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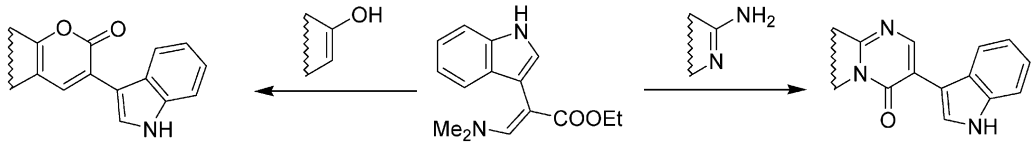
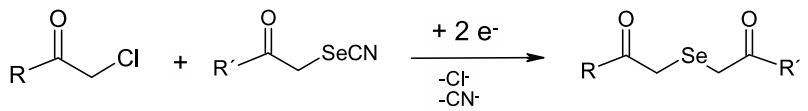
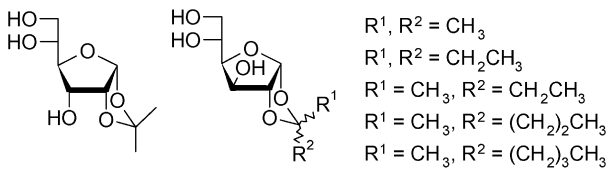
Contents

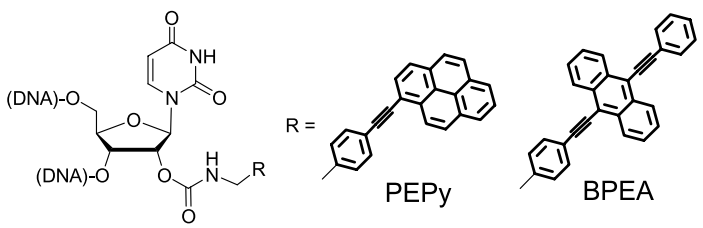


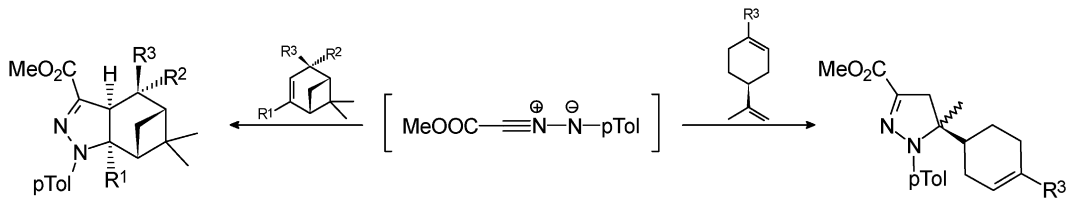


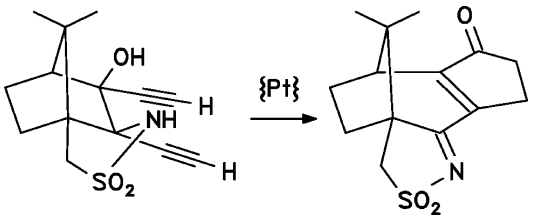
R= C_6H_5, 4-MeO-C_6H_4, 4-Cl-C_6H_4, 4-Br-C_6H_4, 4-Ph-C_6H_4, ^{t}Bu
R′= C_6H_5, 4-MeO-C_6H_4, ^{t}Bu



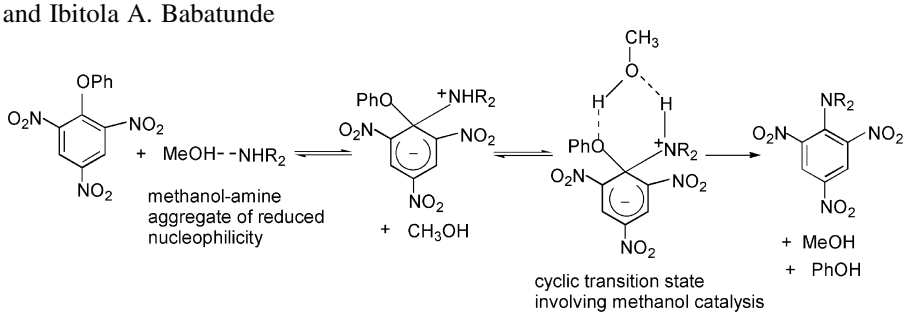
Gluco- and allofuranose derivatives were investigated as potential gelators, showing high gelating ability and result in new consistent class of organogelators. Minimum gelator concentration reaches even 0.03% [g mL^{-1}].

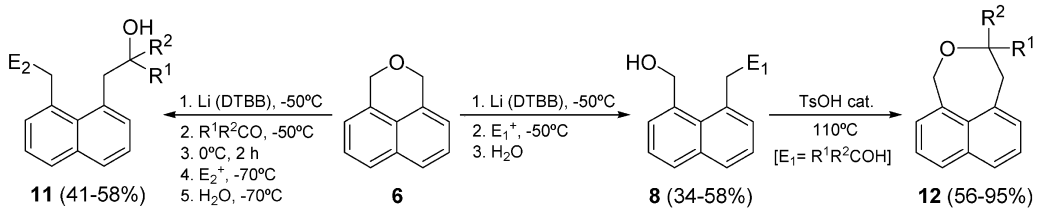


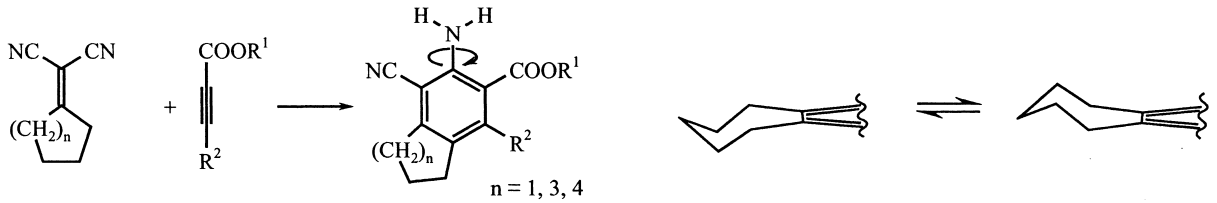


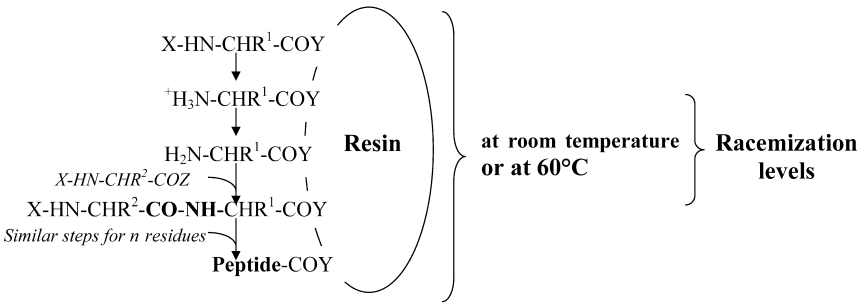


An unprecedented platinum-catalysed cascade reaction leads in one step from a camphor-derived diyne to a tetracyclic cyclopentenone derivative. Its structure was elucidated by NMR spectroscopy and quantum mechanical calculation of the isotropic chemical shifts.



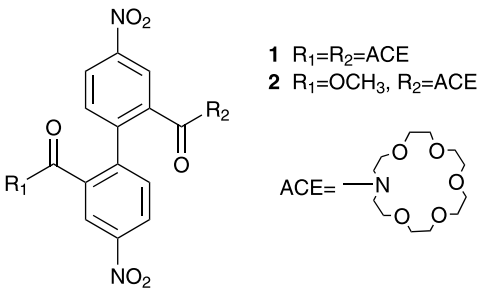


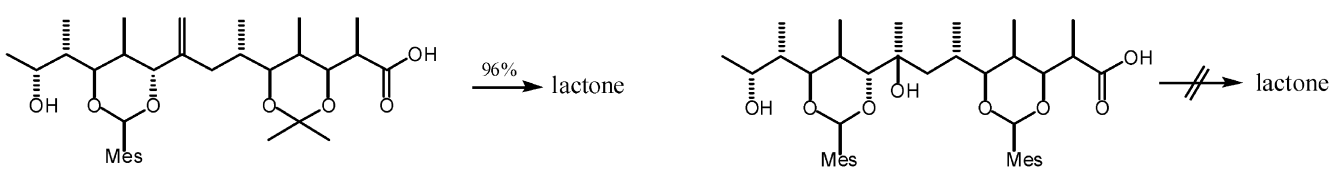


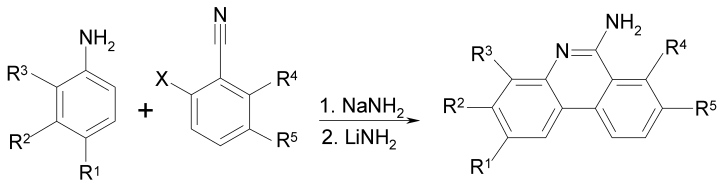


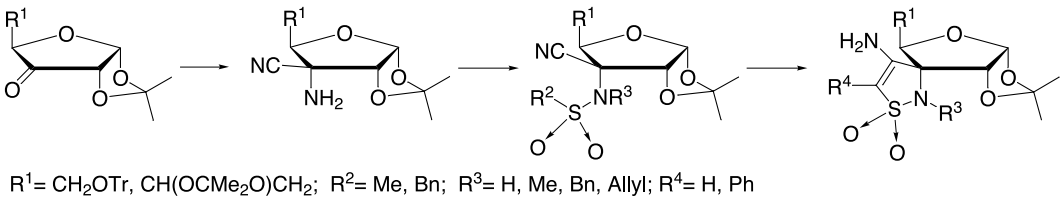
X: urethane-type protecting group; $R^{1,2}$: amino acid side-chains; Y: ester or amide; Z: leaving group.

Extraction and transport experiments with ligands **1** and **2** have shown that the capability of **1** to form a sandwich type 1:1 complex increases both extraction and transport of several cations across a liquid membrane. Under electrochemical conditions 2:1 complexes of **1** are observed.

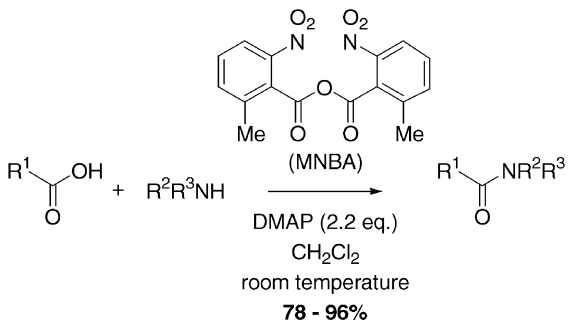


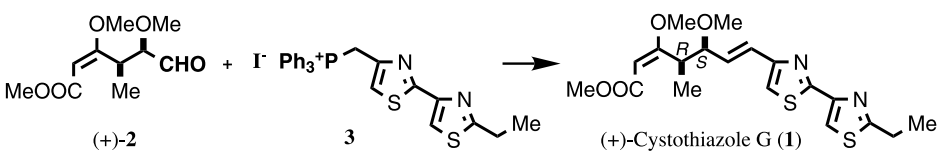




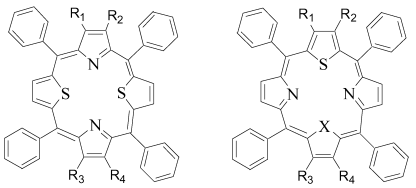


R^1= CH_2OTr, $CH(OCMe_2O)CH_2$; R^2= Me, Bn; R^3= H, Me, Bn, Allyl; R^4= H, Ph





The absolute structure of the isolated cystothiazole G (**1**) was determined based on the chiral synthesis of (**1**) via Wittig condensation of an aldehyde (+)-**2** and phosphonium salt **3**. Thus, the absolute structure of cystothiazole G (**1**) was proved to be (4*R*,5*S*,6(*E*)).



A series of β-pyrrole and β-thiophene substituted porphyrins with N_2S_2 and N_3S porphyrin cores were synthesized and characterized.

OTHER CONTENTS

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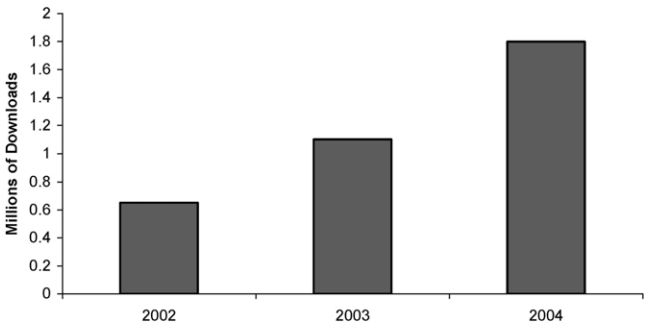

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Investigations into the mechanism of lactamization of lactones yielding in a novel route to biologically active tryptamine derivatives

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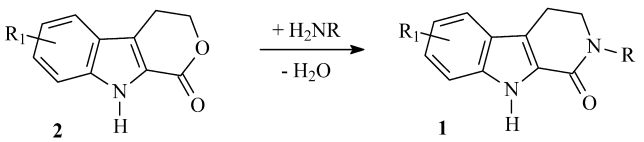
Received 3 February 2004; revised 12 March 2004; accepted 26 March 2004

Abstract—The mechanism of lactamization of corresponding lactones was investigated by means of gas chromatography and synthesis of possible intermediates as references. Lactones react with amines via the amino acid with subsequent elimination of water to the corresponding lactams. In the first step, also hydroxyamides are in equilibrium with the lactones and amines, respectively, which are not able to form the amide though. This mechanism opens a new approach for the synthesis of N^{β}-disubstituted tryptamines.

1. Introduction

The reaction of lactones to lactams is of general importance in organic chemistry. Especially in the field of medicinal chemistry, where this reaction is relevant for the synthesis of versatile intermediates like tetrahydro-9*H*-pyrido[3,4-*b*]indolones (**1**) out of 4,9-dihydropyrano[3,4-*b*]indol-1(3*H*)-ones (**2**) (Scheme 1), for preparation of β-carbolines, serotonin derivatives, and other substances, that are lead substances for drugs for the treatment of diseases of the central nervous system, like psychosis, or are made responsible for causing Parkinson's disease, respectively.[1–3]



Scheme 1. Lactamization of 4,9-dihydropyrano[3,4-*b*]indol-1(3*H*)-ones (**2**) with primary amines yielding tetrahydro-9*H*-pyrido[3,4-*b*]indolones (**1**).

Different relevant experimental data has been found for the course of this reaction: first, Späth and Lintner found that in general lactams are formed with primary amines out of the corresponding lactones at temperatures above 250 °C.[4] Later, this could be proved by the preparation of pyrrolidin-2-ones out of γ-butyrolactones, in which case at temperatures below 180 °C mainly *N*-alkyl-4-hydroxy-butanamide is formed.[5]

The formation of either hydroxy acid amides or lactams is dependent on the applied conditions: *N*-methyl-phthalimidine can be synthesized under high pressure (**3**) out of (2-hydroxymethyl)-*N*-methylbenzamide (**4**). Under normal pressure, the lactone (**5**) and methylamine are formed (Scheme 2).[6] The more basic the amine, the higher are the temperatures to be applied for lactam formation.[4] Also the ring size of the corresponding lactone strongly influences the course of the reaction. Aniline reacts with δ-valerolactone (tetrahydro-2*H*-pyran-2-one) to the hydroxyamide, whereas γ-butyrolactone (dihydrofuran-3(2*H*)-one) gives the lactam.[7] An important hint concerning the mechanism gives the reaction of lactones with secondary amines, in which no cyclization to a lactam is possible. Depending on temperature and reaction time, apart from the hydroxy amide an amino amide is formed, the formation of which could be explained by reaction of an amino acid as intermediate with excess amine.[5,8] Also other experimental data, for example the facts that neither 4-hydroxy-*N*-phenylbutanamide nor compound **6** do form a lactam, leads to the assumption of an amino acid as an intermediate (Scheme 3).[9,10]

The following scheme (Scheme 4) gives an illustration which ways can lead from the lactone (**7**) to the lactam (**8**). For our purposes, we decided to use γ-butyrolactone (**7**) as the reacting lactone and benzylamine (**9**) as the respective amine. Path **a** would mean, that in the first step the hydroxyamide is formed, which could eliminate water to form the

Keywords: Lactamization; Lactones; Amino acids; Hydroxyamides; Tryptamines; Hallocinogens.
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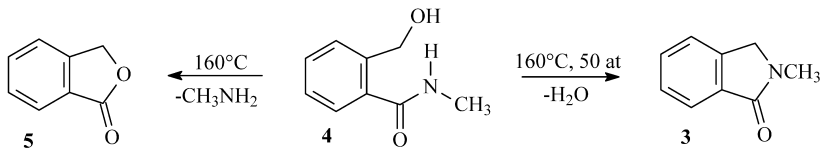
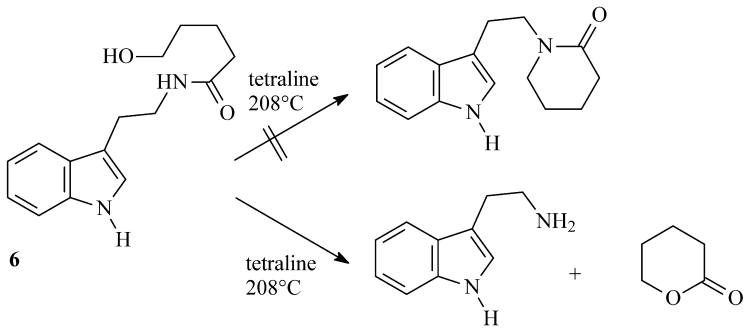
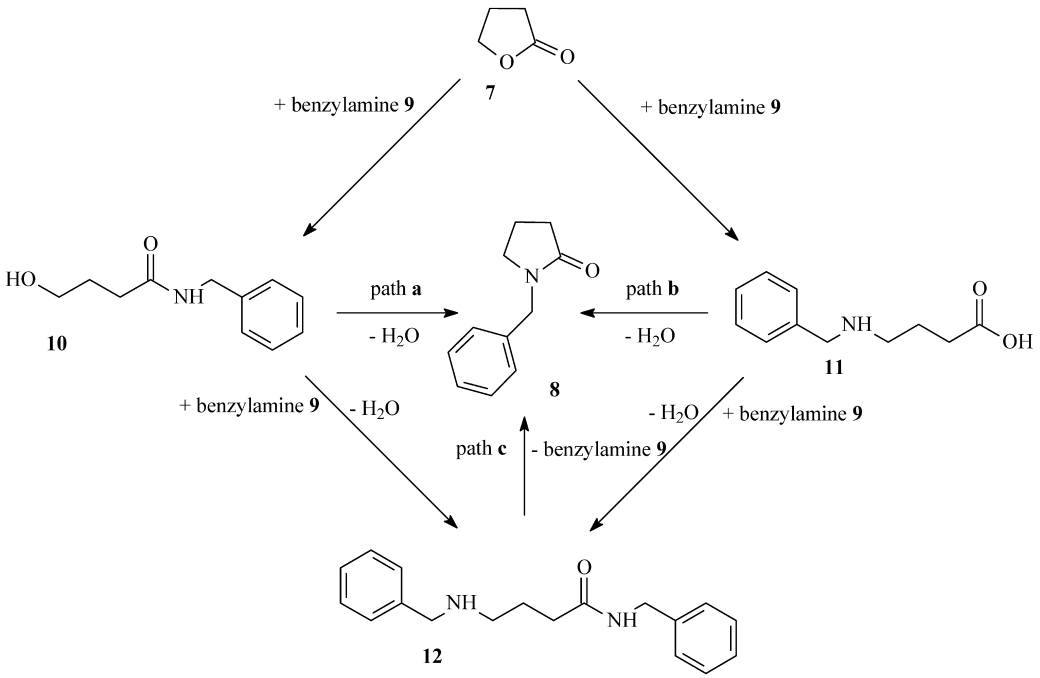


Scheme 2. Dependence of *N*-methyl-phthalimidine (**3**) formation out of (2-hydroxymethyl)-*N*-methylbenzamide (**4**) on the applied pressure.



Scheme 3. 5-Hydroxy-*N*-[2-(1*H*-indol-3-yl)ethyl]pentanamide does not cyclize to the corresponding lactam at elevated temperatures.

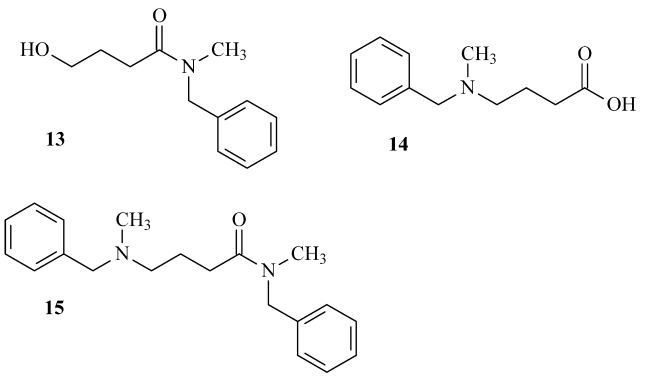


Scheme 4. Different mechanistic routes to the formation of 1-benzylpyrrolidin-2-one (**8**) out of benzylamine (**9**) and γ-butyrolactone (**7**).

lactam. In path **b** addition of the amine leads to an amino acid that cyclizes to the lactam. Both hydroxyamide and amino acid could add a second molecule amine to form the amino amide, that might in turn eliminate amine to give the lactam (path **c**). An interesting possibility to evaluate the mechanism lies in the use of secondary amines to react with the lactone, because the intermediates cannot further react to the lactam.

All possible intermediates and products, that is, hydroxyamide (**10**), amino acid (**11**), amino amide (**12**), and the lactam (**8**) were synthesized, in order to get references to investigate the reaction using gas chromatography. One major problem is of course, that amino acids have never been identified as intermediates, because at the high temperatures necessary for lactamization (and also for gas chromatography) they might directly eliminate water to form the lactam. This should be circumvented by using *N*-benzyl-*N*-methylamine as the amine component, so that no lactams can be formed. Corresponding hydroxyamide (**13**), amino acid (**14**) and amino amide (**15**) should be synthesized as references (Scheme 5).

If indeed the reaction proceeds through the amino acid, it should also be possible to make use of this fact for the synthesis of compounds which are used in medicinal chemistry concerned with drugs for the central nervous system. 4,9-Dihydropyrano[3,4-*b*]indol-1(3*H*)-ones (**2**) could then react to an indole-2-carboxylic acid (**16**), which easily decarboxylates to an N^{β}-disubstituted

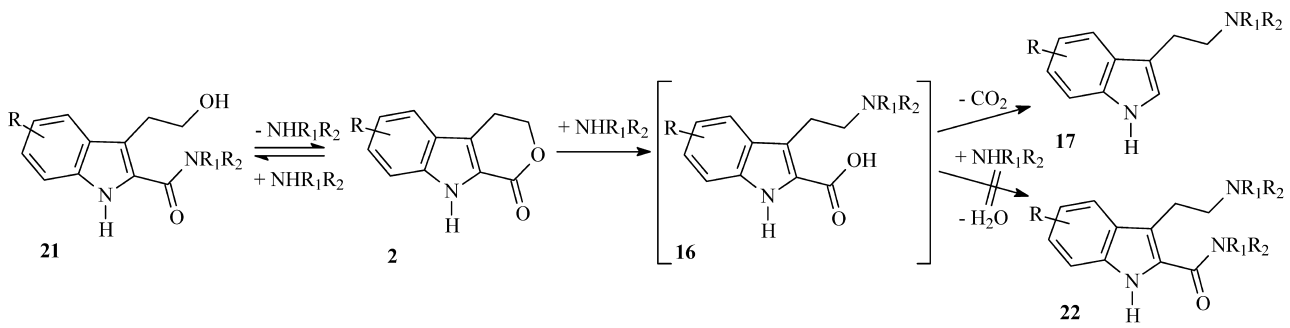


Scheme 5. Possible intermediates for the reaction of *N*-benzyl-*N*-methylamine with γ-butyrolactone (**7**).

Table 1. Gas chromatographic retention times of the compounds specified in Scheme 4 and their silylated derivatives

t_R (min) (silylated compound)	Compound
0.78	γ-Butyrolactone **7**
1.25 (2.84)	Benzylamine **9**
6.45	Lactam **8**
6.45[a] (7.54)	Amino acid **11**
8.15 (8.65)	Hydroxyamide **10**
12.5	Amino amide **12**

[a] The lactam **8**, which is formed under the applied conditions.



Scheme 6. Formation of N^{β}-disubstituted tryptamines (**17**) from 4,9-dihydropyrano[3,4-*b*]indol-1(3*H*)-ones (**2**) and secondary amines.

tryptamine (**17**) (Scheme 6). Also the toxic bufotenin (5-hydroxy-*N*,*N*-dimethyltrytamine) from *Bufo* sp. and the hallucinogenic compound psilocine (4-hydroxy-*N*,*N*-dimethyltryptamine) from *Psilocybe mexicana* (Agaricaceae) belong to this class of compounds, which are derivatives of the neurotransmitter serotonin (5-hydroxy-tryptamine).

2. Results and discussion

The hydroxyamide **10** was synthesized by reaction of γ-butyrolactone with benzylamine at room temperature, the amino acid **11** by hydrolysis of lactam **8** with barium hydroxide solution. The lactam **8** was formed by reaction of γ-butyrolactone with benzylamine at 220 °C. The amino amide **12** was prepared in a two-step synthesis out of 4-chlorobutanoyl chloride: aminolysis with benzylamine (**9**) at room temperature yielded *N*-benzyl-4-chlorobutanamide, which gave the amino amide (**12**) by refluxing with excess benzylamine.

Retention times of the above mentioned compounds were determined with a gas chromatograph (Table 1). Through derivatization with MSTFA (*N*-methyl-trimethylsilyl-trifluoroacetamide) benzylamine **9**, amino acid **11** and hydroxyamide **10** were silylated, so that their retention times changed (Table 1). Silylation was necessary to determine the amino acid, because cyclisation occurs at the temperatures necessary for gas chromatography yielding the lactam **8**. This could be easily proved by heating the amino acid, followed by silylation and gas chromatography. Heating to the melting point (142 °C) and direct cooling yielded 90% lactam, and after heating to 200 °C no amino acid was detectable any more.

For studying the reaction, two equimolar mixtures of γ-butyrolactone and benzylamine were heated for 20 h; one at 150 °C, the other one at 200 °C. After 15 min, 1, 4, 20 h, respectively, samples were taken and chromatographed. It was proved in advance, that the amino acid directly lactamizes, therefore no derivatization was necessary. The changes in the amounts of starting materials and products are given in Figure 1. For estimating the quantitative amounts, the peak area was used. For our qualitative investigation this was precise enough, using naphthalene as an internal standard for determining a correction factor did not improve accuracy, because different reference substances, that are closely related to the respective compounds would have been necessary.

As can be seen from Figure 1, in the 150 °C batch only very low amounts of lactam **8** are formed. The hydroxyamide **10** is rapidly formed. After 1 h the maximum amount is reached, after this time, its amount significantly decreases, while the lactone and the amine peaks, respectively, increase. These results indicate that there might be an equilibrium between the hydroxyamide on the one hand, and lactone and amine on the other hand. In the 200 °C batch, the lactam peak rapidly increases, while the amine and lactone peaks, respectively, decrease. In analogy to the 150 °C batch, the amount of hydroxyamide rapidly increases within the first 15 min, but decreases to almost zero shortly after. This might be either due to the fact, that the hydroxyamide is a direct intermediate, or it exists in equilibrium with the lactone, which can in turn react to

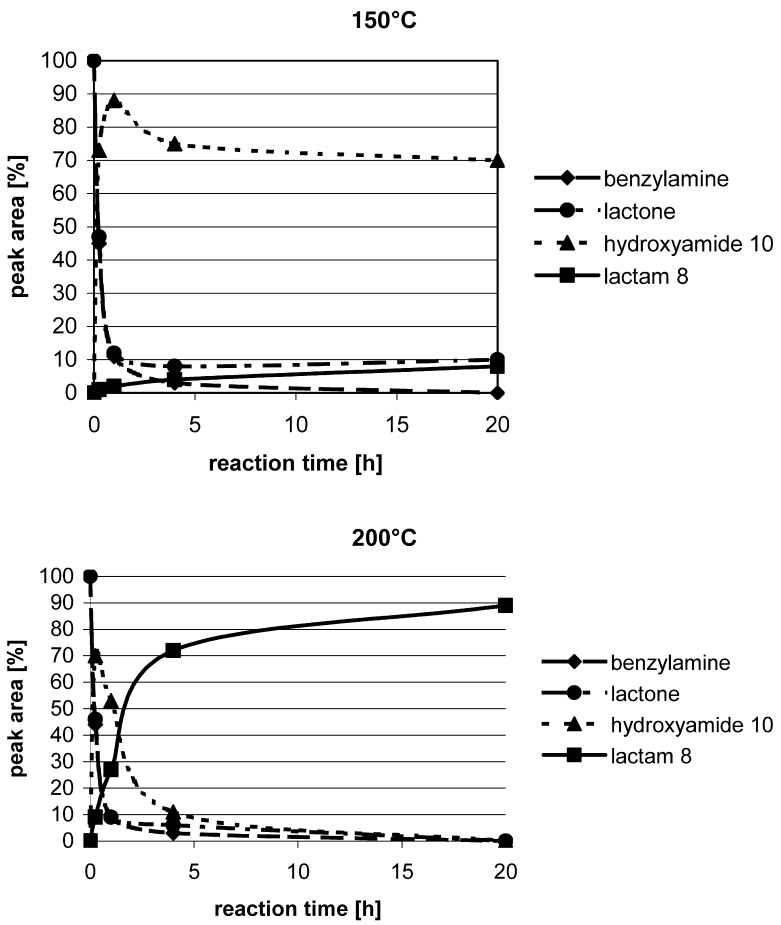


Figure 1. Product formation during the course of reaction of γ-butyrolactone (**7**) and benzylamine (**9**) at different reaction temperatures (150 and 200 °C, respectively) measured by gas chromatography.

another intermediate (the amino acid). It could be easily proved, that hydroxyamide **10** is not a direct intermediate, by heating the hydroxyamide at 200 °C and determine the accruing products. As can be seen from Table 2, small amounts of lactone and amine, respectively, are formed, but even after 20 h only negligible amounts of lactam **8**. Heating the amino amide **12** at 200 °C for 1 h gave the lactam, but large amounts of starting material could be isolated (data not shown). Taking into account this thermal stability of **12**, and the fact, that it could not be determined during the lactamization process, makes it an improbable intermediate.

Table 2. Amounts of products/starting material during the course of pyrolysis of hydroxyamide **10**

t_R (min)	Compound	Area at 200 °C after (%)		
		15 min	1 h	20 h
0.79	γ-Butyrolactone **7**	2.2	3.82	3.16
1.25	Benzylamine **9**	0	0	4.19
6.46	Lactam **8**	0	0	1.83
8.15	Hydroxyamide **10**	83.17	88.27	71.33

We also investigated the reaction of γ-butyrolactone with *N*-benzyl-*N*-methylamine. The reference substances (Scheme 5) were synthesized as follows. Heating γ-butyrolactone with *N*-benzyl-*N*-methylamine for 18 h at 100 °C yielded the hydroxyamide **13**. The amino acid **14** was obtained in two steps out of ethyl 4-bromobutanoate. In the first step the bromine atom was replaced by the *N*-benzyl-*N*-methylamine group, in the second step the ester was hydrolyzed with hydrochloric acid. The corresponding amino amide **15** was also prepared in two steps with *N*-benzyl-*N*-methylamine out of 4-chlorobutanoyl chloride (**16**) via *N*-benzyl-4-bromo-*N*-methylbutanamide (**17**).

Retention times of the respective compounds and their silylated derivatives are given in Table 3. An equimolar mixture of *N*-benzyl-*N*-methylamine and γ-butyrolactone was heated at 200 °C for 7h. Samples were taken after 15 min, 30 min, 2 h, 4 h and 7 h. As can be seen from Figure 2, the starting materials are consumed quite fast; the hydroxyamide **18** is readily formed (it is the kinetically formed product), but disappears rapidly, while the amino

Table 3. Gas chromatographic retention times of the compounds specified in Scheme 5 (reaction of γ-butyrolactone **7** with *N*-benzyl-methylamine) and their silylated derivatives

t_R (min) (silylated compound)	Compound
0.79	γ-Butyrolactone **7**
1.47 (3.13)	*N*-Benzyl-*N*-methylamine
5.85 (7.31)	Amino acid **14**
6.86 (8.75)	Hydroxyamide **13**
10.0	Amino amide **15**

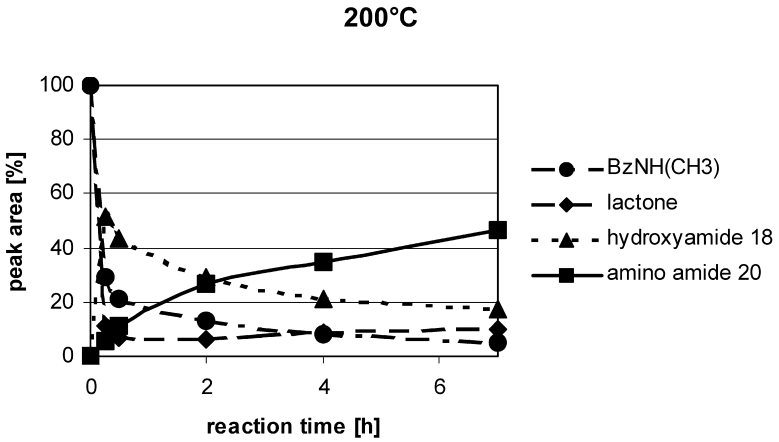


Figure 2. Product formation during the course of reaction of γ-butyrolactone (**7**) and *N*-benzyl-*N*-methylamine at 200 °C measured by gas chromatography.

amide **20** is formed. The formation of an amino amide out of the hydroxyamide does not take place, because heating the hydroxyamide **18** with *N*-benzyl-*N*-methylamine for 3 h at 200 °C gave only low amounts of amino amide, probably via γ-butyrolactone formation, which in turn reacted with *N*-benzyl-*N*-methylamine to the amino amide. Therefore, the amino amide **20** has to be formed via the amino acid **19**, which accrues out of lactone and amine and subsequent reaction with a second amine molecule. The hydroxyamide exists in equilibrium with the starting materials, which are consumed to form the amino amide. In order to prove this assumption, the amino acid **19** was heated with *N*-benzyl-*N*-methylamine at 200 °C. After 30 min, no starting material was found anymore because of complete amino amide formation.

We also examined the reaction of 4,9-dihydropyrano[3,4-*b*]indol-1(3*H*)-ones (**2**) with secondary amines. In this case, hydroxyamide **21**, amino acid **16**, amino amide **22** and trytamines **17** are possible products (Scheme 6). Taking into account the results we obtained with γ-butyrolactone, suppression of formation of the kinetic product **21** is possible by using long reaction times and high temperatures. Decarboxylation occurs faster than reaction with excess amine to the amino amide **22**, therefore reaction of lactones with secondary amines opens a new way for the preparation of the pharmacologically relevant N^{β}-disubstituted tryptamines (**17**), for example, reaction of lactone **2** with *N*-methylaniline leads to *N*-[2-(1*H*-indol-2-yl)ethyl]-*N*-methyl-*N*-phenylamine in 44% yield (Table 4). The formation of tryptamines is of course a further prove for the reaction mechanism previously described. Other different N^{β}-disubstituted tryptamines (**17a**–**p**) were prepared using this reaction, which are listed in Table 4. Compounds **17i** and **17k**, respectively, incorporate a 2-piperidin-4-yl-1*H*-isoindole-1,3(2*H*)-dione moiety, which is a structural feature of the dopaminergic antipsychotic pimocid. They were screened for their affinity to different receptors (Table 5), including the dopamine receptor. Some of these compounds showed high, nanomolar affinities to D_2, α_1 and various serotonin (5-HT) receptors (Table 6).

In summary, the formation of lactams out of lactones goes via the amino acid, but not via the hydroxyamide, which is the kinetic product, that exists in an equilibrium with the starting materials. This information could be used for the preparation of tryptamines, which can be regarded as the amino acid intermediate after decarboxylation.

3. Experimental

3.1. General

Melting points were measured on 'Melting Point Apparatus' by Gallenkamp and are uncorrected. IR spectra were recorded on a Perkin–Elmer '1420'. Elemental analyses were determined with 'CHNO-Analyser Rapid' by Heraeus. ^{1}H NMR spectra were recorded with 'WH-90' (90 MHz, Bruker) and 'AC-200' (200 MHz, Bruker). Chemical shifts (δ) are given in parts per million relative to tetramethylsilane (δ=0.00) as internal standard. Coupling constants (J) are given in Hz. EI mass spectra were measured with MS-30 and MS-50, respectively, A.E.I., Manchester, UK. Analytical thin-layer chromatography (tlc) was conducted on precoated aluminium plates: silica gel 60 F_{254} (Merck Darmstadt, Germany), layer thickness 0.2 mm. For detection iodine vapour or UV light (254 nm), respectively, was used. Silica gel column chromatography utilized silica gel 60 63–200 μm (Baker).

Gas chromatography was performed with Hewlett–Packard model 5890 series II in connection with HP 3396 series II integrator. A capillary column DB-5 (fused silica, 30 m×0.32 mm, film thickness 0.25 μm) by J & W Scientific Inc., California, was applied. Gas supply was adjusted in the following manner: carrier gas hydrogen (15 mL/min); make-up-gas nitrogen (15 mL/min); FID detector: hydrogen (30 mL/min), air (400 mL/min); prepressure: 23 psi; split ratio: 1/20. Injection volume was 1 μL of a 0.1% sample solution. Temperature programme: injector: 200 °C; FID detector: 270 °C; initial temperature: 80 °C (1 min); rate: 15 °C/min; final temperature: 270 °C (2 min). For the reaction of γ-butyrolactone with *N*-benzylmethylamine, conditions were the same, apart from the final temperature [300 °C (2 min)] and the rate (20 °C/min). Silylation was conducted by adding 100 μL MSTFA (*N*-methyl-trimethylsilyl-trifluoroacetamide) and afterwards 200 μL acetonitrile to 1 mg of the respective sample.

Table 4. N^{β}-Disubstituted tryptamines (**17a**–**p**) formed by reaction of 4,9-dihydropyrano[3,4-*b*]indol-1(3*H*)-ones (**2**) with secondary amines

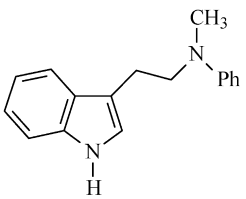
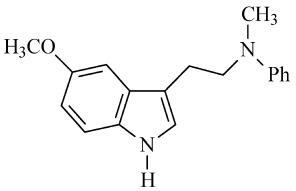
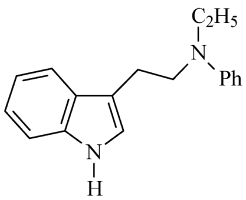
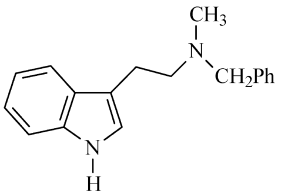
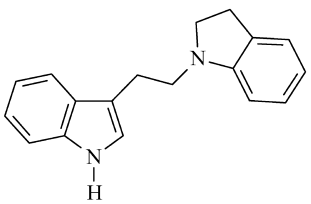
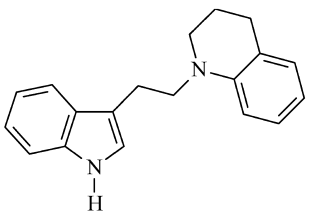
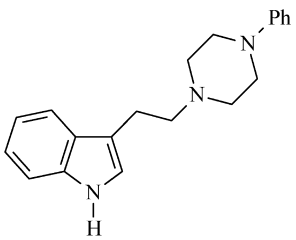
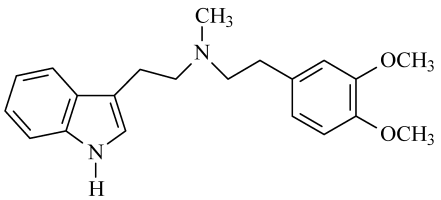
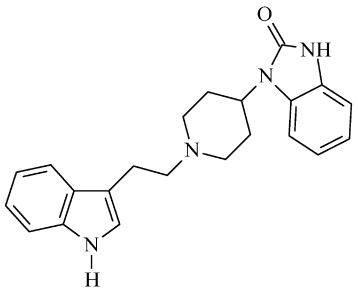
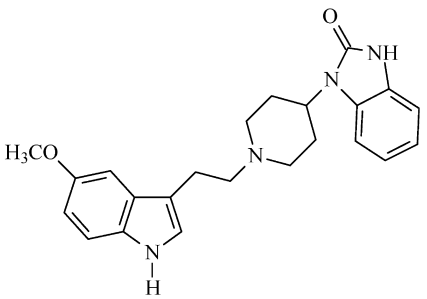
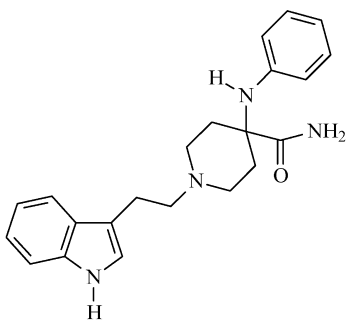
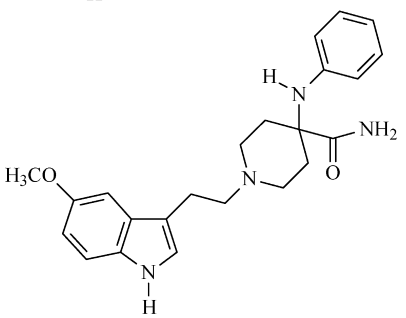
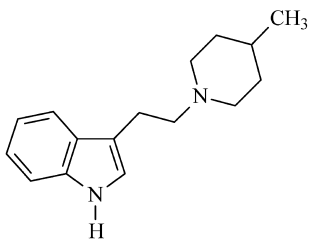
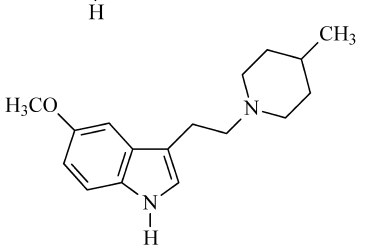
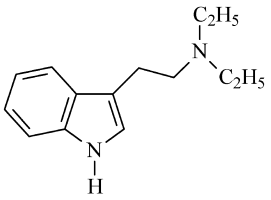
Compound	Compound number	Reaction temperature (°C)	Reaction time (h)	Yield (%)
	**17a**	200	12	44
	**17b**[a]	200	12	38
	**17c**	200	12	41
	**17d**[a]	200	12	18
	**17e**	150	12	16
	**17f**[a]	200	12	33
	**17g**[a]	200	12	47
	**17h**[a]	200	12	37

Table 4 (*continued*)

Compound	Compound number	Reaction temperature (°C)	Reaction time (h)	Yield (%)
	**17i**	200	2	46
	**17k**	200	2	49
	**17l**	200	2	20
	**17m**	200	2	21
	**17n**	180	12	33
	**17o**[a]	180	12	39

(*continued on next page*)

Table 4 (*continued*)

Compound	Compound number	Reaction temperature (°C)	Reaction time (h)	Yield (%)
	**17p**[a]	180	9	39

[a] As the hydrochloride.

This mixture was heated for 15 min at 70 °C. The solution obtained was injected.

3.1.1. *N*-Benzyl-4-hydroxybutanamide (10). To 3.1 mL (40 mmol) of γ-butyrolactone were added 4.4 mL (40 mmol) of benzylamine. The mixture was allowed to stand for 24 h at room temperature. The white precipitate was filtered off and recrystallized from ethanol/diethylether (1/1).

Compound **10**. White, glittering flat crystals (4.35 g, 56% yield). Mp 74–75 °C (lit.[4] mp 74–75 °C). ^{1}H NMR (90 MHz, DMSO-d_6) δ 1.62 (2H, qi, J=8 Hz, C(O)CH_2), 2.16 (2H, t, J=8 Hz, HOCH_2), 3.36 (2H, tt, J=5, 8 Hz, $CH_2CH_2CH_2$), 4.2–4.3 (2H, d, J=6 Hz, benzyl), 4.46 (1H, t, J=5 Hz, OH), 7.22 (5H, m, arom.) ppm. IR (KBr): 1649 cm^{-1} (C=O-valence).

3.1.2. *N*-Benzyl-pyrrolidin-2-one (8). A mixture of 6.2 mL

Table 5. Pharmacological screening results of compounds **17g**, **17i**, **17k**, **17n**, **17o** (N^{β}-disubstituted tryptamines) at different receptors

Receptor/ligand	Decrease in receptor-bound radioactivity (%)					Concentration of test solution (M)
	17g	**17i**	**17k**	**17n**	**17o**	
Adenosine receptors						
^{3}H-CPDPX (A_1)	19	21	14	22	26	10^{-6}
^{3}H-NBTI	9	−62	−50	10	10	10^{-5}
Adrenoceptors						
^{3}H-Prazosin (α_1)	**−98**	**−100**	**−96**	**−92**	−44	10^{-6}
Peptide receptors						
^{3}H-Angiotensin-II	3	3	1	−1	9	10^{-5}
^{3}H-Bradykinin	0	−7	−9	5	2	10^{-5}
^{3}H-Sarafot (ET)	2	−3	3	−1	0	10^{-5}
Dopamine receptors						
^{3}H-Spiperone	**−91**	**−76**	**−72**	−38	−29	10^{-5}
GABA receptor						
^{3}H-Muscimol (GABA-A)	−5	−14	−11	−17	−19	10^{-5}
Glutamate receptors						
^{3}H-MK801	−20	−22	−16	−30	−26	10^{-5}
^{3}H-AMPA	3	−9	10	−9	−14	10^{-5}
ACh receptors						
^{3}H-Cytisin (Nic)	7	−9	−11	−4	−1	10^{-5}
^{3}H-Pirenzipin (M_1)	−5	−29	−29	−9	−11	10^{-6}
Serotonin receptors						
^{3}H-8OH-DPAT (5-HT_{1A})	**−92**	**−71**	**−94**	−32	**−87**	10^{-6}
^{3}H-Ketanserin (5-HT_2)	**−92**	**−100**	**−100**	−48	−49	10^{-6}
^{3}H-Paroxetin (5-HT_{car})	**−95**	**−93**	**−71**	−33	−33	10^{-6}
^{3}H-PDBU (Phorbol)	−3	−13	−4	−11	−4	10^{-5}
^{3}H-Glibenclamide (K^+)	7	−2	−4	0	0	10^{-5}

Bold numbers mark high affinities (i.e. more than −75% decrease).

Table 6. Inhibition constants of compounds **17g**, **17i**, **17k**, **17n**, **17o** (N^{β}-disubstituted tryptamines) at α_1, 5-HT_{1A}, 5-HT_2, and 5-HT_{car} receptors, respectively

Compound	^{3}H-Prazosin (α_1)	^{3}H-8OH-DPAT (5-HT_{1A})	^{3}H-Ketanserin (5-HT_2)	^{3}H-Paroxetin (5-HT_{car})	^{3}H-Spiperone (D_2)
17g	K_I=11 nM	K_I=79.1 nM	K_I=17.2 nM	K_I=146 nM	K_I=101 nM
17i	K_I=3.3 nM	K_I=504 nM	K_I=17.9 nM	K_I=362 nM	Not determined
17k	K_I=6.7 nM	K_I=40.6 nM	K_I=19.1 nM	K_I=101 nM	Not determined
17n	K_I=28 nM	Low affinity	Moderate affinity	Low affinity	Not determined
17o	Moderate affinity	K_I=12.5 nM	Moderate affinity	Low affinity	Not determined

(80 mmol) of γ-butyrolactone and 8.8 mL (80 mmol) of benzylamine was heated for 24 h in a metal bath (bath temperature 220 °C) under reflux and afterwards distilled to yield 11 g of colourless oil (79% yield).

Compound **8**. Bp 124–125 °C/0.8 mm (lit.[4] bp 130–140 °C/1 mm). n_D^{20}=1.5527 (lit.[4] n_D^{20}=1.5520) ^{1}H NMR (90 MHz, DMSO-d_6) δ 1.89 (2H, qui, J=8 Hz, $CH_2CH_2CH_2$), 2.29 (2H, t, J=8 Hz, $C(O)CH_2$), 3.20 (2H, tt, J=8 Hz, CH_2N), 4.37 (2H, s, benzyl), 7.29 (5H, m, arom.) ppm. IR (KBr): 1680 cm^{-1} (C═O-valence).

3.1.3. 4-(Benzylamino)butanoic acid (11). To a solution of 200 mL of 10% aqueous barium hydroxide were added 17.5 g (100 mmol) of *N*-benzyl-pyrrolidin-2-one (**8**). The mixture was heated for 24 h under reflux. After cooling dry ice was added in small pieces until pH=7 was reached. Barium carbonate was filtered off, and the solvent was evaporated to give a solid, which was recrystallized from ethanol/diethylether (1/1) to yield 12 g (62% yield) of white crystals.

Compound **11**. Mp 142 °C (decomp., lit.[11] mp 139 °C). n_D^{20}=1.5527 (lit.[4] n_D^{20}=1.5520) ^{1}H NMR (90 MHz, methanol-d_4) δ 1.87 (2H, qui, J=6 Hz, $CH_2CH_2CH_2$), 2.38 (2H, t, J=6 Hz, CH_2COOH), 3.05 (2H, t, J=6 Hz, CH_2N), 4.13 (2H, s, benzyl), 7.44 (5H, m, arom.) ppm. IR (KBr): 1640 cm^{-1} (C═O-valence).

3.1.4. *N*-Benzyl-4-(benzylamino)butanamide (12). To a solution of 20 mL (183 mmol) of benzylamine in 250 mL of diethylether were added under stirring and ice cooling 10 mL (89 mmol) of 4-chlorobutanoyl chloride. Stirring was continued for 1 h at room temperature. The precipitated amine hydrochloride was filtered off, and the filtrate concentrated under reduced pressure. A white solid (*N*-benzyl-4-chlorobutanamide) was formed (14.8 g, 79% yield), that was recrystallized from acetone/petrolether (40/60) (1/1). Mp 66 °C (lit.[12] mp 68 °C), no further spectroscopic characterization.

A solution of *N*-benzyl-4-chlorobutanamide (11 g, 50 mmol), 11 mL (100 mmol) of benzylamine, and 0.1 g of sodium iodide in 100 mL of ethanol was heated under reflux for 12 h. After cooling, 200 mL of diethylether were added, the precipitated hydrochloride filtered and dried. The hydrochloride was dissolved in 100 mL of water, alkalized with saturated $NaHCO_3$-solution and extracted three times with dichloromethane. Dichloromethane is removed under reduced pressure, and the white solid is recrystallized from diethylether to give 7.2 g (63% yield) of **12** as a white solid.

Compound **12**. Mp 62–63 °C. ^{1}H NMR (90 MHz, DMSO-d_6) δ 1.67 (2H, qui, J=7.5 Hz, $CH_2CH_2CH_2$), 2.18 (2H, t, J=7.5 Hz, CH_2CO), 2.47 (2H, t, J=7.5 Hz, CH_2N), 3.1–3.4 (1H, brd amine-N*H*), 3.64 (2H, s, amine-benzyl), 4.24 (2H, d, J=6 Hz, amide-benzyl), 7.2–7.3 (10H, m, arom.), 8.1–8.4 (1H, brd t, J=6 Hz, amide-N*H*) ppm. IR (KBr): 1640 cm^{-1} (C═O-valence).

3.1.5. *N*-Benzyl-4-hydroxy-*N*-methylbutanamide (13). A mixture of 3.8 mL (50 mmol) of γ-butyrolactone and 6.5 mL (50 mmol) of *N*-benzyl-methylamine were heated at 100 °C (inner temperature) for 18 h. After cooling, the product was purified by column chromatography with dichloromethane as eluent to give 4.5 g (43.5% yield) of a viscous, colourless oil consisting of two conformers.

Compound **13**. ^{1}H NMR (90 MHz, DMSO-d_6) δ 1.7 (2H, qui, J=7 Hz, $CH_2CH_2CH_2$), 2.38 (2H, t, J=7 Hz, CH_2CO) and 2.41 (t, J=7 Hz, CH_2CO, second conformer), 2.8 (3H, s, CH_3) and 2.85 (s, CH_3, second conformer), 4.4–4.5 (1H, brd s, O*H*), 4.5 (2H, s, benzyl) and 4.58 (s, benzyl, second conformer), 3.3–3.5 (2H, m, CH_2OH), 7.12–7.42 (5H, m, arom.) ppm. IR (KBr): 1630 cm^{-1} (C═O-valence).

3.1.6. 4-[Benzyl(methyl)amino]butanoic acid (14) and 14×HCl, respectively. A solution of 11.75 g (50 mmol) of ethyl 4-[benzyl(methyl)amino]butanoate in 50 mL of 2 M hydrochloric acid was heated for 12 h under reflux. The water was removed under reduced pressure and the residue was recrystallized from acetone to give 11.2 g (92% yield) of the hydrochloride of **14**.

Compound **14**×HCl. Mp 173 °C. ^{1}H NMR (90 MHz, DMSO-d_6) δ 1.8–2.0 (2H, m, $CH_2CH_2CH_2$), 2.24 (2H, t, J=8 Hz, CH_2N), 2.55 (3H, s, NCH_3), 2.98 (2H, t, J=8 Hz, CH_2COOH), 4.22 (2H, s, benzyl),7.2–7.4 (5H, m, arom.) ppm. IR (KBr): 1720 cm^{-1} (C═O-valence). Anal. calcd for $C_{12}H_{17}NO_2$: C, 59.14; H, 7.44; N, 5.75. Found: C, 58.65; H, 7.33; N, 5.80.

The hydrochloride **14**×HCl (10 g, 41 mmol) was dissolved in 10 mL of water, alkalized with saturated $NaHCO_3$-solution to pH=7–8, and the water removed under reduced pressure. The residue is suspended three times with 50 mL of acetone and filtered off. Removal of the solvent yielded 8.1 g (i.e., 95% yield related to the hydrochloride) of a colourless, viscous oil, which did not crystallize.

Compound **14**. ^{1}H NMR (90 MHz, DMSO-d_6) δ 1.4–1.9 (2H, m, $CH_2CH_2CH_2$), 2.09 (2H, s, NCH_3), 2.1–2.4 (4H, m, 2×CH_2), 3.47 (2H, s, benzyl),7.31 (5H, m, arom.) ppm. IR (KBr): 1550 cm^{-1} (C═O-valence).

3.1.7. *N*-Benzyl-4-[benzyl(methyl)amino]-*N*-methylbutanamide (15). To a solution of 23.2 mL (180 mmol) of *N*-benzyl-methylamine in 250 mL of diethylether were added under stirring and ice cooling 10 mL (89 mmol) of 4-chlorobutanoyl chloride. Stirring was continued for 1 h at room temperature. The precipitated amine hydrochloride was filtered off, and the filtrate concentrated under reduced pressure. A white solid (*N*-benzyl-4-chloro-*N*-methylbutanamide) was formed (16 g, 80% yield), that was recrystallized from acetone/petrolether (40/60) (1/1) without any further characterization.

A solution of *N*-benzyl-4-chloro-*N*-methylbutanamide (11.3 g, 50 mmol), 13 mL (100 mmol) of *N*-benzyl-methylamine, and 0.1 g of sodium iodide in 100 mL of ethanol was heated under reflux for 12 h. After cooling, the solvent was removed under reduced pressure, the solid dissolved in 100 mL of water. The hydrochloride was dissolved in 100 mL of water, alkalized with saturated $NaHCO_3$-solution and extracted three times with dichloromethane. Dichloromethane is removed under reduced pressure, and the residue

was purified by column chromatography with MeOH/CH_2Cl_2 (1/20) to give a colourless oil (11.6 g, 74% yield related to *N*-benzyl-4-chloro-*N*-methylbutanamide), consisting of two conformation isomers.

Compound **15**. Mp 62–63 °C. ^{1}H NMR (90 MHz, DMSO-d_6) δ 1.5–2.0 (2H, m, $CH_2CH_2CH_2$), 2.07 (3H, s, amine-CH_3) and 2.13 (s, amine-CH_3, second conformer), 2.2–2.6 (4H, m, $CH_2CH_2CH_2$), 2.82 (3H, s, amide-CH_3) and 2.91 (s, amide-CH_3, second conformer), 3.44 (2H, s, amine-benzyl) and 3.49 (s, amine-benzyl, second conformer), 4.5 (2H, s, amide-benzyl) and 4.58 (s, amide-benzyl, second conformer),7.0–7.4 (10H, m, arom.) ppm. IR (KBr): 1640 cm^{-1} (C═O-valence).

The 4,9-dihydropyrano[3,4-*b*]indol-1(3*H*)-ones (**2**) necessary for the following reactions can be either obtained out of δ-valerolactone by reaction with oxalyl ester, followed by reaction with diazotized aniline (Japp–Klingemann-reaction),[13] or out of γ-butyrolactone and oxalyl esters, respectively, following hydrolysis and reaction with phenylhydrazones, as described previously.[14]

3.1.8. General procedure for the preparation of N^{β}-disubstituted tryptamines (17a–p) out of 4,9-dihydropyrano[3,4-*b*]indol-1(3*H*)-ones (2). A mixture of 10 mmol pyrano-indolone (**2**) and 10–40 mmol of the secondary amine were either heated in an autoclave at 200 °C (as indicated in Table 4, in some cases lower temperatures should be applied to avoid by-product formation). After the respective reaction time (see Table 4), the mixture was allowed to cool to room temperature and the excess amine was removed by distillation under reduced pressure. The resulting brown, viscous residue was dissolved in 250 mL of methanol under warming. The insoluble solids were filtered off, the filtrate was concentrated under reduced pressure, and the residue heated for 10 min with refluxing diethylether. Again, the solids were filtered off. Depending on the water solubility of the remaining amine, the filtrate was either treated according to method a) (hardly and non-water-soluble amines) or method b) (water-soluble amines).

(a) The filtrate was extracted two times with 2 N hydrochloric acid, once with water, and dried over Na_2SO_4. The organic phase was treated with 2% hydrochloric acid in ether until the hydrochloride completely precipitated. The suspension was allowed to stand for 30 min, the ether decanted and the residue dried under reduced pressure at 40 °C. To isolate the free base, the hygroscopic hydrochloride was dissolved in 500 mL of boiling water and the hot solution was filtrated. The filtrate is alkalized with 2 N NaOH aqueous solution and extracted three times with diethylether. The organic phase was washed with water, dried over Na_2SO_4, and the ether evaporated. The solid residue was purified by recrystallization from petrolether (40/60)/diethylether (1/1).

(b) The filtrate was extracted three times with water, and dried over Na_2SO_4. The ether was removed under reduced pressure. The product was purified by recrystallization from petrolether/diethylether (1/1). The respective hydrochloride was obtained by adding 2% HCl solution in diethylether to the above filtrate, and filtering the resulting hydrochloride off. The resulting product was purified by recrystallization from methanol/diethylether (1/1).

3.1.9. *N*-[2-(1*H*-Indol-3-yl)ethyl]-*N*-methyl-*N*-phenylamine (17a). *N*-Methylaniline and 4,9-dihydropyrano[3,4-*b*]indol-1(3*H*)-one as starting materials. *Method a*. Beige powder. Mp 140 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.0 (3H, s, NCH_3), 3.08 (2H, t, *J*=7.7 Hz, CH_2CH_2N), 3.72 (2H, t, *J*=7.7 Hz, CH_2CH_2N), 6.7–6.9 (3H, m, $2H_o$, H_p), 7.0 (1H, s, H-2), 7.2–7.5 (5H, m, H-5, H-6, H-7, $2H_m$) ppm. IR (KBr): 3400, 1600, 1500, 1450, 805, 740, 690 cm^{-1}. EI-MS *m*/*z* 250 (M^+). Anal. calcd for $C_{17}H_{18}N_2$: C, 81.56; H, 7.25; N, 11.19. Found: C, 81.20; H, 7.12; N, 11.01.

3.1.10. *N*-[2-(5-Methoxy-1*H*-indol-3-yl)ethyl]-*N*-methyl-*N*-phenylamine (17b). *N*-Methylaniline and 4,9-dihydro-6-methoxy-pyrano[3,4-*b*]indol-1(3*H*)-one as starting materials. *Method a*. Brown hygroscopic crystals. Mp 88–92 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.8 (2H, m, CH_2CH_2N), 3.1 (3H, s, NCH_3), 3.5–3.8 (5H, m, CH_2CH_2N, OCH_3), 6.7 (1H, dd, *J*=10.7, 1.8 Hz, H-6), 7.0 (1H, d, *J*=1.8 Hz, H-4), 7.1 (1H, d, *J*=1.8 Hz, H-2), 7.24 (1H, d, *J*=10.7 Hz, H-7), 7.3–7.6 (5H, m, arom.), 7.6–7.8 (1H, brd, N^+H), 10.77 (1H, brd, indole-N*H*) ppm. IR (KBr): 3420, 3250, 2500, 1600, 1480, 1215, 690 cm^{-1}. Anal. calcd for $C_{18}H_{21}N_2OCl{\times}1.1H_2O$: C, 64.22; H, 6.95; N, 8.32. Found: C, 64.02; H, 6.94; N, 8.46.

3.1.11. *N*-Ethyl-*N*-[2-(1*H*-indol-3-yl)ethyl]-*N*-phenylamine (17c). *N*-Ethylaniline and 4,9-dihydropyrano[3,4-*b*]indol-1(3*H*)-one as starting materials. *Method a*. Beige powder. Mp 99–100 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.07 (3H, t, *J*=7.0 Hz, NCH_2CH_3), 2.94 (2H, t, *J*=7.8 Hz, CH_2CH_2N), 3.34 (2H, q, *J*=7.0 Hz, NCH_2CH_3), 3.52 (2H, t, *J*=7.8 Hz, CH_2CH_2N), 6.57 (1H, t, *J*=7.2 Hz, H_p), 6.7 (2H, d, *J*=8.0 Hz, $2H_o$), 6.95–7.28 (5H, m, H-5, H-6, H-2, $2H_m$), 7.39 (1H, d, *J*=7.5 Hz, H-7), 7.57 (1H, d, *J*=7.5 Hz, H-4), 10.9 (1H, brd, indole-N*H*) ppm. ^{13}C NMR (200 MHz, DMSO-d_6) δ 12.25, 22.9, 44.3, 50.83, 111.48, 111.87, 115.01, 118.22, 118.39, 120.99, 122.81, 127.33, 129.26, 136.36, 147.46 ppm. IR (KBr): 3380, 1580, 1500, 1450, 1350, 740, 690 cm^{-1}. Anal. calcd for $C_{18}H_{20}N_2$: C, 81.78; H, 7.62; N, 10.60. Found: C, 81.59; H, 7.56; N, 10.33.

3.1.12. *N*-Benzyl-*N*-[2-(1*H*-indol-3-yl)ethyl]-*N*-methylamine (17d). *N*-Benzyl-methylamine and 4,9-dihydropyrano[3,4-*b*]indol-1(3*H*)-one as starting materials. *Method b*. The free base was purified by column chromatography with diethylether as eluent, and the product was treated with 2% hydrochloric acid in ether until the hydrochloride completely precipitated. The suspension was allowed to stand for 30 min, the ether decanted and the residue dried under reduced pressure at 40 °C. Brown crystals. Mp 92–95 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.7 (3H, d, *J*=5.0 Hz, NCH_3), 3.1–3.3 (4H, m, CH_2CH_2), 4.2–4.5 (2H, m, benzyl), 6.9–7.1 (2H, m, H-5, H-6), 7.2 (1H, d, *J*=2.5 Hz, H-2), 7.3–7.7 (7H, m, H-7, H-4, arom.), 10.98 (1H, brd, indole-N*H*), 11.17 (1H, brd, N^+H) ppm. IR (KBr): 3200, 2550, 1620, 1450, 740, 700 cm^{-1}. Anal. calcd for $C_{18}H_{21}N_2Cl{\times}0.5H_2O$: C, 69.78; H, 7.10; N, 9.04. Found: C, 69.89; H, 7.36; N, 8.92.

3.1.13. 3-[2-(2,3-Dihydro-1*H*-indol-1-yl)ethyl]-1*H*-indole (17e). Indoline and 4,9-dihydropyrano[3,4-*b*]indol-1(3*H*)-one

as starting materials. *Method a*. Brown powder. Mp 126–128 °C. ^{1}H NMR (90 MHz, DMSO-d_6) δ 2.7–3.1 (4H, m, $CH_2CH_2NCH_2CH_2$), 3.2–3.6 (4H, m, CH_2NCH_2), 6.4–6.7 (2H, t, J=7 Hz, H-5′, H-6′), 6.9–7.3 (5H, m, H-5, H-6, H-2, H-4′, H-7′), 7.38 (1H, dd, J=5.6, 2.6 Hz, H-7), 7.53 (1H, dd, J=5.6, 2.6 Hz, H-4), 10.85 (1H, brd, indole-N*H*) ppm. IR (KBr): 3400, 1605, 1485, 1450, 740 cm^{-1}. Anal. calcd for $C_{18}H_{18}N_2$: C, 82.41; H, 6.92; N, 10.68. Found: C, 82.25; H, 6.95; N, 10.62.

3.1.14. 1-[2-(1*H*-Indol-3-yl)ethyl]-1,2,3,4-tetrahydroquinoline (17f). 1,2,3,4-Tetrahydroquinoline and 4,9-dihydropyrano[3,4-*b*]indol-1(3*H*)-one as starting materials. *Method a*. Peachy powder. Mp 148–149 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.81 (2H, qui, J=6 Hz, CH_2CH_2-CH_2N), 2.65 (2H, t, J=6 Hz, $CH_2CH_2CH_2$), 2.9 (2H, dd, J=10, 5.5 Hz, CH_2CH_2N), 3.25 (2H, t, J=6 Hz, CH_2CH_2-CH_2N), 3.49 (2H, dd, J=10, 5.5 Hz, CH_2CH_2N), 6.45 (1H, t, J=7.5 Hz, H-6′), 6.65 (1H, d, J=8 Hz, H-8′), 6.85 (1H, d, J=7.5 Hz, H-5′), 6.9–7.13 (3H, m, H-5, H-6, H-7′), 7.2 (1H, d, J=2.4 Hz, H-2), 7.34 (1H, d, J=7.5 Hz, H-7), (1H, d, J=7.5 Hz, H-4), 10.85 (1H, brd, indole-N*H*) ppm. IR (KBr): 3400, 1600, 1490, 1450, 740 cm^{-1}. Anal. calcd for $C_{19}H_{20}N_2$: C, 82.57; H, 7.29; N, 10.14. Found: C, 82.83; H, 7.30; N, 10.01.

3.1.15. 3-[2-(4-Methylpiperazin-1-yl)ethyl]-1*H*-indole (17g). 1-Phenylpiperazine and 4,9-dihydropyrano[3,4-*b*]indol-1(3*H*)-one as starting materials. *Method a*. White-beige powder. Mp 130–132 °C (lit.[15] mp 132–134 °C). ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.0–3.5 [8H, m, $N(CH_2CH_2)_2N$], 3.6–3.7 (2H, m, NCH_2CH_2), 3.7–3.9 (2H, m, CH_2N), 6.85 (1H, t, J=6.5 Hz, H-5), 6.95–7.05 (3H, m, 2H$_o$, H$_p$), 7.1 (1H, t, J=6.5 Hz, H-6), 7.2–7.3 (3H, m, H-2, 2H$_m$), 7.35 (1H, t, J=6.5 Hz, H-7), 7.55 (1H, t, J=6.5 Hz, H-4), 11 (1H, brd, indole-N*H*), 11.4 (1H, brd, N^+H) ppm. IR (KBr): 3400, 1600, 1490, 1450, 740 cm^{-1}.

3.1.16. *N*-[2-(3,4-Dimethoxyphenyl)ethyl]-*N*-[2-(1*H*-indol-3-yl)ethyl]-*N*-methylamine (17h). *N*-[2-(3,4-Dimethoxyphenyl)ethyl]-*N*-methylamine and 4,9-dihydropyrano[3,4-*b*]indol-1(3*H*)-one as starting materials. *Method a*. Brown, hygroscopic crystals. Mp 88–90 °C. ^{1}H NMR (200 MHz, DMSO-d_6/D_2O) δ 2.7–2.96 (2H, m, dimethoxyphenyl-CH_2CH_2N), 2.88 (3H, s, NCH_3), 3.05 (2H, m, indole-CH_2CH_2N), 3.15–3.45 (4H, m, $CH_2N(CH_3)CH_2$), 3.6–3.8 (9H, s, s, s, 3×CH_3), 6.55–6.9 (4H, m, H-6, 3H$_{arom.}$), 7.04 (1H, d, J=2 Hz, H-4), 7.16 (1H, s H-2), 7.26 (1H, d, J=9 Hz, H-7), 10.65 (1H, brd, indole-N*H*) ppm. IR (KBr): 1620, 1580, 1510, 1480, 1210, 1020, 800 cm^{-1}. Anal. calcd for $C_{22}H_{29}N_2O_3Cl$×0.5H_2O: C, 63.83; H, 7.31; N, 6.77. Found: C, 63.53; H, 7.46; N, 7.05.

3.1.17. 1-({1-[2-(1*H*-Indol-3-yl)ethyl]piperidin-4-yl}methyl)-1,3-dihydro-2*H*-benzimidazol-2-one (17i). 1-Piperidin-4-yl-1,3-dihydro-2*H*-benzimidazol-2-one and 4,9-dihydropyrano[3,4-*b*]indol-1(3*H*)-one as starting materials. *Method a*. White powder. Mp 249–250 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.65 (2H, d, J=12 Hz, piperidine-CH_2CH_2N), 2.13 (2H, t, J=12 Hz, piperidine-CH_2CH_2N), 2.35 (2H, t, J=12 Hz, piperidine-CH_2CH_2N), 2.64 (2H, t, J=8 Hz, NCH_2CH_2), 2.88 (2H, t, J=8 Hz, NCH_2CH_2), 3.12 (2H, d, J=12 Hz, piperidine-CH_2CH_2N), 4.16 (1H, m, piperidine-C*H*), 6.9–7.1 (5H, m, H-5, 4H$_{arom.}$), 7.1–7.26 (2H, m, H-6, H-2), 7.34 (1H, d, J=7 Hz, H-7), 7.52 (1H, d, J=8.5 Hz, H-4), 10.78 (1H, brd, indole-N*H*), 10.86 (1H, brd, amide-N*H*) ppm. IR (KBr): 1690, 1470, 1370, 1100, 730, 690 cm^{-1}. Anal. calcd for $C_{22}H_{24}N_4O$×0.5H_2O: C, 71.52; H, 6.82; N, 15.17. Found: C, 71.73; H, 6.88; N, 14.88.

3.1.18. 1-({1-[2-(5-Methoxy-1*H*-indol-3-yl)ethyl]piperidin-4-yl}methyl)-1,3-dihydro-2*H*-benzimidazol-2-one (17k). 1-Piperidin-4-yl-1,3-dihydro-2*H*-benzimidazol-2-one and 4,9-dihydro-6-methoxy-pyrano[3,4-*b*]indol-1(3*H*)-one as starting materials. *Method a*. White powder. Mp 249–220 °C. ^{1}H NMR (200 MHz, CD_2Cl_2) δ 1.85 (2H, d, J=12 Hz, piperidine-CH_2CH_2N), 2.25 (2H, t, J=12 Hz, piperidine-CH_2CH_2N), 2.50 (2H, q, J=12 Hz, piperidine-CH_2CH_2N), 2.72 (2H, m, NCH_2CH_2), 2.93 (2H, m, NCH_2CH_2), 3.20 (2H, d, J=12 Hz, piperidine-CH_2CH_2N), 3.86 (3H, s, OCH_3), 4.30 (1H, m, piperidine-C*H*), 6.8 (1H, dd, J=9, 3 Hz, H-6), 7.0–7.18 (5H, m, H-4, 4H$_{arom.}$), 7.2–7.3 (2H, s, d, J=9 Hz, H-2, H-7), 8.08 (1H, brd, indole-N*H*), 9.02 (1H, brd, amide-N*H*) ppm. IR (KBr): 1690, 1480, 1370, 1210, 690 cm^{-1}. Anal. calcd for $C_{23}H_{26}N_4O_2$: C, 70.75; H, 6.71; N, 14.35. Found: C, 70.26; H, 6.97; N, 14.10.

3.1.19. 4-Anilino-1-[2-(1*H*-indol-3-yl)ethyl]piperidine-4-carboxamide (17l). 4-Anilinopiperidine-4-carboxamide and 4,9-dihydropyrano[3,4-*b*]indol-1(3*H*)-one as starting materials. *Method a*. Yellow powder. Mp 127 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.98 (2H, d, J=13 Hz, 2×1H-piperidine-CH_2CH_2N), 2.1–2.5 (4H, m, 2H-piperidine-CH_2CH_2N, 2×1H-piperidine-CH_2CH_2N), 2.65 (2H, t, J=8.5 Hz, CH_2CH_2N), 2.8–3.0 (4H, m, CH_2N, 2×1H-piperidine-NCH_2CH_2), 4.05 (1H, brd, N*H*), 5.42 (2H, brd, CO–NH_2), 6.64 (2H, d, J=7.8 Hz, 2H$_o$), 6.75–6.95 (2H, m, H-5, H$_p$), 7.0–7.25 (4H, m, H-6, H-2, 2H$_m$), 7.35 (1H, d, J=8.3 Hz, H-7), 7.58 (1H, d, J=8.3 Hz, H-4), 8.02 (1H, brd, indole-N*H*) ppm. IR (KBr): 1670, 1600, 1500, 740, 690 cm^{-1}.

3.1.20. 4-Anilino-1-[2-(5-methoxy-1*H*-indol-3-yl)ethyl]-piperidine-4-carboxamide (17m). 4-Anilinopiperidine-4-carboxamide and 9-dihydro-6-methoxy-pyrano[3,4-*b*]indol-1(3*H*)-one as starting materials. *Method b*. White powder. Mp 159 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.98 (2H, d, J=13 Hz, 2×1H-piperidine-CH_2CH_2N), 2.1–2.5 (4H, m, 2H-piperidine-CH_2CH_2N, 2×1H-piperidine-CH_2CH_2N), 2.65 (2H, t, J=8.5 Hz, CH_2CH_2N), 2.8–3.0 (4H, m, CH_2N, 2×1H-piperidine-NCH_2CH_2), 3.84 (3H, s, OCH_3), 4.05 (1H, brd, N*H*), 5.42 (2H, brd, CO–NH_2), 6.64 (2H, d, J=7.8 Hz, 2H$_o$), 6.75–6.95 (2H, m, H-6, H$_p$), 7.0–7.05 (2H, s, s, H-2, H-4), 7.1–7.25 (3H, m, H-7, 2H$_m$), 8.02 (1H, brd, indole-N*H*) ppm. IR (KBr): 3400, 1670, 1600, 800, 690 cm^{-1}. EI-MS *m*/*z* 392 (M^+).

3.1.21. 3-[2-(4-Methylpiperidin-1-yl)ethyl]-1*H*-indole (17n). 4-Methylpiperidine and 9-dihydropyrano[3,4-*b*]indol-1(3*H*)-one as starting materials. *Method b*. White powder. Mp 108 °C (lit.[16] Mp 110 °C). ^{1}H NMR (90 MHz, DMSO-d_6) δ 0.9 (3H, s, CH_3), 1.0–2.7 (9H, m, piperidine-H), 2.8–3.0 (4H, m, CH_2CH_2N), 6.9–7.1 (2H, m, H-5, H-6), 7.2 (1H, d, J=1.8 Hz, H-2), 7.35 (1H, dd, J=7.2, 2 Hz, H-7), 7.62 (1H, dd, J=7.2, 2 Hz, H-4), 11.0 (1H, brd,

indole-N*H*) ppm. IR (KBr): 3400, 3120, 2920, 1625, 1450, 740 cm^{-1}. All the other spectroscopic data were identical with reported data in Ref. 16.

3.1.22. 5-Methoxy-3-[2-(4-methylpiperidin-1-yl)ethyl]-1*H*-indole (17o). 4-Methylpiperidine and 9-dihydro-6-methoxy-pyrano[3,4-*b*]indol-1(3*H*)-one as starting materials. *Method b*. Yellow, hygroscopic crystals. Mp 207–209 °C. ^{1}H NMR (200 MHz, D_2O) δ0.96 (3H, d, J=7 Hz, CH_3), 1.39 (2H, t, J=12 Hz, CHCH_2), 1.62 (1H, m, C*H*), 1.9 (2H, d, J=14 Hz, CHCH_2), 2.86 (2H, t, J=14 Hz, piperidine-N–CH_2), 3.1 (2H, m, CH_2CH_2N), 3.25 (2H, m, CH_2CH_2N), 3.52 (2H, d, J=12 Hz, piperidine-N–CH_2), 3.86 (3H, s, OCH_3), 6.9 (1H, dd, J=9.5, 2.4 Hz, H-6), 7.12 (1H, d, J=2.4 Hz, H-4), 7.22 (1H, s, H-2), 7.43 (1H, d, J=9.5 Hz, H-7) ppm. IR (KBr): 3250, 2930, 2650, 1485, 1450, 1220, 800, 640 cm^{-1}. Anal. calcd for $C_{17}H_{25}N_2OCl\times0.3H_2O$: C, 64.97; H, 8.21; N, 8.92. Found: C, 64.84; H, 8.39; N, 8.79.

3.1.23. *N*,*N*-Diethyl-*N*-[2-(1*H*-indol-3-yl)ethyl]amine (17p). Diethylamine and 9-dihydropyrano[3,4-*b*]indol-1(3*H*)-one as starting materials. *Method b*. White powder. Mp 171 °C (lit.[17] mp 168–169 °C). ^{1}H NMR (90 MHz, D_2O) δ 1.28 (6H, t, J=7.3 Hz, 2×CH_3), 3.0–3.5 (8H, m, CH_2CH_2, 2×NCH_2), 7.1–7.4 (3H, m, H-5, H-6, H-2), 7.5–7.8 (2H, m, H-7, H-4) ppm. IR (KBr): 3200, 2640, 1450, 1430, 745, 700 cm^{-1}. All the other spectroscopic data were identical with reported data in Ref. 17.

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Complexes of benzo-15-crown-5 with protonated primary amines and diamines☆

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Abstract—Three complexes of benzo-15-crown-5 (B15C5) with protonated primary amines $[PhCH_2NH_3(B15C5)](ClO_4)$, $[p\text{-}C_6H_4(CH_2NH_3)_2(B15C5)_2](ClO_4)_2$, and $[(CH_2)_4(NH_3)_2(B15C5)_2](SCN)_2$ were isolated and studied in acetonitrile solutions by NMR, and in the solid state by X-ray crystallography. In all complexes, one B15C5 molecule was bound with each R-NH_3^+ moiety with characteristic small separation of 1.84–1.86 Å between the nitrogen of the R-NH_3^+ group and the O_5 mean plane of the crown residue. No sandwich-type complexes with a 1:2 R-NH_3^+/B15C5 stoichiometry were observed. Binding affinities of B15C5 in acetonitrile were similar for all ammonium cations studied: $K_1=550\pm10\ M^{-1}$ for $[PhCH_2NH_3]^+$; $K_1=1100\pm100$ and $K_2=400\pm30\ M^{-1}$ for $[p\text{-}C_6H_4(CH_2NH_3)_2]^{2+}$; and $K_1=1100\pm100$ and $K_2=300\pm30\ M^{-1}$ for $[H_3N(CH_2)_4NH_3]^{2+}$. The complexation is primarily enthalpy-driven ($\Delta H°=-4.9\pm0.5$ kcal/mol, $\Delta S°=-3.8\pm1.0$ eu for $PhCH_2NH_3^+$–B15C5), as determined by variable temperature ^1H NMR titrations.

1. Introduction

The crown ethers were of a great interest since their discovery had been reported by Pedersen in 1967.[1] The ability of these macrocycles to form non-covalent, H-bonding complexes with ammonium cations has been actively investigated with an eye toward biological applications,[2–4] molecular recognition,[5–12] self-assembly,[13–19] crystal engineering,[20,21] and catalysis.[22] The stoichiometry and stability of these host–guest complexes depend both on the size of the crown ether and on the nature of the ammonium cation (NH_4^+, RNH_3^+, etc).[4,23–25] The numerous studies of 18-crown-6 (18C6) and its derivatives, which have the highest affinity for ammonium cations, invariably showed a 1:1 stoichiometry with both NH_4^+ and RNH_3^+ cations in solution[4,23,24,26] and in the solid state.[27,28–31] A different stoichiometry (2:1) was found in all structurally characterized complexes of 15-crown-5 (15C5) and its derivatives with NH_4^+.[27,32–34] These complexes have a sandwich structure with the NH_4^+ cation placed between two nearly parallel 15C5 residues. The same type of coordination was observed for oxonium ion, isoelectronic to RNH_3^+, which gives $[(H_3O)(B15C5)_2]^+$ complex.[35] The smaller cavity size of 15C5 as compared to 18C6 is generally believed to be responsible for different stoichiometry and structure of their host–guest complexes with NH_4^+.[21,25,27]

Surprisingly, there is little information on stoichiometry and structures of complexes of 15C5 and its derivatives with protonated organic amines. The limited existing data indicate that alkylammonium cations RNH_3^+, unlike NH_4^+, coordinate only one 15C5 molecule.[19,26,36–38] However, it remains unclear whether this stoichiometry is a general property of the interaction between 15C5 and the protonated primary amino group, or whether it results from additional interactions present in all systems studied thus far. To the best of our knowledge, only three crystal structures for the complexes of primary alkylammonium cations (RNH_3^+) with 15C5 or its simple derivatives were reported.[26,36,37] Two of these complexes are head-to-tail dimers of self-complimentary 15C5 derivatives with pendant ammonium arms: 2-ammoniomethyl[15-crown-5][36] and 4′-ammoniomethyl[benzo-15-crown-5][19] (Scheme 1), in which the 1:1 ammonium–crown binding motifs may have been enforced by a 'chelate' effect and additional π-stacking of the benzene rings. In the third structure, a 1:1 complex of substituted monoaza[15-crown-5] with $PhCH(CH_3)NH_3^+$ was reported,[37] but its interpretation is complicated by the presence of bulky substituents in both components of the complex and additional cation–azacrown interactions. Some published data suggested 1:1 stoichiometry[26,38] in solution. These studies were performed in protic polar solvents, where relatively unstable higher-order complexes may have been disfavored due to extensive hydrogen bonding with the solvent. In order to better understand host–guest complexation between 15-crown-5 derivatives

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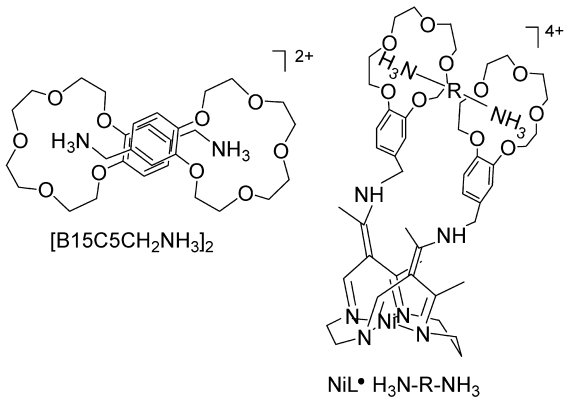
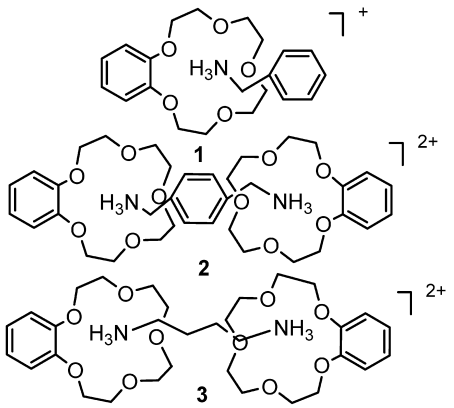


Scheme 1.

and alkylammonium cations, simple systems need to be investigated.

Our interest in the detailed characterization of alkylammonium–B15C5 interactions also stems from our recent finding of shape-selective diammonium binding by a ditopic host containing two B15C5 receptor groups (NiL, Scheme 1). When two B15C5 residues were appended to a metallomacrocyclic platform, the length selectivity of this host in the molecular recognition of alkyldiammonium cations was superior to the selectivity displayed by an analogous B18C6-containing ditopic receptor.[12] Therefore, understanding of alkylammonium and alkyldiammonium binding by simple B15C5 is seen as beneficial for the rational design of functional and selective supramolecular receptors.

Here we report the isolation, acetonitrile solution studies, and crystal structures of three complexes of B15C5 with protonated primary alkylamines: $[PhCH_2NH_3(B15C5)](ClO_4)$ (**1**), $[p\text{-}C_6H_4(CH_2NH_3)_2(B15C5)_2](ClO_4)_2$ (**2**), and $[(CH_2)_4(NH_3)_2(B15C5)_2](SCN)_2$ (**3**) (Scheme 2).



Scheme 2.

2. Results and discussion

2.1. Complex formation between B15C5 and $PhCH_2NH_3^+$

Mixing the B15C5 host with a benzylammonium guest invariably resulted in the crystallization of a 1:1 adduct, regardless of the ratio of components in the starting solution.

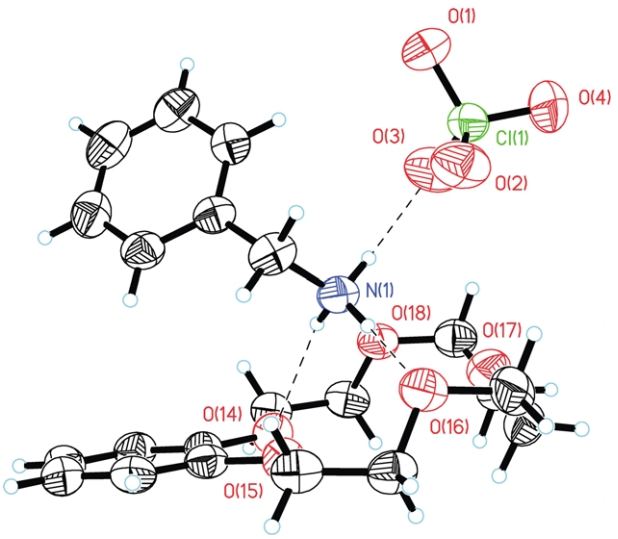


Figure 1. Crystal structure of $[PhCH_2NH_3(B15C5)](ClO_4)$ (**1**). Thermal ellipsoids are drawn at the 50% probability level.

The composition of the solid $[PhCH_2NH_3(B15C5)](ClO_4)$ (complex **1**) was established by elemental analysis and single crystal X-ray diffraction study.

2.1.1. Crystal structure of 1. One view of the molecular structure of **1** is shown in Figure 1. The corresponding crystallographic parameters are given in Table 1. The complexation occurs due to the formation of two hydrogen bonds between the protonated amino group of the ammonium fragment and oxygen atoms O14 and O16 of the crown residue. The metric parameters of the hydrogen bonding are given in Table 2. Oxygen atoms of the crown ether lie in an approximately planar arrangement with mean deviation from the plane of 0.2858(17) Å. The macrocycle adopts a skew conformation (Table 1S, SI), as it does when coordinated to inorganic ammonium, NH_4^+.[27] The N atom of RNH_3^+ group is displaced by 1.842(4) Å from the mean plane defined by the five oxygen atoms of the crown residue. In addition to the two hydrogen bonds with B15C5, the RNH_3^+ cation forms an additional H-bond with the ClO_4^- anion, d(N1···H···O2) is 3.005(5) Å (Fig. 1, Table 2).

Comparing the structural features of complex **1** to other known adducts between 15C5 derivatives and ammonium cations is instructive. In a family of well-known structurally characterized 1:2 sandwich complexes of inorganic NH_4^+ with 15C5 and its derivatives, two hydrogens from the NH_4^+ cation form hydrogen bonds with one crown ether ring, and the remaining two hydrogens are H-bonded to a second macrocycle. In this case the typical distances between ammonium nitrogen and O_5 plane of each crown molecule can be rather large and range from 1.82 to 2.34 Å.[27,32–34,39]

On the other hand, organic primary ammonium cations with functionalized substituents were found to form two NH···O hydrogen bonds with the crown ether host, with the nitrogen atom displaced by 1.89–1.95 Å from the mean O_5 plane.[19,36,37] This short ammonium-crown separation was previously attributed, at least partially, to additional interactions in the host–guest adducts (e.g. 'chelate effect' of two simultaneous crown–ammonium interactions in

Table 1. Crystallographic data for alkylammonium complexes

	Complex		
	1	2	3
Composition	$C_{21}H_{30}ClNO_9$	$C_{18}H_{27}ClNO_9$	$C_{17}H_{26}N_2O_5S$
Formula weight	475.91	436.86	370.46
Space group	P_1(#2)	P_1(#2)	P_1(#2)
a (Å)	10.185(8)	9.4725(13)	8.783(3)
b (Å)	10.403(8)	9.8632(13)	9.602(3)
c (Å)	21.769(17)	11.7661(16)	11.779(3)
α (°)	86.006(16)	90.826(3)	103.607(7)
β (°)	85.321(15)	102.664(3)	91.202(7)
γ (°)	81.085(15)	108.299(3)	91.375(6)
V (Å^3)	2267(3)	1014.2(2)	964.9(5)
Z	4	2	2
d_{calc} (g cm^{-1})	1.394	1.431	1.275
T (K)	293(2)	173(2)	293(2)
μ (mm^{-1})	0.221	0.239	0.196
Final R indices [$I>2\sigma(I)$]	$R1$=0.0709[a], $wR2$=0.1921[b]	$R1$=0.0465[a], $wR2$=0.1160[b]	$R1$=0.0682[a], $wR2$=0.1965[b]
R indices (all data)	$R1$=0.1409[a], $wR2$=0.2561[b]	$R1$=0.0716[a], $wR2$=0.1289[b]	$R1$=0.0851[a], $wR2$=0.2193[b]

[a] $R1=(\sum(F_o-F_c)/\sum(F_o))$.
[b] $wR2=(\sum w(F_o-F_c)^2/\sum w(F_o)^2)^{0.5}$.

Table 2. Hydrogen bonds (length and angles) and perching distance for NH_3^+

Complex	N–H···O (Å)	N–H···O (°)	N–O_5 (Å)	N–H···O(N) anion (Å)	N–H···O(N) anion (°)
1	2.883(4), 3.103(4)	173(4), 151(3)	1.842(4)	3.005(5)	174(4)
2	3.014(3), 2.862(2)	163(2), 167(2)	1.851(2)	3.013(5)	154(2)
3	2.936(2), 2.938(2)	165(3), 168(3)	1.857(2)	2.810(3)	164(2)

self-complementary dimers[19,36] or additional system of hydrogen bonding[37]). One more example of a rather short separation of 1.93 Å was revealed between a secondary dialkylammonium $R_2NH_2^+$ and 15C5 residue by analyzing the crystal structure of $[(H_3O)(B15C5)_2][Me_2NH_2]_2[PMo_{12}O_{40}]\cdot 2(B15C5)$.[35] In this case packing effects could not be ignored, as $Me_2NH_2^+$ cations and crown ether molecules were packed in a 1:1 ratio in the voids of the crystal lattice formed by large polyoxometalate anions.

The small RNH_3^+–15C5 separation found in **1**, where the complex is held together by only two hydrogen bonds, and no significant additional interactions are present, strongly indicates that the distance of 1.8–1.9 Å between the ammonium nitrogen and the 15C5 ring is typical in unconstrained 1:1 adducts. It can also be argued that alkylammonium cations typically form 1:1 adducts with 15C5 derivatives. Indeed, the RNH_3^+ group utilizes two of its hydrogen atoms for H bonding with one crown ether, and the remaining hydrogen is not enough for efficient H-bonding with the second macrocyclic ring. Additionally, the alkyl substituent blocks access to the second crown molecule, thus precluding the formation of sandwich complexes. The larger ammonium-crown separations in NH_4^+-based sandwiches could be due to 'sharing' the singly charged guest between the electronegative regions of two equidistant host molecules.

2.1.2. Solution studies. While X-ray analysis demonstrates the existence of complex **1** in the solid state, the composition and thermodynamic stability of adduct(s) in solution required additional studies.

Mass spectroscopy (ES) of acetonitrile solution of **1** revealed that the 1:1 complex is the predominant species. Two signals were observed in the positive mode, at m/z 376.3 [**1**-ClO_4]$^+$, and m/z 851.2 [(**1**)$_2$-ClO_4]$^+$. No evidence for more complex structures was observed.

When dissolved in CD_3CN at room temperature (0.05 M, saturated solution), complex **1** gives a ^{1}H NMR spectrum with relatively sharp time-averaged signals indicating fast exchange of the complex with its components on the NMR time scale (300 MHz) (Fig. 2). Cooling the solutions down to 238 K caused broadening of the signals (Fig. 2) as well as a significant upfield shift of the methylene resonance of the benzylammonium guest ($\Delta\delta\sim$50 Hz). This clearly indicates a slower rate of exchange and a shift in equilibrium towards complexation. In contrast, heating the solution decreases the yield of **1**, and at 343 K almost no complexation occurred, as seen from Figure 2.

Similar spectral changes (downfield shifts of both the CH_2 and NH_3 protons of the protonated amine) were observed upon dilution of the 0.05 M acetonitrile solution of **1**. The fraction of ammonium groups bound to the crown ether dropped from ~93% to ca. 17% upon 100-fold dilution of the 0.05 M solution of **1** (Fig. 2S, SI).

The stoichiometry of benzylammonium complexation with B15C5 in acetonitrile solution was investigated by Job's method of continuous variations.[40] The maximum yield of the complex was observed at a 1:1 host-to-guest ratio, thus providing strong evidence in favor of a 1:1 stoichiometry of binding in solution (Fig. 3).

Titrations of benzylammonium perchlorate with B15C5 in acetonitrile were performed to determine the association

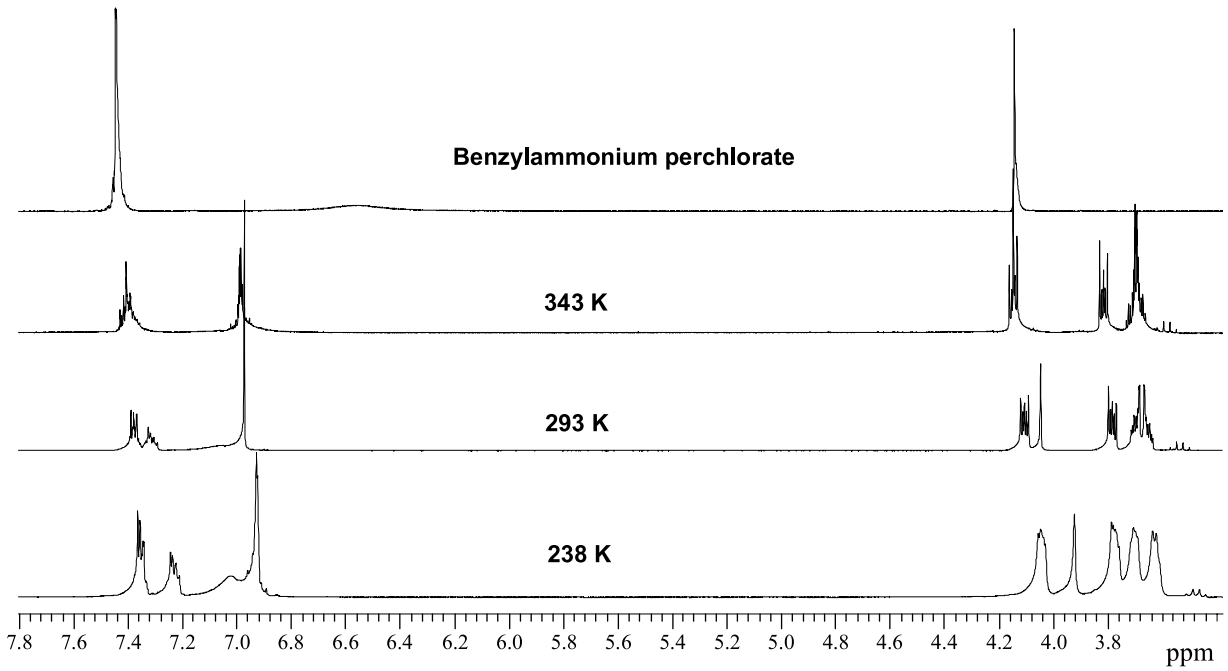


Figure 2. ^{1}H NMR spectra of acetonitrile solution of $[PhCH_2NH_3(B15C5)](ClO_4)$ (**1**) (0.05 M) at different temperatures.

constants at different temperatures. An acetonitrile solution of the B15C5 was gradually added to a solution of alkylammonium and the changes in key cation resonances were monitored. The spectral changes were similar to those shown in Figure 2. The resonance of the ammonium group was shifted significantly downfield ($\Delta\delta_{max}\sim$200 Hz) upon complexation. A drastic change was observed for the aromatic $PhCH_2NH_3^+$ resonance, which was shifted upfiled ($\Delta\delta_{max}\sim$70 Hz) and split into two multiplets in a 3:2 ratio. The resonance of the methylene group of the cation moved by 40 Hz upfield. The protons of B15C5 showed only a slight upfield shift of ca. 10 Hz. Small changes in the ^{1}H NMR spectra of crown ethers upon ammonium binding are common.[19,23]

Binding constants were calculated from the upfield shift of the benzylic (CH_2) protons and the downfield shift of the ammonium protons of $PhCH_2NH_3^+$ in the NMR spectra. The titration data at each temperature were found to fit a 1:1 binding isotherm very well (Fig. 4). Analysis of the data by non-linear least squares as well as the WinEQNMR-program[41] yielded the association constants reported in Table 3. The value of K_1 found in this work is in a good agreement with K_1 for complexation of B15C5 with NH_4^+ in

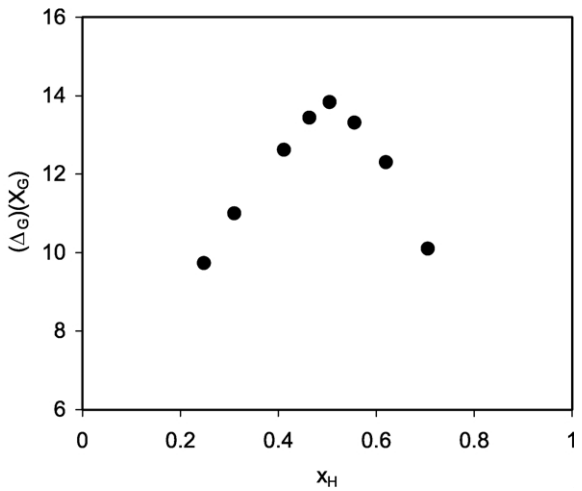


Figure 3. Job's plot for the system containing B15C5 (Host) and benzylammonium cation (Guest) in CD_3CN. The total concentration of the host (H) and guest (G) was kept constant at 2×10^{-2} M. The value of $(\Delta\delta_G)(X_G)$, where X_G denotes molar fraction of the guest, was plotted vs. molar fraction of the host (X_H).

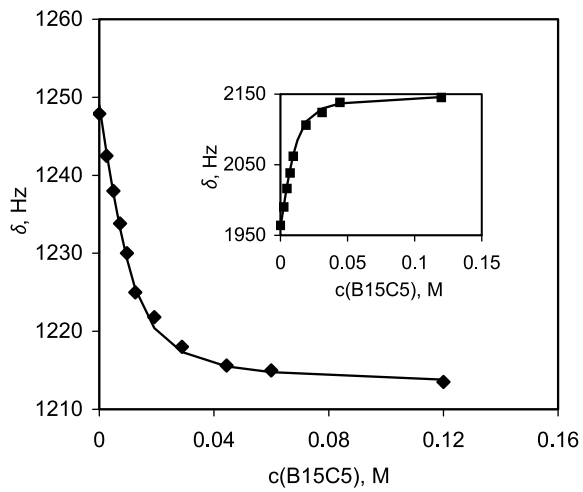


Figure 4. ^{1}H NMR-titration curve of benzylammonium perchlorate (c=12 mM) with B15C5 at 310 K: proton chemical shift of a CH_2 group of ammonium cation vs. concentration of B15C5 added. Inset: proton chemical shift of NH_3 group of $PhCH_2NH_3^+$ cation vs. concentration of added B15C5.

Table 3. Binding constants for complexes of ammonium and alkylammonium cations with B15C5 and 15C5

Guest	Host	T (K)	K_1 (M^{-1})	log K_1	K_2 (M^{-1})	log K_2	Solvent	Method	Reference
$C_6H_5CH_2NH_3^+$	B15C5	310	400±10	2.60			CH_3CN	^1H NMR	This work
	B15C5	298	550±10	2.74			CH_3CN	^1H NMR	This work
	B15C5	288	700±10	2.85			CH_3CN	^1H NMR	This work
	B15C5	280	950±10	2.98			CH_3CN	^1H NMR	This work
$CH_3NH_3^+$	15C5	313		2.61±0.06			EtOH	Potent	38
	15C5	298		3.03±0.11			EtOH	Potent	38
	15C5	283		3.20±0.09			EtOH	Potent	38
	DB15C5	298		0.86±0.14			H_2O	Spectrophot	26
	DB15C5	298		0.95±0.05			H_2O	Spectrophot	26
$C_2H_5NH_3^+$	15C5	313		2.63±0.10			EtOH	Potent	38
	15C5	298		2.76±0.12			EtOH	Potent	38
	15C5	283		3.06±0.07			EtOH	Potent	38
$C_6H_4(CH_2)_2(NH_3)_2^{2+}$	B15C5	298	1100±100	3.04	400±30	2.60	CH_3CN	^1H NMR	This work
	tweezer NiL[a]	298	3×10^3	3.48			CH_3CN	^1H NMR	12
$(CH_2)_4(NH_3)_2^{2+}$	B15C5	298	1100±100	3.04	300±30	2.48	CH_3CN	^1H NMR	This work
	tweezer NiL[a]	298	10^5	5.00			CH_3CN	^1H NMR	12
NH_4^+	B15C5	298		2.16±0.15		1.97±0.2	CH_3CN	Calorim	42
	15C5	298		2.0			CH_3CN	N/a	43
	15C5	298		3.17±0.08			EtOH	Potent	38
	15C5	298		3.50		3.03	MeOH	Calorim	45
	DB15C5	298		0.85±0.09			H_2O	Spectrophot	26

[a] [Ni(4′-aminomethylbenzo-[15-crown-5])$_2$Me$_2$[15]-tetraeneN$_4$]](PF$_6$)$_2$, Scheme 1.

CH_3CN.[42,43] The only previously reported association constants for complexes of alkylammonium cations with 15-crown-5 derivatives were obtained in aqueous[26] or ethanol[38] solutions (Table 3). Surprisingly, these published values of binding constants in protic solvents are comparable to B15C5- RNH_3^+ binding strength in acetonitrile. In order to determine the reasons for this small solvent effect, a more detailed thermodynamic analysis of the $PhCH_2NH_3^+$–B15C5 system was undertaken.

The enthalpic and entropic contributions to host–guest complexation for **1** were determined by Van't Hoff analysis as $\Delta H^\circ = -4.9\pm0.5$ kcal/mol, $\Delta S^\circ = -3.8\pm1.0$ eu (Fig. 5). Our thermodynamic parameters are very close to those obtained in CH_3CN for a 1:1 complex of B15C5 with NH_4SCN,[42] which are reported to be -4.6 ± 0.4 kcal/mol and -5.5 ± 1.4 eu (Table 4). The calculated enthalpy reported here is in reasonable agreement with previously reported values for reactions of 15C5 with alkylammonium ions in ethanolic solution (Table 4).[38,44,45] This agreement is all more apparent when one considers the different dielectric constants and donor/acceptor properties of the solvents.[46] Most direct calorimetric studies of host–guest complex formation between crown ethers and NH_4^+ or RNH_3^+ were performed in aqueous solution, where the enthalpies of complexation of 15C5 and its analogs are too small to be measured.[24,26,47,48] Therefore, any significant ammonium–15C5 binding in water can be attributed to favorable entropy of complexation that results from extensive desolvation of the host and the guest (Table 4). Enthalpy–entropy compensation may be partially responsible for similar binding constants between ammonium cations and 15C5 derivatives in different solvents (Tables 3 and 4).

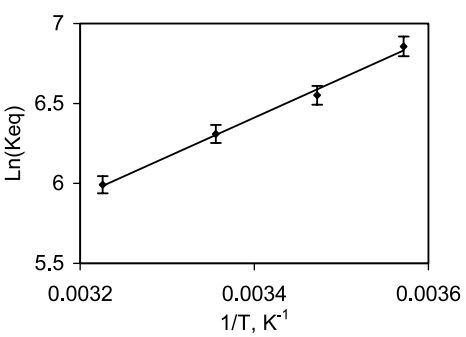


Figure 5. The van't Hoff plot for the formation of [$PhCH_2NH_3$-(B15C5)](ClO$_4$) (**1**) in acetonitrile.

As expected, the complexes between RNH_3^+ and 18-crown-6 derivatives are stronger than their counterparts formed by 15-crown-5 derivatives, as the former adducts are held together by three hydrogen bonds, while the smaller crown ethers in the latter adducts form only two hydrogen bonds with ammonium cations.[23,24] Based on the limited available data, it appears that the substituents in the host or guest molecules exert relatively little influence on complex stability in the ammonium—15-crown-5 systems (Table 3). For larger hosts (18-crown-6 derivatives), the binding affinity for amines followed the series $NH_4^+ > RNH_3^+ > R_2NH^+$.[23] While this general trend still holds for 15C5 derivatives, the difference between NH_4^+ and RNH_3^+ (in protic solvents) is smaller, and sometimes becomes negligible[26,38] (Table 3). This effect may be related to a different number of hydrogen bonds in 18C6 as compared to 15C5 complexes. Similarly, benzo-substituted 18-crown-6 (B18C6 and DB18C6) generally forms weaker complexes than the parent unsubstituted 18C6.[23] This effect is also diminished for a smaller 15C5 ring, where NH_4^+ binding affinity to 15C5 and B15C5 in acetonitrile is similar[42,43] (Table 3).

Our current studies established a 1:1 stoichiometry and fairly high thermodynamic stability of benzylammonium–B15C5 complexation. The complexation in acetonitrile is enthalpically driven. The enthalpic effect is reasonably high, considering that only two hydrogen bonds are responsible for the adduct formation. This may be related to the

Table 4. Enthalpy and entropy values for different complexes of B15C5and 15C5 with ammonium/alkylammonium in different solvents

Alkylamonium/ammonium	Crown ether	ΔH (kcal/mol)	ΔS (cal/mol K)	Solvent	Reference
$C_6H_5(CH_2)NH_3^+$	B15C5	−4.9±0.5	−3.8±1	CH_3CN	This work
NH_4^+	B15C5	−4.6±0.4	−5.5±1.4	CH_3CN	42
NH_4^+	15C5	−4.5±0.5	1.2±0.7	EtOH	38
$CH_3NH_3^+$	15C5	−5.6±0.2	−5.0±1	EtOH	38
$C_2H_5NH_3^+$	15C5	−5.6±0.2	−5.7±1	EtOH	38
NH_4^+	15C5	−2.0		MeOH	45
NH_4^+	B15C5	−0.74		MeOH	45
NH_4^+	15C5	−0.24±0.04	+7.0	H_2O	44

relatively short host–guest contact determined by X-ray crystallography.

2.2. Complex formation between B15C5 and diammonium cations $^+H_3NCH_2C_6H_4CH_2NH_3^+$ and $^+H_3N(CH_2)_4NH_3^+$

The complexes with overall stoichiometry (B15C5)/(diammonium)=2:1 were always isolated, regardless of the ratio of the components in solutions. The formulas were determined by elemental analysis and confirmed by single crystal X-ray diffraction studies.

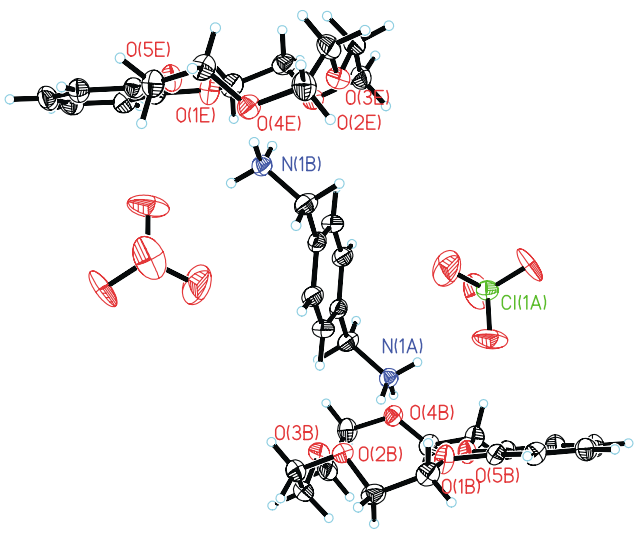


Figure 6. Crystal structure of $[p\text{-}C_6H_4(CH_2NH_3)_2(B15C5)_2](ClO_4)_2$ (**2**). Thermal ellipsoids are drawn at the 50% probability level.

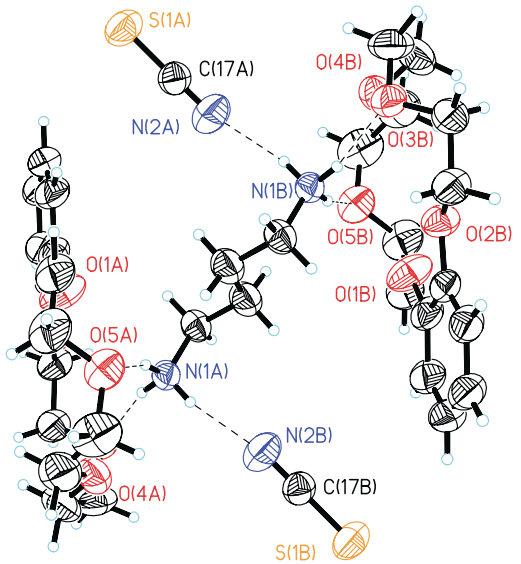


Figure 7. Crystal structure of $[(CH_2)_4(NH_3)_2(B15C5)_2](SCN)_2$ (**3**). Thermal ellipsoids are drawn at the 50% probability level.

2.2.1. X-ray structures of $[p\text{-}C_6H_4(CH_2NH_3)_2(B15C5)_2](ClO_4)_2$ (2), and $[(CH_2)_4(NH_3)_2(B15C5)_2](SCN)_2$ (3).

Both complexes crystallize with two crown ether molecules (one crown ether ring per ammonium group) forming centrosymmetric (C_i) complexes as shown in Figs. 6 and 7. The composition and geometry of each crown ether-ammonium fragment are very similar to that described for the complex **1**.

In complex **2** (Fig. 6) each ammonium group formed three simple H-bonds: two with its crown residue, d(N1···O2)=3.014(3) Å, N1H···O2=163(2)°; d(N1···O4)=2.862(2) Å, N1H···O4=167(2)°, and one with a perchlorate anion, d(N1···O9)=3.013(5) Å, N(1)H1···O9=154(2)°. The crown adopts a conformation in which the five oxygens are nearly coplanar with mean deviation of 0.2739(11) Å from the average O_5 plane. The planes of two crown residues are separated from each other by 9.51 Å while the centroid–centroid distance between O_5 planes is 10.74 Å. Each ammonium ion is perched 1.851(2) Å above the O_5 mean plane.

Substitution of the *p*-xylylenediammonium cation with the less bulky tetramethylenediammonium cation did not affect the geometry of the host–guest interactions in complex **3** (Fig. 7). The complex forms through two hydrogen bonds between each crown residue (involving atoms O3, O5) and its respective ammonium group (Table 2). The H-bond distances are within the usual range: 3.014(3) and 2.862(2) Å (see Table 2). Crown oxygens lie almost in a plane with mean deviation of 0.2527(14) Å. The nitrogen atom of the each ammonium group is 1.857(2) Å above the O_5 plane. Each RNH_3^+ functionality forms the third H-bond with SCN^-: d(N1A–N2)=2.810(3) Å. Centroid–centroid and interplanar separations between two crown rings are 8.29 and 7.44 Å, respectively (Fig. 7).

2.2.2. Solution studies.

The composition of solid complexes **2** and **3**, which have two crown ether residues per diammonium cation, suggests that several different species could co-exist in solution. Mass spectrometry (ES) confirmed this hypothesis. For complex **2**, three signals were observed in positive mode ES: *m/z* 773.4 (100%) corresponding to $[\mathbf{2}\text{-}ClO_4]^+$, *m/z* 505.1 (11%) corresponding to the 1:1 complex ($[\mathbf{2}\text{-B15C5-}(ClO_4)]^+$), and *m/z* 405.2 (47%) corresponding to the 1:1 complex of monoprotonated

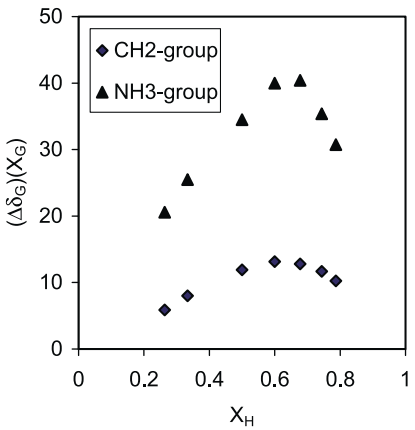


Figure 8. Job's plot for the system containing B15C5 (Host) and *p*-xylylenediammonium salt (Guest) in CD_3CN. The total concentration of the Host (H) and Guest (G) was kept constant at 2×10^{-2} M. The value of $(\Delta\delta_G)(X_G)$, where X_G denotes molar fraction of the guest, was plotted vs. molar fraction of the host (X_H).

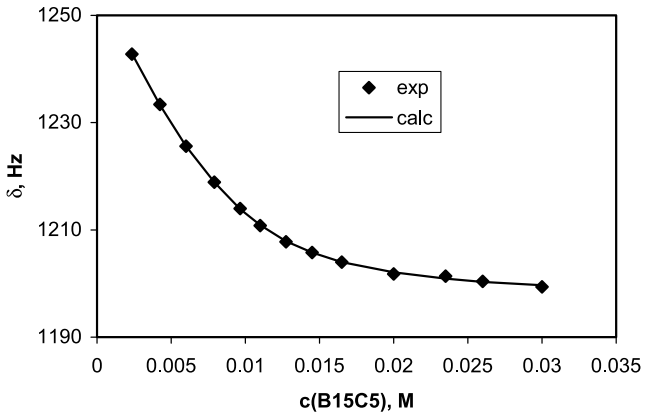


Figure 10. ^{1}H NMR titration curve of *p*-xylylenediammonium perchlorate (0.01 M) with B15C5 in acetonitrile. Experimental points are superimposed with a fit to two-step binding model.

p-xylylenediamine ([**2**-B15C5-H−2(ClO_4^-)]$^+$. Similarly, several signals were found for **3**, including *m*/*z* 357.3 ([**3**-B15C5-H]$^+$), and *m*/*z* 313.7 ([**3**]$^{2+}$).

Like complex **1**, complex **2** exists in fast exchange with its dissociated components in acetonitrile solution on the NMR time scale (Fig. 3S, SI). Upon cooling, the complexation equilibrium is shifted to the right and the rate of exchange between the complex **2** and its components decreases.

Similar behavior was observed for complex **3**, although its low solubility complicated the measurements. Only ca. 20% of the ammonium groups are bound to B15C5 at RT in 0.001 M solution of **3** in CD_3CN. Association is more complete at lower temperatures.

The stoichiometry of the complexation in solution was established by Job's method.[40] Unlike the protonated monoamine, which clearly interacted with just one crown ether molecule (Fig. 3), the protonated diamines bind more than one crown ether residue in solution (Fig. 8). The Job's plot has a broad maximum at ca. 60 mol. % of B15C5, demonstrating the formation of two complexes with [host]/[guest] ratios of 1:1 and 1:2 in acetonitrile solution.

Titration of the diammonium salts with B15C5 at 298 K (Fig. 9) was performed in order to estimate the binding constants for 1:1 and 1:2 complexation. The observed chemical shifts (δ_{obs}) are averages of the chemicals shifts for free diammonium cation and both complexed forms. The ^{1}H NMR chemical shift of the substrate's methylene group ($R\text{-}CH_2NH_3^+$) was monitored during titration and the data were treated using the WinEQNMR program,[41] assuming two-step association scheme:

$$B15C5 + H_3\overset{+}{N}\text{–R–}\overset{+}{N}H_3 \overset{K_1}{\rightleftharpoons} [B15C5]H_3\overset{+}{N}\text{–R–}\overset{+}{N}H_3$$

$$B15C5 + [B15C5]H_3\overset{+}{N}\text{–R–}\overset{+}{N}H_3 \overset{K_2}{\rightleftharpoons}$$

$$[B15C5]H_3\overset{+}{N}\text{–R–}\overset{+}{N}H_3[B15C5]$$

The experimental titration data fit this two-step model very well (Fig. 10, Fig. 4S, 5S, SI), enabling us to calculate the stepwise equilibrium constants, K_1 and K_2 (Table 3), and to estimate the distribution of complex species in solution (Fig. 6S, 7S, SI).

The following values of K_1 and K_2 obtained: 1100±100 and 400±30 M^{-1} for **2**, and 1100±100 and 300±30 M^{-1} for **3** (Table 3). The 1:2 complexes dominate in solution when the B15C5 concentration exceeded of 9.0×10^{-3} M for *p*-xylylenediammonium (0.01 M) and 5.0×10^{-3} M for tetramethylenediammonium (0.0012 M) (Fig. 6S, 7S, SI).

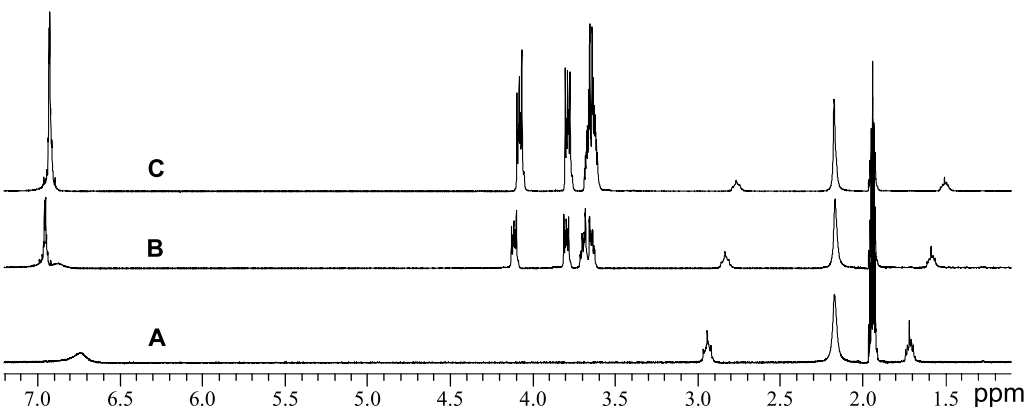


Figure 9. ^{1}H NMR spectra of acetonitrile solutions of tetramethylene diammonium thiocyanate (0.0012 M) (A); with 2 (B); and 6 (C) equiv. B15C5.

The studies described above demonstrate that each ammonium group of the alkyldiammonium ion binds one molecule of B15C5 with an affinity similar to that observed in the $PhCH_2NH_3^+$–B15C5 system. The overall stoichiometry of diammonium–crown interaction, however, is 1:2, which can be clearly seen from the Job's plot shown in Figure 8. This is consistent with the exclusive formation of 1:1 complexes between the protonated monoamine ($PhCH_2NH_3^+$) and B15C5 (Fig. 3), and the lack of the sandwich complex formation in that case. The stepwise equilibrium constants K_1 and K_2 also support a model where the two 'ends' of the alkyldiammonium cations behave independently, with each 'end' binding only one crown ether molecule. The ca. 2–3 fold decrease in K_2 as compared to K_1 is attributed to a statistical factor (the decreased probability of RNH_3^+–crown 'collision').

The length of the linker in $^+H_3N–R–NH_3^+$ (tetramethylene vs. *p*-xylylene) did not influence the association constants for host–guest complexation with B15C5. This result is consistent with data previously reported for the complexation of different alkyldiammonium cations with 18-crown-6.[23]

Very different behavior was exhibited by the ditopic molecular tweezer NiL (Scheme 1, Table 3) in its interaction with diammonium cations: (1) only 1:1 complexes were formed, suggesting an 'end capped', or inclusion, binding mode; (2) binding affinity depended on the length of the linker (length selectivity for inclusion complexation).[12] Since B15C5 alone displays no selectivity with respect to diammonium cation binding, the selectivity of the ditopic host NiL toward $[(CH_2)_2(NH_3)_2]_2^{2+}$ is caused by preorganization of the two receptor fragments attached to a saddle-shaped macrocyclic scaffold. X-ray structural data show that the crown-crown centroid–centroid separation in **3** is about 2.5 Å shorter than the same parameter in **2** (8.29 Å vs. 10.74 Å). Therefore, the best conformation of NiL for $[(CH_2)_2(NH_3)_2]_2^{2+}$ binding has two B15C5 residues separated by ca. 8 Å.

3. Conclusions

X-ray analysis as well as solution studies demonstrate that both mono- and diammonium cations are bound to B15C5 in the same binding mode with a 1:1 ratio of crown residues to R-NH_3^+-groups. Each RNH_3^+ group forms two hydrogen bonds with oxygen atoms of B15C5. No additional significant interactions were identified in host–guest cations **1**–**3**. Nevertheless, the RNH_3^+–B15C5 separation found for all complexes **1**, **2**, and **3** is rather small and falls in the narrow range of 1.8–1.9 Å. It strongly suggests that such small distance is typical in unconstrained 1:1 adducts between RNH_3 and 15-crown-5 derivatives. The absence of sandwich structures, that are common for NH_4^+ complexes with 15C5, suggests that size of the crown ether cavity is not the only factor responsible for the stoichiometry of host–guest complexation. The binding of the second B15C5 moiety to the RNH_3^+ group is prevented by insufficient number of remaining hydrogen atoms, and by steric effects of an alkyl substituent that blocks access to the second crown.

Association constants revealed similar binding affinity for mono- and diammonium cations. The length of the linker in $^+H_3N–R–NH_3^+$ (*p*-xylylenediammonium vs. tetramethylenediammonium) did not affect strength of binding with simple B15C5, in contract to a 30-fold increase in K_{assoc} when ditopic host with two B15C5-functionalities (NiL) binds the shorter diammonium guest ($^+NH_3(CH_2)_4NH_3^+$). Using X-ray data for complexes **2** and **3** we indirectly estimated the ideal interreceptor distance in NiL as ca. 8 Å.

As demonstrated for complex **1**, the complexation between $PhCH_2NH_3^+$ and B15C5 in acetonitrile is enthalpically driven. The enthalpic effect (ca. 5 kcal/mol) is reasonably large, considering that only two hydrogen bonds are responsible for adduct formation.

4. Experimental

4.1. General

Chemicals (reagent grade) and solvents were purchased from Aldrich or Acros and used as received.

Benzo-15-crown-5 was synthesized from catechol and dichlorotetraethylene glycol according to the method described by Izatt et al.[6] The mono- and diammonium guests were prepared as their perchlorate or thiocyanate salts after treatment of the corresponding commercial amine with perchloric or thiocyanic acid in ethanol with further recrystallization from ethanol.

^1H NMR (300.35 MHz) spectra were recorded on a Bruker DPX-300 spectrometer and referenced to the residual solvent peak (^1H δ (CD_3CN)=1.97 ppm).

Mass-spectra were acquired at Proteomics, Cambridge, MA, 02139 (ESMS-MS) or Agilent Technologies, Palo Alto, CA 94303 (ML-MSD-Trap-XCT). Elemental analyses were performed by QTI (Whitehouse, NY 08888).

4.2. X-ray crystallography

Data were collected using a Bruker SMART CCD (charge coupled device) based diffractometer equipped with an LT-2 low-temperature apparatus operating at 173 K (for 1 and 2) and at 298 K for 3. A suitable crystal was chosen and mounted on a glass fiber using Paratone-N oil for the low temperature data collection or epoxy for room temperature data collection. Data were measured using phi and omega scans of 0.3° per frame for 30 s, such that a hemisphere was collected. A total of 1650 frames were collected with a maximum resolution of 0.75 Å. The first 50 frames were recollected at the end of data collection to monitor for decay. Cell parameters were retrieved using SMART[49] software and refined using SAINT,[50] on all observed reflections. Data reduction was performed using the SAINT software,[50] which corrects for Lp. Absorption corrections were applied using SADABS[51] supplied by George Sheldrick. The structures were solved by direct method using the SHELXS-97[52] program and refined by least squares method on F^2 using SHELXL-97[53] incorporated in SHELXTL V6.10.[54] All structures were solved in

the space group P$\bar{1}$ (#2) by analysis of systematic absences. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms (except for those bound to nitrogen atoms) were calculated by geometrical methods and refined according to a riding model.

For complexes **2** and **3** the amine salt molecule lies on an inversion center. The perchlorate anion in **2** was found to be disordered between two orientations; their partial occupancies were refined as 66.5 and 33.5%.

4.3. Equilibrium constant determination

The binding of protonated alkylamines with B15C5 was studied by ^{1}H NMR titration of a guest with the crown in CD_3CN.[12,55] In all cases, concentrations of the reagents were confirmed by integration of appropriate signals.

The stoichiometry of complex formation was determined by Job's method of continuous variations.[40]

Binding constants for 1:1 and 1:2 reversible complexation were calculated from the ^{1}H NMR chemical shift data using the WinEQNMR-program.[41] The binding constant for 1:1 complex between benzylammonium and B15C5 was also calculated by non-linear least-squares fit of the titration curve using MS Excel.[56]

4.3.1. Synthesis of [$PhCH_2NH_3$(B15C5)](ClO_4) (1). Benzylammonium perchlorate (104 mg, 50 mmol) was dissolved in 2 mL of warm methanol and slowly added to 1 mL of a warm solution of crown ether in methanol (134 mg, 50 mmol). The combined solution was filtered and cooled down to 0 °C. Crystallization occurred after 1 day giving 100 mg (21 mmol) of complex **1**. Complex **1** was recrystallized from MeOH before use in further studies. *m/z* (ESMS): 376.3 ([**1**-ClO_4^-]$^+$), 851.2 (2×**1**-ClO_4^-)$^+$. Anal. calcd for $C_{21}H_{30}O_9NCl$: C, 52.98; H, 6.35; N, 2.94; found: C, 52.65; H, 6.20; N, 2.84%. ^{1}H NMR (300 MHz, CH_3CN-d_3) δ: 3.65–3.71 (m, 8H); 3.76–3.79 (m, 4H); 4.00 (s, 2H); 4.07–4.10 (m, 4H); 6.96 (br, 4H); 7.07 (br s, 3H); 7.27–7.35 (m, 2H); 7.36–7.38 (m, 3H). ^{13}C NMR (CH_3CN-d_3) δ: 44.5, 68.90, 68.94, 69.1, 70.0, 115.5, 123.1, 129.9, 130.0, 130.1, 146.7. FT-IR (KBr) ν: 3455, 3200, 3060, 2940, 2880, 1630, 1500, 1451, 1260, 1200, 1107, 930, 850, 748, 557.

The 1:1 complex formed over a wide range of reactant ratios, as evidenced by C, H, N analysis. Very large excess of the crown ether (7-fold and higher) prevented crystallization of any product.

4.3.2. Synthesis of [*p*-$C_6H_4(CH_2NH_3)_2$(B15C5)$_2$](ClO_4)$_2$ (2). *p*-Xylylenediammonium perchlorate (84 mg, 25 mmol) and benzo-15-crown-5 (134 mg, 50 mmol) were separately dissolved in warm methanol (in 3 and 1 mL, respectively) and filtered. Upon slow addition of the amine salt solution to the solution of crown ether, rapid precipitation occurred giving 180 mg (20 mmol) of needle-like crystals. *m/z* (ESMS): 773.4 ([**2**-ClO_4^-]$^+$), 505.1 ([**2**-B15C5-ClO_4^-]$^+$), 405.2 ([**2**-B15C5-H-2ClO_4^-]$^+$). Anal. calcd for $C_{36}H_{54}N_2O_{18}Cl_2$: C, 49.49; H, 6.23; N, 3.20; found: C, 49.43; H, 6.05; N, 3.08. ^{1}H NMR (300 MHz, CH_3CN-d_3) δ: 3.63–3.69 (m, 16H); 3.76–3.79 (m, 8H); 4.03 (s, 4H); 4.07–4.10 (m, 8H); 6.95 (s, 8H); 7.10 (br s, 6H); 7.28 (s, 4H). ^{13}C NMR (CH_3CN-d_3) δ: 44.3, 69.30, 69.33, 69.5, 70.3, 115.8, 123.4, 130.8, 134.9, 149.4. FT-IR (KBr) ν: 3450, 3244, 3170, 3054, 2947, 2925, 2880, 2603, 1629, 1610, 1510, 1451, 1265, 1215, 1107, 1049, 940, 862, 754, 624, 547, 468. The only complex with overall stoichiometry (B15C5)/(diammonium)=2:1 was isolated over wide range of reactant ratios, as evidenced by C, H, N analysis. Large excess of crown ether suppressed crystallization of any product.

4.3.3. Synthesis of [$(CH_2)_4(NH_3)_2$(B15C5)$_2$]($SCN)_2$ (3). Tetramethylenediammonium thiocyanate (52 mg, 25 mmol) was dissolved in 2 mL of warm ethanol and slowly added to 1 mL of a warm ethanolic solution of the crown ether (134 mg, 0.5 mmol). The combined warm solution was filtered. Crystallization occurred upon slow cooling of the solution producing 95 mg (13 mmol) of the complex **3**. *m/z* (ESMS): 357.3 ([**3**-B15C5-H]$^+$), 313.7 ([**3**]$^{2+}$). Anal. calcd for $C_{34}H_{54}N_4O_{10}S_2$: C, 54.96; H, 7.33; N, 7.54; found: C, 54.94; H, 7.08; N, 7.54. ^{1}H NMR (300 MHz, CH_3CN-d_3) δ: 1.50–1.56 (m, 4H); 2.74–2.80 (m, 4H); 3.64–3.71 (m, 16H); 3.78–3.81 (m, 8H); 4.12–4.15 (m, 8H); 6.61–7.00 (br m, 14H). ^{13}C NMR (CH_3CN-d_3) δ: 25.3, 40.4, 69.4, 69.5, 69.9, 70.7, 115.5, 123.2, 149.6. FT-IR (KBr) ν: 3464, 3132, 3060, 2936, 2875, 2060, 1600, 1511, 1455, 1260, 1130, 1080, 1042, 942, 855, 768. The only complex with overall stoichiometry (B15C5)/(diammonium)=2:1 was isolated over wide range of reactant ratios, as evidenced by C, H, N analysis. Large excess of crown ether suppressed crystallization of any product.

5. Supplementary data

Table of average dihedral angles for complexes **1**, **2**, and **3** (Table 1S); ^{1}HNMR spectra (Fig. 2S, 3S), titration curves (Fig. 4S, 5S), plots of distribution of complexed/uncomplexed species in the acetonitrile solution upon titration (Figs. 6S, 7S). Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 229983-229985. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax:+44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

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Preparation of unsymmetrical terphenyls via the nickel-catalyzed cross-coupling of alkyl biphenylsulfonates with aryl Grignard reagents

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Abstract—Unsymmetrical terphenyl derivatives were prepared by sequential transition metal-catalyzed cross-coupling reactions of neopentyl bromobenzenesulfonates with arylboronic acids and arylmagnesium bromides in good yields. Biphenylsulfonates undergo nickel-catalyzed coupling reactions more rapidly than the corresponding benzenesulfonates. The stepwise palladium- and nickel-catalyzed reaction of the bromobenzenesulfonates appears to be a promising and conceptually straightforward route for preparing unsymmetrical terphenyls.

1. Introduction

Transition metal-catalyzed cross-coupling reaction is an extremely powerful tool for constructing carbon–carbon bonds.[1] The palladium- and nickel-catalyzed coupling reactions of aryl halides and pseudohalides with arylboronic acids,[2] arylstannanes,[3] arylzincs,[4] and arylmagnesium halides[5] have been extensively examined to produce unsymmetrical biaryls, which are of great interest due to their biological[6] and optical[7] properties. However, the preparation of unsymmetrical terphenyl derivatives has not been well-known.

Terphenyl derivatives are known to exhibit a variety of optical[8] and electrical[9] properties. They are particularly of interest in the area of liquid crystals.[10] Naturally isolated *p*-terphenyl metabolites[11] including terphenyllin,[12] terferol,[13] and terprenin[14] have potent biological activities. The efforts for substituting terphenyl-based architectures for the alkenyl bridge of stilbene-based compounds[15] and the biphenyl nucleus of biologically active compounds[16] have also been reported.

The most familiar synthetic pathway for symmetric terphenyls is the double C–C cross-coupling reaction of the dihalobenzene derivatives with two equivalents of aryl nucleophiles.[17] Among those processes, the Suzuki–Miyaura reaction is the most preferred due to the stability and low toxicity of the boronic acids as well as the mild reaction conditions. Recently, the double coupling reactions of the phenyl diboronic acids with aryl iodides[18] and aryl distannanes with phenyl bromides[19] for the preparation of symmetric terphenyls were also reported.

The traditional approach for unsymmetrical terphenyls requires the halogenation or boration of the biphenyl intermediates generated by the initial cross-coupling reactions of the two aryl moieties. The brominations of the biaryl compounds obtained by the initial coupling reactions of the aryl halides with arylboronic acids produce bromobiaryl intermediates, which undergo the following Suzuki–Miyaura reactions with arylboronic acids to generate the unsymmetrical terphenyls.[20] An alternative synthetic pathway by generating biphenyboronic acids as intermediates was also reported.[21]

More efficient method for introducing two different aryl groups into a benzene ring is the stepwise chemoselective cross-couplings of aryl compounds containing two dissimilar reactive sites. The order of reactivity for the cross-coupling reaction with organoboronic acids is I>Br>OTf≫Cl. Therefore, the sequential cross-coupling reaction of the bromoiodobenzene derivatives[22] and bromophenyl tosylates[23] with two dissimilar arylboronic acids furnished the unsymmetrical terphenyls. The stepwise cross-coupling reactions of chlorophenylboronic acids with aryl triflates and arylboronic acids[24] as well as the sequential reactions of the bromophenylboronic acids with the iodobenzenes and arylboronic acids[15] also produced the desired unsymmetrical terphenyls. Similarly, the stepwise couplings of aryl Grignard reagents with *p*- and *m*-bromochlorobenzenes

Keywords: Unsymmetrical terphenyls; Cross-coupling; Alkyl biphenylsulfonates.
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catalyzed by non-ligated $NiCl_2$ gave unsymmetrical terphenyls.[25]

It is known that alkylthio and alkylsulfonyl groups, bonded directly to arenes or alkenes, can be substituted for nucleophilic aromatic substitution reactions with aryl and alkyl Grignard reagents in the presence of a Ni(0) catalyst.[26] Chlorobenzene is slightly more reactive than methylthiobenzene in the reaction with BuMgBr in the presence of $dpppNiCl_2$.[27] Therefore, the alkylthio groups were demonstrated to serve as less reactive leaving groups compared to halogens in the preparation of unsymmetrical terphenyls. The chemoselective sequential couplings of chlorophenyl alkyl sulfides with two different arylmagnesium halides gave unsymmetrical terphenyls in moderate yields.[28] The sequential coupling reactions of bis(alkylthio)benzenes, in which the selectivity originates from the difference in the steric effects as a result of the two dissimilar alkylthio groups, have also been reported to give terphenyls in low yields.[29] However, these approaches have not been thoroughly explored presumably due to the inefficient yield and the difficulty in obtaining the appropriate substrates.

Recently, it was reported that the reaction of alkyloxysulfonylarenes with arylmagnesium bromides produced unsymmetrical biaryls in the presence of a nickel catalyst.[30] The alkyloxysulfonyl group acts as an excellent leaving group, while it is not reactive towards the typical palladium catalysts. These results suggested that alkyl bromoarenesulfonates could be used as chemoselective precursors for unsymmetrical terphenyls.

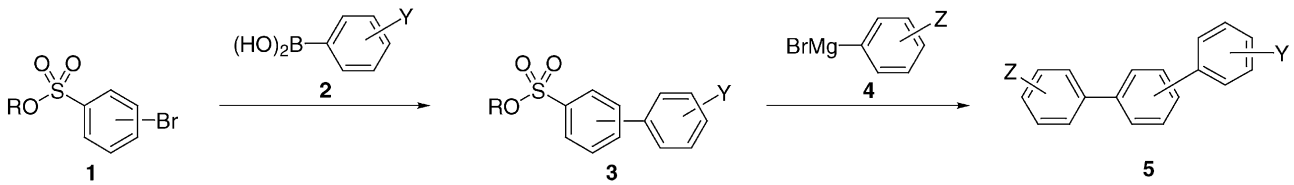
This paper reports our efforts in preparing a variety of unsymmetrical terphenyls via the sequential carbon–carbon cross-coupling reactions of neopentyl bromobenzenesulfonates with arylboronic acids and arylmagnesium bromides (Scheme 1). The results of this study are presented and discussed.

2. Results and discussion

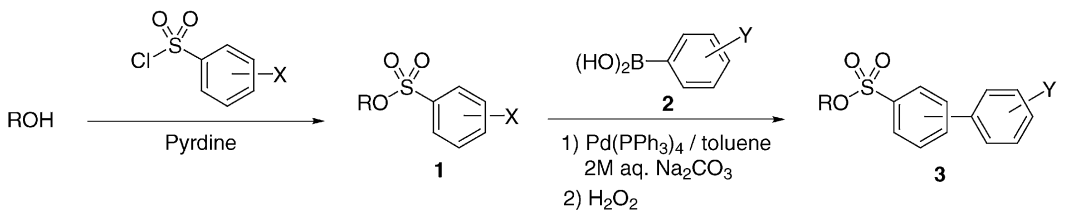
The bromobenzenesulfonates **1** were prepared using a previously reported procedure (Scheme 2).[31] Two types of neopentyl moieties, 2,2-dimethyl-3-phenyl-1-propyl and neopentyl, were selected as alkyl groups in those sulfonates to avoid the competitive substitution and elimination of the arenesulfonate anions in the reactions with the Grignard reagents **4**. All the reactions proceeded well in 68–87% isolated yields. The products **1a**, **1b**, and **1c** were purified by recrystallization from *n*-hexane to give white solids, while **1d** was isolated by column chromatography (Et_2O:*n*-hexane=1:4) as a colorless oil.

Bromobenzenesulfonates **1** underwent a palladium-catalyzed cross-coupling reaction with arylboronic acids **2** smoothly in the presence of sodium carbonate. Since the preparation of biaryls via the reactions of the arylboronic acids with the aryl halides was first reported,[32] the Suzuki–Miyaura reaction has been used successfully to produce biaryl derivatives from a variety of aryl halides and triflates. However, the reaction of the aryl electrophiles containing sulfur-substituents has not been examined intensively.[33] Although a detailed study to optimize the reaction conditions has not been undertaken in this effort, most of the reactions were complete within 6 h at the refluxing temperature of toluene. The displacement of arenesulfonates[34] was not observed under the standard reaction conditions.

The result of the cross-coupling reactions between **1** and **2** is summarized in Table 1. 2,2-Dimethyl-3-phenyl-1-propyl 4-bromobenzenesulfonate (**1a**) reacted rapidly with phenyl- (**2a**), 4-tolyl- (**2b**), and 4-methoxyphenylboronic acid (**2c**) in the presence of $Pd(PPh_3)_4$ to give the corresponding biphenylsulfonates **3a**–**3c** in good yields (entries 1–3). Neopentyl 4-bromobenzenesulfonate (**1b**) showed a similar reactivity with **1a** toward **2b** (entry 4), which shows that the remote alkyl moiety does not influence the reaction.



Scheme 1.

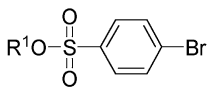
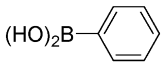
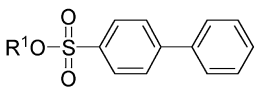
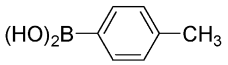
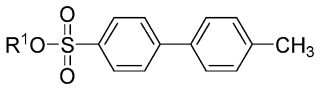
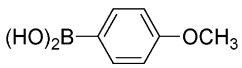
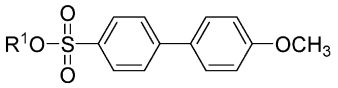
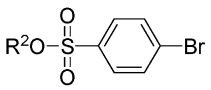
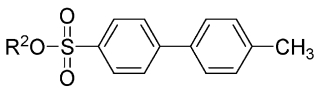
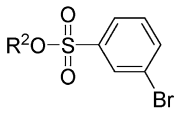
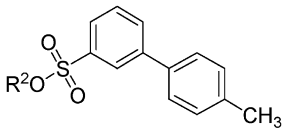
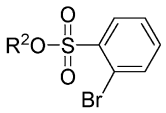
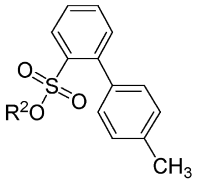
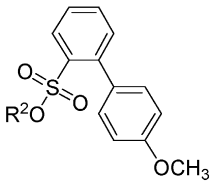


1a: R = 2,2-dimethyl-3-phenyl-1-propyl, X = 4-Br
1b: R = neopentyl, X = 4-Br
1c: R = neopentyl, X = 3-Br
1d: R = neopentyl, X = 2-Br

2a: Y = H
2b: Y = 4-Me
2c: Y = 4-MeO

Scheme 2.

Table 1. Coupling reaction of bromobenzenesulfonates **1** with arylboronic acids **2**

Entry	Bromobenzenesulfonate	Arylboronic acid	Time (h)	Biphenylsulfonate[a]	Yield (%)[b]
1	 **1a**	 **2a**	6	 **3a**	71
2	**1a**	 **2b**	6	 **3b**	73
3	**1a**	 **2c**	6	 **3c**	74
4	 **1b**	**2b**	6	 **3d**	71
5	 **1c**	**2b**	15	 **3e**	67
6	 **1d**	**2b**	6	 **3f**	72
7	**1d**	**2c**	6	 **3g**	69

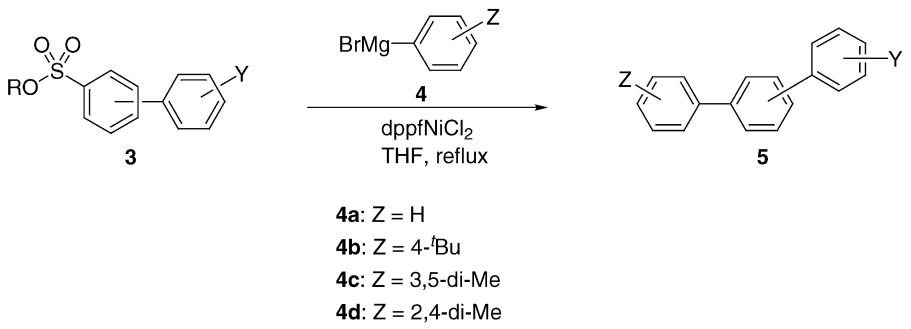
[a] R^1=2,2-dimethyl-3-phenyl-1-propyl, R^2=neopentyl.
[b] Isolated yields based on **1**.

The reaction of 3-bromobenzenesulfonate (**1c**) with **2b** also produced the desired coupling product **3e** in a competitive yield, although it required more time (15 h) for a complete reaction (entry 5). 2-Bromobenzenesulfonate (**1d**) underwent the coupling process with **2b** and **2c** in a similar way as with **1b** (entries 6 and 7). There was no evidence showing that the neighboring neopentyloxysulfonyl group provided a noticeable steric hindrance in the coupling reaction. Products **3a**-**3d** were purified by recrystallization, while **3e**-**3g** was isolated by column chromatography.

The cross-coupling reactions of **3** with **4** were performed in the presence of dppfNiCl$_2$ in refluxing THF, which were previously demonstrated to be the most efficient reaction conditions for the reactions of benzenesulfonates (Scheme 3).[30] Most of those processes proceeded in high yields to give the corresponding unsymmetrical terphenyls **5** via the substitution of the neopentyloxysulfonyl groups. The highest yields were generally obtained when 5 equiv. of **4** were added in two portions, 3 equiv. initially and 2 equiv. after 8 h.

The biphenylsulfonates showed a higher reactivity than the benzenesulfonates under the standard reaction conditions. Most of **3** were completely consumed within 16 h, while the benzenesulfonates typically required almost 30 h for the completion. The faster reaction of more conjugated arenesulfonates has been previously observed in the case of naphthalenesulfonates. The π-electrons appear to play an important role by precomplexing with the catalyst prior to oxidative addition.

The results of the cross-coupling reactions between various **3** and **4** are summarized in Table 2. 2,2-Dimethyl-3-phenyl-1-propyl-4-biphenylsulfonate (**3a**) reacted with phenyl- (**4a**), 4-*tert*-butylphenyl- (**4b**), and 3,5-dimethylphenylmagnesium



Scheme 3.

bromide (**4c**) to generate the unsymmetrical *p*-terphenyls **5a**-**5c** respectively in good yields within 16 h (entries 1–3). There was no significant difference in the reactivities of those nucleophiles. However, the sterically hindered **4d** showed a reduced reactivity. Even though more time (50 h) was allowed, the reaction of **3a** with **4d** generated the coupling product **5d** in only a moderate yield (entry 4).

The coupling reaction of **3b** with **4a**, **4b**, and **4c** also efficiently produced the corresponding unsymmetrical terphenyls **5e**-**5g** within 16 h in refluxing THF (entries 5–7). The methyl group on the 4′-position of biphenylsulfonates did not have any significant effect on the reactivity of those reactions. On the other hand, the reactions of **3c** possessing the 4′-methoxy group with **4a** and **4b** resulted in slightly lower yields, although the products produced by the cleavage of the C–O bond were not detected (entries 8 and 9).[35] The reaction of **3c** with **4d** proceeded slowly, as expected, and gave the reduced yield (entry 10).

The alkyl moiety of the biphenylsulfonates had a significant influence in the progress of the reactions. Neopentyl 4′-methyl-4-biphenylsulfonate (**3d**) required only 3 h for the complete reaction with 3 equiv. of **4a** (entry 11) while **3b** required 16 h for the reaction with 5 equiv. of **4a** (entry 5). This result was quite unexpected considering that both alkyl groups were bulky neopentyl groups. The higher reactivity of the neopentyl compounds compared to the 2,2-dimethyl-3-phenyl-1-propyl substrates was constantly observed in the reactions of **3e**-**3g**.

The bulkiness of the biphenyl moieties also had a great effect on the reactivity of biphenylsulfonates **3**. The reaction of 3-biphenylsulfonate (**3e**) and 2-biphenylsulfonate (**3f**) required 8 and 15 h, respectively for the complete reaction with only 3 equiv. of **4a** (entries 12 and 13). The *ortho*-phenyl group to the alkyloxysulfonyl substituent appears to significantly hinder the approach of the Ni catalyst to C–S bonds. The 4′-methoxy-2-biphenylsulfonate (**3g**), which even contains a slightly deactivating methoxy group, required 16 h for the complete reaction with 5 equiv. of **4b** to produce **5m**, and showed a reduced yield (entry 14). The reaction of **3g** with the sterically hindered **4d** was exceptionally slow in forming the *o*-terphenyl (**5n**) in a lower yield.

Even though no significant levels of byproducts originating from the biphenylsulfonates **3** were observed in this study, the large amount of biphenyls derived by the dimerization of **4** made the purification of **5** by column chromatography difficult in most cases. However, the products **5a**-**5j** could be easily purified by recrystallization from methanol to give white solids.

3. Conclusion

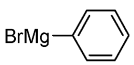
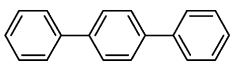
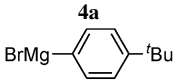
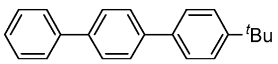
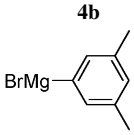
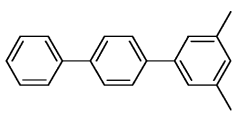
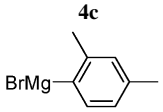
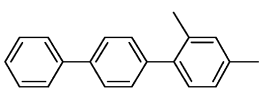
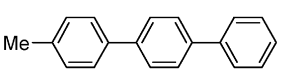
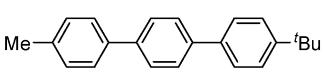
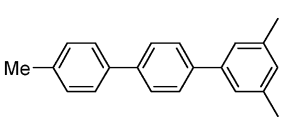
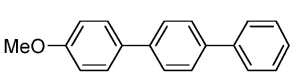
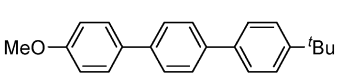
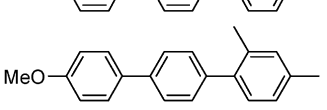
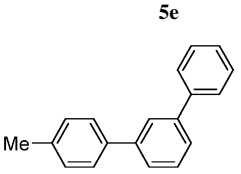
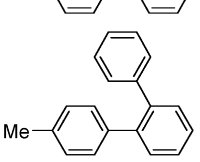
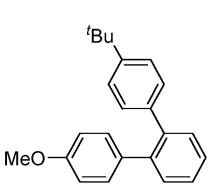
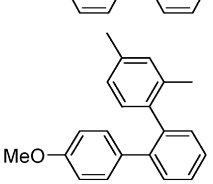
The Suzuki–Miyaura reaction followed by a nickel-catalyzed cross-coupling reaction allows the introduction of two different aryl groups into the benzene nucleus by the sequential substitution of the bromo and neopentyloxysulfonyl groups of the neopentyl bromobenzenesulfonates. The coupling reaction of biphenylsulfonates with the arylmagnesium bromides proceeds faster than the corresponding benzenesulfonates. Neopentyl biphenylsulfonates undergo nickel-catalyzed reactions much faster than the 2,2-dimethyl-3-phenyl-1-propyl biphenylsulfonates. 2-Biphenylsulfonate showed a lower reactivity than the 3- and 4-biphenylsulfonates.

The procedure described in this paper appears to be a promising and conceptually straightforward route to the unsymmetrical terphenyls. The application of this coupling strategy in the solid-phase organic synthesis (SPOS) is also in progress and will be reported in due course.

4. Experimental

All reactions were carried out under an inert atmosphere of Ar. Solvents were distilled from an appropriate drying agent prior to use: toluene from calcium hydride and THF from sodium-benzophenone ketyl. Pyridine was dried over CaH_2 and distilled. ^1H NMR (300 or 500 MHz) and ^{13}C NMR (75 or 125 MHz) were registered in $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. Chemical shifts are reported in δ units (ppm) by assigning TMS resonance in the ^1H spectrum as 0.00 ppm and $CDCl_3$ resonance in the ^{13}C spectrum as 77.2 ppm. All coupling constants, *J*, are reported in hertz (Hz). Analytical and preparative HPLC was performed with an instrument equipped with a UV detector set at 254 nm. Octadecylsilane coated columns, 4.6×250 mm or 20×250 mm, with 5 or 10 μm particle size were used for analytical or preparative runs, respectively. A flow rate of 5 mL/min was used. GC analysis was performed on a bonded 5% phenylpolysiloxane BPX 5 capillary column (SGE, 30 m, 0.32 mm i.d.).

Table 2. Nickel-catalyzed coupling of biphenylsulfonates **3** with aryl Grignard reagents **4**

Entry	Biphenylsulfonate	Grignard reagent	Time (h)	Product	Yield (%)[a]
1	**3a**	 **4a**	16	 **5a**	84
2	**3a**	 **4b**	16	 **5b**	82
3	**3a**	 **4c**	16	 **5c**	80
4	**3a**	 **4d**	50	 **5d**	54
5	**3b**	**4a**	16	 **5e**	81
6	**3b**	**4b**	16	 **5f**	76
7	**3b**	**4c**	16	 **5g**	75
8	**3c**	**4a**	16	 **5h**	73
9	**3c**	**4b**	16	 **5i**	75
10	**3c**	**4d**	50	 **5j**	51
11	**3d**	**4a**[b]	3	**5e**	83
12	**3e**	**4a**[b]	8	 **5k**	75
13	**3f**	**4a**[b]	15	 **5l**	66
14	**3g**	**4b**	16	 **5m**	69
15	**3g**	**4d**	72	 **5n**	38

[a] Isolated yields based on **3**.
[b] 3 equiv. only.

Column chromatography was performed on silica gel 60, 70–230 mesh. Analytical thin-layer chromatography (TLC) was performed using Merck Kieselgel 60 F_{254} precoated plates (0.25 mm) with a fluorescent indicator and visualized with UV light (254 and 365 nm) or by iodine vapor staining. Electron impact (EI, 70 eV) was used as the ionization method for the mass spectrometry. Mass data are reported in mass units (*m*/*z*). Melting points were obtained using a Barnstead/Thermolyne MEL-TEMP apparatus and are uncorrected. 4-Tolyl- (**2b**), 4-methoxyphenylboronic acid (**2c**) were prepared according to a literature procedure.[36] 3,5-Dimethylphenyl- (**4c**, 0.5 M, THF) and 2,4-dimethylphenylmagnesium bromide (**4d**, 0.5 M, THF) were prepared by reacting magnesium turnings with the appropriate organic halides in THF. $DppfNiCl_2$ was prepared according to a literature procedure.[37] [mp 282–283 °C (lit. mp 283–284 °C)]. Phenylboronic acid **2a** and phenyl- (**4a**, 1.0 M, THF) and 4-*tert*-butylphenylmagnesium bromide (**4b**, 2.0 M, Et_2O) were purchased and used as received.

4.1. General procedure for the preparation of neopentyl bromobenzenesulfonates 1

To the alcohol (51.7 mmol) in chloroform (50 mL) at 0 °C, was added pyridine (103 mmol) dropwise over a period of 20 min and bromobenzenesulfonyl chloride (47.0 mmol) in small portions. This reaction mixture was stirred at room temperature for 12 h and diluted with Et_2O and then 0.1% aqueous HCl. The separated organic layer was washed with 0.1% aq. HCl (2×30 mL), water (3×50 mL), and a brine; dried over $MgSO_4$; and concentrated in vacuo. The crude bromobenzenesulfonates **1** were purified by either recrystallization or column chromatography.

4.1.1. 2,2-Dimethyl-3-phenyl-1-propyl 4-bromobenzenesulfonate (1a). The title compound was prepared by the reaction of 2,2-dimethyl-3-phenyl-1-propanol (8.49 g, 51.7 mmol) with *p*-bromobenzenesulfonyl chloride (12.0 g, 47.0 mmol). The crude compound was purified by recrystallization from *n*-hexane to give **1a** (15.7 g, 87%) as a white solid: TLC R_f 0.38 (Et_2O:*n*-hexane=1:4); mp 82–83 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.88 (s, 6H), 2.55 (s, 2H), 3.68 (s, 2H), 6.99–7.03 (m, 2H), 7.19–7.22 (m, 3H), 7.71 (d, *J*=8.7 Hz, 2H), 7.79 (d, *J*=8.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.1 (×2), 35.4, 44.3, 77.7, 126.6, 128.2 (×2), 129.2, 129.7 (×2), 130.6 (×2), 132.9 (×2), 135.3, 137.5; HRMS (EI, 70 eV) Calcd for $C_{17}H_{19}BrO_3S$ (M^+): 382.0238. Found: 382.0231. Anal. Calcd for $C_{17}H_{19}BrO_3S$: C, 53.27; H, 5.00. Found: C, 53.26; H, 4.94.

4.1.2. Neopentyl 4-bromobenzenesulfonate (1b). The title compound was prepared by the reaction of neopentyl alcohol (0.45 g, 5.11 mmol) with *p*-bromobenzenesulfonyl chloride (1.19 g, 4.65 mmol). The crude compound was purified by recrystallization from *n*-hexane to give **1b** (0.47 g, 78%) as a white needle solid: TLC R_f 0.48 (Et_2O:*n*-hexane=1:1); mp 71 °C (lit.[38] mp 70–71 °C); ^1H NMR (300 MHz, $CDCl_3$) δ 0.91 (s, 9H), 3.70 (s, 2H), 7.74 (dd, *J*=8.9, 8.9 Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.1 (×3), 31.8, 80.1, 129.1, 129.6 (×2), 132.8 (×2), 135.4; HRMS (EI, 70 eV) Calcd for $C_{11}H_{15}BrO_3S$ (M^+): 305.9925. Found: 305.9936. Anal. Calcd for $C_{11}H_{15}BrO_3S$: C, 43.01; H, 4.92. Found: C, 42.95; H, 4.89.

4.1.3. Neopentyl 3-bromobenzenesulfonate (1c). The title compound was prepared by the reaction of neopentyl alcohol (0.36 g, 4.09 mmol) with *m*-bromobenzenesulfonyl chloride (0.95 g, 3.72 mmol). The crude compound was purified by recrystallization from *n*-hexane to give **1c** (0.93 g, 81%) as a white solid: TLC R_f 0.51 (Et_2O:*n*-hexane=1:1); mp 44–45 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.92 (s, 9H), 3.72 (s, 2H), 7.45 (dd, *J*=8.1, 7.9 Hz, 1H), 7.79 (ddd, *J*=8.1, 2.0, 1.0 Hz, 1H), 7.85 (ddd, *J*=7.9, 1.7, 1.0 Hz, 1H), 8.06 (dd, *J*=2.0, 1.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.2 (×3), 31.9, 80.3, 123.3, 126.5, 130.9, 130.9, 136.9, 138.1; HRMS (EI, 70 eV) Calcd for $C_{11}H_{15}BrO_3S$ (M^+): 305.9925. Found: 305.9969.

4.1.4. Neopentyl 2-bromobenzenesulfonate (1d). The title compound was prepared by the reaction of neopentyl alcohol (0.57 g, 6.46 mmol) with *o*-bromobenzenesulfonyl chloride (1.5 g, 5.87 mmol). The crude compound was purified by column chromatography (Et_2O:*n*-hexane=1:4) to afford **1d** (1.23 g, 68%) as a viscous colorless oil: TLC R_f 0.56 (Et_2O:*n*-hexane=1:1); ^1H NMR (300 MHz, $CDCl_3$) δ 0.96 (s, 9H), 3.73 (s, 2H), 7.48–7.55 (m, 2H), 7.77–7.82 (m, 1H), 8.09–8.14 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.0 (×3), 31.5, 80.4, 120.6, 127.8, 132.0, 132.2, 134.8, 135.7; HRMS (EI, 70 eV) Calcd for $C_{11}H_{15}BrO_3S$ (M^+): 305.9925. Found: 305.9948.

4.2. General procedure for the preparation of biphenylsulfonates 3

To a solution of **1** (5.22 mmol) and $Pd(PPh_3)_4$ (0.157 mmol, 0.181 g) in toluene (12 mL) was added 2.0 M aqueous Na_2CO_3 (6.0 mL) under an Ar atmosphere. To the resulting mixture was added **2** (5.74 mmol), which was dissolved in ethanol (3 mL). The reaction mixture was heated at reflux for 6 h (15 h for **1c**) with vigorous stirring. Upon cooling to room temperature, 30% hydrogen peroxide (0.3 mL) was added to oxidize the residual boronic acid. The mixture was stirred at room temperature for ca. 1 h and diluted with EtOAc. The organic layer was washed with water and brine; dried over $MgSO_4$; filtered through a small pad of silica gel in a sintered glass filter; and concentrated in vacuo. The biphenylsulfonates **3** were purified by recrystallization and/or column chromatography.

4.2.1. 2,2-Dimethyl-3-phenyl-1-propyl 4-biphenylsulfonate (3a). The title compound was prepared by the reaction of **1a** (2.0 g, 5.2 mmol) with **2a** (0.7 g, 5.7 mmol) in the presence of $Pd(PPh_3)_4$ (184.9 mg, 0.16 mmol) and 2 M aq. Na_2CO_3 (6.0 mL) by using toluene (12.0 mL) as solvent. The crude product was purified by recrystallization from *n*-hexane to give **3a** (1.41 g, 71%) as a white solid: TLC R_f 0.43 (Et_2O:*n*-hexane=1:4); mp 74–75 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.89 (s, 6H), 2.57 (s, 2H), 3.72 (s, 2H), 7.00–7.05 (m, 2H), 7.15–7.21 (m, 3H), 7.42–7.53 (m, 3H), 7.63 (d, *J*=6.7 Hz, 2H), 7.76 (d, *J*=8.7 Hz, 2H), 7.99 (d, *J*=8.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.1 (×2), 35.4, 44.3, 77.4, 126.5, 127.6 (×2), 128.1 (×2), 128.2 (×2), 128.7 (×2), 129.0, 129.4 (×2), 130.7 (×2), 134.7, 137.6, 139.3, 147.0; HRMS (EI, 70 eV) Calcd for $C_{23}H_{24}O_3S$

(M^+): 380.1446. Found: 380.1403. Anal. Calcd for $C_{23}H_{24}O_3S$: C, 72.60; H, 6.36. Found: C, 72.67; H, 6.37.

4.2.2. 2,2-Dimethyl-3-phenyl-1-propyl 4′-methyl-4-biphenylsulfonate (3b). The title compound was prepared by the reaction of **1a** (2.0 g, 5.2 mmol) with **2b** (0.78 g, 5.7 mmol) in the presence of $Pd(PPh_3)_4$ (184.9 mg, 0.16 mmol) and 2 M aq. Na_2CO_3 (6.0 mL) by using toluene (12.0 mL) as solvent. The crude product was purified by recrystallization from *n*-hexane to give **3b** (1.50 g, 73%) as a fluffy white solid: TLC R_f 0.45 (Et_2O:*n*-hexane=1:4); mp 89–91 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.89 (s, 6H), 2.42 (s, 3H), 2.56 (s, 2H), 3.71 (s, 2H), 7.00–7.03 (m, 2H), 7.15–7.19 (m, 3H), 7.31 (d, J=8.1 Hz, 2H), 7.53 (d, J=8.1 Hz, 2H), 7.75 (d, J=8.4 Hz, 2H), 7.97 (d, J=8.4 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.3, 24.1 (×2), 35.4, 44.4, 77.4, 126.5, 127.5 (×2), 127.8 (×2), 128.2 (×2), 128.7 (×2), 130.1 (×2), 130.7(×2), 134.4, 136.4, 137.7, 139.1, 146.9; HRMS (EI, 70 eV) Calcd for $C_{24}H_{26}O_3S$ (M^+): 394.1603. Found: 394.1567. Anal. Calcd for $C_{24}H_{26}O_3S$: C, 73.06; H, 6.64. Found: C, 73.18; H, 6.65.

4.2.3. 2,2-Dimethyl-3-phenyl-1-propyl 4′-methoxy-4-biphenylsulfonate (3c). The title compound was prepared by the reaction of **1a** (2.0 g, 5.2 mmol) with **2c** (0.87 g, 5.7 mmol) in the presence of $Pd(PPh_3)_4$ (184.9 mg, 0.16 mmol) and 2 M aq. Na_2CO_3 (6.0 mL) by using toluene (12.0 mL) as solvent. The crude product was purified by recrystallization from *n*-hexane to give **3c** (1.58 g, 74%) as a white solid: TLC R_f 0.33 (Et_2O:*n*-hexane=1:4); mp 84–85 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.89 (s, 6H), 2.57 (s, 2H), 3.71 (s, 2H), 3.88 (s, 3H), 7.00–7.05 (m, 2H), 7.03 (d, J=9.1 Hz, 2H), 7.16–7.21 (m, 3H), 7.59 (d, J=9.1 Hz, 2H), 7.73 (d, J=8.7 Hz, 2H), 7.96 (d, J=8.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.1 (×2), 35.4, 44.3, 55.5, 77.3, 114.8 (×2), 126.5, 127.4 (×2), 128.2 (×2), 128.7 (×2), 128.8 (×2), 130.7(×2), 131.6, 134.0, 137.6, 146.5, 160.6; HRMS (EI, 70 eV) Calcd for $C_{24}H_{26}O_4S$ (M^+): 410.1552. Found: 410.1597. Anal. Calcd for $C_{24}H_{26}O_4S$: C, 70.22; H, 6.38. Found: C, 70.25; H, 6.33.

4.2.4. Neopentyl 4′-methyl-4-biphenylsulfonate (3d). The title compound was prepared by the reaction of **1b** (2.0 g, 6.5 mmol) with **2b** (0.98 g, 7.2 mmol) in the presence of $Pd(PPh_3)_4$ (231.1 mg, 0.2 mmol) and 2 M aq. Na_2CO_3 (7.0 mL) by using toluene (14.0 mL) as solvent. The crude product was purified by recrystallization from *n*-hexane to give **3d** (1.47 g, 71%) as a white solid: TLC R_f 0.36 (Et_2O:*n*-hexane=1:4); mp 116–118 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.92 (s, 6H), 2.42 (s, 3H), 3.72 (s, 2H), 7.31 (d, J=7.7 Hz, 2H), 7.52 (d, J=8.2 Hz, 2H), 7.74 (d, J=8.9 Hz, 2H), 7.95 (d, J=8.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2, 26.1 (×3), 31.8, 79.8, 127.4 (×2), 127.7 (×2), 128.7 (×2), 130.1 (×2), 134.5, 136.4, 139.1, 146.8; HRMS (EI, 70 eV) Calcd for $C_{18}H_{22}O_3S$ (M^+): 318.1290. Found: 318.1280.

4.2.5. Neopentyl 4′-methyl-3-biphenylsulfonate (3e). The title compound was prepared by the reaction of **1c** (0.4 g, 1.3 mmol) with **2b** (0.19 g, 1.4 mmol) in the presence of $Pd(PPh_3)_4$, (46.22 mg, 0.04 mmol) and 2 M aq. Na_2CO_3 (1.5 mL) by using toluene (10.0 mL) as solvent. The crude product was purified by column chromatography (Et_2O:*n*-hexane=1:4) to give **3e** (0.28 g, 67%) as a white solid: TLC R_f 0.45 (Et_2O:*n*-hexane=1:4); mp 89–90 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.92 (s, 9H), 2.42 (s, 3H), 3.72 (s, 2H), 7.30 (d, J=8.2 Hz, 2H), 7.52 (d, J=8.2 Hz, 2H), 7.61 (t, J=7.7 Hz, 1H), 7.85 (dd, J=7.7, 1.9 Hz, 2H), 8.11 (t, J=1.9 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.4, 26.2 (×3), 31.9, 80.0, 126.2, 126.3, 127.2 (×2), 129.8, 130.0 (×2), 132.1, 136.2, 136.8, 138.6, 142.6; HRMS (EI, 70 eV) Calcd for $C_{18}H_{22}O_3S$ (M^+): 318.1290. Found: 318.1259. Anal. Calcd for $C_{18}H_{22}O_3S$: C, 67.89; H, 6.96. Found: C, 67.75; H, 6.91.

4.2.6. Neopentyl 4′-methyl-2-biphenylsulfonate (3f). The title compound was prepared by the reaction of **1d** (0.49 g, 1.6 mmol) with **2b** (0.25 g, 1.8 mmol) in the presence of $Pd(PPh_3)_4$ (57.78 mg, 0.05 mmol) and 2 M aq. Na_2CO_3 (2.0 mL) by using toluene (10.0 mL) as solvent. The crude product was purified by column chromatography (Et_2O:*n*-hexane=1:4) to give **3f** (0.37 g, 72%) as a colorless oil: TLC R_f 0.47 (Et_2O:*n*-hexane=1:4); ^1H NMR (300 MHz, $CDCl_3$) δ 0.73 (s, 9H), 2.30 (s, 3H), 3.45 (s, 2H), 7.12 (d, J=8.0 Hz, 2H), 7.24 (d, J=8.0 Hz, 2H), 7.28 (dd, J=7.7, 1.3 Hz, 1H), 7.40 (td, J=7.7, 1.3 Hz, 1H), 7.52 (td, J=7.6, 1.2 Hz, 1H), 8.01 (dd, J=7.9, 1.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.3, 26.1 (×3), 31.6, 79.4, 127.7, 128.6, 128.7, 129.5 (×2), 130.1, 133.2 (×2), 135.4, 136.4, 138.1, 142.4; HRMS (EI, 70 eV) Calcd for $C_{18}H_{22}O_3S$ (M^+): 318.1290. Found: 318.1280.

4.2.7. Neopentyl 4′-methoxy-2-biphenylsulfonate (3g). The title compound was prepared by the reaction of **1d** (0.49 g, 1.6 mmol) with **2c** (0.27 g, 1.8 mmol) in the presence of $Pd(PPh_3)_4$ (57.58 mg, 0.05 mmol) and 2 M aq. Na_2CO_3 (2.0 mL) by using toluene (10.0 mL) as solvent. The crude product was purified by column chromatography (Et_2O:*n*-hexane=1:4) to give **3g** (0.37 g, 69%) as a pale yellow oil: TLC R_f 0.28 (Et_2O:*n*-hexane=1:4); ^1H NMR (300 MHz, $CDCl_3$) δ 0.82 (s, 9H), 3.55 (s, 2H), 3.85 (s, 3H), 6.95 (d, J=8.9 Hz, 2H), 7.38 (d, J=8.9 Hz, 2H), 7.36–7.41 (m, 1H), 7.50 (ddd, J=8.1, 7.6, 1.5 Hz, 1H), 7.63 (ddd, J=7.6, 7.6, 1.5 Hz, 1H), 8.11(dd, J=8.1, 1.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.1 (×3), 31.7, 55.5, 79.5, 113.4, 127.6 (×2), 130.0, 130.9(×2), 131.6, 133.3, 133.4, 125.4, 142.2, 159.8; HRMS (EI, 70 eV) Calcd for $C_{18}H_{22}O_4S$ (M^+): 334.1239. Found: 334.1232.

4.3. General procedure for the preparation of unsymmetrical terphenyls 5

To a stirred solution of **3** (0.3 mmol) and $dppfNiCl_2$ (0.015 mmol) in dry THF (6 mL) was slowly added aryl Grignard reagents **4** (0.9 mmol) via syringe at room temperature. This resulted in a color change from dark green to dark brown. The reaction mixture was heated at reflux for ca. 8 h, cooled to room temperature, and an additional 0.6 mmol of **4** was added to the solution. After the resulting mixture was heated at reflux for 8–64 h, cooled to room temperature, and diluted with Et_2O. The organic layer was washed with a 1% aqueous HCl (2×10 mL), water, and brine; dried over $MgSO_4$; and concentrated in vacuo. The product **5a-j** were purified by recrystallization from MeOH to give white solids, and the product **5k-n** were purified by preparative HPLC (CH_3CN:MeOH=2:3).

4.3.1. *p*-Terphenyl (5a). The title compound was prepared by the reaction of **3a** (114.15 mg, 0.30 mmol) with **4a** (1.0 M in THF, 0.9 mL, 0.9 mmol+0.6 mL, 0.6 mmol) in the presence of dppfNiCl$_2$. The crude compound was purified by recrystallization from MeOH to afford **5a** (58.05 mg, 84%) as a white solid: TLC R_f 0.72 (Et$_2$O:*n*-hexane=1:4); mp 211–213 °C [an authentic sample[39] (mp 212–213 °C)]; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.36 (t, J=7.4 Hz, 2H), 7.46 (t, J=7.7 Hz, 4H), 7.64 (d, J=7.2 Hz, 4H), 7.68 (s, 4H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 127.3 (×4), 127.6 (×2), 127.8 (×4), 129.1 (×4), 140.4 (×2), 141.0 (×2).

4.3.2. 4-*tert*-Butyl-*p*-terphenyl (5b). The title compound was prepared by the reaction of **3a** (114.15 mg, 0.30 mmol) with **4b** (2.0 M in THF, 0.45 mL, 0.9 mmol+0.3 mL, 0.6 mmol) in the presence of dppfNiCl$_2$. The crude compound was purified by recrystallization from MeOH to afford **5b** (70.47 mg, 82%) as a white solid: TLC R_f 0.70 (Et$_2$O:*n*-hexane=1:4); mp 180–181 °C; ^{1}H NMR (500 MHz, CDCl$_3$) δ 1.38 (s, 9H), 7.35 (t, J=7.4 Hz, 1H), 7.46 (t, J=7.7 Hz, 2H), 7.49 (d, J=8.3 Hz, 2H), 7.59 (d, J=8.3 Hz, 2H), 7.64 (d, J=7.5 Hz, 2H), 7.67 (s, 4H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 31.5 (×3), 34.7, 126.0 (×2), 127.0 (×2), 127.3 (×2), 127.5, 127.6 (×2), 127.7 (×2), 129.1 (×2), 138.0, 140.1, 140.3, 141.1, 150.7; HRMS (EI, 70 eV) Calcd for $C_{22}H_{22}$ (M^+): 286.1721. Found: 286.1724. Anal. Calcd for $C_{22}H_{22}$: C, 92.26; H, 7.74. Found: C, 92.16; H, 7.69.

4.3.3. 3,5-Dimethyl-*p*-terphenyl (5c). The title compound was prepared by the reaction of **3a** (114.15 mg, 0.30 mmol) with **4c** (0.5 M in THF, 1.8 mL, 0.9 mmol+1.2 mL, 0.6 mmol) in the presence of dppfNiCl$_2$. The crude compound was purified by recrystallization from MeOH to afford **5c** (62.00 mg, 80%) as a white solid: TLC R_f 0.70 (Et$_2$O:*n*-hexane=1:4); mp 88–90 °C; ^{1}H NMR (500 MHz, CDCl$_3$) δ 2.39 (s, 6H), 7.00 (s, 1H), 7.26 (s, 2H), 7.35 (t, J=7.4 Hz, 1H), 7.45 (t, J=7.7 Hz, 2H), 7.64 (d, J=7.7 Hz, 2H), 7.65 (s, 4H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 21.5 (×2), 125.3 (×2), 127.3 (×2), 127.5, 127.7 (×2), 127.8 (×2), 129.1 (×2), 129.3, 138.6 (×2), 140.2, 140.7, 141.0, 141.1; HRMS (EI, 70 eV) Calcd for $C_{20}H_{18}$ (M^+): 258.1409. Found: 258.1403. Anal. Calcd for $C_{20}H_{18}$: C, 92.98; H, 7.02. Found: C, 92.70; H, 7.12.

4.3.4. 2,4-Dimethyl-*p*-terphenyl (5d). The title compound was prepared by the reaction of **3a** (114.15 mg, 0.30 mmol) with **4d** (0.5 M in THF, 1.8 mL, 0.9 mmol+1.2 mL, 0.6 mmol) in the presence of dppfNiCl$_2$. The crude compound was purified by recrystallization from MeOH to afford **5d** (41.85 mg, 54%) as a white solid: TLC R_f 0.68 (Et$_2$O:*n*-hexane=1:4); mp 97–98 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 2.30 (s, 3H), 2.37 (s, 3H), 7.08 (d, J=7.7 Hz, 1H), 7.12 (s, 1H), 7.19 (d, J=7.7 Hz, 1H), 7.32–7.48 (m, 5H), 7.63 (d, J=8.4 Hz, 2H), 7.65 (d, J=8.4 Hz, 2H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 20.6, 21.2, 126.8, 127.0 (×2), 127.3 (×2), 127.5, 129.1 (×2), 130.0 (×2), 130.0, 131.5, 135.5, 137.3, 138.9, 139.7, 141.2, 141.2; HRMS (EI, 70 eV) Calcd for $C_{20}H_{18}$ (M^+): 258.1409. Found: 258.1406. Anal. Calcd for $C_{20}H_{18}$: C, 92.98; H, 7.02. Found: C, 92.84; H, 7.00.

4.3.5. 4-Methyl-*p*-terphenyl (5e). The title compound was prepared by the reaction of **3b** (118.35 mg, 0.30 mmol) with **4a** (1.0 M in THF, 0.9 mL, 0.9 mmol+0.6 mL, 0.6 mmol) in the presence of dppfNiCl$_2$. The crude compound was purified by recrystallization from MeOH to afford **5e** (59.37 mg, 81%) as a white solid: TLC R_f 0.66 (Et$_2$O:*n*-hexane=1:4); mp 209–210 °C; ^{1}H NMR (500 MHz, CDCl$_3$) δ 2.41 (s, 3H), 7.27 (d, J=8.0 Hz, 2H), 7.35 (t, J=7.4 Hz, 1H), 7.46 (t, J=7.6 Hz, 2H), 7.55 (d, J=8.0 Hz, 2H), 7.64 (d, J=7.6 Hz, 2H), 7.66 (s, 4H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 21.2, 127.2 (×2), 127.3 (×2), 127.6 (×2), 127.6, 127.7 (×2), 129.1 (×2), 129.8 (×2), 137.4, 138.1, 140.1, 140.4, 141.1; HRMS (EI, 70 eV) Calcd for $C_{19}H_{16}$ (M^+): 244.1252. Found: 244.1249.

4.3.6. 4-*tert*-Butyl-4″-methyl-*p*-terphenyl (5f). The title compound was prepared by the reaction of **3b** (118.35 mg, 0.30 mmol) with **4b** (2.0 M in THF, 0.45 mL, 0.9 mmol+0.3 mL, 0.6 mmol) in the presence of dppfNiCl$_2$. The crude compound was purified by recrystallization from MeOH to afford **5f** (68.50 mg, 76%) as a pale yellowish solid: TLC R_f 0.68 (Et$_2$O:*n*-hexane=1:4); mp 203–205 °C; ^{1}H NMR (300 MHz, CDCl$_3$) δ 1.37 (s, 9H), 2.41 (s, 3H), 7.27 (d, J=8.7 Hz, 2H), 7.49 (d, J=8.7 Hz, 2H), 7.55 (d, J=8.1 Hz, 2H), 7.59 (d, J=8.6 Hz, 2H), 7.65 (s, 4H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 21.2, 31.5 (×3), 34.7, 126.0 (×2), 126.9 (×2), 127.1 (×2), 127.5 (×2), 127.6 (×2), 129.8 (×2), 137.3, 138.1, 138.2, 139.9, 140.0, 150.6; HRMS (EI, 70 eV) Calcd for $C_{23}H_{24}$ (M^+): 300.1878. Found: 300.1898.

4.3.7. 3,5-Dimethyl-4″-methyl-*p*-terphenyl (5g). The title compound was prepared by the reaction of **3b** (118.35 mg, 0.30 mmol) with **4c** (0.5 M in THF, 1.8 mL, 0.9 mmol+1.2 mL, 0.6 mmol) in the presence of dppfNiCl$_2$. The crude compound was purified by recrystallization from MeOH to afford **5g** (61.29 mg, 75%) as a white solid: TLC R_f 0.72 (Et$_2$O:*n*-hexane=1:4); mp 151–152 °C; ^{1}H NMR (500 MHz, CDCl$_3$) δ 2.39 (s, 6H), 2.40 (s, 3H), 7.00 (s, 1H), 7.25 (s, 2H), 7.26 (d, J=8.1 Hz, 2H), 7.54 (d, J=8.1 Hz, 2H), 7.64 (s, 4H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 21.2, 21.5 (×3), 125.3 (×2), 127.1 (×2), 127.4 (×2), 127.8 (×2),129.1, 129.8 (×2), 137.4, 138.2, 138.6 (×2), 140.2, 140.4, 141.1; HRMS (EI, 70 eV) Calcd for $C_{21}H_{20}$ (M^+): 272.1565. Found: 272.1557. Anal. Calcd for $C_{21}H_{20}$: C, 92.60; H, 7.40. Found: C, 92.43; H, 7.51.

4.3.8. 4-Methoxy-*p*-terphenyl (5h). The title compound was prepared by the reaction of **3c** (123.15 mg, 0.30 mmol) with **4a** (1.0 M in THF, 0.9 mL, 0.9 mmol+0.6 mL, 0.6 mmol) in the presence of dppfNiCl$_2$. The crude compound was purified by recrystallization from MeOH to afford **5h** (57.01 mg, 73%) as a white solid: TLC R_f 0.54 (Et$_2$O:*n*-hexane=1:4); mp 224–225 °C; ^{1}H NMR (500 MHz, CDCl$_3$) δ 3.86 (s, 3H), 7.13 (d, J=8.7 Hz, 2H), 7.35 (t, J=7.4 Hz, 1H), 7.45 (t, J=7.6 Hz, 2H), 7.58 (d, J=8.7 Hz, 2H), 7.61–7.65 (m, 6H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 55.5, 114.5 (×2), 127.3 (×2), 127.4 (×2), 127.5, 127.7 (×2), 128.3 (×2), 129.1 (×2), 133.5, 139.8, 140.0, 141.1; HRMS (EI, 70 eV) Calcd for $C_{19}H_{16}O$ (M^+): 260.1201. Found: 260.1210.

4.3.9. 4-*tert*-Butyl-4″-methoxy-*p*-terphenyl (5i). The title compound was prepared by the reaction of **3c** (123.15 mg, 0.30 mmol) with **4b** (2.0 M in THF, 0.45 mL, 0.9 mmol+0.3 mL, 0.6 mmol) in the presence of dppfNiCl$_2$. The crude compound was purified by recrystallization from

MeOH to afford **5i** (71.19 mg, 75%) as a white solid: TLC R_f 0.55 (Et_2O:*n*-hexane=1:4); mp 236–237 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 1.37 (s, 9H), 3.86 (s, 3H), 6.99 (d, J=8.7 Hz, 2H), 7.48 (d, J=8.3 Hz, 2H), 7.58 (d, J=8.7 Hz, 2H), 7.58 (d, J=8.2 Hz, 1H), 7.62 (d, J=8.2 Hz, 2H), 7.65 (d, J=8.3 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 31.5 (×3), 34.7, 55.5, 114.5 (×2), 126.0 (×2), 126.9 (×2), 127.3 (×2), 127.6 (×2), 128.3 (×2), 133.6, 138.2, 139.6, 139.7, 150.6, 159.5; HRMS (EI, 70 eV) Calcd for $C_{23}H_{24}O$ (M^+): 316.1827. Found: 316.1836. Anal. Calcd for $C_{23}H_{24}O$: C, 87.30; H, 7.64. Found: C, 87.19; H, 7.59.

4.3.10. 2,4-Dimethyl-4″-methoxyterphenyl (5j). The title compound was prepared by the reaction of **3c** (123.15 mg, 0.30 mmol) with **4d** (0.5 M in THF, 1.8 mL, 0.9 mmol+1.2 mL, 0.6 mmol) in the presence of dppfNiCl_2. The crude compound was purified by recrystallization from MeOH to afford **5j** (44.12 mg, 51%) as a white solid: TLC R_f 0.56 (Et_2O:*n*-hexane=1:4); mp 136–137 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 2.30 (s, 3H), 2.38 (s, 3H), 3.86 (s, 3H), 7.00 (d, J=8.7 Hz, 2H), 7.08 (d, J=7.7 Hz, 1H), 7.12 (s, 1H), 7.19 (d, J=7.7 Hz, 1H), 7.37 (d, J=8.7 Hz, 2H), 7.59 (d, J=8.7 Hz, 2H), 7.60 (d, J=8.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.6, 21.2, 55.5, 114.5 (×2), 126.6 (×2), 126.8, 128.3 (×2), 129.9 (×2), 130.0, 131.4, 133.7, 135.5, 137.2, 139.0, 139.3, 140.6, 159.4; HRMS (EI, 70 eV) Calcd for $C_{21}H_{20}O$ (M^+): 288.1514. Found: 288.1522. Anal. Calcd for $C_{21}H_{20}O$: C, 87.46; H, 6.99. Found: C, 87.53; H, 7.06.

4.3.11. 4-Methyl-*p*-terphenyl (5e). The title compound was prepared by the reaction of **3d** (31.84 mg, 0.10 mmol) with **4a** (1.0 M in THF, 0.3 mL, 0.3 mmol) in the presence of dppfNiCl_2. The crude compound was purified by recrystallization from MeOH to afford **5e** (20.28 mg, 83%) as a white solid.

4.3.12. 4-Methyl-(1,1′,3′,1″)-terphenyl (5k). The title compound was prepared by the reaction of **3e** (31.84 mg, 0.1 mmol) with **4a** (1.0 M in THF, 0.3 mL, 0.3 mmol) in the presence of dppfNiCl_2. The crude compound was purified by preparative HPLC (CH_3CN:MeOH=2:3) to afford **5k** (18.32 mg, 75%) as a colorless oil that solidified upon standing to a yellow solid: TLC R_f 0.62 (EtOAc:*n*-hexane=1:4); ^1H NMR (500 MHz, $CDCl_3$) δ 2.41 (s, 3H), 7.27 (d, J=8.0 Hz, 2H), 7.37 (t, J=7.4 Hz, 1H), 7.46 (t, J=7.7 Hz, 2H), 7.50 (d, J=7.5 Hz, 1H), 7.38–7.58 (m, 4H), 7.65 (d, J=8.0 Hz, 2H), 7.79 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.3, 126.1, 126.2, 126.2, 127.3 (×2), 127.5 (×2), 127.6, 129.0 (×2), 129.4, 129.7 (×2), 137.4, 138.5, 141.5, 141.9, 142.0; HRMS (EI, 70 eV) Calcd for $C_{19}H_{16}$ (M^+): 244.1252. Found: 244.1240. Anal. Calcd for $C_{19}H_{16}$: C, 93.40; H, 6.60. Found: C, 93.28; H, 6.57.

4.3.13. 4-Methyl-(1,1′,2′,1″)-terphenyl (5l). The title compound was prepared by the reaction of **3f** (31.84 mg, 0.10 mmol) with **4a** (1.0 M in THF, 0.3 mL, 0.3 mmol) in the presence of dppfNiCl_2. The crude compound was purified by preparative HPLC (CH_3CN:MeOH=2:3) to afford **5l** (16.13 mg, 66%) as a colorless oil that solidified upon standing to a yellow solid: TLC R_f 0.62 (EtOAc:*n*-hexane=1:4); ^1H NMR (300 MHz, $CDCl_3$) δ 2.29 (s, 3H), 7.00–7.03 (m, 4H), 7.13–7.23 (m, 5H), 7.35–7.45 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2, 126.6, 127.5 (×2), 127.7 (×2), 128.1 (×2), 128.9 (×2), 130.0, 130.1 (×2), 130.9 (×2), 136.3, 138.8, 140.8, 142.0; HRMS (EI, 70 eV) Calcd for $C_{19}H_{16}$ (M^+): 244.1252. Found: 244.1247. Anal. Calcd for $C_{19}H_{16}$: C, 93.40; H, 6.60. Found: C, 93.22; H, 6.57.

4.3.14. 4-*tert*-Butyl-4′-methoxy-(1,1′,2′,1″)-terphenyl (5m). The title compound was prepared by the reaction of **3g** (33.44 mg, 0.10 mmol) with **4b** (2.0 M in THF, 0.15 mL, 0.3 mmol+0.1 mL, 0.2 mmol) in the presence of dppfNiCl_2. The crude compound was purified by preparative HPLC (CH_3CN:MeOH=2:3) to afford **5m** (21.83 mg, 69%) as a pale yellow oil that solidified upon standing to a yellow solid: TLC R_f 0.53 (EtOAc:*n*-hexane=1:4); ^1H NMR (300 MHz, $CDCl_3$) δ 1.30 (s, 9H), 3.79 (s, 3H), 6.73–6.78 (m, 2H), 7.04–7.10 (m, 4H), 7.22–7.26 (m, 2H), 7.35–7.43 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 31.4 (×3), 34.5, 55.3, 113.5, 125.1, 127.3 (×2), 127.5 (×2), 129.7, 130.8 (×2), 131.0 (×2), 131.2, 134.4, 138.9, 140.4, 140.7, 149.5, 158.5; HRMS (EI, 70 eV) Calcd for $C_{23}H_{24}O$ (M^+): 316.1827. Found: 316.1844.

4.3.15. 2,4-Dimethyl-4′-methoxy-(1,1′,2′,1″)-terphenyl (5n). The title compound was prepared by the reaction of **3g** (33.44 mg, 0.10 mmol) with **4d** (0.5 M in THF, 0.6 mL, 0.3 mmol+0.4 mL, 0.2 mmol) in the presence of dppfNiCl_2. The crude compound was purified by preparative HPLC (CH_3CN:MeOH=2:3) to afford **5n** (10.96 mg, 38%) as a pale yellow oil that solidified upon standing to a yellow solid: TLC R_f 0.52 (EtOAc:*n*-hexane=1:4); ^1H NMR (300 MHz, $CDCl_3$) δ 1.85 (s, H), 2.30 (s, H), 3.75 (s, 3H), 6.68–6.73 (m, 2H), 6.89 (s, 1H), 6.92 (s, 1H), 6.95 (s, 1H), 7.01–7.06 (m, 3H), 7.31–7.43 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.0, 21.2, 55.3, 113.4, 126.4, 126.9 (×2), 127.6 (×2), 130.1, 130.7 (×2), 131.2 (×2), 134.3, 135.8, 136.7, 138.9, 140.5, 141.0, 158.5; HRMS (EI, 70 eV) Calcd for $C_{19}H_{16}$ (M^+): 288.1514. Found: 288.1556.

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Application of alkyl 3-dimethylamino-2-(1*H*-indol-3-yl)propenoates in the synthesis of 3-heteroarylindoles

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Dedicated to Professor Peter Stanetty, Technical University of Vienna, on the occasion of his 60th anniversary

Abstract—Methyl and ethyl 3-dimethylamino-2-(indol-3-yl)propenoate were prepared from alkyl 3-indoleacetates and *tert*-butoxy-bis(dimethylamino)methane. Upon treatment of these two *N*,*N*-dimethylenaminones with α-heteroarylamines as *N*,*N*-1,3-dinucleophiles, condensed indolylpyrimidones as meridianine analogues were obtained in poor to moderate yields, while intramolecular condensations with *C*,*O*-1,3-dinucleophiles furnished condensed indolylpyranones. Similarly, reaction with hydrazinium chloride led to indolylpyrazolol, while with 3-chloro-6-hydrazinopyridazine only the dimethylamine substitution took place to give the corresponding hydrazone.

1. Introduction

Since their isolation from various marine organisms, aplysinopsins have attracted considerable interest, especially due to their cytotoxicity towards cancer cells and their ability to affect neurotransmitters.[1–16] Recently, the nortopsentins and meridianines, another series of indole alkaloids showing interesting antitumor properties, have been isolated from the sponge *Spongosorites ruetzleri* and from the tunicate *Aplidium meridianum* (Fig. 1).[17–19]

Alkyl 3-(dimethylamino)propenoates and related enaminones play an important role as building blocks for the preparation of many heterocyclic systems, such as fused pyridones, pyrimidones, pyranones, and related fused systems, which are the basic structures of many alkaloids and their synthetic derivatives exhibiting various biological activity. Alkyl 3-(dimethylamino)propenoates and related enaminones have been also employed in the synthesis of functionalised heterocyclic compounds including natural products, and their analogs.[20–25]

Recently, we reported utilisation of various 3-dimethylamino-2-(vinylamino)propenoates and dimethylaminomethylidene substituted heterocycles as reagents for the preparation of aplysinopsins and its analogs.[26–30] In continuation of our work on the synthesis of indole alkaloids, we now report preparation of alkyl 3-dimethylamino-2-(1*H*-indol-3-yl)propenoates **2a–c** and their transformations with *N*,*N*- and *C*,*O*-dinucleophiles into condensed 3-(1*H*-indol-3-yl)-4*H*-pyrimidin-4-ones as the meridianine analogues and into condensed 3-(1*H*-indol-3-yl)-2*H*-pyran-2-ones as the chromene derivatives.

2. Results and discussion

Alkyl (2*E*)-3-dimethylamino-2-(1*H*-indol-3-yl)propenoates **2a–c** were prepared in one step from alkyl 3-indoleacetates **1a–c** and *tert*-butoxy-bis(dimethylamino)methane (Bredereck's reagent). Heating of in DMF under reflux for several hours afforded propenoates **2a–c** in 76–82% yields (Scheme 1).

Compounds **2a** and **2b** reacted with hydrazine hydrochloride (**3a**) in ethanol under reflux to afford the pyrazolol **5** in 82 and 58% yield, respectively. Further reaction of **5** with 1 equiv. of dimethyl acetylenedicarboxylate gave dimethyl 2-[3-hydroxy-4-(1*H*-indol-3-yl)-1*H*-pyrazol-1-yl]but-2-ene-1,4-dioate **7** as a mixture of (*E*)- and (*Z*)-isomer in a ratio of 1:1. Upon treatment of **2a** with 3-chloro-6-hydrazinopyridazine (**3b**), only the substitution of the

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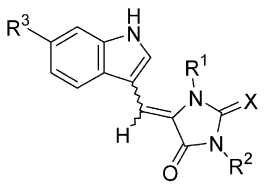
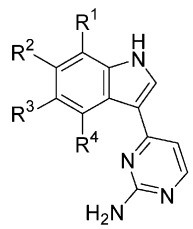




Aplysinopsins

$R^1 = R^2 = Me$, $R^3 = H$, Y = NH

$R^1 = R^2 = Me$, $R^3 = H$, X = O

$R^1 = R^2 = Me$, $R^3 = Br$, X= O

$R^1 = H$, $R^2 = Me$, $R^3 = H$, X = NH

$R^1 = H$, $R^2 = Me$, $R^3 = H$, X = N–Me

$R^1 = H$, $R^2 = Me$, $R^3 = Br$, X = NH

$R^1 = H$, $R^2 = Me$, $R^3 = Br$, X = N–Me

$R^1 = R^2 = R^3 = H$, X = O

$R^1 = R^2 = H$, $R^3 = Br$, X = O

Meridianines

A: $R^1 = OH$, $R^2 = R^3 = R^4 = H$

C: $R^1 = R^3 = R^4 = H$, $R^2 = Br$

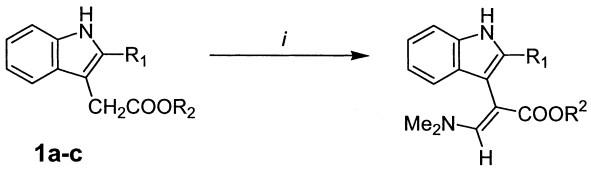
D: $R^1 = R^2 = R^4 = H$, $R^3 = Br$

E: $R^1 = OH$, $R^2 = R^3 = H$, $R^4 = Br$

Figure 1.

dimethylamino group took place to give alkyl 3-[(6-chloropyridazin-3-yl)hydrazono]-2-(1*H*-indol-3-yl)propanoate **8**. On the other hand, reactions of **2a**,**b** with heteroarylamines **9a**–**h** resulted in formation of the cyclocondensation products **10a**–**h**, regardless to the heteroaryl moiety. Thus, heating of the propenoates **2a**,**b** with 2-aminopyridines **9a**–**d**, 3-amino-1*H*-pyrazole-4-carbonitrile (**9e**), 2-aminothiazole (**9f**), 2-aminobenzothiazole (**9g**), and 3-amino-1,2,4-triazole (**9h**) and acetic acid for 1.5–7 h furnished the corresponding 3-heteroaryl-1*H*-indoles **10a**–**h** as the meridianine analogues in 11–95% yields (Scheme 2, Table 1).

By treatment of **2a** with (hetero)cyclic *C*,*O*-dinucleophiles **11a**–**f** in acetic acid under reflux for several hours pyrano[3,2-*c*]pyridin-2-one **12a**, pyrano[2,3-*d*]pyrimidine-2,4,7-trione **12b**, pyrano[4,3-*b*]pyran-2,5-dione **12c**, chromene **12d**,**e**, and pyrano[3,2-*c*]pyrazole derivatives **12f** were obtained in 9–81% yields (Scheme 3).



1a-c **2a-c**

Compound	R^1	R^2	Yield of **2** [%]
1a, **2a**	H	Et	76
1b, **2b**	H	Me	82
1c, **2c**	Me	Me	80

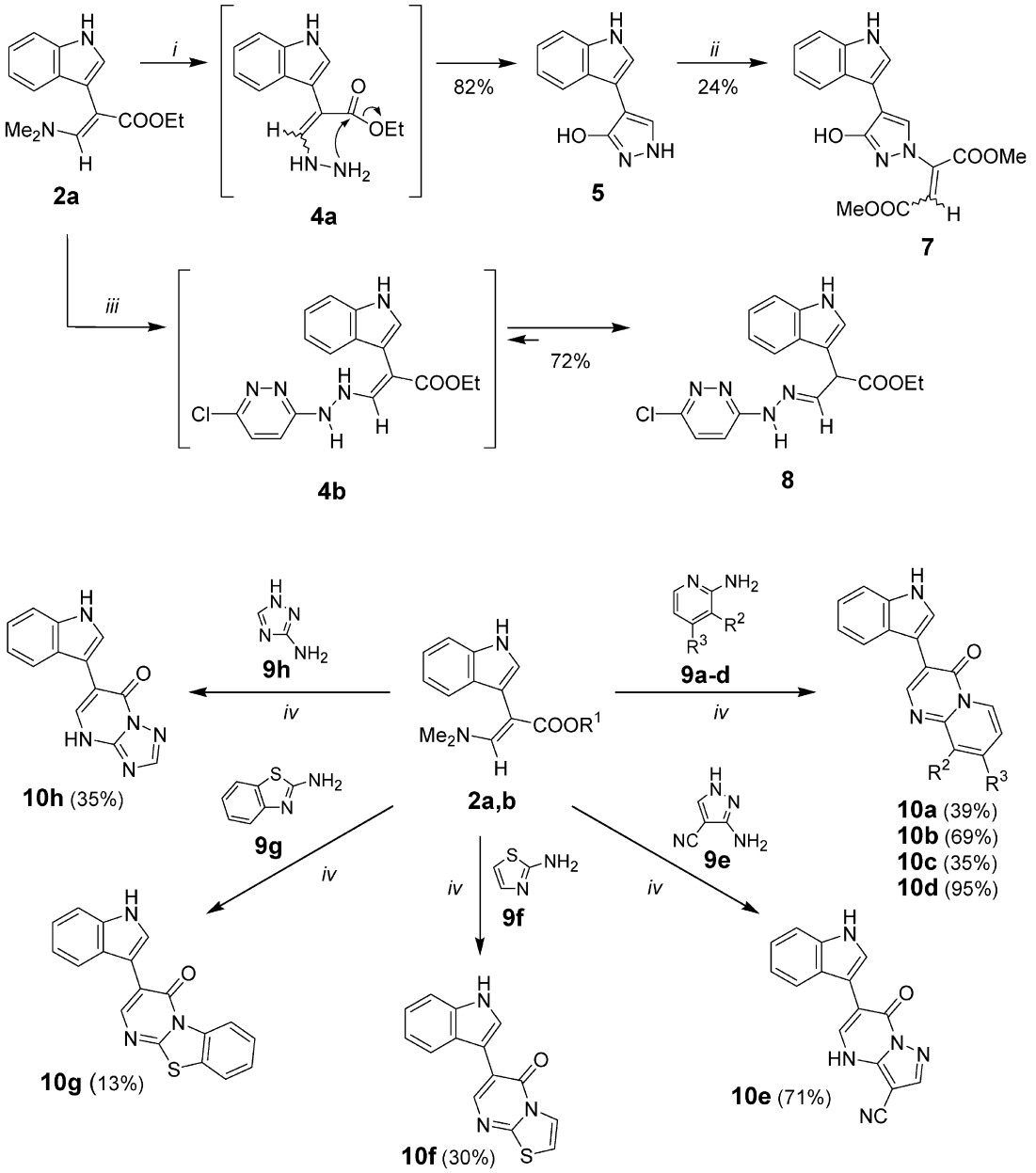
Scheme 1. Reaction conditions: (i) *t*-BuOCH(NMe_2)$_2$, DMF, reflux.

Formation of 3-heteroarylindoles **5**, **10**, and **12** from **2a**,**b** and *N*,*N*-dinucleophiles **3**, **9** and *C*,*O*-dinucleophiles **11** proceeds by initial substitution of the dimethylamino group followed by cyclisation. In the reaction of **2a** with 3-chloro-6-hydrazinopyridazine (**3b**), the intermediate substitution product **4b** was isolated in its hydrazono tautomeric form **7** and further cyclisation did not take place. In all other cases, the intermediate substitution products could not be isolated. Nevertheless, a two step mechanism for formation of indolyl substituted pyrazole **5**, pyrimidones **10**, and pyranones **12** is supported by previously described cyclisations between other closely related 3-(dimethylamino)-propenoates and ambident nucleophiles.[20–25]

3. Structure determination

The structures of all novel compounds were determined by spectroscopic methods (IR, NMR, MS) and by elemental analyses for C, H, and N.

The configuration around the exocyclic C=C double bond in compounds **2b** and **2c** was determined by NMR on the basis of long-range coupling constants ($^3J_{C-H}$) between the methylidene proton (*H*–C(3)) and the carbonyl carbon atom (O=*C*(1)), measured from the antiphase splitting of cross peaks in the HMBC spectrum. Generally, the magnitude of coupling constant $^3J_{C-H}$ for nuclei with *cis*-configuration around the C=C double bond are smaller (2–6 Hz) than that for *trans*-oriented nuclei (8–12 Hz).[28,31–41] The magnitude of coupling constant ($^3J_{C-H}$=5 Hz) showed the (*E*)-configuration around the exocyclic C=C double bond in compounds **2a**,**b** (Fig. 2).



Scheme 2. Reaction conditions: (i) $NH_2NH_2{\cdot}HCl$ (**3a**), EtOH, reflux; (ii) dimethyl acetylenedicarboxylate (**6**), MeOH, reflux; (iii) 3-chloro-6-hydrazinopyridazine (**3b**), EtOH, rt; (iii) heterocyclic amine **9a**–**h**, AcOH, reflux.

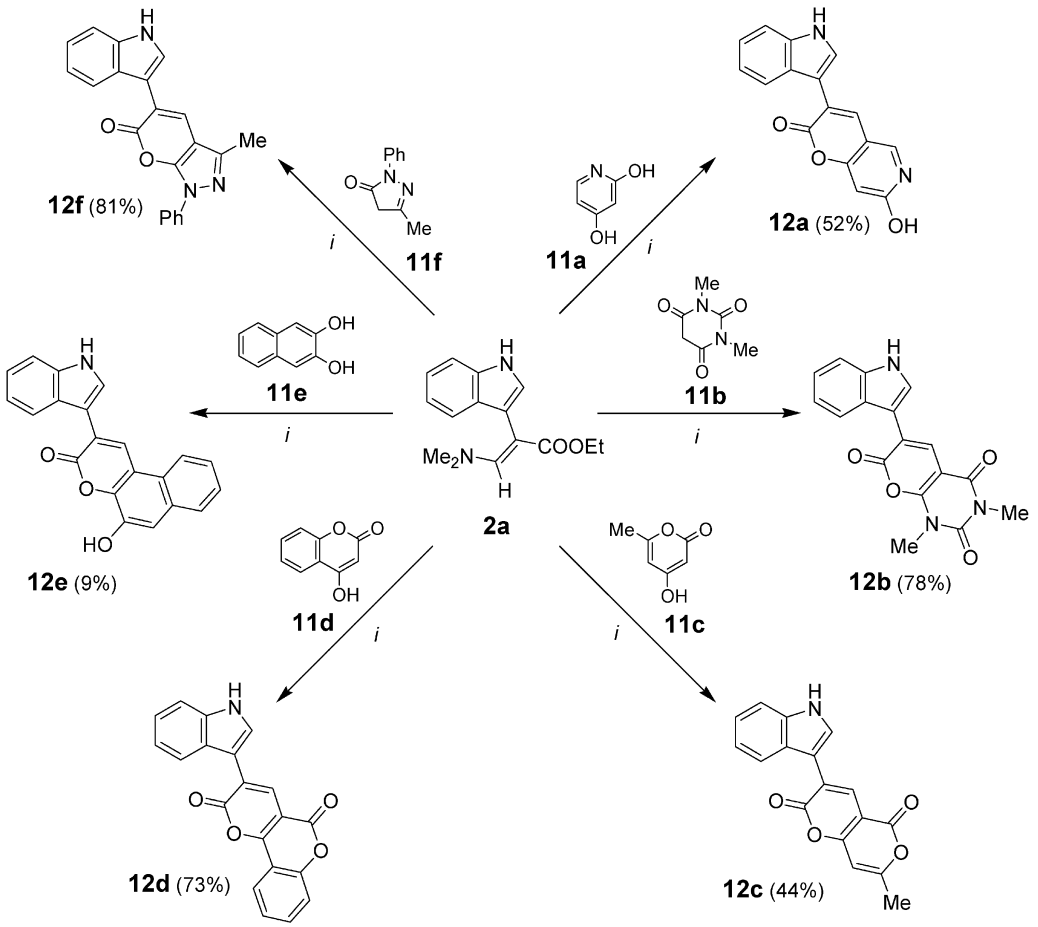
Table 1. 3-Heteroaryl-1*H*-indoles **5**, **7**, **8**, and **10a**–**h**

Reaction	R^1	R^2	R^3	Yield [%]
2a+**3a**→**5**	Et			82
5+**6**→**7**				24
2a+**3b**→**8**	Et			72
2a+**9a**→**10a**	Et	H	H	39
2a+**9b**→**10b**	Et	Me	H	67
2b+**9b**→**10b**	Me	Me	H	69
2a+**9c**→**10c**	Et	OH	H	35
2a+**9d**→**10d**	Et	H	Cl	95
2b+**9d**→**10d**	Me	H	Cl	88
2a+**9e**→**10e**	Et			68
2b+**9e**→**10e**	Me			71
2a+**9f**→**10f**	Et			30
2a+**9g**→**10g**	Et			11
2b+**9g**→**10g**	Me			13
2a+**9h**→**10h**	Et			35
2b+**9h**→**10h**	Me			25

Structure of compound **2b** was additionally determined by X-ray diffraction (Fig. 3).

4. Conclusion

Alkyl 3-dimethylamino-2-(indol-3-yl)propenoates **2a**,**b**, available from alkyl 3-indoleacetates and *tert*-butoxy-bis(dimethylamino)methane, react with ambident nucleophiles, such as hydrazine monohydrochloride (**3a**), α-heteroarylamines (**9a**–**h**), and (hetero)cyclic 1,3-dicarbonyl compound analogues **11a**–**f** to form indolyl substituted pyrazolol **5** and condensed pyrimidones **10a**–**h** and pyranones **12a**–**f**. Condensed indolylpyrimidones **10a**–**h** are structurally closely related to meridianine alkaloids. Thus, indolyl propenoates **2** are suitable reagents for a one step preparation of indolyl substituted heterocycles.



Scheme 3. Reaction conditions: (i) (hetero)cyclic *C*,*O*-dinucleophile **11a**–**f**, AcOH, reflux.

5. Experimental

Melting points were determined on a Kofler micro hot stage. The ^{1}H NMR spectra were obtained on a Bruker Avance DPX 300 at 300 MHz for ^{1}H and 75.5 MHz for ^{13}C nucleus, using DMSO-d_6 and $CDCl_3$ as solvents and TMS as the internal standard. Mass spectra were recorded on an AutoSpecQ spectrometer, IR spectra on a Perkin–Elmer Spectrum BX FTIR spectrophotometer. Microanalyses were performed on a Perkin–Elmer CHN Analyser 2400. Column chromatography (CC) was performed on silica gel (Fluka, silica gel 60, 0.04–0.06 mm).

tert-Butoxy-bis(dimethylamino)methane (Bredereck's reagent), alkyl 3-indoleacetates **1a**–**c**, hydrazine hydrochloride (**3a**), dimethyl acetylenedicarboxylate (**6**), heterocyclic amines **9a**–**h**, and (hetero)cyclic *C*,*O*-dinucleophiles **11a**–**f** are commercially available (Fluka AG). 3-Chloro-6-hydrazinopyridazine (**3b**) was prepared according to the procedure described in the literature.[42]

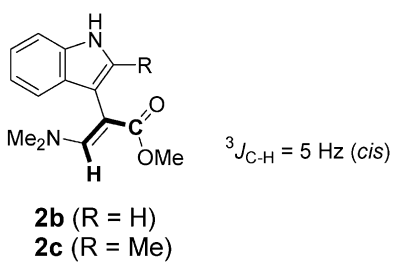


Figure 2.

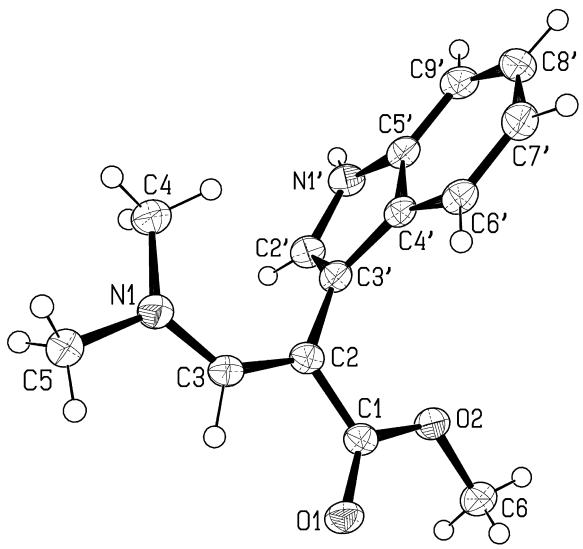


Figure 3. OrtepII view of the asymmetric unit of compound **2b** with labelling of non-hydrogen atoms. (Ellipsoids are at 50% probability level.) The crystallographic data for compound **2b** have been deposited with the Cambridge Crystallographic Data Center as supplementary material with the deposition number: CCDC 228170. These data can be obtained, free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html.

5.1. General procedure for the preparation of alkyl (2*E*)-3-dimethylamino-2-(1*H*-indol-3-yl)propenoates 2a–c

Bredereck's reagent (940 mg, 5.4 mmol) was added to a solution of **1a–c** (4 mmol) in anhydrous DMF (20 ml) and the mixture was heated under reflux for 3–6 h. Volatile components were evaporated in vacuo, the residue was triturated with diethyl ether (~10 ml), the precipitate was collected by filtration, and washed with EtOH–Et_2O to give **2a–c**.

The following compounds were prepared in this manner:

5.1.1. Ethyl (2*E*)-3-dimethylamino-2-(1*H*-indol-3-yl)-propenoate (2a). Prepared from compound **1a** (813 mg, 4 mmol), reflux for 6 h; 785 mg (76%) of a white solid; mp 139–141 °C (Et_2O–EtOH, 1:1). EI-MS: *m*/*z*=258 (M^+). ^{1}H NMR (DMSO-d_6): δ 1.07 (3H, t, *J*=7.2 Hz, C H_3CH_2); 2.62 (6H, s, N(C H_3)$_2$); 3.95 (2H, q, *J*=7.2 Hz, C H_2CH_3); 6.92–7.07 (3H, m, *H*–C(5′), *H*–C(2′) in *H*–C(6′)); 7.23 (1H, dd, *J*=1.1, 7.9 Hz, *H*–C(4′)); 7.32 (1H, dd, *J*=1.1, 7.9 Hz, *H*–C(7′)); 7.62 (1H, s, *H*–C(3)), 10.89 (1H, s, N *H*). ^{13}C NMR (DMSO-d_6): δ 15.5, 42.6, 59.2, 90.0, 110.4, 112.1, 119.3, 120.1, 121.5, 126.3, 130.5, 136.4, 151.1, 170.2. (Found: C, 69.50; H, 6.82; N, 11.06. $C_{15}H_{18}N_2O_2$ requires: C, 69.74; H, 7.02; N, 10.84); ν_{max} (KBr) 1653, 1602, 1550 cm^{-1}.

5.1.2. Methyl (2*E*)-3-dimethylamino-2-(1*H*-indol-3-yl)propenoate (2b). Prepared from compound **1a** (757 mg, 4 mmol), reflux for 4 h; 793 mg (81%) of a white solid; mp 146–148 °C (Et_2O–EtOH, 1:1). EI-MS: *m*/*z*=244 (M^+). ^{1}H NMR (DMSO-d_6): δ 2.61 (6H, s, N(C H_3)$_2$); 3.35 (3H, s, OC H_3); 6.91–7.08 (3H, m, *H*–C(5′), *H*–C(2′) in *H*–C(6′)); 7.21 (1H, dd, *J*=1.1, 7.9 Hz, *H*–C(4′)); 7.32 (1H, dd, *J*=1.1, 7.9 Hz, *H*–C(7′)); 7.60 (1H, s, *H*–C(3)), 10.92 (1H, s, N *H*). ^{13}C NMR (DMSO-d_6): δ 42.6, 51.2, 89.5, 110.4, 112.1, 119.4, 120.0, 121.5, 126.3, 130.6, 136.4, 151.3, 170.8. (Found: C, 68.69; H, 6.82; N, 11.29. $C_{14}H_{16}N_2O_2$ requires: C, 68.83; H, 6.60; N, 11.47); ν_{max} (KBr) 1668, 1584, 1580, 1550 cm^{-1}.

5.1.3. Methyl (2*E*)-3-dimethylamino-2-(2-methyl-1*H*-indol-3-yl)propenoate (2c). Prepared from compound **1c** (813 mg, 4 mmol), reflux for 2 h; 825 mg (80%) of a white solid; mp 142–144 °C (Et_2O–EtOH, 1:1). EI-MS: *m*/*z*=258 (M^+). ^{1}H NMR (DMSO-d_6): δ 2.16 (3H, s, H_3C–C(2′)); 2.61 (6H, s, N(C H_3)$_2$); 3.44 (3H, s, OC H_3); 6.86–6.96 (2H, m, *H*–C(5′), *H*–C(6′)); 7.16 (1H, dd, *J*=1.1, 7.9 Hz, *H*–C(7′)); 7.22 (1H, dd, *J*=1.1, 7.9 Hz, *H*–C(4′)); 7.66 (1H, s, *H*–C(3)), 10.77 (1H, s, N *H*). ^{13}C NMR (DMSO-d_6): δ 12.8, 42.1, 51.2, 89.2, 107.6, 111.1, 119.1, 119.2, 120.6, 131.4, 134.8, 135.9, 151.6, 170.6. (Found: C, 69.51; H, 7.20; N, 10.68. $C_{15}H_{18}N_2O_2$ requires: C, 69.74; H, 7.02; N, 10.84); ν_{max} (KBr) 1664, 1597, 1495 cm^{-1}.

5.1.4. 4-(1*H*-Indol-3-yl)-1*H*-pyrazol-3-ol (5). A mixture of **2a** (129 mg, 0.5 mmol), hydrazine hydrochloride (**3a**, 34 mg, 0.5 mmol), and ethanol (5 ml) was heated under reflux for 3 h. Volatile components were evaporated in vacuo, the residue was triturated with ethanol (~1 ml), and the precipitate was collected by filtration to give **5**. Yield: 82 mg (82%) of a white solid; mp 260 °C (EtOAc, decomp.). EI-MS: *m*/*z*=199 (M^+). ^{1}H NMR (DMSO-d_6): δ 6.99–7.12 (2H, m, *H*–C(5′), *H*–C(6′)), 7.37 (1H, dd, *J*=1.1, 7.9 Hz, *H*–C(7′)), 7.52 (1H, d, *J*=2.6 Hz, *H*–C(2′)), 7.77 (1H, dd, *J*=1.1, 7.9 Hz, *H*–C(4′)), 7.86 (1H, s, *H*–C(5)), 9.88, 10.95, 11.52 (3H, 3s, 1:1:1, 2NH and OH). ^{13}C NMR (DMSO-d_6): δ 100.5, 107.7, 112.2, 112.3, 119.5, 120.6, 121.8, 122.6, 122.7, 126.2, 136.7. (Found: C, 66.19; H, 4.30; N, 20.91. $C_{11}H_9N_3O$ requires: C, 66.32; H, 4.55; N, 21.09); ν_{max} (KBr) 3395, 1652, 1611, 1572, 1524 cm^{-1}.

5.1.5. Dimethyl 2-[3-hydroxy-4-(1*H*-indol-3-yl)-1*H*-pyrazol-1-yl]but-2-ene-1,4-dioate (7). A mixture of **5** (100 mg, 0.5 mmol), dimethyl acetylenedicarboxylate (**6**, 142 mg, 1 mmol), and methanol (5 ml) was heated under reflux for 4 h. Volatile components were evaporated in vacuo, and the residue was purified by CC (EtOAc–cyclohexane, 1:1). Fractions containing the product were combined, and evaporated in vacuo. The solid residue was crystallised from Et_2O to give **7**. Yield: 41 mg (24%) of a red solid; mp 195–200 °C (methanol), *Z*/*E*=50:50. FAB-MS: *m*/*z*=341 (M^+), 342 (MH^+). ^{1}H NMR (DMSO-d_6) *E-isomer*: δ 3.69 (3H, s, COOMe), 3.88 (3H, s, COOMe), 6.49 (1H, s, *H*–C(2)), 7.06–7.17 (2H, m, *H*–C(5″), *H*–C(6″)), 7.43 (1H, dd, *J*=1.1, 7.5 Hz, *H*–C(7″)), 7.66 (1H, d, *J*=2.6 Hz, *H*–C(2″)), 7.76 (1H, dd, *J*=1.1, 7.5 Hz, *H*–C(4″)), 8.58 (1H, s, *H*–C(5′)), 11.29 and 11.62 (2H, 2s, 1:1, NH and OH); *Z-isomer*: 3.69 (3H, s, COOMe), 3.86 (3H, s, COOMe), 6.33 (1H, s, *H*–C(2)), 7.06–7.17 (2H, m, *H*–C(5″), *H*–C(6″)), 7.43 (1H, dd, *J*=1.1, 7.5 Hz, *H*–C(7″)), 7.66 (1H, d, *J*=2.6 Hz, *H*–C(2″)), 7.76 (1H, dd, *J*=1.1, 7.5 Hz, *H*–C(4″)), 8.36 (1H, s, *H*–C(5′)), 11.02 and 11.19 (2H, 2s, 1:1, NH and OH). (Found: C, 59.56; H, 4.69; N, 12.10. $C_{17}H_{15}N_3O_5$ requires: C, 59.82; H, 4.43; N, 12.31); ν_{max} (KBr) 3403, 3062, 2950, 2700, 1740, 1722, 1655 cm^{-1}.

5.1.6. Ethyl 3-[(6-chloropyridazin-3-yl)hydrazono]-2-(1*H*-indol-3-yl)propanoate (8). A mixture of **2a** (129 mg, 0.5 mmol), 3-chloro-6-hydrazinopyridazine (**3b**, 72 mg, 0.5 mmol), and ethanol (5 ml) was stirred at rt for 12 h. Volatile components were evaporated in vacuo, the residue was triturated with ethanol–water, and the precipitate was collected by filtration to give **8**. Yield: 129 mg (72%) of a grayish solid; mp 92–94 °C (ethanol). FAB-MS: *m*/*z*=357 (M^+), 358 (MH^+). ^{1}H NMR (DMSO-d_6): δ 1.18 (3H, t, *J*=6.8 Hz, C H_3CH_2), 4.16 (2H, q, *J*=6.8 Hz, C H_2CH_3), 4.81 (1H, d, *J*=7.2 Hz, *H*–C(2)), 7.01 (1H, dt, *J*=1.1, 8.7 Hz, *H*–C(5′)), 7.10 (1H, dt, *J*=1.1, 8.7 Hz, *H*–C(6′)), 7.26 (1H, d, *J*=2.6 Hz, *H*–C(2′)), 7.39 (1H, dd, *J*=1.1, 8.7 Hz, *H*–C(7′)), 7.40 (1H, d, *J*=9.4 Hz, *H*–C(4″)), 7.56 (1H, dd, *J*=1.1, 8.7 Hz, *H*–C(4′)), 7.62 (1H, d, *J*=9.4 Hz, *H*–C(5″)), 7.75 (1H, d, *J*=7.2 Hz, *H*–C(3)), 11.12 and 11.39 (2H, 2s, 1:1, 2NH). (Found: C, 55.36; H, 4.69; N, 19.03. $C_{17}H_{16}N_5O_2Cl\times1/2H_2O$ requires: C, 55.67; H, 4.67; N, 19.09); ν_{max} (KBr) 1727, 1603, 1527 cm^{-1}.

5.2. General procedure for the preparation of 1*H*-indol-3-yl substituted pyrimidones 10a–h and pyranones 12a–f

A mixture of **2a** (122 mg, 0.5 mmol) or **2b** (129 mg, 0.5 mmol), 1,3-dinucleophile **9**, **11** (0.5 mmol), and acetic acid (100%, 5 ml) was heated under reflux for 1.5–11 h. Volatile components were evaporated in vacuo, the residue

was triturated with ethyl acetate (3 ml), and the precipitate was collected by filtration to give 1*H*-indol-3-yl substituted heterocycle **10**, **12**.

The following compounds were prepared in this manner:

5.2.1. 3-(1*H*-Indol-3-yl)-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (10a). Prepared from **2a** and 2-aminopyridine (**9a**, 47 mg, 0.5 mmol); reflux for 4 h; 51 mg (39%) of a beige foam; mp 235–237 °C (ethyl acetate). EI-MS: *m*/*z*=261 (M^+). ^{1}H NMR (DMSO-d_6): δ 7.10 (1H, dt, *J*=1.1, 7.1 Hz, *H*–C(6′)), 7.17 (1H, dt, *J*=1.1, 7.1 Hz, *H*–C(5′)), 7.37 (1H, d, *J*=6.8 Hz, *H*–C(8)), 7.48 (1H, s, *H*–C(9)), 7.72 (1H, d, *J*=7.1 Hz, *H*–C(7′)), 7.85–7.91 (2H, m, *H*–C(7), *H*–C(4′)), 8.12 (1H, d, *J*=2.6 Hz, *H*–C(2′)), 8.88 (1H, s, *H*–C(2)), 9.10 (1H, d, *J*=7.5 Hz, *H*–C(6)), 11.43 (1H, rs, N *H*). ^{13}C NMR (DMSO-d_6): δ 156.5, 150.6, 149.3, 137.2, 136.1, 127.8, 127.3, 126.9, 126.1, 122.3, 120.8, 120.2, 117.1, 113.4, 112.8, 108.8. (Found: C, 72.25; H, 4.17; N, 15.48. $C_{16}H_{11}N_3O\times1/3H_2O$ requires: C, 71.90; H, 4.40; N, 15.72); EI-HRMS: *m*/*z*=261.091050 (M^+); $C_{16}H_{11}N_3O$ requires: *m*/*z*=261.090212 (M^+); ν_{max} (KBr) 1672, 1630 cm^{-1}.

5.2.2. 3-(1*H*-Indol-3-yl)-8-methyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (10b). Prepared from compound **2a** and 2-amino-4-methylpyridine (54 mg, 0.5 mmol); reflux for 1.5 h; 92 mg (67%) of a beige solid; mp 264–266 °C (ethyl acetate). EI-MS: *m*/*z*=275 (M^+). ^{1}H NMR (DMSO-d_6): δ 2.48 (3H, s, C *H*$_3$), 7.07–7.24 (3H, m, *H*–C(7), *H*–C(5′), *H*–C(6′)), 7.48 (1H, d, *J*=7.9 Hz, *H*–C(6)), 7.53 (1H, s, *H*–C(9)), 7.88 (1H, d, *J*=7.9 Hz, *H*–C(4′)), 8.08 (1H, d, *J*=2.6 Hz, *H*–C(2′)), 8.82 (1H, s, *H*–C(2)), 9.00 (1H, d, *J*=7.1 Hz, *H*–C(7′)), 11.40 (1H, br s, N *H*). (Found: C, 74.25; H, 4.60; N, 14.96. $C_{17}H_{13}N_3O$ requires: C, 74.17; H, 4.76; N, 15.26); ν_{max} (KBr) 1639, 1568 cm^{-1}.

5.2.3. 8-Hydroxy-3-(1*H*-indol-3-yl)-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (10c). Prepared from **2a** and 2-amino-3-hydroxypyridine (**9c**, 55 mg, 0.5 mmol); reflux for 7 h; 48 mg (35%) of a beige solid; mp 226–229 °C (ethyl acetate). EI-MS: *m*/*z*=277 (M^+). ^{1}H NMR (DMSO-d_6): δ 7.09–7.26 (4H, m, *H*–C(5′), *H*–C(6′), *H*–C(7′), *H*–C(7)), 7.48 (1H, d, *J*=7.5 Hz, *H*–C(8)), 7.86 (1H, d, *J*=7.5 Hz, *H*–C(6)), 8.12 (1H, d, *J*=2.6 Hz, *H*–C(2′)), 8.62 (1H, d, *J*=6.8 Hz, *H*–C(4′)), 8.84 (1H, s, *H*–C(2)), 11.44 (1H, rs, N *H*). (Found: C, 69.28; H, 3.95; N, 14.92. $C_{16}H_{11}N_3O_2$ requires: C, 69.31; H, 4.00; N, 15.15); ν_{max} (KBr) 1636, 1576, 1554 cm^{-1}.

5.2.4. 7-Chloro-3-(1*H*-indol-3-yl)-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (10d). Prepared from **2a** and 2-amino-5-chloropyridine (**9d**, 64 mg, 0.5 mmol); reflux for 4 h, 140 mg (95%) of a light yellow solid; mp 247–250 °C (ethyl acetate). EI-MS: *m*/*z*=295 (M^+). ^{1}H NMR (DMSO-d_6): δ 7.12 (1H, dt, *J*=1.1, 6.8 Hz, *H*–C(5′)), 7.18 (1H, dt, *J*=1.1, 6.8 Hz, *H*–C(6′)), 7.49 (1H, d, *J*=6.8 Hz, *H*–C(7′)), 7.73 (1H, s, *H*–C(9)), 7.88 (1H, dd, *J*=2.6, 9.4 Hz, *H*–C(8)), 7.91 (1H, dd, *J*=1.1, 6.8 Hz, *H*–C(4′)), 8.15 (1H, d, *J*=2.6 Hz, *H*–C(6)), 8.89 (1H, s, *H*–C(2)), 9.06 (1H, d, *J*=2.0 Hz, *H*–C(2′)), 11.49 (1H, s, NH). ^{13}C NMR (DMSO-d_6): δ 155.7, 150.1, 147.6, 137.2, 136.4, 128.6, 127.8, 126.0, 125.3, 124.2, 122.4, 120.8, 120.6, 114.4, 112.9, 108.5. (Found: C, 64.66; H, 3.33; N, 14.12. $C_{16}H_{10}N_3O$ requires: C, 64.98; H, 3.41; N, 14.21); ν_{max} (KBr) 1676, 1628, 1565 cm^{-1}.

5.2.5. 6-(1*H*-Indol-3-yl)-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (10e). Prepared from **2a** and 5-amino-1*H*-pyrazole-4-carbonitrile (**9e**, 54 mg, 0.5 mmol); reflux for 2 h; 92 mg (68%) of a beige solid; mp 350–355 °C (DMF–water). EI-MS: *m*/*z*=275 (M^+). ^{1}H NMR (DMSO-d_6): δ 7.05–7.18 (2H, m, *H*–C(5″), *H*–C(6″)), 7.46 (1H, dd, *J*=1.1, 7.9 Hz, *H*–C(4″)), 7.69 (1H, dd, *J*=1.1, 7.9 Hz, *H*–C(7″)), 7.82 (1H, d, *J*=2.2 Hz, *H*–C(2″)), 8.18 (1H, s, *H*–C(5′)), 8.43 (1H, s, =CH), 11.36 (1H, s, N *H*), 13.69 (1H, s, N *H*). ^{13}C NMR (DMSO-d_6): δ 75.1, 107.4, 108.3, 112.7, 113.8, 120.2, 120.4, 122.2, 126.3, 126.6, 136.8, 137.0, 145.2, 146.0, 156.0. (Found: C, 65.42; H, 3.28; N, 23.61. $C_{15}H_9N_5O$ requires: C, 65.45; H, 3.30; N, 25.44); HRMS: *m*/*z*=275.080630 (M^+), $C_{15}H_9N_5O$ requires: *m*/*z*=275.080710 (M^+); ν_{max} (KBr) 1681, 1643, 1602, 1541 cm^{-1}.

5.2.6. 6-(1*H*-Indol-3-yl)-5*H*-[1,3]thiazolo[3,2-*a*]pyrimidin-5-one (10f). Prepared from **2a** and 2-aminothiazole (**9f**, 50 mg, 0.5 mmol); reflux for 5 h, 40 mg (30%) of a light yellow solid; mp 213–217 °C (ethyl acetate). EI-MS: *m*/*z*=267 (M^+), 268 (MH^+). ^{1}H NMR (DMSO-d_6): δ 7.08 (1H, dt, *J*=1.1, 7.9 Hz, *H*–C(5″)), 7.16 (1H, dt, *J*=1.1, 7.9 Hz, *H*–C(6″)), 7.46 (1H, dd, *J*=1.1, 7.9 Hz, *H*–C(7″)), 7.60 (1H, d, *J*=4.9 Hz, *H*–C(6′)), 7.82 (1H, dd, *J*=1.1, 7.9 Hz, *H*–C(4″)), 8.00 (1H, d, *J*=2.6 Hz, *H*–C(2″)), 8.53 (1H, s, *H*–C(5′)), 8.15 (1H, d, *J*=4.9 Hz, *H*–C(7′)), 11.39 (1H, s, N *H*). ^{13}C NMR (DMSO-d_6): δ 107.9, 112.7, 114.5, 114.6, 120.4, 120.7, 122.3, 123.0, 126.1, 127.0, 137.1, 148.8, 157.8, 159.9. (Found: C, 62.78; H, 3.37; N, 15.48. $C_{14}H_9N_3OS$ requires: C, 62.91; H, 3.39; N, 15.72); HRMS: *m*/*z*=267.045950 (M^+). $C_{14}H_9N_3OS$ requires: *m*/*z*=267.046634 (M^+); ν_{max} (KBr) 1659, 1561, 1493 cm^{-1}.

5.2.7. 3-(1*H*-Indol-3-yl)-4*H*-pyrimido[2,1-*b*]-1,3-benzothiazol-4-one (10g). Prepared from **2a** and 2-aminobenzothiazole (**9g**, 75 mg, 0.5 mmol); reflux for 5.5 h; 17 mg (11%) of a greenish solid; mp 276–278 °C (toluene). EI-MS: *m*/*z*=317 (M^+). ^{1}H NMR (DMSO-d_6): δ 7.11 (1H, dt, *J*=1.1, 7.9 Hz, *H*–C(5′)), 7.18 (1H, dt, *J*=1.1, 7.9 Hz, *H*–C(6′)), 7.48 (1H, dd, *J*=3.8, 6.4 Hz, *H*–C(5)), 7.62 (2H, m, *H*–C(6′) in *H*–C(7′)), 7.85 (1H, dd, *J*=3.8, 7.5 Hz, *H*–C(8)), 8.07 (1H, d, *J*=1.9 Hz, *H*–C(2′)), 8.10 (1H, dd, *J*=1.1, 7.9 Hz, *H*–C(7′)), 8.49 (1H, s, *H*–C(2)), 9.09 (1H, dt, *J*=1.1, 7.9 Hz, *H*–C(4′)), 11.48 (1H, s, N *H*). ^{13}C NMR (DMSO-d_6): δ 82.4, 90.6, 107.5, 112.7, 117.6, 120.2, 120.5, 120.7, 122.4, 123.8, 125.4, 127.6, 128.0, 136.9, 147.4, 196.6. (Found: C, 68.28; H, 3.59; N, 12.95. $C_{18}H_{11}N_3OS$ requires: C, 68.12; H, 3.49; N, 13.24); EI-HRMS: *m*/*z*=317.063300 (M^+); $C_{18}H_{11}N_3OS$ requires: 317.062284 (M^+); ν_{max} (KBr) 1654, 1586, 1538, 1501 cm^{-1}.

5.2.8. 6-(1*H*-Indol-3-yl)[1,2,4]triazolo[1,5-*a*]pyrimidin-7(4*H*)-one (10h). Prepared from **2a** and 3-amino-1,2,4-triazole (**9h**, 42 mg, 0.5 mmol); reflux for 5 h, 44 mg (35%) of a light yellow solid; mp 238–243 °C (ethyl acetate). EI-MS: *m*/*z*=251 (M^+). ^{1}H NMR (DMSO-d_6): δ 7.06 (1H, dt, *J*=1.1, 7.5 Hz, *H*–C(5′)), 7.15 (1H, dt, *J*=0.8, 8.3 Hz,

H–C(6′)), 7.45 (1H, dd, *J*=1.1, 8.3 Hz, *H*–C(7′)), 7.69 (1H, dt, *J*=0.8, 7.5 Hz, *H*–C(4′)), 7.78 (1H, d, *J*=2.2 Hz, *H*–C(2′)), 8.22 (1H, s, *H*–C(5)), 8.26 (1H, s, *H*–C(2)), 11.32 (1H, br s, N *H*). (Found: C, 61.41; H, 4.15; N, 27.16. $C_{13}H_9N_5O\times1/4H_2O$ requires: C, 61.05; H, 3.74; N, 27.38); ν_{max} (KBr) 1683, 1635, 1599 cm^{-1}.

5.2.9. 3-(1*H*-Indol-3-yl)-2*H*-pyrano[3,2-*c*]pyridin-2,5(6*H*)-dione (12a). Prepared from **2a** and 2,4-dihydroxypyridine (**11a**, 55 mg, 0.5 mmol); reflux for 7 h; 72 mg (52%) of a beige solid; mp >350 °C (ethyl acetate). EI-MS: *m*/*z*=278 (M^+), 279 (MH^+). ^1H NMR (DMSO-d_6): δ 6.41 (1H, d, *J*=7.4 Hz, *H*–C(8)), 7.19 (2H, m, *H*–C(5′), *H*–C(6′)), 7.50 (1H, dd, *J*=1.1, 6.8 Hz, *H*–C(7′)), 7.57 (1H, d, *J*=7.4 Hz, *H*–C(7)), 7.83 (1H, dd, *J*=1.1, 6.4 Hz, *H*–C(4′)), 8.09 (1H, d, *J*=2.6 Hz, *H*–C(2′)), 8.25 (1H, s, *H*–C(2)), 11.59 and 11.99 (2H, 2s, 1:1, 2N *H*). ^{13}C NMR (DMSO-d_6): δ 98.4, 109.0, 109.9, 113.2, 119.9, 120.7, 121.2, 122.8, 125.8, 128.6, 131.1, 137.3, 160.0, 161.2, 164.6, 172.9. (Found: C, 68.81; H, 3.45; N, 9.92. $C_{16}H_{10}N_2O_3$ requires: C, 69.06; H, 3.62; N, 10.07); HRMS: *m*/*z*=278.068250 (M^+); $C_{16}H_{10}N_2O_3$ requires: 278.069142 (M^+); ν_{max} (KBr) 1725, 1660, 1590, 1551 cm^{-1}.

5.2.10. 6-(1*H*-Indol-3-yl)-1,3-dimethyl-2*H*-pyrano[2,3-*d*]pyrimidine-2,4,7(1*H*,3*H*)-trione (12b). Prepared from **2a** and 1,3-dimethylbarbituric acid (**11b**, 78 mg, 0.5 mmol); reflux for 1.5 h; 162 mg (78%) of a light yellow solid; mp 330–334 °C (ethyl acetate, decomp). EI-MS: *m*/*z*=323 (M^+). ^1H NMR (DMSO-d_6): δ 3.28 (3H, s, C H_3), 3.44 (3H, s, C H_3), 7.18 (2H, m, *H*–C(5′), *H*–C(6′)), 7.49 (1H, dd, *J*=1.5, 6.4 Hz, *H*–C(7′)), 7.73 (1H, dd, *J*=1.1, 6.4 Hz, *H*–C(4′)), 7.93 (1H, d, *J*=2.6 Hz, *H*–C(2′)), 8.13 (1H, s, *H*–C(2)), 11.52 (1H, s, N *H*). (Found: C, 62.94; H, 3.97; N, 12.87. $C_{17}H_{13}N_3O_4$ requires: C, 63.15; H, 4.05; N, 13.00); HRMS: *m*/*z*=323.091500; $C_{17}H_{13}N_3O_4$ requires: *m*/*z*=323.090606); ν_{max} (KBr) 1762, 1715, 1670, 1656 cm^{-1}.

5.2.11. 3-(1*H*-Indol-3-yl)-7-methyl-2*H*,5*H*-pyrano[4,3-*b*]pyrane-2,5-dione (12c). Prepared from compound **2a** and 4-hydroxy-6-methyl-2*H*-pyran-2-one (**11c**, 63 mg, 0.5 mmol); reflux for 1 h; 65 mg (44%) of a greenish solid; mp 330–333 °C (ethyl acetate, decomp). EI-MS: *m*/*z*=293 (M^+). ^1H NMR (DMSO-d_6): δ 2.35 (3H, s, C H_3), 6.69 (1H, s, *H*–C(8)), 7.21 (2H, m, *H*–C(5′), *H*–C(6′)), 7.50 (1H, dd, *J*=2.1, 7.1 Hz, *H*–C(7′)), 7.80 (1H, dd, *J*=2.1, 6.4 Hz, *H*–C(4′)), 8.04 (1H, s, *H*–C(2)), 8.08 (1H, d, *J*=2.6 Hz, *H*–C(2′)), 11.64 (1H, s, N *H*). ^{13}C NMR (DMSO-d_6): δ 20.7, 99.7, 102.7, 108.6, 113.3, 119.9, 121.3, 121.6, 122.9, 125.6, 128.8, 130.4, 137.3, 159.2, 161.2, 162.9, 164.9. (Found: C, 69.62; H, 3.78; N, 4.76. $C_{17}H_{11}NO_4$ requires: C, 69.62; H, 3.78; N, 4.78); HRMS: *m*/*z*=293.068850 (M^+); $C_{17}H_{11}NO_4$ requires: *m*/*z*=293.068808 (M^+); ν_{max} (KBr) 1708, 1592 cm^{-1}.

5.2.12. 3-(1*H*-Indol-3-yl)-2*H*,5*H*-pyrano[3,2-*c*]chromene-2,5-dione (12d). Prepared from **2a** and 4-hydroxycoumarine (**11d**, 81 mg, 0.5 mmol); reflux for 2 h; 120 mg (73%) of an orange solid; mp 181–183 °C (ethyl acetate). EI-MS: *m*/*z*=329 (M^+), 330 (MH^+). ^1H NMR (DMSO-d_6): δ 7.24 (2H, m, *H*–C(5′), *H*–C(6′)), 7.54 (3H, m, *H*–C(7′), *H*–C(10), *H*–C(9)), 7.77 (1H, dt, *J*=1.1, 7.2 Hz, *H*–C(8)), 7.87 (1H, dd, *J*=1.9, 6.8 Hz, *H*–C(4′)), 8.04 (1H, dd, *J*=1.1, 7.2 Hz *H*–C(7)), 8.19 (1H, s, *H*–C(4)), 8.20 (1H, d, *J*=3.0 Hz, *H*–C(2′)), 11.75 (1H, s, N *H*). ^{13}C NMR (DMSO-d_6): δ 105.3, 108.6, 113.4, 114.1, 118.0, 120.0, 121.6, 123.1, 123.4, 125.6, 126.2, 129.4, 130.2, 134.4, 137.5, 153.2, 157.5, 158.9, 159.8, 169.2. (Found: C, 72.83; H, 3.31; N, 4.27. $C_{20}H_{11}NO_4$ requires: C, 72.95; H, 3.37; N, 4.25); HRMS: *m*/*z*=329.069950 (M^+); $C_{20}H_{11}NO_4$ requires: *m*/*z*=329.068808 (M^+); ν_{max} (KBr) 1713, 1628, 1573, 1521 cm^{-1}.

5.2.13. 5-Hydroxy-2-(1*H*-indol-3-yl)-3*H*-benzo[*f*]chromen-3-one (12e). Prepared from **2a** and 2,3-dihydroxynaphtalene (**11f**, 80 mg, 0.5 mmol); reflux for 3 h, 15 mg (9%) of an orange solid; mp 270–274 °C (ethyl acetate, decomp.). EI-MS *m*/*z*=327 (M^+). ^1H NMR (DMSO-d_6): δ 7.24 (2H, m, *H*–C(8), *H*–C(9)), 7.43 (1H, s, *H*–C(1)), 7.54 (3H, m, *H*–C(7), *H*–C(10), *H*–C(4′)), 7.84 (1H, m, *H*–C(5′)), 8.05 (1H, m, *H*–C(6′)), 8.19 (1H, s, *H*–C(6)), 8.49 (1H, m, *H*–C(7′)), 8.97 (1H, s, *H*–C(2′)), 10.56 (1H, s, OH), 11.65 (1H, s, NH). (Found: C, 77.03; H, 3.98; N, 3.94. $C_{21}H_{13}NO_3$ requires: C, 77.05; H, 4.00; N, 4.28); HRMS: *m*/*z*=327.090300 (M^+); $C_{21}H_{13}NO_3$ requires: *m*/*z*=327.089543 (M^+); ν_{max} (KBr) 1679, 1628, 1590, 1570 cm^{-1}.

5.2.14. 5-(1*H*-Indol-3-yl)-1-phenyl-3-methylpyrano[2,3-*c*]pyrazol-6(1*H*)-one (12f). Prepared from **2a** and 1-phenyl-3-methyl-1*H*-pyrazol-5(4*H*)-one (**11f**, 87 mg, 0.5 mmol); reflux for 4 h; 138 mg (81%) of a greenish solid; mp 225–230 °C (ethyl acetate, decomp.). EI-MS: *m*/*z*=341 (M^+). ^1H NMR (DMSO-d_6): δ 3.30 (3H, s, C H_3), 7.12 (1H, dt, *J*=1.1, 7.2 Hz, *H*–C(5′)), 7.18 (1H, dt, *J*=1.1, 6.8 Hz, *H*–C(6′)), 7.41 (1H, dd, *J*=1.1, 6.8 Hz, *H*–C(7′)), 7.48 (1H, dd, *J*=1.1, 7.2 Hz, *H*–C(4′)), 7.57–7.63 (2H, m, 2H od Ph), 7.90–7.85 (4H, m, 3H od Ph in *H*–C(2′)), 8.27 (1H, s, *H*–C(7)), 11.40 (1H, s, NH). (Found: C, 73.80; H, 4.30; N, 12.40. $C_{21}H_{15}N_3O_2$ requires: C, 73.89; H, 4.43; N, 12.31; HRMS: *m*/*z*=341.117550 (M^+); $C_{21}H_{15}N_3O_2$ requires: 341.116427 (M^+); ν_{max} (KBr) 1724, 1580 cm^{-1}.

5.3. X-ray structure determination

The crystallographic data for compound **2b** have been deposited with the Cambridge Crystallographic Data Centre as supplementary material with the deposition number: CCDC 228170. These data can be obtained, free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html.

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The diffraction data for compound **2b** were collected on the Kappa CCD Nonius diffractometer in the Laboratory of Inorganic Chemistry, Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia. We acknowledge with thanks the financial contribution of the Ministry of Science and Technology, Republic of Slovenia

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Electrochemical preparation of α,α′-dicarbonylselenides

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Abstract—A facile preparation of α,α′-dicarbonylselenides has been performed by reaction of α-carbonyl selenocyanates with an enolate, electrogenerated by reduction of a carbon–halogen bond.

1. Introduction

Löwig prepared the first organoselenium compound, diethylselenide, in 1836.[1] However the utility in synthesis of the selenides, remained largely unexploited until the early 1970s.

Selenides are involved in important transformations such as the synthesis of alkanes,[2–4] alkenes[5,6] and alkylhalides.[7,8] Selenides have been shown to act as catalyst for the asymmetric addition of organozinc reagents to aldehydes.[9–11]

The chemistry of macrocyclic ligands containing selenium has attracted much attention due to the fact that the lower electronegativity combined with the greater σ electron-donating properties of Se should yield complexes with interesting structures and redox behavior.[12] Some α-(phenylselenenyl) ketones exhibit glutathione peroxidase (GPx) activity.[13,14] α-(Phenylselenenyl)acetophenone showed catalytic effect of some GPx mimics on the thiol reduction of cytochrome C.[15]

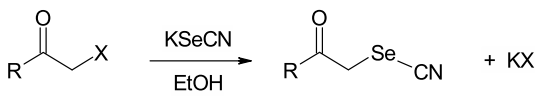
In spite of organoselenium chemistry has been, over the last 25 years, the subject of constant scientific interest and it has been used intensively,[16] relatively few syntheses of selenides have been described. Methyl and phenylalkyl-selenides have been prepared from alkylhalides and selenolates[17] or α-selenoalkyllithiums;[2] from arylhalides and selenolates;[18] from alcohols and arylselenocyanates[8,19] and from alcohols and selenols.[20] Dialkylselenides, including unsymmetrical dialkylselenides, can be synthesized from alkylhalides, Se and NaH using the proper Se/NaH ratio of 1:2.[21] One pot synthesis of ditertiarybutylselenide from the reaction of $SeCl_2$, formed in situ from $SeCl_4$ and Se in THF with ^tBuLi[22] has also been described. Allyl selenides have been synthesized via the reaction of allylbromide with diselenides promoted by the Sm–$BiCl_3$ system in aqueous media[23] and (*E*)-arylvinylselenides were prepared by coupling of (*E*)-vinylselenozirconocenes and diaryl-iodonium salts.[24]

In the present paper we describe the synthesis of α,α′-dicarbonylselenides by reaction of electrogenerated enolates over α-carbonylselenocyanates.

Organoselenium derivatives are no longer systematically classified as toxic and some of them can be bought not only from the chemical companies, but also in several super-markets as a food supplement.[21]

2. Results and discussion

The synthesis of electroactive selenocyanates proceeded by simple nucleophilic displacement of readily available halides with KSeCN (Scheme 1).



R= Ph, 4-$MeOC_6H_4$, tBu
X= Br, Cl

Scheme 1.

In a previous paper we have studied the cathodic reduction of phenacylthiocyanate in aprotic medium leading to an enolate which behaves as nucleophile, in an addition or substitution reaction, or as electrogenerated base.[25] The major product, due to the good leaving group properties of thiocyanate, is the corresponding dimer, obtained by attack

Keywords: Selenides; Cathodic reduction; Phenacyl halides; High dilution; α′-Carbonylselenocyanates.
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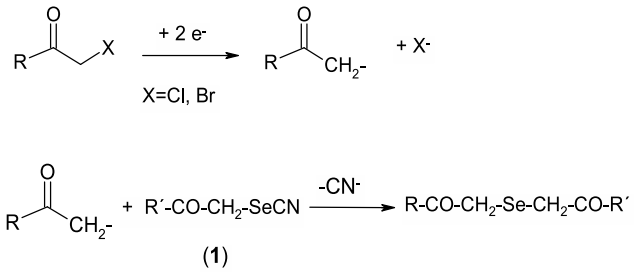
of the electrogenerated anion to another molecule of phenacylthiocyanate.

The electrochemical behavior of phenacylselenocyanate is different. The organic selenocyanates undergo an initial displacement of CN^- by attack of nucleophilic reagents.[26] On the other hand, the α-carbonylthiocyanates do not react with mercury (cathode) but the α-carbonylselenocyanates are destroyed in the presence of mercury.

The aims of our work were to prepare α,α′-dicarbonylselenides by reaction of electrogenerated enolates and α-carbonylselenocyanates.

An additional problem was that α-halocarbonyl compounds are only reduced at a mercury electrode, because at Pt or carbon electrode passivation occurs. To minimize the reaction between mercury and α-carbonylselenocyanate, a solution of it was added in several portions to the cathodic compartment during the electrolysis. At the same time, a solution of the electroactive α-halocarbonyl compound was dropped into the catholyte, where it was immediately reduced under the applied potential conditions. A new drop was added only when the current dropped to zero. This way the electrolysis was carried out under high-diluted conditions on the electroactive substrate.

The first step is the reduction of the carbon–halogen bond to afford the corresponding enolate, which attacks the selenocyanate (**1**) as it is indicated in Scheme 2.



Scheme 2.

It is important to notice that, for the preparation of unsymmetrical selenides, it does not matter whether the reaction is run with R–CO–CH_2–X and R′–CO–CH_2–SeCN or with R′–CO–CH_2–X and R–CO–CH_2–SeCN. In both cases the same selenide is obtained and with similar yields. The corresponding phenones were obtained as main side products together with a small amount of phenacyl diselenides, probably afforded by decomposition of an organomercury derivative formed when the selenocyanates react with the mercury electrode.

3. Experimental

All chemicals were handled in a chemical fume hood to avoid direct physical contact and inhalation of these unpleasant-smelling compounds.

Preparation of phenacylselenocyanate (**1**): a solution of phenacylchloride (30 mmol) in 30 mL EtOH was added dropwise to a KSeCN solution (30 mmol in 30 mL EtOH) under stirring conditions. Once the addition is finished (1 h), the mixture is maintained for half an hour to complete the reaction. Evaporation of the solvent to dryness gave a crude product (over 80% yield), which was washed with water and then recrystallized from EtOH/H_2O (3:2) to afford **1**.

3.1. General electrochemical procedure

The electrochemical reductions were performed under potentiostatic conditions in a concentric cell with two compartments separated by a porous (D3) glass diaphragm and equipped with a magnetic stirrer. A mercury pool (20 cm^2) was used as the cathode, a platinum plate as the anode, and a saturated calomel electrode as the reference. The solvent-supporting electrolyte system (SSE) was nominally anhydrous acetonitrile containing 0.05 M lithium perchlorate. Anhydrous potassium carbonate was added to the anodic compartment for ‘in situ’ neutralization of the generated perchloric acid.

A solution of the electroactive phenacylchloride (1.0 mmol in 10 mL of SSE) was dropped into the cathodic compartment to be electrolyzed at a constant potential of −0.8 V (versus SCE). Simultaneously, a solution of the carbonylselenocyanate (**1**) (1.0 mmol in 20 mL SSE) was added in portions of 4 mL into the catholyte during the electrolysis, **1** being no-electroactive under the applied potential conditions. When the electrochemical process was finished, the solvent in the cathodic solution was removed under reduced pressure. The residue was extracted with ether/water and the organic phase dried over Na_2SO_4 and concentrated by evaporation. The resulting solid was chromatographed on a silica gel (18×3 cm) column, using CH_2Cl_2/hexane (3:2) as eluent and was characterized. Spectroscopic description of all the compounds is given below.

3.1.1. Phenacylselenocyanate (1a). Mp 86–87 °C. (Lit.[27a] 88–89 °C, lit.[27b] 85 °C). IR (KBr) ν(cm^{-1}): 3054, 2944, 2154, 1666, 1592, 1374, 1185, 995, 755, 688. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.96 (s, 2H), 7.54 (t, 2H, J=7.2 Hz), 7.67 (d, 1H, J=7 Hz), 7.96 (d, 2H, J=7 Hz). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 38.7, 102, 128.8, 129.2, 133.8, 134.9, 193.2. MS *m/e* (relative intensity) EI: 227 (M^++6, 1), 225 (M^++4, 3), 223 (M^++2, 2), 221 (M^+, 1), 105 (100), 91 (11), 77 (36), 51 (12).

3.1.2. 4-Methoxyphenacylselenocyanate (1b). Mp 115–117 °C. IR (KBr) ν(cm^{-1}): 3051, 2988, 2934, 2151, 1643, 1603, 1570, 1260, 1172, 997, 821. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.9 (s, 3H), 4.9 (s, 2H), 6.98 (d, 2H, J=9 Hz), 7.92 (d, 1H, J=9 Hz). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 38.7, 55.9, 102, 114.6, 127.0, 165.1, 191.7. MS *m/e* (relative intensity) EI: 255 (M^++4, 4), 253 (M^++2, 3), 251 (M^+, 1), 135 (100), 121 (14), 92 (12), 77 (18), 63 (9). Anal. Calcd for $C_{10}H_9NO_2Se$: C, 47.06; H, 3.53. Found: C, 47.23; H, 3.44.

3.1.3. 3,3-Dimethyl-2-oxo-butylselenocyanate (1f). Mp

94–96 °C. IR (KBr) ν(cm^{-1}): 2971, 2154, 1689, 1367, 1062, 1007, 854. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.23 (s, 9H), 4.53 (s, 2H). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 26.8, 27.2, 37.9, 102, 210. MS *m/e* (relative intensity) EI: 205 (M^++4, 2), 203 (M^++2, 1), 85 (6), 57 (100). Anal. Calcd for $C_7H_{11}NOSe$: C, 40.97; H, 5.36. Found: C, 41.17; H, 5.41.

3.1.4. 2-(Phenacylseleno)acetophenone (2a). 63% yield. Mp 67–69 °C. IR (KBr) ν(cm^{-1}): 3060, 2960, 1672, 1595, 1279, 1184, 750, 684. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.0 (s, 4H), 7.40–7.60 (m, 6H), 8.0 (d, 4H, J=7 Hz). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 28.9, 128.8, 133.6, 135.3, 195.0. MS *m/e* (relative intensity) EI: 315 (M^++4, 4), 316 (M^++2, 2), 314 (M^+, 1), 237 (38), 105 (100), 91 (20), 77 (50), 51 (15). Anal. Calcd for $C_{16}H_{14}O_2Se$: C, 60.57; H, 4.42. Found: C, 60.45; H, 4.60.

3.1.5. 2-(4-Methoxy-phenacylseleno)acetophenone (2b). 57% yield. Mp 48–50 °C. IR (NaCl) ν(cm^{-1}): 3061, 2960, 2839, 1665, 1598, 1275, 1173. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.86 (s, 3H), 3.95 (s, 2H), 3.98 (s, 2H), 6.93 (d, 2H, J=8 Hz), 7.46 (t, 2H, J=7.5 Hz), 7.57 (t, 1H, J=7.5 Hz), 7.9–8.0 (m, 4H). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 28.8, 55.7, 114.1, 128.5, 128.9, 129.0, 131.3, 133.6, 135.5, 164.0, 194.0, 195.3. MS *m/e* (relative intensity) EI: 348 (M^++4, 2), 346 (M^++2, 1), 267 (23), 187 (4), 150 (17), 135 (100), 121 (17), 105 (22), 91 (8), 77 (30), 51 (8). Anal. Calcd for $C_{17}H_{16}O_3Se$: C, 58.79; H, 4.61. Found: C, 59.04; H, 4.58.

3.1.6. 2-(4-Chloro-phenacylseleno)acetophenone (2c). 42% yield. Mp 62–64 °C. IR (KBr) ν(cm^{-1}): 3060, 2923, 1668, 1589, 1338, 1276, 1091. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.95 (s, 2H), 3.98 (s, 2H), 7.4–7.6 (m, 5H) 7.86–8.0 (m, 4H). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 28.7, 29.0, 128.9, 129.0, 129.2, 129.9, 130.3, 133.7, 135.4, 140.1, 193.9, 195.1. MS *m/e* (relative intensity) EI: 354 (M^++6, 3), 352 (M^++4, 7), 350 (M^++2, 3), 237 (140), 154 (177), 156 (73), 141 (269), 139 (803), 113 (113), 11 (34), 105 (1000), 91 (172), 77 (496), 51 (177). Anal. Calcd for $C_{16}H_{13}ClO_2Se$: C, 54.62; H, 3.7. Found: C, 54.77; H, 3.90.

3.1.7. 2-(4-Bromo-phenacylseleno)acetophenone (2d). 62% yield. Mp 34–36 °C. IR (NaCl) ν(cm^{-1}): 3057, 2923, 1666, 1583, 1272, 1070, 1004. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.94 (s, 2H), 3.98 (s, 2H), 7.47 (t, 2H, J=7.4 Hz) 7.55–7.63 (m, 3H), 7.83 (d, 2H, J=8.5 Hz), 7.95 (d, 2H, J=7.4 Hz). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 28.8, 29.2, 128.9, 129.0, 130.4, 132.2, 133.8, 134.2, 135.4, 194.0, 194.9. MS *m/e* (relative intensity) EI: 398 (M^++6, 15), 396 (M^++4, 21), 394 (M^++2, 9), 392 (M^+, 3), 317 (161), 315 (156), 185 (248), 183 (250), 157 (106), 155 (109), 105 (1000), 91 (192), 77 (521), 51 (264). Anal. Calcd for $C_{16}H_{13}BrO_2Se$: C, 48.48; H, 3.28. Found: C, 48.31; H, 3.43.

3.1.8. 2-(4-Phenyl-phenacylseleno)acetophenone (2e). 64% yield. Mp 37–39 °C. IR (NaCl) ν(cm^{-1}): 3060, 1667, 1599, 1274, 1181. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.0 (s, 2H), 3.02 (s, 2H), 7.40–7.50 (m, 5H), 7.53–7.70 (m, 5H), 7.95–8.1 (m, 4H). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 28.9, 127.5, 127.6, 128.5, 128.9, 129.2, 129.5, 133.7, 134.2, 135.5, 139.9, 146.4, 194.8, 195.2. MS *m/e* (relative intensity) EI: 396 (M^++6, 1), 394 (M^++4, 3), 392 (M^++2, 1), 390 (M^+, 1), 313 (25), 196 (24), 181 (100), 167 (25), 152 (71), 105 (72), 77 (61), 51 (28). Anal. Calcd for $C_{22}H_{18}O_2Se$: C, 67.18; H, 4.58. Found: C, 66.90; H, 4.69.

3.1.9. 2-(3,3-Dimethyl-2-oxo-butylseleno)acetophenone (2f). 40% yield. Mp 41–43 °C. IR (NaCl) ν(cm^{-1}): 3052, 2955, 2928, 1724, 1668, 1595, 1275, 1055. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.21 (s, 9H), 3.64 (s, 2H), 3.94 (s, 2H), 7.47 (t, 2H, J=7.6 Hz), 7.57 (t, 1H, J=7.6 Hz), 7.96 (d, 2H, J=7.6 Hz). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 26.9, 27.8, 28.7, 128.5, 128.6, 133.2, 135.0, 194.8, 209.8. MS *m/e* (relative intensity) EI: 298 (M^++4, 6), 296 (M^++2, 3), 294 (M^+, 1), 217 (17), 149 (8), 120 (17), 105 (94), 91 (37), 77 (52), 57 (100), 51 (20). Anal. Calcd for $C_{14}H_{18}O_2Se$: C, 56.56; H, 6.06. Found: C, 56.51; H, 5.89.

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Novel class of saccharide-based organogelators: glucofuranose derivatives as one of the smallest and highly efficient gelators

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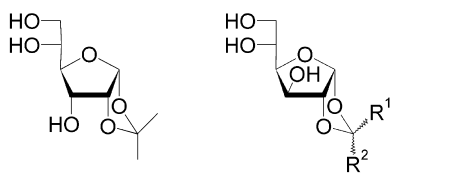
Dedicated to the memory, teachings and wisdom of Professor Aleksander Zamojski

Abstract—Gluco- and allofuranose derivatives, having unprotected three OH groups, were investigated as potential gelators, showing high gelating ability for a variety of organic solvents and result in new consistent class of organogelators. The minimum gelator concentration reaches 0.03%, which is one of the lowest concentrations achieved so far. The correlation between the saccharide crystal structure and its gelating ability was also examined. The SEM pictures of xerogeles obtained from concentrated and diluted gels (1.0–0.03%) show significant differences in μm-scale structure.

In recent years the development of new gelators of organic solvents as well as the investigation of gelating process and gel structure has received much attention.[1] According to the type of driving forces of molecular aggregation, low molecular mass gelators can be classified into two categories: nonhydrogen-bond-based gelators and hydrogen-bond-based gelators. Saccharides, having free OH groups, fall within the latter group. The presence of intermolecular hydrogen bonds is responsible for self-assembling of the gelator molecules which leads to the formation of a fibrous superstructure, as may be observed in the TEM or SEM pictures of xerogels. The structure of a particular fibril can be investigated using small angle X-ray scattering (SAXS). Analysis of the way in which gelator molecules arrange in a bulk crystal may help to predict the gelating ability.[2] Although the last decade brought much progress in the field of low molecular mass organogelators, a proper design of new gelators is still a hard task or very often even a matter of chance. Previously, 4,6-*O*-benzylidene-protected monosaccharides have been extensively studied.[3] The idea was to build a large, consistent library of relatively similar compounds having however, different gelating abilities. This may be regarded as a basic step for understanding the gelating process and combining it with such parameters as molecular conformation, solubility, gelator–solvent and gelator–gelator interactions etc.

In this paper we introduce a new class of potential gelators. These saccharide-based compounds are common in having unprotected 3, 5 and 6 OH groups and a furanose ring (Fig. 1). Compounds **1**–**6** were synthesised according to the general procedure[4,5] and utilized to perform gelating tests. The saccharide was mixed with solvent (~0.5 mL) in a close-capped test tube and the mixture was heated until the solid dissolved (the solvent boiling point was reached during this process). The solution was cooled to room temperature and left for 24 h. The gelator concentration varied from 3 to 1% [g mL^{-1}] or even lower for **3**. Compounds **4**–**6** were obtained as mixtures of two stereoisomers differing from each other in absolute configuration at dioxolane C-2′ carbon atom bridging dioxolane oxygen atoms. Because of difficulties in separation of stereoisomers only preliminary gelating tests were performed, showing that the mixture of stereoisomers has a gelating ability.



1

2: R^1, R^2 = CH_3
3: R^1, R^2 = CH_2CH_3
4: R^1 = CH_3, R^2 = CH_2CH_3
5: R^1 = CH_3, R^2 = $(CH_2)_2CH_3$
6: R^1 = CH_3, R^2 = $(CH_2)_3CH_3$

Figure 1. Structure of monosaccharides **1**–**6**.

Keywords: Monosaccharide; Gel; Organogelators; SEM; Crystal structure.
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Table 1. Organic solvents tested for gelation by **1**–**6**[a]

Solvent	1	2	3	4	5	6
Toluene	P	G	G*	G*	G	G
Benzene	P	G	G*	G*	G	G
p-Xylene	P	G	G*	G*	G	G
Carbon tetrachloride	P	G*	G*	Gp	G	G
Nitrobenzene	P	G	G*			
Chloroform	P	G*	G			
Dibenzyl ether	P	G*	G*			
Cyclohexane	P	G*	P			
n-Hexane	P	P	P			
n-Heptane	P	P	P			
Methanol	S	S	S			
Acetonitrile	S	P	S			
Ethyl acetate	P	P	P			
1,4-Dioxane	S	S	S			
THF	S	S	S			
Water	S	S	S			

[a] Abbreviations used: G=gel at 3.0 wt/vol%, *=gel even under 1.0 wt/vol%, Gp=partial gel, S=solution, P=precipitation.

It is clear from Table 1 that glucofuranose derivatives **2**–**6** act as excellent gelators while allofuranose **1** does not form gels at all. This fits to the previously proposed concept of correlating gelating ability and the way in which molecules pack in the bulk crystal. According to this concept, the tendency to form infinite one-dimensional hydrogen bond-based chains is one of the prerequisites for good gelators.

The crystal structures of **1**[6] and **2**[7] have been determined and are available from the Cambridge Structural Database. Compound **1** exhibits a two-dimensional hydrogen bond network, shown schematically in Figure 2(a), which is characteristic for poor gelators. On the other hand, molecules of **2** are assembled in one-dimensional chains as may be expected for a good gelator. Crystal structures for **3**–**6** are unknown mainly because of difficulties in growing crystals suitable for X-ray analysis.

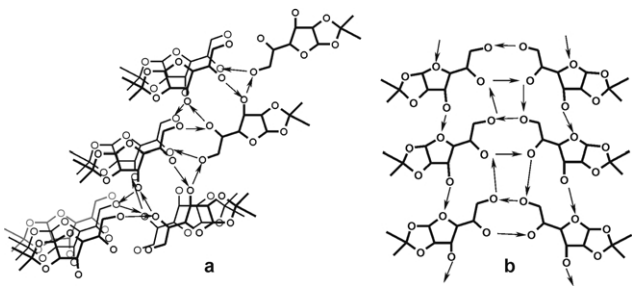

Figure 2. Schematic representation of hydrogen-bond network in **1** (a) and **2** (b). Arrows describe the hydrogen bonds (from donor to acceptor).

Beside the versatility of gelating solvents the wide range of possible concentrations describes the gelator 'quality'. Here **3** can form the gels with CCl_4 in concentrations ranging from 3 to 0.03%. In toluene the minimum gelator concentration (C_{min}) reaches 0.04%. Compared with other gelators (usually the reported C_{min} varied about 0.2% [g mL^{-1}]) these values represent one of the lowest concentrations achieved so far for organic solvents. In terms of molar ratio one molecule of **3** can immobilize almost 10^4 molecules of CCl_4. Only a few similar results can be found in the literature.[8–11] Moreover, a wide range of the gel concentration changing through the two rows of magnitude gives us the unique chance to study the gel structure dependence on gelator concentration. In fact, the preliminary SEM measurements of xerogels obtained from 1.0 and 0.03% gels of **3** in CCl_4 show two completely different structures (Fig. 3). In the case of 1% xerogel the hierarchical structure may be observed (fibrils arranged in 'ribbons').

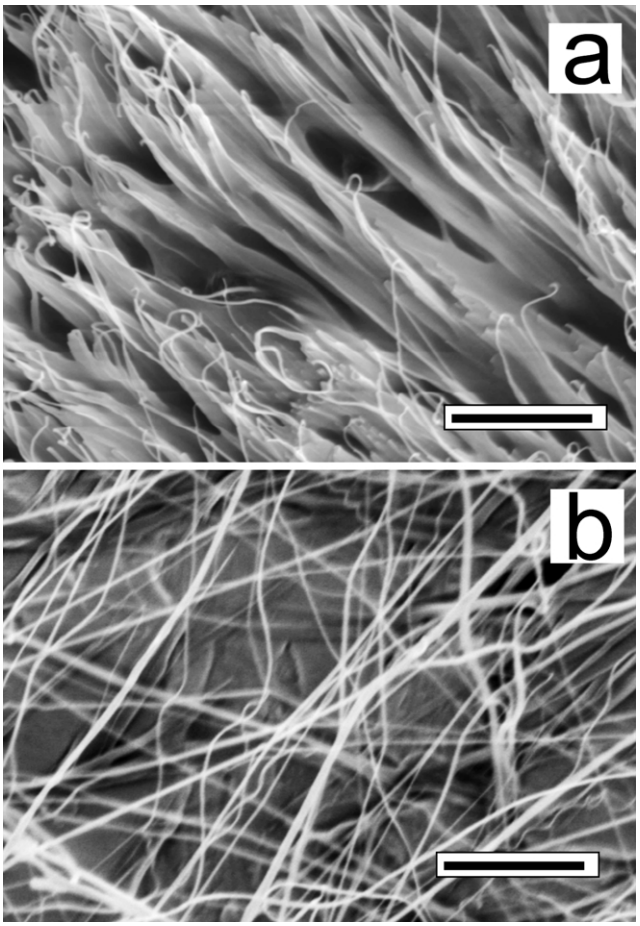

Figure 3. SEM images of the xerogels obtained from **3**/CCl_4 gels in concentration: (a) 1.0%, (b) 0.03% [g mL^{-1}]; scale bar 5 μm.

In conclusion, we have described a new class of low molecular mass organogelators, which may serve as excellent library compounds for the investigation of the gelation phenomenon. In particular, the advantages of this system are: variety of possible homologues among the group of relatively similar compounds, different gelating abilities (both good and poor gelators), wide concentration range of the obtained gels.

1. Experimental

Reagents were used as obtained from supplier. TLC was performed on Silica Gel HF-254 and column chromatography on Silica Gel 230–400 mesh (Merck). NMR spectra were recorded with a Varian AC-200 (200 MHz), Varian Mercury 400BB (400 MHz), and Bruker AM-500 (500 MHz) spectrometers in chloroform-d_1 or chloroform-d_1–methanol-d_4 mixture (approx. 10:1) with Me_4Si as internal standard. High-resolution mass spectra (HR-MS)

were measured with AMD-604 mass spectrometer. Optical rotations were measured with a JASCO DIP-360 automatic polarimeter.

1.1. General procedure

To a mixture of ketone (20–30 equiv.), anhydrous zinc chloride (1.5 equiv.) and 85% phosphoric acid (0.05 equiv.), D-glucose (50 mM) was added and stirred at room temperature for 2–6 days. Undissolved D-glucose was filtered off and washed with a small portion of ketone. The filtrate was made slightly alkaline with 40% aqueous sodium hydroxide, insoluble material was removed by filtration through a Celite pad and washed with acetone. The filtrate was evaporated to dryness and purified by column chromatography.

From 3-pentanone (hexane–ethyl acetate, 9:1, then hexane–ethyl acetate, 1:1, then ethyl acetate as eluents) two products were obtained.

1.1.1. 1,2:5,6-Di-*O*-(1-ethylpropylidene)-α-D-glucofuranose. Faster moving fraction contained the title compound (9%). Mp: 64–65 °C. $[\alpha]_D^{25}$=3.3 (*c* 0.74, chloroform). ^{1}H NMR ($CDCl_3$) δ: 5.98 (d, 1H, $J_{1,2}$=3.7 Hz, H-1), 4.56 (d, 1H, $J_{2,1}$=3.7 Hz, H-2), 4.33 (m, 2H, H-3,5), 4.22 (dd, 1H, $J_{6,6'}$=8.2 Hz, $J_{6,5}$=6.1 Hz, H-6), 4.13 (dd, 1H, $J_{4,3}$=2.9 Hz, $J_{4,5}$=7.7 Hz, H-4), 3.96 (dd, 1H, $J_{6',6}$=8.2 Hz, $J_{6',5}$=5.5 Hz, H-6′), 2.60 (d, 1H, *J*=3.7 Hz, OH), 1.68 (m, 8H, 4×CH_2), 0.93 (m, 12H, 4×CH_3). ^{13}C NMR ($CDCl_3$) δ: 116.48 (C), 114.04 (C), 105.90 (C-1), 86.12, 82.35, 75.99, 74.13, 68.85 (C-6), 30.62 (CH_2), 30.38 (CH_2), 30.13 (CH_2), 29.53 (CH_2), 8.86 (CH_3), 8.61 (CH_3), 8.51 (CH_3). HR-MS (ESI) calcd for $C_{16}H_{28}NaO_6$ $[M+Na]^+$: 339.1778. Found: 339.1794.

1.1.2. 1,2-*O*-(1-Ethylpropylidene)-α-D-glucofuranose (3). Slower moving fraction comprised the title compound: yield 13%. Mp: 125–127 °C. $[\alpha]_D^{25}$=−2.0 (*c* 0.3, chloroform). ^{1}H NMR ($CDCl_3$+CD_3OD) δ: 5.93 (d, 1H, $J_{1,2}$=3.7 Hz, H-1), 4.49 (d, 1H, $J_{2,1}$=3.7 Hz, H-2), 4.30 (d, 1H, $J_{3,4}$=2.7 Hz, H-3), 4.04 (dd, 1H, $J_{4,3}$=2.7 Hz, $J_{4,5}$=7.1 Hz, H-4), 3.96 (m, 1H, H-5), 3.82 (dd, 1H, $J_{6,5}$=3.2 Hz, $J_{6,6'}$=11.6 Hz, H-6), 3.65 (dd, 1H, $J_{6',5}$=5.8 Hz, $J_{6',6}$=11.6 Hz, H-6′), 1.70 (q, 2H, *J*=7.6 Hz, CH_2), 1.56 (q, 2H, *J*=7.5 Hz, CH_2), 0.93 (t, 3H, *J*=7.5 Hz, CH_3), 0.86 (t, 3H, *J*=7.6 Hz, CH_3). ^{13}C NMR ($CDCl_3$+CD_3OD) δ: 116.44 (C), 105.44 (C-1), 86.18, 80.73, 75.58, 70.43, 64.47 (C-6), 30.29 (CH_2), 8.80 (CH_3), 8.49 (CH_3). HR-MS (ESI) calcd for $C_{11}H_{20}NaO_6$ $[M+Na]^+$: 271.1152. Found: 271.1161.

1.1.3. 1,2:5,6-Di-*O*-(1-methylpropylidene)-α-D-glucofuranose. The title compound was obtained from 2-butanone (as a mixture of diastereoisomers). Hexane–ethyl acetate, 7:3 as eluent, yield 28%. ^{1}H NMR ($CDCl_3$) δ: 5.96 (d, $J_{1,2}$=3.6 Hz, H-1), 4.54 (dd, *J*=6.9, 3.7 Hz), 4.33 (m), 3.92–4.28 (m), 2.81 (m), 1.54–1.82 (m), 1.46 (s, C–CH_3), 1.38 (s, C–CH_3), 1.32 (s, C–CH_3), 1.28 (s, C–CH_3), 0.95 (m, CH_2CH_3). ^{13}C NMR ($CDCl_3$) δ: 106.09 (C-1), 105.56 (C-1), 86.38, 85.44, 82.10, 82.01, 81.95, 81.85, 75.72, 74.28, 73.88, 73.57, 68.49 (CH_2), 68.46 (CH_2), 68.32 (CH_2), 68.22 (CH_2), 33.31 (CH_2), 33.10 (CH_2), 33.05 (CH_2), 31.98 (CH_2), 27.30, 25.69, 25.16, 24.29, 8.99 (CH_3), 8.63 (CH_3), 8.57 (CH_3). HR-MS (ESI) calcd for $C_{14}H_{24}NaO_6$ $[M+Na]^+$: 311.1465. Found: 311.1460.

1.1.4. 1,2-*O*-(1-Methylpropylidene)-α-D-glucofuranose (4) (as a mixture of diastereoisomers). 1,2:5,6-Di-*O*-(1-methylpropylidene)-α-D-glucofuranose obtained above (1.05 g, 3.6 mM) was dissolved in methanol (15 mL), 0.8% solution of sulfuric acid (5 mL) was added and stirred for 5 h, neutralized with diethylamine, evaporated to dryness, and purified by column chromatography (ethyl acetate as eluent) to yield 636 mg of the title compound (75%). Mp: 117–118 °C. ^{1}H NMR ($CDCl_3$+CD_3OD) δ (major diastereoisomer): 5.93 (d, 1H, $J_{1,2}$=3.7 Hz, H-1), 4.48 (d, 1H, $J_{2,1}$=3.7 Hz, H-2), 4.28 (d, 1H, $J_{3,4}$=2.7 Hz, H-3), 4.06 (dd, 1H, $J_{4,3}$=2.7 Hz, $J_{4,5}$=8.7 Hz, H-4), 3.94 (m, 1H, H-5), 3.80 (dd, 1H, $J_{6,5}$=3.4 Hz, $J_{6,6'}$=11.6 Hz, H-6), 3.67 (dd, 1H, $J_{6',5}$=6.0 Hz, $J_{6',6}$=11.6 Hz, H-6′), 1.59 (m, 2H, *J*=7.5, 2.0 Hz, CH_2CH_3), 1.43 (s, 3H, CH_3), 0.90 (t, 3H, *J*=7.4 Hz, CH_2CH_3). Minor diastereoisomer: 5.93 (d, 1H, H-1), 4.52 (d, 1H, $J_{2,1}$=3.7 Hz, H-2), 4.30 (d, 1H, $J_{3,4}$=2.8 Hz, H-3), 4.06 (dd, 1H, $J_{4,5}$=5.7 Hz, H-4), 3.94 (m, 1H, H-5), 3.80 (dd, 1H, $J_{6,6'}$=11.6 Hz, H-6), 3.65 (dd, 1H, $J_{6',5}$=6.1 Hz, $J_{6',6}$=11.6 Hz, H-6′), 1.75 (q, 2H, *J*=7.5 Hz, CH_2CH_3), 1.27 (s, 3H, CH_3), 0.97 (t, 3H, *J*=7.5 Hz, CH_2CH_3). ^{13}C NMR ($CDCl_3$+CD_3OD) δ: 114.80 (C), 113.77 (C), 105.64 (C-1), 105.11 (C-1), 86.30, 85.38, 80.69, 80.50, 75.32, 75.27, 70.18, 64.43 (CH_2), 64.40 (CH_2), 33.13 (CH_2), 32.93 (CH_2), 24.69 (CH_3), 23.97 (CH_3), 8.84 (CH_3), 8.44 (CH_3). HR-MS (ESI) calcd for $C_{10}H_{18}NaO_6$ $[M+Na]^+$: 257.0996. Found: 257.0997.

1.1.5. 1,2-*O*-(1-Methylbutylidene)-α-D-glucofuranose (5) (as a mixture of diastereoisomers). The title compound was obtained from 2-pentanone. Hexane–ethyl acetate, 1:1, then ethyl acetate as eluents, yield 7%. Mp: 109–110 °C. ^{1}H NMR ($CDCl_3$+CD_3OD) δ: 5.87 (d, $J_{1,2}$=3.3 Hz, H-1). ^{13}C NMR ($CDCl_3$+CD_3OD) δ: 113.86 (C), 112.90 (C), 105.01 (C-1), 104.46 (C-1), 85.57, 84.70, 80.02, 79.84, 74.83, 74.74, 69.67, 69.59, 63.90 (CH_2), 63.84 (CH_2), 41.96 (CH_2), 41.69 (CH_2), 24.62 (CH_3), 23.80 (CH_3), 17.45 (CH_2), 16.97 (CH_2), 14.02 (CH_3). HR-MS (ESI) calcd for $C_{11}H_{20}NaO_6$ $[M+Na]^+$: 271.1152. Found: 271.1146.

1.1.6. 1,2-*O*-(1-Methylpentylidene)-α-D-glucofuranose (6) (as a mixture of diastereoisomers). The title compound was obtained from 2-hexanone. Hexane–ethyl acetate, 1:1, then ethyl acetate as eluents, yield 3%. Mp: 112–113 °C. ^{1}H NMR ($CDCl_3$+CD_3OD) δ: 5.89 (d, $J_{1,2}$=3.7 Hz, H-1). ^{13}C NMR ($CDCl_3$+CD_3OD) δ: 114.00 (C), 113.07 (C), 105.03 (C-1), 104.50 (C-1), 85.59, 84.72, 80.05, 79.85, 74.90, 74.81, 69.75, 69.66, 63.96 (CH_2), 63.86 (CH_2), 39.47 (CH_2), 39.32 (CH_2), 26.30 (CH_2), 25.85 (CH_2), 24.68 (CH_3), 23.81 (CH_3), 22.67 (CH_2), 22.65 (CH_2), 13.87 (CH_3), 13.81 (CH_3). HR-MS (ESI) calcd for $C_{12}H_{22}NaO_6$ $[M+Na]^+$: 285.1309. Found: 285.1314.

1.2. Gelation test procedure

The gelator (about 15 mg) was mixed in a closed-capped test tube with the appropriate amount of solvent (about 0.5 mL) to result in a concentration of 3% [g mL^{-1}]. The mixture was heated until the solid was dissolved and than about one further minute. By this procedure the solvent bp

was reached. The test tube was cooled in air to 20 °C, left for 24 h at this temperature and then turned upside down. When the gelator formed a gel the whole procedure was repeated with the concentration below 1%. All gels were also examined by using the optical microscope to exclude self-supporting precipitation.

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Oligonucleotides containing new fluorescent 1-phenylethynylpyrene and 9,10-bis(phenylethynyl)anthracene uridine-2′-carbamates: synthesis and properties☆

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Abstract—The synthesis of two novel fluorescent uridine-2′-carbamate phosphoramidites is described. The reagents carrying fluorescent polyaromatic hydrocarbons 1-phenylethynylpyrene (PEPy) or 9,10-bis(phenylethynyl)anthracene (BPEA) are suitable for oligonucleotide synthesis. Prepared oligonucleotide conjugates show strong dye emissions at 401 and 485 nm, but low FRET rate when located in the oligonucleotide duplex. The dyes show considerable compensation of the usual carbamate duplex destabilization. The possible explanation of both effects is binding of PEPy and BPEA to the minor groove of the DNA duplex.

1. Introduction

Oligonucleotides conjugates with fluorescent dyes have numerous and diverse applications in molecular biology, bioorganic chemistry, and medicine. The sugar part of nucleosides, particularly the 2′-position, is an attractive site for modification, for example, for potential antisense applications.[1] Examples of the introduction of hetero- and polyaromatic residues at the 2′ position of nucleosides include dansyl,[2] anthracene,[3] anthraquinone,[4] fluorescein,[5] nalidixic acid,[6] porphyrins,[7] 1,8-naphthalimide,[8] and pyrene.[9] Aromatic ligands attached to the 2′-positions of ribonucleotides in oligonucleotides are placed in the minor groove and can affect duplex stability. Pyrene, a fluorescent tetracyclic aromatic hydrocarbon, is of particular interest because of its abilities to give a response in the fluorescent spectrum upon hybridization of pyrene-labeled oligonucleotides[9b,e–i,k,n] and to form excimers.[9n,o,q] Recently, we developed a convenient method of 2′-carbamate modification of uridine with pyrene,[9n] and non-nucleotide modifying reagents for introducing new fluorescent polyaromatic hydrocarbons 1-phenylethynylpyrene (PEPy) and 9,10-bis(phenylethynyl)anthracene (BPEA) into oligonucleotides.[10] PEPy and BPEA were shown to constitute an energy donor–acceptor pair, and were also able to form excimers. Here, we report the introduction of PEPy and BPEA in the 2′-position of uridine as well as the fluorescent and thermal denaturation properties of modified oligonucleotides.

2. Results and discussion

2.1. Syntheses of phosphoramidites

The first step in the preparation of PEPy-modified uridine-2′-carbamate phosphoramidite **6** is alkynylation of the starting 2′-*O*-(4-iodobenzylaminocarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uridine **1**[9n] with 1-ethynylpyrene in the conditions of Sonogashira coupling ($Pd(PPh_3)_4$/CuI in DMF in the presence of triethylamine)[11] (Scheme 1). The reaction was performed under Ar to reduce the quantity of side product **3** from Glaser acetylene coupling. It is worthy of note, that the reacton mixture should be washed with water many times upon workup to remove the solvent (DMF), otherwise traces of **3** can accompany the main product in the course of column purification on silica gel. The Markiewicz' 3′,5′-*O*-silyl group from **2** was removed with triethylamine trihydrofluoride in THF.[9n] Crystalline nucleoside **4** ($\mathbf{U^P}$) was 5′-*O*-dimethoxytritylated, and then 3′-*O*-phosphitylated using standard methods of nucleoside chemistry.[12] Pyridine (not triethylamine) should be added to the eluent for neutralization of silica gel in chromatographic purification of 5′-*O*-Dmt derivative **5**, because more basic conditions can lead to

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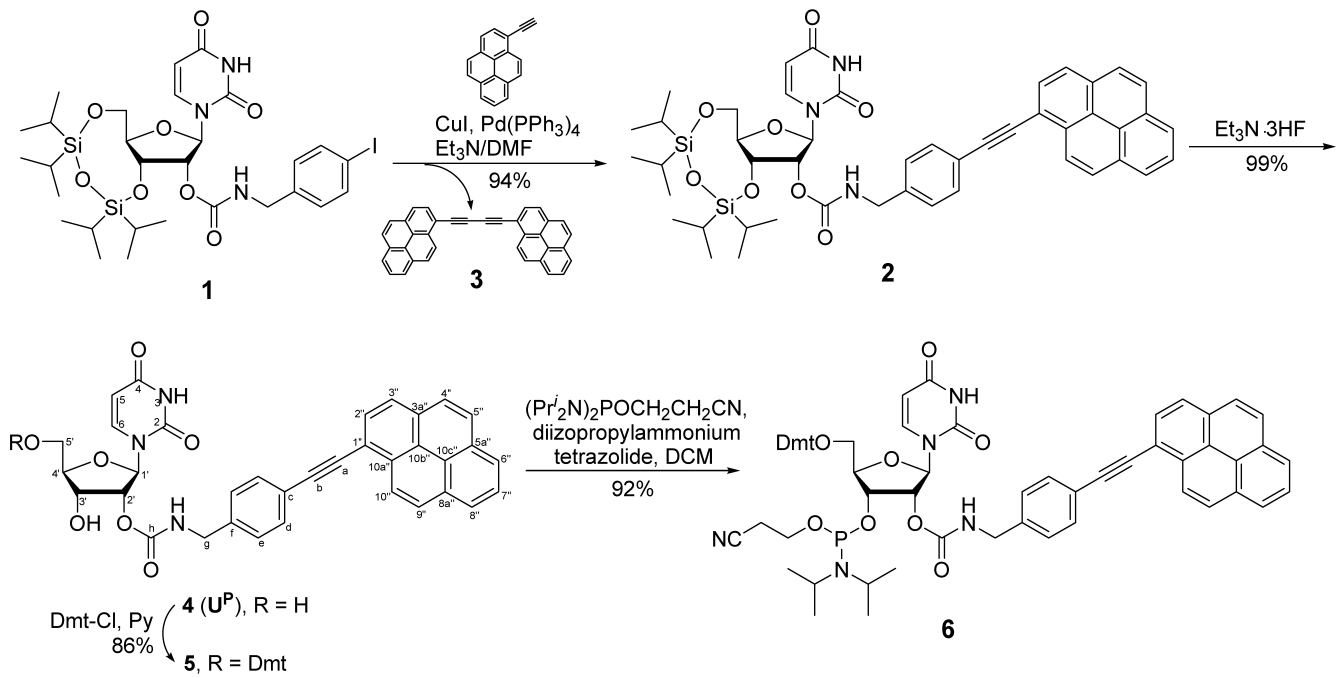
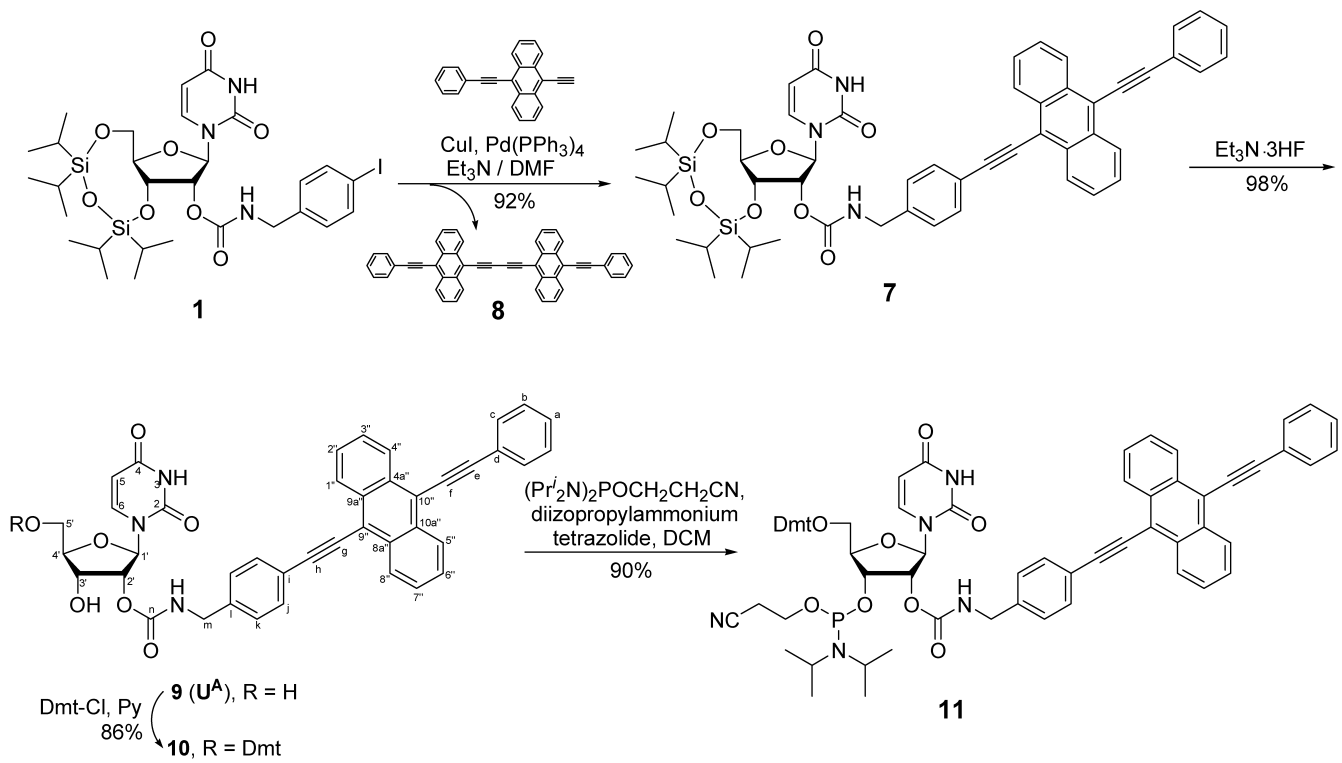


Scheme 1. Synthesis of PEPy uridine-2′-carbamate phosphoramidite **6**.

2′→3′ migration of the carbamoyl group.[9n] A small amount of bis-3′,5′-*O*-Dmt nucleoside **5a** was also isolated. Phosphoramidite **6** was isolated as a solid suitable for prolonged storage (2 years).

It was also tempting to synthesize a similar fluorescent nucleoside which could serve as an energy acceptor for the PEPy nucleoside $\mathbf{U^P}$. BPEA nucleoside $\mathbf{U^A}$ and its 5′-*O*-Dmt-3′-*O*-phosphoramidite derivative **11** were prepared similar to PEPy compounds (Scheme 2). 9-Ethynyl-10-phenylethynylanthracene[10] was used for the assembly of a dye molecule.

The structures of modified nucleosides $\mathbf{U^P}$ and $\mathbf{U^A}$ were confirmed by $^1H-^{13}C$ NMR correlations. The first step in the assignment of ^{13}C NMR spectra was distinguishing the proton-bound carbon atoms taking into account cross-peaks on HMQC spectra. The signals of non-protonated carbons



Scheme 2. Synthesis of BPEA uridine-2′-carbamate phosphoramidite **11**.

Table 1. Properties of modified and unmodified oligodeoxyribonucleotides

#	Sequence, 5′→3′	MALDI MS	Calculated mass $[M+H]^+$
ON01	CTCCCAGGCTCAAAT	4490.4	4496.0
ON02	ATTTGAGCCTGGGAG	4643.8	4643.0
ON03	CTCCCAGGC**U**$^{\mathbf{A}}$CAAAT	4930.4	4930.9
ON04	C**U**$^{\mathbf{A}}$CCCAGGCTCAAAT	4927.8	4930.9
ON05	CTCCCAGGCTCAAA**U**$^{\mathbf{A}}$p[a]	5013.4	5013.4
ON06	CTCCCAGGC**U**$^{\mathbf{P}}$CAAAT	4853.8	4854.9
ON07	C**U**$^{\mathbf{P}}$CCCAGGCTCAAAT	4855.2	4854.9
ON08	C**U**$^{\mathbf{P}}$CCCAGGC**U**$^{\mathbf{P}}$CAAAT	5214.2	5214.0
ON09	CTCCCAGGCTCAAA**U**$^{\mathbf{P}}$p[a]	4935.8	4934.9
ON10	CTCCCAGGCTCAAA**U**$^{\mathbf{P}}$CTGGp[a]	6185.1	4186.1
ON11	A**U**$^{\mathbf{P}}$TTGAGCCTGGGAG	5005.9	5005.9
ON12	AT**U**$^{\mathbf{P}}$TGAGCCTGGGAG	5002.7	5005.9
ON13	ATT**U**$^{\mathbf{P}}$GAGCCTGGGAG	5007.5	5005.9
ON14	CCAGA**U**$^{\mathbf{P}}$TTGAGCCTGGGAG	6229.7	6229.2
ON15	CCAGAT**U**$^{\mathbf{P}}$TGAGCCTGGGAG	6228.7	6229.2
ON16	CCAGATT**U**$^{\mathbf{P}}$GAGCCTGGGAG	6228.2	6229.2

[a] 3′-phosphate.

were assigned using $^1H-^{13}C$ interactions through two, three and four bonds visualized as cross-peaks on HMBC spectra.[†]

2.2. Oligonucleotide synthesis

Fluorescent uridine-2′-carbamate phosphoramidites **6** and **11** were used in solid-phase oligodeoxyribonucleotide synthesis. The coupling time for the modifying reagents was increased to 10 min; the coupling yield was >95%. The sequence chosen was a 15-mer complementary to the 22–36 sequence (**ON02**) of the *trans*-activation responsive region of the human immunodeficiency virus type 1 (HIV-1) TAR RNA (**ON01**).[13] The sequences are given in Table 1. Fluorescent uridine-2′-carbamates were placed instead of internal thymidine (**ON03**, **ON06**, **ON14**-**ON16**), the thymidine located closely to the 5′-end (**ON04**, **ON07**), or at the both sites (**ON08**). Complementary unmodified (**ON02**) and modified (**ON11**-**ON16**) oligodeoxyribonucleotides were also prepared. The integrity and purity of modified oligonucleotides was analyzed by MALDI mass-spectrometry (Fig. 1, Table 1) and HPLC (Fig. 2). An insertion of such bulky moieties into oligonucleotides reduces their mobility on polyacrylamide gel (data not shown) and increases the retention time of conjugates on reverse-phase column (Fig. 2); BPEA containing oligonucleotide **ON03** is considerably more hydrophobic than PEPy labelled oligomer **ON06**.

2.3. Absorbance properties of PEPy and BPEA nucleosides

1-Phenylethynylpyrene[14] and 9,10-bisphenylethynylanthracene[15] have characteristic absorbance spectra. Figure 3 presents the comparison of UV spectra of fluorescent nucleosides $\mathbf{U}^{\mathbf{P}}$ and $\mathbf{U}^{\mathbf{A}}$ in polar solvent (water–ethanol) and Markiewicz-protected nucleosides **2** and **7** in non-polar solvent (DCM). The spectra of both chromophores in DCM have negligible long-wavelength shifts (ca. 2.5–3 nm) in comparison with their spectra in water–ethanol.

The presence of a fluorescent label in the oligonucleotides can be confirmed by UV spectra. Modified oligonucleotides, in addition to the inherent oligonucleotide absorption maximum around 260 nm, show characteristic maxima of the fluorophore, in the region of 350–400 nm (for PEPy) or 420–500 nm (for BPEA) (Fig. 4).

The absorption maxima of the fluorophore are shifted upon changing of the solvent to less polar one. An even greater bathochromic shift was observed for a dye with drastically changed microenvironment. The ‘oligonucleotide-bound’

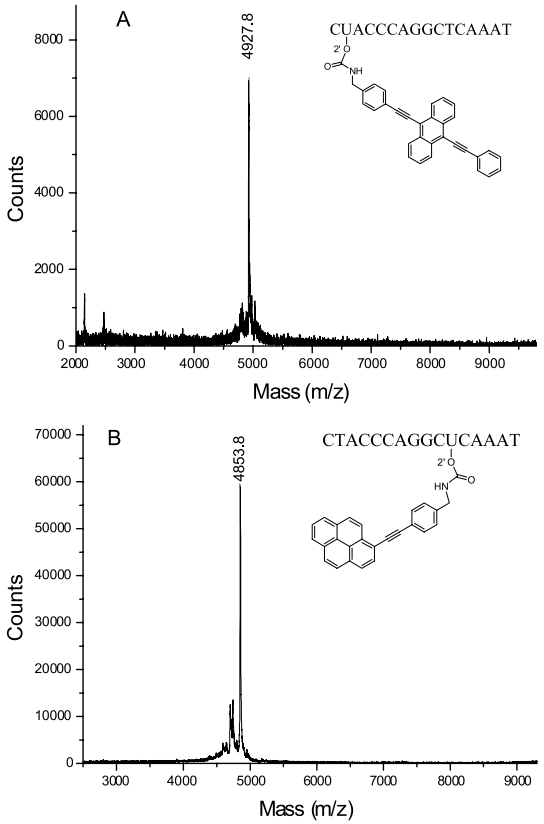


Figure 1. Examples of MALDI-TOF mass spectra: BPEA-modified oligomer **ON04** (A) and PEPy-modified oligomer **ON06** (B).

[†] See Supplementary DataApplication 1.

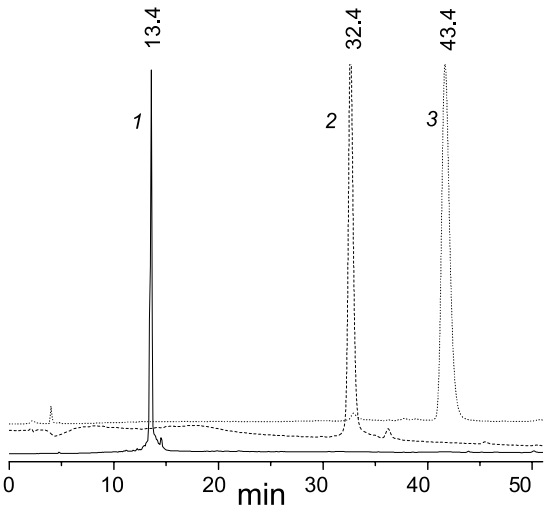


Figure 2. Examples of HPLC profiles of crude oligonucleotides: unmodified 15-mer **ON01** (1), PEPy-modified oligomer **ON06** (2) and BPEA-modified oligomer **ON03** (3). For HPLC conditions see Section 4.

PEPy has a 15 nm shift as compared to the PEPy nucleoside (plot 2 in Fig. 5). This may indicate significant hydrophobic environment of the dye and the decrease in the number of degrees of freedom for the rotational movement, for example, location in the minor groove.

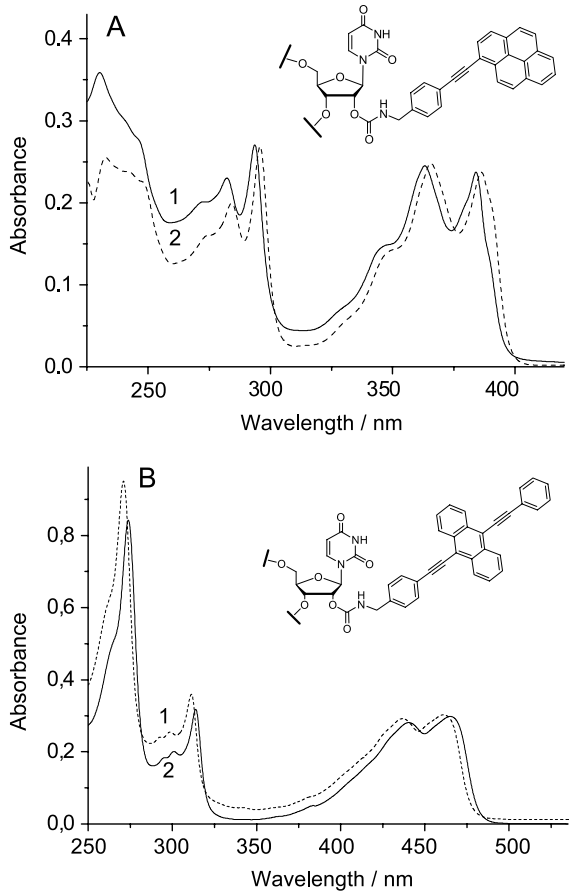


Figure 3. UV spectra of PEPy derivatives (A), normalized at dye absorbance around 360 nm and BPEA derivatives (B), normalized at dye absorbance around 460 nm. Conditions: solution $\mathbf{U}^{\mathbf{P}}$ (A, 1) or $\mathbf{U}^{\mathbf{A}}$ (B, 1) in water/ethanol 1:1 (v/v), solution of **2** (A, 2) or **7** (B, 2) in DCM.

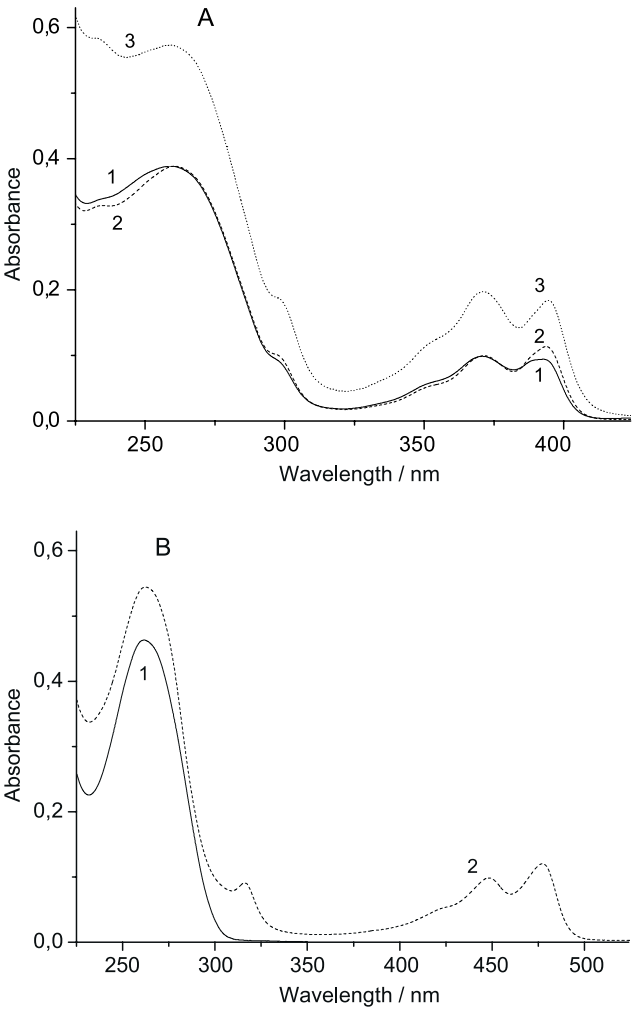


Figure 4. UV spectra of (A) PEPy-modified oligonucleotides **ON06** (1), **ON07** (2), **ON07** (3) in water, normalized by pyrene absorbance, and (B) unmodified **ON01** and BPEA-modified oligonucleotide **ON03**, normalized by oligonucleotide absorbance at 260 nm; ε_{260} for BPEA is approx. 22,000.[16]

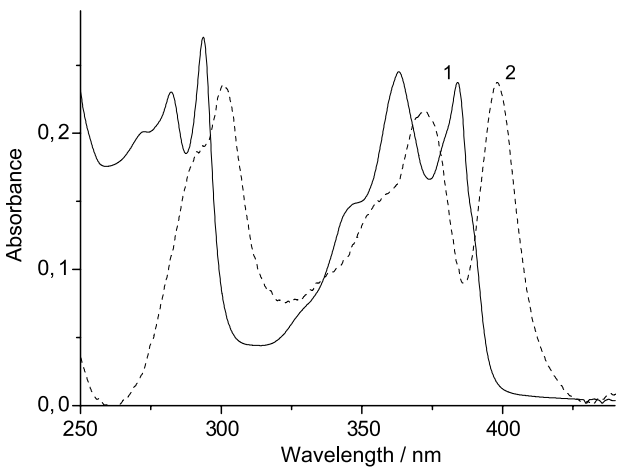


Figure 5. Comparison of the UV spectrum of PEPy-modified nucleoside $\mathbf{U}^{\mathbf{P}}$ in water/ethanol 1:1 (v/v) (1) and the difference between UV spectra of oligonucleotides **ON08** and **ON06** (2) in water, normalized by PEPy absorbance.

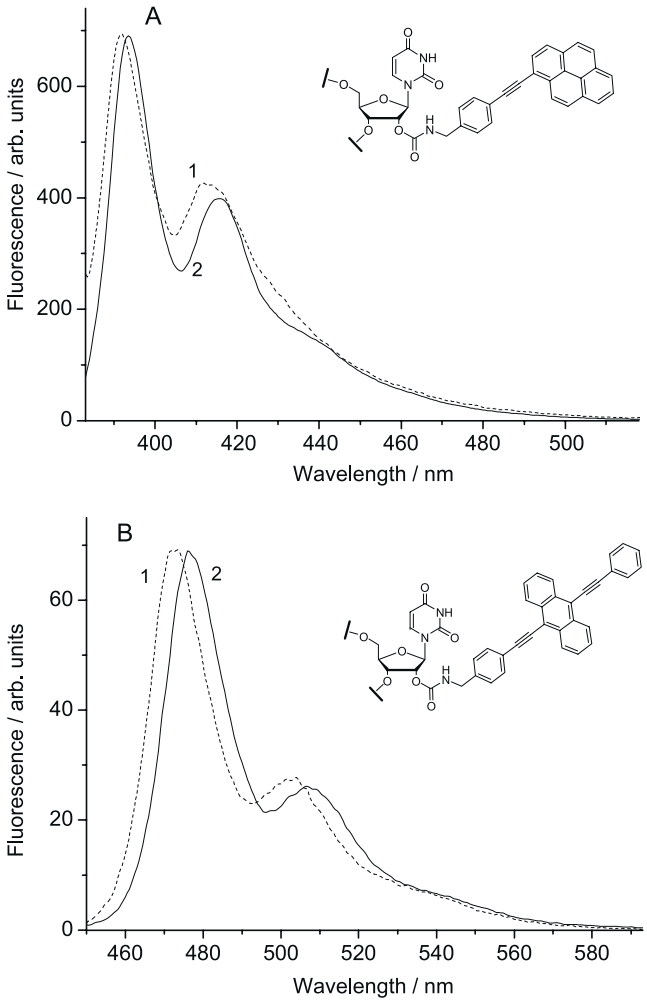


Figure 6. Fluorescence spectra of PEPy (A), and BPEA derivatives (B), normalized at fluorescence maximum. Conditions: solution $\mathbf{U}^{\mathbf{P}}$ (A, 1) or $\mathbf{U}^{\mathbf{A}}$ (B, 1) in water/ethanol 1:1 (v/v), solution **2** (A, 2) or **7** (B, 2) in DCM, hybridization buffer (Section 4).

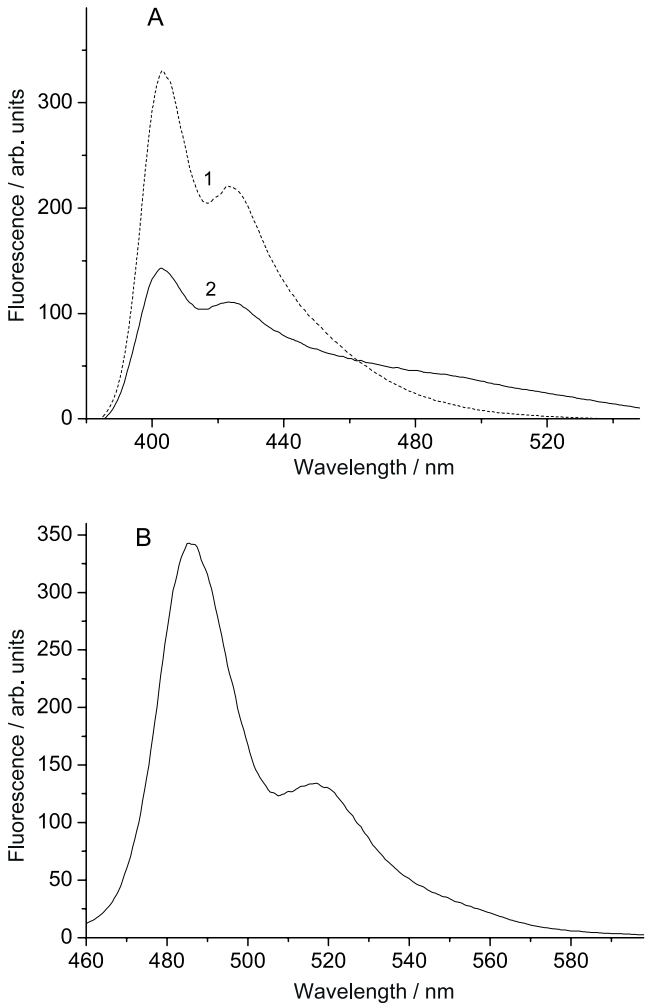


Figure 7. Fluorescence spectra of single stranded (A) PEPy-modified oligonucleotides **ON07** (mono-labeled, 1) and **ON08** (double-labeled, 2), (B) BPEA-modified oligonucleotide **ON04**.

2.4. Fluorescent measurements

The changes in fluorescent spectra of the dyes upon replacement of the solvent are similar to those for absorption. There are negligible long-wave shifts of the fluorescent bands in DCM compared to those in ethanol–water 1:1 solution for both PEPy (about 1.5 nm) and BPEA (about 4 nm) derivatives (Fig. 6).

We studied the luminescent characteristics of the labelled oligonucleotides and their duplexes. The fluorescence spectra were registered in aqueous phosphate buffer (pH 7.0) at the excitation wavelength of 375 nm for PEPy-containing oligonucleotides and 440 nm for BPEA-containing oligonucleotides. As Figure 7 shows, the introduction of a dye residue results in a characteristic monomeric fluorescence with maxima at 401, 423 nm (PEPy, A), and at 485, 516 nm (BPEA, B).

A study of the spectral properties of duplex **ON03×ON11** revealed an irradiative energy transfer. It turned out that BPEA is an energy acceptor for PEPy: hybridization of the above sequence, containing these residues, results in a fluorescence resonance energy transfer (Fig. 8A, plot 3). The effect slightly depends on the distance between fluorophores within duplexes (Fig. 8B).

2.5. Thermal denaturation studies

The negative effect of 2′-carbamate function on the thermal stability of DNA–DNA and DNA–RNA duplexes is well known.[1,9n] Because of the aromatic nature of dyes (PEPy, BPEA) the ‘carbamate’ destabilization is reduced and the melting temperatures remained sufficiently high for spectral studies to be performed (Table 2). This is caused presumably by fluorophore interaction with grooves of DNA duplexes. In cases of near-terminal positions of chromophores duplexes are more stabilized (**ON04×ON11** and **ON07×ON11**). On the other hand, a close proximity of two fluorophores placed in different strands leads to an increase in the melting temperature (cf. **ON03×ON13** and **ON03×ON11** or **ON07×ON13** and **ON07×ON11**). Moreover, the combination of BPEA and PEPy results in better stabilization than two PEPy residues (compare **ON03×ON13** and **ON07×ON13** or **ON03×ON12** and **ON07×ON12** or **ON03×ON11** and **ON07×ON11**).

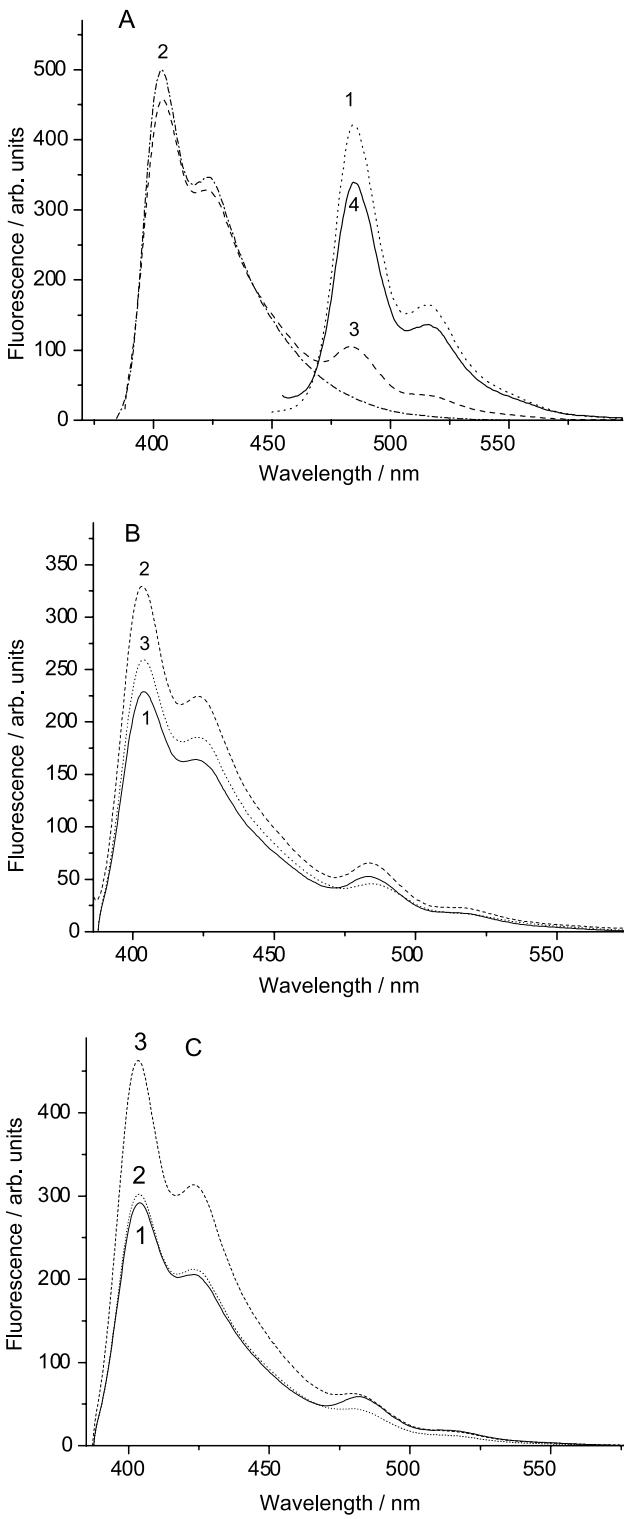


Figure 8. Fluorescence spectra of (A) single stranded BPEA-modified oligonucleotide **ON03** λ_{ex} 440 nm (1), PEPy-modified oligonucleotide **ON11** λ_{ex} 375 nm (2), and their duplex λ_{ex} 375 nm (3), λ_{ex} 440 nm (4); (B) duplexes **ON03×ON11** (1), **ON03×ON12** (2), **ON03×ON13** (3), λ_{ex} 375 nm; (C) duplexes **ON04×ON11** (1), **ON04×ON12** (2), **ON04×ON13** (3), λ_{ex} 375 nm. Conditions: hybridization buffer, concentration of each oligo 10^{-7} M.

Table 2. Thermal denaturation studies of modified oligonucleotides

#	Sequence	T_m (°C)	ΔT_m (°C)
ON01	5′-CTCCCAGGCTCAAAT	57.6	—
ON02	3′-GAGGGTCCGAGTTTA		
ON03	5′-CTCCCAGGC**U**$^{\mathbf{A}}$CAAAT	57.2	−0.4
ON11	3′-GAGGGTCCGAGTT**U**$^{\mathbf{P}}$A		
ON03	5′-CTCCCAGGC**U**$^{\mathbf{A}}$CAAAT	57.5	−0.1
ON12	3′-GAGGGTCCGAGT**U**$^{\mathbf{P}}$TA		
ON03	5′-CTCCCAGGC**U**$^{\mathbf{A}}$CAAAT	59.2	+1.6
ON13	3′-GAGGGTCCGAG**U**$^{\mathbf{P}}$TTA		
ON07	5′-CTCCCAGGC**U**$^{\mathbf{P}}$CAAAT	56.0	−1.6
ON11	3′-GAGGGTCCGAGTT**U**$^{\mathbf{P}}$A		
ON07	5′-CTCCCAGGC**U**$^{\mathbf{P}}$CAAAT	56.1	−1.5
ON12	3′-GAGGGTCCGAGT**U**$^{\mathbf{P}}$TA		
ON07	5′-CTCCCAGGC**U**$^{\mathbf{P}}$CAAAT	57.5	−0.1
ON13	3′-GAGGGTCCGAG**U**$^{\mathbf{P}}$TTA		
ON04	5′-C**U**$^{\mathbf{A}}$CCCAGGCTCAAAT	62.1	+4.5
ON11	3′-GAGGGTCCGAGTT**U**$^{\mathbf{P}}$A		
ON04	5′-C**U**$^{\mathbf{A}}$CCCAGGCTCAAAT	58.0	+0.4
ON12	3′-GAGGGTCCGAGT**U**$^{\mathbf{P}}$TA		
ON04	5′-C**U**$^{\mathbf{A}}$CCCAGGCTCAAAT	60.7	+3.1
ON13	3′-GAGGGTCCGAG**U**$^{\mathbf{P}}$TTA		
ON07	5′-C**U**$^{\mathbf{P}}$CCCAGGCTCAAAT	62.0	+4.4
ON11	3′-GAGGGTCCGAGTT**U**$^{\mathbf{P}}$A		
ON07	5′-C**U**$^{\mathbf{P}}$CCCAGGCTCAAAT	60.8	+3.2
ON12	3′-GAGGGTCCGAGT**U**$^{\mathbf{P}}$TA		
ON07	5′-C**U**$^{\mathbf{P}}$CCCAGGCTCAAAT	59.5	+1.9
ON13	3′-GAGGGTCCGAG**U**$^{\mathbf{P}}$TTA		
ON07	5′-CTCCCAGGC**U**$^{\mathbf{P}}$CAAAT	55.0	−2.6
ON14	3′-GAGGGTCCGAGTT**U**$^{\mathbf{P}}$AGACC		
ON07	5′-CTCCCAGGC**U**$^{\mathbf{P}}$CAAAT	54.7	−2.9
ON15	3′-GAGGGTCCGAGT**U**$^{\mathbf{P}}$TAGACC		
ON07	5′-CTCCCAGGC**U**$^{\mathbf{P}}$CAAAT	55.5	−2.1
ON16	3′-GAGGGTCCGAG**U**$^{\mathbf{P}}$TTAGACC		
ON10	5′-CTCCCAGGCTCAAA**U**$^{\mathbf{P}}$CTGG	63.3	+5.7
ON11	3′-GAGGGTCCGAGTT**U**$^{\mathbf{P}}$A		
ON10	5′-CTCCCAGGCTCAAA**U**$^{\mathbf{P}}$CTGG	58.7	+1.1
ON12	3′-GAGGGTCCGAGT**U**$^{\mathbf{P}}$TA		
ON10	5′-CTCCCAGGCTCAAA**U**$^{\mathbf{P}}$CTGG	58.8	+1.2
ON13	3′-GAGGGTCCGAG**U**$^{\mathbf{P}}$TTA		
ON10	5′-CTCCCAGGCTCAAA**U**$^{\mathbf{P}}$CTGG	62.2	+4.6
ON14	3′-GAGGGTCCGAGTT**U**$^{\mathbf{P}}$AGACC		
ON10	5′-CTCCCAGGCTCAAA**U**$^{\mathbf{P}}$CTGG	59.0	+1.4
ON15	3′-GAGGGTCCGAGT**U**$^{\mathbf{P}}$TAGACC		
ON10	5′-CTCCCAGGCTCAAA**U**$^{\mathbf{P}}$CTGG	58.7	+1.1
ON16	3′-GAGGGTCCGAG**U**$^{\mathbf{P}}$TTAGACC		

3. Summary

The results presented indicate that fluorescent hydrocarbons 1-phenylethynylpyrene (PEPy) and 9,10-bis(phenylethynyl)anthracene (PEPy), when attached through a carbamate spacer to the 2′-position of uridine and directed to the minor groove of DNA duplex, show weak FRET signal in comparison with PEPy–BPEA pair from non-nucleotide reagents. PEPy and BPEA covalently bound to duplexes give increase in T_m thus suggesting the

polyaromatics are interacting with DNA double helix. The possible explanation of the results is that PEPy and BPEA hydrocarbons are bound to the minor groove of DNA and have unfavourable spatial orientation for resonance energy transfer. Dye–DNA interaction in the minor groove can also increase DNA strands affinity and thus reduce the negative effect of 2′-carbamate modification.

4. Experimental

4.1. General

4-Iodobenzylamine, 1-ethynylpyrene were from Lancaster Synthesis; triethylamine trihydrofluoride, triethylamine, were from Aldrich; *N*,*N*-carbonyldiimidazole (CDI) was from Sigma, 4,4′-dimethoxytritylchloride (DmtCl) was from Avocado; bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine was from Fluka. 2′-*O*-(4-Iodobenzylaminocarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uridine,[9n] 9-ethynyl-10-phenylethynyl-anthracene,[10] and diisopropylammonium tetrazolide[12] were prepared as described. DCM was always used freshly distilled over CaH_2. Anhydrous pyridine was from Aldrich; THF was freshly distilled over powdered $LiAlH_4$ and stored over 4 Å molecular sieves under nitrogen. Other solvents were used as received.

500 MHz ^{1}H, 125.7 MHz ^{13}C and 202.4 MHz ^{31}P NMR spectra were measured on a Bruker AC-500 spectrometer. Chemical shifts (δ) for ^{1}H, ^{13}C and ^{31}P are referenced to internal solvent resonances and reported relative to $SiMe_4$ (^{1}H and ^{13}C) and 85% aq. H_3PO_4 (^{31}P). ^{1}H NMR coupling constants are reported in Hz and refer to apparent multiplicities. MALDI-TOF mass spectra were measured on a Voyager-DE BioSpectrometry Workstation (PerSeptive Biosystems) in positive ion mode. Elemental analysis was performed on CHNS-analyser/EA1112 'ThermoFinnigan'. TLC and column chromatographies were carried out with Macherey-Nagel silica gel (ALUGRAM® SIL G/UV$_{254}$ and Kieselgel 60 0.040–0.063 mm). Thermal denaturation experiments with oligonucleotide duplexes were performed on a Perkin–Elmer Lambda 40 UV/VIS Spectrometer with PTP 6 (Peltier Temperature Programmer). Fluorescence spectra were obtained using a Perkin–Elmer LS 50B Luminescence Spectrometer. Thermal denaturation studies for DNA duplexes were done in a buffer containing 100 mM NaCl, 10 mM Na-phosphate, 0.1 mM EDTA, pH 7.0.

4.1.1. 2′-*O*-[4-(Pyren-1-ylethynyl)phenylmethylaminocarbonyl]-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uridine (2). To a solution of 2′-*O*-(4-iodobenzylaminocarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uridine[9n] (2.24 g, 3.0 mmol) in DMF (25 mL), 1-ethynylpyrene (792 mg, 3.5 mmol), CuI (130 mg, 0.68 mmol), $Pd(PPh_3)_4$ (390 mg, 0.34 mmol) and Et_3N (1.0 mL, 7.1 mmol) were added with stirring under argon. After 24 h at room temperature, TLC showed that the reaction is complete. The reaction mixture was diluted with DCM (300 mL) and washed with 0.3 M EDTA–$(NH_4)_2$ (2×200 mL), water (10×200 mL) and 5% citric acid (200 mL), the organic layer was dried and evaporated, and the residue was chromatographed on silica gel in step gradient 0⟶50% EtOAc in $CHCl_3$ (v/v). Yield 2.38 g (94.1%), yellow foam. R_f 0.31 ($CHCl_3$/EtOAc, 1:1). MALDI-TOF MS (matrix 2,4,6-trihydroxyacetophenone (2,4,6-THAP)) 846.87, 868.28. Calcd 844.34 $[M+H]^+$, 866.33 $[M+Na]^+$. ^{1}H NMR ([D$_6$]DMSO): δ 11.41 (s, 1H, H-3), 8.61 (d, 1H, $J_{9'',10''}$=9.1 Hz, H-10″), 8.41–8.21 (m, 7H, H-2″–6″,8″,9″), 8.12 (apparent t, 1H, $J_{6'',7''}=J_{7'',8''}$=7.7 Hz, H-7″), 8.05 (br t, J=6.1 Hz, OCON*H*), 7.70 (m, 3H, H-6, Hd), 7.39 (d, 2H, $J_{d,e}$=8.0 Hz, He), 5.71 (d, 1H, $J_{1',2'}$=1.5 Hz, H-1′), 5.62 (d, 1H, $J_{5,6}$=8.1 Hz, H-5), 5.41 (dd, 1H, $J_{1',2'}$=1.5 Hz, $J_{2',3'}$=5.7 Hz, H-2′), 4.51 (dd, $J_{2',3'}$=5.7 Hz, $J_{3',4'}$=8.1 Hz, 1H, H-3′), 4.27 (m, 2H, J_{NHCH}=6.1 Hz, ^2J=15.8 Hz, NCH_2), 4.11–3.88 (m, 3H, $J_{5'a,5'b}$=12.9 Hz, $J_{4,5'a}$=4.0 Hz, H-4′, H-5′), 1.07–0.97 (m, 28H, Pr^i). Anal. Calcd for $C_{47}H_{53}N_3O_8Si_2$: C, 66.88; H, 6.33; N, 4.98. Found: C, 66.96; H, 6.21; N, 5.01.

4.1.2. 2′-*O*-[4-(9-Phenylethynylanthracen-10-ylethynyl)-phenylmethylaminocarbonyl]-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uridine (7). To a solution of 2′-*O*-(4-iodobenzylaminocarbonyl)-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uridine (0.863 g, 1.157 mmol) in DMF (25 mL), 9-ethynyl-10-(phenylethynyl)anthracene[10] (350 mg, 1.157 mmol), CuI (44 mg, 0.231 mmol), $Pd(PPh_3)_4$ (134 mg, 0.157 mmol) and Et_3N (0.403 mL, 2.89 mmol) were added with stirring under argon for 24 h at room temperature. The workup was as above; the compound was isolated by chromatography on silica gel in step gradient of 0⟶35% EtOAc in $CHCl_3$ (v/v). Yield 0.98 g (92.1%), orange foam. R_f 0.31 ($CHCl_3$/EtOAc, 1:1). MALDI-TOF MS (matrix 2,4,6-THAP) 921.77, 944.23, 960.48. Calcd 921.21 $[M+H]^+$, 943.20 $[M+Na]^+$, 959.30 $[M+K]^+$. ^{1}H NMR ([D$_6$]DMSO): δ 11.41 (s, 1H, H-3), 8.67 (m, 4H, H-1″,4″,5″,8″), 8.07 (br t, 1H, J=6.1 Hz, OCON*H*), 7.89 (m, 2H, Hc), 7.78 (m, 6H, Hj, H-2″,3″,6″,7″), 7.70 (d, 1H, $J_{5,6}$=8.1 Hz, H-6), 7.53 (m, 3H, Ha,b), 7.41 (d, 2H, $J_{j,k}$=8.1 Hz, Hk), 5.71 (br s, 1H, H-1′), 5.60 (m, 1H, H-5), 5.40 (m, 1H, H-2′), 4.52 (dd, 1H, $J_{2',3'}$=5.8 Hz, $J_{3',4'}$= 8.1 Hz, H-3′), 4.27 (m, 2H, J_{NHCH}=6.1 Hz, ^2J=15.8 Hz, NCH_2), 4.11–3.88 (m, 3H, $J_{5'a,5'b}$=12.9 Hz, $J_{4,5'a}$=3.7 Hz, H-4′, H-5′), 1.04–0.98 (m, 28H, Pr^i). Anal. Calcd for $C_{53}H_{57}N_3O_8Si_2$: C, 69.18; H, 6.24; N, 4.57. Found: C, 69.04; H, 6.30; N, 4.62.

4.1.3. 2′-*O*-[4-(Pyren-1-ylethynyl)phenylmethylaminocarbonyl]uridine (4). To a solution of 2′-*O*-[4-(pyren-1-ylethynyl)phenylmethylaminocarbonyl]-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uridine (1.688 g, 2 mmol) in THF (5 mL) in a teflon flask (Nalgene, screw-top) was added triethylamine trihydrofluoride (0.814 mL, 5 mmol) and the mixture was left overnight at room temperature (the completion of deprotection was checked by TLC (15% MeOH in $CHCl_3$, v/v), then diluted with hexane (25 mL). The upper layer was discarded, and the residue (oil) was washed with 1:1 (v/v) toluene–hexane mixture (3×25 mL) by decantation, triturated in absolute ethanol (5 mL), and the crystalline product was filtered off, washed with ethanol (5 mL), diethyl ether (10 mL), and dried in vacuo. Yield 1.193 g (99.1%), yellow crystals. R_f 0.4 ($CHCl_3$/MeOH, 17:3 (v/v)), mp 144–145.5 °C. MALDI-TOF MS (matrix 2,4,6-THAP) 602.78, 625.80. Calcd 602.19 $[M+H]^+$, 624.17 $[M+Na]^+$. ^{1}H NMR ([D$_6$]DMSO): δ 11.38 (s, 1H, H-3, exchangeable with D_2O), 8.62 (d, 1H, $J_{9'',10''}$=9.1 Hz,

H-10″), 8.39–8.21 (m, 7H, H-2″–6″,8″,9″), 8.13 (apparent t, 1H, $J_{6'',7''}=J_{7'',8''}=7.6$ Hz, H-7″), 7.98–7.93 (m, 2H, OCONH, H-6), 7.73 (d, 2H, $J_{e,d}=8.0$ Hz, Hd), 7.37 (d, 2H, $J_{e,d}=8.0$ Hz, He), 6.05 (d, 1H, $J_{1',2'}=5.7$ Hz, H-1′), 5.69 (d, 1H, $J_{5,6}=8.1$ Hz, H-5), 5.56 (d, 1H, $J_{3',OH}=5.1$ Hz, 3′-OH), 5.20 (t, 1H, $J=5.7$ Hz, 5′-OH) (both exchangeable with D_2O), 5.13 (apparent t, 1H, $J_{1'',2''}=J_{2',3'}=5.7$ Hz, H-2′), 4.25 (m 3H, H-3′, NCH_2), 3.94 (m, 1H, H-4′), 3.64 (m, 2H, H-5′). ^{13}C NMR ([D_6]DMSO), δ 43.6 (Cg), 60.8 (C5′), 68.9 (C3′), 74.8 (C2′), 85.4 (C1′), 85.7 (C4′), 87.9 (Ca), 95.2 (Cb), 102.1 (C5), 117.0 (C1″), 120.7 (Cc), 123.3 (C10c″), 123.6 (C10b″), 124.7 (C3″), 124.8 (C10″), 125.9 (2C, C6″, C8″), 126.6 (C4″), 127.1 (C7″), 127.2 (2C, Ce), 128.3 (C5″), 128.7 (C9″), 129.5 (C2″), 130.4, 130.7, 130.8, 130.9 (C3a″, C5a″, C8a″, C10a″), 131.4 (2C, Cd), 140.6 (2C, C6, Cf), 150.5 (C2), 155.4 (Ch), 162.9 (C4). Anal. Calcd for $C_{35}H_{27}N_3O_7$: C, 69.88; H, 4.52; N, 6.98. Found: C, 69.91; H, 4.49; N, 6.92.

4.1.4. 2′-*O*-[4-(9-Phenylethynylanthracen-10-ylethynyl)-phenylmethylaminocarbonyl]uridine (9). Prepared from 2′-*O*-[4-(9-phenylethynylanthracen-10-ylethynyl)phenylmethylaminocarbonyl]-3′,5′-*O*-(tetraisopropyldisiloxan-1,3-diyl)uridine (0.98 g, 1.1 mmol) and triethylamine trihydrofluoride (0.5 mL, 3 mmol) in similar manner to compound **4**. Yield 0.707 g (98%), orange crystals. R_f 0.4 ($CHCl_3$/MeOH, 17:3 (v/v)), mp 165–166 °C. MALDI-TOF MS (matrix 2,4,6-THAP) 679.17, 701.61, 717.64. Calcd 678.71 $[M+H]^+$, 700.69 $[M+Na]^+$, 716.80 $[M+K]^+$. ^1H NMR ([D_6]DMSO): δ 11.38 (s, 1H, H-3, exchangeable with D_2O), 8.69 (m, 4H, $J_{1'',2''}=J_{3'',4''}=J_{5'',6''}=J_{7'',8''}=6.5$ Hz, H-1″,4″,5″,8″), 7.99 (br t, 1H, OCONH), 7.93 (d, 1H, $J_{5,6}=8.1$ Hz, H-6), 7.91 (m, 2H, Hc), 7.85 (d, 2H, $J_{j,k}=8.1$ Hz, Hj), 7.80 (m, 4H, $J_{1'',2''}=J_{3'',4''}=J_{5'',6''}=J_{7'',8''}=6.5$ Hz, H-2″,3″,6″,7″), 7.54 (m, 3H, Ha, Hb), 7.40 (d, 2H, $J_{j,k}=8.1$ Hz, Hk), 6.05 (d, 1H, $J_{1',2'}=5.7$ Hz, H-1′), 5.69 (d, 1H, $J_{5,6}=8.1$ Hz, H-5), 5.61 (d, 1H, $J_{3',OH}=5.1$ Hz, 3′-OH), 5.19 (m, 1H, 5′-OH) (both exchangeable with D_2O), 5.12 (apparent t, 1H, $J_{1',2'}=J_{2',3'}=5.7$ Hz, H-2′), 4.26 (m, 3H, H-3′, NCH_2), 3.93 (m, 1H, H-4′), 3.63 (m, 2H, H-5′). ^{13}C NMR ([D_6]DMSO), δ 43.6 (Cm), 60.8 (C5′), 68.8 (C3′), 74.8 (C2′), 85.4 (2C, C1′, Cg), 85.7 (2C, C4′, Cf), 102.1 (C5), 102.7 (Ce), 102.8 (Ch), 117.3, 117.6 (2C, C9″, C10″), 120.4 (Ci), 122.1 (Cd), 126.7 (4C, C1″, C4″, C5″, C8″), 127.3 (2C, Ck), 127.7 (4C, C2″, C3″, C6″, C7″), 128.8 (2C, Cb), 129.3 (Ca), 131.2 (4C, 4a″, C8a″, C9a″, 10a″), 131.6 (4C, Cj, Cc), 140.6 (C6), 141.0 (Cl), 150.5 (C2), 155.4 (Cn), 162.9 (C4). Anal. Calcd for $C_{41}H_{31}N_3O_7$: C, 72.66; H, 4.61; N, 6.20. Found: C, 72.61; H, 4.55; N, 6.24.

4.1.5. 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[4-(pyren-1-ylethynyl)phenylmethylaminocarbonyl]uridine (5). 2′-*O*-[4-(Pyren-1-ylethynyl)phenylmethylaminocarbonyl]uridine (1.004 g, 1.67 mmol) was coevaporated with toluene (3×20 mL), pyridine (3×20 mL), dissolved in dry pyridine (15 mL), cooled in an ice bath, and DmtCl (623 mg, 1.84 mmol) was added in one portion. After completion of the reaction, the excess of DmtCl was quenched with MeOH (1 mL), and after 10 min the mixture was diluted with $CHCl_3$ (100 mL), washed with water (100 mL), 5% $NaHCO_3$ (100 mL), and water (100 mL), then dried (Na_2SO_4), evaporated, coevaporated with toluene (3×25 mL) and the residue was chromatographed on silica gel column in step gradient of 0.5→1→1.5→2% MeOH in $CHCl_3$/EtOAc 2:1+0.5% pyridine (v/v/v/v). Fractions containing product were combined, evaporated, and the residue was dried in vacuo to afford **5** (1.3 g, 86.2%) as a yellow foam. R_f 0.31 ($CHCl_3$/EtOAc 1:1+1% Et_3N (v/v/v)). MALDI-TOF MS (matrix 2,4,6-THAP) 905.81, 928.04, 944.55. Calcd 904.32 $[M+H]^+$, 926.31 $[M+Na]^+$, 942.28 $[M+K]^+$. ^1H NMR ([D_6]DMSO): δ 11.41 (s, 1H, H-3) (exchangeable with D_2O), 8.62 (d, 1H, $J_{9'',10''}=9.0$ Hz, H-10″), 8.41–8.22 (m, 7H, H-2″–6″,8″,9″), 8.14 (apparent t, 1H, $J_{6'',7''}=J_{7'',8''}=7.5$ Hz, H-7″), 8.00 (br t, 1H, $J=6.1$ Hz, OCONH), 7.73 (m, 3H, H-6,d), 7.43–7.22 (m, 9H, ArH (Dmt)), 6.89 (d, 4H, $J_{d,e}=8.6$ Hz, He), 5.97 (d, 1H, $J_{1',2'}=4.9$ Hz, H-1′), 5.61 (d, 1H, $J_{3',OH}=5.6$ Hz, OH) (exchangeable with D_2O), 5.41 (1H, $J_{5,6}=8.1$ Hz, H-5), 5.23 (apparent t, 1H, $J_{1'2'}=J_{2'3'}=5.2$ Hz, H-2′), 4.37 (m, 1H, H-3′), 4.29 (m, 2H, $NHCH_2$), 4.01 (m, 1H, H-4′), 3.74 (s, 6H, CH_3), 3.32–3.22 (m, 2H, H-5′). Anal. Calcd for $C_{56}H_{45}N_3O_9$: C, 74.40; H, 5.02; N, 4.65. Found: C, 74.62; H, 4.93; N, 4.37. A byproduct, 5′,3′-*O*-bis-(4,4′-dimethoxytrityl)-2′-*O*-[4-(pyren-1-ylethynyl)phenylmethylaminocarbonyl]uridine, was also isolated as a foam (30 mg, 1.5%). ^1H NMR ([D_6]DMSO): δ 11.46 (s, 1H, H-3) (exchangeable with D_2O), 8.63 (d, 1H, $J_{9'',10''}=9.0$ Hz, H-10″), 8.41–8.21 (m, 8H, H-2″–6″,8″,9″, OCONH), 8.14 (apparent t, 1H, $J_{6'',7''}=J_{7'',8''}=7.5$ Hz, H-7″), 7.72 (d, 2H, $J_{a,b}=7.9$ Hz, H-b), 7.52 (d, 1H, $J_{5,6}=8.0$ Hz, H-6), 7.41–7.11 (m, 18H, ArH (Dmt)), 6.86–6.74 (m, 8H, ArH (Dmt)), 6.16 (d, 1H, $J_{1',2'}=6.0$ Hz, H-1′), 5.39 (1H, $J_{5,6}=8.0$ Hz, H-5), 5.10 (apparent t, 1H, $J_{1'2'}=J_{2'3'}=6.0$ Hz, H-2′), 4.34–4.26 (m, 4H, H-3′, H-4′, $NHCH_2$), 3.73 (s, 6H), 3.69 (s, 6H) (CH_3), 3.30 (m, 2H,‡ H-5′).

4.1.6. 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[4-(9-phenylethynylanthracen-10-ylethynyl)phenylmethylaminocarbonyl]uridine (10). Prepared from 2′-*O*-[4-(9-phenylethynylanthracen-10-ylethynyl)phenylmethylaminocarbonyl]uridine (0.68 g, 1.00 mmol) in similar manner to compound **5**. Yield 0.839 g (85.6%), orange foam. R_f 0.4 ($CHCl_3$–EtOAc 1:1+0.5% pyridine (v/v/v)). ^1H NMR ([D_6]DMSO): δ 11.42 (s, 1H, H-3, exchangeable with D_2O), 8.69 (m, 4H, $J_{1'',2''}=J_{3'',4''}=J_{5'',6''}=J_{7'',8''}=6.4$ Hz, H-1″,4″,5″,8″), 8.03 (br t, $J=6.1$ Hz, 1H, OCONH), 7.91 (d, 2H, $J=6.4$ Hz), 7.85 (d, 2H, $J=8.0$ Hz) (Hc,j), 7.80 (m, $J_{1'',2''}=J_{3'',4''}=J_{5'',6''}=J_{7'',8''}=6.4$ Hz, H-2″,3″,6″,7″), 7.73 (d, 1H, $J_{5,6}=8.0$ Hz, H-6), 7.57–7.26 (m, 14H, Ha,b,k, ArH (Dmt)), 6.90 (d, $J=8.5$ Hz, 4H, ArH (Dmt)), 5.98 (d, 1H, $J_{1',2'}=4.5$ Hz, H-1′), 5.63 (d, 1H, $J_{3',OH}=5.0$ Hz, 3′-OH), 5.41 (d, 1H, $J_{5,6}=8.0$ Hz, H-5), 5.11 (apparent t, 1H, $J_{1',2'}=J_{2',3'}=4.5$ Hz, H-2′), 4.40–4.29 (m, 3H, H-3′, NCH_2), 4.02 (m, 1H, H-4′), 3.74 (s, 6H, CH_3), 3.31 (m, 2H,‡ H-5′). Anal. Calcd for $C_{62}H_{49}N_3O_9$: C, 75.98; H, 5.04; N, 4.29. Found: C, 76.21; H, 4.93; N, 4.36. Bis-Dmt-derivative, 5′,3′-*O*-bis-(4,4′-dimethoxytrityl)-2′-*O*-[4-(9-phenylethynylanthracen-10-ylethynyl)phenylmethylaminocarbonyl]uridine, was isolated as a minor byproduct (28 mg, 2.6%). ^1H NMR ([D_6]DMSO): δ 11.45 (s, 0.7H), 11.42 (m, 0.2H), 11.39 (m, 0.1H) (H-3), 8.69 (m, 4H, $J_{1'',2''}=J_{3'',4''}=J_{5'',6''}=J_{7'',8''}=6.5$ Hz, H-1″,4″,5″,8″), 8.03 (br t, 1H, $J=6.1$ Hz, OCONH), 7.91–7.60 (m, 32H, H-2″,3″,6″,7″, H-6, Ha,b,c,j,k, ArH (Dmt)), 6.86–6.74 (m, 8H, ArH (Dmt)),

‡ Calculated value; the signal of water is also present in the region.

6.16 (d, 0.7H, $J_{1',2'}$=6.5 Hz), 6.13 (d, 0.2H, $J_{1',2'}$=6.5 Hz), 6.09 (d, 0.1H, $J_{1',2'}$=6.5 Hz) (H-1′), 5.39 (m, 1H, H-5), 5.11 (apparent t, 1H, $J_{1',2'}=J_{2',3'}$=4.5 Hz, H-2′), 4.36–4.24 (m, 4H, H-3′, H-4′, NCH_2), 3.72 (s, 6H), 3.69 (s, 6H) (CH_3), 3.30 (m, 2H,‡ H-5′).

4.1.7. 3′-*O*-(*N*,*N*-Diisopropylamino-2-cyanoethoxyphosphinyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-[4-(pyren-1-ylethynyl)phenylmethylaminocarbonyl]uridine (6). 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[4-(pyren-1-ylethynyl)-phenylmethylaminocarbonyl]uridine (1.00 g, 1.10 mmol) was coevaporated with dry DCM (2×20 mL), dissolved in dry DCM, diisopropylammonium tetrazolide (285 mg, 1.66 mmol) and bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (0.527 mL, 1.66 mmol) were added, and the mixture was stirred under nitrogen for 2 h. After conversion of the starting compound was complete (monitoring by TLC) the mixture was diluted with $CHCl_3$, washed with 5% $NaHCO_3$ (100 mL), 20% NaCl (100 mL), dried over Na_2SO_4, evaporated to dryness, and the residue was chromatographed on silica gel column in stepwise gradient 5→10→20→25% acetone in $CHCl_3$+1% Et_3N (v/v/v). Fractions containing product were combined, evaporated, and the residue was dried in vacuo to afford **6** (1.3 g, 92%) as a yellow foam. R_f 0.17, 0.23 ($CHCl_3$/EtOAc 1:1+1%Et_3N (v/v/v)). ^{1}H NMR (5% $CDCl_3$ in [D_3]MeCN): δ 8.66 (d, 1H, $J_{9'',10''}$=8.8 Hz, H-10″), 8.33–8.08 (m, 8H, H-2″–9″), 7.74–7.24 (m, 15H, H-6, Ar*H* (Dmt, He,d), OCON*H*), 6.87 (m, 4H, Ar*H* (Dmt)), 6.08 (m, 1H, H-1′), 5.49–5.37 (m, 2H, H-2′,5), 4.64 (m, 1H, H-3′), 4.42–4.33 (m, 2H, NHCH_2), 4.01–4.29 (m, 1H, H-4′), 3.78 (s, 6H, CH_3), 3.75–3.37 (m, 6H,‡ POCH_2, PNC*H*, H-5′), 2.76 (t, 0.9H, *J*=5.9 Hz), 2.51 (t, 1.1H, *J*=5.9 Hz) (CH_2CN, diastereomers), 1.26–1.05 (m, 12H, CHCH_3). ^{31}P NMR (5% $CDCl_3$ in [D_3]MeCN): δ 151.13, 150.99 (diastereomers, ~10:8).

4.1.8. 3′-*O*-(*N*,*N*-Diisopropylamino-2-cyanoethoxyphosphinyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-[4-(9-phenylethynylanthracen-10-ylethynyl)phenylmethylaminocarbonyl]uridine (11). 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[4-(9-phenylethynylanthracen-10-ylethynyl)phenylmethylaminocarbonyl]uridine (0.8 g, 0.82 mmol) was phosphitylated as above with diisopropylammonium tetrazolide (21 mg, 1.23 mmol) and bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (0.39 mL, 1.23 mmol). The compound was chromatographed on silica gel column in stepwise gradient 5→10→20→25% acetone in $CHCl_3$+1% Et_3N (v/v/v). Yield 1.069 g (90.5%). R_f 0.17, 0.23 ($CHCl_3$/EtOAc 1:1+1%Et_3N (v/v/v)). ^{1}H NMR ([D_3]MeCN): δ 8.70 (m, 4H, H-1″,4″,5″,8″), 7.84 (m, 2H), 7.78 (m, 2H) (Hc,j), 7.74 (m, H-2″,3″,6″,7″), 7.67 (m, 1H, H-6), 7.53–7.24 (m, 14H, Ha,b,k, Ar*H* (Dmt)), 6.88 (d, *J*=8.5 Hz, 4H, Ar*H* (Dmt)), 6.08–6.06 (m, 1H, H-1′), 5.44–5.36 (m, 2H, H-5), 4.69–4.59 (m, 1H, H-2′), 4.42–4.01 (m, 4H, H-3′, NCH_2, H-4′), 3.84–3.37 (m, 12H,‡ OCH_3, POCH_2, PNC*H*, H-5′), 2.77 (t, 2H, *J*=5.8 Hz, CH_2CN), 1.28–1.01 (m, 12H, CHCH_3). ^{31}P NMR ([D_3]MeCN): δ 151.00, 150.89 (diastereomers, ~2:1).

4.1.9. Synthesis of oligodeoxynucleotides. The polymer-supported synthesis of oligonucleotides was performed on a ABI 380B DNA/RNA synthesizer, with commercially available 2′-deoxynucleoside phosphoramidites (Cruachem). Oligonucleotides were synthesized starting on a 1 μmol scale. Oligonucleotides were isolated using electrophoresis in 20% denaturing (7 M urea) PAGE in Tris–borate buffer, pH 8.3. The mass of each oligonucleotide was checked by MALDI-TOF mass spectrometry on a Perseptive Biosystems Voyager DE mass spectrometer in positive ion mode using a 1:1 v/v mixture of 2,6-dihydroxyacetophenone (40 mg/mL in MeOH) and aq. diammonium hydrogen citrate (80 mg/mL) as a matrix premixed just before loading the samples onto a plate.

4.2. Supplementary Data

Original ^{1}H, ^{13}C, ^{1}H–^{13}C HMQC and HMBC NMR spectra of nucleosides **4** (**U^P**) and **9** (**U^A**).

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Oxygenated monoterpenes as dipolarophiles for nitrilimine cycloadditions☆

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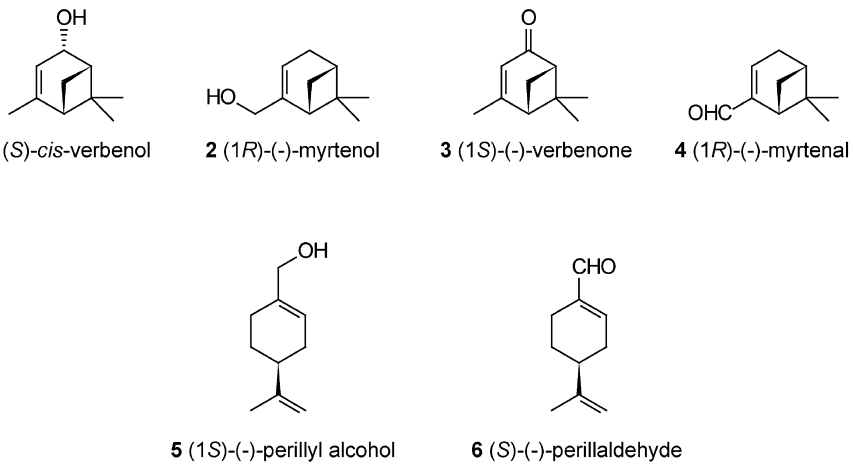
Abstract—A variety of naturally-occurring oxygenated monoterpenes, namely (*S*)-*cis*-verbenol, (1*R*)-(−)-myternol, (1*S*)-(−)-verbenone, (1*R*)-(−)-myternal, (*S*)-(−)-perillyl alcohol, and (*S*)-(−)-perillaldehyde have been submitted to nitrilimine cycloaddition. These processes were fully regio- and stereoselective for four dipolarophiles. In contrast, regioselective but non-stereoselective cycloadditions occurred in the case of two of the monoterpene derivatives. The configurations of the newly-formed stereocentres of the cycloadducts were assigned on the basis of NOE and NOESY experiments.

1. Introduction

Due to their great synthetic value, stereoselective 1,3-dipolar cycloadditions have been the subject of intense research over the last decade.[1] Our recent contributions to this topic have been concerned with the stereoselectivity outcome of nitrilimine cycloadditions in both the inter-,[2] the intra-,[3] and the sequential inter- intramolecular[4] versions. As a result, a wide range of enantiopure bi- or tricyclic systems containing a bridged (or fused) 4,5-dihydropyrazole ring were synthesised,[5] and some of them were found to have with in vitro activity against breast cancer.[6] Following this finding, we decided to submit a variety of naturally-occurring oxygenated monoterpenes,[7] namely (*S*)-*cis*-verbenol **1**, (1*R*)-(−)-myternol **2**, (1*S*)-(−)-verbenone **3**, (1*R*)-(−)-myternal **4**, (*S*)-(−)-perillyl alcohol **5** and (*S*)-(−)-perillaldehyde **6** (Fig. 1) to nitrilimine cycloaddition. The resulting 4,5-dihydropyrazole-containing skeletons may be of biological interest as the mentioned isoprenoids can suppress the proliferation of murine B16 melanoma, human HL-60 leukemia cells and other carcinogenic processes.[8–10] Furthermore, both compounds **1**–**4** containing the pinane



1 (*S*)-*cis*-verbenol **2** (1*R*)-(-)-myrtenol **3** (1*S*)-(-)-verbenone **4** (1*R*)-(-)-myrtenal

5 (1*S*)-(-)-perillyl alcohol **6** (*S*)-(-)-perillaldehyde

Figure 1.

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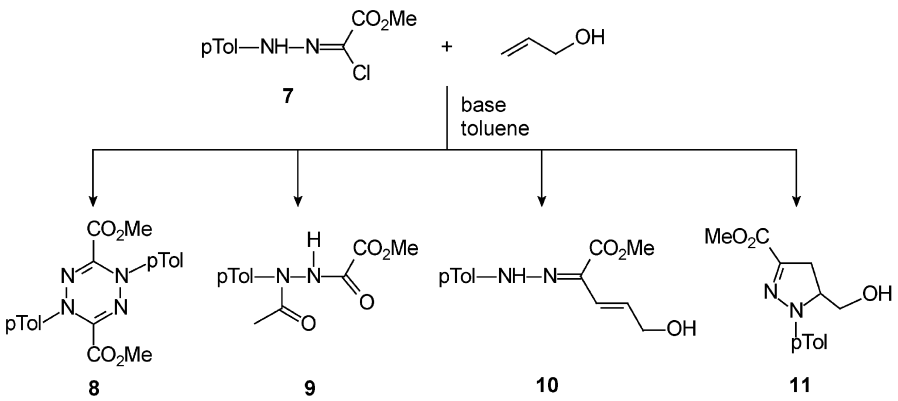


Scheme 1.

Table 1. Reaction between hydrazonoyl chloride **7** and allyl alcohol in dry toluene

Method	Base (equiv.)	Time (h)	Temperature (°C)	Products and yields (%)[a]				
				7	**8**	**9**	**10**	**11**
A	Et_3N (5)	72	25	69	0	—	0	20
B	Et_3N (5)	24	110	0	21	—	0	76[b]
C	Ag_2CO_3 (2)	36	25	17	0	—	0	68[b]
D	AcOCu (1.5)	40	25	47	0	13	6	33
E	AcOAg (1)	3	25	0	0	7	8	84

[a] Isolation yields.
[b] Some amount of tarry material was always formed.

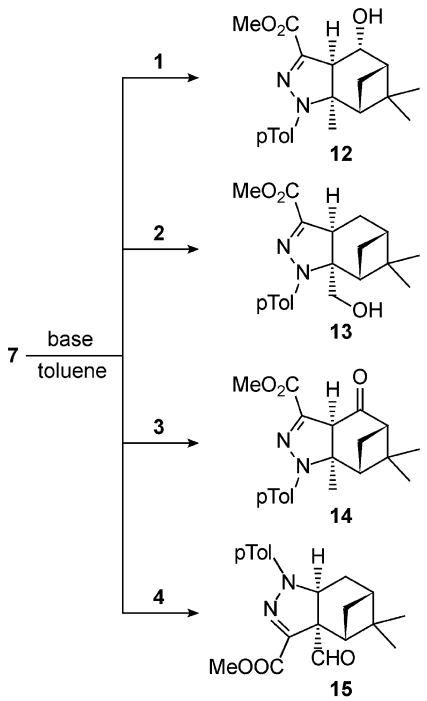
skeleton and limonene derivatives **5**, **6** are interesting dipolarophiles from a mechanistic point of view. In fact, all C=C bonds of such compounds are suitable positions for dipolar attack which can proceed with two opposite orientations onto each diastereoface; hence, both site-, regio and stereoselectivity phenomena are involved in these cycloadditions.

2. Results

The first step of the present work involved a search for the best reaction conditions between hydrazonoyl chloride **7**[11] and monoterpene derivatives **1**–**4** and **5**, **6**. Since some of these terpenes contain the allyl alcohol fragment, we investigated the reaction of hydrazonoyl chloride **7** with an equimolecular amount of allyl alcohol in dry toluene in the presence of a suitable base (Scheme 1), according to methods A–E which are summarised in Table 1. Better isolated yields of the 4,5-dihydropyrazole cycloadduct **11**[12] were obtained using methods B, C and E, although the formation of trivial by-products **8**[12] and **9** could not be entirely suppressed. In the presence of silver acetate (method E), particularly short reaction time and mild conditions paralleled the best isolated yield of **11**. It was also possible to isolate and fully characterise small amounts of the new conjugated hydrazone **10** as a by-product of mechanistic relevance (vide infra).

Next, pinane derivatives **1**–**4** were submitted to cycloaddition with an equimolecular amount of **7** (Scheme 2). Reaction conditions and yields are as presented in Table 2. Structural assignment of cycloadducts **12**–**15** containing the novel bicyclo[3.1.1]heptano[4,5-*c*]pyrazole skeleton are based on analytical and spectral data. As far as ^1H NMR of cycloadducts **12**–**14** is concerned, the chemical shift values of the hydrogen in the 4- position of the 4,5-dihydro pyrazole ring lies in the range 3.31–4.06 δ and are fully consistent with literature data.[13] The ^1H NMR spectrum of cycloadduct **15** shows a doublet of doublets at 4.00 δ. This chemical shift value strongly agrees with that of the hydrogen in the 5- position of the 4,5-dihydropyrazole ring of similar cycloadducts obtained from *N*-phenyl-*C*-ethoxycarbonyl nitrilimine with cyclohexene and norbornene.[13a] These evidences are definitively substantiated by mutual NOE enhancements of the aromatic hydrogens H_c after irradiation of CH_3 (cycloadducts **12** and **14**), CH_2OH (cycloadduct **13**) and H_A (cycloadduct **15**) as it is shown in Figure 2.

The configurations of the newly-formed stereocentres of cycloadducts **12**–**15** were assigned unambiguously upon mutual NOE enhancement of H_A after irradiation of H_B (see Table 3). It is apparent that the mentioned NOE enhancements can occur only in the case of the stereochemical



Scheme 2.

Table 2. Reaction between hydrazonoyl chloride **7** and pinane derivatives **1**–**4** in dry toluene[a,b]

Starting dipolarophile	Base (equiv.)	Time (h)	Temperature (°C)	Products and yields (%)[c]					
				8	**9**	**12**	**13**	**14**	**15**
1	Et_3N (5)	48	110	11	—	23	—	—	—
	Ag_2CO_3 (2)	72	25	43	—	17	—	—	—
	AcOAg (1)	36	25	32	12	20	—	—	—
2	Et_3N (5)	40	110	14	—	—	46	—	—
	Ag_2CO_3 (2)	72	25	38	—	—	28	—	—
	AcOAg (1)	30	25	30	17	—	31	—	—
3	Et_3N (5)	54	110	13	—	—	—	27	—
	Ag_2CO_3 (2)	76	25	21	—	—	—	25	—
	AcOAg (1)	40	25	19	12	—	—	28	—
4	Et_3N (5)	48	110	5	—	—	—	—	37
	Ag_2CO_3 (2)	72	25	30	—	—	—	—	29
	AcOAg (1)	40	25	22	20	—	—	—	35

[a] Some amount of the starting dipolarophile was always recovered (see Section 5).
[b] Some amount of tarry material was always formed.
[c] Isolation yields.

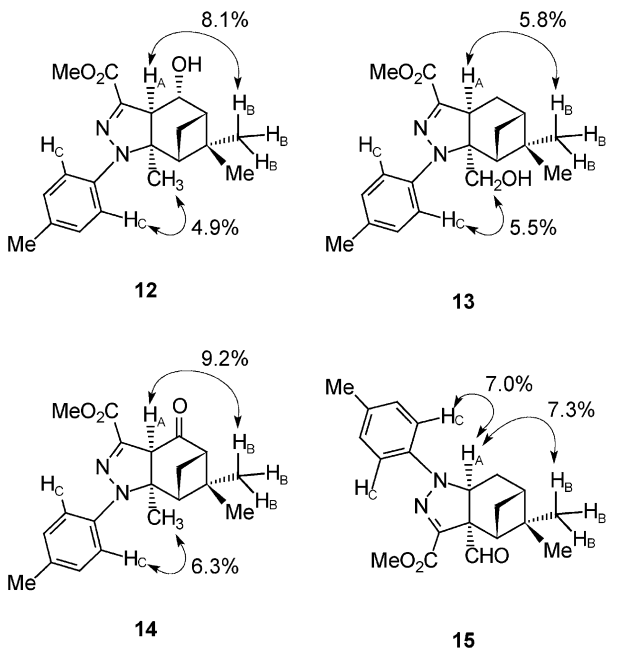


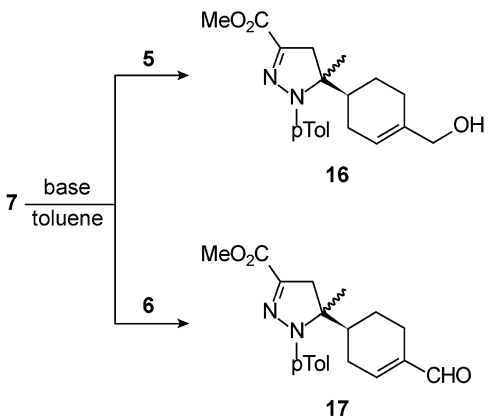
Figure 2.

Table 3. AM1 computed distances between H_A and H_B

Cycloadduct	Computed distances (Å)
12	2.15
13	2.18
14	2.14
15	2.15

arrangement depicted by formulae **12**–**15**. In addition, NOESY experiments performed onto cycloadducts **13** and **14** showed correlation peaks between H_A and H_B. Structural elucidation of cycloadducts **12**–**15** was also performed through computational analysis of their energy minima conformations optimised at the AM1[14] level. The computed distances between H_A and the average position of H_B[15] (Table 3) are rather short and agrees with the experimental NOE enhancements.

Nitrilimine cycloadditions onto the limonene derivatives **5**, **6** (Scheme 3) were accomplished as summarised in Table 4. Both cycloadducts **16** and **17** were obtained as equimolecular mixtures of diastereoisomers which were separated through laborious silica gel column chromatography. It needs to be noted that: (i) all four diastereoisomers crystallised as amorphous powders which precluded any diffractometric analysis, and (ii) NOE spectroscopy was ineffective in determining the proximity of diagnostic hydrogen nuclei due to the unhindered rotation of the cyclohexenyl pendant. It follows that the configurations to C-5 of the 4,5-dihydropyrazoles **16** and **17** remain undetermined.



Scheme 3.

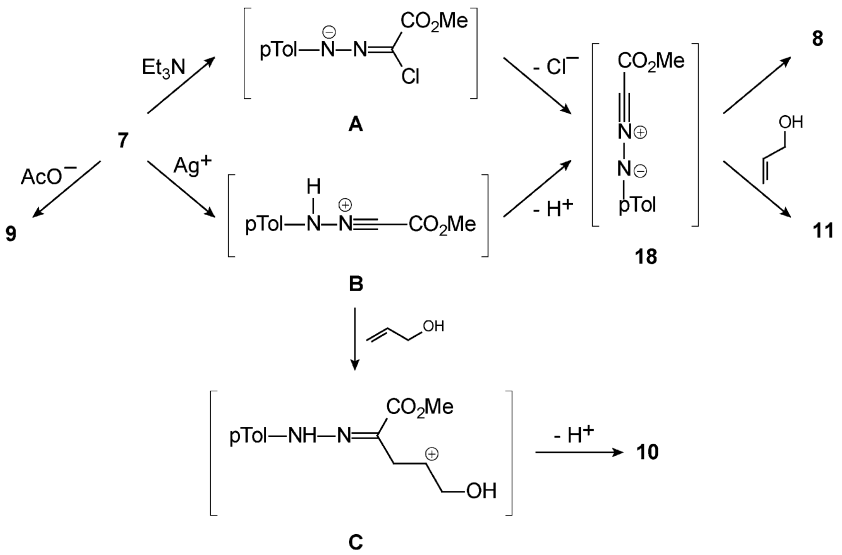
3. Discussion

Nitrilimine generation by treatment of the corresponding hydrazonoyl chloride with silver salts is not unprecedented.[16–18] However, the mechanistic picture outlined in Scheme 4 may be helpful to rationalise the above results. It is likely that the formation of nitrilimine **18** is promoted by the high affinity of the silver ion towards Cl^- via the nitrilium cation **B**. As a concurrent pathway, allyl alcohol capture by the latter gives rise to carbocation **C** which then evolves to **10**. It is reasonable that, due to steric hindrance, dipolarophiles **1** and **2** do not follow this alternative route. On the other hand, it is known that nitrilimine generation under homogeneous conditions proceeds through the

Table 4. Reaction between hydrazonoyl chloride **7** and limonene derivatives **5**, **6** in dry toluene[a,b]

Starting dipolarophile	Base (equiv.)	Time (h)	Temperature (°C)	Products and yields (%)[c]			
				8	**9**	**16**[d]	**17**[d]
5	Et_3N (5)	48	110	16	—	46	—
	Ag_2CO_3 (2)	72	25	12	—	40	—
	AcOAg (1)	36	25	16	14	44	—
6	Et_3N (5)	40	110	14	—	—	48
	Ag_2CO_3 (2)	72	25	10	—	—	41
	AcOAg (1)	30	25	14	14	—	44

[a] Some amount of the starting dipolarophile was always recovered (see Section 5).
[b] Some amount of tarry material was always formed.
[c] Isolated yields.
[d] As equimolecular mixtures of diastereoisomers.



Scheme 4.

aza-anion intermediate **A** according to Huisgen's cycloaddition protocol.[19]

Independent of the reaction conditions, cycloaddition of **18** onto pinane derivatives **1**–**4** were completely regio- and stereoselective (Scheme 2, Table 2). The severe steric hindrance exerted by the two methyl groups placed onto the cyclobutyl ring of the dipolarophiles are responsible for the complete stereoselectivity. In contrast, the observed regioselectivity deserves some comments. It has been recognised that nitrilimine cycloadditions to monosubstituted ethylenes are controlled by the HOMO of the 1,3-dipole,[20] thus leading to 5-substituted-4,5-dihydropyrazoles. This rule is no longer obeyed in the case of 1,2-disubstituted or 1,1,2-trisubstituted ethylenes, whose cycloadditions give regioisomeric mixtures[21] because of the similarity of their LUMO atomic coefficients.[20] However, nitrilimine cycloadditions onto the trisubstituted C=C bond of compounds **1**, **2** gave only 4,5-dihydropyrazoles in which the *N*-terminus of the 1,3-dipole is bonded to the trisubstituted carbon of the dipolarophile fragment. In the present context, steric interactions between the substituents of the 1,3-dipole and that of dipolarophiles **1**, **2** could play a major role in dictating the regioselectivity. On the other hand, regioselectivity onto dipolarophiles **3**, **4** can result from the electronic demands of cycloaddition. In fact, only the cycloadducts in which the *C*-terminus of the 1,3-dipole is bonded to the α- carbon to the carbonyl group were obtained.

As far as limonene derivatives **5**, **6** are concerned, site- and regioselective cycloadditions were found, but these processes were not stereoselective (Scheme 3, Table 4). Steric interactions still play a central role in determining the observed reactivity. It can be noted that: (i) irrespective of electronic activation, only the less substituted C=C bond is involved in the cycloaddition; (ii) 5,5-disubstituted-4,5-dihydropyrazoles are formed, in agreement with the cycloaddition of nitrilimines onto 1,1-disubstituted ethylenes;[20,21] and (iii) the limonene skeleton is not rigid enough to allow stereoselective cycloadditions.

4. Conclusions

Notwithstanding the reactive sites of both pinane and limonene derivatives are sterically crowded, their reactivity towards hydrazonoyl chloride **7** were satisfactory irrespective of the basic agent. Finally, owing to the presence of

reactive functional groups, cycloaddition products are amenable for further functionalisation.

5. Experimental

Melting points were determined with a Büchi apparatus in open tubes and are uncorrected. IR spectra were recorded with a Perkin–Elmer 1725 X spectrophotometer. Mass spectra were determined with a VG-70EQ apparatus. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were taken with a Bruker AMX 300 instrument (in $CDCl_3$ solutions at room temperature). Chemical shifts are given as ppm from tetramethylsilane and *J* values are given in Hz. NOE experiments were performed by setting the following parameters: relaxation delay (d1) 2 s, irradiation power (dl2) 74dB, and total irradiation time (for each signal) 1.8 s. The NOESY experiments were acquired with 1024 data points for 512 increments, without zero-filling. A relaxation delay (d1) of 2 s and a mixing time (d8) of 700 ms (compound **14**) or 800 ms (compound **15**) was used. Optical rotations, $[\alpha]_D^{25}$, were recorded on a Perkin–Elmer 241 polarimeter at the sodium D-line.

Compounds **1**–**4** and **5**, **6** were used as purchased from Aldrich.

Compounds **8** and **11** has been fully characterised previously.[12]

5.1. Reaction between hydrazonoyl chloride 7 and allyl alcohol in dry toluene

Method A. A solution of hydrazonoyl chloride **7** (0.45 g, 2.0 mmol) and allyl alcohol (0.12 g, 2.0 mmol) in dry toluene (20 mL) was treated with triethylamine (1.01 g, 10.0 mmol) and stirred at room temperaure for 72 h. The crude was evaporated under reduced pressure, and then the residue was chromatographed on a silica gel column with ethyl acetate–hexane 2:1. The first fractions contained unreacted **7** (0.31 g, 69%), further elution gave **11** (0.10 g, 20%).

Method B. A solution of the hydrazonoyl chloride **7** (0.45 g, 2.0 mmol) and allyl alcohol (0.12 g, 2.0 mmol) in dry toluene (20 mL) was treated with triethylamine (1.01 g, 1.0 mmol) and refluxed for 24 h. The crude was evaporated under reduced pressure, and then the residue was crystallised from diisopropyl ether giving solid **11** (0.38 g, 76%). The mother liquor was chromatographed on a silica gel column with ethyl acetate–hexane 1:1 affording tetrazine **8** (80 mg, 21%).

Method C. A solution of the hydrazonoyl chloride **7** (0.45 g, 2.0 mmol) and allyl alcohol (0.12 g, 2.0 mmol) in dry toluene (20 mL) was treated with silver carbonate (1.10 g, 4.0 mmol) and stirred in the dark at room temperature for 36 h. The undissolved material was filtered off and washed with ethyl acetate (2×15 mL). The solvent was evaporated, and then the residue was chromatographed on a silica gel column with ethyl acetate–hexane 2:1. The first fractions contained unreacted **7** (77 mg, 17%), further elution gave **11** (0.34 g, 68%).

Method D. A solution of the hydrazonoyl chloride **7** (0.45 g, 2.0 mmol) and allyl alcohol (0.12 g, 2.0 mmol) in dry toluene (20 mL) was treated with copper (I) acetate (0.37 g, 3.0 mmol) and stirred at room temperature for 40 h. The undissolved material was filtered off and washed with ethyl acetate (2×15 mL). The organic layer was washed firstly with 5% aqueous sodium hydrogencarbonate (15 mL), then with water (20 mL). The solvent was evaporated, and then the residue was chromatographed on a silica gel column with ethyl acetate–hexane 2:1. The first fractions contained unreacted **7** (0.21 g, 47%) followed by the hydrazide **9** (65 mg, 13%).

5.1.1. Compound 9. White powder; mp 108 °C (from hexane–benzene); IR (nujol) 3235, 1730, 1720 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.98 (3H, s), 2.37 (3H, s), 3.90 (3H, s), 7.1–7.4 (4H, m), 9.34 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 20.18 (q), 30.12 (q), 43.6 (q), 119.9 (d), 129.2 (d), 131.2 (s), 133.8 (s), 158.6 (s), 159.5 (s), 169.8 (s); MS *m/z* 250 (M^+). Anal. calcd for $C_{12}H_{14}N_2O_4$: C, 57.59; H, 5.64; N, 11.19. Found: C, 57.62; H, 5.69; N, 11.24.

Further elution gave the hydrazone **10** (27 mg, 6%) followed by **11** (0.17 g, 33%).

5.1.2. Compound 10. Thick orange oil; IR (nujol) 3450, 1705 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.90 (1H, br s), 2.32 (3H, s), 3.23 (2H, dd, *J*=3.6, 1.0 Hz), 3.83 (3H, s), 5.02 (1H, dt, *J*=7.8, 3.6 Hz), 6.58 (1H, dt, *J*=7.8, 1.0 Hz) 7.0–7.2 (4H, m), 8.30 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 22.8 (q), 48.8 (t), 54.0 (q), 67.3 (d), 77.2 (d), 118.8 (d), 129.6 (d), 133.1 (s), 134.7 (s), 140.2 (s), 168.2 (s); MS *m/z* 248 (M^+). Anal. calcd for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28. Found: C, 62.93; H, 6.52; N, 11.34.

Method E. A solution of the hydrazonoyl chloride **7** (0.45 g, 2.0 mmol) and allyl alcohol (0.12 g, 2.0 mmol) in dry toluene (20 mL) was treated with silver acetate (0.33 g, 2.0 mmol) and stirred at room temperature for 3 h. The undissolved material was filtered off and washed with ethyl acetate (2×15 mL). The organic layer was washed firstly with 5% aqueous sodium hydrogencarbonate (15 mL), then with water (20 mL). The solvent was evaporated, and then the residue was chromatographed on a silica gel column with ethyl acetate–hexane 2:1. The first fractions contained the hydrazide **9** (35 mg, 7%), further elution gave **10** (40 mg, 8%) followed by **11** (0.42 g, 84%).

5.2. Reaction between hydrazonoyl chloride 7 and monoterpene derivatives 1–4 or 5, 6 in the presence of triethylamine

A solution of hydrazonoyl chloride **7** (0.45 g, 2.0 mmol) and the appropriate monoterpene derivative **1**–**4** or **5**, **6** (2.0 mmol) in dry toluene (20 mL) was treated with triethylamine (1.01 g, 1.0 mmol) and refluxed for the time indicated in Table 2 or 4. The crude was evaporated under reduced pressure and the residue was chromatographed on a silica gel column with ethyl acetate–dichloromethane 9:1. The first fractions contained the starting monoterpene derivative in a 5–15% amount, followed by tetrazine **8**.

In the case of pinane derivatives **1**–**4** further elution gave the corresponding cycloadducts **12**–**15** (see Table 2).

5.2.1. Compound 12. 0.16 g, 23%. Pale yellow powder; mp 79 °C (from diisopropyl ether); $[\alpha]_D^{25}=-28.0$ ($CHCl_3$, $c=0.11$); IR (nujol) 3470, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.19 (3H, s), 1.34 (3H, s), 1.37 (3H, s), 2.10–2.50 (4H, m), 2.29 (3H, s), 3.24 (1H, br s), 3.31 (1H, d, $J=4.4$ Hz), 3.91 (3H, s), 3.95 (1H, dd, $J=12.9$, 4.4 Hz), 7.0–7.2 (4H, m); ^{13}C NMR ($CDCl_3$) δ 21.3 (q), 24.6 (q), 25.0 (q), 27.0 (t), 28.7 (d), 38.2 (s), 44.1 (q), 50.1 (d), 53.0 (d), 58.5 (q), 75.7 (s), 78.7 (d), 119.7 (d), 129.7 (d), 133.4 (s), 139.2 (s), 148.2 (s), 158.8 (s); MS *m/z* 342 (M^+). Anal. calcd for $C_{20}H_{26}N_2O_3$: C, 70.15; H, 7.65; N, 8.18. Found: C, 70.11; H, 7.68; N, 8.22.

5.2.2. Compound 13. Yield 0.32 g, 46%. Pale yellow powder; mp 228 °C (from diisopropyl ether); $[\alpha]_D^{25}=-87.8$ ($CHCl_3$, $c=0.50$); IR (nujol) 3450, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.98 (3H, s), 1.24 (3H, s), 1.70–2.50 (6H, m), 2.24 (3H, s), 3.50 (1H, br s), 3.54 (1H, d, $J=12.2$ Hz), 3.61 (1H, dd, $J=11.3$, 4.6 Hz), 3.70 (1H, d, $J=12.2$ Hz), 3.80 (3H, s), 7.0–7.2 (4H, m); ^{13}C NMR ($CDCl_3$) δ 20.7 (q), 23.9 (q), 27.3 (t), 27.4 (d), 32.7 (t), 37.8 (s), 38.7 (d), 41.0 (d), 46.1 (q), 51.8 (q), 80.8 (s), 119.7 (d), 129.6 (d), 133.3 (s), 139.7 (s), 143.2 (s), 158.8 (s); MS *m/z* 342 (M^+). Anal. calcd for $C_{20}H_{26}N_2O_3$: C, 70.15; H, 7.65; N, 8.18. Found: C, 70.12; H, 7.69; N, 8.24.

5.2.3. Compound 14. Yield 0.18 g, 27%. Yellow powder; mp 85 °C (from diisopropyl ether); $[\alpha]_D^{25}=-89.0$ ($CHCl_3$, $c=0.10$); IR (nujol) 1730, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.05 (3H, s), 1.44 (3H, s), 1.49 (3H, s), 2.30 (3H, s), 2.40–2.80 (4H, m), 3.89 (3H, s), 4.06 (1H, s), 7.0–7.1 (4H, m); ^{13}C NMR ($CDCl_3$) δ 20.7 (q), 24.6 (q), 24.8 (t), 25.2 (q), 27.1 (q), 40.2 (s), 50.3 (d), 52.1 (q), 56.0 (d), 59.8 (q), 61.9 (d), 73.8 (s), 119.8 (d), 130.7 (d), 133.8 (s), 134.2 (s), 139.4 (s), 162.9 (s), 205.1 (s); MS *m/z* 340 (M^+). Anal. calcd for $C_{20}H_{24}N_2O_3$: C, 70.57; H, 7.11; N, 8.23. Found: C, 70.62; H, 7.08; N, 8.29.

5.2.4. Compound 15. Yield 0.25 g, 25%. White powder; mp 76 °C (from diisopropyl ether); $[\alpha]_D^{25}=-28.0$ ($CHCl_3$, $c=0.10$); IR (nujol) 1730, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.82 (3H, s), 1.31 (3H, s), 1.90–2.90 (6H, m), 2.23 (3H, s), 3.90 (3H, s), 4.00 (1H, dd, $J=7.8$, 3.7 Hz), 6.9–7.0 (4H, m), 9.70 (1H, s); ^{13}C NMR ($CDCl_3$) δ 20.7 (q), 23.5 (q), 25.4 (t), 26.4 (q), 31.6 (t), 37.3 (s), 38.4 (d), 41.0 (d), 42.3 (d), 52.1 (q), 81.9 (s), 118.3 (d), 129.5 (d), 133.3 (s), 139.0 (s), 142.4 (s), 162.6 (s), 195.5 (d); MS *m/z* 340 (M^+). Anal. calcd for $C_{20}H_{24}N_2O_3$: C, 70.57; H, 7.11; N, 8.23. Found: C, 70.60; H, 7.13; N, 8.28.

In the case of limonene derivatives **5** and **6** further elution gave the corresponding cycloadducts **16** and **17**, respectively, as equimolecular mixtures of diastereoisomers. Separation of diastereoisomerically pure cycloadducts was achieved by further silica gel column chromatography of the corresponding mixtures **16** and **17** with ethyl acetate–hexane 1:4 (see Table 3).

5.2.5. Compound 16 (first diastereoisomer). Yield 0.16 g, 23%. White powder; mp 55 °C (from methanol); $[\alpha]_D^{25}=-38.0$ ($CHCl_3$, $c=0.12$); IR (nujol) 3460, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.38 (3H, s), 1.65 (1H, br s), 1.70–2.28 (7H, m), 2.30 (3H, s), 2.72 (1H, d, $J=19.4$ Hz), 3.20 (1H, d, $J=19.4$ Hz), 3.84 (3H, s), 4.00 (2H, s), 5.62 (1H, dd, $J=6.7$, 4.5 Hz), 7.0–7.2 (4H, m); MS *m/z* 342 (M^+). Anal. calcd for $C_{20}H_{26}N_2O_3$: C, 70.15; H, 7.65; N, 8.18. Found: C, 70.12; H, 7.63; N, 8.22.

5.2.6. Compound 16 (second diastereoisomer). Yield 0.16 g, 23%. White powder; mp 49 °C (from methanol); $[\alpha]_D^{25}=-9.0$ ($CHCl_3$, $c=0.10$); IR (nujol) 3460, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.48 (3H, s), 1.60 (1H, br s), 1.70–2.28 (7H, m), 2.33 (3H, s), 2.78 (1H, d, $J=19.4$ Hz), 3.21 (1H, d, $J=19.4$ Hz), 3.88 (3H, s), 4.00 (2H, s), 5.68 (1H, dd, $J=6.7$, 4.5 Hz), 7.0–7.2 (4H, m); MS *m/z* 342 (M^+). Anal. calcd for $C_{20}H_{26}N_2O_3$: C, 70.15; H, 7.65; N, 8.18. Found: C, 70.19; H, 7.67; N, 8.21.

5.2.7. Compound 17 (first diastereoisomer). Yield 0.16 g, 24%. White powder; mp 63 °C (from methanol); $[\alpha]_D^{25}=-55.2$ ($CHCl_3$, $c=0.11$); IR (nujol) 2230, 1720, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (3H, s), 1.80–2.40 (7H, m), 2.32 (3H, s), 2.78 (1H, d, $J=17.6$ Hz), 3.18 (1H, d, $J=17.6$ Hz), 3.81 (3H, s), 6.82 (1H, dd, $J=6.9$, 4.4 Hz), 7.0–7.2 (4H, m), 9.40 (1H, s); MS *m/z* 340 (M^+). Anal. calcd for $C_{20}H_{24}N_2O_3$: C, 70.57; H, 7.11; N, 8.23. Found: C, 70.61; H, 7.08; N, 8.27.

5.2.8. Compound 17 (second diastereoisomer). Yield 0.16 g, 24%. White powder; mp 44 °C (from methanol); $[\alpha]_D^{25}=-11.2$ ($CHCl_3$, $c=0.16$); IR (nujol) 2220, 1720, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.51 (3H, s), 1.80–2.40 (7H, m), 2.30 (3H, s), 2.82 (1H, d, $J=17.6$ Hz), 3.20 (1H, d, $J=17.6$ Hz), 3.80 (3H, s), 6.74 (1H, dd, $J=6.9$, 4.4 Hz), 7.0–7.2 (4H, m), 9.44 (1H, s); MS *m/z* 340 (M^+). Anal. calcd for $C_{20}H_{24}N_2O_3$: C, 70.57; H, 7.11; N, 8.23. Found: C, 70.61; H, 7.08; N, 8.27.

5.3. Reaction between hydrazonoyl chloride 7 and monoterpene derivatives 1–4 or 5, 6 in the presence of silver carbonate

A solution of hydrazonoyl chloride **7** (0.45 g, 2.0 mmol) and the appropriate monoterpene derivative **1**–**4** or **5**, **6** (2.0 mmol) in dry toluene (20 mL) was treated with silver carbonate (1.10 g, 4.0 mmol) and stirred in the dark at room temperature for the time indicated in Table 2 or 4. The undissolved material was filtered off and washed with ethyl acetate (2×15 mL). The solvent was evaporated, and then the residue was chromatographed on a silica gel column with ethyl acetate–hexane 2:1. The first fractions contained the starting monoterpene derivative in a 5–15% amount, followed by tetrazine **8**.

In the case of pinane derivatives **2**–**4** further elution gave the corresponding cycloadducts **12**–**15** (see Table 2).

In the case of limonene derivatives **5** and **6** further elution gave the corresponding cycloadducts **16** and **17**, respectively, as equimolecular mixtures of diastereoisomers. Separation of diastereoisomerically pure cycloadducts was achieved by further silica gel column chromatography of the

corresponding mixtures **17** and **18** with ethyl acetate–hexane 1:4 (see Table 3).

5.4. Reaction between hydrazonoyl chloride 7 and monoterpene derivatives 1–4 or 5, 6 in the presence of silver acetate

A solution of hydrazonoyl chloride **7** (0.45 g, 2.0 mmol) and the appropriate monoterpene derivative **1**–**4** or **5**, **6** (2.0 mmol) in dry toluene (20 mL) was treated with silver acetate (0.33 g, 2.0 mmol) and stirred in the dark at room temperature for the time indicated in Table 2 or 4. The undissolved material was filtered off and washed with ethyl acetate (2×25 mL).

The organic layer was washed firstly with 5% aqueous sodium hydrogencarbonate (20 mL), the with water (25 mL). The solvent was evaporated, and then the residue was chromatographed on a silica gel column with ethyl acetate–dichloromethane 9:1. The first fractions contained the starting monoterpene derivative in a 5–15% amount. Further elution gave tetrazine **8** followed by hydrazide **9**.

In the case of pinane derivatives **1**–**4** further elution gave the corresponding cycloadducts **12**–**15** (see Table 2).

In the case of limonene derivatives **5** and **6** further elution gave the corresponding cycloadducts **16** and **17**, respectively, as equimolecular mixtures of diastereoisomers. Separation of diastereoisomerically pure cycloadducts was achieved by further silica gel column chromatography of the corresponding mixtures **16** and **17** with ethyl acetate–hexane 1:4 (see Table 3).

6. Supplementary material

Two colour figures of NOESY spectra of cycloadducts **13** and **14**.

Acknowledgements

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Novel platinum-catalysed cascade reactions: cyclisation, ring expansion and 1,2-oxygen shift reaction of a camphor-derived diyne

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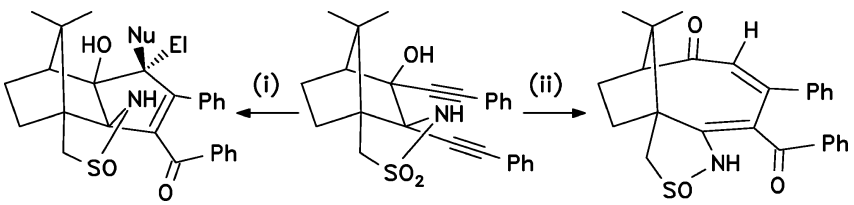
Abstract—2,3-Bis(ethynyl)-3-hydroxy-camphorsultam was converted in one step into a novel tetracyclic cyclopentenone derivative, in an unprecedented platinum-catalysed cascade reaction. In the course of this reaction, cyclisation of the alkynes takes place, together with a ring expansion of the camphor skeleton and 1,2-migration of an oxygen atom. The structure of the unexpected product was analysed in detail by one- and two-dimensional NMR spectroscopy, and validated with the help of quantum mechanical calculations (B3LYP/6-31G** and B3LYP/6-31+G(2df)) of the IR vibrational frequencies and the ^{1}H and ^{13}C isotropic chemical shifts.

1. Introduction

Cascade reactions, that is, multiple functional group transformations in a one-pot reaction, are of increasing importance in organic chemistry.[1–4] This is not only due to the need for more efficient and less labour-intense methodologies for the synthesis of organic compounds, but also consequence of the increasing importance of environmental considerations in chemistry. Catalytic rearrangements are among the most atom efficient cascade reactions as this type of transformation often leads to an increase in complexity of a molecule, without use of any reagents except of a catalyst. All atoms of the starting material are retained in the product, the reactions are thus completely atom economic and occur under production of a minimum amount of waste.[5] The development of new and efficient catalytic rearrangements is still an important issue in organic synthesis, as little is known about the interplay between organic functional groups within a molecule in the presence of a transition metal catalyst, and the synthetic potential such chemistry offers.

Our previous work demonstrated that camphor derived bis-alkynes are prone to undergo cascade reactions due to the high density of functional groups in the molecule and their spacial pre-organisation relative to each other.[6,7] Electrophilic attack results in α-addition of the electrophile and a nucleophile to the internal carbon atom of one of the C≡C bonds, cyclisation with the other alkyne to form a five membered ring, and an unprecedented stereoselective reduction of a sulfonamide to a sulfinamide (see Scheme 1). NMR monitoring allowed for characterisation of the intermediates and provided a sound understanding of the reaction mechanism.[6,7]

Platinum compounds induce a different cascade reaction which results in one step in the formation of compounds whose skeleton resembles to some extend the AB-ring



Scheme 1. (i)=El-Nu; (ii)=cat. $[PtCl_2(PhCN)_2]$.

Keywords: Cycloisomerisation; Platinum; Catalysis; GIAO calculation.
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structure of Taxol. In contrast to the electrophilically induced reactions described above, no intermediates were detected in the Pt-catalysed reaction. Its mechanism therefore still remains unclear.[8]

In a continuation of this work, we became interested in the scope and limitations of this new type of transformation. Derivatives bearing an n-alkyl substituent on the alkynes were shown to provide products analogous to those obtained with aromatically substituted alkynes. Bulky tertiary alkyl substituents, in contrast, lead to a new reaction pattern which is not yet fully understood.[9] By modification of the substrate, we hope to gain more insight into the functional group compatibility of the reaction and mechanistic aspects. This, in turn, will allow for a more general application of this type of reaction to organic synthesis.

Herein, we report on the synthesis of the parent ethynyl-substituted camphor derivative and its platinum-catalysed reaction, together with the structural analysis of the product by NMR spectroscopy and quantum mechanical calculations of spectroscopic data.

2. Results and discussion

The ethynyl group, as the smallest alkyne substituent possible, is expected to be readily involved in cascade reactions because of the lack of any steric hindrance. Additionally, the C≡CH moiety is electronically different from an alkyl or aryl substituted alkyne, and electrophilic attack is therefore expected to occur at the terminal alkyne carbon atom rather than the internal one. This is supposed to lead to a different reaction pattern than the one observed for the phenylacetylene derivative.

2.1. Synthesis of the camphor-derived diynes

The camphor-derived diyne **3** was synthesised via its trimethylsilyl derivative **2**, as shown in Scheme 2. 3-Oxo-camphorsulfonylimine **1** was reacted with two equivalents of $LiCCSiMe_3$ using the protocol previously described for the synthesis of the analogous phenylethynyl substituted compound.[6,8] Removal of the trimethylsilyl groups with Bu_4NF provided the parent bis-ethynyl derivative **3** in good yield. The identity of the products was confirmed by elemental analysis, mass spectrometry, IR and ^1H and ^{13}C NMR spectroscopy.

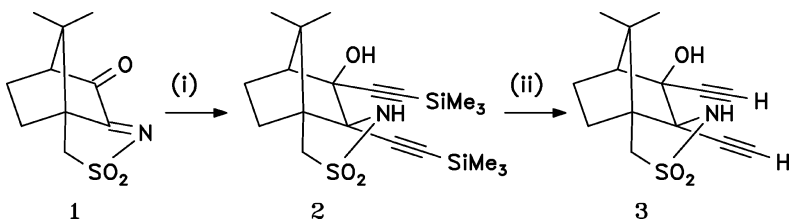
The ethynyl compound **3** is thermally more sensitive than the trimethylsilyl substituted compound **2** or other bis-alkyne derivatives synthesised previously. Its preparation was performed at room temperature, and the product was isolated after a reaction time of 1.5 h, to prevent decomposition of the crude compound to a brown tar, when left overnight. Once purified and crystallised, compound **3** is stable and can be stored at room temperature over a period of at least 6 months.

2.2. Platinum-catalysed cascade reaction of the diyne 3

Compound **3** was reacted in chloroform in the presence of a catalytic amount (2.5 mol%) of $PtCl_2(PhCN)_2$ at 55 °C. The reaction mixture gradually changed colour from yellow to black, suggesting that the platinum compound is reduced to Pt(0) in the course of the reaction. After 6 h of reaction time, the starting material is completely converted into one major product **4** and a small amount of a so far unidentified by-product. The platinum catalyst is essential for the formation of **4**. In its absence, only thermal decomposition to an intractable mixture of uncharacterised compounds takes place.

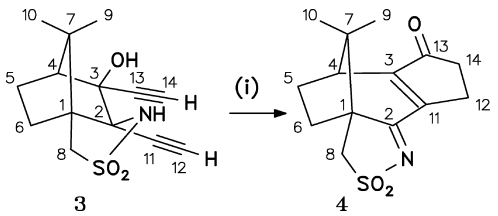
The formation of compound **4** is somewhat unexpected and cannot be deduced from any straightforward reaction mechanism. We have analysed the structure of the product in detail, using standard analytical methods and a combination of two-dimensional NMR experiments. Elemental analysis shows that the compound is an isomer of the starting material. Its fragmentation pattern in the mass spectrum indicates the presence of a sulfonamide or sulfonimine (loss of SO_2, m/z=64). Further loss of 28 mass units suggests either decarbonylation or loss of ethylene. In the IR spectrum, a strong band at 1706 cm^{-1} reveals the presence of a ketone. The strong signal at 1583 cm^{-1} is assigned to the stretching vibration of a C=N bond. The asymmetric and symmetric stretching of the SO_2 group appears at 1335 and 1167 cm^{-1}, respectively.

The ^1H NMR spectrum shows that the hydrocarbon part of the camphor skeleton is unchanged. The CMe_2 bridge appears as two singlets with a low intensity cross peak in the COSY spectrum, due to a small ^4J long range coupling between the two methyl groups. Also the typical spin system of the protons attached to positions 4, 5 and 6 was completely assigned with the help of COSY and NOESY spectra. The CH_2 in position 8 appears as the typical pair of doublets of the diastereotopic protons, indicating that a cyclic sulfonamide or sulfonylimine is still present in the molecule. In addition to these signals, a CH_2–CH_2 unit is detected which neither couples to the protons of the camphor system nor shows any NOE signals to them. ^{13}C NMR confirms the interpretation of the ^1H NMR and additionally reveals the presence of the two quarternary carbon atoms assigned to positions 1 and 7 in the camphor system, one C=O, one C=N, and one tetra-substituted



1 2 3

Scheme 2. (i)=BuLi, $HC{\equiv}CSiMe_3$; (ii)=Bu_4NF.



Scheme 3. (i)=cat. $[PtCl_2(PhCN)_2]$.

alkene moiety. All these structural units can be combined to produce five plausible structures **4** to **8**, shown in Scheme 3 and 4. Compound **7** can be ruled out on grounds of the ^{13}C chemical shifts of the alkene carbon atoms. The electronically unbalanced nature of the C=C bond in **7** would be expected to result in a much larger difference in chemical shift than that found experimentally.

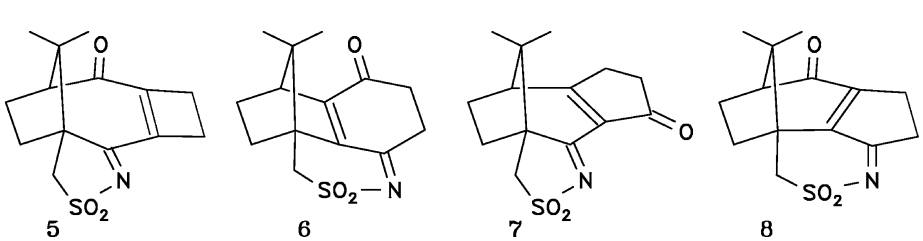
Long-range CH correlation allows for discrimination between the four remaining structures (**4**, **5**, **6**, and **8**). In addition to the expected cross peaks within the hydrocarbon part of the camphor system (i.e., atoms in position 4–10), signals between 12-H and both alkene carbon atoms (C-11 and C-13) appear, which is incompatible with structure **6**. The protons in position 8 correlate to the C=N group, which does not comply with **6** and **8**, and 5-H shows a cross peak to one of the alkene carbon atoms, which speaks against **8**. Therefore, both **6** and **8** can be eliminated as possible structure of the product. A decision between **4** and **5** is more difficult to accomplish, because almost all cross peaks in the HMBC agree with both structures. Only the appearance of a signal between the protons of position 5 and one of the alkene carbons is not compatible with **5** but in favour of **4** as the correct structure of the product. Since there is only one piece of spectroscopic evidence in favour of **4**, we decided to apply computational methods to support our assignment.

2.3. Theoretical support for the structure of product 4

Compounds **4** and **5**, as the most likely structures of the product, were geometry optimised with the DFT functional B3LYP using the basis sets 6-31G** and 6-31+G(2df). Their geometries do not differ significantly with the basis set. In the Hessian matrix, all force constants are real numbers, resulting in positive vibrational frequencies exclusively. The optimised structures are indeed minima on the potential energy surface. Both **4** and **5** are significantly more stable than the starting material **3** (see Table 1). Overall, product **4** is 29.8 kcal/mol more stable than the alternative product **5**. The reaction to produce **4** is therefore thermodynamically highly motivated.

The use of quantum mechanical methods for the calculation of IR spectra is quite established, both in inorganic[10] and organic chemistry,[11] in order to confirm a proposed structure and to distinguish between constitutional isomers. We expected this method to help in the discrimination between **4** and **5**. The calculated harmonic frequencies obtained from the Hessian matrices of the two compounds were scaled by a factor of 0.96 as the commonly accepted value for DFT calculations using the B3LYP functional,[12] and compared with the experimental IR spectrum. Overall, the calculated IR spectra for compounds **4** and **5** are quite similar in general appearance. The vibrational frequencies of characteristic functional groups of **4** appear closer to the experimental values, however, the corresponding data for **5** do not differ drastically, if one takes into account that the choice of the scaling factor might have introduced a systematic error (see Table 2). In **4** the stretching vibration of the C=C bond is very weak and practically falls together with the C=N vibration, which agrees with the experimental spectrum. The calculated spectrum of **5**, in contrast, shows a medium intense C=C stretch in between the C=O and C=N signal. Additionally, **5** displays a medium intense signal at about 590 cm^{-1} due to a skeletal vibration of the carbon framework which can be considered characteristic for this compound. Its absence in the calculated spectrum of **4** as well as in the experimental spectrum gives further evidence for **4** as the correct structure.

Quantum mechanical calculation of NMR chemical shifts is gaining increasing importance in organic chemistry as a tool for structure determination of natural products[13] and even for conformational analysis of organic compounds.[14] For the calculation of the ^1H and ^{13}C NMR isotropic chemical



Scheme 4. Alternative structures of the product of the cascade reaction.

Table 1. Calculated absolute and relative energies of compounds **3**, **4** and **5**

	Absolute energy (a.u.)		Relative energy (kcal/mol)	
	B3LYP/6-31G**	B3LYP/6-31+G(2df)	B3LYP/6-31G**	B3LYP/6-31+G(2df)
3	−1222.114869	−1222.195089	0	0
4	−1222.293767	−1222.371452	−112.25	−110.67
5	−1222.246311	−1222.324048	−82.48	−80.92

Table 2. Selected experimental IR vibrational frequencies and calculated (B3LYP) values of compounds **4** and **5** (cm^{-1})

Expt. data	**4**		**5**		Assignment
	6-31G**	6-31+G(2df)	6-31G**	6-31+G(2df)	
1706	1732	1704	1692	1662	ν (C=O)
1583	1611	1593	1619	1605	ν (C=C)
1583	1582	1574	1550	1542	ν (C=N)
1335	1289	1300	1289	1300	ν_{asym} (SO_2)
1167	1078	1105	1089	1106	ν_{sym} (SO_2)
(−)	(−)	(−)	585	588	Skeletal vibration

Table 3. Experimental and calculated (B3LYP) ^1H and ^{13}C NMR isotropic chemical shifts of compound **4**

^1H NMR			Assignment	^{13}C NMR		
Expt. data	6-31G**	6-31+G(2df)		Expt. data	6-31G**	6-31+G(2df)
0.83	0.77	0.65	9	18.5	19.2	21.7
1.09	0.98	0.97	10	24.5	24.4	25.5
1.51	1.49	1.38	5 *endo*	28.1	31.1	32.9
2.29	2.27	2.21	5 *exo*			
2.05	2.01	1.92	6 *exo*	32.9	35.7	38.0
2.26	2.13	2.12	6 *endo*			
2.66	2.34; 2.35	2.45; 2.45	14	35.7	36.0	38.8
2.88	2.69; 2.71	2.75; 2.75	12	23.6	26.0	28.2
2.91	2.80	2.75	4	43.0	46.3	46.7
3.21	2.58	2.79	8 *exo*	50.1	56.1	55.0
3.26	2.78	2.84	8 *endo*			
			7	49.7	53.6	53.7
			1	64.2	65.7	64.9
			11	154.3	154.7	156.1
			3	159.9	156.3	156.8
			2	178.8	170.1	171.6
			13	206.1	197.6	201.3

Table 4. Calculated (B3LYP) ^1H and ^{13}C NMR isotropic chemical shifts of compound **5**

^1H NMR		Assignment	^{13}C NMR	
6-31G**	6-31+G(2df)		6-31G**	6-31+G(2df)
1.02	0.97	9	19.1	20.9
0.97	0.95	10	29.1	29.3
1.89	1.81	5 *endo*	28.1	30.5
2.28	2.22	5 *exo*		
2.34	2.34	6 *endo*	39.6	41.6
2.22	2.12	6 *exo*		
2.49; 2.75	2.60; 2.76	14	29.1	29.3
2.81;2.82	2.77;2.81	12	28.7	30.2
2.65	2.69	4	65.3	67.6
2.45	2.70	8 *exo*	59.2	57.9
2.77	2.89	8 *endo*		
		7	51.9	51.4
		1	68.3	67.6
		11	144.7	147.6
		13	150.3	153.3
		2	165.7	167.8
		3	189.9	192.6

shifts of **4** and **5**, we applied the GIAO method to the geometry optimised structures obtained with B3LYP/6-31G** and B3LYP/6-31+G(2df). The results are presented in Tables 3 and 4. Figures 1 and 2 show the correlation between experimental and calculated data, the solid line representing the ideal linear relationship $x=y$. Linear regression was performed for all data sets, and the standard deviations, slopes and intercepts of the linear fits are given in Table 5.

Whereas the calculated ^{13}C chemical shifts for **4** compare well with the experimental data, major deviations appear in the data set for compound **5**. Not only atoms C-4, C-14, C-11, C-13, C-2 and C-3 are affected as the ones that differ most in the two structures. Also the hydrocarbon part of the camphor system poorly correlates although this structural unit is the same in both compounds.

The calculated ^1H chemical shifts scatter more than the ^{13}C data, and the results obtained with the 6-31G* * basis set are not satisfactory. With 6-31+G(2df), the linear relationship between experimental and calculated data for **4** is significantly improved. As expected, the calculated chemical shifts of the CH_2-SO_2N group show largest deviation from the experimental values. Even the comparatively large basis set 6-31+G(2df) seems not sufficient to fully cover the influence of the sulfonyl group on the ^1H chemical shifts of protons in its neighbourhood.

Overall, the correlation between experimental and calculated ^1H and ^{13}C chemical shifts is significantly better for compound **4** than for compound **5**, and a clear decision in favour of **4** as the correct structure can be made.

2.4. Mechanistic considerations for the formation of product 4

In our attempts to gain insight into the mechanism of the reaction, its intermediates and the catalytically active species involved, the reaction was carried out in an NMR

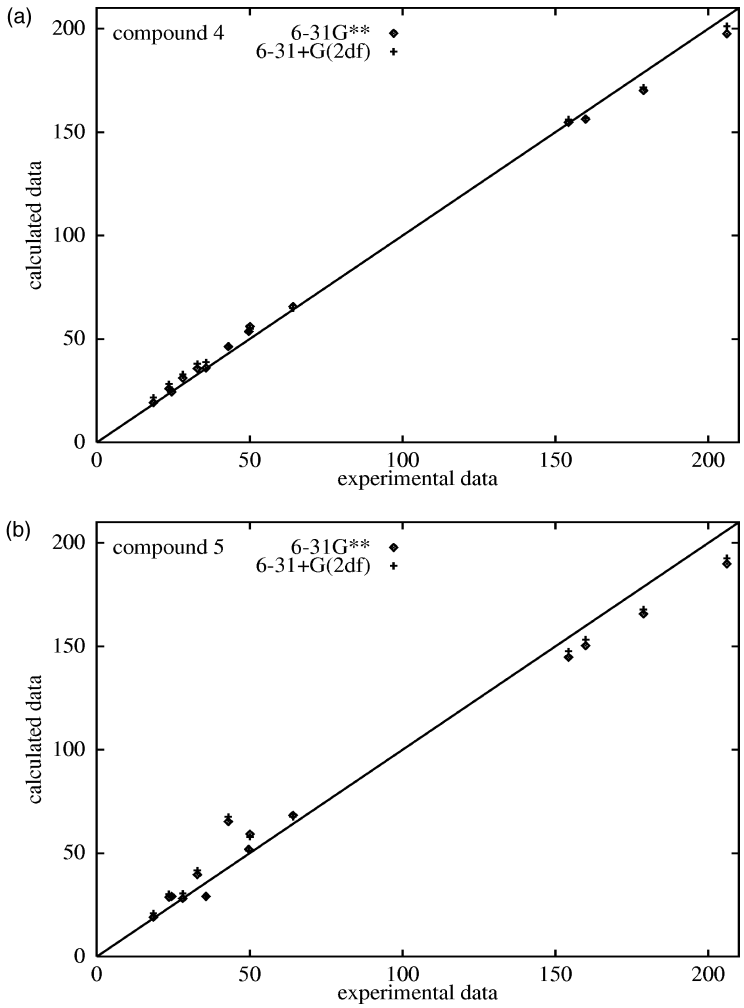


Figure 1. Experimental versus calculated ^{13}C isotropic chemical shifts for compounds **4** (a) and **5** (b).

tube at a temperature of 55 °C, using equimolar amounts of the platinum catalyst. However, the starting material **3** cleanly converted into **4** and no intermediates were detected. In the initial phase of the reaction, a small amount of benzonitrile was released from the platinum complex, and broadening of the OH signal of the starting material was observed. We assume that attack of the platinum occurs from the sterically less hindered side in the neighbourhood of the OH group, and coordination of the substrate causes one benzonitrile ligand to be displaced from the platinum center.

In analogy to the mechanism proposed by Fürstner, and independently by Murai, for related $PtCl_2$ catalysed cycloisomerisations of ene-yne systems, we anticipate that the first step in the catalytic cycle involves π-complexation of one of the alkynes to the platinum center, as shown in Scheme 5. This complex **A** can also be written in its polarised form **B** as an η^1-alkyne complex bearing a positive charge at the internal alkyne carbon atom.[15,16] This resonance structure accounts for the electrophilic nature of Pt(II)-alkyne complexes and their well known reactivity towards nucleophiles.[17] In other ene-yne cycloisomerisations described in the literature, the platinum alkyne complex is then postulated to undergo addition to the tethered alkene to form a coordinated cyclobutene as an intermediate for the observed products.[15,18,19] We believe that this pathway plays a minor role in our reaction because a formal [2+2] addition of the two alkynes would produce a strained structure **C** exhibiting two four membered rings annealed to each other, one of which is a coordinated cyclobutadiene rather that the cyclobutene in the examples of ene-yne cyclisations cited above. We propose a different mechanism in which the hydroxy group in close proximity of the electrophilic carbon atom of **B** efficiently acts as a nucleophile to form an epoxide of the structure **D**, whose resonance forms are shown in Scheme 5 as **D**-**1** to **D**-**6**. Similar alkylidene epoxides were reported as intermediates in the Favorskii rearrangement of α-halo-ketones,[20] and they were shown to be able to undergo cycloaddition reactions[20d] due to the dipolar character of resonance structures of the type **D**-**4** and **D**-**6**. Taking into account that the alkylidene epoxide moiety in **D** is in close proximity to an alkyne, an intramolecular cycloaddition to the C≡C bond is very likely to occur, to provide the five-membered cyclic ketone **E**. Final rearrangement of the strained cyclopropyl ring in a reaction similar to an ene-reaction leads to formation of the carbon skeleton of the final product, from which the platinum is removed in a reductive elimination step to provide **4** and the regenerated catalyst.

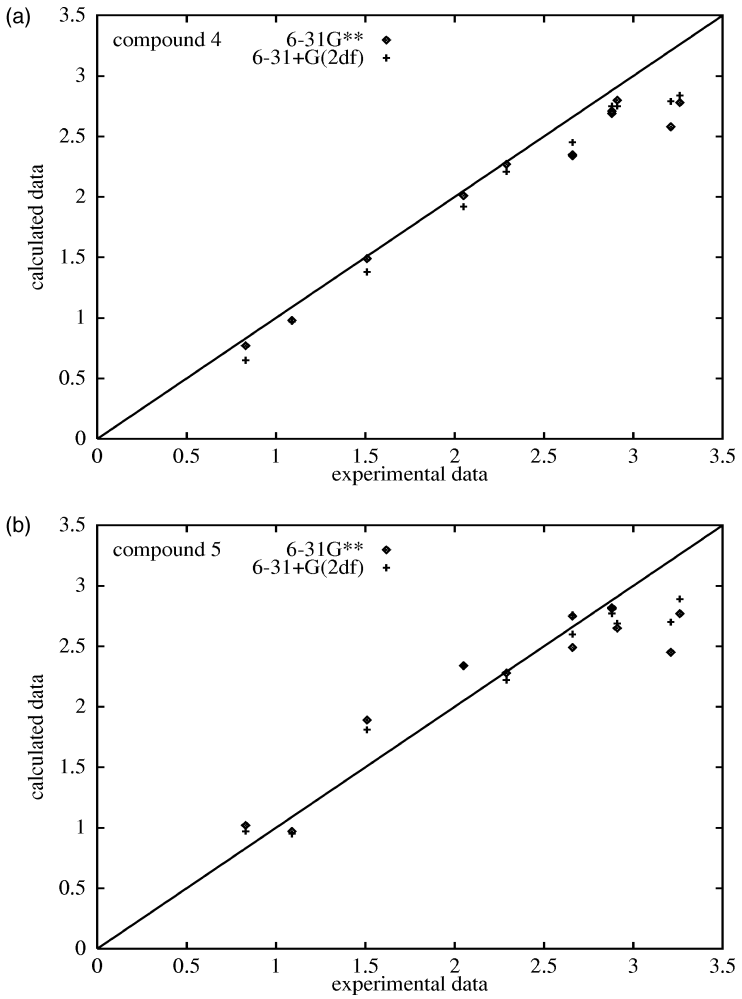


Figure 2. Experimental versus calculated ^{1}H isotropic chemical shifts for compounds **4** (a) and **5** (b).

Table 5. Linear regression data for experimental versus calculated ^{1}H and ^{13}C NMR isotropic chemical shifts of compounds **4** and **5**

	4		**5**	
	6-31G**	6-31+G(2df)	6-31G**	6-31+G(2df)
^{13}C NMR				
R^2	0.9984	0.9990	0.9871	0.9869
Intercept	4.3369	5.4019	8.7706	9.2838
Slope	0.9464	0.9497	0.8848	0.8955
^{1}H NMR				
R^2	0.9566	0.9815	0.8638	0.9246
Intercept	0.1981	0.0075	0.5543	0.3880
Slope	0.8269	0.9116	0.7250	0.8026

3. Concluding remarks

In this work, we have presented a novel platinum-catalysed cascade reaction leading to an isomerisation of a vicinal diyne to a cyclopentenone derivative possessing a new tetracyclic structure. Its tricyclo[6.2.1.$0^{2,6}$]undec-2-ene-3-one substructure has not been previously described. The reaction is highly efficient and leads selectively to a simultaneous transformation of five functional groups in one step. The reaction is technically easy to perform, moreover the starting material is readily accessible and the catalyst is easily prepared, and no precautions with respect to inert atmosphere or dried solvents have to be taken.

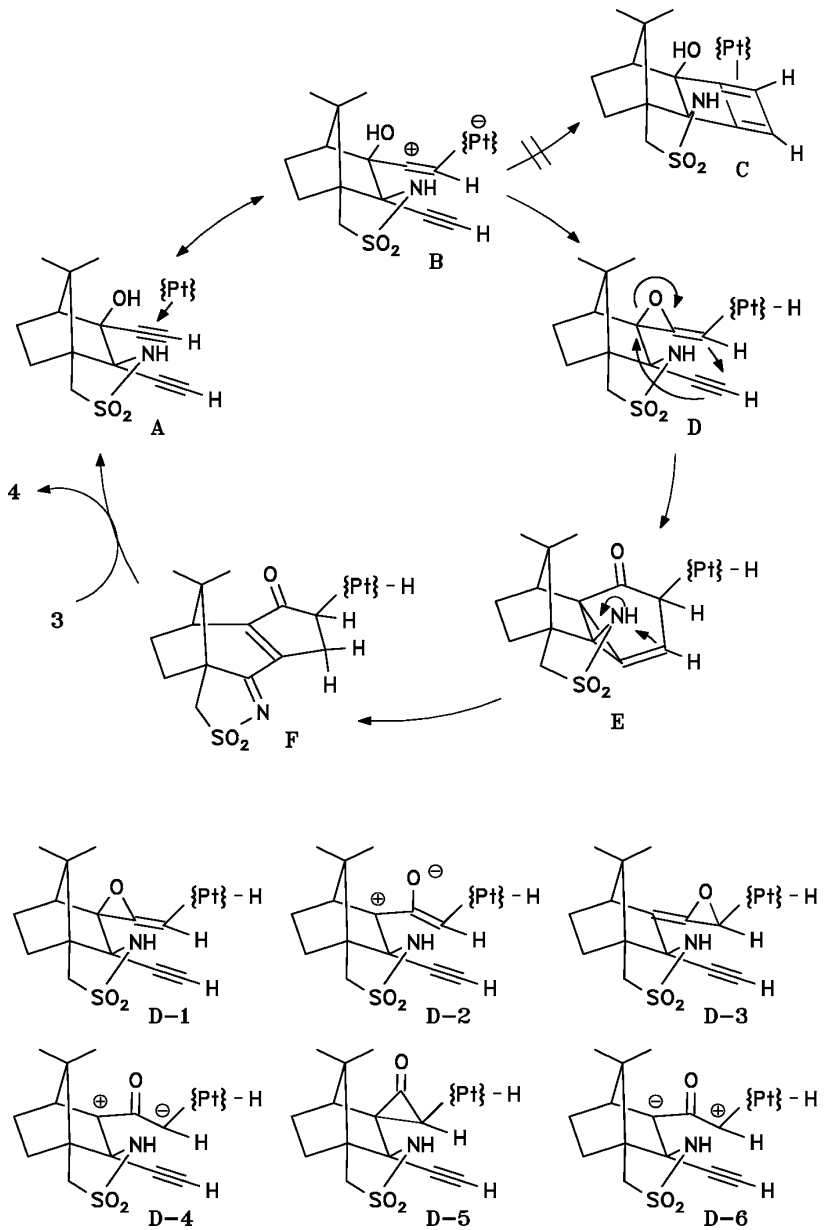
GIAO calculations of ^{1}H and ^{13}C NMR isotropic chemical shifts were applied as very useful tool in the determination of the structure of the product and for further supporting the results obtained by NMR spectroscopy. The computational effort for such kind of a calculation is moderate, since B3LYP/6-31G** already gives good results for ^{13}C NMR data. To reproduce ^{1}H NMR data with sufficient accuracy, a higher level basis set (e.g., 6-31+G(2df)) is necessary.

Overall, a mechanistic pathway of the cascade reaction was proposed, however, more experimental material will be required to fully understand the mechanism, scope and general applicability of the reaction. Work in this sense is currently ongoing in our group.

4. Experimental

4.1. Computational details

Calculations were performed with the programme package GAUSSIAN 03.[21] For visualisation of the results



Scheme 5. Proposed mechanism of the cascade reaction, and resonance structures of intermediate **D**.

MOLDEN[22] was used. Molecular geometries were fully optimized with B3LYP, using the standard basis sets 6-31G** and 6-31+G(2df) for all atoms. IR vibrational frequencies obtained from the Hessian matrix were scaled with a factor of 0.96 as the commonly accepted value for DFT calculations using the B3LYP functional.[12] ^{1}H and ^{13}C chemical shift tensors were calculated using the GIAO method as implemented in Gaussian 03. The calculated chemical shifts are referenced against TMS.

4.2. Materials and instrumentation

3-Oxo-camphorsulfonylimine **1** was prepared according to the literature.[23] C, H, N and S elemental analyses were carried out on a VarioEL III CHNS elemental analyser. Melting points were determined with a Büchi 530 apparatus in open capillaries. For TLC, Merck UV 254 SiO_2-plates have been used. Mass spectra were obtained on a Thermoquest MAT 95XL instrument. Infrared spectra (4000–400 cm^{-1}) were recorded on Perkin Elmer 2000 FTIR and Nicolet Avatar 320 FT-IR instruments in KBr pellets. ^{1}H and ^{13}C one- and two-dimensional NMR experiments were performed on Bruker AM 360 and Bruker DRX 500 spectrometers at ambient temperature. Signals were assigned with the help of COSY, NOESY, HMQC and HMBC spectra.

4.3. Preparation of the camphor-derived bis-diynes

4.3.1. (1a*S*,3a*S*,7*R*)-7-Hydroxy-8,8-dimethyl-1a,7-bis(trimethylsilylethynyl)-1,1a,4,5,6,7-hexahydro-3*H*-3a,6-methano-2,1-benzisothiazole 2,2-dioxide (2). A solution of trimethylsilylacetylene (1.00 g, 10.2 mmol) in dry diethyl ether (10 ml) was cooled in an ice bath. Butyl lithium (1.6 M in hexanes, 6.25 ml, 10 mmol) was added and the reaction mixture was left at room temperature for 30 min, before it

was added dropwise to a suspension of 3-oxo-camphorsulfonylimine **1** (1.14 g, 5 mmol) in dry diethyl ether (10 ml). The reaction mixture was stirred overnight, water (5 ml) was added and the organic phase was separated. The aqueous phase was extracted twice with dichloromethane, and the combined organic phases were dried with Na_2SO_4. After chromatography on SiO_2 (eluent CH_2Cl_2/Et_2O 9:1) the product was obtained as a colourless solid.

Yield is 82%. Anal. Calcd for $C_{20}H_{33}NO_3SSi_2$: C, 56.69; H, 7.85; N, 3.31, S, 7.57. Found: C, 56.78; H, 7.92; N, 3.24; S, 7.63. EI-MS, *m/z*: (M is 423.727) 424 $[M+H]^+$, 359 $[M-SO_2]^+$, 286 $[M-SO_2-SiMe_3]^+$. Mp 178–180 °C. TLC on SiO_2, R_f=0.66 (eluent CH_2Cl_2/Et_2O 9:1). IR spectrum (selected bands), cm^{-1}: 3437 and 3179 s ν (OH and NH), 2167 w ν (C≡C), 1289 and 1077 s ν (SO_2). ^1H NMR spectrum in $CDCl_3$, δ: 0.20 (s, 9H) and 0.21 (s, 9H) (2 $SiMe_3$), 1.00 (s, 3H, 10-H) and 1.44 (s, 3H, 9-H), 1.73 (m, 1H, 6-H *exo*), 1.83 (m, 1H, 5-H *exo*), 1.95 (m, 1H, 5-H *endo*), 2.08 (d, *J*=5.2 Hz, 1H, 4-H), 2.21 (m, 1H, 6-H *endo*), 3.07 (s, br., OH), 3.28 (s, 2H, 8-H), 5.04 (s, br., NH). ^{13}C NMR spectrum in $CDCl_3$, δ: −0.2 ($2SiMe_3$), 23.5 (C-9), 23.9 (C-10), 23.6 (C-5), 28.2 (C-6), 48.8 (C-7), 51.1 (C-8), 56.6 (C-4), 62.0 (C-1), 72.2 (C-2), 81.3 (C-3), 92.9, 95.3, 102.9 and 105.0 (C-11, C-12, C-13 and C-14).

4.3.2. (1a*S*,3a*S*,7*R*)-7-Hydroxy-8,8-dimethyl-1a,7-bis(ethynyl)-1,1a,4,5,6,7-hexahydro-3*H*-3a,6-methano-2,1-benzisothiazole 2,2-dioxide (3). To a solution of **2** (126 mg, 0.3 mmol) in CH_2Cl_2 (2 ml), Bu_4NF (189 mg, 0.6 mmol) was added in small portions over a period of 20 min. The reaction mixture was stirred at room temperature for 1.5 h. The solvent was evaporated at room temperature in a vacuum, and the residue was subjected to chromatography on SiO_2 (eluent CH_2Cl_2/Et_2O 9:1). Crystallisation from CH_2Cl_2/Et_2O provided the product as colourless crystals.

Yield is 66%. Anal. Calcd for $C_{14}H_{17}NO_3S$: C, 60.19; H, 6.13; N, 5.01, S, 11.48. Found: 60.92; H, 6.33; N, 4.92; S, 11.61. EI-MS, *m/z*: (M is 279.360) 280 $[M+H]^+$, 215 $[M-SO_2]^+$. Mp 116–120 °C (dec.). TLC on SiO_2, R_f=0.35 (eluent CH_2Cl_2/Et_2O 9:1). IR spectrum (selected bands), cm^{-1}: 3476 and 3341 s ν (OH and NH), 3282 and 3259 s ν (≡C–H), 2121 s ν (C≡C), 1321 and 1115 s ν (SO_2). ^1H NMR spectrum in $CDCl_3$, δ: 1.00 (s, 3H, 10-H) and 1.44 (s, 3H, 9-H), 1.73 (m, 1H, 6-H *exo*), 1.86 (m, 1H, 5-H *exo*), 1.92 (m, 1H, 5-H *endo*), 2.13 (d, *J*=5.1 Hz, 1H, 4-H), 2.20 (m, 1H, 6-H *endo*), 2.78 (s, 1H) and 2.85 (s, 1H)(2 ≡CH), 3.29 (d, *J*=13.8 Hz, 1H) and 3.34 (d, *J*=13.8 Hz, 1H) (8-H), 3.45 (s, br., OH), 5.18 (s, br., NH). ^{13}C NMR spectrum in $CDCl_3$, δ: 23.4 (C-9), 23.8 (C-10), 23.5 (C-5), 27.9 (C-6), 49.0 (C-7), 51.0 (C-8), 56.2 (C-4), 61.8 (C-1), 71.6 (C-2), 81.2 (C-3), 78.7 and 78.8 (C-12 and C-14), 81.6 and 83.6 (C-11 and C-13).

4.4. Cascade reaction

4.4.1. (3a*S*)-10,10-Dimethyl-1,4,5,6,7,8,9,9b-octahydro-3*H*-3a,6-methanoazuleno[4,5-*c*]iso-thiazol-7-one 2,2-dioxide (4). A solution of **3** (55 mg, 0.2 mmol) and $[PtCl_2(PhCN)_2]$ (2.5 mg, 0.025 mmol, 2.5 mol%) in $CHCl_3$ (3 ml) was heated at 55 °C for 6 h whereupon the colour of the reaction mixture changed from yellow to dark brown. The solvent was evaporated and the product purified by chromatography on SiO_2 (eluent CH_2Cl_2).

Yield is 68%. Anal. Calcd for $C_{14}H_{17}NO_3S$: C, 60.19; H, 6.13; N, 5.01, S, 11.48. Found: 59.74; H, 5.86; N, 4.85; S, 11.58. EI-MS, *m/z*: (M is 279.360) 279 $[M]^+$, 215 $[M-SO_2]^+$, 200 $[M-SO_2-CH_3]^+$, 172 $[M-SO_2-CH_3-CO]^+$. Mp 211 °C. TLC on SiO_2, R_f=0.30 (eluent CH_2Cl_2). IR spectrum (selected bands), cm^{-1}: 3061, 2963 and 2885 ν (C–H), 1706 s ν (C=O), 1583 m ν (C=N, C=C), 1335 and 1167 s ν (SO_2), 743 and 718 m ν (S–N, S–C). ^1H NMR spectrum in $CDCl_3$, δ: 0.83 (s, 3H, 9-H), 1.09 (s, 3H, 10-H), 1.51 (m, 1H, 5-H *endo*), 2.05 (m, 1H, 6-H *endo*), 2.26 (m, 1H, 6-H *exo*), 2.29 (m, 1H, 5-H *exo*), 2.66 (m, 2H, 14-H), 2.88 (m, 2H)(12-H), 2.91 (d, *J*=5.1 Hz, 1H, 4-H), 3.21 (d, *J*=13.6 Hz, 1H, 8-H *exo*) and 3.26 (d, *J*=13.6 Hz, 1H, 8-H *endo*). ^{13}C NMR spectrum in $CDCl_3$, δ: 18.5 (C-9), 23.6 (C-12), 24.5 (C-10), 28.1 (C-5), 32.9 (C-6), 35.7 (C-14), 43.0 (C-4), 49.7 (C-7), 50.1 (C-8), 64.2 (C-1), 154.3 (C-11), 159.9 (C-3), 178.8 (C-2), 206.1 (C-13).

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Rationalization of the conflicting effects of hydrogen bond donor solvent on nucleophilic aromatic substitution reactions in non-polar aprotic solvent: reactions of phenyl 2,4,6-trinitrophenyl ether with primary and secondary amines in benzene–methanol mixtures

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Abstract—The kinetics of the reactions of phenyl 2,4,6-trinitrophenyl ether with piperidine and cyclohexylamine respectively were studied at different amine concentrations in benzene. The reaction of cyclohexylamine was not base-catalysed while that of piperidine was catalysed by one molecule of the nucleophilic amine. Addition of small amounts of hydrogen-bond donor solvent, methanol to the benzene medium of the reactions produced different effects—rate diminution followed by rate increase in one and continuous rate diminution in the other. These effects are compared with that of aniline (previously studied) in which a continuous rate increase was observed. The results are rationalized in terms of the effect of amine-solvent interaction on the nucleophilicity of the amines in addition to some other factors operating through cyclic transition states leading to products. It is evident from the rationalization that the idea of 'dimer nucleophile' in nucleophilic aromatic substitution reactions is erroneous.

1. Introduction

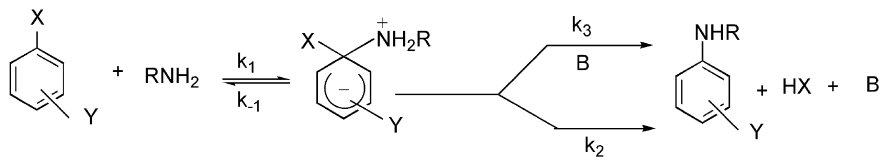
The general mechanism for aromatic bimolecular nucleophilic substitution reactions in all solvents when either primary or secondary amines are the nucleophiles[1,2] is given in Scheme 1.

Application of the steady state hypothesis to Scheme 1 gives Eq. 1,

$$k_A = \frac{k_1(k_2 + k_3[B])}{k_{-1} + k_2 + k_3[B]} \quad (1)$$

where k_A is the observed second-order rate constant and B is either a second molecule of the nucleophile or an added base acting as the catalyst. Specific modifications of the above scheme and equation have, however, been made depending on whether the reaction is taking place in protic, dipolar aprotic[3,4] or non-polar aprotic[5–8] solvents or whether the reaction is catalysed by one or two amine molecules, or an entirely different catalytic entity.

In a previous paper,[9] we subjected to test the claim by Nuldelman and Palleros[10] that the vulnerability of amine–amine hydrogen bonding (dimer) is the factor responsible for the diminishing-rate effect observed on the addition of small amounts of methanol to the reaction of cyclohexylamine with 2,6-dintroanisole in benzene. We found that the addition of small amounts of methanol to the reaction of another primary amine, aniline with another substrate, phenyl 2,4,6-trinitrophenyl ether (**1**) in the same solvent showed no such behaviour, the rate of reaction



Scheme 1.

Keywords: Aprotic solvent; Aromatic substitution reaction; Benzene–methanol mixtures.
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increasing progressively in this case with increasing amounts of methanol. From this, we inferred that the diminution in rate observed by Nuldelman and Palleros could not have been due to the effect of the incursion of methanol on the supposed 'dimerization of the amine'. For, if this were so, the effect should have been more pronounced with the weaker aromatic amine, aniline. We then suggested an alternative interpretation that could explain their results.

In order to have a more general view of the phenomenon in dispute, we decided to examine the reactions of some other amines—primary and secondary, under the same conditions by adding small amounts of methanol to the benzene solutions of their reactions. We have studied the reactions of **1** with piperidine and cyclohexylamine in benzene.

2. Results and discussion

The reactions were studied spectrophotometrically at 29 °C in the presence of varying excesses of the respective amine over the substrate to ensure first-order kinetics. The observed second-order rate constants, k_A were calculated from the first-order rate constants. The reactions in pure solvent, as well as in benzene–methanol mixtures, proceeded straightforwardly to give the expected 2,4,6-trinitrophenylamine and phenol with no side-products.

The effects of methanol addition to the benzene medium of the reactions studied are in Table 1.

The reactions fall into two categories:

(i) Reactions in which addition of small amounts of methanol to the benzene medium diminishes the rate.
   (a) For the base-catalysed reaction of **1** with piperidine, the rate diminution reached a minimum at about 40–50% methanol after which the rate increased almost linearly with the methanol content.
   (b) For the uncatalysed reaction of the same substrate with cylohexylamine, the rate diminution continued to 100% methanol.

(ii) Reaction in which addition of small amounts of methanol to the benzene medium increases the rate continuously.

This was observed in the base-catalysed reaction of the substrate with aniline in benzene.[9]

2.1. Effects of methanol additions

The reaction of **1** with piperidine was observed to be first-order in the substrate and second-order in the amine. The effects of addition of small amounts of methanol to the benzene medium of the reaction at constant amine concentrations are shown in Table 2.

The observed effects of methanol addition on the reactions in the present investigation and also of the previous ones in the literature must be satisfactorily accommodated by a sound reaction mechanism. The effects could be due to the interaction of methanol with (i) the non-polar aprotic solvent or (ii) the substrate or (iii) intermediates on the reaction pathway or (iv) the nucleophile:

(i) Addition of methanol to non-polar aprotic solvent should increase the dielectric constant of the medium thereby increasing the rate of S_NAr reactions due to the extra stabilization of the intermediate first formed from the reaction of the nucleophile with the substrate.
(ii) Complex formation between the substrate and added methanol could be a probable factor. Observations have shown, however, that a particular substrate in a non-polar aprotic medium could display rate

Table 1. Effects of methanol additions to the benzene medium of the reactions studied

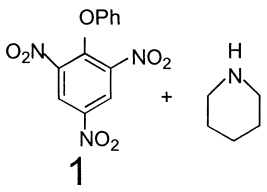
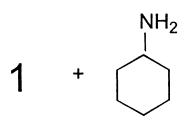
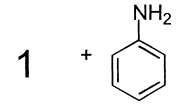
Type of reaction	Base catalysis	Effect of methanol additions
	Catalysed	Initial rate decrease reaching a minimum value then followed by rate increase
	Not catalysed	Continuous rate decrease
	Catalysed	Continuous rate increase[9]

Table 2. Second-order rate constants, k_A for the reaction of **1** with piperidine in benzene and benzene–methanol mixtures at 29 °C. [Substrate]=5×10^{-5} mol dm^{-3}

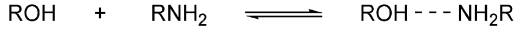
10^3[Pip]/mol dm^{-3}	% MeOH (v/v)	$10k_A$/dm^3 mol^{-1} s^{-1}	10^3[Pip]/mol dm^{-3}	% MeOH (v/v)	$10k_A$/dm^3 mol^{-1} s^{-1}	10^3[Pip]/mol dm^{-3}	% MeOH (v/v)	$10k_A$/dm^3 mol^{-1} s^{-1}
1.0	0	2.06	2.0	50	0.34	4.0	10	0.80
1.0	1	1.12	2.0	60	0.41	4.0	15	0.72
1.0	2	0.83	2.0	75	0.71	4.0	20	0.67
1.0	3	0.58	2.0	90	1.39	4.0	30	0.64
1.0	5	0.36	2.0	100	2.00	4.0	40	0.60
1.0	10	0.21	3.0	0	5.90	4.0	50	0.64
2.0	0	3.97	3.0	1	3.60	4.0	60	0.71
2.0	1	2.35	3.0	2	2.23	4.0	75	1.05
2.0	2	1.45	3.0	3	1.55	4.0	90	1.85
2.0	3	1.04	3.0	5	1.08	4.0	100	2.98
2.0	5	0.68	3.0	10	6.30	5.0	0	9.60
2.0	10	0.41	4.0	0	7.60	5.0	1	5.54
2.0	15	0.35	4.0	1	4.63	5.0	2	3.70
2.0	20	0.33	4.0	2	2.90	5.0	3	2.60
2.0	30	0.32	4.0	3	2.08	5.0	5	1.70
2.0	40	0.32	4.0	5	1.34	5.0	10	1.01

diminution in its reaction with one amine and a continuous rate increase in its reaction with another amine both in the presence of small amounts of methanol. For example, adding methanol to the benzene medium of the reaction of **1** with piperidine (present study) produced a rate diminution while the same addition of methanol produced a continuous rate increase for the reaction of the same substrate with aniline in our previous study.[9]

Also the reaction of 2,4-dinitrofluorobenzene with piperidine by Bernasconi and Zollinger[11] in benzene displayed a rate increase with added methanol while the reaction of the same substrate produced the opposite effect in its reaction with *cis* and *trans*-1,2-diaminocyclohexylamine[12] on the addition of methanol. It can therefore be reasonably assumed that a methanol-substrate interaction is not responsible for the observed diminution in rate in each case.

(iii) Interaction of added methanol with any zwitterionic intermediate formed as in Scheme 1 would assist the simultaneous extraction of a proton and expulsion of the leaving group through hydrogen bonding in the transition state thus leading to an increase in the rate of reaction.

(iv) The formation of aggregates via hydrogen-bonding between amines and hydrogen-bond donor solvent, methanol has been widely studied.[13–15] The methanol molecule acts as a proton donor to the amine resulting in the formation of an aggregate as shown in Scheme 2.



Scheme 2.

The nitrogen atom, having thus used its lone pair of electrons partially for hydrogen-bond formation becomes less nucleophilic compared with the free amine. The amine–methanol aggregate of reduced nucleophilicity can either react with the substrate in the first step of the S_NA_r reaction or be the catalysing entity in the decomposition of the zwitterionic intermediate complex in the second step. The first assumption is obviously more likely, since an uncatalysed reaction in the present study as well as the one reported in literature[11] displayed rate diminution on addition of methanol to the non-polar aprotic medium. The methanol–amine aggregate would slow down the reaction due to its reduced nucleophilicity. It is therefore proposed that the amine–methanol aggregate reacts with the substrate in the first step of the two-step S_NA_r reaction leading to reduced k_1.

2.2. Causes of rate increase and rate diminution

There are only a few cases of rate increase in S_NA_r reactions on addition of methanol to non-polar aprotic solvent. One is our previous study of the reaction of **1** with aniline in benzene[9] and the other is the reaction of 2,4-dinitrofluorobenzene with piperidine by Bernasconi and Zollinger[11] also in benzene.

The common features in these two reactions are:

(a) Base-catalysis with high catalytic effectiveness. This is evident in the base-catalysed reaction of 2,4-dinitrofluorobenzene with piperidine[11] for which the catalytic effectiveness, k_3/k_2=1230 dm^3 mol^{-1} and the base-catalysed reaction of **1** with aniline[7] for which k_3/k_2=1414 dm^6 mol^{-2}.

(b) Susceptibility to hydrogen bonding. It is noteworthy that since a continuous rate increase is only observed for strongly base-catalysed reactions on the addition of methanol to their non-polar aprotic medium, it implies that the factor responsible for this observation comes into play in the second step of the S_NA_r reaction, that is,

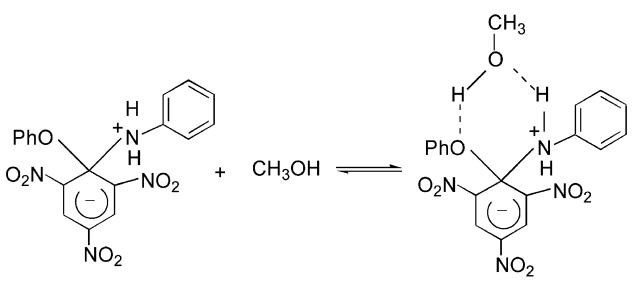


Figure 1.

the decomposition of the zwitterionic intermediate which sometimes involves catalysis.

A close scrutiny of the zwitterionic intermediates involved in the above two reactions reveals a high probability of strong hydrogen bonding in the cyclic transition state involving methanol and either a strong conjugate acid of a weak base (aniline) in the reaction of aniline with **1** (Fig. 1) on the one hand or methanol and a strong hydrogen-bond forming atom (fluorine) as a leaving group in the reaction of 2,4-dinitrofluorobenzene with piperidine (Fig. 2) on the other. Electrophilic catalysis of fluoride ion departure through hydrogen bonding has been demonstrated by Pietra and Fava[16] in the reaction of 1-fluoro-2,4-dinitrobenzene with piperidine in benzene where they observed catalysis by methanol but no catalysis by added triethylamine.

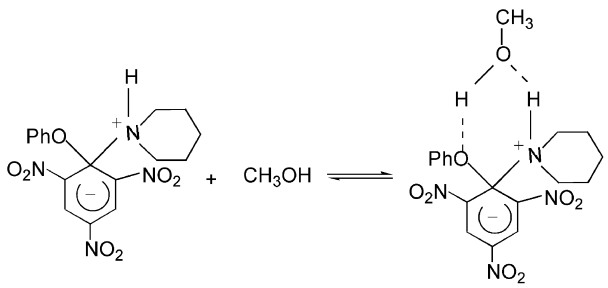


Figure 2.

From the above observations, it is clear that the addition of small amounts of methanol to the non-polar aprotic medium of S_NA_r reactions would produce two effects—(a) decrease in rate due to the reduced necleophilicity of the amine in the formed amine–methanol aggregate and (b) methanol catalysis through hydrogen-bonding.

For S_NA_r reactions in which the first step is rate determining (Scheme 1), $k_2+k_3[B] \gg k_{-1}$ in Eq. 1, giving $k_A=k_1$ and thus, only effect (a) will be observed on addition of methanol because of the reduced nucleophilicity of the attacking amine–methanol aggregate which will be reflected in the reduced value of k_1.

For reactions in which the second step is rate determining, $k_{-1} \gg k_2+k_3[B]$, thus Eq. 1 becomes Eq. 2

$$k_A = \frac{k_1 k_2}{k_{-1}} + \frac{k_1 k_3 [B]}{k_{-1}} \quad (2)$$

and the above two opposing effects (a) and (b) will be in operation because

(i) the first step leading to the formation of the zwitterionic intermediate will be influenced by the reduced nucleophilicity of the resulting nucleophile which is now the amine–methanol aggregate thus causing diminution in rate and

(ii) the second step of the reaction which is the catalytic step is influenced by amine and methanol molecules respectively catalysing through hydrogen-bonding, thus causing an increase in rate.

The observed overall rate would depend on which of the two effects—(i) and (ii) predominates. For reactions in which (i) predominates over (ii), Class A, the observed overall effect is rate diminution and for reactions in which (ii) predominates over (i), Class B, the observed overall effect is rate increase on addition of small amounts of methanol to the aprotic medium.

Thus, the reaction of **1** with piperidine in benzene in the present study and the reaction of 2,6-dinitroanisole with cyclohexylamine in benzene studied by Nudelman and Palleros[10] belong to Class A, while the reaction of **1** with aniline in our previous study[9] belongs to Class B.

It is now clear, therefore, that the explanation given above for Class A reactions is the reason for the rate diminution observed by Nudelman and Palleros in the reaction of 2,6-dinitroanisole with cyclohexylamine in benzene–methanol medium and not our previously suggested explanation of possible reversibility of the reaction.[9,17]

2.3. Mechanism of the base-catalysed reaction

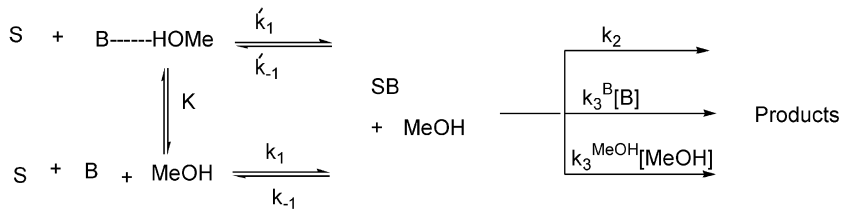
S_NA_r reactions in non-polar aprotic solvents on addition of small amounts of methanol can generally be assumed to involve the attack of the amine–methanol aggregate as well as the free amine on the substrate to produce the zwitterionic intermediate. Since amine–methanol aggregate formation via hydrogen bonding is likely to be a very rapid equilibrium process, three possible routes for conversion of the zwitterionic intermediate to products are proposed.

In Scheme 3, S stands for the substrate, B for the nucleophilic base, B···HOMe for the amine–methanol aggregate, SB for the zwitterionic intermediate, k_3^B and k_3^{MeOH} for the catalytic rate constants for the conversion of the intermediate into products by the base and methanol, respectively.

Since the amine may exist in free or hydrogen-bonded forms as given by Eq. 3,

$$B + MeOH \overset{K}{\rightleftharpoons} B\cdots HOMe \quad (3)$$

the stoichiometric measured concentration, B_{Stoich} will be



Scheme 3.

related to the free base $[B]_{Free}$ by Eq. 4.

$$[B\cdots HOMe] + [B]_{Free} = [B]_{Stoich} \quad (4)$$

From Eqs. 3 and 4, the unmeasurable quantities $[B\cdots HOMe]$ and $[B]_{Free}$ are derived in terms of the measurable quantity $[B]_{Stoich}$.

Application of the steady-state hypothesis to Scheme 3 in terms of the stoichiometric base concentration leads to Eq. 5

$$k_A = \frac{\left(\frac{k_1' K[MeOH]}{1+K[MeOH]} + \frac{k_1}{1+K[MeOH]}\right)\left(k_2 + \frac{k_3^B[B]}{1+K[MeOH]} + k_3^{MeOH}[MeOH]\right)}{k_{-1} + k_{-1}'[MeOH] + k_2 + \frac{k_3^B[B]}{1+K[MeOH]} + k_3^{MeOH}[MeOH]} \quad (5)$$

where [B] is the total (stoichiometric) base concentration and K is the association constant for amine–methanol aggregate formation.

For the base-catalysed reaction, when the second step is rate determining, Eq. 6 holds and Eq. 5 becomes Eq. 7.

$$k_{-1} + k_{-1}'[MeOH] \gg k_2 + \frac{k_3^B[B]}{1+K[MeOH]} + k_3^{MeOH}[MeOH] \quad (6)$$

$$k_A = \frac{\left(\frac{k_1' K[MeOH] + k_1}{1+K[MeOH]}\right)\left(k_2 + \frac{k_3^B[B]}{1+K[MeOH]} + k_3^{MeOH}[MeOH]\right)}{k_{-1} + k_{-1}'[MeOH]} \quad (7)$$

On the assumption that hydrogen bonding with methanol substantially reduces the nucleophilicity of the amine, $k_1 \gg k_1'$, and Eq. 7 then reduces to Eq. 8 which can also be expressed in the form of Eq. 9.

$$k_A = \frac{k_1}{1+K[MeOH]} \times \left(k_2 + \frac{k_3^B[B]}{1+K[MeOH]} + k_3^{MeOH}[MeOH]\right) \quad (8)$$

$$k_A = \frac{k_1 k_2}{k_{-1}(1+K[MeOH])} + \frac{k_1 k_3^B[B]}{k_{-1}(1+K[MeOH]^2)} + \frac{k_1 k_3^{MeOH}[MeOH]}{k_{-1}(1+K[MeOH])} \quad (9)$$

At constant methanol concentration, this equation reduces to Eq. 10

$$k_A = k' + k''[B] \quad (10)$$

where k' and k'' are defined by Eqs. 11 and 12, respectively.

$$k' = \frac{k_1 k_2}{k_{-1}(1+K[MeOH])} + \frac{k_1 k_3^{MeOH}[MeOH]}{k_{-1}(1+K[MeOH])} \quad (11)$$

$$k'' = \frac{k_1 k_3^B[B]}{k_{-1}(1+K[MeOH]^2)} \quad (12)$$

For the reaction of **1** with piperidine, which is catalysed by one amine molecule in benzene, as well as in benzene–methanol mixtures at low methanol concentration, Eq. 9 applies.

The plot of k_A against [amine] for the reaction gave straight lines giving credence to Eq. 10. The values of the intercepts and slopes are listed in Table 3.

The table shows that the intercepts obtained on addition of methanol are all within the proximity of the origin and are, within experimental error, equal to zero. In this reaction, the overall effect of methanol addition is rate diminution. This is because the conjugate acid of the strong base, piperidine (pKa=11.06) is not sufficiently acidic to promote strong hydrogen-bonding with methanol in the cyclic transition state involving the zwitterionic intermediate. The resulting small value of methanol catalytic rate constant, k_3^{MeOH} for the decomposition of the zwitterionic intermediate into products is thus not comparable with the rate diminishing effect of the reduced nucleophilicity of the amine–methanol aggregate.

It is important to note that addition of small amounts of methanol to the reactions of **1** with piperidine (present study) and with aniline (previous study[9]) is expected to produce similar effects of rate diminution in both reactions due to the reduced nucleophilicity of the amine–methanol aggregate. However, the former reaction shows the expected rate decrease while on the contrary the latter reaction shows a continuous rate increase.[9] This is due to the strong methanol catalysis k_3^{MeOH} which comes into play (through a cyclic transition state—Figure 1) in the latter.

Table 3. Values of intercepts and slopes of the plots of k_A against [piperidine] at constant methanol concentrations for the reaction of **1** with piperidine in benzene–methanol at 29 °C

% Methanol	$10^2 k'$/dm^3 mol^{-1} s^{-1}	k''/dm^6 mol^{-1} s^{-1}	r
0	2.13±0.78	187.10±2.35	0.9998
1	1.12±1.44	111.434±4.34	0.9982
2	0.65±0.65	71.90±1.66	0.9990
3	0.46±0.25	50.80±0.76	0.9997
5	0.30±0.35	33.40±1.05	0.9980
10	0.15±0.15	19.90±0.40	0.9990

This is more than enough to compensate for the reduced nucleophilicity of the amine–methanol aggregate because the conjugate acid of the weak base, aniline (p*K*a=4.61) is sufficiently acidic to promote strong hydrogen-bonding with methanol in the cyclic transition state involving the zwitterionic intermediate, hence the rate increase.

The aniline reaction with **1** in benzene and benzene–methanol mixtures is catalysed by two amine molecules and so the corresponding rate Eqs. 8 and 9 involving the free amine and the amine–methanol aggregate would have $[B]^2$ terms instead of [B].

2.4. Effect of temperature

The observations from the study of S_NAr reactions in a non polar aprotic solvent on addition of small amounts of a hydrogen-bond donor solvent, methanol, to the reaction medium have brought into focus the difference in the mechanisms of these reactions in non-polar aprotic and polar protic solvents. The common feature in some of these studies is the parabolic curve sometimes obtained in a plot of the second-order rate constants, k_A against % methanol for some of the base-catalysed reactions (Fig. 3) as observed by us in the present investigation as well as by Nudelman and Palleros.[10,17]

On the left side of these curves, a region in which the medium is predominantly non-polar aprotic is an effect symbolic of a mechanism different from that on the right side which is predominantly polar protic. This is because an increase in the percentage of methanol results in rate diminution on the left while it is rate increase on the right side of the curves. The reaction represented by the left side of the plot (say 3–40% methanol content) was subjected to temperature variation ranging from 15 to 35 °C. It was observed that the rate of the reaction decreased slightly with increasing temperature (Table 4), thus resulting in a small negative activation energy of -1.20 kJ mol^{-1}.

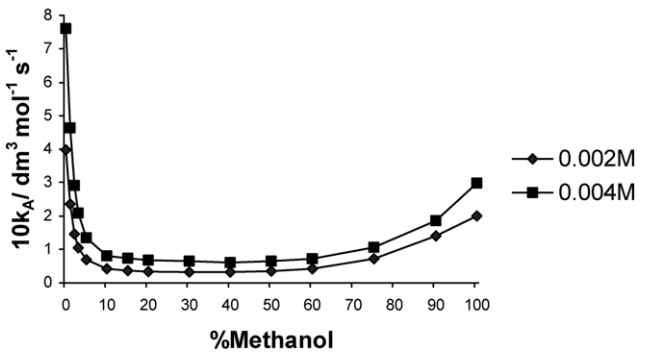


Figure 3. Plots of second-order rate constants k_A against % methanol at constant piperidine concentrations for the reaction of phenyl 2,4,6-trinitrophenyl ether with piperidine in benzene–methanol mixtures at 29 °C.

Similar negative temperature effects have been observed by E. F. Caldin et al.[18] in the reaction of 2,4-dinitrophenol with tri-*n*-octylamine in chlorobenzene and by Banjoko and Ezeani[7] in the reaction of **1** with some substituted anilines in benzene.

The observation in each case has been attributed to hydrogen-bond formation in the transition state as the strength of hydrogen-bonding is known to decrease with increasing temperature.

At the right side of the plot, however, a region where the addition of methanol has considerably increased to make the medium polar (say 60–75% methanol), increase in temperature from 15 to 35 °C led to an increase in rate (Table 4). This thus resulted in a positive activation energy of 3.20 kJ mol^{-1}. This change from negative activation energy to positive activation energy is clearly indicative of a change in mechanism of the reaction in the medium. It could be rightly inferred, therefore, that the reaction represented by the right hand side of the curve does not involve hydrogen-bonding in the transition state. Since the medium is polar in this section, it could now support ionic charges, hence the Specific Base-General Acid (SB-GA) or proton transfer mechanism can be assumed to be operating in this now polar protic medium. Evidence in recent times, however, is in support of a proton transfer mechanism over the widely accepted SB-GA mechanism in polar protic and dipolar aprotic solvents[19,20] when the leaving group is fairly good.

The above observation of a change in mechanism as the medium changes from a non-polar aprotic to a polar protic is a strong support for the cyclic transition state mechanism in non polar aprotic medium. The mechanism thus differs remarkably in this respect from the hetero/homoconjugate mechanism,[21] which does not differentiate between mechanisms in the two media.

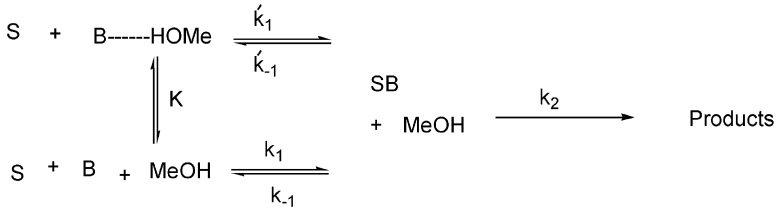
2.5. Mechanism of the uncatalysed reaction

For a reaction that is not base-catalysed and occurs in the presence of small amounts of methanol in non-polar aprotic solvent, Scheme 4 applies.

Application of the steady-state hypothesis to Scheme 4, working in terms of the stoichiometric base concentration,

Table 4. Second-order rate constants for the reaction of **1** with piperidine at constant amine concentration [4×10^{-3} M] in benzene–methanol mixtures at varying temperatures

3% Methanol					60% Methanol				
Temperature (°C)	15	20	25	35	Temperature (°C)	15	20	25	35
$10k_A$/dm^3 mol^{-1} s^{-1}	2.14	2.12	2.10	2.07	$10\,k_A$/dm^3 mol^{-1} s^{-1}	0.66	0.68	0.70	0.73
40% Methanol					75% Methanol				
Temperature (°C)	15	20	25	35	Temperature (°C)	15	20	25	35
$10k_A$/dm^3 mol^{-1} s^{-1}	0.57	0.59	0.61	0.63	$10\,k_A$/dm^3 mol^{-1} s^{-1}	0.94	0.96	0.99	1.05



Scheme 4.

gives the observed overall second-order rate constant, k_A as

$$k_A = \frac{k_2\left(\frac{k_1}{1+K[\mathrm{MeOH}]} + \frac{k'_1K[\mathrm{MeOH}]}{1+K[\mathrm{MeOH}]}\right)}{k_{-1} + k'_{-1}[\mathrm{MeOH}] + k_2}$$

Since the reaction is not base-catalysed, the first step is rate determining and inequality Eq. 13 holds.

$$k_2 \gg k_{-1} + k'_{-1}[\mathrm{MeOH}] \tag{13}$$

$$\therefore k_A = \frac{k_1}{1+K[\mathrm{MeOH}]} + \frac{k'_1K[\mathrm{MeOH}]}{1+K[\mathrm{MeOH}]} \tag{14}$$

The reaction of **1** with cyclohexylamine in benzene is not base-catalysed and so conforms with Scheme 4 and Eq. 14 derived from it. When no methanol is added to the reaction medium, Eq. 14 reduces to

$$k_A = k_1 \tag{15}$$

Table 5 shows the constancy of the second-order rate constant, k_A with [cyclohexylamine] when the reaction is carried out in benzene without methanol, thus giving credence to Eq. 9. Addition of small amounts of methanol to the benzene medium of the reaction showed a sharp decrease in k_A [Table 6] from the initial value of 12.84 $dm^3\ mol^{-1}\ s^{-1}$ in pure benzene to 1.88 $dm^3\ mol^{-1}\ s^{-1}$ in benzene–10% methanol after which the decrease became gradual.

Table 5. Second-order rate constants, k_A for the reaction of phenyl 2,4,6-trinitrophenyl ether with cyclohexylamine (CHA) in benzene at 29 °C

10^4[CHA]/mol dm^{-3}	2.5	3.0	3.5	4.0
k_A/$dm^3\ mol^{-1}\ s^{-1}$	12.83	12.85	12.83	12.84

The experimental data in Table 6 indicate that up to 10% methanol (2.5 mol dm^{-3}), values may be accommodated by the expression for k_A given by Eq. 8. With k_1= 12.84 $dm^3\ mol^{-1}\ s^{-1}$, k'_1 ca. 1.5 $dm^3\ mol^{-1}\ s^{-1}$ and K ca. 7 $dm^3\ mol^{-1}$. At higher methanol concentrations, however, values of rate and equilibrium constants will be expected to be affected by medium effects and so these values will change.

Unlike in the base catalysed reactions, the diminution in rate continued at higher methanol content, reaching a minimum value at 100% methanol (Fig. 4).

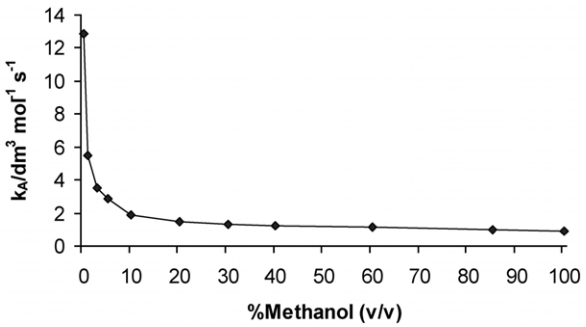


Figure 4. Plot of second-order rate constants, k_A against % methanol at constant cyclohexylamine concentration for the reaction of phenyl 2,4,6-trinitrophenyl ether with cyclohexylamine in benzene–methanol mixtures at 29 °C.

It is worth noting that the value of the second-order rate constant, k_A, in 100% methanol (0.92 $dm^3\ mol^{-1}\ s^{-1}$) is much less than that in 100% benzene (12.84 $dm^3\ mol^{-1}\ s^{-1}$). This is because the first step of the uncatalysed reaction involves the amine–methanol aggregate of reduced nucleophilicity in the former, while in the latter, it involves the free amine molecule with unreduced nucleophilicity.

2.6. Effect of temperature

As the above observation is an unusual one, we felt it worthwhile to investigate the effect of temperature on the reaction. Temperature probe ranging from 15 to 35 °C was therefore, carried out. The rate of the reaction at low methanol content (3% methanol) as well as that at high methanol content (60% methanol) increased appreciably with increase in temperature (Table 7), thus resulting in positive activation energies of 30.49 and 32.11 kJ mol^{-1}, respectively.

The facts to be considered in explaining the above observations are:

(i) diminution in rate on addition of methanol at both low and high methanol contents,
(ii) increase in rate with increase in temperature also at both low and high methanol contents.

Since the reaction is not base catalysed, the first step of the

Table 6. Second-order rate constants for the reaction of **1** with cyclohexylamine (CHA) in benzene–methanol mixtures.at constant cyclohexylamine concentration (0.02 mol^{-1}) at 29 °C [Substrate]=2.5×10^{-5} mol dm^{-3}

% Methanol (v/v)	0	1	3	5	10	20	30	40	60	85	100
k_A ($dm^3\ mol^{-1}\ s^{-1}$)	12.84	5.48	3.51	2.84	1.88	1.50	1.32	1.25	1.14	0.99	0.92

Table 7. Effect of Temperature on the reaction of phenyl-2,4,6-trinitrophenyl ether with cyclohexylamine at constant cyclohexylamine concentration [2.5×10^{-4} M] in benzene–methanol mixtures

3% Methanol					60% Methanol				
Temperature (°C)	15	20	25	35	Temperature (°C)	15	20	25	35
k_A/dm^3 $^{-1}$ s^{-1}	2.04	2.51	3.02	3.76	k_A/dm^3 mol^{-1} s^{-1}	0.61	0.82	1.06	1.52

reaction involving the amine–methanol aggregate of reduced nucleophilicity (as well as that involving the remaining free amine molecules) is rate-determining. It is therefore reasonable to assume that any factor that represses the amine–methanol aggregate formation will lead to an increase in rate while a factor that promotes it will result in rate diminution. Hydrogen-bonds are known to be weakened with an increase in temperature, hence an increase in rate with an increase in temperature is expected at both low and high methanol contents as was indeed observed for the reaction with 3 and 60% methanol content. On the other hand, an increase in methanol content that promotes amine–methanol aggregate formation (of reduced nucleophilicity) should produce rate diminution. The rapid diminution in rate at low methanol content can thus be attributed to the rapid involvement of amine molecules in amine–methanol aggregate formation with the little available methanol molecules and so the concentration of these aggregates increases with additional small amounts of methanol. At reasonably high methanol content, most amine molecules are in the aggregate forms. Further addition of methanol will only result in little increase in their concentration, thus resulting in less rapid diminution in rate, hence the tailing off of the curve (Fig. 4) which reaches a minimum at 100% methanol.

2.7. Deductions

There is no doubt that the factor responsible for the diminution in rate on addition of small amounts of methanol to an S_NA_r reaction in an aprotic solvent of low relative permitivity is the reduced nucleophilicity of the resulting nucleophilic entity. How this comes about, however, is not without some controversy. While from the explanation given earlier, we strongly feel that the reduced nucleophilicity results from the interaction of the amine with the added methanol to form an amine–methanol aggregate, Nudelman and Palleros maintain[10,12,17] that it results from the incursion of the added methanol on the supposed 'amine–amine dimer' thus forming the same amine–methanol aggregate which they termed 'mixed dimer'. It is the contention of these authors that amines exist in aprotic solvents largely as dimers and that it is the dimer that is the nuclophile in S_NA_r reactions. We, on the other hand, believe that in dilute amine solutions as is generally the case in S_NA_r reactions, amines exist largely as free molecules and that it is the free amine molecule that is the nucleophile in S_NA_r reactions. Our contention is buttressed by the fact that the formation constant for aliphatic amine dimers is in the range 0.02–0.1.[21,22a,b] In concentrated amine solutions, however, self-aggregation is likely to occur.

The above two differing views will no doubt result in different interpretations of similar experimental observations. It is remarkable that while the first view is of general applicability, the second one is not. It is difficult, for example, to comprehend how the dimer mechanism would explain the first-order dependence of k_A on the concentration of amines in S_NA_r reactions.

If the dimer, claimed to be the effective nucleophile, reacts with the substrate in the first step of S_NA_r reactions, then, every reaction of the amine should display at least second order dependence of the rate constant k_A on amine concentration, but this is not always the case. For example, the reaction of *n*-butylamine with 2,4,6-trinitrophenyl ether[23] in benzene and that of cyclohexylamine with 2,4-dinitrochlorobenzene,[24] also in benzene, are first order in the amines. The base-catalysed reaction of 2,6-dinitroanisole with cyclohexylamine in toluene[10]which is third-order in amine was explained on the basis of the dimer mechanism but the reaction of the same amine in benzene with 2,4-dinitrochlorobenzene[24] and with **1** (present study, Table 5) are first order in amine. The observation of first-order dependence of k_A on amine concentration in a number of S_NA_r reactions[23–25] in non-polar aprotic solvents shows that the 'dimer nucleophile' mechanism is not of general applicability.

The other notable instance in which the dimer mechanism had been found to be inapplicable is in the base-catalysed reaction of dinitrofluorobenzene with piperidine in benzene by Bernasconi and Zollinger[11] in which added ethanol caused an increase in rate. Nudelman and Mentserrat also carried out the same reaction and made similar observations[12] of rate increase on the addition of ethanol, instead of the rate decrease they expected on the basis of the dimer mechanism. To explain this anomaly, the authors attributed the rate increase to lack of self-association or dimerization of piperidine in benzene, an assertion that is inconsistent with their stand on the dimerization of amines generally. No reason was given for this inconsistency. The authors, however, asserted that the rate increase with small additions of a protic solvent was expected on the basis of hydrogen-bonding assistance to the nucleofuge departure. The above observation, which could not be explained by the dimer mechanism is easily explained on the basis of the cyclic transition state mechanism. This has been extensively dealt with in the section under which S_NA_r reactions exhibiting rate increase on addition of methanol are classified as Class B while those exhibiting rate decrease are classified as Class A. In the second step of the reaction in question, methanol catalysis involving hydrogen-bonding between methanol and the zwitterionic intermediate in the cyclic transition state (Fig. 2), more than compensates for the effect of reduced nucleophilicity of the amine–methanol aggregate operating in the first step of the reaction, hence the rate increase.

3. Conclusion

Addition of hydrogen-bond donor (hbd) solvent to S_NAr reactions involving a substrate and an amine in a non-polar aprotic solvent results in the formation of amine-solvent aggregates of reduced nucleophilicity. The effect should normally result in diminution in rate of reaction but could instead result in an increase in rate if the nature of the zwitterionic intermediate first formed between the substrate and the amine is such that could promote strong hydrogen-bonding between it and the hbd solvent in the cyclic transition state thus leading to its catalytic decomposition into products.

The interpretation of this phenomenon in the literature by a group of co-workers in the field, as being due to the formation of an 'amine–amine dimer' nucleophile is erroneous. That our contention above, is the correct position, is further buttressed by the fact that added hydrogen-bond acceptor (hba) co-solvent, which by implication (or rationalization), should increase the rate of reaction did, in fact, increase the rate. This is due to the increase in the nucleophilicity of the attacking nucleophilic amine through hydrogen-bonding between the amine and the co-solvent. The results of the reactions thus form the basis of our next publication.

4. Experimental

4.1. Materials

The preparation of phenyl 2,4,6-trinitrophenyl ether (**1**) and the purification of benzene and methanol were described previously.[26] Analar piperidine was heated under reflux with sodium wire for 4 h and then distilled. The process was repeated twice and the middle fraction distilling at 106 °C was collected and kept in a desicator, protected from light (lit.[27] bp 105–106 °C). Cyclohexylamine was purified by the same method bp 132 °C (lit.[27] 132–133 °C).

The product of each reaction studied was prepared by standard methods previously described.[28]

N-(2,4,6-Trinitrophenyl) piperidine, mp 100 °C, λ_{max} (C_6H_6) 390 nm.

N-(2,4,6-Trinitrophenyl) cyclohexylamine, mp 90–91 °C, λ_{max} (C_6H_6) 345 nm.

Kinetic procedure. The rates of formation of the products of the reactions were determined spectrophotometrically by the procedure previously described.[26] In some cases, however, the reactions were followed directly in the thermostated cell of the spectrophotometer. The reactions were carried out at 29 °C. For reactions in mixed solvents, the methanol content (v/v) refers to its final volume in the reaction mixture. Optical densities were recorded at the absorption maximum wavelength (λ_{max}) of each product. In all cases the absorption spectrum of the reaction mixture at 'infinity time' corresponded within 2% to the 'mock' infinity prepared by using the respective *N*-(2,4,6-trinitrophenyl)amine obtained as a product of the reaction. The reactions were carried out under conditions of excess of nucleophile over substrate and, in all cases, excellent first-order plots were obtained. The second-order rate constant, k_A were obtained by dividing the first-order rate constants by the amine concentration. All rate determinations were carried out at least in duplicate and the rate constants are accurate to within ±2%.

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Reductive opening of 1*H*,3*H*-benzo[*de*]isochromene: synthesis of 1,8-difunctionalised naphthalenes

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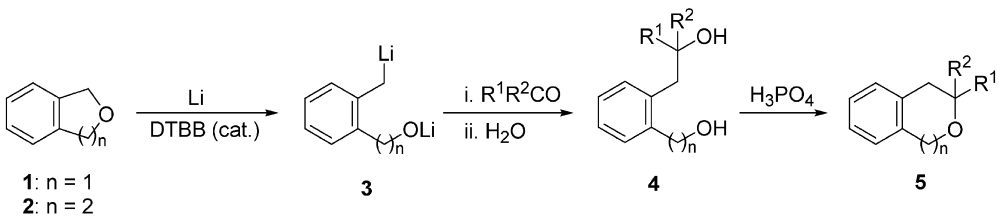
Dedicated to Professor Peter Stannety on the occasion of his 60th birthday

Abstract—The lithiation of 1*H*,3*H*-benzo[*de*]isochromene (**6**) with lithium and a catalytic amount of 4,4′-di-*tert*-butylbiphenyl (DTBB, 5% molar) in THF at −50 °C gives dianionic intermediate **7**, which by reaction with different electrophiles {H_2O, D_2O, ^{t}BuCHO, PhCHO, Me_2CO, $(CH_3CH_2)_2CO$, $[CH_3(CH_2)_4]_2CO$, $(CH_2)_5CO$, $(CH_2)_7CO$, (−)-menthone} at the same temperature followed by hydrolysis leads to functionalised alcohols **8**. If after addition of a carbonyl compound as the first electrophile [^{t}BuCHO, $(CH_2)_5CO$, (−)-menthone], the resulting dialcoholate **9** is allowed to react at 0 °C, a second lithiation takes place to give intermediate **10** which by reaction with a second electrophile [H_2O, ^{t}BuCHO, $(CH_2)_5CO$, CO_2], yields, after hydrolysis, 1,8-difunctionalised naphthalenes **11**. Cyclization under acidic conditions of diols **8e–i** gives oxygen-containing eight-membered heterocycles, which are homologous to the starting material **6**.

1. Introduction

A useful and versatile methodology which can be applied in the preparation of polyfunctionalised organic molecules consists of the reaction of functionalised organometallic compounds[1] with electrophilic reagents. The organolithium intermediates[2] represent a special synthetic case due to their high reactivity under mild reaction conditions, and they can be generated using classical procedures (i.e., halogen–lithium exchange or tin–lithium transmetallation)[3] or through the application of new methods. Amongst the newer methods, the reductive ring opening of heterocycles[4] has shown to be of wide application. Different oxygen-, nitrogen- and sulfur-containing heterocycles of different size (from three to seven-membered rings) have been used to generate the corresponding functionalised organolithium compounds. During the last few years, we have developed an efficient methodology to perform lithiation reactions under very mild reaction conditions, which employs lithium powder and a catalytic amount (<10%) of an arene,[5–8] naphthalene and 4,4′-di-*tert*-butylbiphenyl (DTBB) being the most commonly used.[9] For instance, the application of this reductive methodology to the ring opening of benzofused heterocycles, such as phthalan (**1**)[10] or isochroman (**2**)[10e–n,11] allows the generation of the corresponding functionalised organolithium intermediates **3**, which can be trapped with different electrophiles, for instance carbonyl compounds, to yield diols **4**, suitable precursors of the homologous heterocycles **5** (Scheme 1).

Recently, Azzena et al. reported the use of the mentioned arene catalysed lithiation methodology to study the ring opening of some other benzannelated cyclic ethers and amines and the reaction of the generated lithiated



Scheme 1.

Keywords: Reductive ring opening; Benzoisochromene; DTBB-catalysed lithiation; Electrophilic substitution; Oxygenated heterocycles.
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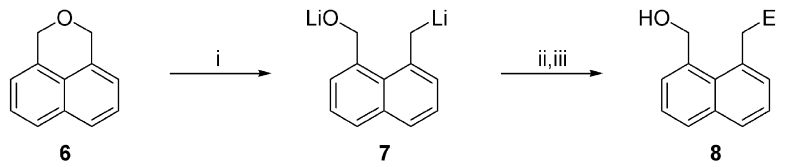


Scheme 2. Reagents and conditions: (i) Li, DTBB (5 mol%), THF, −50 °C, 6 h; (ii) E^+=H_2O, D_2O, ^{t}BuCHO, PhCHO, Me_2CO, $(CH_3CH_2)_2CO$, $[CH_3(CH_2)_4]_2CO$, $(CH_2)_5CO$, $(CH_2)_7CO$, (−)-menthone, −50 °C, 15 min; (iii) H_2O, −50 to 20 °C.

Table 1. Preparation of compounds **8**

Entry	E^+	Product[a]			
		No.	E	Yield (%)[b,c]	R_f[d]
1	H_2O	**8a**	H	58 (>95)	0.46
2	D_2O	**8b**	D	42 (>95)[e]	0.46
3	^{t}BuCHO	**8c**	^{t}BuCHOH	34 (55)	0.33
4	PhCHO	**8d**	PhCHOH	34 (56)	0.16
5	Me_2CO	**8e**	Me_2COH	43 (70)	0.14
6	$(CH_3CH_2)_2CO$	**8f**	$(CH_3CH_2)_2COH$	45 (74)	0.38
7	$[CH_3(CH_2)_4]_2CO$	**8g**	$[CH_3(CH_2)_4]_2COH$	34 (56)	0.58
8	$(CH_2)_5CO$	**8h**	$(CH_2)_5COH$	42 (68)	0.36
9	$(CH_2)_7CO$	**8i**	$(CH_2)_7COH$	43 (70)	0.37
10	(−)-Menthone	**8j**[f]	–	50 (82)	0.37

[a] All products were >95% pure (GLC and 300 MHz ^{1}H NMR).
[b] Isolated yield after column chromatography (silica gel, hexane/ethyl acetate) based on the starting material **6**.
[c] In parenthesis GLC yield of compound **8**.
[d] Silica gel, hexane/ethyl acetate: 2/1.
[e] 55% deuterium from mass spectrometry and ^{13}C NMR.
[f] See Figure 1.

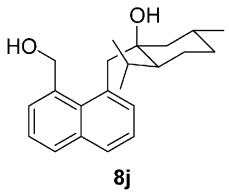


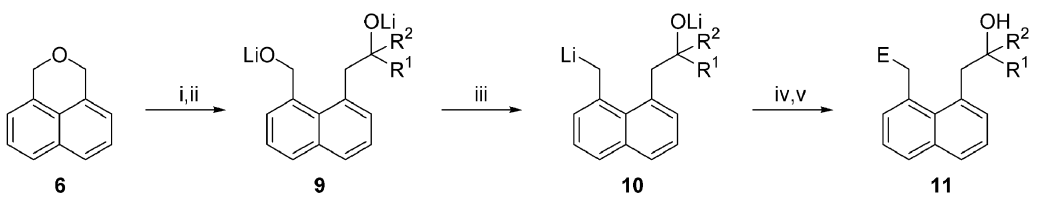
Figure 1.

intermediates mainly with water, deuterium oxide and alkyl bromides, only one reaction with acetone with modest yield being described.[12] In this paper we report the ring opening of 1*H*,3*H*-benzo[*de*]isochromene (**6**) using a DTBB-catalysed lithiation with two synthetic purposes: (a) the monolithiation reaction and trapping with carbonyl compounds as electrophiles to get diols able to be cyclised to the corresponding homologated heterocycles, and (b) the sequential double lithiation in order to introduce two different electrophiles, which for carbonyl compounds also would be able to give the corresponding heterocycles by acidic cyclisation.

2. Results and discussion

Starting material **6** was prepared from commercially available 1,8-naphthalic anhydride by reduction with $LiAlH_4$ in a 3/1 diethyl ether/benzene mixture at 40 °C to give first the corresponding diol[13] which, after treatment with 50% H_3PO_4 at 100 °C, led to the benzoisochromene **6**[12] in 55% overall yield. The reaction of 1*H*,3*H*-benzo[*de*]isochroman (**6**) with an excess of lithium (1/10 molar ratio) in the presence of a catalytic amount of DTBB (5 mol%) in THF at −50 °C led, after 6 h, to a solution of the dianion **7**, which reacted with different electrophiles {H_2O, D_2O, ^{t}BuCHO, PhCHO, Me_2CO, $(CH_3CH_2)_2CO$, $[CH_3(CH_2)_4]_2$-CO, $(CH_2)_5CO$, $(CH_2)_7CO$, (−)-menthone} at the same temperature for 15 min yielding, after hydrolysis with water, the expected functionalised alcohols **8** (Scheme 2 and Table 1). In the case of using (−)-menthone as electrophile, the almost exclusive diastereomer is the one resulting from the attack of the dianionic intermediate **7** to the less hindered face of the carbonyl group (Fig. 1). Temperature of the lithiation step is very important in order to achieve a good selectivity. Thus, the reductive opening of the starting benzoisochromene **6** did not take place at −78 °C in a significant extension in the presence of the same lithiation mixture, the starting heterocycle **6** being recovered after hydrolysis. However, at 0 °C for 1 h, 1,8-dimethylnaphthalene was isolated as the major reaction product. Under these reaction conditions, after reductive opening of the compound **6** giving the dianionic intermediate **7**, a second reductive cleavage of the remaining carbon–oxygen bond took place to give a 1,8-bis(lithiomethyl)naphthalene, which, after hydrolysis with water, led to 1,8-dimethylnaphthalene. This result was in agreement with those reported by Azzena et al.[12] on the lithiation of the compound **6** using as lithiating reagent lithium metal from a 30% weight dispersion in mineral oil in the presence of a catalytic amount of naphthalene (10 mol%).

Taking advantage of the unexpected easy reductive cleavage of the carbon–oxygen bond in the dianionic intermediate **7**, the lithiation of 1*H*,3*H*-benzo[*de*]isochromene (**6**) can be directed to the introduction of two different electrophiles at both benzylic positions in a

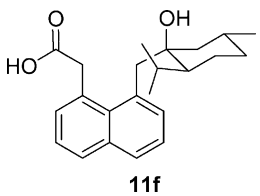


Scheme 3. Reagents and conditions: (i) Li, DTBB (5 mol%), THF, −50 °C, 6 h; (ii) R^1R^2CO=^{t}BuCHO, $(CH_2)_5CO$, (−)-menthone, −50 °C, 15 min; (iii) 0 °C, 2 h; (iv) E^+=H_2O, ^{t}BuCHO, $(CH_2)_5CO$, CO_2, −70 °C, 15 min; (v) H_2O, −70 to 20 °C.

Table 2. Preparation of compounds **11** from compound **6**

Entry	R^1R^2CO	E^+	Product[a]					
			No.	R^1	R^2	E	Yield (%)[b,c]	R_f[d]
1	^{t}BuCHO	H_2O	**11a**	H	^{t}Bu	H	58 (>95)	0.33 (10/1)
2	^{t}BuCHO	^{t}BuCHO	**11b**	H	^{t}Bu	^{t}BuCHOH	49 (80)[e]	0.31 (5/1)
3	^{t}BuCHO	CO_2	**11c**	H	^{t}Bu	CO_2H	42 (>95)	0.18 (2/1)
4	$(CH_2)_5CO$	$(CH_2)_5CO$	**11d**	$(CH_2)_5$		$(CH_2)_5COH$	45 (78)	0.21 (2/1)
5	$(CH_2)_5CO$	CO_2	**11e**	$(CH_2)_5$		CO_2H	43 (74)	0.24 (2/1)
6	(−)-menthone	CO_2	**11f**[f]	—		CO_2H	41 (68)	0.13 (2/1)

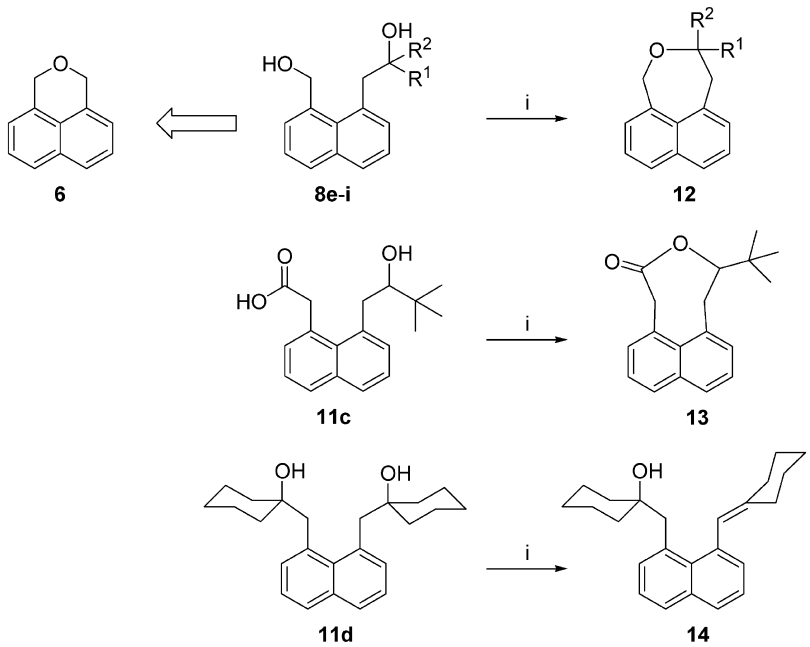
[a] All products were >95% pure (GLC and 300 MHz ^{1}H NMR).
[b] Isolated yield after column chromatography (silica gel, hexane/ethyl acetate) based on the starting material **6**.
[c] In parenthesis GLC yield of compound **11**.
[d] Silica gel, in parenthesis hexane/ethyl acetate ratio.
[e] A ca. 1:1 mixture of diastereomers was obtained (300 MHz ^{1}H NMR).
[f] See Figure 2.



11f

Figure 2.

sequential manner. Thus, after reductive ring opening lithiation to give the dianion **7** (Scheme 2) and reaction with a carbonyl compound as the first electrophile [^{t}BuCHO, $(CH_2)_5CO$, (−)-menthone], the resulting alcoholate **9** was stirred at 0 °C for 2 h, so a second lithiation took place with the excess of lithium still present in the reaction medium to give a new functionalised organolithium compound **10** which, by reaction with a

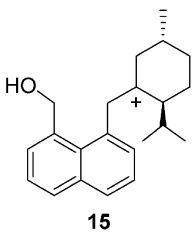


Scheme 4. Reagents and conditions: (i) *p*-TsOH (cat.), MS 4 Å, PhMe, 110 °C, 2 h.

Table 3. Preparation of compounds **12**

Entry	Starting material **8**	Product[a]				
		No.	R^1	R^2	Yield (%)[b,c]	R_f[d]
1	**8e**	**12e**	Me	Me	>95	0.36
2	**8f**	**12f**	CH_3CH_2	CH_3CH_2	>95	0.43
3	**8g**	**12g**	$CH_3(CH_2)_4$	$CH_3(CH_2)_4$	89 (>95)	0.54
4	**8h**	**12h**	$(CH_2)_5$		>95	0.46
5	**8i**	**12i**	$(CH_2)_7$		56 (>95)	0.47

[a] All products were >95% pure (GLC and 300 MHz ^{1}H NMR).
[b] Isolated yield after column chromatography (silica gel, hexane/ethyl acetate) based on the starting material **8**.
[c] In parenthesis GLC yield of compound **12**.
[d] Silica gel, hexane/ethyl acetate: 10/1.



15

Figure 3.

second electrophile [H_2O, ^{t}BuCHO, $(CH_2)_5CO$, CO_2], and final hydrolysis led to 1,8-difunctionalised naphthalenes **11** (Scheme 3 and Table 2). In the case of using prostereogenic carbonyl compounds in both steps, such as pivaldehyde, an almost 1:1 mixture of diastereomers was obtained (Table 2, entry 2).

Finally, intramolecular dehydration of diols **8e–i** by treatment with a catalytic amount of *p*-toluenesulfonic acid in the presence of 4 Å molecular sieves in toluene at 110 °C gave the corresponding oxygen-containing seven-membered heterocycle **12** in very high yields (Scheme 4 and Table 3). In the case of diol **8j** (Fig. 1), a complex mixture of reaction products was obtained, probably involving the most stable tertiary carbenium ion **15** (Fig. 3), which is supposedly involved in this process. It is worthy to note that the whole process **6→12** represents the homologation of the starting heteroycle **6**. Under the same reaction conditions, hydroxyacid **11c** gave the lactone **13** in almost quantitative yield (Scheme 4). On the other hand, the diol **11d** did not lead to the oxygen-containing eight-membered heterocycle giving instead, among other products, the conjugate hydroxyolefin **14** in 63% yield, after column chromatography (Scheme 4).

3. Conclusion

In conclusion, we report here that the reductive opening lithiation of 1*H*,3*H*-benzo[*de*]isochromene (**6**) at −50 °C led to dianionic intermediate **7**, which by reaction with electrophiles and final hydrolysis gave functionalised alcohols **8**. It is worthy to note that it is possible to carry out a sequential double lithiation-reaction with electrophiles to prepare 1,8-difunctionalised naphthalene derivatives **11**.

4. Experimental

4.1. General

All reactions were carried out under an atmosphere of nitrogen in oven-dried glassware, except the preparation of 1*H*,3*H*-benzo[*de*]isochromene (**6**). All reagents were commercially available (Acros, Aldrich) and were used without further purification. Commercially available anhydrous THF (99.9%, water content ≤0.006%, Acros) was used as solvent in all the lithiation reactions. IR spectra were measured (film) with a Nicolet Impact 400 D-FT Spectrometer. NMR spectra were recorded with a Bruker AC-300 or a Bruker ADVANCE DRX-500 using $CDCl_3$ as the solvent. LRMS and HRMS were measured with Shimadzu GC/HS QP-5000 and Finingan MAT95 S spectrometers, respectively. The purity of volatile products and the chromatographic analyses (GLC) were determined with a flame ionisation detector and a 12 m capillary column (0.2 mm diam., 0.33 μm film thickness), using nitrogen (2 mL/min) as carrier gas, $T_{injector}$=275 °C, $T_{detector}$=300 °C, T_{column}=60 °C (3 min) and 60–270 °C (15 °C/min), P=40 kPa. Specific rotations were determined with a Perkin–Elmer 341 digital polarimeter.

4.2. Reductive lithiation of 1*H*,3*H*-benzo[*de*]isochromene (6) and reaction with electrophiles. Preparation of compounds 8

Isolation of compounds **8**. *General procedure*. To a blue suspension of lithium powder (35 mg, 5.0 mmol) and a catalytic amount of DTBB (27 mg, 0.1 mmol; 5 mol%) in dry THF (3 mL) under argon was added dropwise 1*H*,3*H*-benzo[*de*]isochromene (**6**) (85 mg, 0.5 mmol) at −50 °C, and the resulting mixture was stirred for 6 h (monitored by GLC) at the same temperature. Then the corresponding electrophile (1.0 mmol; 0.2 mL in the case of H_2O and D_2O) was added dropwise and stirring was continued for 15 min. After that, the reaction mixture was hydrolysed with water (4 mL), extracted with ethyl acetate (3×10 mL), dried over anhydrous Na_2SO_4 and evaporated (15 Torr). The residue was purified by column chromatography (silica gel, hexane/ethyl acetate) to yield pure products. Yields and R_f values are given in Table 1, physical, analytical and spectroscopic data follow.

4.2.1. 1-Hydroxymethyl-8-methylnaphthalene (8a). White solid, mp 91–92 °C (pentane/dichloromethane) (lit. mp 93–94.5 °C);[14] ν (KBr) 3480–3150 (OH), 3050, 3032 (ArH), 1085 cm^{-1} (CO); δ_H 2.23 (1H, br s, OH), 3.02 (3H, s, CH_3), 5.22 (2H, s, CH_2), 7.35–7.43 (3H, m, ArH), 7.54 (1H, d, J=7.0 Hz, ArH), 7.71–7.74 (1H, m, ArH), 7.82 (1H, d, J=8.1 Hz, ArH); δ_C 24.1 (CH_3), 66.4 (CH_2OH), 124.9, 125.4, 127.9, 128.9, 130.2, 130.4, 131.5, 134.7, 135.6, 137.2 (ArC); *m/z* 172 (M^+, 38%), 155 (11), 154 (69), 153 (100), 152 (20), 143 (11), 141 (13), 129 (13), 128 (31), 127 (10), 115 (19), 77 (16), 76 (36), 63 (13), 51 (12); HRMS: M^+, found 172.0874.$C_{12}H_{12}O$ requires 172.0888.

4.2.2. 8-Deuteromethyl-1-hydroxymethylnaphthalene (8b). White solid, mp 91–92 °C (pentane/dichloromethane); ν (KBr) 3485–3145 (OH), 3048, 3031 (ArH), 1064 cm^{-1} (CO); δ_H 2.46 (1H, br s, OH), 3.02 (2H, s, CH_2D), 5.22 (2H, s, CH_2), 7.35–7.43 (3H, m, ArH), 7.54 (1H, d, J=7.0 Hz, ArH), 7.71–7.81 (1H, m, ArH), 7.84 (1H, d, J=8.1 Hz, ArH); δ_C 23.9 (t, J_{CD}=20.9 Hz), 66.3 (CH_2OH), 124.9, 125.4, 127.8, 128.9, 130.2, 130.4, 131.5, 134.7, 135.6, 137.3 (ArC); *m/z* 173 (M^+, 26%), 172 (24), 155 (40), 154 (99), 153 (100), 152 (19), 143 (11), 142 (12), 141 (13), 129 (28), 128 (45), 127 (16), 116 (12), 115 (25), 77 (24), 76 (29), 75 (11), 64 (11), 63 (21), 51 (19); HRMS: M^+, found 173.0934. $C_{12}H_{11}DO$ requires 173.0950.

4.2.3. 1-[(2-Hydroxy-3,3-dimethyl)butyl]-8-hydroxymethylnaphthalene (8c). Colourless oil; ν (film) 3410–3160 (OH), 3048, 3029 (ArH), 1040 cm^{-1} (CO); δ_H 1.06 [9H, s, $(CH_3)_3C$], 2.83 (2H, br s, 2×OH), 3.38–3.56 (3H, m, CH_2CH), 4.90 (1H, d, J=12.40 Hz, C*H*HOH), 5.35 (1H, d, J=12.40 Hz, CH*H*OH), 7.36–7.44 (3H, m, ArH), 7.49 (1H,

dd, J=6.9, 1.25 Hz, ArH), 7.76–7.84 (2H, m, ArH); δ_C 25.9 [(CH_3)$_3$C], 35.2 [(CH$_3$)$_3$*C*], 36.9 (*C*H$_2$CH), 66.4 (CH$_2$OH), 83.4 (CHOH), 124.9, 125.2, 128.8, 130.7, 130.8, 131.0, 131.9, 135.9, 136.7, 136.9 (ArC); *m*/*z* 240 (M^+-H_2O, 24%), 207 (17), 183 (15), 172 (17), 168 (15), 155 (62), 154 (74), 153 (82), 152 (38), 151 (15), 141 (27), 129 (17), 128 (17), 115 (19), 76 (16), 75 (15), 57 (60), 55 (22), 45 (28), 41 (100); HRMS: M^+-H_2O, found 240.1510. $C_{17}H_{20}O$ requires 240.1514.

4.2.4. 1-Hydroxymethyl-8-[(2-hydroxy-2-phenyl)ethyl]-naphthalene (8d). Colourless oil; ν (film) 3510–3255 (OH), 3057, 3032 (ArH), 1092 cm^{-1} (CO); δ_H 1.70 (2H, br s, 2×OH), 3.65 (1H, dd, J=14.6, 3.9 Hz, C*H*HCH), 3.87 (1H, dd, J=14.6, 9.1 Hz, CH*H*CH), 4.89 (1H, dd, J=9.1, 3.9 Hz), 5.11 (1H, d, J=12.4 Hz, C*H*HOH), 5.37 (1H, d, J=12.4 Hz, CH*H*OH), 7.30–7.46 (6H, m, ArH), 7.57 (1H, d, J=7.0 Hz, ArH), 7.80–7.89 (4H, m, ArH); δ_C 45.3 (*C*H$_2$CH), 66.7 (CH$_2$OH), 77.1 (CHOH), 124.9, 125.2, 125.9, 128.5, 129.0, 129.1, 130.7, 130.9, 131.1, 131.6, 135.0, 136.0, 136.3, 143.9; *m*/*z* 260 (M^+-H_2O, 7%), 172 (15), 155 (14), 154 (100), 153 (76), 152 (23), 107 (10), 79 (11), 77 (18); HRMS: M^+-H_2O, found 260.1202. $C_{19}H_{16}O$ requires 260.1201.

4.2.5. 1-Hydroxymethyl-8-[(2-hydroxy-2-methyl)propyl]naphthalene (8e). Colourless oil; ν (film) 3560–3275 (OH), 3055, 3033 (ArH), 1095 cm^{-1} (CO); δ_H 1.25 [6H, s, (CH$_3$)$_2$C], 2.17 (2H, br s, 2×OH), 3.57 (2H, s, C*H*$_2$COH), 5.18 (2H, s, C*H*$_2$OH), 7.33–7.45 (3H, m, ArH), 7.52–7.55 (1H, m, ArH), 7.78–7.83 (2H, m, ArH); δ_C 29.5 [(*C*H$_3$)$_2$C], 47.7 (*C*H$_2$COH), 66.5 (CH$_2$OH), 72.7 (COH), 124.6, 125.0, 129.2, 129.5, 130.5, 131.4, 132.6, 133.6, 135.8, 137.0 (ArC); *m*/*z* 212 (M^+-H_2O, 9%), 179 (18), 172 (22), 154 (92), 153 (100), 141 (17), 128 (27), 115 (17), 59 (47); HRMS: M^+-H_2O, found 212.1196. $C_{15}H_{16}O$ requires 212.1201.

4.2.6. 1-[(2-Hydroxy-2-ethyl)butyl]-8-hydroxymethylnaphthalene (8f). Colourless oil; ν (film) 3445–3185 (OH), 3050, 3030 (ArH), 1080 cm^{-1} (CO); δ_H 0.90 (6H, t, J=6.9 Hz, 2×CH$_3$), 1.38–1.57 (4H, m, 2×CH$_2$), 2.18 (2H, br s, 2×OH), 3.53 (2H, s, C*H*$_2$COH), 5.17 (2H, s, C*H*$_2$OH), 7.30–7.42 (3H, m, ArH), 7.50–7.53 (1H, m, ArH), 7.76–7.81 (2H, m, ArH); δ_C 8.0 (CH$_3$), 30.0 (CH$_2$), 44.0 (CH$_2$), 66.3 (CH$_2$OH), 76.8 (COH), 124.5, 125.0, 129.1, 130.3, 131.4, 132.5, 132.7, 133.3, 135.8, 137.3 (ArC); *m*/*z* 240 (M^+-H_2O, 1%), 211 (10), 172 (28), 155 (18), 154 (100), 153 (75), 152 (20), 141 (13), 128 (17), 115 (11), 87 (19), 57 (22); HRMS: M^+-H_2O, found 240.1511. $C_{17}H_{20}O$ requires 240.1514.

4.2.7. 1-Hydroxymethyl-8-[(2-hydroxy-2-pentyl)heptyl]-naphthalene (8g). Colourless oil; ν (film) 3430–3120 (OH), 3051, 3029 (ArH), 1105 cm^{-1} (CO); δ_H 0.90 (6H, t, J=6.5 Hz, 2×CH$_3$), 1.28–1.47 (18H, m, 8×CH$_2$, 2×OH), 3.54 (2H, s, C*H*$_2$COH), 5.18 (2H, s, C*H*$_2$OH), 7.28–7.43 (3H, m, ArH), 7.53 (1H, d, J=5.9 Hz, ArH), 7.77–7.81 (2H, m, ArH); δ_C 14.0 (CH$_3$), 22.6, 23.4, 32.3, 38.2, 44.9 (CH$_2$), 66.3 (CH$_2$OH), 77.2 (COH), 124.5, 125.0, 129.2, 130.3, 131.4, 132.5, 132.7, 133.3, 135.8, 137.4 (ArC); *m*/*z* 324 (M^+-H_2O, 9%), 253 (14), 236 (20), 235 (86), 193 (24), 181 (16), 179 (45), 178 (21), 169 (23), 167 (19), 166 (17), 165 (72), 154 (30), 153 (63), 152 (68), 141 (27), 128 (17), 115 (16), 57 (29), 55 (50), 43 (100); HRMS: M^+-H_2O, found 324.2475. $C_{23}H_{32}O$ requires 324.2453.

4.2.8. 1-[(1-Hydroxycyclohexyl)methyl]-8-hydroxymethylnaphthalene (8h). Colourless oil; ν (film) 3535–3140 (OH), 3058, 3036 (ArH), 1045 cm^{-1} (CO); δ_H 1.27–1.93 (12H, m, 5×CH$_2$, 2×OH), 3.54 (2H, s, ArC*H*$_2$COH), 5.16 (2H, s, C*H*$_2$OH), 7.30 (1H, d, J=5.9 Hz, ArH), 7.36–7.42 (2H, m, ArH), 7.52 (1H, d, J=5.8 Hz, ArH), 7.76–7.80 (2H, m, ArH); δ_C 22.0, 25.8, 37.4, 47.2 (CH$_2$), 66.3 (CH$_2$OH), 73.2 (COH), 124.4, 125.0, 129.1, 130.4, 131.6, 132.4, 132.5, 132.9, 135.7, 137.1 (ArC); *m*/*z* 252 (M^+-H_2O, 5%), 172 (19), 165 (20), 155 (15), 154 (100), 153 (75), 152 (36), 141 (12), 128 (19), 115 (15), 81 (18), 55 (30); HRMS: M^+-H_2O, found 252.1503. $C_{18}H_{20}O$ requires 252.1514.

4.2.9. 1-[(1-Hydroxycyclooctyl)methyl]-8-hydroxymethylnaphthalene (8i). Colourless oil; ν (film) 3460–3160 (OH), 3055, 3035 (ArH), 1045 cm^{-1} (CO); δ_H 1.48–1.64 (16H, m, 7×CH$_2$, 2×OH), 3.54 (2H, s, ArC*H*$_2$COH), 5.16 (2H, s, C*H*$_2$OH), 7.29–7.43 (3H, m, ArH), 7.51–7.54 (1H, m, ArH), 7.76–7.81 (2H, m, ArH); δ_C 22.2, 25.0, 28.2, 35.6, 45.5 (CH$_2$), 66.3 (CH$_2$OH), 77.1 (COH), 124.4, 125.1, 129.1, 130.3, 131.6, 132.5, 132.7, 133.2, 135.7, 137.2 (ArC); *m*/*z* 280 (M^+-H_2O, 5%), 155 (16), 154 (100), 153 (53), 152 (15), 55 (11); HRMS: M^+-H_2O, found 280.1834. $C_{20}H_{24}O$ requires 280.1827.

4.2.10. (1*R*,2*S*,5*R*)-1-[(1-Hydroxy-2-isopropyl-5-methyl)-cyclohexyl]methyl-8-hydroxymethylnaphthalene (8j). Colourless oil; ν (film) 3510–3190 (OH), 3055, 3032 (ArH), 1035 cm^{-1} (CO); δ_H 0.64 (3H, d, J=6.55 Hz, C*H*$_3$CH), 0.71–0.97 (m, 3 H), 1.01 (3H, d, J=6.8 Hz, C*H*$_3$CH), 1.02 (3H, d, J=6.8 Hz, C*H*$_3$CH), 1.18–1.28 (2H, m), 1.35–1.49 (1H, m), 1.53–1.60 (1H, m), 1.66–1.70 (1H, m), 2.23 (2H, br s, 2×OH), 2.52–2.61 (1H, m), 2.89 (1H, d, J=14.4 Hz, ArC*H*HCOH), 4.40 (1H, d, J=14.4 Hz, ArCH-*H*COH), 5.22 (2H, s, C*H*$_2$OH), 7.34–7.42 (3H, m, ArH), 7.48–7.51 (1H, m, ArH), 7.76–7.81 (2H, m, ArH); δ_C 18.3 (CH$_3$), 21.3 (CH$_2$), 22.3 (CH$_3$), 24.1 (CH$_3$), 25.8 (CH), 27.8 (CH), 35.1 (CH$_2$), 45.7 (CH$_2$), 47.3 (CH$_2$), 51.4 (CH), 66.4 (CH$_2$OH), 77.2 (COH), 124.5, 124.8, 128.9, 130.3, 130.4, 132.1, 133.1, 134.0, 135.8, 137.5 (ArC); *m*/*z* 308 (M^+-H_2O, 2%), 172 (25), 155 (18), 154 (100), 153 (47), 152 (15), 128 (12), 95 (12), 81 (26), 69 (18), 55 (38); HRMS: M^+-H_2O, found 308.2144. $C_{22}H_{28}O$ requires 308.2140. $[\alpha]_D^{20}=-58.4$ [c=1.0 (CH_2Cl_2)].

4.3. Sequential double lithiation of 1*H*,3*H*-benzo[*de*]isochromene (6) and reaction with electrophiles

Isolation of compounds **11**. *General procedure*. To a blue suspension of lithium powder (35 mg, 5.0 mmol) and a catalytic amount of DTBB (27 mg, 0.1 mmol; 5 mol%) in dry THF (3 mL) under argon was added dropwise 1*H*,3*H*-benzo[*de*]isochromene (**6**) (85 mg, 0.5 mmol) at −50 °C, and the resulting mixture was stirred for 6 h (monitored by GLC) at the same temperature. Then the corresponding carbonyl compound (0.7 mmol) was added dropwise and stirring was continued for 15 min. After that, the reaction mixture was allowed to rise to 0 °C and stirring was

continued for 2 h at this temperature. Then, the reaction mixture was cooled down to −70 °C and a second electrophile (1.0 mmol, 0.2 mL in the case of H_2O, carbon dioxide was bubbled for 15 min) was added dropwise. Finally it was hydrolysed with water (4 mL, 3 M HCl in the case of using carbon dioxide as the second electrophile), extracted with ethyl acetate (3×10 mL), dried over anhydrous Na_2SO_4 and evaporated (15 Torr). The residue was purified by column chromatography (silica gel, hexane/ethyl acetate) to yield pure products. Yields and R_f values are given in Table 2, physical, analytical and spectroscopic data follow.

4.3.1. 1-[(3,3-Dimethyl-2-hydroxy)butyl]-8-methylnaphthalene (11a). Colourless oil; ν (film) 3510–3230 (OH), 3047, 3027 (ArH), 1065 cm^{-1} (CO); δ_H 0.96 [9H, s, $(CH_3)_3C$], 1.72 (1H, br s, OH), 2.83 (3H, s, $ArCH_3$), 3.06 (1H, dd, J=14.0, 10.9 Hz, C*H*HCHOH), 3.41 (1H, dd, J=10.9, 2.1 Hz, CHHC*H*OH), 3.60 (1H, dd, 1H, J=14.0, 2.1 Hz, CH*H*CHOH), 7.15–7.28 (4H, m, ArH), 7.59–7.66 (2H, m, ArH); δ_C 26.1 [(CH_3)$_3$C], 27.0 (CH_3), 35.2 [(CH_3)$_3$*C*], 38.2 (CH_2), 80.1 (CHOH), 124.8, 125.1, 128.3, 128.9, 130.3, 130.7, 132.7, 134.6, 135.9, 136.5 (ArC); *m*/*z* 242 (M^+, 6%), 157 (13), 156 (100), 153 (25), 152 (14), 141 (40), 115 (12), 87 (11), 69 (14), 57 (19); HRMS: M^+, found 242.1665. $C_{17}H_{22}O$ requires 242.1671.

4.3.2. 1,8-Bis(2-hydroxy-3,3-dimethylbutyl)naphthalene (11b). Diastereomeric mixture. Colourless oil; ν (film) 3550–3310 (OH), 3050, 3030 (ArH), 1070 cm^{-1} (CO); δ_H 1.07 [18H, s, 2×(CH_3)$_3$C], 1.46 (2H, br s, 2×OH), 3.08–3.28 (2H, m, 2×C*H*H), 3.36–3.40 (2H, m, 2×CH*H*), 3.60–3.66 (2H, m, 2×CH), 7.32–7.47 (4H, m, ArH), 7.71–7.80 (2H, m, ArH); δ_C 26.0, 26.1 [(CH_3)$_3$C], 35.1, 35.2 [(CH_3)$_3$*C*], 39.2, 39.3 (CH_2), 79.2, 81.0 (CHOH), 129.0, 129.1, 130.9, 131.1, 135.9, 136.1, 136.2, 136.8 (ArC); *m*/*z* 328 (M^+, 0.2%), 242 (10), 167 (11), 165 (10), 156 (10), 155 (16), 154 (11), 153 (25), 141 (10), 57 (54); HRMS: M^+, found 328.2372. $C_{22}H_{32}O_2$ requires 328.2402.

4.3.3. 8-(2-Hydroxy-3,3-dimethylbutyl)-1-naphthylacetic acid (11c). Pale yellow oil; ν (film) 3490–2820 (OH), 3063, 3034 (ArH), 1706 (C=O), 1084 cm^{-1} (CO); δ_H 1.02 [9H, s, $(CH_3)_3C$], 3.12 (1H, dd, J=16.85, 10.9 Hz, C*H*HCHOH), 3.36 (1H, dd, J=16.85, 2.3 Hz, CH*H*CHOH), 3.47 (1H, dd, J=10.8, 2.3 Hz, C*H*OH), 4.19 (1H, d, J=17.7 Hz, C*H*HCO$_2$H), 4.19 (1H, d, J=17.7 Hz, CH*H*CO$_2$H), 7.15 (2H, br s, 2×OH), 7.24–7.38 (4H, m, ArH), 7.72–7.80 (2H, m, ArH); δ_C 25.9 [(CH_3)$_3$C], 35.2 [(CH_3)$_3$*C*], 37.8, 43.0 (CH_2), 81.0 (CHOH), 124.8, 125.1, 129.1, 130.0, 130.2, 131.1, 132.0, 132.2, 135.7, 136.0 (ArC), 177.3 (CO_2H); *m*/*z* 268 (M^+-H_2O, 10%), 183 (13), 182 (86), 181 (14), 165 (17), 155 (29), 154 (59), 153 (100), 152 (71), 151 (18), 57 (51); HRMS: M^+-H_2O, found 268.1464. $C_{18}H_{20}O_2$ requires 268.1463.

4.3.4. 1,8-Bis(1-hydroxycyclohexylmethyl)naphthalene (11d). Colourless oil; ν (film) 3580–3310 (OH), 3055, 3035 (ArH), 1055 cm^{-1} (CO); δ_H 1.43–1.56 (22H, m, 10×CH_2, 2×OH), 3.64 (4H, s, 2×$ArCH_2$), 7.26–7.37 (4H, m, ArH), 7.74 (2H, d, J=8.0 Hz, ArH); δ_C 21.9, 25.9, 37.7, 49.1 (CH_2), 72.4 (COH), 124.4, 129.2, 132.9, 133.7, 134.2, 135.9 (ArC); *m*/*z* 334 (M^+-H_2O, 0.5%), 236 (32), 221 (21), 179 (14), 178 (10), 165 (24), 157 (13), 156 (100), 153 (30), 152 (11), 141 (10), 99 (35), 81 (34), 57 (10), 55 (38); HRMS: M^+-2H_2O, found 316.2184. $C_{24}H_{28}$ requires 316.2191.

4.3.5. 8-(1-Hydroxycyclohexylmethyl)-1-naphthylacetic acid (11e). Pale yellow oil; ν (film) 3645–3110 (OH), 3050 (ArH), 1714 (C=O), 1085 cm^{-1} (CO); δ_H 1.29–1.55 (10H, m, 5×CH_2), 3.30 (2H, s, CH_2COH), 4.53 (2H, s, CH_2CO$_2$H), 7.29–7.40 (4H, m, ArH), 7.76–7.79 (2H, m, ArH); δ_C 21.9, 25.8, 29.7, 37.6, 43.5 (CH_2), 72.3 (COH), 124.5, 124.8, 129.3, 129.9, 131.0, 132.1, 132.7, 132.8, 132.9, 135.9 (ArC), 176.2 (CO_2H); *m*/*z* 280 (M^+-H_2O, 5%), 207 (13), 182 (100), 181 (11), 165 (21), 155 (12), 154 (18), 153 (27), 152 (29), 57 (10), 55 (15); HRMS: M^+-H_2O, found 280.1452. $C_{19}H_{20}O_2$ requires 280.1463.

4.3.6. (1*R*,2*S*,5*R*)-8-[(1-Hydroxy-2-isopropyl-5-methylcyclohexyl)methyl]-1-naphthylacetic acid (11f). Pale yellow oil; ν (film) 3580–2760 (OH), 3040 (ArH), 1709 (C=O), 1090 cm^{-1} (CO); δ_H 0.62 (3H, d, J=6.4 Hz, CH_3CH), 0.73–0.94 (m, 3H), 0.97 (3H, d, J=6.8 Hz, CH_3CH), 1.01 (3H, d, J=6.8 Hz, CH_3CH), 1.22–1.68 (5H, m), 2.45–2.52 (1H, m), 2.77 (1H, d, J=14.6 Hz, C*H*HCOH), 4.06 (1H, d, J=17.8 Hz, C*H*HCO$_2$H), 4.12 (1H, d, J=14.6 Hz, CH*H*COH), 5.10 (1H, d, J=17.8 Hz, CH*H*CO$_2$H), 7.11 (2H, br s, 2×OH), 7.27–7.40 (4H, m, ArH), 7.75–7.80 (2H, m, ArH); δ_C 18.1 (CH_3), 21.0 (CH_2), 22.2 (CH_3), 23.9 (CH_3), 25.7 (CH), 27.6 (CH), 35.0 (CH_2), 43.6 (CH_2), 45.4 (CH_2), 47.4 (CH_2), 51.2 (CH), 76.1 (COH), 124.6, 124.7, 129.1, 130.1, 130.7, 131.9, 132.6, 133.3, 133.7, 135.8 (ArC), 177.9 (CO_2H); *m*/*z* 336 (M^+-H_2O, 0.5%), 183 (14), 182 (100), 154 (27), 153 (35), 152 (11), 55 (14), 44 (12); HRMS: M^+-H_2O, found 336.2070. $C_{23}H_{28}O_2$ requires 336.2089. $[\alpha]_D^{20}=-49.4$ [c=0.95 (CH_2Cl_2)].

4.4. Cyclisation of diols 8 and hydroxyacid 11c

Isolation of compounds **12**, **13** *and* **14**. *General procedure*. To a solution of the corresponding diols **8** and **11d**, or hydroxyacid **11c** (0.1 mmol) in toluene (1.5 mL) a catalytic amount of *p*-toluenesulfonic acid (30 mg) and 4 Å molecular sieves (30 mg) were added. The reaction mixture was heated at 110 °C for 2 h, then toluene was removed by distillation and the resulting residue was hydrolysed with water (mL), extracted with ethyl acetate (3×10 mL), dried over anhydrous Na_2SO_4 and evaporated (15 Torr). The residue was purified by column chromatography (silica gel, hexane/ethyl acetate) to yield pure products. Yields and R_f values are given in Table 3 for compounds **12**, but in the case of compounds **13** and **14**, yields are given in the text, their R_f, analytical and spectroscopic data for all compounds follow.

4.4.1. 3,3-Dimethyl-3,4-dihydro-1*H*-naphtho[1,8-*cd*]-oxepine (12e). Pale yellow oil; ν (film) 3054, 3035 (ArH), 1065 cm^{-1} (CO); δ_H 1.34 [6H, s, $(CH_3)_2$], 3.32 (2H, s, CH_2CO), 5.03 (2H, s, CH_2O), 7.16–7.40 (4H, m, ArH), 7.71 (2H, d, J=8.4 Hz, ArH); δ_C 27.6 (CH_3), 46.9 (CH_2), 67.2 (CH_2O), 77.5 (CO), 124.8, 124.9, 125.4, 125.5, 127.5, 128.2, 128.3, 135.1, 135.6, 139.6 (ArC); *m*/*z* 212 (M^+, 11%), 165 (12), 154 (71), 153 (100), 152 (32), 151 (11); HRMS: M^+, found 212.1217. $C_{15}H_{16}O$ requires 212.1201.

4.4.2. 3,3-Diethyl-3,4-dihydro-1*H*-naphtho[1,8-*cd*]-oxepine (12f). Pale yellow oil; ν (film) 3052, 3036 (ArH), 1071 cm^{-1} (CO); δ_H 0.91 (6H, t, J=7.5 Hz, 2×CH_3), 1.51–1.67 (4H, m, 2×CH_2CH$_3$), 3.27 (2H, s, CH_2CO), 5.02 (2H, s, CH_2O), 7.16–7.22 (2H, m, ArH), 7.25–7.34 (2H, m, ArH), 7.67–7.73 (2H, m, ArH); δ_C 8.3 (CH_3), 28.7 (CH_2), 42.9 (CH_2), 67.0 (CH_2O), 82.0 (CO), 125.0, 125.2, 125.4, 127.4, 128.0, 128.2, 132.4, 135.1, 135.8, 139.7 (ArC); *m/z* 240 (M^+, 5%), 155 (20), 154 (100), 153 (90), 152 (32), 57 (14); HRMS: M^+, found 240.1517. $C_{17}H_{20}O$ requires 240.1514.

4.4.3. 3,3-Dipenthyl-3,4-dihydro-1*H*-naphtho[1,8-*cd*]-oxepine (12g). Colourless oil; ν (film) 3050, 3032 (ArH), 1066 cm^{-1} (CO); δ_H 0.86–0.90 (6H, t, J=6.8 Hz, 2×CH_3), 1.27–1.56 (16H, m, 8×CH_2), 3.27 (2H, s, CH_2CO), 5.02 (2H, s, CH_2O), 7.14–7.23 (2H, m, ArH), 7.30–7.35 (2H, m, ArH), 7.67–7.71 (2H, m, ArH); δ_C 14.0 (CH_3), 22.6, 23.6, 32.4, 36.8, 43.7(CH_2), 66.9 (CH_2O), 81.6 (CO), 125.0, 125.2, 125.5, 127.4, 128.0, 128.1, 132.5, 135.1, 135.8, 139.8 (ArC); *m/z* 324 (M^+, 2%), 155 (25), 154 (100), 153 (44), 152 (13); HRMS: M^+, found 324.2448. $C_{23}H_{32}O$ requires 324.2453.

4.4.4. 3-Cyclohexyl-3,4-dihydro-1*H*-naphtho[1,8-*cd*]-oxepine (12h). Pale yellow oil; ν (film) 3052, 3035 (ArH), 1064 cm^{-1} (CO); δ_H 1.27–1.76 (10H, m, 5×CH_2), 3.29 (2H, s, CH_2CO), 5.05 (2H, s, CH_2O), 7.17–7.36 (4H, m, ArH), 7.68–7.72 (2H, m, ArH); δ_C 22.4, 25.9, 36.1, 45.5 (CH_2), 66.7 (CH_2O), 78.0 (CO), 124.9, 125.3, 125.4, 127.4, 128.0, 128.1, 132.4, 135.1, 135.4, 140.0 (ArC); *m/z* 252 (M^+, 5%), 155 (15), 154 (100), 153 (63), 152 (21); HRMS: M^+, found 252.1537. $C_{18}H_{20}O$ requires 252.1514.

4.4.5. 3-Cyclooctyl-3,4-dihydro-1*H*-naphtho[1,8-*cd*]-oxepine (12i). Colourless oil; ν (film) 3051, 3035 (ArH), 1054 cm^{-1} (CO); δ_H 1.52–1.68 (14H, m, 7×CH_2), 3.27 (2H, s, CH_2CO), 5.00 (2H, s, CH_2O), 7.18 (2H, t, J=6.6 Hz, ArH), 7.26–7.36 (2H, m, ArH), 7.70 (2H, d, J=8.4 Hz, ArH); δ_C 22.4, 25.3, 28.4, 33.9, 44.6 (CH_2), 66.3 (CH_2O), 82.0 (CO), 124.9, 125.3, 125.4, 127.4, 128.05, 128.1, 132.4, 135.1, 135.7, 139.9 (ArC); *m/z* 280 (M^+, 3%), 155 (16), 154 (100), 153 (62), 152 (22), 55 (19); HRMS: M^+, found 280.1812. $C_{20}H_{24}O$ requires 280.1827.

4.4.6. 4-(*tert*-Butyl)-1,2,4,5-tetrahydronaphtho[1,8-*de*]oxocin-2-one (13). Pale yellow oil; R_f 0.50 (hexane/ethyl acetate: 2/1); ν (film) 3055, 3030 (ArH), 1721 (C=O), 1065 cm^{-1} (CO); δ_H 1.12 [9H, s, $(CH_3)_3$C], 3.46 (1H, dd, J=15.9, 6.2 Hz CHHCH), 3.88 (1H, dd, J=15.9, 7.8 Hz, CHHCH), 4.29–4.34 (2H, m, CHO, CHHCO), 4.47 (d, 1H, J=13.9 Hz, CHHCO), 7.37–7.48 (4H, m, ArH), 7.76–7.82 (2H, m, ArH); δ_C 26.2 [(CH_3)$_3$C], 36.8 [(CH_3)$_3$C], 37.0, 44.8 (CH_2), 88.7 (CHO), 125.5, 125.7, 128.8, 129.5, 129.6, 130.6, 130.7, 132.2, 134.1, 135.2 (ArC), 171.7 (CO_2); *m/z* 268 (M^+, 5%), 183 (15), 182 (70), 181 (15), 165 (17), 155 (32), 154 (84), 153 (100), 152 (69), 76 (12), 57 (52); HRMS: M^+, found 268.1483. $C_{18}H_{18}O_2$ requires 268.1463.

4.4.7. 1-Cyclohexylidenemethyl-8-(1-hydroxycyclohexylmethyl)naphthalene (14). Pale yellow oil; R_f 0.79 (hexane/ethyl acetate: 10/1); ν (film) 3570–3270 (OH), 3050, 3030 (ArH), 1060 cm^{-1} (CO); δ_H 1.05–1.64 (18H, m, 9×CH_2), 1.84–1.96 (2H, m, CH_2), 2.98 (1H, d, J=16.5 Hz, ArCHH), 3.09 (1H, d, J=16.5 Hz, ArCHH), 3.41 (1H, br s, OH), 5.33 (1H, br s, ArCH=C), 7.22–7.25 (2H, m, ArH), 7.35–7.41 (2H, m, ArH), 7.66 (2H, d, J=8.1 Hz, ArH); δ_C 21.8, 22.0, 22.5, 23.3, 25.5, 26.5, 35.5, 35.9, 36.5 (CH_2), 77.2 (COH), 124.7, 124.9, 125.3, 125.8, 129.6, 133.0, 134.5, 138.3, 140.1 (C=CH, ArC); *m/z* 316 (M^+-H_2O, 29), 234 (15), 233 (60), 221 (24), 220 (100), 219 (18), 203 (14), 202 (10), 191 (30), 179 (38), 178 (38), 166 (11), 165 (48), 153 (22), 152 (17), 81 (24), 79 (10), 67 (11), 55 (23); HRMS: M^+-H_2O, found 316.2183. $C_{24}H_{28}$ requires 316.2191.

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Restricted rotation of the amino group and ring inversion in highly substituted anilines. A dynamic NMR and computational study

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Abstract—The reaction of cyclic ylidene malononitriles with acetylene (di)carboxylic acid esters led to the production of nine bicyclic systems incorporating highly substituted (5/6) anilines. The free energy of activation ($\Delta G^{\#}$) for the restricted rotation about the aniline–NH_2 bond was experimentally measured in each case and a correlation was evident between the increase in steric strain in the ground state, the electron withdrawing capabilities of the ring substituents, and a reduction in the rotational barrier. For four of the compounds, the slow ring interconversion (chair⥋chair) for the annelated saturated seven-membered ring that formed part of the bicyclic system was also evident. In these four compounds, both dynamic processes were also studied theoretically using ab initio methods whilst the ring interconversion was additionally studied using molecular dynamic simulations. The interconversion between the two stable chair forms was deemed to occur via a conformation series consisting of chair⥋boat⥋twist-boat⥋boat⥋chair.

1. Introduction

As part of our assessment of the suitability of cycloalkylidene malonic acid derivatives as synthetic building blocks for organic compounds, their reaction with acetylene dicarboxylic acid esters have been examined. The reactions of acyclic ylidene malononitriles with acetylene dicarboxylic acid esters yield *m*-toluidine tetracarboxylic acid derivatives[1] and our attention was thus drawn to the reaction of cyclic ylidene malononitriles with acetylene (di)carboxylic acid esters, which lead to bicyclic systems. We have used this reaction to produce the highly substituted anilines **1**–**9** from the cyclic ylidene malononitriles **I**–**IV** [cf. Scheme 1; **IV**=2-(3-methyl-cyclopent-2-enylidene)-malononitrile]. For all of the products **1**–**9**, dynamic behavior in their NMR spectra was evident due to the restricted rotation of the amino group, the free energy ($\Delta G^{\#}$) of which was measured experimentally in each case. The different influences on the barrier for the restricted rotation of an amino group bound to (hetero)aromatic rings (e.g. bulky *ortho* substituents, intra- or intermolecular hydrogen bonds) have been previously discussed in the literature on a number of occasions.[2] One interesting mechanism for altering the rate of internal motion of the amino group is by coordination of the system to a paramagnetic anion. Thus, the rotation of the amino group in the complex of 4-aminopyrimidine with undecatungstocobalto(II)silicate ($[SiW_{11}CoO_{39}]^{6-}$) was found to be much slower than that for free 4-aminopyrimidine, implying that the π-character of the NH_2–C bond increases upon coordination.[3]

In this work we wanted to examine the effects of depleting the electron density (corresponding to a reduction of bond order) in the NH_2–C bond by comparing a set of compounds differing, amongst other things, in their substitution at C-1. Interestingly, compounds **3**–**6** also displayed slow ring inversion of the cycloheptene ring. Both of these dynamic processes were investigated by quantum mechanical calculations for compounds **3**–**6**, and the latter process additionally by molecular dynamics simulations.

2. Results and discussion

2.1. Synthesis

The reaction mechanism for the synthesis of compounds **1**–**9** from **I**–**IV** by reaction with acetylene (di)carboxylic acid esters can be considered to be composed of several distinct, but intuitive steps. The reaction sequence is first initiated by base abstraction of a proton from the

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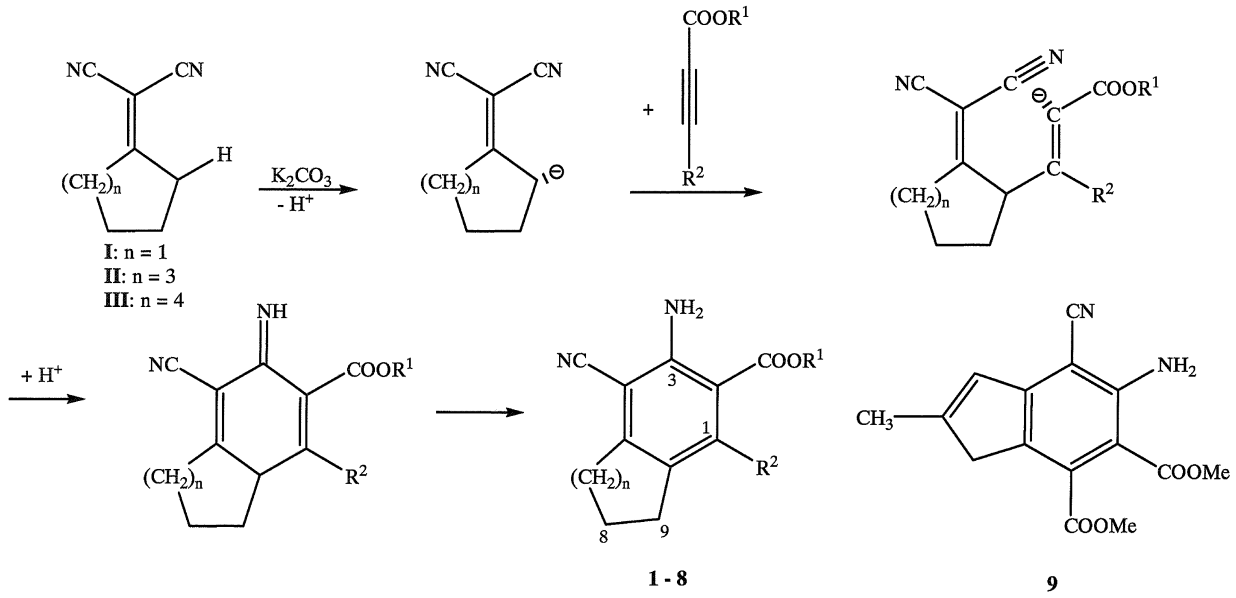
cycloalkylidene malonic acid derivative, followed by nucleophilic attack at the triple bond of the acetylene dicarboxylic acid ester by the resulting anion, and finally, intramolecular nucleophilic ring closure completed by re-protonation and tautomeric rearrangement to the preferred aromatic system. For **1**–**8** this sequence is depicted generally in the Scheme below; however, the reaction is analogous also for compound **9**.

Whilst in principle the reaction is straightforward, yields were found to be variable (high to low) but purification was readily accomplished by recrystallization in all cases. In the cases of reaction with acetylene carboxylic acid esters (to yield compounds **5**, **6**, and **8**), the reactions were found to be highly regiospecific yielding only the product resulting from nucleophilic attack at the alkyne carbon β to the ester functionality. This is presumably due to the greater stability of the intervening carbanion whereby the α carbon formally bears the negative charge which is stabilized by the electron-withdrawing capability of the adjacent ester group.

2.2. Restricted rotation of the amino group

The experimentally-measured barriers to rotation for the amino group, as determined by the decoalescence of the amino protons in the ^{1}H NMR spectra of **1**–**9** upon going down in temperature, are presented in Table 1. The free energies of activation, $\Delta G^{\#}$, were found to be slightly lower in comparison to known values for other *ortho*-substituted anilines possessing the potential for intramolecular hydrogen bonding—as is the case here.

For example, $\Delta G^{\#}$ for the restricted rotation of the amino group in 2-aminoacetophenone was found to be 44±2 kJ/mol,[4] and in 4-substituted 2-nitroanilines the determined values spanned the range 39–52 kJ/mol.[5] The slight lowering of $\Delta G^{\#}$ in these compounds here may be due to the higher degree of substitution (five or six) present in the benzene moiety. In particular, the nitrile group *ortho* to the amino group is able to withdraw electron density from the partial C–NH_2 double bond between the aryl moiety



#	1	2	3	4	5	6	7	8
n	1	1	3	3	3	3	4	4
R^1	Me	Et	Me	Et	Me	Et	Me	Et
R^2	COOMe	COOEt	COOMe	COOEt	H	H	COOMe	H

Atom labelling is given for the seven-membered ring fused compounds **3**-**6**.

Scheme 1.

Table 1. Experimental and calculated activation energies for the restricted rotation of the amino group in **1**–**9**

	1	2	3	4	5	6	7	8	9
T_c (K)	190	191	209	205	224	225	206	229	183
$\Delta G^{\#}$ (kJ/mol)	34.2	34.2	37.7	36.9	40.4	40.5	37.2	41.3	32.8
$\Delta E^{\#}$ (kJ/mol)	—	—	44.2	43.1	46.8	46.7	—	—	—
$\Delta E^{\#}$ (kJ/mol) (CH_2Cl_2)	—	—	34.7	32.5	37.3	37.6	—	—	—

Notes: T_c, coalescence temperature; $\Delta G^{\#}$, experimental free energy of activation as observed for the NH_2 signals; and $\Delta E^{\#}$, energies of activation calculated at the HF/6-31G* level of theory with and without the solvent (CH_2Cl_2).

Table 2. Experimental and calculated energies for the ring inversion in **3**–**6**

	Observed		$\Delta E^{\#}$ (kJ/mol)		$\Delta E^{\#}$ (kJ/mol) (CH_2Cl_2)	
	T_c (K)	$\Delta G^{\#}$ (kJ/mol)	C→B	B→TB	C→B	B→TB
3	204	39.7	42.0/30.6	19.3/10.0 20.5/11.2	41.8/30.3	18.7/9.3 20.5/11.1
4	—	—	42.1/30.6	19.3/11.3 20.5/12.5	42.5/30.7	18.6/11.0 20.5/12.9
5	220	42.8	45.2/30.7	12.1/8.3 17.1/13.3	45.1/30.7	11.6/8.0 17.0/13.4
6	215	41.8	45.2/30.7	12.1/8.3 17.1/13.3	45.3/30.6	11.3/8.1 16.7/13.4

C, chair; B, boat; TB, twist-boat. Notes: T_c, coalescence temperature; $\Delta G^{\#}$, experimental free energy of activation as observed for the NH_2 signals; and $\Delta E^{\#}$, energies of activation calculated at the HF/6-31G* level of theory with and without the solvent (CH_2Cl_2). The first value presented for the calculated values is for the forward reaction, the ensuing value is for the reverse reaction. For the B⟶TB transformation, two possible transformations are possible leading to non-degenerate twist-boat conformers, hence two sets of values. For a comprehension of these available pathways, see Figure 3.

and the NH_2 groups via mesomeric-type mechanisms, thus leading to a smaller free energy of activation as the bond more closely resembles a single bond in character.

The difference in $\Delta G^{\#}$ between compounds bearing a five-membered fused ring (**1**, **2**, and **9**) and those with cycloheptene/cyclooctene moieties (**3**–**8**) probably results from an increased ground state energy in the former set (where $\Delta G^{\#}$ is significantly smaller) as the transition states can be assumed to be very similar for all compounds. It is on the basis that five-membered fused rings are more rigid than their seven- or eight-membered ring analogs that the additional steric strain experienced by the five-membered fused ring systems accounts for the lowered free energy of activation in **1**, **2**, and **9**.

The introduction of an ester group at the *meta* position to the NH_2 group (for the otherwise analogous pairs of compounds **3** and **5**, **4** and **6**, and **7** and **8**) decreases $\Delta G^{\#}$ slightly by 2.7–4.1 kJ/mol. This may also be attributed to electron withdrawal from the aromatic system by the additional π-electron attracting, leading to lower bond order of the C–NH_2 bond and so forth as described above.

Table 3. Mean torsion angles for the conformational states of the cycloheptene moiety in **3**–**6** calculated at the HF/6-31G* level of theory

Angle defined by C atoms	τ (°) ±SD		
	C	B	TB
9a–4a–5–6	66±2	70±1	−31±1
4a–5–6–7	−81±1	−31±2	83±1
5–6–7–8	61±2	−53±1	−45±1
6–7–8–9	−60±1	54±2	−42±2
7–8–9–9a	79±2	29±3	83±1
8–9–9a–4a	−66±3	−69±2	−34±3

C, chair; B, boat; TB, twist-boat.

The barrier to rotation of the amino group in compounds **3**–**6** was also calculated quantum mechanically at the HF/6-31G* level of theory with, and without, inclusion of the solvent (cf. Table 1). The values calculated without inclusion of the solvent were found to be 6.2–6.5 kJ/mol higher than the observed free energies of activation. However, the experimental order of the rotational barriers was, nevertheless, reproduced quite well. Upon inclusion of the solvent, the calculations resulted in a considerable decrease of the calculated energy barrier and in fact, the values were found to be ca. 3 kJ/mol lower than the experimentally measured values for **3**, **5**, and **6**; for **4**, the divergence was greater, 4.4 kJ/mol. Since two ethyl ester groups are spatially more demanding, they impose a greater change in the ground state conformation of **4** in comparison to those of **3**, **5**, and **6**. A slightly different behavior of the solvent continuum model can thus be expected. Here, the ground state conformation of **4** was obviously not well stabilized in the solvent model, thus resulting in a lower predicted activation energy for rotation about the C–NH_2 bond.

2.3. Ring inversion

Upon going down in temperature, splitting of the signals of the CH_2 groups in the ^{1}H NMR spectra for compounds **3**, **5**, and **6** corresponding to positions 5 and 9 was readily observed owing to ring interconversion of the cycloheptene moiety. These dynamic effects were also apparent for **4**, although unfortunately signal splitting did not initiate until −100 °C which precluded experimental quantification. The measured free energies of activation for **3**, **5**, and **6** are presented in Table 2.

The observed values of ca. 40–43 kJ/mol are similar in magnitude to what has been found previously for benzocycloheptene (ca. 46 kJ/mol)[6] or, by the introduction of a

Table 4. Calculated energies ($\Delta E^{\#}$ (kJ/mol)) of the transition state conformations of **3**–**6** at various levels of theory

	HF/3-21G			HF/6-31G*			HF/6-31G* (CH_2Cl_2)		
	C	B	TB	C	B	TB	C	B	TB
3	0.0	8.3	23.2	0.0	11.4	20.7	0.0	11.6	21.0
4	0.0	8.1	20.9	0.0	11.5	19.4	0.0	11.8	19.4
5	0.0	11.3	19.9	0.0	14.5	18.3	0.0	14.4	18.0
6	0.0	11.3	19.9	0.0	14.5	18.3	0.0	14.7	18.0

C, chair; B, boat; TB, twist-boat.

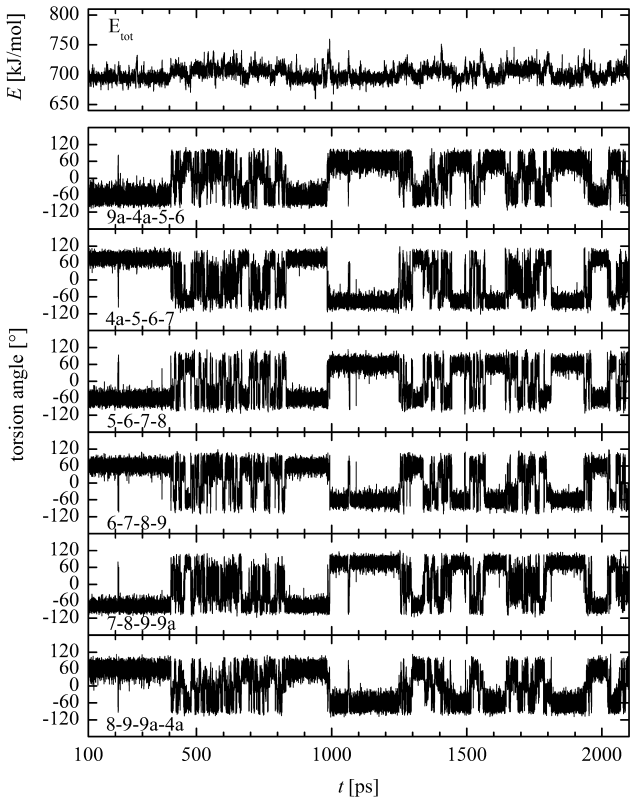


Figure 1. Total energy and torsion angles in the cycloheptene moiety of **5** during MD simulation at 1000 K.

NR group for a CH_2 group, for 2-substituted 2-methyl-2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepines (ca. 46 kJ/mol).[7] However, they are significantly higher than the values found for unsubstituted cycloheptene (ca. 21 kJ/mol)[8], for 5,5-dimethyl-1,3,3,7,7-pentadeuteriocycloheptene (ca. 26 kJ/mol)[9], or for 5,5-difluorocycloheptene (ca. 31 kJ/mol).[10] Thus, it can be inferred that different pathways are probably being taken for the inversion of cycloheptene and benzocycloheptene as outlined by Favini et al.[11] Theoretical calculations at the MP2(full)/6-31G* level of theory for the ring inversion of cycloheptene yielded a value of ca. 22 kJ/mol for the activation energy. Along this inversion pathway, only conformationally coplanar transition states were found. Assuming a twist-boat conformation for the transition state was found to increase the energy to ca. 51 kJ/mol, in discord with experiment.[12]

Quantum chemical calculations of compounds **3**–**6** led to three stable conformational states for the cycloheptene moiety: a chair (C), a boat (B), and a twist-boat (TB). A description of these conformations in terms of mean torsion angles is presented in Table 3. In all cases, a chair conformation was found to be the energetically most favorable conformation (cf. Table 4).

However, MD simulations indicated that both the boat and the twist-boat conformations are incorporated in the ring inversion pathway. For example, the evolution of the torsion angles in the cycloheptene ring system of **5** during a MD simulation run at 1000 K is depicted in Figure 1.

On the basis of the torsion angle data presented in Table 3, the structures produced by MD simulations were assigned to the corresponding conformations (cf. Fig. 2), where the appended + and − signs denote the multiplication of values in Table 3 by +1 and −1, respectively. At 600 K, only boat and twist-boat conformations are present, whereas at 1000 K a significant number of chair conformations are observed in addition to boat and twist-boat conformations. From this it is clear that ring inversion (chair–chair interconversion) only occurs via boat and twist-boat conformations. Furthermore, since boat-twist-boat interconversions are preponderant at 600 K, it implies that chair-boat and chair-twist-boat interconversions must determine

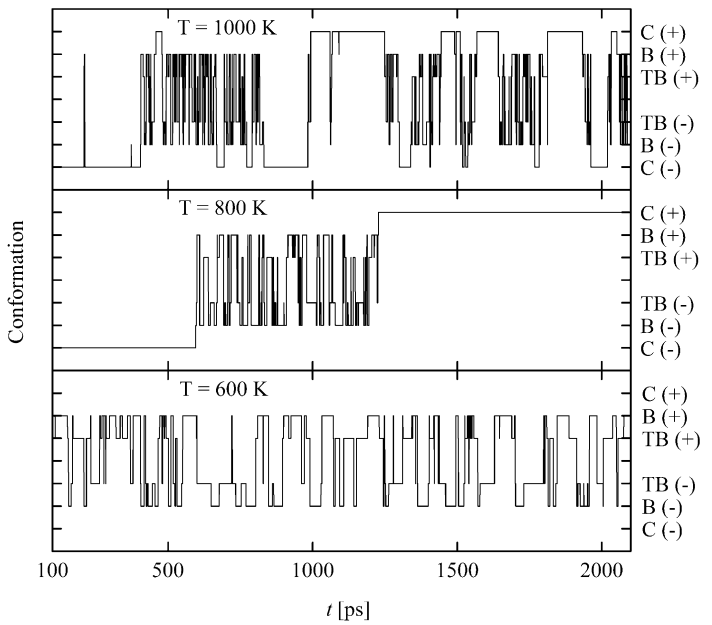


Figure 2. Conformational analysis of the MD simulations at 600, 800, and 1000 K for compound **5**. (C, chair; B, boat; TB, twist-boat; + and − refer to the corresponding conformational values in Table 3 multiplied by +1 and −1, respectively).

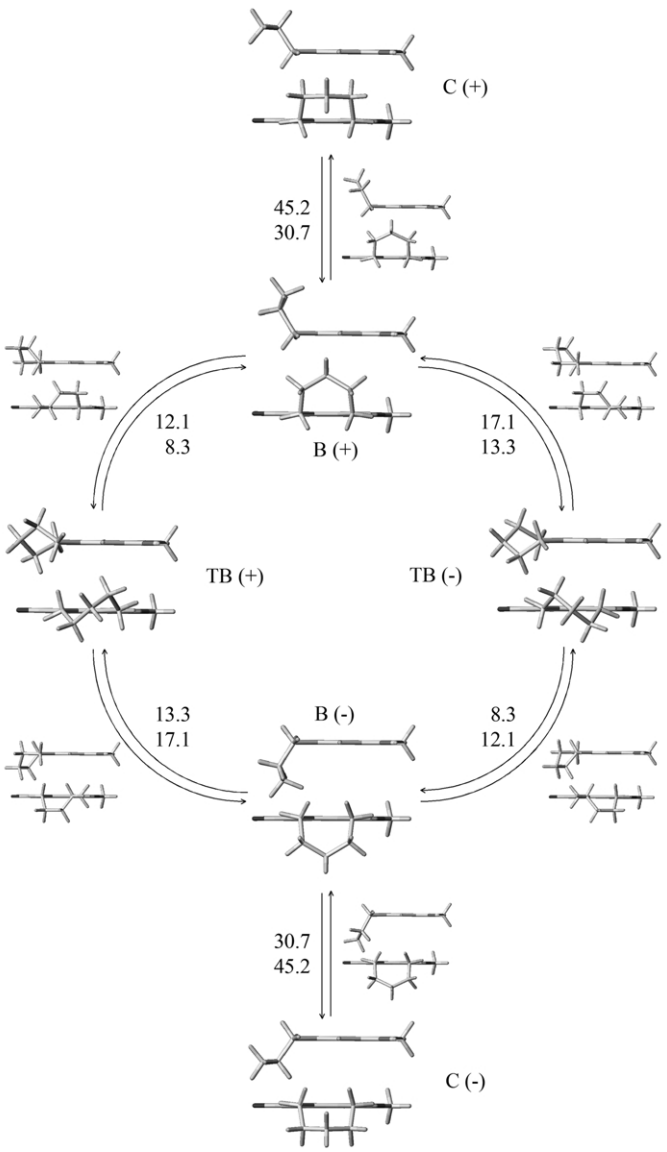


Figure 3. Cycloheptene ring inversion pathway for compound **5** modeled at the HF/6-31G* level of theory. Interconversion steps are labeled with the corresponding energies of activation ($\Delta E^{\#}$ [kJ/mol]); upper numbers: top-to-bottom process, lower numbers: bottom-to-top process.

the experimentally-observed ring inversion behavior of **3**–**6**. Contrary to the inversion pathway in unsubstituted cycloheptenes, here the twist-boat and boat conformations assume a determinant role during the ring interconversion process.

Further quantum chemical calculations were then made to determine the activation energies for the ring interconversion process in **3**–**6** for the relevant conformational processes (chair-boat, boat-twist-boat), and these are presented in Table 2. Since the calculated energies of activation for the chair-boat interconversion processes compare well with the experimental values, it can thus be construed that they allude to the inversion process that is in effect. However, as depicted in Figure 3, the chair⥋chair interconversion evolves via the sequence chair⥋boat⥋twist-boat⥋boat⥋chair. Starting with the chair(+) conformation (in which the carbons 5–9 are above the aromatic ring plane), it first converts to the boat(+) conformation (where the carbons in positions 6–8 remain above the aromatic ring plane). From there, two available pathways deviate to yield two different twist-boat conformations, TB(+) and TB(−), which differ in the positions of carbons 6 and 8 with respect to the aromatic ring plane. For twist-boat(+), C-6 is below, and C-8 is above the plane; for twist-boat(−), these positions are reversed. Both twist-boat conformations, however, can transform into the boat(−) conformation, which is then able to pass through to the chair(−) conformation. The latter conformations are both characterized by C-6, C-7, and C-8 lying below the plane of the aromatic ring.

Importantly, the two boat-twist-boat interconversion pathways differ in activation energy by 5 kJ/mol. This difference can be attributed to an eclipsed interaction between one of the hydrogens on C-5 and the nitrile group in the boat(+)–twist-boat(−) and boat(−)–twist-boat(+) transition state conformations, whereas it is staggered in the boat(+)–twist-boat(+) and boat(−)–twist-boat(−) transition states (cf. Fig. 4).

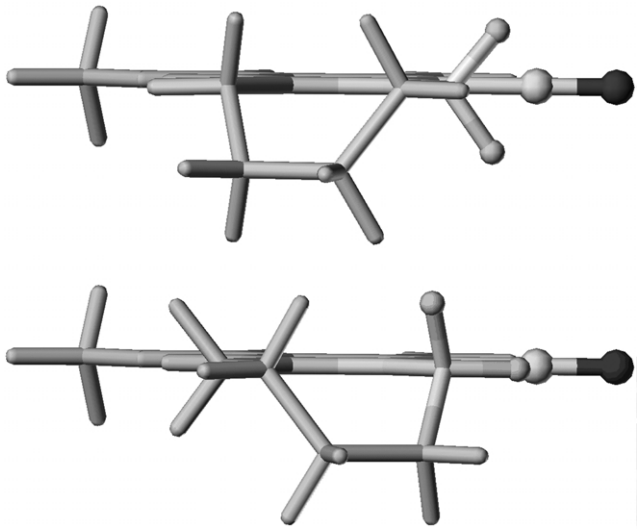


Figure 4. Transition state conformations for the boat(+)–twist-boat(+) (top) and boat(+)–twist-boat(−) (bottom) interconversion processes.

Thus, in going from chair(+)⟶chair(−) a different, but degenerate, route is traversed then for the reverse inversion, chair(−)⟶chair(+).

3. Experimental

3.1. General remarks

IR spectra were recorded on a FTIR 16 PC (Perkin–Elmer). Mass spectra were recorded on an SSQ 710 mass spectrometer (Finnigan MAT) with EI (70 eV). NMR spectra were recorded on an ARX 300 (Bruker) equipped with a VT 1000 temperature control unit using $CDCl_3$ as solvent and TMS (δ=0 ppm) as an internal reference for ^{1}H NMR. ^{13}C NMR spectra were referenced internally to $CDCl_3$ (δ=77 ppm). For dynamic NMR investigations, samples were measured as ca. 0.1 M solutions in CD_2Cl_2. These solutions were prepared anaerobically by five repetitions of a freeze–evacuate–thaw cycle followed by sealing of the tube. The temperatures were taken from a calibration curve determined with a sample of 4% MeOH in CD_3OD.

Quantum chemical calculations were carried out using the ab initio program package Gaussian 98 (Revision A.11.3)[13] on a 32 processor SGI Origin 2000 or a 30 processor Intel Pentium 4 Linux cluster. Geometry optimization was done subsequently at HF/3-21G and HF/6-31G* levels of theory. All energies were calculated without thermodynamic correction. The solvent effect (CH_2Cl_2) was considered using the Self-Consistent Isodensity Polarized Continuum Model (SCIPCM).[14] Results were analyzed and visualized within the molecular modeling package SYBYL 6.9.[15] Molecular dynamics (MD) simulations of single molecules were performed with SYBYL using the Tripos force field[15] at temperatures of 600, 800, and 1000 K. Each MD run consisted of a 100 ps heating period and 2000 ps of data sampling. The step size was 1 fs; snapshots of the trajectory were taken every 50 fs. All bonds to hydrogen atoms were constrained using the SHAKE algorithm.[16]

3.2. Compounds I–IV

The cycloalkylidene malonic acid derivatives **I**,[17] **II**,[18] and **III**[18] were synthesized according to literature. Compound **IV** was synthesized analogously to **II** and **III**.

3.2.1. (3-Methylcyclopent-2-en-1-ylidene)malononitrile (IV). 42.5 g, yield 59%. Mp 88 °C. IR: $\tilde{\nu}$=2222, 1596, 1566 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.18 (s, 3H, CH_3), 2.78 (m, 2H, CH_2), 3.01 (m, 2H, CH_2), 6.56 (s, 1H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ=18.6, 32.4, 37.6, 70.1 [$(CN)_2C$=], 112.7 (CN), 113.2 (CN), 128.1 (CH_3–C=*C*), 176.7 (CH_3*C*=), 185.8 1 [$(CN)_2$C=*C*]. MS (EI): *m*/*z* (%)=144 (62) [M^+], 78 (100). Elemental analysis (%) for $C_9H_8N_2$: Calcd C 74.98, H 5.59, N 19.43; found C 74.75, H 5.64, N 19.52.

3.3. General synthetic procedure for compounds 1–9

Potassium carbonate (0.01 mol) was added to a stirred and cooled (ice-water) methanol solution (25 mL) of the cycloalkylidene malonic acid derivative (0.01 mol) and acetylene dicarboxylic acid ester or ethyl acetylene carboxylate (0.01 mol in either case). The mixture was gradually heated and then refluxed for 30 min. After cooling, the mixture was poured onto ice and left standing overnight. After filtering off and then drying the product, recrystallization was performed using appropriately either methanol or ethanol according to the corresponding alcohol of the ester(s).

3.3.1. Dimethyl 6-amino-7-cyanoindane-4,5-dicarboxylate (1) from I and dimethyl acetylenedicarboxylate (DMAD). 2.14 g, yield 78%. Mp 154–157 °C. IR: $\tilde{\nu}$= 3460, 3354, 2218, 1734, 1702 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ=2.12 (m, 2H, CH_2), 2.82 (t, 2H, CH_2), 3.02 (t, 2H, CH_2), 3.80 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 6.30 (s, 2H, NH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ=22.8, 30.8, 33.5, 52.4, 52.5, 96.0, 107.5, 115.4 (CN), 130.5, 136.1, 150.6, 155.4, 166.6, 168.4. MS (EI): *m*/*z* (%)=274 (38) [M^+], 156 (100). Elemental analysis (%) for $C_{14}H_{14}N_2O_4$: Calcd C 61.31, H 5.14, N 10.21; found C 61.11, H 5.31, N 9.98.

3.3.2. Diethyl 6-amino-7-cyanoindane-4,5-dicarboxylate (2) from I and diethyl acetylenedicarboxylate (DEAD). 1.96 g, yield 65%. Mp 80–82 °C. IR: $\tilde{\nu}$=3457, 3349, 2220, 1736, 1702 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.32 (t, 3H, CH_3), 1.37 (t, 3H, CH_3), 2.12 (m, 2H, CH_2), 2.83 (t, 2H, CH_2), 3.04 (t, 2H, CH_2), 4.36 (2×q, 4H, 2×CH_2), 6.30 (s, 2H, NH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ=13.9, 14.1, 24.3, 30.8, 33.4, 61.4, 61.5, 95.8, 107.6, 115.5 (CN), 130.3, 136.4, 150.7, 155.1, 166.3, 168.0. MS (EI): *m*/*z* (%)=302 (28) [M^+], 227 (100). Elemental analysis (%) for $C_{16}H_{18}N_2O_4$: Calcd C 63.56, H 6.00, N 9.26; found C 63.89, H 5.79, N 9.53.

3.3.3. Dimethyl 3-amino-4-cyano-6,7,8,9-tetrahydro-5*H*-benzo[7]annulene-1,2-dicarboxylate (3) from II and DMAD. 1.15 g, yield 38%. Mp 149–152 °C. IR: $\tilde{\nu}$=3444, 3346, 2220, 1728, 1692 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ=1.68 (m, 6H, 3×CH_2), 2.56 (t, 2H, CH_2), 3.03 (t, 2H, CH_2), 3.85 (s, 3H, CH_3), 3.88 (s, 3H, CH_3), 6.41 (s, 2H, NH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ=26.8, 27.3, 31.0, 31.4, 34.4, 52.3, 52.4, 99.6, 106.1, 116.0 (CN), 128.5, 139.2,

150.0, 154.3, 166.2, 169.3. MS (EI): *m/z* (%)=302 (58) [$M^{+\cdot}$], 184 (100). Elemental analysis (%) for $C_{16}H_{18}N_2O_4$: Calcd C 63.56, H 6.00, N 9.26; found C 63.57, H 6.26, N 8.97.

3.3.4. Diethyl 3-amino-4-cyano-6,7,8,9-tetrahydro-5*H*-benzo[7]annulene-1,2-dicarboxylate (4) from II and DEAD. 1.12 g, yield 34%. Mp 102 °C. IR: $\tilde{\nu}$=3452, 3346, 2220, 1736, 1702 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ=1.35 (t, 3H, CH_3), 1.37 (t, 3H, CH_3), 2.48 (m, 6H, 3×CH_2), 2.61 (t, 2H, CH_2), 2.97 (t, 2H, CH_2), 4.34 (2×q, 4H, 2×CH_2), 6.40 (s, 2H, NH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ=13.5, 13.8, 25.3, 26.8, 30.8, 31.8, 34.5, 61.3, 61.5, 94.1, 108.3, 116.2 (CN), 130.6, 136.9, 151.5, 156.7, 167.1, 168.3. MS (EI): *m/z* (%)=330 (40) [$M^{+\cdot}$], 198 (100). Elemental analysis (%) for $C_{18}H_{22}N_2O_4$: Calcd C 65.43, H 6.71, N 8.48; found C 65.32, H 6.97, N 8.88.

3.3.5. Methyl 3-amino-4-cyano-6,7,8,9-tetrahydro-5*H*-benzo[7]annulene-2-carboxylate (5) from II and ethyl acetylenecarboxylate, recrystallized from MeOH. 1.00 g, yield 41%. Mp 135–137 °C. IR: $\tilde{\nu}$=3438, 3336, 2212, 1692 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ=1.67 (m, 6H, 3×CH_2), 2.65 (t, 2H, CH_2), 2.90 (t, 2H, CH_2), 3.83 (s, 3H, CH_3), 6.40 (s, 2H, NH_2), 7.74 (s, 1H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ=27.1, 28.2, 32.0, 34.5, 35.3, 51.7, 98.3, 108.3, 116.6 (CN), 131.3, 135.6, 150.7, 153.7, 167.4. MS (EI): *m/z* (%)=244 (100) [$M^{+\cdot}$]. Elemental analysis (%) for $C_{14}H_{16}N_2O_2$: Calcd C 68.83, H 6.61, N 11.46; found C 68.73, H 6.63, N 11.55.

3.3.6. Ethyl 3-amino-4-cyano-6,7,8,9-tetrahydro-5*H*-benzo[7]annulene-2-carboxylate (6) from II and ethyl acetylenecarboxylate. 1.08 g, yield 42%. Mp 129–131 °C. IR: $\tilde{\nu}$=3448, 3340, 2212, 1684 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ=1.38 (t, 3H, CH_3), 1.67 (m, 6H, 3×CH_2), 2.71 (t, 2H, CH_2), 3.01 (t, 2H, CH_2), 4.34 (q, 2H, CH_2), 6.43 (s, 2H, NH_2), 7.79 (s, 1H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ=14.3, 27.2, 28.2, 32.1, 34.6, 35.3, 60.7, 98.4, 108.7, 116.7 (CN), 131.3, 135.6, 150.7, 153.6, 167.1. MS (EI): *m/z* (%)=258 (100) [$M^{+\cdot}$]. Elemental analysis (%) for $C_{15}H_{18}N_2O_2$: Calcd C 69.74, H 7.02, N 10.84; found C 70.04, H 7.03, N 11.05.

3.3.7. Dimethyl 3-amino-4-cyano-5,6,7,8,9,10-hexahydrobenzo[8]annulene-1,2-dicarboxylate (7) from III and DMAD. 0.25 g, yield 8%. Mp 116–117 °C. IR: $\tilde{\nu}$=3485, 3352, 2212, 1703 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ=1.36 (m, 8H, 4×CH_2), 2.60 (t, 2H, CH_2), 2.98 (t, 2H, CH_2), 3.86 (s, 3H, CH_3), 3.89 (s, 3H, CH_3), 6.38 (s, 2H, NH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ=25.7, 26.0, 28.4, 30.2, 31.0, 31.3, 52.3, 52.5, 99.6, 107.3, 116.1 (CN), 126.6, 140.0, 150.3, 152.5, 166.4, 169.2. MS (EI): *m/z* (%)=316 (100) [$M^{+\cdot}$]. Elemental analysis (%) for $C_{17}H_{20}N_2O_4$: Calcd C 64.54, H 6.37, N 8.85; found: C 64.77, H 6.19, N 8.84.

3.3.8. Ethyl 3-amino-4-cyano-5,6,7,8,9,10-hexahydrobenzo[8]annulene-2-carboxylate (8) from III and ethyl acetylenecarboxylate. 0.49 g, yield 18%. Mp 98 °C. IR: $\tilde{\nu}$=3486, 3354, 2212, 1702 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ=1.34 (t, 3H, CH_3), 1.44 (m, 8H, 4×CH_2), 2.65 (t, 2H, CH_2), 2.87 (t, 2H, CH_2), 4.12 (q, 2H, CH_2), 6.93 (s, 2H, NH_2), 7.79 (s, 1H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ=13.7, 25.3, 25.7, 29.9, 31.6, 32.5, 35.1, 61.8, 97.3, 108.5, 116.3 (CN), 129.0, 136.3, 151.6, 151.9, 166.7. MS (EI): *m/z* (%)=272 (100) [$M^{+\cdot}$]. Elemental analysis (%) for $C_{16}H_{20}N_2O_2$: Calcd C 70.56, H 7.40, N 10.28; found: C 70.21, H 7.21, N 10.37.

3.3.9. Dimethyl 5-amino-4-cyano-2-methyl-1*H*-indene-6,7-dicarboxylate (9) from IV and DMAD. 0.94 g, yield 33%. Mp 168–170 °C. IR: $\tilde{\nu}$=3456, 3346, 2216, 1734, 1686 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ=2.21 (s, 3H, CH_3), 3.33 (s, 2H, CH_2), 3.85 (s, 3H, CH_3), 3.90 (s, 3H, CH_3), 6.35 (s, 2H, NH_2), 6.62 (s, 1H, CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ=17.2, 41.9 (CH_2), 52.2, 52.5, 90.4, 104.1, 115.6 (CN), 125.5 (H*C*=C), 128.9, 133.9 (HC=*C*)151.1, 154.6, 157.8, 167.1, 168.4. MS (EI): *m/z* (%)=286 (100) [$M^{+\cdot}$]. Elemental analysis (%) for $C_{15}H_{14}N_2O_4$: Calcd C 62.39, H 4.93, N 9.78; found: C 63.13, H 5.17, N 9.61.

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Racemization in stepwise solid-phase peptide synthesis at elevated temperatures

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Abstract—The present work is an attempt to assess racemization in stepwise solid-phase peptide synthesis at elevated temperatures (SPPS-ET), a high-speed approach in which peptide elongation occurs at 55–75 °C. This attempt was based on the notion that a high propensity for this side reaction would hamper employment of this alternative approach and would dampen interest in its further development. Simple peptide models were synthesized using customized protocols for classical SPPS or SPPS-ET. Systematic analyses of the resulting crude peptides by reversed-phase HPLC, ion-exchange HPLC, capillary electrophoresis and electrospray ionization mass spectrometry revealed low diastereomeric byproduct contents. These results indicate that, from the standpoint of racemization, classical SPPS and SPPS-ET protocols were equivalent. Therefore, further studies employing SPPS-ET protocols are justified.

1. Introduction

Due to the remarkable developments witnessed in molecular biology and gene cloning techniques over the last two decades, many in the scientific community had expected that the traditional approaches to peptide chemical synthesis would be used less frequently. However, owing to a variety of factors, this is not likely to happen. The chemical approach allows peptide building from any L- or D-usual or unusual amino acid derivative. This is fully applicable in the synthesis of short- and medium-length modified peptides (amidated, acetylated, branched, partially or fully cyclized, esterified, phosphorylated, alkylated or sulphated). In addition, these modified peptides are required in several biochemical investigations, especially in the exploration of relationship between peptide structure and peptide activity and in the search for new peptidomimetic drugs.[1,2]

In the 1960s, R. B. Merrifield, aiming to accelerate and simplify peptide elongation, introduced stepwise solid-phase peptide synthesis (SPPS).[3] This technique rapidly became the method of choice for the preparation of peptides. Since a synthetic approach such as this allows excess reagents to be removed by filtration without manipulative loss, it has been widely employed for individual or multiple peptide synthesis, as well as in the construction of peptide libraries.[4,5]

Each step of the SPPS process—washing, amino acid coupling (acylation), deprotection, neutralization and acetylation—is typically performed at room temperature. However, peptide chemists have often carried out the coupling step (incorporation of N^{α}-acylated amino acids into growing peptide resins) at higher temperatures. Indeed, in 1986, Tam performed a difficult coupling at 50 °C while constructing the transforming growth factor α on a solid support.[6] Barlos et al. observed that *N*-tritylamino acid benzotriazolyl esters were resistant to racemization during SPPS couplings at 30–80 °C.[7] Three years later, Lloyd et al. attained significant coupling improvement at 60 °C during SPPS of either GFFYCNTTQFFNN or fragment (1–28) of rat anti-natriuretic factor.[8] In the 1990s, many authors performed difficult couplings during SPPS by simply raising reaction temperatures.[9–11]

Conversely, the first attempt to carry out all SPPS cycles at an elevated temperature (herein referred to as SPPS-ET) was not reported until 1994. The authors, Rabinovich and Rivier, used porcine neuropeptide Y (NPY, 36 amino acids) and rat corticotropin releasing factor (CRF, 41 amino acids) sequences as models to propose modifications to conventional SPPS protocols in order to reduce the time required for peptide building.[12] Since they obtained crude peptides of good quality, we performed a systematic study of SPPS-ET over the following years.[13] We examined significant aspects

Keywords: Peptide; Solid-phase synthesis; Elevated temperature; Racemization.
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such as temperature, solvent system, resin chemical stability and swelling properties, coupling agent efficiency and peptide aggregation, as well as quality versus recovery in the crude peptides obtained. The data collected allowed us to propose optimal conditions for manual SPPS-ET of fragment 65–74 of acyl carrier protein [$ACP_{(65-74)}$], a typical aggregating nonapeptide, as well as of unsulphated cholecystokinin-8 (CCK-8) and unsulphated CCK-12 (an octapeptide and a dodecapeptide, both containing troublesome sequences). Two years ago, these successful syntheses and the promising data collected by Rabinovich and Rivier and by our group were favorably reviewed by Rivier and Miranda.[14] Despite this, SPPS-ET has not been used on a routine basis. Many allege that various aspects of the technique have not been sufficiently studied.

Racemization has been a major concern for chemists building biologically active peptides in solution. This undesired reaction is inherent to the chemical method of peptide bond formation and occurs due to creation of an anionic center in the α-carbon atom of activated amino acids in the presence of organic bases.[15,16] In conventional SPPS, the incorporation of most proteinogenic amino acids into the growing peptide chain is achieved with only negligible levels of racemization. This is due to the combined use of urethane-type blocking groups and auxiliary nucleophiles, as well as activating reagents or specific solvents.[15,17–19] On the other hand, it is recognized that protocol modifications may increase the incidence and intensity of this side reaction.

This study is part of our continuing evaluation of SPPS at 60 °C (SPPS-ET). Our aim was to verify whether this modified approach is more susceptible to racemization than is conventional SPPS. Our interest evolved from the notion that, if present, such an aspect would either hamper the use of this alternative synthesis approach or dampen interest in its development. Therefore, manual syntheses of three simple models were carried out using customized protocols for classical SPPS or SPPS-ET. The resulting crude peptides were systematically compared. This is not only the first attempt to measure racemization in SPPS-ET but also an additional demonstration of the benefits that the complementary use of high performance liquid chromatography (HPLC), capillary electrophoresis (CE) and mass spectroscopy (MS) can offer to the field of peptide synthesis.

2. Results and discussion

Figure 1 summarizes the strategy employed in carrying out this investigation. The determination of the peptide recoveries from the resins allowed the comparison between the two synthetic approaches employed from the standpoint of yield. Synthetic isomers helped to identify and estimate the racemized byproducts present in the crude peptides. The selection of simple peptide models (one dipeptide, one tripeptide and one tetrapeptide) was based on the low number of possible optical isomers produced during the building of these peptides, as well as on some simple models previously employed.[16,20–23] In fact, if larger and more complex peptides, such as those previously synthesized by Rabinovich and Rivier and by Varanda and Miranda,[12–14] had been selected, it would have been difficult to separate, identify and quantify the optical isomers contained in the crude peptides. Such complications could even hinder the evaluation of SPPS-ET.

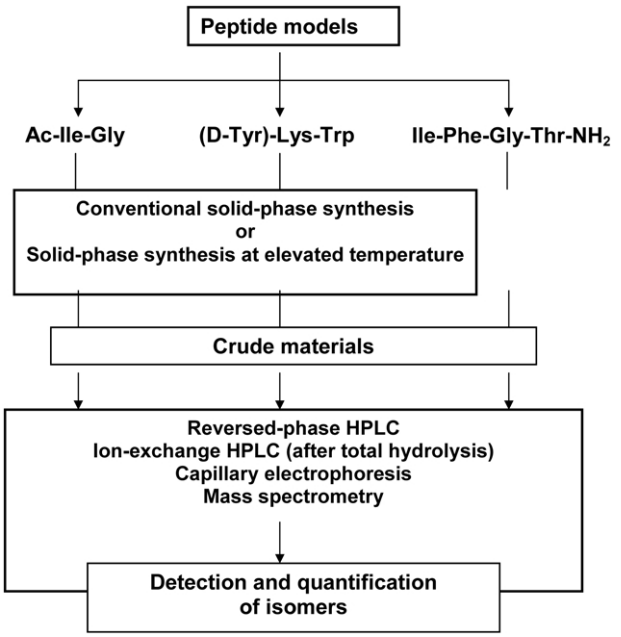

Figure 1. Schematic representation of the systematic study performed.

The peptide Ac-Ile-Gly was selected based on the work of Bodanszky and Conklin, who examined the effect of tertiary amines on racemization incidence during Ac-Ile coupling to Gly-OEt(no:)[22] for quantification of the resulting diastereoisomer (2*R*,3*S*) of L-Ile (2*S*,3*S*), also called D-allo-Ile, the authors employed the Spackman–Stein–Moore method.[24–26]

The choice of (D-Tyr)-Lys-Trp was inspired by a study by Riester and coworkers, who measured racemization in conventional SPPS with the help of CE.[23] The authors performed a systematic analysis using the chosen tripeptide and its seven stereoisomers. Despite persistent LDL and DDL co-elution, they determined that, under the experimental conditions employed for conventional SPPS, the isomer formation rate was only 0.4% per synthesis cycle.

The peptide Ile-Phe-Thr-NH_2 was designed because: (i) when activated under basic conditions, *N*α-acyl-Phe, *N*α-acyl-Ile and *N*α-acyl-Thr tend to racemize; (ii) the incorporation of hindered amino acids such as Ile and Thr to a growing peptide is usually a slow process, allowing their activated forms in solution to have a relatively long life; (iii) the presence of Boc-(D-allo-Ile) in a peptidyl resin is easily confirmed through total hydrolysis followed by IEX-HPLC of the resulting hydrolysate;[26] (iv) the presence of aromatic groups in a peptide enhances its ability to absorb UV light; (v) the simultaneous presence of two hydrophobic amino acids (Phe and Ile) and two hydrophilic amino acids (Gly and Thr) may lead to a tetrapeptide that can be retained in a reversed-phase HPLC column; (vi) the protonation of a free amino group in Ile-Phe-Gly-Thr-NH_2 at low pH permits CE analysis.

The syntheses of these simplistic peptide models were also

considered to be representative of classical SPPS and SPPS-ET of larger sequences and, as such, sufficient for the comparison of these approaches regarding racemization. Indeed, the syntheses performed included different chemical strategies and resins, diverse acylating reagents (*N*α-acyl-amino acids), varying numbers of SPPS cycles and a few potential racemization points. For instance, the synthesis of Ile-Phe-Gly-Thr-NH_2 starting from MBHA[27] consists of four *N*α-amino group deprotections, four *N*α-amino group neutralizations, four acylations (by activated Boc-Ile, Boc-Phe, Boc-Gly and Boc-Thr) and washes corresponding to each step. There are three potential racemization points.

The SPPS-ET procedure was carried out at 60 °C. This was based on our previous syntheses of $ACP_{(65-74)}$ and unsulfated CCK fragments.[13] The choice of 25% DMSO/toluene as solvent mixture was based on the work of Rabinovich and Rivier, as well as on our own previous study.[12–14] Using this synthesis approach, either DIC/HOBt or TBTU/DIPEA, both typical amino acid activating reagents used in classical SPPS,[18] was employed in the syntheses. In order to let racemization occur freely during the coupling reactions, HOBt was not used in SPPS-ET. The crude materials were obtained after final cleavage/full deprotection, allowing for calculation of peptide recoveries from the resins.

The ability of IEX-HPLC, RP-HPLC and CE to effectively separate amino acids, peptides and their derivatives[4,20–22,28,29] is well known. Coupling these techniques with mass spectrometry (LC–MS) has been beneficial in the analysis of detection, quantification, separation, purification and characterization of biologically active peptides.[30–32] Therefore, in order to assess the qualities of the crude peptides obtained, IEX-HPLC, RP-HPLC and CE were all employed. In addition, MS was used to characterize the purified products and standards employed as well as the crude di- and tetrapeptides.

2.1. Ac-Ile-Gly model

The experimental conditions under which Boc-Ile on Gly-phenyl-acetamidomethyl-resin (PAM) and Fmoc-Ile on Gly-*p*-alkoxybenzyl alcohol resin[27] (by Boc or Fmoc strategies,[17] respectively) were coupled are summarized in Table 1. The acylation reactions occurred at high apparent pH (~9.0) in the presence of various coupling reagents.

Table 1. Syntheses of Ac-Ile-Gly

Coupling	SPPS type	Solvent	Coupling reagent	Coupling time (min)	Strategy
1	Conventional	DMF	TBTU	90	*t*-Boc
2	ET	25%DMSO/toluene	TBTU	30	*t*-Boc
3	Conventional	DCM	DIC	90	*t*-Boc
4	ET	25%DMSO/toluene	DIC	30	*t*-Boc
5	ET	NMP	DIC	30	*t*-Boc
6	ET	DMF	DIC	30	*t*-Boc
7	Conventional	DMF	DIC	90	Fmoc
8	ET	25%DMSO/toluene	DIC	30	Fmoc
9	ET	NMP	DIC	30	Fmoc
10	ET	DMF	DIC	30	Fmoc

Conventional—room temperature; ET—elevated temperature (60 °C).

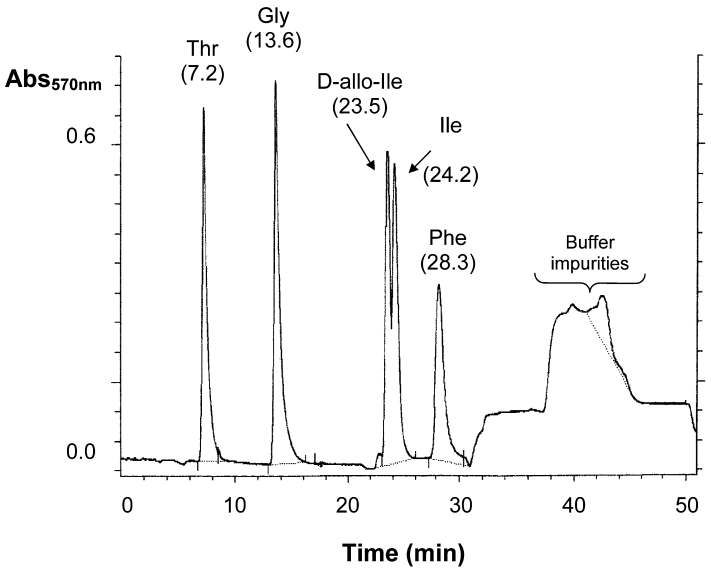

Figure 2. Aminogram of a standard mixture of Thr, Gly, D-allo-Ile, Ile and Phe. Analytical conditions: sodium citrate buffers Na-E™, Na-F™ and Na-D™, a sodium high performance column of 12 cm for amino acid analysis from Beckman Instr. Inc., flow rate of 14 ml/h, complete run cycle of 60 min and detection at wavelengths of 440 and 570 nm.

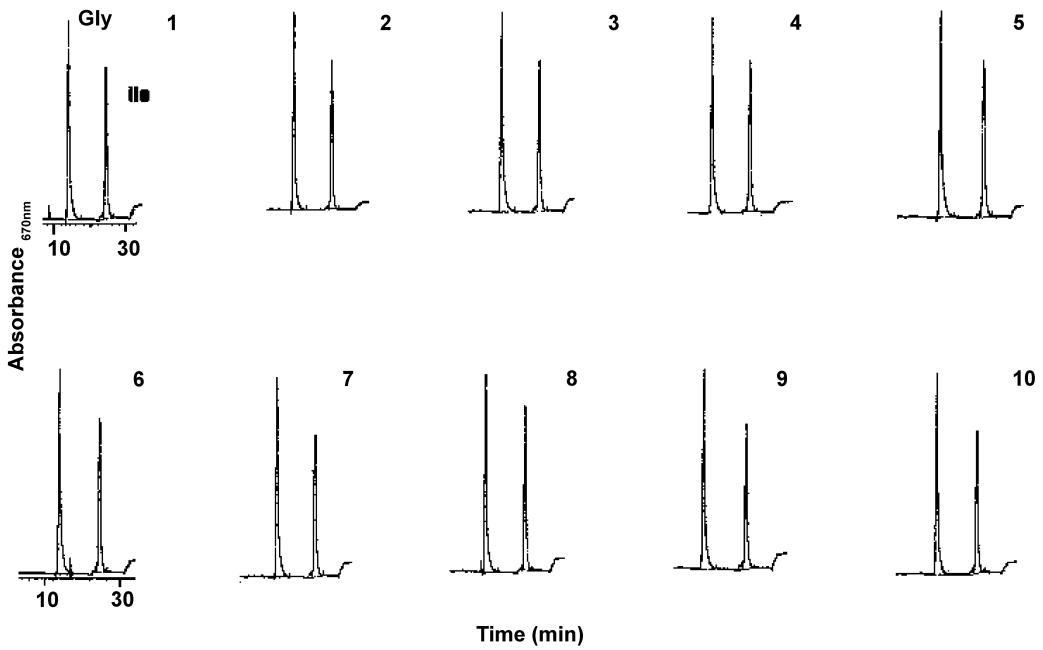

Figure 3. Aminograms of the fully hydrolyzed Ac-Ile-Gly resulting from reactions 1–10 described in Table 1. Analytical conditions: those described in Figure 2.

Having obtained the crude Ac-Ile-Gly from final cleavage/full deprotection, peptide recovery was determined. All values were approximately 0.12, meaning that the synthesis yields were similar. Purification by RP-HPLC followed by mass spectrometry analysis confirmed that the reactions produced the desired dipeptide. As expected, the RP-HPLC analyses of the intact crude peptides revealed that their qualities were very similar (data not shown). Taken together, these results indicated that, in terms of yield and product quality, our customized protocols for classical SPPS and SPPS-ET were comparable, regardless of the chemical strategy, coupling reagent or synthesis approach employed.

Total hydrolysis of the crude Ac-Ile-Gly allowed for IEX-HPLC analysis. For this purpose, the automated amino acid analyzer used was pre-calibrated with a mixture containing 5 nmol of D-allo-Ile and a few other relevant L-amino acids (Thr, Gly, Ile, and Phe). As can be seen in Figure 2, even though Ile and D-allo-Ile are not baseline resolved, an estimated resolution of 0.8–1.0 was achieved. According to Snyder et al., a resolution of 0.8 indicates that both isomers can be collected within 95% purity.[33] With that degree of resolution, if D-allo-Ile were present in the hydrolysates analyzed under the same experimental conditions as those depicted in Figure 2, a second Ile-adjacent peak would have been clearly seen in Figure 3. These results confirm that, regardless of the coupling reagent or chemical strategy used, neither the protocols for conventional SPPS nor those for SPPS-ET promoted substantial formation of the racemized byproducts.

2.2. (D-Tyr)-Lys-Trp model

The crude tripeptides resulting from conventional SPPS and from SPPS-ET on Boc-aminoacyl-PAM[27] again presented very similar peptide recovery (0.33 and 0.28, respectively). Purification of the major components using RP-HPLC followed by mass spectrometry analysis of the purified

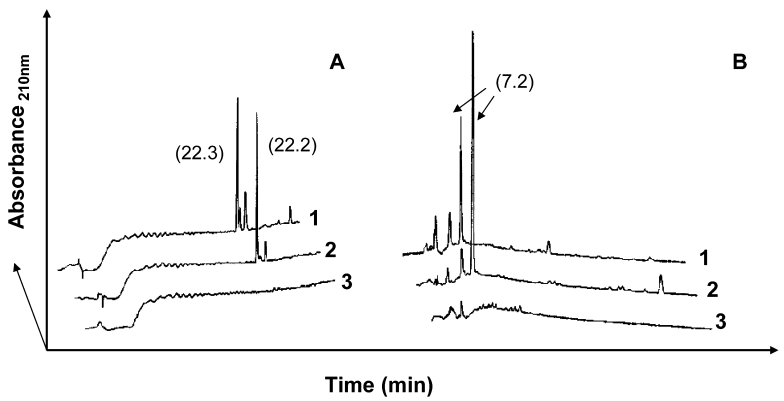

Figure 4. TFA (A) and TEAP (B) RP-HPLC profiles of the crude (D-Tyr)-Lys-Trp resulting from SPPS-ET (1) or from conventional SPPS (2). 3A and 3B are controls. Analytical conditions. For A: solvent 1=0.1% TFA/H_2O, solvent 2=30% MeCN/H_2O 0.09%TFA, linear gradient=5 to 95% B in 30 min. For B: solvent 1=TEAP, solvent 2=30% MeCN/TEAP, gradient=23 to 95% B in 35 min. For both: Vydac C_{18} column (0.4×25.0 cm, 5 μm, 300 Å), flow rate of 1.0 ml/min, wavelength of 210 nm, injected amount of 0.32–0.35 μg.

peptides confirmed that the syntheses were successful. RP-HPLC analyses showed that they were qualitatively different (Fig. 4).

Indeed, CE analyses under experimental conditions established by Riester et al.[23] revealed that the desired DLL product was less abundant in the crude peptide resulting from SPPS-ET (data not shown). This observation led us to question whether or not the contaminants seen (Figs. 4B and 5A) were isomers formed during the acylation of L-Trp-PAM or Lys(2-Cl-Z)-L-Trp-PAM. Therefore, we synthesized the isomers LLL and LDL with conventional SPPS using the Boc strategy and experimental conditions described as appropriate for drastic minimization of racemization (data not shown). Only these two compounds were used as standards because (D-Tyr)-Lys-Trp was built up from a Boc-L-Trp-PAM resin (therefore DLD, DDD, LDD and LLD would not be formed during the peptide elongation) and, under the analytical conditions employed by Riester et al., LDL and DDL isomers were expected to exhibit identical electrophoretic behavior.[23]

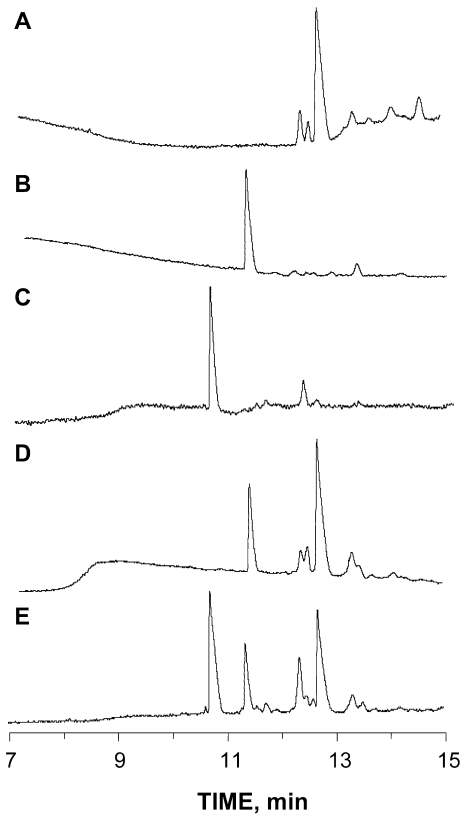

Figure 5. Electropherograms of the crude (D-Tyr)-Lys-Trp resulting from SPPS-ET (A), the standard Tyr-(D-Lys)-Trp (B), the standard Tyr-Lys-Trp (C), the mixture of A and B (D), the mixture of A, B and C (E). Analytical conditions: voltage: 30 kV, buffer: 30 mM Tris plus 10 mM (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid adjusted to pH 2 with citric acid, capillary length: 72 cm (50 cm to detector), capillary diameter: 50 μm, temperature: 20 °C, detection: 210 nm.

Using LLL and LDL isomers and the crude tripeptide resulting from SPPS-ET, CE spiking experiments were performed. As Figure 5 clearly shows, the contaminants did not co-elute with the standards, suggesting that they must result either from acylation of the resin by Boc-L-Trp or from secondary reactions, distinct from racemization, occurring during peptide elongation or cleavage from the resin/full deprotection.

In fact, the chromatograms and spectra obtained from LC/ESI-MS analysis of the crude tripeptides confirmed that the major impurities seen in the SPPS-ET product (Figs. 4B and 5A) are not related to racemization. Instead, they refer to Tyr-Tyr-Lys-Trp, Tyr-Lys-Trp(But), Tyr(Bzl)-Lys(2-Cl-Z)-Trp and Tyr(Bzl)-Lys(2-Cl-Z)-Trp(Bzl). Only trace isomers probably containing D-Trp were detected.

2.3. Ile-Phe-Gly-Thr-NH_2 model

Regarding Ac-Ile-Gly and the tripeptide reported above, the tetrapeptide was synthesized using SPPS-ET or conventional SPPS protocols. An MBHA resin[27] and the *t*-Boc strategy were used.[17] Again, peptide recovery from the solid support were comparable: 0.36 and 0.37 for SPPS-ET and conventional SPPS, respectively. Similar and quite clean profiles were obtained from comparative analysis of the crude tetrapeptides with RP-HPLC using two different solvent systems (data not shown). These results strongly suggested a lack or low percentage of contaminants in the desired LLGlyL product.

Despite this observation, both crude tetrapeptides were chromatographically compared with those of the LDGlyL, DLGlyL and DDGlyL isomers. Again, the standards were pre-prepared using conventional SPPS under experimental conditions in which racemization is drastically minimized (data not shown). In order to prove that the crude Ile-Phe-Gly-Tyr-NH_2 resulting from SPPS-ET (Fig. 6A) was not, or irrelevantly, contaminated by the standard isomers, RP-HPLC spiking experiments were conducted. Similar results were obtained for the crude LLGlyL tetrapeptide resulting from conventional SPPS (data not shown).

The standard tetrapeptides LLGlyD, LDGlyD, DDGlyD and DLGlyD, all containing the L-Thr diastereoisomer (2*R*,3*R*), also known as D-allo-Thr, were not synthesized. This decision was made because no commercial source of the corresponding *t*-Boc-derivative was found, and its preparation from D-allo-Ile is quite expensive. Instead, the crude materials were fully hydrolyzed and the resulting hydrolysates were analyzed using IEX-HPLC followed by amino-acid post-column derivatization with ninhydrin.[24–26]

The aminograms shown in Figures 2 and 7 indicated that, if racemization occurred during the incorporation of Boc-Ile into H-Phe-Gly-Thr(Bzl)-MBHA, the percentage was very low (the detection limit of the automatic analyzer is 1.0×10^{-10} mol). Indeed, as previously stated, significant amounts of D-allo-Ile in the hydrolysates, analyzed under the experimental conditions employed to obtain Figure 2, would have resulted in a second Ile-adjacent peak (Fig. 7). The lack of such a peak led us to rule out significant contamination by DDGlyD and DLGlyD (as well as DLGlyL and DDGlyL) isomers.

Since the L-Thr and D-allo-Thr standards co-eluted under the experimental conditions described in Figure 2, similar conclusions could not be made regarding LLGlyD and LDGlyD contamination. In light of this, the crude peptides

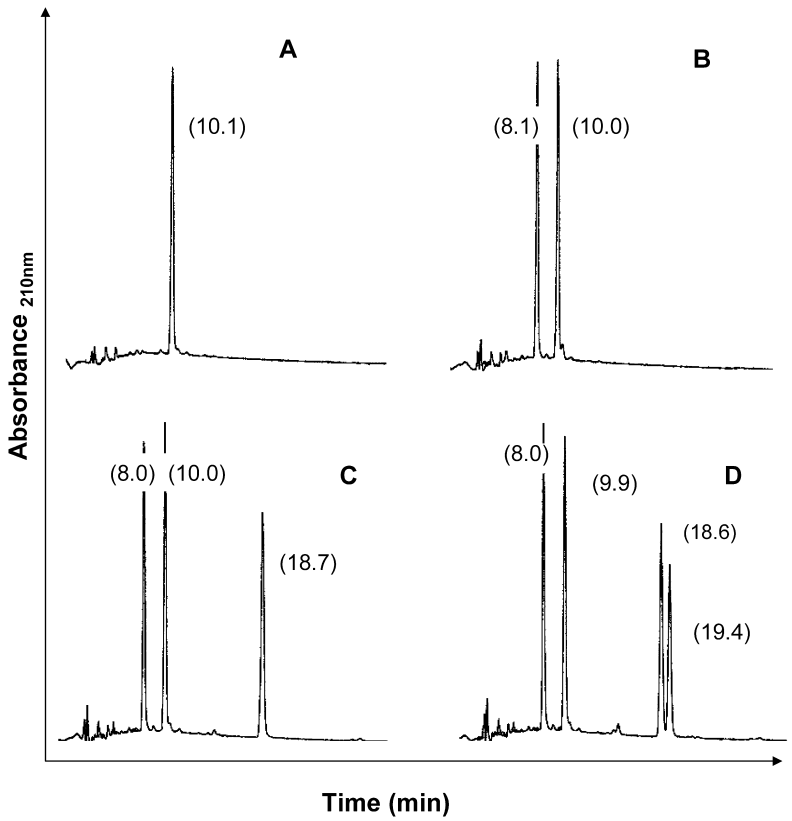

Figure 6. RP-HPLC profiles of the crude Ile-Phe-Gly-Thr-NH_2 resulting from SPPS-ET (A) spiked by some stereoisomers (B–D). B:A (1) and (D-allo-Ile)-(D-Phe)-Gly-Thr-NH_2 (2), C: 1, 2 and (D-allo-Ile)-Phe-Gly-Thr-NH_2 (3), D: 1, 2, 3 and Ile-(D-Phe)-Gly-Thr-NH_2. Analytical conditions: solvent A=TEAP, B=30% MeCN/TEAP, λ=210 nm, flow rate=1.0 ml/min, column=Vydac C_{18} (0.4×25.0 cm, 5 μm, 300 Å), linear gradient=25–70% B in 30 min.

were checked using CE with UV detection, the limits of which usually vary from approximately 10^{-13} to 10^{-15} mol.[34] Prior to that, several analytical scenarios were tested in order to achieve optimized separation between the major products and the possible contaminants (isomers or not). The best results were obtained with 100 mM of phosphoric acid, titrated with 0.1 M of NaOH (max. pH 2.5) containing 10 mM of β-cyclodextrin, at a voltage of 15 kV, a wavelength of 210 nm, an injection time of 4 s (57″ Hg) and a temperature of 20 °C. Figure 8 presents the electropherograms obtained. As can be seen, although the crude peptide product from SPPS at 60 °C is more contaminated than that resulting from classical SPPS, integration of each of its electropherogram peaks yields individual percentages lower than 1%.

The LC/ESI-MS analysis of the crude tetrapeptide resulting from SPPS-ET revealed considerable quantities of the tetrapeptide and of several minor contaminants. Three of them presented masses coincident with that of the desired product. Therefore, it seems reasonable to assume that they are stereoisomers (DDGlyL and LDGlyL isomers may be present). The other contaminants correspond to impurities—deleted and or partially protected sequences such as H-Ile-Phe-Thr-NH_2, H-Phe-Gly-Thr(Bzl)-NH_2, H-Ile-Gly-Thr(Bzl)-NH_2 and H-Ile-Phe-Gly-Thr(Bzl)-NH_2—usually formed in conventional SPPS.

Finally, CE spiking experiments involving large amounts of the crude Ile-Phe-Gly-Thr-NH_2 resulting from SPPS-ET were also performed in an extreme attempt to detect and, if possible, quantify the presence of diastereoisomers. As shown in Figure 9, such crude peptides likely contain very low amounts of DDGlyL and LDGlyL (less than 1%). These data are in agreement with the cleanliness revealed in the RP-HPLC analyses.

These results together with those obtained for the crude Ac-Ile-Gly and (D-Tyr)-Lys-Trp allowed us to draw certain conclusions: (1) in spite of the fact that we did not use LDGlyD and LLGlyD in the CE spiking experiments (performed with the crude Ile-Phe-Gly-Thr-NH_2 resulting from SPPS-ET), there was very little racemization. With the peptides studied and the protocols employed, racemization of less than 1% per cycle occurred using either SPPS-ET or conventional SPPS. This is in agreement with the findings of Riester et al.[23] (in the systematic CE analysis of crude (D-Tyr)-Lys-Trp resulting from classical SPPS) and with the observations recently reported for microwave-assisted stepwise SPPS, an approach based on rapid heating at the molecular level in order to increase peptide coupling. Indeed, according to Erdélyi and Gogoll, no appreciable racemization occurs during TBTU, PyBOP and HATU synthesis of tripeptides that contain hindered amino acids;[35] (2) the short peptide sequences studied do not contain His, Cys and Ser, which are very prone to lose their configuration while being coupled as urethane-protected amino acids even at room temperature (His and Ser were in fact present in the rat CRF synthesized earlier at 75 °C by Rabinovich and Rivier[12] but such a large peptide did not have its isomer content measured). So far the present study has shown that SPPS-ET is indeed safe for peptides containing ‘ordinary’

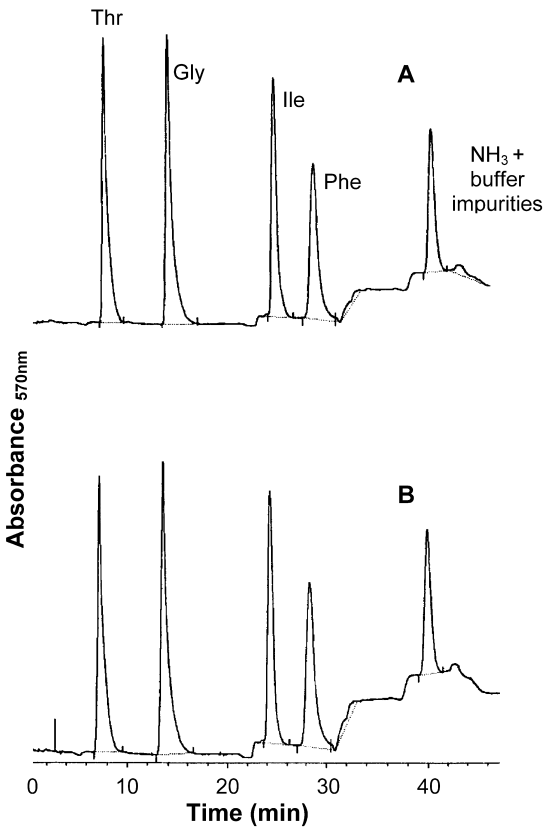

Figure 7. Aminograms of the fully hydrolyzed Ile-Phe-Gly-Thr-NH_2 resulting from conventional SPPS (A) and from SPPS-ET (B). NH_3 results from the hydrolysis process. Analytical conditions: those described in Figure 2.

amino acids; (3) since racemization incidence is dependent on the amino acid derivatives, solvents, bases and activating reagents employed in peptide building,[15] as well as on the coupling time, we anticipate that the amount of isomers formed in SPPS-ET of larger peptides using the protocols described here will be exclusively determined by their sequences and sizes rather than by the experimental conditions employed.

Finally, it is important to emphasize that the present study was designed to evaluate SPPS-ET from the standpoint of racemization propensity. Therefore, the analytical work focused primarily on the possibility of stereoisomers contaminating the crude peptides that resulted from SPPS-ET and classical SPPS. Nevertheless, the LC/ESI-MS results revealed the need for further investigations focused on secondary reactions, distinct from racemization, which may be increased in SPPS-ET. To date, only increased amide dehydration and pGlu formation have been reported.[12,14] On the other hand, we cannot ignore the fact that SPPS at 75 or 60 °C resulted in crude NPY (36 residues), rat CRF (41 residues), $ACP_{(65-74)}$, unsulphated CCK-8 and unsulphated CCK-12 (8 and 12 residues) that presented quite reasonable qualities.[12–14] In order to further estimate the impact caused by our protocols on the quality of chemically complex peptide sequences, we are currently synthesizing medium-sized fragments of unsulphated CCK (troublesome sequences) using both conventional SPPS and SPPS at 60 °C.

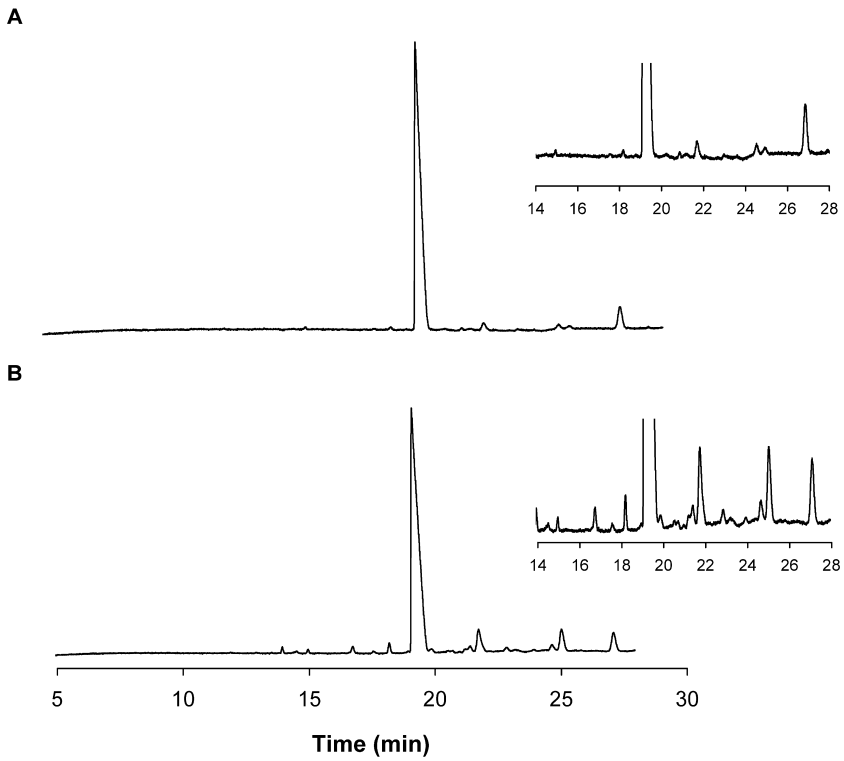

Figure 8. Electropherograms of the crude Ile-Phe-Gly-Thr-NH_2 resulting from conventional SPPS (A) and from SPPS-ET (B). Analytical conditions: voltage: 15 kV, buffer: 100 mM Na_2HPO_4 plus 10 mM of β-cyclodextrin, capillary length: 72 cm (50 cm to detector), capillary diameter: 50 μm, temperature: 20 °C, detection: 210 nm.

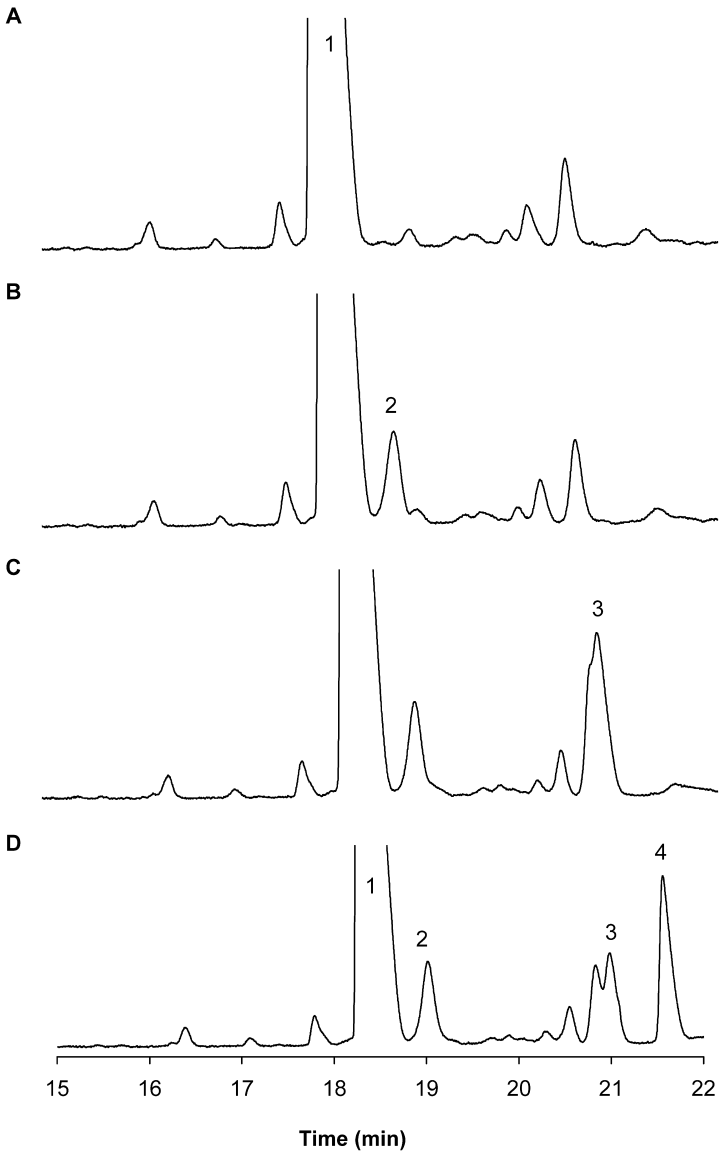

Figure 9. Electropherograms the crude Ile-Phe-Gly-Thr-NH_2 resulting from SPPS at elevated temperature (A) spiked by some stereoisomers (B–D). B: crude (1) and (D-allo-Ile)-(D-Phe)-Gly-Thr-NH_2 (2), C: 1, 2 and (D-allo-Ile)-Phe-Gly-Thr-NH_2 (3), D:1, 2, 3 and Ile-(D-Phe)-Gly-Thr-NH_2. Analytical conditions: voltage: 15 kV, buffer: 100 mM Na_2HPO_4 plus 10 mM of β-cyclodextrin, capillary length: 72 cm (50 cm to detector), capillary diameter: 50 μm; temperature: 20 °C, detection: 210 nm.

3. Conclusion

Because the idea of performing every synthesis cycle at high temperature is relatively recent and has been explored by only a few authors,[12–14] it merits attention. Our results show that, for the peptides studied, SPPS-ET performed at 60 °C with 25% DMSO/toluene and 1 M of DIC in DCM (apparent pH of 8.0) or in TBTU/DIPEA (apparent pH of 9.0) does not promote significant racemization of the incorporating Boc- or Fmoc-amino acids. In addition, racemization levels were comparable to those measured for classical SPPS performed at room temperature with 50% DCM/DMF or pure DMF and 1 M of DIC in DCM/HOBt (apparent pH of 8.0) or in TBTU/DIPEA (apparent pH of 9.5). On the other hand, this finding highlights the urgent need for investigations focused on secondary reactions, distinct from racemization, which may be maximized in SPPS-ET. In summary, the novelty of the present study is that it provides new information on an alternative synthesis approach by describing one of its basic aspects and bringing some others to light.

4. Experimental

4.1. Materials

Dichloromethane (DCM), dimethylsulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF), trifluoroacetic acid (TFA), triethylamine (TEA) and diisopropylethylamine (DIPEA) were of synthesis grade. Methanol (MeOH), isopropanol (*i*PrOH), diisopropyl ether and anisole were of analytical grade. Acetonitrile (MeCN) and TFA were of chromatographic grade. All of the above were acquired from Merck (Darmstadt, Germany) and used as received without further purification. The N^{α}-*tert*-butyloxycarbonyl (Boc)- or N^{α}-fluorenylmethyloxycarbonyl (Fmoc)-L- or D-amino acid derivatives protected or unprotected in their

side-chains—Boc-Gly, Fmoc-Gly, Boc-Phe, Boc-Tyr(2,6-dichlorobenzyl, Dcb), Boc-Asp(cyclohexyl ester, OcHex), Boc-Lys(2-chlorobenzyloxycarbonyl, 2-Cl-Z), Boc-Ser(benzyl, Bzl) and Boc-Thr(Bzl)—were from Bachem (Torrance, CA, USA), Applied Biosystems (Foster City/CA, USA) or Protein Research Foundation (Osaka, Japan). 4-Methylbenzhydrylamine resin (MBHA) was obtained from Applied Biosystems (Foster City, CA, USA). The Boc-Trp-*p*-amino-methyl resin (PAM) and Boc-Ile-PAM were purchased from Bachem (Torrance/CA, USA), as were the coupling reagents diisopropylcarbodiimide (DIC), 1-hydroxybenzotriazole (HOBt) and *O*-benzotriazolyl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU). The hydrogen fluoride (HF) system was from Peptide Institute, Inc. (Osaka, Japan), whereas the HF itself was purchased from Merck (Darmstadt, Germany). Analytical-grade 6 N HCl solution was purchased from Sigma (St. Louis, MO, USA). Other starting materials or reagents were obtained either from Fluka (Buchs, Switzerland), Sigma (St. Louis, MO, USA) or Aldrich Chemical Company, Inc. (Milwaukee, WI, USA). The water employed was purified in a Milli-Q deionizer (Millipore, Bedford/MA, USA).

4.2. Manual peptide synthesis

The peptides were synthesized manually using SPPS-ET or conventional SPPS and the Boc or Fmoc strategy using customized protocols.[13,17]

As usual, each step of the conventional SPPS process was carried out at room temperature. Subsequently, the amino acid derivatives were coupled for 60 min in DCM or DCM/DMF mixtures (1:1, v/v) or in DMF containing equimolar amounts of DIC/HOBt (1:1, eq.: eq.) or of TBTU/DIPEA (1:3, eq.: eq.). The Boc group was removed by soaking for 20 min in 50% DCM/TFA containing 1% anisole, whereas a 10-min soak in 20% piperidine/DMF was used to remove the Fmoc group. Neutralization with 10% TEA/DCM or alternating washes in DCM, DMF, *i*PrOH and MeOH were performed as described by Miranda et al.[4,28] and Varanda and Miranda.[13]

Each SPPS-ET synthesis cycle was carried out at 60 °C. Subsequently, 20–30 min couplings were performed in 25% DMSO/Toluene, DMF or NMP containing DIC or TBTU (equimolar to the amino acid derivative)/DIPEA (3-fold excess). The Boc group was removed by soaking for 10 min in 50% TFA/toluene containing 1% anisole, whereas the Fmoc group was removed with a 5-min soak in 20% piperidine/DMF. The alternating washes with 25% DMSO/toluene, DMF, NMP, *i*PrOH and MeOH were conducted as soon as possible after completion following customized protocols.[4,12,13,28]

In both cases, incomplete couplings were repeated to ensure quantitative yields. Deprotection, coupling and recoupling were monitored using the Kaiser test.[24]

4.3. Peptide detachment from the polymeric support and simultaneous full deprotection

Treatment with HF at 0 °C for 1–1.5 h in the presence of 1% anisole was sufficient for generation of the free unprotected peptides.[4,12,13,28] After complete removal of the HF, the crude peptides were precipitated, washed twice with diisopropyl ether, filtered and extracted with 50% MeCN/water. The aqueous–organic solutions were lyophilized in order to render the powered form of the crude peptides. Recoveries were calculated as milligrams of crude peptides obtained divided by milligrams of peptide-resin submitted to this treatment.

4.4. RP-HPLC analysis and peptide purification

The LDC analytical HPLC system (Thermo Separation Products, San Jose, CA, USA) used consists of a Consta-Metric 3500 pump, a ConstaMetric 3200 pump, a UV–VIS Spectromonitor model 3100 detector, a Rheodyne model 7125 injector (Cotati, CA, USA) and a Waters 745B integrator (Millipore, Milford/CA, USA). This system was coupled to 5-μm, 300 Å, C_{18} Vydac columns (0.46 id×25 cm for analysis or 1.1 id×25 cm for purification. The Separation Group, Inc., Hesperia, CA, USA).

Analysis of the crude synthetic peptides and purification of their major components (always the desired products) were performed employing appropriate linear gradients of two solvents. Solvent A was 0.1% TFA H_2O or TEAP (a mixture of TEA and phosphoric acid, pH 2.5[4,12,13,28,29]) and solvent B was MeCN in water containing 0.09% TFA or in TEAP. In both cases, we used a wavelength of 210 nm and a flow rate of either 1.0 ml/min (for analysis) or 3.0 ml/min (for purification).

4.5. Total peptide hydrolysis and amino acid analysis of the hydrolysates (IEX-HPLC)

The crude or purified peptides were hydrolyzed for 24 h at 110 °C in the presence of 6 N HCl vapor and phenol on a Waters Pico-Tag workstation (Millipore, Milford, CA, USA). After the HCl solution had completely evaporated, the hydrolyzed samples were dissolved in buffer. Subsequently, they were examined in a model 7300 Beckman automated amino acid analyzer (Beckman Instr. Inc, CA, USA), which is an ion-exchange HPLC system employing post-column derivatization of the separated amino acids with ninhydrin in order to detect and quantify them under specific experimental conditions.[24–26] The following passage relates average amino acid contents (found/expected). Ac-Ile-Gly (1–10): Gly, 1.00/1.00; Ile, 0.93/1.00. (D-Tyr)-Lys-Trp (DLL): Tyr, 0.96/1.00; Lys, 1.03/1.00; Trp (not detected in this analysis due to full degradation in 6 N HCl). (D-Tyr)-Lys-Trp optical isomers LLL or LDL: Tyr, 0.96/1.00; Lys, 1.03/1.00; Trp, (not detected in this analysis due to full degradation in 6 N HCl). Ile-Phe-Gly-Thr-NH_2: Ile, 0.86/1,00; Phe, 0.83/1.00; Gly, 1.15/1.00; Thr, 1.15/1.00. Ile-Phe-Gly-Thr-NH_2 optical isomers: LDGlyL: Ile, 0.86/1.00; Phe, 0.83/1.00; Gly, 1.15/1.00; Thr, 1.16/1.00. DLGlyL: D-allo-Ile, 1.12/1.00; Phe, 0.96/1.00; Gly, 1.00/1.00; Thr, 0.93/1.00. DDGlyL: D-allo-Ile, 1.12/1.00; Phe, 0.96/1.00; Gly, 1.00/1.00; Thr, 0.93/1.00.

4.6. Capillary electrophoresis

CE was performed on a model 270A-HT Perkin–Elmer/Applied Biosystems capillary electrophoresis system

(Foster City/CA, USA). Fused silica capillaries of 72 cm×50 μm (50 cm to detector) were used. A potential of 15 or 30 kV was applied. Two buffers were employed. One consisted of 100 mM of Na_2HPO_4 plus 10 mM of β-cyclodextrin[36,37] adjusted to pH 2.5 and the other was composed of 30 mM of Tris plus 10 mM of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (18-C6H) titrated to pH 2.0 with citric acid. Samples were injected hydrodynamically at 5 in. of Hg for 4 s and detected by UV absorbance at 210 nm. The temperature was maintained at 20 °C.

4.7. Peptide characterization through mass spectrometry[38]

After purification, the intact dipeptides, tripeptides and tetrapeptides were analyzed in a Reflex matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer (Bruker Daltonics, Inc., Billerica, MA, USA). As a matrix, α-cyano-4-hydroxycinnamic acid was used in a simple quadrupole ESI/MS instrument (Micromass Ltd., Altrincham, United Kingdom). A 0.1% formic acid/water solution was used as solvent. Found/expected $M+H^+$: Ac-Ile-Gly, 230/231; (D-Tyr)-Lys-Trp, 495/496; Ile-Phe-Gly-Thr-NH_2, 435/436.

The crude peptides were analyzed in a triple quadrupole ESI/MS instrument (Micromass Ltd., Altrincham, United Kingdom) coupled to a RP-HPLC system composed of two Shimadzu LC-10AD pumps, a UV–VIS Shimadzu SDP-10AV detector and a Rheodyne model 7125 injector (Cotati, CA, USA). The experimental conditions employed were the following: solvent A: 0.1% TFA in water, 60% solvent B: acetonitrile/water containing 0.1% TFA, gradient: 5 to 95 of solvent B in 30 min., wavelength of 210 nm, flow rate of 1 ml/min, ionization mode: positive, voltages: 3 kV (capillary) and 37 kV (cone). The software MassLynx™ for Windows NT was used for the data analyses. The results are described in Section 2.

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Bis(crown ethers) derived from biphenyl: extraction and electrochemical properties

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Abstract—Ligands derived from 4,4′-dinitrobiphenyl containing azacrown cavities in the 2,2′ position have been used in extraction and transport experiments. Control experiments with a system containing only one complexing cavity have demonstrated that the capability of forming a sandwich-type complex in the aforementioned ligand not only increases extraction but also the transport across a liquid membrane. Extraction studies have also shown that the complex present in the membrane has a 1:1 stoichiometry with regard to both ligands. Electrochemical studies have also been carried out. The ligand containing two complexing cavities is capable of giving rise to a 2:1 complex under electrochemical conditions.

1. Introduction

Topology of macrocyclic polyethers strongly contributes to their cation complexation. The use of bicyclic structures is an attractive strategy to enhance cation-complexing abilities and selectivities of monocyclic crown ethers. Macrobicyclic polyethers carrying two crown ether moieties at the end of a spacer possess different complexing properties.[1] Usually, these bis(crown ethers) are more likely to form intramolecular sandwich-type complexes with certain cations that are a little larger than the crown ring cavities by means of a cooperative action of two adjacent crown rings. As a result of such a complex formation, the bis(crown ether) exhibits excellent selectivities towards certain cations as compared to the corresponding monocycles analogues.[2,3] On the other hand, bis(crown ether) compounds have also been used as carriers in transport experiments, giving rise to different selectivities and efficiencies. This behaviour can be related to the strong effect of the stability of the cation-carrier complex on the transport efficiency.[4]

Here we report the use of ligands **1** and **2** (Chart 1) in extraction and transport experiments, and the influence of the complex structure in transport efficiency. Additionally, some electrochemical studies on these ligands have been carried out.

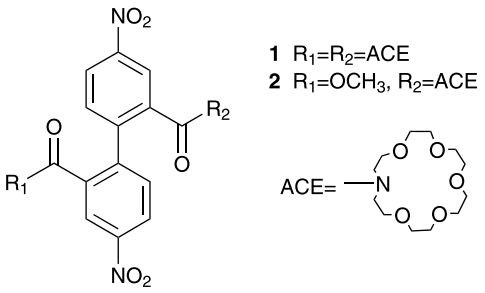

Chart 1.

2. Results and discussion

2.1. Synthesis

Ligands **1** and **2** have been synthesised as described in Scheme 1. The reaction of 4,4′-dinitrodiphenic acid[5] with thionyl chloride gives rise to the corresponding acid chloride that reacts with the commercially available macrocycle 1-aza-18-crown-6 to lead to compound **1**. The asymmetric compound **2** was prepared in a similar way from the monomethylester of the 4,4′-dinitro-diphenic acid (**4**). This ester was prepared from the same acid through the corresponding anhydride (**3**).

2.2. Extraction and transport experiments

Extraction experiments, between chloroform and aqueous phases, with ligands **1** and **2** were carried out following the method described by Cram[6] and the log K_a obtained,

Keywords: Transport; Extraction; Complexation; Azacrown ligands; Electrochemistry.
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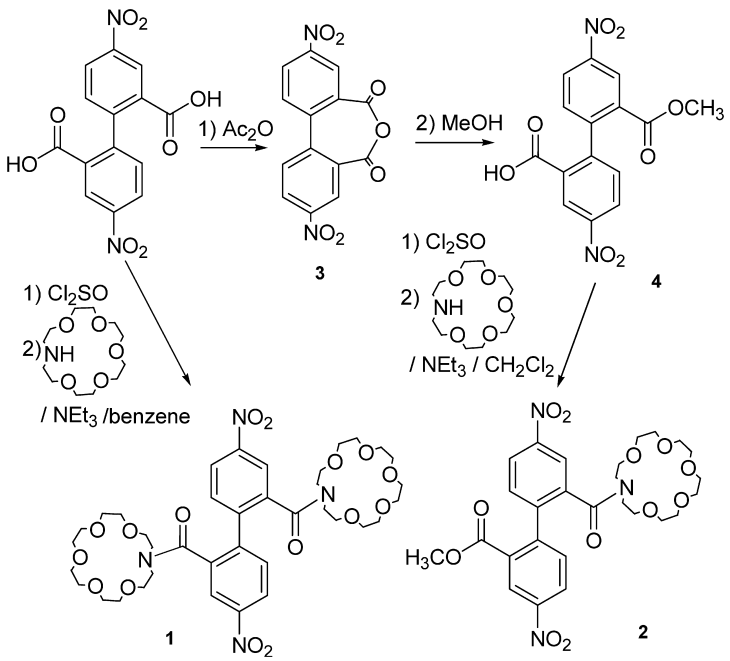


Scheme 1.

Table 1. Extraction experiments with ligands **1** and **2**

Ligand	log K_a				Selectivity			% Extraction			
	Li^+	Na^+	K^+	Cs^+	Na^+/Li^+	Na^+/K^+	Na^+/Cs^+	Li^+	Na^+	K^+	Cs^+
1	5.23	5.35	5.09	5.21	1.32	1.82	1.38	22.0	32.1	27.2	19.3
2	3.42	4.64	4.09	3.16	16.59	3.55	30.20	0.3	8.0	3.5	0.2

assuming a 1/1 stoichiometry of the crown ether and the cation as summarised in Table 1, along with the selectivity ratios Na^+/Li^+, Na^+/K^+ and Na^+/Cs^+, as well as the extraction percentage for each cation.

The bis(crown ether) **1** showed a cation extracting ability, which is greater than those observed in the monocycle compound **2**, probably due to the formation of intramolecular sandwich-type complexes. In specific terms, the complexation of Li^+ and Cs^+ by **1** were enhanced by the log K_a values of 1.81 and 2.05, respectively, as compared to the corresponding monocycle analogue **2** (Fig. 1). The ionic radius of these two cations are too small and too large, respectively, to fit into a single crown cavity, but the simultaneous complexation with both crown subunits allows them to show a large enhancement of the extraction properties.

By contrast, a poor selectivity for alkali cations was observed for the bis(crown ether) when compared with the selectivity observed for the corresponding monocyclic counterpart (Fig. 2). In addition, the results observed for Mg^{2+} are similar to those shown by Li^+, which led us to propose that the ionic radius is one of the most important factors in extraction processes. The low extraction observed for Ca^{2+} may be due to the large K_d shown by the corresponding picrate. Thus, the diffusion gave rise to inaccurate results that have not been included.

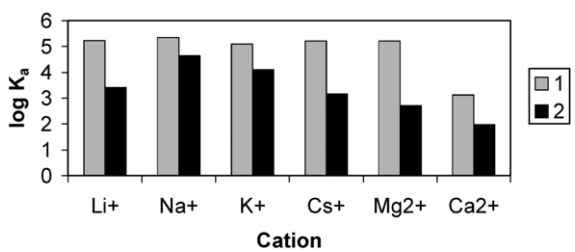


Figure 1. Association constants of ligands **1** and **2**. ($CHCl_3$ at 298 K).

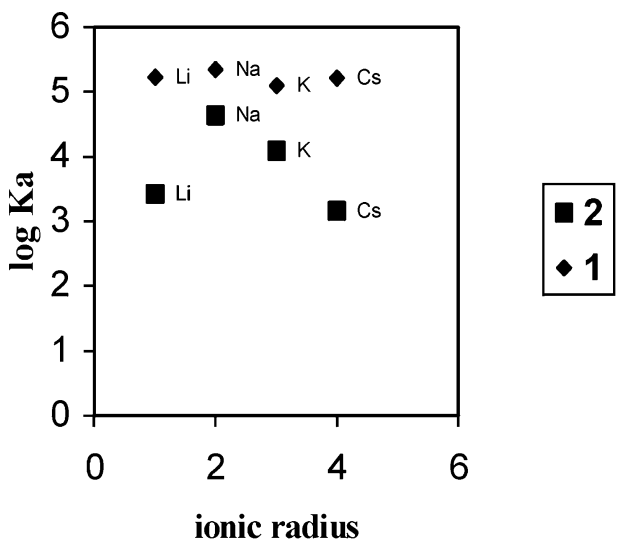


Figure 2. Extraction selectivity for ligands **1** and **2**.

As Figure 3 shows, the slopes are mostly 1 in the log [M] vs log [L] representations (where [M] is the concentration of the cation in the organic phase at the end of the experiment, and where [L] is the concentration of the ligand in the membrane) for the alkali metals studied. These results agree with a 1:1 stoichiometry of the complexes in the organic phase[7] not only with ligand **2** as expected, but also with ligand **1**, as proposed above.

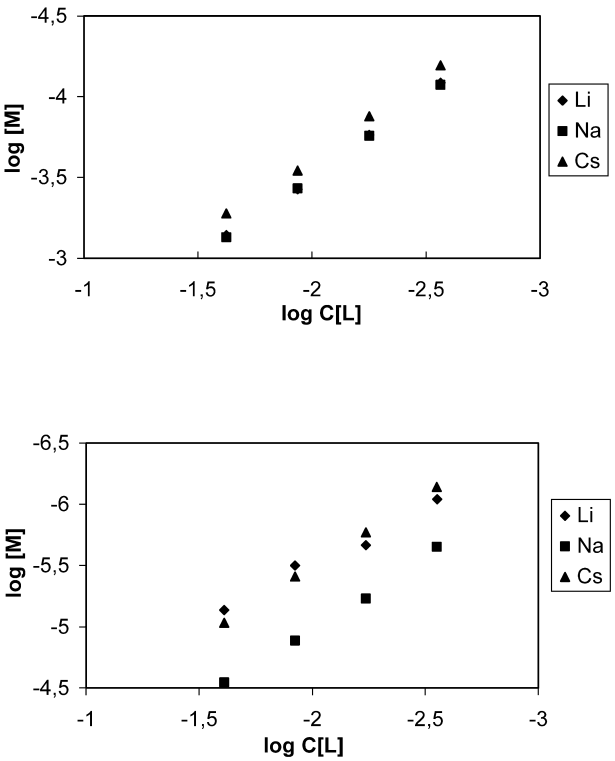


Figure 3. Extraction of LiPic (4.81 mM), NaPic (4.76 mM) and CsPic (2.40 mM) with ligand **1** (top) and **2** (bottom) (concentration of ligands from 23.73 to 2.73 mM).

On the other hand, transport experiments were carried out in a U-tube at 293±1 K.[8] Chloroform was used as the liquid membrane and deionised water was used with both the source and the receiving phase. The concentration of cations was deduced from that of the corresponding picrate anions determined by UV spectroscopy. The results of these experiments are shown in Table 2.

Table 2. Transport efficiency [μM (M^+)/ml (receiving phase) mM (ligand)] through a $CHCl_3$ bulky membrane, 293±1 K after 48 h

Ligand	Transport efficiency			
	Na^+	K^+	Cs^+	Ba^{2+}
1	17.80	10.95	9.56	9.46
2	4.03	2.66	0.99	0.43

These results demonstrate that the stronger the cation complexation is, the more efficiently it is transported. Although Na^+ is the more efficiently transported cation with both ligands, the behaviour of these two ligands is completely different. Thus, ligand **1** is a more efficient carrier than ligand **2** is (around 4.4 times for Na^+, 4.1 for K^+, 9.6 for Cs^+ and 22 for Ba^{2+}) (Fig. 4). These increments

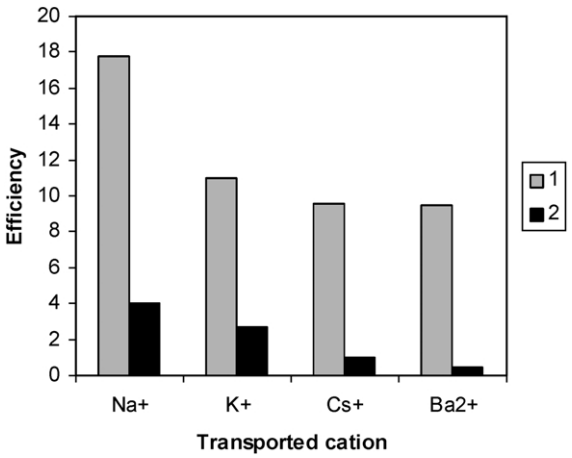


Figure 4. Transport experiments with ligands **1** and **2**.

in transport efficiency cannot only be explained by the mere presence of two cavities in its structure, i.e. ligand **1** transports more than twice ligand **2** does. One explanation for this behaviour could be due a sandwich-type complex formation that allows the cation to be completely encapsulated by the lipophilic part of both coronand subunits (Fig. 5). As expected, the effect of the sandwich-type complex formation in transport is more efficient the larger the cation is (e.g. Cs^+ or Ba^{2+}).

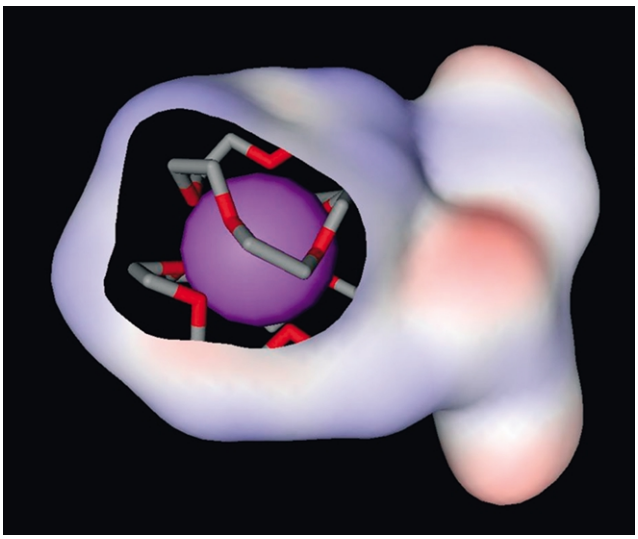


Figure 5. Lipophilic surface in complexes of ligand **1**.

Additionally, experiments have been carried out to know the influence of cation source-phase concentration in transport. Thus, two starting conditions (in the first case cation concentration at the source phase and at the membrane were four times the concentration of the second experiment) were used in NaPic extraction. The results observed demonstrated that there was not a direct correlation between cation concentration and transport, even though the concentration is higher the transport is more highly efficient. However, the effect was different in both ligands. Ligand **2** transported around 5 times the amount in the first experiment than in the second one. By contrast, ligand **1** shows more important increments, this ligand transported around 10 times more cation in the concentrated conditions than in the more diluted source phase (see Table 3).

Table 3. Transport experiment results with ligands **1** and **2**

Picrate salt	ε at 400 nm	Ligand **1**				Ligand **2**			
		c. of H (M)	c. r.p. (M)	c. s.p. (M)	f.e.	c of H (M)	c. r.p. (M)	c. s.p. (M)	f.e.
Li^+	9564	0.0012	2.39×10^{-4}	2.00×10^{-2}	14.43	0.0012	2.63×10^{-5}	2.68×10^{-2}	1.44
Na^+	9532	0.0012	1.58×10^{-4}	1.99×10^{-2}	88.68	0.0012	7.31×10^{-4}	1.70×10^{-2}	40.16
Mg^{2+}	16,820	0.0012	1.20×10^{-4}	2.05×10^{-2}	7.26	0.0012	2.09×10^{-5}	1.87×10^{-2}	1.13
Na^+	9532	0.0003	8.12×10^{-5}	4.94×10^{-3}	17.79	0.0003	1.88×10^{-5}	4.57×10^{-3}	4.03
K^+	10,626	0.0003	4.73×10^{-5}	5.01×10^{-3}	10.95	0.0003	1.25×10^{-5}	4.08×10^{-3}	2.66
Cs^+	10,895	0.0003	3.84×10^{-5}	5.04×10^{-3}	9.55	0.0003	4.68×10^{-6}	4.21×10^{-3}	0.99
Ba^{2+}	19,074	0.0003	3.90×10^{-5}	4.14×10^{-3}	9.45	0.0003	1.99×10^{-6}	3.61×10^{-3}	0.43

c. of H=concentration of host in the membrane; c.r.p.=final concentration of salt at the receiving phase; c.s.p.=final concentration of salt at the source phase; f.e.=factor of efficiency.

2.3. Electrochemical studies

Ligands **1** and **2** present a 4,4′-dinitrobiphenyl subunit which could be considered as a possible electrochemistry switch. Furthermore, electrochemistry has been shown to be an appropriate technique to study the complexation of related ligands with transition and post-transition metals.[9,10] It was for these reasons that we have decided to carry out some electrochemical studies in the presence of Cd^{2+} and Zn^{2+}. These cations were selected in relation to the fact of the most striking contrast that the biological activity of Cd^{2+} has in comparison to the activity of Zn^{2+}. The latter is crucial in a number of important biological processes, whereas Cd^{2+} is one of the most toxic metals.

The cyclic voltammetric (CV) response of the receptors dissolved in MeCN is illustrated in Figure 6(a), as far as **1** is concerned. In the initial cathodic scan, two well-defined reduction peaks appear at −1.02 (C_1) and −1.94 V (C_2), with the first one being preceded by a weak shoulder near to −0.74 V (C_3). In the subsequent anodic scan, an ill-defined shoulder appears at −1.55 V (A_2), followed by two overlapped peaks at −0.88 (A_1) and −0.61 V (A_3). This last peak disappears in CVs if the potential is reversed at potentials close to −1.25 V, suggesting that the peak A_3 corresponds to the oxidation of any species generated during the electrode process C_2 (Fig. 6(b)). The voltammetric response of the ligand **2** in MeCN (0.10 M Bu_4NPF_6) is almost identical to that previously described for **1**.

The observed response can be described on the basis of the well-known electrochemistry of aromatic and nitroaromatic compounds in aprotic solvents, consisting of two successive one-electron transfer processes, yielding an anion radical and a dianion.[11–13] The voltammetric profile, however, depends strongly on the stability of the intermediate radical anion. If this is unstable with respect to a disproportionisation into the dianion and the parent aromatic compound, the voltammogram looks like a single two-electron wave. Such is the case of the studied receptors; here the obtained value of the half-peak width of the process C_1, measured in square wave voltammetrys (SQWVs), 115 mV, is clearly lower than that expected for a reversible one-electron process (126 mV).[14] The overall electrochemical process can be represented as:

$$\mathbf{L} + 2e^- \rightarrow \mathbf{L}^{2-} \quad (1)$$

where **L**=**1**, **2**.

The interaction of **1** with Cd^{2+} ions is illustrated in Figure 7. For 'free' Cd^{2+} (see Fig. 7(a)), a prominent reduction peak appears at −0.73 V (C_4) followed by a tall oxidation peak at

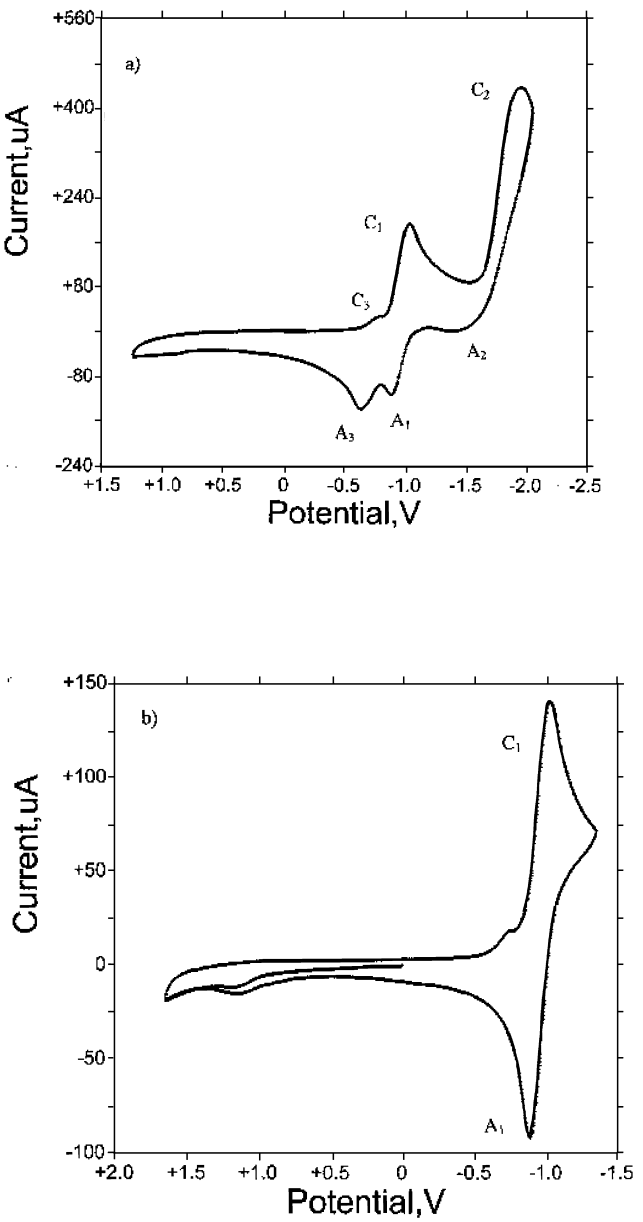


Figure 6. CVs at the GCE of a 2.0 mM solution of **1** in MeCN (0.10 M Bu_4NPF_6). Potential scan rate 200 mV/s. (a) Potential range +1.25/−2.05 V; (b) potential range +1.65/−1.35 V.

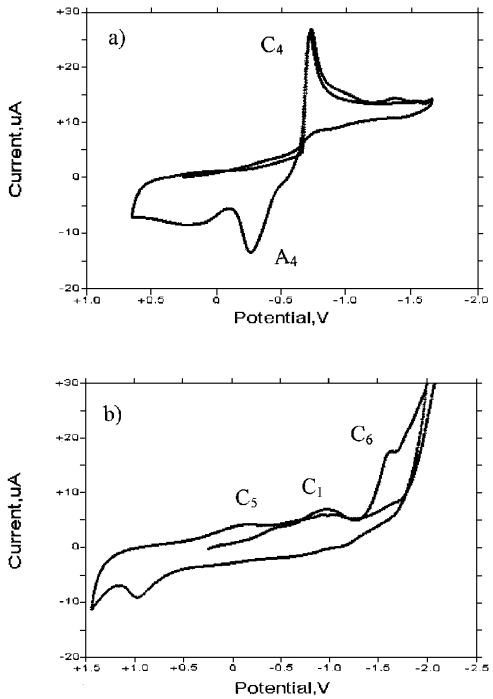


Figure 7. CVs at a Pt electrode of (a) 2.16 mM $Cd(NO3)_2$, (b) id. plus 0.86 mM **1** in MeCN (0.10 M Bu_4NPF_6). Potential scan rate 100 mV/s.

−0.26 V (A_4) which is then followed by an anodic wave close to +0.22 V. The cathodic process corresponds to the two-electron reduction of Cd^{2+} ions to form a non-uniform deposit of Cd metal on the electrode surface, further oxidised to Cd^{2+} in solution (stripping peak A_4), in agreement with the literature.[15]

In the presence of the ligand (see Fig. 7(b)), overlapped peaks at −0.46 (C_5), −0.95 (C_1), −1.64 (C_6) and −1.95 V (C_2) appear, whereas the anodic region becomes ill-defined. As the SQWVs show in Figure 8, the non-coordinated **1** displays isolated peaks C_1 and C_2. On addition of Cd^{2+}, additional peaks appear at −0.49 (C_5) and −1.64 V (C_6) whereas the peak C_1 is resolved into two overlapped peaks at −0.92 and −1.03 V.

The electrode process C_5 can be described in terms of the biphenyl-centred reduction of the complex. This can be represented as:

$$\mathbf{L} + x\mathrm{M}^{2+} + 2^{e-} \rightarrow (\mathrm{M}^{2+})_x(\mathbf{L}^{2-}) \quad (2)$$

Then, the electrode process C_6 can be ascribed to the metal-centred reduction:

$$(\mathrm{M}^{2+})_x(\mathbf{L}^{2-}) + 2x^{e-} \rightarrow x\mathrm{M}^0 + \mathbf{L}^{2-} \quad (3)$$

This process takes place at considerably more negative potentials than those of the reduction of uncomplexed Cd^{2+}, as expected for the electrochemical reduction of metal ions to metal deposits.

The interaction between the ligands and Zn^{2+} follows a scheme similar to that previously described. As shown in the SQWV depicted in Figure 9(a), for Zn^{2+} a prominent reduction peak is recorded at −1.33 V (C_7). By adding increasing amounts of **1** (see Fig. 9(b)), the voltammetric profile becomes similar to that recorded in the Cd^{2+} plus receptor solutions, with a prominent peak near to −0.45 V (C_5) and two overlapped peaks at −0.95 and −1.03 V (C_1). As expected, the reduction of complexed Zn^{2+} to Zn metal occurs at more negative potentials than those of the free Zn^{2+} ions. Also the peak C_6 is absent in SQWVs of the Zn^{2+} plus receptor solutions.

The interaction of **2** with Cd^{2+} and Zn^{2+} gives rise to analogous results that these described for **1**. The most remarkable feature is the appearance of a well-defined reduction peak at −0.45 V (C_5), which accompanies the peaks corresponding to the uncomplexed ligand.

Under the studied conditions, peak current data can be used to determine the stoichiometry and formation constant of the complexes of Cd^{2+} and Zn^{2+} with **1** and **2**, using a generalisation of the molar-ratio method.[16–18] By using the peak currents of C_5, which were measured in solution at constant concentration of M^{2+} and increasing concentrations of each one of the ligands, a 1:1 metal/**2** stoichiometry was obtained as expected, where the formation equilibrium constants were $(5.8\pm0.2)10^3$ and $(4.6\pm0.4)10^3$ (mol dm^{-3}), for Cd^{2+} and Zn^{2+}, respectively.

However, a 2:1 metal/receptor stoichiometry was obtained for **1**, where the formation constants were $(2.8\pm0.2)10^7$ and $(4.1\pm0.4)10^6$ (mol dm^{-3})$^{-2}$, for Cd^{2+}-**1** and Zn^{2+}-**1**, respectively. This stoichiometry must correspond to that of the reduced complexes formed between M^{2+} and the receptor dianion, $\mathbf{L}^{2-}$, resulting from the biphenyl-centred reduction process C_5 (Eq. (2)). The reduction of the biphenyl moiety of **1** gives rise to the formation of a planar

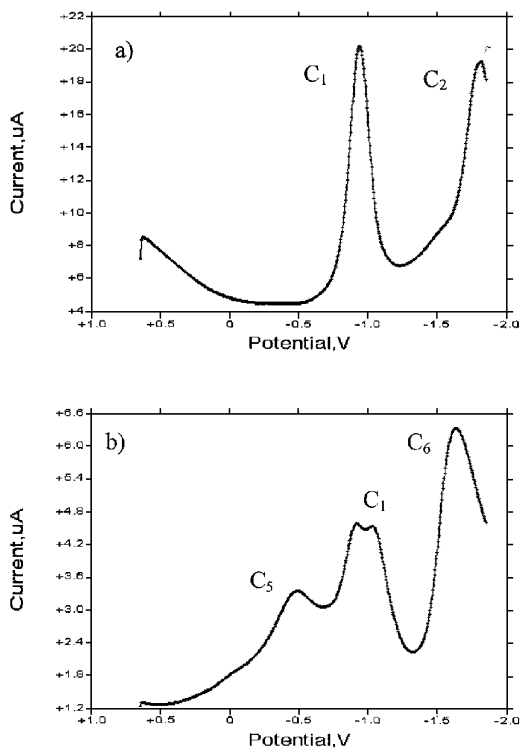
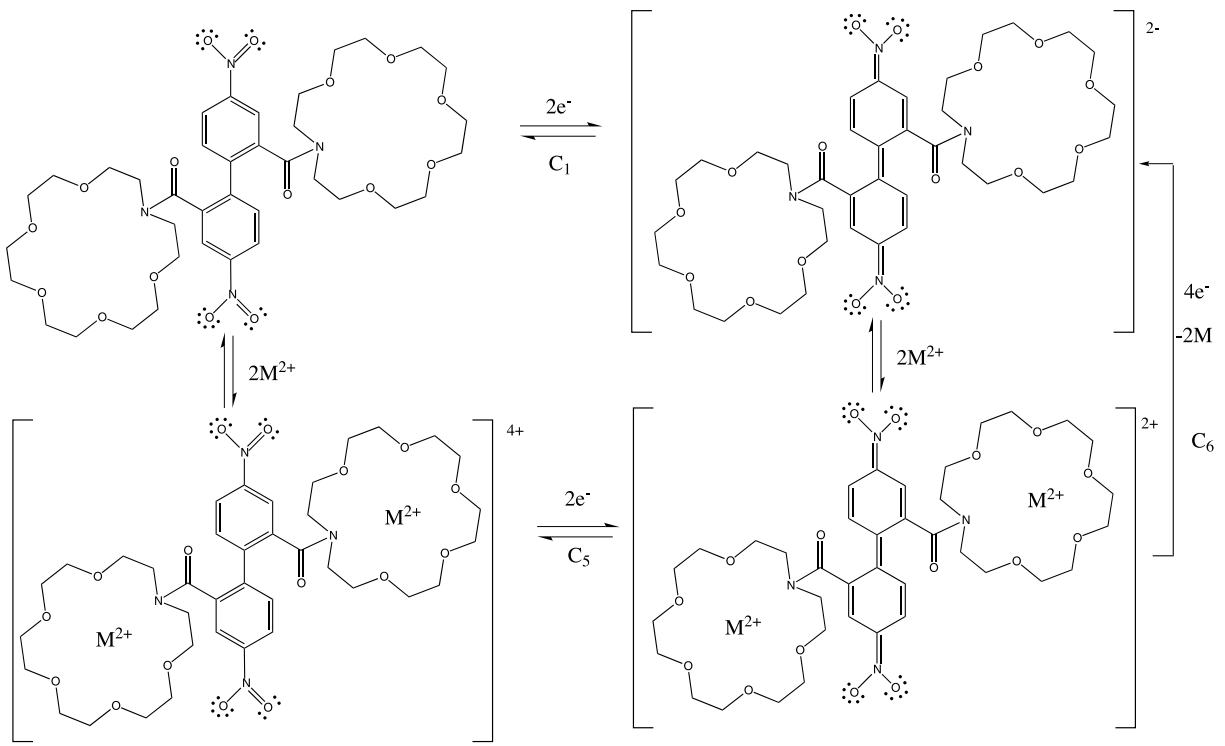


Figure 8. SQWVs at a Pt electrode of (a) 0.48 mM **1**, (b) 2.16 mM Cd^{2+} plus 1.69 mM **1** solutions in MeCN (0.10 M Bu_4NPF_6). Potential step increment 4 mV, square wave amplitude 25 mV, frequency 15 Hz.



Scheme 2.

dianion with the two crown ethers placed faraway from each other, as depicted in Scheme 2. Therefore, there is no place for the formation of a sandwich-type complex, and each of the crowns is coordinated with one metal ion.

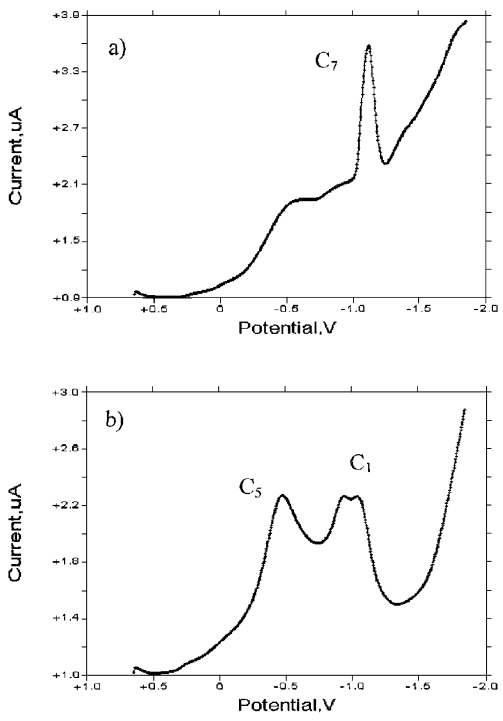


Figure 9. SQWVs at a Pt electrode of: (a) 2.26 mM $Zn(NO_3)_2$, (b) id. plus 1.01 mM **1** solutions in MeCN (0.10 M Bu_4NPF_6). Potential step increment 4 mV, square wave amplitude 25 mV, frequency 15 Hz.

3. Conclusions

The studies carried out with ligands **1** and **2** have demonstrated that the second cavity present in ligand **1** has a strong influence in transport experiments. The sandwich-type complex formation clearly modifies the lipophilic character of this complex, giving rise to an easier transport across the liquid membrane. However, ligand **1** exhibit no selectivity towards the studied cations. By contrast, ligand **2** is less effective in transport, but shows a clear selectivity towards Na^+. In addition, it has been observed that transport efficiency is dependent on the concentration at the source phase.

On the other hand, electrochemical studies showed that ligand **2** is able to complex Cd^{2+} and Zn^{2+} with a 1:1 stoichiometry. Electrochemical results obtained with ligand **1** are more interesting because instead of the sandwich complexes, complexes 2:1 were detected under these conditions. The presence of this type of complexes seems to be related to the coplanarity induced in the biphenyl system by the electrochemical process.

4. Experimental

4.1. General methods

All commercially available reagents were used without further purification. Benzene was dried over sodium. Water sensitive reactions were performed under argon. Column chromatographies were carried out on SDS activated neutral aluminium oxide (0.05–0.2 mm; activity degree 1). Melting points were measured with a Cambridge Instrument and are uncorrected. IR spectra were recorded as KBr pellets on a

Perkin–Elmer 1750 FT-IR and a Bruker Equinox 55 FT-IR. NMR spectra were recorded with Bruker Avance 300/500 and Varian Unity-300/400 spectrometers. Chemical shifts are reported in parts per million downfield from TMS. Spectra were referenced to residual undeuterated solvent. High-resolution mass spectra were taken with a Fisons VG-AUTOSPEC. UV spectra were run at 20 °C (thermostated) on a Shimadzu UV-2102 PC.

4.1.1. Synthesis of 4,4′-dinitro-2,2′-diphenic anhydride acid (3). 4,4′-Dinitro-2,2′-diphenic acid (0.197 g, 0.594 mmol) was stirred at 90 °C in acetic anhydride (40 ml) for 24 h. After removal of the solvent under vacuum, a clear brown oil was obtained. This oil was washed with Et_2O, allowing separation of the soluble starting acid (0.078 g) and its anhydride as a brown powder that was dried under vacuum. (0.119 g, 0.38 mmol, 64% yield). Mp 230–233 °C. HRMS (EI): found M^+ 314.0181, $C_{14}H_6N_2O_7$ requires 314.0175; ν_{max} 1815 (C=O), 1733 (C=O), 1525, 1350 cm^{-1}; δ_H ($CDCl_3$) 8.92 (2H, d, J=2.3 Hz, Ar-H), 8.53 (2H, dd, J_1=8.5 Hz, J_2=2.3 Hz, Ar-H), 7.46 (2H, d, J=8.5 Hz, Ar-H); δ_C (CD_3COCD_3) 166.1 (COOCO), 148.5, 147.0, 131.6, 126.4, 125.3, 124.8 (12 C_{Ar}).

4.1.2. Synthesis of 2-(2-methyloxycarbonyl-4-nitrophenyl)-5-nitrobenzoic acid (4). 4,4′-Dinitro-2,2′-diphenic anhydride acid (0.119 g, 0.38 mmol) was stirred for 5 h in refluxing MeOH (40 ml). Then, the solvent was evaporated under vacuum, to give a dark brown solid. This solid was dissolved in boiling $CHCl_3$ and the hot solution filtered. After evaporation of the solvent under reduced pressure, a white powder (0.132 g, 0.38 mmol) was obtained. Mp 176–180 °C. ν_{max} 2956 (OH), 1728, 1698, 1525, 1350, 1265 cm^{-1}; δ_H ($CDCl_3$) 8.97 (1H, d, J=2.4 Hz, Ar-H), 8.92 (1H, d, J=2.4 Hz, Ar-H), 8.46 (1H, dd, J_1=8.5 Hz, J_2=2.4 Hz, Ar-H), 8.43 (1H, dd, J_1=8.5 Hz, J_2=2.4 Hz, Ar-H), 7.37 (2H, d, J=8.5 Hz, Ar-H), 3.75 (3H, s, OCH_3); δ_C (CD_3COCD_3) 166.13 (COO), 165.83 (COO), 149.54, 149.42, 148.77, 143.70, 132.81, 132.79, 132.08, 131.76, 127.4, 127.45, 126.14 and 125.82 (12 C_{Ar}), 53.25 (OCH_3); MS (EI): M^+ (346, 65%); M^+-1 (345, 100%).

4.1.3. Synthesis of 1. 4,4′-Dinitro-2,2′-diphenic acid (0.063 g, 0.19 mmol) was added to an excess of thionyl chloride (30 ml) and the stirred suspension was heated under reflux until disappearance of the precipitate (ca. 2 h). The resulting solution was concentrated under reduced pressure until all the $SOCl_2$ had been removed. It was dissolved in benzene (30 ml) and dropped over a stirred solution of 1-aza-18-crown-6 (0.105 g, 0.38 mmol) and triethylamine (0.039 g, 0.38 mmol) in benzene (30 ml) under an argon atmosphere. The resulting solution was stirred overnight at room temperature, the reaction mixture filtered and the filtrate then washed with water (3×2 ml), dried over anhydrous Na_2SO_4 and concentrated to yield **1** as a brown oil (0.099 g, 63%). HRMS (EI): found (M^++1) 823.3643; $C_{38}H_{55}N_4O_{16}$ requires 823.3613; ν_{max}: 2867, 1633 (C=O), 1523 (N=O, asym.), 1349 (N=O, sym.), 1113 (CO) cm^{-1}; δ_H ($CDCl_3$) 8.33 (2H, d, J_2=2.7 Hz, Ar-H), 8.29 (2H, dd, J_1=8.4 Hz, J_2=2.7 Hz, Ar-H), 7.74 (2H, dd, J_1=8.4 Hz, Ar-H), 3.7–3.5 (48H, m broad band, $-CH_2-$ linked to O and N); δ_C ($CDCl_3$) 168.24 (s), 147.28 (s), 141.31 (s), 138.17 (d), 131.19 (d), 123.25 (d, s, 2 signals), 71.18 (t), 70.67–70.19 (t, several signals), 68.73 (t), 49.98 (t, CH_2N-), 44.96 (t, CH_2-N–); UV (CH_3CN): ε=17398, λ_{max}=293 nm.

4.1.4. Synthesis of 2. 2-(2-Methyloxycarbonyl-4-nitrophenyl)-5-nitrobenzoic acid (0.509 g, 1.47 mmol) was converted into its acid chloride, as described for the synthesis of **1**. Benzene (30 ml) was added and evaporated to yield 2-(2-methyloxycarbonyl-4-nitrophenyl)-5-nitrobenzoic acid chloride as a solid (0.536 g, 1.47 mmol). It was dissolved in dry CH_2Cl_2 (25 ml) and used in the following step.

To a stirred mixture of 1-aza-18-crown-6 (95%) (0.408 g, 1.47 mmol) and dry triethylamine (99%) (0.150 g, 1.47 mmol) in dry CH_2Cl_2 (20 ml) at 0 °C, the above solution was added dropwise under Ar atmosphere. Then, the mixture was stirred at room temperature and monitored by TLC. After completion of the reaction (12 h), the solution was concentrated under reduced pressure and the crude reaction product was purified by chromatography through a neutral alumina column using CH_2Cl_2/EtOAc (8:2) as eluents to give **2** as a brown oil (0.530 g, 0.887 mmol). (60% yield). HRMS (EI): found M^+ 591.2073. $C_{27}H_{33}N_3O_{12}$ requires 591.2064. ν_{max} 1731 (OC=O), 1634 (NC=O), 1608, 1524 (N=O, asym.), 1349 (N=O, sym.), 1123 cm^{-1}; δ_H ($CDCl_3$) 8.85 (1H, d, J=2.4 Hz, Ar-H), 8.39–8.36 (2H, m, Ar-H), 8.27 (1H, dd, J_1=8.5 Hz, J_2=2.4 Hz, Ar-H), 7.63 (1H, d, J=8.5 Hz, Ar-H), 7.40 (1H, d, J=8.5 Hz, Ar-H), 3.79 (3H, s, OCH_3), 3.65–3.54 (20H, m, $-OCH_2-$), 3.42 (2H, m, $-NCH_2-$), 3.36 (2H, m, $-NCH_2-$); δ_C ($CDCl_3$) 168.20 (CON), 165.23 (–COO), 148.07, 147.66, 145.52, 143.65, 137.35, 133.02, 131.59, 130.78, 126.44, 125.93, 123.50 and 123.11 (12 C_{Ar}), 71.49, 70.95, 70.90, 69.50, 69.00 (10 $-OCH_2-$), 53.29 ($-OCH_3$), 49.91 ($-NCH_2$), 45.19 ($-NCH_2$); UV (CH_3CN): ε=1470, λ_{max}=284 nm.

4.2. Transport experiments

Experiments of transport were carried out across an organic liquid bulky membrane consisting in 15 ml of ethanol-free $CHCl_3$ confined in a 12 mm of diameter U-shaped tube. A known amount of carrier was dissolved in this membrane (see Table 3). Picrate salts were synthesised from picric acid and the corresponding hydroxide of each metal, and most of them were purified by recrystallisation in ethanol/water. Initial solutions of picrate salts in water were prepared: Li^+ (0.02 M), Na^+ (0.02 M), Na^+ (0.005 M), K^+ (0.005 M), Cs^+ (0.005 M), Mg^{2+} (0.02 M) and Ba^{2+} (0.005 M).

Solution of carrier in $CHCl_3$ (15 ml) was transferred to a U-shaped tube, provided with a magnetic stirring bar. Initial solutions of the picrate salts (5 ml) were added to one of the branches of the tube (source phase). 5 ml of pure water was allocated in the other (receiving phase). In addition, one blank experiment for each initial solution was carried out, so as to determine the passive transport, by replacing the 15 ml of carrier solution with 15 ml of pure ethanol-free $CHCl_3$. After 48 h stirring (350 rpm) at 293±1 K, aqueous receiving phase was homogenised and a convenient aliquot was transferred to a 1 cm pathlength quarz UV cell. Distilled water was added to the cell till complete 3 ml of

Table 4. Extraction results with ligands **1** and **2**

Picrate salts	ε at 430 nm in CH_3CN	$K_d \times 10^3$	log K_e		log K_a	
			1	**2**	**1**	**2**
Li^+	8626	1.42 M^{-1}	2.38	0.57	5.23	3.42
Na^+	8975.9	1.74 M^{-1}	2.59	1.88	5.35	4.64
K^+	9251.5	2.55 M^{-1}	2.50	1.50	5.09	4.09
Cs^+	9586.4	5.41 M^{-1}	2.95	0.89	5.21	3.16
Mg^{2+}	9339.4	39.8 M^{-2}	3.81	1.31	5.21	2.71
Ca^{2+}	10,880	$5.41\times10^{+3}$ M^{-2}	3.86	2.70	3.12	1.97

total volume. Absorbance was registered at 400 nm. Factor of efficiency was calculated from (4). In order to determine concentrations, both in the source and in the receiving phase, Beer–Lambert's law was used. Extinction coefficient for each picrate salt in water was determined as in the section of extraction experiments, but using water as the solvent instead of CH_3CN. Values of ε were recorded in Table 4. Experiments of transports were carried out in triplicate and values of factor of efficiency are recorded in Table 3.

$$E = \frac{\dfrac{\mu\text{mol transported cation}}{\text{ml receiving phase}}}{\text{mmol carrier}} \tag{4}$$

4.3. Extraction experiments

The method described by Cram et al.[6] was followed in order to determine values of extractions constants (K_e) and association constants (K_a) for ligands **1** and **2** against several cations in picrates. Calculations were based on absorbance at λ=430 nm of the picric salts in the UV spectra. All the measurements were carried out at 293±1 K (thermostated) on a double beam Shimadzu UV-2102 PC spectrophotometer using a 1 cm pathlength quartz UV- cell. Deionised water and ethanol-free $CDCl_3$ constituted the aqueous and organic phase respectively. Initial 0.015 M solutions in water of monovalent (Li^+, Na^+, K^+) and divalent (Mg^{2+}, Ca^{2+}) cations as picrate salts were prepared, except for Cs^+, which was a 0.0076 M one. Solution of the host was 0.076 M in $CHCl_3$.

Into a 20 ml centrifuge tube was introduced 0.2 ml of the host solution. A volume of 0.5 ml of water was added to one of the tubes. Picrate solution (0.5 ml) was added to each of the others. The tubes were stoppered and shacked vigorously by hand for 3 min. Phases were separated after that by centrifugation.

A 10 µl aliquot of the organic layer was transferred by automatic pipette into a 3.5 ml UV cell. 2.99 ml of CH_3CN were added to complete a total volume of 3 ml. The UV absorption was measured against a cell containing 10 µl of the organic layer at the blank experiment and 2.99 ml of CH_3CN. Absorbance of both cells when they were filled with CH_3CN was balanced by placing the blank one across the reference beam, the sample one across the sample beam and running an automatic baseline correction. Three repetitions were run for a given cation and concentrations were found out using Beer–Lambert's law relationship. Extinction coefficients (ε) in HPLC-quality CH_3CN on the dynamic range of 10^{-5} to 10^6 M were determined using a set of standard different-concentration solutions of each salt and the values were recorded in Table 4.

Values of K_a for monovalent cations were calculated using the model described by Cram.[19,20] Following a similar procedure, K_a for divalent cations were calculated from (6). Values of the constant of distribution (K_d) for alkaline cations were reported by Cram et al.[6] In the case of alkaline-earth cations, K_d were determined following the same experimental procedure as Cram et al., but using (5) instead, which regards the change on the stoichiometry. Values of K_d thereby obtained are recorded in Table 4.

$$L_{CHCl_3} + M^{2+}_{H_2O} + 2A^-_{H_2O} \overset{K_e}{\rightleftarrows} M^{2+}LA^-_{2CHCl_3}$$

$$K_e = \frac{[M^{2+}LA_2^-]_{CHCl_3}}{[L]_{CHCl_3}[M^{2+}]_{H_2O}[A^-]^2_{H_2O}} = \frac{[M^{2+}LA_2^-]_{CHCl_3}}{[L]_{CHCl_3}4[M^{2+}]^3_{H_2O}}$$

$$= \frac{R}{(1-R)4Q^3}$$

being

$$R = \frac{[M^{2+}LA_2^-]_{CHCl_3}}{[L_i]_{CHCl_3}}$$

and

$$Q = [M^{2+}]_{H_2O} = [M^{2+}A^-_{2i}]_{H_2O} - R\left(\frac{V_{CHCl_3}}{V_{H_2O}}\right)[L_i]_{CHCl_3}$$

$$K_d = \frac{[M^{2+}A_2^-]_{CHCl_3}}{4[M^{2+}]^3_{H_2O}} \tag{5}$$

$$L_{CHCl_3} + M^{2+}A^-_{2CHCl_3} \overset{K_a}{\rightleftarrows} M^{2+}LA^-_{2CHCl_3}$$

$$K_a = \frac{[M^{2+}LA_2^-]_{CHCl_3}}{[L]_{CHCl_3}[M^{2+}A_2^-]_{CHCl_3}} = \frac{K_e}{K_d} \tag{6}$$

Where M refers to the metal cation, A to the picrate anion, L to the ligand behaving as a host with only one complexing site, V_{CHCl_3} and V_{H_2O} to the total volume in the organic and the aqueous phases.

4.4. Electrochemical studies

Electrochemical measurements have been performed at 298±1 K in a conventional three-electrode cell under argon atmosphere. Nominal ca. 2.0 mM concentrations of the

ligand were used in dry MeCN using tetrabuthylammonium hexafluorophosphate (0.10 M) as a supporting electrolyte.

Experiments were performed using a BAS CV 50 W equipment using a BAS MF2012 glassy carbon electrode (GCE) (geometrical area 0.071 cm^2), and a BAS MF2014 platinum electrode (geometrical area 0.018 cm^2) as a working electrode. A platinum wire auxiliary electrode and a AgCl (3 M NaCl)/Ag reference electrode separated from the test solution by a salt bridge only containing supporting electrolyte completed the electrode arrangement. The potential of such reference electrode was −35 mV vs the saturated calomel reference electrode (SCE).

Acknowledgements

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Computer-assisted design and lactonization of model *seco*-acid derivatives of lankanolide☆

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Abstract—In some cases, *seco*-acid derivatives (a precursor of macrolactone) did not cyclize to form the corresponding macrolactone. To design easily cyclizable *seco*-acid derivatives of lanaknolide, the conformation of several model *seco*-acids was calculated, and lactonization experiments of the *seco*-acids prepared from oleandomycin were carried out to elucidate the efficiency of the cyclization of the model *seco*-acid. The easily cyclable *seco*-acid was designed to be C8 exomethylene derivative of lankanolide *seco*-acid. On the other hand, *seco*-acid derivative having tertiary alcohol at C8 was predicted not to cyclize to form macrolactone.

1. Introduction

The target molecule, lankanolide **2**, is the aglicone of 14-membered macrolide lankamycin **1**. Lankamycin was isolated in 1960 by Gaumann et al.,[2] and the relative stereostructure of the macrolide was determined in 1972 by Keller-Schlierlein et al.[3] Since then, there has appeared no report of the total synthesis of this macrolide, while synthetic efforts have been carried out.[4] We are interested in the effect of the structure of the *seco*-acid derivative on the efficiency of macrolactonization (Fig. 1). In some cases, *seco*-acid derivatives did not cyclize to give macrolactone derivatives.[5] To avoid such a case, we decided to design the target *seco*-acid derivatives by computer-aided simulation before starting the synthesis.[6] In our design, there are two routes depending on whether before cyclization, C8 is quaternalized (route a) or after cyclization, an asymmetric center at C8 is introduced (route b). Route a is via *seco*-acid **II** which has tertiary alcohol at C8 position. In route b, macrolactone **IV** is oxidized to prepare a tertiary alcohol at C8 after cyclization. The critical point of these two routes will be the reactivity of *seco*-acids **II** and **V** Scheme 1. To predict the reactivity of *seco*-acids **II** and **V**, first, we calculated the conformation of the methyl ester of model *seco*-acid derivatives (**4**, **6** and **8**) corresponding to *seco*-acids **II** and **V**, and the lactones (**3**, **5**, and **7**) (Fig. 2).

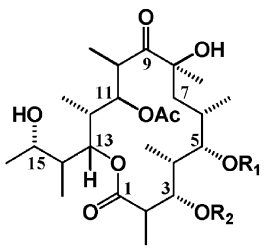

Figure 1. Lankamycin and lankanolide. Lankamycin (**1**): R_1=D-chalcose, R_2=acetylarcanose. Lankanolide (**2**): R_1=H, R_2=H.

2. Results and discussions

2.1. Computer-assisted design of model *seco*-acid

Recently, Goto et al. developed a conformational search method of chain compounds by stepwise bond rotation (conflex).[7] First, we calculated the conformations of *seco*-acid derivatives (**4**, **6**, and **8**) by using 'CONFLEX' and the obtained conformers were classified to conformation cluster (experimental detail was shown in Section 3.4). Similar conformation (differences of all dihedral angle to be compared are less than 10°) is classified in one cluster. The similarity of the clusters between *seco*-acid derivative and the corresponding lactone was also calculated, and the similarity was shown as cluster distance. The more similar the conformation of the cluster to be compared is, the

☆ See Ref. 1.

Keywords: Conformation calculation; *seco*-Acid; Lactonization; Lankanolide.
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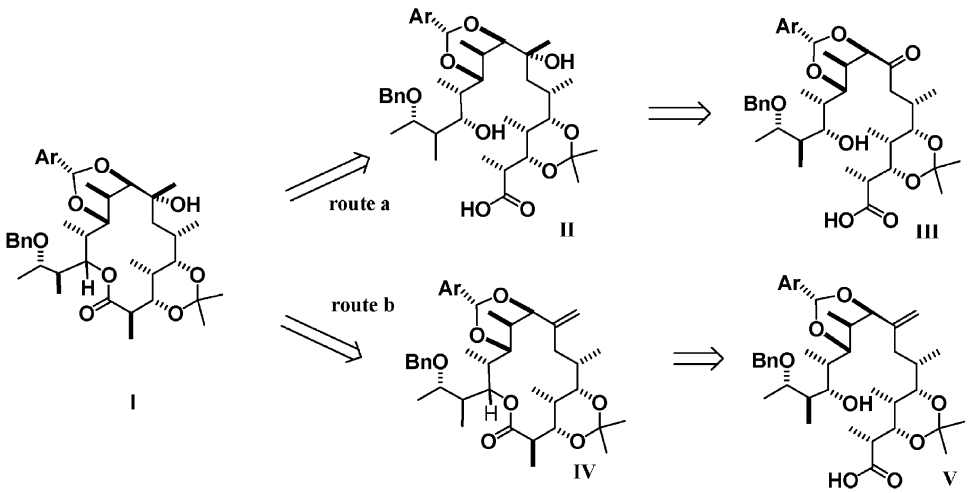


Scheme 1. Retro synthetic analysis of lankanolide.

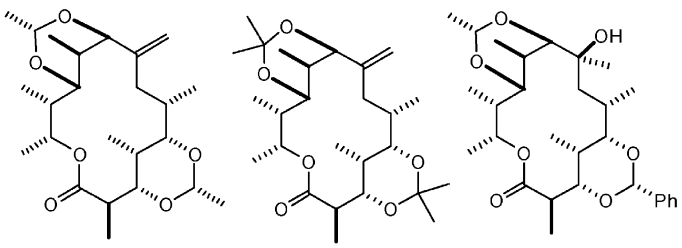


Figure 2. Model *seco*-acids and lactones for conformation calculation.

smaller the conformation distance is.[8] Obtained number of conformers having more than 0.01% population are shown below: 120 for **4**, 256 for **6**, 153 for **8**, 2 for **3**, 4 for **5**, 11 for **7**.

The results were shown in Table 1 (clustering results of conformers generated by conformation search) and Table 2 (cluster distance; cluster similarity between starting *seco*-acid and the corresponding cyclized lactone) and Figure 3. As shown in Figure 3, the simulated conformation of the methyl ester of *seco*-acid **4** showed a conformation cluster (#2, 15.2%) of the *seco*-acid is similar conformation to the corresponding cluster of lactone **3** (conformation distance=8.7).[8,9] On the other hand, the calculated conformation of diacetonide *seco*-acid **6** showed no similar conformation to the major cluster corresponding to lactone **5**. It is well known that the 6-membered ketal of anti-1,3-diol prefers twist-boat type,[10] and the most preferable conformer of 6-membered ketal of anti-1,3-diol (C9, C11) of model *seco*-acid **6** was also twist-boat (Fig. 3). However, most of the conformations of the corresponding lactone **5** was chair type (Fig. 3). Therefore, twist-boat conformer has to change to the more unstable chair conformer before cyclization. However, in the case of *seco*-acid **6**, there is a cluster (#11, population 1.5%) close to the lactone **5**, although population is not high.[9] The simulated conformation of **8** methyl ester also did not contain a similar conformation to the corresponding lactone **7** (conformation distance between the closest conformation of **8** was 49.7). Most of the conformation of model compound **8** is locked as shown in Figure 3, mainly becouse of hydrogen bonding between the tertiary alcohol at C8 and ether oxygen of 6-membered ketal of C3 and C5 diol. Because of the locked

Table 1. Major conformation clusters of model *seco*-acids (**4**, **6**, and **8**) and corresponding lactones (**3**, **5**, and **7**)

	Size[a]	Pop[b] (%)	Size	Pop (%)
Rank	**4**		**3**	
1	126	70.78	4	99.99
2	57	15.18	2	0.01
3	29	3.21		
4	62	2.90		
5	64	2.77		
Rank	**6**		**5**	
1	82	56.2	5	99.7
2	40	16.1	5	0.3
3	19	4.0		
4	28	3.4		
5	4	1.7		
11	14	1.5		
Rank	**8**		**7**	
1	23	99.2	32	99.9
2	2	0.8	27	0.1

[a] Number of conformers comprising a cluster.
[b] Combined percentage population of the component conformers.

Table 2. Cluster distance analysis of the model *seco*-acids and the corresponding lactones

	4	1	2	3	4	5	6	7	8	9	10	
3												
1		72.13	**8.72**	79.26	65.34	84.77	62.89	64.92	47.24	83.94	82.59	
2		81.25	42.25	86.16	72.81	90.3	76.95	75.59	62.56	91.69	87.34	
	6	1	2	3	4	5	6	7	8	9	10	11
5												
1		79.33	71.62	65.82	72.08	47.39	79.47	73.81	82.27	86.14	83.05	**6.44**
2		73.48	89.92	64.46	48.8	11.73	96.1	52.17	69.05	79.96	81.86	33.51
	8	1	2									
7												
1		49.77	48.69									

Close distances (less than 10) are printed in bold letters.

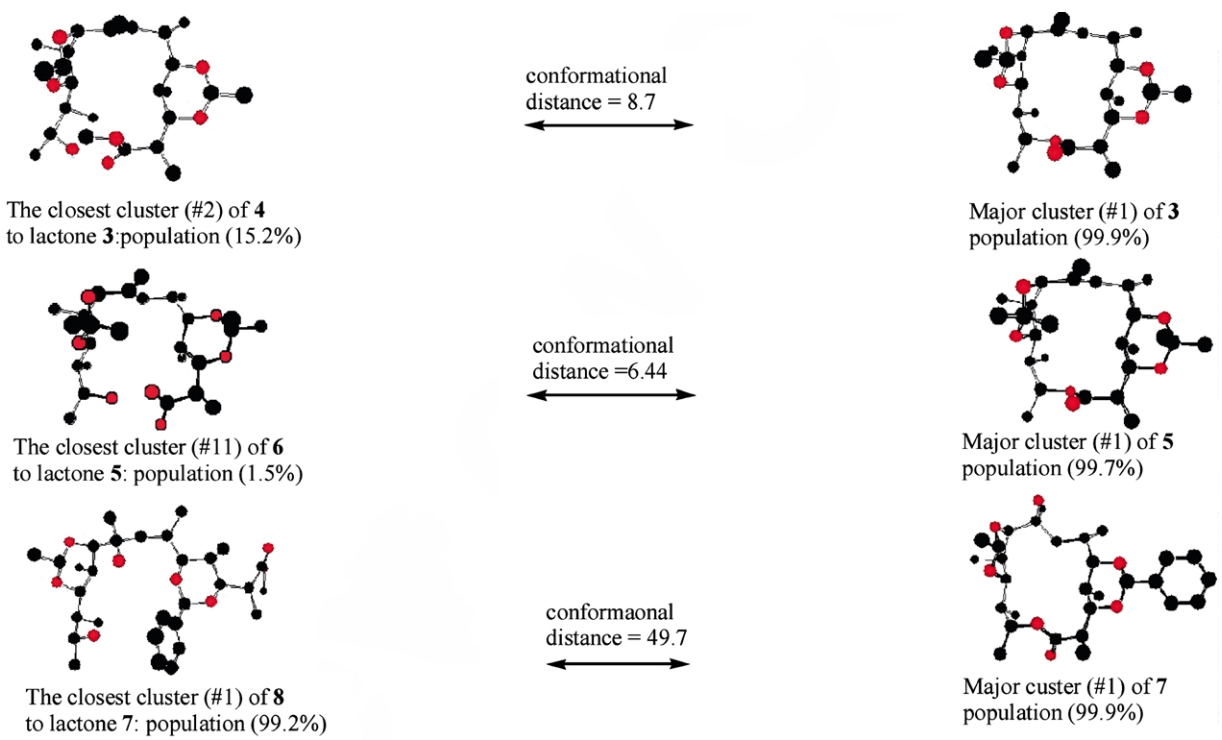
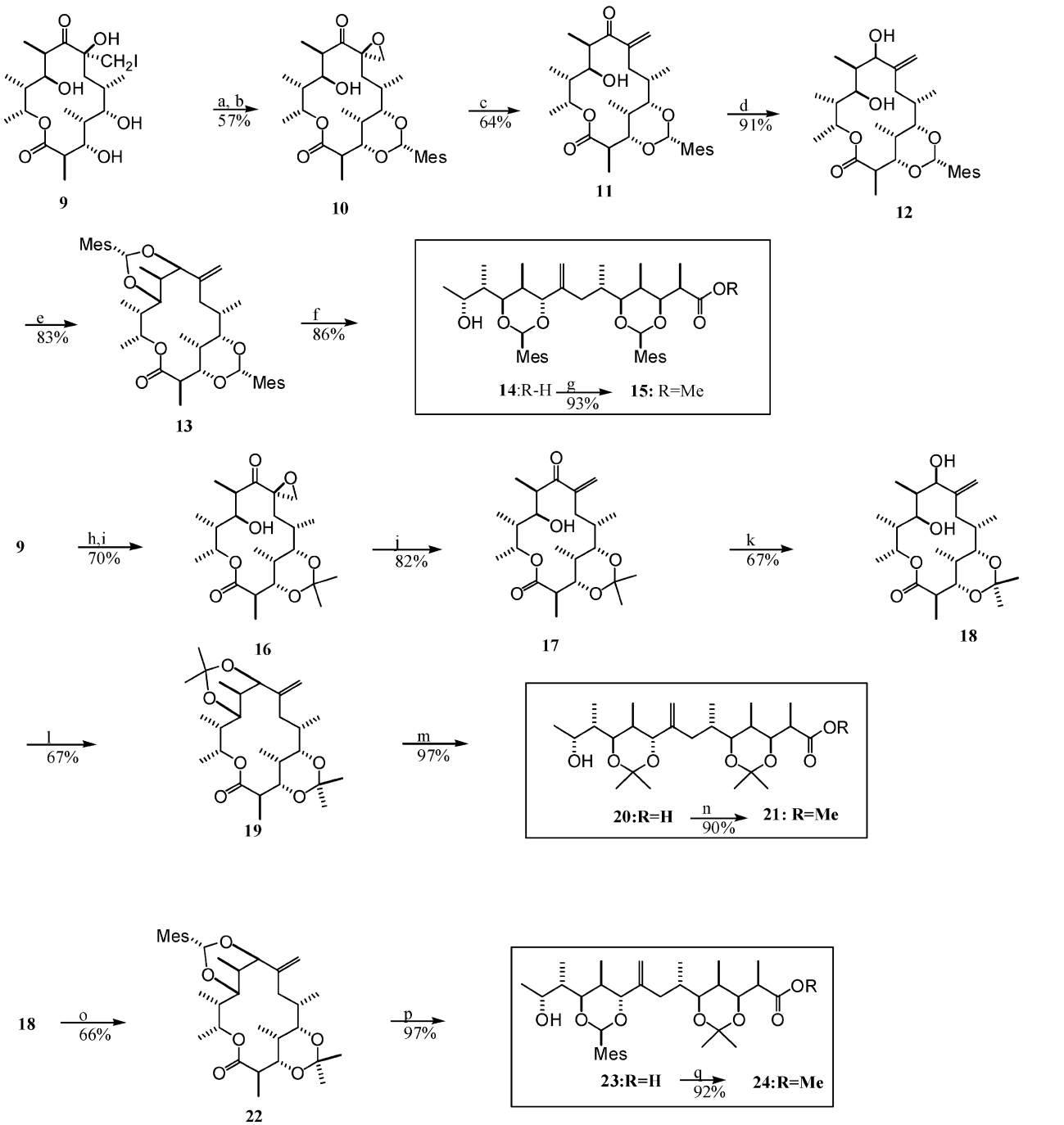


Figure 3. Conformational distance between the cluster of lactone (**3**, **5** and **7**) and the corresponding *seco*-acid (**4**, **6** and **8**).

structure, the reaction point (alcohol at C13 and terminal carboxylic acid) can not approach each other. This result suggests that *seco*-acid **4** having a similar conformation to the corresponding lactone will easily cyclize to form macrolactone.[9] The diacetonide *seco*-acid **6** will cyclize slowly in low yield. The *seco*-acid having a tertiary alcohol at C8 (**8**) can be predicted not to cyclize because unfavorable conformation to cyclize is locked by hydrogen bonding. To test the predicted reactivity of these three types of *seco*-acids, we synthesized several model *seco*-acids, and performed cyclization experiments.

2.2. Synthesis of model *seco*-acids (14, 20, 23, and 32)

seco-Acids (**14**, **20**, **23**, and **32**) were prepared starting from the known intermediate **9** reported by Paterson et al.[11] as shown in Schemes 2 and 3. The iodide **9** was converted to epoxy-ketone **10** via acetalization with mesitaldehyde dimethylacetal and cyclization of iodohydrin with $NaHCO_3$. The epoxide of **10** was deoxygenated to form *exo*-olefin **11**, followed by 1,2-reduction of enone to give diol **12** as a single diastereomer. Acetalization of diol of **12** and hydrolysis of lactone gave *seco*-acid **14**. *seco*-Acid **20** and **23** were synthesized by a similar method. Acetalization of **9** and epoxide formation gave **16** and deoxigenation of **16** gave **17**, then 1,2-reduction afforded diol **18** as a single isomer. The diol of **18** was protected as an acetonide and hydrolyzed with NaOH to form *seco*-acid **20**. Similar treatment of **18** with mesitaldehyde dimethylacetal, and hydrolysis of lactone gave *seco*-acid **23**. *seco*-Acid **32** was also synthesized starting from **9** as shown in Scheme 3. The diol of **9** was protected as a mesitilidene acetal, following reduction of ketone, and the resulting diol was again protected as a mesitilidene acetal to give **28**. The lactone and epoxide were reduced with LAH to form a triol. The primary alcohol of the triol was protected as a TBDMS



Scheme 2. Preparation of model *seco*-acid derivatives **14**, **20** and **23**. Reagents and conditions: (a) $MesCH(OMe)_2$, CSA, CH_2Cl_2, rt, 6 h; (b) 8% aq-$NaHCO_3$, THF, rt, 20 min; (c) $CrCl_2$, acetone–H_2O (2:1), 0 °C, 3 h; (d) $BaBH_4$, $CeCl_3$,THF, −25 °C, 4.5 h; (e) $MesCH(OMe)_2$, CSA, rt, 24 h; (f) 5 N-NaOH, DMSO, 90 °C, 5 h; (g) Me_3SiCHN_2, benene, rt, 1 h; (h) $Me_2C(OMe)_2$, PPTS, CH_2Cl_2, rt, 1 h; (i) 10% aq-$NaHCO_3$, THF, rt, 40 min; (j) $CrCl_2$, acetone–H_2O (2:1), 0 °C, 30 min; (k) $NaBH_4$, $CeCl_3$, −25 °C, 4.5 h; (l) 2-Methoxypropene, PPTS, CH_2Cl_2, 0 °C, 1.25 h; (m) 5 N-NaOH, DMSO, 90 °C, 7 h; (n) Me_3SiCHN_2, benzene, rt, 45 min; (o) $MesCH(OMe)_2$, CH_2Cl_2, CSA, rt, 6 h; (p) 5 N-NaOH, DMSO, 90 °C, 12 h; (q) Me_3SiCHN_2, benzene, rt, 45 min.

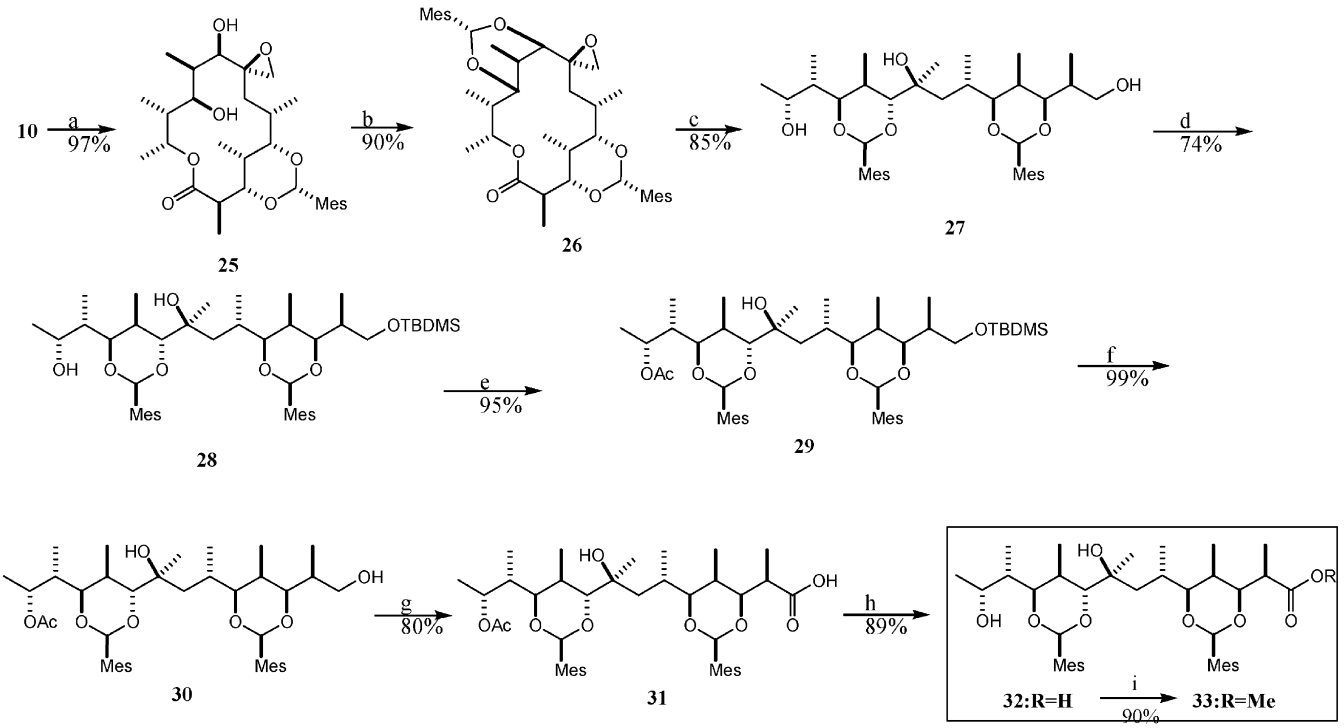
ether, and the secondary alcohol was acetylated to give **29**. Deprotection of TBDMS and Jones oxidation followed by deacetylation gave *seco*-acid **32**.

2.3. Cyclization experiments of the model *seco*-acids

seco-Acids (**14**, **20**, **23**, and **32**) were subjected to macrolactonization. The results of cyclization experiments are shown in Table 3 and Scheme 4. As we predicted, *seco*-acid **14** and **23** cyclize smoothly to give macrolactones **13** and **22** in high yield, even under the normal concentration conditions. On the other hand, *seco*-acid **20** (diacetonide) cyclized sluggishly to form lactone **19** in low yield even under the high dilution condition. *seco*-Acid **32** did not give **34** at all even under the high-dilution condition (Table 3 and Scheme 4).

2.4. Conclusion

The compatibility between computer-simulated reactivity



Scheme 3. Preparation of *seco*-acid derivative **32**. Reagents and conditions: (a) $NaBH_4$, *i*-PrOH-AcOEt (2:1), rt, 20 min. (b) $MesCH(OMe)_2$, CSA, CH_2Cl_2, rt, 5 h. (c) $LiAlH_4$, Et_2O, 30 °C, 2 h. (d) TBDMSCl, Et_3N, DMAP, CH_2Cl_2, rt, 24 h. (e) Ac_2O, Et_3N, DMAP, CH_2Cl_2, rt, 33 h;. (f) n-Bu_4NF, THF, rt, 3.5 h. (g) Jones reagent, acetone, −30 °C, 5 h. (h) 15% NaOH, MeOH, rt, 24 h. (i) Me_3SiCHN_2, benzene, rt, 1 h.

and chemical reactivity is so important that the computer-simulated conformation analysis may predict the reactivity of intramolecular cyclization, and the most preferable model *seco*-acid to synthesize lankanolide is *seco*-acid **23**. In some cases, computer-assisted conformation analysis of model *seco*-acid may complement synthetic design. We are continuing research along this line, and we reported a successful example of total synthesis of lankanolide **2** via the *seco*-acid designed according to the model *seco*-acid **23**.[12]

3. Experimental

3.1. General procedures

All reactions were carried out under argon atmosphere unless otherwise specified. CH_2Cl_2, DMSO and triethylamine were distilled from $CaCl_2$ and stored on molecular sieves. Ether and THF were distilled from sodium-benzophenone ketyl, and used freshly. Optical rotations were measured with a JASCO DIP-370 polarimeter, and P-1030 polarimeter. IR spectra were recorded with a JASO FT/IR-5300 spectorometer. Proton and carbon NMR were recorded with JEOL-EX-270, JEOL-EX-400 and Bruker ARX-500 spectrometers, using tetramethylsilane as an internal standard. Mass spectra were recorded with JEOL JMS-700 TZ and JEOL HMS-HX1100. Column chromatographies were performed on Kanto silicagel 60N (spherical, neutral; 40–100 μm). Merk precoated silicagel $60F_{254}$ plates (0.25 mm thickness) were used for analytical thin-layer chromatography.

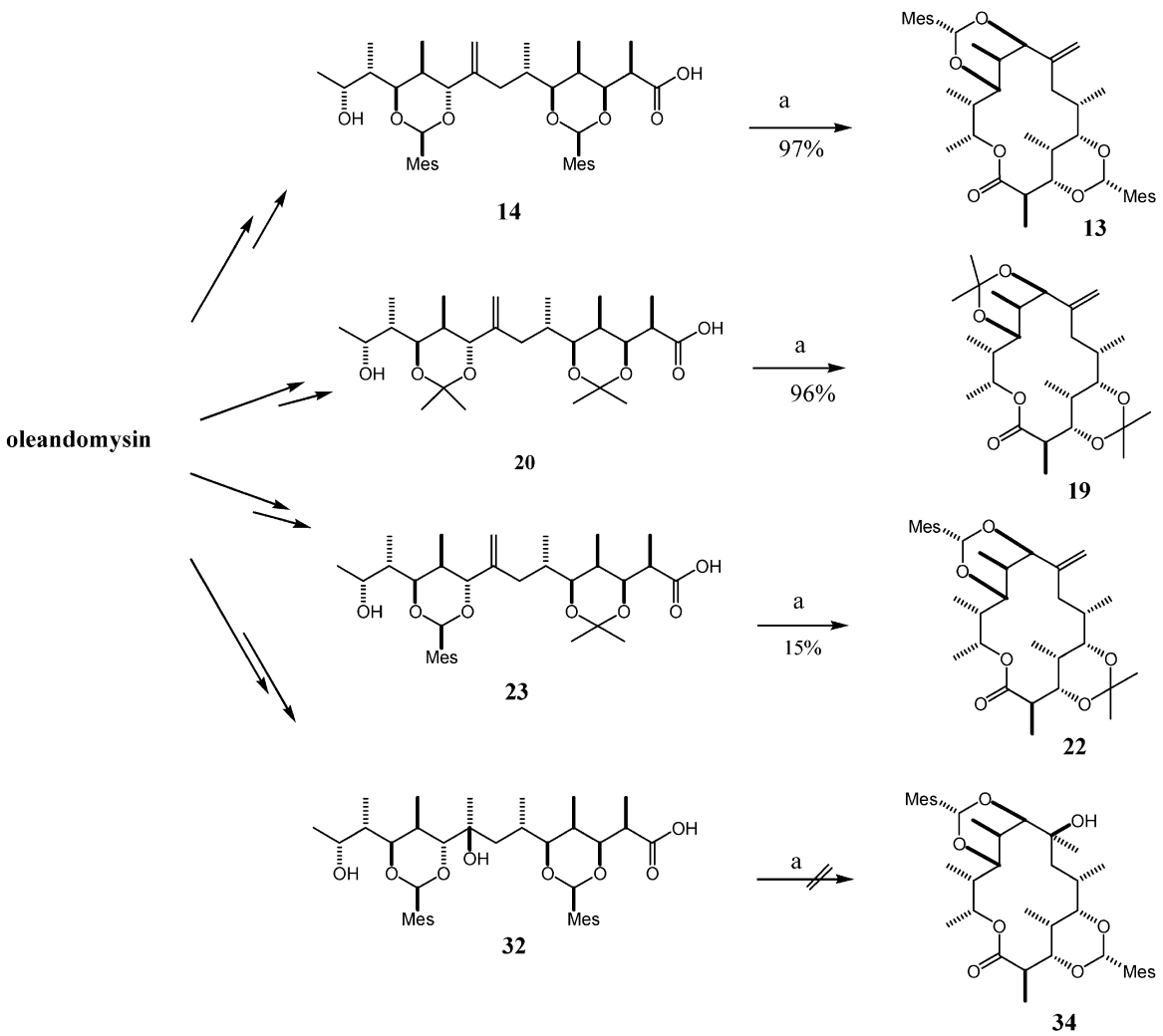
3.2. Preparation of *seco*-acids 14, 20, and 33

3.2.1. 3,5-*O*-(2,4,6-Trimethylbenzylidene)-oleandonolide (10). A solution of **9** (264 mg, 0.51 mmol) and mesitaldehyde dimethylacetal (63 mg, 1.02 mmo0l) and camphorsulfonic acid (3 mg, 13 μmol) in CH_2Cl_2 (4 ml) was stirred for 6 h and then treated with sat. $NaHCO_3$. The mixture was extracted with CH_2Cl_2 and the combined extract was washed with brine, dried over Na_2SO_4, and evaporated to

Table 3. Cyclization reactions of several *seco*-acids

seco-Acid (mM)	Cyclization method[a]	DMAP (mol equiv.)	Reaction temp (°C)	Reaction time (h)	Yield (%)
14 (1.0)	A	2.5	130	43	97
14 (6.6)	B	3	rt	3	82
23 (1.0)	A	2.5	130	11	96
23 (1.0)	B	3	rt	3	81
20 (1.0)	A	2.5	130	43	15
20 (1.0)	B	2.5	rt	43	0
32 (1.0)	A	2.5	130	43	0

[a] A; High-dilution condition: To a 6 mM toluene solution of DMAP in toluene, was added slowly a 2 mM toluene solution of the mixed anhydride prepared from *seco*-acid and 2,4,6-trichlorbenzoyl chloride. B: Normal condition: DMAP was added to a 10 mM toluene solution of the mixed unhydride.



Scheme 4. Effect of C8 functional groups on macrolactonization. Reagents and conditions: (a) to a solution of DMAP was added slowly a dilute solution of the mixed anhydride prepared from *seco*-acid (**14**, **20**, **23** and **32**) and 2,4,6-trichlorobenzoyl chloride.

dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (3:1) to give iodohydrin (187 mg, 57%) as an amorphous solid.

$[\alpha]_D^{22}=-46.0°$ (c=0.98, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 6.81 (2H, s), 5.80 (1H, s), 5.63 (1H, dq, J=2.0, 6.9 Hz), 3.93 (1H, dd, J=10.3, 3.4 Hz), 3.84 (1H, dd, J=6.0, 1.3 Hz), 3.80 (1H, s), 3.69 (1H, t, J=6.0 Hz), 3.59 (1H, dd, J=5.2, 0.9 Hz), 3.58 (1H, t, J=6.5 Hz), 3.53 (1H, d, J=10.3 Hz), 3.42 (1H, d, J=10.3 Hz), 3.12 (1H, q, J=6.9 Hz), 3.11 (1H, d, J=3.0 Hz), 2.84 (1H, dq, J=10.3, 6.7 Hz), 2.57 (1H, m), 2.45 (6H, s), 2.23 (3H, s), 2.21 (1H, m), 2.01 (1H, q, J=6.5 Hz), 1.84 (1H, d, J=14.5 Hz), 1.82 (1H, dd, J=14.5, 6.1 Hz), 1.70–1.58 (2H, m), 1.25 (3H, d, J=6.7 Hz), 1.16 (3H, d, J=6.6 Hz), 1.36 (3H, d, J=6.7 Hz), 1.08 (3H, d, J=7.0 Hz), 1.06 (3H, d, J=7.4 Hz), 0.93 (3H, d, J=7.3 Hz). MS (EI) m/z 644 (M^+). HRMS (EI) m/z Calcd for $C_{30}H_{45}O_7I$ (M^+) 644.2228, found 644.2205.

A cooled (0 °C) mixture of the above iodohydrin (151.2 mg, 0.23 mmol) in THF (25 ml) and 8% aq. $NaHCO_3$ (25 ml) was stirred for 20 min and then treated with phosphate buffer (pH 6.86, 25 ml). After stirring for further 5 min, the mixture was extracted with CH_2Cl_2 and the combined extract was washed with brine and dried over Na_2SO_4 and concentrated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (3:1) to give **10** (118 mg, quant.) as an amorphous solid.

$[\alpha]_D^{25}=-52.0°$ (c=0.77, $CHCl_3$). IR (neat) 1740 cm^{-1}. ^1H NMR (400 MHz, C_6D_6) δ 6.77 (2H, s), 6.06 (1H, dq, J=1.0, 0.6 Hz), 5.98 (1H, s), 4.46 (1H, dq, J=2.0, 6.4 Hz), 4.01 (1H, dd, J=1.5, 7.3 Hz), 3.89 (1H, dd, J=1.5, 10.7 Hz), 2.91 (1H, d, J=4.9, 6.8 Hz), 2.77 (1H, d, J=4.4 Hz), 2.66 (1H, dd, J=2.0, 6.4 Hz), 2.55 (6H, s), 2.29–2.34 (1H, m), 2.30 (1H, d, J=3.9 Hz), 2.13 (3H, s), 1.95 (1H, dd, J=12.2, 15.6 Hz), 1.77–1.88 (1H, m), 1.47–1.55 (1H, m), 1.42 (1H, dd, J=2.0, 15.6 Hz), 1.37 (3H, d, J=6.8 Hz), 1.36 (3H, d, J=6.8 Hz), 1.20 (3H, d, J=6.3 Hz), 1.10 (3H, d, J=6.3 Hz), 1.10 (3H, d, J=6.3 Hz), 1.05 (3H, d, J=7.3 Hz), 0.76 (3H, d, J=6.8 Hz). ^{13}C NMR (100.4 Hz, C_6D_6) δ 204.9, 173.9, 137.9, 137.1, 132.1, 130.1, 127.9, 103.9, 85.2, 70.1, 69.9, 63.5, 47.2, 46.5, 41.7, 33.3, 32.3, 21.0, 20.9, 18.4, 16.6, 13.3, 9.7, 6.4. MS (EI) m/z 516 (M^+). HRMS (EI) m/z Calcd for $C_{30}H_{44}O_7$ (M^+) 516.3087, found 516.3068.

3.2.2. 8,8a-Deoxa-3,5-*O*-(2,4,6-trimethylbenzylidene)-oleandonolide (11). To a cooled (0 °C) solution of **10** (428 mg, 0.83 mmol) in acetone (7 ml) was added dropwise $CrCl_2$ (306 mg, 2.49 mmol) in H_2O 3.5 ml, and the solution was treated with sat. $NaHCO_3$, and extracted with ether. The combined extract was washed with brine and dried over Na_2SO_4 and concentrated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate to give *exo*-olefin **11** (266 mg, 64%) as an amorphous solid.

$[\alpha]_D^{22}=-55.7°$ (c=0.98, $CHCl_3$). IR (neat) 1740, 1702, 1620 cm^{-1}. ^1H NMR (400 MHz, C_6C_6) δ 6.78 (2H, s), 6.05 (1H, s), 5.95 (1H, s), 5.76 (1H, dq, J=1.0, 6.4 Hz), 5.13 (1H, s), 4.03 (1H, dd, J=1.0, 6.4 Hz), 3.80 (1H, dd, J=5.4, 8.8 Hz), 3.68 (1H, dd, J=1.5, 10.8 Hz), 2.89 (1H, dq, J=1.0, 6.8 Hz), 2.82 (1H, dq, J=6.4, 10.8 Hz), 2.71 (1H, d, J=5.4 Hz), 2.56 (6H, s), 2.48–2.56 (1H, m), 2.15 (3H,s), 2.13–2.18 (1H, m), 1.98 (1H,dd, J=6.8, 13.7 Hz), 1.91 (1H, dd, J=2.4, 18.1 Hz), 1.38–1.47 (1H, m), 1.34 (3H, d, J=6.8 Hz), 1.29 (3H, d, J=6.4 Hz), 1.17 (3h, d, 6.4 Hz), 1.15 (3H, d, J=7, 3 Hz), 1.06 (3H, d, J=6.4 Hz), 0.72 (3H, d, J=7.3 Hz). ^{13}C NMR (100.4 MHz, C_6D_6) δ 204.79, 174.5, 146.8, 138.0, 137.1, 131.9, 130.2, 127.9, 121.0, 104.0, 85.2, 80.8, 70.6, 43.6, 42.6, 41.6, 34.0, 33.1, 33.0, 21.0, 20.9, 18.5, 16.6, 13.3, 9.8, 9.0, 6.6. MS (EI) m/z 500 (M^+). HRMS (EI) m/z Calcd for $C_{30}H_{44}O_6$ (M^+) 500.3184, found 500.3137.

3.2.3. (9*R*)-3,5-*O*-(2,4,6-Trimethylbenzylidene)-8,8a-deoxa-9-dehydro-oleandonolide (12). To a cooled (−25 °C) solution of **11** (266 mg, 0.53 mmol) in THF (8 ml) was added $CeCl_3.H_2O$ (102 mg, 0.27 mmol) and after stirring for 30 min, $NaBH_4$ 38 mg, (1.00 mmol) was added to the suspension and the mixture was stirred for 4.5 h at −25 °C and treated with sat. NH_4Cl and extracted with ether. The combined extract was washed with brine and dried over Na_2SO_4 and evaporated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (3:1) to afford diol **12** (230 mg, 91%) as an amorphous solid.

$[\alpha]_D^{23}=-28.0°$ (c=0.98, $CHCl_3$). IR (neat) 1730, 1660, 1620 cm^{-1}. ^1H NMR (400 MHz, C_6C_6) δ 6.80 (1H,s), 6.02 (1H, s), 5.64–5.67 (1H, m), 5.61 (1H, s), 5.14 (1H, s), 4.35 (1H, dd, J=1.5, 114 Hz), 3.75–3.82 (3H, m), 3.56–3.62 (2H, m), 2.78–2.97 (3H, m), 2.59 (6H, s), 2.15 (1H, s), 1.59–1.90 (4H, m), 1.34 (3H, d, J=7.0 Hz), 1.31 (3H, d, J=6.6 Hz), 1.20 (3H, d, J=7.3 Hz), 1.14 (3H, d, J=7.0 Hz), 1.04 (3H, d, J=6.6 Hz), 0.68 (3H, d, J=7.0 Hz). MS (EI) m/z 502 (M^+); HRMS (EI) m/z Calcd for $C_{30}H_{46}O_6$ (M^+) 502.3230, found 502.3322.

3.2.4. (9*R*)-3,5:9,11-Bis-*O*-(2,4,6-trimethylbenzylidene)-8,8a-deoxa-9-dihydro-oleandonolide (13). A solution of the above diol **12** (72 mg, 0.14 mmol) and mesitaldehyde dimethylacetal (82 mg, 0.92 mmol) and camphorsulfonic acid (3 mg) was stirred for 24 h and then treated with sat. $NaHCO_3$. The mixture was extracted with ether and the extract was washed with brine and dried over Na_2SO_4 and evaporated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with benzene hexane (1:1) to hexane and ethyl acetate (9:1) to give dimesitilidene acetal **13** (73 mg, 86%) as an amorphous solid.

$[\alpha]_D^{22}=-67.4°$ (c=1.02, $CHCl_3$). IR (neat) 1720, 1630, 1630 cm^{-1}. ^1H NMR (400 MHz, C_6C_6) δ 6.80 (4H, d, 10.0 Hz), 6.48 (1H, s), 6.15 (1H, s), 5.86 (1H, dq, J=0.9, 6.6 Hz), 5.73 (1H, s), 5.28 (1H,s), 4.43 (1H, dd, J=2.0, 7.0 Hz), 4.36 (1H, br. s). 3.96 (1H, d, J=10.9 Hz), 3.63 (1H, dd, J=1.0, 10.0 Hz), 2.88 (6H, s), 2.84–2.90 (1H, m), 2.73–2.78 (1H, s), 2.57 (1H,s), 2.13 (6H, d, J=6.8 Hz), 1.88–2.01 (2H, m), 1.92 (1H, dd, J=17.5, 12.0 Hz), 1.69 (1H, br d, J=17.5 Hz), 1.50–1.56 (1H, m), 1.40 (3H, d, J=6.6 Hz), 1.39 (3H, d, J=6.8 Hz), 1.33 (3H, d, J=6.6 Hz), 1.23 (3H, d, J=7.1 Hz), 0.91 (3H, d, J=7.3 Hz), 0.77 (3H, d, J=7.3 Hz). MS (EI) m/z (M^+) 630. HRMS (EI) m/z Calcd for $C_{40}H_{56}O_6$ (M^+) 632.4077, found 632.4080.

3.2.5. (9*R*)-3,9:9,11-Bis-*O*-(2,4,6-trimethylbenzylidene)-8,8a-deoxa-9-dihydrooleandonolide *seco*-acid (14). A solution of **13** (175 mg, 0.28 mmol)and 5-N NaOH (1.6 ml, 8 mmol) in DMSO (3.8 ml)was stirred for 5 h at 90 °C and then cooled to room temperature and extracted with ether. The aqueous layer was neutralized with 10% aq. HCl (6 ml). The mixture was extracted with ether and the combined extract was washed with brine and dried over Na_2SO_4 and evaporated to dryness in vacuo. The residue was chromatographed on a silicagel column eluted with hexane and ethyl acetate (1:2) to give *seco*-acid **14** (154 mg, 86%) as an amorphous solid. Because NMR of the *seco*-acid showed peak broadening, the *seco*-acid was characterized as its methyl ester **15**.

$[\alpha]_D^{23}=-57.9°$ (c=0.50, $CHCl_3$). IR (neat) 1720, 1630, 1620 cm^{-1}. MS (EI) m/z 650 (M^+). HRMS (EI) m/z Calcd for $C_{40}H_{58}O_7$ (M^+) 640.4183, found 650.4166.

3.2.6. (9*R*)-3,5:9,11-Bis-*O*-(2,4,6-trimethylbenzylidene)-8,8a-deoxa-9-dihydrooleandonolide *seco*-acid methyl ester (15). To a solution of **14** (12.0 mg, 1.5 μmol) in benzene 1.0 ml) was added MeOH (200 μl) and 10% hexane solution of trimethylsilyldiazomethane (200 μl, 176 μmol) and the solution was stirred for 1 h and then treated with acetic acid and the solution was diluted with ether and washed with sat. $NaHCO_3$ and brine and dried over Na_2SO_4 and concentrated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (4:1) to give methyl ester **15** (11.4 mg, 93%) as an amorphous solid.

^1H NMR (400 MHz, C_6C_6) δ 6.73 (4H, m) 6.15 (1H,s), 5.79 (1H, s), 5.11 (1H,s), 4.29 (1H, br s), 4.20 (1H, dd, J=2.0, 10.3 Hz), 3.98 (1H, dq, J=2.1, 6.6 Hz), 3.93 (1H, dd, J=2.1, 10.1 Hz), 3.35 (3H, s), 3.17 (1H, dd, J=2.1, 9.7 Hz), 3.00 (1H, br dd, J=3.0, 15.0 Hz), 2.87 (1H, dq, J=6.8, 10.1 Hz), 2.54 (3H, s), 2.39 (3H, s), 2.19 (3H, s), 2.10–2.04 (1H, m), 1.00–2.04 (1H, m), 1.78–1.89 (3h, M), 1.32 (3h, D, J=6.8 Hz), 1.28 (3H, d, J=6.8 Hz), 1.14 (3H, d, J=6.8 Hz), 1.00 (3H, d, J=6.6 Hz), 0.80 (3H, d, J=6.8 Hz), 0.66 (3H, d, J=7.1 Hz).

3.2.7. 3,5-*O*-(Isopropylidene)-oleandonolide (16). Compound **16** was prepared following the known method by Paterson et al.[11] To a solution of **9** (130 mg, 0.273 mmol)

and 2,2-dimethoxpropane (6 ml, 48.9 mmol) in CH_2Cl_2 (10 ml) was added PPTS (7 mg, 0.028 mmol) at rt. The solution was stirred for 1 h at rt, then treated with phosphate buffer (pH 7.0), and extracted with CH_2Cl_2. The combined extract was concentrated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (2:1) to give acetonide (105 mg, 75%). To a cooled (0 °C) solution of the acetonide (460 mg, 0.830 mol) in THF (75 ml) was added 10% aq.$NaHCO_3$ (75 ml), and the mixture was stirred for 40 min, then treated with phosphate buffer (pH7.0). The mixture was extracted with CH_2Cl_2 and the combined extract was dried over $MgSO_4$ and concentrated to dryness. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (2:1) to give **16** (355 mg, quantitative yield) as an amorphous solid.

$[\alpha]_D^{23}=-50.1°$ (c=1.20, $CHCl_3$). ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.76 (1H, dq, J=1.2, 6.6 Hz), 4.36 (1H, m), 4.01 (1H, dd, J=1.3, 6.9 Hz), 3.74 (1H, J=1.3, 10.7 Hz), 3.10 (1H, d, J=4.2 Hz), 3.03 (1H, dq, J=1.8, 6.5 Hz), 2.96 (1H, d, J=4.2 Hz), 2.75 (1H, dq, J=6.6, 10.6 Hz), 2.41 (1H, d, J=5.5 Hz), 2.24 (1H. dd, J=12.4, 15.7 Hz), 1.96–2.08 (2H, m), 1.65 (1H, m), 1.42 (3H, s), 1.41 (3H, s), 1.28 (3H, d, J=6.6 Hz), 1.16 (3H, d, J=6.5 Hz), 1.07 (3H, d, J=6.5 Hz), 1.04 (3H, d, J=6.5 Hz), 1.03 (3H, d, J=7.2 Hz), 1.01 (3H, d, J=7.2 Hz).

3.2.8. 8,8a-Deoxa-3,5-*O*-(2,4,6-isopropylidene)-oleandonolide (17). To a cooled (0 °C) solution of **16** (2.03g, 4.76 mmol) in acetone (40 ml) was added dropwise $CrCl_2$ (1.76 g, 14.28 mmol) in H_2O (20 ml). After stirring for 30 min, the solution was treated with sat. $NaHCO_3$, extracted with brine and dried over Na_2SO_4 and evaporated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate to give *exo*-olefin **17** (1.60 g, 82%) as an amorphous solid.

$[\alpha]_D^{24}=-30.9°$ (c=1.00, $CHCl_3$). IR (neat) 1720 cm$^-$ ^{1}H NMR (400 MHz, C_6C_6) δ 6.00 (1H,s), 5.74 (1H, dq, J=1.5, 6.00 Hz), 5.08 (1H, s), 4.10 (1H, dd, J=1.5, 6.2 Hz), 3.72–3.79 (2H, m), 2.72–2.90 (3, m), 1.79–2.36 (5H, m), 1.33 (3H, d, J=6.6 Hz), 1.24 (6H, s), 1.17 (3H, d, J=6.6 Hz), 1.15 (3H, d, J=6.6 Hz), 1.10 (3H, d, J=6.2 Hz), 0.69 (3, d, J=7.0 Hz). MS (EI) m/z 410 (M^+). IHRMS (EI) m/z Calcd for $C_{23}H_{38}O_6$ (M^+) 410.2669, found 410.2643.

3.2.9. (9*R*)-3,5-*O*-Isopropylidene-8,8a-deoxa-9-dihydro-oleandonilide (18). To a cooled (−25 °C) solution of **17** 200 mg, 0.49 mmol) in THF (7.5 ml) was added $CeCl_3$. $7H_2O$ (94 mg, 0.54 mmol) and after stirring for 1 h, $NaBH_4$ 30 mg, (0.79 mmol) was added to the suspension and the mixture was stirred for 4.5 h at −25 °C and treated with sat. NH_4Cl and extracted with ether. The combined extract was washed with brine and dried over Na_2SO_4 and evaporated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (3:1) to afford diol **18** (205 mg, quantitative yield) as an amorphous solid.

$[\alpha]_D^{21}=25.0°$ (c=1.20). ^{1}H NMR (400 MHz, C_6D_6) δ. 5.48 (1H, s), 5.46 (1H, dd, J=6.6, 1.0 Hz), 5.07 (1H, s), 4.33 (1H, dd, J=6.6, 1.0 Hz), 4.00 (1H, br. s), 3.62 (1H, dd, J=10.5, 1.0 Hz), 3.46 (1H, m), 3.37 (1H, d, J=8.0 Hz), 3.03 (1H, d, J=4.0 Hz), 2.63 (1H, dd, J=11.5, 6.8 Hz), 2.50 (1H, m), 2.04 (1H, m), 1.97 (1H, dd, J=17.0, 11.5 Hz), 1.50–1.61 (2H, m), 1.44 (6H, s), 1.25 (3H, d, J=6.8 Hz), 1.12 (3H, d, J=6.8 Hz), 1.03 (3H, d, J=7.3 Hz), 0.98 (3H, d, J=6.8 Hz), 0.98 (3H, d, J=7.3 Hz). ^{13}C NMR (100.4 MHz, C_6D_6), δ 175.8, 147.6, 109.2, 100.6, 72.972.5, 72.1, 69.5, 42.6, 41.6, 35.3, 34.6, 32.4, 32.0, 29.7, 19.9, 18.7, 16.3, 13.2, 9.6, 8.7, 7.7. MS (EI) m/z 412 (M^+). HRMS (EI) m/z Calcd for $C_{23}H_{40}O_6$ (M^+) 412.2825, found 412.2838.

3.2.10. (9*R*)-3,5:9,11-Bis-*O*-isopropylidene-8,8a-deoxa-9-dihydrooleandonolide (19). A solution of the above diol **18** (112 mg, 0.27 mmol) in CH_2Cl_2 was added 2-methoxy-propene (80 ml, 0.81 mmol) and PPTS (20 mg, 85 μmol) at 0 °C and the solution was stirred for 1.25 h and then treated with sat. $NaHCO_3$ and extracted with ether. The combined extract was washed with brine and dried over Na_2SO_4 and concentrated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane ethyl acetate (8:1) to give diacetonide **19** (85 mg, 70%) as an amorphous solid.

$[\alpha]_D^{19}=-26.6°$ (c=0.23, $CHCl_3$). IR (neat) 1730, 1640, 1620 cm^{-1}. ^{1}H NMR (400 MHz, C_6D_6) δ 5.62 (1H, s), 5.47 (1H, dq, J=1.0, 6.8 Hz), 5.22 (1H, br. s), 4.24 (1H, br. s), 4.19 (1H, dd, J=1.5, 6.4 Hz), 3.57 (1H, dd, J=1.0, 10.7 Hz), 3.43 (1H, d, J=9.3 Hz), 2.68 (1H, ddq, J=6.8, 2.5, 10.7 Hz), 2.49–2.56 (1H, m), 2.11 (1h, DD, J=6.5, 13.5 Hz), 1.94–1s.96 (2H, m), 1.69 (1H, dd, J=6.5, 13.5 Hz), 1.49–1.53 (1H, m), 1.45 (3H, s), 1.44 (3H, s), 1.40 (3H.s), 1.29 (3H, s), 1.22 (3H, d, J=6.8 Hz), 1.13 (3H, d, J=6.8 Hz), 1.10 (3H, d, J=6.8 Hz), 1.08 (3H, d, J=6.8 Hz), 1.04, (3H, d, J=6.4 Hz), 0.93 (3H, d, 6.7 Hz). ^{13}C NMR (100.4 Hz, C_6D_6) δ 174.0, 144.8, 113.9, 100.8, 100.2, 80.8, 77.2, 71.9, 69.6, 68.5, 41.0, 40.2, 33.7, 32.5, 32.4, 31.0, 29.8, 29.0, 26.8, 19.7, 18.6, 16.2, 13.0, 11.7, 7.8, 7.4. MS (EI) m/z 452 (M^+). HRMS (EI) m/z Calcd for $C_{26}H_{44}O_6$ (M^+) 452.3138, found 452.3120.

3.2.11. (9*R*)-3,5:9,11-Bis-*O*-isopropilidene-8,8a-deoxa-9-dihydrooleandonolide *seco*-acid (20). A solution of the above diacetonide lactone **19** (121 mg, 0.29 mmol) and 5 N-NaOH (1.7 ml) in DMSO (3.9 ml) was stirred for 7 h at 90 °C. After cooling to room temperature, the solution was extracted with ether. The combined organic layer was washed with sat. $NaHCO_3$ and water and the combined aqueous layer was neutralized with 10% HCl and extracted with ether and the extract was washed with brine and dried over Na_2SO_4 and concentrated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (1:2) to give *seco*-acid **20** (91 mg, 66%) as an amorphous solid. Because NMR spectrum of the *seco*-acid showed peak broadening, the *seco*-acid was characterized as its methyl ester **21**.

3.2.12. (9*R*)-3,5:9,11-Bis-*O*-isopropilidene-9-dihydro-oleandonolide *seco*-acid methyl ester (21). To a solution of **20** (10.0 mg, 21.3 μmol) in benzene 1.0 ml) was added MeOH (200 μl) and 10% hexane solution of trimethyl-silyldiazomethane (200 μl, 176 μmol) and the solution was stirred for 45 min and then treated with acetic acid and the

solution was diluted with ether and washed with sat. $NaHCO_3$ and brine and dried over Na_2SO_4 and concentrated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (4:1) to give methyl ester **21** (10.3 mg, quantitative) as an amorphous solid.

$[\alpha]_D^{21}=-20.0°$ (c=0.78). ^{1}H NMR (400 MHz, C_6D_6) δ 5.08 (1H, br. s), 4.92 (1H, br. s), 4.13 (1H, br. s). 4.06 (1H, dd, J=2.0, 5.0 Hz), 4.02 (1H, dd, J=4.4, 10.7 Hz), 3.87 (1H, d, J=7.3 Hz), 3.37 (3H, s), 3.35 (1H, dd, J=2.0, 10.3 Hz), 3.09 (1H, br. d, J=14.2 Hz), 2.87 (1H, m), 2.22–2.28 (1H, m), 2.05–2.15 (2H, m), 1.78–1.88 (2H, m), 1.64 (1H, dd, J=10.7, 13.7 Hz), 1.49 (3H, s), 1.43 (3H, s), 1.39 (3H, d, J=6.8 Hz), 1.36 (3h, S), 1.30, 3h, S), 1.15 (3H, d, J=6.4 Hz), 1.13 (3H, d, J=7.3 Hz), 0.91 (3H, d, J=6.8 Hz), 0.80 (3H, d, J=6.4 Hz), 0.67 (3H, d, J=7.3 Hz). MS (EI) m/z 484 (M^+). HRMS (EI) m/z Calcd for $C_{27}H_{48}O_7$ (M^+) 484.3400, found 484.3410.

3.2.13. 3,5-*O*-Isopropylidene-9,11-*O*-(2,4,6-trimethylbenzylidene)-(9*R*)-8,8a-deoxa-9-dihydrooleandonolide (22). A solution of diol **18** (205 mg, 0.50 mmol) and mesitaldehyde dimethylacetal (291 mg, 1.50 mmol) and camphorsulfonic acid (3 mg) was stirred for 6 h at room temperature. The solution was treated with sat. $NaHCO_3$ and extracted with CH_2Cl_2 and the combined extract was washed with sat. $NaHCO_3$ and brine and dried over Na_2SO_4 and concentrated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate to give **22** (180 mg, 66%) as an amorphous solid.

$[\alpha]_D^{23}=-39.2°$ (c=0.31, $CHCl_3$). IR (neat) 1720, 1630, 1610 cm^{-1}. ^{1}H NMR (400 MHz, C_6C_6) δ 6.82 (2H, s), 6.44 (1H, s), 5.86 (1H, dq, J=7.0, 1.0 Hz), 5.74 (1H, br. s), 5.28 (1H, br. s), 4.48 (1H, dd, J=2.0, 7.0 Hz), 4.37 (1H, br. s), 3.97 (1H, dd, 1.0, 10.7 Hz), 3.61 (1H, d, J=1.0, 9.8 Hz), 2.88 (6H, s), 2.80–2.85 (1H, m), 2.57–2.63 (1H, m), 2.13 (3H, s), 1.93 (1H, dd, J=6.8, 13.2 Hz), 1.80–1.87 (3H, m), 1.67 (1H, dd, J=17.1, 2.5 Hz), 1.58 (3H, s), 1.52 (1H, m), 1.45 (3H, s), 1.39 (3H, d, J=6.8 Hz), 1.36 (3H, d, J=6.3 Hz), 1.21 (3H, d, J=6.8 Hz), 1.19 (3H, d, J=6.8 Hz), 0.90 (3H, d, J=6.8 Hz), 0.73 (3H, J=7.3 Hz). ^{13}C NMR (100.4 MHz, C_6D_6) δ 174.4, 143.1, 138.1, 137.7, 132.1, 130.2, 128.6, 127.9, 111.8, 100.5, 98.0, 82.4, 78.1, 72.3, 69.3, 41.9, 70.3, 34.1, 32.6, 30.1, 29.5, 20.9, 19.6, 18.2, 16.4, 13.7, 13.1, 7.98, 7.14. MS (EI) m/z 542 (M^+). HRMS (EI) m/z Calcd for $C_{33}H_{50}O_6$ (M^+) 542.3608542, found 542.3608.

3.2.14. (9*R*)-3,5-*O*-Isopropylidene-9,11-*O*-(2,4,6-trimethylbenzylidene)-8,8a-deoxa-9-dihydrooleandonolide *seco*-acid (23). A solution of the **22** 160 mg, 0.30 mmol) and 5 N-NaOH (1.7 ml, 8.5 mmol) was stirred for 12 h at 90 °C, and after cooling to rt, was extracted with ether. The aqueous layer was neutralized with 10% HCl and extracted with ether. The combined extract was washed with water and brine and dried over Na_2SO_4 and concentrated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate to give *seco*-acid **23** 163 mg, 97%) as an amorphous solid. Because NMR spectrum of the *seco*-acid **23** showed peak broadening, the *seco*-acid was characterized as its methyl ester **24**.

$[\alpha]_D^{23}=-51.4°$ (c=1.00, $CHCl_3$). IR (neat) 1710, 1640, 1610 cm^{-1}. MS (EI) m/z 560 (M^+). HRMS (EI) m/z Calcd for $C_{33}H_{52}O_7$ (M^+) 560.3713, found 560.3738.

3.2.15. (9*R*)-3,5-*O*-Isopropylidene-9,11-*O*-(2,4,6-trimethylbenzylidene)-8,8a-deoxa-9-dihydrooleandonolide *seco*-acid methyl ester (24). A solution of **23** (20.0 mg, 35.7 μmol) and MeOH (400 μl) and 10% hexane solution of Me_3SiCHN_2 (400 μl, 35.7 μmol) was stirred for 45 min at room temperature and then treated with acetic acid and the solution was diluted with ether and washed with sat. $NaHCO_3$ and brine and dried over Na_2SO_4 and concentrated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (4:1) to give methyl ester **24** (18 mg, 88%) as an amorphous solid.

$[\alpha]_D^{21}=-49.3°$ (c=0.67, $CHCl_3$). EIMS m/z 574 EIHRMS m/z 574.3852 $(M+H)^+$ [Calcd for $C_{34}H54O_7$ (M^+) 574.3870]. ^{1}H NMR (400 MHz, C_6C_6) δ 6.75 (2H, s), 6.23 (1H, s), 5.21 (1H, s), 5.17 (1H, s), 4.39 (1H, s), 4.25 (1H, dd, J=2.0, 9.8 Hz), 4.01–4.06 (1H, m), 3.99 (1H, dd, J=1.5, 10.3 Hz), 3.36 (1H, s), 3.27 (1H, dd, J=8.3, 1.5 Hz), 2.93 (1H, d, J=14.2 Hz), 2.81 (1H, dq, J=10.0, 6.8 Hz), 2.65 (6H, s), 2.11 (3H,s), 2.07 (1H, dd, J=6.3, 12.7 Hz), 1.85–1.97 (2H, m), 1.69–1.75 (2H, m), 1.38–1.42 (1H, m), 1.35 (3H, d, J=6.8 Hz), 1.31 (3H, s), 1.23 (3H, s), 1.02–1.06 (6H, m), 0.78 (3H, d, J=6.8 Hz), 0.71 (3H, d, J=6.8 Hz). ^{13}C (100.4 MHz, C_6D_6) δ 174.7, 146.5, 137.1, 130.1, 128.6, 127.9, 114.0, 99.3, 81.2, 78.4, 75.7, 67.8, 51.1, 42.5, 40.0, 38.2, 32.8, 32.0, 29.8, 29.2, 20.9, 19.9, 19.6, 15.4, 14.0, 13.8, 9.5, 5.4. MS (EI) m/z 574 (M^+). HRMS (EI) m/z Calcd for $C_{33}H_{52}O_7$ (M^+) 574.3870, found 574.3852.

3.2.16. (9*R*)-9-Dihydro-3,5-*O*-(2,4,6-trimethylbenzylidene)-oleandonolide (25). To a cooled (0 °C) solution of **10** in *iso*-propanol and ethyl acetate (1:2, 48 ml) was added sodium borohydride (60.4 mg, 1.60 mmol), and the solution was stirred for 15 min at room temperature, and treated with sat. NH_4Cl and extracted with CH_2Cl_2 and the combined extract was washed with brine and dried over Na_2SO_4 and concentrated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (2:1) to give **25** (399 mg, 97%) as an amorphous solid.

$[\alpha]_D^{21}=-6.47$ (c=0.66, $CDCl_3$). IR (neat) 1735, 1620 cm^{-1}. ^{1}H NMR (270 MHz, C_6D_6) δ 6.78 (2h, S), 6.08 (1h, s), 5.70 (1H, dq, J=0.8, 6.8 Hz), 4.21 (1H, dd, J=1.1, 7.3 Hz), 4.10 (1H, m), 4.03 (1H, dd, J=1.5, 10.8 Hz), 3.75 (1H, d, J=10.2 Hz), 3.54 (1H, dd, J=3.5, 10.2 Hz), 3.22 (1H, d, J=5.8 Hz), 3.14 (1H, d, J=5.3 Hz), 2.90 (1H, dq, J=6.6, 10.8 Hz), 2.57 (6H, s), 2.32 (1H, d, J=5.3 Hz), 2.21 (1H, m), 2.13 (3H,s), 1.94–2.09 (2H, m), 1.84 (1H, m), 1.66 (1H, dd, J=12.5, 15.6 Hz), 1.37 (3H, d, J=6.6 Hz), 1.36 (3H, d, J=6.8 Hz), 1.17 (3H, d, J=6.7 Hz), 1.11 (3H, d, J=6.8 Hz), 1.02 (3H, d, J=6.8 Hz), 0.74 (3H, d, J=7.0 Hz). MS (EI) m/z 518 (M^+). HRMS (EI) Calcd for $C_{30}H_{46}O_7$ (M^+) 518.3241, found 518.3291.

3.2.17. (9*R*)-9-Dihydro-3,5:9,11-bis-*O*-(2,4,6-trimethylbenzylidene)-oleandonolide (26). A solution of **25** (250 mg, 482 mmol) and mesitaldehyde dimethylacetal (118 mg, 965 mmol) and camphorsulfonic acid (10 mg, 43 mmol) in CH_2Cl_2 (5 ml) was stirred for 5 h at room temperature, and then treated with excess triethylamine. The reaction mixture was concentrated in vacuo and the residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (15:1 to 1:1) to give **26** (188 mg, 90% based on reacted **25**) as an amorphous solid and recovered **25** (75.5 mg, 33%) as an amorphous solid.

$[\alpha]_D^{23}=-75.8$ (c=0.63, $CDCl_3$).IR (neat) 1730 cm^{-1}. ^1H NMR (400 MHz, C_6D_6) δ 7.17 (1H, s), 6.78 (2H, s), 6.77 (2H, s), 6.10 (1H, s), 5.88 (1H, dq, J=0.7, 6.7 Hz), 4.61 (1H, dd, J=0.8, 5.2 Hz),4.22 (1H, dd, J=1.1, 10.8 Hz), 4.01 (1H, dd, J=1.1, 9.3 Hz), 3.78 (1H, s), 2.94 (1H, dq, J=6.8, 10.8 Hz), 2.91 (6H, s), 2.72 (1H, d, J=5.4 Hz), 2.53 (6H, s), 2.20 (1H, m), 2.18 (1H, d, J=5.4 Hz), 2.12 (3H, s), 2.10 (3H, s), 2.05 (1H, m), 1.90–2.00 (2H, m), 1.57 (1H,), 1.44 (3H, d, J=6.8 Hz), 1.41 (3H, d, J=6.8 Hz), 1.38 (3H, d, J=6.8 Hz), 1.13 (3H, d, J=6.4 Hz), 0.94 (3H, d, J=6.7 Hz), 0.88 (3H, d, J=7.3 Hz). MS (EI) m/z 648 (M^+). HRMS (EI) m/z Calcd for $C_{40}H_{56}O_7$ (M^+) 648.4023, found 648.4030.

3.2.18. (2*S*,3*R*,4*S*,5*R*,6*R*,8*S*,10*R*,11*S*,12*R*,13*R*)-2,4,6,8,10,12-Hexamethyl-3,5:9,11-bis-(2,4,6-trimethylbenzylidenedioxy)-tetradecane-1,8,13-triol (27). To a solution of lactone **26** (343 mg, 529 mmol) in ether (10 ml) was added $LiAlH_4$ (60.2 mg, 1.59 mmol) in ether (1 ml), the reaction mixture was stirred for 2 h at 30 °C. To the mixture was added water (60 μl) and 15% NaOH (60 μl) and water (180 μl) consecutively. The reaction mixture was stirred for 1 h, and dried over Na_2SO_4. After stirring over night, an insoluble material was filtered thru a pad of celite and washed with ether. The combined filtrate was concentrated to dryness and the residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (1:1) to give **27** (294 mg, 85%).

^1H NMR (400 MHz, C_6D_6) δ 6.95 (1H, s), 6.73 (2H, s), 6.71 (2H, s), 5.79 (1H, s), 4.64 (1H, dd, J=2.8, 10.8 Hz), 3.94 (1H, m), 3.82 (1H, s), 3.52 (1H, dd, J=2.0, 9.3 Hz), 3.31 (1H, s), 3.26 (1H, dd, J=4.8, 11.0 Hz), 3.20 (1H, dd, J=4.8, 11.0 Hz), 3.09 (1H, dd, J=2.1, 10.1 Hz), 2.78 (1H, d, J=9.5 Hz), 2.51 (6H, s), 2.15 (1H, m), 2.12 (3H, s), 2.06 (1H, m), 1.85 (1H, ddd, J=2.9, 6.9, 10.8 Hz), 1.66–1.79 (2H, m), 1.46 (1H, dd, J=10.8, 13.5 Hz), 1.38 (3H, s), 1.38 (3H, d, J=6.9 Hz), 1.11 (3H, d, J=6.6 Hz), 1.06 (3H, d, J=6.7 Hz), 1.03 (3H, d, J=6.7 Hz), 0.73 (3H, d, J=7.0 Hz), 0.39 (3H, d, J=7.2 Hz). MS (EI) m/z 653 (M^+-1)). HRMS (EI) m/z Calcd for $C_{40}H_{61}O_7$ 653. (M^+-1) 4414, found 653.4408.

3.2.19. (2*R*,3*R*,4*S*,5*R*,6*R*,7*S*,9*R*,10*R*,11*S*,12*R*,13*S*)-14-[(*t*-Butyl)dimetylsilyloxy]-3,5,7,9,11,13-hexamethyl-4,6:10,12-bis-(2,4,6-trimethylbenzylidenedioxy)-tetradecane-2,7-diol (28). A solution of **27** (185 mg, 238 μmol) and triethylamine (66.3 μl, 476 μmol) and *t*-butyldimethylsilyl chloride (59.8 mg, 397 μmol) and DMAP (4 mg, 32.7 μmol) in CH_2Cl_2 (5 ml) was stirred for 32 h at room temperature. The mixture was diluted with CH_2Cl_2 and washed with sat. $NaHCO_3$ and sat. NH_4Cl and brine consecutively, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layer was dried on $MgSO_4$ and the residue was concentrated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (10:1) to give **28** (160 mg, 74%) as an amorphous solid.

^1H NMR (400 MHz, C_6D_6) δ 6.96 (1H, s), 6.73 (2H, s), 6.71 (2H, s), 5.87 (1H, s), 4.65 (1H, dd, J=2.5, 10.3 Hz), 3.94 (1H, m), 3.85 (1H, s), 3.64 (1H, dd, J=2.1, 9.4 Hz), 3.37 (1H, dd, J=4.3, 10.2 Hz), 3.54 (1H, dd, J=4.3, 10.2 Hz), 3.31 (1H, s), 3.22 (1H, dd, J=2.1, 9.7 Hz), 2.89 (1H, d, J=9.8 Hz), 2.69 (6H, s), 2.16 (1H, m), 2.12 (3H, s), 2.11 (3H, s), 2.08 (1H, m), 1.72–1.92 (3H, m), 1.40 (3H, s), 1.40 (3H, d, J=7.0 Hz), 1.14 (3H, d, J=6.8 Hz), 1.14 (3H, d, J=6.8 Hz), 1.12 (3H, d, J=7.2 Hz), 1.02 (9H, s), 0.80 (3H, d, J=7.0 Hz), 0.34 (3H, d, J=7.1 Hz), 0.08 (3H, s), 0.07 (3H, s). MS (EI) m/z 768 (M^+). HRMS (EI) m/z Calcd for $C_{46}H_{76}O_7Si$ (M^+) 768.5360, found 768.5412.

3.2.20. (2*R*,3*R*,4*S*,5*R*,6*R*,7*S*,9*R*,10*R*,11*S*,12*R*,13*S*)-2-Acetoxy-14-[(*t*-butyl)dimethylsilyloxy]-3,5,7,9,11,13-hexamethyl-4,6:10,12-bis-(2,4,6-trimethylbenzylidenedioxy)-tetradecane-7-ol (29). A solution of **28** (137 mg, 178 μmol) and triethylamine (81 μl, 582 μmol) and acetic anhydride (22 μl, 235 μmol) and DMAP (5 mg, 41 μmol) in CH_2Cl_2 was stirred for 33 h at room temperature. The solution was washed with sat. $NaHCO_3$, and sat NH_4Cl, and the combined aqueous layer was extracted with CH_2Cl_2. The combined organic layer was dried on $MgSO_4$ and concentrated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (10:1) to give **29** (137 mg, 95%) as an amorphous solid.

$[\alpha]_D^{21}=-23.2$ (c=1.06, $CDCl_3$).IR (neat) 1740 cm^{-1}. ^1H NMR (270 MHz, C_6D_6) δ 6.80 (2H, s), 6.75 (1H, s), 6.72 (2H, s), 5.89 (1H, s), 5.59 (1H, dq, J=1.7, 6.8 Hz), 4.34 (1H, dd, J=2.2, 10.8 Hz), 3.65 (1H, dd, J=1.5, 9.2 Hz), 3.55 (1H, dd, J=4.1, 10.3 Hz), 3.43 (1H, s), 3.38 (1H, dd, J=4.1, 10.3 Hz),3.37 (1H, s), 3.25 (1H, dd, J=1.5, 10.4 Hz), 2.79 (6H, s), 2.52 (6H, s), 2.17 (1H, m), 2.12 (6H, s), 1.85 (3H, s), 1.78–1.94 (3H, m), 1.62 (1H, m), 1.42 (3H, s), 1.36 (3H, d, J=7.1 Hz), 1.16 (3H, d, J=6.7 Hz), 1.15 (3H, d, J=6.78 Hz), 1.03 1.02 (3H, d, J=7.0 Hz), 0.81 (3H, d, J=6.9 Hz), 0.44 (3H, s), 0.43 (1H, d, J=6.8 Hz), 0.08 (3H, s), 0.07 (3H, s). MS (FAB) m/z 811 (M^++1). HRMS (FAB) m/z Calcd for $C_{48}H_{79}O_8Si$ (M^++1) 811.5540, found 811.5545.

3.2.21. (2*S*,3*R*,4*S*,5*R*,6*R*,8*S*,9*R*,10*R*,11*S*,12*R*,13*R*)-13-Acetoxy-2,4,6,8,10,12-hexamethyl-3,5:9,11-bis-(2,4,6-trimethylbenzylidenedioxy)-tetradecane-1,8-diol (30). To a solution of **29** (131 mg, 161 μmol) in THF (5 ml) was added 1 M THF solution of TBAF (320 μl, 320 μmol), and the solution was stirred for 3.5 h at room temperature and treated with brine and extracted with CH_2Cl_2. The extract was dried on $MgSO_4$ and concentrated to dryness in vacuo. The residue was chromatographed on a silicagel eluting with hexane and ethyl acetate (2:1) to give **30** (111 mg, 99%) as an amorphous solid.

$[\alpha]_D^{21}=-18.8$ (c=1.22, $CDCl_3$). IR (neat) 1740 cm^{-1}. ^1H NMR δ 6.68 (2H, s), 6.74 (1H, s), 6.71 (2H, s), 5.81 (1H, s), 5.59 (1H, dq, J=2.3, 6.7 Hz), 4.34 (1H, dd, J=2.6, 10.3 Hz), 3.52 (1H, dd, J=1.9, 9.8 Hz), 3.43 (1H, s), 3.28 (1H, s), 3.25 (1H, dd, J=4.7, 10.4 Hz), 3.18 (1H, dd, J=4.7, 10.4 Hz), 3.10 (1H, dd, J=1.8, 9.7 Hz), 2.78 (6H, s), 2.52 (6H, s), 2.12 (6H, s), 2.11 (1H, m), 1.91 (1H, dq, J=2.1, 7.0 Hz) 1.85 (3H, s), 1.40 (3H, s), 1.34 (3H, d, J=7.0 Hz), 1.08 (3H, d, J=6.8 Hz), 1.05 (3H, d, J=6.7 Hz), 1.03 (3H, d, J=6.8 Hz), 0.72 (3H, d, J=7.1 Hz), 0.45 (3H, d, J=7.0 Hz). MS (EI) m/z 696 (M^+). HRMS (EI) m/z Calcd for $C_{42}H_{64}O_8$ (M^+) 696.4601, found 696.4622.

3.2.22. (8*S*,9*R*)-13-*O*-Acetyl-3,5-*O*-isopropylidene-9,11-*O*-(2,4,6-trimeethylbenzylidene)-8-hydroxy-8,8a-deoxa-9-dihydro-oleandonolide *seco*-acid (31). To a cooled (−40 °C) solution of **30** (102 mg, 146 μmol) in acetone (3.5 ml) was added 2.67 M Jones reagent (106 μl. 283 μmol) and the solution was stirred for 5 h at −30 °C, then treated with isopropanol and diluted with water, and extracted with CH_2Cl_2. The combined extract was dried on $MgSO_4$ and concentrated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (1:2) to give **31** (83 mg, 80%) as an amorphous solid.

IR (neat) 1745 cm^{-1}. ^1H NMR δ 6.81 (2H, s), 6.72 (3H, s), 5.79 (1H, s), 5.59 (1H, dq, J=2.2, 6.6 Hz), 4.31 (1H, dd, J=2.5, 10.7 Hz), 3.86 (1H, dd, J=2.1, 9.8 Hz), 3.39 (1H, s), 3.09 (1H, dd, J=1.8, 9.7 Hz), 2.86 (1H, dq, J=6.7, 10.6 Hz), 2.78 (6H, s), 2.48 (6H, s), 2.13 (6H, s), 2.02–2.18 (2H, m), 1.83–1.90 (1H, m), 1.87 (3H, s), 1.56–1.79 (2H, m), 1.37 3H, s), 1.34 (3H, d, J=7.1 Hz), 1.29 (3H, d, J=7.0 Hz), 1.17 (3H, d, J=6.8 Hz), 1.03 (3H, d, J=6.6 Hz), 0.67 (3H, d, J=7.1 Hz), 0.45 (3H, d, J=7.1 Hz). MS (EI) m/z 710 (M^+). HRMS (EI) m/z Calcd for $C_{42}H_{62}O_9$ (M^+) 710.4394, found 710.4432.

3.2.23. (8*S*,9*R*)-3,5-*O*-Isopropylidene-9,11-*O*-(2,4,6-trimeethylbenzylidene)-8-hydroxy-8,8a-deoxa-9-dihydro-oleandonolide *seco*-acid (32). To a solution of **31** (55 mg, 77.5 μmol) in MeOH (3 ml) was added 15% NaOH (0.6 ml, 2.25 mol). The reaction mixture was stirred for 24 h and then treated with phosphate buffer (pH7), extracted with ether and the extract was washed with brine and dried over $MgSO_4$ and concentrated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with MeOH and CH_2C_2 (1:50) to give *seco*-acid **32** (48 mg, 93%) as an amorphous solid. Because this *seco*-acid could not be purified completely, analytical data was taken after conversion to its methyl ester **33**.

IR (neat) 1735 cm^{-1}. ^1H NMR (400 MHz, C_6D_6) δ 6.92 (1H, s), 6.74 (2H, s), 6.71 (2H, s), 5.76 (1H, s), 4.62 (1H, dd, J=2.5, 9.7 Hz), 3.96 (1H, dq, J=2.4, 6.6 Hz), 3.83 (1H, dd, J=1.8, 10.2 Hz), 3.29 (1H, s), 3.07 (1H, dd, J=1.8, 10.5 Hz), 2.81 (1H, dq, J=7.0, 10.3 Hz), 2.66 (6H, s), 2.46 (6H, s), 2.12 (3H, s), 2.11 (3H, s), 2.01–2.13 (2H, m), 1.89 (1H, m), 1.82 (1H, dq, J=2.5, 7.0 Hz), 1.34 (3H, d, J=7.1 Hz), 1.28 (3H, d, J=6.9 Hz), 1.13 (3H, d, J=6.9 Hz), 1.11 (3H, d, J=6.6 Hz), 0.67 (3H, d, J=6.8 Hz), 0.35 (3H, d, J=7.0 Hz). MS (EI) m/e 668 (M^+).

3.2.24. (8*S*,9*R*)-3,5-*O*-Isopropylidene-9,11-*O*-(2,4,6-trimeethylbenzylidene)-8-hydroxy-8,8a-d4eoxa-9-dihydro-oleandonolide *seco*-acid methyl ester (33). To a solution of **32** (17 mg, 25.4 μmol) in benzene (1.0 ml) was added MeOH (200 μl) and 10% hexane solution of trimethylsilyldiazomethane (200 μl, 176 μmol) and the solution was stirred for 1 h and then treated with acetic acid and the solution was diluted with ether and washed with sat. $NaHCO_3$ and brine and dried over Na_2SO_4 and concentrated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (4:1) to give methyl ester **33** (15.6 mg, 90%) as an amorphous solid.

$[\alpha]_D^{19}=-16.9$ (c=0.76, $CDCl_3$). IR (neat) 1720 cm^{-1}. ^1H NMR (500 MHz, C_6D_6) 6.95 (1H, s), 6.73 (2H, s), 6.70 (2H, s), 5.76 (1H, s), 4.69 (1H, dd, J=10.6, 2.4 Hz), 3.98 (1H, dd, J=10.1, 6.8 Hz), 3.95 (1H, broad s), 3.69 (1H, s), 3.36 (3H, s), 3.09 (1H, dd, J=10.2, 6.8 Hz), 2.86 (1H, dq, J=6.8, 10.1 Hz), 2.67 (6H, s), 2.48 (6H, s), 2.12 (3H, s), 2.11 (3H, s), 2.10 (1H, m), 1.86 (1H, m), 1.69 (1H, dd, J=14.4, 5.4 Hz), 1.38 (3H, d, J=7.2 Hz), 1.34 (3H, s), 1.29 (3H, d, J=6.8 Hz), 1.13 (3H, d, J=6.9 Hz), 1.11 (3H, d, J=6.6 Hz), 1.00 (1H, dd, J=14.0, 2.7 Hz), 0.66 (3H, d, J=7.1 Hz), 0.37 (3H, d, J=7.1 Hz). ^{13}C NMR (125 MHz, C_6D_6) 174.29, 139.61, 137.57, 136.92, 132.75, 130.80, 130.34, 13o.12, 128.26, 127.44, 102.63, 96.78, 87.67, 86.30, 83.56, 78.70, 79.64, 70.46, 59.97, 51.20, 46.18, 42.64, 39.77, 32.10, 39.14, 29.47, 28.49, 24.16, 20.85, 20.79, 20.60, 18.70, 18.08, 15.30, 15.15, 14.14, 10.93, 6.71. MS (FAB) m/e 683 (M^++1). EXMS (FAB) m/z Calcd for $C_{41}H_{63}O_8$ 683.4544 (M^++1), found 683.4555.

3.3. General methods of lactonization of *seco*-acid 14, 20, 23 and 32

3.3.1. Lactonization under the high dilution condition. To a solution of *seco*-acid (1, 20, 33) (0.047 mmol) in THF (0.95 ml) was added triethylamine (7.2 μl, 52 μmol) and 2,4,6-trichlorobenzoyl chlodide (7.4 μl, 0.047 mmol) and the solution was stirred for 19.5 h and then diluted with toluene (24 ml) and transferred to a syringe. The solution in the syringe was added to a solution of DMAP (139 mg, 1.18 mol) in toluene (20 ml) at 130 °C over 5 h with micro feeder syringe pump. The resulting mixture was stirred for further 6 h at 130 °C. After cooling to rt, the mixture was concentrated to dryness in vacuo and the residue was treated with sat. NH_4Cl and extracted with ether and the combined extract was washed with brine and dried over Na_2SO_4 and concentrated to dryness in vacuo. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (10:1) to give lactone **13** (97%) from**14**, **19** (15%) from **20**, **22** (96%) from **23**, and **34** (0%) from **32**.

3.3.2. Lactonization under the normal condition. To a solution of *seco*-acid (35.7 μmol) in THF (3.5 ml) was added triethylamine (5.5 μl, 40 μmol) and 2,4,6-trichlorobenzoyl chloride (5.6 μl, 36 μmol) at room temperature and the solution was stirred for 17 h and then to the mixture was added DMAP (13.2 mg, 108 μmol), and after stirring for further 3 h the mixture was concentrated to dryness in vacuo and the residue was treated with sat. NH_4Cl and extracted with ether. The combined extract was washed with brine and dried over Na_2SO_4 and concentrated to dryness in vacuo.

The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (10:1) to give lactone **13** (82%), **19** (0%), and **22** (81%).

3.3.3. The attempted lactonization of *seco*-acid 32 (34). To a solution of *seco*-acid **33** (15.4 mg, 23 μmol) in THF (0.5 ml) was added triethylamine (5.2 μl, 34.5 μmol), and 2,4,6-trichlorobenzoyl chloride (5.4 μl, 11.5 μmol), and then the solution was stirred for 20 h at rt, and diluted with toluene (10 ml). The solution was transferred to syringe, added over 8.5 h with a syringe pump to a refluxed solution of DMAP (65 mg, 530 μmol) in toluene (20 ml), the mixture was stirred for 11.5 h under reflux. The mixture was cooled to rt, and concentrated to dryness in vacuo. The residue was treated with sat. NH_4Cl and extracted with ether. The combined extract was washed with brine, dried over $MgSO_4$. The residue was chromatographed on a silicagel column eluting with hexane and ethyl acetate (10:1 to 1:1) to give complicated product and the desired lactone **33** (0%) (Scheme 4).

3.4. Conformation calculations of model *seco*-acids (4, 6 and 8) and corresponding lactones (3, 5, 7), and clustering of conformers

Conformation calculation of model *seco*-acids and the corresponding lactones were carried out by a conformational space searching algorism 'CONFLEX ver.4' working on PC-unix (Linux). Extended MM2 was used as the energy minimizer. The conformers obtained by conformation search were classified into clusters using the single lincage algorithm accelelated by the doubly linked list method.[8a,b] The conformational similarity between a pair of conformers (clusters) A and B was measured by conformational distance, d_{AB}, defined as the root-mean-square difference in the major backbone and ring dihedral angles (Eq. 1):[8c]

$$d_{AB} = \sqrt{\frac{\sum_{i=1}^{17} (w_i^A - w_i^B)^2}{17}} \quad (1)$$

where w_i^A and w_i^B are the *i*-th dihedral angle of conformers A and B, respectively. Seventeen dihedral angle used in Eq. 1 refer to nine backbone bonds along the segment of C3–C4–C5–C6–C7–C8–C9–C10–C11–C12, and to four each along the two dioxane rings, C3–O3–C (acetal or ketal carbon)–O5–C5 and C9–O9–C (acetal or ketal carbon)–O11–C11. The end portions, C3–C2–C1–OMe and C12–C13–OH and OH and phenyl group rotation, was not included in the cluster analysis, although all these bonds were rotated during the conformation search. When a pair of conformers have a conformational distance d_{AB} shorter than 10°, they belong to the same cluster.

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9. Conformations of the 6-membered acetal and ketal of *syn*-1,3-diol (C3,C5) of *seco*-acids **4**, **6** and **8** were shown to be fixed to chair conformation.[10] The substituents (methyl, phenyl or dimethl group) on the 6-membered ring of the *syn*-1,3-diol are far from the backbone chain, we can conclude that the difference in the substituents does not affect the backbone conformation.
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A single step synthesis of 6-aminophenanthridines from anilines and 2-chlorobenzonitriles

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Abstract—Biologically active 6-aminophenanthridines were prepared in a single step procedure: Metal amides in liquid ammonia promoted the condensation of anilines with 2-chloro-benzonitriles. 6-Aminophenanthridines were isolated in moderate yield.

1. Introduction

Prion deseases are neurodegenerative pathologies that include Creutzfeld–Jakob in human, bovine spongiform encephalopathie in cattle and scrapie in sheep. All these disorders are associated with an abnormal conformation of the normal host protein PrP.[1]

Up to now no treatment has demonstrated clinical usefulness.[2] Very recently, we have developed a rapid yeast-based assay to screen for antiprion drugs, which led to the discovery of phenanthridines as new prion inhibitors. In particular, 6-aminophenanthridines (6-AP) displayed the highest inhibition.[3] In our test, 6-APs were found more active than other previously reported inhibitors.[4]

This prompts us to study the preparation of these heterocycles. Phenanthridines can be obtained by cyclisation of various biphenyles: 2-formyl-2′-nitrobiphenyle,[5] 2′iodo-2-isocyanobiphenyle.[6] Other syntheses include cyclisation of *N*-benzylanilides by reaction of hypervalent iodine,[7] condensation of Boc-aniline with 2-chlorobenzaldimines under basic conditions[8] and annelation of tetrahydroquinolin-4-one.[9]

In contrast to the numerous approaches to phenanthridines, very few reports deal with 6-APs. The main routes start from phenanthridinones. Phenanthridinones can be obtained from phenanthridines via rearrangement of the *N*-oxide,[10] from fluorenones by the Schmidt reaction[11] and by Suzuki coupling of 2-Bocaminophenylboronic acid with 2-bromobenzoates.[12] After conversion of phenanthridinone into 6-chlorophenanthridines,[13] 6-AP could then be obtained upon reaction with ammonia.[14]

Recently, a one step synthesis of 6-substituted-phenanthridine has been described. It is based on the condensation of 2 arynes generated from fluoroarenes with one equivalent of nitrile. However this approach does not allow the introduction of various groups on the benzene rings and cannot be applied to the synthesis of unsubstituted 6-amino groups.[15]

2. Results and discussion

2.1. Amination of 6-chlorophenanthridines

At the onset of our work, we tried to prepare 6-APs via 6-chlorophenanthridine[16] using the above mentioned previously reported procedure: 6-chlorophenanthridine was obtained in an overall 48% yield from phenanthridine but exposure to a methanolic solution of ammonia produced only a minor trace of 6-AP. The amination was then successfully achieved by hydrogenation of 6-benzylaminophenanthridine prepared by refluxing 6-chlorophenanthridine in benzylamine (Scheme 1).

2.2. Cyclization of 2-chlorophenylbenzamidine

However, this classical approach was limited. We needed to prepare functionalised 6-APs in order to establish structure–activity relationships. This led us to investigate other routes.

Taking into account that potassium amide in liquid ammonia allowed the cyclisation of the imine of

Keywords: Amidine; Prions; Phenanthridine; Heterocyclisation.
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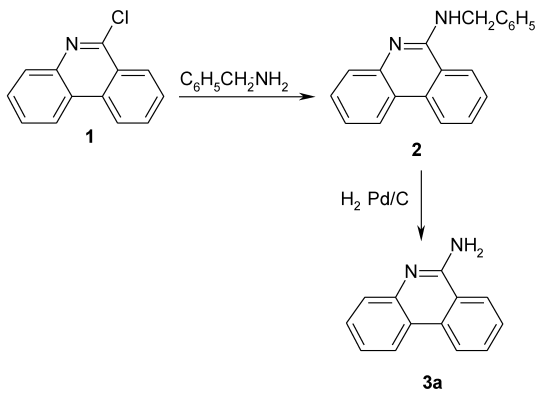
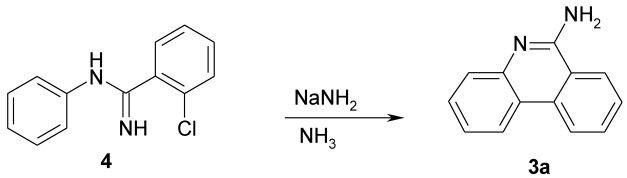


Scheme 1. Amination of 6-chlorophenanthridine.

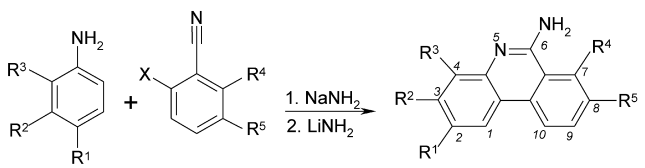
2-chlorobenzaldehyde with naphthylamine into benzo[*c*]-phenanthridine,[17] we have experimented the cyclisation of *N*-phenyl-2-chlorobenzamidine **4**. Addition of the amidine **4** to a sodium amide suspension in liquid ammonia led to the formation of 6-AP **3a** which was separated from unreacted **4** by column chromatography (Scheme 2).



Scheme 2. Cyclisation of 2-chlorophenylbenzamidine.

2.3. Direct condensation of arylamines with 2-halonitriles

In order to get the target compounds in a one step procedure, we have studied the condensation of 2-halobenzonitriles with aryl amines in the presence of several combination of bases: The use of a mixture of sodium and lithium amide was found the most efficient providing single step access to 6-APs. An attempt to use 2-fluorobenzonitrile instead of 2-chlorobenzonitrile was unsuccessful (Scheme 3, Table 1).

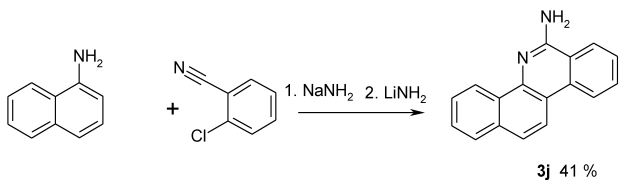


Scheme 3. Condensation of anilines with 2-chlorobenzonitriles.

Table 1. Phenanthridines produced via Scheme 3

Entry	Compounds **3**	R^1	R^2	X	R^3	R^4	R^5	Yield (%)
1	**3a**	H	H	Cl	H	H	H	42
2	**3b**	F	H	Cl	H	H	H	39
3	**3c**	OCH_3	H	Cl	H	H	H	52
4	**3d**	H	F	Cl	H	H	H	44
5	**3e**	H	H	Cl	H	H	Cl	38
6	**3f**	H	H	Cl	H	H	CF_3	36
7	**3g**	OCH_3	H	Cl	OCH_3	H	H	62
8	**3h**	H	H	Cl	H	Cl	H	17
9	**3i**	F	H	Cl	H	H	Cl	33

Similarly, naphthylamine reacted with 2-chlorobenzonitrile to afford the benzo[*c*]phenanthridine **3j** (Scheme 4).



Scheme 4. Preparation of benzo[*c*]phenanthridine.

3. Conclusion

Although the isolated yields of 6-APs were moderate, the presented procedure is simple to carry out, it allowed us to prepare a series of diversely substituted 6-APs, bearing either electro-donating or electro-attractive groups on the aromatic rings.

Efforts are underway to use these easily obtainable 6-APs as synthetic intermediates to prepare other biologically active phenanthridines. Further, the obtention of compound **3j** illustrate the potency of the approach in the preparation of benzophenanthridines which display a broad range of pharmacological activities.[19–21]

4. Experimental

4.1. General

All the starting material used were commercially available except 6-chlorophenanthridine prepared from phenanthridine. ^{1}H and ^{13}C NMR were recorded on a Bruker 400 MHz spectrometer. Structural assignments were achieved by 1D and 2D methods.

4.1.1. 6-Benzylaminophenanthridine (2) from 6-chlorophenanthridine (1). A solution of 6-chlorophenanthridine **1** (4.5 g, 21.1 mmol) in benzylamine 15 mL and tributylamine 2 mL was refluxed 3 h. After concentration under reduced pressure, the residue was chromatographed on silica gel using CH_2Cl_2 containing 0.1% NEt_3 as eluent.

Yield 86%; mp 99 °C; ^{1}H NMR ($CDCl_3$) δ 4.97 (s, 2H, CH_2–C_6H_5); 5.6 (br s, 1H, NH); 7.35 (t, 1H, 2-H); 7.32 (t, 1H, H-8); 7.4 and 7.55 (2m, 5H, C_6H_5); 7.58 (t, 1H,); 7.62 (t,); 7.78 (t, 1H,); 7.82 (t, 1H,); 7.85 (d, 1H, 7-H); 8.37 (d, 1H, 1-H); 8.55 (d, 1H, 10-H). Analysis: calculated for $C_{20}H_{16}N_2$: C, 84.48; H 5.67%; N 9.85%; found: C, 84.35; H 5.81%; N 10.02%.

4.1.2. Debenzylation of 6-benzylaminophenanthridine (2) into 6-aminophenanthridine (3a). To a solution of 6-benzylaminophenanthridine **2** (2.84 g, 10 mmol) in EtOH–AcOH (100 mL, 95:5) was added 5% Pd–C (0.1 g). The mixture was hydrogenated under vigorous stirring (5 atm, 40 °C) for 3 h. After removal by filtration of the catalyst, the solution was concentrated under reduced pressure, diluted in 100 mL H_2O, brought to pH 10 with saturated $NaHCO_3$ and extracted with CH_2Cl_2. The remaining solid crystallized upon trituration with AcOEt.

Yield 19%; mp 187–189 °C; ^{1}H NMR ($CDCl_3$) δ 7.40 (t, 1H, J_{1-2}=J_{2-3}=8.6 Hz, 2-H); 7.58 (t, 1H, J_{7-8}=J_{8-9}=8.5 Hz, 8-H); 7.64 (t, 1H, J_{3-4}=8.6 Hz, 3-H); 7.72 (d, 1H, 4-H); 7.82 (t, 1H, J_{9-10}=8.5 Hz, 9-H); 7.95 (d, 1H, 7-H); 8.38 (d, 1H, 1-H); 8.56 (d, 1H, 10-H) Analysis: calculated for $C_{13}H_{10}N_2$: C, 80.39%; H 5.19%; N 14.42%; found: C, 80.09%; H 5.31%; N 14.56%.

4.1.3. *N*-Phenyl-2-chlorobenzamidine (4). The method used by Daoust and Lessard[18] for the synthesis of phenylbenzamidine was slightly modified: To a solution aniline in 20 mL, toluene, $NaNH_2$ (0.39 g, 10 mmol) was added. After 1h stirring at 50 °C, 2-chlorobenzonitrile was added and the mixture brought to reflux for 2 h. After cooling to 0 °C, NH_4Cl, then water 100 mL were added carefully and the mixture extracted with 3×100 mL CH_2Cl_2. The organic solution was washed with water, dried (Na_2SO_4) and concentrated under reduce pressure. The amidine crystallized from Et_2O as a dark brown solid. Yield 55%; mp 170 °C; ^{1}H NMR ($CDCl_3$) δ 4.80 (br s, 1H, NH); 6.95 (m, 3H, Aro); 7.40 (m, 5H, Aro); 7.45 (d, 1H, Aro); 7.60 (br s, 1H, NH). MS (ES^+) $C_{13}H_{11}N_2Cl$ requires 230 and 232 found: 231, 233 ($M+H^+$, 100%).

4.1.4. Aminophenanthridine 3a from 4. In a flask connected with an efficient condenser, ammonia (100 mL) was introduced followed by $Fe(NO_3)_3$ (0.02 g) and piece by piece Na (1.15 g, 50 mmol). The solution was stirred for 0.5 h during this period, the initial blue colour of the solution of Na gradually turned to grey indicating the complete conversion into $NaNH_2$. *N*-Phenyl-2-chlorobenzamidine **4** was introduced. After 2 h stirring, NH_4Cl (5 g) was added gradually and the mixture was left overnight. Water was added and the mixture was extracted with CH_2Cl_2. The organic layer was washed with water, dried and evaporated. The mixture was applied to a silica gel column and eluted with CH_2Cl_2 then CH_2Cl_2–MeOH (99:1) containing 0.5% NEt_3 as eluent. Unreacted **4** (yield: 22%) was eluted first followed by **3a** (yield 56%).

4.2. Preparation of 6-aminophenanthridines (3) from arylamines and 2-halonitriles

The preparation of the $NaNH_2$ suspension was carried out as described above. To a suspension of $NaNH_2$ in liquid NH_3 (10 mmol, 100 mL) the arylamines **1** in anhydrous Et_2O (10 mmol, 30 mL) were added droplet. The mixture was stirred for 0.5 h. After this time, 2-halobenzonitriles were gradually added. In most cases, the 2-chlorobenzonitriles were added as solids, (2-chlorobenzonitrile was added in anhydrous Et_2O). The mixture was then stirred for 2 h and lithium (0.14 g, 20 mmol) was added in two pieces. The mixture was stirred an additional 2 h, NH_4Cl (2 g) was then added carefully. The reaction was left overnight. After evaporation of ammonia, work up and purification were performed as described above. 6-APs were easily distinguished from benzimidine by their stronger UV absorption on tlc plates. Products **3** were crystallized from AcOEt.

4.2.1. 6-Amino-2-fluorophenanthridine (3b). Mp 132–134 °C; ^{1}H NMR ($CDCl_3$) δ 5.70 (br s, 2H, NH_2); 7.3 (dd, 1H, $J_{3\text{-F}}$=10.1 Hz, J_{1-3}=2.5 Hz, 3-H); 7.65 (t, 1H, J_{8-9}=J_{9-10}=8.5 Hz, 8-H) 7.68 (t, 1H, 3-H); 7.75 (t, 1H, 9-H); 7.85 (d, 1H, 7-H); 7.94 (dd, 1H, 1-H); 8.35 (d, 1-H, 10-H). Analysis: calculated for $C_{13}H_9FN_2$: C, 73.57%; H, 4.27%; N, 13.20%; found: C, 73.76%; H, 4.12%; N, 13.31%.

4.2.2. 6-Amino-2-methoxyphenanthridine (3c). Mp 165–167 °C; ^{1}H NMR ($CDCl_3$) δ 3.90 (s, 3H, OCH_3); (5.20 (br s, 2H, NH_2); 7.12, dd, J_{3-4}=8.5 Hz, J_{1-3}=2 Hz, 3-H); 7.60 (t, 1H, 8-H); 7.66 (d, 1H, 1-H); 7.69 (d, 1H, 4-H); 7.80 (t, 1H, 9-H); 7.95 (d, 1H, 7-H); 8.42 (d, 1H, 10-H). Analysis: calculated for $C_{14}H_{12}N_2O$: C, 74.98%; H, 5.39%; N, 12.49%; found: C, 74.81%; H, 5.45%; N, 12.77%.

4.2.3. 6-Amino-3-fluorophenanthridine (3d). Mp 182–183 °C; ^{1}H NMR ($CDCl_3$) δ 5.75 (br s, 2H, NH_2); 7.15, (td, 1H, $J_{2\text{-F}}$=10.2 Hz, J_{2-4}=2.7 Hz, 2-H); 7.4 (dd,1H, $J_{4\text{-F}}$=10.2 Hz, 4-H); 7.65 (t, 1H, J_{7-8}=J_{8-9}=8.2 Hz, 8-H); 7.84 (t, 1H, J_{9-10}=8.2 Hz, 9-H); 7.94 (d, 1H, 7-H); (8.35, dd, J_{1-2}=8.9 Hz, J_{1-3}=1 Hz, 1-H); 8.48 (d, 1H, 10-H). ^{13}C: 111.5 (d, $J_{3\text{-F}}$=23.3 Hz, C-3); 112.4 (d, $J_{2\text{-F}}$=23.4 Hz, C-2); 123.10, C-10; 123.9, C-7, 124.27 (d, $J_{1\text{-F}}$=8 Hz); 127.51, C-7); 131.40, C-9; 147 (d, J_{C-F}=12 Hz, C_4–*C*–N) 155.89, C-2; 163.22 (d, $J_{3\text{-F}}$=243 Hz, C-3). Analysis: calculated for $C_{13}H_9FN_2$: C, 73.57%; H, 4.27%; N, 13.20%; found: C, 73.41%; H, 4.38%; N, 13.43%.

4.2.4. 6-Amino-8-chlorophenanthridine (3e). Mp 175–180 °C; ^{1}H NMR ($CDCl_3$) δ 6.60 (br s, 2H, NH_2); 7.35 (t, 1H, J_{2-1}=J_{2-3}=8.4 Hz, 2-H); 7.55 (t, 1H, 3-H); 7.65 (d, 1H, J_{3-4}=8.4 Hz, H-4); 7.72 (dd, 1H, J_{9-10}=8 Hz, J_{7-9}=2 Hz, 9-H); 8.18 (d, 1H, 7-H); 8.32 (d, 1H, 1-H); 8.65 (d, 1H, 10-H). Analysis: calculated for $C_{13}H_9ClN_2$: C, 68.28%; H, 3.97%; N, 12.25%; found: 68.44%; H, 4.11%; N, 12.35%.

4.2.5. 6-Amino-8-trifluoromethylphenanthridine (3f). Mp 146–149 °C; ^{1}H NMR ($CDCl_3$) δ 6.60 (br s, 2H, NH_2); 7.35 (t, 1H, J_{1-2}=J_{2-3}=8.3 Hz, 2-H); 7.55 (t, 1H, J_{3-4}=8.3 Hz, 3-H); 7.65 (d, 1H, 4-H); 7.95 (dd, J_{7-9}=2 Hz, 1H, 9-H); 8.15 (d, 1H, J_{7-9}=2 Hz, H-7); 8.32 (d, 1H, 1-H); 8.65 (d, 1H, 10-H). Analysis: calculated for $C_{14}H_9F_3N_2$: C, 64.12%; H, 3.46%; N, 10.68%; found C, 64.10%; H, 3.31%, N, 10.81%.

4.2.6. 6-Amino-2,4-dimethoxyphenanthridine (3g). Mp 138–141 °C; ^{1}H NMR ($CDCl_3$) δ 4.00 and 4.05 (2s, 2×3H, $2OCH_3$); 5.5 (br s, 2H, NH_2); 6.70 (d, 1H, J_{1-3}=2 Hz, 3-H); 7.38 (d, 1H, 1-H); 7.69 (t, 1H; J_{7-8}=J_{8-9}=8.4 Hz, 8-H); 7.82 (t, 1H, 9-H); 8.02 (d, 1H, 7-H) 8.50 (d, 1H, H-10). Analysis: calculated for $C_{15}H_{14}N_2O_2$: C, 70.85%; H, 5.55%; N, 11.02%, found: C, 70.65%; H, 5.45%; N, 10.78%.

4.2.7. 6-Amino-7-chlorophenanthridine (3h). Mp 156–157 °C; ^{1}H NMR ($CDCl_3$) δ 6.37 (br s, 2H, NH_2); 7.37 (t, 1H, J_{2-1}=J_{2-3}=8.3 Hz, 2-H); 7.60 (t, 1H, 3-H); 7.65 (m, 3H, 4-H+8-H+9-H); 8.31 (d, 1H, 1-H); 8.54 (m, 1H, 10-H). Analysis: calculated for $C_{13}H_9ClN_2$: C, 68.28%; H, 3.97%; N, 12.25%; found: C, 68.44%; H, 4.19%; N, 12.17%.

4.2.8. 6-Amino-8-chloro-2-fluorophenanthridine (3i). Mp 187–189 °C; ^{1}H NMR ($CDCl_3$) δ 5.70 (br s, 2H, NH_2); 7.30 (dd, 1H, $J_{2\text{-F}}$=10 Hz, J_{2-1}=8 Hz, 2-H); 7.55 (dd, 1H, 4-H); 7.67 (td, 1H, 9-H); 7.87 (d, 1H, *J*=2 Hz, 7-H) 7.98 (dd,

1-H); 8.35 (d, 1H, 10-H). Analysis: calculated for $C_{13}H_8ClFN_2$: C, 63.30%; H, 3.27%; N, 11.36%; found C, 63.30%; H, 3.15%; N, 11.45%.

4.2.9. 8-Amino-benzo[*c*]phenanthridine (3j). Mp 145–150 °C; ^{1}H NMR ($CDCl_3$) δ 5.45 (br s, 2H, NH_2); 7.50–8.00 (m, 7H); 8.30 (d, 1H); 8.70 (d, 1H); 9.20 (d, 1H). Analysis: calculated for $C_{17}H_{12}N_2$: C, 83.58%; H, 4.95%; N, 11.47%; found C, 83.33%; H, 5.12%; N, 11.23%.

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Synthesis and transformations of [1,2-*O*-isopropylidene-α-D-*erythro* (and α-D-*ribo*)furanose]-3-spiro-3′-(4′-amino-5′H-2′,3′-dihydroisothiazole-1′,1′-dioxide) derivatives

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Abstract—The carbanion-mediated sulfonamide cyclisations (CSIC protocols) of glyco-α-sulfonamidonitriles derived from readily available uloses **1A** and **1B** have been investigated using different bases (potassium carbonate, cesium carbonate, LDA and *n*-BuLi). As a result, a series of enantiomerically pure [1,2-*O*-isopropylidene-α-D-*erythro* (and α-D-*ribo*)furanose]-3-spiro-3′-(4′-amino-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) derivatives have been prepared and isolated in good yields.

1. Introduction

The reactivity of carbanions[1] stabilized by sulfur containing functional groups, one of the most useful and recognized tools for carbon–carbon bond formation in organic synthesis, has been largely reviewed and summarized.[2] Conversely, the literature regarding related carbanions derived from alkanesulfonates and alkanesulfonamides is relatively scarce.[2d] From a synthetic perspective it has been shown that different bases (*n*-BuLi, DBU, etc.) are able to abstract protons from the α-position to sulfur atom in alkanesulfonates (sulfonamides) to give anions that react with different electrophiles, such as alkyl halides, sulfonates, either carbonyl compounds (ketones, esters), nitriles and aromatic activated substrates, in inter-, and intramolecular conversions, to yield substituted alkanesulfonates, alkanesulfonamides and different types of heterocyclic ring systems (Chart 1, Eq. 1 and 2).

We have named, typified and reviewed these types of transformations as CSIC reactions, by taking the initials of the keywords that describe and define the process for both intermolecular (carbanion-mediated sulfonate (or sulfonamide, or sulfoxide or sulfone) intermolecular coupling) and intramolecular (carbanion-mediated sulfonate (or sulfonamide, or sulfoxide or sulfone) intramolecular cyclization) conversions.[3] Recently, we have shown[4] that alkyl and benzylsulfonates, other than mesylates, may also act as the active methylene group in the CSIC reaction to give the corresponding substituted 4-amino-γ-sultones, and that the CSIC conditions can be successfully applied to many alkylsulfonyl nitriles in which the SO_2 group may be attached to either an oxygen or a nitrogen atom.[4]

However, the development and application of the CSIC reaction with alkanesulfonamides located on monosaccharide backbones has never been undertaken. Our interest in carbohydrate chemistry, especially that pertaining to glyco-α-aminonitriles[5] encouraged us to extend our investigations into the CSIC reaction. Very recently, one of us investigated this approach and solved this problem, showing a convenient access to a novel range of precursors for the synthesis of new (aza)nucleosides.[6] Herein we report in full, our studies on the glycoaminocyanation and cyclisation steps to afford the target enantiomerically pure [1,2-*O*-isopropylidene-α-D-*erythro*(and α-D-*ribo*)furanose]-3-spiro-3′-(4′-amino-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) derivatives using synthetic routes which started from either D-xylose or D-glucose. These compounds should be exploited as key intermediates in our current project for the synthesis of new azanucleosides.

Keywords: Carbanion; Sulfonamidonitrile; Organic synthesis; Glyco-α-aminonitrile; CSIC.
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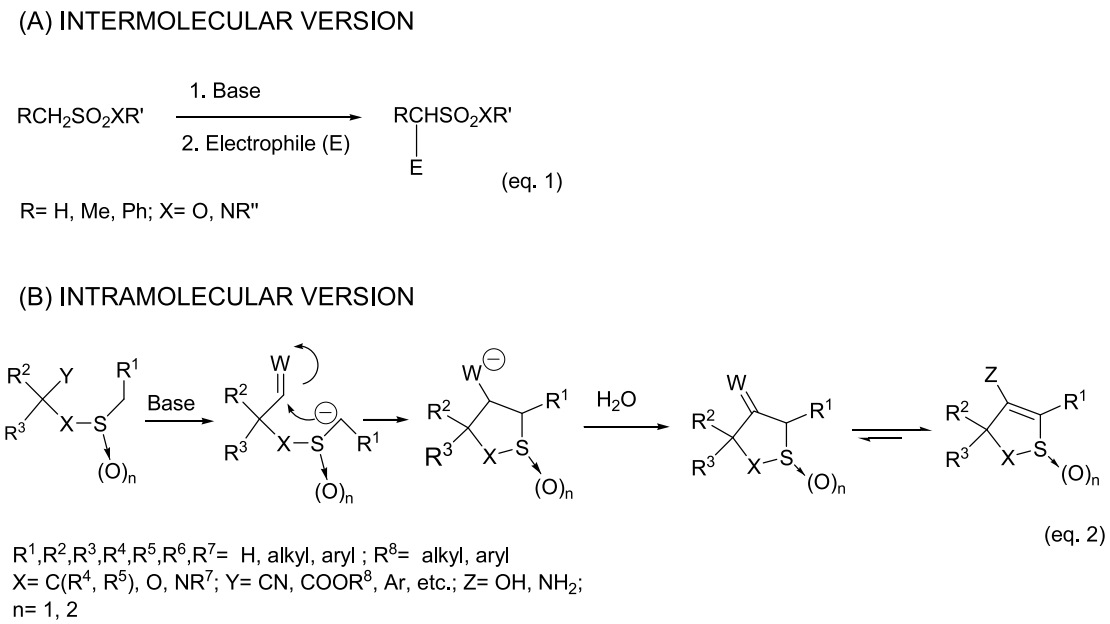


Chart 1. CSIC reaction.

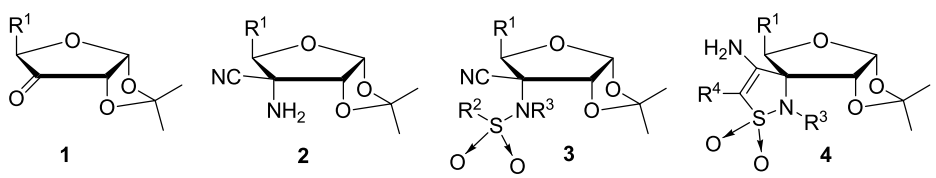
2. Results and discussion

2.1. Synthesis of the precursors and CSIC reactions

At the outset of our project we selected the uloses **1A**[7] and **1B**[8] (Chart 2) with 5-*O*-trityl and 5,6-*O*-isopropylidene protecting groups, respectively, known to be readily cleaved under mild conditions.

Strecker conditions, using NH_3–MeOH and $Ti(OiPr)_4$ as the Lewis acid,[5] applied to the protected *erythro*-pentofuranos-3-ulose derivative **1A**,[7] followed by TMSCN addition gave stereoselectively the α-aminonitrile **2A** (Chart 2) in 96% yield. Compound **2A** on treatment with methanesulfonyl or benzylsulfonyl chloride, in pyridine, using catalytic amounts DMAP, afforded the key methanesulfonamido-nitrile **3Aa** (92%) and the benzylsulfonamidonitrile **3Ab** (93%), respectively (Chart 2). The reaction of precursor **3Aa** with methyl iodide, benzylbromide or allyl bromide in acetone at reflux, in the presence of potassium carbonate, gave the *N*-methylated (**3Ac**), *N*-benzylated (**3Ad**) and the *N*-allylated (**3Ae**) derivatives in 99, 66 and 56% yields, respectively (Chart 2).

Similarly, compound **3Ab**, under the same standard *N*-alkylation conditions, afforded the expected derivatives **3Af–g** in moderate to good chemical yields (31–85%). Very interestingly, during the *N*-allylation of compound **3Ab**, after 17 h at rt, in addition to the expected sulfonamide **3Ah**, a second product (**4Af**) was isolated in 18% yield, and identified as the CSIC resulting molecule from compound **3Ah**. This result suggested that we could possibly perform in situ CSIC reactions on the *N*-alkylated intermediates, using prolonged reaction times. In fact, we were very satisfied to see that compound **3Ab** gave the *N*-methylated (**4Ad**, 95%), *N*-benzylated (**4Ae**, 68%), and the *N*-allylated (**4Af**, 14%) CSICproducts (Chart 2), respectively, after 2 days reaction time, from low to excellent chemical yields.



	R^1
A	CH_2OTr
B	

	R^2	R^3
a	Me	H
b	Bn	H
c	Me	Me
d	Me	Bn
e	Me	Allyl
f	Bn	Me
g	Bn	Bn
h	Bn	Allyl

	R^4	R^3
a	H	Me
b	H	Bn
c	H	Allyl
d	Ph	Me
e	Ph	Bn
f	Ph	Allyll
g	H	H
h	Ph	H

Chart 2.

Alternatively, treatment of the *N*-alkylated methanesulfonamide (**3Ac–e**) and *N*-alkylated benzylsulfonamides (**3Af–h**), with cesium carbonate (1 equiv.), in acetonitrile as solvent, at reflux, in 1–3 h, cleanly afforded compounds **4Aa–c** (46–95%) and **4Ad–f** (48–89%), respectively (Chart 2) (see Section 4).

We have also investigated the direct CSIC reaction on the nitrogen unprotected precursors **3Aa** and **3Ab**. After some experimentation we found that *n*-BuLi or freshly prepared LDA were the bases of choice for this transformation. Accordingly, reaction of sulfonamides **3Aa** and **3Ab** with *n*-BuLi (3 equiv.) (or with LDA, 3 equiv.) at −10 °C, in dry THF, provided the new CSIC reaction products **4Ag** and **4Ah**, respectively, in 98 and 76% yields (Chart 2).

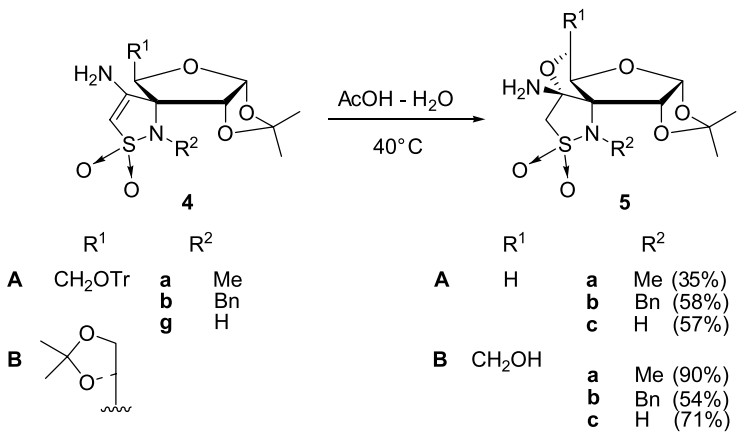
Next, we investigated similar reactivity and experimental methods on related 5,6-isopropylidene containing substrates, starting with the ketone **1B**.[8] These protocols allowed us to prepare the CSIC D-*ribo*-3′-spiro derivatives **4Ba–h**, via the corresponding *N*-alkylated sulfonamides **3Ba–h**, using as the key intermediate α-aminonitrile **2B** (Chart 2) (see Section 4).

All new compounds showed analytical and spectroscopic data in good agreement with the proposed structures (see below).

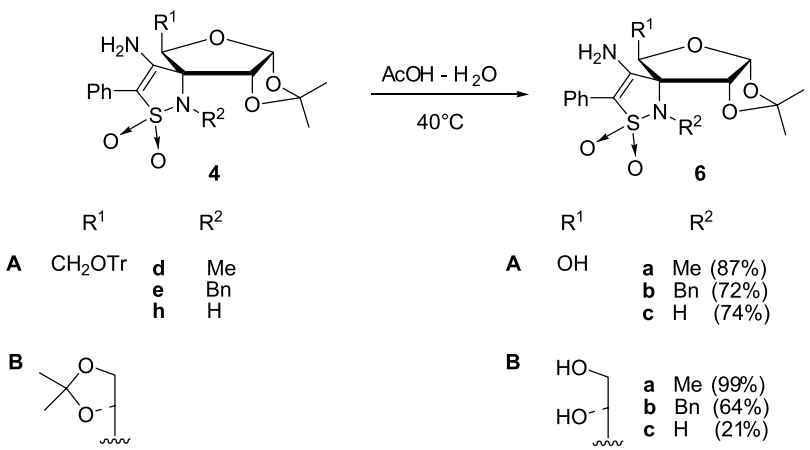
2.2. Acid hydrolyis of the CSIC products

For our current synthetic purposes, it was of interest to investigate the acid hydrolysis of compounds **4A**,**B**. Using aqueous acetic acid at 40 °C for 1–5 h, products **4A(B)a**, **4A(B)b** and **4A(B)g** gave the alcohols (**5A(B)a–c**), obtained after selective hydrolysis of the trityl or the 5,6-isopropylidene groups (Scheme 1). Only one diastereoisomer was detected and isolated. These products were possibly the result of the nucleophilic attack of the free hydroxyl group onto the protonated enamine moiety. The absolute configuration at the quaternary, new formed stereocenter was tentatively assigned as shown, assuming that the nucleophilic attack of the hydroxyl group could only proceed from the rear face of the enamine.

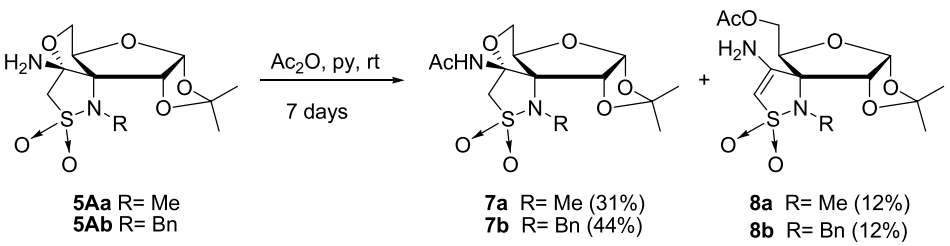
Very interestingly, the analogous 5′-phenyl substituted derivatives **4A(B)d**, **4A(B)e** and **4A(B)h**, under the same conditions, afforded alcohols **6A(B)a–c** (Scheme 2) in good chemical yields, as the only detected products. In no case, we observed similar tetracyclic products as in the hydrolysis of compounds lacking the phenyl ring moiety (see above). It seems that in these substrates the electrons on the enamine nitrogen atom are more delocalized in resonance electronic structures involving the phenyl ring, preventing the intramolecular attack of the hydroxyl group onto the protonated enamine.



Scheme 1. Acid hydrolysis of compounds **4A(B)a**,**b**,**g**.



Scheme 2. Acid hydrolysis of compounds **4A(B)d**,**e**,**h**.



Scheme 3. Acetylation of compounds **5Aa**,**b**.

The structures of products **5** (Scheme 1) and **6** (Scheme 2) have been established by their analytical and spectroscopic data, and after some chemical manipulation.

After acetylation, compounds **5Aa** and **5Ab** afforded monoacetate derivatives **7a/8a**, and **7b/8b**, respectively (Scheme 3). Compounds **7a**,**b** are the monoacetylated derivatives on the aminal precursors, while products **8a**,**b** are the monoacetylated sugar at hydroxyl group in the derivatives where the dihydroisothiazole ring system has been recovered after base-promoted tetrahydrofuran ring opening.

The acetylation of alcohol **5Ba** for 4 h afforded only monoacetate **9** (84%). When the reaction was prolonged for 15 h, only product **10** (60%) was isolated. Finally, acetylation of compound **5Ba**, during 3 days, gave compounds **10** (70%) and **11** (14, 86% pure by ^{1}H NMR analysis) (Scheme 4). These results suggest that in the long-term acetylation of alcohol **5Ba** the key intermediate is the monoacetyl [C6(O)-acetyl] derivative **9**, that gives the bis-acetyl [*N*-acetyl, C6(O)-acetyl] compound **10**, the precursor for the dihydroisothiazole derivative **11**, after base-promoted tetrahydrofuran ring opening followed by acetylation of the resulting free hydroxyl group (Scheme 4).

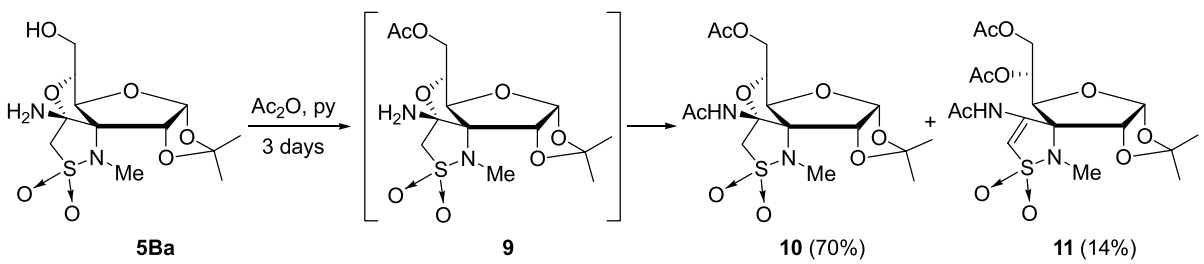
Summarising these results obtained in the acetylation reactions, we were very surprised to see that in the acetylation of compounds **5Aa** (Scheme 3) and **5Ba** (Scheme 4), the resulting minor products **8a**,**b** (12%), compared with **11** (14%), were not acetylated at the nitrogen on the enamine moiety. A careful analysis of the ^{1}H and ^{13}C NMR spectra revealed, for instance, that compound **8a** was actually a monoacetate (2.12 ppm, 3H (CH_3COO); 21.1 (CH_3COO) and 171.0 (CH_3COO) ppm) with a free NH_2 (4.63, s, 2H) group bound to a double bond (H-C5′: 5.62 ppm; C4′ (151.1 ppm); C5′ (95.0 ppm)), in an enamime moiety, while compound **11** has three acetyl groups (2.21, 2.09, 2.07 ppm) with an acetamido (7.65 ppm, br s, CH_3CONH) group bound to carbon C4′ (C4′ (140.1 ppm)-C5′ (108.5 ppm)). The shift to lower field for C5′ has been previously observed by one of us in similar *N*-acylated-2,3-dihydroisothiazole derivatives,[4b] and gives support to this structural assignment, confirmed by the mass spectra and the elemental analyses.

In Scheme 5, we propose a possible mechanism for the formation of compounds **8a**,**b** (Eq. 1). Pyridine promoted abstraction of the acidic Hα-proton in the sulfonamide moiety, followed by ring opening the tetrahydrofuran and intramolecular acetyl migration from the acetamido group to the resulting alkoxy group at C5, via intermediates **I**–**1/I**–**3**, should give the transacetylated compounds **8a**,**b**. The reason why these enamines are not further acetylated remains obscure for us, and work is currently now in progress in laboratory to find an explanation for this apparently anomalous reactivity. However, note that compounds **6Ba**,**b** with a phenyl group at C5′, upon acetylation, only afforded diacetylated derivatives **12a**,**b** in good yield (Scheme 6), showing that in this case the nitrogen in the enamine moiety is also reluctant to undergo acetylation.

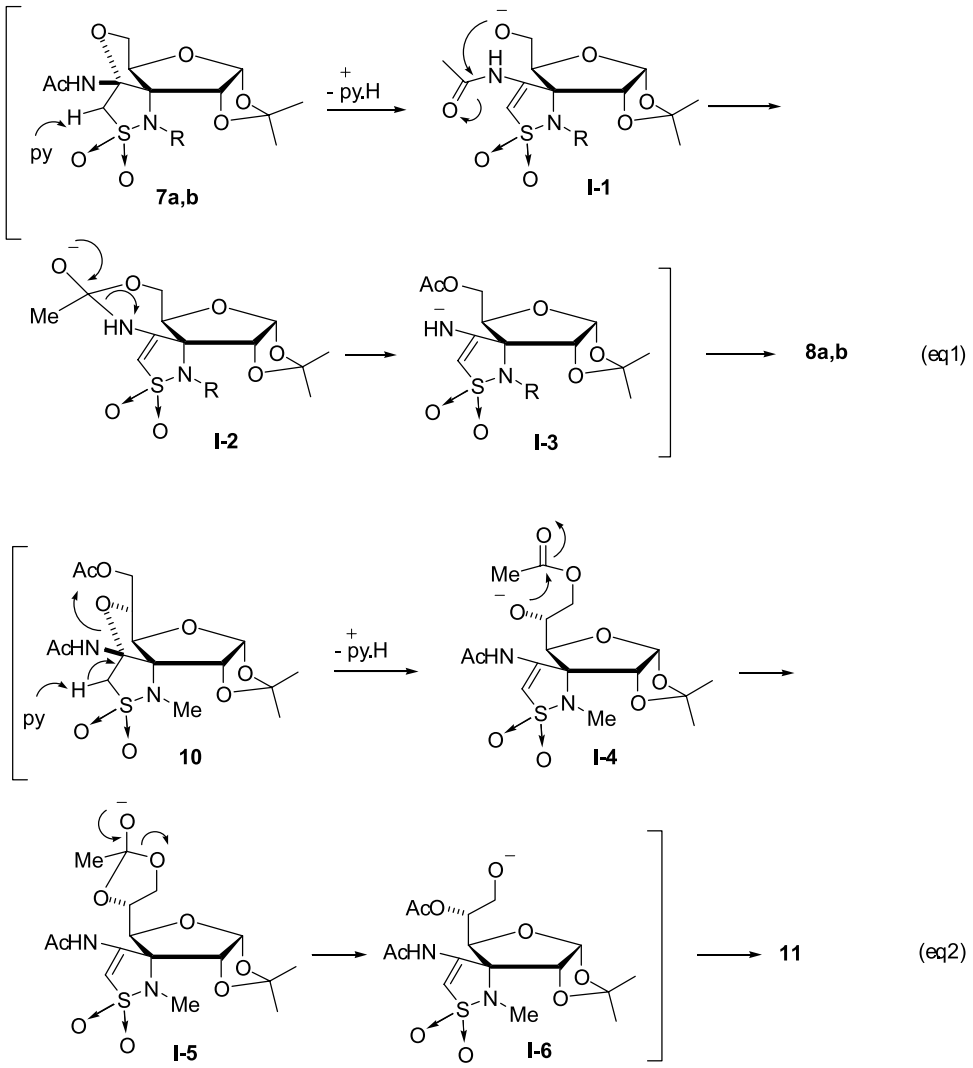
We think that in the case of product **10**, after similar base-promoted tetrahydrofuran ring opening, the exclusive intramolecular acetyl migration, from the acetyl group bound at O–C6 to the resulting secondary alkoxy species at C-5 in intermediate (**I**–**4**), can be explained as shown in Scheme 5 (Eq. 2). In fact, simple inspection with molecular models suggests that a possible intermediate (**I**–**5**) leading to primary alkoxy group (**I**–**6**) is not so sterically constrained, as an analogous intermediate involving the acetyl migration from the acetamido group attached to the spiro heterocyclic ring system. Final intermolecular acetylation process should afford compound **11**.

2.3. Structural analysis and stereochemical considerations

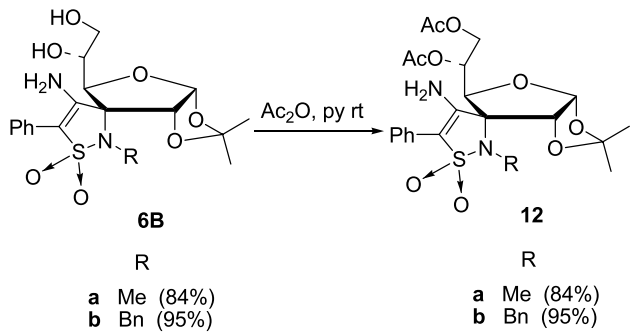
Although the structural analysis of the products obtained in this work has been achieved by extensive NMR spectroscopyic analysis, the assignments of the chemical shifts for



Scheme 4. Long-term acetylation of compound **5Ba**.



Scheme 5.



Scheme 6. Acetylation of compounds **6Ba**,**b**.

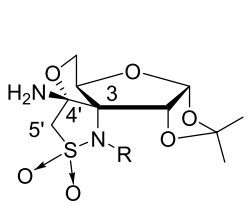
protons and carbons are based on a comparison of spectroscopic data previously reported by us[5,6] and others.[9]

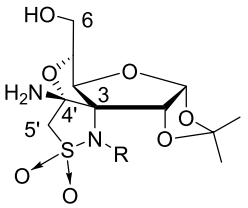
Regarding the different substrates obtained by acid hydrolysis from the CSIC products, in Chart 3 we have shown some of typical chemical shifts observed in the NMR spectra.

Particularly, for compounds **5Ba**–**c** (Scheme 1), we have excluded that the primary hydroxyl group at C-6 could be involved in the ring closure based on the fact that the ^{1}H NMR spectrum of **5Bb** displayed a signal for OH as a triplet (J=5.9 Hz) at 4.71 ppm, that exchangeable with D_2O; in addition, selective irradiation of this signal clearly change the multiplets at 3.16 ppm (H-6a) and 3.00 ppm (H-6b) in two doublet of doublets typical of the AB part of an ABX system. Similar observations were also made on the ^{1}H NMR spectra of compounds **5Ba** and **5Bc**. This is only possible for a structure with the O–C5 oxygen involved in the aminal moiety.

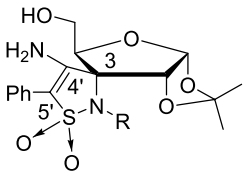
Final confirmation regarding the whole structure on these compounds (**5Ba**–**c**), and the absolute configuration at the new stereocenter at C-4′ were obtained by X-ray analysis of a suitable crystal from of **5Bc** (Fig. 1).

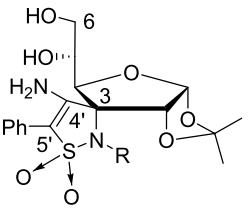
The X-ray molecular structure of compound **5Bc**, shows that the absolute configuration at C4′ is *S*, as previously suggested (Fig. 1). The N···O and O···O distances in the range 2.883(2)–3.121(3) Å and the N···H···O and O···H···O angles in the range 117(2)–174(2)° are in accordance with the formation of four strong hydrogen bonds, two of which are intramolecular and the other two,





5Aa (R=Me)	**5Ab** (R=Bn)	**5Ac** (R=H)	**5Ba** (R=Me)	**5Bb** (R=Bn)	**5Bc** (R=H)
C-3: 79.4	78.5	77.6	C-3: 79.9	80.5	78.3
C-4: 95.5	95.6	96.7	C-4: 96.4	95.9	98.5
C-5: 57.1	58.5	58.9	C-5: 57.4	58.2	60.3
H-5' 3.36/3.58	3.27/3.61	3.27/3.62	OH: 4.83 (t)	4.71(t)	4.99 (t)
$J_{5a,5b}$: 13.2 Hz	13.0 Hz	13.2 Hz	$J_{OH,6}$: 5.9 Hz	5.9 Hz	5.3 Hz





6Aa (R=Me)	**6Ab** (R=Bn)	**6Ac** (R=H)	**6Ba** (R=Me)	**6Bc** (R=Bn)	**6Bc** (R=H)
C-3: 69.7	70.4	68.7	C-3: 70.7	71.6	69.6
C-4: 147.5	147.5	147.4	C-4: 148.2	145.8	149.0
C-5: 102.6	104.4	104.2	C-5: 103.4	107.2	105.7

Chart 3.

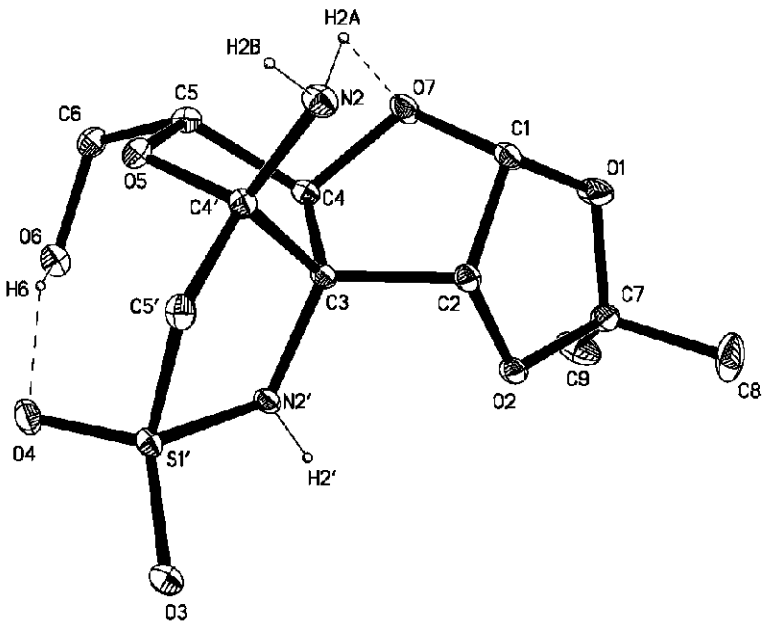


Figure 1. ORTEP view of the molecular unit of **5Bc**, showing intramolecular hydrogen bonds as dotted lines.

Table 1. Hydrogen bonds for **5Bc** (Å and °)

D–H···A	d(D–H)	d(H···A)	d(D···A)	<(DHA)
N(2)–H(2A)···O(7)	0.84(2)	2.65(3)	3.121(3)	117(2)
N(2)–H(2B)···O(6)#1	0.91(3)	2.15(3)	3.054(3)	174(2)
N(2′)–H(2′)···O(6)#2	0.81(3)	2.23(3)	3.001(3)	160(2)
O(6)–H(6)···O(4)	0.87(3)	2.06(3)	2.883(2)	158(2)

Symmetry transformations used to generate equivalent atoms: #1 $x-1, y, z$; #2 $-x+2, y-1/2, -z+1/2$.

intermolecular, whose details are given in Table 1 The intermolecular bonds are extending in two directions giving rise to the formation of folder layers of molecules running to the 'ab' crystal plane (Fig. 2).

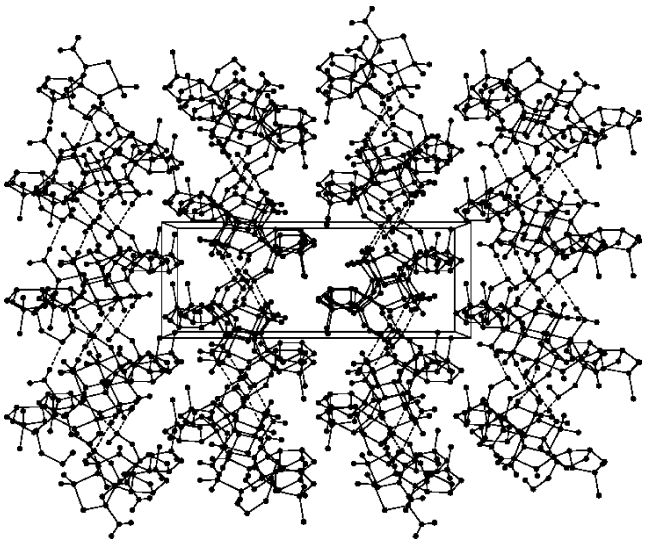


Figure 2. Crystal packing of **5Bc**.

Based on this analysis, and by comparison of the close relationship between the spectroscopic data for **5Bc** with those observed for the analogues **5Ba** and **5Bb** (Chart 3), we confirmed the previously assigned structures for these compounds (Scheme 1).

3. Conclusions

In summary, we have demonstrated that differently α-sulfonamidonitriles derived from commercial carbohydrates are key intermediates in the CSIC reaction leading to useful building blocks incorporating the [1,2-*O*-isopropylidene-α-D-*erythro* (and α-D-*ribo*)furanose]-3-spiro-3′-(5′*H*-4′-amino-2′,3′-dihydroisothiazole-1′,1′-dioxide) structural and functional moiety, and we conclude that:

1. Refluxing of the secondary methanesulfonamides [**3Aa**(**Ba**)] with potassium carbonate as base in acetone, in the presence of the appropriate electrophilic agents, gave the *N*-alkylated products in good yields (56–99%).
2. Under comparable conditions as described above, the benzylsulfonamides [**3Ab**(**Bb**)] gave mixtures of *N*-alkylated and the desired CSIC products in short reaction times. However, extended reaction times in excess of 24 h enabled the CSIC reaction products to be isolated exclusively in good yield.
3. All of the *N*-substituted alkylsulfonamides (**3**) reacted efficiently using cesium carbonate as base in acetonitrile, to give the CSIC products (**4**) in yields ranging from 46 to 99%.
4. *n*-BuLi (or LDA, in THF, at −10 °C) was found to be the most effective base promoting CSIC reactions in *secondary* alkylsufonamides with minimum by-product.
5. The acid hydrolysis of the CSIC adducts **4** gave tetracyclic aminal derivatives implementing the formation of fused tertrahydrofuran rings by nucleophilic attack of the C-5 free hydroxyl group onto the protonated enamine moiety.

4. Experimental

4.1. Materials and methods

Melting points were determined on a digital melting-point apparatus (Electrothermal) and are uncorrected. Optical rotations were recorded in $CHCl_3$, MeOH, acetone or DMSO with a digital polarimeter DIP-370 (JASCO) using a 1 dm cell. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$, acetone-d6, Me_2SO-d6 or MeOD-d3 (internal Me_4Si), respectively, at 300.13 MHz and at 75.47 MHz (Bruker Avance-300). TLC was performed on Silica F254 (Merck) and detection by UV light at 254 nm or by charring with phosphomolybdic–H_2SO_4 reagent. Column chromatography was effected on Silica Gel 60 (Merck, 230 mesh). Acetone, hexane and diethyl ether were distilled before use. Bases and solvents were used as supplied. MeOH–NH_3 is methanol saturated (7 N) with ammonia gas at room temperature. Elemental analyses have been carried out in Madrid (IQOG, CSIC reaction). ^{13}C NMR resonances have been assigned by using standard NMR (DEPT, COSY, HMBQ, HMBC) experiments. Compounds have been recrystallized from ethyl acetate–hexane mixtures. FTIR spectra were obtained on a AVATAR™ 320 neat on KBr plate or using ATR and are reported in cm^{-1}. Mass spectral data were acquiered on a WATERS Micromass ZQ spectrometer or a WATERS Micromass Q-TOFF spectrometer.

4.2. General method for the synthesis of sulfonamides (A)

Methylsulfonyl (or benzylsulfonyl chloride) (3 equiv.) was added dropwise to a solution of the aminonitrile and DMAP (0.5 equiv.) in dry pyridine. The reaction mixture was stirred at rt until the disappearance of the aminonitrile (2–30 h). Then water and EtOAc were added. The organic layer was separated, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by flash chromatography.

4.3. General method for the synthesis for the *N*-alkylation of sulfonamides (B)

To a solution of sulfonamide and K_2CO_3 (1.5 equiv.), in acetone, was added MeI, BnBr or AllBr (2 equiv.). The mixture was refluxed until complete reaction (45 min–28 h). The mixture was filtered through a silica pad and evaporated to dryness. The residue was purified by flash chromatography.

4.4. General method for the one-pot CSIC procedure using K_2CO_3 (C)

To a solution of the sulfonamidonitriles and K_2CO_3 (1.5 equiv.), in acetone, was added MeI, BnBr or AllBr (2 equiv.). The mixture was refluxed until complete reaction (17 h–48 h). The mixture was filtered through Celite and evaporated to dryness. The residue was purified by flash chromatography.

4.5. General method for the CSIC reaction using Cs_2CO_3 (D)

Cs_2CO_3 (1 equiv.) was added to a solution of the sulfonamidonitriles in CH_3CN. The mixture was refluxed until complete reaction and then was filtered through Celite and evaporated to dryness. The residue was purified by flash chromatography.

4.6. General method for the CSIC reaction using *n*-BuLi (E)

To a solution of the sulfonamidonitrile in dry THF, *n*-BuLi (3 equiv., 1.6 M or 2.5 M in hexane) was added. The reaction mixture was stirred for 5 min–20 min at −10 °C. Water and HCl 1 M were added until pH 6. Then, ethyl acetate was added. The organic layer was separated and dried over Na_2SO_4. The solvent was removed under vacuum and the residue was purified by flash chromatography (EtOAc/petroleum ether).

4.7. General method for the acid hydrolysis of acetals (F)

Acetic acid and water (6:4, v/v) were added, and the reaction mixture was stirred at 40 °C for 1 h 10 min–5 h 20 min. Then the solvent was evaporated to dryness and the residue was purified by flash chromatography (EtOAc/petroleum ether) to give the products as indicated.

4.8. General method for acetylations (G)

Pyridine and Ac_2O (1:1, v/v) were added to the compound. The reaction mixture was stirred at room temperature. After complete reaction, the solvent was evaporated to dryness and the residue was purified by flash chromatography eluting with mixtures of hexane/EtOAc.

4.8.1. 3-Amino-3-*C*-cyano-3-deoxy-1,2-*O*-isopropylidene-5-*O*-trityl-α-D-ribofuranose (2A). $Ti(OiPr)_4$ (4.18 mL, 13.9 mmol) was added to a solution of ulose **1A** (5 g, 11.6 mmol) and NH_3 (16.6 mL, NH_3 in MeOH 7 N) in MeOH (23.4 mL). The reaction mixture was stirred at rt for 5 h, then TMSiCN (1.55 mL, 11.6 mmol) was added and the mixture stirred for 12 h. Water (5 mL) and EtOAc were added until oxidation of the titanium residue was complete. The solvent was evaporated to dryness and the crude product was purified by flash chromatography (EtOAc/petroleum ether, 15:85) to yield compound **2A** (5.8 g, 96%) as a colourless solid: mp 163–167 °C; $[\alpha]_D^{25}$ −29 (*c* 1.20, $CHCl_3$); IR (ATR) ν 1479, 1441, 1370, 1205, 1079, 1020, 870, 747, 706 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.50–7.39 [m, 15H, $OC(C_6H_5)_3$], 5.91 (d, $J_{1,2}$=3.6 Hz, 1H, H-1), 4.70 (d, 1H, H-2), 3.86 (dd, $J_{4,5a}$=7.3 Hz, $J_{4,5b}$=5.4 Hz, 1H, H-4), 3.80 (dd, $J_{5a,5b}$=9.7 Hz, 1H, H-5b), 3.54 (dd, 1H, H-5a), 2.04 (s, 2H, NH_2), 1.58 (s, 3H, CH_3), 1.38 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 143.3, 128.6–127.4 [18 C, $OC(C_6H_5)_3$], 118.5 (CN), 113.4 [$OC(CH_3)_2$], 103.9 (C-1), 87.9 [$OC(C_6H_5)_3$], 83.2 (C-2), 79.9 (C-4), 63.3 (C-5), 62.3 (C-3), 26.5 (CH_3), 26.4 (CH_3); MS (APCI+) *m*/*z* 479.33 $[M+Na]^+$, 495.26 $[M+K]^+$, 935.44 $[2M+Na]^+$. Anal. Calcd for $C_{28}H_{28}N_20_4$ (456.43 g/mol): C, 73.66; H, 6.18; N, 6.14. Found: C, 73.55; H, 6.07; N, 6.01.

4.8.2. 3-Amino-3-*C*-cyano-3-deoxy-1,2-*O*-isopropylidene-3-*N*-methanesulfonyl-5-*O*-trityl-α-D-ribofuranose (3Aa). Following the general method (A), CH_3SO_2Cl (0.37 mL, 3.3 mmol) was added dropwise to a solution of **2A** (500 mg, 1.1 mmol) and DMAP (60 mg, 0.55 mmol) in dry pyridine (2.75 mL). The reaction mixture was stirred at room temperature for 5 h. The crude product was submitted to flash chromatography (EtOAc/petroleum ether, 2:8) to yield **3Aa** (540 mg, 92%) as a colourless solid: mp 223–224 °C; $[\alpha]_D^{26}$ −27 (*c* 1.39, $CHCl_3$); IR (ATR) ν 3299, 1381, 1332, 1150, 1054, 879, 751, 704 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.41–7.30 [m, 15H, $OC(C_6H_5)_3$], 5.86 (d, $J_{1,2}$=3.6 Hz, 1H, H-1), 5.61 (s, 1H, NH), 5.07 (d, 1H, H-2), 3.91 (dd, $J_{5a,4}$=8.8 Hz, $J_{4,5b}$=4.8 Hz, 1H, H-4), 3.79 (dd, $J_{5b,5a}$=10.1 Hz, 1H, H-5a), 3.61 (dd, 1H, H-5b), 3.05 (s, 3H, SO_2CH_3), 1.52 (s, 3H, CH_3), 1.25 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 142.7, 128.4–127.6 [18 C, $OC(C_6H_5)_3$], 115.7 (CN), 114.0 [$OC(CH_3)_2$], 104.3 (C-1), 88.6 [$OC(C_6H_5)_3$], 82.4 (C-2), 77.8 (C-4), 63.2 (C-3), 62.7 (C-5), 42.9 (SO_2CH_3), 26.6 (CH_3), 26.3 (CH_3); MS (APCI+) *m*/*z* 557.26 $[M+Na]^+$. Anal. Calcd for $C_{29}H_{30}N_20_6S$ (534.18 g/mol): C, 65.15; H, 5.66; N, 5.24; S, 6.00. Found: C, 64.98; H, 5.61; N, 5.22; S, 6.06.

4.8.3. 3-Amino-3-*C*-cyano-3-deoxy-1,2-*O*-isopropylidene-3-*N*-phenylmethanesulfonyl-5-*O*-trityl-α-D-ribofuranose (3Ab). Following the general method (A), **2A** (4.0 g, 8.76 mmol), DMAP (0.53 g, 4.38 mmol), $C_6H_5CH_2$-SO_2Cl (3.34 g, 17.52 mmol) in pyridine (25 mL) for 2 h gave, after flash chromatograpy (EtOAc/petroleum ether, 2:8), compound **3Ab** (5.0 g, 93%) as a colourless solid: mp 97–99 °C; $[\alpha]_D^{24}$ −40 (*c* 0.47, $CHCl_3$); IR (ATR) ν 3299, 1337, 1162, 1064, 900, 778, 704 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.42–7.29 (m, 20H, 4×C_6H_5), 5.92 (d, $J_{1,2}$=3.6 Hz, 1H, H-1), 5.16 (s, 1H, H-2), 5.10 (d, 1H, NH), 4.53 (d, $J_{A,B}$=13.8 Hz, 1H, H-A, $SO_2CH_2C_6H_5$), 4.34 (d, 1H, H-B, $SO_2CH_2C_6H_5$), 3.86 (dd, $J_{5a,4}$=5.2 Hz, $J_{5a,5b}$=10.3 Hz, 1H, H-5a), 3.70 (dd, 1H, H-4), 3.59 (dd, $J_{5b,4}$=7.3 Hz, 1H, H-5b), 1.59 (s, 3H, CH_3), 1.41 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 143.2–127.9 (24 C, 4×C_6H_5), 116.2 (CN), 114.4 [$OC(CH_3)_2$], 104.8 (C-1), 89.1 [$OC(C_6H_5)_3$], 82.6 (C-2), 78.5 (C-4), 63.3 (C-5), 63.1 (C-3), 60.6 ($SO_2CH_2C_6H_5$), 27.0 (CH_3), 26.8 (CH_3); MS (APCI+) *m*/*z* 633.45 $[M+Na]^+$, 649.37 $[M+K]^+$, 1243.58 $[2M+Na]^+$, 1259.50 $[2M+K]^+$. Anal. Calcd for $C_{35}H_{34}N_20_6S$ (610.21 g/mol): C, 68.83; H, 5.61; N, 4.59; S, 5.25. Found: C, 69.04; H, 5.75; N, 4.68; S, 5.40.

4.8.4. 3-Amino-3-*C*-cyano-3-deoxy-1,2-*O*-isopropylidene-3-*N*-methanesulfonyl-3-*N*-methyl-5-*O*-trityl-α-D-ribofuranose (3Ac). Following the general method (B), a solution of **3Aa** (0.75 g, 1.40 mmol) in acetone (22 mL) was treated with K_2CO_3 (0.29 g, 2.10 mmol) and CH_3I

(0.17 mL, 2.80 mmol). The reaction mixture was refluxed for 2 h to yield, after flash chromatography (EtOAc), compound **3Ac** (0.77 g, 99%) as a colorless oil: $[\alpha]_D^{26}$ +10 (*c* 2.01, $CHCl_3$); IR (ATR) ν 1450, 1349, 1220, 1159, 1034, 958, 709 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.37–7.16 [m, 15H, $OC(C_6H_5)_3$] 5.79 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 4.99 (d, 1H, H-2), 4.48 (dd, $J_{4,5a}$=4.1 Hz, $J_{4,5b}$=5.8 Hz, 1H, H-4), 3.68 (dd, $J_{5a,5b}$=10.7 Hz, 1H, H-5b), 3.38 (dd, 1H, H-5a), 2.81 (s, 3H, SO_2CH_3), 2.8 (s, 3H, NCH_3), 1.47 (s, 3H, CH_3), 1.23 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 142.8, 128.4–127.2 [18 C, $OC(C_6H_5)_3$], 115.3 (CN), 113.0 [$OC(CH_3)_2$], 103.0 (C-1), 87.5 [$OC(C_6H_5)_3$], 84.1 (C-2), 77.6 (C-4), 65.7 (C-3), 63.0 (C-5), 39.7 (SO_2CH_3), 34.4 (NCH_3), 26.2 (CH_3), 26.1 (CH_3); MS (APCI+) *m/z* 571.30 $[M+Na]^+$, 587.42 $[M+K]^+$. Anal. Calcd for $C_{30}H_{32}N_20_6S$ (548.20 g/mol): C, 65.67; H, 5.88; N, 5.11; S, 5.84. Found: C, 65.82; H, 6.10; N, 5.23; S, 5.68.

4.8.5. 3-Amino-3-*N*-benzyl-3-*C*-cyano-3-deoxy-1,2-*O*-isopropylidene-3-*N*-methanesulfonyl-5-*O*-trityl-α-D-ribofuranose (3Ad). Following the general method (B), a solution of **3Aa** (0.39 g, 0.74 mmol) in acetone (10 mL) was treated with K_2CO_3 (0.15 g, 1.11 mmol) and BnBr (0.17 mL, 1.49 mmol). The reaction was refluxed for 19 h to yield after flash chromatography (EtOAc/petroleum ether, 2:8), compound **3Ad** (0.31 g, 66%) as a white solid: mp 66–68 °C; $[\alpha]_D^{24}$ +21 (*c* 0.26, $CHCl_3$); IR (ATR) ν 1441, 1352, 1154, 1029, 747, 699 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.52–7.31 (m, 20H, 4×C_6H_5), 5.95 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 5.16 (d, 1H, H-2), 4.66 (dd, $J_{4,5a}$=5.5 Hz, $J_{4,5b}$=1.9 Hz, 1H, H-4), 4.43 (d, $J_{A,B}$=16.5 Hz, 1H, H-B, $NCH_2C_6H_5$), 4.13 (d, 1H, H-A, $NCH_2C_6H_5$), 3.81 (dd, $J_{5a,5b}$=11.2 Hz, 1H, H-5a), 3.58 (dd, 1H, H-5b), 2.82 (s, 3H, SO_2CH_3), 1.33 (s, 6H, 2×CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 143.4–136.2, 129.2–127.8 [24 C, $OCH_2C_6H_5$, $OC(C_6H_5)_3$], 116.4 (CN), 113.7 [$OC(CH_3)_2$], 103.4 (C-1), 88.1 [$OC(C_6H_5)_3$], 84.8 (C-2), 78.9 (C-4), 67.3 (C-3), 64.5 (C-5), 51.3 ($NCH_2C_6H_5$), 41.4 (SO_2CH_3), 26.9 (CH_3), 26.4 (CH_3); MS (APCI+) *m/z* 647.27 $[M+Na]^+$, 663.26 $[M+K]^+$. Anal. Calcd for $C_{36}H_{36}N_20_6S$ (624.23 g/mol): C, 69.21; H, 5.81; N, 4.48; S, 5.13. Found: C, 69.68; H, 5.80; N, 4.60; S, 5.39.

4.8.6. 3-*N*-Allyl-3-amino-3-*C*-cyano-3-deoxy-1,2-*O*-isopropylidene-3-*N*-methanesulfonyl-5-*O*-trityl-α-D-ribofuranose (3Ae). Following the general method (B), a solution of **3Aa** (1.50 g, 2.80 mmol) in acetone (30 mL) was treated with K_2CO_3 (0.58 g, 4.20 mmol) and AllBr (0.48 mL, 5.60 mmol). The reaction was refluxed for 24 h to yield, after flash chromatography (EtOAc/petroleum ether,1.5:8.5), compound **3Ae** (0.90 g, 56%) as a white solid: mp 167–168 °C; $[\alpha]_D^{30}$ +5 (*c* 0.51, $CHCl_3$); IR (ATR) ν 1485, 1447, 1353, 1159, 1043, 866, 749, 707 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.51–7.31 (m, 15H, 3×C_6H_5), 5.94 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 5.70 (m, 1H, NCH_2-CH=CH_2), 5.14 (m, 2H, NCH_2CH=CH_2), 5.07 (d, 1H, H-2), 4.70 (dd, $J_{4,5a}$=5.6 Hz, $J_{4,5b}$=2.7 Hz, 1H, H-4), 3.87 (m, 1H, H-A, NCH_2CH=CH_2), 3.79 (dd, $J_{5a,5b}$=10.9 Hz, 1H, H-5a), 3.74 (m, 1H, H-B, NCH_2CH=CH_2), 3.46 (dd, 1H, H-5b), 2.98 (s, 3H, SO_2CH_3), 1.63 (s, 3H, CH_3), 1.39 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 143.3–127.8 (18C, 3×C_6H_5), 135.4 (NCH_2CH=CH_2), 118.6 (NCH_2-CH=CH_2), 116.2 (CN), 113.8 [$OC(CH_3)_2$], 103.6 (C-1), 88.0 [$OC(C_6H_5)_3$], 84.3 (C-2), 78.3 (C-4), 67.0 (C-3), 64.1 (C-5), 50.3 (NCH_2CH=CH_2), 41.6 (SO_2CH_3), 26.9 (CH_3), 26.7 (CH_3); MS (APCI+) *m/z* 597.4 $[M+Na]^+$, 613.4 $[M+K]^+$. Anal. Calcd for $C_{32}H_{34}N_20_6S$ (574.21 g/mol): C, 66.88; H, 5.96; N, 4.87; S, 5.58. Found: C, 66.59; H, 5.66; N, 4.58; S, 5.31.

4.8.7. 3-Amino-3-*C*-cyano-3-deoxy-1,2-*O*-isopropylidene-3-*N*-methyl-3-*N*-phenylmethanesulfonyl-5-*O*-trityl-α-D-ribofuranose (3Af). Following the general method (B), **3Ab** (0.80 g, 1.31 mmol), K_2CO_3 (0.27 g, 1.96 mmol), MeI (0.16 mL, 2.62 mmol) in acetone (20 mL) for 1 h 20 min gave, after flash chromatography (EtOAc/petroleum ether, 2:8), compound (**3Af**) (0.70 g, 85%) as a yellow solid: mp 89–91 °C; $[\alpha]_D^{29}$ +37 (*c* 0.50, $CHCl_3$); IR (ATR) ν 1490, 1441, 1353, 1216, 1153, 1032, 883, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.54–7.30 (m, 20H, 4×C_6H_5), 5.97 (d, $J_{1,2}$=3.9 Hz, 1H, H-1), 5.16 (d, 1H, H-2), 4.55 (dd, $J_{4,5a}$=6.9 Hz, $J_{4,5b}$=2.5 Hz, 1H, H-4), 4.36 (s, 2H, $SO_2CH_2C_6H_5$), 3.89 (dd, $J_{5a,5b}$=10.9 Hz, 1H, H-5a), 3.50 (dd, 1H, H-5b), 2.46 (s, 3H, NCH_3), 1.63 (s, 3H, CH_3), 1.39 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 143.7–127.8 (24 C, 4×C_6H_5), 115.9 (CN), 113.7 [$OC(CH_3)_2$], 103.7 (C-1), 88.2 [$OC(C_6H_5)_3$], 85.1 (C-2), 78.5 (C-4), 66.0 (C-3), 64.0 (C-5), 60.3 ($SO_2CH_2C_6H_5$), 36.1 (NCH_3), 26.9 (CH_3), 26.8 (CH_3); MS (APCI+) *m/z* 647.36 $[M+Na]^+$, 663.35 $[M+K]^+$, 1271.53 $[2M+Na]^+$. Anal. Calcd for $C_{36}H_{36}N_20_6S$ (624.23 g/mol): C, 69.21; H, 5.81; N, 4.48; S, 5.13. Found: C, 68.95; H, 5.73; N, 4.64; S, 5.30.

4.8.8. 3-Amino-3-*N*-benzyl-3-*C*-cyano-3-deoxy-1,2-*O*-isopropylidene-3-*N*-phenylmethanesulfonyl-5-*O*-trityl-α-D-ribofuranose (3Ag). Following the general method (B), **3Ab** (0.60 g, 0.98 mmol), K_2CO_3 (0.20 g, 1.47 mmol), BnBr (0.23 mL, 1.96 mmol) in acetone (30 mL) for 4 h gave, after flash chromatography (EtOAc/petroleum ether, 1.5:8.5) compound (**3Ag**) (215 mg, 31%) as a colourless oil and unreacted **3Ab** (255 mg, 42%). **3Ag**: $[\alpha]_D^{29}$ +28 (*c* 0.30, $CHCl_3$); IR (ATR) ν 2959, 2915, 1490, 1447, 1348, 1145, 1085, 1041, 899, 733, 697 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.56–7.33 (m, 25H, 5×C_6H_5), 5.94 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 5.16 (d, 1H, H-2), 4.72 (dd, $J_{4,5a}$=5.9 Hz, $J_{4,5b}$=1.3 Hz, 1H, H-4), 4.37 (d, $J_{A,B}$=13.6 Hz, 1H, H-A, $SO_2CH_2C_6H_5$), 4.23 (d, 1H, H-B, $SO_2CH_2C_6H_5$), 4.14 (d, $J_{A,B}$=16.6 Hz, 1H, H-A, $NCH_2C_6H_5$), 3.82 (m, 2H, H-5a, H-B, $NCH_2C_6H_5$), 3.63 (dd, $J_{5a,5b}$=11.1 Hz, 1H, H-5b), 1.34 (s, 3H, CH_3), 1.19 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 143.6–127.5 (30 C, 5×C_6H_5), 116.6 (CN), 113.7 [$OC(CH_3)_2$], 103.3 (C-1), 88.1 ($OC(C_6H_5)_3$), 84.9 (C-2), 78.6 (C-4), 67.2 (C-3), 64.9 (C-5), 60.2 ($SO_2CH_2C_6H_5$), 52.4 ($NCH_2C_6H_5$), 26.8 (CH_3), 26.1 (CH_3); MS (APCI+) *m/z* 723.4 $[M+Na]^+$, 739.4 $[M+K]^+$. Anal. Calcd for $C_{42}H_{40}N_20_6S$ (700.26 g/mol): C, 71.98; H, 5.75; N, 4.00; S, 4.58. Found: C, 70.68; H, 6.80; N, 4.60; S, 4.69.

4.8.9. 3-*N*-Allyl-3-amino-3-*C*-cyano-3-deoxy-1,2-*O*-isopropylidene-5-*O*-trityl-3-*N*-phenylmethanesulfonyl α-D-ribofuranose (3Ah). Following the general method (B), **3Ab** (0.4 g, 0.65 mmol), K_2CO_3 (0.13 g, 0.98 mmol), AllBr (0.11 mL, 1.31 mmol) in acetone (20 mL) for 17 h gave, after flash chromatography (EtOAc/petroleum ether, 3:7) product (**3Ah**) (211 mg, 49%) as a white solid and 1,2-*O*-isopropylidene-5-*O*-trityl-α-D-*erythro*-pentofuranose-3-

spiro-3′-(2′-*N*-allyl-4′-amino-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (**4Af**) (73 mg, 18%). **3Ah**: mp 97–99 °C; $[\alpha]_D^{32}$ +33 (*c* 0.53, $CHCl_3$); IR (ATR) ν 2921, 2356, 1447, 1348, 1151, 1085, 1036, 871, 751, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.53–7.34 (m, 20H, 4×C_6H_5), 5.97 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 5.58 (m, 1H, NCH_2CH=CH_2), 5.13 (d, 1H, H-2), 5.02 (d, $J_{A,\beta}$=8.2 Hz, 1H, NCH_2CH=CH_2), 4.99 (d, $J_{B,\beta}$=9.9 Hz, 1H, NCH_2-CH=CH_2), 4.77 (dd, $J_{4,5a}$=6.4 Hz, $J_{4,5b}$=1.8 Hz, 1H, H-4), 4.42 (d, $J_{A,B}$=13.7 Hz, 1H, H-A, $SO_2CH_2C_6H_5$), 4.37 (d, 1H, H-B, $SO_2CH_2C_6H_5$), 3.82 (dd, $J_{5a,5b}$=10.9 Hz, 1H, H-5a), 3.47 (m, 3H, H-5b, NCH_2CH=CH_2), 1.66 (s, 3H, CH_3), 1.41 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 143.6–127.8 (24 C, 4×C_6H_5), 136.3 (NCH_2CH=CH_2), 117.8 (NCH_2CH=CH_2), 116.3 (CN), 113.8 [$OC(CH_3)_2$], 103.6 (C-1), 87.9 [$OC(C_6H_5)_3$], 84.5 (C-2), 78.3 (C-4), 66.8 (C-3), 64.5 (C-5), 60.3 ($SO_2CH_2C_6H_5$), 51.6 (NCH_2-CH=CH_2), 26.8 (CH_3), 26.7 (CH_3); MS (APCI+) *m/z* 673.5 $[M+Na]^+$, 689.5 $[M+K]^+$. Anal. Calcd for $C_{38}H_{38}N_20_6S$ (650.25 g/mol): C, 70.13; H, 5.89; N, 4.30; S, 4.93. Found: C, 69.95; H, 5.76; N, 4.29; S, 4.68. **4Af**: oil; $[\alpha]_D^{32}$ +66 (*c* 0.30, $CHCl_3$); IR (ATR) ν 3474, 3353, 2921, 1652, 1448, 1274, 1164, 1079, 909, 876, 733, 703 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.36 (m, 20H, 4×C_6H_5), 5.93 (d, $J_{1,2}$=3.9 Hz, 1H, H-1), 5.83 (m, 1H, NCH_2CH=CH_2), 5.21 (dd, $J_{A,\beta}$=17.3 Hz, $J_{A,B}$=1.3 Hz, 1H, NCH_2CH=CH_2), 5.05 (dd, $J_{B,\beta}$=10.1 Hz, 1H, NCH_2CH=CH_2), 4.73 (d, 1H, H-2), 4.68 (dd, $J_{4,5a}$=2.5 Hz, $J_{4,5b}$=5.6 Hz, 1H, H-4), 4.41 (s, 2H, NH_2), 4.15 (d, $J_{H,\beta}$=6.5 Hz, 2H, NCH_2CH=CH_2), 3.48 (dd, $J_{5a,5b}$=11.3 Hz, 1H, H-5a), 3.36 (dd, 1H, H-5b), 1.69 (s, 3H, CH_3), 1.39 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 146.6 (C-4′), 143.6–126.8 (24 C, 4×C_6H_5), 135.6 (NCH_2CH=CH_2), 118.1 (NCH_2CH=CH_2), 113.7 [$OC(CH_3)_2$], 108.2 (C-5′), 104.2 (C-1), 88.1 [$OC(C_6H_5)_3$], 84.8 (C-2), 76.0 (C-4), 69.8 (C-3), 61.1 (C-5), 44.8 (NCH_2CH=CH_2), 26.7 (CH_3), 26.3 (CH_3); MS (APCI+) *m/z* 673.3 $[M+Na]^+$, 689.2 $[M+K]^+$. Anal. Calcd for $C_{38}H_{38}N_20_6S$ (650.25 g/mol): C, 70.13; H, 5.89; N, 4.30; S, 4.93. Found: C, 69.05; H, 8.04; N, 4.00; S, 4.27.

4.8.10. 1,2-*O*-Isopropylidene-5-*O*-trityl-α-D-*erythro*-pentofuranose-3-spiro-3′-(4′-amino-2′-*N*-allyl-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Ad). Following the general method (C), **3Ab** (200 mg, 0.33 mmol), K_2CO_3 (67 mg, 0.49 mmol) and MeI (0.04 mL, 0.65 mmol) in acetone (10 mL) for 43 h gave, after flash chromatography (EtOAc/petroleum ether, 5.5:4.5), compound **4Ad** (194 mg, 95%) as a white solid: mp 231–232 °C; $[\alpha]_D^{28}$ +28 (*c* 0.15, $CHCl_3$); IR (ATR) ν 3447, 3353, 2921, 1655, 1441, 1260, 1145, 1054, 866, 760, 699 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.47–7.33 (m, 20H, 4×C_6H_5), 5.94 (d, $J_{1,2}$=3.9 Hz, 1H, H-1), 4.72 (d, 1H, H-2), 4.67 (dd, $J_{4,5a}$=3.2 Hz, $J_{4,5b}$=5.8 Hz, 1H, H-4), 4.40 (s, 2H, NH_2), 3.54 (dd, $J_{5a,5b}$=11.2 Hz, 1H, H-5a), 3.38 (dd, 1H, H-5b), 2.94 (s, 3H, NCH_3), 1.68 (s, 3H, CH_3), 1.37 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 146.5 (C-4′), 143.7–126.9 (24 C, 4×C_6H_5), 113.6 [$OC(CH_3)_2$], 107.8 (C-5′), 104.2 (C-1), 88.2 [$OC(C_6H_5)_3$], 85.0 (C-2), 75.4 (C-4), 69.6 (C-3), 61.5 (C-5), 27.4 (NCH_3), 26.6 (CH_3), 26.4 (CH_3); MS (APCI+) *m/z* 647.43 $[M+Na]^+$, 663.41 $[M+K]^+$, 1271.59 $[2M+Na]^+$, 1287.58 $[2M+K]^+$. Anal. Calcd for $C_{36}H_{36}N_20_6S$ (624.23 g/mol): C, 69.21; H, 5.81; N, 4.48; S, 5.13. Found: C, 68.04; H, 6.21; N, 5.00; S, 5.04.

4.8.11. 1,2-*O*-Isopropylidene-5-*O*-trityl-α-D-*erythro*-pentofuranose-3-spiro-3′-(4′-amino-2′-*N*-benzyl-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Ae). Following the general method (C), **3Ab** (500 mg, 0.82 mmol), K_2CO_3 (170 mg, 1.22 mmol) and BnBr (0.19 mL, 1.63 mmol) in acetone (20 mL) for 48 h gave, after flash chromatography (EtOAc/petroleum ether, 3:7), compound **4Ae** (394 mg, 68%) as a white solid: mp 98–100 °C; $[\alpha]_D^{31}$ +91 (*c* 0.50, $CHCl_3$); IR (ATR) ν 1649, 1490, 1447, 1364, 1271, 1211, 1151, 1030, 871, 751, 700 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.31–7.10 (m, 10H, 2×C_6H_5), 5.95 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 4.84 (d, 1H, H-2), 4.75 (s, 2H, $NCH_2C_6H_5$), 4.50 (s, 2H, NH_2), 4.38 (dd, $J_{4,5a}$=2.6 Hz, $J_{4,5b}$=4.2 Hz, 1H, H-4), 3.28 (dd, $J_{5a,5b}$=11.6 Hz, 1H, H-5a), 3.02 (dd, 1H, H-5b), 1.71 (s, 3H, CH_3), 1.42 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 147.6 (C-4′), 143.5–126.9 (30 C, 5×C_6H_5), 113.8 [$OC(CH_3)_2$], 108.0 (C-5′), 104.3 (C-1), 88.1 [$OC(C_6H_5)_3$], 85.2 (C-2), 76.2 (C-4), 70.2 (C-3), 60.4 (C-5), 44.8 ($NCH_2C_6H_5$), 26.9 (CH_3), 26.3 (CH_3); MS (APCI+) *m/z* 723.39 $[M+Na]^+$, 739.31 $[M+K]^+$, 1423.54 $[2M+Na]^+$. Anal. Calcd for $C_{42}H_{40}N_20_6S$ (700.26 g/mol): C, 71.98; H, 5.75; N, 4.00; S, 4.58. Found: C, 71.65; H, 5.37; N, 4.40; S, 4.43.

4.8.12. 1,2-*O*-Isopropylidene-5-*O*-trityl-α-D-*erythro*-pentofuranose-3-spiro-3′-(2′-*N*-allyl-4′-amino-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Af). Following the general method (C), **3Ab** (300 mg, 0.49 mmol), K_2CO_3 (0.10 g, 0.73 mmol) and AllBr (0.08 mL, 0.98 mmol) in acetone (20 mL) for 47 h gave, after flash chromatography (EtOAc/petroleum ether, 4.5:5.5), compound **4Af** (45 mg, 14%).

4.8.13. 1,2-*O*-Isopropylidene-5-*O*-trityl-α-D-*erythro*-pentofuranose-3-spiro-3′-(4′-amino-2′-*N*-methyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Aa). Following the general method (D), Cs_2CO_3 (0.18 g, 0.56 mmol) was added to a solution of **3Ac** (0.31 g, 0.56 mmol) in CH_3CN (5 mL). The reaction mixture was refluxed for 3 h then evaporated to dryness. The residue was purified by flash chromatography (EtOAc/petroleum ether, 6:4) to give **4Aa** (0.28 g, 90%) as a colourless solid: mp 158–160 °C; $[\alpha]_D^{26}$ +13 (*c* 1.01, $CHCl_3$); IR (ATR) ν 1638, 1254, 1210, 1117, 1073, 1024, 871, 706, 682 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.45–7.28 [m, 15H, $OC(C_6H_5)_3$], 5.84 (d, $J_{1,2}$=3.9 Hz, 1H, H-1), 5.43 (s, 1H, H-5′), 4.60 (d, 1H, H-2), 4.52 (t, $J_{4,5}$=5,1 Hz, 1H, H-4), 4.38 (s, 2H, NH_2), 3.40 (d, 2H, H-5), 2.77 (s, 3H, NCH_3), 1.64 (s, 3H, CH_3), 1.34 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.9 (C-4′), 143.2, 128.5–127.1 [18 C, $OC(C_6H_5)_3$], 113.0 [$OC(CH_3)_2$], 103.4 (C-1), 93.2 (C-5′), 87.4 [$OC(C_6H_5)_3$], 84.2 (C-2), 74.8 (C-4), 70.4 (C-3), 60.9 (C-5), 26.4 (NCH_3), 26.0 (CH_3), 25.8 (CH_3); MS (APCI+) *m/z* 571.24 $[M+Na]^+$. Anal. Calcd for $C_{30}H_{32}N_20_6S$ (548.20 g/mol): C, 65.67; H, 5.88; N, 5.11; S, 5.84. Found: C, 65.48; H, 5.67; N, 5.14; S, 5.61.

4.8.14. 1,2-*O*-Isopropylidene-5-*O*-trityl-α-D-*erythro*-pentofuranose-3-spiro-3′-(4′-amino-2′-*N*-benzyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Ab). Following the general method (D), Cs_2CO_3 (0.15 g, 0.47 mmol) was added to a solution of **3Ad** (0.29 g, 0.47 mmol) in CH_3CN (10 mL). The reaction mixture was refluxed for 3 h then

evaporated to dryness. The residue was purified by flash chromatography (EtOAc/petroleum ether, 7:3) to give **4Ab** (0.14 g, 46%) as a yellow oil: $[\alpha]_D^{24}$ +65 (*c* 1.36, $CHCl_3$); IR (ATR) ν 2926, 2362, 1633, 1271, 1110, 1074, 877, 740, 606 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.32 [m, 20H, C_6H_5, $OC(C_6H_5)_3$], 5.86 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 5.44 (s, 1H, H-5′), 4.71 (d, 1H, H-2), 4.58 (d, $J_{A,B}$=15,2 Hz, 1H, H-A, $NCH_2C_6H_5$), 4.51 (d, 1H, H-B, $NCH_2C_6H_5$), 4.42 (s, 2H, NH_2), 4.36 (dd, $J_{4,5a}$=2.7 Hz, $J_{4,5b}$=5.7 Hz, 1H, H-4), 3.21 (dd, $J_{5a,5b}$=11.4 Hz, 1H, H-5a), 3.11 (dd, 1H, H-5b), 1.67 (s, 3H, CH_3), 1.39 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.9 (C-4′), 143.2, 137.5, 129.2–127.6 [24 C, $OCH_2C_6H_5$, $OC(C_6H_5)_3$], 113.8 [$OC(CH_3)_2$], 104.1 (C-1), 94.9 (C-5′), 88.2 [$OC(C_6H_5)_3$], 84.9 (C-2), 76.1 (C-4), 71.5 (C-3), 60.6 (C-5), 44.9 ($NCH_2C_6H_3$), 26.8 (CH_3), 26.3 (CH_3); MS (APCI+) *m*/*z* 647.32 $[M+Na]^+$, 663.32 $[M+K]^+$. Anal. Calcd for $C_{36}H_{36}N_20_6S$ (624.23 g/mol): C, 69.21; H, 5.81; N, 4.48; S, 5.13. Found: C, 69.44; H, 5.57; N, 4.37; S, 5.43.

4.8.15. 1,2-*O*-Isopropylidene-5-*O*-trityl-α-D-*erythro*-pentofuranose-3-spiro-3′-(2′-*N*-allyl-4′-amino-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Ac). Following the general method (D), Cs_2CO_3 (11 mg, 0.34 mmol) was added to a solution of **3Ae** (200 mg, 0.34 mmol) in CH_3CN (5 mL). The reaction mixture was refluxed for 1 h 20 min then evaporated to dryness. The residue was purified by flash chromatography (EtOAc/petroleum ether, 4.5:5.5) to give **4Ac** (190 mg, 95%) as a white solid: mp 115–117 °C; $[\alpha]_D^{29}$ +55 (*c* 0.51, $CHCl_3$); IR (ATR) ν 3452, 3353, 2921, 1644, 1452, 1266, 1211, 1123, 1078, 866, 705 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.47–7.30 (m, 15H, 3×C_6H_5), 5.85 (d, $J_{1,2}$=3.9 Hz, 1H, H-1), 5.75 (m, 1H, NCH_2CH=CH_2), 5.35 (s, 1H, H-5′), 5.19 (d, $J_{A,\beta}$=17.3 Hz, 1H, NCH_2CH=CH_2), 5.03 (d, $J_{B,\beta}$=10.2 Hz, 1H, NCH_2-CH=CH_2), 4.58 (d, 1H, H-2), 4.52 (m, 3H, H-4, NH_2), 3.95 (d, *J*=6.5 Hz, 2H, NCH_2CH=CH_2), 3.41 (dd, $J_{4,5a}$=7.2 Hz, $J_{5a,5b}$=11.1 Hz, 1H, H-5a), 3.33 (dd, $J_{4,5b}$=2.6 Hz, 1H, H-5b), 1.66 (s, 3H, CH_3), 1.36 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.2 (C-4′), 143.7–127.7 (18 C, 3×C_6H_5), 135.3 (NCH_2CH=CH_2), 118.3 (NCH_2-CH=CH_2), 113.6 [$OC(CH_3)_2$], 104.1 (C-1), 94.2 (C-5′), 87.8 [$OC(C_6H_5)_3$], 84.5 (C-2), 76.0 (C-4), 71.3 (C-3), 61.5 (C-5), 44.8 (NCH_2CH=CH_2), 26.6 (CH_3), 26.4 (CH_3); MS (APCI+) *m*/*z* 597.1 $[M+Na]^+$. Anal. Calcd for $C_{32}H_{34}N_20_6S$ (574.21 g/mol): C, 66.88; H, 5.96; N, 4.87; S, 5.58. Found: C, 66.94; H, 6.15; N, 4.69; S, 5.39.

4.8.16. 1,2-*O*-Isopropylidene-5-*O*-trityl-α-D-*erythro*-pentofuranose-3-spiro-3′-(4′-amino-2′-*N*-methyl-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Ad). Following the general method (D), Cs_2CO_3 (0.19 g, 6.05 mmol) was added to a solution of **3Af** (378 mg, 6.05 mmol) in CH_3CN (5 mL). The reaction mixture was refluxed for 1 h then evaporated to dryness. The residue was purified by flash chromatography (EtOAc/petroleum ether, 5.2:4.8) to give **4Ad** (279 mg, 73%).

4.8.17. 1,2-*O*-Isopropylidene-5-*O*-trityl-α-D-*erythro*-pentofuranose-3-spiro-3′-(4′-amino-2′-*N*-benzyl-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Ae). Following the general method (D), Cs_2CO_3 (48 mg, 0.15 mmol) was added to a solution of **3Ag** (105 mg, 0.15 mmol) in CH_3CN (5 mL). The reaction mixture was refluxed for 1 h then evaporated to dryness. The residue was purified by flash chromatography (EtOAc/petroleum ether, 3:7) to give **4Ae** (50 mg, 48%).

4.8.18. 1,2-*O*-Isopropylidene-5-*O*-trityl-α-D-*erythro*-pentofuranose-3-spiro-3′-(2′-*N*-allyl-4′-amino-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Af). Following the general method (D), Cs_2CO_3 (26.3 mg, 0.08 mmol) was added to a solution of **3Ah** (52.6 mg, 0.08 mmol) in CH_3CN (5 mL). The reaction mixture was refluxed for 45 min then evaporated to dryness. The residue was purified by flash chromatography (EtOAc/petroleum ether, 3:7) to give **4Af** (47.2 mg, 89%).

4.8.19. 1,2-*O*-Isopropylidene-5-*O*-trityl-α-D-*erythro*-pentofuranose-3-spiro-3′-(4′-amino-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Ag). Following the general method (E), *n*-BuLi (9 mL, 13 mmol, 1.6 M in hexane) was added to a solution of **3Aa** (200 mg, 3.7 mmol) in THF (30 mL). The reaction mixture was stirred for 10 min at −10 °C. The crude was purified by flash chromatography (EtOAc/petroleum ether, 1:1) to give **4Ag** (2.24 g, 98%) as a colourless solid: mp 93–95 °C; $[\alpha]_D^{25}$ +15 (*c* 1.21, $CHCl_3$); IR (ATR) ν 1633, 1277, 1216, 1125, 1063, 705 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.40–7.23 (m, 15H, $OC(C_6H_5)_3$), 5.86 (d, $J_{1,2}$=4.0 Hz, 1H, H-1), 5.22 (s, 1H, $CHSO_2$), 4.85 (s, 1H, NH), 4.48 (d, 1H, H-2), 4.46 (s, 1H, NH_2), 4.00 (t, $J_{4,5}$=4.8 Hz, 1H, H-4), 3.48 (d, 2H, H-5), 1.51 (s, 3H, CH_3), 1.31 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.1 (C-4′), 143.3, 128.6–127.2 [18 C, $OC(C_6H_5)_3$], 113.5 [$OC(CH_3)_2$], 103.7 (C-1), 94.1 (C-5′), 87.6 [$OC(C_6H_5)_3$], 81.8 (C-2), 79.3 (C-4), 69.3 (C-3), 60.5 (C-5), 26.4 (CH_3), 26.1 (CH_3); MS (APCI+) *m*/*z* 557.20 $[M+Na]^+$. Anal. Calcd for $C_{29}H_{30}N_20_6S$ (534.18 g/mol): C, 65.15; H, 5.66; N, 5.24; S, 6.00. Found: C, 65.06; H, 5.85; N, 5.05; S, 6.20.

4.8.20. 1,2-*O*-Isopropylidene-5-*O*-trityl-α-D-*erythro*-pentofuranose-3-spiro-3′-(4′-amino-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Ah). Following the general method (E), *n*-BuLi (0.98 mL, 2.45 mmol, 2.5 M in hexane) was added to a solution of **3Ab** (500 mg, 0.81 mmol) in THF (10 mL). The reaction mixture was stirred for 20 min at −10 °C. The crude was purified by flash chromatography (EtOAc/petroleum ether, 7:3) to give **4Ah** (380 mg, 76%) as a colourless solid: mp 98–100 °C; $[\alpha]_D^{25}$ +38 (*c* 0.19, $CHCl_3$); IR (ATR) ν 1649, 1479, 1447, 1266, 1140, 1068, 860, 701 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.38 (m, 20H, 4×C_6H_5), 6.04 (d, $J_{1,2}$=3.9 Hz, 1H, H-1), 5.12 (s, 1H, NH), 4.69 (d, 1H, H-2), 4.47 (s, 1H, NH_2), 4.19 (dd, $J_{4,5a}$=3.3 Hz, $J_{4,5b}$=5.2 Hz, 1H, H-4), 3.64 (dd, $J_{5b,5a}$=11.3 Hz, 1H, H-5a), 3.54 (dd, 1H, H-5b), 1.61 (s, 3H, CH_3), 1.41 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 147.0 (C-4′), 143.3–127.1 (24 C, 4×C_6H_5), 114.0 [$OC(CH_3)_2$], 108.1 (C-5′), 104.4 (C-1), 88.3 [$OC(C_6H_5)_3$], 82.5 (C-2), 80.1 (C-4), 68.1 (C-3), 60.8 (C-5), 26.9 (CH_3), 26.6 (CH_3); MS (APCI+) *m*/*z* 633.32 $[M+Na]^+$, 649.31 $[M+K]^+$, 1243.45 $[2M+Na]^+$. Anal. Calcd for $C_{35}H_{34}N_20_6S$ (610.21 g/mol): C, 68.83; H, 5.61; N, 4.59; S, 5.25. Found: C, 69.01; H, 5.90; N, 4.61; S, 5.40.

4.8.21. 3-Amino-3-*C*-cyano-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (2B). Likewise, **1B** (5 g,

19.3 mmol), NH_3 (27.6 mL, NH_3 in MeOH 7 N), $Ti(OiPr)_4$ (6.96 mL, 23.2 mmol) and TMSiCN (2.57 mL, 19.3 mmol) in MeOH (12.4 mL), gave after flash chromatography (EtOAc/petroleum ether, 15:85) compound **2B** (7.1 g, 98%) as a colourless solid: mp 79–82 °C; $[\alpha]_D^{25}$ +5 (*c* 1.2, $CHCl_3$); IR (ATR) ν 3386, 2986, 2932, 1375, 1215, 1164, 1076, 1022, 840 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.84 (d, $J_{1,2}$=3.6 Hz, 1H, H-1), 4.68 (d, 1H, H-2), 4.29 (m, 1H, H-5), 4.10 (dd, $J_{5,6a}$=6.2 Hz, $J_{6a,6b}$=8.9 Hz, 1H, H-6a), 3.92 (dd, $J_{5,6b}$=4.4 Hz, 1H, H-6b), 3.59 (d, $J_{4,5}$=8.9 Hz, 1H, H-4), 2.02 (s, 2H, NH_2), 1.49 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.29 (s, 6H, 2×CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 118.6 (CN), 113.7 [O*C*(CH_3)$_2$], 110.1 [O*C*(CH_3)$_2$], 103.9 (C-1), 83.3 (C-2), 81.6 (C-4), 75.0 (C-5), 67.6 (C-6), 62.7 (C-3), 27.1, 26.8, 26.7, 25.2 (4×CH_3); MS (ES): 285.08 $[M+1]^+$. Anal. Calcd for $C_{13}H_{20}N_20_5$ (284.31 g/mol): C, 54.92; H, 7.09; N, 9.85. Found: C, 54.58; H, 6.87; N, 9.43.

4.8.22. 3-Amino-3-*C*-cyano-3-deoxy-1,2:5,6-di-*O*-isopropylidene-3-*N*-methanesulfonyl-α-D-allofuranose (3Ba). Following the general method (A), **2B** (2 g, 7.1 mmol), DMAP (430 mg, 3.52 mmol), CH_3SO_2Cl (1.63 mL, 21.1 mmol) in pyridine (17.7 mL) for 2 h gave, after flash chromatography (EtOAc/petroleum ether, 25:75), compound **3Ba** (2.03 g, 80%) as a colourless solid: mp 128–131 °C; $[\alpha]_D^{20}$ +13 (*c* 1.04, CH_2Cl_2); IR (ATR) ν 1331, 1210, 1149, 1079, 1038 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.90 (d, $J_{1,2}$=3.6 Hz, 1H, H-1), 5.50 (s, 1H, NH), 5.15 (d, 1H, H-2), 4.37 (ddd, $J_{5,4}$=9.1 Hz, $J_{5,6b}$=6.2 Hz, $J_{5,6a}$=4.3 Hz, 1H, H-5), 4.15 (d, $J_{6a,6b}$=9.1 Hz, 1H, H-6b), 3.96 (d, 1H, H-6a), 3.76 (dd, 1H, H-4), 3.14 (s, 3H, CH_3SO_2), 1.52 (s, 3H, CH_3), 1.43 (s, 6H, 2×CH_3), 1.39 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 116.1 (CN), 114.2 [O*C*(CH_3)$_2$], 110.8 [O*C*(CH_3)$_2$], 104.6 (C-1), 82.4 (C-2), 79.8 (C-4), 74.4 (C-5), 67.5 (C-6), 63.2 (C-3), 43.1 (SO_2CH_3), 26.5 (3×CH_3), 24.6 (CH_3); MS (APCI+) *m*/*z* 385.20 $[M+Na]^+$, 401.18 $[M+K]^+$. Anal. Calcd for $C_{14}H_{22}N_20_7S$ (362.11 g/mol): C, 46.40; H, 6.12; N, 7.73. Found: C, 46.25; H, 5.95; N, 7.61.

4.8.23. 3-Amino-3-*C*-cyano-3-deoxy-1,2;5.6-di-*O*-isopropylidene-3-*N*-phenylmethanesulfonyl-α-D-ribofuranose (3Bb). Following the general method (A), **2B** (0.137 g, 0.14 mmol), DMAP (0.03 g, 0.24 mmol), $C_6H_5CH_2SO_2Cl$ (0.28 g, 1.45 mmol) in dry pyridine (10 mL) for 30 h gave, after flash chromatography (EtOAc/petroleum ether, 8:2) compound **3Bb** (0.127 g, 60%) as a colourless solid: mp 138–139 °C; $[\alpha]_D^{25}$ −16 (*c* 0.35, $CHCl_3$); IR (KBr) ν 3330, 2923, 2853, 1457, 1376, 1338, 1213, 1164, 1082, 1044, 836 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.52–7.36 (m, 5H, $CH_2SO_2C_6H_5$), 5.98 (d, $J_{1,2}$=3.5 Hz, 1H, H-1), 5.31 (d, 1H, H-2), 5.29 (br s, 1H, NH), 4.58 (d, $J_{A,B}$=13.7 Hz, 1H, H-A, $SO_2CH_2C_6H_5$), 4.44 (d, 1H, H-B, $SO_2CH_2C_6H_5$), 4.41 (ddd, $J_{4,5}$=9.34 Hz, $J_{5,6a}$=6.22 Hz, $J_{5,6b}$=3.66 Hz, 1H, H-5), 4.17 (dd, $J_{5,6a}$=6.22 Hz, $J_{6a,6b}$=9.34 Hz, 1H, H-6a), 4.05 (dd, $J_{5,6b}$=3.66 Hz, 1H, H-6b), 3.87 (dd, $J_{4,5}$=9.34 Hz, 1H, H-4), 1.69 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 1,28 (s, 3H, CH_3), 1.07 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 131.0–128.8 ($SO_2CH_2C_6H_5$), 116.4 (CN), 114.5 [O*C*(CH_3)$_2$], 110.9 [O*C*(CH_3)$_2$], 104.6 (C-1), 82.8 (C-2), 79.6 (C-4), 74.5 (C-5), 67.4 (C-6), 63.4 (C-3), 60.1 ($SO_2CH_2C_6H_5$), 26.7, 26.6, 26.4, 24.6, (4×CH_3); MS (APCI+) *m*/*z* 439.1 $[M+1]^+$, 456.1 $[M+NH_4]^+$, 461.1 $[M+Na]^+$, 899.2 $[2M+Na]^+$. Anal. Calcd for $C_{20}H_{26}N_2O_7S$ (438.50 g/mol): C, 55.78; H, 5.98; N, 6.39; S, 7.31. Found: C, 55.98; H, 5.71; N, 6.24; S, 7.12.

4.8.24. 3-Amino-3-*C*-cyano-3-deoxy-1,2:5,6-di-*O*-isopropylidene-3-*N*-methyl-3-*N*-methanesulfonyl-α-D-allofuranose (3Bc). Following the general method (B), a solution of **3Ba** (0.52 g, 1.43 mmol) in acetone (20 mL) was reacted with K_2CO_3 (0.29 g, 2.14 mmol) and CH_3I (0.18 mL, 2.86 mmol). The reaction mixture was refluxed for 45 min to yield, after flash chromatography (EtOAc), compound **3Bc** (0.51 g, 95%) as a colourless solid: mp 127–129 °C; $[\alpha]_D^{23}$ +73 (*c* 0.26, $CHCl_3$); IR (ATR) ν 2992, 2937, 1381, 1350, 1260, 1211, 1157, 1028 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.88 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 5.15 (d, 1H, H-2), 4.48 (m, 1H, H-5), 4.40 (d, $J_{4,5}$=7.6 Hz, 1H, H-4), 4.27 (dd, $J_{6a,6b}$=8.9 Hz, $J_{5,6b}$=6.4 Hz, 1H, H-6b), 4.04 (dd, $J_{5,6a}$=6.0 Hz, 1H, H-6a), 3.17 (s, 3H, NCH_3), 3.10 (s, 3H, SO_2CH_3), 1.58 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.37 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 115.4 (CN), 113.5, 110.4 [2×O*C*(CH_3)$_2$], 103.1 (C-1), 84.9 (C-2), 78.7 (C-4), 74.2 (C-5), 67.8 (C-6), 66.8 (C-3), 40.3 (SO_2CH_3), 35.1 (NCH_3), 26.3 (2×CH_3), 26.0 (CH_3), 24.7 (CH_3); MS (APCI+) *m*/*z* 377.27 $[M+1]^+$, 399.25 $[M+Na]^+$, 415.17 $[M+K]^+$, 775.29 $[2M+Na]^+$. Anal. Calcd for $C_{15}H_{24}N_20_7S$ (376.13 g/mol): C, 47.86; H, 6.43; N, 7.44; S, 8.52. Found: C, 47.58; H, 6.34; N, 7.40; S, 8.55.

4.8.25. 3-Amino-3-*N*-benzyl-3-*C*-cyano-3-deoxy-1,2:5,6-di-*O*-isopropylidene-3-*N*-methanesulfonyl-α-D-allofuranose (3Bd). Following the general method (B), a solution of **3Ba** (1.5 g, 4.14 mmol) in acetone (50 mL) was treated with K_2CO_3 (0.86 g, 6.20 mmol) and BnBr (1.0 mL, 8.3 mmol). The reaction was refluxed for 19 h to yield, after flash chromatography (EtOAc), compound **3Bd** (1.47 g, 78%) as a colourless solid: mp 130–131 °C; $[\alpha]_D^{31}$ +55 (*c* 0.52, $CHCl_3$); IR (ATR) ν 1649, 1337, 1205, 1151, 1030, 819, 697 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.54–7.32 (m, 5H, C_6H_5), 5.82 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 5.17 (d, 1H, H-2), 4.84 (d, $J_{A,B}$=15.8 Hz, 1H, H-A, $NCH_2C_6H_5$), 4.72 (d, 1H, H-B, $NCH_2C_6H_5$), 4.60 (d, $J_{4,5}$=7.4 Hz, 1H, H-4), 4.51 (ddd, 1H, H-5), 4.27 (dd, $J_{5,6a}$=6.3 Hz, $J_{6a,6b}$=8.8 Hz, 1H, H-6a), 4.00 (dd, $J_{5,6b}$=6.7 Hz, 1H, H-6b), 2.92 (s, 3H, SO_2CH_3), 1.42 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.36 (s, 3H, CH_3), 1.32 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 136.6–128.4 (C_6H_5), 116.3 (CN), 113.9 [O*C*(CH_3)$_2$], 110.9 [O*C*(CH_3)$_2$], 103.1 (C-1), 85.4 (C-2), 79.3 (C-4), 75.0 (C-5), 69.6 (C-6), 68.4 (C-3), 52.2 ($NCH_2C_6H_5$), 42.3 (SO_2CH_3), 26.8 (CH_3), 26.5 (CH_3), 26.3 (CH_3), 25.5 (CH_3); MS (APCI+) *m*/*z* 475.34 $[M+Na]^+$, 491.26 $[M+K]^+$, 927.36 $[2M+Na]^+$. Anal. Calcd for $C_{21}H_{28}N_20_7S$ (452.16 g/mol): C, 55.74; H, 6.24; N, 6.19; S, 7.09. Found: C, 55.99; H, 6.50; N, 6.11; S, 7.04.

4.8.26. 3-*N*-Allyl-3-amino-3-*C*-cyano-3-deoxy-1,2:5,6-di-*O*-isopropylidene-3-*N*-methanesulfonyl-α-D-allofuranose (3Be). Following the general method (B), a solution of **3Ba** (1.5 g, 4.14 mmol) in acetone (30 mL) was treated with K_2CO_3 (0.86 g, 6.20 mmol) and AllBr (0.72 mL, 8.28 mmol). The reaction was refluxed for 24 h to yield, after flash chromatography (EtOAc), compound **3Be** (0.79 g, 47%) as a colourless solid: mp 107–108 °C; $[\alpha]_D^{30}$ +63 (*c* 0.54, $CHCl_3$); IR (ATR) ν 2992, 2362, 1381,

1337, 1155, 1033, 961, 860, 824 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.98 (m, 1H, $NCH_2CH{=}CH_2$), 5.81 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 5.19 (m, 2H, $NCH_2CH{=}CH_2$), 5.09 (d, 1H, H-2), 4.50 (d, $J_{4,5}$=7.4 Hz, 1H, H-4), 4.42 (m, 1H, H-5), 4.33 (dd, $J_{A,B}$=8.1 Hz, $J_{B,\beta}$=17.1 Hz, 1H, H-B, $NCH_2CH{=}CH_2$), 4.22 (dd, $J_{5,6a}$=6.3 Hz, $J_{6a,6b}$=8.9 Hz, 1H, H-6a), 4.07 (m, 1H, H-A, $NCH_2CH{=}CH_2$), 3.96 (dd, $J_{5,6b}$=6.5 Hz, 1H, H-6b), 3.08 (s, 3H, SO_2CH_3), 1.54 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.32 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 136.3 (NCH_2-*C*H=CH_2), 118.2 ($NCH_2CH{=}CH_2$), 116.0 (CN), 113.9 [O*C*$(CH_3)_2$], 110.9 [O*C*$(CH_3)_2$], 103.3 (C-1), 84.8 (C-2), 78.8 (C-4), 74.9 (C-5), 68.7 (C-6), 68.3 (C-3), 50.8 ($NCH_2CH{=}CH_2$), 41.8 (SO_2CH_3), 26.8 (CH_3), 26.6 (CH_3), 26.5 (CH_3), 25.4 (CH_3); MS (APCI+) *m/z* 425.5 $[M+Na]^+$, 441.5 $[M+K]^+$. Anal. Calcd for $C_{17}H_{26}N_20_7S$ (402.15 g/mol): C, 50.73; H, 6.51; N, 6.96; S, 7.97. Found: C, 50.54; H, 6.29; N, 6.76; S, 7.96.

4.8.27. 3-Amino-3-*C*-cyano-3-deoxy-1,2;5,6-di-*O*-isopropylidene-3-*N*-methyl-3-*N*-phenylmethanesulfonyl-α-D-ribofuranose (3Bf). Following the general method (B), **3Bb** (74 mg, 0.17 mmol), K_2CO_3 (30 mg, 0.25 mmol), CH_3I (0.02 ml, 0.34 mmol) in acetone (3 mL) for 4 h gave, after flash chromatography (EtOAc/petroleum ether, 9:1), product (**3Bf**) (73 mg, 95%) as a colourless solid: mp 37–39 °C; $[\alpha]_D^{25}$ +101 (*c* 0.37, $CHCl_3$); IR (KBr) ν 3435, 2989, 1456, 1378, 1352, 1215, 1153, 1032 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.53–7.34 (m, 5H, $SO_2CH_2C_6$-*H*$_5$), 5.87 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 5.22 (d, 1H, H-2), 4.58–4.27 (m, 5H, H-5, $SO_2CH_2C_6H_5$, H-6b, H-4), 4.03 (dd, $J_{5,6a}$=6.2 Hz, $J_{6a,6b}$=9.0 Hz, 1H, H-6a), 2.77 (s, 3H, NCH_3), 1.55 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 1.45 (s, 3H, CH_3),1.37 (s, 3H, CH_3) ^{13}C NMR ($CDCl_3$, 50 MHz) δ 131.4–128.3 (6 C, $SO_2CH_2C_6H_5$), 115.6 (CN), 113.5 [$OC(CH_3)_2$], 110.6 [$OC(CH_3)_2$], 103.0 (C-1), 85.7 (C-2), 78.6 (C-4), 74.3 (C-5), 68.2 (C-6), 67.3 (C-3), 60.3 ($SO_2CH_2C_6H_5$), 36.9 (NCH_3), 26.3, 26.2, 26.1, 25.2 (4×CH_3); MS (APCI+) *m/z* 453 $[M+1]^+$, 470 $[M+NH_4]^+$, 475 $[M+Na]^+$, 927 $[2M+Na]^+$. Anal. Calcd. for $C_{21}H_{28}N_2O_7S$ (452.52 g/mol): C, 55.74; H, 6.24; N, 6.19; S, 7.08. Found: C, 56.00; H, 6.09; N, 5.94; S, 6.91.

4.8.28. 3-Amino-3-*N*-benzyl-3-*C*-cyano-3-deoxy-1,2:5,6-di-*O*-isopropylidene-3-*N*-phenylmethanesulfonyl α-D-allofuranose (3Bg). Following the general method (B), **3Bb** (900 mg, 2.05 mmol), K_2CO_3 (0.42 g, 3.07 mmol), BnBr (0.49 mL, 4.10 mmol) in acetone (40 mL) for 4 h gave successively, after flash chromatography (EtOAc/petroleum ether, 2:8), compound (**3Bg**) (172 mg, 16%) as a colourless solid, unreacted **3Bb** (386 mg, 43%), and with (EtOAc/petroleum ether, 7:3), 1,2:5,6-di-*O*-isopropylidene-α-D-ribofuranose-3-spiro-3′-(4′-amino-2′-*N*-benzyl-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (**4Be**) (78 mg, 7%) as a colorless solid. **3Bg**: mp 115–117 °C; $[\alpha]_D^{30}$ +82 (*c* 0.49, $CHCl_3$); IR (ATR) ν 1447, 1348, 1200, 1145, 1033, 866, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.38 (m, 10H, 2×C_6H_5), 5.78 (d, $J_{1,2}$=3.7 Hz, 1H, H-1), 5.15 (d, 1H, H-2), 4.57 (m, 4H, H-4, H-5, $NCH_2C_6H_5$, $SO_2CH_2C_6H_5$), 4.43 (d, $J_{A,B}$=13.7 Hz, 1H, H-B, $SO_2CH_2C_6H_5$), 4.32 (dd, $J_{5,6a}$=5.7 Hz, $J_{6a,6b}$=8.5 Hz, 1H, H-6a), 4.23 (d, $J_{A,B}$=16.4 Hz, 1H, H-B, $NCH_2C_6H_5$), 4.00 (dd, $J_{5,6b}$=6.3 Hz, 1H, H-6b), 1.54 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 1.24 (s, 3H, CH_3), 0.90 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 137.3–128.3 (12 C, 2×C_6H_5), 116.3 (CN), 113.9 [O*C*$(CH_3)_2$], 111.0 [O*C*$(CH_3)_2$], 103.1 (C-1), 85.5 (C-2), 78.9 (C-4), 75.2 (C-5), 69.7 (C-3), 68.6 (C-6), 60.4 ($SO_2CH_2CH_3$), 53.1 ($NCH_2C_6H_5$), 26.7 (2×CH_3), 25.8 (CH_3), 25.6 (CH_3); MS (APCI+) *m/z* 551.31 $[M+Na]^+$, 567.30 $[M+K]^+$. Anal. Calcd for $C_{27}H_{32}N_20_7S$ (528.19 g/mol): C, 61.35; H, 6.10; N, 5.30; S, 6.07. Found: C, 61.24; H, 6.30; N, 5.28; S, 5.95. **4Be**: mp 105–107 °C; $[\alpha]_D^{32}$ +39 (*c* 0.51, $CHCl_3$); IR (ATR) ν 3364, 2975, 1654, 1372, 1215, 1148, 1073, 1022, 877, 731 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.61–7.25 (m, 10H, 2×C_6H_5), 5.82 (d, $J_{1,2}$=3.9 Hz, 1H, H-1), 4.75 (d, 1H, H-2), 4.72 (d, $J_{A,B}$=14.0 Hz, 1H, H-A, $NCH_2C_6H_5$), 4.61 (d, 1H, H-B, $NCH_2C_6H_5$), 4.53 (d, $J_{4,5}$=6.0 Hz, 1H, H-4), 4.36 (s, 2H, NH_2), 4.19 (m, 1H, H-5), 3.97 (dd, $J_{5,6a}$=6.1 Hz, $J_{6a,6b}$=8.3 Hz, 1H, H-6a), 3.90 (dd, $J_{5,6b}$=7.3 Hz, 1H, H-6b), 1.59 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 1.36 (s, 6H, 2×CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 145.2 (C-4′), 137.2–126.9 (12 C, 2×C_6H_5), 113.5 [O*C*$(CH_3)_2$], 110.0 [O*C*$(CH_3)_2$], 107.5 (C-5′), 103.9 (C-1), 84.9 (C-2), 77.1 (C-4), 73.0 (C-5), 71.2 (C-3), 67.1 (C-6), 47.9 ($NCH_2C_6H_5$), 26.7 (CH_3), 26.6 (CH_3), 26.3 (CH_3), 25.7 (CH_3); MS (APCI+) *m/z* 529.41 $[M+1]^+$, 551.38 $[M+Na]^+$, 567.36 $[M+K]^+$, 1079.49 $[M+K]^+$. Anal. Calcd for $C_{27}H_{32}N_20_7S$ (528.19 g/mol): C, 61.35; H, 6.10; N, 5.30; S, 6.07. Found: C, 61.30; H, 6.27; N, 5.42; S, 6.29.

4.8.29. 1,2:5,6-Di-*O*-isopropylidene-α-D-*erythro*-pentofuranose-3-spiro-3′-(4′-amino-2′-*N*-methyl-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Bd). Following the general method (C), **3Bb** (0.50 g, 1.14 mmol), K_2CO_3 (0.23 g, 1.71 mmol) and CH_3I (0.14 mL, 2.28 mmol) in acetone (20 mL) for 48 h gave after flash chromatography (EtOAc/petroleum ether, 3:7), unreacted **3Bb** (43 mg, 8%), (EtOAc/petroleum ether, 7:3), **4Bd** (400 mg, 77%) as a colourless solid. **4Bd**: mp 210–211 °C; $[\alpha]_D^{25}$ +38 (*c* 0.33, $CHCl_3$); IR (KBr) ν 3480, 2930, 1647, 1264, 1147, 1069 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.61–7.40 (m, 5H, C_6H_5), 5.88 (d, $J_{1,2}$=3.9 Hz, 1H, H-1), 4.79 (d, 1H, H-2), 4.52 (d, $J_{4,5a}$=6.4 Hz, 1H, H-4), 4.35 (br s, 2H, NH_2), 4.25 (m, 1H, H-5), 4.11 (dd, $J_{5,6a}$=6.2 Hz, $J_{6a,6b}$=8.2 Hz, 1H, H-6a), 3.98 (dd, $J_{5,6b}$=6.9 Hz, 1H, H-6b), 3.09 (s, 3H, NCH_3), 1.67 (s, 3H, CH_3), 1.44 (s, 3H, CH_3),1.38 (s, 3H, CH_3), 1.36 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 144.9 (C-4′), 129.6–127.0 (C_6H_5), 126.6 (C-5′), 113.5 [O*C*$(CH_3)_2$], 110.1 [O*C*$(CH_3)_2$], 103.5 (C-1), 85.3 (C-2), 75.5 (C-4), 72.9 (C-5), 70.1 (C-3), 67.0 (C-6), 27.7 (NCH_3), 26.3 (2×CH_3), 26.0, 25.4 (2×CH_3); MS (APCI+) *m/z* 453 $[M+1]^+$, 927 $[2M+Na]^+$. Anal. Calcd. for $C_{21}H_{28}N_2O_7S$ (452.52 g/mol): C, 55.74; H, 6.24; N, 6.19; S, 7.08. Found: C, 55.57; H, 5.98; N, 5.99; S, 6.82.

4.8.30. 1,2:5,6-Di-*O*-isopropylidene-α-D-*ribo*-hexofuranose-3-spiro-3′-(4′-amino-2′-*N*-benzyl-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Be). Following the general method (C), **3Ab** (0.5 g, 1.14 mmol), K_2CO_3 (0.23 g, 1.71 mmol) and BnBr (0.27 mL, 2.28 mmol) in acetone (20 mL) for 28 h gave, after flash chromatography (EtOAc/petroleum ether, 7:3), compound **4Be** (374 mg, 62%).

4.8.31. 1,2:5,6-Di-*O*-isopropylidene-α-D-*erythro*-pentofuranose-3-spiro-3′-(2′-*N*-allyl-4′-amino-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Bf). Following the general method (C), **3Bb** (400 mg, 0.91 mmol), K_2CO_3 (0.19 g, 1.36 mmol) and AllBr (0.15 mL, 1.82 mmol) in acetone (20 mL) for 17 h gave after flash chromatography (EtOAc/petroleum ether, 6:4) compound **4Bf** (213 mg, 49%) as a colourless oil: $[\alpha]_D^{32}$ +56 (*c* 0.52, $CHCl_3$); IR (ATR) ν 3463, 3342, 2986, 1655, 1375, 1266, 1212, 1155, 1069, 873 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.42 (m, 5H, C_6H_5), 6.16 (m, 1H, $NCH_2CH{=}CH_2$), 5.84 (d, $J_{1,2}$=3.9 Hz, 1H, H-1), 5.30 (dd, $J_{A,B}$=1.0 Hz, $J_{A,\beta}$=17.0 Hz, 1H, $NCH_2CH{=}CH_2$), 5.14 (dd, $J_{B,\beta}$=10.1 Hz, 1H, $NCH_2CH{=}CH_2$), 4.68 (d, 1H, H-2), 4.46 (s, 2H, NH_2), 4.44 (d, $J_{4,5}$=6.3 Hz, 1H, H-4), 4.20 (m, 1H, H-5), 4.11 (m, 2H, $NCH_2CH{=}CH_2$), 4.03 (dd, $J_{5,6a}$=6.1 Hz, $J_{6a,6b}$=8.3 Hz, 1H, H-6a), 3.92 (dd, $J_{5,6b}$=7.1 Hz, 1H, H-6b), 1.62 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.32 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 145.2 (C-4′), 135.3 ($NCH_2CH{=}CH_2$), 129.8–126.9 (6 C, C_6H_5), 118.2 ($NCH_2CH{=}CH_2$), 113.6 [$OC(CH_3)_2$], 110.1 [$OC(CH_3)_2$], 107.8 (C-5′), 103.9 (C-1), 84.8 (C-2), 76.5 (C-4), 73.0 (C-5), 70.8 (C-3), 67.2 (C-6), 46.4 ($NCH_2CH{=}CH_2$), 26.6 (CH_3), 26.5 (CH_3), 26.3 (CH_3), 25.6 (CH_3); MS (APCI+) *m*/*z* 501.5 $[M+Na]^+$, 517.5 $[M+K]^+$, 979.6 $[2M+Na]^+$. Anal. Calcd for $C_{23}H_{30}N_20_7S$ (478.18 g/mol): C, 57.72; H, 6.32; N, 5.85; S, 6.70. Found: C, 57.77; H, 6.53; N, 5.67; S, 6.61.

4.8.32. 1,2:5,6-Di-*O*-isopropylidene-α-D-*ribo*-hexofuranose-3-spiro-3′-(4′-amino-2′-*N*-methyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Ba). Following the general method (D), Cs_2CO_3 (0.21 g, 0.66 mmol) was added to a solution of **3Bc** (0.25 g, 0.66 mmol) in CH_3CN (4 mL). The reaction mixture was refluxed for 30 min then evaporated to dryness. The residue was purified by flash chromatography (EtOAc/petroleum ether, 7:3) to give **4Ba** (0.10 g, 40%) as a colourless solid: mp 238–240 °C; $[\alpha]_D^{26}$ +45 (*c* 0.18, $CHCl_3$); IR (ATR) ν 3414, 3332, 2981, 1650, 1370, 1260, 1213, 1071 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.82 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 5.64 (s, 1H, H-5′), 4.69 (s, 1H, NH_2), 4.68 (d, 1H, H-2), 4.38 (d, $J_{4,5}$=6.9 Hz, 1H, H-4), 4.19 (q, $J_{5,6a}$=$J_{5,6b}$=6.4 Hz, 1H, H-5), 4.11 (dd, $J_{6b,6a}$=8.3 Hz, 1H, H-6b), 3.98 (dd, 1H, H-6a), 2.99 (s, 3H, NCH_3), 1.63 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.34 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.4 (C-4′), 113.4, 110.1 [2×$OC(CH_3)_2$], 103.2 (C-1), 94.4 (C-5′), 85.1 (C-2), 75.4 (C-5), 72.6 (C-4), 71.4 (C-3), 67.0 (C-6), 27.1 (NCH_3), 26.2 (CH_3), 26.1 (CH_3), 25.9 (CH_3), 25.1 (CH_3); MS (APCI+) *m*/*z* 377.34 $[M+1]^+$, 399.12 $[M+Na]^+$, 415.24 $[M+K]^+$, 775.29 $[2M+Na]^+$. Anal. Calcd for $C_{15}H_{24}N_20_7S$ (376.13 g/mol): C, 47.86; H, 6.43; N, 7.44; S, 8.52. Found: C, 47.64; H, 6.18; N, 7.45; S, 8.37.

4.8.33. 1,2:5,6-Di-*O*-isopropylidene-α-D-*ribo*-hexofuranose-3-spiro-3′-(4′-amino-2′-*N*-benzyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Bb). Following the general method (D), Cs_2CO_3 (0.27 g, 0.83 mmol) was added to a solution of **3Bd** (378 mg, 0.83 mmol) in CH_3CN (5 mL). The reaction mixture was refluxed for 1 h then evaporated to dryness. The residue was purified by flash chromatography (EtOAc/petroleum ether, 9:1) to give **4Bb** (364 mg, 96%) as a colourless solid: mp 88–90 °C; $[\alpha]_D^{32}$ +43 (*c* 0.51, $CHCl_3$); IR (ATR) ν 2981, 2356, 1649, 1370, 1255, 1211, 1130, 1071, 871 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.59–7.30 (m, 5H, $NCH_2C_6H_5$), 5.80 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 5.46 (s, 1H, H-5′), 4.70 (s, 1H, H-2), 4.59 (m, 3H, NH_2, H-A, $NCH_2C_6H_5$), 4.52 (d, $J_{A,B}$=14.1 Hz, 1H, H-B, $NCH_2C_6H_5$), 4.41 (d, $J_{4,5}$=6.5 Hz, 1H, H-4), 4.14 (m, 1H, H-5), 4.05 (dd, $J_{5,6a}$=6.3 Hz, $J_{6a,6b}$=8.3 Hz, 1H, H-6a), 3.95 (dd, $J_{5,6b}$=6.5 Hz, 1H, H-6b), 1.56 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 1.37 (s, 6H, 2×CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.8 (C-4′), 137.4–127.9 (6 C, $NCH_2C_6H_5$), 113.5 [$OC(CH_3)_2$], 110.0 [$OC(CH_3)_2$], 103.7 (C-1), 94.2 (C-5′), 84.8 (C-2), 77.1 (C-4), 72.8 (C-3, C-5), 67.2 (C-6), 47.9 ($NCH_2C_6H_5$), 26.7 (CH_3), 26.5 (CH_3), 26.4 (CH_3), 25.6 (CH_3); MS (APCI+) *m*/*z* 453.57 $[M+1]^+$, 475.34 $[M+Na]^+$, 491.26 $[M+K]^+$, 927.36 $[2M+Na]^+$. Anal. Calcd for $C_{21}H_{28}N_20_7S$ (452.16 g/mol): C, 55.74; H, 6.24; N, 6.19; S, 7.09. Found: C, 56.02; H, 6.30; N, 6.44; S, 7.25.

4.8.34. 1,2:5,6-Di-*O*-isopropylidene-α-D-*ribo*-hexofuranose-3-spiro-3′-(2′-*N*-allyl-4′-amino-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Bc). Following the general method (D), Cs_2CO_3 (216 mg, 0.66 mmol) was added to a solution of **3Be** (267 mg, 0.66 mmol) in CH_3CN (10 mL). The reaction mixture was refluxed for 6 h then evaporated to dryness. The residue was purified by flash chromatography (EtOAc/petroleum ether, 8:2) to give **4Bc** (177 mg, 66%) as a colourless solid: mp 205–207 °C; $[\alpha]_D^{32}$ +40 (*c* 0.50, $CHCl_3$); IR (ATR) ν 3430, 3337, 2981, 1636, 1375, 1249, 1211, 1073, 1031, 873 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.10 (m, 1H, $NCH_2CH{=}CH_2$), 5.81 (d, $J_{1,2}$=3.9 Hz, 1H, H-1), 5.60 (s, 1H, H-5′), 5.28 (dd, *J*=17.2 Hz, 1H, $NCH_2CH{=}CH_2$), 5.14 (dd, *J*=10.1 Hz, 1H, $NCH_2CH{=}CH_2$), 4.87 (d, 2H, NH_2), 4.63 (d, 1H, H-2), 4.31 (d, $J_{4,5}$=6.7 Hz, 1H, H-4), 4.04 (m, 5H, H-5, H-6, $NCH_2CH{=}CH_2$), 1.58 (s, 3H, CH_3), 1.42 (s, 3H, CH_3), 1.34 (s, 3H, CH_3), 1.31 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.9 (C-4′), 135.2 ($NCH_2CH{=}CH_2$), 118.2 ($NCH_2CH{=}CH_2$), 113.6 [$OC(CH_3)_2$], 110.3 [$OC(CH_3)_2$], 103.7 (C-1), 94.3 (C-5′), 84.7 (C-2), 76.5 (C-4), 72.8 (C-5), 72.3 (C-3), 67.3 (C-6), 46.3 ($NCH_2CH{=}CH_2$), 26.7 (CH_3), 26.4 (CH_3), 26.3 (CH_3), 25.5 (CH_3); MS (APCI+) *m*/*z* 425.2 $[M+Na]^+$, 441.2 $[M+K]^+$, 827.3 $[2M+Na]^+$. Anal. Calcd for $C_{17}H_{26}N_20_7S$ (402.15 g/mol): C, 50.73; H, 6.51; N, 6.96; S, 7.97. Found: C, 50.61; H, 6.37; N, 6.84; S, 7.66.

4.8.35. 1,2:5,6-Di-*O*-isopropylidene-α-D-*ribo*-hexofuranose-3-spiro-3′-(4′-amino-2′-*N*-methyl-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Bd). Following the general method (D), Cs_2CO_3 (40 mg, 0.12 mmol) was added to a solution of **3Bf** (46 mg, 0.10 mmol) in CH_3CN (4 mL). The reaction mixture was refluxed for 2 h then evaporated to dryness. The residue was purified by flash chromatography (EtOAc/petroleum ether, 4:6) to give **4Bd** (44 mg, 96%).

4.8.36. 1,2:5,6-Di-*O*-isopropylidene-α-D-*ribo*-hexofuranose-3-spiro-3′-(4′-amino-2′-*N*-benzyl-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Be). Following the general method (D), Cs_2CO_3 (50 mg, 0.15 mmol) was added to a solution of **3Bg** (82 mg, 0.15 mmol) in CH_3CN (5 mL). The reaction mixture was refluxed for 1 h then evaporated to dryness. The residue was purified by

flash chromatography (EtOAc/petroleum ether, 5:5) to give **4Be** (65 mg, 79%).

4.8.37. 1,2:5,6-Di-*O*-isopropylidene-α-D-*ribo*-hexofuranose-3-spiro-3′-(4′-amino-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Bg). Following the general method (E), *n*-BuLi (1.1 mL, 1.65 mmol, 1.6 M in hexane) was added to a solution of **3Ba** (200 mg, 0.55 mmol) in THF (5 mL). The reaction mixture was stirred for 5 min at −10 °C. The crude was flash chromatographed with EtOAc to give **4Bg** (120 mg, 60%) as a colourless solid: mp 230–233 °C; $[\alpha]_D^{25}$ +12 (*c* 0.48, CH_2Cl_2); IR (ATR) ν 1650, 1370, 1266, 1219, 1118, 1068, 1029 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.86 (d, $J_{1,2}$=3.6 Hz, 1H, H-1), 5.54 (s, 1H, NH), 5.05 (s, 1H, SO_2CH), 4.75 (s, 2H, NH_2), 4.60 (d, 1H, H-2), 4.32 (ddt, $J_{5,6b}$=$J_{5,6a}$=5.7 Hz, $J_{4,5}$=7.1 Hz, 1H, H-5), 4.03 (m, 3H, H-4, H-6a, H-6b), 1.55 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 1.30 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.0 (C-4′), 113.7 [O*C*(CH_3)$_2$], 110.3 [O*C*(CH_3)$_2$], 103.7 (C-1), 95.4 (C-5′), 82.9 (C-2), 78.4 (C-4), 73.1 (C-5), 70.4 (C-3), 66.8 (C-6), 26.5 (CH_3), 26.3 (2×CH_3), 25.2 (CH_3); MS (APCI+) *m*/*z* 385.20 $[M+Na]^+$. Anal. Calcd for $C_{14}H_{22}N_20_7S$ (362.11 g/mol): C, 46.40; H, 6.12; N, 7.73; S, 8.85. Found: C, 46.35; H, 5.98; N, 7.64; S, 8.58.

4.8.38. 1,2:5,6-Di-*O*-isopropylidene-α-D-*ribo*-hexofuranose-3-spiro-3′-(4′-amino-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (4Bh). Following the general method (E), *n*-BuLi (1.37 mL, 3.42 mmol, 2.5 M in hexane) was added to a solution of **3Bb** (500 mg, 1.14 mmol) in THF (10 mL). The reaction mixture was stirred for 20 min at −10 °C. The crude was flash chromatographed (EtOAc/petroleum ether, 8:2) to give **4Bh** (404 mg, 80%) as a colourless solid: mp 95–97 °C; $[\alpha]_D^{29}$ +46 (*c* 0.54, $CHCl_3$); IR (ATR) ν 2986, 1644, 1375, 1205, 1145, 1058, 866, 745, 696 cm^{-1}; ^1H NMR (acetone d_6, 300 MHz) δ 7.56–7.39 (m, 5H, C_6H_5), 6.18 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 4.79 (d, 1H, H-2), 4.50 (m, 1H, H-5), 4.30 (d, $J_{4,5}$=4.6 Hz, 1H, H-4), 4.08 (m, 2H, H-6), 1.58 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.30 (s, 3H, CH_3); ^{13}C NMR (acetone d_6, 75 MHz) δ 147.1 (C-4′), 129.4–127.7 (6 C, C_6H_5), 113.3 [O*C*(CH_3)$_2$], 109.2 [O*C*(CH_3)$_2$], 106.7 (C-5′), 104.6 (C-1), 83.0 (C-2), 80.0 (C-4), 73.5 (C-5), 68.8 (C-3), 65.8 (C-6), 26.2 (2×CH_3), 26.0 (CH_3), 25.4 (CH_3); MS (APCI+) *m*/*z* 439.40 $[M+1]^+$, 461.37 $[M+Na]^+$, 477.36 $[M+K]^+$, 899.42 $[2M+Na]^+$, 916.45 $[2M+K]^+$. Anal. Calcd for $C_{20}H_{26}N_20_7S$ (438.15 g/mol): C, 54.78; H, 5.98; N, 6.39; S, 7.31. Found: C, 54.61; H, 5.71; N, 6.53; S, 7.46.

4.8.39. (1*S*,3*R*,7*R*,8*R*,12*S*,14*R*)-12-Amino-5-dimethyl-9-*N*-methyl-10-thia-10,10-dioxide-9-aza-2,4,6,13-tetraoxatetracyclo[6.6.0.0^{3,7}.0^{8,12}]tetradecane (5Aa). Following the general method (F), acetic acid (12 mL) and water (3 mL) were added to the compound **4Aa** (157 mg, 0.28 mmol). After 1 h 10 min and flash chromatography (EtOAc/petroleum ether, 7:3) compound **5Aa** (31 mg, 35%) was isolated as a colourless solid: mp 139–141 °C; $[\alpha]_D^{25}$ +117 (*c* 0.59, $CHCl_3$); IR (ATR) ν 3364, 2915, 1370, 1293, 1235, 1121, 1060 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.77 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 4.97 (d, 1H, H-2), 4.80 (d, $J_{4,5}$=3.1 Hz, 1H, H-4), 4.18 (dd, $J_{5a,5b}$=10.7 Hz, 1H, H-5a), 3.99 (dd, 1H, H-5b), 3.58 (d, $J_{5'a,5'b}$=13.2 Hz, 1H, H-5′b), 3.36 (d, 1H, H-5′a), 3.01 (s, 3H, NCH_3), 2.14 (s, 2H, NH_2), 1.60 (s, 3H, CH_3), 1.36 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 113.0 [O*C*(CH_3)$_2$], 106.4 (C-1), 95.5 (C-4′), 82.4 (C-2), 79.7 (C-4), 79.4 (C-3), 70.3 (C-5), 57.1 (C-5′), 28.3 (NCH_3), 27.1 (CH_3), 26.4 (CH_3); MS (APCI+) *m*/*z* 329.2 $[M+Na]^+$. Anal. Calcd for $C_{11}H_{18}N_20_6S$ (306.09 g/mol): C, 43.13; H, 5.92; N, 9.14; S, 10.47. Found: C, 43.35; H, 5.73; N, 9.07; S, 10.66.

4.8.40. (1*S*,3*R*,7*R*,8*R*,12*S*,14*R*)-12-Amino-9-*N*-benzyl-5-dimethyl-10-thia-10,10-dioxide-9-aza-2,4,6,13-tetraoxatetracyclo[6.6.0.0^{3,7}.0^{8,12}]tetradecane (5Ab). Following the general method (F), acetic acid (16 mL) and water (4 mL) were added to the compound **4Ab** (255 mg, 0.41 mmol). After 1 h and flash chromatography (EtOAc/petroleum ether, 7:3) compound **5Ab** (91 mg, 58%) was isolated as a colourless solid: mp 92–94 °C; $[\alpha]_D^{25}$ +22 (*c* 0.9, $CHCl_3$); IR (KBr) ν 3486, 2928, 1629, 1496, 1457, 1384, 1295, 1211, 1154, 1072, 1021 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.31–7.25 (m, 5H, C_6H_5), 5.65 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 4.99 (d, 1H, H-2), 4.78 (s, 2H, $NCH_2C_6H_5$), 4.37 (d, $J_{4,5b}$=3.5 Hz, 1H, H-4), 3.71 (d, $J_{5a,5b}$=10.0 Hz, 1H, H-5a), 3.61 (d, $J_{5'a,5'b}$=13.0 Hz, 1H, H-5′a), 3.29 (dd, 1H, H-5b), 3.27 (d, 1H, H-5′b), 2.00 (br s, 2H, NH_2), 1.61 (s, 3H, CH_3), 1.30 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 137.6–128.0 (C_6H_5), 112.6 [O*C*(CH_3)$_2$], 106.1 (C-1), 95.6 (C-4′), 81.6 (C-2), 80.3 (C-4), 78.5 (C-3), 70.0 (C-5), 58.5 (C-5′), 44.8 ($NCH_2C_6H_5$), 26.9 (CH_3), 25.9 (CH_3); MS (APCI+) *m*/*z* 383 $[M+1]^+$, 405 $[M+Na]^+$, 765 $[2M+1]^+$, 787 $[2M+Na]^+$. Anal. Calcd for $C_{17}H_{22}N_20_6S$ (382.46 g/mol): C, 53.39; H, 5.80; N, 7.33; S, 8.38. Found: C, 53.12; H, 5.97; N, 7.12; S, 8.42.

4.8.41. (1*S*,3*R*,7*R*,8*R*,12*S*,14*R*)-12-Amino-5-dimethyl-10-thia-10,10-dioxide-9-aza-2,4,6,13-tetraoxatetracyclo-[6.6.0.0^{3,7}.0^{8,12}]tetradecane (5Ac). Following the general method (F), acetic acid (18 mL) and water (12 mL) were added to the compound **4Ag** (1.08 g, 2.02 mmol). After 1 h 30 min and flash chromatography (EtOAc) compound **5Ac** (343 mg, 57%) was isolated as a colourless oil: $[\alpha]_D^{29}$ +42 (*c* 0.69, $CHCl_3$); IR (ATR) ν 2987, 1647, 1378, 1314, 1217, 1164, 1010, 912, 876, 732 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.85 (d, $J_{1,2}$=3.9 Hz, 1H, H-1), 5.67 (s, 1H, NH), 4.90 (d, 1H, H-2), 4.45 (d, $J_{4,5b}$=2.9 Hz, 1H, H-4), 4.13 (d, $J_{5a,5b}$=10.7 Hz, 1H, H-5a), 4.05 (dd, 1H, H-5b), 3.60 (d, $J_{5'a,5'b}$=13.3 Hz, 1H, H-5′a), 3.38 (d, $J_{5'a,NH}$=1.6 Hz, 1H, H-5′b), 2.25 (s, 2H, NH_2), 1.54 (s, 3H, CH_3), 1.37 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 113.2 [O*C*(CH_3)$_2$], 106.4 (C-1), 96.7 (C-4′), 84.1 (C-4), 79.9 (C-2), 77.6 (C-3), 69.6 (C-5), 58.9 (C-5′), 27.3 (CH_3), 27.0 (CH_3); MS (APCI+) *m*/*z* 314.99 $[M+Na]^+$. Anal. Calcd for $C_{10}H_{16}N_20_6S$ (292.07 g/mol): C, 41.09; H, 5.52; N, 9.58; S, 10.97. Found: C, 41.35; H, 5.58; N, 9.64; S, 10.88.

4.8.42. 1,2-*O*-Isopropylidene-α-D-*erythro*-pentofuranose-3-spiro-3′-(4′-amino-2′*N*-methyl-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (6Aa). Following the general method (F), acetic acid (8 mL) and water (2 mL) were added to the compound **4Ad** (374 mg, 0.60 mmol). After 2 h 30 min and flash chromatography (EtOAc) compound **6Aa** (199 mg, 87%) was isolated as a

colourless oil: $[\alpha]_D^{29}$ +42 (*c* 0.34, DMSO); IR (ATR) ν 1633, 1249, 1205, 1140, 1063, 1025, 877 cm^{-1}; ^1H NMR (DMSO d_6, 300 MHz) δ 7.40 (m, 5H, C_6H_5), 6.15 (d, $J_{1,2}$=3.9 Hz, 1H, H-1), 5.79 (s, 2H, NH_2), 5.03 (s, 1H, OH), 4.69 (d, 1H, H-2), 4.48 (dd, $J_{4,5a}$=1.9 Hz, $J_{4,5b}$=7.7 Hz, 1H, H-4) 3.53 (m, 2H, H-5), 2.88 (s, 3H, NCH_3), 1.58 (s, 3H, CH_3), 1.31 (s, 3H, CH_3); ^{13}C NMR (DMSO d_6, 75 MHz) δ 147.5 (C-4′), 129.8-128.3 (6 C, C_6H_5), 112.8 [$OC(CH_3)_2$], 104.3 (C-1), 102.6 (C-5′), 84.7 (C-2), 77.7 (C-4), 69.7 (C-3), 59.7 (C-5), 27.9 (NCH_3), 26.7 (2×CH_3); MS (APCI+) *m/z* 405.0 $[M+Na]^+$, 421.05 $[M+K]^+$, 787.09 $[2M+Na]^+$. Anal. Calcd for $C_{17}H_{22}N_20_60_6S$ (382.12 g/mol): C, 53.39; H, 5.80; N, 7.33; S, 8.38. Found: C, 53.35; H, 5.98; N, 7.52; S, 8.65.

4.8.43. 1,2-*O*-Isopropylidene-α-D-*erythro*-pentofuranose-3-spiro-3′-(4′-amino-2′*N*-benzyl-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (6Ab). Following the general method (F), acetic acid (12 mL) and water (3 mL) were added to the compound **4Ae** (185 mg, 0.40 mmol). After 1 h 30 min and flash chromatography (EtOAc) compound **6Ab** (63 mg, 72%) was isolated as a colourless solid: mp 108–110 °C; $[\alpha]_D^{30}$ +54 (*c* 0.57, DMSO); IR (ATR) ν 1649, 1452, 1375, 1271, 1151, 1068, 1025, 871, 751, 685 cm^{-1}; ^1H NMR (MeOH d_6, 300 MHz) δ 7.45 (m, 10H, 2×C_6H_5), 6.05 (d, $J_{1,2}$=3.9 Hz, 1H, H-1), 4.86 (m, 5H, H-2, NH_2, OH, H-A $NCH_2C_6H_5$), 4.69 (d, $J_{A,B}$=14.8 Hz, 1H, H-B $NCH_2C_6H_5$), 4.57 (dd, $J_{4,5a}$=2.9 Hz, $J_{4,5b}$=7.3 Hz, 1H, H-4), 3.76 (m, 2H, H-5), 1.60 (s, 3H, CH_3), 1.40 (s, 3H, CH_3); ^{13}C NMR (MeOH d_4, 75 MHz) δ 147.5 (C-4′), 137.6–127.2 (12 C, 2×C_6H_5), 113.4 [$OC(CH_3)_2$], 104.4 (2 C, C-1, C-5′), 84.9 (C-2), 78.0 (C-4), 70.4 (C-3), 59.6 (C-5), 46.1 ($NCH_2C_6H_5$), 25.5 (CH_3), 25.2 (CH_3); MS (APCI+) *m/z* 481.10 $[M+Na]^+$, 497.08 $[M+K]^+$, 939.13 $[2M+Na]^+$. Anal. Calcd for $C_{23}H_{26}N_20_60_6S$ (458.15 g/mol): C, 60.25; H, 5.72; N, 6.11; S, 6.99. Found: C, 60.37; H, 5.62; N, 6.04; S, 6.72.

4.8.44. 1,2-*O*-Isopropylidene-α-D-*erythro*-pentofuranose-3-spiro-3′-(4′-amino-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (6Ac). Following the general method (F), acetic acid (8 mL) and water (2 mL) were added to the compound **4Ah** (290 mg, 0.47 mmol). After 50 min and flash chromatography (EtOAc/petroleum ether, 7:3) compound **6Ac** (130 mg, 74%) was isolated as a colourless oil: $[\alpha]_D^{28}$ +24 (*c* 0.37, DMSO); IR (ATR) ν 1644, 1271, 1101, 1063, 1025, 893, 866, 762 cm^{-1}; ^1H NMR (DMSO d_6, 300 MHz) δ 7.43 (m, 5H, C_6H_5), 6.14 (d, $J_{1,2}$=4.0 Hz, 1H, H-1), 5.72 (s, 2H, NH_2), 4.98 (s, 1H, OH), 4.55 (d, 1H, H-2), 4.11 (dd, $J_{4,5a}$=1.5 Hz, $J_{4,5b}$=8.2 Hz, 1H, H-4), 3.69 (m, 1H, H-5a), 3.58 (m, 1H, H-5b), 1.44 (s, 3H, CH_3), 1.31 (s, 3H, CH_3); ^{13}C NMR (DMSO d_6, 75 MHz) δ 147.4 (C-4′), 129.7–128.3 (C_6H_5), 112.7 [$OC(CH_3)_2$], 104.6 (C-1), 104.2 (C-5′), 82.6 (C-2), 82.1 (C-4), 68.7 (C-3), 59.4 (C-5), 27.2 (CH_3), 27.0 (CH_3); MS (APCI+) *m/z* 390.96 $[M+Na]^+$, 759.09 $[2M+Na]^+$. Anal. Calcd for $C_{16}H_{20}N_20_60_6S$ (368.10 g/mol): C, 52.16; H, 5.47; N, 7.60; S, 8.70. Found: C, 52.35; H, 5.78; N, 7.64; S, 8.58.

4.8.45. (1*S*,3*R*,7*R*,8*R*,12*S*,14*R*)-12-Amino-14-hydroxymethylene-5-dimethyl-9-*N*-methyl-10-thia 10,10-dioxide-9-aza-2,4,6,13-tetraoxatetracyclo[6.6.0.0^{3,7}.0^{8,12}]-tetradecane (5Ba). Following the general method (F), acetic acid (24 mL) and water (16 mL) were added to the compound **4Ba** (1.27 g, 3.37 mmol). After 2 h 15 min and flash chromatography (EtOAc/petroleum ether, 9:1) compound **5Ba** (1.03 g, 90%) was isolated as a colourless solid: mp 180–182 °C; $[\alpha]_D^{30}$ +88 (*c* 0.25, MeOH); IR (ATR) ν 1370, 1282, 1260, 1112, 1014, 866 cm^{-1}; ^1H NMR (DMSO d_6, 300 MHz) δ 5.84 (d, $J_{1,2}$=3.6 Hz, 1H, H-1), 4.83 (t, $J_{OH,6}$=5.9 Hz, 1H, OH), 4.78 (d, 1H, H-2), 4.71 (s, 1H, H-4), 4.08 (t, $J_{5,6}$=6.6 Hz, 1H, H-5), 3.57 (d, $J_{5'a,5'b}$=13.8 Hz, 1H, H-5′a), 3.46 (d, 1H, H-5′b), 3.31 (m, 2H, H-6), 2.83 (s, 3H, NCH_3), 1.50 (s, 3H, CH_3), 1.27 (s, 3H, CH_3); ^{13}C NMR (DMSO d_6, 75 MHz) δ 112.5 [$OC(CH_3)_2$], 106.7 (C-1), 96.4 (C-4′), 84.2 (C-5), 82.4 (C-2), 81.1 (C-4), 79.9 (C-3), 61.7 (C-6), 57.4 (C-5′), 27.9 (NCH_3), 27.5 (CH_3), 27.1 (CH_3); MS (APCI+) *m/z* 358.99 $[M+Na]^+$. Anal. Calcd for $C_{12}H_{20}N_20_70_7S$ (336.10 g/mol): C, 42.85; H, 5.99; N, 8.33; S, 9.53. Found: C, 42.71; H, 5.84; N, 8.64; S, 9.61.

4.8.46. (1*S*,3*R*,7*R*,8*R*,12*S*,14*R*)-12-Amino-9-*N*-benzyl-14-hydroxymethylene-5-dimethyl-10-thia-10,10-dioxide-9-aza-2,4,6,13-tetraoxatetracyclo[6.6.0.0^{3,7}.0^{8,12}]tetradecane (5Bb). Following the general method (F), acetic acid (30 mL) and water (20 mL) were added to the compound **4Bb** (2.35 g, 5.2 mmol). After 4 h 30 min and flash chromatography (EtOAc/petroleum ether, 6.5:3.5) compound **5Bb** (1.17 g, 54%) was isolated as a colourless solid: mp 127–129 °C; $[\alpha]_D^{31}$ +31 (*c* 0.33, MeOH); IR (ATR) ν 1375, 1293, 1123, 1058, 1016, 860 cm^{-1}; ^1H NMR (DMSO d_6, 300 MHz) δ 7.41–7.30 (m, 5H, C_6H_5), 5.89 (d, $J_{1,2}$=3.5 Hz, 1H, H-1), 4.87 (d, 1H, H-2), 4.71 (t, $J_{OH,6}$=5.9 Hz, 1H, OH), 4.61 (d, $J_{A,B}$=13.6 Hz, 2H, $NCH_2C_6H_5$), 4.53 (s, 1H, H-4), 4.00 (m, 1H, H-5), 3.61 (d, $J_{5'a,5'b}$=13.7 Hz, 1H, H-5′a), 3.48 (d, 1H, H-5′b), 3.16 (m, 1H, H-6a), 3.00 (m, 1H, H-6b), 1.53 (s, 3H, CH_3), 1.31 (s, 3H, CH_3); ^{13}C NMR (DMSO d_6, 75 MHz) δ 138.2–128.2 (6C, C_6H_5), 112.8 [$OC(CH_3)_2$], 107.2 (C-1), 95.9 (C-4′), 85.0 (C-5), 82.9 (C-4), 82.5 (C-2), 80.5 (C-3), 61.7 (C-6), 58.2 (C-5′), 46.4 ($NCH_2C_6H_5$), 27.8 (CH_3), 27.2 (CH_3); MS (APCI+) *m/z* 413.16 $[M+1]^+$, 435.16 $[M+Na]^+$, 451.12 $[M+K]^+$. Anal. Calcd for $C_{18}H_{24}N_20_70_7S$ (412.13 g/mol): C, 52.42; H, 5.86; N, 6.79; S, 7.77. Found: C, 52.35; H, 5.73; N, 6.69; S, 7.49.

4.8.47. (1*S*,3*R*,7*R*,8*R*,12*S*,14*R*)-12-Amino-14-hydroxymethylene-5-dimethyl-10-thia-10,10-dioxide-9-aza-2,4,6,13-tetraoxatetracyclo[6.6.0.0^{3,7}.0^{8,12}]tetradecane (5Bc). Following the general method (F), acetic acid (18 mL) and water (12 mL) were added to the compound **4Bg** (776 mg, 2.14 mmol). After 2 h 30 min and flash chromatography (EtOAc/petroleum ether, 8:2) compound **5Bc** (495 mg, 71%) was isolated as a colourless solid: mp 198–200 °C; $[\alpha]_D^{30}$ +94 (*c* 0.20, MeOH); IR (ATR) ν 3403, 3364, 2921, 2844, 2356, 1375, 1299, 1167, 1079, 1014, 844 cm^{-1}; ^1H NMR (DMSO d_6, 300 MHz) δ 5.88 (d, $J_{1,2}$=3.7 Hz, 1H, H-1), 4.99 (t, $J_{OH,6}$=5.3 Hz, 1H, OH), 4.66 (d, 1H, H-2), 4.31 (s, 1H, H-4), 4.07 (t, $J_{5,6}$=6.3 Hz, 1H, H-5), 3.49 (m, 3H, H-6, H-5′a), 3.36 (d, $J_{5'a,5'b}$=14.0 Hz, 1H, H-5′b), 1.46 (s, 3H, CH_3), 1.29 (s, 3H, CH_3); ^{13}C NMR (DMSO d_6, 75 MHz) δ 112.3 [$OC(CH_3)_2$], 107.0 (C-1), 98.5 (C-4′), 85.3 (C-4), 83.9 (C-5), 80.2 (C-2), 78.3 (C-3), 61.9 (C-6), 60.3 (C-5′), 27.8 (CH_3), 27.5 (CH_3); MS (APCI+) *m/z* 345.0 $[M+Na]^+$, 361.0 $[M+K]^+$, 667.0 $[2M+Na]^+$. Anal. Calcd for $C_{11}H_{18}N_20_70_7S$ (322.08 g/mol): C, 40.99; H, 5.63; N, 8.69; S, 9.95. Found: C, 40.76; H, 5.83; N, 8.51; S, 9.72.

4.9. X-ray crystal analysis structure of 5Bc

Crystal data: $C_{11}H_{18}N_2O_7S$, molecular mass=322.33, orthorhombic, $P2_12_12_1$, a=7.0092(6) Å, b=10.7175(8) Å, 18.772(2) Å, V=1410.1(2) Å^3, Z=4, D_c=1.518 mg/m^3, $F(000)$=680, μ=0.266 mm^{-1}. Data collection: SMART CCD-BRUKER diffractometer, with graphite-monochromated Mo Kα radiation (λ=0.71073 Å) operating at 50 kV and 20 mA. The intensity data were collected over an hemisphere of the reciprocal space by combination of three exposure sets. Each exposure of 30 s covered 0.3 in ω. Reflection range for the data collection were $2.17°<\theta<28.72°$. The first 50 frames were recollected at the end of the data collection to monitor crystal decay. A total of 8803 reflections were measured and 3319 were considered observed [$I>2(\sigma(I)$ criterium].

Structure solution and refinement: the structure was solved by direct methods. The refinement was done by full matrix least-squares procedures on F^2 (SHELXTL version 5.1).[10] The non-hydrogen atoms were refined anisotropically. The hydrogen atoms were located from Fourier difference and the coordinates was refined except those involved in methyl group that refined as riding on the carbon bonded atom. Final $R(Rw)$ value was 3.71 (8.74). Further crystallographic details for the structure reported in this paper may be obtained from the Cambridge Crystallographic Data Center, on quoting the depository number CCDC- 223921.

4.9.1. 1,2-*O*-Isopropylidene-α-D-*ribo*-hexofuranose-3-spiro-3′-(4′-amino-2′*N*-methy-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (6Ba). Following the general method (F), acetic acid (6 mL) and water (4 mL) were added to the compound **4Bd** (301 mg, 0.66 mmol). After 2 h 15 min, the solvent was eliminated under *vacuum*. The residue was triturated with a solution of EtOAc–petroleum ether to give after filtration **6Ba** (265 mg, 100%) as a colourless solid: mp 115–116 °C; $[\alpha]_D^{28}$ +54 (*c* 0.19, $CHCl_3$); IR (ATR) ν 3419, 2986, 2915, 1645, 1252, 1139, 1047, 1017, 876, 701 cm^{-1}; ^{1}H NMR (DMSO d_6, 300 MHz) δ 7.47–7.35 (m, 5H, C_6H_5), 6.02 (d, $J_{1,2}$=3.9 Hz, 1H, H-1), 5.72 (s, 2H, NH_2), 4.67 (d, 1H, H-2), 4.41 (d, $J_{4,5}$=7.3 Hz, 1H, H-4), 3.74 (m, 1H, H-5), 3.56 (m, 1H, H-6a) 3.42 (m, 1H, H-6b), 2.90 (s, 3H, NCH_3), 1.55 (s, 3H, CH_3), 1.30 (s, 3H, CH_3); ^{13}C NMR (DMSO d_6, 75 MHz) δ 148.2 (C-4′), 129.7–128.5 (6 C, C_6H_5), 112.8 [$OC(CH_3)_2$], 103.4 (2 C, C-1, C-5′), 85.9 (C-2), 75.4 (C-4), 70.7 (C-3), 70.4 (C-5), 64.1 (C-6), 28.2 (NCH_3), 26.8 (CH_3), 26.7 (CH_3); MS (APCI+) *m*/*z* 413.1 [M+1]$^+$, 435.1 [M+Na]$^+$, 847.1 [2M+Na]$^+$. Anal. Calcd for $C_{18}H_{24}N_2O_7S$ (412.13 g/mol): C, 52.42; H, 5.86; N, 6.79; S, 7.77. Found: C, 52.57; H, 5.62; N, 6.81; S, 7.58.

4.9.2. 1,2-*O*-Isopropylidene-α-D-*ribo*-hexofuranose-3-spiro-3′-(4′-amino-2′*N*-benzyl-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (6Bb). Following the general method (F), acetic acid (6 mL) and water (4 mL) were added to the compound **4Be** (220 mg, 0.41 mmol). After 1 h 10 min and flash chromatography (EtOAc/petroleum ether, 5:5) compound **6Bb** (131 mg, 64%) was isolated as a colourless solid: mp 91–92 °C; $[\alpha]_D^{28}$ −0.3° (*c* 0.08, $CHCl_3$); IR (ATR) ν 2356, 1737, 1644, 1370, 1216, 1145, 1058, 1014, 866 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.59–7.37 (m, 10H, 2×C_6H_5), 5.82 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 4.83 (d, $J_{A,B}$=14.4 Hz, 1H, H-A, $NCH_2C_6H_5$), 4.78 (d, 1H, H-2), 4.56 (d, 1H, H-B, $NCH_2C_6H_5$), 4.51 (s, 2H, NH_2), 4.44 (d, $J_{4,5}$=8.9 Hz, 1H, H-4), 3.83 (m, 2H, H-5, H-6a), 3.65 (dd, $J_{5,6b}$=6.7 Hz, $J_{6a,6b}$=11.6 Hz, 1H, H-6b), 1.57 (s, 3H, CH_3), 1.34 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 146.1 (C-4′), 136.9–126.3 (12 C, 2×C_6H_5), 113.7 [$OC(CH_3)_2$], 107.2 (C-5′), 103.6 (C-1), 84.7 (C-2), 76.3 (C-4), 71.6 (C-3), 69.8 (C-5), 64.7 (C-6), 48.0 ($NCH_2C_6H_5$), 26.4 (2×CH_3); MS (APCI+) *m*/*z* 489.2 [M+1]$^+$, 511.1 [M+Na]$^+$, 527.1 [M+K]$^+$. Anal. Calcd for $C_{24}H_{28}N_2O_7S$ (488.16 g/mol): C, 59.00; H, 5.78; N, 5.73; S, 6.56. Found: C, 59.27; H, 5.81; N, 5.84; S, 6.79.

4.9.3. 1,2-*O*-Isopropylidene-α-D-*ribo*-hexofuranose-3-spiro-3′-(4′-amino-5′-phenyl-5′*H*-2′,3′-dihydroisothiazole-1′,1′-dioxide) (6Bc). Following the general method (F), acetic acid (18 mL) and water (12 mL) were added to the compound **4Bh** (279 mg, 0.63 mmol). After 5 h 20 min, the solvent was eliminated under vacuum. The residue was triturated with a solution of EtOAc–petroleum ether to give after filtration **6Bc** (53.5 mg, 21%) as a colourless solid: mp 104–106 °C; $[\alpha]_D^{34}$ +4 (*c* 0.06, MeOH); IR (ATR) ν 1644, 1249, 1151, 1101, 1036, 860 cm^{-1}; ^{1}H NMR (MeOH d_4, 300 MHz) δ 7.57–7.43 (m, 5H, C_6H_5), 6.03 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 4.71 (s, 1H, H-2), 4.18 (d, $J_{4,5}$=8.2 Hz, 1H, H-4), 4.04 (m, 1H, H-5), 3.77 (dd, $J_{5,6a}$=2.9 Hz, $J_{6a,6b}$=11.7 Hz, 1H, H-6a) 3.65 (dd, $J_{5,6b}$=5.7 Hz, 1H, H-6b), 1.61 (s, 3H, CH_3), 1.40 (s, 3H, CH_3); ^{13}C NMR (MeOH d_4, 75 MHz) δ 149.0 (C-4′), 132.0–127.5 (6C, C_6H_5), 113.4 [$OC(CH_3)_2$], 105.7 (C-5′), 104.3 (C-1), 83.7 (C-2), 78.1 (C-4), 70.7 (C-5), 69.6 (C-3), 63.9 (C-6), 25.6 (CH_3); MS (APCI+) *m*/*z* 421.05 [M+Na]$^+$. Anal. Calcd for $C_{17}H_{22}N_2O_7S$ (398.11 g/mol): C, 51.25; H, 5.57; N, 7.03; S, 8.05. Found: C, 51.35; H, 5.60; N, 7.34; S, 8.23.

4.9.4. Acetylation of compound 5Aa. Following the general method (G), pyridine (1 mL) and Ac_2O (0.5 mL) were added to compound **5Aa** (53 mg, 0.17 mmol). The reaction mixture is stirred at room temperature for 7 days. The solvent was removed under vacuo with toluene and the residue was flash chromatographed (MeOH/CH_2Cl_2, 1:99) to give successively **7a** (19 mg, 31%) and **8a** (7 mg, 12%) as white solids. **7a**: mp 103–104 °C; $[\alpha]_D^{20}$ +58 (*c* 0.10, acetone); IR (ATR) ν 3344, 2360, 2338, 1675, 1514, 1296, 1056 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.83 (s, 1H, N*H*$COCH_3$), 5.70 (d, $J_{1,2}$=3.7 Hz, 1H, H-1), 5.05 (d, 1H, H-2), 5.00 (d, $J_{5'a,5'b}$=13.6 Hz, 1H, H-5′a), 4.87 (d, $J_{4,5b}$=2.8 Hz, 1H, H-4), 4.30 (d, $J_{5a,5b}$=10.6 Hz, 1H, H-5a), 4.20 (dd, 1H, H-5b), 3.40 (d, 1H, H-5′b), 3.00 (s, 3H, NCH_3), 2.10 (s, 3H, $COCH_3$), 1.60 (s, 3H, CH_3), 1.35 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.2 (CO), 113.5 [$OC(CH_3)_2$], 105.8 (C-1), 95.0 (C-4′), 82.7 (C-3), 81.8 (C-2), 79.0 (C-3), 72.8 (C-5), 50.7 (C-5′), 27.8 (NCH_3), 27.2 (CH_3), 26.5 (CH_3); MS (APCI+) *m*/*z* 371.3 [M+Na]$^+$, 387.2 [M+K]$^+$. Anal. Calcd for $C_{13}H_{20}N_2O_7S$ (348.10 g/mol): C, 44.82; H, 5.79; N, 8.04; S, 9.20. Found: C, 44.75; H, 5.73; N, 8.05; S, 9.37. **8a**: mp 219–220 °C; $[\alpha]_D^{20}$ +12 (*c* 0.05, acetone); IR (ATR) ν 2359, 2336, 1737, 1644, 1240, 1128, 1059, 1017 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.88 (d, $J_{1,2}$=3.9 Hz, 1H, H-1), 5.62 (s, 1H, H-5′), 4.72 (d, 1H, H-2), 4.69 (dd, $J_{4,5a}$=4.2 Hz,

$J_{4,5b}$=7.4 Hz, 1H, H-4), 4.63 (s, 2H, NH_2), 4.26 (m, 2H, H-5), 3.02 (s, 3H, NCH_3), 2.12 (s, 3H, $OCOCH_3$), 1.66 (s, 3H, CH_3), 1.37 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.0 (CO), 151.1 (C-4′), 114.0 [O*C*$(CH_3)_2$], 104.1 (C-1), 95.0 (C-5′), 84.7 (C-2), 73.3 (C-4), 71.1 (C-3), 61.7 (C-5), 27.3 (NCH_3), 26.5 (CH_3), 26.3 (CH_3), 21.1 ($OCOCH_3$); MS (APCI+) *m/z* 371.3 $[M+Na]^+$, 387.2 $[M+K]^+$, 719.3 $[2M+Na]^+$, 735.2 $[2M+K]^+$. Anal. Calcd for $C_{13}H_{20}N_20_7S$ (348.10 g/mol): C, 44.82; H, 5.79; N, 8.04; S, 9.20. Found: C, 44.68; H, 5.84; N, 8.18; S, 9.34.

4.9.5. Acetylation of compound 5Ab. Following the general method (G), pyridine (2 mL) and Ac_2O (1 mL) were added to compound **5Ab** (89 mg, 0.23 mmol). The reaction mixture is stirred at room temperature for 16 h. The solvent was removed under vacuo with toluene and the residue was flash chromatographed (MeOH/CH_2Cl_2, 1:99) to give successively **7b** (44 mg, 44%) and **8b** (12 mg, 12%) as white solids. **7b**: mp 205–206 °C; $[\alpha]_D^{20}$ +9 (*c* 0.20, acetone); IR (ATR) ν 3245, 2356, 2339, 1651, 1531, 1314, 1155, 1070, 1016, 837 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.43–7.28 (m, 5H, C_6H_5), 5.90 (s, 1H, N*H*$COCH_3$), 5.70 (d, $J_{1,2}$=3.7 Hz, 1H, H-1), 5.18 (d, 1H, H-2), 4.87 (m, 3H, H-5′a, NC$H_2C_6H_5$), 4.53 (d, $J_{4,5b}$=3.5 Hz, 1H, H-4), 4.00 (d, $J_{5a,5b}$=10.6 Hz, 1H, H-5a), 3.72 (dd, 1H, H-5b), 3.93 (d, $J_{5'a,5'b}$=13.7 Hz, 1H, H-5′b), 2.05 (s, 3H, $NCOCH_3$), 1.68 (s, 3H, CH_3), 1.36 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.4 (CO), 137.8–128.4 (C_6H_5), 113.5 [O*C*$(CH_3)_2$], 106.1 (C-1), 95.0 (C-4′), 82.3 (C-4), 81.0 (C-2), 80.3 (C-4), 72.7 (C-5), 52.7 (C-5′), 45.4 (O*C*$H_2C_6H_5$), 27.4 (CH_3), 26.4 (CH_3), 24.7 (NCO*C*H_3); MS (APCI+) *m/z* 447.3 $[M+Na]^+$, 463.3 $[M+K]^+$, 871.3 $[2M+Na]^+$. Anal. Calcd for $C_{19}H_{24}N_20_7S$ (424.13 g/mol): C, 53.76; H, 5.70; N, 6.60; S, 7.55. Found: C, 53.75; H, 5.62; N, 6.73; S, 7.88. **8b**: mp 90–91 °C; $[\alpha]_D^{20}$ +17 (*c* 0.10, acetone); IR (ATR) ν 2354, 2336, 1741, 1648, 1230, 1128, 1017, 873 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.54–7.31 (m, 5H, C_6H_5), 5.87 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 5.61 (s, 1H, H-5′), 4.82 (d, 1H, H-2), 4.79 (m, 2H, NC$H_2C_6H_5$), 4.72 (s, 2H, NH_2), 4.43 (dd, $J_{4,5a}$=8.0 Hz, $J_{4,5b}$=2.3 Hz, 1H, H-4), 4.05 (dd, $J_{5a,5b}$=12.4 Hz, 1H, H-5a), 3.94 (dd, 1H, H-5b), 1.96 (s, 3H, $OCOCH_3$), 1.71 (s, 3H, CH_3), 1.34 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.8 (CO), 151.3 (C-4′), 137.5–128.1 (C_6H_5), 114.2 [O*C*$(CH_3)_2$], 104.2 (C-1), 94.5 (C-5′), 84.7 (C-4), 74.7 (C-4), 71.3 (C-3), 61.9 (C-5), 45.0 (N*C*$H_2C_6H_5$), 26.8 (CH_3), 26.2 (CH_3), 21.0 ($OCOCH_3$); MS (APCI+) *m/z* 447.3 $[M+Na]^+$, 463.3 $[M+K]^+$, 871.3 $[2M+Na]^+$. Anal. Calcd for $C_{19}H_{24}N_20_7S$ (424.13 g/mol): C, 53.76; H, 5.70; N, 6.60; S, 7.55. Found: C, 53.66; H, 5.81; N, 6.64; S, 7.84.

4.9.6. Acetylation of compound 5Ba. Following the general method (G), pyridine (1 mL) and Ac_2O (1 mL) were added to compound **5Ba** (50 mg, 0.15 mmol). The reaction mixture was stirred at rt for 4 h. The solvent was removed under vacuo with toluene and the residue was flash chromatographed (hexane/AcOEt, 1:1) to give **9** (47 mg, 84%) as a colorless solid and **10** (1 mg, 1%). **9**: mp 211–213 °C; $[\alpha]_D^{25}$ +107 (*c* 0.90, $CHCl_3$); IR. (KBr) ν 3448, 3362, 2987, 2939, 1738, 1633, 1294, 1249,1022 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.84 (d, $J_{1,2}$=3.9 Hz, 1H, H-1), 5.00 (d, $J_{1,2}$=3.9 Hz, 1H, H-2), 4.72 (s, 1H, H-4), 4.42 (t, *J*=5.8 Hz), 4.22 (dd, $J_{5,6a}$=5.8 Hz, $J_{6a,6b}$=11.6 Hz, 1H, H-6a), 4.03 (dd, $J_{5,6b}$=5.7 Hz, $J_{6a,6b}$=11.6 Hz, 1H, H-6b), 3.67 (d, $J_{5'a,5'b}$=13.3 Hz, 1H, H-5′a), 3.34 (d, $J_{5'a,5'b}$=13.3 Hz, 1H, H-5′b), 3.06 (s, 3H, N–CH_3), 2.20 (br s, 2H, NH_2), 2.12 (s, 3H, CH_3CO), 1.62 (s, 3H, CH_3), 1.37 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.6 (CO), 112.6 [O*C*$(CH_3)_2$O], 106.4 (C-1), 95.9 (C-4′), 81.4 (C-2), 80.9 (C-4), 80.6 (C-5), 79.1 (C-3), 62.8 (C-6), 57.2 (C-5′), 27.8 (NCH_3), 26′6, 25.9 [OC(*C*$H_3)_2$O], 20.8 (CH_3CO); MS (APCI+) *m/z* 379.2 $[M+1]^+$, 401.2 $[M+Na]^+$, 757.2 $[2M+1]^+$, 779.3 $[2M+Na]^+$. Anal. Calcd for $C_{14}H_{22}N_2O_8S$ (378.40 g/mol): C, 44.44; H, 5.86; N, 7.40; S, 8.47. Found: C, 44.74; H, 5.64; N, 7.31; S, 8.59. Following the general method (G), pyridine (1 mL) and Ac_2O (1 mL) were added to compound **5Ba** (30 mg, 0.079 mmol). The reaction mixture was stirred at rt for 15 h. The solvent was removed under vacuo with toluene and the residue was flash chromatographed (hexane/AcOEt, 1:1) to give **10** (20 mg, 60%) and unreacted **5Ba** (10 mg, 33%). **10**: mp 59–61 °C; $[\alpha]_D^{25}$ +83 (*c*0.27, $CHCl_3$); IR(KBr) ν 3435, 2963, 1744, 1685, 1519, 1376, 1311, 1262, 1157, 1098. 1024, 802 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.03 (br s, 1H, NH), 5.73 (d, $J_{1,2}$=3.9 Hz, 1H, H-1), 5.10 (d, $J_{1,2}$=3.9 Hz, 1H, H-2), 4.76 (d, $J_{5a,5b}$= 14.1 Hz, 1H, H-5′a), 4.74 (s, 1H, H-4), 4.54 (t, $J_{5,6a}$=6.1 Hz, 1H, H-5), 4.25 (dd, $J_{5,6a}$=6.3 Hz, $J_{6a,6b}$=11.9 Hz, 1H, H-6a), 4.09 (dd, $J_{5,6b}$=5.6 Hz, $J_{6a,6b}$=11.9 Hz, 1H, H-6b), 3.57 (d, $J_{5a,5b}$=14.1 Hz, 1H, H-5′b), 3.04 (s, 3H, NCH_3), 2.10 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 1.59 (s, 3H, CH_3), 1.33 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.8 (CO), 170.5 (CO), 113.3 [O*C*$(CH_3)_2$O], 106.2 (C-1), 95.1 (C-4′), 83.3 (C-5), 82.5 (C-3), 80.8 (C-2), 80.7 (C-4), 62.7 (C-6), 51.9 (C-5′), 27.9 (NCH_3), 27.0, 26.3 [OC(*C*$H_3)_2$O], 24.5 (CH_3CON), 21.0 (CH_3COO); MS (APCI+) *m/z* 421.3 $[M+1]^+$, 443.2 $[M+Na]^+$, 863.3 $[2M+1]^+$. Anal. Calcd for $C_{16}H_{24}N_2O_9S$ (420.44 g/mol): C, 45.71; H, 5.75; N, 6.66; S, 7.63. Found: C, 45.66; H, 5.69; N, 6.51; S, 7.96. Following the general method (G), **5Ba** (48 mg, 0.14 mmol) was treated with pyridine (1 mL) and Ac_2O (1 mL), at rt for 3 days, gave, after flash chromatography (hexane: AcOEt, 1:1), compounds **10** (41 mg, 70%) and **11** (9 mg, 14%, 86% pure). **11**: amorphous; IR (KBr) ν 3449, 2990, 1752, 1637, 1498, 1377, 1249, 1216, 1114, 1054 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.65 (br s, 1H, NH), 7.39 (s, 1H, H-5′), 5.85 (d, $J_{1,2}$=3.9 Hz, 1H, H-1), 5.22 (m, $J_{5,6a}$=2.4 Hz, $J_{5,6b}$=5.8 Hz 1H, H-5), 4.67 (d, $J_{1,2}$=3.8 Hz, 1H, H-2), 4.61 (d, *J*=10.1 Hz 1H, H-4), 4.55 (dd, $J_{5,6a}$=2.4 Hz, $J_{6a,6b}$=12.2 Hz, 1H, H-6a), 4.09 (dd, $J_{5,6b}$=5.8 Hz, $J_{6a,6b}$=12.2 Hz, 1H, H-6b), 3.04 (s, 3H, NCH_3), 2.21 (s, 3H, CH_3CO), 2.09 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 1.65 (s, 3H, CH_3), 1.35 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.6 (CO), 169.6 (CO), 168.3 (CO), 140.1 (C-4′), 114.1 [O*C*$(CH_3)_2$O], 108.5 (C-5′), 102.8 (C-1), 85.0 (C-2), 77.4 (C-3), 71.3 (C-4), 67.6 (C-5), 63.6 (C-6), 26.8 (NCH_3), 26′0, 25.9 [OC(*C*$H_3)_2$O], 24.7 (CH_3CO), 20.7 (CH_3CO), 20.6 (CH_3CO); MS (APCI+) *m/z* 463.3 $[M+1]^+$, 480.3 $[M+NH_4]^+$, 485.3 $[M+Na]^+$, 947.3 $[2M+Na]^+$.

4.9.7. Acetylation of compound 6Ba. Following the general method (G), pyridine (2 mL) and Ac_2O (1 mL) were added to the compound **6Ba** (75 mg, 0.18 mmol). After 12 h and flash chromatography (EtOAc/petroleum ether, 5:5) product **12a** (75 mg, 84%) was isolated as a

colorless solid: mp 126–127 °C; $[\alpha]_D^{20}$ +54 (*c* 0.25, $CHCl_3$); IR (ATR) ν 3356, 2356, 2327, 1733, 1648, 1379, 1256, 1213, 1133, 1058, 1034 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.41 (m, 5H, C_6H_5), 5.86 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 5.86 (ddd, $J_{4,5}$=10.0 Hz, $J_{5,6a}$=2.4 Hz, $J_{5,6b}$=5.9 Hz, 1H, H-5), 4.77 (d, 1H, H-2), 4.65 (d, 1H, H-4), 4.55 (dd, $J_{6a,6b}$=12.1 Hz, 1H, H-6a), 4.46 (s, 2H, NH_2), 4.11 (dd, 1H, H-6b), 3.09 (s, 3H, NCH_3), 2.12 (s, 3H, $OCOCH_3$), 2.07 (s, 3H, $OCOCH_3$), 1.65 (s, 3H, CH_3), 1.36 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.0, 170.5 (2×CO), 144.7 (C-4′), 129.9–126.4 (6C, C_6H_5), 113.9 [$OC(CH_3)_2$], 108.2 (C-5′), 103.5 (C-1), 85.9 (C-4), 71.7 (C-2), 70.5 (C-3), 68.2 (C-5), 64.2 (C-6), 27.8 (NCH_3), 26.7 (CH_3), 26.4 (CH_3), 21.5 ($OCOCH_3$), 21.1 ($OCOCH_3$); MS (APCI+) *m/z* 519.1 $[M+Na]^+$, 534.9 $[M+K]^+$. Anal. Calcd for $C_{22}H_{28}N_20_9S$ (496.15 g/mol): C, 53.22; H, 5.68; N, 5.64; S, 6.46. Found: C, 53.30; H, 5.47; N, 5.42; S, 6.29.

4.9.8. Acetylation of compound 6Bb. Following the general method (G), pyridine (1 mL) and Ac_2O (0.5 mL) were added to the compound **6bB** (50 mg, 0.10 mmol). After 12 h and flash chromatography (EtOAc/petroleum ether, 4.5:5.5) product **12b** (55 mg, 95%) was isolated as a colorless solid: mp 94–95 °C; $[\alpha]_D^{20}$ +78 (*c* 0.25, $CHCl_3$); IR (ATR) ν 2355, 2331, 1743, 1659, 1494, 1372, 1240, 1216, 1155, 1056, 1023 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.64–7.38 (m, 10H, 2×C_6H_5), 5.88 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 5.39 (ddd, $J_{4,5}$=9.9 Hz, $J_{5,6a}$=2.3 Hz, $J_{5,6b}$=5.8 Hz, 1H, H-5), 4.85 (d, 1H, H-2), 4.75 (d, $J_{A,B}$=14.2 Hz, 1H, H-A $NCH_2C_6H_5$), 4.69 (d, H-4), 4.59 (d, 1H, H-B $NCH_2C_6H_5$), 4.54 (dd, $J_{6a,6b}$=12.1 Hz, 1H, H-6a), 4.41 (s, 2H, NH_2), 4.05 (dd, 1H, H-6b), 2.22 (s, 3H, $OCOCH_3$), 2.08 (s, 3H, $OCOCH_3$),1.60 (s, 3H, CH_3), 1.39 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.0–170.6 (2×CO), 144.3 (C-4′), 136.7–126.3 (12C, 2×C_6H_5), 113.8 [$OC(CH_3)_2$], 108.5 (C-5′),103.5 (C-1), 85.6 (C-2), 73.1 (C-4), 71.3 (C-3), 68.4 (C-5), 64.2 (C-6), 48.2 ($NCH_2C_6H_5$), 26.6 (CH_3), 26.4 (CH_3), 21.8 ($OCOCH_3$), 21.1 ($OCOCH_3$); MS (APCI+) *m/z* 595.1 $[M+Na]$,$^+$ 611.1 $[M+K]$.$^+$ Anal. Calcd. for $C_{28}H_{32}N_2O_9S$ (572.18 g/mol): C, 58.73; H, 5.63; N, 4.89; S, 5.60. Found: C, 58.57; H, 5.72; N, 4.74; S, 6.82.

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The effective use of substituted benzoic anhydrides for the synthesis of carboxamides

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Abstract—Various carboxamides are synthesized from the corresponding carboxylic acids and amines with high product-selectivities using 2-methyl-6-nitrobenzoic or 2,4,6-trichlorobenzoic anhydride in the presence of 4-(dimethylamino)pyridine.

1. Introduction

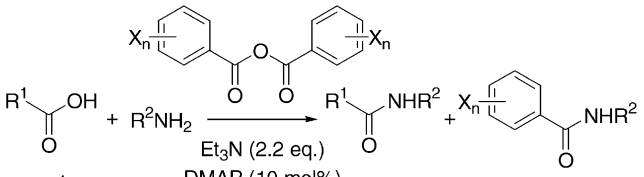
Recently, we reported several useful methods for the synthesis of carboxylic acid derivatives using substituted benzoic anhydrides under acidic or basic conditions.[1,2] For example, nearly equimolar amounts of carboxylic acids and alcohols or ω-hydroxycarboxylic acids react in the presence of 2-methyl-6-nitrobenzoic anhydride (MNBA) with basic catalysts such as 4-(dimethylamino)pyridine (DMAP) to produce the corresponding carboxylic esters or lactones in high yields.[2] During the reaction process using MNBA, the intermediary mixed anhydride was initially formed and it functions as a reactive acylating reagent of alcohols to give the desired carboxylic esters in high yields with high product-selectivities. In this report, we would describe an effective method for the accelerated synthesis of carboxamides[3,4] including sterically-hindered compounds using substituted benzoic anhydrides by basic promoters.

2. Results and discussion

2.1. Amidation reaction via mixed-anhydrides using benzoic anhydrides

First, benzoic or several substituted benzoic anhydrides were screened for the reaction of 3-phenylpropanoic acid (**1**) with 3-phenylpropylamine (**2**) as shown in Table 1. The desired carboxamide, 3-phenyl-*N*-(3-phenylpropyl)propanamide (**3**), was obtained in 55% yield along with the 10% formation of the undesired benzamide by the addition of **2** to the reaction mixture of **1** and benzoic anhydride (entry 1). When 4-methoxybenzoic anhydride was employed for the model case, the amount of undesirable benzamide increased to 34% (entry 2). The addition of electron withdrawing substituents, such as the trifluoromethyl or cyano group, on the 4-position of the aromatic moiety increased the reactivity of the anhydride but the selectivity was not satisfactory (entries 3 and 4). Although the reaction using 2,6-dichlorobenzoic anhydride afforded **3** in medium yield as listed in entry 5, an excellent chemical yield of **3** and relatively good product-selectivity were obtained when using 2,4,6-trichlorobenzoic anhydride (TCBA, entry 6). As shown in entry 7, MNBA also

Table 1. Isolated yields of carboxamide **3** and ratios of **3** to by-products



Entry	X_n	Yield of **3** (%)	Yield of **BP** (%)	**3/BP**[a]
1[b]	H	55	10	5.5/1
2[b]	4-MeO	48	34	1.4/1
3[b]	4-CF_3	52	12	4.3/1
4[b]	4-CN	77	5	15/1
5[b]	2,6-Cl_2	64	8	8.0/1
6[b]	2,4,6-Cl_3	97	3	32/1
7[b]	2-Me-6-NO_2	83	1	83/1
8[c]	2,4,6-Cl_3	63	25	2.5/1
9[c]	2-Me-6NO_2	84	14	6.0/1

[a] Determined by ^{1}H NMR using a crude mixture.
[b] **2** (1.0 equiv.) was added to the mixture of **1** (1.0 equiv.) and benzoic anhydride or substituted ones (1.2 equiv.).
[c] TCBA or NMBA (1.2 equiv.) was added to the mixture of **1** (1.0 equiv.) and **2** (1.0 equiv.).

Keywords: Carboxamides; 2-Methyl-6-nitrobenzoic anhydride; 2,4,6-Trichlorobenzoic anhydride; Mixed-anhydrides; 4-(Dimethylamino)pyridine.
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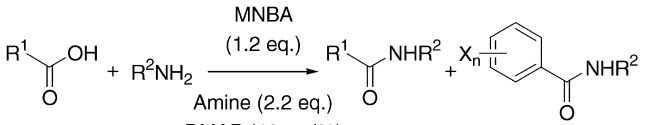
functions as a suitable benzoic anhydride to produce the desired carboxamide although the yield was somewhat lower than that of entry 6. Furthermore, TCBA or MNBA was added to the reaction mixture of **1** and **2** in order to determine the best structure of the benzoic part of the anhydride for maintaining high product-selectivity. According to this procedure, it was revealed that the reaction using MNBA gave preferable product-selectivity compared to that obtained by the reaction using TCBA (entries 8 and 9).

Next, some bases were screened for the reaction of **1** with **2** by the promotion of MNBA as shown in Table 2. When the reaction was carried out using a 2.2 molar amount of triethylamine in the absence of DMAP, the product selectivity significantly decreased as compared to that of the DMAP catalyzed reaction (entries 1 and 2). Furthermore, by employing other typical bases, such as *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), poor product-selectivities and low yields of the desired amide **3** were obtained (entries 3 and 4). On the other hand, the yield of **3** increased to 92% without any loss of the high product-selectivity when the present reaction was performed in the presence of a 2.2 molar amount of DMAP as a stoichiometric base (entry 5). It was found that the use of a stoichiometric amount of 4-pyrrolidinopyridine (PPY) was also effective for this reaction and the desired amide **3** was obtained in excellent yield with high chemoselectivity (entry 6).

Table 2. Isolated yields of carboxamide **3** and ratios of **3** to by-products



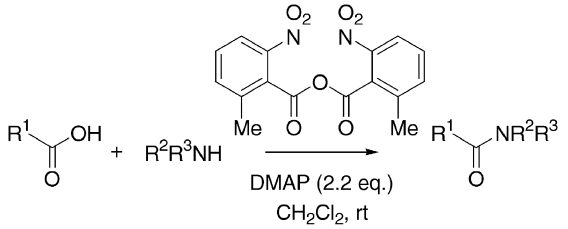
Entry	Base	Yield of **3** (%)	Yield of **BP** (%)	**3/BP**[a]
1[b]	Et_3N	20	68	0.29/1
2	Et_3N	84	14	6.0/1
3	TMEDA	47	47	1/1
4	DBU	16	64	0.25/1
5[c]	DMAP	92	trace	141/1
6[d]	PPY	93	1.0	93/1

[a] Determined by ^1H NMR using a crude mixture.
[b] The reaction was carried out in the absence of DMAP.
[c] The reaction was carried out in the presence of DMAP (2.2 equiv.) without any tertiary amine.
[d] The reaction was carried out in the presence of PPY (2.2 equiv.) without any tertiary amine.

The amidation proceeded at room temperature in several other polar solvents, such as DMI (95%, **3/BP**=32/1), DMF (92%, 50/1), MeCN (91%, 85/1), THF (83%, 7.7/1) and $MeNO_2$ (49%, 64/1) under the same reaction conditions as entry 5 in the Table 2.

Table 3 shows the yields for a variety of carboxamides including ones derived from bulky substrates. The reactions of **1** with benzylamine (**4**), diphenylmethylamine (**6**), 1-phenylethylamine (**8**), 1-adamantanamine (**10**), benzylmethylamine (**12**), piperidine (**14**) and aniline (**16**) proceeded to form the corresponding coupling products in high yields (entries 3–9). It is noteworthy that the undesired benzamide was not produced at all except for entry 1. 2-Phenylpropanoic acid (**18**), a 2-branched carboxylic acid, also reacted with **2** to form the desired 2-phenyl-*N*-(3-phenylpropyl)propanamide (**19**) in high yield (entry 10). The amidation of **18** with other typical amines including bulky ones gave the corresponding carboxamides in good to excellent yields with perfect product-selectivities (entries 11–17).

Table 3. Isolated yields of various carboxamides



Entry	Carboxylic acid	Amine	Product	Yield (%)
1[a]	**1**	**2**	**3**	92[b]
2[a,c]	**1**	**2**	**3**	84[d]
3[a,c]	**1**	$PhCH_2NH_2$ (**4**)	**5**	87[d]
4[a]	**1**	Ph_2CHNH_2 (**6**)	**7**	91[d]
5[e]	**1**	$PhCHMeNH_2$ (**8**)	**9**	82[d]
6[f]	**1**	1-Adamantyl-NH_2 (**10**)	**11**	96[d]
7[e]	**1**	$PhCH_2NHMe$ (**12**)	**13**	96[d]
8[e]	**1**	Piperidine (**14**)	**15**	81[d]
9[e]	**1**	$PhNH_2$ (**16**)	**17**	78[d]
10[e]	PhCHMeCOOH (**18**)	**2**	**19**	94[d]
11[e]	**18**	**4**	**20**	90[d]
12[a]	**18**	**6**	**21**	90[d]
13[e]	**18**	**8**	**22**	88[d]
14[f]	**18**	**10**	**23**	92[d]
15[e]	**18**	**12**	**24**	93[d]
16[e]	**18**	**14**	**25**	82[d]
17[e]	**18**	**16**	**26**	85[d]

[a] Amines (1.0 equiv.) were added to the mixture of carboxylic acids (1.2 equiv.) and MNBA (1.2 equiv.).
[b] **3/BP**=141/1.
[c] The reaction was carried out at −78 °C.
[d] Carboxamide/benzamide=>200/1.
[e] Amines (1.0 equiv.) were added to the mixture of carboxylic acids (1.1 equiv.) and MNBA (1.1 equiv.).
[f] Amines (1.0 equiv.) were added to the mixture of carboxylic acids (1.3 equiv.) and MNBA (1.3 equiv.).

3. Conclusion

It is noted that the present reaction provides a convenient and high-yielding method for the preparation of carboxamides from nearly equimolar amounts of free carboxylic acids and amines which involve bulky alkyl groups. The experimental procedure is quite simple and almost pure carboxamides are obtained just by mixing the substrates at room temperature.

4. Experimental

4.1. General methods

All reactions were carried out under argon atmosphere in dried glassware. Dichloromethane was distilled from

diphosphorus pentoxide, then calcium hydride, and dried over MS 4 Å. Thin layer chromatography was performed on Wakogel B5F. All melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded with tetramethylsilane (TMS) or chloroform (in chloroform-*d*) as internal standard.

4.2. Starting materials

All reagents were purchased from Tokyo Kasei Kogyo Co., Ltd, Kanto Chemical Co., Inc. or Aldrich Chemical Co., Inc., and used without further purification unless otherwise noted. 2-Methyl-6-nitrobenzoic anhydride (MNBA) was purchased from Tokyo Kasei Kogyo Co., Ltd (TCI, M1439) or synthesized from 2-methyl-6-nitrobenzoic acid.[2d]

4.3. Typical experimental procedure for the amidation reaction

A typical experimental procedure is described for the reaction of 3-phenylpropanoic acid (**1**) with 3-phenylpropylamine (**2**); to a solution of DMAP (59.6 mg, 0.488 mmol) in dichloromethane (2.0 mL) were added MNBA (91.6 mg, 0.266 mmol) and **1** (40.0 mg, 0.266 mmol). After having been stirred for 5 min, a solution of **2** (30.0 mg, 0.222 mmol) in dichloromethane (1.0 mL) was added. The reaction mixture was stirred for 14 h at room temperature and then saturated aqueous sodium hydrogencarbonate was added. The mixture was extracted with dichloromethane, and the organic layer was washed with water and brine, dried over sodium sulfate. After filtration of the mixture and evaporation of the solvent, the crude product was purified by thin layer chromatography to afford 54.6 mg (92%) of 3-phenyl-*N*-(3-phenylpropyl)-propanamide (**3**).

4.3.1. 3-Phenyl-*N*-(3-phenylpropyl)propanamide (3). Mp 57 °C. IR (KBr) 3260, 1640, 1540 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=7.35–7.10 (m, 10H), 5.46 (br s, 1H), 3.15 (dt, *J*=6.2, 7.0 Hz, 2H), 2.85 (t, *J*=7.7 Hz, 2H), 2.48 (t, *J*=7.7 Hz, 2H), 2.34 (t, *J*=7.7 Hz, 2H), 1.67 (tt, *J*=7.0, 7.7 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ=172.0, 141.3, 140.7, 128.4, 128.3, 128.2, 128.2, 126.1, 125.8, 39.0, 38.3, 33.1, 31.6, 30.9. HR MS: Calcd for $C_{18}H_{22}NO$ (M+H$^+$) 268.1701, found 268.1700.

4.3.2. *N*-Benzyl-3-phenylpropanamide[5] (5). Mp 80 °C. IR (KBr) 3290, 1650, 1540 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=7.30–7.09 (m, 10H), 5.68 (br s, 1H), 4.36 (d, *J*=5.6 Hz, 2H), 2.96 (t, *J*=7.6 Hz, 2H), 2.48 (t, *J*=7.6 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ=171.8, 140.7, 138.1, 128.6, 128.5, 128.4, 127.7, 127.4, 126.2, 43.5, 38.4, 31.7.

4.3.3. *N*-(Diphenylmethyl)-3-phenylpropanamide (7). Mp 149 °C. IR (KBr) 3340, 1640, 1540 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=7.33–7.06 (m, 15H), 6.28–6.18 (m, 1H), 6.01 (br s, 1H), 3.02 (t, *J*=7.5 Hz, 2H), 2.58 (t, *J*=7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ=171.0, 141.3, 140.6, 140.6, 128.6, 128.6, 128.5, 128.4, 128.4, 128.3, 127.4, 127.4, 126.3, 56.8, 38.4, 31.6. HR MS: Calcd for $C_{22}H_{22}NO$ (M+H$^+$) 316.1701, found 316.1699.

4.3.4. 3-Phenyl-*N*-[(1*S*)-1-phenylethyl]propanamide[4a] (9). 98% ee. Mp 92 °C. IR (KBr): 3280, 1640, 1560 cm^{-1}. $[\alpha]_D^{21}$=−63.6° (c 1.03, EtOH). ^{1}H NMR ($CDCl_3$) δ=7.21–7.00 (m, 10H), 5.90 (br s, 1H), 4.97 (quin, *J*=7.6 Hz, 1H), 2.83 (t, *J*=7.3 Hz, 2H), 2.35 (t, *J*=7.3 Hz, 2H), 1.28 (d, *J*=7.6 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ=171.1, 143.1, 140.7, 128.4, 128.4, 128.3, 127.1, 126.1, 126.0, 48.4, 38.4, 31.7, 21.5. HR MS: Calcd for $C_{17}H_{20}NO$ (M+H$^+$) 254.1545, found 254.1542.

4.3.5. *N*-1-Adamantyl-3-phenylpropanamide (11). Mp 125 °C. IR (KBr) 3250, 1640, 1540 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=7.31–7.19 (m, 5H), 5.00 (br s, 1H), 2.92 (t, *J*=7.6 Hz, 2H), 2.37 (t, *J*=7.6 Hz, 2H), 2.04 (br s, 3H), 1.92 (br d, 6H), 1.65 (br s, 6H). ^{13}C NMR ($CDCl_3$) δ=171.1, 141.1, 128.4, 127.1, 126.1, 51.8, 41.5, 39.5, 36.3, 31.8, 29.4. HR MS: Calcd for $C_{19}H_{26}NO$ (M+H$^+$) 284.2014, found 284.2015.

4.3.6. *N*-Benzyl-*N*-methyl-3-phenylpropanamide (13). A mixture of two stereoisomers A and B. IR (neat) 1640 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=7.33–7.06 (m, 10H, A+B), 4.59 (s, 2aH, A), 4.45 (s, 2bH, B), 3.05–2.96 (m, 2H, A+B), 2.70–2.64 (m, 2H, A+B), 2.94 (s, 3bH, B), 2.84 (s, 3aH, A). ^{13}C NMR ($CDCl_3$) δ=172.5 (B), 172.2 (A), 141.3, 141.2, 137.3, 136.4, 128.8, 128.8, 128.5, 128.4, 127.9, 127.9, 127.5, 127.5, 127.2, 127.2, 126.1, 126.0, 53.1 (B), 50.8 (A), 35.3 (A), 34.9 (B), 34.7 (A), 33.9 (B), 31.5 (B), 31.3 (A). HR MS: Calcd for $C_{17}H_{20}NO$ (M+H$^+$) 254.1545, found 254.1541.

4.3.7. 3-Phenylpropanoylpiperidine[6] (15). IR (neat) 1640 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=7.24–7.11 (m, 5H), 3.55 (br t, *J*=5.3 Hz, 2H), 3.32 (br t, *J*=5.3 Hz, 2H), 2.96 (t, *J*=8.0 Hz, 2H), 2.61 (t, *J*=8.0 Hz, 2H), 1.65–1.45 (br m, 6H). ^{13}C NMR ($CDCl_3$) δ=170.2, 141.4, 128.3, 128.3, 125.9, 46.5, 42.6, 35.0, 31.5, 26.3, 25.4, 24.4.

4.3.8. *N*-Phenyl-3-phenylpropanamide[7] (17). Mp 98 °C. IR (neat) 3320, 1660, 1530 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=7.73 (br s, 1H), 7.42 (d, *J*=7.8 Hz, 2H), 7.27–7.14 (m, 7H), 7.05 (t, *J*=7.4 Hz, 1H), 2.98 (t, *J*=7.8 Hz, 2H), 2.60 (t, *J*=7.8 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ=170.8, 140.5, 137.8, 128.8, 128.5, 128.2, 126.2, 124.2, 120.1, 39.1, 31.5.

4.3.9. 2-Phenyl-*N*-(3-phenylpropyl)propanamide[4a] (19). Mp 93 °C. IR (KBr): 3250, 1640, 1560 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=7.34–7.05 (m, 10H), 5.44 (br s, 1H), 3.50 (q, *J*=7.3 Hz, 1H), 3.24–3.15 (m, 2H), 2.51 (br t, 2H), 1.76–1.69 (m, 2H), 1.50 (d, *J*=7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ=174.1, 141.4, 141.3, 128.8, 128.3, 128.2, 127.5, 127.2, 125.9, 47.0, 39.1, 33.0, 31.0, 18.4. HR MS: Calcd for $C_{18}H_{21}NONa$ (M+Na$^+$) 290.1521, found 290.1495.

4.3.10. *N*-Benzyl-2-phenylpropanamide[4a] (20). Mp 78 °C. IR (KBr): 3280, 1640, 1550 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=7.32–7.09 (m, 10H), 6.02 (br s, 1H), 4.34 (dd, *J*=14.9, 5.9 Hz, 1H), 4.30 (dd, *J*=14.9, 5.9 Hz, 1H), 3.57 (q, *J*=7.1 Hz, 1H), 1.51 (d, *J*=7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ=174.1, 141.3, 138.4, 128.8, 128.5, 127.6, 127.4, 127.2, 127.2, 47.0, 43.4, 18.5. HR MS: Calcd for $C_{16}H_{17}NONa$ (M+Na$^+$) 262.1208, found 262.1210.

4.3.11. *N*-(Diphenylmethyl)-2-phenylpropanamide[4a] (21). Mp 134 °C. IR (KBr): 3280, 1640, 1540 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=7.37–6.95 (m, 15H), 6.19–6.00 (m, 1H), 3.59 (q, *J*=7.1 Hz, 1H), 1.48 (d, *J*=7.1 Hz, 3H). ^{13}C NMR

($CDCl_3$) δ=173.1, 141.5, 141.3, 141.2, 128.8, 128.5, 128.3, 127.5, 127.4, 127.3, 127.2, 127.1, 127.0, 56.7, 46.8, 18.3. HR MS: Calcd for $C_{22}H_{21}NONa$ ($M+Na^+$) 338.1521, found 338.1528.

4.3.12. (2*RS*)-2-Phenyl-*N*-[(1*SR*)-1-phenylethyl]propanamide[4a] (22a). Mp 127 °C. IR (KBr): 3350, 1640, 1540 cm^{-1}. ^1H NMR ($CDCl_3$) δ=7.37–7.19 (m, 10H), 5.56 (br d, 1H), 5.09 (quin, *J*=6.9 Hz, 1H), 3.53 (q, *J*=7.1 Hz, 1H), 1.51 (d, *J*=7.1 Hz, 3H), 1.34 (d, *J*=6.9 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ=173.2, 143.2, 141.4, 128.9, 128.6, 127.6, 127.2, 127.2, 126.0, 48.7, 47.1, 21.5, 18.6. HR MS: Calcd for $C_{17}H_{19}NONa$ ($M+Na^+$) 276.1365, found 276.1374.

4.3.13. (2*RS*)-2-Phenyl-*N*-[(1*RS*)-1-phenylethyl]propanamide[4a] (22b). Mp 127 °C. IR (KBr): 3240, 1640, 1540 cm^{-1}. ^1H NMR ($CDCl_3$) δ=7.34–7.17 (m, 8H), 7.08 (dd, *J*=7.7, 1.2 Hz, 2H), 5.59 (d, *J*=7.1 Hz, 1H), 5.08 (quin, *J*=7.1 Hz, 1H), 3.57 (q, *J*=7.3 Hz, 1H), 1.51 (d, *J*=7.3 Hz, 3H), 1.39 (d, *J*=7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ=173.1, 143.2, 141.3, 128.8, 128.5, 127.6, 127.2, 127.1, 125.7, 48.6, 47.1, 21.9, 18.4. HR MS: Calcd for $C_{17}H_{19}NONa$ ($M+Na^+$) 276.1365, found 276.1320.

4.3.14. *N*-1-Adamantyl-2-phenylpropanamide[4a] (23). Mp 136 °C. IR (KBr): 3300, 1640, 1550 cm^{-1}. ^1H NMR ($CDCl_3$) δ=7.34–7.22 (m, 5H), 5.11 (br s, 1H), 3.45 (q, *J*=7.1 Hz, 1H), 2.02 (br s, 3H), 1.90 (br d, 6H), 1.63 (br t, 6H), 1.46 (d, *J*=7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ=173.2, 142.1, 128.7, 127.5, 127.0, 51.7, 47.8, 41.4, 36.3, 29.4, 18.7. HR MS: Calcd for $C_{19}H_{25}NONa$ ($M+Na^+$) 306.1834, found 306.1841.

4.3.15. *N*-Benzyl-*N*-methyl-2-phenylpropanamide[4a] (24). A mixture of two stereoisomers A and B. IR (neat): 1640 cm^{-1}. ^1H NMR ($CDCl_3$) δ=7.31–7.15 (m, 8H, A+B), 7.01 (d, *J*=7.3 Hz, 2H, A+B), 4.66 (d, *J*=14.6 Hz, 1aH, A), 4.65 (d, *J*=16.7 Hz, 1bH, B), 4.54 (d, *J*=14.6 Hz, 1aH, A), 4.24 (d, *J*=16.7 Hz, 1bH, B), 3.92 (q, *J*=6.8 Hz, 1aH, A), 3.87 (q, *J*=6.8 Hz, 1bH, B), 2.93 (s, 3bH, B), 2.79 (s, 3aH, A), 1.49 (d, *J*=6.8 Hz, 3aH, A), 1.46 (d, *J*=6.8 Hz, 3bH, B). ^{13}C NMR ($CDCl_3$) δ=174.1 (B), 173.7 (A), 141.9, 141.7, 137.4, 136.6, 128.8, 128.8, 128.8, 128.4, 127.8, 127.4, 127.3, 127.2, 127.1, 126.8, 126.7, 126.2, 52.9 (B), 51.1 (A), 43.4 (A), 43.1 (B), 34.7 (A), 34.2 (B), 20.9 (B), 20.8 (A). HR MS: Calcd for $C_{17}H_{19}NONa$ ($M+Na^+$) 276.1365, found 276.1349.

4.3.16. 2-Phenylpropanoylpiperidine[4a] (25). IR (neat): 1640 cm^{-1}. ^1H NMR ($CDCl_3$) δ=7.33–7.19 (m, 5H), 3.88 (q, *J*=6.8 Hz, 1H), 3.70–3.35 (br m, 4H), 1.52–1.37 (m, 6H), 1.44 (d, *J*=6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ=171.7, 142.4, 128.8, 127.2, 126.6, 43.2, 43.2, 25.7, 24.5, 20.8. HR MS: Calcd for $C_{14}H_{19}NONa$ ($M+Na^+$) 240.1365, found 240.1377.

4.3.17. *N*-Phenyl-2-phenylpropanamide[8] (26). Mp 136 °C. IR (neat): 3360, 1660, 1540 cm^{-1}. ^1H NMR ($CDCl_3$) δ=7.47 (br s, 1H), 7.42 (d, *J*=7.8 Hz, 2H), 7.34–7.20 (m, 7H), 7.04 (t, *J*=7.3 Hz, 1H), 3.70 (q, *J*=7.0 Hz, 1H), 1.56 (d, *J*=7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ=172.5, 140.9, 137.9, 129.0, 128.8, 127.6, 127.4, 124.2, 119.8, 47.9, 18.5.

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(+)-Cystothiazole G: isolation and structural elucidation

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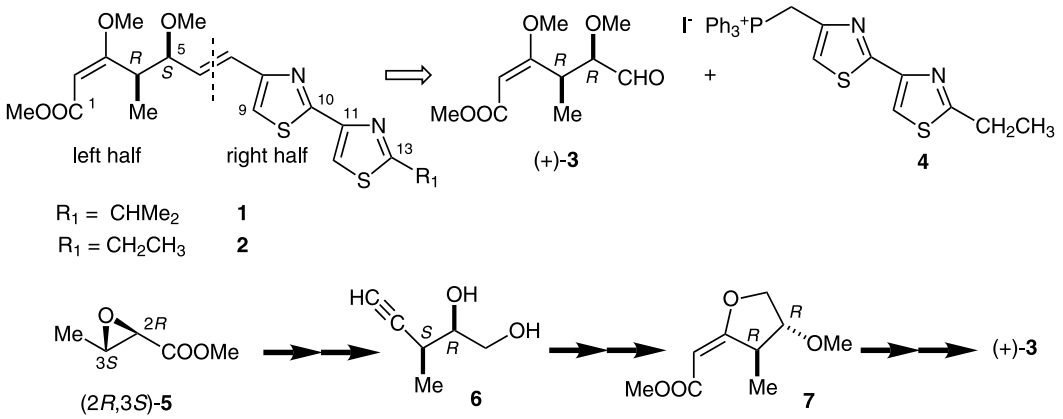
Abstract—Palladium-catalyzed cyclization-methoxycarbonylation of (2*R*,3*S*)-3-methylpent-4-yne-1,2-diol (**6**) derived from (2*R*,3*S*)-epoxybutanoate **5** followed by methylation gave the tetrahydro-2-furylidene acetate (−)-**7**, which was converted to the left-half aldehyde (+)-**3**. A Wittig reaction between (+)-**3** and the phosphoranylide derived from the bithiazole-type phosphonium iodide **4** using lithium bis(trimethylsilyl)amide afforded the (+)-cystothiazole G (**2**), whose spectral data were identical with those of the natural product (+)-**2**. Thus, the stereochemistry of cystothiazole G (**2**) was proved to be (4*R*,5*S*,6(*E*)).

1. Introduction

Recently, we reported the structural elucidation and the total synthesis of an antifungal substance named cystothiazole A (**1**)[1,2] from the myxobacterium *Cystobacter fuscus* strain AJ-13278 by using an inhibition assay against the phytopathogenic fungus, *Phytophthora capsici*. Further investigation of a large-scale culture of this strain resulted in the isolation of additional cystothiazole analogs, cystothiazole G (**2**).[3] This compound showed also inhibitory activity against *P. capsici*. More recently, further close analogues of cystothiazole G (**2**) named melithiazol H (**2**) have been isolated by a German group from another myxobacterium, *Myxococcus stipitatus*, strain Mx s64, although the absolute structure of melithiazol H (**2**) was not determined.[4] This paper describes the isolation, determination of the absolute structure based on the total synthesis, and biological activity of cystothiazole G (**2**) (Scheme 1).

A further search for additional cystothiazoles afforded 0.5 mg of a new member, cystothiazole G (**2**) ($[\alpha]_D^{24}=+108$ ($c=0.039$, $CHCl_3$)) from the extracts of a large-scale fermentation. The structure of cystothiazole G (**2**) was



Scheme 1.

Keywords: (+)-Cystothiazole G; Cyclization–methoxycarbonylation; Chiral synthesis.
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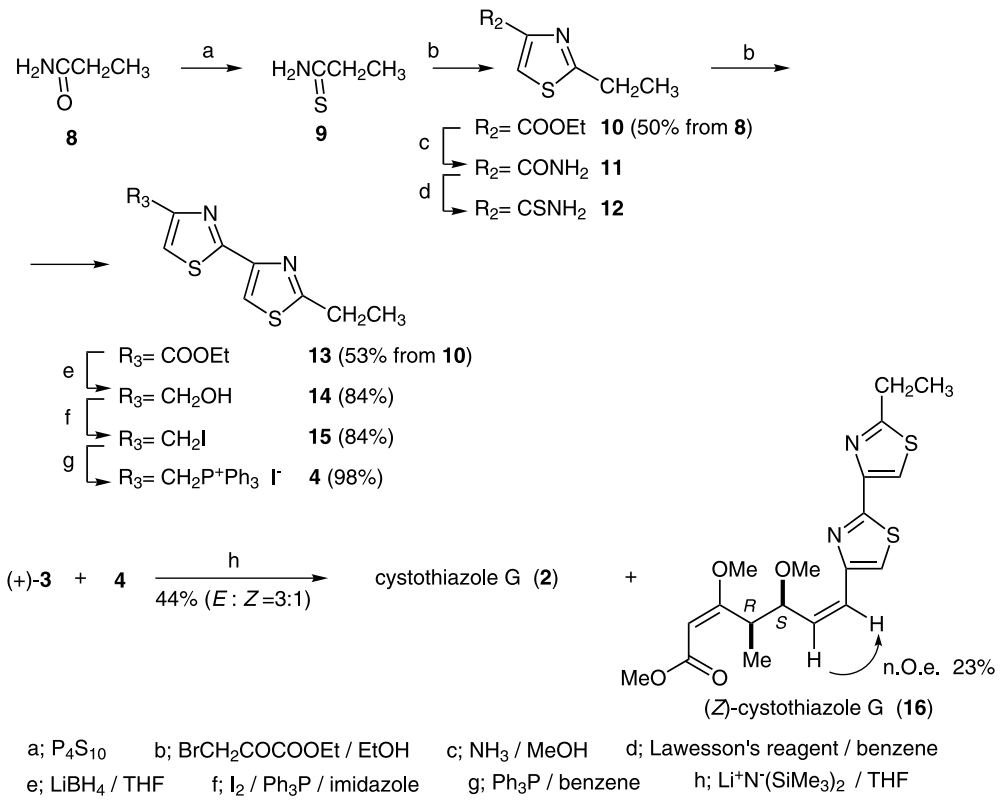

Scheme 2.

elucidated by comparison with the spectral data for cystothiazole A (**1**). The ^{1}H NMR data were similar to those for cystothiazole A (**1**) except for the presence of the signals due to an ethyl group observed at δ 1.43 (t, J=7.6 Hz, 3H) and 3.09 (q, J=7.6 Hz, 2H) and for the absence of the signals due to the isopropyl group of cystothiazole A (**1**). This NMR information as well as the molecular formula that is less than that of cystothiazole A (**1**) by CH_2 suggest that cystothiazole G (**2**) is 14-demethylcystothiazole A. A similar specific rotation to that of cystothiazole A (**1**) suggests that the absolute stereochemistry of cystothiazole G (**2**) is the same as that of cystothiazole A (**1**). Retrosynthetically, the synthesis of **2** can be achieved by Wittig condensation of the left-half aldehyde **3** and the right-half phosphonium iodide **4**. The synthesis of chiral aldehyde **3** was achieved in the total synthesis of cystothiazole A (**1**).[2] Palladium-catalyzed cyclization–methoxycarbonylation of (2*R*,3*S*)-3-methylpent-4-yne-1,2-diol (**6**) derived from (2*R*,3*S*)-epoxybutanoate (**5**) followed by methylation gave the tetrahydro-2-furylideneacetate **7**, which was converted to the left-half aldehyde (+)-**3**.[2] The synthesis of the right part **4** is shown in Scheme 2.

Treatment of propionamide (**8**) with P_4S_{10} gave a propionthioamide (**9**), which was reacted with α-bromopyruvate to afford a mono-thiazole ester **10** in 50% overall yield from **8**. Treatment of **10** with NH_3/MeOH followed by thioamidation with Lawesson's reagent yielded a thioamide **12**, which was reacted with α-bromopyruvate to afford a bithiazole ester **13** in 53% overall yield from **10**. $LiBH_4$ reduction (alcohol **14**: 84% yield) of **13** followed by treatment with I_2/Ph_3P/imidazole provided an iodide **15** in 84% yield. The reaction of **15** and triphenylphosphine gave a phosphonium salt **4** in 98% yield, which was condensed with (+)-**3** in the presence of lithium bis(trimethylsilyl)amide in THF to afford a mixture ((+)-(*E*)-**2**/(+)-(*Z*)-**16**=3:1) of olefins in 44% yield. Both isomers were isolated by preparative silica-gel thin-layer chromatography to provide (+)-**2** (colorless needles from *n*-hexane/AcOEt (20:1), mp 121–122 °C, $[\alpha]_D^{21}$=+108.8 (c=1.025, $CHCl_3$)) and (+)-**16** ($[\alpha]_D^{24}$=+215.9 (c=1.09, $CHCl_3$)). The (*Z*)-geometry of (+)-**16** was confirmed by the NOE enhancement for the olefinic protons (23%). The physical data of the synthetic (+)-**2** were identical with those ($[\alpha]_D^{24}$=+108 (c=0.039, $CHCl_3$), ^{1}H NMR($CDCl_3$)) of the isolated cystothiazole G (+)-**2**. Thus, the stereochemistry of cystothiazole G (**2**) was proved to be (4*R*,5*S*,6(*E*)). ^{1}H NMR (CD_3OD) data of the synthetic (+)-**2** were also identical with those of Melithiazole H (**2**), while the absolute structure of Melithiazole H (**2**) was not determined because of no information with respect to the specific rotation. The antifungal activity of the natural cystothiazole G (**2**) against the phytopathogenic fungus, *P. capsici*, was evaluated by using a paper disc assay method as reported previously.[1] The minimum dose applied on a paper disc to inhibit the fungal growth was 1 μg/disc. The synthetic cystothiazole G (**2**) also showed the activity at a similar level of dosage (0.2 μg/disc). According to our recent studies on antifungal tests using the phytopathogenic fungus, *P. capsici*, synthetic cystothiazole A (**1**) ((4*R*,5*S*)-**1**) showed activity up to a dose of 0.04 μg/disc. However, not only the enantiomer ((4*S*,5*R*)-**1**) but also the two diastereomers ((4*S*,5*S*)-**1** and (4*R*,5*R*)-**1**) did not show any antifungal activity up to 100 μg/disc. This result was not

expected at all, because all the stereoisomers possess the β-methoxyacrylate unit that is regarded as the binding site to the target molecules.[5]

In conclusion, palladium-catalyzed cyclization–methoxycarbonylation of (2*R*,3*S*)-3-methylpent-4-yne-1,2-diol (**6**) derived from (2*R*,3*S*)-epoxybutanoate **5** followed by methylation gave the tetrahydro-2-furylidene acetate (−)-**7**, which was converted to the left-half aldehyde (+)-**3**. A Wittig reaction between (+)-**3** and the phosphoranylide derived from the bithiazole-type phosphonium iodide **4** using lithium bis(trimethylsilyl)amide afforded the synthetic (+)-cystothiazole A (**2**), whose spectral data were identical with those of the natural product (+)-**2**. The stereochemistry of cystothiazole G (**2**) was proved to be (4*R*,5*S*,6(*E*)).

2. Experimental

2.1. General

All melting points were measured on a Yanaco MP-3S micro melting point apparatus and are uncorrected. ^{1}H- and ^{13}C NMR spectra were recorded on JEOL AL 400 spectrometer in $CDCl_3$. Carbon substitution degrees were established by DEPT pulse sequence. High-resolution mass spectra (HR-MS) and the fast atom bombardment mass spectra (FAB-MS) were obtained with a JEOL JMS 600H spectrometer. IR spectra were recorded with a JASCO FT/IR-300 spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. All evaporations were performed under reduced pressure. For column chromatography, silica gel (Kieselgel 60) was employed.

2.2. Isolation of cystothiazole G (2)

A large-scale fermentation (150 L culture) and fractionation to obtain the fractions of the first silica gel column chromatograph were reported in the literature.[1] The most polar part (950–1150 mL) of the hexane/EtOAc (4:1) fractions and the EtOAc fraction (400 mL) were combined (2.5 g), and a portion (1.35 g) was subjected to silica gel medium-pressure liquid chromatography (Develosil Lop 60, Nomura Chemical; 128 min linear gradient from 1 to 33% acetone in toluene, 5 mL/min) to give seven fractions. The first fraction eluted from 1 to 12% acetone (98.2 mg) was separated by preparative HPLC (Develosil ODS-10 (20 i.d.×250 mm), Nomura Chemical; 60% MeCN, 7 mL/min, detected at 254 nm) to give cystothiazole G (**2**) (0.5 mg, t_R=62.5 min) as a solid. Cystothiazole G (**2**): $[\alpha]_D^{24}$=+108 (c=0.039, $CHCl_3$); UV (MeOH) λ_{max} 226 (ε 33,400), 244 (ε 34,000), 312 (ε 12,500) nm, IR ($CHCl_3$): 1717, 1700, 1624, 1150, 1094 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.22 (3H, d, J=6.9 Hz), 1.43 (3H, t, J=7.6 Hz), 3.09 (2H, q, J=7.6 Hz), 3.33 (3H, s), 3.60 (3H, s), 3.66 (3H, s), 3.81 (1H, t, J=7.6 Hz), 4.17 (1H, dq, J=7.6, 6.9 Hz), 4.97 (1H, s), 6.41 (1H, dd, J=7.6, 15.7 Hz), 6.58 (1H, d, J=15.7 Hz), 7.09 (1H, s), 7.84 (1H, s). ^{13}C NMR ($CDCl_3$): δ 14.1, 14.1, 26.9, 39.8, 50.8, 55.5, 57.0, 84.4, 91.1, 115.0, 115.2, 125.6, 131.7, 148.9, 154.5, 162.4, 167.7, 173.6, 176.7. HR-MS (FAB) (m/z): calcd for $C_{19}H_{25}O_4N_2S_2$ (M^++1): 409.1256. Found: 409.1238.

2.3. 2-Ethylthiazole-4-carboxylic acid ethyl ester (10)

To a solution of phosphorus pentasulfide (P_4S_{10}; 5.1 g, 11.47 mmol) in ether (40 mL) was added propionamide **8** (8.39 g, 114.7 mmol) and the whole mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with brine and extracted with ether. The organic layer was dried over $MgSO_4$ and evaporated to give a crude **9**. A mixture of the crude **9** and ethyl α-bromopyruvate (22.39 g, 114.5 mmol) in EtOH (100 mL) was stirred at reflux for 15 min. The reaction mixture was evaporated, diluted with AcOEt, and washed with 7% aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated to give a crude oil, which was chromatographed on silica gel (200 g, *n*-hexane/AcOEt=15:1) to afford colorless compound **10** (10.81 g, 51% overall yield from **8**).

Compound **10**. IR (KBr): 1719 cm^{-1}; ^{1}H NMR: δ 1.41 (3H, t, J=7.2 Hz), 1.41 (3H, t, J=7.6 Hz), 3.10 (2H, q, J=7.6 Hz), 4.42 (2H, q, J=7.2 Hz), 8.06 (1H, s). ^{13}C NMR: δ 14.3, 14.4, 27.1, 61.4, 126.8, 146.8, 161.5, 173.8. MS (FAB) m/z: 186 (M^++1).

2.4. 2′-Ethyl[2,4′]bithiazolyl-4-carboxylic acid ethyl ester (13)

A mixture of **10** (6.0 g, 32.38 mmol) and NH_3 saturated EtOH (100 mL) in a sealed tube was stood for 3 days at room temperature. After cooling, the reaction mixture was evaporated to afford a crude amide **11**. To a solution of crude **11** in benzene (100 mL) was added Lawesson's reagent (6.47 g, 16 mmol) and the whole mixture was stirred for 1 h at reflux. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. The organic layer was evaporated to give a crude thioamide **12**. To a solution of the crude thioamide **12** and ethyl α-bromopyruvate (6.05 g, 31 mmol) in absolute EtOH (200 mL) was stirred for 1 h at reflux. The reaction mixture was evaporated, diluted with 7% aqueous $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. The organic layer was evaporated to give a crude residue, which was chromatographed on silica gel (60 g, *n*-hexane/AcOEt=15:1) to afford **13** (4.6 g, 53%). Recrystallization of **13** from *n*-hexane provided colorless needles **13**.

Compound **13**. Mp 82–83 °C; IR (KBr): 1712 cm^{-1}; ^{1}H NMR: δ 1.43 (3H, t, J=7.2 Hz), 1.44 (3H, t, J=6.8 Hz), 3.09 (2H, q, J=7.6 Hz), 4.45 (2H, q, J=7.2 Hz), 8.03 (1H, s), 8.16 (1H, s). ^{13}C NMR: δ 14.0, 14.4, 26.9, 61.5, 116.6, 127.6, 147.9, 147.9, 161.5, 163.6, 173.7. Anal. Calcd for $C_{11}H_{12}N_2O_2S_2$: C, 49.23; H, 4.51; N, 10.44. Found: C, 49.28; H, 4.49; N, 10.45. MS (FAB) m/z: 269 (M^++1).

2.5. 2′-Ethyl[2,4′]bithiazolyl-4-methanol (14)

A mixture of **13** (2.0 g, 7.45 mmol) and $LiBH_4$ (0.492 g, 22.6 mmol) in THF (30 mL) was stirred for 50 min at room temperature. The reaction mixture was diluted with H_2O (10 mL) and the whole was stirred for 5 h at the same temperature. The reaction mixture was extracted with AcOEt and washed with brine, and dried over $MgSO_4$. The organic layer was evaporated to give a crude residue,

which was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=5:1) to afford **14** (1.43 g, 84%). Recrystallization of **14** from *n*-hexane–AcOEt provided colorless needles **14**.

Compound **14**. Mp 94–96 °C; IR (KBr): 3223 cm^{-1}; ^1H NMR: δ 1.43 (3H, d, J=7.6 Hz), 3.08 (2H, q, J=7.6 Hz), 4.82 (2H, s), 7.20 (1H, s), 7.87 (1H, s). ^{13}C NMR: δ 14.1, 26.9, 60.8, 115.2, 115.6, 148.4, 157.1, 163.5, 173.8. Anal. Calcd for $C_9H_{10}N_2OS_2$: C, 47.76; H, 4.45; N, 12.38. Found: C, 47.68; H, 4.51; N, 12.33. MS (FAB) m/z: 227 (M^++1).

2.6. 2′-Ethyl[2,4′]bithiazolyl-4-methyleneiodide (15)

To a mixture of **14** (1.22 g, 5.39 mmol), triphenylphosphine (1.56 g, 5.95 mmol) and imidazole (0.550 g, 8.1 mmol) in THF (25 mL) was added I_2 (1.51 g, 5.94 mmol) under argon atmosphere and the whole mixture was stirred for 30 min at room temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. The organic layer was evaporated to give a crude residue, which was chromatographed on silica gel (20 g, *n*-hexane/AcOEt=30:1) to afford **15** (1.53 g, 84%). Recrystallization of **22** from *n*-hexane provided colorless needles **15**.

Compound **15**. Mp 119–121 °C; IR (KBr): 1533, 1498, 1432 cm^{-1}; ^1H NMR: δ 1.43 (3H, t, J=7.6 Hz), 3.08 (2H, q, J=7.6 Hz), 4.56 (2H, s), 7.25 (1H, s), 7.86 (1H, s). ^{13}C NMR: δ −1.53, 14.1, 26.9, 115.6, 116.7, 148.5, 154.0, 163.1, 173.7. Anal. Calcd for $C_9H_9IN_2S_2$: C, 32.15; H, 2.70; N, 8.33. Found: C, 32.15; H, 2.90; N, 8.00. MS (FAB) m/z: 337 (M^++1).

2.7. 2′-Ethyl[2,4′]bithiazolyl-4-methylenetriphenylphosphonium iodide (4)

A mixture of **15** (1.40 g, 4.16 mmol) and triphenylphosphine (2.74 g, 10.4 mmol) in benzene (14 mL) was stirred for 8 h at reflux. After cooling, the resulting colorless powder **4** (2.46 g, 98%) was obtained by filtration.

Compound **4**. Mp 265–267 °C; ^1H NMR: δ 1.40 (3H, t, J=7.6 Hz), 3.04 (2H, q, J=7.6 Hz), 5.51 (2H, d, J=14 Hz), 7.26 (1H, s), 7.63–7.84 (15H, m), 8.08 (1H, d, J=3.2 Hz). Anal. Calcd for $C_{27}H_{24}IN_2PS_2$: C, 54.18; H, 4.04; N, 4.68. Found: C, 54.35; H, 4.11; N, 4.57. MS (FAB) m/z: 471 (M^+−I).

2.8. Wittig condensation of (+)-3 and 4

To a solution of **4** (0.527 g, 0.88 mmol) in THF (5 mL) was added lithium bis(trimethylisilyl)amide (1 M solution in THF, 0.88 mL, 0.88 mmol) at 0 °C under argon atmosphere and the whole mixture was stirred for 20 min at the same temperature. A solution of (+)-**3** (0.095 g, 0.44 mmol) in THF (2 mL) was added to the above reaction mixture at 0 °C and the whole mixture was stirred for 15 min at the same temperature. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and evaporated to afford a crude product which was chromatographed on silica gel (10 g, *n*-hexane/AcOEt=20:1) to give a mixture ((*E*)/(*Z*)=3:1) of **2**. This mixture was subjected to thin-layer chromatography (silica-gel, *n*-hexane/AcOEt=5:1) to afford (+)-**2** (59.3 mg, 33%) and a colorless oil (+)-**16** (19.8 mg, 11%). Recrystallization of (+)-**2** from *n*-hexane/AcOEt (20:1) provided colorless needles **2**.

Compound (+)-**2**. Mp 121–122 °C; $[\alpha]_D^{21}$=+108.8 (c=1.025, $CHCl_3$); IR (KBr): 3110, 2977, 1710, 1618, 1144, 1079 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.22 (3H, d, J=6.8 Hz), 1.43 (3H, t, J=7.6 Hz), 3.09 (2H, q, J=7.6 Hz), 3.32 (3H, s), 3.60 (3H, s), 3.66 (3H, s), 3.81 (1H, t, J=7.8 Hz), 4.17 (1H, dq, J=7.6, 6.8 Hz), 4.97 (1H, s), 6.41 (1H, dd, J=7.7, 15.7 Hz), 6.57 (1H, d, J=15.7 Hz), 7.08 (1H, s), 7.84 (1H, s). ^1H NMR (CD_3OD): δ 1.26 (3H, d, J=6.8 Hz), 1.46 (3H, t, J=7.6 Hz), 3.12 (2H, q, J=7.6 Hz), 3.37 (3H, s), 3.66 (3H, s), 3.66 (3H, s), 3.83 (1H, t, J=8.4 Hz), 4.23 (1H, dq, J=8.4, 6.8 Hz), 5.08 (1H, s), 6.39 (1H, dd, J=8.4, 15.6 Hz), 6.62 (1H, d, J=15.6 Hz), 7.40 (1H, s), 8.04 (1H, s). ^{13}C NMR (CD_3OD): δ 14.6, 15.1, 27.6, 41.2, 51.2, 56.2, 57.1, 85.8, 92.0, 116.7, 117.0, 126.8, 132.3, 149.4, 155.2, 163.8, 169.3, 175.1, 177.5. $C_{19}H_{24}N_2O_4S_2$: C, 55.86; H, 5.92; N, 6.86. Found: C, 55.85; H, 6.03; N, 6.69. MS (FAB) m/z: 409 (M^++1).

Compound (+)-**16**. $[\alpha]_D^{24}$=+215.9 (c=1.09, $CHCl_3$); IR ($CHCl_3$): 1705, 1620 cm^{-1}; ^1H NMR (CD_3OD): δ 1.22 (3H, d, J=6.8 Hz), 1.41 (3H, t, J=7.6 Hz), 3.08 (2H, q, J=7.6 Hz), 3.29 (3H, s), 3.34 (3H, s), 3.62 (3H, s), 4.16 (1H, dq, J=6.8, 9.6 Hz), 4.96 (1H, s), 5.20 (1H, t, J=9.6 Hz), 5.49 (1H, dd, J=9.6, 12.0 Hz), 6.57 (1H, d, J=12.0 Hz), 7.42 (1H, s), 7.93 (1H, s). ^{13}C NMR (CD_3OD): δ 14.5, 15.2, 27.6, 40.7, 51.2, 55.7, 56.8, 80.1, 91.8, 116.5, 119.7, 126.4, 133.3, 149.8, 154.6, 162.9, 169.5, 175.4, 177.8. HR-MS (FAB) (m/z): calcd for $C_{19}H_{25}O_4N_2S_2$ (M^++1): 409.1256. Found: 409.1276.

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Synthesis of β-pyrrole and β-thiophene substituted 21,23-dithia and 21-monothiaporphyrins

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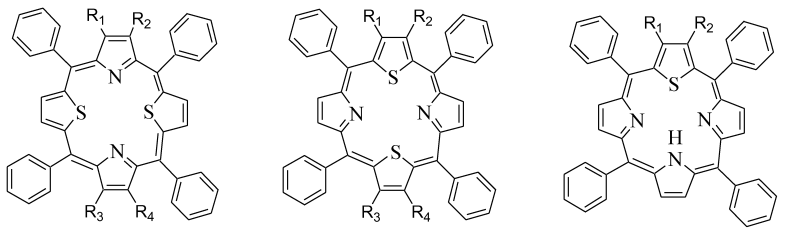
Abstract—A series of β-pyrrole and β-thiophene substituted porphyrins with N_2S_2 and N_3S porphyrin cores were synthesized and characterized. The introduction of substituents at β-pyrrole and β-thiophene carbons resulted in significant shifts in ^{1}H NMR, absorption and fluorescence maxima. These effects were attributed to alteration of the porphyrin ring current caused by substituents at β-positions.

1. Introduction

Porphines have two potential positions: *meso*- and β-positions at which substituents can be introduced. The electronic properties of porphyrins can be tuned at will by introducing suitable substituents at *meso*- or at β-positions. Generally, most of the porphyrins synthesized possess aryl groups at *meso* positions because they can be synthesized easily and they are very stable to handle for various applications.[1] Further introduction of substituents on *meso*-aryl groups does not much alter the electronic properties of the porphyrin ring since the substituents are not in direct conjugation with the porphyrin macrocycle. However, the introduction of substituents at the β-positions alters the electronic properties tremendously since these substituents are in direct conjugation with the porphyrin ring.[2] There are several reports on β-substituted porphyrins with an N_4 core. Both electron withdrawing and electron releasing substituents has been introduced and detailed spectral and electrochemical properties investigated.[3] Porphyrins bearing electron withdrawing substituents such as $-$Br, $-NO_2$ at β-pyrrole carbons have considerable potential as catalysts for epoxidation of alkenes and hydroxylation of alkanes.[4] Although there is extensive literature on β-substitution of porphyrins with an N_4 core, except a couple of reports by us,[5] there is no other report on β-substituted core modified porphyrins. The core-modified porphyrins resulting from the replacement of pyrrole with heterocycles such as thiophene, furan, selenophene and tellurophene have received less attention in spite of their novel properties such as stabilization of metals in unusual oxidation states.[6] We have been interested in the synthesis and the chemistry of new core-modified porphyrins for use in bioorganic and materials chemistry applications[7] and in this paper we



R_1, R_2, R_3, R_4 = Br : **1**

R_1, R_2, R_3, R_4 = Ph : **2**

R_1, R_3 = Ph; R_2, R_4 = NO_2 : **3**

R_1, R_3 = CH_3; R_2, R_4 = H : **4**

R_1, R_2, R_3, R_4 = CH_3 : **5**

R_1, R_3 = Ph; R_2, R_4 = H : **6**

R_1, R_2, R_3, R_4 = Ph : **7**

R_1 = CH_3, R_2 = H : **8**

R_1, R_2 = CH_3 : **9**

R_1 = Ph, R_2 = H : **10**

R_1, R_2 = Ph : **11**

Chart 1.

Keywords: β-Substituted porphyrins; Core-modified porphyrins; Bathochromic shifts; Non-planarity.
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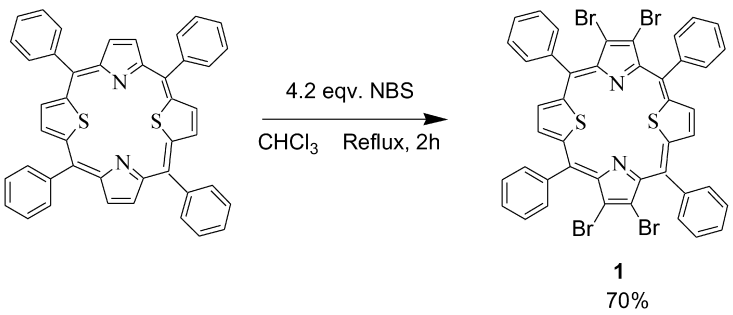


Scheme 1. Synthetic scheme for β-pyrrole substituted 21,23-dithiaporphyrin **1**.

report the synthesis and characterization of a series of β-substituted thiaporphyrins with N_2S_2 and N_3S porphyrin cores. The thiaporphyrins have two types of β-positions: β-pyrroles and β-thiophenes at which the substituents can be introduced. We successfully synthesized β-substituted thiaporphyrins in which the substituents were introduced at β-pyrrole as well as β-thiophene carbons independently (Chart 1) and investigated the effect of substituents on the spectral properties of the porphyrins.

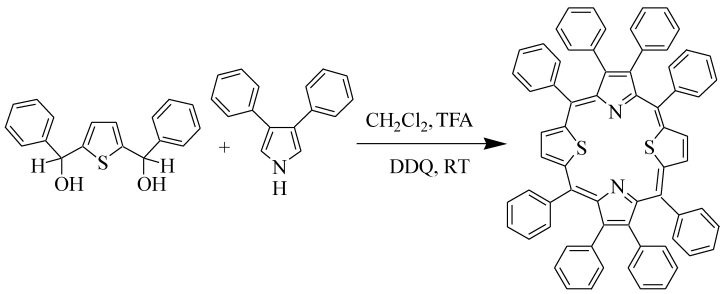
2. Results and discussion

2.1. β-Pyrrole substituted 21,23-dithiaporphyrins

Our initial goals were to introduce bromines at β-pyrrole carbons and use them as synthons for the synthesis of other β-substituted dithiaporphyrins via Suzuki coupling. Thus, we investigated the possibility of tetrabrominating the 5,10,15,20-tetraphenyl-21,23-dithiaporphyrin (S_2TPP). Treatment of S_2TPP with 4.2 equiv. of *N*-bromosuccinimide in chloroform gave (β-Br)$_4S_2$TPP, **1**, in 70% yield (Scheme 1). The tetrabrominated porphyrin, **1**, was purified by silica gel column using dichloromethane and characterized with ^{1}H NMR, mass, absorption and emission spectroscopies. The absence of the pyrrole signal in ^{1}H NMR indicated that the hydrogens were replaced by bromines at the β-positions of two pyrrole rings. The β-thiophene protons appeared as singlet indicating the symmetric nature of **1**. The thiophene protons in **1** experienced an upfield shift of 0.26 ppm compared to S_2TPP due to the change in ring current caused by the bromines at β-pyrrole carbons. The mass showed characteristic M^+ and $M-Br_n$ (n=1–4) peaks. The introduction of bromines at β-pyrrole carbons resulted in large red shifts and broadening of absorption bands (Table 1). This is because of the presence of bulky substituents at the β-pyrrole carbons which reduce the energy gap between HOMO and LUMO. The emission bands of **1** also experienced similar red shifts compared to S_2TPP. However, **1** was more weakly fluorescent than S_2TPP because of the presence of heavy halogens at the β-pyrrole carbons. To introduce aryl groups at β-pyrrole carbons, **1** was reacted with substituted phenyl boronic acids under Suzuki coupling conditions. This resulted in a complicated mixture of products and no attempts were made to separate and identify the products. We then diverted our attention to synthesize 3,4-disubstituted pyrroles for using them to synthesize β-substituted porphyrins. The 3,4-disubstituted pyrrole synthesis available in the literature is generally a multi-step process with low yields. However, Ono et al.[8] recently reported an easy and faster method of synthesizing 3,4-disubstituted pyrroles and then converting them to β-substituted porphyrins. Thus we prepared 3,4-diphenyl pyrrole following their method and condensed it with 2,5-bis(α-hydroxymethylphenyl)thiophene using $BF_3{\cdot}OEt_2$ in chloroform to obtain (β-Ph)$_4S_2$TPP, **2**, in 25% yield (Scheme 2). Ono et al.[8] reported that 3,4-diphenyl pyrrole was not reactive enough to form porphyrins with aromatic aldehydes. Hence they formylated and reduced it to the hydroxymethyl group and tetramerized it under acidic conditions to form porphyrins. However, in the present case the 3,4-diphenyl pyrrole reacted easily. The reactive functionality of 2,5-bis(hydroxymethyphenyl)thiophene[9] helped in the formation of **2** under normal porphyrin

Table 1. Absorption and emission data of porphyrins **1**–**11** along with S_2TPP and STPPH in toluene

Porphyrin	Soret band λ_{max} (nm) ($\varepsilon\times10^{-4}$)	Absorption Q-bands, λ_{max} (nm) ($\varepsilon\times10^{-4}$)				Fluorescence λ_{max} (λ_{ex}=440 nm)		
		IV	III	II	I	$Q_{(0,0)}$	$Q_{(0,1)}$	ϕ
S_2TPP	435 (25.0)	519 (2.60)	547 (0.70)	633 (0.22)	696 (0.45)	706	781	0.0076
1	440 (19.2)	521 (1.9)	—	633 (0.15)	698 (0.22)	740	—	$<10^{-4}$
2	440 (21.2)	519 (1.9)	—	637 (0.22)	700 (0.31)	735	—	0.0009
3	449 (18.1)	530 (1.70)	—	649 (0.18)	717 (0.23)	802	—	$<10^{-4}$
4	438 (17.5)	520 (2.07)	—	637 (0.13)	701 (0.32)	706	—	0.0015
5	446 (16.9)	527 (1.80)	—	646 (0.25)	714 (0.31)	744	—	0.0005
6	444 (13.0)	524 (1.37)	—	640 (0.13)	707 (0.21)	722	—	0.0020
7	454 (2.90)	524 (0.69)	—	654 (0.17)	715 (0.12)	736	—	0.0003
STPPH	429 (18.7)	513 (1.71)	547 (0.44)	618 (0.19)	678 (0.30)	678	760	0.0168
8	428 (23.7)	513 (2.07)	546 (sh)	614 (0.32)	674 (0.34)	682	—	0.0044
9	430 (15.9)	516 (1.43)	550 (sh)	618 (0.21)	678 (0.41)	693	—	0.0025
10	433 (14.3)	517 (1.37)	549 (sh)	619 (0.17)	680 (0.19)	688	—	0.0036
11	433 (17.2)	517 (2.65)	549 (sh)	619 (0.46)	679 (0.42)	693	—	0.0014



Scheme 2. Synthetic scheme for β-pyrrole substituted 21,23-dithiaporphyrin **2**.

forming conditions. The porphyrin **2** was characterized by ^{1}H NMR, mass, absorption and emission spectroscopies. In ^{1}H NMR, the pyrrole protons were absent as expected. The thiophene protons were also upfield shifted by 0.44 ppm compared to S_2TPP.[6] The product was further confirmed by the presence of the molecular ion peak in the mass spectrum. The absorption (Fig. 1) and emission bands (Fig. 2) were broadened and also red shifted compared to S_2TPP[6] (Table

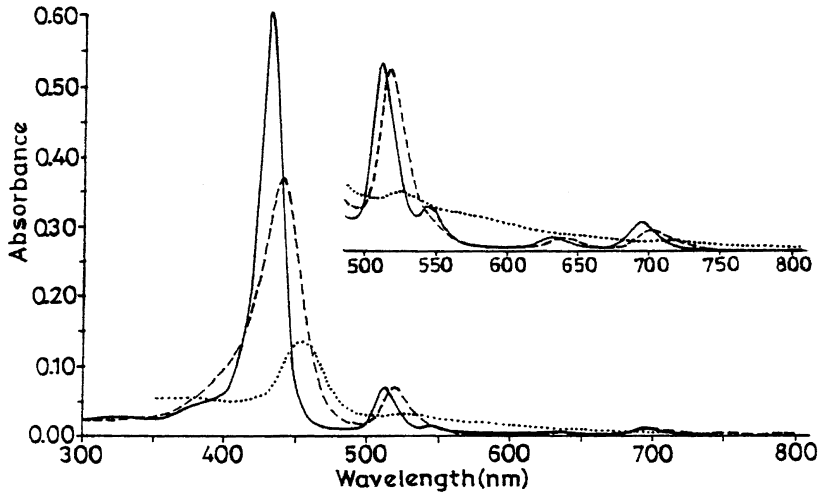


Figure 1. UV–visible spectra S_2TPP (—), **2** (- - -) and **7** (···) recorded in toluene.

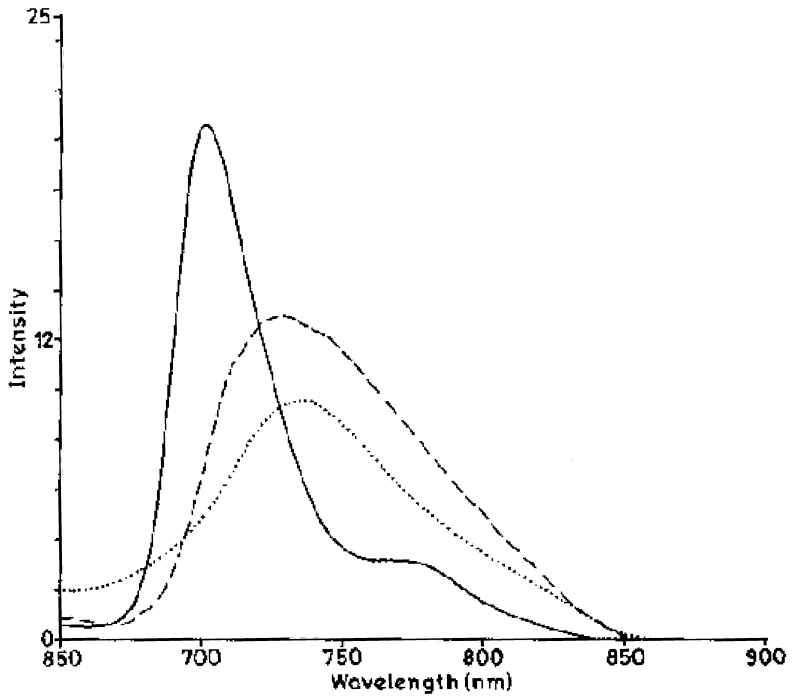
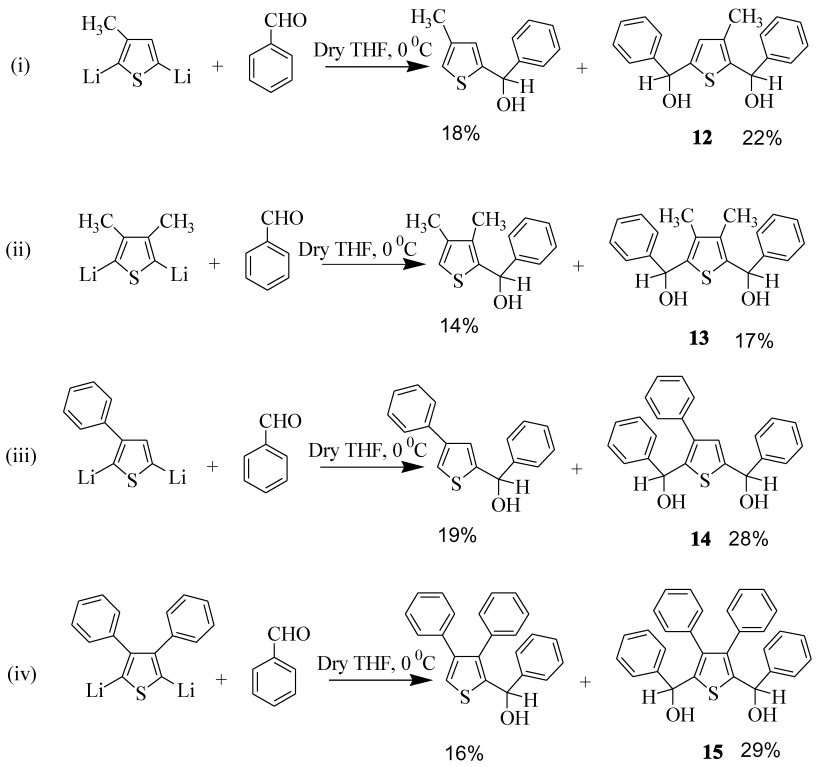


Figure 2. Fluorescence spectra of S_2TPP (—), **2** (- - -) and **7** (···) recorded in toluene at λ_{ex}=440 nm.

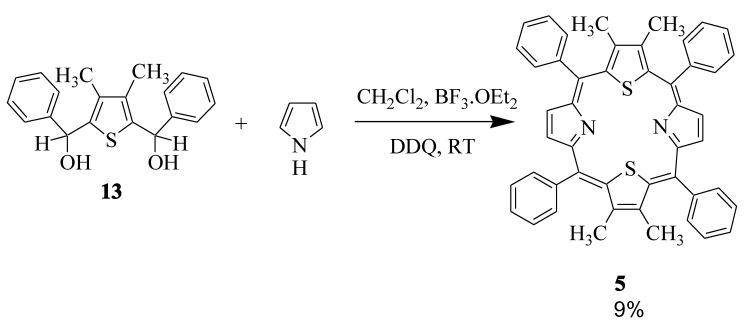


Scheme 3. Synthetic scheme for diols **12**, **13**, **14** and **15**.

1) indicating that the phenyl substituents at β-pyrrole carbons alter the energy levels. The molar absorption coefficients and fluorescence yields of **1** and **2** were reduced compared to S_2TPP. Similarly we prepared 3-phenyl-4-nitropyrrole following Ono's method[8] and condensed it with 2,5-bis(α-hydroxymethylphenyl) thiophene in the presence of $BF_3{\cdot}OEt_2$ in chloroform to obtain (β-Ph)(β-NO_2)S_2TPP, **3**, in 4.8% yield. The crude porphyrin **3** was purified by silica gel column chromatography using dichloromethane as an eluent. The ^{1}H NMR spectrum of **3** varied with the concentration since porphyrins with electron withdrawing substituents have a high tendency to aggregate. The mass spectrum showed an M^+ ion peak, hence confirming the product. The absorption and emission bands of **3** were broader and also red shifted with lower absorption coefficients and quantum yields than **1** and **2**.

2.2. β-Thiophene substituted 21,23-dithiaporphyrins

The substituents at the β-pyrrole carbons altered the porphyrin ring current which was reflected in the observed spectral properties. However, there is no report to understand the effect of substituents on ring current if the substituents are present at β-thiophene carbons instead of β-pyrrole carbons. Our preliminary study[5c] indicated that the substituents at β-thiophene carbons alter the porphyrin ring more effectively when compared to the same substituents at the β-pyrrole carbons. Thus we synthesized N_2S_2 porphyrins **4** and **5** containing two and four methyl groups, respectively, at the β-thiophene carbons. The required thiophene precursors, 3-methyl thiophene and 3,4-dimethyl thiophene were synthesized following known methodology.[10] The unknown diols **12** and **13** were synthesized as shown in Scheme 3 following Ulman and Manassen's method.[9] The 3-methyl or 3,4-dimethyl thiophene was first reacted with *n*-butyl lithium in *n*-hexane in the presence of TMEDA, followed by treatment with benzaldehyde in THF at 0 °C. The tlc analysis indicated the formation of diol **12** or **13** with small amounts of mono-ol and unreacted benzaldehyde. The diols **12** and **13** were purified by silica gel column chromatography using petroleum ether/ethyl acetate and to afford **12** as a semi-white solid in 22% and **13** as a



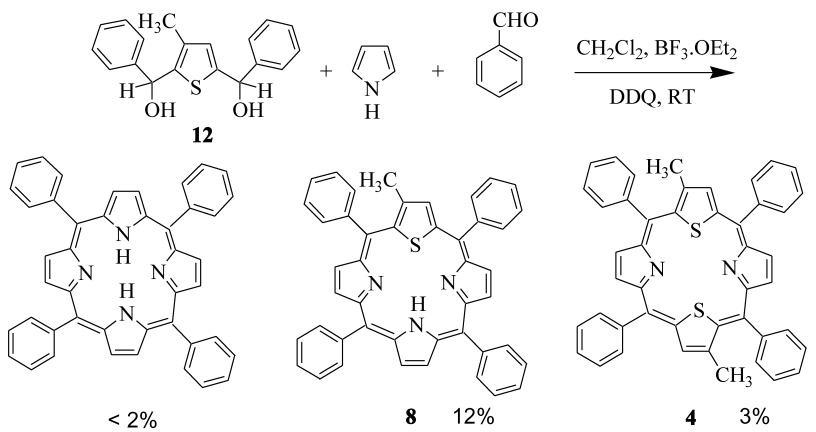
Scheme 4. Synthetic scheme for β-thiophene substituted 21,23-dithiaporphyrin **5**.

crystalline white solid in 17% yield. The N_2S_2 porphyrins **4** and **5** were synthesized by condensing the appropriate diol **12** and **13**, respectively, with pyrrole in CH_2Cl_2 in the presence of $BF_3{\cdot}OEt_2$ at room temperature, followed by oxidation with DDQ (Scheme 4). The crude porphyrins **4** and **5** were purified by silica gel column chromatography using petroleum ether/CH_2Cl_2 and the pure crystalline purple solids of porphyrins **4** and **5** were obtained in 8–9% yields. The porphyrins **4** and **5** were characterized by NMR, mass, absorption and fluorescence spectroscopies. The ^{1}H NMR spectrum of **4** in which one methyl group is present at the β-thiophene carbons showed a very interesting feature. The two thiophene protons, one on each thiophene of **4**, appeared as a singlet which was upfield shifted compared to S_2TPP.[6] Unlike in S_2TPP where pyrrole protons appear as a singlet, the pyrrole protons of **4** appeared as four separate signals: two doublets and two singlets indicating that the protons were not equivalent. The pyrrole protons of **4** were upfield shifted compared to S_2TPP. In porphyrin **5**, the thiophene protons disappeared due to the substitution of thiophene hydrogens by methyl groups. The pyrrole protons in **5** appeared as singlet and upfield shifted than pyrrole protons of S_2TPP. The absorption and emission bands of **4** and **5** were shifted bathochromically with low absorption coefficients and fluorescence yields compared to S_2TPP (Table 1). However, the magnitude of red shifts was larger for **5** than **4** due to the presence of more methyl groups at the β-thiophene carbons in **5** than in **4**.

The porphyrins with phenyl groups substituted at β-thiophene carbons **6** and **7** were synthesized using corresponding unknown diols **14** and **15**, respectively. The diols **14** and **15** were synthesized using 3-phenylthiophene and 3,4-diphenylthiophene, respectively, under identical *n*-butyl lithium conditions mentioned for diols **12** and **13** (Scheme 3). The diols **14** and **15** were purified by silica gel column chromatography and afforded as white solids in 28–29% yields. To prepare porphyrins **6** and **7**, 1 equiv. of diol **14** and **15**, respectively, was condensed with 1 equiv. of pyrrole under mild acidic conditions followed by oxidation with DDQ. The crude porphyrinic mixture was subjected twice to silica gel column chromatography to provide **6** in 4.8% and **7** in 2.3% yields as purple solids. The porphyrins **6** and **7** were confirmed by ^{1}H NMR and the molecular ion peak in mass spectra. In ^{1}H NMR, porphyrin **6** showed a singlet for thiophene protons which was upfield shifted by 0.11 ppm compared to S_2TPP. Porphyrin **7** did not show any thiophene signal as expected due to the presence of phenyl groups at β-thiophenes. The pyrrole protons in **6** appeared as three separate signals because of the unsymmetric substitution pattern whereas the pyrrole protons in **7** appeared as singlet, due to the symmetric substitution. Furthermore, the pyrrole protons in **7** experienced the maximum upfield shifts compared to any other β-substituted 21,23-dithiaporphyrin reported in this paper. This suggests that the four phenyl groups at β-thiophene carbons in **7** altered the porphyrin ring current more effectively compared to 21,23-dithiaporphyrins having four phenyl substituents at β-pyrrole carbons or any other β-substituted 21,23-dithiapophyrins. The absorption (Fig. 1) and emission bands (Fig. 2) of **7** also exhibited maximum red shifts (Table 1) and reduction in absorption coefficients and fluorescence yield.

2.3. β-Thiophene substituted 21-monothiaporphyrins

The thiophene diols **12** and **13** were also used to synthesize β-thiophene substituted 21-monothiaporphyrins **8** and **9**, respectively. The condensation of 1 equiv. of **12** with 2 equiv. of benzaldehyde and 3 equiv. of pyrrole gave a crude porphyrin mixture (Scheme 5). The tlc analysis showed the formation of three porphyrins: *meso*-5,10,15,20-tetraphenyl-β-2,12-dimethyl-21,23-dithiaporphyrin (N_2S_2 porphyrin **4**), the required *meso*-5,10,15,20-tetraphenyl-β-2-methyl-21-monothiaporphyrin (N_3S porphyrin **8**) and *meso*-5,10,15,20-tetraphenyl porphyrin (N_4 porphyrin). The mixture of three porphyrins was separated by silica gel column chromatography and the desired N_3S porphyrin **8** was collected as the second band using petroleum ether/CH_2Cl_2 in 12% yield. Similarly the condensation of 1 equiv. of diol **13** with 2 equiv. of benzaldehyde and 3 equiv. of pyrrole followed by column chromatography gave porphyrin **9** in 12% yield. Both porphyrins **8** and **9** were characterized by NMR, mass, absorption and fluorescence spectroscopic techniques. The thiophene proton of **8** in ^{1}H NMR appeared as a singlet and it was upfield shifted by 0.35 ppm compared to β-unsubstituted *meso*-5,10,15,20-tetraphenyl-21-monothiaporphyrin (STPPH).[6] The thiophene protons of **9** were absent as expected. The pyrrole protons of **8** and **9** appeared as five and three separate signals, respectively, and they were slightly upfield shifted compared to STPPH. The absorption spectra of porphyrins **8** and **9**



Scheme 5. Synthetic scheme for β-thiophene substituted 21- monothiaporphyrin **8**.

showed four Q-bands and one Soret band with negligible shifts in peak maxima compared to STPPH (Table 1).

Porphyrins **10** and **11** were synthesized similarly by condensing 1 equiv. of diol **14** and **15**, respectively, with 2 equiv. of benzaldehyde and 3 equiv. of pyrrole under porphyrin forming conditions. The crude porphyrins were subjected to silica gel column chromatography and pure porphyrins **10** and **11** were obtained in ~6% yields. In ^{1}H NMR, the thiophene proton appeared as singlet in **10** and it was absent in **11** due to substitution by phenyl groups. The pyrrole protons appeared as three signals in both **10** and **11** indicating the low symmetric nature of porphyrins. The absorption and emission peaks also experienced slight red shifts with a reduction in intensity compared to STPPH (Table 1).

3. Conclusions

In conclusion, we have prepared a series of β-pyrrole and β-thiophene substituted thiaporphyrins with N_2S_2 and N_3S porphyrin cores. The introduction of substituents at β-pyrrole and β-thiophene carbons alters the electronic properties of the porphyrins. The magnitude of the effect depends on the number of substituents. The substituents at β-positions of thiaporphyrins cause upfield shifts of thiophene and pyrrole protons in ^{1}H NMR, bathochromic shifts in absorption bands, and large red shifts with reduction in quantum yields of fluorescence bands compared to unsubstituted thiaporphyrin counterparts. We are presently investigating the metallation and electrochemical properties of these porphyrins.

4. Experimental

4.1. Data for compounds

4.1.1. *meso*-5,10,15,20-Tetraphenyl-β-7,8,17,18-tetrabromo-21,23-dithiaporphyrin (1). 5,10,15,20-Tetraphenyl-21,23-dithiaporphyrin (80 mg, 0.123 mmol) was dissolved in 20 ml of $CHCl_3$ in 100 ml one-necked round bottomed flask and 4.2 equiv. of freshly recrystallized *N*-bromosuccinimide (336 mg, 0.516 mmol) was added to it. The reaction mixture was refluxed for 2 h. The progress of the reaction was monitored with tlc and absorption spectroscopy. The solvent was removed on a rotary evaporator and the crude compound was purified by silica gel column chromatography using CH_2Cl_2 as eluent (74 mg, 70%). Mp >300 °C ^{1}H NMR ($CDCl_3$, δ in ppm) 7.80 (m, 12H, Ar), 8.06 (m, 8H, Ar), 9.39 (s, 4H, β-thiophene). ^{13}C NMR ($CDCl_3$, δ in ppm) 14.21, 29.45, 32.01, 113.03, 127.92, 133.62, 140.09, 149.30. LD-MS $C_{44}H_{24}N_2S_2Br_4$ calcd mass, 964.4. Obsd *m*/*z* 964.7 (M^+), 885.7 (M−1Br), 805.9 (M−2Br), 725.2 (M−3Br), 645 (M−4Br). Anal. calcd: C, 54.80; H, 2.51; N, 2.90. Found: C, 54.93; H, 2.42; N, 2.78. IR (neat, ν (cm^{-1})) 3276, 2934, 2861, 741.

4.1.2. *meso*-5,10,15,20-Tetraphenyl-β-7,8,17,18-tetraphenyl-21,23-dithia-porphyrin (2). 2,5-Bis(α-hydroxyphenylmethyl)thiophene (100 mg, 0.337 mmol) and 3,4-diphenylpyrrole (80 mg, 0.365 mmol) were dissolved in 25 ml CH_2Cl_2 in a 100 ml one-necked round bottomed flask fitted with an argon bubbler. Trifluoroacetic acid (96.3 μl) was added to initiate the condensation. The reaction mixture was stirred under argon at room temperature. The progress of the reaction was monitored using absorption spectroscopy. Aliquots of the reaction mixture were taken at regular intervals and oxidized with DDQ and the formation of the porphyrin was checked using absorption spectroscopy. The reaction was very slow and it took 10 h for completion. DDQ (100 mg, 0.440 mmol) was then added and the reaction mixture was stirred in air for 2 h. The solvent was removed under vacuum and the crude compound was dissolved in minimum amount of CH_2Cl_2 to prepare slurry powder by adding silica gel. The slurry was loaded on a silica gel column and eluted with CH_2Cl_2. The desired compound eluted very slowly and was collected and subjected to second silica gel column chromatography using CH_2Cl_2 as solvent to afford **2** in 25% yield. Mp >300 °C. ^{1}H NMR ($CDCl_3$, δ in ppm) 6.90 (m, 8H, Ar), 7.06 (m, 4H, Ar), 7.21 (m, 8H, Ar), 7.52 (m, 8H, Ar), 7.71 (m, 8H, Ar), 7.80 (m, 4H, Ar), 9.23 (m, 4H, β-thiophene). LD-MS $C_{68}H_{44}N_2S_2$ calcd mass, 953.2. Obsd *m*/*z* 953.9 (M^+). Anal. calcd: C, 85.68; H, 4.65; N, 2.94. Found: C, 85.84; H, 4.56; N, 3.03. IR (KBr, ν (cm^{-1})) 3420, 3230, 2963, 741.

4.1.3. *meso*-5,10,15,20-Tetraphenyl-β-7,17-diphenyl-β-8,18-dinitro-21,23-dithia porphyrin (3). In a 500 ml one-necked round bottomed flask, 2,5-bis(hydroxyphenylmethyl) thiophene (500 mg, 1.687 mmol) and 2-nitro-3-phenylpyrrole (400 mg, 2.123 mmol) were dissolved in 250 ml $CHCl_3$ and argon was purged for 10 min. $BF_3{\cdot}OEt_2$ (660 ml of 2.5 M stock solution) was added and the reaction mixture was stirred for 1 h under argon at room temperature. The formation of porphyrin was confirmed with absorption spectroscopy by taking small amounts of reaction mixture and oxidizing with DDQ in toluene. DDQ (425 mg, 1.872 mmol) was added and the reaction mixture was stirred in air for 2 h. The solvent was removed on a rotary evaporator and the crude compound was purified twice by silica gel column chromatography using CH_2Cl_2 as solvent to afford a dark purple solid (35 mg, 4.6%). Mp >300 °C ^{1}H NMR ($CDCl_3$, δ in ppm) 7.10–7.30 (m, 30H, Ar), 7.50–7.70 (m, 4H, β-thiophene). LD-MS $C_{56}H_{34}N_4S_2O_4$ calcd mass 891.0, obsd *m*/*z* 892.7. Anal. calcd: C, 75.49; H, 3.85; N, 6.29. Found: C, 75.86; H, 3.79; N, 6.58; IR (KBr, ν (cm^{-1})) 3414, 2915, 1550, 741.

4.1.4. 2,5-Bis(hydroxymethylphenyl)-3-methylthiophene (12). In a 100 ml three-necked round bottomed flask fitted with a gas inlet tube, a reflux condenser and a rubber septum, dried and distilled *n*-hexane (10 ml) was placed. *N*,*N*′,*N*″,*N*‴-Tetramethylethylene diamine (TMEDA) (0.42 ml, 0.32 g, 2.76 mmol) and *n*-butyl lithium (4 ml of a 1.6 M solution in hexane) were injected into the stirred solution. 2-Methylthiophene (0.200 g, 2.04 mmol) was injected and the solution was refluxed gently for 1 h. In another three-necked 100 ml round bottomed flask fitted with gas inlet and outlet tube and septum, benzaldehyde (0.53 ml, 0.550 g, 5.20 mmol) was dissolved in 10 ml dry THF. The solution was cooled in an ice bath and nitrogen was bubbled for 15 min. The 2,5-dilithio-3-methylthiophene suspension from the first flask was added to

benzaldehyde solution in THF through siphon apparatus over a period of 10 min. After the addition was complete, the mixture was allowed to attain room temperature. An ice cold saturated NH_4Cl solution was added and the mixture was then extracted with ether (3×50 ml). The organic layers were combined, washed with brine and dried over anhydrous Na_2SO_4. The crude product obtained on evaporation of the solvent was subjected to silica gel column chromatography using petroleum ether/ethyl acetate mixture (4:1), which afforded the pure diol **12** as a semi-white solid (140 mg, 22%). Mp 125–127 °C. ^{1}H NMR ($CDCl_3$, δ in ppm) 2.10 (s, 3H, CH_3), 2.42 (s, 2H, OH), 5.51 (s, 2H, CH), 7.18 (1H, thiophene) 7.32–7.51 (m, 10H, phenyl). ^{13}C NMR ($CDCl_3$, δ in ppm) 65.15, 70.70, 79.73, 126.81, 128.95, 139.59. ES-MS $C_{19}H_{18}O_2S$ calcd av. mass, 310.4. Obsd *m/z* 293.0 (M−OH). Anal calcd: C, 73.52; H, 5.84. Found: C, 73.97; H, 6.03. IR (KBr, ν (cm^{-1})) 3395 (OH), 2925, 1715, 1649, 1458, 1268, 1185, 1008, 750, 696, 530.

4.1.5. 2,5-Bis(hydroxymethylphenyl)-3,4-dimethylthiophene (13). 2,5-Dilithio-3,4-dimethyl thiophene was synthesized by lithiating 3,4-dimethyl thiophene (500 mg, 4.46 mmol) with *n*-butyl lithium (8.0 ml of 1.6 M solution in hexane) in dry hexane (25 ml) in the presence of TMEDA (1.6 ml, 1.24 g, 10.7 mmol) under similar reaction conditions as mentioned for compound **12**. The dilithiated salt of 3,4-dimethyl thiophene was then added to the solution of benzaldehyde (1.20 ml, 1.24 g, 11.6 mmol) in dry THF (25 ml) followed by similar work-up as mentioned for compound **12** gave crude diol **13**. Column chromatography on silica gel using petroleum ether/ethyl acetate mixture (4:1) and afforded pure diol **13** as a white solid (248 mg, 17%). Mp 141–143°C. ^{1}H NMR ($CDCl_3$, δ in ppm) 2.05 (s, 6H, $-CH_3$), 2.32 (s, 2H, −OH), 6.01 (s, 2H, −CH), 7.24–7.41 (m, 10H, phenyl). ^{13}C NMR ($CDCl_3$, δ in ppm) 13.11, 71.03, 126.35 127.71, 128.38, 134.32, 139.63, 142.68. FAB-MS $C_{20}H_{20}O_4S$ calcd av. mass, 324.4. Obsd *m/z* 324.0. Anal calcd: C, 74.04; H, 6.21. Found: C, 74.36; H, 6.53. IR (KBr, ν (cm^{-1})) 3293 (OH), 3062, 3027, 2915, 2860, 1493, 1452, 1198, 1080, 1007, 914, 852, 697, 582.

4.1.6. 2,5-Bis(hydroxymethylphenyl)-3-phenylthiophene (14). 2,5-Dilithio-3-phenyl thiophene was synthesized by stirring 3-phenyl thiophene (0.320 g, 2.00 mmol) with *n*-butyl lithium (4.0 ml of 1.6 M solution in hexane) in dry diethylether (15 ml) at room temperature. The dilithiated salt of 3-phenyl thiophene was added to the solution of benzaldehyde (0.460 ml, 0.439 g, 4.14 mmol) in dry THF (15 ml) followed by similar work-up as mentioned for compound **12** gave crude diol **14**. Column chromatography on silica gel using petroleum ether/ethyl acetate mixture (3:1) as eluent afforded pure **14** as a white solid (210 mg, 28%). Mp 128–130 °C ^{1}H NMR ($CDCl_3$, δ in ppm) 2.40 (s, 2H, −OH), 5.93 (d, *J*=7.2 Hz, 1H −CH), 6.04 (s, 1H, −CH), 6.75 (s, 1H, β-thiophene), 7.30–7.61 (m, 15H, phenyl). ^{13}C NMR ($CDCl_3$, δ in ppm) 19.60, 49.30, 59.52, 97.06, 128.05, 129.71, 129.97, 130.79, 131.53. ES-MS $C_{24}H_{20}O_2S$ calcd av. mass, 372.4. Obsd *m/z* 355.1 (M−OH). Anal calcd: C, 77.39; H, 5.41. Found: C, 77.52; H, 5.76. IR (KBr, ν (cm^{-1})) 3314 (OH), 3057, 3028, 2876, 1600, 1495, 1447, 1285, 1194, 1155, 1008, 847, 700, 533.

4.1.7. 2,5-Bis(hydroxymethylphenyl)-3,4-diphenylthiophene (15). 3,4-Diphenyl thiophene (0.236 g, 1.00 mmol) and *n*-butyl lithium (2 ml of a 1.6 M solution in hexane) were stirred in dry diethylether (10 ml) for 1 h at room temperature to get the dilithio salt of 3,4-diphenyl thiophene. The dilithiated salt of 3,4-diphenyl thiophene was added to the solution of benzaldehyde (0.230 ml, 0.243 g, 2.30 mmol) in 10 ml THF solution followed by similar work as mentioned for compound **12**. Column chromatography on silica gel using petroleum ether/ethyl acetate mixture (3:1) afforded pure **15** as a white solid (0.130 g, 29%). Mp 130–131 °C. ^{1}H NMR ($CDCl_3$, δ in ppm) 2.32 (s, 2H, OH), 5.95 (s, 2H, CH), 7.04 (t, *J*=2.2 Hz, 4H, phenyl), 7.25–7.35 (m, 16H, phenyl). ^{13}C NMR ($CDCl_3$, δ in ppm) 70.80, 126.28, 127.04, 127.23, 127.73, 127.92, 128.38, 130.20, 135.48, 143.10. ES-MS $C_{30}H_{24}O_2S$ calcd av. mass, 448.5. Obsd *m/z* 449.0. Anal calcd: C, 80.33; H, 5.39. Found: C, 80.56; H, 5.44. IR (KBr, ν (cm^{-1})) 3348 (OH), 3060, 3028, 2920, 1602, 1492, 1449, 1274, 1133, 1010, 912, 768, 731, 700, 640.

4.1.8. *meso*-5,10,15,20-Tetraphenyl-β-2,12-dimethyl-21,23-dithiaporphyrin (4). A stream of argon was passed through dichloromethane (30 ml) in a 100 ml round-bottomed flask for 10 min. The diol **12** (0.100 g, 0.320 mmol) and pyrrole (25 μl, 0.360 mmol) were added and argon purging was continued for an additional 10 min. $BF_3{\cdot}OEt_2$ (20 μl of 2.5 M stock solution in CH_2Cl_2) was added and the reaction mixture was stirred for 1 h. The progress of the reaction was monitored by TLC and absorption spectroscopy. After 2 h stirring, DDQ (50 mg, 0.220 mmol) was added and the reaction was stirred in air for additional 1 h. The solvent was removed in vacuo and the crude compound was purified by silica column using petroleum ether/CH_2Cl_2 (3:7) mixture as eluent. The desired compound **4** was obtained as a purple solid (18 mg, 8.6%). Mp >300 °C. ^{1}H NMR ($CDCl_3$, δ in ppm) 2.87 (s, 6H, CH_3), 7.72–7.80 (m, 12H, *m*,*p*-phenyl), 8.03–8.06 (m, 4H, *o*-phenyl), 8.20–8.22 (m, 4H, *o*-phenyl), 8.37 (s, 1H, β-pyrrole.), 8.44 (d, *J*=4.57 Hz, 1H, β-pyrrole), 8.56 (d, *J*=4.39 Hz, 1H, β-pyrrole), 8.63 (s, 1H, β-pyrrole), 9.40 (s, 2H, β-thiophene). ^{13}C NMR ($CDCl_3$, δ in ppm) 20.29, 29.78, 42.90, 127.71, 128.69, 133.42, 134.42, 134.38, 139.54. FAB-MS $C_{46}H_{32}N_2S_2$ calcd av. mass, 678.8. Obsd *m/z* 677.0. Anal. calcd: C, 81.62; H, 4.77; N, 4.14. Found: C, 81.91; H, 4.53; N, 3.89. IR (KBr, ν (cm^{-1})) 3396, 2922, 2857, 703.

4.1.9. *meso*-5,10,15,20-Tetraphenyl-β-2,3,12,13-tetramethyl-21,23-dithiaporphyrin (5). A solution of diol **13** (0.100 g, 0.306 mmol) and pyrrole (25 μl, 0.360 mmol) in 30 ml CH_2Cl_2 was treated with $BF_3{\cdot}OEt_2$ (10 μl of 2.5 M stock solution) under an argon atmosphere to initiate the condensation. After 1 h, DDQ (0.040 g, 0.176 mmol) was added and stirring was continued for an additional 1 h. Column chromatography on silica with petroleum ether/CH_2Cl_2 (3:7) gave solid purple colored porphyrin **5** (20 mg, 9%). Mp >300 °C ^{1}H NMR ($CDCl_3$, δ in ppm) 2.61 (s, 12H, CH_3), 7.74 (m, 12H, phenyl), 8.12 (m, 8H, phenyl), 8.26 (s, 4H, β-pyrrole). ^{13}C NMR ($CDCl_3$, δ in ppm) 18.44, 29.87, 32.10, 127.23, 127.35, 132.19, 133.75, 143.07, 144.67. ES-MS $C_{48}H_{36}N_2S_2$ calcd av. mass: 704.9. Obsd *m/z* 704.8. Anal. calcd: C, 81.78; H, 5.15; N, 3.97. Found: C, 81.42, H, 5.27, N, 3.95. IR (KBr, ν (cm^{-1})) 3435, 3059, 2929, 703.

4.1.10. *meso*-5,10,15,20-Tetraphenyl-β-2,12-diphenyl-21,23-dithiaporphyrin (6). In a 100 ml round bottomed flask, thiophene diol **14** (0.200 g, 0.534 mmol) and pyrrole (40 μl, 0.038 g, 0.56 mmol) were mixed in 50 ml CH_2Cl_2 under an argon atmosphere. $BF_3{\cdot}OEt_2$ (40 μl of 2.5 M stock solution) was added and the reaction was stirred for 1 h at room temperature. DDQ (90 mg, 0.396 mmol) was added and the reaction mixture was stirred in air for an additional 1 h. The solvent was removed on a rotary evaporator and the crude compound was purified twice using silica gel column chromatography with CH_2Cl_2 as solvent to afford **6** as a purple solid (21 mg, 4.8%). Mp >300 °C. ^{1}H NMR ($CDCl_3$, δ in ppm) 7.28–7.34 (m, 4H, Ar), 7.42–7.58 (m, 6H, Ar), 7.72–7.84 (m, 12H, Ar), 8.21–8.38 (m, 8H, Ar), 8.48 (d, *J*=5.20 Hz, 2H, β-pyrrole), 8.53 (d, *J*=4.80 Hz, β-pyrrole), 8.66 (s, 1H, β-pyrrole), 9.56 (s, 2H, β-thio-phene). ^{13}C NMR ($CDCl_3$, δ in ppm) 29.89, 127.98, 128.33, 129.14, 129.51, 130.06, 131.18, 133.37, 134.69, 135.60, 136.44, 140.41. ES-MS $C_{56}H_{36}N_2S_2$ calcd av. mass, 801.0. Obsd *m/z* 801.3. Anal. calcd: C, 83.97; H, 4.53; N, 3.50: Found: C, 83.65; H, 4.67; N, 3.77. IR (KBr, ν (cm^{-1})) 3414, 3059, 2929, 697.

4.1.11. *meso*-5,10,15,20-Tetraphenyl-β-2,3,12,13-tetraphenyl-21,23-dithiaporphyrin (7). In a 100 ml round bottomed flask, diol **15** (0.200 g, 0.446 mmol) and pyrrole (40 μl, 0.038 g, 0.5 mmol) were dissolved in 45 ml CH_2Cl_2 and argon was purged for 10 min. $BF_3{\cdot}OEt_2$ (40 μl of 2.5 M stock solution) was added and the reaction was stirred for 1 h under argon at room temperature. DDQ (80 mg, 0.352 mmol) was added and the reaction mixture was stirred for 1.5 h in air. The solvent was removed on a rotary evaporator and the crude compound was purified twice by silica gel column chromatography using CH_2Cl_2 as solvent to give **7** as a purple solid (10 mg, 2.3%). Mp >300 °C. ^{1}H NMR ($CDCl_3$, δ in ppm) 7.04–7.91 (m, 40H, phenyl), 8.10 (s, 4H, β-pyrrole). ^{13}C NMR ($CDCl_3$, δ in ppm) 14.31, 29.88, 128.66, 130.41, 132.06, 138.55. ES-MS $C_{68}H_{44}N_2S_2$ calcd av. mass: 953.2. Obsd *m/z*. 953.33. Anal. calcd: C, 85.68; H, 4.65; N, 2.94. Found: C, 85.37; H, 4.93; N, 3.13. IR (KBr, ν (cm^{-1})) 3321, 3063, 2931, 703.

4.1.12. *meso*-5,10,15,20-Tetraphenyl-β-2-methyl-21-monothiaporphyrin (8). A solution of diol **12** (312 mg, 1 mmol), benzaldehyde (0.220 ml, 2.05 mmol) and pyrrole (212 mg, 3.17 mmol) in 100 ml of CH_2Cl_2 were treated with $BF_3{\cdot}OEt_2$ (30 μl of 2.5 M stock solution) and stirred for 1 h under an argon atmosphere. DDQ (180 mg, 0.792 mmol) was added and reaction mixture was stirred for 1 h in air. The solvent was removed under reduced pressure. The TLC analysis of crude product showed the formation of three porphyrins as expected. The crude compound was loaded on silica and eluted with a mixture of petroleum ether/dichloromethane. The desired compound was collected as the second band using petroleum ether/CH_2Cl_2 (1:1). The solvent was removed on a rotary evaporator to afford **8** as a dark purple crystalline solid (78 mg, 12%). Mp >300 °C. ^{1}H NMR ($CDCl_3$, δ in ppm) −2.56 (s, 1H, NH), 2.93 (s, 3H, CH_3), 7.77–7.80 (m, 12H, *m*,*p*-phenyl), 8.03–8.20 (m, 8H, *o*-phenyl), 8.43 (d, *J*=4.57 Hz, 1H, β-pyrrole), 8.52 (d, *J*=4.57 Hz, 1H, β-pyrrole.), 8.58 (d, *J*=4.57 Hz, 1H, β-pyrrole), 8.65 (d, *J*=4.57 Hz, 1H, β-pyrrole), 8.88 (s, 2H, β-pyrrole), 9.46 (s, 1H, β-thiophene). ^{13}C NMR ($CDCl_3$, δ in ppm) 14.33, 20.47, 29.90, 126.77, 127.18, 127.63, 128.01, 128.69, 132.87, 133.47, 134.33, 134.54, 134.91, 135.71, 137.87, 139.11, 141.39, 142.60, 143.79, 145.98, 154.99. FAB-MS $C_{45}H_{31}N_3S$ calc av. mass, 645.8. Obsd *m/z*: 646. Anal. calcd: C, 83.69; H, 4.84; N, 6.51. Found: C, 83.93; H, 5.05; N, 6.30. IR (KBr, ν (cm^{-1})) 3325, 3039, 2923, 707.

4.1.13. *meso*-5,10,15,20-Tetraphenyl-β-2,3-dimethyl-21-monothiaporphyrin (9). Diol **13** (200 mg, 0.617 mmol), benzaldehyde (140 μl, 148 mg, 1.40 mmol) and pyrrole (140 μl, 135 mg, 2.02 mmol) in CH_2Cl_2 (70 ml) were treated with $BF_3{\cdot}OEt_2$ (30 μl of 2.5 M stock solution) under an argon atmosphere. After 1 h, DDQ (110 mg, 0.484 mmol) was added and the reaction mixture was stirred for 1 h in air. Chromatography on silica gel with petroleum ether/CH_2Cl_2 (6:4) gave the desired compound **9** as a purple solid which moved as the second band (50 mg, 12%). Mp >300 °C. ^{1}H NMR ($CDCl_3$, δ in ppm) −2.37 (s, 1H, –NH), 2.80 (s, 6H, $-CH_3$), 7.72–7.77 (m, 12H, *m*,*p*-phenyl), 8.08–8.21 (m, 8H, *o*-phenyl), 8.45 (d, *J*=4.39 Hz, 2H, β-pyrrole), 8.52 (d, *J*=4.39 Hz, 2H, β-pyrrole), 8.87 (m, 2H, β-pyrrole), ^{13}C NMR ($CDCl_3$, δ in ppm) 18.36, 29.79, 32.02, 53.49, 123.72, 126.66, 127.16, 128.38, 129.99, 133.17, 133.87, 134.46, 138.78, 142.51, 144.94, 154.48, 158.80. ES-MS $C_{46}H_{33}N_3S$ calc av. mass, 659.8, obsd *m/z* 660. Anal. calcd: C, 83.73; H, 5.04; N, 6.37. Found: C, 84.11; H, 5.32; N, 6.16. IR (KBr, ν (cm^{-1})) 3322, 3059, 2929, 710.

4.1.14. *meso*-5,10,15,20-Tetraphenyl-β-2-phenyl-21-monothiaporphyrin (10). 2,5-Bis(hydroxymethylphenyl)-3-phenylthiophene **14** (250 mg, 0.679 mmol), benzaldehyde (150 μl, 1.42 mmol) and pyrrole (150 μl, 2.17 mmol) were dissolved in 60 ml CH_2Cl_2. $BF_3{\cdot}OEt_2$ (40 μl of 2.5 M stock solution) was added to initiate the condensation and the reaction mixture was stirred for 1 h. DDQ (0.115 g, 0.509 mmol) was added and the reaction left stirring for 1 h in air. The solvent was removed on a rotary evaporator and the crude porphyrin was purified by silica gel column chromatography with petroleum ether/CH_2Cl_2 (1:1) to give the desired porphyrin **10** as a purple solid (30 mg, 6.3%). Mp >300 °C. ^{1}H NMR ($CDCl_3$, δ in ppm) −2.51 (s, 1H, NH), 7.10–7.30 (m, 5H, aryl), 7.44 (m, 2H, *o*-phenyl), 7.75–7.83 (m, 12H, *m*,*p*-phenyl), 8.21–8.26 (m, 6H, *o*-phenyl), 8.49 (s, 2H, β-pyrrole), 8.62 (d, *J*=4.80 Hz, 2H, β-pyrrole), 8.70 (d, *J*=3.60 Hz, 1H, β-pyrrole), 8.92 (s, 1H, β-pyrrole), 9.65 (s, 1H, β-thiophene). ^{13}C NMR ($CDCl_3$, δ in ppm) 29.88, 123.72, 124.29, 126.82, 127.61, 128.05, 130.89, 132.54, 133.56, 134.37, 134.71, 135.13, 138.68, 139.65, 140.44, 141.21, 142.46, 144.47, 149.54, 155.09, 157.80. ES-MS $C_{50}H_{33}N_3S$ calcd av. mass, 707.8, obsd *m/z* 708.3. Anal. calcd: C, 84.84; H, 4.70; N, 5.94. Found: C, 84.98; H, 4.92; N, 6.12. IR (KBr, ν (cm^{-1})) 3332, 3059, 3923, 703.

4.1.15. *meso*-5,10,15,20-Tetraphenyl-β-2,3-diphenyl-21-monothiaporphyrin (11). 2,5-Bis(hydroxymethyl)-3,4-diphenylthiophene **15** (0.080 g, 0.178 mmol), benzaldehyde (40 μl, 0.398 mmol) and pyrrole (40 μl, 0.579 mmol) were dissolved in 30 ml CH_2Cl_2. $BF_3{\cdot}OEt_2$ (40 μl of 2.5 M stock solution) was added to initiate the cyclization and reaction mixture was stirred for 1 h. DDQ (0.115 g, 0.506 mmol) was added and stirred the reaction mixture in air for an additional 1 h. The solvent was removed on a rotary

evaporator and the crude porphyrin mixture was purified by column chromatography with petroleum ether/CH_2Cl_2 (1:4) to afford the desired porphyrin **11** as a purple solid (9 mg, 6.4%). Mp >300 °C. ^{1}H NMR ($CDCl_3$, δ in ppm) −2.29 (s, 1H, NH), 6.80–7.40 (m, 22H, aryl), 7.66–7.80 (m, 8H, aryl), 8.20 (m, 3H, β-pyrrole) 8.32 (m, 1H, β-pyrrole), 8.46 (m, 1H, β-pyrrole), 8.88 (s, 1H, β-pyrrole). ^{13}C NMR ($CDCl_3$, δ in ppm) 14.44, 22.70, 29.37, 29.70, 31.93, 123.54, 125.78, 125.94, 126.46, 126.64, 126.81, 127.83, 128.69, 132.03, 134.08, 134.43, 138.56, 139.11, 141.17, 141.73, 142.31, 150.01, 155.09, 159.18. ES-MS $C_{56}H_{37}N_3S$ calcd av. mass, 783.9. Obsd *m/z* 784.3. Anal. calcd: C, 85.79; H, 4.76; N, 5.36. Found: C, 85.96; H, 4.89; N, 5.54. (KBr, ν (cm^{-1})) 3319, 3053, 2858, 702.

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