H, dd, J_1=15.5 Hz, J_2=7.5 Hz, H-14), 2.08–2.03 (1H, m, H-7), 2.05 (3H, s, Ac), 2.03 (3H, s, Ac), 1.87 (3H, s, H-18), 1.65–1.20 (3H, m, H-7 and H-6), 1.13 (3H, s, H-17 or H-16), 1.11 (3H, s, H-16 or H-17), 1.05 (3H, s, H-19). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 212.12 (C, C-4), 172.51 (C-1′), 169.51 (C, Ac), 168.64 (C, Ac), 166.68 (C, Ar), 165.09 (C, Ar), 145.82 (C, C-11), 139.28 (C, Ar), 137.51 (C, C-12), 135.00 (C, Ar), 132.63 (CH, Ar), 131.65 (CH, Ar), 130.29 (C, Ar), 129.75 (CH, Ar), 128.78 (CH, Ar), 128.33 (CH, Ar), 128.00 (CH, Ar), 127.11 (CH, Ar), 127.06 (CH, Ar), 127.04 (CH, Ar), 81.68 (CH, C-13), 76.34 (CH, C-9), 76.17 (CH_2, C-20), 74.97 (C, C-15), 73.49 (CH, C-2′), 72.58 (CH_2, C-5), 69.11 (CH, C-10), 68.85 (C, C-1), 67.95 (CH, C-2), 54.38 (CH, C-3′), 50.28 (CH, C-3), 42.28 (C, C-8), 36.46 (CH_2, C-14), 32.36 (CH_2, C-7), 27.48 (CH_3, C-16 or C-17), 25.25 (CH_3, C-16 or C-17), 21.82 (CH_2, C-6), 20.86 (CH_3, Ac), 20.68 (CH_3, Ac), 18.14 (CH_3, C-19), 12.05 (CH_3, C-18). IR (film) ν_{max}: 3450, 2921, 1730, 1663, 1517, 1486, 1454, 1372, 1233, 1100, 1030. HRMS (ESI-TOF high acc) calcd for $C_{47}H_{53}NO_{13}Na$ (MH^+) 862.3409, found 862.3416. $[\alpha]_D^{25}$ −1.9 (*c* 0.53, ethyl acetate).

3.2. Biological assays

Tubulin test was performed according to the described procedure.[30]

3.2.1. Cell culture and determination of cell viability. The cells of the rat glioma cell line C6 (ATCC) were seeded in

96-well flat-bottom plates (2×10^4 cells/well) and incubated with paclitaxel or compound **26** for 48 h. The cells were cultivated at 37 °C in a humidified atmosphere with 5% CO_2, in a HEPES-buffered RPMI 1640 cell culture medium supplemented with 5% fetal calf serum, 2 mmol L-glutamine, 50 μmol 2-mercaptoethanol, 10 mmol sodium pyruvate and penicillin/streptomycin (all from Sigma, St. Louis, MO). Cell viability was analyzed by staining the viable cells with crystal violet, or by measuring the mitochondrial-dependent reduction of 3-[4,5-dimethyl thiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) to formazan. Both crystal violet and MTT test were performed exactly as previously described,[31] and the results were presented as % viability relative to untreated control.

3.3. Modeling

All calculations including energy minimization, conformational searches, and Glide Docking were performed in Maestro (Schördinger Inc.).[22] With the exception of the free radical intermediate **30**, 10,000-step MMFF/GBSA/H_2O conformational searches were performed for taxane analogs within 7.0 kcal/mol of the global minimum. For C-5 of the C–O–C•–C radical unit of intermediate **30** (Scheme 3 and Fig. 2), a set of provisional force field parameters were employed in a 10,000-step gas phase MM3* conformational search.

A local modification of the refined electron crystallographic complex of PTX/β-tubulin[32] was made prior to docking. Thus, Arg284 on the M-loop was relocated from the electron crystallographic position so as to form a hydrogen bond with PTX's C-10 acetyl group. Without this modification, the Glide method (Schrödinger Inc.)[34,22] docks the taxane structures in an inverted binding pose that directs the two C-13 side chain phenyl rings out into solvent instead of deep within the hydrophobic pocket. With the M-loop modified protein, however, Glide is able to accurately predict the binding mode for PTX conformation as observed in the refined electron crystallographic complex.[32] Accordingly, taxane analogs **22** and **26** were constructed with the C-13 T-taxane geometry and the *seco*-C,D ring conformer illustrated in Figure 3 followed by rigid Glide Docking into the complex. The best docking poses were chosen on the basis of the Emodel scoring function together with visualization to ensure reasonable binding modes.

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21. As MM3* does not incorporate force field parameters for the C–O–C$^{\bullet}$–C radical unit, we employed a set of generic parameters.
22. http://www.schrodinger.com/Products/macromodel.html.
23. The two lowest energy conformations of **30** (Scheme 3) fall within a 0.9 kcal/mol energy window. Both faces of C-5 are open to attack by Br$^{\bullet}$ within the two conformers.
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Access to a new family of medium ring aromatic lactones

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Abstract—We report a new method for preparation of hydroxyacids as precursors of benzolactones using a simple and an efficient electrochemical step. This gives in only four steps six- to eleven-membered lactones with high isolated yields from conveniently substituted aryl bromides. The lactonisation was performed according to the Yamamoto's process.

1. Introduction

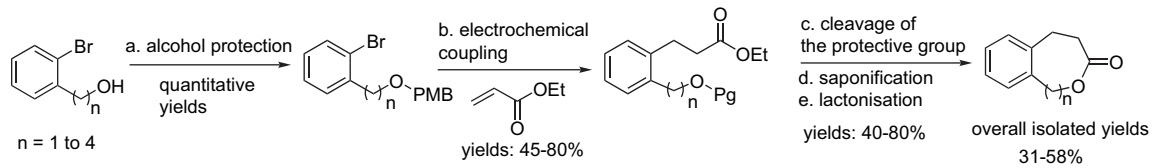
The development of new methods for the construction of hetero- and carbo-cyclic medium ring systems has been a long standing goal to organic chemists. Among the targeted structures, medium ring lactones are of interest as backbones of many bioactive compounds like antibiotics.[1] Their access is staying difficult despite number of cyclisation methods.[2] Our current investigations have focused on the preparation of medium ring benzolactones, which are core structures of compounds like Salicylihalamide A, a potential anticancer drug.[3] As a general feature, these benzolactones have a non-conjugated carbonyl, and therefore, are not obtainable through conventional methods. We have already reported a method for preparation of the precursor hydroxyacid, using an efficient electrochemical C–C bond forming step (Scheme 1).[4]

2. Results and discussion

In this approach, the starting material bears the alcohol function, while the carboxylic group is introduced through the electrochemical step. This method was used to prepare seven- to ten-membered benzolactones in good yields. However, this strategy requires five steps from the alcohol, which may also be eventually commercially unavailable, and including the protection–deprotection of the alcohol (respectively, steps **a** and **c** in Scheme 1).

To improve the efficiency of the synthesis, we thought of avoiding the alcohol protection step. This can be done by having a carbonyl group as an alcohol precursor. Based on this, the carbonyl group should be first introduced, that requires it being compatible with the electrochemical step, or, alternatively, introduced through the electrochemical step conducted on a starting compound bearing a carboxylic group. These two pathways have been explored and compared in this study. The first run involved 2-bromophenylacetone as the starting material in the electrochemical coupling with ethyl acrylate. As shown in Scheme 2, the unexpected bicyclic compound **1** was only formed as a result of a tandem reaction involving the favoured nucleophilic addition to the carbonyl (Scheme 2). Such a ring forming tandem reaction has already been observed,[5] and cannot be avoided whatever the reaction parameters are.

In view of this result, we chose to reverse the order of insertion of the functional groups, i.e., first the carboxylic group by a chemical step if the starting compound is not commercially available, then the carbonyl in the electrochemical coupling. We checked this route with the ester **2** and methylvinylketone (MVK) (Scheme 3). We obtained the formation of the products **3** and **4**. However, the reduction product **4** is



Scheme 1.

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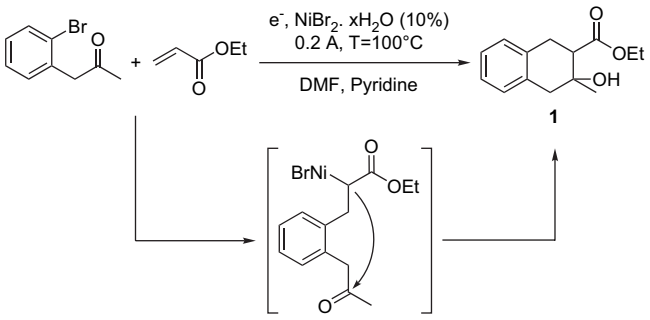


Scheme 2.

the major isolated compound (43%). Interestingly, no cyclisation has occurred.

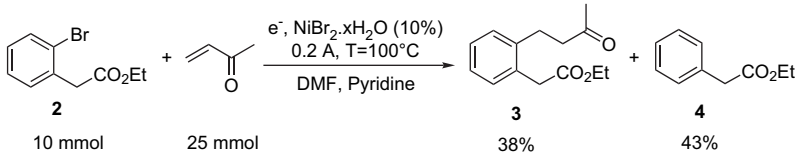
Compound **5**, which was prepared by a Wittig reaction, was next involved in the electrochemical coupling with MVK. The purpose for this study was to check whether the conjugated double bond would react or not in the reaction conditions, for the reason that, it could be necessary to keep it as such to have it in the final compound, or simply not to have it reduced in the early stage, but later along with the ketone group (see below). Actually, this reaction ended with the formation of a 1/1 mixture (GC) of **6** and **7** (not isolated), as reported in Scheme 4.

To obtain **6** selectively, and without **7**, we tuned several reaction parameters (solvent DMF–ACN instead of DMF–Pyridine, temperature, etc.) and notably found that the use of a large amount of MVK (ca. 10 equiv instead of 2.5 vs ArX) as well as of a higher amount of nickel catalyst by generating nickel salts from the anode, either stainless steel or (64/36) iron/nickel rod, can quite favourably improve the yield and the selectivity. The effect of these parameters can be understood on the basis of the mechanism given in Scheme 5.

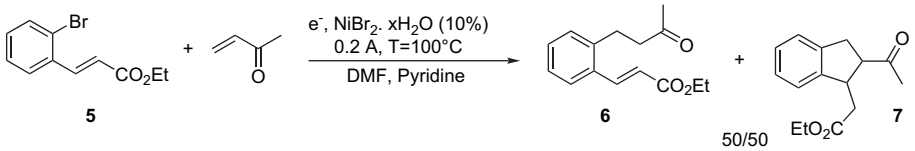
The formation of **7** along with **6** indicates that, as shown in Scheme 5, the key intermediate (I) can reversibly evolve into intermediate (II) by addition to the C–C double bond. In addition, (II) can be assumed to be at least as stable as (I). This would account for the formation of **7** in significant amount, and for the need for some more nickel catalysts than usually required in order to improve the yield of **6**. Similar effects have already been mentioned.[6] So, having a large excess of MVK as both reagent and ligand to shift the equilibrium back to (I) enabled to obtain **6** in 63% isolated yield, and without even traces of compound **7**.

According to this procedure, we could develop a new approach to the aimed benzolactones in a shorter sequence than the one in the previous method.[4] This is summarised in Scheme 6.

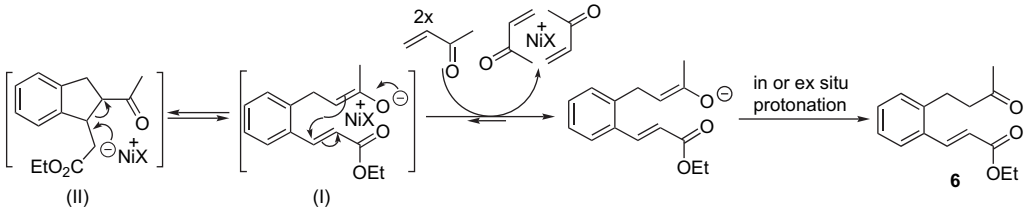
We first prepared a series of commercially unavailable *ortho*-bromoesters (ethyl *ortho*-bromophenylalkenoates) from either *ortho*-bromo-2-phenylacetic acid (for n=1 and n'=0) or *ortho*-bromobenzaldehyde (for n=0, 1 and n'=0–2). Thus, compound **2** was prepared by esterification of the corresponding acid.[7] Compounds corresponding to n=0, 1 and n'=0–2 were prepared by Wittig or Wadsworth–Emmons methods as described by Gibson[8] (**5** and **10**), Jordis[9] (**9**) and Touchard[10] (**8**). Also, to examine the possibility of having medium ring lactones with double C–C bonds, we have prepared the (*E*)-(**5**) and (*Z*)-(**8**) isomers of ethyl 3-(2-bromophenyl)prop-2-enoate.



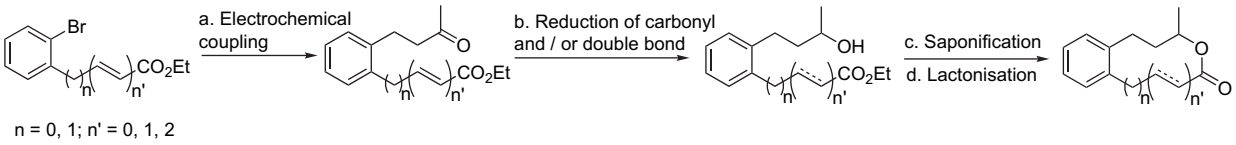
Scheme 3.



Scheme 4.

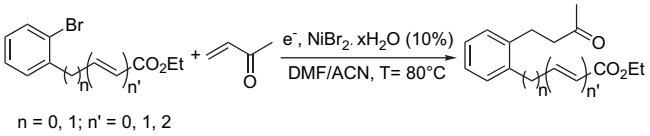


Scheme 5.



Scheme 6.

The next step was the electrochemical coupling with MVK (Scheme 7) using reaction conditions described above. Yields are given in Table 1.



Scheme 7.

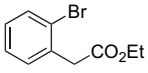
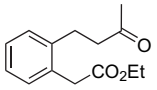
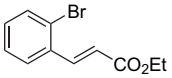
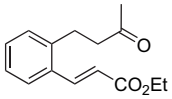
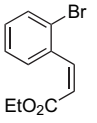
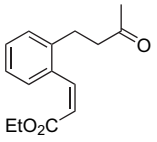
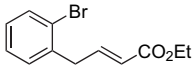
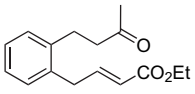
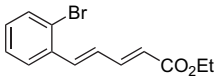
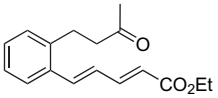
Isolated yields are good and show that the steric *ortho* effect has only a slight influence on the results in agreement with our previous results.[5] No internal tandem addition was observed whatever the starting ester was. Of the two stereoisomers **5** and **8** (see Table 1, entries 2 and 3) the *Z*-isomer gives a lower yield along with the formation of more of the reduced product of the C-halogen bond (ArH). Access to primary alcohols would require acrolein as substrate. However, previous investigations have shown that reactions involving acrolein are unsuccessful, and that its diethyl acetal can be used instead.[11] This gives the expected aldehyde **14**, after hydrolysis of the adduct, with a yield in agreement with our previous investigations[11] (Scheme 8).

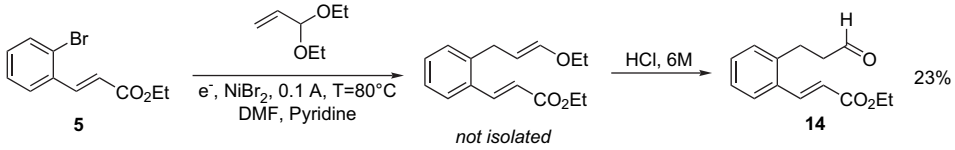
Hydroxyesters were obtained by reduction of the carbonyl. The keto-esters containing a C–C double bond can also be reduced selectively either at the carbonyl of the ketone (reduction by $NaBH_4$)[12] or at both the carbonyl and the C–C double bond by reaction of $NiCl_2/NaBH_4$ in MeOH, at 0 °C to room temperature, as described by Narisada.[13] Results are shown in Table 2.

The two final steps were conducted (Scheme 9) as in our previous paper.[4] Hydroxyesters were converted to the corresponding hydroxyacids by saponification in KOH–dioxane at reflux (quantitative yields).[14] Lactones were obtained from hydroxyacids by the efficient Yamamoto's process[15] using $Sc(OTf)_3$ (10%) and *p*-$(NO_2C_6H_4CO)_2O$ (2 equiv) in acetonitrile.

Yields for the lactonisation step are given in Table 3.

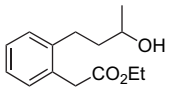
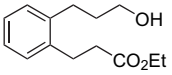
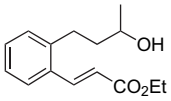
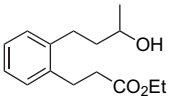
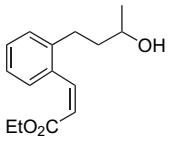
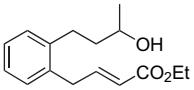
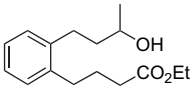
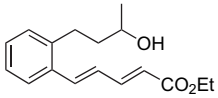
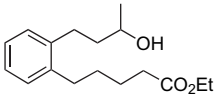
Table 1. Nickel-catalysed arylation of methylvinylketone with *ortho*-bromoarylester

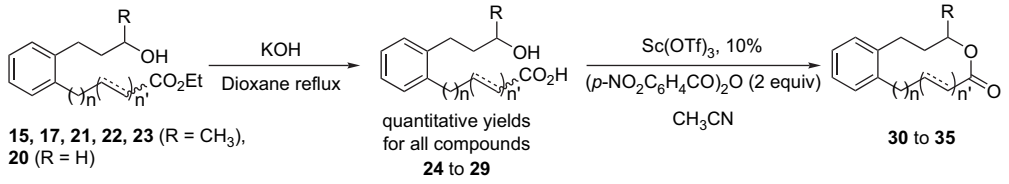
Entry	*ortho*-Bromoarylester	Compound number	Product	Compound number	Isolated yield (%)
1		**2**		**3**	65
2		**5**		**6**	63
3		**8**		**11**	50
4		**9**		**12**	84
5		**10**		**13**	63



Scheme 8.

Table 2. Reductions of keto-esters by $NaBH_4$ or $NaBH_4/NiCl_2$

Entry	Hydroxyester obtained by $NaBH_4$ reduction in methanol at 0 °C			Hydroxyester obtained by $NiCl_2/NaBH_4$ reduction in methanol at 0 °C		
	Compound		Isolated yield (%)	Compound		Isolated yield (%)
1		**15**	70		**20**	75
2		**16**	95		**21**	Quantitative
3		**17**	Quantitative	—		—
4		**18**	Quantitative		**22**	Quantitative
5		**19**	93		**23**	90



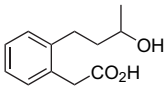
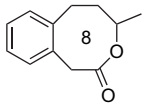
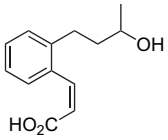
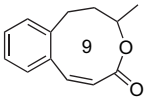
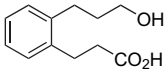
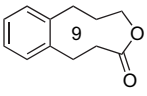
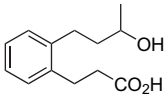
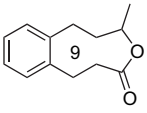
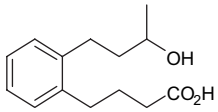
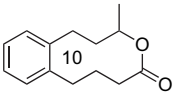
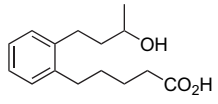
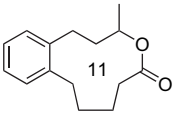
15, 17, 21, 22, 23 (R = CH_3), **20** (R = H)

quantitative yields for all compounds **24** to **29**

30 to **35**

Scheme 9.

Table 3. Lactonisation of hydroxyacids

Entry	Hydroxyacid	Compound number	Benzolactone	Compound number	Isolated yield (%)
1		**24**		**30**	53
2		**25**		**31**	66
3		**26**		**32**	66
4		**27**		**33**	74
5		**28**		**34**	65
6		**29**		**35**	60

Thus, we prepared six benzolactones with ring size from 8 to 11. Regarding the possible presence of a C–C double bond in the medium-size lactone ring, it comes out that the cyclisation was only observed from the *Z*-isomer (**25**) leading to a nine-membered lactone, whereas no cyclisation has occurred from the corresponding *E*-isomer. These benzolactones, which have methyl group on lactone ring, are racemic. No attempt to perform enantioselective reduction was made so far. The obtained yields range from 53 to 74%, and no trend can be found to explain the observed differences in yields, though the lowest yield is obtained in the formation of a eight-membered lactone, as mentioned in our previous study.[4] Most of the prepared compounds, either precursors or lactones are new compounds.

3. Experimental

3.1. General

All reagents and supporting electrolytes were used as obtained commercially. All reactions were performed under an inert atmosphere (argon) unless otherwise indicated. An iron rod was used as the anode. The cathode was made of nickel foam.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 (200 MHz) or AVANCE 300 (300 MHz) spectrometer at room temperature, except for **33** and **34** at 70 °C. Regarding the ^{13}C data, some of aromatic signal are missing. This might be a consequence of the overlapping of certain signal. Infrared spectra were recorded on a Perkin Elmer Spectrum BX II spectrometer. Mass spectra (electron impact) were obtained on a Thermoquest GCQ spectrometer coupled to a Finnigan-GCQ chromatograph with a CPSIL5CB/MS capillary column. High-resolution mass spectra and elemental analyses were performed by 'Service Central d'Analyses du CNRS, Lyon'.

3.1.1. Ethyl 2-(2-bromophenyl)ethanoate (2). RN: [2178-24-7].[16]

3.1.2. Ethyl (*E*)-3-(2-bromophenyl)prop-2-enoate (5) and ethyl (*E*)-4-(2-bromophenyl)but-2-enoate (9). See in Ref. 4, respectively, for compounds **11** and **12**.

3.1.3. Ethyl (*E*,*E*)-5-(2-bromophenyl)penta-2,4-dienoate (10). Triethyl phosphonocrotonate (9.31 mL, 42 mmol) was added to a stirred suspension of sodium hydride (1.5 g, 39 mmol) in THF (50 mL) at 0 °C to give a white foam. The mixture was allowed to warm to room temperature for 30 min then recooled in an ice bath and 2-bromobenzaldehyde (5.55 mL, 30 mmol) was added as a solution in THF (70 mL). After 20 min the reaction mixture was allowed to warm to room temperature and stirred for 1 h. Saturated aqueous ammonium chloride (50 mL) was then added to the mixture. Diethyl ether was added and the combined layers were washed with water (3×25 mL), dried ($MgSO_4$) and the solvent removed under vacuum to give **10**. Colorless oil, 5.71 g, 68%, purification by column chromatography (silica gel, pentane–Et_2O, 90/10). ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 7.63–7.57 (m, 2H, ArH), 7.51 (dd, J=11.1 and J=15.3 Hz, 1H), 7.35–7.26 (m, 2H, ArH), 7.19–7.09 (m, 1H, ArH), 6.82 (dd, J=11.1 Hz and J=15.4 Hz, 1H), 6.05 (d, J=15.4 Hz, 1H), 4.27 (q, J=7.1 Hz, 2H), 1.35 (t, J=7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 166.7, 144.1, 138.5, 135.7, 133.3, 130.1, 128.7, 127.6, 127.0, 124.6, 122.5, 60.5, 14.4. EIMS *m*/*z* (% relative abundance): 282 (19), 280 (21), 209 (23), 208 (17), 207 (26), 206 (16), 156 (20), 129 (31), 128 (100), 127 (13). IR ν_{max} in $CHCl_3$ solution (cm^{-1}): 3065, 2979, 1707, 1627. HRMS (ESI) *m*/*z* Calcd for $C_{13}H_{14}BrO_2$ (M+H): 281.0177; Found: 281.0192.

3.1.4. Ethyl (*Z*)-3-(2-bromophenyl)prop-2-enoate (8). In a 500-mL flask were added under argon K_2CO_3 (4 g, 29 mmol), 18-crown-6 (18C6) (0.76 g, 1.44 mmol) and 300 mL of chlorobenzene. The mixture was stirred at room temperature for 3 h before being cooled to 0 °C. The phosphonate (5.06 g, 15.24 mmol) and the aldehyde (3.4 mL, 14.5 mmol) were then added. The mixture was maintained at 0 °C until the work-up. The reaction mixture was quenched with 50 mL saturated NH_4Cl and the organic phase washed by saturated NH_4Cl and water until neutrality. It was then dried over Na_2SO_4 and the solvent removed under vacuum. The *Z*/*E*-mixture was purified by gel chromatography (pentane–Et_2O, 95/5). Colorless oil, 3.27 g, 98%; RN: [99134-35-7]. ^{1}H NMR (Acetone-d_6, 300 MHz, δ ppm): 7.63–7.19 (m, 4H, ArH), 7.13 (d, J=12.2 Hz, 1H), 6.05 (d, J=12.2 Hz, 1H), 4.15 (q, J=7.1 Hz, 2H), 1.21 (t, J=7.1 Hz, 3H). ^{13}C NMR (Acetone-d_6, 75 MHz, δ ppm): 165.5, 142.5, 135.9, 132.2, 130.8, 129.9, 126.6, 123.1, 121.8, 60.3, 14.0. EIMS *m*/*z* (% relative abundance): 257 (<10), 255 (<10), 175 (57), 147 (100), 103 (34), 102 (24). IR ν_{max} in $CHCl_3$ solution (cm^{-1}): 3029, 3021, 2925, 2940, 1719, 1637.

3.2. General procedure for the arylation of electron-deficient olefins

Under argon, in an undivided cell equipped with a nickel grid (area 40 cm^2) as the cathode and a Fe/Ni (64/36) rod as the anode, tetrabutylammonium bromide (0.34 mmol) and tetrabutylammonium iodide (0.21 mmol) as supporting electrolytes were dissolved in a mixture of DMF (25 mL) and ACN (25 mL). 1,2-Dibromoethane (0.1 mmol) was introduced. After a short electrolysis run at constant current density (0.2 A) and at room temperature over 15 min, the activated olefin (100 mmol), $NiBr_2 \cdot 3H_2O$ (1 mmol) was added and the reaction mixture was heated at 80 °C. The electrosynthesis was run at current density (0.2 A) 10 min after, the aryl bromide (10 mmol) was added. The reaction was monitored by GC and stopped after the aryl bromide was consumed. The mixture was then hydrolysed with hydrochloric acid (1 N, 30 mL) and diluted with diethyl ether (2×50 mL) and the combined organic layers were washed with water and saturated NaCl solution, then dried over $MgSO_4$. The oil thus obtained was purified by column chromatography (silica gel, pentane–ether, 90/10 eluent) to give the desired compound.

3.2.1. Ethyl 2-[2-(3-oxobutyl)phenyl]ethanoate (3). Colorless oil, 1.52 g, 65%. ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 7.27–7.17 (m, 4H, ArH), 4.18 (q, J=7.2 Hz, 2H), 3.70 (s, 2H), 2.97–2.92 (m, 2H), 2.81–2.76 (m, 2H), 2.18 (s, 3H), 1.29 (t, J=7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz,

δ ppm): 207.8, 171.7, 139.7, 132.5, 130.7, 129.2, 127.6, 126.5, 60.9, 44.5, 38.6, 30.0, 26.6, 14.2. EIMS *m/z* (% relative abundance): 234 (2), 216 (21), 189 (12), 188 (78), 170 (25), 161 (15), 146 (17), 145 (100), 144 (36), 143 (50), 142 (26), 131 (11), 129 (11), 128 (10), 117 (63), 116 (12), 115 (32), 91 (17). IR ν_{max} in $CHCl_3$ solution (cm^{-1}): 3036, 2941, 1719, 1604, 1493. HRMS (ESI) *m/z* Calcd for $C_{14}H_{19}O_3$ (M+H): 235.1334; Found: 235.1329.

3.2.2. Ethyl (*E*)-3-[2-(3-oxobutyl)phenyl]prop-2-enoate (6). Colorless oil, 1.55 g, 63%. ^1H NMR ($CDCl_3$, 300 MHz, δ ppm): 8.00 (d, *J*=15.8 Hz, 1H), 7.71–7.24 (m, 4H, ArH), 6.45 (d, *J*=15.8 Hz, 1H), 4.23 (q, *J*=7.0 Hz, 2H), 3.03–2.98 (m, 2H), 2.80–2.74 (m, 2H), 2.12 (s, 3H), 1.30 (t, *J*=7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 206.8, 166.1, 141.4, 141.0, 132.9, 130.1, 129.8, 126.7, 126.6, 119.8, 59.9, 44.1, 28.9, 26.6, 13.7. EIMS *m/z* (% relative abundance): 246 (3), 201 (17), 200 (18), 172 (30), 158 (16), 157 (100), 131 (11), 130 (17), 129 (60), 128 (15), 115 (17). IR ν_{max} in $CHCl_3$ solution (cm^{-1}): 3068, 2985, 2940, 2905, 2876, 1704, 1634, 1601, 1368. HRMS (ESI) *m/z* Calcd for $C_{15}H_{19}O_3$ (M+H): 247.1334; Found: 247.1335.

3.2.3. Ethyl (*Z*)-3-[2-(3-oxobutyl)phenyl]prop-2-enoate (11). Colorless oil, 1.23 g, 50%. ^1H NMR ($CDCl_3$, 300 MHz, δ ppm): 7.57–7.19 (m, 5H, ArH), 6.09 (d, *J*=12.0 Hz, 1H), 4.11 (q, *J*=7.1 Hz, 2H), 2.93–2.88 (m, 2H), 2.77–2.71 (m, 2H), 2.16 (s, 3H), 1.17 (t, *J*=7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 207.9, 165.9, 142.8, 138.5, 129.2, 128.6, 126.7, 125.8, 122.0, 60.2, 44.2, 30.1, 27.5, 14.0. EIMS *m/z* (% relative abundance): 201 (16), 200 (20), 172 (17), 158 (17), 157 (100), 131 (10), 130 (12), 129 (62), 128 (19), 115 (20). IR ν_{max} in $CHCl_3$ solution (cm^{-1}): 3032, 2987, 2962, 1713, 1634. HRMS (ESI) *m/z* Calcd for $C_{15}H_{19}O_3$ (M+H): 247.1334; Found: 247.1347.

3.2.4. Ethyl (*E*)-4-[2-(3-oxobutyl)phenyl]but-2-enoate (12). Colorless oil, 2.19 g, 84% mixture of *E*/*Z* (80/20). ^1H NMR ($CDCl_3$, 300 MHz, δ ppm): 7.25–7.17 (m, 4H, ArH), 7.09 (dt, J_{trans}=15.6 Hz, *J*=6.4 Hz, 1H), 5.75 (dt, J_{trans}=15.6 Hz, *J*=1.7 Hz, 1H), 4.13 (q, *J*=7.1 Hz, 2H), 3.62 (dd, *J*=6.4 Hz, *J*=1.7 Hz, 2H), 2.92–2.75 (m, 4H), 2.11 (s, 3H), 1.24 (t, *J*=7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 206.3, 165.6, 147.5, 139.7, 135.8, 129.9, 129.3, 127.0, 126.4, 121.9, 59.7, 43.8, 35.0, 29.0, 26.1, 13.7. EIMS *m/z* (% relative abundance): 260, 215 (14), 214 (88), 196 (10), 181 (14), 173 (11), 171 (17), 169 (14), 157 (49), 156 (100), 145 (11), 144 (13), 143 (22), 129 (83), 128 (83), 127 (10), 115 (24). IR ν_{max} in $CHCl_3$ solution (cm^{-1}): 3072, 2983, 1715, 1603, 1491. HRMS (ESI) *m/z* Calcd for $C_{16}H_{20}O_3Na$ (M+Na) 283.1310; Found: 283.1311.

3.2.5. Ethyl (*E*,*E*)-5-[2-(3-oxobutyl)phenyl]penta-2,4-dienoate (13). Colorless oil, 1.72 g, 63%. ^1H NMR (Acetone-d_6, 200 MHz, δ ppm): 7.62–7.14 (m, 6H, ArH), 6.95 (dd, *J*=15.4 and 10.9 Hz, 1H), 6.00 (d, J_{trans}=15.3 Hz, 1H), 4.12 (q, *J*=7.2 Hz, 2H), 2.97–2.89 (m, 2H), 2.79–2.69 (m, 2H), 2.04 (s, 3H), 1.21 (t, *J*=7.2 Hz, 3H). ^{13}C NMR (Acetone-d_6, 50 MHz, δ ppm): 207.1, 167.6, 145.8, 141.2, 138.5, 135.6, 130.9, 129.9, 128.7, 127.6, 126.9, 122.2, 60.7, 45.2, 29.9, 27.8, 14.7. EIMS *m/z* (% relative abundance): 272 (15), 229 (16), 214 (39), 198 (23), 186 (10), 185 (23), 183 (25), 181 (33), 180 (24), 169 (23), 168 (47), 165 (16), 155 (40), 153 (16), 142 (17), 141 (100), 129 (20), 128 (18), 115 (28). IR ν_{max} in $CHCl_3$ solution (cm^{-1}): 3032, 2984, 2875, 1708, 1610. HRMS (ESI) *m/z* Calcd for $C_{17}H_{20}O_3Na$ (M+Na): 295.1310; Found: 295.1299.

3.2.6. Ethyl (*E*)-3-[2-(3-oxopropyl)phenyl]prop-2-enoate (14). Under argon, in an undivided cell equipped with a nickel grid (area 40 cm^2) as the cathode and a Fe/Ni (64/36) rod as the anode, tetrabutylammonium bromide (0.34 mmol) and tetrabutylammonium iodide (0.21 mmol) as supporting electrolytes were dissolved in a mixture of DMF (50 mL) and pyridine (5 mL). 1,2-Dibromoethane (0.1 mmol) was introduced after a short electrolysis run at constant current density (0.2 A) and at room temperature over 15 min, the olefin (30 mmol), $NiBr_2 \cdot 3H_2O$ (0.75 mmol) and the aryl bromide (10 mmol) were added, and the reaction mixture heated at 80 °C. The electrosynthesis was run at current density (0.1 A). The reaction was monitored by GC and stopped after the aryl bromide was consumed. The mixture was then hydrolysed with hydrochloric acid (6 N, 30 mL) and diluted with diethyl ether (2×50 mL) and the combined organic layers were washed with water and saturated NaCl solution, then dried over $MgSO_4$. The oil thus obtained was purified by column chromatography (silica gel, pentane–ether, 70/30 eluent) to give **14**. Colorless oil, 0.54 g, 23%. ^1H NMR ($CDCl_3$, 300 MHz, δ ppm): 9.77 (s, 1H), 7.98 (d, *J*=15.8 Hz, 1H), 7.56–7.20 (m, 4H, ArH), 6.37 (d, *J*=15.8 Hz, 1H), 4.25 (q, *J*=7.1 Hz, 2H), 3.10–3.04 (m, 2H), 2.75–2.69 (m, 2H), 1.32 (t, *J*=7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 200.8, 166.8, 141.4, 139.9, 133.0, 130.2, 129.9, 127.0, 126.8, 120.2, 60.4, 44.9, 25.4, 14.3. EIMS *m/z* (% relative abundance): 231 (27), 204 (19), 203 (100), 202 (34), 178 (17). IR ν_{max} in $CHCl_3$ solution (cm^{-1}): 3068, 2979, 2828, 1711. HRMS (ESI) *m/z* Calcd for $C_{14}H_{17}O_3$ (M+H): 233.1178; Found: 233.1175.

3.3. Reduction of carbonyl function only

To a solution of product (1 equiv, 7 mmol, 1.64 g) in methanol (30 mL) was added $NaBH_4$ (1 equiv, 0.27 g) portion by portion at 0 °C. The reaction mixture was stirred for 3 h at room temperature and then quenched with aqueous saturated sodium hydrogen carbonate (10 mL). The mixture was extracted with ethyl acetate (3×20 mL). The organic layers were dried over $MgSO_4$ and evaporated. Purification was done by column chromatography on silica gel (eluent: pentane–ethyl acetate system) to give the desired hydroxyesters.

3.3.1. Ethyl 2-[2-(3-hydroxybutyl)phenyl]ethanoate (15). Colorless oil, 1.14 g, 70%, purification by column chromatography (silica gel, pentane–ethyl acetate, 70/30). ^1H NMR ($CDCl_3$, 300 MHz, δ ppm): 7.28–7.17 (m, 4H, ArH), 4.18 (q, *J*=7.1 Hz, 2H), 3.87–3.83 (m, 1H), 3.72 (s, 2H), 2.84–2.68 (m, 3H), 1.79–1.72 (m, 2H), 1.29 (t, *J*=7.1 Hz, 3H), 1.26 (d, *J*=6.7 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 172.1, 140.8, 132.4, 130.6, 129.4, 127.5, 126.2, 67.2, 61.0, 40.1, 38.7, 28.9, 23.5, 14.2. EIMS *m/z* (% relative abundance): 237 (<10), 218 (29), 192 (16), 190 (23), 175

(12), 172 (22), 149 (10), 147 (14), 146 (28), 145 (100), 144 (93), 143 (50), 131 (27), 130 (24), 129 (82), 128 (12), 119 (21), 118 (29), 117 (49), 116 (13), 115 (20), 105 (31), 104 (14), 103 (10), 91 (26). IR ν_{max} in $CHCl_3$ solution (cm^{-1}): 3474, 3037, 2970, 2875, 1727, 1603, 1493. HRMS (ESI) *m/z* Calcd for $C_{14}H_{21}O_3$ (M+H): 237.1491; Found: 237.1496.

3.3.2. Ethyl (*E*)-3-[2-(3-hydroxybutyl)phenyl]prop-2-enoate (16). Colorless oil, 0.94 g, 95%, purification by column chromatography (silica gel, pentane–ethyl acetate, 70/30). ^{1}H NMR (Acetone-d_6, 200 MHz, δ ppm): 8.15 (d, J=15.8 Hz, 1H), 7.84–7.34 (m, 4H), 6.57 (d, J=15.8 Hz, 1H), 4.35 (q, J=7.1 Hz, 2H), 3.94–3.83 (m, 1H), 3.11–2.90 (m, 2H), 2.1 (br s, 1H, H_{OH}), 1.84–1.73 (m, 2H), 1.42 (t, J=7.1 Hz, 3H), 1.31 (d, J=5.9 Hz, 3H). ^{13}C NMR (Acetone-d_6, 50 MHz, δ ppm): 167.0, 143.2, 142.6, 142.4, 133.6, 130.8, 127.4, 127.2, 120.1, 67.0, 60.7, 42.0, 24.0, 23.8, 14.5. EIMS *m/z* (% relative abundance): 248 (<5), 230 (8), 203 (12), 185 (14), 169 (12), 160 (14), 159 (31), 158 (19), 157 (52), 156 (52), 155 (15), 147 (12), 145 (15), 144 (26), 143 (34), 142 (88), 141 (21), 132 (11), 131 (40), 130 (47), 129 (100), 128 (23), 117 (33), 116 (33), 115 (37). IR ν_{max} in $CHCl_3$ solution (cm^{-1}): 3462, 3036, 2932, 2876, 1694, 1600, 1484. HRMS (ESI) *m/z* Calcd for $C_{15}H_{20}O_3Na$ (M+Na): 271.1310; Found: 271.1303.

3.3.3. Ethyl (*Z*)-3-[2-(3-hydroxybutyl)phenyl]prop-2-enoate (17). Colorless oil, 1.08 g, quantitative yield, purification by column chromatography (silica gel, pentane–ethyl acetate, 70/30). ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 7.59–7.17 (m, 5H, ArH and olefinic H), 6.06 (d, J=12.0 Hz, 1H), 4.09 (q, J=7.1 Hz, 2H), 3.83–3.76 (m, 1H), 2.77–2.65 (m, 2H), 2.56 (br s, 1H, H_{OH}), 1.75–1.68 (m, 2H), 1.21 (d, J=6.2 Hz, 3H), 1.15 (t, J=7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 166.2, 143.1, 139.8, 134.9, 129.2, 128.8, 128.6, 125.4, 121.1, 67.1, 60.2, 39.8, 29.8, 23.6, 13.9. EIMS *m/z* (% relative abundance): 185 (25), 169 (12), 159 (13), 158 (20), 157 (45), 156 (43), 155 (12), 147 (14), 144 (22), 143 (13), 142 (34), 141 (21), 131 (26), 130 (21), 129 (100), 128 (27), 117 (17), 116 (20), 115 (40). IR ν_{max} in $CHCl_3$ solution (cm^{-1}): 3474, 2970, 2931, 1713, 1633. HRMS (ESI) *m/z* Calcd for $C_{15}H_{21}O_3$ (M+H): 249.1491; Found: 249.1492.

3.3.4. Ethyl (*E*)-4-[2-(3-hydroxybutyl)phenyl]but-2-enoate (18). Colorless oil, 1.04 g, quantitative yield, *E*/*Z* (80/20) purification by column chromatography (silica gel, pentane–ethyl acetate, 70/30). ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 7.50–7.11 (m, 5H), 5.77 (dt, J_{trans}=15.6 Hz, J=1.7 Hz, 1H), 4.21 (q, J=7.1 Hz, 2H), 3.93–3.80 (m, 1H), 3.60 (dd, J=6.4 Hz, J=1.7 Hz, 2H), 2.92–2.61 (m, 2H), 2.03 (br s, 1H, H_{OH}), 1.83–1.70 (m, 2H), 1.33–1.25 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 166.7, 147.6, 140.4, 135.5, 130.0, 129.5, 127.1, 126.3, 122.2, 67.6, 60.3, 40.4, 35.5, 28.9, 23.6, 14.2. EIMS *m/z* (% relative abundance): 262, 216 (18), 215 (12), 198 (11), 183 (18), 173 (11), 171 (16), 170 (39), 169 (38), 157 (26), 156 (29), 155 (16), 145 (15), 144 (11), 143 (18), 141 (29), 131 (13), 130 (23), 129 (100), 128 (61), 127 (12), 117 (19), 115 (27), 91 (11). IR ν_{max} in $CHCl_3$ solution (cm^{-1}): 3608, 3504, 3020, 2970, 2874, 1712, 1602, 1490. HRMS (ESI) *m/z* Calcd for $C_{16}H_{22}O_3Na$ (M+Na): 285.1467; Found: 285.1470.

3.3.5. Ethyl (*E*,*E*)-5-[2-(3-hydroxybutyl)phenyl]penta-2,4-dienoate (19). Colorless oil, 0.79 g, 93%, purification by column chromatography (silica gel, pentane–ether, 70/30). ^{1}H NMR (Acetone-d_6, 300 MHz, δ ppm): 7.51 (dd, J=15.3 Hz, J=11.1 Hz, 1H), 7.45 (d, J_{trans}=15.3 Hz, 1H), 7.69–7.22 (m, 4H), 7.03 (dd, J=15.3 Hz, J=11.1 Hz, 1H), 6.00 (d, J_{trans}=15.3 Hz, 1H), 4.19 (q, J=7.2 Hz, 2H), 3.84–3.76 (m, 1H), 3.02–2.76 (m, 3H), 1.71–1.63 (m, 2H), 1.27 (t, J=7.2 Hz, 3H), 1.18 (d, J=6.1 Hz, 3H). ^{13}C NMR (Acetone-d_6, 75 MHz, δ ppm): 167.2, 145.9, 142.4, 138.7, 135.2, 130.8, 129.7, 128.2, 127.1, 126.5, 121.8, 67.1, 60.6, 41.9, 29.9, 24.2, 14.6. EIMS *m/z* (% relative abundance): 274 (10), 228 (20), 200 (14), 185 (14), 183 (26), 182 (22), 181 (21), 170 (40), 169 (27), 168 (25), 167 (46), 157 (18), 156 (16), 155 (25), 153 (28), 144 (16), 143 (58), 142 (66), 141 (100), 132 (13), 129 (43), 128 (43), 117 (12), 116 (12), 115 (44). IR ν_{max} in $CHCl_3$ solution (cm^{-1}): 3510, 2971, 2875, 1707, 1600. HRMS (ESI) *m/z* Calcd for $C_{17}H_{23}O_3$ (M+H): 275.1647; Found: 275,1641.

3.4. Typical procedure for reduction

To a solution of product (1 equiv, 5.7 mmol) and $NiCl_2$ (5.7 mmol) in methanol (50 mL) was added $NaBH_4$ (7 equiv, 39.9 mmol) portionwise at 0 °C. The reaction mixture was stirred for 3 h at room temperature and then quenched with aqueous saturated sodium hydrogen carbonate (20 mL). The mixture was extracted with ethyl acetate (3×20 mL). The organic layers were dried over $MgSO_4$ and evaporated. Purification was done by column chromatography on silica gel (pentane–ethyl acetate system, eluent) to give the desired hydroxyl-esters.

3.4.1. Ethyl 3-[2-(3-hydroxypropyl)phenyl]propanoate (20). See Ref. 4 (compound **6c**), 0.97 g, 75%. RN: [136416-11-0].[4]

3.4.2. Ethyl 3-[2-(3-hydroxybutyl)phenyl]propanoate (21). Colorless oil, 1.42 g, quantitative yield, purification by column chromatography (silica gel, pentane–ethyl acetate, 50/50). ^{1}H NMR (Acetone-d_6, 200 MHz, δ ppm): 7.31–7.20 (m, 4H, ArH), 4.20 (q, J=7.0 Hz, 2H), 3.94–3.86 (m, 1H), 3.75 (br s, 1H, H_{OH}), 3.12–2.66 (m, 6H), 1.85–1.74 (m, 2H), 1.35–1.28 (m, 6H). ^{13}C NMR (Acetone-d_6, 50 MHz, δ ppm): 173.0, 141.3, 139.2, 130.0, 129.6, 127.1, 126.6, 67.1, 60.6, 41.7, 35.8, 28.2, 24.1, 23.9, 14.4. EIMS *m/z* (% relative abundance): 250 (3), 232 (55), 187 (24), 186 (78), 185 (10), 179 (11), 171 (23), 160 (12), 159 (18), 158 (63), 157 (15), 156 (11), 146 (14), 145 (40), 144 (56), 143 (75), 142 (21), 133 (24), 131 (37), 130 (27), 129 (100), 128 (53), 118 (20), 117 (79), 116 (31), 115 (52), 105 (21), 104 (10), 91 (28). IR ν_{max} in $CHCl_3$ solution (cm^{-1}): 3473, 3034, 2980, 2875, 1724, 1603, 1491. Anal. Calcd for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86; O, 19.17. Found: C, 71.77; H, 8.84; O, 19.30.

3.4.3. Ethyl 4-[2-(3-hydroxybutyl)phenyl]butanoate (22). Colorless oil, 0.575 g, quantitative yield, purification by column chromatography (silica gel, pentane–ethyl acetate, 50/50). ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 7.31–7.16 (m, 4H, ArH), 4.19 (q, J=7.1 Hz, 2H), 3.97–3.86 (m, 1H), 2.92–2.61 (m, 4H), 2.43 (t, J=7.1 Hz, 2H), 2.01–1.90

(m, 3H, H_{OH}), 1.81–1.71 (m, 2H), 1.33–1.28 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 173.8, 140.1, 139.3, 129.4, 129.3, 126.6, 126.0, 67.7, 60.5, 40.9, 33.9, 32.0, 28.7, 26.4, 23.7, 14.3. EIMS *m/z* (% relative abundance): 264, 246 (49), 201 (42), 200 (72), 199 (14), 185 (17), 182 (17), 177 (12), 172 (16), 160 (21), 159 (47), 158 (65), 157 (27), 156 (12), 147 (22), 146 (26), 145 (60), 144 (19), 143 (54), 142 (15), 132 (14), 131 (90), 130 (54), 129 (100), 128 (17), 118 (20), 117 (82), 116 (17), 115 (37), 105 (22), 91 (36). IR ν_{max} in $CHCl_3$ solution (cm^{-1}): 3055, 2968, 1727, 1603, 1491. HRMS (ESI) *m/z* Calcd for $C_{16}H_{25}O_3$ (M+H): 265.1804; Found: 265.1802.

3.4.4. Ethyl 5-[2-(3-hydroxybutyl)phenyl]pentanoate (23). Colorless oil, 1.61 g, 90%, purification by column chromatography (silica gel, pentane–ethyl acetate, 50/50). ^1H NMR ($CDCl_3$, 300 MHz, δ ppm): 7.31–7.15 (m, 4H, ArH), 4.17 (q, J=7.1 Hz, 2H), 3.96–3.86 (m, 1H), 2.89–2.64 (m, 4H), 2.43–2.38 (m, 2H), 2.16 (br s, 1H, H_{OH}), 1.83–1.62 (m, 6H), 1.30 (d, J=6.2 Hz, 3H), 1.30 (t, J=7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 173.9, 140.0, 139.9, 129.3, 129.2, 126.1, 126.0, 67.7, 60.4, 40.8, 34.2, 32.3, 30.7, 28.8, 25.0, 23.7, 14.3. EIMS *m/z* (% relative abundance): 278, 260 (47), 215 (17), 214 (28), 186 (19), 185 (11), 173 (14), 171 (18), 158 (11), 146 (15), 145 (100), 144 (34), 143 (26), 131 (54), 130 (20), 129 (59), 128 (20), 117 (48), 115 (21), 105 (22), 91 (22). IR ν_{max} in $CHCl_3$ solution (cm^{-1}): 3517, 3017, 2937, 2869, 1728, 1602, 1490. HRMS (ESI) *m/z* Calcd for $C_{17}H_{26}O_3Na$ (M+Na): 301.1780; Found: 301.1780.

3.5. Typical procedure for saponification

To a solution of product (7 mmol) in dioxane–water (1:1, v:v, 70 mL) was added a 20% solution of potassium hydroxide in water (24 mmol). The reaction mixture was refluxed for 16 h and cooled to room temperature, then H_2SO_4 was added until pH=1 was reached. The mixture was extracted with ether (3×25 mL). The combined organic layers were dried ($MgSO_4$) and evaporated to leave a crude product.

3.5.1. 2-[2-(3-Hydroxybutyl)phenyl]ethanoic acid (24). Colorless oil, 0.73 g, quantitative yield. ^1H NMR (Acetone-d_6, 300 MHz, δ ppm): 7.26–7.13 (m, 4H, ArH), 3.83–3.71 (m, 1H), 3.71 (s, 2H), 2.86–2.65 (m, 2H), 1.73–1.66 (m, 2H), 1.19 (d, J=6.2 Hz, 3H). ^{13}C NMR (Acetone-d_6, 75 MHz, δ ppm): 172.4, 141.3, 133.1, 130.6, 129.2, 127.1, 125.7, 66.4, 40.4, 37.7, 28.9, 23.1. IR ν_{max} in $CHCl_3$ solution (cm^{-1}): 3406, 3200–2800, 2968, 2646, 1712, 1603, 1493. HRMS (ESI) *m/z* Calcd for $C_{12}H_{17}O_3$ (M+H): 209.1178; Found: 209.1190.

3.5.2. (*Z*)-3-[2-(3-Hydroxybutyl)phenyl]prop-2-enoic acid (25). Colorless oil, 0.884 g, 91%. ^1H NMR (Acetone-d_6, 300 MHz, δ ppm): 7.35–7.10 (m, 5H, ArH), 6.07 (d, J=12.2 Hz, 1H), 3.80–3.70 (m, 1H), 2.98–2.62 (m, 2H), 1.70–1.62 (m, 2H), 1.15 (d, J=6.2 Hz, 3H). ^{13}C NMR (Acetone-d_6, 75 MHz, δ ppm): 166.4, 142.2, 140.5, 134.9, 129.2, 128.7, 128.2, 125.1, 121.3, 66.2, 40.2, 29.7, 23.1. IR pellet of KBr (ν cm^{-1}): 3200–2800, 1703, 1364. HRMS (ESI) *m/z* Calcd for $C_{13}H_{17}O_3$ (M+H): 221.1178; Found: 221.1174.

3.5.3. 3-[2-(3-Hydroxypropyl)phenyl]propanoic acid (26). See Ref. 4 (compound **7c**).

3.5.4. 3-[2-(3-Hydroxybutyl)phenyl]propanoic acid (27). Colorless oil, 0.475 g, 97%. ^1H NMR (Acetone-d_6, 200 MHz, δ ppm): 7.06–6.92 (m, 4H, ArH), 3.73–3.64 (m, 1H), 2.85–2.40 (m, 6H), 1.61–1.49 (m, 2H), 1.06 (d, J=6.2 Hz, 3H). ^{13}C NMR (Acetone-d_6, 50 MHz, δ ppm): 174.7, 141.2, 139.4, 130.0, 129.6, 127.1, 126.7, 67.4, 41.5, 35.6, 28.2, 23.9, 23.8. IR pellet of KBr (ν cm^{-1}): 3387, 3040, 2968, 2875, 1711, 1604, 1491. HRMS (ESI) *m/z* Calcd for $C_{13}H_{18}O_3Na$ (M+Na): 245.1154; Found: 245.1141.

3.5.5. 4-[2-(3-Hydroxybutyl)phenyl]butanoic acid (28). Colorless oil, 0.45 g, quantitative yield. ^1H NMR (Acetone-d_6, 300 MHz, δ ppm): 7.99–6.96 (m, 4H, ArH), 3.82–3.76 (m, 1H), 2.99–2.62 (m, 4H), 2.39 (t, J=7.3 Hz, 2H), 1.92–1.82 (m, 2H), 1.71–1.63 (m, 2H), 1.19 (d, J=6.2 Hz, 3H). ^{13}C NMR (Acetone-d_6, 300 MHz, δ ppm): 173.9, 140.5, 139.5, 129.2, 126.0, 125.8, 66.5, 41.1, 33.0, 31.6, 28.6, 26.5, 23.1. IR pellet of KBr (ν cm^{-1}): 3200–2800, 3018, 2967, 1710, 1604, 1491. HRMS (ESI) *m/z* Calcd for $C_{14}H_{21}O_3$ (M+H): 237.1491; Found: 237.1496.

3.5.6. 5-[2-(3-Hydroxybutyl)phenyl]pentanoic acid (29). Colorless oil, 1.61 g, 90%. ^1H NMR (Acetone-d_6, 300 MHz, δ ppm): 7.19–7.08 (m, 4H, ArH), 3.89–3.83 (m, 1H), 2.89–2.67 (m, 4H), 2.38–2.33 (m, 2H), 1.75–1.63 (m, 6H), 1.23 (d, J=6.0 Hz, 3H). ^{13}C NMR (Acetone-d_6, 75 MHz, δ ppm): 174.6, 140.3, 140.0, 129.2, 129.17, 125.9, 125.8, 66.8, 40.9, 33.3, 32.1, 30.7, 28.6, 24.8, 23.1. IR pellet of KBr (ν cm^{-1}): 3200–2800, 3018, 2937, 2870, 1705, 1604, 1490. HRMS (ESI) *m/z* Calcd for $C_{15}H_{22}O_3Na$ (M+Na): 273.1467; Found: 273.1458.

3.6. Preparation of *p*-nitrobenzoic anhydride

To a mixture of *p*-nitrobenzoic acid (3.34 g, 20 mmol) and *p*-nitrobenzoic chloride (3.71 g, 20 mmol) in dichloromethane (50 mL) was added pyridine (2.02 mL, 25 mmol) dropwise at 0 °C. The reaction mixture was stirred for 15 h at room temperature and then quenched with cold water (20 mL). The mixture was extracted with dichloromethane several times. The organic layers were dried over $MgSO_4$ and evaporated. The crude product was purified by recrystallisation from dichloromethane–hexane to afford *p*-nitrobenzoic anhydride (5.4 g, 85% yield).

3.7. Typical procedure for lactonisation

p-Nitrobenzoic anhydride (506 mg, 1.6 mmol) was dissolved in dry acetonitrile (340 mL), and a cloudy solution of scandium triflate (1.6 mL, 0.16 mmol, 0.1 M) in acetonitrile was added to the solution at room temperature under argon. A solution of hydroxycarboxylic acid (20 mL, 0.8 mmol, 0.08 M) in THF was slowly added with a syringe pump over 15 h to the mixed solution at reflux under argon, and the reaction mixture was stirred for an additional 5 h at reflux. After being cooled to room temperature, the solution was quenched with aqueous saturated sodium hydrogen carbonate (8 mL). The resulting mixture was concentrated under reduced

pressure and extracted with diethyl ether twice. The organic layers were dried over magnesium sulfate, filtered and concentrated under vacuum. Purification was done by flash column chromatography on silica gel to give the desired lactone.

3.7.1. 2-[2-(3-Hydroxybutyl)phenyl]ethanoic acid, ζ lactone (30). Colorless oil, 0.08 g, 53%, purification by flash column chromatography (silica gel, pentane–Et_2O, 90/10). ^1H NMR ($CDCl_3$, 300 MHz, δ ppm): 7.30–7.14 (m, 4H, ArH), 4.84–4.79 (m, 1H), 4.12 (d, AB system $\Delta\nu$/J=7.2 Hz, J=14.4 Hz, 1H), 3.78 (d, AB system $\Delta\nu$/J=7.2 Hz, J=14.4 Hz, 1H), 2.97–2.90 (m, 2H), 2.05–1.86 (m, 2H), 1.40 (d, J=6.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz, δ ppm): 173.1, 139.3, 133.3, 130.3, 129.8, 127.9, 127.2, 77.1, 39.7, 38.6, 33.0, 22.4. EIMS *m*/*z* (% relative abundance): 190 (<10), 148 (17), 146 (31), 131 (79), 118 (11), 117 (41), 115 (30), 105 (14), 104 (100), 103 (17), 91 (25), 78 (37). IR ν_{max} in $CHCl_3$ solution (cm^{-1}): 3024, 2971, 2931, 1728, 1603, 1493. HRMS (ESI) *m*/*z* Calcd for $C_{12}H_{15}O_2$ (M+H): 191.1072; Found: 191.1064.

3.7.2. (*Z*)-3-[2-(3-Hydroxybutyl)phenyl]prop-2-enoic acid, η lactone (31). Colorless oil, 0.097 g, 0.66%, purification by flash column chromatography (silica gel, pentane–Et_2O, 90/10). ^1H NMR (DMF-d_7, 300 MHz, δ ppm): 7.38–7.20 (m, 5H, ArH), 6.15 (d, J=12.2 Hz, 1H), 5.24–5.13 (m, 1H), 2.79–2.72 (m, 1H), 2.47–2.35 (m, 2H), 1.90–1.78 (m, 1H), 1.10 (d, J=6.5 Hz, 3H). ^{13}C NMR (DMF-d_7, 75 MHz, δ ppm): 168.1, 142.8, 142.3, 134.6, 131.0, 130.6, 128.9, 125.5, 121.7, 71.0, 34.7, 29.75, 21.8. EIMS *m*/*z* (% relative abundance): 202 (31), 174 (24), 161 (11), 160 (37), 159 (40), 158 (16), 157 (31), 156 (100), 147 (15), 146 (25), 145 (12), 143 (13), 142 (62), 141 (18), 133 (12), 132 (46), 131 (52), 130 (27), 129 (58), 128 (41), 127 (13), 118 (34), 117 (16), 116 (24), 115 (60), 104 (10), 103 (13), 91 (12), 89 (12). IR ν_{max} in CH_2Cl_2 solution (cm^{-1}): 3058, 2984, 2935, 1705. HRMS (ESI) *m*/*z* Calcd for $C_{13}H_{15}O_2$ (M+H): 203.1072; Found: 203.1068.

3.7.3. 3-[2-(3-Hydroxypropyl)phenyl]propanoic acid, η lactone (32). See Ref. 4 (compound **8c**).

3.7.4. 3-[2-(3-Hydroxybutyl)phenyl]propanoic acid, η lactone (33). Colorless oil, 0.121 g, 74%, purification by flash column chromatography (silica gel, pentane–Et_2O, 90/10). ^1H NMR (DMF-d_7 70 °C, 300 MHz, δ ppm): 7.39–7.20 (m, 4H, ArH), 4.93–4.85 (m, 1H), 3.41–3.32 (m, 1H), 2.99–3.12 (m, 2H), 2.66–2.78 (m, 2H), 2.54–2.63 (m, 1H), 2.34–2.42 (m, 1H), 2.07–2.14 (m, 1H), 1.38 (d, J=6.4 Hz, 3H). ^{13}C NMR (DMF-d_7, 70 °C, 75 MHz, δ ppm): 173.9, 142.5, 138.7, 130.8, 130.6, 126.9, 126.2, 70.9, 38.9, 37.3, 30.6, 27.1, 20.7. EIMS *m*/*z* (% relative abundance): 205 (13), 204 (10), 187 (12), 186 (66), 171 (13), 158 (11), 157 (15), 146 (13), 145 (29), 144 (100), 143 (29), 142 (12), 133 (15), 131 (44), 130 (13), 129 (55), 128 (33), 118 (42), 117 (99), 116 (22), 115 (73), 104 (15), 91 (34), 77 (11). IR ν_{max} in CH_2Cl_2 solution (cm^{-1}): 3058, 2929, 2856, 1728, 1604, 1492. HRMS (ESI) *m*/*z* Calcd for $C_{13}H_{17}O_2$ (M+H): 205.1229; Found: 205.1233.

3.7.5. 4-[2-(3-Hydroxybutyl)phenyl]butanoic acid, θ lactone (34). Colorless oil, 0.113 g, 65%, purification by flash column chromatography (silica gel, pentane–Et_2O, 90/10). ^1H NMR (DMF-d_7 70 °C, 300 MHz, δ ppm): 7.25–7.11 (m, 4H, ArH), 4.68–4.52 (m, 1H), 2.98–2.65 (m, 4H), 2.32–2.02 (m, 5H), 1.94–1.82 (m, 1H), 1.18 (d, J=6.2 Hz, 3H). ^{13}C NMR (DMF-d_7, 70 °C, 75 MHz, δ ppm): 172.8, 140.7, 139.2, 129.4, 128.8, 125.7, 125.6, 70.8, 36.8, 33.5, 28.3, 27.5, 26.7, 20.3. EIMS *m*/*z* (% relative abundance): 218 (21), 201 (17), 200 (100), 185 (16), 160 (11), 159 (10), 158 (50), 157 (21), 146 (17), 145 (68), 144 (18), 143 (79), 132 (11), 131 (88), 130 (62), 129 (59), 128 (16), 118 (27), 117 (81), 116 (16), 115 (45), 105 (12), 104 (10), 91 (33), 78 (15). IR ν_{max} in CH_2Cl_2 solution (cm^{-1}): 3017, 2977, 2932, 2867, 1725, 1492. HRMS (ESI) *m*/*z* Calcd for $C_{14}H_{19}O_2$ (M+H): 219.1385; Found: 219.1393.

3.7.6. 5-[2-(3-Hydroxybutyl)phenyl]pentanoic acid, ι lactone (35). Colorless oil, 0.11 g, 60%, purification by flash column chromatography (silica gel, pentane–Et_2O, 90/10). ^1H NMR (Acetone-d_6, 300 MHz, δ ppm): 7.18–7.07 (m, 4H, ArH), 5.11–5.02 (m, 1H), 3.03–2.96 (m, 2H), 2.62–2.47 (m, 3H), 2.33–2.25 (m, 1H), 1.92–1.75 (m, 5H), 1.53–1.35 (m, 1H), 1.27 (d, J=6.5 Hz, 3H). ^{13}C NMR (Acetone-d_6, 75 MHz, δ ppm): 172.8, 140.6, 139.9, 129.8, 129.3, 125.9, 125.8, 69.5, 36.4, 33.1, 29.9, 29.2, 25.5, 23.7, 18.1. EIMS *m*/*z* (% relative abundance): 232 (37), 214 (17), 185 (10), 157 (12), 156 (14), 146 (14), 145 (100), 144 (16), 143 (30), 132 (10), 131 (78), 130 (32), 129 (48), 128 (18), 117 (51), 116 (12), 115 (33), 105 (13), 91 (31). IR ν_{max} in CH_2Cl_2 solution (cm^{-1}): 3061, 2871, 1723, 1603, 1492. HRMS (ESI) *m*/*z* Calcd for $C_{15}H_{21}O_2$ (M+H): 233.1542; Found: 233.1554.

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Optically pure *trans*-2,3-disubstituted *N*-sulfinyl aziridines. Regio- and stereoselective opening mediated by the sulfinyl group

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Abstract—A new entry to optically pure *trans*-2,3-disubstituted *N*-sulfinyl aziridines starting from 1,2-aminosulfides, involving formation of a sulfonium salt intermediate followed by intramolecular nucleophilic attack by the sulfinamide nitrogen atom, is reported. The regio- and stereoselective opening of the aziridine ring can be achieved by anchimeric assistance of the sulfinyl group.

1. Introduction

Optically active aziridines are versatile building blocks that have found widespread use in organic synthesis.[1] Additionally, the aziridine nucleus is also included in some natural and biologically active products, such as mitomycins and azinomycins,[2] and has been used as ligands and auxiliaries in asymmetric synthesis.[1a,f,3]

Asymmetric methods for aziridine synthesis are mainly based on nitrene transfer or equivalents to olefins and carbene/carbenoids transfer to imines.[4] Alkene aziridination has allowed high enantioselectivities with cinnamate esters, chromene derivatives, and styrene but simple alkenes resulted in low enantioselectivity.[4c,5] The addition of chiral metallocarbenes to imines has been less successful.[6] Best results have been achieved by addition of ethyl diazoacetate to imines in the presence of chiral boron Lewis acid catalysts to afford *cis*-aziridines (up to 99% ee).[7] Recently, enantiopure *trans*-2-ethenylaziridines have been synthesized from allenyl zinc species and chiral *N*-sulfinylimines.[8] The use of *S*-chiral sulfinylimines with achiral bromoenolates[9] and α-halomethyl phosphonates[10] has also been studied, but only in the first case high levels of stereocontrol are observed.

The addition of achiral sulfonium and sulfoxonium ylides to enantiopure sulfinylimines has been described but only moderate diastereoselectivities were achieved.[11] As alternative method, aziridination of *N*-sulfonylimines with chiral ylides derived from the camphor skeleton[12] and Eliel's oxathiane[13] to afford *cis*-alkynylaziridines in moderate enantioselectivity and cis/trans mixtures in up to 99.9% ee has also been reported. Aggarwal et al. have developed one of the best catalytic imine aziridination procedure in which ylides were generated in situ from diazocompounds or, more safely, from the corresponding tosyl hydrazone salts and a chiral sulfide.[14] The procedure afforded aziridines in high ee with diastereoselectivities depending on the activating group on nitrogen (cis/trans ratio of ca. 1:3 and 1:6 using *N*-SES[14a,b] and *N*-TcBoc aldimines,[14c] respectively). The asymmetric aziridination of imines via aza-Darzens reaction has also been reported. Thus, addition of a chiral bromoacylsultam to imines afforded *cis*- or *trans*-aziridines with a high levels of diastereoselectivity.[15]

As most of these methods are quite efficient to prepare *cis*-aziridines whereas the trans selective aziridination remains less settled, indirect routes involving preparation and cyclization of enantiomerically enriched 1,2-amino alcohols[16] or 1,2-aminosulfides[17] stand as the most viable alternatives to prepare these isomers.

Recently, we have reported a highly stereoselective one-step asymmetric synthesis of *anti*-1,2-disubstituted 1,2-aminosulfide derivatives[18] (Scheme 1). We now report an efficient method to prepare *trans*-2,3-disubstituted aziridines starting from these aminosulfides. Moreover, as the resulting aziridines have a sulfinyl group at the appropriated position to act as an anchimeric assistant in the opening reactions of the aziridine rings, we have studied the ability of this group

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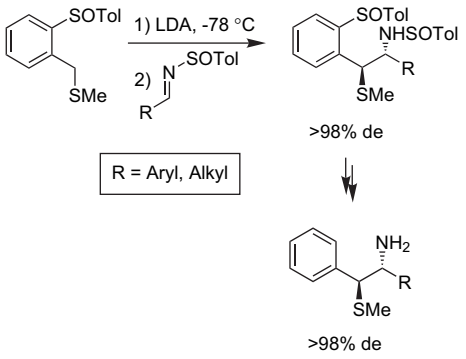


Scheme 1.

in controlling the regio- and stereoselectivity of these reactions. This anchimeric assistance has been reported in some reactions such as the hydrolysis of nitriles[19] and the addition of halogens to double bonds.[20]

2. Results and discussion

Aminosulfides **1a–e** and **1g** were synthesized as single diastereoisomers[18] by reaction of the lithium (*S*)-α-(methylthio)-2-(*p*-tolylsulfinyl)benzyl carbanion with (*S*)-*N*-*p*-tolylsulfinyl aldimines. For compound **1f**, a 96:4 mixture of two stereoisomers in 65% yield was achieved (see Section 4).

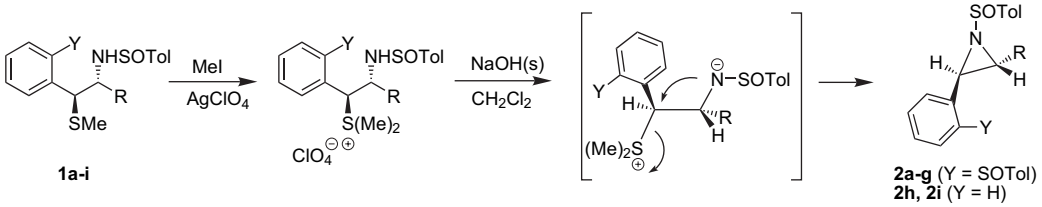
Aminosulfides **1a–g** were transformed into aziridines through a one pot procedure. This method involves the methylation at sulfenyl sulfur with MeI in the presence of $AgClO_4$. The resulting sulfonium salts were isolated simply by filtration of the crude reaction mixture through a pad of Celite followed by washing with CH_2Cl_2 and evaporate to dryness. Sulfonium salts were then transformed to the corresponding aziridines by cyclization with powered NaOH and quenching the reaction mixture with water. The overall process afforded analytically pure aziridines in moderate to high yield after one chromatographic purification. The scope of the aziridine synthesis is summarized in Table 1.

A range of 2,3-disubstituted aziridines bearing aromatic and heteroaromatic substituents of varying steric demand and electronic effects were accessed as single stereoisomers (de>98%; entries 1–6). No other isomer could be detected by NMR (300 MHz) from the reaction crudes. In addition, the synthesis of 2-alkyl-3-arylaziridine **2g** was carried out with analogous result (entry 7). The moderate yields obtained for **2b** and **2f** (entries 2 and 6) were due to the low solubility of the corresponding sulfonium salts in CH_2Cl_2. The use of more efficient solvents did not allow removing residual silver salts that partially inhibited the further cyclization step.

Hydrogenolysis of the Ar–SOTol bond can be made under mild conditions with organolithium compounds[21] and it was possible to remove the chiral auxiliary before cyclization step. It has been illustrated by C-desulfinylation of **1a** and **1g** with *t*-BuLi (1.8 equiv) followed by quenching with saturated NH_4Cl to afford the *N*-sulfinyl aminosulfides **1h** and **1i**, respectively, in nearly quantitative yield. Remarkably, the N-desulfinylation does not occur under these conditions. Subsequent intramolecular cyclization of the **1h** and **1i** takes place under the experimental conditions described above, affording 2,3-diphenyl and 2-butyl-3-phenyl *N*-sulfinyl aziridines, **2h** and **2i**, respectively, in good yields (entries 8 and 9). It suggests that the sulfinyl group at the aromatic ring has no significant role in the cyclization process.

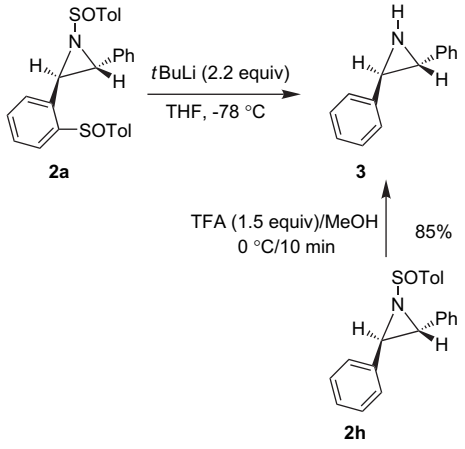
Although S_N1 and S_N2 processes are possible from benzylsulfonium salts,[22] the isolation of only one diastereoisomer in all the substrates shown in Table 1 suggests that cyclization must occur with inversion of the configuration at C-2 according to an internal S_N2 process (see scheme in Table 1). The intramolecular attack of the sulfinamide anion to the benzyl carbon must be much faster than heterolytic cleavage of the C–S bond to generate the benzylic carbocation. This is expected for aminosulfides **1a–g**, bearing a sulfinyl group, but it is also the case for **1h** and **1i** lacking of deactivating group at the ring. Moreover, the complete stereoselectivity observed in these reactions indicates that no base catalyzed epimerization occurred and the intermediate did not revert back to the corresponding ylide and imine.

Table 1. Synthesis of *N*-sulfinyl aziridines **2a–i**



Entry	Substrate	Y	R	Aziridine (% isolated yield)	de (%)
1	**1a**	SOTol	Ph	(2*R*,3*R*)-(−)-**2a** (78)	>98
2	**1b**	SOTol	*o*-BrC_6H_4	(2*R*,3*R*)-(+)-**2b** (43)	>98
3	**1c**	SOTol	*p*-$MeOC_6H_4$	(2*R*,3*R*)-(−)-**2c** (65)	>98
4	**1d**	SOTol	*p*-CNC_6H_4	(2*R*,3*R*)-(−)-**2d** (65)	>98
5	**1e**	SOTol	2-Naphthyl	(2*R*,3*R*)-(−)-**2e** (76)	>98
6	**1f**	SOTol	2-Pyridyl	(2*R*,3*R*)-(−)-**2f** (45)	>98
7	**1g**	SOTol	*n*-Bu	(2*R*,3*R*)-(+)-**2g** (75)	>98
8	**1h**	H	Ph	(2*R*,3*R*)-(+)-**2h** (77)	>98
9	**1i**	H	*n*-Bu	(2*R*,3*R*)-(+)-**2i** (76)	>98

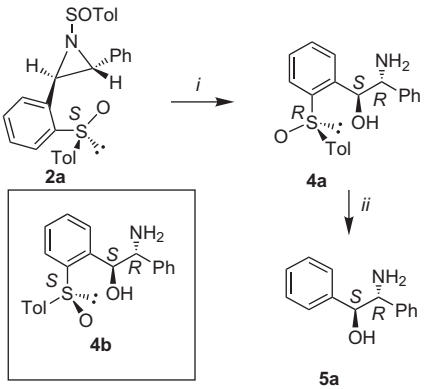
The trans-configuration for the aziridines **2a–i** was based on the reaction mechanism and clearly confirmed by the coupling constants of the vicinal hydrogens H-2 and H-3 of 4.1–4.7 Hz.[23] The absolute configuration was established for (2*R*,3*R*)-**2a** and for (2*R*,3*R*)-**2h** by chemical correlation with the known (+)-(*R*,*R*)-*trans*-2,3-diphenylaziridine **3**.[24] C-desulfinylation and N-desulfinylation of **2a**, in one pot reaction, take place in almost quantitative yield by reaction with *t*-BuLi (2.2 equiv). For **2h** the hydrolysis of the N–S bond was carried out by reaction with TFA (1.5 equiv)/MeOH without ring-opening (Scheme 2). The obtained aziridine **3** exhibited the same specific optical rotation as that reported in the literature,[24] so that it was considered to be enantiopure. These two reactions illustrate the way to obtain NH-aziridines from the *N*-sulfinyl aziridines **2a–i** shown in Table 1. It is remarkable that the cleavage of the N–S bond with *t*-BuLi is possible for **2a** but not for **1a**.[18] The similar behavior observed in all the cyclization reactions, all of them evolving in a completely stereoselective manner, suggests that the absolute configuration for compounds **2b–i** is identical to that determined for **2a**.



Scheme 2.

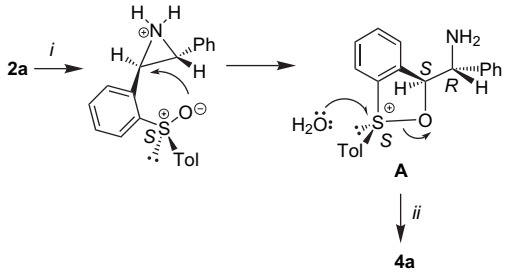
In order to study the influence of the sulfinyl group on the course of the opening ring we choose compound **2a**. According to the electronic effects of the aromatic rings, the nucleophilic processes evolving through unimolecular mechanisms (S_N1) should mainly take place on the benzyl carbon joined to the Ph group with epimerization. By contrast, reactions involving S_N2 mechanisms would be completely stereoselective but moderately regioselective. We first studied the reaction of **2a** with TFA in MeOH followed by reaction with aqueous HCl and neutralization. Only one aminoalcohol, **4a**, was formed under these conditions, which indicates that the opening of the aziridine ring of **2a** has occurred with a complete control of the regio- and stereoselectivity (Scheme 3).

The absolute configuration of the aliphatic chiral carbons at **4a** was established by chemical correlation with the (1*S*,2*R*)-2-amino-1,2-diphenylethane-1-ol **5a**.[25] This correlation was performed by reaction of **4a** with Ra–Ni.[26] The configuration at sulfur was established as *R* by comparison of the NMR data of **4a** with those of the **4b** (Scheme 3), previously obtained.[27] As they are different, both compounds should be diastereoisomers, which means that they differ in the configuration at sulfur.



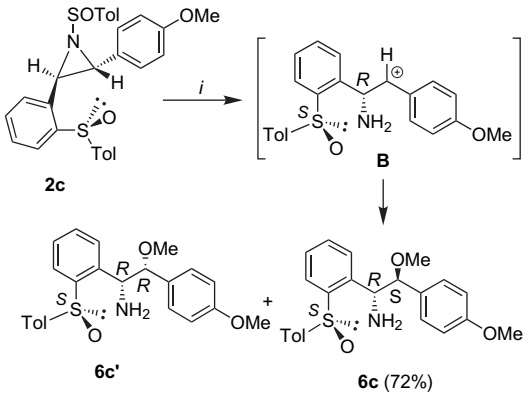
Scheme 3. Reagents and conditions: *i*. (1) TFA (1.5 equiv), MeOH, 0 °C, 15 min, (2) HCl(aq) 1 M followed by neutralization; *ii*. Ni–Ra, THF.

In order to explain these results, the anchimeric assistance of the sulfinyl group in the opening of the aziridine ring must be assumed. After the hydrolysis of the N–S bond by TFA, the protonation at aziridinic nitrogen must be followed by the intramolecular attack of the sulfinyl oxygen to form an oxysulfonium intermediate **A** (Scheme 4). The attack of the nucleophile (H_2O) to the sulfur of this intermediate, maybe catalyzed by the protonation at oxygen with HCl, would determine the formation of the sulfoxide **4a** with (*R*)-configuration at sulfur.



Scheme 4. Reagents and conditions: *i*. TFA (1.5 equiv), MeOH, 0 °C, 15 min; *ii*. HCl(aq) 1 M.

Finally we have studied the reaction of **2c** with TFA in methanol. In this case, a 80:20 mixture of two β-aminoethers, **6c** and **6′c**, was obtained (Scheme 5). They were purified by chromatography, but only the major one (**6c**) could be obtained in diastereomerically pure form. Regiochemistry as well as stereochemistry of **6c** have been determined by ^1H NMR. The major isomer (**6c**) exhibits a coupling constant in benzene (6.8 Hz), which increases in DMSO (8.0 Hz). It suggests that population of the conformation with and *anti* relationship between the coupled protons increases when intramolecular associations of the OH and NH_2 groups (hydrogen bonds) are minimized by the solvent. This behavior is only expected for the *erythro* isomer with the opposite configuration at the chiral carbons. Regiochemistry of **6c** was deduced from NOESY experiments performed in DMSO (Fig. 1) and benzene. It is remarkable that the proton joined to the oxygenated carbon exhibits a lower chemical shift than the one joined to the nitrogenated carbon, which can only be explained as a consequence of the anisotropic effect of the S–O bond, which reinforces the regiochemistry postulated for **6c**.



Scheme 5. Reagents and conditions: *i*. (1) TFA (1.5 equiv), MeOH, 0 °C, 15 min.

This is the typical result expected for S_N1 processes involving stable benzyl carbocations stabilized by the *p*-OMe group that can be attacked by the solvent, methanol, from any of its diastereotopic faces, affording **6c** and **6′c**. The low stability of the carbocations derived from **2a** determines that they cannot be spontaneously formed from the protonated species shown in Scheme 4. It is also remarkable that nucleophilic intramolecular attack of the sulfinyl oxygen to the carbocation **B** (it would yield the alcohol instead of the OMe derivative) is slower than the attack of the solvent. It suggests that the formation of a five-membered ring like **A** in Scheme 4 is much easier than the formation of a six-membered ring that must be formed from **B** of Scheme 5.

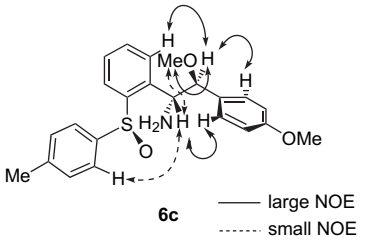


Figure 1. Effects of NOEs observed for NOESY experiments from **6c**.

3. Conclusion

In summary a new entry to enantiopure *trans*-*N*-sulfinyl aziridines starting from enantiopure 1,2-aminosulfides is reported. It involves their conversion into the corresponding 2-aminosulfonium salts followed by the completely stereoselective elimination of the sulfur moiety according to an internal S_N2 process. The anchimeric assistance of the sulfinyl group allows the complete regio- and stereocontrolled opening of some *ortho*-sulfinylphenyl aziridines in acidic medium.

4. Experimental

4.1. General

Dry solvents and liquid reagents were distilled under argon just prior to use. THF was distilled from sodium and benzophenone ketyl, CH_2Cl_2 was dried over P_2O_5, and diisopropylamine over KOH. *n*-BuLi (1.6 M solution in hexane) were purchased from Âcros. All reaction vessels, after being flame-dried, were kept under argon. Reactions were monitored by TLC on commercially available precoated plates (Merck silica gel 60 F_{254}). Column chromatography was performed by using Silica Gel Merck 60 (230–400 mesh) and *Varian* SCX column. Melting points were measured using a Gallemkamp apparatus in open capillary tubes and are uncorrected. Optical rotations were measured with a 141 Perkin–Elmer polarimeter. ^{1}H NMR spectra (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) spectra were performed with a Bruker AC-300 spectrometer. Chemical shifts (δ) are given in parts per million, relative to TMS, coupling constants (J) in hertz. Mass spectra were measured by electron impact (EI, 70 eV) or FAB with a VG AutoSpec spectrometer.

4.2. Synthesis of [1*R*,2*S*,(*S*)*S*]-*N*-{1-pyridyl-2-[(*S*)-2-(*p*-toluenesulfinyl)phenyl]-2-(methylthio)ethyl}-*p*-toluene sulfinamide,[18] 1f

A solution of *n*-BuLi (0.60 mmol, 1.6 M in hexane, 1.2 equiv) was added over ^{i}Pr$_2$NH (0.89 mmol, 1.8 equiv) in THF (3 ml) at 0 °C. After 45 min stirring, the mixture was cooled at −78 °C and then a solution of (*S*)-α-methylthio-2-(*p*-tolylsulfinyl)toluene (138 mg, 0.50 mmol, 1.0 equiv) in THF (2 ml) was added. After 5 min stirring, *N*-(2-pyridinemethylidene)-*p*-toluene sulfinamide[28] (244 mg, 1.0 mmol, 2.0 equiv) dissolved in THF (4 ml) was added at −78 °C. When the reaction was completed (5 min), the mixture was hydrolyzed at that temperature with saturated aqueous NH_4Cl (2 ml) and extracted with CH_2Cl_2 (3×10 ml). The combined organic extracts were dried with $MgSO_4$ and the solvent was removed under reduced pressure to afford a mixture of epimers in C_1, in 96:4 ratio. The residue was purified by flash-column chromatography (AcOEt/hexane 4:1) to give pure aminosulfide **1f** (60%): $[\alpha]_D^{20}$ +18.4 (*c* 0.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.63 (dd, 1H, *J* 4.6, 1.5, H-arom.), 8.11 and 7.66 (2dd, 2H, *J* 7.2, 1.5, H-arom.), 7.69–7.57 (m, 3H, H-arom.), 7.33 (dd, 1H, *J* 7.7, 0.9, H-arom.), 7.26–7.22 (m, 1H, H-arom.), 7.60, 6.94, 6.87, and 6.50 (2 AA′BB′ systems, 8H, H-arom.), 4.90 (d, 1H, *J* 10.5, H-C2), 4.68 (t, 1H, *J* 10.5, H-C1), 4.39 (d, 1H, *J* 10.5, –NH), 2.34 and 2.11 (2s, 6H, 2CH_3C$_6$H$_4$–), 1.63 (s, 3H, CH_3S–); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.2, 144.5, 141.8, 141.4, 140.6 and 139.6 (C-arom.), 139.7, 136.5, 131.8, 129.6 (2C), 129.0 (2C), 128.9, 128.2, 126.1 (2C), 125.0 (2C), 124.5, 123.4 and 123.1 (CH-arom.), 65.1 and 49.7 (C-1 and C-2), 21.2 (2CH_3C$_6$H$_4$–), 14.8 (CH_3S–). HRMS calcd for $C_{28}H_{28}N_2O_2S_3$: 520.1313; found, 520.1307.

4.3. General procedure for the reactions of aziridination summarized in Table 1

To a solution of 1,2-aminosulfides [(**1a**–**i**), 0.1 mmol] in methyliodide (1 ml) was added silver perchlorate (20.8 mg, 0.1 mmol). The mixture was stirred for 3 h at room temperature and then filtered through a pad of Celite, the filtrate was washed with CH_2Cl_2. The solvent was evaporated to give the required salt, as a white solid, which was used without further purification. To a solution of the appropriate salt (1 mmol) in CH_2Cl_2 (5 ml) was added powered

KOH (4.1 mg, 0.062 mmol). The mixture was stirred for 1 h at room temperature. The reaction was hydrolyzed with 4 ml of water, and the mixture was extracted with CH_2Cl_2 (3×4 ml). The organic layer was dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by flash-column chromatography.

4.3.1. *trans*-(2*R*,3*R*)-2-Phenyl-3-[(*S*)-2-(*p*-toluenesulfinyl)-phenyl]-1-[(*S*)-(*p*-toluenesulfinyl)]aziridine, 2a. Eluent for chromatography: hexane/Et_2O 1:4. Yield: 78%; white syrup; $[\alpha]_D^{20}$ −23.6 (*c* 0.9, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 8.02 (dd, 1H, *J* 7.8, 0.9, H-arom.), 7.56–7.19 (m, 12H, H-arom.), 4.22 and 4.14 (2d, 2H, *J* 4.4, H-C2 and H-C3), 2.36 and 2.35 (2s, 6H, 2C$H_3C_6H_4$–); ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.1, 142.6, 142.0, 141.6, 141.0, 133.8 and 132.7 (C-arom.), 130.9, 130.1, 129.4, 129.3, 128.5, 128.4, 128.3, 126.4, 124.8 and 124.5 (CH-arom.), (C-2 and C-3 are missing), 21.4 and 21.3 (2$CH_3C_6H_4$–). HRMS calcd for $C_{21}H_{18}NOS$ (M^+−SOTol): 332.1109; found, 332.1106.

4.3.2. *trans*-(2*R*,3*R*)-2-(*o*-Bromophenyl)-3-[(*S*)-2-(*p*-toluenesulfinyl)phenyl]-1-[(*S*)-(*p*-toluenesulfinyl)]aziridine, 2b. Eluent for chromatography: hexane/Et_2O 2:1. Yield: 43%; white syrup; $[\alpha]_D^{20}$ +48.3 (*c* 0.2, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.96 (d, 1H, *J* 7.7, H-arom.), 7.59, 7.58, 7.45, and 7.44 (2 AA′BB′ systems, 8H, H-arom.), 7.56–7.50 (m, 1H, H-arom.), 7.34–7.17 (m, 6H, H-arom.), 4.33 and 4.29 (2d, 2H, *J* 4.7, H-C2 and H-C3), 2.38 (s, 6H, C$H_3C_6H_4$–); ^{13}C NMR (75 MHz, $CDCl_3$): δ 154.2, 151.8, 142.9, 142.2, 142.0, 141.6, 141.0 and 132.3 (C-arom.), 132.4, 131.1, 130.1 (2C), 129.9, 129.6, 129.4 (2C), 128.7, 127.4, 126.4 (2C), 124.8 and 124.6 (3C, CH-arom.), (C-2 and C-3 are missing), 21.4 (2$CH_3C_6H_4$–). HRMS calcd for $C_{21}H_{17}BrNOS$ (M^+−SOTol): 410.0214; found, 410.0219.

4.3.3. *trans*-(2*R*,3*R*)-2-(*p*-Methoxyphenyl)-3-[(*S*)-2-(*p*-toluenesulfinyl)phenyl]-1-[(*S*)-(*p*-toluenesulfinyl)]aziridine, 2c. Eluent for chromatography: hexane/Et_2O 1:2. Yield: 65%; yellow syrup; $[\alpha]_D^{20}$ −44.1 (*c* 0.5, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.98 (d, 1H, *J* 7.8, H-arom.), 7.45 (dd, 4H, *J* 8.0, 6.1, H-arom.), 7.34 (dd, 4H, *J* 6.4, 2.5, H-arom.), 7.23–7.13 (m, 5H, H-arom.), 6.92 (d, part of AA′BB′ system, 2H, H-arom.), 4.27 and 4.00 (2d, 2H, *J* 4.1, H-C2 and H-C3), 3.83 (s, 3H, CH_3O–C_6H_4–), 2.36 and 2.35 (2s, 6H, 2C$H_3C_6H_4$–). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.7, 144.8, 142.0, 141.6, 141.5, 141.2, 141.0 and 133.4 (C-arom.), 132.0, 131.0, 130.8 (2C), 130.5, 130.2 (2C), 129.4 (2C), 128.0, 126.2 (2C), 124.8 (2C) and 114.3 (2C, CH-arom.), 55.2 ($CH_3OC_6H_4$–), (C2 and C3 are missing), 21.4 (2$CH_3C_6H_4$–). HRMS calcd for $C_{22}H_{20}NO_2S$ (M^+−SOTol): 362.1215; found, 362.1225.

4.3.4. *trans*-(2*R*,3*R*)-2-(*p*-Cyanophenyl)-3-[(*S*)-2-(*p*-toluenesulfinyl)phenyl]-1-[(*S*)-(*p*-toluenesulfinyl)]aziridine, 2d. Eluent for chromatography: hexane/Et_2O 1:2. Yield: 65%; white syrup; $[\alpha]_D^{20}$ −27.0 (*c* 0.6, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.97 (d, 1H, *J* 7.8, H-arom.), 7.65 (d, part of AA′BB′ system, 2H, H-arom.), 7.59–7.29 (m, 5H, H-arom.), 7.44, 7.43, 7.23, and 7.22 (2 AA′BB′ systems, 8H, H-arom.), 4.21 and 4.17 (2d, 2H, *J* 4.1, H-C2 and H-C3), 2.37 and 2.36 (2s, 6H, C$H_3C_6H_4$–); ^{13}C NMR (75 MHz, $CDCl_3$): δ 143.2, 142.3, 142.1, 142.0, 140.6, 140.0, 133.8 and 133.2 (C-arom.), 132.3, 131.1, 130.1, 129.7, 129.6, 128.9, 128.6, 126.4, 124.7 and 124.5 (CH-arom.), 59.9 and 41.9 (C-2 and C-3), 21.4 and 21.2 (2$CH_3C_6H_4$–). HRMS calcd for $C_{22}H_{17}N_2OS$: (M^+−SOTol): 357.1062; found, 357.1054.

4.3.5. *trans*-(2*R*,3*R*)-2-(2-Naphthyl)-3-[(*S*)-2-(*p*-toluenesulfinyl)phenyl]-1-[(*S*)-(*p*-toluenesulfinyl)]aziridine, 2e. Eluent for chromatography: hexane/AcOEt 1:1. Yield: 76%; white syrup; $[\alpha]_D^{20}$ −91.5 (*c* 0.6, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 8.04 (dd, 1H, *J* 7.8, 1.0, H-arom.), 7.85, 7.19, and 7.20 (part of AA′BB′ system, 4H, H-arom.), 7.56–7.30 (m, 15H, H-arom.), 4.32 and 4.30 (2d, 2H, *J* 4.3, H-C2 and H-C3), 2.35 and 2.34 (2s, 6H, 2C$H_3C_6H_4$–); ^{13}C NMR (75 MHz, $CDCl_3$): δ 145.1, 142.7, 142.1, 141.6, 141.0, 133.2, 133.0, 132.7 and 131.4 (C-arom.), 131.0, 130.1, 129.5, 129.4, 128.7, 128.3, 128.1, 128.0, 127.7, 126.5, 126.4, 125.3, 124.8 and 124.6 (CH-arom.), (C-2 and C-3 are missing), 21.4 (2$CH_3C_6H_4$–). HRMS calcd for $C_{25}H_{20}$ NOS (M^+−SOTol): 382.1266; found, 382.1252.

4.3.6. *trans*-(2*R*,3*R*)-2-(Pyridyl)-3-[(*S*)-2-(*p*-toluenesulfinyl)phenyl]-1-[(*S*)-(*p*-toluenesulfinyl)]aziridine, 2f. Eluent for chromatography: hexane/Et_2O 1:2. Yield: 45%; white syrup; $[\alpha]_D^{20}$ −46.3 (*c* 0.3, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.97 (d, 1H, *J* 7.9, H-arom.), 7.71 (dd, 1H, *J* 7.9, 1.7, H-arom.), 7.50–7.29 (m, 5H, H-arom.), 7.48, 7.45, 7.20, and 7.19 (2 AA′BB′ systems, 8H, H-arom.), 4.48 and 4.19 (2d, 2H, *J* 4.1, H-C2 and H-C3), 2.35 (s, 6H, C$H_3C_6H_4$–); ^{13}C NMR (75 MHz, $CDCl_3$): δ 154.9, 144.9, 142.5, 141.9, 141.6, 140.9 and 132.7 (C-arom.), 139.5, 136.7, 131.0 (3C), 130.1, 129.4 (2C), 128.6, 126.2 (2C), 124.8 (2C), 124.3, 123.4 and 123.2 (CH-arom.), (C2 and C3 are missing), 21.4 (2$CH_3C_6H_4$–).

4.3.7. *trans*-(2*R*,3*R*)-2-Butyl-3-[(*S*)-2-(*p*-toluenesulfinyl)-phenyl]-1-[(*S*)-(*p*-toluenesulfinyl)]aziridine, 2g. Eluent for chromatography: hexane/Et_2O 1:3. Yield: 75%; colorless oil; $[\alpha]_D^{20}$ +27.2 (*c* 0.3, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.87 (dd, 1H, *J* 7.7, 1.1, H-arom.), 7.49, 7.45, 7.25, and 7.18 (2 AA′BB′ systems, 8H, H-arom.), 7.36 (dt, 1H, *J* 7.7, 1.1, H-arom.), 7.25 and 6.75 (2d, 2H, *J* 7.7, H-arom.), 3.81 (d, 1H, *J* 4.1, H-C3), 2.74 (dt, 1H, *J* 6.4, 4.1, H-C2), 2.38 and 2.35 (2s, 6H, 2C$H_3C_6H_4$–), 2.07–1.38 (3m, 6H, $CH_3(CH_2)_3$–), 0.96 (t, 1H, *J* 7.2, C$H_3(CH_2)_3$–); ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.4, 142.8, 141.7, 141.4, 141.3 and 134.8 (C-arom.), 130.9, 130.1, 129.3, 128.5, 126.7, 125.8, 124.7 and 124.0 (CH-arom.), 49.8 and 33.9 (C-2 and C-3), 30.3, 28.2 and 22.4 ($CH_3(CH_2)_3$–), 21.4 and 21.3 (2$CH_3C_6H_4$–), 13.9 ($CH_3(CH_2)_3$). HRMS calcd for $C_{19}H_{22}NOS$ (M^+−SOTol): 312.1422; found, 312.1420.

4.3.8. *trans*-[2*R*,3*R*,(*S*)*S*]-2,3-Diphenyl-1-(*p*-toluenesulfinyl)aziridine, 2h. Eluent for chromatography: hexane/Et_2O 4:1. Yield: 77%; white syrup; $[\alpha]_D^{20}$ +117.9 (*c* 0.2, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.60 (d, 1H, *J* 8.2, H-arom.), 7.45–7.32 (m, 8H, H-arom.), 7.23 (d, 1H, *J* 8.2, H-arom.), 4.17 (s, 2H, H-C2 and H-C3), 2.36 (s, 3H, C$H_3C_6H_4$–); ^{13}C NMR (75 MHz, $CDCl_3$): δ 143.0, 141.3 and 134.7 (C-arom.), 129.4 (2C), 128.4 (3C), 128.1 (3C) and 124.9 (2C, CH-arom.), 43.7 (C2 and C3), 21.3 ($CH_3C_6H_4$–).

4.3.9. *trans*-[2*R*,3*R*,(*S*)*S*]-2-Butyl-3-phenyl-1-(*p*-toluenesulfinyl)aziridine, 2i. Eluent for chromatography: hexane/Et_2O 4:1. Yield: 76%; colorless oil; $[\alpha]_D^{20}$ +16.0 (*c* 0.6, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.61 and 7.22 (AA′BB′ system, 4H, H-arom.), 7.26–7.16 (m, 3H, H-arom.), 7.12 (dd, 2H, *J* 7.7, 2.2, H-arom.), 3.67 (d, 1H, *J* 4.1, H-C3), 2.75–2.70 (m, 1H, H-C2), 2.10–1.21 (2m, 6H, $-(CH_2)_3CH_3$), 0.95 (t, 3H, *J* 7.1, $-(CH_2)_3CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 143.2, 141.2 and 137.1 (C-arom.), 129.4 (2C), 128.2 (2C), 127.3, 126.8 (2C) and 125.0 (2C, CH-arom.), 49.2 and 37.5 (C2 and C3), 30.4, 28.2 and 22.3 ($-CH_2)_3CH_3$), 21.3 ($CH_3C_6H_4$–), 14.0 ($-(CH_2)_3CH_3$).

4.4. Representative procedure for C–S desulfinylation

To a stirred solution of **1a** and **1h** (0.12 mmol) in THF (2 ml) was added *t*-BuLi (0.15 ml, 0.22 mmol, 1.5 M in hexane, 1.8 equiv). When the reaction was completed (5 min), the mixture was hydrolyzed with saturated aqueous NH_4Cl (1 ml) and extracted with CH_2Cl_2 (3×3 ml). The combined organic layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by flash-column chromatography.

4.4.1. [1*R*,2*S*,(*S*)*S*]-*N*-[1,2-(Diphenyl)-2-(methylthio)ethyl]-*p*-toluene sulfinamide, 1h. This product was obtained from **1a**. Eluent for chromatography: hexane/Et_2O 1:1; quantitative yield; colorless syrup; spectroscopic and optical rotation data of compound **1h** are coincident with those previously reported: $[\alpha]_D^{20}$ +155.0 (*c* 0.7, $CHCl_3$) [lit.[18]=+155.8 (*c* 0.5, $CHCl_3$)]; ^1H NMR (300 MHz, $CDCl_3$): δ 7.33–7.21 (m, 7H, H-arom.), 7.20–7.15 (m, 3H, H-arom.), 7.00–6.98 (m, 2H, H-arom.), 4.76 (dd, 1H, *J* 6.1, 6.5, H-C1), 4.75 (br s, 1H, –NH), 4.21 (d, 1H, *J* 6.5, H-C2), 2.31 (s, 3H, $CH_3C_6H_4$–), 1.84 (s, 3H, CH_3S–).

4.4.2. [1*R*,2*S*,(*S*)*S*]-*N*-[1-Butyl-2-phenyl-2-(methylthio)ethyl]-*p*-toluene sulfinamide, 1i. This product was obtained from **1g**. Eluent for chromatography: hexane/Et_2O 4:1; quantitative yield; colorless oil; $[\alpha]_D^{20}$ +180.6 (*c* 0.7, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.53 and 7.40 (AA′BB′ system, 4H, H-arom.), 7.34–7.23 (m, 5H, H-arom.), 4.26 (d, 1H, *J* 9.4, H-C2), 4.18 (d, 1H, *J* 5.0, –NH), 3.65–3.56 (m, 1H, H-C1), 2.39 (s, 3H, $CH_3C_6H_4$–), 1.96 (s, 3H, CH_3S–), 1.51–0.92 (m, 6H, $CH_3(CH_2)_3$–), 0.78 (t, 1H, *J* 7.0, $CH_3(CH_2)_3$–); ^{13}C NMR (75 MHz, $CDCl_3$): δ 142.0, 141.0 and 139.2 (C-arom.), 129.2 (2C), 128.9 (2C), 128.3 (2C), 127.2 and 125.7 (2C, CH-arom.), 59.4 and 59.3 (C-1 and C-2), 32.1, 27.8 and 22.1 ($CH_3(CH_2)_3$–), 21.4 and 21.2 ($2CH_3C_6H_4$–), 15.0 and 13.8 (CH_3S– and $CH_3(CH_2)_3$). HRMS calcd for $C_{19}H_{22}NOS$: 312.1422; found, 312.1421.

4.4.3. (+)-(2*R*,3*R*)-2,3-Diphenylaziridine, 3. This product was obtained from **2a** following the procedure described above but using 2.2 equiv of *t*-BuLi. The product was purified by SCX column chromatography. Yield: 75%; colorless syrup; $[\alpha]_D^{20}$ +320 (*c* 0.5, $CHCl_3$) [lit.[24]=328.8 (*c* 1.25, $CHCl_3$)]; spectroscopic data of compound **3** are coincident with those previously reported:[24] ^1H NMR (300 MHz, $CDCl_3$): δ 7.29–7.40 (m, 10H, H-arom.), 3.12 (s, 2H, H-C2 and H-C3), 1.70 (br s, 1H, –NH).

4.5. Representative procedure N–S desulfinylation

Method A: To a stirred solution of aziridine **2a** (0.05 mmol) in methanol (1 ml) was added TFA (6.3 μl, 0.08 mmol, 1.5 equiv). After the mixture was stirred for 15 min at 0 °C, the solvent was evaporated, and the residue was filtered through SCX column chromatography. The reaction mixture was diluted in methanol (1 ml) and then HCl (0.5 ml, 1 N) was added. After 1 h, concentrated NH_3 was added until the solution was brought to pH 10. The solvent was evaporated, and the residue was purified by SCX column chromatography to afford the corresponding aminoalcohol.

4.5.1. (1*R*,2*S*)-2-Amine-2-phenyl-1-[(*R*)-(2-*p*-toluenesulfinyl)phenyl]-ethanol, 4a. Eluent for chromatography: NH_3/methanol (7 M). Yield: 70%; colorless oil; $[\alpha]_D^{20}$ +40 (*c* 0.3, EtOH); ^1H NMR (300 MHz, $CDCl_3$): δ 7.84 (d, 1H, *J* 7.4, H-arom.), 7.77 (d, 1H, *J* 6.7, H-arom.), 7.60 (dd, 1H, *J* 7.4, 6.7, H-arom.), 7.48–7.32 (m, 6H, H-arom.), 7.26–7.17 (m, 4H, H-arom.), 4.93 (br s, 1H, HO-C1), 4.84 and 4.44 (2d, 2H, *J* 8.3, H-C1 and H-C2), 2.36 (s, 3H, $CH_3C_6H_4$–). HRMS calcd for $C_{21}H_{22}NO_2S$ (M^++1): 352.1365; found, 352.1341.

Method B: To a stirred solution of aziridine **2c** (0.05 mmol) in methanol (1 ml) was added TFA (6.3 μl, 0.08 mmol, 1.5 equiv). After the mixture was stirred for 15 min at 0 °C, the solvent was evaporated, and the residue was purified by flash-column chromatography (CH_2Cl_2/MeOH 95:5) to give a mixture of amines **6c** and **6′c** in a 80:20 ratio (yield 90%). Only the major product could be isolated as pure compound.

4.5.2. (1*R*,2*S*)-2-Methoxy-2-(*p*-methoxyphenyl)-1-[(*S*)-(2-*p*-toluenesulfinyl)phenyl]-ethylamine, 6c. White syrup; $[\alpha]_D^{20}$ −127.1 (*c* 0.7, $CHCl_3$); ^1H NMR (300 MHz, DMSO-d_6): δ 7.56 (m, 2H, H-arom.), 7.43–7.32 (m, 2H, H-arom.), 7.48 and 7.28 (AA′BB′ system, 4H, $CH_3C_6H_4$–), 7.01 and 6.75 (AA′BB′ system, 4H, H-arom.), 4.46 (d, 1H, *J* 7.98, H-C1), 4.19 (d, 1H, *J* 7.98, H-C2), 3.67 (s, 3H, CH_3O–C_6H_4–), 3.12 (s, 3H, CH_3O-C2), 2.29 (s, 3H, $CH_3C_6H_4$–); ^{13}C NMR (75 MHz, DMSO-d_6): δ 158.8, 144.0, 143.2, 141.0 and 140.3 (C-arom.), 130.7 (CH-arom.), 130.4 (C-arom.), 130.1 and 128.8 (2C, CH-arom.), 128.4 and 128.3 (2C, CH-arom.), 125.1 (2C), 123.2 and 113.5 (2C, CH-arom.), 87.6 (C-2), 56.5 and 56.4 (C-1 and CH_3O-C-2), 55.1 ($CH_3OC_6H_4$–), 21.0 ($CH_3C_6H_4$–). ^1H NMR (300 MHz, C_6D_6): δ 8.21 (m, 1H, H-arom.), 7.66 (m, 3H, H-arom.), 7.24–7.09 (m, 4H, H-arom.), 6.85 (m, 4H, H-arom.), 4.89 (br s, 1H, H-C1), 4.30 (d, 1H, *J* 6.80, H-C2), 3.35 (s, 3H, CH_3O–C_6H_4–), 3.20 (s, 3H, CH_3O-C2), 1.97 (s, 3H, $CH_3C_6H_4$–). HRMS calcd for $C_{23}H_{26}NO_3S$ (M^++1): 396.1627; found, 396.1616.

4.5.3. (1*R*,2*R*)-2-Methoxy-2-(*p*-methoxyphenyl)-1-[(*S*)-(2-*p*-toluenesulfinyl)phenyl]-ethylamine, 6′c. White syrup; ^1H NMR (300 MHz, $CDCl_3$), described from a mixture of compounds **6c** and **6′c**: δ 7.96 and 7.60 (2dd, 2H, *J* 7.5, 1.7, H-arom.), 7.55–7.37 (m, 2H, H-arom.), 7.48, 7.22, 7.11, and 6.86 (2 AA′BB′ systems, 8H, H-arom.), 4.58 and

4.30 (2d, 2H, *J* 6.3, H-C1 and H-C2), 3.81 (s, 3H, CH_3O–C_6H_4–), 3.13 (s, 3H, CH_3O-C2), 2.34 (s, 3H, C$H_3C_6H_4$–).

4.6. Representative procedure for C–S desulfinylation with Ni–Ra

A solution of compound **4a** (0.05 mmol) in THF (1 ml) was added to a suspension of activated Raney nickel (350 mg) in THF (1 ml). The mixture was stirred for 2 h, and the crude was purified by SCX column to afford the aminoalcohol **5a**.

4.6.1. (1*S*,2*R*)-2-Amino-1,2-diphenylethanol, 5a. Yield: 68%; $[\alpha]_D^{20}$ +6.9 (*c* 0.5, EtOH) [lit.[25]=+7.0 (*c* 0.6, EtOH)]; spectroscopic data of compound **5a** are coincident with those previously reported:[25] ^{1}H NMR (300 MHz, CD_3OD): δ 7.30–7.19 (m, 10H, H-arom.), 4.81 and 4.04 (2d, 2H, *J* 6.1, H-C1 and H-C2).

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Efficient synthesis of pyrroles and 4,5,6,7-tetrahydroindoles via palladium-catalyzed oxidation of hydroxy-enamines

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Abstract—Facile and one-pot synthetic route of poly-substituted pyrroles and 4-oxo-4,5,6,7-tetrahydroindoles is established, which consists of three steps: (1) palladium-catalyzed oxidation of hydroxy-enamines by using tetrakis(triphenylphosphine)palladium and mesityl bromide oxidation system, (2) intramolecular cyclization, and (3) dehydration.

1. Introduction

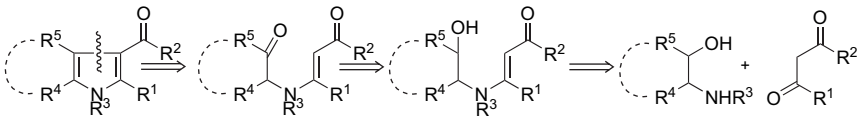
Highly functionalized pyrroles are subunits of considerable importance in heme, chlorophyll, bile pigments, vitamin B_{12}, and marine source-derived pyrrole alkaloids.[1] Since Knorr reported the first synthesis of pyrroles,[2] a series of papers have been published on the syntheses of substituted pyrroles.[3] For example, the pyrrole synthesis by the Lewis acid-mediated reaction of readily available 2-acetoxypropanal *N*,*N*-dimethylhydrazone with cyclic and open chain silyl enol ethers was reported by Enders et al.,[1] low valent titanium-mediated approach to pyrroles by Fürstner et al.,[4] pyrrole synthesis via [2+3] cycloaddition reactions of *S*-methyl *N*-(benzotriazol-1-ylmethyl)thioamide with α,β-unsaturated esters, ketones, nitriles, and vinylpyridines by Pindur and Adam,[5] and convenient synthesis of 2-cyanopyrroles from isocyanoacetonitrile by Adamczyk and Reddy.[6] 4-Substituted indole nucleus is also an important subunit in a wide range of biologically active natural products, whose construction has been a topic of interest for many years.[7]

There are a few reports on the palladium-catalyzed oxidation of hydroxyl groups such as efficient oxidation of alcohols with CCl_4 in the presence of bases by using palladium acetate or palladium chloride,[8] palladium-catalyzed oxidation of secondary alcohols by the use of bromobenzene as an oxidant,[9–11] and palladium-catalyzed oxidation of primary and secondary alcohols to carbonyl compounds under phase transfer catalyst conditions.[12] In the present paper, we describe an efficient synthetic procedure of poly-substituted pyrroles and 4-oxo-4,5,6,7-tetrahydroindoles, by the palladium-catalyzed oxidation of hydroxy-enamines to the corresponding pyrroles and indoles, part of which has been previously reported in our communication.[13]

2. Results and discussion

Our synthetic strategy to prepare pyrroles and indoles via the palladium-catalyzed oxidation is shown in Scheme 1. Poly-substituted pyrroles and 4-oxo-4,5,6,7-tetrahydroindoles are to be synthesized by the oxidation of β-hydroxy-enamines, which can be obtained by the condensation of amino alcohols and carbonyl compounds.

First, β-hydroxy-enamines **3** were prepared by condensation of amino alcohol **2** with β-ketoester or β-diketone **1** in the presence of 4 Å molecular sieves, in moderate to good yields.



Scheme 1.

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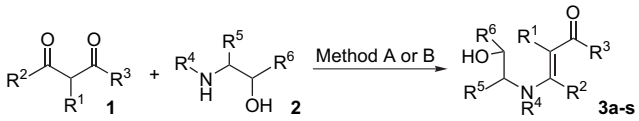
Table 1. Preparation of β-hydroxy-enamines



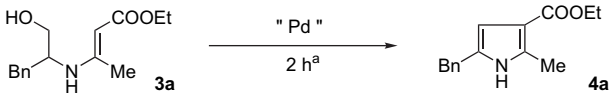
Entry	Ketones (**1**)			β-Amino alcohols (**2**)			Methods[a]	Products (**3**) (yields %)[b]
	R^1	R^2	R^3	R^4	R^5	R^6		
1	H	Me	OEt	H	Bn	H	A	**3a** (96)
2	H	Me	OEt	H	Ph	H	A	**3b** (97)
3	H	Me	OEt	H	^{i}Pr	H	A	**3c** (93)
4	H	Me	OEt	H	H	H	A	**3d**[15] (73)
5	H	Me	OEt	H	H	Me	A	**3e** (84)
6	H	Me	OEt	H	H	Ph	A	**3f** (99)
7	H	Me	Me	H	Me	H	A	**3g** (77)
8	H	Ph	OEt	H	Bn	H	B[c]	**3h** (50)
9	H	Me	OEt	Me	H	H	B	**3i** (52)
10	H	Me	OEt	–$(CH_2)_3$–		H	A	**3j** (97)
11	–$(CH_2)_3$–		OEt	H	Bn	H	A	**3k** (54)
12	–$(CH_2)_4$–		OEt	H	Bn	H	B	**3l** (97)
13	H	Me	OEt	H		–$(CH_2)_4$–	A	**3m** (93)
14	H	–$(CH_2)_3$–		H	H	H	B[d,e]	**3n** (60)
15	H	–$(CH_2)_3$–		H	Ph	H	A[f]	**3o**[16] (80)
16	H	–$(CH_2)_3$–		H	H	Ph	B[d,g]	**3p**[17] (78)
17	H	–$(CH_2)_3$–		H	Me	H	B[d,h]	**3q** (91)
18	H	–$(CH_2)_3$–		H	H	Me	B[d,i]	**3r** (84)
19	H	–$(CH_2)_3$–		H		–$(CH_2)_4$–	B[d,j]	**3s** (89)

[a] Method A: a THF solution (20 mL) of ketone (7.9 mmol), β-amino alcohol (9.4 mmol), and 4 Å molecular sieves (5.0 g) was stirred at room temperature for 7 days under argon. Method B: a benzene solution (30 mL) of ketone (11.7 mmol), β-amino alcohol (14.9 mmol), and 4 Å molecular sieves (15.0 g) was refluxed for 12 h under argon.
[b] Isolated yields.
[c] *p*-TsOH·H_2O was employed.
[d] THF was used as the solvent instead of benzene.
[e] Reaction time: 6 h.
[f] Reaction time: 144 h.
[g] Reaction time: 6.5 h.
[h] Reaction time: 2 h.
[i] Reaction time: 4 h.
[j] Reaction time: 7 h.

Amino alcohol **2** was obtained commercially or easily synthesized by lithium aluminum hydride reduction of amino acids.[14] Preparation of various β-hydroxy-enamines **3a–s** under several different reaction conditions are summarized in Table 1. Then, **3a** was converted to **4a**. Different reaction conditions examined were as summarized in Table 2. Palladium catalyst, aryl halide, and base were all shown to be indispensable components for the reaction (entries 8, 18, and 24 in Table 2). Entries 1–3 in Table 2 demonstrated that better results were to be obtained, when the reaction temperature was at 150 °C. Entries 5–7 may also support the conclusion; so all the reactions were performed at about 150 °C. As regards the solvent, DMF was found to be more efficient (entries 1 vs 4). As regards base component, potassium carbonate at the ratio between the base and β-hydroxy-enamines of 2:1 was found to give generally better results. Potassium hydroxide, potassium acetate, triethylamine, and silver carbonate were found to be less efficient (entries 1 vs 11–14 in Table 2). As regards the aryl halide component, bromobenzene gave better results than iodobenzene or chlorobenzene (entries 1 vs 15 and 16 in Table 2). According to Tamaru et al.,[9–11] mesityl bromide is an efficient oxidant in the reaction of this type. In our experiment, mesityl bromide was also shown to be the aryl halide component giving the highest yield (entry 17 in Table 2). As regards the amount of aryl halide to be used, in the case of bromobenzene, its ratio to β-hydroxy-enamine was shown to be above 1:1 (entries 1 vs 19–22 in Table 2). As regards palladium catalyst, palladium(II) acetate and palladium(II) chloride were also effective (entries 25–28 in Table 2). The Swern, PCC, and PDC oxidation of β-hydroxy-enamine **3a** led to the decomposition of **3a**. In the series of experiments presently performed, the optimum reaction conditions for the preparation of **4a** are given in entry 17 of Table 2.

Then, poly-substituted pyrroles and 4-oxo-4,5,6,7-tetrahydroindoles were prepared via palladium-catalyzed oxidation of various β-hydroxy-enamines as shown in Tables 3 and 4, and Scheme 2. Palladium-catalyzed oxidation of β-hydroxy-enamines **3a–h** gave the corresponding poly-substituted pyrroles (**4a–h**) in moderate to good yields (entries 1–8 in Table 3), except in the case of **3i**, in which R^1 is not a hydrogen but a methyl group and the nucleophilic activity of *N*-substituted β-hydroxy-enamine **3i** is considered to be lower (entry 9 in Table 3). As shown in Table 4, these β-hydroxy-enamines **3n–q** gave 4-oxo-4,5,6,7-tetrahydroindoles (**4n–q**) in 52–85% yields, which are important intermediates for the synthesis of 4-substituted indoles such as ergot alkaloids. Palladium-catalyzed oxidation of β-hydroxy-enamines **3j**, **3m**, and **3s** gave **4j** (27%), **4m** (85%), and **4s** (99%), respectively (Scheme 2). Analogous oxidation of **3k–l** were shown to produce a number of products, as demonstrated in TLC.

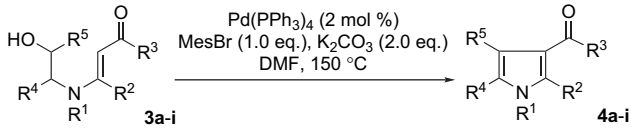
Table 2. Preparation of **4a** via palladium-catalyzed oxidation under several reaction conditions



Entry	Pd catalyst (mol %)	ArX (equiv)	Base (equiv)	Solvent	Reaction temperature (°C)	Yield (%)[b]
1	$Pd(PPh_3)_4$ (2.0)	PhBr (1.0)	K_2CO_3 (2.0)	DMF	150	55
2	$Pd(PPh_3)_4$ (2.0)	PhBr (1.0)	K_2CO_3 (2.0)	DMF	120	46
3	$Pd(PPh_3)_4$ (2.0)	PhBr (1.0)	K_2CO_3 (2.0)	DMF	100	10
4	$Pd(PPh_3)_4$ (2.0)	PhBr (1.0)	K_2CO_3 (2.0)	DMSO	150	18
5	$Pd(PPh_3)_4$ (2.0)	PhBr (1.0)	K_2CO_3 (2.0)	Toluene	Reflux	38
6	$Pd(PPh_3)_4$ (2.0)	PhBr (1.0)	K_2CO_3 (2.0)	1,4-Dioxane	Reflux	14
7	$Pd(PPh_3)_4$ (2.0)	PhBr (1.0)	K_2CO_3 (2.0)	THF	Reflux	None
8	$Pd(PPh_3)_4$ (2.0)	PhBr (1.0)	None	DMF	150	None
9	$Pd(PPh_3)_4$ (2.0)	PhBr (1.0)	K_2CO_3 (1.0)	DMF	150	37
10	$Pd(PPh_3)_4$ (2.0)	PhBr (1.0)	$KHCO_3$ (2.0)	DMF	150	44
11	$Pd(PPh_3)_4$ (2.0)	PhBr (1.0)	KOH (2.0)	DMF	150	8
12	$Pd(PPh_3)_4$ (2.0)	PhBr (1.0)	AcOK (2.0)	DMF	150	22
13	$Pd(PPh_3)_4$ (2.0)	PhBr (1.0)	Et_3N (2.0)	DMF	150	None
14	$Pd(PPh_3)_4$ (2.0)	PhBr (1.0)	Ag_2CO_3	DMF	150	None
15	$Pd(PPh_3)_4$ (2.0)	PhCl (1.0)	K_2CO_3 (2.0)	DMF	150	22
16	$Pd(PPh_3)_4$ (2.0)	PhI (1.0)	K_2CO_3 (2.0)	DMF	150	17
17	$Pd(PPh_3)_4$ (2.0)	MesBr (1.0)	K_2CO_3 (2.0)	DMF	150	82
18	$Pd(PPh_3)_4$ (2.0)	None	K_2CO_3 (2.0)	DMF	150	None
19	$Pd(PPh_3)_4$ (2.0)	PhBr (0.25)	K_2CO_3 (2.0)	DMF	150	19
20	$Pd(PPh_3)_4$ (2.0)	PhBr (0.5)	K_2CO_3 (2.0)	DMF	150	28
21	$Pd(PPh_3)_4$ (2.0)	PhBr (2.0)	K_2CO_3 (2.0)	DMF	150	58
22	$Pd(PPh_3)_4$ (2.0)	PhBr (4.0)	K_2CO_3 (2.0)	DMF	150	59
23	$Pd(PPh_3)_4$ (0.8)	MesBr (1.0)	K_2CO_3 (2.0)	DMF	150	78
24	None	PhBr (1.0)	K_2CO_3 (2.0)	DMF	150	None
25	$Pd(OAc)_2$ (3.0)	MesBr (1.0)	K_2CO_3 (2.0)	DMF	150	63
26	$Pd(OAc)_2$ (3.0)	MesBr (1.0)	K_2CO_3 (2.0)	DMF	150	72
27	$Pd(OAc)_2$ (5.0)	MesBr (1.0)	K_2CO_3 (2.0)	DMF	150	74
28	$PdCl_2$ (3.0)	MesBr (1.0)	K_2CO_3 (2.0)	DMF	150	76

[a] The starting material **3a** was consumed after 2 h.
[b] Isolated yields.

Table 3. Preparation of pyrroles (**4a–i**)

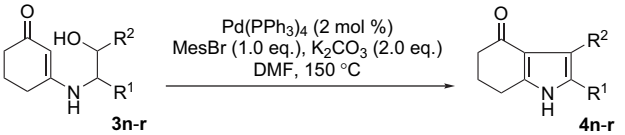


Entry	β-Hydroxy-enamine **3**	R^1	R^2	R^3	R^4	R^5	Reaction time (h)	Pyrroles **4** (yield %)[a]
1	**3a**	H	Me	OEt	Bn	H	2	**4a** (82)
2	**3b**	H	Me	OEt	Ph	H	5	**4b**[18] (67)
3	**3c**	H	Me	OEt	^{i}Pr	H	4	**4c** (83)
4	**3d**	H	Me	OEt	H	H	1	**4d**[19] (74)
5	**3e**	H	Me	OEt	H	Me	3	**4e**[20] (76)
6	**3f**	H	Me	OEt	H	Ph	5	**4f**[21] (80)
7	**3g**	H	Me	OMe	Me	H	3.5	**4g**[22] (57)
8	**3h**	H	Ph	OEt	Bn	H	4	**4h** (84)
9	**3i**	Me	Me	OEt	H	H	2	**4i**[23] (36)

[a] Isolated yields.

A plausible mechanism involved in this series of reaction is schematically shown in Figure 1. Oxidative addition of palladium(0) catalyst to aryl bromide produces palladium(II) adduct, which with β-hydroxy-enamine gives an alkoxy palladium(II). Finally, β-elimination of the palladium catalyst proceeded to give a carbonyl compound. Intramolecular cyclization of the carbonyl compound thus obtained, the subsequent dehydration, and isomerization of the protons provide pyrroles and indoles. Palladium(0) catalyst is then reproduced via reductive elimination of palladium species.

Table 4. Preparation of 4-oxo-4,5,6,7-tetrahydroindoles

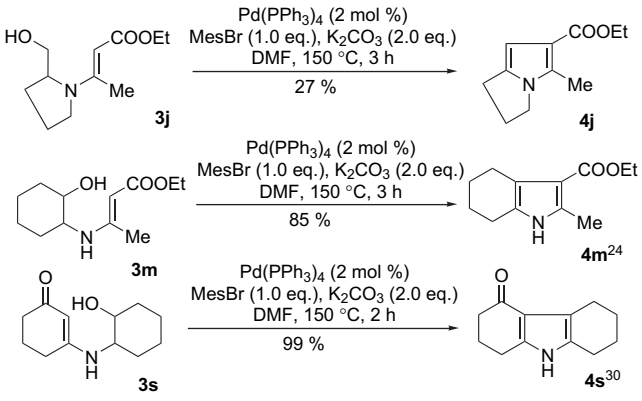


Entry	β-Hydroxy-enamine **3**	R^1	R^2	Reaction time (h)	4-Oxo-4,5,6,7-tetrahydro-indoles **4** (yield %)[a]
1	**3n**	H	H	4	**4n**[25] (63)
2	**3o**	Ph	H	2.5	**4o**[26] (65)
3	**3p**	H	Ph	7	**4p**[27] (52)
4	**3q**	Me	H	2	**4q**[28] (85)
5	**3r**	H	Me	1	**4r**[29] (60)

[a] Isolated yields.

3. Conclusion

The present study describes an efficient and facile synthesis of pyrroles and 4,5,6,7-tetrahydroindoles from hydroxy-enamines. In this procedure, the starting materials, i.e., hydroxy-enamines, are prepared easily from commercially available amino alcohols, and the final products are produced in a fewer steps in moderate to good yields. Of the compounds produced in the present method, 4-oxo-4,5,6,7-tetrahydroindoles are quite useful intermediates for the synthesis of biologically active natural products such as ergot alkaloids.



Scheme 2.

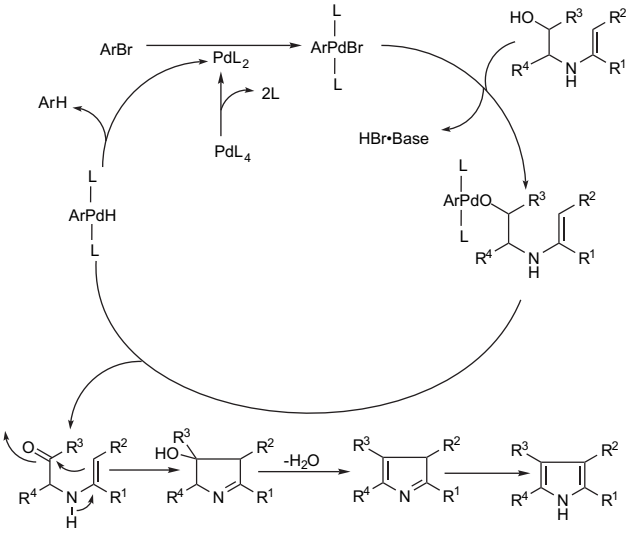


Figure 1. Plausible reaction mechanism.

4. Experimental

4.1. General methods

Melting points were measured in Yanagimoto micro melting point apparatus and are recorded uncorrected. Infrared spectra (cm^{-1}) were recorded on a Japan Spectroscopic Co. A-100 and Mass spectra were recorded on a Hitachi M-80B or Fisons VG Auto Spec instrument. ^1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were obtained on a Varian Gemini A-300 and the chemical shifts are given in parts per million downfield from the internal standard (TMS). Flash chromatography and MPLC were performed by using Merck silica gel Kieselgel 60® (230–400 mesh) or Merck Aluminum oxide 90® (70–230 mesh) column and preparative thin layer chromatography (PTLC) with Merck Kieselgel 60 F_{254} precoated glass plates (thickness 0.25 or 0.50 mm). All solvents were of commercial grade, which were distilled and dried with sodium benzophenone ketyl (tetrahydrofuran (THF), 1,4-dioxane, toluene, and benzene) or with CaH_2 (DMF, DMSO, and CH_2Cl_2). Lithium bases (^{n}BuLi, ^{s}BuLi, and ^{t}BuLi) were purchased from Aldrich Chemical Co., Ltd. All other reagents were of the highest available grade and used as purchased.

4.2. General procedure for the preparation of β-hydroxy-enamines

Method A: 4 Å MS (5 g) and THF solution (10 mL) of β-dicarbonyl compound (7.9 mmol, 1.0 equiv) were added to a dry THF solution (20 mL) of β-amino alcohol (9.4 mmol, 1.2 equiv). The reaction mixture was stirred at room temperature for 7 day under an argon atmosphere. After filtration through a short pad of Celite® 545, the solvent was evaporated in vacuo to give a residue, which was purified by aluminum oxide flash chromatography (eluting with EtOAc) to give a β-amino alcohol (**3a–g**, **3j–k**, **3m**, and **3o**).

Method B: 4 Å MS (5 g) and THF solution (10 mL) of β-dicarbonyl compound (11.7 mmol, 1.0 equiv) were added to a dry benzene solution (30 mL) of β-amino alcohol (14.9 mmol, 1.2 equiv). The reaction mixture was refluxed for 12 h under an argon atmosphere. After filtration through a short pad of Celite® 545, the solvent was evaporated in vacuo to give a residue, which was purified by aluminum oxide flash chromatography eluting with EtOAc to give a β-amino alcohol (**3h–i**, **3l**, **3n**, and **3p–s**).

4.2.1. Compound 3a. Pale yellow oil; IR (film) 3420 (OH), 3290 (NH), 1610 (C═O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.66 (1H, br d, 9.6), 7.31–7.15 (5H, m), 4.37 (1H, s), 4.08 (2H, q, 7.1), 3.76–3.56 (3H, m), 2.86 (1H, dd, 13.5, 5.2), 2.69 (1H, dd, 13.5, 7.9), 2.24 (1H, br s), 1.62 (3H, s), 1.24 (3H, t, 7.1); MS (EI, *m/z*) 263 (M^+). Anal. Calcd for $C_{15}H_{21}NO_3$: C, 68.41; H, 8.04; N, 5.32. Found: C, 68.49; H, 8.09; N, 5.31.

4.2.2. Compound 3b. Pale yellow oil; IR (film) 3420 (OH), 3300 (NH), 1650 (C═O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.20 (1H, br d, 8.4), 7.40–7.25 (5H, m), 4.70–4.60 (1H, m), 4.55 (1H, s), 4.13 (2H, q, 7.1), 3.86 (1H, dd, 11.3, 4.5), 3.77 (1H, dd, 11.3, 7.0), 1.82 (3H, s), 1.28 (3H, t, 7.1); MS (EI, *m/z*) 249 (M^+). Anal. Calcd for $C_{14}H_{19}NO_3$: C, 67.44; H, 7.68; N, 5.62. Found: C, 67.40; H, 7.75; N, 5.62.

4.2.3. Compound 3c. Pale yellow oil; IR (film) 3420 (OH), 3300 (NH), 1650 (C═O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.62 (1H, br d, 9.4), 4.47 (1H, s), 4.10 (2H, qd, 7 0.9), 3.65–3.76 (1H, m), 3.50–3.60 (1H, m), 3.30–3.42 (1H, m), 1.95 (3H, s), 1.67 (1H, br s), 1.26 (3H, t, 7.1), 0.96 (6H, d, 6.9); HRMS (EI, *m/z*) Calcd for $C_{11}H_{21}NO_3$ (M^+): 215.1521. Found: 215.1532.

4.2.4. Compound 3e. Colorless prisms, mp 63–65 °C (hexane); IR (KBr) 3420 (OH), 3300 (NH), 1620 (C═O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.74 (1H, br s), 4.49 (1H, s), 4.09 (2H, q, 7.1), 3.92 (1H, m), 3.27 (1H, ddd, 13.5, 6.2, 4.0), 3.16 (1H, ddd, 13.5, 7.3, 6.4), 1.99 (1H, br d), 1.93 (3H, s), 1.25 (3H, t, 7.1), 1.23 (3H, t, 7.1); MS (EI, *m/z*) 187 (M^+). Anal. Calcd for $C_9H_{17}NO_3$: C, 57.73; H, 9.15; N, 7.48. Found: C, 57.77; H, 9.15; N, 7.59.

4.2.5. Compound 3f. Colorless needles, mp 91–93 °C (hexane); IR (KBr) 3450 (OH), 3300 (NH), 1640 (C═O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.88 (1H, br s), 7.40–7.27 (5H, m), 4.85–4.79 (1H, m), 4.48 (1H, s), 4.09 (2H, q, 7.1), 3.43 (1H, dd, 6.4, 4.7), 3.42 (1H, dd, 7.5, 6.2), 2.38 (1H, br d), 1.86 (3H, s), 1.24 (3H, t, 7.1); MS (EI, *m/z*)

249 (M^+). Anal. Calcd for $C_{14}H_{19}NO_3$: C, 67.44; H, 7.68; N, 5.62. Found: C, 67.34; H, 7.70; N, 5.77.

4.2.6. Compound 3g. Pale yellow oil; IR (KBr) 3320 (NH), 1615 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 10.8 (1H, br s), 4.96 (1H, s), 3.73 (1H, m), 3.66 (1H, m), 3.53 (1H, dd, 11.1, 7.4), 2.42 (1H, br s), 1.99 (3H, s), 1.98 (3H, s), 1.19 (3H, d, 6.6); MS (EI, *m/z*) 249 (M^+). HRMS (EI, *m/z*) Calcd for $C_8H_{15}NO_2$ (M^+): 157.1103. Found: 157.1095.

4.2.7. Compound 3h. Pale yellow oil; IR (KBr) 3450 (OH), 3300 (NH), 1735 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.50 (1H, br d, 10.2), 7.37–7.20 (6H, m), 7.04–6.92 (4H, m), 4.57 (1H, s), 4.16 (2H, q, 7.1), 3.64–3.44 (3H, m), 2.79 (1H, dd, 13.5, 5.5), 2.51 (1H, dd, 13.5, 7.8), 2.30 (1H, br s), 1.29 (3H, t, 7.1); MS (EI, *m/z*) 325 (M^+). Anal. Calcd for $C_{20}H_{23}NO_3$: C, 73.82; H, 7.12; N, 4.30. Found: C, 73.53; H, 7.31; N, 4.25.

4.2.8. Compound 3i. Pale yellow oil; IR (KBr) 3430 (OH), 1680, 1660 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.63 (1H, s), 4.09 (2H, q, 7.1), 3.77 (2H, q, 5.7), 3.46 (2H, t, 5.7), 2.95 (3H, s), 2.49 (3H, s), 1.51 (1H, br t), 1.25 (3H, t, 7.1); MS (EI, *m/z*) 187 (M^+). Anal. Calcd for $C_9H_{17}NO_3$: C, 57.73; H, 9.15; N, 7.48. Found: C, 57.79; H, 9.06; N, 7.43.

4.2.9. Compound 3j. Pale yellow oil; IR (KBr) 3450 (OH), 3300 (NH), 1740 (C=O), 1660 (C=C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.58 (1H, s), 4.08 (2H, td, 7.1, 1.3), 3.98–3.88 (1H, m), 3.66 (1H, dd, 10.9, 3.9), 3.50 (1H, dd, 10.9, 3.5), 3.33–3.21 (2H, m), 2.50 (3H, s), 2.10–1.91 (4H, m), 1.78 (1H, br s), 1.25 (3H, t, 7.1); MS (EI, *m/z*) 213 (M^+). Anal. Calcd for $C_{11}H_{19}NO_3$: C, 61.94; H, 8.98; N, 6.57. Found: C, 62.15; H, 9.05; N, 6.61.

4.2.10. Compound 3k. Pale yellow oil; IR (KBr) 3425 (OH), 3320 (NH), 1650 (C=O), 1590 (C=C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.46 (1H, br d, 8.7), 7.30–7.10 (5H, m), 4.14 (2H, q, 7.1), 3.70–3.49 (3H, m), 2.85 (1H, dd, 13.5, 5.2), 2.71 (1H, dd, 13.5, 7.5), 2.50 (1H, br s), 2.49–2.35 (3H, m), 2.10–1.95 (1H, m), 1.80–1.50 (2H, m), 1.27 (3H, t, 7.1); MS (EI, *m/z*) 213 (M^+). HRMS (EI, *m/z*) Calcd for $C_{17}H_{23}NO_3$ (M^+): 289.1678. Found: 289.1690.

4.2.11. Compound 3l. Pale yellow oil; IR (film) 3450 (OH), 3290 (NH), 1720 (C=O), 1610 (C=C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 9.04 (1H, br d, 10.2), 7.32–7.14 (5H, m), 4.13 (2H, q, 7.1), 3.82–3.68 (1H, m), 3.65 (1H, dd, 11.0, 4.2), 3.53 (1H, dd, 11.0, 6.8), 2.83 (1H, dd, 13.5, 6.0), 2.70 (1H, dd, 13.5, 7.7), 2.26–2.12 (4H, m), 1.88–1.78 (2H, m), 1.56–1.32 (3H, m), 1.28 (3H, t, 7.1); MS (EI, *m/z*) 303 (M^+). Anal. Calcd for $C_{18}H_{25}NO_3$: C, 71.25; H, 8.31; N, 4.62. Found: C, 71.08; H, 8.47; N, 4.52.

4.2.12. Compound 3m. Colorless oil; IR (film) 3450 (OH), 3300 (NH), 1740 (C=O), 1610 (C=C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.51 (1H, br d, 9.4), 4.48 (1H, s), 4.09 (2H, q, 7.1), 3.42–3.33 (1H, m), 3.23–3.10 (1H, m), 2.30 (1H, br s), 2.10–2.02 (1H, m), 1.98 (3H, s), 1.95–1.87 (1H, m), 1.80–1.67 (2H, m), 1.29 (4H, m), 1.26 (3H, t, 7.1); MS (EI, *m/z*) 227 (M^+). Anal. Calcd for $C_{12}H_{21}NO_3$: C, 63.41; H, 9.31; N, 6.16. Found: C, 63.43; H, 9.45; N, 6.14.

4.2.13. Compound 3n. Colorless needles, mp 102–106 °C (MeCN); IR (film) 3260 (OH, NH), 1680 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.74 (1H, br s), 5.10 (1H, s), 3.81 (2H, td, 5.3, 2.4), 3.22 (2H, td, 5.0, 5.0), 2.37 (2H, t, 6.3), 2.30 (2H, t, 6.5), 1.95 (2H, m); MS (EI, *m/z*) 155 (M^+). Anal. Calcd for $C_8H_{13}NO_2$: C, 61.91; H, 8.44; N, 9.03. Found: C, 61.89; H, 8.42; N, 9.09.

4.2.14. Compound 3q. Pale yellow oil; IR (film) 3300 (OH, NH), 1550 (C=C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.15 (1H, s), 3.74–3.68 (1H, m), 3.63–3.54 (2H, m), 2.37–2.28 (4H, m), 1.95 (2H, m), 1.20 (3H, d, 6.3); HRMS (EI, *m/z*) Calcd for $C_9H_{15}NO_2$ (M^+): 169.1103. Found: 169.1106.

4.2.15. Compound 3r. Pale yellow amorphous solid, mp 81–82 °C ($CHCl_3$); IR (film) 3300 (OH, NH), 1550 (C=C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.44–5.22 (1H, br s), 5.10 (1H, s), 4.04–3.98 (1H, s), 3.17 (1H, ddd, 13.1, 6.0, 3.0), 2.96 (1H, ddd, 13.1, 8.5, 4.4), 2.36 (1H, t, 6.2), 2.30 (2H, t, 6.5), 1.95 (2H, m), 1.25 (3H, d, 6.3); HRMS (EI, *m/z*) Calcd for $C_9H_{15}NO_2$ (M^+): 169.1103. Found: 169.1107.

4.2.16. Compound 3s. Pale yellow needles, mp 198–199 °C (hexane/AcOEt); IR (film) 3300 (OH, NH), 1580 (C=C) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.80 (1H, d, 7.1), 4.86 (1H, s), 4.64 (1H, d, 4.9), 3.36–3.20 (1H, m), 3.04–2.93 (1H, m), 2.36–2.26 (2H, m), 2.08–2.02 (2H, m), 1.92–1.80 (2H, m), 1.77 (2H, m), 1.66–1.52 (2H, m), 1.30–0.98 (4H, m); MS (EI, *m/z*) 209 (M^+). Anal. Calcd for $C_{12}H_{19}NO_2$: C, 68.86; H, 9.15; N, 6.69. Found: C, 68.82; H, 9.07; N, 6.59.

4.3. General procedure for the preparation of pyrrole derivatives

A mixture of $Pd(PPh_3)_4$ (0.025 g, 0.02 mmol, 2 mol %), K_2CO_3 (0.27 g, 2.0 mmol), mesityl bromide (0.20 g, 1.0 mmol), β-hydroxy-enamine (1.0 mmol), and dry DMF (5 mL) was heated at 150 °C for 2 h under an argon atmosphere. After cooling, H_2O (20 mL) was added to the mixture, which was extracted with Et_2O (3×20 mL). The extract was then dried over Na_2SO_4, filtered, and evaporated in vacuo to give a residue, which was purified by silica gel flash chromatography (eluting with hexane–EtOAc system) to give the pyrrole derivatives (**4a–j** and **4m–r**).

4.3.1. Compound 4a. Colorless prisms, mp 106–107 °C (hexane); R_f 0.16 (hexane/AcOEt=4:1); IR (KBr) 3270 (NH), 1660 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.77 (1H, s), 7.36–7.16 (5H, m), 6.32 (1H, d, 2.9), 4.25 (2H, q, 7.1), 3.88 (2H, s), 2.45 (3H, s), 1.33 (3H, t, 7.1); MS (EI, *m/z*) 243 (M^+). Anal. Calcd for $C_{15}H_{17}NO_2$: C, 74.05; H, 7.04; N, 5.76. Found: C, 73.99; H, 6.94; N, 5.72.

4.3.2. Compound 4c. Colorless prisms, mp 66–68 °C (hexane); R_f 0.17 (hexane/AcOEt=4:1); IR (KBr) 3030 (NH), 1690 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.90 (1H, br s), 6.23 (1H, dd, 3.0, 0.9), 4.25 (2H, q, 7.1), 2.83 (1H, m), 2.50 (3H, s), 1.33 (3H, t, 7.1), 1.24 (6H, d, 6.9); MS (EI, *m/z*) 195 (M^+). Anal. Calcd for $C_{11}H_{17}NO_2$: C, 67.66; H, 8.78; N, 7.17. Found: C, 67.67; H, 8.72; N, 7.21.

4.3.3. Compound 4h. Colorless prisms, mp 107–108 °C (hexane); R_f 0.37 (hexane/AcOEt=4:1); IR (KBr) 3300 (NH), 1660 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.99 (1H, br s), 7.57–7.51 (2H, m), 7.41–7.29 (5H, m), 7.28–7.21 (3H, m), 6.52 (1H, dd, 3.0, 0.9), 4.20 (2H, q, 7.1), 3.98 (2H, s), 1.25 (3H, t, 7.1); MS (EI, *m/z*) 305 (M^+). Anal. Calcd for $C_{20}H_{19}NO_2$: C, 78.66; H, 6.27; N, 4.59. Found: C, 78.56; H, 6.27; N, 4.70.

4.3.4. Compound 4j. Colorless oil; R_f 0.24 (hexane/AcOEt=4:1); IR (film) 1695 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.17 (1H, d, 1.1), 4.24 (2H, q, 7.1), 3.82 (2H, t, 7.1), 2.80 (2H, t, 7.4), 2.47 (2H, m), 2.46 (3H, s), 1.32 (3H, t, 7.1); MS (EI, *m/z*) 193 (M^+). Anal. Calcd for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82; N, 7.25. Found: C, 68.46; H, 7.78; N, 7.05.

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Process research on arylnaphthalene lignan aza-analogues: a new palladium-catalyzed benzannulation of α,β-bisbenzylidenesuccinic acid derivatives

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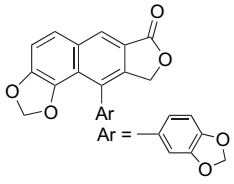
Abstract—The discovery of a new Pd-catalyzed benzannulation reaction of bisbenzylidenesuccinimide during process research on arylnaphthalene lignan aza-analogues is described. An extension of the Pd-catalyzed benzannulation to the regiospecific synthesis of various arylnaphthalene lignan aza-analogues utilizing classical Stobbe condensation is included.

1. Introduction

The objective of process chemistry in the pharmaceutical industry is to develop an efficient synthetic process for a new drug candidate that is cost effective, safe and easy to apply, environmentally friendly, and robust enough for large-scale preparation, to support both preclinical and clinical studies and also commercial purpose. In order to realize such a process, chemists utilize state-of-the-art methods of organic synthesis. In addition, a new method of synthesis discovered during process chemistry research can contribute to organic synthesis. This relationship is mutually beneficial to both fields of chemistry. In this paper, we disclose a new Pd-catalyzed benzannulation reaction[1] of α,β-bisbenzylidenesuccinic acid derivatives discovered during process research on 1-arylnaphthalene lignan aza-analogues.[2]

1-Arylnaphthalene lignans[3] occur widely in nature and have several regioisomers as a structural feature. Helioxanthin **1**, Justicidin E **2**, and Taiwanin C **3** are representative, bearing a 'curved' 7,8-methylenedioxynaphthalene or 'straight' 6,7-methylenedioxynaphthalene and 'up' 3-lactone or 'down' 2-lactone carbonyl moiety. Since each regioisomer varies in its bioactivities,[3b–d] analogues, particularly nitrogen-containing types, have been studied as potent candidates. For example, hydrazide compound **4** of antiviral activity,[4a] cyanonaphthalene **5** as a 5-lipoxygenase inhibitor,[4b] and amide analogue **7** for PDE-V inhibitor[4c] have been reported to date.



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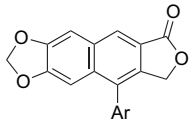

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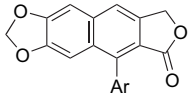
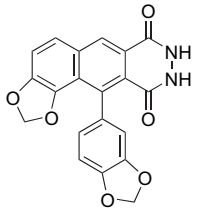


3 (Taiwanin C)



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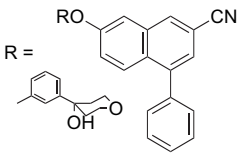

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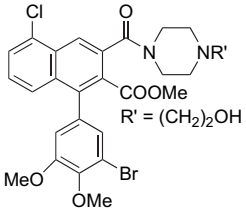

6

Keywords: Lignans; Palladium and compounds; Naphthalenes; Biaryls.
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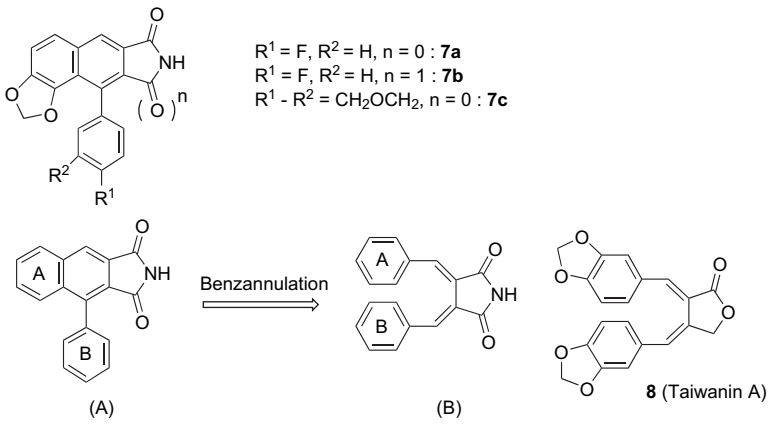


Scheme 1.

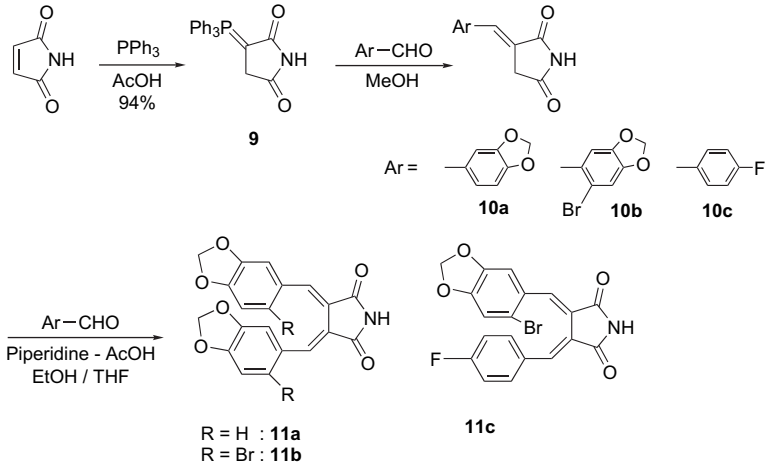
1-Arylnaphthalene lignan analogues[2] **7a–c**, with a 'curved' 7,8-methylenedioxynaphthalene and 'up' lactam or imide moiety, have been studied in Takeda Pharmaceutical Co. as new drug candidates. A number of methods have been developed to construct the arylnaphthalene lignan skeleton, represented by the key reaction of the strategy, intermolecular[5] or intramolecular Diels–Alder reactions,[6] conjugate addition reactions,[7] biaryl coupling reactions,[8] and a benzannulation reaction.[9] However, to achieve the regiospecific synthesis of the target compounds **7a–c**, we decided to explore a new method based on the strategy shown in Scheme 1, in which the target arylnaphthalene (A) would be prepared via the benzannulation[10] of bisbenzylidenesuccinimide (B). The substrate (B) is also of interest as an aza-analogue of the natural lignan Taiwanin A **8**, having antithrombotic activity.[11]

2. Results and discussion

2.1. Discovery of a Pd-catalyzed benzannulation reaction of α,β-bisbenzylidenesuccinimide during process research on arylnaphthalene lignan analogues

Initially, the cyclization of bisbenzylidenesuccinimide was studied under various conditions. As shown in Scheme 2, bisbenzylidenesuccinimide **11a–c** were prepared by the Wittig reaction[12] using a stable phosphorane **9** with a benzaldehyde and the Knoevenagel reaction of monobenzylidenesuccinimide **10a–c** with a benzaldehyde. In the process, as well as symmetrical type **11a–b**, unsymmetrical bisbenzylidenesuccinimide **11c** was prepared as a single isomer (Table 1) independent of the order in which 6-bromopiperonal and 4-fluorobenzaldehyde were used in the two condensation reactions. Since the Wittig reaction using a stable phosphorane gives the product of the (*E*)-configuration, this result supports the notion that a bisbenzylidenesuccinic acid derivative usually forms an (*E*)/(*E*)-type isomer.[13]

Next, the thermal cyclization of symmetrical bisbenzylidenesuccinimide **11a** in DMF was studied. While the heating of the DMF solution at 140 °C under an atmosphere of nitrogen gave dihydronaphthalene **12**, the corresponding conditions under ambient atmosphere provided naphthalene **13** with dehydrogenation. To avoid the reaction at the 6-position of the 3,4-piperonyl group, we investigated the thermal cyclization of unsymmetrical bisbenzylidenesuccinimide **11c** bearing bromine as a protective group. A complex reaction mixture with a small amount of the desired naphthalene **14** was given under a higher temperature (190 °C in NMP) condition. On the other hand, to



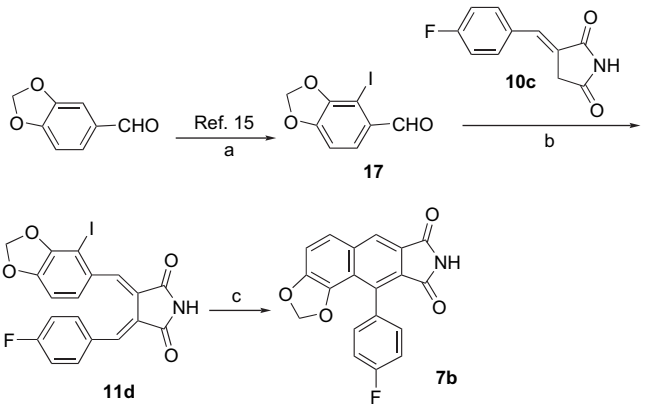
Scheme 2.

Table 1. Syntheses of mono- or bisbenzylidenesuccinimide **10a–c**, **11a–c**

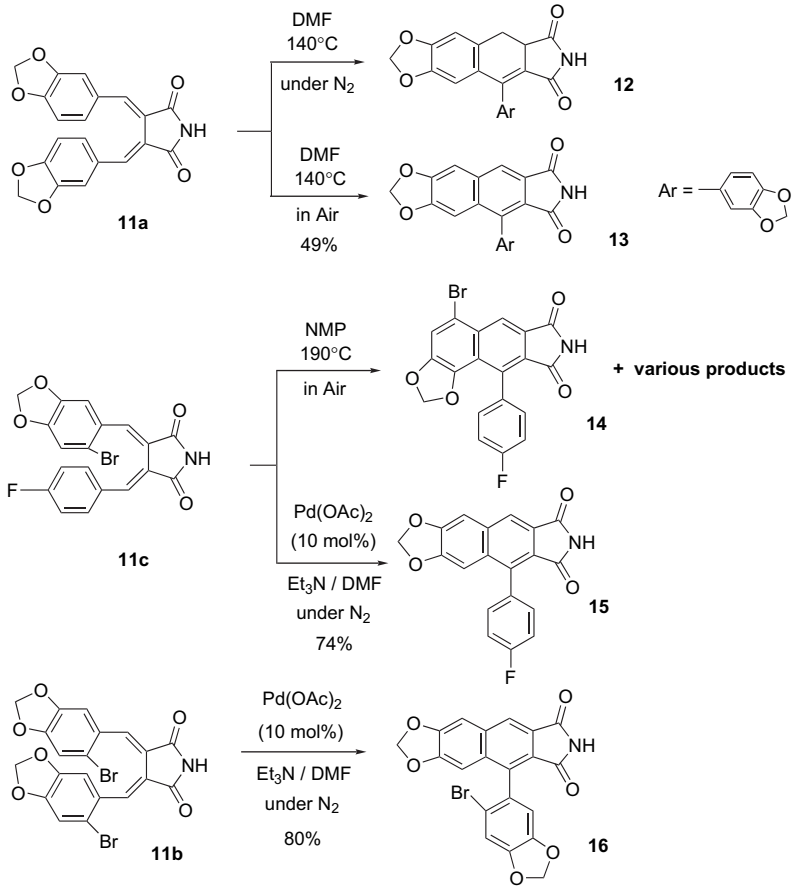
Compound	Yield (%)	Purity (%) by HPLC	^{1}H NMR signal of benzylidene moiety
10a	88	99.8	7.31 (1H, t, *J*=2.3 Hz)
10b	82	99.4	7.53 (1H, t, *J*=2.3 Hz)
10c	83	99.9	7.39 (1H, t, *J*=2.2 Hz)
11a	83	98.9	7.49 (2H, s)
11b	82	94.2	7.43 (2H, s)
11c (via **10b**)	80	93.4	7.44 (1H, s) and 7.59 (1H, s)
11c (via **10c**)	81	98.1	The same as above

accelerate the dehydrogenation, stoichiometric use of $Pd(OAc)_2$ in AcOH for the cyclization afforded naphthalene **15** with dehydrobromination. This surprising result inspired us to try a catalytic Heck reaction with $Pd(OAc)_2$ (10 mol %)/Et_3N in DMF at 100 °C, for the cyclization of **11c** to give naphthalene **15** in 74% yield. In addition, the Pd-catalyzed reaction of the symmetrical bisbenzylidene form **11b** provided naphthalene **16** with only monodehydrobromination. To our knowledge, there had been no report regarding the generation of benzene ring using the intramolecular Heck reaction of an arylhalide or a vinylhalide with the conjugated 1,3-diene system before our preliminary report[1a] for the synthesis of Helioxanthin based on the discovery of a Pd-catalyzed benzannulation of bisbenzylidensuccimide derivatives. Therefore, our discovery will contribute to general synthesis[14] of naphthalene derivatives (Scheme 3).

Based on the discovery of the Pd-catalyzed benzannulation reaction, we have achieved the regiospecific synthesis of 'curved' 7,8-methylenedioxy-substituted naphthalene **7b** in combination with directed ortho metalation. Thus, a key intermediate, 2-iodopiperonal **17**, prepared by Young's metalation synthesis[15] was condensed with monobenzylidenesuccinimide **10c** to give bisbenzylidenesuccinimide **11d**, which was then converted to naphthalene **7b** by the Pd-catalyzed benzannulation. In the process, each product was easily isolated in high purity only by crystallization suitable for a large-scale preparation (Scheme 4).



Scheme 4. Reagents and conditions: (a) *n*-BuLi, *N*,*N*,*N*′-trimethylethylenediamine, DME–THF then *n*-BuLi, I_2, 46%; (b) piperidine, AcOH, EtOH, 79%; (c) $Pd(OAc)_2$ (10 mol %), Et_3N (2.2 equiv), DMF, 110 °C; 80 min, 78%.

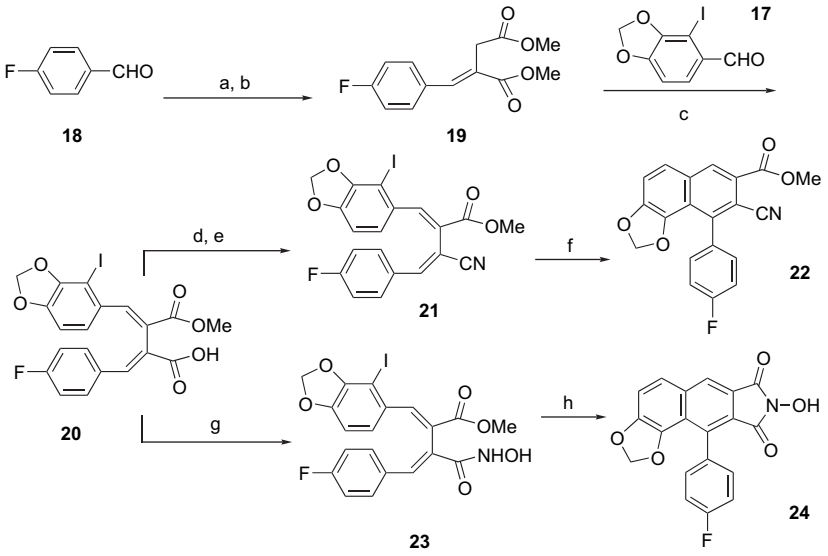


Scheme 3.

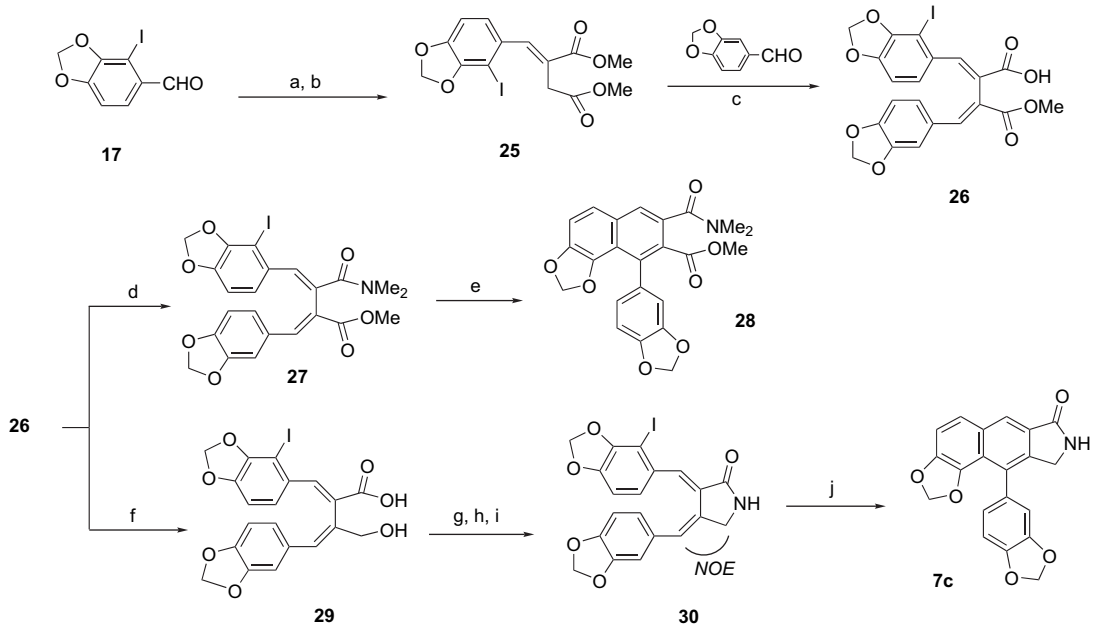
2.2. Extension of the new Pd-catalyzed benzannulation to the regiospecific synthesis of various arylnaphthalene lignan analogues

Next our objective is to extend the Pd-catalyzed benzannulation to the synthesis of other aza-analogues utilizing classical Stobbe condensation in two stages. The first Stobbe condensation of diethyl succinate with 4-fluorobenzaldehyde **18** gave the monobenzylidene form **19**. The second reaction of **19** with 2-iodopiperonal **17** provided bisbenzylidene halfester **20** as a key intermediate, in which the ester moiety of **19** distant from the reacting carbon was selectively saponified in a typical Stobbe condensation manner.[10a–c] Conversion of the carboxyl moiety of halfester **20** to a cyano group via dehydration of the corresponding amide yielded the bisbenzylidene form **21**, which was then converted into 2-cyanonaphthalene **22** by subsequent Pd-catalyzed benzannulation. Halfester **20** was also transformed to the corresponding hydroxamic acid derivative **23**, which was cyclized to furnish naphthoimide **24** by sequential hydroxyimide formation and the Pd-catalyzed benzannulation (Scheme 5).

By changing the order in which the 2-iodopieronylidene moiety is installed into Stobbe's halfester, the regiospecific synthesis of different substituted aza-analogues can be achieved. Indeed, as shown in Scheme 6, the first Stobbe's condensation of diethyl succinate with 2-iodopiperonal **17**



Scheme 5. Reagents and conditions: (a) $(CH_2COOEt)_2$, NaOMe, MeOH then aq NaOH, 36%; (b) MeOH, H_2SO_4, 94%; (c) NaOMe, MeOH, 74%; (d) $(COCl)_2$, cat. DMF, THF then aq NH_3, 91%; (e) $(COCl)_2$, cat. DMF, THF, 91%; (f) $Pd(OAc)_2$ (10 mol %), K_2CO_3 (2.2 equiv), NMP, 110 °C, 50 min, 32%; (g) $(COCl)_2$, cat. DMF, THF then NH_2OH, 68%; (h) $Pd(OAc)_2$ (10 mol %), Et_3N (2.2 equiv), DMF, 110 °C, 45 min, 69%.



Scheme 6. Reagents and conditions: (a) $(CH_2COOEt)_2$, NaOMe, MeOH, aq NaOH, 62%; (b) MeOH, H_2SO_4, 84%; (c) NaOMe, MeOH, 82%; (d) $(COCl)_2$, cat. DMF, THF then Me_2NH, 67%; (e) $Pd(OAc)_2$ (20 mol %), AcOK (2.2 equiv), NMP, 9%; (f) $LiEt_3BH$, THF, 67%; (g) EtI, K_2CO_3, DMF; (h) PBr_3, MePh; (i) aq NH_3, THF–MeOH, 35% based on **29**; (j) $Pd(OAc)_2$ (10 mol %), AcOK (2.2 equiv), NMP, 110 °C, 40 min, 48% (HPLC yield 72%).

gave the monobenzylidene **25**, which was then converted into bisbenzylidene halfester **26** by the second condensation with piperonal. Since halfester **26** bore a carboxyl moiety the near 2-iodopieronylidene group, conversion of the carboxyl into a *N*,*N*-dimethylamide group and subsequent Pd-catalyzed benzannulation introduced the amide moiety as a nitrogen-containing substituent to the 3-position of naphthalene derivative **28**. As well as compound **21**, the acyclic bisbenzylidene **27** gave lower yield of the Pd-catalyzed benzannulation than the bisbenzylidenesuccinimides. Moreover, the super-hydride reduction[16] of an ester moiety of halfester **26** afforded the hydroxy form **29**, which was transformed into lactam **30** by a sequence of esterization, bromination, and amination. The geometrical configuration of α,β-bisbenzylidene-γ-lactam **30** was confirmed as (*E*,*E*)-configuration, on the basis of the NOESY spectrum study focusing on a relationship between benzylidene and lactone protone. The bisbenzylidenelactam **30** was cyclized by a Pd catalyst to provide the lactam analogue **7c** bearing an 'up' carbonyl moiety.

Discussion about the possible mechanism of the novel benzannulation reaction of (*E*,*E*)-α,β-bisbenzylidene-γ-lactam **30** inspired us to recognize the role of its 1,3-diene system. The following reaction mechanism including two conceivable processes is proposed in Scheme 7. In the first process, the oxidative addition of palladium(0) into the C–I bond of **30** generates σ-arylpalladium complex **31** based on the (*E*,*E*)-type isomer. Despite the steric hindrance of the other aryl group, the stable palladium(II) complex with the 1,3-diene system (square planar complex) accelerates *syn* insertion of an intramolecular alkenyl double bond into the C–Pd bond, to give δ-dihydronaphthalenylpalladium complex **32**. Then, the palladium(II) species **32** smoothly undergoes *syn* β-hydride elimination to yield the naphthalene product **7a**. In the other process, (*E*,*E*)-type σ-arylpalladium complex **31** is isomerized to (*E*,*Z*)-type σ-arylpalladium complex **33** due to the delocalized π-electron system on the 1,3-diene complex with the palladium(II) species. The subsequent *syn* insertion of an intramolecular alkenyl double bond to the C–Pd bond provides σ-dihydronaphthalenylpalladium complex **34**. Although the reaction intermediate **34** does not undergo *syn* β-hydride elimination to yield the naphthalene **7a**, **34** is converted into π-allylpalladium complex **35**, which isomerizes into σ-dihydronaphthalenylpalladium complex **32** allowing *syn* β-hydride elimination.

3. Conclusion

In conclusion, a new Pd-catalyzed benzannulation of bisbenzylidenesuccinimides has been discovered during process research on arylnaphthalene lignan analogues. Furthermore, an extension of the benzannulation to the regiospecific synthesis of various arylnaphthalene lignan analogues has been achieved utilizing classical Stobbe condensation.

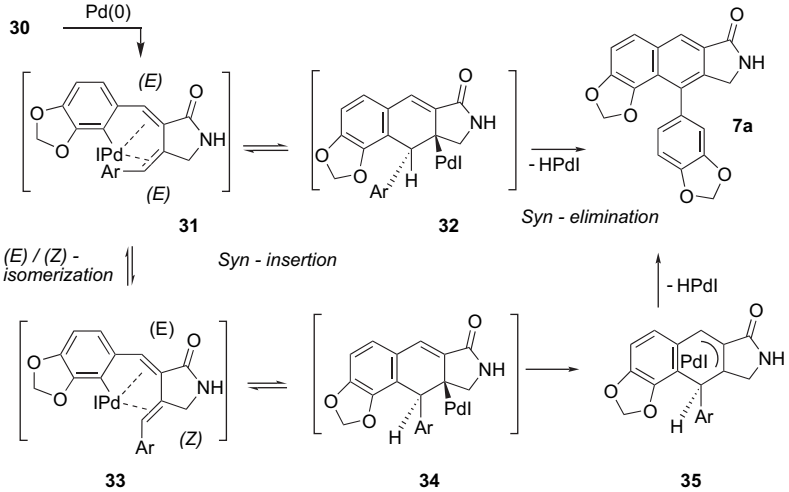
4. Experimental

4.1. General remarks

Melting points were recorded on a Yanagimoto micro melting apparatus and are uncorrected. IR spectra were recorded on a Horiba FT-210 spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker DPX-300 spectrometer. Elemental analyses and mass spectra were analyzed by Takeda Analytical Research Ltd. HPLC was performed on a YMC-Pack ODS-A302 column (150 mm×4.6 mm I.D.) with 0.05 M KH_2PO_4 in water and acetonitrile (50:50) at 25 °C. Detection was effected with a Hitachi spectrophotometric detector at 254 nm.

4.2. Pd-catalyzed benzannulation reaction of α,β-bisbenzylidenesuccinimide

4.2.1. 3-(Triphenylphosphoranylidene)pyrrolidine-2,5-dione 9. A mixture of maleimide (66.6 g, 688 mmol), triphenylphosphine (180 g, 686 mmol), and acetone (670 ml) was heated under reflux for 1 h. After cooling, the resulting precipitate was filtered, washed with acetone (200 ml), and dried in vacuo at 40 °C to give **9** (233 g, yield 94%) as a colorless solid, which was used for the next step without further purification.



Scheme 7.

4.2.2. 3-(1,3-Benzodioxol-5-ylmethylene)pyrrolidine-2,5-dione 10a (method A). A mixture of phosphorane **9** (2.35 g, 6.66 mmol), piperonal (1.00 g, 6.66 mmol), and methanol (20 ml) was heated under reflux for 1 h and 10 min, then cooled at room temperature. The resulting crystal was filtered, washed with methanol (5 ml), and dried in vacuo at 40 °C to give **10a** (1.36 g, yield 88%) as a colorless crystalline powder: mp 230 °C (decomposed), ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 3.61 (2H, d, J=2.3 Hz), 6.10 (2H, s), 7.01 (1H, d, J=8.1 Hz), 7.1–7.2 (2H, m), 7.30 (1H, t, J=2.3 Hz), 11.4 (1H, br), IR (ATR, cm^{-1}): 1695, 1645, 1498, 1180, 1027, MS (EI, m/z): (M^+) 231; Elemental analysis: calcd for $C_{12}H_9NO_4$, C: 62.34, H: 3.92, N: 6.06, found C: 62.15, H: 3.78, N: 6.07.

4.2.3. 3-[(6-Bromo-1,3-benzodioxol-5-yl)methylene]pyrrolidine-2,5-dione 10b. Following the method A, phosphorane **9** (4.71 g, 13.1 mmol) was treated with 6-bromo-3,4-methylenedioxybenzaldehyde (3.00 g, 13.1 mmol) in methanol (60 ml) for 1.5 h under reflux to give **10b** (3.34 g, yield 82%) as a pale yellow powder: mp 265 °C (decomposed), ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 3.65 (2H, d, J=2.3 Hz), 6.16 (2H, s), 7.25 (1H, s), 7.39 (1H, s), 7.53 (1H, t, J=2.3 Hz), 11.5 (1H, br), IR (ATR, cm^{-1}): 1765, 1695, 1501, 1348, 1205, 1038, MS (EI, m/z): (M+1) 311, (M) 309; Elemental analysis: calcd for $C_{12}H_8BrNO_4$, C: 46.48, H: 2.60, N: 4.52, found C: 46.56, H: 2.67, N: 4.27.

4.2.4. 3-(4-Fluorobenzylidene)pyrrolidine-2,5-dione 10c. Following the method A, phosphorane **9** (29.0 g, 80.6 mmol) was treated with 4-fluorobenzaldehyde (10.0 g, 80.6 mmol) in methanol (100 ml) for 1.5 h under reflux to give **10c** (13.9 g, yield 83%) as a colorless crystalline powder: mp 210 °C (decomposed), ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 3.64 (2H, d, J=2.2 Hz), 7.28–7.34 (2H, m), 7.39 (1H, t, J=2.2 Hz), 7.67–7.72 (2H, m), 11.4 (1H, br), IR (ATR, cm^{-1}): 1765, 1707, 1650, 1510, 1350, 1184, MS (EI, m/z): (M^+) 205; Elemental analysis: calcd for $C_{11}H_8NO_2F$, C: 64.39, H: 3.93, N: 6.83, found C: 64.28, H: 3.95, N: 6.86.

4.2.5. 3,4-Bis(1,3-benzodioxol-5-ylmethylene)pyrrolidine-2,5-dione 11a (method B). A mixture of compound **10a** (350 mg, 1.51 mmol), piperonal (227 mg, 1.51 mmol), and piperidine (129 mg, 1.51 mmol) in ethanol (3.5 ml) and tetrahydrofuran (3.5 ml) was heated under reflux for 18 h. The reaction mixture was then allowed to stand at room temperature, and the resulting crystals were filtered and washed with ethanol/tetrahydrofuran (1:1, 1.2 ml) and dried in vacuo at 40 °C to give **11a** (456 mg, yield 83%) as a yellow crystalline powder: mp 249 °C (decomposed), ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 5.90 (4H, s), 6.30 (2H, d, J=1.2 Hz), 6.62 (2H, d, J=8.1 Hz), 6.65 (2H, dd, J=8.1 and 1.2 Hz), 7.49 (2H, s), 11.5 (1H, br), IR (ATR, cm^{-1}): 1692, 1485, 1246, 1037, 925, MS (EI, m/z): (M^+) 363; Elemental analysis: calcd for $C_{20}H_{13}NO_6$, C: 66.12, H: 3.61, N: 3.86, found C: 65.89, H: 3.76, N: 3.87.

4.2.6. 3,4-Bis[(6-bromo-1,3-benzodioxol-5-yl)methylene]pyrrolidine-2,5-dione 11b. Following the method B, compound **10b** (1.50 g, 4.84 mmol) was treated with 6-bromo-3,4-methylenedioxybenzaldehyde (1.11 g, 4.83 mmol), piperidine (411 mg, 4.83 mmol), acetic acid (290 mg, 4.83 mmol), and ethanol (30 ml) for 11 h under reflux to give **11b** (2.07 g, yield 82%) as a yellow crystalline powder: mp 245 °C (decomposed), ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 5.96 (4H, s), 5.97 (2H, s), 7.24 (2H, s), 7.38 (2H, s), 11.7 (1H, br), IR (ATR, cm^{-1}): 1755, 1702, 1471, 1263, 1116, 1034, MS (EI, m/z): (M+2) 521, (M) 519; Elemental analysis: calcd for $C_{20}H_{11}NO_6Br_2$, C: 46.10, H: 2.13, N: 2.69, found C: 45.86, H: 2.13, N: 2.72.

4.2.7. 3-[(6-Bromo-1,3-benzodioxol-5-yl)methylene]-4-(4-fluorobenzylidene)pyrrolidine-2,5-dione 11c. Following the method B, compound **10b** (1.50 g, 4.84 mmol) was treated with 4-fluorobenzaldehyde (0.813 g, 6.55 mmol), piperidine (0.558 g, 6.55 mmol), acetic acid (0.393 g, 6.55 mmol), and ethanol (30 ml) for 6 h under reflux to give the title compound (1.60 g, yield 80%) as a yellow crystalline powder: mp 244 °C (decomposed), ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 5.84 (2H, s), 6.07 (2H, s), 6.77–6.83 (2H, m), 6.94–6.99 (2H, m), 7.18 (2H, s), 7.44 (1H, s), 7.59 (1H, s), 11.72 (1H, br), IR (KBr, cm^{-1}): 1760, 1706, 1473, 1421, 1238, 833, 648, MS (EI, m/z): (M+2) 417, (M) 415; Elemental analysis: calcd for $C_{19}H_{11}NO_4BrF$, C: 54.83, H: 2.66, N: 3.37, found C: 54.53, H: 2.55, N: 3.25.

Following the method B, compound **10c** (2.69 g, 13.1 mmol) was treated with 6-bromo-3,4-methylenedioxybenzaldehyde (3.00 g, 13.1 mmol), piperidine (1.12 g, 13.1 mmol), acetic acid (0.787 g, 13.1 mmol), and ethanol (53.8 ml) to give the title compound (4.41 g, yield 81%).

4.2.8. 9-(1,3-Benzodioxol-5-yl)-5*H*-[1,3]benzodioxolo[5,6-*f*]isoindole-6,8(5a*H*,7*H*)-dione 12. A mixture of compound **11a** (6.00 g, 16.5 mmol) and *N*,*N*-dimethylformamide (120 ml) was heated at 140 °C for 96 h under a nitrogen atmosphere. The reaction mixture was allowed to stand at room temperature, and then ethyl acetate (180 ml), tetrahydrofuran (60 ml), and H_2O (180 ml) were added. The aqueous layer was separated and extracted with ethyl acetate (2×60 ml). The combined organic layer was washed with H_2O and concentrated in vacuo to give crude compound **12**, which indicated a 66% peak area on HPLC analysis. The residue was purified with silica-gel column chromatography (eluent: hexane/acetate) and washed with ethanol and ethyl acetate to give the title compound (0.63 g, yield 16%) as a pale yellow crystalline powder: mp 261–263 °C, ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 2.79 (1H, t, J=15.7 Hz), 3.07 (1H, dd, J=15.3 and 6.5 Hz), 3.66 (1H, dd, J=16.6 and 6.5 Hz), 5.9–6.1 (2H, m), 6.09 (2H, d, J=3.9 Hz), 6.33 (1H, s), 6.5–7.0 (2H, m), 6.96 (1H, d, J=8.0 Hz), 7.05 (1H, s), 11.2 (1H, br), ^{13}C NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 28.03, 40.79, 101.11, 101.51, 107.57, 108.28, 109.05, 122.58, 128.38, 129.19, 131.63, 142.58, 146.08, 146.52, 147.41, 148.04, 167.65, 176.43, IR (ATR, cm^{-1}): 1759, 1702, 1484, 1236, 1032, 639, MS (EI, m/z): (M^+) 363; Elemental analysis: calcd for $C_{20}H_{13}NO_6$, C: 66.12, H: 3.61, N: 3.86, found C: 65.91, H: 3.55, N: 3.90, HPLC purity: 98.5%.

4.2.9. 5-(1,3-Benzodioxol-5-yl)-6*H*-[1,3]benzodioxolo[5,6-*f*]isoindole-6,8(7*H*)-dione 13. A mixture of compound **11a** (350 mg, 0.963 mol) and *N*,*N*-dimethylformamide (7 ml) was heated at 140 °C for 20 h under an

ambient atmosphere. The reaction mixture was then allowed to stand at room temperature, ethyl acetate (15 ml) and H_2O (15 ml) were added. The aqueous layer was separated and extracted with ethyl acetate. The combined organic layers were washed with H_2O, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was washed with ethyl acetate and then ethanol to give the title compound (170 mg, yield 49%) as a colorless crystalline powder: mp 299 °C (decomposed), ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 6.13 (2H, d, J=11.6 Hz), 6.22 (2H, d, J=1.6 Hz), 6.80 (1H, dd, J=7.9 and 1.6 Hz), 6.9–7.0 (2H, m), 7.05 (1H, d, J=7.9 Hz), 7.73 (1H, s), 8.22 (1H, s), 11.2 (1H, br), IR (ATR, cm^{-1}): 1767, 1722, 1469, 1253, 1039, MS (EI, *m*/*z*): (M^+) 361; Elemental analysis: calcd for $C_{20}H_{11}NO_6 \cdot 0.5H_2O$, C: 64.87, H: 3.27, N: 3.78, found C: 65.27, H: 3.20, N: 3.84.

4.2.10. 5-Bromo-10-(4-fluorophenyl)-7*H*-[1,3]benzodioxolo[4,5-*f*]isoindole-7,9(8*H*)-dione 14. A mixture of compound **11c** (2.00 g, 4.81 mmol) and *N*-methylpyrrolidone (20 ml) was heated at 190 °C for 9.5 h under an ambient atmosphere. The reaction mixture was then allowed to stand at room temperature, before ethyl acetate, tetrahydrofuran, and 1 M HCl were added to it. The aqueous layer was separated and extracted with ethyl acetate. The combined organic layers were washed with 1 M HCl and then H_2O, dried over anhydrous $MgSO_4$, and concentrated in vacuo to give crude compound **14**, which indicated a 13% peak area on HPLC analysis. The residue was purified with silica-gel column chromatography (eluent: hexane/acetate) and recrystallized from hexane and ethyl acetate to give the title compound (36.3 mg, yield 1.8%) as a yellow crystalline powder: mp 305 °C (decomposed), ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 5.98 (2H, s), 6.94–7.25 (2H, m), 7.39–7.44 (2H, m), 8.06 (1H, s), 8.43 (1H, s), 11.4 (1H, br), IR (ATR, cm^{-1}): 1712, 1278, 1134, 1061, 635, 499, MS (EI, *m*/*z*): (M+2) 415, (M) 413; Elemental analysis: calcd for $C_{19}H_9BrFNO_4$, C: 55.10, H: 2.19, N: 3.38, found C: 54.89, H: 2.29, N: 3.11, HPLC purity: 96.7%.

4.2.11. 5-(4-Fluorophenyl)-6*H*-[1,3]benzodioxolo[5,6-*f*]isoindole-6,8(7*H*)-dione 15 (method C). A mixture of compound **11b** (1.00 g, 2.40 ml), $Pd(OAc)_2$ (53.9 mg, 0.24 mmol), triethylamine (291 mg, 2.88 mmol), and *N*,*N*-dimethylformamide (20 ml) was stirred at room temperature under a nitrogen atmosphere for 1 h and the resulting mixture was heated at 100 °C for 1 h. After the reaction mixture had stood at room temperature, 1 M HCl (20 ml) was added to give a precipitate. The solid was filtered, washed with ethanol/tetrahydrofuran (1:1), and dried in vacuo at 40 °C to give the title compound (593 mg, yield 74%) as a colorless crystalline powder: mp 328 °C (decomposed), ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 5.98 (2H, s), 6.59 (1H, s), 7.04–7.20 (4H, m), 7.47 (1H, s), 8.03 (1H, s), 11.2 (1H, br), IR (ATR, cm^{-1}): 1765, 1704, 1463, 1244, 1037, 750, 640, MS (EI, *m*/*z*): (M^+) 335; Elemental analysis: calcd for $C_{19}H_{10}FNO_4$, C: 68.06, H: 3.01, N: 4.18, found C: 67.93, H: 2.94, N: 4.09.

4.2.12. 5-(6-Bromo-1,3-benzodioxol-5-yl)-6*H*-[1,3]benzodioxolo[5,6-*f*]isoindole-6,8(7*H*)-dione 16. Following the method C, compound **11b** (1.56 g, 3 mmol) was treated with $Pd(OAc)_2$ (67.4 mg, 0.3 mmol), triethylamine (353 mg, 3.6 mmol), and *N*,*N*-dimethylformamide (31.2 ml) for 2 h at 100 °C to give the title compound (1.06 g, yield 80%) as a pale yellow crystalline powder: mp 301 °C (decomposed), ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 6.19 (2H, d, J=13.0 Hz), 6.24 (2H, s), 6.76 (1H, s), 6.97 (1H, s), 7.39 (1H, s), 7.73 (1H, s), 8.31 (1H, s), 11.3 (1H, br), IR (ATR, cm^{-1}): 1706, 1464, 1231, 1024, 640, 437, MS (EI, *m*/*z*): (M+2) 441, (M) 439, HRMS: calcd for $C_{20}H_{10}NO_6Br$, 438.9691, found 438.9689.

4.2.13. 2-Iodo-3,4-methylenedioxybenzaldehyde (2-iodopiperonal) 17. (The following procedure is a modification of Young's 2-iodopiperonal synthesis.)

To a stirred mixture of *N*,*N*,*N*′-trimethylethyldiamine (29.4 g, 288 mmol) and 1,2-dimethoxyethane (DME) (180 ml) was added 1.6 M *n*-BuLi in hexanes (180 ml, 288 mmol) at 0–10 °C under a nitrogen atmosphere. After further stirring at the same temperature for 1 h, a DME (540 ml) solution of piperonal (35.1 g, 234 mmol) was added to the mixture. After more stirring at 0–10 °C for 1 h, the resulting mixture was diluted with tetrahydrofuran (360 ml), followed by 1.6 M *n*-BuLi in hexanes (225 ml, 360 mmol). The mixture was stirred at room temperature for 4.5 h, and iodine (107 g, 421 mmol) was added portionwise from −60 to −70 °C. The reaction mixture was then warmed up to room temperature for 22 h, and satd aq $Na_2S_2O_3$ (350 ml) was added to it. The aqueous layer was separated and extracted with ethyl acetate (175 ml+90 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The concentrated residue was recrystallized from ethanol (320 ml) to give the title compound as the first crop (28.2 g). The second crop (1.7 g) was obtained from the mother liquor (yield 46%) as a colorless crystalline powder: ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 6.25 (2H, s), 7.07 (1H, d, J=8.2 Hz), 7.47 (1H, d, J=8.2 Hz), 9.80 (1H, s), MS (EI): (M^+) 276; Elemental analysis: calcd for $C_8H_5O_3I$, C: 34.81, H: 1.83, I: 45.98, found C: 34.85, H: 1.80, I: 46.01.

4.2.14. 3-(4-Fluorobenzylidene)-4-[(4-iodo-1,3-benzodioxol-5-yl)methylene]pyrrolidine-2,5-dione 11d. Following the method B, compound **10c** (4.15 g, 18.1 mmol) was treated with 2-iodopiperonal (5.00 g, 13.1 mmol), piperidine (1.54 g, 18.1 mmol), acetic acid (1.09 g, 18.1 mmol), and ethanol (83 ml) under reflux for 4.5 h to give the title compound (6.62 g, yield 79%) as a colorless crystalline powder: ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 6.03–6.13 (4H, m), 6.73–6.78 (2H, m), 6.88–6.92 (2H, m), 7.44 (1H, s), 7.56 (1H, s), 11.7 (1H, br), IR (ATR, cm^{-1}): 1701, 1453, 1227, 1173, 1038, 936, 446, MS (EI, *m*/*z*): (M^+) 463, HRMS: calcd for $C_{19}H_{11}NO_4FI$, 462.9717, found 462.9734; Elemental analysis: calcd for $C_{19}H_{11}FNO_4I \cdot 0.5H_2O$, C: 48.33, H: 2.56, N: 2.97, found C: 48.56, H: 2.34, N: 2.90.

4.2.15. 10-(4-Fluorophenyl)-7*H*-[1,3]benzodioxolo[4,5-*f*]isoindole-7,9(8*H*)-dione 7b. A mixture of compound **11d** (6.57 g, 14.2 ml), $Pd(OAc)_2$ (319 mg, 1.42 mmol), triethylamine (3.16 g, 31.2 mmol), and *N*,*N*-dimethylformamide (131 ml) was stirred at room temperature under a nitrogen atmosphere for 1 h and the resulting mixture was heated at 110 °C for 1 h and 20 min. After the reaction

mixture had stood at room temperature, its insoluble part was filtered out and washed with *N*,*N*-dimethylformamide (50 ml). To the combined filtrate and washing were added ethyl acetate (260 ml) and 5% aq $Na_2S_2O_3 \cdot 5H_2O$ (140 ml). The aqueous layer was separated and extracted with ethyl acetate (130 ml+65 ml). The combined organic layers were washed with H_2O (2×200 ml) and concentrated in vacuo. The residue was washed with tetrahydrofuran/EtOH (1:4, 25 ml) and dried in vacuo at 40 °C to give the title compound (3.70 g, yield 78%) as a yellow crystalline powder: mp 285 °C (decomposed), ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 5.72 (2H, s), 6.95–7.01 (2H, m), 7.17–7.22 (2H, m), 7.35 (1H, d, J=8.6 Hz), 7.20 (1H, d, J=8.6 Hz), 8.17 (1H, s), 11.1 (1H, br), IR (ATR, cm^{-1}): 1756, 1716, 1284, 1348, 1057, 637, 447, MS (EI, *m*/*z*): (M^+) 335; Elemental analysis: calcd for $C_{19}H_{10}FNO_4 \cdot 0.5H_2O$, C: 66.28, H: 3.22, N: 4.07, found C: 66.49, H: 3.30, N: 4.08, HPLC purity: 96.8%.

4.3. Pd-catalyzed benzannualtion to regiospecific syntheses of various arylnaphthalene lignan analogues (Schemes 5 and 6)

4.3.1. Dimethyl 2-(4-fluorobenzylidene)succinate 19. To a stirred mixture of diethyl succinate (52.0 g, 299 ml), 28% NaOMe in methanol (43.4 g, 225 mmol), and methanol (87 ml) was added dropwise 4-fluorobenzaldehyde (18.6 g, 150 mmol) in methanol (46 ml) under reflux and the resulting mixture was heated for 2 h under reflux, then treated with 2 M-NaOH (298 ml) for 2 h at the same condition. After standing at room temperature, the reaction mixture was concentrated in vacuo and the resulting residue was recrystallized from ethyl acetate to give 2-(4-fluorobenzylidene)-succinic acid (8.16 g) as colorless crystals. The second crop (4.04 g) was obtained from the mother liquor of the first crystals (total yield 36%) as a colorless crystalline powder: ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 3.36 (2H, s), 7.26–7.31 (2H, m), 7.45–7.50 (2H, m), 7.73 (1H, s), 12.5 (2H, br), IR (ATR, cm^{-1}): 1673, 1309, 1218, 916, 829, 537, MS (EI, *m*/*z*): (M^+) 224; Elemental analysis: calcd for $C_{11}H_9O_4F$, C: 58.93, H: 4.05, found C: 59.14, H: 4.00.

To a stirred mixture of 2-(4-fluorobenzylidene)succinic acid (12.1 g, 54.0 mmol) and methanol (242 ml) was added concd H_2SO_4 (5.30 g, 54.0 mmol) and refluxed for 18 h. After cooling, the reaction mixture was concentrated in vacuo. To the residue was added H_2O (121 ml) and extracted with ethyl acetate (121 ml+61 ml). The combined organic layers were washed with H_2O (187 ml×2), dried over anhydrous $MgSO_4$, and concentrated in vacuo to give the title compound as a pale yellow oil (12.8 g, yield 94%), which was used for the next step without further purification: ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 3.51 (2H, s), 3.64 (3H, s), 3.75 (3H, s), 7.26–7.32 (2H, m), 7.47–7.52 (2H, m), 7.81 (1H, s), IR (Neat, cm^{-1}): 2954, 1739, 1712, 1602, 1508, 1436.

4.3.2. 2-(4-Fluorobenzylidene)-4-(4-iodo-1,3-benzodioxol-5-yl)-3-(methoxycarbonyl)but-3-enoic acid 20. To a stirred mixture of compound **19** (12.7 g, 46.3 mmol), compound **17** (12.8 g, 46.3 mmol), and methanol (254 ml) was added 28% NaOMe in methanol (21.4 g, 111 mmol) and refluxed for 3.5 h. After the cooling of the reaction mixture, 6 M HCl (37 ml, 222 mmol) was added. The precipitate was filtered and washed with H_2O (254 ml) to give the title compound (18.1 g, yield 82%). The second crop (497 mg) was obtained from the mother liquor of the first crystals (total yield 74%) as a pale yellow crystalline powder: mp 209 °C (decomposed), ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 3.70 (3H, s), 6.08 (2H, s), 6.8–6.9 (2H, m), 7.1–7.2 (2H, m), 7.3–7.4 (2H, m), 7.63 (1H, s), 7.68 (1H, s), 12.8 (1H, br), IR (KBr, cm^{-1}): 1700, 1683, 1600, 1232, 1047, 773, MS (EI, *m*/*z*): (M^+) 496; Elemental analysis: calcd for $C_{20}H_{14}O_6FI$, C: 48.41, H: 2.84, found C: 48.49, H: 2.87.

4.3.3. Methyl 3-cyano-4-(4-fluorophenyl)-2-[(4-iodo-1,3-benzodioxol-5-yl)methylene]but-3-enoate 21. To a stirred mixture of compound **20** (16.4 g, 33.0 mmol), tetrahydrofuran (328 ml), and *N*,*N*-dimethylformamide (3.3 ml), thionyl chloride (41 ml) was added dropwise at room temperature and the resulting mixture was heated for 2 h under reflux. After cooling, the reaction mixture was added dropwise to a stirred mixture of 28% aq NH_3 (328 ml) and tetrahydrofuran (164 ml) at ice-cold temperature. After further stirring at the same temperature for 2 h, concd HCl (250 ml) and water (400 ml) were added and the reaction mixture was extracted with ethyl acetate (100 ml×2 and 50 ml). The combined organic layers were concentrated in vacuo and the concentrated residue was washed with methanol (50 ml) to give methyl 3-(aminocarbonyl)-4-(4-fluorophenyl)-2-[(4-iodo-1,3-benzodioxol-5-yl)methylene]but-3-enoate (14.9 g, yield 91%) as a pale yellow crystalline powder: ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 3.67 (3H, s), 6.08 (2H, s), 6.87 (1H, d, J=8.8 Hz), 7.03 (1H, d, J=8.8 Hz), 7.0–7.3 (5H, m), 7.38 (1H, s), 7.46 (1H, br), 7.71 (1H, s), IR (ATR, cm^{-1}): 1702, 1674, 1460, 1228, 1047, 772, MS (EI, *m*/*z*): (M^+) 495; Elemental analysis: calcd for $C_{20}H_{15}FINO_5$, C: 48.50, H: 3.05, N: 2.83, found C: 48.49, H: 3.28, N: 2.68.

To a stirred mixture of the above amide (14.7 g, 29.6 mmol), tetrahydrofuran (294 ml), and *N*,*N*-dimethylformamide (4.4 ml), oxalyl chloride (14.7 ml) was added dropwise at room temperature and the resulting mixture was stirred at room temperature for 3 h. To the reaction mixture was added H_2O (394 ml) and extracted with ethyl acetate (149 ml×2). The combined organic layer was washed with H_2O (300 ml×2), dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was washed with methanol to give the title compound **21** (12.8 g, yield 91%) as a pale yellow crystalline powder: mp 173–177 °C, ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 3.66 (3H, s), 6.16 (2H, s), 6.96 (1H, d, J=8.8 Hz), 7.04 (1H, d, J=8.8 Hz), 7.1–7.3 (2H, m), 7.4–7.5 (2H, m), 7.71 (1H, s), 7.84 (1H, s), IR (KBr, cm^{-1}): 2208, 1718, 1598, 1457, 1216, 925, MS (EI, *m*/*z*): (M^+) 477; Elemental analysis: calcd for $C_{20}H_{13}FINO_4$, C: 50.34, H: 2.75, N: 2.94, found C: 50.53, H: 2.84, N: 2.64.

4.3.4. Methyl 8-cyano-9-(4-fluorophenyl)naphtho[1,2-*d*][1,3]dioxole-7-carboxylate 22. A mixture of compound **21** (12.1 g, 25.4 mmol), $Pd(OAc)_2$ (570 mg, 2.54 mmol), potassium carbonate (7.73 g, 55.9 mmol), and *N*-methylpyrrolidone (242 ml) was stirred at room temperature under N_2 for 1 h and then heated at 110 °C for 4 h. The reaction mixture

was allowed to stand at room temperature, and any insoluble matter was filtered off and washed with *N*-methylpyrrolidone (30 ml). The filtrate and washing were combined, washed with satd $Na_2S_2O_3$ (300 ml) and H_2O (300 ml), dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified with silica-gel column chromatography to give the title compound as a pale yellow crystalline powder (2.80 g, 32%): mp 224 °C (decomposed), ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 3.58 (3H, s), 6.35 (2H, s), 7.3–7.4 (2H, m), 7.4–7.5 (2H, m), 7.62 (1H, d, J=8.8 Hz), 7.95 (1H, d, J=8.8 Hz), 8.76 (1H, s), IR (KBr, cm^{-1}): 2217, 1708, 1459, 1274, MS (EI, *m*/*z*): (M^+) 349; Elemental analysis: calcd for $C_{20}H_{12}FNO_4$, C: 68.77, H: 3.46, N: 4.01, found C: 68.56, H: 3.41, N: 3.93.

4.3.5. Methyl 4-(4-fluorophenyl)-3-[(hydroxyamino)carbonyl]-2-[(4-iodo-1,3-benzodioxol-5-yl)methylene]but-3-enoate 23 (method D). To a stirred mixture of compound **20** (2.00 g, 4.03 mmol), tetrahydrofuran (20 ml), chloroform (20 ml), and *N*,*N*-dimethylformamide (0.4 ml), oxalyl chloride (5 ml) was added dropwise at room temperature. After being stirred at room temperature for 5 h, the reaction mixture was concentrated in vacuo. A tetrahydrofuran (50 ml) solution of the above residue was added dropwise to a stirred mixture of hydroxylamine hydrochloride (2.44 g, 40.3 mmol), tetrahydrofuran (20 ml), and satd $NaHCO_3$ (20 ml) at ice-cold temperature. After further stirring for 1 h at the same temperature, 6 M HCl (20 ml) was added to the reaction mixture and extracted with ethyl acetate twice. The combined organic layer was washed with H_2O, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was washed with a mixture of ethyl acetate and *n*-hexane to give the title compound (1.40 g, yield 68.0%) as a pale yellow crystalline powder: mp 206 °C (decomposed), ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 3.68 (3H, s), 6.08 (2H, s), 6.8–6.9 (1H, m), 7.0–7.3 (6H, m), 7.74 (1H, s), 8.94 (1H, br), 10.8 (1H, br), IR (KBr, cm^{-1}): 1695, 1656, 1506, 1461, 1226, MS (EI, *m*/*z*): (M^+) 511; Elemental analysis: calcd for $C_{20}H_{15}FINO_6$, C: 46.99, H: 2.96, N: 2.74, found C: 47.11, H: 2.91, N: 2.64.

4.3.6. 10-(4-Fluorophenyl)-8-hydroxy-7*H*-[1,3]benzodioxolo[4,5-*f*]isoindole-7,9(8*H*)-dione 24 (method E). A mixture of compound **23** (164 mg, 0.321 mmol), $Pd(OAc)_2$ (7.2 mg, 0.0321 mmol), triethylamine (71.4 mg, 0.706 mmol), and *N*,*N*-dimethylformamide (3.3 ml) was stirred for 1 h at room temperature and then at 110 °C for 45 min. The reaction mixture was allowed to stand at room temperature, and all insoluble matter was filtered off and washed with *N*,*N*-dimethylformamide. To the combined filtrate and washing was added aq $Na_2S_2O_3$ and extracted with ethyl acetate three times. The combined organic layers were washed with H_2O twice, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The resulting residue was washed with methanol and dried in vacuo at 40 °C to give the title compound (78.1 mg, yield 69%) as a yellow crystalline powder: mp 256 °C (decomposed), ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 5.95 (2H, s), 7.1–7.3 (2H, m), 7.4–7.5 (2H, m), 7.58 (1H, d, J=8.6 Hz), 7.93 (1H, d, J=8.6 Hz), 8.45 (1H, s), 10.8 (1H, br), IR (ATR, cm^{-1}): 1714, 1508, 1290, 1068, 880, 723, MS (EI, *m*/*z*): (M^+) 351, HRMS: calcd for $C_{19}H_{10}NO_5F$, 351.0543, found 351.0548.

4.3.7. Dimethyl 2-[(4-iodo-1,3-benzodioxol-5-yl)methylene]succinate 25. To a stirred mixture of compound **17** (26.7 g, 96.7 mmol), diethyl succinate (33.6 g, 193 mmol), and methanol (135 ml) was added 28% NaOMe in methanol (28.0 g, 145 mmol) and the resulting mixture was refluxed for 3 h and 25 min. NaOH (2 M, 267 ml) was then added and the mixture was refluxed for 3 h. After cooling, the reaction mixture was concentrated in vacuo. To the residue were added H_2O (134 ml) and concd HCl (134 ml) and the mixture was extracted with ethyl acetate (267 ml+134 ml×2). The combined organic layers were washed with water (300 ml×2), dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was washed with ethyl acetate (67 ml) to give 2-[(4-iodo-1,3-benzodioxol-5-yl)methylene]succinic acid (22.6 g, 62%) as a colorless crystalline powder: ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 3.24 (2H, s), 6.14 (2H, s), 6.87 (1H, d, J=8.0 Hz), 7.00 (1H, d, J=8.0 Hz), 7.56 (1H, s), 12.6 (2H, br), IR (KBr, cm^{-1}): 2918, 1734, 1697, 1460, 1284, MS (FAB, *m*/*z*): ($M-H)^-$ 375.

To a stirred mixture of 2-[(4-iodo-1,3-benzodioxol-5-yl)-methylene]succinic acid (22.5 g, 59.8 mmol) and methanol (225 ml) was added concd H_2SO_4 (5.87 g, 59.8 mmol) and the resulting mixture was refluxed for 15 h and 40 min. After cooling, the reaction mixture was concentrated in vacuo. To the residue was added H_2O (200 ml) and extracted with ethyl acetate (300 ml+150 ml×2). The combined organic layers were washed with H_2O (300 ml×2), dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was washed with methanol (170 ml) and dried in vacuo at 40 °C to give the title compound (20.2 g, 84%) as a colorless crystalline powder: mp 130–133 °C, ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 3.39 (2H, s), 3.62 (3H, s), 3.76 (3H, s), 6.15 (2H, s), 6.85 (1H, d, J=8.0 Hz), 6.98 (1H, d, J=8.0 Hz), 7.62 (1H, s), IR (KBr, cm^{-1}): 2958, 1718, 1460, 1429, 1373, 1333, 1230, 1180, MS (EI, *m*/*z*): (M^+) 404; Elemental analysis: calcd for $C_{14}H_{13}IO_6$, C: 41.61, H: 3.24, I: 31.40, found C: 41.45, H: 3.27, I: 31.55.

4.3.8. 4-(1,3-Benzodioxol-5-yl)-2-[(4-iodo-1,3-benzodioxol-5-yl)methylene]-3-(methoxycarbonyl)but-3-enoic acid 26. To a stirred mixture of compound **25** (15.0 g, 37.1 mmol), piperonal (6.13 g, 40.8 mmol), and methanol (150 ml) was added 28% NaOMe in methanol (17.2 g, 89.0 mmol) and refluxed for 5 h. After the mixture was cooled, concd HCl (14.8 ml, 178 mmol) was added. The precipitate was filtered and washed with H_2O (60 ml) to give the title compound (15.9 g, yield 82%) as a pale yellow crystalline powder: mp 207 °C (decomposed), ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 3.66 (3H, s), 6.04 (2H, s), 6.08 (2H, d, J=2.7 Hz), 6.79 (1H, d, J=8.2 Hz), 6.83 (1H, d, J=8.2 Hz), 6.87–6.97 (3H, m), 7.58 (1H, s), 7.73 (1H, s), 12.9 (1H, br), IR (KBr, cm^{-1}): 1716, 1672, 1460, 1230, MS (EI, *m*/*z*): (M^+) 522; Elemental analysis: calcd for $C_{21}H_{15}IO_8 \cdot 0.5H_2O$, C: 47.48, H: 3.04, I: 23.89, found C: 47.64, H: 2.94, I: 23.80.

4.3.9. Methyl 2-(1,3-benzodioxol-5-ylmethylene)-3-[(dimethylamino)carbonyl]-4-(4-iodo-1,3-benzodioxol-5-yl)-but-3-enoate 27. Following the method D, compound **26** (3.00 g, 5.74 mmol) was treated with tetrahydrofuran

(30 ml), *N*,*N*-dimethylformamide (0.6 ml), and oxalyl chloride (7.59 ml, 57.4 mmol) to give the corresponding acid chloride, which was treated with dimethylamine hydrochloride (4.68 g, 57.4 mmol), satd $NaHCO_3$ (60 ml), and tetrahydrofuran (45 ml) to provide the title compound (3.05 g, yield 97%) as a pale yellow crystalline powder: mp 190–191 °C, ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 2.8–3.2 (6H, br), 3.70 (3H, s), 6.01 (4H, s), 6.4–6.7 (2H, m), 6.7–6.9 (3H, m), 7.17 (1H, s), 7.48 (1H, s), IR (ATR, cm^{-1}): 1689, 1630, 1449, 1170, 1040, 932, MS (EI, *m*/*z*): (M^+) 549; Elemental analysis: calcd for $C_{23}H_{20}NO_7I$, C: 50.29, H: 3.67, N: 2.55, found C: 50.22, H: 3.58, N: 2.62.

4.3.10. Methyl 9-(1,3-benzodioxol-5-yl)-7-[(dimethylamino)-carbonyl]naphtho[1,2-*d*][1,3]dioxole-8-carboxylate 28. Following the method E, compound **27** (1.10 g, 2 mmol) was treated with $Pd(OAc)_2$ (89.8 mg, 0.4 mmol), AcOK (432 mg, 4.4 mmol), and *N*-methylpyrrolidone (22 ml) at 110 °C for 4 h, which was followed by purification with column chromatography (eluent: hexanes/AcOEt) and crystallization from aq methanol, to give the title compound (74.8 mg, yield 8.9%) as a pale yellow crystalline powder: ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 2.64 (3H, s), 2.85 (3H, s), 3.67 (3H, s), 6.00 (2H, s), 6.15 (2H, s), 6.8–7.0 (3H, s), 7.25 (1H, d, *J*=8.6 Hz), 7.47 (1H, d, *J*=8.6 Hz), 7.81 (1H, s), IR (ATR, cm^{-1}): 1737, 1617, 1459, 1223, 1200, 1111, MS (EI, *m*/*z*): (M^+) 421; Elemental analysis: calcd for $C_{23}H_{19}NO_7 \cdot 0.5H_2O$, C: 64.18, H: 4.68, N: 3.08, found C: 64.17, H: 4.82, N: 3.17, HPLC purity: 99.6%.

4.3.11. 4-(1,3-Benzodioxol-5-yl)-3-(hydroxymethyl)-2-[(4-iodo-1,3-benzodioxol-5-yl)methylene]-but-3-enoic acid 29. Under a nitrogen atmosphere, compound **29** (2.00 g, 3.83 mmol) was suspended in tetrahydrofuran (6 ml) and cooled at 0–10 °C. To the suspension was added dropwise 1.0 M $LiBHEt_3$ in tetrahydrofuran (25.2 ml, 25.2 mmol), maintaining the temperature below 10 °C, and stirred at this temperature for 1 h. To the reaction mixture were added 50% aq acetic acid (3 ml) and H_2O. The resultant mixture was extracted with ethyl acetate three times. The combined organic layers were washed with H_2O, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was crystallized from ethyl acetate/*n*-hexane (2:1) to give the title compound (1.26 g, yield 67%) as a pale yellow crystalline powder: ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 3.8–4.0 (3H, m), 5.97 (2H, s), 6.10 (2H, s), 6.57 (1H, s), 6.69 (1H, dd, *J*=8.2 and 1.6 Hz), 6.7–6.9 (3H, m), 7.25 (1H, d, *J*=8.2 Hz), 7.63 (1H, s), IR (KBr, cm^{-1}): 3384, 1691, 1459, 1255, 1232, MS (FAB): (M−H) 493; Elemental analysis: calcd for $C_{20}H_{15}IO_7$, C: 48.60, H: 3.06, I: 25.68, found C: 48.38, H: 3.13, I: 25.31 and 25.42.

4.3.12. 4-(1,3-Benzodioxol-5-ylmethylene)-3-[(4-iodo-1,3-benzodioxol-5-yl)methylene]pyrrolidin-2-one 30. A mixture of compound **29** (1.2 g), iodoethane (2.83 g, 18.2 mmol), K_2CO_3 (1.26 g, 9.10 mmol), and *N*,*N*-dimethylformamide (8.18 ml) was stirred at room temperature for 2 h. To the reaction mixture was added H_2O (50 ml) and extracted with ethyl acetate (100 ml+50 ml+50 ml). The combined organic layers were washed with H_2O (50 ml), dried over anhydrous $MgSO_4$, and concentrated in vacuo to leave an oil.

To the residual oil was added toluene (24 ml), followed by PBr_3 (657 mg), and the resulting mixture was stirred at room temperature for 1 h. To the reaction mixture was added H_2O (50 ml) and extracted with ethyl acetate (50 ml) twice. The combined organic layers were washed with H_2O (50 ml), dried over anhydrous $MgSO_4$, and concentrated in vacuo to leave an oil.

To the residual oil were added tetrahydrofuran (12 ml), 28% aq NH_3 (24 ml), and methanol (12 ml) and the resulting mixture was stirred at room temperature for 11 h. After the reaction mixture was concentrated in vacuo, ethyl acetate (50 ml) and H_2O (50 ml) were added to the residue. The aqueous layer was separated and extracted with ethyl acetate (50 ml). The combined organic layers were washed with H_2O (50 ml), dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified with silica-gel column chromatography (eluent: toluene/ethyl acetate) to give the title compound as a yellow solid (405 mg, yield 35%): ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 4.07 (2H, s), 5.84 (2H, s), 5.99 (1H, d, *J*=1.3 Hz), 6.02 (2H, s), 6.15 (1H, d, *J*=8.2 Hz), 6.19 (1H, d, *J*=8.2 Hz), 6.37 (1H, dd, *J*=1.3 and 8.1 Hz), 6.50 (1H, d, *J*=8.1 Hz), 6.53 (1H, s), 7.12 (1H, s), 8.41 (1H, br s), IR (KBr, cm^{-1}): 1697, 1454, 1249, 1037, MS (EI, *m*/*z*): (M+) 475, HRMS: calcd for $C_{20}H_{14}NO_5I$, 474.9914, found 474.9917, HPLC purity: 98.7%.

4.3.13. 10-(1,3-Benzodioxol-5-yl)-8,9-dihydro-7*H*-[1,3]benzodioxolo[4,5-*f*]isoindol-7-one 7c. A mixture of compound **30** (300 mg, 0.631 mmol), $Pd(OAc)_2$ (14.2 mg, 0.0631 mmol), AcOK (136 mg, 1.39 mmol), and *N*-methylpyrrolidone (6 ml) was stirred at room temperature for 1 h and the resulting mixture was heated at 110 °C for 40 min. After the reaction mixture had stood at room temperature, insoluble matter was filtered off and washed with *N*-methylpyrrolidone (3 ml) and ethyl acetate (10 ml). To the combined filtrate and washing was added aq $Na_2S_2O_3$ (35 ml) and extracted with ethyl acetate (20 ml+15 ml+15 ml). The combined organic layers were washed with 1 M HCl (2×30 ml) and brine (2×30 ml), and concentrated in vacuo. The residue was washed with tetrahydrofuran/ethanol (1:1, 0.5 ml) and dried in vacuo at 40 °C to give the title compound as yellow crystals (106 mg, yield 48%): mp: 250–251 °C (lit.[2] mp: 252–254 °C), ^{1}H NMR (DMSO-d_6, TMS, 300 MHz) δ (ppm): 4.20 (2H, s), 5.95 (2H, dd, *J*=6.3 and 0.9 Hz), 6.09 (2H, dd, *J*=14.1 and 0.8 Hz), 6.87 (1H, dd, *J*=7.9 and 1.6 Hz), 6.96 (1H, d, *J*=7.9 Hz), 7.00 (1H, d, *J*=1.6 Hz), 7.43 (1H, d, *J*=8.7 Hz), 7.95 (1H, d, *J*=8.7 Hz), 8.29 (1H, s), 8.58 (1H, br), IR (KBr, cm^{-1}): 1701, 1635, 1495, 1460, 1439, 1272, MS (EI, *m*/*z*): (M^+) 347; Elemental analysis: calcd for $C_{20}H_{13}NO_5 \cdot 0.75H_2O$, C: 66.57, H: 4.19, N: 3.88, found C: 66.65, H: 4.18, N: 4.16, HPLC purity: 96.9%.

The palladium-catalyzed reaction of compound **30** (25 mg) was carried out as before. The reaction mixture was assayed by HPLC under the following conditions to indicate a 72% yield of compound **7c**.

HPLC conditions: YMC-Pack ODS-A302 column (150 mm×4.6 mm I.D.) with 0.05 M KH_2PO_4 in water and acetonitrile (60:40) at 25 °C. Detection was effected with a Hitachi spectrophotometric detector at 254 nm.

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New dioxadiaza-, trioxadiaza- and hexaaza-macrocycles containing dibenzofuran units

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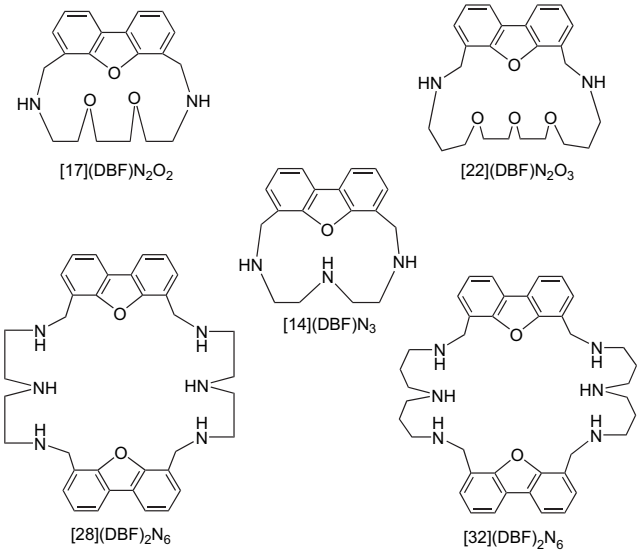
Abstract—New dioxadiaza-, trioxadiaza-, and hexaaza-macrocycles containing rigid dibenzofuran groups (DBF) were prepared by a convenient synthetic route in high yields. The structures of the macrocycles were unequivocally established by electrospray mass spectrometry (ESIMS) studies together with NMR spectroscopy, with the exception of [14](DBF)N_3. The structures of the copper complex of [14](DBF)N_3 and of the diprotonated form of [22](DBF)N_2O_3 were determined by single crystal X-ray diffraction. Conformational analyses on the free macrocycles [14](DBF)N_3 and [22](DBF)N_2O_3 as well as on their larger counterparts containing two DBF units were undertaken in order to understand the synthetic findings.

1. Introduction

In recent years there have been an increasing interest in the design, synthesis and use of diverse macrocyclic receptors capable of selective recognition of metal ions, anions and neutral molecules,[1–4] since their cavity sizes, shapes and components can be readily modified. These features have led to the development of many applications, from catalysis to mediated transport, from sensors or devices and to molecular machines or pharmacology.[1–6]

We are interested in developing simple synthetic methods to prepare polyaza- or polyoxapolyaza-macrocyclic compound having rigid 4,6-dibenzofuran (DBF) units and flexible spacers able to recognize metal ions or organic substrates. The DBF group is not a common building block, nevertheless it has already been used to prepare supramolecular compounds or devices as a spacer in rigid macrocycles,[7,8] catenanes,[8] cavitands,[9,10] calixarenes[11] and rigidly preorganised clefts or as the central part of tweezers.[9,10,12–19] Most of the known compounds contain the 2,8- or 3,7-substituted DBF fragments.

In the present work, five novel polyaza- or polyoxadiaza-macrocycles incorporating the DBF structural fragments, [17](DBF)N_2O_2, [22](DBF)N_2O_3, [14](DBF)N_3, [28](DBF)$_2N_6$ and [32](DBF)$_2N_6$ (see Scheme 1), were synthesized by a [1+1] or [2+2] condensation of different linear amines with 4,6-dibenzofurandicarbaldehyde. All the prepared macrocycles display an important structural feature



Scheme 1.

Keywords: Macrocycles; Dibenzofuran derivatives; X-ray structures; [2+2] Condensation.
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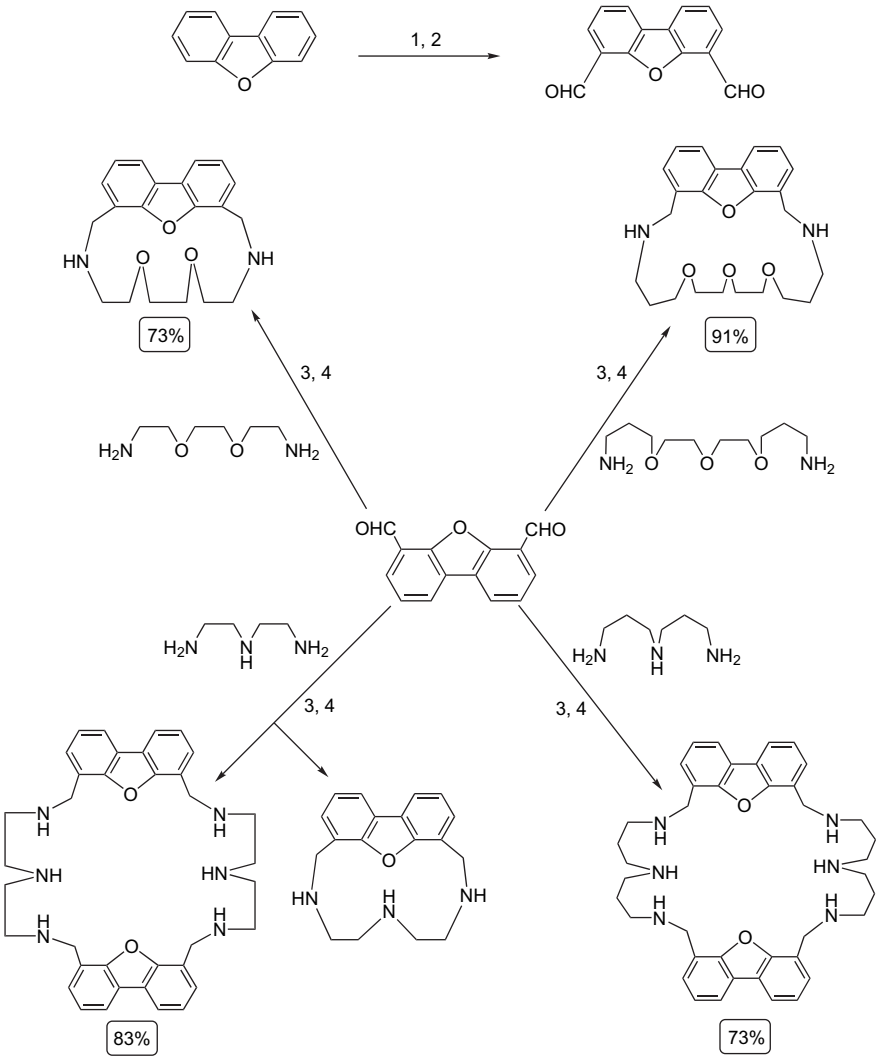
one or two rigid DBF moieties coupled at the 4 and 6 positions with flexible polyoxadiaza or polyaza spacers. The largest macrocycles, [22](DBF)N_2O_3, [28](DBF)$_2N_6$ and [32](DBF)$_2N_6$, are capable of coordinating one or two metal ions, while the smallest ones [14](DBF)N_3 and [17](DBF)N_2O_2 can only form mononuclear metal complexes. On the other hand, the hexaprotonated forms of [28](DBF)$_2N_6$ and [32](DBF)$_2N_6$ as well as their dimetal complexes are receptors for dianion substrates leading to the formation of stable supermolecules. The binding studies will be provided in future publications.

2. Results and discussion

2.1. Synthesis

The compounds [17](DBF)N_2O_2 and [22](DBF)N_2O_3 were prepared by the [1+1] cyclocondensation of 4,6-dibenzofurandicarbaldehyde with the appropriate diamine, 2,2′-ethylenedioxy-bis(ethylamine) and 4,7,10-trioxa-1,13-tridecanediamine, respectively, while [28](DBF)$_2N_6$ and [32](DBF)$_2N_6$ resulted from the [2+2] cyclocondensation with diethylenetriamine and *N*-(3-aminopropyl)-1,3-propanediamine, respectively. In all cases low temperature was used and the desired macrocycles were obtained in high yield after reduction of the corresponding Schiff bases with $NaBH_4$ (see Scheme 2). Several attempts to obtain the larger macrocycles [34](DBF)$_2N_4O_4$ and [44](DBF)$_2N_4O_6$ via [2+2] condensation using the corresponding polyoxadiamine were undertaken without success, in spite of the use of experimental conditions favouring thermodynamically the formation of these larger molecules, low temperature and high dilution. The resulting products ([1+1] or [2+2]) were distinguished by mass spectrometry.

The macrocycles [17](DBF)N_2O_2 and [22](DBF)N_2O_3 were isolated as white powders from ethanolic hydrobromic acid solutions, while pure [28](DBF)$_2N_6$ and [32](DBF)$_2N_6$ were obtained by recrystallization of the crude product from ethanol. The macrocycle [14](DBF)N_3 found in a small amount, as a side-product of the synthesis of [28](DBF)$_2N_6$, was isolated from the liquor mother ethanol solutions as a copper(II) complex by addition of $CuSO_4$. The X-ray single crystal structure of this complex was determined showing definitely that the [1+1] product was also formed, see below. Then the synthesis of [14](DBF)N_3 as a single product was attempted in conditions favouring the [1+1]



Scheme 2. (1) Et_2O, TMEDA, *n*-BuLi, reflux; (2) DMF, 0 °C, then rt 24 h; (3) EtOH, 0 °C and (4) $NaBH_4$, rt.

product, but surprisingly in spite of our best efforts all the synthetic routes always yielded the larger macrocycle as the main product.

Condensation of 4,6-dibenzofurandicarbaldehyde with the aromatic spacer *m*-xylylenediamine or different aliphatic polyamines having longer chains, such as 3,3′-ethylenediiminodipropylamine and triethylenetetramine, was also carried out in the experimental conditions described above. However the work-up of these syntheses revealed the presence of mixtures of polymers and other macrocycles and the isolation of the expected macrocycles was impossible.

The dioxadiamine and trioxadiamine mainly yielded to [1+1] cyclocondensation products while both triamines seem to favour the formation of larger macrocycles via [2+2] condensation. This synthetic finding led us to think that intramolecular bonding interactions, such as hydrogen bonds, play a role in the preorganization of different intermediate compounds involved in the final stage of cyclocondensation process determining the synthetic route followed (we recall to this point bellow).

Other approaches involving one-pot reaction for the synthesis of similar macrocycles require a template or high dilution. However, these synthetic routes yield in general to complicated work-up, more difficult product purifications, and waste of solvents.[20–22]

2.2. Electrospray mass spectrometry studies

The ESI mass spectra acquired in positive polarity mode using methanol solutions of [17](DBF)N_2O_2, [22](DBF)N_2O_3, [28](DBF)$_2N_6$ and [32](DBF)$_2N_6$ showed peaks corresponding to the monoprotonated ligands. In the spectra of [17](DBF)N_2O_2 and [28](DBF)$_2N_6$ *m*/*z* peaks (363.3 and 613.1, respectively) corresponding to the sodium adducts of the macrocycles are present. A low intensity *m*/*z* peak at 296.0 was also observed in the [28](DBF)$_2N_6$ mass spectrum that can be assigned to the $[M+2H]^{2+}$ diprotonated form. The intensity of this peak was increased when 0.1% formic acid was added to the macrocycle dissolved in methanol. The spectrum of [32](DBF)$_2N_6$ also contains one weak peak corresponding to the double protonated ion $[M+2H]^{2+}$ (*m*/*z* 324).

2.3. X-ray crystallographic studies

In the X-ray crystal structure discrete units of $\{H_2[22](DBF)N_2O_3\}^{2+}$ and two independent PF_6^- anions are held together by hydrogen-bonding interactions between the fluoride atoms and the two N–H binding sites leading to the formation of the supermolecule as shown in Figure 1. The dimensions of all hydrogen-bonding interactions found are listed in Table 1. Both PF_6^- anions are located outside of the macrocyclic cavity. The first PF_6^- anion is involved in only one N···H–F hydrogen bond with a N···F distance of 2.18 Å, while the second one is involved in a bifurcated

Table 1. Dimensions of the hydrogen bonds of **1** and **2**

Donor–H···acceptor	H···A/Å	D···A/Å	D–H···A/°
$\{H_2[22](DBF)N_2O_3\}\cdot 2PF_6$ (**1**)			
Intermolecular			
N(16)–H(16A)···F(13)	2.18	2.926(2)	140
N(30)–H(30A)···F(21)	2.02	2.888(2)	161
N(30)–H(30A)···F(23)	2.34	3.059(2)	136
Intramolecular			
N(16)–H(16B)···O(20)	2.15	2.741(2)	123
N(16)–H(16B)···O(23)	2.02	2.863(2)	156
N(30)–H(30B)···O(26)	2.12	2.833(2)	136
N(16)–H(16A)···O(26)	2.44	3.044(2)	125
$[Cu([14](DBF)N_3)(H_2O)_2](SO_4)\cdot 3H_2O$ (**2**)			
N(16)–H(16)···O(500)	2.04	2.940(11)	170
N(19)–H(19)···O(21) [2-*x*,1-*y*,1-*z*]	2.31	3.120(10)	149
N(22)–H(22)···O(23)	2.58	3.177(10)	124
N(22)–H(22)···O(300) [2-*x*,1-*y*,1-*z*]	2.46	3.226(11)	142
O(100)–H(101)···O(300)	1.99(5)	2.745(11)	155(4)
O(100)–H(102)···O(22)	2.15(6)	2.743(10)	130(6)
O(200)–H(201)···O(24) [2-*x*,2-*y*,1-*z*]	1.98(4)	2.681(8)	146(4)
O(200)–H(202)···O(23)	1.95(4)	2.718(10)	159(5)
O(300)–H(301)···O(500)	2.24(7)	2.885(12)	136(7)
O(300)–H(302)···O(21) [2-*x*,1-*y*,1-*z*]	1.99(8)	2.765(10)	159(8)
O(400)–H(401)···O(23)	2.09(6)	2.890(10)	167(10)
O(400)–H(402)···O(22) [2-*x*,2-*y*,1-*z*]	2.19(9)	2.976(11)	161(11)
O(500)–H(501)···O(400) [2-*x*,2-*y*,1-*z*]	2.04(8)	2.817(13)	158(8)

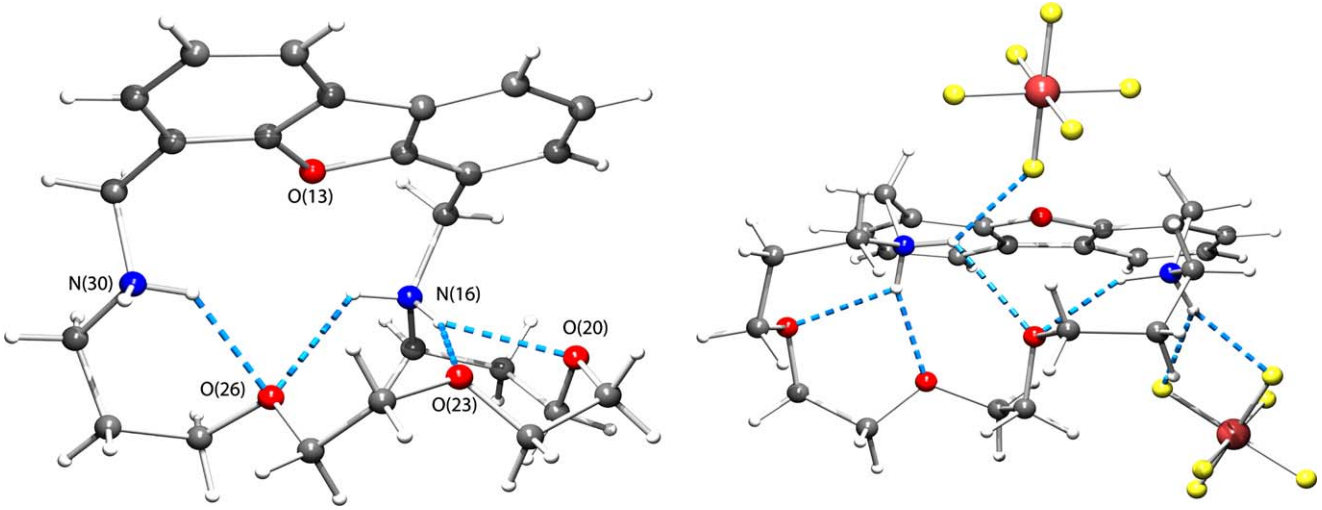


Figure 1. Structure of the supramolecular association found in solid state for $\{H_2[22](DBF)N_2O_3\}\cdot 2PF_6$. Molecular structure of $\{H_2[22](DBF)N_2O_3\}^{2+}$ with the labelling scheme adopted (left) and the supramolecular interaction between $\{H_2[22](DBF)N_2O_3\}^{2+}$ and PF_6^- anion (right).

hydrogen-bonding interaction with a N–H binding site of the second nitrogen donor atom, the H···F distances being 2.02 and 2.34 Å. Three N–H binding sites are directed toward the electron lone pairs of three oxygen donors leading to the formation of four intramolecular N–H···O hydrogen bonds with H···O distances of 2.02, 2.12, 2.15 and 2.44 Å. These hydrogen-bonding interactions are certainly important for the stabilization of the conformation presented in Figure 1 (left) for the protonated receptor.

The X-ray single crystal diffraction of the Cu(II) complex with [14](DBF)N_3 is built up from an asymmetric unit composed of one $[Cu([14](DBF)N_3)(H_2O)_2]^{2+}$ cation (1), one SO_4^{2-} as the counter-ion and three water molecules. The molecular structure of $[Cu([14](DBF)N_3)(H_2O)_2]^{2+}$ with the labelling scheme adopted is presented in Figure 2. The copper atom shows a distorted octahedral coordination environment with the equatorial plane determined by the three nitrogen donor atoms with Cu–N distances ranging from 2.106(7) to 2.138(7) Å and an oxygen atom from a water molecule with a Cu–O of 2.037(6) Å. The axial positions are occupied by oxygen atoms from the DBF moiety and of the second coordination water molecule at Cu–O distances of 2.059(6) and 2.352(5) Å, respectively. The copper centre is 0.110(3) Å away from the equatorial coordination plane towards the apical water molecule. This geometric arrangement is achieved with the folding of the macrocycle along the N(22)–N(16) axis leading to a dihedral angle between the DBF moiety and the plane defined by the three nitrogen donor atoms of 75.8(3)°. Selected bond length angles subtended at the copper centre are given in Table 2. The longest Cu–O apical distance reveals a tetragonal distortion, which is expected for metal complexes with d^9 electronic configuration. The Cu–N distances found are within the typical values reported for azamacrocylic complexes with copper bonded to N_{sp^3} nitrogen donor.[23]

In the crystal structure the ionic species $[Cu([14](DBF)N_3)(H_2O)_2]^{2+}$ and SO_4^{2-}, and water solvent molecules are assembled by an extensive 1-D chain of hydrogen bonds along the [010] base vector as shown in Figure 2 (right). Their molecular dimensions are listed in Table 1 together with those found for $\{H_2[22](DBF)N_2O_3\}\cdot 2PF_6$. The SO_4^{2-} anions link directly three adjacent $[Cu([14](DBF)N_3)(H_2O)_2]^{2+}$ cations through the five independent hydrogen bonds with two N–H groups (H···O= 2.31 and 2.58 Å), two with equatorial coordinated water molecule (H···O=1.98(4) and 1.95(4) Å) and one with axial coordinated water (H···O=2.15(6) Å). This pattern of hydrogen bonds leads to the formation of 1-D network, which is completed by multiple hydrogen bonds between water bridges composed of three water molecules with the third N–H binding site (H···O=2.04 Å), axial coordinated water molecule (H···O=1.99(5) Å) and SO_4^{2-} anions with H···O distances ranging from 1.99(8) to 2.19(9) Å. The O···H distances within the water bridge are 2.04(8) and 2.24(7) Å.

Table 2. Selected bond lengths (Å) and angles (°) for $[Cu([14](DBF)N_3)(H_2O)_2]^{2+}$

Cu–O(13)	2.352(5)	Cu–O(100)	2.059(6)
Cu–O(200)	2.037(6)	Cu–N(16)	2.106(7)
Cu–N(19)	2.123(7)	Cu–N(22)	2.138(7)
N(16)–Cu–N(22)	160.6(2)		
O(100)–Cu–O(13)	172.9(2)	O(200)–Cu–N(19)	174.6(3)
O(200)–Cu–O(100)	92.0(2)	O(200)–Cu–N(16)	100.4(2)
O(100)–Cu–N(16)	101.8(3)	O(100)–Cu–N(19)	92.0(3)
N(16)–Cu–N(19)	82.5(3)	O(200)–Cu–N(22)	93.7(3)
O(100)–Cu–N(22)	91.0(3)	N(19)–Cu–N(22)	82.5(3)
O(200)–Cu–O(13)	84.7(2)	N(16)–Cu–O(13)	85.0(2)
N(19)–Cu–O(13)	91.0(2)	N(22)–Cu–O(13)	83.0(2)

To the best of our knowledge, the two structures reported here represent the first two examples of oxaazamacrocycles incorporating DBF moieties in their skeletons. Indeed a search on Cambridge Data Base[24] retrieved only 11 crystal structures of macrocyclic compounds with BDF moieties. In seven of them, the BDF fragment is bridging two porphyrin platforms used to anchor two metal transition centres (refcodes ATIREA, INUWOD, LIRSEK, LIRSIO

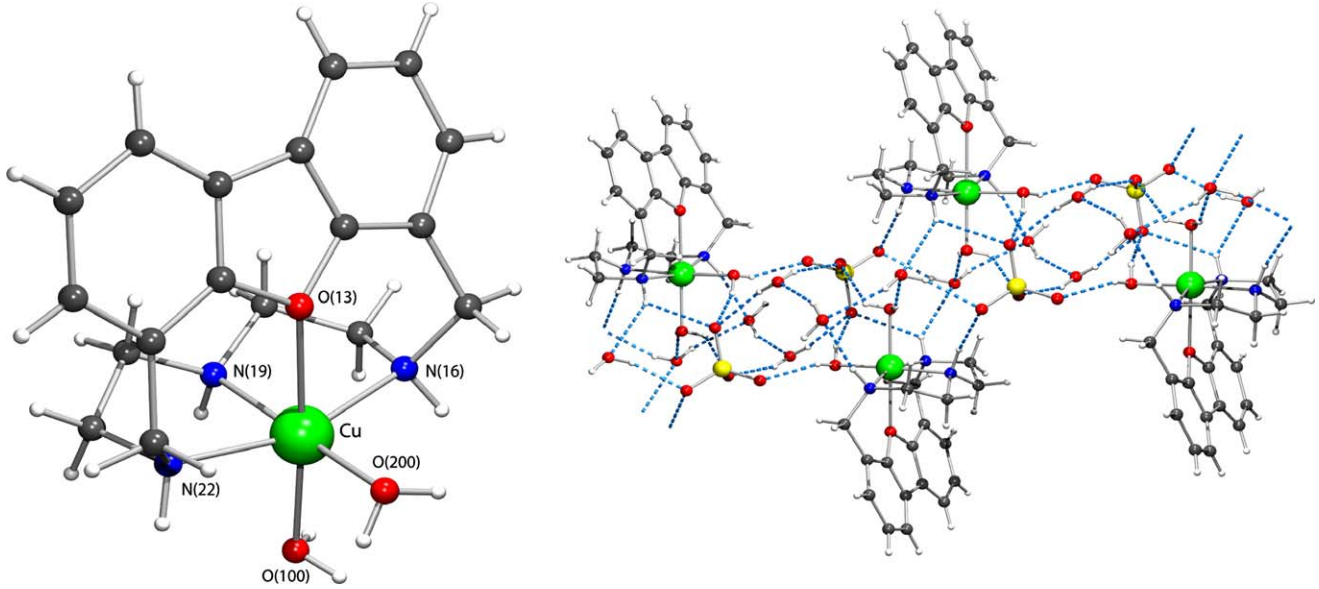


Figure 2. X-ray single crystal structure of $[Cu([14](DBF)N_3)(H_2O)_2](SO_4)\cdot 3H_2O$. Molecular structure of $[Cu([14](DBF)N_3)(H_2O)_2]^{2+}$ with the labelling scheme adopted (left) and crystal packing diagram showing the 1-D network of N–H···O hydrogen bonds (right).

and LOMLAA) or not (refcodes MOBVOO and OFOSIL). In the remaining four compounds, the macrocycle incorporates one (refcode AFILUW), three (refcodes JUSXOK and JUSYAX) or four (JUSXIE) coupled DBF structural units. The compound, with the refcode JUSYAX is the unique azamacrocycle and displays a rigid structure with three-DBF units head connected by a small dimethyl-ethylenodiamine spacer.

2.4. Molecular modelling

In order to give a further insight on the influence of structural features in the cyclocondensation process, i.e., the formation of smaller or larger macrocycles, conformational analyses were undertaken for [14](DBF)N_3 and [22](DBF)N_2O_3 and their larger counterpart ones [28](DBF)$_2N_6$ and [44](DBF)$_2N_4O_6$ via molecular dynamics quenching methods using AMBER-8 software package.[25] Atomic parameters were taken from the GAFF force field[26] and the atomic partial charges for neutral species were calculated with the AM1-BCC method.[27] The structures of all four macrocycles were heated in the gas phase at 2000 K for 1 ns using a time step of 1 fs. Snapshots were saved for every 0.1 ps leading to trajectory files containing 10 000 frames, which were subsequently energy-minimized using an appropriate house script.

For [14](DBF)N_3 the lowest energy structure was found at 78.9 kcal mol^{-1} and displays a folded conformation with the DBF unit making with the plane of the nitrogen donors a dihedral angle of 71.9°. The two N–H groups adjacent to DBF unit are at a short O···H distances of 2.31 Å from the oxygen donor leading to two N–H···O hydrogen bonds with angles 131°. Furthermore, both N–H groups also form N–H···N hydrogen bonds with the N–H group opposite to DBF unit having a N···H distances of 2.16 Å and the N–H···N angles of 117°. Thus, the simultaneous occurrence of four intramolecular hydrogen-bonding contacts leads to a lowest energy conformation with an apparent rigid structure. The first roughly planar energy conformation is only 0.95 kcal mol^{-1} above the minimum energy conformation. The N–H group opposite to the oxygen donor is involved with it in a strong hydrogen-bonding with a H···O distance of 2.19° and N–H···O angle of 175°.

The first three conformations of [28](DBF)$_2N_6$ with energy ranging between 145.1 and 147.1 kcal mol^{-1} exhibit the aromatic DBF unit in an almost parallel arrangement, as shown in Figure 3, which suggests the presence of a π–π bonding interaction. Furthermore, all three conformations show at least a single hydrogen bond interaction involving a N–H group and an oxygen donor from the DBF unit. For example, the lowest energy conformation exhibits a N–H group pointing to the oxygen from a DBF unit at H···O distance of 2.37 Å giving a N–H···O angle of 129°. In addition, this conformation reveals four N–H···N hydrogen-bonding interactions. Three of them are hydrogen bonds between neighbour N–H groups with H···N distances ranging from 2.41 to 2.57 Å. The remaining one with a H···N distance of 2.01 Å and a N–H···N angle of 160° is the most strong and occurs between two N–H groups from different spacers suggesting that they play an important role in the stabilization of the lowest energy conformation. It is interesting to note that short N–H···N hydrogen-bonding contacts are also present in the next lowest energy conformations.

The lowest five energy conformations found for [22](DBF)N_2O_3 have energies between 42.3 and 44.9 kcal mol^{-1} with the DBF unit tilted relatively to the N_2O_3 plane and all display two hydrogen bonds. In three of them, the N–H···O intramolecular bonding contacts with H···O distances ranging from 2.07 to 2.36 Å occur only between N–H groups and ether oxygen donors of the spacer as observed in the crystal structure of $\{H_2[22](DBF)N_2O_3\}^{2+}$ (see above). The fourth conformation has an energy of 44.1 kcal mol^{-1} and the hydrogen bonds involve the oxygen donors from the spacer and the DBF unit with H···O distances of 1.96 and 2.30 Å, respectively. These conformational types are illustrated in Figure 4, which shows the lowest energy structures of [22](DBF)N_2O_3 and [44](DBF)$_2N_4O_6$. As would be expected the largest macrocycle [44](DBF)$_2N_4O_6$ has enough flexibility to adopt very different conformations with similar energies. Indeed the two lowest energy conformations have different arrangements of the two DBF units. In the first, these units have an almost perpendicular spatial disposition, while in the second they are almost parallel making dihedral angles of ca. 90° and 0°, respectively. As a consequence of the perpendicular orientation of two units in the first conformation, one C–H hydrogen from one DBF unit is directed towards the aromatic ring of the second one at a distance of 2.58 Å, which suggests the presence of edge to face C–H(σ)···π interaction. The dimensions of this macrocycle also allow the establishment of isolated hydrogen bonds without any apparent steric strain, as happens in the second lowest energy conformation, which has a short H···O contact of 2.40 Å. Both polyoxapolyaza-macrocycles, [22](DBF)N_2O_3 and [44](DBF)$_2N_4O_6$, do not show N–H···N hydrogen-bonding interactions in

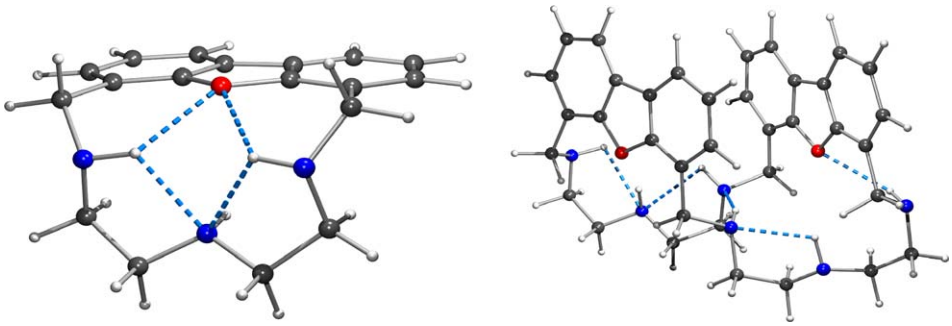


Figure 3. Structures of the lowest energy conformations found with GAFF force field[26] for [14](DBF)N_3 (left) and [28](DBF)$_2N_6$ (right).

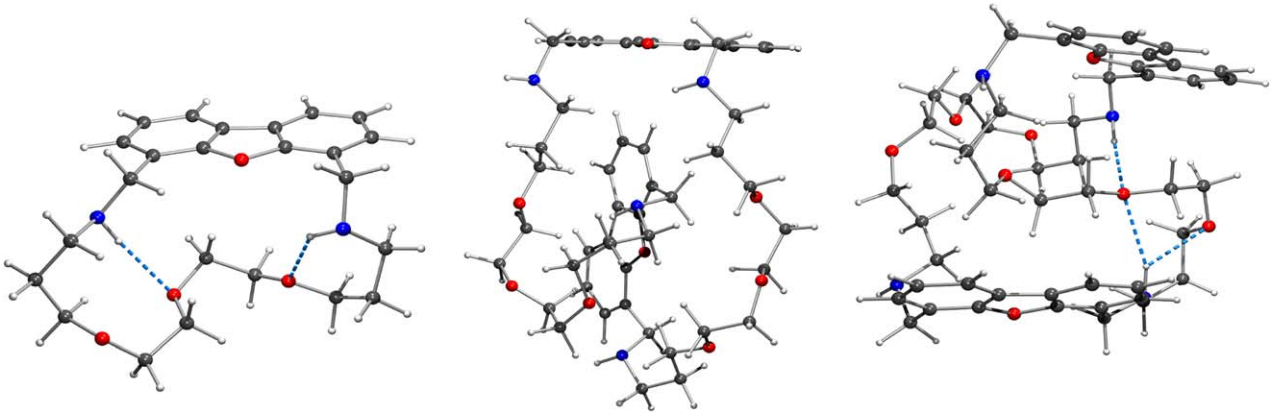


Figure 4. Lowest energy conformations for [22](DBF)N_2O_3 (left) and [44](DBF)$_2N_4O_6$ (centre and right).

all the low energy minimized structures found by conformational analyses, which is in contrast with what was observed for the two polyaza-macrocycles, [14](DBF)N_3 and [28](DBF)$_2N_6$.

2.5. Conclusions

A new series of polyoxapolyaza- or polyaza-macrocyclic receptors containing 4,6-dibenzofuran units was prepared in high yield with a convenient synthetic route, which did not require high dilution or template techniques. The electrospray ionization mass spectrometry ESIMS technique was successfully used for preliminary analysis and determination of the stoichiometry of the macrocyclic compounds. The X-ray single crystal structure of the copper complex with [14](DBF)N_3 confirmed that the [1+1] condensation of 4,6-dibenzofurandicarbaldehyde with diethylenetriamine also occurred.

It is interesting to note that the condensation of 4,6-dibenzofurandicarbaldehyde with different diamines, at the same reaction conditions, led preferentially to [1+1] macrocycles in certain cases and to [2+2] in others. Indeed the dioxa- and trioxa-diamines used to undergo [1+1] condensations while both triamines led to [2+2] ones. The crystal structure of the protonated [22](DBF)N_2O_3 macrocycle displays three intramolecular N–H···O hydrogen-bonding interactions suggesting that the formation of the free macrocycle via [1+1] cyclocondensation can be favoured. In order to bring some light to this point the structural preferences of the macrocycles [14](DBF)N_3, [22](DBF)N_2O_3, [28](DBF)$_2N_6$ and [44](DBF)$_2N_4O_6$ were investigated through conformational analysis using the GAFF force field. The smaller macrocycles in their lowest energy structures have two N–H···O hydrogen bonds. In [14](DBF)N_3, these interactions are accomplished by two extra N–H···N hydrogen bonds leading to an apparent rigid structure. These facts suggest that intramolecular hydrogen-bonding interactions N–H···O and/or N–H···N can be present in the [1+1] cyclocondensation process, pre-organizing the corresponding intermediate in an almost rigid arrangement for the final cyclization step of the smaller macrocycles. Multiple N–H···N bonding interactions were observed in [28](DBF)$_2N_6$ suggesting that the formation of this larger macrocycle is favoured relatively to the smaller counterpart by the cooperative effect induced by this set of hydrogen bonds. In contrast, this type of interaction was not observed for the polyoxapolyaza-macrocycles, [22](DBF)N_2O_3 and [44](DBF)$_2N_4O_6$, and only the first one was obtained. Furthermore, the theoretical calculations also indicated that in the gas phase, other factors can contribute to the final cyclization product, as happens with [28](DBF)$_2N_6$, in which the intramolecular arrangement suggests the presence of π–π stacking interactions. Of course, the molecular modelling studies described here are somewhat simplistic as only the structural features were considered, relegating other factors certainly important for the formation of these macrocycles, such as solvation and entropy changes. Studies of the metal coordination and supramolecular properties of these novel polyaza- and polyoxapolyaza-macrocycles are in progress in our laboratories.

3. Experimental

3.1. Reagents

The compounds 4,6-dibenzofuran, 2,2′-ethylenedioxy-bis-(ethylamine), 4,7,10-trioxa-1,13-tridecanediamine, diethylenetriamine and *N*-(3-aminopropyl)-1,3-propanediamine were obtained from a commercial supplier and used as received unless noted , 4,6-dibenzofurandicarbaldehyde was prepared by reported method.[28] For mass spectra acquisition LC–MS grade solvents were used. The reference used for the ^{1}H NMR measurements in D_2O was the 3-(trimethylsilyl)-propanoic acid-d_4-sodium salt and in $CDCl_3$ the solvent itself (at 7.26 ppm). For ^{13}C NMR spectra in D_2O, 1,4-dioxane (signal at 67.15 ppm) was used as an internal reference.

3.2. Synthesis of [17](DBF)N_2O_2

The 4,6-dibenzofurandicarbaldehyde (0.5 g, 2.23 mmol) was suspended in ethanol (150 cm^3) and the mixture left stirring in an ice-bath for half an hour. To this solution, 2,2′-ethylenedioxy-bis(ethylamine) (0.33 g, 2.23 mmol) in ethanol (50 cm^3) was added dropwise during 3–4 h, and left stirring for overnight at 0 °C. The 4,6-dibenzofurandicarbaldehyde dissolved in the ethanol solution during the reaction. At the end the yellow solution became completely transparent. Then $NaBH_4$ (0.7 g, 18.5 mmol) was directly added into the same reactor and the solution was left overnight at room temperature. Ethanol was removed and the remaining grey product was dissolved in water (50 cm^3).

The pH of the aqueous solution was increased to 12 with NaOH and extraction with chloroform (5×30 cm^3) was carried out. The organic phases were combined, dried with Na_2SO_4 and completely evaporated under *vacuum* to obtain a yellow oil. This oil was dissolved in ethanol (30 cm^3) and 48% hydrobromic acid (about 2 cm^3). The desired product was precipitated in the form of a white HBr-salt. Yield: 73.7%, mp 261–262 °C (dec). ^{1}H NMR (D_2O): δ 3.35 (br s, 4H), 3.71 (br s, 4H), 3.83 (s, 4H), 4.54 (s, 4H), 7.42 (t, J=7.4 Hz, 2H), 7.50 (d, J=7.1 Hz, 2H) and 8.09 (d, J=7.4 Hz, 2H). ^{13}C NMR (D_2O): δ 48.1, 49.2, 67.5, 72.4, 117.2, 125.4, 126.6, 126.7, 132.0 and 156.4. Found: C, 47.87; H, 5.52; N, 5.65%. Calcd for $C_{20}H_{24}N_2O_3\cdot2HBr$: C, 47.83; H, 5.22; N, 5.58%. IR (KBr pellets, cm^{-1}): 3428, 2934, 2794, 1437, 1422, 1189, 1130, 1104, 785, 746. ESIMS, *m*/*z* 341.2 [M+H]$^+$.

3.3. Synthesis of [22](DBF)N_2O_3

A similar procedure to the one described above was employed to prepare [22](DBF)N_2O_3 from 4,6-dibenzofurandicarbaldehyde (0.5 g, 2.23 mmol) and 4,7,10-trioxa-1,13-tridecanediamine (0.49 g, 2.23 mmol). After extraction the organic phases were combined, dried with Na_2SO_4 and completely evaporated under *vacuum* to obtain the yellow oil. This oil was dissolved in ethanol (30 cm^3), and 48% hydrobromic acid (about 2 cm^3) was added. The desired product was precipitated in the form of a white HBr-salt. Yield: 91.4%, mp 281–282 °C (dec). ^{1}H NMR (D_2O): δ 1.97 (t, J=5.9 Hz, 4H), 3.26 (t, J=7.1 Hz, 4H), 3.58 (m, 12H), 4.50 (s, 4H), 7.40 (t, J=7.5 Hz, 2H), 7.48 (d, J=7.5 Hz, 2H) and 8.05 (d, J=7.5 Hz, 2H). ^{13}C NMR (D_2O): δ 27.8, 47.7, 48.1, 70.5, 71.8, 71.9, 117.1, 125.5, 126.6, 126.7, 131.9 and 156.5. Found: C, 50.43; H, 6.42; N, 5.03%. Calcd for $C_{24}H_{32}N_2O_4\cdot2HBr$: C, 50.19; H, 5.97; N, 4.88%. IR (KBr pellets, cm^{-1}): 3437, 2950, 2798, 1437, 1425, 1201, 1109, 845, 781, 748. ESIMS, *m*/*z* 413.0 [M+H]$^+$.

3.4. Synthesis of [28](DBF)$_2N_6$ and of the complex [Cu([14](DBF)N_3)(H_2O)$_2$](SO_4)$^{2-}\cdot3H_2O$

A similar procedure to the one described above was employed to prepare [28](DBF)$_2N_6$ from 4,6-dibenzofurandicarbaldehyde (0.5, 2.23 mmol) and diethylenetriamine (0.23 g, 2.23 mmol). After evaporation of the solvent from extraction, the recrystallization of the residue from ethanol provided 0.55 g (83.4%) of [28](DBF)$_2N_6$ as a white solid. Mp 187–189 °C (dec). ^{1}H NMR ($CDCl_3$): δ 1.90 (br s, 6H), 2.76 (s, 16H), 4.14 (s, 8H), 7.25 (t, J=7.5 Hz, 4H), 7.32 (d, J=6.9 Hz, 4H) and 7.82 (d, J=7.5 Hz, 4H). ^{13}C NMR ($CDCl_3$): δ 48.6, 48.8, 49.2, 119.5, 122.8, 124.2, 124.3, 127.3 and 154.5. Found: C, 73.0; H, 7.18; N, 14.17%. Calcd for $C_{36}H_{42}N_6O_2$: C, 73.19; H, 7.17; N, 14.23%. IR (KBr pellets, cm^{-1}): 3427, 3281, 2922, 2833, 1432, 1185, 845, 770, 747. ESIMS, *m*/*z* 591.1 [M+H]$^+$.

An aqueous solution of $CuSO_4\cdot5H_2O$ (0.04 mmol, 0.01 g) was directly added to the ethanolic mother solution of [28](DBF)$_2N_6$ and the mixture was stirred for 1 h. The solvent was removed under *vacuum*. The solid was dissolved in a mixture of acetonitrile/methanol (1:1). Blue crystals were formed in two weeks by slow evaporation of the solvent at room temperature.

3.5. Synthesis of [32](DBF)$_2N_6$

A similar procedure to the one described above was employed to prepare [32](DBF)$_2N_6$ from 4,6-dibenzofurandicarbaldehyde (0.5 g, 2.23 mmol) and *N*-(3-aminopropyl)-1,3-propanediamine (0.30 g, 2.23 mmol). After evaporation of the solvent resulting from the extraction, the recrystallization of the residue from ethanol provided 0.53 g (73.4%) of [32](DBF)$_2N_6$ as a white solid. Mp 172–173 °C (dec). ^{1}H NMR ($CDCl_3$): δ 1.72 (t, J=6 Hz, 14H), 2.68 (t, J=6.9 Hz, 8H), 2.72 (t, J=6.9 Hz, 8H), 4.15 (s, 8H), 7.28 (t, J=7.5 Hz, 4H), 7.38 (d, J=6.9 Hz, 4H) and 7.82 (d, J=7.2 Hz, 4H). ^{13}C NMR ($CDCl_3$): δ 30.2, 47.7, 48.4, 48.5, 119.4, 122.8, 124.1, 124.4, 127.0 and 154.3. Found: C, 71.17; H, 8.11; N, 12.30%. Calcd for $C_{40}H_{50}N_6O_2\cdot1.5H_2O$: C, 71.29; H, 7.93; N, 12.47%. IR (KBr pellets, cm^{-1}): 3409, 3262, 2929, 2825, 1432, 1417, 1185, 845, 777, 749. ESIMS, *m*/*z* 647.1 [M+H]$^+$.

3.6. Synthesis of {H_2[22](DBF)N_2O_3}$\cdot2PF_6$

The compound [22](DBF)N_2O_3 (0.1 g) was dissolved in water/methanol 1:1 (10 cm^{-3}) and HPF_6 (50%) was added until pH 4–5. The solvent was removed under *vacuum*. The solid was dissolved in acetonitrile/ethanol/H_2O (2:2:1). White crystals were formed in few weeks by slow evaporation of the solvent at room temperature.

3.7. Mass spectrometry assays

The compounds were diluted in methanol to a concentration of $1–10\times10^{-6}$ M. Mass spectra for all the compounds have been acquired in the positive polarity mode, after their direct injection into the mass spectrometer using a syringe pump. Mass spectra for compound [17](DBF)N_2O_2 has been acquired at *m*/*z* ranging from 250 to 800 in a LCQ ion trap mass spectrometer equipped with ESI source. The following tune conditions were used: ion spray voltage, 4 kV (positive mode) and temperature of the heated capillary, 250 °C. Nitrogen was used as sheath gas at a flow rate of 20 arbitrary units. Mass spectra for the compounds [22](DBF)N_2O_3, [28](DBF)$_2N_6$ and [32](DBF)$_2N_6$ have been acquired at *m*/*z* ranging from 250 to 1000 in an Esquire ion trap mass spectrometer equipped with ESI source and run by Esquire control. The following tune conditions were used: ion spray voltage, 4 kV (positive mode) and temperature of the heated capillary, 250 °C. Nitrogen was used as drying gas at a flow rate of 5 L/min and at a constant pressure of 15 psi.

3.8. Crystallography

X-ray data for **1** were collected at 150 K on a X-Calibur CCD system. The crystal was positioned at 50 mm from the CCD and 330 frames were measured each for 10 s.

Data for **2** were collected at 298 K on a MAR research Image plate system, respectively. The crystal of **2** was positioned at 70 mm from the image plate. Using an appropriate counting time 95 frames were taken at 2° intervals. Data analysis for **2** was performed with the XDS program.[29] Both data collections were carried out with Mo Kα radiation. The pertinent crystallographic data are given in Table 3.

Table 3. Room temperature crystal data and pertinent refinement details for compounds **1** and **2**

Compound	**1**	**2**
Molecular formula	$\{H_2[22](DBF)N_2O_3\}\cdot 2PF_6$	$[Cu([14](DBF)N_3)(H_2O)_2](SO_4)\cdot 3H_2O$
Empirical formula	$C_{24}H_{34}F_{12}N_2O_4P_2$	$C_{18}H_{31}CuN_3O_{10}S$
M_w	704.47	545.06
Crystal system	Monoclinic	Triclinic
Space group	$P2_1/a$	$P\bar{1}$
a/[Å]	11.4202(10)	9.360(11)
b/[Å]	14.4733(11)	10.223(13)
c/[Å]	18.0700(16)	12.870(15)
α/[°]	(90)	88.64(1)
β/[°]	95.634(7)	68.79(1)
γ/[°]	(90)	80.22(1)
V/[Å^3]	2972.3(4)	1130(2)
Z	4	2
Mg/m^3	1.574	1.601
X-ray system	X-Calibur-CCD	Mar-image plate
μ/[mm^{-1}]	0.256	1.119
Reflections collected	18271	6909
Unique reflections, [R_{int}]	7975[0.0302]	4020[0.0435]
Final R indices		
R_1, wR_2 [$I>2\sigma I$]	0.0421, 0.1040	0.0961, 0.1884
R_1, wR_2 (all data)	0.1015, 0.1101	0.1160, 0.1969
Largest diff. peak and hole	0.317, −0.257	0.521, −0.815

The structures of both compounds were solved by direct methods and by subsequent difference Fourier syntheses and refined by full matrix least squares on F^2 using the SHELX-97 system programs.[30] Anisotropic thermal parameters were used for all non-hydrogen atoms. The hydrogen atoms bonded to carbon and nitrogen atoms were included in refinement at calculated positions while hydrogen atoms of the water molecules were located from difference Fourier maps and refined with O–H distances and H–O–H angles constrained to 0.82 Å and 104.5°, respectively. The thermal movement of hydrogen atoms was described using isotropic parameters equivalent to 1.2 times to that atoms, which were attached. The residual electronic density for both compounds, less than 1 eÅ^{-3}, was within expected values. The molecular diagrams presented were drawn with graphical package software PLATON software package.[31]

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.06.073.

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Cu(I)-catalyzed asymmetric oxidative cross-coupling of 2-naphthol derivatives

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Abstract—The asymmetric oxidative coupling reaction between 2-naphthol or binaphthol derivatives and 3-hydroxy-2-naphthoate derivatives with the copper(I)-(*S*)-(−)-isopropylidenebis(4-phenyl-2-oxazoline) catalyst was carried out. The reaction proceeded in a highly cross-coupling selective manner (≤99.7%) to produce the binaphthyl or quaternaphthyl derivative in good yield (≤92%) with enantioselectivity of up to 74%.

1. Introduction

The 1,1′-bi-2-naphthol derivatives bearing an axially dissymmetric structure have been widely used in asymmetric synthesis, catalysis, and resolution.[1] The oxidative coupling reaction of the 2-naphthol derivatives is a facile and effective method for their synthesis, and many studies on the homo- or self-coupling reaction with chiral metal catalysts, such as Cu(I), Ru(II), and V(IV), affording a symmetrical binaphthol framework have been reported.[2] In contrast, there have been few reports on the synthesis of the binaphthol derivatives possessing an unsymmetrical structure through the catalytic cross-coupling reaction.[3] Recently, we found that the oxidative coupling reaction between two differently substituted 2-naphthol derivatives using the CuCl-(*S*)-2,2′-isopropylidenebis(4-phenyl-2-oxazoline) [(*S*)Phbox] catalyst (Fig. 1) at room temperature under an O_2 atmosphere proceeded in a highly cross-coupling selective manner, up to 99.7%.[4] In addition, this system was successfully applied to the asymmetric oxidative cross-coupling polymerization of the methyl 6,6′-dihydroxy-2,2′-binaphthalene-7-carboxylate leading to the polybinaphthol derivative with a number average molecular weight of 1.2×10^4, which consisted of the cross-coupling unit of 96%.[5]

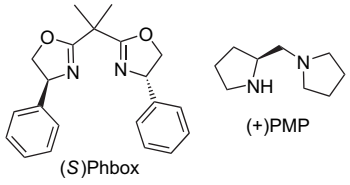

Figure 1. Chiral ligands.

On the other hand, studies on the synthesis of the oligobinaphthols, such as the ter- and quaternaphthyl derivatives, by the oxidative coupling are also available.[6] They are often prepared by the cross-coupling reaction, e.g., the ternaphthyl skeleton is constructed by coupling between the binaphthyl and naphthyl compounds, and an excess amount of one substrate to the other is generally used to maximize the yield of the desired cross-coupling oligonaphthyl product.

In this study, further investigations on the highly selective oxidative cross-coupling reaction of 2-naphthols with the copper-bisoxazoline catalyst, and its extension to the facile synthesis of the quaternaphthyl derivatives through the double oxidative cross-coupling reaction using a stoichiometric amount of the binaphthol derivatives and 2-naphthol in the ratio of 1:2.

2. Results and discussion

To optimize the reaction conditions, the oxidative cross-coupling (OCC) with the (*S*)Phbox ligand was examined using 2-naphthol **1a** and methyl 3-hydroxy-2-naphthoate **2a** as substrates (Scheme 1). The results of the OCC using CuCl-(*S*)Phbox by changing the catalyst molar ratio are listed in Table 1. In a previous report, the reaction using 0.2 equiv of the catalyst to the substrates for 3 h resulted in an 87% yield with a cross-coupling selectivity (*Y*) of 95.8% and an enantioselectivity of 10% ee (*S*) (entry 1).[4]

Keywords: Binaphthol; Cross-coupling; Asymmetric oxidative coupling.
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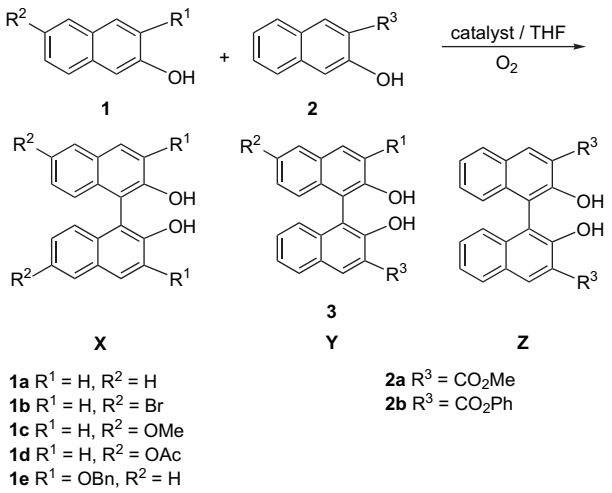


Scheme 1.

Table 1. Asymmetric OCC between **1a** and **2a**[a]

Entry	[CuCl-(*S*)Phbox]/[**1**+**2**]	Time (h)	Coupling ratio **X**:**Y**:**Z**[b]	Cross-coupling product **Y**	
				Yield (%)[c]	ee (%)[d]
1	0.2	3	0.3:95.8:3.9	87	10 (*S*)
2	0.1	6	0:97.4:2.6	82	8 (*S*)
3	0.05	9	0:98.5:1.5	81	7 (*S*)
4	0.01	24	0:99.6:0.4	79	8 (*S*)

[a] [**1a**]/[**2a**]=1:1, [**1a**]=0.18 M, temp=rt, O_2 atmosphere.
[b] Ratio of isolated yields.
[c] Isolated yield.
[d] Determined by HPLC analysis (Chiralpak AD).

With the decreasing molar ratio of the catalyst, the cross-coupling selectivity slightly increased with almost no decrease in the yield, as well as the enantioselectivity of the cross-coupling product. The OCC with 1.0 mol % of CuCl-(*S*)Phbox catalyst showed the highest cross-coupling selectivity of 99.6% (entry 4).

The reaction with various copper salts (1.0 mol %) in THF at room temperature was also conducted (Table 2). In every catalyst system, the cross-coupling bond formation preferentially took place. However, the yield and stereoselectivity were significantly reduced during the reaction with the copper(II) salt (entry 1). The counter-anion of the copper salt

Table 2. Asymmetric OCC between **1a** and **2a**[a]

Entry	CuX	Time (h)	Coupling ratio **X**:**Y**:**Z**[b]	Cross-coupling product **Y**	
				Yield (%)[c]	ee (%)[d]
1	$CuCl_2$	48	0:98.1:1.9	21	1 (*R*)
2	CuBr	24	0:99.1:0.9	76	3 (*S*)
3	CuI	24	0:97.9:2.1	66	1 (*S*)
4	CuOTf	24	0:97.2:2.8	41	7 (*S*)
5	$[(CH_3CN)_4Cu]PF_6$	24	0:95.5:4.5	53	5 (*R*)

[a] [CuX]/[(*S*)Phbox]/[**1a**]/[**2a**]=0.010:0.013:0.5:0.5, [**1a**]=0.18 M, temp=rt, O_2 atmosphere.
[b] Ratio of isolated yields.
[c] Isolated yield.
[d] Determined by HPLC analysis (Chiralpak AD).

Table 3. Asymmetric OCC between **1** and **2**[a]

Entry	**1**	**2**	Coupling ratio **X**:**Y**:**Z**[b]	Cross-coupling product **Y**		
				3	Yield (%)[c]	ee (%)[d]
1	**1b**	**2a**	5.2:88.7:6.1	**3b**	74	8 (*S*)
2	**1c**	**2b**	0:99.7:0.3	**3c**	87	16 (*S*)
3	**1d**	**2a**	0:99.7:0.3	**3d**	94	10 (*S*)
4	**1d**	**2b**	0:97.3:2.7	**3e**	76	46 (*R*)
5	**1e**	**2b**	12.0:86.3:1.7	**3f**	71	67 (*R*)
6[e]	**1e**	**2b**	11.1:87.0:1.9	**3f**	70	70 (*R*)
7[f]	**1e**	**2b**	13.5:85.5:1.0	**3f**	67	73 (*R*)
8[g]	**1e**	**2b**	12.8:85.1:2.1	**3f**	44	74 (*R*)

[a] [CuCl]/[(*S*)Phbox]/[**1**]/[**2**]=0.010:0.013:0.50:0.50, [**1**]=0.18 M, temp=rt, time=24 h, O_2 atmosphere.
[b] Ratio of isolated yields.
[c] Isolated yield.
[d] Determined by HPLC analysis (Chiralpak AS or AD).
[e] [CuCl]/[(*S*)Phbox]/[**1**]/[**2**]=0.05:0.06:0.50:0.50, temp=0 °C, 48 h.
[f] [CuCl]/[(*S*)Phbox]/[**1**]/[**2**]=0.05:0.06:0.50:0.50, temp=−20 °C, 72 h.
[g] [CuCl]/[(*S*)Phbox]/[**1**]/[**2**]=0.1:0.13:0.50:0.50, temp=−40 °C, 72 h.

also significantly affected the catalyst activity and stereocontrol.

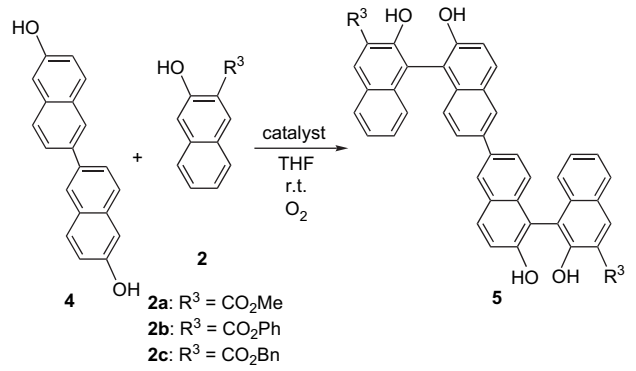
Table 3 shows the results of the OCC between the 2-naphthol derivatives **1** and 3-hydroxy-2-naphthoate derivatives **2** using the CuCl-(*S*)Phbox catalyst (1.0 mol %). The reaction of 6-substituted-2-naphthol **1c**, **1d** demonstrated the excellent cross-coupling selectivity of 99.7% (entries 2 and 3), whereas the reaction of 2-naphthol having a substituent at the 3-position **1e** showed the highest enantioselectivity of 67% ee (*R*) (entry 3). By lowering the reaction temperature to −40 °C, the enantioselectivity was further improved to 74% ee (*R*), although the yield of the cross-coupling product was reduced (entry 6).

The results of the OCC between the 6,6′-dihydroxy-2,2′-binaphthalene **4** and **2a** (**4**:**2**=1:2 ratio) with various copper complexes of (+)-1-(2-pyrrolidinylmethyl)pyrrolidine [(+)-PMP] (Fig. 1), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA), and (*S*)Phbox, in THF at room temperature under an O_2 atmosphere are listed in Table 4 (Scheme 2). The stereoselectivity of the quaternaphthyl derivatives was determined by circular dichroism (CD)[7] and high-performance-liquid-chromatography (HPLC) analyses. The reaction with CuCl-(+)PMP hardly produced any coupling product (entry 1), whereas the quaternaphthyl **5a** was obtained in

Table 4. Asymmetric OCC between **4** and **2**[a]

Entry	Catalyst	**2**	Time (h)	Quaternaphthyl **5**		
				5	Yield (%)[b]	*RR*:*RS*:*SS*[c]
1	CuCl-(+)PMP	**2a**	24	**5a**	<1	—
2	CuCl(OH)-TMEDA	**2a**	10	**5a**	32	19:62:19
3	CuCl-(*S*)Phbox	**2a**	5	**5a**	85	11:45:44
4[d]	CuCl-(*S*)Phbox	**2a**	24	**5a**	80	9:42:49
5[e]	CuCl-(*S*)Phbox	**2a**	48	**5a**	92	10:43:47
6	CuCl-(*S*)Phbox	**2b**	5	**5b**	60	40:48:12
7	CuCl-(*S*)Phbox	**2c**	5	**5c**	79	12:47:41

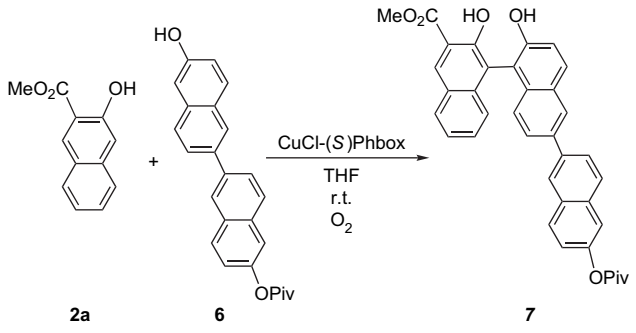
[a] Conditions: [CuCl]/[diamine]/[**4**]/[**2a**]=0.20:0.25:0.33:0.66, [**4**]=0.12 M, solvent=THF, O_2 atmosphere, temp=rt.
[b] Isolated yield.
[c] Determined by HPLC (Chiralpak AD) and CD measurements.
[d] [CuCl]/[diamine]/[**4**]/[**2a**]=0.010:0.013:0.33:0.66.
[e] Reaction temp=−20 °C.



Scheme 2.

a low yield (32%) as a mixture of *dl* and *meso* during the reaction with the CuCl(OH)-TMEDA catalyst for 10 h (entry 2). In marked contrast, the CuCl-(*S*)Phbox catalyst effectively produced the double cross-coupling product **5a** in good yield (85%, reaction time=5 h), accompanied by the sole homo-coupling by-product of **2a** in 8% yield (entry 3). Therefore, the cross-coupling selectivity was calculated to be 95.5%, whose value is quite consistent with that observed for the coupling reaction between **1a** and **2a**. The stereoselectivity (*RR*/*RS*/*SS*) of the obtained **5a** was determined to be 11:45:44, that is, *R*/*S*=33.5:66.5 [33% ee (*S*)]. When 1.5 mol % of the catalyst to **2a** was used, **5a** was again obtained in a good yield [80%, 40% ee (*S*), entry 4]. The reaction at the lower temperature of −20 °C for 48 h produced the excellent yield of **5a** of 92% and a high cross-coupling selectivity of 99% (yield of the homo-coupling product: 2%, entry 5).

The model reaction using the mono-pivaloylated compounds **6** and **2a** (1:1) was also carried out [[**6**]/[**2a**]/[CuCl]/[(*S*)Phbox]=0.5/0.5/0.2/0.25, rt, 5 h] (Scheme 3). The cross-coupling product **7** was obtained in 87% yield with a cross-coupling selectivity of 95.8% and an enantioselectivity of 42% ee (*S*). These results are roughly comparable to those observed for the double cross-coupling process. Accordingly, the second cross-coupling reaction should be slightly affected by the structure formed during the first cross-coupling process.



Scheme 3.

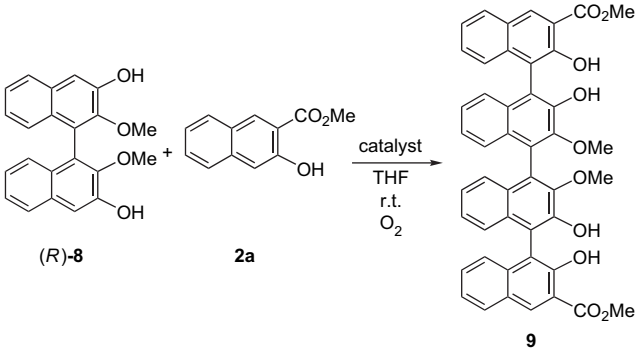
The OCC of the phenyl ester **2b** or benzyl ester **2c** with **4** also afforded a quaternaphthyl in a moderate or good yield, whereas the stereoselectivity for the cross-coupling reaction was the opposite of each other, i.e., 28% ee (*R*) for the former and 29% ee (*S*) for the latter (entries 6 and 7). The structure of the ester groups on the 2-naphthol significantly affected the coupling stereochemistry.

Table 5. Asymmetric OCC between (*R*)-**8** and **2a**[a]

Entry	Catalyst	Time (h)	Quaternaphthyl **9**	
			Yield (%)[b]	*RRR*:*RRS*:*SRS*[c]
1	CuCl(OH)-TMEDA	24	45	45:45:10
2	CuCl-(*S*)Phbox	6	65	56:38:6
3[d]	CuCl-(*S*)Phbox	24	60	62:34:4
4[d]	CuCl-(*R*)Phbox	24	56	15:35:50

[a] Conditions: [CuCl]/[diamine]/[**8**]/[**2a**]=0.20:0.25:0.33:0.66, [**8**]=0.12 M, solvent=THF, O_2 atmosphere, temp=rt.
[b] Isolated yield.
[c] Ratio of isolated yield.
[d] [CuCl]/[diamine]/[**8**]/[**2a**]=0.010:0.013:0.33:0.66.

Table 5 summarizes the results of the OCC between **2a** and the chiral 1,1′-binaphthyl derivative, (*R*)-**8**, (**2a**:**8**=2:1 ratio) with the Cu(I)-diamine catalyst (Scheme 4). The absolute configuration of the quaternaphthyls **9** was assigned on the basis of the spectral pattern and absorption intensity at around 240, 260, and 340 nm for the CD, in addition to the NMR analysis.[4,6d,h]



Scheme 4.

The reaction with the CuCl(OH)-TMEDA catalyst afforded a quaternaphthyl **9** in the low yield of 45%, and the *R*-structure is predominantly formed [*RRR*/*RRS*/*SRS*=45:45:10, 35% ee (*R*), entry 1]. The CuCl-(*S*)Phbox catalyst produced **9** in the yield of 65%, which is also rich in the *R*-configuration [*RRR*/*RRS*/*SRS*=56:38:6, 50% ee (*R*), entry 2]. The OCC with 1.5 mol % of CuCl-(*S*)Phbox catalyst also afforded **9** in a 60% yield with a stereoselectivity of 62:34:4 [58% ee (*R*), entry 3]. The reaction with the (*R*)Phbox catalyst produced a quaternaphthyl with an *S*-selectivity [35% ee (*S*), entry 4]. Accordingly, the *R*-structure was preferentially constructed using the CuCl-(*S*)Phbox, and this selectivity is consistent with that observed for the reaction between the 3-alkoxy-2-naphthols and 3-hydroxy-2-naphthoates.[4] The stereoselectivity was influenced by the structure of the binaphthol used as a substrate, as well as the ligand.

During the reaction using the CuCl-(*S*)Phbox catalyst, the cross-coupling bond formation predominantly proceeded. It is postulated that the naphthol bearing an ester group

may act as an acceptor molecule due to its electron-deficient character, while the one-electron oxidation should be preferentially promoted on the other substrate, the 2-naphthols, to generate an intermediate radical species, and then the cross-coupling reaction may selectively take place.[3e,4] The cross-coupling- or enantioselectivity increased to some extent with a decrease in the molar ratio of the catalyst. These may be due to the fact that the minor coupling of the intermediate radical generated from the 3-hydroxy-2-naphthoates in a less stereoselective manner occurs and produces the cross- and homo-coupling compounds during the reaction with a higher catalyst loading.

3. Conclusion

In conclusion, the CuCl-(*S*)Phbox is a very effective catalyst for the oxidative coupling reaction between two differently substituted 2-naphthols leading to the cross-coupling product with a high selectivity. In addition, the quaternaphthyl derivatives can be directly prepared from stoichiometric amounts of the binaphthols and 2-naphthols (1:2 in feed) by the double oxidative cross-coupling reaction. The yields of the cross-coupling products, cross-coupling selectivity, and stereoselectivity were significantly affected by the structure of the substrates and copper salts.

4. Experimental

4.1. General

^{1}H and ^{13}C NMR spectra were measured on a Varian Unity Inova (500 MHz for ^{1}H) or Mercury 200 (200 MHz for ^{1}H) spectrometer. The infrared (IR) spectra were recorded on a Horiba FT-720 spectrometer. The mass (MS) spectra were obtained using a JEOL AX505H. The optical rotation was measured on a Jasco P-1010 polarimeter at 25 °C. The circular dichroism (CD) spectra were obtained with a Jasco J-720WI apparatus. The high-performance-liquid-chromatography (HPLC) analyses were performed on a Jasco 986-PU chromatograph equipped with UV (Jasco 970-UV) and polarimetric (Jasco OR-990) detectors at room temperature.

4.2. General procedure for oxidative cross-coupling

The 2-naphthols **1** and 3-hydroxy-2-naphthoates **2** were added to a mixture of CuCl and a diamine in THF ([**1**+**2**]=0.35 M, [Cu(I)]/[diamine]/[**1**]/[**2**]=0.010:0.012:0.50:0.50). After room-temperature stirring under an O_2 atmosphere, the reaction mixture was diluted with $CHCl_3$ and then washed with 1 N HCl. The organic solutions were then dried over $MgSO_4$. Filtration and concentration afforded the crude products. Purification was accomplished by silica gel column chromatography that produced the binaphthol derivatives.

Compound **3d**: ^{1}H NMR (500 MHz, $CDCl_3$) 10.84 (s, 1H, –OH), 8.73 (s, 1H, aromatic), 7.95–7.86 (s, 2H, aromatic), 7.58–6.97 (m, 7H, aromatic), 4.99 (s, 1H, –OH), 4.08 (s, 3H, $-CH_3$), 2.32 (s, 3H, $-CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) 170.26, 169.76, 154.85, 151.37, 146.59, 137.25, 133.96, 131.49, 130.23, 129.83, 129.79, 129.42, 127.29, 126.11, 124.71, 124.56, 121.55, 118.93, 118.54, 114.28, 114.20, 114.18, 52.90, 21.18; IR (KBr, cm^{-1}) 3208, 2954, 1756, 1677, 1604, 1504, 1340, 1321, 1207, 1149; $[\alpha]_D^{25}$ −12 (*c* 1.0, THF) for 10% ee (*S*) (Chiralpak AD-H column, hexane/2-propanol=3:1, 0.50 mL/min, 245 nm UV detector), t_R=22.6 min for (*S*) and t_R=24.3 min for (*R*). Mass (FAB): 403 *m*/*z* $[M]^+$. Anal. Calcd for $C_{24}H_{18}O_6$: C, 71.64; H, 4.51. Found: C, 71.51; H, 4.58.

Compound **3e**: ^{1}H NMR (500 MHz, $CDCl_3$) 10.54 (s, 1H, –OH), 9.00 (s, 1H, aromatic), 8.02–7.87 (s, 2H, aromatic), 7.59–6.99 (m, 12H, aromatic), 4.97 (s, 1H, –OH), 2.32 (s, 3H, $-CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) 169.77, 168.71, 155.01, 151.35, 150.03, 146.64, 137.66, 134.68, 131.46, 130.68, 130.03, 129.91, 129.79, 129.46, 127.40, 126.68, 126.08, 124.82, 121.65, 121.59, 118.99, 118.55, 114.59, 114.58, 114.07, 113.80, 21.18; IR (KBr, cm^{-1}) 3268, 1756, 1689, 1602, 1504, 1338, 1315, 1274, 1205, 1145; $[\alpha]_D^{25}$ +63 (*c* 1.0, THF) for 46% ee (*R*) (Chiralpak AD-H column, hexane/2-propanol=3:1, 0.50 mL/min, 245 nm UV detector), t_R=23.0 min for (*R*) and t_R=25.5 min for (*S*). Mass (FAB): 465 *m*/*z* $[M]^+$. Anal. Calcd for $C_{29}H_{20}O_6$: C, 74.99; H, 4.34. Found: C, 74.91; H, 4.52.

Compound **5a**: ^{1}H NMR (200 MHz, $CDCl_3$) 10.87 (s, 2H, –OH), 8.74 (s, 2H, aromatic), 8.13 (s, 2H, aromatic), 8.01–7.93 (m, 4H, aromatic), 7.60–7.13 (m, 12H, aromatic), 4.98 (s, 2H, –OH), 4.08 (s, 6H, $-CH_3$); IR (KBr, cm^{-1}) 3434, 1679, 1625, 1502, 1442, 1340, 1322, 1226, 1209, 1151; $[\alpha]_D^{25}$ −70 (*c* 1.0, THF) for the mixture (*RR*/*RS*/*SS*=11:45:44) (Chiralpak AD-H column, hexane/EtOH=1:1, 0.45 mL/min, 245 nm UV detector), t_R=36.2 min for (*RR*) and t_R=66.4 min for (*RS*) and t_R=127.9 min for (*SS*). Mass (FAB): *m*/*z* 687 $[M]^+$. Anal. Calcd for $C_{44}H_{30}O_8$: C, 76.96; H, 4.40. Found: C, 76.96; H, 4.51.

Compound **5b**: ^{1}H NMR (200 MHz, $CDCl_3$) 10.57 (s, 2H, –OH), 9.01 (s, 2H, aromatic), 8.14 (s, 2H, aromatic), 8.05–7.97 (m, 4H, aromatic), 7.63–7.17 (m, 22H, aromatic), 5.00 (s, 2H, –OH); IR (KBr, cm^{-1}) 3434, 1689, 1625, 1596, 1336, 1315, 1203, 1187, 1151, 1133; $[\alpha]_D^{25}$ +73 (*c* 1.0, THF) for the mixture (*RR*/*RS*/*SS*=40:48:12) (Chiralpak AD-H column, hexane/EtOH=1:1, 0.65 mL/min, 245 nm UV detector), t_R=26.1 min for (*RR*) and t_R=51.7 min for (*RS*) and t_R=95.0 min for (*SS*). Mass (FAB): *m*/*z* 811 $[M]^+$. Anal. Calcd for $C_{54}H_{34}O_8$: C, 79.99; H, 4.23. Found: C, 79.99; H, 4.28.

Compound **5c**: ^{1}H NMR (200 MHz, $CDCl_3$) 10.86 (s, 2H, –OH), 8.77 (s, 2H, aromatic), 8.12 (s, 2H, aromatic), 8.01–7.92 (m, 4H, aromatic), 7.60–7.37 (m, 18H, aromatic), 7.26–7.13 (m, 4H, aromatic), 5.51 (s, 4H, $-CH_2-$), 4.96 (s, 2H, –OH); IR (KBr, cm^{-1}) 3434, 1675, 1596, 1500, 1338, 1311, 1274, 1205, 1153, 1089; $[\alpha]_D^{25}$ −50 (*c* 1.0, THF) for the mixture (*RR*/*RS*/*SS*=12:47:41) (Chiralpak AD-H column, hexane/EtOH=1:1, 0.50 mL/min, 245 nm UV detector), t_R=64.3 min for (*RR*) and t_R=111.9 min for (*RS*) and t_R=127.6 min for (*SS*). Mass (FAB): *m*/*z* 839 $[M]^+$. Anal. Calcd for $C_{56}H_{38}O_8$: C, 80.18; H, 4.57. Found: C, 80.17; H, 4.66.

Compound **7**: ^{1}H NMR (200 MHz, $CDCl_3$) 10.89 (s, 1H, –OH), 8.75 (s, 1H, aromatic), 8.19–8.20 (m, 7H, aromatic), 7.65–7.54 (m, 2H, aromatic), 7.43–7.38 (m, 3H, aromatic), 7.27–7.17 (m, 3H, aromatic), 5.03 (s, 1H, –OH), 4.07 (s, 3H, CH_3), 1.41 (s, 9H, $-CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) 177.25, 170.30, 154.94, 151.66, 148.76, 138.27, 137.36, 135.83, 133.96, 132.82, 132.74, 131.67, 130.57, 130.23, 129.89, 129.57, 129.52, 128.07, 127.37, 126.56, 126.35, 126.34, 125.57, 125.27, 124.72, 124.57, 121.55, 118.25, 118.18, 114.37, 114.34, 114.00, 52.92, 39.15, 27.18; IR (KBr, cm^{-1}) 3426, 2969, 1718, 1681, 1604, 1506, 1442, 1284, 1205, 1145; $[\alpha]_D^{25}$ −38 (*c* 1.0, THF) for 42% ee (*S*) (Chiralpak AD-H column, hexane/2-propanol=2:1, 0.50 mL/min, 245 nm UV detector), t_R=29.6 min for (*R*) and t_R=61.0 min for (*S*). Mass (FAB): 571 *m/z* $[M]^+$. Anal. Calcd for $C_{37}H_{30}O_6$: C, 77.88; H, 5.30. Found: C, 77.84; H, 5.22.

Compound (*R*,*R*,*R*)-**9**: Mp >300 °C; pale-yellow prisms (recrystallized from *n*-hexane/$CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) 10.93 (s, 2H, –OH), 8.75 (s, 2H, aromatic), 7.99–7.94 (m, 2H, aromatic), 7.54–7.49 (m, 2H, aromatic), 7.42–7.37 (m, 4H, aromatic), 7.24–7.18 (m, 8H, aromatic), 6.18 (s, 2H, –OH), 4.10 (s, 6H, CH_3), 3.59 (s, 6H, $-CH_3$); ^{13}C NMR (50 MHz, $CDCl_3$) 170.56, 154.25, 146.29, 145.82, 137.14, 133.20, 130.72, 129.88, 129.64, 129.12, 127.30, 126.52, 125.73, 124.86, 124.64, 124.42, 124.09, 123.08, 116.61, 116.05, 114.25, 61.24, 52.81; IR (KBr, cm^{-1}) 3496, 1675, 1504, 1436, 1413, 1398, 1340, 1317, 1222, 1151; $[\alpha]_D^{25}$ +179 (*c* 1.0, THF). Mass (FAB): 746 *m/z* $[M]^+$. Anal. Calcd for $C_{46}H_{34}O_{10}$: C, 73.99; H, 4.59. Found: C, 74.00; H, 4.58.

Compound (*R*,*R*,*S*)-**9**: Mp >300 °C; pale-yellow needles (recrystallized from *n*-hexane/$CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) 10.92 (s, 1H, –OH), 10.77 (s, 1H, –OH), 8.75 (s, 2H, aromatic), 7.99–7.94 (m, 2H, aromatic), 7.54–7.36 (m, 8H, aromatic), 7.26–7.20 (m, 6H, aromatic), 6.22 (s, 1H, –OH), 6.15 (s, 1H, –OH), 4.09 (s, 3H, $-CH_3$), 4.08 (s, 3H, $-CH_3$), 3.62 (s, 3H, $-CH_3$), 3.55 (s, 3H, $-CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) 170.56, 170.48, 154.27, 154.24, 146.40, 146.33, 145.90, 145.82, 137.24, 137.15, 133.17, 133.15, 130.79, 130.70, 129.87, 129.86, 129.68, 129.60, 129.16, 129.11, 127.29, 127.23, 126.51, 126.03, 125.67, 125.58, 124.96, 124.90, 124.89, 124.69, 124.36, 124.34, 124.10, 124.07, 123.08, 122.71, 116.66, 116.64, 116.18, 115.91, 114.26, 114.24, 61.28, 61.17, 52.80, 52.77; IR (KBr, cm^{-1}) 3494, 1681, 1504, 1446, 1396, 1338, 1317, 1224, 1211, 1153; $[\alpha]_D^{25}$ +66 (*c* 1.0, THF). Mass (FAB): 746 *m/z* $[M]^+$. Anal. Calcd for $C_{46}H_{34}O_{10}$: C, 73.99; H, 4.59. Found: C, 73.99; H, 4.57.

Compound (*S*,*R*,*S*)-**9**: Mp >300 °C; pale-yellow needles (recrystallized from *n*-hexane/THF); ^{1}H NMR (200 MHz, $CDCl_3$) 10.76 (s, 2H, –OH), 8.75 (s, 2H, aromatic), 7.99–7.95 (m, 2H, aromatic), 7.49–7.40 (m, 8H, aromatic), 7.27–7.20 (m, 6H, aromatic), 6.20 (s, 2H, –OH), 4.09 (s, 6H, $-CH_3$), 3.58 (s, 6H, $-CH_3$); ^{13}C NMR (50 MHz, $CDCl_3$) 170.50, 154.26, 146.43, 145.91, 137.26, 133.12, 130.77, 129.84, 129.68, 129.13, 127.23, 126.04, 125.54, 124.99, 124.82, 124.28, 124.09, 122.65, 116.71, 116.04, 114.26, 61.20, 52.77; IR (KBr, cm^{-1}) 3502, 1679, 1502, 1446, 1396, 1338, 1317, 1224, 1211, 1151; $[\alpha]_D^{25}$ −55 (*c* 1.0, THF). Mass (FAB): 746 *m/z* $[M]^+$. Anal. Calcd for $C_{46}H_{34}O_{10}$: C, 73.99; H, 4.59. Found: C, 73.98; H, 4.60.

Acknowledgements

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Supplementary data

CD spectra of **5a–c**, **7**, and **9** are available. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.06.069.

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Stilbene like carbazole dimer-based electroluminescent materials

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Abstract—Two carbazole dimers (**1** and **10**) were synthesized from 9-ethyl-9*H*-carbazole-3-carbaldehyde and 6-bromo-9-ethyl-9*H*-carbazole-3-carbaldehyde by McMurry C–C coupling reaction. Palladium(0)-catalyzed C–N coupling reactions of **10** and various diarylamines result in the formation of stable carbazole derivatives in good yields. These compounds are fluorescent in blue to yellow region with moderate to good quantum yields. Also, they are thermally stable and capable of hole-transporting due to the presence of the carbazole moieties. The electroluminescent devices fabricated using **1**, **2**, and **3** as hole-transporters/emitters with a bilayer structure ITO/**Cpd**/TPBI or Alq_3/LiF/Al (**Cpd**=**1**, **2**, and **3**) exhibit good performance (e.g., η_{ext}=1.0–2.1%; η_p=0.9–1.9 lm/W; η_c=2.4–4.8 cd/A at a current density of 100 mA/cm^2).

1. Introduction

Considerable progress has been made on organic light emitting diodes (OLEDs) since the seminal report by Tang et al., on the device with a double-layer structure.[1] Now OLEDs have become visible in consumer electronics such as car stereos, mobile phones, and digital cameras. Appropriate choosing of the electron- and hole-transporting materials is necessary in order to have high performance small molecules[2] or polymeric[3] OLEDs. The durability of the devices, which depends on the thermal and morphological stability of the organic films, is also a key factor in affecting the device performances. We have been interested in the development of new materials for OLED application. We found that carbazolyl unit is beneficial to raise both glass transition temperature and thermal stability of luminescent chromophores.[4] Further, it is relatively easy to functionalize the carbazolyl moiety at the 3-, 6-, or 9-positions for tuning its physical properties. A variety of carbazole derivatives, including small molecules,[4] dendrimers,[5] or polymers,[6] which served as hosts,[7] dopants,[8] hole-transporters,[9] or emitters with a wide color range,[10] have been reported. Recent developments in carbazole derivatives and their application as advanced amorphous materials for photorefractive systems, organic light emitting diodes as well as other optoelectronic devices are investigated.[11] Earlier we synthesized a series of compounds containing a carbazolyl core encapsulated with peripheral arylamines. These materials were found to be green or blue emitting and capable of hole-transporting.[4] In this report, we further integrated two carbazole moieties by McMurry C–C coupling reaction. For the sake of tuning the emission color in visible light region or altering energy level to adjust the ability of hole injection, the stilbene like carbazole dimer was followed by encapsulation with arylamines. Although, the stilbene-containing materials[11,12] and oligomeric carbazolyl compounds[13] were reported for electroluminescent applications, to our knowledge, no stilbene like carbazole dimer-based electroluminescent materials have been reported so far. Double-layer EL devices using these compounds as hole-transporting layer and emitting layer and Alq_3 [tris(8-quinolinolato)aluminum][14] or TPBI [1,3,5-tris(*N*-phenyl-benzimidazol-2-yl)benzene][15] as electron-transporting layer were fabricated by vacuum deposition.

2. Results and discussion

The compounds for electroluminescent study are depicted in Figure 1. Scheme 1 illustrates the synthesis of these new compounds. 9-Ethyl-9*H*-carbazole-3-carbaldehyde (**8**) was obtained starting from ethylation of carbazole followed by treating the resulting 9-ethyl-9*H*-carbazole with $POCl_3$ in DMF via Vilsmeier–Haack reaction[16] in 65% yield. Compound **8** was further treated with NBS (*N*-bromosuccinimide) to give 6-bromo-9-ethyl-9*H*-carbazole-3-carbaldehyde (**9**), which reacted with titanium(IV) chloride and zinc powder to afford **10** in good yield (85%). Finally, palladium-catalyzed coupling reactions[17] of **10** with arylamines using $Pd(OAc)_2$/P(*t*-Bu)$_3$ as the catalyst and *t*-BuONa as the base resulted in efficient C–N bond formation to give

Keywords: Carbazole dimmer; Electroluminescent; McMurry C–C coupling reaction.
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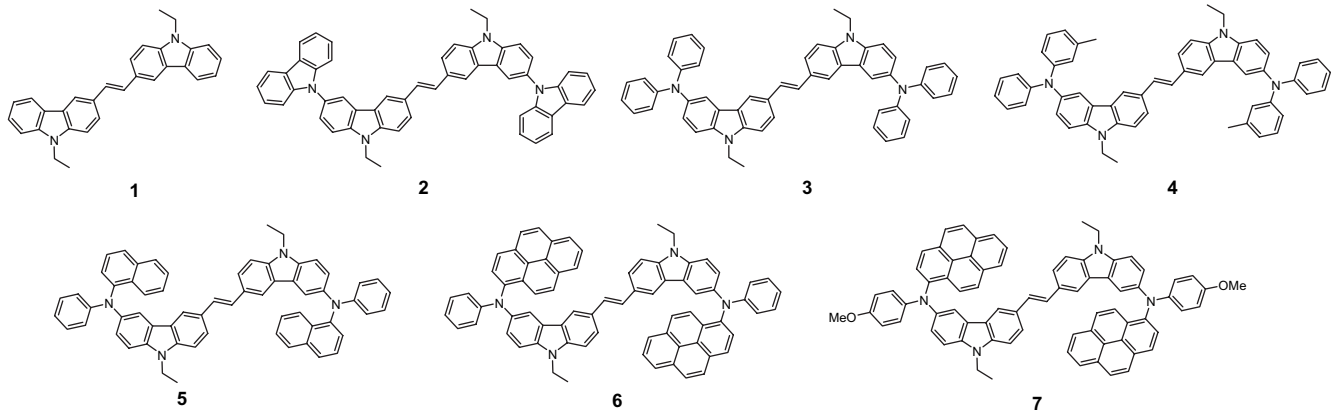
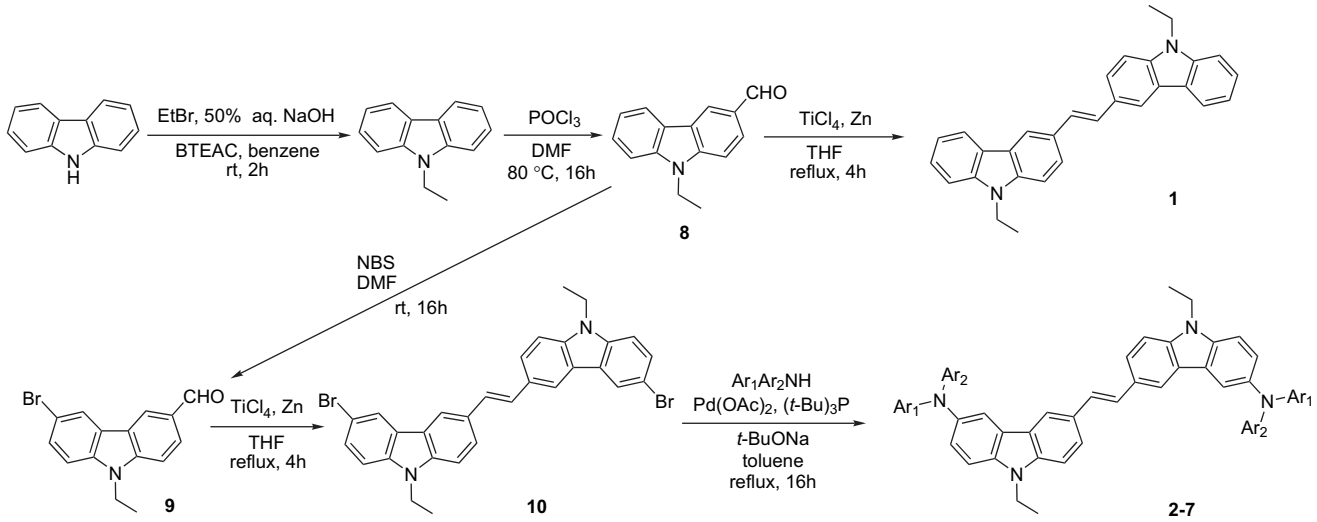


Figure 1. Structure of the compounds.



Scheme 1.

diaminocarbazole dimers **2**–**7**. It must be mentioned that the reactions readily occurred at lower temperature (ca. 80 °C) to form both *Z*- and *E*-isomers. However, higher reaction temperature (reflux in toluene) gave predominantly *E*-form in 65 to 80% yields. All the target products were soluble in common organic solvents such as CH_2Cl_2, $CHCl_3$, or THF so that all these diaminocarbazole dimers can be easily purified by flash column chromatography. Compounds **1**–**7** were fully characterized by ^1H and ^{13}C NMR spectroscopies, mass spectroscopy, and elementary analyses. The thermal properties of the compounds were determined by DSC (differential scanning calorimetry) and TGA (thermogravimetric analysis) measurements (Table 1). All the compounds except **5** and **7** are crystalline. No crystallization and

Table 1. Physical properties of the compounds

Compd	λ_{abs},[a] nm	λ_{em},[a] nm (Φ_f,[b] %)	λ_{em},[c] nm (Φ_f,[b] %)	λ_{em},[d] nm	T_m/T_d,[e] °C	E_{ox} (ΔE_p),[f] mV	HOMO, eV	LUMO, eV
1	308, 342	411 (40)	407 (44)	447	218/268	245 (92), 555 (82)	5.05	2.05
2	294, 344	416 (45)	412 (49)	451	335/403	370 (55), 676 (i)	5.17	2.17
3	309, 346	434 (18)	422 (10)	463	284/349	201 (120), 755 (i)	5.00	2.05
4	310, 347	441 (20)	421 (8)	468	207/370	190 (68), 764 (i)	4.99	2.04
5	306, 344	507 (9)	454 (8)	472	na/382	198 (142), 769 (i)	5.00	2.07
6	321, 356	549 (16)	503 (33)	523	295/403	192 (144), 690 (i)	4.99	2.33
7	334, 361	560 (20)	516 (35)	545	na/468	107 (114), 601 (i)	4.91	2.34

Oxidation potential reported is adjusted to the potential of ferrocene ($E_{1/2}$=245 mV vs $Ag/AgNO_3$), which was used as an internal reference. Conditions of cyclic voltammetric measurements: glassy carbon working electrode; $Ag/AgNO_3$ reference electrode. Scan rate: 100 mV/s. Electrolyte: tetrabutylammonium hexafluorophosphate; i, irreversible process. HOMO calculated from CV potentials using ferrocene as standard [HOMO=4.8+($E_{ox}-E_{Fc}$)]. LUMO derived from the relationship E_g, HOMO−LUMO, where E_g was obtained from optical spectroscopy; na: not available.

[a] Recorded in dichloromethane solutions.

[b] Quantum yield was measured with reference to Coumarin 1 (99%) in ethyl acetate. Refractive index change due to the use of different solvent correction was applied in the calculation.

[c] Recorded in toluene solutions.

[d] Film samples.

[e] Decomposition temperature (T_d) corresponds to the 5% weight loss.

[f] Recorded in dichloromethane solutions.

melting were noticed for these compounds after fast cooling of the melt. However, no glass transition can be detected. The thermal decomposition temperatures of these compounds range from 268 to 468 °C. Introduction of arylamines into the central carbazole dimer greatly enhanced the decomposition temperature (T_d) of the materials. This effect is prominent when the carbazole (**2**) or pyrenyl amine (**6** and **7**) is introduced at 3- and 3′-positions.

The photophysical properties of the carbazole dimer derivatives were examined by UV–vis and fluorescence spectroscopies in both dichloromethane and toluene solutions. The absorption and emission spectra of the compounds in dichloromethane were shown in Figure 2. In general, each of these compounds has absorption at ~350 nm, which was assigned to π–π* transition of the carbazole dimer core. In addition, there exist localized π–π* transition due to end-capping arylamines such as carbazole, diphenylamine, *N*-phenylnaphthalen-1-amine, or *N*-phenylpyren-1-amine. Compounds **6** and **7**, which have pyrene moieties display an extra band at the longer wavelength due to pyrenyl amine. These compounds emit blue to yellow light with emission maxima ranging from 416 to 560 nm in dichloromethane solution. The emission of **2** is almost identical to the emission of the central carbazole dimer (compound **1**), possibly due to the weak donating ability of the carbazolyl moiety and the noncoplanarity between the carbazolyl moiety and central core. All other compounds, **3**–**7**, have better conjugation between the periphery arylamines and the central core, and exhibit longer λ_{em}. No solvatochromic effect in the absorption spectra was observed. However, there is significant dipolar character in the excited state of the compounds **5**–**7** in view of the large positive solvatochromic effect in more polar solvent ($\lambda_{em}(CH_2Cl_2)-\lambda_{em}$(toluene)>45 nm).

From cyclic voltammetric measurements and square wave voltammetric methods, the electrochemical properties of the compounds were studied and the redox potentials of the compounds are compiled in Table 1. Compound **1** exhibits two reversible one-electron redox waves, which indicates that there exist some electronic communications between the two carbazole moieties and cause them to be oxidized individually.[18] In addition, compounds **2**–**7**, in which the carbazole dimer was incorporated with arylamines, exhibit two reversible one-electron redox waves, which are barely resolvable and an irreversible two-electron wave due to the oxidation of the peripheral diarylamines and the central carbazole dimer (Table 1 and Fig. 3), respectively. The first oxidation potential decreases (**2**>**3**>**5**>**4**>**6**>**7**) in accordance with the electron donating ability of the substituent at the nitrogen atoms of the peripheral amines. Lack of electronic communication between the two

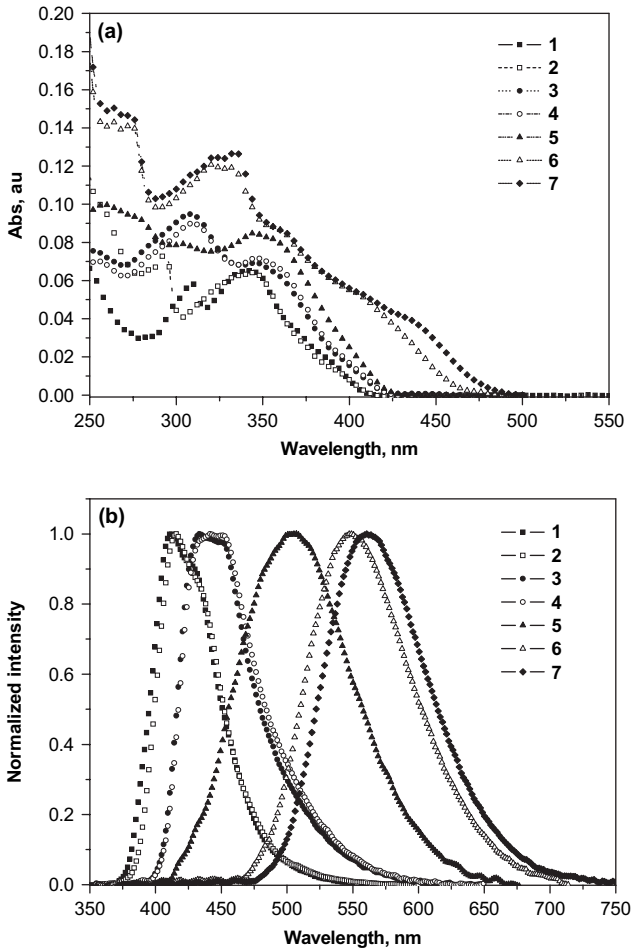


Figure 2. (a) Absorption and (b) emission spectra of compounds **1**–**7** in CH_2Cl_2. The excitation wavelength in emission spectra is 345 nm.

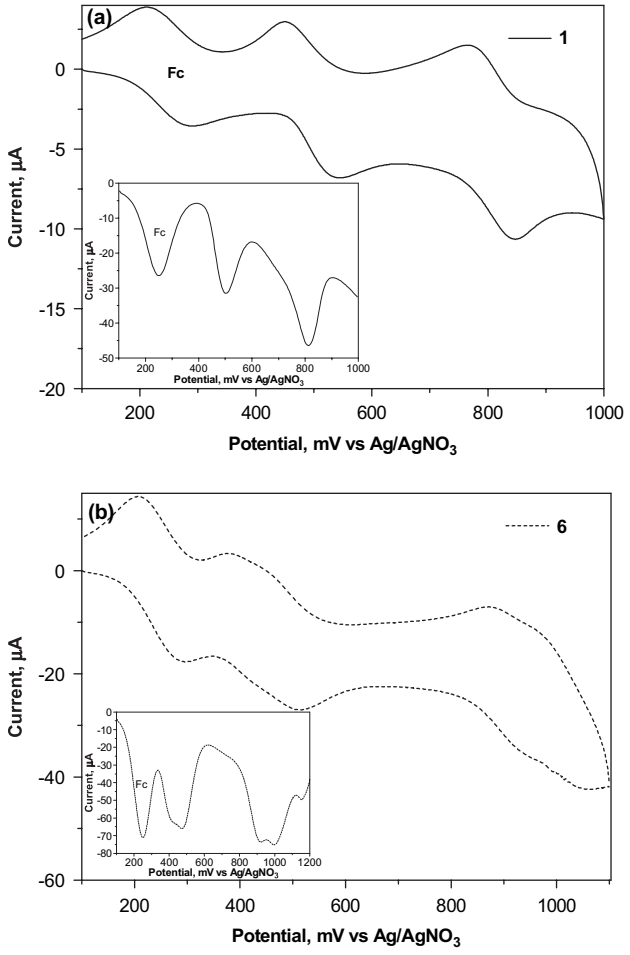


Figure 3. Cyclic voltammograms of (a) **1** and (b) **6** recorded in CH_2Cl_2 (scan rate 100 mV/s). Inset: different pulse voltammograms in the oxidization region.

peripheral amines in these compounds is in contrast to the 3,6-diarylamine substituted carbazoles (*carb*) we reported earlier, and can be attributed to the longer distance between the two peripheral amines.[4] In *carb* compounds, the two peripheral amines are electronically communicated and two one-electron oxidation waves are observed. The first oxidation potential of the carb compounds is, therefore, more cathodic than that of the compounds in this study.

Double-layer EL devices using compounds **1**, **2**, and **3** as the hole-transporting (HTL) as well as emitting layer and TPBI (1,3,5-tris(*N*-phenylbenzimidazol-2-yl)benzene) (device **I**) or Alq_3 (tris(8-quinolinolato)aluminum) (device **II**) as electron-transporting layer (ETL) were fabricated with the structure: ITO/**Cpd** (40 nm)/TPBI or Alq_3 (40 nm)/LiF (1 nm)/Al (200 nm) (**Cpd**=**1**, **2**, or **3**). Relative energy alignment of the constituents is shown in Figure 4 and the device performance parameters are collected in Table 2. Device **I** containing compounds **1** and **2** emitted blue light characteristic of the carbazole dimer derivatives while the device containing **3** emitted greenish blue light, which possibly resulted from the exciplex generated at the interface between the compound and TPBI layer. The electroluminescent (EL) spectra were shown in Figure 5. Such an outcome may be due to the higher hole mobility of **3** than **1** or **2**, which facilitated the carrier recombination to occur between the two layers.[19] In order to verify this argument, device **III** of the

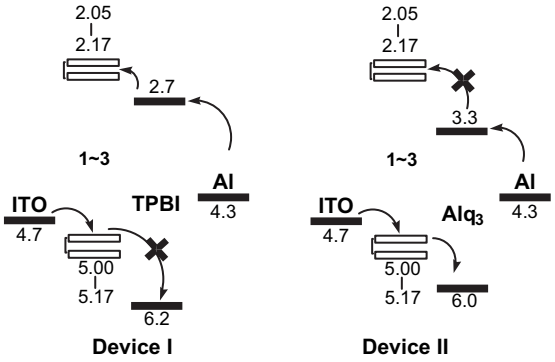


Figure 4. Energy alignment of the constituents in device **I** and device **II**.

Table 2. Electroluminescence data for the devices **I** and **II**[a]

	1 TPBI/Alq_3	**2** TPBI/Alq_3	**3** TPBI/Alq_3
V_{on}, V	3.0/2.5	4.0/3.5	3.0/2.5
L_{max}, cd/m^2	8703/36913	5533/28526	10817/24494
λ_{em}, nm	454/522	454/520	484/526
CIE (*x*, *y*)	0.17, 0.19/0.30, 0.56	0.16, 0.15/0.30, 0.54	0.20, 0.31/0.32, 0.56
fwhm, nm	82/94	74/96	92/98
$\eta_{ext,max}$, %	2.25/1.47	2.41/1.56	1.31/1.03
$\eta_{p,max}$, lm/W	1.92/2.91	1.95/3.05	2.34/1.75
$\eta_{c,max}$, cd/A	3.26/4.74	2.93/4.96	2.77/3.31
L, cd/m^2 (*)	3100/4347	2334/4776	2549/3306
η_{ext}, % (*)	2.14/1.47	1.93/1.51	1.21/1.02
η_p, lm/W (*)	1.31/1.88	0.88/1.79	1.14/1.35
η_c, cd/A (*)	3.10/4.74	2.35/4.78	2.56/3.31

[a] The measured values are given in the order of devices **I** and device **II**. L_{max}, maximum luminance; *L*, luminance; V_{on}, turn-on voltage; *V*, voltage; $\eta_{ext,max}$, maximum external quantum efficiency; $\eta_{p,max}$, maximum power efficiency; $\eta_{c,max}$, maximum current efficiency; η_{ext}, external quantum efficiency; η_p, power efficiency; η_c, current efficiency; fwhm, full width at half maximum; *, at a current density of 100 mA/cm^2. V_{on} was obtained from the *x*-intercept of log (luminance) versus applied voltage plot.

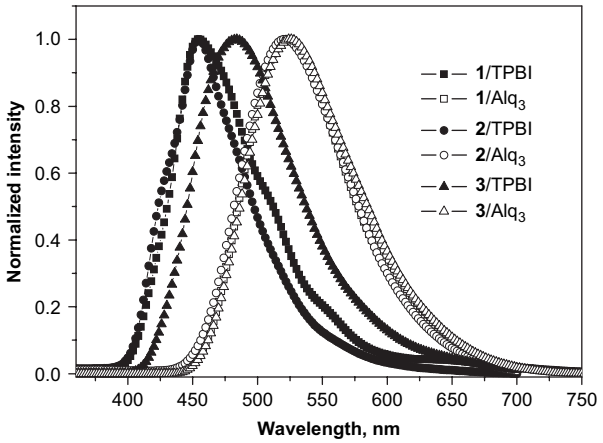


Figure 5. EL curves for device **I** and device **II** containing **1**, **2**, and **3**.

configuration ITO/**3** (60 nm)/TPBI (20 nm)/LiF (1 nm)/Al (200 nm) was fabricated. The thicker HTL and thinner ETL led to better balance of electrons and holes in the device **III**. Consequently, the EL spectrum of the device **III** matched with the film spectrum of **3** at an operating voltage of 6 V (Fig. 6). As the applied voltage increased, more holes reached the interface of HTL and ETL and more exciplexes were generated (Inset of Fig. 6). At a voltage of 12 V, the EL spectrum was nearly superimposable with that of the device **I** operated at 6 V. Such an explanation was further supported by the similar emission spectrum between device **I** and a mixed film of TPBI and **3**, where the exciplex of them should surely generate. Device **II** containing **1**, **2**, or **3** emitted green light from Alq_3. We believe that the HOMO energy gap (ΔE=1.03−1.20 eV) between HTL and ETL in the device **I** is sufficiently large to effectively block the injection of holes from the **Cpd** layer to the TPBI layer. In contrast, the smaller HOMO energy gap (ΔE=1.00–0.83 eV) between HTL and ETL allowed easier injection of holes from **Cpd** into the Alq_3 layer in the device **II**. This result is consistent with our previous studies in arylamine-containing carbazole derivatives.[4]

The current–voltage (I–V), luminance–current (L–I), and current efficiency–current density curves of devices **I** and **II** were shown in Figures 7–9. The relative low turn-on

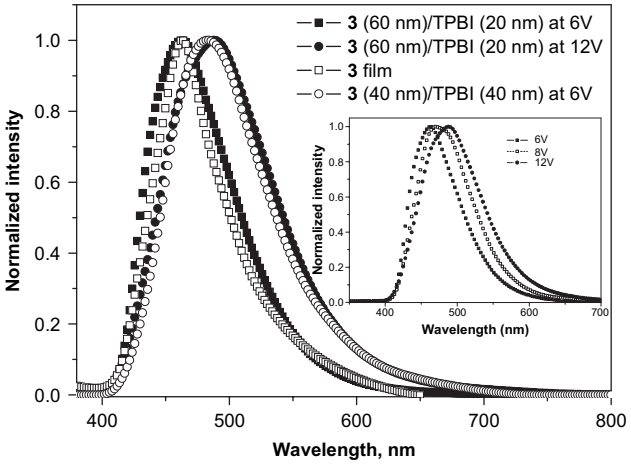


Figure 6. Comparison of EL and film PL curves for **3**. Inset: EL spectra of the device ITO/**3** (60 nm)/TPBI (20 nm)/LiF/Al at 6, 8, and 12 V.

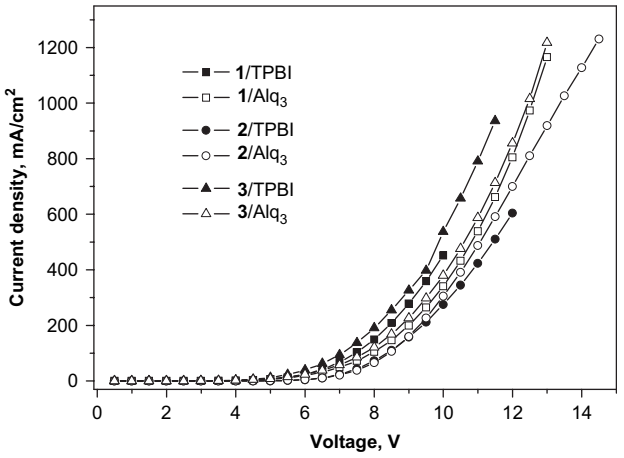


Figure 7. I–V curves for device **I** and device **II** containing compounds **1**, **2**, and **3**.

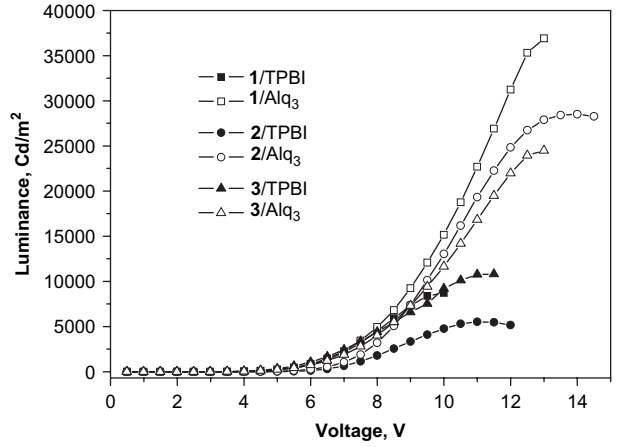


Figure 8. L–V curves for device **I** and device **II** containing **1**, **2**, and **3**.

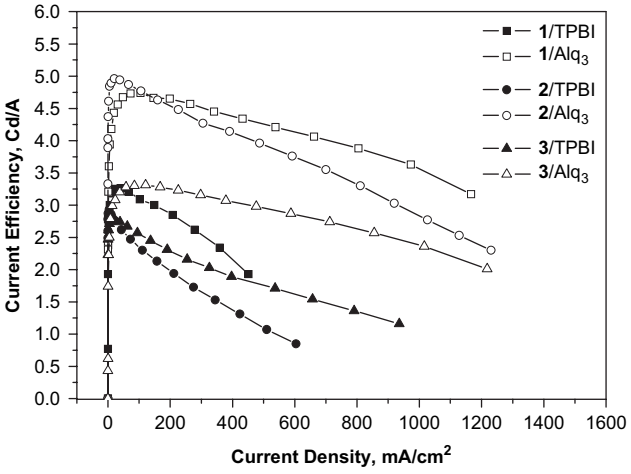


Figure 9. Current efficiency–current density curves for device **I** and device **II** containing **1**, **2**, and **3**.

and operating voltages in all devices **I** and **II** makes them promising for better performances. For blue emitting device **I**, the Commission International de l'Éclairage (CIE) coordinates (x, y) of **1**, **2**, and **3** are located at (0.17, 0.19), (0.16, 0.15), and (0.20, 0.31), respectively. They also exhibit good performance at operating voltage (e.g., L=2334–3100 cd/m^2; η_{ext}=1.2–2.1%; η_p=0.9–1.3 lm/W; η_c=2.4–3.1 cd/A at a current density of 100 mA/cm^2). For green-emitting device **II**, the CIE coordinates (x, y) are almost superimposable ((0.30, 0.56), (0.30, 0.54), and (0.32, 0.56) for **1**, **2** and **3**, respectively). The performance without optimization appear to be approximately equal (e.g., L_{max}= 24494–36913 cd/m^2; $\eta_{ext,\ max}$=1.0–1.6%; $\eta_{p,\ max}$=1.8–3.1 lm/W; $\eta_{c,\ max}$=3.3–5.0 cd/A) compared with the standard device of the structure ITO/α-NPD (40 nm)/Alq_3 (40 nm)/LiF (1 nm)/Al (150 nm) referred in previous literature.[20]

3. Conclusions

To summarize, we have prepared a series of stilbene like carbazolyl derivatives by incorporating diarylamines at the peripheries of a carbazole dimer unit via McMurry C–C coupling reaction followed by palladium(0)-catalyzed C–N bond formation. The arylamine substituents significantly enhance the thermal stability of the carbazole dimer core. By altering the peripheral amines, the emitted color of these materials can be tuned from blue to yellow with moderate to good quantum yields. Double-layer devices using **1**–**3** as hole-transporting and emitting layer were fabricated. These devices exhibit very promising performance in blue or green region when TPBI and Alq_3 were selected as electron-transporting layers.

4. Experimental

4.1. General

Unless otherwise specified, all the reactions were carried out under nitrogen atmosphere using standard Schlenk techniques. Solvents were dried by standard procedures. All column chromatography was performed with the use of silica gel (230–400 mesh, Macherey-Nagel GmbH & Co.) as stationary phase. The ^{1}H NMR spectra were recorded on a Bruker AMX400 spectrometer. Electronic absorption spectra were measured in toluene, dichloromethane, and acetonitrile using a Cary 50 Probe UV–vis spectrophotometer. Emission spectra were recorded by a Jasco FP-6500 fluorescence spectrometer. Emission quantum yields were measured in various organic solvents by standard methods with reference to Coumarin 1 in acetonitrile. Cyclic voltammetry experiments were performed with a CHI-621B electrochemical analyzer. All measurements were carried out at room temperature with a conventional three-electrode configuration consisting of platinum working and auxiliary electrodes and a nonaqueous $Ag/AgNO_3$ reference electrode. The $E_{1/2}$ values were determined as $1/2(E_p^a+E_p^c)$, where E_p^a and E_p^c were the anodic and cathodic peak potentials, respectively. The solvent in all experiments was CH_2Cl_2 and the supporting electrolyte was 0.1 M tetrabutylammonium perchlorate. DSC measurements were carried out using a Perkin–Elmer 7 series thermal analyzer at a heating rate of 10 °C/min. TGA measurements were performed on a Perkin–Elmer TGA7 thermal analyzer. FAB-mass spectra were collected on a JMS-700 double focusing mass spectrometer (JEOL, Tokyo, Japan) with a resolution of 8000 (5% valley definition). For FAB-mass spectra, the source accelerating

voltage was operated at 10 kV with a Xe gun, using 3-nitrobenzyl alcohol as the matrix. Elementary analyses were performed on a Perkin–Elmer 2400 CHN analyzer.

4.2. Synthesis

6-Bromo-9-ethyl-9*H*-carbazole-3-carbaldehyde (**9**) was synthesized by following literature procedures.[21] (*E*)-1,2-Bis(9-ethyl-9*H*-carbazol-3-yl)ethene (**1**) and (*E*)-1,2-bis(6-bromo-9-ethyl-9*H*-carbazol-3-yl)ethene (**10**) were synthesized by similar procedure. Only the preparation of **10** will be described in detail.

4.2.1. (*E*)-1,2-Bis(6-bromo-9-ethyl-9*H*-carbazol-3-yl)-ethene (10). A mixture of 6-bromo-9-ethyl-9*H*-carbazole-3-carbaldehyde (6.04 g, 20 mmol) and zinc powder (3.92 g, 60 mmol) in a 250 mL two-neck flask was added dry THF. Titanium(IV) chloride (3.29 mL, 30 mmol) was added dropwise to the flask immersed in an ice bath. The ice bath was removed after addition was complete, and the reaction mixture was refluxed under nitrogen for 4 h. After being cooled, the solution was added to ice water and extracted with dichloromethane. The organic layer was collected, washed with brine, and dried over anhydrous $MgSO_4$. After filtration and removal of the solvent, the crude product was further recrystallized from CH_2Cl_2/hexane to give **1** as a pale yellow solid. Yield: 85%. ^{1}H NMR (δ, $CDCl_3$): 1.43 (t, J=3.6 Hz, 6H), 4.34 (q, J=7.2 Hz, 4H), 7.26 (d, J=8.6 Hz, 2H), 7.31 (s, 2H), 7.38 (d, J=8.5 Hz, 2H), 7.53 (dd, J=8.6, 1.9 Hz, 2H), 7.72 (dd, J=8.5, 1.6 Hz, 2H), 8.18 (d, J=1.2 Hz, 2H), 8.23 (d, J=1.9 Hz, 2H). FABMS (*m*/*z*): 571.9 (M^+).

4.2.2. (*E*)-1,2-Bis(9-ethyl-9*H*-carbazol-3-yl)ethene (1). White solids. Yield: 90%. ^{1}H NMR (δ, $CDCl_3$): 1.44 (t, J=3.6 Hz, 6H), 4.37 (q, J=7.2 Hz, 4H), 7.23 (d, J=8.6 Hz, 2H), 7.35 (s, 2H), 7.39 (d, J=8.5 Hz, 4H), 7.44 (t, J=7.4 Hz, 2H), 7.71 (dd, J=8.5, 1.6 Hz, 2H), 8.13 (d, J=7.6 Hz, 2H), 8.26 (d, J=1.9 Hz, 2H). ^{13}C NMR ($CDCl_3$): 140.3, 139.4, 129.2, 127.0, 125.7, 124.3, 123.3, 123.0, 120.5, 118.9, 118.3, 108.6, 108.5, 37.6, 13.9. FABMS (*m*/*z*): 414.1 (M^+). Anal. Calcd for $C_{30}H_{26}N_2$: C, 86.92; H, 6.32; N, 6.76. Found: C, 86.50; H, 6.28; N, 6.37.

(*E*)-1,2-Bis(9-ethyl-3,9′-bi(9*H*-carbazol)-6-yl)ethene (**2**), (*E*)-6,6′-(ethene-1,2-diyl)bis(9-ethyl-*N*,*N*-diphenyl-9*H*-carbazol-3-amine) (**3**), (*E*)-6,6′-(ethene-1,2-diyl)bis(9-ethyl-*N*-phenyl-*N*-*m*-tolyl-9*H*-carbazol-3-amine) (**4**), (*E*)-6,6′-(ethene-1,2-diyl)bis(9-ethyl-*N*-(naphthalen-1-yl)-*N*-phenyl-9*H*-carbazol-3-amine) (**5**), (*E*)-6,6′-(ethene-1,2-diyl)bis(9-ethyl-*N*-phenyl-*N*-(pyren-1-yl)-9*H*-carbazol-3-amine) (**6**), and (*E*)-6,6′-(ethene-1,2-diyl)bis(9-ethyl-*N*-(4-methoxyphenyl)-*N*-(pyren-1-yl)-9*H*-carbazol-3-amine) (**7**) were synthesized by a similar procedure as described for **2**.

4.2.3. (*E*)-1,2-Bis(9-ethyl-3,9′-bi(9*H*-carbazol)-6-yl)-ethene (2). The mixture of carbazole (0.37 g, 2.2 mmol), compound **1** (0.57 g, 1.0 mmol), sodium *tert*-butoxide (0.29 g, 3.0 mmol), $Pd(OAc)_2$ (9.0 mg, 0.040 mmol), tri(*tert*-butyl)phosphine (11 mg, 0.040 mmol), and dry toluene (20 mL) were refluxed under nitrogen for 16 h. After cooling, it was quenched with water and extracted with dichloromethane. The combined organic layer was washed with brine solution and dried over anhydrous $MgSO_4$. After filtration and removal of the solvent, the crude product was further purified by column chromatography on silica gel using dichloromethane/hexane=1/4 as the eluant to yield **2** as a pale yellow solid. Yield: 75%. ^{1}H NMR (δ, $CDCl_3$): 1.52 (t, J=3.6 Hz, 6H), 4.45 (q, J=7.2 Hz, 4H), 7.25–7.29 (m, 4H), 7.31 (s, 2H), 7.36–7.38 (m, 8H), 7.45 (d, J=9.6 Hz, 2H), 7.58 (dd, J=9.5, 8.6 Hz, 4H), 7.74 (dd, J=8.5, 1.6 Hz, 2H), 8.16 (d, J=7.7 Hz, 4H), 8.21 (s, 2H), 8.25 (s, 2H). ^{13}C NMR ($CDCl_3$): 141.9, 140.1, 139.4, 129.6, 129.0, 127.1, 125.8, 125.3, 125.0, 123.9, 123.1, 123.0, 120.3, 120.2, 119.7, 119.5, 119.4, 118.5, 110.5, 109.8, 109.5, 109.0, 38.0, 14.0. FABMS (*m*/*z*): 744.2 (M^+). Anal. Calcd for $C_{54}H_{40}N_4$: C, 87.07; H, 5.41; N, 7.52. Found: C, 86.80; H, 5.29; N, 7.47.

4.2.4. (*E*)-6,6′-(Ethene-1,2-diyl)bis(9-ethyl-*N*,*N*-diphenyl-9*H*-carbazol-3-amine) (3). Pale yellow solid. Yield: 79%. ^{1}H NMR (δ, $CDCl_3$): 1.47 (t, J=3.6 Hz, 6H), 4.35 (q, J=7.2 Hz, 4H), 6.95 (t, J=6.8 Hz, 4H), 7.12 (d, J=7.9 Hz, 8H), 7.22 (d, J=7.9 Hz, 8H), 7.25 (s, 2H), 7.29–7.38 (m, 6H), 7.65 (dd, J=8.5, 1.6 Hz, 2H), 7.91 (s, 2H), 8.11 (s, 2H). ^{13}C NMR ($CDCl_3$): 148.7, 139.9, 139.6, 137.6, 129.2, 129.0, 126.8, 125.5, 124.6, 123.9, 123.0, 122.7, 121.5, 118.9, 118.3, 109.4, 108.7, 37.8, 14.0. FABMS (*m*/*z*): 748.5 (M^+). Anal. Calcd for $C_{54}H_{44}N_4$: C, 86.60; H, 5.92; N, 7.48. Found: C, 86.20; H, 6.24; N, 7.30.

4.2.5. (*E*)-6,6′-(Ethene-1,2-diyl)bis(9-ethyl-*N*-phenyl-*N*-*m*-tolyl-9*H*-carbazol-3-amine) (4). Pale yellow solid. Yield: 80%. ^{1}H NMR (δ, $CDCl_3$): 1.45 (t, J=3.6 Hz, 6H), 2.23 (s, 6H), 4.33 (q, J=7.2 Hz, 4H), 6.77 (d, J=8.6 Hz, 2H), 6.88–6.93 (m, 6H), 7.07–7.13 (m, 6H), 7.19 (d, J=7.9 Hz, 4H), 7.22 (s, 2H), 7.26–7.36 (m, 6H), 7.63 (dd, J=8.5, 1.6 Hz, 2H), 7.89 (s, 2H), 8.10 (s, 2H). ^{13}C NMR ($CDCl_3$): 148.5, 139.1, 137.1, 131.5, 128.9, 128.7, 125.8, 123.9, 123.2, 122.5, 122.2, 120.4, 119.8, 119.7, 109.2, 108.5, 37.7, 21.5, 14.0. FABMS (*m*/*z*): 776.4 (M^+). Anal. Calcd for $C_{56}H_{48}N_4$: C, 86.56; H, 6.23; N, 7.21. Found: C, 86.48; H, 6.31; N, 6.88.

4.2.6. (*E*)-6,6′-(Ethene-1,2-diyl)bis(9-ethyl-*N*-(naphthalen-1-yl)-*N*-phenyl-9*H*-carbazol-3-amine) (5). Yellow solid. Yield: 70%. ^{1}H NMR (δ, $CDCl_3$): 1.52 (t, J=3.6 Hz, 6H), 4.45 (q, J=7.2 Hz, 4H), 6.84–6.91 (m, 6H), 7.15 (t, J=7.9 Hz, 4H), 7.19 (s, 2H), 7.26–7.37 (m, 10H), 7.42–7.50 (m, 4H), 7.60 (dd, J=8.5, 1.6 Hz, 2H), 7.73 (d, J=8.2 Hz, 2H), 7.88 (d, J=8.2 Hz, 2H), 7.91 (d, J=1.9 Hz, 2H), 8.04 (d, J=1.2 Hz, 2H), 8.08 (d, J=8.6 Hz, 2H). ^{13}C NMR ($CDCl_3$): 150.1, 144.5, 140.7, 139.9, 137.1, 135.3, 131.1, 129.0, 128.9, 128.3, 126.8, 126.4, 126.2, 126.0, 125.8, 124.6, 124.4, 123.9, 123.6, 123.0, 119.9, 119.6, 118.3, 116.8, 115.2, 109.1, 108.6, 37.7, 14.0. FABMS (*m*/*z*): 848.4 (M^+). Anal. Calcd for $C_{62}H_{48}N_4$: C, 87.70; H, 5.70; N, 6.60. Found: C, 88.09; H, 5.95; N, 6.37.

4.2.7. (*E*)-6,6′-(Ethene-1,2-diyl)bis(9-ethyl-*N*-phenyl-*N*-(pyren-1-yl)-9*H*-carbazol-3-amine) (6). Yellow solid. Yield: 77%. ^{1}H NMR (δ, $CDCl_3$): 1.42 (t, J=3.6 Hz, 6H), 4.28 (q, J=7.2 Hz, 4H), 6.87 (t, J=7.3 Hz, 2H), 6.95 (d, J=7.7 Hz, 4H), 7.14 (s, 2H), 7.17 (d, J=7.7 Hz, 4H), 7.26–7.29 (m, 4H), 7.34 (dd, J=8.7, 2.1 Hz, 2H), 7.55 (dd, J=8.4, 1.3 Hz, 2H), 7.86 (d, J=8.2 Hz, 2H), 7.90–7.96 (m, 8H), 8.03 (s, 4H), 8.08 (d, J=7.5 Hz, 2H), 8.12–8.16

(m, 4H), 8.26 (d, J=9.2 Hz, 2H). ^{13}C NMR ($CDCl_3$): 150.2, 141.9, 140.9, 139.9, 137.0, 131.3, 131.1, 129.1, 129.0, 127.6, 127.3, 126.8, 126.1, 126.0, 125.0, 124.9, 123.7, 122.9, 120.1, 119.9, 118.2, 116.9, 109.2, 108.6, 37.7, 13.9. FABMS (m/z): 996.3 (M^+). Anal. Calcd for $C_{74}H_{52}N_4$: C, 89.13; H, 5.26; N, 5.62. Found: C, 88.80; H, 5.48; N, 5.52.

4.2.8. (*E*)-6,6′-(Ethene-1,2-diyl)bis(9-ethyl-*N*-(4-methoxy-phenyl)-*N*-(pyren-1-yl)-9*H*-carbazol-3-amine) (7). Yellow solid. Yield: 65%. ^{1}H NMR (δ, $CDCl_3$): 1.38 (t, J=3.6 Hz, 6H), 3.75 (s, 6H), 4.26 (q, J=7.2 Hz, 4H), 6.74 (d, J=7.7 Hz, 4H), 6.99 (d, J=7.7 Hz, 4H), 7.12 (s, 2H), 7.15–7.26 (m, 6H), 7.54 (dd, J=8.4, 1.3 Hz, 2H), 7.80 (d, J=8.2 Hz, 2H), 7.83–7.96 (m, 8H), 8.03 (s, 4H), 8.05–8.13 (m, 6H), 8.26 (d, J=9.2 Hz, 2H). ^{13}C NMR ($CDCl_3$): 136.5, 131.4, 131.2, 128.9, 128.6, 127.3, 126.6, 126.5, 126.1, 125.9, 125.0, 124.8, 124.7, 123.9, 123.2, 123.0, 122.8, 120.5, 118.2, 115.3, 114.5, 109.1, 108.5, 55.5, 37.7, 14.0. FABMS (m/z): 1056.2 (M^+). Anal. Calcd for $C_{76}H_{56}N_4O_2$: C, 86.34; H, 5.34; N, 5.30. Found: C, 85.91; H, 5.65; N, 5.46.

4.3. OLEDs fabrication and measurements

Electron-transporting materials TPBI and Alq_3 were synthesized according to literature procedures and were sublimed twice prior to use. Pre-patterned ITO substrates with an effective individual device area of 3.14 mm^2 were cleaned as described in a previous report.[22] Double-layer EL devices using carbazole derivatives as the hole-transport layer and TPBI or Alq_3 as the electron-transport layer were fabricated. For comparison, a typical device using NPB (1,4-bis(1-naphthylphenylamino)biphenyl) as the hole-transporting layer was also fabricated. All devices were prepared by vacuum deposition of 40 nm of the hole-transporting layer, followed by 40 nm of TPBI or Alq_3, and then 1 nm of LiF and 200 nm of Al were deposited as the cathode. I–V curve was measured on a Keithley 2400 source meter in an ambient environment. Light intensity was measured with a Newport 1835 optical meter.

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N-Biphenyl thioureas as carboxylate receptors. Effect of the ligand substituents on the geometry of the complexes

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Abstract—Six new biphenyl thiourea derivatives have been prepared to be used in carboxylate sensing. Experiments carried out with these ligands have demonstrated that the type of interaction with TBA carboxylates is strongly dependent on the substituents in the thiourea moiety. These interactions go from the formation of 1:1 hydrogen-bonded complexes to acid–base reactions. In addition, different geometries have been observed for the complexes being dependent on the conformations of the free ligands in solution.

1. Introduction

There is currently a great interest in the development of sensors for carboxylate anions due to their presence in a variety of biomolecules and particularly in amino acids.[1] Many of the carboxylate binding sites in these systems contain either one[2] or two[3] urea or thiourea subunits as hydrogen-bond donor groups. There has been much discussion about the thiourea (urea)–carboxylate bonding motif. In general it is assumed that thioureas bind to carboxylates through a double hydrogen bond involving both N–H fragments of the thiourea and both carboxylate oxygens in a Y-type bidentated complex (Chart 1).[3b,4] However this geometry should not be proposed in general because other factors such as conformational equilibria[4e,5] or dimerization processes[6] should also be considered.

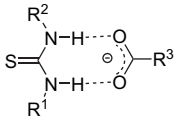

Chart 1.

In our group we have been interested in the use of 4,4′-disubstituted biphenyls as signalling units in cation and anion sensing[7] and of thioureas as binding sites for anion recognition.[8] We report herein the synthesis of neutral carboxylate receptors **1**–**4** based on thioureas bearing a 4′-nitrobiphenyl group attached to one N atom, as well as receptors **5** and **6** with a biphenyl substituent on the thiourea moiety. These receptors have been tested with various aromatic carboxylates with different basicities as well as with fluoride and acetate anions. Our experiments allow us to affirm that the complexes formed between thiourea receptors and carboxylate anions do not always show a Y-type geometry. By contrary, with several ligands the carboxylate group is only bound to one NH group of the thiourea as it has been previously described for amide-based ligands.[9] In addition, in some cases the process is not a real complexation but an acid–base reaction.[10]

2. Results and discussion

2.1. Synthesis and conformational studies of receptors 1–6

The structures of the new receptors **1**–**6** are shown in Scheme 1. Biphenyl thiourea derivatives were prepared from 4-amino-4′-nitrobiphenyl (**7**)[11] or 4-aminobiphenyl (**8**) and the corresponding isothiocyanates (**9**–**12**) in refluxing THF. 4-Amino-4′-nitrobiphenyl (**7**) was prepared by nitration of 4-nitrobiphenyl with nitric acid, followed by partial reduction to the *p*-aminonitro compound by reaction with aqueous sodium hydrogen sulfide.[12] 4-Aminobiphenyl (**8**) was obtained by reduction of 4-nitrobiphenyl with aqueous NaHS.

All compounds were characterized by NMR and MS. For ligands **1**, **3**, **4** and **6**, only one resonance is observed for each proton in the ^{1}H NMR spectra in DMSO-d_6, indicating that there is only one predominant thiourea rotamer in solution, or a fast equilibrium between different conformations

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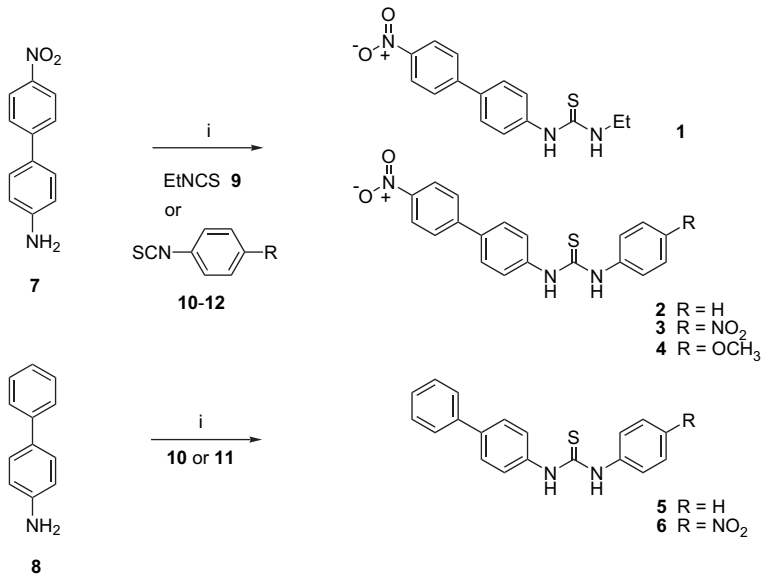

Scheme 1. Synthesis of receptors **1**–**6**. (i) Et_3N, THF, reflux.

(Fig. 1a). By contrast, the ^{1}H NMR spectra in DMSO-d_6 of ligands **2** and **5**, with a phenyl group attached to one nitrogen atom of the thiourea moiety are more complicated (Fig. 1b), showing at least two sets of signals for each ligand. These results indicate that two different conformations of the thioureas are present in solution, with slow *E*–*Z* rotameric interconversion rates on the NMR time-scale. In order to know which are the conformations present in solution in each case additional NMR experiments were carried out. Thus NOE experiments showed that thiourea **2** exists in DMSO solution as a mixture of *E*,*Z* and *Z*,*E* rotamers **2a** and **2b** (Chart 2).

Similar studies carried out with ligand **3** suggest that some degree of aggregation in solution occurs under the experimental conditions in contrast with that observed in ureas.[13] This self-association seems to situate the aromatic rings in

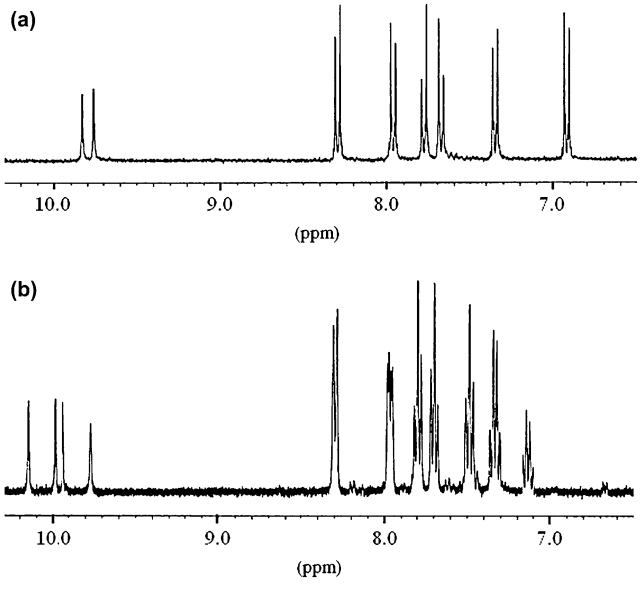

Figure 1. N–H and aromatic ^{1}H NMR signals of: (a) **4** and (b) **2** in DMSO-d_6.

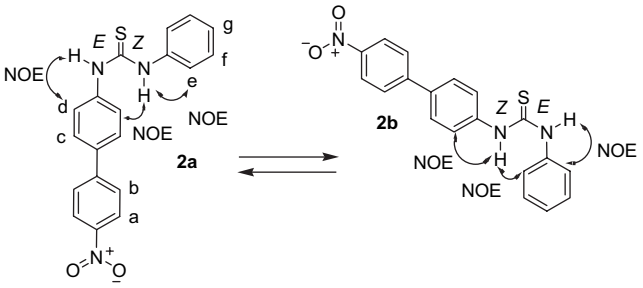

Chart 2. Main conformations of **2** in DMSO-d_6 solution.

the way shown in Chart 3 according to the NOE signals observed in the NMR experiments. In addition, when solvent was changed from DMSO-d_6 to the less polar acetone-d_6 a much more complex ^{1}H NMR spectrum was obtained reflecting the presence of a main conformation in addition to several rotamers and/or aggregates. This self-association through hydrogen-bonding is probably inhibited in the two rotamers of **2**.

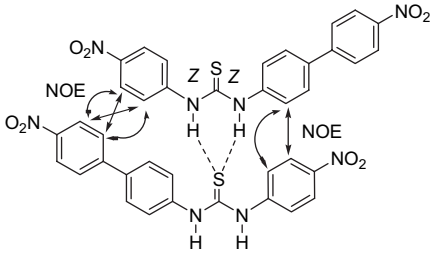

Chart 3. Proposed self-assembled dimer of **3** in DMSO-d_6 solution.

2.2. Complexation studies

To study the ability of these ligands to act as sensors for carboxylates, a series of aromatic carboxylates (benzoate, *p*-methylbenzoate, *o*- and *p*-nitrobenzoate and *o*- and *p*-methoxybenzoate, all as their tetrabutylammonium (TBA)

salts) was studied, in order to evaluate the effect of the different substituents on the aromatic ring in the complexation constants. We also decided to test acetate and fluoride anions.

Aromatic carboxylates were prepared from the corresponding carboxylic acids and TBA hydroxide in DMSO. Benzoate, acetate and fluoride TBA salts were commercially available.

2.2.1. UV–vis studies. The anion binding ability of receptors **1**–**6** was evaluated by UV–vis titration of each receptor with the appropriate anion in DMSO solution. Changes in the UV spectra agree with the expected results. Thus, in the presence of the same anion, larger changes were observed with thioureas containing two aromatic substituents (**2**–**6**) than with thiourea derivative **1** (Fig. 2).

The presence of an electron withdrawing nitro group at the *para* position of the phenyl ring makes ligand **3** acid enough to experiment deprotonation reactions under some experimental conditions.[10] The deprotonation was characterized by the presence of a new band in the UV spectrum at 464 nm. As it was expected this band does not appear in the presence of both *p*-nitrobenzoate and *o*-nitrobenzoate

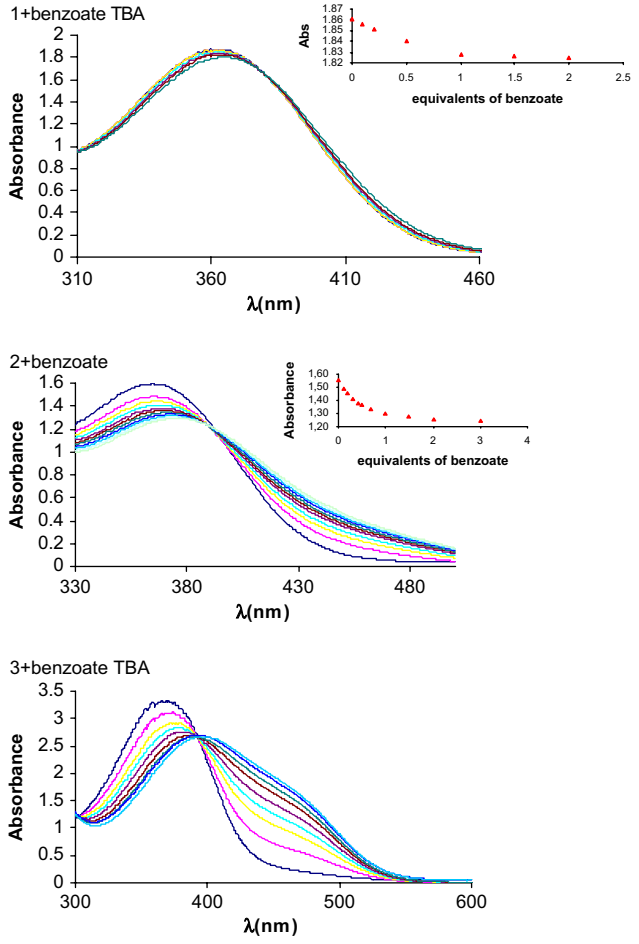

Figure 2. UV–vis absorption spectrophotometric titration of ligands **1**, **2** and **3** with TBA benzoate in DMSO at 25 °C. Inset: stoichiometry determination for **1**+TBA benzoate at 360.5 nm and **2**+TBA benzoate at 360 nm.

because of their lower basicity. Complexation constants evaluated in these experiments are shown in Table 1. These experiments in addition to other carried out by using ^{1}H NMR in DMSO-d_6 demonstrated that all the complexes have a 1:1 stoichiometry (Fig. 2).

If we try to correlate the observed binding constants with the basicity of the aromatic carboxylates, two different behaviours are observed, depending on the thiourea receptor. With ligands **1** and **4** a linear correlation is observed between the acid strengths of the *para* substituted benzoic acids and the binding constants of the corresponding complexes, following a Hammet-type behaviour (see Fig. 3). Thus, for ligand **1** the highest binding constant is observed with the more basic *p*-methoxybenzoate anion (log K 4.5) whereas the lowest binding constant is observed for *p*-nitrobenzoate (log K 3.1). In anion complexation generally for higher anion basicity, stronger complexation constants are observed.[14] This relationship indicates that the complexation of the anions to the receptor **1** is free from steric effects due to the substituent on the phenyl ring, and only electronic factors should be taken into account.

In contrast, with ligands **2** and **5**, there is no apparent relationship between basicities and binding constants. As we observe in Table 1 for receptor **2**, similar association constants are observed for *p*-nitro and *p*-methoxybenzoate (log K 3.4 and 3.6), whereas the highest log K is observed for benzoate anion (5.0), with no substituent on the phenyl ring. One explanation to this behaviour can be found in the previously described conformational equilibrium shown by these ligands. Thus, the determined values correspond not only to the complexation process but to the overall equilibrium between both conformations of the free ligand and their corresponding complexes.

Finally, ligands **3** and **6** experiment deprotonation reactions (except in the presence of *p*- and *o*-nitrobenzoate) and the values obtained with the more basic carboxylates are related to this acid–base process and for this reason has a very similar value for each ligand.

These receptors were also tested against acetate and fluoride anions, which are more basic than the previous aromatic carboxylate anions (Table 2). A 'naked-eye' colour change was observed during most of these titrations.[15] The strongest colour changes were observed for F^- anion, which is the strongest base under these conditions (see Fig. 4).

The results indicate that an acid–base reaction is taking place, resulting in deprotonation of the receptor. In fact, when 3 equiv of fluoride was added to ligand **3** in DMSO-d_6 both a broad signal in the ^{1}H NMR spectrum at 15.7 ppm and a peak at −145.5 ppm in the ^{19}F NMR appear, which can be assigned to the HF_2^- species.[7a] In addition, the band at 464 nm observed in the UV spectrum also confirms the deprotonation reaction with these more basic anions (Fig. 5). This deprotonation process was confirmed by additional experiments with ligand **3** and TBA hydroxide. An instantaneous orange colour was observed upon addition of base, and the UV–vis spectra were very similar to those obtained in the presence of fluorides or acetate anions (see Supplementary data). As it was expected the effect was stronger

Table 1. Log *K* values for receptors **1–6** with aromatic carboxylates, in DMSO at 25 °C[a]

Anion[b]	Receptor					
	1	**2**	**3**	**4**	**5**	**6**
$C_6H_5COO^-$	3.8±0.1	5.0±0.2	5.2±0.4	4.4±0.4	4.0±0.3	5.8±0.5
p-$NO_2C_6H_4COO^-$	3.1±0.2	3.4±0.2	3.2±0.2	3.1±0.3	4.4±0.2	3.5±0.1
p-$MeC_6H_4COO^-$	4.1±0.3	4.4±0.2	5.3±0.4	4.8±0.3	4.1±0.4	5.9±0.6
p-$MeOC_6H_4COO^-$	4.5±0.3	3.6±0.3	5.2±0.4	5.8±0.4	3.7±0.1	5.8±0.3
o-$NO_2C_6H_4COO^-$	3.6±0.2	3.2±0.2	4.1±0.4	3.4±0.2	3.5±0.1	3.5±0.1
o-$MeOC_6H_4COO^-$	5.2±0.4	4.5±0.3	5.3±0.4	5.0±0.4	2.7±0.4	5.6±0.4

[a] The results were calculated by UV–vis titration.
[b] All anions were used as their TBA salts.

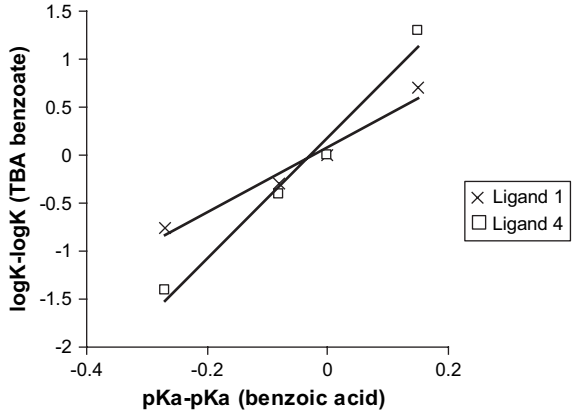

Figure 3. Correlation of the acid dissociation constants of benzoic acids with the binding constants between benzoate anions and receptors **1** and **4**.

Table 2. Log *K* values for the interaction of receptors **1–6** with fluoride and acetate anion, in DMSO at 25 °C[a]

Anion[b]	Receptor					
	1	**2**	**3**	**4**	**5**	**6**
CH_3COO^-	4.3±0.4	—	6.0±0.5	4.6±0.6	5.3±0.5	6.5±0.5
F^-	4.1±0.1	10.4±0.2	—	5.5±0.4	4.6±0.1	6.7±0.4

[a] The results were calculated by UV–vis titration.
[b] All anions were used as their TBA salts.

with ligand **3** than with ligand **6** due to the second nitro group present in ligand **3**, which makes the receptor more acidic.

Figure 4. Colour changes observed on addition of TBA salts (10 equiv) to a DMSO solution of receptor **3**. Left to right: no addition, fluoride, acetate, *o*-methoxybenzoate and *o*-nitrobenzoate.

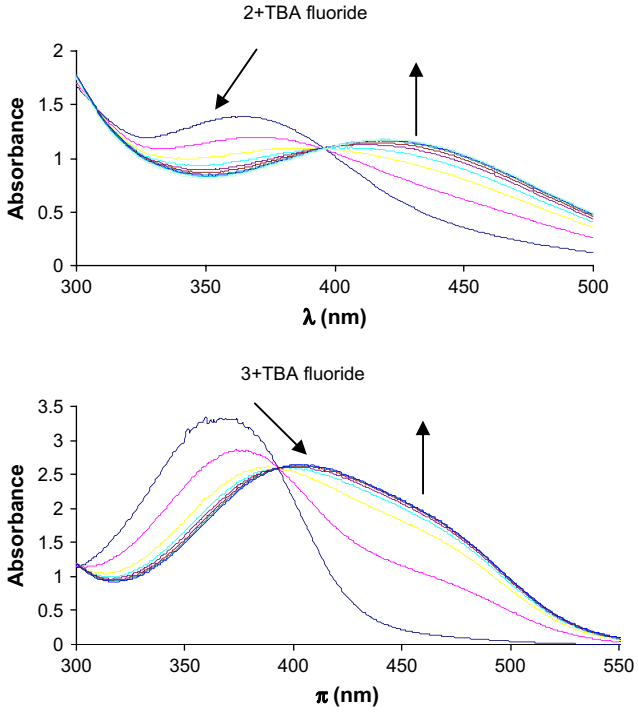

Figure 5. UV–vis absorption spectrophotometric titration of ligands **2** and **3** with TBA fluoride in DMSO at 25 °C.

2.2.2. ^{1}H NMR studies. In order to get some information about the structure of the complex in solution, ^{1}H NMR studies were undertaken. Figures. 6 and 7 show the ^{1}H NMR spectra in DMSO-d_6 of ligands **1** and **2** in the presence of 1 equiv of TBA benzoate. As it can be seen, the NH signals corresponding to ligand **1** are shifted downfield after the anion addition. The $\Delta\delta$ showed by both signals are very similar (2.43 ppm for H_α and 2.35 ppm for H_β) what agrees with a Y-type complex involving both thiourea hydrogens. Similar complexes can be proposed for ligand **4**. With ligands **3** and **6** the NH signals do not appear in the ^{1}H NMR spectrum, which is also coherent with the deprotonation previously proposed.[10]

Finally, ligands **2** and **5** showed a clearly different behaviour similar to what was observed with the free ligands. Addition of TBA *p*-methoxybenzoate to DMSO solutions of **2** or **5** gave rise in each case to two different 1:1 complexes, corresponding very likely to the two different thiourea rotamers originally present in solution. Thus, as it can be seen in Figure 7 in the case of ligand **2**, four signals are observed for the N–H hydrogen, these signals, as it was expected are

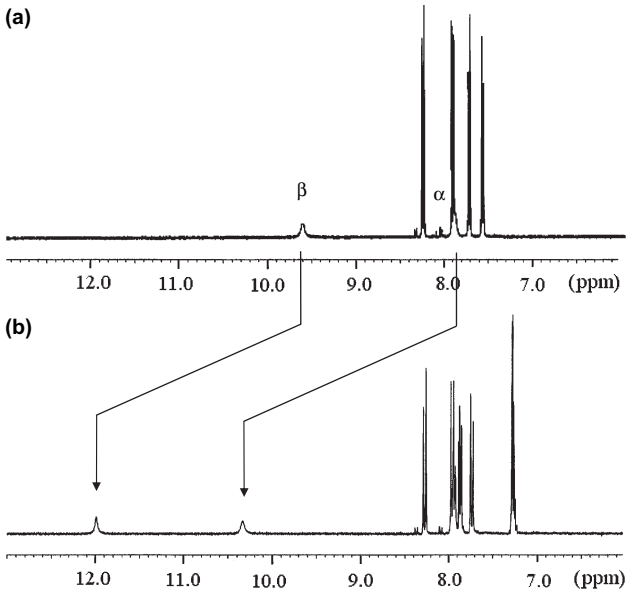

Figure 6. ^{1}H NMR aromatic and amide zone in DMSO-d_6 of: (a) ligand **1** and (b) ligand **1**+1 equiv of TBA benzoate.

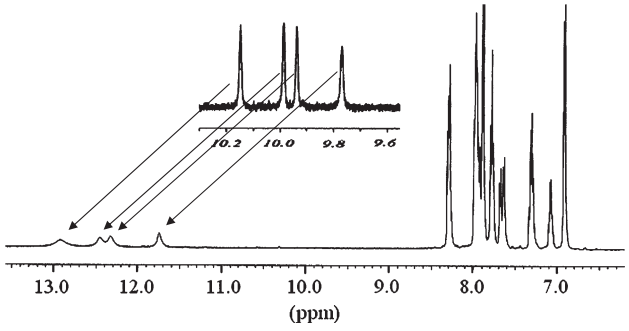

Figure 7. ^{1}H NMR aromatic and amide zone in DMSO-d_6 of ligand **2**+1 equiv of TBA *p*-methoxybenzoate. Inset: NH zone of the free ligand.

downfield shifted as a consequence of the complexation. In addition, NOE′s experiments suggest for one of the complexes the structure shown in Figure 8, which is also in accordance with the structure obtained by PCModel.[16] Of course the system is present in the solution in a dynamic equilibrium containing the four possible complexes. This is in good agreement with the strong shifting observed for the four NH signals in the NMR spectrum. Anyway, what is conclusive is that the two conformations observed in the ligand are also present in the complex. This fact excludes a Y-type complex in this case.

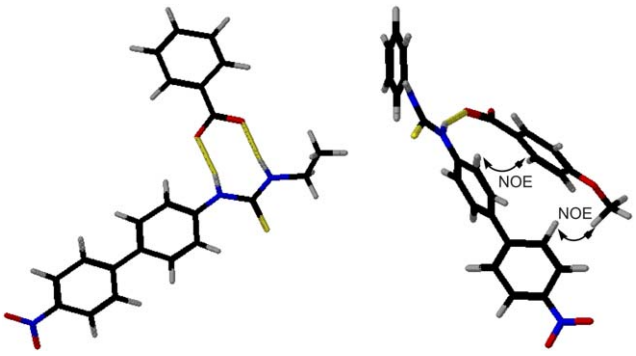

Figure 8. Structural proposal based on PC model 8.0[16] for the complexes between ligands **1** with benzoate (Y-type complex) and **2** with *p*-methoxybenzoate (open complex).

3. Conclusion

A series of biphenyl substituted thioureas have been prepared and their ability to bind aromatic carboxylates has been evaluated by UV–vis titration and NMR experiments. These studies allow us to establish that the geometry of the complexes formed between carboxylate and thiourea receptors is strongly dependent on the substituent in the thiourea group and on their conformational behaviour.[17] Thus, when the ligand is mainly in a *Z,Z* conformation, a Y-type complex can be postulated. By contrast, when other rotamers are present in the solution the geometry of the 1:1 complex can be different with the carboxylate group only bound by one oxygen atom to the more acidic NH atom. Finally, when the thiourea NH groups are acidic enough to give rise to acid–base reactions, strong colour changes are observed with the most basic anions.

4. Experimental

4.1. General procedures and materials

4-Amino-4′-nitrobiphenyl, **7**, was prepared as previously reported, by nitration of 4-nitrobiphenyl with nitric acid followed by partial reduction of the isolated product with sodium hydrogen sulfide.[12] All other reagents were commercially available, and were used without purification. Triethylamine was freshly distilled from CaH_2. THF was distilled from Na/benzophenone under Ar prior to use. Column chromatography was performed with silica gel 60 (230–400 mesh, Merck). Silica gel 60 F254 (Merck) plates were used for TLC. ^{1}H and ^{13}C NMR spectra were recorded with the deuterated solvent as the lock and residual solvent as the internal reference. High-resolution mass spectra (FAB) were recorded in the positive ion mode. UV–vis spectra were recorded using a 1 cm path length quartz cuvette. All measurements were carried out at 293 K (thermostatted).

4.2. Syntheses

4.2.1. *N*-Ethyl-*N*′-(4′-nitro[1,1′-biphenyl]-4-yl)thiourea, 1. Ethylisothiocyanate, **9** (0.24 mL, 2.8 mmol) and dry Et_3N (0.2 mL, 1.4 mmol) were slowly added to a 60 °C solution of 4-amino-4′-nitrobiphenyl, **7** (0.30 g, 1.4 mmol) in THF (3 mL). The mixture was refluxed overnight and then was allowed to reach room temperature. The resulting yellow precipitate was filtered off and dried in vacuum, to give **1** (0.173 g, 42%) as a yellow powder. Mp: 214–216 °C. IR (KBr): 3370 (NHEt), 3145 (NHAr), 2986 (Ar), 1594 (C=C), 1510 (N=O), 1524 (N=O), 1344 (C=S), 850 (C–N) cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.67 (br s, 1H, NH), 8.29 (d, J=8.9 Hz, 2H), 7.95 (br s, 1H, NH), 7.94 (d, J=8.9 Hz, 2H), 7.76 (d, J=8.6 Hz, 2H), 7.64 (d, J=8.6 Hz, 2H), 3.52 (q, J=7.0 Hz, 2H), 1.15 (t, J=7.0 Hz, 3H). ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 180.0, 146.2, 146.1, 140.6, 132.6, 127.4, 127.2, 124.1, 122.7, 38.7, 14.1. Anal. calcd for $C_{15}H_{15}N_3SO_2$: C, 59.78%; H, 5.02%;

N, 13.94%; S, 10.62%. Found: C, 59.15%; H, 4.94%; N, 13.66%; S, 10.23%. HRMS (FAB): calcd for $C_{15}H_{15}N_3SO_2$: 301.116; found: 301.094.

4.2.2. *N*-(4′-Nitro[1,1′-biphenyl]-4-yl)-*N*′-phenylthiourea, 2. In a similar way, **2** was prepared from **7** (1.4 g, 6.09 mmol), phenylisothiocyanate, **10** (0.75 mL, 6.3 mmol) and Et_3N (0.9 mL, 6.3 mmol) in refluxing THF (12 mL) for 4 h. The yellow precipitate was filtered off and purified by column chromatography on silica gel (CH_2Cl_2) to yield **2** as a pale yellow solid (1.47 g, 70%). Mp 175–177 °C. IR: 3203 (NH), 1348 (C=S), 1594 (NO_2), 1513 (C=C), 830, 730. ^{1}H NMR (DMSO-d_6, 400 MHz): (mixture of conformers) δ 10.16, 10.00, 9.94, 9.78 (2NH), 8.30 (d, J=8 Hz, 2H), 7.98 (m, 2H), 7.80 (m, 2H), 7.70 (m, 2H), 7.49 (m, 2H), 7.34 (m, 2H), 7.14 (m, 1H). ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 179.4, 146.3, 146.1, 140.6, 140.5, 139.4, 139.3, 133.3, 133.1, 128.5, 128.4, 127.4, 127.3, 124.5, 124.4, 124.1, 123.6, 123.5. HRMS (FAB) calcd for $C_{19}H_{15}N_3O_2S$: 349.406; found: 349.401.

4.2.3. *N*-(4′-Nitro[1,1′-biphenyl]-4-yl)-*N*′-(4-nitrophenyl)-thiourea, 3. This compound was prepared from **7** (1.5 g, 7.0 mmol), *p*-nitrophenylisothiocyanate, **11** (1.26 g, 7.0 mmol) and Et_3N (1.0 mL, 7.0 mmol) in THF (20 mL) as described above. The solvent was partially evaporated, giving rise to an orange precipitate, which was filtered off and dried in vacuum (1.49 g, 54%). Mp 217–219 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.50 (br d, 2H, 2NH), 8.30 (d, J=9.0 Hz, 2H), 8.22 (d, J=9.2 Hz, 2H), 7.97 (d, J=9.0 Hz, 2H), 7.87–7.80 (m, 4H), 7.69 (d, J=8.7 Hz, 2H). ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 179.1, 146.4, 146.2, 146.0, 142.4, 140.0, 133.8, 127.5, 127.4, 124.4, 124.2, 123.7, 121.7. HRMS (FAB) calcd for $C_{19}H_{15}N_3O_2$: 394.400; found: 394.403.

4.2.4. *N*-(4′-Nitro[1,1′-biphenyl]-4-yl)-*N*′-(4-methoxyphenyl)thiourea, 4. *p*-Methoxy phenylisothiocyanate **12** (0.25 mL, 1.8 mmol), Et_3N (0.2 mL, 0.14 mmol) and TBAF (1 M in THF, 20 μL) were added to a refluxing solution of **7** (0.39 g, 1.8 mmol) in THF (15 mL). After 20 h of refluxing, another equivalent of **12** was added to the reaction mixture, and the reflux was maintained for another 20 h. The solvent was evaporated and the crude product was purified by column chromatography on silica gel (hexane–EtAcO) to give **4** as a yellow solid (0.27 g, 40%). Mp 178–182 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.83 (s, 1H, NH), 9.76 (s, 1H, NH), 8.29 (d, J=9.0 Hz, 2H), 7.96 (d, J=9.0 Hz, 2H), 7.78 (d, J=9.0 Hz, 2H), 7.68 (d, J=9.0 Hz, 2H), 7.35 (d, J=9.0 Hz, 2H), 6.92 (d, J=9.0 Hz, 2H), 3.75 (s, 3H). ^{13}C NMR (DMSO-d_6, 100.5 MHz): δ 179.7, 156.6, 146.3, 146.1, 140.7, 133.3, 133.0, 132.0, 127.3, 126.0, 124.1, 123.5, 113.7, 55.2. HRMS (FAB) calcd for $C_{20}H_{17}N_3O_3S$: 379.543; found: 379.547.

4.2.5. *N*-(1,1′-Biphenyl-4-yl)-*N*′-phenylthiourea, 5. Phenylisothiocyanate (0.22 mL, 1.77 mmol) and Et_3N (0.26 mL) were added dropwise to a 70 °C solution of **8** (0.30 g, 1.77 mmol) in THF (10 mL) and the mixture was refluxed for 48 h. The solvent was evaporated under reduced pressure, and the residue was treated with a 1:1 mixture of hexane–ether to give a white precipitate, which was filtered off and dried in vacuum to yield **5** as a white powder (0.39 g, 86%). Mp 158–160 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.91 and 9.87, 9.80 (3×s, 2H, 2NH), 7.70–7.10 (m, 14H). ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 179.6, 139.7, 139.5, 139.4, 136.0, 129.3, 129.0, 128.4, 127.2, 126.6, 126.4, 124.4, 123.6. HRMS (FAB) calcd for $C_{19}H_{16}N_2S$: 304.103; found: 304.104.

4.2.6. *N*-(1,1′-Biphenyl-4-yl)-*N*′-(4-nitrophenyl)thiourea, 6. Reaction of **8** (0.30 g, 1.5 mmol), **11** (0.27 g, 1.5 mmol) and Et_3N (0.26 mL) in refluxing THF (6 mL) for 24 h, gave **6** (0.48 g, 91%) as a white powder. Mp 184–187 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.43 (br s, 2H, 2NH), 8.22 (d, J=9.0 Hz, 2H), 7.86 (d, J=9.0 Hz, 2H), 7.70–7.58 (m, 6H), 7.47 (t, J=7.9 Hz, 2H) and 7.35 (t, J=7.2 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 179.1, 142.3, 139.6, 138.4, 136.6, 129.0, 127.3, 126.8, 126.5, 126.4, 124.4, 123.9, 121.6. HRMS (FAB): calcd for $C_{19}H_{15}N_3O_2S$, 350.0963; found: 350.0970.

4.3. Binding studies

Binding constants of ligands **1**–**6** toward tetrabutylammonium carboxylates were evaluated by UV–visible titrations in DMSO. Typically, 10^{-4} M solutions of the receptors in DMSO (3 mL) were titrated by adding 2 μL aliquots of the envisaged carboxylates (as their TBA salts) in DMSO and registering the UV–visible spectrum after each addition. Log K_c was calculated by fitting all spectrophotometric titration curves with the SPECFIT program.[18]

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Phloroglucinols, depsidones and xanthones from the twigs of *Garcinia parvifolia*

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Abstract—Seven phloroglucinols, named parvifoliols A–G (**1**–**7**), two depsidones, named parvifolidones A, B (**8**, **9**), and three xanthones, named parvifolixanthones A–C (**10**–**12**), were isolated from the twigs of *Garcinia parvifolia* along with seven known compounds: garcidepsidone B, mangostinone, rubraxanthone, dulxanthone D, 1,3,5,6-tetrahydroxyxanthone, norathyriol, and (2*E*,6*E*,10*E*)-(+)-4β-hydroxy-3-methyl-5β-(3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl)cyclohex-2-en-1-one. Their structures were proposed on the basis of spectroscopic data. The antibacterial and antioxidation activities were evaluated.

1. Introduction

Plants of the genus *Garcinia*, widely distributed in tropical Africa, Asia, New Caledonia, and Polynesia, have yielded an abundance of biologically active and structurally intriguing natural products.[1,2] As part of our ongoing research program on the identification of antibacterial constituents from plants in the genus *Garcinia*, we have investigated the twigs of *Garcinia parvifolia* belonging to the Guttiferae family. Phytochemical investigation on the latex,[3] leaves,[4] and bark[5] of *G. parvifolia* resulted in the isolation of ten xanthones and four depsidones. In this paper, we describe the isolation and characterization of seven phloroglucinols, parvifoliols A–G (**1**–**7**), two depsidones, parvifolidones A, B (**8**, **9**), and three xanthones, parvifolixanthones A–C (**10**–**12**), along with seven known compounds, garcidepsidone B,[4] mangostinone,[6] rubraxanthone,[3] dulxanthone D,[7] 1,3,5,6-tetrahydroxyxanthone,[8] norathyriol,[9] and (2*E*,6*E*,10*E*)-(+)-4β-hydroxy-3-methyl-5β-(3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl)cyclohex-2-en-1-one[10] from the twigs of *G. parvifolia*. The isolated compounds were examined for the antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and the antioxidation effect based on the scavenging activity study of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical.

2. Results and discussion

Parvifoliol A (**1**) was isolated as a colorless gum whose molecular formula $C_{19}H_{26}O_5$ was deduced by HREIMS. The UV absorption bands at 223, 268, and 317 nm indicated the presence of an aromatic moiety while the IR spectrum showed hydroxyl (3433 cm^{-1}) and conjugated ester carbonyl (1662 cm^{-1}) stretching bands. The ^1H NMR spectrum (Table 1) displayed resonances for one aromatic proton (δ 6.07, s), one geranyl unit [δ 5.16 (1H, mt, J=6.9 Hz), 5.07 (1H, mt, J=6.9 Hz), 3.26 (2H, d, J=6.9 Hz), 2.00 (4H, m), 1.76 (3H, s) 1.64 (3H, s), and 1.58 (3H, s)], and two methoxyl groups (δ 4.03, s and 3.83, s). The carbonyl carbon resonance at δ 170.0 together with its HMBC correlation with the methoxy protons at δ 4.03 established the presence of a methyl ester group. The geranyl unit, the other methoxyl group (δ 3.83), and the aromatic proton were located at C-3 (δ 93.5), C-4 (δ 164.2), and C-5 (δ 91.6), respectively, on the basis of HMBC correlations from the methylene protons (H_2-7, δ 3.26) of the geranyl unit to C-2 (δ 158.6), C-3, and C-4, from the methoxy protons to C-4, and from the aromatic proton to C-1 (δ 109.0), C-3, C-4, and C-6 (δ 160.8). Signal enhancement of H-5 and H_2-7 after irradiation at 4-OMe in the NOEDIFF experiment

Keywords: *Garcinia parvifolia*; Phloroglucinols; Depsidones; Xanthones; Antibacterial activity; Antioxidant activity.
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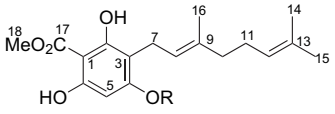
Table 1. ^{1}H and ^{13}C NMR data of compounds **1–4**

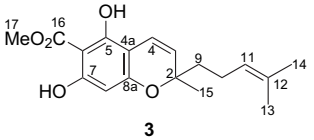
Position	1		2		3		4	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	—	109.0, s	—	93.7, s	—	—	—	—
2	—	158.6, s	5.99, s	162.3, s	—	80.2, s	—	73.7, s
3	—	93.5, s	—	106.0, s	5.41, d, 10.0	124.7, d	4.73, t, 8.7	91.0, d
4	—	164.2, s	—	162.3, s	6.65, dd, 10.0, 0.6	116.4, d	3.03, d, 8.7	26.7, t
4-OMe	3.83, s	55.6, q	—	—	—	—	—	—
4a	—	—	—	—	—	102.1, s	—	105.1, s
5	6.07, s	91.6, d	6.00, s	96.0, d	—	161.0, s	—	167.1, s
6	—	160.8, s	—	162.3, s	—	93.4, s	—	93.0, s
7	3.26, d, 6.9	21.5, t	3.35, d, 7.2	21.6, t	—	161.0, s	—	167.1, s
8	5.16, mt, 6.9	122.5, d	5.23, mt, 7.2	121.7, d	5.96, br s	96.5, d	6.00, s	90.8, d
8a	—	—	—	—	—	161.0, s	—	167.1, s
9	—	134.8, s	—	138.7, s	1.69, m	41.7, t	1.60, m	36.7, t
10	2.00, m	39.8, t	2.07, m	39.7, t	2.06, m	22.6, t	2.11, m	21.9, t
11	2.00, m	26.8, t	2.07, m	26.4, t	5.08, mt, 7.0	123.9, d	5.12, mt, 7.2	124.0, d
12	5.07, mt, 6.9	124.5, d	5.05, mt, 6.9	123.8, d	—	131.8, s	—	132.2, s
13	—	131.4, s	—	132.0, s	1.57, s	17.6, q	1.63, s	17.7, q
14	1.58, s	17.7, q	1.59, s	17.7, q	1.66, s	25.7, q	1.68, s	25.7, q
15	1.64, s	25.7, q	1.67, s	25.6, q	1.39, s	27.1, q	1.29, s	22.7, q
16	1.76, s	16.0, q	1.80, s	16.2, q	—	169.8, s	—	169.8, s
17	—	170.0, s	—	170.0, s	4.03, s	52.5, q	4.03, s	52.4, q
18-OMe	4.03, s	52.4, q	4.04, s	52.4, q	—	—	—	—

confirmed the assigned location. The carbon chemical shifts of C-2 and C-6 established the attachment of hydroxyl groups at these carbons. Consequently, the methyl ester moiety was linked at C-1. Parvifoliol A was thus determined to be **1**.

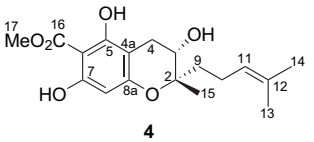


1 R = Me
2 R = H

Parvifoliol B (**2**) was isolated as a colorless gum. The molecular formula $C_{18}H_{24}O_5$ determined by HREIMS showed that it was lower than that of **1** by one methylene unit. The UV and IR bands were almost identical to those of **1**. The ^{1}H and ^{13}C NMR spectra (Table 1) were similar to those of **1** except for the disappearance of the aromatic methoxyl resonance in **2**, indicating the presence of a hydroxyl group instead of the methoxyl group at C-4 (δ 162.3) in **2**. The identical location of the geranyl side chain and the aromatic proton to that of **1** was confirmed by the following HMBC correlations: 2-OH (δ 5.99, s)/C-1 (δ 93.7), C-2 (δ 162.3), and C-3 (δ 106.0), H_2-7 (δ 3.35, d, J=7.2 Hz)/C-2, C-3, and C-4, and H-5 (δ 6.00, s)/C-1, C-3, C-4, and C-6 (δ 162.3). Consequently, parvifoliol B had the structure **2**.



3

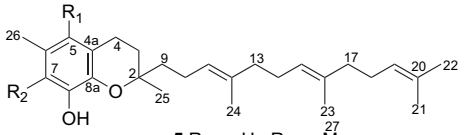


4

Parvifoliol C (**3**) was isolated as a colorless gum and the molecular formula of **3** was established as $C_{18}H_{22}O_5$ by HREIMS. The UV spectrum revealed bands at 227, 254, 262, 278, and 333 nm while the IR spectrum was similar to that of **1**. The ^{1}H NMR data (Table 1) were similar to those of **1** except for the fact that the geranyl resonances in **1** were replaced by signals of two *cis*-olefinic protons of the chromene ring [δ 6.65 (dd, J=10.0 and 0.6 Hz) and 5.41 (d, J=10.0 Hz)], one 4-methyl-3-pentenyl unit [δ 5.08 (1H, mt, J=7.0 Hz), 2.06 (2H, m), 1.69 (2H, m), 1.66 (3H, s), and 1.57 (3H, s)], and one oxyquaternary methyl group (δ 1.39, s) in **3**. ^{3}J HMBC correlations between the higher field olefinic proton (H-3, δ 5.41) with C-4a (δ 102.1) and C-9 (δ 41.7) of the 4-methyl-3-pentenyl unit and C-15 (δ 27.1) of the oxyquaternary methyl group established the fusion of the chromene ring at C-4a and C-8a (δ 161.0) with an ether linkage at C-8a and also revealed the linkage of both 4-methyl-3-pentenyl unit and Me-15 at C-2 of the chromene ring. Signal enhancement of H-4 (δ 6.65), H_2-9 (δ 1.69), and Me-15 (δ 1.39), upon irradiation of H-3 in the NOEDIFF experiment, supported the assignment. In addition, H-8 (δ 5.96, br s) showed a zig-zag coupling with H-4 in the COSY spectrum. Parvifoliol C was thus assigned to be **3**.

Parvifoliol D (**4**) was isolated as a colorless gum. The molecular formula was determined to be $C_{18}H_{24}O_6$ by HREIMS. The UV and IR absorption bands were similar to those of **1**. The ^{1}H NMR data (Table 1) revealed the replacement of the *cis*-olefinic proton resonances of the chromene unit in **3** with resonances of one oxymethine proton (δ 4.73, t, J=8.7 Hz) and methylene protons (δ 3.03, d, J=8.7 Hz) in **4**. ^{3}J HMBC correlations of the oxymethine proton/C-9 (δ 36.7) and C-15 (δ 22.7) as well as those of the methylene protons/C-2 (δ 73.7) and C-8a (δ 167.1) established the location of these protons at C-3 and C-4 of the chroman unit, respectively. The relative orientation of the methyl group at C-2 and the hydroxyl group at C-3 was assigned as trans since irradiation of H-3 enhanced the signal intensity of

Me-15 in the NOEDIFF experiment. Therefore, parvifoliol D had the structure **4**.



5 R_1 = H, R_2 = Me
6 R_1 = R_2 = H
7 R_1 = Me, R_2 = OH
28

Parvifoliol E (**5**), isolated as a colorless gum, had the molecular formula $C_{28}H_{42}O_2$ determined by HREIMS. It exhibited UV absorption bands at 208, 227, and 298 nm while a hydroxyl stretching band (3420 cm^{-1}) was the only significant absorption observed in the IR spectrum. The ^{1}H NMR spectrum (Table 2) displayed resonances of one aromatic proton (δ 6.36, br s), one hydroxyl group (δ 4.35, br s), four methylene protons of a chroman ring [δ 2.67 (2H, t, J=6.6 Hz) and 1.75 (2H, m)], one 4,8,12-trimethyltrideca-3,7,11-trienyl unit [δ 5.12 (3H, m), 2.12 (2H, m), 2.07 (6H, m), 1.96 (2H, m), 1.66 (1H, m), 1.54 (1H, m), 1.68 (3H, s), 1.61 (3H, s), 1.60 (3H, s), and 1.58 (3H, s)], two aromatic methyl groups (δ 2.13, 6H, s), and one oxyquaternary methyl group (δ 1.26, 3H, s). The presence of the 4,8,12-trimethyltrideca-3,7,11-trienyl moiety was established by COSY, HMQC, and HMBC correlations. The singlet aromatic proton at δ 6.36 was assigned as H-5, according to its ^3J HMBC correlations with C-4 (δ 22.3), C-7 (δ 125.8), C-8a (δ 145.7), and C-26 (δ 11.8). In the HMQC spectrum, C-4 and C-26 were correlated with H_2-4 (δ 2.67) of the chroman ring and Me-26 (δ 2.13), respectively. These results also indicated the attachment of Me-26 at C-6 (δ 121.7) and the fusion of the chroman ring at C-4a (δ 118.2) and C-8a. The HMBC cross peaks between the other methylene protons (H_2-3, δ 1.75) of the chroman ring and C-4a, C-9 (δ 39.8), and C-25 (δ 24.0) confirmed the fusion of the chroman ring at C-4a with an ether linkage at C-8a and further established the linkage of both the oxyquaternary methyl and 4,8,12-trimethyltrideca-3,7,11-trienyl groups at C-2 of the chroman skeleton. This assigned location was supported by signal enhancement of H_2-3 and H_2-10 after irradiation of Me-25 in NOEDIFF experiment. The remaining aromatic methyl group was assigned at C-7 (δ 125.8) on the basis of the chemical shifts of C-8 (δ 146.3) and C-8a, which indicated the presence of two adjacent oxy-substituents. Thus, parvifoliol E was determined to be **5**.

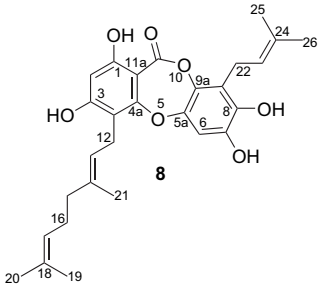
Parvifoliol F (**6**), isolated as a colorless gum, had the molecular formula $C_{27}H_{40}O_2$ assigned by HREIMS. It exhibited UV and IR absorption bands almost identical to those of **5**. The ^{1}H NMR spectrum (Table 2) was similar to that of **5** except that resonances for the singlet aromatic proton and one of the aromatic methyl groups in **5** were replaced by signals of two *meta*-aromatic protons [δ 6.47 (d, J=2.7 Hz) and 6.37 (d, J=2.7 Hz)] in **6**. The higher field aromatic proton was attributed to H-5 according to its ^3J HMBC correlations with C-4 (δ 22.5), C-7 (δ 115.7), C-8a (δ 146.0), and C-26 (δ 16.0). The other *meta*-aromatic proton was then located at C-7. Signal enhancement of both H-5 and H-7 after irradiation of Me-26 in the NOEDIFF experiment supported the position of two *meta*-aromatic protons. Accordingly, parvifoliol F was characterized as **6**.

Table 2. ^{1}H and ^{13}C NMR data of compounds **5–7**

Position	**5**		**6**		**7**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	—	75.2, s	—	75.3, s	—	75.0, s
3	1.75, m	31.4, t	1.76, m	31.4, t	1.68, m	31.3, t
4	2.67, t, 6.6	22.3,[a] t	2.69, t, 6.6	22.5, t	2.32, m	20.6, t
4a	—	118.2, s	—	121.3, s	—	117.3, s
5	6.36, br s	112.2, d	6.37, d, 2.7	112.6, d	—	115.2, s
6	—	121.7, s	—	127.4, s	—	122.2, s
7	—	125.8, s	6.47, d, 2.7	115.7, d	—	144.7, s
8	4.35, br s	146.3, s	4.45, br s	147.8, s	4.44, br s	126.9, s
8a	—	145.7, s	—	146.0, s	—	145.9, s
9	1.66, m, 1.54, m	39.8, t	1.69, m, 1.53, m	39.7, t	1.59, m	39.8, t
10	2.07, m	22.2,[a] t	2.12, m	22.2, t	2.11, m	22.2, t
11	5.12, m	124.4,[b] d	5.11, m	124.3,[a] d	5.10, m	124.2, d
12	—	135.1,[c] s	—	135.1,[b] s	—	135.2, s
13	2.12, m	39.8, t	1.97, m	39.7, t	2.00, m	39.7, t
14	2.07, m	26.6,[d] t	2.07, m	26.6, t	2.07, m	26.8,[a] t
15	5.12, m	124.4,[b] d	5.11, m	124.4,[a] d	5.14, m	124.2, d
16	—	135.0,[c] s	—	135.0,[b] s	—	135.0, s
17	1.96, m	39.7, t	1.97, m	39.7, t	2.00, m	39.7, t
18	2.07, m	26.8,[d] t	2.01, m	26.8, t	2.07, m	26.7,[a] t
19	5.12, m	124.2,[b] d	5.11, m	124.3,[a] d	5.10, m	124.4, d
20	—	131.3, s	—	131.3, s	—	131.2, s
21	1.61, s	17.7, q	1.60, s	17.7, q	1.60, br s	17.7, q
22	1.68, s	25.7, q	1.68, s	25.7, q	1.68, s	25.7, q
23	1.58, s	16.0, q	1.59, s	15.9, q	1.59, s	15.9,[b] q
24	1.60, s	15.9, q	1.58, s	16.0, q	1.59, s	16.0,[b] q
25	1.26, s	24.0, q	1.26, s	24.3, q	1.26, s	23.8, q
26	2.13, s	11.8,[e] q	2.12, s	16.0, q	2.20,[a] s	12.3, q
27	2.13, s	11.9,[c] q	—	—	—	—
28	—	—	—	—	2.19,[a] s	12.0, q

[a,b,c,d,e] Chemical shifts with the same index in the same column may be interchanged.

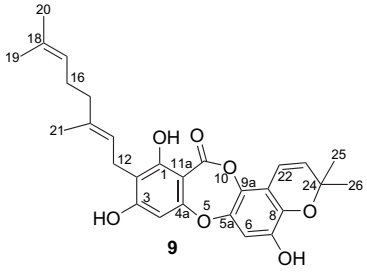
Parvifoliol G (**7**) was isolated as a colorless gum. The HREIMS showed the molecular formula $C_{28}H_{42}O_3$, which was higher than that of **5** by one oxygen atom. Its UV and IR absorption bands were almost identical to those of **5**. The ^{1}H NMR data (Table 2) were similar to those of **5** except for the disappearance of the aromatic proton in **7**, suggesting the replacement of the aromatic proton with a hydroxyl group. The HMBC correlations from H_2-4 (δ 2.32, m) to C-4a (δ 117.3), C-5 (δ 115.2), and C-8a (δ 145.9), and from Me-28 (δ 2.19 or 2.20) to C-4a, C-5, and C-6 (δ 122.2) revealed the linkage of Me-28 at C-5. The other aromatic methyl group (Me-26, δ 2.20 or 2.19) was attached at C-6 on the basis of HMBC correlations of Me-26/C-5, C-6, and C-7 (δ 144.7). The oxygenated C-8 resonated at much higher field (δ 126.9) due to the shielding effect of two *ortho* oxy-substituents at C-7 and C-8a. The structure of parvifoliol G was thus assigned to be **7**.



8

The molecular formula of parvifolidone A (**8**), isolated as a yellow gum, was established as $C_{28}H_{32}O_7$ by HREIMS. It exhibited UV and IR absorption bands similar to those of garcidepsidone B.[4] The ^{1}H, ^{13}C NMR (Table 3), and HMBC data were similar to those of garcidepsidone B except for the differences in the HMBC correlations observed in left hand ring. ^3J HMBC correlations of the aromatic proton (δ 6.29, s)/C-4 (δ 111.2) and C-11a (δ 99.3) and those of the methylene protons (δ 3.57, d, J=6.9 Hz) of the geranyl unit/C-3 (δ 162.6) and C-4a (δ 158.7) established the attachment of the aromatic proton and the geranyl side chain at C-2 (δ 100.9) and C-4, respectively. Signal enhancement of H_2-12 and Me-21 (δ 1.86, s) in the NOEDIFF experiment after irradiation of H-6 (δ 6.69, s) confirmed the close proximity of H-6 and the geranyl group. Therefore, parvifolidone A was determined to be **8**.

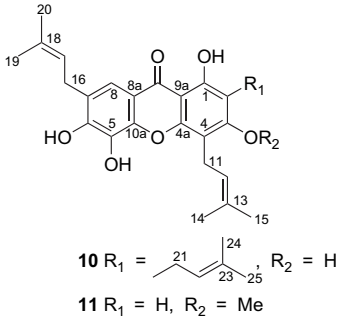
Parvifolidone B (**9**) was isolated as a yellow gum whose molecular formula was determined as $C_{28}H_{30}O_7$ by HREIMS. The UV and IR absorption bands indicated the presence of the depsidone chromophore. The ^{1}H NMR data (Table 3) were similar to those of garcidepsidone B.[4] The differences were proton resonances in the right hand ring. The prenyl resonances in garcidepsidone B were replaced by characteristic signals of the dimethylchromene ring [δ 6.76 (1H, d, J=10.2 Hz), 5.75 (1H, d, J=10.2 Hz), and 1.46 (6H, s)] in **9**. The HMBC correlations from the *cis*-olefinic proton at δ 6.76 (H-22) to C-8 (δ 136.5) and C-9a (δ 132.6) and from the other *cis*-olefinic proton (H-23) to C-9 (δ 113.8) established the fusion of the dimethylchromene ring at C-8 and C-9 with an ether linkage at C-8. Consequently, parvifolidone B had the depsidone structure **9**.



9

Table 3. ^{1}H and ^{13}C NMR data of compounds **8** and **9**

Position	**8**		**9**	
	δ_H	δ_C	δ_H	δ_C
1	10.72, s	163.2, s	11.36, s	162.4, s
2	6.29, s	100.9, d	—	110.8, s
3	6.23, br s	162.6, s	6.26, br s	162.4, s
4	—	111.2, s	6.29, s	100.5, d
4a	—	158.7, s	—	160.1, s
5a	—	143.4, s	—	142.2, s
6	6.69, s	105.4, d	6.69, s	106.4, d
7	5.53, br s	142.0, s	5.39, br s	143.1, s
8	5.59, br s	139.8, s	—	136.5, s
9	—	120.0, s	—	113.8, s
9a	—	135.9, s	—	132.6, s
11	—	168.3, s	—	168.5, s
11a	—	99.3, s	—	98.5, s
12	3.57, d, 6.9	22.4, t	3.41, d, 7.2	22.0, t
13	5.20, mt, 6.9	121.3, d	5.23, mt, 7.2	120.7, d
14	—	139.4, s	—	140.3, s
15	2.13, m	39.7, t	2.08, m	39.7, t
16	2.13, m	26.3, t	2.08, m	26.3, t
17	5.05, mt, 6.9	123.6, d	5.04, mt, 6.6	123.6, d
18	—	132.2, s	—	132.3, s
19	1.60, s	17.7, q	1.59, s	17.7, q
20	1.67, s	25.7, q	1.68, s	25.7, q
21	1.86, s	16.4, q	1.80, s	16.2, q
22	3.57, d, 6.9	23.9, t	6.76, d, 10.2	116.2, d
23	5.25, mt, 6.9	120.2, d	5.75, d, 10.2	132.1, d
24	—	136.7, s	—	77.2, s
25	1.83, s	18.0, q	1.46, s	27.7, q
26	1.76, s	25.8, q	1.46, s	27.7, q



10 R_1 = , R_2 = H

11 R_1 = H, R_2 = Me

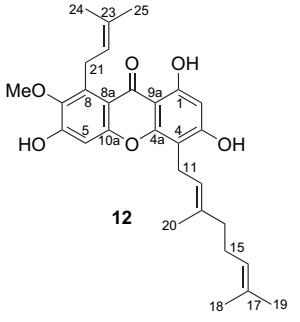
Parvifolixanthone A (**10**), isolated as a yellow gum, had the molecular formula $C_{28}H_{32}O_6$ determined by HREIMS. It exhibited UV absorption bands of a xanthone chromophore at 256, 286, and 329 nm while hydroxyl and conjugated carbonyl absorption bands were observed at 3346 and 1641 cm^{-1}, respectively, in the IR spectrum. The ^{1}H NMR spectrum (Table 4) displayed signals of one chelated hydroxyl group (δ 13.32, s), one aromatic proton (δ 7.58, s), three prenyl units: unit 1 [δ 5.28 (1H, mt, J=7.2 Hz),

Table 4. ^{1}H and ^{13}C NMR data of compounds **10–12**

Position	**10**		**11**		**12**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	13.32, s	158.6, s	13.12, s	162.2, s	13.35, s	161.7, s
2	—	108.9, s	6.37, s	94.4, d	6.25, s	98.5, d
3	6.43, s	160.3, s	—	163.6, s	5.97, br s	138.8, s
3-OMe	—	—	3.91, s	56.1, q	—	—
4	—	105.2, s	—	107.3, s	—	104.1, s
4a	—	152.6, s	—	153.8, s	—	154.0, s
5	5.67, br s	130.1, s	5.61, s	130.1, s	6.88, s	101.6, d
6	6.15, br s	147.7, s	6.12, s	147.8, s	6.33, br s	155.8, s
7	—	125.3, s	—	125.4, s	—	142.7, s
7-OMe	—	—	—	—	3.82, s	62.1, q
8	7.58, s	117.1, d	7.60, s	117.1, d	—	137.1, s
8a	—	113.4, s	—	113.3, s	—	112.2, s
9	—	180.7, s	—	180.7, s	—	182.4, s
9a	—	102.9, s	—	103.0, s	—	104.1, s
10a	—	143.7, s	—	143.8, s	—	154.6, s
11	3.52, d, 6.6	22.0, t	3.49, d, 5.7	21.7, t	3.53, d, 6.6	21.6, t
12	5.25, mt, 6.6	122.3, d	5.22, br s	122.9, d	5.28, mt, 6.6	121.3, d
13	—	133.5, s	—	131.7, s	—	138.8, s
14	1.85, s	18.0, q	1.85, s	17.9, q	2.09, m	39.7, t
15	1.73, s	25.7, q	1.71, s	25.6, q	2.09, m	26.4, t
16	3.41, d, 7.5	28.5, t	3.42, d, 7.5	28.4, t	5.03, mt, 6.6	123.7, d
17	5.34, mt, 7.5	121.1, d	5.35, mt, 7.5	121.1, d	—	124.3, s
18	—	134.4, s	—	134.3, s	1.60, d, 0.9	18.0, q
19	1.75, s	17.9, q	1.76, s	17.9, q	1.66, d, 0.9	25.7, q
20	1.77, s	25.9, q	1.77, s	25.8, q	1.89, d, 0.9	18.2, q
21	3.46, d, 7.2	21.6, t	—	—	4.10, d, 6.0	26.6, t
22	5.28, mt, 7.2	121.4, d	—	—	5.27, mt, 6.0	123.1, d
23	—	135.8, s	—	—	—	135.7, s
24	1.85, s	18.0, q	—	—	1.83, d, 1.2	18.2, q
25	1.78, d, 1.2	25.8, q	—	—	1.70, d, 1.2	25.8, q

3.46 (2H, d, J=7.2 Hz), 1.85 (3H, s), and 1.78 (3H, d, J=1.2 Hz)], unit 2 [δ 5.25 (1H, mt, J=6.6 Hz), 3.52 (2H, d, J=6.6 Hz, 2H), 1.85 (3H, s), and 1.73 (3H, s)], and unit 3 [δ 5.34 (1H, mt, J=7.5 Hz), 3.41 (2H, d, J=7.5 Hz), 1.77 (3H, s), and 1.75 (3H, s)], and three hydroxyl groups [δ 6.43 (s), 6.15 (br s), and 5.67 (br s)]. The ^{13}C NMR, DEPT, and HMQC data indicated that **10** consisted of 15 quaternary, four methine, three methylene, and six methyl carbons. The location of all substituents was established by HMBC data as follows. The chelated hydroxyl group was placed at C-1 (δ 158.6), a *peri*-position of the xanthone carbonyl group, and gave cross peaks with C-1, C-2 (δ 108.9), and C-9a (δ 102.9). ^{3}J HMBC correlations between the methylene protons (H_2-21, δ 3.46) of the prenyl unit 1 and C-1 and C-3 (δ 160.3) and those between the methylene protons (H_2-11, δ 3.52) of the prenyl unit 2 and C-3 and C-4a (δ 152.6) established the attachment of the prenyl units 1 and 2 at C-2 and C-4 (δ 105.2), respectively. The singlet aromatic proton at δ 7.58 was attributed to H-8 on the basis of the proton chemical shift and ^{3}J HMBC correlations of H-8/C-6 (δ 147.7), C-9 (δ 180.7), and C-10a (δ 143.7). The prenyl unit 3 was linked at C-7 due to HMBC correlations between the methylene protons (H_2-16, δ 3.41) with C-6, C-7 (δ 125.3), and C-8 (δ 117.1). Furthermore, HMBC correlations from the hydroxy proton at δ 6.43 to C-2, C-3, and C-4 and from the hydroxy proton at δ 6.15 to C-5, C-6, and C-7 established the linkage of these hydroxyl groups at C-3 and C-6, respectively. Thus, the remaining hydroxy proton at δ 5.67 belonged to the C-5 hydroxyl group. Signal enhancement of H-12 and H_2-21 in the NOEDIFF experiment upon irradiation of 3-OH and that of H_2-16 after irradiation of H-8 supported the assigned location of all prenyl substituents. Therefore, parvifolixanthone A was characterized as **10**.

The molecular formula of parvifolixanthone B (**11**), isolated as a yellow gum, was deduced as $C_{24}H_{26}O_6$ by HREIMS. The UV and IR absorption bands similar to those of **10** indicated that **11** had a xanthone chromophore. The ^{1}H NMR spectrum (Table 4) was similar to that of **10** except that one of the prenyl resonances in **10** was replaced by a singlet signal of an aromatic proton at δ 6.37 in **11**. This proton was attributed to H-2 due to HMBC correlations of H-2/C-1 (δ 162.2), C-3 (δ 163.6), C-4 (δ 107.3), and C-9a (δ 103.0). An additional methoxyl group resonating at δ 3.91 in the ^{1}H NMR spectrum of **11** was located at C-3 according to its HMBC correlation with C-3. Signal enhancement observed between H-2 and the methoxy protons in the NOEDIFF experiment confirmed the assignment. Parvifolixanthone B was thus determined to be **11**.



Parvifolixanthone C (**12**), isolated as a yellow gum, had the molecular formula $C_{29}H_{34}O_6$ determined by HREIMS. The

UV and IR absorption revealed the presence of a xanthone chromophore. The ^{1}H NMR data (Table 4) were similar to those of dulxanthone D[7] except that one of the aromatic protons on the right hand ring in dulxanthone D was replaced by the geranyl signal in **12**. The geranyl unit was located at C-4 (δ 104.1) on the basis of HMBC correlations between methylene protons H_2-11 (δ 3.53, d, J=6.6 Hz) with C-3 (δ 138.8), C-4, and C-4a (δ 154.0). Accordingly, parvifolixanthone C had the tetraoxygenated xanthone structure **12**.

All isolated compounds except for **1**, **4**, **9**, 1,3,5,6-tetrahydroxyxanthone, and (2*E*,6*E*,10*E*)-(+)-4β-hydroxy-3-methyl-5β-(3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl)cyclohex-2-en-1-one for which insufficient material was available were tested for antibacterial activity against MRSA. Rubraxanthone, previously reported as a strong antibacterial substance against both normal and penicillin-resistant strains,[3] showed the best antibacterial activity with a minimum inhibitory concentration (MIC) value of 19.5 μM while parvifoliol B (**2**) displayed much less activity with a MIC value of 100 μM. However, both exhibited much weaker antibacterial activity than the standard vancomycin (a MIC value of 0.6 μM). The remaining compounds were inactive against MRSA. In addition, the isolated compounds were examined for antioxidant effect with DPPH assay as xanthones and phloroglucinols have been reported as radical scavengers.[11] Parvifoliol E (**5**) showed high radical scavenging potency with a 50% inhibitory concentration (IC_{50}) value of 0.02 μM, which was much lower than that of the reference 2,6-di-*tert*-butyl-4-hydroxyanisole (BHT) having an IC_{50} value of 0.13 μM. The less active compounds were norathyriol and parvifoliol F (**6**) of which IC_{50} values were 0.08 and 0.10 μM, respectively. Garcidepsidone B gave an equal IC_{50} value to BHT while **7**, **8**, **10**, **11**, and **13** exhibited weaker antioxidant activity than BHT with IC_{50} values in the range of 0.18–0.38 μM. Other compounds gave no activity.

The genus *Garcinia* is known to be rich in a variety of compounds, for example, polyprenylated xanthones[5] and benzophenones.[12] However, only twelve depsidones,[4,13–16] three phloroglucinols of tocotrienol type,[11,17] and one methyl ester of a benzopyran derivative[18] have been isolated from *Garcinia* plants. We now add new members to the lists of compounds of these types. This is also the first report on the isolation of methyl esters of phloroglucinols from the genus *Garcinia*.

3. Experimental

3.1. General experimental procedures

Infrared spectra (IR) were determined on a Perkin–Elmer 783 FTS165 FTIR spectrometer. Ultraviolet (UV) absorption spectra were determined by using MeOH on a Shimadzu UV-160A spectrophotometer. Optical rotations were measured on a Jasco P-1020 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a 300 MHz Bruker FTNMR Ultra Shield™ spectrometer. Mass spectra were obtained on a MAT 95 XL mass spectrometer (Thermofinnigan). Thin-layer chromatography (TLC) and precoated TLC were performed on silica gel GF_{254} (Merck). Column chromatography (CC) was performed on silica gel (Merck) type 100 (70–230 Mesh ASTM) eluted either with gradient system A (CH_2Cl_2–MeOH) or B (light petroleum–EtOAc) or on Sephadex LH-20 eluted with MeOH or on reverse-phase silica gel C-18 eluted with a gradient of MeOH–H_2O, unless otherwise stated. Light petroleum had bp 40–60 °C.

3.2. Plant material

The twigs of *G. parvifolia* were collected at Trang Province, Thailand. A voucher specimen is deposited in the Herbarium of the Department of Biology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

3.3. Extraction and isolation

The dried and chopped twigs of *G. parvifolia* (2.4 kg) were extracted with MeOH (7 L) for 5 days at room temperature three times. Filtration and subsequent evaporation of the combined MeOH extracts to dryness in vacuo afforded a dark brown gum (102.5 g), which was subjected to silica gel CC with a gradient of $CHCl_3$–hexane followed by a gradient of $CHCl_3$–MeOH to give 11 fractions (A–K). Fraction B (299.6 mg) was further purified by silica gel CC with a gradient of $CHCl_3$–light petroleum to afford **1** (3.6 mg) and **3** (9.3 mg). Fraction C (283.9 mg) was then separated by silica gel CC with gradient system B to give **5** (18.3 mg) and **7** (21.9 mg). Fraction E (1.06 g), upon purification with Sephadex LH-20 CC, yielded three subfractions (E1–E3). Subfraction E3 was further purified by repeated Sephadex LH-20 CC to give **2** (12.9 mg) and **4** (3.5 mg). Fraction G (3.20 g) was subjected to silica gel CC using gradient system A to yield seven subfractions (G1–G7). Subfraction G2 (203.0 mg) was purified by Sephadex LH-20 CC to give **6** (44.3 mg), **10** (17.9 mg) and **11** (9.2 mg). Subfractions G3 (182.8 mg), G5 (238.3 mg), and G6 (148.2 mg) were subjected to Sephadex LH-20 CC to yield **12** (4.8 mg), and (2*E*,6*E*,10*E*)-(+)-4β-hydroxy-3-methyl-5β-(3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraenyl)cyclohex-2-en-1-one (3.6 mg) from subfractions G3, G5, and G6, respectively. Fraction I (3.05 g) was purified by silica gel CC with gradient system A to give four subfractions (I1–I4). Subfraction I2 contained **9** (3.2 mg). Subfraction I3 (206.3 mg) was separated by silica gel CC using gradient system A to afford **8** (34.5 mg). Subfraction I4 (523.8 mg) was subjected to silica gel CC with acetone–light petroleum (1:9) to yield mangostinone (6.6 mg), rubraxanthone (11.3 mg), dulxanthone D (5.3 mg), and garcidepsidone B (9.2 mg). Fraction J (1.64 g) was purified by reverse-phase silica gel CC to afford three subfractions (J1–J3). Subfraction J2 (132.5 mg) was further separated by Sephadex LH-20 CC to afford 1,3,5,6-tetrahydroxyxanthone (2.1 mg) and norathyriol (5.3 mg).

3.3.1. Parvifoliol A (1). Colorless gum; UV (MeOH) λ_{max} (log ε) 223 (4.36), 268 (4.15), 317 (3.40) nm; IR (neat) ν_{max} 3433, 1662 cm^{-1}; HREIMS *m*/*z* [M]$^+$ 334.1788 (calcd for $C_{19}H_{26}O_5$, 334.1780); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 1.

3.3.2. Parvifoliol B (2). Colorless gum; UV (MeOH) λ_{max} (log ε) 224 (4.38), 271 (4.20), 315 (3.42) nm; IR (neat) ν_{max} 3433, 1660 cm^{-1}; HREIMS *m*/*z* [M]$^+$ 320.1625 (calcd for $C_{18}H_{24}O_5$, 320.1624); ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 1.

3.3.3. Parvifoliol C (3). Colorless gum; $[\alpha]_D^{29}$ −36.4 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 227 (3.17), 254 (4.39), 262 (4.49), 278 (3.25), 333 (2.82) nm; IR (neat) ν_{max} 3431, 1668 cm^{-1}; HREIMS *m/z* $[M]^+$ 318.1474 (calcd for $C_{18}H_{22}O_5$, 318.1462); ^1H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 1.

3.3.4. Parvifoliol D (4). Colorless gum; $[\alpha]_D^{29}$ −21.2 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 232 (4.16), 272 (4.37), 311 (2.32) nm; IR (neat) ν_{max} 3430, 1662 cm^{-1}; HREIMS *m/z* $[M]^+$ 336.1564 (calcd for $C_{18}H_{24}O_6$, 336.1573); ^1H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 1.

3.3.5. Parvifoliol E (5). Colorless gum; $[\alpha]_D^{29}$ −3.2 (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.23), 227 (3.14), 298 (3.09) nm; IR (neat) ν_{max} 3420 cm^{-1}; HREIMS *m/z* $[M]^+$ 410.3184 (calcd for $C_{28}H_{42}O_2$, 410.3185); ^1H NMR ($CDCl_3$, 300 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 2.

3.3.6. Parvifoliol F (6). Colorless gum; $[\alpha]_D^{29}$ +26.2 (*c* 0.31, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.17), 223 (3.21), 297 (2.41) nm; IR (neat) ν_{max} 3387 cm^{-1}; HREIMS *m/z* $[M]^+$ 396.3015 (calcd for $C_{27}H_{40}O_2$, 396.3028); ^1H NMR ($CDCl_3$, 300 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 2.

3.3.7. Parvifoliol G (7). Colorless gum; $[\alpha]_D^{29}$ +53.0 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 207 (4.24), 226 (3.11), 301 (3.10) nm; IR (neat) ν_{max} 3431 cm^{-1}; HREIMS *m/z* $[M]^+$ 426.3116 (calcd for $C_{28}H_{42}O_3$, 426.3134); ^1H NMR ($CDCl_3$, 300 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 2.

3.3.8. Parvifolidone A (8). Yellow gum; UV (MeOH) λ_{max} (log ε) 223 (3.59), 276 (3.43), 319 (2.82) nm; IR (neat) ν_{max} 3373, 1656 cm^{-1}; HREIMS *m/z* $[M]^+$ 480.2110 (calcd for $C_{28}H_{32}O_7$, 480.2143); ^1H NMR ($CDCl_3$, 300 MHz), see Table 3; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 3.

3.3.9. Parvifolidone B (9). Yellow gum; UV (MeOH) λ_{max} (log ε) 221 (4.09), 272 (3.16), 281 (3.15), 327 (2.59) nm; IR (neat) ν_{max} 3363, 1657 cm^{-1}; HREIMS *m/z* $[M]^+$ 478.1993 (calcd for $C_{28}H_{30}O_7$, 478.1986); ^1H NMR ($CDCl_3$, 300 MHz), see Table 3; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 3.

3.3.10. Parvifolixanthone A (10). Yellow gum; UV (MeOH) λ_{max} (log ε) 256 (4.46), 286 (3.68), 329 (3.60) nm; IR (neat) ν_{max} 3346, 1641 cm^{-1}; HREIMS *m/z* $[M]^+$ 464.2197 (calcd for $C_{28}H_{32}O_6$, 464.2199); ^1H NMR ($CDCl_3$, 300 MHz), see Table 4; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 4.

3.3.11. Parvifolixanthone B (11). Yellow gum; UV (MeOH) λ_{max} (log ε) 236 (4.19), 254 (4.48), 285 (3.65), 328 (3.60) nm; IR (neat) ν_{max} 3410, 1644 cm^{-1}; HREIMS *m/z* $[M]^+$ 410.1751 (calcd for $C_{24}H_{26}O_6$, 410.1729); ^1H NMR ($CDCl_3$, 300 MHz), see Table 4; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 4.

3.3.12. Parvifolixanthone C (12). Yellow gum; UV (MeOH) λ_{max} (log ε) 241 (4.01), 257 (3.82), 317 (3.15), 359 (2.59) nm; IR (neat) ν_{max} 3394, 1646 cm^{-1}; HREIMS *m/z* $[M]^+$ 478.2371 (calcd for $C_{29}H_{34}O_6$, 478.2350); ^1H NMR ($CDCl_3$, 300 MHz), see Table 4; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 4.

3.4. Antibacterial activity testing

MICs were determined by the agar microdilution method.[19] The test substances were dissolved in DMSO (Merck, Germany). Serial two-fold dilutions of the test substances were mixed with melted Mueller–Hinton agar (Difco) in the ratio of 1:100 in microtiter plates with flat-bottomed wells (Nunc, Germany). Final concentration of the test substances in agar ranged from 200 to 0.39 μg/mL. MRSA isolated from a clinical specimen, Songklanakarin Hospital, was used as test strain. Inoculum suspensions (10 μL) were spotted on agar-filled wells. The inoculated plates were incubated at 35 °C for 18 h. MICs were recorded by reading the lowest substance concentration that inhibited visible growth. Growth controls were performed on agar containing DMSO.

3.5. Free radical scavenging activity

This was carried out according to that of Yen and Hsieh.[20] To different concentrations of a sample in methanol (0.5 mL each) was added 1 mL of a methanolic solution of 0.2 mM DPPH. After mixing thoroughly, the mixture was allowed to stand in the dark for 30 min and the absorbance at 523 nm was measured using methanol for the baseline correction. The results were then compared with that of the control prepared as above but without any sample. Radical scavenging activity was expressed as percentage and was calculated using the following formula: %Scavenging= $[(A_{control}-A_{sample})/A_{control}]\times 100$. For each sample, the result was presented as IC_{50} (sample concentration that produced 50% scavenging of DDPH radical).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.06.059.

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Bis-4*H*-imidazoles–tetraazafulvalenes–2,2′-biimidazoles: three variations of one redox system

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Abstract—The reversibility of the two-electron reduction of tetraazafulvalenes **1** could be confirmed by employing cyclovoltammetric measurements. However, attempts to oxidize these systems electrochemically, as well as by oxidizing agents, failed. In contrast, the bis-vinylogous derivatives **2** proved to be multi-step redox systems showing two-reversible reduction as well as oxidation waves. The chemically initiated oxidation of **2** yielded the bis-4*H*-imidazoles **6**. In the presence of an excess of the oxidizing agent they dimerized and formed the deep blue colored derivative **7a**. Treatment of the phenylogous systems **8** with sodium dithionite provided a new entry to quinomethides of type **9**, which can be stabilized towards oxygen by cyclization reaction to give the pigment-like bis-urea **11**. Derivative **9** represents the SEM form of this four-step redox system and thus can finally be reduced to yield the tetraaminosubstituted biimidazoles **12**. Based on these findings, the close correlation among bis-4*H*-imidazoles (OX), tetraazafulvalenes (SEM), and tetraaminosubstituted biimidazoles (RED) could be demonstrated. Due to the fact that tetraazafulvalenes constitute stable closed-shell SEM systems, their intense UV–vis absorptions can now be explained and related to their redox behavior.

1. Introduction

Electrochromic behavior is mainly based on two-step redox systems for which Hünig and others have developed a new principle.[1,2] In the field of advanced materials, the so-called violenes,[3] which are the radical ions $SEM^{+/-}$, always represent the species with the longest wavelength absorptions and highest extinction coefficients within the redox system. Their color intensity is closely related to the thermodynamic stability for which the semiquinone formation constant (K_{SEM}) is the most commonly applied value. Recently, the synthesis and electrochemical characterization of boratetraazapentalenes derived from 4*H*-imidazoles **1** was reported.[4] The radical anions of this new class of electrophores show unusual high values of K_{SEM} (up to 10^{15}). Since radical reactions often have low activation barriers, these open-shell species tend to decompose more rapidly than closed-shell systems. We therefore focused our work on the development of redox systems in which the radical SEM state is replaced by closed-shell moieties.

Tetraazafulvalenes **2** and their bis-vinylogous derivative **3** represent a novel class of cross-conjugated heterocyclic compounds for which different syntheses have been developed in our laboratories. Due to the secondary arylamino groups, further derivatization can be realized by a large variety of alkylation/acylation, cyclization, and cross-coupling procedures.[5–8] Their long wavelength absorptions in the UV–vis spectra together with high extinction coefficients ($\log \varepsilon > 4$) make them promising candidates for the construction of functional dyes.[9]

In principle, the electron-poor heterocyclic part of **2** enables them to be the electronic counterpart of tetrathiafulvalenes and in addition, offers the ability for the synthesis of charge-transfer complexes with such donor molecules.[10] On the other hand, tetraazafulvalenes can easily be reduced to yield 4,4′,5,5′-tetraaminosubstituted 2,2′-biimidazoles **6**. These electron-rich heterocycles can also be regarded as leuco-forms of **2** and immediately reoxidize in the presence of air oxygen to its starting material (Scheme 1). The greenish fluorescent derivatives **6** can be stabilized under strictly anaerobic conditions by acylation reactions with Boc_2O or $CF_3CH_2SO_2Cl$.[11]

2. Results and discussion

Due to its inherent merocyanine type system, **3** is supposed to behave as a multi-step redox system according to Scheme 1. We could recently[11] demonstrate that tetraazafulvalenes **3** behave as electrophores, which can easily be switched between oxidized and reduced form. In the first step, most probably the radical anion would be generated. The second

Keywords: bis-4*H*-Imidazoles; Tetraazafulvalenes; Biimidazoles; Four-electron redox system; Quinomethides.
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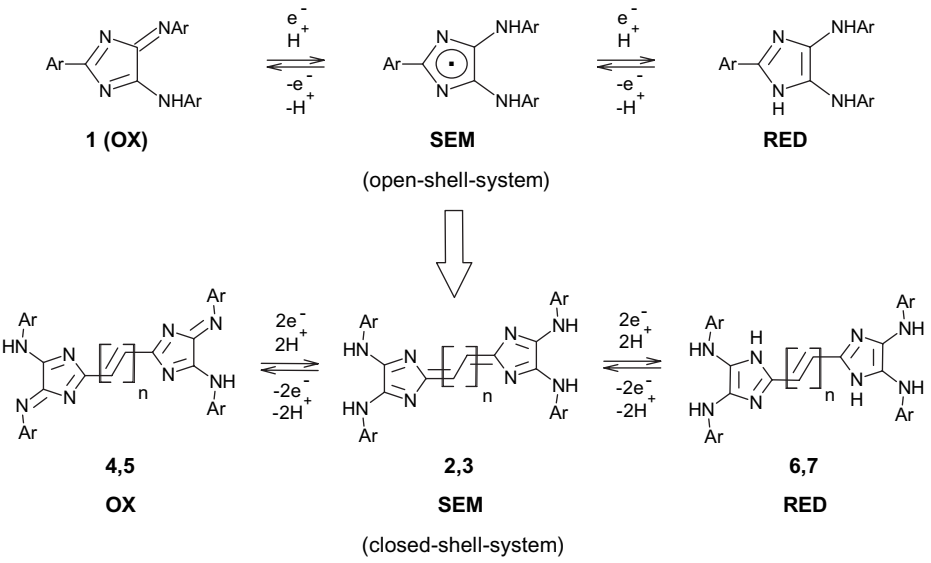


Scheme 1. Compounds **2**, **4**, and **6**: n=0; **3**, **5**, and **7**: n=1.

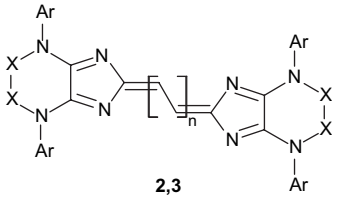
electron transfer step leads to the formation of the dianion, which is immediately protonated by water to yield the 2,2′-biimidazoles of type **7**. Now, both consecutive single-electron transfer processes could be recorded by electrochemical measurements. Employing difference pulse polarographic measurements, two peaks can be clearly ascribed to two different single-electron transfer steps. The quasi-reversibility of the reduction was confirmed by cyclovoltammetric measurements ($\Delta E_{RED,OX}$[1,2]>0.059 V). The redox potentials and semiquinone formation constants K_{SEM} of selected tetraazafulvalenes are listed in Table 1. Compared to other acceptor molecules such as tetracyanoquinodimethane (TCNQ) or *N*,*N*′-dicyanoquinodiimines (DCNQI)[12] with K_{SEM} values in the range of 10^7–10^{11}, the tetraazafulvalenes show rather small parameters (10^2–10^{11}).

The data also demonstrates the small influence of the substituted aryl groups, the bonding mode of the two imidazoles (n=0,1), as well as the nature of an additional ring fusion on the reduction potential.

The oxidation reaction of **2** provides the possibility of obtaining derivatives of type **4**, in which two 4*H*-imidazoles are directly connected via a single bond. The cyclovoltammogram of **2b** (Ar=4-Tol) showed two-irreversible oxidation waves at positive potentials. These findings were in agreement with the results obtained by using a series of oxidation reagents. All attempts to chemically oxidize **2** yielded polymeric material and only small amounts of isocyanides and derivatives of parabanic acid. The latter resulted from a direct oxidative cleavage of the central double bond of **2**. The independent synthesis of **4** starting from oxalyl chloride and oxalic amidines[13] was unsuccessful and proved to be an additional evidence for the instability of the bis-4*H*-imidazoles **4**.

In further experiments we therefore studied the redox behavior of the easily accessible tetraazafulvadienes **3**. Such vinylogous derivatives should show a similar redox chemistry as their parent compound **2**. The cyclic voltammograms, as well as the difference pulse polarographic measurements

Table 1. Reduction potentials E^1_{RED}, E^2_{RED}, and K_{SEM} of **2** and **3**

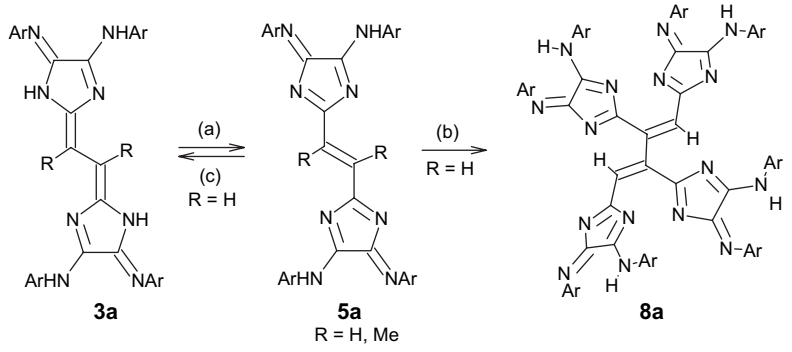


Compound	n	Ar	X–X	E^1_{RED} (V)	E^2_{RED} (V)	K_{SEM}
2a	0	4-*t*-C_4H_9–C_6H_4	H, H	−0.669	−0.786	9.6E+001
2b	0	4-CH_3–C_6H_4	H, H	−0.775	−1.050	1.5E+005
2c	0	4-I–C_6H_4	H, H	−0.648	−0.781	1.8E+002
2d	0	4-Br–C_6H_4	H, H	−0.762	−0.961	2.4E+003
2e	0	4-Cl–C_6H_4	H, H	−0.861	−1.133	4.1E+004
2f	0	4-*t*-C_4H_9–C_6H_4	–CH=CH–	−0.570	−1.251	3.5E+011
2g	0	3-CF_3–C_6H_4	–C=C– (CN CN)	−1.375	−1.625	1.7E+004
2h	0	4-*t*-C_4H_9–C_6H_4	>CH(OEt)	−0.762	−1.050	7.6E+004
3a	1	4-*t*-C_4H_9–C_6H_4	H, H	−0.654	−1.043	3.9E+006
3b	1	4-Br–C_6H_4	H, H	−0.693	−0.825	1.7E+002

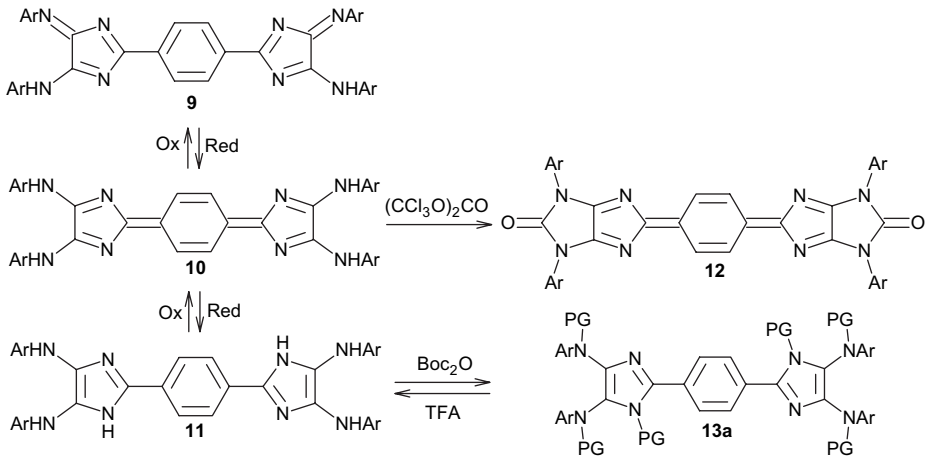
of **3a** and **3b**, reveal two completely reversible reduction waves, which correspond to two single-electron transfer steps. This reduction of **3** yielded their leuco-forms **7**, which could also be obtained by ultrasound irradiation of **3** in THF in the presence of small amounts of aqueous sodium dithionite. Employing metallic lithium, this chemically induced reduction of derivatives **3** was already used for the preparation of stilbenoid biimidazoles.[5] However, in derivatives **3**, two-reversible oxidation waves (**3a**: E^1_{OX}=0.566 V, E^2_{OX}=0.879 V; **3b**: E^1_{OX}=0.664 V, E^2_{OX}=0.908 V) promise an entry to stable oxidation products of type **5** (Scheme 1), which are not accessible via tetraazafulvalenes **2**.

The chemical initiated oxidation of **3** has also been succeeded by using several oxidizing agents (DDQ, Cu^{2+}/pyridine, iodosobenzene bis-trifluoroacetate, 1,4-benzoquinone, cerium(IV) ammonium nitrate). In all cases studied the oxidation products were purified by column chromatography and identified as bis-4*H*-imidazoles **5**. These deep red compounds are stable up to 50 °C; at higher temperatures and in the presence of an excess of oxidizing agent they were transformed into deep blue compounds. By means of MS and NMR spectroscopy, derivative **8a** (Ar=4-*t*-BuPh) was characterized as a dimerization product derived from the corresponding bis-4*H*-imidazole **5a**. This somewhat surprising dimerization probably took place via cationic radical intermediates[14] formed by oxidation of the exocyclic methine groups of **5** (R=H). Derivatives in which the methine hydrogens are replaced by methyl groups (R=CH_3)[14] did not show such oxidative dimerization reactions (Scheme 2).

We have now expanded our research to consider the redox chemistry of bifunctional, 'phenylogous' 4*H*-imidazoles in order to combine the experimental findings of 4*H*-imidazoles[4] with those of tetraazafulvalenes. These molecules can easily be obtained via two different pathways[13,15] and are stable crystalline compounds. Due to their low solubilities in solvents used for CV measurements, only chemically induced redox reactions were studied. Thus, upon treatment of bis-4*H*-imidazoles **9** with sodium dithionite, the color of the solution turned from red to blue. Purification of the reaction mixture by flash chromatography under argon yielded deep blue stable substances, but upon exposure to air a fast reoxidation to the starting material was observed. The spectral data (NMR and MS) of **10** was in agreement with the structural assignment presented in Scheme 3. The UV–vis spectrum of **10** exhibited a strong absorption at long wavelengths (λ_{max} (log ε): **10a**: 634 (4.4); **10b**: 633 (4.2)), which is characteristic for such quinoid compounds.[16–19] Since no radical species could be detected by ESR, the quinoid structure of **10** could be achieved by electron coupling of both parts of the peripheric reduced heterocycles. Derivative **10** constitutes the first tetraaminosubstituted quinomethide and can be regarded as a hybrid between tetraazafulvalenes and quinodimethides. The cyclization of **10** with triphosgene formed the deep purple bis-urea **12**, which showed pigment-like properties and was stable towards oxygen. The reduction



Scheme 2. Compounds **3a**, **5a**, and **8a**: Ar=4-*t*-C_4H_9–Ph; Reaction conditions: (a) 1 equiv DDQ, 5 min, rt, yield 45%; (b) 1 equiv DDQ, 18 h, rt, yield 50% and (c) 0.06 M $Na_2S_2O_4$, ultrasound irradiation, 15 min, rt, yield 92%.



Scheme 3. Compounds **9**–**12a**: Ar=4-*t*-C_4H_9–Ph; **b**: Ar=4-*n*C_6H_{13}–Ph; **13a**: Ar=4-*t*-C_4H_9–Ph, PG=Boc.

of **10** with an excess of sodium dithionite finally lead to phenylogous 2,2′-biimidazoles of type **11** (Scheme 3).

These derivatives possess two-electron-rich 4,5-diaminoimidazoles and are therefore only stable if air is strictly excluded. The stabilization of **11a** can be realized via acylation reaction with Boc_2O to yield the greenish fluorescent derivative **13a**. The deprotection of this conserved leuco-form with trifluoroacetic acid reproduced the bis-imidazole **11a**, which reoxidized immediately in the presence of air to revert back to **9a**. No formation of stable charge-transfer complexes was observed with para benzoquinone. In a smooth reaction, only oxidation took place and yielded the starting material **9**.

3. Experimental

3.1. General

All reactions were monitored by TLC, carried out on 0.25 mm Merck silica gel plates ($60F_{254}$) using UV light. ^1H and ^{13}C NMR spectra were recorded with a Bruker DRX 400 or Bruker AC 250 spectrometer. Melting points are measured with a Galen TM3 apparatus and are uncorrected. UV–vis spectra were recorded on a Perkin–Elmer Lambda 19 spectrophotometer. MS spectra were taken from measurements on a Finnigan MAT SAQ 710 mass spectrometer. Elemental analyses were carried out in-house with an automatic analyzer LECO CHNS 932. The tetraazafulvalenes **2**[6,8] and **3**[14,20,21] were prepared according to literature procedures. Electrochemical measurements were carried out with a Metrohm 663VA Stand using mercury or platinum electrodes and tetrabutylammoniumhexafluorophosphate as conductive salt.

3.1.1. (*syn*, *anti*)-5,5′-Diethoxy-4,6,4′,6′-tetrakis(4-*tert*-butylphenyl)-5,6,5′,6′-tetrahydro-4*H*,4′*H*-[2,2′]bis(imidazo[4,5]imidazolylidene) (2h). Yield: 52%, metallic green crystals, mp>300 °C; ^1H NMR (250 MHz, THF): δ 1.01 (t, J=6.9 Hz, 6H), 1.36 (s, 36H), 3.35 (d, J=7.8 Hz, 4H), 7.48 (d, J=8.7 Hz, 8H), 8.04 (d, J=8.7 Hz, 8H); ^{13}C NMR (250 MHz, THF): δ 11.5, 28.5, 31.8, 53.2, 100.5, 114.6, 123.5, 132.5, 143.9, 155.0, 157.8; MS (DCI with H_2O): *m/e* (%): 833 $(M+1)^+$ (100), 786 (21), 775 (11), 728 (31), 671 (10), 480 (98), 428 (25), 333 (48), 186 (13), 147 (61), 133 (42), 57 (28); UV–vis (THF) λ_{max} (log ε): 431 (4.4), 458 (4.7), 490 (4.8) nm; Fluorescence (THF, 340 nm): $\lambda_{max,em}$: 509, 562 nm; Anal. calcd for $C_{52}H_{64}N_8O_2$: C, 74.97; H, 7.74; N, 13.45. Found: C, 74.90; H, 7.79; N, 13.49.

3.2. Reduction of the tetraazafulvalenes of type (3)[11]

3.2.1. 2-[2-[4,5-Bis-(4-*tert*-butylphenylamino)-1*H*-imidazol-2-yl]-vinyl]-N^4,N^5-bis-(4-*tert*-butylphenyl)-1*H*-imidazol-4,5-diamine (7a). Yield: 94%, brownish solid; ^1H NMR (250 MHz, THF): δ 1.19 (s, 36H), 6.55 (d, J=8.7 Hz, 8H), 7.01 (d, J=8.7 Hz, 8H); UV–vis (THF) λ_{max}: 387 nm; Fluorescence (THF, 340 nm) $\lambda_{max,em}$: 515 nm; $C_{48}H_{60}N_8$ (748.5).

3.2.2. 2-[2-[4,5-Bis-(4-bromophenylamino)-1*H*-imidazol-2-yl]-vinyl]-N^4,N^5-bis-(4-bromophenyl)-1*H*-imidazol-4,5-diamine (7b). Yield: 92%, brownish solid; ^1H NMR (250 MHz, THF): δ 7.06 (d, J=8.8 Hz, 8H), 6.53 (d, J=8.8 Hz, 8H); UV–vis (THF) λ_{max}: 405 nm; Fluorescence (THF, 340 nm) $\lambda_{max,em}$: 534 nm; $C_{32}H_{24}Br_4N_8$ (840.2).

3.3. General procedure for the oxidation of tetraazafulvalenes of type (3)

A mixture of **3** (0.1 mmol), 30 ml of THF, and 0.1 mmol DDQ was stirred at rt for about 10 min. The solvent was removed in vacuo and the crude product **5** was purified by column chromatography (SiO_2, toluene/acetone 9:1).

5a: Yield: 45%, red solid, mp 260 °C (decomp.); ^1H NMR (250 MHz, THF): δ 1.37 (s, 36H), 7.49 (m, 16H), 8.06 (s, 2H); ^{13}C NMR (250 MHz, THF): δ 28.8, 32.4, 117.6, 123.2, 123.4, 123.6, 126.0, 126.8, 134.6, 135.6, 141.3, 147.8; MS (DCI with H_2O): *m/e* (%): 745 $(M)^+$ (1), 613 (4), 508 (50), 466 (46), 389 (10), 353 (12), 307 (30), 251 (6), 176 (14), 160 (66), 150 (100), 134 (44), 94 (30); UV–vis (THF) λ_{max} (log ε): 451 (4.2), 479 (4.2), 533 (4.2) nm; CV: E^1_{OX}=0.566 V, E^2_{OX}=0.879 V; $C_{48}H_{56}N_8$ (745.04).

5b: Yield: 42%, red solid, mp 258 °C; ^1H NMR (250 MHz, THF): δ 6.91 (s, 2H), 7.08–7.20 (m, 16H); ^{13}C NMR (250 MHz, THF): δ 117.5, 122.3, 123.1, 124.9, 125.0, 127.9, 128.7, 131.4, 137.2, 137.4, 147.0, 147.7, 151.7; MS (CI): *m/e* (%): 837 $(M+1)^+$ (4), 803 (35), 558 (50), 471 (60), 429 (66), 413 (100), 349 (38), 309 (40), 291 (61), 223 (18), 81 (24); UV–vis (THF) λ_{max} (log ε): 231 (4.1), 288 (4.2), 526 (4.3) nm; CV: E^1_{OX}=0.664 V, E^2_{OX}=0.908 V; $C_{32}H_{20}N_8Br_4$ (836.34).

3.4. Procedure for the preparation of the dimer (8a)

Compound **5a** (0.05 mmol) was dissolved in 20 ml of THF. After addition of 0.05 mmol DDQ, the reaction mixture was stirred at rt for 18 h. After removing the solvent in vacuo, the dimer **8a** was purified by column chromatography (SiO_2, toluene/acetone 9:1).

8a: Yield: 50%, dark blue solid, mp 254 °C; ^1H NMR (250 MHz, THF): δ 1.21 (s, 18H), 1.29 (s, 18H), 1.32 (s, 18H), 1.36 (s, 18H), 6.83 (d, J=8.5 Hz, 4H), 7.06 (d, J=8.5 Hz, 4H), 7.12 (d, J=8.5 Hz, 4H), 7.17 (d, J=8.5 Hz, 4H), 7.28 (d, J=8.5 Hz, 4H), 7.36 (d, J=8.5 Hz, 4H), 7.40 (d, J=8.5 Hz, 4H), 7.85 (d, J=8.5 Hz, 4H), 8.44 (s, 2H), 8.64 (s, 2H); ^{13}C NMR (250 MHz, THF): δ 30.5, 33.5, 117.7, 118.2, 119.3, 125.2, 135.4, 137.1, 145.3, 148.4, 150.4, 157.2; MS (DCI with H_2O): *m/e* (%): 1490 $(M)^+$ (80), 1342 (100), 1130 (10), 985 (4), 894 (12), 833 (30), 721 (8), 508 (14), 231 (8), 217 (8), 131 (8); UV–vis (THF) λ_{max} (log ε): 228 (4.9), 286 (4.7), 529 (4.5), 571 (4.7), 623 (4.6) nm; $C_{96}H_{114}N_{16}$ (1490.08).

3.5. Reduction of bis-4*H*-imidazoles (9) to quinomethides (10)

Derivative **9** (0.1 mmol) dissolved in THF was reduced with a 0.06 M sodium dithionite solution (1 equiv). The deep blue colored quinomethides **10** were obtained after removing the solvent in vacuo.

10a: Yield: 94%, dark blue solid; ^{1}H NMR (400 MHz, THF): δ 1.27 (s, 36H), 6.84 (d, J=8.8 Hz, 8H), 7.31 (d, J=8.8 Hz, 8H), 8.06 (s, 4H); HRMS (DEI): m/z calcd for $C_{52}H_{60}N_8$ $(M)^+$: 796.4941, found: 796.5030; UV–vis (THF) λ_{max} (log ε): 634 (4.4) nm.

10b: Yield: 95%, dark blue solid; ^{1}H NMR (250 MHz, THF): δ 0.78 (t, J=8.0 Hz, 12H), 1.20 (m, 24H), 1.45 (m, 16H), 6.55 (d, J=8.5 Hz, 8H), 6.77 (d, J=8.5 Hz, 8H), 7.97 (s, 4H); HRMS (DEI): m/z calcd for $C_{60}H_{76}N_8$ $(M)^+$: 908.6035, found: 908.6040; UV–vis (THF) λ_{max} (log ε): 633 (4.0) nm.

3.6. Cyclization of the quinomethides (10) with triphosgene to bis-urea derivatives (12)

Compound **10** (0.1 mmol) and 0.25 mmol of triphosgene were dissolved under argon in 20 ml of dry toluene. The reaction mixture was then heated under reflux for 5 h. After completion of the reaction the cyclic bis-urea derivatives were separated by addition of a mixture of water/acetone (1:1) as deep purple pigment-like solids, which were filtered off and dried in vacuo.

12a: Yield: 30%, purple, metallic-shining microcrystals, mp 280 °C (decomp.); ^{1}H NMR (250 MHz, THF): δ 1.29 (s, 36H), 7.05 (s, 4H), 7.42 (d, J=8.5 Hz, 8H), 7.54 (d, J=8.5 Hz, 8H); MS (DCI with H_2O): m/e (%): 849 $(M)^+$ (22), 793 (2), 424 (6), 379 (10), 323 (2), 176 (100), 159 (26), 123 (28), 109 (22), 93 (15); IR (KBr): 2963, 2906, 2868, 1754, 1624, 1517, 1437, 1406, 1266, 1107, 1088, 1034, 978, 877, 831, 802, 693, 667 cm^{-1}; UV–vis (THF) λ_{max}: 475, 556 nm; $C_{54}H_{56}N_8O_2$ (848.45).

12b: Yield: 34%, purple, metallic-shining microcrystals, mp 300 °C (decomp.); ^{1}H NMR (400 MHz, THF): δ 0.92 (t, J=8.0 Hz, 12H), 1.36 (m, 32H), 2.70 (t, J=8.0 Hz, 8H), 7.38 (d, J=8.5 Hz, 8H), 8.05 (d, J=8.5 Hz, 8H), 8.15 (s, 4H); MS (DCI with H_2O): m/e (%): 961 $(M+1)^+$ (4), 745 (12), 584 (16), 407 (12), 309 (8), 204 (100), 178 (59), 132 (27), 106 (13); IR (KBr): 2922, 2854, 1751, 1621, 1512, 1484, 1403, 975 cm^{-1}; UV–vis (THF) λ_{max} (log ε): 246 (4.4), 560 (4.8) nm; $C_{62}H_{72}N_8O_2$ (960.58).

3.7. Reduction of the quinomethides (10) to biimidazoles (11)

Derivative **10** (0.1 mmol), dissolved in 20 ml of THF was reduced with 5 ml of 0.06 M solution of sodium dithionite. The reaction mixture was irradiated with ultrasound for 10 min at rt. The biimidazoles **11** were obtained as yellow solids by removing the solvent in vacuo.

11a: Yield: 96%, yellow solid; ^{1}H NMR (250 MHz, THF): δ 1.24 (s, 36H), 6.82 (d, J=8.3 Hz, 8H), 7.29 (d, J=8.3 Hz, 8H), 8.04 (s, 4H); UV–vis (THF) λ_{max} (log ε): 317 (4.4), 370 (4.5) nm; $C_{52}H_{62}N_8$ (798.48).

11b: Yield: 97%, yellow solid; ^{1}H NMR (250 MHz, THF): δ 0.79 (t, J=8.0 Hz, 12H), 1.20 (m, 24H), 1.45 (m, 16H), 6.51 (d, J=8.5 Hz, 8H), 7.09 (d, J=8.5 Hz, 8H), 7.863 (s, 4H); UV–vis (THF) λ_{max} (log ε): 317 (4.3), 375 (4.4) nm; $C_{60}H_{78}N_8$ (910.60).

3.8. Derivative (13a) was prepared according to the literature procedure[11]

13a: Yield: 15%, brownish solid; ^{1}H NMR (250 MHz, THF): δ 1.17 (s, 18H), 1.22 (s, 36H), 1.29 (s, 36H), 7.24 (d, J=8.8 Hz, 8H), 7.70 (m, 12H); MS (micro-ESI, acetone/methanol): m/e (%): 1399 $(M)^+$ (36), 1299 (12), 1199 (14), 1100 (8), 1001 (6), 901 (8), 800 (4), 775 (8), 623 (10), 471 (20), 413 (100), 301 (10); IR (KBr): 2966, 2871, 1758, 1717, 1611, 1515, 1477, 1392, 1367, 1325, 1302, 1145, 1107, 836 cm^{-1}; UV–vis (THF) λ_{max}: 271, 296, 335 nm.

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Tetrahedron

Tröger's base scaffold in racemic and chiral fashion as a spacer for bisdistamycin formation. Synthesis and DNA binding study

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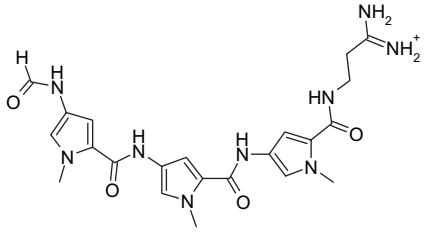
Abstract—'Head-to-head' oligo-*N*-methylpyrrole peptide dimers linked by a methano[1,5]diazocin scaffold are presented in racemic as well as chiral fashion. Their DNA binding activities were assayed on calf thymus DNA, poly(dA-dT)$_2$, and poly(dC-dG)$_2$ by NMR and ECD spectroscopies, and fluorescence probe displacement assay. The presented dimers prefer AT sequences, but show higher affinity to poly(dC-dG)$_2$ than distamycin A. The (4*R*,9*R*) configuration of methanodiazocin bridge was found to be better suited for interaction with ct-DNA and poly(dA-dT)$_2$ than (4*S*,9*S*) configuration.

1. Introduction

The low molecular weight, sequence selective agents that interact with double-stranded DNA have been studied over four decades.[1] These molecules are mainly based on natural products, which bind to the minor groove of DNA. One of the most studied minor groove binders is distamycin A (Fig. 1, **1**)[2] and its analogues.[1,3–5]

Distamycin A is naturally occurring antibiotic agent that can reversibly bind to the minor groove of DNA by hydrogen and van der Waals bonds, and electrostatic interactions. Solution NMR spectroscopy[6,7] and X-ray diffraction[8] studies established a strong preference of distamycin A for adenine–thymine (AT) rich sequences containing at least four AT base pairs. In addition, extension of the number of pyrrole units in distamycin A analogues increased the sequence specificity for longer tracts of AT rich DNA,[9,10] and replacing *N*-methylpyrrole (Py) by *N*-methylimidazole (Im) ring changed the selectivity from AT to the cytosine–guanosine (CG) base pair.[11,12] NMR[13,14] and footprinting[15,16] experiments showed that distamycin A and its homologues can bind DNA as monomers as well as antiparallel dimers.



1

Figure 1.

Based on this understanding, covalently linked oligopeptide dimers have been prepared, which were coupled in antiparallel 'head-to-tail' fashion with various linkers.[4,5,17–19] These covalent linkages of two oligopeptides form the ligands with a U-shape motif, which binds to DNA with both increased affinity and specificity, as compared to the unlinked oligopeptides. For instance, dimer ImPyPy-PyPyPy linked by γ-aminobutyric acid binds to 5′-TGTTA-3′ with more than 2 orders of magnitude greater affinity than the unlinked oligopeptides, ImPyPy and PyPyPy.[17]

The parallel 'tail-to-tail'[20] and 'head-to-head'[21–30] linked oligopeptide dimers have been designed as bidentate DNA binders. In the case of 'head-to-head' dimeric oligopeptides, it was found that conformationally constrained cycloalkane and *trans*-alkenic linkers[28] prove higher effectivity for their bidentate binding to DNA than flexible polymethylene linked chains.[26,27] In addition, the stereochemistry of the linkers is also important and can control the binding.[23,29] To the best of our knowledge, only two examples of DNA binding studies of 'head-to-head' dimeric oligopeptides

Keywords: Tröger's base; Distamycin dimer; Enantioselective binding; Dissymmetry factor; VCD.
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with an optically active linker have been reported to date.[23,29] And the problem of ideal shape and stereochemistry of linker for bidentate binding is still unclear.

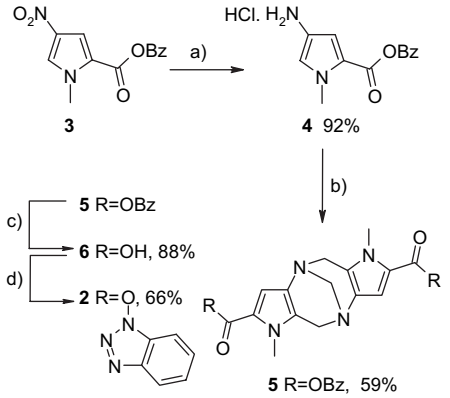
In order to obtain structural information and further insights into chiral discrimination in the binding of optically active 'head-to-head' dimeric oligopeptides to DNA, we report in this study a constrained system of dimeric oligo-*N*-methylpyrrole peptides linked by the methano[1,5]diazocin scaffold, which fashions Tröger's base (TB) derivatives. The unique V-shape geometry (81–104°) of TB and the inherent chirality have been used in the construction of various receptors.[31–33] Moreover, some heterocyclic TB derivatives including phenanthroline and acridine interact specifically and enantioselectively with DNA.[34–36] The design of presented oligo-*N*-methylpyrrole peptide dimers also involved the width of linkers, which was established to be approximately 8 Å. The size of linkers should not cause steric restraints on binding of proposed dimers to the minor groove of DNA that is 10 Å wide.[37] In addition, the groove width in DNA–distamycin complexes was determined from 7 to 14 Å.[13,37,38] We rationalized that the interconnection of two distamycin analogues with methanodiazocin scaffold of TB could afford enantio-selective bidentate minor groove binders.

2. Results and discussion

2.1. Synthesis

As we described previously,[39] direct preparation of such compounds by methanodiazocin bridging of the corresponding amino-monomers is unsuccessful because degradation effects predominate. The synthetic strategy is based on the extension of central 4,9-methano-1,6-dimethyl-4,5,9,10-tetrahydro-1*H*,6*H*-dipyrrolo-[3,2-*b*:3′,2′-*f*][1,5]diazocin-2,7-dicarboxylate core via amide coupling of active ester precursor **2** and amino-arm.

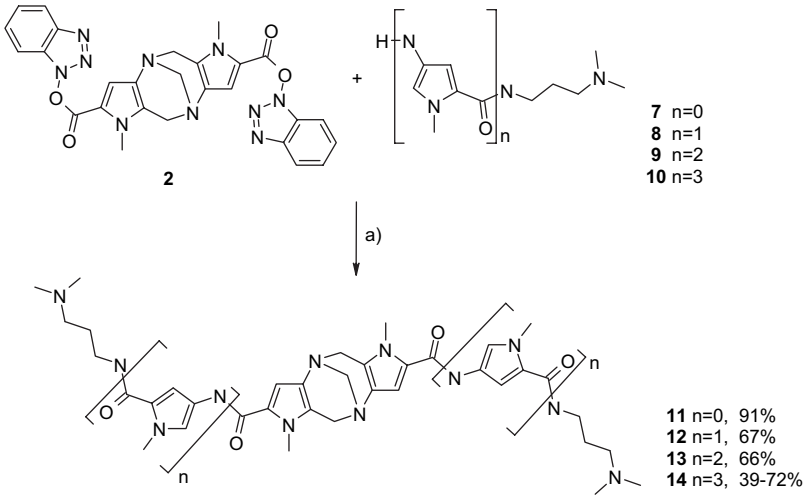
Active ester precursor **2** was obtained in four steps by reaction sequences shown in Scheme 1. Benzyl 1-methyl-4-nitropyrrole-2-carboxylate (**3**) was prepared as described elsewhere.[40] The following reduction[41,42] of nitro group was achieved by nickel boride (Ni_2B) to give amine hydrochloride[43] **4**. Treatment of **4** with aqueous solution of formaldehyde regioselectively gave TB analogue **5**.[39] Catalytic hydrogenation of benzyl ester groups of **5** yielded dicarboxylic derivative **6** that was subsequently converted to active ester **2** by its reaction with 1-hydroxy benzotriazole (HOBt) and *N*,*N*′-dicyclohexylcarbodiimide (DCC).



Scheme 1. Reagents and conditions: (a) Ni_2B, aqueous concd HCl, methanol, 70 °C; (b) aqueous concd H_2CO, aqueous concd HCl, ethanol, rt; (c) H_2, Pd/C, DMF, rt; (d) DCC, HOBt, DMF, CH_2Cl_2, rt.

The active ester **2** was then used for the preparation of methanodiazocin bridged dimeric oligo-*N*-methylpyrrole derivatives via amide protocol with amines[40] **7–10** (see Scheme 2). Products **11–14** were separated from reaction mixtures by column chromatography on silica with 5% concd aqueous ammonia in methanol as an eluent. The most effective DNA-binder **14** was then prepared in both optically pure forms (4*R*,9*R*)-**14** and (4*S*,9*S*)-**14** by a similar synthetic method.

Enantiomeric synthesis came from an analogue of benzyl ester **5**, 1-phenylethyl ester **15** that is easily obtainable in the optically pure state as we have recently described.[42] Conversion of 1-phenylethyl esters **15a** and **15b** (Fig. 2) into active esters (4*R*,9*R*)-**2** and (4*S*,9*S*)-**2** were performed without separation of acid **6** due to racemization hazard of methanodiazocin bridge. Note that acid (4*R*,9*R*)-**6** and (4*S*,9*S*)-**6** were also prepared due to better band assignment in IR



Scheme 2. Reagents and conditions: (a) DMF, amine, 60 °C.

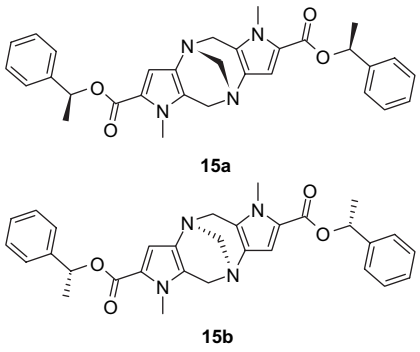


Figure 2.

and vibrational CD (VCD) spectra of (4*R*,9*R*)-**14** and (4*S*,9*S*)-**14**. The optical stability of active esters (4*R*,9*R*)-**2** and (4*S*,9*S*)-**2**, as well as dimers (4*R*,9*R*)-**14** and (4*S*,9*S*)-**14** was confirmed by ECD (see Fig. 3, ECD spectra of final dimer (4*R*,9*R*)-**14** and (4*S*,9*S*)-**14**) and optical rotation measurements of both enantiomers.

The structures of all prepared compounds were verified by detailed analyses of ^{1}H, ^{13}C, 1D NOESY, gHSQC, gHMBC, and gCOSY NMR experiments. The optically pure acids (4*R*,9*R*)-**6** and (4*S*,9*S*)-**6** and dimers (4*R*,9*R*)-**14** and (4*S*,9*S*)-**14** were then investigated by VCD that is a powerful tool for the description of chiral systems, like prediction of conformation of macromolecules,[44–47] determination of absolute configuration of molecules,[48] and interaction studies[49,50] of molecules as well as macromolecules.[51]

The racemate **14** and enantiomers (4*S*,9*S*)-**14** and (4*R*,9*R*)-**14** provide identical IR absorption spectra in the measured region 1800–1100 cm^{-1} (see Fig. 4B). The VCD spectra of (4*S*,9*S*)-**14** and (4*R*,9*R*)-**14** are nearly symmetrical (mirror-image) with respect to baseline. The baseline was obtained as VCD spectrum of racemate **14** and it was close to zero. The characteristic vibrations of pyrroles and amide I (1800–1350 cm^{-1}) are well separated from the characteristic vibrations of methanodiazocin bridge [52] (1350–1100 cm^{-1}).

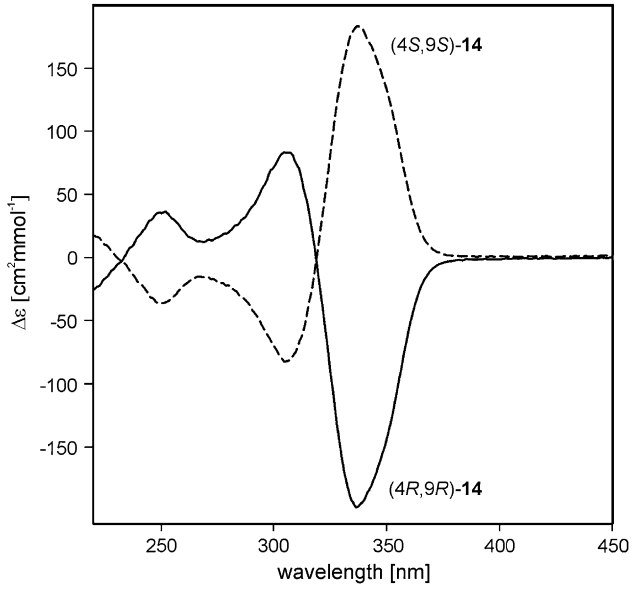


Figure 3. ECD spectra of final dimer (4*R*,9*R*)-**14** and (4*S*,9*S*)-**14**.

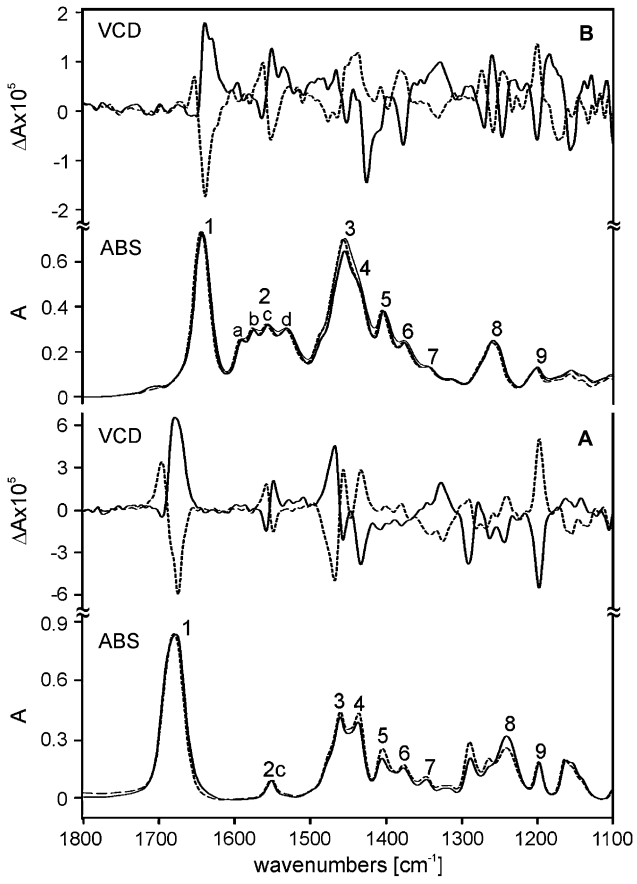


Figure 4. VCD and IR absorption spectra of (A) (4*S*,9*S*)-**6** (dashed line) and (4*R*,9*R*)-**6** (solid line), and (B) (4*S*,9*S*)-**14** (dashed line) and (4*R*,9*R*)-**14** (solid line), and IR absorption of **14** racemate (solid thin line) in $(CD_3)_2SO$.

However, we were not able to resolve the vibration of *N*-methylpyrroles that are bridged by methanodiazocin, and the vibrations of *N*-methylpyrroles from the tripeptidic arms. Moreover, the bands of the methanodiazocin bridge are overlapped by the bands of *N*-methylpyrroles and *N*,*N*-dimethylaminopropyl terminals (see Table 1 and Fig. 4B, Peak no. 4, 7, and 8).

Table 1. Characteristic IR vibrations of acid **6** and dimer **14** in $(CD_3)_2SO$, 1100–1800 cm^{-1} spectral region

Band No.	Wavenumbers [cm^{-1}]		Characteristic vibrations
	Acid **6**	Dimer **14**	
1	1680	1643	C=O stretch: –COOH group (for **6**), amide I (for **14**)
2a		1591	C=C stretch: pyrrole
2b		1575	C=C stretch: pyrrole
2c	1550	1557	C=C stretch: pyrrole of central core
2d		1531	C=C stretch: pyrrole
3	1461	1455	CH_3 asymmetric bend
4	1435	1432	CH_2 bend (scissor)
5	1404	1404	Ring vibration: pyrrole
6	1376	1376	CH_3 symmetric bend
7	1344	1344	CH_3 and CH_2 deformation
8	1241	1259	C–N stretch: methanodiazocin bridge, *N*-methylpyrrole groups+ *N*,*N*-dimethylamino group (for **14**)
9	1201	1201	C–N stretch: methanodiazocin bridge

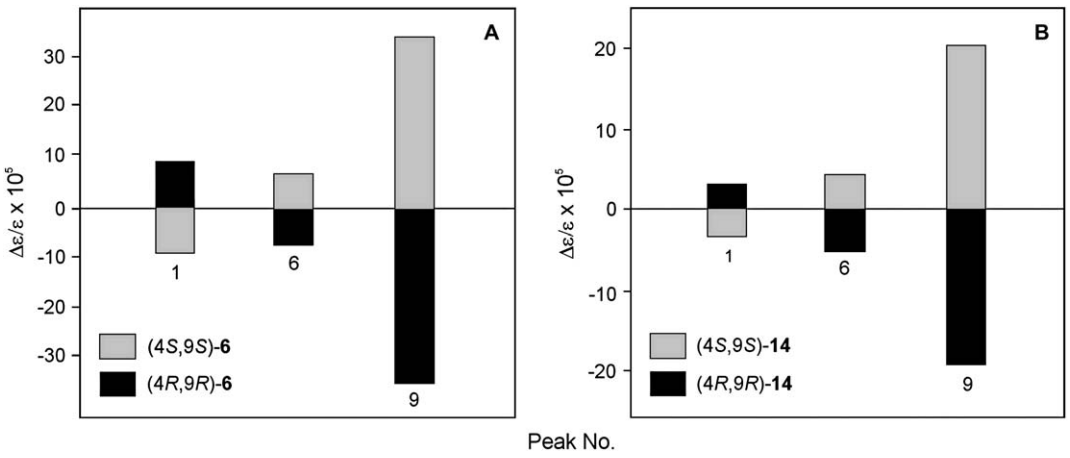


Figure 5. VCD dissymmetry factor $\Delta\varepsilon/\varepsilon$ of selected bands of (A) **6** and (B) **14**.

IR and VCD spectra of enantiomers of **6**, the compound representing the di-*N*-methylpyrrolomethanodiazocin core, were studied in parallel to elucidate the assignment of vibration bands of **14** (see Fig. 4 and Table 1). Enantiomers (4*S*,9*S*)-**6** and (4*R*,9*R*)-**6** also provide identical IR absorption spectra and mirror-image VCD spectra as observed for (4*S*,9*S*)-**14** and (4*R*,9*R*)-**14**.

The absorption band at 1557 cm^{-1} (2c) was assigned to *N*-methylpyrroles of the central core of **14** because this band is also present in spectra of **6** at 1550 cm^{-1} (2c) and the VCD pattern corresponding to these absorption bands are almost the same. The other bands (2a, 2b, and 2d) were assigned to *N*-methylpyrroles of the tripeptide arms of **14** and do not possess VCD signals. The bands at 1680 and 1643 cm^{-1} (1) were assigned to C═O vibration of carboxylic group of **6** and the amide group of **14**, respectively. The CH_3 bend vibrations (3, 6, and 7), the CH_2 bend vibrations (4 and 7), the ring vibration of pyrroles (5), and C–N vibrations (8 and 9) were observed in the absorption spectra of acid **6** as well as dimer **14**.

2.2. Asymmetry transfer evolution

Dissymmetry factor[53,54] (ratio of intensity of VCD signal and the intensity of corresponding IR signal, $\Delta\varepsilon/\varepsilon$) values calculated for characteristic vibrations were used to describe the distribution of asymmetry in both molecules **6** and **14**. Generally, $\Delta\varepsilon/\varepsilon$ is the highest for chiral center and decreases with increasing distance from the chiral center. In other words, the $\Delta\varepsilon/\varepsilon$ values of selected vibrations referred to the $\Delta\varepsilon/\varepsilon$ value of chiral center, relative $\Delta\varepsilon/\varepsilon$ values represent the transfer of asymmetry from chiral center to the rest of molecule. Dissymmetry factors of **6** (Fig. 5A) and **14** (Fig. 5B) were calculated for characteristic vibrations of methanodiazocin bridge, C═O, and methyl groups, which are located in the chiral center and in a different distance from the chiral core.

The maximal $\Delta\varepsilon/\varepsilon$ value was observed for the C–N stretching vibrations of the chiral methanodiazocin bridge (9) in the spectra of **6** and **14**, as expected. The $\Delta\varepsilon/\varepsilon$ values of C═O stretch (1) and CH_3 symmetrical bend (6) of **6** are almost the same and reach about one quarter of the $\Delta\varepsilon/\varepsilon$ value of methanodiazocin bridge (9). For dimer **14**, the $\Delta\varepsilon/\varepsilon$ value of amide I stretch (1) and the CH_3 symmetrical bend (6) are given by summation[†] of contributions of individual amide I and methyl groups, respectively. Both values, therefore, represent the average of individual contributions. They reach only one fifth of the $\Delta\varepsilon/\varepsilon$ value of methanodiazocin bridge (9).

The transfer of asymmetry from methanodiazocin bridge to the *N*-methylpyrrole tripeptide arms of **14** can be clarified by comparing the relative $\Delta\varepsilon/\varepsilon$ values of C═O stretch (1) and CH_3 symmetrical bend (6) referred to the $\Delta\varepsilon/\varepsilon$ value of band no. 9 for **14** versus the same values obtained for **6**. There are two possible extreme situations. Firstly, the asymmetry in **14** is transferred only to the first amide group of the tripeptidic arms, then the decrease of *relative* $\Delta\varepsilon/\varepsilon$ values (1 and 6) of **14** versus **6** should be approximately 80%. Secondly, the asymmetry in **14** is transferred to all amide groups of tripeptidic arms equally, then the *relative* $\Delta\varepsilon/\varepsilon$ values (1 and 6) should be the same for **6** and **14**. The approximately 20% reduction of *relative* $\Delta\varepsilon/\varepsilon$ values (1 and 6) of **14** compared to **6** indicates a descending but important influence of the chiral methanodiazocin bridge on the optical activity in the *N*-methylpyrrole tripeptidic arms of **14**. On the other hand, partially differentiated vibrations of the *N*-methylpyrrole units of **14** (2a, 2b, 2c and 2d), showing only one VCD signal for *N*-methylpyrroles of the central core (2c) of **14**, is not consistent with the distribution of asymmetry far from the di-*N*-methylpyrrolomethanodiazocin core.

In summary, the asymmetry in both **6** and **14** is definitely transported up to the carboxyl and first amide, respectively. Distribution of asymmetry to the rest of dimer **14** is then ambiguous.

[†] The IR and VCD signals of all present amide groups as well as methyl groups are not resolved and are given by summation of all appropriate signals. This is evident from different ratio between the IR signal intensity of the methyl groups and the methanodiazocin bridge in the spectra of **6** and **14**. While the IR signal of **6** at 1376 cm^{-1} is as intensive as the IR signal of **6** at 1201 cm^{-1} and the IR signal of **14** at 1376 cm^{-1} is three times more intense than IR signal of **14** at 1201 cm^{-1}.

2.3. Binding study

DNA binding activity of dimers **11**–**14** was assayed on DNAs by NMR and ECD spectroscopies, and using a fluorescence probe displacement assay in deutero-water and deutero-water/deutero-methanol mixture (2:1), and cacodylate buffer, respectively.

NMR experiments were only performed with **11** and **12**, because the solubility of the other dimers (**13** and **14**) is limited and these dimers form aggregates in water as well as in the mixture of water and methanol. Addition of calf thymus (ct) DNA to the solution of **11** led to distension (line broadening) of all signals of **11** in ^{1}H spectrum. The presence of ct-DNA has the biggest influence on the signals of methylene protons of methanodiazocin bridge. Distension of signals of **11** indicates an intermediate rate of exchange between the complexed and free state of **11**. In addition, the hydrogen nuclei of **11** relaxed faster in the presence of ct-DNA. Generally, shorter relaxation times signify a decrease in the degree of freedom of molecules, which is consistent with the complex formation of **11** and ct-DNA. The same results were achieved for dimer **12** at considerable (approximately 10 times) lower ratio of ct-DNA and ligand.

On the other hand, dimers **11** and **12** did not provide the induced ECD band above 300 nm upon their addition to ct-DNA (see Fig. 6) that is typical for binding of **1** and its analogues to minor groove of ct-DNA.[55,56] In addition, the intensity of the induced ECD band is proportional to the strength of binding. In the presence of ct-DNA, only dimers **13** and **14** provided new ECD bands above 300 nm. The magnitude of the positive ECD band was more than three times higher in the case of dimer **14** compared to **13**. These results are in agreement with the previous observation that the strength of binding of distamycin related compounds

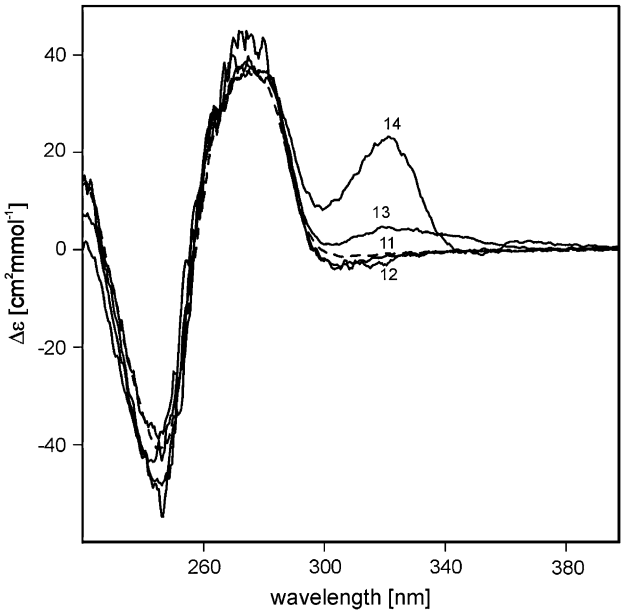


Figure 6. ECD spectra of **11–14** in the presence of ct-DNA (solid lines) and the spectrum of pure ct-DNA (dashed line).

to DNA increases with the number of *N*-methylpyrrole units present in the oligopeptides.[20]

The ECD signal of ct-DNA, the positive and negative bands at 274 and 245 nm, is typical for the B-form of DNA[57] and did not change on adding dimer **11**–**14** to ct-DNA (see Fig. 6). So, the interaction between B-DNA and **13**, and **14** does not significantly influence the structure of ct-DNA.

The following ECD spectroscopic measurements were done for the interactions of dimer **12**–**14** with poly(dA-dT)$_2$ and poly(dC-dG)$_2$, respectively. As expected, **13**-poly(dA-dT)$_2$ and **14**-poly(dA-dT)$_2$ exhibited the induced ECD band above 300 nm. In addition, dramatic changes in the induced ECD band of **14**-poly(dA-dT)$_2$ were observed at higher drug concentration (c(**14**):c(poly(dA-dT)$_2$), ~1:4). This indicates a two-step binding mode of **14** to poly(dA-dT)$_2$. The intensity of the induced ECD band in the spectrum of **12**-poly(dA-dT)$_2$ and **14**-poly(dC-dG)$_2$ was low and reached less than one sixth of the intensity of ECD band corresponding to **13**-poly(dA-dT)$_2$ or **14**-poly(dA-dT)$_2$. The addition of **12** and **13** to the poly(dC-dG)$_2$ did not lead to induction of the ECD band above 300 nm.

The binding stoichiometry (n_b, number of dimer molecules bound per nucleotide base pair) of **14** to ct-DNA and poly(dA-dT)$_2$ was determined by ECD spectral titrations of DNA with **14**. The n_b was determined as n (the ratio of c(**14**) to c(DNA)) at the break in the plot of the intensity of the induced ECD signal at selected wavelength against the n. The n_b values (~0.15 for ct-DNA and ~0.12 for poly(dA-dT)$_2$) indicate that DNA saturates at about one molecule of **14** per six nucleotide base pairs in the case of ct-DNA and one molecule of **14** per about eight nucleotide base pairs in the case of poly(dA-dT)$_2$. The number of base pairs bonded by dimer **14** is lower than one would expect for bidentate binding mode of such compound to DNA. In addition, a similar n_b value was previously obtained for the interaction of **1** with poly(dA-dT)$_2$.[28] It appears that dimers **12**–**14** bind to DNA either through one of their *N*-methylpyrrole peptide arms and partial contacts of the linker or through the terminal segments of both *N*-methylpyrrole peptide arms only.

The quantification of binding strength of dimers **12**–**14** to DNA was done by the competition between compounds **1**, **12**–**14**, and ethidium bromide (EB).[58] Displacement of ethidium bromide from ct-DNA, poly(dA-dT)$_2$, and poly(dC-dG)$_2$ was accompanied by a decrease in the fluorescence intensity measured at 595 nm. The apparent binding constants (K_{app}) were calculated from K_{EB}[EB]=K_{app}[drug], where [drug] is the concentration of **12**–**14** at a 50% reduction of fluorescence and K_{EB} is known.[59] The K_{app} values for **1** are close to the data obtained by previous measurements.[27,60] The K_{app} values for **12**–**14** calculated by this way (see Table 2) are consistent with the results obtained by ECD spectroscopy. The almost identical K_{app} values of **12** to all types of studied DNAs show that dimer **12** binds DNA without any selectivity. Dimers **13** and **14** showed lower affinity for ct-DNA and poly(dA-dT)$_2$ than **1**, however, they bound significantly stronger to poly(dC-dG)$_2$. Nevertheless, both compounds exhibit a preference for AT sequences compared to GC sequences. The strength

Table 2. Apparent binding constants, K_{app} ($\times 10^5$ M^{-1})

Compound	ct-DNA	poly(dA-dT)$_2$	poly(dC-dG)$_2$	poly(dA-dT)$_2$/ poly(dC-dG)$_2$
1	140±4	346±3	2.0±0.2	173
12	2.0±0.5	2.4±0.5	1.8±0.4	—
13	33±3	34±4	20±2	1.7
14	99±4	148±4	40±3	3.7
(4*R*,9*R*)-**14**	112±4	191±4	42±3	4.5
(4*S*,9*S*)-**14**	90±3	128±3	36±2	3.5

of binding to DNAs and the preference for AT sequences increases with the increasing number of pyrrole units in the dimer. A decrease in the binding affinity and selectivity of methanodiazocin linked distamycin analogues compared to distamycin itself confirms the conclusion from the binding stoichiometry determination, the nonbidentate binding of dimers **12**–**14** to DNA. On the other hand, distamycin A and netropsin exhibit higher binding affinity for ct-DNA than 'head-to-head' bisnetropsin analogues with a 1,2-cyclopropanedicaraboxamide linker that binds to DNA with a bidentate binding mode.[28] Nevertheless, all the data evaluation indicates that the rigid methanodiazocin bridge is not an optimal spacer molecule for the construction of bidentate minor groove binders based on 'head-to-head' bisdistamycin derivatives.

The stereochemical control of minor groove binding by the methanodiazocin bridge on *N*-methylpyrrole oligopeptide dimer was studied with the optically pure forms of the most effective binding dimer **14**. The ECD spectral titrations suggested a similar affinity of (4*R*,9*R*)-**14** and (4*S*,9*S*)-**14** to all studied DNAs. The ECD spectra of (4*R*,9*R*)-**14**-poly(dC-dG)$_2$ and (4*S*,9*S*)-**14**-poly(dC-dG)$_2$ after subtraction of poly(dC-dG)$_2$ contributions were nearly symmetrical (mirror-image) with respect to base lines. This 'spectral symmetry' explains the small intensity of the ECD band above 300 nm at titration of poly(dC-dG)$_2$ with racemic **14**. Fluorescence probe displacement assay shows that enantiomer (4*R*,9*R*)-**14** has a slightly higher affinity for all studied DNAs than enantiomer (4*S*,9*S*)-**14** (Table 2). In addition, enantiomer (4*R*,9*R*)-**14** also exhibited slightly higher discrimination for poly(dA-dT)$_2$ versus poly(dC-dG)$_2$ sequences than enantiomer (4*S*,9*S*)-**14**. The '*R* enantiomeric discrimination' has also been described by Lown and co-workers on a netropsin dimer linked by *trans*-1,2-cyclopropane.[29]

3. Conclusion

We have described new bisdistamycin analogues with a TB scaffold in racemic as well as chiral fashion. DNA binding studies of such compounds showed that the 'head-to-head' linking of two *N*-methylpyrrole oligopeptides by the methanodiazocin scaffold caused decreasing affinity and selectivity of binding to AT sequences, and increases affinity to CG sequences compared to distamycin A. Moreover, the directionality in the binding can be controlled by the stereochemistry of our linker. In the case of the most effective binding dimer **14**, the (4*R*,9*R*)-form is better suited for interaction with all studied DNAs than the (4*S*,9*S*)-form.

4. Experimental

4.1. General

Thin-layer chromatography was performed on Merck Silica gel 60 F_{254} TLC plates. For column chromatography the neutral silica gel SiliTech 32–63, 60 Å (ICN Biomedicals) was used. ^{1}H, ^{13}C, 1D NOESY, gHSQC, gHMBC, and gCOSY NMR spectra were obtained with Varian Gemini 300 HC (300.1 MHz for ^{1}H NMR and 75.5 MHz for ^{13}C NMR spectra) at 23 °C in $CDCl_3$ or $(CD_3)_2SO$. Correlation NMR techniques were applied for the assignment of chemical shifts to atoms of the molecules. The chemical shifts are given in parts per million relative to $(CH_3)_4Si$. For the sake of clarity in the assignment of NMR spectra, the pyrrole rings of the dimer **12**–**14** are numbered from methanodiazocin bridge to *N*,*N*-dimethylaminopropyl terminal. Mass spectra were recorded with a VG Analytical ZAB-EQ spectrometer and Biflex mass spectrometer (MALDI-MS).

Calf thymus DNA (42% C+G, mean molecular mass ~20 MDa) was prepared and characterized as described previously.[61] Homopolymers poly(dA-dT)$_2$ and poly(dC-dG)$_2$ were purchased from Sigma. The DNA concentrations are expressed per base pairs.

The ECD spectra were measured at a resolution of 1 nm using Jasco-810 spectrophotometer using 0.1 cm path length and three accumulations. The concentration of **11**–**14** was 0.2 mM. The DNA concentration was 1.6 mM. Cacodylate/NaCl (0.02/0.1 M) buffer of pH 7.4 was used as a solvent.

Fluorescence measurements were performed on a Shimadzu RF 40 spectrofluorophotometer using a 1 cm quartz cell at room temperature. The DNA–EB complexes were excited at 546 nm and the fluorescence was measured at 595 nm. To the solution of EB and DNA (10 mM Tris pH 7.4, 1 mM EDTA, 1.3 μM EB, and 3.9 μM DNA) were added aliquots of a 1 mM stock solution of **12**–**14** and the fluorescence was measured after each addition until the fluorescence was reduced to 50%.

The VCD and IR absorption spectra were measured at a resolution of 4 cm^{-1} using Bruker FTIR IFS 66/S spectrometer equipped with a VCD/IRRAS module PMA 37.[62] Demountable cell composed of 50 μm Teflon spacer and CaF_2 windows were used. VCD spectra were obtained as the average of 25 blocks of scans, each block counts 3680 scans (20 min). Concentrations of **6** and **14** were 0.16 and 0.04 M ($(CD_3)_2SO$), respectively. The concentration was chosen so that the absorbance maxima did not exceed value of about 0.8 for 50 μm path length in the spectral region 1800–1100 cm^{-1}.

4.2. Benzyl 1-methyl-4-nitropyrrole-2-carboxylate (3)

To a solution of 1-methyl-4-nitro-2-trichloroacetylpyrrole (30.02 g, 110.57 mmol) in THF (160 ml), a mixture of benzyl alcohol (30 ml, 290 mmol) and natrium hydride (0.50 g, 21.7 mmol) in THF (40 ml) was added at 0 °C. The resulting suspension was warmed to room temperature and stirred overnight. The reaction mixture was neutralized with $TsOH \cdot H_2O$ (yellow color changed to white) and the

insoluble part was filtered off. The filtrate was concentrated in vacuo and the residue was diluted with petroleum ether (300 ml), which caused crystallization of **3**. Pale yellow crystals of product **3** (25.12 g, 87%) were filtered off, washed with petrolether (3×100 ml), cold methanol (2×50 ml), water (2×50 ml) and again with methanol (2×50 ml), and dried in vacuo.

Mp 109–111 °C (lit.[40] mp 112–113 °C); ^{1}H NMR ($CDCl_3$) δ 3.97 (3H, s, NCH_3), 5.29 (2H, s, CH_2), 7.32–7.43 (5H, m, C*H*-Ph), 7.44 (1H, d, *J*=2.2, C*H*CCO), 7.59 (1H, d, *J*=2.2, C*H*NCH$_3$); ^{13}C NMR ($CDCl_3$) δ 37.83 (N*C*H$_3$), 66.38 (*C*H$_2$), 112.76 (*C*HCCO), 128.05 (*o*-*C*H of Ph), 122.68 (*C*CO), 127.66 (*C*HNCH$_3$), 128.34 (*p*-*C*H of Ph), 128.55 (*m*-*C*H of Ph), 135.09 (*C*NO$_2$), 135.31 (*ipso*-*C* of Ph), 159.36 (*C*O).

4.3. Benzyl 1-methyl-4-aminopyrrole-2-carboxylate hydrochloride (4)

Freshly prepared nickel boride Ni_2B (15.00 g) and **3** (15.00 g, 57.63 mmol) were added to methanol (390 ml) and 1 M hydrochloric acid (130 ml). The resulting suspension was stirred at 70 °C till **3** was no longer present in the reaction mixture (followed by TLC). For each 1 h, 2 ml of concd hydrochloric acid were slowly added to the reaction mixture. The reaction mixture was filtered and the filter cake was washed with methanol (3×50 ml). All liquid portions were combined and concentrated in vacuo to 100 ml (mainly to remove methanol), which caused crystallization of **4** (10.52 g, 68%) as pale brown crystals. A second crop of product **4** (3.64 g, 24%) was obtained by concentration of mother liquor.

Mp 213–217 °C (decomp.); ^{1}H NMR ((CD$_3$)$_2$SO) δ 3.84 (3H, s, NCH_3), 5.23 (2H, s, CH_2), 6.83 (1H, d, *J*=2.2, C*H*CCO), 7.27 (1H, d, *J*=2.2, C*H*NCH$_3$), 7.28–7.42 (5H, m, C*H*-Ph), 10.25 (3H, br s, $^+$NH_3); ^{13}C NMR ((CD$_3$)$_2$SO) δ 36.50 (N*C*H$_3$), 65.15 (*C*H$_2$), 111.63 (*C*HCCO), 113.88 (*C*NH$_3$), 120.56 (*C*CO), 123.97 (*C*HNCH$_3$), 127.76 (*o*-*C*H of Ph), 127.96 (*p*-*C*H of Ph), 128.40 (*m*-*C*H of Ph), 136.13 (*ipso*-*C* of Ph), 159.36 (*C*O); MS (FAB) *m*/*z* (%): 231 (100) [M$^+$−Cl], 243 (10) [M$^+$−HCl+Na].

4.4. Dibenzyl-4,9-methano-1,6-dimethyl-4,5,9,10-tetrahydro-1*H*,6*H*-dipyrrolo-[3,2-*b*:3′,2′-*f*][1,5]diazocin-2,7-dicarboxylate (5)

Hydrochloride **4** (5.00 g, 18.75 mmol) was dissolved in ethanol (50 ml), and then formaldehyde (7.5 ml, 36% aqueous solution) and concd HCl (7.5 ml) were added. The reaction mixture was stirred for 24 h at room temperature and then concentrated under reduced pressure to one half of the original volume, diluted with water (100 ml), and finally basified with ammonia solution. The basic mixture (pH 14) was extracted with dichloromethane (3×50 ml) and the combined organic layers were dried over anhydrous $MgSO_4$ and evaporated to dryness. The residue was crystallized (dichloromethane/diethyl ether) to give benzyl ester **5** (2.75 g, 59%) as colorless crystals.

Mp 182–185 °C; ^{1}H NMR ($CDCl_3$) δ 3.64 (6H, s, NCH_3), 3.92 (2H, d, *J*=15.9, *endo* C*H*HN), 4.09 (2H, s, NCH_2N), 4.36 (2H, d, *J*=16.5, *exo* CH*H*N), 5.24 (4H, s, CH_2Ph), 6.77 (2H, s, C*H*CCO), 7.36 (10H, m, C*H*-Ph); ^{13}C NMR ($CDCl_3$) δ 32.56 (N*C*H$_3$), 52.21 (C*C*H$_2$N), 65.31 (*C*H$_2$Ph), 68.90 (N*C*H$_2$N), 110.29 (*C*HCCO), 119.76 (*C*CO), 127.18 (*C*CH$_2$N), 127.86 (*o*-*C*H of Ph), 127.97 (*p*-*C*H of Ph), 128.46 (*m*-*C*H of Ph), 131.24 (*C*NCH$_2$), 136.50 (*ipso*-*C* of Ph), 160.90 (*C*O).

4.5. 4,9-Methano-1,6-dimethyl-4,5,9,10-tetrahydro-1*H*,6*H*-dipyrrolo-[3,2-*b*:3′,2′-*f*][1,5]diazocin-2,7-dicarboxylic acid (6)

To a solution of benzyl ester **5** (0.490 g, 0.99 mmol) in dry DMF (15 ml), a catalyst (5% Pd/C, 50 mg) was added. The reaction mixture was treated under hydrogen atmosphere (101.3 kPa) at room temperature for 3 h. The catalyst was filtered off and the filtrate was evaporated in vacuo to dryness. Brownish residue was stirred with methanol (3 ml) for 3 h and the mixture was then filtered to give acid **6** (275 mg, 88% yield) as a white powder.

Mp>200 °C (decomp.); ^{1}H NMR ((CD$_3$)$_2$SO) δ 3.56 (6H, s, NCH_3), 3.89 (2H, d, *J*=16.6, *endo* C*H*HN), 3.94 (2H, s, NCH_2N), 4.27 (2H, d, *J*=16.5, *exo* CH*H*N), 6.53 (2H, s, C*H*CCO); ^{13}C NMR ((CD$_3$)$_2$SO) δ 32.82 (N*C*H$_3$), 51.52 (C*C*H$_2$N), 68.28 (N*C*H$_2$N), 109.67 (*C*HCCO), 119.59 (*C*CO), 127.23 (*C*CH$_2$N), 130.95 (*C*NCH$_2$), 162.11 (*C*O); MS (FAB) *m*/*z* (%): 317 (100) [M$^+$+H]; elemental analysis calcd (%) for $C_{15}H_{16}N_4O_4$ (316.32): C 56.96, H 5.10, N 17.71; found: C 56.87, H 5.51, N 17.81.

4.6. Bis(benzotriazol-1-yl)-4,9-methano-1,6-dimethyl-4,5,9,10-tetrahydro-1*H*,6*H*-dipyrrolo-[3,2-*b*:3′,2′-*f*]-[1,5]diazocin-2,7-dicarboxylate (2)

A solution of acid **6** (100 mg, 0.32 mmol) with HOBt (120 mg, 0.889 mmol) and DCC (165 mg, 0.800 mmol) in dichloromethane (15 ml) and DMF (2 ml) was stirred for 12 h at room temperature. The insoluble part was filtered off and the filtrate was evaporated. The residue was chromatographed on silica (gradient from dichloromethane/diethyl ether 7:3 to diethyl ether) to give active ester **2** (115 mg, 66%), which was further purified by precipitation (dichloromethane/diethyl ether).

Mp>220 °C (decomp.); ^{1}H NMR ($CDCl_3$) δ 3.70 (6H, s, NCH_3), 4.09 (2H, d, *J*=16.8, *endo* C*H*HN), 4.20 (2H, s, NCH_2N), 4.51 (2H, d, *J*=16.8, *exo* CH*H*N), 7.22 (2H, s, C*H*CCO), 7.36–7.58 (6H, m, C*H*-Ar), 8.06 (2H, d, *J*=8.3, C*H*CNO); ^{13}C NMR ($CDCl_3$) δ 32.94 (N*C*H$_3$), 52.60 (C*C*H$_2$N), 68.77 (N*C*H$_2$N), 108.30 (*C*HCHCNN), 113.16 (*C*HCCO), 113.86 (*C*CO), 120.35 (*C*HCNO), 124.61 (*C*HCHCNO), 128.48 (*C*HCHCNN), 128.95 (*C*NN), 131.47 (*C*CH$_2$N), 132.51 (*C*CH$_2$N), 143.32 (*C*NO), 156.50 (CO); MS (FAB) *m*/*z* (%): 551 (65) [M$^+$+H], 416 (100) [M$^+$−C$_6$H$_4$N$_3$O]; elemental analysis calcd (%) for $C_{27}H_{22}N_{10}O_4$ (550.54): C 58.91, H 4.03, N 25.44; found: C 58.82, H 4.18, N 25.30.

4.6.1. Compound (4*R*,9*R*)-2. 1-Phenylethyl ester (*R*)(4*R*,9*R*)(*R*)-**15a** (504 mg, 0.961 mmol) was dissolved in DMF (10 ml), and then TEA (600 μl) and catalyst (5% Pd/C, 97 mg) were added. The reaction mixture was treated

under hydrogen atmosphere (101.3 kPa) at room temperature for 3 h. The catalyst was filtered off and the filtrate was diluted with dichloromethane (50 ml), and then HOBt (600 mg, 4.445 mmol) and DCC (800 mg, 3.879 mmol) were added. The reaction mixture was stirred for 12 h at room temperature and then evaporated in vacuo to dryness. Dichloromethane (15 ml) was added to the residue and the insoluble part was filtered off. Filtrate was chromatographed on silica (gradient from dichloromethane/diethyl ether 7:3 to diethyl ether) to give active ester (4*R*,9*R*)-**2** (400 mg, 83%), which was further purified by precipitation (dichloromethane/diethyl ether).

Compound (4*R*,9*R*)-**2** has identical NMR spectra as **2**; $[\alpha]_D^{20}$ +62 (*c* 0.156 g/100 ml DMF).

4.6.2. Compound (4*S*,9*S*)-2. Enantiomer (4*S*,9*S*)-**2** was prepared by the same synthetic procedure as (4*R*,9*R*)-**2**. Reduction of **15b** (200 mg, 17.3 mmol) by 5% Pd/C (40 mg) followed by reaction with HOBt (240 mg, 1.778 mmol) and DCC (320 mg, 1.551 mmol) gave active ester (4*S*,9*S*)-**2** (150 mg, 78%).

Compound (4*S*,9*S*)-**2** has identical NMR spectra as **2**; $[\alpha]_D^{20}$ –59 (*c* 0.409 g/100 ml DMF).

4.7. *N*,*N*′-Bis(dimethylaminopropan-3-yl)-4,9-methano-1,6-dimethyl-4,5,9,10-tetrahydro-1*H*,6*H*-dipyrrolo-[3,2-*b*:3′,2′-*f*][1,5]diazocin-2,7-dicarboxamide (11)

N,*N*-Dimethyl-propane-1,3-diamine **7** (2 ml) was added to a DMF (3 ml) solution of TB active ester (150 mg, 272 μmol) and the mixture was stirred for 5 h at room temperature. The reaction mixture was evaporated to dryness in vacuo and the residue was separated by column chromatography (concd aqueous ammonia/methanol 5:95) to give desired amide **11** (120 mg, 91%) as white powder.

Mp>220 °C (decomp.); ν_{max} (KBr)/cm^{-1} 3350 (NH), 1629 (C═O), 1560 and 1535 (pyrrole, ring vib.), and 1198 (C–N); ^{1}H NMR ((CD$_3$)$_2$SO) δ 1.55 (4H, quintet, *J*=7.2, C*H*$_2$CH$_2$N), 2.12 (12H, s, N(C*H*$_3$)$_2$), 2.23 (4H, t, *J*=7.2, CH$_2$C*H*$_2$N), 3.12 (4H, m, C*H*$_2$NH), 3.52 (6H, s, NC*H*$_3$), 3.75 (2H, d, *J*=16.2, *endo* C*H*HN), 3.89 (2H, s, NC*H*$_2$N), 4.25 (2H, d, *J*=16.2, *exo* CH*H*N), 6.46 (2H, s, C*H*CCO), 7.84 (2H, t, *J*=5.6, N*H*); ^{13}C NMR ((CD$_3$)$_2$SO) δ 27.17 (*C*H$_2$CH$_2$N), 32.03 (NCH$_3$), 36.78 (*C*H$_2$NH), 45.00 (N(*C*H$_3$)$_2$), 51.40 (C*C*H$_2$N), 56.80 (CH$_2$*C*H$_2$N), 68.47 (N*C*H$_2$N), 104.75 (*C*HCCO), 122.84 (*C*CO), 124.24 (*C*CH$_2$N), 130.39 (*C*NCH$_2$), 161.36 (*C*O); MS (FAB) *m*/*z* (%): 485 (100) [M$^+$+H]; elemental analysis calcd (%) for C$_{25}$H$_{40}$N$_8$O$_2$ (484.65): C 61.96, H 8.32, N 23.12; found: C 61.79, H 8.38, N 23.03.

4.8. General protocol for the preparation of dimers 12–14

The solution of amine (**8**–**10**) and TB active ester **2** in DMF was stirred for 12 h at 60 °C. The reaction mixture was evaporated to dryness in vacuo and the residue was separated by column chromatography (concd aqueous ammonia/methanol 5:95) to give corresponding TB distamycin dimer (**12**–**14**).

4.9. *N*,*N*′-Bis(5-[3-dimethylaminopropyl]aminocarbonyl-1-methylpyrrol-3-yl)-4,9-methano-1,6-dimethyl-4,5,9,10-tetrahydro-1*H*,6*H*-dipyrrolo-[3,2-*b*:3′,2′-*f*]-[1,5]diazocin-2,7-dicarboxamide (12)

Reaction of amine **8** (176 mg, 787 μmol) with active ester **2** (170 mg, 309 μmol) gave dimer **12** (150 mg, 67%) as a light yellow powder.

ν_{max} (KBr)/cm^{-1} 3400 (NH), 1636 (C═O), 1578 and 1533 (pyrrole, ring vib.), and 1198 (C–N); ^{1}H NMR ((CD$_3$)$_2$SO) δ 1.59 (4H, quintet, *J*=6.9, C*H*$_2$CH$_2$N), 2.15 (12H, s, N(C*H*$_3$)$_2$), 2.26 (4H, t, *J*=6.6, CH$_2$C*H*$_2$N), 3.16 (4H, q, *J*=6.2, C*H*$_2$NH), 3.58 (6H, s, NC*H*$_3$-Py$_0$), 3.76 (3H, s, NC*H*$_3$-Py$_1$), 3.83 (2H, d, *J*=16.2, *endo* C*H*HN), 3.95 (2H, s, NC*H*$_2$N), 4.32 (2H, d, *J*=16.2, *exo* CH*H*N), 6.68 (2H, s, C*H*CCO-Py$_0$), 6.76 (2H, d, *J*=1.9, C*H*CCO-Py$_1$), 7.12 (2H, d, *J*=1.7, C*H*NCH$_3$-Py$_1$), 8.06 (2H, t, *J*=5.6, CON*H*-Py$_1$), 9.65 (2H, s, CON*H*-Py$_0$); ^{13}C NMR ((CD$_3$)$_2$SO) δ 27.02 (*C*H$_2$CH$_2$N), 32.18 (N*C*H$_3$-Py$_0$), 35.95 (N*C*H$_3$-Py$_1$), 37.00 (*C*H$_2$NH), 45.01 (N(*C*H$_3$)$_2$), 51.52 (C*C*H$_2$N), 56.95 (CH$_2$*C*H$_2$N), 68.50 (N*C*H$_2$N), 103.91 (*C*HCCO-Py$_1$), 105.52 (*C*HCCO-Py$_0$), 117.64 (*C*HNCH$_3$), 122.11 (*C*NH-Py$_1$), 122.65 (*C*CO-Py$_0$), 123.01 (*C*CO-Py$_1$), 125.00 (*C*CH$_2$N), 130.56 (*C*NCH$_2$), 158.60 (*C*O-Py$_0$), 161.21 (*C*O-Py$_1$); MS (MALDI) *m*/*z* (%): 729 (100) [M$^+$+H]; elemental analysis calcd (%) for C$_{37}$H$_{52}$N$_{12}$O$_4$ (728.90): C 60.97, H 7.19, N 23.06; found: C 60.84, H 7.27, N 23.02.

4.10. *N*,*N*′-Bis(5-[{5-(3-dimethylaminopropyl)aminocarbonyl-1-methylpyrrol-3-yl}aminocarbonyl]-1-methylpyrrol-3-yl)-4,9-methano-1,6-dimethyl-4,5,9,10-tetrahydro-1*H*,6*H*-dipyrrolo-[3,2-*b*:3′,2′-*f*][1,5]diazocin-2,7-dicarboxamide (13)

Reaction of amine **9** (138 mg, 398 μmol) with active ester **2** (90 mg, 163 μmol) gave dimer **13** (105 mg, 66%) as a yellow powder.

ν_{max} (KBr)/cm^{-1} 3400 (NH), 1640 (C═O), 1581 and 1531 (pyrrole, ring vib.), and 1198 (C–N); ^{1}H NMR ((CD$_3$)$_2$SO) δ 1.60 (4H, quintet, *J*=7.0, C*H*$_2$CH$_2$N), 2.16 (12H, s, N(C*H*$_3$)$_2$), 2.27 (4H, t, *J*=7.0, CH$_2$C*H*$_2$N), 3.17 (4H, m, C*H*$_2$NH), 3.60 (6H, s, NC*H*$_3$-Py$_0$), 3.77 (6H, s, NC*H*$_3$-Py$_2$), 3.81 (6H, s, NC*H*$_3$-Py$_1$), 3.85 (2H, d, *J*=16.5, *endo* C*H*HN), 3.96 (2H, s, NC*H*$_2$N), 4.34 (2H, d, *J*=16.0, *exo* CH*H*N), 6.69 (2H, s, C*H*CCO-Py$_0$), 6.81 (2H, d, *J*=1.8, C*H*CCO-Py$_2$), 6.98 (2H, d, *J*=1.8, C*H*CCO-Py$_1$), 7.16 (2H, d, *J*=1.8, C*H*NCH$_3$-Py$_2$), 7.18 (2H, d, *J*=1.8, C*H*NCH$_3$-Py$_1$), 8.07 (2H, t, *J*=5.5, CON*H*-Py$_2$), 9.72 (2H, s, CON*H*-Py$_0$), 9.87 (2H, s, CON*H*-Py$_1$); ^{13}C NMR ((CD$_3$)$_2$SO) δ 26.98 (*C*H$_2$CH$_2$N), 32.22 (N*C*H$_3$-Py$_0$), 35.97 (N*C*H$_3$-Py$_2$), 36.10 (N*C*H$_3$-Py$_1$), 36.99 (*C*H$_2$NH), 44.96 (N(*C*H$_3$)$_2$), 51.55 (C*C*H$_2$N), 56.92 (CH$_2$*C*H$_2$N), 68.52 (N*C*H$_2$N), 104.03 (*C*HCCO-Py$_2$), 104.58 (*C*HCCO-Py$_1$), 105.57 (*C*HCCO-Py$_0$), 117.77 (*C*HNCH$_3$-Py$_2$), 118.31 (*C*HNCH$_3$-Py$_1$), 122.15 (*C*NH-Py$_1$+Py$_2$), 122.64 (*C*CO-Py$_0$), 122.79 (*C*CO-Py$_1$), 122.99 (*C*CO-Py$_2$), 125.07 (*C*CH$_2$N), 130.59 (*C*NCH$_2$), 158.46 (CO-Py$_1$), 158.65 (CO-Py$_0$), 161.23 (*C*O-Py$_2$); MS (MALDI) *m*/*z* (%): 973 (100) [M$^+$+H], 995 (10) [M$^+$+Na]; elemental analysis calcd (%) for C$_{49}$H$_{64}$N$_{16}$O$_6$ (973.16): C 60.48, H 6.63, N 23.03; found: C 60.37, H 6.72, N 23.00.

4.11. *N*,*N*′-Bis(5-[{5-[{5-(3-dimethylaminopropyl)-aminocarbonyl-1-methylpyrrol-3-yl}aminocarbonyl]-1-methylpyrrol-3-yl}aminocarbonyl]-1-methylpyrrol-3-yl)-4,9-methano-1,6-dimethyl-4,5,9,10-tetrahydro-1*H*,6*H*-dipyrrolo-[3,2-*b*:3′,2′-*f*][1,5]diazocin-2,7-dicarboxamide (14)

Reaction of amine **10** (310 mg, 662 μmol) with active ester **2** (150 mg, 272 μmol) gave dimer **14** (106 mg, 39%) as a yellow powder.

ν_{max} (KBr)/cm^{-1} 3400 (NH), 1640 (C═O), 1581 and 1531 (pyrrole, ring vib.), and 1198 (C–N); ^{1}H NMR ((CD$_3$)$_2$SO) δ 1.63 (4H, quintet, J=6.9, CH_2CH$_2$N), 2.22 (12H, s, N(CH_3)$_2$), 2.35 (4H, t, J=6.9, CH$_2$CH_2N), 3.19 (4H, m, CH_2NH), 3.62 (6H, s, NCH_3-Py$_0$), 3.80 (6H, s, NCH_3-Py$_3$), 3.87 (14H, m, 2×NCH_3-(Py$_1$+Py$_2$)+*endo* CHHN), 3.98 (2H, s, NCH_2N), 4.36 (2H, d, J=16.0, *exo* CHHN), 6.72 (2H, s, CHCCO-Py$_0$), 6.84 (2H, s, CHCCO-Py$_3$), 7.01 (2H, s, CHCCO-Py$_1$), 7.04 (2H, s, CHCCO-Py$_2$), 7.18 (2H, s, CHNCH$_3$-Py$_3$), 7.20 (2H, s, CHNCH$_3$-Py$_2$), 7.23 (2H, s, CHNCH$_3$-Py$_1$), 8.08 (2H, t, J=5.1, CONH-Py$_3$), 9.73 (2H, s, CONH-Py$_0$), 9.89 (2H, s, CONH-Py$_2$), 9.94 (2H, s, CONH-Py$_1$); ^{13}C NMR ((CD$_3$)$_2$SO) δ 26.78 (CH_2CH$_2$N), 32.21 (NCH$_3$-Py$_0$), 35.96 (NCH$_3$-Py$_3$), 36.10 (NCH$_3$-Py$_1$+Py$_2$), 36.84 (CH$_2$NH), 44.73 (N(CH$_3$)$_2$), 51.53 (CCH$_2$N), 56.73 (CH$_2$CH$_2$N), 68.53 (NCH$_2$N), 104.09 (CHCCO-Py$_3$), 104.64 (CHCCO-Py$_{1\ or\ 2}$), 104.71 (CHCCO-Py$_{1\ or\ 2}$), 105.58 (CHCCO-Py$_0$), 117.81 (CHNCH$_3$-Py$_3$), 118.36 (CHNCH$_3$-Py$_2$), 118.43 (CHNCH$_3$-Py$_1$), 122.17 (CNH-Py$_{1-3}$+Py$_{1-3}$), 122.20 (CNH-Py$_{1-3}$), 122.63 (CCO-Py$_0$), 122.77 (CCO-Py$_1$+Py$_2$), 122.95 (CCO-Py$_3$), 125.07 (CCH$_2$N), 130.58 (CNCH$_2$), 158.48 (CO-Py$_{1\ or\ 2}$), 158.51 (CO-Py$_{1\ or\ 2}$), 158.65 (CO-Py$_0$), 161.27 (CO-Py$_3$); MS (MALDI) *m*/*z* (%): 1217 (100) [M$^+$+H], 615 (90) [M$^+$-C$_{31}$H$_{39}$N$_9$O$_4$]; elemental analysis calcd (%) for C$_{61}$H$_{76}$N$_{20}$O$_8$ (1217.42): C 60.18, H 6.29, N 23.01; found: C 60.04, H 6.34, N 22.84.

4.11.1. Compound (4*R*,9*R*)-14. Enantiomer (4*R*,9*R*)-**14** was prepared using general procedure. Reaction of amine **10** (165 mg, 352 μmol) with active ester (4*R*,9*R*)-**2** (80 mg, 145 μmol) gave dimer (4*R*,9*R*)-**14** (91 mg, 51%) as a yellow powder.

Compound (4*R*,9*R*)-**14** has identical NMR spectra as **14**; $[\alpha]_D^{20}$ −182 (*c* 0.060 g/100 ml DMF).

4.11.2. Compound (4*S*,9*S*)-14. Enantiomer (4*S*,9*S*)-**14** was prepared using general procedure. Reaction of amine **10** (170 mg, 363 μmol) with active ester (4*S*,9*S*)-**2** (80 mg, 145 μmol) gave dimer (4*S*,9*S*)-**14** (128 mg, 72%) as a yellow powder.

Compound (4*S*,9*S*)-**14** has identical NMR spectra as **14**; $[\alpha]_D^{20}$ +183 (*c* 0.055 g/100 ml DMF).

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.06.056.

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Synthesis of glutamic acid and glutamine peptides possessing a trifluoromethyl ketone group as SARS-CoV 3CL protease inhibitors

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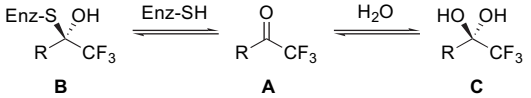
Abstract—Trifluoromethyl-β-amino alcohol **11** [(4*S*)-*tert*-butyl 4-amino-6,6,6-trifluoro-5-hydroxyhexanoate] was synthesized in five steps starting from Cbz-L-Glu-OH **5** where the key step involved the introduction of the trifluoromethyl (CF_3) group to oxazolidinone **7**, resulting in the formation of silyl ether **8** [(4*S*,5*S*)-benzyl 4-(2-(*tert*-butoxycarbonyl)ethyl)-5-(trifluoromethyl)-5-(trimethylsilyloxy)oxazolidine-3-carboxylate]. Compound **11** was then converted into four tri- and tetra-glutamic acid and glutamine peptides (**1**–**4**) possessing a CF_3-ketone group that exhibited inhibitory activity against severe acute respiratory syndrome coronavirus protease (SARS-CoV $3CL^{pro}$).

1. Introduction

In May 2003, two groups reported that a novel coronavirus (CoV) was the causative agent of severe acute respiratory syndrome (SARS).[1,2] CoV encodes a chymotrypsin-like protease ($3CL^{pro}$) that plays a pivotal role in the replication of the virus.[3] $3CL^{pro}$ is functionally analogous to the main picornavirus protease $3C^{pro}$ and both are cysteine proteases with a catalytic dyad (Cys-145 and His-41) in the active site, with Cys as the nucleophile and His as the general base.[4,5] Although a global SARS crisis was avoided in 2003 and the infection was contained, it is still a matter of necessity to find compounds that can inhibit SARS-CoV in case that the disease might re-emerge.

Compounds containing a trifluoromethyl ketone (CF_3-ketone) moiety form an important group of biologically useful fluorinated molecules[6] that can be used as protease inhibitors, as first described by Abeles et al.[7] The CF_3 group next to the carbonyl group thermodynamically stabilizes the hemi-ketal form relative to the ketone form, thus making the carbonyl prone to nucleophilic substitution by water, the active site Ser hydroxyl or Cys thiol group present in serine or cysteine proteases. Nucleophilic attack by the active site thiol in SARS-CoV $3CL^{pro}$ would convert the CF_3-ketone **A** to the tetrahedral adduct **B** (Scheme 1), which is believed to mimic the substrate–enzyme intermediate formed during substrate peptide-bond hydrolysis. Since adduct **B** is relatively stable, compound **A** would behave as a protease inhibitor,[8] suggesting that compounds containing a CF_3-ketone moiety may play an important role as $3CL^{pro}$ inhibitors. CF_3-ketone **A** also forms a relatively stable hydrate adduct **C** upon reacting with water. A unique and conservative recognition of the substrate's Gln residue at the P_1 site has been identified in the CoV cysteine protease family.[9] Therefore, a Gln-derived CF_3-ketone residue would contribute to the activity of SARS-CoV $3CL^{pro}$ inhibitors. Based on these considerations, a new synthetic method for forming Gln and Glu derivatives possessing a CF_3-ketone moiety was developed and this strategy was used in the synthesis of four peptides (compounds **1**–**4**).



B **A** **C**

Scheme 1. Trifluoromethyl ketone adducts.

Keywords: Trifluoromethyl ketone; Protease inhibitors; Severe acute respiratory syndrome coronavirus protease (SARS-CoV $3CL^{pro}$).
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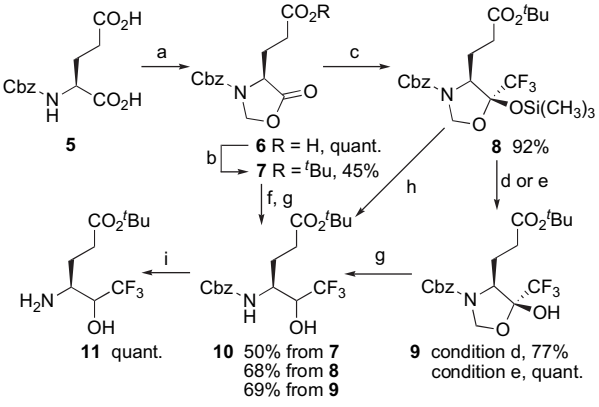
2. Results and discussion

2.1. Synthesis of trifluoromethyl-β-amino alcohol 11

The target compounds were envisioned being synthesized in two parts, viz, the peptide part and β-amino alcohol **11** containing the CF_3 unit. These two parts would then be coupled together and further elaborated to the desired target compounds. The synthesis of the key compound **11** started with oxazolidinone acid **6** prepared from Cbz-L-Glu-OH (**5**) under conditions described by Moore et al.[10] The resulting acid **6** was then converted to *tert*-butyl ester **7** (45%) that was expediently converted to silyl ether **8** (92% yield) (Scheme 2), which was isolated as a single diastereomer as determined by ^1H and ^{13}C NMR analyses, by utilizing a literature method.[11,12] The depicted stereochemistry for compound **8** is based on literature precedence for a very similar compound in which the addition of the CF_3 anion is *anti* to the side chain.[11] Product **8** was then readily desilylated upon treatment with tetrabutylammonium fluoride (TBAF) giving alcohol **9** in 77% yield.



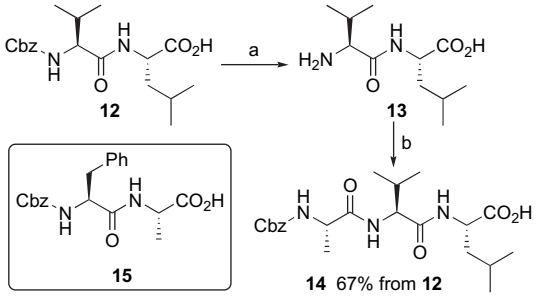
Scheme 2. Synthesis of β-amino alcohol **11**. Reagents and conditions: (a) paraformaldehyde, *p*-TsOH·H_2O, toluene, reflux, 1.67 h; (b) *t*-BuOH, EDC·HCl, DMAP, Et_3N, THF, rt, 16 h; (c) CsF, $CF_3Si(CH_3)_3$, THF, amb. temp, sonication, 2 h; (d) TBAF, THF, 0 °C–rt, 0.5 h; (e) MeOH/water (9:1), rt, 3 h; (f) CsF, $CF_3Si(CH_3)_3$, THF, sonication, amb. temp, 2 h then water, sonication, amb. temp, 0.5 h; (g) $NaBH_4$, MeOH, rt, 21 h; (h) $NaBH_4$, MeOH, rt, 16 h; (i) H_2, Pd/C (10%), MeOH, rt, 16 h.

We observed that compound **8** was partly converted to the desilylated product **9** when exposed to air. The cause of the partial protio-desilylation might be due to the moisture-sensitive nature of compound **8**. In the patent literature, there is one report of desilylation occurring upon stirring similar compounds in methanol,[13] most likely caused by water present in the methanol. For our substrate, we found that this method only proceeded when the reaction was carried out on a small scale (20 mg or less). However, by adding water to the methanol [methanol/water (9:1 v/v)], substrate **8** could be fully converted to compound **9** after 3 h stirring at room temperature (Scheme 2).

Compound **7** was also converted to the corresponding alcohol **9** (72% yield) in a one-pot reaction by adding small amount of water to the reaction mixture of intermediate **8** followed by sonication for an additional half hour. Finally, the desired alcohol **10** was obtained by treating compound **9** with $NaBH_4$ in methanol at room temperature. This gave target compound **10** as a ca. 4.5:1 mixture of diastereomers, as determined by ^1H and ^{13}C NMR analyses, in 69% yield. Among the different synthetic routes tried, treating a methanol solution of silyl ether **8** with $NaBH_4$ seems to be an efficient route to synthesize alcohol **10**. Under these conditions, we obtained the desired compound **10** in 68% yield (Scheme 2). Finally, the protecting group within substrate **10** could be easily cleaved off by hydrogenation over Pd/C (10%) affording alcohol **11** in quantitative yield.

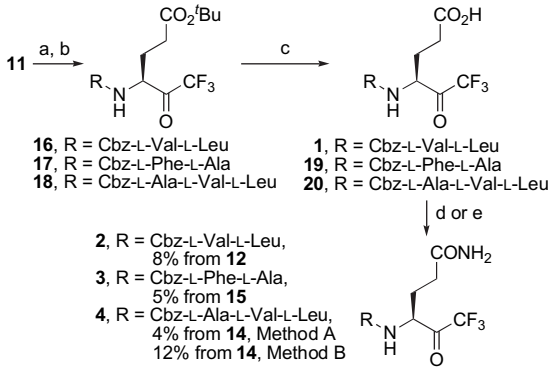
2.2. Synthesis of glutamic acid and glutamine peptides with a CF_3-ketone unit

With compound **11** prepared, focus could now shift toward the synthesis of the acid component coupling partners, namely peptides **12**, **14**, and **15**. Protected dipeptides **12** and **15** could be prepared following literature procedures[14,15] while tripeptide **14** could be prepared from dipeptide **12** as outlined in Scheme 3. The Cbz group within compound **12** could be removed using standard hydrogenation conditions, thus giving dipeptide **13** that was used directly in the next step. Coupling compound **13** with Cbz-L-Ala-OSu[16] afforded dipeptide **14** in 67% yield over the two steps.



Scheme 3. Synthesis of tripeptide **14** from dipeptide **12**. Reagents and conditions: (a) H_2, Pd/C (10%), MeOH/water/AcOH (9.5:5:1), rt, 2 h; (b) Cbz-L-Ala-OSu,[16] Et_3N, DMF, 0 °C–rt, 16 h.

Coupling of peptide **12** with β-amino alcohol **11** gave the expected amide (Scheme 4) that was used directly in the next step affording ketone **16**. Peptides **14** and **15** were subjected to the exactly same reaction sequences giving ketones **17** and **18**.



Scheme 4. The final steps toward the target compounds. Reagents and conditions: (a) peptide (**12**, **14** or **15**), HOBt, EDC·HCl, DMF, 0 °C–rt, 21 h; (b) Dess–Martin periodinane, CH_2Cl_2, rt, 19 h; (c) TFA, CH_2Cl_2, rt, 16 h; (d) HOBt, EDC·HCl, ammonia solution (28% aq solution), DMF, 16 h (Method A); (e) Boc_2O, NH_4HCO_3, pyridine, 1,4-dioxane, rt, 23 h (Method B).

Treating compound **16** with trifluoroacetic acid (TFA) resulted in clean removal of the *tert*-butyl group forming tripeptide **1**. Examination of the ^{13}C NMR spectrum did reveal that inhibitor **1** exists predominantly as the hydrate form in $CDCl_3$ (containing one drop of DMSO-d_6). ^{19}F NMR analysis of the same sample not only showed that the hydrate form was the dominant tautomer in the sample but that the two other possible tautomeric forms of tripeptide **1** were also present in small amount.[17] The equilibrium between the different tautomeric forms of this compound might shift depending on solvent. Due to the small amount of compound available, it was decided to study this in more detail by using a simpler model compound (vide infra).

Compounds **17** and **18** were subjected to the exactly same reaction conditions as ester **16** affording peptides **19** and **20**. The remaining crude tripeptide **1** and peptides **19** and **20** were subjected directly to the coupling conditions outlined in Scheme 4 (Method A), thus giving products **2**–**4** in 8, 5, and 4% yield over the four steps, respectively, after HPLC purification. The low chemical yield for the target compounds is a result of the last reaction sequence that seems to be very inefficient giving rise to many side products. In an effort to improve the yield for the last step, compound **4** was prepared by a mixed anhydride strategy using a slightly modified literature procedure (Method B).[18] By such means, we were able to improve the overall yield of inhibitor **4**, from peptide **14**, from 4 to 12%.

^{19}F NMR studies of the three glutamine peptides showed that compounds **2** and **4** only existed in the cyclic form while tripeptide **3** was a ca. 3.3:1 mixture of the cyclic and keto forms in $CDCl_3$.[19] Recently, similar observations were reported for glutamine fluoromethyl ketones by Cai et al.[20] Previously, there have also been reports that glutaminal compounds mostly exist as the hemiaminal in organic solvent.[21,22]

2.3. Synthesis of model Glu-CF_3 compounds

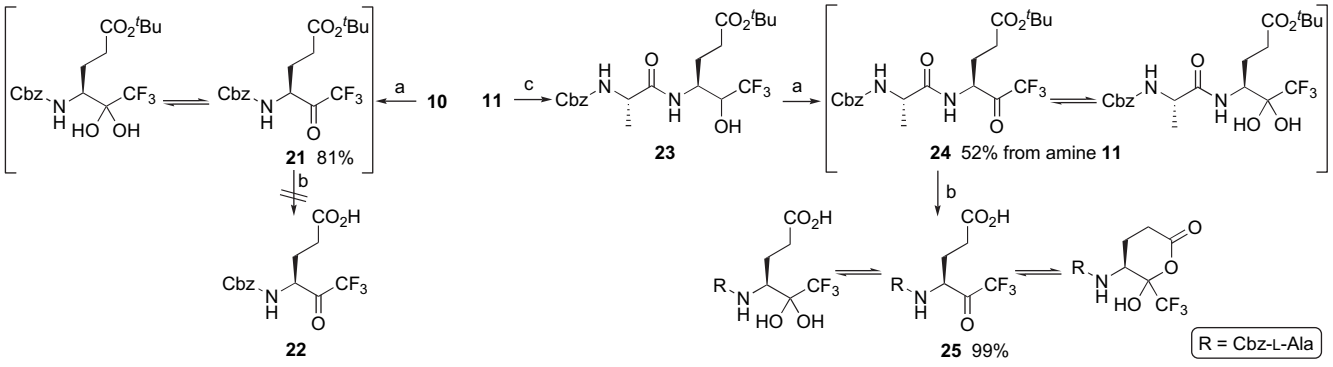
As previously noted, target peptide **1** was predominantly present in the hydrate form in $CDCl_3$. However, as alluded to in the previous section, this might differ depending on the solvent used for the NMR studies. Therefore, we decided to synthesize acid **22**, which is a much simpler molecule than the real system but, nevertheless, thought to be a good model for this study. To this end, alcohol **10** was converted to ketone **21** in 81% yield and as a ca. 2:1 mixture of the keto and hydrate forms as evident from ^{19}F and ^{13}C NMR analyses (Scheme 5). Attempts to convert compound **21** to the free acid **22** only resulted in the formation of decomposition products.

The lack of stability for our desired model compound forced us to use a slightly more complex acid for these studies. Compound **25** was synthesized in a three-step process, as outlined in Scheme 5, by first coupling Cbz-L-Ala-OH with amine **11**. This gave the desired alcohol **23**, which was directly oxidized to ketone **24** (52% yield over the two steps). From the ^{13}C and ^{19}F NMR analyses of this ketone, it became evident that the ketone exists as a ca. 7:3 mixture of the hydrate and keto forms. The rather unstable ketone **24** was then deprotected giving dipeptide **25** in almost quantitative yield in ca. 90% purity as determined by HPLC analysis. The ^{13}C NMR spectrum suggested that compound **25** exists mostly as the cyclic hemiacetal in $CDCl_3$ (resonance shifts from >170 to 75 ppm). This was also the case when the ^{13}C NMR spectrum was obtained for the same sample in CD_3OD.

NMR studies of model compounds **21**, **24**, and **25** in the predominant keto, hydrate, and hemiacetal forms, respectively, supported our assignment of compound **1** as existing mainly in the hydrate form in $CDCl_3$. This evidence was derived from the ^{19}F and ^{13}C NMR spectra of ketones **21** and **24** that were both present as a mixture of the keto and hydrate forms.[23] The work with the model compound also suggests that the form these acids appear in solution is highly solvent- and concentration-dependent.

2.4. Inhibitory activity of synthesized compounds

The inhibitory activity of the target compounds against SARS-CoV 3CL^{pro} was tested using a fluorescence-based peptide cleavage assay (Table 1).[23] We originally thought that the glutamine peptides (compounds **2**–**4**) would be the more potent inhibitors in these assays. However, the glutamate-possessing inhibitor **1** was the most potent of the group. The conformation that these compounds exist in during their interaction with the active site of SARS-CoV 3CL^{pro} is believed to contribute to binding affinity. Cai and co-workers found that their Gln fluoromethyl ketones exhibited low activity in their assays, a fact which they explained by referring to that their inhibitors predominantly exist in the cyclic form as evident from NMR studies.[20] Indeed, the cyclic form is



Scheme 5. Attempted synthesis of model compound **22** and synthesis of model compound **25**. Reagents and conditions: (a) Dess–Martin periodinane, CH_2Cl_2, rt, 16 h; (b) TFA, CH_2Cl_2, rt, 16 h; (c) HOBt, EDC·HCl, Cbz-L-Ala-OH, DMF, rt, 21 h.

Table 1. Inhibitory activity of peptides against the SARS-CoV 3CL^{pro}

Compound	Structure	K_i (μM)
1	Cbz-Val-Leu-Glu-CF_3	116.1±13.6
2	Cbz-Val-Leu-Gln-CF_3	>1000
3	Cbz-Phe-Ala-Gln-CF_3	844.4±120.3
4	Cbz-Ala-Val-Leu-Gln-CF_3	134.5±31.6

not expected to interact effectively with the active site of SARS-CoV.[20] The Gln compounds synthesized in our study were also found to be mainly in the cyclic form which may explain the low biological activity for these compounds. However, the Glu inhibitor **1** was found to mainly exist in the hydrate form, which is a form that most likely will interact more effectively with the active site.

3. Conclusion

A simple five-step procedure for the synthesis of β-amino alcohol **11** containing a CF_3 group was developed. This alcohol was further elaborated into four tri- and tetra-Glu and Gln peptides. Compounds **1** and **4** were found to be moderate SARS-CoV 3CL^{pro} inhibitors. Current work is focused on the co-crystallization of compounds **1** and **4** with SARS-CoV 3CL^{pro} in an attempt to elucidate their mode of action.

4. Experimental

4.1. General procedures

Melting points were measured on a Yanagimoto micro hot-stage apparatus and are uncorrected. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on either a JEOL JNM-AL300 spectrometer operating at 300 MHz for proton and 75 MHz for carbon, or a Varian UNITY INOVA 400NB spectrometer operating at 400 MHz for proton and 101 MHz for carbon. Chemical shifts were recorded as δ values in parts per million (ppm) downfield from tetramethylsilane (TMS). Fluorine (^{19}F) NMR spectra were recorded on a Varian UNITY INOVA 400 spectrometer operating at 376 MHz for fluorine. Fluorine NMR spectra were referenced externally to C_6F_6 at 0.00 ppm. Low-resolution mass spectra (ESI) were recorded on a Finnigan SSQ-7000 spectrophotometer. Low- and high-resolution mass spectra (FAB) were recorded on a JEOL JMS-SX102A spectrometer equipped with JMA-DA7000 data system. Low- and high-resolution mass spectra (CI) were recorded on a JEOL JMS-GCmate. Optical rotations were measured with a Horiba High-speed Accurate Polarimeter SEPA-300 at the sodium-D line (589 nm) at the concentrations (c, g 100 mL^{-1}). The measurements were carried out between 22 and 28 °C in a cell with path length (l) of 0.5 dm. Specific rotations $[\alpha]_D$ are given in 10^{-1} deg cm^2 g^{-1}. Preparative HPLC was carried out on a C18 reverse phase column (20×250 mm; YMC Pack ODS SH343-5) with a binary solvent system (a linear gradient of CH_3CN and aq TFA (0.1%) at a flow rate of 5.0 mL min^{-1}), detected at 230 nm. Analytical HPLC was performed using a C18 reverse phase column (4.6×150 mm; YMC Pack ODS AM302) with a binary solvent system (a linear gradient of CH_3CN and aq TFA (0.1%) at a flow rate of 0.9 mL min^{-1}), detected at 230 nm. The t_R given for the target compounds are obtained from analytical HPLC. Solvents used for HPLC were of HPLC grade and all other chemicals were of analytical grade or better.

4.1.1. (*S*)-3-[3-(Benzyloxycarbonyl)-5-oxooxazolidin-4-yl]propanoic acid 6.[10] This compound was synthesized according to the procedure in Ref. 10. $[\alpha]_D^{26}$ +80.5 (c 3.9, MeOH) {lit.[24] $[\alpha]_D^{25}$ +73 (c 2.35, MeOH)}.

4.1.2. (*S*)-*tert*-Butyl-3-[3-benzyloxycarbonyl-5-oxooxazolidin-4-yl]propanoate 7. DMAP (428 mg, 3.50 mmol) and EDC·HCl (1.83 g, 9.56 mmol) were added to a stirred solution of oxazolidinone acid **6** (2.26 g, 7.78 mmol) and *t*-BuOH (2.2 mL, 23.0 mmol) in THF (80 mL) at room temperature. The reaction mixture was then stirred for 5 min before triethylamine (1.1 mL, 7.89 mmol) was added dropwise. The reaction mixture was then stirred for 16 h before being diluted with EtOAc (100 mL) and washed with citric acid (2×50 mL of a 5% aq solution), $NaHCO_3$ (2×50 mL of a 5% aq solution) and brine (2×50 mL) before being dried (Na_2SO_4). Filtration and concentration under reduced pressure gave a light-yellow oil, which was subjected to flash chromatography (silica, hexane→hexane/EtOAc 9:1→4:1 gradient eluent). Concentration of the relevant fractions (R_f 0.3 in hexane/EtOAc 4:1) gave the title compound **7**[25] (1.22 g, 45%) as a clear, colorless oil: $[\alpha]_D^{25}$ +63.2 (c 3.86, EtOH) {lit.[25] $[\alpha]_D^{22}$ +27.9 (c 1.58, EtOH)}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41–7.30 (m, 5H), 5.54 (br s, 1H), 5.22 (d, J=4.8 Hz, 1H), 5.19 (s, 2H), 4.37 (t, J=5.3 Hz, 1H), 2.37–2.11 (m, 4H), 1.43 (s, 9H); MS (ESI+) m/z 372 (M$^+$+Na, 100%).

4.1.3. (4*S*,5*S*)-Benzyl 4-[2-(*tert*-butoxycarbonyl)ethyl]-5-trifluoromethyl-5-(trimethylsilyloxy)oxazolidine-3-carboxylate 8. Cesium fluoride (87.8 mg, 0.58 mmol) and (trifluoromethyl)trimethylsilane (0.73 mL, 4.94 mmol) were added to a solution of oxazolidinone **7** (986.0 mg, 3.98 mmol) in dry THF (20 mL) maintained under an argon atmosphere. The reaction mixture was then sonicated for 2 h at ambient temperature before being diluted with EtOAc (40 mL). The resulting solution was washed with water (1×20 mL) and brine (1×20 mL) before being dried ($MgSO_4$). Filtration and concentration under reduced pressure gave the title compound **8** (1.80 g, 92%) as a clear, yellow oil, which was >95% pure (as judged by ^{1}H NMR analysis): $[\alpha]_D^{28}$ +37.8 (c 1.26, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.27 (m, 5H), 5.41–5.29 (m, 1H), 5.14 (s, 2H), 4.83 (br s, 1H), 4.37 (br s, 1H), 2.32 (app. br s, 2H), 1.94 (6, J=7.0 Hz, 1H), 1.79 (app. br s, 1H), 1.41 (s, 9H), 0.20 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.0, 153.9, 135.7, 128.5, 128.2, 127.9, 122.2 (q, J_{C-F}=287.3 Hz), 102.2 (br), 80.3, 77.8, 67.8, 59.1, 31.7, 28.0, 23.9, 1.0; MS (CI+) m/z 492 (M$^+$+H, 1%), 436 (10), 401 (4), 392 (6), 334 (7),107 (7), 91 (100), 57 (47); HRMS (CI+): calcd for $C_{22}H_{33}NO_6F_3Si$ (M$^+$+H) 492.2029, found 492.2030.

4.1.4. (4*S*,5*R*)-Benzyl 4-[2-(*tert*-butoxycarbonyl)ethyl]-5-trifluoromethyl-5-hydroxyoxazolidine-3-carboxylate 9. Method A: TBAF (0.11 mL of a 1 M solution in THF, 0.11 mmol) was added dropwise to a stirred solution of compound **8** (44.5 mg, 0.091 mmol) in THF (2.0 mL) at 0 °C. The reaction mixture was then allowed to heat to room temperature and stirred for 0.5 h before being diluted with

EtOAc (15 mL). The organic phase was washed with water (2×10 mL) and brine (1×10 mL) before being dried ($MgSO_4$). Filtration and concentration under reduced pressure gave a yellow oil, which was subjected to flash chromatography (silica, hexane/EtOAc 4:1 eluent). Evaporation of the relevant fractions (R_f 0.2) gave the title alcohol **9** (29.3 mg, 77%) as a clear, yellow oil: $[\alpha]_D^{26}$ +41.0 (*c* 0.97, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.30 (m, 5H), 5.34 (br s, 1H), 5.17 (s, 2H), 4.88 (d, *J*=4.8 Hz, 1H), 4.40 (t, *J*=6.7 Hz, 1H), 2.40 (t, *J*=7.1 Hz, 2H), 2.14–1.97 (m, 2H), 1.43 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.5, 153.9, 135.6, 128.6, 128.4, 128.0, 122.4 (q, J_{C-F}=286.1 Hz), 101.1 (q, J_{C-F}=33.2 Hz), 81.4, 77.9, 67.9, 58.3, 31.2, 28.0, 22.8; MS (CI+) *m*/*z* 420 (M^++H, 2%), 364 (15), 91 (100); HRMS (CI+): calcd for $C_{19}H_{25}NO_6F_3$ (M^++H) 420.1634, found 420.1633. Method B: A solution of silyl ether **8** (129.3 mg, 0.26 mmol) in MeOH (4.5 mL) and water (0.5 mL) was stirred at room temperature for 3 h. The reaction mixture was then concentrated under reduced pressure to give the title alcohol **9** (110.2 mg, quant.), which was identical, in all respects, with the material obtained by Method A. The product was >95% pure (as judged by ^{1}H NMR analysis).

4.1.5. One-pot synthesis of compound 9 from compound 7. Method C: Cesium fluoride (37.9 mg, 0.25 mmol) and (trifluoromethyl)trimethylsilane (0.31 mL, 2.02 mmol) were added to a solution of oxazolidinone **7** (427.0 mg, 1.72 mmol) in dry THF (9.0 mL) maintained under an argon atmosphere. The reaction mixture was then sonicated for 2 h at ambient temperature before water (0.30 mL) was added and the reaction mixture was sonicated for an additional 0.5 h. The reaction mixture was then diluted with EtOAc (30 mL) and washed with water (1×10 mL) and brine (1×10 mL) before being dried ($MgSO_4$). Filtration and concentration under reduced pressure gave the title alcohol **9** (520.8 mg, 72%), which was identical, in all respects, with the material obtained via the stepwise method.

4.1.6. (4*S*)-*tert*-Butyl 4-(benzyloxycarbonyl)amino-6,6,6-trifluoro-5-hydroxyhexanoate 10. Sodium borohydride (0.76 g, 20.09 mmol) was added to a stirred solution of alcohol **9** (1.09 g, 2.60 mmol) in THF (70 mL) under an atmosphere of argon. The resulting reaction mixture was stirred for 23 h before being quenched by addition of water (10 mL). The water phase was then extracted with EtOAc (3×30 mL) and the combined organic fractions were dried ($MgSO_4$). Filtration and concentration under reduced pressure gave a light-yellow oil, which was subjected to flash chromatography (silica, hexane/EtOAc 3:1 eluent). Concentration of the relevant fractions (R_f 0.2) gave the title alcohol **10** (702.1 mg, 69%) as a clear, viscous, colorless oil and as a ca. 4.5:1 mixture of diastereomers (as judged by ^{1}H and ^{13}C NMR analyses): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.23 (m, 5H), 5.58 (d, *J*=9.3 Hz, 0.17H), 5.54 (d, *J*=9.3 Hz, 0.83H), 5.10 (br s, 0.83H), 5.05 (s, 1.67H), 4.87 (br s, 0.17H), 4.62 (s, 0.33H), 4.09–3.82 (m, 2H), 2.29 (t, *J*=7.4 Hz, 2H), 1.97–1.74 (m, 2H), 1.41 (s, 7.4H), 1.40 (s, 1.6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.1, 173.0, 156.5 (9), 156.5 (5), 136.0, 135.9, 128.4, 128.3, 128.1, 128.0, 127.9, 127.7, 127.5, 126.9, 124.4 (q, J_{C-F}=283.2 Hz), 81.0 (4), 81.0 (1), 71.9 (q, J_{C-F}=29.4 Hz), 70.6 (q, J_{C-F}=30.1 Hz), 67.0, 66.9, 51.2, 49.6, 31.8, 31.6, 27.8, 27.2, 23.7; MS (CI+) *m*/*z* 392 (M^++H, 3%), 336 (27), 292 (12), 91 (100); HRMS (CI+): calcd for $C_{18}H_{25}NO_5F_3$ (M^++H) 392.1684, found 392.1689.

4.1.7. Synthesis of compound 10 from compound 8. Sodium borohydride (78.8 mg, 2.08 mmol) was added to a stirred solution of silyl ether **8** (89.0 mg, 0.18 mmol) in MeOH (5.0 mL) at room temperature. The reaction mixture was then stirred at room temperature for 16 h before being quenched by addition of water (5.0 mL). The water phase was extracted with EtOAc (3×15 mL) and the combined organic fractions were dried ($MgSO_4$). Filtration and concentration under reduced pressure gave a light-yellow oil, which was subjected to flash chromatography (silica, hexane/EtOAc 3:1 eluent). Concentration of the relevant fractions (R_f 0.2) gave the title alcohol **10** (48.3 mg, 68%) as a viscous colorless oil and as a ca. 4.5:1 mixture of diastereomers (as judged by ^{1}H and ^{13}C NMR analyses). The material obtained via this method was identical, in all respects, with the material obtained via the reduction of alcohol **9**.

4.1.8. (4*S*)-*tert*-Butyl 4-amino-6,6,6-trifluoro-5-hydroxyhexanoate 11. Amine **10** (204.3 mg, 0.52 mmol) and Pd/C (10%) (21.0 mg) were stirred vigorously for 16 h in MeOH (6.0 mL) under an atmosphere of H_2. The reaction mixture was then diluted with MeOH (10 mL) and filtered through a plug of Celite® and washed afterwards with MeOH (3×10 mL). Concentration of the filtrate under reduced pressure gave the title amine **11** (134.3 mg, quant.) as a white solid: mp 93–95 °C and as a ca. 4.5:1 mixture of diastereomers (as judged by ^{1}H and ^{13}C NMR analyses): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.50–4.20 (br s, 3H), 4.10–3.85 (m, 1H), 3.54 (app. br s, 0.18H), 3.39 (app. br s, 0.82H), 2.55–2.29 (m, 2H), 2.17–1.85 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.7, 172.2, 125.3 (q, J_{C-F}=284.0 Hz), 125.2 (q, J_{C-F}=283.4 Hz), 81.0, 80.8, 71.4 (q, J_{C-F}=28.6 Hz), 70.2 (q, J_{C-F}=29.4 Hz), 51.6, 48.3, 32.3, 32.0, 29.7, 28.0 (2), 27.9 (9); MS (CI+) *m*/*z* 258 (M^++H, 12%), 242 (8), 202 (36), 102 (100); HRMS (CI+): calcd for $C_{10}H_{19}NO_3F_3$ (M^++H) 258.1317, found 258.1319. Anal. Calcd for $C_{10}H_{18}F_3NO_3\cdot 0.5H_2O$: C, 45.11; H, 7.19; N, 5.26. Found: C, 45.27; H, 6.79; N, 5.44.

4.1.9. *N*-Benzyloxycarbonyl-L-valyl-L-leucine 12.[14] This compound was synthesized according to the procedure in Ref. 14: mp 134–136 °C (lit.[4] mp 135–137 °C); $[\alpha]_D^{25}$ −20.5 (*c* 0.47, CH_2Cl_2) {lit.[4] $[\alpha]_D^{20}$ −24.0 (*c* 0.49, CH_2Cl_2)}. Anal. Calcd for $C_{19}H_{28}N_2O_5$: C, 62.62; H, 7.74; N, 7.69. Found: C, 62.82; H, 7.94; N, 7.87.

4.1.10. *N*-Benzyloxycarbonyl-L-alanyl-L-valyl-L-leucine 14. Protected dipeptide **12** (1.00 g, 2.74 mmol) and Pd/C (10%) (100.0 mg) were stirred vigorously in a mixture of MeOH (9.5 mL), water (5.0 mL), and acetic acid (1.0 mL) under an atmosphere of H_2 for 2 h. The reaction mixture was then filtered through a plug of Celite® and washed afterwards with MeOH (3×10 mL). Concentration under reduced pressure gave peptide **13**[26] (500.0 mg), which was used directly in the next step without further purification. A solution of *N*-hydroxysuccinimide ester of Cbz-L-Ala-OH[16] (694.0 mg, 2.17 mmol) in DMF (5.0 mL) was added dropwise to a solution of amine **13** (500.0 mg) and triethylamine (0.606 mL, 4.34 mmol) in DMF (10 mL) maintained at 0 °C

over the course of 30 min. The reaction mixture was then allowed to heat to room temperature and stirred for 16 h before being concentrated under reduced pressure. The resulting substrate was dissolved in EtOAc (40 mL) and washed with citric acid (2×20 mL of a 5% aq solution) and brine (1×20 mL) before being dried (Na_2SO_4). Filtration and concentration under reduced pressure gave a light-yellow solid, which was recrystallized from hexane/EtOAc to give the title protected peptide **14**[27] (800.0 mg, 67% over the two steps) as a white solid: mp 194–195 °C; $[\alpha]_D^{25}$ −52.4 (*c* 0.71, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38–7.30 (m, 5H), 6.99 (br d, *J*=7.3 Hz, 1H), 6.65 (d, *J*=7.3 Hz, 1H), 5.47 (br d, *J*=6.6 Hz, 1H), 5.11 (s, 2H), 4.56–4.49 (m, 1H), 4.26 (app. t, *J*=7.9 Hz, 2H), 3.49–3.40 (m, 1H), 2.17–1.54 (m, 3H), 1.3 (d, *J*=7.0 Hz, 3H), 0.95–0.88 (m, 12H) (one signal due to OH in COOH could not be discerned); ^{13}C NMR (75 MHz, $CDCl_3$+one drop of DMSO-d_6) δ 174.0, 172.3, 170.5, 155.6, 136.1, 128.1, 127.7, 66.3, 58.0, 50.4, 40.8, 33.6, 30.5, 22.4, 21.5, 18.9, 17.6 (one signal obscured or overlapping); MS (FAB+) *m*/*z* 436 (M+H, 7%), 305 (8), 222 (7), 91 (100); HRMS (FAB+): calcd for $C_{22}H_{40}N_3O_6$ (M^++H) 436.2448, found 236.2451. Anal. Calcd for $C_{22}H_{33}N_3O_6$: C, 60.67; H, 7.64; N, 9.65. Found: C, 60.89; H, 7.91; N, 9.88.

4.1.11. *N*-Benzyloxycarbonyl-L-phenylalanyl-L-alanine 15.[15] This compound was synthesized according to the procedure in Ref. 15: mp 157–159 °C (lit.[15] mp 157–158 °C); $[\alpha]_D^{24}$ −8.1 (*c* 0.65, EtOH) {lit.[15] $[\alpha]_D^{25}$ −9.5 (*c* 1.0, EtOH)}.

4.1.12. General procedure for the synthesis of (*S*)-4-[*N*-(benzyloxycarbonyl)-L-valyl-L-leucyl]amino-6,6,6-trifluoro-5-oxohexanoic acid 1, (*S*)-4-[*N*-(benzyloxycarbonyl)-L-phenylalanyl-L-alanyl]amino-6,6,6-trifluoro-5-oxohexanoic acid 19, and (*S*)-4-[*N*-(benzyloxycarbonyl)-L-alanyl-L-valyl-L-leucyl]amino-6,6,6-trifluoro-5-oxohexanoic acid 20. Coupling: HOBt (59.6 mg, 0.39 mmol) and EDC·HCl (80.4 mg, 0.42 mmol) were added to a stirred solution of the relevant protected peptide (0.39 mmol) in DMF (6.0 mL) at 0 °C. The reaction mixture was then stirred for 15 min before amine **11** (100.0 mg, 0.39 mmol) dissolved in DMF (6.0 mL) was added dropwise. The reaction mixture was then allowed to heat to room temperature and stirred for 21 h before DMF was removed under reduced pressure. The resulting residue was diluted with EtOAc (30 mL) and washed with citric acid (2×10 mL of a 5% aq solution), $NaHCO_3$ (2×10 mL of a 5% aq solution), and brine (2×10 mL) before being dried (Na_2SO_4). Filtration and concentration under reduced pressure gave the desired compound in quantitative yield. The crude product, which contained small amounts of impurities, was used in the next step without further purification. All products had satisfactory low-resolution mass spectra. Oxidation: Dess–Martin periodinane (439 mg, 1.04 mmol) was added to a stirred solution of the relevant peptide from the previous step in CH_2Cl_2 (15.0 mL) at 0 °C. The resulting reaction mixture was then allowed to heat to room temperature and stirred for 19 h before being filtered through a plug of Celite® and washed afterwards with EtOAc (3×15 mL). Concentration under reduced pressure gave the desired compound as a yellow oil. The material was used in the next step without further purification. All products had satisfactory low-resolution mass spectra. Deprotection: TFA (0.115 mL, 1.55 mmol) was added dropwise to a stirred solution of the relevant compound from the previous step in CH_2Cl_2 (4.0 mL) at 0 °C. The reaction mixture was then allowed to heat to room temperature and stirred for 16 h before being concentrated under reduced pressure. The crude product was used directly in the next step without further purification except for a small amount of the crude peptide **1**, which was purified at this stage in order to provide a sample for biological assaying.

4.1.13. (*S*)-4-[*N*-(Benzyloxycarbonyl)-L-valyl-L-leucyl]-amino-6,6,6-trifluoro-5-oxohexanoic acid 1. Part of the resulting yellow oil was subjected to preparative HPLC purification in order to provide a sample for biological testing. Concentration of the relevant fractions (t_R 23.7 min) gave the title compound **1** (5.9 mg) as a white solid and as a ca. 6:1 mixture of the hydrate and keto forms (as judged by ^{19}F NMR analysis) and the hydrate form existed as a ca. 1:1 mixture of rotamers (as judged by ^{13}C NMR analysis). Trace amounts of the cyclic form of this compound could also be seen by ^{19}F NMR: mp 172–173 °C; $[\alpha]_D^{22}$ −15.6 (*c* 0.28, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$+one drop of DMSO-d_6) δ 7.40–7.20 (m, 6H), 6.00 (dd, *J*=8.3 and 24.3 Hz, 1H), 5.1 (app. dd, *J*=12.1 and 16.5 Hz, 2H), 4.48–4.44 (m, 1H), 4.22–4.13 (m, 1H), 4.01 (q, *J*=8.1 Hz, 1H), 3.80–2.70 (br s, 2H), 2.35 (app. s, 2H), 2.23–1.88 (m, 3H), 1.72–1.47 (m, 3H), 0.96–0.88 (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$+one drop of DMSO-d_6) δ 175.9, 175.8, 174.4, 173.8, 172.0, 156.9, 156.7, 136.0, 128.4, 128.2, 128.1, 94.0 (q, J_{C-F}=29.8 Hz), 67.2, 67.0, 60.9, 60.8, 53.8, 53.6, 52.0, 51.9, 30.7, 30.6, 30.4, 24.5 (4), 24.4 (7), 23.1, 22.9, 22.8, 21.5, 21.4, 19.1, 17.7 (signal due to CF_3 group carbon could not be discerned); ^{19}F NMR (376 MHz, $CDCl_3$+one drop of DMSO-d_6) δ −74.8 (cyclic), −76.4 (keto), −76.5 (keto), −81.8 (hydrate), −81.9 (hydrate), −82.1 (hydrate), −82.2 (hydrate). (The appearance of four signals for the hydrate form of this compound in ^{19}F NMR is probably due to partial racemization over time at the α position of this compound. The extent of racemization at the time ^{19}F NMR was measured was less than 10%.[28]) MS (FAB+) *m*/*z* 546 (M^++H, 5%), 502 (1), 412 (1), 347 (4), 91 (100); HRMS (FAB+): calcd for $C_{25}H_{35}N_3O_7F_3$ (M^++H) 546.2427, found 546.2421. Anal. Calcd for $C_{25}H_{34}F_3N_3O_7 \cdot 1/4CF_3COOH \cdot H_2O$: C, 51.73; H, 6.17; N, 7.10. Found: C, 51.79; H, 6.34; N, 7.13.

4.1.14. (*S*)-4-[*N*-(Benzyloxycarbonyl)-L-phenylalanyl-L-alanyl]amino-6,6,6-trifluoro-5-oxohexanoic acid 19. MS (ESI−) *m*/*z* 550 (M−H, 64%), 442 (100).

4.1.15. (*S*)-4-[*N*-(Benzyloxycarbonyl)-L-alanyl-L-valyl-L-leucyl]amino-6,6,6-trifluoro-5-oxohexanoic acid 20. MS (ESI−) *m*/*z* 615 (M−H, 100%), 507 (60).

4.1.16. General procedure for the synthesis of (*S*)-4-[*N*-(benzyloxycarbonyl)-L-valyl-L-leucyl]amino-6,6,6-trifluoro-5-oxohexanamide 2, (*S*)-4-[*N*-(benzyloxycarbonyl)-L-phenylalanyl-L-alanyl]amino-6,6,6-trifluoro-5-oxohexanamide 3, and (*S*)-4-[*N*-(benzyloxycarbonyl)-L-alanyl-L-valyl-L-leucyl]amino-6,6,6-trifluoro-5-oxohexanamide 4. Method A: HOBt (25.0 mg, 0.16 mmol) and EDC·HCl (31.0 mg, 0.16 mmol) were added to a stirred solution of the relevant peptide from the previous step in DMF (7.0 mL) at 0 °C. The resulting reaction mixture

was then stirred for 15 min before ammonia solution (31.0 μL of a 28% aq solution) was added dropwise. The reaction mixture was then allowed to heat to room temperature and stirred for 16 h before DMF was removed under reduced pressure. The residue thus obtained was then dissolved in EtOAc (20 mL) and washed with citric acid (2×10 mL of a 5% aq solution), $NaHCO_3$ (2×10 mL of a 5% aq solution), and brine (2×10 mL) before being dried (Na_2SO_4). Filtration and concentration under reduced pressure gave the crude product, which was purified by preparative HPLC. Concentration of the relevant fractions gave the desired compounds in the yields stated below.

4.1.17. (*S*)-4-[*N*-(Benzyloxycarbonyl)-L-valyl-L-leucyl]-amino-6,6,6-trifluoro-5-oxohexanamide 2. Concentration of the relevant fractions (t_R 24.2 min) gave the title compound **2** (16.9 mg, 8% from acid **12**) as a white solid and as a ca. 1:1 mixture of rotamers (as judged by ^1H, ^{13}C, and ^{19}F NMR analyses): mp 112–113 °C; $[\alpha]_D^{26}$ +32.0 (*c* 0.07, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.45 (br d, *J*=8.1 Hz, 0.5H), 7.39–7.31 (m, 5H), 7.08 (app. br d, *J*=8.1 Hz, 0.5H), 6.83 (br s, 0.5H), 6.52 (br s, 0.5H), 6.48–6.41 (m, 1H), 5.38 (app. br d, *J*=5.7 Hz, 0.5H), 5.30 (app. br d, *J*=5.7 Hz, 0.5H), 5.12 (s, 2H), 4.66 (app. br s, 0.5H), 4.47 (app. br s, 1H), 4.38 (app. br d, *J*=6.6 Hz, 0.5H), 3.93 (app. br s, 1H), 2.66–2.43 (m, 2H), 2.22–1.45 (m, 11H), 0.97–0.88 (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 172.1, 172.0, 171.8 (4), 171.8 (0), 171.7 (6), 157.0, 135.6, 128.7, 128.6, 128.5, 128.2, 128.1, 67.8, 67.6, 61.3, 61.2, 52.3, 47.0, 40.0, 39.6 (4), 39.6 (0), 30.3, 24.8, 24.7, 22.8, 22.7, 21.6, 19.2, 19.1, 17.9, 17.8 (signal due to CF_3 group carbon and signal due to the carbon adjacent to the CF_3 group could not be discerned); ^{19}F NMR (376 MHz, $CDCl_3$) δ −82.7 (cyclic), −83.3 (cyclic); MS (ESI+) *m/z* 567 (M^++Na, 100%), 545 (M^++H, 5); HRMS (FAB+): calcd for $C_{25}H_{36}N_4O_6F_3$ (M^++H) 545.2587, found 545.2591. Anal. Calcd for $C_{25}H_{35}F_3N_4O_6 \cdot 1/4CF_3COOH \cdot 1/4H_2O$: C, 53.03; H, 6.24; N, 9.70. Found: C, 53.26; H, 6.35; N, 9.79.

4.1.18. (*S*)-4-[*N*-(Benzyloxycarbonyl)-L-phenylalanyl-L-alanyl]amino-6,6,6-trifluoro-5-oxohexanamide 3. Concentration of the relevant fractions (t_R 21.9 min) gave the title compound **3** (8.0 mg, 5% from acid **15**) as an off-white solid and as a ca. 1:1 mixture of rotamers (as judged by ^{13}C and ^{19}F NMR analyses) and as a ca. 3.3:1 mixture of the cyclic and keto forms (as judged by ^{19}F NMR): mp 108–111 °C; $[\alpha]_D^{27}$ −6.8 (*c* 0.24, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.38–7.07 (m, 14.5H), 5.47 (br s, 0.5H), 5.08–4.94 (m, 2H), 4.64–4.22 (m, 3H), 3.19–2.85 (m, 2H), 2.44 (app. br s, 2H), 2.11–1.77 (m, 2H), 1.28 (app. br s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.3 (3), 172.2 (8), 172.0 (2), 171.9 (7), 156.7, 156.6, 156.5, 135.7, 129.1, 128.9, 128.8, 128.6, 128.5, 128.3, 128.2, 128.0, 127.4, 67.6, 67.4, 56.5, 56.3, 49.2, 46.5, 45.8, 37.9, 37.8, 37.6, 29.6, 28.8, 22.8, 17.8, 17.1 (signal due to CF_3 group carbon and signal due to the carbon adjacent to the CF_3 group could not be discerned); ^{19}F NMR (376 MHz, $CDCl_3$) δ −76.2 (keto), −82.9 (cyclic), −83.2 (cyclic); MS (FAB+) *m/z* 573 (M^++Na, 7%), 551 (M^++H, 5); HRMS (FAB+): calcd for $C_{26}H_{30}N_4O_6F_3$ (M^++H) 551.2117, found 551.2114.

4.1.19. (*S*)-4-[*N*-(Benzyloxycarbonyl)-L-alanyl-L-valyl-L-leucyl]amino-6,6,6-trifluoro-5-oxohexanamide 4. Concentration of the relevant fractions (t_R 25.0 min) gave the title compound **4** (8.5 mg, 4% from acid **14**) as a white solid and as a ca. 1:1 mixture of rotamers (as judged by ^{13}C and ^{19}F NMR analyses): mp 140–141 °C; $[\alpha]_D^{27}$ −4.5 (*c* 0.11, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.38–7.36 (m, 3H), 7.33–7.30 (m, 2H), 7.13 (app. br d, *J*=8.4 Hz, 0.8H), 7.06–6.96 (m, 1.2H), 6.62 (app. br d, *J*=4.4 Hz, 0.8H), 6.49 (app. br d, *J*=4.4 Hz, 0.2H), 6.20 (s, 0.8H), 6.10 (s, 0.2H), 5.28 (br s, 0.8H), 5.24 (br s, 0.2H), 5.13 (d, *J*=4.0 Hz, 2H), 4.69 (dt, *J*=3.6 Hz, 0.2H), 4.64–4.56 (m, 0.2H), 4.54 (dt, *J*=3.6 Hz, 0.8H), 4.50–4.43 (m, 0.8H), 4.16–4.01 (m, 2H), 3.70–3.30 (br s, 0.2H), 2.64–2.14 (m, 4H), 1.95–1.90 (m, 1H), 1.87–1.76 (m, 1H), 1.45 (d, *J*=7.1 Hz, 3H), 0.96 (d, *J*=6.6 Hz, 6H), 0.91–0.86 (m, 6H) (the signal for three protons were obscured by the signal for residual water in the sample); ^1H NMR (400 MHz, $CDCl_3$+one drop of DMSO-d_6) δ 7.38–7.31 (m, 5H), 7.07–6.99 (m, 3H), 6.81 (app. br s, 1H), 6.42–6.23 (m, 2H), 5.11 (s, 2H), 4.55–4.39 (m, 2H), 4.19–4.08 (m, 2H), 3.70–3.30 (br s, 1H), 2.56–2.41 (m, 2H), 1.94–1.86 (m, 1H), 1.77–1.53 (m, 4H), 1.39 (d, *J*=7.1 Hz, 3H), 0.98–0.89 (m, 12H); ^{19}F NMR (376 MHz, $CDCl_3$+one drop of DMSO-d_6) δ −83.2 (cyclic), −83.3 (cyclic); MS (ESI+) *m/z* 654 (M^++K, 35%), 638 (M^++Na, 100), 616 (M^++H, 68); HRMS (FAB+): calcd for $C_{28}H_{41}N_5O_7F_3Na$ (M^++Na) 638.2778, found 638.2783.

4.1.20. (*S*)-4-[*N*-(Benzyloxycarbonyl)-L-alanyl-L-valyl-L-leucyl]amino-6,6,6-trifluoro-5-oxohexanamide 4. Method B: Pyridine (0.133 mL, 1.64 mmol) was added dropwise to a stirred solution of peptide **20** (39.1 mg) and di-*tert*-butyl dicarbonate (23.6 mg, 0.18 mmol) in 1,4-dioxane (13 mL) under an argon atmosphere at room temperature. Ammonium bicarbonate (324 mg, 4.10 mmol) was then added to the resulting solution and the reaction mixture was stirred at room temperature for 23 h before being diluted with EtOAc (20 mL). The organic phase was washed with citric acid (1×10 mL of a 5% aq solution), $NaHCO_3$ (1×10 mL of a 5% aq solution), and brine (1×10 mL) before being dried (Na_2SO_4). Filtration and concentration under reduced pressure gave a light-yellow solid, which was purified by preparative HPLC. Concentration of the relevant fractions (t_R 25.4 min) gave the title compound **4** (8.4 mg, 12% from peptide **14**), which was identical, in all respects, with the material obtained via Method A.

4.1.21. (*S*)-*tert*-Butyl 4-(benzyloxycarbonyl)amino-6,6,6-trifluoro-5-oxohexanoate 21. Dess–Martin periodinane (220.0 mg, 0.52 mmol) was added to a stirred solution of alcohol **10** (91.8 mg, 0.24 mmol) in CH_2Cl_2 (5.0 mL) at room temperature. The reaction mixture was then stirred for 16 h before being filtered through a plug of Celite® and washed afterwards with EtOAc (3×5 mL). The filtrate was concentrated under reduced pressure to give a light-yellow crude product, which was purified by flash chromatography (silica, hexane/EtOAc/triethylamine 50:49.6:0.4). Concentration of the relevant fractions (R_f 0.56 in hexane/EtOAc 1:1) gave the title compound **21** (74.4 mg, 81%) as a clear oil and as a ca. 2:1 mixture of the keto and hydrated forms (as judged by ^{19}F and ^{13}C NMR analyses): $[\alpha]_D^{25}$ +3.6 (*c* 0.73, MeOH); ^1H NMR (300 MHz, $CDCl_3$) δ 7.38–7.31 (m, 5H), 5.57 (br d,

J=7.7 Hz, 0.5H), 5.46 (br d, J=7.7 Hz, 0.5H), 5.11 (s, 2H), 4.88–4.78 (m, 0.5H), 3.96–3.86 (m, 0.5H), 2.42–2.09 (m, 3H), 1.97–1.86 (m, 1H), 1.43 (s, 4.5H), 1.42 (s, 4.5H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.3 (q, J_{C-F}=34.7 Hz), 173.5, 171.8, 157.9, 155.8, 135.7, 128.6, 128.5 (0), 128.4 (8), 128.3, 128.2, 128.1, 128.0, 127.7, 127.6 (4), 127.5 (8), 127.0, 123.1 (q, J_{C-F}=288.6 Hz), 115.5 (q, J_{C-F}=292.3 Hz), 94.2 (q, J_{C-F}=30.5 Hz), 81.5, 81.3, 67.5, 67.4, 55.5, 55.0, 31.8, 30.9, 27.9, 25.3, 23.2; ^{19}F NMR (376 MHz, $CDCl_3$) δ −76.5 (keto), −82.3 (hydrate); MS (ESI−) m/z 388 (M−H, 70%), 280 (100).

4.1.22. (*S*)-*tert*-Butyl 4-[*N*-(benzyloxycarbonyl)-L-alanyl]amino-6,6,6-trifluoro-5-oxohexanoate 24. HOBt (31.8 mg, 0.21 mmol) and EDC·HCl (43.6 mg, 0.23 mmol) were added to a stirred solution of Cbz-L-Ala-OH (44.1 mg, 0.198 mmol) in DMF (3.0 mL) at 0 °C. The reaction mixture was then stirred for 15 min before amine **11** (49.0 mg, 0.19 mmol) dissolved in DMF (2.0 mL) was added dropwise. The reaction mixture was then allowed to heat to room temperature and stirred for 21 h before DMF was removed under reduced pressure. The residue thus obtained was dissolved in EtOAc (20 mL) and washed with citric acid (2×10 mL of a 5% aq solution), $NaHCO_3$ (2×10 mL of a 5% aq solution), and brine (2×10 mL) before being dried (Na_2SO_4). Filtration and concentration under reduced pressure gave the title compound **23** (66.8 mg) as a clear, yellow oil. The material was used directly in the next step without further purification: MS (FAB+) m/z 485 (M^++Na, 5%), 463 (M^++H, 10), 407 (32), 363 (18), 91 (100); HRMS (FAB+): calcd for $C_{21}H_{30}N_2O_6F_3$ 463.2056, found 463.2061.

Dess–Martin periodinane (138.8 mg, 0.33 mmol) was added to a stirred solution of alcohol **23** (66.8 mg) in CH_2Cl_2 (3.0 mL) at room temperature. The reaction mixture was then stirred for 16 h before being diluted with EtOAc (10 mL) and filtered through a plug of Celite® and washed with EtOAc (3×10 mL). Concentration under reduced pressure gave a light-yellow oil, which was subjected to flash chromatography (silica, hexane/EtOAc/Et_3N 50:49.8:0.2 eluent). Concentration of the relevant fractions (R_f 0.2 in hexane/EtOAc 1:1) gave the title compound **24** (45.7 mg, 52% over the two steps) as a clear, colorless oil and as a ca. 7:3 mixture of the hydrate and keto forms (as judged by ^{1}H and ^{19}F NMR analyses) and both tautomers exist as a ca. 1:1 mixture of rotamers (as judged by ^{1}H, ^{13}C, and ^{19}F NMR analyses): $[\alpha]_D^{24}$ −9.1 (c 2.02, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56–7.27 (m, 5H), 7.14 (app. br d, J=6.6 Hz, 0.3H), 7.03 (app. d, J=8.4 Hz, 0.3H), 6.08–5.42 (br m, 1.7H), 5.12–5.05 (m, 2H), 4.90 (br s, 0.3H), 4.38–4.06 (m, 2H), 2.42–2.11 (m, 3H), 1.98–1.84 (m, 1H), 1.44 (s, 4H), 1.42 (4) (s, 4H), 1.42 (1) (s, 1H), 1.39–1.34 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.6 (q, J_{C-F}=34.7 Hz), 189.5 (q, J_{C-F}=34.7 Hz), 175.0, 174.8, 173.6, 173.2, 172.8, 172.3, 172.1, 156.3, 156.2, 156.0, 136.0, 135.9, 135.8, 128.5, 128.3, 128.2 (0), 128.1 (6), 128.0 (7), 128.0 (3), 128.0 (1), 123.1 (q, J_{C-F}=288.8 Hz), 115.5 (q, J_{C-F}=292.6 Hz), 94.3 (q, J_{C-F}=30.9 Hz), 94.2 (q, J_{C-F}=30.5 Hz), 81.7, 81.6, 81.3, 81.2, 67.3, 67.1, 53.9, 53.8, 53.7, 50.9, 50.7, 50.1, 31.8, 30.9 (2), 30.9 (0), 29.7, 27.9, 24.8, 23.4, 23.2, 18.4, 18.1; ^{19}F NMR (376 MHz, $CDCl_3$) δ −76.5 (keto), −82.0 (hydrate), −82.1 (hydrate) (one signal obscured or overlapping); MS (ESI−) m/z 459 (M−H, 38%), 351 (100).

4.1.23. 4-[*N*-(Benzyloxycarbonyl)-L-alanyl]amino-6,6,6-trifluoro-5-oxohexanoic acid 25. TFA (0.10 mL, 1.30 mmol) was added dropwise to a solution of ester **24** (40.0 mg, 0.087 mmol) in CH_2Cl_2 (5 mL) at room temperature. The reaction mixture was then stirred at room temperature for 24 h before being concentrated under reduced pressure to give the title compound **25** (34.9 mg, crude yield 99%) as a yellow oil and ca. 90% pure (as judged by HPLC analysis) and as a ca. 6:2:1 mixture of cyclic, keto, and hydrate forms (as judged by ^{19}F NMR analysis) and the cyclic form existed as a ca. 1:1 mixture of rotamers (as judged by ^{19}F NMR analysis): $[\alpha]_D^{24}$ +10.5 (c 0.77, $CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 7.38–7.25 (m, 5H), 5.08 (s, 2H), 4.18 (q, J=7.3 Hz, 1H), 3.04–2.86 (m, 1H), 2.58 (t, J=6.5 Hz, 1H), 1.38 (d, J=7.3 Hz, 3H), 1.46–1.23 (m, 2H) (signal for one proton was obscured by the signal for methanol); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.5, 176.9, 155.9, 136.0, 128.5, 128.2, 128.1, 122.4 (q, J_{C-F}=283.1 Hz), 75.0 (q, J_{C-F}=31.7 Hz), 67.1, 49.5, 34.0, 29.7, 27.7, 18.3; ^{13}C NMR (101 MHz, CD_3OD) δ 176.5, 176.0, 158.4, 138.2, 129.4, 129.0, 128.8, 75.8 (q, J_{C-F}=30.1 Hz), 67.5, 50.8, 35.3, 28.2, 17.9 (signal due to CF_3 group carbon could not be discerned and one signal was obscured or overlapping); ^{19}F NMR (376 MHz, $CDCl_3$) δ −74.6 (2) (cyclic), −74.6 (4) (cyclic), −76.2 (keto), −82.2 (1) (hydrate), −82.2 (3) (hydrate), −82.3 (0) (hydrate), −82.3 (2) (hydrate). (The appearance of four signals for the hydrate form of this compound in ^{19}F NMR is probably due to partial racemization over time at the α position of this compound. The extent of racemization at the time ^{19}F NMR was measured was less than 5%.[28]) MS (ESI−) m/z 403 (M−H, 46%), 222 (69), 199 (100).

4.2. Enzyme inhibitory assay

The inhibitory assay was performed using a commercially available fluorogenic substrate Dabcyl-KTSAVLQSGFRKME-Edans (Genesis Biotech, Taiwan) corresponding to the N-terminal autocleavage site of SARS 3CL^{pro}.[29] The change in fluorescence intensity was monitored in a Cary Eclipse fluorescence spectrophotometer (Varian) with 355 and 538 nm for excitation and emission wavelengths, respectively. Kinetic measurements were performed at 25 °C in buffer containing 10 mM sodium phosphate (pH 7.4), 10 mM sodium chloride, 1 mM EDTA, and 1 mM TCEP. The inhibition constant, K_i, was determined by measuring the apparent kinetic parameters at a constant substrate concentration with varying inhibitor concentrations (0–1 mM). The protease (final concentration of 1 mM) was incubated with inhibitor for 10 min at room temperature and the reaction was initiated by adding the substrate (a volume corresponding to a final concentration of 5 mM in the reaction mixture). The dependence of activity on the inhibitor concentration was analyzed in a manner similar to what was reported earlier.[30] Briefly, the kinetic parameters were determined by global nonlinear regression analysis to the equation.

$$v_I/v_0 = V_{max}[S]/\{[S] + K_m(1 + [I]/K_i)\}$$

where v_I and v_0 are the rate of substrate cleavage in the presence and absence of inhibitor, respectively. V_{max} is the

maximal rate, [*S*] is the substrate concentration, [*I*] is the inhibitor concentration, and K_m is the Michaelis constant.[31]

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Supplementary data

NMR spectra for all new compounds **1**, **2**, **3**, **4**, **8**, **9**, **10**, **11**, **21**, **24**, and **25**, and HPLC chromatograms of compounds **1**, **2**, **3**, and **4**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.06.052.

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Tetrahedron

Carboxylic acid to amide hydrogen bonding. 10-Oxo-semirubins

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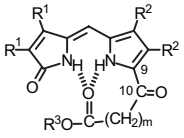
Abstract—Using their amide (and pyrrole) groups, dipyrrinones act as hydrogen bonding receptors for carboxylic acids, as found in a large number of 10-oxo-semirubins (**1**–**6**). The latter can be synthesized readily by Friedel–Crafts coupling of 9-*H* dipyrrinones with half-ester acid chlorides or diacid dichlorides of α,ω-dicarboxylic acids, ranging from C_2 to C_{10}. With ω-oxo-alkanoic acid chains of C_5 or $\geq C_5$, intramolecular hydrogen bonding is observed. With acid chains $<C_5$ hydrogen bonding is not observed. Uncharacteristically (for dipyrrinones), 10-oxo-dipyrrinone acids (**1**–**6**) and their corresponding esters (**1e**–**6e**) remain monomeric in hydrogen bond promoting solvents.

1. Introduction

Dipyrrinones[1] (Fig. 1A) are the component units and chromophores of bilirubin (Fig. 1B), the yellow-orange pigment of jaundice.[2] Previous studies showed that they tend to form intermolecularly hydrogen-bonded dimers (Fig. 1C) in the crystal[3,4] and in nonpolar solvents.[4,5] The association constant is surprisingly large (K_{assoc}~$3–4\times10^4$ at 23 °C) in $CDCl_3$,[5] given that simpler amide-to-amide hydrogen-bonded dimers have K_{assoc}~10^2 in $CHCl_3$.[6] In various dipyrrinones and their esters,[5] and in bilirubin dimethyl ester,[7] hydrogen-bonded dimers (Fig. 1C) prevail in nonpolar solvents. In bilirubin, however, the pigment is monomeric in solution[7] and in the crystal.[8] Its dipyrrinones are hydrogen-bonded intramolecularly to the opposing propionic CO_2H groups (Fig. 1D), and the resulting half-opened book, or ridge-tile-shaped conformation is greatly stabilized.[9] The requirements for intramolecular hydrogen bonding in bilirubin are: (1) a dipyrrinone and (2) a carboxylic acid with six carbons, as counted from dipyrrinone with nine carbons—as shown in [6]-semirubin (Fig. 1E),[10] the model for one-half bilirubin. [6]-Semirubin and its 10-oxo analog (a model for 10-oxo-bilirubin, a proposed bilirubin metabolite[11]) were shown to be monomeric in $CHCl_3$, which obeyed Beer's law and exhibited NOEs between the CO_2H and the lactam N*H*—all evidence for intramolecular hydrogen bonding.[10]

Later studies of [10]- and [20]-semirubins, and 10-oxo-[10]-semirubin showed even with very long carboxylic acid chains attached to C(9), the dipyrrinone chromophore is still strongly hydrogen-bonded intramolecularly to the CO_2H terminus.[12] Interestingly, the various [6]- and [10]-semirubin esters formed *intermolecularly* hydrogen-bonded dimers (as in Fig. 1C) in $CHCl_3$, but the corresponding 10-oxo-semirubin esters were monomers.[10,12] In the following, we report the syntheses of shorter chain 10-oxo-semirubins and their esters (see Structures below), along with their solution properties and hydrogen bonding.



10-Oxo-[n]-semirubins
(n=m+2)

Acid	[n]	m	R^1	R^2	R^3	R^3	Ester
1	2	0	Et	Et	H	Et	**1e**
2	4	2	Et	Et	H	Me	**2e**
3	5	3	Et	Et	H	Me	**3e**
4	6	4	Me	Me	H	Et	**4e**
5	7	5	Me	Me	H	Me	**5e**
6	10	8	Me	Me	H	Me	**6e**

Structures

2. Results and discussion

2.1. Synthesis

The syntheses of 10-oxo-semirubins **1**–**6** and their esters **1e**–**6e** are direct and short, assuming the availability of the required precursors 9-*H* dipyrrinones **7** and **8**.[13,14] The half-ester acid chloride (or diacid dichloride in the synthesis of **2**, **3**, and **6**) were obtained from the appropriate α,ω-dicarboxylic acid and reacted under Friedel–Crafts conditions (Scheme 1) with the relevant dipyrrinone: in cold CH_2Cl_2 and in the presence of anhydrous $AlCl_3$ (for **2**–**5e**) or $SnCl_4$ (for **1e** and **6**). Thus, **6** was obtained from **7** directly in 72% yield following aqueous acid work-up and purification by

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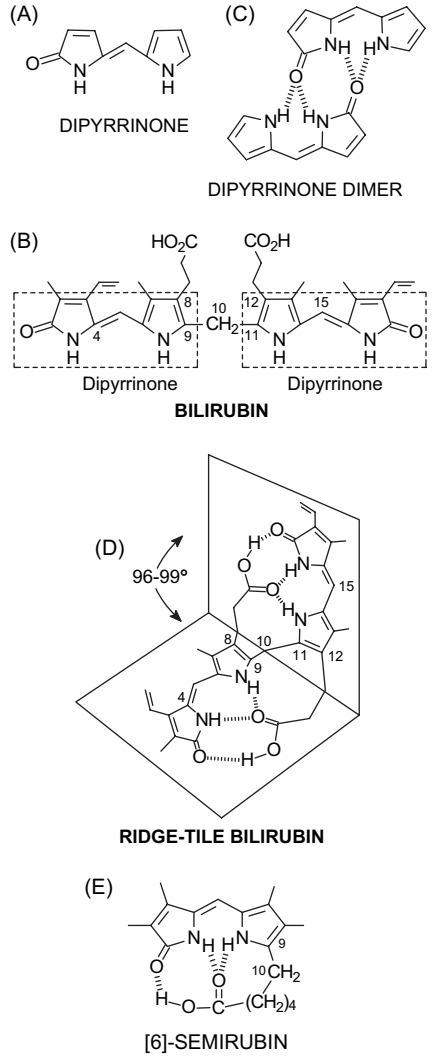


Figure 1. (A) Dipyrrinone chromophore. (B) Linear representation of bilirubin. (C) Dipyrrinone planar, hydrogen-bonded dimer. (D) The most stable conformation of bilirubin is not linear but it is shaped like a half-opened book, like a ridge-tile and stabilized by intramolecular hydrogen bonding. (E) Intramolecularly hydrogen-bonded dipyrrinone analog of bilirubin, called [6]-semirubin (where [6]=number of carbon atoms in the acid chain).

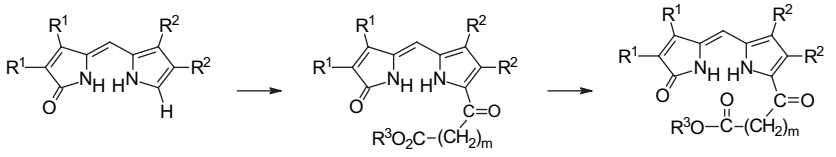
radial chromatography.[12] Its methyl ester (**6e**) was obtained in 93% yield following Fischer esterification.[12] Dipyrrinone **7** also served as precursor for **4e** and **5e′**, with yields of 64 and 40%, respectively. Saponification led to **4** and **6** in 81 and 88% yields.

To achieve improved solubility of 10-oxo-semirubins in $CHCl_3$, dipyrrinone **8**, with ethyl groups replacing methyls, was used as precursor. Thus, reaction of **8** with (i) monoethyl oxalyl chloride gave **1e** in 69% purified yield;[15] (ii) succinyl dichloride gave **2** in 22% purified yield; and (iii) glutaryl dichloride gave **3** in 35% purified yield. Ester **1e** was saponified easily and in high yield (70%) to the corresponding acid (**1**).[15]

2.2. Molecular structure

The constitutional structures of **1**–**6** and **1e**–**6e** (see Structures) follow from the structure of the well-known dipyrrinone starting materials (**7**[13] and **8**[14]) and from the method of synthesis. They were confirmed by their ^{13}C NMR spectra. Consistent with the postulated structures, 10-oxo-[2]-semirubin (**1**), 10-oxo-[4]-semirubin (**2**), 10-oxo-[5]-semirubin (**3**), 10-oxo-[6]-semirubin (**4**), 10-oxo-[7]-semirubin (**5**), and 10-oxo-[10]-semirubin (**6**) and their esters **1e**–**6e** show chemical shifts (Table 2) characteristic of the dipyrrinone core and the ω-oxo-alkanoic acid/ester fragment. The carbon resonances of acids **2**–**6** are scarcely distinguished from their corresponding esters in $(CD_3)_2SO$ (Table 1), except that the ester carbonyl is ∼1 ppm higher field than the acid, and an OCH_3 resonance is present.

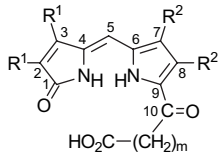
The carbon resonances of ring carbon at positions 2, 4, and 6 and the methyls or methylenes of **2**–**6** (Table 1) and **2e**–**6e** (Table 2) in $CDCl_3$ differ slightly from those in $(CD_3)_2SO$, while in $CDCl_3$ ring carbon at position 3 of **2**–**6** is more deshielded by ∼1 ppm. Ring carbon at position 9 of **4**–**6** and **3e**–**6e** is more shielded by ∼2 ppm in $CDCl_3$ than in $(CD_3)_2SO$. Larger differences appear at C(5), C(7), and C(8), which are ∼3–4 ppm more deshielded in $CDCl_3$. Of particular interest are the lactam C(1) and CO_2H carbonyl resonances of acids **2**–**6**, which are much more deshielded in $CDCl_3$ than in $(CD_3)_2SO$; yet the differences are only



	R^1	R^2			R^1	R^2	R^3	m			R^1	R^2	R^3	m
7	Me	Me	a	**4e,5e′**	Me	Me	Et	4,5	b	**4,5**	Me	Me	H	4,5
7	Me	Me	c	**6**	Me	Me	H	8	d	**6e**	Me	Me	Me	8
8	Et	Et	e	**1e**	Et	Et	Et	0	b	**1**	Et	Et	H	0
8	Et	Et	f	**2**	Et	Et	H	2	d	**2e**	Et	Et	Me	2
8	Et	Et	f	**3**	Et	Et	H	3	d	**3e**	Et	Et	Me	3

Scheme 1. Reagents and conditions: (a) $AlCl_3$+$EtO_2C(CH_2)_mCOCl$; (b) NaOH; (c) $SnCl_4$+$ClOC(CH_2)_8COCl$; (d) CH_3OH, H_2SO_4; (e) $SnCl_4$+EtO_2CCOCl; (f) $AlCl_3$+$ClOC(CH_2)_mCOCl$. [**5e′** is an ethyl ester, from which **5** is prepared by step (b). Compound **5e** is prepared from **5** by step (d).]

Table 1. ^{13}C NMR chemical shifts[a] of 10-oxo-semirubins **1–6**, in $CDCl_3$ and $(CD_3)_2SO$



	m	R^1	R^2
1	0	Et	Et
2	2	Et	Et
3	3	Et	Et
4	4	Me	Me
5	5	Me	Me
6	8	Me	Me

Carbon	Chemical shifts in $CDCl_3$						Chemical shifts in $(CD_3)_2SO$					
	1	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**
1	173.5	175.6	175.0	175.8	175.8	175.6	175.0	172.3	172.3	172.6	172.7	172.7
2	143.1	134.6	134.1	134.6	134.8	135.4	135.7	134.0	134.0	126.7	126.7	126.7
3	147.4	148.3	148.4	142.7	142.7	142.4	147.1	146.9	146.9	141.7	141.7	141.7
4	130.8	130.0	130.3	127.3	127.4	125.2	135.8	132.1	132.0	135.5	135.5	135.9
5	95.6	98.7	98.7	100.0	99.2	98.8	94.8	95.9	95.9	96.2	96.2	96.2
6	139.2	133.7	133.0	130.5	131.4	131.6	129.4	128.8	128.8	125.7	128.3	128.3
7	135.1	132.5	132.2	128.7	128.0	128.3	125.7	127.8	127.7	122.7	122.7	122.8
8	126.9	128.3	127.9	125.3	125.5	124.4	132.3	129.3	129.5	128.4	125.6	125.6
9	135.0	131.2	131.5	127.9	127.9	127.6	131.7	131.7	131.7	130.3	130.3	130.3
10	166.3	187.9	189.7	191.1	192.3	190.6	172.5	188.1	189.3	189.6	189.7	189.9
10^1	—	34.4	39.4	39.8	39.9	[b]	—	33.8	37.7	38.8		[c]
10^2	—	28.8	20.7	23.0	30.2	[b]	—	27.8	19.2	24.3	24.5	[c]
10^3	—	—	33.4	25.2	21.6	[b]	—	—	33.0	23.3	23.5	[c]
10^4	—	—	—	31.8	24.6	[b]	—	—	—	33.6	28.4	[c]
10^5	—	—	—	—	33.9	[b]	—	—	—	—	33.6	[c]
CO_2H	164.0	179.3	179.3	180.4	180.5	178.9	166.7	174.1	174.3	174.4	174.4	174.5
2^1-CH_2/CH_3	18.0	18.0	18.0	8.4	8.6	8.4	16.3	16.9	16.9	8.4	8.4	8.4
2^2-CH_3	15.5	15.7	15.8	—	—	—	16.2	15.6	15.6	—		—
3^1-CH_2/CH_3	17.3	17.3	17.4	9.8	10.1	9.9	16.8	16.5	16.5	9.5	9.5	9.6
3^2-CH_3	14.6	15.5	15.6	—	—	—	15.7	15.5	15.5	—		—
7^1-CH_2/CH_3	17.2	17.0	17.1	9.1	9.4	9.3	16.2	16.4	16.5	9.1	9.1	9.1
7^2-CH_3	13.8	14.0	14.0	—	—	—	15.3	13.7	13.7	—		—
8^1-CH_2/CH_3	19.2	18.6	18.7	11.0	11.4	11.5	17.7	17.9	17.9	11.2	11.2	11.3
8^2-CH_3	16.3	16.7	16.8	—		—	13.5	16.3	16.3	—		—

[a] δ, parts per million downfield from $(CH_3)_4Si$ for 10^{-2} M solution.
[b] Carbons 10^1–10^8, in order: 39.3, 33.9, 27.5, 27.3, 26.9, 23.43, 23.37 ppm.
[c] Carbons 10^1–10^8, in order: 33.7, 29.47, 28.9, 28.8, 28.7, 28.6, 28.55, 28.50 ppm.

small in their esters, **2e–6e**. As in earlier studies, the contrasting behavior of **2–6** (vs **2e–6e**) suggests intramolecular hydrogen bonding in the acids.

2.3. Molecularity in solution

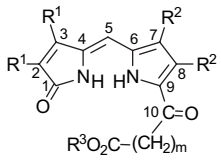
In order to assess whether **1–6** and **1e–6e** are monomeric in $CHCl_3$ solution, we determined their molecular weights by vapor pressure osmometry (VPO) over a molal concentration range $1.6–6.1\times10^{-3}$ mol/kg. The calibration standard was benzil (fw=210, $MW_{obs}=220\pm15$ g/mol), and the molecular weights determined for the compounds of this work are summarized in Table 3. The data indicate that all of the 10-oxo-semirubins, as well as their esters, are monomeric in $CHCl_3$ solution, and all obey Beer's law. In contrast, ordinary dipyrrinones and semirubin esters tend strongly toward dimerization. The differing behavior is apparently due to the presence and orientation of the oxo group: the C=O is probably oriented *anti* to the pyrrole NH,[4] leaving the alkyl chain oriented *syn* to the pyrrole NH and thus preventing intermolecular hydrogen bonding.

2.4. ^1H NMR and hydrogen bonding

syn-Z-Dipyrrinone N–H ^1H NMR chemical shifts in $(CD_3)_2SO$ all are typically very similar.[1,10,12] The 10-oxo-semirubins and their esters are no exception, as the data in Table 4 show. Thus the lactam and pyrrole NHs have similar chemical shifts, with the pyrrole NH being slightly more deshielded due to the presence of the nearby 10-oxo group.[4] In $CDCl_3$ solvent, however, where both intra- and intermolecular bonding is promoted, major differences in the NH chemical shifts are seen.

Dipyrrinones are avid participants in hydrogen bonding.[1,4,5,10,12,16–18] Diagnostic behavior and typical hydrogen bonding of this pattern are found in the planar dimer motif (Fig. 1C); the intrinsic N–H ^1H NMR chemical shifts of the lactam and pyrrole hydrogens of the monomer (δ ~8 ppm) become strongly deshielded to, approximately, 11 and 10 ppm, respectively, in nonpolar solvents such as $CDCl_3$.[4,5,17,19] However, when the dipyrrinones engage in hydrogen bonding with CO_2H groups, whether intermolecularly (Fig. 1C)[18] or intramolecularly[10,12] (Fig. 1E), the NH chemical shifts are relatively more shielded, especially the pyrrole NH (~9 ppm), and to a lesser degree the lactam NH (~10.5).[10,12,18] Similar chemical shifts are also found in tetrapyrroles such as bilirubin.[9,18,20] Consistent with these data for NH chemical shifts where the dipyrrinone is hydrogen-bonded to a CO_2H group, we observe lactam NH chemical shifts of 10.4–10.7 ppm and pyrrole NH chemical shifts of ~9.2 ppm for 10-oxo-semirubins **3–6** in $CDCl_3$. Though, we cannot strictly rule out the possibility that one or more H_2O molecules might intervene between the dipyrrinone moiety and the remote carboxylic acid group, special care was taken to exclude traces of water from the samples and

Table 2. ^{13}C NMR chemical shifts[a] of 10-oxo-semirubin esters **1e–6e** in $CDCl_3$ and $(CD_3)_2SO$



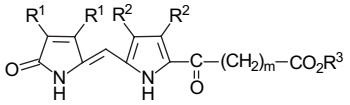
	m	R^1	R^2	R^3
1e	0	Et	Et	Et
2e	2	Et	Et	Me
3e	3	Et	Et	Me
4e	4	Me	Me	Et
5e	5	Me	Me	Me
6e	8	Me	Me	Me

Carbon	Dipyrrinone chemical shifts in $CDCl_3$						Dipyrrinone chemical shifts in $(CD_3)_2SO$					
	1e	**2e**	**3e**	**4e**	**5e**	**6e**	**1e**	**2e**	**3e**	**4e**	**5e**	**6e**
1	173.1	173.5	173.2	173.1	174.1	172.7	173.3	172.3	172.3	172.6	172.7	172.7
2	140.7	135.4	134.5	136.8	136.7	135.4	136.2	134.2	134.0	135.5	135.5	126.7
3	147.4	147.6	147.7	141.8	142.1	141.7	147.2	146.9	146.9	141.7	141.4	141.7
4	132.4	133.4	130.4	128.4	131.4	130.3	136.3	129.1	129.5	126.7	126.7	135.5
5	95.9	96.9	96.5	96.8	97.2	96.2	94.7	95.9	95.9	96.2	96.2	96.2
6	137.8	133.6	134.4	130.9	128.5	126.7	129.6	128.8	127.8	122.7	128.3	128.3
7	134.5	130.1	132.9	129.1	123.9	122.8	125.6	128.0	128.8	125.7	122.8	122.8
8	128.1	129.3	128.9	123.5	129.3	128.3	132.6	131.8	131.7	128.3	125.6	125.6
9	130.1	130.1	129.8	126.3	126.3	125.6	132.6	132.3	132.1	130.3	130.3	130.3
10	168.6	188.0	189.5	189.7	190.3	189.8	172.5	187.8	189.1	189.5	189.7	189.8
10^1	—	34.3	39.1	39.7	40.1	[b]	—	33.7	37.5	38.7	38.9	[c]
10^2	—	28.6	20.9	23.9	29.2	[b]	—	27.6	19.1	24.2	28.3	[c]
10^3	—	—	33.0	24.7	24.3	[b]	—	—	32.7	23.2	23.4	[c]
10^4	—	—	—	34.2	25.1	[b]	—	—	—	33.4	24.4	[c]
10^5	—	—	—	—	34.1	[b]	—	—	—	—	33.2	[c]
CO_2R	164.1	174.1	175.7	175.6	174.3	173.3	165.0	173.1	173.2	172.8	173.3	173.3
OCH_2/CH_3	62.9	52.2	53.0	60.3	51.6	51.1	62.0	51.3	51.2	59.6	51.1	51.2
CH_2CH_3	13.8	—	—	14.2		—	13.6	—	—	14.1	—	—
2^1-CH_2/CH_3	14.9	18.0	18.0	8.6	8.8	8.4	16.9	16.9	16.9	8.3	8.4	8.4
2^2-CH_3	17.3	16.1	15.8	—		—	16.2	15.6	15.6	—	—	—
3^1-CH_2/CH_3	18.0	17.5	17.4	9.9	10.0	9.6	16.4	16.4	16.5	9.5	9.6	9.6
3^2-CH_3	15.4	15.6	15.6	—		—	15.8	13.7	13.7	—	—	—
7^1-CH_2/CH_3	17.2	17.1	17.5	9.5	9.6	9.1	16.3	16.5	16.5	9.1	9.1	9.1
7^2-CH_3	14.2	14.0	14.0	—		—	15.3	15.5	15.5	—	—	—
8^1-CH_2/CH_3	18.9	18.9	18.7	11.7	11.9	11.2	17.4	17.9	17.9	11.2	11.2	11.2
8^2-CH_3	16.4	16.6	16.7	—		—	13.7	16.3	16.3	—	—	—

[a] δ, parts per million downfield from $(CH_3)_4Si$ for 10^{-2} M solutions.
[b] Carbons 10^1–10^8, in order: 40.2, 34.1, 29.4, 29.3, 29.1, 24.9, 24.5 ppm.
[c] Carbons 10^1–10^8, in order: 39.4, 23.8, 28.8, 28.7, 28.6, 28.4, 28.4, 33.3 ppm.

$CDCl_3$ solvent. In the absence of such procedures, the OH and NH resonances were somewhat broadened. We can rule out intermolecular hydrogen bonding between dipyrrinones and CO_2H groups of 10-oxo-semirubins **3–6**, as it has been reported for xanthobilirubic acid[18] because VPO studies indicate that they are monomeric in $CHCl_3$.

Table 3. Molecular weights determined by vapor pressure osmometry[a] and Beer's law behavior in chloroform solution for 10-oxo-dipyrrinones **1–6** and their esters **1e–6e**



Compound	*m*	R^1	R^2	R^3	FW[b]	MW[c]	Conc. range[d]	Beer's law[e]
1	0	Et	Et	H	344	386±11	$2.0–5.8\times10^{-3}$	✓
2	2	Et	Et	H	372	403±13	$1.8–5.6\times10^{-3}$	✓
3	3	Et	Et	H	386	398±5	$1.7–5.2\times10^{-3}$	✓
4	4	Me	Me	H	344	364±30	$2.2–6.1\times10^{-3}$	✓
5′	5	Me	Et[f]	H	386	381±11	$1.6–5.1\times10^{-3}$	✓
6	8	Me	Me	H	400	411±10	$1.6–5.7\times10^{-3}$	✓
1e	0	Et	Et	Et	372	374±7	$1.9–5.4\times10^{-3}$	✓
2e	2	Et	Et	Me	386	396±9	$1.9–5.6\times10^{-3}$	✓
3e	3	Et	Et	Me	400	384±36	$1.8–5.6\times10^{-3}$	✓
4e	4	Me	Me	Et	372	373±10	$1.7–5.5\times10^{-3}$	✓
5e	5	Me	Me	Et	386	385±4	$1.9–5.4\times10^{-3}$	✓
6e	8	Me	Me	Me	414	455±25	$2.0–5.0\times10^{-3}$	✓

[a] Calibrated with benzil (FW=210, measured MW=220±15) at 45 °C.
[b] Formula weight.
[c] Molecular weight in g/mol.
[d] mol/kg.
[e] Obeys Beer's Law (✓).
[f] Semirubin **5** was replaced in the VPO study with the more soluble analog **5′**.

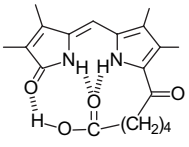
Table 4. Comparison of the dipyrrinone NH and CO_2H ^1H NMR chemical shifts[a] in $CDCl_3$ and $(CD_3)_2SO$ solvents[b]

10-Oxo-semirubin	δ (ppm) in $CDCl_3$			δ (ppm) in $(CD_3)_2SO$		
	Lactam	Pyrrole	CO_2H	Lactam	Pyrrole	CO_2H
1	11.83	10.01	14.34	11.14	10.52	[c]
2	10.4	9.2	[c]	10.4	10.7	12.1
3	10.57	9.39	13.16	10.36	10.77	12.04
4	10.66	9.21	12.80	10.33	10.75	11.99
5	10.74	9.10	13.09	10.34	10.75	11.97
6	10.40	9.22	12.03	10.35	10.74	11.95
1e	10.54	7.55	—	11.24	10.44	—
2e	9.37	8.24	—	10.3	10.73	—
3e	9.72	8.59	—	10.33	10.75	—
4e	9.14	8.05	—	10.32	10.74	—
5e	9.52	9.17	—	10.33	10.73	—
6e	9.28	8.48	—	10.34	10.73	—

[a] δ, Downfield from Me_4Si.
[b] Run as 10^{-2} M solutions in $(CD_3)_2SO$ and ~3×10^{-3} M solutions in $CDCl_3$.
[c] Not observed.

Although, the presence of C(10) carbonyl group might be expected to cause some differences in the NH chemical shifts in **3–6** relative to those of dipyrrinones with alkyl groups at C(9), the shielding of the pyrrole NH is typical of a dipyrrinone hydrogen-bonded to a carboxylic acid, as it is the chemical shift of the lactam NH. Thus, on the basis of the ^1H NMR NH chemical shifts it seems probable that the 10-oxo-semirubins **3–6** are strapped into a conformation shown in Figure 2 for **4**. Although the lactam, pyrrole, and carboxylic acid proton chemical shifts of **1** (and **1e**) differ greatly from **2–6** (and **2e–6e**), this is apparently a consequence of the acid group being directly conjugated with the dipyrrinone chromophore in **1** (and **1e**). Intramolecular hydrogen bonding is impossible in **1** (and **1e**), and intermolecular hydrogen bonding apparently does not occur, since VPO indicates only monomers in $CHCl_3$ solution.

The pyrrole and lactam NH chemical shifts of the 10-oxo-semirubin esters (**2e** and **6e**) in $CDCl_3$ are unusually shielded to 9.2 and 8.5 ppm, respectively. In contrast, monomeric dipyrrinones have corresponding NH chemical shifts at 7.7 and 8.1 ppm,[5] and intermolecularly hydrogen-bonded dipyrrinones exhibit corresponding chemical shifts more deshielded to ~11.2 and ~10.2 ppm,[4] and intramolecular hydrogen bonding also causes deshielding NH resonances.[7,10,12] VPO studies of **1e–6e** indicate that the monomers in $CHCl_3$ solution (Table 3), unusual for dipyrrinone esters, and their ^1H NMR NH chemical shifts in $CDCl_3$ do not correlate with either *intra* or *inter*molecular hydrogen bonding, e.g., the observed lactam NH chemical shift of **4e** (9.14 ppm) lies between that of a typical nonhydrogen-bonded dipyrrinone monomer (~7.7 ppm) and a hydrogen-bonded dimer (~11.2 ppm). One might expect the presence of the ester group to weaken any intramolecular hydrogen bonding of the ester carbonyls to the dipyrrinone NHs, thereby causing them to move upfield, thus explaining the atypical NH chemical shifts of **3e–6e**. The 8.5 ppm chemical shift of the pyrrole NH is indicative of an *anti* orientation of the C(10) carbonyl relative to the pyrrole NH,[4] suggesting that the ω-oxo-ester chain interferes with intermolecular hydrogen bonding but orients the ester chain for limited intramolecular hydrogen bonding. One may find a parallel for the dependence of the NH chemical shift on the orientation of the ketone carbonyl group in certain pyrryl ketones. For example, when the carbonyl is *anti* to the pyrrole NH, as in *tert*-butyl 2-(3,4-dimethylpyrryl) ketone, the NH chemical shift is 8.6 ppm, but when the carbonyl group is *syn*, as in *tert*-butyl 2-pyrryl ketone, it lies at 9.5 ppm.[21]



4

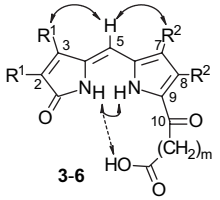
Figure 2. Intramolecularly hydrogen-bonded 10-oxo-[6]-semirubin (**4**).

2.5. Conformation and NOE

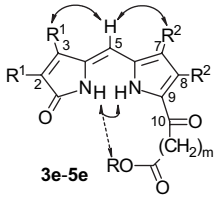
The structural assignment, particularly the *syn-Z*-configuration of the C(4) exocyclic double bond of the dipyrrinone moiety in **1–6** and **1e–6e** was confirmed by the observation of strong nuclear Overhauser effects (NOEs) in $CDCl_3$ between the lactam and pyrrole NHs, and moderate NOEs between the C(5)–H and the C(3) and C(7) methyls (or methylenes of the ethyls) (Fig. 3). Since we were interested in evidence for hydrogen bonding, the relative orientation of the alkanoic acid group and the dipyrrinone terminus was of considerable interest. Their close proximity in **3–6** and **3e–5e** was confirmed by NOEs observed between the carboxylic acid hydrogens and the lactam NHs. The data indicate a proximal spatial relationship between the carboxylic acid and lactam groups in **3–6** that is consistent with the intramolecular hydrogen bonding motif shown in the structural representation of Figures 1E and 2. Taken collectively, the NOE data are consistent with the VPO data, which show that **1–6** (and **1e–6e**) are monomeric in $CDCl_3$.

2.6. Molecular dynamics calculations

In support of the conclusions reached (above) by NMR spectroscopic analysis, molecular dynamics calculations[22] of 10-oxo-[5]-semirubin (**3**) and 10-oxo-[10]-semirubin (**6**) show that these compounds prefer intramolecularly hydrogen-bonded conformations (Fig. 4), which are computed to lie



3-6



3e-5e

Figure 3. Selected $^1H\{^1H\}$-NOEs found in 10-oxo-semirubins **3–6** and their esters (**3e–5e**) in $CDCl_3$ solvent are indicated by curved double-headed arrows. The dotted arrows signify weak NOEs.

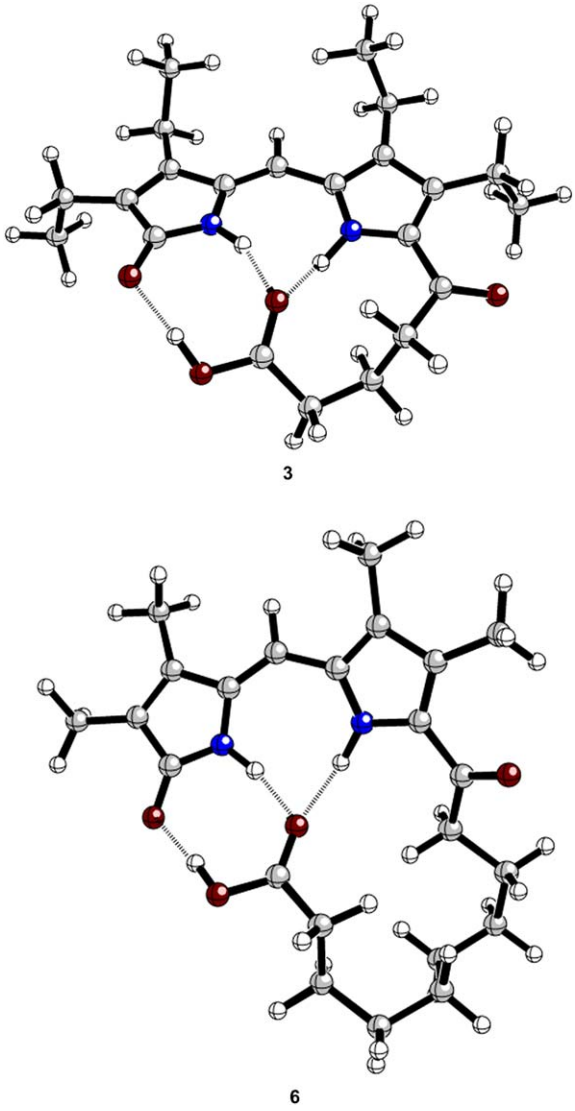


Figure 4. Energy-minimum structures of **3** and **6** from molecular dynamics calculations using Sybyl, Ref. 22.

some 12–13 kcal/mol lower in energy than the nonhydrogen-bonded forms. The intramolecularly hydrogen-bonded conformations have computed molecular parameters similar to those found in the dipyrrinones of bilirubin and mesobilirubin.[9,23,24] The dipyrrinone moieties of **3** and **6** are only slightly twisted, with C(4)–C(5)–C(6)–N torsion angles of ~15° and 22°, respectively.

2.7. Optical spectra

The UV–vis spectral data for **1**–**6** and **1e**–**6e** in solvents with a wide range of polarity are given in Table 5. The long wavelength bands of 10-oxo-semirubins (**1**–**6**) and their esters (**1e**–**6e**) have nearly the same λ_{max} in polar solvents, except λ_{max} of the acids that is shifted bathochromically by 10–15 nm in nonpolar solvents, solvents likely to promote hydrogen bonding. Smaller wavelength shifts attend the spectra of the esters over the range of solvents used. While the spectral shifts do not unambiguously confirm an intramolecularly hydrogen-bonded structure for **1**–**9**, they lend support to this conclusion, based on NMR spectral analysis and VPO studies, and they are consistent with the ability of the 10-oxo-semirubin acids of this study to adopt a unique conformational structure in nonpolar solvents.

3. Concluding comments

The presence of an oxo group in **1**–**6** and **1e**–**6e** does little to inhibit intramolecular hydrogen bonding and appears to inhibit dimer formation. From VPO measurements, it was found that 10-oxo-semirubins and their esters are monomeric in $CHCl_3$, a solvent that promotes hydrogen bonding. The preferred *anti* orientation of the oxo group[4] of 10-oxo-semirubins directs the alkanoic chain toward the dipyrrinone NHs, thereby promoting intramolecular hydrogen bonding in the case of acids (but probably to a lesser extent in the esters). The *anti* orientation of the oxo group effectively inhibits the formation of dipyrrinone dimers of the type shown in Figure 1C.

4. Experimental

4.1. General procedures

All UV–vis spectra were recorded on a Perkin–Elmer λ-12 spectrophotometer, and vapor pressure osmometry (VPO) measurements were performed using an Osmomat 070 (Gonotec, Berlin, Germany) in $CHCl_3$ at 45 °C with benzil as calibration standard. Nuclear magnetic resonance (NMR) spectra were obtained on a GE QE-300 spectrometer operating at 300 MHz, or on a Varian Unity Plus 500 MHz spectrometer in $CDCl_3$ solvent (unless otherwise specified). Chemical shifts δ were reported in parts per million referenced to the residual $CHCl_3$; ^1H signal at 7.26 ppm and ^{13}C signal at 77.0 ppm. To ensure anhydrous samples and solvent in the ^1H NMR experiments, the samples were dried under vacuum in a drying pistol at refluxing ethanol or toluene temperature and using P_2O_5 desiccant. The $CDCl_3$ solvent was stored over CaH_2 after having been passed through a column of Woelm basic Al_2O_3 (super Act 1). Heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) spectra were used to assign ^{13}C NMR spectra. Melting points were taken on a Mel Temp capillary apparatus and are uncorrected. Combustion analyses were carried out by Desert Analytics, Tucson, AZ. Analytical thin layer chromatography was carried out on J.T. Baker silica gel IB-F plates (125 μm layers). Flash chromatography was carried out using Woelm silica gel F, thin layer chromatography grade. Radial chromatography was carried out on Merck silica gel PF_{254} with gypsum preparative layer grade, using a Chromatotron (Harrison Research, Palo Alto, CA). Spectral data were obtained in spectral grade solvents (Aldrich or Fisher). Dichloromethane, methanol, tetrahydrofuran, hexane, and 2-propanol were obtained from Fisher, and anhydrous stannic chloride, aluminum chloride, diacid chloride of succinic acid, and the half ethyl ester acid chloride of oxalic acid were obtained from Acros.

Deuterated chloroform and dimethyl sulfoxide were obtained from Cambridge Isotope Laboratories. Mono-ester acid chlorides of adipic and pimelic acids were synthesized

Table 5. Solvent dependence of UV–vis data for 10-oxo-semirubins **1–6** and esters **1e–6e**

	λ_{max} (ε_{max})[a]				
	C_6H_6	$CHCl_3$	CH_3OH	CH_3CN	$(CH_3)_2SO$
1	420 (19,450) 440 (19,450)	436 (18,000)	403 (23,300) 421 (21,400)	425 (17,500)	407 (24,800) 427 (23,800)
2	423 (31,750) 402 (32,700)	420 (29,000) 399 (31,750)	417 (29,000) 396 (32,700)	410 (23,950) 389 (29,950)	420 (32,250) 398 (34,550)
3	423 (44,050) 401 (38,250)	422 (42,000) 400 (38,250)	418 (31,700) 396 (34,550)	411 (26,350) 390 (30,050)	420 (32,500) 398 (34,550)
4	421 (19,200) 400 (20,600)	421 (27,500) 400 (28,600)	411 (22,200) 393 (27,200)	408 (16,300) 386 (21,700)	413 (17,800) 400 (20,600)
5	400 (29,150) 422 (29,150)	400 (29,150) 421 (27,950)	394 (28,550) —	388 (25,850) —	396 (28,250) 416 (24,300)
6	422 (19,800) 399 (21,600)	422 (26,300) 399 (28,500)	416 (24,300) 393 (28,400)	409 (18,300) 387 (23,400)	412 (19,800) 400 (21,700)
1e	424 (23,250)	427 (25,150)	424 (27,500) 410 (27,100)	412 (24,650)	432 (30,350) 411 (27,050)
2e	396 (27,800) 417 (23,400)	397 (28,250) 418 (23,400) sh	395 (32,650) 416 (28,250)	388 (29,750) 410 (23,400) sh	397 (32,150 419 (29,750)
3e	395 (34,850) 419 (35,900)	397 (33,750) 420 (32,100)	396 (32,100) 416 (28,300)	389 (29,950) 409 (24,500)	397 (32,650) 419 (29,950)
4e	415 (16,700) 395 (22,900)	415 (19,100) 394 (24,200)	412 (22,100) 393 (26,600)	406 (18,300) 384 (24,000)	414 (16,700) 395 (22,900)
5e	393 (23,300) 416 (16,000)	393 (23,650) 414 (17,850)	394 (29,500) 413 (24,050)	386 (27,850) 406 (21,500)	395 (30,200) 416 (25,500)
6e	419 (17,500) 397 (22,600)	419 (21,600) 398 (24,200)	418 (25,100) 393 (27,900)	409 (19,800) 386 (25,600)	414 (17,700) 396 (23,900)

[a] λ_{max} in nanometer, ε_{max} in L mol^{-1} cm^{-1}.

from the corresponding diacids (m=4 and 5) by standard literature procedures.[25] Eicosanedioyl dichloride was prepared by standard methods from eicosanedioic acid. (4*Z*)-2,3,7,8-Tetramethyl-10*H*-dipyrrin-1-one (**7**)[13] and (4*Z*)-2,3,7,8-tetraethyl-10*H*-dipyrrin-1-one (**8**)[14] were prepared as described in the literature. The syntheses of **1/1e**,[15] **4/4e**,[10] and **6/6e**[12] were reported previously.

4.1.1. (4*Z*)-9-(Carboethoxymethanoyl)-2,3,7,8-tetraethyl-(10*H*)-dipyrrin-1-one (1e). Prepared as described in the literature.[15] Mp 152–153 °C [lit.[15] mp 152–153 °C]; IR (NaCl, thin film) ν: 3310, 2477, 2933, 2873, 1737, 1702, 1682, 1638, 1213 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.16 (m, 9H), 1.22 (t, J=7.69 Hz, 3H), 1.43 (t, J=6.95 Hz, 3H), 2.4 (q, J=7.69 Hz, 2H), 2.54 (m, 4H), 2.81 (q, J=7.69 Hz, 2H), 4.39 (q, J=6.69 Hz, 2H), 5.94 (s, 1H), 7.56 (br s, 1H), 10.54 (br s, 1H) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz) and UV–vis data are given in Table 2 and Table 5, respectively.

4.1.2. (4*Z*)-9-(3-Carbomethoxypropanoyl)-2,3,7,8-tetramethyl-(10*H*)-dipyrrin-1-one (2e). Acid **2** (40 mg, 0.11 mmol) was dissolved in CH_3OH (25 mL), then 10% sulfuric acid (5 mL) was added slowly, and the solution was heated to reflux for 1 h. The solution was cooled to room temperature and taken up in CH_2Cl_2 and washed with satd aq $NaHCO_3$ (2×50 mL). The organic layer was separated and dried with Na_2SO_4 and the solvent was removed. The residue was crystallized with hexane–CH_2Cl_2 to give 35 mg (85%) of pure **2**. Mp 148 °C; IR (NaCl, film) ν: 3325, 2968, 1742, 1673, 1651, 1225, 1164, 946 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.13 (t, J=7.76 Hz, 3H), 1.14 (t, J=7.76 Hz, 3H), 1.21 (m, 6H), 2.39 (q, J=7.76 Hz, 2H), 2.52 (q, J=7.76 Hz, 2H), 2.54 (q, J=7.3 Hz, 2H), 2.77 (q, J=6.85 Hz, 2H), 2.78 (q, J=6.85 Hz, 2H), 3.14 (t, J=6.85 Hz, 2H), 3.73 (s, 3H), 5.94 (s, 1H), 8.24 (br, 1H), 9.37 (br, 1H) ppm; ^{13}C NMR (DMSO-d_6, 125 MHz) and UV–vis data are given in Table 2 and Table 5, respectively.

Anal. Calcd for $C_{22}H_{30}O_4N_2$ (386): C, 68.37; H, 7.82; N, 7.25. Found: C, 68.15; H, 7.64; N, 7.02.

4.1.3. (4*Z*)-9-(4-Carboethoxybutanoyl)-2,3,7,8-tetraethyl-(10*H*)-dipyrrin-1-one (3e). As in the preparation of **2e** (above), **3** (40 mg, 0.1 mmol) was converted to its methyl ester to give 31 mg (76%) of pure **3e**. Mp 96–98 °C; IR (NaCl, film) ν: 3275, 2968, 2935, 1739, 1674, 1645, 1462, 1436, 1162 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 1.13 (t, J=7.52 Hz, 3H), 1.15 (t, J=7.52 Hz, 3H), 1.17 (t, J=7.26 Hz, 3H), 1.22 (t, J=7.52 Hz, 3H), 2.04 (m, 2H), 2.39 (q, J=7.78 Hz, 2H), 2.53 (m, 6H), 2.8 (m, 4H), 3.87 (s, 3H), 5.96 (s, 1H), 8.59 (br, 1H), 9.72 (br, 1H) ppm; ^{13}C NMR (DMSO-d_6, 125 MHz) and UV–vis data are given in Table 2 and Table 5, respectively.

Anal. Calcd for $C_{23}H_{32}O_4N_2$ (400): C, 68.97; H, 8.05; N, 6.99. Found: C, 68.99; H, 7.95; N, 6.99.

4.1.4. (4*Z*)-9-(6-Carboethoxyhexanoyl)-2,3,7,8-tetramethyl-(10*H*)-dipyrrin-1-one (5e). As in **2e** and **3e**, **5** (83 mg, 0.22 mmol) was converted to its methyl ester to give 59 mg (68%) of pure **5e**. Mp 147–149 °C; IR (NaCl,

film) ν: 3339, 2949, 1739, 1656, 1436, 1170, 760, 693 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.4 (m, 2H), 1.68 (m, 4H), 1.93 (s, 3H), 2.07 (s, 3H), 2.11 (s, 3H), 2.3 (s, 3H), 2.31 (t, J=7.69 Hz, 2H), 2.79 (t, J=7.32 Hz, 2H), 3.66 (s, 3H), 5.94 (s, 1H), 8.71 (br, 1H), 9.36 (br, 1H) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz) and UV–vis data are given in Table 2 and Table 5, respectively.

Anal. Calcd for $C_{21}H_{28}O_4N_2$ (372): C, 67.72; H, 7.58; N, 7.52. Found: C, 67.44; H, 7.41; N, 7.39.

4.1.5. (4*Z*)-9-(6-Carboethoxyhexanoyl)-2,3,7,8-tetramethyl-(10*H*)-dipyrrin-1-one (5e′). In a 1 L round bottom flask equipped with a magnetic stir bar and drying tube, anhyd $AlCl_3$ (3.0 g, 22.5 mmol) was dissolved in CH_2Cl_2 (300 mL). The solution was cooled in an ice bath for 30 min at which time monoethyl pimeloyl chloride (3.04 g, 14.7 mmol) was added in one portion to the solution. The solution was stirred and cooled for 5 min, then a solution of **7** (1.0 g, 4.6 mmol) in CH_2Cl_2 (200 mL) was added, and cooling was continued for 30 min (ice bath) followed by stirring overnight at room temperature. The solution was poured into a 2 L beaker filled with 400 mL of ice-water, and the mixture was stirred for 30 min. The organic layer was removed, and the aq layer was extracted with CH_2Cl_2 (3×100 mL). The combined organic layers were washed with H_2O (3×200 mL), dried over anhyd Na_2SO_4, and the solvent was removed (roto-vap). The residue was purified by radial chromatography (2% MeOH in CH_2Cl_2) and then crystallized to give 0.67 g (40%) of yellow crystals. Mp 129–130 °C; IR (NaCl, thin film) ν: 3341, 2939, 1735, 1656, 1436, 1248, 1171, 759, 694 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.24 (t, J=7.32 Hz, 3H), 1.42 (m, 2H), 1.69 (m, 4H), 1.74 (s, 3H), 2.07 (s, 3H), 2.11 (s, 3H), 2.3 (m, 5H), 2.79 (t, J=7.33 Hz, 2H), 4.11 (q, J=6.96 Hz, 2H), 5.94 (s, 1H), 8.8 (br s, 1H), 9.39 (br s, 1H) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 8.8, 9.7, 10.1, 11.9, 14.5, 24.3, 25.1, 29.2, 34.4, 40.1, 60.4, 97.4, 124.1, 126.3, 128.3, 129.3, 131.3, 136.4, 142.2, 174, 174.2, 190.3 ppm.

Anal. Calcd for $C_{22}H_{30}O_4N_2$ (386): C, 68.37; H, 7.82; N, 7.25. Found: C, 68.16; H, 7.73; N, 7.22.

4.1.6. (4*Z*)-9-(Carboxymethyl)-2,3,7,8-tetraethyl-(10*H*)-dipyrrin-1-one (1). Prepared in 70% yield as described in the literature.[15] Mp 154–158 °C (dec [lit.[15] mp 154–158 °C]); IR (NaCl, thin film) ν: 3165, 3162, 2962, 1682, 1686, 1650, 1272 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.17 (m, 12H), 2.41 (q, J=7.33 Hz, 2H), 2.59 (m, 4H), 2.82 (q, J=7.33 Hz, 2H), 6.13 (s, 1H), 10 (br s, 1H), 11.83 (br s, 1H) ppm; ^{13}C NMR (DMSO-d_6, 125 MHz) and UV–vis data are given in Table 1 and Table 5, respectively.

4.1.7. (4*Z*)-9-(2-Carboxyethyl)-2,3,7,8-tetramethyl-(10*H*)-dipyrrin-1-one (2). In a 250 mL round bottom flask equipped with a stir bar and drying tube, anhyd $AlCl_3$ (1.0 g, 7.5 mmol) was dissolved in CH_2Cl_2 (50 mL). The mixture was cooled in an ice bath for 30 min. To the mixture was added succinyl dichloride (0.5 mL, 0.1 mmol) and the mixture was cooled for an additional 10 min. A solution of **8** (40 mg, 0.15 mmol) in 20 mL of CH_2Cl_2 was added in one portion to the reaction mixture. The reaction mixture was stirred in the ice bath for 10 min and stirred for 72 h at room temperature. The reaction mixture was poured into 100 mL of 10% aq HCl and ice. The mixture was stirred for 1 h and the organic layer was removed from the aqueous layer. The aqueous layer was extracted with CH_2Cl_2 (3×50 mL) and the combined organic layers were washed with water (4×100 mL) and dried (Na_2SO_4). The solvent was removed (roto-vap), and the residue was purified by radial chromatography (eluting with 3% MeOH in CH_2Cl_2) and crystallized from hexane–CH_2Cl_2 to give 13 mg (22%) of yellow crystals of pure **2**. Mp 148–150 °C; IR (NaCl, thin film) ν: 3267, 2968, 2932, 1684, 1653, 1463, 1434, 1262, 1164 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ: 1.09 (t, J=7.3 Hz, 3H), 1.12 (t, J=7.3 Hz, 3H), 1.14 (t, J=7.3 Hz, 3H), 1.2 (t, J=7.76 Hz, 3H), 2.37 (q, J=7.76 Hz, 2H), 2.52 (q, J=7.76 Hz, 2H), 2.54 (q, J=7.76 Hz, 2H), 2.72 (t, J=5.93 Hz, 2H), 2.74 (q, J=7.76 Hz, 2H), 3.32 (t, J=6.39 Hz, 2H), 6.04 (s, 1H), 9.25 (br s, 1H), 10.36 (br s, 1H) ppm; ^{13}C NMR (DMSO-d_6, 125 MHz) and UV–vis data are given in Table 1 and Table 5, respectively.

Anal. Calcd for $C_{21}H_{28}O_4N_2$ (372): C, 67.72; H, 7.58; N, 7.52. $C_{21}H_{28}O_4N_2 \cdot \frac{1}{4}\ H_2O$ (377): C, 66.91; H, 7.62; N, 7.43. Found: C, 67.29; H, 7.50; N, 7.41.

4.1.8. (4*Z*)-9-(3-Carboxypropyl)-2,3,7,8-tetraethyl-(10*H*)-dipyrrin-1-one (3). In a 250 mL round bottom flask equipped with a magnetic stir bar and drying tube, anhyd $AlCl_3$ (1.0 g, 7.5 mmol) was dissolved in CH_2Cl_2 (50 mL). The mixture was cooled (ice bath) while the diacid chloride of glutaric acid was added. The solution was cooled for an additional 10 min and a solution of **8** (200 mg, 0.73 mmol) in CH_2Cl_2 (50 mL) was added in one portion. The solution was stirred at room temperature for 23 h. The mixture was then poured into ice-water (300 mL) and stirred for 1 h. The organic layer was separated from the aqueous layer and the aqueous layer was extracted with CH_2Cl_2 (3×75 mL). The organic layers were combined and washed with H_2O (3×100 mL) and dried over anhyd Na_2SO_4. The solvent was removed (roto-vap), and the residue was purified by radial chromatography (3% MeOH–CH_2Cl_2). The purified residue was crystallized from hexane–CH_2Cl_2 to give 100 mg (35%) of pure **3**. Mp 212–213 °C; IR (NaCl, thin film) ν: 3295, 2968, 2935, 1719, 1654, 1462, 1273, 1196 cm^{-1}; ^1H-NMR ($CDCl_3$, 500 MHz) δ: 1.14 (m, 9H), 1.21 (t, J=7.76 Hz, 3H), 2.13 (p, J=7.3 Hz, 2H), 2.55 (m, 6H), 2.75 (q, J=7.3 Hz, 2H), 2.92 (t, J=7.76 Hz, 2H), 6.06 (s, 1H), 9.39 (br s, 1H), 10.57 (br s, 1H), 13.16 (br s, 1H) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) and UV–vis data are given in Table 1 and Table 5, respectively.

Anal. Calcd for $C_{22}H_{30}O_4N_2$ (386): C, 68.37; H, 7.82; N, 7.25. Found: C, 68.33; H, 7.74; N, 7.52.

4.1.9. (4*Z*)-9-(6-Carboxyhexyl)-2,3,7,8-tetramethyl-(10*H*)-dipyrrin-1-one (5). In a 250 mL round bottom flask equipped with a magnetic stir bar was dissolved 10-oxo-semirubin ethyl ester **5e′** (200 mg, 0.52 mmol) in THF (100 mL). To the mixture was added 2 M aq NaOH (20 mL) and the mixture was held at reflux for 3 h. The warm solution was poured into ice-water (100 mL) and stirred while 10% aq HCl was slowly added until the pH of the mixture was ~1. The acidic solution was extracted with CH_2Cl_2 (3×50 mL), and the combined organic extracts

were washed with H_2O (200 mL) and dried over Na_2SO_4 (anhyd). The solvent was removed (roto-vap). The crude product was washed with cold CH_2Cl_2 to give 150 mg (81%) of pure **5**. Mp 215–216 °C; IR (NaCl, film) ν: 3272, 2967, 1698, 1683, 1652, 1458, 1267, 1164, 434 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.17 (m, 12H), 1.8 (m, 2H), 1.94 (m, 2H), 2.4 (q, J=7.69 Hz, 2H), 2.55 (m, 4H), 2.8 (q, J=7.69 Hz, 2H), 2.86 (m, 4H), 6.1 (s, 1H), 9.2 (br s, 1H), 10.8 (br s, 1H), 12.9 (br s, 1H) ppm; ^{13}C NMR ($CDCl_3$, 75 MHz) and UV–vis data are given in Table 1 and Table 5, respectively.

Anal. Calcd for $C_{20}H_{26}O_4N_2$ (358): C, 67.02; H, 7.31; N, 7.82. Found: C, 66.78; H, 7.22; N, 7.66.

4.1.10. (4*Z*)-9-(6-Carboxyhexyl)-7,8-diethyl-2,3-dimethyl-(10*H*)-dipyrrin-1-one (5′). In a 300 mL round bottom flask equipped with a magnetic stir bar and drying tube anhyd $AlCl_3$ (1.0 g, mmol) was dissolved in CH_2Cl_2 (100 mL). The solution was cooled in an ice bath for 30 min at which time monoethyl pimeloyl chloride (1.00 g, 4.85 mmol) was added in one portion to the solution. The solution was stirred and cooled for 5 min, then a solution of (4*Z*)-7,8-diethyl-2,3-dimethyl-(10*H*)-dipyrrin-1-one (0.50 g, 2.1 mmol) in CH_2Cl_2 (100 mL) was added, and cooling was continued for 30 min (ice bath) followed by stirring overnight at room temperature. The solution was poured into a 1 L beaker filled with 200 mL of ice-water, and the mixture was stirred for 30 min. The organic layer was removed and the aqueous layer was extracted with CH_2Cl_2 (3×75 mL). The combined organic layers were washed with brine (3×200 mL), dried over anhyd Na_2SO_4, and the solvent was removed (roto-vap). The residue was purified by radial chromatography (2% MeOH in CH_2Cl_2) and then crystallized to give 0.30 g (35%) of yellow crystals that were used directly in the next step. Mp 118–120 °C; IR (NaCl, film) ν: 2965, 2931, 2870, 1735, 1670, 1654, 1457, 1437, 1257, 1173 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ: 1.14 (t, J=7.3 Hz, 3H), 1.2 (t, J=7.3 Hz, 3H), 1.24 (t, J=7.3 Hz, 3H), 1.39 (p, J=7.3 Hz, 2H), 1.65 (p, J=7.3 Hz, 2H), 1.73 (m, 2H), 1.92 (s, 1H), 2.11 (s, 1H), 2.29 (t, J=7.76 Hz, 2H), 2.53 (q, J=7.3 Hz, 2H), 2.75 (q, J=7.3 Hz, 2H), 2.85 (t, J=7.3 Hz, 2H), 4.11 (q, J=6.85 Hz, 2H), 5.96 (s, 1H), 9.26 (br s, 1H), 9.48 (br s, 1H) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 8.9, 10.1, 14.5, 16.3, 16.7, 17.5, 18.9, 24.3, 25.1, 29.1, 34.4, 39.3, 60.4, 97.2, 128.3, 128.8, 130.4, 130.6, 132.7, 136.3, 142.4, 174, 174.1, 190.3 ppm.

The above compound (100 mg, 0.24 mmol) was saponified for 3 h as for **5**. The residue was crystallized from CH_2Cl_2 to give 55 mg (59%) of pure **5′**. Mp 210–212 °C; IR (NaCl, film) ν: 2964, 1718, 1659, 1622, 1435, 1406, 1267, 1251, 1200, 1172, 995 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ: 1.15 (t, J=7.3 Hz, 3H), 1.16 (t, J=7.3 Hz, 3H), 1.57 (m, 2H), 1.7 (p, J=6.85 Hz, 2H), 1.85 (p, J=7.3 Hz, 2H), 1.93 (s, 1H), 2.14 (s, 1H), 2.48 (t, J=5.48 Hz, 2H), 2.55 (q, J=7.76 Hz, 2H), 2.79 (q, J=7.3 Hz, 2H), 2.9 (t, J=7.3 Hz, 2H), 6.09 (s, 1H), 9.09 (br s, 1H), 10.78 (br s, 1H), 13.2 (br s, 1H) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 8.6, 10.1, 15.6, 16.7, 17.4, 18.6, 21.3, 24.6, 30.3, 33.9, 39.7, 99.1, 127.2, 127.3, 130.9, 131.6, 134.2, 134.4, 142.8, 175.8, 180.8, 192 ppm.

Anal. Calcd for $C_{22}H_{27}O_4N_2$ (383): C, 68.37; H, 7.82; N, 7.25. Found: C, 68.29; H, 7.89; N, 7.28.

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Synthesis and binding ability of bile acid-based receptors for recognition of flavin analogues

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Abstract—Novel cholaphanes **6a**,**b**, based on lithocholic and deoxycholic acids, were synthesised through **3a**,**b** by a sequence of reactions involving Cs-salt methodology of macrocyclisation. Cholaphanes **6a**,**b** and acyclic steroidal receptors **3a**,**b** bind flavin analogues via three hydrogen bonds in $CHCl_3$.

1. Introduction

Enzymes containing riboflavin as cofactor play very important roles in cellular metabolism such as dehydrogenation of NAD(P)H and D-amino acids, hydroxylation of aromatic substrates, activation of molecular oxidation, etc.[1] The major reason for this is the unique chemical property of the isoalloxazine hetero-aromatic system present in riboflavin, which undergoes reversible oxidation–reduction involving one- or two-electron transfer. The apoprotein of the enzyme acts as a modulator to change its reduction potential. Considerable efforts have been made to design model systems to understand the role of the hydrogen-bond interactions and their effect on the regulation of the flavin activity.[2]

Rotello and Cooke have studied the effect of hydrogen bond interaction on the redox potential of isoalloxazine derivatives in aprotic solvents using synthetic receptors based on 2,6-diaminopyridine to reproduce the specific hydrogen bond patterns present in flavoenzymes.[3] The results show that in addition to hydrogen bonding, the hydrophobicity of the system also plays a significant role in the stabilisation of the flavin radical anion. Yano and co-workers have utilised melamine derivatives bearing a guanidinium ion to show the effect of the hydrogen bond and electrostatic interactions of guanidinium ion on the redox potential of isoalloxazine derivatives.[4]

To investigate the effect of these factors in more detail, we have designed and synthesised various acyclic and cyclic bile acid-based 2,6-diaminopyridine systems and studied their binding behaviour with isoalloxazine derivatives. Although bile acids have been used for the design of supramolecular hosts for carbohydrates,[5] nucleic acid bases[6] and for anion recognition,[7] receptor molecules based on bile acid for flavin coenzyme have not been reported so far. These systems provide well-defined binding sites in terms of hydrogen bond and hydrophobic interactions for the regulation of the binding and reduction potential of isoalloxazine–receptor interactions.

2. Results and discussion

Cholaphanes **6a**,**b** involving head-to-head combination of bile acids **1a**,**b** were synthesised through a five-step synthetic route (Scheme 1). For the synthesis of **6b**, the 3α- and 12α-hydroxy groups of deoxycholic acid (3α,12α-dihydroxy-5β-cholan-24-oic acid) were first protected by formylation with formic acid (100%). Attempts to condense the formylated compound with 2,6-diaminopyridine in the presence of DCC proved futile. However, condensation of the acid chloride of 3α,12α-*O*-diformyldeoxycholic acid **2b** with 2,6-diaminopyridine in a molar ratio 2.2:1 in dry THF yielded *N*,*N*′-bis(3α,12α-*O*-diformyldeoxycholyl)-2,6-diaminopyridine **3b** in 77% yield. Hydrolysis of the diamide with LiOH in THF–H_2O resulted in *N*,*N*′-bisdeoxycholyl-pyridine-2,6-diamine **4b** in 80% yield. The selective bromoacetylation of both the equatorial 3-OH groups was achieved in 70% yield by stirring a mixture of the hydrolysed product (1 equiv), bromoacetyl bromide (2.0 equiv) and K_2CO_3 in dry $CHCl_3$ for 10 min. The crucial cyclisation step was accomplished by using the Cs-salt methodology.[8] The synthetic route leading to cholaphane **6a** is similar to the one described above except the bromoacetylation step, where no selective bromoacetylation was required.

Keywords: Cholaphanes; Flavoenzyme; Flavin mimic; Isoalloxazines; Molecular recognition.
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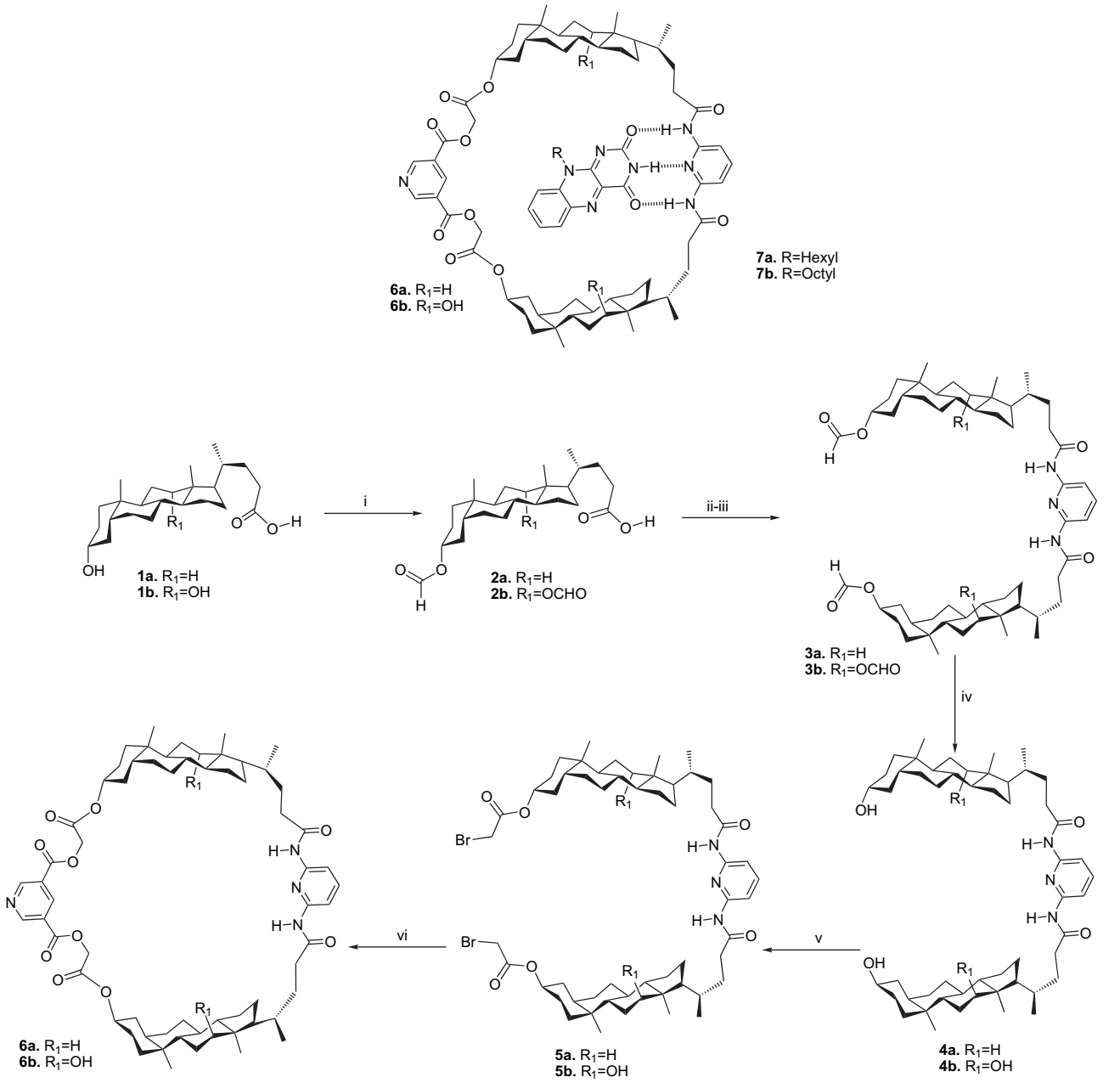

Scheme 1. Reagents and conditions (and yields): (i) HCOOH, 60 °C, 4 h, (98%); (ii) $SOCl_2$, benzene, 4 h, reflux, (~100%); (iii) 2,6-diaminopyridine, triethylamine, THF, 0–5 °C, 12 h, (80%) for **3a** and (77%) for **3b**; (iv) LiOH, THF–H_2O, rt, 24 h, (84%) for **4a** and (80%) for **4b**; (v) $BrCH_2COBr$, anhydrous K_2CO_3, $CHCl_3$, 55–60 °C, 10 min, (85%) for **5a** and (70%) for **5b**; (vi) bis-caesium pyridine 2,6-dicarboxylate, DMF, 12 h, rt, (69%) for **6a** and (68%) for **6b**.

Flavin analogues (Fig. 1) were prepared by the selective monoalkylation of 1,2-phenylenediamine followed by cyclisation of the 2-amino-*N*-alkylanilines with alloxan in acidic conditions resulting in 10-hexyl- and 10-octylisoalloxazines **7a**,**b**.[9] Binding behaviour of the receptors (**3a**,**b**, **6a**,**b**) with **7a**,**b** was examined by ^{1}H NMR spectroscopy. As a typical example, the complexation between receptor **3a** and flavin analogue **7a** was studied by ^{1}H NMR titration experiment in $CDCl_3$, with receptor **3a** (0.01 M) against various aliquots of 0.06 M solution of **7a**. The chemical shifts of the amide protons were recorded at each concentration until saturation of chemical shifts was observed. Each titration was carried out in duplicate. Analysis of the saturation data with WinEQNMR software, a nonlinear regression curve-fitting program,[10] revealed 1:1 complexation with a binding constant of 600 M^{-1}. Job's plot further confirmed the 1:1 binding with maximum complexation at 0.5 mole fraction.

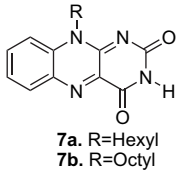

Figure 1. Flavin analogues.

The binding constants for the complexation of the receptors with flavin analogues are listed in Table 1. In case of acyclic receptors **3a**,**b**, **7a** shows better binding behaviour than **7b**.

Table 1. Binding constants K_a (M^{-1}) for complexation of flavin analogues with bile acid-based receptors[a]

Receptor	Flavin analogues, K_a	
	7a	**7b**
3a	600	250
3b	400	240
6a	60	50
6b	110	110

[a] Determined in $CDCl_3$ at 25 °C, errors estimated to be ≤10%.

This may be because of the better fit of **7a** with receptors **3a**,**b** due to the smaller size of the hexyl group. Also, cholaphanes **6a**,**b** have smaller binding constants than receptors **3a**,**b**. The smaller K_a values of the cholaphanes **6a**,**b** as compared to **3a**,**b** can be attributed to the steric hindrances faced by the flavin analogues towards cholaphanes during complexation. Moreover, cholaphane **6b** having two hydroxyl groups at 12α- and 12α′-positions shows larger binding constant than cholaphane **6a**, which may be due to the additional hydrogen bond interactions.

3. Conclusion

In conclusion, we have developed the synthesis of novel cholaphanes in view of mimicking the specific hydrogen bond patterns present in the flavoenzyme. The ability of the cholaphanes to bind to flavin analogues was overshadowed by the acyclic diamides because of steric hindrance; however, they showed a notable ability to bind flavin analogues. We feel that such mimicry has not been exploited before. The cholaphanes can also be converted into 1,4-dihydropyridine system by standard methods. The structural features of these macrocyclic steroidal dihydropyridine systems help to bind flavin analogues and may mimic the role played by oxidoreductases using the NADH–flavin coupled system. Work in this direction is underway and will be reported in due course.

4. Experimental

4.1. General

Melting points are uncorrected. IR spectra were recorded on a Nicolet Protégé 460 FTIR Spectrometer, using potassium bromide pellets. ^1H and ^{13}C NMR spectra were recorded on a Bruker Spectrospin DPX 300. Tetramethylsilane was used as internal reference and the chemical shifts are expressed as displacement (δ) in parts per million downfield from tetramethylsilane. High-resolution mass spectra (ES) were recorded on a VG-Fisons 'Autospec' spectrometer. Column chromatography was carried out using Spectrochem silica gel 230–400 mesh for flash chromatography. The solid compounds were dried under vacuum in the presence of P_2O_5.

4.1.1. 3α-*O*-Formyllithocholic acid 2a. Lithocholic acid (3α-hydroxy-5β-cholan-24-oic acid) **1a** (3 g, 7.96 mmol) was dissolved in 20 ml of 100% formic acid. The solution was stirred at 60 °C for 4 h. The reaction mixture was cooled to room temperature and was added dropwise to water (100 ml) with stirring to get white precipitate. The precipitate was filtered and vacuum dried to give 3.15 g of **2a** (98%). Mp 127–128 °C; IR ν_{max} (KBr)/cm^{-1} 3446, 2948, 1725, 1450, 1182; ^1H NMR (300 MHz, $CDCl_3$, TMS) δ 0.65 (s, 3H, 18-Me), 0.91 (br s, 6H, 21-Me and 19-Me), 1.08–2.40 (28H, steroidal H), 4.85 (m, 1H, 3β-H), 8.04 (s, 1H, –OCHO); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ 12.02, 18.21, 20.81, 23.29, 24.14, 26.27, 26.60, 26.95, 28.12, 30.72, 31.00, 32.18, 34.55, 34.93, 35.27, 35.76, 40.09, 40.41, 41.88, 42.72, 55.92, 56.43, 74.41, 160.84, 180.39; ES-HRMS calcd for ($C_{25}H_{40}O_4$·Na) 427.2824, found 427.2823.

4.1.2. *N*,*N*′-Bis(3α-*O*-formyllithocholyl)-pyridine-2,6-diamine 3a. Freshly distilled thionyl chloride (1 ml) was added dropwise to a solution of **2a** (4 g, 9.9 mmol) in 20 ml of dry benzene and a drop of DMF at 0 °C. The reaction mixture was stirred at 60 °C for 4 h and then evaporated to dryness in vacuo. Dry benzene (10 ml) was added and the syrup evaporated twice to completely remove leftover thionyl chloride. The acid chloride was dissolved in dry THF (10 ml) and added dropwise to a solution of 2,6-diaminopyridine (0.44 g, 4.03 mmol) and triethylamine (1.4 ml) in dry THF (15 ml) at 0 °C. After the reaction was completed, the solution was concentrated, which was then extracted with chloroform, dried, and evaporated to dryness. The residue was purified by flash chromatography (elution with EtOAc–hexane 1:6) to give 2.84 g of **3a** (80%). Mp 105–107 °C; IR ν_{max} (KBr)/cm^{-1} 3326, 2937, 2863, 1720, 1588, 1507; ^1H NMR (300 MHz, $CDCl_3$, TMS) δ 0.65 (s, 6H, 18-Me), 0.93 (s, 6H, 19-Me), 0.96 (br s, 6H, 21-Me), 1.08–2.44 (56H, steroidal H), 4.85 (m, 2H, 3β-H), 7.67–7.72 (m, 3H, 2×–NHCO– and Py-4-H), 7.89 (d, 2H, *J*=8.1 Hz, Py-3,5-H), 8.04 (s, 2H, –OCHO); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ 12.07, 18.40, 20.83, 23.30, 24.18, 26.29, 26.63, 26.96, 28.24, 30.88, 31.41, 32.20, 34.75, 34.94, 35.44, 35.77, 40.13, 40.43, 41.88, 42.76, 56.02, 56.46, 74.39, 109.37, 140.99, 149.43, 160.77, 171.97; ES-HRMS calcd for ($C_{55}H_{83}N_3O_6$·H)$^+$ 882.6360, found 882.6361.

4.1.3. *N*,*N*′-Bislithocholyl-pyridine-2,6-diamine 4a. To a solution of **3a** (2 g, 2.26 mmol) in THF–H_2O (10:1, 25 ml), was added LiOH (0.2 g, 4.65 mmol). The solution was stirred at room temperature for 12 h. The solution was evaporated and the residue was extracted with chloroform, dried over sodium sulfate and purified by flash chromatography (elution with EtOAc–hexane 1:5) to give 1.57 g of **4a** (84%). Mp 132–134 °C; IR ν_{max} (KBr)/cm^{-1} 3422, 2934, 1683, 1585; ^1H NMR (300 MHz, $CDCl_3$, TMS) δ 0.58 (s, 6H, 18-Me), 0.84 (s, 6H, 19-Me), 0.88 (d, 6H, *J*=6.1 Hz, 21-Me), 1.0–2.3 (56H, steroidal H), 3.56 (m, 2H, 3β-H), 7.53 (s, 2H, 2×–NHCO–), 7.62 (t, 1H, *J*=8.0 Hz, Py-4-H), 7.82 (d, 2H, *J*=8.0 Hz, Py-3,5-H); ^{13}C NMR (75 MHz, DMSO, TMS) δ 11.90, 18.31, 20.42, 23.28, 23.87, 26.17, 26.90, 27.76, 30.37, 31.24, 33.19, 34.21, 35.03, 35.39, 36.28, 38.85, 38.94, 40.12, 41.53, 42.28, 55.62, 56.10, 69.88, 108.89, 139.81, 150.37, 172.59; ES-HRMS calcd for ($C_{53}H_{83}N_3O_4$·H)$^+$ 826.6462, found 826.6434.

4.1.4. *N*,*N*′-Bis(3α-*O*-bromoacetyllithocholyl)-pyridine-2,6-diamine 5a. Compound **4a** (1.5 g, 1.81 mmol) was stirred at 55–60 °C in dry chloroform (20 ml) until it was

completely dissolved. Anhydrous K_2CO_3 was then added. To this, a solution of bromoacetyl bromide (1.09 g, 5.44 mmol) in dry chloroform (10 ml) was added. After 10 min, the heating was stopped, ice-cold water (20 ml) was added and the organic layer was separated, which was subsequently dried over Na_2SO_4 and evaporated. The crude product was purified by flash chromatography (elution with EtOAc–hexane 1:6) to give 1.65 g of **5a** (85%). Mp 152–154 °C; IR ν_{max} (KBr)/cm^{-1} 3337, 2936, 1734, 1702, 1586, 1284; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 0.65 (s, 6H, 18-Me), 0.86 (s, 6H, 19-Me), 0.93 (d, 6H, J=6.2 Hz, 21-Me), 1.08–2.43 (56H, steroidal H), 3.8 (s, 4H, –$COCH_2$Br), 4.79 (m, 2H, 3β-H), 7.52 (s, 2H, 2×–NHCO–), 7.69 (t, 1H, J=7.9 Hz, Py-4-H), 7.88 (d, 2H, J=7.9 Hz, Py-3,5-H); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ 12.04, 18.39, 20.81, 23.25, 24.15, 26.34, 26.94, 28.23, 29.66, 30.22, 31.38, 31.89, 31.96, 34.62, 34.87, 35.42, 35.73, 40.09, 40.38, 41.84, 42.73, 56.02, 56.42, 76.6, 109.31, 140.88, 149.40, 166.49, 171.91; ES-HRMS calcd for $(C_{57}H_{85}N_3O_6Br_2\cdot H)^+$ 1066.4883, found 1066.4932.

4.1.5. Bis(3α-*O*-hydroxyacetyllithocholyl)-pyridine-2,6-diamine cyclic 3,5-pyridine dicarboxylate (lithocholaphane) 6a. Compound **5a** (0.99 g, 0.92 mmol) was dissolved in 250 ml of dry DMF and to this was added an equivalent amount of bis-caesium 3,5-pyridine dicarboxylate (0.39 g, 0.92 mmol). The reaction mixture was stirred at room temperature for 12 h. Then, DMF was evaporated under vacuo. The crude product obtained was dissolved in chloroform (30 ml) and washed with brine (10 ml), dried (Na_2SO_4), and evaporated to dryness under vacuum. The impure cholaphane was then purified by flash chromatography (elution with EtOAc–hexane 1:3) to give 0.75 g of **3a** (69%). Mp 175–177 °C; IR ν_{max} (KBr)/cm^{-1} 3369, 2936, 1741, 1702, 1586, 1287, 1205; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 0.59 (s, 6H, 18-Me), 0.85 (s, 6H, 19-Me), 0.88 (d, 6H, J=6.2 Hz, 21-Me), 1.01–2.29 (56H, steroidal H), 4.72 (m, 2H, 3β-H), 4.80 (s, 4H, –$COCH_2$–), 7.57 (s, 2H, 2×–NHCO–), 7.62 (t, 1H, J=8.0 Hz, Py-4-H), 7.81 (d, 2H, J=8.0 Hz, Py-3,5-H), 8.91 (s, 1H, Py′-4-H), 9.42 (s, 2H, Py′-2,6-H); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ 11.99, 18.47, 20.84, 23.21, 24.07, 26.19, 26.35, 26.81, 28.20, 31.01, 31.86, 33.44, 34.47, 34.88, 35.65, 40.16, 40.53, 41.76, 42.69, 54.88, 56.52, 56.69, 62.30, 76.58, 109.24, 125.37, 138.66, 140.92, 149.43, 154.76, 163.76, 163.65, 172.04; ES-HRMS calcd for $(C_{64}H_{88}N_4O_{10}\cdot H)^+$ 1073.6579, found 1073.6625.

4.1.6. 3α,12α-*O*-Diformyldeoxycholic acid 2b. Deoxycholic acid (3α,12α-dihydroxy-5β-cholan-24-oic acid) **1b** (3 g, 7.64 mmol) was dissolved in 20 ml of 100% formic acid. The solution was stirred at 60 °C for 4 h. The reaction mixture was cooled to room temperature and was added dropwise to water (100 ml) with stirring to get white precipitate. The precipitate was filtered and vacuum dried to give 3.35 g of **2b** (98%). Mp 173–175 °C; IR ν_{max} (KBr)/cm^{-1} 3446, 2948, 1725, 1721, 1450, 1182; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 0.75 (s, 3H, 18-Me), 0.84 (d, 3H, J=6.2 Hz, 21-Me), 0.92 (s, 3H, 19-Me), 1.08–2.39 (26H, steroidal H), 4.85 (m, 1H, 3β-H), 5.25 (m, 1H, 12β-H), 8.03 (s, 1H, –OCHO), 8.13 (s, 1H, –OCHO); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ 12.34, 17.44, 22.93, 23.44, 25.74, 25.90, 26.47, 26.78, 27.34, 30.48, 30.95, 32.09, 34.03, 34.20, 34.67, 34.78, 35.97, 41.73, 45.01, 47.35, 49.25, 73.21, 76.00, 160.60, 160.86, 180.09; ES-HRMS calcd for $(C_{26}H_{40}O_6\cdot K)$ 487.2462, found 487.2464.

4.1.7. *N*,*N*′-Bis(3α,12α-*O*-diformyldeoxycholyl)-pyridine-2,6-diamine 3b. Acid chloride of **2b** (4 g, 8.91 mmol) was prepared following the method described for the synthesis of **3a**. The acid chloride was dissolved in dry THF (10 ml) and added dropwise to a solution of 2,6-diaminopyridine (0.44 g, 4.03 mmol) and triethylamine (1.4 ml) in dry THF (15 ml) at 0 °C. The reaction was worked up as described above for **2b** and the residue obtained was purified by flash chromatography (elution with EtOAc–hexane 1:6) to give 3.02 g of **3b** (77%). Mp 154–155 °C; IR ν_{max} (KBr)/cm^{-1} 3334, 2946, 2869, 1722, 1586, 1506; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 0.75 (s, 6H, 18-Me), 0.87 (d, 6H, J=6.0 Hz, 21-Me), 0.93 (s, 6H, 19-Me), 1.04–2.42 (52H, steroidal H), 4.84 (m, 2H, 3β-H), 5.26 (s, 2H, 12β-H), 7.59 (s, 2H, 2×–NHCO–), 7.69 (t, 1H, J=8.0 Hz, Py-4-H), 7.88 (d, 2H, J=8.0 Hz, Py-3,5-H), 8.03 (s, 2H, –OCHO), 8.14 (s, 2H, –OCHO); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ 12.36, 17.60, 22.91, 23.43, 25.75, 25.80, 26.46, 26.74, 27.40, 30.56, 31.02, 32.07, 33.96, 34.18, 34.62, 34.88, 35.58, 41.70, 45.02, 47.45, 49.24, 74.04, 75.98, 109.32, 141.08, 149.24, 160.54, 160.59, 171.79; ES-HRMS calcd for $(C_{57}H_{83}N_3O_{10}\cdot H)^+$ 970.6157, found 970.6161.

4.1.8. *N*,*N*′-Bisdeoxycholyl-pyridine-2,6-diamine 4b. To a solution of **3b** (2 g, 2.06 mmol) in THF–H_2O (10:1, 25 ml), was added LiOH (0.4 g, 9.3 mmol). The solution was stirred at room temperature for 24 h. The solution was evaporated and the residue was extracted with chloroform, dried over sodium sulfate and purified by flash chromatography (elution with EtOAc–hexane 1:3) to give 1.42 g of **4b** (80%). Mp 176–178 °C; IR ν_{max} (KBr)/cm^{-1} 3420, 2932, 1680, 1586; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 0.63 (s, 6H, 18-Me), 0.83 (s, 6H, 19-Me), 0.94 (d, 6H, J=6.0 Hz, 21-Me), 1.04–2.5 (52H, steroidal H), 3.54 (m, 2H, 3β-H), 3.9 (s, 2H, 12β-H), 7.60 (t, 1H, J=7.8 Hz, Py-4-H), 7.72 (d, 2H, J=7.8 Hz, Py-3,5-H), 8.91 (s, 2H, 2×–NHCO–); ^{13}C NMR (75 MHz, DMSO, TMS) δ 13.27, 17.89, 23.24, 23.89, 24.33, 26.57, 27.55, 27.80, 29.42, 31.01, 32.15, 33.74, 34.13, 34.62, 35.94, 36.47, 37.07, 42.41, 46.81, 48.28, 70.78, 71.86, 79.29, 108.78, 139.07, 151.18, 173.22; ES-HRMS calcd for $(C_{53}H_{83}N_3O_6\cdot H)^+$ 858.6360, found 858.6346.

4.1.9. *N*,*N*′-Bis(3α-*O*-bromoacetyldeoxycholyl)-pyridine-2,6-diamine 5b. Compound **4b** (1.5 g, 1.74 mmol) was stirred at 55–60 °C in dry chloroform (30 ml) until it was completely dissolved. Anhydrous K_2CO_3 was then added dropwise. To this, a solution of bromoacetyl bromide (0.7 g, 3.48 mmol) in dry chloroform (15 ml) was added. After 10 min, the heating was stopped, ice-cold water (20 ml) was added and the organic layer was separated, which was subsequently dried over Na_2SO_4 and evaporated. The crude product was purified by flash chromatography (elution with EtOAc–hexane 1:4) to give 1.33 g of **5b** (70%). Mp 162–165 °C; IR ν_{max} (KBr)/cm^{-1} 3423, 2939, 1734, 1585, 1287; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 0.69 (s, 6H, 18-Me), 0.93 (s, 6H, 19-Me), 1.01 (d, 6H, J=5.8 Hz, 21-Me), 1.08–2.44 (52H, steroidal H), 3.79 (s, 4H, –$COCH_2$Br), 4.00 (s, 2H, 12β-H), 4.78 (m, 2H, 3β-H), 7.61 (s, 2H, 2×–NHCO–),

7.69 (t, 1H, J=7.9 Hz, Py-4-H), 7.88 (d, 2H, J=7.9 Hz, Py-3,5-H); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ 12.68, 17.41, 19.28, 22.97, 23.51, 25.90, 26.19, 26.80, 27.41, 28.69, 30.45, 31.11, 33.60, 34.02, 34.44, 34.66, 35.00, 35.86, 41.74, 46.42, 47.10, 48.20, 71.37, 76.48, 109.26, 140.98, 149.21, 166.74, 171.94; ES-HRMS calcd for $(C_{57}H_{85}N_3O_8Br_2\cdot H)^+$ 1098.4782, found 1098.4810.

4.1.10. Bis(3α-*O*-hydroxyacetyldeoxycholyl)-pyridine-2,6-diamine cyclic 3,5-pyridine dicarboxylate (deoxycholaphane) 6b. Compound **5b** (0.8 g, 0.72 mmol) was dissolved in 250 ml of dry DMF and to this was added an equivalent amount of bis-caesium 3,5-pyridine dicarboxylate (0.30 g, 0.72 mmol). The reaction mixture was stirred at room temperature for 12 h. The reaction was worked up following the method described above for **5a**. The impure cholaphane obtained was purified by flash chromatography (elution with EtOAc–hexane 1:2) to give 0.54 g of **6b** (68%). Mp 170–171 °C; IR ν_{max} (KBr)/cm^{-1} 3423, 2936, 1740, 1702, 1586, 1288, 1207; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 0.69 (s, 6H, 18-Me), 0.91 (s, 6H, 19-Me), 1.01 (d, 6H, J=5.6 Hz, 21-Me), 1.08–2.40 (52H, steroidal H), 3.98 (s, 2H, 12β-H), 4.83 (m, 2H, 3β-H), 4.88 (s, 4H, –$COCH_2$–), 7.69 (t, 1H, J=7.9 Hz, Py-4-H), 7.79–7.84 (m, 4H, Py-3,5-H and 2×–NHCO–), 8.99 (s, 1H, Py′-4-H), 9.50 (s, 2H, Py′-2,6-H); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ 11.67, 16.58, 21.98, 22.54, 24.94, 25.28, 25.74, 26.41, 27.93, 29.83, 30.81, 32.12, 32.63, 32.98, 33.53, 33.65, 34.81, 40.68, 44.75, 45.37, 47.60, 61.28, 71.99, 75.27, 108.46, 124.42, 137.69, 139.95, 148.51, 153.85, 162.91, 165.72, 171.37; ES-HRMS calcd for ($C_{64}H_{88}N_4O_{12}$) 1104.6399, found 1104.6387.

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Supplementary data

^{13}C and ^{1}H NMR spectra of bile acid derivatives, binding isotherms and mass spectra are provided in supplementary data. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.06.029.

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Microwave-assisted regioselective synthesis of natural 6-prenylpolyhydroxyisoflavones and their hydrates with hypervalent iodine reagents

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Abstract—Microwave-assisted oxidative rearrangement of 3′-iodotetraalkoxychalcones with hypervalent iodine such as [hydroxy(tosyloxy)-iodo]benzene or [bis(trifluoroacetoxy)iodo]benzene, followed by microwave-mediated hydrolysis and in situ cyclization of the resultant acetals gave 6-iodotrialkoxyisoflavones. Pd(0)-catalyzed coupling reaction of the 6-iodoisoflavones with 2-methyl-3-butyn-2-ol under microwave irradiation gave 6-alkynylisoflavones, whose hydrogenation gave the respective hydrates of wighteone, lupisoflavone and derrubone. Wighteone (**1a**), lupisoflavone (**1b**) and derrubone (**1c**) were obtained by dehydration of their respective hydrates under microwave irradiation.

1. Introduction

In the 10 years since the appearance of the first paper on organic synthesis under microwave dielectric heating, the field has expanded dramatically.[1–4] Chemistry in the 21st century is increasingly being called upon to develop green chemistry methods as it attempts to deal with the scientific challenges of protecting the human health and the environment from the hazards posed by chemical processes. Considerable research efforts to use microwave for organic synthesis have been expended over the last two decades. This is because microwave minimizes the formation of unwanted by-products, and it reduces the need for organic solvents to a minimum or can even be used under solvent-free conditions.[5,6] Our present study will report on the total synthesis of some physiologically important prenylisoflavones under microwave dielectric heating with environmentally-friendly hypervalent iodine reagents and minimal use of solvents. Isoflavone derivatives are widely distributed in nature and are very important as precursors of prenylisoflavones and pterocarpans.[7,8] In addition, they exhibit phytoalexin, antifungal, anti-inflammatory and anticancer properties.[9–11] Recent studies have shown that some isoflavones have excellent health-promoting effects.[12] Hence, isoflavones and their derivatives have been receiving considerable attention in the fields of preventive medicine, food supplements, agrochemicals and cosmetics in recent years. Soy isoflavones show oxidative metabolism properties in humans in vitro and in vivo.[13] However, very recent studies[14] have also indicated that some soy isoflavones such as genistein and/or daidzein induced cancers of reproductive organs of female rodents. Despite these findings, there is growing research interest in isoflavonoids due to their health-related properties.

Wighteone, which has a strong antifungal property, was first isolated from healthy leaves of *Lupinus albus* together with luteone in 1976, but its structure was not fully identified at the time.[15] The following year (1977), wighteone was isolated from fungus-inoculated stems of *Glycine wightii* as a phytoalexin and the structure was assigned to be 5,7,4′-trihydroxy-6-(3-methyl-2-butenyl)isoflavone (**1a**) on the basis of spectroscopic method.[9] Wighteone was also isolated as erythrinin B from the bark of *Erythrina variegata*[16] and, together with luteone, from the roots of white lupin.[17,18] Moreover, wighteone was metabolized in a culture of *Aspergillus flavus* to be transformed into wighteone hydrate as a major metabolite, whose structure was determined as 5,7,4′-trihydroxy-6-(3-hydroxy-3-methylbutyl)isoflavone (**2a**) by spectroscopic analysis.[19] Synthesis of **1a** and **2a** by classical heating method has been reported earlier.[20] The isomer [lupiwighteone=5,7,4′-trihydroxy-8-(3-methyl-2-butenyl)-isoflavone] of wighteone has also been synthesized by conventional method.[21,22] But, we report here the first total synthesis of wighteone (**1a**) and wighteone hydrate (**2a**) under microwave irradiation (MWI). Lupisoflavone, a new

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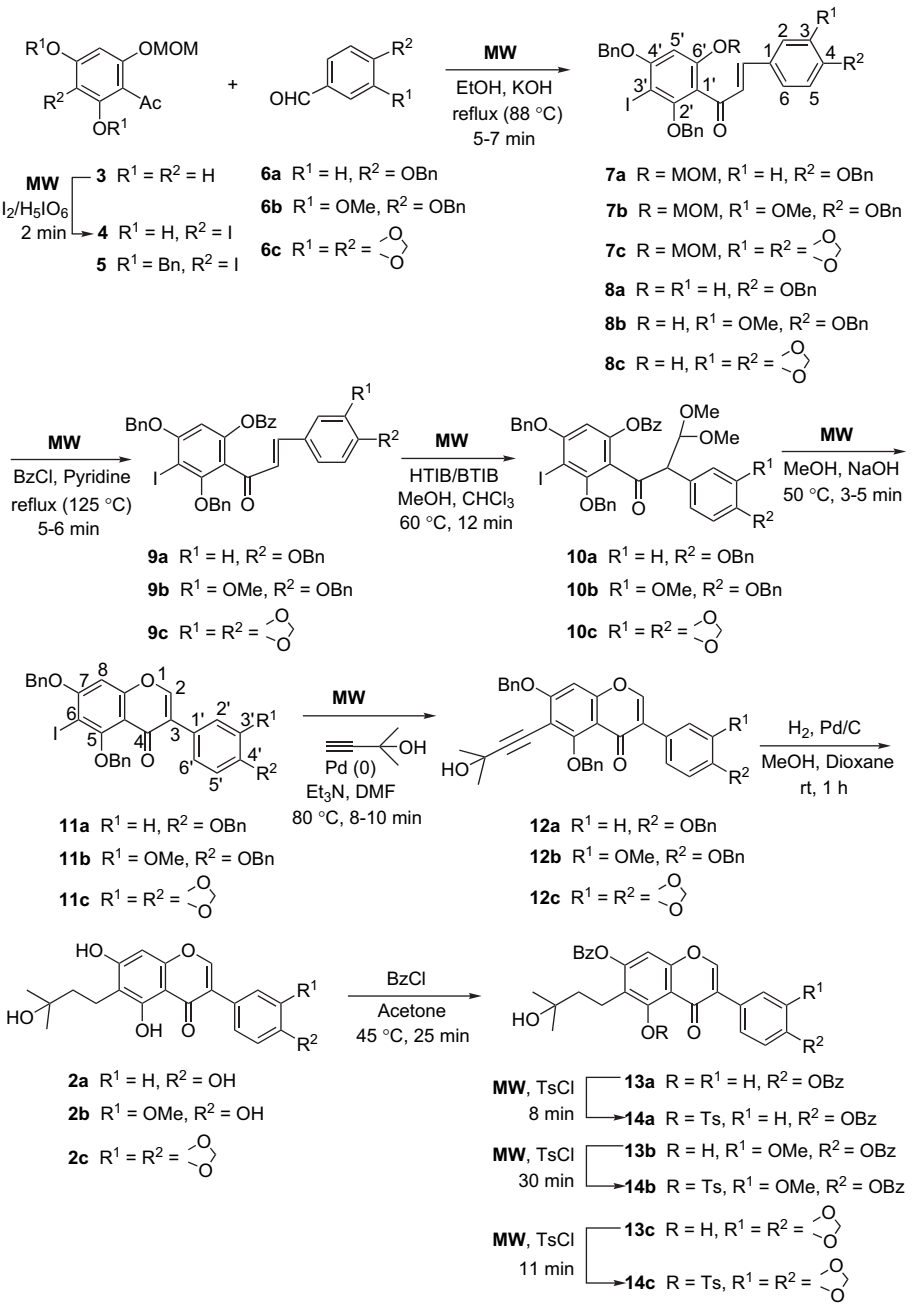


Scheme 1.

prenylated isoflavone, was isolated as a minor constituent from the leaf extract of white lupin (*L. albus* L.) and the structure was deduced as 5,7,4′-trihydroxy-6-(3-methyl-2-butenyl)-3′-methoxyisoflavone (**1b**) with the help of spectroscopic analyses.[17,18] Lupisoflavone shows moderate antifungal activity.[17] Furthermore, lupisoflavone induces the conversion of both the C_1 and C_2 cell wall isoperoxidases to the C_5 isoperoxidases, which possess scopoletin-peroxidase activity.[23] This is a unique characteristic of lupisoflavone to bring about the conformational change of these cell wall enzymes. Neither partial nor total synthesis of lupisoflavone has yet been reported by either conventional or MWI methods. Derrubone was isolated from the root of the Indian tree *Derris Robosta*.[24] Structural investigation of derrubone and its analogues (especially robustic acid, robustone and derrustone) was carried out by chemical and spectroscopic methods.[24–27] From degradative and spectroscopic analyses, the structure of derrubone was found to be 5,7-dihydroxy-6-(3-methyl-2-butenyl)-3′,4′-methylenedioxyisoflavone (**1c**). Synthesis of derrubone has been reported, however, the yield obtained was very low.[28] Moreover, the report lacked spectroscopic data to establish the structure of derrubone except for the melting point. Therefore, we report here the first total synthesis of **1b** and **1c** under MWI.

The regioselective and direct introduction of an alkenyl or alkyl group at the 6-position of the isoflavone skeleton is relatively difficult, as it consists of many protections and consequent deprotections, and the easy isomerization of 6-alkylpolyhydroxyisoflavones into 8-alkylpolyhydroxyisoflavones

by bases.[29,30] Generally, isoflavones are synthesized by oxidative rearrangement of chalcones with thallium(III) nitrate trihydrate, Tl(III)$(NO_3)_3\cdot 3H_2O$ (TTN).[31,32] Compound **2a** was also synthesized by oxidative rearrangement of the corresponding 3′-iodochalcone with TTN under conventional heating in low yield.[33] These results show the limit and scope of TTN as an oxidizing reagent of chalcones. Moreover, TTN is toxic, expensive and adversely affects the environment. Recently, it has been reported that hypervalent iodine reagents such as [hydroxy(tosyloxy)iodo]benzene (HTIB)[34] and [bis(trifluoroacetoxy)iodo]benzene (BTIB)[35] have become more useful for the oxidative rearrangement of chalcones. We were able to achieve far better results by using hypervalent iodine reagents as oxidizing agents for the conversions of chalcones to acetals and isoflavones.[20,36] Unlike TTN, hypervalent iodine reagents are environmentally-friendly and have the added benefits of being easier to prepare and handle.[37] The use of MW-technique with hypervalent iodine reagents was not only accelerated reaction pathways but also very advantageous from both the economical and the environmental standpoints. We do report here on the first total syntheses of **1a**, **2a**, **1b**, 5,7,4′-trihydroxy-6-(3-hydroxy-3-methylbutyl)-3′-methoxyisoflavone (**2b**), **1c** and 5,7-dihydroxy-6-(3-hydroxy-3-methylbutyl)-3′,4′-methylenedioxyisoflavone (**2c**) from their corresponding 3′-iodochalcones using hypervalent iodine reagents under MWI, a better synthetic route considering green chemistry (Scheme 1).

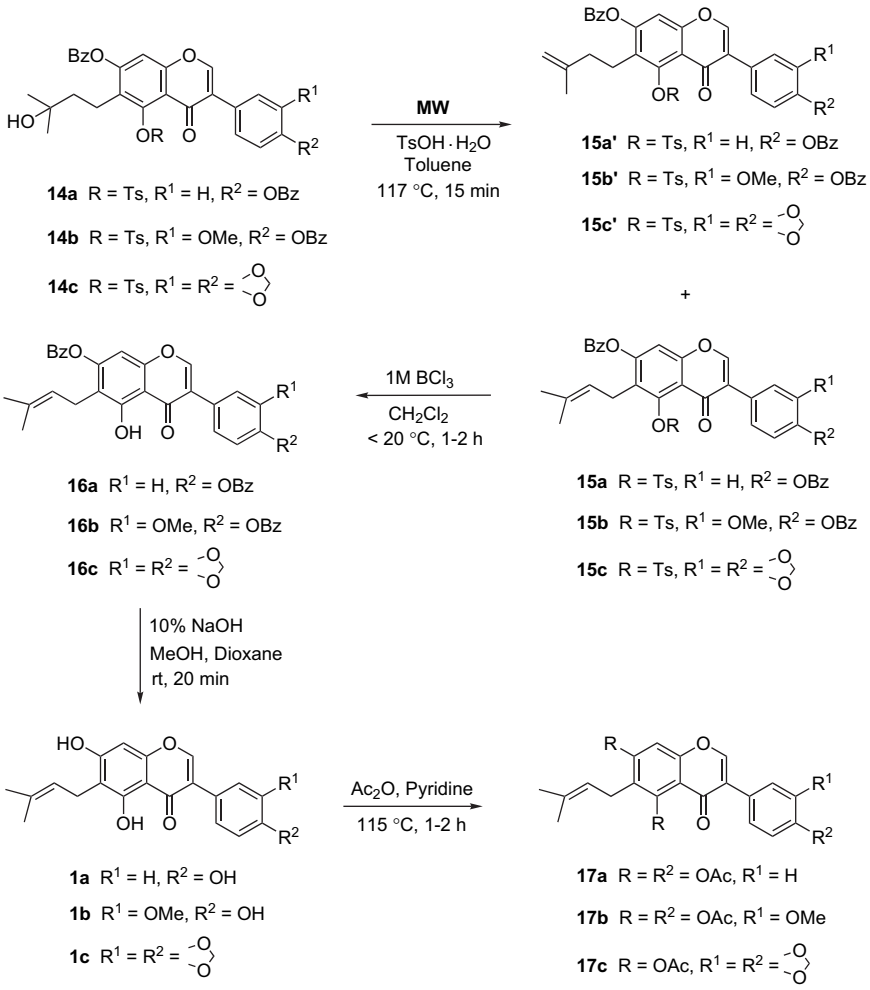
2. Results and discussion

Microwave-assisted regioselective introduction of iodine at the 3′-position of 6′-methoxymethoxyacetophenone **3**, obtained by the catalytic hydrogenolysis (5% Pd/C) of 2′,4′-bis(benzyloxy)-6′-methoxymethoxyacetophenone, was carried out with iodine and periodic acid[33,38] under temperature controlled MWI for 2 min to give the desired 3′-iodoacetophenone **4** in 96% yield. The benzylation of compound **4** with benzyl chloride in the presence of K_2CO_3 in dimethylformamide (DMF) gave 2′,4′-bis(benzyloxy)-3′-iodoacetophenone **5** in 82% yield. Microwave-mediated cross aldol condensations of **5** with different aromatic aldehydes such as 4-benzyloxybenzaldehyde (**6a**), 4-benzyloxy-3-methoxybenzaldehyde (**6b**) and 3,4-methylenedioxybenzaldehyde (**6c**) in the presence of alcoholic KOH solution from 5 to 7 min gave the 6′-methoxymethoxychalcones **7a–c** as crude semisolids, respectively. 6′-Hydroxychalcones **8a–c** were obtained from their respective crude compounds **7a–c** by concd HCl-mediated hydrolysis in a mixture of methanol and chloroform in more than 85% yields (via two steps from **5**). The separate treatment of **8a–c** with benzoyl chloride in pyridine under MWI from 5 to 6 min afforded 6′-benzoyloxychalcones **9a–c** in 91, 98 and 89% yields, respectively. The separate oxidative rearrangement of **9a–c** with HTIB in methanol under MWI for 12 min gave the respective crude acetals **10a–c**, which were liable to be unstable through silica gel column chromatography (decomposition takes place). The structures of **10a–c** were confirmed by ^{1}H NMR [δ: 3.0 and 3.22, CH(OCH_3)$_2$]. The subsequent hydrolysis of **10a–c** with 20% NaOH and in situ ring closure under MWI from 3 to 5 min afforded the desired 6-iodoisoflavones **11a–c** in 74, 59 and 46% yields (via two steps from their corresponding 6′-benzoyloxychalcones **9a–c**),

Table 1. ^{1}H NMR (400 MHz, CD_3COCD_3) data for 6-prenyl- and alkylisoflavones **1a**, **1b**, **1c** (wighteone, lupisoflavone, derrubone) and **2a**, **2b**, **2c** (wighteone hydrate, lupisoflavone hydrate, derrubone hydrate)[a]

Compound	2-H	8-H	2′-H	6′-H	3′-H	5′-H	Me	CH_2	=CH	OCH_3/OCH_2O	OH
1a	8.14s	6.49s	7.46d, J=8.7 [1H]	7.46d, J=8.7 [1H]	6.90d, J=8.7 [1H]	6.90d, J=8.7 [1H]	1.65s, 1.78s	3.37d, J=7.1	5.28t, J=7.1		13.32s
Natural product[19] (**1a**)	8.15s	6.49s	7.45d, J=8.8 [1H]	7.45d, J=8.8 [1H]	6.90d, J=8.8 [1H]	6.90d, J=8.8 [1H]	1.65s, 1.78s	3.37br d, J=7.1	5.28br t, J=7.1		13.32s
2a	8.15s	6.47s	7.46d, J=8.7 [1H]	7.46d, J=8.7 [1H]	6.91d, J=8.7 [1H]	6.91d, J=8.7 [1H]	1.26s [6H]	1.71m, 2.78m			8.43s, 13.32s
Natural product[19] (**2a**)	8.14s	6.47s	7.45d, J=8.8 [1H]	7.45d, J=8.8 [1H]	6.91d, J=8.8 [1H]	6.91d, J=8.8 [1H]	1.26s [6H]	1.71m, 2.78m			13.31s
1b	8.19s	6.50s	7.25d, J=2.0 [1H]	7.05dd, J=8.3, 2.0 [1H]		6.89d, J=8.3 [1H]	1.65s, 1.78s	3.36d, J=7.1	5.27br t	3.88s, OCH_3 [3H]	9.70s, 13.35s
Natural product[17] (**1b**)	8.19s	6.52s	7.25d, J=2.4 [1H]	7.06dd, J=8.3, 2.4 [1H]		6.89d, J=8.3 [1H]	1.65s, 1.78s	3.36d, J=7.1	5.27br t	3.89s, OCH_3 [3H]	13.35s
2b	8.18s	6.47s	7.25d, J=2.0 [1H]	7.05dd, J=8.3, 2.0 [1H]		6.89d, J=8.3 [1H]	1.26s [6H]	1.71m, 2.78m		3.88s, OCH_3 [3H]	13.34s
1c[b]	8.19s	6.50s	7.15d, J=1.7 [1H]	7.06dd, J=8.1, 1.7 [1H]		6.90d, J=8.1 [1H]	1.65s, 1.78s	3.35d, J=7.1	5.27br t	6.04s, OCH_2O [2H]	9.75s, 13.25s
2c	8.18s	6.47s	7.15d, J=1.7 [1H]	7.06dd, J=8.1, 1.7 [1H]		6.90d, J=8.1 [1H]	1.26s [6H]	1.71m, 2.77m		6.04s, OCH_2O [2H]	13.24s

[a] s: Singlet; d: doublet; t: triplet; dd: double doublet; m: multiplet; br: broad.
[b] The NMR of the natural derrubone (**1c**) is not available in the literature. So, comparison could not be made with the natural sample.



Scheme 2.

respectively. In a similar manner, the oxidative rearrangement of **9a–c** was also carried out with BTIB under MWI for 12 min and the resultant acetals **10a–c** were cyclized with 20% NaOH to give **11a–c** in 69, 25 and 32% yields, respectively. Microwave-assisted coupling reaction of **11a–c** with 2-methyl-3-butyn-2-ol in the presence of Pd(0)[39,40] in a mixture of triethylamine and DMF from 8 to 10 min gave 6-(3-hydroxy-3-methylbutynyl)isoflavones **12a–c** in 78, 64 and 68% yields, respectively. The quantitative catalytic hydrogenation and hydrogenolysis of **12a–c** with 5% Pd/C in a mixture of methanol and 1,4-dioxane at room temperature afforded 6-(3-hydroxy-3-methylbuyl)isoflavones **2a–c** in 88, 93 and 87% yields, respectively. It has been mentioned earlier that compound **2a** (wighteone hydrate) is a natural product although the other two compounds **2b** and **2c** are not yet obtained as natural products. The spectral data and other physical properties of **2a** were identical with those of the natural sample of wighteone hydrate[19] (see Table 1, Section 4). Exhaustive benzoylation of **2a** with benzoyl chloride under MWI gave a mixture (about 1:1) of 4′,7-bis(benzoyloxy)isoflavone **13a** and 4′,5,7-tris(benzoyloxy)-6-(3-hydroxy-3-methylbutyl)isoflavone. On the other hand, the exhaustive benzoylation of 6-alkylpolyhydroxyisoflavones with bases in prolonged reaction time causes their isomerization to 8-alkylpolyhydroxyisoflavones by conventional heating method.[29,30,33] Therefore, the partial benzoylation of **2a–c** was achieved in acetone at 45 °C for 25 min to give the 5-hydroxyisoflavones **13a–c** in 85, 86 and 91% yields, respectively. The failure of exhaustive benzoylation of compounds **2a–c** is presumably due to the non-bonding interaction of 5-OH with C-4 carbonyl oxygen. Tosylation of the 5-hydroxyisoflavones **13a–c** with TsCl under MWI from 8 to 30 min in acetone gave 5-tosyloxyisoflavones **14a–c** in 89, 93 and 94% yields, respectively Scheme 2.

Compound **14a** was dehydrated with $TsOH \cdot H_2O$ in a solution of acetic acid and dry toluene under MWI for 15 min[†] to give a mixture of the desired 6-prenylisoflavone **15a** and the regioisomer, 6-(3-methyl-3-butenyl)isoflavone **15a′**. The dehydration of the other compounds **14b** and **14c** was also carried out in a similar manner to give the respective 6-prenylisoflavones **15b** and **15c** with a slight amount of their corresponding regioisomers **15b′** and **15c′**. The ^1H NMR spectra of each of the isomeric alkenyl mixtures (**15a** and **15a′**, **15b** and **15b′**, **15c** and **15c′**) showed that the unwanted regioisomers (**15a′–c′**) were less than 5% in

[†] The total reaction time was observed to be 30 min. But, it took 15 min for the reaction mixture to get reflux.

every case. The formation of the 6-alkenylisoflavones **15a–c** as major products can easily be understood by their ^{1}H NMR spectra [peaks due to $CH_2CH{=}C(CH_3)_2$ at δ: 3.37 (2H, d) and $CH_2CH_2C(CH_3){=}CH_2$ at δ: 4.51 and 4.62 (each 1H, s)]. The same dehydration under classical heating conditions, which was reported in our previous papers,[20,33] led to the formation of the unwanted regioisomer in about 28% yield. It is difficult to remove the regioisomer from the mixture by the usual physical methods. But, in the case of the microwave method, the unwanted regioisomer, which is less than 5%, is very easy to remove by usual physical methods such as recrystallization. It is clear from our data that microwave heating has advantages over the classical heating in that it reduces reaction time and solvent quantity and also on account of its very high regioselectivity, which is explained below.

2.1. High regioselectivity of MW dehydration—formation of 6-prenylisoflavone as major product

It has been reported that microwave dielectric heating and non-thermal effects play an important role in the regio-, chemo- and stereoselectivity, however, it is worth noting that there is no concrete clarification of these observations.[4] Without exception, we were able to achieve very high regioselectivity of the MW dehydration of compounds **14a–c**. Each of the dehydrations led to the formation of the required 6-prenylisoflavones **15a–c** as major products (in some cases, one product exclusively) and far less or almost no regioisomers **15a′–c′**. This increased selectivity is the most important factor in MW-synthesis, because the desired product was obtained, rather than the unwanted regioisomer. The possible explanation for the formation of 6-prenylisoflavones as major products lies in the fact that, due to its powers, MW provides elevated heating rates and accelerated reaction times. We used toluene as solvent for the dehydration and the reflux temperature was observed to be 117 °C, which was higher than its conventional boiling point (110 °C). Under such elevated heating conditions, the thermodynamically-controlled products, 6-prenylisoflavones, predominated over the kinetically-controlled regioisomers. The unwanted regioisomer, thermally labile, is converted into the more stable 6-prenylisoflavone due to such elevated heating rates.

The detosylation of **15a–c** with 1 M BCl_3 solution in dichloromethane at room temperature gave the respective 5-hydroxyisoflavones **16a–c** in 91, 94 and 79% yields, respectively. The hydrolysis of **16a–c** with 10% NaOH in a mixture of methanol and 1,4-dioxane at room temperature gave 5,7,4′-trihydroxy-6-(3-methyl-2-butenyl)isoflavone (wighteone) (**1a**) in 72%, 5,7,4′-trihydroxy-6-(3-methyl-2-butenyl)-3′-methoxyisoflavone (lupisoflavone) (**1b**) in 62% and 5,7-dihydroxy-6-(3-methyl-2-butenyl)-3′,4′-methylenedioxyisoflavone (derrubone) (**1c**) in 79% yields, respectively. The spectral data and other physical properties of **1a**, **1b** and **1c** were identical with those of the natural samples of wighteone,[19] lupisoflavone[17] and derrubone,[24] respectively (see Table 1, Section 4). On the basis of these results, the structures of wighteone, lupisoflavone and derrubone were confirmed for the first time by the MW-synthesis of **1a**, **1b** and **1c**, respectively. 6-Prenylisoflavones **1a–c** were converted into their respective acetate derivatives **17a–c**.

3. Conclusion

For the total synthesis of biologically important 6-prenylisoflavones **1a–c**, MWI technique was employed successfully. MW-synthesis was much more advantageous over the conventional method with regard to reaction time, solvent quantity and product yield. By using the MWI method in our synthesis, moreover, we were able to achieve very high regioselectivity compared to the results obtained under conventional heating. And though wighteone was obtained in a slight mixture with its regioisomer (5%), the other two compounds (lupisoflavone and derrubone) were obtained with a very small amount of regioisomers under microwave dehydration. This high regioselective synthesis of prenylisoflavones under microwave conditions is very important as it gave clean product and avoided the need for arduous regioisomeric separation.

4. Experimental

4.1. General

All the melting points were taken on a Yanaco MP-J3 micro melting-point apparatus and were uncorrected. The ^{1}H NMR spectra were recorded with a JEOL EX-400 spectrophotometer (400 MHz) using tetramethylsilane (TMS) as the internal standard. The IR spectra were obtained on an FT/IR-460 Plus (JASCO) spectrophotometer using KBr pellets. The UV spectra were obtained on a Hitachi U-2000 spectrophotometer. Elemental analyses were obtained on a Yanaco CHN corder model MT-5. A microwave oven (650 W and 2.45 GHz, modified properly by fitting a condenser and a thermo-sensor through the holes made in the roof; Shikoku Instrumentation Co., Ltd, Japan) was used as a reaction apparatus. Column chromatography and thin-layer chromatography (TLC) were carried out with Kieselgel 60 (70–230 mesh) and Kieselgel 60 F_{254} (Merck).

4.1.1. 2′,4′-Dihydroxy-6′-methoxymethoxyacetophenone (3). The palladium/carbon catalyzed hydrogenolysis of 2′,4′-bis(benzyloxy)-6′-methoxymethoxyacetophenone[30] (4.80 g, 12.2 mmol), which was synthesized by methoxymethylation of 2′,4′-bis(benzyloxy)-6′-hydroxyacetophenone, in a mixture of MeOH (100 ml) and AcOEt (100 ml) was carried out at 20 °C until the uptake of hydrogen ceased. The solvent was removed under reduced pressure and the resulting compound was recrystallized from a mixture of AcOEt and hexane to give **3** (2.48 g, 95%) as colourless crystals, mp 117–119 °C. ^{1}H NMR ($CDCl_3$) δ: 2.65 (3H, s, $COCH_3$), 3.52 (3H, s, OCH_3), 5.25 (2H, s, OCH_2O), 6.04 (1H, d, J=2.4 Hz, Ar–H), 6.14 (1H, d, J=2.4 Hz, Ar–H), 13.79 (1H, s, $C_{2'}$–OH); Anal. Calcd for $C_{10}H_{12}O_5$: C, 56.60; H, 5.70. Found: C, 56.61; H, 5.60.

4.1.2. MW-synthesis of 2′,4′-dihydroxy-3′-iodo-6′-methoxymethoxyacetophenone (4). Compound **3** (2.50 g, 11.8 mmol) was dissolved in ethanol (40 ml), followed by the successive addition of iodine (1.49 g, 5.87 mmol) and periodic acid (542 mg, 2.37 mmol in water, 5 ml). The reaction mixture was irradiated under MW for 2 min at 45 °C. Cooling and diluting the reaction mixture with water gave a crystalline solid, which was recrystallized from a mixture

of AcOEt and hexane to give **4** (3.85 g, 96%) as a pale yellow solid, mp 162–164 °C. ^{1}H NMR ($CDCl_3$) δ: 2.68 (3H, s, $COCH_3$), 3.51 (3H, s, OCH_3), 5.27 (2H, s, OCH_2O), 6.0 (1H, s, $C_{4'}$–OH), 6.43 (1H, s, $C_{5'}$–H), 14.95 (1H, s, $C_{2'}$–OH); Anal. Calcd for $C_{10}H_{11}IO_5$: C, 35.52; H, 3.28. Found: C, 35.32; H, 3.17.

4.1.3. 2′,4′-Bis(benzyloxy)-3′-iodo-6′-methoxymethoxyacetophenone (5). A solution of benzyl chloride (4.10 g, 32.4 mmol) in DMF (5 ml) was added slowly to a mixture of **4** (5.0 g, 14 mmol) and K_2CO_3 (10.0 g, 72.3 mmol) in DMF (50 ml) under nitrogen. The reaction mixture was heated at 70 °C for 1 h, and then cooled to room temperature, and extracted with $CHCl_3$. The extract was washed with 5% HCl and water and dried (Na_2SO_4) after which the solvent was removed. The residue was recrystallized from a mixture of AcOEt and MeOH to give **5** (6.30 g, 82%) as colourless needles, mp 98–99 °C. ^{1}H NMR ($CDCl_3$) δ: 2.47 (3H, s, $COCH_3$), 3.46 (3H, s, OCH_3), 4.97 (2H, s, $PhCH_2$), 5.15 (2H, s, OCH_2O), 5.18 (2H, s, $PhCH_2$), 6.65 (1H, s, $C_{5'}$–H), 7.32–7.61 (10H, m, Ar–H×10); Anal. Calcd for $C_{24}H_{23}IO_5$: C, 55.61; H, 4.47. Found: C, 55.66; H, 4.48.

4.1.4. MW-synthesis of 4,2′,4′-tris(benzyloxy)-3′-iodo-6′-methoxymethoxychalcone (7a) and 4,2′,4′-tris(benzyloxy)-6′-hydroxy-3′-iodochalcone (8a). A mixture of **5** (5.0 g, 9.6 mmol) and **6a** (2.66 g, 12.5 mmol) was dissolved in alc. KOH (5.40 g, 96.2 mmol in 100 ml EtOH). The reaction mixture was irradiated under MW for 6 min (1 min×6 times irradiation, 1–2 min interval/irradiation), and monitored by TLC to establish completion. The reaction mixture was neutralized with 10% HCl and extracted with $CHCl_3$, and then the solvent was removed under reduced pressure to give a yellow semisolid mass of **7a**, which was hydrolyzed with concd HCl in a mixture of MeOH (60 ml) and $CHCl_3$ (60 ml) at 40 °C for 1 h. The hydrolyzed mixture was allowed to cool to room temperature, extracted with $CHCl_3$, washed with water and dried (Na_2SO_4). The solvent was removed under reduced pressure to give a solid mass, which was recrystallized from a mixture of $CHCl_3$ and AcOEt to afford **8a** (5.99 g, 93%, two steps yield from **5**) as a yellow solid, mp 138–140 °C. ^{1}H NMR ($CDCl_3$) δ: 4.85, 5.10 and 5.21 (each 2H, s, $PhCH_2$), 6.42 (1H, s, $C_{5'}$–H), 6.82 (2H, d, J=8.7 Hz, C_3– and C_5–H), 7.18–7.52 (17H, m, Ar–H×17), 7.83 and 7.88 (each 1H, d, J=15.4 Hz, =CH), 13.77 (1H, s, $C_{6'}$–OH); Anal. Calcd for $C_{36}H_{29}IO_5$: C, 64.68; H, 4.37. Found: C, 64.81; H, 4.53.

4.1.5. MW-synthesis of 4,2′,4′-tris(benzyloxy)-6′-benzoyloxy-3′-iodochalcone (9a). Benzoyl chloride (1.73 g, 12.3 mmol) was slowly added to a mixture of **8a** (5.50 g, 8.23 mmol) in pyridine (45 ml). The reaction mixture was irradiated incessantly under MW at 125 °C for 5 min. After cooling, it was extracted with $CHCl_3$, washed with 5% HCl and water and dried (Na_2SO_4). After removal of the solvent, a pale yellow crude mass was obtained. The crude was purified on silica gel column chromatography ($CHCl_3$/hexane; 3:2) to give **9a** as a fluffy crystalline solid (5.75 g, 91%), mp 47–48 °C. ^{1}H NMR ($CDCl_3$) δ: 4.99, 5.07 and 5.21 (each 2H, s, $PhCH_2$), 6.76 (1H, s, $C_{5'}$–H), 6.88 (1H, d, J=15.8 Hz, =CH), 6.89 (2H, d, J=8.7 Hz, C_2– and C_6–H), 7.27–7.63 (20H, m, Ar–H×20), 8.04 (2H, d, J=8.7 Hz, C_3– and C_5–H), 8.08 (1H, d, J=15.8 Hz, =CH); Anal. Calcd for $C_{43}H_{33}IO_6$: C, 66.85; H, 4.31. Found: C, 66.68; H, 4.45.

4.1.6. MW-synthesis of 1-[6-benzoyloxy-2,4-bis(benzyloxy)-3-iodophenyl]-2-(4-benzyloxyphenyl)-3,3-dimethoxypropan-1-one (10a) and 5,7,4′-tris(benzyloxy)-6-iodoisoflavone (11a). Compound **9a** (5.50 g, 7.12 mmol) was dissolved in a mixture of MeOH (50 ml) and $CHCl_3$ (20 ml), followed by the addition of HTIB (4.12 g, 10.5 mmol). The reaction mixture was irradiated under MW for 12 min (2 min×6 times irradiation, 1–2 min interval/irradiation) at 60 °C. The excess HTIB was decomposed with 5% Na_2SO_3 solution (1.5 ml) and then the reaction mixture was extracted with $CHCl_3$, washed with water and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave crude acetal **10a** (6.50 g) as a semisolid mass. This crude mass was dissolved in a mixture of MeOH (40 ml) and $CHCl_3$ (10 ml), followed by the addition of 20% NaOH (32 ml) and irradiated under MW at 50 °C for 5 min. The reaction mixture was neutralized with 10% HCl, extracted with $CHCl_3$, washed with water and dried (Na_2SO_4). The solvent was removed under reduced pressure to give a yellow solid. The crude solid was purified by column chromatography (CH_2Cl_2/hexane; 3:1) and further recrystallized from a mixture of AcOEt and MeOH (1:1) to give 6-iodoisoflavone **11a** (3.51 g, 74%, two steps yield from **8a**), mp 154–156 °C. ^{1}H NMR ($CDCl_3$) δ: 5.07, 5.10 and 5.26 (each 2H, s, $PhCH_2$), 6.74 (1H, s, C_8–H), 7.02 (2H, d, J=8.3 Hz, $C_{3'}$– and $C_{5'}$–H), 7.31–7.49 (15H, m, Ar–H×15), 7.76 (2H, d, J=8.3 Hz, $C_{2'}$– and $C_{6'}$–H), 7.81 (1H, s, C_2–H); Anal. Calcd for $C_{36}H_{27}IO_5$: C, 64.87; H, 4.08. Found: C, 64.65; H, 4.22.

Acetal **10a**: ^{1}H NMR ($CDCl_3$) δ: 3.0 and 3.22 (each 3H, s, OCH_3), 4.92, 5.02 and 5.11 (each 2H, s, $PhCH_2$), 4.78 and 4.95 (each 1H, d, J=10.2 Hz, CH), 6.59 (1H, s, Ar–H), 7.05 (2H, d, J=8.7 Hz, Ar–H×2), 7.11 (2H, d, J=8.6 Hz, Ar–H×2), 7.13–7.71 (24H, m, Ar–H×24).

The similar treatment of compound **9a** (2.11 g, 2.97 mmol) with BTIB (1.91 g, 4.44 mmol) under MWI for 12 min (2 min×6 times irradiation, 1–2 min interval/irradiation) at 60 °C gave crude acetal **10a**, which was cyclized with 20% NaOH under MWI for 5 min to give **11a** (1.25 g, 69%).

4.1.7. MW-synthesis of 5,7,4′-tris(benzyloxy)-6-(3-hydroxy-3-methyl-1-butynyl)isoflavone (12a). Compound **11a** (3.50 g, 5.25 mmol) was dissolved in DMF (15 ml), followed by the successive addition of Et_3N (60 ml), $PdCl_2$ (46 mg, 0.25 mmol), PPh_3 (120 mg, 0.457 mmol) and CuI (44 mg, 0.23 mmol) and finally 2-methyl-3-butyn-2-ol (1.53 ml, 15.7 mmol). The reaction mixture was irradiated under MW at 80 °C under nitrogen for 8 min (1 min×8 times irradiation, 1–2 min interval/irradiation), and then cooled to room temperature. The cool mixture was filtered through a sintered glass using Celite and the filtrate was extracted with AcOEt. The AcOEt extract was washed with 5% HCl and water and dried (Na_2SO_4). The solvent was removed under reduced pressure and the resulting solid was chromatographed on silica gel column (CH_2Cl_2/AcOEt; 9:1) and further recrystallized from a mixture of AcOEt and Me_2CO (2:1) to give **12a** as a colourless crystalline solid (2.55 g, 78%), mp 170–171 °C. ^{1}H NMR ($CDCl_3$) δ: 1.50

(6H, s, $CH_3\times2$), 5.10 (2H, s, $PhCH_2$), 5.20 (4H, s, $PhCH_2\times2$), 6.71 (1H, s, C_8–H), 7.02 (2H, d, J=8.7 Hz, $C_{3'}$– and $C_{5'}$–H), 7.66 (2H, d, J=8.7 Hz, $C_{2'}$– and $C_{6'}$–H), 7.29–7.52 (15H, m, Ar–H×15), 7.79 (1H, s, C_2–H); Anal. Calcd for $C_{41}H_{34}O_6$: C, 79.08; H, 5.50. Found: C, 79.11; H, 5.68.

4.1.8. 5,7,4′-Trihydroxy-6-(3-hydroxy-3-methylbutyl)isoflavone (wighteone hydrate) (2a). Compound **12a** (1.0 g, 1.6 mmol) was hydrogenolyzed over 5% Pd/C (120 mg) in a mixture of methanol (35 ml) and dioxane (35 ml) until the uptake of hydrogen ceased. The resulting compound was recrystallized from a mixture of MeOH and Me_2CO to give **2a** (504 mg, 88%) as a colourless solid, mp 230–232 °C (lit.[19] 225–228 °C). ^{1}H NMR (see Table 1); IR (KBr) ν_{max} 3340, 3300, 2920, 1620, 1500, 1450, 1220, 1058 cm^{-1}; UV λ_{max} nm (log ε) (MeOH): 265sh (4.41), 214 (4.29), (+$AlCl_3$) 269 (4.37), (+NaOAc) 335.5 (4.1), 274.5sh (4.39), 231sh (4.45); Anal. Calcd for $C_{20}H_{20}O_6$: C, 67.41; H, 5.66. Found: C, 67.61; H, 5.80.

4.1.9. 7,4′-Bis(benzoyloxy)-5-hydroxy-6-(3-hydroxy-3-methylbutyl)isoflavone (13a). A mixture of **2a** (650 mg, 1.82 mmol), benzoyl chloride (0.48 ml, 4.1 mmol) and K_2CO_3 (1.40 g, 10.1 mmol) in acetone (25 ml) was heated at 45 °C under nitrogen for 25 min. Filtered off K_2CO_3, and removal of the solvent under reduced pressure gave a residue, which was extracted with AcOEt, washed with 5% HCl and water and dried (Na_2SO_4). The solvent was removed under reduced pressure and the resulting compound was recrystallized from a mixture of CH_2Cl_2 and Me_2CO to give **13a** (880 mg, 85%) as a colourless solid, mp 160–161 °C. ^{1}H NMR ($CDCl_3$) δ: 1.20 (6H, s, $CH_3\times2$), 1.74 and 2.77 (each 2H, m, CH_2), 6.90 (1H, s, C_8–H), 7.34 (2H, d, J=8.5 Hz, $C_{3'}$– and $C_{5'}$–H), 7.25–7.68 (10H, m, Ar–H×10), 8.01 (1H, s, C_2–H), 8.24 (2H, d, J=8.5 Hz, $C_{2'}$– and $C_{6'}$–H), 13.13 (1H, s, $C_{5'}$–OH); Anal. Calcd for $C_{34}H_{28}O_8$: C, 72.33; H, 5.00. Found: C, 72.45; H, 5.10.

4.1.10. MW-synthesis of 7,4′-bis(benzoyloxy)-6-(3-hydroxy-3-methylbutyl)-5-tosyloxyisoflavone (14a). A mixture of **13a** (500 mg, 0.885 mmol), tosyl chloride (290 mg, 1.52 mmol) and K_2CO_3 (1.29 g, 9.33 mmol) in acetone (35 ml) was irradiated under MW for 8 min (2 min×4 times irradiation, 1–2 min interval/irradiation). The reaction mixture was cooled to room temperature, and neutralized with 5% HCl and then extracted with AcOEt, washed with water and dried (Na_2SO_4). After removal of the solvent, the obtained crude solid was recrystallized from a mixture of $CHCl_3$ and Me_2CO (10:3) to give **14a** (566 mg, 89%) as colourless needles, mp 178–181 °C. ^{1}H NMR ($CDCl_3$) δ: 1.14 (6H, s, $CH_3\times2$), 1.33 (1H, br s, OH), 1.74 and 2.82 (each 2H, m, CH_2), 2.42 (3H, s, Ar–CH_3), 7.25–7.74 (15H, m, Ar–H×15), 7.90 (1H, s, C_2–H), 7.96 (2H, d, J=8.5 Hz, $C_{3'}$– and $C_{5'}$–H), 8.23 (2H, d, J=8.5 Hz, $C_{2'}$– and $C_{6'}$–H); Anal. Calcd for $C_{41}H_{34}O_{10}S$: C, 68.51; H, 4.77. Found: C, 68.75; H, 4.81.

4.1.11. MW-synthesis of 7,4′-bis(benzoyloxy)-6-(3-methyl-2-butenyl)-5-tosyloxyisoflavone (15a). To a solution of **14a** (1.0 g, 1.4 mmol) in dry toluene (15 ml) was added $TsOH\cdot H_2O$ (2.38 ml of a 5.24×10^{-1} M solution in acetic acid). The reaction mixture was refluxed under MWI at 117 °C for 15 min. After cooling, the reaction mixture was extracted with ether, washed with 5% $NaHCO_3$ and water and dried (Na_2SO_4). After removal of the solvent, the obtained crude mass was chromatographed on silica gel column ($CHCl_3$ as a solvent) to give 6-alkenylisoflavone as a crystalline solid. The ^{1}H NMR spectrum showed that it was a mixture of 6-(3-methyl-2-butenyl)isoflavone **15a** and the regioisomer, 6-(3-methyl-3-butenyl)isoflavone **15a′** (**15/15a′**=95:5). The isomeric mixture was recrystallized twice from a mixture of $CHCl_3$ and Me_2CO (5:1) to give **15a** (720 mg, 74% from **14a**) as a crystalline solid, mp 202–204 °C. ^{1}H NMR ($CDCl_3$) δ: 1.41 and 1.46 (each 3H, s, CH_3), 2.40 (3H, s, Ar–CH_3), 3.36 (2H, d, J=6.5 Hz, CH_2), 4.96 (1H, t, J=6.5 Hz, =CH), 7.25–7.70 (13H, m, Ar–H×13), 7.89 (1H, s, C_2–H), 7.92–8.25 (6H, m, Ar–H×6); Anal. Calcd for $C_{41}H_{32}O_9S$: C, 70.27; H, 4.60. Found: C, 70.05; H, 4.72.

4.1.12. 7,4′-Bis(benzoyloxy)-5-hydroxy-6-(3-methyl-2-butenyl)isoflavone (16a). Compound **15a** (400 mg, 0.571 mmol) was dissolved in dry CH_2Cl_2 (10 ml), followed by the addition of BCl_3 (0.60 ml, 1 M solution in CH_2Cl_2) in an ice bath. The reaction mixture was stirred below 20 °C under nitrogen for 2.5 h. The resulting mixture was quenched with saturated NH_4Cl solution and extracted with CH_2Cl_2, washed with water and dried (Na_2SO_4). The solvent was removed under reduced pressure and the obtained compound was purified on silica gel column chromatography ($CHCl_3$ as a solvent) and further recrystallized from AcOEt to give **16a** (285 mg, 91%) as a colourless crystalline solid, mp 192–194 °C. ^{1}H NMR ($CDCl_3$) δ: 1.58 and 1.60 (each 3H, s, CH_3), 3.39 (2H, d, J=6.8 Hz, CH_2), 5.17 (1H, t, J=6.8 Hz, =CH), 6.87 (1H, s, C_8–H), 7.32–7.67 (10H, m, Ar–H×10), 8.00 (1H, s, C_2–H), 8.20–8.24 (4H, m, Ar–H×4), 13.10 (1H, s, C_5–OH); Anal. Calcd for $C_{34}H_{26}O_7$: C, 74.71; H, 4.79. Found: C, 74.57; H, 4.91.

4.1.13. 5,7,4′-Trihydroxy-6-(3-methyl-2-butenyl)isoflavone (wighteone) (1a). Compound **16a** (180 mg, 0.329 mmol) was dissolved in a mixture of methanol (3 ml) and dioxane (3 ml), followed by the addition of 10% NaOH (2 ml). The reaction mixture was stirred at 25 °C for 20 min. The resulting mixture was neutralized with 2% HCl and the organic layer was evaporated under reduced pressure. The obtained residue was extracted with AcOEt, washed with water and dried (Na_2SO_4). The solvent was removed under reduced pressure to give a solid mass, which was chromatographed on silica gel column (AcOEt/$CHCl_3$; 1:6) and the resulting compound was recrystallized from a mixture of $CHCl_3$ and AcOEt to give the 6-prenylisoflavone **1a** (80 mg, 72%) as a pale yellow crystalline solid, mp 205–207 °C (lit.[19] 206–208 °C). ^{1}H NMR (see Table 1); IR (KBr) ν_{max} 3365, 3240, 2930, 1650, 1615, 1510, 1215, 1065, 818 cm^{-1}; UV λ_{max} nm (log ε) (MeOH): 266sh (4.45), 214 (4.38), (+$AlCl_3$) 268.5sh (4.41), (+NaOAc) 341 (3.93), 275.5 (4.43), 229sh (4.70); Anal. Calcd for $C_{20}H_{18}O_5$: C, 70.99; H, 5.36. Found: C, 70.88; H, 5.52.

4.1.14. 5,7,4′-Triacetoxy-6-(3-methyl-2-butenyl)isoflavone (17a). Acetylation of **1a** (40 mg, 0.11 mmol) was achieved by acetic anhydride/pyridine method at 115 °C for 2 h. The obtained gummy mass was chromatographed on silica gel column ($CHCl_3$/hexane; 5:1) and further

recrystallized from a mixture of $CHCl_3$ and hexane to give **17a** (38 mg, 71%) as a colourless crystalline solid, mp 173–175 °C. ^{1}H NMR ($CDCl_3$) δ: 1.67 and 1.75 (each 3H, s, CH_3), 2.31, 2.35 and 2.43 (each 3H, s, $COCH_3$), 3.25 (2H, br d, CH_2), 5.01 (1H, br t, =CH), 7.13 (2H, d, J=8.6 Hz, $C_{3'}$– and $C_{5'}$–H), 7.21 (1H, s, C_8–H), 7.49 (2H, d, J=8.6 Hz, $C_{2'}$– and $C_{6'}$–H), 7.86 (1H, s, C_2–H). Anal. Calcd for $C_{26}H_{24}O_8$: C, 67.23; H, 5.21. Found: C, 67.35; H, 5.32.

4.1.15. MW-synthesis of 4,2′,4′-tris(benzyloxy)-3′-iodo-3-methoxy-6′-methoxymethoxychalcone (7b) and 4,2′,4′-tris(benzyloxy)-6′-hydroxy-3′-iodo-3-methoxychalcone (8b). A mixture of **5** (4.40 g, 8.48 mmol) and **6b** (2.46 g, 10.2 mmol) was dissolved in alc. KOH (3.30 g, 58.8 mmol in 60 ml EtOH). The reaction mixture was irradiated under MW for 7 min (1 min×7 times irradiation, 1–2 min interval/irradiation), and monitored by TLC to establish completion. A similar treatment of the reaction mixture (as in the case of compound **8a**) gave **8b** (5.65 g, 95%, two steps yield from **5**) as a yellow solid, mp 133–134 °C. ^{1}H NMR ($CDCl_3$) δ: 3.66 (3H, s, OCH_3), 4.82 (2H, s, $PhCH_2$), 5.20 (4H, s, $PhCH_2$×2), 6.42 (1H, s, $C_{5'}$–H), 6.76 (1H, d, J=8.3 Hz, C_5–H), 6.82 (1H, d, J=1.7 Hz, C_2–H), 6.91 (1H, dd, J=8.3 and 1.7 Hz, C_6–H), 7.15–7.52 (15H, m, Ar–H×15), 7.81 and 7.86 (each 1H, d, J=15.3 Hz, =CH), 13.77 (1H, s, $C_{6'}$–OH); Anal. Calcd for $C_{37}H_{31}IO_6$: C, 63.62; H, 4.47. Found: C, 63.47; H, 4.63.

4.1.16. MW-synthesis of 4,2′,4′-tris(benzyloxy)-6′-benzoyloxy-3′-iodo-3-methoxychalcone (9b). Benzoyl chloride (1.56 g, 11.2 mmol) was slowly added to a mixture of **8b** (6.0 g, 8.6 mmol) in pyridine (55 ml). The reaction mixture was irradiated incessantly under MW at 125 °C for 5 min. The reaction mixture was worked up in a similar manner (as in the case of compound **9a**) to give **9b** (6.79 g, 98%) as a fluffy crystalline solid, mp 65–68 °C. ^{1}H NMR ($CDCl_3$) δ: 3.83 (3H, s, OCH_3), 4.98, 5.17 and 5.20 (each 2H, s, $PhCH_2$), 6.76 (1H, s, $C_{5'}$–H), 6.79 (1H, d, J=8.3 Hz, C_5–H), 6.91 (1H, dd, J=8.3 and 1.7 Hz, C_6–H), 6.89 (1H, d, J=15.8 Hz, =CH), 6.94 (1H, d, J=1.7 Hz, C_2–H), 6.96 (1H, d, J=15.8 Hz, =CH), 7.25–7.59 (20H, m, Ar–H×20); Anal. Calcd for $C_{44}H_{35}IO_7$: C, 65.84; H, 4.58. Found: C, 65.84; H, 4.43.

4.1.17. MW-synthesis of 1-[6-benzoyloxy-2,4-bis(benzyloxy)-3-iodophenyl]-2-(4-benzyloxy-3-methoxyphenyl)-3,3-dimethoxypropan-1-one (10b) and 5,7,4′-tris(benzyloxy)-6-iodo-3′-methoxyisoflavone (11b). Compound **9b** (7.0 g, 8.7 mmol) was dissolved in a mixture of MeOH (50 ml) and $CHCl_3$ (10 ml), followed by the addition of HTIB (5.46 g, 13.9 mmol). The reaction mixture was irradiated under MW for 12 min (2 min×6 times irradiation, 1–2 min interval/irradiation) at 60 °C. The reaction mixture was worked up in a similar manner (as in the case of compound **10a**) to give crude acetal **10b**. This crude mass was hydrolyzed and cyclized in a similar manner (as in the case of compound **11a**) to give isoflavone **11b** (3.53 g, 59%, two steps yield from **8b**), mp 198–200 °C. ^{1}H NMR ($CDCl_3$) δ: 3.92 (3H, s, OCH_3), 5.06, 5.20 and 5.26 (each 2H, s, $PhCH_2$), 6.75 (1H, s, C_8–H), 6.92 (1H, d, J=7.8 Hz, $C_{5'}$–H), 6.94 (1H, dd, J=7.8 and 1.9 Hz, $C_{6'}$–H), 7.16 (1H, d, J=1.9 Hz, $C_{2'}$–H), 7.26–7.53 (15H, m, Ar–H×15), 7.81 (1H, s, C_2–H); Anal. Calcd for $C_{37}H_{29}IO_6$: C, 63.80; H, 4.22. Found: C, 63.63; H, 4.26.

Acetal **10b**: ^{1}H NMR ($CDCl_3$) δ: 3.0 and 3.22 (each 3H, s, OCH_3), 4.68 and 4.78 (each 1H, d, J=10.2 Hz, CH), 5.10 (2H, s, $PhCH_2$), 5.16 and 5.20 (each 2H, s, $PhCH_2$), 6.56 (1H, d, J=8.3 Hz, Ar–H), 6.60 (1H, dd, J=8.3 and 1.7 Hz, Ar–H), 6.65 (1H, d, J=1.7 Hz, Ar–H), 6.71–7.70 (21H, m, Ar–H×21).

The similar treatment of compound **9b** (180 mg, 0.224 mmol) with BTIB (145 mg, 0.337 mmol) under MWI for 12 min (2 min×6 times irradiation, 1–2 min interval/irradiation) at 60 °C gave crude acetal **10b**, which was cyclized with 20% NaOH under MWI for 5 min to give **11b** (39 mg, 25%).

4.1.18. MW-synthesis of 5,7,4′-tris(benzyloxy)-6-(3-hydroxy-3-methyl-1-butynyl)-3′-methoxyisoflavone (12b). Compound **11b** (1.50 g, 2.15 mmol) was dissolved in DMF (12 ml), followed by the successive addition of Et_3N (40 ml), $PdCl_2$ (30 mg, 0.16 mmol), PPh_3 (70 mg, 0.26 mmol) and CuI (44 mg, 0.23 mmol) and finally 2-methyl-3-butyn-2-ol (0.85 ml, 8.7 mmol). The similar treatment of the reaction mixture under MWI for 10 min (2 min×5 times irradiation, 1–2 min interval/irradiation) (as in the case of compound **12a**) gave **12b** as a colourless crystalline solid (0.89 g, 64%), mp 151–153 °C. ^{1}H NMR ($CDCl_3$) δ: 1.51 (6H, s, CH_3×2), 3.92 (3H, s, OCH_3), 5.20 (6H, s, $PhCH_2$×3), 6.72 (1H, s, C_8–H), 6.90 (1H, d, J=8.3 Hz, $C_{5'}$–H), 6.94 (1H, dd, J=8.3 and 1.7 Hz, $C_{6'}$–H), 7.16 (H, d, J=1.9 Hz, $C_{2'}$–H), 7.26–7.52 (15H, m, Ar–H×15), 7.79 (1H, s, C_2–H); Anal. Calcd for $C_{42}H_{36}O_7$: C, 77.28; H, 5.56. Found: C, 77.13; H, 5.49.

4.1.19. 5,7,4′-Trihydroxy-6-(3-hydroxy-3-methylbutyl)-3′-methoxyisoflavone (lupisoflavone hydrate) (2b). Compound **12b** (1.0 g, 1.5 mmol) was hydrogenolyzed over 5% Pd/C (150 mg) in a mixture of methanol (45 ml) and dioxane (45 ml) until the uptake of hydrogen ceased. The resulting compound was recrystallized from a mixture of MeOH and Me_2CO to give **2b** (550 mg, 93%) as a colourless solid, mp 220–223 °C. ^{1}H NMR (see Table 1); IR (KBr) ν_{max} 3443, 3083, 2966, 1665, 1519, 1464, 1208, 1057 cm^{-1}; UV λ_{max} nm (log ε) (MeOH): 269 (4.34), 219 (4.3), (+$AlCl_3$) 267sh (4.36), 219 (4.31), (+NaOAc) 334 (3.93), 277 (4.36), 234sh (4.24); Anal. Calcd for $C_{21}H_{22}O_7$: C, 65.28; H, 5.74. Found: C, 65.22; H, 5.61.

4.1.20. 7,4′-Bis(benzoyloxy)-5-hydroxy-6-(3-hydroxy-3-methylbutyl)-3′-methoxyisoflavone (13b). A mixture of **2b** (480 mg, 1.24 mmol), benzoyl chloride (0.52 ml, 4.5 mmol) and K_2CO_3 (1.71 g, 12.4 mmol) in acetone (30 ml) was heated at 45 °C under nitrogen for 25 min. The reaction mixture was worked up in a similar manner (as in the case of compound **13a**) to give **13b** (642 mg, 86%) as colourless needles, mp 182–183 °C. ^{1}H NMR ($CDCl_3$) δ: 1.21 (6H, s, CH_3×2), 1.74 and 2.77 (each 2H, m, CH_2), 3.86 (3H, s, OCH_3), 6.90 (1H, s, C_8–H), 7.10–7.71 (13H, m, Ar–H×13), 8.03 (1H, s, C_2–H), 13.16 (1H, s, C_5–OH); Anal. Calcd for $C_{35}H_{30}O_9$: C, 70.70; H, 5.09. Found: C, 70.57; H, 5.17.

4.1.21. MW-synthesis of 7,4′-bis(benzoyloxy)-6-(3-hydroxy-3-methylbutyl)-3′-methoxy-5-tosyloxyisoflavone (14b). A mixture of **13b** (500 mg, 0.840 mmol), tosyl chloride (257 mg, 1.34 mmol) and K_2CO_3 (1.16 g, 8.32 mmol) in acetone (20 ml) was irradiated under MW for 30 min (3 min×10 times irradiation, 1–2 min interval/irradiation). The reaction mixture was worked up in a similar manner (as in the case of compound **14a**) to give compound **14b** as a colourless crystalline solid (585 mg, 93%), mp 170–171 °C. ^{1}H NMR ($CDCl_3$) δ: 1.13 (6H, s, CH_3×2), 1.71 and 2.79 (each 2H, m, CH_2), 2.42 (3H, s, CH_3–Ar), 3.86 (3H, s, OCH_3), 7.02–7.82 (18H, m, Ar–H×18), 7.92 (1H, s, C_2–H); Anal. Calcd for $C_{42}H_{36}O_{11}S$: C, 67.37; H, 4.85. Found: C, 67.19; H, 4.96.

4.1.22. MW-synthesis of 7,4′-bis(benzoyloxy)-6-(3-methyl-2-butenyl)-3′-methoxy-5-tosyloxyisoflavone (15b). To a solution of **14b** (430 mg, 0.574 mmol) in dry toluene (20 ml) was added $TsOH\cdot H_2O$ (1.40 ml of a 5.24×10^{-1} M solution in acetic acid). The reaction mixture was irradiated incessantly under MW at 117 °C for 15–20 min. The similar work up of the reaction mixture (as in the case of compound **15a**) gave the 6-alkenylisoflavone. The ^{1}H NMR spectrum showed that it was a mixture of 6-(3-methyl-2-butenyl)isoflavone **15b** and the regioisomer, 6-(3-methyl-3-butenyl)isoflavone **15b′** (**15b**/**15b′**=99:1). The isomeric mixture was recrystallized from a mixture of $CHCl_3$ and AcOEt to give **15b** (359 mg, 86% from **14b**) as a crystalline solid, mp 170–172 °C. ^{1}H NMR ($CDCl_3$) δ: 1.39 and 1.46 (each 3H, s, CH_3), 2.39 (3H, s, CH_3–Ar), 3.35 (2H, d, J=6.3 Hz, CH_2), 3.86 (3H, s, OCH_3), 4.95 (1H, br t, =CH), 7.02–7.70 (18H, m, Ar–H×18), 7.91 (1H, s, C_2–H); Anal. Calcd for $C_{42}H_{34}O_{10}S$: C, 69.03; H, 4.69. Found: C, 68.80; H, 4.77.

4.1.23. Synthesis of 7,4′-bis(benzoyloxy)-5-hydroxy-6-(3-methyl-2-butenyl)-3′-methoxyisoflavone (16b). Compound **15b** (200 mg, 0.273 mmol) was dissolved in dry CH_2Cl_2 (10 ml), followed by the addition of BCl_3 (0.25 ml, 1 M solution in CH_2Cl_2) in an ice bath. The similar treatment and work up of the reaction mixture (as in the case of compound **16a**) gave the 6-alkenylisoflavone **16b** (148 mg, 94%) as a colourless crystalline solid, mp 153–155 °C. ^{1}H NMR ($CDCl_3$) δ: 1.58 and 1.60 (each 3H, s, CH_3), 3.39 (2H, d, J=6.6 Hz, CH_2), 3.88 (3H, s, OCH_3), 5.16 (1H, br t, =CH), 6.87 (1H, s, C_8–H), 7.10–7.70 (13H, m, Ar–H×13), 8.02 (1H, s, C_2–H), 13.13 (1H, s, C_5–OH); Anal. Calcd for $C_{35}H_{28}O_8$: C, 72.91; H, 4.89. Found: C, 72.97; H, 5.12.

4.1.24. Synthesis of 5,7,4′-trihydroxy-6-(3-methyl-2-butenyl)-3′-methoxyisoflavone (lupisoflavone) (1b). Compound **16b** (130 mg, 0.225 mmol) was dissolved in a mixture of methanol (3 ml) and dioxane (3 ml), followed by the addition of 10% NaOH (1 ml, 2.5 mmol). The similar work up of the reaction mixture (as in the case of compound **1a**) gave 6-prenylisoflavone **1b** (52 mg, 62%) as a pale yellow crystalline solid, mp 161–163 °C. ^{1}H NMR (see Table 1); IR (KBr) ν_{max} 3435, 3085, 2949, 1649, 1517, 1459, 1205, 1068 cm^{-1}; UV λ_{max} nm ($\log\varepsilon$) (MeOH): 267sh (4.39), 220 (4.36), (+$AlCl_3$) 341 (3.97), 274sh (4.39), (+NaOAc) 338 (4.02), 276sh (4.45); Anal. Calcd for $C_{21}H_{20}O_6$: C, 68.47; H, 5.47. Found: C, 68.39; H, 5.31.

4.1.25. 5,7,4′-Triacetoxy-6-(3-methyl-2-butenyl)-3′-methoxyisoflavone (17b). Acetylation of **1b** (50 mg, 0.13 mmol) was achieved in a similar manner (as in the case of compound **17a**) to give **17b** (54 mg, 80%) as a colourless crystalline solid, mp 154–156 °C. ^{1}H NMR ($CDCl_3$) δ: 1.67 and 1.75 (each 3H, s, CH_3), 2.33, 2.35 and 2.43 (each 3H, s, $COCH_3$), 3.30 (2H, br d, CH_2), 3.86 (3H, s, OCH_3), 5.01 (1H, br t, =CH), 6.97 (1H, dd, J=8.3 and 1.7 Hz, $C_{6'}$–H), 7.06 (1H, d, J=8.3 Hz, $C_{5'}$–H), 7.12 (1H, d, J=1.7 Hz, $C_{2'}$–H), 7.22 (1H, s, C_8–H), 7.87 (1H, s, C_2–H). Anal. Calcd for $C_{27}H_{26}O_9$: C, 65.68; H, 5.25. Found: C, 65.93; H, 4.95.

4.1.26. MW-synthesis of 2′,4′-bis(benzyloxy)-3′-iodo-3,4-methylenedioxy-6′-methoxymethoxychalcone (7c) and 2′,4′-bis(benzyloxy)-6′-hydroxy-3′-iodo-3,4-methylenedioxychalcone (8c). A mixture of **5** (4.0 g, 7.7 mmol) and **6c** (1.60 g, 10.6 mmol) was dissolved in alc. KOH (3.0 g, 53 mmol in 50 ml EtOH). The reaction mixture was irradiated under MW for 6 min (2 min×3 times irradiation, 1–2 min interval/irradiation), and monitored by TLC to establish completion. A similar treatment of the reaction mixture (as in the case of compound **8a**) gave **8c** (4.15 g, 89%, two steps yield from **5**) as a yellow solid, mp 161–163 °C. ^{1}H NMR ($CDCl_3$) δ: 4.86 and 5.21 (each 2H, s, $PhCH_2$), 5.99 (2H, s, O–CH_2–O), 6.42 (1H, s, $C_{5'}$–H), 6.69 (1H, d, J=1.7 Hz, C_2–H), 6.71 (1H, d, J=7.8 Hz, C_5–H), 6.89 (1H, dd, J=8.05 and 1.7 Hz, C_6–H), 7.24–7.52 (10H, m, Ar–H×10), 7.76 and 7.81 (each 1H, d, J=15.3 Hz, =CH), 13.69 (1H, s, $C_{6'}$–OH); Anal. Calcd for $C_{30}H_{23}IO_6$: C, 59.42; H, 3.82. Found: C, 59.29; H, 3.97.

4.1.27. MW-synthesis of 2′,4′-bis(benzyloxy)-6′-benzoyloxy-3′-iodo-3,4-methylenedioxychalcone (9c). Benzoyl chloride (1.21 g, 8.52 mmol) was slowly added to a mixture of **8c** (4.0 g, 6.6 mmol) in pyridine (40 ml). The reaction mixture was irradiated incessantly under MW at 125 °C for 6 min. The reaction mixture was worked up in a similar manner (as in the case of compound **9a**) to give **9c** (4.10 g, 89%) as a fluffy crystalline solid. ^{1}H NMR ($CDCl_3$) δ: 4.99 and 5.21 (each 2H, s, $PhCH_2$), 5.98 (2H, s, O–CH_2–O), 6.73 (1H, s, $C_{5'}$–H), 6.75–6.94 (3H, m, Ar–H×3), 7.24–7.52 (15H, m, Ar–H×15), 7.53 and 7.56 (each 1H, d, J=17.5 Hz, =CH); Anal. Calcd for $C_{37}H_{27}IO_7$: C, 62.55; H, 3.83. Found: C, 62.56; H, 3.97.

4.1.28. MW-synthesis of 1-[6-benzoyloxy-2,4-bis(benzyloxy)-3-iodophenyl]-2-(3,4-methylenedioxyphenyl)-3,3-dimethoxypropan-1-one (10c) and 5,7-bis(benzyloxy)-6-iodo-3′,4′-methylenedioxyisoflavone (11c). Compound **9c** (2.0 g, 2.8 mmol) was dissolved in a mixture of MeOH (25 ml) and $CHCl_3$ (6 ml), followed by the addition of HTIB (1.76 g, 4.48 mmol). The reaction mixture was irradiated under MW for 12 min (2 min×6 times irradiation, 1–2 min interval/irradiation) at 60 °C. The reaction mixture was worked up in a similar manner (as in the case of compound **10a**) to give crude acetal **10c** as a semisolid mass. This crude mass was hydrolyzed and cyclized under MWI for 5 min in a similar way (as in the case of compound **11a**) to give iodoisoflavone **11c** (0.78 g, 46%, two steps yield from **8c**), mp 205–206 °C. ^{1}H NMR ($CDCl_3$) δ: 5.07 and 5.26 (each 2H, s, $PhCH_2$), 5.99 (2H, s, O–CH_2–O), 6.75 (1H, s, C_8–H), 6.85 (1H, d, J=7.8 Hz, $C_{5'}$–H), 6.92 (1H,

dd, J=8.05 and 1.7 Hz, $C_{6'}$–H), 7.08 (1H, d, J=1.7 Hz, $C_{2'}$–H), 7.26–7.57 (10H, m, Ar–H×10), 7.80 (1H, s, C_2–H); Anal. Calcd for $C_{30}H_{21}IO_6$: C, 59.62; H, 3.49. Found: C, 59.36; H, 3.62.

Acetal **10c**: ^{1}H NMR ($CDCl_3$) δ: 3.03 and 3.22 (each 3H, s, OCH_3), 4.70 and 4.79 (each 1H, d, J=10.2 Hz, CH), 4.98 and 5.11 (each 2H, s, $PhCH_2$), 5.84 (2H, s, O–CH_2–O), 6.48 (1H, s, Ar–H), 6.50 (1H, d, J=7.8 Hz, Ar–H), 6.57 (1H, dd, J=7.8 and 1.7 Hz, Ar–H), 6.67 (1H, d, J=1.7 Hz, Ar–H), 7.25–7.65 (15H, m, Ar–H×15).

The similar treatment of compound **9c** (2.01 g, 2.82 mmol) with BTIB (1.89 g, 4.39 mmol) under MWI (2 min×6 times irradiation, 1–2 min interval/irradiation) at 60 °C gave crude acetal **10c**, which was cyclized under MWI for 6 min to give **11c** (547 g, 32%).

4.1.29. MW-synthesis of 5,7-bis(benzyloxy)-6-(3-hydroxy-3-methyl-1-butynyl)-3′,4′-methylenedioxyisoflavone (12c). Compound **11c** (2.0 g, 3.3 mmol) was dissolved in DMF (12 ml), followed by the successive addition of Et_3N (55 ml), $PdCl_2$ (30 mg, 0.16 mmol), PPh_3 (82 mg, 0.31 mmol) and CuI (32 mg, 0.16 mmol) and finally 2-methyl-3-butyn-2-ol (1.2 ml, 16 mmol). The similar treatment of the reaction mixture under MWI (2 min×4 times irradiation, 1–2 min interval/irradiation) (as in the case of compound **12a**) gave **12c** as a colourless crystalline solid (1.26 g, 68%), mp 188–190 °C. ^{1}H NMR ($CDCl_3$) δ: 1.50 (6H, s, CH_3×2), 5.20 and 5.21 (each 2H, s, $PhCH_2$), 5.98 (2H, s, O–CH_2–O), 6.71 (1H, s, C_8–H), 6.85 (1H, d, J=8.0 Hz, $C_{5'}$–H), 6.92 (1H, dd, J=8.0 and 1.7 Hz, $C_{6'}$–H), 7.08 (1H, d, J=1.7 Hz, $C_{2'}$–H), 7.25–7.52 (10H, m, Ar–H×10), 7.78 (1H, s, C_2–H); Anal. Calcd for $C_{35}H_{28}O_7$: C, 74.99; H, 5.03. Found: C, 74.75; H, 4.97.

4.1.30. 5,7-Dihydroxy-6-(3-hydroxy-3-methylbutyl)-3′,4′-methylenedioxyisoflavone (derrubone hydrate) (2c). Compound **12c** (500 mg, 0.895 mmol) was hydrogenolyzed over 5% Pd/C (80 mg) in a mixture of methanol (25 ml) and dioxane (25 ml) until the uptake of hydrogen ceased. The similar treatment of the reaction mixture (as in the case of compound **2a**) gave **2c** as a colourless crystalline solid (300 mg, 87%), mp 186–187 °C. ^{1}H NMR (see Table 1); IR (KBr) ν_{max} 3429, 3090, 2972, 2892, 1654, 1572, 1490, 1253, 1061 cm^{-1}; UV λ_{max} nm (log ε) (MeOH): 338 (3.87), 272sh (4.35), 219 (4.31), (+$AlCl_3$) 381 (3.0), 267sh (4.33), (+NaOAc) 292 (3.27), 233sh (4.45); Anal. Calcd for $C_{21}H_{20}O_7$: C, 65.62; H, 5.24. Found: C, 65.40; H, 5.29.

4.1.31. Synthesis of 7-benzoyloxy-5-hydroxy-6-(3-hydroxy-3-methylbutyl)-3′,4′-methylenedioxyisoflavone (13c). A mixture of **2c** (700 mg, 1.82 mmol), benzoyl chloride (0.25 ml, 2.2 mmol) and K_2CO_3 (2.51 g, 18.2 mmol) in acetone (25 ml) was heated at 45 °C under nitrogen for 25 min. The similar treatment of the reaction mixture (as in the case of compound **13a**) gave **13c** as a colourless crystalline solid (809 mg, 91%), mp 156–158 °C. ^{1}H NMR ($CDCl_3$) δ: 1.20 (6H, s, CH_3×2), 1.71 and 2.74 (each 2H, m, CH_2), 6.01 (2H, s, O–CH_2–O), 6.84 (1H, d, J=8.0 Hz, $C_{5'}$–H), 6.90 (1H, s, C_8–H), 6.94 (1H, dd, J=8.0 and 1.7 Hz, $C_{6'}$–H), 7.05 (1H, d, J=1.7 Hz, $C_{2'}$–H), 7.26–7.70 (5H, m, Ar–H×5), 7.94 (1H, s, C_2–H), 13.15 (1H, s, C_5–OH); Anal. Calcd for $C_{28}H_{24}O_8$: C, 68.85; H, 4.95. Found: C, 68.77; H, 4.85.

4.1.32. MW-synthesis of 7-benzoyloxy-6-(3-hydroxy-3-methylbutyl)-3′,4′-methylenedioxy-5-tosyloxyisoflavone (14c). A mixture of **13c** (800 mg, 1.63 mmol), tosyl chloride (468 mg, 2.45 mmol) and K_2CO_3 (2.50 g, 18.1 mmol) in acetone (25 ml) was irradiated incessantly under MW for 11 min. The reaction mixture was worked up in a similar manner (as in the case of compound **14a**) to give compound **14c** as a colourless crystalline solid (990 mg, 94%), mp 183–184 °C. ^{1}H NMR ($CDCl_3$) δ: 1.13 (6H, s, CH_3×2), 1.71 and 2.74 (each 2H, m, CH_2), 2.43 (3H, s, Ar–CH_3), 6.0 (2H, s, O–CH_2–O), 6.85 (1H, s, C_8–H), 6.84 (1H, d, J=8.0 Hz, $C_{5'}$–H), 6.87 (1H, dd, J=8.0 and 1.7 Hz, $C_{6'}$–H), 6.97 (1H, d, J=1.7 Hz, $C_{2'}$–H), 7.26–7.71 (9H, m, Ar–H×9), 7.83 (1H, s, C_2–H); Anal. Calcd for $C_{35}H_{30}O_{10}S$: C, 65.41; H, 4.71. Found: C, 65.29; H, 4.67.

4.1.33. MW-synthesis of 7-benzoyloxy-6-(3-methyl-2-butenyl)-3′,4′-methylenedioxy-5-tosyloxyisoflavone (15c). To a solution of **14c** (400 g, 0.622 mmol) in dry toluene (20 ml) was added $TsOH\cdot H_2O$ (1.16 ml of a 5.24×10^{-1} M solution in acetic acid). The reaction mixture was irradiated incessantly under MW at 117 °C (refluxing) for 20 min. The reaction mixture was worked up in a similar manner (as in the case of compound **15a**) to give the 6-alkenylisoflavone **15c** as a crystalline solid. The ^{1}H NMR spectrum showed that it was a mixture of 6-(3-methyl-2-butenyl)isoflavone **15c** and the regioisomer, 6-(3-methyl-3-butenyl)isoflavone **15c′** (**15c/15c′**=99:1). The isomeric mixture was recrystallized from a mixture of $CHCl_3$ and Me_2CO (5:1) to give **15c** (328 mg, 85% from **14c**) as a crystalline solid, mp 157–158 °C. ^{1}H NMR ($CDCl_3$) δ: 1.40 and 1.45 (each 3H, s, CH_3), 2.41 (3H, s, Ar–CH_3), 3.34 (2H, d, J=6.5 Hz, CH_2), 4.94 (1H, t, J=6.5 Hz, =CH), 6.0 (2H, s, O–CH_2–O), 6.83–7.96 (13H, m, Ar–H×13), 7.81 (1H, s, C_2–H); Anal. Calcd for $C_{35}H_{28}O_9S$: C, 67.63; H, 4.52. Found: C, 67.59; H, 4.41.

4.1.34. 7-Benzoyloxy-5-hydroxy-6-(3-methyl-2-butenyl)-3′,4′-methylenedioxyisoflavone (16c). Compound **15c** (400 mg, 0.640 mmol) was dissolved in dry CH_2Cl_2 (10 ml), followed by the addition of BCl_3 (0.37 ml, 1 M solution in CH_2Cl_2) in an ice bath. The similar treatment and work up of the reaction mixture (as in the case of compound **16a**) gave the 6-alkenylisoflavone **16c** (240 mg, 79%) as a colourless crystalline solid, mp 115–117 °C. ^{1}H NMR ($CDCl_3$) δ: 1.57 and 1.59 (each 3H, s, CH_3), 3.38 (2H, d, J=6.8 Hz, CH_2), 5.15 (1H, t, J=6.8 Hz, =CH), 6.01 (2H, s, O–CH_2–O), 6.84 (1H, s, C_8–H), 6.88 (1H, d, J=8.0 Hz, $C_{5'}$–H), 6.95 (1H, dd, J=8.0 and 1.7 Hz, $C_{6'}$–H), 7.05 (1H, d, J=1.7 Hz, $C_{2'}$–H), 7.25–7.69 (5H, m, Ar–H×5), 7.93 (1H, s, C_2–H), 13.13 (1H, s, C_5–OH); Anal. Calcd for $C_{28}H_{22}O_7$: C, 71.48; H, 4.71. Found: C, 71.62; H, 4.81.

4.1.35. Synthesis of 5,7-dihydroxy-6-(3-methyl-2-butenyl)-3′,4′-methylenedioxyisoflavone (derrubone) (1c). Compound **16c** (150 mg, 0.318 mmol) was dissolved in a mixture of methanol (3 ml) and dioxane (3 ml), followed by the addition of 10% NaOH (1.1 ml). The similar treatment and work up of the reaction mixture (as in the case of compound **1a**) gave 6-prenylisoflavone **1c** (92 mg, 79%) as a pale

yellow crystalline solid, mp 210–211 °C (lit.[24] 210–212 °C). ^{1}H NMR (see Table 1); IR (KBr) ν_{max} 3443, 3083, 2925, 2858, 1647, 1506, 1436, 1245, 1056 cm^{-1}; UV λ_{max} nm (log ε) (MeOH): 341 (4.03), 274sh (4.39), 223 (4.40), (+$AlCl_3$) 266sh (4.41), 222 (4.37), (+NaOAc) 341 (4.07), 276 (4.41), 234sh (4.45); Anal. Calcd for $C_{21}H_{18}O_6$: C, 68.85; H, 4.95. Found: C, 68.63; H, 4.98.

4.1.36. 5,7-Diacetoxy-6-(3-methyl-2-butenyl)-3′,4′-methylenedioxyisoflavone (17c). Acetylation of **1c** (60 mg, 0.16 mmol) was achieved in a similar manner (as in the case of compound **17a**) to give **17c** (65 mg, 88%) as a colourless crystalline solid, mp 204–205 °C. ^{1}H NMR ($CDCl_3$) δ: 1.67 and 1.75 (each 3H, s, CH_3), 2.34 and 2.43 (each 3H, s, $COCH_3$), 3.25 (2H, br d, CH_2), 5.01 (1H, br t, =CH), 5.98 (2H, s, O–CH_2–O), 6.83 (1H, d, J=8.0 Hz, $C_{5'}$–H), 6.86 (1H, dd, J=8.0 and 1.7 Hz, $C_{6'}$–H), 6.99 (1H, d, J=1.7 Hz, $C_{2'}$–H), 7.20 (1H, s, C_8–H), 7.83 (1H, s, C_2–H). Anal. Calcd for $C_{25}H_{22}O_8$: C, 66.66; H, 4.92. Found: C, 66.49; H, 4.87.

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Stereoselective synthesis of fluoroalkenes via (*Z*)-2-fluoroalkenyliodonium salts

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Abstract—Stereoselective synthesis of fluoroalkenes is described. (*Z*)-2-Fluoro-1-alkenyl(phenyl)iodonium tetrafluoroborates (**1**) were synthesized stereoselectively in good yields by Michael-type addition of HF to 1-alkynyl(phenyl)iodonium tetrafluoroborates (**2**) with a commercially available HF reagent, hydrofluoric acid or Et_3N–3HF. Pd-catalyzed cross-coupling reactions using **1** gave (*Z*)-2-fluoro-1-alkene derivatives in moderate yields. The treatment of **1** with KI in the presence of a catalytic amount of CuI gave (*Z*)-2-fluoro-1-iodo-1-alkenes (**3**). Pd-catalyzed cross-coupling reactions of **3** gave better results than that of **1**, and a variety of (*Z*)-2-fluoro-1-alkene derivatives were synthesized in good yields.

1. Introduction

Fluorinated analogues of natural compounds have attracted the interest of biological and medicinal chemists, because the introduction of a fluorine atom into a natural product can dramatically enhance the biological activity.[1] However, organofluorine compounds are scarce in nature; therefore, they have to be synthesized by fluorination of organic compounds or by using building-block methodology with readily available fluorine-containing substrates.[2] When a fluorine atom is introduced into a carbon–carbon double bond of a biologically active compound, the regio- and stereoselective introduction of the fluorine atom is important because the bioactivity is strongly dependent on the position and stereochemistry of the fluorine atom.[3] The most popular approach to the stereoselective preparation of fluoroalkenes[4] is the Horner–Wadsworth–Emmons reaction using fluoroorganophosphonates with carbonyl compounds; however, a mixture of stereoisomers is generally formed.[5] On the other hand, Pd-catalyzed cross-coupling reaction using alkenyl halides or metals is often employed as a powerful tool to obtain further complex alkenes stereoselectively. Therefore, a cross-coupling reaction using fluoroalkenyl halides or metals would be a versatile method for the stereoselective synthesis of fluoroalkenes. However, the cross-coupling method has been adequately developed because the stereoselective synthesis of fluoroalkenyl halides or metals is difficult. Recently, we reported the stereoselective syntheses of various (*E*)-2-fluoro-1-alkene derivatives by Pd-catalyzed cross-coupling reactions using (*E*)-2-fluoro-1-alkenyl-(*p*-tolyl)iodonium salts, which were prepared from terminal alkynes and *p*-iodotoluene difluoride.[6] Hence, we turned our attention into the stereoselective synthesis of (*Z*)-2-fluoro-1-alkene derivatives. Ochiai et al. reported that (*Z*)-2-fluoro-1-alkenyl(phenyl)iodonium salts (**1**)[7] were stereoselectively prepared by Michael-type addition of a fluoride anion to the corresponding 1-alkynyl(phenyl)iodonium salts (**2**)[8] with CsF; however, the yields were only 15–20% due to the low nucleophilicity of the fluoride anion. Although the simplest reagent for an HF addition is hydrogen fluoride, it requires special equipment, technique, and know-how to use for organic synthesis due to the high toxicity and explosive reactivity to organic compounds. In ordinary laboratories, amine–*n*HF[9] and hydrofluoric acid are commonly used as convenient HF reagents instead of hydrogen fluoride. We found that the HF addition of **2** with these HF reagents smoothly proceeded to afford **1** effectively.[10] In this report, we would like to present the details of the stereoselective synthesis of **1** and their utilization to the synthesis of (*Z*)-2-fluoro-1-alkene derivatives by Pd-catalyzed cross-coupling reactions.

2. Results and discussions

2.1. Stereoselective synthesis of (*Z*)-2-fluoroalkenyliodonium salts (1)

Initially, we employed 1-dodecynyl(phenyl)iodonium tetrafluoroborate (**2a**) as a simple starting material and attempted an HF addition using Et_3N–*n*HF (Table 1). Although

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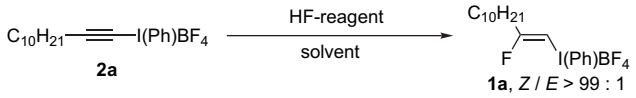
Table 1. HF addition of **2a** with Et_3N–*n*HF or aq HF

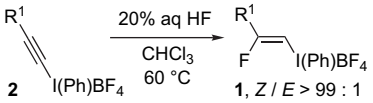


Entry	HF reagent	Solvent	Temp (°C)	Time (h)	Yield (%)
1	Et_3N–5HF	CH_2Cl_2	rt	24	0[a]
2	Et_3N–3HF	CH_2Cl_2	rt	96	71
3	Et_3N–2HF	CH_2Cl_2	rt	44	32[b]
4	Et_3N–3HF	Neat	rt	78	71
5	Et_3N–3HF	Neat	40	8	72
6	Et_3N–3HF	Neat	60	0.75	67[c]
7	46% aq HF	$CHCl_3$	60	84	81
8	30% aq HF	$CHCl_3$	60	8	82
9	20% aq HF	$CHCl_3$	60	6	84
10	10% aq HF	$CHCl_3$	60	5	71[c]

[a] Starting material **2a** was recovered unchanged.
[b] Tri-fluorinated compound, 1,2,2-trifluorododecane (**4**), was obtained in 26% yield.
[c] Small amount of **4** was observed after the reaction.

Et_3N–5HF was inert to **2a** in dichloromethane at room temperature (entry 1), a more nucleophilic fluorinating reagent, Et_3N–3HF, reacted slowly with **2a** to give (*Z*)-2-fluoro-1-dodecenyl(phenyl)iodonium tetrafluoroborate (**1a**) in 71% yield after 96 h (entry 2). The ^1H NMR of the crude reaction mixture indicated that the HF addition proceeded with excellent stereoselectivity (*Z*/*E*>99:1). When **2a** was treated with a more nucleophilic reagent, Et_3N–2HF, further Michael addition of fluoride anion to **1a** occurred to produce 1,2,2-trifluorododecane (**4**) in 26% yield, and the yield of **1a** was reduced to 32% (entry 3). When fluorination of **2a** was carried out with Et_3N–3HF without dichloromethane, the reaction time was reduced to 78 h (entry 4). The HF addition reaction proceeded more effectively at 40 °C (entry 5), but the formation of a small amount of tri-fluorinated compound **4** was observed at 60 °C (entry 6). Next, we attempted the HF addition using hydrofluoric acid, which is commonly used in a laboratory as a simple and cost effective HF reagent. Although commercially available 46% hydrofluoric acid required 84 h at 60 °C to consume **2a** completely, the desired product **1a** was obtained in high yield (entry 7).

Table 2. Synthesis of **1**



1	R^1	Time (h)	Yield (%)
b	Ph	8	43
c	*t*-Bu	12	84
d	(*cyclo*-C_6H_{11})-CH_2	12	74
e	Cl-$(CH_2)_9$	6	80
f	*t*-Bu-CO-$(CH_2)_8$	6	72
g	*i*-PrO_2C-$(CH_2)_8$	6	76

We found that the HF addition reaction was more effectively carried out with diluted hydrofluoric acid (entries 8–10).[11] Finally, the best result was obtained by using 20% hydrofluoric acid, and **1a** was synthesized in 84% yield with excellent stereoselectivity (*Z*/*E*>99:1) (entry 9).[12]

Under the same reaction conditions, 1-alkynyl(phenyl)iodonium salts **2**, which have a *n*-alkyl or a sterically hindered alkyl group, were converted into the corresponding (*Z*)-2-fluoro-1-alkenyliodonium salts **1** in good yields (Table 2). Unfortunately, 2-phenylethynyl(phenyl)iodonium tetrafluoroborate (**2b**) gave the desired product **1b** in lower yield because the starting material **2b** was somewhat labile under the reaction conditions, although **1b** was isolated as a stable white solid.

2.2. Stereoselective synthesis of (*Z*)-2-fluoro-1-alkene derivatives by Pd-catalyzed cross-coupling reaction using (*Z*)-2-fluoroalkenyliodonium salts (1)

First of all, we tried the methoxycarbonylation of **1a** in the presence of $PdCl_2$ with CO in methanol.[6b,13,14] The methoxycarbonylation completed in 2 h at room temperature to give the desired product, methyl (*Z*)-3-fluoro-2-tridecenoate (**5a**) in 73% yield; however, methyl benzoate (**6**, 8%) and (*Z*)-2-fluoro-1-iodo-1-dodecene (**3a**, 9%) were also formed by the methoxycarbonylation of the phenyl group instead of the fluoroalkenyl group on the starting material (Fig. 1).

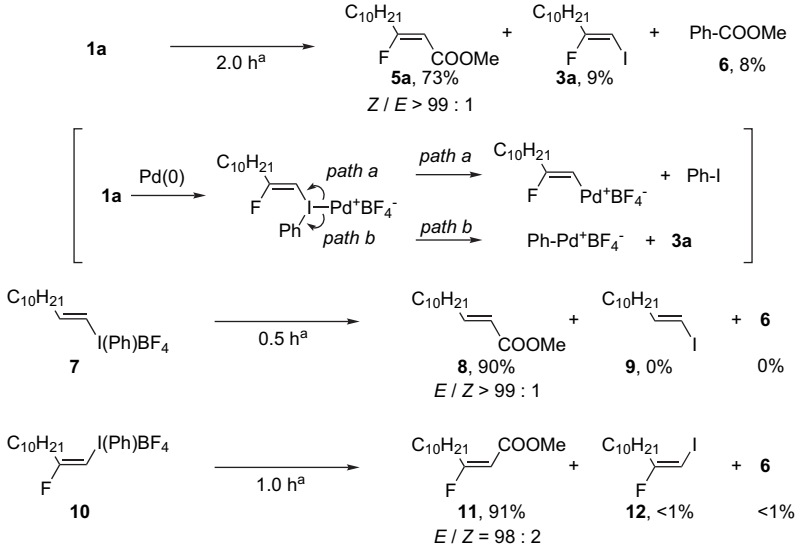
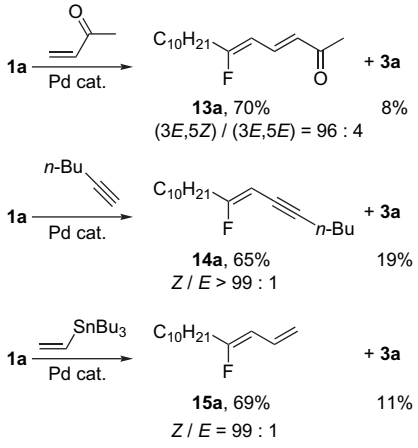


Figure 1. Methoxycarbonylation of **1a**, **7**, and **10**. [a]Reagents and conditions: $PdCl_2$ 2 mol %, CO 1 atm, $NaHCO_3$ 1 equiv, MeOH, rt.

When a nonfluorinated starting material, (*E*)-1-dodecenyl-(phenyl)iodonium tetrafluoroborate (**7**), was subjected to the reaction conditions, the methoxycarbonylation proceeded much faster than that of **1a** to give methyl (*E*)-2-tridecenoate (**8**, 90%) without the formation of (*E*)-1-iodo-1-dodecene (**9**) and **6**.[14] Interestingly, the methoxycarbonylation of (*E*)-2-fluoro-1-dodecenyl(phenyl)iodonium tetrafluoroborate (**10**), which has an alkyl group on the cis-position to the iodonio group, proceeded faster than that of **1a** to give methyl (*E*)-3-fluoro-2-tridecenoate (**11**, 91%) with only a trace amount of (*E*)-2-fluoro-1-iodo-1-dodecene (**12**) and **6**.[6b] Generally, the Pd-catalyzed methoxycarbonylation of an (*E*)-alkenyliodide proceeds faster than that of the (*Z*)-isomer.[15] Hence, we found that the cis-bonded vinylic fluorine atom to the iodonio group disturbed '*path a*', which gave (*Z*)-fluoroalkenylpalladium intermediate, and it caused to produce the phenylpalladium intermediate by '*path b*'; however, the effect of the fluorine atom is unclear now.[14,16,17]

Similarly, Heck reaction,[6c,d,16] Sonogashira reaction,[4k,6f,17] and Stille reaction[6d,18] of **1a** gave the desired (*Z*)-2-fluoro-1-alkene derivatives **13a**–**15a** in moderate yields, but the formation of **3a** was observed in all cases (Scheme 1). Unfortunately, we couldn't suppress the formation of **3a** by modification of the reaction conditions; therefore, we decided to use (*Z*)-2-fluoro-1-iodo-1-alkenes **3** to the Pd-catalyzed cross-coupling reactions instead of (*Z*)-2-fluoro-1-alkenyliodonium salts **1**.

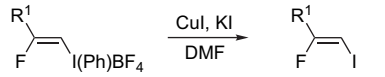


Scheme 1. Pd-catalyzed cross-coupling reactions using **1a**.

2.3. Synthesis of (*Z*)-2-fluoro-1-iodo-1-alkenes (3) from (*Z*)-2-fluoroalkenyliodonium salts (1)

The transformation of alkenyliodonium salts to iodoalkenes with CuI and KI was first reported by Ochiai et al.[7,14b] They proposed that the substitution reaction of iodine for iodonio group can be catalyzed by CuI; however, excess amount of CuI and KI were used in their procedure. We tried the synthesis of **3a** from **1a** with a catalytic amount of CuI and a stoichiometric amount of KI, and confirmed that the reaction well proceeded with 5 mol % of CuI to give **3a** in good yield (Table 3, entry 2), although no reaction occurred without CuI (entry 3). Under the reaction conditions listed in entry 2, a variety of (*Z*)-fluoroiodoalkenes **3** were synthesized from **1** in good yields with retention of the stereochemistry (entries 4–8).

Table 3. Synthesis of **3** from **1**[a]



Entry	**3**	R^1	Time (h)	Yield (%)
1[b]	**a**	$C_{10}H_{21}$	12	87
2	**a**	$C_{10}H_{21}$	36	89
3[c]	**a**	$C_{10}H_{21}$	24	0
4	**b**	Ph	3	87
5	**d**	(*cyclo*-C_6H_{11})-CH_2	36	88
6	**e**	Cl-$(CH_2)_9$	36	92
7	**f**	*t*-Bu-CO-$(CH_2)_8$	36	91
8	**g**	*i*-PrO_2C-$(CH_2)_8$	36	91

[a] Unless otherwise mentioned, reactions were carried out with 0.5 mmol of **1**, 5 mol % of CuI, and 0.5 mmol of KI in DMF (0.125 M) at rt.
[b] CuI (0.5 mmol) was used.
[c] KI (0.75 mmol) was used in the absence of CuI.

2.4. Pd-catalyzed cross-coupling reaction using (*Z*)-2-fluoro-1-iodo-1-alkene (3)

Then, we attempted the Pd-catalyzed cross-coupling reactions using (*Z*)-2-fluoro-1-iodo-1-dodecene (**3a**) and (*Z*)-α-fluoro-β-iodostyrene (**3b**). By Pd-catalyzed cross-coupling reactions, such as methoxycarbonylation, Heck reaction, Stille reaction, Sonogashira reaction, and Suzuki–Miyaura reaction[19] using (*Z*)-2-fluoro-1-iodoalkenes **3**, a variety of (*Z*)-2-fluoro-1-alkene derivatives (**5** and **13**–**17**) were synthesized stereoselectively in good yields (Table 4). By using our methodology for the fluoroalkenes synthesis, we have succeeded in the stereoselective synthesis of the fluorinated analogues of insect sex pheromones and reported in a recent paper.[20]

3. Conclusions

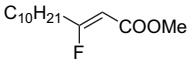
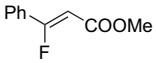
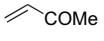
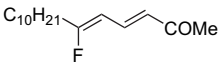
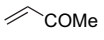
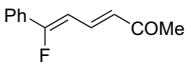
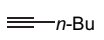
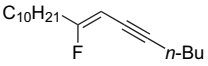
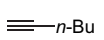
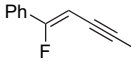
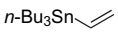
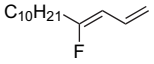
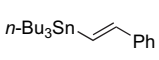
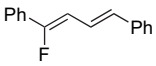
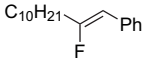
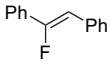
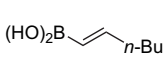
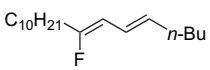
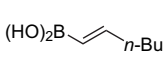
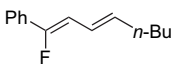
(*Z*)-2-Fluoro-1-alkenyl(phenyl)iodonium salts **1** were stereoselectively synthesized in good yields by an HF addition to 1-alkynyl(phenyl)iodonium salts **2** with diluted hydrofluoric acid or Et_3N–3HF. Pd-catalyzed cross-coupling reactions using **1** gave (*Z*)-2-fluoro-1-alkene derivatives in fair yields. The transformation of **1** to (*Z*)-2-fluoro-1-iodo-1-alkenes **3** was performed with a catalytic amount of CuI and a stoichiometric amount of KI. By Pd-catalyzed cross-coupling reactions of **3**, various (*Z*)-2-fluoro-1-alkene derivatives were stereoselectively synthesized in good yields. We previously reported that (*E*)-2-fluoro-1-alkenyliodonium salts were stereoselectively synthesized by the reaction of terminal alkynes with *p*-iodotoluene difluoride and their application to the stereoselective synthesis of (*E*)-2-fluoro-1-alkenes. Hence, we developed an efficient methodology for the highly stereoselective synthesis of (*E*)- and (*Z*)-2-fluoro-1-alkene derivatives from terminal alkynes via the fluoroalkenyliodonium salts.

4. Experimental

4.1. General

The chemical shifts, δ, of ^{1}H NMR (400 MHz), ^{19}F NMR (376 MHz), and ^{13}C NMR (100 MHz) spectra were referred

Table 4. Pd-catalyzed cross-coupling reactions **3a** and **3b**

Substrate	Coupling reagent	Product		Yield (%)	*Z*/*E*
3a	CO, MeOH		**5a**	88	>99:1
3b	CO, MeOH		**5b**	77	>99:1
3a			**13a**	77	98:2 (3*E*,5*Z*)/(3*E*,5*E*)
3b			**13b**	76	96:4 (3*E*,5*Z*)/(3*E*,5*E*)
3a			**14a**	88	>99:1
3b			**14b**	83	>99:1
3a			**15a**	86	>99:1
3b			**15b**	83	>99:1(1*Z*,3*E*)/(1*E*,3*E*)
3a	Ph-B(OH)$_2$		**16a**	88	>99:1
3b	Ph-B(OH)$_2$		**16b**	85	>99:1
3a			**17a**	81	>99:1 (5*E*,7*Z*)/(5*E*,7*E*)
3b			**17b**	72	>99:1(1*Z*,3*E*)/(1*E*,3*E*)

to TMS (^{1}H, ^{13}C) and $CFCl_3$ (^{19}F). Et_3N–2HF was prepared as CH_2Cl_2 solution by the addition of Et_3N to Et_3N–3HF in CH_2Cl_2 before use. Commercial $CHCl_3$ was distilled before use. $Pd(PPh_3)_4$,[21] (*E*)-1-dodecenyl(phenyl)iodonium tetrafluoroborate (**7**),[14b] Et_3N–5HF,[22] tributylvinylstannane,[23] tributylstyrylstannane,[24] and (*E*)-1-hexenylboronic acid[25] were prepared according to the literatures. 1-Alkynyl(phenyl)-iodonium tetrafluoroborates (**2**) were prepared from the corresponding terminal alkynes by our method.[8c] The spectral data for **1a**,[10] **1c–g**,[10] **2b**,[26] **3a**,[10] **5a**,[10] **11**,[6b] **12**,[6a] and **14a**[10] were reported in the literatures.

4.2. Synthesis of (*Z*)-2-fluoro-1-dodecenyl(phenyl)iodonium tetrafluoroborate (1a) by the reaction of 2a with Et_3N–3HF

In a Teflon™ PFA vessel were placed 1-dodecynyl(phenyl)-iodonium tetrafluoroborate (**2a**) (228 mg, 0.5 mmol) and Et_3N–3HF (805 mg, 5 mmol) at room temperature, and the mixture was stirred at 40 °C for 8 h. The reaction mixture was poured into water (100 ml) and extracted with CH_2Cl_2 (10 ml) four times. The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting viscous oil was dissolved in CH_2Cl_2 (1 ml) and a white suspension was formed by the addition of hexane (40 ml). The white suspension was left in a refrigerator for 2 h and clear upper liquid was removed by decantation. The remained white solid was washed with hexane (5 ml) again, separated by decantation, and dried in vacuo to give (*Z*)-2-fluoro-1-dodecenyl(phenyl)iodonium tetrafluoroborate (**1a**)[10] (72%, 171 mg, 0.36 mmol, *Z*/*E*>99:1).

4.3. Synthesis of 1a by the reaction of 2a with hydrofluoric acid

In a Teflon™ PFA vessel were placed **2a** (228 mg, 0.5 mmol), $CHCl_3$ (2 ml), and a 20% hydrofluoric acid (500 mg, 5 mmol) at room temperature, and the mixture was vigorously stirred at 60 °C for 6 h. The reaction mixture was poured into a 0.5 M aq $NaBF_4$ (20 ml) and extracted with CH_2Cl_2 (10 ml) four times. The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting viscous oil was dissolved in CH_2Cl_2 (1 ml) and a white suspension was formed by the addition of hexane (40 ml). The white suspension was left in a refrigerator for 2 h and the clear upper liquid was removed by decantation. The remained precipitate was washed with hexane (40 ml) again, separated from hexane by decantation, and dried in vacuo to give pure **1a** (84%, 200 mg, 0.42 mmol, *Z*/*E*>99:1).

4.4. (*Z*)-2-Fluoro-2-phenylethenyl(phenyl)iodonium tetrafluoroborate (1b)

Mp 136.5–137.0 °C, δ_H (DMSO-d_6) 7.53–7.77 (8H, m), 7.94 [1H, d, $^3J_{H-F(olefin)}$ 37.5 Hz, 1-H], 8.17 (2H, d, *J* 8.03 Hz); δ_F (DMSO-d_6) −83.80 [1F, d, $^3J_{H-F(olefin)}$ 37.5 Hz, 2-F]; δ_C (DMSO-d_6) 80.4 (d, $^2J_{C-F}$ 21.5 Hz, 1-C), 115.4, 125.9 (2C, d, $^3J_{C-F}$ 7.4 Hz, *ortho*), 127.4 (d, $^2J_{C-F}$ 28.0 Hz, *ipso*), 129.3 (2C), 131.8 (2C), 132.0, 132.5, 135.0 (2C), 164.6 (d, $^1J_{C-F}$ 261.8 Hz, 2-C); ν (KBr)/cm^{-1} 3113, 1625, 1575, 1496, 1470, 1445, 1290, 1084, 1037, 987, 796, 768, 740, 677, 651, 634, 603, 521; [HR FABMS Calcd for $C_{14}H_{11}FI$ (M−BF_4): 324.9890. Found: M^+−BF_4, 324.9868]. Found: C, 40.63; H, 2.67%. Calcd for $C_{14}H_{11}BF_5I$: C, 40.82; H, 2.69%.

4.5. 1-Dodecynyl(phenyl)iodonium tetrafluoroborate (2a)

Mp 41.5–42.2 °C, δ_H ($CDCl_3$) 0.88 (3H, t, *J* 7.1 Hz, 12-H), 1.19–1.39 (14H, m), 1.55–1.63 (2H, m, 4-H), 2.65 (2H, t, *J* 7.1 Hz, 3-H), 7.53–8.07 (5H, m, Ph); δ_C ($CDCl_3$) 14.1, 16.0, 20.8, 22.6, 27.6, 28.7, 28.9, 29.2, 29.3, 29.5, 31.8, 114.1, 114.6, 132.7 (2C), 132.9, 133.8 (2C); ν (KBr)/cm^{-1} 3051, 2924, 2853, 2168, 1470, 1441, 1069, 1038, 987, 738, 678, 650; [HR FABMS Calcd for $C_{18}H_{26}I$ (M−BF_4): 369.1079. Found: M^+−BF_4, 369.1096]. Found: C, 47.42; H, 5.81%. Calcd for $C_{18}H_{26}BF_4I$: C, 47.40; H, 5.75%.

4.6. 3,3-Dimethyl-1-butynyl(phenyl)iodonium tetrafluoroborate (2c)

Mp 189.6–190.5 °C, δ_H ($CDCl_3$) 1.33 (9H, s, ^{t}Bu), 7.55–8.05 (5H, m, Ph); δ_C ($CDCl_3$) 15.7, 29.9 (3C), 30.2, 114.8, 121.4, 132.77 (2C), 132.83, 133.5 (2C); ν (KBr)/cm^{-1} 3097, 2977, 2932, 2871, 2192, 2155, 1560, 1470, 1446, 1366, 1286, 1253, 1051, 921, 743, 675, 645; [HR FABMS Calcd for $C_{12}H_{14}I$ (M−BF_4): 285.0140. Found: M^+−BF_4, 285.0146]. Found: C, 38.55; H, 3.74%. Calcd for $C_{12}H_{14}BF_4I$: C, 38.75; H, 3.79%.

4.7. 3-Cyclohexyl-1-propynyl(phenyl)iodonium tetrafluoroborate (2d)

Mp 86.2–87.2 °C, δ_H ($CDCl_3$) 0.94–1.30 (5H, m), 1.56–1.77 (6H, m), 2.57 (2H, d, *J* 6.6 Hz, 3-H), 7.54–8.07 (5H, m, Ph); δ_C ($CDCl_3$) 16.4, 25.8 (2C), 25.8, 28.5, 32.6 (2C), 36.9, 113.6, 114.8, 132.8 (2C), 132.9, 133.7 (2C); ν (KBr)/cm^{-1} 3086, 2928, 2849, 2185, 1562, 1473, 1446, 1417, 1327, 1275, 1070, 891, 765, 738, 676, 650; [HR FABMS Calcd for $C_{15}H_{18}I$ (M−BF_4): 325.0453. Found: M^+−BF_4, 325.0470]. Found: C, 43.83; H, 4.42%. Calcd for $C_{15}H_{18}BF_4I$: C, 43.73; H, 4.40%.

4.8. 11-Chloro-1-undecynyl(phenyl)iodonium tetrafluoroborate (2e)

Mp 47.7–48.4 °C, δ_H ($CDCl_3$) 1.21–1.44 (10H, m), 1.55–1.63 (2H, m, 4-H), 1.72–1.79 (2H, m, 10-H), 2.64 (2H, t, *J* 7.3 Hz, 3-H), 3.53 (2H, t, *J* 6.8 Hz, 11-H), 7.52–8.07 (5H, m, Ph); δ_C ($CDCl_3$) 15.9, 20.9, 26.8, 27.6, 28.69, 28.73, 28.8, 29.2, 32.6, 45.2, 114.2, 114.6, 132.8 (2C), 132.9, 133.8 (2C); ν (KBr)/cm^{-1} 3050, 2992, 2925, 2854, 2166, 1562, 1470, 1441, 1305, 1051, 988, 740, 679, 650; [HR FABMS Calcd for $C_{17}H_{23}ClI$ (M−BF_4): 389.0533. Found: M^+−BF_4, 389.0545]. Found: C, 42.91; H, 4.76%. Calcd for $C_{17}H_{23}BClF_4I$: C, 42.85; H, 4.86%.

4.9. 2-(10,10-Dimethyl-9-oxoundecanyl)ethynyl-(phenyl)iodonium tetrafluoroborate (2f)

Oil, δ_H ($CDCl_3$) 1.13–1.37 (17H, m), 1.51–1.63 (4H, m), 2.47 (2H, t, *J* 7.1 Hz, 10-H), 2.65 (2H, t, *J* 7.1 Hz, 3-H), 7.53–8.07 (5H, m, Ph); δ_C ($CDCl_3$) 16.0, 20.8, 23.7, 26.4 (3C), 27.5, 28.5, 28.6, 29.0, 29.1, 36.4, 44.1, 114.0, 114.6, 132.7 (2C), 132.9, 133.8 (2C), 216.4; ν (neat)/cm^{-1} 3093, 2930, 2857, 2182, 1702, 1469, 1445, 1366, 1067, 985, 740, 676; [HR FABMS Calcd for $C_{21}H_{30}IO$ (M−BF_4): 425.1341. Found: M^+−BF_4, 425.1344]. Found: C, 49.19; H, 5.91%. Calcd for $C_{21}H_{30}BF_4IO$: C, 49.25; H, 5.90%.

4.10. 10-Isopropoxycarbonyl-1-decynyl(phenyl)-iodonium tetrafluoroborate (2g)

Oil, δ_H ($CDCl_3$) 1.20–1.39 (14H, m), 1.56–1.61 (4H, m), 2.25 (2H, t, *J* 7.3 Hz, 10-H), 2.65 (2H, t, *J* 7.1 Hz, 3-H), 4.97–5.03 (1H, m, ^{i}Pr), 7.54–8.07 (5H, m, Ph); δ_C ($CDCl_3$) 16.3, 20.5, 21.6 (2C), 24.6, 27.2, 28.3, 28.4, 28.61, 28.64, 34.4, 67.3, 113.1, 114.3, 132.4 (2C), 132.7, 133.8 (2C), 173.4; ν (neat)/cm^{-1} 3090, 3062, 2980, 2932, 2857, 2182, 1726, 1691, 1469, 1445, 1375, 1182, 1107, 985, 741, 676; [HR FABMS Calcd for $C_{20}H_{28}IO_2$ (M−BF_4): 427.1134. Found: M^+−BF_4, 427.1134]. Found: C, 46.50; H, 5.43%. Calcd for $C_{20}H_{28}BF_4IO_2$: C, 46.72; H, 5.49%.

4.11. Synthesis of (*Z*)-2-fluoro-1-iodo-1-dodecene (3a) from 1a

To a DMF solution (4 ml) of **1a** (238 mg, 0.5 mmol) were added CuI (4.8 mg, 0.025 mmol) and KI (83 mg, 0.5 mmol), and the mixture was stirred at room temperature for 36 h. The reaction mixture was poured into 3 M aq NH_4Cl (15 ml) and extracted with ether (10 ml) three times. The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. In order to remove the generated iodobenzene, the reaction mixture was kept at 40 °C and 0.01 mmHg for 1 h. The product **3a**[10] was isolated by column chromatography (silica gel, hexane) in 89% yield (139 mg, *Z*/*E*>99:1).

4.12. (*Z*)-2-Fluoro-1-iodo-2-phenylethene (3b)

Oil, δ_H ($CDCl_3$) 6.08 [1H, d, $^3J_{H-F(olefin)}$ 34.4 Hz, 1-H], 7.36–7.52 (5H, m, Ph); δ_F ($CDCl_3$) −90.62 [1F, d, $^3J_{H-F(olefin)}$ 34.4 Hz]; δ_C ($CDCl_3$) 53.4 (d, $^2J_{C-F}$ 28.8 Hz, 1-C), 124.6 (2C, d, $^3J_{C-F}$ 5.8 Hz, *ortho*), 128.7 (2C, d, $^4J_{C-F}$ 1.7 Hz, *meta*), 129.8, 130.9 (d, $^2J_{C-F}$ 28.9 Hz, *ipso*), 163.1 (d, $^1J_{C-F}$ 251.9 Hz, 2-C); ν (neat)/cm^{-1} 3095, 3058, 1629, 1574, 1495, 1445, 1281, 1187, 1034, 1015, 791, 768, 741, 687, 606; [HR EIMS Calcd for C_8H_6FI (M): 247.9498. Found: M^+, 247.9480]. Found: C, 38.78; H, 2.48%. Calcd for C_8H_6FI: C, 38.74; H, 2.44%.

4.13. (*Z*)-3-Cyclohexyl-2-fluoro-1-iodo-1-propene (3d)

Oil, δ_H ($CDCl_3$) 0.85–1.30 (5H, m), 1.52–1.62 (6H, m), 2.22 (2H, dd, $^3J_{H-F}$ 20.0, *J* 7.0 Hz, 3-H), 5.14 [1H, d, $^3J_{H-F(olefin)}$

34.6 Hz, 1-H]; δ_F ($CDCl_3$) −79.22 [1F, d, $^3J_{H-F(olefin)}$ 34.6 Hz]; δ_C ($CDCl_3$) 26.0 (2C), 26.2, 32.7 (2C), 34.9, 40.6 (d, $^2J_{C-F}$ 26.4 Hz, 3-C), 51.3 (d, $^2J_{C-F}$ 26.4 Hz, 1-C), 165.5 (d, $^1J_{C-F}$ 261.0 Hz, 2-C); ν (neat)/cm^{-1} 3091, 2924, 2851, 1655, 1448, 1425, 1285, 1242, 1193, 1119, 962, 939, 896, 871, 746; [HR EIMS Calcd for $C_9H_{14}FI$ (M): 268.0124. Found: M^+, 268.0136].

4.14. (*Z*)-11-Chloro-2-fluoro-1-iodo-1-undecene (3e)

Oil, δ_H ($CDCl_3$) 1.30–1.54 (12H, m), 1.73–1.80 (2H, m, 4-H), 2.33 (2H, dt, $^3J_{H-F}$ 16.6, *J* 7.3 Hz, 3-H), 3.53 (2H, t, *J* 6.8 Hz, 11-H), 5.17 [1H, d, $^3J_{H-F(olefin)}$ 34.7 Hz, 1-H]; δ_F ($CDCl_3$) −79.87 [1F, dt, $^3J_{H-F}$ 16.6, $^3J_{H-F(olefin)}$ 34.7 Hz]; δ_C ($CDCl_3$) 25.8, 26.8, 28.6, 28.8, 29.0, 29.2, 32.6, 32.7 (d, $^2J_{C-F}$ 27.2 Hz, 3-C), 45.1, 50.6 (d, $^2J_{C-F}$ 27.2 Hz, 1-C), 166.6 (d, $^1J_{C-F}$ 261.0 Hz, 2-C); ν (neat)/cm^{-1} 3092, 2929, 2855, 1656, 1464, 1429, 1257, 1116, 875, 748, 724, 650; [HR EIMS Calcd for $C_{11}H_{19}ClFI$ (M): 332.0204. Found: M^+, 332.0178].

4.15. (*Z*)-12,12-Dimethyl-2-fluoro-1-iodo-11-oxo-1-tridecene (3f)

Oil, δ_H ($CDCl_3$) 1.13 (9H, s), 1.20–1.36 (8H, m), 1.48–1.58 (4H, m), 2.33 (2H, dt, $^3J_{H-F}$ 16.8, *J* 7.3 Hz, 3-H), 2.47 (2H, t, *J* 7.3 Hz, 10-H), 5.17 [1H, d, $^3J_{H-F(olefin)}$ 34.6 Hz, 1-H]; δ_F ($CDCl_3$) −79.85 [1F, dt, $^3J_{H-F}$ 16.8, $^3J_{H-F(olefin)}$ 34.6 Hz]; δ_C ($CDCl_3$) 23.8, 25.8, 26.4 (3C), 28.6, 29.0, 29.2, 29.3, 32.7 (d, $^2J_{C-F}$ 26.4 Hz, 3-C), 36.4, 44.1, 50.6 (d, $^2J_{C-F}$ 26.4 Hz, 1-C), 166.6 (d, $^1J_{C-F}$ 261.8 Hz, 2-C), 216.0; ν (neat)/cm^{-1} 3092, 2930, 2855, 1704, 1656, 1476, 1464, 1365, 1259, 1117, 1067, 988, 875, 747; [HR EIMS Calcd for $C_{15}H_{26}FIO$ (M): 368.1012. Found: M^+, 368.1039].

4.16. (*Z*)-10-Isopropoxycarbonyl-2-fluoro-1-iodo-1-decene (3g)

Oil, δ_H ($CDCl_3$) 1.22–1.30 (14H, m), 1.48–1.64 (4H, m), 2.26 (2H, t, *J* 7.8 Hz, 10-H), 2.33 (2H, dt, $^3J_{H-F}$ 16.6, *J* 7.6 Hz, 3-H), 4.96–5.05 (1H, m), 5.17 [1H, d, $^3J_{H-F(olefin)}$ 34.6 Hz, 1-H]; δ_F ($CDCl_3$) −79.93 [1F, dt, $^3J_{H-F}$ 16.6, $^3J_{H-F(olefin)}$ 34.6 Hz]; δ_C ($CDCl_3$) 21.8 (2C), 24.9, 25.8, 28.6, 28.97 (2C), 29.03, 32.7 (d, $^2J_{C-F}$ 27.3 Hz, 3-C), 34.6, 50.7 (d, $^2J_{C-F}$ 27.2 Hz, 1-C), 67.3, 166.6 (d, $^1J_{C-F}$ 261.0 Hz, 2-C), 173.3; ν (neat)/cm^{-1} 3092, 2979, 2931, 2856, 1730, 1657, 1467, 1373, 1252, 1181, 1146, 1111, 962, 876, 824, 748; [HR EIMS Calcd for $C_{14}H_{24}FIO_2$ (M): 370.0805. Found: M^+, 370.0818].

4.17. 1,2,2-Trifluorododecane (4)

Oil, δ_H ($CDCl_3$) 0.88 (3H, t, *J* 6.8 Hz, 12-H), 1.27–1.56 (16H, m), 1.87–2.00 (2H, m, 3-H), 4.42 (2H, dt, $^3J_{H-F}$ 11.4, $^2J_{H-F}$ 46.6 Hz, 1-H); δ_F ($CDCl_3$) −234.95 to −235.29 (1F, m), −109.44 to −109.64 (2F, m); δ_C ($CDCl_3$) 14.1, 21.5 (t, $^3J_{C-F}$ 4.1 Hz, 4-C), 22.7, 29.3 (3C), 29.4, 29.6, 31.9, 33.0 (t, $^2J_{C-F}$ 23.9 Hz, 3-C), 81.5 (dt, $^2J_{C-F}$ 37.1, $^1J_{C-F}$ 177.6 Hz, 1-C), 121.1 (dt, $^2J_{C-F}$ 22.3, $^1J_{C-F}$ 241.2 Hz, 2-C); ν (neat)/cm^{-1} 2960, 2926, 2856, 1466, 1381, 1280, 1196, 1137, 1060, 928; [HR EIMS Calcd for $C_{12}H_{23}F_3$ (M): 224.1752. Found: M^+, 224.1768].

4.18. Synthesis of methyl (*Z*)-3-fluoro-2-tridecenoate (5a) from 1a

In a glass round-bottom flask fitted with a balloon (3 L) were placed $PdCl_2$ (1.8 mg, 0.01 mmol), $NaHCO_3$ (42 mg, 0.5 mmol), and MeOH (4 ml). After complete replacement of the atmosphere in the flask with CO, the balloon was filled with CO. Then a MeOH solution (1 ml) of **1a** (238 mg, 0.5 mmol) was added and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into 3 M aq NH_4Cl (15 ml) and extracted with ether (10 ml) three times. The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The product **5a**[10] was isolated by column chromatography (silica gel, hexane–diethyl ether) in 73% yield (89 mg, *Z*/*E*>99:1). Oil, δ_H ($CDCl_3$) 0.88 (3H, t, *J* 7.1 Hz, 13-H), 1.21–1.37 (14H, m), 1.52–1.59 (2H, m, 5-H), 2.26 (2H, dt, $^3J_{H-F}$ 17.3, *J* 7.6 Hz, 4-H), 3.72 (3H, s, OMe), 5.18 [1H, d, $^3J_{H-F(olefin)}$ 33.1 Hz, 2-H]; δ_F ($CDCl_3$) −79.53 [1F, dt, $^3J_{H-F}$ 17.3, $^3J_{H-F(olefin)}$ 33.1 Hz]; δ_C ($CDCl_3$) 14.1, 22.7, 25.5, 28.8, 29.2, 29.3, 29.4, 29.5, 31.9, 33.0 (d, $^2J_{C-F}$ 24.1 Hz, 4-C), 51.3, 98.4 (d, $^2J_{C-F}$ 6.0 Hz, 2-C), 164.3, 172.4 (d, $^1J_{C-F}$ 281.1 Hz, 3-C); ν (neat)/cm^{-1} 2951, 2926, 2855, 1736, 1685, 1466, 1436, 1349, 1278, 1217, 1137, 1033, 889, 833, 722; [HR EIMS Calcd for $C_{13}H_{22}FO$ (M−OMe): 213.1655. Found: M^+−OMe, 213.1648]. Found: C, 68.78; H, 10.42%. Calcd for $C_{14}H_{25}FO_2$: C, 68.82; H, 10.31%.

Under the same reaction conditions, methyl (*E*)-2-tridecenoate (**8**) (90%, *E*/*Z*>99:1) and methyl (*E*)-3-fluoro-2-tridecenoate (**11**) (91%, *Z*/*E*=2:98) were prepared from (*E*)-1-dodecenyl(phenyl)iodonium tetrafluoroborate (**7**) and (*E*)-2-fluoro-1-dodecenyl(phenyl)iodonium tetrafluoroborate (**10**), respectively.

4.19. Synthesis of 5a from 3a

In a round-glass flask fitted with a balloon (3 L) were placed $PdCl_2(PPh_3)_2$ (7.0 mg, 0.01 mmol), Et_3N (50 mg, 0.5 mmol), and MeOH (5 ml). After complete replacement of the atmosphere in the flask with CO, the balloon was filled with CO, and **3a** (156 mg, 0.5 mmol) was added into the flask. After stirring at 60 °C for 48 h, the reaction mixture was poured into 3 M aq NH_4Cl (15 ml) and extracted with ether (10 ml) three times. The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The product **5a** was isolated by column chromatography (silica gel, hexane–ether) in 88% yield (107 mg, *Z*/*E*>99:1).

The methoxycarbonylations of **9** and **12** were carried out under the same reaction conditions.

4.20. Methyl (*Z*)-3-fluoro-3-phenyl-2-propenoate (5b)

Prepared from **3b** as described for **5a** in 77% yield (*Z*/*E*>99:1). Oil, δ_H ($CDCl_3$) 3.80 (3H, s, COOMe), 5.92 [1H, d, $^3J_{H-F(olefin)}$ 33.4 Hz, 2-H], 7.42–7.68 (5H, m, Ph); δ_F ($CDCl_3$) −96.25 [1F, d, $^3J_{H-F(olefin)}$ 33.4 Hz]; δ_C ($CDCl_3$) 51.6, 96.8 (d, $^2J_{C-F}$ 7.4 Hz, 2-C), 125.6 (2C, $^3J_{C-F}$ 8.2 Hz, *ortho*), 128.9 (2C), 130.5 (d, $^2J_{C-F}$ 25.60 Hz, *ipso*), 131.6, 164.5, 166.4 (d, $^1J_{C-F}$ 277.6 Hz, 3-C); ν (neat)/cm^{-1} 3090, 2997, 2952, 2844, 1727, 1662, 1496, 1450, 1435, 1339,

1285, 1192, 1167, 1057, 1004, 828, 770, 688; [HR EIMS Calcd for $C_{10}H_9FO_2$ (M): 180.0586. Found: M^+, 180.0586].

4.21. (*E*)-1-Dodecenyl(phenyl)iodonium tetrafluoroborate (7)

Mp 36.0–36.5 °C, δ_H ($CDCl_3$) 0.88 (3H, t, *J* 7.1 Hz, 12-H), 1.19–1.32 (14H, m), 1.41–1.48 (2H, m, 4-H), 2.31–2.36 (2H, m, 3-H), 6.79 (1H, d, *J* 13.7 Hz, 1-H), 6.99 (1H, dt, *J* 7.3, 13.7 Hz, 2-H), 7.48–8.02 (5H, m, Ph); δ_C ($CDCl_3$) 14.1, 22.6, 27.6, 28.9, 29.2, 29.3, 29.4, 29.5, 31.8, 35.3, 96.5, 109.6, 132.4 (2C), 132.7, 135.6 (2C), 155.4; ν (KBr)/cm^{-1} 3052, 3002, 2918, 2850, 1469, 1444, 1067, 988, 739; [HR FABMS Calcd for $C_{18}H_{28}I$ ($M-BF_4$): 371.1236. Found: M^+-BF_4, 371.1220]. Found: C, 46.84; H, 6.03%. Calcd for $C_{18}H_{28}BF_4I$: C, 47.19; H, 6.16%.

4.22. Synthesis of (*E*)-2-fluoro-1-dodecenyl(phenyl)-iodonium tetrafluoroborate (10)

To a CH_2Cl_2 solution (6 ml) of 1-dodecyne (332 mg, 2 mmol) was added an Et_3N–5HF solution (22 ml) of *p*-iodotoluene difluoride (768 mg, 3 mmol) at 0 °C and the mixture was stirred at 0 °C for 2 h. The reaction mixture was poured into water (30 ml) and extracted with CH_2Cl_2 (20 ml) three times. The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting crude (*E*)-2-fluoro-1-dodecenyl(phenyl)iodonium fluoride was dissolved in acetonitrile (10 ml) with $AgBF_4$ (779 mg, 4 mmol) and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured into water (30 ml) and extracted with CH_2Cl_2 (20 ml) three times. The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting viscous oil was dissolved in CH_2Cl_2 (1 ml) and a white suspension was formed by the addition of hexane (40 ml). The white suspension was left in a refrigerator for 2 h and clear upper liquid was removed by decantation. The remained white solid was washed with hexane (5 ml) again, separated by decantation, and dried in vacuo to give pure compound **10** (43%, 400 mg, 0.84 mmol, *E*/*Z*>99:1). Mp 71.8–72.4 °C, δ_H ($CDCl_3$) 0.88 (3H, t, *J* 6.8 Hz, 12-H), 1.17–1.30 (14H, m), 1.47–1.54 (2H, m, 4-H), 2.79 (2H, dt, *J* 7.6, $^3J_{H-F}$ 22.2 Hz, 3-H), 6.72 [1H, d, $^3J_{H-F(olefin)}$ 14.4 Hz, 1-H], 7.46–7.98 (5H, m, Ph); δ_F ($CDCl_3$) −65.89 [1F, dt, $^3J_{H-F(olefin)}$ 14.4, $^3J_{H-F}$ 22.2 Hz]; δ_C ($CDCl_3$) 14.1, 22.6, 25.8, 28.9, 29.2, 29.26, 29.32, 29.5, 31.8, 32.2 (d, $^2J_{C-F}$ 23.9 Hz, 3-C), 78.3 (d, $^2J_{C-F}$ 47.9 Hz, 1-C), 112.1, 132.3 (2C), 132.5, 134.5 (2C), 176.2 (d, $^1J_{C-F}$ 286.5 Hz, 2-C); ν (KBr)/cm^{-1} 3045, 2925, 2854, 1638, 1467, 1440, 1303, 1071, 993, 877, 797, 736, 684; [HR FABMS Calcd for $C_{18}H_{27}FI$ ($M-BF_4$): 389.1142. Found: M^+-BF_4, 389.1154]. Found: C, 45.47; H, 5.57%. Calcd for $C_{18}H_{27}BF_5I$: C, 45.41; H, 5.72%.

4.23. Synthesis of (3*E*,5*Z*)-6-fluoro-3,5-hexadecadien-2-one (13a) from 1a

To a mixture of $Pd(OAc)_2$ (5.6 mg, 0.025 mmol) and KI (4.2 mg, 0.025 mmol) in DMF (1.5 ml) were added 1.2 M aq $NaHCO_3$ (0.5 ml, 0.60 mmol) and methyl vinyl ketone (88 mg, 1.25 mmol) at room temperature. The reaction mixture was then cooled to −20 °C and a DMF solution (1 ml) of **1a** (238 mg, 0.5 mmol) was added. After stirring for 12 h at −20 °C, the reaction mixture was poured into 3 M aq NH_4Cl (15 ml), and extracted with ether (10 ml) three times. The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The product **13a** was isolated by column chromatography (silica gel, hexane–diethyl ether) in 70% yield [89 mg, (3*E*,5*Z*)/(3*E*,5*E*)=96:4]. Oil, δ_H ($CDCl_3$) 0.88 (3H, t, *J* 7.1 Hz, 16-H), 1.23–1.35 (14H, m), 1.54–1.57 (2H, m, 8-H), 2.25–2.33 (5H, m), 5.44 [1H, dd, *J* 11.2, $^3J_{H-F(olefin)}$ 33.4 Hz, 5-H], 6.04 (1H, d, *J* 15.9 Hz, 3-H), 7.42 (1H, dd, *J* 11.2, 15.9 Hz, 4-H); δ_F ($CDCl_3$) −92.13 [1F, dt, $^3J_{H-F}$ 17.7, $^3J_{H-F(olefin)}$ 33.4 Hz]; δ_C ($CDCl_3$) 14.1, 22.6, 25.9, 26.7, 28.9, 29.2, 29.3, 29.4, 29.5, 31.9, 32.5 (d, $^2J_{C-F}$ 24.0 Hz, 7-C), 105.5 (d, $^2J_{C-F}$ 11.5 Hz, 5-C), 128.8 (d, $^4J_{C-F}$ 3.2 Hz, 3-C), 135.5 (d, $^3J_{C-F}$ 6.6 Hz, 4-C), 167.4 (d, $^1J_{H-F}$ 274.2 Hz, 6-C), 198.7; ν (neat)/cm^{-1} 3057, 2951, 2926, 2855, 1695, 1659, 1599, 1466, 1361, 1254, 1134, 982, 866, 722; [HR FABMS Calcd for $C_{16}H_{27}FO$ ($M-BF_4$): 254.2046. Found: M^+-BF_4, 254.2037].

4.24. Synthesis of 13a from 3a

To a DMF solution (2.5 ml) of $Pd(PPh_3)_4$ (57.8 mg, 0.05 mmol) were added Et_3N (505 mg, 5 mmol), methyl vinyl ketone (88 mg, 1.25 mmol), and **3a** (156 mg, 0.5 mmol) at room temperature and the mixture was stirred at 60 °C for 4 h. The reaction mixture was poured into 3 M aq NH_4Cl (15 ml) and extracted with ether (10 ml) three times. The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The product **13a** was isolated by column chromatography (silica gel, hexane–diethyl ether) in 77% yield (98 mg, *Z*/*E*=98:2).

4.25. (3*E*,5*Z*)-6-Fluoro-6-phenyl-3,5-hexadien-2-one (13b)

Prepared from **3b** as described for **13a** in 76% yield (*Z*/*E*=96:4). Mp 89.2–90.0 °C, δ_H ($CDCl_3$) 2.35 (3H, s, Me), 6.24 (1H, d, *J* 15.9 Hz, 3-H), 6.27 [1H, dd, *J* 11.2, $^3J_{H-F(olefin)}$ 33.2 Hz, 5-H], 7.42–7.65 (6H, m); δ_F ($CDCl_3$) −108.44 [1F, d, $^3J_{H-F(olefin)}$ 33.2 Hz]; δ_C ($CDCl_3$) 27.0, 104.8 (d, $^2J_{C-F}$ 13.3 Hz, 5-C), 124.9 (2C, d, $^3J_{C-F}$ 7.4 Hz, *ortho*), 128.8 (2C, d, $^4J_{C-F}$ 2.5 Hz, *meta*), 130.3 (d, $^3J_{C-F}$ 4.1 Hz, 3-C), 130.5, 130.7 (d, $^2J_{C-F}$ 26.4 Hz, *ipso*), 135.3 (d, $^3J_{C-F}$ 5.8 Hz, 4-C), 161.8 (d, $^1J_{C-F}$ 265.1 Hz, 6-C), 198.4; ν (KBr)/cm^{-1} 1658, 1631, 1363, 1292, 1257, 1008, 976, 768, 692; [HR FABMS Calcd for $C_{12}H_{11}FO$ (M): 190.0794. Found: M^+, 190.0808].

4.26. Synthesis of (*Z*)-8-fluoro-7-octadecen-5-yne (14a) from 1a

A DMF solution (5 ml) of $Pd(OAc)_2$ (5.6 mg, 0.025 mmol) and PPh_3 (13.1 mg, 0.05 mmol) was stirred at room temperature for 10 min and then CuI (15.2 mg, 0.08 mmol), hex-1-yne (49 mg, 0.6 mmol), Et_3N (76 mg, 0.75 mmol), and a DMF solution (1 ml) of **1a** (238 mg, 0.5 mmol) were added. After stirring for 15 min at room temperature, the reaction mixture was poured into 3 M aq NH_4Cl (15 ml), and extracted with ether (10 ml) three times. The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The product **14a**[10] was isolated by

column chromatography (silica gel, hexane) in 65% yield (86 mg, *Z*/*E*>99:1).

4.27. Synthesis of 14a from 3a

A mixture of $Pd(OAc)_2$ (5.6 mg, 0.025 mmol) and PPh_3 (13 mg, 0.05 mmol) in DMF (5 ml) was stirred at room temperature for 10 min and then CuI (15 mg, 0.08 mmol), hex-1-yne (62 mg, 0.75 mmol), Et_3N (150 mg, 1.5 mmol), and **3a** (156 mg, 0.5 mmol) were added. After stirring at 30 °C for 2 h, the reaction mixture was poured into 3 M aq NH_4Cl (15 ml), and extracted with ether (10 ml) three times. The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The product **14a** was isolated by column chromatography (silica gel, hexane) in 88% yield (117 mg, *Z*/*E*>99:1).

4.28. (*Z*)-1-Fluoro-1-phenyl-1-octen-3-yne (14b)

Prepared from **3b** as described for **14a** in 83% yield (*Z*/*E*> 99:1). Oil, δ_H ($CDCl_3$) 0.94 (3H, t, *J* 7.3 Hz, 8-H), 1.44–1.61 (4H, m), 2.40–2.44 (2H, m, 5-H), 5.57 [1H, dt, $^5J_{H-H}$ 2.4, $^3J_{H-F(olefin)}$ 33.4 Hz, 2-H], 7.34–7.54 (5H, m, Ph); δ_F ($CDCl_3$) −106.77 [1F, d, $^3J_{H-F(olefin)}$ 33.4 Hz]; δ_C ($CDCl_3$) 13.6, 19.5, 22.0, 30.8, 73.1 (d, $^4J_{C-F}$ 3.3 Hz, 4-C), 87.6 (d, $^2J_{C-F}$ 16.6 Hz, 2-C), 97.8 (d, $^3J_{C-F}$ 5.8 Hz, 3-C), 123.9 (2C, d, $^3J_{C-F}$ 7.4 Hz, *ortho*), 128.6 (2C, d, $^4J_{C-F}$ 1.6 Hz, *meta*), 129.6, 131.3 (d, $^2J_{C-F}$ 26.4 Hz, *ipso*), 164.2 (d, $^1J_{C-F}$ 258.6 Hz, 1-C); ν (neat)/cm^{-1} 3058, 2958, 2932, 2872, 2221, 1643, 1496, 1448, 1326, 1286, 1038, 1018, 830, 760, 688; [HR EIMS Calcd for $C_{14}H_{15}F$ (M): 202.1158. Found: M^+, 202.1148].

4.29. Synthesis of (*Z*)-4-fluoro-1,3-tetradecadiene (15a) from 1a

To a DMF solution (2 ml) of $Pd(PPh_3)_4$ (28.9 mg, 0.025 mmol) were added a DMF solution (1 ml) of **1a** (238 mg, 0.5 mmol) and tributylvinylstannane (174 mg, 0.55 mmol) at room temperature. After stirring at room temperature for 96 h, the reaction mixture was poured into 3 M aq NH_4Cl (15 ml), and extracted with ether (10 ml) three times. The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The product **15a** was isolated by column chromatography (silica gel, hexane) in 69% yield (73 mg, *Z*/*E*=99:1). Oil, δ_H ($CDCl_3$) 0.88 (3H, t, *J* 7.1 Hz, 14-C), 1.23–1.35 (14H, m), 1.47–1.54 (2H, m, 6-H), 2.19 (2H, dt, *J* 7.6, $^3J_{H-F}$ 17.5 Hz, 5-H), 4.95 (1H, d, *J* 10.5 Hz, 1-H), 5.10 (1H, dd, *J* 1.7, 17.1 Hz, 1-H), 5.25 [1H, dd, *J* 10.5, $^3J_{H-F(olefin)}$ 35.6 Hz, 3-H], 6.59 (1H, dt, *J* 10.5, 17.1 Hz, 2-H); δ_F ($CDCl_3$) −103.74 [1F, dt, $^3J_{H-F}$ 17.5, $^3J_{H-F(olefin)}$ 35.6 Hz]; δ_C ($CDCl_3$) 14.1, 22.7, 26.1, 29.0, 29.3 (2C), 29.5, 29.6, 31.9, 32.0 (d, $^2J_{C-F}$ 25.6 Hz, 5-C), 106.9 (d, $^2J_{C-F}$ 11.5 Hz, 3-C), 114.6 (d, $^4J_{C-F}$ 3.3 Hz, 1-C), 128.7 (d, $^3J_{C-F}$ 6.6 Hz, 2-C), 161.1 (d, $^1J_{C-F}$ 266.6 Hz, 4-C); ν (neat)/cm^{-1} 3088, 2955, 2926, 2855, 1684, 1467, 1418, 1133, 994, 899, 861; [HR EIMS Calcd for $C_{14}H_{25}F$ (M): 212.1940. Found: M^+, 212.1933].

4.30. Synthesis of 15a from 3a

To a DMF solution (3 ml) of $PdCl_2(PPh_3)_2$ (25 mg, 0.035 mmol) were added **3a** (156 mg, 0.5 mmol) and tributylvinylstannane (270 mg, 0.85 mmol) at room temperature. The reaction mixture was stirred at 60 °C for 0.5 h, then poured into 3 M aq NH_4Cl (15 ml), and extracted with ether (10 ml) three times. The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The product **15a** was isolated by column chromatography (silica gel, hexane) in 86% yield (91 mg, *Z*/*E*>99:1).

4.31. (1*Z*,3*E*)-1-Fluoro-1,4-diphenyl-1,3-butadiene (15b)

Prepared from **3b** with tributylstyryltin as described for **15a** in 83% yield (*Z*/*E*>99:1). Mp 132.5–133.0 °C, δ_H ($CDCl_3$) 6.29 [1H, dd, *J* 11.0, $^3J_{H-F(olefin)}$ 34.8 Hz, 2-H], 6.66 (1H, d, *J* 15.8 Hz, 4-H), 7.21–7.60 (11H, m); δ_F ($CDCl_3$) −118.26 [1F, d, $^3J_{H-F(olefin)}$ 34.8 Hz]; δ_C ($CDCl_3$) 106.9 (d, $^2J_{C-F}$ 13.3 Hz, 2-C), 120.9 (d, $^3J_{C-F}$ 5.0 Hz, 3-C), 123.9 (2C, d, $^3J_{C-F}$ 7.4 Hz, *ortho*), 126.5 (2C), 127.7, 128.6 (2C), 128.7 (2C), 128.9, 132.0 (d, $^3J_{C-F}$ 26.4 Hz, *ipso*), 132.3 (d, $^4J_{C-F}$ 3.3 Hz, 4-C), 137.3, 157.0 (d, $^1J_{C-F}$ 255.3 Hz, 1-C); ν (KBr)/cm^{-1} 3060, 3033, 3020, 2997, 1634, 1488, 1444, 1320, 1280, 994, 965, 863, 748, 687, 653, 617; [HR EIMS Calcd for $C_{16}H_{13}F$ (M): 224.1001. Found: M^+, 224.1005].

4.32. Synthesis of (*Z*)-2-fluoro-1-phenyl-1-dodecene (16a) from 3a

To a mixture of $PdCl_2(PPh_3)_2$ (18 mg, 0.025 mmol) and phenylboronic acid (73 mg, 0.6 mmol) in benzene (5 ml) were added 2 M aq K_2CO_3 (0.3 ml, 0.6 mmol) and **3a** (156 mg, 0.5 mmol) at room temperature. After stirring at 80 °C for 1.5 h, the reaction mixture was poured into 3 M aq NH_4Cl (15 ml), and extracted with diethyl ether (10 ml) three times. The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The product **16a** was isolated by column chromatography (silica gel, hexane) in 88% yield (112 mg, *Z*/*E*>99:1). Oil, δ_H ($CDCl_3$) 0.88 (3H, t, *J* 6.7 Hz, 12-H), 1.21–1.63 (16H, m), 2.31 (2H, dt, *J* 7.6, $^3J_{H-F}$ 18.3 Hz, 3-H), 5.45 [1H, d, $^3J_{H-F(olefin)}$ 39.5 Hz], 7.17–7.47 (5H, m, Ph); δ_F ($CDCl_3$) −101.25 [1F, dt, $^3J_{H-F}$ 18.3, $^3J_{H-F(olefin)}$ 39.5 Hz]; δ_C ($CDCl_3$) 14.1, 22.7, 26.4, 28.8, 29.1, 29.2, 29.3, 29.5, 29.6, 31.9, 108.0 (d, $^2J_{C-F}$ 28.9 Hz, 1-C), 126.6 (2C), 128.4 (3C), 134.4 (d, $^3J_{C-F}$ 14.1 Hz, *ipso*), 162.8 (d, $^1J_{C-F}$ 253.1 Hz, 2-C); ν (neat)/cm^{-1} 3059, 3026, 2926, 2854, 1691, 1496, 1466, 1346, 1149, 912, 882, 831, 751, 693; [HR EIMS Calcd for $C_{18}H_{27}F$ (M): 262.2097. Found: M^+, 262.2094].

4.33. (*Z*)-2-Fluoro-1,2-diphenylethene (16b)

Prepared from **3b** as described for **16a** in 85% yield (*Z*/*E*>99:1). Mp 92.5–93.2 °C, δ_H ($CDCl_3$) 6.31 [1H, d, $^3J_{H-F(olefin)}$ 39.5 Hz, 2-H], 7.23–7.65 (10H, m); δ_F ($CDCl_3$) −114.78 [1F, d, $^3J_{H-F(olefin)}$ 39.5 Hz]; δ_C ($CDCl_3$) 105.8 (d, $^2J_{C-F}$ 9.9 Hz, 2-C), 124.3 (2C, d, $^3J_{C-F}$ 7.4 Hz, *ortho*), 127.3 (2C, d, $^4J_{C-F}$ 2.5 Hz, *meta*), 128.6 (3C), 128.9, 129.0 (2C), 132.9 (d, $^2J_{C-F}$ 28.1 Hz, *ipso*), 133.7 (d, $^3J_{C-F}$ 3.3 Hz, *ipso*), 157.2 (d, $^1J_{C-F}$ 258.5 Hz, 1-C); ν (KBr)/cm^{-1} 3089, 3054, 3020, 1653, 1494, 1449, 1333, 1282, 1199, 1077, 1033, 1011, 913, 830, 762, 687, 626; [HR EIMS Calcd for $C_{14}H_{11}F$ (M): 198.0845. Found: M^+, 198.0845].

4.34. Synthesis of (5*E*,7*Z*)-8-fluoro-5,7-octadecadiene (17a) from 3a

To a mixture of $Pd(PPh_3)_4$ (29 mg, 0.025 mmol) and (*E*)-hex-1-enylboronic acid (77 mg, 0.6 mmol) in benzene (5 ml) was added an EtOH solution (0.5 ml) of KOH (56 mg, 1 mmol) and **3a** (156 mg, 0.5 mmol) at room temperature. After stirring for 1 h at 80 °C, the reaction mixture was poured into 3 M aq NH_4Cl (15 ml), and extracted with diethyl ether (10 ml) three times. The combined organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The product **17a** was isolated by column chromatography (silica gel, hexane) in 83% yield (111 mg, (5*Z*,7*E*)/(5*E*,7*E*)>99:1). Oil, δ_H ($CDCl_3$) 0.86–0.91 (6H, m), 1.21–1.51 (20H, m), 2.05–2.21 (4H, m), 5.17 [1H, dd, *J* 10.7, $^3J_{H-F(olefin)}$ 36.3 Hz, 7-H], 5.57 (1H, dt, *J* 6.8, 15.6 Hz, 5-H), 6.22–6.29 (1H, m, 6-H); δ_F ($CDCl_3$) −106.88 [1F, dt, $^3J_{H-F}$ 17.7, $^3J_{H-F(olefin)}$ 36.3 Hz]; δ_C ($CDCl_3$) 13.9, 14.1, 22.2, 22.7, 26.2, 29.0, 29.3, 29.4, 29.5, 29.6, 31.9, 32.0 (d, $^2J_{C-F}$ 26.4 Hz, 9-C), 32.2, 32.5, 106.3 (d, $^2J_{C-F}$ 12.3 Hz, 7-C), 121.7 (d, $^3J_{C-F}$ 5.8 Hz, 6-C), 132.3, 159.2 (d, $^1J_{C-F}$ 260.2 Hz, 8-C); ν (neat)/cm^{-1} 3039, 2956, 2925, 2855, 1685, 1635, 1466, 1137, 969, 850, 722; [HR EIMS Calcd for $C_{18}H_{33}F$ (M): 268.2566. Found: M^+, 268.2561].

4.35. (1*Z*,3*E*)-1-Fluoro-1-phenyl-1,3-octadiene (17b)

Prepared from **3b** as described for **17a** in 72% yield [(1*Z*,3*E*)/(1*E*,3*E*)>99:1]. Oil, δ_H ($CDCl_3$) 0.92 (3H, t, *J* 7.1 Hz, 8-H), 1.30–1.46 (4H, m), 2.17 (2H, dt, *J* 7.1, 7.1 Hz, 5-H), 5.83 (1H, dt, *J* 7.1, 15.3 Hz, 4-H), 6.05 [1H, dd, *J* 10.7, $^3J_{H-F(olefin)}$ 35.6 Hz, 2-H], 6.48 (1H, dd, *J* 10.7, 15.3 Hz, 3-H), 7.27–7.55 (5H, m, Ph); δ_F ($CDCl_3$) −121.19 [1F, d, $^3J_{H-F(olefin)}$ 35.6 Hz]; δ_C ($CDCl_3$) 13.9, 22.3, 31.4, 32.8, 106.7 (d, $^2J_{C-F}$ 14.1 Hz, 2-C), 122.1 (d, $^3J_{C-F}$ 5.8 Hz, 3-C), 123.7 (2C, d, $^3J_{C-F}$ 7.4 Hz, *ortho*), 128.4 (2C), 128.5, 132.4 (d, $^2J_{C-F}$ 27.2 Hz, *ipso*), 135.7 (d, $^4J_{C-F}$ 3.3 Hz, 4-C), 155.1 (d, $^1J_{C-F}$ 251.9 Hz, 1-C); ν (neat)/cm^{-1} 3036, 2957, 2927, 2858, 1653, 1627, 1599, 1495, 1448, 1322, 1281, 994, 969, 761, 688; [HR EIMS Calcd for $C_{14}H_{17}F$ (M): 204.1314. Found: M^+ 204.1313].

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