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Contents

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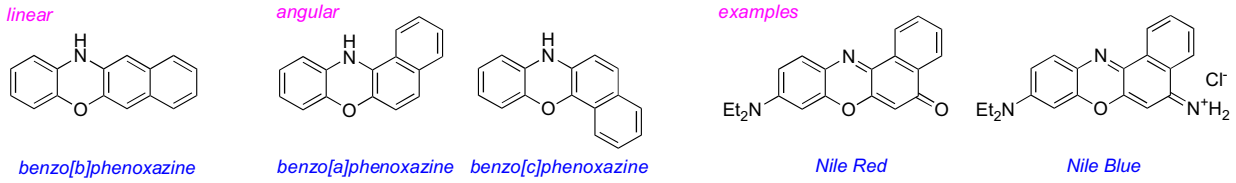
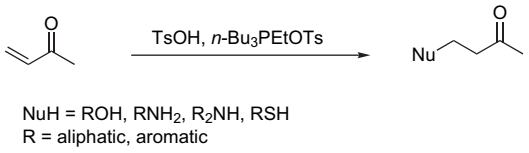
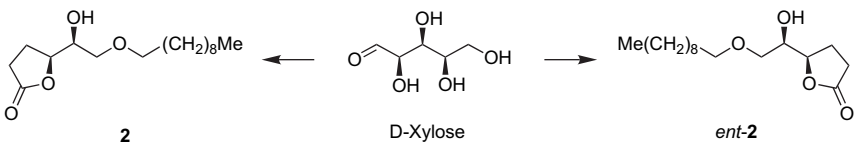
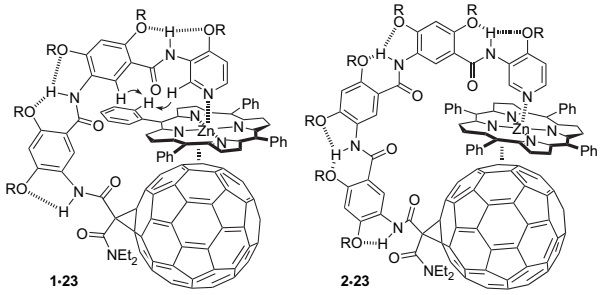


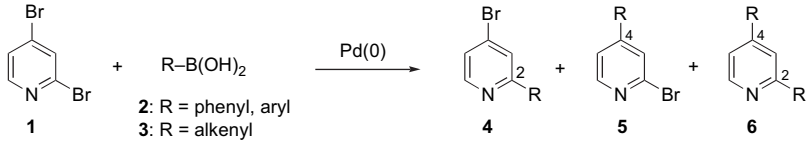
Syntheses and spectroscopic properties of benzophenoxazine fluorescent dyes are reviewed from the perspective of their potential applications as probes in biotechnology, especially with respect to intracellular imaging.

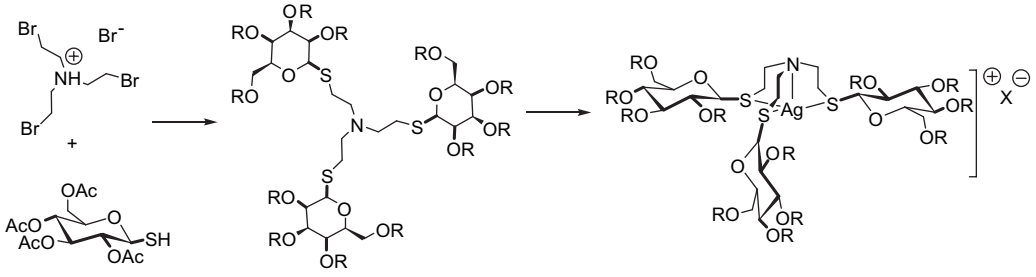
ARTICLES

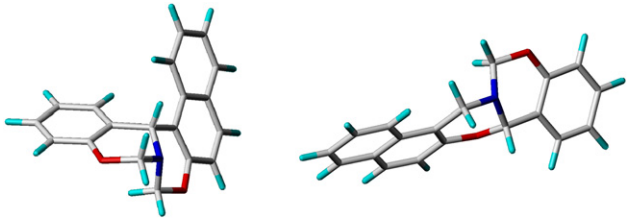


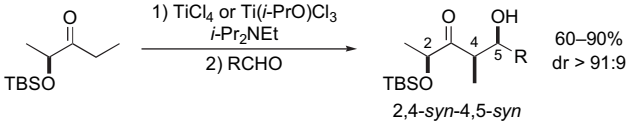


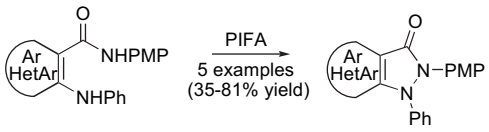




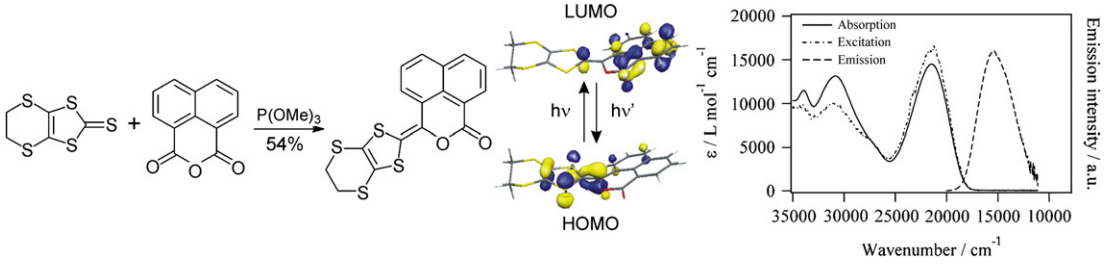


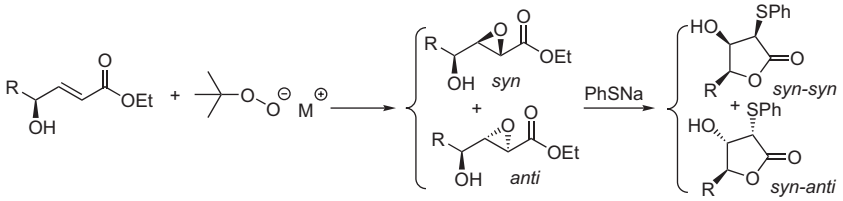


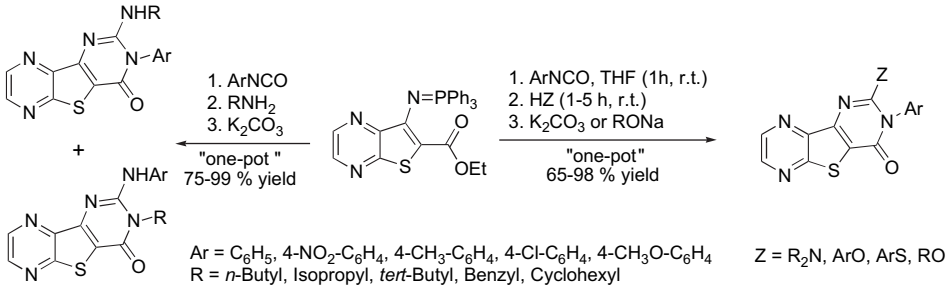


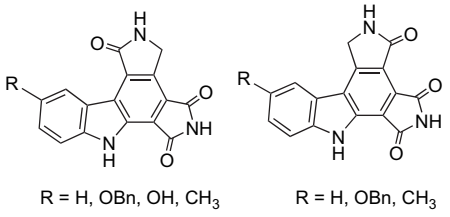


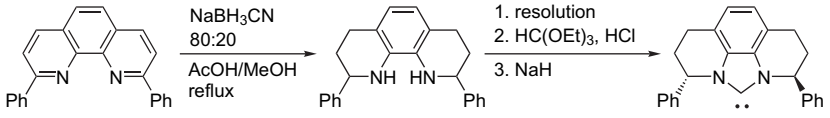
The title compounds are obtained by an intramolecular PIFA-mediated N–N bond construction.

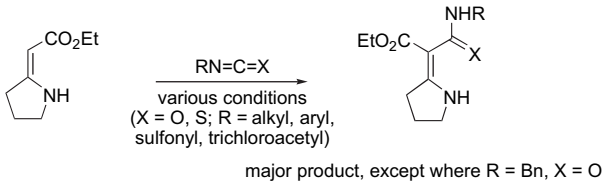




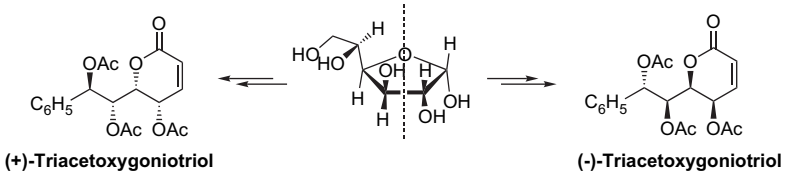




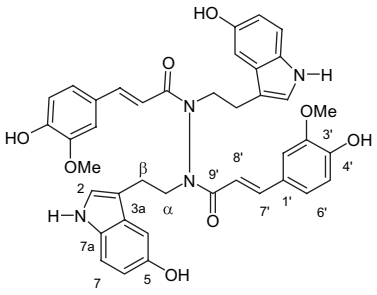


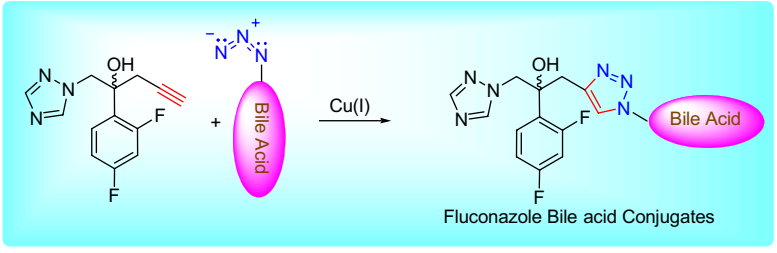


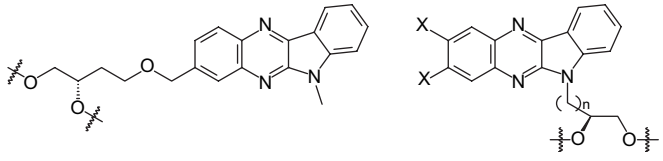
The title reactions give predominantly the *C*-acyl products rather than the previously-reported *N*-acyl products.

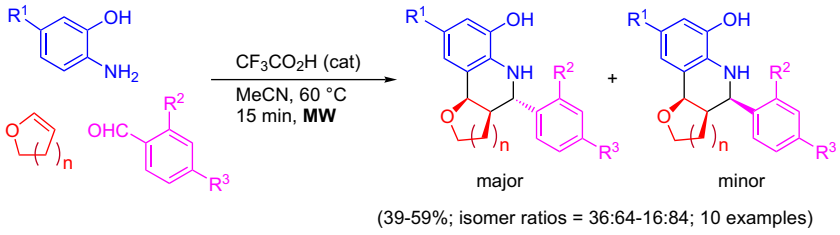


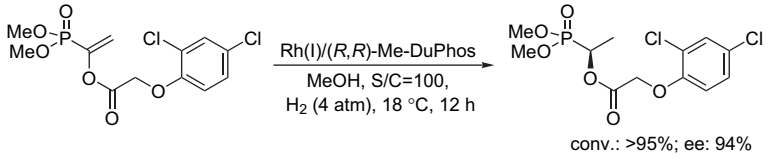
Montamine, a unique dimeric indole alkaloid, isolated from the seeds of *Centaurea montana*, displayed significant in vitro anticancer activity in the MTT assay using the CaCo2 colon cancer cell line (IC_{50}=43.9 μM).

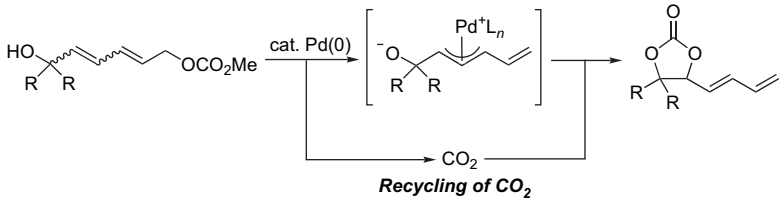


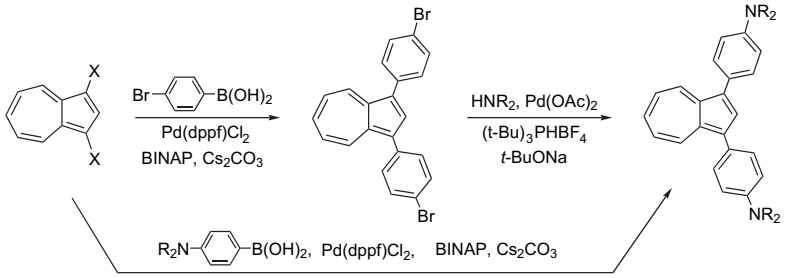


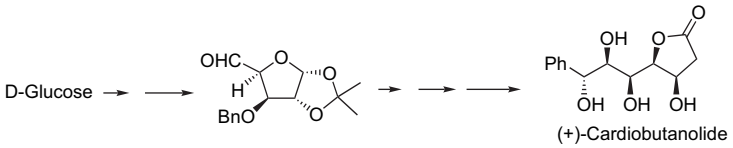




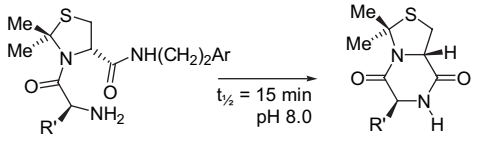


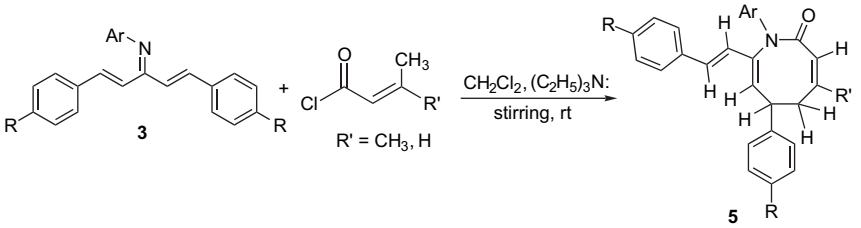




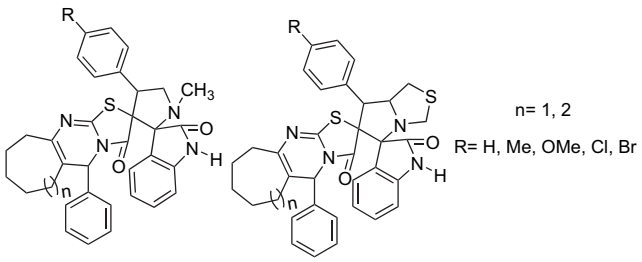


A formal total synthesis of (+)-cardiobutanolide has been accomplished from D-glucose, a readily available precursor.





A facile single step synthesis of novel azocinone derivatives involving tandem [2+2] cycloaddition and Cope rearrangement in the reactions of cross-conjugated azatrienes with vinyl/isopropenyl ketenes supported by theoretical calculations, is reported.



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Benzophenoxazine-based fluorescent dyes for labeling biomolecules

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Contents

1. Introduction

Meldola's Blue **1**, Nile Red **2**, and Nile Blue **3** have some desirable attributes as fluorescent probes.[3] Dyes **2** and **3** have reasonably high fluorescence quantum yields in apolar solvents and they fluoresce at reasonably long wavelengths. Nile Red, in particular, fluoresces far more strongly in apolar media than in polar ones, and the fluorescent emission shows a large bathochromic (i.e., red-) shift in polar media, hence it can be used as a probe for environment polarity;[4–6] these characteristics may be attributes for some applications, but limitations for others. None of these dyes are significantly soluble in aqueous media, and their quantum yields are dramatically reduced. One of the big challenges in the production of fluorescent dyes is to produce water-soluble probes that fluoresce strongly in aqueous media, particularly above 600 nm or at even longer wavelengths. Motivation for research in this area is drawn from needs for intracellular, tissue, and whole organism imaging where near-IR dyes are far more conspicuous than ones emitting at 550 nm or less.[7]

The phenoxazine skeleton may be extended by adding fused benzene rings to the *a–c* or *h–j* faces. Benzophenoxazines of this type are 'angular'[1] or 'linear' depending on the orientation of the ring fusion, as illustrated in Figure 1a.

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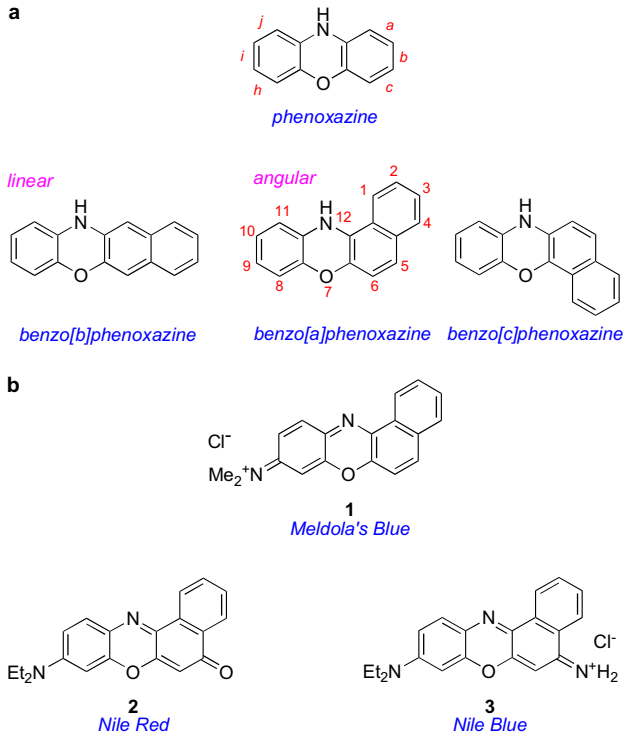


Figure 1. (a) Phenoxazines and benzophenoxazines; (b) structures of the three best known fluorescent dyes in this class.

Substituents that freely donate and/or accept electron density on benzophenoxazine cores can, in some orientations, give fluorescent compounds. The first notably fluorescent compound to be discovered in this class was Meldola's Blue **1**, but Nile Red **2**[2] and Nile Blue **3**[2] are far more frequently used in contemporary science.

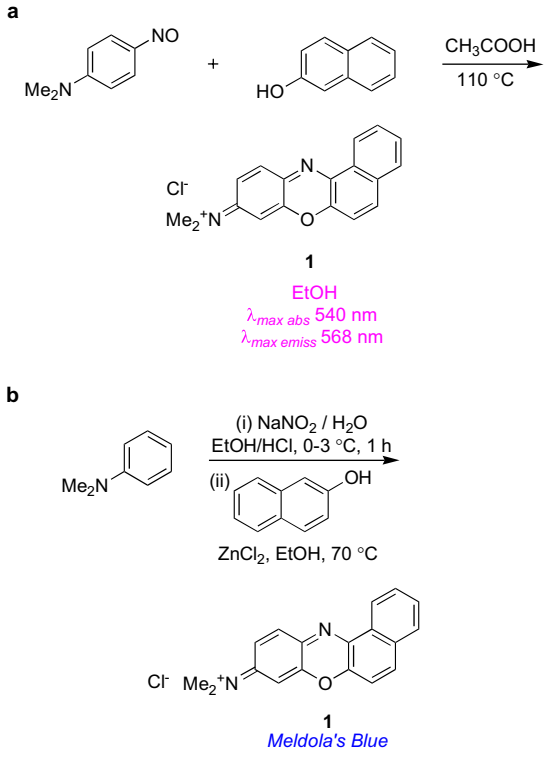
The objective of this review is to summarize the state of the art of benzophenoxazine dyes in such a way that all readers can understand quickly what has been done to develop useful probes in this category. Inspired readers should also be able to use this summary to design research approaches that are not yet explored but would lead to useful endpoints.

2. Meldola's Blue

This dye is used in textiles, paper, and paints, mainly as a pigment. It is not a particularly useful fluorescent dye for labeling proteins because of its poor water solubility. Further, its fluorescence is weak in all common media (e.g., EtOH) and does not give a clear indication of the surrounding polarity. However, Meldola's Blue has been used as a component in redox sensors[16] for detection of materials such as NADH,[8] pyruvates,[9] hydrogen peroxide,[10] glucose,[11] and 3-hydroxybutyrate.[12] It has also been used in electrochemical experiments involving DNA wherein the dye mediates electron transport.[13]

2.1. Syntheses

The original (1879) synthesis of Meldola's Blue involved condensation of a nitroso compound with 2-naphthol at elevated temperatures (Scheme 1a).[14] Details of the reaction conditions and the yield were not given. Subsequently, Meldola's Blue has been made with a variety of counter ions via reactions involving different Lewis acids (Scheme 1b).[15] Counter ion modifications have been used to modulate the electronic properties of solid materials for applications not directly related to labeling biomolecules.



Scheme 1. (a) Original synthesis of Meldola's Blue; (b) a more recent approach.

2.2. Photophysical properties

Phenoxazines without strongly electron withdrawing or donating substituents have unexceptional absorbance characteristics, and they are not particularly fluorescent compounds. Fusion of a benzene ring onto the heterocycle does not alter this situation dramatically. Meldola's Blue has one dimethylamino substituent and the heterocyclic core is oxidized. These changes in the composition of the heterocycle shift its absorption and emission characteristics into a useful range: $\lambda_{max\ abs}$ 540 nm, $\lambda_{max\ emiss}$ 568 nm EtOH. This dye is not noted for its solvatochromic properties.[17]

3. Nile Red derivatives

3.1. Introduction

Nile Red has a neutral oxidized phenoxazine system, i.e., it is a phenoxazinone. The 9-diethylamino substituent is able to donate electron density into the carbonyl group across the ring; this electronic arrangement probably accounts for its highly fluorescent properties (Fig. 2).

The water-solubility of Nile Red is extremely poor, but in other solvents its fluorescence maxima and intensity are good indicators of the dye's environment-polarity. As

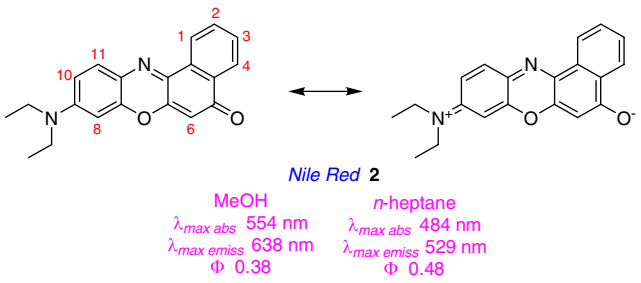
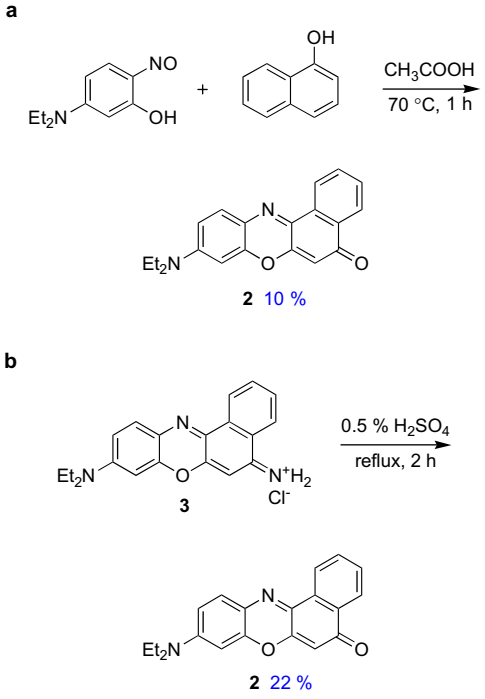


Figure 2. Electron delocalization in Nile Red.

mentioned above, this solvatochromic effect is such that polar media cause a red-shift but decreased fluorescence intensity. This decreased fluorescence intensity is probably due to self-quenching of the dye in face-to-face aggregates. Consequently, this dye is particularly useful for studying lipids and events that involve impregnation of the dye in apolar media. Surprisingly, very few water-soluble analogs of Nile Red have been reported, and only limited fluorescence data have been given for those.

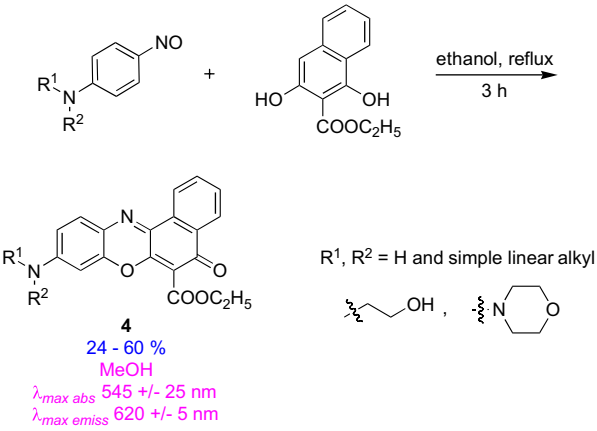
3.2. Syntheses

The first synthesis of Nile Red was a condensation reaction of a nitrosophenol (Scheme 2a). The patent literature indicates that other solvent systems can be used. It is also possible to prepare Nile Red via hydrolysis of Nile Blue as indicated in Scheme 2b. The product is usually isolated via chromatography on silica.



Scheme 2. (a) Original synthesis of Nile Red; (b) synthesis from Nile Blue.

Substituted or modified Nile Red derivatives may be prepared via variations of the syntheses above, i.e., *de novo* methods, or by functionalizing Nile Red itself. For instance, a *de novo* approach was used to prepare the 6-carboxyethyl derivatives **4** (reaction 1). These are potentially interesting since hydrolysis of the ester would give a carboxylic acid for attachment to biomolecules; however, this does not appear to have been attempted yet.[19] The patent literature also describes a synthesis of the 2-carboxy Nile Red derivative.[20]



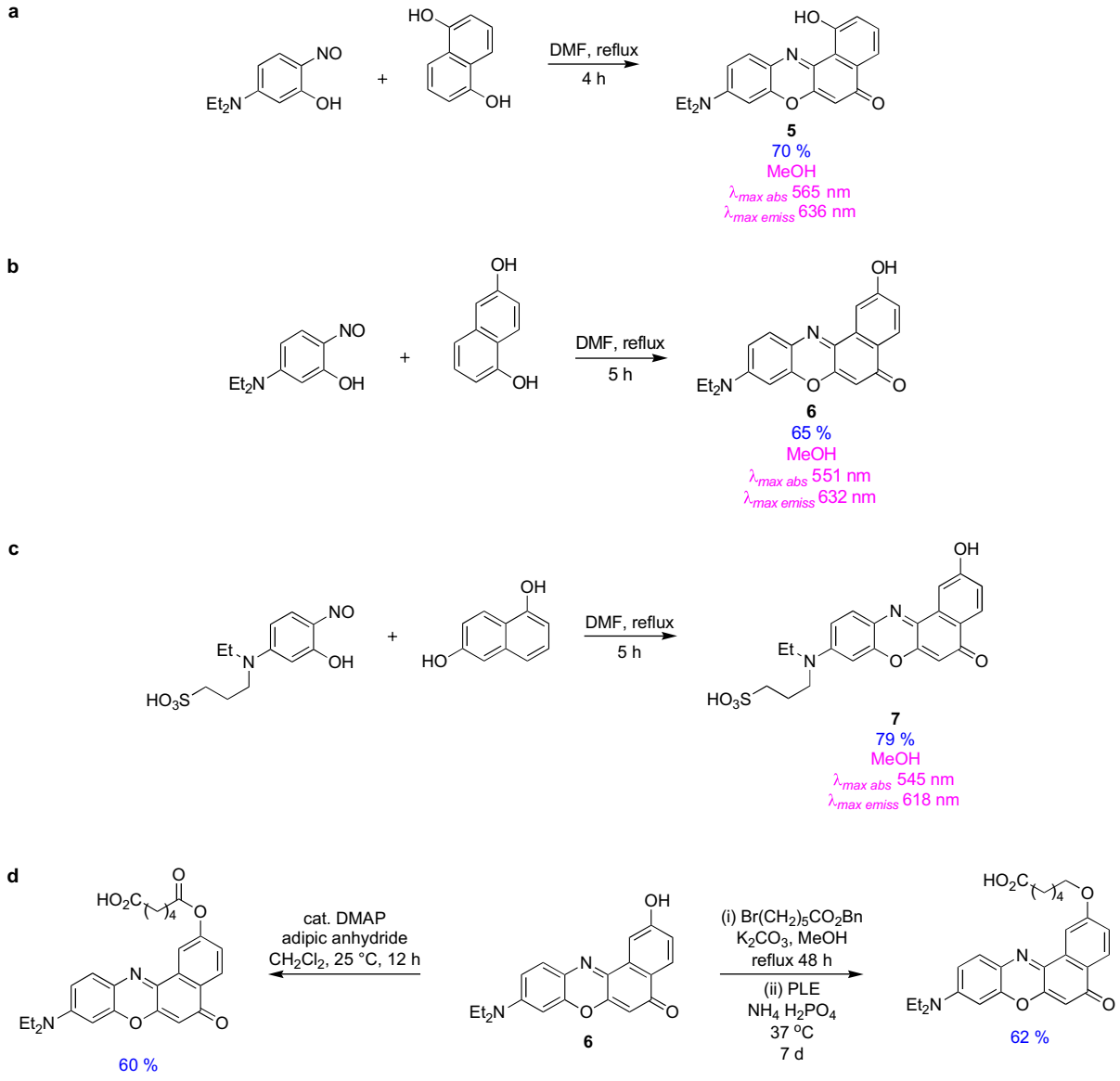
(1)

De novo syntheses of 1- and 2-hydroxy Nile Red, compounds **5** and **6**, respectively, have also been performed, and the products are easily modified via other reactions.[21,6] Thus, Briggs and co-workers at Amersham prepared the parent hydroxy compounds as shown in Scheme 3. Some of the reactions used to derivatize these materials are also shown. In some ways the hydroxyl group of the hydroxy Nile Reds **5** and **6** is an inconvenience because the spectroscopic properties of the dye under physiological conditions become highly pH dependent. However, this phenol is useful as a functional group to incorporate a handle for attachment to biomolecules (Scheme 3d).

A series of fluorinated phenoxazines (not shown) and benzophenoxazines (Scheme 4) have been prepared via sequential S_NAr substitutions reactions of fluorinated aromatics.[22] The 6-fluoro substituent in the compound shown below changes its spectroscopic properties slightly (see below) and, presumably its pH dependence.

The so-called 'FLAsH dyes' feature bisarsenic(3+)-based dye precursors that are non-fluorescent, presumably due to rapid quenching of the excited state via intramolecular energy transfer. However, the disposition of the arsenic dyes is such that they are thought to react with dicysteine units engineered into modified proteins that are expressed within cells. This type of reaction has at least two effects, it: (i) alters the oxidation potential of the arsenic centers and (ii) restricts rotations about the C–As bonds. For whatever reason, structural changes such as this render the dye-protein complex fluorescent. Only proteins within the cell that have the special arrangement of Cys-residues are likely to become labeled in this way, so the approach allows for highly selective visualization of the engineered protein (Fig. 3).

The original FLAsH dyes were fluorescein derivatives.[23] A range of bisarsenic derivatives on different dye skeletons



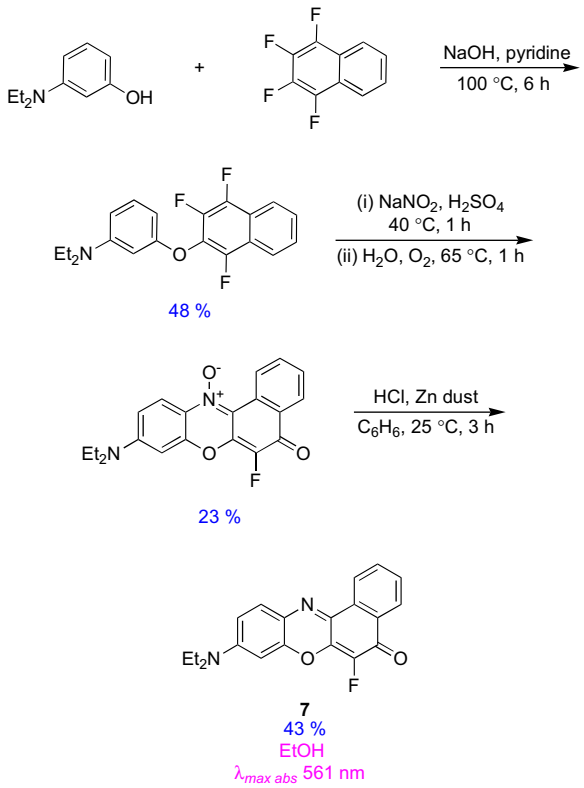
Scheme 3. (a) Synthesis of 1-hydroxy Nile Red; (b) synthesis of 2-hydroxy Nile Red; and (c) some derivatization reactions of 1-hydroxy Nile Red.

has been prepared for evaluation, but only fluorescein and Nile Red derivatives have emerged as useful. This is because several parameters must be controlled tightly if this type of experiment will work. For instance, the As-to-As distance must match the disposition of the target thiols, the ethanedithiol (EDT) concentration in the cell is critical, and the dye must permeate into the cell.

The FLAsH Nile Red derivative **8** was prepared as indicated in Scheme 5. This involves a standard condensation reaction to form the benzophenoxazine core, but without the *N*,*N*-diethyl substituents of Nile Red. These were omitted to allow more space for manipulations at the 6- and 8-positions. FLAsH dye **8** gives less fluorescent enhancement on binding than similar fluorescein dyes, but it emits at a longer wavelength (604 nm) and that, as mentioned before, is a more transparent region of the spectrum for intracellular imaging. Calcium-induced conformational changes of appropriately modified, intracellular calmodulin have been followed using FLAsH dye **8**.[24]

Scheme 6 describes syntheses of two more classical thiol-selective dyes, ones that rely on S_N2 displacement of iodide from iodoacetyl groups.[25] Thus probes **9** and **10** were prepared from a Nile Red derivative and from a 2-hydroxy Nile Red compound, respectively. Curiously, when these were complexed to a particular Cys-residue in maltose binding protein, dye **9** showed a three-fold enhancement of fluorescence, while the emission from **10** was *reduced* by a factor of five. The authors explain this by proposing that **10** is less constrained when bound to protein.

Two interesting questions arise from the work on Nile Red derivatives that is described above. First, would water-soluble derivatives of Nile Red have high quantum yields in aqueous media? As already stated, most Nile Red derivatives do not fluoresce strongly in polar media, but this could be due to aggregation effects that might be avoided if the dye has some intrinsic water solubility. Second, do water-soluble Nile Red derivatives also show the pronounced bathochromic shift in polar media, that is, observed for



Scheme 4. Preparation of 6-fluoro Nile Red.

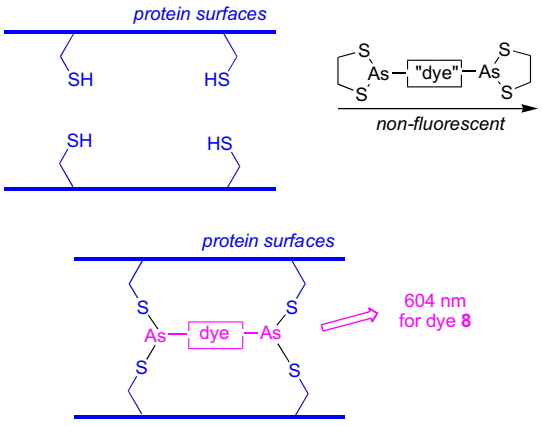
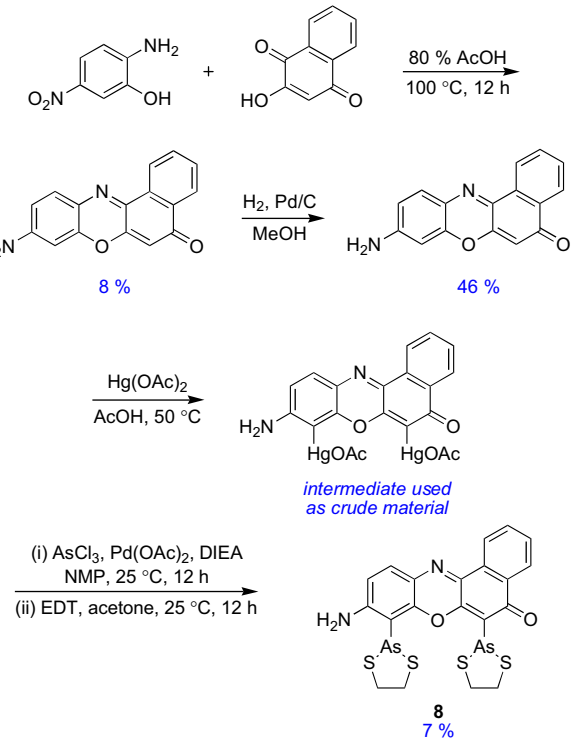


Figure 3. Conceptual basis of fluorescent arsenic dyes.

compounds in the series with little or no significant aqueous solubility?

No significantly water-soluble Nile Red derivatives have been prepared to answer the questions posed above until recent work[18] from our laboratories. Classical condensation routes were used to prepare the three Nile Red derivatives **11**–**13** (Scheme 7). These were designed to be water soluble, but, surprisingly, the diol/phenol **11** was not, even in aqueous base. The other two compounds do have very good water solubilities. Their spectroscopic properties are discussed in the next sub-section; but the data shown in Table 3 show that the answers to both these questions were affirmative for compounds **12** and **13**.

Some researchers may be interested in access to more sophisticated analogs of Nile Red. One obvious approach



Scheme 5. Preparation of the FLAsH system **8**.

would be to prepare iodo-, bromo-, or chloro-substituted compounds then elaborate them via organometallic couplings. There have been reports of attempted halogenation of Nile Red, but the regioselectivity and extent of the halogenations proved hard to control and mixtures were produced.[26–28]

3.3. Spectroscopic properties

Table 1 summarizes spectroscopic data for Nile Red in different solvents, all taken from the same source.[4] These data show decreased fluorescence intensity of Nile Red correlates much more with hydrogen bonding than with solvent polarity, and the magnitude of this difference is best appreciated from the graphical presentation of only the emission wavelengths and intensities that is given in Figure 4. However, the bathochromic shift seems to be a function of solvent polarity, consistent with stabilization of relatively polar excited states.

A similar study featuring emission and absorption maxima, quantum yields, and solvent polarities was performed for

Table 1. Solvent dependency of emission intensities and wavelengths for Nile Red **2**

Solvent	$\lambda_{max\ abs}$ (nm)	$\lambda_{max\ emiss}$ (nm)	Relative fluorescence intensity
Water	591	657	18
EtOH	559	629	355
Acetone	536	608	687
$CHCl_3$	543	595	748
iso-Amyl acetate	517	584	690
Xylene	523	565	685
n-Dodecane	492	531	739
n-Heptane	484	529	585

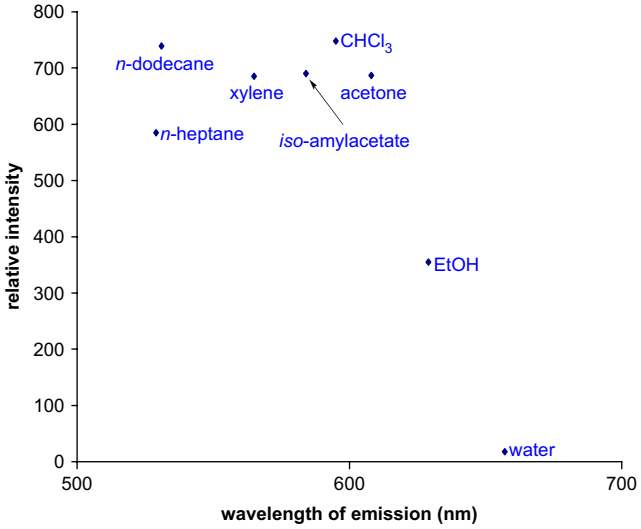


Figure 4. Solvent dependency of emission intensities and wavelengths for Nile Red **2**.

Table 2. Solvent dependency of quantum yield and wavelengths for 2-hydroxy Nile Red **6**

Solvent	$\lambda_{max\ abs}$ (nm)	$\lambda_{max\ emiss}$ (nm)	Quantum yield (Φ)
Cyclohexane	514	528	0.51
Dibutyl ether	514	555	0.66
Toluene	525	568	0.76
Acetone	528	608	0.78
EtOH	544	634	0.58
1,2-Ethanediol	584	655	0.40
CF_3CH_2OH	587	653	0.24
$(CF_3)_2CHOH$	612	670	0.09

2-hydroxy Nile Red **6** (Table 2). Selected data from this study are shown in Figure 5. Just as with Nile Red, polar hydrogen-bonding solvents correlate with reduced quantum yields and significant bathochromic shifts.

Fluorescence lifetimes contribute to two important physical parameters of fluorescent dyes. Dyes with long fluorescent lifetimes can emit strongly because non-radiative processes are relatively slow, and because intersystem crossing to triplet states is less competitive.

Fluorescence lifetimes for Nile Red in different solvents have been measured (Table 3). These data show that the fluorescence lifetime of Nile Red is 3.65 ns (EtOH)[29] in comparison to fluorescein (4.25 ns, EtOH)[30] and tetramethylrhodamine (i.e., rhodamine 6-G, 3.99 ns, EtOH).[30] The fluorescent lifetime of Nile Red does not seem to vary much with solvent polarity, but it is extremely sensitive to H-bonding. In hydrogen-bonding solvents the fluorescence lifetime of Nile Red decreases dramatically.

As stated above, the bias of this review is to highlight potential applications in biotechnology, especially for intracellular imaging. There are two common approaches to long wavelength dyes for imaging in tissues. The first, and most obvious, is to prepare analogs of known dyes with extended conjugated systems. Secondly, two-photon absorption can be used,[31] in which two long wavelength photons absorbed by a molecule promote it to an excited state that then emits

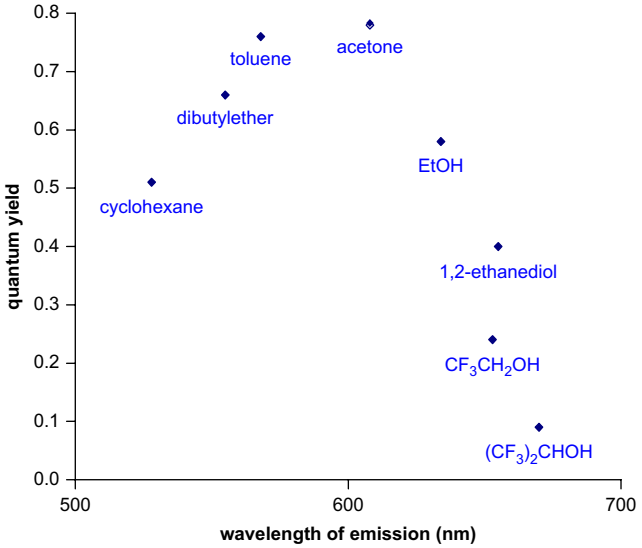


Figure 5. Solvent dependency of quantum yield and wavelengths for 2-hydroxy Nile Red **6**.

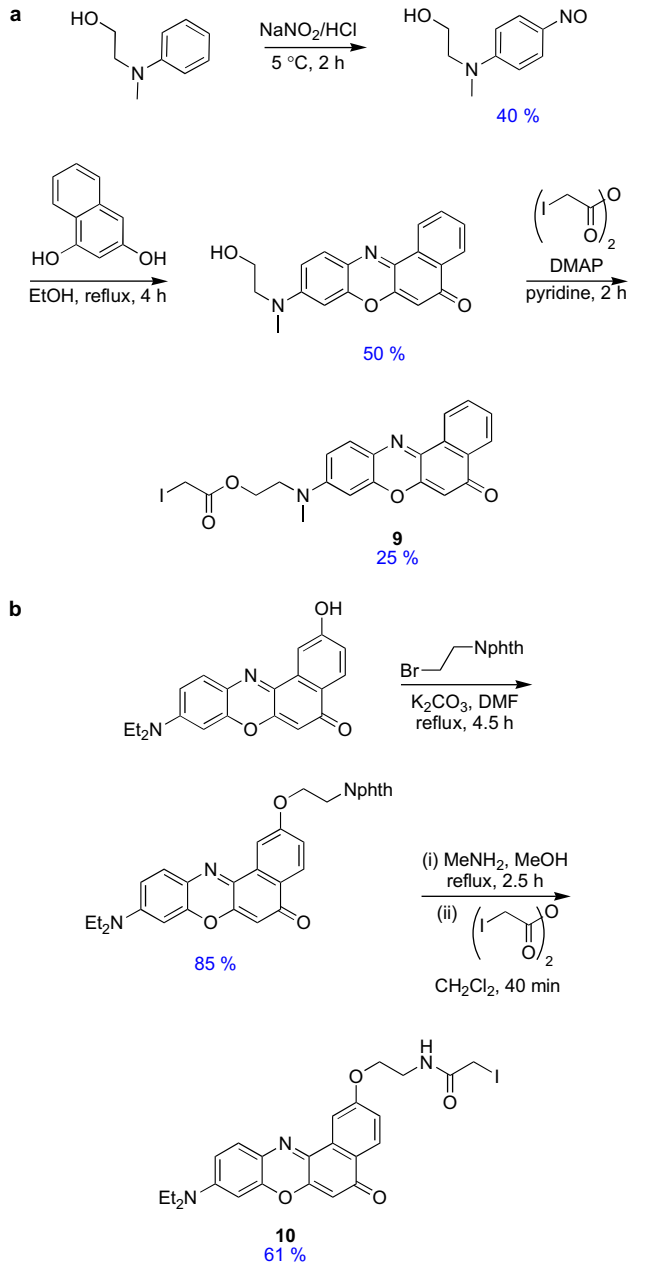
Table 3. Spectroscopic properties of water-soluble Nile Red derivatives **11–13** in different solvents

Dye	$\lambda_{max\ abs}$ (nm)	$\lambda_{max\ emiss}$ (nm)	Quantum yield (Φ)	Solvent
11	542	631	0.56	EtOH
12	520	632	0.43	EtOH
12	560	648	0.33	Phos[a] 7.4
12	556	647	0.07	Bor[b] 9.0
13	548	632	0.42	EtOH
13	558	652	0.37	Phos[a] 7.4
13	556	650	0.18	Bor[b] 9.0

[a] pH 7.4 Phosphate buffer.
[b] pH 9.0 Borate buffer.

a single photon of higher energy. This is a way to excite intracellular or tissue samples at a wavelength that is more transparent to these media. The dependence of two-photon absorption on the intensity of laser beam allows for high spatial selectivity by focusing the laser beam on the target cell and thus preventing any damage to adjacent cells. Relatively few dyes are suitable for practical experiments using two-photon excitation because most do not absorb two long wavelength photons efficiently, i.e., they have poor two-photon cross-sections. Unfortunately, Nile Red has a relatively poor two-photon cross-section.[31]

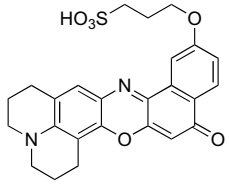
One issue with two-photon excitation experiments is that the emitted light is of a short wavelength compared to the excitation source, and this might not be in a convenient region to permeate out of cells of other tissues, and for detection. One strategy to circumvent this problem is to arrange a fluorescence energy transfer system (FRET) featuring a donor with a large two-photon cross-section and an acceptor with a more convenient, longer wavelength, and emission maxima. 2-Hydroxy Nile Red derivatives are being used in such systems. Thus a series of compounds (of which **14** and **15** are two of the simplest; Fig. 6) have been prepared toward this end; and donor-to-acceptor energy transfer efficiencies of more than 70% were observed (Fig. 5).[32,33] These compounds presumably do not have the solubility and size characteristics that render them suitable for a range of



Scheme 6. Two thiol-selective dye electrophiles: (a) a Nile Red derivative; (b) a 2-hydroxy Nile Red derivative.

applications in biotechnology, so further developments in this area would be opportune.

Finally, there is the possibility that the fluorescence of Nile Red is somewhat attenuated if the excited state of the benzophenoxazinone core is reduced via electron transfer from the 9-amino substituent. We mention this because the possibility has been explored via AM1 calculations.[34] These gauge the degree of charge transfer in a planar state relative to a twisted one in which the amine lone pair is not disposed to electron donation to the heterocycle. The degree of electron transfer from the 9-amino substituent will be dependent on the conformation *and* on the reduction potential of the heterocycle in its excited state. If intramolecular charge transfer was a major pathway for quenching the fluorescence of Nile Red derivatives, it would be expected that compound **16** would be less fluorescent than Nile Red itself; this dye itself has not been prepared.



16
more or less fluorescent than Nile Red?

4. Nile Blue

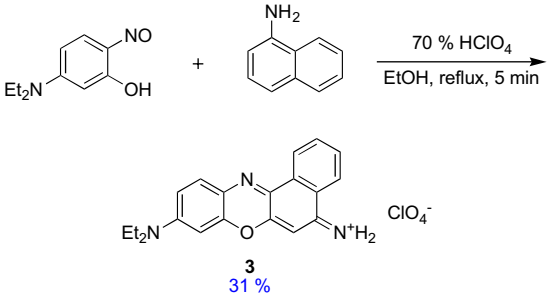
4.1. Introduction

Nile Blue has a positively charged, oxidized, phenoxazine system, i.e., it is a phenoxazinium; the conspicuous difference between this and Nile Red **2** is that the latter is neutral. Both dyes have a 9-diethylamino substituent to donate electron density across the ring, but Nile Red **2** and Blue **3** have different electron acceptors, a carbonyl and an iminium group, respectively (Fig. 7).

One obvious consequence of the difference in charges for Nile Red **2** and Blue **3** is that water-solubility of Nile Blue is significantly better. Like its Red cousin, the fluorescence maxima and intensity of Nile Blue are good indicators of the polarity of the dye's environment. This solvatochromic effect gives a red-shift in polar media, as would be expected for stabilization of a more charged excited state. The intensity of the fluorescence of **3** in water is about 0.01; this is a small value but, in comparison with the lack of fluorescence of Nile Red in aqueous media, this is significant.

4.2. Syntheses

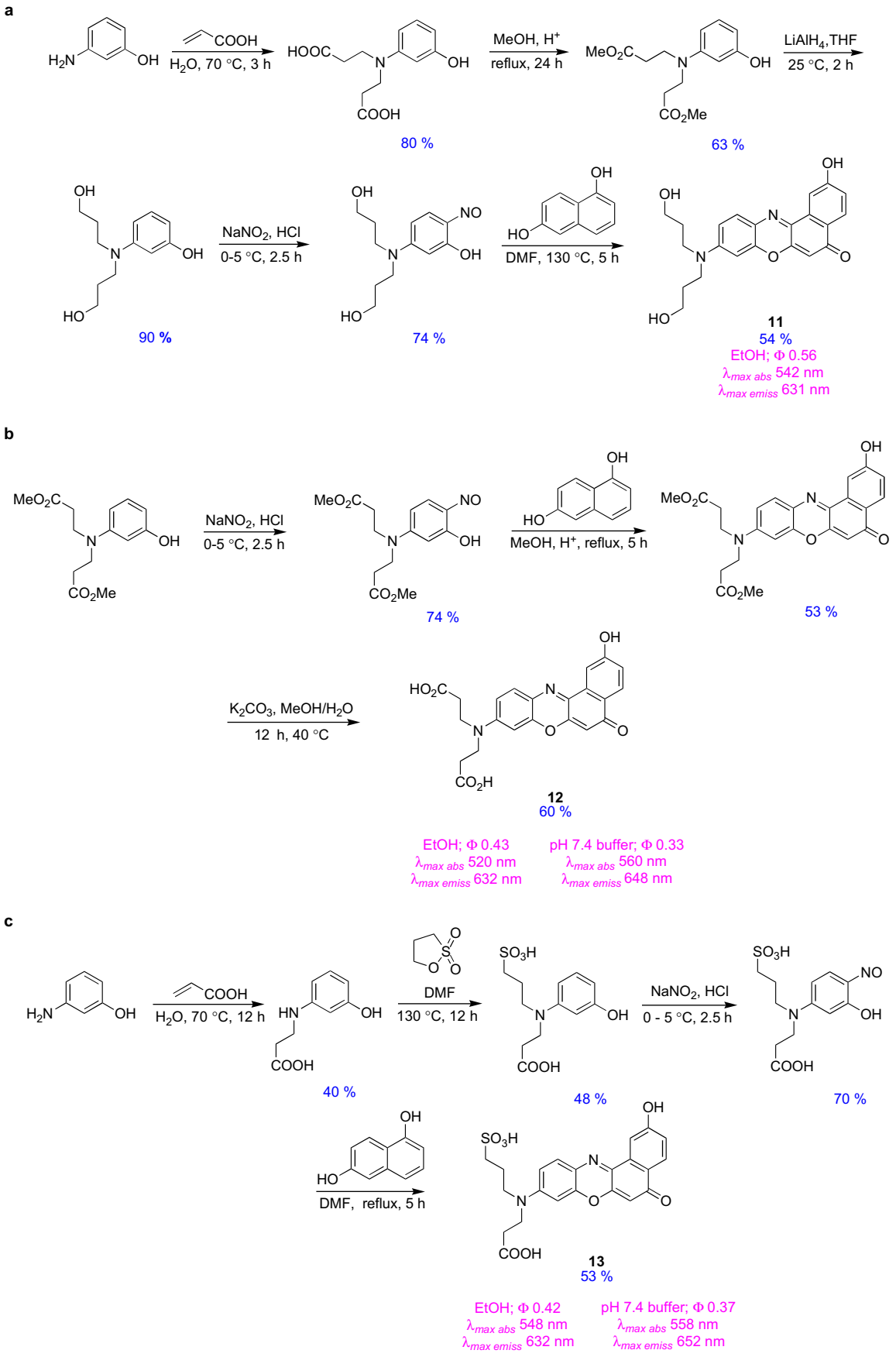
The original synthesis of Nile Blue[2] involved condensation of 5-amino-2-nitrosophenol with 1-aminonaphthalene in acetic acid, but the yield was only 3%. However, use of perchloric acid in ethanol gives a significantly higher yield (reaction 2).[35]



3
31 %

(2)

De novo syntheses of Nile Blue derivatives involve use of similar, but substituted starting materials. Scheme 8 describes syntheses of dyes **17**–**20** that incorporate 8-hydroxy julolidine.[35] This amine is a 'privileged fragment' in dye syntheses because it holds the nitrogen lone pair in



Scheme 7. Synthesis of 2-hydroxy Nile Red derivatives featuring the following functionalities: (a) a diol; (b) a dicarboxylic acid; and (c) a carboxylic acid sulfonic acid combination.

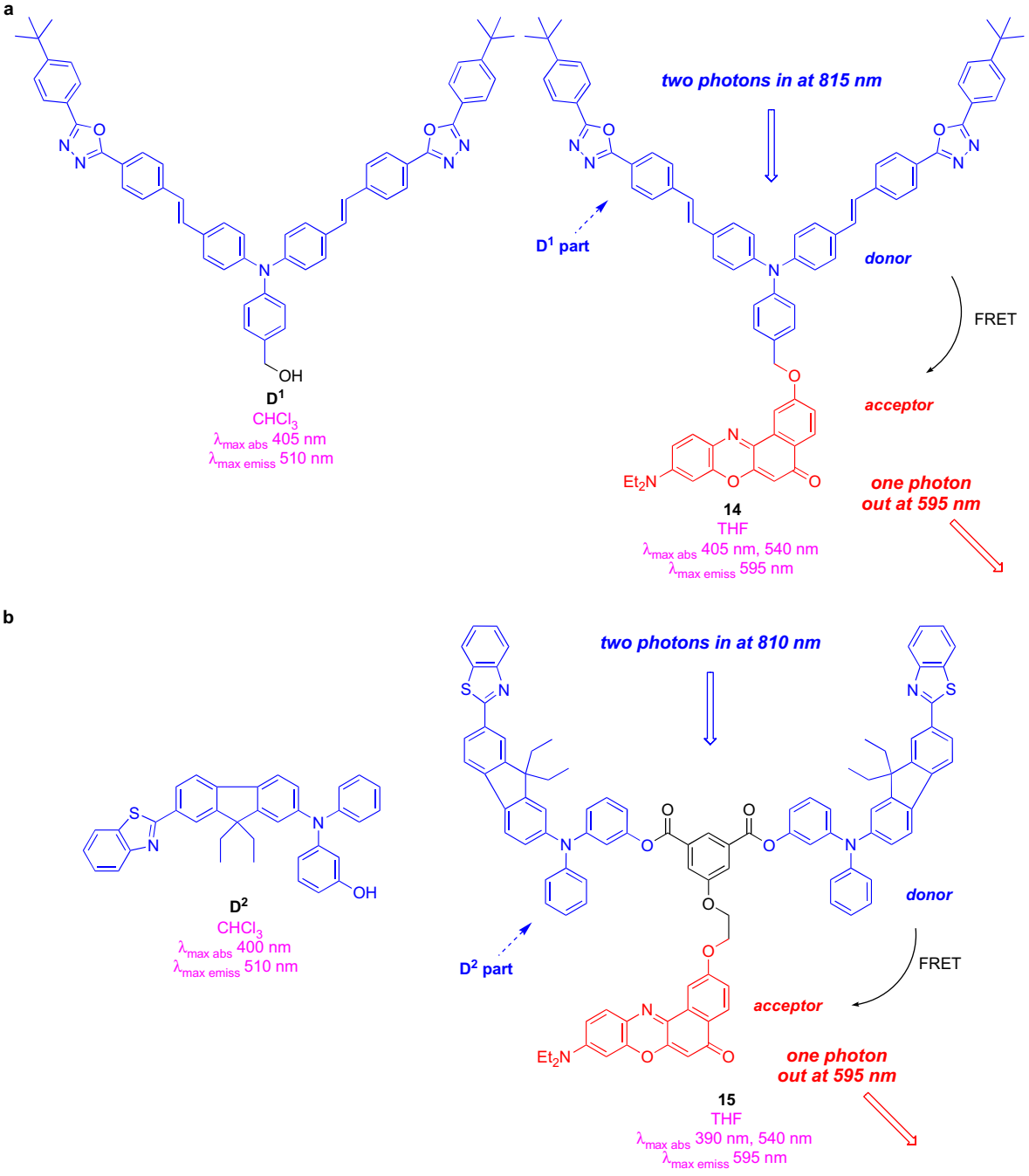


Figure 6. Two-photon-donor acceptor FRET cassettes: (a) **D**^{1} is a two-photon donor used to make cassette **14**; (b) cassette **15** is made from **D**^{2}.

conjugation with the aromatic rings. This alters the reactivity of the starting material; in fact, nitrosylated 8-hydroxyjulolidine was insufficiently reactive in this synthesis so Hartmann and co-workers modified the synthon to include the reactive azo-functionality as shown. Unfortunately, the julolidine fragment is quite hydrophobic too, so the product dyes have very limited solubility in aqueous systems.

An early synthesis of Nile Red featured hydrolysis of a Nile Blue derivative (see Scheme 2b). This implies that Nile Blue derivatives have finite hydrolytic stability, and this could be a drawback in some situations. Dyes **17**–**20** are much less vulnerable to this mode of decomposition because of their fused cyclic structures that hold the amines in place.

Nile Blue derivatives with enhanced water-solubilities and/or groups for bioconjugation can be made from amines with appropriate *N*-substituents. For instance, Scheme 9 shows preparations of dyes **21**,[36,37] **22**/**23**,[38,39] and **24**/**25**[40] in which sulfonic acid groups enhance water solubilities and carboxylic acid groups could potentially be activated and reacted with amines on biological molecules. The syntheses of **24** and **25** are shorter and higher yielding than other syntheses of water-soluble Nile Blue derivatives.

Compounds **22** and **23** are the 'EVO Blue' dyes.[38,39] These have been claimed to be more stable than rhodamine and BODIPY dyes under acidic conditions. This enhanced acid stability was suggested to be useful in the context of high

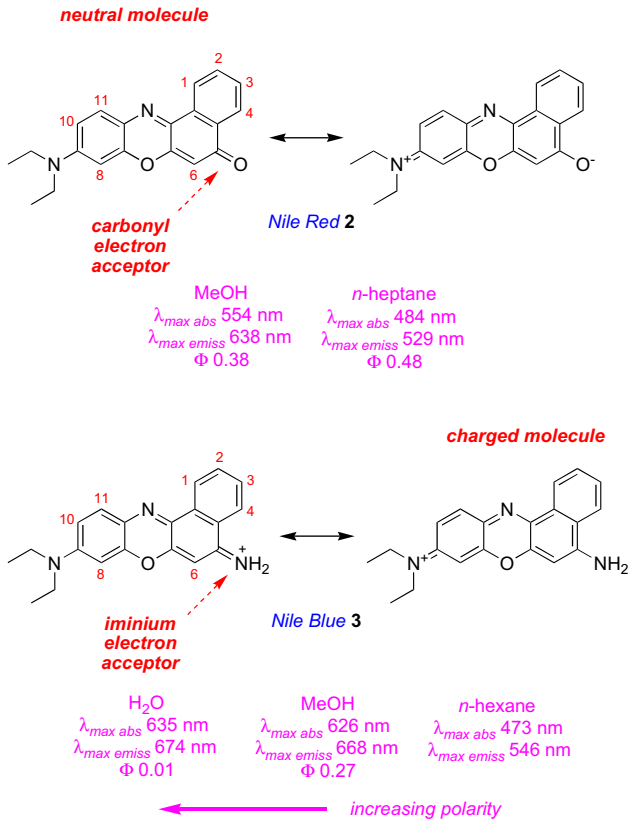


Figure 7. Structures of Nile-Red and Blue contrasted.

throughput screening and solid phase syntheses that involve cleavage from resins by acids.

Other approaches to water-soluble Nile Blue derivatives have involved modification of the parent dye after the heterocyclic framework was assembled. For instance, Scheme 10a shows a patent procedure for alkylation of the 5-amino/iminium substituent; we note that chlorosulfonic acid is an unusual choice for the ester hydrolysis reaction. In the second example, Scheme 10b, an amino anthracene was used to prepare a derivative of Nile Blue that has an extended aromatic system. This modification gave a probe with absorbance and fluorescence emission shifted to the red. Unfortunately, the material **27** was probably not a pure compound since the degree of sulfonation, the regiochemistry, and the chemical/quantum yields were not given.[41]

Direct iodination of Nile Blue is possible, and the 6-iodo derivative **28** can be prepared from this (Scheme 11). However, chromatography was required, the yield of the iodinated product was low, and slight variation of the conditions can lead to formation of other regioisomers. Similar considerations apply to the corresponding bromination reaction, except the product yield was better. Conversely, the *de novo* synthesis of the 2-iodo derivative **30** is unambiguous with respect to the regioisomer formed, but column chromatography is still required and the yield is also low. The diiodide **31** can be formed by a second iodination of the 2-iodide. These halogenated derivatives are potentially useful for forming more sophisticated derivatives, but they were originally prepared as possible photosensitizers to initiate processes that are destructive to carcinogenic cells.[42,43] These photosensitizers promoted to the excited singlet state decays to the triplet state and generates singlet oxygen, which is considered to be responsible for its potent activity toward cancer cells.

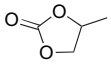
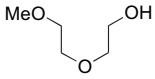
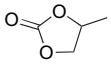
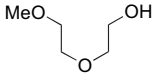
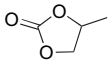
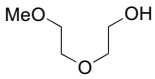
4.3. Spectroscopic properties

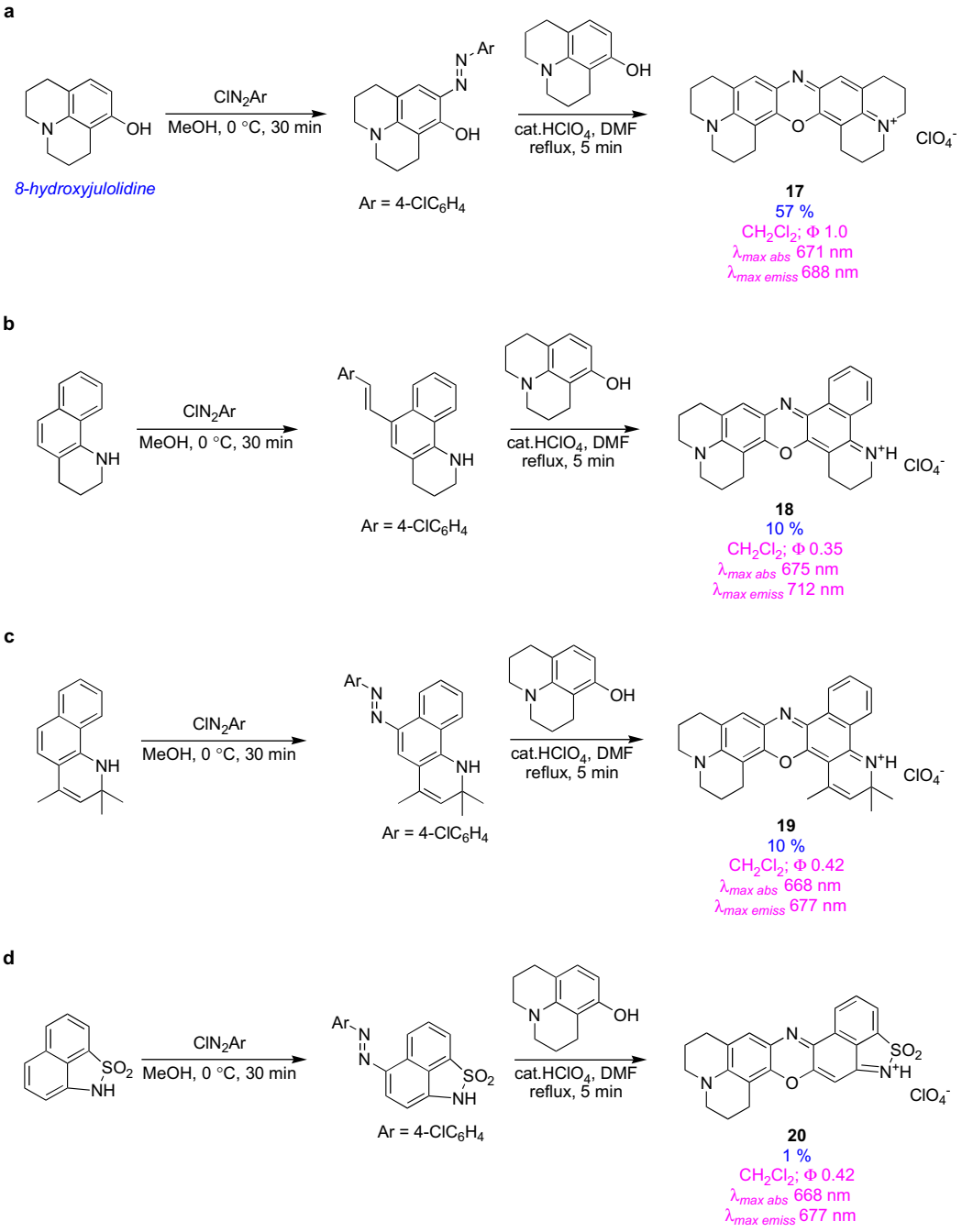
Like Nile Red **2**, Nile Blue shows progressively longer absorption and emission maxima as the solvent polarity is increased. However, the Stokes' shifts observed for the red probe **2** in different solvents is far greater; this parameter for the Blue compound can still be exceptionally high (almost 100 nm), but in polar solvents it is reduced to around 40 nm (Table 4).[44]

Table 4. UV absorption and fluorescence emission maxima of Nile Blue **3** in different solvents

Solvent	$\lambda_{max\ abs}$ (nm)	$\lambda_{max\ emiss}$ (nm)
Toluene	493	574
4-Chlorobenzene	503	576
Acetone	499	596
DMF	504	598
$CHCl_3$	624	647
1-Butanol	627	664
2-Propanol	627	665
EtOH	628	667
MeOH	626	668
Water	635	674
1.0 N HCl, pH 1.0	457	556
0.1 N NaOH, pH 11.0	522	668
NH_4OH, pH 13.0	524	668

Table 5. Study of effect of different anions on spectral properties of Nile Blue in different solvents

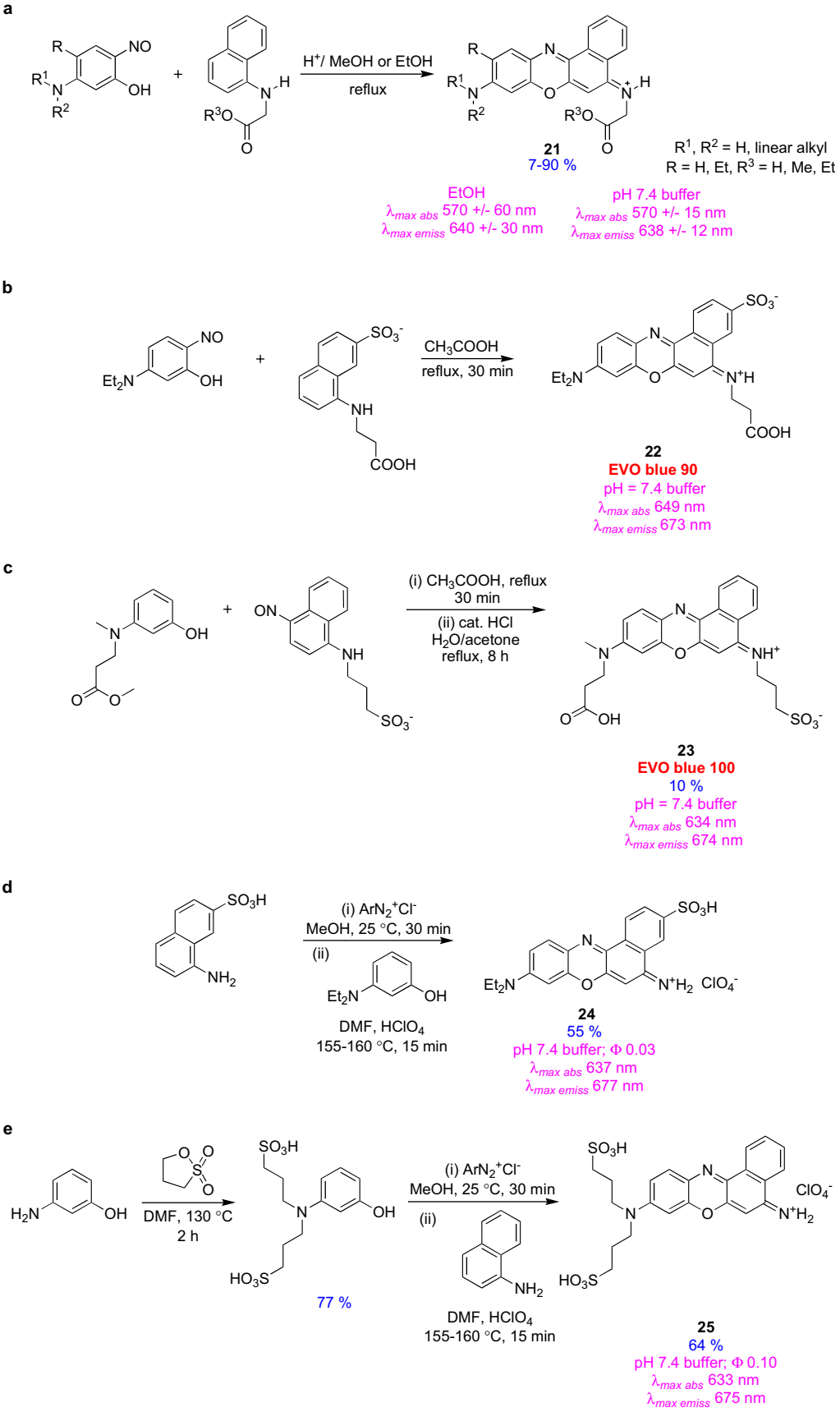
Anion	Solvent	$\lambda_{max\ abs}$ (nm)	$\lambda_{max\ emiss}$ (nm)
Chloride	EtOH	628	689
		632	—
		634	694
Acetate	EtOH	628	689
		632	687
		634	700
Benzoate	EtOH	628	691
		632	690
		634	702
iso-Butanoate	EtOH	628	691
Hydroxide	EtOH	515	661



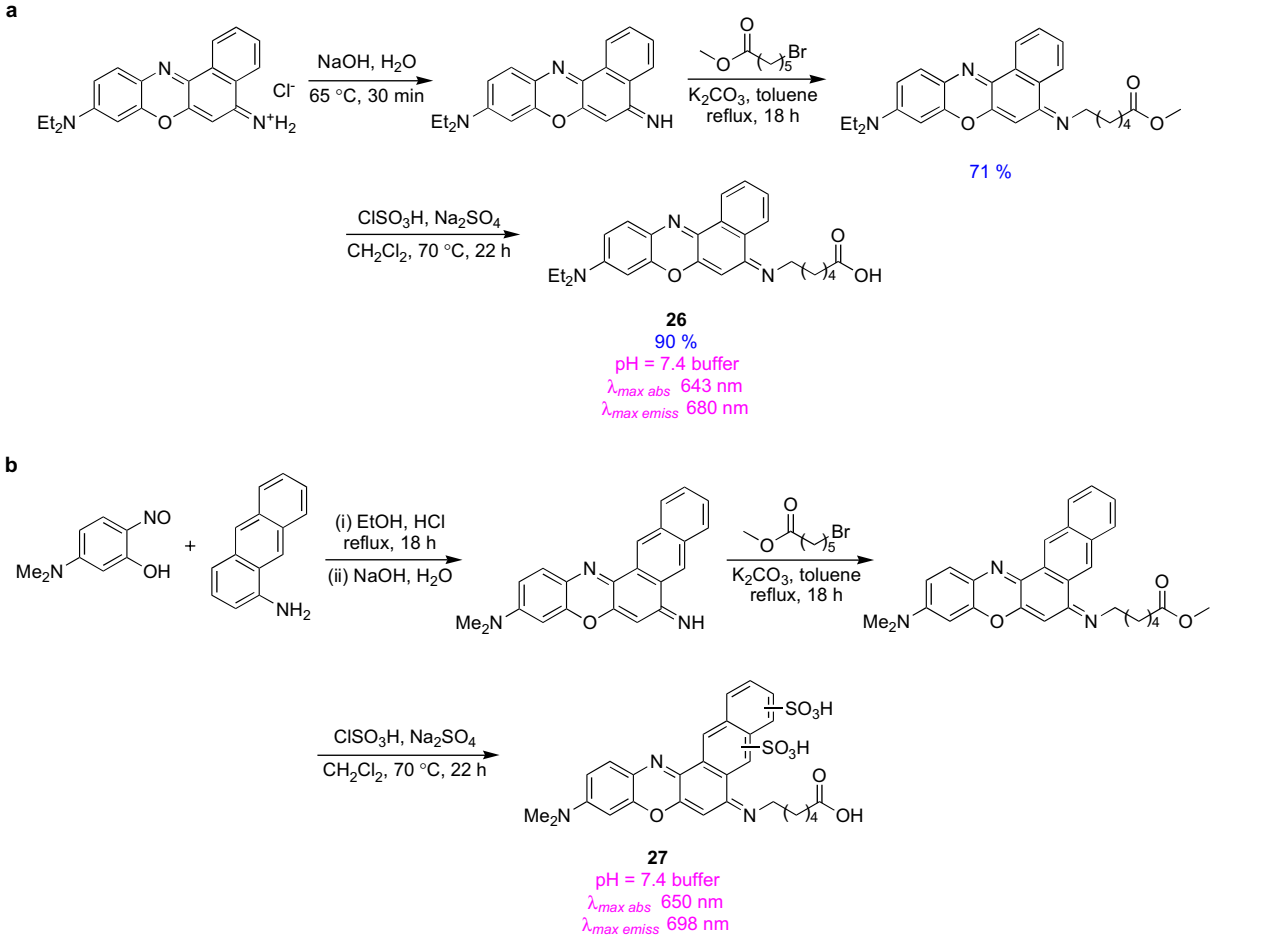
Scheme 8. Syntheses of Nile Blue derivatives from 8-hydroxyjulolidine.

Table 4 also reveals that the spectroscopic characteristics of Nile Blue are pH dependent. This is because under basic conditions the iminium group will be deprotonated, whereas under strongly acidic conditions the 5-amino might even become protonated.[45] Curiously, the spectroscopic properties of Nile Blue are somewhat dependent on the counter ion used.[46] We speculate that this could even be a reflection on intimate ion pairing influencing the solvent sphere of the dye (Table 5).

The fluorescence lifetime of Nile Blue **3** in ethanol has been measured at 1.42 ns. This is shorter than the corresponding value of Nile Red (see above; 3.65 ns). The lifetime of Nile Blue is relatively invariant at dilute concentrations (10^{-3}–10^{-8} mol dm^{-3}) but changes as the concentration is increased, and in different solvents. This is probably an indication of the interdependence of the spectroscopic properties and the degree of aggregation in solution. In support of this assertion, we note that the lifetimes do not seem to be significantly impacted by the viscosity of the medium, but they are temperature dependent.[47] As far as we are aware, the two-photon cross-section of Nile Blue has not been reported.



Scheme 9. Syntheses of Nile Blue derivatives with water solubilizing *N*-substituents.



Scheme 10. Preparation of water-soluble Nile Blue derivatives: (a) with only carboxylic side chain; (b) with carboxylic acid side chain and two additional sulfonic acid groups.

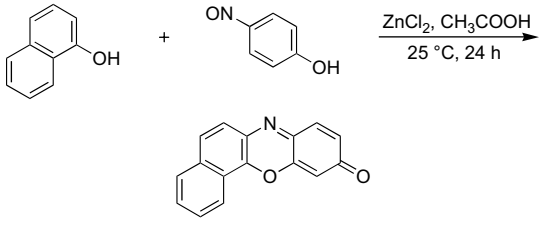
5. Other benzophenoxazine dyes

5.1. Introduction

There is no obvious reason why benzo[*a*]phenoxazines should be more fluorescent than similar compounds with different ring fusion patterns. Our interpretation of the literature is that other phenoxazines with appropriate substituents have certainly been less well studied as fluorescence probes, and are probably less synthetically accessible.

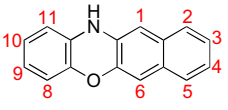
5.2. Syntheses

The benzo[*c*]phenoxazine **32** has been prepared via a route that is similar to those used for the Nile compounds **2** and **3**. 1-Naphthol is used in this synthesis (reaction 3)[46] rather than 2-naphthol, the isomer used for **2** and **3**. This change is necessary to obtain the different ring fusion, but it also may account for the very poor yield. This is because of the well-known reduced reactivity for 1-napthol at the 2-position in electrophilic substitution reactions, relative to the greater tendency for 2-napthol to react at the 1-position. To the best of our knowledge, the fluorescent properties of **32** have not been reported in any depth; indeed, the molecule lacks an electron donor in conjugation with the carbonyl to give it the type of extended oscillating dipole that seems to be common for fluorescent molecules.



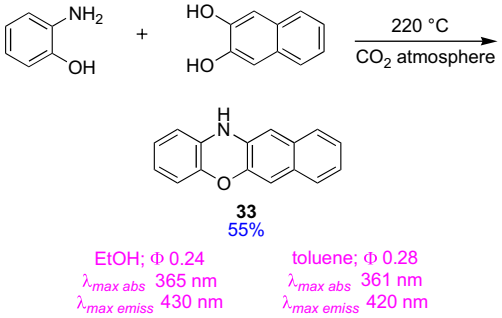
(3)

Benzo[*b*]phenoxazines are linear. Substituted derivatives of these are numbered according to the system shown below.



The linear system **33** has been prepared via the high temperature condensation process shown in reaction 4.[48] This has absorption and fluorescence emission properties that are characteristic of an extended aromatic heterocycle.

Like **32**, this compound does not have substituents that would allow it to be reduced to a phenoxazinone or phenoxazinium form.



(4)

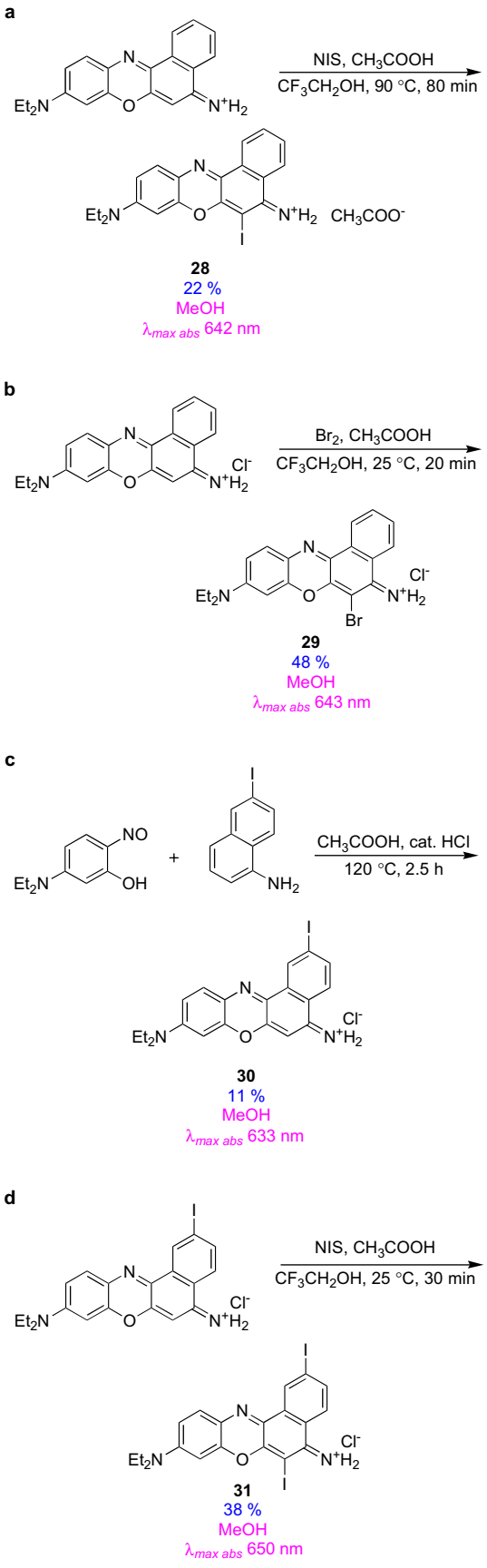
Some nitro and amino derivatives of benzo[*b*]phenoxazines have been reported in literature, and Scheme 12 shows them.[49] Parts a and b show nitrosylation/oxidation reactions that can be used to prepare nitro-substituted derivatives **35** and **36**. Predictably, these are not particularly fluorescent compounds. However, the 1,9-diaminobenzo[*b*]phenoxazinium **37** has the potential to be strongly fluorescent. Unfortunately, it was neutralized to **38** *then* the fluorescence properties were recorded.

6. Conclusions

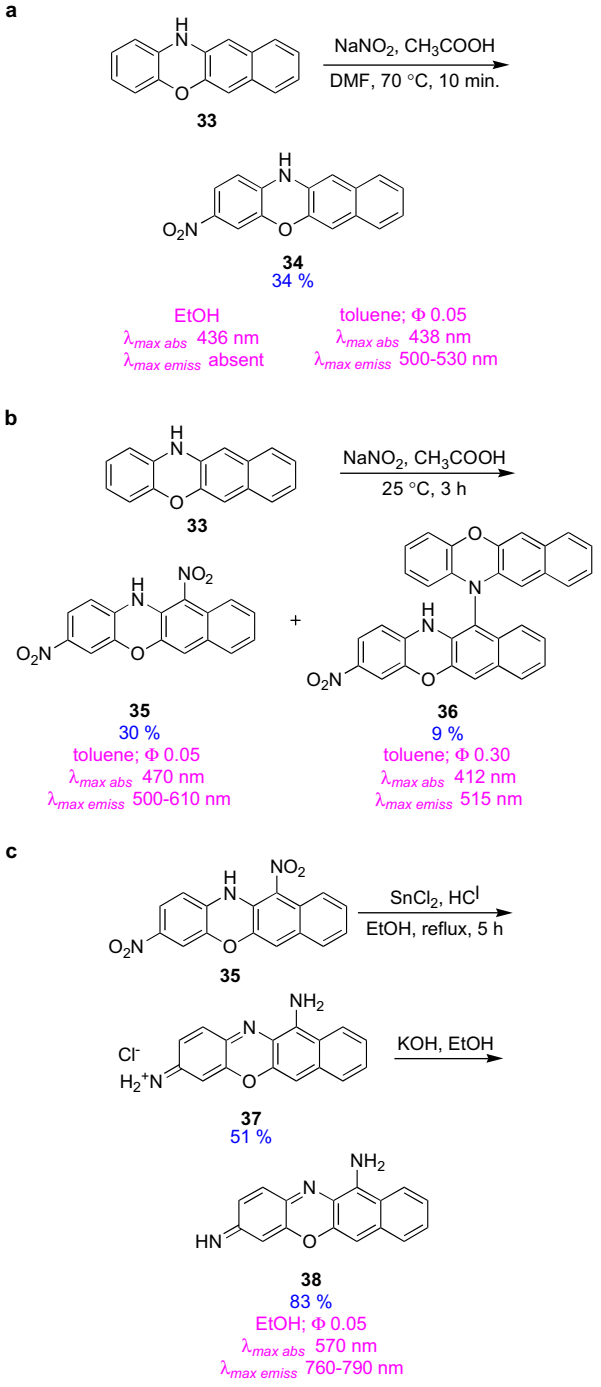
Current knowledge of fluorescent benzophenoxazine-derived probes is based almost on the benzo[*a*]phenoxazine ring fusion series. Almost all the syntheses feature high temperature condensation methods, and not contemporary synthetic methods like, for example, Buchwald–Hartwig couplings to introduce amine substituents. In fact, many of the synthetic methods reported are based on the procedures that are now over a century old. This, and the prevalence of patent literature in this area, mean that many of the experimental procedures presented are difficult to follow, and complete spectroscopic data is rarely recorded.

Nile Red and its derivatives have some interesting spectroscopic properties (long wavelength emissions, large Stokes' shifts) but most compounds in this series have limited water solubilities. There are, however, some recent efforts to make modified compounds to redress this. Nile Blue and its derivatives tend to be more water soluble.

Relatively little work has been done to modify benzophenoxazine dyes so that they emit even further to the red: the longest wavelength emission in the existing probes is about 700 nm. To this end, it would be useful to have access to more functionalized compounds that can be prepared easily on a gram scale, like 2-hydroxy Nile Red **6**. Other modifications might be used to give derivatives with enhanced extinction coefficients (these tend to be 10,000 or less), or improved two-photon cross-sections. These types of developments would be facilitated by more detailed studies of fundamental reactions that can be used to modify these compounds, e.g., halogenation and nitration.



Scheme 11. Preparation of halogenated Nile Blue derivatives: (a) 6-iodo; (b) 6-bromo; (c) 2-iodo; and (d) 2,6-diiodo.



Scheme 12. Syntheses of benzo[*b*]phenoxazine derivatives: (a) 9-nitro; (b) 1,9-dinitro and 9-nitro-1,12-bis(benzo[*b*]phenoxazine); and (c) 1-amino-9-iminobenzo[*b*]phenoxazine.

As far as we can see, there is no good reason why virtually all fluorescent dyes in this series are benzo[*a*]phenoxazine derivatives, and not any other ring fusion. This appears to be merely a question of synthetic availability.

Overall, benzophenoxazine-based probes are an intriguing subset of the fluorescent dye toolbox. They have some obvious drawbacks for applications in biotechnology, but the developments that need to be made to make more useful labels of this type are reasonably well defined.

Acknowledgements

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Biographical sketch

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The ionic liquid ethyltri-*n*-butylphosphonium tosylate as solvent for the acid-catalysed hetero-Michael reaction

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Abstract—A new and convenient method for the acid-catalysed Michael addition reactions of alcohols, thiols and amines to methyl vinyl ketone has been developed using the ionic liquid ethyltri-*n*-butylphosphonium tosylate. The reaction conditions are mild and obviate the need for toxic and expensive Lewis acid catalysts, offering advantages over more commonly used systems.

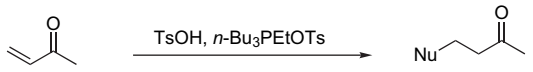
1. Introduction

The Michael addition[1] is a powerful reaction for the formation of carbon–carbon and carbon–hetero atom bonds.[2] Both Michael- and hetero-Michael additions are widely used in inter- and intra-molecular reactions to give products, which include important building blocks for many biologically active molecules. Hetero-Michael addition of amines to α,β-unsaturated carbonyl compounds gives β-amino ketones, which are attractive for their use as synthetic intermediates of anticancer agents, antibiotics and other drugs.[3] Michael addition of a thiol to an α,β-unsaturated ketone results in the formation of a sulfur–carbon bond; this is a key reaction in the synthesis of biologically active compounds such as the calcium antagonist diltiazem.[4] Similarly, β-oxy ketones are also important in organic synthesis.[5] Moreover the β-amino, β-thio and β-oxy ketone functionalities occur in many natural products.[6]

The uncatalysed addition reaction of alcohols to α,β-unsaturated ketones proceeds very slowly and gives only moderate yields of the β-oxy ketones. Other methods most commonly employed use red mercury oxide and boron trifluoride etherate as catalysts, which are both toxic.[7a,b] These methods also require working under an inert atmosphere. Other methods widely used are the acid- or base-catalysed reaction of the neat reagents but in this case the reaction mixture needs to be neutralised very carefully, otherwise the ethers decompose on distillation. A significant drawback to this method is that the reaction is difficult to control and can be very violent thus requiring cooling or the use of an inert solvent. The Mannich reaction is a classic method for the preparation of β-amino ketones[8a] but this reaction has serious disadvantages, including drastic reaction conditions and long reaction times.[8a–d] In contrast, the aza-Michael addition route usually requires acid- or base-catalysis to activate one of the substrates.[2,9] High costs coupled with environmental issues due to the large amount of acidic effluents being generated are drawbacks associated with Lewis acid-catalysed aza-Michael reactions.[10] Thiols are typically difficult to use in the presence of Lewis acidic metals since they are known to poison these catalysts. In the thia-Michael reaction, the thiols are generally activated by deprotonation.[11]

The past five years have seen concerted efforts to develop effective, economical and environmentally friendly methodologies for the hetero-Michael addition. Some of these exciting developments include the use of an azaphosphatrane nitrate salt,[12] $CeCl_3 \cdot 7H_2O$/NaI,[13] Nafion® SAC-13,[14] $Bi(NO_3)_3$,[15] $Bi(OTf)_3$,[16] $InBr_3$,[17] $Cu(BF_4)_2$,[18] and strong Brønsted acids.[19] There are also examples of the use of ammonium ionic liquids as catalysts and/or solvents for the hetero-Michael addition of thiols,[20] and aliphatic amines.[21]

With a view to establishing more practical reaction conditions, as well as facilitating the recovery of the products, we have investigated the application of the phosphonium ionic liquid, ethyltri-*n*-butylphosphonium tosylate (*n*-Bu_3PEtOTs) in the acid-catalysed hetero-Michael addition of a series of alcohols, thiols and amines to methyl vinyl ketone (MVK) as the prototypical Michael acceptor (Scheme 1). This method obviates the need for expensive metal catalysts and strong bases, and instead uses cheap and readily available reagents.



NuH = ROH, RNH_2, R_2NH, RSH
R = aliphatic, aromatic

Scheme 1. Acid-catalysed hetero-Michael addition in *n*-Bu_3PetOTs.

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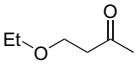
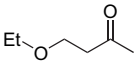
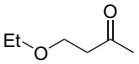
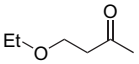
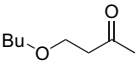
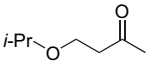
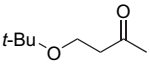
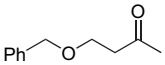
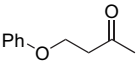
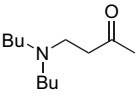
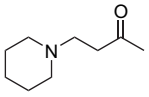
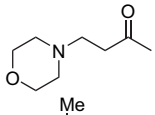
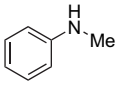
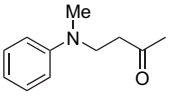
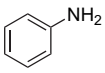
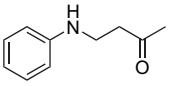
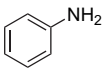
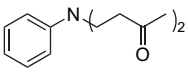
Phosphonium tosylates have proven to be effective solvents for organic synthesis, having the advantages of high thermal stability, tolerance towards air and moisture and low vapour pressures. Phosphonium tosylates are cheap, non-corrosive and some are solid at room temperature, making them easy to handle and to separate from the reaction products.[22,23]

2. Results and discussion

Ethyltri-*n*-butylphosphonium tosylate (*n*-$Bu_3PEtOTs$) was chosen because its melting point permits reactions at relatively low temperatures. It also crystallises readily, making it easy to separate from the product and recycle. In addition, it is economically and readily synthesised from commercially available starting materials (tri-*n*-butylphosphine and ethyl tosylate) at low cost. Initially the hetero-Michael reaction was attempted in the ionic liquid without any catalyst, however, no detectable yield of product was observed. *p*-Toluenesulfonic acid monohydrate ($TsOH\cdot H_2O$) was chosen as the catalyst because it is the conjugate acid of the solvent anion. The optimum quantity for the reaction was established as 2% by systematically varying the quantity of the catalyst and monitoring the yield of the product obtained.

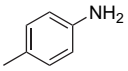
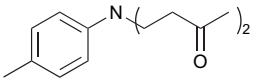
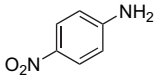
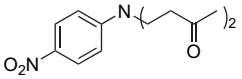
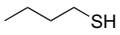
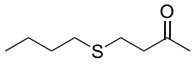
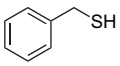
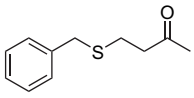
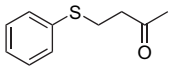
Selected results are summarised in Table 1. The reaction with the aliphatic alcohols, which are weak nucleophiles, (Entries 1–8) gave yields ranging from 12–75%, which compare well with other methods.[7,14] Varying the ratio of donor and acceptor did not affect the yields significantly (Entries 1–3). In our adaptation, the best isolated yield (59%) for the reaction with ethanol (Entry 3) compares well with the

Table 1. Hetero-Michael addition to methyl vinyl ketone (MVK)

Entry	Michael donor	MVK/Michael donor	Time [h]	Product	Yield (%)[a] (isolated)	Reference
1	EtOH	1:1	3		62[24]	27
2	EtOH	1.5:1	3		67	27
3	EtOH	1:1.5	3		66 (59)	27
4	EtOH	1:1	6		64	27
5	*n*-BuOH	1:1	3		76 (63)	28
6	*i*-PrOH	1:1	3		52 (45)	29
7	*t*-BuOH	1:1	3		22 (12)	30
8	$C_6H_5CH_2OH$	1:1	3		80 (75)	19
9	C_6H_5OH	1:1	3		100	26
10	Bu_2NH	1:1	1		82	31
11		1:1	1		83	32
12		1:1	1		80	31
13		1:1	3		90	12
14		1:1	3		95[b]	33
15		2.1:1	3		100 (98)	34

(*continued*)

Table 1. (*continued*)

Entry	Michael donor	MVK/Michael donor	Time [h]	Product	Yield (%)[a] (isolated)	Reference
16		2.1:1	3		100 (98)	34
17		2.1:1	3		100 (98)	33
18		1:1	3		95 (90)	20a
19		1:1	3		100 (96)	14
20		1:1	3		100	12

Reactions were conducted at 40 °C on a 5.0 mmol scale.
[a] Yield determined by ^{1}H NMR.
[b] A small amount (2%) of the bis-addition product was also obtained.

yields for the $BF_3 \cdot OEt_2$-catalysed reaction (52–77%)[7a] and is only slightly lower than the reaction catalysed with sodium ethanolate (71%).[24] The only method giving significantly higher yields (95%) is the acid-catalysed reaction of MVK in an excess of ethanol.[25] The reactions with *n*-butanol (Entry 5) and benzyl alcohol (Entry 8) were similar to those of the other primary alcohols and good yields of 63% and 75% were obtained. The reaction of benzyl alcohol compares well with the Brønsted acid-catalysed reaction where 72% yield was obtained after 48 h.[19] In comparison to the primary alcohols, the yield for isopropanol (Entry 6) was marginally lower (~45%) but still superior to the known base-catalysed (12–20%)[24] and the $BF_3 \cdot OEt_2$-catalysed reactions (34%).[7] Unsurprisingly, *tert*-butanol (Entry 7) gave lower yield (22%), as expected for a hindered nucleophile. A yield was not quoted for the equivalent $BF_3 \cdot OEt_2$/HgO-method.[7] In comparison to the pyridine-catalysed reaction of phenol (Entry 9) with MVK at 100 °C, the $Bu_3PEtOTs$/TsOH system was more efficient giving 100% conversion in only 3 h.[26] These oxo-Michael reactions in ethyltri-*n*-butylphosphonium tosylate cannot be compared with the corresponding reactions in imidazolium ionic liquids since there are no reports of oxo-Michael reactions in this class of ionic liquids.

A broad range of aliphatic and aromatic amines were also effective nucleophiles, generating 4-aminobutanone derivatives (Entries 10–17). The reactions were fast and excellent yields were obtained (82–98%) with both primary and secondary amines. These results compare very well with the recently reported improved method for the reaction of di-*n*-butylamine with MVK, involving Lewis acid catalysis by a $CeCl_3 \cdot 7H_2O$/NaI system supported in SiO_2, which gave the addition products in 84% yield after 5 h.[13] Whilst the yield is comparable with that obtained in the $Bu_3PEtOTs$/TsOH system (82%) the reaction time was greatly extended. Furthermore, the workup involved extraction, washing and flash chromatography, which are unnecessary with the reactions in the phosphonium ionic liquid. Similarly the reactions of piperidine and morpholine gave 4-piperidin-1-yl-butan-2-one and 4-morpholinobutan-2-one in 83% and 80% yields, respectively, which compares favourably with the reaction catalysed by the azaphosphatrane nitrate where yields of 88% and 90% were obtained after 20 h reaction time.[12] The $Cu(acac)_2$-catalysed reaction in the ionic liquid [bmim]BF_4, appears to be the most efficient for the reactions of aliphatic amines giving high yields in 10–60 min.[21b] Excellent yields were also obtained with aromatic amines (Entries 12–14) and these are superior to the yields obtained with strong Brønsted acids.[19]

We found that both aliphatic and aromatic thiols reacted equally well with methyl vinyl ketone in the $Bu_3PEtOTs$/TsOH system and the Michael adducts were obtained in excellent yields (95–100%) (Entries 18, 20). This is a favourable contrast with the Brønsted acid catalyst, Tf_2NH, which was used in acetonitrile as the solvent.[19] Thiophenol could not be used as a reactant in that system and a yield of only 66% was obtained after 1 h for benzyl mercaptan.[19] The yields were similar to those obtained with bismuth triflate.[16] While the reaction of thiophenol in the ammonium ionic liquid, 1-pentyl-3-methylimidazolium bromide was rapid and the Michael adduct was isolated in 75% yield after only 45 min,[20b] the reaction catalysed by an azaphosphatrane nitrate salt resulted in 95% yield after 40 h.[12] There appears to be an advantage in using ionic liquids as solvents/catalysts for this reaction.

3. Conclusion

We have developed a new method for the hetero-Michael addition of alcohols, thiols and amines to methyl vinyl ketone, which uses very small amounts of a non-aqueous, non-volatile and inexpensive acid as catalyst. The yields are good, even when equimolar amounts of the reagents are used. In addition, we are avoiding some of the problems associated with the other methods recently developed. The reaction conditions are mild and there is no need to work under an inert atmosphere.

The role of the ionic liquid in the hetero-Michael reaction is not yet understood and further investigations are necessary. These results show that the phosphonium ionic liquid acts differently to the ammonium-based ionic liquids in that both aliphatic and aromatic amines reacted equally well in the phosphonium ionic liquid while only aliphatic amines have been successful Michael donors in ammonium ionic liquids.[21] The oxo-Michael reaction has not been reported in ammonium ionic liquids and therefore comparisons cannot be made, however, the thia-Michael reaction has been well studied and excellent yields were reported, which are comparable to the results obtained in this study.[20]

4. Experimental

4.1. General

Chemicals were obtained from Aldrich or Lancaster. Methyl vinyl ketone and aniline were distilled prior to use, all other materials were used as received. ^{1}H and ^{13}C NMR spectra were recorded at 270 and 68 MHz, respectively, on a Jeol GX270 spectrometer. All spectra were measured in $CDCl_3$ as the solvent and the chemical shifts were referenced to tetramethylsilane (TMS) as an internal standard (0 ppm). The ^{31}P NMR spectrum was recorded at 121 MHz on a Bruker AM-300 spectrometer at 121 MHz in $CDCl_3$ as the solvent and phosphoric acid as the external standard. The IR spectrum was recorded using a KBr disc on a Nicolet 140 FTIR spectrometer in the range 4000–400 cm^{-1}. The accurate mass measurement was carried out at the EPSRC National Mass Spectrometry Service Centre, Chemistry Department, University of Wales, Swansea. The melting point was recorded on a Reichert hot-stage melting point apparatus and is uncorrected.

4.2. Preparation of ethyltri-*n*-butylphosphonium tosylate

Ethyltri-*n*-butylphosphonium tosylate was prepared by heating a solution of tri-*n*-butylphosphine (11 g, 54 mmol) and ethyl tosylate (11 g, 54 mmol) in dry toluene (40 mL) at 100 °C under a nitrogen atmosphere for 16 h. The solvent was evaporated to give a white solid, which was suspended in dry ether, filtered and washed with dry ether to furnish the product (96%) as a white solid, mp 73–76 °C (lit.: 74.5–75.2 °C),[22a] IR ν_{max}/cm^{-1} (KBr) 3086w (CH), 3050w (CH), 3021w (CH), 2950s (CH), 2929s (CH), 2865s (CH), 1465m (P–C), 1129vs (S═O), 1043s, 1014s, 815s, 710s, 681s, 579s; δ_H ($CDCl_3$) 0.91 (9H, t, *J* 7, 3×CH_3(CH$_2$)$_3$P), 1.22 (3H, dt, *J* 18 and 8, CH_3CH$_2$P), 1.42–1.56 (12H, m, 3×CH$_3$(CH_2)$_2$CH$_2$P), 2.17–2.39 (6H, m, 3×CH$_3$(CH$_2$)$_2$CH_2P), 2.32 (3H, s, CH_3C$_6$H$_5$), 2.33 (2H, dt, *J* 13 and 8, CH$_3$CH_2P), 7.11 (2H, d, *J* 8, *H*-3 and 3′ of tolyl), 7.16 (2H, d, *J* 8, *H*-2 and 2′ of tolyl); δ_C ($CDCl_3$) 6.1 (d, *J* 5, *C*H$_3$CH$_2$P), 12.6 (d, *J* 50, CH$_3$*C*H$_2$P), 13.5 (3C, d, *J* 7, *C*H$_3$(CH$_2$)$_3$P), 18.3 (3C, d, *J* 47, CH$_3$CH$_2$CH$_2$*C*H$_2$P), 21.3 (*C*H$_3$C$_6$H$_5$), 23.7 (3C, d, *J* 4, *C*H$_2$CH$_2$P), 23.9 (3C, d, *J* 13, *C*H$_2$CH$_2$CH$_2$P), 126.1 (C-3 and 3′ of tolyl), 128.4 (C-2 and 2′ of tolyl), 138.9 (C-4 of tolyl), 144.5 (C-1 of tolyl); δ_P +35.2; *m*/*z* (EI) 231.2236 (M^+, $C_{14}H_{32}P$ requires 231.2236), 171.0109 (M^-, $C_7H_7SO_3$ requires 171.0121).

4.3. Representative procedure

In a typical reaction methyl vinyl ketone (350 mg, 5 mmol), ethanol (230 mg, 5 mmol), *p*-toluenesulfonic acid (20 mg, 0.1 mmol) and ionic solvent *n*-Bu$_3$PEtOTs (2 g) were placed in a 25 mL round-bottomed flask fitted with a condenser and a magnetic stirrer bar. The mixture results in a homogeneous solution on heating at 40 °C and the reaction mixture was stirred at this temperature for 3 h. The product was isolated by Kugelrohr distillation or extraction with diethyl ether (3×10 mL) followed by filtration through a silica plug to give 4-ethoxybutan-2-one; δ_H ($CDCl_3$) 1.18 (3H, t, *J* 7, CH_3CH$_2$–O), 2.19 (3H, s, CH_3CO), 2.69 (2H, t, *J* 6, O–CH$_2$CH_2CO), 3.49 (2H, q, *J* 7, CH$_3$CH_2–O), 3.68 (2H, t, *J* 6, O–CH_2CH$_2$CO);[27] δ_C ($CDCl_3$) 15.0 (*C*H$_3$CH$_2$–O), 30.3 (*C*H$_3$C═O), 43.7 (O–CH$_2$*C*H$_2$CO), 65.4 (O–*C*H$_2$CH$_2$CO), 66.3 (CH$_3$*C*H$_2$–O), 207.2 (*C*═O).[27]

Similarly, all the products of the hetero-Michael reactions were analysed by ^{1}H NMR spectroscopy and the data compared with the literature (Table 1).

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Enantiodivergent synthesis of muricatacin related lactones from D-xylose based on the latent symmetry concept: preparation of two novel cytotoxic (+)- and (−)-muricatacin 7-oxa analogs

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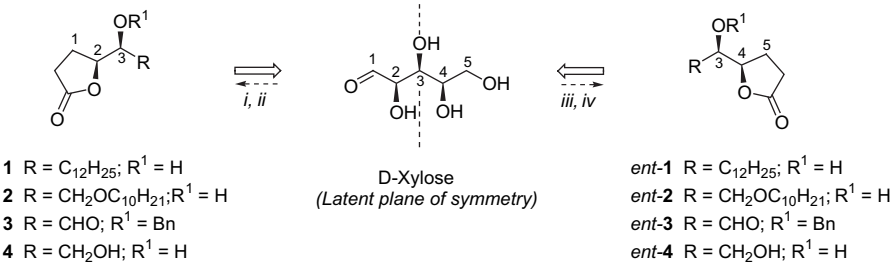
Abstract—Enantiodivergent formal synthesis of (+)- and (−)-muricatacins from D-xylose has been accomplished through utilization of the latent plane of symmetry present in the starting monosaccharide. This approach was extended to the preparation of two novel (+)- and (−)-muricatacin 7-oxa analogs (**2** and *ent*-**2**, respectively), which showed in vitro antitumor activity toward some human malignant cells. The analog *ent*-**2** showed a powerful antiproliferative activity against the K562 cell line, being 36-fold more potent than the standard cytotoxic agent, doxorubicin. Compound **2**, however, showed a powerful cytotoxic activity against HL-60 cells, being more than 17-fold more potent with respect to the reference compound.

1. Introduction

Development of efficient chemical pathways that allow the preparation of both enantiomers of compounds of biomedicinal interest is an important goal in the synthetic organic chemistry. Enantiomers of biologically active natural products often exhibit improved potencies or even novel activities altogether. For example, the unnatural (−)-enantiomer of the antitumor, antibiotic roseophilin is 2–10 times more potent than the natural (+)-isomer in cytotoxicity assays.[1] Numerous nucleoside analogs of natural D-configuration displayed a potent antitumor or antiviral activity, but most of them were found to be too toxic for general clinical applications. On the contrary, the corresponding L-configuration counterparts are not recognized by normal cellular enzymes and are less toxic to normal cells.[2] A number of biologically active natural products comprised of both (+)- and (−)-enantiomers have been described.[3,4] One such molecule that has attracted considerable attention since its isolation from the seeds of the tropical plant *Anona muricata*[4] is muricatacin (5-hydroxy-4-heptadecanolide), an acetogenin derivative that shows strong cytotoxic activity against certain human tumor cell lines. The isolated sample was a mixture of enantiomers **1** and *ent*-**1** (Scheme 1) with the (−)-(*R*,*R*)-isomer



Scheme 1. Enantiodivergent strategy for the preparation of (+)- and (−)-muricatacin related lactones by chirality transfer from D-xylose (sugar numbering). (i) 'CH_2CO_2R'—introduction at C-1 followed by γ-lactonization, (ii) oxidative glycol cleavage of the C_4–C_5 bond, (iii) 'CH_2CO_2R'—elongation at C-5 followed by γ-lactonization, (iv) C_1–C_2 glycol cleavage.

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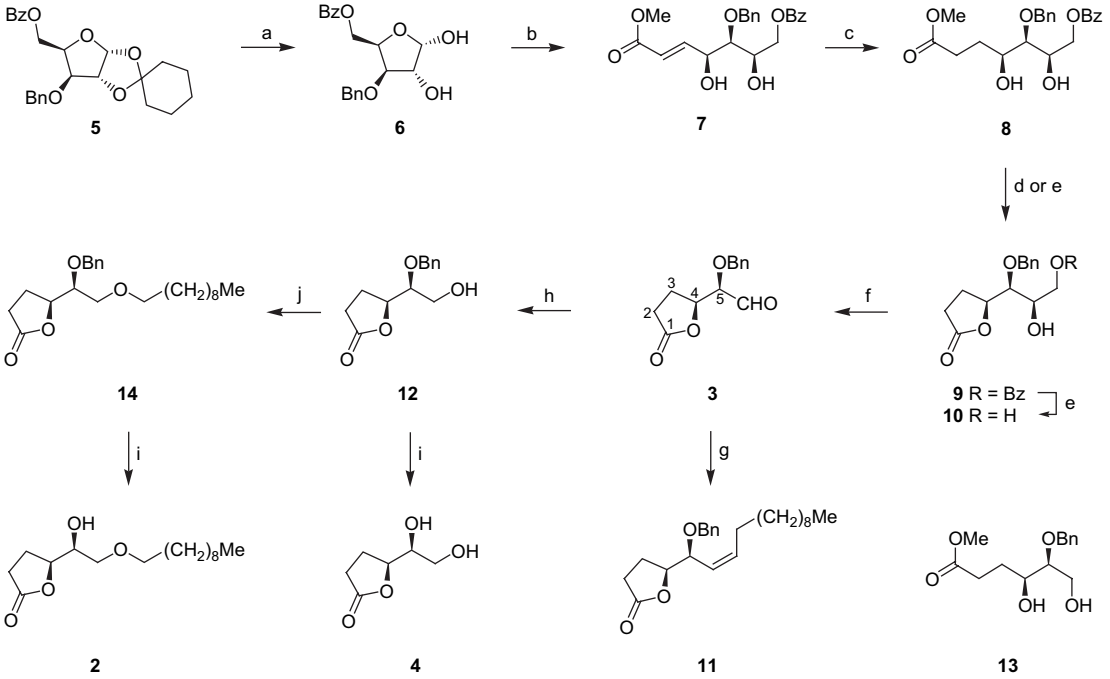
ent-**1** being predominant (ee, ca. 25%). Both (+)- and (−)-muricatacins show similar antitumor potency,[4,5] and not surprisingly were the object of numerous synthetic efforts. Syntheses of (+)- and/or (−)-muricatacin from various non-carbohydrate precursors have been reported,[5,6] along with a number of carbohydrate based approaches,[7,8] most being target oriented. Hence, development of new and flexible strategy that would enable the preparation of not only both enantiomers **1** and *ent*-**1**, but also a variety of their isosteric analogs is still demanded. A number of muricatacin stereoisomers and analogs have also been synthesized,[9,10] but only a few have been evaluated for their antitumor activity.[10] Herein we report a novel general approach to the enantiodivergent formal synthesis of (+)- and (−)-muricatacins from D-xylose[11] based on the latent symmetry concept,[12] as well as the preparation and antitumor screening of two novel (+)- and (−)-muricatacin 7-oxa analogs **2** and *ent*-**2**.

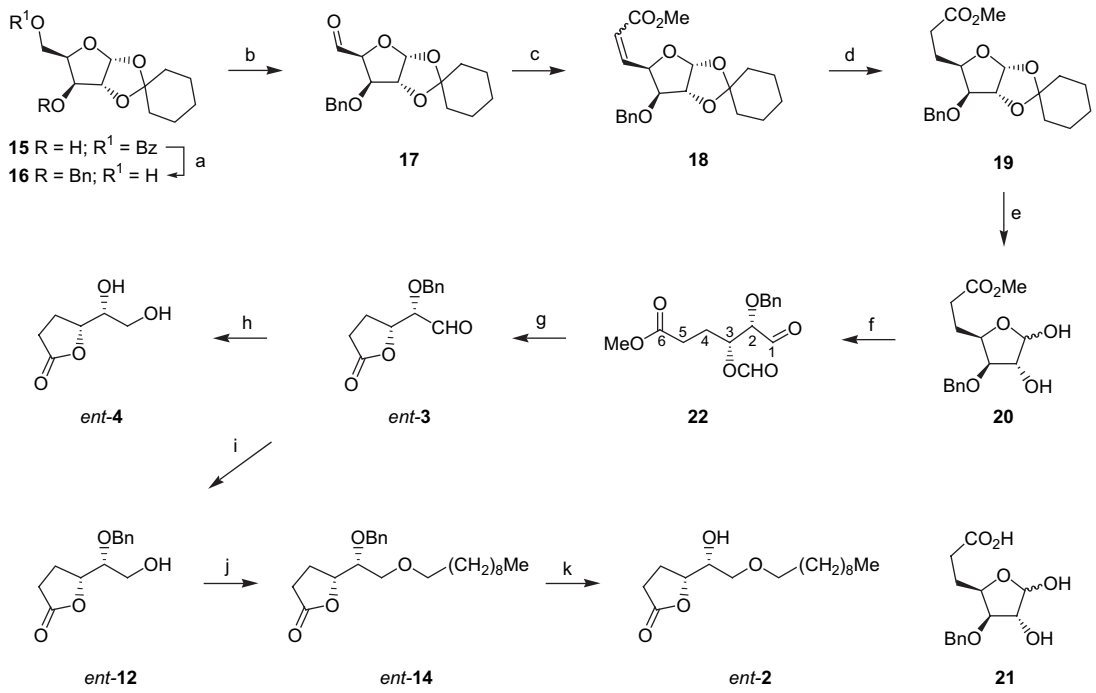
2. Results and discussion

Our enantiodivergent strategy relies on the synthesis of both enantiomeric forms of aldehydo-lactone **3** from D-xylose. As outlined in Scheme 1, (+)-muricatacin (**1**) might be prepared by a sequence that will ensure the introduction of the C-2 and C-3 stereocenters of D-xylose into the target structure **1** via the aldehydo-lactone **3**. It was further assumed that the intermediate **3** should be available from a suitably protected D-xylose derivative through the following several key steps: (i) 'CH_2CO_2R'—introduction at C-1 followed by γ-lactonization and (ii) oxidative glycol cleavage of the C_4–C_5 bond. Due to the latent plane of symmetry present in the starting monosaccharide an alternative sequence, which involves (iii) 'CH_2CO_2R'—elongation at C-5 followed by γ-lactonization and (iv) C_1–C_2 glycol cleavage in a suitably protected D-xylose derivative, should provide access to the aldehydo-lactone *ent*-**3** bearing the C_3–C_4 chiral segment of D-xylose. It was further assumed that both intermediates **3** and *ent*-**3** could be converted to the targets **1** and *ent*-**1** by a Wittig elongation/catalytic reduction process. Alternatively, the chiral synthons **3** and *ent*-**3** may be first converted to the dihydroxylactones **4** and *ent*-**4** and finally to the targets **1** and *ent*-**1** via a known two-step sequence.[8] Moreover, we have also planned to adopt this enantiodivergent strategy for the preparation of both enantiomeric forms of hitherto unknown muricatacin 7-oxa analogs **2** and *ent*-**2** via the hydroxylactones **12** (Scheme 2) and *ent*-**12** (Scheme 3).

The formal synthesis of **1**, along with the preparation of corresponding 7-oxa-analog **2** is summarized in Scheme 2. The sequence started from 5-*O*-benzoyl-3-*O*-benzyl-1,2-*O*-cyclohexylidene-α-D-xylofuranose (**5**), which is prepared from D-xylose in four steps.[13] Hydrolytic removal of the cyclohexylidene protective group in **5** with aq acetic acid, gave the corresponding lactol **6**. Wittig olefination of **6** with methyl(triphenylphosphoranylidene)-acetate in DMF took place stereoselectively to afford the (*E*)-unsaturated ester **7** (83%) as the only isolable product. The *E*-selectivity of this step was essential, because it is well known that similar (*Z*)-α,β-unsaturated esters rapidly undergo a sequential lactonization/Michael ring-closure process.[16] Catalytic hydrogenation of **7** over PtO_2 in ethanol yielded the corresponding saturated ester **8**, which upon treatment with aq trifluoroacetic acid gave hydroxylactone **9** in excellent yield. Sodium methoxide O-debenzoylation of **9** furnished a moderate yield of dihydroxylactone **10**. By using the last two-step sequence ester **8** was converted to lactone **10** in 51% overall yield. However, we found that direct treatment of **8** with sodium methoxide in methanol provides the desired intermediate **10** in significantly higher yield (82%).



Scheme 2. (a) 7:3 AcOH/H_2O, reflux, 5.5 h, 85%; (b) $Ph_3P/CHCO_2Me$, DMF, 60–70 °C, 3.5 h, 84%; (c) H_2/PtO_2, EtOH, rt, 16 h, 75%; (d) 2:1 TFA/H_2O, rt, 2.5 h, 92%; (e) NaOMe, MeOH, rt, 1.5 h, 55% from **9**, 82% from **8**; (f) aq $NaIO_4$, silica gel, CH_2Cl_2, rt, 1 h, 89%; (g) $[Ph_3PCH_2(CH_2)_9Me]^+Br^-$, LiHMDS, THF, −78 °C→rt, 72 h, 7%; (h) (i) $NaBH_4$, MeOH, 0 °C→rt, 1.5 h, (ii) TFA, 2 h, 73%; (i) H_2-Pd/C, EtOAc, rt, 19 h, 69% of **4**, 82% of **2**; (j) $C_{10}H_{21}Br$, Ag_2O, AgOTf, Et_2O, reflux, 7.5 h, 80%.



Scheme 3. (a) (i) BnBr, NaH, DMF, 0 °C→rt, 2.5 h, (ii) NaOMe, MeOH, rt, 2 h, 89%; (b) DCC, anhyd H_3PO_4, Py, DMSO, rt, 3.5 h, 83%; (c) $Ph_3P/CHCO_2Me$, CH_2Cl_2, N_2, rt, 2 h, 97%; (d) H_2/PtO_2, EtOH, rt, 19 h, 92%; (e) 1:1 AcOH/H_2O, reflux, 1.5 h, 64% of **20**, 7% of **21**; (f) aq $NaIO_4$, silica gel, CH_2Cl_2, rt, 1.5 h, 98%; (g) 2:1 TFA/H_2O, rt, 1.5 h, 77% from **20**; (h) (i) $NaBH_4$, MeOH, 0 °C→rt, 2.5 h, (ii) TFA, 1 h, (iii) H_2-Pd/C, 19 h, 71%; (i) (i) $NaBH_4$, MeOH, 0 °C→rt, 2 h, (ii) TFA, 1 h, 68% from **20**; (j) $C_{10}H_{21}Br$, Ag_2O, AgOTf, Et_2O, reflux, 5.5 h, 71%; (k) H_2/PtO_2, EtOH, rt, 19 h, 87%.

Oxidative cleavage of the diol functionality in **10** was achieved by treatment with $NaIO_4$-impregnated wet silica in dichloromethane, whereby the aldehydo-lactone **3** was obtained. In the light of its stereochemical features the molecule **3** fully corresponds to the chiral lactone core of (+)-muricatacin (**1**).

With the requisite intermediate **3** in hand, we next focused on its C_{11}-elongation in order to elaborate the muricatacin side chain. According to the initial plan, Wittig olefination of aldehyde **3** with the appropriate C_{11}-ylide should enable us to resolve this problem. However, reaction of **3** with $Ph_3P{=}CH(CH_2)Me$ results in complex mixtures of products under a variety of experimental conditions, possibly because of the electrophilic nature of the lactone moiety. The best experimental protocol, which provides a poor yield (7%) of the desired olefin **11**, involved a reaction of aldehyde **3** with the Wittig reagent generated in situ from undecyltriphenylphosphonium bromide and LiHMDS in THF at −78 °C.[17] Disappointingly, all attempts to improve the outcome of this transformation were unsuccessful. We were therefore forced to find an alternative methodology for elaboration of the muricatacin side chain. According to the plan presented in Scheme 1, conversion of aldehyde **3** to the corresponding diol **4** represents a possible alternative route for completion of the synthesis.

The preparation of **4** began with the synthesis of the primary alcohol **12** from dihydroxylactone **10**. Oxidative cleavage of the terminal diol in **10** provided the aldehydo-lactone **3**, which was isolated in pure form after the usual work-up and used in the next step without further purification. Subsequent reduction of crude **3** with sodium borohydride gave the expected primary alcohol **12** along with an equal amount of ester **13**, as established from ^1H NMR of crude reaction mixture.[18] The mixture was not separated, but was further treated with aq trifluoroacetic acid to complete the lactonization of ester **13** into lactone **12**. The intermediate **12** was thus obtained in a 73% overall yield with respect to the starting compound **10** (based on recovered intermediate **3**). Catalytic hydrogenolysis of **12** (10% Pd/C) furnished the known diol **4**, which was recently used a convenient intermediate for the preparation of conformationally constrained analogs of diacylglycerol.[19] The ^1H and ^{13}C NMR spectral data and the optical rotation of diol **4** thus obtained were in full agreement with reported values.[19] Compound **4** can be converted to (+)-muricatacin according to the reported procedure.[8]

Hydroxylactone **12** also represents a divergent intermediate for the preparation of (+)-muricatacin 7-oxa analog **2**. Thus, O-alkylation of **12** with decyl bromide gave the corresponding 7-*O*-decyl derivative **14** (80%), which was subsequently *O*-debenzylated, under the conditions similar to those already used for the conversion of **12** to **4**. (+)-Muricatacin 7-oxa-analog **2** was thus obtained in 82% yield.

The 5-*O*-benzoyl-1,2-*O*-cyclohexylidene-α-D-xylofuranose (**15**), readily available from D-xylose,[14,15] was used as a starting material for the formal synthesis of (−)-muricatacin (*ent*-**1**), as well as for the preparation of its 7-oxa-analog *ent*-**2** (Scheme 3). Compound **15** was converted to the primary alcohol **16** through a simple one-pot procedure, which involved 3-O-benzylation of **15** (BnBr, NaH, DMF) followed by 5-O-debenzoylation of **5** (NaOMe, MeOH). The intermediate **16** was thus obtained in 89% overall yield with respect to **15**. Oxidation of the primary hydroxyl group in **16** gave the unstable[20] aldehyde **17** (83%), which upon treatment with methyl(triphenylphosphoranylidene)-acetate

in dry dichloromethane afforded the expected unsaturated ester **18** as a 2:1 mixture of the corresponding *Z*- and *E*-isomers. Catalytic hydrogenation of **18**, followed by hydrolytic removal of the cyclohexylidene protective group in **19**, gave a 64% yield of the corresponding lactol **20** (based on recovered **19**), accompanied with a small amount of the carboxylic acid **21** (7%). In an alternative procedure the crude mixture obtained after hydrolysis of **19** was treated with an ethereal solution of diazomethane to convert the carboxylic acid **21** to the ester **20**. In this way, the required intermediate **20** was obtained in 81% yield. Oxidative cleavage of purified diol **20** with sodium periodate on silica gel afforded the formate **22**, which upon treatment with aq trifluoroacetic acid yielded the γ-lactone *ent*-**3**, with absolute configuration of both stereocenters corresponding to (−)-muricatacin (*ent*-**1**). Spectral data (^{1}H and ^{13}C NMR) and physical constants ($[\alpha]_D$ and R_f) of *ent*-**3** thus obtained were in full agreement with values recorded for the opposite enantiomer **3**.

The intermediate *ent*-**3** was converted to dihydroxylactone *ent*-**4** by the newly developed one-pot procedure comprised of previous sodium borohydride reduction of *ent*-**3** to the corresponding primary alcohol (not shown in the reaction scheme), followed by a subsequent hydrogenolytic removal of benzyl ether protective group in the intermediate under the acidic conditions (10% Pd/C, 2:1 TFA/MeOH). This procedure provided the desired intermediate *ent*-**4** in 71% overall yield. The ^{1}H and ^{13}C NMR spectral data, as well as the value of optical rotation for *ent*-**4** were fully consistent with those reported previously.[8] Since the conversion of diol *ent*-**4** to (−)-muricatacin through a three-step sequence has been previously reported by Saniere et al.,[8] the preparation of *ent*-**4** formally represents a novel synthesis of (−)-muricatacin (*ent*-**1**) from D-xylose.

Moreover, the aldehydo-lactone *ent*-**3** was converted to the (−)-muricatacin 7-oxa-analog (*ent*-**2**) through a three-step sequence similar to that already used for the conversion of **3** to **2**. Thus, sodium borohydride reduction of the aldehyde group in *ent*-**3** gave the primary alcohol *ent*-**12**, which was subsequently converted to the 7-*O*-decyl derivative *ent*-**14** after reaction with decyl bromide in refluxing ether, in presence of Ag_2O and AgOTf as catalysts. Hydrogenolytic removal of benzyl ether protective group in *ent*-**14** furnished the target *ent*-**2** to be ready for biological testing.

2.1. Evaluation of cytotoxic activity

Compounds **2** and *ent*-**2** were evaluated for their in vitro cytotoxicity against human myelogenous leukemia K562, promyelocytic leukemia HL-60, human T-cell leukemia (JURKAT), cervix carcinoma HeLa, and estrogen receptor positive breast adenocarcinoma MCF-7 cell line. Cytotoxic activity was evaluated by using MTT assay,[21] after exposure of cells to the tested compounds for 48 h. Standard cytotoxic agent doxorubicin (DOX) was used as a positive control in this assay. The results are presented in Table 1.

Compound **2** showed a powerful antiproliferative activity against the HL-60 cell line, being over 17-fold more potent with respect to doxorubicin. This analog was also active against the K562 and JURKAT cells, but the respective IC_{50} values were more than 5- and 12-fold higher with respect to those observed for the reference compound, DOX. The opposite enantiomer *ent*-**2** showed a powerful cytotoxic activity against K562 cells being 36-fold more potent than doxorubicin. Against the HL-60 cell line, this compound exhibited a significant antiproliferative activity, which was 35% higher than that observed for doxorubicin. This analog was also active against the JURKAT, HeLa, and MCF-7 cells but with respective IC_{50} values being over 2-, 20- and 40-fold higher than those observed for the reference compound.

Table 1. In vitro cytotoxicity of **2**, *ent*-**2**, and DOX

Compds	IC_{50}, μM[a]				
	K562	HL-60	JURKAT	HeLa	MCF-7
2	1.96	0.26	4.89	>100	>100
ent-**2**	0.01	2.96	1.06	23.58	31.55
DOX	0.36	4.62	0.39	1.17	0.75

[a] IC_{50} is the concentration of compound required to inhibit the cell growth by 50% compared to an untreated control.

3. Conclusions

In conclusion, a new and general strategy for the synthesis of enantiopure 5-hydroxyalkylbutan-4-olides by chirality transfer from D-xylose has been developed. The synthetic pathway that furnished with the preparation of (+)- and (−)-5,6-dihydroxy-4-hexanolides **4** and *ent*-**4** formally represents a new enantiodivergent synthesis of (+)- and (−)-muricatacins from D-xylose. This approach has been applied for the preparation of hitherto unknown (+)- and (−)-muricatacin 7-oxa analogs **2** and *ent*-**2**, which showed powerful antiproliferative activities against some malignant cells. In addition to providing access to both enantiomers of **1** and **2**, this enantiodivergent approach is flexible and straightforward. It uses non-expensive reagents and a readily available starting material. These advantages make the synthetic methodology suitable for easy preparation of a variety of muricatacin analogs in both enantiomeric series for biological evaluation.

4. Experimental

4.1. General methods

Melting points were determined on a Büchi 510 apparatus and were not corrected. Optical rotations were measured on P 3002 (Krüss) and Polamat A (Zeiss, Jena) polarimeters in chloroform solutions at room temperature. IR spectra were recorded with Specord 75 (Carl–Zeiss) and Nexus 670 (Thermo Nicolet, DTGS-detector) IR spectrophotometers. NMR spectra were recorded on a Bruker AC 250 E instrument and the chemical shifts (δ-scale) are expressed in parts per million values downfield from tetramethylsilane. Chemical ionization mass spectra were recorded on Finnigan-MAT 8230 spectrometer with isobutane as a reagent gas. TLC was performed on DC Alufolien Kieselgel 60 F_{254} (E. Merck). Flash column chromatography was performed using Kieselgel 60 (0.040–0.063 mm, E. Merck). All organic extracts were dried with anhydrous Na_2SO_4. Organic solutions were concentrated in a rotary evaporator under diminished pressure at a bath temperature below 35 °C.

4.1.1. 5-*O*-Benzoyl-3-*O*-benzyl-D-xylofuranose (6). A solution of **5** (3.644 g, 8.58 mmol) in 70% aq AcOH was stirred for 5.5 h at reflux. After the mixture cooled to room temperature it was concentrated by co-distillation with toluene and the residue purified by flash column chromatography (3:2 toluene/EtOAc), to afford pure **6** (2.517 g, 85%) as a colorless solid. Recrystallization from CH_2Cl_2/hexane gave an analytical sample **6** as colorless needles, mp 59–60 °C, $[\alpha]_D^{23}$ −18.1→+4.2 (24 h, *c* 1.0, $CHCl_3$), R_f=0.68 (Et_2O), anomeric ratio: α/β=3:1 (from ^1H NMR spectrum recorded immediately after dissolution of the sample). IR (KBr): ν_{max} 3420 (OH), 1720 (C=O), 1600 (Ph). ^1H NMR ($CDCl_3$+D_2O): δ 4.05 (br s, 0.25H, $J_{2,3}$=1.3, $J_{3,4}$=3.5 Hz, H-3β), 4.09 (dd, 0.75H, $J_{2,3}$=2.9, $J_{3,4}$=4.8 Hz, H-3α), 4.24 (dd, 0.75H, $J_{1,2}$=3.8 Hz, H-2α), 4.33 (br s, 0.25H, H-2β), 4.41–4.78 (m, 4H, PhCH_2, H-4β, H-4α, H-5β and H-5α), 5.27 (br s, 0.25H, H-1β), 5.56 (d, 0.75H, $J_{1,2}$=3.8 Hz, H-1α), 7.18–8.09 (m, 10H, 2×Ph). ^{13}C NMR ($CDCl_3$): δ 63.61 (C-5α), 64.12 (C-5β), 71.94 (Ph*C*H_2-α), 72.51 (Ph*C*H_2-β), 75.02 (C-2α), 76.35 (C-4α), 77.50 (C-2β), 79.08 (C-4β), 82.07 (C-3β), 82.85 (C-3α), 96.26 (C-1α), 103.36 (C-1β), 127.49, 127.59, 127.73, 127.82, 127.89, 128.19, 128.28, 128.39, 128.46, 128.56, 129.61, 129.72, 129.98, 133.05 and 137.34 (2×Ph), 166.49 (C=O). MS (CI): *m*/*z* 401 (M^+−H+C_4H_{10}), 383 (M^+−OH+C_4H_{10}), 345 (MH^+), 327 (M^+−OH), 237 (M^+−OBn). Anal. Found: C, 66.53; H, 6.06. Calcd for $C_{19}H_{20}O_6$: C, 66.27; H, 5.85.

4.1.2. Methyl 7-*O*-benzoyl-5-*O*-benzyl-2,3-dideoxy-D-*xylo*-hept-2-enonate (7). To a solution of **6** (1.985 g, 5.76 mmol) in dry DMF (37 mL) was added Ph_3P=$CHCO_2Me$ (2.508 g, 7.5 mmol). The mixture was stirred for 3.5 h at 60–70 °C and then evaporated. The residue was purified by flash column chromatography (Et_2O) to afford pure **7** (1.948 g, 84%) as a colorless syrup, $[\alpha]_D^{23}$ −13.8 (*c* 1.0, $CHCl_3$), R_f=0.5 (4:1 $^iPr_2O$/EtOAc). IR (KBr): ν_{max} 3450 (OH), 1700 (C=O), 1620 (C=C), 1600 (Ph). ^1H NMR ($CDCl_3$): δ 3.33 (br s, 2H, exchangeable with D_2O, 2×OH), 3.63 (t, 1H, $J_{4,5}$=$J_{5,6}$=3.8 Hz, H-5), 3.74 (s, 3H, CO_2Me), 4.15 (m, 1H, H-6), 4.36 (dd, 1H, $J_{6,7a}$=4.8, $J_{7a,7b}$=11.5 Hz, H-7a), 4.46 (dd, 1H, $J_{6,7b}$=6.8, $J_{7a,7b}$=11.5 Hz, H-7b), 4.62 (m, 1H, H-4), 4.64 and 4.72 (2×d, 2H, J_{gem}=11.2 Hz, PhCH_2), 6.20 (dd, 1H, $J_{2,4}$=1.9, $J_{2,3}$=15.6 Hz, H-2), 7.07 (dd, 1H, $J_{3,4}$=4.4, $J_{2,3}$=15.6 Hz, H-3), 7.22–8.06 (m, 10H, 2×Ph). ^{13}C NMR ($CDCl_3$): δ 51.68 (CO_2Me), 65.82 (C-7), 70.23 (C-6), 71.19 (C-4), 74.75 (Ph*C*H_2), 79.96 (C-5), 121.25 (C-2), 128.21, 128.29, 128.37, 128.52, 129.49, 129.61, 133.22 and 136.97 (2×Ph), 147.39 (C-3), 166.63 and 166.81 (C-1 and Ph*C*=O). MS (CI): *m*/*z* 401 (MH^+), 369 (M^+−OMe). Anal. Found: C, 66.25; H, 5.88. Calcd for $C_{22}H_{24}O_7$: C, 65.99; H, 6.04.

4.1.3. Methyl 7-*O*-benzoyl-5-*O*-benzyl-2,3-dideoxy-D-*xylo*-heptonate (8). A solution of **7** (0.554 g, 1.38 mmol) in EtOH (11 mL) was hydrogenated over PtO_2 (8 mg) for 16 h at room temperature. The mixture was filtered and the catalyst washed with EtOH. The organic solution was evaporated and the residue was purified by flash column chromatography (9:1 $^iPr_2O$/EtOAc) to afford pure **8** (0.419 g, 75%) as a colorless syrup, $[\alpha]_D^{23}$ −12.8 (*c* 1.0, $CHCl_3$), R_f=0.36 (4:1 $^iPr_2O$/EtOAc). IR (film): ν_{max} 3470 (OH), 1720 (C=O), 1600 (Ph). ^1H NMR ($CDCl_3$): δ 1.89 (m, 2H, 2×H-3), 2.49 (m, 2H, 2×H-2), 2.72 (d, 1H, $J_{4,OH}$=6.5 Hz, exchangeable with D_2O, OH-4), 3.06 (d, 1H, $J_{6,OH}$=5.9 Hz, exchangeable with D_2O, OH-6), 3.48 (dd, 1H, $J_{4,5}$=4.0, $J_{5,6}$=3.1 Hz, H-5), 3.67 (s, 3H, CO_2Me), 3.88 (m, 1H, H-4), 4.17 (m, 1H, H-6), 4.41 (dd, 1H, $J_{6,7a}$=5.0, $J_{7a,7b}$=11.5 Hz, H-7a), 4.47 (dd, 1H, $J_{6,7b}$=6.7, $J_{7a,7b}$=11.5 Hz, H-7b), 4.73 (s, 2H, PhCH_2), 7.28–8.09 (m, 10H, 2×Ph). ^{13}C NMR ($CDCl_3$): δ 29.0 (C-3), 30.49 (C-2), 51.65 (CO_2Me), 66.12 (C-7), 70.22 (C-6), 71.15 (C-4), 75.11 (Ph*C*H_2), 80.9 (C-5), 128.16, 128.38, 128.42, 128.47, 128.56, 129.63, 133.15 and 137.39 (2×Ph), 166.56 (Ph*C*O), 174.27 (*C*O_2Me). MS (CI): *m*/*z* 403 (MH^+), 371 (M^+−OMe). Anal. Found: C, 66.02; H, 6.21. Calcd for $C_{22}H_{26}O_7$: C, 65.66; H, 6.51.

4.1.4. 7-*O*-Benzoyl-5-*O*-benzyl-2,3-dideoxy-D-*xylo*-heptono-1,4-lactone (9). A solution of **8** (0.127 g, 0.31 mmol) in a mixture of TFA (2 mL) and water (1 mL) was stirred at room temperature for 2.5 h. The volatiles were removed by co-distillation with toluene and the residue purified by flash column chromatography (4:1 CH_2Cl_2/EtOAc) to give pure **9** (0.106 g, 92%) as a colorless oil, $[\alpha]_D^{23}$ −19.3 (*c* 0.99, $CHCl_3$), R_f=0.46 (4:1 CH_2Cl_2/EtOAc). IR (film): ν_{max} 3450 (OH), 1780 (C=O, lactone), 1710 (C=O, Bz), 1600 (Ph). ^1H NMR ($CDCl_3$): δ 1.82–2.41 (m, 2H, 2×H-3), 2.61 (m, 2H, 2×H-2), 3.15 (s, 1H, exchangeable with D_2O, OH), 3.60 (dd, 1H, $J_{4,5}$=5.8, $J_{5,6}$=3.1 Hz, H-5), 4.11 (m, 1H, H-6), 4.37 (dd, 1H, $J_{6,7a}$=5.2, $J_{7a,7b}$=11.5 Hz, H-7a), 4.49 (dd, 1H, $J_{6,7b}$=6.7, $J_{7a,7b}$=11.5 Hz, H-7b), 4.73 and 4.85 (2×d, 2H, J_{gem}=11.6 Hz, PhCH_2), 4.82 (m, 1H, H-4), 7.29–8.05 (m, 10H, 2×Ph). ^{13}C NMR ($CDCl_3$): δ 24.52 (C-3), 28.24 (C-2), 65.85 (C-7), 69.06 (C-6), 74.18 (Ph*C*H_2), 79.73 (C-5), 80.83 (C-4), 127.91, 128.0, 128.15, 128.25, 128.32, 129.46, 133.08 and 137.21 (2×Ph), 166.4 (Ph*C*O), 176.89 (C-1). MS (CI): *m*/*z* 371 (MH^+).

4.1.5. 5-*O*-Benzyl-2,3-dideoxy-D-*xylo*-heptono-1,4-lactone (10). *Procedure A*: a solution of **9** (0.107 g, 0.29 mmol) in 0.09 M methanolic NaOMe (0.6 mL, 0.06 mmol) was stirred for 1 h at room temperature. An additional amount of 0.09 M NaOMe in MeOH (0.3 mL, 0.03 mmol) was added to the reaction mixture and stirring was continued for the next 1 h at room temperature. The mixture was neutralized with 1 M AcOH in MeOH (0.09 mL, 0.09 mmol) and evaporated. Flash column chromatography (4:1→1:4 CH_2Cl_2/EtOAc) of the residue gave pure **10** (0.043 g, 55%) as a colorless syrup, R_f=0.31 (EtOAc).

Procedure B: a solution of **8** (1.458 g, 3.62 mmol) in 0.09 M methanolic NaOMe (6.9 mL, 0.63 mmol) was stirred for 1.5 h at room temperature, then acidified with 2:1 aq TFA (0.08 mL) and concentrated by co-distillation with toluene. Flash column chromatography (EtOAc) of the residue gave pure **10** (0.7932 g, 82%) as a colorless oil, $[\alpha]_D^{23}$ +3.0 (*c* 1.06, $CHCl_3$), R_f=0.31 (EtOAc). IR (film): ν_{max} 3400 (OH), 1750 (C=O), 1610 (Ph). ^1H NMR ($CDCl_3$+D_2O): δ 1.91 and 2.25 (2×m, 2H, 2×H-3), 2.35 (m, 2H, 2×H-2), 3.49 (t, 1H, $J_{4,5}$=$J_{5,6}$=4.9 Hz, H-5), 3.60 (dd, 1H, $J_{6,7a}$=5.2, $J_{7a,7b}$=11.6 Hz, H-7a), 3.67 (dd, 1H, $J_{6,7b}$=5.2, $J_{7a,7b}$=11.6 Hz, H-7b), 3.81 (m, 1H, H-6), 4.62–4.83 (m, 3H, PhCH_2 and H-4), 7.24–7.4 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): δ 24.49 (C-3), 28.24 (C-2), 63.54 (C-7), 70.98 (C-6), 74.41 (Ph*C*H_2), 80.70 (C-5), 80.73 (C-4), 128.09,

128.17, 128.5 and 137.43 (Ph), 177.32 (C-1). MS (CI): *m/z* 267 (MH^+). Anal. Found: C, 63.43; H, 6.63. Calcd for $C_{14}H_{18}O_5$: C, 63.15; H, 6.81.

4.1.6. 6-Aldehydo-5-*O*-benzyl-2,3-dideoxy-L-*threo*-hexono-1,4-lactone (3). To a solution of **10** (0.793 g, 2.98 mmol) in CH_2Cl_2 (11 mL) was added Kieselgel 60 (6.0 g, 0.063–0.2 nm) and 0.65 M aq $NaIO_4$ (6.0 mL, 3.90 mmol). The resulting heterogeneous mixture was vigorously stirred for 1 h at room temperature then filtered and evaporated. Flash column chromatography (2:1 toluene/EtOAc), followed by crystallization from CH_2Cl_2/hexane gave an analytical sample **3** (0.621 g, 89%) as colorless needles, mp 93–94 °C, $[\alpha]_D^{23}$ +135.8 (*c* 1.08, $CHCl_3$), R_f=0.3 (2:1 toluene/EtOAc). IR (film): ν_{max} 1770 (C=O, lactone), 1720 (CH=O). ^{1}H NMR ($CDCl_3$): δ 1.92–2.68 (m, 4H, 2×H-2 and 2×H-3), 3.78 (dd, 1H, $J_{4,5}$=2.7, $J_{5,6}$=1.5 Hz, H-5), 4.58 (d, 1H, J_{gem}=11.9 Hz, PhC*H*a), 4.74–4.85 (m, 2H, H-4 and PhC*H*b), 7.33 (m, 5H, Ph), 9.68 (d, 1H, H-6). ^{13}C NMR ($CDCl_3$): δ 23.38 (C-3), 27.61 (C-2), 73.33 (Ph*C*H_2), 78.69 (C-4), 83.88 (C-5), 128.2, 128.52, 128.69 and 136.76 (Ph), 176.57 (C-1), 201.57 (C-6). MS (CI): *m/z* 469 ($2M^+$+H), 235 (MH^+). Anal. Found: C, 66.30; H, 6.00. Calcd for $C_{13}H_{14}O_4$: C, 66.66; H, 6.02.

4.1.7. (*Z*)-6-*C*-Decylidene-5-*O*-benzyl-2,3-dideoxy-L-*threo*-hexono-1,4-lactone (11). To a stirred suspension of undecyltriphenylphosphonium bromide (0.716 g, 1.44 mmol) in dry THF (5 mL) was added a 1 M solution of LiHMDS in dry THF (1.44 mL, 1.44 mmol) dropwise over a period of 5 min at 0 °C under N_2. The bright red solution was stirred for another 15 min, cooled to −78 °C, and a solution of the aldehyde **3** (0.1698 g, 0.72 mmol) in dry THF (1.5 mL) was instantly added. The mixture was stirred for 3 h at −78 °C, while it was allowed to warm to room temperature. The light yellow mixture was stirred at room temperature for 72 h, then quenched with 10% aq NH_4Cl (5 mL), and extracted with Et_2O. The combined organic phases were washed with brine, dried, and evaporated. Flash column chromatography of the residue (2:1 toluene/EtOAc) gave pure (*Z*)-olefin **11** (0.0175 g, 7%) as a colorless oil, $[\alpha]_D^{23}$ +40.2 (*c* 0.74, $CHCl_3$), R_f=0.45 (9:1 toluene/EtOAc). IR (film): ν_{max} 1780 (C=O). ^{1}H NMR ($CDCl_3$): δ 0.89 (t, 3H, *J*=6.4 Hz, Me), 1.07–1.60 (m, 16H, 8×CH_2), 1.95–2.67 (m, 6H, H-2, H-3 and H-8), 4.24 (dd, 1H, $J_{4,5}$=5.4, $J_{5,6}$=9.4 Hz, H-5), 4.38 and 4.66 (2×d, 2H, J_{gem}=12.0 Hz, PhCH_2), 4.55 (m, 1H, H-4), 5.41 (m, 1H, $J_{5,6}$=9.4, $J_{6,7}$=11.4 Hz, H-6), 5.80 (m, 1H, $J_{6,7}$=11.4, $J_{7,8}$=7.8 Hz, H-7), 7.25–7.43 (m, 5H, Ph). ^{1}H NOE contact: H-6 and H-7. ^{13}C NMR ($CDCl_3$): δ 14.07 (Me), 22.65, 23.80, 28.07, 28.30, 28.31, 29.45, 29.52, 29.58 and 31.88 (9×CH_2, C-2 and C-3), 70.05 (Ph*C*H_2), 75.25 (C-5), 81.77 (C-4), 124.81 (C-6), 127.66, 128.36 and 138.09 (Ph), 137.39 (C-7), 177.20 (C-1). MS (CI): *m/z* 373 (MH^+).

4.1.8. 5-*O*-Benzyl-2,3-dideoxy-L-*threo*-hexono-1,4-lactone (12). To a cooled (0 °C) and stirred solution of **3** (0.122 g, 0.52 mmol) in MeOH (1.2 mL) was added $NaBH_4$ (0.0197 g, 0.52 mmol) in two portions, and the resulting suspension was stirred at 0 °C for 1.5 h. TFA (2 mL) was then added and the mixture was stirred for additional 2 h while it was allowed to warm to room temperature. The volatiles were removed by co-distillation with toluene and the residue purified by flash column chromatography (4:1 CH_2Cl_2/EtOAc). The unchanged aldehyde **3** (0.015 g, 12%) was first eluted, followed by pure **12** (0.082 g, 73% on the basis of the recovered intermediate **3**) that was isolated as a colorless oil, $[\alpha]_D^{23}$ +22.9 (*c* 1.09, $CHCl_3$), R_f=0.24 (4:1 CH_2Cl_2/EtOAc). IR (film): ν_{max} 3450 (OH), 1770 (C=O). ^{1}H NMR ($CDCl_3$): δ 1.87–2.32 (m, 2H, 2×H-3), 2.36–2.85 (m, 3H, 2×H-2 and OH), 3.50 (m, 1H, H-5), 3.70 (dd, 1H, $J_{5,6a}$=4.9, $J_{6a,6b}$=11.6 Hz, H-6a), 3.79 (dd, 1H, $J_{5,6b}$=5.3, $J_{6a,6b}$=11.6 Hz, H-6b), 4.65 and 4.73 (2×d, 2H, J_{gem}=11.6 Hz, PhCH_2), 4.70 (m, 1H, H-4), 7.28–7.45 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): δ 24.08 (C-3), 28.22 (C-2), 60.93 (C-6), 72.99 (Ph*C*H_2), 80.22 (C-4), 80.51 (C-5), 127.79, 127.86, 128.4 and 137.71 (Ph), 177.36 (C=O). MS (CI): *m/z* 473 ($2M^+$+H), 237 (MH^+).

4.1.9. 2,3-Dideoxy-L-*threo*-hexono-1,4-lactone (4). A solution of **12** (0.082 g, 0.35 mmol) in EtOAc (1.7 mL) was hydrogenated over 10% Pd/C (0.041 g) for 18 h at room temperature. The mixture was filtered and the catalyst washed successively with EtOAc and MeOH. The combined organic solutions were evaporated and the residue was purified by flash column chromatography (EtOAc) to afford pure **4** (0.035 g, 69%) as a colorless syrup, $[\alpha]_D$ +58.0 (*c* 2.01, MeOH), lit.[19] $[\alpha]_D$ +58.18 (*c* 2.03, MeOH), R_f=0.22 (EtOAc). IR (film): ν_{max} 3384 (OH), 1759 (C=O). ^{1}H NMR ($CDCl_3$): δ 2.26 (m, 2H, 2×H-3), 2.57 (m, 2H, 2×H-2), 3.46–3.84 (m, 4H, H-5, 2×H-6 and OH), 4.14 (br s, 1H, OH), 4.59 (m, 1H, H-4). ^{13}C NMR ($CDCl_3$): δ 23.9 (C-3), 28.45 (C-2), 63.27 (C-6), 73.56 (C-5), 80.77 (C-4), 178.21 (C-1).

4.1.10. 5-*O*-Benzyl-6-*O*-decyl-2,3-dideoxy-L-*threo*-hexono-1,4-lactone (14). To a solution of **12** (0.101 g, 0.43 mmol) in dry Et_2O (2 mL) were added successively Ag_2O (0.249 g, 1.07 mmol), AgOTf (0.028 g, 0.11 mmol), and $C_{10}H_{21}Br$ (0.44 mL, 2.13 mmol). The mixture was stirred under reflux for 7.5 h, then diluted with CH_2Cl_2 (10 mL), filtered, and evaporated. The residue was purified by flash column chromatography (CH_2Cl_2) to give pure **14** (0.129 g, 80%) a colorless oil, $[\alpha]_D^{23}$ +30.2 (*c* 1.05, $CHCl_3$), R_f=0.24 (CH_2Cl_2). IR (film): ν_{max} 1779 (C=O). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H, Me), 1.16–1.65 (m, 16H, 8×CH_2), 1.93–2.31 (m, 2H, 2×H-3), 2.32–2.67 (m, 2H, 2×H-2), 3.44 (t, 2H, *J*=6.6 Hz, 2×H-8), 3.60 (m, 3H, H-5 and 2×H-6), 4.60 and 4.77 (2×d, 2H, J_{gem}=11.8 Hz, PhCH_2), 4.70 (m, 1H, H-4), 7.24–7.41 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): δ 13.97 (Me), 22.52, 23.89, 25.98, 28.18, 29.17, 29.29, 29.41, 29.45, 29.49 and 31.74 (8×CH_2, C-2 and C-3), 69.61 (C-6), 71.64 (C-8), 72.77 (Ph*C*H_2), 78.9 (C-5), 79.67 (C-4), 127.65, 127.70, 128.26 and 137.90 (Ph), 177.34 (C-1). MS (CI): *m/z* 377 (MH^+). Anal. Found: C, 73.18; H, 9.50. Calcd for $C_{23}H_{36}O_4$: C, 73.37; H, 9.64.

4.1.11. 6-*O*-Decyl-2,3-dideoxy-L-*threo*-hexono-1,4-lactone (2). A solution of **14** (0.084 g, 0.22 mmol) in EtOAc (1.7 mL) was hydrogenated over 10% Pd/C (0.042 g) for 19 h at room temperature. The suspension was filtered through a Celite pad and washed with EtOAc. The combined filtrates were evaporated and the residue purified by flash column chromatography (CH_2Cl_2 → 9:1 CH_2Cl_2/EtOAc) to afford pure **2** (0.051 g, 82%) as a colorless solid. Recrystallization from $CHCl_3$/hexane gave an analytical sample **2**, mp

42 °C, $[\alpha]_D^{23}$ +33.0 (*c* 0.83, $CHCl_3$), R_f=0.21 (9:1 CH_2Cl_2/EtOAc). IR (film): ν_{max} 3427 (OH), 1737 (C=O). ^1H NMR ($CDCl_3$): δ 0.85 (t, 3H, *J*=6.4 Hz, Me), 1.13–1.63 (m, 16H, 8×CH_2), 2.24 (m, 2H, 2×H-3), 2.39–2.72 (m, 2H, 2×H-2), 2.87 (br s, 1H, exchangeable with D_2O, OH), 3.44 (t, 2H, *J*=6.6 Hz, 2×H-8), 3.51 (d, 2H, $J_{5,6}$=5.8 Hz, 2×H-6), 3.78 (br s, 1H, H-5), 4.56 (td, 1H, $J_{4,5}$=3.4, $J_{3,4}$=7.0 Hz, H-4). ^{13}C NMR ($CDCl_3$): δ 13.99 (Me), 22.56, 23.74, 25.96, 28.27, 29.2, 29.45, 29.47 and 31.78 (8×CH_2, C-2 and C-3), 71.12 (C-6), 71.69 (C-8), 71.94 (C-5), 79.79 (C-4), 177.53 (C-1). MS (CI): *m/z* 573 ($2M^+$+H), 287 (MH^+). Anal. Found: C, 66.99; H, 10.74. Calcd for $C_{16}H_{30}O_4$: C, 67.10; H, 10.56.

4.1.12. 3-*O*-Benzyl-1,2-*O*-cyclohexylidene-α-D-xylofuranose (16). To a cooled (0 °C) and stirred solution of **15** (2.603 g, 7.78 mmol) in anhydrous DMF (39 mL) were added successively NaH (0.403 g, 13.4 mmol) and BnBr (1.4 mL, 11.77 mmol), and the mixture was stirred for 2.5 h at room temperature. The mixture was cooled to 0 °C and treated with 0.1 M NaOMe in MeOH (15.6 mL, 1.56 mmol) while stirring for 2 h at room temperature. The mixture was neutralized with 1 M AcOH in MeOH (1.56 mL, 1.56 mmol), evaporated by co-distillation with toluene, and the residue purified by flash column chromatography (9:1 CH_2Cl_2/EtOAc) to afford pure **16** (2.22 g, 89%) as a colorless oil, $[\alpha]_D^{23}$ −57.7 (*c* 0.99, $CHCl_3$), R_f=0.32 (9:1 CH_2Cl_2/EtOAc). IR (film): ν_{max} 3450 (OH). ^1H NMR ($CDCl_3$): δ 1.32–1.77 (m, 10H, 5×CH_2 from C_6H_{10}), 2.33 (br s, 1H, exchangeable with D_2O, OH), 3.84 (dd, 1H, $J_{4,5a}$=4.6, $J_{5a,5b}$=11.8 Hz, H-5a), 3.96 (dd, 1H, $J_{4,5b}$=5.0 Hz, $J_{5a,5b}$=11.8 Hz, H-5b), 4.03 (d, 1H, $J_{3,4}$=3.4 Hz, H-3), 4.28 (m, 1H, H-4), 4.50 and 4.73 (2×d, 2H, J_{gem}=12.0 Hz, PhCH_2), 4.65 (d, 1H, $J_{1,2}$=3.8 Hz, H-2), 6.00 (d, 1H, $J_{1,2}$=3.8 Hz, H-1), 7.28–7.44 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): δ 23.53, 23.81, 24.83, 35.78 and 36.42 (5×CH_2 from C_6H_{10}), 60.92 (C-5), 71.86 (PhCH_2), 80.0 (C-4), 81.99 (C-2), 82.86 (C-3), 104.61 (C-1), 112.44 (Cq from C_6H_{10}), 127.63, 128.09, 128.59 and 137.11 (Ph). MS (CI): *m/z* 642 ($2M^+$+H), 321 (MH^+). Anal. Found: C, 65.01; H, 6.61. Calcd for $C_{18}H_{22}O_6$: C, 64.66; H, 6.63.

4.1.13. 3-*O*-Benzyl-1,2-*O*-cyclohexylidene-α-D-*xylo*-pentadialdo-1,4-furanose (17). To a stirred solution of **16** (2.935 g, 9.16 mmol) and DCC (5.67 g, 27.48 mmol) in anhydrous DMSO (14.6 mL), were added dry pyridine (0.36 mL; 5 mmol) and 0.82 M solution of anhydrous H_3PO_4 in DMSO (5.5 mL, 4.5 mmol). The resulting reaction mixture was stirred at room temperature for 3.5 h and then diluted with EtOAc (50 mL). A solution of oxalic acid (2.31 g, 10.15 mmol) in MeOH (7 mL) was added and the resulting suspension was washed successively with 10% aq NaCl (100 mL) and 10% aq $NaHCO_3$ (50 mL). The organic layer was separated and the aqueous solution extracted with EtOAc (2×30 mL). The combined organic solution was washed with brine, dried, and concentrated by co-distillation with toluene. Flash column chromatography (9:1 toluene/EtOAc) of the residue gave pure **17** (2.429 g, 83%) as an unstable pale yellow syrup, $[\alpha]_D^{23}$ −41.4 (*c* 0.95, $CHCl_3$), R_f=0.27 (9:1 toluene/EtOAc). IR (film): ν_{max} 1720 (CH=O). ^1H NMR ($CDCl_3$): δ 1.34–1.76 (m, 5×CH_2 from C_6H_{10}), 4.37 (d, 1H, $J_{3,4}$=3.7 Hz, H-3), 4.49 and 4.62 (2×d, 2H, J_{gem}=11.9 Hz, PhCH_2), 4.58 (dd, 1H, $J_{3,4}$=3.7, $J_{4,5}$=1.5 Hz, H-4), 4.66 (d, 1H, $J_{1,2}$=3.5 Hz, H-2), 6.14 (d, 1H, $J_{1,2}$=3.5 Hz, H-1), 7.27–7.41 (m, 5H, Ph), 9.69 (d, 1H, CHO). ^{13}C NMR ($CDCl_3$): δ 23.53, 23.81, 24.75, 35.85 and 36.67 (5×CH_2 from C_6H_{10}), 72.36 (PhCH_2), 81.76 (C-2), 83.92 (C-3), 84.58 (C-4), 105.8 (C-1), 113.31 (Cq from C_6H_{10}), 127.66, 128.11, 128.51 and 136.69 (Ph), 200.07 (CH=O). MS (CI): *m/z* 637 ($2M^+$+H), 319 (MH^+).

4.1.14. Methyl 3-*O*-benzyl-5,6-dideoxy-1,2-*O*-cyclohexylidene-α-D-*xylo*-heptofuranuronate (19). A mixture of **17** (2.429 g, 7.63 mmol) and Ph_3P=$CHCO_2Me$ (6.412 g, 19.18 mmol) in anhydrous CH_2Cl_2 (92 mL) was stirred under N_2 at room temperature for 2 h and then evaporated. The residue was purified by flash column chromatography (9:1 toluene/EtOAc) to give **18** (2.767 g, 97%) as a 2:1 mixture of corresponding *Z*- and *E*-isomers. A solution of **18** (2.767 g, 7.39 mmol) in EtOH (55 mL) was hydrogenated over PtO_2 (0.029 g) for 19 h at room temperature. The mixture was filtered and the catalyst washed with EtOH. The organic solution was evaporated and the residue was purified by flash column chromatography (15:1 toluene/EtOAc) to afford pure **19** (2.571 g, 92%) as a colorless oil, $[\alpha]_D^{23}$ −38.6 (*c* 1.02, $CHCl_3$), R_f=0.31 (15:1 toluene/EtOAc), IR (film): ν_{max} 1740 (C=O). ^1H NMR ($CDCl_3$): δ 1.33–1.77 (m, 5×CH_2 from C_6H_{10}), 2.04 (m, 2H, 2×H-5), 2.40 (m, 2H, 2×H-6), 3.66 (s, 3H, CO_2Me), 3.82 (d, 1H, $J_{3,4}$=3.1 Hz, H-3), 4.18 (ddd, 1H, $J_{3,4}$=3.1 Hz, $J_{4,5a}$=5.6, $J_{4,5b}$=8.4 Hz, H-4), 4.50 and 4.71 (2×d, 2H, J_{gem}=12.0 Hz, PhCH_2), 4.61 (d, 1H, $J_{1,2}$=3.9 Hz, H-2), 5.91 (d, 1H, $J_{1,2}$=3.9 Hz, H-1), 7.25–7.37 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): δ 23.51 (C-5), 23.81, 23.82, 24.86, 35.68 and 36.29 (5×CH_2 from C_6H_{10}), 30.56 (C-6), 51.41 (CO_2Me), 71.67 (PhCH_2), 79.12 (C-4), 81.82 (C-2), 82.19 (C-3), 104.22 (C-1), 111.97 (Cq from C_6H_{10}), 127.57, 127.80, 128.38 and 137.50 (Ph), 173.57 (C-7). MS (CI): *m/z* 377 (MH^+), 345 (M^+−OMe).

4.1.15. Methyl 3-*O*-benzyl-5,6-dideoxy-D-*xylo*-heptofuranuronate (20) and 3-*O*-benzyl-5,6-dideoxy-D-*xylo*-heptofuranuronic acid (21). *Procedure A*: a solution of **19** (1.322 g, 3.51 mmol) in 50% aq AcOH (38 mL) was heated under reflux for 2 h and then concentrated by co-distillation with toluene. The residue was purified by flash column chromatography (1:1 toluene/EtOAc). Eluted first was the unchanged starting compound **19** (0.142 g, 11%). Eluted second was the pure **20** (0.596 g, 64% on the basis of the recovered starting material **19**) as a colorless syrup, R_f=0.24 (1:1 toluene/EtOAc). Final eluting of the column with EtOAc gave pure **21** (0.074 g, 7%) as a colorless syrup, $[\alpha]_D^{23}$ −4.1 (*c* 0.9, EtOH), R_f=0.44 (999:1 EtOAc/AcOH). IR (film): ν_{max} 3395 (OH), 1713 (C=O). ^1H NMR (acetone-d_6): δ 1.84–2.60 (m, CH_2-5, CH_2-6α and β), 3.87–3.92 (m, H-3α and β), 4.14–4.32 (m, H-2 and H-4α and β), 4.50 and 4.83 (2×d, 2H, PhCH_2), 5.05 (s, H-1β), 5.34 (d, $J_{1,2}$=4.3 Hz, H-1α), 7.21–7.48 (m, 5H, Ph). ^{13}C NMR (acetone-d_6): δ 25.62, 26.39, 30.72 and 30.91 (C-5, C-6α and β), 72.02 and 72.23 (PhCH_2 α and β), 76.23, 77.70, 80.03 and 80.22 (C-2 and C-4α and β), 84.66 (C-3β), 85.01 (C-3α), 96.63 (C-1α), 104.14 (C-1β), 128.22, 128.33, 128.34, 128.46, 129.02, 129.07, 139.28 and 139.51 (Ph), 174.73 and 174.82 (C=O, α and β).

Procedure B: a solution of **19** (1.103 g, 2.9 mmol) in 50% aq AcOH (31 mL) was heated under reflux for 2 h and then

concentrated by co-distillation with toluene. The residue was dissolved in ether (9 mL) and treated for 1.5 h at room temperature with an ethereal solution of diazomethane (9 mL), generated from *N*-methyl-*N*′-nitro-*N*-nitroso guanidine (0.532 g) and 5 M NaOH (2.4 mL). The mixture was evaporated and the residue purified by flash column chromatography (1:1 toluene/EtOAc). Eluted first was the unchanged starting compound **19** (0.149 g, 13%). Eluted second was the pure **20** (0.607 g, 81% on the basis of the recovered starting material **19**) as a colorless syrup, R_f=0.24 (1:1 toluene/EtOAc), $[\alpha]_D^{23}$ +2.0 (*c* 1.08, $CHCl_3$), anomeric ratio: α/β=3:1 (from ^{1}H NMR). IR (film): ν_{max} 3400 (OH), 1720 (C=O). ^{1}H NMR ($CDCl_3$+D_2O): δ 1.96 (m, 2H, 2×H-5α and β), 2.41 (m, 2H, 2×H-6), 3.66 (s, 2.25H, CO_2Me-α), 3.71 (s, 0.75H, CO_2Me-β), 3.84 (dd, 0.25H, $J_{2,3}$=1.2, $J_{3,4}$=4.3 Hz, H-3β), 3.88 (dd, 0.75H, $J_{2,3}$=2.6, $J_{3,4}$=4.6 Hz, H-3α), 4.16–4.32 (m, 2H, H-2 and H-4), 4.54 and 4.71 (2×d, 1.5H, J_{gem}=12.0 Hz, PhCH_2-α), 4.55 and 4.70 (2×d, 0.5H, J_{gem}=11.7 Hz, PhCH_2-β), 5.30 (s, 0.25H, H-1β), 5.44 (d, 0.75H, $J_{1,2}$=4.3 Hz, H-1α), 7.30–7.41 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): δ 24.6 (C-5α), 25.51 (C-5β), 30.53 (C-6α), 30.7 (C-6β), 51.62 (CO_2Me-α), 51.65 (CO_2Me-β), 71.79 (PhCH_2-α), 72.47 (PhCH_2-β), 75.57 (C-2β), 77.63 (C-2α), 77.89 (C-4β), 80.45 (C-4α), 82.56 (C-3β), 83.67 (C-3α), 95.59 (C-1α), 102.9 (C-1β), 127.61, 127.78, 127.87, 128.19, 128.4, 128.59, 136.94 and 137.74 (Ph), 174.06 (C-7). MS (CI): *m*/*z* 557 ($2M^+-OH-H_2O$), 297 (MH^+), 279 (M^+-OH). Anal. Found: C, 61.08; H, 6.66. Calcd for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80.

4.1.16. Methyl 2-*O*-benzyl-4,5-dideoxy-3-*O*-formyl-D-*threo*-hexuronate (22) and 6-aldehydo-5-*O*-benzyl-2,3-dideoxy-D-*threo*-hexono-1,4-lactone (*ent*-3). To a solution of **20** (0.576 g, 1.94 mmol) in CH_2Cl_2 (7.8 mL) were added Kieselgel 60 (3.88 g, 0.063–0.2 nm) and 0.65 M aq $NaIO_4$ (3.9 mL, 2.53 mmol). The resulting heterogeneous mixture was vigorously stirred for 1.5 h at room temperature, then filtered, and evaporated by co-distillation with toluene to afford crude **22** (0.561 g, 98%) as a yellow oil, which was used in the next step without further purification. A minor part of crude **22** was purified by flash column chromatography (49:1 CH_2Cl_2/EtOAc) to afford pure **22** as a colorless syrup, $[\alpha]_D^{23}$ −15.1 (*c* 1.13, $CHCl_3$), R_f=0.28 (49:1 CH_2Cl_2/EtOAc). IR (film): ν_{max} 3460 (OH), 1740–1720 (C=O). ^{1}H NMR ($CDCl_3$): δ 2.06 (m, 2H, 2×H-4), 2.30 (m, 2H, 2×H-5), 3.65 (s, 3H, CO_2Me), 3.90 (dd, 1H, $J_{1,2}$=1.0, $J_{2,3}$=3.7 Hz, H-2), 4.58 and 4.80 (2×d, 2H, J_{gem}=11.8 Hz, PhCH_2), 5.36 (td, 1H, $J_{2,3}$=3.7, $J_{3,4a}$=$J_{3,4b}$=7.0 Hz, H-3), 7.25–7.40 (m, 5H, Ph), 8.03 (s, 1H, OC*H*O), 9.60 (d, 1H, $J_{1,2}$=1.0 Hz, H-1). ^{13}C NMR ($CDCl_3$): δ 25.56 (C-4), 29.58 (C-5), 51.76 (CO_2Me), 71.07 (C-3), 73.42 (PhCH_2), 82.87 (C-2), 128.32, 128.45, 128.64 and 136.34 (Ph), 160.11 (OCHO), 172.67 (C-6), 200.37 (C-1). MS (CI): *m*/*z* 295 (MH^+), 267 (MH^+-CO). A solution of crude ester **22** (0.561 g) in a mixture of TFA (9.1 mL) and water (4.55 mL) was stirred for 1.5 h at room temperature and then concentrated by co-distillation with toluene. The residue was chromatographed on a column of flash silica (2:1 toluene/EtOAc) to give pure *ent*-**3** (0.35 g, 77% from **20**) as a white solid. Recrystallization from CH_2Cl_2/hexane gave an analytical sample as colorless needles, mp 93–94 °C, $[\alpha]_D^{23}$ −129.8 (*c* 1.0, $CHCl_3$), R_f=0.3 (2:1 toluene/EtOAc). IR, NMR, and mass spectral data of thus obtained product *ent*-**3** were consistent with those recorded for the (+)-enantiomer **3** (Section 4.1.6). Anal. Found: C, 66.23; H, 6.03. Calcd for $C_{13}H_{14}O_4$: C, 66.66; H, 6.02.

4.1.17. 2,3-Dideoxy-D-*threo*-hexono-1,4-lactone (*ent*-4). To a solution of *ent*-**3** (0.111 g; 0.47 mmol) in MeOH (1.1 mL) was added $NaBH_4$ (0.02 g; 0.52 mmol) in portions at 0 °C. The reaction mixture was stirred for 1.5 h at 0 °C and then for 1 h at room temperature. One more equivalent of $NaBH_4$ (0.02 g; 0.52 mmol) was added and stirring was continued for an additional 1 h at room temperature. After cooling to 0 °C, TFA (2 mL) was added and the reaction mixture was stirred for an additional 1 h. To the mixture was finally added 10% Pd/C (0.056 g) and the resulting suspension was hydrogenated for 19 h at room temperature. The mixture was filtered and the catalyst washed with methanol. The organic solution was concentrated by co-distillation with toluene and methanol. The residue was purified by flash column chromatography (4:1 CH_2Cl_2/EtOAc) to afford pure *ent*-**4** (0.048 g; 71%) as a colorless syrup, $[\alpha]_D$ −40.0 (*c* 2.07, MeOH), lit.[8] $[\alpha]_D$ −43.3 (*c* 0.9, MeOH), R_f=0.22 (EtOAc). Spectral data of *ent*-**4** were consistent with those recorded for the opposite enantiomer **4** (Section 4.1.9), as well as with the reported values.[8]

4.1.18. 5-*O*-Benzyl-2,3-dideoxy-D-*threo*-hexono-1,4-lactone (*ent*-12). To a solution of **20** (0.699 g, 2.36 mmol) in CH_2Cl_2 (9.5 mL) were added Kieselgel 60 (4.72 g, 0.063–0.2 nm) and 0.65 M aq $NaIO_4$ (4.7 mL, 3.06 mmol). The mixture was vigorously stirred at room temperature for 45 min, filtered, and the precipitate washed with CH_2Cl_2. The combined organic solutions were dried and evaporated to give chromatographically homogenous sample **22**. A solution of crude **22** (0.665 g) in a mixture of TFA (10.8 mL) and water (5.4 mL) was stirred at room temperature for 1.5 h. The mixture was concentrated by co-distillation with toluene to give chromatographically homogenous lactone *ent*-**3** (0.598 g) as a colorless solid. To a cooled (0 °C) and stirred solution of crude *ent*-**3** (0.598 g) in MeOH (5.6 mL) was added $NaBH_4$ (0.095 g, 2.52 mmol) and the mixture was stirred for 1.5 h at 0 °C. One more equivalent of $NaBH_4$ (0.095 g, 2.52 mmol) was added and stirring was continued for an additional 1 h at room temperature. After cooling to 0 °C, TFA (11.2 mL) was added and the reaction mixture was stirred for an additional 1 h while it was allowed to warm to room temperature. The mixture was concentrated by co-distillation with toluene and the residue was purified by flash column chromatography (4:1 CH_2Cl_2/EtOAc). Eluted first was the unchanged intermediate *ent*-**3** (0.092 g, 17% with respect to **20**). Eluted second was the pure product *ent*-**12** (0.314 g, 68% from **20**, on the basis of the recovered *ent*-**3**), $[\alpha]_D^{23}$ −23.1 (*c* 1.05, $CHCl_3$), R_f=0.24 (4:1 CH_2Cl_2/EtOAc). Spectral data of *ent*-**12** were consistent with those recorded for the opposite enantiomer **12** (Section 4.1.8).

4.1.19. 5-*O*-Benzyl-6-*O*-decyl-2,3-dideoxy-D-*threo*-hexono-1,4-lactone (*ent*-14). A mixture of *ent*-**12** (0.054 g, 0.23 mmol), Ag_2O (0.133 g, 0.57 mmol), AgOTf (0.015 g, 0.06 mmol), and $C_{10}H_{21}Br$ (0.24 mL, 1.15 mmol) in anhydrous Et_2O (0.55 mL) was heated under reflux for 5.5 h. After the mixture cooled to room temperature it was diluted with CH_2Cl_2 (5 mL), filtered, and evaporated. Flash column chromatography (CH_2Cl_2/EtOAc) of the residue gave pure

ent-**14** (0.061 g, 71%) as a colorless oil, $[\alpha]_D^{23}$ −35.1 (*c* 0.92, $CHCl_3$), R_f=0.24 (CH_2Cl_2). Spectral data of *ent*-**14** were consistent with those recorded for (+)-enantiomer **14** (Section 4.1.10). Anal. Found: C, 73.03; H, 9.38. Calcd for $C_{23}H_{36}O_4$: C, 73.37; H, 9.64.

4.1.20. 6-*O*-Decyl-2,3-dideoxy-D-*threo*-hexono-1,4-lactone (*ent*-2). A solution of *ent*-**14** (0.061 g; 0.16 mmol) in EtOAc (1.25 mL) was hydrogenated over 10% Pd/C (0.031 g) following the same methodology as described above (procedure in Section 4.1.11), to afford pure *ent*-**2** (0.040 g, 87%), mp 42 °C, $[\alpha]_D$ −33.0 (*c* 0.83, $CHCl_3$), R_f=0.21 (9:1 CH_2Cl_2/EtOAc). Spectral data of *ent*-**2** were consistent with those recorded for (+)-enantiomer **2** (Section 4.1.11). Anal. Found: C, 67.01; H, 10.67. Calcd for $C_{16}H_{30}O_4$: C, 67.10; H, 10.56.

4.2. In vitro antitumor assay

Antitumor activity was evaluated by the tetrazolium colorimetric MTT assay. The assay is based on cleavage of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to formazan by mitochondrial dehydrogenases in viable cells. Exponentially growing cells were harvested, counted by trypan blue exclusion, and plated into 96-well microtiter plates (Costar) at optimal seeding density of 10^4 (K562, HL-60, JURKAT) or 5×10^3 (HeLa, MCF-7) cells per well to assure logarithmic growth rate throughout the assay period. Viable cells were plated in a volume of 90 μL per well, and preincubated in complete medium at 37° C for 24 h to allow cell stabilization prior to the addition of substances. Tested compounds, at ten the required final concentration, in growth medium (10 μL/well) were added to all wells except to the control ones and microplates were incubated for 24 h. The wells containing cells without tested compounds were used as control. Three hours before the end of incubation period, 10 μL of MTT solution was added to each well. MTT was dissolved in the medium at 5 mg/mL and filtered to sterilize and remove a small amount of insoluble residue present in some batches of MTT. Acidified 2-propanol (100 μL of 0.04 M HCl in 2-propanol) was added to each well and mixed thoroughly to dissolve the dark blue crystals. After a few minutes at room temperature to ensure that all crystals were dissolved, the plates were read on a spectrophotometer plate reader (Multiscan MCC340, Labsystems) at 540/690 nm. The wells without cells containing complete medium and MTT only acted as blank. The compound cytotoxicity was expressed as the IC_{50} (50% inhibitory concentration).

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Foldamer-based pyridine–fullerene tweezer receptors for enhanced binding of zinc porphyrin

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Abstract—This paper reports the design and synthesis of a new series of hydrogen bonding-mediated foldamer-derived tweezer receptors that are used for efficient complexation of zinc porphyrin guest. One end of the rigidified aromatic amide backbone is incorporated with one fullerene unit, while another end is connected to one pyridine or imidazole unit. The ^{1}H NMR, UV–vis, and fluorescent investigations in chloroform revealed that, due to the intramolecular hydrogen bonding-driven preorganized folded conformation, the fullerene and pyridine units of the receptors are located with suitable spatial separation and consequently able to co-complex zinc porphyrin with remarkably increased stability. In contrast, the imidazole-incorporated receptor displays a weakened binding affinity possibly due to structural mismatching and large steric hindrance. The association constants of the complexes of the new receptors with zinc porphyrin have been determined.

1. Introduction

Development of synthetic receptors for efficient recognition of special molecule or ion requires high structural and binding-site complementarity between the receptor and guest.[1] In order to achieve high binding stability and selectivity, more than one binding site is usually needed to be introduced in the receptors and the binding sites should also be located with suitable distance and orientation. Covalently bonded molecular tweezers represent one class of structurally unique receptors for many site-matching guests.[2] Nevertheless, the synthesis of these receptors is usually of low efficiency or is time-consuming, and in many cases their structural modifications are also difficult.[3] Therefore, it is of importance to develop new, simple approaches for designing tweezer-styled receptors.

We recently reported a new strategy for developing a new generation of assembling tweezers by making use of hydrogen bonding-induced aromatic amide oligomers as backbones.[4,5] Two or more zinc porphyrin or pyridine units have been introduced to rationally designed folded backbones for efficient complexation of fullerene or porphyrin guests by cooperative two-point interaction.[6] In this paper, we report the synthesis of a new series of foldamer-derived fullerene and pyridine-incorporated tweezer receptors that can efficiently complex zinc porphyrin in chloroform.[7]

Keywords: Molecular recognition; Foldamer; Hydrogen bonding; Fullerene; Porphyrin.
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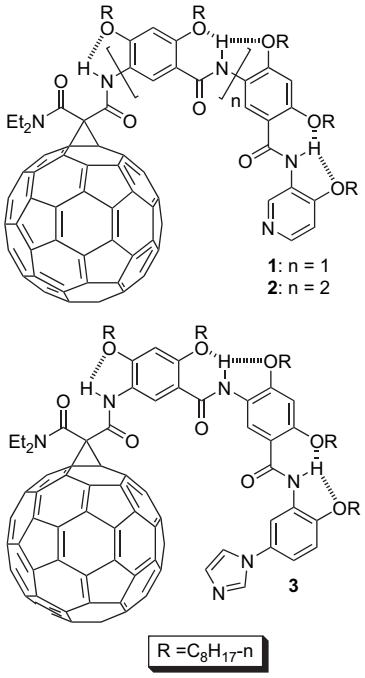
2. Results and discussion

Three foldamer-based receptors **1**–**3** have been synthesized, which were designed on the basis of recent reports that intramolecular three-centered hydrogen bonding can induce linear aromatic amide oligomers to adopt folded or other rigidified conformation.[5,8] The pyridine or imidazole unit was incorporated because they are good nitrogen ligands for coordination with metallated porphyrins,[9] and fullerene was introduced to the receptors because important π–π stacking has been revealed between fullerene and metallated porphyrin.[10]

The synthetic route for compound **1** is shown in Scheme 1. Thus, compound **4** was first nitrated in concentrated sulfuric acid to give **5**[11] in 72% yield. The latter was converted into **6** in 70% yield (two steps) by reacting with phosphorus pentachloride in 1,2-dichloroethane, followed by treatment of the chloride intermediate with *n*-octanol. Palladium-catalyzed hydrogenation of compound **6** in methanol generated amine **7** in 90% yield. With **7** available, the coupling reaction of aniline **8**[12] with acyl chloride **9**[13] in dichloromethane was performed, which produced compound **10** in 80% yield. The intermediate was again hydrogenated to give **11** in 96% yield. Aniline **11** was then reacted with 3-(diethylamino)-3-oxopropanoic acid[14] in dichloromethane in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) to afford intermediate **12** in 80% yield. Compound **12** was then hydrolyzed with LiOH in aqueous methanol and THF to afford **13** in 90% yield. The acid was reacted with **7** in chloroform also with DCC as coupling reagent to produce **14** in 78% yield. Finally, treatment of intermediate **14** with fullerene in

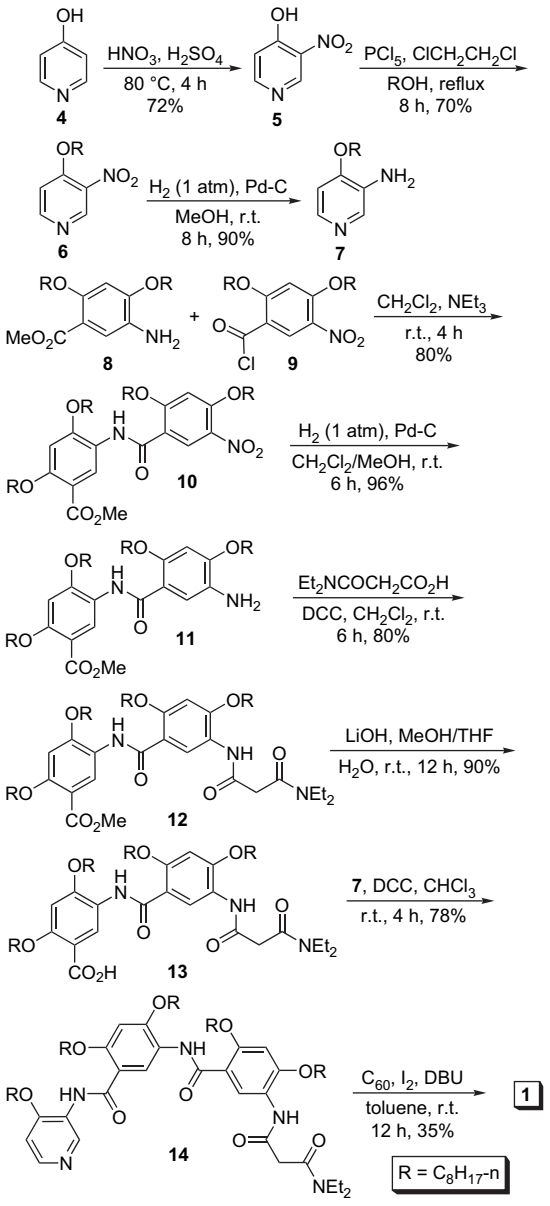


toluene at room temperature in the presence of iodine and 7,11-diazabicyclo[5.4.0]undec-11-ene (DBU) afforded **1** in 35% yield.

For the synthesis of compound **2** (Scheme 2), compound **15** was first prepared in 85% yield from the reaction of **7** with **9** in refluxing chloroform and triethylamine. The intermediate then underwent palladium-catalyzed hydrogenation in dichloromethane and methanol to afford **16** in 96% yield. Compound **16** was reacted with **13** in chloroform in the presence of DCC to afford **17** in 78% yield. Finally, compound **17** was treated with fullerene in the presence of iodine and DBU in toluene to give **2** in 20% yield.

The synthetic route for compound **3** is provided in Scheme 3. Compound **18**[15] was first treated with iodine and silver sulfate in methanol to give **19** in 90% yield. The iodide was then coupled with imidazole in hot DMF in the presence of potassium carbonate, cupric iodide, and proline to afford **20** in 70% yield. Palladium-catalyzed hydrogenation of compound **20** in methanol and dichloromethane produced **21** in 96% yield. The aniline derivative was then coupled with **13** in chloroform in the presence of DCC to afford **22** in 70% yield. Finally, the intermediate was reacted with fullerene and iodine in toluene in the presence of DBU to give **3** in 18% yield.

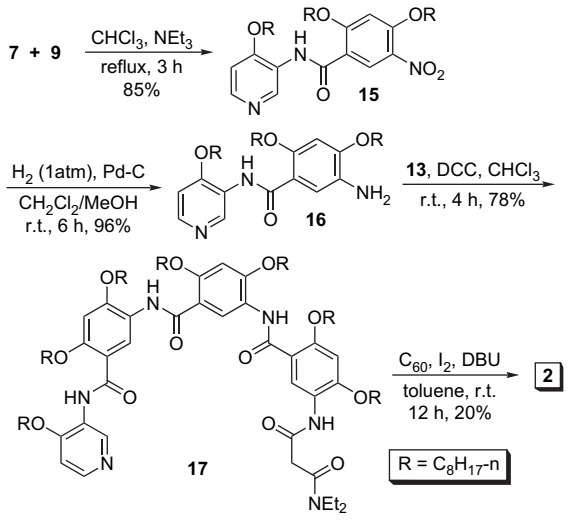
The ^{1}H NMR spectra of compounds **1**–**3** and their precursors **14**, **17**, and **22** in $CDCl_3$ are provided in Figure 1. The signals of the NH protons have been assigned by the D_2O exchange experiments. The great difference between the chemical shifts of the signals of the receptors and their precursors may be attributed to the large shielding effect of the fullerene unit in the receptors. Because the rigidified crescent secondary structure of the aromatic amide backbones in the receptors has been previously established,[16] it is reasonable to



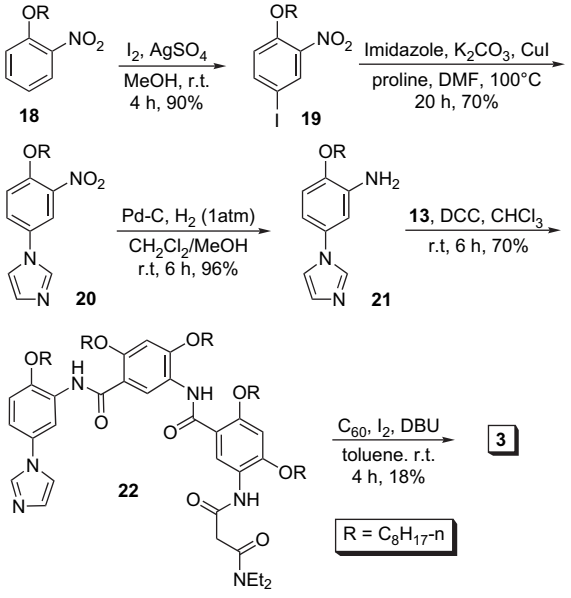
Scheme 1.

assume that the present fullerene- and nitrogen ligand-appended compounds also adopt folded conformation.

Adding zinc porphyrin **23** to the solution of **1** in $CDCl_3$ caused important shifting of several signals of both compounds (Figs. 1b–e), suggesting that important complexation occurs between them. Similar results were also observed for the solution of **2** and **23** in $CDCl_3$. Quantitative complexing behaviors of **1** and **2** with zinc porphyrin **23** in chloroform were then investigated by the UV–vis spectroscopy. The plots of the change of the UV–vis absorbance of **23** with the incremental addition of **1** and **2** are shown in Figures 2 and 3. Remarkable hypochromic effect was exhibited for the Soret band of **23**, which also supports strong intermolecular coordination. The UV–vis titration spectra of both systems displayed a clear isosbestic point for the Soret band and the Q-band, suggesting a 1:1 binding mode.[9] The association



Scheme 2.



Scheme 3.

constants (K_{assoc}) of complexes **1**·**23** and **2**·**23** in chloroform were determined by fitting their UV–vis titration data to a 1:1 binding mode,[6,17] which gave a value of approximately 7.6×10^3 and 1.2×10^4 M^{-1}, respectively. On the basis of the identical titration method, the K_{assoc} values of complexes **14**·**23** and **17**·**23** in chloroform have been determined to be ca. 1.0×10^3 and 1.4×10^3 M^{-1}, respectively. The UV–vis titration spectra of **23** with **17** are shown in Figure 4 as an example. These values are pronouncedly lowered than those of the corresponding complexes of the fullerene-appended foldamers. Considering the remarkably large size of the fullerene unit, the increase in the stability of the complexes of **1** and **2**, relative to that of the corresponding fullerene-free ligands, suggests that important π–π stacking interaction forms between **23** and the fullerene units in **1** and **2**. The increased binding stability reflects that this stacking and the intermolecular zinc–pyridine coordination join together to promote the formation of a 'two-point'-bound

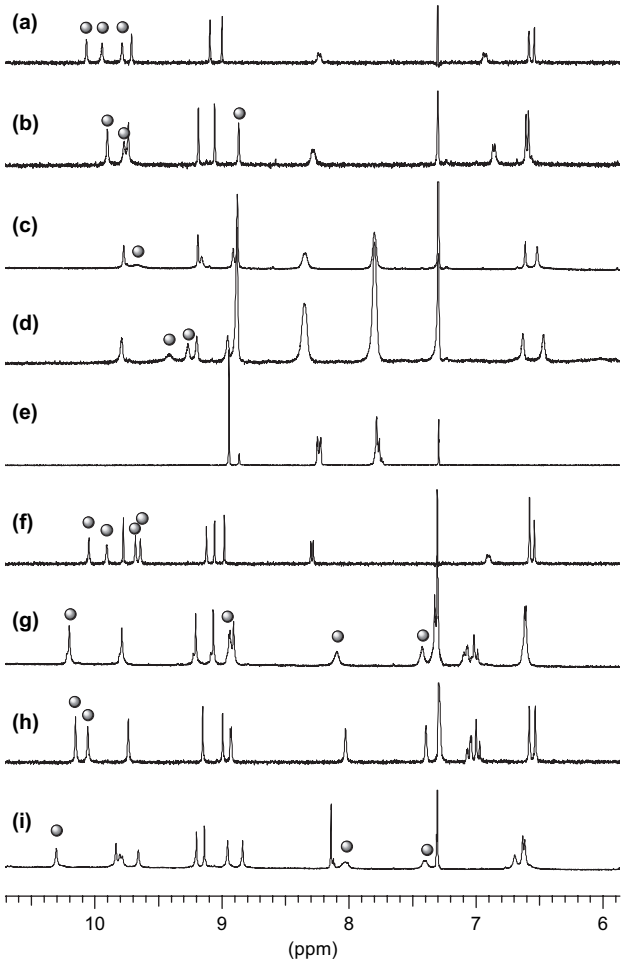


Figure 1. Partial ^{1}H NMR spectrum of (a) **14**, (b) **1**, (c) **1**+**23** (1:0.5), (d) **1**+**23** (1:1), (e) **23**, (f) **17**, (g) **2**, (h) **22**, and (i) **3** in $CDCl_3$ at 25 °C (6 mM). The labeled peaks are those of the amide protons.

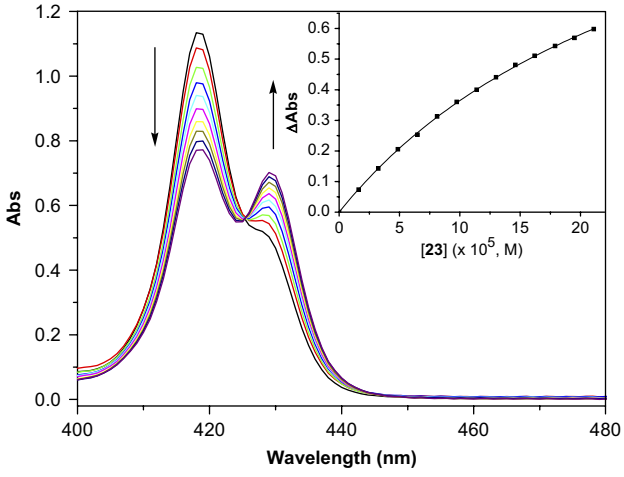


Figure 2. The change of the absorption spectra of **23** (2.8×10^{-6} M) with the addition of **1** (0–70 equiv) in chloroform at 25 °C (inset: plot of the absorption of **23** at 422 nm vs [**1**]).

complex, as shown in Figure 5. The higher binding stability of complex **2**·**23** might reflect a better spatial orientation of the pyridine and fullerene units of **2** for cooperative binding of zinc porphyrin **23**.

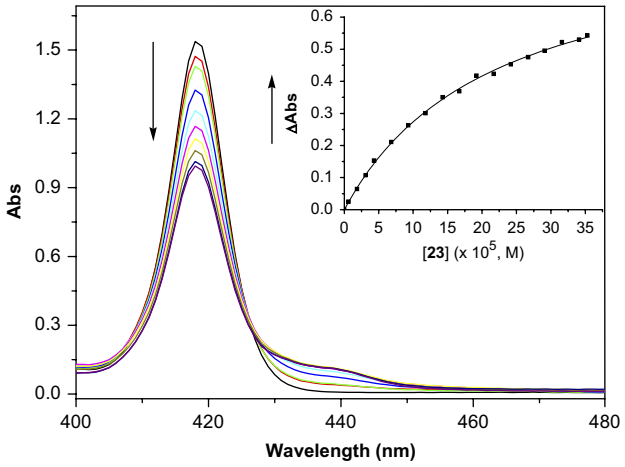


Figure 3. The change of the absorption spectra of **23** (2.8×10^{-6} M) with the addition of **2** (0–130 equiv) in chloroform at 25 °C (inset: plot of the absorption of **23** at 422 nm vs [**2**]).

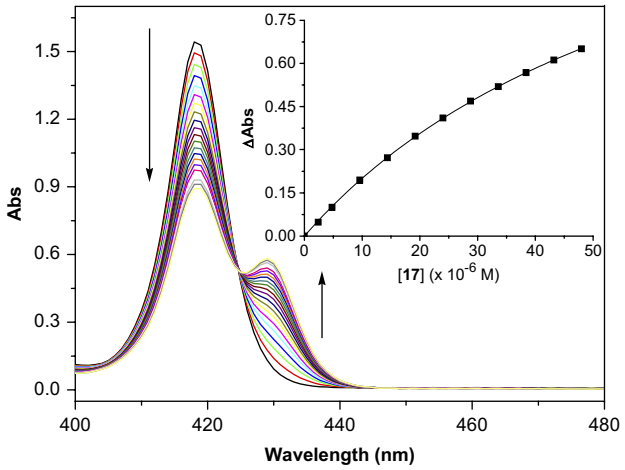
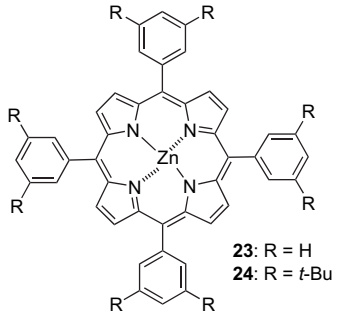


Figure 4. The change of the absorption spectra of **17** (2.8×10^{-6} M) with the addition of **23** (0–65 equiv) in chloroform at 25 °C (inset: plot of the absorption of **23** at 422 nm vs [**17**]).



The strong binding affinity of **1** and **2** toward **23** also caused efficient quenching of the emission of **23**. Fluorescent titration experiments were therefore also carried out in chloroform, which gave rise to a K_{assoc} of ca. 7.8×10^3 and 1.3×10^4 M^{-1} for complexes **1**·**23** and **2**·**23**, respectively. These values are consistent with the results obtained by the UV–vis experiments. As an example, the fluorescent titration results for compound **2** are shown in Figure 6.

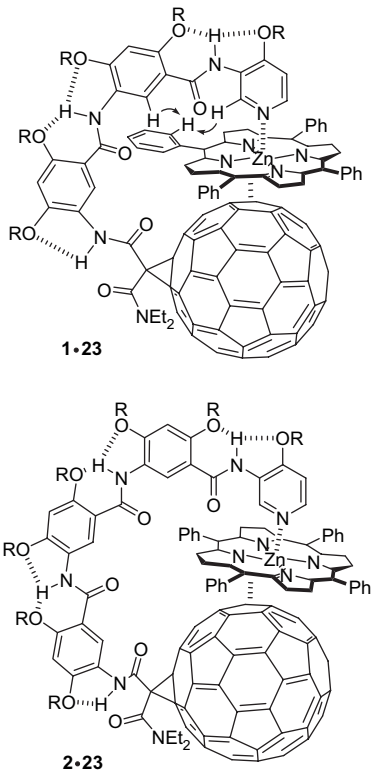


Figure 5. Proposed structures for 'two-point'-bound complexes **1**·**23** (the observed intermolecular NOE connections are shown) and **2**·**23**.

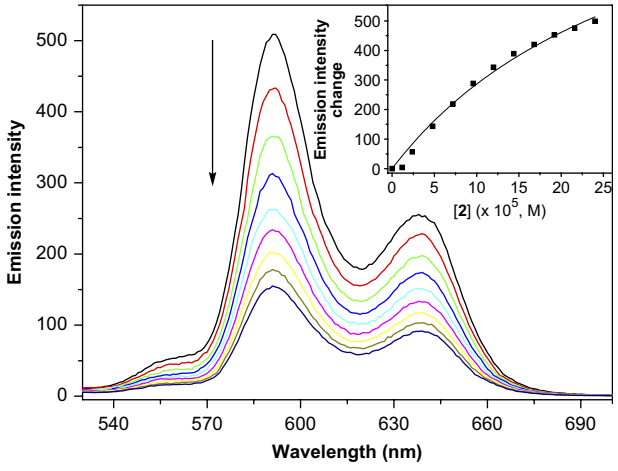


Figure 6. The change of the fluorescent spectra of **23** (1.2×10^{-6} M) with the addition of **2** (0–17 equiv) in chloroform at 25 °C (inset: plot of the emission change of **23** at 422 nm vs [**2**]).

Under similar experimental conditions, addition of **1** or **2** to the solution of **24** of larger size in chloroform did not cause obvious change of the UV–vis spectrum of **24**, implying that there is no important interaction. In contrast, UV–vis titration experiments performed in chloroform revealed important interaction between **24** and **17**, which corresponded to a K_{assoc} of ca. 1.2×10^3 M^{-1}. These results can be explained by considering the increased steric repulsion between **24** and the large fullerene units in **1** and **2**,

which retards the possible intermolecular π–π stacking and coordination interaction.

It has been established that imidazole is stronger than pyridine as ligand for zinc porphyrin.[9] Surprisingly, adding **3** to the solution of **23** in chloroform only led to slight hypochromism of the Soret band of the latter in the UV–vis spectrum (Fig. 7), which corresponded to a K_{assoc} of ca. $1.4\times10^2\ M^{-1}$ for complex **3·23**. In contrast, the K_{assoc} of complex **22·23** in the same solvent was determined by UV–vis titration experiments (Fig. 8) to be ca. $6.0\times10^3\ M^{-1}$. This value is comparable to that of many of the imidazole–zinc porphyrin complexes[9] but is remarkably higher than that of complex **3·23**. These observations may also be attributed to the great spatial hindrance of the fullerene unit in **3**, which obstructs the approach of **23** to the imidazole unit of **3** as shown in Figure 9. In contrast, the imidazole of fullerene-free **22** could efficiently coordinate to **23** of smaller size by adopting the separated conformation as shown in Figure 9 to form stable complex.

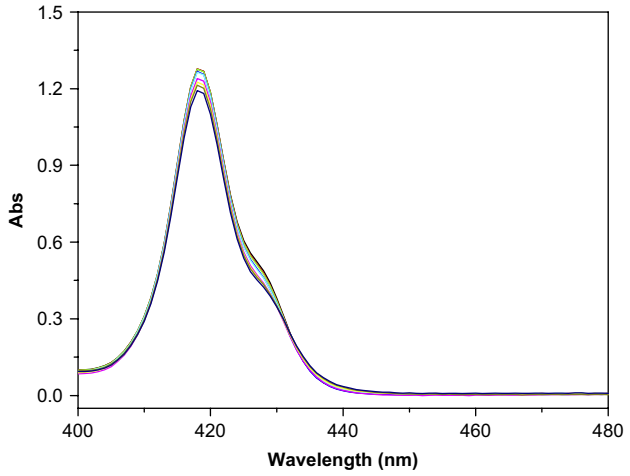


Figure 7. The change of the absorption spectra of **23** (2.8×10^{-6} M) with the addition of **3** (0–100 equiv) in $CDCl_3$ at 25 °C.

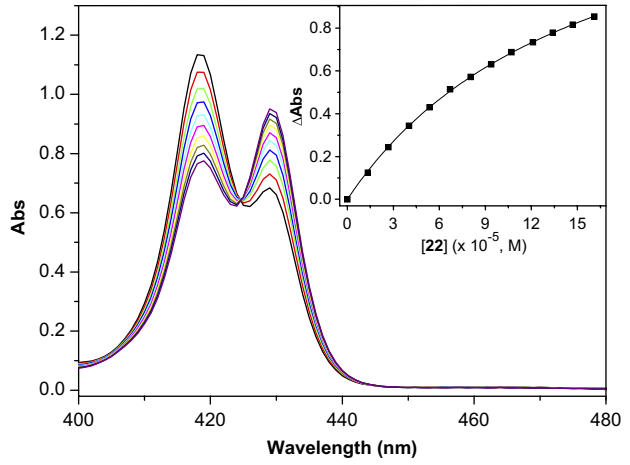


Figure 8. The change of the absorption spectra of **23** (2.8×10^{-6} M) with the addition of **22** (0–65 equiv) in chloroform at 25 °C (inset: plot of the absorption of **23** at 422 nm vs [**22**]).

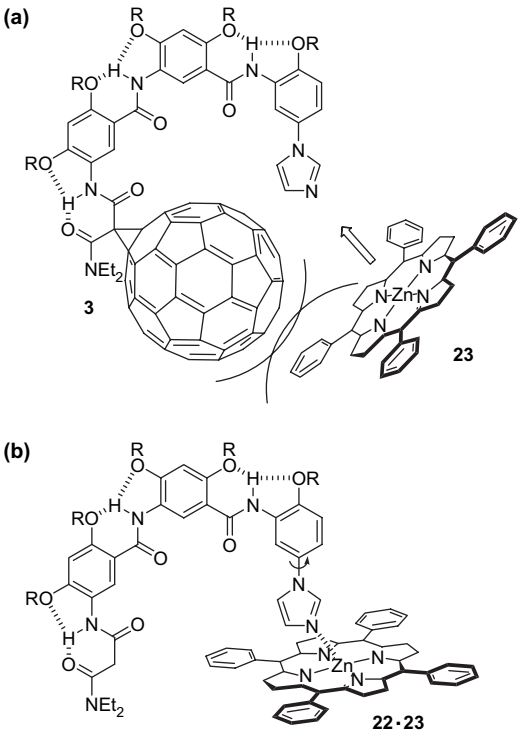


Figure 9. (a) Proposed repulsion of the fullerene unit in **3** toward zinc porphyrin **23**. (b) The structure of complex **22·23**.

3. Conclusion

In summary, we have reported the synthesis of a new series of foldamers, which are incorporated with one fullerene and one pyridine unit, at the two ends of their aromatic amide backbone. The ^{1}H NMR, UV–vis, and fluorescent investigations in chloroform have revealed that the new rigidified molecules are able to efficiently complex zinc porphyrin as a result of cooperative coordination and π–π stacking interactions. A 'two-point' binding mode has been proposed for the new complexes. The stability of the new series of complexes is sensitive to the steric effect and, as a result, very weak complexation has been revealed for zinc porphyrin and imidazole-incorporated receptor of similar structure. The result demonstrates that hydrogen bonding-induced artificial secondary structures are new versatile assembling building blocks for molecular recognition and supramolecular chemistry.

4. Experimental section

4.1. General methods

The ^{1}H NMR spectra were recorded on 500, 400 or 300 MHz spectrometer in the indicated solvents. Chemical shifts are expressed in parts per million using residual solvent protons as internal standards. Chloroform (7.26 ppm) was used as an internal standard for chloroform-*d*. Elemental analysis was carried out at the SIOC analytical center. Unless otherwise indicated, all commercially available materials were used as received. All solvents were dried before use following standard procedures. All reactions were carried out under

an atmosphere of nitrogen. Silica gel (1–4 μm) was used for column chromatography.

4.1.1. Compound 6. A suspension of compound **5**[11] (5.00 g, 35.7 mmol) and phosphorus pentachloride (7.90 g, 42.6 mmol) in 1,2-dichloroethane (35 mL) was heated under reflux until a clear solution was formed. The solution was cooled to 35 °C and *n*-octanol (55 mL) was added dropwise. The mixture was heated again under reflux for 1 h and then cooled to room temperature. The precipitate formed was filtered and washed with cold water and ethanol. The crude product was purified by recrystallization from acetonitrile to give **6** as a white solid (6.30 g, 70%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.01 (s, 1H), 8.61 (d, J=5.7 Hz, 1H), 7.02 (d, J=5.7 Hz, 1H), 4.20 (t, J=5.7 Hz, 2H), 1.86 (t, J=5.7 Hz, 2H), 1.52–1.29 (m, 10H), 0.90 (t, J=6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 158.5, 154.3, 146.8, 136.8, 109.1, 70.1, 31.7, 29.1, 29.0, 28.5, 25.6, 22.5, 14.0. MS (EI): *m*/*z* 253 $[M+H]^+$. HRMS (EI): calcd for $C_{13}H_{20}N_2O_3$ $[M-NH_2]^+$: 235.1447. Found: 235.1455.

4.1.2. Compound 7. A suspension of **6** (5.06 g, 20.0 mmol) and Pd–C (5%, 0.26 g) in methanol (200 mL) was stirred under the atmosphere of hydrogen gas (1 atm) at room temperature for 8 h. The solid was filtered off and the filtrate concentrated in vacuo. The resulting residue was subjected to flash chromatography (CH_2Cl_2/AcOEt 20:1) to give compound **7** as a pale yellow solid (4.00 g, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.00 (s, 1H), 7.95 (d, J=5.7 Hz, 1H), 6.68 (d, J=5.4 Hz, 1H), 4.04 (t, J=6.3 Hz, 2H), 3.74 (br, 2H), 1.87–1.83 (m, 2H), 1.47–1.25 (m, 10H), 0.89 (t, J=7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 157.0, 137.6, 130.4, 124.1, 106.6, 70.3, 31.6, 29.1, 29.0, 28.5, 25.7, 22.5, 14.0. MS (EI): *m*/*z* 222 $[M]^+$. HRMS (EI): calcd for $C_{13}H_{22}N_2O$: 222.1732. Found: 222.1742.

4.1.3. Compound 10. To a stirred solution of compound **8**[12] (3.26 g, 8.00 mmol) and triethylamine (1.00 g, 10.0 mmol) in dichloromethane (50 mL) was added a solution of **9** (3.52 g, 8.00 mmol) in dichloromethane (25 mL). The solution was stirred at room temperature for 4 h and then washed with diluted hydrochloric acid (20 mL), water (2×30 mL), brine (30 mL) and dried over sodium sulfate. Upon removal of the solvent under reduced pressure, the resulting residue was purified by column chromatography (dichloromethane/methanol 30:1) to give **10** as a yellow solid (6.50 g, 80%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.63 (s, 1H), 8.94 (s, 1H), 8.92 (s, 1H), 6.54 (s, 1H), 6.49 (s, 1H), 4.26 (t, J=7.2 Hz, 2H), 4.13–4.08 (m, 4H), 4.01 (t, J=6.6 Hz, 2H), 3.86 (s, 3H), 1.97–1.81 (m, 6H), 1.48–1.25 (m, 30H), 0.89–0.83 (m, 9H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 165.7, 160.8, 160.5, 157.0, 156.5, 152.3, 141.6, 133.6, 131.2, 124.9, 120.8, 114.8, 98.3, 97.9, 70.4, 70.1, 70.0, 69.0, 51.6, 31.9, 31.8, 31.7, 31.6, 29.6, 29.3, 29.2, 29.1, 29.07, 28.8, 28.2, 25.9, 25.8, 25.7, 22.6, 22.6, 22.5, 14.0. MS (MALDI): *m*/*z* 813.6 $[M+H]^+$, 835.5 $[M+Na]^+$. HRMS (MALDI): calcd for $C_{47}H_{76}N_2O_9Na$ $[M+Na]^+$: 835.5449. Found: 835.5443.

4.1.4. Compound 11. A suspension of compound **10** (4.07 g, 5.00 mmol) and Pd–C (5%, 0.25 g) in dichloromethane and methanol (50 mL, 1:1) was stirred under the atmosphere of hydrogen gas (1 atm) at room temperature for 6 h. The solid was filtered and the filtrate concentrated under reduced pressure. The crude product was subjected to flash chromatography (dichloromethane/methanol 30:1 v/v) to give **11** as a pale yellow solid (3.76 g, 96%). The compound was unstable in air and used for the next step without further characterization.

4.1.5. Compound 12. To a solution of compound **11** (2.35 g, 3.00 mmol) and 3-(diethylamino)-3-oxopropanoic acid (0.48 g, 3.00 mmol) in dichloromethane (50 mL) was added DCC (0.68 g, 3.3 mmol). The solution was stirred at room temperature for 6 h and the solid formed was filtrated. The solvent was then removed under reduced pressure and the resulting residue was purified by column chromatography (dichloromethane/methanol 20:1 v/v) to give **12** as a white solid (2.22 g, 80%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.99 (s, 1H), 9.76 (s, 1H), 8.95 (s, 1H), 8.90 (s, 1H), 6.43 (s, 2H), 4.10–3.92 (m, 8H), 3.78 (s, 3H), 3.43–3.33 (m, 4H), 1.85–1.78 (m, 8H), 1.50–1.09 (m, 46H), 0.82–0.76 (m, 12H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 167.9, 163.9, 156.6, 154.0, 152.6, 152.4, 124.7, 121.8, 112.3, 98.5, 97.4, 70.4, 70.1, 51.5, 42.7, 40.8, 40.7, 31.7, 29.3, 29.2, 25.9, 22.6, 14.4, 14.0, 12.9. MS (MALDI-TOF): *m*/*z* 924 $[M+H]^+$, 946 $[M+Na]^+$, 962 $[M+K]^+$. HRMS (MALDI-TOF): calcd for $C_{54}H_{90}N_3O_9$ $[M+H]^+$: 924.6677. Found: 924.6671.

4.1.6. Compound 13. To a solution of compound **12** (2.00 g, 2.02 mmol) in THF (40 mL), methanol (10 mL), and water (10 mL) was added lithium hydroxide monohydrate (1.00 g, 40 mmol). The mixture was stirred at room temperature for 12 h and then acidified with dilute hydrochloric acid to pH=3. The mixture was concentrated under reduced pressure to ca. 10 mL and then stayed until no precipitate was formed. The solid was filtered, washed with cold water thoroughly, and then dried in vacuo. The crude product obtained was purified by recrystallization from ethyl acetate to give **13** as a white solid (1.76 g, 90%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.06 (s, 1H), 9.75 (s, 1H), 9.13 (s, 1H), 8.94 (s, 1H), 6.51 (s, 1H), 6.45 (s, 1H), 4.21–4.03 (m, 8H), 3.49 (s, 2H), 3.45–3.40 (m, 4H), 1.89–1.87 (m, 8H), 1.57–1.13 (m, 46H), 0.89–0.82 (m, 12H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 164.9, 164.8, 162.8, 154.7, 153.6, 153.2, 126.7, 114.4, 97.3, 96.7, 70.7, 70.3, 69.4, 69.2, 43.6, 31.7, 31.7, 31.6, 29.4, 29.3, 29.2, 29.1, 29.0, 25.9, 25.8, 22.7, 22.6, 14.1, 14.0. MS (MALDI-TOF): *m*/*z* 910 $[M+H]^+$, 932 $[M+Na]^+$, 948 $[M+K]^+$. HRMS (MALDI-TOF): calcd for $C_{53}H_{88}N_3O_9$ $[M+H]^+$: 910.6521. Found: 910.6515.

4.1.7. Compound 14. A suspension of compound **13** (0.91 g, 1.00 mmol), **7** (0.22 g, 1.00 mmol), and DCC (0.23 g, 1.10 mmol) in chloroform (25 mL) was stirred at room temperature for 4 h. The solid formed was removed by filtration and the filtrate concentrated under reduced pressure. The resulting residue was subjected to column chromatography (dichloromethane/methanol 15:1 v/v) to afford **14** as a white solid (0.85 g, 78%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.09 (s, 1H), 9.99 (s, 1H), 9.79 (s, 1H), 9.70 (s, 1H), 9.03 (s, 1H), 8.99 (s, 1H), 8.18 (d, J=6.0 Hz, 1H), 6.98 (br s, 1H), 6.56 (s, 1H), 6.50 (s, 1H), 4.32–4.03 (m, 10H), 3.47 (s, 2H), 3.45–3.36 (m, 4H), 1.92–1.85 (m, 10H), 1.56–1.13 (m, 56H), 0.87–0.82 (m, 15H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 169.8, 163.0, 162.8, 162.3, 155.2, 154.3, 153.9, 152.9, 152.4, 144.8, 126.3, 125.2, 122.6, 121.4, 115.3, 114.9,

109.9, 106.3, 97.7, 97.4, 70.5, 69.1, 69.0, 68.8, 61.5, 41.8, 31.9, 31.8, 29.4, 29.3, 29.2, 28.9, 25.9, 25.8, 25.7, 22.6, 21.6, 14.1. MS (MALDI-TOF): *m/z* 1148 $[M+H]^+$, 1136 $[M+Na]^+$. HRMS (MALDI-TOF): calcd for $C_{66}H_{108}N_5O_9$ $[M+H]^+$: 1114.8147. Found: 1114.8142.

4.1.8. Compound 1. A solution of compound **14** (0.22 g, 0.20 mmol), fullerene (0.14 g, 0.20 mmol), and iodine (50 mg, 0.20 mmol) in dry toluene was heated under reflux for 10 min and then cooled to room temperature. DBU (0.034 mL) was added with a syringe and the mixture stirred for 12 h. Upon removal of the solvent in vacuo, the resulting residue was subjected to column chromatography (toluene/dichloromethane 20:1) to give compound **1** as a purple solid (0.11 g, 35%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.93 (s, 1H), 9.78 (s, 1H), 9.72 (s, 1H), 9.15 (s, 1H), 9.04 (s, 1H), 8.85 (s, 1H), 8.25 (t, J=2.4 Hz, 1H), 6.88 (dd, J_1=1.2 Hz, J_2=6.3 Hz, 1H), 6.57 (s, 1H), 6.55 (s, 1H), 4.22–4.06 (m, 12H), 3.78 (br s, 2H), 2.02–1.86 (m, 12H), 1.46–1.30 (m, 54H), 0.89–0.84 (m, 15H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 162.6, 159.3, 158.8, 158.7, 154.9, 154.5, 145.3, 145.2, 145.1, 144.8, 144.7, 144.6, 143.8, 143.0, 142.9, 142.8, 142.3, 142.1, 141.1, 76.7, 76.6, 76.5, 31.8, 31.7, 29.6, 29.4, 29.3, 29.2, 29.1, 25.9, 22.6, 22.5, 14.1, 14.0. MS (MALDI-TOF): *m/z* 1854 $[M+Na]^+$. HRMS (MALDI-TOF): calcd for $C_{126}H_{105}N_5O_9Na$ $[M+Na]^+$: 1854.7310. Found: 1854.7805.

4.1.9. Compound 15. To a solution of compound **7** (1.11 g, 5.00 mmol) and triethylamine (0.8 mL, 8.00 mmol) in chloroform (80 mL) was added a solution of compound **9** (2.20 g, 5.00 mmol) in chloroform (20 mL). The mixture was heated under reflux for 3 h and then cooled to room temperature. After workup, the crude product was purified by column chromatography (dichloromethane/AcOEt 10:1) to afford **15** as a white solid (2.51 g, 80%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.73 (s, 1H), 9.65 (s, 1H), 8.91 (s, 1H), 8.28 (d, J=5.4 Hz, 1H), 6.86 (d, J=5.4 Hz, 1H), 6.54 (s, 1H), 4.29 (t, J=6.9 Hz, 2H), 4.21–4.12 (m, 4H), 2.00–1.84 (m, 6H), 1.49–1.29 (m, 30H), 0.89–0.86 (m, 9H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 160.9, 160.6, 156.8, 153.7, 145.9, 142.5, 133.6, 131.4, 125.3, 114.2, 106.4, 97.9, 70.5, 70.2, 68.9, 31.7, 31.6, 31.7, 29.3, 29.2, 29.1, 29.0, 28.8, 25.8, 25.7, 22.6, 22.5, 14.7, 14.4, 14.0. MS (ESI): *m/z* 628 $[M+H]^+$. HRMS (ESI): calcd for $C_{36}H_{58}N_3O_6$ $[M+H]^+$: 628.4300. Found: 628.4320.

4.1.10. Compound 16. It was prepared as a white solid in 96% yield by the palladium-catalyzed hydrogenation of **15** according to the procedure described above for **11**. ^{1}H NMR (CD_3OD, 400 MHz): δ 9.81 (s, 1H), 8.55 (d, J=6.9 Hz, 1H), 8.24 (s, 1H), 7.78 (d, J=6.9 Hz, 1H), 7.02 (s, 1H), 4.60 (t, J=6.9 Hz, 2H), 4.51 (t, J=6.9 Hz, 2H), 4.31 (t, J=6.6 Hz, 2H), 2.02–1.90 (m, 6H), 1.56–1.27 (m, 30H), 0.93–0.85 (m, 9H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 164.7, 162.1, 160.8, 159.0, 140.1, 132.9, 130.0, 128.9, 115.3, 114.4, 111.2, 100.5, 73.9, 72.6, 71.9, 33.4, 33.4, 31.0, 30.9, 30.8, 30.9, 30.8, 30.8, 30.5, 30.1, 27.3, 27.1, 24.2, 24.1, 14.8. MS (ESI): *m/z* 598 $[M+H]^+$, 638 $[M+K]^+$. HRMS (ESI): calcd for $C_{36}H_{60}N_3O_4$ $[M+H]^+$: 598.4507. Found: 598.4578.

4.1.11. Compound 17. It was prepared as a white solid (78%) from the reaction of compounds **13** and **16** according to the procedure described above for **12**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.04 (s, 1H), 10.02 (s, 1H), 9.88 (s, 1H), 9.75 (s, 1H), 9.65 (s, 1H), 9.61 (s, 1H), 9.09 (s, 1H), 9.02 (s, 1H), 8.95 (s, 1H), 8.25 (d, J=5.4 Hz, 1H), 6.87 (d, J=5.4 Hz, 1H), 6.53 (s, 2H), 6.49 (s, 1H), 4.20–4.03 (m, 14H), 3.46 (s, 2H), 3.43–3.35 (m, 4H), 1.91–1.80 (m, 20H), 1.45–1.12 (m, 70H), 0.88–0.82 (m, 21H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 167.8, 163.8, 163.0, 162.9, 154.1, 153.9, 153.7, 153.6, 152.8, 152.7, 152.5, 145.0, 142.4, 142.3, 126.3, 126.1, 126.0, 125.1, 122.6, 122.3, 121.6, 115.5, 115.1, 114.9, 106.1, 97.9, 97.6, 97.4, 70.4, 70.3, 69.1, 69.0, 68.7, 42.8, 40.8, 31.8, 31.7, 29.6, 29.4, 29.3, 29.2, 29.1, 29.0, 28.8, 25.8, 25.7, 22.6, 14.4, 14.0, 12.9. MS (MALDI-TOF): *m/z* 1490 $[M+H]^+$, 1513 $[M+Na]^+$, 1529 $[M+K]^+$. HRMS (MALDI-TOF): calcd for $C_{89}H_{145}N_6O_{12}$ $[M+H]^+$: 1490.0921. Found: 1490.0915.

4.1.12. Compound 2. It was prepared as a purple solid (20%) from the reaction of compound **17** with fullerene according to the procedure described above for the preparation of **1**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.27 (s, 1H), 9.80 (s, 1H), 9.75 (s, 1H), 9.62 (s, 1H), 9.17 (s, 1H), 9.10 (s, 1H), 8.92 (s, 1H), 8.80 (s, 1H), 8.10 (s, 1H), 7.97 (br, 1H), 7.36 (br s, 1H), 6.64 (s, 1H), 6.58 (s, 1H), 6.54 (s, 1H), 4.61 (br, 2H), 4.64 (br, 2H), 4.23–4.11 (m, 14H), 1.86–1.90 (m, 20H), 1.47–1.26 (m, 6H), 0.86–0.77 (m, 21H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 158.6, 154.3, 148.5, 145.2, 145.1, 144.7, 144.6, 144.5, 143.8, 143.1, 143.0, 142.9, 142.2, 142.0, 140.9, 138.4, 128.5, 126.9, 125.0, 121.2, 120.5, 110.4, 97.6, 70.6, 70.4, 69.5, 69.2, 63.9, 56.0, 43.1, 31.8, 31.8, 31.7, 29.6, 29.5, 29.4, 29.3, 29.2, 25.9, 25.7, 25.5, 22.7, 22.6, 14.2, 14.0. MS (MALDI-TOF): *m/z* 2208 $[M+H]^+$, 2230 $[M+Na]^+$, 2246 $[M+K]^+$. HRMS (MALDI-TOF): calcd for $C_{149}H_{144}N_6O_{12}$ $[M+H]^+$: 2208.0764. Found: 2208.0759.

4.1.13. Compound 19. To a stirred solution of compound **18** (5.00 g, 20.0 mmol) in methanol (100 mL) were added iodine (5.12 g, 20.0 mmol) and silver sulfate (6.26 g, 20.0 mmol). The suspension was stirred for 1 h and then the solid was filtered off. The filtrate was concentrated under reduced pressure and the resulting residue triturated in ethyl acetate (100 mL). The organic phase was washed with water (50 mL×2), brine (50 mL) and dried over sodium sulfate. Upon removal of the solvent under reduced pressure, the resulting residue was recrystallized from ethyl acetate to give **19** as a dark brown solid (6.79 g, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.08 (d, J=2.1 Hz, 1H), 7.76 (dd, J_1=2.4 Hz, J_2=8.7 Hz, 1H), 6.83 (d, J=8.7 Hz, 1H), 4.06 (t, J=6.6 Hz, 2H), 1.83–1.76 (m, 2H), 1.59–1.27 (m, 10H), 0.87 (t, J=6.9 Hz, 3H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 152.3, 142.4, 133.7, 116.5, 80.2, 69.9, 31.7, 29.2, 29.1, 28.8, 25.8, 25.7, 22.6, 14.0. MS (EI): *m/z* 377 $[M]^+$. HRMS (EI): calcd for $C_{14}H_{20}NO_3I$: 377.0488. Found: 377.0488.

4.1.14. Compound 20. A suspension of compound **19** (3.75 g, 10.0 mmol), imidazole (0.80 g, 12.0 mmol), pottasium carbonate (3.45 g, 25.0 mmol), cupric iodide (0.10 g, 0.50 mmol), and proline (0.10 g, 1.00 mmol) in DMF (20 mL) was stirred at 100 °C for 40 h and then concentrated under reduced pressure. The residue was washed with water and the solid filtered. The solid was triturated in ethyl acetate (100 mL). The organic phase was washed with water

(50 mL), brine (50 mL) and dried over sodium sulfate. After the solvent was removed under reduced pressure, the crude product was purified by column chromatography (dichloromethane/methanol 100:1) to give **20** as a yellow solid (2.20 g, 70%). ^{1}H NMR (CD_3COCD_3, 400 MHz): δ 8.10 (s, 1H), 8.09 (s, 1H), 7.90 (dd, J_1=3.3 Hz, J_2=9.0 Hz, 1H), 7.63 (d, J=1.5 Hz, 1H), 7.52 (d, J=9.0 Hz, 1H), 7.13 (s, 1H), 4.28 (t, J=6.6 Hz, 2H), 1.52 (m, 2H), 1.37–1.31 (m, 10H), 0.89 (t, J=6.9 Hz, 3H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 151.7, 139.8, 135.7, 130.8, 129.7, 127.1, 119.1, 118.5, 115.7, 70.2, 70.0, 31.7, 29.1, 29.1, 28.8, 26.0, 25.7, 22.6, 14.1. MS (EI): *m/z* 317 $[M]^+$. HRMS (EI): calcd for $C_{17}H_{24}N_3O_3$ $[M+H]^+$: 318.1818. Found: 318.1822.

4.1.15. Compound 21. It was prepared as a pale yellow solid (96%) from the palladium-catalyzed hydrogenation of **20** following the procedure described above for **11**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.63 (br, 1H), 7.29 (br, 2H), 6.88 (br, 1H), 6.73 (d, J=8.1 Hz, 2H), 6.66 (d, J=8.1 Hz, 1H), 6.11 (br, 2H), 3.93 (t, J=6.3 Hz, 2H), 1.77–1.70 (m, 2H), 1.39–1.14 (m, 10H), 0.82 (t, J=6.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 146.8, 138.2, 129.1, 119.9, 111.4, 110.7, 107.9, 68.8, 31.7, 29.2, 26.0, 22.6, 14.1. MS (EI): *m/z* 287 $[M]^+$. HRMS (EI): calcd for $C_{17}H_{25}N_3O$: 287.1998. Found: 287.2004.

4.1.16. Compound 22. It was prepared as a pale yellow solid (70%) from the reaction of compounds **13** and **21** according to the procedure described above for **14**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.14 (s, 1H), 10.04 (s, 1H), 9.72 (s, 1H), 9.13 (s, 1H), 8.97 (s, 1H), 8.91 (d, J=3.0 Hz, 1H), 7.99 (s, 1H), 7.36 (s, 1H), 7.26 (s, 1H), 7.00 (dd, J_1=3.0 Hz, J_2=11.4 Hz, 1H), 6.95 (d, J=11.4 Hz, 1H), 6.54 (s, 1H), 6.49 (s, 1H), 4.21–4.03 (m, 10H), 3.46 (s, 2H), 3.44–3.34 (m, 4H), 1.93–1.84 (m, 10H), 1.58–1.12 (m, 50H), 0.89–0.82 (m, 15H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 167.8, 164.0, 163.6, 162.9, 154.0, 153.7, 152.7, 152.6, 147.3, 130.3, 125.7, 124.9, 122.7, 121.7, 115.7, 114.9, 114.8, 114.4, 111.7, 97.6, 97.2, 77.7, 76.6, 76.3, 70.5, 70.3, 69.3, 69.1, 69.0, 53.4, 42.8, 40.8, 40.6, 31.9, 31.7, 29.7, 29.3, 29.2, 29.1, 25.8, 22.6, 14.4, 14.0, 12.9. MS (MALDI-TOF): *m/z* 1179 $[M+H]^+$, 1201 $[M+Na]^+$. HRMS (MALDI-TOF): calcd for $C_{70}H_{111}N_6O_9$ $[M+H]^+$: 1179.8335. Found: 1179.8407.

4.1.17. Compound 3. It was prepared as a purple solid (18%) from the reaction of **22** with fullerene according to the procedure described above for the preparation of **1**. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 10.19 (s, 1H), 9.77 (s, 1H), 9.20 (s, 1H), 9.05 (s, 1H), 8.92 (s, 1H), 8.88 (s, 1H), 8.06 (br, 1H), 7.39 (s, 1H), 7.29 (s, 1H), 7.05 (d, J=11 Hz, 1H), 6.96 (d, J=8.7 Hz, 1H), 6.58 (s, 1H), 6.57 (s, 1H), 4.23–4.08 (m, 12H), 3.68 (br, 2H), 1.93–1.85 (m, 10H), 1.46–1.27 (m, 50H), 0.86 (t, J=3 Hz, 15H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 158.6, 148.5, 145.6, 145.5, 145.3, 145.2, 144.8, 144.7, 144.6, 143.8, 143.1, 143.0, 142.9, 142.2, 142.1, 140.9, 138.5, 126.9, 125.0, 121.2, 120.9, 120.5, 114.4, 110.4, 69.6, 63.9, 56.0, 31.8, 31.7, 31.5, 29.6, 29.5, 29.4, 29.2, 29.1, 29.0, 25.9, 25.8, 22.6, 14.3, 14.0. MS (MALDI-TOF): *m/z* 1898 $[M+H]^+$, 1920 $[M+Na]^+$, 1936 $[M+K]^+$. HRMS (MALDI-TOF): calcd for $C_{130}H_{109}N_6O_9$ $[M+H]^+$: 1897.8256. Found: 1897.8250.

The method for the determination of association constants has been reported in previous papers.[6]

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Regioselectivity in alkenyl(aryl)-heteroaryl Suzuki cross-coupling reactions of 2,4-dibromopyridine. A synthetic and mechanistic study

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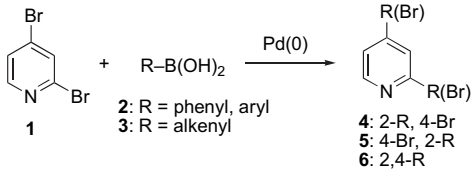
Abstract—2,4-Dibromopyridine undergoes a regioselective Suzuki cross-coupling reaction at position 2 with several alkenyl(aryl) boronic acids to render 4-bromo-2-carbon substituted pyridines, difficult to be prepared otherwise, in good yields under palladium catalysis, either $Pd(PPh_3)_4$/TlOH or $Pd_2dba_3/PCy_3/K_3PO_4$ at 25 °C. This behavior is explained on the basis of the electrophilic character of both C–Br bonds, being their relative reactivity in 2,4-dibromopyridine similar to that in the corresponding monobromopyridines. In addition, the dicoupled compound **6** is not formed through a double oxidative addition of 2,4-dibromopyridine to $Pd(PPh_3)$.

1. Introduction

As part of ongoing studies in our laboratories concerning complex pyridine-containing molecules, we have recently published an efficient and versatile stereoselective synthesis of the ocular pigment A2E, a pyridinium bisretinoid fluorophore.[1] The synthetic procedure that consisted in the substitution of both bromides in 2,4-dibromopyridine by means of two sequential selective palladium-catalyzed cross-coupling reactions, selectively introduced an organic substituent only at position 2 of the pyridine ring, followed by a second one at position 4.[2] This result prompted us to extend the applicability of this methodology and to study the scope of the processes. In addition, the 2,4-substituted pyridyl moiety is the structural motif in numerous pharmaceuticals and natural products.[3] While 4-alkenyl(aryl)-2-bromopyridines can be easily prepared from 4-bromopyridine followed by simple introduction of a bromo substituent at position 2, there is no straightforward method to 4-bromo-2-carbon substituted pyridines.

The coupling between an organic electrophile and an alkenyl-(aryl)boronic acid, known as Suzuki reaction, is amongst the most outstanding reactions in organic synthesis.[4] Several studies regarding selective palladium-catalyzed reactions of dihalopyridines, mainly 2,5-, 2,3-, 2,6-, and 3,5-disubstituted, have appeared in the literature during the last decade.[5] The observed selectivity was attributed either to the different electrophilicities of the carbon atoms or to the lower reactivity of the monosubstitution product, because of electronic or steric factors. However, the use of 2,4-dibromopyridine in regioselective cross-couplings is scarce and, to the best of our knowledge, there are only two examples, a Suzuki coupling with 3-bromophenylboronic acid that gave the 2-coupled compound in a moderate 24% yield[6a] and a palladium-catalyzed amination with a poor regioselectivity.[6b]

In this work we report a study on the regioselectivity of palladium-catalyzed cross-coupling reactions between aryl and alkenyl boronic acids (Suzuki reaction) and 2,4-dibromopyridine (Scheme 1). The ^{13}C NMR spectrum of 2,4-dibromopyridine shows that the chemical shifts of the carbon centers with bromine atoms attached (C^2 and C^4) differ slightly (142.5 and 133.9 ppm, respectively). Therefore, oxidative addition to Pd(0) could preferentially occur at one carbon center, presumably C^2, ensuring positional selective coupling reactions as could be expected for an S_N2-like process.



Scheme 1. Suzuki coupling of 2,4-dibromopyridine (**1**) and boronic acids **2** and **3**.

Keywords: 2,4-Dibromopyridine; Suzuki reaction; Regioselectivity; Palladium catalysis.
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2. Results and discussion

To investigate the regioselectivity and efficiency of different systems for the Suzuki cross-coupling reaction of 2,4-dibromopyridine (**1**)[7] and arylboronic acids (**2**)[8] to afford 2-aryl-4-bromopyridine, the reaction with phenylboronic acid (**2a**) was chosen as a model. The selectivity is given as the ratio among the three possible products, the monocoupled regioisomers, **4** and **5**, and the dicoupled product **6**.

We have used Pd(0) and Pd(II) species as catalysts, and polar and apolar solvents in aqueous or anhydrous conditions. The results are shown in Table 1 and the identification of the cross-coupling products was done either by comparison with published data[9] and/or by NMR experiments.

When $Pd(OAc)_2$ was utilized as pre-catalyst in the presence of bulky and electron rich phosphines, [(2-biphenylyl)di-*tert*-butylphosphine (2-BDBP)[10] and PCy_3[11]], known to accelerate the oxidative addition step, with different anhydrous bases (KF, K_2CO_3, Cs_2CO_3) we found out that the conversions at 25 °C or 50 °C were still low in the presence of 2.0 or 2.4 equiv of phenylboronic acid. The dicoupled compound **6a** was the major product even if the reaction was run with 1.0 equiv of phenylboronic acid (Table 1, entry 1). The proportion of **6a**, much higher than the statistical one, is difficult to explain solely on the basis of reactivity arguments.

If instead Pd_2dba_3 was the palladium source, the outcome was different depending upon the phosphine. While PCy_3 gave a very selective catalyst in spite of the presence of 2.5 equiv of **2a** (entry 3), 2-BDBP with 1.0 equiv of **2b** at 25 °C yielded the dicoupled compound **6b** as the major product (entry 5). It is also interesting to point out that the 4-coupled compound **5a** was obtained in higher ratio than the 2-coupled **4a** when 2-BDBP was used, either with $Pd(OAc)_2$ or with Pd_2dba_3 (Table 1, entries 5–7).[12]

When the reaction was carried out with $Pd(PPh_3)_4$ in combination with 3 M K_2CO_3 in toluene a selectivity of 6:1:0.5 was obtained (entry 8), but 2.0 equiv of boronic acid and temperatures up to 50 °C were necessary to achieve complete conversion which, on the other hand, caused a reduction of the selectivity (**4a/5a/6a**, 6:1:1.5) (entry 9). However, $Pd(PPh_3)_4$ in the presence of 10% aq TlOH in THF at 25 °C (entry 10) gave a very good selectivity, ratio **4a/5a/6a** 18:1:1, with total conversion after 12–24 h.[13]

In order to establish whether electronic effects of *p*-substituted arylboronic acids **2** (X=MeO, Me, F, CHO, CO_2Me) are important for this cross-coupling reaction, we run the reaction with 2,4-dibromopyridine in the presence of Pd(0) species [$Pd(PPh_3)_4$, aq TlOH, and THF, and Pd_2dba_3, PCy_3, K_3PO_4, and dioxane] at 25 °C. From the results shown in Table 1 it is possible to infer that either electron donating groups, such as MeO or Me, or fluoride (entries 11–13) on the boronic acid did not influence much the final result, while electron withdrawing groups, such as CHO or CO_2Me, prevented the cross-coupling to occur (entries 14 and 15).[14]

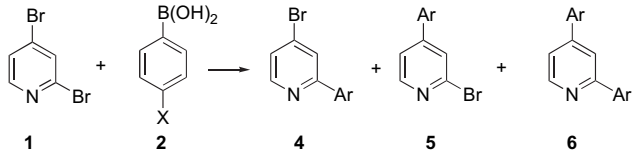
Thus, two catalytic systems, namely $Pd(PPh_3)_4$ in the presence of aqueous TlOH in THF and Pd_2dba_3/PCy_3 in anhydrous conditions (K_3PO_4 in dioxane), were found to be highly selective to obtain 4-bromo-2-arylpyridines through a Suzuki coupling of 2,4-dibromopyridine (**1**) and arylboronic acids (**2**) at 25 °C. On the other hand, if the aim is to prepare symmetrical 2,4-disubstituted pyridines, the reaction conditions will be Pd_2dba_3, (2-biphenylyl)di-*tert*-butylphosphine, $K_3PO_4 \cdot 1.5H_2O$ with an excess of boronic acid (Table 1, entries 6 and 7). Accordingly, two important ligands in luminescence devices,[15] 2,4-diphenylpyridine (**6a**) and 2,4-di(methoxyphenyl)pyridine (**6b**), were obtained in very good yields, 85 and 82%, respectively.

An important goal would be to prepare 2-alkenyl-4-bromopyridines (**4**) in one step from 2,4-dibromopyridine (**1**). For that purpose, we used boronic acids **3g**, **3h**, and **3i**[16] under both catalytic systems [$Pd(PPh_3)_4$, aq TlOH, and THF, and Pd_2dba_3, PCy_3, K_3PO_4, and dioxane] at 25 °C and the results are shown in Table 2.

Interestingly, boronic acids **3g**, **3h**, and **3i** presented an almost total selectivity for position 2 when the coupling was run with $Pd(PPh_3)_4$ and TlOH in THF (Table 2, entries 1, 3, and 4).

However, when the reaction was performed with Pd_2dba_3/PCy_3 in the presence of 1.2 equiv of boronic acid **3g** the selectivity dropped to 5.5:1:1.5 (entry 2), lower than the one for arylboronic acids **2** (Table 1). This result indicates that there is a significant competition among 2,4-dibromopyridine and the monocoupled compounds for the boronic acid present in the reaction mixture.

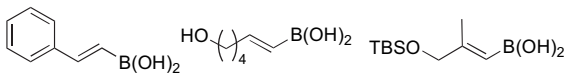
Table 1. Reaction of 2,4-dibromopyridine (**1**) with phenylboronic acid (**2a**)



Entry	**2** (equiv)	Reaction conditions[a]	*T* (°C)	**4**:**5**:**6**[b]	Yield[c] (Conv)[d]
1	**2a** (1.2)	$Pd(OAc)_2$, Cs_2CO_3, PCy_3, dioxane	25	2:1:7	— (35)
2	**2a** (1.0)	Pd_2dba_3, K_3PO_4,	25	11:0.3:1	43 (60)
3	**2a** (2.5)	PCy_3, dioxane	25	7:0:1	69 (>98)
4	**2b** (2.5)		25	7:0:1	72 (>98)
5	**2b** (1.0)	Pd_2dba_3, $K_3PO_4 \cdot 1.5H_20$,	25	1:1.2:15	— (46)
6	**2b** (2.5)	2-BDBP, toluene	40	1:1.2:26	82 (>98)
7	**2a** (2.5)		40	0:0:1	85 (>98)
8	**2a** (1.2)	$Pd(PPh_3)_4$, aq K_2CO_3,	50	6:1:0.5[e]	31 (75)
9	**2a** (2.0)	toluene	50	6:1:1.5[e]	40 (>98)
10	**2a** (1.2)	$Pd(PPh_3)_4$, aq TlOH,	25	18:1:1[e]	67 (>98)
11	**2b** (1.2)	THF	25	15:1:1[e]	64 (>98)
12	**2c** (1.2)		25	17:1:1[e]	60 (>98)
13	**2d** (1.2)		25	15:1:1.5[e]	68 (>98)
14	**2e** (1.2)		25	—	— (0)
15	**2f** (1.2)		25	—	— (0)

2-BDBP: (2-biphenylyl)di-*tert*-butylphosphine.
[a] $Pd(PPh_3)_4$, 8–15 mol %; $Pd(OAc)_2$, 1–5 mol %; Pd_2dba_3, 1.5–10 mol %.
[b] Calculated by ^1H NMR of the crude mixture.
[c] Yield of the pure major product.
[d] Conversion calculated by ^1H NMR of the crude mixture.
[e] 4,4′-Dibromo-2,2′-bipyridine was detected in variable amounts.

Table 2. Coupling of 2,4-dibromopyridine (**1**) and alkenylboronic acids **3g–i**



3g **3h** **3i**

Entry	**3** (equiv)	Reaction conditions	**4**:**5**:**6**[a]	Yield (%)[b]
1	**3g** (1.2)	$Pd(PPh_3)_4$, aq TlOH, THF	17:1:<1	71
2	**3g** (1.2)	Pd_2dba_3, K_3PO_4, PCy_3, dioxane	5.5:1:1.5	40[c]
3	**3h** (1.2)	$Pd(PPh_3)_4$, aq TlOH, THF	15:1:0	77
4	**3i**[d] (1.7)[e]	$Pd(PPh_3)_4$, aq TlOH, THF	16:1:0	75

[a] Calculated by ^{1}H NMR of the crude reaction mixture.
[b] Yield of the pure major product.
[c] Conversion (81%) calculated by ^{1}H NMR of the crude reaction mixture.
[d] Prepared in situ.
[e] Effective equivalents (1.2 equiv) considering boronic acid dimer and protodeboronation by ^{1}H NMR.

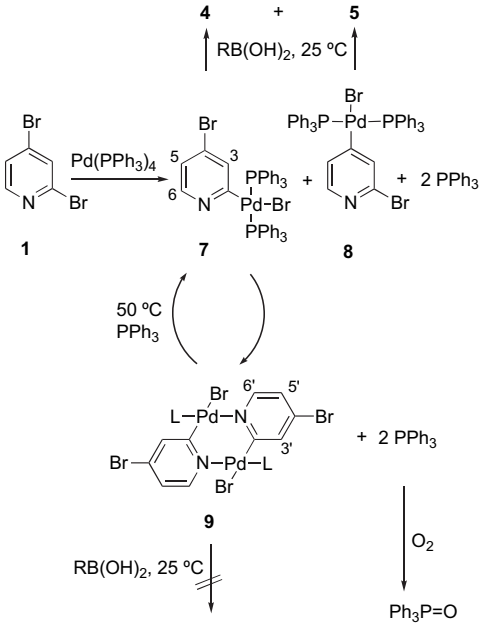
Due to the instability problems of compound **3i**, caused by the methyl group cis to the boronic acid moiety, an important protodeboronation process was detected and, hence, a bigger excess of **3i** was needed (entry 4).[17] Thus, when 1.7 equiv (1.2 effective, as determined by ^{1}H NMR) of **3i** were used, compound **4i**, key intermediate in the synthesis of A2E,[2] was obtained in good yield.

We demonstrated the selectivity of the coupling by means of two-dimensional NMR experiments (^{1}H and ^{13}C correlations, HSQC and HMBC) with compounds **3h** and **3i**. All the other compounds showed the same behavior, with (H^6, H^5, and H^3) pyridyl-proton shifts of the 4-coupled product **5** at higher field.

So, 2-alkenyl-4-bromopyridines were obtained in good yields (71–77%) when 2,4-dibromopyridine was treated with alkenyl boronic acids in the presence of $Pd(PPh_3)_4$/TlOH in THF at 25 °C by means of a highly regioselective cross-coupling process.

In order to get some insights into the mechanism of the process, we studied the reaction of 2,4-dibromopyridine and $Pd(PPh_3)_4$ in toluene by ^{1}H and ^{31}P NMR. Thus, treatment of 2,4-dibromopyridine with 1.0 equiv of $Pd(PPh_3)_4$ in toluene at 25 °C under oxygen-free conditions for 16 h lead to a mixture of both σ-palladium complexes **7** and **8** in 81% yield along with traces of a compound identified as the dimeric complex **9** (Scheme 2).

To the best of our knowledge, this is the first time that the formation of complex **7** has been observed by ^{1}H and ^{31}P NMR and MS. The ^{1}H NMR of σ-palladium complex **7** (Fig. 1b) shows the pyridine proton upfield shifted (7.40, 6.65, and 6.22 ppm for H^6, H^3, and H^5, respectively) compared to 2,4-dibromopyridine signals (Fig. 1a) due to an anisotropic shielding effect of the triphenylphosphine ligand as well as by back donation from palladium to the pyridine ring via the carbon atom. The oxidative addition of Pd-complex **7** undergoes a rapid conversion into the Pd-dinuclear-complex **9**, whose structure has unequivocally been established by X-ray crystallography, through an attack of the basic free pyridyl nitrogen on the neighboring metal, followed by elimination of one phosphine (Scheme 2). ^{1}H NMR of the dinuclear complex **9** shows H^3 and H^5 at 6.73 ppm, while the H^6 proton resonance appears as a multiplet at 8.32 ppm,



Scheme 2. Reaction of 2,4-dibromopyridine (**1**) with 1.0 equiv of $Pd(PPh_3)_4$.

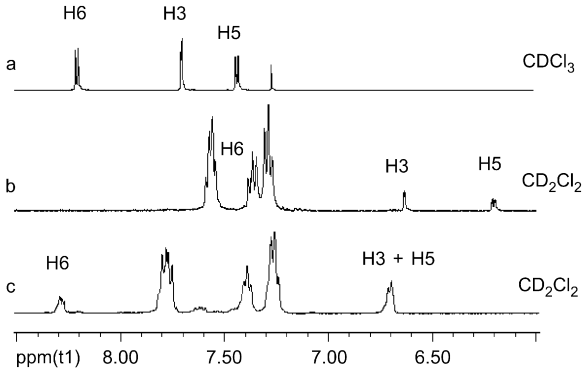


Figure 1. ^{1}H NMR spectra: (a) 2,4-dibromopyridine (**1**); (b) complex **7**; (c) dinuclear complex **9**.

upfield for a nitrogen coordinated to a metal atom indicating that the deshielding effect induced by the coordination is partially counteracted by the palladium back donation (Fig. 1c). Its ^{13}C NMR shows two characteristic resonances at 189.1 and 151.7 ppm for C^2–Pd and C^6, respectively. The tendency of the 2-carbon bonded pyridine to coordinate another metal center through the nitrogen atom is in accordance with its high basicity.[18]

The X-ray structure of complex **9** shows a boat conformation for the six-membered ring containing both Pd and N. Each palladium atom exhibits a square-planar geometry with bromide and phosphine trans to carbon and nitrogen, respectively (Fig. 2).

The ^{31}P NMR spectrum of the reaction of 2,4-dibromopyridine and $Pd(PPh_3)_4$ (Fig. 3) showed four signals. The signal at 22.65 ppm corresponds to the monomeric complex **7** and the presence of only one signal indicates that both phosphine ligands are equivalent. The resonance at downfield shift, 23.86 ppm (Fig. 3a) was assigned to the 2-bromopyrid-4-yl palladium complex **8**, not observed in ^{1}H NMR due to

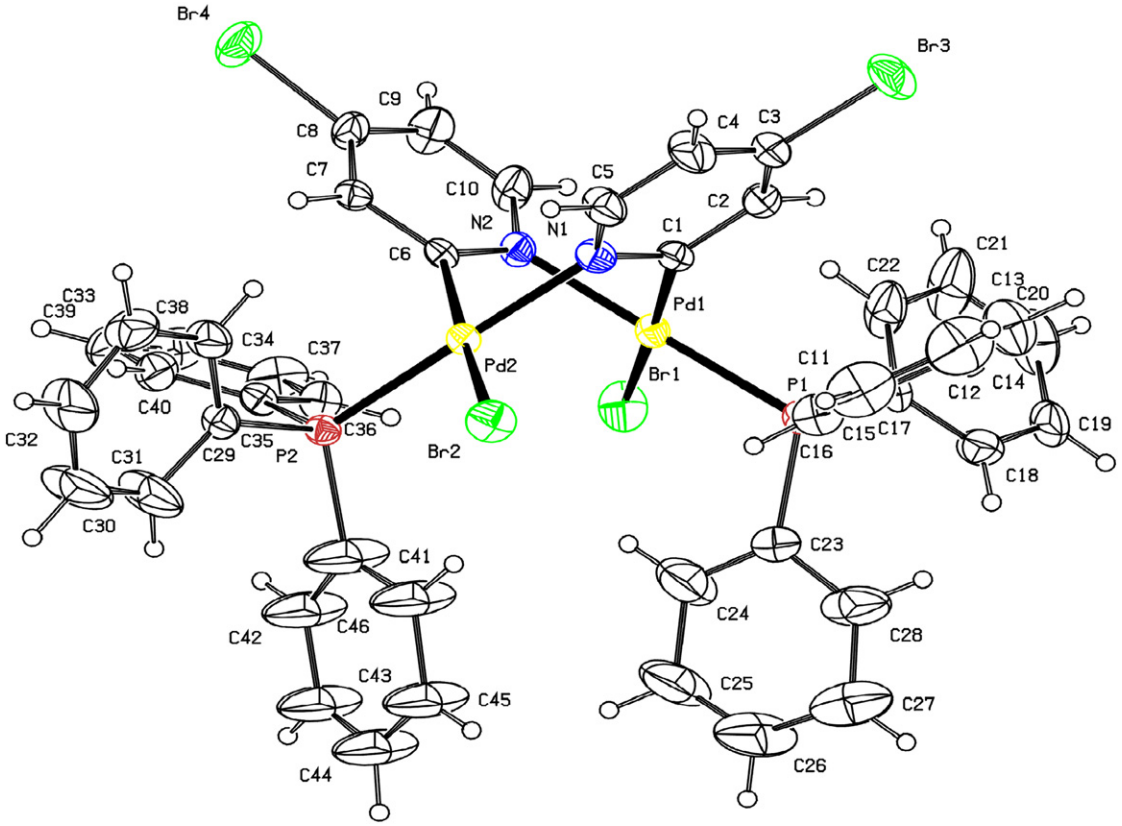


Figure 2. ORTEP representation of complex **9**.

either detection limits or signal-overlapping. The ratio between complex **7** and complex **8** in the oxidative addition determines the selectivity of the cross-coupling process. After 1 h, another signal started to appear at 30.45 ppm, much higher field than in the monomeric complex, and is due to the dimeric complex **9** (Fig. 3b).

The equilibrium between monomer complex **7** and dinuclear complex **9** in toluene shifts to the monomer upon addition of triphenylphosphine at 50 °C but no change is observed at 25 °C. Traces of air speed up the transformation of the mononuclear complex (22.21 ppm) into the dinuclear complex (30.45 ppm) (Scheme 2; Fig. 3b) due to the oxidation of PPh_3 (−4.85 ppm) to PPh_3O (24.70 ppm).[19]

Aiming to determine the formation pathway of the 2,4-disubstituted pyridine **6**, an equimolar mixture of both

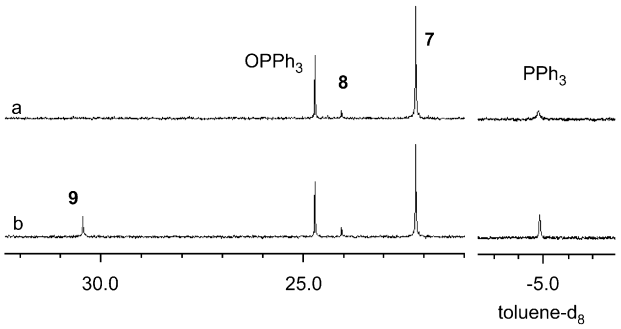


Figure 3. ^{31}P NMR spectra of the reaction of **1** with $Pd(PPh_3)_4$: (a) t=1 h; (b) t=11 h.

monobromopyridines, **4a** and **5a**, was treated with $Pd(PPh_3)_4$ (50 mol %), $PhB(OH)_2$ (100 mol %), and 3 M K_2CO_3 (100 mol %) in toluene for 15 h at 50 °C. The NMR analysis of the resulting reaction mixture showed that 2-bromo-4-phenylpyridine **5a** reacted seven fold faster than the corresponding 4-bromopyridine **4a**, in agreement with the obtained regioselectivity (Table 1, entry 8). The electrophilic character of the C–Br bond does not change much in the monobromo derivatives **4a** and **5a** compared to 2,4-dibromopyridine (**1**) as shown by their ^{13}C NMR shifts (Fig. 4). Therefore, their reactivity should be similar to that of **1** and, as expected, the selectivity dropped to 3.5:0:1 when the reaction was run in the presence of $Pd(PPh_3)_4$ and TlOH in THF with 1.2 equiv of boronic acid for longer periods.[20]

The presence of compound **5a**, even in those cases with excess of boronic acid, together with the reported observation that potentially bifunctional 2,6-dichloropyridine adds oxidatively only to one molecule of $Pd(PPh_3)_4$,[18a] could indicate that the dicoupled compound is formed mainly through the monocoupled derivatives and not by double oxidative addition on 2,4-dibromopyridine (**1**). When 2,4-dibromopyridine

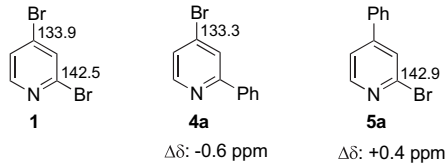


Figure 4. ^{13}C NMR chemical shifts for mono- and dibromopyridines.

was treated with 2.0 equiv of $Pd(PPh_3)_4$ at 25 °C, the corresponding 2,4-dimetallated pyridine was not detected but the signals corresponding to mono- and di-palladium complexes **7** and **9** (δ=22.21 and 30.44 ppm, respectively) along with triphenylphosphine oxide (δ=24.70 ppm), $Pd(PPh_3)_4$ (δ=24.39 ppm), and the lower field signal, 24.05 ppm, assigned to the 2-bromopyrid-4-yl palladium complex **8**.[21]

To summarize, we have carried out effective regioselective Suzuki cross-couplings between 2,4-dibromopyridine and several alkenyl(aryl) boronic acids that furnished 4-bromo-2-carbon substituted pyridines, difficult to be prepared otherwise, in good yields under palladium catalysis, either $Pd(PPh_3)_4$/TlOH or $Pd_2dba_3/PCy_3/K_3PO_4$ at 25 °C. Also, we have shown that the C–Br bond at position 2 of the pyridine ring is more reactive than at position 4 and the observed low TON could be due to the formation of a dinuclear palladium complex from the mononuclear σ-alkenyl palladium complex. In addition we have concluded that the dicoupled compound **6** is not formed through a double oxidative addition of 2,4-dibromopyridine to Pd(0) species.

3. Experimental section

3.1. General

Reagents and solvents were purchased as reagent-grade and used without further purification unless otherwise stated. Solvents were dried according to published methods[22] and distilled before use. All reactions were performed in oven-dried or flame-dried glassware under an inert atmosphere of Ar unless otherwise stated. NMR spectra were recorded in a Bruker AMX400 (400.13 MHz and 100.61 MHz for proton and carbon, respectively) spectrometer at 298 K with residual solvent peaks as internal reference and the chemical shifts are reported in δ (ppm), coupling constants J are given in (hertz) and expressed as follows: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet. ^{13}C-multiplicities assigned with DEPT experiments and COSY, HMBC, and HSQC methods were used to establish atom connectivities. Electronic impact ionization (EI) and fast atom bombardment (FAB) mass spectra were recorded on a VG-Autospec M instrument. Infrared spectra (IR) were obtained on a JASCO FT/IR-4200 infrared spectrometer. Peaks are quoted in wave numbers (cm^{-1}) and their relative intensities are reported as follows: s=strong, m=medium, w=weak. Melting points (mp) were taken on a Stuart Scientific apparatus. Elemental analysis was performed using a Fisons EA-1108 analyzer.

3.2. General methods for Suzuki reactions

Method A: in a Schlenk flask, a solution of $Pd(PPh_3)_4$ (0.08 or 0.24 equiv) and aryl(vinyl)boronic acid (1.2–2.0 equiv) in THF (0.2 M) was thoroughly degassed. Then, a solution of 2,4-dibromopyridine (1.0 equiv) in THF (0.4 M) was added dropwise followed by a previously degassed 10% aq TlOH solution (3.8 equiv). The reaction mixture was stirred at 25 °C until no reaction progress was observed either by TLC or by ^{1}H NMR, 4–24 h. It was then diluted with CH_2Cl_2 and filtered through a plug of Celite®. The filtrate was washed with saturated $NaHCO_3$ aqueous solution, dried (Na_2SO_4), and concentrated to dryness.

Method B: in a Schlenk flask, a solution of $Pd(PPh_3)_4$ (0.15 equiv) and aryl(vinyl)boronic acid (2.0 equiv) in toluene (0.14 M) was degassed via three 'freeze–thaw' cycles. Then, a solution of 2,4-dibromopyridine (1.0 equiv) in toluene (0.35 M) was added dropwise followed by 3 M aq K_2CO_3 (2.0 equiv). The resulting mixture was heated at 50 °C, followed as in method A. After cooling down to 25 °C, water was added and the mixture was extracted with diethyl ether. The combined organic extracts were washed with water, dried (Na_2SO_4), and concentrated to dryness.

Method C: in a Schlenk flask, a mixture of Pd_2dba_3 (0.10 equiv), PCy_3 (0.20 equiv), and aryl (vinyl)boronic acid (1.0–2.4 equiv) in dioxane (0.2 M) was thoroughly degassed. Then, a solution of 2,4-dibromopyridine (1.0 equiv) in dioxane (0.2 M) was added dropwise, followed by K_3PO_4 (2.0 equiv). The reaction mixture was stirred at 25 °C for 26–72 h. It was then diluted with Et_2O and filtered through a plug of Celite®. The filtrate was dried (Na_2SO_4) and concentrated to dryness.

Method D: in a Schlenk flask, a mixture of Pd_2dba_3 (0.015 equiv), (2-biphenylyl)di-*tert*-butylphosphine (0.06 equiv), $K_3PO_4 \cdot 1.5H_2O$ (2.4 equiv), and aryl (vinyl)boronic acid (1.0–2.5 equiv) in toluene (0.3 M) was degassed via three 'freeze-thaw' cycles. Then, a solution of 2,4-dibromopyridine (1.0 equiv) in toluene (0.6 M) was added dropwise. The reaction mixture was stirred at 25–40 °C for 24–42 h. It was then diluted with Et_2O and filtered through a plug of Celite®. The filtrate was dried (Na_2SO_4) and concentrated to dryness.

3.2.1. 4-Bromo-2-phenylpyridine (4a). Following method A, 2,4-dibromopyridine (**1**) (50 mg, 0.21 mmol) with phenylboronic acid (**2a**) (31 mg, 0.25 mmol) in the presence of $Pd(PPh_3)_4$ (19 mg, 0.02 mmol) and 10% aq TlOH solution (1.8 mL, 0.80 mmol) in THF (1.5 mL), for 24 h at 25 °C, afforded, after purification by flash chromatography (SiO_2, 92:8 hexane/EtOAc), 33 mg (67%) of 4-bromo-2-phenylpyridine (**4a**)[9a] as a yellow oil, 2 mg (4%) of 2-bromo-4-phenylpyridine (**5a**) as a white solid (mp 64 °C, hexane/CH_2Cl_2, lit.[9c] 65–66 °C), and 2 mg (4%) of 2,4-diphenylpyridine (**6a**) as a yellow solid (mp 67–68 °C, hexane/CH_2Cl_2, lit.[9d] oil). Data of **4a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (dd, J=5.3 Hz, 1.7 Hz, 1H, H^5), 7.44–7.51 (m, 3H, $H^{3'}$ and $H^{4'}$), 7.90 (d, J=1.7 Hz, 1H, H^3), 7.96–7.98 (m, 2H, $H^{2'}$), 8.51 (d, J=5.3 Hz, 1H, H^6) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 123.8 (CH), 125.1 (CH), 126.9 (2CH), 128.7 (2CH), 129.5 (CH), 133.3 (C), 137.9 (C), 150.2 (CH), 158.8 (C) ppm. IR (NaCl) ν 3041 (d, C–H), 1566 (f), 1544 (f), 1497 (d), 1460 (m), 1443 (f), 1378 (f), 1094 (d), 1072 (d), 1053 (m), 873 (d), 823 (m), 772 (f), 729 (m), 692 (f), 633 (m), 590 (m). MS (EI$^+$) m/z (%) 236 ([M+1]$^+$ [^{81}Br], 12), 235 (M$^+$ [^{81}Br], 98), 234 ([M+1]$^+$ [^{79}Br], 21), 233 (M$^+$ [^{79}Br], 100), 155 (9), 154 (70), 153 (10). HRMS (EI$^+$) calcd for $C_{11}H_8N^{81}Br$, 234.9820 and $C_{11}H_8N^{79}Br$, 232.9840; found, 234.9828 and 232.9844. Data of **5a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.52 (m, 4H, H^5–$H^{3'}$–$H^{4'}$), 7.59–7.61 (m, 2H, $H^{2'}$), 7.70 (br s, 1H,

H^3), 8.40 (d, J=5.1 Hz, 1H, H^6) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 120.8 (CH), 125.8 (CH), 127.0 (2CH), 129.2 (2CH), 129.6 (CH), 136.7 (C), 142.9 (C), 150.4 (CH), 151.3 (C) ppm. IR (NaCl) ν 3057 (d, C–H), 1585 (f), 1529 (f), 1457 (m), 1369 (m), 1125 (d), 1078 (m), 844 (d), 786 (d), 760 (f), 699 (m), 612 (d). MS (EI^+) m/z (%) 236 ($[M+1]^+$ [^{81}Br], 6), 235 (M^+ [^{81}Br], 48), 234 ($[M+1]^+$ [^{79}Br], 6), 233 (M^+ [^{79}Br], 49), 155 (13), 154 (100), 153 (13). HRMS (EI^+) calcd for $C_{11}H_8{}^{81}BrN$, 234.9820 and $C_{11}H_8{}^{79}BrN$, 232.9840; found, 234.9821 and 232.9837. Data of **6a**: ^1H NMR (400 MHz, $CDCl_3$) δ 7.42 (dd, J=5.1 Hz, 1.7 Hz, 1H, H^5), 7.44–7.55 (m, 6H, $2H^{3'}$–$2H^{3''}$–$H^{4'}$–$H^{4''}$), 7.68 (m, 2H, $2H^{2''}$), 7.94 (br s, 1H, H^3), 8.11 (m, 2H, $2H^{2'}$), 8.76 (d, J=5.1 Hz, 1H, H^6) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 118.5 (CH), 120.0 (CH), 126.9 (CH), 128.6 (CH), 128.8 (CH), 128.9 (CH), 138.2 (C), 139.2 (C), 149.0 (C), 149.9 (CH), 157.8 (C) ppm. IR (NaCl) ν 3059 (d, C–H), 1594 (f), 1542 (m), 1497 (d), 1470 (m), 1445 (d), 1391 (m), 1075 (d), 887 (d), 844 (d), 760 (f), 737 (m), 693 (f), 641 (d), 611 (d). MS m/z (%) 232 ($[M+1]^+$, 3), 231 (M^+, 100), 230 (82), 202 (5), 154 (4), 102 (3). HRMS calcd for $C_{17}H_{13}N$, 231.1048; found, 231.1043.

Following method C, 2,4-dibromopyridine (**1**) (0.25 g, 1.05 mmol) with phenylboronic acid (**2a**) (0.31 g, 2.53 mmol) in the presence of Pd_2dba_3 (0.10 g, 0.10 mmol), PCy_3 (0.06 g, 0.21 mmol), and K_3PO_4 (0.45 g, 2.11 mmol) in dioxane (10.5 mL), for 36 h at 25 °C, afforded, after purification by flash chromatography (SiO_2, 92:8 hexane/EtOAc), 173 mg (72%) of **4a** and 25 mg (10%) of **6a**.

3.2.2. 2,4-Diphenylpyridine (6a). Following method D, 2,4-dibromopyridine (**1**) (75 mg, 0.32 mmol) with phenylboronic acid (**2a**) (96 mg, 0.79 mmol) in the presence of Pd_2dba_3 (4 mg, 5×10^{-3} mmol), (2-biphenylyl)di-*tert*-butylphosphine (6 mg, 0.02 mmol), and $K_3PO_4\cdot1.5H_2O$ (182 mg, 0.76 mmol) in toluene (1.5 mL), for 24 h at 40 °C, afforded, after purification by flash chromatography (SiO_2, 85:15 hexane/EtOAc), 63 mg (85%) of 2,4-diphenylpyridine (**6a**).

3.2.3. 4-Bromo-2-(*p*-methoxyphenyl)pyridine (4b). Following method A, 2,4-dibromopyridine (**1**) (100 mg, 0.42 mmol) with *p*-methoxyphenylboronic acid (**2b**) (76 mg, 0.50 mmol) in the presence of $Pd(PPh_3)_4$ (114 mg, 0.10 mmol) and 10% aq TlOH solution (3.6 mL, 1.60 mmol) in THF (3.0 mL), for 12 h at 25 °C, afforded, after purification by flash chromatography (SiO_2, 85:15 hexane/EtOAc), 71 mg (64%) of 4-bromo-2-(*p*-methoxyphenyl)pyridine (**4b**) as a white solid (mp 37–38 °C, hexane/CH_2Cl_2), 5 mg (4%) of 2-bromo-4-(*p*-methoxyphenyl)pyridine (**5b**) as a white solid (mp 57 °C, hexane/CH_2Cl_2, lit.[23] 54–55 °C), and 5 mg (4%) of 2,4-bis(*p*-methoxyphenyl)pyridine (**6b**) as a white solid (mp 158 °C, hexane/CH_2Cl_2, lit.[24] 152–153 °C). Data of **4b**: ^1H NMR (400 MHz, $CDCl_3$) δ 3.83 (s, 3H, OMe), 6.97 (d, J=8.9 Hz, 2H, $H^{3'}$), 7.30 (dd, J=5.1 Hz, 1.6 Hz, 1H, H^5), 7.81 (br s, 1H, H^3), 7.92 (d, J=8.9 Hz, 2H, $H^{2'}$), 8.43 (d, J=5.1 Hz, 1H, H^6) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.2 (CH_3), 114.1 (2CH), 122.8 (CH), 124.3 (CH), 128.2 (2CH), 130.4 (C), 133.2 (C), 150.1 (CH), 158.4 (C), 160.8 (C) ppm. IR (NaCl) ν 2957 (w, C–H), 2932 (w, C–H), 2836 (w, C–H). MS (EI^+) m/z (%) 266 ($[M+1]^+$ [^{81}Br], 12), 265 (M^+ [^{81}Br], 97), 264 ($[M+1]^+$ [^{79}Br], 14), 263 (M^+ [^{79}Br], 100), 250 ($[M-CH_3]^+$ [^{81}Br], 21), 248 ($[M-CH_3]^+$ [^{79}Br], 22). HRMS (EI^+) calcd for $C_{12}H_{10}{}^{81}BrNO$, 264.9925 and $C_{12}H_{10}{}^{79}BrNO$, 262.9946; found, 264.9926 and 262.9938. Anal. Calcd (%): C 54.57, H 3.82; N 5.30; found: C 54.98, H 3.83, N 5.37. Data of **5b**: ^1H NMR (400 MHz, $CDCl_3$) δ 3.86 (s, 3H, OMe), 7.00 (d, J=8.7 Hz, 2H, $H^{3'}$), 7.41 (dd, J=5.1 Hz, 1.5 Hz, 1H, H^5), 7.56 (d, J=8.7 Hz, 2H, $H^{2'}$), 7.65 (br s, 1H, H^3), 8.35 (d, J=5.1 Hz, 1H, H^6) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.4 (CH_3), 114.7 (CH), 120.2 (2CH), 125.1 (CH), 128.2 (2CH), 128.8 (C), 142.9 (C), 150.2 (CH), 150.7 (C), 161.0 (C) ppm. IR (NaCl) ν 2934 (w, C–H), 2837 (w, C–H), 1609 (m), 1584 (s), 1520 (s), 1459 (m), 1372 (m), 1293 (m), 1253 (s), 1182 (m), 1120 (m), 1081 (m), 1047 (m), 987 (w), 822 (s), 753 (w), 686 (w), 570 (w). MS (EI^+) m/z (%) 266 ($[M+1]^+$ [^{81}Br], 10), 265 (M^+ [^{81}Br], 87), 264 ($[M+1]^+$ [^{79}Br], 12), 263 (M^+ [^{79}Br], 92), 185 (13), 184 (100), 169 (37), 153 (10), 141 (29), 140 (27), 114 (16), 92 (28), 69 (55). HRMS (EI^+) calcd for $C_{12}H_{10}{}^{81}BrNO$, 264.9925 and $C_{12}H_{10}{}^{79}BrNO$, 262.9946; found, 264.9927 and 262.9946. Data of **6b**: ^1H NMR (400 MHz, $CDCl_3$) δ 3.87 (s, 6H, 2×OMe), 7.02 (m, 4H, $H^{3'}$ and $H^{3''}$), 7.34 (dd, J=5.2 Hz, 1.4 Hz, 1H, H^5), 7.64 (d, J=8.7 Hz, 2H, $H^{2''}$), 7.83 (br s, 1H, H^3), 8.01 (d, J=8.9 Hz, 2H, $H^{2'}$), 8.65 (d, J=5.2 Hz, 1H, H^6) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.3 ($2CH_3$), 114.1 (CH), 114.5 (CH), 117.3 (CH), 119.0 (CH), 128.1 (CH), 128.18 (CH), 128.23 (CH), 130.9 (C), 132.2 (C), 148.6 (C), 149.9 (CH), 157.6 (C), 160.4 (2C). IR (NaCl) ν 3023 (w, C–H), 2959 (w, C–H), 2839 (w, C–H), 1607 (m), 1541 (m), 1517 (s), 1466 (w), 1425 (w), 1382 (w), 1306 (m), 1284 (w), 1250 (s), 1183 (s), 1116 (m), 1043 (s), 1020 (s), 828 (s), 814 (s), 755 (m), 540 (w). MS (EI^+) m/z (%) 292 ($[M+1]^+$, 3), 291 (M^+, 100), 276 (10), 248 (4), 205 (4), 204 (4), 146 (4), 124 (3), 108 (2). HRMS (EI^+) calcd for $C_{19}H_{17}NO_2$, 291.1259; found, 291.1254. Anal. Calcd for $C_{19}H_{17}NO_2$: C 78.33, H 5.88, N 4.81; found: C 78.35, H 5.95, N 4.92.

Following method C, 2,4-dibromopyridine (**1**) (0.25 g, 1.05 mmol) with *p*-methoxyphenylboronic acid (**2b**) (0.38 g, 2.53 mmol) in the presence of Pd_2dba_3 (0.10 g, 0.10 mmol), PCy_3 (0.06 g, 0.21 mmol), and K_3PO_4 (0.45 g, 2.11 mmol) in dioxane (10.5 mL), for 26 h at 25 °C, afforded, after purification by flash chromatography (SiO_2, 85:15 hexane/EtOAc), 0.20 g (72%) of 4-bromo-2-(*p*-methoxyphenyl)pyridine (**4b**) and 31 mg (10%) of 2,4-bis(*p*-methoxyphenyl)pyridine (**6b**).

3.2.4. 2,4-Bis(*p*-methoxyphenyl)pyridine (6b). Following procedure D, 2,4-dibromopyridine (**1**) (0.25 g, 1.05 mmol) with *p*-methoxyphenylboronic acid (**2b**) (0.40 g, 2.62 mmol) in the presence of Pd_2dba_3 (14 mg, 0.2 mmol), (2-biphenylyl)di-*tert*-butylphosphine (19 mg, 0.06 mmol), and $K_3PO_4\cdot1.5H_2O$ (0.60 g, 2.53 mmol) in toluene (3.0 mL), for 42 h at 40 °C, afforded, after purification by flash chromatography (SiO_2, 75:25 hexane/EtOAc), 251 mg (82%) of **6b**.

3.2.5. 4-Bromo-2-(*p*-methylphenyl)pyridine (4c). Following method A, 2,4-dibromopyridine (**1**) (75 mg, 0.32 mmol) with *p*-methylphenylboronic acid (**2c**) (52 mg, 0.38 mmol) in the presence of $Pd(PPh_3)_4$ (89 mg, 0.08 mmol) and 10% aq TlOH solution (2.7 mL, 1.22 mmol) in THF (2.3 mL), for 12 h at 25 °C, afforded, after purification by flash chromatography (SiO_2, 95:5 hexane/EtOAc), 48 mg

(60%) of 4-bromo-2-(*p*-methylphenyl)pyridine (**4c**) as a white solid (mp 61 °C, hexane/CH_2Cl_2), 3 mg (3%) of 2-bromo-4-(*p*-methylphenyl)pyridine[25] (**5c**) and 3 mg (3%) of 2,4-bis(*p*-methylphenyl)pyridine[26] (**6c**). Data of **4c**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.41 (s, 3H, Me), 7.28 (d, J=7.8 Hz, 2H, H$^{2'}$), 7.36 (dd, J=5.1 Hz, 1.8 Hz, 1H, H^5), 7.86–7.88 (m, 3H, H^3 and 2H$^{3'}$), 8.48 (d, J=5.1 Hz, 1H, H^6) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.3 (CH_3), 123.5 (CH), 124.9 (CH), 126.8 (CH), 129.6 (CH), 133.4 (C), 135.2 (C), 139.7 (C), 150.2 (CH), 158.8 (C) ppm. FTIR (neat) ν 3009 (w, C–H), 2939 (w, C–H), 2909 (w, C–H), 2851 (w, C–H). MS (EI$^+$) m/z (%) 250 ([M+1]$^+$ [^{81}Br], 13), 249 (M$^+$ [^{81}Br], 95), 248 ([M+1]$^+$ [^{79}Br], 39), 247 (M$^+$ [^{79}Br], 100). HRMS (EI$^+$) calcd for $C_{12}H_{10}N^{81}Br$, 248.9976 and $C_{12}H_{10}N^{79}Br$, 246.9997; found, 248.9978 and 246.9997. Anal. Calcd (%): C 58.09, H 4.06, N 5.65; found: C 58.11, H 4.03, N 5.69.

3.2.6. 4-Bromo-2-(*p*-fluorophenyl)pyridine (4d). Following method A, 2,4-dibromopyridine (**1**) (27 mg, 0.11 mmol) with *p*-fluorophenylboronic acid (**2d**) (18 mg, 0.13 mmol) in the presence of $Pd(PPh_3)_4$ (29 mg, 0.02 mmol) and 10% aq TlOH solution (0.9 mL, 0.40 mmol) in THF (1.5 mL), for 16 h at 25 °C, afforded, after purification by flash chromatography (SiO_2, 95:5 hexane/EtOAc), 19 mg (68%) of 4-bromo-2-(*p*-fluorophenyl)pyridine (**4d**) as an oil. Data of **4d**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (t, J=8.6 Hz, 2H, H$^{3'}$) 7.39 (dd, J=5.2 Hz, 1.6 Hz, 1H, H^5), 7.85 (d, J=1.6 Hz, 1H, H^3), 7.96 (dd, J=8.6 Hz, 5.4 Hz, 2H, H$^{2'}$), 8.48 (d, J=5.2 Hz, 1H, H^6) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 115.8 (d, J_{C-F}=21.6 Hz, 2CH), 123.5 (CH), 125.1 (CH), 128.9 (d, J_{C-F}=8.5 Hz, 2CH), 133.5 (C), 134.2 (d, J_{C-F}=3.3 Hz, C), 150.3 (CH), 157.8 (C), 163.8 (d, J_{C-F}=249.7 Hz, C) ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ 112.0 ppm. MS (EI$^+$) m/z (%) 254 ([M+1]$^+$ [^{81}Br], 9), 253 (M$^+$ [^{81}Br], 92), 252 ([M+1]$^+$ [^{79}Br], 13), 251 (M$^+$ [^{79}Br], 100). HRMS (EI$^+$) calcd for $C_{11}H_7{}^{81}BrFN$, 252.9725 and $C_{11}H_7{}^{79}BrFN$, 250.9746; found, 252.9730 and 250.9737.

3.2.7. 4-Bromo-2-[(1*E*)-2-phenylethenyl]pyridine (4g). Following method A, 2,4-dibromopyridine (**1**) (100 mg, 0.42 mmol) with (*E*)-2-phenylvinylboronic acid (**3g**) (0.15 g, 1.01 mmol) in the presence of $Pd(PPh_3)_4$ (38 mg, 0.03 mmol) and 10% aq TlOH solution (3.6 mL, 1.60 mmol) in THF (3.0 mL), for 21 h at 25 °C, afforded, after purification by flash chromatography (SiO_2, 90:10 hexane/EtOAc), 79 mg (72%) of 4-bromo-2-[(1*E*)-2-phenyleth-1-enyl]pyridine (**4g**) as a white solid (mp 51 °C, hexane/CH_2Cl_2) and traces of a compound identified as 2,4-bis[(1*E*)-2-phenyleth-1-enyl]pyridine (**6g**) (mp 174 °C, hexane/CH_2Cl_2, lit.[27] 175 °C). Data of **4g**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (d, J=16.1 Hz, 1H, H$^{1'}$ or H$^{2'}$), 7.29–7.40 (m, 4H, ArH), 7.54–7.58 (m, 3H, ArH), 7.65 (d, J=16.1 Hz, 1H, H$^{1'}$ or H$^{2'}$), 8.40 (d, J=5.2 Hz, 1H, H^6) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 124.9 (CH), 125.0 (CH), 126.4 (CH), 127.1 (CH), 128.6 (CH), 128.7 (CH), 133.0 (C), 134.2 (CH), 136.0 (C), 150.1 (CH), 156.9 (C) ppm. MS (EI$^+$) m/z (%) 261 ([M+1]$^+$ [^{81}Br], 20), 260 (M$^+$ [^{81}Br], 98), 259 ([M+1]$^+$ [^{79}Br], 21), 258 (M$^+$ [^{79}Br], 100). HRMS (EI$^+$) calcd for $C_{13}H_{10}N^{81}Br$, 259.9898 and $C_{13}H_{10}N^{79}Br$, 257.9918; found, 259.9894 and 257.9912. Data of **6g**: ^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.09 (d, J=16.3 Hz, 1H, H$^{1''}$), 7.23 (d, J=16.1 Hz, 1H, H$^{1'}$), 7.29 (d, J=5.2 Hz, 1H, H^5), 7.3–7.4 (m, 7H, ArH and H$^{2''}$), 7.51 (s, 1H, H^3), 7.59 (d, J=7.5 Hz, 2H, Ar*H*), 7.62 (d, J=7.5 Hz, 2H, Ar*H*), 7.72 (d, J=16.1 Hz, 1H, H$^{2'}$), 8.54 (d, J=5.2 Hz, 1H, H^6) ppm. ^{13}C NMR (100.63 MHz, CD_2Cl_2) δ 119.7 (CH), 120.1 (CH), 126.6 (CH), 127.6 (CH), 127.7 (CH), 128.5 (CH), 128.9 (CH), 129.31 (CH), 129.34 (CH), 129.4 (CH), 133.3 (CH), 133.6 (CH), 136.9 (C), 137.3 (C), 145.9 (C), 150.4 (CH), 156.5 (C) ppm. FTIR (neat) ν 3025 (d, C–H). MS (EI$^+$) m/z (%) 284 ([M+1]$^+$, 6), 283 (M$^+$, 37), 282 ([M−1]$^+$, 100). HRMS calcd for $C_{21}H_{17}N$, 283.1361; found, 283.1348.

3.2.8. 4-Bromo-2-[(1*E*)-6-hydroxyhexenyl]pyridine (4h). Following method A, 2,4-dibromopyridine (**1**) (100 mg, 0.42 mmol) with (*E*)-6-hydroxyhex-1-en-1-ylboronic acid (**3h**) (73 mg, 0.51 mmol) in the presence of $Pd(PPh_3)_4$ (39 mg, 0.03 mmol) and 10% aq TlOH solution (3.6 mL, 1.60 mmol) in THF (3.0 mL), for 24 h at 25 °C, afforded, after purification by flash chromatography (SiO_2, 40:60 hexane/EtOAc), 83 mg (77%) of 4-bromo-2-[(1*E*)-6-hydroxyhex-1-enyl]pyridine (**4h**) as a yellow oil and 5 mg (5%) of 2-bromo-4-[(1*E*)-6-hydroxyhex-1-enyl]pyridine (**5h**) as an oil. Data of **4h**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52–1.62 (m, 4H, H$^{4'}$ and H$^{5'}$), 2.21 (br s, 1H, OH), 2.27 (c, J=7.0 Hz, 2H, H$^{3'}$), 3.64 (t, J=6.2 Hz, 2H, H$^{6'}$), 6.39 (dt, J=15.7 Hz, 1.4 Hz, 1H, H$^{1'}$), 6.72 (dt, J=15.7 Hz, 7.0 Hz, 1H, H$^{2'}$), 7.23 (dd, J=5.3 Hz, 1.8 Hz, 1H, H^5), 7.39 (d, J=1.8 Hz, 1H, H^3), 8.28 (d, J=5.3 Hz, 1H, H^6) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.9 (CH_2, C$^{4'}$), 32.1 (CH_2, C$^{5'}$), 32.4 (CH_2, C$^{3'}$), 62.4 (CH_2, C$^{6'}$), 124.1 (CH, C^3), 124.7 (CH, C^5), 128.9 (CH, C$^{1'}$), 133.1 (C, C^4), 137.4 (CH, C$^{2'}$), 149.9 (CH, C^6), 157.4 (C, C^2) ppm. FTIR (NaCl) ν 3500–3000 (br, O–H), 2932 (s, C–H), 2860 (m, C–H), 1652 (m), 1568 (s), 1542 (s), 1464 (m), 1385 (m), 1063 (w), 972 (m), 876 (w), 818 (w), 690 (m). MS (EI$^+$) m/z (%) 258 ([M+1]$^+$ [^{81}Br], 2), 257 (M$^+$ [^{81}Br], 14), 256 ([M+1]$^+$ [^{79}Br], 6), 255 (M$^+$ [^{79}Br], 12). HRMS (EI$^+$) calcd for $C_{11}H_{14}NO^{81}Br$, 257.0238 and $C_{11}H_{14}NO^{79}Br$, 255.0259; found, 257.0228 and 255.0257. Data of **5h**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51–1.65 (m, 4H, H$^{4'}$ and H$^{5'}$), 2.29 (m, 2H, H$^{3'}$), 3.69 (t, J=6.1 Hz, 2H, H$^{6'}$), 6.28 (d, J=15.8 Hz, 1H, H$^{1'}$), 6.49 (dt, J=15.8 Hz, 6.9 Hz, 1H, H$^{2'}$), 7.15 (dd, J=5.2 Hz, 1.4 Hz, 1H, H^5), 7.39 (br s, 1H, H^3), 8.24 (d, J=5.2 Hz, 1H, H^6) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.0 (CH_2), 32.2 (CH_2), 32.7 (CH_2, C$^{3'}$), 62.6 (CH_2, C$^{6'}$), 119.7 (CH, C^5), 124.7 (CH, C^3), 126.8 (CH, C$^{1'}$), 137.6 (CH, C$^{2'}$), 142.8 (C, C^2), 148.0 (C, C^4), 150.1 (CH, C^6) ppm. MS (EI$^+$) m/z (%) 258 ([M+1]$^+$ [^{81}Br], 0.8), 257 (M$^+$ [^{81}Br], 6), 256 ([M+1]$^+$ [^{79}Br], 3). HRMS (EI$^+$) calcd for $C_{11}H_{14}NO^{81}Br$, 257.0238 and $C_{11}H_{14}NO^{79}Br$, 255.0259; found, 257.0230 and 255.0257.

3.2.9. 4-Bromo-2-[(1*E*)-3-*tert*-butyldimethylsilyloxy-2-methylpropenyl]pyridine (4i). To a cold (−78 °C) solution of (*E*)-*tert*-butyl(3-iodo-2-methylallyloxy)dimethylsilane[28] (0.45 g, 1.44 mmol) in THF (4.0 mL) in a Schlenk flask was added dropwise *t*BuLi (1.8 mL, 1.7 M in pentane, 3.03 mmol) and the mixture was stirred at −78 °C for 30 min. Then, $B(OiPr)_3$ (0.7 mL, 2.88 mmol) was added dropwise and the mixture was stirred at 0 °C for 2 h. At this time, following method A, $Pd(PPh_3)_4$ (78 mg, 0.07 mmol), a solution of 2,4-dibromopyridine (**1**) (0.19 g,

0.80 mmol) in THF (4.0 mL) and a 10% aq TlOH (7.0 mL, 3.2 mmol) solution were added sequentially and the mixture was stirred at 25 °C until complete disappearance of dibromopyridine **1** as observed by TLC and NMR (10 h). The crude mixture was diluted with Et_2O, filtered through a Celite® plug, and washed with an aqueous saturated $NaHCO_3$ solution. The organic layer was dried over Na_2SO_4 (anhyd) and concentrated in vacuo. The residue was purified by column chromatography on silica gel (96:4 hexane/EtOAc) affording 0.20 g (75%) of 4-bromo-2-[(1*E*)-3-*tert*-butyldimethylsilyloxy-2-methylprop-1-enyl]pyridine **4i** as a yellow oil and 13 mg (5%) of 2-bromo-4-[(1*E*)-3-*tert*-butyldimethylsilyloxy-2-methylprop-1-enyl]pyridine **5i** as a yellow oil. Data of **4i**: ^{1}H NMR (CD_2Cl_2) δ 0.12 (s, 6H, $Si(CH_3)_2$), 0.95 (s, 9H, *t*Bu), 2.05 (br s, 3H, CH_3), 4.19 (d, J=0.7 Hz, 2H, $H^{3'}$), 6.51 (m, 1H, $H^{1'}$), 7.26 (dd, J=5.3 Hz, 1.8 Hz, 1H, H^5), 7.39 (d, J=1.8 Hz, 1H, H^3), 8.38 (d, J=5.3 Hz, 1H, H^6) ppm. ^{13}C NMR (CD_2Cl_2) δ −5.1 (CH_3, $Si(CH_3)_2$), 15.8 (CH_3, CH_3–$C^{2'}$), 18.9 (C, C-*t*Bu), 26.3 (CH_3, *t*Bu), 68.5 (CH_2, $C^{3'}$), 121.7 (CH, $C^{1'}$), 124.3 (CH, C^5), 127.6 (CH, C^3), 132.9 (C, C^4), 145.1 (C, $C^{2'}$), 150.4 (CH, C^6), 159.2 (C, C^2) ppm. FTIR (neat) ν 2929 (w, C–H), 2855 (w, C–H). MS (EI$^+$) *m*/*z* (%) 343 (M$^+$ [^{81}Br], 21), 341 (M$^+$ [^{79}Br], 21), 286 ([M−*t*Bu]$^+$ [^{81}Br], 100), 284 ([M−*t*Bu]$^+$ [^{79}Br], 95). HRMS (EI$^+$) calcd for $C_{15}H_{24}NOSi^{81}Br$, 343.0790 and $C_{15}H_{24}NOSi^{79}Br$, 341.0810; found, 343.0794 and 341.0810. Data of **5i**: ^{1}H NMR ($CDCl_3$) δ 0.11 (s, 6H, $Si(CH_3)_2$), 0.95 (s, 9H, *t*Bu), 1.83 (s, 3H, CH_3), 4.17 (s, 2H, $H^{3'}$), 6.44 (s, 1H, $H^{1'}$), 7.12 (dd, J=5.1 Hz, 1.2 Hz, 1H, H^5), 7.35 (br s, 1H, H^3), 8.28 (d, J=5.1 Hz, 1H, H^6) ppm. ^{13}C NMR ($CDCl_3$) δ −5.4 (CH_3, $Si(CH_3)_2$), 15.2 (CH_3, CH_3–$C^{2'}$), 18.4 (C, C–*t*Bu), 25.9 (CH_3, *t*Bu), 67.5 (CH_2, $C^{3'}$), 119.5 (CH, $C^{1'}$), 122.7 (CH, C^5), 127.5 (CH, C^3), 142.3 (C, C^2), 143.7 (C, $C^{2'}$), 148.7 (C, C^4), 149.7 (CH, C^6) ppm. FTIR (neat) ν 2951 (w, C–H), 2929 (w, C–H), 2855 (w, C–H), 1660 (w). MS (EI$^+$) *m*/*z* (%) 343 (M$^+$ [^{81}Br], 2), 342 ([M−1]$^+$ [^{81}Br], 11), 341 (M$^+$ [^{79}Br], 2), 340 ([M−1]$^+$ [^{79}Br], 11), 300 (100), 298 (98), 286 ([M−*t*Bu]$^+$ [^{81}Br], 25), 284 ([M−*t*Bu]$^+$ [^{79}Br], 25). HRMS (EI$^+$) calcd for $C_{15}H_{24}NOSi^{81}Br$, 343.0790 and $C_{15}H_{24}NOSi^{79}Br$, 341.0810; found, 343.0781 and 341.0801.

3.2.10. *trans*-Bromo(4-bromopyrid-2-yl-κ*C*^{2})bis(triphenylphosphane)palladium(II) (7) and di-μ-(4-bromopyrid-2-yl)-κ*N*:κ*C*^{2}-bis[bromotriphenylphosphanepalladium(II)] (9). To a thoroughly degassed solution of $Pd(PPh_3)_4$ (0.36 g, 0.32 mmol) in toluene (0.5 mL), a solution of 2,4-dibromopyridine (**1**) (75 mg, 0.32 mmol) in toluene (0.5 mL) was added dropwise. The reaction mixture was stirred at 25 °C for 16 h. Then, the solvent was removed under reduced pressure; the residue was titrated with ether yielding 225 mg (81%) of complex **7** along with complex **8**. After crystallization from CH_2Cl_2/hexane, complex **9** was obtained as a crystalline solid (mp>190 °C, decomp.). Data of mononuclear complex **7**: ^{1}H NMR (400 MHz, CD_2Cl_2) δ 6.22 (dd, J=5.3 Hz, 1.9 Hz, 1H, H^5), 6.65 (d, J=1.9 Hz, 1H, H^3), 7.29–7.35 (m, 12H, PPh_3), 7.37–7.41 (m, 6H, PPh_3), 7.40 (d, J=5.3 Hz, 1H, H^6), 7.57–7.61 (m, 12H, PPh_3) ppm. ^{31}P NMR (162 MHz, CD_2Cl_2) δ 22.6 ppm. MS (FAB$^+$) *m*/*z* (%) 870 (8), 868 (9), 788 (54), 632 (64), 630 (82). HRMS (FAB$^+$) calcd for $C_{41}H_{34}NP_2{}^{79}Br_2{}^{106}Pd$, 865.9568; found, 865.9576. Data of mononuclear complex **8**: ^{31}P NMR (162 MHz, CD_2Cl_2) δ 23.86 ppm. Data of dinuclear complex **9**: ^{1}H NMR (400 MHz, CD_2Cl_2) δ 6.74 (m, 4H, 2×H^5 and 2×H^3), 7.27–7.33 (m, 12H, PPh_3), 7.41–7.44 (m, 12H, PPh_3), 7.78–7.85 (m, 12H, PPh_3), 8.31 (d, J=5.9 Hz, 1H, Ar*H*), 8.32 (d, J=5.4 Hz, 1H, Ar*H*) ppm. ^{13}C NMR (100 MHz, CD_2Cl_2) δ 123.1 (d, $^3J_{C-P}$=3.0 Hz, CH), 129.0 (d, $^3J_{C-P}$=10.9 Hz, CH), 131.2 (d, $^4J_{C-P}$=2.0 Hz, CH), 131.2 (d, $^1J_{C-P}$=52.3 Hz, C), 135.6 (d, $^2J_{C-P}$=11.6 Hz, CH), 151.7 (CH), 189.1 (C) ppm. ^{31}P NMR (162 MHz, CD_2Cl_2) δ 30.2 ppm. MS (FAB$^+$) *m*/*z* (%) 1211 (0.6), 1131 (1), 788 (64), 630 (89).

3.3. Crystal data and structure refinement for 9

Crystallographic data were collected on a Bruker Smart 1000 CCD diffractometer at 20 °C using graphite monochromated Mo Kα radiation (λ=0.71073 Å), and were corrected for Lorentz and polarization effects. The frames were integrated with the Bruker SAINT[29] software package and the data were corrected for absorption using the program SADABS.[30] The structures were solved by direct methods using the program SHELXS97.[31] All non-hydrogen atoms were refined with anisotropic thermal parameters by full-matrix least-squares calculations on F^2 using the program SHELXL97. Hydrogen atoms were inserted at calculated positions and constrained with isotropic thermal parameters. Drawings were produced with PLATON.[32] Empirical formula: $C_{46}H_{36}Br_4N_2P_2Pd_2$; formula weight: 1211.15; temperature: 293(2) K; crystal system: triclinic (*P*-1); unit cell dimensions: a=11.1679(9) Å, b=13.0847(10) Å, c=18.1885(14) Å; α=71.522(2)°, β=81.415(2)°, γ=73.8510(10)°; volume: 2415.9(3) Å^3; Z=2; density (calculated): 1.665 Mg/m^3; absorption coefficient=4.150 mm^{-1}. F(000)=1176; crystal size=0.40×0.12×0.06 mm^3; independent reflections 10085 [R(int)=0.0349]; data/restraints/parameters=10085/0/475; final R [I>2σ(I)]: R_1=0.0579, wR_2=0.1599; R indices (all data): R_1=0.1004, wR_2=0.1731.[33]

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Supplementary data

^{31}P NMR of **1** with 2.0 equiv of $Pd(PPh_3)_4$. Copy of ^{1}H and ^{13}C NMR spectra of all new compounds. HMBC for **4h**, **5h**, **4i**, and **5i**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.09.040.

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12. Since mechanistic studies on the Suzuki coupling reaction of 2,4-dibromopyridine and phenylboronic acid in the presence of $Pd(PPh_3)_4$ showed that the selectivity follows the pattern for the oxidative addition step, a cautiously tentative interpretation for this finding could be that, unexpectedly, this phosphine reversed the oxidative addition selectivity between 2- and 4-C–Br bonds. The same behavior was observed in pentafluoropyridine with $[Pd(PCy_3)_2]$, see: Jasim, N. A.; Perutz, R. N.; Whitwood, A. C.; Braun, T.; Izundu, J.; Neumann, B.; Rothfeld, S.; Stammler, H.-G. *Organometallics* **2004**, *23*, 6140–6149.
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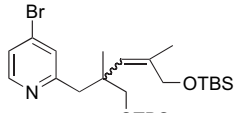

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Silver(I) complexes based on novel tripodal thioglycosides: synthesis, structure and antimicrobial activity

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Abstract—The reaction of tris(2-bromoethyl)amine hydrobromide with sugar thiols or thioacetates leads to the formation of novel carbohydrate substituted tripodal NS_3 ligands. Complexation with silver(I) ions gives stable complexes. NMR, X-ray, MS and EXAFS studies indicate their mononuclear C_3-symmetric structure. The highly water soluble complexes formed from the unprotected ligands show a wide spectrum of effective antimicrobial activities and their use lowers the cytotoxic and antiproliferative activities compared to the free silver salts.

1. Introduction

Silver(I) complexes received attention because of their often displayed antimicrobial activity.[1–6] Furthermore, their anticancer[7–11] and antiviral[12] effects and the possible use of the radioisotope ^{111}Ag in radioimmunotherapy attracted attention.[13] Most of the antimicrobially active compounds displayed a slow release of silver ions, but stability and solubility in water are desired for their application. Carbohydrates are increasingly used to implement such properties to obtain bioactive metal complexes.[14–18] However, the chemistry of sugars together with silver(I) ions so far is limited to the detection of reducing sugars based on the reduction to silver (Tollen's reagent), the structural characterisation of a silver(I) complex of lactobionate,[19] the complexation of D-glucono-δ-lactone[20] and β-D-glucopyranosyl-thiol[21] and the study of the antiviral activity of silver(I) glycoporphyrin derivatives.[22]

Benzyl- and vinyl-substituted ethylene-bridged NS_3-open chain ligands have been reported recently to form stable Ag^I complexes in which the silver ions are not completely encapsulated and can be attacked by competing ligands to undergo transmetallation.[23] Although thioglycosides are very active intermediates—for instance in oligosaccharide synthesis where they are used as glycosyl donors especially in connection with metal salts as activators to transfer the sugar moiety to glycosyl acceptors[24]—we report herein the formation of stable silver(I) complexes derived from novel tripodal thioglycosidic ligands.

2. Results and discussion

The acetyl-protected compounds **5** and **6** were prepared by the reaction of tris(2-bromoethyl)amine hydrobromide **1**[25] with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-thiol **2** and 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-thiol **3**[26] in DMF and triethylamine. The corresponding mannose derivative **7** was synthesised in a one-pot reaction in DMF starting from 2,3,4,6-tetra-*O*-acetyl-α-D-thioacetyl-mannopyranose **4**,[27] in situ hydrolysis of the thioacetyl group by stirring with diethylamine at 0 °C and subsequent addition of triethylamine and the bromide **1**. Cleavage of the acetyl groups could not be carried out with conventional methods. Under basic conditions the removal of the formed salts from the extremely water soluble ligands **8**–**10** was not possible. Treatment with acids for the ester hydrolysis caused decomposition of the ligands. Only in a 1:1 mixture of water and ethanol using a hydroxyl group loaded DOWEX-resin the reaction proceeds smoothly (Scheme 1). After filtration over silica gel and evaporation the pure unprotected substances

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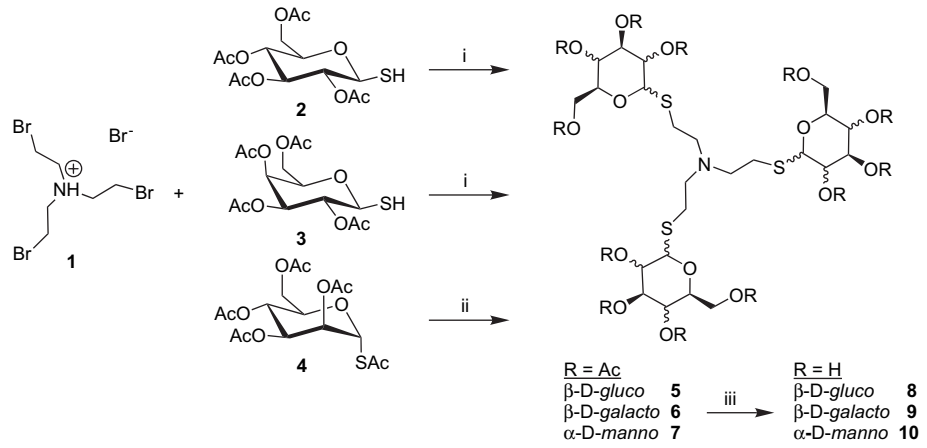
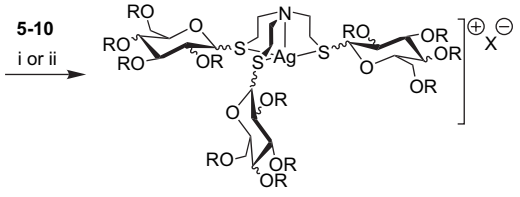


Scheme 1. Synthesis of the tripodal ligands: (i) NEt_3, DMF, 12 h, room temperature; (ii) $HNEt_2$, DMF, 0 °C, 15 min and then NEt_3, 12 h, room temperature; (iii) DOWEX-OH^-, H_2O/EtOH, 12 h, 60 °C.

8–**10** could be obtained. All synthesised ligands show in solution C_3-symmetry. Only one set of signals of one 'arm' occurs in the ^1H and ^{13}C NMR spectra. Besides the signals typical for the respective sugar residue the ethylene protons show a multiplet from 2.7 to 2.8 ppm and two ^{13}C-signals around 55 ppm (–*C*H_2–N–) and 30 ppm (–*C*H_2–S–).

To determine the influence of the anions complexation of **5**–**7** was carried out in ethanol with silver(I) nitrate and silver(I) hexafluorophosphate by refluxing the mixtures for 15 min under light exclusion, **8**–**10** were stirred together with the appropriate silver salt in water overnight (Scheme 2). Degradation of the thioglycosides was not observed under these conditions and after filtration and evaporation the complexes were obtained as white powders. Only refluxing especially the water soluble complexes **17**–**22** for a longer time with an excess of silver salt resulted in the formation of dark precipitates and a mixture of side products. Also the NMR samples show the formation of a silver mirror when standing for several days. From the solid substances only the α-mannose derived compounds became yellow when stored over several weeks. ESI-MS of the compounds indicate the complete formation of the complexes. No signals for the free ligands were observed. The acetyl-protected substances **11**–**16** gave almost single peaks at *m*/*z* 1296 for the cations of the general formula $[AgL]^+$. All water soluble complexes **17**–**22** show besides the molpeak at *m*/*z* 792 the same characteristic degradation pattern under ESI conditions.



		β-D-*gluco*	β-D-*galacto*	α-D-*manno*
R = OAc	X = NO_3^-	**11**	**12**	**13**
	X = PF_6^-	**14**	**15**	**16**
R = H	X = NO_3^-	**17**	**18**	**19**
	X = PF_6^-	**20**	**21**	**22**

Scheme 2. Synthesis of the silver(I) complexes: (i) $AgNO_3$ and (ii) $AgPF_6$; for R=Ac: reflux in ethanol, 15 min; for R=H: stirring in water, room temperature, overnight.

NMR measurements in acetone-d_6 or $CDCl_3$ (**11**–**16**) and D_2O (**17**–**22**) clearly indicate the C_3-symmetric structure of all complexes in solution. The ethylene protons around the silver ion are not equal anymore and show four separate signals at around 2.6 ppm, 2.9 ppm, 3.0 ppm and 3.2 ppm. The corresponding ^{13}C-signals were shifted to 35 ppm (–*C*H_2—S–) and 50 ppm (–*C*H_2–N–). The shifts and the separation of the peaks are only depending on the sugar residue and the used solvent. Almost no influence of the anion was observed so that an ionic structure of the complexes in solution can be assumed.

Recrystallisation of **11** from ethanol resulted in the formation of crystals suitable for single crystal X-ray structural analysis. Figure 1 shows the ORTEP view of one of the three molecules found in the asymmetric unit. The Ag^I ion is trigonal-pyramidally coordinated by the ligand and deflected out of the plane defined by the three sulfur atoms away from the nitrogen atom at the top of the trigonal pyramid.

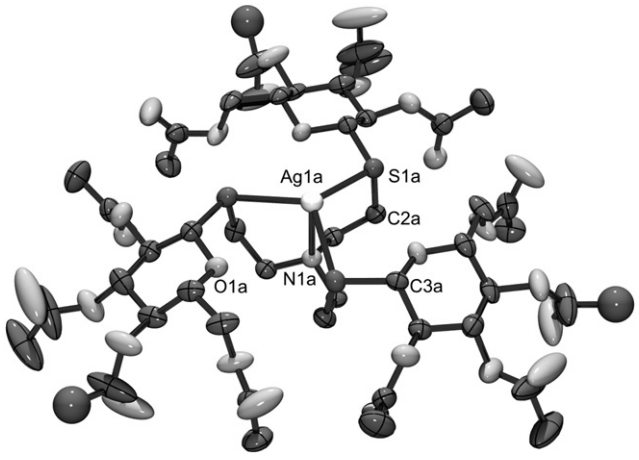


Figure 1. ORTEP view (50% probability) of one of the molecules in the structure of **11**. H-atoms and the coordinating nitrate anion are omitted for clarity. Selected distances [Å] and angles [°]: Ag1a-S1a 2.6806(13), Ag1a-N1a 2.607(7), N1a-Ag1a-S1a 77.48(3), S1a-Ag1a-S1a 115.43(2).

Additionally, in the solid state one nitrate anion (not shown in Fig. 1) is weakly coordinated to the silver ion (Ag1a-O1na 2.594(16)).

To verify the structure of the deprotected complexes EXAFS measurements have been carried out using complex **11** as a standard. The k^3-weighted EXAFS oscillations, $k^3\chi(k)$, resemble each other, suggesting a similar coordination structure of the Ag^I ions. The $k^3\chi(k)$ values were fitted with sum of two back-scattering contributions, namely, Ag–N ($k^3\chi(k)_N$) and Ag–S ($k^3\chi(k)_S$). The back-scattering amplitude and phase shift function, $F_i(k)$ and $\Phi_i(k)$, for single scattering pathways were estimated by means of ab initio self-consistent calculation using FEFF 8.2.[28] No empirical parameters are used in the analysis of EXAFS oscillation. Table 1 lists the best-fitted parameters, interatomic distances r, the coordination number N and the Debye–Waller factor σ. The parameters for **17**(BN) are close to those for **11**(BN). Therefore, **17** should have a similar coordination structure like **11**. The interatomic distances r_N and r_S for **11**(BN) are slightly shorter than that derived from X-ray crystallography. The amplitude of EXAFS oscillation of **17**(H_2O) is smaller than that for solid phase. However, the contribution ratio between Ag–N and Ag–S, i.e., N_N/N_S value, is 0.78 which is almost the same as for the solid state (0.83). The reduced EXAFS oscillation of the sample in aqueous solution may be caused by the difference in the amplitude reduction factor S_0^2. Consequently, the unprotected complex **17** has a similar coordination structure to that of the protected complex **11** and keeps this structure even in aqueous solution.

Table 1. Structural parameters derived from EXAFS analysis

Sample	Shell	N	r (Å)	dE	σ	R (%)
11(BN)[a]	Ag–N	2.9	2.684	−1.528	0.090	2.999
	Ag–S	3.9	2.600	−0.552	0.119	
17(BN)[a]	Ag–N	2.9	2.651	1.255	0.070	1.938
	Ag–S	3.5	2.560	1.886	0.105	
17(H_2O)[b]	Ag–N	1.4	2.508	1.721	0.177	0.981
	Ag–S	1.8	2.545	3.098	0.113	

[a] Boron nitrate pellets.
[b] Aqueous solution.

The antimicrobial activities of the obtained water soluble complexes **17**–**22** against bacteria, yeasts and fungi were examined qualitatively by agar diffusion tests (Table 2). Whereas the free ligands **8**–**10** show no activity the silver complexes possess a wide spectrum of effective antibacterial and antifungal activities. They inhibit the growth of Gram-positive and Gram-negative bacteria (*Bacillus subtilis*, sensitive and multiresistant (MRSA) *Staphylococcus aureus*, vancomycin resistant (VRE) *Enterococcus faecalis*, *Mycobacterium vaccae*, *Escherichia coli*, *Pseudomonas aeruginosa*), yeasts (*Candida albicans*, *Candida glabrata*) and fungi (*Penicillium notatum*, *Aspergillus fumigatus*, *Fusarium oxysporum*), but not of silver non-sensitive *Aspergillus terreus*.

Additionally compounds were evaluated by the determination of MIC values for selected organisms (Table 3). With MIC values of 15.6–62.5 μM the compounds exhibit effective activities against bacteria in the same range of those observed for water soluble silver complexes based on L-histidine and (*S*)-2-pyrrolidone-5-carboxylic acid,[6] but moderate compared to 0.05–0.8 μM for ciprofloxacin under the same conditions. For Gram-negative bacteria a two times higher activity of the complexes is observed compared to the silver salts.

The experiments show that the effects are independent from the used sugar residue or anion. Three mechanisms for the inhibition by aqueous silver(I) ions have been proposed, interference with electron transport, binding to DNA and interaction with the cell membrane.[29] Consistently with other studies the mechanism of the antimicrobial action has to be related to the replacement of the ligands in the silver(I)

Table 3. Antibacterial activities evaluated by minimal inhibition concentration of selected compounds[a]

	17	**19**	**20**	**23**	$AgNO_3$	$AgPF_6$
E. coli	15.6	15.6	15.6	15.6	31.3	46.9
P. aeruginosa	15.6	23.4	15.6	15.6	23.4	31.3
E. faecalis	62.5	62.5	62.5	62.5	62.5	62.5
M. vaccae	15.6	15.6	15.6	15.6	31.3	46.9

[a] MIC values in μmol/l.

Table 2. Qualitative determination of antimicrobial activity by agar diffusion tests (diameter of inhibition zones in mm)

	8	**9**	**10**	**17**	**18**	**19**	**20**	**21**	**22**	$AgNO_3$	$AgPF_6$	Ciprofloxacin	Amphoter. B[a]	Nystatin
B. subtilis	0	0	0	14	13	14	14	13.5	13.5	12.5	11.5	28	—	—
S. aureus	0	0	0	14p	14p	14.5p	15p	14.5p	14.5	13p	12p	19	—	—
S. aureus MRSA	0	0	0	14.5P	14P	14.5P	14.5P	14.5P	14P	12.5P	12P	0	—	—
E. faecalis VRE	0	0	0	12	12	13	13	13	13	12	12	15	—	—
M. vaccae	0	0	0	14	13.5	14	14.5	14	14	12.5	12	21	—	—
E. coli	0	0	0	16	15	16	16.5	15	15	13	12.5	21/29p	—	—
P. aeruginosa	0	0	0	13.5p	14p	14p	14P	13p	14p	12p	11.5p	24.5	—	—
S. salmomicolor	0	0	0	17A	17	18P	17A	18A	16A	12P	12P	—	20P	—
C. albicans	0	0	0	17p	16.5p	15p	16p	16p	15	11	10	—	20.5	—
P. notatum	0	0	0	25	24	24	25	25	24	15	14.5	—	16/19p	—
A. fumigatus	0	0	0	19p	15P	19p	15p	18P	17P	12/14p	12A	—	—	22[b]
F. oxysporum	0	0	0	19P	15P	17P	15P	18P	16P	12p	12P	—	—	18[c]
A. terreus	0	0	0	0	0	0	0	0	0	11p	0	—	—	19[d]

p—Colonies in the inhibition zone; P—many colonies in the inhibition zone; A—indication of inhibition zone.
[a] 10 μg/ml.
[b] 50 μg/ml.
[c] 200 μg/ml.
[d] 100 μg/ml.

complexes by proteins of the microbes as sulfur donor ligands.[30,31] As a key factor the strength of binding of the Ag^I ions by N/S/O donors has been determined. Generally compounds with weaker bonds such as Ag–N and Ag–O bonds show a wider spectrum of antimicrobial activities. In our case the silver is bound only weakly to the thio-ether functions, which allow further replacement with biological ligands.

The antiproliferative and cytotoxic activities of the ligands **8–10** and the complexes **17–22** were determined using the cell lines K-562 (human chronic myeloid leukaemia) and L-929 (mouse fibroblast) for antiproliferative effects and HeLa (human cervix carcinom) for cytotoxic effects (Table 4). The results show that the carbohydrate based complexes exhibit only half of the cytotoxic and antiproliferative activities than the appropriate silver salt. No sugar specific action has been observed.

Table 4. Antiproliferative and cytotoxic activities[a]

Compound	Antiproliferative effect		Cytotoxicity
	L-929[b]	K-562[b]	HeLa[c]
17	5.7	2.2	43.1
18	5.7	3.0	45.4
19	5.7	2.6	44.2
20	7.7	3.6	54.3
21	10.0	2.9	79.5
22	8.6	3.0	68.0
$AgNO_3$	2.4	1.2	24.1
$AgPF_6$	5.9	2.4	30.5

[a] Ligands **8–10** show no effects up to 292 mmol/l.
[b] GI_{50} in μmol/l.
[c] CC_{50} in μmol/l.

3. Conclusion

In conclusion, we could develop highly practical synthetic methods to obtain new sugar substituted tripodal NS_3 ligands. Their complexation with silver(I) ions leads to stable C_3-symmetric complexes with defined mononuclear structure. The solubility of the Ag^I complexes is influenced by the sugar moieties and their residues. Independently from the sugar residue the water soluble silver compounds show a wide range of effective antimicrobial activities against bacteria, yeast and fungi. The use of the carbohydrate based substances enhances the antimicrobial activity and reduces the cytotoxicity and antiproliferative activities compared to the free silver(I) salts.

4. Experimental

4.1. General

All reagents and solvents were purchased from commercial sources and used as received. NMR spectra were measured on a JEOL JMTC-400/54/SS, ESI-MS were carried out on a JEOL JMS-T100LC, elemental analyses on a Perkin–Elmer PE2400 Series II CHNS/O Analyzer (Nara Institute of Science and Technology).

4.1.1. Tris[2-(2,3,4,6-tetra-*O*-acetyl-β-D-thio-glucopyranosyl)ethyl]amine (5). 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-thiol (8.79 g, 24.1 mmol) was dissolved in 50 ml DMF and 4.7 ml triethylamine were added. At 0 °C under stirring 3.33 g (8.02 mmol) of tris(2-bromoethyl)amine hydrobromide were added and the solution was allowed to warm up to room temperature and stirred overnight. DMF was evaporated, the residue extracted with ethyl acetate and water. After drying the organic phase with sodium sulfate, ethyl acetate was removed and the raw product was recrystallised from ethanol to yield 8.70 g (91%) colourless needles. ^{1}H NMR (400 MHz, $CDCl_3$): 4.55 (d, 3H, H-1, $J_{1,2}$ 10.0 Hz), 5.24 (t, 3H, H-2, $J_{2,3}$ 9.5 Hz), 5.08 (t, 3H, H-3, $J_{3,4}$ 9.8 Hz), 5.03 (t, 3H, H-4, $J_{4,5}$ 9.8 Hz), 3.77 (ddd, 3H, H-5), 4.26 (dd, 3H, H-6, $J_{5,6}$ 4.6 Hz, $J_{6,6'}$ 12.4 Hz), 4.14 (dd, 3H, H-6′, $J_{5,6'}$ 2.2 Hz), 2.71–2.80 (m, 12H, 6×CH_2), 2.01, 2.03, 2.07, 2.09 (4s, 36H, CH_3-acetyl). ^{13}C NMR ($CDCl_3$): 170.3, 169.9, 169.2 (*C*O-acetyl), 83.07 (*C*1), 75.77 (*C*2), 73.71 (*C*3), 69.66 (*C*4), 68.20 (*C*5), 62.04 (*C*6), 53.70 (N–*C*H_2–), 27.58 (S–*C*H_2–), 20.7 (*C*H_3-acetyl). ESI-MS *m*/*z* (%): 1210.44 (100) $[M+Na]^+$. Anal. Calcd for $C_{48}H_{69}NO_{27}S_3$: C, 48.52; H, 5.85; N, 1.18. Found: C, 48.45; H, 5.86; N, 1.25%.

4.1.2. Tris[2-(2,3,4,6-tetra-*O*-acetyl-β-D-thio-galactopyranosyl)ethyl]amine (6). 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-thiol (3.432 g, 9.426 mmol) was reacted with 1.303 g (3.142 mmol) tris(2-bromoethyl)amine hydrobromide and 3.808 g triethylamine in DMF analogous to **5**. After extraction with ethyl acetate and water the organic phase was dried over sodium sulfate, filtered and ethyl acetate was evaporated. Chromatographic cleaning over silica gel with ethyl acetate/hexane 2:1 (R_f 0.5) yields 1.45 g (40%) of colourless solid. ^{1}H NMR (400 MHz, $CDCl_3$): 4.54 (d, 3H, H-1, $J_{1,2}$ 9.8 Hz), 5.21 (t, 3H, H-2, $J_{2,3}$ 10.0 Hz), 5.07 (dd, 3H, H-3, $J_{3,4}$ 3.0 Hz), 5.44 (d, 3H, H-4), 3.99 (t, 3H, H-5, $J_{5,6}$ 6.7 Hz), 4.13 (m, 6H, H-6, H-6′), 2.77 (m, 12H, 6×CH_2), 1.99, 2.05, 2.07, 2.16 (4s, 36H, CH_3-acetyl). ^{13}C NMR ($CDCl_3$): 170.1, 169.9, 169.8, 169.4 (*C*O-acetyl), 83.73 (*C*1), 74.35 (*C*2), 71.72 (*C*3), 67.23 (*C*4), 67.31 (*C*5), 61.35 (*C*6), 54.10 (N–*C*H_2–), 27.89 (S–*C*H_2–), 20.8, 20.7 (*C*H_3-acetyl). ESI-MS *m*/*z* (%): 1210.37 (100) $[M+Na]^+$. Anal. Calcd for $C_{48}H_{69}NO_{27}S_3$: C, 48.52; H, 5.85; N, 1.18. Found: C, 48.11; H, 5.87; N, 1.24%.

4.1.3. Tris[2-(2,3,4,6-tetra-*O*-acetyl-α-D-thio-mannopyranosyl)ethyl]amine (7). 2,3,4,6-Tetra-*O*-acetyl-α-D-thioacetyl-mannose (1 g, 2.46 mmol) was dissolved in 20 ml DMF and 180 mg (2.46 mmol) diethylamine were added drop wise at 0 °C. After stirring for 15 min an excess of triethylamine (1 ml) and 343 mg (0.82 mmol) tris(2-bromoethyl)amine hydrobromide were added and the solution was stirred overnight at room temperature. DMF was evaporated and the residue extracted with ethyl acetate and water. After drying the organic phase with sodium sulfate, ethyl acetate was evaporated and the raw product cleaned by column chromatography. ^{1}H NMR (400 MHz, $CDCl_3$): 5.33–5.28 (m, 9H, H-1, H-2, H-4), 5.26 (dd, 3H, H-3, $J_{3,4}$ 3.2 Hz, $J_{2,3}$ 10.0 Hz), 4.35–4.29 (m, 6H, H-5, H-6, $J_{5,6}$ 5.0 Hz, $J_{5,6'}$ 1.9 Hz), 4.11 (dd, 3H, H-6′, $J_{6,6'}$ 12.0 Hz), 2.80–2.62 (m, 12H, 6×CH_2), 1.99, 2.05, 2.10, 2.16 (4s, 36H, CH_3-acetyl). ^{13}C NMR ($CDCl_3$): 170.2, 169.6, 169.4 (*C*O-acetyl), 82.57 (*C*1), 69.30 (*C*2), 71.03 (*C*3), 69.12 (*C*4), 66.32 (*C*5), 62.40 (*C*6), 53.83 (N–*C*H_2–), 29.28 (S–*C*H_2–), 20.95, 20.85, 20.76, 20.68 (*C*H_3-acetyl). ESI-MS *m*/*z* (%): 1210.35 (100)

[M+Na]$^+$. Anal. Calcd for $C_{48}H_{69}NO_{27}S_3$: C, 48.52; H, 5.85; N, 1.18. Found: C, 48.33; H, 5.93; N, 1.25%.

4.1.4. Tris[2-(β-D-thio-glucopyranosyl)ethyl]amine (8). Tris[2-(2,3,4,6-tetra-*O*-acetyl-β-D-thio-glucopyranosyl)ethyl]-amine **5** (870 mg, 0.73 mmol) and 4.9 g of OH^--loaded DOWEX were dissolved in 45 ml of a mixture of water and ethanol 1:1. The reaction mixture was stirred at 60 °C until no starting material could be detected by TLC. Filtration and evaporation gave the raw product as colourless foam. It was cleaned by filtration over silica gel with methanol yielding 332 mg (60%). ^{1}H NMR (400 MHz, D_2O, DSS): 4.53 (d, 3H, H-1, $J_{1,2}$ 9.8 Hz), 3.31 (t, 3H, H-2, $J_{2,3}$ 8.8 Hz), 3.39 (t, 3H, H-3, $J_{3,4}$ 8.8 Hz), 3.44–3.50 (m, 6H, H-4, H-5), 3.89 (d, 3H, H-6, $J_{6,6'}$ 12.2 Hz), 3.69 (dd, 3H, H-6′, $J_{5,6'}$ 5.6 Hz), 2.86 (m, 12H, 6×CH_2). ^{13}C NMR (D_2O, DSS): 87.98 (*C*1), 82.44 (*C*2), 79.65 (*C*3), 74.77 (*C*4), 72.06 (*C*5), 63.45 (*C*6), 56.02 (N–*C*H_2–), 29.15 (S–*C*H_2–). ESI-MS *m*/*z* (%): 706.25 (100) [M+Na]$^+$. Anal. Calcd for $C_{24}H_{45}NO_{15}S_3\cdot 3H_2O$: C, 39.07; H, 6.97; N, 1.90. Found: C, 38.99; H, 7.00; N, 1.98%.

4.1.5. Tris[2-(β-D-thio-galactopyranosyl)ethyl]amine (9). Similar to **8**. ^{1}H NMR (400 MHz, D_2O, DSS): 4.47 (d, 3H, H-1, $J_{1,2}$ 9.5 Hz), 3.55 (t, 3H, H-2, $J_{2,3}$ 9.8 Hz), 3.64 (dd, 3H, H-3, $J_{3,4}$ 3.4 Hz), 3.96 (d, 3H, H-4), 3.68–3.78 (m, 9H, H-5, H-6, H-6′), 2.86 (m, 12H, 6×CH_2). ^{13}C NMR (D_2O, DSS): 88.51 (*C*1), 81.52 (*C*2), 76.39 (*C*3), 71.33 (*C*4), 72.12 (*C*5), 63.65 (*C*6), 55.98 (N–*C*H_2–), 29.18 (S–*C*H_2–). ESI-MS *m*/*z* (%): 706.25 (100) [M+Na]$^+$. Anal. Calcd for $C_{24}H_{45}NO_{15}S_3$: C, 42.15; H, 6.63; N, 2.05. Found: C, 39.85; H, 6.77; N, 2.11%.

4.1.6. Tris[2-(α-D-thio-mannopyranosyl)ethyl]amine (10). Similar to **8**. ^{1}H NMR (400 MHz, D_2O, DSS): 5.31 (s, 3H, H-1), 4.04 (d, 3H, H-2, $J_{2,3}$ 3.0 Hz), 3.77 (m, 6H, H-3, H-6′), 3.65 (t, 3H, H-4, $J_{3,4}$ 9.6 Hz, $J_{4,5}$ 9.6 Hz), 3.99 (m, 3H, H-5, $J_{5,6'}$ 6.1 Hz), 3.88 (d, 3H, H-6, $J_{6,6'}$ 12.2 Hz), 2.76–2.92 (m, 12H, 6×CH_2). ^{13}C NMR (D_2O, DSS): 87.51 (*C*1), 75.63 (*C*2), 74.19 (*C*3), 73.55 (*C*4), 69.61 (*C*5), 63.39 (*C*6), 55.25 (N–*C*H_2–), 30.16 (S–*C*H_2–). ESI-MS *m*/*z* (%): 706.25 (100) [M+Na]$^+$. Anal. Calcd for $C_{24}H_{45}NO_{15}S_3$: C, 42.15; H, 6.63; N, 2.05. Found: C, 40.94; H, 6.81; N, 2.12%.

4.1.7. Tris[2-(2,3,4,6-tetra-*O*-acetyl-β-D-thio-glucopyranosyl)ethyl]-amine-silver(I)-nitrate (11). Compound **5** (300 mg, 0.252 mmol) in 10 ml ethanol was mixed with 42.6 mg (0.252 mmol) of silver(I) nitrate and refluxed for 15 min. After filtration the ethanol was evaporated and the residue recrystallised from ethanol yielding 280 mg (82%) of colourless crystals suitable for single crystal X-ray analysis. ^{1}H NMR (400 MHz, $CDCl_3$): 4.63 (d, 3H, H-1, $J_{1,2}$ 9.8 Hz), 5.24 (t, 3H, H-2, $J_{2,3}$ 9.3 Hz), 5.17 (t, 3H, H-3, $J_{3,4}$ 9.8 Hz), 5.09 (t, 3H, H-4, $J_{4,5}$ 9.8 Hz), 3.86 (d, 3H, H-5), 4.02 (d, 3H, H-6, $J_{6,6'}$ 12.0 Hz), 4.50 (dd, 3H, H-6′, $J_{5,6'}$ 2.2 Hz), 3.20 (d, 3H, C*H*, *J* 12.9 Hz), 2.90 (t, 3H, C*H*, *J* 12.3 Hz), 2.82 (t, 3H, C*H*, *J* 12.5 Hz), 2.44 (d, 3H, C*H*, *J* 12.9 Hz), 2.06, 2.05, 2.00, 1.98 (4s, 36H, CH_3-acetyl). ^{13}C NMR ($CDCl_3$): 169.8, 169.3 (*C*O-acetyl), 82.19 (*C*1), 77.10 (*C*2), 73.87 (*C*3), 67.74 (*C*4), 67.02 (*C*5), 60.67 (*C*6), 51.70 (N–*C*H_2–), 26.8 (S–*C*H_2–), 20.6, 20.5 (*C*H_3-acetyl). ESI-MS *m*/*z* (%): 1296.34 (100) [M]$^+$. Anal. Calcd for $C_{48}H_{69}AgN_2O_{30}S_3$: C, 42.45; H, 5.12; N, 2.06. Found: C, 42.18; H, 5.00; N, 2.19%.

4.1.8. Tris[2-(2,3,4,6-tetra-*O*-acetyl-β-D-thio-galactopyranosyl)ethyl]-amine-silver(I)-nitrate (12). Compound **6** (100 mg, 0.084 mmol) and 14.2 mg $AgNO_3$ were dissolved in 10 ml ethanol and the solution was heated up for 15 min. It was filtered and the solvent was evaporated to give a colourless powder. ^{1}H NMR (400 MHz, $CDCl_3$): 4.68 (d, 3H, H-1, $J_{1,2}$ 9.8 Hz), 5.22 (t, 3H, H-2, $J_{2,3}$ 10.0 Hz), 5.08 (dd, 3H, H-3, $J_{3,4}$ 3.3 Hz), 5.45 (d, 3H, H-4), 3.95 (m, 3H, H-5), 4.18–4.23 (m, 6H, H-6, H-6′), 2.14–3.12 (m, 12H, 6×CH_2), 1.98, 2.04, 2.11, 2.18 (4s, 36H, CH_3-acetyl). ^{13}C NMR ($CDCl_3$): 169.9, 169.4 (*C*O-acetyl), 82.24 (*C*1), 75.09 (*C*2), 71.44 (*C*3), 66.98 (*C*4), 66.49 (*C*5), 60.60 (*C*6), 51.00 (N–*C*H_2–), 27.40 (S–*C*H_2–), 20.7 (*C*H_3-acetyl). ESI-MS *m*/*z* (%): 1296.33 (100) [M]$^+$. Anal. Calcd for $C_{48}H_{69}AgN_2O_{30}S_3$: C, 42.45; H, 5.12; N, 2.06. Found: C, 40.82; H, 5.27; N, 2.08%.

4.1.9. Tris[2-(2,3,4,6-tetra-*O*-acetyl-α-D-thio-mannopyranosyl)ethyl]-amine-silver(I)-nitrate (13). Similar to **12**. ^{1}H NMR (400 MHz, $CDCl_3$): 5.46 (s, 3H, H-1), 5.29–5.36 (m, 9H, H-2, H-3, H-4), 4.20–4.35 (m, 6H, H-5, H-6), 4.11 (dd, 3H, H-6′, $J_{5,6'}$ 1.7 Hz, $J_{6,6'}$ 10.0 Hz), 3.06–2.74 (m, 12H, 6×CH_2), 2.19, 2.10, 2.06, 2.03 (4s, 36H, CH_3-acetyl). ^{13}C NMR ($CDCl_3$): 170.2, 170.0, 169.6, 169.4 (*C*O-acetyl), 81.34 (*C*1), 71.31 (*C*2), 70.40 (*C*3), 68.86 (*C*4), 65.91 (*C*5), 62.01 (*C*6), 51.05 (N–*C*H_2–), 29.39 (S–*C*H_2–), 20.99, 20.76 (*C*H_3-acetyl). ESI-MS *m*/*z* (%): 1296.23 (100) [M]$^+$. Anal. Calcd for $C_{48}H_{69}AgN_2O_{30}S_3$: C, 42.45; H, 5.12; N, 2.06. Found: C, 42.07; H, 5.18; N, 2.10%.

4.1.10. Tris[2-(2,3,4,6-tetra-*O*-acetyl-β-D-thio-glucopyranosyl)ethyl]-amine-silver(I)-hexafluorophosphate (14). Similar to **11**, with 100 mg (0.084 mmol) of **5** and 21.2 mg (0.084 mmol) of $AgPF_6$. Compound **14** directly precipitates from solution to yield 96 mg colourless solid. ^{1}H NMR (400 MHz, acetone-d_6): 4.63 (m, 6H, H-1, H-3), 5.31 (t, 3H, H-2, $J_{2,3}$ 9.3 Hz), 5.02 (t, 3H, H-4, $J_{4,5}$ 10.0 Hz), 4.08 (ddd, 3H, H-5, $J_{5,6}$ 5.4 Hz, $J_{5,6'}$ 2.2 Hz), 4.23 (dd, 3H, H-6, $J_{6,6'}$ 12.4 Hz), 4.14 (dd, 3H, H-6′), 3.37 (m, 3H, C*H*), 3.18 (m, 3H, C*H*), 2.99 (m, 3H, C*H*), 2.84 (m, 3H, C*H*), 2.13, 2.08, 2.03, 1.99 (4s, 36H, CH_3-acetyl). ^{13}C NMR (acetone-d_6): 170.4, 169.9 (*C*O-acetyl), 84.34 (*C*1), 77.10 (*C*2), 73.87 (*C*3), 70.72 (*C*4), 68.51 (*C*5), 62.77 (*C*6), 51.91 (N–*C*H_2–), 31.28 (S–*C*H_2–), 20.5 (*C*H_3-acetyl). ESI-MS *m*/*z* (%): 1296.19 (100) [M]$^+$. Anal. Calcd for $C_{48}H_{69}AgF_6NO_{27}PS_3$: C, 40.01; H, 4.83; N, 0.97. Found: C, 39.44; H, 5.01; N, 1.11%.

4.1.11. Tris[2-(2,3,4,6-tetra-*O*-acetyl-β-D-thio-galactopyranosyl)ethyl]-amine-silver(I)-hexafluorophosphate (15). Similar to **11**. ^{1}H NMR (400 MHz, $CDCl_3$): 4.75 (d, 3H, H-1, $J_{1,2}$ 9.8 Hz), 5.14 (t, 3H, H-2, $J_{2,3}$ 10.0 Hz), 5.07 (dd, 3H, H-3, $J_{3,4}$ 2.9 Hz), 5.44 (d, 3H, H-4), 3.90 (m, 3H, H-5), 4.15–4.20 (m, 6H, H-6, H-6′), 3.20 (t, 3H, C*H*, *J* 13.0 Hz), 3.05 (d, 3H, C*H*, *J* 14.4 Hz), 2.90 (t, 3H, C*H*, *J* 12.3 Hz), 2.69 (d, 3H, C*H*, *J* 12.9 Hz), 1.99, 2.05, 2.08, 2.28 (4s, 36H, CH_3-acetyl). ^{13}C NMR ($CDCl_3$): 170.2, 169.6, 169.8, 169.4 (*C*O-acetyl), 86.48 (*C*1), 75.18 (*C*2), 71.25 (*C*3), 67.06 (*C*4, *C*5), 61.73 (*C*6), 50.57 (N–*C*H_2–), 34.48 (S–*C*H_2–), 20.7 (*C*H_3-acetyl). ESI-MS *m*/*z* (%): 1296.22 (100) [M]$^+$. Anal. Calcd for $C_{48}H_{69}AgF_6NO_{27}PS_3$: C, 40.01; H, 4.83; N, 0.97. Found: C, 40.69; H, 4.88; N, 0.98%.

4.1.12. Tris[2-(2,3,4,6-tetra-*O*-acetyl-α-D-thio-mannopyranosyl)ethyl]-amine-silver(I)-hexafluorophosphate (16). Ligand **7** (395 mg, 0.333 mmol) and 84.1 mg (0.333 mmol) of $AgPF_6$ were dissolved in 30 ml ethanol by refluxing the mixture for 15 min. The solution was allowed to cool to room temperature. Overnight crystals were formed. They were collected and recrystallised from ethanol. ^{1}H NMR (400 MHz, $CDCl_3$): 5.40–5.36 (m, 9H, H-1, H-2, H-4), 5.29 (dd, 3H, H-3, $J_{3,4}$ 2.9 Hz, $J_{2,3}$ 10.0 Hz), 4.31–4.23 (m, 6H, H-5, H-6), 4.11 (dd, 3H, H-6′, $J_{6,6'}$ 10.0 Hz), 3.12–2.99 (m, 6H, 3×CH_2), 2.83 (s, 6H, 3×CH_2), 2.21, 2.11, 2.06 (3s, 36H, CH_3-acetyl). ^{13}C NMR ($CDCl_3$): 170.3, 169.2 (*C*O-acetyl), 81.34 (*C*1), 70.85 (*C*2), 71.28 (*C*3), 69.20 (*C*4), 65.49 (*C*5), 61.89 (*C*6), 49.27 (N–*C*H_2–), 30.50 (S–*C*H_2–), 20.91, 20.83, 20.73 (*C*H_3-acetyl). ESI-MS *m*/*z* (%): 1296.23 (100) $[M]^+$. Anal. Calcd for $C_{48}H_{69}AgF_6NO_{27}PS_3$: C, 40.01; H, 4.83; N, 0.97. Found: C, 39.49; H, 4.89; N, 1.07%.

4.1.13. Tris[2-(β-D-thio-glucopyranosyl)ethyl]-amine-silver(I)-nitrate (17). Compound **8** (277 mg, 0.405 mmol) and 68.5 mg (0.405 mmol) $AgNO_3$ were dissolved in water (5 ml) and the solution was stirred overnight. After filtration the solvent was evaporated. By diffusion of acetone into a aqueous solution of the raw product 310 mg (89%) of colourless needles were obtained. ^{1}H NMR (400 MHz, D_2O, DSS): 4.71 (d, 3H, H-1, $J_{1,2}$ 9.8 Hz), 3.31 (t, 3H, H-2, $J_{2,3}$ 9.3 Hz), 3.40 (t, 3H, H-3, $J_{3,4}$ 9.5 Hz), 3.47–3.52 (m, 6H, H-4, H-5), 3.96 (d, 3H, H-6, $J_{6,6'}$ 12.2 Hz), 3.72 (dd, 3H, H-6′, $J_{5,6'}$ 6.1 Hz), 3.25 (d, 3H, C*H*, *J* 12.2 Hz), 3.06 (d, 3H, C*H*, *J* 15.1 Hz), 2.84–2.99 (m, 3H, C*H*), 2.61 (d, 3H, C*H*, *J* 13.7 Hz). ^{13}C NMR (D_2O, DSS): 88.88 (*C*1), 82.63 (*C*2), 79.51 (*C*3), 75.38 (*C*4), 71.85 (*C*5), 63.53 (*C*6), 53.30 (N–*C*H_2–), 34.57 (S–*C*H_2–). ESI-MS *m*/*z* (%): 792.16 (100) $[M]^+$, 630.09 (60) $[M-C_6H_{10}O_5]^+$, 468.03 (70) $[M-2(C_6H_{10}O_5)]^+$, 305.97 (95) $[M-3(C_6H_{10}O_5)]^+$. Anal. Calcd for $C_{24}H_{45}AgN_2O_{18}S_3$: C, 33.77; H, 5.31; N, 3.28. Found: C, 32.90; H, 5.68; N, 3.07%.

4.1.14. Tris[2-(β-D-thio-galactopyranosyl)ethyl]-amine-silver(I)-nitrate (18). Similar to **17**. ^{1}H NMR (400 MHz, D_2O, DSS): 4.66 (d, 3H, H-1, $J_{1,2}$ 10.0 Hz), 3.54 (t, 3H, H-2, $J_{2,3}$ 9.5 Hz), 3.80–3.66 (m, 12H, H-3, H-5, 2×H-6), 3.99 (s, 3H, H-4), 3.22 (m, 3H, CH_2), 3.06 (d, 3H, CH_2), 2.90 (m, 9H, 3×CH_2). ^{13}C NMR (D_2O, DSS): 89.70 (*C*1), 81.59 (*C*2), 76.09 (*C*3), 71.30 (*C*4), 72.43 (*C*5), 63.86 (*C*6), 55.98 (N–*C*H_2–), 29.18 (S–*C*H_2–). ESI-MS *m*/*z* (%): 792.19 (100) $[M]^+$, 630.12 (40) $[M-C_6H_{10}O_5]^+$, 468.05 (40) $[M-2(C_6H_{10}O_5)]^+$, 305.98 (50) $[M-3(C_6H_{10}O_5)]^+$. Anal. Calcd for $C_{24}H_{45}AgN_2O_{18}S_3$: C, 33.77; H, 5.31; N, 3.28. Found: C, 33.33; H, 5.74; N, 3.07%.

4.1.15. Tris[2-(α-D-thio-mannopyranosyl)ethyl]-amine-silver(I)-nitrate (19). Similar to **17**. ^{1}H NMR (400 MHz, D_2O, DSS): 5.35 (s, 3H, H-1), 4.14 (s, 3H, H-2), 3.85 (dd, 3H, H-3, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 9.5 Hz), 3.72 (t, 3H, H-4, $J_{3,4}$ 9.6 Hz, $J_{4,5}$ 9.6 Hz), 3.97 (t, 3H, H-5, $J_{5,6'}$ 7.3 Hz), 3.90 (d, 3H, H-6, $J_{6,6'}$ 12.4 Hz), 3.78 (dd, 3H, H-6′), 2.76–3.08 (m, 12H, 6×CH_2). ^{13}C NMR (D_2O, DSS): 85.15 (*C*1), 76.77 (*C*2), 73.72 (*C*3), 73.37 (*C*4), 69.33 (*C*5), 63.21 (*C*6), 50.80 (N–*C*H_2–), 30.96 (S–*C*H_2–). ESI-MS *m*/*z* (%): 792.20 (70) $[M]^+$, 630.13 (30) $[M-C_6H_{10}O_5]^+$, 468.06 (50) $[M-2(C_6H_{10}O_5)]^+$, 305.98 (100) $[M-3(C_6H_{10}O_5)]^+$. Anal. Calcd for $C_{24}H_{45}AgN_2O_{18}S_3$: C, 33.77; H, 5.31; N, 3.28. Found: C, 31.46; H, 5.55; N, 3.31%.

4.1.16. Tris[2-(β-D-thio-glucopyranosyl)ethyl]-amine-silver(I)-hexafluorophosphate (20). Compound **8** (313 mg, 0.458 mmol) together with 115.7 mg (0.458 mmol) $AgPF_6$ was stirred in 20 ml water at room temperature overnight. The solution was filtered and solvent evaporated to give 385 mg (90%) white solid. ^{1}H NMR (400 MHz, D_2O, DSS): 4.71 (d, 3H, H-1, $J_{1,2}$ 9.8 Hz), 3.31 (t, 3H, H-2, $J_{2,3}$ 9.3 Hz), 3.40 (t, 3H, H-3, $J_{3,4}$ 9.5 Hz), 3.47–3.55 (m, 6H, H-4, H-5), 3.96 (d, 3H, H-6, $J_{6,6'}$ 12.0 Hz), 3.72 (dd, 3H, H-6′, $J_{5,6'}$ 6.1 Hz), 3.25 (d, 3H, C*H*, *J* 12.2 Hz), 3.06 (d, 3H, C*H*, *J* 15.1 Hz), 2.88–2.99 (m, 3H, C*H*), 2.60 (d, 3H, C*H*, *J* 13.2 Hz). ^{13}C NMR (D_2O, DSS): 88.98 (*C*1), 82.65 (*C*2), 79.53 (*C*3), 75.47 (*C*4), 71.87 (*C*5), 63.55 (*C*6), 53.28 (N–*C*H_2–), 34.66 (S–*C*H_2–). ESI-MS *m*/*z* (%): 792.17 (100) $[M]^+$, 630.10 (60) $[M-C_6H_{10}O_5]^+$, 468.04 (70) $[M-2(C_6H_{10}O_5)]^+$, 305.97 (95) $[M-3(C_6H_{10}O_5)]^+$. Anal. Calcd for $C_{24}H_{45}AgF_6NO_{15}PS_3$: C, 30.78; H, 4.84; N, 1.50. Found: C, 29.02; H, 5.13; N, 1.42%.

4.1.17. Tris[2-(β-D-thio-galactopyranosyl)ethyl]-amine-silver(I)-hexafluorophosphate (21). Similar to **20**. ^{1}H NMR (400 MHz, D_2O, DSS): 4.68 (d, 3H, H-1, $J_{1,2}$ 9.8 Hz), 3.53 (t, 3H, H-2, $J_{2,3}$ 9.5 Hz), 3.84–3.74 (m, 9H, H-5, 2×H-6), 3.67 (dd, 3H, H-3, $J_{3,4}$ 2.3 Hz), 3.99 (d, 3H, H-4), 3.26 (t, 3H, C*H*, *J* 12.5 Hz), 3.06 (d, 3H, C*H*, *J* 14.9 Hz), 2.93 (t, 6H, C*H*, *J* 16.0 Hz), 2.61 (d, 3H, C*H*, *J* 13.2 Hz). ^{13}C NMR (D_2O, DSS): 89.87 (*C*1), 81.61 (*C*2), 76.06 (*C*3), 72.47 (*C*4), 71.31 (*C*5), 63.92 (*C*6), 53.36 (N–*C*H_2–), 35.02 (S–*C*H_2–). ESI-MS *m*/*z* (%): 792.20 (90) $[M]^+$, 630.13 (70) $[M-C_6H_{10}O_5]^+$, 468.06 (80) $[M-2(C_6H_{10}O_5)]^+$, 305.98 (100) $[M-3(C_6H_{10}O_5)]^+$. Anal. Calcd for $C_{24}H_{45}AgF_6NO_{15}PS_3$: C, 30.78; H, 4.84; N, 1.50. Found: C, 29.28; H, 4.97; N, 1.38%.

4.1.18. Tris[2-(α-D-thio-mannopyranosyl)ethyl]-amine-silver(I)-hexafluorophosphate (22). Similar to **20**. ^{1}H NMR (400 MHz, D_2O, DSS): 5.35 (s, 3H, H-1), 4.14 (s, 3H, H-2), 3.85 (dd, 3H, H-3, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 9.5 Hz), 3.72 (t, 3H, H-4, $J_{3,4}$ 9.6 Hz, $J_{4,5}$ 9.6 Hz), 3.97 (t, 3H, H-5, $J_{5,6'}$ 6.6 Hz), 3.90 (d, 3H, H-6, $J_{6,6'}$ 12.0 Hz), 3.78 (dd, 3H, H-6′), 2.71–3.08 (m, 12H, 6×CH_2). ^{13}C NMR (D_2O, DSS): 85.12 (*C*1), 76.77 (*C*2), 73.73 (*C*3), 73.38 (*C*4), 69.33 (*C*5), 63.21 (*C*6), 50.73 (N–*C*H_2–), 30.93 (S–*C*H_2–). ESI-MS *m*/*z* (%): 792.20 (80) $[M]^+$, 630.13 (20) $[M-C_6H_{10}O_5]^+$, 468.06 (40) $[M-2(C_6H_{10}O_5)]^+$, 305.98 (100) $[M-3(C_6H_{10}O_5)]^+$. Anal. Calcd for $C_{24}H_{45}AgF_6NO_{15}PS_3$: C, 30.78; H, 4.84; N, 1.50. Found: C, 28.76; H, 4.99; N, 1.39%.

4.2. Crystal structure of 11

The intensity data for the compound were collected on a Nonius KappaCCD diffractometer, using graphite-monochromated Mo Kα radiation. Data were corrected for Lorentz and polarisation effects, but not for absorption effects.[32,33] The structures were solved by direct methods (SHELXS)[34] and refined by full-matrix least squares techniques against Fo^2 (SHELXL-97).[35] The hydrogen atoms were included at calculated positions with fixed thermal parameters. All non-hydrogen atoms of the cations were refined anisotropically.[35] ORTEP and POV-ray were used for structural representation.

$[C_{48}H_{69}AgNO_{27}S_3]^+$ NO_3^-, Mr=1358.10 g mol^{-1}, colourless prism, size 0.04×0.04×0.03 mm^3, trigonal, space group *P*3, *a*=19.0275(3), *b*=19.0275(3), *c*=14.4848(3) Å, *V*=4541.6(1) Å^3, *T*=−90 °C, *Z*=3, ρ_{calcd}=1.490 g cm^{-3}, μ (Mo Kα)=5.26 cm^{-1}, *F*(000)=2118, 32,600 reflections in *h*(−23/24), *k*(−24/24), *l*(−18/16), measured in the range 1.87°≤Θ≤27.47°, completeness Θ_{max}=99.7%, 13,470 independent reflections, R_{int}=0.039, 11,036 reflections with $F_o>4\sigma(F_o)$, 736 parameters, 1 restraints, $R1_{obs}$=0.054, wR^2_{obs}=0.137, $R1_{all}$=0.071, wR^2_{all}=0.148, GOOF=1.024, Flack-parameter 0.01(2), largest difference peak and hole: 0.865/−0.652 eÅ^{-3}. CCDC 291388 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336 033; or deposit@ccdc.cam.ac.uk).

4.3. Extended X-ray absorption fine structure (EXAFS)

Measurements were performed at beam line 10B of the Photon Factory of the High Energy Acceleration Research Organization (KEK-PF), Tsukuba, Japan. A channel-cut Si(311) monochromator was used. The ring current was 300–450 mA, and the storage ring was operated with electron energy of 2.5 GeV. The experiments at the Ag K edge (25,516.5 eV) were carried out at room temperature in the transmission mode. The samples are prepared as BN pellets of **11** and **17** for solid state, and aqueous solution of **17** using a cell having pathlength of 2 cm.

4.4. Antimicrobial activity

Antimicrobial activity was determined qualitatively by agar diffusion tests according to literature.[36] A suspension (100 µl) of the test organism with a density of McFarland standard 0.5 was inoculated into 32 ml of sterile melted agar medium and poured into Petri dishes. Holes of 9 mm in diameter were cut in the agar and filled with 50 µl of a 0.2 mM solution of the compound. Inhibition zones were read after overnight incubation.

Antimicrobial activity of the selected compounds was studied quantitatively by the determination of minimal inhibitory concentrations (MICs) according to the NCCLS guidelines[37] using the broth microdilution method.[38] The bacteria were grown overnight at 37 °C in Mueller–Hinton broth (MHB) (Difco). A 50 µl compound solution of 2 mM were serially diluted by a factor of two with MHB. Then the wells were inoculated with 50 µl of test organisms to give a final concentration of 5×10^5 CFU/ml. After microtiter plates were incubated at 37 °C for 24 h, the MIC values were read with a Nepheloscan Ascent 1.4 automatic plate reader (Labsystems, Vantaa, Finland) as the lowest dilution of antibiotic allowing no visible growth. *C. albicans* and *C. glabrata* were grown in yeast nitrogen base (Difco) supplemented with 1% glucose and incubated at 30 °C.

4.5. Antiproliferative and cytotoxic activities

Antiproliferative and cytotoxic activities of the compounds were determined as described using the cell lines K-562 (human chronic myeloid leukaemia) and L-929 (mouse fibroblast) for antiproliferative effects and HeLa (human cervix carcinom) for cytotoxic effects.[39]

Cells of established suspended cell lines K-562 (DSM ACC 10) and adherent L-929 (DSM ACC 2) were cultured in RPMI 1640 medium (GIBCO BRL 42402-016), supplemented with 25 µg/ml gentamicin sulfate (BioWhittaker 17-528Z), 10% heat inactivated foetal bovine serum (GIBCO BRL 10500-064), and L-glutamine (GIBCO BRL 25030-024) at 37 °C in high density polyethylene flasks (NUNC 156340).

HeLa (DSM ACC 57) cells were grown in RPMI 1640 culture medium (GIBCO BRL 21875-034) supplemented with 25 µg/ml gentamicin sulfate (BioWhittaker 17-528Z), and 10% heat inactivated foetal bovine serum (GIBCO BRL 10500-064) at 37 °C in high density polyethylene flasks (NUNC 156340).

The compounds were assayed against cell lines K-562 (human chronic myeloid leukaemia) and L-929 (mouse fibroblast) for their antiproliferative effects. The adherent cells of L-929 were harvested at the logarithmic growth phase after soft trypsinization, using 0.25% trypsin in PBS containing 0.02% EDTA (Biochrom KG L2163).

For each experiment with K-562, L-929, and HeLa approximately 10,000 cells were seeded with 0.1 ml RPMI 1640 (GIBCO BRL 21875-034), containing 25 µg/ml gentamicin sulfate (BioWhittaker 17-528Z), but without HEPES, per well of the 96-well microplates (K-562: NUNC 163320, L-929, HeLa: NUNC 167008).

For the cytotoxic assay HeLa cells were preincubated for 48 h without the test substances. The dilutions of the compounds were carried out carefully on the monolayers of HeLa cells after the preincubation time.

Cells of L-929, K-562, and HeLa were incubated for 72 h at 37 °C in a humidified atmosphere and 5% CO_2.

Suspension cultures of K-562 in microplates were analysed by an electronic cell analyser system CASY 1 (SCHÄRFE, Reutlingen, Germany). The software for data evaluation CASYSTAT (SCHÄRFE) offers a fast graphical evaluation of the measurement parameters, for example, as diagrams of cell diameter distributions, overlays of different curves, and cell volume distributions. The principles of measurement and evaluation of data were described. The 0.2 ml content of each well in the microplate was diluted 1:50 with CASYTON (NaCl: 7.93 g/l; Na_2EDTA: 0.38 g/l; KCl: 0.4 g/l; $NaH_2PO_4\cdot 1H_2O$: 0.22 g/l; $NaH_2PO_4\cdot 2H_2O$: 2.45 g/l; NaF: 0.3 g/l; SCHÄRFE). Every count/ml was automatically calculated from the arithmetic mean of three successive counts of 0.4 ml each. From the dose–response curves the GI_{50} values (concentration which inhibited cell growth by 50%) were calculated with CASYSTAT. The GI_{50} value was defined as being where the concentration–response curve intersected the 50% line, determined by means of the cell counts/ml, compared to control. The essential parameters for the estimation of growth inhibition and for changes in diameter distribution curves are expressed as diagrams.

The adherent L-929 and HeLa cells were fixed by glutaraldehyde and stained with a 0.05% solution of methylene blue for 15 min. After gentle wash the stain was eluted with 0.2 ml of 0.33 N HCl in the wells. The optical densities were measured at 630 nm in SUNRISE microplate reader (TECAN). Comparisons of the different values were performed with software Magellan (TECAN).

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Synthesis and conformational analysis of naphth[1′,2′:5,6][1,3]oxazino[3,2-*c*][1,3]benzoxazine and naphth[1′,2′:5,6][1,3]oxazino[3,4-*c*][1,3]benzoxazine derivatives

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Abstract—A new functional group, the hydroxy group, was inserted into a Betti base by reaction with salicylaldehyde, and the naphthoxazine derivatives thus obtained were converted by ring-closure reactions with formaldehyde, acetaldehyde, propionaldehyde or phosgene to the corresponding naphth[1′2′:5,6][1,3]oxazino[3,2-*c*][1,3]benzoxazine derivatives. Further, the conformational analysis of these polycyclic compounds by NMR spectroscopy and an accompanying molecular modelling are reported; especially, both quantitative anisotropic ring current effects of the aromatic moieties in these compounds and steric substituent effects were employed to determine the stereochemistry of the naphthoxazinobenzoxazine derivatives.

1. Introduction

The Betti reaction is a convenient method with which to prepare α-aminobenzylnaphthol derivatives. Earlier, this three-component modified Mannich reaction with 2-naphthol, benzaldehyde and ammonia resulted in 1,3-diphenylnaphthoxazine, which on subsequent hydrolysis, gave the desired 1-α-aminobenzyl-2-naphthol (Betti base, **1**, cf. Scheme 1).[1,2] The reaction can be extended by using substituted benzaldehydes[3–5] or formaldehyde[3] instead of benzaldehyde, and 1-naphthol[6] instead of 2-naphthol.

Replacement of ammonia with chiral amines led to non-racemic *N*-substituted Betti base derivatives, which opened up a new area of application of these chiral aminonaphthols as ligands in asymmetric transformations.[7–10]

In spite of the two potentially reactive functional groups, relatively few publications have appeared in this field. In our previous studies on the ring-closing behaviour of these versatile synthons, mainly reactions with aldehydes, phosgene and oxocarboxylic acids were carried out. Through the use of salicylaldehyde, the functionalization of the Betti bases was partially resolved. In this way, trifunctional Betti base derivatives could be prepared.[11]

Our present aim was to insert a new functional group (e.g., a hydroxy group) into Betti base derivatives, and to transform the reaction products via another ring-closure reaction to naphthoxazinobenzoxazine derivatives. A further aim was the conformational analysis of these polycyclic compounds by NMR spectroscopy and an accompanying molecular modelling. This spectroscopic and theoretical study involved the quantitative determination of the anisotropic/ring current effects of the aromatic ring moieties and steric substituent effects to reveal ^{1}H chemical shift differences due to the stereochemistry of the naphthoxazinobenzoxazine derivatives.

2. Results and discussion

2.1. Syntheses

The Betti base can be functionalized with the hydroxy group in two different ways. One possibility is the reaction of Betti base **1** with salicylaldehyde (cf. Scheme 1). On NMR spectroscopic analysis in $CDCl_3$ at 300 K, however, the naphthoxazine derivative (**2**) thus obtained proved to be a three-component tautomeric mixture. The tautomeric ratios

Keywords: Conformational analysis; Naphthoxazinobenzoxazine; NMR; Ring current effect; Steric substituent effect; Molecular modelling.
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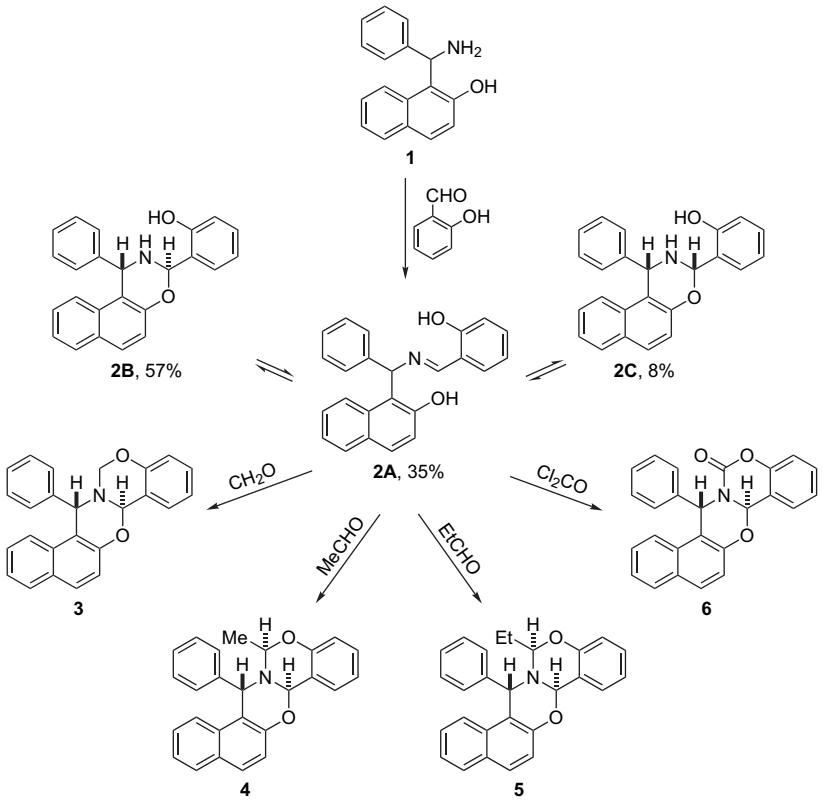


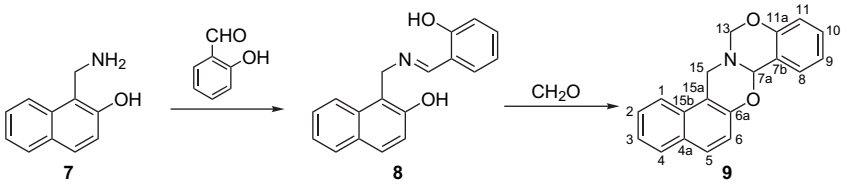
Scheme 1.

are depicted in Scheme 1. Compound **2** could be converted by the ring-closure reactions with formaldehyde,[11] acetaldehyde,[11] propionaldehyde or phosgene to the desired naphth[1′,2′:5,6][1,3]oxazino[3,2-*c*][1,3]benzoxazine derivatives **3**–**6**. The relative configurations of **3**–**6** are also depicted in Scheme 1; no minor diastereomers were detected even in the crude products. Similar high diastereoselectivity has often been observed in the analogous ring closures of aminoalcohols, and explained as a result of kinetic control governing the second ring closures of the tautomeric cyclic intermediates.[12,13]

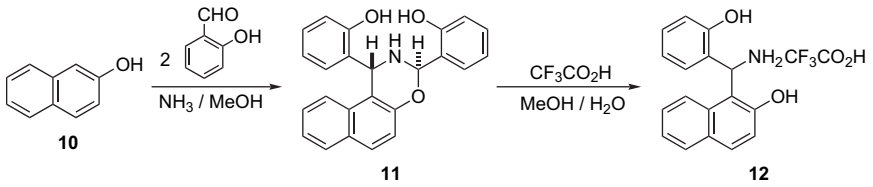
Condensation of 1-aminomethyl-2-naphthol **7** with salicylaldehyde led to the Schiff base **8**, which could be easily converted into the unsubstituted naphth[1′,2′:5,6][1,3]-oxazino[3,2-*c*][1,3]benzoxazine derivative **9** (cf. Scheme 2).[11]

For the syntheses of the analogous naphth[1′,2′:5,6]-[1,3]oxazino[3,4-*c*][1,3]benzoxazine derivatives, the *ortho*-functionalized Betti base derivative **12** was prepared from 2-naphthol **10**, salicylaldehyde and ammonia. Subsequent acidic hydrolysis, with HCl, of the initially isolated naphthoxazine derivative **11** (cf. Scheme 3) failed; however, only the acidic hydrolysis of **11** with TFA led to the desired aminobenzylnaphthol trifluoroacetate **12**. The *ortho*-functionalized Betti base derivative **12** readily decomposes; for this reason, it was used as the trifluoroacetate in the further transformations.

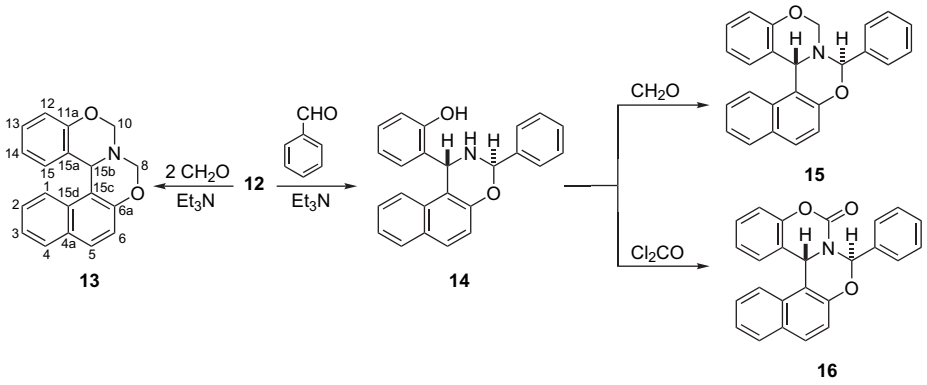
The ring-closure reaction of **12** with 2 equiv of formaldehyde led to the parent naphth[1′,2′:5,6][1,3]oxazino[3,4-*c*][1,3]benzoxazine **13** (cf. Scheme 4). For substitution of the ring system at positions 8 and 10, the aminonaphthol derivative **12** was first treated with an equivalent amount of benzaldehyde. Naphthoxazine **14** was then reacted with formaldehyde or phosgene and yielded the desired 8-phenylnaphth[1′,2′:5,6][1,3]oxazino[3,4-*c*][1,3]benzoxazine **15** and 8-phenylnaphth[1′,2′:5,6][1,3]oxazino[3,4-*c*][1,3]-benzoxazin-10-one **16**, respectively (cf. Scheme 4). NMR measurements indicated that the naphthoxazinobenzoxazine derivatives **15** and **16** were obtained with practically full stereoselectivity; relative configurations are depicted in Scheme 4.



Scheme 2.



Scheme 3.



Scheme 4.

2.2. Conformational analysis

$^{1}H/^{13}C$ NMR study of the analogous compounds **13**–**16**, corroborated by the results of parallel ab initio quantum chemical calculations, revealed that the naphtho-bound (unsaturated) oxazine ring prefers the 7,15b-*twisted-chair* conformation. Similarly, the corresponding benzo-bound (unsaturated) oxazine ring in **13** and **15** prefers the 11,15b-*twisted-chair* conformation. Ab initio calculations of all different configurations and their corresponding conformations led to local minima, but they were drastically higher in energy than the global energy minimum structures of **13**–**16** (for **13**, see Fig. 1).

In the preferred conformation, the C-15a–C-15b bond is trans to the nitrogen lone pair. In the unsubstituted derivative **13**, this conformation is proved by the NMR results: it is the only one with the possibility of 'W' coupling ($^{4}J_{H,H}$) between H-15b and H-8_{eq}, which is confirmed experimentally. The NOE enhancements given in Table 1 corroborate the present 9*S**,15b*R** configuration.

For the 8-phenyl-substituted analogue **15**, a similar global energy minimum structure was calculated. The additional phenyl substituent in position 8 is found in the *equatorial* position, as proved by NMR spectroscopy: only in this conformation is 'W' coupling possible but this time two 'W' couplings ($^{4}J_{C,H}$, C-15c–H-10_{eq}; *i*-C_{Ph}–H-15b) were found in the HMBC NMR spectra. Table 1 gives the relevant

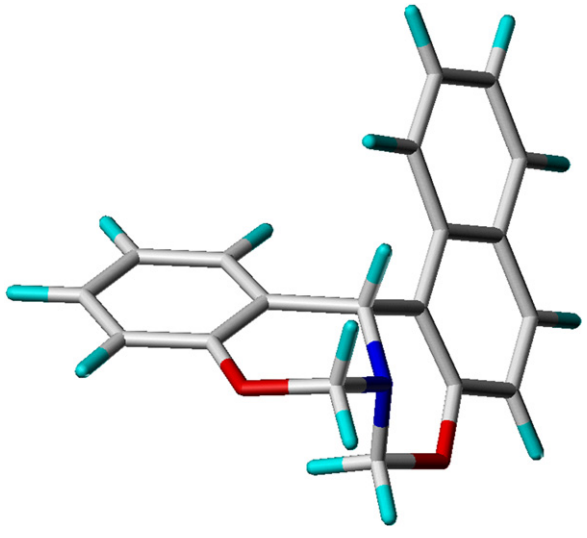


Figure 1. Global energy minimum structure of (9*S**,15b*R**)-**13**.

Table 1. Calculated distances and observed NOEs in compounds **9**, **13** and **15**

Compound	Protons studied	Distances calculated	NOEs found (+) or not detected (−)
13	H-15b···H-8_{eq}	4.094	−
	H-15b···H-8_{ax}	3.681	−
	H-15b···H-10_{ax}	2.345	+
	H-15b···H-10_{eq}	3.573	−
15	H-10_{eq}···H-8	3.215	+
	H-10_{ax}···H-8	3.825	−
	H-15b···H-10_{ax}	2.304	+
	H-15b···H-10_{eq}	3.676	−
9	H-7a···H-15_{ax}	2.624	+
	H-7a···H-15_{eq}	3.668	−
	H-13_{ax}···H-15_{ax}	3.931	−
	H-13_{eq}···H-15_{ax}	3.465	−
	H-13_{ax}···H-15_{eq}	3.035	+
	H-13_{eq}···H-15_{eq}	2.248	+

calculated distances and observed NOEs, which confirm the calculated 8*S**,9*S**,15b*R** structure of **15**.

In the carbonyl analogue **16**, two configurations (*R***S** and *R***R**) as energy minima structures were found from the ab initio calculations, differing in energy by 5.27 kcal/mol (22.06 kJ/mol). Crucial 'W' couplings are not possible in either configuration, and were not observed. In the more stable configuration (*R***S**), the rotation of the phenyl substituent is unhindered (cf. Fig. 2); in the corresponding *R***R** configuration, however, strong hindrance of this rotation by the adjacent carbonyl group is expected. A variable-temperature NMR study gave no exchange phenomena. The suggested *R***S** configuration of **16** could finally be characterized by the trans position of H-15b and H-8 with a distance of 4.007 Å in the calculated global energy minimum structure. In the corresponding isomer (*R***R**)-**16** these protons are cis-positioned, the distance is only 3.131 Å, and the corresponding NOE between these protons could not be observed. Thus, it could be concluded that **16** exists in the *R***S** configuration; the benzo-bound oxazine ring prefers an 11,15b *boat* conformation (cf. Fig. 2).

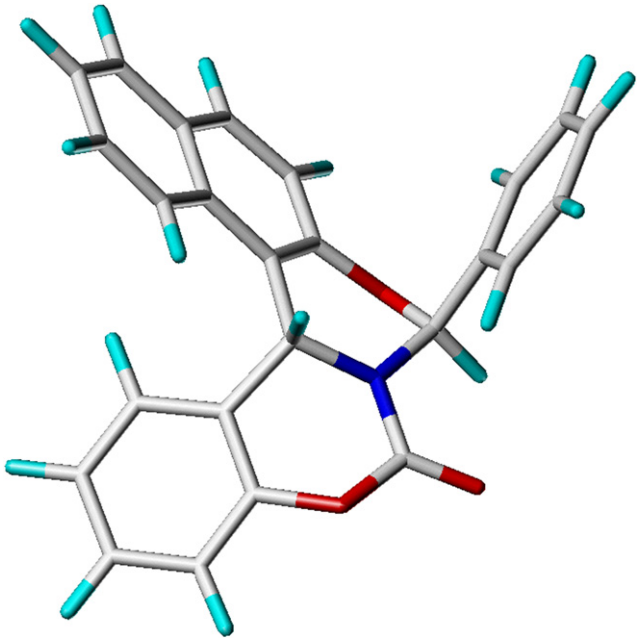


Figure 2. Global energy minimum structure of (8*S**,15b*R**)-**16**.

In the naphth[1′,2′:5,6]-[1,3]oxazino[3,2-*c*][1,3]benzoxazine derivatives **3**–**6** and **9**, the naphtho-bound oxazine ring was found to have a 7,15-*twisted-chair* conformation; with the exception of **6** the corresponding benzo-bound oxazine ring has a 7a,12-*twisted-chair* conformation. The ab initio calculated global energy minimum structure of **9** is depicted in Figure 3. The existence of this stereochemistry in **9** is unequivocally corroborated by the NMR spatial information (the NOEs found are given in Table 1). An assumed nitrogen inversion could not be confirmed; variable-temperature NMR measurements down to −100 °C did not reveal any dynamic effects.

The ab initio calculations on **3** led to the global energy minimum structure with the phenyl substituent in an *axial* position. This is proved by the NOEs between H-15 and H-13, similar to the spatial information in the previous structure: only an *equatorial* H-15 is close enough to the two H-13 atoms to furnish the NOE obtained. Thus, this compound can be described as (7a*R**, 14*S**,15*S**)-**3**.

The corresponding ab initio calculations on **4** and **5** yielded the same result: the phenyl substituent prefers the *axial* conformation. The alkyl substituents on the oxazine ring moiety, however, were *equatorial*. These methyl and ethyl conformations are corroborated by the corresponding NOEs between H-15 and these alkyl substituents (cf. Fig. 4).

Finally, in the global energy minimum structure of **6**, the phenyl substituent on C-15 was found to be *axial* (as in **4** and **5**). The *equatorial* position of the phenyl would lead to strong steric hindrance with the nearby carbonyl group. In this structure, the benzo-bound oxazine ring is nearly planar and involves almost planar nitrogen atom bonding. Supporting information from NMR spectroscopy, such as stereospecific couplings or characteristic NOEs, could not be obtained for **6** because of the strong overlapping of the relevant proton absorptions.

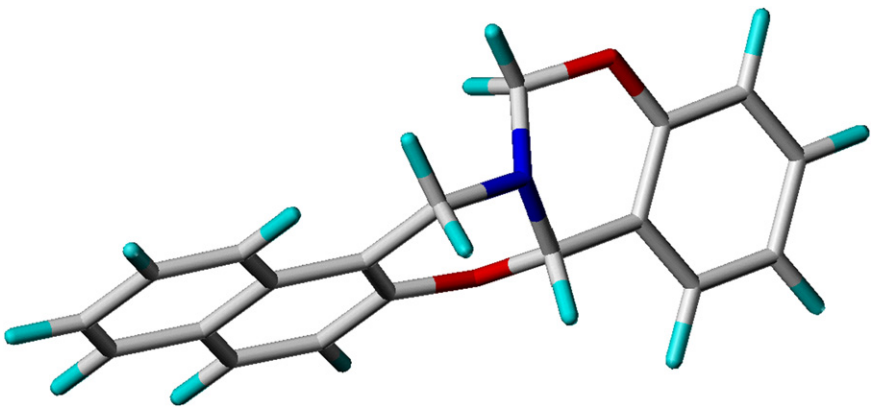


Figure 3. Global energy minimum structure of (7a*S**,14*R**)-**9**.

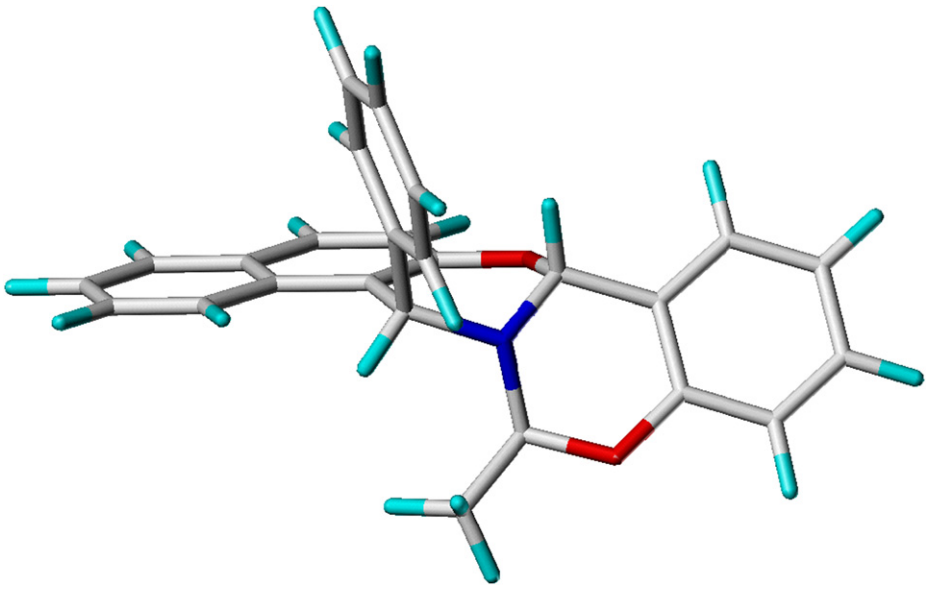


Figure 4. Global energy minimum structure of (7a*S**,13*R**,14*S**,15*S**)-**4**.

2.3. Anisotropic and steric effects

The most striking difference between the structures of the two series of compounds is the relative position of the annelated naphthalene and benzene rings. Both rings have strong ring current effects, which should mainly influence the chemical shift differences of the aromatic protons in the two series of compounds. The chemical shifts of the relevant protons are given in Table 2 together with the values calculated theoretically (i) by our method of examining only the ring current effects[14] of the aromatic moieties in the two series of compounds, and (ii) by exclusive GIAO[15] calculations.

Comparison of the experimental and theoretically calculated chemical shift differences between the relevant protons (e.g., H-15 in **13** with H-9 in **9**, etc.—cf. Table 2) demonstrates excellent agreement in both direction (high- or low-field) and amount; thus, the ^{1}H NMR spectra of corresponding compounds in the two series are correctly described by the ab initio calculations.

The coincidence between the experimental chemical shift differences and the calculated ones resulting from the present anisotropic ring current effects of both naphthyl and phenyl(s) in the corresponding molecules exclusively is less satisfactory. The sign of the differences (ring current effect influences to high- or low-field) is correct, but the values calculated in this way for the ring current effects are generally too large. Other effects on ^{1}H chemical shifts must therefore be responsible, and this was studied by a critical NBO/NCS analysis of both orbital occupations and partitions to chemical shifts (vide infra). Nevertheless, a number of conformationally relevant conclusions can be drawn.

In all three analogues examined with respect to ^{1}H chemical shift differences, δ(H-15) in series 1 is strongly high-field shifted with respect to H-8 in series 2 (cf. Figs. 1 and 3). As the reason, therefore, the ring current effect of the naphthyl moiety can readily be identified (strongly shielding H-15 in **13** with respect to the δ value of H-8 in **9**). Instead, the second proton examined, H-1 in the naphthalene moieties, is found to be low-field shifted in series 1 with respect to the position in the analogues of series 2. Both the experimental and the ring current effect on the ^{1}H chemical shifts, however, differ in size, though the direction is correctly illustrated. This is interesting, because the influence of the ring current effects on the ^{1}H chemical shifts during the stereochemical analysis usually proves to be perfect.[16] Only if strong steric hindrance is present can the ring current effect alone not explain the chemical shift differences.[14] In the pair **13**–**9**, for instance, the anisotropic ring current effect of the phenyl ring on H-1 is nearly zero; H-1 in **13** is positioned at the border between the high- and low-field ring current effects, as depicted in Figure 5.

Table 2. Experimental and calculated differences in chemical shift (δ) of selected pairs of protons in the two series of compounds studied

Compounds	H atoms	$\Delta\delta_{exp}$/ppm	$\Delta\delta_{calc}$/ppm (ring current effects)	$\Delta\delta_{calc}$/ppm (GIAO)	$\Delta\delta_{calc}$/ppm (total substituent effect of naphthyl/phenyl)
13–9	15–8	−0.45	−0.95	−0.28	−0.67
	1–1	+0.26	+0.04	+0.37	+0.09
15–3	15–8	−0.32	−0.72	−0.15	—
	1–1	+0.58	+0.51	+0.53	—
16–6	15–8	−1.0	−1.58	−0.81	−1.33
	1–1	+0.04	+0.81	−0.04	+0.20

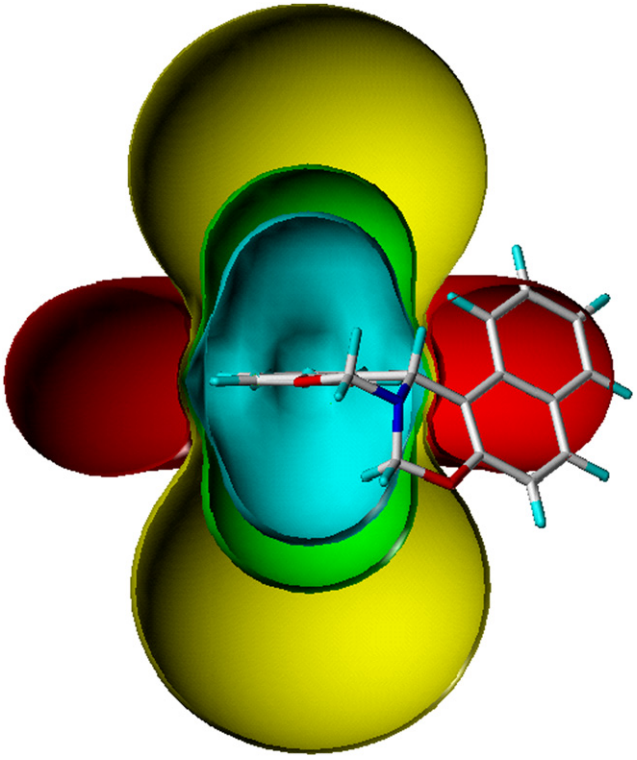


Figure 5. Ring current effect of the phenyl ring in **13**.

In order to examine the presence of steric hindrance, partially covering the ring current effect, a critical NBO/NCS analysis of the steric hindrance differences between the corresponding derivatives in the two series of compounds was performed. The results are included in Table 2; the total partitions of naphthyl/phenyl to the chemical shifts of H-15/H-8 and H-1/H-1 in **13–9** and **16–6** were examined (the effects were not studied in the comparison of **15** and **13** because of the additional phenyl substituent on C-8). These partitions summarize the overall substituent effects of the naphthyl/phenyl moieties on the corresponding ^{1}H chemical shifts (including ring current effect partitions) and are found to be in much better accordance with both the experimental and the GIAO-calculated ^{1}H chemical shifts, corroborating the correct estimations. The differences between the ring current effect and the partitions of the overall naphthyl/phenyl ring systems originate from other substituent effects, steric hindrance, and the differences between the total substituent effects and the experimental/GIAO-calculated ^{1}H chemical shifts originate from the rest of the molecules.

The present study corroborates the importance of both anisotropic ring current effects and steric effects of nearby structural moieties in determining ^{1}H chemical shift differences of otherwise similar organic compounds.

3. Experimental

3.1. General

Melting points were determined on a Kofler micro melting apparatus and are uncorrected. Merck Kieselgel 60F_{254} plates were used for TLC.

The NMR spectra were recorded in $CDCl_3$ (unless specified as DMSO-d_6) solution in 5 mm tubes, at room temperature, on a BRUKER AVANCE 500 spectrometer at 500.17 (^{1}H) and 125.78 (^{13}C) MHz, with the deuterium signal of the solvent as the lock and TMS as the internal standard for ^{1}H or the solvent as the internal standard for ^{13}C. All spectra (^{1}H, ^{13}C, *gs*-H,H-COSY, *gs*-HMQC, *gs*-1D-HMQC, *gs*-HMBC and NOESY) were acquired and processed with the standard BRUKER software.

Geometry optimizations were performed without restrictions, using the Gaussian 03[17] program package. Density functional theory calculations were carried out at the B3LYP/6-31G**[18,19] level of theory. Different starting conformations were created and the results were analyzed and displayed by using the molecular modelling program SYBYL7.0.[20] Different local energy minima conformations were selected to analyze the relative stability and the geometrical parameter.

The ring current effects were calculated with the GIAO method at the HF/6-31G* level of theory, based on the calculation of NICS.[21] The studied molecules were placed at the centre of a grid ranging from −10 to 10 Å (step width 0.5 Å), resulting in a cube with 68921 lattice points. At these lattice points the magnetic shielding was calculated, and the values were transformed into SYBYL[20] contour files and displayed.

The anisotropy values in Table 2 result from NICS calculations. The ring current effects of all aromatic moieties at the positions of the studied atoms were added. Table 2 contains the differences in these values of the corresponding atoms in these pairs of molecules. Chemical shifts were calculated at the B3LYP/6-31G** level of theory, using the GIAO method.

Various platforms were used for the calculations, e.g., SGI Octane, SGI Origin workstations or Linux cluster.

Compounds **1**,[3] **2**,[11] **3**,[11] **4**,[11] **7**[3] and **8**[11] were prepared according to procedures known in the literature.

During our investigations, the assignments of the chemical shifts of **3** and **4**, which were given in Ref. 11, had to be revised. For this reason, both assignments are given, with the values obtained above. *Compound* ***3***: ^{1}H NMR δ 7.76 (m, 2H, H-4 and H-5), 7.32 (m, 10H, H-1, H-2, H-3, H-8, H-10 and Ph), 7.13 (d, J=8.9 Hz, 1H, H-6), 6.97 (m, 2H, H-9 and H-11), 5.61 (s, 1H, H-7a), 5.53 (s, 1H, H-15), 4.96 (d, J=−7.1 Hz, 1H, H-13) and 4.92 (d, J=−7.1 Hz, 1H, H-13); ^{13}C NMR δ 153.1 (C-11a), 150.5 (C-6a), 141.3 (*i*-Ph), 131.8 (C-15b), 130.6 (C-10), 129.7 (C-5), 129.2 (*o*-Ph), 129.0 (2C, C-4a, C-8), 128.6 (C-4), 128.4 (*m*-Ph), 127.7 (*p*-Ph or C-2), 126.8 (C-2 or *p*-Ph), 123.5 (C-3 or C-1), 122.6 (C-1 or C-3), 121.1 (C-9), 120.1 (C-7b), 119.9 (C-11), 118.9 (C-6), 111.1 (C-15a), 78.5 (C-7a, $^1J_{C,H}$= 167.4 Hz), 77.7 (C-13, $^1J_{C,H}$=158.6, 155.1 Hz) and 57.7 (C-15, $^1J_{C,H}$=136.2 Hz). *Compound* ***4***: ^{1}H NMR δ 7.79 (d, J=7.4 Hz, 1H, H-4), 7.76 (d, J=9.0 Hz, 1H, H-5), 7.41 (d, J=7.8 Hz, 1H, H-1), 7.34 (m, 2H, H-2 and H-3), 7.28 (m, 7H, H-8, H-10 and Ph), 7.12 (d, J=8.9 Hz, 1H, H-6), 6.95 (dt, J=7.5, 0.9 Hz, 1H, H-9), 6.91 (d, J=8.0 Hz, 1H,

H-11), 5.84 (s, 1H, H-15), 5.64 (s, 1H, H-7a), 5.10 (q, J=5.5 Hz, 1H, H-13) and 1.73 (d, J=5.6 Hz, 3H, Me); ^{13}C NMR δ 153.5 (C-11a), 151.2 (C-6a), 141.6 (*i*-Ph), 131.8 (C-15b), 130.4 (C-10), 129.7 (C-5), 129.2 (*o*-Ph), 128.9 (C-4a), 128.7 (2C, C-4, C-8), 128.4 (*m*-Ph), 127.5 (*p*-Ph), 127.0 (C-2), 123.5 (C-3), 122.1 (C-1), 120.8 (C-9), 120.0 (C-7b), 118.9 (C-6), 116.6 (C-11), 110.7 (C-15a), 80.9 (C-13, $^{1}J_{C,H}$=155.8 Hz), 79.2 (C-7a, $^{1}J_{C,H}$=166.9 Hz), 54.4 (C-15, $^{1}J_{C,H}$=137.2 Hz) and 19.6 (Me).

3.1.1. (7a*R,13*R**,15*S**)-13-Ethyl-15-phenylnaphth[1′,2′:5,6][1,3]oxazino[3,2-*c*][1,3]benzoxazine (5).** A mixture of compound **2** (0.50 g, 1.41 mmol) and propionaldehyde (0.13 g, 2.12 mmol) was stirred in $CHCl_3$ (25 mL) at room temperature until the TLC revealed no more starting material (20 h). The solvent was then removed under reduced pressure (the NMR spectra of the crude product showed no minor diastereomers) and the residue was crystallized from *n*-hexane–Et_2O (2:1), and recrystallized from *n*-hexane–*i*-Pr_2O (1:1). Yield: 0.41 g (74%). Mp 229–231 °C. ^{1}H NMR δ 7.79 (d, J=7.6 Hz, 1H, H-4), 7.78 (d, J=9.0 Hz, 1H, H-5), 7.44 (d, J=8.2 Hz, 1H, H-1), 7.35 (m, 2H, H-2 and H-3), 7.29 (m, 7H, H-8, H-10 and Ph), 7.12 (d, J=8.9 Hz, 1H, H-6), 6.95 (t, J=7.5 Hz, 1H, H-9), 6.93 (d, J=8.4 Hz, 1H, H-11), 5.86 (s, 1H, H-15), 5.65 (s, 1H, H-7a), 4.91 (t, J=5.0 Hz, 1H, H-13), 2.03 (m, 2H, CH_2) and 1.21 (t, J=7.3 Hz, 3H, Me); ^{13}C NMR δ 153.6 (C-11a), 151.2 (C-6a), 141.6 (*i*-Ph), 131.9 (C-15b), 130.4 (C-10), 129.7 (C-5), 129.2 (2C, *o*-Ph), 128.8 (C-4a), 128.7 (2C, C-4 and C-8), 128.4 (2C, *m*-Ph), 127.5 (*p*-Ph or C-3), 127.0 (C-2), 123.5 (C-3 or *p*-Ph), 122.0 (C-1), 120.7 (C-9), 119.9 (C-7b), 118.9 (C-6), 116.6 (C-11), 110.9 (C-15a), 84.3 (C-13, $^{1}J_{C,H}$=155.5 Hz), 79.6 (C-7a, $^{1}J_{C,H}$=167.3 Hz), 53.9 (C-15, $^{1}J_{C,H}$=137.3 Hz), 25.2 (CH_2) and 8.0 (Me). Anal. Calcd for $C_{27}H_{23}NO_2$: C, 82.42; H, 5.89; N, 3.56. Found: C, 83.03; H, 5.92; N, 3.62.

3.1.2. (7a*R,15*S**)-15-Phenylnaphth[1′,2′:5,6][1,3]oxazino[3,2-*c*][1,3]benzoxazin-13-one (6).** Naphthoxazine **2** (0.40 g, 1.13 mmol) was suspended in toluene–H_2O (20:20 mL), and Et_3N (0.25 g, 2.5 mmol) and phosgene (2.2 mL; 20% in toluene, 2.26 mmol) were added. The mixture was stirred at room temperature for 15 h, and EtOAc (40 mL) and H_2O (40 mL) were then added. The organic layer was separated, dried (Na_2SO_4) and evaporated. The oily residue crystallized on treatment with *n*-hexane (20 mL). The crystalline product was filtered off and recrystallized from *n*-hexane–*i*-Pr_2O (1:1). Yield: 0.28 g (65%). Mp 256–259 °C. ^{1}H NMR δ 7.79 (m, 2H, H-4 and H-5), 7.44 (m, 5H, H-1, H-8, H-10 and *o*-Ph), 7.33 (m, 5H, H-2, H-3, *m*- and *p*-Ph), 7.28 (s, 1H, H-15), 7.23 (t, J=7.5 Hz, 1H, H-9), 7.14 (d, J=9.0 Hz, 1H, H-6), 7.13 (d, J=8.0 Hz, 1H, H-11) and 6.25 (s, 1H, H-7a); ^{13}C NMR δ 151.0 (C-6a), 149.2 (C-11a), 148.3 (C-13), 140.0 (*i*-Ph), 131.5 (C-10), 131.2 (C-15b), 130.2 (C-5), 129.5 (C-4a), 128.9 (4C, *o*- and *m*-Ph), 128.6 (2C, C-4 and *p*-Ph), 127.8 (C-8), 127.1 (C-2), 124.7 (C-9), 124.2 (C-3), 123.4 (C-1), 118.2 (C-6), 116.4 (C-11), 115.1 (C-7b), 112.1 (C-15a), 76.7 (C-7a, $^{1}J_{C,H}$=167.2 Hz) and 54.8 (C-15, $^{1}J_{C,H}$=144.0 Hz). Anal. Calcd for $C_{25}H_{17}NO_3$: C, 79.14; H, 4.52; N, 3.69. Found: C, 79.85; H, 4.61; N, 3.65.

3.1.3. Naphth[1′,2′:5,6][1,3]oxazino[3,2-*c*][1,3]benzoxazine (9). To a solution of **8** (0.4 g, 1.44 mmol) in $CHCl_3$ (30 mL), 40% aqueous formaldehyde (0.5 mL) was added. The mixture was stirred at room temperature for 4 h, during which the TLC revealed no more starting material. The solvent was evaporated off at reduced pressure, and the residue was crystallized with *n*-hexane and recrystallized from *n*-hexane–*i*-Pr_2O (3:1). Yield: 0.30 g (72%). Mp 176–177 °C. ^{1}H NMR δ 7.78 (d, J=8.1 Hz, 1H, H-4), 7.67 (d, J=8.9 Hz, 1H, H-5), 7.62 (d, J=8.4 Hz, 1H, H-1), 7.51 (t, J=7.6 Hz, 1H, H-2), 7.42 (d, J=7.2 Hz, 1H, H-8), 7.38 (t, J=7.2 Hz, 1H, H-3), 7.32 (t, J=7.9 Hz, 1H, H-10), 7.08 (d, J=8.9 Hz, 1H, H-6), 7.04 (t, J=7.4 Hz, 1H, H-9), 6.96 (d, J=8.2 Hz, 1H, H-11), 5.78 (s, 1H, H-7a), 5.05 (d, J=−7.2 Hz, 1H, H-13), 4.85 (d, J=−17.2 Hz, 1H, H-15$_{ax}$), 4.73 (d, J=−7.2 Hz, 1H, H-13) and 4.29 (d, J=−17.2 Hz, 1H, H-15$_{eq}$); ^{13}C NMR δ 153.2 (C-11a), 150.2 (C-6a), 130.9 (C-15b), 130.6 (C-10), 129.0 (C-4a), 128.7 (2C, C-4 and C-8), 128.6 (C-5), 126.8 (C-2), 123.8 (C-3), 121.2 (C-9), 120.9 (C-1), 119.9 (C-7b), 118.8 (C-6), 116.9 (C-11), 111.0 (C-15a), 82.5 (C-7a, $^{1}J_{C,H}$=165.4 Hz), 77.5 (C-13, $^{1}J_{C,H}$=158.6, 155.5 Hz) and 45.6 (C-15, $^{1}J_{C,H(eq)}$=136.0 Hz, $^{1}J_{C,H(ax)}$=141.5 Hz). Anal. Calcd for $C_{19}H_{15}NO_2$: C, 78.87; H, 5.23; N, 4.84. Found: C, 78.76; H, 5.26; N, 4.86.

3.1.4. 1,3-Di(2-hydroxyphenyl)-2,3-dihydro-1*H*-naphth[1,2-*c*][1,3]oxazine (11). To a solution of 2-naphthol (**10**, 5.77 g, 40 mmol) in MeOH (80 mL) was added the appropriate aromatic salicylaldehyde (9.76 g, 80 mmol) and 20% methanolic ammonia solution (10 mL). The mixture was left to stand at ambient temperature for two days, during which a yellow crystalline product (**11**) separated out. The crystals were filtered off and washed with cold MeOH (2×40 mL). Yield: 8.8 g (60%). Mp 138–141 °C. The NMR spectra showed a mixture of ring (r) and chain (c) tautomers with an approximate ratio of 0.25:1. ^{1}H NMR (DMSO-d_6): δ 10.26 (br), 8.80 (s, 1H, N=CH[c]), 8.03 (d, J=7.3 Hz, 1H), 7.93 (m, 2.5H), 6.66–7.48 (m), 5.97 (s, 0.5H, H-1r and H-3r) and 4.52 (s, 1.25H, OH/NH); ^{13}C NMR (DMSO-d_6): δ 163.5 (N=CH), 160.9, 160.6, 155.3, 154.8, 154.0, 136.7, 132.9, 132.4, 132.2, 130.8, 130.3, 129.7, 129.5, 128.9, 128.7, 128.6, 128.5, 127.4, 126.6, 126.2, 123.6, 123.3, 122.6, 122.4, 119.7, 119.4, 119.2, 119.0, 118.7, 117.9, 117.4, 116.9, 115.6, 78.8 (C-3r), 64.2 (CH–N[c]) and 48.5 (C-1r). Anal. Calcd for $C_{24}H_{19}NO_3$: C, 78.03; H, 5.18; N, 3.79. Found: C, 77.96; H, 5.21; N, 3.81.

3.1.5. 1-α-Amino(2-hydroxyphenyl)methyl-2-naphthol trifluoroacetate (12). Naphthoxazine **11** (5 g, 10.8 mmol) was suspended in MeOH (60 mL), and H_2O (60 mL) and TFA (10 mL) were added. The mixture was stirred at 80 °C for 5 h. The solvents were evaporated off and the residue was crystallized from EtOAc. Yield: 3.84 g (93%). Mp 176–179 °C. ^{1}H NMR δ 11.04, 10.55, 8.51 (3br s, 5H, OH, NH), 7.94 (d, J=8.6 Hz, 1H, H-8), 7.87 (d, J=8.9 Hz, 1H, H-4), 7.85 (d, J=8.3 Hz, 1H, H-5), 7.48 (dt, J=7.6, 1.3 Hz, H-7), 7.32 (m, 3H, H-3, H-6 and H-6′), 7.17 (dt, J=7.7, 1.6 Hz, H-4′), 6.95 (dd, J=8.1, 0.9 Hz, H-3′), 6.75 (dt, J=7.5, 1.0 Hz, H-5′) and 6.38 (s, 1H, Ar-CH); ^{13}C NMR δ 158.0 (q, $^{2}J_{C,F}$=31.0 Hz, COOH), 154.8 (C-2′), 153.6 (C-2), 131.9 (C-8a), 130.4 (C-4), 129.7 (C-4′), 129.0 (C-6′), 128.6 (C-5), 128.1 (C-4a), 127.1 (C-7), 123.0 (C-6), 122.9 (C-1′), 121.7 (C-8), 119.0 (C-5′), 118.6 (C-3),

117.3 (q, $^1J_{C,F}$=300.2 Hz, CF_3), 115.4 (C-3′), 113.5 (C-1) and 46.6 (CH–NH_2). Anal. Calcd for $C_{19}H_{16}F_3NO_4$: C, 60.16; H, 4.25; N, 3.69. Found: C, 60.32; H, 4.28; N, 3.71.

3.1.6. Naphth[1′,2′:5,6][1,3]oxazino[3,4-*c*][1,3]benzoxazine (13). A mixture of compound **12** (0.4 g, 1.05 mmol), Et_3N (0.12 g, 1.16 mmol) and 40% formaldehyde solution (0.18 g) in MeOH (25 mL) was stirred at room temperature for 24 h. The white precipitate that formed was filtered off and recrystallized from MeOH. Yield: 0.24 g (79%). Mp 109–112 °C. ^{1}H NMR δ 7.88 (d, J=8.5 Hz, 1H, H-1), 7.82 (d, J=8.1 Hz, 1H, H-4), 7.74 (d, J=8.8 Hz, 1H, H-5), 7.53 (t, J=8.3 Hz, 1H, H-2), 7.40 (t, J=7.1 Hz, 1H, H-3), 7.13 (t, J=7.7 Hz, 1H, H-13), 7.11 (d, J=8.9 Hz, 1H, H-6), 6.97 (d, J=7.8 Hz, 1H, H-15), 6.80 (dd, J=8.2, 0.8 Hz, 1H, H-12), 6.68 (t, J=7.7 Hz, 1H, H-14), 5.94 (s, 1H, H-15b), 5.51 (d, J=−10.7 Hz, 1H, H-10_{ax}), 5.14 (d, J=−10.7 Hz, 1H, H-10_{eq}), 5.04 (d, J=−7.6 Hz, 1H, H8_{ax}) and 4.97 (dd, J=−7.6, 1.1 Hz, 1H, H8_{eq}); ^{13}C NMR δ 151.5 (C-11a), 150.2 (C-6a), 132.7 (C-15d), 129.7 (C-5), 129.3 (C-15), 129.0 (C-4a), 128.8 (C-4 or C-13), 128.6 (C-13 or C-4), 126.9 (C-2), 123.6 (C-3), 122.8 (C-1), 122.3 (C-15a), 120.6 (C-14), 118.7 (C-6), 116.5 (C-12), 114.2 (C-15c), 80.5 (C-10, $^1J_{C,H(eq)}$=179.8 Hz, $^1J_{C,H(ax)}$=181.0 Hz), 76.9 (C-8, $^1J_{C,H(eq)}$=157.9 Hz, $^1J_{C,H(ax)}$=155.0 Hz) and 52.5 (C-15b). Anal. Calcd for $C_{19}H_{15}NO_2$: C, 78.87; H, 5.23; N, 4.84. Found: C, 78.97; H, 5.31; N, 4.79.

3.1.7. 1-(2-Hydroxyphenyl)-3-phenyl-2,3-dihydro-1*H*-naphth[1,2-*c*][1,3]oxazine (14). A mixture of compound **12** (1 g, 2.64 mmol), Et_3N (0.3 g, 3.0 mmol) and benzaldehyde (0.28 g, 2.64 mmol) in MeOH (25 mL) was stirred at room temperature for 24 h. The white precipitate that separated out was filtered off and washed with MeOH. Yield: 0.87 g (93%). Mp 190–193 °C. ^{1}H NMR δ 7.81 (m, 2H, H-6 and H-7), 7.54 (d, J=6.8 Hz, 2H, H-2″), 7.40 (m, 6H, H-8, H-9, H-10, H-3″ and H-4″), 7.22 (m, 2H, H-5, H-4′), 7.03 (d, J=8.1 Hz, 1H, H-3′), 6.67 (t, J=7.7 Hz, 1H, H-5′), 6.62 (d, J=7.6 Hz, 1H, H-6′), 5.90 (s, 1H, H-1) and 5.81 (s, 1H, H-3); ^{13}C NMR δ 156.1 (C-2′), 151.7 (C-4a), 138.0 (C-1″), 131.8 (C-10a), 131.0 (C-6′), 129.9 (C-6), 129.5 (C-4′), 128.9 (C-4″), 128.8 (C-6a), 128.6 (3C, C-7 and C-3″), 127.2 (C-9), 125.9 (C-2″), 124.9 (C-1′), 123.8 (C-8), 122.7 (C-10), 119.8 (C-5′), 119.2 (C-5), 117.5 (C-3′), 112.6 (C-10b), 81.7 (C-3) and 52.5 (C-1). Anal. Calcd for $C_{24}H_{19}NO_2$: C, 81.56; H, 5.42; N, 3.96. Found: C, 81.72; H, 5.45; N, 3.95.

3.1.8. (8*R,15b*S**)-8-Phenylnaphth[1′,2′:5,6][1,3]oxazino[3,4-*c*][1,3]benzoxazine (15).** To a solution of **14** (0.4 g, 1.1 mmol) in $CHCl_3$ (30 mL), 40% aqueous formaldehyde (0.5 mL) was added. The mixture was stirred at room temperature for 4 h, during which the TLC revealed no more starting material. The solvent was evaporated off at reduced pressure, and the residue was crystallized with Et_2O and recrystallized from i-Pr_2O (30 mL). Yield: 0.38 g (94%). Mp 216–219 °C. ^{1}H NMR δ 7.90 (d, J=8.4 Hz, 1H, H-1), 7.84 (d, J=8.0 Hz, 1H, H-4), 7.76 (d, J=8.9 Hz, 1H, H-5), 7.61 (m, 2H, *o*-Ph), 7.54 (t, J=8.2 Hz, 1H, H-2), 7.47 (m, 3H, *m*- and *p*-Ph), 7.41 (t, J=8.0 Hz, 1H, H-3), 7.17 (t, J=7.3 Hz, 1H, H-13), 7.14 (d, J=8.9 Hz, 1H, H-6), 7.00 (d, J=7.7 Hz, 1H, H-15), 6.86 (dd, J=8.2, 1.1 Hz, 1H, H-12), 6.72 (t, J=7.7 Hz, 1H, H-14), 6.13 (s, 1H, H-15b), 5.92 (s, 1H, H-8), 5.26 (d, J=−10.8 Hz, 1H, H-10_{ax}) and 4.61 (d, J=−10.8 Hz, 1H, H-10_{eq}); ^{13}C NMR δ 152.4 (C-11a), 150.9 (C-6a), 136.3 (*i*-Ph), 132.6 (C-15d), 129.9 (*p*-Ph), 129.7 (C-5), 129.2 (C-15), 129.0 (C-4a), 128.9 (C-13), 128.7 (3C, C-4 and *m*-Ph), 128.4 (*o*-Ph), 127.0 (C-2), 123.6 (C-3), 122.8 (C-1), 122.7 (C-15a), 120.6 (C-14), 118.7 (C-6), 116.4 (C-12), 113.9 (C-15c), 79.4 (C-10, $^1J_{C,H(eq)}$=164.0 Hz, $^1J_{C,H(ax)}$=160.7 Hz), 86.2 (C-8, $^1J_{C,H}$=157.2 Hz) and 54.2 (C-15b, $^1J_{C,H}$=141.4 Hz). Anal. Calcd for $C_{25}H_{19}NO_2$: C, 82.17; H, 5.24; N, 3.83. Found: C, 82.25; H, 5.26; N, 3.85.

3.1.9. (8*R,15b*S**)-8-Phenylnaphth[1′,2′:5,6][1,3]oxazino[3,4-*c*][1,3]benzoxazin-10-one (16).** Naphthoxazine **14** (0.40 g, 1.13 mmol) was suspended in toluene–H_2O (20:20 mL), and Et_3N (0.25 g, 2.5 mmol) and phosgene (2.2 mL; 20% in toluene, 2.26 mmol) were added. The mixture was stirred at room temperature for 72 h, and EtOAc (40 mL) and H_2O (40 mL) were then added. The organic layer was separated, dried (Na_2SO_4) and evaporated. The oily residue crystallized on treatment with Et_2O (20 mL). The crystalline product was filtered off and recrystallized from i-Pr_2O (15 mL). Yield: 0.25 g (58%). Mp 270–272 °C. ^{1}H NMR δ 7.81 (d, J=7.0 Hz, 1H, H-4), 7.78 (d, J=9.3 Hz, 1H, H-5), 7.48 (d, J=7.2 Hz, 1H, H-1), 7.41 (m, 4H, H-2, H-3 and *o*-Ph), 7.29 (m, 6H, H-6, H-12, H-13, *m*- and *p*-Ph), 7.15 (s, 1H, H-8), 6.88 (t, J=7.0 Hz, 1H, H-14), 6.44 (d, J=7.8 Hz, 1H, H-15) and 6.36 (s, 1H, H-15b); ^{13}C NMR δ 152.3 (C-10), 151.7 (C-11a), 150.8 (C-6a), 136.0 (*i*-Ph), 131.1 (C-15d), 130.3 (C-5), 129.7 (C-4a), 129.3 (C-13), 128.9 (C-4), 128.8 (*m*-Ph), 128.5 (*p*-Ph), 127.1 (C-2), 126.1 (3C, C-15, *o*-Ph), 124.6 (C-3), 124.4 (C-14), 123.4 (C-15a), 122.7 (C-1), 120.2 (C-6), 116.7 (C-12), 111.8 (C-15c), 81.7 (C-8, $^1J_{C,H}$=164.4 Hz) and 48.9 (C-15b, $^1J_{C,H}$=143.4 Hz). Anal. Calcd for $C_{25}H_{17}NO_3$: C, 79.14; H, 4.52; N, 3.69. Found: C, 79.26; H, 4.55; N, 3.71.

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Stereoselective titanium-mediated aldol reactions of (*S*)-2-*tert*-butyldimethylsilyloxy-3-pentanone

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Abstract—Titanium-mediated aldol reactions based on (*S*)-2-*tert*-butyldimethylsilyloxy-3-pentanone, a lactate-derived chiral ketone, provide the corresponding 2,4-*syn*-4,5-*syn* adducts in high yields and diastereomeric ratios with a wide array of achiral and chiral aldehydes. Furthermore, spectroscopic studies of intermediates involved in the process have permitted to propose a mechanism that accounts for the experimental results.

1. Introduction

Aldol reactions involving chiral ketones represent one of the most efficient and versatile methodologies for the stereoselective construction of carbon–carbon bonds.[1] Particularly, pioneering studies by Heathcock et al.[2] and Masamune et al.[3] earlier revealed the synthetic potentiality of α-hydroxy ketones such as **1** and **2** (Fig. 1) and paved the way for the development of a plethora of asymmetric processes based on the reactivity of lithium and boron enolates.[4–8] This chemistry was significantly enriched by Evans discovery that tetrachlorotitanium enolates, generated directly from ketones, participate in highly selective aldol reactions.[9] In the case of ketone **2**, the stereoselectivity of the titanium-mediated aldol reaction was found to be comparable to that reported for the analogous boron-mediated process.[9b] Unfortunately, most of these approaches were bound to chiral ketones containing bulky groups (*tert*-butyl and cyclohexyl in **1** and **2**, respectively, in Fig. 1), which limited their applicability in synthesis. However, Paterson nicely established that the aforementioned steric requirements are not essential and that dicyclohexylborinates from lactate-derived ketones **3** and **4** (Fig. 1) furnish highly stereoselective aldol transformations provided that the hydroxyl protecting group and the enolization procedures are suitably chosen.[10–12] Then, the corresponding aldol adducts can be manipulated to give access to a wide range of molecular architectures present in natural polyoxygenated metabolites.[13] More recently, Denmark has disclosed highly stereoselective aldol processes involving trichlorosilyl enolates from ketone **5** (Fig. 1) and chiral phosphoramides, having proved that the α-chiral center rules the stereochemical outcome of the aldol addition.[14]

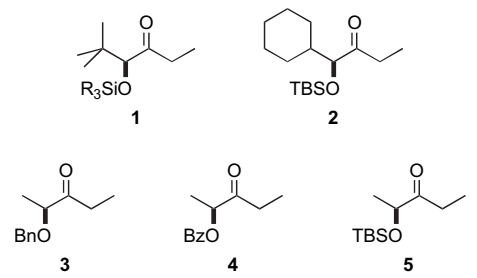

Figure 1.

These findings provided us the impetus for launching several years ago a project devoted to the study of aldol reactions based on chiral α-hydroxy ketones[15] and the application of the resulting adducts to the synthesis of polypropionate motifs embedded in natural products. Taking advantage of the high reactivity displayed by titanium enolates, we have developed highly stereoselective aldol reactions based on protected lactate-derived ketones.[16–18] Herein, we disclose an efficient titanium-mediated aldol methodology based on (*S*)-2-*tert*-butyldimethylsilyloxy-3-pentanone (**5**).[19]

2. Results and discussion

(*S*)-2-*tert*-Butyldimethylsilyloxy-3-pentanone (**5**) can be prepared through acylation of EtM (M=Li, MgBr) organometallic species with amides derived from commercially available (*S*) lactate esters. Remarkably, enantiomerically pure ketone **5** is routinely prepared in multigram scale

Keywords: Aldol reactions; Asymmetric reactions; Titanium enolates; Chiral ketone.
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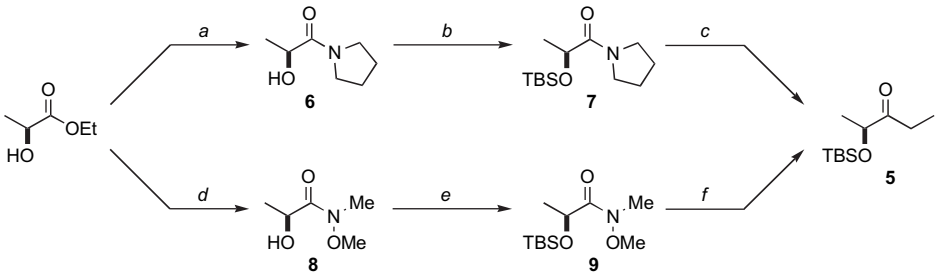


Scheme 1. Reagents and conditions: (a) pyrrolidine, rt; (b) TBSCl, Et_3N, cat DMAP, rt, THF, 94%; (c) EtLi, −78 °C, THF, 84%; (d) MeONHMe·HCl, *i*-PrMgCl, 76%; (e) TBSCl, Et_3N, cat DMAP, rt, THF, 97%; (f) EtMgBr, 0 °C, THF, 92%.

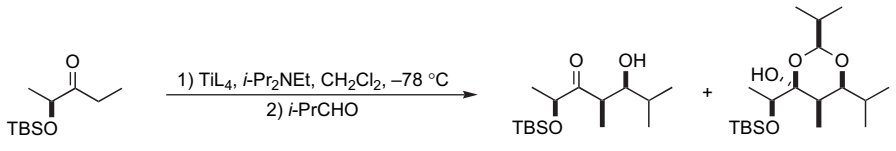
following a synthetic sequence based on *N*-acyl pyrrolidine **6** as shown in Scheme 1. Indeed, the high nucleophilicity of the pyrrolidine permits to obtain quantitatively the hydroxy amide **6** in a free solvent process at rt; then, the crude reaction mixture is submitted to standard silylation conditions and, finally, the resulting silyloxy amide **7** is treated with EtLi to afford the desired ketone **5** in high overall yield (78%).[15c] Alternatively, it can be also prepared in good yields (68%) via Weinreb amide **8**[10] (Scheme 1).[20] Eventually, the corresponding enantiomer, (*R*)-2-*tert*-butyldimethylsilyloxy-3-pentanone (*ent*-**5**), can be likewise obtained from the commercially available (*R*) isobutyl lactate.[15c]

With a straightforward and reliable supply of the required ketone **5** in hand, we began the study of the titanium-mediated aldol reactions with a survey of different Lewis acids. Taking advantage of the experimental procedure reported by Evans, we initially carried out the enolization with several titanium Lewis acids (1.1 equiv) and *i*-Pr_2NEt (1.1 equiv) for 1.5 h at −78 °C and the resulting enolate was allowed to react with isobutyraldehyde (1.2 equiv) for 2 h at the same temperature. The results are summarized in Table 1.

As expected, the reactivity of the titanium Lewis acids decreases as the number of alkoxy groups bound to the metal increases, to the point that ketone **5** can be recovered unaltered when Ti(*i*-PrO)$_3$Cl is used (see entry 5 in Table 1). Interestingly, all the other Lewis acids (see entries 1–4 in Table 1) afforded aldol **10a** as a single diastereomer (dr 97:3 by ^{1}H NMR), which proved that bulky groups on the ketone are not required to achieve highly diastereoselective processes. We were particularly entrusted with $TiCl_4$ and Ti(*i*-PrO)Cl_3, since they could be easily handled and afforded good yields. Further optimization of the process involving both Lewis acids revealed that the enolization as well as the reaction time were pretty fast and could be reduced to 30 min (compare entries 2, 6, and 8 and 3, 7, and 9, respectively).[21] Additionally, we observed the formation of variable amounts (5–10%) of hemiacetal **11a**, which was presumably responsible that some of the abovementioned reactions did not go to completion satisfactorily. Thus, slightly higher and more reproducible yields were obtained using 1.5 equiv of isobutyraldehyde (see entries 10 and 11 in Table 1), although we are aware that good yields can also be achieved with 1.2 equiv.[22]

Next, these experimental procedures were successfully generalized to other aliphatic, aromatic, and α,β-unsaturated aldehydes. As results summarized in Table 2 prove, the corresponding 2,4-*syn*-4,5-*syn* aldols **10** were obtained in high yields and with excellent diastereomeric ratios for a wide array of aliphatic aldehydes, even in the case of sterically undemanding acetaldehyde (see entries 4 and 13 in Table 2). Remarkably, Ti(*i*-PrO)Cl_3-mediated aldol reactions afforded slightly better diastereomeric ratios than the corresponding $TiCl_4$ counterparts, being in most cases higher than 95:5.

Table 1. Titanium-mediated aldol reaction of ketone **5** with isobutyraldehyde

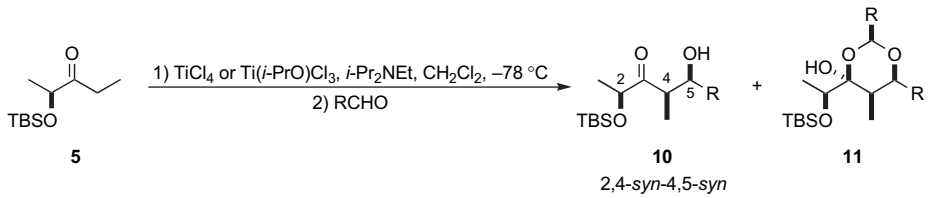


5 **10a** **11a**

Entry	Lewis acid	Enolization time (h)	Reaction time (h)	Aldehyde equivalents	Yield of **10** (%)[a]
1	$TiBr_4$	1.5	2	1.2	52 (17)
2	$TiCl_4$	1.5	2	1.2	83 (5)
3	Ti(*i*-PrO)Cl_3	1.5	2	1.2	76 (14)
4	Ti(*i*-PrO)$_2Cl_2$	1.5	2	1.2	35 (50)
5	Ti(*i*-PrO)$_3$Cl	1.5	2	1.2	— (90)
6	$TiCl_4$	0.5	2	1.2	80 (<5)
7	Ti(*i*-PrO)Cl_3	0.5	2	1.2	74 (15)
8	$TiCl_4$	0.5	0.5	1.2	80 (6)
9	Ti(*i*-PrO)Cl_3	0.5	0.5	1.2	80 (10)
10	$TiCl_4$	0.5	0.5	1.5	85 (—)
11	Ti(*i*-PrO)Cl_3	0.5	0.5	1.5	85 (<5)

[a] Isolated yield. In parentheses are the isolated yields of unreacted ketone.

Table 2. $TiCl_4$- and Ti(*i*-PrO)Cl_3-mediated aldol reactions of ketone **5**



Entry	Lewis acid	Aldehyde	R	dr[a]	Yield **10** (%)[b]
1	$TiCl_4$	**a**	$(CH_3)_2CH$	97:3	85 (82)[c]
2	$TiCl_4$	**b**	$(CH_3)_2CHCH_2$	95:5	81
3	$TiCl_4$	**c**	$CH_3CH_2CH_2$	95:5	85
4	$TiCl_4$	**d**	CH_3	91:9	58[d]
5	$TiCl_4$	**e**	Ph	93:7[e]	82
6	$TiCl_4$	**f**	4-NO_2Ph	95:5[e]	82
7	$TiCl_4$	**g**	4-MeOPh	92:8[e]	80[f]
8	$TiCl_4$	**h**	H_2C=$CH(CH_3)$	97:3	85 (90)[g]
9	$TiCl_4$	**i**	(*E*) CH_3CH=CH	96:4	80
10	Ti(*i*-PrO)Cl_3	**a**	$(CH_3)_2CH$	97:3	85 (85)[c]
11	Ti(*i*-PrO)Cl_3	**b**	$(CH_3)_2CHCH_2$	97:3	78
12	Ti(*i*-PrO)Cl_3	**c**	$CH_3CH_2CH_2$	96:4	86
13	Ti(*i*-PrO)Cl_3	**d**	CH_3	93:7	60[d]
14	Ti(*i*-PrO)Cl_3	**e**	Ph	93:7[e]	81
15	Ti(*i*-PrO)Cl_3	**f**	4-NO_2Ph	95:5[e]	80
16	Ti(*i*-PrO)Cl_3	**g**	4-MeOPh	92:8[e]	80[f]
17	Ti(*i*-PrO)Cl_3	**h**	H_2C=$CH(CH_3)$	97:3	81
18	Ti(*i*-PrO)Cl_3	**i**	(*E*) CH_3CH=CH	96:4	79

[a] Determined by ^{1}H NMR.
[b] Isolated yield (dr≥95:5).
[c] Scale=4 mmol.
[d] Thirty percent of hemiacetal **11d** isolated.
[e] Determined by HPLC.
[f] Overall isolated yield.
[g] Scale=3 mmol.

Stereoselectivity was slightly eroded in the case of aromatic aldehydes, especially for the electron rich *p*-methoxybenzaldehyde (**g**), which afforded low diastereomeric ratios (dr 92:8) both with $TiCl_4$ and Ti(*i*-PrO)Cl_3. The reasons for this behavior are still unclear. Finally, outstanding diastereoselectivities (dr≥96:4) were also achieved for α,β-unsaturated aldehydes with both Lewis acids.

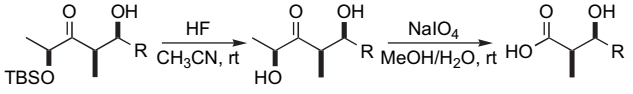
Otherwise, several reactions were easily scaled-up without observing any loss of diastereoselectivity or yield (see entries 1, 8, and 10 in Table 2).

Noteworthy, formation of hemiacetals **11** was routinely observed in the case of aliphatic aldehydes, **a**–**d**. Particularly important was the case of acetaldehyde-derived **11d** (R=Me in Table 2), which turned out to be rather stable and could be isolated more easily than in other cases (see entries 4 and 13 in Table 2).

Relative 4,5-*syn* stereochemistry of aldols **10** was assigned by means of the analysis of diagnostic coupling constants in ^{1}H NMR ($^3J_{4,5}$<4.0 Hz) and chemical shifts in ^{13}C NMR (δ_{Me4}<11 ppm).[23,24] However, absolute stereochemistry required further attention. Hence, removal of protecting groups from representative aldols **10a** and **10e** afforded dihydroxy ketones **12**, which were subsequently converted into the corresponding β-hydroxy carboxylic acids **13** in good overall yield (Table 3). Spectroscopic and physical data of **13** match with those previously reported in the literature and confirmed the configuration assigned to aldols **10**.

The ability of ketone **5** to rule the stereochemical outcome of such substrate-controlled aldol reactions was next challenged in double asymmetric processes[26] confronting titanium enolates from **5** to chiral β- and α-hydroxy aldehydes as **14** (and *ent*-**14**)[27] and **15** (and *ent*-**15**)[28] as shown in Scheme 2. In this context, Evans et al. documented that $TiCl_4$-mediated aldol addition of 2-methyl-3-pentanone to chiral α-methyl-β-hydroxy aldehydes provides the corresponding *anti*-Felkin adducts in moderate stereoselectivity.[29] In addition, preliminary studies carried out in our group proved that the parallel process associated to chiral α-hydroxy aldehyde **15** favors the corresponding Felkin adduct.[16b] Thus, it was not surprising to observe that matched pairs

Table 3. Correlation to β-hydroxy acids



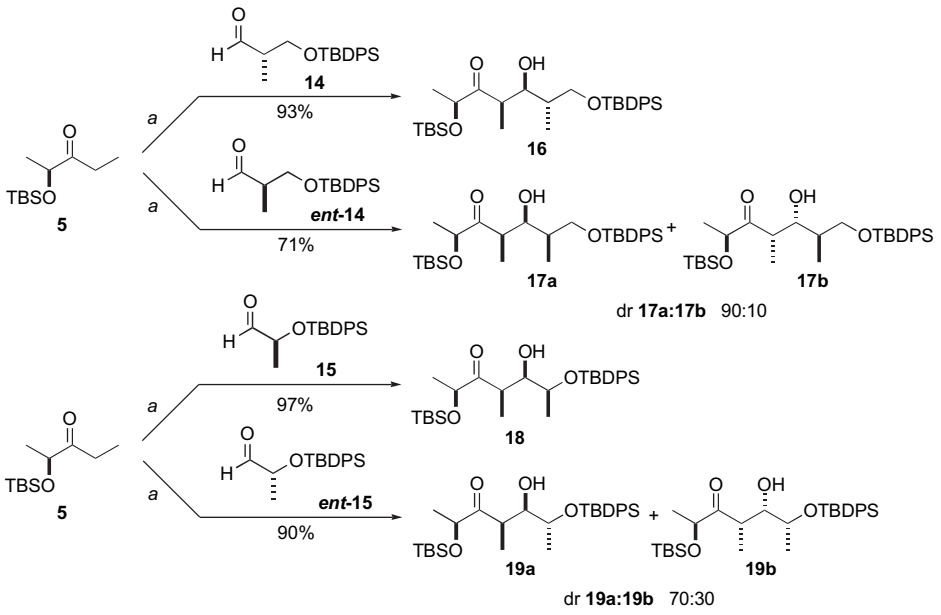
10a R = *i*-Pr **10e** R = Ph
12a R = *i*-Pr **12e** R = Ph
13a R = *i*-Pr **13e** R = Ph

13a $[\alpha]_D$=+11.4 (*c* 0.65, $CHCl_3$) Heathcock[2d] $[\alpha]_D$=+9.1 (*c* 2.2, $CHCl_3$); Palomo[25] $[\alpha]_D$=+10.8 (*c* 1.0, $CHCl_3$)

13e $[\alpha]_D$=+28.8 (*c* 1.0, $CHCl_3$) Heathcock[2d] $[\alpha]_D$=+28.5 (*c* 1.2, $CHCl_3$); Palomo[25] $[\alpha]_D$=+28.5 (*c* 1.0, $CHCl_3$)

Entry	Hydroxy acid	R	Overall yield (%)[a]
1	**13a**	*i*-Pr	67
2	**13e**	Ph	82

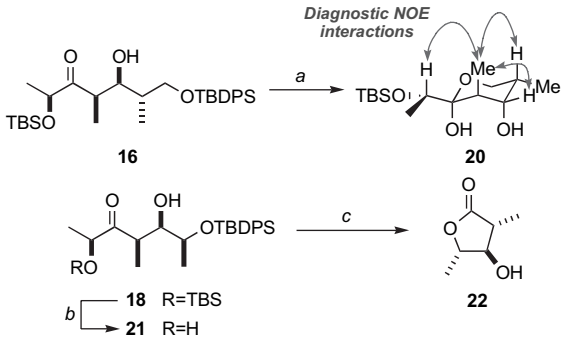
[a] Isolated yield from aldol **10**.



Scheme 2. Reagents and conditions: (a) (i) $TiCl_4$, *i*-Pr_2NEt, CH_2Cl_2, −78 °C; (ii) chiral aldehyde.

involving aldehydes **14** and **15** afforded a single diastereomer in high yields (Scheme 2).[30] For β-hydroxy aldehyde *ent*-**14**, a still synthetically useful mixture (71% yield, dr 90:10) of *syn* diastereomers was obtained irrespective of the Lewis acid ($TiCl_4$ or Ti(*i*-PrO)Cl_3) employed. However, ketone **5** hardly overcame the stereochemical bias imparted by the lactate-derived aldehyde *ent*-**15** and a 70:30 mixture of diastereomers was obtained in 90% yield.

The configuration of aldols **16** and **18** was confirmed through the selective removal of the silicon-protecting groups. Indeed, selective cleavage of TBDPS group[31] in **16** afforded hemiacetal **20** (Scheme 3), which was carefully analyzed by 1D and 2D NMR techniques. Alternatively, deprotection of TBS group in **18** provided dihydroxy ketone **21** (Scheme 3), which was, in turn, oxidized with $NaIO_4$ and TBDPS-deprotected to give lactone **22**, whose ^1H NMR and ^{13}C NMR spectra matched those reported in the literature.[2b] The configuration of major diastereomers in the case of mismatched pairs (**17a** and **19a**) was accounted for the dominant trend due to chiral ketone **5**.



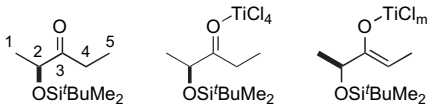
Scheme 3. Reagents and conditions: (a) TBAF, AcOH, DMF, rt, 52%; (b) HF, CH_3CN, rt, 84%; (c) (i) $NaIO_4$, MeOH/H_2O 2:1, rt; (ii) HF, CH_3CN, rt, 45%.

Once established the outline for highly stereoselective reactions, a deeper understanding of the mechanism of the process was considered desirable. Unfortunately, there is a lack of information concerning the structure of titanium enolates. To date, only one crystal X-ray analysis has been reported,[32] theoretical approaches are scarce[33] and spectroscopic studies on titanium enolates in solution[34] have provided a poor knowledge about the aggregation state or the coordination sphere of the titanium.[35] Indeed, it is still unknown if they must be considered as real enolates or alternatively as ate-complexes, the role of the amine or the distribution of ligands around the metal.

Regarding these elusive issues, Evans et al. speculated that ketone-derived titanium enolates exist as aggregated complexes with the amine intimately associated with the enolate, possibly through ion pairing.[9b] This model has been mostly held along mechanistic discussions involving titanium enolates and could be safely applied to our system. However, it was deemed worthwhile to get a more accurate picture of the species implicated in the process. Hence, NMR studies on putative intermediates of the process were carried out in CD_2Cl_2 at −78 °C.

In the beginning, it was easy to get both ^1H NMR and ^{13}C NMR spectra of ketone **5** in CD_2Cl_2 at low temperatures, which turned out to be very similar to those previously registered at rt in $CDCl_3$ (for ^1H NMR chemical shifts see Table 4). Unfortunately, subsequent study of the $TiCl_4$–ketone complex **23** proved troublesome because silicon-protecting group resulted to be too sensitive to the Lewis acid. Finally, careful addition of a solution of $TiCl_4$ to a stock solution of ketone **5** at −78 °C and quick analysis of the resulting mixture allowed us to observe significant downfield shifts for nuclei close to the carbonyl and the ether groups (see Table 4) and a common peak broadening, which suggests a dynamic behavior. At last, ^1H NMR spectra of the ensuing enolate showed a main set of broad signals and

Table 4. ^{1}H NMR (300 MHz) chemical shifts (CD_2Cl_2, −78 °C) of ketone **5** and intermediates **23** and **24**



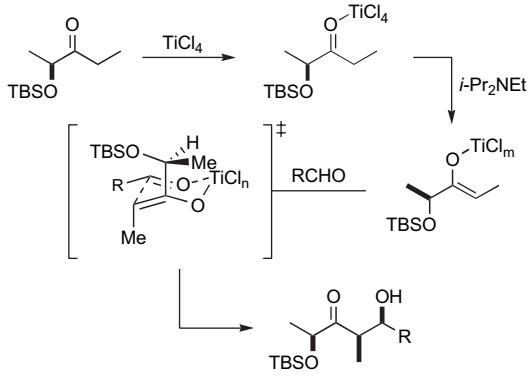
5 **23** **24**

Entry	Nucleus	δ in **5**[a]	δ in **23**[a]	δ in **24**[a]
1	H1	1.17	1.40	1.35
2	H2	4.07	4.90	4.60
3	H4	2.51, 2.63	3.00	5.30
4	H5	0.87	1.05	1.95
5	Si–Me	−0.01	0.10	−0.01
6	Si–*t*Bu	0.82	0.87	0.81

[a] Chemical shifts (δ) are quoted in parts per million referenced to $CHDCl_2$ (δ 5.31).

a close inspection of its 2D NOESY NMR revealed cross-peaks between C═CH and OTBS group. Hence, these evidences support a *Z* geometry and an antiperiplanar arrangement of both C–O bonds for the enolate **24** (see Table 4 and Scheme 4).

These pieces of evidence and the models currently accepted permit to propose the mechanism for the process represented in Scheme 4. As shown, enolization of a chelated $TiCl_4$–ketone complex provides the corresponding *Z*-enolate, which evolves through a cyclic six-membered transition state. In such scenario, the antiperiplanar distribution of both TBSO–C and C–OTi bonds would act as the key element that determines the stereochemical outcome of the reaction since the preferred chair transition state places the less sterically demanding substituent (H vs Me) of the Cα stereocenter pointing toward the inside of the ring, as it has been previously proposed for similar systems.[2d,3b,10,36]



Scheme 4.

This mechanism is consistent with the stereochemical outcome of reactions involving both achiral and chiral aldehydes. Indeed, chiral α-methyl-β-hydroxy aldehydes show the usual *anti*-Felkin bias according to the model established by Roush.[37] Likewise, α-hydroxy aldehydes afford the expected Felkin adducts, although in this case the Cornforth paradigm is the theoretical model[38] that more accurately describes the asymmetric induction in aldol additions to α-alkoxy aldehydes.[39,40]

3. Conclusions

In summary, we have proved that titanium enolates from lactate-derived chiral ketone **5** can be easily obtained by direct enolization with $TiCl_4$/*i*-Pr_2NEt and Ti(*i*-PrO)Cl_3/*i*-Pr_2NEt. Furthermore, they participate in highly stereoselective aldol processes with a wide scope of achiral and chiral aldehydes that afford the corresponding 2,4-*syn*-4,5-*syn* diastereomers in good yields irrespective of the titanium Lewis acid used in the enolization. Finally, spectroscopic studies of the intermediates involved in this process have permitted to propose a mechanism that accounts for the experimental results.

4. Experimental

4.1. General

Melting points were taken on an Electrothermal apparatus and have not been corrected. Specific rotations were determined at 20 °C on a Perkin–Elmer 241 MC polarimeter. IR spectra were recorded on either a Perkin–Elmer 681 or a Nicolet 510 FT spectrometer and only the more representative frequencies (cm^{-1}) are reported. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.4 MHz) spectra were recorded on a Varian Unity Plus 300 spectrometer; ^{1}H NMR (400 MHz) and ^{13}C NMR (100.6 MHz) spectra were recorded on a Varian Mercury; ^{1}H NMR (500 MHz) spectra were recorded on a Varian Unity Inova 500 spectrometer; chemical shifts (δ) are quoted in parts per million and referenced to internal TMS for ^{1}H NMR and $CDCl_3$ (δ 77.0) for ^{13}C NMR; data are reported as follows: s, singlet; d, doublet; t, triplet; q, quartet; hep, heptuplet; m, multiplet; br, broad; coupling constants (*J*) are quoted in hertz; where appropriate, 2D techniques were also used to assist in structural elucidation. Low resolution chemical ionization mass spectra (MS) were recorded on an HP-5988 A spectrometer. High resolution mass spectra (HRMS) were obtained from the Centro de Apoio Cientifico Tecnoloxico a Investigacion (C.A.C.T.I.), Universidad de Vigo. HPLC was carried out with a BGY 126 (250×4 mm) column [silica gel Spherisorb S3W] with a 0.9 mL min^{-1} flux. Flash chromatography was performed on SDS silica gel (35–70 μm). Analytical thin-layer chromatography was carried out on Merck Kieselgel 60 F_{254} plates. The following solvents and reagents were purified and dried according to the standard procedures: CH_2Cl_2, THF, Et_2O, DMF, *i*-Pr_2NEt. All other reagents were used as received.

4.2. Preparation of (*S*)-2-*tert*-butyldimethylsilyloxy-3-pentanone (5)

4.2.1. (*S*)-2-*tert*-Butyldimethylsilyloxy-*N*-methoxy-*N*-methylpropanamide (9). A solution of TBSCl (2.56 g, 17.0 mmol) in THF (5+1 mL) was added via canula to a solution of **8**[10] (1.51 g, 11.3 mmol), Et_3N (3.9 mL, 27.8 mmol) and a catalytic amount of DMAP in THF (10 mL) at 0 °C under N_2. The resulting mixture was stirred at 0 °C for 10 min at rt for two days. It was then diluted with Et_2O (150 mL), washed with 1 M HCl (50 mL), satd $NaHCO_3$ (50 mL), and brine (50 mL). The organic layer was dried ($MgSO_4$) and concentrated. The resulting oil was purified by flash chromatography (from hexanes/EtOAc 90:10

to 60:40), which afforded 2.72 g (11.0 mmol, 97%) of (*S*)-2-*tert*-butyldimethylsilyloxy-*N*-methoxy-*N*-methylpropanamide (**9**). Colorless oil; R_f=0.65 (hexanes/EtOAc 60:40); $[\alpha]_D$ −24.6 (*c* 1.0, $CHCl_3$); IR (film): *ν* 2956, 2933, 1686; ^{1}H NMR ($CDCl_3$, 300 MHz) *δ* 4.68 (1H, q, *J*=6.6, C*H*OTBS), 3.70 (3H, s, OCH_3), 3.21 (3H, br s, NCH_3), 1.33 (3H, d, *J*=6.6, CH_3CHOTBS), 0.91 (9H, s, SiC(CH_3)$_3$), 0.11 (3H, s, SiCH_3), 0.08 (3H, s, SiCH_3); ^{13}C NMR ($CDCl_3$, 75.4 MHz) *δ* 66.6 (br), 61.2, 32.8 (br), 25.8, 20.9, 18.3, −4.7, −5.0.

4.2.2. (*S*)-2-*tert*-Butyldimethylsilyloxy-3-pentanone (5). A 2 M solution of EtMgCl (10.9 mL, 21.8 mmol) in Et_2O was added dropwise to a solution of **9** (2.69 g, 10.9 mmol) in THF (140 mL) at 0 °C under N_2 and the resulting mixture was stirred at 0 °C for 1 h. The reaction was quenched by the addition of satd NH_4Cl (80 mL). The organic layer was washed with satd NH_4Cl (50 mL) and H_2O (50 mL). The aqueous layers were extracted with Et_2O (2×100 mL) and the combined organic extracts were dried ($MgSO_4$) and concentrated carefully (*caution: concentration in vacuo has to be carried out carefully in order to prevent losses of product*). The resulting oil was purified by flash chromatography (hexanes/Et_2O 90:10), which afforded 2.16 g (10.0 mmol, 92% yield) of (*S*)-2-*tert*-butyldimethylsilyloxy-3-pentanone (**5**).[15c]

4.3. General procedure of titanium-mediated aldol reactions from ketone 5

4.3.1. Using $TiCl_4$. Neat $TiCl_4$ (0.12 mL, 1.1 mmol) is added slowly to a solution of ketone **5** (216 mg, 1.0 mmol) in CH_2Cl_2 (5 mL) at −78 °C under N_2. The resulting yellow mixture is stirred for 3–4 min and *i*-Pr_2NEt (0.19 mL, 1.1 mmol) is added dropwise. The resulting dark red solution is stirred for 30 min at −78 °C and, after the dropwise addition of freshly distilled aldehyde (1.5 equiv), stirring is continued for 30 min at −78 °C. The reaction is quenched by the addition of satd NH_4Cl (5 mL) and vigorously stirred at rt. The mixture is diluted with Et_2O, washed with H_2O, satd $NaHCO_3$, and brine. The aqueous phases are extracted with Et_2O, and the combined organic extracts are dried ($MgSO_4$) and concentrated. The resulting oil is analyzed by ^{1}H NMR or HPLC and purified by flash chromatography (hexanes/EtOAc or CH_2Cl_2).

Note—p-nitrobenzaldehyde was added as a solution in CH_2Cl_2 (0.5 mL+0.5 mL) using 4 mL of solvent for the enolization. Methacrolein was used as received.

4.3.2. Using Ti(*i*-PrO)Cl_3. Freshly distilled Ti(*i*-PrO)$_4$ (83 μL, 0.28 mmol) is added dropwise to a solution of $TiCl_4$ (92 μL, 0.84 mmol) in CH_2Cl_2 (1 mL) at 0 °C under N_2. The resulting yellow mixture is stirred for 10 min at 0 °C, diluted with CH_2Cl_2 (1 mL) and stirred for 10 min at rt. Then, the ensuing colorless solution is added dropwise (it is rinsed with 2×0.5 mL) via canula for 10–15 min to a solution of ketone **5** (216 mg, 1.0 mmol) in CH_2Cl_2 (2 mL) at −78 °C under N_2, followed by *i*-Pr_2NEt (0.19 mL, 1.1 mmol). The resulting dark red solution is stirred for 30 min at −78 °C and, after the dropwise addition of freshly distilled aldehyde (1.5 equiv), stirring is continued for 30 min at −78 °C. The reaction mixture is quenched and worked-up as in the previous case.

4.3.3. (2*S*,4*R*,5*S*)-2-*tert*-Butyldimethylsilyloxy-5-hydroxy-4,6-dimethyl-3-heptanone (10a). Colorless oil; R_f=0.2 (hexanes/EtOAc 9:1); $[\alpha]_D$ +24.7 (*c* 0.89, $CHCl_3$); IR (film): *ν* 3530 (br), 1702; ^{1}H NMR ($CDCl_3$, 500 MHz) *δ* 4.19 (1H, q, *J*=7.0, C*H*OTBS), 3.42 (1H, dd, *J*=8.8, *J*=2.4, C*H*OH), 3.32 (1H, qd, *J*=7.2, *J*=2.4, COC*H*CH$_3$CHOH), 1.72–1.62 (1H, m, C*H*(CH$_3$)$_2$), 1.32 (3H, d, *J*=7.0, CH_3CHOTBS), 1.11 (3H, d, *J*=7.2, COCHCH_3CHOH), 1.00 (3H, d, *J*=6.6, CH_3), 0.90 (9H, s, SiC(CH_3)$_3$), 0.82 (3H, d, *J*=6.8, CH_3), 0.07 (3H, s, SiCH_3), 0.06 (3H, s, SiCH_3); ^{13}C NMR ($CDCl_3$, 100.6 MHz) *δ* 219.8 (C), 76.1 (CH), 74.6 (CH), 41.3 (CH), 30.4 (CH), 25.7 (CH$_3$), 21.4 (CH$_3$), 19.4 (CH$_3$), 18.8 (CH$_3$), 18.0 (C), 9.3 (CH$_3$), −4.7 (CH$_3$), −5.0 (CH$_3$); HRMS (+FAB): *m*/*z* calcd for $[M+H]^+$ $C_{15}H_{33}O_3Si$: 289.2207; found: 289.2199.

4.3.4. (2*S*,4*R*,5*S*)-2-*tert*-Butyldimethylsilyloxy-5-hydroxy-4,7-dimethyl-3-octanone (10b). Colorless oil; R_f=0.3 (hexanes/EtOAc 9:1); $[\alpha]_D$ +2.7 (*c* 2.1, $CHCl_3$); IR (film): *ν* 3500 (br), 1720; ^{1}H NMR ($CDCl_3$, 500 MHz) *δ* 4.21 (1H, q, *J*=6.8, C*H*OTBS), 3.94 (1H, ddd, *J*=9.3, *J*=4.1, *J*=2.9, C*H*OH), 3.08 (1H, qd, *J*=7.2, *J*=2.9, COC*H*CH$_3$CHOH), 1.85–1.70 (1H, m, C*H*(CH$_3$)$_2$), 1.55–1.42 (1H, m, CH_xH$_y$), 1.34 (3H, d, *J*=6.8, CH_3CHOTBS), 1.14 (3H, d, *J*=7.2, COCHCH_3CHOH), 1.15–1.00 (1H, m, CH$_x$$H_y$), 0.92 (9H, s, SiC(C$H_3$)$_3$), 0.92 (3H, d, *J*=6.8, CH_3), 0.91 (3H, d, *J*=6.5, CH_3), 0.10 (3H, s, SiCH_3), 0.09 (3H, s, SiCH_3); ^{13}C NMR ($CDCl_3$, 75.4 MHz) *δ* 219.2, 74.6, 68.8, 44.5, 43.1, 25.7, 24.5, 23.4, 22.0, 21.3, 18.0, 10.1, −4.6, −5.0; HRMS (+FAB): *m*/*z* calcd for $[M+H]^+$ $C_{16}H_{35}O_3Si$: 303.2355; found: 303.2362.

4.3.5. (2*S*,4*R*,5*S*)-2-*tert*-Butyldimethylsilyloxy-5-hydroxy-4-methyl-3-octanone (10c). Colorless oil; R_f= 0.2 (hexanes/EtOAc 9:1); $[\alpha]_D$ +9.2 (*c* 1.35, $CHCl_3$); IR (film): *ν* 3475 (br), 1711; ^{1}H NMR ($CDCl_3$, 500 MHz) *δ* 4.19 (1H, q, *J*=6.9, C*H*OTBS), 3.85–3.80 (1H, m, C*H*OH), 3.10 (1H, qd, *J*=7.2, *J*=2.8, COC*H*CH$_3$CHOH), 1.55–1.40 (2H, m, CHOHCH_2), 1.35–1.20 (2H, m, CH_2CH$_3$), 1.31 (3H, d, *J*=6.9, CH_3CHOTBS), 1.11 (3H, d, *J*=7.2, COCHCH_3CHOH), 0.91 (3H, t, *J*=7.2, CH$_2$CH_3), 0.90 (9H, s, SiC(CH_3)$_3$), 0.07 (6H, s, Si(CH_3)$_2$); ^{13}C NMR ($CDCl_3$, 75.4 MHz) *δ* 219.1, 74.5, 70.6, 44.1, 36.1, 25.7, 21.3, 19.2, 18.0, 14.0, 9.9, −4.7, −5.0; HRMS (+FAB): *m*/*z* calcd for $[M+H]^+$ $C_{15}H_{33}O_3Si$: 289.2199; found: 289.2195.

4.3.6. (2*S*,4*R*,5*S*)-2-*tert*-Butyldimethylsilyloxy-5-hydroxy-4-methyl-3-hexanone (10d). Colorless oil; R_f= 0.15 (hexanes/EtOAc 9:1); $[\alpha]_D$ +16.8 (*c* 1.0, $CHCl_3$); IR (film): *ν* 3462 (br), 1713; ^{1}H NMR ($CDCl_3$, 300 MHz) *δ* 4.21 (1H, q, *J*=6.9, C*H*OTBS), 4.06 (1H, qd, *J*=6.4, *J*=3.4, C*H*OH), 3.08 (1H, qd, *J*=7.2, *J*=3.4, COC*H*CHOH), 1.34 (3H, d, *J*=6.9, CH_3CHOTBS), 1.15 (3H, d, *J*=7.2, COCHCH_3CHOH), 1.15 (3H, d, *J*=6.4, CHOHCH_3), 0.92 (9H, s, SiC(CH_3)$_3$), 0.10 (3H, s, SiCH_3), 0.09 (3H, s, SiCH_3); ^{13}C NMR ($CDCl_3$, 75.4 MHz) *δ* 218.6, 74.5, 67.1, 45.5, 25.6, 21.1, 20.0, 18.0, 10.2, −4.7, −5.1; HRMS (+FAB): *m*/*z* calcd for $[M+H]^+$ $C_{13}H_{29}O_3Si$: 261.1886; found 261.1892.

4.3.7. (1*R*,2*R*,4*S*)-4-*tert*-Butyldimethylsilyloxy-1-hydroxy-2-methyl-1-phenyl-3-pentanone (10e). Colorless oil; R_f=0.15 (hexanes/EtOAc 9:1); HPLC (hexanes/*i*-PrOH 99:1) t_R=5.2 min (minor diastereomer, t_R=5.6 min); $[\alpha]_D$ +4.2 (*c* 1.0, $CHCl_3$); IR (film): ν 3500 (br), 1713; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.34–7.30 (4H, m, Ar*H*), 7.26–7.21 (1H, m, Ar*H*), 5.03 (1H, d, *J*=3.7, C*H*OH), 4.14 (1H, q, *J*=6.9, C*H*OTBS), 3.37 (1H, qd, *J*=7.2, *J*=3.7, COC*H*CH_3CHOH), 1.27 (3H, d, *J*=6.9, C*H*$_3$CHOTBS), 1.05 (3H, d, *J*=7.2, COCHC*H*$_3$CHOH), 0.90 (9H, s, SiC(C*H*$_3$)$_3$), 0.08 (3H, s, SiC*H*$_3$), 0.06 (3H, s, SiC*H*$_3$); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 218.7, 141.7, 128.2, 127.2, 125.9, 74.6, 72.8, 46.9, 25.7, 21.0, 18.0, 10.4, −4.7, −5.0; HRMS (+FAB): *m*/*z* calcd for $[M+H]^+$ $C_{18}H_{31}O_3Si$: 323.2042; found: 323.2045.

4.3.8. (1*R*,2*R*,4*S*)-4-*tert*-Butyldimethylsilyloxy-1-hydroxy-2-methyl-1-(4-nitrophenyl)-3-pentanone (10f). Colorless oil; R_f=0.30 (CH_2Cl_2); HPLC (hexanes/EtOAc 97.5:2.5) t_R=35.3 min (minor diastereomer, t_R=50.1 min); $[\alpha]_D$ −0.6 (*c* 1.1, $CHCl_3$); IR (film): ν 3512 (br), 1710, 1524, 1349; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.25–8.15 (2H, m, Ar*H*), 7.55–7.45 (2H, m, Ar*H*), 5.17 (1H, br s, C*H*OH), 4.23 (1H, q, *J*=6.9, C*H*OTBS), 3.41 (1H, qd, *J*=7.2, *J*=2.9, COC*H*CH_3CHOH), 1.35 (3H, d, *J*=6.9, C*H*$_3$CHOTBS), 1.03 (3H, d, *J*=7.2, COCHC*H*$_3$CHOH), 0.92 (9H, s, SiC(C*H*$_3$)$_3$), 0.11 (6H, s, Si(C*H*$_3$)$_2$); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 218.8, 149.1, 147.2, 126.7, 123.5, 74.7, 71.9, 46.3, 25.7, 21.4, 18.0, 9.8, −4.6, −5.0; HRMS (+FAB): *m*/*z* calcd for $[M+H]^+$ $C_{18}H_{30}NO_5Si$: 368.1893; found: 368.1882.

4.3.9. (1*R*,2*R*,4*S*)-4-*tert*-Butyldimethylsilyloxy-1-hydroxy-1-(4-methoxyphenyl)-2-methyl-3-pentanone (10g). Colorless oil; R_f=0.25 (CH_2Cl_2); $[\alpha]_D$ +1.7 (*c* 1.0, $CHCl_3$); HPLC (hexanes/*i*-PrOH 99:1) t_R=6.5 min (minor diastereomer, t_R=8.0 min); IR (film): ν 3504 (br), 1713; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.28–7.20 (2H, m, Ar*H*), 6.92–6.84 (2H, m, Ar*H*), 4.99 (1H, dd, *J*=4.0, *J*=1.8, C*H*OH), 4.13 (1H, q, *J*=6.8, C*H*OTBS), 3.80 (3H, s, OC*H*$_3$), 3.35 (1H, qd, *J*=7.1, *J*=4.0, COC*H*CH_3CHOH), 1.27 (3H, d, *J*=6.8, C*H*$_3$CHOTBS), 1.08 (3H, d, *J*=7.1, COCHC*H*$_3$CHOH), 0.92 (9H, s, SiC(C*H*$_3$)$_3$), 0.09 (3H, s, SiC*H*$_3$), 0.08 (3H, s, SiC*H*$_3$); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 218.5, 158.8, 134.0, 127.1, 113.6, 74.6, 72.6, 55.2, 47.1, 25.7, 20.9, 18.0, 10.8, −4.7, −5.0; HRMS (+FAB): *m*/*z* calcd for $[M+H]^+$ $C_{19}H_{33}O_4Si$: 353.2148; found: 353.2141.

4.3.10. (2*S*,4*R*,5*R*)-2-*tert*-Butyldimethylsilyloxy-5-hydroxy-4,6-dimethyl-6-hepten-3-one (10h). Colorless oil; R_f=0.1 (hexanes/EtOAc 94:6); $[\alpha]_D$ +12.4 (*c* 1.0, $CHCl_3$); IR (film): ν 3500 (br), 1702, 1653; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.12–5.09 (1H, m, C=C*H*$_x$H$_y$), 4.95–4.93 (1H, m, C=CH$_x$*H*$_y$), 4.32 (1H, br s, C*H*OH), 4.21 (1H, q, *J*=6.9, C*H*OTBS), 3.30 (1H, qd, *J*=7.2, *J*=2.8, COC*H*CH_3CHOH), 1.67–1.65 (3H, m, C*H*$_3$C=CH$_2$), 1.28 (3H, d, *J*=6.9, C*H*$_3$CHOTBS), 1.06 (3H, d, *J*=7.2, C*H*$_3$CHOTBS), 0.91 (9H, s, SiC(C*H*$_3$)$_3$), 0.08 (6H, s, Si(C*H*$_3$)$_2$); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 219.2, 143.2, 111.8, 74.6, 73.2, 41.8, 25.7, 21.3, 19.6, 18.0, 9.6, −4.6, −5.0; HRMS (+FAB): *m*/*z* calcd for $[M+H]^+$ $C_{15}H_{31}O_3Si$: 287.2042; found: 287.2035.

4.3.11. (2*S*,4*R*,5*S*,6*E*)-2-*tert*-Butyldimethylsilyloxy-5-hydroxy-4-methyl-6-octen-3-one (10i). Yellowish oil; R_f=0.2 (hexanes/EtOAc 9:1); $[\alpha]_D$ −6.3 (*c* 1.3, $CHCl_3$); IR (film): ν 3475 (br), 1709, 1640; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.72 (1H, dqd, *J*=15.7, *J*=6.5, *J*=1.2, HC=C*H*CH_3), 5.44 (1H, ddq, *J*=15.7, *J*=6.5, *J*=1.7, *H*C=CHC*H*$_3$), 4.36–4.32 (1H, m, C*H*OH), 4.21 (1H, q, *J*=6.9, C*H*OTBS), 3.19 (1H, qd, *J*=7.2, *J*=3.8, COC*H*CH_3CHOH), 1.70 (3H, ddd, *J*=6.5, *J*=1.7, *J*=0.9, HC=CHC*H*$_3$), 1.30 (3H, d, *J*=6.9, C*H*$_3$CHOTBS), 1.11 (3H, d, *J*=7.2, COCHC*H*$_3$CHOH), 0.90 (9H, s, SiC(C*H*$_3$)$_3$), 0.07 (6H, s, Si(C*H*$_3$)$_2$); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 217.7, 130.7, 127.7, 74.6, 72.2, 45.2, 25.7, 21.0, 18.0, 17.7, 11.1, −4.6, −5.0; HRMS (+FAB): *m*/*z* calcd for $[M+H]^+$ $C_{15}H_{31}O_3Si$: 287.2042; found: 287.2050.

4.3.12. Hemiacetal from *i*-PrCHO (11a). Colorless oil; R_f=0.6 (hexanes/EtOAc 9:1); $[\alpha]_D$ −22.7 (*c* 0.6, $CHCl_3$); IR (film): ν 3525, 2957, 2929; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.84 (1H, d, *J*=4.5, OCHO), 3.83 (1H, q, *J*=6.3, C*H*OTBS), 3.64 (1H, s, OH), 3.59 (1H, dd, *J*=9.7, *J*=2.0, OC*H*CH(CH$_3$)$_2$), 1.85–1.60 (3H, m, C*H*CH$_3$ and 2×C*H*(CH$_3$)$_2$), 1.13 (3H, d, *J*=6.0, C*H*$_3$), 1.03 (3H, d, *J*=6.3, C*H*$_3$), 0.98 (3H, d, *J*=6.9, C*H*$_3$), 0.96 (3H, d, *J*=6.9, C*H*$_3$), 0.93 (9H, s, SiC(C*H*$_3$)$_3$), 0.89 (3H, d, *J*=6.6, C*H*$_3$), 0.83 (3H, d, *J*=6.9, C*H*$_3$), 0.14 (3H, s, SiC*H*$_3$), 0.12 (3H, s, SiC*H*$_3$); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 98.5, 98.3, 80.1, 71.2, 33.7, 32.4, 28.7, 25.8, 19.8, 18.1, 17.3, 17.2, 16.9, 16.4, 6.9, −4.6, −4.9.

4.3.13. Hemiacetal from CH_3CHO (11d). Colorless oil; R_f=0.5 (hexanes/EtOAc 9:1); $[\alpha]_D$ −22.9 (*c* 1.3, $CHCl_3$); IR (film): ν 3527, 2933; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.27 (1H, q, *J*=5.2, OC*H*O), 4.34 (1H, qd, *J*=6.6, *J*=2.1, C(OH)–CHC*H*CH$_3$), 3.78 (1H, q, *J*=6.3, C*H*OTBS), 3.63 (1H, s, O*H*), 1.40 (1H, qd, *J*=6.8, *J*=2.1, C(OH)C*H*(CH$_3$)CH), 1.26 (3H, d, *J*=5.2, OCH(C*H*$_3$)O), 1.13 (3H, d, *J*=6.6, C(OH)CHCHC*H*$_3$), 1.07 (3H, d, *J*=6.3, C*H*$_3$CHOTBS), 0.90 (3H, d, *J*=6.8, C(OH)CH(C*H*$_3$)CH), 0.89 (9H, s, SiC(C*H*$_3$)$_3$), 0.08 (3H, s, SiC*H*$_3$), 0.07 (3H, s, SiC*H*$_3$); ^{1}H NMR (C_6D_6, 400 MHz) δ 5.41 (1H, q, *J*=5.2, OC*H*O), 4.50 (1H, qd, *J*=6.5, *J*=2.2, C(OH)CHC*H*CH$_3$), 3.79 (1H, q, *J*=6.2, C*H*OTBS), 3.64 (1H, s, O*H*), 1.34 (3H, d, *J*=5.2, OCH(C*H*$_3$)O), 1.31 (1H, qd, *J*=6.8, *J*=2.2, C(OH)–C*H*(CH$_3$)CH), 1.04 (3H, d, *J*=6.5, C(OH)CHCHC*H*$_3$), 1.01 (3H, d, *J*=6.2, C*H*$_3$CHOTBS), 0.94 (9H, s, SiC(C*H*$_3$)$_3$), 0.83 (3H, d, *J*=6.8, C(OH)CH(C*H*$_3$)CH), 0.09 (3H, s, SiC*H*$_3$), 0.07 (3H, s, SiC*H*$_3$); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 98.4, 91.9, 71.2, 70.1, 36.7, 25.8, 20.7, 18.2, 18.0, 16.7, 6.7, −4.6, −5.1.

4.4. General procedure for TBS removal

A 48% aq solution of HF (3 equiv) is added dropwise to a 0.2 M solution of aldol **10** in CH_3CN at rt. The reaction mixture is stirred for 1 h and diluted with CH_2Cl_2. The organic layer is washed with satd $NaHCO_3$, dried ($MgSO_4$) and concentrated. The resulting oil is purified by flash chromatography (hexanes/EtOAc), which affords dihydroxy ketones **12** in 74–87% yield.

4.4.1. (2*S*,4*R*,5*S*)-2,5-Dihydroxy-4,6-dimethyl-3-heptanone (12a). Yield: 74%. Colorless oil; R_f=0.25 (hexanes/

EtOAc 60:40); $[\alpha]_D$ +69.9 (*c* 1.5, $CHCl_3$); IR (film): ν 3438 (br), 2967, 1708; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.45 (1H, q, *J*=7.0, CH_3CHOH), 3.58 (1H, dd, *J*=8.0, *J*=3.2, $COCHCHOH$), 2.99 (1H, qd, *J*=7.2, *J*=3.2, $COCHCHOH$), 1.71 (1H, dhep, *J*=8.0, *J*=6.6, $CH(CH_3)_2$), 1.42 (3H, d, *J*=7.0, CH_3CHOH), 1.19 (3H, d, *J*=7.2, $COCHCH_3$), 1.03 (3H, d, *J*=6.6, $CH(CH_3)_2$), 0.90 (3H, d, *J*=6.6, $CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 217.7, 75.7, 71.2, 42.8, 30.4, 19.9, 19.1, 18.7, 10.4; MS (CI–NH_3) *m*/*z*: $[M+NH_4]^+$=192 (100).

4.4.2. (1*R*,2*R*,4*S*)-1,4-Dihydroxy-2-methyl-1-phenyl-3-pentanone (12e). Yield: 87%. Colorless oil; R_f=0.20 (hexanes/EtOAc 60:40); $[\alpha]_D$ +42.1 (*c* 1.1, $CHCl_3$); IR (film): ν 3409 (br), 3063, 2979, 1710; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.40–7.20 (5H, m, Ar*H*), 5.06 (1H, dd, *J*=5.4, *J*=2.2, $CHOHPh$), 4.32 (1H, qd, *J*=7.1, *J*=5.1, CH_3CHOH), 3.35 (1H, d, *J*=5.1, CH_3CHOH), 3.07 (1H, qd, *J*=7.1, *J*=5.4, $COCHCH_3CHOH$), 2.84 (1H, br s, $CHOHPh$), 1.17 (3H, d, *J*=7.1, $COCHCH_3CHOH$), 1.09 (3H, d, *J*=7.1, CH_3CHOH); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 216.3, 141.7, 128.3, 127.8, 126.1, 73.2, 71.5, 48.6, 19.1, 12.3; MS (CI–NH_3) *m*/*z*: $[M+NH_4]^+$=226 (100).

4.5. General procedure for oxidation of α-hydroxy ketones

A mixture of dihydroxy ketone **12** (1 mmol) and $NaIO_4$ (1.46 g, 10 mmol) in 2:1 MeOH/H_2O (10 mL) is stirred for 1 h at rt. It is diluted with Et_2O (10 mL), cooled to 0 °C, and 1 M HCl is slowly added to reach pH 1. The mixture is partitioned with Et_2O (10 mL) and H_2O (10 mL). The organic layer is separated and the aqueous layer is thoroughly extracted with Et_2O (4×10 mL). The combined ethereal extracts are dried ($MgSO_4$) and concentrated. The resulting oil is purified by flash chromatography (CH_2Cl_2/MeOH 95:5), which affords hydroxy acids **13** in 90–95% yield.

4.5.1. (2*R*,3*S*)-3-Hydroxy-2,4-dimethylpentanoic acid (13a). Yield: 90%. Viscous colorless oil; R_f=0.10 (CH_2Cl_2/MeOH 95:5); $[\alpha]_D$ +11.4 (*c* 0.65, $CHCl_3$) [lit.[2d] $[\alpha]_D$ +9.1 (*c* 2.2, $CHCl_3$); lit.[25] $[\alpha]_D$ +10.8 (*c* 1.0, $CHCl_3$)]; IR (film): ν 3500–2750 (br), 1711; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.40 (2H, br s, 2×O*H*), 3.64 (1H, dd, *J*=8.1, *J*=3.6, C*H*OH), 2.71 (1H, qd, *J*=7.1, *J*=3.6, HOOCC*H*), 1.73 (1H, dhep, *J*=8.1, *J*=6.7, $CH(CH_3)_2$), 1.21 (3H, d, *J*=7.1, $HOOCCHCH_3$), 1.02 (3H, d, *J*=6.7, CH_3CHCH_3), 0.89 (3H, d, *J*=6.7, CH_3CHCH_3); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 181.6, 76.9, 41.8, 30.6, 19.0, 18.7, 9.7; MS (CI–NH_3) *m*/*z*: $[M+NH_4]^+$=164 (100), $[M+H]^+$=147 (16).

4.5.2. (2*R*,3*R*)-3-Hydroxy-2-methyl-3-phenylpropanoic acid (13e). Yield: 95%. White solid; mp=76–77 °C [lit.[25] mp=78–79 °C]; R_f=0.10 (CH_2Cl_2/MeOH 95:5); $[\alpha]_D$ +28.8 (*c* 1.0, $CHCl_3$) [lit.[2d] $[\alpha]_D$ +28.5 (*c* 1.27, $CHCl_3$); lit.[25] $[\alpha]_D$ +28.5 (*c* 1.0, $CHCl_3$)]; IR (film): ν 3500–2750 (br), 1708; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.40–7.20 (5H, m, Ar*H*), 6.40 (2H, br s, 2×O*H*), 5.17 (1H, d, *J*=3.9, C*H*OH), 2.84 (1H, qd, *J*=7.1, *J*=3.9, $COCHCH_3$), 1.15 (3H, d, *J*=7.1, $COCHCH_3$); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 180.9, 141.0, 128.4, 127.7, 125.9, 73.4, 46.2, 10.3; MS (CI–NH_3) *m*/*z*: $[M+NH_4]^+$=198 (100).

4.6. Spectroscopic data for aldol adducts from chiral aldehydes

4.6.1. (2*S*,4*R*,5*S*,6*S*)-2-*tert*-Butyldimethylsilyloxy-7-*tert*-butyldiphenylsilyloxy-5-hydroxy-4,6-dimethyl-3-heptanone (16). Yellowish oil; R_f=0.35 (hexanes/EtOAc 90:10); $[\alpha]_D$ +10.4 (*c* 1.05, $CHCl_3$); IR (film): ν 3516 (br), 1702; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.70–7.65 (4H, m, Ar*H*), 7.45–7.35 (6H, m, Ar*H*), 4.26 (1H, q, *J*=6.8, C*H*OTBS), 3.89 (1H, dd, *J*=9.2, *J*=2.4, C*H*OH), 3.81 (1H, dd, *J*=9.6, *J*=4.8, $CH_xH_yOTBDPS$), 3.74 (1H, dd, *J*=9.6, *J*=4.8, $CH_xH_yOTBDPS$), 3.27 (1H, qd, *J*=7.2, *J*=2.4, $COCHCHOH$), 1.82–1.72 (1H, m, $CHCH_2OTBDPS$), 1.33 (3H, d, *J*=6.8, $CH_3CHOTBS$), 1.13 (3H, d, *J*=7.2, $COCH(CH_3)CHOH$), 1.05 (9H, s, $SiC(CH_3)_3$), 0.92 (9H, s, $SiC(CH_3)_3$), 0.91 (3H, d, *J*=6.8, $CH(CH_3)CH_2OTBDPS$), 0.09 (3H, s, $SiCH_3$), 0.08 (3H, s, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 218.4 (C), 135.6 (CH), 133.5 (C), 133.4 (C), 129.6 (CH), 127.6 (CH), 74.4 (CH), 72.6 (CH), 66.9 (CH_2), 41.8 (CH), 37.7 (CH), 26.9 (CH_3), 25.7 (CH_3), 21.3 (CH_3), 19.3 (C), 18.0 (C), 13.6 (CH), 9.1 (CH), −4.6 (CH_3), −4.9 (CH_3); HRMS (+ESI): calcd for $[M+Na]^+$ $C_{31}H_{50}O_4Si_2Na$: 565.3140; found: 565.3159.

4.6.2. (2*S*,4*R*,5*R*,6*S*)-2-*tert*-Butyldimethylsilyloxy-6-*tert*-butyldiphenylsilyloxy-5-hydroxy-4-methyl-3-heptanone (18). Yellowish oil; R_f=0.15 (hexanes/EtOAc 96:4); $[\alpha]_D$ +9.9 (*c* 1.2, $CHCl_3$); IR (film): ν 3500 (br), 1710; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.75–7.60 (4H, m, Ar*H*), 7.45–7.35 (6H, m, Ar*H*), 4.19 (1H, q, *J*=6.7, C*H*OTBS), 3.83 (1H, dd, *J*=6.4, *J*=4.7, C*H*OH), 3.81–3.76 (1H, m, C*H*OTBDPS), 3.41 (1H, qd, *J*=7.0, *J*=4.7, $COCHCHOH$), 1.32 (3H, d, *J*=6.7, $CH_3CHOTBS$), 1.09 (3H, d, *J*=7.0, $COCH(CH_3)CHOH$), 1.06 (9H, s, $SiC(CH_3)_3$), 1.02 (3H, d, *J*=6.0, $CH(OTBDPS)CH_3$), 0.91 (9H, s, $SiC(CH_3)_3$), 0.05 (3H, s, $SiCH_3$), 0.04 (3H, s, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 217.3 (C), 135.9 (CH), 135.8 (CH), 134.3 (C), 133.2 (C), 129.8 (CH), 129.6 (CH), 127.7 (CH), 127.4 (CH), 75.1 (CH), 74.5 (CH), 69.7 (CH), 41.4 (CH), 27.1 (CH_3), 25.8 (CH_3), 21.3 (CH_3), 19.2 (C), 19.1 (CH_3), 18.1 (C), 11.7 (CH_3), −4.7 (CH_3), −4.8 (CH_3); HRMS (+FAB): calcd for $[M+Na]^+$ $C_{30}H_{48}O_4Si_2Na$: 551.2989; found: 551.2979.

4.7. Synthesis of hemiacetal 20 from 16

A 0.025 M solution of TBAF·$3H_2O$ and AcOH in DMF (4.3 mL, 108 μmol) was added to a 10 mL round bottom flask containing **16** (117 mg, 215 μmol) and the resulting mixture was stirred for 3.5 h at rt. Then, it was diluted with Et_2O (30 mL) and H_2O (30 mL), and the organic layer was washed with brine (25 mL), dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography (from hexanes to hexanes/EtOAc 85:15), which afforded 17 mg (56 μmol, 52% yield) of oil. Spectroscopic analysis of this oil revealed that the main component (9:1 mixture) was cyclic hemiacetal **20**. Colorless oil; R_f=0.15 (hexanes/EtOAc 85:15); IR (film): ν 3510 (br), 2931, 1115, 1038, 1014; ^1H NMR (C_6D_6, 400 MHz) δ 3.92 (1H, d, *J*=10.9, CHO*H*), 3.65 (1H, t, *J*=11.8, CH_xH_yO), 3.58 (1H, q, *J*=6.3, C*H*OTBS), 3.51 (1H, s, O*H*), 3.48–3.39 (1H, m, C*H*OH), 3.21 (1H, dd, *J*=11.8, *J*=5.5, CH_xH_yO), 1.92 (1H, qd, *J*=7.2, *J*=2.7, O_2CCHCH_3), 1.79–1.67 (1H, m,

C*H*CH_2O), 0.96 (3H, d, J=6.3, CH_3CHOTBS), 0.93 (9H, s, SiC(CH_3)$_3$), 0.88 (3H, d, J=6.8, CHC$H_3$$CH_2O$), 0.63 (3H, d, J=7.2, O_2CCHCH_3), 0.07 (3H, s, SiCH_3), 0.05 (3H, s, SiCH_3); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.94–3.90 (1H, m, CHO*H*), 3.71 (1H, t, J=11.8, CH_xH$_y$O), 3.70 (1H, s, O*H*), 3.66 (1H, q, J=6.3, C*H*OTBS), 3.52–3.45 (1H, m, C*H*OH), 3.41 (1H, dd, J=11.8, J=5.4, C$H_x$$H_y$O), 2.10–1.90 (2H, m, 2×C*H*CH_3), 1.10 (3H, d, J=6.3, CH_3CHOTBS), 0.94 (3H, d, J=7.1, CHCH_3), 0.88 (9H, s, SiC(CH_3)$_3$), 0.88 (3H, d, J=6.8, CHCH_3), 0.07 (3H, s, SiCH_3), 0.05 (3H, s, SiCH_3); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ 99.0, 75.0, 71.1, 61.4, 37.9, 29.1, 25.8, 18.2, 17.4, 12.8, 12.7, −4.6, −5.1.

4.8. Synthesis of lactone 22 from 18

A mixture of **18** (201 mg, 0.38 mmol) and 48% aq HF (45 μL, 1.25 mmol) in CH_3CN (4.5 mL) was stirred at rt under N_2 for 45 min. Then, it was diluted with CH_2Cl_2 (100 mL), washed with satd $NaHCO_3$ (2×50 mL), dried (Na_2SO_4), and concentrated. The purification of the residue by flash chromatography (hexanes/EtOAc 4:1) afforded 133 mg (0.32 mmol, 84%) of (2*S*,4*R*,5*R*,6*S*)-6-*tert*-butyldiphenylsilyloxy-2,5-dihydroxy-4-methyl-3-pentanone (**21**). Next, a mixture of **21** (127 mg, 306 μmol) and $NaIO_4$ (635 mg, 3 mmol) in MeOH/H_2O 2:1 (3.5 mL) was stirred at rt for 1.5 h, diluted with CH_2Cl_2 (50 mL), and washed with 0.5 M HCl (2×10 mL). The aqueous layers were extracted with CH_2Cl_2 (2×10 mL) and the combined extracts were dried (Na_2SO_4) and concentrated, which provided a brownish oil (123 mg) that was used in the next step without further purification. A mixture of this oil and 48% aq HF (110 μL, 3.2 mmol) in CH_3CN (1.5 mL) was stirred at rt under N_2 for 60 h. Then, it was diluted with CH_2Cl_2 (100 mL), washed with satd $NaHCO_3$ (2×50 mL), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (hexanes/EtOAc 2:1), which afforded 18 mg (138 μmol, 45% yield over two steps) of (2*R*,3*R*,4*S*)-3-hydroxy-2,4-dimethylbutyrolactone (**22**). Brown solid; mp= 59.0–60.5 °C; R_f=0.10 (hexanes/EtOAc 2:1); $[\alpha]_D$ −23.5 (*c* 1.0, $CHCl_3$); IR (KBr): ν 3463 (br), 1746; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.22 (1H, dq, J=7.6, J=6.1, COOC*H*), 3.76–3.67 (1H, m, C*H*OH), 2.75 (1H, br s, O*H*), 2.60 (1H, dq, J=9.2, J=7.2, C*H*COO), 1.46 (3H, d, J=6.1, COOCHCH_3), 1.31 (3H, d, J=7.2, OOCCHCH_3); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 176.7, 80.5, 80.0, 43.9, 18.0, 12.5.

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An advantageous synthesis of new indazolone and pyrazolone derivatives

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Abstract—The synthesis of new indazolone and pyrazolone derivatives starting from methyl anthranilate type substrates is presented. This general approach constitutes a novel and advantageous alternative for the synthesis of the target heterocycles, which implies the use of the environmentally friendly oxidizer PIFA. The synthetic design includes the oxidation of *N*-arylamides by the hypervalent iodine reagent to the corresponding *N*-acylnitrenium ions, which can be intramolecularly trapped by an amine moiety to furnish the title compounds by formation of a new N–N single bond.

1. Introduction

In spite of the limited occurrence of the indazole skeleton as a structural motif in natural products—to the best of our knowledge *nigellicine*[1] and *nigellidine*[2] (see Fig. 1) are the only examples found in the literature—an intensive effort for the development of efficient synthetic routes toward the preparation of these types of alkaloids has been carried out. This interest is mainly fueled by the promising pharmaceutical activities that they show. In fact, since benzydamine, the first non-steroidal anti-inflammatory drug (NSAID) bearing an indazole subunit, was commercialized in 1960,[3] many reports on the anti-inflammatory,[4] antipyretic,[5] analgesic,[6] and antihyperlipidemic[7] properties of these kinds of compounds, particularly of indazol-3-one derivatives, have appeared in the literature. Additionally, other simplified related structures, such as pyrazolones, have recently caught the attention of the medicinal community because of their bioactivity as inhibitors of tumor necrosis factor-α (TNF-α) production.[8] Nevertheless, despite the existence of a wide range of routes for the synthesis of the target heterocycles, an examination of the literature reveals that the chemistry involved in their preparation often lacks useful generality, entails certain inconvenient reagents, and implies, in some cases, the use of either expensive reagents or starting materials.[9]

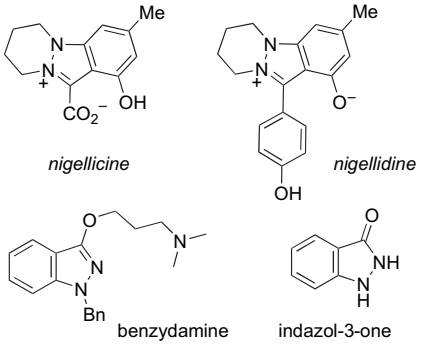

Figure 1. Selected examples of indazole derivatives.

One of our ongoing research lines deals with the search for novel applications of the hypervalent iodine reagent PIFA [phenyliodine(III)bis-(trifluoroacetate)] in heterocyclic synthesis,[10] a tactic that has found efficient applications in the synthesis of a number of compounds, which might be appealing for medicinal purposes.[11] Among them, we have recently reported an alternative access to the indazolone skeleton **1** through a PIFA-mediated oxidative cyclization.[12] This novel approach, disclosed in Scheme 1, is based on the ability of PIFA to oxidize adequately substituted amides to the corresponding *N*-acylnitrenium intermediates.[13] Then, in the presence of an amine moiety, an intramolecular attack leads to the construction of new N–N linkages affording the desired heterocycle.

In our preliminary communication we were intrigued by both the best experimental conditions to carry out the reaction, and the structural requirements of the substrates for an efficient transformation. This optimization process led to the conclusion that the cyclization protocol was restricted to aromatic amides and substituted nucleophilic amines, a result that is coherent with the mechanistic proposal shown

Keywords: Hypervalent iodine; *N*-Acylnitrenium; Indazolone; Pyrazolone.
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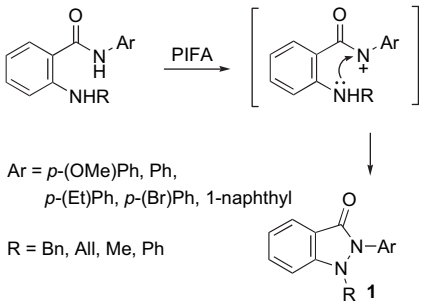
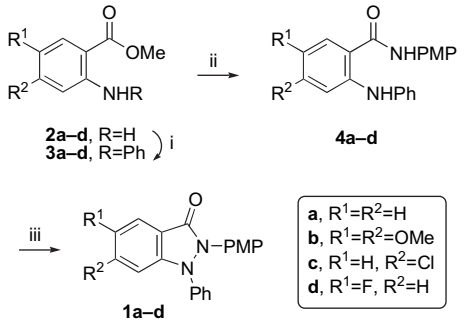


Scheme 1. Designed strategy for the synthesis of indazolones of type **1**.

above for this transformation. Additionally, the multiple and interesting pharmacological activities that these derivatives can display suggest that additional bioactivities are likely to be found when similar systems are tested. Now, in this paper, the success of the described methodology for the synthesis of related structures will be examined. Thus, herein we would like to report the application of the PIFA-mediated N–N bond forming process to the synthesis of a series of indazolone derivatives in which the benzo-moiety is replaced by other motifs, such as either substituted arene rings or the heterocyclic thiophene system. In addition, the construction of the pyrazolone nucleus, which is present in important groups of therapeutic agents, is also reported following this protocol.

2. Results and discussion

Once the *para*-methoxyphenyl group (PMP) had been selected as the proper neighboring group to stabilize the corresponding *N*-acylnitrenium ions,[14] and the phenyl group as an adequate substituent to make the amine functionality nucleophilic enough to attack such intermediates, a series of substrates **4a–d** were chosen to construct the target indazolone derivatives. These precursors were obtained (see Scheme 2) in a two-step synthesis from commercially available methyl anthranilates **2a–d** by a palladium-catalyzed *N*-arylation reaction using bromobenzene as the arylating agent,[15] followed by the direct transformation of the resulting aminoesters **3a–d** into the desired amides **4a–d** by a $AlMe_3$-mediated aminolysis reaction.[16] Next, we studied the behavior of these aromatic amides with the trivalent iodine reagent PIFA under the cyclization conditions previously optimized by our research group.[12]



Scheme 2. Reagents and conditions: (i) PhBr, $Pd(OAc)_2$, Xantphos, Cs_2CO_3, toluene, 100 °C, sealed tube; (ii) $AlMe_3$, *para*-anisidine, CH_2Cl_2, reflux; (iii) PIFA (0.01 M), CH_2Cl_2, TFA, 0 °C.

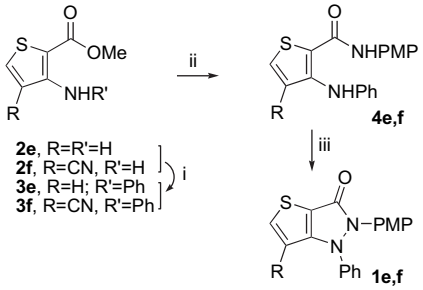
Table 1. Synthetic details for the transformation of **2a–d** to **1a–d**

Entry	**3a–d** (%)[a]	**4a–d** (%)[b]	**1a–d** (%)[a]
1	**3a** (93)	**4a** (69)	**1a** (61)
2	**3b** (85)	**4b** (68)	**1b** (0)
3	**3c** (71)	**4c** (70)	**1c** (81)
4	**3d** (85)	**4d** (70)	**1d** (77)

[a] Isolated yields after purification by flash-chromatography.
[b] Isolated yields after purification by crystallization from Et_2O.

As shown in Table 1, the success of the proposed PIFA-mediated cyclization process revealed a strong dependence on the nature of the substituents in the aryl ring. Thus, it proved to be suitable for unsubstituted substrates (entry 1) and for substrates bearing electron-withdrawing groups such as chlorine (entry 3) and fluorine (entry 4) affording the desired indazolones **1a**, **1c**, and **1d** in 61, 81, and 77% yields, respectively. In contrast, the cyclization process failed when it was tested on the dimethoxy-substituted amide **4b** (entry 2). In this case, the desired indazolone **1b** was not even detected, and a complex mixture of products was obtained instead. Therefore, these results suggest that the required PIFA-promoted oxidation of the amide functionality is precluded with respect to an oxidation process that would take place on highly enriched aromatic rings (i.e., **4b**) failing, hence, to afford the desired heterocycle.

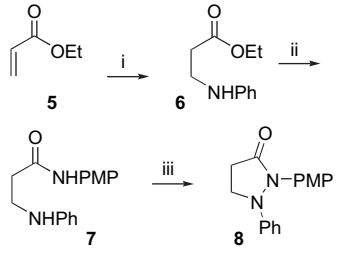
As mentioned above, in order to investigate the extension of the presented methodology to other fused heterocyclic systems, we also faced the synthesis of the related thieno-fused pyrazolone derivatives **1e**,**f** by a common sequence as shown in Scheme 3. Thus, methyl 3-amino-2-thiophenecarboxylates **2e**,**f** were submitted to a palladium-catalyzed *N*-arylation process affording satisfactorily the corresponding *N*-phenyl derivatives **3e**,**f**, which were next efficiently transformed into the desired aromatic amides **4e**,**f**. On treatment with the hypervalent iodine reagent PIFA, it was observed that even after application of a complete array of experimental conditions (by modifying solvents, temperature, and additives), amide **4e** could never furnish the corresponding bicycle **1e**, and complete degradation of the starting material was observed. Although synthetically discouraging, this result could be anticipated considering the similar electronic nature of the 3,4-dimethoxyphenyl and thienyl systems. For that reason, we also experimented the behavior of amide **4f** under our PIFA-promoted cyclization conditions. In this particular case, the desired



Scheme 3. Reagents and conditions: (i) PhBr, $Pd(OAc)_2$, Xantphos, Cs_2CO_3, toluene, 100 °C, sealed tube (69% for **3e**, 57% for **3f**); (ii) $AlMe_3$, *para*-anisidine, CH_2Cl_2, reflux (82% for **4e**, 68% for **4f**); (iii) PIFA (0.01 M), CH_2Cl_2, TFA, 0 °C (0% for **1e**, 61% for **1f**).

thieno-fused pyrazolone **1f** was obtained in a nice 61% yield. Apparently, the decrease of the oxidation potential of the thiophene ring due to the presence of the electron-withdrawing cyano group allows nitrogen oxidation and, therefore, the cyclization reaction to take place.[17]

Finally, taking into account the already described favorable results, we decided to test the presented oxidative process on a linear amide to determine its suitability for the construction of the simple pyrazolone skeleton. In this case (see Scheme 4) the synthesis of the required *para*-methoxyphenylamide **7** was accomplished by following a known[18] aza-Michael reaction on ethyl acrylate **5**, followed by a $AlMe_3$-promoted amidation protocol on the so-obtained derivative **6**. Next, when amide **7** was submitted to the action of PIFA, the desired pyrazolone derivative **8** was obtained in a moderate 35% yield using trifluoroethanol as solvent, instead of CH_2Cl_2/TFA as for the previous examples, to attain complete conversion of the starting material.



Scheme 4. Reagents and conditions: (i) $PhNH_2$, $FeCl_3 \cdot 7H_2O$, H_2O, room temperature (72%); (ii) $AlMe_3$, *para*-anisidine, CH_2Cl_2, reflux (72%); (iii) PIFA (0.01 M), TFEA, 0 °C (35%).

3. Conclusions

A practical and facile approach to the synthesis of *N*,*N*-disubstituted indazolone and pyrazolone derivatives has been developed. Our method features the succeeding intramolecular trapping of *N*-acylnitrenium ions, which are generated by the oxidative action of PIFA on aromatic amides, by amines functionalities providing, hence, a novel and versatile route for the construction of the title heterocycles through the formation of new N–N linkages under rather mild experimental conditions. Furthermore, a study on the pharmacological activity that these derivatives may exhibit is currently in progress.

4. Experimental

4.1. General

All reagents were purchased and used as received. Melting points were measured using open glass capillaries and are uncorrected. Infrared spectra were recorded as KBr plates or as thin films and peaks are reported in cm^{-1}. Only representative absorptions are given. NMR spectra were recorded on a 300 (300 MHz for ^1H and 75.4 MHz for ^{13}C) instrument at 20 °C. Chemical shifts (δ) were measured in ppm relative to chloroform (δ=7.26 for ^1H or 77.00 for ^{13}C) as internal standard. Coupling constants, *J*, are reported in hertz. DEPT experiments were used to assist with the assignation of the signals. HRMS spectra were measured by using a Waters GCT Mass Spectrometer.

4.2. Typical procedure for the Pd-catalyzed *N*-arylation reaction. Preparation of esters 3a–f

4.2.1. Synthesis of methyl *N*-phenylanthranilate (3a). A solution of commercially available methyl anthranilate (**2a**) (0.70 mL, 5.29 mmol), bromobenzene (0.47 mL, 4.41 mmol), $Pd(OAc)_2$ (20.2 mg, 0.09 mmol), Xantphos (104 mg, 0.18 mmol), and Cs_2CO_3 (2.01 g, 6.17 mmol) in toluene (18 mL) was heated at 100 °C in a sealed tube. After 3 h the reaction mixture was filtered and the so-obtained solid residue was washed with CH_2Cl_2 (3×15 mL). Then, the resulting organic layer was concentrated in vacuo and the crude product was purified by flash-chromatography (CH_2Cl_2) to afford the *N*-phenyl derivative **3a** (93%) as yellowish oil.[12]

4.2.2. Methyl 4,5-dimethoxy-2-phenylaminobenzoate (3b). According to the typical procedure, ester **3b** was obtained from commercially available methyl 2-amino-4,5-dimethoxybenzoate (**2b**) in 85% yield as a white solid after purification by column chromatography (CH_2Cl_2) followed by crystallization from hexanes: mp 139–140 °C (hexanes); ^1H NMR ($CDCl_3$) δ 3.77 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.81 (s, 1H, H_{arom}), 7.02–7.07 (m, 1H, H_{arom}), 7.21–7.35 (m, 4H, H_{arom}), 7.40 (s, 1H, H_{arom}), 9.41 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 51.3, 55.6, 56.1 (OCH_3), 97.4 (CH), 103.4 (C), 112.7, 121.3, 122.8, 129.2 (CH), 140.8, 141.2, 143.9, 154.3 (C), 168.2 (CO); IR (KBr) 3273 (NH), 1661 (CO); MS (EI) *m*/*z* (%) 287 (M^+, 42), 272 (50), 255 (37), 240 (45), 212 (100), 184 (25); HRMS calcd for $C_{16}H_{17}NO_4$: 287.1158, found: 287.1158.

4.2.3. Methyl 4-chloro-2-phenylaminobenzoate (3c). According to the typical procedure, ester **3c** was obtained from commercially available methyl 2-amino-4-chlorobenzoate (**2c**) in 71% yield as a yellow oil after purification by column chromatography (CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 3.90 (s, 3H, OCH_3), 6.66–6.67 (m, 1H, H_{arom}), 6.69–7.41 (m, 6H, H_{arom}), 7.89 (d, *J*=8.6, 1H, H_{arom}), 9.56 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 51.8 (OCH_3), 109.9 (C), 113.2, 117.1, 123.2, 124.4, 129.5, 132.9 (CH), 139.7, 140.5, 149.0 (C), 168.3 (CO); IR (film) 3308 (NH), 1684 (CO); MS (EI) *m*/*z* (%) 263 (M^++2, 77), 261 (M^+, 93), 231 (91), 229 (100), 201 (71); HRMS calcd for $C_{14}H_{12}ClNO_2$: 261.0557, found: 261.0554.

4.2.4. Methyl 5-fluoro-2-phenylaminobenzoate (3d). According to the typical procedure, ester **3d** was obtained from commercially available methyl 2-amino-5-fluorobenzoate (**2d**) in 85% yield as a yellow oil after purification by column chromatography (CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 4.04 (s, 3H, OCH_3), 7.17–7.26 (m, 2H, H_{arom}), 7.35–7.39 (m, 3H, H_{arom}), 7.46–7.51 (m, 2H, H_{arom}), 7.77–7.81 (m, 1H, H_{arom}), 9.46 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 51.8 (OCH_3), 112.1 (d, *J*=6.8, C), 115.5 (d, *J*=7.1, CH), 116.6 (d, *J*=23.5, CH), 121.6 (d, *J*=23.0, CH), 121.8, 123.3, 129.3 (CH), 140.8 (C), 144.3 (d, *J*=1.3, C), 155.6 (d, *J*=236.1, C), 167.7 (d, *J*=3.0, CO); IR (film) 3319 (NH), 1690 (CO); MS (EI) *m*/*z* (%) 245 (M^+, 9), 213 (24), 185

(100); HRMS calcd for $C_{14}H_{12}FNO_2$: 245.0852, found: 245.0853.

4.2.5. Methyl 3-phenylaminothiophene-2-carboxylate (3e). According to the typical procedure, ester **3e** was obtained from commercially available methyl 3-aminothiophene-2-carboxylate (**2e**) in 69% yield as a yellowish solid after purification by column chromatography (CH_2Cl_2) followed by crystallization from Et_2O: mp 66–67 °C (Et_2O); ^1H NMR ($CDCl_3$) δ 3.88 (s, 3H, OCH_3), 7.04–7.20 (m, 4H, H_{arom}), 7.31–7.37 (m, 3H, H_{arom}), 8.81 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 51.3 (OCH_3), 102.8 (C), 117.8, 120.2, 122.9, 129.3, 131.7 (CH), 141.3, 151.3 (C), 165.0 (CO); IR (KBr) 3331 (NH), 1667 (CO); MS (EI) *m/z* (%) 233 (M^+, 44), 201 (100), 173 (32); HRMS calcd for $C_{12}H_{11}NO_2S$: 233.0511, found: 233.0519.

4.2.6. Methyl 4-cyano-3-phenylaminothiophene-2-carboxylate (3f). According to the typical procedure, aminoester **3f** was obtained from commercially available methyl 3-amino-4-cyanothiophene-2-carboxylate (**2f**) in 57% yield as a white solid after purification by column chromatography (CH_2Cl_2) followed by crystallization from Et_2O: mp 159–160 °C (Et_2O); ^1H NMR ($CDCl_3$) δ 3.89 (s, 3H, OCH_3), 7.19–7.26 (m, 3H, H_{arom}), 7.36–7.41 (m, 2H, H_{arom}), 7.89 (s, 1H, H_{arom}), 8.62 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 51.9 (OCH_3), 103.2 (C), 105.3 (C), 112.8 (CN), 123.9, 125.9, 129.1, 141.3 (CH), 139.4, 151.1 (C), 163.9 (CO); IR (KBr) 3331 (NH), 2228 (CN), 1667 (CO); MS (EI) *m/z* (%) 258 (M^+, 75), 226 (100), 197 (53), 154 (22), 120 (40), 77 (62); HRMS calcd for $C_{13}H_{10}N_2O_2S$: 258.0463, found: 258.0467.

4.3. Typical procedure for the amidation reaction. Preparation of *para*-methoxyphenylamides 4a–f and 7

4.3.1. Synthesis of *N*-(4-methoxyphenyl)-2-phenylaminobenzamide (4a). A solution of $AlMe_3$ (8.81 mmol, 2.0 M in toluene) was added dropwise to a cooled (0 °C) suspension of *para*-anisidine (1.08 g, 8.81 mmol) in CH_2Cl_2 (45 mL). When the addition was complete, the reaction mixture was allowed to warm to room temperature and stirring was continued for 45 min until the gas evolution ceased. Then, a solution of methyl *N*-phenylanthranilate (**3a**) (1.00 g, 4.40 mmol) in CH_2Cl_2 (8 mL) was added and the mixture was heated under reflux overnight. The reaction mixture was cooled to room temperature and was carefully quenched with 5% aq HCl (20 mL). The organic layer was separated and the aqueous layer extracted with CH_2Cl_2 (3×15 mL). The combined organic extracts were washed with a saturated aqueous solution of $NaHCO_3$ (15 mL) and brine (15 mL). Then, the organic layer was dried over sodium sulfate, filtered, and the solvent was evaporated at reduced pressure. The resulting residue was purified by crystallization from Et_2O to afford benzamide **4a** (69%) as a white solid.[12]

4.3.2. 4,5-Dimethoxy-*N*-(4-methoxyphenyl)-2-phenylaminobenzamide (4b). According to the typical procedure, benzamide **4b** was obtained from *N*-phenylaminoester **3b** in 69% yield as a white solid after purification by crystallization from Et_2O: mp 122–123 °C (Et_2O); ^1H NMR ($CDCl_3$) δ 3.71 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 6.76–6.93 (m, 4H, H_{arom}), 6.99–7.02 (m, 2H, H_{arom}), 7.19–7.36 (m, 5H, H_{arom}), 8.44 (br s, 1H, NH), 8.96 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 55.3, 55.8, 56.5 (OCH_3), 102.5, 111.8 (CH), 113.3 (C), 114.0, 118.7, 121.6, 122.8, 129.4 (CH), 130.8, 139.2, 142.7, 142.9, 152.4, 156.2 (C), 166.4 (CO); IR (KBr) 3313 (NH), 1638 (CO); MS (EI) *m/z* (%) 378 (M^+, 7), 256 (27), 212 (100), 184 (25), 154 (14); HRMS calcd for $C_{22}H_{22}N_2O_4$: 378.1580, found: 378.1579.

4.3.3. 4-Chloro-*N*-(4-methoxyphenyl)-2-phenylaminobenzamide (4c). According to the typical procedure, benzamide **4c** was obtained from *N*-phenylaminoester **3c** in 70% yield as a white solid after purification by crystallization from Et_2O: mp 144–145 °C (Et_2O); ^1H NMR ($CDCl_3$) δ 3.80 (s, 3H, OCH_3), 6.70–6.74 (m, 1H, H_{arom}), 6.89 (d, J=8.7, 2H, H_{arom}), 7.05–7.28 (m, 3H, H_{arom}), 7.31–7.46 (m, 6H, H_{arom}), 7.77 (br s, 1H, NH), 9.35 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 55.4 (OCH_3), 114.2, 114.5 (CH), 116.0 (C), 117.6, 121.7, 122.8, 123.5, 128.6, 129.4 (CH), 130.1, 138.7, 140.2, 147.2, 156.9 (C), 167.1 (CO); IR (KBr) 3308 (NH), 1637 (CO); MS (EI) *m/z* (%) 354 (M^++2, 4), 352 (M^+, 11), 230 (24), 195 (100), 167 (70); HRMS calcd for $C_{20}H_{17}ClN_2O_2$: 352.0979, found: 352.0982.

4.3.4. 5-Fluoro-*N*-(4-methoxyphenyl)-2-phenylaminobenzamide (4d). According to the typical procedure, benzamide **4d** was obtained from *N*-phenylaminoester **3d** in 70% yield as a white solid after purification by crystallization from Et_2O: mp 144–145 °C (Et_2O); ^1H NMR ($CDCl_3$) 3.81 (s, 3H, OCH_3), 6.90 (d, J=8.9, 2H, H_{arom}), 6.97–7.11 (m, 4H, H_{arom}), 7.28–7.36 (m, 4H, H_{arom}), 7.43 (d, J=8.8, 2H, H_{arom}), 7.98 (br s, 1H, NH), 8.54 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 55.4 (OCH_3), 114.0 (d, J=23.4, CH), 119.0 (d, J=30.0, CH), 114.1, 118.9, 119.5 (CH), 121.1 (d, J=5.7, C), 122.1, 122.8, 129.3 (CH), 130.2, 140.9, 142.0, 156.8 (C), 155.7 (d, J=239.0, C), 166.2 (CO); IR (KBr) 3308 (NH), 1643 (CO); MS (EI) *m/z* (%) 336 (M^+, 65), 214 (86), 185 (99), 123 (100), 108 (80), 77 (26); HRMS calcd for $C_{20}H_{17}FN_2O_2$: 336.1274, found: 336.1273.

4.3.5. *N*-(4-Methoxyphenyl)-3-phenylaminothiophene-2-carboxamide (4e). According to the typical procedure, amide **4e** was obtained from *N*-phenylaminoester **3e** in 82% yield as a white solid after purification by crystallization from Et_2O: mp 136–138 °C (Et_2O); ^1H NMR ($CDCl_3$) δ 3.80 (s, 3H, OCH_3), 6.88–6.90 (m, 2H, H_{arom}), 7.00–7.04 (m, 1H, H_{arom}), 7.13–7.43 (m, 9H, H_{arom}+NH), 9.40 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 55.4 (OCH_3), 114.1, 119.6, 119.7, 122.4, 122.9, 127.5, 129.2 (CH), 105.6, 130.4, 141.8, 150.1, 156.6 (C), 163.2 (CO); IR (KBr) 3296 (NH), 1590 (CO); MS (EI) *m/z* (%) 324 (M^+, 46), 201 (95), 173 (81), 158 (65), 123 (100), 108 (72), 77 (53); HRMS calcd for $C_{18}H_{16}N_2O_2S$: 324.0932, found: 324.0936.

4.3.6. 4-Cyano-*N*-(4-methoxyphenyl)-3-phenylaminothiophene-2-carboxamide (4f). According to the typical procedure, amide **4f** was obtained from *N*-phenylaminoester **3f** in 68% yield as a white solid after purification by crystallization from Et_2O: mp 133–134 °C (Et_2O); ^1H NMR ($CDCl_3$) δ 3.76 (s, 3H, OCH_3), 6.80 (d, J=8.8, 2H, H_{arom}), 6.98 (d, J=7.9, 2H, H_{arom}), 7.08 (t, J=7.3, 1H, H_{arom}), 7.27–7.31 (m, 4H, H_{arom}), 7.87 (s, 1H, H_{arom}), 7.88 (br s, 1H, NH), 8.41 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 55.3 (OCH_3), 107.7 (C), 112.9 (CN), 114.0, 119.5, 122.6,

123.7, 129.3 (CH), 129.7 (C), 137.7 (CH), 141.6, 145.3, 156.8 (C), 163.2 (CO); IR (KBr) 3284 (NH), 2250 (CN), 1596 (CO); MS (EI) *m/z* (%) 349 (M^+, 46), 227 (89), 198 (59), 172 (11), 131 (16), 123 (100), 108 (69); HRMS calcd for $C_{19}H_{15}N_3O_2S$: 349.0885, found: 349.0883.

4.3.7. *N*-(4-Methoxyphenyl)-3-phenylaminopropionamide (7). According to the typical procedure, amide **7** was obtained from ethyl 3-phenylaminopropionate[18] (**6**) in 72% yield as a white solid after purification by crystallization from Et_2O: mp 100–101 °C (Et_2O); ^1H NMR ($CDCl_3$) δ 2.62 (t, J=5.8, 2H, CH_2), 3.54 (t, J=5.8, 2H, CH_2), 3.77 (s, 3H, OCH_3), 6.66–6.85 (m, 5H, H_{arom}), 7.17–7.37 (m, 5H, H_{arom}+NH), 7.54 (br s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 36.2, 40.1 (CH_2), 55.4 (OCH_3), 113.3, 113.9, 118.0, 121.9, 129.3 (CH), 130.6, 147.5, 156.3 (C), 170.0 (CO); IR (KBr) 3296 (NH), 1655 (CO); MS (EI) *m/z* (%) 270 (M^+, 20), 165 (29), 123 (41), 108 (43), 106 (100), 77 (67), 51 (31); HRMS calcd for $C_{16}H_{18}N_2O_2$: 270.1368, found: 270.1364.

4.4. Typical procedure for the PIFA-mediated cyclization. Preparation of indazol-3-ones 1a,c,d and pyrazolones 1f and 8

4.4.1. 2-(4-Methoxyphenyl)-1-phenyl-1,2-dihydro-3*H*-indazol-3-one (1a). A solution of PIFA (202 mg, 0.46 mmol) in 46 mL of CH_2Cl_2 was added at 0 °C to a solution of benzamide **4a** (100 mg, 0.32 mmol) and TFA (0.08 mL, 0.94 mmol) in 32 mL of the same solvent, and the new solution was stirred for 1 h. Then, the solvent was evaporated at reduced pressure and the resulting residue was purified by column chromatography (hexanes/EtOAc, 1:1) followed by crystallization from hexanes to afford indazolone **1a** as a white solid (61%).[12]

4.4.2. 6-Chloro-2-(4-methoxyphenyl)-1-phenyl-1,2-dihydro-3*H*-indazol-3-one (1c). According to the general procedure, indazolone **1c** was obtained from benzamide **4c** in 81% yield as a white solid after purification by column chromatography (hexanes/EtOAc, 1:1) followed by crystallization from hexanes: mp 142–143 °C (hexanes); ^1H NMR ($CDCl_3$) δ 3.71 (s, 3H, OCH_3), 6.84 (d, J=8.9, 2H, H_{arom}), 7.14–7.39 (m, 7H, H_{arom}), 7.42 (d, J=8.9, 2H, H_{arom}), 7.85–7.87 (m, 1H, H_{arom}); ^{13}C NMR ($CDCl_3$) δ 55.2 (OCH_3), 112.2, 114.1 (CH), 116.4 (C), 123.9, 124.5, 125.3, 125.4, 127.8 (CH), 128.1 (C), 129.6 (CH), 139.0, 141.0, 149.9, 157.9 (C), 161.6 (CO); IR (KBr) 1690 (CO); MS (EI) *m/z* (%) 352 (M^++2, 18), 350 (M^+, 70), 335 (100), 307 (78), 279 (37); HRMS calcd for $C_{20}H_{15}ClN_2O_2$: 350.0822, found: 350.0817.

4.4.3. 5-Fluoro-2-(4-methoxyphenyl)-1-phenyl-1,2-dihydro-3*H*-indazol-3-one (1d). According to the general procedure, indazolone **1d** was obtained from benzamide **4d** in 77% yield as a pale brown oil after purification by column chromatography (hexanes/EtOAc, 1:1); ^1H NMR ($CDCl_3$) δ 3.73 (s, 3H, OCH_3), 6.84 (d, J=8.8, 2H, H_{arom}), 7.08–7.11 (m, 1H, H_{arom}), 7.22–7.25 (m, 4H, H_{arom}), 7.32–7.37 (m, 2H, H_{arom}), 7.58–7.61 (m, 3H, H_{arom}); ^{13}C NMR ($CDCl_3$) δ 55.1 (OCH_3), 109.2 (d, J=24.1, CH), 113.8 (d, J=8.0, CH), 114.0 (CH), 118.7 (d, J=9.0, C), 121.1 (d, J=26.0, CH), 124.4, 125.3, 127.7 (CH), 127.9 (C), 129.5 (CH), 141.5, 146.1, 157.9 (C), 158.9 (d, J=243.1, C), 161.5 (CO); IR (film) 1684 (CO); MS (EI) *m/z* (%) 334 (M^+, 73), 319 (75), 263 (93), 184 (100), 157 (62); HRMS calcd for $C_{20}H_{15}FN_2O_2$: 334.1118, found: 334.1113.

4.4.4. 6-Cyano-2-(4-methoxyphenyl)-1-phenylthieno[3,2-*c*]pyrazol-3-one (1f). According to the general procedure, pyrazolone **1f** was obtained from amide **4f** in 61% yield as a brownish oil after purification by column chromatography (hexanes/EtOAc, 1:1); ^1H NMR ($CDCl_3$) δ 3.74 (s, 3H, OCH_3), 6.85 (d, J=8.6, 2H, H_{arom}), 7.33–7.35 (m, 7H, H_{arom}), 8.20 (s, 1H, H_{arom}); ^{13}C NMR ($CDCl_3$) δ 55.3 (OCH_3), 97.8, 111.6 (C), 114.3 (CH), 115.3 (CN), 125.8, 126.6 (CH), 127.7 (C), 129.4, 129.6, 146.3 (CH), 138.7, 154.9, 158.1 (C), 158.7 (CO); IR (film) 2290 (CN), 1678 (CO); MS (EI) *m/z* (%) 347 (M^+, 72), 332 (97), 219 (22), 131 (49), 123 (11), 69 (100); HRMS calcd for $C_{19}H_{13}N_3O_2S$: 347.0728, found: 347.0728.

4.4.5. 2-(4-Methoxyphenyl)-1-phenylpyrazol-3-one (8). According to the general procedure, but using TFEA as solvent instead of the combination of CH_2Cl_2/TFA, pyrazolone **8** was obtained from amide **7** in 35% yield as a white solid after purification by column chromatography (hexanes/EtOAc, 1:1) followed by crystallization from hexanes: mp 99–100 °C (Et_2O); ^1H NMR ($CDCl_3$) δ 2.70 (t, J=7.1, 2H, CH_2), 3.75 (s, 3H, OCH_3), 3.99 (t, J=7.1, 2H, CH_2), 6.83 (d, J=8.7, 2H, H_{arom}), 6.96–7.06 (m, 3H, H_{arom}), 7.24–7.30 (m, 2H, H_{arom}), 7.72 (d, J=8.7, 2H, H_{arom}); ^{13}C NMR ($CDCl_3$) δ 31.3, 54.9 (CH_2), 55.3 (OCH_3), 113.9, 118.4, 120.0, 123.5, 129.1 (CH), 131.4, 149.7, 156.2 (C), 171.1 (CO); IR (KBr) 1696 (CO); MS (EI) *m/z* (%) 268 (M^+, 80), 212 (57), 135 (90), 118 (96), 77 (100), 51 (38); HRMS calcd for $C_{16}H_{16}N_2O_2$: 268.1212, found: 268.1218.

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Supplementary data

Supplementary data associated with this article, which include ^1H NMR and ^{13}C NMR spectra of all new compounds, can be found in the online version, at doi:10.1016/j.tet.2006.09.031.

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Preparation and characterization of 3-(4,5-ethylenedithio-1,3-dithiol-2-ylidene)naphthopyranone: a luminescent redox-active donor–acceptor compound

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Abstract—A new 1,3-dithiol-2-ylidene substituted naphthopyranone **2** has been synthesized and characterized. UV–vis spectroscopic and cyclic voltammetry results, interpreted on the basis of density functional theory, show that **2** displays an intramolecular charge-transfer transition and acts like a donor–acceptor (D–A) system. Furthermore, a weak fluorescence originating from the excited charge-transfer state is observed.

1. Introduction

Naphthalene and especially perylene derivatives are known to exhibit interesting conducting and optical properties and they have found applications, for instance as xerographic dyes or in organic photovoltaic solar cells.[1,2] In most of these compounds, the naphthalene or perylene units act as electron-acceptors, which have, as in the case of naphthalenetetracarboxylic dianhydride (NDA) or naphthaldiimide (NDI), electron affinities in the range of tetracyano-*p*-quinodimethane (TCNQ).[2] Remarkably, the perylene unit by itself is also known as one of the earliest donors used in the preparation of highly conducting organic solids.[3] However, the relative instability of the perylene cation, the low solubility, and the absence of peripheral interactions greatly limit the preparation of conducting materials.[3]

In contrast, tetrathiafulvalene (TTF) and its derivates feature unique π-donor properties and they are successfully used as versatile building blocks for charge-transfer salts, which give rise to a multitude of organic conductors and superconductors.[4,5] Furthermore, the capacity to form persistent cation radical and dication species upon oxidation, leads to the formation of mixed-valence systems.[5] As a consequence, TTF derivatives are frequently used as donor units in donor–acceptor (D–A) ensembles, which are of prime interest due to their potential applications in molecular electronics and optoelectronics.[6]

The combination or extension of the naphthalene or perylene units with TTF or 1,3-dithiol-2-ylidene moieties is expected to yield materials with interesting donor–acceptor and/or conducting properties. Very recently, dyads and triads containing one or two TTF units covalently linked through a σ-spacer to perylenediimide (PDI) and NDI have been reported.[7,8] The possibility to develop light intensity dependent molecular electronic switches with these systems has already been demonstrated in a porphyrin–PDI–porphyrin triad.[9] In the case of a TTF–PDI dyad, a reversible modulation of the emission fluorescence intensity in solution by either electron or energy transfer has been achieved.[7] This dyad can therefore be considered as a new kind of molecular redox switch with a delayed optical response.[7]

However, to date neither an example of a TTF unit linked by a π-conjugated spacer to a naphthalene or perylene moiety, nor a naphthalene or perylene core extended by 1,3-dithiol-2-ylidene units has been reported. In the latter case, analogous systems such as furano-quinonoid extended TTFs[10]

Keywords: Naphthopyranone; TTF; Donor–acceptor compound; Cyclic voltammetry; TDDFT calculations; Luminescence.
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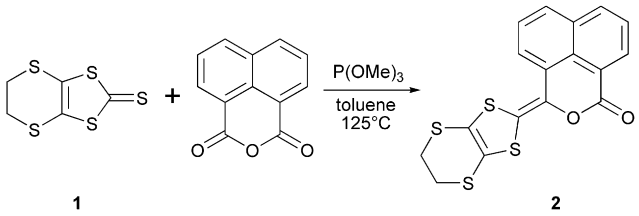
provide the basic synthetic strategy for the preparation of such compounds. Along this line, we describe herein the synthesis and characterization of 1,3-dithiol-2-ylidene substituted naphthopyranone **2**. Structural, spectroscopic, and electrochemical investigations on **2** have been performed and were rationalized on the basis of time-dependent density functional theory (TDDFT).

2. Results and discussion

2.1. Synthesis

Following the procedure for the preparation of furano-quinonoid extended TTFs,[10] compound **2** was obtained in a reasonable yield (54%) from the trimethylphosphite-mediated coupling reaction of 1,8-naphthalic anhydride with 4,5-ethylenedithio-1,3-dithiole-2-thione **1** (Scheme 1). Compound **2** has been fully characterized by NMR, elemental analysis, IR, and MS as listed in Section 4, as well as by single crystal X-ray analysis.



Scheme 1. Synthesis of compound **2**.

2.2. Crystal structure

Compound **2** crystallizes in the monoclinic space group $P2_1$ with one molecule per asymmetric unit. The molecular structure with selected bond distances is shown in Figure 1.

The structural parameters of the planar naphthopyranone unit determined for **2** are comparable with those found for 1,8-naphthalic anhydride.[12] The 4,5-ethylenedithio-1,3-dithiol-2-ylidene entity exhibits a nearly planar geometry typical for this heterocycle in its neutral state.[10a] The whole molecule adopts a conformation, which allows a reduction in steric hindrance between the sulfur atom (S4) of the 1,3-dithiole ring and the hydrogen atom (H15A) of the naphthalene system (interatomic distance S4···H15A is 2.446 Å). The torsion angles C5–C6–C16–C15 and S4–C5–C6–C16 are 12.6° and 2.9°, respectively. Similarly, the bond angles C5–C6–C16 (129.81°) and S4–C5–C6 (125.92°) also reflect this steric repulsion. In the crystal lattice, the molecules are stacked along the *a*-axis in a herringbone type arrangement (Fig. 2). They are linked by some unconventional C–H···O hydrogen bonds but no close S···S contacts can be found.

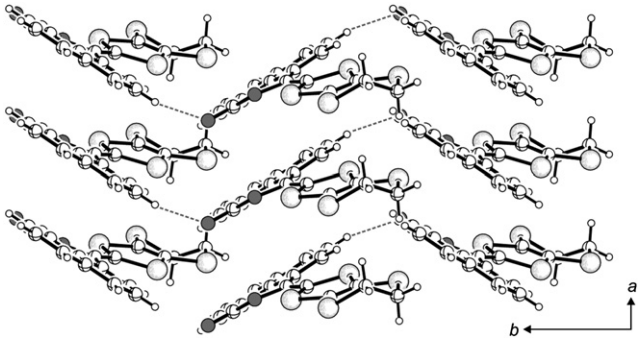


Figure 2. *ab* Projection of the crystal structure of **2**. Stacking of molecules along the *a*-axis in a herringbone type arrangement. Unconventional C–H···O bonds are depicted as dotted lines.

2.3. Electrochemical measurements

The solution redox properties of compound **2** were investigated in dichloromethane by cyclic voltammetry (CV). The cyclic voltammogram is presented in Figure 3. Two quasi-reversible redox processes ($E^{1/2}_{red1}=-1.63$ V, $E^{1/2}_{ox1}=0.74$ V, $\Delta E_{red\text{-}ox}=2.37$ V) and one irreversible oxidation ($E_{peak}=1.27$ V) were observed. The first reduction process corresponds to the reduction of the naphthalene unit. The first oxidation process corresponds to the formation of a

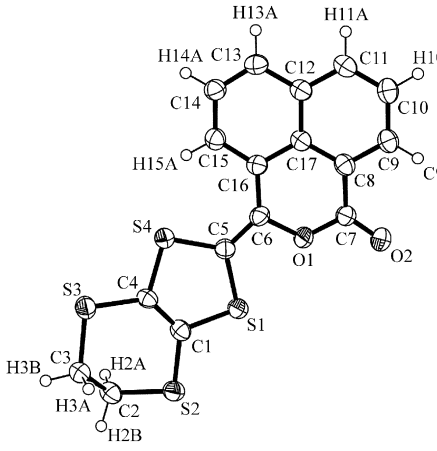


Figure 1. ORTEP[11] representation of the asymmetric unit of **2** (ellipsoids are drawn at 50% probability). Selected bond length [Å]: C5–C6 1.343(7); C5–S1 1.759(5); C5–S4 1.752(5); C6–O1 1.407(6); C7–O1 1.369(6); C7–O2 1.202(6); C1–C4 1.326(7); C1–S1 1.758(5); C4–S4 1.751(5); C1–S2 1.740(5); C4–S3 1.758(4).

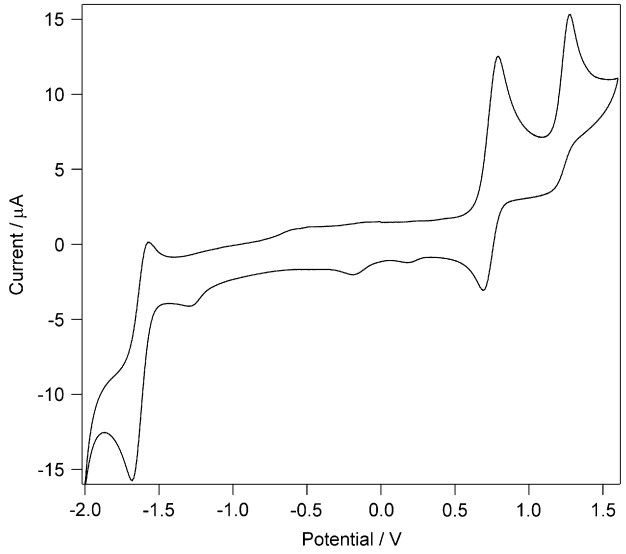


Figure 3. Cyclic voltammogram of **2**, measured under N_2 in CH_2Cl_2 versus Ag/AgCl at room temperature at a scan rate of 100 mV s^{-1}, using 0.1 M Bu_4NPF_6 as electrolyte and Pt as working electrode.

radical cationic species and the second irreversible oxidation correlates with the formation of a thermodynamically unstable dicationic state.

2.4. Photophysical properties and TDDFT calculations

The UV–vis spectrum of **2** shows three strong absorption bands at 33,900 cm^{-1} (294 nm), 30,800 cm^{-1} (324 nm), and 21,500 cm^{-1} (465 nm). In addition, compound **2** shows fluorescence in CH_2Cl_2 with a maximum at 15,700 cm^{-1} (636 nm) and a quantum yield of around 0.5% at room temperature. In Figure 4, the absorption, luminescence and the corresponding excitation spectra are depicted. The latter agrees well with the absorption spectrum of **2**, suggesting that the fluorescence is indeed due to compound **2** and not due to an impurity or a photoproduct. The large Stokes shift of 5800 cm^{-1}, indicating substantial nuclear rearrangements in the excited state, is in line with the comparatively low quantum yield.

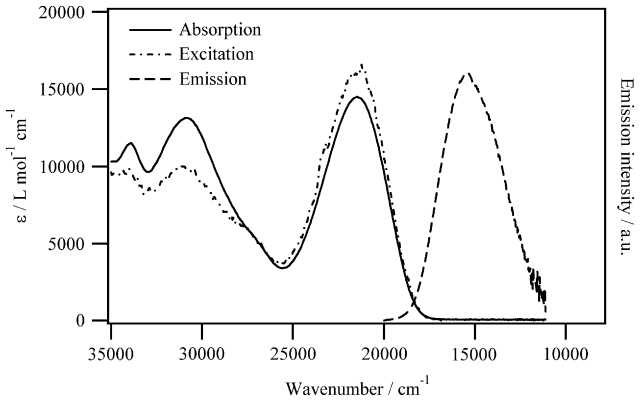


Figure 4. Luminescence (dashed), excitation (dashed-dotted), and absorption (solid) spectra of **2** in CH_2Cl_2 ($c=7.4\times10^{-6}$ M, $\lambda_{ex}=465$ nm, $\lambda_{em}=640$ nm, room temperature).

Moreover, and quite remarkably, the charge-transfer energy calculated from the electrochemical data (Section 2.3) is 2.37 V (≈19,100 cm^{-1}), which is almost the same as the zero-point energy of the charge-transfer excited state evaluated from the crossing-point of the absorption and fluorescence spectra (18,350 cm^{-1}).

Compound **2** can be chemically oxidized to its radical cation $\mathbf{2}^{\bullet+}$ by $FeCl_3$ or $[Fe(bipy)_3](PF_6)_3$. The decrease of the absorption band at 21,500 cm^{-1} upon oxidation goes simultaneously with the appearance of new absorption bands at 19,000 cm^{-1} (526 nm), 15,500 cm^{-1} (645 nm), and 10,900 cm^{-1} (917 nm) together with isosbestic points at 20,000 cm^{-1} and 24,600 cm^{-1}, as shown in Figure 5.

Moreover, in the oxidized form $\mathbf{2}^{\bullet+}$, the fluorescence is completely quenched.

In order to rationalize the electronic absorption spectrum of **2** and of the oxidized species $\mathbf{2}^{\bullet+}$, TDDFT calculations with the B3LYP functional were performed for the low-lying excited states. For comparison, excitation energies have been computed both with the experimental geometry of the neutral **2** and with B3LYP optimized geometries of the neutral and the singly oxidized gas phase species using Ahlichs valence double zeta (VDZ),[13] triple-zeta (TZV) basis sets,[14] and polarization functions[15] with the ORCA[16] implementation of the time-depending DFT method.[17] However, only minor differences could be observed. Therefore, only the excitation energies and absorption intensities of the neutral and the singly oxidized form that have been calculated with the experimental geometry using Ahlichs VDZ basis set[13] are shown in Figure 6 and listed in Table 1. They are in good agreement with the experimental data.

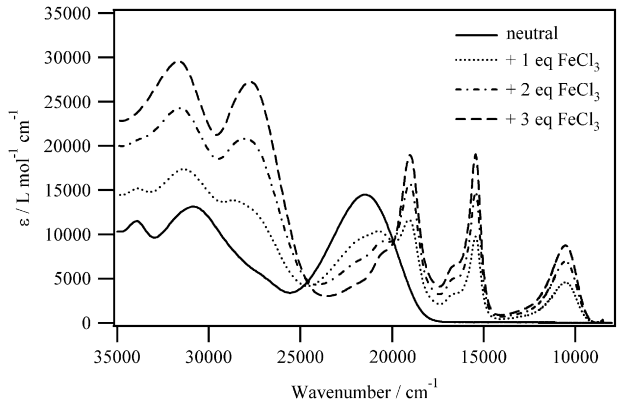


Figure 5. Absorption spectra of **2** in CH_2Cl_2 ($c=2\times10^{-5}$ M) before and after the addition of up to 3 equiv $FeCl_3$.

The HOMO of **2** is a π orbital centered on the 1,3-dithiol-2-ylidene subunit, designated in Figure 6 as orbital 95. In contrast, the LUMO of **2** (orbital 96) is more centered on the naphthopyranone subunit. Therefore, the lowest energy transition corresponds to an intramolecular charge-transfer transition.

Another reason that supports the charge-transfer nature of the absorption band at 21,500 cm^{-1} can be deduced by relating the spectroscopic data with the redox potentials by means of the theory developed by Marcus and Hush.[18,19] This theory correlates a charge-transfer transition with the energetic barrier to thermally activated electron transfer (ΔG^0), which can be estimated from the difference between the redox potentials of the donor and acceptor centers ($\Delta E_{red\text{-}ox}$).[19]

The geometric differences for the ground and excited states represent an asymmetric situation, in which the energy of the charge-transfer transition, E_{abs}, is equal to the energy difference between the initial and final states, plus the reorganizational energy λ (Fig. 7, Eq. 1).

$$E_{abs} = \Delta G^0 + \lambda \quad (1)$$

Therefore, a reorganizational energy $\lambda \approx 2400$ cm^{-1} for the absorption band at 21,500 cm^{-1} can be calculated from the CV data. The energy λ can be correlated to $\Delta\nu_{1/2}$, the bandwidth at half-height of the absorption band, using Eq. 2.[20]

$$\Delta\nu_{1/2} = 48.06\lambda^{1/2} \quad (2)$$

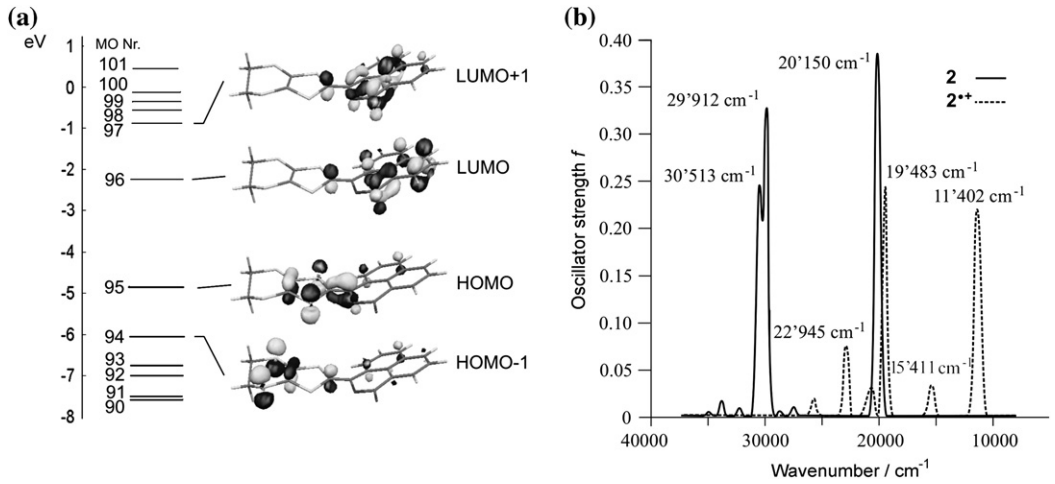


Figure 6. (a) MO scheme for **2**. The HOMO–LUMO excitation essentially corresponds to an intramolecular charge-transfer transition from the 1,3-dithiol-2-ylidene unit to the naphthopyranone part; (b) electronic transitions for **2** and $\mathbf{2}^{\bullet+}$ calculated by TDDFT,[17] B3LYP functional, Ahlrichs VDZ basis set,[13] and ORCA.[16]

Table 1. TDDFT energies, oscillator strengths (*f*) and assignments of the electronic transitions of **2** (geometry of X-ray structure) and $\mathbf{2}^{\bullet+}$ (adopting the X-ray geometry of **2** without change), B3LYP functional, Ahlrichs SVP basis set,[13] ORCA[16,17]

2				$\mathbf{2}^{\bullet+}$			
Transition energy (cm^{-1})	*f*	Assignment		Transition energy (cm^{-1})	*f*	Assignment	
20,150 (*21,500*)	0.40	95→96	89%	11,402 (*10,900*)	0.22	*94→95	90%
		95→97	6%			95→96	7%
27,563	0.01	94→96	64%	15,411 (*15,500*)	0.03	*93→95	85%
		95→97	28%			95→96	7%
28,758	0.01	95→98	30%	18,079	0.00	*92→95	96%
		95→99	31%				
		95→100	30%				
29,912 (*30,800*)	0.33	95→98	39%	19,483 (*19,000*)	0.25	95→96	47%
		95→97	28%			*91→95	16%
		94→96	18%			94→96	12%
						*93→95	9%
30,513 (*33,900*)	0.24	95→97	28%	20,711 (*20,500*)	0.03	*91→95	77%
		95→98	20%			95→96	5%
		95→99	17%			94→96	5%
		95→100	12%				
32,262	0.01	95→100	47%	21,160 (*20,500*)	0.02	*90→95	93%
		95→99	39%				
		92→96	6%				
33,796	0.02	93→96	65%	22,945 (*22,000*)	0.08	94→96	50%
		90→96	13%			95→96	20%
						93→96	10%
34,941	0.01	90→96	56%	25,794	0.02	*89→95	59%
		92→96	27%			94→96	12%
		93→96	6%			95→97	8%

Transitions for $\mathbf{2}^{\bullet+}$, which are not present for **2** because of the doubly filled orbital (95) are denoted by an asterisk (*). Experimental data are given in parentheses.

With this relationship, a theoretical bandwidth of 2350 cm^{-1} can be calculated, which compares well with that one observed experimentally (2200 cm^{-1}).

3. Conclusions

The preparation of a 1,3-dithiol-2-ylidene substituted naphthopyrano compound has been demonstrated. The interpretation of the spectroscopic and cyclic voltammetric data on the basis of time-dependent density functional theory revealed that compound **2** acts as a donor–acceptor (D–A) system. The nature of the strong absorption band at 21,500 cm^{-1} (465 nm) could be assigned to a charge-transfer transition. The corresponding charge-transfer luminescence exhibits a large Stokes shift of 5800 cm^{-1}. Despite the low quantum efficiency of 0.5%, it represents a rare example of luminescence from such a D–A dyad. Therefore, the preparation of a naphthopyrano extended TTF or the attachment of 1,3-dithiol-2-ylidene units to NDA and perylenedicarboxylic anhydride is further investigated in expectation of interesting D–A properties.

4. Experimental

4.1. General considerations

The compound 4,5-ethylenedithio-1,3-dithiole-2-thione **1** was prepared according to the literature procedure.[21] All

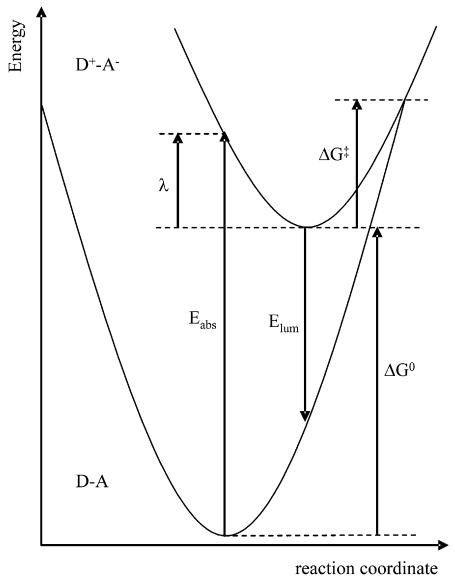


Figure 7. Potential energy surface of a charge-transfer transition in the case of D–A+E_{abs}→D^+–A^-. E_{abs}: optical transition energy, λ: reorganizational energy, ΔG^0: free energy term, $\Delta G^{\ddagger}$: dynamic thermal parameter.[20]

other chemicals and solvents were purchased from commercial sources and were used without further purification. Absorption spectra were recorded on a Cary 50 Bio UV–vis spectrophotometer and on a Bruker IFS66/S NIR spectrophotometer at room temperature. Emission and excitation spectra were measured on a Horiba Fluorolog 3. The solutions were degassed by bubbling nitrogen through them for 20 min before measuring. Compound **2** was measured as a CH_2Cl_2 solution and the oxidation agent $FeCl_3$ was added as a CH_3CN solution.

4.2. Synthesis

4.2.1. 3-(4,5-Ethylenedithio-1,3-dithiol-2-ylidene)-1*H*,3*H*-naphtho[1,8-*cd*]pyran-1-one (2). Compound **1** (0.26 g, 1.2 mmol) and 1,8-naphthalic anhydride (0.15 g, 0.8 mmol) were suspended in 20 ml of dry toluene under N_2. Then, 1.8 ml of $P(OMe)_3$ was added and the yellowish suspension was refluxed for 3 h at 125 °C. The solvent was evaporated from the red mixture by distillation. Purification of the reddish brown solid by column chromatography eluting with a gradient of 0–100% EtOAc in CH_2Cl_2 yielded **2** as red solid. Yield: 0.15 g (54%). Crystals were grown by layer diffusion. A CH_2Cl_2 solution of **2** was superposed by a MeOH layer. Mp 230–233 °C. Anal. Calcd for $C_{17}H_{10}O_2S_4$: C, 54.52; H, 2.69. Found: C, 54.51; H, 2.82. ^{1}H NMR (CD_2Cl_2) δ: 3.30 (s, 4H), 7.29 (dd, J^1=7.4 Hz, J^2=0.8 Hz, 1H), 7.61 (t, J=7.8 Hz, 1H), 7.62 (t, J=7.7 Hz, 1H), 7.72 (d, J=7.9 Hz, 1H), 8.08 (dd, J^1=8.3 Hz, J^2=1.1 Hz, 1H), 8.28 (dd, J^1=7.3 Hz, J^2=1.1 Hz, 1H). ^{13}C NMR (THF-d_8) δ: 30.20, 30.38, 121.42, 123.57, 123.72, 126.49, 127.84, 128.15, 129.03, 129.64, 138.96, 134.60. IR (KBr, cm^{-1}): 3435, 2924, 1735, 1513, 1372, 1352, 1251, 1183, 1148, 1127, 1090, 829, 777, 763, 743, 518. MS (EI) *m*/*z* 374 (M^+).

4.3. X-ray crystallography

An orange rod-like crystal of compound **2** was mounted on a Stoe Mark II-Imaging Plate Diffractometer System[22] equipped with a graphite-monochromator. Data collection were performed at −100 °C using Mo Kα radiation (λ=0.71073 Å). One hundred and twenty exposures (6 min per exposure) were obtained at an image plate distance of 135 mm, φ=0° and 0°<ω<180° with the crystal oscillating through 1.5° in ω. The resolution was D_{min}−D_{max} 23.99−0.82 Å. The structure was solved by direct methods using the program SHELXS-97[23] and refined by full matrix least squares on F^2 with SHELXL-97.[24] All hydrogen atoms were included in calculated positions and treated as riding atoms using SHELXL-97 default parameters. All non-hydrogen atoms were refined anisotropically. An empirical absorption correction was applied using DELrefABS (PLATON03,[25] T_{min}=0.168, T_{max}=0.640). Crystal data have been deposited at the Cambridge Crystallographic Data Centre, reference CCDC 612152. Copy of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

4.4. Computational details

Electronic excitation energies and absorption intensities of the neutral and of the singly ionized form of **2** have been computed using the program package ORCA.[16] The hybrid B3LYP functional and both Ahlrichs valence double zeta (VDZ)[13] and, alternatively for comparison, triple-zeta (TZV) basis sets,[14] and polarization functions[15] have been utilized along with the ORCA implementation of the time-depending DFT method.[17] Excitation energies have been computed both with the experimental geometry of the neutral **2** and with B3LYP optimized geometries of the neutral **2**, and the singly ionized gas phase species $\mathbf{2}^{\bullet+}$.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.09.032.

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Diastereoselective synthesis of γ-hydroxy α,β-epoxyesters and their conversion into β-hydroxy α-sulfenyl γ-butyrolactones

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Abstract—The diastereoselectivity of the nucleophilic epoxidation of γ-hydroxy-α,β-unsaturated esters has been studied. The γ-hydroxy-α,β-unsaturated esters were obtained through treatment of ethyl (*E*)-4-oxo-2-butenoate with the corresponding Grignard reagent and were used as a racemic mixture. The resulting γ-hydroxy α,β-epoxyesters were treated with thiophenol for transformation into α-phenylsulfanyl trisubstituted γ-butyrolactones. The *syn*,*syn*-lactones isomerize easily in basic media into the *syn*,*anti* structures. In order to explain this interconversion, a retroaldol–aldol sequence has been proposed and a sulfur–oxygen interaction has been invoked to explain the *syn* stereochemical preference of the α-sulfured aldols resulting from the intramolecular aldol reaction.

1. Introduction

α,β-Epoxyesters are versatile functionalities in organic synthesis since they can be converted into interesting synthetic compounds through the opening of the oxirane ring.[1] The most convenient method for their preparation is through epoxidation of unsaturated esters using a hydroperoxide in the presence of a base.[2] A deeper understanding of the stereoselectivity of the epoxidation of unsaturated esters would increase the synthetic applications of these intermediates. We previously reported the influence of solvent and temperature on the epoxidation of γ-hydroxy-α,β-unsaturated esters[3] and now wish to report a general study of this reaction including a correction of the previous stereochemical assignment of some of the resulting epoxides.

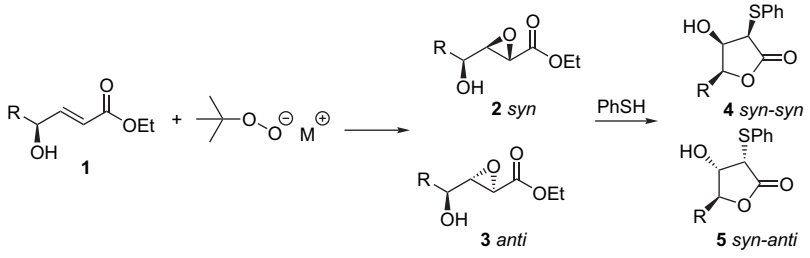
In this paper we also show that the thiophenol-mediated transformation of the γ-hydroxy α,β-epoxyesters into α-phenylsulfanyl γ-butyrolactones **4/5** was useful for the stereochemical determination of the preceding epoxyesters (Scheme 1). Trisubstituted γ-butyrolactones are an interesting family of compounds,[4] which could be then obtained starting from chiral γ-hydroxy α,β-epoxyesters.

The stereoselectivity of the epoxidation reactions was measured as the ratio between *syn*/*anti* diastereomers **2** and **3** (Scheme 1) and must be interpreted as a conjugate addition to an unsaturated ester modulated by a stereocenter in the γ-position.[5]

2. Results

2.1. Preparation of substrates

We wanted to study the selectivity of epoxidation of γ-hydroxy-α,β-unsaturated esters with a range of R alkyl



Scheme 1. General scheme of reactions.

Keywords: Diastereoselective epoxidation; Epoxyesters; Lactonization; γ-Butyrolactones.
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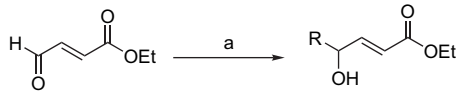
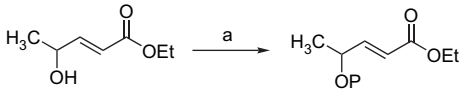
groups. Commonly γ-hydroxy-α,β-unsaturated esters are obtained in enantiopure form through Wittig–Horner reaction of chiral aldehydes or enzymatic resolutions of the corresponding racemic mixtures,[6] we synthesized the γ-hydroxy-α,β-unsaturated esters through treatment of ethyl (*E*)-4-oxo-2-butenoate[3] with the corresponding Grignard reagent and they were used as a racemic mixture in the epoxidation process (Scheme 2).



1a, R=Ph
1b, R=Me
1c, R=*i*-Propyl
1d, R=*i*-Pentyl
1e, R=*n*-Butyl
1f, R=Chx
1g, R=*p*-MeOPh
1h, R=*t*-Butyl

Scheme 2. Preparation of γ-hydroxy-α,β-unsaturated esters: (a) RMgBr, THF, −78 °C–0 °C.

We also prepared *O*-protected α,β-unsaturated esters in order to study the influence of the hydroxyl protecting group on the epoxidation. These compounds were synthesized through protection of compound **1b** via standard conditions (Scheme 3).



1b

6a, P=TPS
6b, P=TIPS
6c, P=MOM

Scheme 3. Protection of γ-hydroxy-α,β-unsaturated esters: (a) P–Cl, base.

2.2. Epoxidation of γ-hydroxy-α,β-unsaturated ethyl esters

Esters **1** were epoxidized using lithium *tert*-butylperoxide as the oxidizing reagent in THF as solvent at −20 °C. Table 1 shows that the diastereomeric ratios are similar for all conditions examined, furnishing the **2** *syn* isomer as the major product. The *syn*/*anti* assignment for **2b/3b** represents a correction to our previous work,[3] an explanation for which is provided subsequently.

These results showed that stereoselectivity does not depend on the nature of the pendant R alkyl group for all γ-hydroxy-α,β-unsaturated esters examined.

In order to study the influence of the temperature over the stereoselectivity, we carried out the epoxidation reaction of compound **1b** at different temperatures (Table 2).

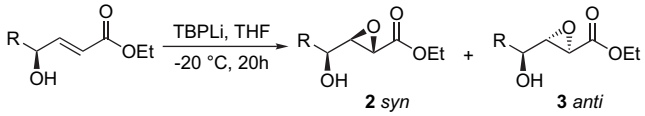
Table 2 shows that there is no temperature dependence since the diastereomeric ratios at different temperatures in a range between −80 and 50 °C are same within experimental error.

Similar $J_{3,4}$ coupling constants were observed for diastereomers *syn* α,β-epoxyesters **2a–h** and also for *anti* α,β-epoxyesters **3a–h** (Table 3). For *syn* isomers, $J_{3,4}$ values were between 3.5 and 4.5 Hz whilst for the *anti* form, $J_{3,4}$ ranged from 2.5 to 3.5 Hz.

Thus, the measurement of $J_{3,4}$ represented a convenient method for the stereochemical assignment of these compounds whenever both isomers were available.

We also epoxidized compound **1b** by using oxidants other than lithium *tert*-butylperoxide (Table 4).

Table 1. Epoxidation of γ-hydroxy-α,β-unsaturated esters using lithium *tert*-butylperoxide

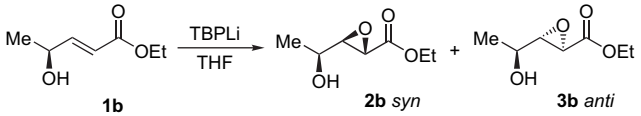


2 *syn* **3** *anti*

Entry	Substrate	R	*T* (°C)/*t* (h)	**2**:**3**[a]	Yield (%)
1	**1a**	Ph	−20/20	80:20	78
2	**1b**	Me	−20/20	70:30	60
3	**1c**	*i*-Pr	−20/20	80:20	55
4	**1d**	*i*-Pent	−20/20	78:22	44
5	**1e**	*n*-Bu	−20/20	81:19	48
6	**1f**	Chx	−20/15	76:24	47
7	**1g**	*p*-MeOPh	−20/15	77:23	69
8	**1h**	*t*-Bu	−20/72	70:30	41

[a] Ratio measured by ^{13}C NMR of the crude reaction mixtures.

Table 2. Epoxidation of **1b** at different temperatures

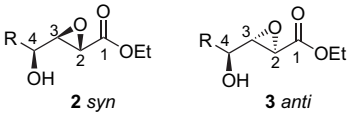


1b **2b** *syn* **3b** *anti*

Entry	*T* (°C)/*t* (h)	**2b**:**3b**[a]	Yield (%)
1	−80/72	73:27	30
2	−60/46	78:22	55
3	−40/24	77:23	54
4	−20/20	70:30	60
5	0/14	74:26	55
6	25/3	78:22	52
7	50/5	76:24	45

[a] Ratio measured by ^{13}C NMR of the crude reaction mixtures.

Table 3. Coupling constants of epoxyalcohols



2 *syn* **3** *anti*

R	$J_{3,4}$ **2** *syn* (Hz)	$J_{3,4}$ **3** *anti* (Hz)
Ph	4.5	2.5
Me	4.2	3.1
i-Pr	4	3
i-Pent	4	3.5
n-Bu	4	[a]
Chx	4	3
p-MeOPh	4.5	2.5
t-Bu	3.5	2.5

[a] Coupling constant could not be measured because of overlapping signals.

Table 4. Epoxidation of ester **1b** using other conditions

Entry	Reagent	Solvent	*T* (°C)/*t*	**2b**:**3b**[b]	Yield (%)
1	TBHP, EtLi	THF	−20/20 h	70:30	60
2	TBHP, EtLi, 12-cr-4[a]	THF	−20/60 h	81:19	39
3	TBHP, EtLi, TMEDA[a]	THF	−20/20 h	88:12	50[c]
4	TBHP, EtLi	THF/HMPA	−20/20 h	88:12	42
5	TBHP, EtLi	THF/DMF	−20/20 h	85:15	51
6	TBHP, Et_2Zn[a]	CH_2Cl_2	0 °C/120 h	20:80	15[c]
7	TBHP, Bu_2Mg	THF	−20/20 h	—	N.R.
8	TBHP, NaH	THF	Rt/1.5 h	56:44	22
9	TBHP, NaH	THF	−20/20 h	79:21	18
10	TBHP, NaH	THF	−80/72 h	68:32	25
11	TBHP, KH	THF	−20/20 h	62:38	30
12	TBHP, Ti(O-*i*-Pr)$_4$	CH_2Cl_2	0/4 days	40:60	74[c]
13	TBHP, EtLi, Ti(O-*i*-Pr)$_4$	THF	Rt/6 days	—	N.R.
14	CMHP, EtLi	THF	−20/20 h	77:23	65
15	*m*-CPBA	CH_2Cl_2	−20/4 days	67:33	70[c]
16	*m*-CPBA, K_2CO_3	CH_2Cl_2	Rt/20 h	—	N.R.
17	TBHP, EtLi	Hexanes	−20/20 h	46:54	25
18	TBHP, EtLi	Toluene	−20/20 h	68:32	53

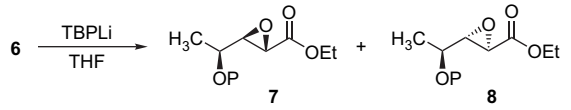
[a] 12-Cr-4 (2.5 equiv) was used in entry 2; 2 equiv of TMEDA were used in entry 3; 1.1 equiv of Et_2Zn were used in entry 6.
[b] Ratio measured by ^{13}C NMR of the crude reaction mixtures.
[c] Starting material was recovered: 31% for entry 3; 29% for entry 6; 50% for entry 12; 25% for entry 15.

If the reaction was carried out using lithium *tert*-butylperoxide in the presence of a cation scavenger (entries 2 and 3) or in a more polar solvent (entries 4 and 5), then a better selectivity was observed. On the other hand, in the alkaline peroxides series, potassium gave poorer stereoselectivity than either lithium or sodium (entries 1 and 8–11). The *anti* isomer predominated when zinc[7] peroxide was used, as opposed to the selectivity achieved using alkaline cations. Magnesium[8] (entry 7) was not reactive. When titanium isopropoxide (entry 12) was used, starting material was recovered and stereoselectivity was poor, furnishing the *anti* isomer as the major product. The reaction using lithium *tert*-butylperoxide and titanium isopropoxide (entry 13) did not give reaction product.

Lithium cumylperoxide gave the same result as lithium *tert*-butylperoxide (entry 14). The rate of the epoxidation using *m*-CPBA[9] (entry 15) was slower than with the other oxidants, affording the *syn* isomer as the major one. When *m*-CPBA was used in the presence of potassium carbonate[10] (entry 16), only starting material was recovered.

If the reaction was carried out using lithium *tert*-butylperoxide in non-polar solvents (entries 17 and 18) then a poor selectivity was observed.

Table 5. Epoxidation of *O*-protected-γ-hydroxy-α,β-unsaturated esters



Entry	*T* (°C)/*t*	**6**	**7**:**8**[a]	Yield (%)
1	Rt/5 days	**6a**	55:45	50
2	2/10 days	**6b**	81:19	56
3	−20/100 h	**6b**	84:16	75[b]
4	−20/5 days	**6c**	63:37	47[b]

[a] Ratio measured by ^{13}C NMR of the crude reaction mixtures.
[b] Starting material was recovered: 50% for entry 3; 44% for entry 4.

O-Protected unsaturated esters **6** were subjected to epoxidation using lithium *tert*-butylperoxide (Table 5).

All the *O*-protected esters (**6a**–**c**) were less reactive than free compound **1b** since the epoxidation reactions took longer time (a few days for all cases). The triisopropylsilyl protecting group gave the best selectivity, furnishing the *syn* isomer **7** as the major one.

Studies to rationalize the stereoselectivity of these epoxidation reactions are currently underway in our laboratory.

2.3. Synthesis of γ-butyrolactones

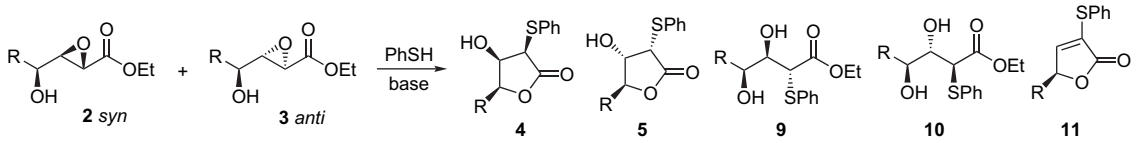
The γ-hydroxy α,β-epoxyesters **2/3** were treated with thiophenol in the presence of a base resulting in the opening of the oxirane ring. The products derived from these reactions were the corresponding γ-butyrolactones **4/5** or the diols **9/10** depending on the conditions and the substrate (Table 6).

When epoxides **2a/3a** (from entry 1, Table 1) were treated with thiophenol in the presence of triethylamine at room temperature for 1 h, only diols **9a/10a** were obtained (entry 1, Table 6) but with longer time, partial cyclization of diols furnished lactones **4a/5a** (entry 2, Table 6) in 1:1 ratio. This mixture of diols and lactones was directly subjected to acid treatment, furnishing a mixture of diastereomeric lactones with lactone **4a** being the major one (Table 7).

When epoxides **2b/3b** (from entry 2, Table 1) were treated with thiophenol and triethylamine in acetonitrile, diol **9b**, lactones **4b/5b** in 1:1 ratio, and butenolide **11b** derived from dehydration of the lactones were obtained (entry 3, Table 6). When the reaction was performed in methanol instead of acetonitrile for longer time (entry 4), only lactones were obtained and, surprisingly, **5b** was the major lactone, thus showing opposite stereoselection to **2a/3a**.

A similar result was obtained using Sharpless conditions with sodium thiophenolate buffered with thiophenol[11] (entry 5).

Table 6. Treatment of epoxyesters **2/3** with thiophenol



Entry	Substrate	*T* (°C)/*t*	Reagents	4	5	9	10	11	Yield (%)
1	**2a/3a** (R=Ph)	Rt/1 h	PhSH/Et_3N/CH_3CN			**9a** (75)	**10a** (25)		65
2	**2a/3a** (R=Ph)	Rt/16 h	PhSH/Et_3N/CH_3CN	**4a** (25)	**5a** (25)	**9a** (50)			—[a]
3	**2b/3b** (R=Me)	0/45′	PhSH/Et_3N/CH_3CN	**4b** (19)	**5b** (23)	**9b** (28)		**11b** (30)	43
4	**2b/3b** (R=Me)	Rt/6 h	PhSH/Et_3N/MeOH	**4b** (32)	**5b** (68)				55
5	**2b/3b** (R=Me)	Rt/2 h	PhSH/PhSNa/THF	**4b** (37)	**5b** (63)				50
6	**2b/3b** (R=Me)	rt/1 h	PhSH/PhSNa/THF	**4b** (21)	**5b** (33)	**9b** (46)			—[a]
7	**2b/3b** (R=Me)	−40/80′	PhSH/PhSNa/THF			**9b** (72)	**10b** (28)		67
8	**2c/3c** (R=*i*-Pr)	Rt/22 h	PhSH/Et_3N/CH_3CN	**4c** (19)	**5c** (17)	**9c** (57)		**11c** (7)	45
9	**2c/3c** (R=*i*-Pr)	Rt/1 h	PhSH/PhSNa/THF	**4c** (33)	**5c** (67)				89
10	**2c** (R=*i*-Pr)	Rt/2 h	PhSH/PhSNa/THF	**4c** (40)	**5c** (60)				70
11	**2e/3e** (R=*n*-Bu)	Rt/2 h	PhSH/PhSNa/THF	**4e** (34)	**5e** (66)				66
12	**2f/3f** (R=Chx)	Rt/2 h	PhSH/PhSNa/THF	**4f** (34)	**5f** (66)				77

[a] Crude oil was directly subjected to acid cyclization (see Table 6).

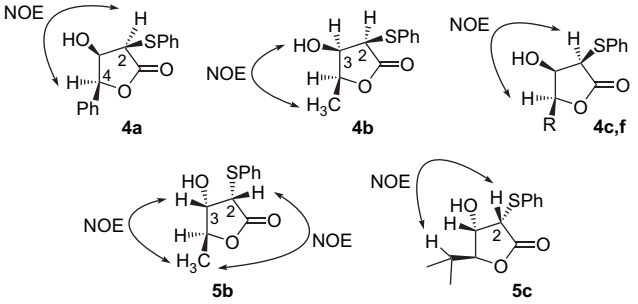
When this reaction was conducted for shorter time (entry 6), diol **9b** and a mixture of lactones were obtained and at low temperature (entry 7) diols **9b/10b** were obtained, furnishing **9b** as the major one. The mixture of diols and lactones obtained from entry 6 was directly subjected to acid treatment (Table 6), furnishing **4b/5b** but **4b** being the major product, a result opposite to that for entries 4 and 5.

The results obtained with epoxides **2c/3c** were similar to **2b/3b**. When epoxides **2c/3c** (entry 3, Table 1) were treated with thiophenol and triethylamine in acetonitrile, then diol **9c** and lactones **4c/5c** were obtained along with butenolide **11c** (entry 8). Using sodium thiophenolate, the result was also similar to **2b/3b**, yielding lactones **4c/5c** (entry 9). Curiously, a mixture of **4c/5c** was obtained when the substrate was pure major epoxyester (entry 10).

The reaction of epoxides **2e/3e** and **2f/3f** (entries 5 and 6, Table 1) with sodium thiophenolate gave a similar result to the other epoxides, furnishing a mixture of lactones **4/5** in 34:66 ratio for both cases (entries 11 and 12).

2.4. Stereochemical determination of lactones 4/5 and diols 9/10

The stereochemical assignment of the lactones was performed by NOE experiments. Lactone **4a** gave NOE between H-2 and H-4 whilst **5a** did not. Lactone **5b** gave NOE between the methyl and H-2 and between the methyl and H-3, whilst the lactone **4b** gave NOE between the methyl and OH but not between the methyl and H-2. **4c** and **4f** gave NOE between the H-2 and H-4 whilst **5c** H-2 gave NOE with the isopropylic hydrogen.



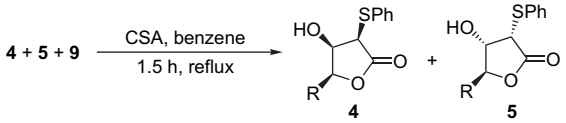
The X-ray crystal structures of **4a–c** confirmed definitively the stereochemistry of *syn*,*syn*-lactones.[12] The conformation of all three γ-butyrolactones in the X-ray structures is very similar, having the R alkyl groups and the thiophenyl substituents in equatorial position and the hydroxyl groups in axial position (Fig. 1).

In comparing the shifts of the ring protons and ring ^{13}C nuclei for all lactones (Table 8) some similarities were observed: *syn*,*anti*-lactones **5** show H2 upfield shift with respect to the *syn*,*syn* **4**, while H3 and H4 appear downfield in **5** with respect to **4**, and C2 appears shifted upfield for **5** while C4 appears downfield in **5** in relation to **4**.[13]

The resulting diols from these reactions were transformed into cyclic carbonates: the mixture of diols **9a/10a** (from entry 1, Table 6) and **9b/10b** (from entry 7, Table 6) was submitted to reaction with triphosgene giving carbonates **12a/13a** and **12b/13b**, respectively (Table 9).

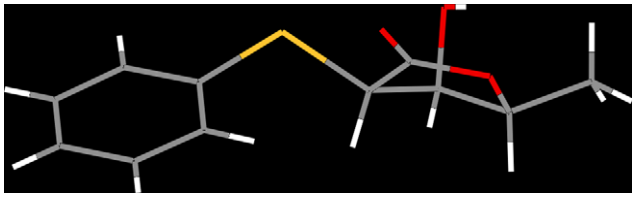
The stereochemistry of the carbonates was assigned by NMR: coupling constants ($J_{4,5}$ for **13**, higher than for **12**) and by NOE experiments.

Table 7. Cyclization of diol **9** in acidic media

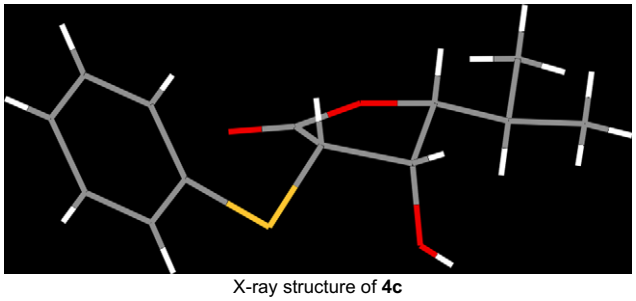


Entry	Substrate	**4**:**5**	Yield (%)
1	Entry 2, Table 6	76:24	57
2	Entry 6, Table 6	65:35	45

X-ray structure of **4a**



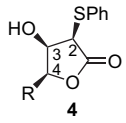
X-ray structure of **4b**



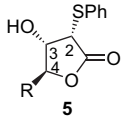
X-ray structure of **4c**

Figure 1. X-ray structures of lactones **4a**, **4b**, and **4c**.

Table 8. ^{1}H and ^{13}C NMR shifts of lactones **4** and **5**



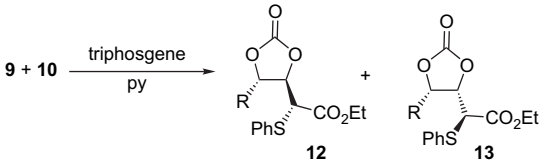
4



5

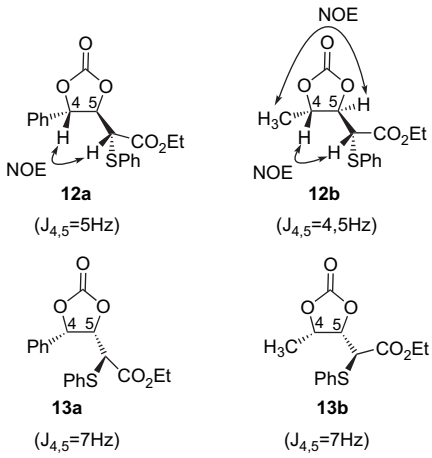
Lactone	δ H2	δ H3	δ H4	δ C2	δ C3	δ C4
4a (R=Ph)	4.25	4.46	5.37	56.5	83.0	71.4
5a (R=Ph)	3.89	4.39	5.50	52.2	83.3	75.5
4b (R=Me)	4.24	4.24	4.51	57.3	79.0	69.9
5b (R=Me)	3.81	4.31	4.65	53.0	78.7	74.7
4c (R=*i*-Pr)	4.25	4.31	3.86	57.6	88.3	68.6
5c (R=*i*-Pr)	3.80	4.35	4.06	53.5	88.4	74.0
4e (R=*n*-Bu)	4.18	4.20	4.26	56.9	83.2	69.4
5e (R=*n*-Bu)	3.73	4.23	4.35	52.9	83.2	74.3
4f (R=Chx)	4.16	4.23	3.88	57.5	87.1	68.3
5f (R=Chx)	3.72	4.29	4.06	53.3	87.0	73.8

Table 9. Cyclization of diols **9/10**



12 **13**

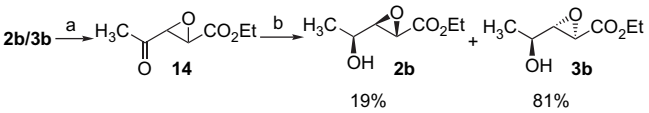
Entry	Diols (**9**/**10**)	**12**	**13**	Yield (%)
1	Entry 1, Table 6	**12a** (75)	**13a** (25)	65
2	Entry 7, Table 6	**12b** (72)	**13b** (28)	42



12a ($J_{4,5}$=5Hz) **12b** ($J_{4,5}$=4,5Hz)

13a ($J_{4,5}$=7Hz) **13b** ($J_{4,5}$=7Hz)

We have also assured the stereochemical assignment of **2b/3b** by reduction of epoxyketone **14** using zinc borohydride. As expected, the reduction of an epoxyketone using zinc borohydride gave the *anti* isomer as the major one[14] (Scheme 4).

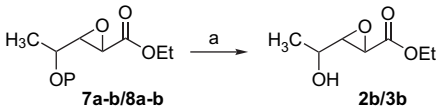


Scheme 4. Reduction of compound **14**: (a) Dess–Martin, 70%; (b) $Zn(BH_4)_2$, 75%.

The NMR spectra of the major isomer were identical to the minor in the **2b/3b** mixture derived from the epoxidation (entry 2, Table 1). The major diastereomer in **2b/3b** mixture was the *syn* one, correcting the stereochemical assignment we previously published.[3]

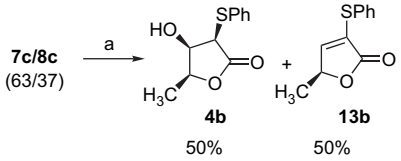
2.5. Stereochemical determination of *O*-protected epoxides

The stereochemistry of the *O*-silyl protected epoxides (**7a–c/8a–c**) was established through deprotection affording the known epoxides **2b/3b** (Scheme 5).



Scheme 5. Deprotection of *O*-silyl protected epoxides: (a) TBAF, THF.

In case of the *O*-methoxymethyl protected epoxides, the stereochemistry was established through treatment with thiol and Lewis acid,[15] which furnished lactone **4b** and butenolide **13b** (Scheme 6).

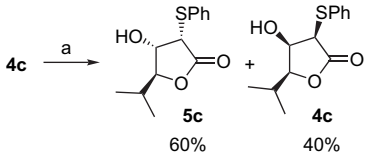


Scheme 6. Deprotection of epoxides **7c/8c**: (a) PhSH, $BF_3 \cdot Et_2O$, 70%.

3. Discussion

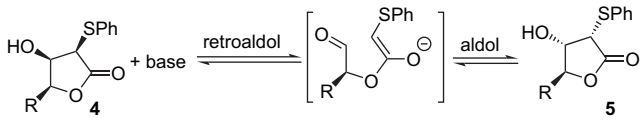
We were unable to make a definitive assignment for the stereochemistry of epoxides **2b/3b** since carbonates and lactones obtained in acidic media would yield as the major epoxide **2b** (same stereoselectivity as epoxides **2a/3a**) but for lactones obtained in basic media, the major isomer would be **3b**, as can also be said for **2c/3c**.

We believe that an isomerization of lactone **4** into lactone **5** occurs during the opening of the epoxides under basic conditions (entries 4 and 5, 9–12 of Table 6). To confirm this speculation, pure lactone **4c** was treated with triethylamine at room temperature and a 4:6 mixture of **4c** and **5c** was obtained (Scheme 7).



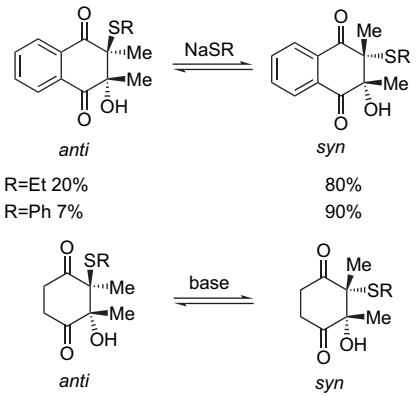
Scheme 7. Basic isomerization of lactone **4c**: (a) Et_3N, THF, 25 °C, 13 h.

The most plausible mechanism for the isomerization is a retroaldol–aldol sequence (Scheme 8). The retroaldol reaction of **4** is forced by the steric hindrance of the substituents into *syn*,*syn* relationship into the cyclic structure, then the resulting enolate–aldehyde intermediate gives an aldol cyclization to furnish a thermodynamic mixture of lactones. The major product is the more thermodynamically stable one, **5**, having substituents in *syn*,*anti* relationship.



Scheme 8. Retroaldol–aldol sequence.

A retroaldol–aldol sequence has been invoked by some authors to explain the isomerization of β-hydroxy α-sulfenyl carbonylic compounds.[16] The two examples shown in Scheme 9 are taken from the literature[16a,b] and both represent isomerizations of *anti* β-hydroxy α-sulfenyl cyclic ketones into the *syn* ones.



Scheme 9. Isomerization of β-hydroxy α-sulfenyl carbonylic compounds.

Both lactones **4** and **5** show a 2,3-*syn* relationship as the *syn*-ketones in Scheme 9.

This tendency of α-hydroxy thiols derived from an intramolecular aldol reaction to be *syn* could be explained by a sulfur–oxygen interaction;[17] this interaction over the enolate–aldehyde would orientate the aldehyde and the sulfured enolate in the *syn* position. A similar sulfur–oxygen interaction has been invoked between the carbonylic oxygen and the thiolic sulfur of 2-mercaptoacetophenones.[17b] The stereochemical outcome in the intramolecular aldol reaction would then be controlled by two factors: a steric factor about 3,4-*anti* relationship in **5** and an S–O interaction about 2,3-*syn* relationship in both **4** and **5**.

4. Conclusions

The stereoselectivity in the nucleophilic epoxidation of γ-hydroxy-α,β-unsaturated esters has been studied. The resulting *syn*/*anti* α,β-epoxyesters have been converted into trisubstituted α-phenylsulfanyl γ-butyrolactones using thiophenol. The *syn*,*syn*-lactones isomerize easily in basic media into the *syn*,*anti* structures. In order to explain this interconversion, a retroaldol–aldol sequence has been proposed and a sulfur–oxygen interaction has been invoked to explain the *syn* stereochemical preference of the α-sulfured aldols resulting from the intramolecular aldol reaction.

5. Experimental section

5.1. General experimental methods

All solvents used in reactions were freshly distilled from appropriate drying agents before use. ^{1}H NMR spectra and ^{13}C NMR spectra were measured in $CDCl_3$ (^{1}H, 7.24 ppm; ^{13}C 77.0 ppm) solution at 30 °C on a 300 MHz Mercury Varian or a 500 MHz Innova Varian NMR spectrometer at the Serveis Centrals d'Instrumentació Científica de la Universitat Jaume I. Mass spectra were measured in a QTOF I (quadrupole–hexapole-TOF) mass spectrometer with an orthogonal Z-spray-electrospray interface (Micromass, Manchester, UK). IR spectra were recorded as oily films on NaCl plates on a Perkin–Elmer 2000 FTIR spectrometer. EM Science Silica Gel 60 was used for column chromatography while TLC was performed with E. Merck precoated plates (Kieselgel 60, F_{254}, 0.25 mm). Unless otherwise specified, all reactions were carried out under argon atmosphere with magnetic stirring.

5.1.1. General experimental procedure for the syntheses of esters 1. To a liquid N_2/acetone cold solution of ethyl (*E*)-4-oxo-2-butenoate (65.1 mmol) in THF (150 mL) was added alkyl magnesium chloride solution (26 mL, 78.1 mmol) drop wise under N_2 atmosphere for a period of 5 min. The resulting mixture was left to warm up to room temperature for 1 h and then quenched with satd aq NH_4Cl solution and extracted with Et_2O (3×40 mL), the organic layers were washed (brine), dried (Na_2SO_4), and concentrated. The crude oil was purified through chromatography (silica-gel, hexanes/EtOAc (8:2) and (7:3)) to afford an oil.

Spectroscopic data for **1a** (yield=29%): ^{1}H NMR ($CDCl_3$) δ 7.32–7.24 (6H, m), 6.98 (1H, dd, J=15.5, 5.5 Hz), 6.09 (1H, dd, J=15.5, 1.5 Hz), 5.30 (1H, m), 4.13 (2H, q, J=7.5 Hz), 2.00 (1H, br s), 1.21 (3H, t, J=7.5 Hz). ^{13}C NMR ($CDCl_3$) δ 166.52, 148.37, 141.02, 128.93, 128.46, 126.63, 120.49, 73.68, 60.55, 14.26 ppm. IR (NaCl) ν 3467, 3064, 3032, 2983, 2937, 2905, 1779, 1717, 1656, 1494, 1455, 1369, 1304, 1175, 1097, 1032, 700 cm^{-1}. HRMS *m/z* calcd for $C_{12}H_{14}O_3Na$ [M+Na$^+$]: 229.0841, found: 229.0814.

Spectroscopic data for **1b** (yield=72%): ^{1}H NMR ($CDCl_3$) δ 6.88 (1H, dd, J=15.8, 4.8 Hz), 5.94 (1H, dd, J=15.6, 1.7 Hz), 4.39 (1H, m), 4.12 (2H, q, J=7.1 Hz), 3.03 (1H, s), 1.26 (3H, d, J=6.6 Hz), 1.22 (1H, t, J=7.1 Hz). ^{13}C NMR ($CDCl_3$) δ 166.76, 151.32, 119.34, 66.84, 60.40, 22.48, 14.07 ppm. IR (NaCl) ν 3429, 2983, 1722, 1448, 1369, 1307, 1185, 1039, 980, 868 cm^{-1}. HRMS *m/z* calcd for $C_7H_{12}O_3$ [M+H$^+$]: 145.0865, found: 145.0828; calcd for $C_7H_{11}O_3Na$ [M+Na$^+$]: 167.0684, found: 167.0488.

Spectroscopic data for **1c** (yield=45%): ^{1}H NMR ($CDCl_3$) δ 6.92 (1H, dd, J=15.8, 5.1 Hz), 6.00 (1H, dd, J=15.6, 1.7 Hz), 4.17 (2H, q, J=7.1 Hz), 4.06 (1H, td, J=5.3, 0.9 Hz), 1.96 (1H, br s), 1.80 (1H, ddd, J=6.8, 6.6, 5.9 Hz), 1.26 (3H, t, J=7.1 Hz), 0.92 (3H, d, J=6.9 Hz), 0.91 (3H, d, J=6.9 Hz). ^{13}C NMR ($CDCl_3$) δ 166.54, 148.91, 121.13, 75.91, 60.43, 33.65, 18.24, 17.43, 14.21 ppm. IR (NaCl) ν 3483, 2964, 2934, 2876, 1722, 1656, 1467, 1370, 1274, 1179, 1036, 873 cm^{-1}. HRMS *m/z* calcd for $C_9H_{16}O_3Na$ [M+Na$^+$]: 195.0997, found: 195.0976.

Spectroscopic data for **1d** (yield=22%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.89 (1H, dd, J=15.6, 4.8 Hz), 5.97 (1H, dd, J=15.6, 1.5 Hz), 4.21 (1H, dq, J=5.4, 1.8 Hz), 4.14 (2H, q, J=6.9 Hz), 2.61 (1H, br s), 1.52 (3H, m), 1.26 (2H, m), 1.24 (3H, t, J=7.2 Hz), 0.84 (3H, d, J=6.6 Hz), 0.83 (1H, d, J=6.6 Hz). ^{13}C NMR ($CDCl_3$) δ 166.71, 150.49, 119.99, 71.24, 60.40, 34.47, 34.19, 27.92, 22.45, 22.36, 14.13 ppm. IR (NaCl) ν 3449, 2957, 1720, 1655, 1467, 1369, 1275, 1177 cm^{-1}. HRMS *m/z* calcd for $C_{11}H_{20}O_3Na$ [M+Na$^+$]: 223.1310, found: 223.1288.

Spectroscopic data for **1e** (yield=44%): ^{1}H NMR ($CDCl_3$) δ 6.93 (1H, dd, J=15.5, 5 Hz), 6.02 (1H, dd, J=15.6, 1.5 Hz), 4.29 (1H, m), 4.20 (2H, q, J=7.1 Hz), 1.82 (1H, d, J=4.2 Hz), 1.59 (2H, m), 1.33 (2H, m), 1.29 (3H, t, J=7.2 Hz), 0.9 (3H, t, J=7.0 Hz). ^{13}C NMR ($CDCl_3$) δ 166.59, 150.21, 120.20, 71.19, 60.45, 36.41, 27.34, 22.56, 14.25, 13.94 ppm. IR (NaCl) ν 3449, 2959, 2872, 1721, 1656, 1466, 1369, 1305, 1275, 1179, 1040, 985 cm^{-1}. HRMS *m/z* calcd for $C_{10}H_{18}O_3Na$ [M+Na$^+$]: 209.1154, found: 209.1141.

Spectroscopic data for **1f** (yield=25%): ^{1}H NMR ($CDCl_3$) δ 6.94 (1H, dd, J=16, 5.5 Hz), 6.01 (1H, dd, J=15.5, 1.5 Hz), 4.19 (1H, q, J=7 Hz), 4.07 (1H, td, J=5.5, 1.5 Hz), 1.97 (1H, br s), 1.62–1.81 (6H, m), 1.4 (1H, m), 1.28 (3H, t, J=7.5 Hz), 0.99–1.33 (4H, m). ^{13}C NMR ($CDCl_3$) δ 166.54, 149.25, 120.95, 75.44, 60.40, 43.58, 36.41, 28.83, 27.98, 26.31, 26.06, 26.01, 14.20 ppm. IR (NaCl) ν 3460, 2925, 2855, 1715, 1658, 1450, 1369, 1276, 1176, 1113, 1039, 985, 893, 869, 733 cm^{-1}. HRMS *m/z* calcd for $C_{12}H_{20}O_3Na$ [M+Na$^+$]: 235.1310, found: 235.1302.

Spectroscopic data for **1g** (yield=31%): ^{1}H NMR ($CDCl_3$) δ 7.19 (2H, d, J=8.5 Hz), 6.97 (1H, dd, J=15.5, 5.0 Hz), 6.82 (2H, d, J=15.5 Hz), 6.06 (1H, d, J=15.5 Hz), 5.19 (1H, d, J=4.5 Hz), 4.12 (2H, q, J=7.5 Hz), 3.37 (1H, br s), 1.23 (3H, t, J=7.5 Hz). ^{13}C NMR ($CDCl_3$) δ 166.57, 159.60, 148.83, 133.19, 127.98, 120.02, 114.19, 73.04, 60.48, 55.31, 14.18 ppm. IR (NaCl) ν 3468, 2982, 2838, 1719, 1655, 1610, 1586, 1513, 1465, 1369, 1304, 1251, 1173, 1095, 1034, 982, 834, 775 cm^{-1}. HRMS *m/z* calcd for $C_{13}H_{16}O_4Na$ [M+Na$^+$]: 259.0946, found: 259.0954.

Spectroscopic data for **1h** (yield=19%): ^{1}H NMR ($CDCl_3$) δ 6.98 (1H, dd, J=16, 5.5 Hz), 6.00 (1H, dd, J=15.5, 1.5 Hz), 4.15 (2H, q, J=7.5 Hz), 3.90 (1H, dd, J=5.5, 1.5 Hz), 1.25 (3H, t, J=7.5 Hz), 0.9 (9H, s). ^{13}C NMR ($CDCl_3$) δ 166.51, 147.84, 121.78, 78.90, 60.35, 35.46, 25.65, 14.15 ppm. IR (NaCl) ν 3497, 2964, 2907, 2873, 1770, 1722, 1655, 1479, 1369, 1280, 1164, 1112, 1038, 871, 774 cm^{-1}. HRMS *m/z* calcd for $C_{10}H_{19}O_3$ [M+H$^+$]: 187.1334, found: 187.1307.

5.1.2. General experimental procedure for the epoxidation of esters 1. To a −78 °C cold THF (3.5 mL) was added TBHP (3.3 M in toluene[18]) (2.2 mmol) and then ethyllithium (0.5 M in benzene/cyclohexane (9:1)[19]) (1.61 mmol). The resulting mixture was stirred at −78 °C for 15 min and then a solution of compound **1** (1.46 mmol) in THF (2 mL) was added drop wise and then the mixture was left at −20 °C (fridge) for 20 h. Then solid Na_2SO_3 (120 mg) was added in one portion and stirred for 15 min, then diluted with satd aq NH_4Cl solution and extracted with Et_2O (3×30 mL), the organic layers were washed (brine), dried (Na_2SO_4), and concentrated. The crude oil was purified through chromatography (silica-gel, hexanes/EtOAc (7:3) and (6:4)).

Spectroscopic data for **2a/3a**: ^{1}H NMR ($CDCl_3$) δ 7.22–7.34 (5H, m, majoritary and minoritary), 4.78 (1H, d, J=2.5 Hz, minoritary), 4.58 (1H, d, J=4.5 Hz, majoritary), 4.14 (2H, m, majoritary and minoritary), 3.63 (1H, d, J=2 Hz, minoritary), 3.50 (1H, d, J=2 Hz, majoritary), 3.41 (1H, dd, J=4.5, 2 Hz, majoritary), 3.35 (1H, t, J=2.5 Hz, minoritary), 1.23 (3H, t, J=6.5 Hz, majoritary and minoritary). ^{13}C NMR ($CDCl_3$) δ 168.71 (minoritary), 168.49 (majoritary), 139.42 (majoritary), 139.67 (minoritary), 128.74, 128.70, 128.49, 128.43, 126.47, 126.39, 72.18 (majoritary), 70.40 (minoritary), 61.75 (majoritary), 61.70 (minoritary), 60.88 (majoritary), 60.64 (minoritary), 51.10 (majoritary), 49.81 (minoritary), 13.99 (majoritary and minoritary) ppm. IR (NaCl) ν 3467, 3058, 3024, 2976, 2926, 2853, 1732, 1452, 1372, 1205, 1093, 1066, 1025, 904, 760, 701 cm^{-1}. HRMS *m/z* calcd for $C_{12}H_{14}O_4Na$ [M+Na$^+$]: 245.079, found: 245.0790.

Spectroscopic data for **2b/3b**: ^{1}H NMR ($CDCl_3$) δ 4.21 (2H, m, majoritary and minoritary), 3.97 (1H, dq, J=6.4, 3.1 Hz), 3.97 (1H, dq, J=6.4, 3.1 Hz, minoritary), 3.78 (1H, dq, J=6.4, 4.2 Hz, majoritary), 3.48 (1H, d, J=2.0 Hz, minoritary), 3.43 (1H, d, J=2.0 Hz, majoritary), 3.20 (1H, m, minoritary), 3.18 (1H, dd, J=4.2, 2.0 Hz, majoritary), 1.28

(3H, d, J=6.6 Hz, majoritary and minoritary), 1.26 (1H, d, J=6.4 Hz, majoritary), 1.25 (1H, d, J=6.2 Hz, minoritary). ^{13}C NMR ($CDCl_3$) δ 168.97 (minoritary), 168.75 (majoritary), 65.96 (majoritary), 64.31 (minoritary), 61.65 (majoritary and minoritary), 61.49 (majoritary), 61.09 (minoritary), 50.88 (majoritary), 49.73 (minoritary), 19.80 (majoritary), 18.75 (minoritary), 14.00 (majoritary and minoritary) ppm. IR (NaCl) ν 3477, 2983, 2921, 1738, 1656, 1452, 1372, 1285, 1202, 1028, 978, 901, 806, 720 cm^{-1}. HRMS m/z calcd for $C_7H_{12}O_4Na$ [M+Na$^+$]: 183.0633, found: 183.0625.

Spectroscopic data for **2c/3c**: ^{1}H NMR ($CDCl_3$) δ 4.21 (2H, m, majoritary and minoritary), 3.63 (1H, dd, J=5.0, 3.0 Hz, minoritary), 3.53 (1H, d, J=1.5 Hz, minoritary), 3.45 (1H, d, J=2.0 Hz, majoritary), 3.35 (1H, d, J=4.0, 6.5 Hz, majoritary), 3.26 (1H, dd, J=4.0, 2.0 Hz, majoritary), 3.24 (1H, dd, J=3.0, 2.0 Hz, minoritary), 1.87 (2H, m, majoritary and minoritary), 1.29 (3H, t, J=7.0 Hz, majoritary and minoritary), 1.00 (3H, d, J=6.5 Hz, majoritary and minoritary), 0.98 (3H, d, J=6.5 Hz, majoritary and minoritary). ^{13}C NMR ($CDCl_3$) δ 169.09 (minoritary), 168.89 (majoritary), 74.29 (majoritary), 72.31 (minoritary), 61.75 (majoritary), 61.69 (minoritary), 59.34 (minoritary), 59.22 (minoritary), 50.81 (majoritary), 49.73 (minoritary), 32.69 (majoritary), 31.61 (minoritary), 18.46 (majoritary), 18.75 (minoritary), 17.92 (majoritary), 17.34 (minoritary), 14.12 (majoritary and minoritary) ppm. IR (NaCl) ν 3497, 2964, 2877, 1732, 1468, 1371, 1285, 1244, 1199, 1028, 905 cm^{-1}. HRMS m/z calcd for $C_9H_{16}O_4Na$ [M+Na$^+$]: 211.0946, found: 211.0926.

Spectroscopic data for **2d/3d**: ^{1}H NMR ($CDCl_3$) δ 4.17 (2H, m, majoritary and minoritary), 3.74 (1H, dq, J=4.0, 3.5 Hz, minoritary), 3.53 (1H, dq, J=4.0, 4.0 Hz, majoritary), 3.46 (1H, d, J=2.0 Hz, minoritary), 3.42 (1H, d, J=2.5 Hz, majoritary), 3.16 (1H, dd, J=4.0, 2.0 Hz, majoritary), 3.15 (minoritary, signal overlapped with majoritary), 2.11 (1H, br s), 1.46–1.58 (4H, m, majoritary and minoritary), 1.32 (1H, m, majoritary and minoritary), 1.24 (3H, t, J=7.0 Hz, majoritary and minoritary), 0.84 (3H, d, J=7.0 Hz, majoritary and minoritary), 0.83 (3H, d, J=7.0 Hz, majoritary and minoritary). ^{13}C NMR ($CDCl_3$) δ 169.09 (minoritary), 168.81 (majoritary), 69.93 (majoritary), 68.33 (minoritary), 61.66 (majoritary), 61.64 (minoritary), 60.73 (majoritary), 60.45 (minoritary), 50.89 (majoritary), 49.69 (minoritary), 34.23 (majoritary), 34.10 (minoritary), 32.30 (majoritary), 31.26 (minoritary), 28.01 (minoritary), 27.93 (majoritary), 22.46 (majoritary), 22.34 (minoritary), 14.03 (majoritary and minoritary) ppm.

Spectroscopic data for **2e/3e**: ^{1}H NMR ($CDCl_3$) δ 4.21 (2H, m, majoritary and minoritary), 3.71 (1H, m, minoritary), 3.58 (1H, m, majoritary), 3.49 (1H, d, J=1.8 Hz, minoritary), 3.44 (1H, d, J=2.0 Hz, majoritary), 3.17 (1H, dd, J=4.0, 2.0 Hz, majoritary), 3.17 (minoritary, signal overlapped with majoritary), 1.58 (2H, m), 1.33 (4H, m), 1.27 (3H, t, J=7.1 Hz, majoritary and minoritary), 0.88 (1H, t, J=7.1 Hz, majoritary and minoritary). ^{13}C NMR ($CDCl_3$) δ 169.04 (minoritary), 168.80 (majoritary), 69.66 (majoritary), 67.99 (minoritary), 61.68 (majoritary), 61.65 (minoritary), 60.74 (majoritary), 60.45 (minoritary), 50.89 (majoritary), 49.63 (minoritary), 34.14 (majoritary), 33.05 (minoritary), 27.32 (majoritary), 27.20 (minoritary), 22.59 (minoritary), 22.53 (majoritary), 14.05 (majoritary and minoritary), 13.87 (majoritary and minoritary) ppm. IR (NaCl) ν 3480, 2934, 2860, 1738, 1633, 1468, 1372, 1200, 1031, 959, 906, 809, 748 cm^{-1}. HRMS m/z calcd for $C_{10}H_{18}O_4Na$ [M+Na$^+$]: 225.1103, found: 225.1109; calcd for $C_{10}H_{18}O_4K$ [M+K$^+$]: 241.842, found: 241.0849.

Spectroscopic data for **2f/3f**: ^{1}H NMR ($CDCl_3$) δ 4.18 (2H, m, majoritary and minoritary), 3.56 (1H, dd, J=5.5, 3.0 Hz, minoritary), 3.46 (1H, d, J=2.0 Hz, minoritary), 3.39 (1H, d, J=2.0 Hz, majoritary), 3.28 (1H, dd, J=6.5, 4.0 Hz, majoritary), 3.21 (1H, dd, J=4.0, 2.0 Hz, majoritary), 3.19 (1H, dd, J=3.0, 2.0 Hz, minoritary), 1.97 (1H, br s), 1.58–1.87 (5H, m, majoritary and minoritary), 1.50 (1H, m, majoritary and minoritary), 1.24 (3H, t, J=7.5 Hz, majoritary and minoritary), 0.95–1.22 (5H, m, majoritary and minoritary). ^{13}C NMR ($CDCl_3$) δ 169.10 (minoritary), 168.84 (majoritary), 73.77 (majoritary), 71.90 (minoritary), 61.67 (majoritary), 61.63 (minoritary), 59.43 (majoritary), 59.22 (minoritary), 50.90 (majoritary), 49.78 (minoritary), 42.30 (majoritary), 41.43 (minoritary), 28.77 (minoritary), 28.71 (majoritary), 28.43 (majoritary), 27.78 (minoritary), 26.28 (minoritary), 26.24 (majoritary), 26.08 (minoritary), 25.96 (minoritary), 25.94 (majoritary), 25.84 (majoritary), 14.07 (majoritary and minoritary), 13.87 (majoritary and minoritary) ppm. IR (NaCl) ν 3484, 2928, 2855, 1737, 1450, 1371, 1282, 1199, 1096, 1030, 906, 735 cm^{-1}. HRMS m/z calcd for $C_{12}H_{20}O_4Na$ [M+Na$^+$]: 251.1259, found: 251.1255; calcd for $C_{12}H_{20}O_4K$ [M+K$^+$]: 267.0999, found: 267.1006.

Spectroscopic data for **2g/3g**: ^{1}H NMR ($CDCl_3$) δ 7.33 (2H, d, J=9.0 Hz, majoritary), 7.30 (2H, d, J=8.5 Hz, minoritary), 6.90 (2H, d, J=8.5 Hz, majoritary and minoritary), 4.86 (1H, d, J=2.5 Hz, minoritary), 4.61 (1H, d, J=4.0 Hz, majoritary), 4.20 (2H, m, majoritary and minoritary), 3.68 (1H, d, J=2 Hz, minoritary), 3.54 (1H, d, J=1.5 Hz, majoritary), 3.45 (1H, dd, J=4.5, 2 Hz, majoritary), 3.39 (1H, dd, J=2.5, 2.0 Hz, minoritary), 2.55 (1H, br s, majoritary), 1.84 (1H, br s, minoritary), 1.27 (3H, t, J=7.0 Hz, majoritary), 1.26 (3H, t, J=7.0 Hz, minoritary). ^{13}C NMR ($CDCl_3$) δ 168.75 (minoritary), 168.56 (majoritary), 159.83 (minoritary), 159.74 (majoritary), 131.67 (majoritary), 130.68 (minoritary), 127.90 (minoritary), 127.81 (majoritary), 114.18 (majoritary and minoritary), 71.79 (majoritary), 70.03 (minoritary), 61.76 (majoritary), 61.72 (minoritary), 60.93 (majoritary), 60.63 (minoritary), 55.30 (majoritary and minoritary), 51.10 (majoritary), 49.82 (minoritary), 14.04 (majoritary and minoritary) ppm. IR (NaCl) ν 3483, 3058, 2985, 2939, 2909, 2839, 1740, 1613, 1586, 1515, 1466, 1444, 1421, 1372, 1304, 1225, 1202, 1178, 1113, 1096, 1069, 1032, 964, 907, 836, 737, 703 cm^{-1}. HRMS m/z calcd for $C_{13}H_{16}O_5Na$ [M+Na$^+$]: 275.0895, found: 275.0855; calcd for $C_{13}H_{16}O_5K$ [M+K$^+$]: 291.0635, found: 291.0657.

Spectroscopic data for **2h/3h**: ^{1}H NMR ($CDCl_3$) δ 6.98 (1H, dd, J=16, 5.5 Hz), 6.00 (1H, dd, J=15.5, 1.5 Hz), 4.15 (2H, q, J=7.5 Hz), 3.90 (1H, dd, J=5.5, 1.5 Hz), 1.25 (3H, t, J=7.5 Hz), 0.9 (9H, s). ^{13}C NMR ($CDCl_3$) δ 166.51, 147.84, 121.78, 78.90, 60.35, 35.46, 25.65, 14.15 ppm. IR (NaCl) ν 3497, 2964, 2907, 2873, 1770, 1722, 1655, 1479, 1369, 1280, 1164, 1112, 1038, 871, 774 cm^{-1}. HRMS m/z calcd for $C_{10}H_{18}O_4Na$ [M+Na$^+$]: 225.1103, found:

225.1103; calcd for $C_{10}H_{18}O_4Na$ [M+K$^+$]: 241.0842, found: 241.0882.

5.1.2.1. 4-(*tert*-Butyl-diphenyl-silanyloxy)-pent-2-enoic acid ethyl ester 6a. To an ice-bath cold solution of compound **1b** (266 mg, 1.85 mmol) in DMF (5 mL) was added imidazole (138 mg, 2.03 mmol) and then *tert*-butyldiphenylsilylchloride (528 μL, 2.03 mmol). The resulting mixture was stirred at room temperature (21 °C) for 70 h and then was quenched with brine and extracted with Et_2O (3×30 mL), the organic layers were washed (brine), dried (Na_2SO_4), and concentrated. The crude oil was purified through chromatography (silica-gel, hexanes/EtOAc (9:1) to afford 542 mg (77%) of an oil. ^{1}H NMR ($CDCl_3$) δ 7.76 (2H, dd, J=7.7, 1.3 Hz), 7.71 (2H, dd, J=8.1, 1.5 Hz), 7.41 (6H, m), 6.99 (1H, dd, J=15.6, 4.6 Hz), 6.10 (1H, dd, J=15.6, 1.5 Hz), 4.54 (1H, m), 4.24 (2H, m), 1.32 (3H, t, J=7.2 Hz), 1.20 (1H, d, J=6.4 Hz), 1.17 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 166.42, 151.16, 135.66, 135.62, 133.85, 133.29, 129.66, 127.53, 127.50, 119.16, 68.59, 60.02, 26.88, 23.18, 19.10, 14.13 ppm. IR (NaCl) ν 3072, 3050, 2960, 2859, 1722, 1428, 1369, 1295, 1157, 1112, 978, 822, 741, 703 cm^{-1}. HRMS m/z calcd for $C_{23}H_{30}O_3SiNa$ [M+Na$^+$]: 405.1862, found: 405.1822.

5.1.2.2. 4-Triisopropylsilanyloxy-pent-2-enoic acid ethyl ester 6b. To an ice-bath cold solution of compound **1b** (582 mg, 4.04 mmol) in DMF (12 mL) was added imidazole (358 mg, 5.25 mmol) and then triisopropylsilylchloride (980 μL, 4.44 mmol). The resulting mixture was stirred at room temperature (21 °C) for 42 h and then was quenched with brine and extracted with Et_2O (3×30 mL), the organic layers were washed (brine), dried (Na_2SO_4), and concentrated. The crude oil was purified through chromatography (silica-gel, hexanes/EtOAc (9:1)) to afford 1 g (83%) of an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.88 (1H, dd, J=15.3, 4.2 Hz), 5.95 (1H, dd, J=15.3, 1.8 Hz), 4.51 (1H, dq, J=6.3, 1.8 Hz), 4.12 (2H, q, J=6.9 Hz), 1.23 (3H, d, J=6.3 Hz), 1.22 (3H, t, J=6.3 Hz), 1.01 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 166.59, 151.99, 118.92, 67.81, 60.07, 23.84, 17.92, 17.90, 14.13, 12.24 ppm. IR (NaCl) ν 2930, 2868, 1723, 1465, 1369, 1294, 1159, 1094, 883 cm^{-1}. HRMS m/z calcd for $C_{16}H_{32}O_3SiNa$ [M+Na$^+$]: 323.2018, found: 323.1956; calcd for $C_{16}H_{33}O_3Si$ [M+H$^+$]: 301.2199, found: 301.2172.

5.1.2.3. 4-Methoxymethoxy-pent-2-enoic acid ethyl ester 6c. To an ice-bath cold solution of compound **1b** (730 mg, 5.1 mmol) in CH_2Cl_2 (16 mL) were added diisopropylethylamine (3.55 ml, 20.4 mmol), chloromethyl methyl ether (1.55 ml, 20.4 mmol), and then 4-dimethylaminopyridine (464 mg, 3.8 mmol). The resulting mixture was stirred at room temperature (23 °C) for 20 h and then was quenched with brine and extracted with CH_2Cl_2 (3×30 mL), the organic layers were washed (brine), dried (Na_2SO_4), and concentrated. The crude oil was purified through chromatography (silica-gel, hexanes/EtOAc (8:2) and (7:3)) to afford 460 mg (48%) of an oil. ^{1}H NMR ($CDCl_3$) δ 6.58 (1H, dd, J=15.5, 6.0 Hz), 5.72 (1H, dd, J=15.5, 1.5 Hz), 4.35 (2H, s), 4.08 (2H, dq, J=7.0, 7.0 Hz), 3.92 (2H, q, J=7.5 Hz), 3.08 (s, 3H), 1.03 (3H, d, J=6.5 Hz), 1.01 (3H, t, J=7.0 Hz). ^{13}C NMR ($CDCl_3$) δ 165.45, 148.31, 120.36, 93.91, 70.49, 59.63, 54.56, 19.92, 13.61 ppm. IR (NaCl) ν 2981, 2976, 2824, 1718, 1659, 1449, 1370, 1299, 1272, 1158, 1099, 1033, 982, 919, 869, 734 cm^{-1}. HRMS m/z calcd for $C_9H_{16}O_4Na$ [M+Na$^+$]: 211.0946, found: 211.0919.

5.1.3. General experimental procedure for the epoxidation of esters 6. To a −78 °C cold THF (3.5 mL) was added TBHP (3.3 M in toluene[18]) (600 μL, 1.97 mmol) and then ethyllithium (0.5 M in benzene/cyclohexane (9:1)) (2.9 mL, 1.45 mmol), gas evolution was observed. The resulting mixture was stirred at −78 °C for 15 min and then a solution of compound **6** (1.31 mmol) in THF (2 mL) was added drop wise and then the mixture was stirred at the desired temperature for the desired time (see Table 4). Then solid Na_2SO_3 (110 mg) was added in one portion and stirred for 15 min, then diluted with satd aq NH_4Cl solution and extracted with Et_2O (3×30 mL), the organic layers were washed (brine), dried (Na_2SO_4), and concentrated. The crude oil was purified through chromatography (silica-gel, hexanes/EtOAc (7:3)) to afford an oil.

Spectroscopic data for **7a/8a**: ^{1}H NMR ($CDCl_3$) δ 7.61 (4H, m), 7.27 (6H, m), 4.11 (2H, m), 3.67 (1H, m), 3.23 (1H, d, J=2 Hz), 3.17 (1H, d, J=5.2 Hz), 3.13 (1H, d, J=1.5 Hz), 3.06 (1H, dd, J=4.5, 1.5 Hz), 1.18 (3H, t, J=7 Hz), 1.08 (3H, d, J=6.5 Hz), 1.01(1H, d, J=6.5 Hz), 0.98 (9H, m). ^{13}C NMR ($CDCl_3$) δ 169.02, 168.92, 135.90, 135.83, 135.81, 135.32, 134.80, 133.97, 133.77, 133.32, 133.14, 129.82, 129.77, 129.73, 129.56, 127.66, 127.65, 127.60, 68.89, 67.38, 61.94, 61.53, 61.48, 61.30, 50.99, 50.77, 26.91, 26.85, 26.57, 20.55, 19.76, 19.23, 19.18, 18.99, 14.09, 14.07 ppm. IR (NaCl) ν 3072, 2960, 2932, 2892, 2858, 1737, 1472, 1428, 1391, 1374, 1305, 1197, 1113, 1029, 998, 822, 740, 702 cm^{-1}. HRMS m/z calcd for $C_7H_{12}O_4Na$ [M+Na$^+$]: 421.1811, found: 421.1844.

Spectroscopic data for **7b/8b**: ^{1}H NMR ($CDCl_3$) δ 4.20 (2H, m, majoritary and minoritary), 3.97 (1H, dq, J=6.5, 4.0 Hz, minoritary), 3.80 (1H, dq, J=5.5, 6.5 Hz, majoritary), 3.42 (1H, d, J=2.0 Hz, minoritary), 3.31 (1H, d, J=2.0 Hz, majoritary), 3.19 (1H, dd, J=5.5, 2.0 Hz, majoritary), 3.10 (1H, dd, J=4.0, 2.0 Hz), 1.27 (3H, t, J=7.5 Hz, minoritary), 1.26 (3H, t, J=7.0 Hz, majoritary), 1.23 (3H, d, J=6.5 Hz, majoritary), 1.23 (3H, d, J=6.5 Hz, minoritary), 1.04 (21H, m, majoritary and minoritary). ^{13}C NMR ($CDCl_3$) δ 169.28 (minoritary), 168.97 (majoritary), 68.41 (majoritary), 66.07 (minoritary), 62.29 (majoritary), 61.63 (minoritary), 61.53 (majoritary), 61.48 (minoritary), 50.79 (majoritary), 50.52 (minoritary), 20.48 (minoritary), 20.33 (majoritary), 17.95, 17.91 (majoritary and minoritary), 14.06 (majoritary and minoritary), 12.29 (majoritary and minoritary) ppm. IR (NaCl) ν 2944, 2887, 2860, 1755, 1465, 1373, 1284, 1244, 1196, 1170, 1122, 1099, 1059, 1031, 999, 907, 883, 827, 761, 681 cm^{-1}. HRMS m/z calcd for $C_{16}H_{32}SiO_4Na$ [M+Na$^+$]: 339.1968, found: 339.1966.

Spectroscopic data for **7c/8c**: ^{1}H NMR ($CDCl_3$) δ 4.64 (1H, d, J=7.0 Hz, minoritary), 4.55 (1H, d, J=7.0 Hz, minoritary), 4.54 (1H, d, J=7.0 Hz, majoritary), 4.51 (1H, d, J=7.0 Hz, majoritary), 4.12 (2H, m, majoritary and minoritary), 3.55 (1H, dq, J=6.5, 5.0 Hz, majoritary), 3.54 (1H, dq, J=6.5, 6.0 Hz, majoritary), 3.33 (1H, d, J=2.0 Hz, majoritary), 3.26 (3H, s, minoritary), 3.23 (3H, s), 3.22 (1H, d,

J=2.0 Hz, minoritary), 3.13 (1H, dd, J=6.0, 2.0 Hz, minoritary), 3.05 (1H, dd, J=5.0, 2.0 Hz, majoritary), 1.19 (3H, t, J=6.5 Hz, majoritary and minoritary), 1.16 (3H, d, J=6.5 Hz, majoritary and minoritary). ^{13}C NMR ($CDCl_3$) δ 168.77 (majoritary), 168.66 (minoritary), 95.61 (majoritary), 95.07 (minoritary), 71.67 (minoritary), 71.14 (majoritary), 61.65 (minoritary), 61.53 (majoritary), 60.57 (minoritary), 59.96 (majoritary), 55.43 (majoritary), 55.30 (minoritary), 51.44 (majoritary), 50.14 (minoritary), 17.75 (majoritary), 17.04 (minoritary), 14.04 (majoritary), 14.02 (minoritary) ppm. HRMS m/z calcd for $C_9H_{16}O_5Na$ [M+Na$^+$]: 227.0895, found: 227.0874.

5.1.4. General experimental procedure for the treatment of 2 with sodium thiophenolate to furnish 4/5. An ice-bath cold suspension of sodium hydride (60% in mineral oil) (1.12 mmol) in THF (1 mL) was treated with thiophenol (2.25 mmol). The mixture was stirred at room temperature for 15 min and then a solution of the epoxyester **2** (0.75 mmol) in THF (1 mL) was added drop wise and the mixture was stirred at room temperature for the required time (see Table 5). Then brine was added and extracted with Et_2O (3×20 mL), the organic layers were washed (brine), dried (Na_2SO_4), and concentrated. The crude oil was purified through chromatography (silica-gel, hexanes/EtOAc (7:3) and (6:4)).

Spectroscopic data for **4a**: ^{1}H NMR ($CDCl_3$) δ 7.50–7.53 (m, 2H), 7.35–7.45 (8H, m), 5.47 (1H, d, J=3.0 Hz), 4.56 (1H, ddd, J=4.0, 3.0, 2.0 Hz), 4.35 (1H, d, J=4.0 Hz), 2.37 (1H, d, J=2.0 Hz). ^{13}C NMR ($CDCl_3$) δ 172.63, 133.04, 132.59, 129.57, 129.05, 128.71, 128.59, 126.52, 82.98, 71.38, 56.64 ppm. IR (NaCl) ν 3460, 3060, 2916, 1951, 1886, 1752, 1581, 1498, 1456, 1438, 1326, 1290, 1214, 1179, 1110, 1024, 994, 954, 942, 815, 790, 701, 634 cm^{-1}. HRMS m/z calcd for $C_{16}H_{14}O_3SNa$ [M+Na$^+$]: 309.0562, found: 309.0580 (recrystallized from hexanes/EtOAc, mp 151.5–154.5 °C).

Spectroscopic data for **5a**: ^{1}H NMR ($CDCl_3$) δ 7.48–7.50 (m, 2H), 7.24–7.35 (8H, m), 5.49 (1H, d, J=4.0 Hz), 4.38 (1H, dd, J=4.0, 1.0 Hz), 3.89 (1H, d, J=1.0 Hz), 2.20 (1H, s). ^{13}C NMR ($CDCl_3$) δ 173.05, 132.93, 132.54, 131.21, 129.53, 129.12, 128.98, 128.89, 126.31, 83.27, 75.49, 52.17 ppm. IR (NaCl) ν 3389, 3053, 2987, 1776, 1440, 1265, 1156, 1070, 1023, 909, 650 cm^{-1}. HRMS m/z calcd for $C_{16}H_{14}O_3SNa$ [M+Na$^+$]: 309.0562, found: 309.0562 (recrystallized from hexanes/EtOAc, mp 142.5–144.3 °C).

Spectroscopic data for **4b**: ^{1}H NMR ($CDCl_3$) δ 7.33–7.57 (m, 5H), 4.51 (1H, dq, J=6.5, 3 Hz), 4.24 (2H, m), 2.75 (1H, s), 1.50 (3H, d, J=6.0 Hz). ^{1}H NMR (C_6D_6) δ 7.19 (2H, dd, J=6, 3.5 Hz), 6.90 (3H, t, J=6 Hz), 3.50 (1H, dq, J=6.5, 3 Hz), 3.41 (1H, d, J=5 Hz), 3.24 (1H, m), 2.11 (1H, d, J=2.5 Hz), 1.09 (3H, d, J=6.5 Hz). ^{13}C NMR ($CDCl_3$) δ 172.94, 132.41, 129.68, 128.71, 78.99, 69.86, 57.31, 14.13 ppm. IR (NaCl) ν 3381, 2921, 2860, 1752, 1630, 1436, 1174, 1113 cm^{-1}. HRMS m/z calcd for $C_{11}H_{12}O_3SNa$ [M+Na$^+$]: 247.0405, found: 247.0376.

Spectroscopic data for **5b**: ^{1}H NMR ($CDCl_3$) δ 7.33–7.57 (m, 5H), 4.65 (1H, dq, J=6.6, 4.4 Hz), 4.31 (1H, dd, J=4.4, 2.8 Hz), 3.81 (1H, d, J=2.8 Hz), 1.40 (3H, d, J=6.6 Hz). ^{13}C NMR ($CDCl_3$) δ 172.94, 133.21, 132.26, 129.53, 129.50, 128.87, 78.66, 74.74, 53.02, 13.68 ppm. IR (NaCl) ν 3399, 2922, 2853, 1759, 1721, 1623, 1439, 1313, 1259, 1113, 1052 cm^{-1}. HRMS m/z calcd for $C_{11}H_{12}O_3SNa$ [M+Na$^+$]: 247.0405, found: 247.0412.

Spectroscopic data for **4c**: ^{1}H NMR ($CDCl_3$) δ 7.52–7.54 (2H, m), 7.33–7.36 (3H, m), 4.31 (1H, m), 4.24 (1H, d, J=4.0 Hz), 3.86 (1H, dd, J=10.0, 2.5 Hz), 2.75 (1H, s), 2.27 (1H, m), 1.11 (3H, d, J=7.0 Hz), 0.99 (3H, d, J=6.5 Hz). ^{13}C NMR ($CDCl_3$) δ 172.21, 132.37, 132.00, 129.68, 128.67, 88.34, 68.55, 57.64, 27.64, 19.83, 17.51 ppm. IR (NaCl) ν 3423, 2965, 1760, 1471, 1440, 1392, 1339, 1171, 1072, 1026, 873, 778, 745, 690 cm^{-1}. HRMS m/z calcd for $C_{13}H_{16}O_3SNa$ [M+Na$^+$]: 275.0718, found: 275.0761 (recrystallized from hexanes/EtOAc, mp 147.8–148.5 °C).

Spectroscopic data for **5c**: ^{1}H NMR ($CDCl_3$) δ 7.52–7.54 (2H, m), 7.33–7.36 (3H, m), 4.35 (1H, m), 4.06 (1H, dd, J=10.0, 3.0 Hz), 3.80 (1H, s), 2.42 (1H, d, J=5.0 Hz), 2.15 (1H, m), 1.10 (3H, d, J=6.5 Hz), 0.95 (3H, d, J=7.0 Hz). ^{13}C NMR ($CDCl_3$) δ 173.51, 132.83, 131.58, 129.49, 128.76, 88.43, 73.96, 53.50, 27.01, 19.86, 17.62 ppm. IR (NaCl) ν 3449, 3060, 2965, 2876, 1759, 1583, 1471, 1440, 1391, 1370, 1340, 1199, 1173, 1121, 1070, 1024, 955, 916, 821, 778, 747, 690 cm^{-1}. HRMS m/z calcd for $C_{13}H_{16}O_3SNa$ [M+Na$^+$]: 275.0718, found: 275.0721.

Spectroscopic data for **4e**: ^{1}H NMR ($CDCl_3$) δ 7.45–7.49 (m, 2H), 7.27–7.30 (3H, m), 4.26 (1H, m), 4.20 (1H, m), 4.18 (1H, d, J=4.0 Hz), 2.70 (1H, s), 1.73–1.90 (2H, m), 1.29–1.39 (4H, m), 0.86 (3H, t, J=7.5 Hz). ^{13}C NMR ($CDCl_3$) δ 172.17, 132.38, 132.06, 129.67, 128.67, 82.70, 69.20, 57.24, 28.27, 27.39, 22.54, 13.91 ppm. IR (NaCl) ν 3449, 3060, 2958, 2872, 1759, 1583, 1467, 1440, 1307, 1177, 1089, 1024, 938, 832, 745, 691 cm^{-1}. HRMS m/z calcd for $C_{14}H_{18}O_3SNa$ [M+Na$^+$]: 289.0874, found: 289.0856.

Spectroscopic data for **5e**: ^{1}H NMR ($CDCl_3$) δ 7.44–7.45 (2H, m), 7.24–7.35 (3H, m), 4.35 (1H, m), 4.23 (1H, m), 3.89 (1H, d, J=1.0 Hz), 2.90 (1H, s), 1.59–1.85 (2H, m), 1.22–1.40 (4H, m), 0.83 (3H, t, J=7.0 Hz). ^{13}C NMR ($CDCl_3$) δ 173.72, 132.83, 131.58, 129.49, 128.76, 83.22, 74.20, 52.87, 27.70, 27.52, 22.42, 13.84 ppm. IR (NaCl) ν 3441, 3059, 2959, 2921, 2859, 1748, 1583, 1479, 1439, 1343, 1274, 1187, 1123, 996, 945, 739, 690 cm^{-1}. HRMS m/z calcd for $C_{14}H_{18}O_3SNa$ [M+Na$^+$]: 289.0874, found: 289.0848.

Spectroscopic data for **4f**: ^{1}H NMR ($CDCl_3$) δ 7.45–7.47 (2H, m), 7.27–7.30 (3H, m), 4.23 (1H, m), 4.16 (1H, d, J=4.5 Hz), 3.88 (1H, dd, J=10.5, 2.5 Hz), 2.70 (1H, s), 0.83–2.03 (11H, m). ^{13}C NMR ($CDCl_3$) δ 172.16, 132.43, 131.97, 129.68, 128.69, 87.10, 68.33, 57.47, 36.58, 30.16, 27.62, 26.32, 25.35, 25.27 ppm. IR (NaCl) ν 3470, 3055, 2910, 2854, 1751, 1439, 1265, 1173, 1097, 1001, 944, 895, 704 cm^{-1}. HRMS m/z calcd for $C_{16}H_{20}O_3SNa$ [M+Na$^+$]: 315.1031, found: 315.1040.

Spectroscopic data for **5f** (partially contaminated with **4f**): ^{1}H NMR ($CDCl_3$) δ 7.46–7.50 (2H, m), 7.28–7.30 (3H, m), 4.30 (1H, m), 4.06 (1H, dd, J=10.5, 3.5 Hz), 3.72 (1H,

s), 0.85–2.00 (12H, m). ^{13}C NMR ($CDCl_3$) δ 172.16, 132.92, 131.59, 129.52, 128.81, 86.85, 73.86, 53.29, 36.08, 30.08, 27.82, 26.23, 25.37, 25.28 ppm. IR (NaCl) ν 3443, 3060, 2923, 2854, 1750, 1650, 1439, 1261, 1224, 1189, 1097, 1017, 735, 687 cm^{-1}. HRMS *m/z* calcd for $C_{16}H_{20}O_3SNa$ [M+Na$^+$]: 315.1031, found: 315.1026.

5.1.5. General experimental procedure for the cyclization of 9/10 into carbonates 12/13. An ice-bath cold solution of diols **9/10** (0.44 mmol) in THF (11 mL) was treated with pyridine (0.22 mmol) and triphosgene (0.48 mmol). The mixture was refluxed for 7.5 h. Then brine was added and extracted with Et_2O (3×20 mL), the organic layers were washed (brine), dried (Na_2SO_4), and concentrated. The crude oil was purified through chromatography (silica-gel, hexanes/EtOAc (8:2) and (7:3)).

Spectroscopic data for **12a**: ^{1}H NMR ($CDCl_3$) δ 7.13–7.50 (10H, m), 5.60 (1H, d, *J*=4.5 Hz), 4.87 (1H, dd, *J*=8.0, 4.5 Hz), 4.11 (2H, m), 3.81 (1H, d, *J*=7.5 Hz), 1.16 (1H, t, *J*=7.5 Hz). IR (NaCl) ν 3064, 2983, 1815, 1735, 1593, 1458, 1440, 1368, 1312, 1267, 1158, 1069, 1020, 952, 863, 765, 737 cm^{-1}. HRMS *m/z* calcd for $C_{19}H_{18}O_5SNa$ [M+Na$^+$]: 381.0773, found: 381.0758.

Spectroscopic data for **13a**: ^{1}H NMR ($CDCl_3$) δ 7.06–7.38 (10H, m), 5.86 (1H, d, *J*=7.0 Hz), 5.22 (1H, dd, *J*=10.5, 7.0 Hz), 4.11 (2H, m), 3.31 (1H, d, *J*=10.5 Hz), 1.16 (1H, t, *J*=7.5 Hz). IR (NaCl) ν 3063, 2982, 1810, 1728, 1594, 1475, 1441, 1369, 1264, 1157, 1020, 952, 862, 752, 698 cm^{-1}. HRMS *m/z* calcd for $C_{19}H_{18}O_5SNa$ [M+Na$^+$]: 381.0773, found: 381.0765.

Spectroscopic data for **12b/13b**: ^{1}H NMR ($CDCl_3$) δ 7.19–7.49 (5H, m, **12b** and **13b**), 4.83 (1H, dq, *J*=7.0, 6.5 Hz, **13b**), 4.73 (1H, dq, *J*=6.5, 4.5 Hz, **12b**), 4.53 (1H, d, *J*=8.0, 4.5 Hz, **12b**), 4.45 (1H, d, *J*=10.5, 7.0 Hz, **13b**), 4.14 (2H, m, **12b** and **13b**), 3.70 (1H, d, *J*=8.0 Hz, **12b**), 3.63 (1H, d, *J*=10.5 Hz, **13b**), 1.52 (3H, d, *J*=6.5 Hz, **12b**), 1.31 (3H, d, *J*=6.5 Hz, **13b**), 1.19 (1H, t, *J*=7.0 Hz, **12b**), 1.18 (1H, t, *J*=7.0 Hz, **13b**). IR (NaCl) ν 3060, 2955, 2854, 1805, 1732, 1466, 1377, 1259, 1157, 1069, 746, 685 cm^{-1}. HRMS *m/z* calcd for $C_{14}H_{16}O_5SNa$ [M+Na$^+$]: 319.0616, found: 319.0582.

5.1.5.1. 3-Acetyl-oxirane-2-carboxylic acid ethyl ester 14. An ice-bath cold solution of compound **1b** (163 mg, 1.02 mmol) in CH_2Cl_2 (4 mL) was treated with pyridine (463 μL, 5.72 mmol) and Dess–Martin periodinane (574 mg, 1.53 mmol). The mixture was stirred at room temperature (21 °C) for 1.5 h. Then satd aq $NaHCO_3/Na_2S_2O_3$ solution was added, diluted with Et_2O, then extracted with Et_2O (3×20 mL), the organic layers were washed (brine), dried (Na_2SO_4), and concentrated. The crude oil was purified through chromatography (silica-gel, hexanes/EtOAc (8:2) and (7:3)) to afford 113 mg (70%) of an oil. ^{1}H NMR ($CDCl_3$) δ 4.27 (2H, m), 3.63 (1H, d, *J*=2.0 Hz), 3.59 (1H, d, *J*=2.0 Hz), 2.12 (3H, s), 1.32 (3H, t, *J*=7.0 Hz). ^{13}C NMR ($CDCl_3$) δ 202.56, 166.71, 62.38, 57.86, 51.83, 24.49, 14.07 ppm. IR (NaCl) ν 2983, 2915, 1745, 1718, 1459, 1365, 1310, 1202, 1095, 1030, 874, 806 cm^{-1}. HRMS *m/z* calcd for $C_7H_{10}O_4Na$ [M+Na$^+$]: 181.0477, found: 181.0462.

5.1.6. Experimental procedure for the reduction of 14. An ice-bath cold solution of compound **14** (48 mg, 0.30 mmol) in Et_2O (4 mL) was treated with zinc borohydride solution[20] (0.17 M in Et_2O) (18 ml, 3 mmol). The mixture was stirred cold with an ice-bath for 30 min. Then satd aq NH_4Cl solution was added and extracted with Et_2O (3×20 mL), the organic layers were washed (brine), dried (Na_2SO_4), and concentrated. The crude oil was purified through chromatography (silica-gel, hexanes/EtOAc (7:3) and (6:4)) to afford 36 mg (75%) of an oil.

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A practical one-pot procedure for the synthesis of pyrazino[2′,3′:4,5]thieno[3,2-*d*]pyrimidinones by a tandem aza-Wittig/heterocumulene-mediated annulation strategy

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Abstract—A simple one-pot and efficient method is described for the synthesis of pyrazino[2′,3′:4,5]thieno[3,2-*d*]pyrimidinone derivatives **6** via a tandem aza-Wittig/heterocumulene-mediated annulation process. The iminophosphorane **3** reacted with aryl isocyanates, followed by heterocyclization on addition of secondary amines to give the corresponding guanidine intermediates **5**, which were cyclized in the presence of a catalytic amount of potassium carbonate to tricyclic compounds **6**. Similarly, iminophosphorane **3** reacts with phenols, thiophenol, or ROH to give 2-aryl(alkyl)oxy(thio)pyrazino[2′,3′:4,5]thieno[3,2-*d*]pyrimidinone derivatives **7** in good yields. The corresponding carbodiimide **4c** and guanidine-type intermediate compounds **5** could be isolated and characterized, thus confirming the suggested reaction pathway. However, two isomeric pyrazinothienopyrimidinones **8** and **9** may be produced in the reaction of iminophosphorane **3** with aromatic isocyanates and subsequent reaction with primary amines in the presence of a catalytic amount of potassium carbonate. The effects of the nucleophiles and isocyanates on the regioselectivity of the cyclization have been investigated.

1. Introduction

The development of efficient and mild methods for heterocyclic compound synthesis represents a broad area of organic chemistry.[1] Structures containing such units often play an essential role because of their biological activity, particularly in cancer and virus researches.[2] Among these heterocycles, thienopyrimidine derivatives are an important class of heterocyclic compounds in pharmaceutical discovery research.[3] Also, some derivatives of thienodipyrimidines have been synthesized and some of them show good antitumor activity.[4]

Whereas pyridine annelated sulfur-containing heterocycles have been studied extensively,[5] comparatively little is known about aza-analogue systems in which an *S*-heterocycle is fused to a pyrazine nucleus. During the last years, we reported the synthesis of substituted heterocycles containing the pyridothienopyrimidine and pyridazinothienopyrimidine skeletons with the aim of finding compounds with anti-inflammatory and antihistaminic activities.[6] In a search of the literature it is surprising that their isosteres pyrazinothienopyrimidines, moreover isosteres of quinoxalinepyrimidines, have been practically ignored.[7]

Following this research line and in continuation of our work on the studies on *S*- and *N*-heterocyclic compounds, we describe here a convenient approach to substituted pyrazinothienopyrimidinone derivatives **I** as isosteres of pharmaceutically relevant pyridothienopyrimidines[8] as well as their use as appropriate 1,10-phenanthroline-like ligands toward transition metals (Fig. 1). Derivatives **I** are of considerable interest as potential biologically active compounds or pharmaceuticals.

In the development of strategies for the preparation of heterocycles the aza-Wittig reaction has proved to be exceptionally useful and great progress has been made in the field of heterocyclic compounds by the aza-Wittig methodology.

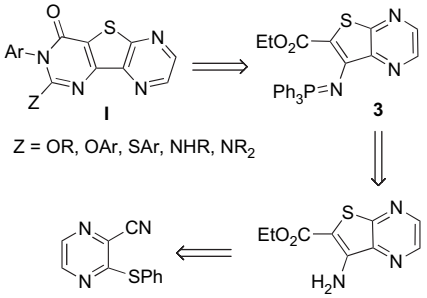

Figure 1. Retrosynthetic pathway for synthesis of the pyrazinothienopyrimidinone derivatives **I**.

Keywords: Pyrazinothienopyrimidinone; Aza-Wittig; Heterocumulene.
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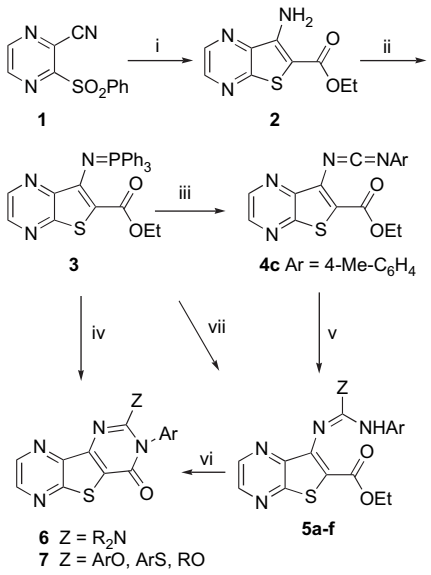
Both inter- and intramolecular version of the aza-Wittig reaction has assumed increasing importance for the specific construction of many heterocyclic compounds, in particular nitrogen heterocyclic compounds.[9] The intramolecular aza-Wittig reaction is a powerful tool for the synthesis of five–seven membered nitrogen heterocycles[10] and the intermolecular aza-Wittig reaction followed by electrocyclization, intramolecular cycloaddition, or heterocyclization, the tandem aza-Wittig, and cyclization sequence has been utilized for the synthesis of many important nitrogen heterocycles.[11]

Heteroaryliminophosphoranes, derived from *C*-aminoheterocycles, have proved to be very versatile building blocks for the construction of fused heterocondensed systems.[12] In our ongoing efforts to synthesize biologically active compounds as potential therapeutic agents based on heterocyclization reactions of azahexatriene compounds, we have previously published the synthesis of fused pyrimidines based on the tandem aza-Wittig heterocumulene-mediated annulation strategy.[13] As a continuation of our work on the aza-Wittig-type methodology, we report here a simple, general, and effective strategy for the preparation of substituted derivatives of the pyrazino[2′,3′:4,5]thieno[3,2-*d*]pyrimidine ring system employing *N*-heteroaryl iminophosphoranes as a conveniently accessible precursor. Pyrimidothienopyrazines were obtained in a one-pot reaction of the corresponding iminophosphorane of heteroaromatic β-enamino ester **2** with isocyanates, followed by heterocyclization on addition of nucleophilic reagents HZ. Dihydropyrimido-annulation occurs via a heterocumulene moiety, available from the reaction of the iminophosphorane and an isocyanate, which on addition of the corresponding nucleophile undergoes ring closure by nucleophilic attack of the adjacent *Z* group to give a six-membered heterocyclic ring.

2. Preparation of pyrazino[2′,3′:4,5]thieno[3,2-*d*]-pyrimidinones

The starting compound for the aza-Wittig reaction and heterocyclization sequence was prepared from the readily available ethyl 3-aminothieno[2,3-*b*]pyrazine-2-carboxylate **2**. The synthesis of a set of pyrazinothienopyrimidinone derivatives **6** and **7** is depicted in Scheme 1.

The initial reaction of 3-benzenesulfonylpyrazine-2-carbonitrile **1**[14] with ethyl 2-mercaptoacetate, in the presence of an equimolecular amount of sodium carbonate, gave an excellent yield (97%) of pyrazine carboxylate **2**. The key iminophosphorane **3** was obtained, in 97% yield, by a modified Kirsanov reaction of the β-enamino ester **2** with in situ generated dichlorotriphenylphosphorane using a hexachloroethane–triphenylphosphine–triethylamine reagent system.[15] Reaction of iminophosphorane **3** with several aromatic isocyanates, followed by heterocyclization on addition of secondary amines in the presence of a catalytic amount of K_2CO_3, resulted in the formation of triphenylphosphine oxide and the corresponding triheterocyclic pyrazino[2′,3′:4,5]-thieno[3,2-*d*]pyrimidin-4(3*H*)ones **6a–j** directly in excellent yields (85–98%). Aniline also reacted directly with the iminophosphorane **3** and phenylisocyanate to afford **6k** in 86% yield. The mechanism for these conversions involves an initial



Scheme 1. Reagents and conditions: (i) $HSCH_2CO_2Et$, Na_2CO_3, EtOH, reflux; (ii) C_2Cl_6, PPh_3, NEt_3, toluene, 100 °C, sealed tube; (iii) 4-MeC_6H_4NCO, THF, room temperature; (iv) ArNCO, THF (1–2 h, room temperature), HZ (5 h, room temperature), K_2CO_3, or NaOR (1 h, reflux); (v) secondary amine, THF, room temperature; (vi) K_2CO_3, acetone, reflux; (vii) ArNCO, THF (1–2 h, room temperature), HZ (5 h, room temperature).

aza-Wittig reaction between the iminophosphorane and the isocyanate to give the highly reactive carbodiimide intermediates which, in turn, were conveniently converted by a one-pot procedure into the corresponding heterocycles **6**, via initial addition of an amine to the carbodiimide cumulenic system followed by intramolecular hetero conjugate addition annulation, by simply heating in acetone in the presence of catalytic K_2CO_3. The results are listed in Table 1. Similarly, iminophosphorane **3** reacted with isocyanates and phenol, thiophenol, substituted phenols, or ROH in the presence of catalytic K_2CO_3 or Na^+RO^- to give the 2-aryl(alkyl)oxy(thioxy)pyrazinothienopyrimidinones **7** in satisfactory to good yields (65–85%).

The participation of carbodiimide **4** as an intermediate in this process has been confirmed experimentally: the treatment of iminophosphorane **3** with *p*-tolylisocyanate in dry THF resulted in a conversion of the former into ethyl 3-(*p*-tolylimino-methyleneamino)thieno[2,3-*b*]pyrazine-6-carboxylate **4c**. Likewise, reaction of iminophosphorane **3** with aryl isocyanates and secondary amines at room temperature resulted in the formation of triphenylphosphine oxide and

Table 1. 2-Dialkylaminopyrazino[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-ones **6a–6k**

Compd	Ar	Z	Yield (%)	Mp (°C)
6a	C_6H_5	NEt_2	92	215–216
6b	4-CH_3O–C_6H_4	NEt_2	90	194–196
6c	4-CH_3–C_6H_4	NEt_2	91	185–187
6d	4-Cl–C_6H_4	NEt_2	94	230–231
6e	4-NO_2–C_6H_4	NEt_2	93	224–225
6f	4-Cl–C_6H_4	Morpholino	86	232–233
6g	C_6H_5	Morpholino	98	289–290
6h	C_6H_5	Thiomorpholino	85	292–293
6i	C_6H_5	Pyrrolidino	90	244–245
6j	C_6H_5	Piperidino	85	220–221
6k	C_6H_5	C_6H_5NH	86	254–255

the corresponding guanidine-type intermediate derivatives **5a–5f** in good yields (70–85%) (Table 2). It is clear that compounds **5** are the key intermediates for the processes. In the presence of anhydrous potassium carbonate, the separated **5a–f** underwent intramolecular heterocyclization across the electrophilic ester functionality to give the fused pyrimidines **6**. Direct cyclization of the initially formed carbodiimide via a 1,3-OMe migration followed by electrocyclization (Wamhoff's pyrimido annelation)[16] was not observed.

Similarly, iminophosphorane **3** reacted with isocyanates and phenol, thiophenol, substituted phenols, or ROH in the presence of catalytic K_2CO_3 or Na^+RO^- to give the 2-aryl(alkyl)-oxy(thioxy)pyrazinothienopyrimidinones **7** in satisfactory to good yields (65–85%). The one-pot formation of aryloxy- and arylthioxy pyrazino[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-ones **7** (Table 3) was carried out by the reaction of iminophosphorane **3** with phenylisocyanate, followed by heterocyclization on addition of phenols or thiophenols in the presence of catalytic potassium carbonate. Irrespective of the fact whether the substituents on the phenols were electron-withdrawing or electron-releasing groups, the cyclization was completed smoothly at room temperature. Meanwhile, 4-ethoxy- or 4-methoxy-pyrazino[2′,3′:4,5]-thieno[3,2-*d*]pyrimidin-4(3*H*)-one **7f** or **7g** was obtained in satisfactory yield when the reaction took place in the presence of catalytic sodium ethoxide or sodium methoxide, respectively. The formation of **7** can be also rationalized in terms of an initial nucleophilic addition of phenoxide, thiophenoxide, or alkoxide to the carbodiimide **4** to give the intermediate **5**, which cyclizes to affords **7** (Scheme 1).

Carbodiimide compound **4c**, guanidine compounds **5a–5f**, and fused 2-substituted pyrimidinones **6** and **7** were characterized from their microanalyses, spectroscopic, and mass spectrometric data. The EI-mass spectra show the expected molecular ion peaks in moderate to high intensity and the fragmentation pattern is in accord with the proposed structure. The IR spectra of the guanidine-type intermediates **5a–5f** showed a strong absorption at ν=3280–3320 cm^{-1} attributed to the NH group, while in the ^1H NMR spectra, the NH proton appears at 5.70–6.13 ppm as a broad singlet, in addition to the set of signals due to the ethoxy group. Also, the ^{13}C NMR spectra showed signals between 14.3–14.5 and 60.7–61.4 ppm due to the ethoxy groups. After heterocyclization, the spectra of pyrazinothienopyrimidones **6** and **7** did not include those type of signals.

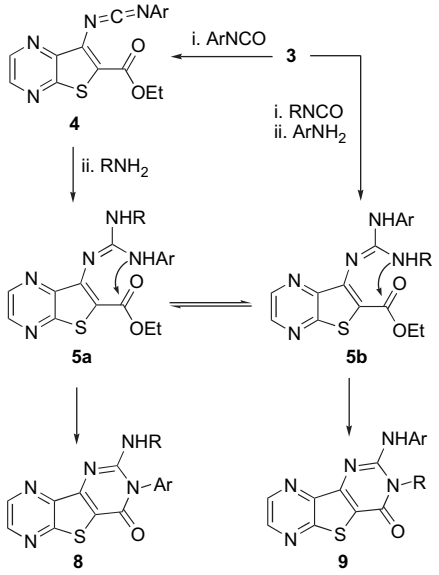
Table 2. Guanidine-type intermediate compounds **5a–5f**

Compd	Ar	Z	Time (h)	Yield (%)	Mp (°C)
5a	C_6H_5	NEt_2	3	70	106–107
5b	4-CH_3O–C_6H_4	NEt_2	4	85	194–195
5c	4-CH_3–C_6H_4	NEt_2	5	84	194–195
5d	4-Cl–C_6H_4	NEt_2	4	75	155–156
5e	4-NO_2–C_6H_4	NEt_2	1	80	182–183
5f	4-Cl–C_6H_4	Morpholino	4	70	160–161

Table 3. 2-Aryl(alkyl)oxypyrazino[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-ones **7a–7g**

Compd	Ar	Z	Yield (%)	Mp (°C)
7a	C_6H_5	C_6H_5O	68	232–234
7b	C_6H_5	C_6H_5S	85	226–227
7c	C_6H_5	4-NO_2–C_6H_4O	78	143–145
7d	C_6H_5	4-$(CH_3)_3C$–C_6H_4O	67	228–230
7e	C_6H_5	4-Benzyloxy-C_6H_4O	70	203–205
7f	C_6H_5	EtO	65	202–204
7g	C_6H_5	MeO	72	204–206

Two isomeric pyrazino[2′,3′:4,5]thieno[3,2-*d*]pyrimidinones **8** and **9** may be produced in the reaction of iminophosphorane **3** with primary amines via a guanidine-type intermediate **5**. In addition, these isomeric pyrazinothienopyrimidones, **8** and **9**, may be produced in the treatment of the heteroaryl-imino-phosphorane **3** with ArNCO/RNH_2 or RNCO/$ArNH_2$ (Scheme 2).



Scheme 2.

We decided to explore the effects of the structural variations on isocyanates and primary amines in the ratio of the formation of compounds **8** and **9**. First, a comparative study of the one-pot procedure for the cyclization of **3** was carried out with phenylisocyanate and substituted primary amines. The ratio of **8**:**9** compounds is strongly influenced by the alkyl substituent at the primary amine. The general conditions in Scheme 1 are used and the results are listed in Table 4. The results showed that the selectivity of the reaction was found markedly dependent on the nature of the primary amine employed. A helpful generalization is that whenever

Table 4. Selectivity and yields in the reaction of **3** with phenylisocyanate and primary amines

Entry	Compd	R	Ar	Yield (%)[a]	Mp (°C)
1	**8a**	*n*-Butyl	C_6H_5	57	197–198
2	**9a**	*n*-Butyl	C_6H_5	19	99–101
3	**8b**	Benzyl	C_6H_5	55	113–115
4	**9b**	Benzyl	C_6H_5	44	175–176
5	**8c**	*iso*-Propyl	C_6H_5	80	228–230
6	**9c**	*iso*-Propyl	C_6H_5	0	
7	**8d**	Cyclohexyl	C_6H_5	95	208–210
8	**9d**	Cyclohexyl	C_6H_5	0	
9	**8e**	*tert*-Butyl	C_6H_5	82	231–232
10	**9e**	*tert*-Butyl	C_6H_5	0	

[a] Unoptimized yield of analytically pure isolated product.

the primary amines are heavily substituted, that is, R=*iso*-propyl, *tert*-butyl, or cyclohexyl, these reactions afforded only **8**, compound **9** not being formed (Table 4, entries 5–10). However, when the primary amine is less hindered, e.g., R=*n*-butyl or R=benzyl, the selectivity is less satisfactory, affording a 75:25 and 55:45 ratios of **8**:**9** compounds, respectively, ratios determined by ^{1}H NMR analysis of the mixture of reaction (Table 4, entries 1–4).

The most important factor in the formation of products **8** and **9** can be ascribed mainly to the large difference in cyclization rates due to the steric hindrance around the Ph and R groups.[13,17] Although the carbodiimide **4** is mainly coplanar due to the resonance effect, it is immediately obvious that the amine would approach essentially by the opposite direction of the COOEt group due to the steric hindrance to form the guanidine-type intermediate **5a** (Scheme 2). However, **5a** may convert to **5b** through C–N single bond rotation and this offers the opportunity that both guanidine-type intermediates, **5a** and **5b**, are suitable to cyclize: **5a** for the arylamine group and **5b** by the alkylamino group to form **8** and **9**, respectively. Steric hindrance between alkyl group and ester group would explain the regioselectivity of the reaction. Thus, the bulkier R group could rationalize the only formation of the final products **8c**–**8e** (Table 4, entries 5, 7, and 9). In these cases, the initially formed **5a** more easily undergoes cyclization to give **8** than to divert to **5b** to give **9**, due the greater steric hindrance between the iso-propyl, cyclohexyl, or *tert*-butyl group and the ester group in the guanidine-type intermediate **5b**. However, with *n*-butyl or benzylamine, the minor steric hindrance between the alkyl group and the ester group may convert more easily **5a** to **5b** with subsequent cyclization of **5a** and **5b** by the arylamino or alkylamino group, respectively, to yield a mixture of the pyrazinothienopyrimidines **8a/9a** and **8b/9b** (Table 4, entries 1–4).

Though steric effects are of greatest importance in governing the relative ratios between compounds **8** and **9** and may determine, which product is formed in these processes, it is also noteworthy that there are other electronic effects that are of importance on the selectivity of the cyclization reaction. In this manner, in an effort to explore the electronic effects on the selectivity of the reaction, at the same time, to achieve diversity in terms of substitution, aryl isocyanates bearing electron-withdrawing or electron-releasing group were also subjected to the reaction conditions with iminophosphorane **3** and primary amines. To the best of our knowledge, no precedent has been reported in this way.

Armed with the results from Table 4, we examined the scope of the one-pot tandem reaction sequence for phenylisocyanate, 4-nitrophenylisocyanate, and 4-methylisocyanate varying the primary amine. Interesting enough, the results from Table 5 seem to suggest that the selectivity of the reaction is particularly sensitive to electronic effects, as soon as the ratio between compounds **8** and **9** was found to have a pronounced dependence on the relative nucleophilicities of the NHAr groups. Not surprisingly, the effect of nucleophilicity of amine groups on the ratio of the formation of compounds **8c**:**9c**, **8d**:**9d**, and **8e**:**9e** will not be important (Table 5, entries 7–15). In terms of precedent discussion, this high regioselectivity is in agreement with the explanation that the bulkier R group exerts a directing steric effect affording a single cyclized product **8**. However, entries 1–6 show the effect of varying the NHAr group in Scheme 2, allowing us to deduce that their relative nucleophilicity is responsible for these changes in the selectivity of the reaction and will affect the resulting product distribution when *n*-butyl or benzylamine is employed in the one-pot reaction. The most significant difference was observed in the reaction with aryl isocyanates bearing electron-withdrawing groups. Thus, the reaction of **3** with phenylisocyanate/*n*-butylamine or phenylisocyanate/benzylamine proceeded giving the corresponding compounds **8**:**9** in high yields, but little selectivity was observed (Table 5, entries 1 and 4), whereas, in contrast to this, the use of 4-nitrophenylisocyanate/*n*-butylamine and 4-nitrophenylisocyanate/benzylamine resulted in a dramatic change in the reaction selectivity, so that after the reaction the isomer **9** is present in the reaction mixtures with a 95:5 and 90:10 ratios, respectively, ratios determined by NMR spectroscopy (Table 5, entries 2 and 5). One can assume that the powerful electron-withdrawing nitro group decreases the electron density on nitrogen and it reduces strongly the nucleophilicity of the NHAr group. Then, the initially formed guanidine-type intermediate **5a** does not undergo cyclization to give **8** but then could divert conveniently to **5b** making the cyclization mainly by the most reactive butyl or benzylamine (Scheme 2). Moreover, for a comparison of the method, reaction of iminophosphorane **3** with isocyanates bearing electron-releasing groups was carried out. In this reaction the electron-releasing alkyl groups increase the electron density on nitrogen and the initially formed **5a** more easily supports cyclization to give **8** than to divert to **5b** to give **9**. In this manner, the *p*-tolylisocyanate gave a very high **8**-selectivity compared to that of phenylisocyanate (Table 5, entries 3 and 6). Last, the nucleophilicity dependence of the NHR group on the selectivity of the reaction was examined with phenylisocyanate/*p*-nitroaniline or *p*-nitrophenylisocyanate/aniline. As can be seen, in agreement with our previous results, we found that the reaction proceeded in high yield and with complete regioselectivity. In this case, guanidine intermediates **5a** and **5b** (Scheme 2) are the same sterically hindered making the cyclization to progress only by the strong nucleophilic amine

Table 5. Selectivity and yields in the reaction of **3** with ArCNO and primary amines

Entry	R	Ar	**8**/**9**[a]	Yield of isolated products (%)	
1	*n*-Butyl	C_6H_5	70:30	**8a**: 57	**9a**: 19
2	*n*-Butyl	4-NO_2–C_6H_4	5:95		**9a$_1$**: 82
3	*n*-Butyl	4-CH_3–C_6H_4	95:5	**8a$_2$**: 89	
4	Benzyl	C_6H_5	55:45	**8b**: 55	**9b**: 44
5	Benzyl	4-NO_2–C_6H_4	10:90		**9b$_1$**: 85
6	Benzyl	4-CH_3–C_6H_4	90:10	**8b$_2$**: 82	
7	*iso*-Propyl	C_6H_5	100:0	**8c**: 75	
8	*iso*-Propyl	4-NO_2–C_6H_4	95:5	**8c$_1$**: 81	
9	*iso*-Propyl	4-CH_3–C_6H_4	100:0	**8c$_2$**: 98	
10	Cyclohexyl	C_6H_5	100:0	**8d**: 95	
11	Cyclohexyl	4-NO_2–C_6H_4	100:0	**8d$_1$**: 84	
12	Cyclohexyl	4-CH_3–C_6H_4	100:0	**8d$_2$**: 88	
13	*tert*-Butyl	C_6H_5	100:0	**8e**: 82	
14	*tert*-Butyl	4-NO_2–C_6H_4	100:0	**8e$_1$**: 93	
15	*tert*-Butyl	4-CH_3–C_6H_4	100:0	**8e$_2$**: 85	
16	Phenyl	4-NO_2–C_6H_4	100:0	**8f**: 98	

[a] Ratios determined by ^{1}H NMR analysis of the mixture of compounds **8**/**9**. We were not able to separate the isomers **8a$_1$**, **8b$_1$**, **9a$_2$**, **9b$_2$**, and **9c$_1$**.

group to obtain **8f** (Table 5, entry 16; Ar=phenyl, R=4-nitrophenyl in Scheme 2) in excellent yield with total selectivity.

Compounds **8** and **9** were characterized from their spectral data, and the comparison of ^{1}H NMR spectra between **8** and **9** led us to confirm the structure of products without difficulty. In particular, in the ^{1}H NMR spectra of **8**, the corresponding proton of NH displays a characteristic triplet or doublet multiplicity due to coupling with the methylene or methine protons adjacent to the nitrogen atom. Its chemical shift is 3.91–4.73, more shielded than the one in PhNH of **9**.[18] For example, the ^{1}H NMR spectrum of **8a** shows the signals of NH at 4.27 as a triplet and NCH_2 at 3.52–3.63 as a multiplet. When the sample was treated with D_2O, its NCH_2 showed the signal as a triplet with disappearance of signals of NH absorption, which suggests the existence of an NHBu group in **8a**. The ^{1}H NMR spectrum of compound **9a** did not include the characteristic triplet corresponding to NH–Bu proton, but shows the NCH_2 signal at 4.29 as triplet and a new singlet signal at δ=6.66 due to the NHPh group, confirming the selective formation of pyrimido compounds **9** in this case. We were able to obtain suitable crystals of compounds **8** and **9** for X-ray study, which corroborated our earlier assignments (see Section 4). For example, Figures 2 and 3 show the molecular structure of compounds **8a** and **9a$_1$**, respectively. Interestingly, the results revealed a nearly coplanar disposition of the NHR group, in **8a**, and the NHAr group, in **9a$_1$**, with the three heterocyclic rings of the pyrazinothienopyrimidine system, whereas the angle between the NAr group in **8a** (NR in **9a$_1$**) and the triheterocyclic fused ring is approximately of 90°.

In summary, one-pot aza-Wittig/Heterocumulene-mediated annulation methodology provides an efficient protocol for preparing the functionalized pyrazino[2′,3′:4,4]thieno[3,2-*d*]pyrimidinone derivatives, with variable substituents at the pyrimidine ring, from the ethyl 3-(triphenylphosphoranylideneamino)thieno[2,3-*b*]pyrazine-6-carboxylate. The one-pot sequence can be extended to secondary amines, phenols, thiophenols, or ROH. In the case of primary amines, a highly regioselective cyclization has been observed and explored. Some advantages, such as the readily available reagents, one-pot procedure in high yields, mild reaction conditions, easy control of regioselectivity, and straightforward product isolation, make this annulation strategy attractive and practical for these previously unreported pyrazinothienopyrimidinones of considerable interest as potential biologically active compounds as well as pharmaceuticals.

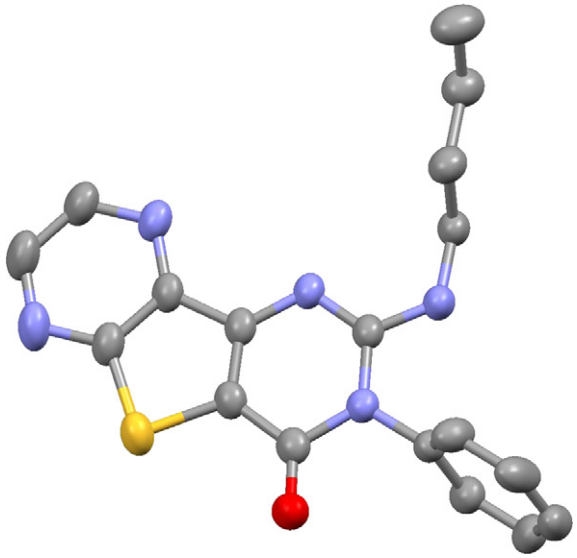


Figure 2. X-ray structure of compound **8a** (30% thermal probability ellipsoids). Hydrogen atoms have been omitted for clarity.

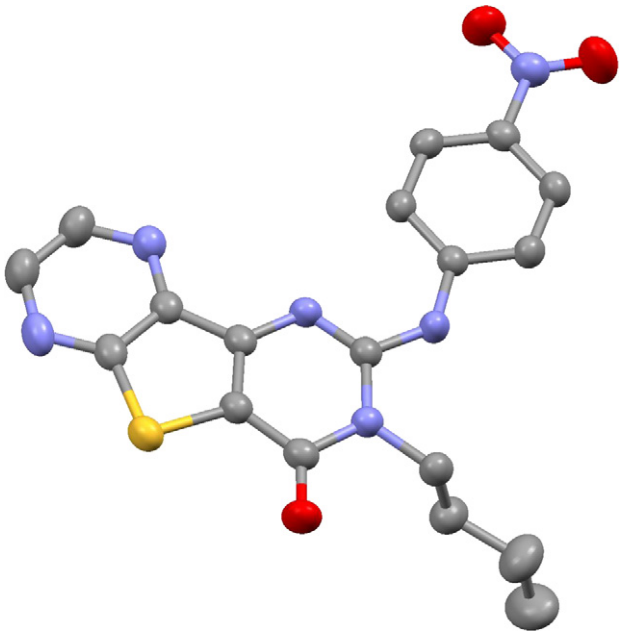


Figure 3. X-ray structure of compound **9a$_1$** (50% thermal probability ellipsoids). Hydrogen atoms have been omitted for clarity.

3. Experimental

3.1. General

NMR spectra were recorded at 200 or 300 MHz for ^{1}H and 50 or 75 MHz for ^{13}C. Chemical shifts are reported in parts per million (δ) relative to internal Me_4Si. IR spectra were recorded as potassium bromide disks. Melting points were obtained on a capillary melting point apparatus and are uncorrected. All reagents used were commercial grade chemicals from freshly opened containers. Microanalyses for C, H, N, and S were performed by the elemental analyses general service of the University of A Coruña.

3.1.1. Ethyl 3-aminothieno[2,3-*b*]pyrazine-2-carboxylate (2). To a solution of 3-benzenesulfonylpyrazine-2-carbonitrile **1**[14] (2.00 g, 8.2 mmol) in EtOH/THF (5:1, v/v) (120 mL) was added Na_2CO_3 (1.06 g, 10 mmol) and the resultant reaction mixture was stirred at reflux temperature for 1 h. After cooling at room temperature, was extracted with dichloromethane (3×200 mL) and the organic extracts were combined and dried over sodium sulfate. The methylene dichloride solution was concentrated to dryness, and the residual material was recrystallized from ethanol to give **2** (1.80 g, 97%); mp 120–121 °C (lit.[7a] mp 114–116 °C).

3.1.2. Ethyl 3-(triphenylphosphoranylideneamino)-thieno[2,3-*b*]pyrazine-6-carboxylate (3). To a mixture of

2 (1.00 g, 6.65 mmol), triphenylphosphine (2.62 g, 10 mmol), and hexachloroethane (1.37 g, 10 mmol) in dry toluene (40 mL), triethylamine (2.4 mL, 16.50 mmol) was added dropwise. The reaction mixture was heated at 100 °C in a sealed tube for 48 h. After cooling, the precipitate obtained was filtered off, washed with water, and recrystallized from ethanol, to give **3** (2.00 g, 97%) as a yellow solid: mp 174–176 °C; IR (KBr) ν 1690 (C═O), 1175 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.44 (t, J=7.1 Hz, 3H), 4.44 (q, J=7.1 Hz, 2H), 7.35–7.54 (m, 15H), 8.58 (d, J=2.4 Hz, 1H), 8.62 (d, J=2.4 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.7, 60.4, 128.0, 128.3, 131.2, 131.3, 132.7, 132.9, 133.5, 138.5, 138.8, 142.5, 144.9, 148.3, 155.2, 163.9. ^{31}P NMR (81 MHz, $CDCl_3$) δ 7.37; MS (EI) m/z 483 (M^+, 55), 410 (58). Anal. Calcd for $C_{27}H_{22}N_3O_2PS$: C, 67.07; H, 4.59; N, 8.69; S, 6.63. Found: C, 67.13; H, 4.51; N, 8.57; S, 6.80.

3.1.3. Ethyl 3-(*p*-tolylimino-methylenamino)thieno[2,3-*b*]pyrazine-6-carboxylate (4c). To a solution of **3** (0.15 g, 0.31 mmol) in THF (3 mL) was added *p*-tolylisocyanate (0.05 g, 0.37 mmol) and the mixture was stirred at room temperature for 3–5 h. The solvent was evaporated and the solid obtained was purified by flash chromatography using hexane/CH_2Cl_2 (1:1; v/v) as eluent. Yield 53%; yellow solid; mp 105–106 °C; IR (KBr) ν 2140 (C═N), 1710 (C═O), 1580, 1570, 1505 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.43 (t, J=7.3 Hz, 3H), 2.35 (s, 3H), 4.45 (q, J=7.3 Hz, 2H), 7.06–7.29 (m, 4H), 8.71 (d, J=2.4 Hz, 1H), 8.66 (d, J=2.4 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.3, 21.0, 62.0, 122.2, 124.6, 129.5, 130.0, 131.6, 133.7, 135.0, 135.8, 142.3, 144.0, 154.3, 161.5. Anal. Calcd for $C_{17}H_{14}N_4O_2S$: C, 60.34; H, 4.17; N, 16.56; S, 9.48. Found: C, 60.53; H, 4.31; N, 16.46; S, 9.31.

3.1.4. Ethyl thieno[2,3-*b*]pyrazine-6-carboxylates (5). The appropriate isocyanate (0.37 mmol) was added to a solution of iminophosphorane **3** (0.15 g, 0.31 mol) in THF (3 mL). The mixture was stirred at room temperature for 1–2 h until the iminophosphorane had disappeared (TLC monitored) and it was therefore treated with an appropriate amine (0.37 mmol). The resultant solution was stirred at room temperature for 1–5 h. The solvent was evaporated, ether (5 mL) was added, and the mixture was stirred at room temperature for 0.5 h. The solid formed was filtered off and purified by crystallization from ethanol or flash chromatography to give the corresponding compounds **5**.

3.1.5. Ethyl 2-(*N*′,*N*′-diethyl-*N*″-phenylguanidino)-thieno[2,3-*b*]pyrazine-6-carboxylate (5a). Yield 70%; yellow solid; mp 106–107 °C; IR (KBr) ν 3320 (NH), 1635 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.31 (t, J=7.3 Hz, 6H), 1.38 (t, J=7.3 Hz, 3H), 3.59 (q, J=7.3 Hz, 4H), 4.35 (q, J=7.3 Hz, 2H), 6.59–6.70 (m, 3H), 6.78–6.89 (m, 3H), 8.47 (d, J=2.2 Hz, 1H), 8.61 (d, J=2.2 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.5, 14.5, 43.1, 60.7, 119.3, 122.1, 128.3, 129.2, 141.3, 142.8, 145.0, 152.7, 155.1, 163.0. MS (EI) m/z 397 (M^+, 24), 324 (58). Anal. Calcd for $C_{20}H_{23}N_5O_2S$: C, 60.43; H, 5.83; N, 17.62; S, 8.07. Found: C, 60.33; H, 6.01; N, 17.56; S, 8.22.

3.1.6. Ethyl 2-[*N*′,*N*′-diethyl-*N*″-(4-methoxyphenyl)guanidino]thieno[2,3-*b*]pyrazine-6-carboxylate (5b). Yield 85%; yellow solid; mp 194–195 °C; IR (KBr) ν 3320 (NH), 1635 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.29 (t, J=6.8 Hz, 6H), 1.39 (t, J=6.8 Hz, 3H), 3.56 (q, J=6.8 Hz, 4H), 3.58 (s, 3H), 4.35 (q, J=6.8 Hz, 2H), 6.35–6.45 (m, 3H), 6.66–6.70 (m, 2H), 8.47 (d, J=2.4 Hz, 1H), 8.60 (d, J=2.4 Hz, 1H); MS (EI) m/z 427 (M^+, 20), 354 (60), 203 (100). Anal. Calcd for $C_{21}H_{25}N_5O_3S$: C, 59.00; H, 5.89; N, 16.38; S, 7.50; Found: C, 58.93; H, 5.71; N, 16.51; S, 7.72.

3.1.7. Ethyl 2-[*N*′,*N*′-diethyl-*N*″(4-methylphenyl)guanidino]thieno[2,3-*b*]pyrazine-6-carboxylate (5c). Yield 84%; yellow solid; mp 194–195 °C; IR (KBr) ν 3320 (NH), 1635; ^1H NMR (200 MHz, $CDCl_3$) δ 1.30 (t, J=7.3 Hz, 6H), 1.39 (t, J=7.3 Hz, 3H), 2.15 (s, 3H), 3.57 (q, J=7.3 Hz, 4H), 4.35 (q, J=7.3 Hz, 2H), 6.50 (br, 1H), 6.60–6.67 (m, 4H), 6.78–6.89 (m, 3H), 8.47 (d, J=2.2 Hz, 1H), 8.61 (d, J=2.2 Hz, 1H). Anal. Calcd for $C_{21}H_{25}N_5O_2S$: C, 61.29; H, 6.12; N, 17.02; S, 7.79. Found: C, 61.44; H, 6.00; N, 17.16; S, 7.62.

3.1.8. Ethyl 2-[*N*′,*N*′-diethyl-*N*″-(4-chlorophenyl)guanidino]thieno[2,3-*b*]pyrazine-6-carboxylate (5d). Yield 75%; yellow solid; mp 155–156 °C; IR (KBr) ν 3280 (NH), 1710 (C═O) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.30 (t, J=7.3 Hz, 6H), 1.39 (t, J=7.3 Hz, 3H), 3.57 (q, J=7.3 Hz, 4H), 4.35 (q, J=7.3 Hz, 2H), 5.70 (br, 1H), 6.66 (s, 2H), 6.81–6.85 (m, 2H), 8.51 (d, J=2.4 Hz, 1H), 8.62 (d, J=2.2 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.5, 14.4, 43.1, 60.9, 120.2, 126.8, 128.3, 139.1, 141.4, 143.1, 148.2, 152.6, 155.2; MS (EI) m/z 433/431 (M^+, 11/28), 358 (39). Anal. Calcd for $C_{20}H_{22}ClN_5O_2S$: C, 55.61; H, 5.13; Cl, 8.21; N, 16.21; S, 7.42. Found: C, 55.43; H, 5.21; Cl, 8.34; N, 16.39; S, 7.25.

3.1.9. Ethyl 2-[*N*′,*N*′-diethyl-*N*″-(4-nitrophenylguanidino]thieno[2,3-*b*]pyrazine-6-carboxylate (5e). Yield 80%; yellow solid; mp 182–183 °C; IR (KBr) ν 3280 (NH), 1680 (C═O) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$,) δ 1.30 (t, J=7.3 Hz, 6H), 1.40 (t, J=7.3 Hz, 3H), 3.58 (q, J=7.3 Hz, 4H), 4.37 (q, J=7.3 Hz, 2H), 6.63 (br, 3H), 7.75 (d, J=9.1, 2H), 8.53 (d, J=2.2 Hz, 1H), 8.64 (d, J=2.2 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.6, 14.3, 43.4, 61.4, 117.8, 124.6, 140.9, 141.6, 143.4, 149.7, 154.9, 163.4; MS (EI) m/z 442 (M^+, 14), 413 (5), 369 (53), 220 (100). Anal. Calcd for $C_{20}H_{22}N_6O_4S$: C, 54.29; H, 5.01; N, 18.99; S, 7.25. Found: C, 54.43; H, 5.19; N, 18.79; S, 7.13.

3.1.10. Ethyl 2-[(4-chlorophenylamino)-morpholin-1-yl-methyleneamino]thieno[2,3-*b*]pyrazine-6-carboxylate (5f). Yield 70%; yellow solid; mp 160–161 °C; IR (KBr) ν 3300 (NH), 1700 (C═O) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.38 (t, J=7.3 Hz, 3H), 3.51–3.62 (m, 4H), 3.66–3.77 (m, 4H), 4.35 (q, J=7.3 Hz, 2H), 6.13 (br, 1H), 6.86–7.01 (m, 4H), 8.54 (d, J=2.2 Hz, 1H), 8.63 (d, J=2.2 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.4, 47.2, 61.2, 66.4, 120.6, 127.5, 128.7, 138.9, 141.7, 143.3, 152.3, 155.2, 162.8; MS (EI) m/z 447/445 (M^+, 8/21), 372 (24). Anal. Calcd for $C_{20}H_{20}ClN_5O_3S$: C, 53.87; H, 4.52; Cl, 7.95; N, 15.71; S, 7.19. Found: C, 53.97; H, 4.61; Cl, 8.14; N, 15.59; S, 7.05.

3.2. General procedure for the synthesis of 2-dialkylylamino-3-phenylpyrazino[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one 6a–6k

To a solution of iminophosphorane **3** (0.15 g, 0.31 mmol) in dry THF (3 mL) was added the appropriate isocyanate (0.37 mmol) at room temperature. The mixture was stirred at room temperature for 1–2 h until the iminophosphorane had disappeared (TLC monitored) and it was therefore treated with an appropriate secondary amine or aniline (0.37 mmol). The resultant solution was stirred at room temperature for 1–5 h. The solvent was evaporated, the residue was treated with a catalytic amount of K_2CO_3, acetone (3 mL) was added, and the mixture was refluxed for 1 h. The solvent was removed under reduced pressure, and the residue was subjected to chromatography on silica gel eluting with a hexanes/ethyl acetate gradient from 5 to 40% ethyl acetate to give **6** as a yellow solid.

3.2.1. 2-Diethylamino-3-*N*-phenylpyrazino[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (6a). Yield 92%; mp 215–216 °C; IR (KBr) ν 1685 (C=O), 1530 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.92 (t, 6H, J=7.3 Hz, 6H), 3.25 (q, J=7.3 Hz, 4H), 7.35–7.58 (m, 5H), 8.69 (d, J=2.2 Hz, 1H), 8.85 (d, J=2.2 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.5, 45.3, 128.5, 128.6, 129.2, 137.6, 142.5, 143.8, 148.9, 158.0, 158.7, 159.9; MS (EI) *m*/*z* 351 (M$^+$, 20), 322 (100). Anal. Calcd for $C_{18}H_{17}N_5OS$: C, 61.52; H, 4.88; N, 19.93; S, 9.12. Found: C, 61.43; H, 4.81; N, 20.06; S, 9.31.

3.2.2. 2-Diethylamino-3-*N*-(4-methoxyphenyl)pyrazino-[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (6b). Yield 90%; mp 194–196 °C; IR (KBr) ν 1690 (C=O), 1525 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.95 (t, J=7.3 Hz, 6H), 3.26 (q, J=7.3 Hz, 4H), 3.87 (s, 3H), 7.03–7.29 (m, 4H), 8.68 (d, J=2.4 Hz, 1H), 8.84 (d, J=2.4 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.6, 45.3, 55.5, 114.5, 118.3, 129.5, 130.1, 142.4, 143.8, 144.2, 148.9, 158.3, 158.6, 159.4, 160.2; MS (EI) *m*/*z* 381 (M$^+$, 13), 352 (46). Anal. Calcd for $C_{19}H_{19}N_5O_2S$: C, 59.82; H, 5.02; N, 18.36; S, 8.41. Found: C, 60.03; H, 5.01; N, 18.26; S, 8.31.

3.2.3. 2-Diethylamino-3-*N*-(4-methylphenyl)pyrazino-[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (6c). Yield 91%; mp 185–187 °C; IR (KBr) ν 1670 (C=O), 1520 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.93 (t, J=6.8 Hz, 6H), 2.43 (s, 3H), 3.26 (q, J=6.8 Hz, 4H), 7.25–7.33 (m, 4H), 8.68 (d, J=2.4 Hz, 1H), 8.84 (d, J=2.4 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.6, 21.2, 45.3, 128.2, 129.9, 134.9, 138.5, 142.4, 143.8, 144.1, 148.9, 158.2, 158.7, 160.1; MS (EI) *m*/*z* 365 (M$^+$, 22), 336 (100). Anal. Calcd for $C_{19}H_{19}N_5OS$: C, 62.44; H, 5.24; N, 19.16; S, 8.77. Found: C, 62.63; H, 5.21; N, 19.26; S, 8.61.

3.2.4. 2-Diethylamino-3-*N*-(4-chlorophenyl)pyrazino-[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (6d). Yield 94%; mp 230–231 °C; IR (KBr) ν 1680 (C=O), 1530 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.96 (t, J=6.8 Hz, 6H), 3.25 (q, J=6.8 Hz, 4H), 7.30–7.52 (m, 4H), 8.70 (d, J=2.4 Hz, 1H), 8.85 (d, J=2.4 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.5, 45.3, 118.5, 129.5, 129.9, 134.5, 135.9, 142.4, 142.6, 144.0, 149.0, 157.8, 158.7, 159.9; MS (EI) *m*/*z* 387/385 (M$^+$, 7/20), 356 (100). Anal. Calcd for $C_{18}H_{16}ClN_5OS$: C, 56.03; H, 4.18; Cl, 9.19; N, 18.15; S, 8.31. Found: C, 56.14; H, 4.21; Cl, 9.33; N, 17.99; S, 8.51.

3.2.5. 2-Diethylamino-3-*N*-(4-nitrophenyl)pyrazino-[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (6e). Yield 93%; mp 224–225 °C; IR (KBr) ν 1670 (C=O), 1520 cm^{-1}; ^{1}H NMR (200 MHZ, $CDCl_3$) δ 0.98 (t, J=7.3 Hz, 6H), 3.25 (q, J=7.3 Hz, 4H), 7.63, 8.41 (m, 4H), 8.72 (d, J=2.2 Hz, 1H), 8.88 (d, J=2.2 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.5, 45.2, 124.5, 142.8, 143.1, 143.8, 144.2, 147.2, 149.1, 157.2, 158.7, 159.2; MS (EI) *m*/*z* 396 (M$^+$, 39), 367 (85). Anal. Calcd for $C_{18}H_{16}N_6O_3S$: C, 54.54; H, 4.07; N, 21.20; S, 8.09. Found: C, 54.73; H, 4.01; N, 20.26; S, 8.24.

3.2.6. 3-*N*-(4-Chlorophenyl)-2-(4-morpholinyl)pyrazino-[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (6f). Yield 86%; mp 232–233 °C; IR (KBr) ν 1670 (C=O), 1520 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.23–3.32 (m, 4H), 3.50–3.54 (m, 4H), 7.40, 7.54 (m, 4H), 8.72 (d, J=2.2 Hz, 1H), 8.87 (d, J=2.2 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 49.4, 65.8, 120.0, 129.5, 129.7, 134.8, 135.1, 142.7, 143.7, 144.2, 148.5, 157.4, 158.5, 159.1; MS (EI) *m*/*z* 401/399 (M$^+$, 36/100), 313 (57). Anal. Calcd for $C_{18}H_{14}ClN_5O_2S$: C, 54.07; H, 3.53; Cl, 8.87; N, 17.51; S, 8.02. Found: C, 54.13; H, 3.41; Cl, 9.08; N, 17.46; S, 7.91.

3.2.7. 2-(4-Morpholinyl)-3-*N*-phenylpyrazino[2′,3′:4,5]-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (6g). Yield 98%; mp 289–290 °C; IR (KBr) ν 1701 (C=O), 1520, 1490, 1265 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.26–3.36 (m, 4H), 3.40–3.50 (m, 4H), 7.41–7.62 (m, 5H), 8.71 (d, J=2.4 Hz, 1H), 8.86 (d, J=2.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 49.4, 65.8, 119.9, 128.4, 128.9, 129.3, 136.7, 142.6, 144.0, 148.5, 157.6, 158.5, 159.3; MS (EI) *m*/*z* 365 (M$^+$, 5), 279 (10), 77 (100). Anal. Calcd for $C_{18}H_{15}N_5O_2S$: C, 59.16; H, 4.14; N, 19.17; S, 8.78. Found: C, 58.97; H, 4.20; N, 19.01; S, 8.62.

3.2.8. 3-*N*-Phenyl-2-(4-thiomorpholinyl)pyrazino-[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (6h). Yield 85%; mp 292–293 °C; IR (KBr) ν 1702 (C=O), 1530, 1449, 1383 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.30–2.40 (m, 4H), 3.50–3.70 (m, 4H), 7.30–7.65 (m, 5H), 8.71 (d, J=2.4 Hz, 1H), 8.86 (d, J=2.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.4, 51.9, 120.1, 128.7, 128.8, 129.4, 137.0, 142.6, 144.1, 148.4, 158.4, 159.4; MS (EI) *m*/*z* 397 (M$^+$, 4), 279 (25), 203 (15). Anal. Calcd for $C_{18}H_{15}N_5OS_2$: C, 56.67; H, 3.96; N, 18.36; S, 16.81. Found: C, 56.43; H, 4.03; N, 18.36; S, 16.77.

3.2.9. 3-*N*-Phenyl-2-(1-pyrrolidinyl)pyrazino[2′,3′:4,5]-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (6i). Yield 90%; mp 244–245 °C; IR (KBr) ν 1703 (C=O), 1570, 1443, 1348 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.70–1.82 (m, 4H), 3.16–3.25 (m, 4H), 7.35–7.57 (m, 5H), 8.66 (d, J=2.4 Hz, 1H), 8.81 (d, J=2.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.4, 50.6, 128.7, 129.2, 137.2, 142.2, 143.8, 144.2, 150.0, 155.3, 158.9, 159.7; MS (EI) *m*/*z* 349 (M$^+$, 20), 294 (13), 77 (100). Anal. Calcd for $C_{18}H_{15}N_5OS$: C, 61.87; H, 4.33; N, 20.04; S, 9.18. Found: C, 61.73; H, 4.34; N, 19.91; S, 9.07.

3.2.10. 3-*N*-Phenyl-2-(1-piperidinyl)pyrazino[2′,3′:4,5]-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (6j). Yield 85%; mp 220–221 °C; IR (KBr) ν 1700 (C=O), 1602, 1547, 1520, 1390 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20–1.50 (m, 6H), 3.10–3.40 (m, 4H), 7.30–7.56 (m, 5H), 8.66 (d, J=2.4 Hz, 1H), 8.82 (d, J=2.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.9, 24.8, 50.4, 119.2, 128.4, 128.5, 129.1, 137.4, 142.5, 143.9, 144.0, 148.8, 158.6, 158.7, 159.6; MS (EI) m/z 363 (M^+, 20). Anal. Calcd for $C_{19}H_{17}N_5OS$: C, 62.79; H, 4.71; N, 19.27; S, 8.82. Found: C, 62.63; H, 4.63; N, 19.16; S, 8.78.

3.2.11. 2-Phenyl-3-*N*-phenylaminopyrazino[2′,3′:4,5]-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (6k). Yield 86%; mp 254–255 °C; IR (KBr) ν 3404 (NH), 1682 (C=O), 1595, 1556, 1537, 1516 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.24 (s, 1H), 7.08–7.13 (m, 1H), 7.31–7.37 (m, 2H), 7.47–7.57 (m, 4H), 7.59–7.71 (m, 3H), 8.65 (d, J=2.3 Hz, 1H), 8.79 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 117.2, 120.8, 124.4, 128.8, 129.0, 130.6, 131.0, 133.7, 137.2, 142.4, 143.8, 149.3, 150.3, 158.3, 158.4; MS (EI) m/z 371 (M^+, 55). Anal. Calcd for $C_{20}H_{13}N_5OS$: C, 64.68; H, 3.53; N, 18.86; S, 8.63. Found: C, 64.49; H, 3.53; N, 18.66; S, 8.65.

3.3. General procedure for the synthesis of 3-aryloxy-2-phenylpyrazino[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one 7a–7e

To a solution of iminophosphorane **3** (0.15 g, 0.31 mmol) in dry THF (3 mL) was added phenylisocyanate (0.37 mmol) at room temperature. The mixture was stirred at room temperature for 1–2 h until the iminophosphorane had disappeared (TLC monitored) and after treating with an appropriate substituted phenol or thiophenol (0.37 mmol), a catalytic amount of K_2CO_3 was added and the resultant solution was refluxed for 1 h. The solvent was evaporated under reduced pressure, the solid residue was treated with ether (5 mL), and the mixture was stirred at room temperature for 0.5 h. The solid formed was filtered off and purified by chromatography on silica gel eluting with a dichloromethane/ethyl acetate gradient from 10 to 30% ethyl acetate to give **7** as a yellow solid.

3.3.1. 3-*N*-Phenyl-2-phenoxypyrazino[2′,3′:4,5]-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (7a). Yield 68%; mp 232–234 °C; IR (KBr) ν 1687 (C=O), 1600, 1564, 1534, 1505, 1487 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18–7.32 (m, 3H), 7.37–7.50 (m, 4H), 7.51–7.65 (m, 3H), 8.69 (d, J=2.1 Hz, 1H), 8.80 (d, J=2.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 121.0, 126.3, 127.9, 129.5, 129.7, 129.8, 142.8, 144.1; MS (FAB) m/z 373 (MH^+, 98), 279 (13). Anal. Calcd for $C_{20}H_{12}N_4O_2S$: C, 64.50; H, 3.25; N, 15.04; S, 8.61. Found: C, 64.42; H, 3.11; N, 14.95; S, 8.56.

3.3.2. 3-*N*-Phenyl-2-phenylthiopyrazino[2′,3′:4,5]-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (7b). Yield 85%; mp 226–227 °C; IR (KBr) ν 1677 (C=O), 1516, 1493, 1340, 1233, 1182 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43–7.55 (m, 5H), 7.57–7.70 (m, 5H), 8.65 (d, J=2.2 Hz, 1H), 8.76 (d, J=2.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 127.8, 128.9, 129.4, 130.1, 130.2, 130.6, 135.4, 143.0, 143.9; MS (FAB) m/z 389 (MH^+, 100), 279 (16). Anal. Calcd for $C_{20}H_{12}N_4OS_2$: C, 61.84; H, 3.11; N, 14.42; S, 16.51. Found: C, 61.72; H, 2.99; N, 14.39; S, 16.45.

3.3.3. 2-(4-Nitrophenoxy)-3-*N*-phenylpyrazino[2′,3′:4,5]-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (7c). Yield 78%; mp 143–145 °C; IR (KBr) ν 1699 (C=O), 1569, 1538, 1524, 1487, 1345, 1260, 1218 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.49 (m, 4H), 7.53–7.65 (m, 3H), 8.28–8.50 (m, 2H), 8.72 (d, J=2.3 Hz, 1H), 8.82 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 121.9, 125.6, 127.8, 129.8, 129.9, 133.9, 143.1, 144.3; MS (FAB) m/z 418 (MH^+, 50), 371 (19), 279 (100). Anal. Calcd for $C_{20}H_{11}N_5O_4S$: C, 57.55; H, 2.66; N, 16.78; S, 7.68. Found: C, 57.45; H, 2.82; N, 16.73; S, 7.52.

3.3.4. 3-(4-*tert*-Butylphenoxy)-2-*N*-phenylpyrazino-[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (7d). Yield 67%; mp 228–230 °C; IR (KBr) ν 1690 (C=O), 1567, 1542, 1503, 1354, 1268, 1215 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (s, 9H), 7.13–7.16 (m, 2H), 7.40–7.45 (m, 4H), 7.50–7.63 (m, 3H), 8.70 (d, J=2.1 Hz, 1H), 8.81 (d, J=2.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 31.4, 120.3, 127.7, 126.7, 127.9, 129.4, 134.5, 142.8, 144.1; MS (EI) m/z 348 (M^+, 45), 294 (43). Anal. Calcd for $C_{24}H_{20}N_4O_2S$: C, 67.27; H, 4.70; N, 13.07; S, 7.48. Found: C, 67.11; H, 4.53; N, 12.95; S, 7.33.

3.3.5. 3-(4-Benzyloxyphenoxy)-2-*N*-phenylpyrazino-[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (7e). Yield 70%; mp 203–205 °C; IR (KBr) ν 1660 (C=O), 1568, 1547, 1535, 1520, 1497, 1446, 1354, 1195 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.06 (s, 2H), 6.90–7.20 (m, 4H), 7.25–7.80 (m, 10H), 8.69 (d, J=2.1 Hz, 1H), 8.81 (d, J=2.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 70.4, 115.7, 122.0, 127.5, 127.9, 128.1, 128.6, 129.5, 129.7, 142.8, 144.0; MS (FAB) m/z 479 (MH^+, 24), 279 (52). Anal. Calcd for $C_{27}H_{18}N_4O_3S$: C, 67.77; H, 3.79; N, 11.71; S, 6.70. Found: C, 67.52; H, 3.61; N, 11.65; S, 6.58.

3.3.6. 3-Alkoxy-2-phenylpyrazino[2′,3′:4,5]thieno[3,2-*d*]-pyrimidin-4(3*H*)-one 7f and 7g. To a solution of iminophosphorane **3** (0.15 g, 0.31 mmol) in THF (3 mL) was added the appropriate isocyanate (0.37 mmol) at room temperature. The mixture was stirred at room temperature for 1–2 h until the iminophosphorane had disappeared (TLC monitored). The solvent was evaporated, ROH (4 mL) was added to dissolve the solid, and then NaOR (0.40 mmol) was added and the resultant mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure, and the residue was subjected to chromatography on silica gel eluting with a dichloromethane/ethyl acetate 90:10 (v/v).

Compound **7f**: yield 65%; yellow solid; mp 202–204 °C; IR (KBr) ν 1692 (C=O), 1563, 1541, 1515, 1373, 1335, 1196 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (t, J=7.1 Hz, 3H), 4.65 (q, J=7.1 Hz, 2H), 7.28–7.33 (m, 2H), 7.48–7.60 (m, 3H), 8.73 (d, J=2.3 Hz, 1H), 8.88 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 66.0, 127.9, 129.2, 129.4, 142.7, 144.1; MS (EI) m/z 324 (M^+, 44). Anal. Calcd for $C_{16}H_{12}N_4O_2S$: C, 59.25; H, 3.73; N, 17.27; S, 9.89. Found: C, 59.13; H, 3.61; N, 17.12; S, 9.79.

Compound **7g**: yield 72%; yellow solid; mp 204–206 °C; IR (KBr) ν 1693 (C=O), 1569, 1543, 1517, 1487, 1449, 1353, 1265, 1194 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.14 (s, 3H), 7.25–7.35 (m, 2H), 7.45–7.65 (m, 3H), 8.73 (d, J=2.3 Hz, 1H), 8.88 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.8, 127.9, 129.3, 129.5, 134.3, 142.7, 143.6, 144.1, 147.7, 156.8, 158.3, 158.7; MS (FAB) m/z 311 (MH^+, 68). Anal. Calcd for $C_{15}H_{10}N_4O_2S$: C, 58.05; H, 3.25; N, 18.05; S, 10.33. Found: C, 57.93; H, 3.38; N, 18.12; S, 10.19.

3.4. General procedure for the synthesis of pyrazinothienopyrimidinones 8 and 9

The appropriate isocyanate (phenyl-, 4-nitrophenyl-, or 4-methylphenylisocyanate) (0.37 mmol) was added to a solution of iminophosphorane **3** (0.15 g, 0.31 mmol) in THF (3 mL) at room temperature. The mixture was stirred at room temperature for 1–2 h until the iminophosphorane had disappeared (TLC monitored) and it was then treated with the appropriate primary amine (0.37 mmol). The resultant solution was stirred at room temperature for 5 h. The solvent was evaporated, the solid residue was treated with a catalytic amount of K_2CO_3, acetone (3 mL) was added, and the mixture was refluxed for 1 h. The solvent was evaporated under reduced pressure, and the residue was subjected to chromatography on silica gel eluting with a hexanes/ethyl acetate gradient from 5 to 40% ethyl acetate to give **8/9** as a yellow solid.

3.4.1. 2-*n*-Butylamino-3-*N*-phenylpyrazino[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (8a). Yield 57%; mp 197–198 °C; IR (KBr) ν 3435 (NH), 1676 (C=O), 1589, 1564, 1548, 1530, 1523, 1486 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J=7.3 Hz, 3H), 1.20–1.35 (m, 2H), 1.42–1.56 (m, 2H), 3.52–3.63 (m, 2H), 4.27 (t, J=5.0 Hz, 1H, NH), 7.32–7.40 (m, 2H), 7.53–7.69 (m, 3H), 8.67 (d, J=2.3 Hz, 1H), 8.81 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7, 19.9, 31.1, 40.0, 128.6, 130.6, 130.8, 134.0, 142.2, 143.7, 144.0, 150.2, 153.5, 158.5, 158.6; MS (EI) m/z 351 (M^+, 25), 294 (85). Anal. Calcd for $C_{18}H_{17}N_5OS$: C, 61.52; H, 4.88; N, 19.93; S, 9.12. Found: C, 61.37; H, 4.95; N, 19.92; S, 8.92.

3.4.2. 3-*N*-*n*-Butyl-2-phenylaminopyrazino[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (9a). Yield 19%; mp 99–101 °C dec; IR (KBr) ν 3354 (NH), 1674 (C=O), 1657, 1537, 1523, 1494, 1472, 1456, 1444 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.04 (t, J=7.3 Hz, 3H), 1.45–1.60 (m, 2H), 1.80–1.95 (m, 2H), 4.29 (t, J=7.7 Hz, 2H), 6.66 (s, 1H, NH), 7.14–7.23 (m, 1H), 7.40–7.50 (m, 2H), 7.63–7.72 (d, 2H), 8.79 (d, J=2.3 Hz, 1H), 8.86 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7, 20.3, 29.8, 41.9, 121.6, 124.8, 129.3, 137.8, 142.4, 143.8, 150.6, 158.7; MS (EI) m/z 351 (M^+, 25), 294 (45). Anal. Calcd for $C_{18}H_{17}N_5OS$: C, 61.52; H, 4.88; N, 19.93; S, 9.12. Found: C, 61.34; H, 4.99; N, 19.83; S, 8.95.

3.4.3. 2-Benzylamino-3-*N*-phenylpyrazino[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (8b). Yield 55%; mp 113–115 °C; IR (KBr) ν 3249 (NH), 1681 (C=O), 1552, 1522, 1493, 1339 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 4.63 (t, J=5.3 Hz, 1H, NH), 4.83 (d, J=5.3 Hz, 2H), 7.20–7.35 (m, 5H), 7.35–7.45 (m, 2H), 7.50–7.70 (m, 3H), 8.69 (d, J=2.3 Hz, 1H), 8.83 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 46.3, 127.4, 127.7, 128.6, 128.8, 130.5, 130.9, 133.9, 137.5, 142.3, 143.9, 150.1, 153.4, 158.6, 158.7; MS (EI) m/z 385 (M^+, 5), 279 (3), 106 (25), 91 (100). Anal. Calcd for $C_{21}H_{15}N_5OS$: C, 65.44; H, 3.92; N, 18.17; S, 8.32; Found: C, 65.30; H, 4.01; N, 18.07; S, 8.16.

3.4.4. 3-*N*-Benzyl-2-phenylaminopyrazino[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (9b). Yield 44%; mp 175–176 °C; IR (KBr) ν 3311 (NH), 1655 (C=O), 1595, 1536, 1461, 1455, 1446 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.55 (s, 2H), 6.73 (s, 1H, NH), 7.05–7.15 (m, 1H), 7.30–7.55 (m, 9H), 8.66 (d, J=2.3 Hz, 1H), 8.76 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 45.5, 121.0, 124.5, 126.8, 129.0, 129.1, 129.8, 134.0, 137.6, 142.4, 143.9, 149.2, 150.9, 158.7, 159.1; MS (EI) m/z 385 (M^+, 25), 294 (15). Anal. Calcd for $C_{21}H_{15}N_5OS$: C, 65.44; H, 3.92; N, 18.17; S, 8.32. Found: C, 65.33; H, 4.01; N, 18.05; S, 8.15.

3.4.5. 2-Isopropylamino-3-*N*-phenylpyrazino[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (8c). Yield 80%; mp 228–230 °C; IR (KBr) ν 3416 (NH), 1679 (C=O), 1545, 1522, 1508, 1485 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.15 (d, J=6.5 Hz, 6H), 4.11 (d, J=7.7 Hz, 1H, NH), 4.47–4.62 (m, 1H), 7.33–7.35 (m, 2H), 7.54–7.65 (m, 3H), 8.65 (d, J=2.3 Hz, 1H), 8.80 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.7, 44.0, 115.2, 128.6, 130.2, 130.8, 134.0, 142.2, 143.7, 144.1, 150.2, 152.7, 158.5, 158.7; MS (EI) m/z 337 (M^+, 40), 294 (100). Anal. Calcd for $C_{17}H_{15}N_5OS$: C, 60.52; H, 4.48; N, 20.76; S, 9.50. Found: C, 60.48; H, 4.55; N, 20.56; S, 9.41.

3.4.6. 2-Cyclohexylylamino-3-*N*-phenylpyrazino[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (8d). Yield 95%; mp 208–210 °C; IR (KBr) ν 3271 (NH), 1685 (C=O), 1532, 1517, 1487, 1479, 1452 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.00–1.13 (m, 3H), 1.31–1.49 (m, 5H), 1.88–1.92 (m, 2H), 4.20–4.28 (m, 2H), 7.30–7.32 (m, 2H), 7.47–7.60 (m, 3H), 8.59 (d, J=2.3 Hz, 1H), 8.75 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.9, 25.2, 32.4, 49.6, 114.9, 128.4, 130.1, 130.6, 133.9, 142.0, 143.5, 143.8, 150.1, 152.6, 158.3, 158.5; MS (EI) m/z 377 (M^+, 10), 294 (95). Anal. Calcd for $C_{20}H_{19}N_5OS$: C, 63.64; H, 5.07; N, 18.55; S, 8.49; Found: C, 63.56; H, 4.97; N, 18.49; S, 8.47.

3.4.7. 2-*tert*-Butylamino-3-*N*-phenylpyrazino[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (8e). Yield 82%; mp 231–232 °C; IR (KBr) ν 3432 (NH), 1683 (C=O), 1561, 1545, 1521, 1485 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.48 (s, 9H), 4.12 (s, 1H, NH), 7.33–7.37 (m, 2H), 7.61–7.64 (m, 3H), 8.66 (d, J=2.3 Hz, 1H), 8.85 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.9, 53.2, 128.6, 130.3, 130.9, 143.6, 145.0; MS (EI) m/z 351 (M^+, 5), 294 (30). Anal. Calcd for $C_{18}H_{17}N_5OS$: C, 61.52; H, 4.88; N, 19.93; S, 9.12. Found: C, 61.49; H, 4.73; N, 20.06; S, 9.25.

3.4.8. 3-*N*-*n*-Butyl-2-(4-nitrophenylamino)pyrazino-[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one ($9a_1$). Yield 82%; mp 234–235 °C; IR (KBr) ν 3423 (NH), 1676

(C=O), 1538, 1502, 1404 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.06 (t, J=7.3 Hz, 3H), 1.48–1.63 (m, 2H), 1.80–1.96 (m, 2H), 4.34 (t, J=7.7 Hz, 2H), 7.03 (s, 1H, NH), 7.30–7.37 (m, 2H), 7.87–7.99 (m, 2H), 8.71 (d, J=2.3 Hz, 1H), 8.84 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7, 20.2, 30.1, 42.1, 120.1, 125.3, 142.7, 143.5, 144.2, 158.3; MS (EI) m/z 396 (M^+, 15), 339 (25). Anal. Calcd for $C_{18}H_{16}N_6O_3S$: C, 54.54; H, 4.07; N, 21.20; S, 8.09. Found: C, 54.31; H, 4.21; N, 21.45; S, 8.14.

3.4.9. 2-*n*-Butylamino-3-*N*-(4-methylphenyl)pyrazino-[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one ($8a_2$). Yield 89%; mp 163–165 °C; IR (KBr) ν 3256 (NH), 1611 (C=O), 1562, 1524, 1509, 1480, 1464, 1435 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J=7.3 Hz, 3H), 1.22–1.34 (m, 2H), 1.44–1.54 (m, 2H), 2.45 (s, 3H), 3.53–3.60 (m, 2H), 4.35 (t, J=5.1 Hz, 1H, NH), 7.21–7.23 (m, 2H), 7.39–7.42 (m, 2H), 8.65 (d, J=2.3 Hz, 1H), 8.80 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7, 19.9, 21.3, 31.1, 42.0, 115.3, 128.2, 131.2, 131.5, 140.5, 142.1, 143.7, 144.1, 150.1, 153.6, 158.5, 158.7; MS (EI) m/z 365 (M^+, 10), 308 (30). Anal. Calcd for $C_{19}H_{19}N_5OS$: C, 62.44; H, 5.24; N, 19.16; S, 8.77. Found: C, 62.51; H, 5.39; N, 19.02; S, 8.75.

3.4.10. 3-*N*-Benzylamino-2-(4-nitrophenylamino)pyrazino-[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one ($9b_1$). Yield 85%; mp 113–115 °C; IR (KBr) ν 3393 (NH), 1676 (C=O), 1610, 1541, 1503, 1464, 1413 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.61 (s, 2H), 7.01 (s, 1H, NH), 7.42–7.57 (m, 5H), 7.58–7.65 (m, 2H), 8.21–8.28 (m, 2H), 8.74 (d, J=2.3 Hz, 1H), 8.86 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 45.8, 119.7, 125.3, 127.0, 130.0, 130.2, 133.7, 142.8, 144.3; MS (EI) m/z 430 (M^+, 10), 279 (3), 91 (20). Anal. Calcd for $C_{21}H_{14}N_6O_3S$: C, 58.60; H, 3.28; N, 19.52; S, 7.45. Found: C, 58.58; H, 3.39; N, 19.43; S, 7.39.

3.4.11. 2-Benzylamino-3-*N*-(4-methylphenyl)pyrazino-[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one ($8b_2$). Yield 82%; mp 218–220 °C; IR (KBr) ν 3336 (NH), 1685 (C=O), 1553, 1522, 1509, 1452, 1343 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.43 (s, 3H), 4.73 (t, J=5.2 Hz, 1H, NH), 4.83 (d, J=5.2 Hz, 2H), 7.20–7.35 (m, 7H), 7.36–7.44 (m, 2H), 8.68 (d, J=2.3 Hz, 1H), 8.81 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.3, 46.2, 115.9, 127.4, 127.6, 128.2, 128.7, 131.0, 131.6, 137.5, 140.7, 142.3, 143.8, 144.1, 150.0, 153.6, 158.6, 158.8; MS (EI) m/z 399 (M^+, 20), 91 (35). Anal. Calcd for $C_{22}H_{17}N_5OS$: C, 66.15; H, 4.29; N, 17.53; S, 8.03. Found: C, 66.24; H, 4.08; N, 17.64; S, 8.17.

3.4.12. 2-Isopropylamino-3-*N*-(4-nitrophenyl)pyrazino-[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one ($8c_1$). Yield 81%; mp>300 °C; IR (KBr) ν 3350 (NH), 1685 (C=O), 1545, 1520, 1480, 1390 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.21 (d, J=6.5 Hz, 6H), 3.91 (d, J=7.8 Hz, 1H, NH), 4.45–4.70 (m, 1H), 7.58–7.65 (m, 2H), 8.50–8.56 (m, 2H), 8.71 (d, J=2.3 Hz, 1H), 8.86 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.8, 44.5, 115.1, 126.1, 130.4, 139.9, 142.5, 144.1, 148.8, 151.7; MS (EI) m/z 382 (M^+, 63), 339 (100), 324 (17), 203 (33). Anal. Calcd for $C_{17}H_{14}N_6O_3S$: C, 53.40; H, 3.69; N, 21.98; S, 8.39. Found: C, 53.49; H, 3.53; N, 21.76; S, 8.25.

3.4.13. 2-Isopropylamino-3-*N*-(4-methylphenyl)pyrazino-[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one ($8c_2$). Yield 98%; mp 217–219 °C; IR (KBr) ν 3414 (NH), 1676 (C=O), 1665, 1552, 1547, 1522, 1507, 1482, 1466, 1422, 1409 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.16 (d, J=6.5 Hz, 6H), 2.46 (s, 3H), 4.18 (d, J=7.9 Hz, 1H, NH), 4.45–4.75 (m, 1H), 7.17–7.25 (m, 2H), 7.40–7.45 (m, 2H), 8.65 (d, J=2.3 Hz, 1H), 8.81 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.3, 22.7, 43.9, 115.2, 128.2, 131.2, 132.5, 140.5, 142.2, 143.7, 144.1, 150.2, 152.9, 158.6, 158.8; MS (EI) m/z 351 (M^+, 40), 308 (100), 91 (25). Anal. Calcd for $C_{18}H_{17}N_5OS$: C, 61.52; H, 4.88; N, 19.93; S, 9.12. Found: C, 61.45; H, 4.97; N, 20.03; S, 9.17.

3.4.14. 2-Cyclohexylylamino-3-*N*-(4-nitrophenyl)pyrazino-[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one ($8d_1$). Yield 84%; mp 246–248 °C; IR (KBr) ν 3429 (NH), 1690 (C=O), 1547, 1530, 1346 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.06–1.24 (m, 3H), 1.35–1.50 (m, 3H), 1.73–1.92 (m, 2H), 1.99–2.04 (m, 2H), 3.97 (d, J=8.0 Hz, 1H, NH), 4.25–4.38 (m, 1H), 7.57–7.65 (m, 2H), 8.48–8.56 (m, 2H), 8.71 (d, J=2.3 Hz, 1H), 8.86 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.3, 25.3, 32.8, 50.4, 126.1, 130.4, 140.0, 142.5, 144.1, 148.8, 150.6, 151.7; MS (EI) m/z 422 (M^+, 5), 339 (30), 203 (30), 123 (30), 99 (52). Anal. Calcd for $C_{20}H_{18}N_6O_3S$: C, 56.86; H, 4.29; N, 19.89; S, 7.59. Found: C, 56.69; H, 4.13; N, 19.76; S, 7.46.

3.4.15. 2-Cyclohexylylamino-3-*N*-(4-methylphenyl)pyrazino[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one ($8d_2$). Yield 88%; mp 233–235 °C; IR (KBr) ν 3416 (NH), 1686 (C=O), 1546, 1532, 1520, 1485, 1347 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.00–1.23 (m, 3H), 1.36–1.65 (m, 5H), 1.90–2.05 (m, 2H), 2.48 (s, 3H), 4.20–4.35 (m, 2H), 7.19–7.26 (m, 2H), 7.39–7.47 (m, 2H), 8.67 (d, J=2.3 Hz, 1H), 8.82 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.3, 24.2, 25.4, 32.8, 49.9, 115.2, 128.2, 131.3, 131.5, 140.5, 142.2, 143.6, 144.1, 150.2, 153.0, 158.6, 158.9; MS (EI) m/z 391 (M^+, 10), 308 (100). Anal. Calcd for $C_{21}H_{21}N_5OS$: C, 64.43; H, 5.41; N, 17.89; S, 8.19. Found: C, 64.33; H, 5.42; N, 17.69; S, 8.12.

3.4.16. 2-*tert*-Butylamino-3-*N*-(4-nitrophenyl)pyrazino-[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one ($8e_1$). Yield 93%; mp 227–229 °C; IR (KBr) ν 3415 (NH), 1690 (C=O), 1596, 1556, 1545, 1523, 1518, 1484 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.50 (s, 9H), 3.99 (s, 1H, NH), 7.58–7.61 (m, 2H), 7.48–7.51 (m, 2H), 8.68 (d, J=2.3 Hz, 1H), 8.86 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.0, 53.8, 126.0, 130.4, 140.3, 142.6, 143.9, 144.1, 148.7, 150.3, 151.0, 158.4, 158.7; MS (EI) m/z 396 (M^+, 15), 339 (90). Anal. Calcd for $C_{18}H_{16}N_6O_3S$: C, 54.54; H, 4.07; N, 21.20; S, 8.09. Found: C, 54.43; H, 4.19; N, 21.14; S, 8.19.

3.4.17. 2-*tert*-Butylamino-3-*N*-(4-methylphenyl)pyrazino-[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one ($8e_2$). Yield 85%; mp 224–226 °C; IR (KBr) ν 3428 (NH), 1681 (C=O), 1557, 1545, 1520, 1484, 1456 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.43 (s, 3H), 4.29 (s,

1H, NH), 7.18–7.21 (m, 2H), 7.33–7.41 (m, 2H), 8.62 (d, J=2.3 Hz, 1H), 8.81 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2, 28.8, 53.0, 115.1, 128.1, 131.4, 140.3, 142.2, 143.4, 144.2, 149.8, 152.2, 158.4, 158.9; MS (EI) m/z 365 (M^+, 10), 308 (50). Anal. Calcd for $C_{19}H_{19}N_5OS$: C, 62.44; H, 5.24; N, 19.16; S, 8.77. Found: C, 62.49; H, 5.39; N, 19.13; S, 8.91.

3.4.18. 3-*N*-(4-Nitrophenyl)-2-phenylaminopyrazino-[2′,3′:4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (8f). Yield 98%; mp 254–256 °C; IR (KBr) ν 3380 (NH), 1609 (C=O), 1572, 1542, 1537, 1520, 1503, 1492, 1487, 1455, 1413 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.54 (s, 1H, NH), 7.49–7.52 (m, 2H), 7.68–7.79 (m, 3H), 7.81–7.84 (m, 2H), 8.24–8.27 (m, 2H), 8.75 (d, J=2.3 Hz, 1H), 8.90 (d, J=2.3 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 119.7, 125.2, 128.8, 131.2, 131.4, 133.3, 142.9, 143.1, 143.4, 143.6, 144.3, 148.6, 149.0, 158.1, 158.4; MS (EI) m/z 416 (M^+, 100), 370 (20). Anal. Calcd for $C_{20}H_{12}N_6O_3S$: C, 57.69; H, 2.90; N, 20.18; S, 7.70. Found: C, 57.50; H, 2.78; N, 20.33; S, 7.82.

4. Crystallographic material

Crystallographic data (excluding structural factors) for **8a**, **8c$_1$**, and **9a$_1$** have been deposited in the Cambridge Crystallographic Data Center as supplementary publication numbers CCDC 614690, CCDC 614691, and CCDC 614692 for **8a**, **8c$_1$**, and **9a$_1$**, respectively. Copies of the data may be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 1223 33603 or e-mail: deposit@ccdc.cam.ac.uk).

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Synthesis, in vitro antiproliferative activities, and Chk1 inhibitory properties of dipyrrolo[3,4-*a*:3,4-*c*]carbazole-triones

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Abstract—The syntheses of dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,4,6-triones and dipyrrolo[3,4-*a*:3,4-*c*]carbazole-3,4,6-triones are reported. These compounds can be considered as granulatimide analogues in which a maleimide replaces the imidazole moiety and a five-membered lactam ring replaces the upper maleimide. The Chk1 inhibitory properties of the more soluble compounds have been evaluated and their in vitro antiproliferative activities toward three tumor cell lines: murine leukemia L1210, and human colon carcinoma HT29 and HCT116. Due to their insolubility, the biological activities of the other compounds in this series could not be evaluated. All the tested compounds proved to be potent Chk1 inhibitors.

1. Introduction

The carbazole framework is found in many biologically active compounds. Some of them, such as rebeccamycin, are topoisomerase I inhibitors. Others, such as staurosporine and UCN-01, are inhibitors of kinases.[1–3] Granulatimide and isogranulatimide, natural compounds isolated from an ascidian, as well as staurosporine and UCN-01, indolocarbazole compounds isolated from cultures of *Streptomyces*, or synthetic compounds such as SB-218078 have triggered considerable interest as cell cycle G2 checkpoint inhibitors (Fig. 1).[4–7] In the cell division cycle, the G2 checkpoint is activated in response to DNA damage. Its role consists in blocking the cell cycle to allow time for DNA repair. In more than 60% of cancer cells, the G1 checkpoint is lacking, due to mutations of the *p53* gene. In the *p53*-mutated cells, only the G2 ckeckpoint provides cancer cells with an opportunity to repair their DNA after damage. Accordingly, combining a DNA damaging agent with a G2 checkpoint inhibitor will force selectively cancer cells into a premature and lethal mitosis due to an accumulation of DNA lesions.[8–10] The Chk1 kinase plays a major role in the G2 checkpoint regulation.[11,12] Therefore, Chk1 inhibitors are relevant targets for the conception of agents that are able to kill selectively cancer cells without causing damage to healthy cells. Granulatimide, isogranulatimide, staurosporine, UCN-01, and SB-218078 were found to be efficient Chk1 inhibitors. All of them possess a carbazole moiety, with an upper heterocycle containing an imide or a lactam function. Moreover, in staurosporine, UCN-01, and SB-218078, a carbohydrate-like heterocycle is linked to both indole nitrogens.

The crystal structures of SB-218078, staurosporine, UCN-01,[13] and isogranulatimide[14] in complex with Chk1 kinase, have been determined. These compounds are ATP-competitive Chk1 inhibitors. In the structures of the four complexes,

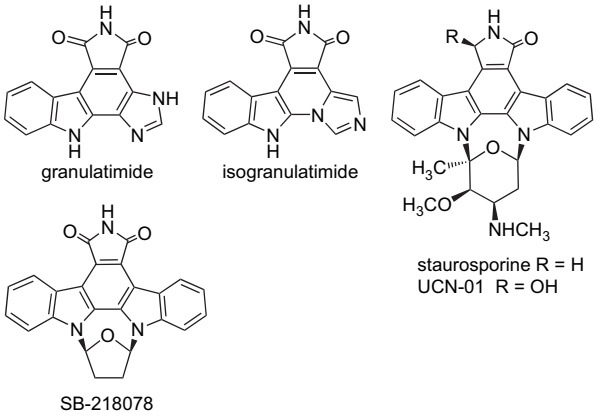

Figure 1.

Keywords: Granulatimide; Dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,4,6-trione; Dipyrrolo[3,4-*a*:3,4-*c*]carbazole-3,4,6-trione; Antitumor agents; Chk1 inhibitors.
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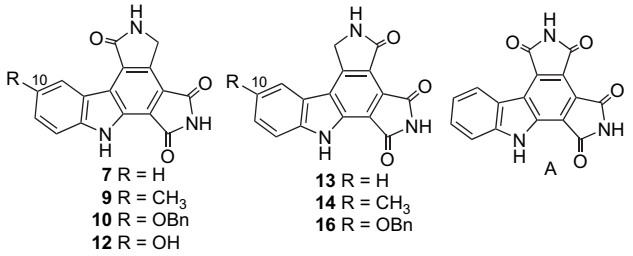


Figure 2.

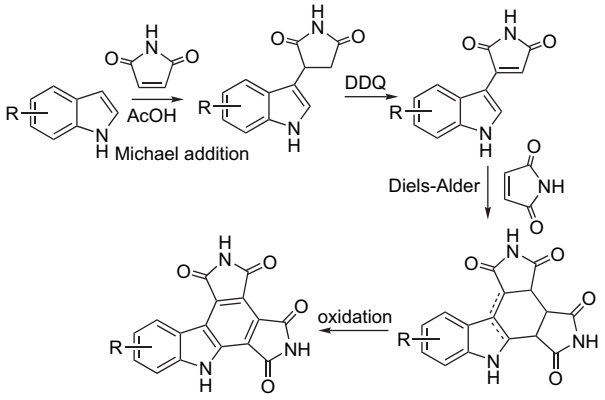
two hydrogen bonds between the inhibitors and the ATP binding site of the enzyme are conserved: the first one between the NH of the upper heterocycle and the carbonyl oxygen of Glu[85], and the second one between the oxygen of the carbonyl group of the lactam or imide function of the drug and the amide nitrogen of Cys[87]. Granulatimide and isogranulatimide isomers, and structurally related compounds bearing modified heterocycles, have been recently synthesized.[15–24]

In this paper, we describe the syntheses of dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,4,6-triones and dipyrrolo[3,4-*a*:3,4-*c*]carbazole-3,4,6-triones (Fig. 2). Compared with granulatimide and isogranulatimide, the imidazole has been replaced by a maleimide and the upper heterocycle contains a lactam function, like in staurosporine, instead of the imide function present in granulatimide and isogranulatimide. Moreover, several substituents have been introduced in the 10-position of the indole moiety. Some compounds in this series proved to be extremely insoluble, therefore, their biological activities could not be evaluated. For the most soluble compounds, the Chk1 inhibitory activities and the cytotoxicities toward three tumor cell lines: murine leukemia L1210, and human colon carcinoma HT29 and HCT116 were evaluated.

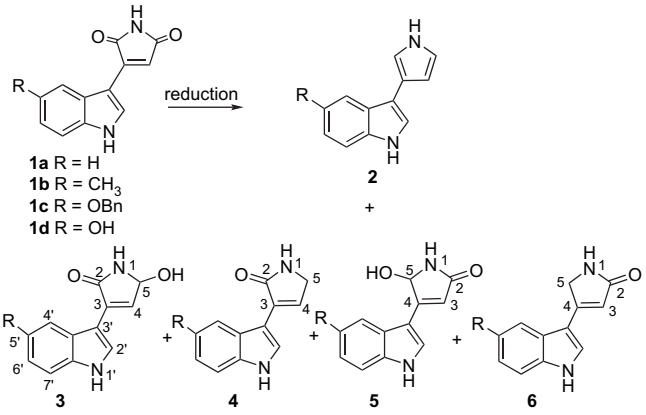
2. Results and discussion

2.1. Chemistry

In previous works,[22–24] we described the four-step synthesis of bis-imides granulatimide analogues (Scheme 1). A similar synthetic scheme was applied for the synthesis of



Scheme 1.



Scheme 2.

dipyrrolo[3,4-*a*:3,4-*c*]carbazole-triones. The reduction of the 3-(indol-3-yl)-maleimides intermediates led to lactams and hydroxylactams, from which the synthesis was completed.

Hydroxy and methyl substituents were introduced in the 10-position because in the bis-imide series, these substitutions led to the most efficient Chk1 inhibitors.

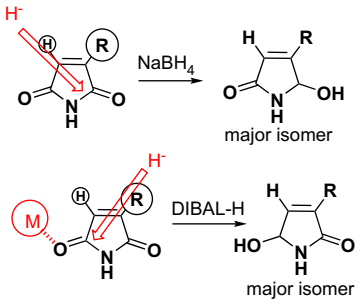
3-(Indol-3-yl)-maleimides **1a–d** (R=H, OBn, OH, and CH_3) were prepared as previously described[22,24] in two steps from the corresponding substituted indoles via a Michael addition with maleimide followed by dehydrogenation of the Michael adduct using DDQ. The corresponding lactams and hydroxylactams were obtained by reduction of the 3-(indol-3-yl)-maleimides (Scheme 2).

Depending on the reducing agent ($LiAlH_4$, $NaBH_4$ or DIBAL-H) and the substituent on the indole moiety, important variations were observed in the yields of compounds **2–6** (Table 1). Indeed, Mase et al.[25] showed that the regioselectivity of the reduction of monosubstituted maleimides using $NaBH_4$ was due to the approach of the hydride anion from the less hindered carbonyl group. Therefore, the hydride anion attacks the more hindered carbonyl group. When using DIBAL-H, the inverted regioselectivity is explained by the complexation effect of the carbonyl group with an aluminum atom preferably coordinated to the less hindered carbonyl group. Therefore, the hydride anion approaches from the more hindered carbonyl group and attacks

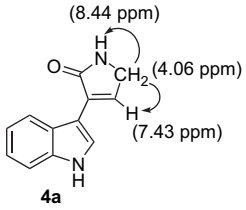
Table 1. Percentages of compounds **2**, **3**, **4**, **5**, and **6** obtained by reduction of **1a–d** using $LiAlH_4$, $NaBH_4$ or DIBAL-H and ratio for the reductions on Cl/Cm (Cl: less hindered carbon, Cm: more hindered carbon)

Starting product	Reducing agent	Compounds (%)					Reduction ratio on Cl/Cm (%)	
		2	**3**	**4**	**5**	**6**		
1a	$LiAlH_4$	9	6	11	23	19	29	71
	$NaBH_4$	0	11	0	60	0	16	84
	DIBAL-H	0	17	33	15	0	77	23
1b	$LiAlH_4$	1	0	11	17	21	22	78
	DIBAL-H	0	54	11	19	0	78	22
1c	$LiAlH_4$	0	0	6	24	40	8	92
	DIBAL-H	0	42	13	13	0	81	19
1d	$LiAlH_4$	0	0	12	33	0	27	73
	DIBAL-H	0	0	28	15	0	65	35

the less hindered carbonyl group (Scheme 3). In compounds **3** and **4**, the less hindered carbonyl group has been reduced, whereas in compounds **5** and **6**, the more hindered carbonyl group has been reduced. With the less bulky $LiAlH_4$, the complexation of both carbonyl groups with an aluminum atom may occur, but the hydride anion approaches very probably more quickly from the less hindered side. The structure of compounds **4a** was assigned from NMR NOESY correlations between the two methylene protons and the NH of the lactam function and the vicinal ethylenic proton (Scheme 4). In compound **6a**, no NOESY correlations were observed between the protons of the methylene group and the ethylenic proton. The H_4 of compound **4a** is shifted at 7.43 ppm, whereas the H_3 of compound **6a** is shifted at 6.26 ppm. Based on these NMR data, the structures of compounds **3a** and **5a** were assigned (**3a**: H_4 at 7.14 ppm, **5a**: H_3 at 6.17 ppm). By analogy with the unsubstituted compounds **3a**–**6a**, the structures of the analogues substituted in 5′-position on the indole moiety were assigned from the chemical shifts of the ethylenic protons.

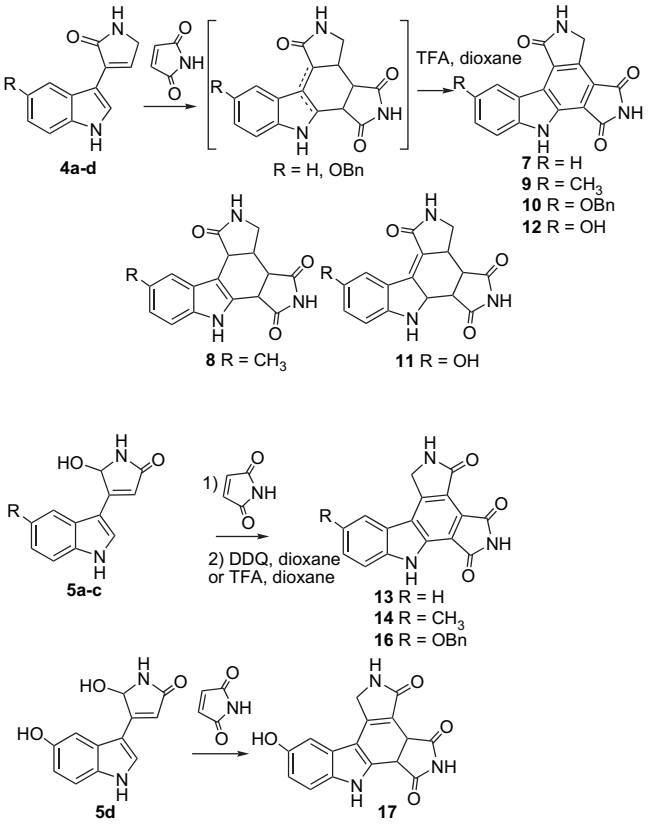


Scheme 3.



Scheme 4.

The next step was a Diels–Alder cycloaddition with maleimide. In previous studies, it was observed that the Diels–Alder cycloaddition carried out between 3-indolyl-maleimide and maleimide could lead, according to the treatment, filtration or chromatography on silicagel, to indoline or indole isomers.[22,26] When the Diels–Alder reaction was performed from **4a** and **4c**, the mixture of isomers could not be separated. The oxidation yielding to the final aromatic compounds **7** and **10** was carried out on the isomeric mixture. With lactams **4b** and **4d**, the indole intermediate **8** and the indoline intermediate **11** were isolated. The position of the double bond was determined from ^1H NMR data. Indeed, an indole and an imide NH are usually shifted at about 11–12 ppm, whereas an indoline and a lactam NH are shifted at about 7–9 ppm. In compounds **8**, two exchangeable protons are shifted at 11.12, and 11.52 ppm whereas in compound **11**, only one exchangeable proton was shifted at 10.94 ppm. Oxidation of the intermediates in dioxane in the presence of TFA gave lactams **7**, **9**, **10**, and **12** (Scheme 5).



Scheme 5.

Diels–Alder reactions performed from hydroxy-lactams **3a–d** did not lead to the cycloadducts, whereas from hydroxy-lactams **5a–d**, the cycloaddition occurred with the loss of a water molecule. With compounds **5c** and **5d**, the indole intermediates **15** and **17** could be isolated. Oxidation of the intermediates in dioxane, either in the presence of TFA or with DDQ, led to the required lactams **13**, **14**, and **16**. In spite of various modifications of the oxidation procedure of intermediate **17** (in dioxane in the presence of TFA from 6 to 20 equiv from 60 °C to 80 °C or with DDQ 2 equiv in dioxane at room temperature), the required aromatized compound could not be obtained. Concomitant with the aromatization, oxidation of the lactam heterocycle to imide was observed.

Cycloadditions between maleimide and lactams **6** did not lead to the required cycloadducts. With lactam **6** in which R=H, depending on the solvents used, either a double Diels–Alder reaction or a Diels–Alder reaction followed by a Michael addition with a second molecule of maleimide occurred.

2.2. Chk1 inhibitory activities

The Chk1 inhibitory activities could only be determined with compounds **7**, **10**, **12**, **13**, and intermediate **17** and were compared with those of granulatimide, isogranulatimide, and bis-imide analogue **A** (Fig. 2) (Table 2).[27] Due

Table 2. Percentages of Chk1 inhibition at a drug concentration of 10 μM, IC_{50} values (μM) toward Chk1; in vitro antiproliferative activities against three tumor cell lines: murine leukemia L1210, and human HT29 and HCT116 colon carcinoma (IC_{50} μM)

Compound	% of Chk1 inhibition at 10 μM	IC_{50} Chk1 (μM)	L1210	HCT116	HT29
Granulatimide	93.9	0.08	2.8	6.1	5.7
Isogranulatimide	89.7	0.44	10	13	13.7
A	94.4	0.02	32.7	nd	9.7
7	85.4	0.05	nd	nd	nd
10	69.8	1	25.9	28.3	36.4
12	95.7	0.01	54.5	63.5	41.8
13	71.7	0.37	47.0	58.9	>100
17	78.6	2.83	>50	43.7	49.7

to the insolubility of compounds **9**, **14**, and **16**, their Chk1 inhibitory activities could not be evaluated. Compounds **7**, unsubstituted at the 10-position, and compound **12** bearing a hydroxy group, are stronger Chk1 inhibitors than granulatimide and isogranulatimide. Interestingly, when the carbonyl of the lactam heterocycle is oriented toward the indole moiety, the compounds seem to be more efficient Chk1 inhibitors than those in which the carbonyl of the lactam heterocycle is oriented toward the imide heterocycle (compare **7** and **13**). These results are not completely surprising since, in the crystal structures of staurosporine, UCN-01, and isogranulatimide in complex with Chk1, the carbonyl on the left of the upper heterocycle accepts a hydrogen bond from the amide nitrogen of Cys^{87}, whereas no hydrogen bond is formed with the carbonyl located on the right. However, the strong Chk1 inhibitory activity of compound **13** could be due to a different position of the molecule in the ATP binding site allowing the formation of the two fundamental hydrogen bonds with Glu^{85} and Cys^{87}. In this orientation, the imide heterocycle would be positioned on the left and the indole moiety would lie on the right. No significant differences are observed between the Chk1 inhibitory activities of lactam **7** and imide **A**. Compared with unsubstituted compound **7**, compound **12** substituted with a hydroxy group is a stronger Chk1 inhibitor.

2.3. In vitro antiproliferative activities

The cytotoxicities of the soluble compounds were evaluated toward three tumor cell lines: murine leukemia L1210, and human colon carcinoma HT29 and HCT116 and compared with those of granulatimide, isogranulatimide, and compound **A** (Table 2). Compared with granulatimide and isogranulatimide, all the lactams tested are considerably less active, their cytotoxicities are in the same range as those of imide **A**. A checkpoint inhibitor is not expected to be cytotoxic by itself. However, the weak in vitro antiproliferative activities of the new compounds described in this paper suggests a possible instability of these compounds in the biological medium.

3. Conclusion

In conclusion, this work reports the synthesis of pyrrolo[3,4-*a*:3,4-*c*]carbazole-1,4,6-tetraones and pyrrolo[3,4-*a*:3,4-*c*]-carbazole-3,4,6-tetraones. These compounds are structurally related to the Chk1 inhibitor granulatimide. Their upper heterocycle contains a lactam function like in staurosporine. All the new compounds are potent Chk1 inhibitors suggesting that the orientation of the carbonyl group of the upper heterocycle, either toward the indole moiety or toward the maleimide unit, could modify the positioning of the drug in the active site of the kinase. Their weak cytotoxicity could be due to a limited penetration into the cells or to a degradation in the biological media. This hypothesis is currently under investigation.

4. Experimental

4.1. Chemistry

IR spectra were recorded on a Perkin–Elmer 881 spectrometer (ν in cm^{-1}). NMR spectra were performed on a Bruker AVANCE 400 and AVANCE 500 (chemical shifts δ in parts per million, the following abbreviations are used: singlet (s), broad singlet (br s), doublet (d), doubled doublet (dd), triplet (t), doubled triplet (dt), multiplet (m), pseudo quadruplet (pq), tertiary carbons (C tert), and quaternary carbons (C quat). The signals were assigned from ^1H–^1H COSY, HSQC, and HMBC NMR correlations. Low-resolution mass spectra (ESI+ and APCI+) and HRMS were determined on a MS Hewlett Packard instrument. Chromatographic purifications were performed by flash silicagel Geduran SI 60 (Merck) 0.040–0.063 mm column chromatography.

4.2. Typical procedure for the reduction using $LiAlH_4$

To a solution of 3-(indol-3-yl)-maleimide (200 mg, 0.94 mmol) in THF (40 mL) was added dropwise a 1 M solution of $LiAlH_4$ in Et_2O (6 mL) at room temperature. The mixture was stirred for 3 days. After cooling to 0 °C, water (14 mL) was added. The mixture was acidified to pH 2 with 2 M HCl (2 mL). After extraction with EtOAc, the organic phase was washed with saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$ and the solvent was removed. The residue was purified by flash chromatography (eluent: from EtOAc/cyclohexane 1:1 to EtOAc/MeOH 9:1).

4.3. Typical procedure for the reduction using $NaBH_4$

To a solution of 3-(indol-3-yl)-maleimide (2.4 mmol) in THF (50 mL) was added sodium borohydride (90 mg, 2.4 mmol) in portions. The mixture was stirred at room temperature for 24 h. After cooling to 0 °C, water was added. After extraction with EtOAc, the organic phase was dried over $MgSO_4$, the solvent was removed and the residue was purified by flash chromatography (eluent: from cyclohexane/EtOAc 1:1 to EtOAc/MeOH 9:1).

4.4. Typical procedure for the reduction using DIBAL-H

To a solution of 3-(indol-3-yl)-maleimide (0.94 mmol) in THF (70 mL) at −78 °C was added dropwise a 1 M solution of DIBAL-H in toluene (2.3 mL). The mixture was stirred for 1.5 h at −78 °C, then a 1 M solution of DIBAL-H in toluene (2.3 mL) was added. The mixture was stirred at −78 °C for 6 h. Then the mixture was warmed to 0 °C and saturated aqueous $NaHCO_3$ was added dropwise. After extraction

with EtOAc, the organic phase was dried over $MgSO_4$, the solvent was removed and the residue was purified by flash chromatography (eluent: from EtOAc/cyclohexane 1:1 to EtOAc/MeOH 9:1).

4.4.1. 3-(1*H*-Indol-3-yl)-1*H*-pyrrole (2a). Yellow solid. Mp 65 °C. IR (KBr) ν_{NH} 2924 cm^{-1}. HRMS (ESI+) $[M+H]^+$ calcd for $C_{12}H_{11}N_2$ 183.0922, found 183.0926.

^1H NMR (400 MHz, DMSO-d_6): 6.38 (1H, s), 6.79 (1H, s), 7.04 (1H, dt, J_1=7.0 Hz, J_2=1.0 Hz), 7.09–7.10 (2H, m), 7.37 (1H, d, J=8.0 Hz), 7.40 (1H, d, J=2.5 Hz), 7.77 (1H, d, J=8.0 Hz), 10.73 (1H, br s), 10.91 (1H, s).

^{13}C NMR (100 MHz, DMSO-d_6): 106.1, 111.4, 113.4, 117.8, 118.5, 119.5, 120.6, 120.8 (C tert), 111.6, 117.4, 125.4, 136.5 (C quat).

4.4.2. 3-(1*H*-Indol-3-yl)-1*H*-5-hydroxy-2,5-dihydro-pyrrol-2-one (3a). Orange-brown solid. Mp >300 °C. IR (KBr) $\nu_{C=C}$ 1632 cm^{-1}, $\nu_{C=O}$ 1705 cm^{-1}, ν_{NH-OH} 3200–3500 cm^{-1}. Mass (ESI+) $[M+Na]^+$ 237.

^1H NMR (400 MHz, DMSO-d_6): 5.59 (1H, d, J=9.0 Hz, H_5), 5.99 (1H, d, J=9.0 Hz, OH), 7.14 (1H, s, H_4), 7.18 (1H, dt, J_1=8.0 Hz, J_2=1.0 Hz), 7.21 (1H, dt, J_1=8.0 Hz, J_2=1.0 Hz), 7.51 (1H, d, J=8.0 Hz), 7.94 (1H, d, J=8.0 Hz), 8.28 (1H, d, J=2.5 Hz), 8.65 (1H, s), 11.46 (1H, s).

^{13}C NMR (100 MHz, DMSO-d_6): 78.2 (CHOH), 106.0, 125.5, 130.6, 136.1 (C quat), 112.0, 119.7, 119.9, 121.7, 126.5, 133.8 (C tert), 171.7 (C=O).

4.4.3. 3-(1*H*-Indol-3-yl)-1*H*-2,5-dihydro-pyrrol-2-one (4a). Orange-brown solid. Mp 205–207 °C. IR (KBr) $\nu_{C=C}$ 1628 cm^{-1}, $\nu_{C=O}$ 1678 cm^{-1}, ν_{NH} 3284 cm^{-1}. HRMS (ESI+) $[M+H]^+$calcd for $C_{12}H_{11}N_2O$ 199.0871, found 199.0880.

^1H NMR (400 MHz, DMSO-d_6): 4.06 (2H, s, CH_2), 7.15 (1H, dt, J_1=8.0 Hz, J_2=1.0 Hz, $H_{5'}$), 7.24 (1H, dt, J_1=8.0 Hz, J_2=1.0 Hz, $H_{6'}$), 7.43 (1H, d, J=1.5 Hz, H_4), 7.49 (1H, d, J=8.0 Hz, $H_{7'}$), 7.93 (1H, d, J=8.0 Hz, $H_{4'}$), 8.28 (1H, d, J=2.5 Hz, $H_{2'}$), 8.44 (1H, s, NH_1), 11.36 (1H, s, NH_{indole}).

^{13}C NMR (100 MHz, DMSO-d_6): 46.0 (CH_2), 106.8, 125.5 (C quat), 130.6, 136.0 (C quat), 111.8, 119.3, 119.6, 121.5, 125.5 (C tert arom), 131.8 (CH lactam), 173.1 (C=O).

4.4.4. 4-(1*H*-Indol-3-yl)-1*H*-5-hydroxy-2,5-dihydro-pyrrol-2-one (5a). Orange-brown solid. Mp 207 °C. IR (KBr) $\nu_{C=C}$ 1608 cm^{-1}, $\nu_{C=O}$ 1662 cm^{-1}, ν_{NH} 3271 cm^{-1}. HRMS (ESI−) $[M-H]^-$ calcd for $C_{12}H_9N_2O_2$ 213.0664, found 213.0667.

^1H NMR (400 MHz, DMSO-d_6): 5.80 (1H, dd, J_1=9.5 Hz, J_2=1.0 Hz, H_5), 6.17 (1H, d, J=1.0 Hz, H_3), 6.22 (1H, d, J=9.5 Hz, OH), 7.17 (1H, dt, J_1=8.0 Hz, J_2=1.0 Hz), 7.19 (1H, dt, J_1=8.0 Hz, J_2=1.0 Hz), 7.47 (1H, d, J=8.0 Hz), 7.84 (1H, d, J=3.0 Hz), 7.85 (1H, d, J=8.0 Hz), 8.16 (1H, s), 11.69 (1H, s).

^{13}C NMR (100 MHz, DMSO-d_6): 80.5 (CHOH), 108.0, 125.3, 136.6, 154.4 (C quat), 112.1, 113.2, 120.1, 120.6, 122.1, 128.5 (C tert), 172.8 (C=O).

4.4.5. 4-(1*H*-Indol-3-yl)-1*H*-2,5-dihydro-pyrrol-2-one (6a). Brown solid. Mp 255 °C. IR (KBr) $\nu_{C=C}$ 1610 cm^{-1}, $\nu_{C=O}$ 1649 cm^{-1}, ν_{NH} 3255 cm^{-1}. HRMS (ESI−) $[M-H]^-$ calcd for $C_{12}H_9N_2O$ 197.0715, found 197.0722.

^1H NMR (400 MHz, DMSO-d_6): 4.42 (2H, s, CH_2), 6.26 (1H, d, J=1.0 Hz, H_3), 7.15 (1H, dt, J_1=8.0 Hz, J_2=1.0 Hz, $H_{5'}$), 7.19 (1H, dt, J_1=8.0 Hz, J_2=1.0 Hz, $H_{6'}$), 7.46 (1H, d, J=8.0 Hz, $H_{7'}$), 7.84 (3H, m, $H_{2'}$, $H_{4'}$, NH_1), 11.69 (1H, d, J=1.0 Hz, NH_{indole}).

^{13}C NMR (100 MHz, DMSO-d_6): 48.3 (CH_2), 109.0, 124.8, 136.9, 152.7 (C quat), 112.1, 114.4, 119.9, 120.6, 122.1, 126.7 (C tert), 175.0 (C=O).

4.4.6. 3-(5-Methyl-1*H*-indol-3-yl)-1*H*-pyrrole (2b). Green solid. Mp 110 °C. IR (KBr) ν_{NH} 3423 cm^{-1}. HRMS (ESI+) $[M+H]^+$ calcd for $C_{13}H_{13}N_2$ 197.1079, found 197.1084.

^1H NMR (400 MHz, DMSO-d_6): 2.44 (3H, s), 6.39 (1H, pq, J=2.0 Hz), 6.82 (1H, pq, J=2.0 Hz), 6.94 (1H, dd, J_1=8.0 Hz, J_2=1.5 Hz), 7.11 (1H, pq, J=2.0 Hz), 7.28 (1H, d, J=8.0 Hz), 7.37 (1H, d, J=2.5 Hz), 10.80 (1H, s, NH), 10.74 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 21.3 (CH_3), 106.1, 111.0, 113.3, 117.7, 119.1, 120.7, 122.3 (C tert), 111.1, 117.6, 125.7, 126.9, 134.9 (C quat).

4.4.7. 3-(5-Methyl-1*H*-indol-3-yl)-1*H*-5-hydroxy-2,5-dihydro-pyrrol-2-one (3b). Off-white solid. Mp >300 °C. IR (KBr) $\nu_{C=C}$ 1633 cm^{-1}, $\nu_{C=O}$ 1697 cm^{-1}, $\nu_{NH,OH}$ 3200–3500 cm^{-1}. HRMS (ESI+) $[M+H-H_2O]^+$ calcd for $C_{13}H_{11}N_2O$ 211.0871, found 211.0871.

^1H NMR (400 MHz, DMSO-d_6): 2.48 (3H, s), 5.59 (1H, d, J=7.5 Hz), 5.99 (1H, d, J=8.5 Hz, OH), 7.04 (1H, t, J=8.0 Hz), 7.14 (1H, s, H_4), 7.38 (1H, d, J=8.0 Hz), 7.73 (1H, s), 8.25 (1H, d, J=3.0 Hz), 8.65 (1H, s, NH), 11.32 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 21.3 (CH_3), 78.3 (CHOH), 105.6, 125.8, 128.7, 130.8, 134.5 (C quat), 111.6, 119.5, 123.3, 126.5, 133.5 (C tert), 171.8 (C=O).

4.4.8. 3-(5-Methyl-1*H*-indol-3-yl)-1*H*-2,5-dihydro-pyrrol-2-one (4b). Ochre solid. Mp >300 °C. IR (KBr) $\nu_{C=C}$ 1606 cm^{-1}, $\nu_{C=O}$ 1649 cm^{-1}, ν_{NH} 3100–3300 cm^{-1}. HRMS (ESI+) $[M+Na]^+$ calcd for $C_{13}H_{12}N_2ONa$ 235.0847, found 235.0853.

^1H NMR (400 MHz, DMSO-d_6): 2.47 (3H, s), 4.05 (2H, s), 7.02 (1H, dd, J_1=8.0 Hz, J_2=1.0 Hz), 7.36 (1H, d, J=8.0 Hz), 7.41 (1H, d, J=1.5 Hz, H_4), 7.71 (1H, br s), 8.22 (1H, d, J=2.5 Hz), 8.40 (1H, s, NH), 11.21 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 21.3 (CH_3), 45.9 (CH_2N), 106.3, 125.7, 128.3, 130.8, 134.4 (C quat), 111.5, 119.3, 123.1, 125.5, 131.5 (C tert), 173.2 (C=O).

4.4.9. 4-(5-Methyl-1*H*-indol-3-yl)-1*H*-5-hydroxy-2,5-dihydro-pyrrol-2-one (5b). Off-white solid. Mp 104 °C. IR (KBr) $\nu_{C=C}$ 1615 cm^{-1}, $\nu_{C=O}$ 1682 cm^{-1}, ν_{NH} 3274, 3398 cm^{-1}. HRMS (ESI+) $[M+Na]^+$ calcd for $C_{13}H_{12}N_2O_2Na$ 251.0796, found 251.0805.

^1H NMR (400 MHz, DMSO-d_6): 2.47 (3H, s), 5.83 (1H, dd, J_1=9.5 Hz, J_2=1.5 Hz), 6.21 (1H, d, J=1.0 Hz, H_3), 6.23 (1H, d, J=9.5 Hz), 7.05 (1H, dd, J_1=8.0 Hz, J_2=1.0 Hz), 7.39 (1H, d, J=8.0 Hz), 7.70 (1H, br s), 7.82 (1H, d, J=2.5 Hz), 8.16 (1H, s, NH), 11.59 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 21.2 (CH_3), 80.5 (CHOH), 107.6, 125.6, 129.4, 134.9, 154.5 (C quat), 111.7, 113.0, 119.8, 123.6, 128.4 (C tert), 172.9 (C=O).

4.4.10. 4-(5-Methyl-1*H*-indol-3-yl)-1*H*-2,5-dihydro-pyrrol-2-one (6b). Off-white solid. Mp 140 °C. IR (KBr) $\nu_{C=C}$ 1605 cm^{-1}, $\nu_{C=O}$ 1649 cm^{-1}, ν_{NH} 3035–3430 cm^{-1}. HRMS (ESI+) $[M+H]^+$ calcd for $C_{13}H_{13}N_2O$ 213.1028, found 213.1027.

^1H NMR (400 MHz, DMSO-d_6): 2.46 (3H, s), 4.43 (2H, s), 6.28 (1H, d, J=1.0 Hz, H_3), 7.05 (1H, dd, J_1=8.0 Hz, J_2=1.0 Hz), 7.37 (1H, d, J=8.0 Hz), 7.69 (1H, s), 7.81 (1H, s), 7.82 (1H, s), 11.58 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 21.2 (CH_3), 48.3 (CH_2N), 108.6, 125.1, 129.4, 135.1, 152.8 (C quat), 111.8, 114.1, 119.6, 123.6, 126.7 (C tert), 175.1 (C=O).

4.4.11. 3-(5-Benzyloxy-1*H*-indol-3-yl)-1*H*-5-hydroxy-2,5-dihydro-pyrrol-2-one (3c). Off-white solid. Mp >200 °C (decomposition). IR (KBr) $\nu_{C=C}$ 1631 cm^{-1}, $\nu_{C=O}$ 1706 cm^{-1}, $\nu_{NH,OH}$ 3100–3500 cm^{-1}. HRMS (ESI+) $[M+H]^+$ calcd for $C_{19}H_{17}N_2O_3$ 321.1239, found 321.1256.

^1H NMR (400 MHz, DMSO-d_6): 5.22 (2H, s, CH_2), 5.58 (1H, dt, J_1=9.0 Hz, J_2=2.0 Hz, H_5), 5.98 (1H, d, J=9.0 Hz, OH), 6.94 (1H, dd, J_1=9.0 Hz, J_2=2.0 Hz), 7.12 (1H, t, J=1.5 Hz), 7.36–7.46 (5H, m), 7.55 (2H, d, J=7.0 Hz), 8.25 (1H, d, J=3.0 Hz), 8.63 (1H, s, NH), 11.33 (1H, d, J=2.5 Hz, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 69.9 (CH_2O), 78.3 (CHOH), 103.4, 112.3, 112.6, 127.1, 127.6 (3C), 128.3 (2C), 133.5 (C tert), 105.9, 125.9, 130.6, 131.3, 137.8, 153.2 (C quat), 171.8 (C=O).

4.4.12. 3-(5-Benzyloxy-1*H*-indol-3-yl)-1*H*-2,5-dihydro-pyrrol-2-one (4c). Brown solid. Mp 185 °C. IR (KBr) $\nu_{C=C}$ 1620 cm^{-1}, $\nu_{C=O}$ 1680 cm^{-1}, ν_{NH} 3100–3500 cm^{-1}. Mass (ESI+) $[M+K]^+$ 343, $[M+Na]^+$ 327.

^1H NMR (400 MHz, DMSO-d_6): 4.05 (2H, s), 5.20 (2H, s), 6.92 (1H, dd, J_1=9.0 Hz, J_2=2.0 Hz), 7.33–7.47 (6H, m), 7.54 (2H, d, J=7.5 Hz), 8.23 (1H, d, J=2.5 Hz), 8.41 (1H, s, NH), 11.22 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 46.0 (CH_2N), 69.9 (CH_2OBn), 103.4, 112.0, 112.4, 126.1, 127.6, 127.7 (2C), 128.3 (3C), 131.3 (C tert), 106.7, 125.8, 130.6, 131.2, 137.8, 153.0 (C quat), 173.1 (C=O).

4.4.13. 4-(5-Benzyloxy-1*H*-indol-3-yl)-1*H*-5-hydroxy-2,5-dihydro-pyrrol-2-one (5c). Off-white solid. Mp 215–216 °C. IR (KBr) $\nu_{C=C}$ 1613 cm^{-1}, $\nu_{C=O}$ 1681 cm^{-1}, $\nu_{NH,OH}$ 3040–3663 cm^{-1}. HRMS (ESI+) $[M+H]^+$ calcd for $C_{19}H_{17}N_2O_3$ 321.1239, found 321.1242.

^1H NMR (400 MHz, DMSO-d_6): 5.23 (2H, s), 5.81 (1H, d, J=9.5 Hz), 6.22 (1H, s), 6.23 (1H, d, J=9.0 Hz), 6.95 (1H, d, J=9.0 Hz), 7.34–7.46 (5H, m), 7.54 (2H, d, J=7.5 Hz), 7.83 (1H, s), 8.17 (1H, s), 11.61 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 69.7 (CH_2OBn), 80.5 (CHOH), 103.5, 111.4, 111.9, 112.8, 127.6 (3C), 128.3 (2C), 129.0 (C tert), 107.9, 125.8, 131.7, 137.7, 153.6, 154.3 (C quat), 173.0 (C=O).

4.4.14. 4-(5-Benzyloxy-1*H*-indol-3-yl)-1*H*-2,5-dihydro-pyrrol-2-one (6c). Off-white solid. Mp 235–237 °C. IR (KBr) $\nu_{C=C}$ 1606 cm^{-1}, $\nu_{C=O}$ 1651 cm^{-1}, ν_{NH} 3200–3430 cm^{-1}. HRMS (ESI+) $[M+H]^+$ calcd for $C_{19}H_{17}N_2O_2$ 305.1290, found 305.1297.

^1H NMR (400 MHz, DMSO-d_6): 4.41 (2H, s), 5.23 (2H, s), 6.29 (1H, s, H_3), 6.94 (1H, d, J=9.0 Hz), 7.34–7.46 (5H, m), 7.54 (2H, d, J=7.5 Hz), 7.82 (2H, s), 11.59 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 48.3, 69.7 (CH_2), 103.2, 103.5, 112.9, 104.0, 114.0, 127.2, 127.6 (2C), 128.3 (2C), 129.0 (C tert), 108.9, 125.3, 132.0, 137.7, 152.7, 153.6 (C quat), 175.2 (CO).

4.4.15. 4-(5-Hydroxy-1*H*-indol-3-yl)-1*H*-5-hydroxy-2,5-dihydro-pyrrol-2-one (5d). Brown solid. Mp 102–104 °C. IR (KBr) $\nu_{C=C}$ 1614 cm^{-1}, $\nu_{C=O}$ 1678 cm^{-1}, $\nu_{NH,OH}$ 2981–3599 cm^{-1}. HRMS (ESI+) $[M+Na]^+$ calcd for $C_{12}H_{10}N_2O_3Na$ 253.0589, found 253.0595.

^1H NMR (400 MHz, DMSO-d_6): 5.81 (1H, d, J=10.0 Hz), 5.93 (1H, s), 6.23 (1H, d, J=10.0 Hz, OH), 6.75 (1H, d, J=9.0 Hz), 7.14 (1H, s), 7.31 (1H, dd, J_1=9.0 Hz, J_2=1.0 Hz), 7.78 (1H, s), 8.16 (1H, s), 8.97 (1H, s), 11.49 (1H, s, NH_{indole}).

^{13}C NMR (100 MHz, DMSO-d_6): 80.5 (CHOH), 104.2, 111.9, 112.0, 112.6, 128.8 (C tert), 107.2, 126.3, 130.9, 152.1, 154.9 (C quat), 172.8 (C=O).

4.4.16. 3-(5-Hydroxy-1*H*-indol-3-yl)-1*H*-2,5-dihydro-pyrrol-2-one (4d). Brown solid. Mp 180 °C. IR (KBr) $\nu_{C=C}$ 1618 cm^{-1}, $\nu_{C=O}$ 1672 cm^{-1}, ν_{NH} 3100–3550 cm^{-1}. Mass (APCI+) $[M+H]^+$ 215.

^1H NMR (400 MHz, DMSO-d_6): 4.04 (2H, s), 6.72 (1H, d, J=9.0 Hz), 7.20 (1H, s), 7.21 (1H, s), 7.27 (1H, d, J=9.0 Hz), 8.18 (1H, s), 8.39 (1H, s), 8.84 (1H, br s), 11.08 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 45.9 (CH_2), 103.9, 111.6, 112.1, 125.8, 130.5 (C tert), 106.0, 126.3, 130.4, 131.0, 151.4 (C quat), 173.2 (C=O).

4.4.17. 2*H*,5*H*,7*H*-1,3,4,6-Tetrahydro-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,4,6-trione (7). A mixture of maleimide

(244 mg, 2.51 mmol) and compound **4a** (100 mg, 0.505 mmol) in xylene (14 mL) was refluxed for 3 days. After cooling, the yellow precipitate was filtered off washed with xylene and dried. The yellow solid (144 mg, 0.491 mmol, 97% yield) corresponds to an isomeric mixture of Diels–Alder adducts. The mixture of isomers (84 mg, 0.286 mmol) in dioxane (22 mL) was refluxed for 36 h in the presence of trifluoroacetic acid (293 μL). After evaporation, EtOAc was added to the residue. The mixture was filtered off. The solid was successively washed with saturated aqueous $NaHCO_3$, brine, and EtOAc to give compound **7** as a yellow-brown solid (22 mg, 0.075 mmol, 27% yield).

Mp >310 °C. IR (KBr) $\nu_{C=O}$ 1690, 1700, 1720 cm^{-1}, ν_{NH} 3387 cm^{-1}. HRMS (ESI+) $[M+H]^+$ calcd for $C_{16}H_{10}N_3O_3$ 292.0722, found 292.0745.

^1H NMR (400 MHz, DMSO-d_6): 4.77 (2H, s), 7.34 (1H, t, J=7.5 Hz), 7.61 (1H, t, J=8.0 Hz), 7.73 (1H, d, J=7.5 Hz), 9.08 (1H, s), 9.25 (1H, d, J=8.0 Hz), 11.40 (1H, s), 12.37 (1H, s).

Due to its insolubility, the ^{13}C NMR spectrum could not be recorded.

4.4.18. 10-Methyl-2*H*,5*H*,7*H*-1,3,3a,3b,4,6,6a,11c-octahydro-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,4,6-trione (8). A mixture of maleimide (179 mg, 1.84 mmol) and compound **4b** (78 mg, 0.37 mmol) in xylene (10 mL) was refluxed for 3 days. After cooling, the mixture was filtered off and the solid residue was washed with xylene then dried to give **8** as an off-white solid (116 mg, 0.37 mmol, 100% yield).

Mp >295 °C. IR (KBr) $\nu_{C=O}$ 1715, 1777 cm^{-1}, ν_{NH} 3061–3684 cm^{-1}. HRMS (ESI+) $[M+H]^+$ calcd for $C_{17}H_{16}N_3O_3$ 310.1192, found 310.1178.

^1H NMR (400 MHz, DMSO-d_6): 2.37 (3H, s), 2.82 (1H, t, J=9.5 Hz), 3.11 (1H, t, J=9.0 Hz), 3.25 (1H, m), 3.51 (1H, t, J=7.0 Hz), 3.56 (1H, d, J=7.0 Hz), 4.38 (1H, d, J=8.5 Hz), 6.92 (1H, d, J=8.0 Hz), 7.28 (1H, d, J=8.5 Hz), 7.58 (1H, s, NH), 7.75 (1H, s), 11.12 (1H, s, NH), 11.52 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 21.3 (CH_3), 41.3 (CH_2N), 35.6, 38.6, 40.3, 40.7 (CH), 110.7, 120.2, 122.8 (C tert), 102.2, 125.9, 126.3, 126.7, 135.3 (C quat), 175.1, 177.0, 178.5 (C=O).

4.4.19. 10-Methyl-2*H*,5*H*,7*H*-1,3,4,6-tetrahydro-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,4,6-trione (9). A solution of **8** (104 mg, 0.34 mmol) in dioxane (11 mL) and trifluoroacetic acid (266 μL) was stirred at 80 °C for 48 h. After evaporation, water was added to the residue, the mixture was filtered off, the solid was washed with water and with small amounts of EtOAc to give **9** as an orange solid (80 mg, 0.263 mmol, 78% yield).

Mp >300 °C. IR (KBr) $\nu_{C=O}$ 1716, 1758 cm^{-1}, ν_{NH} 3208–3664 cm^{-1}. HRMS (ESI+) $[M+H]^+$ calcd for $C_{17}H_{12}N_3O_3$ 306.0879, found 306.0894.

^1H NMR (400 MHz, DMSO-d_6): 2.50 (3H, s), 4.75 (2H, s), 7.43 (1H, d, J=8.0 Hz), 7.61 (1H, d, J=8.0 Hz), 9.05 (2H, s), 11.36 (1H, s), 12.23 (1H, s).

Due to its insolubility, the ^{13}C NMR spectrum could not be recorded.

4.4.20. 10-Benzyloxy-2*H*,5*H*,7*H*-1,3,4,6-tetrahydro-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,4,6-trione (10). A mixture of maleimide (122 mg, 1.25 mmol) and compound **4c** (74 mg, 0.25 mmol) in xylene (6 mL) was refluxed for 18 h. After cooling, the mixture was filtered off and the solid residue was washed with CH_2Cl_2 then was dried to give an orange solid as a mixture of isomers (97 mg, 0.25 mmol, quantitative yield).

The mixture of isomers (90 mg, 0.23 mmol), dioxane (6 mL) and trifluoroacetic acid (2.2 mmol, 172 μL) was stirred at 80 °C for 48 h. After evaporation, water was added to the residue, the mixture was filtered off and the solid residue was washed repeatedly with water and small amounts of EtOAc to give **10** (61 mg, 0.15 mmol, 65% yield) as a dark red solid.

Mp >300 °C. IR (KBr) $\nu_{C=C}$ 1617 cm^{-1}, $\nu_{C=O}$ 1722, 1774 cm^{-1}, ν_{NH} 3100–3550 cm^{-1}. HRMS (ESI+) $[M+Na]^+$ calcd for $C_{23}H_{15}N_3O_4Na$ 420.0960, found 420.0974.

^1H NMR (400 MHz, DMSO-d_6): 4.76 (2H, s), 5.21 (2H, s), 7.33–7.65 (7H, m), 8.95 (1H, d, J=2.0 Hz), 9.07 (1H, s), 11.37 (1H, s), 12.21 (1H, s).

Due to its insolubility, the ^{13}C NMR spectrum could not be recorded.

4.4.21. 10-Hydroxy-2*H*,5*H*,7*H*-1,3,3a,3b,4,6,6a,11c-octahydro-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,4,6-trione (11). A mixture of maleimide (40 mg, 0.48 mmol) and **4d** (92 mg, 0.43 mmol) in xylene (5 mL) was refluxed for 24 h. After cooling, the mixture was filtered off and the solid residue was washed with CH_2Cl_2 then was dried to give **11** (40 mg, 0.13 mmol, 30% yield) as a dark orange solid.

Mp >300 °C. IR (KBr) $\nu_{C=O}$ 1691, 1763 cm^{-1}, $\nu_{NH,OH}$ 3300–3550 cm^{-1}. Mass (ESI+) $[M+H]^+$ 312.

^1H NMR (400 MHz, DMSO-d_6): 2.94 (1H, m), 3.27 (1H, t, J=8.0 Hz), 3.47 (1H, t, J=9.0 Hz), 3.61 (1H, t, J=10.0 Hz), 4.23–4.29 (2H, m), 6.28 (1H, s), 6.59 (1H, d, J=8.0 Hz), 6.63 (1H, d, J=8.0 Hz), 7.83 (1H, s), 8.06 (1H, s), 8.67 (1H, s), 10.94 (1H, s).

^{13}C NMR (100 MHz, DMSO-d_6): 41.1 (CH_2N), 36.2, 42.7, 60.5, 70.0 (CH), 110.4, 113.2, 119.8 (C tert arom), 119.5, 122.8, 143.1, 148.8, 149.8 (C quat arom), 168.4, 175.7, 178.4 (C=O).

4.4.22. 10-Hydroxy-2*H*,5*H*,7*H*-1,3,4,6-tetrahydro-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-1,4,6-trione (12). A solution of **11** (80 mg, 0.257 mmol) in dioxane (8 mL) was stirred at 80 °C for 48 h in the presence of trifluoroacetic acid (2.56 mmol, 200 μL). After evaporation, water was added to the residue, the mixture was filtered off and the solid residue was washed successively with water and small amounts

of EtOAc to give **12** (52 mg, 0.169 mmol, 66% yield) as a red solid.

Mp >300 °C. IR (KBr) $\nu_{C=O}$ 1711 cm^{-1}, $\nu_{NH,OH}$ 3200–3672 cm^{-1}. HRMS (ESI+) $[M+H]^+$ calcd for $C_{16}H_{10}N_3O_4$ 308.0671, found 308.0672.

^1H NMR (400 MHz, DMSO-d_6): 4.75 (2H, s), 7.11 (1H, dd, J_1=9.0 Hz, J_2=2.0 Hz), 7.54 (1H, d, J=9.0 Hz), 8.66 (1H, d, J=2.0 Hz), 9.02 (1H, s), 9.27 (1H, s), 11.33 (1H, s), 12.06 (1H, s).

Due to its insolubility, the ^{13}C NMR spectrum could not be recorded.

4.4.23. 2*H*,5*H*,7*H*-1,3,4,6-Tetrahydro-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-3,4,6-trione (13). A mixture of **5a** (241 mg, 1.126 mmol) and maleimide (131 mg, 1.35 mmol) in xylene (20 mL) was refluxed for 4 days. After filtration, the solid residue was washed with water to give an isomeric mixture of the Diels–Alder adducts as a brown solid (254 mg, 0.86 mmol, 77% yield).

A solution of the isomeric mixture (60 mg, 0.205 mmol) in dioxane (7 mL) was refluxed for 21 days in the presence of trifluoroacetic acid (900 μL). After evaporation, EtOAc was added to the residue. The mixture was filtered off, and the solid was successively washed with saturated aqueous $NaHCO_3$, water, and EtOAc to give **13** as a brown solid (32 mg, 0.110 mmol, 54% yield).

Mp >300 °C. IR (KBr) $\nu_{C=O}$1710, 1720, 1780 cm^{-1}, ν_{N-H} 3000–3500 cm^{-1}. Mass (ESI+) $[M+H]^+$ 292. HRMS (ESI+) $[M+Na]^+$ calcd for $C_{16}H_9N_3O_3Na$ 314.0542, found 314.0556.

^1H NMR (400 MHz, DMSO): 5.03 (2H, s), 7.41 (1H, t, J=7.5 Hz), 7.63 (1H, t, J=7.5 Hz), 7.78 (1H, d, J=8.0 Hz), 8.12 (1H, d, J=8.0 Hz), 8.76 (1H, s, NH), 11.21 (1H, s, NH), 12.46 (1H, s, NH). Due to its insolubility, the ^{13}C NMR spectrum could not be recorded.

4.4.24. 10-Methyl-2*H*,5*H*,7*H*-1,3,4,6-tetrahydro-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-3,4,6-trione (14). A mixture of compound **5b** (120 mg, 0.53 mmol) and maleimide (61 mg, 0.62 mmol) in xylene (5 mL) was refluxed for 3 days. After filtration, the solid was washed with water and dried to give an isomeric mixture of the Diels–Alder adducts (161 mg, 0.52 mmol, 100% yield) as a brown solid.

A solution of the isomeric mixture (150 mg, 0.49 mmol) in dioxane (16 mL) was refluxed for 48 h in the presence of trifluoroacetic acid (2.2 mL). After evaporation, EtOAc was added to the residue. The mixture was filtered off to give **14** (75 mg, 0.24 mmol, 46% yield) as a brown solid.

Mp >300 °C. IR (KBr) $\nu_{C=O}$ 1719, 1770 cm^{-1}, ν_{NH} 3100–3550 cm^{-1}. HRMS (ESI+) $[M+Na]^+$ calcd for $C_{17}H_{11}N_3O_3Na$ 328.0698, found 328.0681.

^1H NMR (400 MHz, DMSO-d_6): 5.01 (2H, s), 7.45 (1H, d, J=8.5 Hz), 7.66 (1H, d, J=8.0 Hz), 7.92 (1H, s), 8.76 (1H, s, NH), 11.18 (1H, s, NH), 12.34 (1H, s, NH).

Due to its insolubility, the ^{13}C NMR spectrum could not be recorded.

4.4.25. 10-Benzyloxy-2*H*,5*H*,7*H*-1,3,3a,3b,4,6,6a,11c-octahydro-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-3,4,6-trione (15). A mixture of **5c** (90 mg, 0.28 mmol) and maleimide (34 mg, 0.35 mmol) in xylene (7 mL) was refluxed for 48 h. After filtration, the solid was washed with water and dried to give **15** (102 mg, 0.26 mmol, 91% yield) as a brown-orange solid.

Mp >300 °C. IR (KBr) $\nu_{C=O}$ 1719, 1778 cm^{-1}, ν_{NH} 3100–3550 cm^{-1}. HRMS (ESI+) $[M+H]^+$ calcd for $C_{23}H_{18}N_3O_4$ 400.1297, found 400.1285.

^1H NMR (400 MHz, DMSO-d_6): 4.33–4.39 (2H, m), 4.59 (1H, d, J=11.0 Hz), 4.64 (1H, d, J=17.5 Hz), 5.16 (2H, s), 6.89 (1H, dd, J_1=9.0 Hz, J_2=2.0 Hz), 7.13 (1H, d, J=2.0 Hz), 7.36–7.52 (6H, m), 7.97 (1H, s, NH), 11.72 (1H, s, NH), 11.73 (1H, s, NH).

^{13}C NMR (100 MHz, DMSO-d_6): 45.2, 69.6 (CH_2), 42.3, 46.7 (CH), 102.4, 111.2, 113.1, 127.6 (2C), 127.7, 128.3 (2C) (C tert), 105.2, 115.7, 123.1, 132.1, 133.0, 137.4, 148.3, 153.4 (C quat), 172.3, 177.3, 177.5 (CO).

4.4.26. 10-Benzyloxy-2*H*,5*H*,7*H*-1,3,4,6-tetrahydro-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-3,4,6-trione (16). A mixture of compound **15** (50 mg, 0.12 mmol) and DDQ (28 mg, 0.12 mmol) in dioxane (5 mL) was stirred overnight. After removal of the solvent, the residue was washed successively with water, EtOAc, and small amounts of THF. Compound **16** (19 mg, 0.05 mmol, 40% yield) was isolated as a brown solid.

Mp >300 °C. IR (KBr) $\nu_{C=C}$ 1617 cm^{-1}, $\nu_{C=O}$ 1722, 1774 cm^{-1}, ν_{NH} 3100–3500 cm^{-1}. Mass (ESI+) $[M+H]^+$ 398. HRMS (ESI+) $[M+Na]^+$ calcd for $C_{23}H_{15}N_3O_4Na$ 420.0960, found 420.0955.

^1H NMR (400 MHz, DMSO-d_6): 5.03 (2H, s), 5.29 (2H, s), 7.36–7.70 (8H, m), 8.79 (1H, s, NH), 11.19 (1H, s, NH), 12.33 (1H, s, NH).

Due to its insolubility, the ^{13}C NMR spectrum could not be recorded.

4.4.27. 10-Hydroxy-2*H*,5*H*,7*H*-1,3,3b,4,6,6a-hexahydro-dipyrrolo[3,4-*a*:3,4-*c*]carbazole-3,4,6-trione (17). A mixture of **5d** (120 mg, 0.52 mmol) and maleimide (61 mg, 0.62 mmol) in xylene (14 mL) was refluxed for 48 h. After filtration, the solid was washed with water and dried to give **17** (129 mg, 0.42 mmol, 81% yield) as an off-white solid.

Mp >300 °C. IR (KBr) $\nu_{C=O}$ 1708, 1778 cm^{-1}, $\nu_{NH,OH}$ 3200–3550 cm^{-1}. Mass (ESI+) $[M+H]^+$ 310, $[M+Na]^+$ 332. HRMS (ESI+) $[M+Na]^+$ calcd for $C_{16}H_{11}N_3O_4Na$ 332.0647, found 332.0664.

^1H NMR (400 MHz, DMSO-d_6): 4.32 (1H, d, J=18.0 Hz), 4.36 (1H, d, J=11.0 Hz), 4.54 (1H, d, J=19.0 Hz), 4.57 (1H, d, J=11.0 Hz), 6.69 (1H, dd, J_1=9.0 Hz, J_2=2.0 Hz),

6.84 (1H, d, J=2.0 Hz), 7.29 (1H, d, J=9.0 Hz), 7.88 (1H, s, NH), 8.96 (1H, s), 11.57 (1H, s, NH), 11.71 (1H, s, NH).

Due to its insolubility, the ^{13}C NMR spectrum could not be recorded.

4.5. Chk1 inhibitory assays

Human Chk1 full-length enzyme with an N-terminal GST sequence was either purchased from Upstate Biochemicals (No. 14-346) or purified from extracts of Sf9 cells infected with a baculovirus encoding GST-Chk1. Assays for compound testing were based upon the method described by Davies et al.[28]

4.6. Growth inhibition assays

Tumor cells were provided by American Type Culture Collection (Frederik, MD, USA). They were cultivated in RPMI 1640 medium (Life Science technologies, Cergy-Pontoise, France) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10 mM HEPES buffer (pH=7.4). Cytotoxicity was measured by the microculture tetrazolium assay as described.[29] Cells were continuously exposed to graded concentrations of the compounds for four doubling times, then 15 μL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added to each well and the plates were incubated for 4 h at 37 °C. The medium was then aspirated and the formazan solubilized by 100 μL of DMSO. Results are expressed as IC_{50}, concentration, which reduced by 50% the optical density of treated cells with respect to untreated controls.

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Reduction of substituted 1,10-phenanthrolines as a route to rigid chiral benzimidazolylidenes

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Abstract—Variously substituted 1,10-phenanthrolines are reduced to octahydrophenanthrolines in moderate to good yields with $NaBH_3CN$ in acetic acid/methanol. The exact solvent composition is important to avoid the formation of tetrahydrophenanthrolines and *N*-alkylated by-products, and to optimize the formation of octahydrophenanthrolines. Resolution of a racemic reduction product gives an enantiomerically pure C_2-symmetric diamine from which the corresponding rigid benzimidazolylidene is prepared, whereas reduction of chiral phenanthrolines derived from bicyclic ketones affords diastereomerically pure diamines, which may also be converted to benzimidazolylidenes.

1. Introduction

N-Heterocyclic carbenes (NHCs) with an imidazolidine framework can be classified into one of three general structural types: (a) saturated imidazolinylidenes (e.g., **1**, Fig. 1), (b) unsaturated imidazolylidenes (**2**), and (c) benzimidazolylidenes (**3**).[1] Imidazolylidenes and imidazolinylidenes are being extensively examined as reagents for asymmetric synthesis, although benzimidazolylidenes (**3**) have not been scrutinized to the same degree.[2] This may be because current synthetic approaches used to prepare benzimidazole-based NHCs are more limited, making the preparation of chiral analogs a challenging endeavor. Examples of chiral benzimidazolium salts that have been reported include α-methylbenzyl derivatives by Diver et al.[3,4] (**3a**,**b**, Fig. 2), acetonides **4** and **5** by Marshall et al.,[5] and Benaglia et al.,[6] and binaphthyl **6** by Duan et al.[7]

The preceding chiral NHCs are typically prepared by the introduction of one or two chiral substituents α to nitrogen by aryl amination,[3] or by quaternization[8,9] of the imidazole nitrogen. While analogous methods have been used to prepare chiral imidazolinylidene (**1**) and imidazolylidene NHCs (**2**),[10] existing methodology is less applicable for the preparation of chiral benzimidazolylidenes.[2] For example, chirality in the backbone of benzimidazolylidenes (**3**), such as in Grubbs' imidazolinylidene[11] **1**, is precluded by the presence of a fused aromatic ring. In addition, condensation of chiral amines with glyoxal, as in the preparation imidazolylidene **2**,[12,13] is not possible for benzimidazolylidenes. As a result, the current state-of-the-art for the synthesis of chiral benzimidazolylidenes such as **3** involves double Pd-catalyzed aryl amination of 1,2-dibromobenzene, followed by formylative ring closure.[3,4] The use of 2-nitrophenyl isocyanide, which holds promise for the synthesis of non-chiral benzimidazolylidenes, may not be adaptable to chiral derivatives.[14]

The main limitation of established routes to chiral benzimidazolylidenes is that chirality often occurs in freely-rotating acyclic pendant groups (**3a**,**b**), or in remote positions (**4**) that may not give well-defined chiral environments in catalytic processes (Fig. 2).[15] Consequently, chiral benzimidazolylidenes remain underrepresented as potential reagents for asymmetric synthesis, even though their electronic characteristics are intermediate between imidazolylidenes (**2**) and

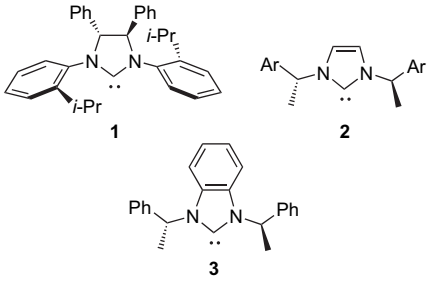

Figure 1. Examples of imidazolinylidenes (**1**), imidazolylidenes (**2**), and benzimidazolylidenes (**3**).

Keywords: Phenanthrolines; *N*-Heterocyclic carbenes; Benzimidazolylidenes; Reduction; Diastereoselective.
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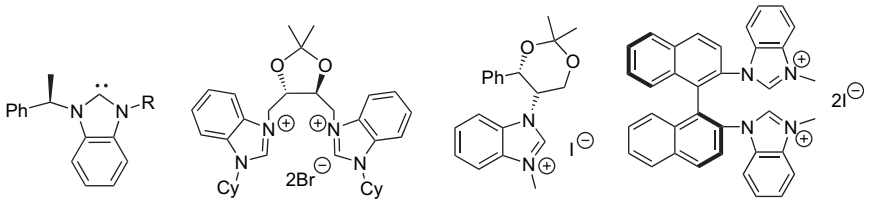


3a, R = CH(Me)Ph
3b, R = Ph
4 **5** **6**

Figure 2. Examples of chiral benzimidazolylidenes.

imidazolinylidenes (**1**).[3] In fact, benzimidazolylidenes have the advantage of being electronically tuneable by the introduction of substituents *para* to nitrogen on the aromatic ring.[16]

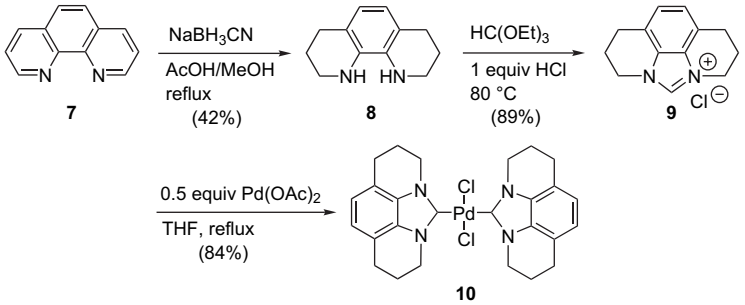
Given that free NHCs are often prepared by deprotonation of azolium salts,[8,9] new approaches toward the preparation of chiral benzimidazolium salts may lead to their structural diversification and increase their utility. The structural diversification of chiral benzimidazolylidenes is warranted considering that they have shown promise recently as organocatalysts[17,1c] and in Rh-catalyzed reductions,[7] and because they are vastly outnumbered[2] in comparison to imidazol(in)ylidenes for which desirable structural trends are emerging.[1]

Substituted 1,10-phenanthrolines have a rich history as ligands for transition-metal catalysis, but the majority of reported compounds retain the aromaticity of the entire tricyclic core.[18] Less attention has been directed toward the use of reduced phenanthrolines as precursors for new ligands.[19] It is known that nitrogen containing π-deficient heteroaromatics (pyridines, quinolines) undergo reduction to piperidines or tetrahydroquinolines under a wide range of conditions,[20,21] including the use of borohydrides in acid.[22] The use of borohydride reagents for the reduction of variously substituted 1,10-phenanthrolines to octahydrophenanthrolines has not been investigated, but is attractive in that the diamine products may serve as precursors to rigid benzimidazolium salts after treatment with orthoesters.

Previously, we have demonstrated the feasibility of this approach by synthesizing a catalytically active bis(benzimidazolylidene)palladium complex (**10**) in three steps from fully aromatic 1,10-phenanthroline (Scheme 1).[23,1a] The key step in the preparation of this ligand involved a convenient reduction of the pyridyl rings in 1,10-phenanthroline with sodium cyanoborohydride in refluxing AcOH/MeOH to provide octahydro-1,2,3,4,7,8,9,10-phenanthroline **8** in useful yield. This method precluded the need for high-pressure hydrogenation to prepare diamine **8**.[24] It was envisioned that the tetracyclic framework exemplified by **9** may serve as a prototype for the preparation of rigid chiral benzimidazolylidenes containing stereogenic centers α to nitrogen, but without freely-rotating pendant groups. In order to realize this potential, two questions first required an answer regarding the methodology: (a) could sterically encumbered phenanthrolines bearing substituents at the 2-, 2,9- and 2,3-positions (Scheme 4) be reduced with equal facility to the corresponding octahydrophenanthrolines using $NaBH_3CN$ in AcOH, and (b) could substituted phenanthrolines bearing chiral moieties be diastereoselectively reduced to provide stereochemically pure diamines for the preparation of structurally rigid benzimidazolylidenes. Herein we report the results of these studies.

2. Results and discussion

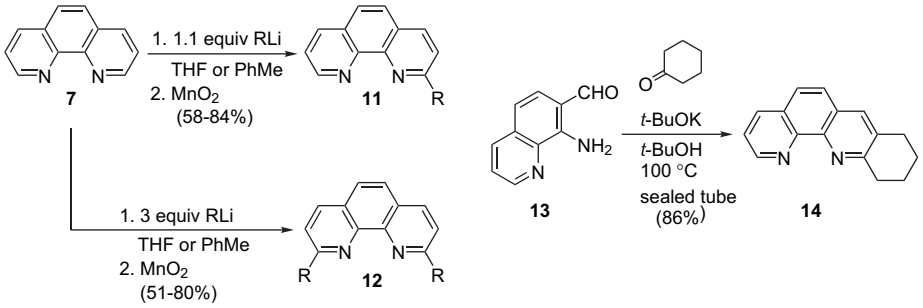
To answer the first question regarding the scope of the reduction conditions on substrates with greater steric demand than **7**, a series of 2-, 2,9- and 2,3-substituted 1,10-phenanthrolines were prepared by established methods such as nucleophilic aromatic substitution–oxidation for 2- and 2,9-derivatives, or de novo synthesis for 2,3-derivatives. Thus, treatment of **7** with 1.1 equiv of MeLi, BuLi, *i*-PrLi, PhLi or *t*-BuLi, in THF or toluene, followed by MnO_2 oxidation of the intermediate dihydro adducts, provided the required 2-substituted phenanthrolines **11** (Scheme 2).[25,26] Similarly, the 2,9-disubstituted phenanthrolines **12** were prepared by treatment of **7** with 3 equiv of MeLi, BuLi, *i*-PrLi or PhLi.[27] The 2,3-disubstituted derivatives were prepared via the Friedländer[28,29] reaction, as in the condensation of quinoline **13** with cyclohexanone.[30,31]



7 **8** **9**

10

Scheme 1. A benzimidazolylidene derived from octahydrophenanthroline.



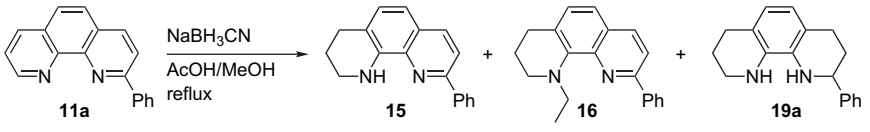
Scheme 2. Preparation of 2-, 2,9- and 2,3-substituted phenanthrolines.

2.1. Reduction of achiral 2-, 2,9- and 2,3-substituted phenanthrolines

Early investigations into the reduction of substituted compounds focused on optimizing the conditions required to reduce 2-phenyl-1,10-phenanthroline (**11a**, R=Ph) to the corresponding octahydrophenanthroline. Reduction of **11a** with $NaBH_3CN$ in 50:50 AcOH/MeOH gave tetrahydrophenanthroline **15** in 60% yield in which only the unsubstituted pyridyl ring was reduced (Scheme 3). In an attempt to force the reduction of the substituted ring, the reaction was repeated in neat AcOH. Under these conditions, only *N*-ethyltetrahydrophenanthroline **16** was isolated in 57% yield. These results mirror similar observations made by Gribble and Heald[22] in the $NaBH_4$/AcOH mediated reductive alkylation of tetrahydroquinoline **17** (Scheme 3), which afforded *N*-ethyltetrahydroquinoline (**18**). At first, the exclusive formation of **16** in neat AcOH implied that it would not be possible to reduce the phenyl-substituted ring under any conditions using $NaBH_3CN$. However, a series of experiments in which the solvent composition was varied by 10% increments from 50:50 AcOH/MeOH to neat AcOH revealed an interesting trend. Whereas products **15** and **16** were isolated exclusively using 50:50 AcOH/MeOH or neat AcOH, respectively, a mixture of **15** (43%) and the desired 2-phenyloctahydrophenanthroline **19a** (21%) was isolated using 60:40 AcOH/MeOH as the solvent medium. Using 70:30 AcOH/MeOH gave **15** and **19a** in 21 and 30% yields, respectively, while 80:20 AcOH/MeOH afforded predominantly the desired **19a** in 57% yield. Raising the acetic acid concentration to 90:10 resulted in a significant decrease in the yield of **19a** (18%), along with concomitant formation of **16** (17%).

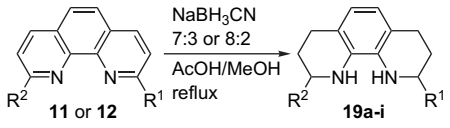
The preceding results indicate that the formation of tetrahydrophenanthroline **15** is relatively facile, but that two competing reactions occur on the initially formed intermediate depending on the proportion of acid present. In neat acetic acid, ethylation of the newly formed piperidyl nitrogen appears to proceed faster than reduction of the phenyl-substituted pyridyl ring, whereas utilizing proportionately less acetic acid (80%) allows reduction of the substituted pyridyl ring to predominate. The fact that *N*-ethylation inhibits further reduction of **16** may be tentatively attributed to the greater basicity of the *N*-ethyl piperidyl nitrogen compared to the remaining pyridyl nitrogen.

Based on the experiments performed on **11a**, the remaining 2- and 2,9-substituted phenanthrolines were subjected to reduction using a solvent mixture of either 70:30 or 80:20 AcOH/MeOH. Yields of racemic 2-substituted octahydrophenanthrolines ranged from 57% for 2-Me to 70% for 2-*i*-Pr (**19a–d**, Scheme 4). The 2-*tert*-Butyl-octahydrophenanthroline (**19e**) was produced in lower 23% yield, indicating a steric limitation in the reduction of these systems with $NaBH_3CN$.[22] When 2,9-disubstituted derivatives were subjected to the same reduction conditions, octahydrophenanthrolines **19f–i** were produced in yields of 62–73% as mixtures of *meso* and *rac* isomers. Reduction of 8,9,10,11-tetrahydro-benzo[*b*][1,10]phenanthroline (**14**) afforded the corresponding octahydrophenanthroline **20** as a mixture of *syn* and *anti* isomers in 54% yield. Thus, with the exception of **19e**, useful yields of octahydrophenanthrolines may be obtained by treatment of achiral 2-, 2,9- and 2,3-substituted phenanthrolines with $NaBH_3CN$ in AcOH/MeOH.

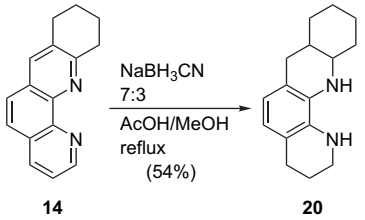


AcOH:MeOH	yld, % **15**	yld, % **16**	yld, % **19a**
50:50	60	0	0
60:40	43	0	21
70:30	21	0	30
80:20	<5	0	57
90:10	0	17	18
100:0	0	57	0

Scheme 3. Effect of solvent in the reduction of 2-phenyl-1,10-phenanthroline to 2-phenyl-1,2,3,4,7,8,9,10-octahydro-1,10-phenanthroline (**19a**).



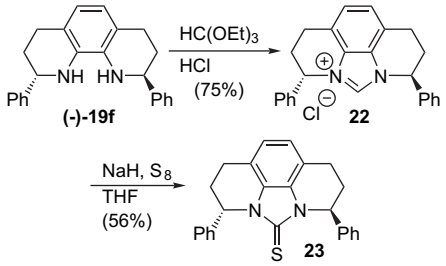
Compound	R^1	R^2	Prod.	yld, % **19**
11a	Ph	H	**19a**	57
11b	Me	H	**19b**	57
11c	*n*-Bu	H	**19c**	63
11d	*i*-Pr	H	**19d**	70
11e	*t*-Bu	H	**19e**	23
12a	Ph	Ph	**19f**	63
12b	Me	Me	**19g**	73
12c	*n*-Bu	*n*-Bu	**19h**	65
12d	*i*-Pr	*i*-Pr	**19i**	62



Scheme 4. Reduction of variously 2-, 2,9- and 2,3-substituted phenanthrolines.

2.2. Resolution of 2,9-diphenyloctahydrophenanthroline and NHC synthesis

Recently, Herrmann et al. have reported an expedient resolution of *rac*-2,2′-bipiperidine as a cyclic chiral phosphorus(V) adduct, prepared in one pot from PCl_3, (−)-menthol, and elemental sulfur.[32] This procedure is potentially applicable for the resolution of C_2-symmetric 2,9-substituted octahydrophenanthrolines (**19f–i**). As a test case, *meso*/*rac*-2,9-diphenyloctahydrophenanthroline (**19f**) was treated with PCl_3 which, following addition of (−)-menthol and S_8, afforded the analogous cyclic adducts **21a–c** (Scheme 5) as a mixture of three diastereomers in approximately equal amounts, as determined by ^{31}P NMR spectroscopy. Recrystallization of the crude mixture of adducts **21a–c** allowed for the purification of two of these diastereomers (**21a**,**b**), which were individually converted back to the free diamines **19f** with $LiAlH_4$ in refluxing THF. Of the separated diamines, the product derived from **21a** had a large negative optical



Scheme 6. A C_2-symmetric benzimidazolylidene derived from (−)-**19f** trapped as a thiourea (**23**).

rotation,[33] while the product derived from **21b** was optically inactive, indicating that it was *meso*-**19f**. Treatment of (−)-**19f** with triethyl orthoformate and HCl furnished the rigid C_2-symmetric benzimidazolium salt **22**, which was deprotonated and trapped with elemental sulfur to give thiourea **23**, thus indicating the generation of the putative free NHC (Scheme 6). The stereochemistry of **21a** was determined by X-ray crystallographic analysis (Fig. 3).

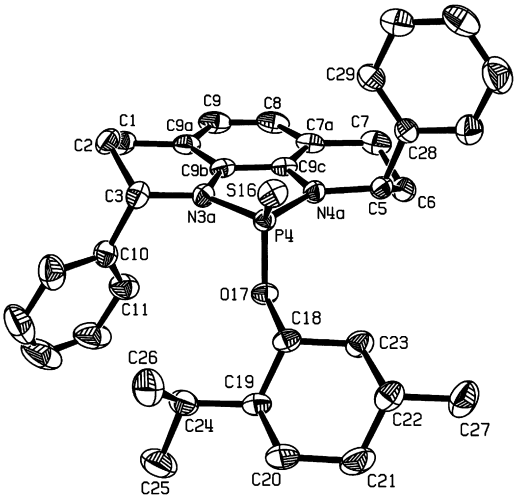
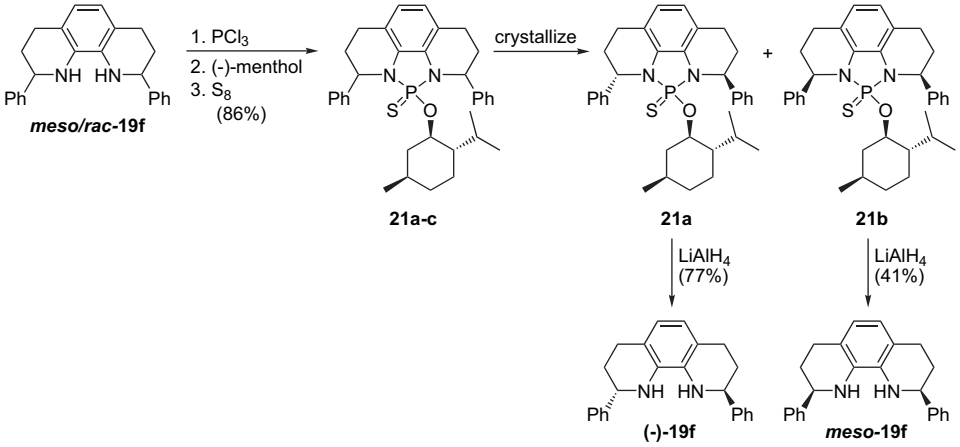


Figure 3. ORTEP® plot of **21a** at 30% probability. Hydrogen atoms are omitted for clarity.



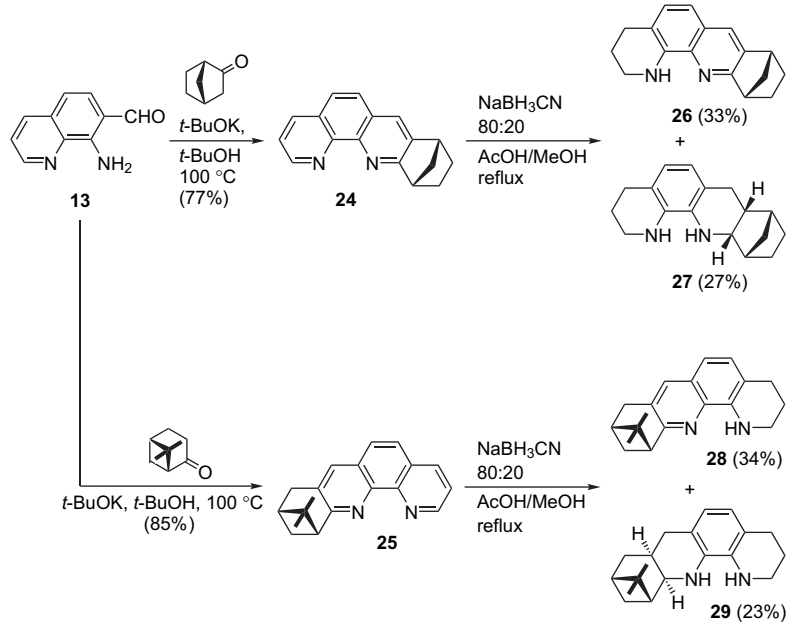
Scheme 5. Separation of (−)-**19f** and *meso*-**19f** via covalent diastereomers.

2.3. Benzimidazolylidenes derived from chiral 2,3-substituted phenanthrolines

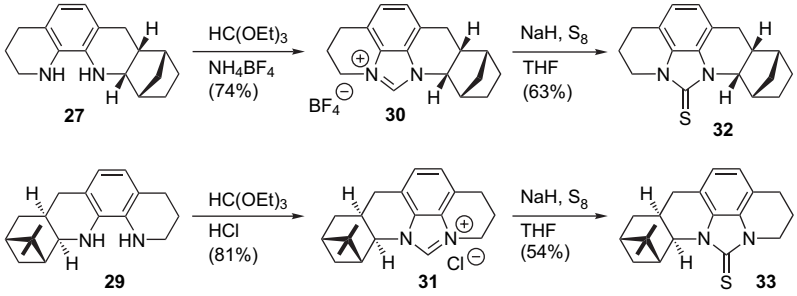
The introduction of chiral moieties into otherwise flat phenanthrolines normally involves de novo construction of the phenanthroline from chiral ketones,[34] analogous to the preparation of achiral cyclohexanone adduct **14**. Judicious selection of chiral bicyclic ketones with *endo* and *exo* faces would be expected to provide a degree of stereoselectivity during subsequent reduction to the octahydrophenanthrolines. This would avert the potentially tedious task of separating *syn* and *anti* diastereomers, as was the case in the reduction of **14**. For example, the bicyclic adduct derived by the Friedländer condensation of **13** with (+)-norcamphor (**24**, Scheme 7) would be expected to undergo hydride attack and protonation primarily from the *exo* face to afford the *syn* diastereomer. Similarly, reduction of the (+)-nopinone adduct[35] **25** ought to occur mainly from the '*endo*' face because of the presence of *exo* geminal methyl groups, also resulting in the selective formation of a *syn* diastereomer.

These predictions were borne out experimentally. Treatment of the norcamphor-derived phenanthroline **24** with $NaBH_3CN$ in 80:20 AcOH/MeOH provided the tetrahydrophenanthroline **26** (33%), along with the desired octahydrophenanthroline **27** (27%) *as a single diastereomer*, as determined by ^1H and ^{13}C NMR analyses. Reduction of the (+)-nopinone adduct **25** under the same conditions afforded the analogous products **28** and **29**. Examination of the ^1H and ^{13}C NMR spectra established that the reduction had been completely stereoselective, with only one product being discernible. All attempts to increase the yield[36] of **27** and **29** by performing the reduction in neat acetic acid resulted only in the formation of the corresponding *N*-ethylated tetrahydrophenanthrolines, as observed previously for **11a**.

Nevertheless, diamines **27** and **29** readily cyclized upon treatment with triethyl orthoformate and 1 equiv of acid (NH_4BF_4 or HCl) to the rigid chiral benzimidazolium salts **30** and **31** (Scheme 8). Deprotonation of these salts with NaH in the presence of elemental sulfur afforded thioureas **32** and **33** via the putative free NHCs. Spectroscopic analysis confirmed that the stereochemical integrity of the benzimidazolium salts was retained in the corresponding thioureas. Moreover, COSY and NOESY[37] experiments established the *syn* relative stereochemistry of **32** and **33**, as depicted



Scheme 7. Preparation and reduction of chiral 2,3-substituted phenanthrolines.



Scheme 8. Preparation of rigid chiral benzimidazolium salts and sulfur trapping experiments of their derived NHCs.

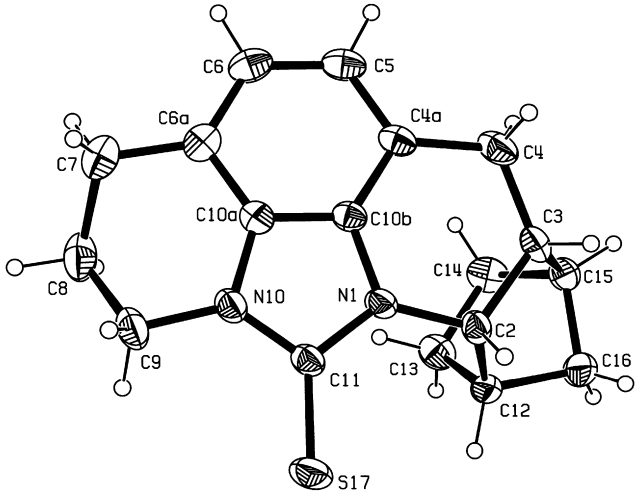


Figure 4. ORTEP® plot of **32** at 30% probability.

in Scheme 8, indicating that reduction of phenanthrolines **24** and **25** had occurred from the *exo* and *endo* faces, respectively. Crystals of **32** suitable for X-ray crystallographic analysis were grown and the molecular structure (Fig. 4) was obtained to verify the stereochemical assignment made by the COSY and NOESY experiments.[38]

3. Conclusions

It has been demonstrated that variously 2-, 2,9- and 2,3-substituted phenanthrolines may be reduced to octahydrophenanthrolines in useful yields with $NaBH_3CN$ in refluxing 7:3 or 8:2 AcOH/MeOH. Resolution of 2,9-diphenyloctahydrophenanthroline (**19f**) as a chiral phosphorus(V) adduct[33] gives access to a rigid C_2-symmetric benzimidazolylidene by deprotonation of the benzimidazolium salt (**22**). The reduction methodology may be extended to chiral phenanthrolines bearing bicyclic moieties, resulting in a high degree of diastereoselectivity by virtue of predominant *exo* (**24**) or *endo* (**25**) approach of the reagents (albeit in lower yields). The resulting optically active diamines may be cyclized to provide rigid chiral benzimidazolium salts, from which it is possible to generate the putative free NHCs with NaH, as indicated by trapping experiments with sulfur. The relative stereochemistry of thioureas **32** and **33** was established by COSY and NOESY NMR experiments, and for **32** it was further confirmed by X-ray analysis.[37]

We are currently exploring the resolution of C_2-symmetric amines **19h–i**, as well as the preparation of structurally more diverse 2- and 2,9-substituted octahydrophenanthrolines for the synthesis of additional NHCs in this series. Carbene precursors **22**, **30**, and **31**, and others derived by this approach, are under investigation as organocatalysts, and as ligands for transition-metal catalysis. A preliminary experiment has shown that ligand **22** and $Pd(dba)_2$ catalyze the formation of 1,3-dimethyl-3-phenyloxindole in an unoptimized 48% ee via the putative NHC–Pd complex. Previous chiral NHC-mediated syntheses of this compound have given 57 and 67% ee.[39] The outcome of this, and other studies, will be reported in due course.

4. Experimental

4.1. General

All reagents were purchased from Aldrich, Fisher Scientific, Acros or Strem and used as received unless otherwise indicated. Tetrahydrofuran (THF) was freshly dried and distilled over sodium/benzophenone ketyl under an atmosphere of nitrogen. Toluene was distilled over sodium under a nitrogen atmosphere. Dichloromethane was distilled over CaH_2 under an atmosphere of nitrogen. Organolithium reagents were titrated against *N*-benzylbenzamide[40] to a blue endpoint. All reactions were performed under argon in flame- or oven-dried glassware using syringe–septum cap techniques unless otherwise indicated. Column chromatography was performed on Silicycle silica gel 60 (70–230 mesh). NMR spectra were obtained on a Bruker Avance 300 or Avance 600 instrument and are referenced to TMS or to the residual proton signal of the deuterated solvent for ^1H spectra, and to the carbon multiplet of the deuterated solvent for ^{13}C spectra according to values given in *Spectrometric Identification of Organic Compounds, Seventh Edition*, pp 200 and 240. FTIR spectra were recorded on an ATI Mattson Research Series spectrometer. Low- and high-resolution mass spectral data were obtained on a Kratos Concept 1S Double Focusing spectrometer. Optical rotations were measured on a Rudolph Research Autopol III automatic polarimeter. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA, USA. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected.

4.1.1. 2-Isopropyl-1,10-phenanthroline (11d). A stirred solution of anhyd 1,10-phenanthroline (1.00 g, 5.55 mmol) in PhMe (40 mL) at ambient temperature under argon was treated with a solution of isopropyllithium[41] (3.61 mL, 1.69 M in THF, 6.10 mmol). The resulting dark red solution was stirred for 16 h, cooled to 0 °C, and worked-up by addition of water (20 mL). The organic layer was separated and the remaining aqueous layer was extracted with CH_2Cl_2 (3×10 mL). The combined organic layer was treated with MnO_2[42] (4 g, 46 mmol) and stirred for 30 min, after which anhyd $MgSO_4$ was added, and stirring continued for an additional 30 min. The resulting mixture was filtered and concentrated in vacuo. Column chromatography (neutral alumina, 69:30:1 hexanes/EtOAc/Et_3N, R_f=0.33) afforded **11d** (745 mg, 60%) as a pale yellow oil; IR (KBr, neat): ν_{max} 3045, 2963, 2928, 2869 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.22 (dd, 1H, J=4.3, 1.6 Hz), 8.22–8.16 (m, 2H), 7.74 (d, 1H, J=8.9 Hz), 7.69 (d, 1H, J=8.8 Hz), 7.61–7.56 (m, 2H), 3.68 (septet, 1H, J=7.0 Hz), 1.46 (d, 6H, J=7.0 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 168.4, 150.1, 146.0, 145.2, 136.4, 135.9, 128.6, 127.0, 126.3, 125.4, 122.4, 119.9, 37.5, 23.0; EIMS [m/z (%)]: 222 (M^+, 44), 207 (100); HRMS (EI) calcd for $C_{15}H_{14}N_2$: 222.1157, found: 222.1161.

4.1.2. 2,9-Diisopropyl-1,10-phenanthroline (12d). A stirred solution of anhyd 1,10-phenanthroline (1.00 g, 5.55 mmol) in PhMe (40 mL) at ambient temperature under argon was treated with a solution of isopropyllithium (9.9 mL, 1.69 M in THF, 16.6 mmol). The resulting dark red solution was stirred for 16 h, cooled to 0 °C, and worked-up by addition of water (20 mL). The organic layer was

separated and the remaining aqueous layer was extracted with CH_2Cl_2 (3×10 mL). The combined organic layer was treated with MnO_2 (4 g, 46 mmol) and stirred for 30 min, after which anhyd $MgSO_4$ was added, and stirring continued for an additional 30 min. The resulting mixture was filtered and concentrated in vacuo. Column chromatography (silica gel, 85:15 hexanes/EtOAc, R_f=0.22) afforded **12d** (753 mg, 51%) as a pale yellow oil; IR (KBr): ν_{max} 3041, 2963, 2928, 2869 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.13 (d, 2H, J=8.3 Hz), 7.67 (s, 2H), 7.54 (d, 2H, J=8.4 Hz), 3.57 (septet, 2H, J=6.9 Hz), 1.48 (d, 12H, J=7.0 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 167.7, 145.1, 136.3, 127.2, 125.3, 120.0, 37.2, 22.8; EIMS [m/z (%)]: 264 (M^+, 44), 249 (49); HRMS (EI) calcd for $C_{18}H_{20}N_2$: 264.1626, found: 264.1619.

4.1.3. (+)-2,3-(Bicyclo[2.2.1]heptanyl)-1,10-phenanthroline (24). A solution of aminoaldehyde **13** (600 mg, 3.49 mmol) and (+)-norcamphor[43] (386 mg, 3.50 mmol) in saturated *t*-BuOK/*t*-BuOH (6 mL) was heated to 100 °C in a sealed tube for 5 h. After cooling to room temperature, the solvent was removed in vacuo, and the residue was taken up in CH_2Cl_2 (20 mL). The organic phase was washed with water (3×5 mL), brine, dried over anhyd Na_2SO_4, and concentrated in vacuo. Column chromatography (neutral alumina, 98:2 EtOAc/Et_3N, R_f=0.43) gave phenanthroline **24** (658 mg, 77%) as a pale yellow solid; mp>200 °C (sublimes); $[\alpha]_D^{18}$ +78.4 (c 1.0, $CHCl_3$); IR (KBr): ν_{max} 2973, 2869, 1499, 1383 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.21–9.19 (m, 1H), 8.25 (dd, 1H, J=8.1, 1.0 Hz), 7.88 (s, 1H), 7.75 (ABq, 2H), 7.59 (dd, 1H, J=8.1, 4.5 Hz), 3.89 (s, 1H), 3.62 (s, 1H), 2.17–2.09 (m, 2H), 2.02–1.98 (m, 1H), 1.79–1.76 (m, 1H), 1.54 (t, 1H, J=7.2 Hz), 1.36 (t, 1H, J=6.9 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 170.7, 149.8, 145.8, 143.6, 142.1, 136.0, 128.0, 127.7, 126.0, 126.2, 124.8, 122.1, 47.5, 45.6, 42.4, 27.1, 25.5; EIMS [m/z (%)]: 246 (M^+, 93), 218 (100); HRMS (EI) calcd for $C_{17}H_{14}N_2$: 246.1157, found: 246.1158; Anal. Calcd for $C_{17}H_{14}N_2$: C, 82.91; H, 5.73. Found: C, 82.67; H, 5.78.

4.1.4. (1*R*,5*S*)-(2,3-*b*)-Pineno-1,10-phenanthroline (25). Prepared by a modification of the procedure of Thummel et al.[30] A solution of aminoaldehyde **13** (1.00 g, 5.81 mmol) and (+)-nopinone (803 mg, 5.81 mmol) in saturated *t*-BuOK/*t*-BuOH (10 mL) was heated to 100 °C in a sealed tube for 5 h. After cooling to room temperature, the solvent was removed in vacuo and the residue was taken up in CH_2Cl_2 (20 mL). The organic phase was washed with water (3×10 mL), brine, dried over anhyd Na_2SO_4, and concentrated in vacuo. Column chromatography (neutral alumina, 60:40 hexanes/EtOAc, R_f=0.18) gave phenanthroline **25** (1.35 g, 85%) as a pale yellow solid; mp 167–169 °C (lit.[30] 168–170 °C); ^1H NMR (300 MHz, $CDCl_3$): δ 9.18–9.16 (m, 1H), 8.24–8.21 (m, 1H), 7.95 (s, 1H), 7.73 (ABq, 2H), 7.57 (dd, 1H, J=8.1, 4.5 Hz), 3.62 (t, 1H, J=5.4 Hz), 3.20 (s, 2H), 2.85–2.82 (m, 1H), 2.43–2.41 (m, 1H), 1.48 (s, 3H), 1.41 (d, 1H, J=9.9 Hz), 0.73 (s, 3H).

4.1.5. *rac*-2-Methyl-1,2,3,4,7,8,9,10-octahydro-1,10-phenanthroline (19b). *Typical procedure*: a round-bottomed flask containing a rapidly stirred solution of **11b** (550 mg, 2.83 mmol) in glacial acetic acid (18 mL) and methanol (7 mL) was treated with an equal mass of $NaBH_3CN$ (550 mg, 8.8 mmol) in portions over 5 min. A reflux condenser was attached and the resulting deep red mixture heated to reflux. After 2 h, an additional 550 mg portion of $NaBH_3CN$ was added, a process that was repeated every 2 h for 4 more hours (2.2 g of $NaBH_3CN$ was added in total). Two hours after the last addition of $NaBH_3CN$, a color change from deep red to orange was observed, indicating completion of the reduction. The solution was cooled to room temperature and the majority of the methanol was removed on a rotary evaporator in vacuo. The resulting mixture was treated with 6 M aq NaOH (50 mL) and the whole was extracted with CH_2Cl_2 (4×10 mL). The combined organic extract was washed with water, brine, dried over anhyd Na_2SO_4, filtered, and concentrated in vacuo. Column chromatography (silica gel, 85:15 hexanes/EtOAc, R_f=0.24) gave diamine **19b** (315 mg, 57%) as a clear oil; IR (KBr, neat): ν_{max} 3342, 2924, 2843, 1581, 1485, 1331, 1255 cm^{-1}; ^1H NMR (600 MHz, acetone-d_6): δ 6.25 (d, 1H, J=7.2 Hz), 6.23 (d, 1H, J=9.0 Hz), 3.65 (br, 1H), 3.56 (br, 1H), 3.29–3.23 (m, 3H), 2.82–2.71 (m, 1H), 2.65–2.57 (m, 3H), 1.89–1.84 (m, 1H), 1.80 (quintet, 2H, J=6.0 Hz), 1.48–1.41 (m, 1H), 1.20 (d, 3H, J=6.3 Hz); ^{13}C NMR (150.9 MHz, acetone-d_6): δ 133.6, 133.0, 119.9, 119.4, 119.0, 118.8, 48.4, 43.1, 31.3, 27.9, 27.6, 23.3, 22.8; EIMS [m/z (%)]: 202 (M^+, 100); HRMS (EI) calcd for $C_{13}H_{18}N_2$: 202.1470, found: 202.1466.

4.1.6. *rac*-2-Phenyl-1,2,3,4,7,8,9,10-octahydro-1,10-phenanthroline (19a). According to the *typical procedure*, a solution of **11a** (256 mg, 1.00 mmol) in glacial acetic acid (8 mL) and MeOH (2 mL) was treated with $NaBH_3CN$ (256 mg, 4.07 mmol), and the reaction mixture was heated to reflux. Three subsequent additions of $NaBH_3CN$ (256 mg each) were made every 2 h for over 6 h total. Standard workup and column chromatography (silica gel, 90:10 hexanes/EtOAc, R_f=0.15) gave diamine **19a** (150 mg, 57%) as a colorless solid; mp 90–91 °C; IR (KBr): ν_{max} 3347, 3283, 3023, 2935, 2922, 2839, 1614, 1581, 1492, 1439, 1331, 1252 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6): δ 7.41 (d, 2H, J=7.6 Hz), 7.32 (t, 2H, J=8.7 Hz), 7.25 (t, 1H, J=7.2 Hz), 6.31–6.28 (ABq, 2H), 4.39 (d, 1H, J=8.9 Hz), 3.95 (br, 1H), 3.92 (br, 1H), 3.31–3.22 (m, 2H), 2.85–2.80 (m, 2H), 2.70–2.65 (m, 2H), 2.58 (dt, 1H, J=15.6, 5.6 Hz), 2.03–2.01 (m, 1H), 1.91–1.85 (m, 1H), 1.81 (quintet, 2H, J=6.0 Hz); ^{13}C NMR (75.5 MHz, acetone-d_6): δ 146.4, 133.5, 132.9, 129.1, 127.8, 127.4, 120.3, 119.3, 119.2, 118.8, 57.1, 43.1, 31.8, 28.0, 27.2, 23.3; EIMS [m/z (%)]: 264 (M^+, 100), 187 (15); HRMS (EI) calcd for $C_{18}H_{20}N_2$: 264.1626, found: 264.1627.

4.1.7. *rac*-2-*n*-Butyl-1,2,3,4,7,8,9,10-octahydro-1,10-phenanthroline (19c). According to the *typical procedure*, a solution of **11c** (365 mg, 1.54 mmol) in glacial acetic acid (7 mL) and MeOH (3 mL) was treated with $NaBH_3CN$ (365 mg, 5.8 mmol), and the reaction mixture was heated to reflux. Three subsequent additions of $NaBH_3CN$ (365 mg each) were made every 2 h for over 6 h total. Standard workup and column chromatography (silica gel, 90:8:2 hexanes/EtOAc/Et_3N, R_f=0.67) gave diamine **19c** (236 mg, 63%) as a pale yellow oil; IR (KBr, neat): ν_{max} 3337, 2926, 2853, 1583, 1486, 1348, 1252 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6): δ 6.27–6.22 (ABq, 2H), 3.77 (br, 1H), 3.50 (br, 1H), 3.27–3.24 (m, 2H), 3.14–3.12 (m, 1H), 2.77–2.55

(m, 4H), 1.93–1.86 (m, 1H), 1.81 (quintet, 2H, J=6.0 Hz), 1.58–1.30 (m, 7H), 0.92 (t, 3H, J=7.2 Hz); ^{13}C NMR (75.5 MHz, acetone-d_6): δ 133.5, 133.1, 120.0, 119.7, 119.0, 118.8, 52.8, 43.1, 37.2, 29.2, 28.7, 27.9, 27.4, 23.6, 23.3, 14.4; EIMS [m/z (%)]: 244 (M^+, 55), 187 (100); HRMS (EI) calcd for $C_{16}H_{24}N_2$: 244.1939, found: 244.1935.

4.1.8. *rac*-2-Isopropyl-1,2,3,4,7,8,9,10-octahydro-1,10-phenanthroline (19d). According to the *typical procedure*, a solution of **11d** (105 mg, 0.47 mmol) in glacial acetic acid (7 mL) and MeOH (3 mL) was treated with $NaBH_3CN$ (105 mg, 1.67 mmol), and the reaction mixture was heated to reflux. Three subsequent additions of $NaBH_3CN$ (105 mg each) were made every 2 h for over 6 h total. Standard workup and column chromatography (silica gel, 88:10:2 hexanes/EtOAc/Et_3N, R_f=0.22) gave diamine **19d** (76 mg, 70%) as a pale yellow oil; IR (KBr, neat): ν_{max} 3339, 3037, 2954, 2927, 2870, 2841, 1583, 1485, 1437, 1347, 1256 cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6): δ 6.26 (d, 1H, J=7.8 Hz), 6.23 (d, 1H, J=8.1 Hz), 3.81 (br, 1H), 3.48 (br, 1H), 3.31–3.18 (m, 2H), 2.98–2.92 (m, 1H), 2.72–2.62 (m, 4H), 1.90–1.69 (m, 4H), 1.55–1.50 (m, 1H), 1.00 (d, 3H, J=6.9 Hz), 0.98 (d, 3H, J=6.9 Hz); ^{13}C NMR (75.5 MHz, acetone-d_6): δ 133.8, 133.1, 120.1, 119.8, 119.0, 118.8, 58.5, 43.1, 33.3, 27.9, 27.6, 25.5, 23.3, 19.0, 18.6; EIMS [m/z (%)]: 230 (M^+, 28), 187 (100); HRMS (EI) calcd for $C_{15}H_{22}N_2$: 230.1783, found: 230.1795.

4.1.9. *rac*-2-*tert*-Butyl-1,2,3,4,7,8,9,10-octahydro-1,10-phenanthroline (19e). According to the *typical procedure*, a solution of **11e** (171 mg, 0.75 mmol) in glacial acetic acid (8 mL) and MeOH (2 mL) was treated with $NaBH_3CN$ (177 mg, 2.82 mmol), and the reaction mixture was heated to reflux. Three subsequent additions of $NaBH_3CN$ (177 mg each) were made every 2 h for over 6 h total. Standard workup and column chromatography (silica gel, 90:10 hexanes/EtOAc, R_f=0.32) gave diamine **19e** (42 mg, 23%) as a colorless solid; mp 71–72 °C; IR (KBr): ν_{max} 3346, 3300, 2955, 2933, 2851, 1614, 1582, 1475, 1429, 1335 cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6): δ 6.27 (d, 1H, J=7.2 Hz), 6.24 (d, 1H, J=7.8 Hz), 3.82 (br, 1H), 3.44 (br, 1H), 3.28–3.21 (m, 2H), 2.86 (dt, 1H, J=10.8, 2.4 Hz), 2.79–2.56 (m, 4H), 1.99–1.93 (m, 1H), 1.83–1.74 (m, 2H), 1.53–1.41 (m, 1H), 0.99 (s, 9H); ^{13}C NMR (75.5 MHz, acetone-d_6): δ 134.3, 133.2, 120.3, 120.0, 119.0, 118.7, 62.2, 43.1, 34.1, 28.3, 27.9, 26.3, 24.2, 23.3; EIMS [m/z (%)]: 244 (M^+, 18), 187 (100); HRMS (EI) calcd for $C_{16}H_{24}N_2$: 244.1939, found: 244.1942.

4.1.10. *meso*/*rac*-2,9-Diphenyl-1,2,3,4,7,8,9,10-octahydro-1,10-phenanthroline (19f). According to the *typical procedure*, a solution of **12a** (250 mg, 0.75 mmol) in glacial acetic acid (8 mL) and MeOH (2 mL) was treated with $NaBH_3CN$ (250 mg, 3.98 mmol), and the reaction mixture was heated to reflux. Three subsequent additions of $NaBH_3CN$ (250 mg each) were made every 2 h for over 6 h total. Standard workup and column chromatography (silica gel, 96:4 hexanes/EtOAc, R_f=0.38) gave diamine **19f** (161 mg, 63%), a pale yellow solid, as a mixture of stereoisomers; mp 107–110 °C; IR (KBr): ν_{max} 3339, 3030, 2944, 2919, 2835, 1580, 1473, 1420, 1335, 1252 cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6): δ 7.39 (d, 4H, J=7.0 Hz), 7.30 (t, 4H, J=7.0 Hz), 7.22 (d, 2H, J=7.2 Hz), 6.35 (s, 2H), 4.43–4.40 (m, 2H), 4.25 (br, 2H), 2.91–2.75 (m, 4H), 2.61 (dt, 2H, J=15.9, 5.2 Hz), 1.97–1.88 (m, 2H); ^{13}C NMR (75.5 MHz, acetone-d_6): δ 146.3, 132.8, 129.0, 127.7, 127.4, 119.6, 118.9, 57.0, 31.4, 26.9; EIMS [m/z (%)]: 340 (M^+, 54); HRMS (EI) calcd for $C_{24}H_{24}N_2$: 340.1939, found: 340.1942; Anal. Calcd for $C_{24}H_{24}N_2$: C, 84.67; H, 7.11. Found: C, 84.35; H, 7.10.

4.1.11. *meso*/*rac*-2,9-Dimethyl-1,2,3,4,7,8,9,10-octahydro-1,10-phenanthroline (19g). According to the *typical procedure*, a solution of neocuproine **12b** (217 mg, 1.00 mmol) in glacial acetic acid (3.5 mL) and MeOH (1.5 mL) was treated with $NaBH_3CN$ (217 mg, 3.45 mmol), and the reaction mixture was heated to reflux. Three subsequent additions of $NaBH_3CN$ (217 mg each) were made every 2 h for over 6 h total. Standard workup and column chromatography (silica gel, 92:8 PhMe/Et_3N, R_f=0.22) gave **19g** (158 mg, 73%), a colorless solid, as a 1:1 mixture of *meso* and *rac* stereoisomers; mp 63–65 °C; IR (KBr): ν_{max} 3324, 3036, 2959, 2918, 2839, 1582, 1480, 1446, 1334, 1255 cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6): δ 6.23 (s, 2H), 3.64 (br, 1H), 3.55 (br, 1H), 3.31–3.23 (m, 2H), 2.78–2.54 (m, 4H), 1.87–1.82 (m, 2H), 1.48–1.38 (m, 2H), 1.18 (d, 3H, J=6.3 Hz), 1.17 (d, 3H, J=6.3 Hz); ^{13}C NMR (75.5 MHz, acetone-d_6): δ 133.1, 133.0, 119.4, 119.3, 118.8, 118.7, 48.4, 48.3, 31.2, 27.5, 27.5, 22.9, 22.7; FABMS [m/z (%)]: 216 (M^+, 100); HRMS (FAB) calcd for $C_{14}H_{20}N_2$: 216.1626, found: 216.1619.

4.1.12. *meso*/*rac*-2,9-Di-*n*-butyl-1,2,3,4,7,8,9,10-octahydro-1,10-phenanthroline (19h). According to the *typical procedure*, a solution of **12c** (236 mg, 0.81 mmol) in glacial acetic acid (7 mL) and MeOH (3 mL) was treated with $NaBH_3CN$ (236 mg, 3.76 mmol), and the reaction mixture was heated to reflux. Three subsequent additions of $NaBH_3CN$ (236 mg each) were made every 2 h for over 6 h total. Standard workup and column chromatography (silica gel, 94:5:1 hexanes/EtOAc/Et_3N, R_f=0.14) gave **19h** (156 mg, 65%), a colorless oil, as a 1:1 mixture of *meso* and *rac* stereoisomers; IR (KBr, neat): ν_{max} 3339, 3038, 2953, 2926, 2869, 2855, 1584, 1482, 1438, 1347, 1258 cm^{-1}; ^{1}H NMR (600 MHz, acetone-d_6): δ 6.26 (s, 1H), 6.25 (s, 1H), 3.57 (br, 1H), 3.48 (br, 1H), 3.16–3.11 (m, 2H), 2.79–2.67 (m, 2H), 2.63–2.59 (m, 2H), 1.92–1.89 (m, 2H), 1.57–1.34 (m, 12H), 0.92 (t, 6H, J=6.9 Hz); ^{13}C NMR (75.5 MHz, acetone-d_6): δ 133.2, 133.2, 120.0, 119.9, 119.0, 118.8, 52.9, 52.8, 37.2, 37.1, 29.1, 29.0, 28.9, 28.7, 23.6, 23.6, 14.4; EIMS [m/z (%)]: 300 (M^+, 72), 243 (89), 239 (100); HRMS (EI) calcd for $C_{20}H_{32}N_2$: 300.2565, found: 300.2561.

4.1.13. *meso*/*rac*-2,9-Diisopropyl-1,2,3,4,7,8,9,10-octahydro-1,10-phenanthroline (19i). According to the *typical procedure*, a solution of **12d** (233 mg, 0.88 mmol) in glacial acetic acid (8 mL) and MeOH (2 mL) was treated with $NaBH_3CN$ (233 mg, 3.71 mmol), and the reaction mixture was heated to reflux. Three subsequent additions of $NaBH_3CN$ (233 mg each) were made every 2 h for over 6 h total. Standard workup and column chromatography (silica gel, 94:5:1 hexanes/EtOAc/Et_3N, R_f=0.22) gave a diamine **19i** (149 mg, 62%), a colorless solid, as an unequal mixture of *meso* and *rac* stereoisomers; mp 49–50 °C; IR (KBr): ν_{max} 3352, 3041, 2959, 2915, 2873, 2836, 1586, 1483, 1432, 1336, 1261 cm^{-1}; ^{1}H NMR (300 MHz,

acetone-d_6): δ 6.27 (s, 2H), 3.45 (br, 2H), 2.92 (br, 2H), 2.70–2.63 (m, 4H), 1.90–1.80 (m, 2H), 1.78–1.69 (m, 2H), 1.60–1.44 (m, 2H), 1.02 (d, 6H, J=6.9 Hz), 0.98 (d, 6H, J=6.6 Hz); ^{13}C NMR (75.5 MHz, acetone-d_6): δ 133.6, 133.5, 120.2, 118.9, 118.8, 58.6, 58.5, 33.3, 33.1, 27.5, 27.4, 25.4, 25.3, 19.3, 19.0, 18.7, 18.4; EIMS [m/z (%)]: 272 (M^+, 33), 229 (79), 126 (100), 98 (90); HRMS (FAB) calcd for $C_{18}H_{28}N_2$: 272.2252, found: 272.2242.

4.1.14. *syn/anti*-1,2,3,4,7,7a,8,9,10,11,11a,12-Dodecahydro-benzo[*b*][1,10]phenanthroline (20). According to the *typical procedure*, a solution of **14** (117 mg, 0.50 mmol) in glacial acetic acid (3.5 mL) and MeOH (1.5 mL) was treated with $NaBH_3CN$ (117 mg, 1.86 mmol), and the reaction mixture was heated to reflux. Three subsequent additions of $NaBH_3CN$ (117 mg each) were made every 2 h for over 6 h total. Standard workup and column chromatography (silica gel, 90:10 hexanes/EtOAc, R_f=0.33) gave diamine **20** (66 mg, 54%), a pale yellow solid, as a mixture of *syn* and *anti* isomers; mp 95–105 °C; IR (KBr): ν_{max} 3345, 3033, 2921, 2851, 1581, 1487, 1433 cm^{-1}; ^{1}H NMR (600 MHz, acetone-d_6, major diastereomer as determined by HSQC): δ 6.26 (s, 2H), 3.73 (br, 1H), 3.49 (br, 1H), 3.26–3.22 (m, 2H), 2.84–2.80 (m, 1H), 2.73 (td, 1H, J=10.8, 3.6 Hz), 2.63 (t, 2H, J=6.6 Hz), 2.52 (dd, 1H, J=15.6, 5.4 Hz), 2.36 (dd, 1H, J=16.2, 11.4 Hz), 1.99–1.96 (m, 1H), 1.81–1.70 (m, 5H), 1.40–1.31 (m, 4H); ^{13}C NMR (150.9 MHz, acetone-d_6, major diastereomer as determined by HSQC and DEPT): δ 133.2, 132.8, 119.9, 119.8, 119.0, 118.7, 57.2, 43.0, 38.6, 35.6, 34.3, 32.9, 27.9, 26.8, 25.6, 23.3; EIMS [m/z (%)]: 242 (M^+, 100); HRMS (EI) calcd for $C_{16}H_{22}N_2$: 242.1783, found: 242.1777.

4.2. 4-[(1*R*,2*S*,5*R*)-Menthyloxy)]-3,5-diphenyl-1,2,3,5,6,7-hexahydro-3a,4a-diaza-4-phosphacyclopenta[*def*]phenanthrene 4-sulfide (21a–c)

A solution of **19f** (1.25 g, 3.67 mmol) and dimethylaniline (2.6 mL, 20.2 mmol) in CH_2Cl_2 (13 mL) under argon was treated with PCl_3 (0.32 mL, 3.7 mmol), added dropwise by syringe. The resulting yellow solution darkened and evolved heat for 15 min, at which point a reflux condenser was attached and the mixture heated to reflux under argon. After 1 h, (−)-menthol (573 mg, 3.67 mmol) was added in one portion from the top of the condenser and reflux was continued. After 1 h, S_8 (1.18 g, 36.7 mmol) was added in one portion and the mixture was refluxed for an additional 25 min. The solvent was removed in vacuo and the residue was suspended in 8 M aq HCl (25 mL). The aqueous phase was extracted with Et_2O (3×25 mL), and the combined organic extract was dried over anhyd Na_2SO_4, filtered, and concentrated in vacuo. Column chromatography (silica gel, first with hexanes to remove S_8, then 96:4 hexanes/EtOAc) gave **21a–c** (1.76 g, 86%), a pale yellow solid, as an approximately 1:1:1 mixture of three stereoisomers as determined by ^{31}P NMR [δ 69.7 (**21a**), 64.4 (**21b**), 66.4 (**21c**)]. The mixture of stereoisomers was suspended in abs MeOH (115 mL) and heated to reflux for 4 h. Hot filtration of the mixture afforded enriched **21a** (404 mg). Recrystallization from pentane/CH_2Cl_2 gave the pure **21a** diastereomer (353 mg). The filtrate was concentrated to approximately half the original volume in vacuo and standing at room temperature afforded crystals of **21b** (100 mg), which were collected by filtration. Concentration of the remaining mother liquor gave a residue that was enriched in **21c**; attempts to recrystallize **21c** from several solvents did not afford **21c** completely free of **21a** and **21b**.

4.2.1. Compound 21a. Pale yellow solid; mp 225–227 °C (pentane/CH_2Cl_2); $[\alpha]_D^{18}$ −249 (c 1.0, $CHCl_3$); X-ray analysis (CCDC 612643) was performed on a colorless plate fragment (0.40×0.21×0.10 mm), which was obtained by recrystallization from pentane/CH_2Cl_2. $C_{34}H_{41}N_2OPS$: M=556.74 g/mol, orthorhombic, $P2_12_12_1$, a=6.5541(4) Å, b=21.0909(13) Å, c=21.9930(14) Å, V=3040.1(3) Å^3, Z=4, D_c=1.216 g/cm^3, $F(000)$=1192, T=295(2) K. Data were collected on a Bruker APEX CCD system with graphite monochromated Mo Kα radiation (λ=0.71073 Å), 25,619 data were collected. The structure was solved by Patterson and Fourier (SHELXTL) and refined by full-matrix least squares on F^2 resulting in final R, R_w, and GOF [for 6612 data with $F>2\sigma(F)$] of 0.0583, 0.0733, and 1.621, respectively, for solution using the 3*S*,5*S* model, Flack parameter=0.00(6); IR (KBr): ν_{max} 3054, 3029, 2948, 2919, 2865, 1471, 1289, 1152, 1110 cm^{-1}; ^{31}P NMR (121.5 MHz, $CDCl_3$): δ 69.7; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31–7.11 (m, 10H), 6.57 (s, 2H), 5.34–5.28 (m, 1H), 5.18–5.13 (m, 1H), 4.20 (qd, 1H, J=10.5, 4.2 Hz), 2.62–2.57 (m, 1H), 2.52–2.39 (m, 2H), 2.34–2.06 (m, 5H), 1.60–1.58 (m, 2H), 1.47–1.35 (m, 2H), 1.18–1.07 (m, 1H), 1.02–0.98 (m, 1H), 0.94 (d, 3H, J=6.9 Hz), 0.88–0.67 (m, 3H), 0.30 (d, 3H, J=6.9 Hz), 0.25 (d, 3H, J=6.9 Hz); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 142.9, 141.3, 128.5, 128.4, 128.1, 127.1, 127.0, 126.4, 126.3, 117.85, 117.82, 117.25, 117.21, 116.8, 116.7, 80.9 (d, $^2J_{^{13}C-^{31}P}$ = 8.8 Hz), 52.69, 52.66, 47.31, 47.25, 44.2, 33.9, 31.5, 30.3, 30.2, 29.91, 29.87, 24.4, 22.3, 22.2, 20.9, 19.52, 19.48, 15.2; EIMS [m/z (%)]: 556 (M^+, 12), 418 (100); HRMS (EI) calcd for $C_{34}H_{41}N_2OPS$: 556.2677, found: 556.2674.

4.2.2. Compound 21b. Pale yellow solid; mp 230–231 °C (MeOH/$CHCl_3$); $[\alpha]_D^{18}$ −17 (c 0.23, $CHCl_3$); IR (KBr): ν_{max} 3050, 3026, 2927, 2867, 2857, 1470, 1291, 1168, 1110 cm^{-1}; ^{31}P NMR (243 MHz, $CDCl_3$): δ 64.4; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.32–7.28 (m, 4H), 7.25–7.21 (m, 4H), 7.19 (d, 2H, J=7.8 Hz), 6.59 (s, 2H), 5.11–5.10 (m, 1H), 5.06–5.05 (m, 1H), 4.29 (qd, 1H, J=10.8, 4.8 Hz), 2.61–2.57 (m, 2H), 2.49–2.42 (m, 2H), 2.32–2.26 (m, 2H), 2.19–2.10 (m, 4H), 1.67–1.65 (m, 2H), 1.46–1.42 (m, 1H), 1.35–1.31 (m, 1H), 1.09–0.96 (m, 2H), 0.94 (d, 3H, J=7.2 Hz), 0.93 (d, 3H, J=6.6 Hz), 0.90 (d, 3H, J=7.2 Hz), 0.87–0.80 (m, 1H); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 141.5, 141.2, 128.5, 128.4, 128.33, 128.26, 128.2, 127.0, 126.9, 126.4, 126.1, 118.2, 117.2 117.1, 117.04, 117.00, 80.0 (d, $^2J_{^{13}C-^{31}P}$ = 9.1 Hz), 52.53, 52.50, 52.47, 52.44, 48.0, 47.9, 31.5, 30.52, 30.48, 29.90, 29.87, 25.7, 22.7, 22.1, 21.0, 20.3, 20.0, 16.1; EIMS [m/z (%)]: 556 (M^+, 15), 418 (100); HRMS (EI) calcd for $C_{34}H_{41}N_2OPS$: 556.2677, found: 556.2672.

4.2.3. (−)-(2*S*,9*S*)-19f. A stirred solution of **21a** (181 mg, 0.32 mmol) in THF (4 mL) under Ar was treated with $LiAlH_4$ (143 mg, 3.58 mmol) and heated at reflux for 100 min. The mixture was cooled in an ice bath and Et_2O (10 mL) was added. The mixture was treated sequentially with water (0.14 mL), 10% aq NaOH solution (0.14 mL),

and water (0.43 mL). The precipitated aluminum salts were removed by filtration through a pad of Celite in a sintered funnel and washed thoroughly with small aliquots of Et_2O. The filtrate was dried over anhyd Na_2SO_4, filtered, and concentrated in vacuo. Column chromatography (silica gel, 96:4 hexanes/EtOAc, R_f=0.40) gave (−)-**19f** (85 mg, 77%) as a pale yellow solid; mp 147–149 °C; $[\alpha]_D^{18}$ −287 (*c* 1.38, acetone); ^{1}H NMR (300 MHz, acetone-d_6): δ 7.41 (d, 4H, *J*=7.2 Hz), 7.29 (t, 4H, *J*=6.9 Hz), 7.21 (d, 2H, *J*=8.4 Hz), 6.35 (s, 2H), 4.46–4.42 (m, 2H), 4.24 (br, 2H), 2.94–2.75 (m, 4H), 2.62 (td, 2H, *J*=15.9, 4.8 Hz), 1.97–1.88 (m, 2H); ^{13}C NMR (75.5 MHz, acetone-d_6): δ 146.4, 133.2, 129.0, 127.7, 127.5, 119.7, 119.0, 57.2, 32.1, 27.3.

4.2.4. *meso*-19f. A stirred solution of **21b** (32 mg, 0.057 mmol) in THF (1 mL) under Ar was treated with $LiAlH_4$ (25 mg, 0.63 mmol) and heated at reflux for 45 min. The mixture was cooled in an ice bath and treated sequentially with water (0.03 mL), 10% aq NaOH solution (0.03 mL), and water (0.08 mL). The precipitated aluminum salts were removed by filtration through a pad of Celite in a sintered funnel and washed thoroughly with small aliquots of Et_2O. The filtrate was dried over anhyd Na_2SO_4, filtered, and concentrated in vacuo. Column chromatography (silica gel, 97:3 hexanes/EtOAc, R_f=0.40) gave *meso*-**19f** (8 mg, 41%) as a pale yellow solid; mp 97–99 °C; $[\alpha]_D^{18}$ 0; ^{1}H NMR (300 MHz, acetone-d_6): δ 7.37 (d, 4H, *J*=7.2 Hz), 7.29 (t, 4H, *J*=6.9 Hz), 7.22 (d, 2H, *J*=7.2 Hz), 6.34 (s, 2H), 4.46–4.42 (m, 2H), 4.25 (br, 2H), 2.86–2.75 (m, 4H), 2.61 (td, 2H, *J*=15.9, 5.1 Hz), 2.00–1.92 (m, 2H); ^{13}C NMR (75.5 MHz, acetone-d_6): δ 146.4, 132.9, 129.1, 127.8, 127.4, 119.6, 119.0, 57.0, 31.4, 27.0.

4.2.5. (−)-(3*S*,5*S*)-Diphenyl-1,2,3,5,6,7-hexahydro-3a-aza-cyclopenta[*def*]phenanthren-4a-azonium chloride (22). A stirred solution of diamine (−)-**19f** (48 mg, 0.14 mmol) in $(EtO)_3CH$ (4 mL) was treated with concentrated HCl solution (12 μL, 0.14 mmol) under argon and heated to 80 °C. After 1 h, the septum was removed and the mixture heated at 80 °C for an additional 2 h, then cooled to ambient temperature. Et_2O (6 mL) was added to induce precipitation of the product, which was collected on a Hirsch funnel, washed with cold ether, and dried under high vacuum to give **22** (41 mg, 75%) as a beige powder; mp 244–245 °C; $[\alpha]_D^{18}$ −205 (*c* 1.0, $CHCl_3$); IR (KBr): ν_{max} 3155, 3035, 2926, 2854, 1629, 1499, 1454, 1175 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.46 (s, 1H), 7.48–7.23 (m, 12H), 6.28 (br, 2H), 3.20–3.18 (m, 2H), 3.08–3.05 (m, 2H), 2.66 (br, 2H), 2.52 (br, 2H); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 138.7, 136.8, 129.5, 129.3, 128.3, 127.0, 124.6, 123.0, 60.0, 31.9, 21.7; FABMS [*m*/*z* (%)]: 351 (M−Cl$^-$, 100), HRMS (FAB) calcd for $C_{25}H_{23}N_2$: 351.1861, found: 351.1807.

4.2.6. (−)-(3*S*,5*S*)-Diphenyl-1,2,3,5,6,7-hexahydro-3a,4a-diazacyclopenta[*def*]phenanthrene-4-thione (23). A stirred suspension of **22** (34 mg, 0.09 mmol) and S_8 (3 mg, 0.1 mmol) in THF (3 mL) under argon was treated with NaH (6 mg, 0.14 mmol, 60% dispersion in mineral oil) and stirred for 50 min. The reaction mixture was worked-up with water (3 mL) and the product was extracted with Et_2O (4×1.5 mL). The combined organic extract was dried over anhyd Na_2SO_4, filtered, and concentrated in vacuo. Gradient column chromatography (silica gel, 90:10 hexanes/EtOAc, then 80:20 hexanes/EtOAc, R_f=0.12) gave **23** (19 mg, 56%) as a colorless solid; mp>255 °C; $[\alpha]_D^{18}$ −285 (*c* 0.59, $CHCl_3$); IR (KBr): ν_{max} 3034, 2952, 2922, 2892, 2857, 1480, 1382, 1360, 1328 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31–7.20 (m, 6H), 6.98–6.94 (m, 6H), 5.89 (t, 2H, *J*=3.0 Hz), 2.85–2.77 (dt, 2H, *J*=16.2, 3.6 Hz), 2.73–2.62 (m, 2H), 2.49–2.43 (m, 4H); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 166.8, 139.5, 128.7, 128.3, 127.4, 125.8, 120.2, 118.4, 54.9, 30.7, 19.6; EIMS [*m*/*z* (%)]: 382 (M$^+$, 100), HRMS (EI) calcd for $C_{25}H_{22}N_2S$: 382.1504, found: 382.1499.

4.3. Reduction of norcamphor adduct 24

According to the *typical procedure*, a solution of **24** (96 mg, 0.39 mmol) in glacial acetic acid (4 mL) and MeOH (1 mL) was initially treated with $NaBH_3CN$ (96 mg, 1.53 mmol), and the reaction mixture was heated to reflux. Three subsequent additions of $NaBH_3CN$ (96 mg each) were made every 2 h for over 6 h total. Standard workup and column chromatography (silica gel, 9:1 hexanes/EtOAc) gave, sequentially, tetrahydrophenanthroline **26** (32 mg, 33%, R_f=0.30) and octahydrophenanthroline **27** (27 mg, 27%, R_f=0.16).

4.3.1. Compound 26. Pale yellow solid; mp 95–96 °C; $[\alpha]_D^{18}$ +79 (*c* 1.0, acetone); IR (KBr): ν_{max} 3404, 3048, 2936, 2923, 2868, 2812, 1517, 1490, 1335 cm^{-1}; ^{1}H NMR (600 MHz, acetone-d_6): δ 7.64 (s, 1H), 6.99 (d, 1H, *J*=8.4 Hz), 6.85 (d, 1H, *J*=8.4 Hz), 6.02 (br, 1H), 3.50–3.47 (m, 3H), 3.35 (br, 1H), 2.84 (t, 2H, *J*=6.6 Hz), 2.05–2.02 (m, 2H), 1.98 (quin, 2H, *J*=6.0 Hz), 1.83–1.81 (m, 1H), 1.70 (d, 1H, *J*=9.0 Hz), 1.32–1.27 (m, 2H); ^{13}C NMR (150.9 MHz, acetone-d_6): δ 167.6, 141.7, 139.9, 136.1, 128.2, 127.4, 126.2, 116.1, 113.9, 47.3, 46.0, 42.9, 41.9, 28.2, 27.7, 26.5, 22.9; EIMS [*m*/*z* (%)]: 250 (M$^+$, 100), 221 (21); HRMS (EI) calcd for $C_{17}H_{18}N_2$: 250.1470, found: 250.1475.

4.3.2. Compound 27. Colorless glass; $[\alpha]_D^{18}$ −37.9 (*c* 0.71, acetone); IR (KBr): ν_{max} 3423, 3033, 2941, 2870, 1093 cm^{-1}; ^{1}H NMR (300 MHz, acetone-d_6): δ 6.34 (d, 1H, *J*=7.8 Hz), 6.27 (d, 1H, *J*=7.8 Hz), 4.03 (br, 1H), 3.31 (dd, 1H, *J*=10.3, 3.0 Hz), 3.28–3.22 (m, 2H), 2.84 (br, 1H), 2.68 (t, 2H, *J*=6.5 Hz), 2.41 (dd, 1H, *J*=13.4, 6.4 Hz), 2.29–2.22 (m, 2H), 2.15–2.10 (m, 2H), 1.92–1.86 (m, 1H), 1.80 (quin, 2H, *J*=5.2 Hz), 1.68–1.64 (m, 1H), 1.48–1.47 (m, 1H), 1.39–1.37 (m, 1H), 1.33–1.28 (m, 1H), 1.25–1.18 (m, 1H); ^{13}C NMR (150.9 MHz, acetone-d_6): δ 136.0, 132.5, 125.2, 120.9, 119.4, 117.8, 57.4, 43.20, 43.18, 41.8, 41.3, 38.8, 28.4, 28.1, 23.6, 23.4, 21.0; EIMS [*m*/*z* (%)]: 254 (M$^+$, 100), 185 (44), 84 (80); HRMS (EI) calcd for $C_{17}H_{22}N_2$: 254.1783, found: 254.1781.

4.4. Reduction of nopinone adduct 25

According to the *typical procedure*, a solution of **25** (100 mg, 0.36 mmol) in glacial acetic acid (4 mL) and MeOH (1 mL) was initially treated with $NaBH_3CN$ (100 mg, 1.59 mmol), and the reaction mixture was heated to reflux. Three subsequent additions of $NaBH_3CN$ (100 mg each) were made every 2 h for over 6 h total. Standard workup and column chromatography (silica gel, 95:5 hexanes/EtOAc) gave, sequentially, tetrahydrophenanthroline **28** (35 mg, 34%,

R_f=0.21) and octahydrophenanthroline **29** (24 mg, 23%, R_f=0.07).

4.4.1. Compound 28. Off-white solid; mp 155–157 °C; $[\alpha]_D^{18}$ +23.0 (*c* 1.0, $CHCl_3$); IR (KBr): ν_{max} 3407, 2968, 2951, 2927, 2833, 1568, 1514, 1489, 1381, 1348, 1325 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6): δ 7.73 (s, 1H), 6.99 (d, 1H, *J*=8.1 Hz), 6.84 (d, 1H, *J*=8.1 Hz), 6.03 (br, 1H), 3.49–3.45 (m, 2H), 3.09–3.08 (m, 2H), 3.02 (t, 1H, *J*=5.4 Hz), 2.86–2.75 (m, 2H), 2.39–2.33 (m, 1H), 2.02–1.94 (m, 2H), 1.44 (s, 3H), 1.30 (d, 2H, *J*=9.6 Hz), 0.65 (s, 3H); ^{13}C NMR (75.5 MHz, acetone-d_6): δ 164.1, 141.4, 135.2, 134.5, 128.7, 128.4, 127.6, 116.0, 113.1, 51.9, 41.8, 41.0, 40.1, 31.6, 31.3, 27.7, 26.4, 22.8, 21.7; EIMS [*m*/*z* (%)]: 278 (M^+, 100); HRMS (EI) calcd for $C_{19}H_{22}N_2$: 278.1783, found: 278.1787.

4.4.2. Compound 29. Off-white solid; mp 103–105 °C; $[\alpha]_D^{18}$ −73.0 (*c* 1.0, acetone); IR (KBr): ν_{max} 3421, 3298, 2933, 2856, 1473 cm^{-1}; ^1H NMR (300 MHz, acetone-d_6): δ 6.36 (d, 1H, *J*=7.5 Hz), 6.27 (d, 1H, *J*=7.5 Hz), 4.00–3.50 (br, 2H), 3.51 (dd, 1H, *J*=9.6, 4.4 Hz), 3.23 (m, 2H), 2.75 (dd, 1H, *J*=13.7, 7.0 Hz), 2.67 (t, 2H, *J*=6.6 Hz), 2.60–2.20 (m, 5H), 1.94 (m, 1H), 1.79 (quin, 2H, *J*=5.5 Hz), 1.66 (dd, 1H, *J*=13.8, 8.4 Hz), 1.32 (d 1H, *J*=8.6 Hz), 1.21 (s, 3H), 1.04 (s, 3H); ^{13}C NMR (150.9 MHz, acetone-d_6): δ 136.8, 132.4, 124.2, 120.7, 119.6, 117.7, 59.5, 47.3, 43.1, 42.5, 39.2, 34.7, 34.4, 30.6, 28.5 (2C), 28.1, 23.4, 23.3; EIMS [*m*/*z* (%)]: 282 (M^+, 100), 278 (49), 211 (39); HRMS (EI) calcd for $C_{19}H_{26}N_2$: 282.2096, found: 282.2093.

4.4.3. Benzimidazolium tetrafluoroborate 30. A solution of diamine **27** (77 mg, 0.30 mmol) in $(EtO)_3CH$ (10 mL) was treated with NH_4BF_4 (32 mg, 0.30 mmol). A reflux condenser was attached and the mixture was stirred at 80 °C under argon for 13 h. After cooling to room temperature, ether (10 mL) was added to induce precipitation of the product, which was collected by Büchner filtration on medium porosity (slow-flow) filter paper. After washing with ether, the product was dried thoroughly under high vacuum to give **30** (78 mg, 74%) as a colorless solid; mp 62–63 °C; $[\alpha]_D^{18}$ +22.4 (*c* 0.58, $CHCl_3$); IR (KBr): ν_{max} 3134, 2995, 2922, 2870, 1510, 1084 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 9.33 (s, 1H), 7.28 (d, 1H, *J*=7.4 Hz), 7.26 (d, 1H, *J*=8.0 Hz), 4.91 (dd, 1H, *J*=10.5, 5.4 Hz), 4.68–4.60 (m, 2H), 3.21 (dd, 1H, *J*=18.9, 9.7 Hz), 3.09–3.05 (m, 3H), 2.99–2.95 (m, 2H), 2.44–2.42 (m, 1H), 2.42–2.35 (m, 2H), 1.84 (d, 1H, *J*=9.9 Hz), 1.65 (d, 1H, *J*=10.3 Hz), 1.42–1.31 (m, 2H), 1.14–1.09 (m, 1H), 0.34–0.29 (m, 1H); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 138.3, 127.4, 127.2, 124.4, 123.9, 122.3, 121.2, 55.5, 45.0, 43.4, 43.3, 38.7, 36.8, 22.7 (2C), 22.2, 21.8, 21.7; FABMS [*m*/*z* (%)]: 265 ($M-BF_4^-$, 100); HRMS (FAB) calcd for $C_{18}H_{21}N_2$: 265.1705, found: 265.1691.

4.4.4. Benzimidazolium chloride 31. A solution of diamine **29** (40 mg, 0.14 mmol) in $(EtO)_3CH$ (3 mL) was treated with concentrated HCl solution (12 μL, 0.14 mmol). A reflux condenser was attached and the mixture was stirred at 80 °C under argon for 15 h, after which the condenser was removed and heating was continued in air for an additional 2 h. After cooling to room temperature, the solvent was carefully decanted and the precipitated residue washed repeatedly with Et_2O and dried thoroughly under high vacuum to give **31** (39 mg, 81%) as a hygroscopic amorphous solid foam; mp>250 °C; $[\alpha]_D^{19}$ <+1 (*c* 0.5, acetone); IR (KBr): ν_{max} 3088, 2994, 2917, 2866, 1634, 1509, 1470, 1329 cm^{-1}; ^1H NMR (600 MHz, acetone-d_6): δ 11.59 (br, 1H), 7.40 (d, 1H, *J*=7.0 Hz), 7.35 (d, 1H, *J*=7.1 Hz), 5.25 (br, 1H), 4.86 (br, 1H), 4.63 (br, 1H), 3.42–3.36 (m, 3H), 3.10 (m, 2H), 2.93 (d, 1H, *J*=15.9 Hz), 2.42–2.39 (m, 2H), 2.32 (br, 1H), 2.19 (quin, 1H, *J*=7.6 Hz), 1.97 (br, 1H), 1.91 (d, 1H, *J*=10.5 Hz), 1.68 (t, 1H, *J*=13.1 Hz), 1.27 (s, 3H), −0.05 (s, 3H); ^{13}C NMR (150.9 MHz, acetone-d_6): δ 128.8, 128.4, 125.4, 124.8, 124.1, 122.2, 58.3, 46.0, 45.7, 41.9, 40.4, 32.4, 27.9, 27.7, 27.6, 26.0, 24.1, 23.6, 19.1; FABMS [*m*/*z* (%)]: 293 ($M-Cl^-$, 100); HRMS (FAB) calcd for $C_{20}H_{25}N_2$: 293.2018, found: 293.2075.

4.4.5. Thiourea 32. A flame-dried 25 mL round-bottom flask under argon was charged with tetrafluoroborate **30** (20 mg, 0.057 mmol), sodium hydride (3.7 mg, 60% dispersion in mineral oil, 0.091 mmol), and S_8 (1.8 mg, 0.057 mmol). Dry THF (2 mL) was added and the resulting suspension was stirred at ambient temperature for 45 min. The reaction mixture was worked-up with water (2 mL) and the product extracted with Et_2O (3×2 mL). The combined organic layer was washed with water (2 mL), brine (2 mL), dried over anhyd Na_2SO_4, filtered, and concentrated in vacuo. Column chromatography (silica gel, 5:1 hexanes/EtOAc, R_f=0.34) gave **32** (10.5 mg, 63%) as a colorless powder; mp 156–158 °C; $[\alpha]_D^{20}$ +130.7 (*c* 0.82, $CHCl_3$); X-ray analysis (CCDC 617690) was performed on a pale yellow prism fragment (0.30×0.27×0.19 mm), which was obtained by crystallization from MeOH. $C_{18}H_{20}N_2S$: *M*=296.42 g/mol, triclinic, $P\bar{1}$, *a*=9.067(5) Å, *b*=9.5590(6) Å, *c*=10.3151(6) Å, α=63.723(1)°, β=65.662(1)°, γ=76.558(1)°, *V*=729.03(7) $Å^3$, *Z*=2, D_c=1.350 g/cm^3, *F*(000)=316, *T*=295(2) K. Data were collected on a Bruker APEX CCD system with graphite monochromated Mo Kα radiation (λ=0.71073 Å), 6057 data were collected. The structure was solved by Patterson and Fourier (SHELXTL) and refined by full-matrix least squares on F^2 resulting in final *R*, R_w, and GOF [for 3294 data with $F>2\sigma(F)$] of 0.0648, 0.1083, and 1.934, respectively; IR (KBr): ν_{max} 2951, 2875, 1493, 1406, 1365 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 6.89 (d, 1H, *J*=7.8 Hz), 6.85 (d, 1H, *J*=7.5 Hz), 4.50 (dd, 1H, *J*=10.5, 3.9 Hz), 4.15–4.02 (m, 2H), 3.60 (s, 1H), 3.05–2.77 (m, 5H), 2.32 (s, 1H), 2.26–2.16 (m, 2H), 1.72 (d, 1H, *J*=10.2 Hz), 1.54 (d, 1H, *J*=10.2 Hz), 1.32–1.21 (m, 3H), 0.63–0.55 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 166.6, 128.2, 128.0, 120.1, 119.5, 117.5, 117.3, 53.6, 44.0, 41.6, 40.1, 38.4, 37.4, 23.3, 23.0, 22.8, 22.2, 21.7; EIMS [*m*/*z* (%)]: 296 (M^+, 100); HRMS (EI) calcd for $C_{18}H_{20}N_2S$: 296.1347, found: 296.1355.

4.4.6. Thiourea 33. A flame-dried 25 mL round-bottom flask under argon was charged with vacuum-dried salt **31** (72 mg, 0.22 mmol), sodium hydride (13.8 mg, 60% dispersion in mineral oil, 0.35 mmol), and S_8 (7.1 mg, 0.22 mmol). Dry THF (7 mL) was added and the resulting suspension was stirred at ambient temperature for 2.5 h. The reaction mixture was worked-up with water (3 mL) and the product extracted with Et_2O (3×3 mL). The combined organic layer was washed with water (2 mL), brine (2 mL), dried over anhyd Na_2SO_4, filtered, and concentrated in vacuo. Column

chromatography (silica gel, 5:1 hexanes/EtOAc, R_f=0.35) gave **33** (38.3 mg, 54%) as a clear colorless glass; $[\alpha]_D^{19}$ +125 (*c* 0.5, $CHCl_3$); IR (NaCl, thin film): ν_{max} 2997, 2937, 1493, 1373, 1352 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 6.88 (ABq, 2H), 4.81 (dd, 1H, *J*=7.2, 5.4 Hz), 4.30 (q, 1H, *J*=5.7 Hz), 4.05 (t, 2H, *J*=5.7 Hz), 3.24–3.12 (m, 1H), 3.06–2.98 (m, 1H), 2.93–2.80 (m, 2H), 2.68 (d, 1H, *J*=16.2 Hz), 2.33–2.26 (m, 1H), 2.22–2.03 (m, 3H), 1.97–1.91 (m, 1H), 1.80–1.68 (m, 2H), 1.25 (s, 3H), 0.17 (s, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 166.3, 129.1, 128.0, 120.3, 120.0, 117.7, 117.1, 57.0, 41.8, 41.5, 40.6, 39.0, 32.6, 28.1, 27.6 (2C), 25.2, 23.3, 22.9, 18.8; EIMS [*m*/*z* (%)]: 324 (M^+, 95), 291 (75), 203 (100); HRMS (EI) calcd for $C_{20}H_{24}N_2S$: 324.1660, found: 324.1653.

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Supplementary data

^{13}C NMR data for all new compounds, ^{31}P NMR spectra for **21a** and **21b**, and COSY/NOESY experiments for **32** and **33**. This material is available via the Internet at http://www.sciencedirect.com. CCDC 612643 and 617690 contain the supplementary crystallographic data for compounds **21a** and **32**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.09.023.

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Acylation of alkylidenepyrrolidines with heterocumulenes—a reinvestigation

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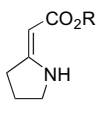
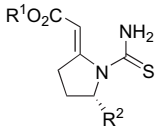
Abstract—The reactions of alkylidenepyrrolidine esters with isocyanates generally favour C-acylation, except in the case of benzyl isocyanate. Reactions with alkyl isocyanates are slow, and require forcing conditions. Reactions with isothiocyanates give exclusively the C-acylated products.

1. Introduction

Alkylidenepyrrolidines **1** are versatile heterocyclic ambident nucleophiles, which have been extensively used in organic synthesis.[1] During the course of our studies towards the synthesis of the batzelladine alkaloids,[2] we had occasion to undertake a three-component coupling reaction of an alkylidenepyrrolidine with an aldehyde and a silyl isothiocyanate.[3] Since the stereoselectivity in this reaction was modest, we sought an understanding of the mechanism in order to optimise this. For this study, we required a range of compounds of general structure **2** in order to study the stereoselectivity of their reactions with aldehydes. The N-acylation of alkylidenepyrrolidines has been reported with a range of alkyl and aryl isocyanates.[4] However, when we attempted to repeat some of the reactions in this report, we observed somewhat different results. In order to verify the trends observed during this work, a number of additional heterocumulenes were also investigated. We now report our results herein.



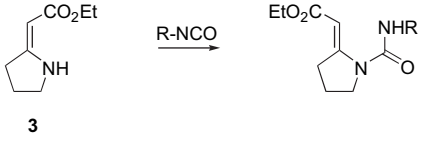
1



2

2. Results and discussion

The publication by Tronche[4] states that in general, all isocyanates used gave an approximately 70:30 mixture of products favouring N-acylation. With alkyl isocyanates, reactions were carried out in pyridine at room temperature for 2 h, while reactions with aryl isocyanates were undertaken in benzene at reflux (12 h). The examples reported are shown in Scheme 1.

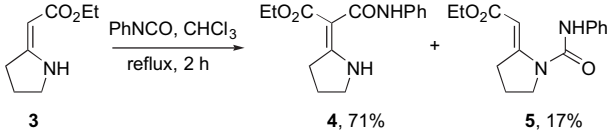


3

R = Me, *n*-Bu, *n*-C_6H_{13}, *c*-C_6H_{11}, Bn, Ph, 3-ClC_6H_4, 4-ClC_6H_4, 4-$CH_3C_6H_4$

Scheme 1.

In our hands, reaction of phenyl isocyanate with (*Z*)-alkylidenepyrrolidine **3** in chloroform under reflux gave a 3.4:1 crude mixture of C- and N-acylated products **4** and **5**. The isolated yields approximately reflect this regioselectivity (Scheme 2). At room temperature in the same solvent, a 2.1:1 mixture of the same compounds was obtained. When benzene (reflux) was used as solvent, a 5.2:1 mixture was obtained, while THF (also at reflux) gave the highest selectivity at 7:1. In all cases, the C-acylated product **4** predominated.



3 **4**, 71% **5**, 17%

Scheme 2.

All compounds in our study have been fully characterised (^{1}H NMR, ^{13}C NMR+DEPT, IR, MS, HRMS). The ^{1}H NMR

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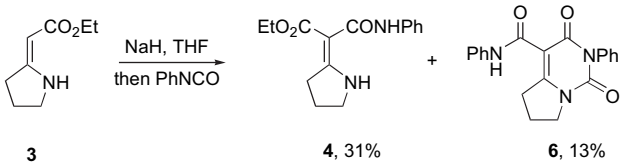
and melting point data which we have obtained for compound **5** are entirely in line with those reported by Tronche.[4] In an attempt to favour N-acylation, compound **3** was deprotonated with sodium hydride prior to reaction with phenyl isocyanate. However, this gave only a mixture of compound **4** and the novel heterocycle **6** (Scheme 3). Clearly the formation of compound **6** requires N-acylation, but this could occur after the C-acylation. In no case have we been able to obtain a mixture favouring the N-acylated product **5**.

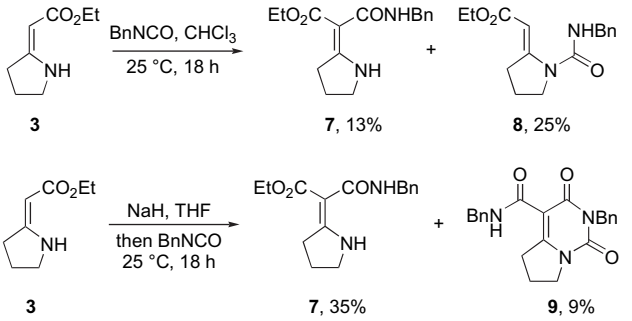


3 **4**, 31% **6**, 13%

Scheme 3.

In contrast, reaction of alkylidenepyrrolidine **3** with benzyl isocyanate proceeds to give a 2:1 mixture of N-acylated compound **8** and C-acylated compound **7**, exactly as reported.[4] We chose to carry out this reaction in chloroform rather than pyridine, and obtained poor conversion (Scheme 4). Upon heating, the overall yields were dramatically improved at the expense of regioselectivity (1:1). Deprotonation of the alkylidenepyrrolidine with sodium hydride gave good selectivity for C-acylation (5.3:1). In this case, the bicyclic compound **9** was also formed (9% yield), while the low conversion meant that compound **8** was not actually isolated.

At this point we felt that we were beginning to establish a trend, and that alkyl isocyanates would give predominantly N-acyl products while aryl isocyanates would give predominantly C-acyl products. In order to verify this, reactions were carried out with cyclohexyl and butyl isocyanates. In both

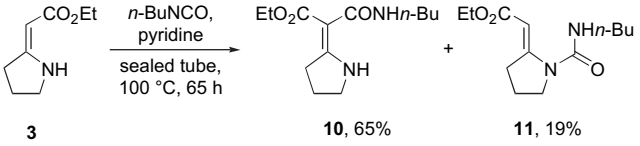


3 **7**, 13% **8**, 25%

3 **7**, 35% **9**, 9%

Scheme 4.

cases, no reaction was observed in either chloroform or pyridine at room temperature. With butyl isocyanate, upon heating for 65 h at 100 °C, C-acylation was again found to predominate (Scheme 5).



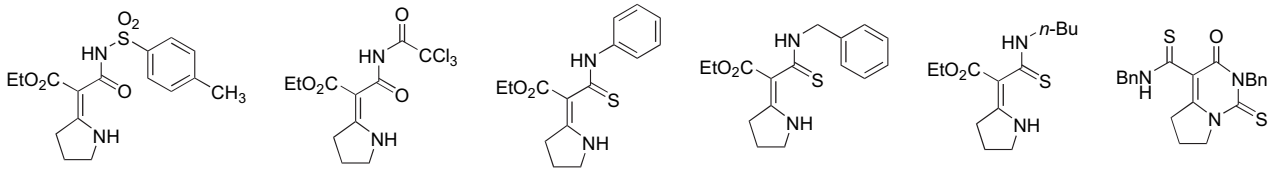
3 **10**, 65% **11**, 19%

Scheme 5.

Following on from these results, a number of other isocyanates and some isothiocyanates were investigated in this reaction. The results are summarised in Table 1. Of these other compounds, only 4-methylphenyl isocyanate was found to produce any of the N-acylated product, this being the minor compound produced. As we have previously discussed,[2] we believe that the data reported by Tronche for the N-acylated

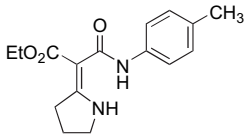
Table 1. Reactions of alkylidenepyrrolidine **3** with a range of heterocumulenes

Entry	Heterocumulene	Conditions	Ratio C:N acylation	Products (% isolated yield)
1	PhNCO	$CHCl_3$, 25 °C, 18 h	2.1:1	**4** (53%); **5** (20%)
2	PhNCO	$CHCl_3$, reflux, 2 h	3.4:1	**4** (71%); **5** (17%)
3	PhNCO	Benzene, reflux, 2 h	5.2:1	Not purified
4	PhNCO	THF, reflux, 2 h	7:1	Not purified
5	PhNCO	NaH, THF, 18 h	1:0	**4** (31%); **6** (13%)
6	BnNCO	$CHCl_3$, 25 °C, 18 h	1:2	**7** (13%); **8** (25%)
7	BnNCO	$CHCl_3$, reflux, 20 h	1:1	**7** (39%); **8** (50%)
8	BnNCO	NaH, THF, 18 h	5.3:1	**7** (35%); **9** (9%)
9	*n*-BuNCO	Pyridine, 100 °C, 65 h	2.3:1	**10** (65%); **11** (19%)
10	4-MeC_6H_4NCO	$CHCl_3$, reflux, 2 h	2.3:1	**12** (56%); **13** (23%)
11	TsNCO	$CHCl_3$, 25 °C, 2 h	1:0	**14** (94%)
12	$Cl_3CCONCO$	$CHCl_3$, 25 °C, 18 h	1:0	**15** (51%)
13	PhNCS	$CHCl_3$, reflux, 18 h	1:0	**16** (58%)
14	BnNCS	$CHCl_3$, reflux, 18 h	1:0	**17** (66%)
15	*n*-BuNCS	Pyridine, 100 °C, 46 h	1:0	**18** (89%)
16	BnNCS	NaH, THF, 18 h	1:0	**17** (38%); **19** (25%)

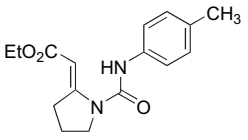


14 **15** **16** **17** **18** **19**

product **13** are actually more consistent with the C-acylated product **12**, so that our results are actually in agreement. In particular, the melting point reported for compound **13** is close to that which we have measured for compound **12**. In all other cases, the C-acylated product was formed exclusively, and in each case as a single double-bond isomer according to the spectroscopic data. The double-bond geometry is presumed to be that shown as a result of more favourable hydrogen bonding.[5]

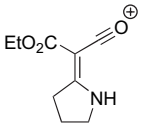


12

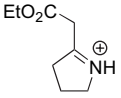


13

With the benefit of hindsight, it is straightforward to distinguish the C- and N-acyl compounds by mass spectrometry (electrospray or APCI). The former all give a strong peak at *m*/*z* 182 corresponding to fragment **20**, while the latter all give a peak at *m*/*z* 156, which presumably corresponds to **21**.

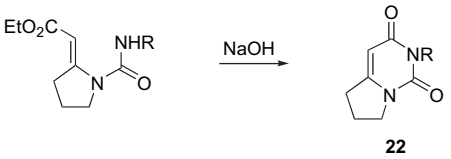


20



21

Tronche's group subsequently reported the formation of pyrrolo[1,2-*c*]pyrimidines **22** as shown in Scheme 6.[6] All of these compounds presented plausible NMR and analytical data, and it is difficult to see how any of these compounds could have been formed from the C-acyl isomers. We therefore have no doubt that the N-acyl compounds were indeed formed, although particularly in the case of R=*n*-Bu and *c*-C_6H_{13}, we are unable to reproduce their formation. The alkylidenepyrrolidine literature shows numerous examples of subtle reactivity,[7] so it seems possible that impurities present in either Tronche's or our own starting materials could affect the regioselectivity.



22

R = Me, *n*-C_4H_9, *n*-C_6H_{13}, *c*-C_6H_{11}, Bn, Ph

Scheme 6.

The other factor which could affect the regiochemical outcome is the double-bond geometry in the alkylidenepyrrolidine. Earlier reports[8] from the group of Tronche state that the (*Z*)-alkylidenepyrrolidine **3** gives exclusively the C-acylation product with methyl and phenyl isocyanates, while an unspecified mixture of (*E*) and (*Z*) isomers leads to the 70:30 mixture favouring N-acylation. In their 1988 paper,[4] Tronche and co-workers do state that a mixture of double-bond isomers was used. However, they prepared alkylidenepyrrolidine **3** by an Eschenmoser sulfide contraction, which is known to strongly favour the (*Z*)-alkylidenepyrrolidine,[1] and indeed the authors state in their experimental section that the (*Z*) isomer **3** was isolated in 46% yield, while the (*E*) isomer was unstable and was not isolated under these conditions.[4] Assuming that the (*Z*) isomer **3** and (*E*) isomer give C- and N-acylation products respectively, it is difficult to see how a mixture favouring the (*Z*) isomer could possibly give a 70:30 mixture favouring N-acylation. We are only aware of a single unequivocal example of the synthesis of the (*E*) isomer of a simple NH alkylidenepyrrolidine.[9] While many authors do draw the (*E*) isomer, it seems likely that this is for convenience, and generally no comment is made about the double-bond geometry. In the cases where the (*E*) isomer has been drawn and spectroscopic data are presented, all of these compounds appear to be the (*Z*) isomer.

Based on these observations, we can offer no conclusive explanation for the discrepancies between our own results and those of Tronche. Nevertheless, our results have proven to be reproducible and consistent over a range of heterocumulenes, and with batches of alkylidenepyrrolidine **3** prepared on a number of different occasions. We therefore feel that the results reported herein represent the norm for the acylation of alkylidenepyrroldines with heterocumulenes.

3. Experimental

3.1. General

All reactions were carried out under an atmosphere of dry nitrogen. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin–Elmer 1600 FTIR spectrophotometer. Mass spectra were recorded on a Fisons VG Platform II spectrometer and on a Micromass Q-TOF Micro spectrometer. NMR spectra were recorded on a Bruker DPX 400 spectrometer operating at 400 MHz for ^{1}H and at 100 MHz for ^{13}C at 25 °C. All chemical shifts are reported in parts per million downfield from TMS. Coupling constants (*J*) are reported in hertz. Multiplicity in ^{13}C NMR was obtained using the DEPT pulse sequence. Flash chromatography was performed using Matrex silica 60 35–70 μm. Compound **3** was prepared according to a literature method.[10]

3.1.1. *N*-Phenyl-2-pyrrolidin-(2*E*)-ylidene-malonamic acid ethyl ester (4) and (1-phenylcarbamoyl-pyrrolidin-(2*E*)-ylidene)-acetic acid ethyl ester (5). To a solution of (*Z*)-pyrrolidin-2-ylidene-acetic acid ethyl ester **3** (106 mg, 0.7 mmol) in $CHCl_3$ (6 mL) was added phenyl isocyanate (89 μL, 0.8 mmol), and the mixture stirred at 25 °C for 18 h. The solvent was then removed in vacuo. The resulting orange oil was purified by column chromatography (eluting with ethyl acetate/hexane 4.5:7) to give compound **4** (R_f=0.8) (99 mg, 53%) and compound **5** (R_f=0.37) (38 mg, 20%).

Data for compound **4**: yellow solid, mp 76–78 °C; ν_{max} (CH_2Cl_2)/cm^{-1} 3236, 1650, 1614; δ_H (400 MHz; $CDCl_3$) 11.44 (1H, br s, CH_2NH), 11.29 (1H, br s, PhNH), 7.50 (2H, d, *J* 7.6, aromatic CH), 7.21 (2H, apparent t, *J* 7.9, aromatic CH), 6.95 (1H, t, *J* 7.4, aromatic CH), 4.14 (2H, q, *J* 7.1, $CO_2CH_2CH_3$), 3.52 (2H, t, *J* 7.7, CH_2NH), 3.11 (2H, t, *J* 7.9, $CH_2C{=}C$), 1.91 (2H, m, CH_2CH_2NH), 1.25 (3H, t, *J* 7.1, $CO_2CH_2CH_3$); δ_C (100 MHz; $CDCl_3$) 174.9 (CH_2CNH), 169.9 ($C{=}O$), 168.9 ($C{=}O$), 139.2 (aromatic C), 128.7 (aromatic CH), 123.0 (aromatic CH), 120.6 (aromatic CH), 87.0 ($COCCO_2Et$), 59.9 ($CO_2CH_2CH_3$), 47.7 (CH_2NH), 36.5 ($CH_2C{=}C$), 21.3 (CH_2CH_2NH), 14.6 ($CO_2CH_2CH_3$); *m*/*z* (ES^+) 297.2 (MNa^+, 7%), 275.3 (MH^+, 2), 183.1 (28), 182.1 (100), 154.0 (99), 138.0 (98); HRMS (ES^+) calcd for $C_{15}H_{19}N_2O_3$ (MH^+) 275.1396, found 275.1373.

Data for compound **5**: colourless solid, mp 127–129 °C (lit.[4] mp 128 °C); ν_{max} (CH_2Cl_2)/cm^{-1} 3377, 1689, 1660, 1594; δ_H (400 MHz; $CDCl_3$) 7.31 (2H, d, *J* 7.7, aromatic CH), 7.22 (2H, apparent t, *J* 7.9, aromatic CH), 7.01 (1H, t, *J* 7.4, aromatic CH), 6.95 (1H, br s, NHPh), 6.34 (1H, apparent d, *J* 1.6, CH=C), 4.03 (2H, q, *J* 7.1, $CO_2CH_2CH_3$), 3.64 (2H, t, *J* 7.1, CH_2NCO), 3.12 (2H, apparent dt, *J* 1.6, 7.8, $CH_2C{=}C$), 1.87 (2H, apparent quintet, *J* 7.4, CH_2CH_2NCO), 1.16 (3H, t, *J* 7.1, $CO_2CH_2CH_3$). δ_C (100 MHz; $CDCl_3$) 168.9 (ester $C{=}O$), 158.2 (alkene C), 152.1 (urea $C{=}O$), 137.5 (aromatic C), 129.0 (aromatic CH), 124.3 (aromatic CH), 120.7 (aromatic CH), 95.6 (alkene CH), 59.4 (CO_2CH_2), 49.4 (CH_2NCO), 31.9 ($CH_2C{=}C$), 21.1 (CH_2CH_2NCO), 14.5 (CH_3CH_2OCO); *m*/*z* (ES^+) 275 (MH^+, 55%), 156 (100); HRMS (ES^+) calcd for $C_{15}H_{19}N_2O_3$ (MH^+) 275.1396, found 275.1397.

3.1.2. 1,3-Dioxo-2-phenyl-1,2,3,5,6,7-hexahydro-pyrrolo[1,2-*c*]pyrimidine-4-carboxylic acid phenylamide (6). To a stirred suspension of sodium hydride (60% dispersion in oil, 35 mg, 1.5 mmol) in dry THF (15 mL), was added dropwise a solution of (*Z*)-pyrrolidin-2-ylidene-acetic acid ethyl ester **3** (207 mg, 1.3 mmol) in THF (5 mL) at 0 °C. The mixture was then stirred for 2 h at 25 °C. Phenyl isocyanate (159 mg, 1.3 mmol) was added, and the mixture stirred for 18 h at rt. The reaction mixture was quenched with saturated NH_4Cl solution (30 mL), the organic layer extracted, and the aqueous layer washed with DCM (3×30 mL). The combined organic washings were washed with brine (2×50 mL), dried over $MgSO_4$, and the solvent removed in vacuo. The residue was recrystallised from ethanol to give the *title compound* (60 mg, 13%) as a pale yellow solid, mp 253–255 °C; ν_{max} (CH_2Cl_2)/cm^{-1} 3239, 1705, 1682, 1591, 1437; δ_H (400 MHz; $CDCl_3$) 11.12 (1H, br s, PhNHCO), 7.60–7.35 (5H, m, aromatic CH), 7.30–7.15 (4H, m, aromatic CH), 7.00 (1H, t, *J* 7.3, aromatic CH), 4.00 (2H, t, *J* 7.5, CH_2NCO), 3.77 (2H, t, *J* 7.9, $CH_2C{=}C$), 2.17 (2H, apparent quintet, *J* 7.7, CH_2CH_2NCO); δ_C (100 MHz; $CDCl_3$) 164.9 ($C{=}O$), 163.5 ($C{=}O$), 160.6 ($C{=}O$), 147.8 (CH_2CNCO), 137.2 (aromatic C), 133.4 (aromatic C), 128.7 (aromatic CH), 128.3 (aromatic CH), 127.9 (aromatic CH), 127.1 (aromatic CH), 123.0 (aromatic CH), 119.3 (aromatic CH), 100.3 (NHCO–*C*–CO), 48.2 (CH_2NCO), 33.4 ($CH_2C{=}C$), 19.4 (CH_2CH_2NCO); *m*/*z* (ES^+) 370.3 (MNa^+, 92%), 348.3 (MH^+, 100), 273.2 (44), 255.2 (95); HRMS (ES^+) calcd for $C_{20}H_{18}N_3O_3$ (MH^+) 348.1348, found 348.1343. Purification of the filtrate by flash column chromatography gave compound **4** (113 mg, 31%) (data as above).

3.1.3. *N*-Benzyl-2-pyrrolidin-(2*E*)-ylidene-malonamic acid ethyl ester (7) and (1-benzylcarbamoyl-pyrrolidin-(2*E*)-ylidene)-acetic acid ethyl ester (8). To a solution of (*Z*)-pyrrolidin-2-ylidene-acetic acid ethyl ester **3** (103 mg, 0.7 mmol) in $CHCl_3$ (6 mL) was added benzyl isocyanate (80 μL, 0.7 mmol), and the mixture stirred at 25 °C for 18 h. The solvent was then removed in vacuo and the resulting orange oil purified by column chromatography (eluting with ethyl acetate/hexane 3:7) giving, in order of elution, compound **7** (R_f=0.41) (25 mg, 13%) and compound **8** (R_f=0.18) (48 mg, 25%).

Data for compound **7**: yellow solid, mp 95–98 °C; ν_{max} (Nujol)/cm^{-1} 3408, 1639, 1594; δ_H (400 MHz; $CDCl_3$) 11.40 (1H, s, CH_2NH), 9.51 (1H, m, $PhCH_2NH$), 7.27–7.18 (5H, m, aromatic CH), 4.43 (2H, d, *J* 5.7, $PhCH_2NH$), 4.09 (2H, q, *J* 7.1, $CO_2CH_2CH_3$), 3.50 (2H, t, *J* 7.4, CH_2NH), 3.09 (2H, t, *J* 7.9, $CH_2C{=}C$), 1.92 (2H, apparent quintet, *J* 7.7, CH_2CH_2NH), 1.22 (3H, t, *J* 7.1, $CO_2CH_2CH_3$); δ_C (100 MHz; $CDCl_3$) 174.4 (CH_2CNH), 170.5 ($C{=}O$), 169.7 ($C{=}O$), 139.6 (aromatic C), 128.5 (aromatic CH), 127.3 (aromatic CH), 126.8 (aromatic CH), 86.6 ($COCCO_2Et$), 59.5 ($CO_2CH_2CH_3$), 47.5 ($PhCH_2NH$), 43.0 (CH_2NH), 36.2 ($CH_2C{=}C$), 21.4 (CH_2CH_2NH), 14.6 ($CO_2CH_2CH_3$); *m*/*z* (ES^+) 311 ($MNa+H_2O^+$, 26%), 289 (MH^+, 10), 243 (5), 182 (100); HRMS (ES^+) calcd for $C_{16}H_{22}N_2O_3$ (MH^+) 289.1552, found 289.1548.

Data for compound **8**: colourless solid, mp 88–92 °C, lit.[4] mp 86 °C; ν_{max} (Nujol)/cm^{-1} 3357, 1669, 1604; δ_H (400 MHz; $CDCl_3$) 7.28–7.14 (5H, m, aromatic CH), 6.41 (1H, apparent t, *J* 1.7, CH=C), 5.32 (1H, br s, $NHCH_2Ph$), 4.38 (2H, d, *J* 5.6, $NHCH_2Ph$), 4.02 (2H, q, *J* 7.1, $CO_2CH_2CH_3$), 3.54 (2H, t, *J* 7.1, CH_2NCO), 3.11 (2H, apparent dt, *J* 1.7, 7.8, $CH_2C{=}CH$), 1.87 (2H, apparent quintet, *J* 7.5, CH_2CH_2NCO), 1.16 (3H, t, *J* 7.1, $CO_2CH_2CH_3$); δ_C (100 MHz; $CDCl_3$) 169.1 (ester $C{=}O$), 158.3 (CH_2CNCO), 154.5 (urea $C{=}O$), 158.5 (aromatic C), 128.7 (aromatic CH), 127.7 (aromatic CH), 127.5 (aromatic CH), 95.0 ($CH{=}C$), 59.2 ($CO_2CH_2CH_3$), 49.1 ($NHCH_2Ph$), 44.5 (CH_2NCO), 31.8 ($CH_2C{=}C$), 21.1 (CH_2CH_2NCO), 14.5 ($CO_2CH_2CH_3$); *m*/*z* (ES^+) 329 (M+Na+H_2O, 65%), 261 (38), 156 (100), 128 (97); HRMS (ES^+) calcd for $C_{16}H_{22}N_2O_3$ (MH^+) 289.1552, found 289.1548.

3.1.4. 1,3-Dioxo-2-benzyl-1,2,3,5,6,7-hexahydro-pyrrolo[1,2-*c*]pyrimidine-4-carboxylic acid benzylamide (9). To a stirred suspension of sodium hydride (60% dispersion in oil, 45 mg, 1.1 mmol) in dry THF (15 mL), was added dropwise a solution of (*Z*)-pyrrolidin-2-ylidene-acetic acid ethyl ester **3** (159 mg, 1.0 mmol) in THF (5 mL) at 0 °C. The mixture was then stirred for 2 h at 25 °C. Benzyl isocyanate (0.13 mL, 1.0 mmol) was added, and the mixture stirred for 18 h at 25 °C. The reaction mixture was quenched with saturated NH_4Cl solution (30 mL), the organic layer was separated and the aqueous layer washed with CH_2Cl_2 (3×30 mL). The combined organic layers were washed with brine (2×50 mL), dried over $MgSO_4$, and the solvent

removed in vacuo. The resulting orange solid was purified by column chromatography (eluting with 3:7 ethyl acetate/hexane) giving, in order of elution, compound **9** (R_f=0.57) (36 mg, 9%) and compound **7** (R_f=0.41) (103 mg, 35%) (data as above).

Data for compound ***9***: yellow solid, mp 117–120 °C; ν_{max} (CH_2Cl_2)/cm^{-1} 3248, 1705, 1646, 1623, 1545, 1442; δ_H (400 MHz; $CDCl_3$) 11.10 (1H, br s, CH_2NHCO), 7.31 (4H, apparent t, *J* 7.6, aromatic CH), 7.26–7.12 (6H, m, aromatic CH), 5.04 (4H, apparent s, 2×PhCH_2N), 3.64 (2H, t, *J* 7.6, CH_2NCO), 3.39 (2H, t, *J* 8.0, CH_2C=C), 2.06 (2H, apparent quintet, *J* 7.8, CH_2CH_2NCO); δ_C (100 MHz; $CDCl_3$) 177.2 (Cl_3CC=O), 165.2 (C=O), 162.4 (C=O), 151.6 (CH_2CNCO), 137.6 (aromatic C), 137.6 (aromatic C), 128.5 (aromatic CH), 128.4 (aromatic CH), 128.2 (aromatic CH), 127.4 (aromatic CH), 127.3 (aromatic CH), 88.5 (NHCO–C–CO), 48.2 (CH_2NCO), 44.19, 44.16 (2×PhCH_2N), 35.3 (CH_2C=C), 20.8 (CH_2CH_2NCO); *m/z* (ES$^+$) 376 (MH$^+$, 100%), 310 (32), 238 (23), 123 (66); HRMS (ES$^+$) calcd for $C_{22}H_{22}N_3O_3$ (MH$^+$) 376.1661, found 376.1657.

3.1.5. *N*-Butyl-2-pyrrolidin-(2*E*)-ylidene-malonamic acid ethyl ester (10) and (1-*n*-butylcarbamoyl-pyrrolidin-(2*E*)-ylidene)-acetic acid ethyl ester (11). To a solution of (*Z*)-pyrrolidin-2-ylidene-acetic acid ethyl ester **3** (147 mg, 0.9 mmol) in pyridine (0.5 mL) was added butyl isocyanate (128 μL, 1.1 mmol). The solution was stirred at 100 °C in a sealed tube for 65 h, after which time the solvent was removed under reduced pressure. The resulting dark brown oil was purified by column chromatography (eluting with ethyl acetate/hexane 4:7), to give compound **10** (R_f=0.33) (156 mg, 65%) and compound **11** (R_f=0.11) (45 mg, 19%) both as pale yellow oils.

Data for compound ***10***: pale yellow oil; ν_{max} (CH_2Cl_2)/cm^{-1} 3314, 1654, 1598; δ_H (400 MHz; $CDCl_3$) 11.45 (1H, br s, CH_2NH), 9.10 (1H, br s, CH_2NHCO), 4.10 (2H, q, *J* 7.1, CO_2CH_2), 3.51 (2H, t, *J* 7.4, CH_2NH), 3.21 (2H, apparent q, *J* 6.5, CH_2NHCO), 3.08 (2H, t, *J* 7.9, CH_2C=C), 1.93 (2H, apparent quintet, *J* 7.7, CH_2CH_2NH), 1.46 (2H, apparent quintet, *J* 7.3, $CH_3CH_2CH_2$), 1.31 (2H, apparent sextet, *J* 7.4, $CH_3CH_2CH_2$), 1.23 (3H, t, *J* 7.1, $CO_2CH_2CH_3$), 0.85 (3H, t, *J* 7.3, $CH_3CH_2CH_2$); δ_C (100 MHz; $CDCl_3$) 174.1 (CH_2CNH), 170.3 (C=O), 169.6 (C=O), 86.5 (COCCO$_2$Et), 59.3 (CO_2CH_2), 47.3 (CH_2NH), 38.6 (CH_2NHCO), 36.0 (CH_2C=C), 31.8 ($CH_3CH_2CH_2$), 21.3 (CH_2CH_2NH), 20.3 ($CH_3CH_2CH_2$), 14.4 ($CO_2CH_2CH_3$), 13.8 ($CH_3CH_2CH_2$); *m/z* (APCI) 255 (MH$^+$, 24%), 182 (100); HRMS (ES$^+$) calcd for $C_{13}H_{23}N_2O_3$ (MH$^+$) 255.1709, found 255.1704.

Data for compound ***11***: pale yellow oil; ν_{max} (CH_2Cl_2)/cm^{-1} 3348, 1646, 1565; δ_H (400 MHz; $CDCl_3$) 6.28 (1H, br s, alkene CH), 4.97 (1H, br s, NHCON), 4.04 (2H, q, *J* 7.1, CO_2CH_2), 3.56 (2H, t, *J* 7.1, CH_2NCO), 3.21 (2H, apparent q, *J* 6.6, CH_2NHCO), 3.13 (2H, t, *J* 7.7, CH_2C=C), 1.89 (2H, apparent quintet, *J* 7.4, CH_2CH_2NCO), 1.47 (2H, apparent quintet, *J* 7.4, $CH_3CH_2CH_2$), 1.29 (2H, apparent sextet, *J* 7.4, $CH_3CH_2CH_2$), 1.18 (3H, t, *J* 7.1, $CO_2CH_2CH_3$), 0.87 (3H, t, *J* 7.3, $CH_3CH_2CH_2$); δ_C (100 MHz; $CDCl_3$) 169.0 (ester C=O), 158.4 (CH_2CNCO), 154.4 (urea C=O), 94.3 (CHCCO$_2$Et), 59.2 (CO_2CH_2), 49.2 (CH_2NCO), 40.4 (CH_2NHCO), 32.0 (CH_2C=C), 31.9 ($CH_3CH_2CH_2$), 21.1 (CH_2CH_2NH), 20.9 ($CH_3CH_2CH_2$), 14.5 ($CO_2CH_2CH_3$), 13.8 ($CH_3CH_2CH_2$); *m/z* (APCI) 273 (M+H$_3$O$^+$, 42%), 255 (MH$^+$, 19%), 227 (16), 156 (100); HRMS (ES$^+$) calcd for $C_{13}H_{23}N_2O_3$ (MH$^+$) 255.1709, found 255.1704.

3.1.6. 2-Pyrrolidin-(2*E*)-ylidene-*N*-*p*-tolyl-malonamic acid ethyl ester (12) and (1-*p*-tolylcarbamoyl-pyrrolidin-(2*E*)-ylidene)-acetic acid ethyl ester (13). To a solution of (*Z*)-pyrrolidin-2-ylidene-acetic acid ethyl ester **3** (161 mg, 1 mmol) in $CHCl_3$ (6 mL) was added *p*-tolyl isocyanate (130 μL, 1 mmol), and the solution stirred under reflux for 2 h. The solvent was removed in vacuo to produce a yellow oil, which solidified on standing. The yellow solid was purified by column chromatography (eluting with EtOAc/hexane 4.5:7) giving, in order of elution, compound **12** (R_f=0.76) (168 mg, 56%) and compound **13** (R_f=0.29) (69 mg, 23%).

Data for compound ***12***: pale yellow solid, mp 150–153 °C; ν_{max} (solution)/cm^{-1} 3216, 1649, 1619; δ_H (400 MHz; $CDCl_3$) 11.40 (1H, br s, CH_2NH), 11.25 (1H, br s, ArNH), 7.37 (2H, apparent d, *J* 7.4, aromatic CH), 7.01 (2H, apparent d, *J* 7.4, aromatic CH), 4.12 (2H, q, *J* 7.1, CO_2CH_2), 3.49 (2H, t, *J* 7.4, CH_2NH), 3.08 (2H, t, *J* 7.9, CH_2C=C), 2.20 (3H, s, CH_3Ar), 1.88 (2H, apparent quintet, *J* 7.7, CH_2CH_2NH), 1.23 (3H, t, *J* 7.1, $CO_2CH_2CH_3$); δ_C (100 MHz; $CDCl_3$) 174.8 (CH_2CNH), 169.9 (C=O), 168.8 (C=O), 136.5 (aromatic C), 132.5 (aromatic C), 129.3 (aromatic CH), 120.4 (aromatic CH), 87.0 (COCCO$_2$Et), 59.8 (CO_2CH_2), 47.6 (CH_2NH), 36.5 (CH_2C=C), 21.3 (CH_2CH_2NH), 20.9 (CH_3Ar), 14.6 ($CO_2CH_2CH_3$); *m/z* (ES$^+$) 311 (MNa$^+$, 30%), 289 (MH$^+$, 30), 182 (100); HRMS (ES$^+$) calcd for $C_{16}H_{22}N_2O_3$ (MH$^+$) 289.1552, found 289.1537.

Data for compound ***13***: colourless solid, mp 132–135 °C, lit.[4] mp 155 °C; ν_{max} (CH_2Cl_2)/cm^{-1} 3448, 1694, 1609; δ_H (400 MHz; $CDCl_3$) 7.25 (2H, apparent d, *J* 8.3, aromatic CH), 7.04 (2H, apparent d, *J* 8.3, aromatic CH), 6.77 (1H, s, NHAr), 6.31 (1H, apparent t, *J* 1.6, CH=C), 4.04 (2H, q, *J* 7.1, CO_2CH_2), 3.66 (2H, t, *J* 7.1, CH_2NCO), 3.15 (2H, apparent dt, *J* 1.7, 7.8, CH_2C=C), 2.24 (3H, s, CH_3Ar), 1.90 (2H, apparent quintet, *J* 7.4, CH_2CH_2NCO), 1.17 (3H, t, *J* 7.1, $CO_2CH_2CH_3$); δ_C (100 MHz; $CDCl_3$) 168.8 (ester C=O), 158.1 (CH_2CNCO), 152.1 (urea C=O), 134.8 (aromatic C), 134.0 (aromatic CH), 129.5 (aromatic CH), 120.7 (aromatic CH), 95.4 (CH=C), 59.3 (CO_2CH_2), 49.4 (CH_2NCO), 32.0 (CH_2C=C), 21.1 (CH_2CH_2NCO), 20.8 (CH_3Ar), 14.5 ($CO_2CH_2CH_3$); *m/z* (ES$^+$) 311 (MNa$^+$, 22%), 289 (MH$^+$, 8), 156 (100), 110 (34); HRMS (ES$^+$) calcd for $C_{16}H_{20}N_2O_3Na$ (MNa$^+$) 311.1372, found 311.1356.

3.1.7. 3-Oxo-2-pyrrolidin-(2*E*)-ylidene-3-(toluene-4-sulfonylamino)-propionic acid ethyl ester (14). To a solution of (*Z*)-pyrrolidin-2-ylidene-acetic acid ethyl ester **3** (102 mg, 0.7 mmol) in $CHCl_3$ (6 mL) was added *p*-tosyl isocyanate (100 μL, 0.7 mmol) and the mixture stirred at reflux for 2 h. The solvent was removed in vacuo to yield the *title compound* (220 mg, 94%) as a white crystalline solid, without need for further purification, mp 154–158 °C; ν_{max} (Nujol)/cm^{-1} 3447, 1644, 1594; δ_H (400 MHz; $CDCl_3$) 12.20

(1H, s, CH_2NH), 11.30 (1H, s, SO_2NHCO), 7.83 (2H, apparent d, J 8.3, aromatic CH), 7.18 (2H, apparent d, J 8.3, aromatic CH), 4.09 (2H, q, J 7.1, $CO_2CH_2CH_3$), 3.46 (2H, t, J 7.5, CH_2NH), 3.05 (2H, t, J 7.9, CH_2C=C), 2.29 (3H, s, CH_3Ar), 1.89 (2H, apparent quintet, J 7.7, CH_2CH$_2$NH), 1.20 (3H, t, J 7.1, $CO_2CH_2CH_3$); δ_C (100 MHz; $CDCl_3$) 175.9 (CH_2CNH), 169.5 (C=O), 167.7 (C=O), 143.9 (aromatic C), 137.4 (aromatic C), 129.3 (aromatic CH), 128.1 (aromatic CH), 86.5 (COCCO$_2$Et), 60.5 ($CO_2CH_2CH_3$), 48.1 (CH_2NH), 36.6 (CH_2C=C), 21.6 (CH_3Ar), 20.8 (CH_2CH_2NH), 14.4 ($CO_2CH_2CH_3$); m/z (ES^+) 353 (MH^+, 34%), 182 (100); HRMS (ES^+) calcd for $C_{16}H_{21}N_2O_5S$ (MH^+) 353.1171, found 353.1166.

3.1.8. 3-Oxo-2-pyrrolidin-(2*E*)-ylidene-3-(2,2,2-trichloro-acetylamino)-propionic acid ethyl ester (15). To a stirred solution of (*Z*)-pyrrolidin-2-ylidene-acetic acid ethyl ester **3** (63 mg, 0.4 mmol) was added trichloroacetyl isocyanate (48 μL, 0.4 mmol) and the mixture stirred for 18 h at 25 °C. The solvent was then removed in vacuo. The resulting yellow oil was purified by column chromatography (eluting with ethyl acetate/hexane 4:7) to give the *title compound* (R_f=0.25) (72 mg, 51%) as an off-white solid, mp 110–112 °C; ν_{max} (solution)/cm^{-1} 3197, 1750, 1664, 1614; δ_H (400 MHz; $CDCl_3$) 13.38 (1H, br s, Cl_3CCO–NH–CO), 11.31 (1H, br s, CH$_2$NH), 4.20 (2H, q, J 7.1, CO_2CH_2), 3.66 (2H, t, J 7.5, CH_2NH), 3.20 (2H, t, J 7.9, CH_2C=C), 2.04 (2H, apparent quintet, J 7.8, CH_2CH$_2$NH), 1.28 (3H, t, J 7.1, $CO_2CH_2CH_3$); δ_C (100 MHz; $CDCl_3$) 176.6 (Cl_3CCONH), 174.9 (CH_2CNH), 169.8 (ester C=O), 167.8 (NHCOC=C), 93.2 (Cl_3C), 87.8 (COCCO$_2$Et), 60.8 (CO_2CH_2), 48.4 (CH_2NH), 36.9 (CH_2C=C), 20.9 (CH_2CH_2NH), 14.4 ($CO_2CH_2CH_3$); m/z (ES^+) 343 (MH^+, 18%) (+consistent isotopomer peaks), 182 (100); HRMS (ES^+) calcd for $C_{11}H_{14}N_2O_4{}^{35}Cl_3$ (MH^+) 343.0019, found 343.0015.

3.1.9. Phenylthiocarbamoyl-(2*Z*)-pyrrolidin-2-ylidene-acetic acid ethyl ester (16). To a stirred solution of (*Z*)-pyrrolidin-2-ylidene-acetic acid ethyl ester **3** (60 mg, 0.4 mmol) in $CHCl_3$ (6 mL) was added phenyl isothiocyanate (46 μL, 0.4 mmol), and the mixture stirred for 20 h under reflux. The solvent was removed in vacuo and the resulting brown solid recrystallised from aqueous ethanol to yield the *title compound* (65 mg, 58%) as colourless needles, mp 118–120 °C; ν_{max} (solution)/cm^{-1} 3176, 1649, 1248; δ_H (400 MHz; $CDCl_3$) 13.28 (1H, br s, CH$_2$NH), 12.47 (1H, br s, PhNH), 7.38 (2H, d, J 7.6, aromatic CH), 7.31 (2H, apparent t, J 7.9, aromatic CH), 7.16 (1H, t, J 7.4, aromatic CH), 4.18 (2H, q, J 7.2, CO_2CH_2), 3.64 (2H, t, J 7.4, CH_2NH), 3.15 (2H, t, J 7.8, CH_2C=C), 1.98 (2H, apparent quintet, J 7.6, CH_2CH$_2$NH), 1.28 (3H, t, J 7.2, $CO_2CH_2CH_3$); δ_C (100 MHz; $CDCl_3$) 190.2 (thioamide C=S), 174.1 (CH_2CNH), 170.2 (ester C=O), 139.7 (aromatic C), 128.6 (aromatic CH), 126.3 (aromatic CH), 126.2 (aromatic CH), 95.0 (CSCCO), 60.4 (CO_2CH_2), 48.0 (CH_2NH), 37.8 (CH_2C=C), 21.9 (CH_2CH_2NH), 14.4 ($CO_2CH_2CH_3$); m/z (ES^+) 291 (MH^+, 29%), 198 (100); HRMS (ES^+) calcd for $C_{15}H_{19}N_2O_2S$ (MH^+) 291.1167, found 291.1172.

3.1.10. Benzylthiocarbamoyl-(2*Z*)-pyrrolidin-2-ylidene-acetic acid ethyl ester (17). To a solution of (*Z*)-pyrrolidin-2-ylidene-acetic acid ethyl ester **3** (155 mg, 1 mmol) in $CHCl_3$ (6 mL) was added benzyl isothiocyanate (160 μL, 1.2 mmol), and the solution was stirred under reflux for 23 h. The mixture was allowed to cool, and the solvent was removed in vacuo. The resulting dark orange oil was purified by column chromatography (eluting with ethyl acetate/hexane 1:4) to give the *title compound* (R_f=0.41) (204 mg, 66%) as buff waxy solid, mp 83–87 °C; ν_{max} (solution)/cm^{-1} 3197, 1639, 1268; δ_H (400 MHz; $CDCl_3$) 13.05 (1H, br s, CH$_2$NH), 12.22 (1H, br s, PhCH$_2$NH), 7.18–7.11 (5H, m, aromatic CH), 4.79 (2H, apparent d, J 4.9, PhCH_2NH), 4.08 (2H, q, J 7.2, CO_2CH_2) 3.59 (2H, t, J 7.3, CH_2NH), 3.09 (2H, t, J 7.8, CH_2C=C), 1.93 (2H, apparent quintet, J 7.6, CH_2CH$_2$NH), 1.20 (3H, t, J 7.2, $CO_2CH_2CH_3$); δ_C (100 MHz; $CDCl_3$) 190.0 (thioamide C=S), 173.4 (CH_2CNH), 169.9 (ester C=O), 137.7 (aromatic C), 128.7 (aromatic CH), 127.9 (aromatic CH), 127.3 (aromatic CH), 94.3 (CSCCO$_2$Et), 60.1 (CO_2CH_2), 49.1 (PhCH_2NH), 47.8 (CH_2NH), 37.5 (CH_2C=C), 21.9 (CH_2CH_2NH), 14.4 ($CO_2CH_2CH_3$); m/z (ES^+) 305 (MH^+, 100%), 259 (24), 198 (78); HRMS (ES^+) calcd for $C_{16}H_{21}N_2O_2S$ (MH^+) 305.1324, found 305.1300.

3.1.11. *n*-Butylthiocarbamoyl-(2*Z*)-pyrrolidin-2-ylidene-acetic acid ethyl ester (18). To a solution of (*Z*)-pyrrolidin-2-ylidene-acetic acid ethyl ester **3** (160 mg, 1.0 mmol) in pyridine (0.5 mL) was added butyl isothiocyanate (149 μL, 1.2 mmol). The solution was stirred at 100 °C in a sealed tube for 46 h, after which time the solvent was removed under reduced pressure. The resulting dark orange solid was recrystallised from aqueous ethanol to give the *title compound* (240 mg, 89%) as an orange solid, mp 53–56 °C; ν_{max} (CH_2Cl_2)/cm^{-1} 3176, 1633, 1257; δ_H (400 MHz; $CDCl_3$) 13.05 (1H, br s, CH$_2$NH), 10.92 (1H, br s, CH$_2$NHCS), 4.13 (2H, q, J 7.2, CO_2CH_2), 3.59 (2H, apparent q, J 7.3, CH_2NHCS), 3.58 (2H, t, J 7.3, CH_2NH), 3.08 (2H, t, J 7.8, CH_2C=C), 1.94 (2H, apparent quintet, J 7.6, CH_2CH$_2$NH), 1.58 (2H, apparent quintet, J 7.3, $CH_3CH_2CH_2$), 1.36 (2H, apparent sextet, J 7.4, $CH_3CH_2CH_2$), 1.24 (3H, t, J 7.2, $CO_2CH_2CH_3$), 0.88 (3H, t, J 7.3, $CH_3CH_2CH_2$); δ_C (100 MHz; $CDCl_3$) 189.5 (thiocarbamoyl C=S), 173.1 (CH_2CNH), 169.9 (ester C=O), 94.0 (CSCCO$_2$Et), 60.0 (CO_2CH_2), 47.6 (CH_2NH), 44.7 (CH_2NHCS), 37.4 (CH_2C=C), 30.3 ($CH_3CH_2CH_2$), 21.8 (CH_2CH_2NH), 20.4 ($CH_3CH_2CH_2$), 14.3 ($CO_2CH_2CH_3$), 13.8 ($CH_3CH_2CH_2$); m/z (APCI) 271 (MH^+, 74%), 225 (26), 198 (100), 156 (19); HRMS (ES^+) calcd for $C_{13}H_{23}N_2O_2S$ (MH^+) 271.1480, found 271.1476.

3.1.12. 2-Benzyl-3-oxo-1-thioxo-1,2,3,5,6,7-hexahydro-pyrrolo[1,2-*c*]pyrimidine-4-carbothioic acid benzylamide (19). To a stirred suspension of sodium hydride (60% dispersion in oil, 18 mg, 0.44 mmol) in dry THF (10 mL), was added dropwise a solution of (*Z*)-pyrrolidin-2-ylidene-acetic acid ethyl ester **3** (62 mg, 0.4 mmol) in THF (5 mL) at 0 °C. The mixture was then stirred for 2 h at 25 °C. Benzyl isothiocyanate (53 μL, 1.0 mmol) was added, and the mixture stirred for 18 h at 25 °C. The reaction mixture was quenched with saturated NH_4Cl solution (30 mL), the organic layer separated, and the aqueous layer extracted with CH_2Cl_2 (3×30 mL). The combined organic layers were washed with brine (2×50 mL), dried over $MgSO_4$, and the solvent removed in vacuo. The resulting dark orange solid was purified by column chromatography

(eluting with ethyl acetate/hexane 3:7) to give, in order of elution, compound **17** (R_f=0.68) (46 mg, 38%) (data as above) and the *title compound* (R_f=0.45) (42 mg, 25%) as a pale solid, mp 130–132 °C; ν_{max} (CH_2Cl_2)/cm^{-1} 3337, 1664, 1288; δ_H (400 MHz; $CDCl_3$) 14.0 (1H, br s, $PhCH_2NHCS$), 7.45–7.05 (10H, m, aromatic CH), 6.45 (2H, s, one of $PhCH_2N$), 5.73 (2H, s, one of $PhCH_2N$), 3.74 (2H, t, *J* 7.4, CH_2NCS), 3.55 (2H, t, *J* 7.9, $CH_2C{=}C$), 2.17 (2H, apparent quintet, *J* 7.7, CH_2CH_2NCO); δ_C (100 MHz; $CDCl_3$) 191.0 (C=S), 183.4 (C=S), 176.6 (C=O), 157.8 (alkene C), 135.6 (aromatic C), 135.5 (aromatic C), 127.3 (aromatic CH), 127.2 (aromatic CH), 126.5 (aromatic CH), 126.1 (aromatic CH), 125.6 (aromatic CH), 125.6 (aromatic CH), 102.8 (alkene C), 56.0 (CH_2N), 50.5 (CH_2N), 47.9 (CH_2N), 37.1 (CH_2), 20.1 (CH_2); *m/z* (ES^+) 408 (MH^+, 100%), 383 (28), 303 (22), 238 (29), 182 (58); HRMS (ES^+) calcd for $C_{22}H_{22}N_3OS_2$ (MH^+) 408.1204, found 408.1208.

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RCM/PCC oxidation strategy for synthesis of functionalized cyclic α,β-unsaturated lactones: synthesis of (+)-triacetoxygoniotriol and its diastereomers

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Abstract—A novel methodology leading to the synthesis of (+)-triacetoxygoniotriol **2** from D-glucose is described. Construction of the core six-membered α,β-unsaturated lactone moiety involved ring closing metathesis (RCM) followed by a PCC oxidation. Later exploiting the pseudo-symmetry of D-glucose three other diastereomers of triacetoxygoniotriol were synthesized using the developed methodology.

1. Introduction

Styryllactones, isolated from the stem bark of *Goniothalamus gigeanteus* (*Annanoceae*) have interesting heterocyclic skeletons.[1] They show significant murine toxicity toward lymphocytic leukemia systems. Goniotriol **1a** is one of the important members of styryllactone family. It showed significant cytotoxicity in the potato disc test and brine shrimp test. Interestingly both enantiomers of goniotriol occur in nature and its stereochemistry varies with the source of extraction.[2] Syntheses of (+)-goniotriol adopting both chiral pool and enantioselective approaches (including our own approach) and its analogues have been reported and their bioactivities were studied.[3,4] Also, synthesis of other styryllactones from goniotriol has been reported. Taking into account the various approaches reported in the literature we embarked on developing a novel unified approach for synthesis of various diastereomers of (+)-goniotriol from D-glucose. In our approach, which is described here we constructed the core α,β-unsaturated lactone moiety by using RCM and PCC oxidation of dialkenyl derivatives. We also showed that the allyl group, which is generally known to be a protection group could be used as a masked acrylic ester moiety during our synthesis. Herein, we wish to report complete details of our approach along with the synthesis of both the enantiomers of goniotriol, thus demonstrating the versatility of our approach (Fig. 1).

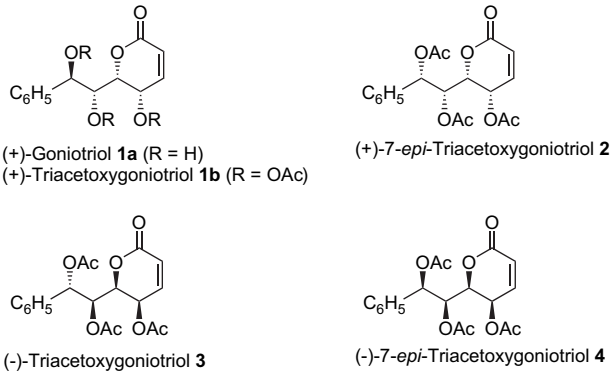

Figure 1.

2. Results and discussion

Retrosynthetic analysis of our approach for the synthesis of goniotriols **1**–**4** is depicted in Scheme 1. The recognition that the core six-membered α,β-unsaturated lactone moiety of the goniotriol could be constructed by ring closing metathesis followed by PCC oxidation guided our planning from the outset. In the synthetic direction (−)-triacetoxygoniotriols **3** and **4** could be synthesized from RCM product of dialkene **5** by a PCC oxidation reaction.[5] Dialkene **5** could originate from the acetonide **6** by incorporation of the required phenyl group. Finally the acetonide **6** can be synthesized from diol **7**, which in turn could be obtained from D-glucose by known steps.[6]

Our synthesis commenced as outlined in Scheme 2. Dialkenyl acetonide **6** was subjected to hydrolysis using acetic

Keywords: Styryllactones; Goniotriol; RCM and PCC oxidation.
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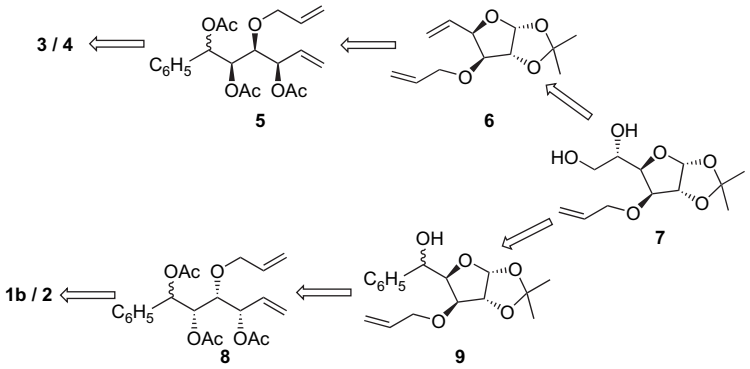
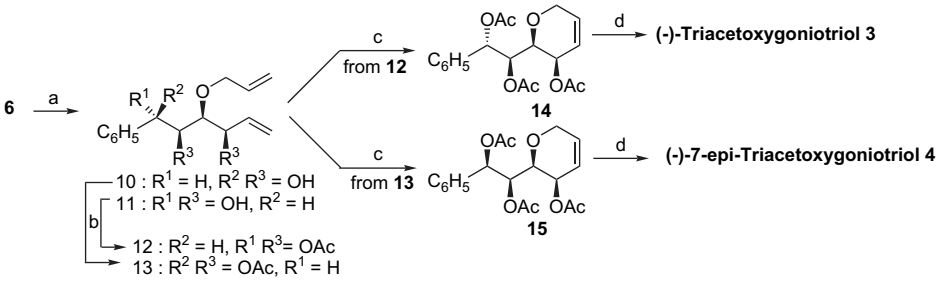


Scheme 1.

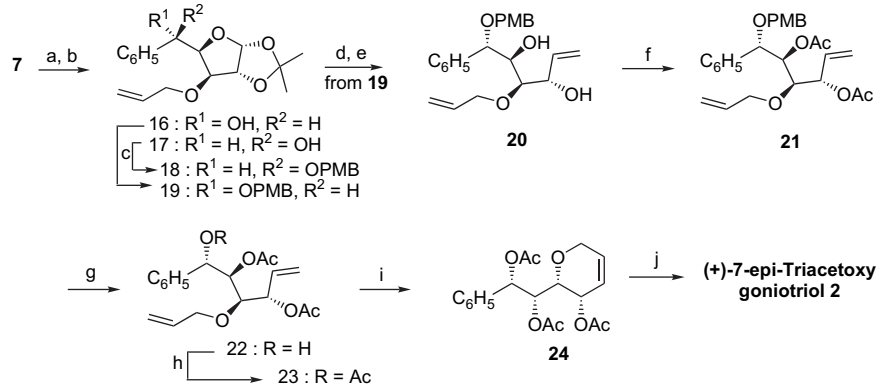


Scheme 2. (a) (i) CH_3CO_2H–H_2O (4:1), reflux, 12 h (86% yield); (ii) PhMgBr, THF, 0 °C to rt, 30 h (74% yield, a:b=3:1); (b) Ac_2O, Et_3N, DMAP, 24 h (88% yield); (c) Grubb's catalyst (5 mol %), DCM, rt, 24 h (90% yield); (d) PCC, Py, DCM, reflux, 8 h (64% yield).

acid in water at 90 °C to furnish an inseparable mixture of anomeric diols in 86% yield. Diols were subjected to a Grignard reaction with phenyl magnesium bromide in dry THF at 0 °C resulting in an inseparable mixture of diastereomeric triols **10** and **11** in 3:1 ratio. The diastereomeric diols on acetylation followed by column chromatography afforded essentially pure triacetates **12** and **13** in 49% combined yield (for two steps). The stereochemistry of the major diastereomer **13** was deduced by the literature analogy and was later unambiguously corroborated by the single crystal X-ray structure of (−)-7-*epi*-triacetoxygoniotriol **4**, which was derived from **13**.[4,7] Treatment of triacetate **12** to ring closing metathesis conditions using Grubb's catalyst [benzylidene-bis(tri-cyclohexylphosphine)-dichlororuthenium (5 mol %)] in dry DCM yielded allyl ether **15** in 90% yield. The allylic methylene moiety in ether **15** was oxidized with 3 equiv of PCC and pyridine in dry DCM furnished the required styryllactone, [7*R*,6*S*,5*S*,4*R*]-7-*epi*-triacetoxy-(−)-goniotriol **4**.[5] *It is appropriate to mention that this is the first example wherein such an oxidation was conducted on densely functionalized intermediate*. The minor isomer **12** was converted into (−)-triacetoxygoniotriol **3** under the same conditions.

In order to synthesize (+)-triacetoxygoniotriols **1b** and **2**, which are enantiomers of **3** and **4**, respectively, we decided to exploit the *pseudo*-symmetry of D-glucose. It was envisioned that the intermediate generated by the addition of phenyl magnesium bromide onto the aldehyde generated by the oxidative cleavage of diol **7** could be converted to triacetoxydialkene **8**, which is enantiomeric to triacetoxydialkene **5**. Intermediate **8** could be converted to triacetoxygoniotriols **1b** and **2** using the conditions described earlier.

Accordingly, the synthesis of (+)-7-*epi*-triacetoxygoniotriol **2** is outlined in Scheme 3. The aldehyde generated by the oxidative cleavage of diol **7** using $NaIO_4$ in MeOH, when allowed to react with phenyl magnesium bromide in dry THF at 0 °C resulted in the formation of the diastereomeric alcohols **16** and **17** in a 8:2 ratio with 77% overall yield. The stereochemistry at the C-5 center in two diastereomers was established by the literature analogy (Fig. 2).[8] The proton on C-3 in the case of alcohol **16** (major diastereomer) having L-*ido* configuration appeared approximately δ 0.4 upfield than that in the alcohol **17** with the D-*gluco* configuration due to the anisotropy of the phenyl moiety. The benzylic alcohol in **16** was then converted to PMB ether **19** using standard reaction conditions. Conversion of the PMB ether **19** to dialkene **20** involved a two-step reaction sequence of acid hydrolysis followed by Wittig reaction. While the Wittig reaction preceded without any complication, albeit moderate yield, the acid hydrolysis was complicated as a result of cleavage of PMB group under the hydrolysis conditions. The most efficient protocol entailed conducting acidic hydrolysis using 4 M HCl in THF at 55 °C and halting the reaction prior to completion (52% yield based on the isolated yield of the anomeric diols after column chromatography; 88% based on the recovered **19**). The recovered diol was resubjected to acidic hydrolysis. Acidic hydrolysis under prolonged reaction conditions or use of strong acids resulted in low yields of the desired product. Peracetylation of diol **20** gave diacetate **21**, which was then exposed to DDQ in moist CH_2Cl_2 to afford alcohol **22** in overall 88% yield. It was then converted into the triacetate **23**, followed by a ring closing metathesis reaction using Grubb's catalyst gave allyl ether **24** in 92% yield. Oxidation of the allyl ether **24** using PCC and pyridine in dry DCM furnished the required lactone in



Scheme 3. (a) $NaIO_4$, MeOH–H_2O (10:1), 0 °C, 4 h (88% yield); (b) PhMgBr, THF, 0 °C to rt, 12 h (74% yield, a:b=8:2); (c) NaH, PMBCl, Bu_4NI (cat), DMF, 0 °C to rt, 18 h (quant); (d) 4 M HCl–THF (1:4), 55 °C, 4 h (88%); (e) $Ph_3P{=}CH_2$, THF, −10 °C to rt, 24 h (55% yield); (f) Ac_2O, Et_3N, DMAP, 0 °C to rt, 24 h (quant); (g) DDQ, moist DCM, rt, 2 h (88%); (h) Ac_2O, Et_3N, DMAP, 0 °C to rt, 24 h (quant); (i) Grubb's catalyst (5%) DCM, rt, 24 h (92% yield); (j) PCC, C_5H_5N, DCM, reflux, 7 h (65% yield).

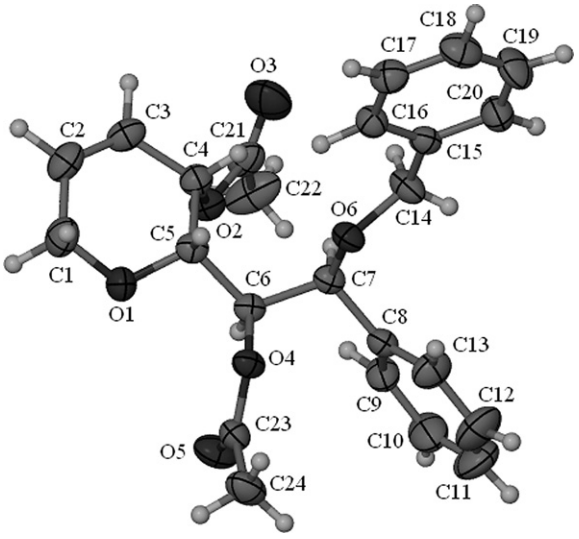


Figure 2.

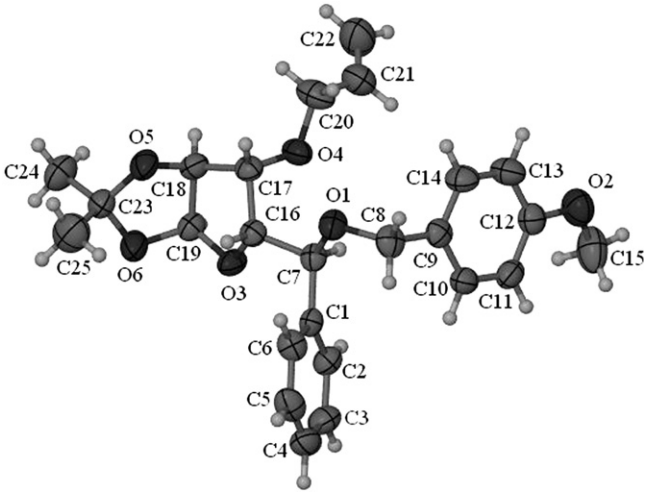


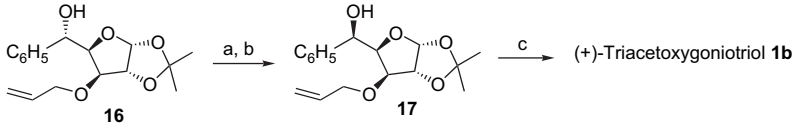
Figure 3.

65% yield. The (+)-7-*epi*-triacetoxygoniotriol thus obtained showed same optical rotation but with opposite sign as that of (−)-7-*epi*-triacetoxygoniotriol **4** ($[\alpha]_D^{22}$ +134.1 (*c* 0.2, MeOH)).[4] The absolute stereochemistry of (−)-7-*epi*-triacetoxygoniotriol **4** was further confirmed from single crystal X-ray of the 8-*O*-benzyl derivative of the compound **24**.[8]

The reversal of the stereochemistry at the C-6 center of **16** was accomplished by first oxidizing the benzylic alcohol in acetonide **16** followed by reduction with $NaBH_4$ at 0 °C.[9] Single crystal X-ray structure of the PMB ether of **17** unambiguously ascertained the stereochemistry at the benzylic position (Fig. 3).[10] Alcohol **17** was later converted to (+)-triacetoxygoniotriol **1b** using the same reaction sequence as described in Scheme 4.

3. Conclusion

Synthesis of natural products containing six-membered α,β-unsaturated lactones as a core feature using RCM reactions has been reported.[11] These approaches involve intramolecular RCM reactions of appropriately substituted acrylyl alkenylic esters. However, in some cases acrylyl esters are known to be reluctant to undergo a ring closing metathesis reaction due to the formation of stable seven-membered metal chelates formed between the ester carbonyls and the intermediate metal carbene species.[12] In the present case we have used allyl ether as a masked acrylic ester moiety to overcome such difficulties. Since allyl ethers withstand many harsher reaction conditions the developed



Scheme 4. (a) PDC, DCM, rt, 4 h (80% yield); (b) $NaBH_4$, 0 °C, 4 h (90% yield, 9:1 (**17**:**16**)); (c) Scheme 2c–j.

methodology offers an attractive alternative to acrylyl ester derived synthesis of cyclic lactones. Also D-glucose offers possibility of manipulating the stereochemistry around a given carbon atom at a time. Hence it is possible to synthesize all stereoisomers of goniotriol from D-glucose using the developed methodology.

4. Experimental

4.1. General

Optical rotations were measured with a JASCO DIP-370 digital polarimeter using a sodium lamp (λ=589 nm) at 24 °C. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using Me_4Si as an internal standard on a Varian spectrometer. Thin-layer chromatography was performed on E. Merck glass plates silica gel sheets (Silica Gel F_{254}) and visualized under UV light and/or stained with ceric ammonium molybdate–aqueous H_2SO_4 solution. Column chromatography was carried out on silica gel (E. Merck 230–400 mesh). All solvents were distilled before use.

4.1.1. 2,4,5-Tri-*O*-acetyl-3-*O*-allyl-5,6-dideoxy-1-*O*-phenyl-hex-1-enitol 12/13. Dialkene **6** (0.5 g, 2.2 mmol) was dissolved in aqueous AcOH (10 ml, 1:4) and refluxed for 12 h. Removal of the solvents in vacuo yielded a yellow oil, which was then dissolved in DCM (10 ml) and washed with satd aqueous $NaHCO_3$ solution (5 ml). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The resultant crude diol was dissolved in dry THF (10 ml) and was added drop wise (15 min) to a solution of phenyl magnesium bromide (2 g, 11.0 mmol) in THF (15 ml) at 0 °C. After stirring for 2 h at 0 °C and then for 30 h at rt, cold 1 M HCl (5 ml) was added and extracted with EtOAc (10 ml). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. Flash column chromatography (1:1, hexane–EtOAc) afforded triols **10/11** (0.36 g, 1.36 mmol, 74%) as a 3:1 mixture of diastereomers. The triols **10** and **11** (0.36 g, 1.36 mmol) were dissolved in Et_3N (2 ml) at 0 °C and DMAP (20 mg) was added. Acetic anhydride (0.83 g, 8.1 mmol) was added and the resultant mixture was stirred for 24 h at rt. Et_3N was evaporated and flash column chromatography of the resultant crude mixture afforded triacetoxydialkenes **12** and **13** (0.46 g, 1.20 mmol, 88% yield) in 3:1 ratio, respectively, as a pale viscous oil. HRMS (FAB) *m/z* 391.1741 (MH^+, $C_{21}H_{27}O_7$, requires 391.1757).

Compound **13**: 0.35 g, $[\alpha]_D^{22}$ +8.5 (*c* 2, $CHCl_3$); ν_{max} (liquid film) 3120, 1743, 1643, 1604, 1434, 1335, 1130 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38–7.30 (m, 5H, Ph), 5.95–5.81 (m, 2H, C*H*=CH_2), 5.86 (d, *J*=8.0 Hz, 1H, PhC*H*(OAc)), 5.40–5.35 (m, 2H, C*H*(OAc) and AcOC*H*CH=CH_2), 5.30–5.19 (m, 4H, CH=CH_2), 4.25 (dd, *J*=4.5, 13.0 Hz, 1H, OCH_2CH=CH_2), 4.10 (dd, *J*=5.5, 13.0 Hz, 1H, OCH_2CH=CH_2), 3.69 (dd, *J*=3.0, 7.5 Hz, 1H, C*H*Oallyl), 2.09 (s, 3H, OA*c*), 2.02 (s, 3H, OA*c*), 1.81 (s, 3H, OA*c*). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.0, 169.5, 169.4, 136.6, 134.5, 132.4, 128.8, 128.4, 127.9, 118.5, 118.2, 77.7, 74.3, 73.9, 73.4, 72.3, 21.4, 21.3, 20.8.

^{1}H NMR of compound **23** was similar to that of compound **13** ($[\alpha]_D^{22}$ −8.4 (*c* 2, $CHCl_3$)).

Compound **12**: 0.10 g, $[\alpha]_D^{22}$ −18.5 (*c* 2, $CHCl_3$); ν_{max} (liquid film) 3110, 1740, 1638, 1610, 1444, 1342 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.36–7.28 (m, 5H, Ph), 6.03 (d, *J*=8.0 Hz, 1H, PhC*H*(OAc)), 5.94–5.81 (m, 2H, C*H*=CH_2), 5.46 (q, *J*=3.5 Hz, 1H, CH=CH_2), 5.42–5.40 (m, 1H, CH=CH_2), 5.30 (d, *J*=2.0 Hz, 1H, CH=CH_2), 5.27 (d, *J*=2.0 Hz, 1H, CH=CH_2), 5.24 (dd, *J*=2.0, 7.0 Hz, 1H, OC*H*CH=CH_2), 5.19 (dd, *J*=2.0, 10.5 Hz, 1H, C*H*OAc), 4.15 (dd, *J*=5.0, 10.0 Hz, 1H, OCH_2CH=CH_2), 3.89 (dd, *J*=4.0, 10.0 Hz, 1H, OCH_2CH=CH_2), 3.51 (dd, *J*=3.0, 7.0 Hz, 1H, C*H*Oallyl), 2.06 (s, 3H, OA*c*), 2.05 (s, 3H, OA*c*), 2.03 (s, 3H, OA*c*). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.1, 169.6, 169.4, 136.6, 134.4, 132.4, 128.5, 128.4, 128.0, 118.5, 118.3, 77.8, 74.5, 73.6, 73.4, 72.5, 21.6, 21.3, 20.6.

^{1}H NMR of (8*R*) diastereomer of compound **23** was similar to that of compound **12** ($[\alpha]_D^{22}$ +18.4 (*c* 2, $CHCl_3$)).

4.1.2. 1,2,4-Tri-*O*-acetyl-3,7-anhydro-5,6-dideoxy-1-*O*-phenylhept-5-enitol 14/15. To a degassed solution of dialkene **12/13/23** (0.10 g, 0.25 mmol) in anhydrous DCM (3 ml) was added Grubb's catalyst (10 mg, 0.012 mmol) and stirred for 24 h at rt. The reaction mixture was diluted with DCM (2 ml) and air was bubbled through the solution to deactivate the catalyst. The reaction mixture was filtered through Celite and concentrated in vacuo. Flash column chromatography (10% EtOAc in hexane) afforded pyran **14/15/24** (82 mg, 0.23 mmol, 90% yield) as a colorless semi solid. HRMS (FAB) *m/z* 362.3737 (MH^+, $C_{19}H_{22}O_7$, requires 362.3729).

Compound **15**: $[\alpha]_D^{22}$ −152.1 (*c* 0.2, MeOH); ν_{max} (liquid film) 3080, 1742, 1646, 1430, 1335 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.38–7.31 (m, 5H), 6.04–5.98 (m, 3H), 5.52 (t, *J*=5.4 Hz, 1H), 5.00–4.95 (m, 1H), 4.30 (d, *J*=19.8 Hz, 1H), 4.08 (d, *J*=19.8 Hz, 1H), 3.55 (dd, *J*=2.5, 5.5 Hz, 1H), 2.09 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.3, 169.6, 136.5, 131.8, 128.6, 126.9, 122.2, 74.2, 73.5, 73.1, 66.1, 65.5, 21.0, 20.9, 20.8.

^{1}H NMR of compound **24** was similar to that of compound **15** ($[\alpha]_D^{22}$ +152.1 (*c* 0.2, MeOH)).

Compound **14**: $[\alpha]_D^{22}$ +90.2 (*c* 0.2, MeOH); ν_{max} (KBr) 3100, 1740, 1644, 1440, 1320 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.38–7.29 (m, 5H), 6.04–5.96 (m, 2H), 5.83 (d, *J*=5.5 Hz, 1H), 5.52 (d, *J*=6.0 Hz, 1H), 5.01–4.97 (m, 1H), 4.33 (d, *J*=18.0 Hz, 1H), 4.10 (d, *J*=18.0 Hz, 1H), 3.57 (dd, *J*=4.0, 8.0 Hz, 1H), 2.09 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.2, 169.8, 136.5, 131.6, 128.9, 126.7, 122.0, 74.1, 73.5, 73.2, 66.2, 65.3, 21.1, 20.9, 20.7.

^{1}H NMR of (8*R*) diastereomer of compound **24** was similar to that of compound **15** ($[\alpha]_D^{22}$ −90.0 (*c* 0.2, MeOH)).

4.1.3. General procedure for the oxidation of cyclic allyl ethers to lactones. To a solution of pyran **14/15/24** (80 mg,

0.20 mmol) in anhydrous DCM were added PCC (142 mg, 0.6 mmol) and pyridine (0.1 ml) and refluxed for 7 h. The solvent was evaporated and ethyl ether (5 ml) was added and filtered through Celite. The solvent was evaporated and flash column chromatography (5–20% EtOAc in hexane, gradient elution) afforded triacetoxygoniotriol **3/4/2** (54.5 mg, 65% yield) as a white solid. HRMS (FAB) *m/z* 376.1158 (MH^+, $C_{19}H_{20}O_8$, requires 376.1164).

7-*epi*-7,6,4-Triacetoxy-(−)-goniotriol **4**: $[\alpha]_D^{22}$ −135.0 (*c* 0.2, MeOH); ν_{max} (KBr) 3100, 1742, 1640, 1448, 1336 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.34 (m, 5H), 6.93 (dd, *J*=5.4, 9.5 Hz, 1H), 6.16 (d, *J*=9.5 Hz, 1H), 6.05 (d, *J*=6.2 Hz, 1H), 5.62 (dd, *J*=5.1, 6.2 Hz, 1H), 5.24 (dd, *J*=3.2, 5.1 Hz, 1H), 4.43 (dd, *J*=3.2, 5.1 Hz, 1H), 2.1 (s, 6H), 2.08 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.1, 169.4, 169.2, 140.3, 129.3, 129.2, 127.2, 124.7, 100.1, 75.7, 74.0, 71.8, 62.9, 21.1, 21.0.

^1H NMR of 7-*epi*-7,6,4-triacetoxy-(+)-goniotriol **2** was similar to that of compound **4** ($[\alpha]_D^{22}$ +135.0 (*c* 0.2, MeOH)).

7,6,4-Triacetoxy-(−)-goniotriol **3**: $[\alpha]_D^{22}$ −121 (*c* 0.8, MeOH); ν_{max} (KBr) 3124, 1742, 1644, 1440, 1327 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.42–7.33 (m, 5H), 6.93 (dd, *J*=6.0, 10.2 Hz, 1H), 6.16 (d, *J*=9.8 Hz, 1H), 5.96 (d, *J*=5.2 Hz, 1H), 5.76 (dd, *J*=4.8, 7.1 Hz, 1H), 5.30 (dd, *J*=3.2, 6.0 Hz, 1H), 4.53 (dd, *J*=3.2, 6.8 Hz, 1H), 2.10 (s, 3H), 2.08 (s, 3H), 2.01 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.2, 169.4, 169.1, 140.4, 129.4, 129.0, 127.3, 124.5, 100.3, 76.1, 74.2, 71.6, 62.7, 21.3, 21.1.

^1H NMR of 7,6,4-triacetoxy-(+)-goniotriol **1b** was similar to that of compound **3** ($[\alpha]_D^{22}$ +121 (*c* 0.8, MeOH)).

4.1.4. 1,2-*O*-(1-Methylethyledene)-3-*O*-allyl-5-*C*-phenyl-α-D-*xylo*-furanose 16/17. To vigorously stirred slurry of silica gel (10 g, Acme 60–120 mesh) in DCM (100 ml) a solution of $NaIO_4$ (5.83 g, 22.27 mmol in 15 ml water) was added and continued stirring at rt for 10 min after which a solution of diol **7** (4 g, 18.18 mmol) in methanol (20 ml) was added. The reaction mixture was further stirred for 4 h and solids were filtered. Solvents were evaporated to give the aldehyde as a colorless oil, which was dried in vacuo over P_2O_5 (88% yield). The resultant crude aldehyde was dissolved in anhydrous THF (60 ml) and the solution was added drop wise (30 min) to a solution of phenyl magnesium bromide (6.8 g, 37.8 mmol) in anhydrous THF (150 ml) at 0 °C. After stirring for 4 h at 0 °C and then 12 h at rt, cold 1 M HCl (25 ml) was added and extracted with EtOAc (30 ml). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. Flash chromatography (1:1, hexane–EtOAc) afforded diastereomeric alcohols **16/17** (3.9 g, 12.8 mmol, 74% yield) as 8:2 (**16**:**17**) mixtures of diastereomers as a yellow oil separable by column chromatography. HRMS (FAB) *m/z* 329.1350 (MNa^+, $C_{17}H_{22}O_5Na$, requires 329.1365).

(5*S*)-Isomer **16**: $[\alpha]_D^{22}$ −5.0 (*c* 1, MeOH); ν_{max} (liquid film) 3480–3060 (br), 1610, 1434, 1335, 1130 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.49–7.34 (m, 5H, Ph), 6.03 (d, *J*=6.5 Hz, 1H, OC*H*O), 5.92–5.78 (m, 1H, C*H*=CH_2), 5.35–5.20 (m, 2H, CH=CH_2), 5.06 (dd, *J*=3.0, 13.0 Hz, 1H, PhC*H*(OH), 4.57 (d, *J*=6.0 Hz, 1H, C*H*OCMe_2), 4.33 (dd, *J*=6.0, 13.0 Hz, 1H, PhCH(OH)C*H*), 4.04 (dd, *J*=5.5, 12.5 Hz, 1H, O$CH_2$$CHCH_2$), 3.77 (dd, *J*=5.5, 12.5 Hz, 1H, O$CH_2$$CHCH_2$), 3.50 (d, *J*=5.0 Hz, 1H, C*H*Oallyl), 2.89 (d, *J*=2.5 Hz, 1H, O*H*), 1.51 (s, 3H, C(CH_3)$_2$), 1.33 (s, 3H, C(CH_3)$_2$). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 140.0, 133.8, 128.6, 128.4, 127.5, 118.0, 112.1, 105.5, 84.9, 82.4, 82.2, 72.8, 71.1, 27.0, 26.5.

(5*R*)-Isomer **17**: $[\alpha]_D^{22}$ −34.9 (*c* 1, MeOH); ν_{max} (liquid film) 3450–3050 (br), 1620, 1424, 1340, 1140 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.42–7.35 (m, 4H, Ph), 7.31–7.26 (m, 1H, Ph), 6.02 (d, *J*=5.5 Hz, 1H, OC*H*O), 5.95–5.86 (m, 1H, C*H*=CH_2), 5.32 (d, *J*=17.0 Hz, 1H, CH=CH_2), 5.25 (d, *J*=10.5 Hz, 1H, CH=CH_2), 5.11 (t, *J*=6.5 Hz, 1H, PhC*H*(OH)), 4.55 (d, *J*=2.5 Hz, 1H, C*H*OCMe_2), 4.33 (t, *J*=2.5 Hz, 1H, PhCH(OH)C*H*), 4.16–4.12 (m, 1H, OCH_2CH=CH_2), 3.96–3.92 (m, 1H, OCH_2CH=CH_2), 3.88–3.86 (m, 1H, C*H*Oallyl), 3.51 (d, *J*=7.0 Hz, 1H, O*H*), 1.47 (s, 3H, C(CH_3)$_2$), 1.31 (s, 3H, C(CH_3)$_2$). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 141.5, 133.4, 128.6, 127.8, 126.2, 118.7, 105.4, 83.1, 82.5, 81.9, 72.4, 71.3, 26.9, 26.4.

4.1.5. 1,2-*O*-(1-Methylethyledene)-5-*O*-(4-methoxybenzyl)-3-*O*-allyl-5-*C*-phenyl-5-*O*-α-D-*xylo*-furanoses 18 and 19. To pentane-washed NaH (0.16 g, 6.5 mmol) in anhydrous DMF (5 ml) was added alcohol **16/17** (1 g, 3.2 mmol) at 0 °C and stirred for 15 min. 4-Methoxybenzylchloride (0.77 g, 4.9 mmol) and tetrabutylammonium iodide (20 mg) were added and the resultant reaction mixture was stirred for 18 h at rt. HCl (1 M, 10 ml) was added slowly and extracted twice with DCM (10 ml). The organic layers were collected dried and concentrated in vacuo. Flash column chromatography (10% EtOAc in hexane) afforded acetonide **18/19** (1.40 g, 3.13 mmol, 98% yield) as a colorless oil. HRMS (FAB) *m/z* 449.1938 (MNa^+, $C_{25}H_{30}O_6Na$, requires 449.1940).

(5*S*)-Isomer **18**: $[\alpha]_D^{22}$ +18.2 (*c* 1, $CHCl_3$); ν_{max} (liquid film) 3110, 1634, 1595, 1410, 1340, 1044 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.42–7.35 (m, 5H, Ph), 7.23 (d, *J*=9.0 Hz, 2H, *Ph* of PMB), 6.83 (d, *J*=8.0 Hz, 2H, *Ph* of PMB), 6.02 (d, *J*=4.0 Hz, 1H, OC*H*O), 5.76–5.68 (m, 1H, C*H*=CH_2), 5.22 (d, *J*=17.5 Hz, 1H, CH=CH_2), 5.14 (d, *J*=10.5 Hz, 1H, CH=CH_2), 4.68 (d, *J*=9.0 Hz, 1H, PhC*H*(OPMB)), 4.48–4.45 (m, 2H, C*H*OCMe_2, PhCH(OPMB)C*H*), 4.49 (d, *J*=11.5 Hz, 1H, OCH_2PhOMe), 4.32 (d, *J*=11.5 Hz, 1H, OCH_2PhOMe), 3.82 (dd, *J*=5.0, 12.0 Hz, 1H, O$CH_2$$CHCH_2$), 3.77 (s, 3H, O*Me*), 3.48 (dd, *J*=6.0, 12.5 Hz, 1H, O$CH_2$$CHCH_2$), 3.20 (d, *J*=3.5 Hz, 1H, C*H*Oallyl), 1.50 (s, 3H, CMe_2), 1.29 (s, 3H, CMe_2). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.1, 138.7, 133.9, 130.7, 129.5, 128.4, 117.6, 113.7, 111.7, 105.7, 84.1, 82.2, 81.6, 79.9, 70.9, 70.2, 55.4, 27.0, 26.4.

(5*R*)-Isomer **19**: $[\alpha]_D^{22}$ −45.0 (*c* 1, MeOH); ν_{max} (liquid film) 3120, 1640, 1602, 1420, 1344, 1045 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.46–7.31 (m, 5H, Ph), 7.18 (d, *J*=14.5 Hz, 2H, *Ph* of PMB), 6.84 (d, *J*=15.0 Hz, 2H, *Ph* of PMB), 5.80–5.86 (m, 1H, C*H*=CH_2), 5.84 (d, *J*=6.5 Hz, 1H, OC*H*O), 5.34–5.20 (m, 2H, CH=CH_2), 4.65 (d, *J*=15.0 Hz, 1H, PhC*H*(OPMB)), 4.55 (d, *J*=6.5 Hz, 1H, C*H*OCMe_2), 4.31 (d, *J*=18.5 Hz, 1H,

OCH_2Ph(OMe$_2$)), 4.27 (d, J=5.0 Hz, 1H, PhCH(OPMB)CH), 4.20 (d, J=17.5 Hz, 1H, OCH_2-Ph(OMe)), 4.16 (d, J=9.0 Hz, 1H, OCH_2CHCH$_2$), 4.10 (d, J=4.0 Hz, 1H, CHOallyl), 4.06 (dd, J=9.0, 21.0 Hz, 1H, OCH_2CHCH$_2$), 3.79 (s, 3H, OMe), 1.39 (s, 3H, CMe_2), 1.26 (s, 3H, CMe_2). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.4, 139.8, 134.6, 130.6, 129.7, 128.6, 128.2, 128.1, 117.6, 114.0, 111.7, 105.3, 83.1, 82.5, 81.8, 78.2, 71.8, 70.3, 55.5, 27.0, 26.5.

4.1.6. 3-*O*-Allyl-5,6-dideoxy-1-*O*-(4-methoxybenzyl)-1-*O*-phenyl-hex-5-enitol (6*S*)-20 and (6*R*)-20. To acetonide **18/19** (0.3 g, 0.7 mmol) dissolved in THF (3 ml) was added 4 M HCl (2 ml) and the resultant mixture was heated to 55 °C for 4 h. The reaction mixture was cooled, neutralized with satd aqueous $NaHCO_3$ solution (5 ml) and extracted twice with EtOAc (5 ml). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. Flash column chromatography (10–30% gradient elution) afforded diol (0.13 g, 52%) as anomeric mixture and acetonide **18/19** (0.10 g, 44%) was recovered. The diol (0.1 g, 0.2 mmol) was dissolved in anhydrous THF (0.5 ml) and added drop wise to methylenetriphenylphosphine ylide (prepared from methyltriphenylphosphonium bromide (0.46 g, 1.3 mmol) and *n*-BuLi (0.79 ml of 1.6 M in hexane, 1.2 mmol) at −5 °C) at −10 °C. The reaction mixture was stirred at −10 °C for 2 h and then at rt for 24 h, then quenched with cold satd aqueous NH_4Cl (2 ml), and extracted twice with EtOAc (5 ml). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. Flash column chromatography (10–20% EtOAc in hexane, gradient elution) afforded pure dialkene (6*S*)-**20**/(6*R*)-**20** (54.6 mg, 1.4 mmol, 55% yield) as a colorless liquid. HRMS (FAB) *m*/*z* 407.1826 (MNa^+, $C_{23}H_{28}O_5Na$, requires 407.1835).

Compound (6*S*)-**20**: $[\alpha]_D^{22}$ −88.0 (*c* 1, $CHCl_3$); ν_{max} (liquid film) 3450–3100 (br), 1638, 1610, 1450, 1342, 1145 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.42–7.36 (m, 5H), 7.23 (d, J=14.0 Hz, 2H), 6.90 (d, J=12.0 Hz, 2H), 5.90–5.83 (m, 2H), 5.40–5.18 (m, 4H), 4.43–4.37 (m, 2H), 4.32 (t, J=10.5 Hz, 1H), 4.20 (d, J=19.0 Hz, 1H), 4.19 (d, J=19.0 Hz, 1H), 4.07 (dd, J=9.0, 20.5 Hz, 1H), 3.83 (s, 3H), 3.82–3.76 (m, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.6, 139.3, 137.4, 134.9, 130.0, 128.8, 128.5, 128.1, 117.4, 117.3, 114.0, 81.4, 80.3, 75.2, 74.6, 74.5, 70.2, 55.5.

Compound (6*R*)-**20**: $[\alpha]_D^{22}$ +47.0 (*c* 2, $CHCl_3$); ν_{max} (liquid film) 3500–3100 (br), 1640, 1610, 1440, 1135, 1095 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.42–7.35 (m, 5H), 7.20 (d, J=8.5 Hz, 2H), 6.87 (d, J=8.0 Hz, 2H), 5.93–5.85 (m, 1H), 5.83–5.76 (m, 1H), 5.33–5.21 (m, 2H), 5.15 (d, J=10.0 Hz, 1H), 4.57 (d, J=7.5 Hz, 1H), 4.41 (d, J=11.5 Hz, 1H), 4.31 (m, 1H), 4.23 (d, J=11.0 Hz, 1H), 4.23–4.17 (m, 2H), 3.95–3.91 (m, 2H), 3.81 (s, 3H), 3.05–3.02 (m, 1H), 2.99 (br s, 1H), 2.67 (br s, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.6, 138.4, 138.0, 135.0, 130.0, 128.9, 128.7, 128.2, 117.0, 116.7, 114.1, 81.7, 80.8, 76.1, 73.8, 70.6, 55.5.

4.1.7. 2,4-*O*-Acetyl-3-*O*-allyl-5,6-dideoxy-1-*O*-(4-methoxybenzyl)-1-*O*-phenyl-hex-5-enitol 21 and its (6*R*) diastereomer. To a mixture of diol **20** (50 mg, 0.13 mmol) and DMAP (7 mg) in Et_3N (0.5 ml) at 0 °C was added acetic anhydride (53 mg, 0.52 mmol). The resultant mixture was stirred for 24 h at rt. Et_3N was evaporated and flash column chromatography of the resultant crude mixture gave diacetate (60 mg, 0.12 mmol, 98% yield) as a pale yellow oil. HRMS (FAB) *m*/*z* 491.2052 (MNa^+, $C_{27}H_{32}O_7Na$, requires 491.2045).

(6*S*)-Isomer **21**: $[\alpha]_D^{22}$ −15.5 (*c* 2, $CHCl_3$); ν_{max} (liquid film) 3180, 1742, 1610, 1550, 1320 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.39–7.28 (m, 5H), 7.20 (d, J=8.0 Hz, 2H), 6.87 (d, J=8.0 Hz, 2H), 5.93–5.87 (m, 1H), 5.85–5.78 (m, 1H), 5.35 (t, J=6.5 Hz, 1H), 5.24 (d, J=17.0 Hz, 1H), 5.20 (d, J=17.0 Hz, 1H), 5.18–5.16 (m, 3H), 4.57 (d, J=9.5 Hz, 1H), 4.37 (d, J=11.5 Hz, 1H), 4.24–4.18 (m, 2H), 4.05 (dd, J=4.5, 12.0 Hz, 1H), 3.98 (d, J=8.5 Hz, 1H), 3.80 (s, 3H), 2.07 (s, 3H), 1.70 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.0, 169.1, 159.1, 138.0, 135.0, 132.6, 130.0, 129.9, 128.6, 128.4, 118.4, 117.3, 114.0, 78.8, 74.7, 74.5, 73.9, 70.3, 55.5, 21.4, 20.8.

(6*R*)-Isomer **21**: $[\alpha]_D^{22}$ +32.5 (*c* 2, $CHCl_3$); ν_{max} (liquid film) 3210, 1740, 1605, 1520, 1142 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.39–7.30 (m, 5H), 7.19 (d, J=9.0 Hz, 2H), 6.87 (d, J=8.5 Hz, 2H), 5.95–5.80 (m, 2H), 5.35–5.30 (m, 2H), 5.24–5.19 (m, 2H), 5.14 (dd, J=4.0, 8.5 Hz, 1H), 4.60 (d, J=5.0 Hz, 1H), 4.43 (d, J=11.5 Hz, 1H), 4.16 (d, J=12 Hz, 2H), 4.12 (dd, J=6.0, 12.5 Hz, 1H), 3.93 (dd, J=6.0, 12.5 Hz, 1H), 3.82 (s, 3H), 3.47 (t, J=5.0 Hz, 1H), 2.08 (s, 3H), 2.00 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.2, 170.0, 159.4, 138.0, 134.7, 133.0, 130.4, 130.2, 129.5, 128.8, 128.6, 128.0, 118.1, 117.1, 114.0, 79.5, 79.0, 74.9, 74.0, 73.9, 70.4, 55.5, 21.3, 21.2.

4.1.8. General procedure for PMB cleavage: synthesis of 2-4-di-*O*-acetyl-3-*O*-allyl-5,6-dideoxy-1-*O*-phenyl-hex-5-enitol 22 and its (6*R*)-diastereomer. To a stirred solution of diacetate **21** (55 mg, 0.11 mmol) in wet DCM (0.2 ml) was added DDQ (35.0 mg, 0.15 mmol) and stirred for 2 h at rt. DCM (2 ml) and satd aqueous $NaHCO_3$ (1 ml) were added to the reaction mixture and extracted. The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. Flash column chromatography afforded alcohol (6*S*)-isomer **22**/(6*R*)-isomer **22** (34 mg, 0.096 mmol, 88% yield) as a colorless oil. HRMS (FAB) *m*/*z* 371.1486 (MNa^+, $C_{19}H_{24}O_6Na$, requires 371.1471).

(6*S*)-Isomer **22**: $[\alpha]_D^{22}$ −32.0 (*c* 2, $CHCl_3$); ν_{max} (liquid film) 3550–3100 (br), 1740, 1605, 1450, 1140 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41–7.30 (m, 5H), 6.03–5.93 (m, 1H), 5.82–5.73 (m, 1H), 5.42–5.29 (m, 2H), 5.27–5.22 (m, 2H), 5.17–5.12 (m, 1H), 4.97–4.95 (m, 1H), 4.32–4.28 (m, 1H), 4.19–4.11 (m, 1H), 3.81 (dd, J=2.5, 7.0 Hz, 1H), 2.10 (d, J=4.5 Hz, 1H), 2.06 (s, 3H), 1.92 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.9, 169.7, 140.5, 134.4, 132.5, 128.6, 128.3, 126.7, 118.7, 118.2, 78.5, 74.3, 74.1, 73.3, 21.3, 21.1.

(6*R*)-Isomer **22**: $[\alpha]_D^{22}$ +28.0 (*c* 2, $CHCl_3$); ν_{max} (liquid film) 3500–3100 (br), 1738, 1610, 1450, 1245 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.37–7.29 (m, 5H), 5.91–5.86 (m, 2H), 5.51–5.48 (m, 1H), 5.32–5.28 (m, 2H), 5.20 (dd, J=2.0, 11.0 Hz, 1H), 4.97 (d, J=5.5 Hz, 1H), 4.22–4.18 (m, 1H), 4.15–4.10 (m, 2H), 4.02–3.98 (m, 1H), 3.58 (d,

J=4.5 Hz, 1H), 2.06 (s, 3H), 1.99 (s, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.4, 170.5, 140.4, 134.4, 132.5, 128.8, 126.7, 119.6, 118.3, 117.7, 117.5, 79.1, 75.3, 73.8, 60.6, 21.3, 21.1.

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Montamine, a unique dimeric indole alkaloid, from the seeds of *Centaurea montana* (Asteraceae), and its in vitro cytotoxic activity against the CaCo2 colon cancer cells

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Abstract—Reversed-phase HPLC analysis of the methanol extract of the seeds of *Centaurea montana* afforded a flavanone, montanoside (**4**), six epoxylignans, berchemol (**7**), berchemol 4′-*O*-β-D-glucoside (**5**), pinoresinol (**10**), pinoresinol 4-*O*-β-D-glucoside (**8**), pinoresinol 4,4′-di-*O*-β-D-glucoside (**6**), pinoresinol 4-*O*-apiose-(1→2)-β-D-glucoside (**9**), two quinic acid derivatives, *trans*-3-*O*-*p*-coumaroylquinic acid (**1**), *cis*-3-*O*-*p*-coumaroylquinic acid (**2**), and eight indole alkaloids, tryptamine (**3**), *N*-(4-hydroxycinnamoyl)-5-hydroxytryptamine (**11**), *cis*-*N*-(4-hydroxycinnamoyl)-5-hydroxytryptamine (**12**), centcyamine (**16**), *cis*-centcyamine (**17**), moschamine (**13**), *cis*-moschamine (**14**) and a dimeric indole alkaloid, montamine (**15**). While the structures of two new compounds, montanoside (**4**) and montamine (**15**), were established unequivocally by UV, IR, MS and a series of 1D and 2D NMR analyses, all known compounds were identified by comparison of their spectroscopic data with literature data. The antioxidant properties of these compounds were assessed by the DPPH assay, and their toxicity towards brine shrimps and cytotoxicity against CaCo-2 colon cancer cells were evaluated by the brine shrimp lethality and the MTT cytotoxicity assays, respectively. The novel dimer, montamine (**15**), showed significant in vitro anticolon cancer activity (IC_{50}=43.9 μM) while that of the monomer, moschamine (**13**), was of a moderate level (IC_{50}=81.0 μM).

1. Introduction

Centaurea montana (family: Asteraceae alt. Compositae), an erect plant with large, reddish, blue centre flower heads, is native to Australia, Belgium and Italy, and also cultivated in many countries of the world.[1] While a number of flavonoids,[2–5] acetylenes and a lignan, arctigenin,[5] have previously been reported from the aerial parts of *C. montana*,[2–5] to our knowledge, no report on the isolation of any plant secondary metabolites from the seeds or any pharmacological properties of this plant is available to date. Many species of the genus *Centaurea* have long been used in traditional medicine to cure various ailments, e.g., diabetes, diarrhoea, rheumatism, malaria, hypertension, etc., and a variety of secondary metabolites have been reported from different species of this genus.[6] As a part of our ongoing phytochemical investigation on the species of the genus *Centaurea*,[6–11] we now report on the isolation, structure elucidation and bioactivity of a series of compounds, including a new flavanone named, montanoside (**4**), six epoxylignans, berchemol (**7**), berchemol 4′-*O*-β-D-glucoside (**5**), pinoresinol (**10**), pinoresinol 4-*O*-β-D-glucoside (**8**), pinoresinol 4,4′-di-*O*-β-D-glucoside (**6**), pinoresinol 4-*O*-apiose-(1→2)-β-D-glucoside (**9**), two quinic acid derivatives, *trans*-3-*O*-*p*-coumaroylquinic acid (**1**), *cis*-3-*O*-*p*-coumaroylquinic acid (**2**), and eight indole alkaloids, tryptamine (**3**), *N*-(4-hydroxycinnamoyl)-5-hydroxytryptamine (**11**), *cis*-*N*-(4-hydroxycinnamoyl)-5-hydroxytryptamine (**12**), centcyamine (**16**), *cis*-centcyamine (**17**), moschamine (**13**), *cis*-moschamine (**14**) and a novel dimer montamine (**15**) from the seeds of *C. montana*.

2. Results and discussion

Reversed-phase preparative HPLC analysis of the methanol extract of the seeds of *C. montana* afforded a new flavanone,

Keywords: *Centaurea montana*; Asteraceae; DPPH assay; Cytotoxicity; MTT assay; Colon cancer; Brine shrimp lethality assay; Lignan; Flavanone; Indole alkaloids; Dimer.
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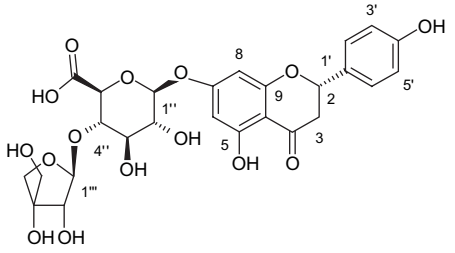
montanoside (**4**), six epoxylignans, berchemol (**7**), berchemol 4′-*O*-β-D-glucoside (**5**), pinoresinol (**10**), pinoresinol 4-*O*-β-D-glucoside (**8**), pinoresinol 4,4′-di-*O*-β-D-glucoside (**6**), pinoresinol 4-*O*-apiose-(1→2)-β-D-glucoside (**9**), two quinic acid derivatives, *trans*-3-*O*-*p*-coumaroylquinic acid (**1**), *cis*-3-*O*-*p*-coumaroylquinic acid (**2**), and eight indole alkaloids, tryptamine (**3**), *N*-(4-hydroxycinnamoyl)-5-hydroxytryptamine (**11**), *cis*-*N*-(4-hydroxycinnamoyl)-5-hydroxytryptamine (**12**), centcyamine (**16**), *cis*-centcyamine (**17**), moschamine (**13**), *cis*-moschamine (**14**), and a novel dimer, montamine (**15**). The spectroscopic data of the known lignans (**5**–**10**), quinic acid derivatives (**1** and **2**) and alkaloids (**3**, **11**–**14**, **16** and **17**) were in good agreement with respective literature data.[12–22] The structures of the novel compounds, montanoside (**4**) and montamine (**15**), were established unequivocally by UV, MS and a series of 1D and 2D NMR analyses.

The ESIMS spectrum of **4** showed the *pseudo*molecular ion peak at *m*/*z* 603 $[M+Na]^+$ suggesting Mr=580. The HRCIMS gave the *pseudo*molecular ion at *m*/*z* 598.1769 $[M+NH_4]^+$ (calculated 598.1771 for $C_{26}H_{32}NO_{15}$). The UV absorptions at 213, 252 and 343 nm and the IR absorption band (1679 cm^{-1}) for a conjugated carbonyl were indicative of a flavanone skeleton.[23,24] The ^1H and ^{13}C NMR spectra (Table 1) of **4** showed the presence of one methylene [δ_H 3.12 (dd, J=13.0, 17.0 Hz) and 2.73 (dd, J=2.0, 17.0 Hz); δ_C 42.4], one oxymethine [δ_H 5.36 (dd, J=2.0, 13.0 Hz); δ_C 79.0] and two methines [δ_H 6.15 (d, J=2.0 Hz); δ_C 95.0 and 6.11 (d, J=2.0 Hz); δ_C 96.5] of the ring A, and four methines in a group of two chemically equivalent protons [δ_H 7.28 (d, J=8.4 Hz) and 6.77 (d, J=8.4 Hz)] of the ring B. These data also supported **1** being a flavanone.[25] The presence of a 1,4-disubstituted benzene ring system was evident from these signals, which was further confirmed by cross peaks between H-2′/6′ and H-3′/5′ in the ^1H–^1H COSY spectrum. The ^1H–^{13}C HMBC correlations between H-6 (δ_H 6.11) and C-8 (δ_C 95.0) and C-10 (δ_C 104.0), and H-8 (δ_H 6.15) and C-6 (δ_C 96.5), C-7 (δ_C 164.0) and C-10 (δ_C 104.0) further confirmed the flavanone skeleton. The ^1H and ^{13}C NMR spectra (Table 1) also revealed signals representing two sugar moieties. A doublet at δ_H 5.05 (d, J=7.2 Hz) and additional signals (δ_H 3.47–3.72) in the ^1H NMR implied that one of the sugars was a β-glucose derivative and the carbon signal at δ 173.0 in the ^{13}C NMR confirmed that it was a β-D-glucuronic acid moiety.[26] Five more ^{13}C NMR signals at δ 109.0, 79.9, 77.5, 74.0 and 65.0 could be assigned to the carbons of another sugar, apiose.[27] A ^3J ^1H–^{13}C long-range coupling between the anomeric proton of apiose δ_H 5.38 (H-1″) and δ_C 75.0 (C-4″) in the HMBC spectrum confirmed that the apiose was connected to glucuronic acid at C-4″. The presence of apiose/glucuronic acid was also confirmed by the loss of 309 mass units from the molecular mass of the compound in the ESIMS spectrum. The ^3J ^1H–^{13}C long-range HMBC correlation between δ_H 5.05 (H-1″) and δ_C 164.0 (C-7) confirmed that the glucuronic acid moiety was connected to C-7 of the flavanone skeleton. The specific optical rotation for **4** was found to be −48°. Literature review showed that all natural (−)-flavanones possess a *S* configuration at C-2.[28,29] Comparing with other flavanones of established absolute configuration measured by using circular dichroism spectroscopy, **4** was considered having *S* configuration at C-2 due to its levorotatory nature.[28,29] Thus, **4** was determined as a flavanone derivative, and named montanoside. To the best of our knowledge this is a new natural product.

Table 1. ^1H NMR (chemical shift, multiplicity, coupling constant J in hertz), ^{13}C NMR data and long-range HMBC correlations for montanoside (**4**)

Carbon number	Chemical shift δ in ppm		HMBC ($^1H \rightarrow {}^{13}C$)	
	^1H[a]	^{13}C[a]	^2J	^3J
2	5.36, dd, 2.0, 13.0	79.0	—	—
3	3.12, dd, 13.0, 17.0 2.73, dd, 2.0, 17.0	42.5	C-4	C-1′
4	—	196.0	—	—
5		162.0	—	—
6	6.11, d, 2.0	96.5	—	C-8, C-10
7		164.0	—	—
8	6.15, d, 2.0	95.0	C-7	C-6, C-10
9	—	162.0	—	—
10	—	104.0	—	—
1′	—	130.0	—	—
2′	7.28, d, 8.4	129.0	C-3′	C-2, C-4′, C-6′
3′	6.77, d, 8.4	115.1	C-4′	C-1′, C-5′
4′	—	158.0	—	—
5′	6.77, d, 8.4	115.1	C-4′	C-1′, C-3′
6′	7.28, d, 8.4	129.0	C-5′	C-2, C-2′, C-4′
1″	5.05, d, 7.2	99.0	—	C-7
2″	3.47, m	73.0	C-3″	—
3″	3.61, m	77.8	—	—
4″	3.72, t, 9.2	75.0	—	—
5″	3.65, m	78.0	—	C-1″
6″		173.0	—	—
1‴	5.38, d, 1.0	109.0	C-2‴	C-4″, C-3‴
2‴	3.89, m	77.5		C-4‴, C-5‴
3‴	—	79.9	—	—
4‴	3.91, m	74.0	—	C-5‴
5‴	3.47, m	65.0	C-3‴	C-4‴

[a] ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) in CD_3OD.



4

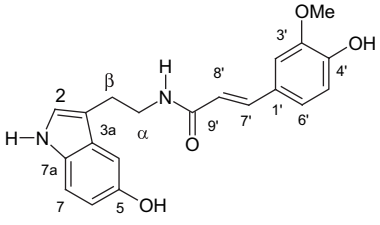
The UV and IR spectral data of **15** indicated the presence of a serotonin moiety conjugated with a cinnamic acid derivative substructure, exactly similar to that of moschamine (**13**).[6] The ^{13}C NMR spectrum of **15** (Table 2) displayed 20 carbons. The DEPT-135 indicated the presence of two methylenes (δ_C 41.0, 25.0), nine methines (δ_C 141.0, 121.0, 126.0, 117.5, 115.0, 111.5, 111.2, 111.1, 110.0), seven quarternary (δ_C 149.0, 148.0, 146.0, 133.0, 129.5, 129.0, 111.0), one carbonyl carbon (δ_C 172.0) and a methoxy group (δ 55.5). These carbon signals and the ^1H NMR data (Table 2) also supported the fact that **15** was composed of a serotonin derived substructure and a feruloyl moiety like the known alkaloid moschamine (**13**), and was further confirmed by the ^1H–^1H COSY and ^1H–^{13}C HMBC experiments. The ^1H–^1H COSY spectrum revealed four different spin systems: H-7↔H-6↔H-4, $H_2\alpha \leftrightarrow H_2\beta$, H-7′↔H-8′ and H–H-6′↔H-5′ and the ^1H–^{13}C HMBC spectrum showed key correlations between H-2 to C-3, C-3a and

Table 2. ^{1}H NMR (chemical shift, multiplicity, coupling constant J in hertz), ^{13}C NMR data and long-range HMBC correlations for montamine (**15**)

Carbon number	Chemical shift δ in ppm		HMBC (^{1}H→^{13}C)	
	^{1}H[a]	^{13}C[a]	^2J	^3J
2	6.93, s	126.0	C-3	C-3a, C-7a
3	—	111.0	—	—
3a	—	129.5	—	—
4	6.83, d, 2	111.2	C-5	C-7a
5	—	148.0	—	—
6	6.82, dd, 2, 8	111.1	C-5	C-7a
7	7.26, d, 8	111.5	C-7a	C-3a, C-5
7a	—	133.0	—	—
β-CH_2	2.27, m 2.19, m	25.0	C-2, C-α-CH_2	C-3
α-CH_2	2.86, m 2.77, m	41.0	C-β-CH_2	C-3, C-9′
1′		129.0	—	—
2′	6.99, d, 2	110.0	—	C-6, C-4′, C-7′
3′	—	146.0	—	—
4′	—	149.0	—	—
5′	6.70, d, 8.4	115.0	—	C-1′, C-3′
6′	6.91, dd, 2, 8.4	121.0	—	C-2′, C-4′, C-7′
7′	7.27, d, 15.6	141.0	—	C-2′, C-6′, C-9′
8′	6.31, d, 15.6	117.5	C-9′	C-1′
9′	—	172.0	—	—
OCH_3 (3′)	3.78, s	55.5	—	C-3′

[a] ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) in CD_3OD.

C-7a, H-4 and C-5 and C-7a, H-6 and C-5 and C-7a, H-7 and C-3a and C-5, H-8′ and C-1′ and C-9′. All spectroscopic data suggested that **15** possessed a moschamine (**13**) type substructure. However, the α-CH_2 and β-CH_2 protons of **15** gave rise to significantly different resonances in the ^{1}H NMR spectrum compared to those of **13** (Table 3). For moschamine (**13**), the signals for α-CH_2 and β-CH_2 protons were observed at δ_H 3.53 (t, J=7.2 Hz) and 2.88 (t, J=7.2 Hz), whereas they were found at more upfield region δ_H 2.86, m and 2.27, m, respectively, for **15**. The ESIMS spectrum showed that **15** had a molecular mass of 702 instead of 352. The HRESIMS gave $[M+Na]^+$ at 725.2587 (required 725.2587), counted for the molecular formula $C_{40}H_{38}N_4O_8$. The HRESIMS and ^{1}H NMR spectra confirmed that **15** was a symmetrical dimer of moschamine (**13**), formed through an N–N linkage. The N–N linkage could be either of these three: (a) between two ring N of the indole skeleton; (b) one ring N of the indole skeleton and the other from the serotonin side chain N; or (c) two N from the serotonin side chain. As only one set of ^{1}H and ^{13}C NMR data were observed for **15**, option (b) could be ruled out because it would generate an asymmetric dimer. Options (a) and (c) could generate symmetrical dimers, but only option (a) could explain the upfield shift of the methylene resonances in the ^{1}H NMR spectrum. The upfield shift was due to the fact that in **15**, the formation of a N–N dimer brought these methylenes into close proximity of the aromatic electron clouds, and the rotation about N–N bond was somewhat restricted. A trivial name, montamine, has been proposed for this dimer. Although the N–N dimer formation between two tryptamine/serotonin derivatives is not common, there is precedence of such dimer formation in the case of the indole alkaloid, schischkiniin, which was isolated from *Centaurea schischkinii*.[7] Montamine (**15**), isolated from *C. montana*, is a new natural product.



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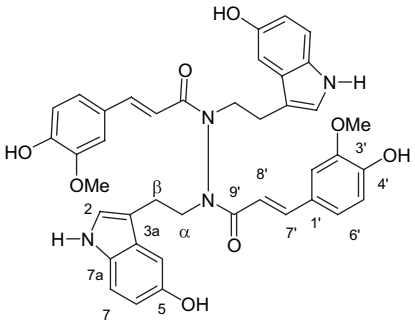

15

The DPPH assay[30] is an easy and straightforward method for determining the free radical scavenging property of a compound. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant, which can donate an electron to DPPH, the purple colour, which is typical of the free DPPH radical, decays and the change in absorbance at 517 nm is monitored spectrophotometrically. All the compounds (**1**–**15**) showed low to moderate levels of free radical scavenging activity (IC_{50}=16.0×10^{-2}–2.02×10^{-3} mg/mL) (Table 4). Among eight alkaloids (**3** and **11**–**17**), moschamine (**13**), montamine (**15**) and centcyamine (**16**) showed the most prominent antioxidant property, which could be attributed to the presence of the highest number of phenolic hydroxyl groups (four –OH) in the molecule (Table 4).

The brine shrimp lethality assay, which has been proven to be an effective and rapid assay method to screen compounds for potential general toxicity and cytotoxic activity,[31,32] was used to determine the general toxicity of compounds **1**–**17**. The LD_{50} values of quinic acid derivatives and epoxylignans were between 6.5×10^{-2} and 10.3×10^{-2} mg/mL (Table 4),

Table 3. Major differences in NMR data of **13** and **15**

Molecule	Group	^{1}H	^{13}C	HMBC (^{1}H→^{13}C)	HRESIMS $[M+Na]^+$
Moschamine (**13**)[6]	α-CH_2	3.53, t, 7.2	40.3	C-2, C-9′	375.1321
	β-CH_2	2.88, t, 7.2	25.2	C-2	
Montamine (**15**)	α-CH_2	2.86, m 2.77, m	41.0	C-3, C-9′, C-β-CH_2	725.25869
	β-CH_2	2.27, m 2.19, m	25.0	C-2, C-3, C-α-CH_2	

Table 4. Antioxidant (DPPH assay) and cytotoxic (MTT assay) activities, and brine shrimp toxicity (Brine Shrimp Lethality assay) of compounds **1–17**

Compounds	Antioxidant activity IC_{50} (mg/mL)	Cytotoxicity IC_{50} (μM)	Brine shrimp toxicity LD_{50} (mg/mL)
1	7.6×10^{-2}	146.4	7.8×10^{-2}
2	10.0×10^{-2}	325.0	10.3×10^{-2}
3	6.4×10^{-2}	198.0	8.3×10^{-2}
4	5.0×10^{-2}	153.4	7.2×10^{-3}
5	2.1×10^{-2}	1260.0	10.3×10^{-2}
6	$>5\times10^{-2}$	843.2	7.2×10^{-1}
7	3.2×10^{-2}	833.0	3.1×10^{-2}
8	3.6×10^{-2}	705.0	6.8×10^{-2}
9	3.0×10^{-2}	1130.0	8.3×10^{-2}
10	1.4×10^{-2}	233.0	6.5×10^{-2}
11	1.6×10^{-2}	125.0	7.5×10^{-2}
12	4.8×10^{-2}	411.0	8.2×10^{-2}
13	2.2×10^{-3}	81.0	20×10^{-3}
14	4.5×10^{-3}	213.0	19.2×10^{-3}
15	3.6×10^{-2}	43.9	3.5×10^{-3}
16	2.8×10^{-3}	82.2	15×10^{-3}
17	3.2×10^{-3}	213.0	13.8×10^{-3}
Methanol extract of *Centaurea montana*	32.7×10^{-2}	56.4	62.5×10^{-2}
Quercetin	2.88×10^{-5}	—	—
Podophyllotoxin	—	—	2.79×10^{-3}

and montanoside (**4**), moschamine (**13**) and montamine (**15**) were found to be the most toxic of all the test compounds towards brine shrimp ($LD_{50}=7.2\times10^{-3}$, 20×10^{-3}, 3.5×10^{-3} mg/mL, respectively). The toxicity of these compounds (**4**, **13** and **15**) was comparable to that of the positive control podophyllotoxin ($LD_{50}=2.79\times10^{-3}$ mg/mL), a well known cytotoxic lignan.

The in vitro cytotoxicities of all the compounds isolated and characterised in this work were determined by the MTT assay against colon cancer cell line, CaCo-2 (Table 4).[33] The dimeric indole alkaloid, montamine (**15**), exhibited significant in vitro anticancer activity with an IC_{50} value of 43.9 μM. It is interesting to note that the dimerisation of moschamine (**13**) leading to the formation of montamine (**15**) increased the cytotoxicity two-fold. The unique structural features of **15** can certainly be exploited as a template for generating compounds with enhanced anticancer activity. The new flavanone, montanoside (**4**) displayed low levels of cytotoxicity with an IC_{50} value of 153.4 μM. However, all the isolated epoxylignans demonstrated low levels of activity against colon cancer cells in vitro. Among the epoxylignans, the presence of a sugar moiety in the molecule tends to reduce significantly the anticancer activity of these compounds. It can be assumed that the presence of a sugar group may prevent the effective transport of these compounds through the cell membrane, hence their reduced biological activities. It is noteworthy that the degree of brine shrimp toxicity displayed by the test compounds in the brine shrimp lethality assay corresponded well with the cytotoxic potentials of these compounds observed in the MTT assay using colon cancer cell line.

3. Conclusion

Compounds (**1–17**) isolated and identified from the seeds of *C. montana* showed various levels of activities in the DPPH, the brine shrimps lethality and the MTT cytotoxicity assays. However, the most significant finding is the discovery of the novel dimeric indole alkaloid, montamine (**15**), which exhibited significant in vitro anticancer activity against the CaCo2 cell line with an IC_{50} value of 43.9 μM.

4. Experimental

4.1. General procedures

UV spectra were obtained in MeOH using a Hewlett–Packard 8453 UV–vis spectrometer. MS analyses were performed on a Quattro II triple quadrupole instrument. NMR spectra were recorded in CD_3OD on a Varian Unity INOVA 400 MHz NMR spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C) using the residual solvent peaks as internal standard. HPLC separation was performed using a Dionex prep-HPLC system coupled with Gynkotek GINA50 autosampler and Dionex UVD340S Photo-Diode-Array detector and/or A JASCO PU-1580 Intelligent HPLC Pump, coupled with JASCO DG-1580-53 Degasser and JASCO LG-1580-02 Ternary Gradient Unit. A Luna C_{18} preparative (10 μm, 250 mm×21.2 mm) and/or a Luna C_{18} semi-preparative HPLC column (5 μm, 250 mm×10 mm) were used. Sep-Pak Vac 35 cc (10 g) C_{18} cartridge (Waters) was used for pre-HPLC fractions. HMBC spectra were optimised for a long-range J_{H-C} of 9 Hz and the NOESY experiment was carried out with a mixing time of 0.8 s.

4.2. Plant material

The seeds of *C. montana* were collected from B & T, World Seeds Sarl, Paguignan, 34210 Olonzac, France. A voucher specimen PHSH80006 has been retained in the herbarium of the Plant and Soil Science Department, University of Aberdeen, Scotland (ABD).

4.3. Extraction and isolation of compounds

Ground seeds of *C. montana* (100 g) were Soxhlet-extracted, successively, with *n*-hexane, dichloromethane and methanol (MeOH) (1 L each). The MeOH extract was fractionated by solid phase extraction method using a Sep-Pak C_{18} (10 g) cartridge eluting with a step gradient: 30, 40, 60, 80 and 100% MeOH in water (200 mL each). Preparative-HPLC (eluted with a linear gradient-water/MeCN=90:10–60:40 over 50 min followed by 40% MeCN for 10 min, 20 mL/min) of the Sep-Pak fraction, which was eluted with 30% MeOH, yielded seven fractions: **F1** (30.4 mg, t_R=6.1 min), **F2** (78.2 mg, t_R=11.2 min), **F3** (50.9 mg, t_R=12.2 min), **F4** (60.0 mg, t_R=18.2 min), **F5** (925.0 mg, t_R=19.0 min), **F6** (45.4 mg, t_R=26.5 min) and **F7** (68.2 mg, t_R=27.3 min). Following the same HPLC procedure, 40% Sep-Pak fraction of the MeOH extract yielded compound **10** (14.1 mg, t_R=31.5 min) in addition to previous seven fractions. Compounds **1–4** (7.0 mg, t_R=9.0 min; 4.5 mg, t_R=10.0 min; 3.0 mg, t_R=16.0 min and 2.0 mg; t_R=23.0 min, respectively), **5**,**6** (12.3 mg, t_R=42.0 min and 9.8 mg, t_R=70.0 min, respectively), **9** (19.9 mg, t_R=48.0 min) and **11–14** (3.5 mg, t_R=40.5 min; 4.5 mg, t_R=49.0 min; 6.0 mg, t_R=56.0 min and 22.5 mg, t_R=67.0 min, respectively) were further purified by semi-prep HPLC (isocratic elution

with MeCN in water, 2.0 mL/min), respectively, from fractions **F1** (10% MeCN in water), **F2** (12% MeCN in water), **F4** (17% MeCN in water) and **F6** (20% MeCN in water). From fraction **F5**, compounds **7** (25.0 mg, t_R=74.0 min) and **8** (20.0 mg, t_R=84.0 min) were obtained by prep-HPLC (isocratic elution with 15% MeCN in water, 20 mL/min). Similar prep-HPLC purification of the 60% Sep-Pak fraction produced two fractions **F8** (32.0 mg, t_R=17.5 min) and **F9** (33.0 mg, t_R=19.8 min). **F8** was further purified by semi-prep HPLC (isocratic elution with 25% MeCN in water, 2.0 mL/min) to obtain **15** (4.0 mg, t_R=92.0 min). However, **16** (5.0 mg, t_R=31.0 min) and **17** (4.0 mg, t_R=34.0 min) were obtained from fraction **F9** by prep-HPLC (isocratic elution with 30% MeCN in water, 20 mL/min).

4.3.1. Montanoside (4). Yellow amorphous (yield 0.002%); 5.0 mg; $[\alpha]_D^{23}$ −48 (*c* 0.021, MeOH); UV λ_{max} (MeOH): 213, 252, 343; IR ν_{max} (neat): 3459, 1679, 1246 cm^{-1}; ESIMS *m/z* 603 $[M+Na]^+$, 307, $[glucose+apiose+2H]^+$, 271 $[M-glucose-apiose]^+$, 104, 60; HRCIMS *m/z* 598.1769 $[M+NH_4]^+$ (calculated 598.1771 for $C_{26}H_{32}NO_{15}$); ^1H NMR (400 MHz, CD_3OD): see Table 1; ^{13}C NMR (100 MHz, CD_3OD): see Table 1.

4.3.2. Montamine (15). Gum (yield 0.004%); UV λ_{max} (MeOH): 213, 271, 274 nm; IR ν_{max} (neat): 3434, 3380, 2362, 1652, 1590, 1516, 1460, 1366, 1270, 1201, 1120, 1032 cm^{-1}; HRESIMS: 725.2587 $[M+Na]^+$ (calculated 725.2587 for $C_{40}H_{38}N_4O_8Na$); ^1H NMR: see Table 2; ^{13}C NMR: see Table 2.

4.4. Free radical scavenging activity: DPPH assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH), molecular formula $C_{18}H_{12}N_5O_6$, was obtained from Fluka Chemie AG, Bucks. Quercetin was obtained from Avocado Research Chemicals Ltd, Shore road, Heysham, Lancs. The method used by Takao et al.[30] was adopted with appropriate modifications. DPPH (4 mg) was dissolved in MeOH (50 mL) to obtain a concentration of 80 μg/mL.

4.4.1. Qualitative assay. Test compounds (**1–17**) were applied on a TLC plate and sprayed with DPPH solution using an atomiser. The plate was allowed to develop for 30 min. The colour change (purple on white) was noted.

4.4.2. Quantitative assay. Test compounds (**1–17**) were dissolved in MeOH to obtain a concentration of 0.5 mg/mL each. Dilutions were made to obtain concentrations of 5×10^{-2}, 5×10^{-3}, 5×10^{-4}, 5×10^{-5}, 5×10^{-6}, 5×10^{-7}, 5×10^{-8}, 5×10^{-9}, 5×10^{-10} mg/mL. Diluted solutions (1.00 mL each) were mixed with DPPH (1.00 mL) and allowed to stand for 30 min for any reaction to occur. The UV absorbance was recorded at 517 nm. The experiment was performed in triplicate and the average absorption was noted for each concentration. The same procedure was followed for the positive control, quercetin, a well known natural antioxidant.

4.5. Brine shrimp lethality assay

Shrimp eggs were purchased from The Pet Shop, Kittybrewster Shopping Complex, Aberdeen, UK. The bioassay was conducted following the procedure described by Meyer et al.[31] The eggs were hatched in a conical flask containing 300 mL artificial seawater. The flasks were well aerated with the aid of an air pump and kept in a water bath at 29–30 °C. A bright light source was left on and the nauplii hatched within 48 h. The compounds **1–17** were dissolved in 20% aq DMSO to obtain a concentration of 1 mg/mL. These were serially diluted two times and seven different concentrations were obtained. A solution of each concentration (1 mL) was transferred into clean sterile universal vials with pipette, and aerated seawater (9 mL) was added. About 10 nauplii were transferred into each vial with pipette. A check count was performed and the number alive after 24 h was noted. LD_{50} values were determined using the Probit analysis method.[32]

4.6. MTT cytotoxicity assay

CaCo2 cells were maintained in Earle's minimum essential medium (Sigma), supplemented with 10% (v/v) foetal calf serum (Labtech Int.), 2 mM L-glutamine (Sigma), 1% (v/v) non-essential amino acids (Sigma), 100 IU/mL penicillin and 100 μg/mL streptomycin (Sigma). Exponentially growing cells were plated at 2×10^4 cells cm^{-2} into 96-well plates and incubated for 72 h before the addition of drugs. Stock solution of compounds was initially in DMSO or H_2O and further diluted with fresh complete medium.

The growth-inhibitory effects of the compounds (**1–17**) were measured using standard tetrazolium MTT assay.[33] After 72 h of incubation at 37 °C, the medium was removed and 100 μL of MTT reagent (1 mg/mL) in serum free medium was added to each well. The plates were incubated at 37 °C for 4 h. At the end of the incubation period, the medium was removed and pure DMSO (200 μL) was added to each well. The metabolised MTT product dissolved in DMSO was quantified by reading the absorbance at 560 nm on a micro plate reader (Dynex Technologies, USA). The IC_{50} values were calculated from the equation of the logarithmic line determined by fitting the best line (Microsoft Excel) to the curve formed from the data. The IC_{50} value was obtained from the equation y=50 (50% value).

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References and notes

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Design and synthesis of fluconazole/bile acid conjugate using click reaction

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Abstract—Novel fluconazole/bile acid conjugates were designed and their regioselective synthesis was achieved in very high yield via Cu(I) catalyzed intermolecular 1,3-dipolar cycloaddition. These new molecules showed good antifungal activity against *Candida* species.

1. Introduction

The incidence of life threatening fungal infections has tremendously increased in last two decades due to greater use of immunosuppressive drugs, prolonged use of broad-spectrum antibiotics, wide-spread use of indwelling catheters, and also in cancer and AIDS patients.[1] The presently marketed antifungal drugs are either highly toxic (amphotericin-B) or becoming ineffective due to appearance of resistant strains (flucytosine and azoles).[2] Azole antifungals are strong inhibitors of lanosterol 14α-demethylase, which is major component of fungal cell membrane.[3] Fluconazole (Fig. 1) is an orally effective, potent, and safe triazole based antifungal drug, with favorable pharmacokinetic characteristics and low toxicity.[4] Due to the emergence of new fungal pathogens, resistant to fluconazole, great efforts have been made to modify the chemical structure of fluconazole, in order to broaden its antifungal activity and increase its potency.[5]

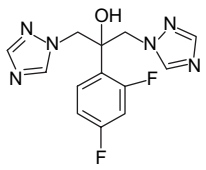
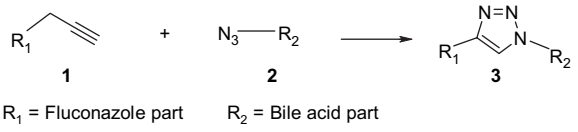


Figure 1. Fluconazole.

In recent years bile acid structures have become increasingly important in a number of fields, such as pharmacology, biomimetic, and supramolecular chemistry.[6] Bile acid transporters have been shown to accept and carry a variety of analogues that are derivatized at different positions of bile acids.[7] They have been used as absorption enhancers and as new cholesterol lowering agents.[7a] A common feature of bile acid derived antimicrobials is its potential to exhibit facially amphiphilic nature, due to polar hydroxyl groups on one face and nonpolar hydrophobic methyl groups on the other face.[8] Polyene macrolide amphotericin-B, peptide antimicrobial agent polymixin B, and squalamine in the cyclic form show such amphiphilicity and function as ionophores.[9]

Bioconjugation has recently emerged as a fast growing technology that affects almost every discipline of life science. It aims at the ligation of two or more molecules to form new complexes with the combined properties of its individual components.[10] In continuation of our work on bile acids,[11] we designed new bioconjugates **3** (Scheme 1) of bile acids



Scheme 1.

having amphiphilic nature as amphotericin-B and pharmacophore of fluconazole, linked together with 1,2,3-triazole, which may be viewed as an isoster of one of the 1,2,4-triazole component of fluconazole. 1,2,3-Triazole moieties are attractive connecting units, since they are stable to metabolic degradation and capable of hydrogen bonding, which can be favorable in binding of biomolecular targets and for solubility.[12] 1,2,3-Triazole moiety does not occur in nature, although the synthetic molecules containing 1,2,3-triazole unit shows diverse biological activities including antibacterial, herbicidal, fungicidal, antiallergic, and anti-HIV.[13]

Keywords: Bile acid; Fluconazole/bile acid conjugate; Click chemistry; Antifungal agent; 1,2,3-Triazole.
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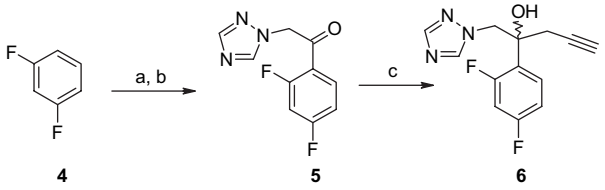
1,3-Dipolar cycloaddition of terminal acetylene and organic azides has been a method of choice for the synthesis of 1,2,3-triazoles.[14]

2. Results and discussion

In our approach to synthesize these new molecules, we considered performing Huisgen (click) reaction to connect fluconazole part containing terminal alkyne **1** and bile acid containing terminal azide **2**, in the presence of Cu(I) catalyst to form fluconazole/bile acid conjugate **3** (Scheme 1). Accordingly, we synthesized 2-(2,4-difluorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)pent-4-yn-2-ol **6** by propargylation of the corresponding ketone **5**[5b] by using propargyl bromide and zinc dust to obtain racemic compound **6** (Scheme 2).

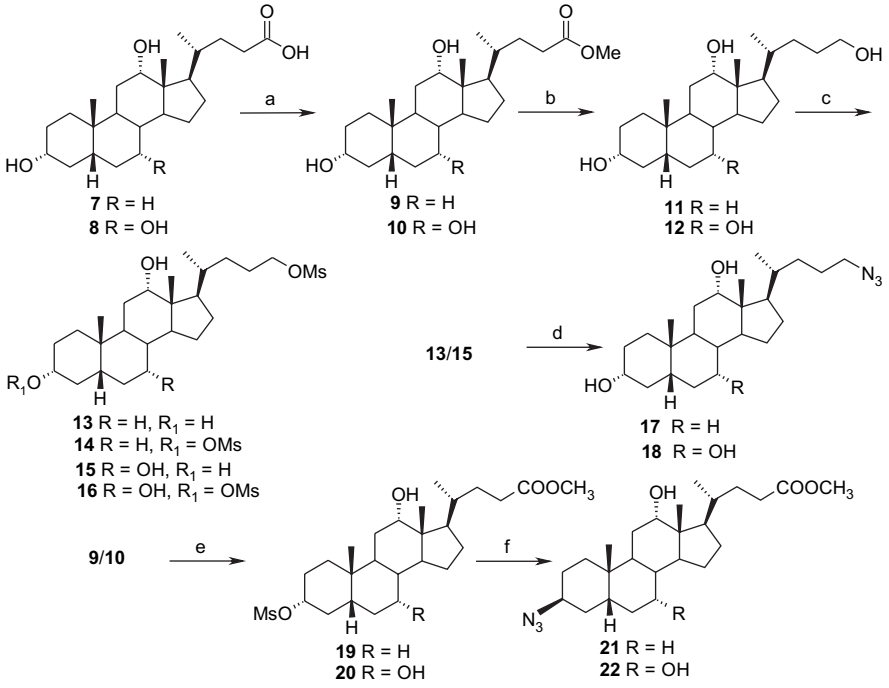


Scheme 2. Reagents and conditions: (a) $AlCl_3$, 1,2-dichloroethane, chloroacetyl chloride, 25 °C, 7 h; (b) 1,2,4-triazole, $NaHCO_3$, toluene, reflux, 4 h (overall 55% in a+b step); (c) Zn, propargyl bromide, DMF/THF, 25 °C, 5 h, 95%.

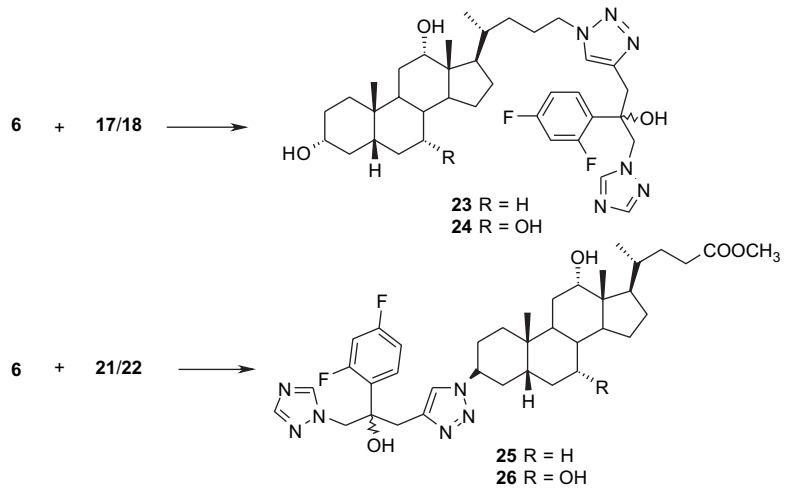
The compound **6** constitutes an alkyne component. In the proton NMR spectrum of compound **6**, the acetylenic proton was identified as triplet at δ 2.06 ppm and doublet of doublet at δ 2.87 ppm obtained due to β methylene. In the ^{13}C NMR spectrum it showed intricacies of various ^{19}F–^{13}C coupling as in fluconazole molecule.[15] The presence of acetylenic group was also evident from IR spectrum wherein the absorption due to acetylenic group was observed at 3307 cm^{-1}.

C-24-Azido bile acid derivatives **17** and **18** were synthesized from the corresponding mesyl compounds **13**[11a] and **15** (Scheme 3). IR of these compounds showed absorption due to azido group at 2100 cm^{-1}. In proton NMR spectrum, resonance corresponding to C-24-methylene group was observed at δ 3.24 ppm. Similarly, C-3-azido bile acid derivatives **21** and **22** were synthesized according to the literature procedures[16] with small modifications. The presence of azido group of the compounds **21** and **22** was also confirmed by IR spectrum. All the four compounds are well characterized by ^1H NMR, ^{13}C NMR, mass, and elemental analyses. The cycloaddition of **6** to azido compound **17** was attempted under previously reported conditions[11a] with copper sulfate and sodium ascorbate in *t*-BuOH/H_2O. This reaction failed at low temperature and at 50–60 °C, the reaction was slow and after 3 days the product **23** was obtained in 10% yield along with starting material. Although the result was encouraging, the reaction condition needed to be optimized. Among the various reaction conditions, microwave assisted Cu(I) catalyzed reaction was found to be suitable for this conjugation.[17] Under microwave irradiation compound **6** was reacted with C-24-azide **17** in DMF/H_2O using catalytic amount of Cu(I) to give fluconazole/bile acid conjugate **23** as a diastereomeric mixture in 92% yield (Scheme 4).

In the proton NMR spectrum of compound **23**, resonance corresponding to C-24-methylene protons was identified at δ 3.15 ppm and 3.48 ppm (two doublets) and the proton of 1,2,3-triazole was noticed at δ 7.19 ppm. Methylene at C-4 position of 1,2,3-triazole was identified at δ 4.20 ppm. In the IR spectrum, compound **23** showed absorption due to



Scheme 3. Reagents and conditions: (a) *p*-TsOH/MeOH, 25 °C, 24 h, 95–96%; (b) LAH/THF, 25 °C, 2 h, 93–97%; (c) (i) **11**, MsCl, Et_3N, CH_2Cl_2, 0 °C, 10 min or (ii) **12**, MsCl, pyridine, 0 °C, 10 min; (d) NaN_3, DMF, 60 °C, 3 h, 93–94%; (e) MsCl, Et_3N, CH_2Cl_2, 0 °C, 10 min; (f) NaN_3, DMF, 60 °C, 3 h, 90–91%.



Scheme 4. Reagents and conditions: $CuSO_4 \cdot 5H_2O$ (5 mol %), sodium ascorbate (40 mol %), DMF/H_2O (9:1), microwave, 5 min, 90–95%.

hydroxyl group at 3391 cm^{-1}. In addition, it gave satisfactory elemental analysis and in mass spectrum it showed molecular ion peak at 667.34 (M+1).

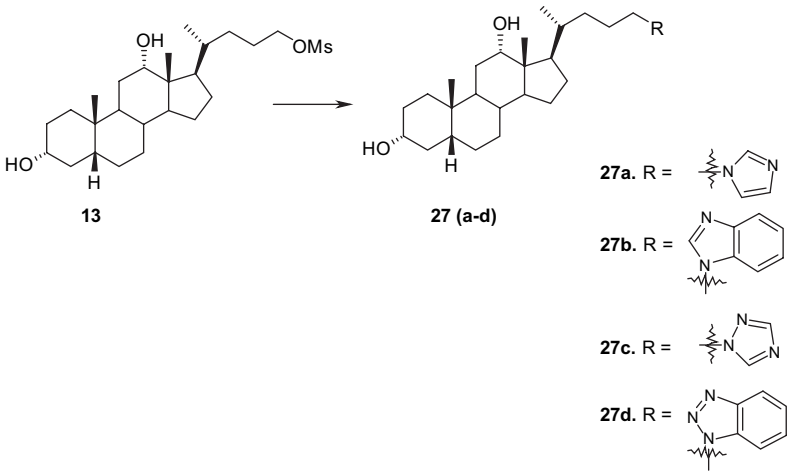
We then extrapolated the ligation protocol successfully to other bile acid derived azides, **18**, **21**, and **22** and synthesized fluconazole/bile acid conjugates **24**, **25**, and **26** (Scheme 4). All these fluconazole/bile acid bioconjugates showed very good antifungal activity against *Candida* species (Table 1).

For the comparison of biological activity we synthesized bile acid azoles conjugates **27a–d** containing imidazole, benzimidazole, triazole, and benzotriazole at C-24 position from C-24-monomesyl compound **13** (Scheme 5). However,

Table 1. In vitro antifungal activity for compounds **23**, **24**, **25**, **26**, and **27a–d**

Compound	Inhibitory concentration in μg/mL against											
	1		2		3		4		5		6	
	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}
23	3.12	2.11	12.5	11.34	6.25	6.11	12.5	10.58	>50	>50	6.25	5.82
24	6.25	4.58	50	44.76	25	12.34	25	24.82	>50	>50	6.25	5.16
25	6.25	5.72	25	22.23	25	23.34	25	22.48	>50	>50	6.25	5.48
26	6.25	3.44	50	48.42	3.12	2.64	25	21.46	>50	>50	3.12	2.18
27a	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
27b	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
27c	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
27d	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
Amphotericin-B	0.12	0.09	0.06	0.04	0.12	0.08	0.12	0.09	0.5	0.38	0.12	0.11
Fluconazole	0.5	0.13	1.0	0.46	2.0	1.06	1.0	0.63	2.0	1.06	1.0	0.21

1. *Candida albicans*, 2. *Cryptococcus neoformans*, 3. *Sporothrix schenckii*, 4. *Trichophyton mentagrophytes*, 5. *Aspergillus fumigatus*, 6. *Candida parapsilosis* (ATCC-22019).



Scheme 5. Reagents and conditions: **RH**, NaH, DMF, 25 °C, 12–20 h, 75–95%.

all these compounds showed very weak antifungal activity (Table 1).

3. Conclusion

In summary we have designed and synthesized fluconazole/bile acid conjugates at C-3 and C-24 positions of bile acids under microwave assisted Cu(I) catalyzed cycloaddition reaction. This reaction gave fluconazole/bile acid conjugates, linked with 1,4-disubstituted 1,2,3-triazole regioselectively, in excellent yield and in less reaction time. These new molecules showed very good antifungal activity against *Candida albicans*, *Sporothrix schenckii*, and *Candida parapsilosis* having MIC ranging from 3.12 to 6.25 μg/mL. It is thought that in this biological activity, bile acid part acts as a drug carrier and fluconazole part acts as an inhibitor of 14α-demethylase enzymes in fungal cell. Work to clarify the role of bile acid is underway in our laboratory. This is the first report of use of 'click chemistry' for the modification of fluconazole.

4. Experimental

4.1. General experimental techniques and apparatus

TLC was performed on precoated silica gel F_{254} plates (0.25 mm; E. Merck) and product(s) and starting material(s) were detected by either viewing under UV light or treating with an ethanolic solution of phosphomolybdic acid or anisaldehyde spray followed by heating. Column chromatography was performed on neutral deactivated aluminum oxide. Optical rotations were obtained on Bellingham & Stanley ADP-220 Polarimeter. Specific rotations ($[\alpha]_D$) are reported in deg/dm and the concentration (*c*) is given in g/100 mL in the specified solvent. Infrared spectra were recorded in $CHCl_3$ as a solvent on Schimadzu 8400 series FTIR instrument. ^{1}H NMR spectra were recorded on a Brucker AC-200 and 400 spectrometers at 200.13 and 400.13 MHz and ^{13}C NMR spectra were recorded on a Brucker AC-200 at 50.32 MHz. The chemical shifts are given in parts per million relative to tetramethylsilane. Mass spectra were recorded on LC–MS/MS-TOF API QSTAR PULSAR spectrometer, and samples were introduced by infusion method using Electrospray Ionisation Technique. Elemental analyses were performed by CHNS-O EA 1108-Elemental analyser, Carloerba Instrument (Italy) or Elementar vario EL (Germany) and were within ±0.4% of calculated values. Melting points were determined on a Thermonik Campbell melting point apparatus and were uncorrected. Microwave irradiation was carried out in an open glass vessel using a domestic microwave oven (800 W, BPL-make). Standard work up: after extraction of all the reactions, the organic extracts were washed with water and brine and dried over anhydrous Na_2SO_4 and concentrated in vacuum.

4.2. Synthesis of terminal acetylene

4.2.1. 1-(2,4-Difluorophenyl)-2-(1*H*-1,2,4-triazol-1-yl) ethanone (5). Compound **5** was synthesized from **4** using literature procedure.[5b] White solid, yield 55% (overall in a+b step); mp=104–106 °C (lit.[5b] 103–105 °C); IR (cm^{-1}): 1703, 1614, 1593; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.59 (d, *J*=3.54 Hz, 2H), 6.93–7.10 (m, 2H), 7.99–8.11 (m, 2H), 8.21 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 58.2 (dd, $^4J_{CF}$=14.09 Hz), 104.8 (dd, $^2J_{CF}$=25.66 Hz), 112.9 (dd, $^2J_{CF}$=21.63 Hz, $^4J_{CF}$=3.02 Hz), 118.8 (dd, $^2J_{CF}$=14.09 Hz, $^4J_{CF}$=3.52 Hz), 132.9 (dd, $^3J_{CF}$=4.52, 10.81 Hz), 144.8, 151.7, 163.0 (dd, $^1J_{CF}$=256.38 Hz, $^3J_{CF}$=13.08 Hz), 166.6 (dd, $^1J_{CF}$=259.9 Hz, $^3J_{CF}$=12.58 Hz), 187.6 (d, $^3J_{CF}$=5.53 Hz). Anal. Calcd for $C_{10}H_7F_2N_3O$: C, 53.82; H, 3.16; F, 17.03; N, 18.83. Found: C, 54.10; H, 3.02; F, 16.87; N, 18.71; MS (LC–MS) *m/z*: 224.57 (M+1), 246.57 (M+23 for Na).

4.2.2. 2-(2,4-Difluorophenyl)-1-(1*H*-1,2,4-triazole-1-yl)-pent-4-yn-2-ol (6). The ketone **5** (0.500 g, 2.24 mmol) and propargyl bromide (4 mL, 6.73 mmol) were dissolved in a mixed solvent DMF/THF 1:1 (10 mL). To this well stirred solution, activated zinc dust (washed with 2% HCl and water and dried in vacuum) (0.439 g, 6.73 mmol) was slowly added at room temperature.[18] After 5 min exothermic reaction brought itself to reflux, which was allowed to attain 25 °C. The whole reaction mixture was then stirred for 5 h at 25 °C. Ice-cold HCl solution (5%) was added to the reaction mixture and it was extracted with EtOAc, and washed with water and brine. Solvent was evaporated under reduced pressure to afford crude product, which was purified by column chromatography on silica gel (5% MeOH/DCM) to produce compound **6** (0.551 g) as a white solid.

Yield 95%; mp=145–146 °C; IR (cm^{-1}): 3272, 3137; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.06 (t, *J*=2.65 Hz, 1H), 2.86 (dd, *J*=16.01, 2.65 Hz, 1H ABX pattern), 2.92 (dd, *J*=18.01, 2.65 Hz, 1H ABX pattern), 4.13 (br s, 1H, OH), 4.72 and 4.82 (two d, *J*=14.01 Hz, 2H AB pattern), 6.73–6.87 (m, 2H), 7.50–7.59 (m, 1H), 7.87 (s, 1H), 8.20 (s, 1H); ^{13}C NMR ($CDCl_3$+CD_3OD, 50 MHz): δ 29.1 (d, $^4J_{CF}$=5.03 Hz), 56.3 (d, $^4J_{CF}$=5.03 Hz), 71.9, 73.4 (d, $^3J_{CF}$=4.53 Hz), 78.1, 103.9 (dd, $^2J_{CF}$=26.17, 27.17 Hz), 111.1 (dd, $^2J_{CF}$=20.63 Hz, $^4J_{CF}$=3.52 Hz), 124.2 (dd, $^2J_{CF}$=13.08 Hz, $^4J_{CF}$=3.52 Hz), 129.6 (dd, $^3J_{CF}$=6.04, 9.56 Hz), 144.2, 150.4, 158.6 (dd, $^1J_{CF}$=246.58 Hz, $^3J_{CF}$=12.08 Hz), 162.6 (dd, $^1J_{CF}$=249.59 Hz, $^3J_{CF}$=12.08 Hz). Anal. Calcd for $C_{13}H_{11}F_2N_3O$: C, 59.31; H, 4.21; F, 14.43; N, 15.96. Found: C, 59.45; H, 4.13; F, 14.31; N, 15.87; MS (LC–MS) *m/z*: 264.06 (M+1), 286.05 (M+23 for Na).

4.3. Synthesis of terminal azide

4.3.1. Synthesis of methyl 3α,12α-dihydroxy-5β-cholane-24-oate (9) and methyl 3α,7α,12α-trihydroxy-5β-cholane-24-oate (10). Compounds **9** and **10** were synthesized in overall good yield starting from cholic acid **7** and deoxycholic acid **8** using the literature procedure.[11e]

4.3.1.1. Methyl 3α,12α-dihydroxy-5β-cholane-24-oate (9). White solid; mp=82–105 °C (lit.[19a] 70–108 °C); IR (cm^{-1}): 3385, 1728; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.68 (s, 3H, CH_3-18), 0.91 (s, 3H, CH_3-19), 0.98 (d, *J*=6.36 Hz, 3H, CH_3-21), 3.62 (m, 1H, CH-3), 3.67 (s, 3H), 3.98 (br s, 1H, CH-12); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.6, 17.1, 23.0, 23.6, 26.0, 27.1, 27.4, 28.4, 29.6, 30.1,

30.8, 31.0, 33.4, 34.0, 35.2, 35.9, 36.2, 42.0, 46.3, 47.0, 48.0, 51.4, 71.4, 72.9, 174.7.

4.3.1.2. Methyl 3α,7α,12α-trihydroxy-5β-cholane-24-oate (10). White solid; mp=155–156 °C (lit.[19b] 155–156 °C); IR (cm^{-1}): 3376, 1731; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.68 (s, 3H, CH_3-18), 0.89 (s, 3H, CH_3-19), 0.98 (d, *J*=5.69 Hz, 3H, CH_3-21), 3.57 (m, 1H, CH-3), 3.67 (s, 3H), 3.89 (br s, 1H, CH-7), 4.01 (br s, 1H, CH-12); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.8, 17.7, 22.8, 23.6, 26.6, 27.7, 28.5, 30.0, 31.4, 31.5, 35.2, 35.2, 35.7, 35.8, 39.9, 39.9, 42.0, 42.0, 46.8, 47.3, 51.8, 68.8, 72.2, 73.4, 175.2.

4.3.2. Synthesis of 3α,12α,24-trihydroxy-5β-cholane (11) and 3α,7α,12α,24-tetrahydroxy-5β-cholane (12). Compounds **11** and **12** were synthesized in overall good yield starting from methyl esters of cholic acid **9** and deoxycholic acid **10** using the literature procedure.[19c]

4.3.2.1. 3α,12α,24-Trihydroxy-5β-cholane (11). White solid; mp=123–124 °C (lit.[19d] 107–114 °C, lit.[19e] 123 °C); IR (cm^{-1}): 3257; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.69 (s, 3H), 0.91 (s, 3H), 0.99 (d, *J*=6.87 Hz, 3H), 3.61 (m, 3H), 4.00 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.7, 17.7, 23.1, 23.7, 26.2, 27.2, 27.6, 28.5, 29.4, 30.4, 31.8, 33.6, 34.1, 35.3, 35.4, 36.1, 36.4, 42.1, 46.5, 47.5, 48.2, 63.4, 71.8, 73.3.

4.3.2.2. 3α,7α,12α,24-Tetrahydroxy-5β-cholane (12). Mp=236–238 °C (lit.[19c] 236.5–238 °C); IR, ^1H NMR, and ^{13}C NMR spectroscopic data are consistent that reported in literature.[19c]

4.3.3. Synthesis of 3α,12α-dihydroxy 24-mesyloxy-5β-cholane (13) and 3α,24-dimesyloxy-12α-hydroxy-5β-cholane (14). To a solution of **11** (2.000 g 5.28 mmol) in dry CH_2Cl_2 (20 mL) was added triethylamine (1.5 mL, 0.56 mmol) at 0 °C. Methane sulfonyl chloride (0.53 mL, 6.86 mmol in 10 mL CH_2Cl_2) was added dropwise in 10 min at 0 °C, and ice was added to the reaction mixture immediately after addition was complete. The reaction mixture was extracted with CH_2Cl_2. Organic layer was washed with $NaHCO_3$, water, and brine. Solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (0.5% MeOH/CH_2Cl_2) to obtain pure products **13** (1.805 g) and **14** (0.615 g).

4.3.3.1. 3α,12α-Dihydroxy 24-mesyloxy-5β-cholane (13). White solid; mp=78 °C; $[\alpha]_D^{26}$ +93.04 (*c* 0.2, $CHCl_3$); IR (cm^{-1}): 3419, 1416, 1448 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.68 (s, 3H, CH_3-18), 0.91 (s, 3H, CH_3-19), 0.99 (d, *J*=6.26 Hz, 3H, CH_3-21), 3.00 (s, 3H), 3.60 (m, 1H, CH-3), 3.97 (s, 1H, CH-12), 4.19 (t, *J*=6.66 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.6, 17.4, 23.0, 23.6, 25.9, 26.0, 27.0, 27.5, 28.6, 30.3, 31.4, 33.5, 34.0, 35.0, 35.2, 35.9, 36.3, 37.3, 42.0, 46.4, 47.3, 48.1, 70.5, 71.6, 73.0. Anal. Calcd for $C_{25}H_{44}O_5S$: C, 65.75; H, 9.71; S, 7.02. Found: C, 65.45; H, 9.53; S, 7.28; MS (LC–MS) *m/z*: 457.19 (M+1), 479.13 (M+23 for Na).

4.3.3.2. 3α,24-Dimesyloxy-12α-hydroxy-5β-cholane (14). White solid; mp=67–68 °C; $[\alpha]_D^{27}$ +43.25 (*c* 2.7, $CHCl_3$); IR (cm^{-1}): 3566; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.68 (s, 3H, CH_3-18), 0.92 (s, 3H, CH_3-19), 1.00 (d, *J*=6.26 Hz, 3H, CH_3-21), 3.00 (s, 3H), 3.01 (s, 3H), 4.00 (s, 1H, CH-12), 4.21 (t, *J*=6.66 Hz, 2H), 4.65 (m, 1H, CH-3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.6, 17.4, 22.7, 23.4, 25.7, 25.8, 26.6, 27.4, 27.5, 28.5, 31.2, 33.1, 33.4, 33.7, 34.7, 34.9, 35.7, 37.2, 38.7, 41.9, 46.3, 47.1, 47.9, 70.6, 72.7, 82.7. Anal. Calcd for $C_{26}H_{46}O_7S_2$: C, 58.39; H, 8.67; S, 11.99. Found: C, 58.22; H, 8.39; S, 12.16; MS (LC–MS) *m/z*: 557.32 (M+23 for Na).

4.3.4. Synthesis of 3α,7α,12α-trihydroxy 24-mesyloxy-5β-cholane (15) and 3α,24-dimesyloxy-7α,12α-dihydroxy-5β-cholane (16). To a solution of **12** (0.394 g, 1 mmol) in dry pyridine (5 mL), methane sulfonyl chloride (0.12 mL, 1.5 mmol) was added to the reaction mixture. After 10 min, ice was added and it was extracted with EtOAc. Organic layer was washed with cold water, cold-HCl (5%), water, and brine and dried over Na_2SO_4. Solvent was evaporated under reduced pressure and the crude product was purified by column chromatography (10% MeOH/CH_2Cl_2) to obtain pure **15** (0.329 g) and pure **16** (0.125 g).

4.3.4.1. 3α,7α,12α-Trihydroxy 24-mesyloxy-5β-cholane (15). White solid; mp=79–81 °C; $[\alpha]_D^{26}$ +68.49 (*c* 0.9, $CHCl_3$); IR (cm^{-1}): 3408; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.68 (s, 3H, CH_3-18), 0.89 (s, 3H, CH_3-19), 0.99 (d, *J*=6.44 Hz, 3H, CH_3-21), 3.01 (s, 3H), 3.44 (m, 1H, CH-3), 3.84 (s, 1H, CH-7), 3.97 (s, 1H, CH-12), 4.21 (t, *J*=6.57 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.3, 17.5, 20.8, 22.3, 23.1, 25.8, 26.2, 27.5, 28.0, 29.6, 30.1, 31.3, 34.5, 34.7, 35.1, 37.4, 39.2, 39.3, 41.3, 41.5, 46.3, 46.9, 68.4, 70.8, 71.9, 73.1. Anal. Calcd for $C_{25}H_{44}O_6S$: C, 63.52; H, 9.38; S, 6.78. Found: C, 63.21; H, 9.12; S, 6.53; MS (LC–MS) *m/z*: 473.91 (M+1), 495.89 (M+23 for Na).

4.3.4.2. 3α,24-Dimesyloxy-7α,12α-dihydroxy-5β-cholane (16). White solid; mp=82–84 °C; $[\alpha]_D^{25}$ +29.85 (*c* 0.8, $CHCl_3$); IR (cm^{-1}): 3434; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.69 (s, 3H, CH_3-18), 0.91 (s, 3H, CH_3-19), 0.99 (d, *J*=6.44 Hz, 3H, CH_3-21), 2.99 (s, 3H), 3.01 (s, 3H), 3.87 (br s, 1H), 4.00 (s, 1H, CH-12), 4.21 (t, *J*=6.69 Hz, 2H), 4.51 (m, 1H, CH-3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.3, 17.5, 22.1, 22.7, 23.0, 26.3, 27.4, 27.8, 28.0, 31.3, 34.2, 34.4, 34.7, 35.1, 35.9, 37.2, 38.7, 39.2, 41.3, 41.6, 46.3, 47.1, 68.0, 70.7, 72.8, 83.0. Anal. Calcd for $C_{26}H_{46}O_8S_2$: C, 56.70; H, 8.42; S, 11.64. Found: C, 56.84; H, 8.26; S, 11.81; MS (LC–MS) *m/z*: 573.51 (M+23 for Na).

4.3.5. Synthesis of 3α,12α-dihydroxy 24-azido-5β-cholane (17) and 3α,7α,12α-trihydroxy 24-azido-5β-cholane (18). To a solution of **13** (0.300 g, 0.66 mmol) in dry DMF (10 mL), sodium azide (0.214 g, 3.28 mmol) was added and stirring was continued at 60–65 °C for 3–5 h. The reaction mixture was allowed to cool to room temperature. It was then poured into ice-cold water (30 mL) and extracted with EtOAc. The organic extract was washed with cold water and brine. Solvent was evaporated under reduced pressure to afford crude product **17**, which was purified by column chromatography on silica gel (10% EtOAc/hexane) to produce pure compound **17** as a white solid (0.247 g).

4.3.5.1. 3α,12α-Dihydroxy 24-azido-5β-cholane (17). White solid, yield 94%; mp=126 °C; $[\alpha]_D^{28}$ +40.57 (*c* 1.4,

$CHCl_3$); IR (cm^{-1}): 2090, 3409; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.69 (s, 3H, CH_3-18), 0.91 (s, 3H, CH_3-19), 0.99 (d, J=6.65 Hz, 3H, CH_3-21), 3.24 (t, J=7.05 Hz, 2H), 3.62 (m, 1H, CH-3), 4.00 (br s, 1H, CH-12); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.7, 17.6, 23.1, 23.6, 25.6, 26.1, 27.1, 27.5, 28.7, 30.5, 32.9, 33.7, 34.1, 35.3, 36.1, 36.5, 42.2, 46.5, 47.5, 48.3, 51.9, 71.7, 73.2. Anal. Calcd for $C_{24}H_{41}N_3O_2$: C, 71.42; H, 10.24; N, 10.41. Found: C, 71.19; H, 10.46; N, 10.38; MS (LC–MS) *m/z*: 404.88 (M+1), 426.83 (M+23 for Na).

4.3.5.2. 3α,7α,12α-Trihydroxy 24-azido-5β-cholane (18). Compound **18** was prepared by similar procedure from compound **15**. White solid, yield 93%; mp=160 °C; $[\alpha]_D^{25}$ +31.46 (*c* 0.8, $CHCl_3$); IR (cm^{-1}): 2098, 3410; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.68 (s, 3H), 0.89 (s, 3H), 0.99 (d, J=6.32 Hz, 3H), 3.25 (t, J=6.57 Hz, 2H), 3.52 (m, 1H), 3.87 (br s, 1H), 4.00 (br s, 1H), 4.50 (br s, 3H, OH); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.4, 17.6, 22.4, 23.1, 25.6, 26.2, 27.6, 28.1, 30.2, 32.8, 34.6, 34.7, 35.3, 35.3, 39.3, 39.4, 41.4, 41.5, 46.3, 47.1, 51.9, 68.4, 71.8, 73.1. Anal. Calcd for $C_{24}H_{41}N_3O_3$: C, 68.70; H, 9.85; N, 10.01. Found: C, 68.65; H, 9.69; N, 9.94; MS (LC–MS) *m/z*: 420.24 (M+1), 442.23 (M+23 for Na).

4.3.6. Synthesis of methyl-3α-mesyloxy-12α-hydroxy-5β-cholane-24-oate (19) and methyl-3α-mesyloxy-7α-12α-dihydroxy-5β-cholane-24-oate (20). Compounds **19** and **20** were prepared from compounds **9** and **10** by using similar procedure as used for the preparation of compound **13**.

4.3.6.1. Methyl-3α-mesyloxy-12α-hydroxy-5β-cholane-24-oate (19). White solid, yield 89%; mp=62–63 °C; $[\alpha]_D^{27}$ +45.77 (*c* 4.1, $CHCl_3$); IR (cm^{-1}): 1728, 3549; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.67 (s, 3H), 0.91 (s, 3H), 0.97 (d, J=6.06 Hz, 3H), 2.99 (s, 3H), 3.65 (s, 3H), 3.98 (br s, 1H), 4.64 (m, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.3, 16.8, 22.5, 23.3, 25.6, 26.5, 27.1, 27.3, 28.3, 30.5, 30.6, 32.9, 33.1, 33.5, 34.5, 34.8, 35.5, 38.4, 41.7, 46.1, 46.7, 47.6, 51.1, 72.3, 82.5, 174.3. Anal. Calcd for $C_{26}H_{44}O_6S$: C, 64.43; H, 9.15; S, 6.62. Found: C, 64.58; H, 9.02; S, 6.73; MS (LC–MS) *m/z*: 485.23 (M+1), 507.22 (M+23 for Na).

4.3.6.2. Methyl-3α-mesyloxy-7α-12α-dihydroxy-5β-cholane-24-oate (20). White solid, yield 87%; mp=83–85 °C; $[\alpha]_D^{28}$ +29.98 (*c* 0.9, $CHCl_3$); IR (cm^{-1}): 1728, 3460; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.69 (s, 3H), 0.91 (s, 3H), 0.99 (d, J=6.06 Hz, 3H), 2.99 (s, 3H), 3.67 (s, 3H), 3.88 (br s, 1H), 4.00 (br s, 1H), 4.51 (m, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.4, 17.2, 22.1, 23.0, 26.3, 27.4, 27.8, 28.0, 30.7, 30.9, 34.1, 34.4, 34.7, 35.1, 35.9, 38.7, 39.3, 41.3, 41.6, 46.4, 47.0, 51.4, 68.0, 72.8, 82.9, 174.7. Anal. Calcd for $C_{26}H_{44}O_7S$: C, 62.37; H, 8.86; S, 6.40. Found: C, 62.23; H, 8.92; S, 6.23; MS (LC–MS) *m/z*: 501.07 (M+1), 523.17 (M+23 for Na).

4.3.7. Synthesis of methyl-3β-azido-12α-hydroxy-5β-cholane-24-oate (21) and methyl-3β-azido-7α,12α-dihydroxy-5β-cholane-24-oate (22). The compounds **19** and **20** were reacted with NaN_3 (3 equiv) in DMF for 4 h at 80–90 °C to give compounds **21** and **22**.[20]

4.3.7.1. Methyl-3β-azido-12α-hydroxy-5β-cholane-24-oate (21). White solid, yield 91%; mp=127–128 °C (lit.[16a] 128 °C); $[\alpha]_D^{27}$ +41.34 (*c* 0.8, $CHCl_3$); IR (cm^{-1}): 1728, 2102, 3503; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.68 (s, 3H), 0.94 (s, 3H), 0.97 (d, J=6.20 Hz, 3H), 3.66 (s, 3H), 3.94 (br s, 1H), 3.99 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.7, 17.3, 23.5, 23.5, 24.5, 25.9, 26.4, 27.4, 28.8, 30.1, 30.5, 30.8, 31.0, 33.2, 34.4, 35.0, 35.8, 37.2, 46.5, 47.3, 48.3, 51.4, 58.7, 73.1, 174.6. Anal. Calcd for $C_{25}H_{41}N_3O_3$: C, 69.57; H, 9.57; N, 9.74. Found: C, 69.47; H, 9.90; N, 9.66; MS (LC–MS) *m/z*: 432.26 (M+1), 454.25 (M+23 for Na).

4.3.7.2. Methyl-3β-azido-7α,12α-dihydroxy-5β-cholane-24-oate (22). White solid, yield 90%; mp=169–170 °C (lit.[16b] 157 °C); $[\alpha]_D^{27}$ +22.45 (*c* 1.16, MeOH) (lit.[16b] +23.7); IR (cm^{-1}): 1728, 2098, 3439; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.70 (s, 3H), 0.93 (s, 3H), 0.97 (d, J=6.06 Hz, 3H), 3.67 (s, 3H), 3.86–3.89 (br s, 2H), 3.99 (br s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.4, 17.2, 22.7, 23.2, 24.5, 26.0, 27.4, 28.3, 30.4, 30.8, 31.0, 33.0, 34.2, 35.1, 35.2, 36.7, 39.3, 41.7, 46.5, 47.2, 51.5, 58.7, 68.4, 73.0, 174.7. Anal. Calcd for $C_{25}H_{41}N_3O_4$: C, 67.08; H, 9.23; N, 9.39. Found: C, 67.21; H, 9.18; N, 9.31; MS (LC–MS) *m/z*: 448.24 (M+1), 470.22 (M+23 for Na).

4.4. General procedure for cycloaddition (23–26)

The alkyne **6** (1 equiv) and the azide **17**, **18**, **21**, or **22** (1.3 equiv) were dissolved in DMF/H_2O 4:1 (5 mL). To this solution, $CuSO_4 \cdot 5H_2O$ (0.05 equiv) and sodium ascorbate (0.40 equiv) were added. The reaction mixture was placed in a domestic microwave reactor and irradiated for 5 min at 415 W. The reaction mixture was cooled, ice was added, and it was then extracted with EtOAc. The extract was washed with water and brine. Solvent was evaporated under reduced pressure and crude product was purified by column chromatography on silica gel using 5% MeOH/CH_2Cl_2 system to obtain fluconazole/bile acid conjugates **23**, **24**, **25**, or **26** linked with 1,4-disubstituted 1,2,3-triazole.

4.4.1. 3α,12α-Dihydroxy-24-[(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-1*H*-1,2,3-triazol-1-yl)]-5β-cholane (23). White solid, yield 95%; mp=169–171 °C; IR (cm^{-1}): 1597, 1618, 3391; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.65 (s, 3H), 0.90–0.93 (6H), 3.15 and 3.48 (two doublets, J=14.90 Hz, 2H, adjacent to C-4 end of 1,2,3-triazole), 3.62 (m, 1H), 3.95 (br s, 1H), 4.20 (t, J=6.82 Hz, 2H), 4.53–4.74 (two doublets, J=14.15 Hz, 2H), 5.55 (1H, OH), 6.70–6.80 (m, 2H), 7.19 (br s, 1H), 7.33–7.46 (m, 1H), 7.81 (br s, 1H), 8.16 (br s, 1H). Anal. Calcd for $C_{37}H_{52}F_2N_6O_3$: C, 66.64; H, 7.86; F, 5.70; N, 12.60. Found: C, 66.81; H, 7.77; F, 5.51; N, 12.55; MS (LC–MS) *m/z*: 667.34 (M+1).

4.4.2. 3α,7α,12α-Trihydroxy-24-(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-1*H*-1,2,3-triazol-1-yl)-5β-cholane (24). White solid, yield 93%; mp=118–121 °C; IR (cm^{-1}): 1597, 1618, 3404; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.65 (s, 3H), 0.88 (s, 3H), 0.93 (d, J=6.52 Hz, 3H), 3.17 (d, J=14.15 Hz, 1H adjacent to 1,2,3-triazole), 3.42–3.49 (m, 2H, C3-H and 1H adjacent

to 1,2,3-triazole), 3.83 (br s, 1H), 3.94 (br s, 1H), 4.19 (m, 2H), 4.58 and 4.72 (two doublets, J=14.06 Hz, 2H, adjacent to 1,2,4-triazole), 6.69–6.78 (m, 2H), 7.20 (br s, 1H), 7.42 (m, 1H), 7.83 (br s, 1H), 8.20 (br s, 1H). Anal. Calcd for $C_{37}H_{52}F_2N_6O_4$: C, 65.08; H, 7.68; F, 5.56; N, 12.31. Found: C, 64.93; H, 7.66; F, 5.39; N, 12.45; MS (LC–MS) *m/z*: 683.29 (M+1), 705.27 (M+23 for Na).

4.4.3. Methyl-3β-[(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-1*H*-1,2,3-triazol-1-yl)]-12α-hydroxy-5β-cholane-24-oate (25). White solid, yield 90%; mp=183–185 °C; IR (cm^{-1}): 1618, 1728, 3362; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.67 (s, 3H), 0.80 (s, 3H), 0.96 (d, J=5.93 Hz, 3H), 3.19 and 3.54 (two doublets, J=16.29 Hz, 2H, adjacent to 1,2,3-triazole), 3.65 (s, 3H), 4.00 (br s, 1H), 4.54 (br s, 1H), 4.73 (br s, 2H, adjacent to 1,2,4-triazole), 6.63–6.80 (m, 2H), 7.23–7.35 (m, 2H), 7.86 (br s, 1H), 8.46 (br s, 1H). Anal. Calcd for $C_{38}H_{52}F_2N_6O_4$: C, 65.68; H, 7.54; F, 5.47; N, 12.09. Found: C, 65.43; H, 7.67; F, 5.24; N, 11.93; MS (LC–MS) *m/z*: 695.35 (M+1), 717.31 (M+23 for Na).

4.4.4. Methyl-3β-[(4-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-1*H*-1,2,3-triazol-1-yl)]-7α-12α-dihydroxy-5β-cholane-24-oate (26). White solid, yield 91%; mp=172–174 °C; IR (cm^{-1}): 1618, 1730, 3420; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.69 (s, 3H), 0.79 (s, 3H), 0.99 (d, J=6.27 Hz, 3H), 3.12 and 3.51 (two doublets, J=14.35 Hz, 2H, adjacent to 1,2,3-triazole), 3.66 (s, 3H), 3.87 (br s, 1H), 4.00 (br s, 1H), 4.46 (br s, 1H, C3-H), 4.63–4.72 (two doublets, J=14.30 Hz, 2H, adjacent to 1,2,4-triazole), 6.66–6.75 (m, 2H), 7.21 (br s, 1H), 7.36 (br s, 1H), 7.83 (br s, 1H), 8.30 (br s, 1H). Anal. Calcd for $C_{38}H_{52}F_2N_6O_5$: C, 64.21; H, 7.37; F, 5.35; N, 11.82. Found: C, 64.10; H, 7.29; F, 5.19; N, 11.77; MS (LC–MS) *m/z*: 711.29 (M+1), 733.26 (M+23 for Na).

4.5. General procedure for 27a–d

Azole compound **RH** (2 mmol) and NaH (3 mmol) were stirred in dry DMF (3 mL) at 0 °C for 20 min. To this mixture compound **13** (1 mmol) in DMF (2 mL) was added dropwise at 0 °C. The reaction mixture was allowed to warm to 25 °C and stirred for 24 h at this temperature. Ice was added to the reaction mixture and it was extracted with EtOAc. The extract was washed with water and brine, and solvent was evaporated under reduced pressure to afford crude product, which was purified by column chromatography on silica gel (5% MeOH/CH_2Cl_2) to produce compounds **27a–d** in 75–95% yield.

4.5.1. 3α,12α-Dihydroxy-24-(1*H*-imidazol-1-yl)-5β-cholane (27a). White solid, yield 75%; mp=215–216 °C; $[\alpha]_D^{25}$ +50.30 (*c* 0.7, $CHCl_3$); IR (cm^{-1}): 3300, 1510; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.67 (s, 3H), 0.91 (s, 3H), 0.97 (d, J=6.26 Hz, 3H), 3.62 (m, 1H), 3.90 (t, J=7.44 Hz, 2H), 3.98 (br s, 1H), 6.91 (br s, 1H), 7.06 (br s, 1H), 7.48 (br s, 1H); ^{13}C NMR ($CDCl_3$+CD_3OD, 50 MHz): δ 12.3, 17.1, 22.7, 22.7, 23.4, 25.9, 26.9, 27.3, 27.4, 28.4, 29.6, 32.4, 33.3, 33.9, 35.0, 35.0, 35.8, 41.9, 46.7, 47.4, 47.7, 48.6, 71.1, 72.6, 118.8, 128.1, 136.5. Anal. Calcd for $C_{27}H_{44}N_2O_2$: C, 75.65; H, 10.35; N, 6.54. Found: C, 75.77; H, 10.21; N, 6.43; MS (LC–MS) *m/z*: 429.30 (M+1), 451.26 (M+23 for Na).

4.5.2. 3α,12α-Dihydroxy-24-(1*H*-benzo[*d*]imidazol-1-yl)-5β-cholane (27b). White solid, yield 91%; mp=118 °C; $[\alpha]_D^{27}$ +42.77 (*c* 1.0, $CHCl_3$); IR (cm^{-1}): 3346, 1643, 1616; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.65 (s, 3H), 0.90 (s, 3H), 0.95 (d, J=6.60 Hz, 3H), 3.60 (m, 1H), 3.96 (br s, 1H), 4.13 (t, J=6.60 Hz, 2H), 7.26–7.32 (m, 2H), 7.38–7.41 (m, 1H), 7.79–7.82 (m, 1H), 7.90 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.5, 17.4, 23.0, 23.5, 26.0, 26.4, 27.0, 27.4, 28.7, 30.4, 32.9, 33.5, 34.0, 35.1, 35.2, 35.9, 36.3, 42.0, 45.4, 46.3, 47.0, 48.1, 71.4, 72.8, 109.5, 120.1, 121.9, 122.7, 133.7, 142.7, 143.5. Anal. Calcd for $C_{31}H_{46}N_2O_2$: C, 77.78; H, 9.69; N, 5.85. Found: C, 77.64; H, 9.54; N, 5.77; MS (LC–MS) *m/z*: 479.26 (M+1), 501.20 (M+23 for Na).

4.5.3. 3α,12α-Dihydroxy-24-(1*H*-1,2,4-triazol-1-yl)-5β-cholane (27c). White solid, yield 95%; mp=196–197 °C; $[\alpha]_D^{26}$ +45.33 (*c* 0.7, $CHCl_3$); IR (cm^{-1}): 3421; ^1H NMR ($CDCl_3$, 200 MHz): δ 0.66 (s, 3H), 0.90 (s, 3H), 0.98 (d, J=6.26 Hz, 3H), 3.61 (m, 1H), 3.97 (br s, 1H), 4.13 (t, J=6.65 Hz, 2H), 7.94 (s, 1H), 8.05 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.4, 17.1, 22.8, 23.4, 25.9, 26.2, 26.9, 27.4, 28.3, 26.6, 32.2, 33.3, 33.8, 35.0, 35.0, 35.7, 35.7, 41.8, 46.1, 46.8, 47.8, 50.1, 71.2, 72.7, 142.6, 151.0. Anal. Calcd for $C_{26}H_{43}N_3O_2$: C, 72.68; H, 10.09; N, 9.78. Found: C, 72.49; H, 10.00; N, 9.67; MS (LC–MS) *m/z*: 430.49 (M+1), 452.44 (M+23 for Na).

4.5.4. 3α,12α-Dihydroxy-24-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-5β-cholane (27d). White solid, yield 92%; mp=98–101 °C; $[\alpha]_D^{26}$ +47.82 (*c* 0.5, $CHCl_3$); IR (cm^{-1}): 3417; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.65 (s, 3H), 0.90 (s, 3H), 0.95 (d J=6.59 Hz, 3H), 3.61 (m, 1H), 3.95 (br s, 1H), 4.61 (t, J=7.33 Hz, 2H), 7.34–7.40 (m, 1H), 7.46–7.55 (m, 2H), 8.06 (d, J=8.06 Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 12.6, 17.5, 23.0, 23.6, 26.1, 26.4, 27.1, 27.4, 28.6, 30.4, 32.8, 33.6, 34.0, 35.0, 35.2, 36.0, 36.4, 42.1, 46.4, 47.2, 48.1, 48.6, 71.6, 73.0, 109.2, 119.9, 126.0, 127.0, 132.9, 144.2. Anal. Calcd for $C_{30}H_{45}N_3O_2$: C, 75.11; H, 9.46; N, 8.76. Found: C, 75.26; H, 9.39; N, 8.71; MS (LC–MS) *m/z*: 480.53 (M+1), 502.49 (M+23 for Na).

4.6. Biological evaluation procedure

4.6.1. MIC and IC_{50} determination. Minimum inhibitory concentration of compounds was tested according to standard microbroth dilution technique as per NCCLS guidelines.[21] Briefly, testing was performed in flat bottom 96 well tissue culture plates (CELLSTAR® Greiner bio-one GmbH, Germany) in RPMI 1640 medium buffered with MOPS (3-[*N*-morpholino]propanesulfonic acid) (Sigma Chem. Co., MO, USA) for fungal strains and in Muller Hinton broth (Titan Biotech Ltd, India) for bacterial strains. The concentration range of tested compounds was 50–0.36 and 32–0.0018 μg/mL for standard compounds. The plates were incubated in a moist chamber at 35 °C and absorbance at 492 nm was recorded on VersaMax microplate reader (Molecular Devices, Sunnyvale, USA) after 48 h for *C. albicans* and *C. parapsilosis*, 72 h for *Aspergillus fumigatus*, *S. schenckii*, and *Cryptococcus neoformans*, and 96 h for *Trichophyton mentagrophytes*. MIC was determined as 90% inhibition of growth with respect to the growth control and IC_{50} was the concentration at which 50% growth

inhibition was observed by using SOFTmax Pro 4.3 Software (Molecular Devices, Sunnyvale, USA).

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Supplementary data

^{1}H NMR, ^{13}C NMR, DEPT, and spectral chart of compounds **5**, **6**, **13**–**26**, and **27a**–**d**, and LC–MS of compounds **23**–**26**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.09.021.

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Intercalating nucleic acids (INAs) containing insertions of 6*H*-indolo[2,3-*b*]quinoxaline

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Abstract—6*H*-Indolo[2,3-*b*]quinoxaline was studied as a covalently bound heteroaromatic intercalator. Six monomers were synthesized and incorporated into DNA oligonucleotides. Through a study of linker length dependence it was concluded that the linker between the oligo and the intercalator must consist of at least five C atoms in order to stabilize a DNA duplex. An intercalator with a 2′-deoxy-D-riboside linker to the oligo could also stabilize a DNA/RNA duplex, while (*S*)-4-(6-methylindolo[2,3-*b*]quinoxalin-3-ylmethoxy)-butane-1,2-diol was able to stabilize both DNA/DNA, DNA/RNA and a DNA/LNA duplex. Mismatch studies revealed a huge sensitivity to the C–C mismatch at the 5′-site of the intercalator.

1. Introduction

The term *intercalation* was first used by Lerman,[1] who had conducted a number of physical studies on interactions of DNA with planar aromatic compounds, to describe the non-covalent binding of a planar polyaromatic molecule between the nucleobases of DNA. When intercalation occurs, the torsion angles in the sugar-phosphodiester backbone of DNA are changed in order to accommodate the intercalating aromatic moiety. This causes an unwinding of the DNA helix with a maximum helix increase of 3.4 Å, which can be measured by viscosity and sedimentation of the DNA solution.[1] The degree of unwinding varies depending on the intercalator and the DNA sequence.[2–4] Normally the helix increase is less than the maximal 3.4 Å, because of other effects such as bending of the duplex around the site of intercalation. X-ray studies on intercalation were first provided by Wang et al.[5] Classical intercalators such as proflavine, acridine, ethidium bromide, daunomycin[6] and actinomycin[7,8] are known for their anti-tumour activity by inhibition of topoisomerases,[9,10] but the group of intercalators has expanded heavily over the years and now includes mono-, bis- and trisintercalators. For recent reviews, see Martinez and Chacón-Garcia,[11] Braña et al.[12] and Graves and Velea.[13]

We have used the term *intercalating nucleic acids* to define an oligonucleotide with an intercalating pseudonucleotide inserted with a covalent bond. Important factors to be considered while using intercalating nucleic acids for hybridization are the structure of the backbone, and the length of the linker and intercalator.[14] We use a vicinal dihydroxy system to incorporate the intercalator as a bulge in the DNA backbone, because it creates a distance between the intercalator and the neighbouring nucleobases of ca. 3.4 Å. Furthermore it introduces additional flexibility into the backbone. Previously we have focused on pyrene as the intercalating moiety and have achieved significant discrimination between DNA and RNA,[15] as the DNA duplex was stabilized while the RNA duplex was destabilized. The fluorescence properties of the pyrene intercalator confirmed its intercalation rather than groove binding in duplexes.[16] These findings led to commercialization of intercalating nucleic acids containing bulge insertions of (*R*)-1-*O*-(1-pyrenylmethyl)glycerol (INA™) in order to exploit the technology in diagnostics, e.g., DNA methylation screening.[17]

For this study we were looking for a heteroaromatic intercalator in order to achieve higher affinity towards ssRNA. Ren et al.[18] had done some studies on the binding affinity of small molecules to an RNA:DNA hybrid using a poly rA:poly dT assay. Among the 84 tested compounds, of which five compounds showed a potential, they found the best one to be Ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole (**1**),[19] a naturally occurring alkaloid, known for its promising anti-tumour activities.[20]

6*H*-Indolo[2,3-*b*]quinoxaline (**2**) can be seen as an aza analogue of Ellipticine. It is a well-examined and

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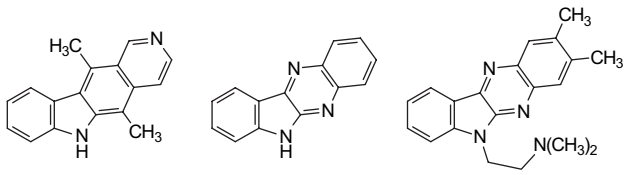


Figure 1. Structures of Ellipticine (**1**), 6*H*-Indolo[2,3-*b*]quinoxaline (**2**) and B-220 (**3**).

well-described compound, primarily by Bergman and co-workers.[21–23] It has nitrogen atoms in the third ring instead of the 5,11-methyl groups in Ellipticine. Several analogues of 6*H*-indolo[2,3-*b*]quinoxaline have been synthesized, e.g., B-220 (2,3-dimethyl-6-(2-dimethylaminoethyl)-6*H*-indolo[2,3-*b*]quinoxaline, **3**), which have shown high antiviral activity[24,25] (Fig. 1).

Ellipticine (**1**) as well as B-220 (**3**) have been used as non-covalent intercalators.[21,22,26–29] Furthermore, Arimondo et al.[30] observed a stronger stabilization of a poly(dAdT)·poly(dAdT) duplex for compound **2** than for its corresponding pyridopyrazino[2,3-*b*]indole analogues. It was therefore of interest to us to test 6*H*-indolo[2,3-*b*]quinoxaline as an intercalating nucleic acid. We also chose indolo[2,3-*b*]-quinoxaline as the intercalator for this study because of its direct synthesis.

In this paper we present the synthesis of six intercalating nucleic acids with indolo[2,3-*b*]quinoxaline as the intercalator (Fig. 2) and their evaluation by thermal stability measurements and fluorescence spectroscopy.

For monomers **4**–**7** the covalently bound linker was attached to N-6, which is the same position as the dimethylaminoethyl side chain that is attached in B-220 (**3**). Monomer **8** has a 2′-deoxy-D-ribose as the connector to the backbone of the DNA strand. It was synthesized in order to evaluate the flexibility of the acyclic linkers versus the natural furanose sugar linkage. The site of linker connection to the intercalator was changed in monomer **9** in order to investigate whether this could lead to better base stacking as the intercalator would be positioned differently in the duplex.

In this study we have investigated the dependence of linker length on the stabilities of DNA/DNA, DNA/RNA and DNA/LNA duplexes by varying the linker.

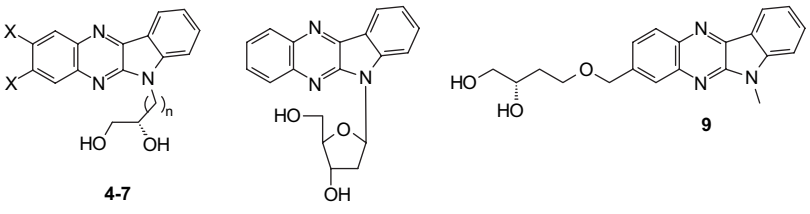
2. Results and discussion

2.1. Chemistry

The commercially available ((*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-yl)-methanol was mesylated in CH_2Cl_2/Et_3N according to the procedure described by Kim et al.[31] to give methanesulfonic acid (*R*)-2,2-dimethyl-1,3-dioxolane-4-ylmethyl ester (**10**).[32] Enantiomerically pure methanesulfonic acid 2-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-ethyl ester (**11**)[33] was obtained from (*S*)-malic acid in four steps. (*S*)-Malic acid was converted to dimethyl (*S*)-malate according to Mori and Ikunaka.[34] The diester was reduced with $LiAlH_4$ to (*S*)-1,2,4-butanetriol and protected with an isopropylidene group as described by Hayashi et al.[35] Mesylation of 2-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-ethanol with methanesulfonyl chloride was carried out in either pyridine according to Augustyns et al.[33] or CH_2Cl_2/Et_3N according to Kim et al.[31] Using pyridine as the solvent gave **11** in 51% yield, while the latter gave **11** in 99% yield. Enantiomerically pure methanesulfonic acid 3-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-propyl ester (**12**) was obtained from L-glutamic acid in four steps. L-Glutamic acid was converted to (*S*)-5-oxotetrahydrofuran-2-carboxylic acid as described by Herdeis.[36] Reduction with $LiAlH_4$ according to Brunner and Lautenschalger[37] yielded (*S*)-1,2,5-pentanetriol. This was protected with an isopropylidene group and mesylated as described above to give **12**.[38]

6*H*-Indolo[2,3-*b*]quinoxaline (**2**) and its 2,3-dichloro derivative (**13**) were easily prepared by condensation of isatin with 1,2-phenylene diamine in glacial acetic acid according to Schunck's method.[39,40] For the alkylation, **2** and **13** were deprotonated (at N-6) with NaH giving red anions. Following the procedure of Cassel et al.[41] they were then reacted with the mesylated alcohols **10** and **11** in refluxing DMF for 48 h in the presence of the phase-transfer catalyst tetra-*n*-butylammonium bromide (TBAB) giving **14b** and **c** in yields of 35–50%. A similar yield of **14d** was achieved without addition of TBAB,[42] whereas this procedure afforded **14a** in 76% yield using a long reaction time of three days.

The alkylation is supposed to take place on N-6 and this molecule will be similar to B-220 (**3**). However, there is a risk of forming an isomer due to alkylation on N-5. Bergman and co-workers[22,43] reported a 4:1 ratio of the 6-substituted versus 5-substituted derivative, by using Knotz's method.[44]



	n	X
4	1	H
5	1	Cl
6	2	H
7	3	H

Figure 2.

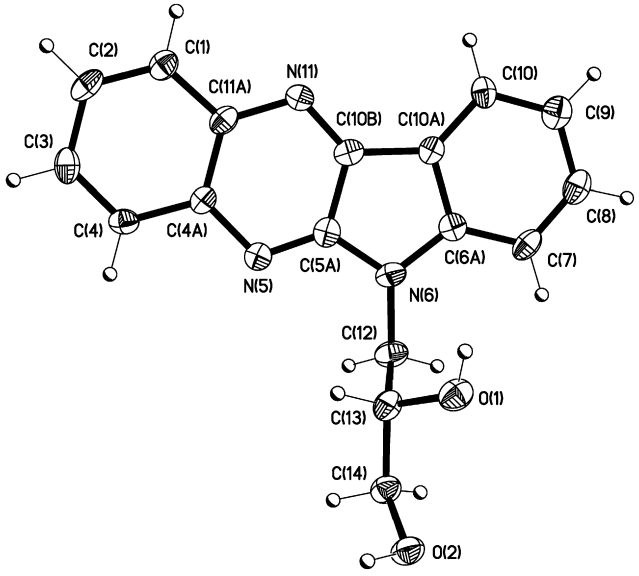
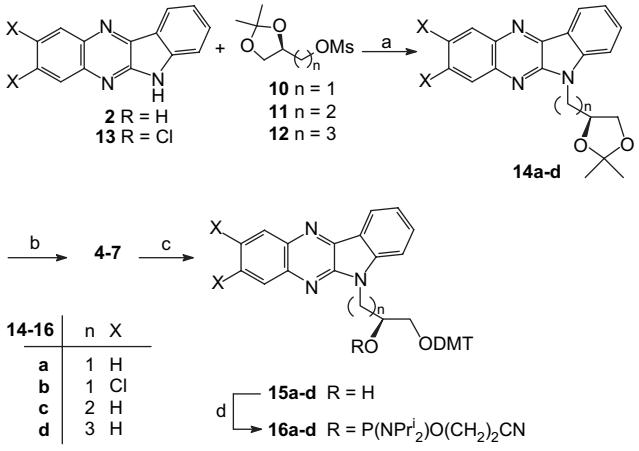


Figure 3. One independent molecule in the asymmetric unit of the X-ray structure of **4**. Displacement ellipsoids are shown at the 30% probability level for non-H atoms.

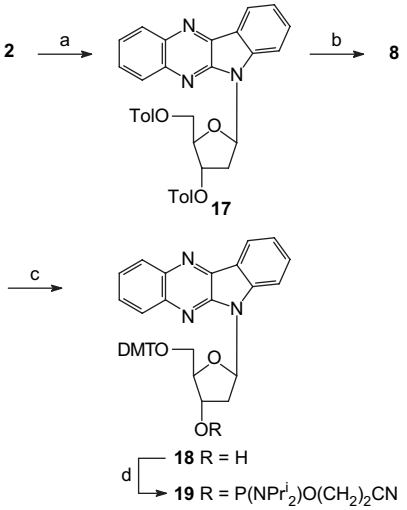
However, we did not isolate the N-5 alkylated isomer. The N-6 alkylation was proven by obtaining a synchrotron X-ray structure of the corresponding alcohol **4**, a step further in the synthetic route (see Figs. 2 and 3 and Scheme 1).



Scheme 1. (a) NaH, TBAB, DMF, 35–76%; (b) AcOH/H_2O (4:1), 67–93%; (c) DMT–Cl, pyr or CH_2Cl_2/Et_3N, 24–83%; (d) NC(CH$_2$)$_2$OP(N^iPr$_2$)$_2$, *N*,*N*-diisopropylammonium tetrazolide, CH_2Cl_2, 61–84%.

Treatment of **14a–d** with 80% aq acetic acid gave the diols **4–7**. These were DMT-protected using DMT–chloride in pyridine or in CH_2Cl_2/Et_3N and then converted to the phosphoramidites **16a–d** using 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphane in CH_2Cl_2.

We also synthesized an analogue having a 2′-deoxy-D-riboside as the backbone linker (Scheme 2). 2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-pentofuranosyl chloride was prepared according to Rolland et al.[45] and coupled with 6*H*-indolo-[2,3-*b*]quinoxaline (**2**) using NaH in DMF according to the procedure described above.[41] α- and β-Isomers were separated by dissolving the mixture in MeCN by which the β-isomer precipitated. Extraction with $CHCl_3$ isolated the α-isomer. Assignment of α,β-configuration was done by 2D NMR and NOE. Deprotection of the toluoyl groups were carried out using sodium methoxide in methanol as described by Abdel-Megied et al.[46] yielding β-6-(2′-deoxy-ribose)-indolo[2,3-*b*]quinoxaline (**8**). DMT-protection of the diol and conversion to the phosphoramidite (**19**) were carried out as described above.



Scheme 2. (a) 2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-pentofuranosyl chloride, NaH, TBAB, DMF, 32%; (b) CH_3ONa, MeOH, 73%; (c) DMT–Cl, CH_2Cl_2/Et_3N, 58%; (d) NC(CH$_2$)$_2$OP(N^iPr$_2$)$_2$, *N*,*N*-diisopropylammonium tetrazolide, CH_2Cl_2, 21%.

Having made **4–7** with three different linker lengths and a sugar moiety (**8**), we also found it interesting to change the connection site of the intercalator to the linker. This was done in order to position the intercalator in a different way inside the duplex and thereby to investigate, whether better base stacking could be obtained. At the same time the linker length was further increased.

6*H*-Indolo[2,3-*b*]quinoxaline-3-carboxylic acid (**20**) was synthesized by condensation of isatin with 3,4-diaminobenzoic acid in glacial acetic acid according to Schunck's method.[39,40] As the diamino compound is not symmetric, there was a possibility of regioisomers. However, we did not isolate the regioisomeric 6*H*-indolo[2,3-*b*]quinoxaline-2-carboxylic acid. The regioselectivity has previously been reported by Varma and Khan.[47] However, we found it more convincing to prove the structure **20** by an X-ray structure of the corresponding alcohol **23**, a few steps further in the synthetic route (see Fig. 4 and Scheme 3).

In order to avoid alkylation on N-6, when alkylating the new site (at the 3 position), the N-6 position was blocked with a methyl group using CH_3I in DMSO in the presence of powdered potassium hydroxide. This yielded a 3:2 mixture of 6-methylindolo[2,3-*b*]quinoxaline-3-carboxylic acid methyl ester (**21**) and the carboxylic acid (**22**). A small sample of the crude product was separated into pure compounds. The rest was reduced to the corresponding alcohol (6-methyl-indolo[2,3-*b*]quinoxalin-3-yl)methanol (**23**) using $LiAlH_4$ in THF. Badger and Nelson[48] reported a 40% yield of the 6-methylated compound and 12% of the 5-methylated by

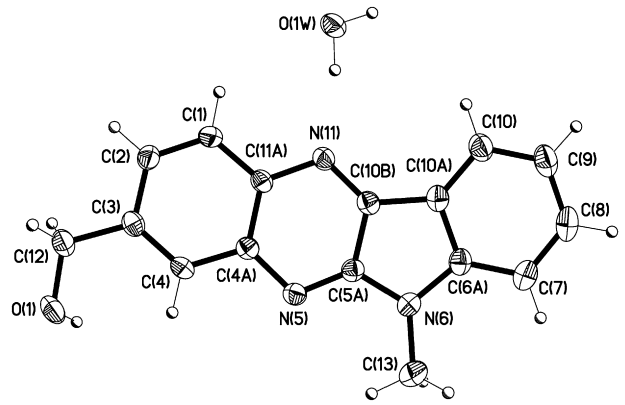
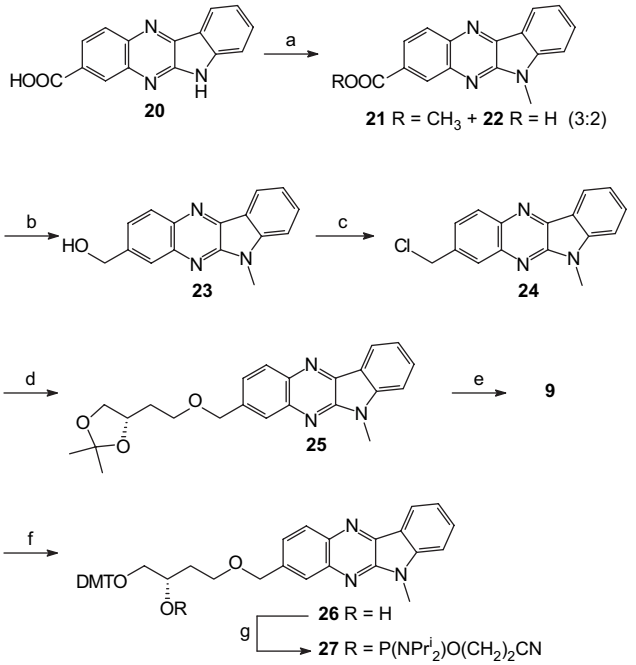


Figure 4. X-ray structure of **23**·H_2O showing displacement ellipsoids at the 50% probability level for non-H atoms.



Scheme 3. (a) CH_3I, KOH, DMSO; (b) $LiAlH_4$, THF, 44% (over two steps); (c) $SOCl_2$, CH_2Cl_2, 89%; (d) (*S*)-2,2-dimethyl-1,3-dioxolane-4-ethanol, KOH, toluene, 67%; (e) AcOH/H_2O (4:1); (f) DMT–Cl, CH_2Cl_2/Et_3N, 55%; (g) NC(CH$_2$)$_2$OP(*N*^{i}Pr$_2$)$_2$, *N*,*N*-diisopropylammonium tetrazolide, CH_2Cl_2, 47%.

using 1 equiv of KOH and CH_3I in ethanol. We used an excess of both KOH and CH_3I and DMSO as solvent, which according to Zegar et al.[22] should give a 95:5 ratio, but we isolated only the 6-methylated compound, which was also proven by the X-ray structure of **23**·H_2O (Fig. 4).

In order to have a more reactive leaving group for the subsequent alkylation reaction, the alcohol **23** was converted to 3-chloromethyl-6-methylindolo[2,3-*b*]quinoxaline (**24**) using thionyl chloride in CH_2Cl_2 according to the procedure described by Bair et al.[49] Following the procedure of Tirosh et al.,[50] **24** was reacted with 2-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-ethanol in toluene in the presence of powdered potassium hydroxide to give **25**. Subsequent treatment of **25** with 80% acetic acid as described above, followed by DMT-protection and phosphitylation gave **27** (Scheme 3).

The DMT-protected phosphoramidites of the intercalating nucleic acid monomers **4**–**9** were incorporated into two different DNA oligonucleotides using the same coupling times (2 min) in the oligo synthesis as was used for the amidites of natural nucleosides. The monomers **4**–**9** were inserted as a bulge in a 12-mer highly conserved HIV-1 strand,[51] which was modified around the site of intercalation (C-G switch) according to Christensen et al.[14,15] They were also inserted as a bulge or as an end-positioned intercalating pseudonucleotide in an 11-mer oligonucleotide earlier described by Filichev et al.[52] All modified oligonucleotides were confirmed by MALDI-TOF analysis with a variation of m/z ±3.

2.2. Thermal melting studies

The project was designed to study the linker length dependence of the intercalating nucleic acids. As it is seen from Table 1, intercalators with short linkers (**4**–**6**) were destabilizing the DNA duplex, whereas stabilization was obtained when a longer linker (**7**) or a sugar moiety (**8**) is used. Introduction of chloro substituents in the 2 and 3 positions of the 6*H*-indolo[2,3-*b*]quinoxaline (**5**) gave a marginal improvement compared to **4**. Highest stabilization was obtained for **9**, which had the longest linker, and the linker attached to C-3 instead of N-6. It is believed that the shorter linker is unable to position the intercalator optimally for base stacking in the duplex without disturbance of the backbone.

In comparison with previous studies[14] of pyrene and anthracene intercalators inserted in the same sequence and with the same linker length, 6*H*-indolo[2,3-*b*]quinoxaline (**9**) gives a higher ΔT_m (+4.5 °C vs +2.5 °C for pyrene and −1.3 °C for anthracene).

Shifting the intercalator to the complementary strand (Table 1, bottom) was expected to give higher T_m as better stacking is normally obtained when the intercalator is neighbouring two guanines in the same strand rather than two cytosines. This was also observed with the linkers **4**–**7**. However, the opposite effect is the case, when the acyclic linker is replaced with the 2-deoxyribose (**8**) or the elongated

Table 1. Melting temperatures of duplexes with **4**, **5**, **6**, **7**, **8** and **9** inserted as a bulge

	X=	—	**4**	**5**	**6**	**7**	**8**	**9**
	Sequences	T_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)
ODN1	5′-AGCTTG–GTTGAG-3′							
ODN2	3′-TCGAAC*X*CAACTC-5′	49.6	−9.0	−8.3	−1.0	+1.7	+4.0	+4.5
ODN3	5′-AGCTTG*X*GTTGAG-3′							
ODN4	3′-TCGAAC–CAACTC-5′	49.6	−6.0	−5.7	−1.9	+4.3	+2.2	−0.9

ΔT_m is the difference in T_m between duplexes with an intercalator (*X*) inserted and the corresponding unmodified duplex.

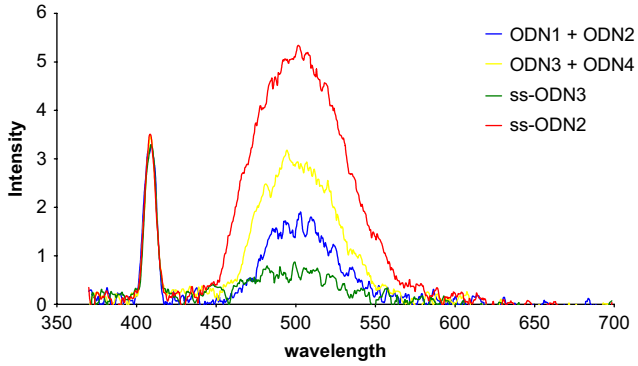


Figure 5. Fluorescence spectra of **9** inserted in **ODN2** and **ODN3**.

linker/intercalator system **9**. For **9**, a destabilization of the duplex was even observed contrary to what is seen, when the linker is connected to N-6 and when pyrene is used as the intercalator.[14] For the same linker length, shifting the pyrene intercalator to the complementary strand resulted in further stabilization of the duplex (ΔT_m=3.6 °C).[14] With the linker attached to C-3 of indolo[2,3-*b*]quinoxaline, the fourth ring of the intercalator can reach deeply into the duplex, which is not the case with pyrene because of its shape. It is thus anticipated that the intercalator **9** is placed nearer to the nucleobases of the complementary strand than those of its 'own' strand and in this way it is capable of stacking to guanines in the opposite strand in a better way.

This hypothesis is supported by fluorescence spectroscopy (Fig. 5). Weak fluorescence was observed for the ss-DNA containing insertions of **9** between two guanines. When **9** was inserted between two cytosines in the ss-DNA, the fluorescence was stronger. Upon hybridization to the complementary strand, the monomer fluorescence of **9** inserted between the two cytosines was quenched. To the contrary, enhancement of fluorescence was observed when the ss-DNA containing insertions of **9** between two guanines was hybridized to the complementary strand. This implies that the intercalator in the first case makes base stacking with the neighbouring guanines in the opposite strand, which also leads to stabilization of the duplex.

The intercalators were also placed opposite to each other in the two strands of the duplex in a zipping manner (Table 2). We observed a significant difference between **4** and **5** as the 2,3-dichloro substituted **5** was destabilizing the duplex by −4.4 °C, while **4** gave a destabilization of −11.5 °C. Compound **7** was marginally stabilizing the duplex. The most important result was obtained with the 2′-deoxyribose linkage **8**, which gave a significant stabilization. This result is remarkable, because previous studies[53] on two pyrenes directly opposing each other in the same duplex sequence resulted in destabilization. This has also been the case in other studies with INA[54] with two pyrenes opposing each other in a slightly different DNA sequence. The stabilization of **8** is most likely due to zipping of the two indolo[2,3-*b*]quinoxaline intercalating moieties. Also **9** was stabilizing the duplex when two intercalating moieties were inserted opposite to each other. In this case zipping may be facilitated by the longer linker.

For further investigation on indolo[2,3-*b*]quinoxaline as an intercalator, monomers **4**–**9** were incorporated into a 11-mer oligonucleotide[52] either in a region of A·T base pairs in the middle of the strand or as an end positioned pseudonucleotide. Thermal stability measurements were made against DNA, RNA and LNA[55] strands (Table 3).

Also for this sequence the dependence on linker length was obvious. When incorporated in the middle of the sequence, **4**, **5** and **6** destabilized the DNA/DNA duplex. A linker with n=3 (**7**) was necessary in order to achieve stabilization. The RNA/DNA duplex stabilization was only observed for the deoxyribose linked intercalators **8** and **9** with the longest linker attached to C-3.

Incorporation of 5′-end pseudonucleotide gave stabilization to all the duplexes and for all the investigated linkers. It is seen that the chloro substituents do not lead to further stabilization of the duplex when compared with **4**. However, it is noteworthy that **8**, which gives the highest T_m when incorporated into the middle of the DNA duplex, gives a lower T_m than **7** and **9** when incorporated at the 5′-end of a duplex. This might be due to the greater flexibility of the acyclic linker.

For the affinity studies towards LNA, we did not use a fully modified duplex but incorporated three thymines with LNA (T^L) into a DNA duplex. LNA locks the sugar ring in a north-type conformation, resulting in an A-type duplex, which is typically seen for RNA/RNA duplexes. If the duplex is not fully modified with LNA monomers, there will only be A-type duplex around the insertions. In the rest of the duplex—away from LNA monomers—there will be the B-type structure like in DNA/DNA duplexes. However, in this case, with three LNA insertions evenly distributed, it is reasonable to anticipate an A-type structure of the duplex as revealed by NMR structure determination of a similar LNA/DNA duplex.[56,57] Except for **9** all intercalating nucleic acid monomers destabilized the DNA/LNA duplex when they were inserted as a bulge in the middle of the sequence. It is interesting to observe the discrimination of **7** and **8** among DNA/LNA, DNA/RNA and DNA/DNA duplexes. Compound **7** stabilized the DNA/DNA duplex, but destabilized the DNA/LNA and DNA/RNA duplexes, even though the latter was only marginally destabilized (ΔT_m=−0.7 °C).

Table 2. Melting temperatures of duplexes with zipping insertions of **4**, **5**, **7**, **8** and **9**

	X=	—	**4**	**5**	**7**	**8**	**9**
	Sequences	T_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)
ODN3	5′-AGCTTG*X*GTTGAG-3′						
ODN2	3′-TCGAAC*X*CAACTC-5′	49.6	−11.5	−4.4	+0.3	+9.7	+6.6

ΔT_m is the difference in T_m between duplexes with an intercalator (*X*) inserted and the corresponding unmodified duplex.

Table 3. Comparison of DNA/DNA, DNA/RNA and DNA/LNA duplexes with **4**, **5**, **7**, **8** and **9** inserted as a bulge or at the end of the duplex

X=	—	**4**	**5**	**6**	**7**	**8**	**9**
Sequences	T_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)	ΔT_m (°C)
5′-TGTGAT–ATGCT-3′ 3′-ACACTA*X*TACGA-5′	42.4	−9.9	−6.8	−1.9	2.4	4.6	3.4
5′-TGTGATATGCT-3′ 3′-ACACTATACGA*X*-5′	42.4	2.4	2.4	—	3.8	3.4	4.6
5′-UGUGAU–AUGCU-3′ 3′-ACACTA*X*TACGA-5′	39.5	−13.4	−11.8	—	−0.7	1.8	2.3
5′-UGUGAUAUGCU-3′ 3′-ACACTATACGA*X*-5′	39.5	0.7	0.4	—	1.7	1.5	1.6
5′-TGT^{L}GAT^L–AT^{L}GCT-3′ 3′-ACA CTA *X*TA CGA-5′	56.7	−15.6	−16.9	−9.9	−4.4	−4.6	1.6
5′-TGT^{L}GAT^L–AT^{L}GCT-3′ 3′-ACA CTA TAC GA*X*-5′	56.7	2.1	2.9	—	3.5	3.1	4.6

T^L denotes locked nucleotide of thymine. ΔT_m is the difference in T_m between duplexes with an intercalator (*X*) inserted and the corresponding unmodified duplex.

Table 4. Melting temperatures of mismatched sequences with **4**, **7**, **8** and **9** inserted as a bulge

Sequence: 5′-AGC TTZ *Y*TT GAG-3′
3′-TCG AAC *X* CAACTC-5′

	X=		—		**4**		**7**		**8**		**9**	
	Z	*Y*	T_m (°C)	ΔT_m (°C)	T_m (°C)	ΔT_m (°C)	T_m (°C)	ΔT_m (°C)	T_m (°C)	ΔT_m (°C)	T_m (°C)	ΔT_m (°C)
Wild type	G	G	49.6		40.6		51.3		53.6		54.1	
Mut. 1	G	C	21.7	−27.9	17.9	−22.7	20.4	−30.9	22.3	−31.3	37.1	−17.0
Mut. 2	G	A	33.6	−16.0	28.3	−12.3	31.6	−19.7	34.7	−18.9	38.5	−15.6
Mut. 3	G	T	32.4	−17.2	28.4	−12.2	30.5	−20.8	33.9	−19.7	40.2	−13.9
Mut. 4	C	G	28.2	−21.4	30.3	−10.3	35.3	−16.0	37.3	−16.3	37.2	−16.9
Mut. 5	A	G	31.1	−18.5	30.5	−10.1	36.4	−14.9	38.4	−15.2	38.4	−15.7
Mut. 6	T	G	32.2	−17.4	30.5	−10.1	37.2	−14.1	39.7	−13.9	38.1	−16.0

ΔT_m is the difference in T_m between the matched sequence and the mismatched.

Compound **8** stabilized both DNA/DNA and DNA/RNA duplexes, but destabilized the DNA/LNA duplex. Compound **9**, which was stabilizing the investigated DNA/DNA, DNA/RNA and DNA/LNA duplexes, is currently being examined for stabilization of Three Way Junctions (TWJ).

Finally mismatch studies were carried out (Table 4). The specificity for hybridization was measured by the difference in the melting temperature between the fully complementary duplex and the duplex where one mismatch has been introduced. In general, when a mismatch was introduced at the 3′-site of the intercalator, this proved to be less sensitive than the unmodified oligo, while a greater sensitivity was observed when the mismatch was introduced at the 5′-site of the intercalator. However, for the C–C mismatch at the 5′-site of the intercalator, monomers **7** and **8** caused a drop in melting temperatures up to ca. 30 °C.

3. Conclusion

From the study of linker length dependence we conclude that the linker must, as a minimum, have a length corresponding to **7**, when used as a covalently bound intercalator. Shorter linkers destabilize the duplexes as they are not able to position the intercalator optimally for base stacking without disturbing the backbone. Introduction of chloro substituents in the 2,3 positions of 6*H*-indolo[2,3-*b*]quinoxaline did not increase stabilization significantly and therefore 2,3-dichloro-6*H*-indolo[2,3-*b*]quinoxaline was not coupled to any of the longer linkers.

In comparison studies of a DNA/DNA, DNA/RNA and a DNA/LNA duplex the linker length dependence was also obvious. When inserted in an A·T region in the middle of a 11-mer sequence, only **9** could stabilize DNA/LNA, while **8** and **9** stabilized DNA/RNA. Stabilization of a DNA/DNA duplex could be achieved using **7**, **8** or **9**.

In mismatch studies we found the intercalator to be less sensitive to mismatch at the 3′-site than observed for the unmodified duplex and more sensitive to the 5′-site of the intercalator. With insertions of the 6*H*-indolo[2,3-*b*]quinoxaline containing monomers **7** and **8** the maximum drop in melting temperature was ca. 30 °C for a C–C mismatch.

4. Experimental

4.1. General

NMR spectra were recorded on a Varian Gemini 2000 NMR spectrometer at 300 MHz for ^{1}H, 75 MHz for ^{13}C and 121.5 MHz for ^{31}P with TMS as an internal standard for ^{1}H NMR, deuterated solvents $CDCl_3$ (δ 77.00), CD_2Cl_2 (δ 53.80), CD_3OD (δ 49.00), DMSO (δ 39.44) for ^{13}C NMR,

and 85% H_3PO_4 as an external standard for ^{31}P NMR. MALDI mass spectra were recorded on a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (Ionspec, Irvine, CA). For accurate ion mass determinations, the $[M+H]^+$ or $[M+Na]^+$ ion was peak matched using ions derived from the 2,5-dihydroxybenzoic acid matrix. IR spectra were recorded on a Perkin–Elmer 1720 Infrared Fourier Transform Spectrometer. Melting points were determined on a Büchi melting point apparatus. Silica gel (0.040–0.063 mm) used for column chromatography and analytical silica gel TLC plates 60 F_{254} were purchased from Merck. UV-light or a stain of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O/Ce_2(SO_4)_3$ (50:1) in 5% sulfuric acid was used for visualization. Solvents used for column chromatography were distilled prior to use, while reagents were used as purchased. Petroleum ether (PE): bp 60–80 °C. NMR assignment follows standard nucleoside style, that is the carbon next to the intercalator is assigned C-1′.

4.2. General procedure for alkylation of 2 and 13, method A[41]

NaH (60% in oil, 1.5 equiv) was added portionwise to a solution of 6*H*-indolo[2,3-*b*]quinoxaline[39,40] (**2**, 0.139 g, 0.63 mmol) or 2,3-dichloro-6*H*-indolo-[2,3-*b*]quinoxaline[58] (**13**, 0.250 g, 0.87 mmol) in dry DMF (40 mL). After stirring at rt for 30 min, TBAB (0.2 equiv) was added and the reaction mixture was stirred for additional 30 min. The mesylated alcohol **10** or **11** (2 equiv) was added dropwise. The reaction mixture was stirred at 140 °C for 48 h. After cooling, DMF was evaporated off under reduced pressure. The residue was treated with water and extracted three times with $CHCl_3$. The combined organic phases were dried ($MgSO_4$) and the solvent evaporated off. The product was purified by silica gel column chromatography using EtOAc/PE (25:75 v/v) as an eluent.

4.2.1. 2,3-Dichloro-6-((*S*)-2,2-dimethyl-1,3-dioxolan-4-ylmethyl)-6*H*-indolo[2,3-*b*]quinoxaline (14b). Yield: 0.122 g (35%) as a yellow solid, R_f 0.94 (EtOAc/PE 2:3), mp 131 °C. ^1H NMR ($CDCl_3$): δ 1.32, 1.38 (2×s, 6H, 2×CH_3), 3.97 (dd, 1H, *J*=5.5 Hz, 8.6 Hz, H-3′), 4.15 (dd, 1H, *J*=5.5 Hz, 8.6 Hz, H-3′), 4.52 (dd, 1H, *J*=5.5 Hz, 14.6 Hz, H-1′), 4.61 (dd, 1H, *J*=5.5 Hz, 14.6 Hz, H-1′), 4.69 (quintet, 1H, *J*=5.5 Hz, H-2′), 7.40 (t, 1H, *J*=7.4 Hz, H_{arom}), 7.61–7.74 (m, 2H, H_{arom}), 8.19 (s, 1H, H_{arom}), 8.36 (s, 1H, H_{arom}), 8.39 (d, 1H, *J*=7.9 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 25.17, 26.70 (2×CH_3), 44.49 (C-1′), 67.24 (C-3′), 74.36 (C-2′), 109.10 ($C(CH_3)_2$), 110.70, 119.15, 121.64, 122.81, 128.29, 131.62, 132.87, 138.10, 139.18, 145.23 (C_{arom}). HRMS (MALDI): *m/z* calcd for $C_{20}H_{18}Cl_2N_3O_2^+$ (MH^+): 402.0770, found 402.0783.

4.2.2. 6-[2-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)ethyl]-6*H*-indolo[2,3-*b*]quinoxaline (14c). Yield: 0.110 g (50%) as a yellow oil, R_f 0.76 (EtOAc/PE 1:4). ^1H NMR ($CDCl_3$): δ 1.32 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 2.13–2.25 (m, 2H, H-2′), 3.57 (dd, 1H, *J*=7.1 Hz, 8.0 Hz, H-4′), 4.01 (dd, 1H, *J*=6.0 Hz, 8.0 Hz, H-4′), 4.11–4.19 (m, 1H, H-3′), 4.52–4.70 (m, 2H, H-1′), 7.37 (m, 1H, H_{arom}), 7.56 (d, 1H, *J*=8.2 Hz, H_{arom}), 7.64–7.77 (m, 3H, H_{arom}), 8.11 (dd, 1H, *J*=1.2 Hz, 8.2 Hz, H_{arom}), 8.29 (dd, 1H, *J*=1.2 Hz, 8.2 Hz, H_{arom}), 8.46 (d, 1H, *J*=7.2 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 25.57, 27.05 (2×CH_3), 32.60 (C-2′), 38.36 (C-1′), 69.18 (C-4′), 73.47 (C-3′), 109.10 ($C(CH_3)_2$), 109.54, 119.42, 120.91, 122.66, 125.97, 127.75, 128.71, 129.32, 130.94, 139.29, 140.08, 140.52, 144.45, 145.47 (C_{arom}). HRMS (MALDI): *m/z* calcd for $C_{21}H_{22}N_3O_2^+$ (MH^+): 348.1706, found 348.1705.

4.3. General procedure for alkylation of 2 and 13, method B[42]

NaH (60% in oil, 1.2 equiv) was added in two portions to a solution of 6*H*-indolo-[2,3-*b*]quinoxaline[39,40] (**2**, 0.230 g, 1 mmol) in dry DMF (20 mL). The reaction mixture was heated to 80 °C for 30 min, before the mesylated alcohol **10** (0.405 g, 2 mmol, 2 equiv) or **12** (0.250 g, 1 mmol, 1 equiv) was added dropwise. The reaction mixture was stirred at 80 °C overnight (**14d**) or for three days (**14a**). The mixture was partitioned between CH_2Cl_2 and brine. The organic phase was dried ($MgSO_4$), and the solvent evaporated by co-evaporation with dry xylene. The product was purified by silica gel column chromatography using EtOAc/PE (25:75 v/v) as an eluent.

4.3.1. 6-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-ylmethyl)-6*H*-indolo[2,3-*b*]quinoxaline (14a). Yield: 0.268 g (76%) as a yellow solid, R_f 0.44 (EtOAc/PE 1:1), mp 135 °C. ^1H NMR ($CDCl_3$): δ 1.33 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 4.00 (dd, 1H, *J*=5.5 Hz, 8.6 Hz, H-3′), 4.13 (dd, 1H, *J*=6.0 Hz, 8.6 Hz, H-3′), 4.54 (dd, 1H, *J*=5.5 Hz, 14.4 Hz, H-1′), 4.65 (dd, 1H, *J*=5.5 Hz, 14.4 Hz, H-1′), 4.70 (quintet, 1H, *J*=5.5 Hz, H-2′), 7.38 (m, 1H, H_{arom}), 7.62–7.78 (m, 4H, H_{arom}), 8.11 (dd, 1H, *J*=1.5 Hz, 8.3 Hz, H_{arom}), 8.31 (dd, 1H, *J*=1.5 Hz, 8.3 Hz, H_{arom}), 8.46 (dd, 1H, *J*=0.7 Hz, 7.8 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 25.20, 26.68 (2×CH_3), 44.31 (C-1′), 67.32 (C-3′), 74.49 (C-2′), 109.76 ($C(CH_3)_2$), 110.41, 119.47, 121.14, 122.53, 126.13, 127.75, 128.50, 129.25, 130.90, 139.40, 139.97, 140.43, 144.89, 145.80 (C_{arom}). HRMS (MALDI): *m/z* calcd for $C_{20}H_{19}N_3O_2Na^+$ (MNa^+): 356.1369, found 356.1377.

4.3.2. 6-[3-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)propyl]-6*H*-indolo[2,3-*b*]quinoxaline (14d). Yield: 0.132 g (35%) as a yellow oil. ^1H NMR ($CDCl_3$): δ 1.34 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 1.63–1.73 (m, 2H, H-3′), 2.02–2.10 (m, 2H, H-2′), 3.47 (t, 1H, *J*=8.0 Hz, H-5′), 3.99 (dd, 1H, *J*=5.9 Hz, 8.0 Hz, H-5′), 4.14–4.19 (quintet, 1H, *J*=5.9 Hz, H-4′), 4.52 (dd, 2H, *J*=6.7 Hz, 13.4 Hz, H-1′), 7.38 (t, 1H, *J*=′7.6 Hz, H_{arom}), 7.47 (d, 1H, *J*=8.3 Hz, H_{arom}), 7.64–7.77 (m, 3H, H_{arom}), 8.12 (dd, 1H, *J*=1.5 Hz, 8.3 Hz, H_{arom}), 8.29 (dd, 1H, *J*=1.5 Hz, 8.3 Hz, H_{arom}), 8.47 (d, 1H, *J*=7.6 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 24.83 (C-2′), 25.64, 26.90 (2×CH_3), 30.66 (C-3′), 41.12 (C-1′), 69.23 (C-5′), 75.50 (C-4′), 108.84 ($C(CH_3)_2$), 109.41, 119.46, 120.82, 122.72, 125.91, 127.75, 128.68, 129.29, 130.90, 139.25, 139.93, 140.54, 144.24, 145.58 (C_{arom}). HRMS (MALDI): *m/z* calcd for $C_{22}H_{23}N_3O_2Na^+$ (MNa^+): 384.1682, found 384.1691.

4.4. General procedure for isopropylidene deprotection

Compounds **14a–d** were stirred in acetic acid/H_2O (4:1, 50 mL) at rt overnight. The solvent was evaporated under reduced pressure.

4.4.1. (*S*)-3-(Indolo[2,3-*b*]quinoxalin-6-yl)-propane-1,2-diol[59] (4). Yield: 0.194 g (93%) as yellow crystals (obtained from 0.237 g, 0.71 mmol of **14a**), R_f 0.32 (4% MeOH/CH_2Cl_2), mp 215 °C. IR (KBr): 3338 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.52 (d, 2H, J=5.3 Hz, H-3′), 4.14 (m, 1H, H-2′), 4.45 (dd, 1H, J=7.8 Hz, 14.4 Hz, H-1′), 4.56 (dd, 1H, J=4.4 Hz, 14.4 Hz, H-1′), 4.89 (br s, 1H, OH), 5.03 (br s, 1H, OH), 7.41 (t, 1H, J=7.7 Hz, H_{arom}), 7.71–7.86 (m, 4H, H_{arom}), 8.12 (dd, 1H, J=8.3 Hz, 1.1 Hz, H_{arom}), 8.28 (dd, 1H, J=8.3 Hz, 1.1 Hz, H_{arom}), 8.38 (d, 1H, J=7.7 Hz, H_{arom}). ^{13}C NMR (DMSO-d_6): δ 44.93 (C-1′), 63.96 (C-3′), 69.60 (C-2′), 111.14, 118.54, 120.77, 121.91, 125.94, 127.50, 128.88, 129.04, 131.11, 138.58, 139.63, 139.89, 145.09, 145.44 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{17}H_{15}N_3O_2Na^+$ (MNa^+): 316.1056, found 316.1054.

4.4.2. (*S*)-3-(2,3-Dichloro-indolo[2,3-*b*]quinoxalin-6-yl)-propane-1,2-diol (5). Yield: 0.080 g (92%) as a yellow solid (obtained from 0.097 g, 0.24 mmol of **14b**), R_f 0.50 (5% MeOH/$CHCl_3$), mp 212 °C. ^1H NMR (DMSO-d_6): δ 3.50 (t, 2H, J=5.4 Hz, H-3′), 4.07–4.12 (m, 1H, H-2′), 4.41 (dd, 1H, J=7.9 Hz, 14.2 Hz, H-1′), 4.51 (dd, 1H, J=4.2 Hz, 14.2 Hz, H-1′), 4.83 (t, 1H, J=5.5 Hz, OH), 4.97 (d, 1H, J=5.3 Hz, OH), 7.42 (m, 1H, H_{arom}), 7.80 (m, 3H, H_{arom}), 8.33–8.36 (m, 2H, H_{arom}). ^{13}C NMR (DMSO-d_6): δ 44.49 (C-1′), 63.91 (C-3′), 69.44 (C-2′), 111.43, 118.01, 121.28, 122.28, 128.10, 129.58, 131.92, 137.13, 138.85, 145.19 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{17}H_{14}Cl_2N_3O_2^+$ (MH^+): 362.0457, found 362.0472.

4.4.3. (*S*)-4-(Indolo[2,3-*b*]quinoxalin-6-yl)-butane-1,2-diol (6). Yield: 0.065 g (67%) as a yellow oil (obtained from 0.110 g, 0.32 mmol of **14c**), R_f 0.20 (EtOAc/PE 1:4). ^1H NMR (DMSO-d_6): δ 1.74–1.87 (m, 1H, H-2′), 2.07–2.18 (m, 1H, H-2′), 3.26–3.57 (m, 3H, H-3′, H-4′), 4.50–4.67 (m, 2H, H-1′), 4.83 (br s, 2H, 2×OH), 7.42 (m, 1H, H_{arom}), 7.71–7.86 (m, 4H, H_{arom}), 8.14 (dd, 1H, J=8.4 Hz, 1.1 Hz, H_{arom}), 8.27 (dd, 1H, J=8.4 Hz, 1.1 Hz, H_{arom}), 8.39 (d, 1H, J=7.7 Hz, H_{arom}). ^{13}C NMR (DMSO-d_6): δ 32.22 (C-2′), 38.26 (C-1′), 65.81 (C-4′), 69.04 (C-3′), 110.43, 118.54, 120.88, 122.18, 125.99, 127.51, 128.95, 129.08, 131.37, 138.59, 139.56, 139.93, 144.35, 144.90 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{18}H_{18}N_3O_2^+$ (MH^+): 308.1393, found 308.1395.

4.4.4. (*S*)-5-(Indolo[2,3-*b*]quinoxalin-6-yl)-pentane-1,2-diol (7). Yield: 0.103 g (88%) as yellow crystals (obtained from 0.132 g, 0.37 mmol of **14d**). ^1H NMR (CD_3OD): δ 1.30–1.39 (m, 1H, H-3′), 1.44–1.50 (m, 1H, H-3′), 1.79–1.94 (m, 2H, H-2′), 3.20–3.30 (m, 2H, H-5′), 3.52 (m, 1H, H-4′), 4.25 (m, 2H, H-1′), 7.11–8.14 (m, 8H, H_{arom}). ^{13}C NMR (CD_3OD): δ 25.78 (C-2′), 31.61 (C-3′), 42.32 (C-1′), 67.23 (C-5′), 72.80 (C-4′), 111.03, 119.77, 122.04, 123.43, 127.18, 128.47, 129.44, 129.94, 132.53, 139.48, 140.67, 141.44, 145.71, 146.50 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{19}H_{19}N_3O_2Na^+$ (MNa^+): 344.1369, found 344.1363.

4.4.5. 6-(2′-Deoxy-3′,5′-di-*O*-(*p*-toluoyl)-β-D-ribofuranosyl)-indolo[2,3-*b*]quinoxaline (17). NaH (60% in oil, 0.274 g, 6.8 mmol, 1.5 equiv) was added portionwise to a solution of 6*H*-indolo[2,3-*b*]quinoxaline (**2**, 1.0 g, 4.6 mmol, 1 equiv) in dry DMF (40 mL) under N_2. After stirring at rt for 30 min, TBAB (0.30 g, 0.9 mmol, 0.2 equiv) was added and the reaction mixture was stirred for another 30 min. 2-Deoxy-3,5-di-*O*-(*p*-toluoyl)-α-D-pentofuranosyl chloride (1.64 g, 4.2 mmol, 0.9 equiv) was added dropwise. The reaction mixture was stirred at rt for 48 h. The solvent was evaporated off under reduced pressure, and the residue was purified by silica gel column chromatography using EtOAc/PE (40:60 v/v) as an eluent. α- and β-Isomers were separated by dissolving the mixture in MeCN by which the β-isomer precipitated. Extraction with $CHCl_3$ isolated the α-isomer.

Yield: 0.77 g (32%) as a yellow solid. ^1H NMR ($CDCl_3$): δ 2.40, 2.46 (2×s, 6H, 2×CH_3), 2.65 (ddd, 1H, J=14.3 Hz, 6.6 Hz, 2.9 Hz, H-2′), 3.76 (dt, J=14.3 Hz, 6.6 Hz, H-2″), 4.65 (q, 1H, J=3.7 Hz, H-4′), 4.74 (dd, 1H, J=11.9 Hz, 4.3 Hz, H-5′), 4.94 (dd, 1H, J=11.9 Hz, 3.7 Hz, H-5′), 6.08 (dt, 1H, J=7.4 Hz, 6.6 Hz, H-3′), 7.10 (t, 1H, J=6.6 Hz, H-1′), 7.17 (d, 2H, J=8.0 Hz, H_{arom}), 7.31 (d, 2H, J=8.0 Hz, H_{arom}), 7.35–7.42 (m, 2H, H_{arom}), 7.68–7.80 (m, 3H, H_{arom}), 7.94 (d, 2H, J=8.0 Hz, H_{arom}), 8.05 (d, 2H, J=8.0 Hz, H_{arom}), 8.16 (dd, 1H, J=8.1 Hz, 1.5 Hz, H_{arom}), 8.29 (dd, 1H, J=8.1 Hz, 1.5 Hz, H_{arom}), 8.46 (dd, 1H, J=8.1 Hz, 1.5 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 21.68, 21.74 (2×CH_3), 34.73 (C-2′), 63.95 (C-5′), 74.64 (C-3′), 81.42 (C-4′), 83.43 (C-1′), 111.61, 120.33, 121.83, 122.72, 126.66, 126.73, 126.92, 128.05, 129.10, 129.12, 129.26, 129.69, 129.84, 130.94, 139.61, 140.02, 142.85, 143.87, 144.31, 145.18 (C_{arom}), 166.15, 166.29 (2×C═O). HRMS (MALDI): m/z calcd for $C_{35}H_{29}N_3O_5Na^+$ (MNa^+): 594.1999, found 594.2005.

4.4.6. 6-(2-Deoxy-β-D-ribofuranosyl)-indolo[2,3-*b*]quinoxaline (8). Sodium (0.056 g, 1 mmol) was dissolved in methanol (20 mL), and **17** (0.383 g, 0.67 mmol) was added. The reaction mixture was stirred at rt for three days. The solution was neutralized with NH_4Cl (s), and stirred for another 30 min. The mixture was filtered and product was purified by silica gel column chromatography using 5% MeOH in CH_2Cl_2 as an eluent.

Yield: 0.165 g (73%) as a yellow solid, R_f 0.35 (8% MeOH/CH_2Cl_2), mp 226 °C. IR (KBr): 3368 cm^{-1}. ^1H NMR (DMSO-d_6): δ 2.23 (ddd, 1H, J=13.0 Hz, 6.3 Hz, 2.5 Hz, H-2′), 3.04–3.14 (m, 1H, H-2″), 3.72 (dd, 1H, J=11.6 Hz, 4.5 Hz, H-5′), 3.82 (dd, 1H, J=11.6 Hz, 3.9 Hz, H-5″), 3.99 (q, 1H, J=3.9 Hz, H-4′), 4.61–4.64 (m, 1H, H-3′), 5.23 (br s, 1H, OH), 5.42 (br s, 1H, OH), 7.01 (dd, 1H, J=8.7 Hz, 6.3 Hz, H-1′), 7.46 (t, 1H, J=7.7 Hz, H_{arom}), 7.72–7.88 (m, 3H, H_{arom}), 8.09 (dd, 2H, J=8.3 Hz, 1.2 Hz, H_{arom}), 8.28 (dd, 1H, J=8.3 Hz, 1.2 Hz, H_{arom}), 8.40 (d, 1H, J=7.7 Hz, H_{arom}). ^{13}C NMR (DMSO-d_6): δ 36.91 (C-2′), 61.76 (C-5′), 70.81 (C-3′), 82.92 (C-4′), 87.10 (C-1′), 112.72, 119.28, 121.64, 122.10, 126.63, 127.44, 128.98, 129.20, 131.32, 138.78, 139.32, 139.79, 142.71, 144.61 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{19}H_{17}N_3O_3Na^+$ (MNa^+): 358.1162, found 358.1151.

4.5. General procedure for DMT-protection of diols 4–8

The diols were dissolved in either dry pyridine (20 mL) (**5** and **6**) or CH_2Cl_2 (20 mL) and Et_3N (1 mL) (**4**, **7** and **8**), and DMT–chloride (1.1–1.5 equiv) were added. The

reaction mixture was stirred at rt for 36 h and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using EtOAc/cyclohexane/Et_3N (33:65:2 v/v/v) as an eluent.

4.5.1. (*S*)-(4,4′-Dimethoxytriphenylmethyloxy)-3-indolo-[2,3-*b*]quinoxalin-6-yl-propan-2-ol (15a). Yield: 0.147 g (44%) as a yellow oil (obtained from 0.164 g, 0.56 mmol of **4**). ^{1}H NMR ($CDCl_3$): δ 2.81 (br s, 1H, OH), 3.16 (dd, 1H, J=6.7 Hz, 9.5 Hz, H-3′), 3.37 (dd, 1H, J=5.1 Hz, 9.5 Hz, H-3′), 3.73, 3.78 (2×s, 6H, 2×OCH_3), 4.39 (m, 1H, H-2′), 4.57 (dd, 1H, J=6.0 Hz, 14.6 Hz, H-1′), 4.64 (dd, 1H, J=3.7 Hz, 14.6 Hz, H-1′), 6.72 (d, 1H, J=8.7 Hz, H_{arom}), 6.81 (d, 4H, J=9.1 Hz, H_{arom}), 7.14–7.40 (m, 9H, H_{arom}), 7.48–7.72 (m, 4H, H_{arom}), 8.01 (dd, 1H, J=8.2 Hz, 1.3 Hz, H_{arom}), 8.25 (dd, 1H, J=8.2 Hz, 1.3 Hz, H_{arom}), 8.41 (d, 1H, J=7.6 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 46.19 (C-1′), 55.14 (2×OCH_3), 64.91 (C-3′), 70.32 (C-2′), 86.42 (C_{DMT}), 110.05, 113.03, 113.12, 119.38, 121.12, 122.50, 126.12, 126.77, 127.02, 127.43, 127.78, 127.99, 129.10, 129.24, 129.93, 130.96, 135.68, 135.81, 139.20, 139.62, 140.15, 144.71, 144.82, 145.94, 158.42, 158.59 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{38}H_{33}N_3O_4Na^+$ (MNa^+): 618.2363, found 618.2379.

4.5.2. (*S*)-1-(4,4′-Dimethoxytriphenylmethyloxy)-3-(2,3-dichloro-indolo[2,3-*b*]quinoxalin-6-yl)-propan-2-ol (15b). Yield: 0.200 g (62%) as yellow foam (obtained from 0.175 g, 0.48 mmol of **5**). ^{1}H NMR ($CDCl_3$): δ 3.01 (br s, 1H, OH), 3.24 (dd, 1H, J=6.1 Hz, 9.7 Hz, H-3′), 3.37 (dd, 1H, J=5.1 Hz, 9.7 Hz, H-3′), 3.76, 3.79 (2×s, 6H, 2×OCH_3), 4.38–4.41 (m, 1H, H-2′), 4.53–4.56 (m, 2H, H-1′), 6.74 (d, 4H, J=8.7 Hz, H_{arom}), 6.82 (d, 1H, J=8.7 Hz, H_{arom}), 7.16–7.69 (m, 11H, H_{arom}), 8.06 (s, 1H, H_{arom}), 8.28 (s, 1H, H_{arom}), 8.59 (d, 1H, J=4.1 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 45.87 (C-1′), 55.04, 55.15 (2×OCH_3), 65.12 (C-3′), 70.01 (C-2′), 86.47 (C_{DMT}), 110.31, 113.06, 113.11, 118.86, 121.51, 122.75, 123.69, 126.84, 127.01, 127.81, 127.99, 129.11, 129.54, 129.93, 131.65, 132.86, 135.64, 135.93, 137.68, 138.56, 140.85, 144.60, 145.10, 149.76, 158.47 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{38}H_{32}Cl_2N_3O_4^+$ (MH^+): 664.1764, found 664.1791.

4.5.3. (*S*)-1-(4,4′-Dimethoxytriphenylmethyloxy)-4-indolo[2,3-*b*]quinoxalin-6-yl-butan-2-ol (15c). Yield: 0.206 g (83%) as a yellow oil (obtained from 0.125 g, 0.41 mmol of **6**). ^{1}H NMR ($CDCl_3$): δ 1.71–2.22 (m, 2H, H-2′), 3.04–3.19 (m, 3H, H-3′, H-4′), 3.55 (br s, 1H, OH), 3.73, 3.79 (2×s, 6H, 2×OCH_3), 4.46 (m, 1H, H-1′), 4.80 (m, 1H, H-1′), 6.71–6.86 (m, 4H, H_{arom}), 7.11–7.53 (m, 11H, H_{arom}), 7.66–7.78 (m, 3H, H_{arom}), 8.09 (dd, 1H, J=8.1 Hz, 1.5 Hz, H_{arom}), 8.31 (dd, 1H, J=8.1 Hz, 1.5 Hz, H_{arom}), 8.49 (d, 1H, J=7.3 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 32.92 (C-2′), 37.97 (C-1′), 55.13, 55.22 (2×OCH_3), 67.29 (C-3′, C-4′), 85.98 (C_{DMT}), 109.45, 113.00, 119.51, 121.12, 122.79, 126.11, 126.66, 127.03, 127.50, 127.68, 127.74, 127.81, 128.09, 128.97, 129.11, 129.35, 129.92, 131.18, 135.99, 139.30, 139.45, 139.87, 144.37, 144.76, 158.33 (C_{arom}).

4.5.4. (*S*)-1-(4,4′-Dimethoxytriphenylmethyloxy)-5-indolo[2,3-*b*]quinoxalin-6-yl-pentan-2-ol (15d). Yield: 0.068 g (34%) as a yellow oil (obtained from 0.103 g, 0.32 mmol of **7**), R_f 0.13 (EtOAc/cyclohexane 1:2). ^{1}H NMR ($CDCl_3$): δ 1.51–1.59 (m, 2H, H-3′), 1.97–2.13 (m, 2H, H-2′), 3.01 (dd, 1H, J=6.8 Hz, 9.3 Hz, H-5′), 3.12 (dd, 1H, J=3.7 Hz, 9.3 Hz, H-5′), 3.74 (s, 6H, 2×OCH_3), 3.91–3.93 (m, 1H, H-4′), 4.48–4.57 (m, 2H, H-1′), 6.74 (d, 4H, J=8.8 Hz, H_{arom}), 7.17 (dd, 2H, J=2.5 Hz, 6.5 Hz, H_{arom}), 7.23 (d, 6H, J=8.8 Hz, H_{arom}), 7.35 (d, 2H, J=7.4 Hz, H_{arom}), 7.45 (d, 1H, J=8.1 Hz, H_{arom}), 7.63–7.73 (m, 3H, H_{arom}), 8.07 (dd, 1H, J=8.1 Hz, 1.6 Hz, H_{arom}), 8.29 (dd, 1H, J=8.1 Hz, 1.6 Hz, H_{arom}), 8.47 (d, 1H, J=7.4 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 24.95 (C-2′), 30.30 (C-3′), 41.28 (C-1′), 55.12 (2×OCH_3), 67.44 (C-5′), 70.89 (C-4′), 85.90 (C_{DMT}), 109.48, 113.00, 119.41, 120.81, 122.68, 125.88, 126.69, 127.65, 127.71, 128.00, 128.71, 129.23, 129.91, 130.96, 135.86, 135.91, 139.16, 140.03, 140.33, 144.30, 144.72, 145.60, 158.35 (C_{arom}).

4.5.5. 6-[2-Deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-ribofuranosyl]-indolo[2,3-*b*]quinoxaline (18). Yield: 0.148 g (58%) as a yellow oil (obtained from 0.134 g, 0.40 mmol of **8**), R_f 0.35 (4% MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 2.36–2.46 (m, 2H, H-2′), 3.43–3.56 (m, 2H, H-5′), 3.72 (s, 6H, 2×OCH_3), 4.16 (m, 1H, H-4′), 5.02 (m, 1H, H-3′), 6.71 (m, 4H, H_{arom}), 7.00 (t, 1H, J=7.1 Hz, H-1′), 7.15–7.43 (m, 11H, H_{arom}), 7.66–7.71 (m, 2H, H_{arom}), 7.80 (d, 1H, J=7.6 Hz, H_{arom}), 7.92 (dd, 1H, J=7.6 Hz, 2.2 Hz, H_{arom}), 8.26 (dd, 1H, J=7.6 Hz, 2.2 Hz, H_{arom}), 8.44 (dd, 1H, J=7.6 Hz, 2.2 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 37.21 (C-2′), 55.15 (2×OCH_3), 63.64 (C-5′), 72.69 (C-3′), 83.09 (C-4′), 84.98 (C-1′), 86.46 (C_{DMT}), 111.98, 113.05, 120.11, 121.61, 122.57, 126.44, 126.80, 127.77, 127.98, 128.18, 128.72, 129.13, 130.04, 130.09, 130.97, 135.72, 135.79, 139.38, 140.02, 140.33, 143.12, 144.65, 145.19, 158.42, 158.45 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{40}H_{35}N_3O_5Na^+$ (MNa^+): 660.2469, found 660.2450.

4.6. General procedure for synthesis of phosphoramidites

The DMT-protected compound (**15a–d**, **18**) was mixed with diisopropylammoniumtetrazolide (1.7 equiv) and dissolved in dry CH_2Cl_2 (10 mL). 2-Cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphane (2.5 equiv) was added, and the reaction mixture was stirred at rt overnight. The solvent was evaporated off under reduced pressure, and the residue was purified by silica gel column chromatography using EtOAc/cyclohexane/Et_3N (49:49:2 v/v/v) as an eluent.

4.6.1. Phosphoramidite of (*S*)-1-(4,4′-dimethoxytriphenylmethyloxy)-3-indolo[2,3-*b*]quinoxalin-6-yl-propan-2-ol (16a). Yield: 0.078 g (61%) as a yellow oil (obtained from 0.095 g, 0.16 mmol of **15a**), R_f 0.58 (EtOAc/cyclohexane 1:1). ^{31}P NMR ($CDCl_3$): δ 149.96, 150.27. HRMS (MALDI): m/z calcd for $C_{47}H_{50}N_5O_5PNa^+$ (MNa^+): 818.3442, found 818.3401.

4.6.2. Phosphoramidite of (*S*)-1-(4,4′-dimethoxytriphenylmethyloxy)-3-(2,3-dichloro-indolo[2,3-*b*]quinoxalin-6-yl)-propan-2-ol (16b). Yield: 0.146 g (66%) as a yellow oil (obtained from 0.170 g, 0.26 mmol of **15b**), R_f 0.64 (EtOAc/cyclohexane 1:1). ^{1}H NMR ($CDCl_3$): δ 0.74–1.28 (m, 12H, 4×CH_3), 2.06, 2.32 (2×t, 2H, J=6.6 Hz, CH_2CN),

3.22–3.60 (m, 6H, H-3′, OCH_2CH$_2$CN, 2×CH(CH$_3$)$_2$), 3.77 (s, 6H, 2×OCH$_3$), 4.07–4.16 (m, 1H, H-2′), 4.62–4.67 (m, 2H, H-1′), 6.76–6.84 (m, 4H, H$_{arom}$), 7.16–7.69 (m, 12H, H$_{arom}$), 8.12–8.41 (m, 3H, H$_{arom}$). ^{13}C NMR (CDCl$_3$): δ 20.09 (CH$_2$CN), 24.28, 24.40, 24.46, 24.54 (4×CH(CH$_3$)$_2$), 42.73, 42.92 (2×CH(CH$_3$)$_2$), 44.42 (C-1′), 55.17 (2×OCH$_3$), 57.75 (OCH$_2$CH$_2$CN), 64.87 (C-3′), 70.63 (C-2′), 86.28 (C$_{DMT}$), 110.57, 113.02 (C$_{arom}$), 119.08 (CN), 121.29, 122.76, 126.76, 127.73, 128.11, 128.27, 129.10, 129.70, 129.98, 131.50, 132.60, 135.79, 135.98, 137.83, 139.27, 144.71, 145.10, 145.93, 158.43 (C$_{arom}$). ^{31}P NMR (CDCl$_3$): δ 150.20, 150.34. HRMS (ESI): *m/z* calcd for C$_{47}$H$_{48}$N$_5$O$_5$Cl$_2$PNa$^+$ (MNa$^+$): 886.2662, found 886.2684.

4.6.3. Phosphoramidite of (*S*)-1-(4,4′-dimethoxytriphenylmethyloxy)-4-indolo[2,3-*b*]quinoxalin-6-yl-butan-2-ol (16c). Yield: 0.206 g (84%) as a yellow oil (obtained from 0.185 g, 0.30 mmol of **15c**). ^{1}H NMR (CDCl$_3$): δ 1.04–1.26 (m, 12H, 4×CH$_3$), 2.23–2.38 (m, 2H, H-2′), 2.40, 2.59 (2×t, 2H, J=6.5 Hz, CH$_2$CN), 3.10, 3.24 (2×dd, 1H, J=6.2 Hz, 9.2 Hz, H-4′), 3.36 (m, 1H, H-4′), 3.55–3.71 (m, 4H, OCH_2CH$_2$CN, 2×CH(CH$_3$)$_2$), 3.77, 3.79 (2×s, 6H, 2×OCH$_3$), 4.51–4.61 (m, 2H, H-1′), 4.17–4.22 (m, 1H, H-3′), 6.76–6.84 (m, 4H, H$_{arom}$), 7.16–7.76 (m, 14H, H$_{arom}$), 8.04 (m, 1H, H$_{arom}$), 8.29 (dd, 1H, J=8.1 Hz, 1.3 Hz, H$_{arom}$), 8.47 (d, 1H, J=7.6 Hz, H$_{arom}$). ^{31}P NMR (CDCl$_3$): δ 149.47, 149.70.

4.6.4. Phosphoramidite of (*S*)-1-(4,4′-dimethoxytriphenylmethyloxy)-5-indolo[2,3-*b*]quinoxalin-6-yl-pentan-2-ol (16d). Yield: 0.066 g (73%) as a yellow oil (obtained from 0.068 g, 0.11 mmol of **15d**), R_f 0.58 (EtOAc/cyclohexane 1:1). ^{1}H NMR (CDCl$_3$): δ 1.69–2.00 (m, 2H, H-3′), 2.34, 2.64 (2×t, 2H, J=6.5 Hz, CH$_2$CN), 2.90 (dd, 1H, J=6.2 Hz, 9.2 Hz, H-5′), 3.01–3.15 (m, 1H, H-5′), 3.41–3.51, 3.56–3.70 (2×m, 4H, OCH_2CH$_2$CN, 2×CH(CH$_3$)$_2$), 3.73 (s, 6H, 2×OCH$_3$), 3.97–4.06 (m, 1H, H-4′), 4.47–4.53 (m, 2H, H-1′), 6.71 (m, 4H, H$_{arom}$), 7.13–7.75 (m, 14H, H$_{arom}$), 8.07 (m, 1H, H$_{arom}$), 8.29 (dd, 1H, J=8.1 Hz, 1.2 Hz, H$_{arom}$), 8.48 (d, 1H, J=7.6 Hz, H$_{arom}$). ^{13}C NMR (CDCl$_3$): δ 20.29 (CH$_2$CN), 23.71 (C-2′), 24.36, 24.46, 24.55, 24.66 (4×CH$_3$), 30.85 (C-3′), 41.35 (C-1′), 42.87, 43.04 (2×CH(CH$_3$)$_2$), 55.12 (2×OCH$_3$), 58.09 (OCH$_2$CH$_2$CN), 65.66 (C-5′), 72.70 (C-4′), 85.76 (C$_{DMT}$), 109.52, 112.91, 117.61, 119.44, 120.75, 122.66, 125.85, 126.55, 126.62, 127.62, 127.77, 127.81, 128.04, 128.11, 128.65, 129.31, 129.95, 130.91, 136.00, 139.21, 140.01, 144.34, 144.46, 144.86, 158.26, 158.31 (C$_{arom}$). ^{31}P NMR (CDCl$_3$): δ 149.08, 149.60. HRMS (MALDI): *m/z* calcd for C$_{49}$H$_{54}$N$_5$O$_5$PNa$^+$ (MNa$^+$): 846.3755, found 846.3778.

4.6.5. Phosphoramidite of 6-[2-deoxy-5-*O*-(4,4′-dimethoxytriphenylmethyl)-β-D-ribofuranosyl]-indolo[2,3-*b*]quinoxaline (19). Yield: 0.033 g (21%) as a yellow oil (obtained from 0.119 g, 0.19 mmol of **18**). ^{1}H NMR (CDCl$_3$): δ 1.19–1.28 (m, 12H, 4×CH$_3$), 2.47, 2.63 (2×t, 2H, J=6.5 Hz, CH$_2$CN), 3.41–3.92 (m, 8H, H-2′, H-5′, OCH_2CH$_2$CN, 2×CH(CH$_3$)$_2$), 3.73 (2×s, 6H, 2×OCH$_3$), 4.30 (m, 1H, H-4′), 5.04–5.18 (m, 1H, H-3′), 6.66–6.72 (m, 4H, H$_{arom}$), 7.01–7.43 (m, 12H, H-1′, H$_{arom}$), 7.70 (m, 2H, H$_{arom}$), 7.89–7.96 (m, 2H, H$_{arom}$), 8.27 (dd, 1H, J=7.6 Hz, 2.2 Hz, H$_{arom}$), 8.43–8.46 (m, 1H, H$_{arom}$). ^{13}C NMR (CDCl$_3$): δ 20.33 (CH$_2$CN), 24.52, 24.54, 24.60, 24.67 (4×CH(CH$_3$)$_2$), 36.39 (C-2′), 43.21, 43.32 (2×CH(CH$_3$)$_2$), 55.15 (2×OCH$_3$), 58.53 (OCH$_2$CH$_2$CN), 62.98 (C-5′), 73.32 (C-3′), 83.29 (C-4′), 84.73 (C-1′), 86.30 (C$_{DMT}$), 112.29, 112.97 (C$_{arom}$), 117.45 (CN), 120.14, 121.58, 122.47, 126.37, 126.76, 127.68, 127.93, 128.31, 128.68, 129.21, 130.17, 130.97, 135.70, 135.74, 135.78, 135.81, 139.45, 140.04, 143.11, 144.64, 145.22, 158.39 (C$_{arom}$). ^{31}P NMR (CDCl$_3$): δ 149.63, 150.02. HRMS (MALDI): *m/z* calcd for C$_{49}$H$_{52}$N$_5$O$_6$PNa$^+$ (MNa$^+$): 860.3547, found 860.3578.

4.6.6. 6-Methylindolo[2,3-*b*]quinoxaline-3-carboxylic acid methyl ester (21). 6*H*-Indolo[2,3-*b*]quinoxaline-3-carboxylic acid[58] (**20**, 2.50 g, 9.5 mmol) was suspended in dry DMSO (25 mL) and powdered KOH (1.85 g, 33 mmol, 3.5 equiv) was added. The reaction mixture turned red, as it was stirred for 15 min CH$_3$I (5.11 g, 36 mmol, 3.8 equiv) was added through a syringe. The reaction mixture was stirred at rt for 48 h. N_2 was bubbled through the reaction mixture in order to get rid of excess of CH$_3$I. H_2O (50 mL) was added, and the compound precipitated. The yellow powder was filtered, washed with water and dried in vacuo. A small sample was separated for analysis by silica gel column chromatography using EtOAc/PE 1:1 as an eluent. The rest was used for the next step without further purification. The ratio between the ester and the carboxylic acid was 3:2.

Yellow solid, R_f 0.53 (EtOAc/PE 1:1), mp 180 °C. ^{1}H NMR (CDCl$_3$): δ 3.87, 4.00 (2×s, 6H, 2×CH$_3$), 7.35 (d, 2H, J=7.3 Hz, H$_{arom}$), 7.66 (t, 1H, J=7.3 Hz, H$_{arom}$), 8.21 (m, 2H, H$_{arom}$), 8.37 (d, 1H, J=7.2 Hz, H$_{arom}$), 8.73 (s, 1H, H$_{arom}$). ^{13}C NMR (CDCl$_3$): δ 27.39 (CH$_3$), 52.34 (OCH$_3$), 109.20, 118.88, 121.10, 122.91, 125.31, 129.30, 129.61, 130.30, 131.65, 139.45, 141.12, 141.38, 145.32, 145.88 (C$_{arom}$), 166.71 (COOR). HRMS (MALDI): *m/z* calcd for C$_{17}$H$_{14}$N$_3$O$_2^+$ (MH$^+$): 292.1081, found 292.1071.

4.6.7. 6-Methylindolo[2,3-*b*]quinoxaline-3-carboxylic acid (22). Yellow solid, R_f 0.42 (EtOAc/PE 1:1), mp 192 °C. ^{1}H NMR (DMSO-d_6): δ 3.78 (s, 3H, CH$_3$), 7.34 (t, 1H, J=7.5 Hz, H$_{arom}$), 7.57 (d, 1H, J=8.0 Hz, H$_{arom}$), 7.71 (t, 1H, J=7.5 Hz, H$_{arom}$), 7.93–8.14 (m, 2H, H$_{arom}$), 8.22 (d, 1H, J=7.5 Hz, H$_{arom}$), 8.46 (s, 1H, H$_{arom}$). ^{13}C NMR (DMSO-d_6): δ 27.30 (CH$_3$), 110.06, 117.98, 120.96, 122.26, 124.95, 129.04, 129.28, 130.29, 131.79, 138.78, 140.21, 140.64, 145.04, 145.17 (C$_{arom}$), 166.96 (COOH). HRMS (MALDI): *m/z* calcd for C$_{16}$H$_{11}$N$_3$O$_2^+$ (MH$^+$): 278.0924, found 278.0922.

4.6.8. (6-Methylindolo[2,3-*b*]quinoxalin-3-yl)methanol (23). LiAlH$_4$ (0.65 g, 17.1 mmol) was suspended in dry THF (20 mL) at 0 °C under N_2. The crude mixture of **21** and **22** was dissolved in dry THF (20 mL) and added dropwise using a syringe. The icebath was removed and the reaction was gently refluxed for 5 h. The reaction was quenched at 0 °C by carefully adding 1 mL H_2O, 1 mL of a 15% aq solution of NaOH and 3 mL of H_2O. The reaction mixture was filtered and washed with ethanol. The solvent was evaporated off under reduced pressure, and the residue was purified by silica gel column chromatography using 5% MeOH/CHCl$_3$ as an eluent.

Yield: 1.10 g (44% over two steps) as yellow crystals, R_f 0.34 (5% MeOH/$CHCl_3$), mp 175 °C. IR (KBr): 3371 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.89 (s, 1H, CH_3), 4.80 (d, 2H, J=5.4 Hz, CH_2), 5.53 (t, 1H, OH), 7.39 (t, 1H, J=7.7 Hz, H_{arom}), 7.66–7.80 (m, 3H, H_{arom}), 8.03 (s, 1H, H_{arom}), 8.20 (d, 1H, J=8.7 Hz, H_{arom}), 8.35 (d, 1H, J=7.7 Hz, H_{arom}). ^{13}C NMR (DMSO-d_6): δ 27.36 (CH_3), 62.77 (CH_2), 110.01, 118.44, 120.78, 121.81, 123.78, 124.91, 128.61, 130.96, 137.59, 138.80, 139.83, 143.71, 144.44, 145.16 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{16}H_{14}N_3O^+$ (MH^+): 264.1131, found 264.1128.

4.6.9. 3-Chloromethyl-6-methylindolo[2,3-*b*]quinoxaline (24). (6-Methylindolo[2,3-*b*]quinoxalin-3-yl)methanol (**23**, 0.500 g, 1.9 mmol) was suspended in a mixture of pyridine (0.23 mL) and CH_2Cl_2 (10 mL) and the suspension was cooled to 0 °C. $SOCl_2$ (0.25 mL) was added slowly with a syringe. The reaction was stirred at rt overnight. The reaction mixture was poured into water (20 mL) and CH_2Cl_2 (10 mL) was added. The two-phase system was stirred for 30 min, after which the phases were separated. The organic phase was washed with 5% $NaHCO_3$ (aq) (2×25 mL) and satd aq NaCl (2×25 mL) and dried using $MgSO_4$. The solvent was removed under reduced pressure yielding a yellow solid, which was purified by silica gel column chromatography using 1% MeOH/CH_2Cl_2 as eluent.

Yield: 0.478 g (89%) as a yellow solid, R_f 0.50 (1% MeOH/CH_2Cl_2), mp 204–205 °C. ^1H NMR (CD_2Cl_2): δ 3.84 (s, 3H, CH_3), 4.78 (s, 2H, CH_2), 7.30 (t, 1H, J=7.4 Hz, H_{arom}), 7.40 (d, 1H, J=8.2 Hz, H_{arom}), 7.59–7.64 (m, 2H, H_{arom}), 8.00 (s, 1H, H_{arom}), 8.13 (d, 1H, J=8.6 Hz, H_{arom}), 8.32 (d, 1H, J=7.9 Hz, H_{arom}). ^{13}C NMR (CD_2Cl_2): δ 27.76 (CH_3), 46.57 (CH_2), 109.83, 119.34, 121.52, 122.92, 126.71, 127.44, 129.66, 129.87, 131.80, 138.66, 138.84, 140.54, 140.79, 145.60 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{16}H_{13}ClN_3^+$ (MH^+): 282.0973, found 282.0795.

4.6.10. 3-[2-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)ethoxymethyl]-6-methylindolo[2,3-*b*]quinoxaline (25). To a solution of 2-((*S*)-2,2-dimethyl-1,3-dioxolan-4-yl)-ethanol (0.180 g, 1.23 mmol) in dry toluene (15 mL) was added pulverized KOH (0.736 g, 13.0 mmol) followed by 3-chloromethyl-6-methylindolo[2,3-*b*]quinoxaline (**24**, 0.205 g, 0.73 mmol). A Dean–Stark apparatus was filled with dry toluene in the side arm and used, while refluxing the reaction mixture for 48 h. H_2O (10 mL) was added and the organic phase was washed with H_2O (3×10 mL), dried using $MgSO_4$ and evaporated under reduced pressure yielding a yellow oil, which was purified by silica gel column chromatography using 2% MeOH/CH_2Cl_2 as an eluent.

Yield: 0.190 g (67%) as a yellow oil, R_f 0.46 (4% MeOH/CH_2Cl_2). ^1H NMR ($CDCl_3$): δ 1.37, 1.41 (2×s, 6H, 2×CH_3), 1.91–1.97 (m, 2H, H-2′), 3.59–3.64 (m, 1H, H-3′), 3.69 (t, 2H, J=6.8 Hz, H-1′), 3.94 (s, 3H, NCH_3), 4.08–4.13 (m, 1H, H-4′), 4.26–4.30 (m, 1H, H-4″), 4.76 (s, 2H, CH_2), 7.34–7.43 (m, 2H, H_{arom}), 7.62–7.70 (m, 2H, H_{arom}), 7.97–8.21 (m, 1H, H_{arom}), 8.25 (d, 1H, J=8.6 Hz, H_{arom}), 8.44 (d, 1H, J=7.7 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 25.78, 26.95 ($C(CH_3)_2$), 27.48 (CH_3), 33.92 (C-2′), 67.35 (C-1′), 69.67 (C-4′), 72.85 (CH_2), 73.80 (C-3′), 108.58 ($C(CH_3)_2$), 109.14, 119.35, 120.93, 122.54, 125.43, 125.76, 129.40, 130.90, 137.90, 138.67, 139.35, 140.39, 144.84, 145.68 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{23}H_{25}N_3O_3Na^+$ (MNa^+): 414.1788, found 414.1776.

4.6.11. (*S*)-4-(6-Methylindolo[2,3-*b*]quinoxalin-3-ylmethoxy)-butane-1,2-diol (9). The same procedure was used as for compounds **4**–**7**.

Quantitative yield. Yellow oil. ^1H NMR (DMSO-d_6): δ 1.53–1.62, 1.80–1.90 (2×m, 2H, H-2′), 3.23–3.32 (m, 2H, H-4′), 3.66 (t, 2H, J=6.5 Hz, H-1′), 3.82–3.95 (m, 1H, H-3′), 3.89 (s, 3H, NCH_3), 4.51 (br s, 2H, 2×OH), 4.73 (s, 2H, CH_2), 7.39 (t, 1H, J=7.3 Hz, H_{arom}), 7.62–7.83 (m, 3H, H_{arom}), 7.99–8.13 (m, 1H, H_{arom}), 8.19 (d, 1H, J=8.6 Hz, H_{arom}), 8.33 (d, 1H, J=7.6 Hz, H_{arom}). ^{13}C NMR (DMSO-d_6): δ 27.40 (CH_3), 33.67 (C-2′), 66.04 (C-1′), 67.13 (C-4′), 68.44 (C-3′), 71.47 (CH_2), 110.09, 118.40, 120.83, 121.89, 124.94, 125.31, 128.83, 131.09, 137.77, 139.10, 139.68, 139.90, 144.58, 145.21 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{20}H_{21}N_3O_3Na^+$ (MNa^+): 374.1475, found 374.1462.

4.6.12. (*S*)-1-(4,4′-Dimethoxytriphenylmethyloxy)-4-(6-methylindolo[2,3-*b*]quinoxalin-3-ylmethoxy)-butan-2-ol (26). The same procedure was used as for DMT-protection of compounds **4**–**8**.

Yield: 0.149 g (55%) as a yellow oil, ^1H NMR ($CDCl_3$): δ 1.80–1.86 (m, 2H, H-2′), 3.13–3.17, 3.68–3.73 (2×m, 4H, H-1′, H-4′), 3.76 (s, 6H, 2×OCH_3), 3.96 (s, 3H, NCH_3), 4.02–4.12 (m, 1H, H-3′), 4.74 (s, 2H, CH_2), 6.81 (d, 4H, J=8.7 Hz, H_{arom}), 7.19–7.29 (m, 3H, H_{arom}), 7.32 (d, 4H, J=8.7 Hz, H_{arom}), 7.36–7.46 (m, 4H, H_{arom}), 7.59 (dd, 1H, J=8.5 Hz, 1.8 Hz, H_{arom}), 7.70 (t, 1H, J=7.0 Hz, H_{arom}), 7.98–8.20 (m, 1H, H_{arom}), 8.25 (d, 1H, J=8.7 Hz, H_{arom}), 8.46 (d, 1H, J=7.8 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 27.50 (CH_3), 33.53 (C-2′), 55.16 (OCH_3), 67.33 (C-1′), 68.14 (C-4′), 69.43 (C-3′), 72.95 (CH_2), 85.97 (C_{DMT}), 109.16, 113.06, 120.88, 120.95, 122.58, 125.47, 125.85, 126.72, 127.77, 128.14, 129.42, 130.02, 130.93, 136.05, 138.67, 139.21, 140.38, 144.85, 158.41 (C_{arom}). HRMS (MALDI): m/z calcd for $C_{20}H_{39}N_3O_5Na^+$ (MNa^+): 676.2782, found 676.2757.

4.6.13. Phosphoramidite of (*S*)-1-(4,4′-dimethoxytriphenylmethyloxy)-4-(6-methylindolo[2,3-*b*]quinoxalin-3-ylmethoxy)-butan-2-ol (27). The same procedure was used as for phosphitylation of compounds **15a**–**d**.

Yield: 0.061 g (47%) as a yellow oil, R_f 0.52 (EtOAc/cyclohexane 1:1). ^1H NMR ($CDCl_3$): δ 1.04–1.17 (m, 12H, 4×CH_3), 2.38, 2.53 (2×t, 2H, J=6.5 Hz, CH_2CN), 1.92–1.99 (m, 1H, H-2′), 2.08–2.17 (m, 1H, H-2′), 3.02–3.07, 3.20–3.23 (2×m, 2H, H-4′), 3.49–3.72 (m, 6H, H-1′, OCH_2CH_2CN, 2×$CH(CH_3)_2$), 3.76 (s, 6H, 2×OCH_3), 3.98 (s, 3H, NCH_3), 4.19–4.22 (m, 1H, H-3′), 4.70–4.74 (m, 2H, CH_2), 6.77–6.82 (m, 4H, H_{arom}), 7.16–7.29 (m, 4H, H_{arom}), 7.34 (d, 4H, J=8.8 Hz, H_{arom}), 7.40 (t, 1H, J=7.5 Hz, H_{arom}), 7.46 (dd, 2H, J=2.9 Hz, 7.3 Hz, H_{arom}), 7.62 (m, 1H, H_{arom}), 7.71 (t, 1H, J=7.3 Hz, H_{arom}), 8.06 (d, 1H, J=8.6 Hz, H_{arom}), 8.25 (dd, 1H, J=1.0 Hz, 8.6 Hz, H_{arom}), 8.47 (d, 1H, J=7.5 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$): δ 21.03 (CH_2CN), 24.43, 24.54, 24.60, 24.73 (4×CH_3),

26.90 (CH_3), 33.84 (C-2′), 42.33, 42.81 (2×*C*H(CH_3)$_2$), 55.15 (2×OCH_3), 58.23 (O*C*H_2CH_2CN), 64.61 (C-1′), 66.31 (C-4′), 67.08 (C-3′), 72.72 (CH_2), 85.86 (C_{DMT}), 109.19, 112.97 (C_{arom}), 119.33 (CN), 120.95, 122.57, 125.56, 125.77, 126.59, 127.67, 128.24, 129.34, 130.16, 130.92, 136.19, 138.03, 139.27, 140.44, 144.06, 144.99, 158.41 (C_{arom}). ^{31}P NMR ($CDCl_3$): δ 148.57, 149.06. HRMS (MALDI): *m*/*z* calcd for $C_{50}H_{56}N_5O_6PNa^+$ (MNa^+): 876.3860, found 876.3865.

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-609476 (**4**) and CCDC-609477 (**23**·H_2O). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44 1223 36033 or e-mail: deposit@ccdc.cam.ac.uk).

4.7. ODN and INA syntheses, purification and measurement of melting temperatures

The ODN and INA syntheses were carried out on an Expedite™ Nucleic Acid Synthesis System Model 8909 from Applied Biosystems. The 6*H*-indolo[2,3-*b*]quinoxaline amidits **16a–d**, **19** and **27** were dissolved in dry MeCN (if necessary a 1:1 mixture of dry MeCN and dry CH_2Cl_2), as a 0.075 M solution and inserted into the growing oligonucleotide chain using the same conditions as for normal nucleotide couplings (2 min coupling). The ODNs were synthesized with DMT and purified on a Waters Prep LC 4000 HPLC with a Waters Prep LC controller and a Waters 2487 Dual λ Absorbance detector on a Waters Xterra™ MS C_{18} column. Buffer A [950 mL of 0.1 M NH_4HCO_3 and 50 mL of MeCN (pH=9.0)] and buffer B [250 mL of 0.1 M NH_4HCO_3 and 750 mL of MeCN (pH=9.0)]. Gradients: 5 min 100% A, linear gradient to 70% B in 30 min, 2 min with 70% B, linear gradient to 100% B in 8 min and then 100% A in 15 min (product peak at ~35 min). The ODNs were DMT-deprotected in 80% aq acetic acid (100 μL) for 20 min, diluted with 1 M sodium acetate (150 μL), and precipitated from abs ethanol (600 μL). All modified ODNs were confirmed by MALDI-TOF analysis on a Voyager Elite Bio Spectrometry Research Station from Perceptive Biosystems.

Melting temperature measurements were performed on a Perkin–Elmer Lambda 20 UV/VIS spectrometer fitted with a PTP-6 temperature programmer. Melting temperature (T_m) measurements were determined in a 1 mM EDTA, 10 mM $Na_2HPO_4·2H_2O$, 140 mM NaCl buffer at pH=7.0 for 1.5 μM of each strand. The melting temperature was determined as the maximum of the first derivative plots of the melting curve and are with an uncertainty of ±1.0 °C as determined by repetitive experiments.

4.8. Fluorescence measurements

Fluorescence measurements were performed on a Perkin–Elmer LS-55 luminescence spectrometer fitted with a Julabo F25 temperature controller by an excitation at 360 nm and detection at 370–700 nm. All measurements were conducted at 10 °C in a 140 mM NaCl, 10 mM sodium phosphate, 1 mM EDTA buffer at pH=7.0 with a concentration of 1.5 μM of each strand.

4.9. Single-crystal X-ray diffraction

Crystals of **4** and **23**·H_2O were obtained from solutions in MeOH/CH_2Cl_2, layered with hexane. X-ray diffraction data for **4** were collected at Beamline I911-3 at the MAX-II storage ring, MAX-lab, University of Lund, Sweden (λ=0.750 Å). Diffraction data for **23**·H_2O were collected locally with a Bruker Nonius X8APEX-II instrument. Molecule **4** crystallizes in the non-centrosymmetric space group $P2_1$ with two molecules in the asymmetric unit. The ring systems adopt a centrosymmetric arrangement (space group $P2_1/n$), but the centrosymmetry is broken by the chiral side-chains. It was not possible to determine the absolute structure: the *S* enantiomer was assigned on the basis of the chemistry and Friedel opposites were merged as equivalent data in the final cycles of refinement.

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Acid-mediated three-component aza-Diels–Alder reactions of 2-aminophenols under controlled microwave heating for synthesis of highly functionalized tetrahydroquinolines. Part 9: Chemistry of aminophenols☆

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Abstract—The aza-Diels–Alder reactions of two 2-aminophenols in combination with six substituted benzaldehydes and two electron-rich cyclic alkenes were investigated under controlled microwave heating. The reactions were carried out in the presence of a catalytic amount of CF_3CO_2H in MeCN at 60 °C for 15 min, affording highly functionalized 8-hydroxy-1,2,3,4-tetrahydroquinolines in 39–59% isolated yields and in 36:64–16:84 diastereomer ratios in favor of the trans isomers. The microwave-heated three-component aza-Diels–Alder reactions completed in significantly reduced reaction time to give the same level of chemical yield and diastereomer ratio obtained from the room temperature reaction.

1. Introduction

1,2,3,4-Tetrahydroquinoline[1] is an important heterocyclic scaffold of medicinal and therapeutical interests. Among the currently available synthetic methodologies, the aza-Diels–Alder reaction is the most enabling and versatile approach to functionalized 1,2,3,4-tetrahydroquinolines.[2] The reaction can be carried out in one-pot fashion starting from an aniline, an aldehyde, and an electron-rich alkene, which is known as one of the three-component reactions.[2b] Activation of *N*-arylaldimines toward cycloadditions with alkenes is required. Lanthanide triflates, other Lewis acids,[3,4] and protic acids[5] are the catalysts of choice. In recent years miscellaneous promoters have been reported, including molecular iodine, perchlorates, montmorillonite clay, Selectfluor™ fluorinating reagent, and polymer-supported benzotriazole.[6] The aza-Diels–Alder reactions have been reported to proceed under photochemical conditions,[7] and in aqueous media,[8] fluorinated solvents,[9] and ionic liquids.[10] Moreover, it has been tailored for solid-phase synthesis[11] and used as the key step in alkaloid synthesis.[12] During the past few years, we have established methodologies for diversity-oriented synthesis of indoles,[13] benzofurans,[14] 1,4-benzoxazines,[15a–c] and dibenz[*b*,*f*][1,4]oxazepines[15d] by using readily available 2-aminophenols as the starting materials. In connection with our interest in microwave-assisted organic synthesis,[13c,15,16] we have successfully combined the Ugi four-component reaction (U-4CR) of 2-aminophenols with microwave chemistry for high-throughput synthesis of heterocycles.[15c,d] In this article, we report on aza-Diels–Alder reactions of 2-aminophenols under controlled microwave heating[17] for synthesis of highly functionalized 8-hydroxy-1,2,3,4-tetrahydroquinolines.[18]

2. Results and discussion

Although substituted anilines have been extensively used in the three-component aza-Diels–Alder reactions, there are very limited examples of 1,2,3,4-tetrahydroquinolines synthesized via the aza-Diels–Alder reactions of 2-aminophenols.[4c,h,o,p] Kobayashi and co-worker applied 2-aminophenol-derived *N*-arylaldimines in asymmetric aza-Diels–Alder reactions catalyzed by a chiral Yb complex.[4c] The phenolic hydroxy group proved to be essential for high asymmetric induction. Qian and co-workers reported the $GdCl_3$-catalyzed one-pot aza-Diels–Alder reaction of 2-aminophenol (**1a**) with benzaldehyde (**2a**) and 3,4-dihydro-2*H*-pyran (**3a**) in MeCN at room temperature for 90 min to afford the pyrano[3,2-*c*]quinolines **4a** and **5a** in 60% combined yield and in a 35:65 isomer ratio (Scheme 1).[4h] Similar reaction using 2,3-dihydrofuran (**3b**) as the alkene

☆ For Part 8, see Ref. 15d.
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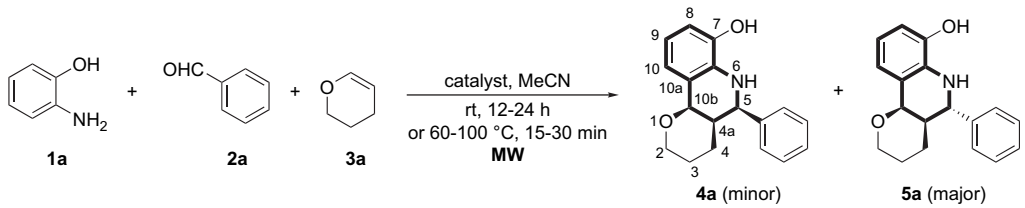

Scheme 1. Three-component aza-Diels–Alder reaction of 2-aminophenol **1a**.

component furnished the corresponding furo[3,2-*c*]quinolines in 67% combined yield and in a 48:52 isomer ratio. As compared to other anilines used by Qian et al.,[4h] lower chemical yields were observed for the aza-Diels–Alder reaction of 2-aminophenol (**1a**). In another report by Batey and co-worker, <20% yield was obtained for the aza-Diels–Alder reaction of 2-aminophenol (**1a**) with 2-ethoxytetrahydrofuran and cyclopentadiene catalyzed by $Dy(OTf)_3$ in MeCN at room temperature for 24 h.[4p] We began our investigation with the aza-Diels–Alder reaction of **1a** with **2a** and **3a** as shown in Scheme 1. Some results on optimization of reaction conditions under controlled microwave heating along with the room temperature entries are summarized in Table 1.

We first examined the room temperature reaction in MeCN in the presence of CF_3CO_2H (Table 1, entries 1 and 2). The adducts **4a** and **5a** were isolated in 43% and 57% yields by using 1.1 equiv (12 h) and 0.11 equiv (24 h) of the acid, respectively. The isomer ratio of **4a**:**5a** was 18:82 in both trials. An improved yield of 65% was obtained for the reaction conducted by using 11 mol % of $Yb(OTf)_3$ as the catalyst, but the isomer ratio of **4a**:**5a** decreased to 36:64 (Table 1, entry 3). The latter results are consistent with those of Qian et al. by employing 20 mol % of $GdCl_3$.[4h] We then performed the same aza-Diels–Alder reaction in MeCN in the presence of 0.01–0.11 equiv of CF_3CO_2H with microwave heating at 60–100 °C for 15–30 min (Table 1, entries 4–8). The reactions at 60 °C afforded better yields. After adjusting the reactant ratio of **1a**:**2a**:**3a** to 1.2:1.2:1.0, the adducts **4a** and **5a** were produced in 59% yield and in 18:82 ratio with microwave irradiation at 60 °C for 15 min in the presence of 0.13 equiv of CF_3CO_2H (Table 1, entry 9). Unfortunately, the microwave-assisted reactions with $Yb(OTf)_3$ catalyst gave inferior yields and isomer ratios (Table 1, entries 10–12).

In Kobayashi's[4c] and Qian's[4h] studies on aza-Diels–Alder reactions involving 2-aminophenol (**1a**), only two aromatic aldehydes, 1-naphthaldehyde and benzaldehyde (**2a**), were used. With the optimized reaction conditions given in the entry 9 of Table 1, we carried out the microwave-assisted aza-Diels–Alder reactions by combination of two aminophenols **1a**,**b**, six aromatic aldehydes **2a–f**, and two cyclic alkenes **3a**,**b** (Scheme 2). According to the results listed in Table 2, the reactions of 3,4-dihydro-2*H*-pyran (**3a**) generally gave higher yields (48–59%) and stereoselectivities (24:76–16:84) (Table 2, entries 1–4, 9, and 10) as compared to those of 2,3-dihydrofuran (**3b**). The latter afforded the

Table 1. Optimization of reaction conditions for three-component aza-Diels–Alder reaction of 2-aminophenol **1a**[a]

Entry	Conditions	Catalyst[b]	Combined yield (%)[c]	Ratio of **4a**:**5a**[d]
1	**1a**:**2a**:**3a**=1.0:1.1:1.2, rt, 12 h	CF_3CO_2H (1.1 equiv)	43	18:82
2	**1a**:**2a**:**3a**=1.0:1.1:1.2, rt, 24 h	CF_3CO_2H (0.11 equiv)	57	18:82
3	**1a**:**2a**:**3a**=1.0:1.1:1.2, rt, 24 h	$Yb(OTf)_3$ (0.11 equiv), $MgSO_4$	65 (60)[e]	36:64 (35:65)[e]
4	**1a**:**2a**:**3a**=1.0:1.1:1.2, 60 °C, 15 min	CF_3CO_2H (0.11 equiv)	46	17:83
5	**1a**:**2a**:**3a**=1.0:1.1:1.2, 60 °C, 30 min	CF_3CO_2H (0.11 equiv)	51	23:77
6	**1a**:**2a**:**3a**=1.0:1.1:1.2, 80 °C, 15 min	CF_3CO_2H (0.11 equiv)	43	25:75
7	**1a**:**2a**:**3a**=1.0:1.1:1.2, 100 °C, 30 min	CF_3CO_2H (0.11 equiv)	<33	21:79
8	**1a**:**2a**:**3a**=1.0:1.1:1.2, 100 °C, 15 min	CF_3CO_2H (0.01 equiv)	28	25:75
9	**1a**:**2a**:**3a**=1.2:1.2:1.0, 60 °C, 15 min	CF_3CO_2H (0.13 equiv)	59	18:82
10	**1a**:**2a**:**3a**=1.0:1.1:1.2, 60 °C, 30 min	$Yb(OTf)_3$ (0.11 equiv), 4 Å MS	52	41:59
11	**1a**:**2a**:**3a**=1.0:1.1:1.2, 60 °C, 30 min	$Yb(OTf)_3$ (0.11 equiv)	25	46:54
12	**1a**:**2a**:**3a**=1.2:1.2:1.0, 100 °C, 15 min	$Yb(OTf)_3$ (0.13 equiv)	39	40:60

[a] All microwave-assisted reactions were carried out on a technical microwave reactor with temperature and pressure controlling capacity.
[b] The catalyst loading was calculated according to the amount of **1a** used. The final concentrations of the catalyst were in the range of 30–33 mM except for entries 1 and 8.
[c] Isolated combined yields of **4a** and **5a**.
[d] Calculated according to the isolated weights of **4a** and **5a**.
[e] Data in the parentheses are quoted from Ref. 4h and were obtained with 20 mol % $GdCl_3$ and $MgSO_4$ in MeCN at room temperature for 90 min.

Scheme 2. Microwave-assisted synthesis of highly functionalized 1,2,3,4-tetrahydroquinolines **4** and **5**.

Table 2. Microwave-assisted one-pot synthesis of 8-hydroxy-1,2,3,4-tetrahydroquinolines[a]

Entry	2-Aminophenol (**1**)	Aldehyde (**2**)	Alkene (**3**)	Combined yield (%)[b]	Ratio of **4**:**5**[c]
1	**1a**: R^1=H	**2a**: R^2=R^3=H	**3a**: *n*=2	**4a+5a**: 59 (60)[e]	18:82 (35:65)[e]
2	**1b**: R^1=5-Me	**2a**: R^2=R^3=H	**3a**: *n*=2	**4b+5b**: 48	22:78
3	**1a**: R^1=H	**2b**: R^2=H, R^3=F	**3a**: *n*=2	**4c+5c**: 52	18:82
4	**1a**: R^1=H	**2c**: R^2=Cl, R^3=H	**3a**: *n*=2	**4d+5d**: 51	16:84
5	**1a**: R^1=H	**2a**: R^2=R^3=H	**3b**: *n*=1	**4e+5e**: 41 (67)[e]	29:71 (48:52)[e]
6	**1a**: R^1=H	**2d**: R^2=H, R^3=Cl	**3b**: *n*=1	**4f+5f**: 41	36:64[d]
7	**1b**: R^1=5-Me	**2a**: R^2=R^3=H	**3b**: *n*=1	**4g+5g**: 39	31:69
8	**1a**: R^1=H	**2e**: R^2=CO_2Me, R^3=H	**3b**: *n*=1	**4h+5h**: 40	25:75[d]
9	**1a**: R^1=H	**2e**: R^2=CO_2Me, R^3=H	**3a**: *n*=2	**4i+5i**: 56	16:84
10	**1a**: R^1=H	**2f**: R^2=NO_2, R^3=H	**3a**: *n*=2	**4j+5j**: 51	24:76

[a] A 1.2:1.2:1.0 mixture of **1**:**2**:**3** in MeCN along with 0.13 equiv of CF_3CO_2H to **1** was heated with microwave irradiation at 60 °C for 15 min. All microwave-assisted reactions were carried out on a technical microwave reactor with temperature and pressure controlling capacity.
[b] Isolated combined yields of **4** and **5**.
[c] Calculated according to the isolated weights of **4** and **5**.
[d] Inseparable mixture. The ratio was determined by ^{1}H NMR spectrum.
[e] Data in the parentheses are quoted from Ref. 4h and were obtained with 20 mol % $GdCl_3$ and $MgSO_4$ in MeCN at room temperature for 60–90 min.

products in 39–41% yields and in 36:64–25:75 isomer ratios (Table 2, entries 5–8). The diastereomers **4** and **5** could be separated in most cases and the stereochemistry of the known pairs of compounds **4a/5a** and **4e/5e** was assigned by comparision with the reported spectral data.[4h] For other new compounds, their stereochemistry was suggested analogously. In the cases of **4f/5f** and **4h/5h**, inseparable mixtures were obtained (Table 2, entries 6 and 8). Their ratios were estimated by ^{1}H NMR spectra (see Supplementary data). We tried the three-component reaction of **1b**, **3a**, and *p*-anisaldehyde under the optimized conditions, but the product yield was very low (data not shown). The result was consistent with the early finding of Baudelle and co-workers[5a] that electron-rich aromatic aldehydes were not advantageous for the CF_3CO_2H-mediated aza-Diels–Alder reactions with aniline and 3,4-dihydro-2*H*-pyran. Moreover, the reaction using 2-amino-4-chlorophenol as the amine component gave the pair of adducts in ca. 30% yield in total (data not shown). Due to low yields of the aza-Diels–Alder reactions of substituted 2-aminophenols, a further detail evaluation was not attempted.

3. Conclusion

In summary, we have established the microwave reaction conditions for the CF_3CO_2H-catalyzed three-component aza-Diels–Alder reactions involving 2-aminophenols as the amine building blocks. As compared to 24 h required for the room temperature reaction, the microwave-assisted version can be done at 60 °C for 15 min. In general, electron-deficient aromatic aldehydes provided the adducts in 39–59% isolated yields and in 36:64–16:84 isomer ratios in favor of the trans isomer. Despite the limitation in substrate scope, the above described aza-Diels–Alder reactions of 2-aminophenols under controlled microwave heating provide a high-throughput access to highly functionalized 8-hydroxy-1,2,3,4-tetrahydroquinolines.

4. Experimental

4.1. General methods and the microwave reactor

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 (400 or 500 MHz for ^{1}H and 100 or 125 MHz for ^{13}C, respectively) with $CHCl_3$ or DMSO as the internal reference. IR spectra were taken on an FT-IR spectrophotometer. Mass spectra (MS) were measured by the +ESI or −ESI method. Melting points are uncorrected. Silica gel plates pre-coated on glass were used for thin-layer chromatography using UV light, or 7% ethanolic phosphomolybdic acid and heating as the visualizing methods. Silica gel was used for flash column chromatography. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials. Reagents were obtained commercially and used as received. All microwave-assisted reactions were carried out on an Emrys creator from Personal Chemistry AB (now under *Biotage AB*, Uppsala, Sweden) with temperature measured by an IR sensor. The microwave-assisted reaction time is the hold time at the final temperature.

4.2. General procedure for microwave-assisted synthesis of pyrano(or furo)[3,2-*c*]quinolines 4a–j and 5a–j

To a 10-mL pressurized process vial were added sequentially 2-aminophenol (**1**, 1.2 mmol), aldehyde (**2**, 1.2 mmol), MeCN (4 mL), CF_3CO_2H (10 μL, 0.13 mmol), and 3,4-dihydro-2*H*-pyran (**3a**, 1.0 mmol) or 2,3-dihydrofuran (**3b**, 1.0 mmol). The loaded vial was then sealed with a cap containing a silicon septum, and put into the microwave cavity and heated at 60 °C for 15 min (very high mode with maximum power input of 75 W). EtOAc (5 mL) was added to the reaction mixture and the organic layer was washed with saturated aqueous $NaHCO_3$ (5 mL×2). Then the aqueous phase was extracted with EtOAc (5 mL). The combined organic layer was washed with saturated aqueous NH_4Cl and brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with EtOAc and petroleum ether (60–90 °C) to afford **4a–j** and **5a–j**. The structures and yields of the products are given in Scheme 2 and Table 2.

4.2.1. (4a*R,5*R**,10b*R**)-3,4,4a,5,6,10b-Hexahydro-7-hydroxy-5-phenyl-2*H*-pyrano[3,2-*c*]quinoline (4a).[4h]** A white crystalline solid; mp 210–212 °C (EtOAc–hexane) (lit.,[4h] 218–219 °C); R_f=0.58 (25% EtOAc in hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.24 (br s, 1H), 7.45–7.26 (m, 5H), 6.75 (d, *J*=7.6 Hz, 1H), 6.61 (d, *J*=7.6 Hz, 1H), 6.53 (t, *J*=8.0 Hz, 1H), 5.25 (d, *J*=4.8 Hz, 1H), 4.64 (br s,

1H), 4.51 (br s, 1H), 3.47 (d, J=11.2 Hz, 1H), 3.30–3.20 (m, 1H), 2.04 (br s, 1H), 1.36 (br s, 3H), 1.11 (br s, 1H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.22 (m, 5H), 7.00 (t, J=4.4 Hz, 1H), 6.59 (d, J=4.4 Hz, 1H), 5.32 (d, J=4.8 Hz, 1H), 5.30–5.03 (br s, 1H), 4.62 (s, 1H), 4.30–4.05 (br s, 1H), 3.55 (d, J=10.0 Hz, 1H), 3.39 (t, J=11.6 Hz, 1H), 2.12 (br s, 1H), 1.75–1.17 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.1, 141.2, 134.3, 128.3 (×2), 127.4, 126.8 (×2), 121.2, 119.9, 117.2, 113.2, 72.9, 60.8, 59.1, 38.9, 25.4, 18.1; HRMS (+ESI) calcd for $C_{18}H_{20}NO_2$ ($M+H^+$), 282.1489; found, 282.1486.

4.2.2. (4a*R,5*S**,10b*R**)-3,4,4a,5,6,10b-Hexahydro-7-hydroxy-5-phenyl-2*H*-pyrano[3,2-*c*]quinoline (5a).**[4h] A white crystalline solid; mp 188–190 °C (EtOAc–hexane) (lit.,[4h] 190–191 °C); R_f=0.45 (25% EtOAc in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.30 (m, 5H), 6.85 (br s, 1H), 6.56 (br s, 2H), 5.50–4.70 (br s, 1H), 4.69 (d, J=9.6 Hz, 1H), 4.60–4.20 (br s, 1H), 4.42 (s, 1H), 4.13–4.10 (m, 1H), 3.73 (td, J=11.2, 2.0 Hz, 1H), 2.12–2.09 (m, 1H), 1.89–1.81 (m, 1H), 1.69–1.61 (m, 1H), 1.51–1.47 (m, 1H), 1.36–1.33 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.2, 142.0, 134.6, 128.5 (×2), 127.8 (×2), 127.7, 122.8, 120.7, 116.4, 114.8, 74.6, 68.6, 54.5, 36.8, 24.0, 22.0; HRMS (+ESI) calcd for $C_{17}H_{20}NO_2$ ($M+H^+$), 282.1489; found, 282.1487.

4.2.3. (4a*R,5*R**,10b*R**)-3,4,4a,5,6,10b-Hexahydro-7-hydroxy-9-methyl-5-phenyl-2*H*-pyrano[3,2-*c*]quinoline (4b).** A pale-yellow oil; R_f=0.59 (25% EtOAc in hexane); IR (KBr) 3394 (br), 2923, 1597, 1491, 1452, 1042 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.17 (s, 1H), 7.434–7.272 (m, 5H), 6.57 (s, 1H), 6.45 (s, 1H), 5.20 (d, J=5.2 Hz, 1H), 4.57 (s, 1H), 4.33 (s, 1H), 3.47–3.44 (m, 1H), 3.28–3.25 (m, 1H), 2.14 (s, 3H), 2.02 (br s, 1H), 1.36 (br s, 3H), 1.10–1.08 (m, 1H); MS (+ESI) m/z 296 ($M+H^+$, 100).

4.2.4. (4a*R,5*S**,10b*R**)-3,4,4a,5,6,10b-Hexahydro-7-hydroxy-9-methyl-5-phenyl-2*H*-pyrano[3,2-*c*]quinoline (5b).** A white crystalline solid; mp 180–182 °C (EtOAc–hexane); R_f=0.49 (25% EtOAc in hexane); IR (KBr) 3388, 3265 (br), 2944, 2859, 1525, 1456, 1254, 1026 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.10 (s, 1H), 7.41–7.28 (m, 5H), 6.45 (s, 1H), 6.44 (s, 1H), 4.75–4.30 (br s, 1H), 4.53 (d, J=15.2 Hz, 1H), 4.24 (d, J=2.4 Hz, 1H), 3.86 (d, J=11.2 Hz, 1H), 3.56 (t, J=10.4 Hz, 1H), 2.11 (s, 3H), 1.97–1.94 (m, 1H), 1.75–1.57 (m, 2H), 1.29–1.27 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 143.7, 143.4, 132.0, 128.9 (×2), 128.1 (×2), 128.0, 124.8, 121.7, 121.1, 114.8, 73.9, 67.5, 54.8, 38.9, 24.4, 22.4, 20.9; MS (+ESI) m/z 318 ($M+Na^+$, 45), 296 ($M+H^+$, 100); HRMS (+ESI) calcd for $C_{19}H_{22}NO_2$ ($M+H^+$), 296.1645; found, 296.1639.

4.2.5. (4a*R,5*R**,10b*R**)-5-(2′-Fluorophenyl)-3,4,4a,5,6,10b-hexahydro-7-hydroxy-2*H*-pyrano[3,2-*c*]quinoline (4c).** A white solid; R_f=0.45 (25% EtOAc in hexane); IR (KBr) 3398, 3254 (br), 2959, 1578, 1486, 1437, 1260 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.30 (s, 1H), 7.62 (t, J=6.8 Hz, 1H), 7.37–7.17 (m, 3H), 6.74 (d, J=7.2 Hz, 1H), 6.61 (d, J=7.6 Hz, 1H), 6.54 (t, J=7.2 Hz, 1H), 5.21 (d, J=5.6 Hz, 1H), 4.88 (s, 1H), 4.56 (s, 1H), 3.60–3.40 (m, 1H), 3.28–3.22 (m, 1H), 2.08 (br s, 1H), 1.38 (br s, 3H), 1.09 (br s, 1H); MS (+ESI) m/z 300 ($M+H^+$, 100).

4.2.6. (4a*R,5*S**,10b*R**)-5-(2′-Fluorophenyl)-3,4,4a,5,6,10b-hexahydro-7-hydroxy-2*H*-pyrano[3,2-*c*]quinoline (5c).** A white crystalline solid; mp 178–180 °C (EtOAc–hexane); R_f=0.36 (25% EtOAc in hexane); IR (KBr) 3400 (br), 2934, 1489, 1262, 1051 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (s, 1H), 7.49 (t, J=7.6 Hz, 1H), 7.37–7.17 (m, 3H), 6.63 (t, J=4.8 Hz, 2H), 6.46–6.42 (m, 1H), 4.88–4.84 (m, 2H), 4.31 (d, J=3.2 Hz, 1H), 3.84 (d, J=6.8 Hz, 1H), 3.56 (t, J=6.4 Hz, 1H), 2.02 (d, J=8.0 Hz, 1H), 1.67 (d, J=8.4 Hz, 2H), 1.31 (d, J=8.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.2, 163.8, 147.8, 138.5, 134.2 (d, J_{C-F}=12.5 Hz), 133.7 (d, J_{C-F}=8.4 Hz), 129.2 (d, J_{C-F}=3.1 Hz), 125.4, 124.9, 120.4, 119.8 (d, J_{C-F}=22.5 Hz), 117.8, 77.6, 71.2, 51.8 (br), 42.1, 28.5, 26.7; MS (+ESI) m/z 300 ($M+H^+$, 100). Anal. calcd for $C_{18}H_{18}FNO_2$: C, 72.22; H, 6.06; N, 4.68. Found: C, 72.10; H, 6.15; N, 4.63.

4.2.7. (4a*R,5*R**,10b*R**)-5-(4′-Chlorophenyl)-3,4,4a,5,6,10b-hexahydro-7-hydroxy-2*H*-pyrano[3,2-*c*]quinoline (4d).** A white solid; R_f=0.45 (25% EtOAc in hexane); IR (KBr) 3396, 3211 (br), 2953, 1589, 1486, 1248, 1092 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.30 (s, 1H), 7.44 (dd, J=12.8, 8.4 Hz, 4H), 6.74 (d, J=7.6 Hz, 1H), 6.61 (d, J=7.6 Hz, 1H), 6.53 (t, J=7.6 Hz, 1H), 5.22 (d, J=5.6 Hz, 1H), 4.62 (s, 1H), 4.58 (s, 1H), 3.48–3.45 (m, 1H), 3.28–3.22 (m, 1H), 2.02 (br s, 1H), 1.40–1.25 (m, 3H), 1.06 (br s, 1H); MS (−ESI) m/z 316 ($M+2-H^+$, 33), 314 ($M-H^+$, 100).

4.2.8. (4a*R,5*S**,10b*R**)-5-(4′-Chlorophenyl)-3,4,4a,5,6,10b-hexahydro-7-hydroxy-2*H*-pyrano[3,2-*c*]quinoline (5d).** A white crystalline solid; mp 184–186 °C (EtOAc–hexane); R_f=0.35 (25% EtOAc in hexane); IR (KBr) 3390, 3312 (br), 2939, 2852, 1508, 1488, 1259, 1088 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.21 (s, 1H), 7.42 (s, 4H), 6.61 (d, J=7.6 Hz, 2H), 6.42 (t, J=7.6 Hz, 1H), 4.79 (br s, 1H), 4.53 (d, J=10.4 Hz, 1H), 4.28 (d, J=2.8 Hz, 1H), 3.84 (d, J=11.6 Hz, 1H), 3.57 (t, J=10.8 Hz, 1H), 1.96–1.93 (m, 1H), 1.72–1.60 (m, 2H), 1.28–1.26 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 143.7, 142.5, 134.3, 132.3, 130.0 (×2), 128.8 (×2), 121.4, 121.0, 116.2, 113.7, 73.6, 67.4, 54.0, 38.6, 24.3, 22.4; MS (+ESI) m/z 318 ($M+2+H^+$, 33), 316 ($M+H^+$, 100). Anal. calcd for $C_{18}H_{18}ClNO_2$: C, 68.46; H, 5.75; N, 4.44. Found: C, 68.40; H, 5.85; N, 4.49.

4.2.9. (3a*R,4*R**,9b*R**)-6-Hydroxy-4-phenyl-2,3,3a,9b-tetrahydro-2*H*-furo[3,2-*c*]quinoline (4e).**[4h] A white crystalline solid; mp 200–201 °C (EtOAc–hexane) (lit.,[4h] 188–189 °C); R_f=0.37 (25% EtOAc in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.30 (m, 5H), 6.99 (d, J=6.8 Hz, 1H), 6.69–6.62 (m, 2H), 5.32 (d, J=8.0 Hz, 1H), 5.20–5.10 (br s, 1H), 4.68 (d, J=2.4 Hz, 1H), 4.31 (br s, 1H), 3.84 (td, J=8.4, 3.2 Hz, 1H), 3.73 (ddd, J=8.0, 8.0, 8.0 Hz, 1H), 2.84–2.77 (m, 1H), 2.29–2.18 (m, 1H), 1.58–1.52 (m, 1H); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.37 (br s, 1H), 7.49–7.27 (m, 5H), 6.69 (d, J=7.6 Hz, 1H), 6.61 (d, J=7.6 Hz, 1H), 6.53 (t, J=8.0 Hz, 1H), 5.15 (d, J=8.0 Hz, 1H), 4.59 (d, J=2.8 Hz, 1H), 4.48 (s, 1H), 3.57 (dd, J=8.4, 5.2 Hz, 2H), 2.78–2.68 (m, 1H), 2.02–1.92 (m, 1H), 1.37–1.30 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 144.1, 143.0, 134.6, 128.7 (×2), 127.5, 126.7 (×2), 123.1, 120.4, 117.8, 112.9, 75.6, 66.0, 56.6, 45.3, 24.7; MS (+ESI) m/z 290 ($M+Na^+$, 100), 268 ($M+H^+$, 68).

4.2.10. (3a*R,4*S**,9b*R**)-6-Hydroxy-4-phenyl-2,3,3a,9b-tetrahydro-2*H*-furo[3,2-*c*]quinoline (5e).**[4h] A white crystalline solid; mp 150–152 °C (EtOAc–hexane) (lit.,[4h] 155–156 °C); R_f=0.31 (25% EtOAc in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47–7.34 (m, 5H), 7.04–7.02 (m, 1H), 6.65–6.62 (m, 2H), 5.21 (br s, 1H), 4.64 (d, *J*=4.8 Hz, 1H), 4.55 (br s, 1H), 4.04 (ddd, *J*=8.4, 8.4, 8.4 Hz, 1H), 3.85 (ddd, *J*=8.4, 8.4, 8.4 Hz, 1H), 3.76 (d, *J*=11.6 Hz, 1H), 2.52–2.46 (m, 1H), 2.05–1.98 (m, 1H), 1.77–1.70 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.7, 141.5, 134.8, 128.5 (×2), 128.3 (×2), 128.0, 122.7, 120.2, 117.6, 114.1, 76.3, 65.0, 57.3, 43.1, 28.6; MS (+ESI) *m*/*z* 290 (M+Na$^+$, 65), 268 (M+H$^+$, 100).

4.2.11. (3a*R,4*R**,9b*R**)-4-(2′-Chlorophenyl)-6-hydroxy-2,3,3a,9b-tetrahydro-2*H*-furo[3,2-*c*]quinoline (4f) and (3a*R**,4*S**,9b*R**)-4-(2′-chlorophenyl)-6-hydroxy-2,3,3a,9b-tetrahydro-2*H*-furo[3,2-*c*]quinoline (5f).** A white crystalline solid of 36:64 mixture of two inseparable diastereomers **4f** and **5f**; mp 227–229 °C (EtOAc–hexane); R_f=0.35 (25% EtOAc in hexane); IR (KBr) 3400, 3210 (br), 2887, 1595, 1512, 1487, 1233, 1026 cm^{-1}; MS (+ESI) *m*/*z* 326 (M+2+Na$^+$, 13), 324 (M+Na$^+$, 42), 304 (M+2+H$^+$, 26), 302 (M+H$^+$, 100). Anal. calcd for $C_{17}H_{16}ClNO_2$: C, 67.66; H, 5.34; N, 4.64. Found: C, 67.57; H, 5.29; N, 4.62. *NMR data assigned for **4f***: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40 (br s, 1H), 7.79 (d, *J*=8.0 Hz, 1H), 7.50–7.32 (m, 3H), 6.73–6.52 (m, 3H), 5.16 (d, *J*=7.6 Hz, 1H), 4.90 (d, *J*=2.0 Hz, 1H), 4.58 (s, 1H), 3.60–3.56 (m, 2H), 2.90–2.80 (m, 1H), 2.08–1.92 (m, 1H), 1.37–1.20 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.5, 144.0, 138.7, 136.0, 133.9, 133.2, 132.6, 131.8, 127.3, 124.6, 122.2, 117.1, 79.4, 70.2, 57.7, 45.9, 29.0. *NMR data assigned for **5f***: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40 (s, 1H), 7.66 (d, *J*=8.0 Hz, 1H), 7.50–7.32 (m, 3H), 6.78 (d, *J*=7.2 Hz, 1H), 6.73–6.52 (m, 2H), 4.83 (s, 1H), 4.50 (d, *J*=4.8 Hz, 1H), 4.28 (d, *J*=10.4 Hz, 1H), 3.87 (ddd, *J*=8.4, 8.4, 8.4 Hz, 1H), 3.71 (dt, *J*=8.4, 5.6 Hz, 1H), 2.50–2.44 (m, 1H), 2.08–1.92 (m, 1H), 1.59–1.52 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.3, 143.9, 139.0, 137.9, 134.0, 133.8, 133.7, 132.2, 125.9, 124.7, 121.3, 117.6, 79.8, 69.1, 56.4, 47.0, 32.7.

4.2.12. (3a*R,4*R**,9b*R**)-6-Hydroxy-8-methyl-4-phenyl-2,3,3a,9b-tetrahydro-2*H*-furo[3,2-*c*]quinoline (4g).** A white solid; R_f=0.42 (25% EtOAc in hexane); IR (KBr) 3415, 3239 (br), 2920, 1588, 1516, 1455, 1241, 1039 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.31 (s, 1H), 7.48–7.26 (m, 5H), 6.50 (s, 1H), 6.44 (s, 1H), 5.10 (d, *J*=8.0 Hz, 1H), 4.52 (s, 1H), 4.31 (s, 1H), 3.58–3.54 (m, 2H), 2.77–2.67 (m, 1H), 2.12 (s, 3H), 1.99–1.93 (m, 1H), 1.34–1.30 (m, 1H); MS (+ESI) *m*/*z* 282 (M+H$^+$, 100).

4.2.13. (3a*R,4*S**,9b*R**)-6-Hydroxy-8-methyl-4-phenyl-2,3,3a,9b-tetrahydro-2*H*-furo[3,2-*c*]quinoline (5g).** A white crystalline solid; mp 204–206 °C (EtOAc–hexane); R_f=0.40 (25% EtOAc in hexane); IR (KBr) 3393, 3215 (br), 2884, 1523, 1455, 1255, 1023 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H), 7.47–7.30 (m, 5H), 6.59 (s, 1H), 6.50 (s, 1H), 4.52 (s, 1H), 4.43 (d, *J*=5.2 Hz, 1H), 3.86 (ddd, *J*=8.0, 8.0, 8.0 Hz, 1H), 3.67–3.63 (m, 1H), 3.60 (d, *J*=11.2 Hz, 1H), 2.39–2.34 (m, 1H), 2.15 (s, 3H), 1.92–1.87 (m, 1H), 1.57–1.52 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 144.1, 142.8, 132.7, 128.9 (×2), 128.7 (×2), 128.2, 125.8, 122.0, 120.8, 114.5, 76.0, 64.8, 57.6, 43.4, 29.0, 21.0; MS (+ESI) *m*/*z* 282 (M+H$^+$, 100). Anal. calcd for $C_{18}H_{19}NO_2$: C, 76.84; H, 6.81; N, 4.98. Found: C, 76.87; H, 6.94; N, 5.08.

4.2.14. (3a*R,4*R**,9b*R**)-6-Hydroxy-4-[(4′-methoxycarbonyl)-phenyl]-2,3,3a,9b-tetrahydro-2*H*-furo[3,2-*c*]-quinoline (4h) and (3a*R**,4*S**,9b*R**)-6-hydroxy-4-[(4′-methoxycarbonyl)-phenyl]-2,3,3a,9b-tetrahydro-2*H*-furo[3,2-*c*]quinoline (5h).** A pale-yellow gum of 25:75 mixture of two inseparable diastereomers **4h** and **5h**; R_f=0.23 (25% EtOAc in hexane). *Partial NMR data assigned for **4h***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, *J*=8.0 Hz, 2H), 6.82 (d, *J*=7.2 Hz, 1H), 6.62 (d, *J*=4.4 Hz, 1H), 5.23 (d, *J*=7.6 Hz, 1H). An enriched sample of **5h** was obtained by recrystallization of the mixture. *Compound **5h***: IR (KBr) 3375 (br), 2952, 1722, 1279 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J*=8.0 Hz, 2H), 7.52 (d, *J*=7.6 Hz, 2H), 7.01 (br s, 1H), 6.62 (br s, 2H), 6.45–6.20 (br s, 1H), 4.64 (d, *J*=4.8 Hz, 1H), 4.59 (br s, 1H), 4.04 (ddd, *J*=8.0, 8.0, 8.0 Hz, 1H), 3.94 (s, 3H), 3.89–3.82 (m, 1H), 3.79 (d, *J*=11.2 Hz, 1H), 2.50–2.43 (m, 1H), 2.04–1.98 (m, 1H), 1.75–1.73 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 147.0, 142.7, 134.5, 129.9 (×2), 129.8, 128.3 (×2), 122.9, 120.5, 117.8, 114.0, 76.1, 65.1, 57.2, 52.2, 43.3, 28.6; MS (−ESI) *m*/*z* 324 (M−H$^+$, 100); HRMS calcd for $C_{19}H_{19}NO_4Na$ (M+Na$^+$), 348.1206; found, 348.1201.

4.2.15. (4a*R,5*R**,10b*R**)-3,4,4a,5,6,10b-Hexahydro-7-hydroxy-5-[(4′-methoxycarbonyl)phenyl]-2*H*-pyrano[3,2-*c*]quinoline (4i).** A pale-yellow solid; R_f=0.29 (25% EtOAc in hexane); IR (KBr) 3388 (br), 2949, 1719, 1280, 1026 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.28 (s, 1H), 7.97 (d, *J*=8.0 Hz, 2H), 7.58 (d, *J*=8.8 Hz, 2H), 6.75 (d, *J*=7.6 Hz, 1H), 6.64–6.51 (m, 2H), 5.25 (d, *J*=5.6 Hz, 1H), 4.70 (d, *J*=2.0 Hz, 1H), 4.69 (d, *J*=7.6 Hz, 1H), 3.86 (s, 3H), 3.47 (d, *J*=11.6 Hz, 1H), 3.27–3.23 (m, 1H), 2.14–2.12 (m, 1H), 1.35 (br s, 3H), 1.03 (br s, 1H); MS (−ESI) *m*/*z* 338 (M−H$^+$, 100).

4.2.16. (4a*R,5*S**,10b*R**)-3,4,4a,5,6,10b-Hexahydro-7-hydroxy-5-[(4′-methoxycarbonyl)phenyl]-2*H*-pyrano[3,2-*c*]quinoline (5i).** A pale-yellow solid; mp 164–166 °C; R_f=0.22 (25% EtOAc in hexane); IR (KBr) 3404 (br), 2926, 1721, 1508, 1437, 1282, 1259 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (d, *J*=8.5 Hz, 2H), 7.48 (d, *J*=8.0 Hz, 2H), 6.82 (d, *J*=3.5 Hz, 2H), 6.55–6.51 (m, 2H), 4.70 (d, *J*=10.5 Hz, 1H), 4.41 (s, 1H), 4.13–4.08 (m, 1H), 3.92 (s, 3H), 3.72 (dd, *J*=11.5, 9.5 Hz, 1H), 2.09–2.07 (m, 1H), 1.84–1.81 (m, 1H), 1.67–1.64 (m, 1H), 1.42–1.34 (m, 2H) (OH not found); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.4, 148.0, 142.5, 134.3, 130.1 (×2), 129.7, 128.1 (×2), 122.8, 121.1, 117.0, 114.8, 74.5, 68.7, 54.7, 52.4, 39.1, 24.2, 22.3; MS (+ESI) *m*/*z* 340 (M+H$^+$, 100); HRMS calcd for $C_{20}H_{21}NO_4$ (M+H$^+$), 340.1543; found, 340.1543.

4.2.17. (4a*R,5*R**,10b*R**)-3,4,4a,5,6,10b-Hexahydro-7-hydroxy-5-(4′-nitrophenyl)-2*H*-pyrano[3,2-*c*]quinoline (4j).** A yellow solid; R_f=0.32 (25% EtOAc in hexane); IR (KBr) 3414, 3188 (br), 2949, 1517, 1349 cm^{-1}; ^{1}H NMR

(400 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.23 (d, J=8.8 Hz, 2H), 7.71 (d, J=8.4 Hz, 2H), 6.73 (d, J=7.6 Hz, 1H), 6.61 (d, J=7.6 Hz, 1H), 6.54 (t, J=7.6, 7.6 Hz, 1H), 5.24 (d, J=7.0 Hz, 1H), 4.82 (s, 1H), 4.75 (s, 1H), 3.29–3.21 (m, 2H), 2.10 (br s, 1H), 1.34 (br s, 3H), 0.99 (br s, 1H); MS (−ESI) m/z 651 (2M−H$^+$, 100), 325 (M−H$^+$, 69).

4.2.18. (4a*R,5*S**,10b*R**)-3,4,4a,5,6,10b-Hexahydro-7-hydroxy-5-(4′-nitrophenyl)-2*H*-pyrano[3,2-*c*]quinoline (5j).** A yellow crystalline solid; mp 200–203 °C (EtOAc–hexane); R_f=0.24 (25% EtOAc in hexane); IR (KBr) 3463 (br), 2933, 1508, 1346, 1256 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H), 8.21 (d, J=8.8 Hz, 2H), 7.67 (d, J=8.4 Hz, 2H), 6.63 (d, J=7.2 Hz, 2H), 6.44 (t, J=7.6 Hz, 1H), 5.06 (s, 1H), 4.66 (d, J=9.2 Hz, 1H), 4.29 (d, J= 2.8 Hz, 1H), 3.83 (d, J=10.8 Hz, 1H), 3.58 (t, J=9.6 Hz, 1H), 2.03–1.99 (m, 1H), 1.74–1.62 (m, 2H), 1.33–1.22 (m, 2H); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (d, J=8.8 Hz, 2H), 7.62 (d, J=9.2 Hz, 2H), 6.88 (d, J=6.8 Hz, 1H), 6.65–6.58 (m, 2H), 5.10 (br s, 1H), 4.81 (d, J=10.8 Hz, 1H), 4.43 (d, J=2.8 Hz, 1H), 4.42 (d, J=12.0 Hz, 1H), 4.13–4.09 (m, 1H), 3.74 (td, J=11.2, 2.0 Hz, 1H), 2.14–2.10 (m, 1H), 1.88–1.70 (m, 2H), 1.45–1.35 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.9, 147.3, 143.9, 134.1, 129.3 (×2), 124.0 (×2), 121.2, 120.9, 116.4, 113.8, 73.1, 67.0, 54.5, 38.7, 24.3, 22.6; MS (−ESI) m/z 651 (2M−H$^+$, 100), 325 (M−H$^+$, 11). Anal. calcd for $C_{18}H_{18}N_2O_4$: C, 66.25; H, 5.56; N, 8.58. Found: C, 66.04; H, 5.70; N, 8.51.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.09.012.

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Efficient catalytic asymmetric synthesis of α-substituted phenyloxyacetyloxy and aroyloxy phosphonates

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Abstract—Optically active α-substituted phenyloxyacetyloxy and aroyloxy phosphonates have been synthesized via catalytic asymmetric hydrogenation of the corresponding prochiral α,β-unsaturated phosphonates using Rh(I)/(*R*,*R*)-Me-DuPhos as the catalyst in methanol at 18 °C. The asymmetric hydrogenation reaction exhibits excellent enantioselectivity with enantiomeric excesses from 91 to 96%.

1. Introduction

The biological importance of phosphonates was recognized over 45 years ago.[1] Among them, α-substituted phosphonates are particularly important in connection with their remarkable biological activities. They have been widely used as enzyme inhibitors,[2] antibacterial agents,[3] anti-HIV agents,[4] botryticides,[5] and haptens for catalytic antibodies.[6] For these reasons, the synthesis of α-substituted phosphonates and their functionalized derivatives is an important objective.[7] The absolute configuration of α-substituted phosphonates can affect their biological activity,[8] thus stimulating interest in the asymmetric synthesis of these compounds. For example, intensive efforts have been made in the asymmetric synthesis of optically active α-amino and α-hydroxy phosphonates in recent years.[9] Enantioselective and catalytic processes are especially attractive. Elegant examples include enantioselective synthesis of α-hydroxy and/or α-amino phosphonates via asymmetric hydrogenation,[10] asymmetric borane reduction,[11] asymmetric dihydroxylation and aminohydroxylation,[12] and asymmetric hydrophosphonylation.[13]

Although numerous methods are available for the asymmetric synthesis of α-hydroxy and α-amino phosphonates, there are no reports concerning the straightforward route to asymmetric synthesis of α-substituted phenyloxyacetyloxy and aroyloxy phosphonates, two kinds of important derivatives of α-hydroxy phosphonates. Recently, we have synthesized a series of racemic α-substituted phosphonates and examined their biological activities.[14] It has been found that α-substituted phenyloxyacetyloxy and aroyloxy phosphonates, for example, dimethyl α-(2,4-dichlorophenoxyacetoxy) ethyl phosphonate, are good inhibitors of pyruvate dehydrogenases and exhibit potent herbicidal activities.[14] Considering the importance of chirality for biologically active molecules, synthesis of optically active α-substituted phenoxyacetyloxyl and aroyloxy phosphonates is of great interest. Based on the pioneering work of Burk and Imamoto,[10] we herein describe the catalytic asymmetric synthesis of α-substituted phenyloxyacetyloxy and aroyloxy phosphonates via asymmetric hydrogenation of the corresponding prochiral α,β-unsaturated phosphonates using Rh(I)/(*R*,*R*)-Me-DuPhos complex as the catalyst.

2. Results and discussion

The α-substituted α,β-unsaturated phosphonates are synthesized by a two-step procedure as shown in Scheme 1. The Arbuzov reaction of acyl chlorides **2** and trialkyl phosphates **1** gave α-keto phosphonates **3**, which were then treated with various aroyl chlorides or substituted phenoxyacetyl chlorides in the presence of Et_3N from 0 °C to room temperature to exclusively afford *E*-α-substituted α,β-unsaturated phosphonates **4a**–**4w** in 56–95% isolated yields.[15] To our knowledge, the α,β-unsaturated phosphonates **4a**–**4w** are novel and first synthesized.

Keywords: Asymmetric catalysis; Chirality; Phosphonate; Hydrogenation.
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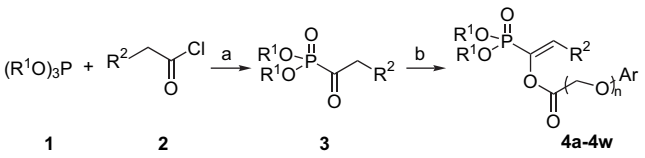


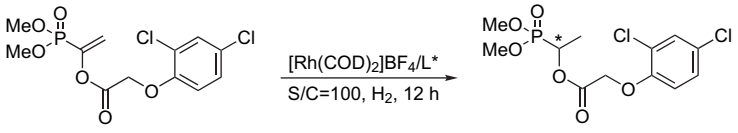
R^1=Me or Et; R^2=H or Me; Ar=phenyl, substituted phenyl, thiophenyl, or furfuryl; n=0 or 1.
(a) 0 °C-rt, 12 h. (b) Substituted aroyl or phenoxyacetyl chlorides, Et_3N, THF, 0 °C-rt, 3 h.

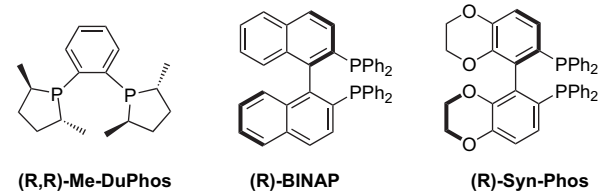
Scheme 1.

In the search for an efficient catalyst, a variety of chiral bisphosphine ligands and a monodentate phosphorus ligand have been screened by using $[Rh(COD)_2]BF_4$ as the catalyst precursor and the results are summarized in Table 1. With dimethyl α-(2,4-dichlorophenoxyacetoxy) vinyl phosphonate **4a** as a representative substrate, the asymmetric hydrogenation was initially effected in methanol with hydrogen pressure of 4 atm using the in situ generated chiral Rh(I)-complexes (Table 1, entries 1–5). The ligands employed played an important role in this transformation, with Me-DuPhos being an excellent ligand (Table 1, entry 1). Under these reaction conditions, the asymmetric hydrogenation could be completed within 12 h at 18 °C with excellent conversion (>95%) and enantiomeric excess (ee, 94%). Although (*R*)-*syn*-Phos and (4*S*,5*S*)-DIOP were also effective as added ligands for this rhodium(I)-catalyzed hydrogenation, the results were generally inferior to those of (*R*,*R*)-Me-DuPhos (Table 1, entries 1, 3, and 4). Compared to other bidentate phosphine, (*R*)-BINAP is almost ineffective (Table 1, entry 2), which is consistent with other asymmetric hydrogenations of enol derivatives.[16] Hydrogenation of **4a** with Rh-(*R*)-MA-Me catalyst under analogous reaction conditions resulted in only poor conversion (Table 1, entry 5). With dichloromethane as the solvent and under higher pressure of H_2, the chiral monodentate ligand, (*R*)-MA-Me, can be used for this reaction, but it is not as effective as (*R*,*R*)-Me-DuPhos in terms of either activity or stereoselectivity (Table 1, entry 6). The method to generate the chiral catalyst has no obvious effects on either reactivity or enantioselectivity, and the reaction gave almost identical results, irrespective of the use of the in situ generated catalyst or commercially available one (Table 1, entries 1 vs 7). Note that $[Rh(COD)_2]BF_4$/(*R*,*R*)-Me-DuPhos and [(COD)Rh-(*S*,*S*)-Me-DuPhos]OTf constitute the same stereochemical environment, although the respective absolute configurations are different (Table 1, entries 1 and 7).

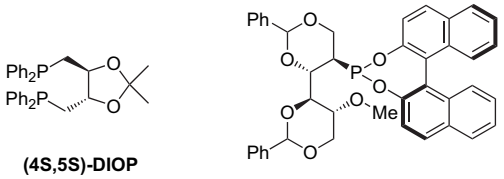
Table 2 summarizes some results of the effect of varying the reaction conditions. Using the $[Rh(COD)_2]BF_4$/(*R*,*R*)-Me-DuPhos catalyst system, we observed that the enantioselectivity of the reaction depended on the solvents employed (Table 2). Although the catalytic hydrogenation went to completion in both CH_2Cl_2 and THF, the reaction led to 78 and 88% ee, respectively (Table 2, entries 2 and 3). Almost no reaction was observed in toluene probably due to the little solubility of the catalyst in toluene (Table 2, entry 4).

Table 1. Ligands screening on the asymmetric hydrogenation





(R,R)-Me-DuPhos (R)-BINAP (R)-Syn-Phos

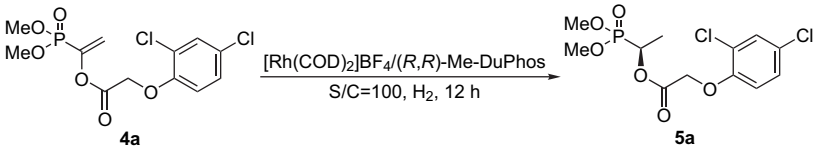


(4S,5S)-DIOP (R)-MA-Me

Entry	Pressure (atm)	Solvent	Ligand	Conv.[a] (%)	ee[b] (%)
1	4	MeOH	(*R*,*R*)-Me-DuPhos	>95	94
2	4	MeOH	(*R*)-BINAP	<5	nd
3	4	MeOH	(*R*)-*syn*-Phos	50	32
4	4	MeOH	(4*S*,5*S*)-DIOP	59	16
5	4	MeOH	(*R*)-MA-Me	<5	nd
6	10	CH_2Cl_2	(*R*)-MA-Me	64	36
7	4	MeOH	(*S*,*S*)-Me-DuPhos[c]	>95	−94

[a] Conversions were determined by ^1H NMR.
[b] Enantiomeric excesses were determined by chiral HPLC on a Daicel Chiralcel OJ-H column.
[c] Commercially available [(COD)Rh-(*S*,*S*)-Me-DuPhos]OTf as the catalyst.

Table 2. Investigation of reaction conditions on the asymmetric hydrogenation



Entry	Pressure (atm)	Temp (°C)	Solvent	Conv.[a] (%)	ee[b] (%)
1	4	18	MeOH	>95	94
2	4	18	CH_2Cl_2	>95	78
3	4	18	THF	>95	88
4	4	18	Toluene	<5	nd
5	4	50	MeOH	>95	94
6	1.3	18	MeOH	>95	94
7	10	18	MeOH	>95	94
8[c]	4	18	MeOH	15	93

[a] Conversions were determined by ^{1}H NMR.
[b] Enantiomeric excesses were determined by chiral HPLC on a Daicel Chiralcel OJ-H column.
[c] S/C=500:1.

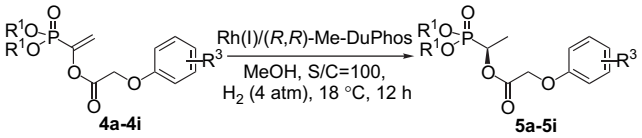
Changing the hydrogen pressure to 1.3 or 10 atm resulted in identical results (Table 2, entries 6 and 7 vs 1). Increasing the reaction temperature from 18 to 50 °C did not affect the reaction (Table 2, entries 1 and 5). Reducing the catalyst loading to S/C=500:1 resulted in much lower conversion, only 15%. However, it did not decrease the ee value very much (Table 2, entry 8).

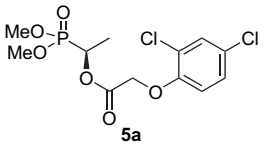
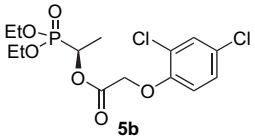
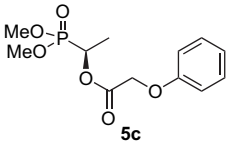
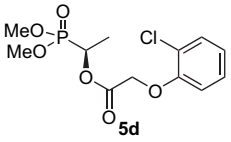
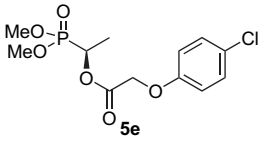
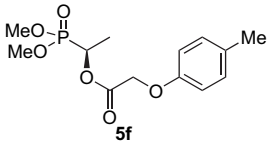
The asymmetric hydrogenation of a series of substituted α,β-unsaturated phosphonates was then performed using 1 mol % of in situ generated Rh(I)-catalyst in MeOH at 4 atm of H_2 for 12 h at 18 °C and the results are summarized in Tables 3 and 4. This rhodium-catalyzed hydrogenation exhibits broad substrate scope and excellent levels of enantioselectivity from 91 to 96% ee.

Our main objective was to develop a practical route to a diverse range of optically active α-substituted ethyl phosphonates **5a**–**5u**. Table 3 shows the results of the synthesis of chiral aroyloxyacetyloxylethyl phosphonates via asymmetric hydrogenation. Substrates with *O*,*O*-dimethyl or *O*,*O*-diethyl were hydrogenated with excellent enantiomeric excesses ranging from 91 to 96% ee. The hydrogenation reaction took place very well regardless of various electron-withdrawing or electron-donating substituent on the different positions of the aromatic ring. The biological study showed that the hydrogenation product, for example, (*S*)-diethyl α-(2,4-dichlorophenoxyacetoxy) ethyl phosphonate **5b** exhibited potent herbicidal activity.

Burk and co-workers have demonstrated that the Me-DuPhos-Rh is capable of hydrogenating enolbenzoates with some examples.[10c] In order to examine the diversity of the substrates, we have synthesized a variety of α,α-disubstituted- and α,α,β-trisubstituted vinyl phosphonates with different substituents on the benzene ring as well as some substrates with *O*- or *S*-heterocycles and subjected them to our standard reaction conditions. It was found that the substituted benzoxyl alkyl phosphonates **5j**–**5p** with electron-donating or electron-withdrawing group on the aromatic ring were also obtained with excellent ee values (Table 4, entries 1–7). The asymmetric hydrogenation of heterocyclic substrates went smoothly to afford the expected

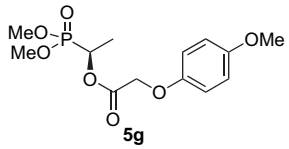
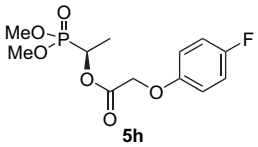
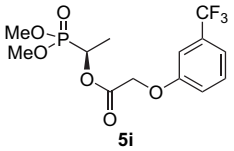
Table 3. Results of the catalytic asymmetric hydrogenation of various α-substituted phenyloxyacetyloxy-α,β-unsaturated phosphonates



Entry	Product	Conv.[a] (%)	ee[b] (%)	Config.[c]
1	 **5a**	>95	94	*S*-(+)
2	 **5b**	>95	96	(*S*)-(+)
3	 **5c**	>95	93	(*S*)-(+)
4	 **5d**	>95	93	(*S*)-(+)
5	 **5e**	>95	95	(*S*)-(+)
6	 **5f**	>95	91	(*S*)-(+)

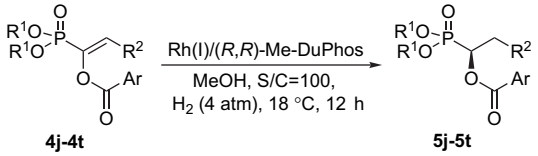
(*continued*)

Table 3. (*continued*)

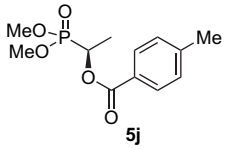
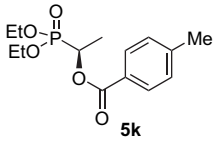
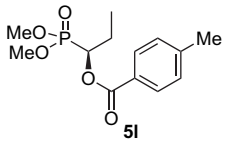
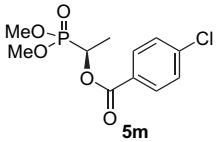
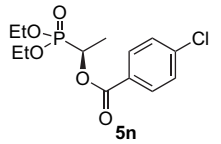
Entry	Product	Conv.[a] (%)	ee[b] (%)	Config.[c]
7	 **5g**	91	95	(*S*)-(+)
8	 **5h**	>95	94	(*S*)-(+)
9	 **5i**	>95	92	(*S*)-(+)

[a] Conversions were determined by ^{1}H NMR.
[b] Enantiomeric excesses were determined by chiral HPLC on a Daicel Chiralcel OJ-H or AS-H column.
[c] Configurations were assigned on the basis of correlation between HPLC elution order, optical rotation, and catalyst configuration relative to the known compound **6a**.

Table 4. Results of the catalytic asymmetric hydrogenation of various α-substituted aroyloxy-α,β-unsaturated phosphonates

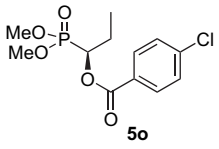
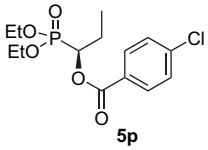
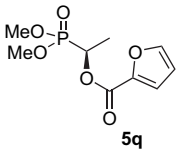
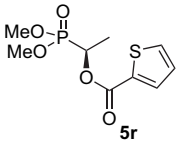
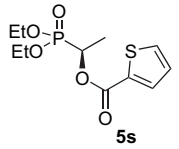
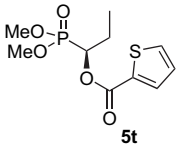


4j-4t **5j-5t**

Entry	Product	Conv.[a] (%)	ee[b] (%)	Config.[c]
1	 **5j**	>95	95	(*S*)-(−)
2	 **5k**	>95	95	(*S*)-(−)
3	 **5l**	>95	94	(*S*)-(+)
4	 **5m**	>95	95	(*S*)-(−)
5	 **5n**	>95	96	(*S*)-(−)

(*continued*)

Table 4. (*continued*)

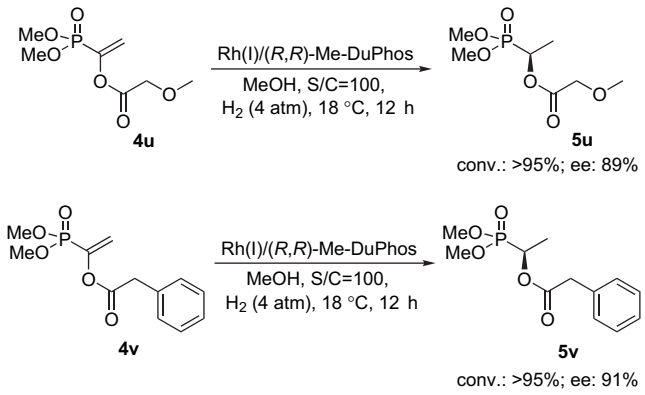
Entry	Product	Conv.[a] (%)	ee[b] (%)	Config.[c]
6	 **5o**	>95	91	(*S*)-(+)
7	 **5p**	>95	92	(*S*)-(+)
8	 **5q**	>95	96	(*S*)-(−)
9	 **5r**	>95	95	(*S*)-(−)
10	 **5s**	>95	95	(*S*)-(−)
11[d]	 **5t**	>95	94	(*S*)-(+)

[a] Conversions were determined by ^{1}H NMR.
[b] Enantiomeric excesses were determined by chiral HPLC on a Daicel Chiralcel OJ-H column.
[c] Configurations were assigned on the basis of correlation between HPLC elution order, optical rotation, and catalyst configuration relative to the known compound **6a**.
[d] Under 10 atm H_2 pressure.

products **5q**–**5t** in great conversions with excellent enantioselectivities (Table 4, entries 8–11). Although the strong thiophilicity of transition metals might make the catalytic reaction ineffective,[17] the catalytic hydrogenation of **4r**, **4s**, and **4t** was carried out in more than 95% conversions under mild conditions, respectively. α,α,β-Trisubstituted-α,β-unsaturated phosphonates **4t** were only partially hydrogenated under the optimized reaction conditions after 12 h, and this is perhaps due to a combination of steric or electronic effect.[10a] However, with higher H_2 pressure (10 atm), **4t** goes to completion smoothly (Table 4, entry 11).

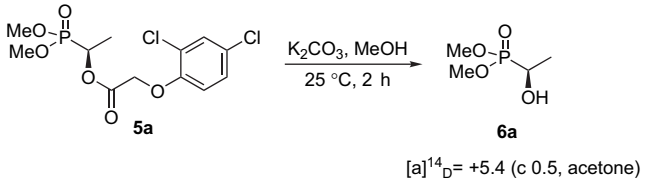
In the case of **4u** and **4v**, the reaction gave similarly high conversion but a slightly low enantioselectivity (89 and 91% ee, respectively, Scheme 2).

The α-substituted phenyloxyacetyloxy and aroyloxy phosphonates **5** can be simply deprotected using K_2CO_3 in MeOH at room temperature for 2 h to afford the corresponding



Scheme 2.

α-hydroxy phosphonates **6**. In order to determine the absolute configuration of the hydrogenation products, **5a** was converted to the previously reported compound **6a**, as shown in Scheme 3. The $[\alpha]_D$ value of **6a** is the same as the one reported in the literature,[17] which indicates that the absolute configuration of the hydrogenation products is *S* configuration.



Scheme 3.

3. Conclusion

In summary, we have synthesized a series of optically active phenoxyacetyloxyl and aroyloxy phosphonates in high enantiomeric purity via catalytic asymmetric hydrogenation using Rh(I)/(*R*,*R*)-Me-DuPhos as the catalytic system in methanol and most of the target compounds including their precursors are novel. The methodology described herein represents one of the most straightforward routes to these compounds.

4. Experimental

4.1. General procedure for the preparation of α,β-unsaturated phosphonates (the preparation of 4a is representative)

Acetyl chloride (2.5 mL, 35 mmol) was cooled to 0 °C in an oven-dried flask fitted with an addition funnel. Trimethyl phosphite (5 mL, 42 mmol) was added dropwise. A balloon was used to compensate for the released methyl chloride. When the addition was complete, the reaction was warmed to room temperature and stirred overnight. The mixture was concentrated under vacuum to remove volatile impurities and then the crude material was taken directly to next step. THF (40 mL) and 2,4-dichlorophenoxy acetyl chloride (5 mL, 28.2 mmol) were added to the crude product, and the system was chilled to 0 °C. Et_3N (4.5 mL) was dissolved in 20 mL of THF, and this solution was then added slowly to the reaction mixtures. After stirred for 45 min, the reaction mixture was warmed up to room temperature and stirred for another 3 h. The reaction mixture was diluted with EtOAc (200 mL), which was washed with saturated $NaHCO_3$ (100 mL×3), brine (75 mL), and then dried with $MgSO_4$. The solvent was removed in vacuo to give yellow oil, which was purified on silica gel (EtOAc:petroleum ether=1:1) to give 5.93 g of **4a** as white solid in 59% isolated yield.

4.1.1. *O*,*O*-Dimethyl α-(2,4-dichlorophenoxyacetoxy) vinyl phosphonate (4a). White solid; yield 59%; mp 78–80 °C; IR (KBr) 1799, 1636, 1587, 1487, 1252, 1144, 1024, 814, 800 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, 1H, *J*=2.4 Hz), 7.20 (dd, 1H, *J*=8.8 and 2.4 Hz), 6.86 (d, 1H, *J*=8.8 Hz), 6.11 (dd, 1H, *J*=6.8 and 2.4 Hz), 5.86 (dd, 1H, *J*=34.4 and 2.4 Hz), 4.86 (s, 2H), 3.76 (d, 6H, *J*=11.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 53.2, 53.3, 65.9, 114.3, 114.9, 122.2, 122.5, 124.3, 127.5, 127.7, 130.5, 143.3, 145.6, 152.2, 166.0, 166.1; ^{31}P NMR (162 MHz, $CDCl_3$) δ 9.70; MS (EI) *m*/*z* 354 (M^+-1), 355 (M^+), 357 (M^++2). Anal. Calcd for $C_{12}H_{13}Cl_2O_6P$: C, 40.59; H, 3.69. Found: C, 40.38; H, 3.76.

4.1.2. Diethyl α-(2,4-dichlorophenoxyacetoxy) vinyl phosphonate (4b). Pale yellow liquid; yield 75%; IR (KBr) 2984, 1784, 1630, 1585, 1480, 1263, 1161, 1022, 801 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, 1H, *J*=2.4 Hz), 7.19 (dd, 1H, *J*=8.8 and 2.4 Hz), 6.86 (d, 1H, *J*=8.8 Hz), 6.10 (dd, 1H, *J*=6.8 and 2.4 Hz), 5.81 (dd, 1H, *J*=34.4 and 2.4 Hz), 4.85 (s, 2H), 4.17–4.09 (m, 4H), 1.33 (t, 6H, *J*=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0, 16.1, 57.7, 66.0, 115.0, 121.4, 121.6, 124.4, 127.5, 127.7, 130.5, 144.6, 146.8, 152.3, 166.1; ^{31}P NMR (162 MHz, $CDCl_3$) δ 6.75; MS (EI) *m*/*z* 382 (M^+-1), 383 (M^+), 385 (M^++2). Anal. Calcd for $C_{14}H_{17}Cl_2O_6P$: C, 43.88; H, 4.47. Found: C, 43.48; H, 4.76.

4.1.3. Dimethyl α-phenoxyacetoxy vinyl phosphonate (4c). Little yellow solid; yield 68%; mp 57–59 °C; IR (KBr) 2969, 1778, 1630, 1599, 1589, 1492, 1445, 1378, 1260, 1161, 1017, 946, 840, 797, 755, 695, 586, 532 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.32 (t, 2H, *J*=7.8 Hz), 7.02 (t, 1H, *J*=7.2 Hz), 6.94 (d, 2H, *J*=8.0 Hz), 6.13 (dd, 1H, *J*=10.8 and 2.2 Hz), 5.85 (dd, 1H, *J*=34.6 and 2.2 Hz), 4.80 (s, 2H), 3.76 (d, 6H, *J*=11.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 53.45, 53.51, 65.1, 114.8, 122.2, 122.4, 122.6, 129.8, 145.6, 157.6, 166.8; ^{31}P NMR (162 MHz, $CDCl_3$) δ 10.16; MS (EI) *m*/*z* 286 (M^+), 287 (M^++1). Anal. Calcd for $C_{12}H_{15}O_6P$: C, 50.36; H, 5.28. Found: C, 50.12; H, 5.35.

4.1.4. Dimethyl α-(2-chlorophenoxyacetoxy) vinyl phosphonate (4d). Colorless liquid; yield 64%; IR (KBr) 2958, 1785, 1589, 1485, 1449, 1258, 1197, 1084, 1043 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40 (dd, 1H, *J*=8.0 and 1.6 Hz), 7.23 (t, 1H, *J*=1.2 Hz), 6.98 (t, 1H, *J*=1.6 Hz), 6.91 (dd, 1H, *J*=4.0 and 1.2 Hz), 6.12 (dd, 1H, *J*=11.0 and 2.4 Hz), 5.86 (dd, 1H, *J*=34.8 and 2.4 Hz), 4.88 (s, 2H), 3.77–3.72 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 53.50, 53.51, 65.9, 114.1, 112.4, 122.6, 123.1, 123.5, 127.9, 130.8, 143.4, 145.6, 153.3, 166.2; ^{31}P NMR (162 MHz, $CDCl_3$) δ 10.08; MS (EI) *m*/*z* 320 (M^+-1), 321

(M^+), 322 (M^++1). Anal. Calcd for $C_{12}H_{14}ClO_6P$: C, 44.95; H, 4.40. Found: C, 44.75; H, 4.40.

4.1.5. Dimethyl α-(4-chlorophenoxyacetoxy) vinyl phosphonate (4e). Little yellow solid; yield 80%; mp 32–34 °C; IR (KBr) 2957, 1784, 1596, 1493, 1446, 1266, 1151, 1027, 834, 801, 640, 509 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.26 (d, 2H, J=11.2 Hz), 6.87 (d, 2H, J=11.2 Hz), 6.11 (dd, 1H, J=10.6 and 2.4 Hz), 5.85 (dd, 1H, J=34.6 and 2.4 Hz), 4.77 (s, 2H), 3.77 (d, 6H, J=11.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 53.3, 53.4, 65.0, 115.99, 122.2, 122.4, 126.9, 129.3, 129.4, 143.0, 156.0, 166.3; ^{31}P NMR (162 MHz, $CDCl_3$) δ 9.12; MS (EI) m/z 320 (M^+). Anal. Calcd for $C_{12}H_{14}ClO_6P$: C, 44.95; H, 4.40. Found: C, 44.60; H, 4.74.

4.1.6. Dimethyl α-(4-methylphenoxyacetoxy) vinyl phosphonate (4f). Little yellow liquid; yield 87%; IR (KBr) 2958, 1786, 1613, 1512, 1445, 1250, 1149, 1032 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.10 (d, 2H, J=8.4 Hz), 6.83 (d, 2H, J=8.4 Hz), 6.12 (dd, 1H, J=11.0 and 2.2 Hz), 5.85 (dd, 1H, J=36.6 and 2.2 Hz), 4.76 (s, 2H,), 3.78–3.75 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 53.37, 53.43, 65.2, 114.4, 114.6, 122.3, 122.6, 130.1, 131.4, 143.3, 145.6, 145.6, 155.5, 166.9; ^{31}P NMR (162 MHz, $CDCl_3$) δ 10.19; MS (EI) m/z 300 (M^+), 301 (M^++1). Anal. Calcd for $C_{13}H_{17}O_6P$: C, 52.00; H, 5.71. Found: C, 52.44; H, 5.48.

4.1.7. Dimethyl α-(4-methoxyphenoxyacetoxy) vinyl phosphonate (4g). Yellow liquid; yield 75%; IR (KBr) 2958, 1762, 1507, 1458, 1202, 1034 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.90–6.83 (m, 4H), 6.12 (dd, 1H, J=10.8 and 2.4 Hz), 5.84 (dd, 1H, J=34.6 and 2.2 Hz), 4.74 (s, 2H), 3.80–3.74 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 53.2, 53.3, 53.6, 65.8, 114.6, 115.8, 122.1, 122.4, 143.2, 145.4, 151.6, 154.6, 166.6; ^{31}P NMR (162 MHz, $CDCl_3$) δ 9.16; MS (EI) m/z 316 (M^+). Anal. Calcd for $C_{13}H_{17}O_7P$: C, 49.37; H, 5.42. Found: C, 49.02; H, 5.47.

4.1.8. Dimethyl α-(4-fluorophenoxyacetoxy) vinyl phosphonate (4h). Yellow liquid; yield 79%; IR (KBr) 2960, 1785, 1507, 1443, 1204, 1033, 831 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.00 (t, 2H, J=7.6 Hz), 6.91–6.88 (m, 2H), 6.11 (dd, 1H, J=10.6 and 2.4 Hz), 5.85 (dd, 1H, J=34.4 and 2.4 Hz), 4.76 (s, 2H), 3.77 (d, 6H, J=11.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 53.4, 53.5, 65.7, 116.0, 116.1, 116.3, 122.3, 122.5, 143.4, 145.6, 153.8, 156.9, 159.3, 166.7; ^{31}P NMR (162 MHz, $CDCl_3$) δ 10.14; MS (EI) m/z 304 (M^+), 305 (M^++1). Anal. Calcd for $C_{12}H_{14}FO_6P$: C, 47.38; H, 4.64. Found: C, 47.49; H, 4.60.

4.1.9. Dimethyl α-(3-trifluoromethylphenoxyacetoxy) vinyl phosphonate (4i). Little yellow liquid; yield 62%; IR (KBr) 2962, 2859, 1757, 1594, 1494, 1457, 1331, 1170, 1127, 1066 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.42 (t, 1H, J=8.0 Hz), 7.30–7.28 (m, 1H), 7.16–7.12 (m, 2H), 6.13 (dd, 1H, J=11.2 and 2.4 Hz), 5.85 (dd, 1H, J=34.4 and 2.4 Hz), 4.84 (s, 2H), 3.76 (d, 6H, J=11.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 53.4, 53.5, 65.1, 111.69, 116.72, 118.3, 118.91, 118.95, 119.8, 122.4, 122.5, 122.7, 125.2, 130.4, 132.0, 132.3, 143.4, 145.6, 157.7, 166.28, 166.30; ^{31}P NMR (162 MHz, $CDCl_3$) δ 10.04; MS (EI) m/z 354 (M^+), 355 (M^++1). Anal. Calcd for $C_{13}H_{14}F_3O_6P$: C, 44.08; H, 3.98. Found: C, 44.19; H, 4.05.

4.1.10. Dimethyl α-(4-methylbenzoxy) vinyl phosphonate (4j). Pale yellow liquid; yield 87%; IR (KBr) 2956, 2854, 1739, 1612, 1578, 1457, 1265, 1176, 1025, 836, 801, 748, 686 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, 2H, J=8.4 Hz), 7.28 (d, 2H, J=8.0 Hz), 6.18 (dd, 1H, J=10.8 and 2.2 Hz), 5.92 (dd, 1H, J=34.2 and 2.2 Hz), 3.82 (d, 6H, J=11.2 Hz), 2.44 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.6, 53.18, 53.23, 121.9, 122.2, 126.0, 129.4, 130.3, 144.0, 144.9, 146.3, 164.3; ^{31}P NMR (162 MHz, $CDCl_3$) δ 10.33; MS (EI) m/z 270 (M^+). Anal. Calcd for $C_{12}H_{15}O_5P$: C, 53.34; H, 5.60. Found: C, 53.49; H, 5.44.

4.1.11. Diethyl α-(4-methylbenzoxy) vinyl phosphonate (4k). Pale yellow liquid; yield 78%; IR (KBr) 2984, 1740, 1611, 1577, 1478, 1393, 1267, 1198, 1019, 975, 798, 748, 687 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, 2H, J=8.4 Hz), 7.28 (d, 2H, J=8.4 Hz), 6.17 (dd, 1H, J=11.2 and 2.0 Hz), 5.90 (dd, 1H, J=35.2 and 2.0 Hz), 4.22–4.16 (m, 4H), 2.44 (s, 3H), 1.34 (t, 6H, J=6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.36, 16.42, 21.90, 63.1, 63.2, 121.4, 121.7, 126.1, 129.5, 130.3, 144.9, 147.2, 164.2; ^{31}P NMR (162 MHz, $CDCl_3$) δ 7.46; MS (EI) m/z 299 (M^++1). Anal. Calcd for $C_{14}H_{19}ClO_5P$: C, 56.37; H, 6.42. Found: C, 56.56; H, 6.15.

4.1.12. (*E*)-Dimethyl α-(4-methylbenzoxy) propenyl phosphonate (4l). White solid; yield 88%; mp 36–39 °C; IR (KBr) 2960, 2856, 1737, 1662, 1612, 1446, 1262, 1179, 1017, 806, 752, 688 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, 2H, J=8.0 Hz), 7.29 (d, 2H, J=8.4 Hz), 6.71–6.63 (m, 1H), 3.78 (d, 6H, J=11.2 Hz), 2.44 (s, 3H), 1.74 (dd, 3H, J=6.8 and 2.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 12.3, 21.8, 53.15, 53.20, 125.8, 129.4, 130.3, 135.1, 135.4, 139.2, 141.8, 144.8, 163.5; ^{31}P NMR (162 MHz, $CDCl_3$) δ 11.72; MS (EI) m/z 284 (M^+). Anal. Calcd for $C_{13}H_{17}O_5P$: C, 54.93; H, 6.03. Found: C, 54.84; H, 5.84.

4.1.13. Dimethyl α-(4-chlorobenzoxy) vinyl phosphonate (4m). Colorless liquid; yield 95%; IR (KBr) 2959, 1735, 1633, 1595, 1402, 1263, 1191, 1013, 834, 751, 680 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (dd, 2H, J=6.8 and 2.0 Hz), 7.47 (dd, 2H, J=6.8 and 2.0 Hz), 6.18 (dd, 1H, J=10.8 and 2.0 Hz), 5.93 (dd, 1H, J=34.2 and 2.4 Hz), 3.82 (d, 6H, J=11.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 53.25, 53.30, 122.1, 122.3, 127.2, 128.8, 129.1, 131.5, 131.7, 140.6, 144.0, 146.2, 163.5; ^{31}P NMR (162 MHz, $CDCl_3$) δ 10.32; MS (EI) m/z 290 (M^+-1), 291 (M^+). Anal. Calcd for $C_{11}H_{12}ClO_5P$: C, 45.46; H, 4.16. Found: C, 45.48; H, 4.13.

4.1.14. Diethyl α-(4-chlorobenzoxy) vinyl phosphonate (4n). Colorless liquid; yield 82%; IR (KBr) 2984, 1743, 1633, 1593, 1488, 1401, 1264, 1195, 1016, 850, 754, 680 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, 2H, J=8.8 Hz), 7.46 (d, 2H, J=8.4 Hz), 6.17 (dd, 1H, J=11.2 and 2.0 Hz), 5.90 (dd, 1H, J=34.8 and 2.0 Hz), 4.21–4.16 (m, 4H), 1.34 (t, 6H, J=6.8 Hz); ^{13}C NMR (100 MHz,

$CDCl_3$) δ 16.06, 16.13, 62.98, 63.03, 121.3, 121.5, 127.4, 129.1, 131.6, 140.5, 145.0, 147.3, 163.5; ^{31}P NMR (162 MHz, $CDCl_3$) δ 7.36; MS (EI) *m/z* 319 (M^+). Anal. Calcd for $C_{13}H_{16}ClO_5P$: C, 48.99; H, 5.06. Found: C, 49.04; H, 4.97.

4.1.15. (*E*)-Dimethyl α-(4-chlorobenzoxy) propenyl phosphonate (4o). Colorless liquid; yield 86%; IR (KBr) 2956, 1740, 1660, 1593, 1488, 1402, 1260, 1174, 1023, 799, 754, 682 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.05 (dd, 2H, *J*=6.8 and 2.0 Hz), 7.47 (dd, 2H, *J*=6.8 and 2.0 Hz), 6.71–6.61 (m, 1H), 3.80 (d, 6H, *J*=11.2 Hz), 1.75 (dd, 3H, *J*=6.8 and 2.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.8, 12.0, 52.98, 53.03, 127.1, 129.1, 131.7, 135.1, 135.3, 137.1, 139.4, 140.5, 162.8; ^{31}P NMR (162 MHz, $CDCl_3$) δ 11.33; MS (EI) *m/z* 305 (M^+), 307 (M^++2). Anal. Calcd for $C_{12}H_{14}ClO_5P$: C, 47.31; H, 4.63. Found: C, 47.26; H, 4.73.

4.1.16. (*E*)-Diethyl α-(4-chlorobenzoxy) propenyl phosphonate (4p). White solid; yield 56%; mp 52–55 °C; IR (KBr) 2992, 1734, 1655, 1593, 1488, 1403, 1256, 1148, 1092, 1009, 976, 801, 751, 545 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.05 (dd, 2H, *J*=6.8 and 2.0 Hz), 7.47 (dd, 2H, *J*=6.8 and 2.0 Hz), 6.67–6.63 (m, 1H), 4.19–4.11 (m, 4H), 1.73 (dd, 3H, *J*=7.2 and 2.4 Hz), 1.32 (t, 6H, *J*=6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 12.3, 16.28, 16.34, 31.5, 62.7, 127.2, 129.0, 131.6, 134.3, 134.6, 140.3, 162.6; ^{31}P NMR (162 MHz, $CDCl_3$) δ 8.44; MS (EI) *m/z* 332 (M^+-1), 333 (M^+), 336 (M^++3). Anal. Calcd for $C_{14}H_{18}ClO_5P$: C, 50.54; H, 5.45. Found: C, 50.28; H, 5.59.

4.1.17. Dimethyl α-(2-furancarbonyl oxo) vinyl phosphonate (4q). Colorless liquid; yield 73%; IR (KBr) 2959, 2856, 1750, 1577, 1473, 1394, 1294, 1174, 1031, 884, 837 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.32 (s, 1H), 6.58–6.57 (m, 1H), 6.19 (dd, 1H, *J*=11.0 and 2.0 Hz), 5.93 (dd, 1H, *J*=34.8 and 2.0 Hz), 3.82 (d, 6H, *J*=11.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 53.2, 53.3, 112.2, 119.9, 122.1, 122.3, 142.9, 143.1, 145.4, 147.4, 155.6, 154.6, 166.6; ^{31}P NMR (162 MHz, $CDCl_3$) δ 9.29; MS (EI) *m/z* 247 (M^++1), 248 (M^++2). Anal. Calcd for $C_9H_{11}O_6P$: C, 43.91; H, 4.50. Found: C, 43.73; H, 4.67.

4.1.18. Dimethyl α-(2-thiophenecarbonyl oxo) vinyl phosphonate (4r). Waxy solid; yield 85%; IR (KBr) 2957, 1732, 1630, 1522, 1462, 1415, 1267, 1197, 1027, 837, 793, 740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, 1H, *J*=3.2 Hz), 7.67 (d, 1H, *J*=4.4 Hz), 7.16 (t, 1H, *J*=4.4 Hz), 6.18 (dd, 1H, *J*=11.2 and 2.4 Hz), 5.94 (dd, 1H, *J*=34.4 and 2.4 Hz), 3.83 (d, 6H, *J*=11.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 53.28, 53.33, 121.8, 122.0, 128.0, 131.8, 133.9, 134.0, 134.97, 135.03, 143.7, 146.0, 159.4; ^{31}P NMR (162 MHz, $CDCl_3$) δ 9.74; MS (EI) *m/z* 262 (M^+), 263 (M^++1). Anal. Calcd for $C_9H_{11}O_5PS$: C, 41.22; H, 4.23. Found: C, 41.48; H, 4.26.

4.1.19. Diethyl α-(2-thiophenecarbonyl oxo) vinyl phosphonate (4s). Colorless liquid; yield 76%; IR (KBr) 2984, 1732, 1630, 1522, 1477, 1415, 1267, 1194, 1026, 851, 795, 740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, 1H, *J*=3.6 Hz), 7.66 (d, 1H, *J*=4.8 Hz), 7.16 (t, 1H, *J*=4.4 Hz), 6.18 (dd, 1H, *J*=10.8 and 2.0 Hz), 5.91 (dd, 1H, *J*=34.4 and 2.0 Hz), 4.19 (m, 4H), 1.35 (t, 3H, *J*=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.06, 16.12, 121.5, 121.7, 128.2, 132.1, 134.0, 135.1, 144.8, 147.0, 159.6; ^{31}P NMR (162 MHz, $CDCl_3$) δ 7.25; MS (EI) *m/z* 290 (M^+), 291 (M^++1). Anal. Calcd for $C_{11}H_{15}O_5PS$: C, 45.52; H, 5.21. Found: C, 45.88; H, 5.16.

4.1.20. (*E*)-Dimethyl α-(2-thiophenecarbonyl oxo) propenyl phosphonate (4t). White solid; yield 92%; mp 84–85 °C; IR (KBr) 2954, 1737, 1664, 1523, 1414, 1259, 1149, 1011, 801, 752, 740, 541 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.92 (dd, 1H, *J*=3.6 and 0.8 Hz), 7.67 (dd, 1H, *J*=4.8 and 1.6 Hz), 7.17 (dd, 1H, *J*=4.8 and 4.0 Hz), 6.72–6.64 (m, 1H), 3.80 (d, 6H, *J*=11.2 Hz), 1.77 (dd, 3H, *J*=6.8 and 2.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 12.3, 53.29, 53.34, 128.5, 132.0, 134.2, 135.3, 135.7, 136.0, 137.1, 139.4, 159.3; ^{31}P NMR (162 MHz, $CDCl_3$) δ 11.38; MS (EI) *m/z* 276 (M^+), 277 (M^++1). Anal. Calcd for $C_{10}H_{13}O_5PS$: C, 43.48; H, 4.74. Found: C, 43.60; H, 4.81.

4.1.21. Dimethyl α-(methoxyacetoxy) vinyl phosphonate (4u). Colorless liquid; yield 80%; IR (KBr) 2959, 1775, 1630, 1456, 1376, 1263 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.11 (dd, 1H, *J*=10.4 and 2.3 Hz), 5.84 (dd, 1H, *J*=34.8 and 2.3 Hz), 4.2 (s, 2H), 3.79 (d, 6H, *J*=11.2 Hz), 3.49 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 53.4, 53.5, 59.6, 69.5, 122.2, 122.4, 143.4, 145.7, 168.1; ^{31}P NMR (162 MHz, $CDCl_3$) δ 10.11; MS (EI) *m/z* 224 (M^+), 225 (M^++1). Anal. Calcd for $C_7H_{13}O_6P$: C, 37.51; H, 5.85. Found: C, 37.32; H, 5.59.

4.1.22. Dimethyl α-(phenyl acetoxy) vinyl phosphonate (4v). Colorless liquid; yield 68%; IR (KBr) 3032, 2956, 1738, 1225, 1031, 934 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.36–7.27 (m, 5H), 6.06 (dd, 1H, *J*=11.4 and 2.2 Hz), 5.77 (dd, 1H, *J*=35.2 and 2.4 Hz), 3.80–3.61 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.9, 53.1, 53.2, 121.7, 122.0, 127.4, 128.6, 129.2, 132.8, 143.6, 145.8, 168.9; ^{31}P NMR (162 MHz, $CDCl_3$) δ 9.59; MS (EI) *m/z* 270 (M^+). Anal. Calcd for $C_{12}H_{15}O_5P$: C, 53.34; H, 5.60. Found: C, 53.38; H, 5.66.

4.2. Typical procedure for the asymmetric hydrogenation (the preparation of 5a is representative)

In a glove box, the Rh/Me-DuPhos complex was made in situ by mixing $[Rh(COD)_2]BF_4$ (2.0 mg, 0.005 mmol) and (*R*,*R*)-Me-DuPhos (1.7 mg, 0.0055 mmol) in MeOH (2.0 mL). After the mixture was stirred at room temperature for 10 min, substrate **4a** (178 mg, 0.5 mmol) in MeOH (2.0 mL) was added by a syringe. The hydrogenation was performed at room temperature under 4 atm H_2 for 12 h. After carefully releasing the excess hydrogen, the conversion was determined by ^1H NMR analysis and it was found more than 95%. The reaction mixtures were concentrated and then passed through a silica gel plug using ethyl acetate and petroleum ether (3:1) as an eluant to give pure product **5a** (159 mg, 89% yield). The enantiomeric excess (ee value) was determined by comparison of the enantiomerically enriched sample to the racemate on chiral HPLC.

4.2.1. (*S*)-Dimethyl α-(2,4-dichlorophenoxyacetoxy) ethyl phosphonate (5a). Pale yellow liquid; 94% ee; $[\alpha]_D^{14}$ +15.2 (*c* 2.00, $CHCl_3$); IR (KBr) 2957, 1741, 1586, 1476, 1456,

1266, 1178, 1021, 836, 802 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, 1H, J=2.4 Hz), 7.16 (dd, 1H, J=8.8 and 2.4 Hz), 6.77 (d, 1H, J=8.8 Hz), 5.37 (p, 1H, J=7.2 Hz), 4.74 (s, 2H), 3.77 (t, 6H, J=10.4 Hz), 1.49 (dd, 3H, J=16.6 and 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.0, 53.4, 64.4, 66.1, 114.8, 124.1, 127.2, 127.5, 130.3, 152.2, 167.0; ^{31}P NMR (162 MHz, $CDCl_3$) δ 22.46; MS (EI) m/z 356 (M^+-1), 357 (M^+), 358 (M^++1). Anal. Calcd for $C_{12}H_{15}Cl_2O_6P$: C, 40.36; H, 4.23. Found: C, 40.58; H, 4.34. Enantiomeric excess determination: HPLC, UV 202 nm, Chiralcel OJ-H, 1 mL/min, 10% 2-propanol/90% hexane, (*R*) t_1=48.57 min; (*S*) t_2=55.41 min.

4.2.2. (*S*)-Diethyl α-(2,4-dichlorophenoxyacetoxy) ethyl phosphonate (5b). Pale yellow liquid; 96% ee; $[\alpha]_D^{14}$ +15.1 (*c* 3.10, $CHCl_3$); IR (KBr) 2984, 1741, 1478, 1265, 1191, 1022, 801 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, 1H, J=2.4 Hz), 7.17 (dd, 1H, J=8.8 and 2.4 Hz), 6.79 (d, 1H, J=8.8 Hz), 5.36 (p, 1H, J=7.2 Hz), 4.74 (s, 2H), 4.19–4.09 (m, 4H), 1.49 (dd, 3H, J=16.4 and 7.2 Hz), 1.31 (q, 6H, J=8.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.1, 16.5, 63.0, 65.0, 66.3, 66.7, 114.9, 124.3, 127.7, 130.4, 152.3, 167.2; ^{31}P NMR (162 MHz, $CDCl_3$) δ 19.96; MS (EI) m/z 385 (M^+), 387 (M^++2), 388 (M^++3). Anal. Calcd for $C_{14}H_{19}Cl_2O_6P$: C, 43.66; H, 4.97. Found: C, 43.68; H, 4.77. HPLC, UV 293 nm, Chiralcel OJ-H, 0.5 mL/min, 10% 2-propanol/90% hexane, (*R*) t_1=36.87 min; (*S*) t_2=43.63 min.

4.2.3. (*S*)-Dimethyl α-phenoxyacetoxy ethyl phosphonate (5c). Pale yellow liquid; 93% ee; $[\alpha]_D^{14}$ +12.9 (*c* 1.40, $CHCl_3$); IR (KBr) 2958, 1769, 1600, 1496, 1457, 1249, 1178, 1048, 833 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.29–7.24 (m, 2H), 6.97 (t, 1H, J=7.4 Hz), 6.95–6.86 (m, 2H), 5.38 (p, 1H, J=7.2 Hz), 4.66 (s, 2H), 3.77–3.72 (m, 6H), 1.48 (dd, 3H, J=16.8 and 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.1, 53.47, 53.53, 64.2, 65.2, 65.9, 114.7, 122.0, 129.7, 157.7, 168.0, 168.1; ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.3; MS (EI) m/z 287 (M^+-1), 287 (M^+), 289 (M^++1). Anal. Calcd for $C_{12}H_{17}O_6P$: C, 50.00; H, 5.94. Found: C, 50.35; H, 5.87. Enantiomeric excess determination: HPLC, UV 205 nm, Chiralcel OJ-H, 0.8 mL/min, 20% 2-propanol/80% hexane, (*S*) t_1=22.70 min; (*R*) t_2=26.16 min.

4.2.4. (*S*)-Dimethyl α-(2-chlorophenoxyacetoxy) ethyl phosphonate (5d). Yellow liquid; 93% ee; $[\alpha]_D^{14}$ +13.1 (*c* 1.20, $CHCl_3$); IR (KBr) 2958, 1769, 1589, 1485, 1449, 1378, 1246, 1189, 1407, 833 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.39 (dd, 1H J=7.8 and 1.6 Hz), 7.21–7.19 (m, 1H), 6.98–6.94 (m, 1H), 6.86–6.84 (m, 1H), 5.44–5.37 (m, 1H), 4.78 (s, 2H), 3.80–3.73 (m, 6H), 1.51 (dd, 3H, J=16.8 and 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.2, 53.6, 64.5, 65.2, 65.5, 66.1, 114.0, 114.7, 116.1, 122.9, 123.4, 127.8, 129.6, 129.7, 130.8, 153.4, 167.5, 167.5; ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.09; MS (EI) m/z 322 (M^+-1), 323 (M^+), 324 (M^++1). Anal. Calcd for $C_{12}H_{16}ClO_6P$: C, 44.67; H, 5.00. Found: C, 44.54; H, 4.94. Enantiomeric excess determination: HPLC, UV 205 nm, Chiralcel AS-H, 0.5 mL/min, 20% 2-propanol/80% hexane, (*S*) t_1=21.26 min; (*R*) t_2=23.20 min.

4.2.5. (*S*)-Dimethyl α-(4-chlorophenoxyacetoxy) ethyl phosphonate (5e). Yellow liquid; 95% ee; $[\alpha]_D^{14}$ +15.7 (*c* 1.30, $CHCl_3$); IR (KBr) 2958, 1768, 1493, 1247, 1188, 1049, 830 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.25 (dd, 2H, J=7.0 and 2.0 Hz), 6.84 (dd, 2H, J=6.8 and 2.4 Hz), 5.40 (p, 1H, J=8.0 Hz), 4.67 (s, 2H), 3.81–3.76 (m, 6H), 1.51 (dd, 3H, J=16.8 and 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.1, 53.4, 53.5, 53.6, 64.3, 65.4, 66.0, 116.1, 127.0, 129.6, 156.3, 167.65, 167.72; ^{31}P NMR (162 MHz, $CDCl_3$) δ 22.96; MS (EI) m/z 322 (M^+-1), 323 (M^+), 324 (M^++1). Anal. Calcd for $C_{12}H_{16}ClO_6P$: C, 44.67; H, 5.00. Found: C, 44.65; H, 4.95. Enantiomeric excess determination: HPLC, UV 205 nm, Chiralcel AS-H, 0.5 mL/min, 20% 2-propanol/80% hexane, (*S*) t_1=20.93 min; (*R*) t_2=26.53 min.

4.2.6. (*S*)-Dimethyl α-(4-methylphenoxyacetoxy) ethyl phosphonate (5f). Colorless liquid; 91% ee; $[\alpha]_D^{14}$ +14.8 (*c* 1.38, $CHCl_3$); IR (KBr) 2957, 1769, 1512, 1447, 1291, 1248, 1178, 1048 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.06 (d, 2H, J=8.0 Hz), 6.77 (d, 2H, J=8.0 Hz), 5.38 (p, 1H, J=7.2 Hz), 4.63 (s, 1H), 3.78–3.71 (m, 6H), 2.26 (s, 3H), 1.50 (dd, 3H, J=16.8 and 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.1, 20.6, 64.1, 65.4, 65.8, 114.5, 130.1, 131.3, 155.6, 168.1, 168.2; ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.40; MS (EI) m/z 322 (M^+-1), 302 (M^+), 303 (M^++1). Anal. Calcd for $C_{13}H_{19}O_6P$: C, 51.66; H, 6.34. Found: C, 51.58; H, 6.25. Enantiomeric excess determination: HPLC, UV 205 nm, Chiralcel OJ-H, 0.8 mL/min, 20% 2-propanol/80% hexane, (*S*) t_1=21.60 min; (*R*) t_2=27.69 min.

4.2.7. (*S*)-Dimethyl α-(4-methoxyphenoxyacetoxy) ethyl phosphonate (5g). Pale yellow liquid; 95% ee; $[\alpha]_D^{14}$ +12.2 (*c* 0.74, $CHCl_3$); IR (KBr) 2958, 1769, 1059, 1446, 1244, 1183, 1032, 831 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.87–6.82 (m, 4H), 5.41 (p, 1H, J=7.2 Hz), 4.64 (s, 1H), 3.81–3.77 (m, 6H), 1.51 (dd, 3H, J=16.8 and 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.2, 53.56, 53.62, 55.8, 64.2, 65.9, 66.2, 114.8, 116.0, 152.0, 154.8, 168.4; ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.41; MS (EI) m/z 318 (M^+-1), 319 (M^+). Anal. Calcd for $C_{13}H_{19}O_7P$: C, 49.06; H, 6.02. Found: C, 48.66; H, 5.78. Enantiomeric excess determination: HPLC, UV 205 nm, Chiralcel AS-H, 0.7 mL/min, 40% 2-propanol/60% hexane, (*S*) t_1=13.93 min; (*R*) t_2=20.14 min.

4.2.8. (*S*)-Dimethyl α-(4-fluorophenoxyacetoxy) ethyl phosphonate (5h). Pale yellow liquid; 94% ee; $[\alpha]_D^{14}$ +13.3 (*c* 1.24, $CHCl_3$); IR (KBr) 2959, 2856, 1769, 1507, 1447, 1249, 1186, 1031, 831 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.01–6.97 (m, 2H), 6.88–6.84 (m, 2H), 5.40 (p, 1H, J=7.2 Hz), 4.66 (s, 2H), 3.81–3.76 (m, 6H), 1.51 (dd, 3H, J=16.8 and 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.2, 53.48, 53.54, 53.6, 64.3, 66.0, 116.0, 116.1, 116.3, 153.9, 156.8, 159.2, 167.9, 168.0; ^{31}P NMR (162 MHz, $CDCl_3$) δ 22.3; MS (EI) m/z 306 (M^+), 307 (M^++1). Anal. Calcd for $C_{12}H_{16}FO_6P$: C, 47.07; H, 5.27. Found: C, 46.80; H, 5.14. Enantiomeric excess determination: HPLC, UV 205 nm, Chiralcel OJ-H, 0.8 mL/min, 20% 2-propanol/80% hexane, (*S*) t_1=20.76 min; (*R*) t_2=21.98 min.

4.2.9. (*S*)-Dimethyl α-(3-trifluoromethylphenoxyacetoxy) ethyl phosphonate (5i). Yellow liquid; 92% ee; $[\alpha]_D^{14}$ +10.3 (*c* 1.10, $CHCl_3$); IR (KBr) 2960, 2857, 1769, 1495, 1457,

1332, 1182, 1126, 1068, 834 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40 (t, 1H, J=8.0 Hz), 7.28 (d, 1H, J=8.0 Hz), 7.12–7.08 (m, 2H, J=7.6 Hz), 5.41 (p, 1H, J=7.2 Hz), 4.74 (s, 1H), 3.81–3.76 (m, 6H), 1.51 (dd, 3H, J=16.8 and 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.1, 53.4, 53.5, 53.57, 53.63, 64.4, 65.3, 66.1, 111.6, 111.7, 118.3, 118.7, 118.8, 122.6, 125.3, 130.4, 132.0, 132.3, 157.9, 167.47, 167.54; ^{31}P NMR (162 MHz, $CDCl_3$) δ 22.9; MS (EI) m/z 356 (M^+), 357 (M^++1). Anal. Calcd for $C_{13}H_{16}F_3O_6P$: C, 43.83; H, 4.53. Found: C, 43.92; H, 4.39. Enantiomeric excess determination: HPLC, UV 205 nm, Chiralcel AS-H, 0.5 mL/min, 20% 2-propanol/80% hexane, (*S*) t_1=13.92 min; (*R*) t_2=15.20 min.

4.2.10. (*S*)-Dimethyl α-(4-methylbenzoxy) ethyl phosphonate (5j). Colorless liquid; 95% ee; $[\alpha]_D^{14}$ −19.61 (*c* 2.38, $CHCl_3$); IR (KBr) 2957, 2854, 1725, 1612, 1456, 1266, 1179, 1029, 832, 805, 753, 690 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, 2H, J=8.0 Hz), 7.21 (d, 2H, J=8.0 Hz), 5.51 (p, 1H, J=7.2 Hz), 3.82 (dd, 6H, J=10.4 and 4.4 Hz), 2.37 (s, 3H), 1.55 (dd, 3H, J=8.8 and 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.2, 21.7, 53.3, 53.6, 63.5, 65.2, 126.6, 129.2, 129.9, 144.2, 165.3; ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.86; MS (EI) m/z 271 (M^+-1), 272 (M^+), 273 (M^++1). Anal. Calcd for $C_{12}H_{17}O_5P$: C, 52.94; H, 6.29. Found: C, 53.15; H, 6.33. HPLC, UV 246 nm, Chiralcel OJ-H, 0.5 mL/min, 10% 2-propanol/90% hexane, (*S*) t_1=25.46 min; (*R*) t_2=28.38 min.

4.2.11. (*S*)-Diethyl α-(4-methylbenzoxy) ethyl phosphonate (5k). Pale yellow liquid; 95% ee; $[\alpha]_D^{14}$ −22.38 (*c* 1.82, $CHCl_3$); IR (KBr) 2985, 1724, 1613, 1266, 1023, 968, 799, 752 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, 2H, J=8.0 Hz), 7.22 (d, 2H, J=8.0 Hz), 5.49 (p, 1H, J=7.2 Hz), 4.19–4.12 (m, 4H), 2.38 (s, 3H), 1.55 (dd, 3H, J=16.8 and 7.2 Hz), 1.28 (t, 6H, J=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.3, 16.5, 21.7, 62.7, 63.0, 63.9, 65.6, 126.8, 129.2, 129.9, 144.2, 165.5; ^{31}P NMR (162 MHz, $CDCl_3$) δ 21.38; MS (EI) m/z 300 (M^+). Anal. Calcd for $C_{14}H_{21}ClO_5P$: C, 56.00; H, 7.05. Found: C, 56.24; H, 7.15. HPLC, UV 246 nm, Chiralcel OJ-H, 0.5 mL/min, 10% 2-propanol/90% hexane, (*S*) t_1=14.30 min; (*R*) t_2=16.40 min.

4.2.12. (*S*)-Dimethyl α-(4-methylbenzoxy) propanyl phosphonate (5l). Colorless liquid; 94% ee; $[\alpha]_D^{14}$ +8.63 (*c* 1.16, $CHCl_3$); IR (KBr) 2957, 2854, 1725, 1613, 1457, 1256, 1178, 1026, 835, 753 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, 2H, J=8.0 Hz), 7.26 (d, 2H, J=8.0 Hz), 5.48 (m, 1H), 3.79 (d, 6H, J=10.8 Hz), 2.42 (s, 3H), 2.06–1.99 (m, 2H), 1.03 (t, 3H, J=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.3, 21.8, 23.1, 53.3, 53.6, 68.3, 67.0, 126.6, 129.4, 130.1, 144.4, 165.5; ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.15; MS (EI) m/z 286 (M^+). Anal. Calcd for $C_{13}H_{19}O_5P$: C, 54.54; H, 6.69. Found: C, 54.32; H, 6.81. HPLC, UV 246 nm, Chiralcel OJ-H, 0.5 mL/min, 10% 2-propanol/90% hexane, (*S*) t_1=21.95 min; (*R*) t_2=23.66 min.

4.2.13. (*S*)-Dimethyl α-(4-chlorobenzoxy) ethyl phosphonate (5m). Pale yellow liquid; 95% ee; $[\alpha]_D^{14}$ −19.6 (*c* 1.20, $CHCl_3$); IR (KBr) 2956, 1734, 1594, 1455, 1401, 1265, 1177, 1025, 832, 802, 759, 685 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, 2H, J=8.4 Hz), 7.44 (d, 2H, J=8.4 Hz), 5.54 (p, 1H, J=7.6 Hz), 3.82 (dd, 6H, J=10.6 and 2.4 Hz), 1.60 (dd, 3H, J=16.8 and 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.1, 53.3, 55.5, 63.9, 65.6, 127.7, 128.5, 128.8, 129.8, 131.2, 133,4, 139.9, 164.4; ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.48; MS (EI) m/z 292 (M^+−1), 293 (M^+), 294 (M^++1), 295 (M^++2). Anal. Calcd for $C_{11}H_{14}ClO_5P$: C, 45.14; H, 4.82. Found: C, 45.46; H, 4.93. HPLC, UV 246 nm, Chiralcel OJ-H, 0.5 mL/min, 10% 2-propanol/90% hexane, (*S*) t_1=26.32 min; (*R*) t_2=28.40 min.

4.2.14. (*S*)-Diethyl α-(4-chlorobenzoxy) ethyl phosphonate (5n). Yellow liquid; 96% ee; $[\alpha]_D^{14}$ −14.49 (*c* 1.72, $CHCl_3$); IR (KBr) 2985, 1730, 1594, 1488, 1400, 1265, 1174, 1026, 795, 685 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, 2H, J=8.4 Hz), 7.40 (d, 2H, J=8.4 Hz), 5.48 (p, 1H, J=7.6 Hz), 4.20–4.13 (m, 4H), 1.56 (dd, 3H, J=16.8 and 7.2 Hz), 1.29 (t, 6H, J=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.2, 16.5, 62.9, 63.0, 64.4, 66.1, 128.0, 128.9, 131.3, 139.9, 164.6; ^{31}P NMR (162 MHz, $CDCl_3$) δ 21.02; MS (EI) m/z 321 (M^+). Anal. Calcd for $C_{13}H_{18}ClO_5P$: C, 48.69; H, 5.66. Found: C, 48.57; H, 5.95. HPLC, UV 246 nm, Chiralcel OJ-H, 0.5 mL/min, 10% 2-propanol/90% hexane, (*S*) t_1=14.79 min; (*R*) t_2=17.13 min.

4.2.15. (*S*)-Dimethyl α-(4-chlorobenzoxy) propanyl phosphonate (5o). Colorless liquid; 91% ee; $[\alpha]_D^{14}$ +7.14 (*c* 2.46, $CHCl_3$); IR (KBr) 2957, 1730, 1594, 1488, 1458, 1401, 1259, 1175, 1030, 833, 758, 684 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, 2H, J=8.4 Hz), 7.40 (d, 2H, J=8.4 Hz), 5.45–5.40 (m, 1H), 3.76 (d, 6H, J=10.6 Hz), 2.04–1.93 (m, 2H), 1.00 (t, 3H, J=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.2, 10.4, 23.0, 53.3, 53.4, 68.7, 70.3, 127.7, 128.9, 131.3, 140.0, 164.8; ^{31}P NMR (162 MHz, $CDCl_3$) δ 22.79; MS (EI) m/z 307 (M^+), 308 (M^++1), 309 (M^++2). Anal. Calcd for $C_{12}H_{16}ClO_5P$: C, 47.00; H, 5.26. Found: C, 47.24; H, 4.99. HPLC, UV 246 nm, Chiralcel OJ-H, 0.5 mL/min, 10% 2-propanol/90% hexane, (*S*) t_1=21.15 min; (*R*) t_2=23.25 min.

4.2.16. (*S*)-Diethyl α-(4-chlorobenzoxy) propanyl phosphonate (5p). Colorless liquid; 92% ee; $[\alpha]_D^{14}$ +6.55 (*c* 2.28, $CHCl_3$); IR (KBr) 2981, 1730, 1594, 1488, 1400, 1256, 1094, 1024, 971, 757 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, 2H, J=8.4 Hz), 7.45 (d, 2H, J=8.4 Hz), 5.42 (m, 1H), 4.18–4.13 (m, 4H), 2.00 (m, 2H), 1.31 (q, 6H, J=7.2 Hz), 1.03 (t, 3H, J=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.4, 10.5, 16.6, 23.0, 62.9, 69.1, 70.8, 127.9, 129.0, 131.3, 134.0, 165.0; ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.26; MS (EI) m/z 333 (M^+−2), 335 (M^+), 337 (M^++2). Anal. Calcd for $C_{14}H_{20}ClO_5P$: C, 50.23; H, 6.02. Found: C, 50.49; H, 5.86. HPLC, UV 246 nm, Chiralcel OJ-H, 0.5 mL/min, 10% 2-propanol/90% hexane, (*S*) t_1=12.42 min; (*R*) t_2=13.35 min.

4.2.17. (*S*)-Dimethyl α-(2-furancarbonyl oxo) ethyl phosphonate (5q). Pale yellow liquid; 96% ee; $[\alpha]_D^{14}$ −18.6 (*c* 0.68, $CHCl_3$); IR (KBr) 2959, 2856, 1733, 1579, 1474, 1395, 1323, 1294, 1246, 1179, 1117, 1049, 939, 825 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, 1H, J=1.6 Hz), 7.26 (d, 1H, J=3.6 Hz), 6.54 (dd, 1H, J=3.6 and 1.6 Hz), 5.56–5.48 (m, 1H), 3.87–3.79 (m, 6H), 1.60 (dd, 3H, J=16.8 and 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.3,

53.5, 53.6, 53.7, 53.8, 63.9, 65.6, 112.2, 119.1, 143.9, 147.1, 157.4, 157.5; ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.71; MS (EI) m/z 248 (M^+-1), 249 (M^++1). Anal. Calcd for $C_9H_{13}O_6P$: C, 43.56; H, 5.28. Found: C, 43.81; H, 5.19. Enantiomeric excess determination: HPLC, UV 254 nm, Chiralcel OJ-H, 0.8 mL/min, 10% 2-propanol/90% hexane, (*S*) t_1=20.35 min; (*R*) t_2=25.83 min.

4.2.18. (*S*)-Dimethyl α-(2-thiophenecarbonyl oxo) ethyl phosphonate (5r). Colorless liquid; 95% ee; $[\alpha]_D^{14}$ −29.6 (*c* 3.64, $CHCl_3$); IR (KBr) 2957, 1720, 1524, 1456, 1417, 1260, 1180, 1029, 830, 795, 749 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, 1H, *J*=3.6 Hz), 7.60 (d, 1H, *J*=4.8 Hz), 7.12 (t, 1H, *J*=4.4 Hz), 5.50 (p, 1H, *J*=7.6 Hz), 3.86–3.80 (M, 6H), 1.58 (dd, 3H, *J*=16.4 and 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.3, 53.4, 53.7, 64.2, 65.9, 128.0, 132.8, 133.3, 143.3, 140.0, 161.1; ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.21; MS (EI) m/z 263 (M^+-1), 264 (M^+), 265 (M^++1). Anal. Calcd for $C_9H_{13}O_5PS$: C, 40.91; H, 4.96. Found: C, 41.13; H, 4.61. HPLC, UV 246 nm, Chiralcel OJ-H, 0.5 mL/min, 10% 2-propanol/90% hexane, (*S*) t_1=53.43 min; (*R*) t_2=63.24 min.

4.2.19. (*S*)-Diethyl α-(2-thiophenecarbonyl oxo) ethyl phosphonate (5s). Yellow liquid; 95% ee; $[\alpha]_D^{14}$ −28.7 (*c* 1.12, $CHCl_3$); IR (KBr) 2985, 1717, 1524, 1417, 1362, 1257, 1094, 1046, 1024, 970 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86–7.85 (m, 1H), 7.61 (dd, 1H, *J*=6.8 and 1.2 Hz), 7.14-7.11 (m, 1H), 5.48 (p, 1H, *J*=7.6 Hz), 4.25–4.17 (m, 4H), 1.59 (dd, 3H, *J*=16.4 and 7.2 Hz), 1.34 (t, 6H, *J*=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.4, 16.57, 16.63, 16.7, 62.9, 63.0, 63.2, 64.6, 66.3, 128.1, 133.0, 134.2, 161.0, 161.1; ^{31}P NMR (162 MHz, $CDCl_3$) δ 21.25; MS (EI) m/z 291 (M^+-1), 292 (M^+). Anal. Calcd for $C_{11}H_{17}O_5PS$: C, 45.20; H, 5.86. Found: C, 45.60; H, 5.59. Enantiomeric excess determination: HPLC, UV 254 nm, Chiralcel OJ-H, 0.8 mL/min, 10% 2-propanol/90% hexane, (*S*) t_1=8.98 min; (*R*) t_2=10.70 min.

4.2.20. (*S*)-Dimethyl α-(2-thiophenecarbonyl oxo) propanyl phosphonate (5t). Yellow liquid; 94% ee; $[\alpha]_D^{14}$ +2.79 (*c* 1.60, $CHCl_3$); IR (KBr) 2957, 2854, 1718, 1523, 1416, 1362, 1254, 1096, 1051 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (dd, 1H, *J*=3.6 and 1.2 Hz), 7.62 (dd, 1H, *J*=4.8 and 1.2 Hz), 7.14 (dd, 1H, *J*=4.8 and 3.6 Hz), 5.45–5.39 (m, 1H), 3.84–3.79 (m, 6H), 2.06–1.95 (m, 2H), 1.05 (t, 3H, *J*=7.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.3, 10.4, 23.1, 53.36, 53.42, 53.65, 53.72, 68.8, 70.5, 128.1, 132.7, 133.4, 134.4, 161.4; ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.18; MS (EI) m/z 278 (M^+), 279 (M^++1). Anal. Calcd for $C_{10}H_{15}O_5PS$: C, 43.16; H, 5.43. Found: C, 43.00; H, 5.21. Enantiomeric excess determination: HPLC, UV 246 nm, Chiralcel OJ-H, 1 mL/min, 10% 2-propanol/90% hexane, (*S*) t_1=17.23 min; (*R*) t_2=21.65 min.

4.2.21. (*S*)-Dimethyl α-(methoxyacetoxy) ethyl phosphonate (5u). Pale yellow liquid; 89% ee; $[\alpha]_D^{14}$ +22.1 (*c* 0.88, $CHCl_3$); IR (KBr) 2959, 1764, 1455, 1247, 1186, 1128, 1049, 946, 834 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.43–5.31 (m, 1H), 4.10 (s, 2H), 3.84–3.73 (m, 6H), 3.47 (s, 3H), 1.52 (dd, 3H, *J*=16.4 and 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.2, 53.47, 53.53, 59.5, 63.7, 65.4, 69.7, 169.26, 169.33; ^{31}P NMR (162 MHz, $CDCl_3$) δ 23.71; MS (EI) m/z 226 (M^+), 227 (M^++1), 228 (M^++2). Anal. Calcd for $C_7H_{15}O_6P$: C, 37.17; H, 6.69. Found: C, 36.88; H, 6.49. Enantiomeric excess determination: HPLC, UV 205 nm, Chiralcel AS-H, 0.5 mL/min, 20% 2-propanol/80% hexane, (*S*) t_1=15.44 min; (*R*) t_2=16.38 min.

4.2.22. (*S*)-Dimethyl α-(phenylacetoxy) ethyl phosphonate (5v). Colorless liquid; 91% ee; $[\alpha]_D^{14}$ +7.71 (*c* 0.74, $CHCl_3$); IR (KBr) 2957, 2854, 1745, 1455, 1247, 1148, 1049, 832 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.27 (m, 5H), 5.30 (p, 1H, *J*=7.6 Hz), 3.72–3.68 (m, 8H), 1.47 (dd, 3H, *J*=16.4 and 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.2, 41.4, 53.4, 53.5, 63.9, 65.6, 127.5, 128.8, 129.5, 133.6, 170.5; ^{31}P NMR (162 MHz, $CDCl_3$) δ 24.1; MS (EI) m/z 272 (M^+). Anal. Calcd for $C_{12}H_{17}O_5P$: C, 52.94; H, 6.29. Found: C, 52.89; H, 6.14. Enantiomeric excess determination: HPLC, UV 205 nm, Chiralcel AD-H, 0.5 mL/min, 20% 2-propanol/80% hexane, (*S*) t_1=10.09 min; (*R*) t_2=10.88 min.

4.2.23. (*S*)-Dimethyl (1-hydroxyethyl) phosphonate 6a. A purified sample of **5a** (530 mg, 1.5 mmol) was combined with anhydrous K_2CO_3 (620 mg, 3 equiv) in MeOH. The reaction mixture was stirred for 2 h at room temperature. MeOH was removed by evaporation and the residue was passed through a silica gel column using ethyl acetate as an eluant to give pure product **6a** (50 mg, 22% yield) as colorless oil. Spectral data matched with those reported in the literature.[18]

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.09.011.

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Palladium-catalyzed carbon dioxide elimination–fixation reaction of 6-methoxycarbonyloxy-2,4-hexadien-1-ols

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Abstract—Cyclic carbonates substituted with 1,3-butadienyl moiety were synthesized by a palladium-catalyzed reaction of dienylic carbonates including a carbon dioxide elimination–fixation process. The reaction proceeded via a migration–isomerization of the resulting π-allylpalladium intermediates to afford *trans*-1,3-dienyl-substituted cyclic carbonates in a selective manner.

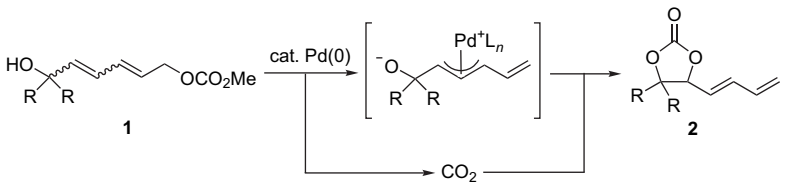
1. Introduction

Fixation of carbon dioxide into organic substances represents an attractive area of study in both organic and green chemistry.[1] Palladium-catalyzed reactions are one of the common methods to fix external carbon dioxide into organic compounds.[2] Recently, we have developed a novel type of palladium-catalyzed reaction using propargylic carbonates with phenols, which involves a CO_2-recycling process.[3] The reaction proceeds via a carbon dioxide elimination–fixation step to afford phenoxy-substituted cyclic carbonates.[3a–3c] This process can be successfully applied to a palladium-catalyzed reaction using allylic carbonates.[3d] In these reactions, the formation of π-allylpalladium intermediate followed by fixation of CO_2 is a key step. We sought to determine whether a CO_2-recycling process could apply for conjugated dienylic carbonates. Although there are many examples about the reaction of allylic compounds by palladium catalyst,[4] only a few examples have been reported about the reactivity for conjugated dienylic compounds.[5] We report here a palladium-catalyzed reaction of 6-methoxycarbonyloxy-2,4-hexadien-1-ols **1** to produce 1,3-dienyl-substituted cyclic carbonates **2** via a CO_2-recycling process (Scheme 1).

2. Results and discussion

The dienylic carbonates **1** for the palladium-catalyzed reactions were synthesized as follows (Scheme 2). The ketones **3a–d** were subjected to the nucleophilic addition of siloxy enyne (*E*)-**4**[6] in the presence of BuLi leading to the corresponding acetylenic alcohols, which were desilylated with TBAF to afford diols (*E*)-**5a–d**. *trans*-Selective reduction of the alkyne moiety using $LiAlH_4$ followed by treatment with methyl chloroformate to produce the dienylic carbonates (*E*,*E*)-**1a–d**. Similarly, dienylic benzoate (*E*,*E*)-**6** was prepared from **5a** in two steps.

We also prepared geometric isomers of (*E*,*E*)-**1a** possessing (*E*,*Z*)-, (*Z*,*E*)-, and (*Z*,*Z*)-olefinic moieties (Scheme 3). Addition of siloxy enyne (*Z*)-**4**, which was easily prepared from



Scheme 1.

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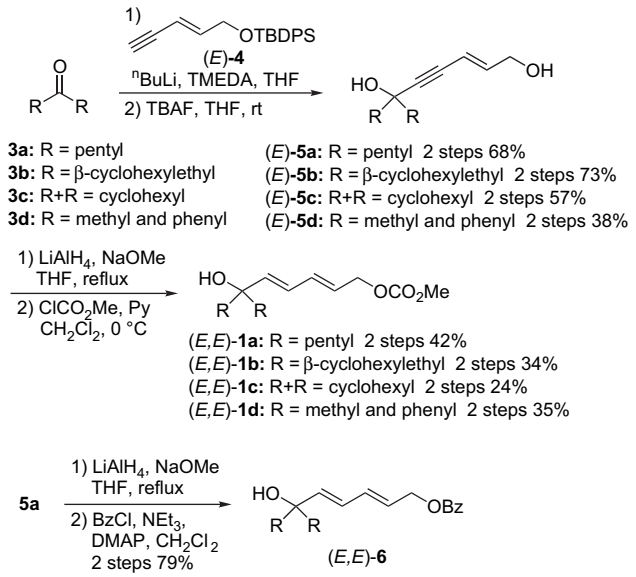
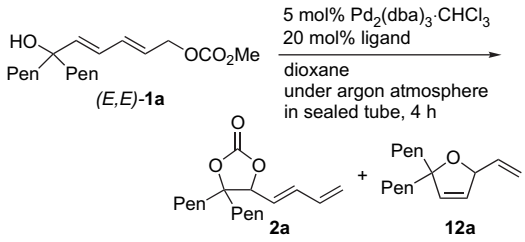


Scheme 2.

Table 1. Initial attempts for the reaction of (*E*,*E*)-**1a**[a]

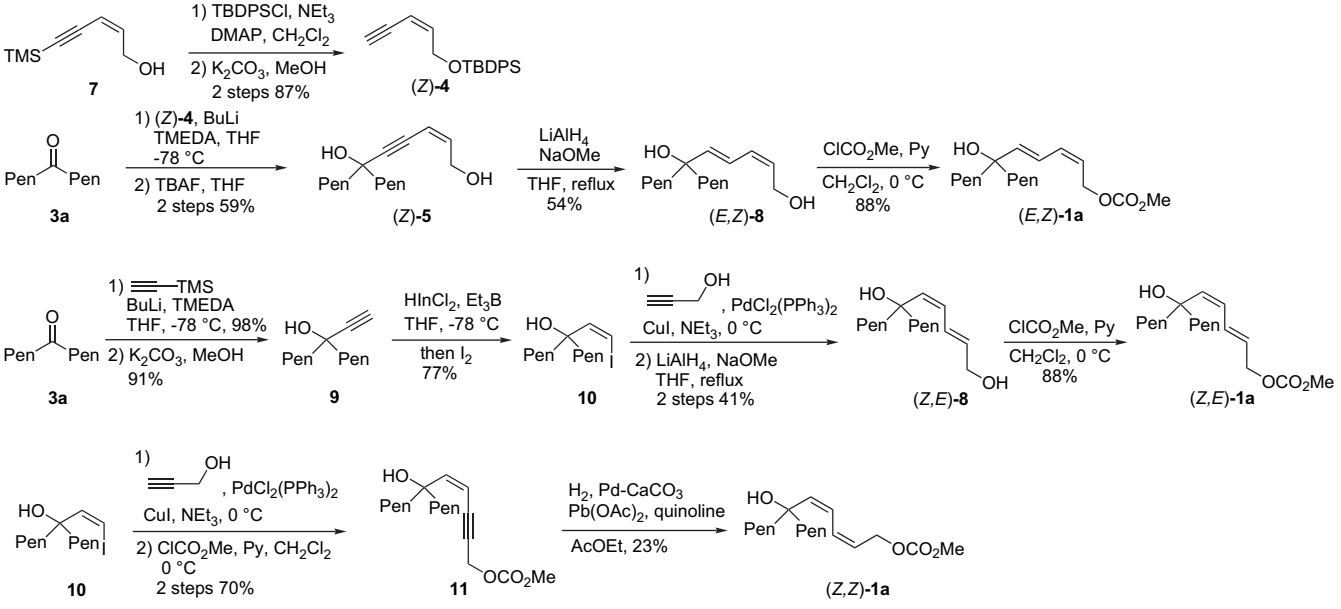


Entry	Ligand	Temp (°C)	Product's yield (%)	
			2a	**12a**
1	dppe	50	57	—
2	dppp	50	15	—
3	dppf	50	15	—
4	dppm	50	7	60
5[b]	PPh_3	50	4	56
6	dppv	50	58	27
7	dppv	80	28	58
8	dppv	100	22	74

[a] Pen=pentyl.
[b] Ligand (40 mol %) was used.

known enyne **7**,[7] to dipentylketone (**3a**) followed by desilylation yielded the diol (*Z*)-**5**. Conversion of (*Z*)-**5** by the same procedure described for (*E*)-**5a** gave dienylic carbonate (*E*,*Z*)-**1a** in a stereoselective manner. Isomers (*Z*,*E*)-**1a** and (*Z*,*Z*)-**1a** were prepared from (*Z*)-iodoalkene **10**. After the formation of propargylic alcohol **9** from **3a**, **10** was prepared by regio- and stereoselective hydroindation–iodolysis.[8] The compound **10** was then subjected to Sonogashira reaction with propargyl alcohol followed by $LiAlH_4$ reduction to provide the coupled product (*Z*,*E*)-**8**, which was transformed to dienylic carbonate (*Z*,*E*)-**1a**. The substrate (*Z*,*Z*)-**1a** was obtained by Lindlar reduction of a propargylic carbonate **11**, derived from **10** in two steps.

Our initial attempt at palladium-catalyzed reaction of dienylic carbonate begins with (*E*,*E*)-**1a** (Table 1). When (*E*,*E*)-**1a** was subjected to the reaction, treated with 5 mol % $Pd_2(dba)_3\cdot CHCl_3$ and 20 mol % 1,2-bis(diphenylphosphino)ethane (dppe) in dioxane at 50 °C under an argon atmosphere in a sealed tube, cyclic carbonate **2a** having a (*E*)-1,3-dienyl group was produced in 57% yield (entry 1). The yield of **2a** was decreased to 15% on the reaction in the presence of 1,3-bis(diphenylphosphino)propane (dppp) and 1,1′-bis(diphenylphosphino)ferrocene (dppf) (entries 2 and 3). Interestingly, vinyl-substituted dihydrofuran **12a** was predominantly yielded when bis(diphenylphosphino)-methane (dppm) and triphenylphosphine were used as a ligand (entries 4 and 5). The best result was obtained by carrying out the reaction in the presence of 1,2-bis(diphenylphosphino)ethylene (dppv) (58% yield for **2a**, 27% yield for **12a**) (entry 6). It was also found that the ratio of **2a** to **12a** was changed by altering the reaction temperature (entries 7 and 8). Accordingly, the yield of **2a** decreased to 22%, and that of **12a** increased to 74% by the reaction at 100 °C (entry 8).

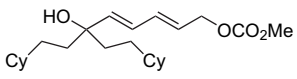
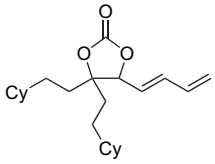
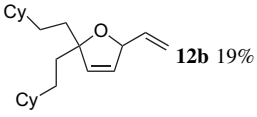
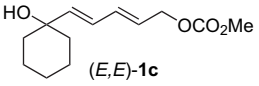
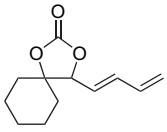
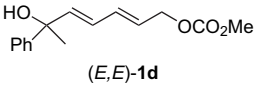
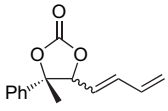
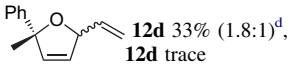
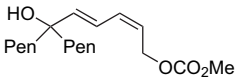
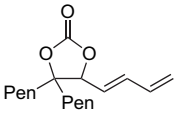
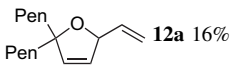
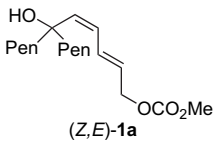
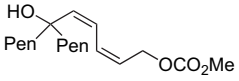


Scheme 3.

Dienylic carbonates having various substituents were next subjected to the palladium-catalyzed reaction conditions described above (Table 2). Substrate (*E*,*E*)-**1b** with dicyclohexylethyl substitution underwent the reaction to give cyclic carbonate **2b** in 50% yield along with dihydrofuran **12b** in 19% yield (entry 1). Reaction of substrate (*E*,*E*)-**1c** containing a cyclohexanol moiety provided the corresponding product **2c** in 68% yield (entry 2). Dienylic carbonate (*E*,*E*)-**1d**, which has unsymmetric substituents, was transformed to cyclic carbonates **2d** (1.8:1 mixture, 34% total yields) and dihydrofurans **12d** (1.8:1 mixture, 33% total yields) as the diastereomeric mixture (entry 3). Although the conversion of (*E*,*E*)-**1d** to **2d** was unsatisfied, the yield could be improved to 72% (3.5:1 mixture) when the process was conducted under a CO_2 atmosphere (entry 4). Substrate (*E*,*Z*)-**1a**, which is a geometric isomer of (*E*,*E*)-**1a**, also reacted with palladium catalyst to give cyclic carbonate **2a** in 70% yield with dihydrofuran **12a** (entry 5). Similarly, reactions using other isomers (*Z*,*E*)-**1a** and (*Z*,*Z*)-**1a** resulted in the production of **2a** along with **12a** in moderate yields, respectively (entries 6 and 7). In these reactions, the olefinic stereochemistries in the resulting cyclic carbonates **2a**–**d** are all *E*, and the results indicate that the reaction proceeds via a thermodynamically favorable common intermediate.

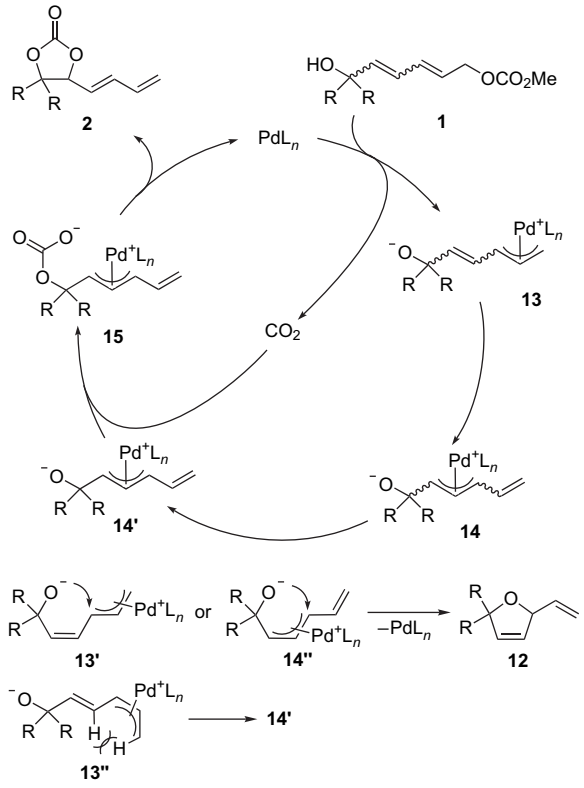
A plausible mechanism for the formation of cyclic carbonates **2** and dihydrofurans **12** is shown in Scheme 4. In this process, the palladium catalyst initially promotes decarboxylation of the conjugated dienylic carbonate **1** to generate the π-allylpalladium complex **13** and CO_2. The complex **13** would be equilibrated to the intermediates **14**, in which the π-allylpalladium is migrated to the internal allylic position. The intermediates **14** would be further transformed to the most thermodynamically favorable *syn*-configurated isomer **14′** via the π–σ–π isomerization process.[4] Finally, fixation of CO_2 followed by cyclization of the resulting intermediate **15** produces the cyclic carbonates **2**. The selective formation of (*E*)-1,3-dienyl-substituted cyclic carbonate **2a** regardless of the olefinic geometry of the substrate **1a** supports that all the reactions have occurred via the common intermediate **14′**. On the other hand, dihydrofurans **12** would be yielded from the direct cyclization of *anti*-configurated π-allyl isomer **13′** or **14″**. At higher reaction temperatures, it is expected that the direct cyclization would proceed prior to the fixation of CO_2 resulting in the selective formation of dihydrofurans **12** (entries 7 and 8 in Table 1).[9] The best yield for **2** was observed from the substrate (*E*,*Z*)-**1a** (entry 5 in Table 2). For this reason, the initially formed π-allylintermediate **13″** from (*E*,*Z*)-**1a** would be relatively unstable

Table 2. Reactions using various substituted dienylic carbonates[a]

Entry	Dienylic carbonate **1**	Product's yield	
		2	**12**
1[b]	 (*E*,*E*)-**1b**	 **2b** 50%	 **12b** 19%
2[b]	 (*E*,*E*)-**1c**	 **2c** 68%	
3[b] 4[c,e]	 (*E*,*E*)-**1d**	 **2d** 34% (1.8:1)[d], **2d** 72% (3.5:1)[d]	 **12d** 33% (1.8:1)[d], **12d** trace
5[c]	 (*E*,*Z*)-**1a**	 **2a** 70%	 **12a** 16%
6[c]	 (*Z*,*E*)-**1a**	**2a** 53%	**12a** 31%
7[c]	 (*Z*,*Z*)-**1a**	**2a** 47%	**12a** 26%

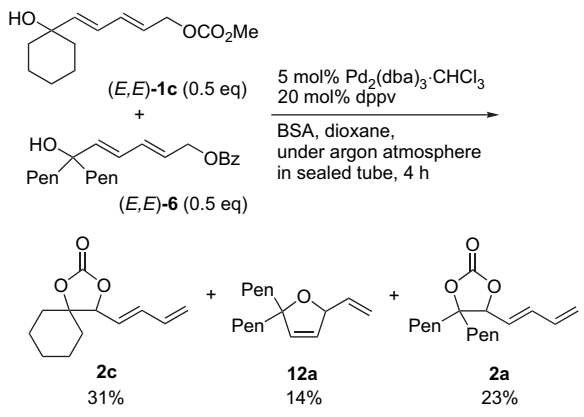
[a] All reactions were carried out with 5 mol % $Pd_2(dba)_3 \cdot CHCl_3$ and 20 mol % ligand in dioxane at 50 °C for 4–12 h.
[b] dppe was used as a ligand.
[c] dppv was used as a ligand.
[d] The stereochemistries of each product are not determined.
[e] The reaction was carried out under CO_2 atmosphere.

because of the allylic strain, which would cause the fast conversion to the intermediate **14′** resulting in the efficient production of cyclic carbonates **2**. The increased yield of **12** from the reaction of (*Z*,*E*)-**1a** (entry 6 in Table 2) indicates that initially formed π-allyl intermediate **13′** or **14″** from this substrate would facilitate the direct cyclization to give **12**. Although the reason for the observed specificity for the production of **2** and **12** depending on the ligand is not clear, it is proposed that this CO_2 elimination–fixation process is sensitive for the steric and electronic property of the phosphine ligand.



Scheme 4. Proposed reaction mechanism for the production of **2** and **12**.

To examine whether CO_2 dissociates from the substrate in the reaction, a crossover experiment with allylic carbonate (*E*,*E*)-**1c** and allylic benzoate (*E*,*E*)-**6** was next performed (Scheme 5). Reaction of an equimolar mixture of (*E*,*E*)-**1c** and (*E*,*E*)-**6** with palladium catalysis in the presence of *N*,*O*-bis(trimethylsilyl)acetamide (BSA)[10] gave cyclic carbonate **2a** in 23% yield, which was derived from (*E*,*E*)-**6**, along with the formation of (*E*,*E*)-**1c**-derived cyclic carbonate **2c** and (*E*,*E*)-**6**-derived dihydrofuran **12a** in 31% and 14% yield, respectively. It has been clear that **2a** arises by reaction of in situ generated CO_2 formed by decarboxylation of (*E*,*E*)-**1c**.



Scheme 5. Crossover experiment using carbonate (*E*,*E*)-**1c** and benzoate (*E*,*E*)-**6**.

3. Conclusion

In conclusion, we have developed a methodology for the synthesis of 1,3-dienyl-substituted cyclic carbonates by a CO_2-recycling reaction of dienylic carbonates with a palladium catalyst. This reaction proceeded via a successive migration–isomerization of the resulting π-allylpalladium intermediate as well as CO_2 elimination–fixation process. Cyclic carbonates are attractive and important compounds in a variety of chemical research fields, and this reaction would provide a new protocol for the synthesis of cyclic carbonates having a 1,3-dienyl substituent. Synthetic applications of the obtained products and further studies about this type of reactions are now in progress.

4. Experimental

4.1. General

All nonaqueous reactions were carried out under a positive atmosphere of argon and nitrogen in dried glassware unless otherwise indicated. Materials were obtained from commercial suppliers and used without further purification except when otherwise noted. Solvents were dried and distilled according to standard protocol. The phrase ‘residue upon workup’ refers to the residue obtained when the organic layer was separated and dried over anhydrous $MgSO_4$ and the solvent was evaporated under reduced pressure.

4.1.1. (*E*)-6-Hydroxy-1,1-dipentyl-4-hexen-2-yn-1-ol [(*E*)-5a]. To a stirred solution of siloxy enyne (*E*)-**4** (1.5 g, 4.7 mmol) and TMEDA (1.1 mL, 7.05 mmol) in THF (25 mL) was added dropwise a 1.60 M solution of BuLi in hexane (4.4 mL, 7.05 mmol) at −78 °C. After stirring was continued for 2 h at −78 °C, a solution of the 6-undecanone (**3a**) (1.4 mL, 7.05 mmol) in THF (5 mL) was added dropwise to this solution, and stirring was continued for 4 h at the same temperature. The reaction mixture was diluted with water and extracted with Et_2O. The combined extracts were washed with aqueous NH_4Cl and saturated aqueous NaCl. The residue upon workup was chromatographed on silica gel with hexane–AcOEt (95:5 v/v) as eluent to give the alcohol. To a stirred solution of alcohol in THF (30 mL) was added dropwise a 1.0 M TBAF in THF (9.6 mL, 9.6 mmol) at rt. After stirring was continued for 24 h at the same temperature. The reaction mixture was diluted with water and extracted with Et_2O. The combined extracts were washed with aqueous $NaHCO_3$ and saturated NaCl. The residue upon workup was chromatographed on silica gel with hexane–AcOEt (80:20 v/v) as eluent to give diol (*E*)-**5a** [814 mg, 68% from (*E*)-**4**] as a yellow oil; IR (neat) 3304, 2934, 2860 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$)

δ 0.96 (6H, t, J=6.6 Hz), 1.31–1.68 (17H, m), 1.90 (1H, s), 4.22 (2H, dd, J=3.6 and 6.4 Hz), 5.78 (1H, td, J=3.6 and 15.9 Hz), 6.23 (1H, td, J=6.4 and 15.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8, 22.4, 23.7, 31.8, 41.8, 62.4, 71.5, 82.0, 92.9, 109.9, 141.8; MS m/z 181 [$M^+-71(C_5H_{11})$]; HRMS m/z calcd for $C_{11}H_{17}O_2$ [$M^+-71(C_5H_{11})$]: 181.1228, found 181.1221.

4.1.2. (*E*)-6-Hydroxy-1,1-bis(2-cyclohexylethyl)-4-hexen-2-yn-1-ol [(*E*)-5b]. Yield 73% (for two steps); colorless oil; IR (neat) 3329, 2920, 2851 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84–0.96 (4H, m), 1.11–1.24 (10H, m), 1.33–1.41 (4H, m), 1.63–1.74 (14H, m), 4.18–4.26 (2H, m), 5.78 (1H, d, J=15.1 Hz), 6.23 (1H, td, J=5.1 and 15.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.2, 26.5, 31.6, 33.2, 37.8, 39.2, 62.6, 71.7, 82.0, 93.1, 110.0, 141.7; MS m/z 301 [$M^+-31(CH_2OH)$]; HRMS m/z calcd for $C_{21}H_{33}O$ [$M^+-31(CH_2OH)$]: 301.2531, found 301.2525.

4.1.3. 1-{(*E*)-(5-Hydroxy-3-hexen-1-yl)}-cyclohexanol [(*E*)-5c]. Yield 57% (for two steps); yellow oil; IR (neat) 3461, 2931, 2856 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19–1.94 (10H, m), 2.51 (1H, br s), 2.68 (1H, s), 4.20 (2H, dd, J=1.5 and 4.8 Hz), 5.79 (1H, dt, J=1.5 and 15.9 Hz), 6.24 (1H, dt, J=4.8 and 15.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.1, 25.0, 39.8, 62.3, 62.6, 69.0, 82.1, 93.5, 109.9, 141.9; MS m/z 180 (M^+); HRMS m/z calcd for $C_{11}H_{16}O_2$ (M^+): 180.1151, found 180.1150.

4.1.4. 6-Hydroxy-1-methyl-1-phenyl-4-hexen-2-yn-1-ol [(*E*)-5d]. Yield 38% (for two steps); yellow oil; IR (neat) 3287, 2984, 2927, 2862 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (3H, s), 1.43 (1H, s), 2.33 (1H, s), 4.23 (2H, dd, J=1.8 and 5.1 Hz), 5.84 (1H, td, J=1.8 and 15.9 Hz), 6.31 (1H, td, J=5.1 and 15.9 Hz), 7.27–7.40 (3H, m), 7.64–7.67 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 33.2, 62.8, 70.3, 82.7, 93.2, 109.7, 124.9, 127.7, 128.3, 142.3, 145.5; MS m/z 187 [$M^+-15(CH_3)$]; HRMS m/z calcd for $C_{12}H_{11}O_2$ [$M^+-15(CH_3)$]: 187.0759, found 187.0747.

4.1.5. (2*E*,4*E*)-1,1-Dipentyl-6-methoxycarbonyloxy-2,4-hexadien-1-ol [(*E*,*E*)-1a]. To a stirred suspension of LAH (30.4 mg, 0.8 mmol) and NaOMe (86.4 mg, 1.6 mmol) in THF (20 mL) was added dropwise the solution of diol (*E*)-**5a** (100.0 mg, 0.4 mmol) in THF (5 mL) at 0 °C. After refluxing for 3 h, the reaction mixture was treated with the minimum amount of cold water, and extracted with AcOEt. The combined extracts were washed with aqueous $NaHCO_3$ and saturated NaCl. The residue upon workup was chromatographed on silica gel with hexane–AcOEt (95:5 v/v) as eluent to give the dienylic alcohol. To a stirred solution of this dienylic alcohol and pyridine (97.1 μL, 1.2 mmol) in CH_2Cl_2 (20 mL) was added dropwise methyl chloroformate (34.0 μL, 0.44 mmol) at 0 °C, and stirring was continued for 1 h at the same temperature. The reaction mixture was diluted with water and extracted with AcOEt. The combined extracts were washed with aqueous NH_4Cl and saturated NaCl. The residue upon workup was chromatographed on silica gel with hexane–AcOEt (70:30 v/v) as eluent to give dienylic carbonate (*E*,*E*)-**1a** [52.6 mg, 42% from (*E*)-**5a**] as a colorless oil; IR (neat) 3477, 2932, 2858, 1732 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (6H, t, J=6.9 Hz), 1.25–1.51 (17H, m), 3.79 (3H, s), 4.66 (2H, d, J=6.6 Hz), 5.70 (1H, d, J=15.0 Hz), 5.74 (1H, td, J=6.6 and 14.7 Hz), 6.23 (1H, dd J=10.2 and 15.0 Hz), 6.34 (1H, dd, J=14.7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 22.5, 23.1, 32.2, 41.0, 54.7, 68.1, 75.2, 124.9, 126.7, 134.8, 142.0, 155.8; MS m/z 241 [$M^+-71(C_5H_{11})$]; HRMS m/z calcd for $C_{13}H_{21}O_4$ [$M^+-71(C_5H_{11})$]: 241.1440, found 241.1432.

4.1.6. (2*E*,4*E*)-1,1-Bis(2-cyclohexylethyl)-6-methoxycarbonyloxy-2,4-hexadien-1-ol [(*E*,*E*)-1b]. Yield 34% (for two steps); yellow oil; IR (neat) 3479, 2922, 2851, 1747 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85–0.87 (4H, m), 1.11–1.24 (10H, m), 1.45–1.50 (4H, m), 1.51–1.69 (13H, m), 3.79 (3H, s), 4.66 (2H, d, J=6.6 Hz), 5.71 (1H, d, J=15.1 Hz), 5.78 (1H, dd, J=6.6 and 14.9 Hz), 6.20 (1H, dd, J=10.5 and 15.1 Hz), 6.32 (1H, dd, J=10.5 and 14.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.2, 26.5, 30.8, 33.2, 33.3, 38.0, 38.3, 54.6, 68.1, 75.1, 124.8, 126.8, 134.8, 142.0, 155.7; MS m/z 374 [$M^+-18(H_2O)$]; HRMS m/z calcd for $C_{24}H_{38}O_2$ [$M^+-18(H_2O)$]: 374.2821, found 374.2818.

4.1.7. 1-{(1*E*,3*E*)-(5-Methoxycarbonyloxy-1,3-hexadienyl)}-cyclohexanol [(*E*,*E*)-1c]. Yield 24% (for two steps); yellow oil; IR (neat) 3400, 2932, 2856, 1732 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25–1.32 (2H, m), 1.49–1.68 (9H, m), 3.78 (3H, s), 4.65 (2H, dd, J=0.96 and 6.6 Hz), 5.76 (1H, td, J=6.6 and 14.6 Hz), 5.87 (1H, d, J=14.6 Hz), 6.24–6.35 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.7, 25.2, 37.5, 54.6, 67.9, 71.2, 125.2, 125.9, 134.8, 143.3, 155.6; MS m/z 240 (M^+); HRMS m/z calcd for $C_{13}H_{20}O_4$ (M^+): 240.1361, found 240.1347.

4.1.8. (2*E*,4*E*)-6-Methoxycarbonyloxy-1-methyl-1-phenyl-2,4-hexadien-1-ol [(*E*,*E*)-1d]. Yield 35% (for two steps); yellow oil; IR (neat) 3443, 2976, 2956, 1747 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.68 (3H, s), 1.90 (1H, s), 3.78 (3H, s), 4.69 (2H, dd, J=1.0 and 6.8 Hz), 5.79 (1H, dt, J=6.8 and 14.9 Hz), 6.04 (1H, d, J=14.7 Hz), 6.27 (1H, dd, J=10.4 and 14.7 Hz), 6.34 (1H, t, J=10.4 Hz), 7.23–7.27 (1H, m), 7.32–7.36 (2H, m), 7.42–7.46 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.5, 54.7, 67.9, 74.3, 125.2, 126.3, 126.7, 127.2, 128.4, 134.3, 142.0, 146.4, 155.7; MS m/z 247 [$M^+-15(CH_3)$]; HRMS m/z calcd for $C_{14}H_{15}O_4$ [$M^+-15(CH_3)$]: 247.0970, found 247.0953.

4.1.9. (2*E*,4*E*)-1,1-Dipentyl-6-benzoyloxy-2,4-hexadien-1-ol [(*E*,*E*)-6]. To a stirred suspension of LAH (96.4 mg, 2.54 mmol) and NaOMe (274 mg, 5.08 mmol) in THF (25 mL) was added dropwise the solution of diol (*E*)-**5a** (257 mg, 1.27 mmol) in THF (5 mL) at 0 °C. After refluxing for 3 h, the reaction mixture was treated with the minimum amount of cold water, and extracted with AcOEt. The combined extracts were washed with aqueous $NaHCO_3$ and saturated NaCl. The residue upon workup was chromatographed on silica gel with hexane–AcOEt (95:5 v/v) as eluent to give the dienylic alcohol. To a stirred solution of the resulting dienylic alcohol, triethylamine (0.35 mL, 2.54 mmol) and a catalytic amount of DMAP in CH_2Cl_2 (20 mL) was added dropwise benzoyl chloride (0.15 mL, 1.27 mmol) at 0 °C, and stirring was continued for 1 h at the same temperature. The reaction mixture was diluted with water and extracted with AcOEt. The combined

extracts were washed with aqueous NH_4Cl and saturated NaCl. The residue upon workup was chromatographed on silica gel with hexane–AcOEt (80:20 v/v) as eluent to give dienyl allylic benzoate (*E*,*E*)-**6** [358 mg, 79% from (*E*)-**5a**] as a colorless oil; IR (neat) 3416, 2932, 2860, 1715 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.27–1.63 (16H, m), 4.86 (2H, d, J=6.3 Hz), 5.75 (2H, d, J=15.3 Hz), 5.87 (1H, td, J=6.6 and 14.7 Hz), 6.26 (1H, dd, J=10.5 and 14.7 Hz), 6.39 (1H, dd, J=10.5 and 15.3 Hz), 7.41–7.47 (2H, m), 7.53–7.59 (1H, m), 8.05–8.08 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 22.5, 23.1, 32.2, 41.1, 65.2, 75.2, 125.8, 126.9, 128.4, 129.7, 130.3, 133.0, 134.1, 141.6, 166.5; MS *m*/*z* 340 [$M^+-18(H_2O)$]; HRMS *m*/*z* calcd for $C_{23}H_{32}O_2$ [$M^+-18(H_2O)$]: 340.2402, found 340.2383.

4.1.10. (*Z*)-5-*tert*-Butyldiphenylsilyloxy-3-penten-1-yne [(*Z*)-4]. To a stirred solution of (*Z*)-allylic alcohol **7** (308 mg, 2.0 mmol), triethylamine (0.56 mL, 4.0 mmol) and a catalytic amount of DMAP in CH_2Cl_2 (20 mL) was added TBDPSCl (0.627 mL, 2.4 mmol) at 0 °C, and stirring was continued for 1 h at rt. The reaction mixture was diluted with water and extracted with AcOEt. The combined extracts were washed with aqueous NH_4Cl and saturated aqueous NaCl. The residue upon workup was chromatographed on silica gel with hexane–AcOEt (95:5 v/v) as eluent to give TBDPS ether. To a stirred solution of the formed TBDPS ether in methanol (20 mL) was added K_2CO_3 (332 mg, 2.4 mmol) at rt. After stirring was continued for 5 h at rt, the reaction mixture was diluted with water and extracted with Et_2O. The combined extracts were washed with saturated NaCl. The residue upon workup was chromatographed on silica gel with hexane–AcOEt (90:10 v/v) as eluent to give silyl ether (*Z*)-**4** (559.7 mg, 87% from **7**) as a colorless oil; IR (neat) 3294, 2932, 2856 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.05 (9H, s), 3.00 (1H, s), 4.49 (2H, dd, J=1.6 and 6.0 Hz), 5.45 (1H, ddd, J=1.6, 3.6 and 10.8 Hz), 6.17 (1H, dt, J=6.0 and 10.8 Hz), 7.53–7.44 (6H, m), 7.64–17.69 (4H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.2, 26.9, 62.4, 79.3, 82.8, 108.0, 127.6, 129.6, 133.5, 135.5, 144.4; MS *m*/*z* 320 (M^+); HRMS *m*/*z* calcd for $C_{21}H_{24}OSi$ (M^+): 320.1596, found 320.1578.

4.1.11. (*Z*)-6-Hydroxy-1,1-dipentyl-4-hexen-2-yn-1-ol [(*Z*)-5]. To a stirred solution of silyl ether (*Z*)-**4** (352 mg, 1.1 mmol) and TMEDA (0.25 mL, 1.65 mmol) in THF (20 mL) was added dropwise a 1.60 M solution of BuLi in hexane (1.0 mL, 1.65 mmol) at −78 °C. After stirring was continued for 2 h at −78 °C, a solution of the 6-undecanone (**3a**) (0.34 mL, 1.65 mmol) in THF (5 mL) was added dropwise to this solution, and stirring was continued for 4 h at the same temperature. The reaction mixture was diluted with water and extracted with Et_2O. The combined extracts were washed with aqueous NH_4Cl and saturated aqueous NaCl. The residue upon workup was chromatographed on silica gel with hexane–AcOEt (90:10 v/v) as eluent to give propargylic alcohol. To a stirred solution of the formed propargylic alcohol in THF (30 mL) was added dropwise a 1.0 M TBAF in THF (2.2 mL, 2.2 mmol) at rt. After stirring was continued for 24 h at the same temperature. The reaction mixture was diluted with water and extracted with Et_2O. The combined extracts were washed with aqueous $NaHCO_3$ and saturated NaCl. The residue upon workup was chromatographed on silica gel with AcOEt–hexane (20:80 v/v) as eluent to give diol (*Z*)-**5** (162 mg, 59% from (*Z*)-**4**) as a yellow oil; IR (neat) 3333, 2934, 2860 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.91 (6H, t, J=6.9 Hz), 1.26–1.69 (16H, m), 1.94 (1H, s), 2.05 (1H, s), 4.40 (2H, dd, J=1.5 and 5.7 Hz), 5.64 (1H, td, J=1.5 and 10.8 Hz), 6.08 (1H, td, J=5.7 and 10.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 22.5, 23.8, 31.8, 41.8, 60.8, 71.7, 79.8, 98.6, 110.4, 141.4; MS *m*/*z* 181 [$M^+-71(C_5H_{11})$]; HRMS *m*/*z* calcd for $C_{11}H_{17}O_2$ [$M^+-71(C_5H_{11})$]: 181.1229, found 181.1229.

4.1.12. (2*E*,4*Z*)-6-Hydroxy-1,1-dipropyl-2,4-hexadien-1-ol [(*E*,*Z*)-8]. To a stirred suspension of LAH (22.8 mg, 0.60 mmol) and NaOMe (64.8 mg, 1.20 mmol) in THF (15 mL) was added dropwise the solution of diol (*Z*)-**5** (75.7 mg, 0.30 mmol) in THF (5 mL) at 0 °C. After refluxing for 3 h, the reaction mixture was treated with the minimum amount of cold water, and extracted with AcOEt. The combined extracts were washed with aqueous $NaHCO_3$ and saturated NaCl. The residue upon workup was chromatographed on silica gel with hexane–AcOEt (95:5 v/v) as eluent to give dienylic alcohol (*E*,*Z*)-**8** (41.5 mg, 54%) as a colorless oil; IR (neat) 3360, 2955, 2860 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.26–1.33 (12H, m), 1.49–1.55 (4H, m), 1.78 (1H, s), 2.08 (1H, s), 4.33 (2H, d, J=6.9 Hz), 5.35 (1H, td, J=6.9 and 11.1 Hz), 5.73 (1H, d, J=15.0 Hz), 6.10 (1H, t, J=11.1 Hz), 6.51 (1H, dd, J=11.1 and 15.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 22.5, 23.1, 32.2, 41.0, 58.6, 75.3, 122.5, 128.8, 130.4, 142.1; MS *m*/*z* 183 [$M^+-71(C_5H_{11})$]; HRMS *m*/*z* calcd for $C_{11}H_{19}O_2$ [$M^+-71(C_5H_{11})$]: 183.1385, found 183.1366.

4.1.13. (2*E*,4*Z*)-1,1-Dipentyl-6-methoxycarbonyloxy-2,4-hexadien-1-ol [(*E*,*Z*)-1a]. To a stirred solution of dienylic alcohol (*E*,*Z*)-**8** (40.7 mg, 0.16 mmol) and pyridine (38.8 μL, 0.48 mmol) in CH_2Cl_2 (5 mL) was added dropwise methyl chloroformate (13.6 μL, 0.18 mmol) at 0 °C, and stirring was continued for 1 h at the same temperature. The reaction mixture was diluted with water and extracted with AcOEt. The combined extracts were washed with aqueous NH_4Cl and saturated NaCl. The residue upon workup was chromatographed on silica gel with hexane–AcOEt (80:20 v/v) as eluent to give dienylic carbonate (*E*,*Z*)-**1a** (44.0 mg, 88%) as a colorless oil; IR (neat) 3485, 2931, 2860, 1730 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.88 (6H, t, J=7.2 Hz), 1.27–1.34 (11H, m), 1.45–1.54 (6H, m), 3.79 (3H, s), 4.83 (2H, d, J=7.2 Hz), 5.52 (1H, td, J=7.2 and 10.2 Hz), 5.78 (1H, d, J=15.0 Hz), 6.21 (1H, dd, J=10.2 and 11.2 Hz), 6.51 (1H, dd, J=11.2 and 15.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 22.5, 23.1, 32.2, 41.0, 54.7, 63.8, 75.3, 122.1, 122.7, 133.0, 143.5, 155.9; MS *m*/*z* 237 [$M^+-75(OCO_2CH_3)$]; HRMS *m*/*z* calcd for $C_{16}H_{29}O$ [$M^+-75(OCO_2CH_3)$]: 237.2218, found 237.2208.

4.1.14. 3-Pentyl-1-octyn-3-ol (9). To a stirred solution of trimethylsilyl acetylene (7.1 mL, 50.0 mmol) and TMEDA (7.5 mL, 50.0 mmol) in THF (225 mL) was added dropwise a 1.60 M BuLi in hexane (31.3 mL, 50.0 mmol) at −78 °C. After stirring was continued for 2 h at −78 °C, a solution of the 6-undecanone (**3a**) (5.1 mL, 25.0 mmol) in THF (25.0 mL) was added dropwise to this solution, and stirring was continued for 4 h at the same temperature. The reaction

mixture was diluted with water and extracted with AcOEt. The combined extracts were washed with aqueous NH_4Cl and saturated NaCl. The residue upon workup was chromatographed on silica gel with hexane–AcOEt (90:10 v/v) as eluent to give propargylic alcohol (6.58 g, 98%) as a colorless oil. To a stirred solution of the above propargylic alcohol (3.30 g, 12.3 mmol) in methanol (120 mL) was added K_2CO_3 (2.04 g, 14.8 mmol) at rt. After stirring was continued for 5 h at rt, the reaction mixture was diluted with water and extracted with Et_2O. The combined extracts were washed with saturated NaCl. The residue upon workup was chromatographed on silica gel with hexane–AcOEt (90:10 v/v) as eluent to give alkyne **9** (2.20 g, 91%) as a colorless oil; IR (neat) 3400, 3310, 2935, 3862 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.93 (6H, t, J=6.8 Hz), 1.26–1.39 (8H, m), 1.46–1.54 (4H, m), 1.61–1.69 (4H, m), 1.88 (1H, s), 2.43 (1H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 13.9, 22.5, 23.7, 31.9, 41.7, 71.0, 72.0, 87.0; MS m/z 125 [$M^+-71(C_5H_{11})$]; HRMS calcd for $C_8H_{13}O$ [$M^+-71(C_5H_{11})$]: 125.1004, found 125.0985.

4.1.15. (*Z*)-1,1-Dipentyl-3-iode-2-propen-1-ol (10). To a stirred suspension of anhydrous indium trichloride (747 mg, 3.4 mmol) in THF (25 mL) was added dropwise DIBAL (1.0 M hexane solution, 3.3 mL, 3.3 mmol) at −78 °C. After stirring was continued for 30 min at −78 °C, a solution of alkyne **9** (491 mg, 2.5 mmol) in THF (5 mL) and triethylborane (1.0 M hexane solution 0.5 mL, 0.5 mmol) were added to this suspension, and stirring was continued for 2.5 h at the same temperature. Iodine (1.9 g, 7.5 mmol) was then added to the reaction mixture, and the mixture was continuously stirred for 30 min at −78 °C. The reaction mixture was poured into saturated $NaHCO_3$ solution and sodium thiosulfate solution was added. The product was extracted with Et_2O, and the combined extracts were washed with saturated NaCl. The residue upon workup was chromatographed on silica gel with hexane–AcOEt (90:10 v/v) as eluent to give alkenyl iodide **10** (577 mg, 77%) as a colorless oil; IR (neat) 3467, 2930, 2954 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.89 (6H, t, J=6.8 Hz), 1.24–1.43 (12H, m), 1.54–1.71 (4H, m), 2.12 (1H, s), 6.25 (1H, d, J=8.5 Hz), 6.49 (1H, d, J=8.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 23.2, 32.3, 40.6, 75.5, 77.3, 144.4; MS m/z 253 [$M^+-71(C_5H_{11})$]; HRMS m/z calcd for $C_8H_{14}OI$ [$M^+-71(C_5H_{11})$]: 253.0089, found 253.0074.

4.1.16. (2*Z*,4*E*)-6-Hydroxy-1,1-dipropyl-2,4-hexadien-1-ol [(*Z*,*E*)-8]. To a suspension of CuI (16.2 mg, 85.0 μmol), $PdCl_2(PPh_3)_2$ (59.7 mg, 85.0 μmol) in triethylamine (20 mL) was added propargyl alcohol (0.14 mL, 1.7 mmol) at 0 °C. After stirring was continued for 30 min at 0 °C, a solution of alkenyl iodide **10** (551 mg, 1.7 mmol) in triethylamine (5 mL) was added to this solution, and stirring was continued for 1 h at the same temperature. The reaction mixture was poured into saturated NH_4Cl solution and extracted with AcOEt. The combined extracts were washed with saturated NaCl. The residue upon workup was chromatographed on silica gel with hexane–AcOEt (70:30 v/v) as eluent to give enyne. To a stirred suspension of LAH (129 mg, 3.4 mmol) and NaOMe (367 mg, 6.8 mmol) in THF (20 mL) was added dropwise the solution of the produced enyne in THF (5 mL) at 0 °C. After refluxing for 3 h, the reaction mixture was treated with the minimum amount of cold water, and extracted with AcOEt. The combined extracts were washed with aqueous $NaHCO_3$ and saturated NaCl. The residue upon workup was chromatographed on silica gel with toluene–AcOEt (90:10 v/v) as eluent to give dienylic alcohol (*Z*,*E*)-**8** (165.9 mg, 41% from **10**) as a yellow oil; IR (neat) 3360, 2955, 2860 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (6H, t, J=7.1 Hz), 1.22–1.30 (10H, m), 1.43–1.57 (8H, m), 4.20 (2H, d, J=6.1 Hz), 5.30 (1H, d, J=11.7 Hz), 5.77 (1H, td, J=6.1 and 15.2 Hz), 6.01 (1H, t, J=11.7 Hz), 7.14 (1H, dd, J=11.5 and 15.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 23.5, 32.4, 42.4, 63.6, 77.5, 128.3, 128.7, 133.4, 136.3; MS m/z 183 [$M^+-71(C_5H_{11})$]; HRMS m/z calcd for $C_{11}H_{19}O_2$ [$M^+-71(C_5H_{11})$]: 183.1385, found 183.1373.

4.1.17. (2*Z*,4*E*)-1,1-Dipentyl-6-methoxycarbonyloxy-2,4-hexadien-1-ol [(*Z*,*E*)-1a]. To a stirred solution of dienylic alcohol (*Z*,*E*)-**8** (166 mg, 0.65 mmol) and pyridine (0.16 mL, 1.95 mmol) in CH_2Cl_2 (10 mL) was added dropwise methyl chloroformate (60.2 μL, 0.78 mmol) at 0 °C, and stirring was continued for 1 h at the same temperature. The reaction mixture was diluted with water and extracted with AcOEt. The combined extracts were washed with aqueous NH_4Cl and saturated NaCl. The residue upon workup was chromatographed on silica gel with hexane–AcOEt (95:5 v/v) as eluent to give dienylic carbonate (*Z*,*E*)-**1a** (178 mg, 88%) as a yellow oil; IR (neat) 3520, 2932, 2860, 1747 cm^{-1}; ^1H NMR (400 MHz, C_6D_6) δ 0.43 (6H, t, J=6.8 Hz), 0.71–0.95 (17H, m), 2.86 (3H, s), 4.08 (2H, dd, J=1.2 and 6.6 Hz), 4.66 (1H, d, J=12.0 Hz), 5.13 (1H, dt, J=6.6 and 15.4 Hz), 5.45 (1H, t, J=11.7 Hz), 7.07 (1H, ddd, J=1.2, 11.7 and 15.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 22.5, 23.2, 32.2, 42.3, 54.7, 68.4, 77.6, 127.1, 128.4, 132.1, 137.9, 155.8; MS m/z 237 [$M^+-75(OCO_2CH_3)$]; HRMS m/z calcd for $C_{16}H_{29}OI$ [$M^+-75(OCO_2CH_3)$]: 237.2219, found 237.2231.

4.1.18. (*Z*)-1,1-Dipentyl-6-methoxycarbonyloxy-2-hexen-4-yn-1-ol (11). To a suspension of CuI (13.8 mg, 72.5 μmol) and $PdCl_2(PPh_3)_4$ (50.9 mg, 72.5 μmol) in triethylamine (10 mL) was added propargyl alcohol (0.12 mL, 1.45 mmol) at 0 °C. After stirring was continued for 30 min at 0 °C, a solution of alkenyl iodide **10** (471 mg, 1.45 mmol) in triethylamine (5 mL) was added to this solution, and stirring was continued for 1 h at the same temperature. The reaction mixture was poured into saturated NH_4Cl solution and extracted with AcOEt. The combined extracts were washed with saturated NaCl. The residue upon workup was chromatographed on silica gel with hexane–AcOEt (70:30 v/v) as eluent to give enyne. To a stirred solution of the formed enyne and pyridine (0.35 mL, 4.35 mmol) in CH_2Cl_2 (20 mL) was added dropwise methyl chloroformate (0.11 mL, 1.45 mmol) at 0 °C, and stirring was continued for 1 h at the same temperature. The reaction mixture was diluted with water and extracted with AcOEt. The combined extracts were washed with aqueous NH_4Cl and saturated NaCl. The residue upon workup was chromatographed on silica gel with hexane–AcOEt (85:15 v/v) as eluent to give propargylic carbonate **11** (315 mg, 70% from **10**) as a yellow oil; IR (neat) 3562, 2932, 2860, 1747 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.88 (6H, t, J=6.9 Hz), 1.24–1.41 (12H, m), 1.43–1.56 (4H, m), 2.82 (1H, s), 3.81 (3H, s),

4.86 (2H, d, J=2.4 Hz), 5.56 (1H, td, J=2.4 and 12.0 Hz), 5.93 (1H, d, J=12.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8, 22.4, 23.0, 32.1, 41.0, 54.9, 55.9, 76.4, 83.5, 88.8, 105.8, 150.6, 155.0; MS m/z 310 (M^+); HRMS m/z calcd for $C_{18}H_{30}O_4$ (M^+): 310.2144, found 310.02115.

4.1.19. (2*Z*,4*E*)-1,1-Dipentyl-6-methoxycarbonyloxy-2,4-hexadien-1-ol [(*Z*,*Z*)-1a]. To a stirred solution of propargylic carbonate **11** (79.1 mg, 0.23 mmol) in AcOEt (2.6 mL) was added Pd/$CaCO_3$ (14.0 mg) poisoned with lead and quinoline (5.6 μL) at rt. The reaction flask was purged with H_2. After stirring was continued for 4 h at same temperature. The reaction mixture was filtered through a short pad of Celite. The residue upon workup was chromatographed on silica gel with hexane–AcOEt (95:5 v/v) as eluent to give dienylic carbonate (*Z*,*Z*)-**3a** (16.0 mg, 23%) as a yellow oil; IR (neat) 3498, 2955, 2860, 1747 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (6H, t, J=6.8 Hz), 1.21–1.34 (11H, m), 1.54–1.58 (6H, m), 3.79 (3H, s), 4.81 (2H, dd, J=1.5 and 7.1 Hz), 5.44 (1H, d, J=12.0 Hz), 5.58 (1H, td, J=7.1 and 12.0 Hz), 6.27 (1H, t, J=12.0 Hz), 7.13 (1H, tt, J=1.5 and 12.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 23.4, 32.3, 42.4, 54.8, 63.2, 77.6, 123.1, 124.0, 129.3, 138.9, 155.6; MS m/z 294 [$M^+-18(H_2O)$]; HRMS m/z calcd for $C1_8H_{30}O_3$ [$M^+-18(H_2O)$]: 294.2195, found 294.2179.

4.2. General procedure for the palladium-catalyzed reaction of dienylic carbonate 1. Reaction of (*E*,*E*)-1a (entry 6 in Table 1)

To a stirred solution of dienylic carbonate [(*E*,*E*)-**1a**] (34.3 mg, 0.11 mmol) in dioxane (1.1 mL) were added $Pd_2(dba)_3\cdot CHCl_3$ (5.7 mg, 5.5 μmol) and dppv (8.7 mg, 22.0 μmol) in a sealed tube at rt. After stirring was continued for 4 h at 50 °C, the reaction mixture was concentrated and the residue was chromatographed on silica gel with hexane–AcOEt (90:10 v/v) as eluent to give cyclic carbonate **2a** (17.8 mg, 58%) and dihydrofuran **12a** (7.0 mg, 27%) as a colorless oil, respectively.

4.2.1. (3*E*)-1,2-Carbonyldioxy-1,1-dipentyl-hexadiene (2a). Yield 58%; colorless oil; IR (neat) 2931, 2870, 1790 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (6H, t, J=6.8 Hz), 1.26–1.75 (16H, m), 4.80 (1H, d, J=7.6 Hz), 5.27 (1H, d, J=9.2 Hz), 5.35 (1H, d, J=14.7 Hz), 5.67 (1H, dd, J=7.6 and 14.4 Hz), 6.33–6.41 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8, 22.3, 22.5, 22.5, 31.8, 31.8, 33.2, 36.3, 84.5, 88.3, 120.9, 124.3, 135.1, 136.8, 154.2; MS m/z 236 [$M^+-44(CO_2)$]; HRMS m/z calcd for $C_{16}H_{28}O$ [$M^+-44(CO_2)$]: 236.2140, found 236.2134.

4.2.2. 2,2-Dipentyl-5-vinyl-2,5-dihydrofuran (12a). Yield 27%; yellow oil; IR (neat) 2930, 2860, 1468 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (6H, t, J=6.9 Hz), 1.26–1.37 (12H, m), 1.50–1.61 (4H, m), 5.09 (1H, dd, J=1.2 and 10.2 Hz), 5.16 (1H, dd, J=1.8 and 7.2 Hz), 5.25 (1H, dd, J=1.2 and 17.1 Hz), 5.60 (1H, dd, J=1.8 and 6.0 Hz), 5.61 (1H, d, J=6.0 Hz), 5.80 (1H, ddd, J=7.2, 10.2 and 17.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 22.5, 22.6, 23.6, 24.0, 32.3, 32.3, 39.8, 40.1, 87.1, 93.5, 115.7, 128.4, 133.4, 139.1; MS m/z 236 (M^+); HRMS m/z calcd for $C_{16}H_{28}O$ (M^+): 236.2140, found 236.2133.

4.2.3. (3*E*)-1,2-Carbonyldioxy-bis(2-cyclohexylethyl)-hexadiene (2b). Yield 50%; yellow oil; IR (neat) 2922, 2851, 1801 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87–0.94 (4H, m), 1.11–1.33 (12H, m), 1.62–1.78 (14H, m), 4.77 (1H, d, J=7.6 Hz), 5.28 (1H, d, J=9.5 Hz), 5.36 (1H, d, J=14.9 Hz), 5.64 (1H, dd, J=7.6 and 14.9 Hz), 6.32–6.42 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.1, 26.4, 30.2, 30.2, 30.6, 33.0, 33.0, 33.1, 33.2, 33.8, 37.7, 37.8, 84.5, 88.5, 120.8, 124.4, 135.1, 136.7, 154.2; MS m/z 360 (M^+); HRMS m/z calcd for $C_{23}H_{36}O_3$ (M^+): 360.2655, found 360.2642.

4.2.4. 2,2-Bis-(2-cyclohexyl-ethyl)-5-vinyl-2,5-dihydrofuran (12b). Yield 19%; colorless oil; IR (neat) 2922, 2851, 1448 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78–0.81 (4H, m), 1.07–1.22 (12H, m), 1.41–1.63 (14H, m), 5.02 (1H, dd, J=1.2 and 10.2 Hz), 5.07 (1H, dd, J=1.0 and 7.3 Hz), 5.17 (1H, dd, J=1.2 and 17.4 Hz), 5.58 (1H, dd, J=2.0 and 6.1 Hz), 5.61 (1H, d, J=6.1 Hz), 5.72 (1H, ddd, J=7.3, 10.2, and 17.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.4, 26.4, 26.7, 31.5, 32.0, 33.4, 33.4, 33.5, 37.1, 37.6, 38.2, 38.2, 87.1, 93.5, 115.5, 128.3, 133.4, 139.0; MS m/z 316 (M^+); HRMS m/z calcd for $C_{22}H_{36}O$ (M^+): 316.2767, found 316.2758.

4.2.5. (3*E*)-1,2-Carbonyldioxy-(1-spirocyclohexyl)-3,5-hexadiene (2c). Yield 68%; yellow oil; IR (neat) 3026, 2928, 1800 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21–1.30 (1H, m), 1.39–1.46 (1H, m), 1.55–1.58 (6H, m), 1.60–1.92 (2H, m), 4.64 (1H, d, J=7.8 Hz), 5.27 (1H, d, J=5.6 and 9.3 Hz), 5.35 (1H, d, J=5.6 and 15.6 Hz), 5.65 (1H, dd, J=7.8 and 14.2 Hz), 6.31–6.42 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.5, 22.1, 24.7, 31.5, 35.4, 85.7, 85.7, 120.1, 123.9, 134.9, 136.6, 153.9; MS m/z 208 (M^+); HRMS m/z calcd for $C_{12}H_{16}O_3$ (M^+): 208.1100, found 208.1061.

4.2.6. Carbonyldioxy-1-methyl-1-phenyl-(3*E*)-hexadiene (2d). Yield 34% (1.8:1 diastereomeric mixture); major product: yellow oil; IR (neat) 2983, 2930, 1798 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.68 (3H, s), 4.96 (1H, d, J=7.8 Hz), 5.33 (1H, d, J=9.5 Hz), 5.39 (1H, d, J=15.8 Hz), 5.80 (1H, dd, J=8.0 and 14.4 Hz), 6.37–6.48 (2H, m), 7.32–7.44 (5H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.1, 86.4, 86.7, 121.5, 122.8, 123.8, 128.9, 129.0, 134.8, 137.9, 140.9, 153.4; MS m/z 230 (M^+); HRMS m/z calcd for $C_{14}H_{14}O_3$ (M^+): 230.0943, found 230.0936. Minor product: yield 12%; yellow oil; IR (neat) 2982, 2927, 1798 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.88 (3H, s), 4.93 (1H, dd, J=8.5 and 18.1 Hz), 4.96 (1H, d, J=8.5 Hz), 5.16 (1H, dd, J=1.2 and 10.4 Hz), 5.28 (1H, dd, J=1.2 and 17.1 Hz), 6.08 (1H, td, J=10.4 and 17.1 Hz), 6.29 (1H, dd, J=10.4 and 18.1 Hz), 7.20–7.24 (2H, m), 7.32–7.41 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.7, 86.7, 87.2, 120.9, 124.9, 125.1, 128.4, 128.5, 134.7, 136.9, 137.6, 153.9; MS m/z 230 (M^+); HRMS m/z calcd for $C_{14}H_{14}O_3$ (M^+): 230.0943, found 230.0936.

4.2.7. 2-Methyl-2-phenyl-5-vinyl-2,5-dihydrofuran (12d). Yield 33% (1.8:1 diastereomeric mixture); colorless oil; IR (neat) 2923, 2852, 1462 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65 (1.92H, s), 1.69 (1.08H, s), 5.10 (0.64H, dt, J=1.2 and 10.2 Hz), 5.17 (0.36H, dt, J=1.2 and 10.2 Hz),

5.26 (1H, m), 5.31–5.37 (1H, m), 5.72 (1H, m), 5.80 (0.64H, ddd, J=7.3, 10.0 and 17.3 Hz), 5.90 (0.36H, ddd, J=7.0, 9.5 and 17.3 Hz), 6.04 (1H, m), 7.23 (1H, m), 7.30–7.34 (2H, m), 7.40–7.45 (2H, m); MS m/z 186 (M^+); HRMS m/z calcd for $C_{13}H_{14}O$ (M^+): 186.1044, found 186.1040.

4.3. Crossover experiment of (*E*,*E*)-1a and (*E*,*E*)-6

To a stirred solution of dienylic carbonate (*E*,*E*)-**1c** (35.4 mg, 0.15 mmol) and dienylic benzoate (*E*,*E*)-**6** (53.7 mg, 0.15 mmol) in dioxane (3.0 mL) were added $Pd_2(dba)_3 \cdot CHCl_3$ (15.5 mg, 15.0 μmol), dppv (23.8 mg, 0.06 mmol), and BSA (55.6 μL, 0.23 mmol) in a sealed tube at rt. After stirring was continued for 4 h at 50 °C, the reaction mixture was concentrated and the residue was chromatographed on silica gel with hexane–AcOEt (95:5 v/v) as eluent to give **2a** (9.5 mg, 23%), **2c** (9.6 mg, 31%), and dihydrofuran **12a** (5.0 mg, 14%) as a colorless oil, respectively.

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Synthesis of *N*,*N*,*N*′,*N*′-tetrasubstituted 1,3-bis(4-aminophenyl)azulenes and their application to a hole-injecting material in organic electroluminescent devices

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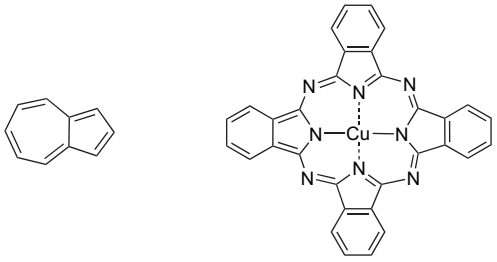
Abstract—After a preliminary search of the reaction conditions for the Suzuki–Miyaura cross-coupling of haloazulenes with arylboronic acids, the title compounds were synthesized either by the direct coupling reaction between 1,3-dihaloazulene and the corresponding *N*,*N*-disubstituted 4-aminophenylboronic acids or by a two-step sequence involving the cross-coupling with 4-bromophenylboronic acid and subsequent Pd-catalyzed amination. Application of the title diamines to a hole-injecting material in organic electroluminescent devices was carried out to provide their prominent characteristics as a novel durable, non-cyanine and non-polyamine substance without color fade. The diamine derivatives, extended by an ethynyl unit between the azulenyl core and the 4-aminophenyl moiety, were also synthesized and found, unfortunately, unsuitable for vacuum deposition in preparing a multilayer composite.

1. Introduction

Azulene (**1**) constructed by two odd membered rings, an isomer of naphthalene, appertains to a typical non-alternant hydrocarbon (Chart 1).[1] It appears blue in color because of its relatively narrow HOMO–LUMO gap and the reduced mutual repulsion between the unpaired electrons at the first excited state based on the nature of the coefficients of the HOMO and LUMO inherently accompanied with non-alternant hydrocarbons,[2] and the color has fascinated physical and synthetic organic chemists for a long time.[3] While azulene and its derivatives were used as anti-inflammatory and antiulcer agents extensively in the fields of medicinal chemistry for a long time,[4] their application in material science has been less pronounced despite their attractive color. There is only scattered literature on their applications in material science. There are some reports on azulene-containing polymers as a conductive material[5] and an amperometric biosensor,[6] azulene derivatives as non-linear optics[7] and near-infrared quencher.[8] Independently, we have studied the synthesis of the title azulene derivatives and applied them to a material in organic electroluminescent (organic EL) devices.[9]



azulene, 1 CuPc, 2

Chart 1.

Organic EL devices have received much attention in recent years because of their application in flat and thin panel displays, overcoming the drawbacks of contemporary electronic displays.[10,11] Recent interest in developing practical EL devices for long-term use with high power-efficiency is focused on those with a multilayered structure, which

Keywords: Azulenes; Suzuki–Miyaura reaction; Amination; Organic electroluminescent device; Hole-injecting material.
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‡ M. Oda for the molecular design, syntheses and physical properties.

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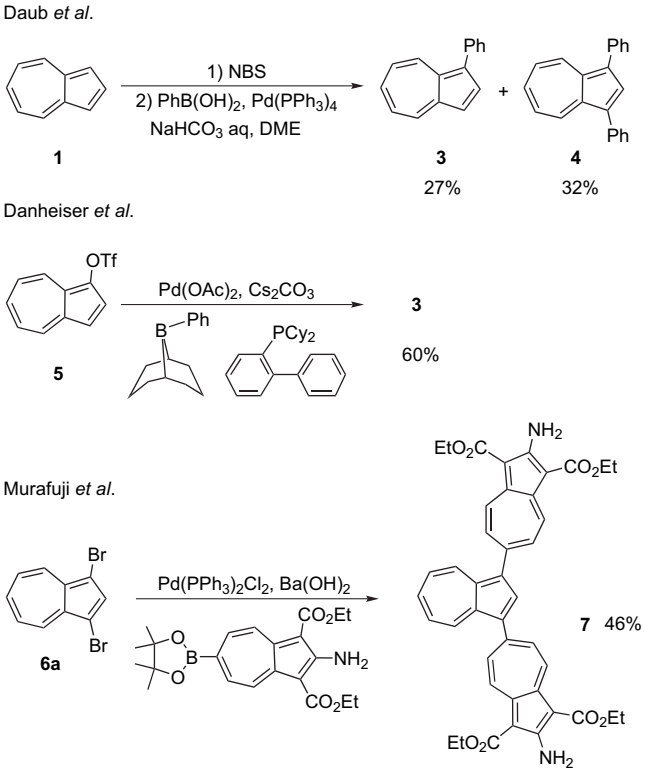
comprises hole-injecting, hole-transporting, light-emitting, hole-blocking, and electron-injecting layers between the indium-tin-oxide (ITO) electrode and the cathode. A hole-injecting layer (HIL) has been used to intermediate between the ITO electrode and a hole-transporting layer (HTL) to demonstrate greater operational half-life time compared with that without HIL.[12] Several materials, such as copper phthalocyanine (CuPc, **2**)[12c] and aryl-substituted tetraamine,[12a,b,e] were reported for this purpose. Among these, CuPc was introduced by Kodak researchers and was a widely used material. However, CuPc itself has strong absorption in a visible light range so that the device containing it shows color fade, particularly at a range of 550–700 nm, depending on the thickness. Azulene has relatively high HOMO and low LUMO energy levels and is colored, but its extinction coefficient for visible absorption is small. Thus, we undertook a design where CuPc could be replaced by an azulene derivative with less number of amino substituents. In this paper, we disclose the synthetic study of the cross-coupling between haloazulenes and arylboronic acids, and describe the full detailed synthesis of the title diamines containing an azulene as a core π-electron chromophore and their application to the HIL materials, which overcomes this shortcoming of CuPc, as a novel non-phthalocyanine and non-polyamine substance.

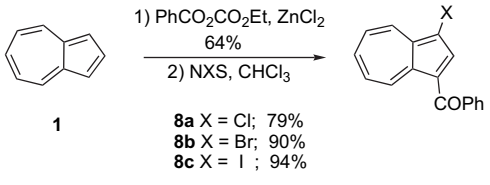
2. Results and discussion

2.1. Synthesis of 1-aryl-3-benzolylazulenes as preliminary experiments for the Suzuki–Miyaura cross-coupling reaction of haloazulenes

In 1997, Daub et al. reported the first example of the Suzuki–Miyaura cross-coupling reaction[13] of bromoazulenes (Scheme 1).[14] After bromination of azulene with *N*-bromosuccinimide, the products were subjected to the Suzuki–Miyaura cross-coupling reaction with phenylboronic acid and $Pd(PPh_3)_4$ as a catalyst to give a mixture of 1-phenyl- and 1,3-diphenylazulenes (**3** and **4**) in yields of 27 and 32%, respectively. Later, Danheiser et al. found efficient coupling with azulenyl triflate and *B*-phenyl-9-BBN[15] and Murafuji et al. disclosed the double cross-coupling of azulenylboronic acids and 1,3-dibromoazulene (**6a**) in better yields than that of **4** reported by Daub et al.,[16] though both methods required expensive reagents and the manipulation of azulene substrates and the yields were not necessarily satisfactory.[17] Since we focused on an efficient Suzuki–Miyaura coupling of 1,3-dihaloazulenes for the synthesis of HTL materials, we had begun with a preliminary search of the reaction conditions for the cross-coupling between haloazulenes and arylboronic acids. We chose 1-benzoyl-3-haloazulenes (**8**) as an initial substrate to avoid any obstacles on the process because an electron-withdrawing substituent like a benzoyl group is known to accelerate the oxidative addition of the palladium(0) reagent in Suzuki–Miyaura coupling. The substrates **8a–c** were prepared by halogenation of 1-benzoylazulene (**7**) with *N*-halosuccinimide (Scheme 2). We applied various reaction conditions with $Pd(PPh_3)_4$, $Pd(PPh_3)_2Cl_2$, and $Pd(dppf)Cl_2$[18] as a catalyst and phenylboronic acid for **8**. The results are shown in Table 1. At first, we used bromide **8b** as a substrate (entries 1–6). The reaction under the conditions with **8b**, $Pd(dppf)Cl_2$, BINAP, and Cs_2CO_3[19] (entry 5) provided the product **9** in a good yield, and iodide **8c** under the same conditions was transformed to **9** also in a good yield (entry 8) but not chloride **8a** (entry 7). Although, we applied the Fu's reaction conditions[20] for chloride **8a** with $Pd_2(dba)_3$,[21] $(t\text{-Bu})_3PHBF_4$, and CsF, the yield of **9** was as low as 20% (entry 9). We also examined the reactions of **8b** and **c** with various arylboronic acids under the conditions of the entries 5 and 8 in Table 1. The results are shown in Table 2. Satisfactory yields of the corresponding aryl-substituted products were found either from **8b** or **c**. The yields from **8c** were slightly better than those from **8b** except in the case of the 4-dibenzofurylboronic acid. With this knowledge of the satisfactory reaction conditions with $Pd(dppf)Cl_2$, BINAP, and Cs_2CO_3 in hand, synthesis of 1,3-diarylazulenes from 1,3-dihaloazulenes was undertaken next.



Scheme 1.

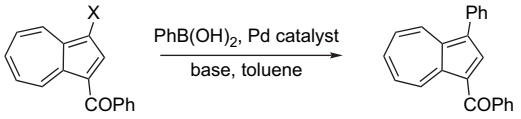


Scheme 2.

2.2. Synthesis of various 1,3-diarylazulenes including the title azulenes

From 1,3-dibromo- and 1,3-diiodoazulenes, **6a** and **b**, 1,3-diarylazulenes, **4** and **11**, were synthesized under the reaction conditions with $Pd(dppf)Cl_2$, BINAP, and Cs_2CO_3 (Table 3). In general the yields from iodide **6b** are better than those from bromide **6a** except in the case of the reaction

Table 1. The Suzuki–Miyaura cross-coupling of 1-benzoyl-3-haloazulenes with phenylboronic acid under various conditions

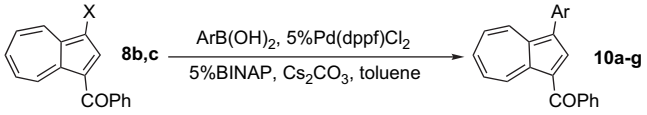


8a-c **9**

Entry	X (**8**)	Pd catalyst and additive[a]	Base[b]	Reaction time (h)[c]	Yield of **9** (%)[d]
1	Br (**8b**)	$Pd(PPh_3)_4$	Cs_2CO_3	2	55
2	Br (**8b**)	$Pd(PPh_3)_2Cl_2$, PPh_3	Cs_2CO_3	1	55
3	Br (**8b**)	$Pd(dppf)Cl_2$	Cs_2CO_3	16	74
4	Br (**8b**)	$Pd(dppf)Cl_2$	K_2CO_3	30	38
5	Br (**8b**)	$Pd(dppf)Cl_2$, BINAP	Cs_2CO_3	2	87
6	Br (**8b**)	$Pd(dppf)Cl_2$, BINAP	K_2CO_3	2	46
7	Cl (**8a**)	$Pd(dppf)Cl_2$, BINAP	Cs_2CO_3	4	40
8	I (**8c**)	$Pd(dppf)Cl_2$, BINAP	Cs_2CO_3	2	90
9	Cl (**8a**)	$Pd_2(dba)_3$, $(t\text{-Bu})_3PHBF_4$, CsF[e]	Cs_2CO_3	26	20

[a] Pd catalyst (5 mol %) and 2 equiv of phenylboronic acid to **8** were used.
[b] Base (4 equiv) was used.
[c] Reaction was done in refluxing toluene.
[d] Isolated yield after chromatographic purification.
[e] Pd catalyst and the ligand (12 mol %), and 2 equiv of phenylboronic acid were used. The reaction was carried out at 90 °C in dioxane as a solvent.

Table 2. The Suzuki–Miyaura cross-coupling of 1-benzoyl-3-haloazulenes with various arylboronic acids under the conditions with $Pd(dppf)Cl_2$, BINAP, and Cs_2CO_3 in refluxing toluene[a]



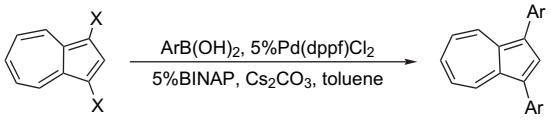
Entry	X (**8**)	Ar	Reaction time (h)	Product	Yield (%)[b]
1	Br (**8b**)	*t*-Bu	3	**10a**	63
2	I (**8c**)	*t*-Bu	3	**10a**	74
3	Br (**8b**)		3	**10b**	89
4	I (**8c**)		3	**10b**	97
5	Br (**8b**)	Me_2N	3.5	**10c**	61
6	I (**8c**)	Me_2N	3.5	**10c**	65
7	Br (**8b**)		1.5	**10d**	81
8	I (**8c**)		1.5	**10d**	86
9	Br (**8b**)		1.5	**10e**	80
10	I (**8c**)		1.5	**10e**	92
11	Br (**8b**)	O	1	**10f**	86
12	I (**8c**)	O	1	**10f**	73
13	Br (**8b**)	S	1	**10g**	82
14	I (**8c**)	S	1	**10g**	83

[a] Pd catalyst and BINAP (5 mol %), 2 equiv of arylboronic acid to **8**, and 4 equiv of Cs_2CO_3 were used.
[b] Isolated yield after chromatographic purification.

with 4-(*N*,*N*-dimethylamino)phenylboronic acid, which required a longer reaction time (entry 8). Although some yields of 1,3-diarylazulenes **11** from **6b** (entries 2, 6, and 10) were good, other yields were relatively low. In these double Suzuki–Miyaura reactions, thus, the second couplings are assumed to be comparatively deactivated by the first aryl substituents.

We obtained the amine-substituted 1,3-diphenylazulene derivatives **11c–e**, all of which we had designed as primitive hole-injecting materials. For these diamines, we also applied a stepwise method, which involves the cross-coupling with 4-bromophenylboronic acid (**12**) and subsequent Pd-catalyzed amination (Scheme 3). The synthetic intermediate, 1,3-bis(4-bromophenyl)azulene (**11f**), was prepared by the Suzuki–Miyaura coupling under similar reaction conditions in 18% yield from **6a** or 53% yield from **6b**. The amination[22] of **11f** using $Pd(OAc)_2$ as a catalyst was carried out under Fu's reaction conditions with $(t\text{-Bu})_3PHBF_4$ as a ligand and *t*-BuONa as a base in toluene.[20] In a case where volatile dimethylamine was used, the reaction was carried out in a sealed tube to give 72% yield of **11c**. Other derivatives,

Table 3. The Suzuki–Miyaura cross-coupling of 1,3-dihaloazulenes with various arylboronic acids under the conditions with $Pd(dppf)Cl_2$, BINAP, and Cs_2CO_3 in refluxing toluene[a]

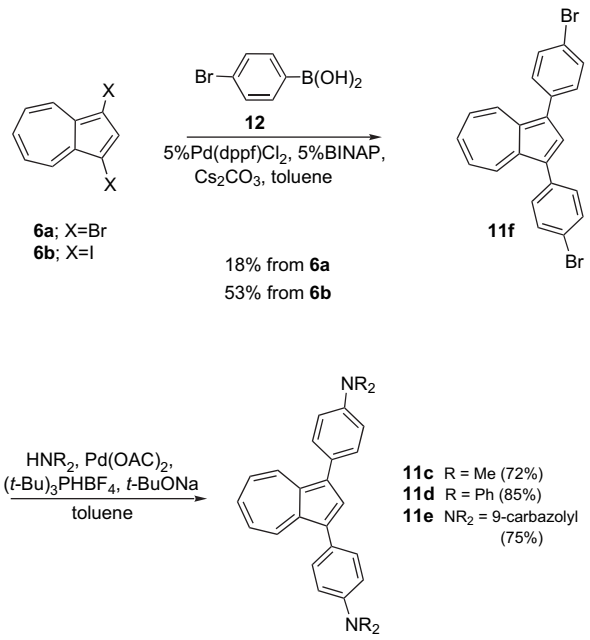


6a,b **4, 11a-e**

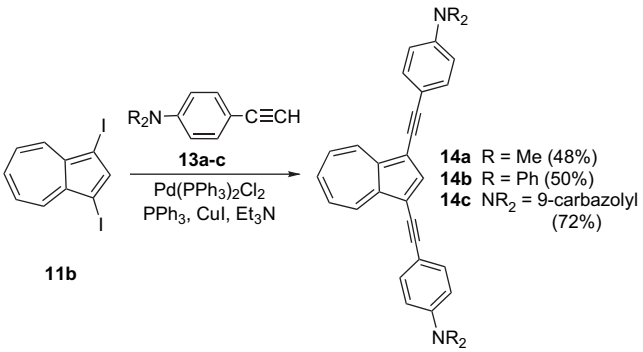
Entry	X (**6**)	Ar	Reaction time (h)	Product	Yield of **9** (%)[b]
1	Br (**6a**)	Ph–	2	**4**	61
2	I (**6b**)	Ph–	2	**4**	90
3	Br (**6a**)	4-Ph–C_6H_4–	3	**11a**	7
4	I (**6b**)	4-Ph–C_6H_4–	4	**11a**	35
5	Br (**6a**)	1-Naphthyl–	2	**11b**	43
6	I (**6b**)	2-Naphthyl–	2	**11b**	75
7	Br (**6a**)	4-(Me_2N)–C_6H_4–	24	**11c**	23
8	I (**6b**)	4-(Me_2N)–C_6H_4–	24	**11c**	10
9	Br (**6a**)	4-(Ph_2N)–C_6H_4–	2	**11d**	40
10	I (**6b**)	4-(Ph_2N)–C_6H_4–	1	**11d**	80
11	Br (**6a**)	4-Cz–C_6H_4–[c]	2	**11e**	22
12	I (**6b**)	4-Cz–C_6H_4–[c]	2	**11e**	24

[a] Pd catalyst and BINAP (5 mol %), 3 equiv of arylboronic acid to **6**, and 4 equiv of base were used.
[b] Isolated yield after chromatographic purification.
[c] Cz means a 9-carbazolyl group.

11d and **11e**, were obtained with the corresponding amines by conventional heating under atmosphere of argon in 85 and 75% yields, respectively. Although the two-step yields are not attractive as compared with those of the direct method, the amination can provide a divergent protocol for preparing a variety of the amine derivatives from a single intermediate. We also prepared similar diamine derivatives, **14a–c**, which are extended by an ethynyl unit between phenyl and the azulene moieties, by the Sonogashira reaction[23] of **11b** with the corresponding acetylene compounds, **13a–c**, as shown in Scheme 4.



Scheme 3.



Scheme 4.

2.3. Structure and physical properties of *N*,*N*,*N*′,*N*′-tetrasubstituted 1,3-bis(4-aminophenyl)- and 1,3-bis(4-aminophenylethynyl)azulenes

The structures of three *N*,*N*,*N*′,*N*′-tetrasubstituted 1,3-bis(4-aminophenyl)azulenes and three *N*,*N*,*N*′,*N*′-tetrasubstituted 1,3-bis(4-aminophenylethynyl)azulenes were characterized by spectroscopic and combustion analyses. Signals in the ^1H and ^{13}C NMR spectra were assigned by correlations based on their two-dimensional H–H COSY, NOESY, HMQC, and HMBC spectra; the results are shown in Figure 1. A higher field shift of azulenyl protons in the ^1H NMR spectra of **11c** and **14a** compared with those of the others may be ascribed to a stronger electron-donating effect of the dimethylamino group. All of them were isolated as green solids and have mainly three strong absorption bands at 230–250, 320–350, and 370–410 nm, and a broad band at 600–700 nm. The latter band in a visible range has small extinction coefficients of ≈200, which are far weaker than that of CuPc. The results are promising for overcoming the color fade observed in the device using CuPc (vide infra). Electrochemical oxidation of **11c–e** and **14a–c** was examined by cyclic voltammetry in dichloromethane containing 0.1 M tetrabutylammonium perchlorate. The diamines **11c–e** showed two oxidation potentials (E_{ox}^1 and E_{ox}^2) and the differences between them are more than 0.38 V, which are large enough for the purpose of organic EL applications. The diamines **14b**,**c** were very sensitive to electrochemical oxidation. While diamines **14b**,**c** provided quasi-reversible voltammograms with one oxidation potential,[24] the diamine **14a** did not show any clear oxidation potential in its voltammogram and a significant amount of an insoluble substance was observed at the electrode surface after scans. The HOMO energy levels of these compounds were estimated from the first oxidation potentials (Table 4).[25] Those of **11c** and **d** are slightly less than that of CuPc, that of **11e** is the same, and those of **14b**,**c** are slightly greater. It should be noted that the HOMO energy levels of **11c** and **d** intermediate between those of HTL materials, such as TPD (5.4–5.5 eV),[26] α-NPD (5.4 eV),[27] TPTE (5.3 eV),[28,29] and the work function of the ITO electrode (4.6–5.0 eV),[12b] and that of **11e** is the same as that of TPTE, suggesting that these compounds can be used as HIL materials in organic EL devices (Chart 2).[30] Since the diamine derivatives, **14a–c**, were found to decompose during vacuum deposition in OLED fabrication besides possessing slightly greater HOMO energy levels than some of the HTL materials, only the derivatives, **11c–e**, were further examined for application to HIL material.

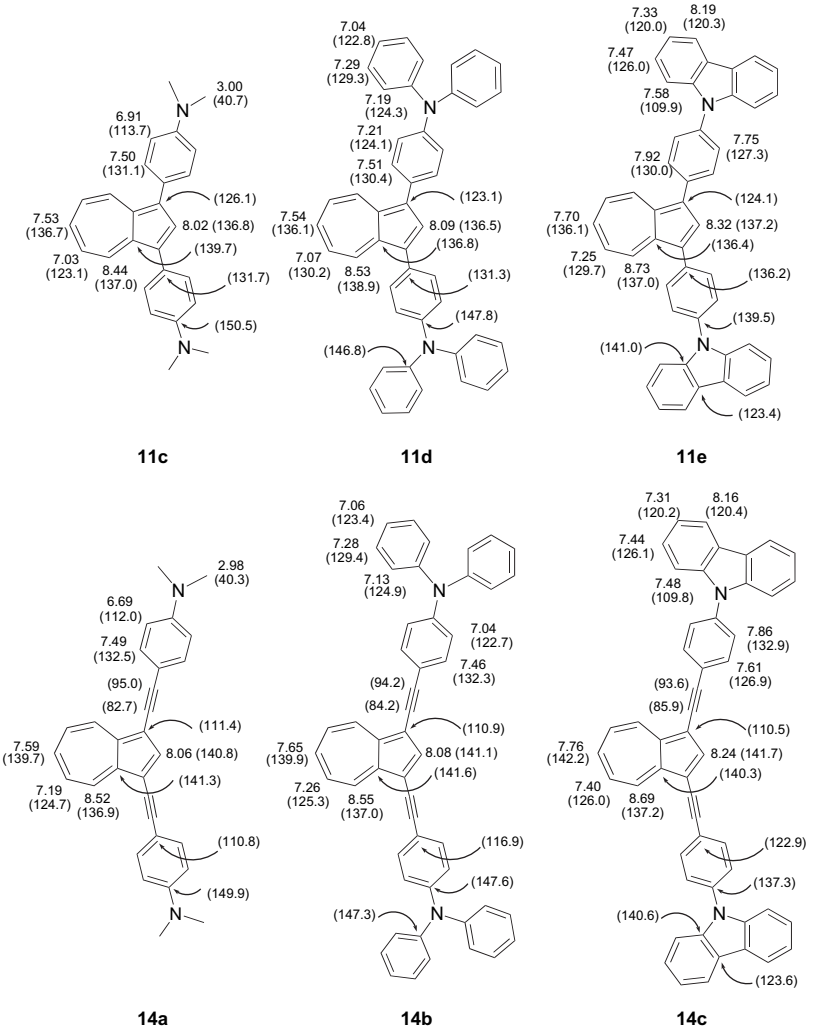


Figure 1. Assignment of ^{1}H and ^{13}C chemical shifts. Carbon shifts are in parentheses.

2.4. Application of *N*,*N*,*N*′,*N*′-tetrasubstituted 1,3-bis(4-aminophenyl)azulenes as a hole-injecting material in organic EL devices

Transmittance of visible light through the films of **11c–e** on quartz was first examined. Reduction of the transmittance with the 100 nm film of CuPc was very clear. Similarly, transmittance of visible light through the thin film (100 nm) of **11c–e** on quartz was compared with that of CuPc. While only 20–30% of the light in the 500–800 nm range passed through the film of CuPc, more than 80% of the light in all of the visible range passed through the film of **11c–e**. A thinner film was also examined; reduction of less than 15% of the initial light in the range through the thin film (10 nm) of **11c–e** was observed, 20–30% of the light was diminished through that of CuPc. As expected from their visible absorption spectra, indeed color fade was not observed with the thin film of **11c–e**. Then, an application for EL devices was investigated with the multilayered structure **A**, which emits green light, depicted in Figure 2. The initial characteristics, the CIE chromatocity coordinate, EL peak, and half-life time are shown in Table 5. There is no clear difference in CIE chromatocity coordinate and EL peak between the HIL materials. Although, the initial luminance with **11c–e** was slightly lower than that with CuPc, the half-life time with **11c–e** was much longer than that of CuPc. While operation with a low initial voltage retards degradation of a device in general,[12d] the device with **11c** unusually shows higher initial voltage and longer half-life time. Probably the relatively greater hole drift mobility of **11c** compared with those of **11d** and **e**[31] may overcome its defect. Since reduction of

Table 4. Oxidation potentials and estimated HOMO energy levels of CuPc, **11c–e**, and **14a–c**

Compounds	$E_{ox\ 1/2}$ (V vs Fc/Fc^{+})[a]		HOMO (eV)[b]
	E_{ox}^{1}	E_{ox}^{2}	
CuPc	0.46		5.3
11c	0.42	1.02	5.2
11d	0.38	0.94	5.2
11e	0.51	0.89	5.3
14a	—[c]	—[c]	—
14b	0.62	—[c]	5.4
14c	0.55	—[c]	5.4

[a] Corrected values from the E_{ox} (V vs SCE) by subtracting the E_{ox} value of ferrocene (0.48 V in dichloromethane, 0.50 V in *N*,*N*-dimethylformamide) in the same conditions. CuPc was measured in *N*,*N*-dimethylformamide and others were in dichloromethane.

[b] HOMO energy values were obtained from oxidation potentials (E_{ox}^{1}) against the value of ferrocene and calculated by taking the HOMO energy value of ferrocene to be 4.8 eV with respect to zero energy level.

[c] E_{ox} was not clearly observed.

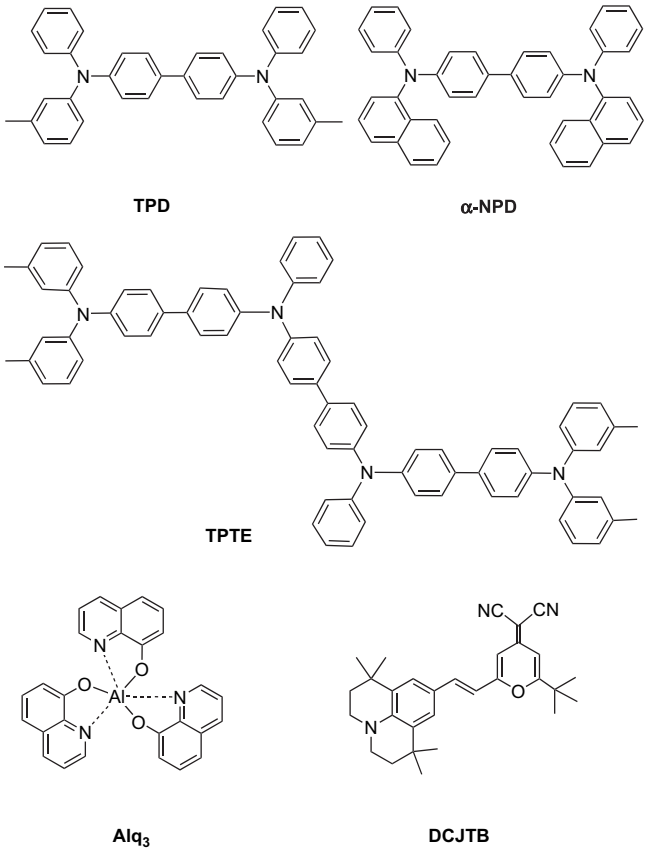


Chart 2. Structures of the dopant and hole-transporting and light-emitting materials used in our study.

light at a range of 500–800 nm through the thin films of **11c–e** was clearly shown, we also examined another multilayered structure **B**, which emitted the orange light by using DCJTB[32] as a dopant, depicted in Figure 2. The characteristics of the luminescence are shown in Table 6. Although we have not checked the half-life time with this structure, the device with **11c** indicated the most effective performance among them. With these results in hand, we now are

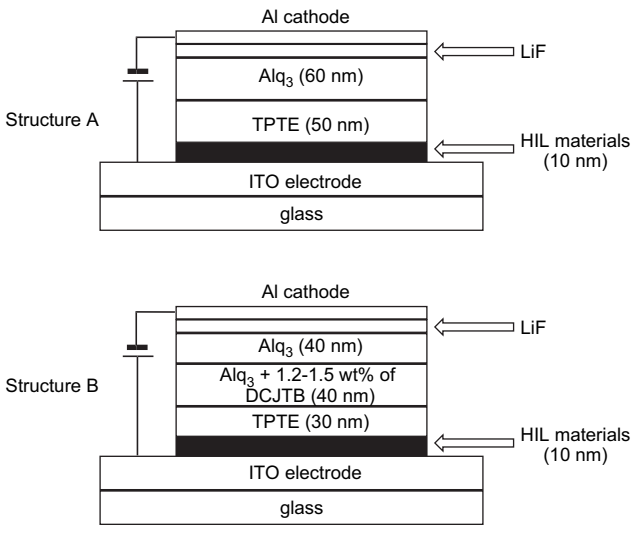


Figure 2. The investigated organic EL architectures.

Table 5. The characteristics of the EL devices (structure **A**) with CuPc and **11c–e** as HIL materials at 11 mA cm^{-2}

HIL materials	Initial luminance (cd cm^{-2})	Initial voltage (eV)	CIE[a] chromaticity coordinates		EL peak (nm)	Half-life time (h)
			x	*y*		
11c	578	8.42	0.336	0.524	526	630
11d	567	5.52	0.356	0.531	535	400
11e	543	5.09	0.355	0.534	538	600
CuPc	602	7.35	0.335	0.534	533	150

[a] Commission Internationale de L'Éclairage.

Table 6. The characteristics of the EL devices (structure **B**) with CuPc and **11c–e** as HIL materials at 11 mA cm^{-2}

HIL materials	Initial luminance (cd cm^{-2})	Initial voltage (eV)	CIE[a] chromaticity coordinates		EL peak (nm)
			x	*y*	
11c	444	9.95	0.610	0.379	620
11d	238	10.05	0.619	0.373	621
11e	299	6.71	0.609	0.384	614
CuPc	221	8.11	0.617	0.375	618

[a] Commission Internationale de L'Éclairage.

advancing to the development of a more practical application, which will be reported elsewhere in due course.

3. Summary

After a preliminary search of the reaction conditions for the Suzuki–Miyaura cross-coupling of haloazulenes with various arylboronic acids, bases, palladium catalysts, and additive ligands, the title compounds have been synthesized in moderate yields either by the direct coupling reaction between 1,3-dihaloazulene and the corresponding *N*,*N*-disubstituted 4-aminophenylboronic acids under the conditions with $Pd(dppf)Cl_2$, BINAP, and Cs_2CO_3 in refluxing toluene or by a two-step sequence involving the cross-coupling with 4-bromophenylboronic acid and subsequent $Pd(OAc)_2$-catalyzed amination with $(t\text{-Bu})_3PHBF_4$ and *t*-BuONa. We have also synthesized the derivatives extended by an ethynyl group between the azulenyl core and the aminophenyl group, *N*,*N*,*N*′,*N*′-tetrasubstituted 1,3-bis(4-aminophenylethynyl)azulenes **14a–c**, as a candidate for HIL material. Unfortunately, the latter derivatives were not appropriate for a fabrication process by vacuum deposition. Application of these title diamines, **11c–e**, to HIL material in organic electroluminescent devices was made to provide their prominent characteristics as a novel durable, non-cyanine, and non-polyamine substance without color fade.

4. Experimental

4.1. General

Melting points were measured on a Yanaco MP-3 and are uncorrected. IR spectra were recorded on a Perkin–Elmer Spectrum RX I spectrometer. UV spectra were measured on a Shimadzu UV-1600 spectrometer. ^1H and ^{13}C NMR were

recorded with tetramethylsilane as an internal standard on a JEOL α400 or JEOL δECP-600 NMR. Mass spectra were measured on a JMS-700 mass spectrometer. Cyclic voltammograms were recorded on a Yanako P1100 instrument. Column chromatography was done with either Merck Kieselgel 60 Art 7734 or Wako Activated alumina. Toluene was purified by distillation from calcium hydride under a nitrogen atmosphere. Diphenylamine, dimethylamine aqueous solution, and carbazole were purchased from Tokyo Kasei Ind. Co., cesium carbonate was purchased from Kanto Chem. Inc., and BINAP (*rac*-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl), tetrakis(triphenylphosphine)palladium, bis-(triphenylphosphine)palladium dichloride, tris(dibenzylideneacetone)dipalladium, and [1,1′-bis(diphenylphosphino)-ferrocene]dichloropalladium (1:1 complex with dichloromethane) were purchased from Aldrich Co. and were used without purification. Various arylboronic acids were also purchased from Aldrich Co., except 4-[9-(carbazolyl)]-phenylboronic acid, which was prepared by the reported method.[33] Sodium *tert*-butoxide, palladium acetate, and copper iodide were purchased from Wako Chem. Co. and were used without purification. The ethynyl compounds **13a**,[34] **b**,[35] and **c**[36] were prepared from disubstituted 4-haloanilines by palladium-catalyzed Sonogashira coupling with trimethylsilylacetylene, followed by deprotection with K_2CO_3 in methanol according to their reported methods.[37] 1,3-Dihaloazulenes, **6a** and **b**, were prepared from azulene with *N*-iodo- and *N*-bromosuccinimides according to a literature procedure[38] and were purified by alumina chromatography before use.

4.2. Synthesis of 1-benzoyl-3-haloazulenes (8)

To a solution of 1-benzoylazulene (2.00 mmol), prepared by the method of Sugihara et al.,[39] in 10 ml of chloroform at 0 °C was added 3.00 mmol of *N*-halosuccinimide in several portions. After being stirred at 40 °C for 3 h, the reaction mixture was concentrated and the residue was purified by alumina column chromatography. Elution with chloroform–hexane (3:7) gave **8** as solids. Analytical samples were obtained by recrystallization from hexane–dichloromethane. Compound **8a** (X=Cl) (79%): green solids, mp 70–72 °C. ^{1}H NMR ($CDCl_3$) δ=9.69 (d, J=9.8 Hz, 1H), 8.59 (d, J=9.8 Hz, 1H), 7.97 (s, 1H), 7.91 (t, J=9.8 Hz, 1H), 7.84–7.81 (dm, J=6.8 Hz, 2H), 7.67–7.56 (m, 3H), 7.53–7.49 (m, 2H) ppm; ^{13}C NMR ($CDCl_3$) δ=192.0, 140.9, 140.7, 140.0, 139.9, 139.7, 138.6, 136.2, 131.4, 129.5, 129.4, 128.2, 127.9, 122.5, 116.9; IR (KBr) ν_{max}=1655m, 1627s, 1595s, 1576m, 1561w, 1543m, 1525w, 1508w, 1498m, 1476w, 1458m, 1449w, 1417s, 1385s, 1342m, 1288m, 1237s, 1179, 1119w, 1026w, 977w, 958w, 919w, 867w, 801w, 781m, 742s, 706s, 671m, 637m, 587w, 565w cm^{-1}; UV–vis (CH_2Cl_2) λ_{max}=242 (log ε=4.46), 280 (4.44), 317 (4.59), 393 (4.15), 406sh (4.14), 559 (2.88), 562 (2.88), 608sh (2.74), 650 (2.30) nm; MS (70 eV) *m*/*z* (rel int): 268 (M^+, 27), 267 (M^+-1, 17), 266 (M^+, 81), 265 (M^+-1, 6), 231 (M^+-1, 12), 202 (15), 191 (32), 190 (12), 189 (100), 161 (17), 126 (40). Anal. Calcd for $C_{17}H_{11}ClO$: C, 76.55; H, 4.16. Found: C, 76.78; H, 4.30.

Compound **8b** (X=Br) (90%): green microprizms, mp 93–96 °C (lit.[40] 88 °C). ^{1}H NMR ($CDCl_3$) δ=9.70 (d, J=10.0 Hz, 1H), 8.57 (d, J=10.0 Hz, 1H), 8.05 (s, 1H), 7.92 (t, J=10.0 Hz, 1H), 7.84–7.82 (dm, J=6.8 Hz, 2H), 7.67–7.57 (m, 3H), 7.53–7.49 (m, 2H) ppm; ^{13}C NMR ($CDCl_3$) δ=191.9, 142.8, 140.8, 140.74, 140.71, 140.4, 139.6, 137.9, 131.5, 129.6, 129.5, 128.2, 127.9, 123.9, 104.6; IR (KBr) ν_{max}=1625s, 1594s, 1574s, 1533m, 1492s, 1456s, 1414m, 1379m, 1333m, 1285m, 1236m, 1178m, 1116m, 1072w, 1024m, 977w, 956w, 932w, 904m, 866m, 854m, 800m cm^{-1}; UV–vis (CH_2Cl_2) λ_{max}=236 (log ε=4.42), 244 (4.45), 274sh (4.40), 282 (4.44), 307sh (4.51), 318 (4.57), 393 (4.11), 404 (4.10), 521sh (2.64), 555 (2.72), 581sh (2.66), 662sh (2.07) nm; MS (70 eV) *m*/*z* (rel int): 313 (M^++1, 18), 312 (M^+, 95), 311 (M^++1, 22), 310 (M^+, 100), 235 (90), 233 (91), 207 (10), 205 (11), 126 (84), 77 (19).

Compound **8c** (X=I) (94%): green solids, mp 109–111 °C. ^{1}H NMR ($CDCl_3$) δ=9.68 (d, J=9.8 Hz, 1H), 8.49 (d, J=9.8 Hz, 1H), 8.17 (s, 1H), 7.93 (t, J=9.8 Hz, 1H), 7.84–7.81 (dm, J=6.8 Hz, 2H), 7.72–7.56 (m, 3H), 7.53–7.49 (m, 2H) ppm; ^{13}C NMR ($CDCl_3$) δ=191.9, 148.8, 144.1, 141.5, 140.7 (2C), 140.5, 138.8, 131.5, 129.9, 129.5, 128.3, 128.2, 126.3, 75.3; IR (KBr) ν_{max}=1654w, 1636w, 1616s, 1588w, 1573m, 1545w, 1529m, 1509w, 1491m, 1447m, 1414m, 1364m, 1329w, 1282m, 1233m, 1173w, 1129w, 1079w, 1036w, 1019m, 953w, 929w, 898m, 875w, 859s, 795w, 775m, 747s, 732w, 703m, 665m, 619w cm^{-1}; UV–vis (CH_2Cl_2) λ_{max}=231 (log ε=4.53), 284 (4.50), 321 (4.58), 395 (4.15), 407 (4.15), 413 (3.97), 552 (2.90), 590sh (2.82), 638sh (2.28) nm; MS (70 eV) *m*/*z* (rel int): 358 (M^+, 100), 282 (10), 281 (86), 253 (10), 202 (18), 126 (49), 77 (12). Anal. Calcd for $C_{17}H_{11}IO$: C, 57.01; H, 3.10. Found: C, 57.37; H, 3.25.

4.3. The Suzuki–Miyaura cross-coupling reactions of 1-benzoyl-3-haloazulenes (8)

A mixture of **8** (0.300 mmol), Cs_2CO_3 (1.20 mmol), and arylboronic acid (0.600 mmol) in 10 ml of toluene was evacuated well. And either 0.015 mmol of the palladium catalyst or the same amount of both the palladium catalyst and an additive was added to the mixture. The flask was evacuated and refilled with argon five times. The mixture was heated at 110 °C until the halogen substrate was almost consumed. Then the resulting reaction mixture was poured into water and extracted with ether (30 ml×3). The combined organic layer was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel to give the product.

Compound **9** (90%): 1-benzoyl-3-phenylazulene, dark purple microcrystals, mp 187–191 °C. ^{1}H NMR ($CDCl_3$) δ=9.77 (d, J=10.0 Hz, 1H), 9.71 (d, J=10.0 Hz, 1H), 8.17 (s, 1H), 7.90–7.84 (m, 3H), 7.66–7.47 (m, 9H), 7.34 (m, J=7.4 Hz, 1H) ppm; ^{13}C NMR ($CDCl_3$) δ=192.9, 142.2, 142.3, 141.3, 140.6, 140.3, 139.6, 137.3, 136.3, 131.2, 130.8, 129.7, 129.6, 129.1, 128.7, 128.1, 127.7, 127.0, 123.9 ppm; IR (KBr) ν_{max}=3050w, 3022w, 1617, 1594s, 1571s, 1524s, 1490s, 1421m, 1390m, 1365m, 1303w, 1236s, 1177w, 1113w, 1074w, 1018m, 910m, 894m cm^{-1}; UV–vis (CH_2Cl_2) λ_{max}=238 (log ε=4.36), 256 (4.36), 296 (4.53), 316 (4.45), 407 (3.99), 515 (2.56), 556 (2.66), 600 (2.55) nm; MS (70 eV) *m*/*z* (rel int): 309 (M^++1, 24), 308

(M^+, 100), 232 (11), 231 (62), 203 (10), 202 (45), 126 (3), 77 (5). Anal. Calcd for $C_{23}H_{16}O$: C, 89.58; H, 5.23. Found: C, 89.48; H, 5.44.

Compound **10a** (74%): 1-benzoyl-3-(4-*tert*-butylphenyl)-azulene, green solids, mp 155–158 °C. ^{1}H NMR ($CDCl_3$) δ=9.75 (d, J=9.8 Hz, 1H), 8.74 (d, J=9.8 Hz, 1H), 8.16 (s, 1H), 7.88 (d, J=8.4 Hz, 2H), 7.85 (t, J=9.2 Hz, 1H), 7.62 (t, J=9.8 Hz, 1H), 7.58–7.45 (m, 4H), 7.52 (s, 4H), 1.39 (s, 9H) ppm; ^{13}C NMR ($CDCl_3$) δ=192.9, 150.0, 142.43, 142.40, 141.3, 140.6, 140.2, 139.5, 137.4, 133.4, 131.2, 130.8, 129.6, 129.4, 129.0, 128.1, 127.6, 125.7, 123.8, 34.6, 31.4 ppm; UV–vis (CH_2Cl_2) λ_{max}=236 (log ε=4.44), 258 (4.40), 284 (4.50), 297 (4.58), 320 (4.46), 410 (4.03), 522 (2.60), 561 (2.69), 614 (2.52) nm; IR (KBr) ν_{max}=3023w, 2962w, 2868w, 1619s, 1595m, 1574m, 1531m, 1501m, 1446s, 1423s, 1389s, 1368s, 1299m, 1269m, 1235s, 1187m, 1174m, 1157m, 1099m, 1018m, 913m, 872m, 840s, 802s, 745s, 699s, 639s, 571m cm^{-1}; MS (70 eV) *m*/*z* (rel int): 365 (M^++1, 29), 364 (M^+, 100), 350 (19), 349 (63), 287 (8), 257 (9), 228 (6), 202 (11), 105 (23), 77 (9). Anal. Calcd for $C_{27}H_{24}O$: C, 88.97; H, 6.64. Found: C, 88.80; H, 6.79.

Compound **10b** (97%): 1-benzoyl-3-(4-biphenylyl)azulene, dark green needless, mp 133–136 °C. ^{1}H NMR ($CDCl_3$) δ=9.78 (d, J=9.8 Hz, 1H), 8.77 (d, J=9.8 Hz, 1H), 8.21 (s, 1H), 7.90 (d, J=7.4 Hz, 2H), 7.88 (t, J=9.8 Hz, 1H), 7.74 (d, J=8.4 Hz, 2H), 7.62–7.69 (m, 5H), 7.46–7.60 (m, 6H), 7.38 (tm, J=7.4 Hz, 1H) ppm; ^{13}C NMR ($CDCl_3$) δ=192.9, 142.6, 142.3, 141.2, 140.7, 146.0, 140.4, 139.8, 139.6, 137.4, 135.3, 131.3, 130.3, 130.1, 129.6, 129.2, 128.9, 128.2, 127.8, 127.5, 127.4, 127.1, 124.0 ppm; UV–vis (CH_2Cl_2) λ_{max}=237sh (log ε=4.49), 244 (4.52), 309 (4.72), 409 (4.04), 516sh (2.58), 560 (2.65), 610sh (2.54) nm; IR (KBr) ν_{max}=3054w, 3029w, 2925w, 2852w, 1618s, 1596m, 1576m, 1531w, 1509w, 1488w, 1446m, 1424s, 1388m, 1361m, 1238s, 1184w, 1159w, 1019w, 913m, 846m, 807m, 766m, 746s, 727m, 696s, 656m cm^{-1}; MS (70 eV) *m*/*z* (rel int): 385 (M^++1, 34), 384 (M^+, 100), 307 (44), 278 (19), 277 (17), 276 (20), 252 (6), 202 (10), 138 (3), 77 (7). Anal. Calcd for $C_{29}H_{20}NO$: C, 90.60; H, 5.24. Found: C, 90.52; H, 5.39.

Compound **10c** (65%): 1-benzoyl-3-{4-(*N*,*N*-dimethylamino)phenyl}azulene, brown solids, mp 146–149 °C. ^{1}H NMR ($CDCl_3$) δ=9.70 (d, J=9.6 Hz, 1H), 8.68 (d, J=9.6 Hz, 1H), 8.12 (s, 1H), 7.88 (d, J=6.8 Hz, 2H), 7.79 (t, J=9.6 Hz, 1H), 7.58–7.67 (m, 7H), 6.88 (d, J=6.8 Hz, 2H), 3.02 (s, 6H) ppm; ^{13}C NMR ($CDCl_3$) δ=192.3, 150.8, 142.8, 142.3, 142.1, 141.4, 140.7, 139.7, 138.4, 132.1, 131.9, 131.0, 130.2, 129.3, 129.0, 128.2, 127.2, 124.4, 113.6, 40.6 ppm; UV–vis (CH_2Cl_2) λ_{max}=229 (log ε=4.35), 248 (4.38), 300 (4.63), 322 (4.75), 423 (3.86), 578 (2.65) nm; IR (KBr) ν_{max}=3022w, 2886w, 2803w, 1610s, 1573m, 1540s, 1507m, 1447m, 1420s, 1388s, 1349s, 1237s, 1200m, 1161m, 1019m, 945m, 912m, 823s cm^{-1}; MS (70 eV) *m*/*z* (rel int): 352 (M^++1, 39), 351 (M^+, 100), 274 (11), 258 (7), 246 (7), 231 (6), 202 (28), 137 (9), 105 (8), 77 (11). Anal. Calcd for $C_{25}H_{21}NO$: C, 85.44; H, 6.02; N, 3.99. Found: C, 85.36; H, 6.11; N, 4.07.

Compound **10d** (86%): 1-benzoyl-3-(1-naphthyl)azulene, purple solids, mp 180–182 °C. ^{1}H NMR ($CDCl_3$) δ=9.86 (d, J=9.7 Hz, 1H), 8.28 (d, J=9.7 Hz, 1H), 8.22 (s, 1H), 7.96–7.90 (m, 4H), 7.86 (t, J=9.7 Hz, 1H), 7.69 (t, J=9.7 Hz, 1H), 7.66 (d, J=8.8 Hz, 1H), 7.61–7.46 (m, 6H), 7.35–7.43 (m, 2H) ppm; ^{13}C NMR ($CDCl_3$) δ=192.9, 144.0, 142.5, 142.1, 141.2, 140.2, 139.6, 137.7, 134.0, 133.8, 132.9, 131.2, 129.6, 129.3, 129.0, 128.7, 128.4, 128.1, 127.9, 127.7, 126.2, 126.1, 125.9, 125.4, 123.8 ppm; UV–vis (CH_2Cl_2) λ_{max}=233 (log ε=4.68), 247sh (4.61), 279 (4.62), 297sh (4.62), 304sh (4.67), 315 (4.71), 388sh (4.12), 405 (4.17), 514sh (2.70), 550 (2.82), 602sh (2.65) nm; IR (KBr) ν_{max}=3049w, 2370w, 2344w, 1621s, 1586m, 1574m, 1520m, 1425s, 1375s, 1303m, 1259m, 1236s, 1161m, 1132m, 1006m, 908m, 821s, 809s, 780m, 748s, 711m, 699m, 660m cm^{-1}; MS (70 eV) *m*/*z* (rel int): 359 (M^++1, 43), 358 (M^+, 100), 281 (48), 253 (35), 252 (75), 126 (10), 105 (6), 77 (7). Anal. Calcd for $C_{27}H_{18}O$: C, 90.47; H, 5.06. Found: C, 90.25; H, 5.33.

Compound **10e** (92%): 1-benzoyl-3-(2-naphthyl)azulene, dark purple plates, mp 116–118 °C. ^{1}H NMR ($CDCl_3$) δ=9.79 (d, J=10.0 Hz, 1H), 8.80 (d, J=10.0 Hz, 1H), 8.27 (s, 1H), 8.02 (s, 1H), 7.96 (d, J=8.8 Hz, 1H), 7.93–7.86 (m, 5H), 7.71 (dd, J=8.4, 1.6 Hz, 1H), 7.65 (t, J=10.0 Hz, 1H), 7.59–7.49 (m, 6H) ppm; ^{13}C NMR ($CDCl_3$) δ=192.9, 142.5, 141.3, 140.8, 140.4, 139.7, 137.4, 133.8, 133.6, 132.4, 131.3, 130.8, 129.6, 128.4, 128.2, 128.1, 127.9, 127.8, 126.4, 126.0, 124.0 ppm; UV–vis (CH_2Cl_2) λ_{max}=229 (log ε=4.17), 256 (3.81), 290 (4.05), 304sh (4.02), 317 (3.99), 393sh (4.02), 398 (4.55), 409 (3.56), 516 (2.63), 518sh (2.65), 552 (2.71), 602 (2.55), 611sh (2.59), 749 (0.752) nm; IR (KBr) ν_{max}=3051w, 2371w, 2343w, 1615s, 1594m, 1572m, 1524m, 1501m, 1444m, 1421s, 1393s, 1332m, 1304m, 1234s, 1203m, 1176m, 1016m, 957m, 920m, 863m, 833s, 747s, 709s, 662s, 639m, 572m cm^{-1}; MS (70 eV) *m*/*z* (rel int): 359 (M^++1, 29), 358 (M^+, 100), 281 (49), 253 (18), 252 (54), 126 (10), 77 (7). Anal. Calcd for $C_{27}H_{18}O$: C, 90.47; H, 5.06. Found: C, 90.27; H, 5.27.

Compound **10f** (86%): 1-benzoyl-3-(4-dibenzofuryl)azulene, purple plates, mp 204–205 °C. ^{1}H NMR ($CDCl_3$) δ=9.85 (d, J=9.9 Hz, 1H), 8.63 (d, J=9.9 Hz, 1H), 8.43 (s, 1H), 8.01 (d, J=7.7 Hz, 1H), 7.99 (d, J=7.5 Hz, 1H), 7.96 (d, J=7.5 Hz, 2H), 7.89 (t, J=9.9 Hz, 1H), 7.69 (t, J=9.9 Hz, 1H), 7.62 (d, J=7.5 Hz, 1H), 7.56 (t, J=7.5 Hz, 1H), 7.52–7.46 (m, 5H), 7.44 (t, J=7.5 Hz, 1H), 7.37 (t, J=7.5 Hz, 1H) ppm; ^{13}C NMR ($CDCl_3$) δ=192.8, 156.1, 154.0, 143.6, 142.6, 141.23, 141.16, 140.3, 139.7, 137.7, 131.3, 129.8, 129.5, 128.7, 128.1, 127.8, 127.3, 124.8, 124.7, 124.3, 124.2, 123.1, 120.9, 120.8, 119.5 ppm; UV–vis (CH_2Cl_2) λ_{max}=242 (log ε=4.60), 252sh (4.56), 291 (4.58), 313 (4.47), 394sh (3.90), 405 (3.92), 516sh (2.49), 550 (2.57), 599sh (2.42) nm; IR (KBr) ν_{max}=3050w, 2369w, 2344w, 1617s, 1575m, 1525m, 1449s, 1418s, 1388s, 1366s, 1307m, 1240s, 1185s, 1128m, 1018m, 940m, 840s, 814s, 748s, 710s, 651s, 611m, 563m cm^{-1}; MS (70 eV) *m*/*z* (rel int): 399 (M^++1, 32), 398 (M^+, 100), 322 (14), 321 (55), 292 (29), 265 (11), 263 (24) 237 (5), 199 (4), 161 (4), 132 (4), 105 (4), 77 (10). Anal. Calcd for $C_{19}H_{18}O_2$: C, 87.42; H, 4.55. Found: C, 87.56; H, 4.73.

Compound **10g** (83%): 1-benzoyl-3-(4-dibenzothienyl)azulene, purple powder, mp 194–201 °C. ^{1}H NMR ($CDCl_3$) δ=9.87 (d, J=10.0 Hz, 1H), 8.54 (d, J=10.0 Hz, 1H), 8.41 (s, 1H), 8.21 (dm, J=7.6 Hz, 2H), 7.95 (dm, J=7.2 Hz, 2H), 7.91 (t, J=10.0 Hz, 1H), 7.80 (dm, J=8.0 Hz, 1H), 7.71 (t, J=10.0 Hz, 1H), 7.63–7.44 (m, 8H) ppm; ^{13}C NMR ($CDCl_3$) δ=192.9, 142.5, 142.4, 141.1, 140.1, 140.9, 140.5, 139.9, 139.5, 137.6, 136.2, 135.9, 131.4, 131.3, 129.7, 129.6, 128.8, 128.5, 128.2, 127.9, 126.9, 124.8, 124.5, 124.1, 122.7, 121.8, 120.5 ppm; UV–vis (CH_2Cl_2) λ_{max}=239 (log ε=4.66), 257sh (4.50), 282sh (4.45), 291 (4.47), 305sh (4.44), 392sh (3.90), 403 (3.92), 547 (2.65), 605sh (2.44) nm; IR (KBr) ν_{max}=3048w, 1617s, 1589m, 1574m, 1523m, 1509w, 1499w, 1480w, 1444s, 1420s, 1397s, 1358s, 1298m, 1241s, 1210m, 1166m, 1131m, 1099w, 1018m, 937m, 845m, 822m, 796m, 752s, 717m, 703m, 676m, 651m, 619m, 578w, 536w, 433w cm^{-1}; MS (70 eV) m/z (rel int): 415 (M^++1, 32), 414 (M^+, 100), 338 (13), 337 (50), 309 (12), 308 (41), 306 (8), 276 (4), 207 (6), 169 (7), 154 (8), 77 (4). Anal. Calcd for $C_{29}H_{18}OS$: C, 84.03; H, 4.38. Found: C, 84.06; H, 4.55.

4.4. The Suzuki–Miyaura cross-coupling reactions of 1,3-dihaloazulenes (6)

To a solution of 0.500 mmol of 1,3-dihaloazulene in 10 ml of toluene were added 1.50 mmol of arylboronic acid, 15.6 mg (0.025 mmol) of BINAP, 20.4 mg (0.025 mmol) of Pd(dppf)$Cl_2\cdot CH_2Cl_2$, and 489 mg (1.50 mmol) of Cs_2CO_3. This flask was evacuated and refilled with argon five times. The mixture was heated at 110 °C for several hours and then the resulting reaction mixture was poured into water and extracted with ether (30 ml×3). The combined organic layer was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by chromatography to give a coupling product.

Compound **4** (90%): 1,3-diphenylazulene, green needles, mp 114–116 °C (lit. 100–102 °C;[14] 117–118 °C[41]). ^{1}H NMR ($CDCl_3$) δ=8.55 (d, J=9.8 Hz, 2H), 8.12 (s, 1H), 7.65 (d, J=7.2 Hz, 4H), 7.58 (t, J=9.8 Hz, 1H), 7.51 (t, J=7.2 Hz, 4H), 7.37 (tm, J=7.4 Hz, 2H), 7.12 (t, J=9.8 Hz, 2H) ppm.

Compound **11a** (35%): 1,3-bis(4-biphenylyl)azulene, green needles, mp 243–245 °C. ^{1}H NMR ($CDCl_3$) δ=8.62 (d, J=9.8 Hz, 2H), 8.21 (s, 1H), 7.72 (m, 12H), 7.61 (t, J=9.9 Hz, 1H), 7.49 (t, J=7.4 Hz, 4H), 7.38 (t, J=7.4 Hz, 2H), 7.16 (t, J=9.8 Hz, 2H) ppm; ^{13}C NMR ($CDCl_3$) δ=140.9, 139.3, 136.9, 139.1, 137.1, 136.3, 130.2, 128.8, 127.6, 127.4, 127.3, 127.1, 123.7 ppm; IR (KBr) ν_{max}=3033m, 2928w, 1594m, 1578s, 1570s, 1561s, 1544s, 1525m, 1509m, 1498w, 1482s, 1459m, 1425m, 1399m, 1365m, 1224w, 1076w, 1004m, 938w, 872w, 845s, 765s, 739s, 724s, 691s, 576m, 488w, 461w cm^{-1}; UV–vis (CH_2Cl_2) λ_{max}=228 (log ε=4.41), 240sh (4.37), 269sh (4.41), 315 (4.73), 388 (4.05), 483 (1.00), 571sh (1.39), 622 (1.51), 680sh (1.39), 762sh (0.81) nm; MS (70 eV) m/z (rel int): 433 (M^++1, 38), 432 (M^+, 100), 430 (1), 415 (1), 352 (3), 339 (2), 326 (2), 276 (5), 252 (2), 216 (9), 202 (1), 152 (1), 77 (1). Anal. Calcd for $C_{34}H_{24}$: C, 94.35; H, 5.72. Found: C, 94.41; H, 5.59.

Compound **11b** (75%): 1,3-bis(1-naphthyl)azulene, blue solids, mp 66–69 °C. ^{1}H NMR (DMSO-d_6 at 100 °C)[42] δ=8.17 (s, 1H), 8.14 (d, J=9.8 Hz, 2H), 7.90–8.05 (m, 4H), 7.78 (d, J=8.0 Hz, 2H), 7.70 (t, J=9.8 Hz, 1H), 7.65–7.70 (m, 4H), 7.54 (d, J=8.0 Hz, 2H), 7.45 (t, J=8.0 Hz, 2H), 7.19 (t, J=9.8 Hz, 2H) ppm; ^{13}C NMR (DMSO-d_6 at 100 °C) δ=139.4, 138.7, 137.2, 135.6, 133.8, 133.2, 132.2, 128.2, 127.8, 127.3, 126.9, 125.7, 125.3, 125.0, 123.2 ppm; UV–vis (CH_2Cl_2) λ_{max}=742sh (log ε=2.33), 662sh (2.87), 608 (2.96), 553sh (2.78), 384sh (3.37), 352sh (3.68), 311sh (3.93), 293 (4.06), 279sh (3.99), 268 (3.97), 229 (4.32) nm; IR (KBr) ν_{max}=3040m, 2949m, 2923m, 2852m, 1569s, 1543m, 1524w, 1507m, 1459m, 1437m, 1379m, 1354m, 1318w, 1261m, 1230w, 1158w, 1112m, 1009m, 967w, 947w, 925w, 801s, 776s, 741s, 647m, 579w, 553w cm^{-1}; MS (70 eV) m/z (rel int): 381 (M^++1, 34), 380 (M^+, 100), 379 (13), 378 (9), 377 (12), 376 (13), 363 (9), 253 (6), 252 (15), 188 (7), 182 (7), 149 (3), 111 (3), 97 (3), 69 (4). Anal. Calcd for $C_{30}H_{20}\cdot 1/3CH_2Cl_2$: C, 89.12; H, 5.10. Found: C, 89.21; H, 5.10.[43]

Compound **11c** (23%): 1,3-bis[4-(*N*,*N*-dimethylamino)phenyl]azulene, green microcrystals, mp 152–154 °C. ^{1}H NMR ($CDCl_3$) δ=8.44 (d, J=9.8 Hz, 2H), 8.02 (s, 1H), 7.53 (t, J=9.8 Hz, 1H), 7.50 (dm, J=8.6 Hz, 4H), 7.03 (t, J=9.8 Hz, 2H), 6.91 (dm, J=8.6 Hz, 4H), 3.00 (s, 12H, CH_3) ppm; ^{13}C NMR ($CDCl_3$) δ=150.5, 139.7, 137.0, 136.8, 136.7, 131.7, 131.1, 126.1, 123.1, 113.7, 40.7 ppm; IR (KBr) ν_{max}=3021w, 2916m, 2882m, 2848m, 2798m, 1610s, 1562m, 1533s, 1502s, 1477m, 1443m, 1383m, 1353s, 1225s, 1195m, 1167m, 1125m, 1062m, 946s, 867m, 820s, 737s, 724s, 572m, 553m, 536m cm^{-1}; UV–vis (CH_2Cl_2) λ_{max}=236 (log ε=4.42), 279 (4.54), 312 (4.78), 384 (4.23), 657 (2.51) nm; MS (70 eV) m/z (rel int): 366 (M^+, 100), 350 (21), 183 (11). Anal. Calcd for $C_{26}H_{26}N_2$: C, 85.21; H, 7.15; N, 7.64. Found: C, 85.45; H, 7.24; N, 7.70.

Compound **11d** (80%): 1,3-bis[4-(*N*,*N*-diphenylamino)phenyl]azulene, green needles, mp 279–281 °C. ^{1}H NMR ($CDCl_3$) δ=8.53 (d, J=10.0 Hz, 2H), 8.09 (s, 1H), 7.54 (t, J=10.0 Hz, 1H), 7.51 (d, J=8.8 Hz, 4H), 7.29 (t, J=8.0 Hz, 8H), 7.21 (d, J=8.8 Hz, 4H), 7.19 (d J=8.0 Hz, 8H), 7.07 (t, J=10.0 Hz, 2H), 7.04 (t, J=8.0 Hz, 4H) ppm; ^{13}C NMR ($CDCl_3$) δ=147.8, 146.3, 138.9, 136.8, 136.5, 136.1, 131.3, 130.4, 130.2, 129.3, 124.4, 124.1, 123.1, 122.8 ppm; IR (KBr) ν_{max}=3034m, 3020m, 2956m, 2924s, 2852s, 1589s, 1570m, 1561m, 1543m, 1524m, 1509m, 1491s, 1459s, 1449m, 1439m, 1421m, 1408m, 1377m, 1364m, 1314m, 1271s, 1218m, 1174m, 1153m, 1109m, 1074m, 1027m, 936w, 896w, 871w, 840m, 749s, 694s, 670m, 619m, 573w, 548w, 528w, 511m, 497m cm^{-1}; UV–vis (CH_2Cl_2) λ_{max}=230 (log ε=4.53), 244sh (4.51), 303 (4.71), 330 (4.71), 347sh (4.63), 395sh (4.63), 577sh (2.32), 636 (2.45), 698sh (2.33) nm; MS (70 eV) m/z (rel int): 614 (M^+, 100), 307 (15), 167 (13). Anal. Calcd for $C_{46}H_{34}N_2\cdot 0.8H_2O$: C, 87.81; H, 5.70; N, 4.45. Found: C, 87.76; H, 5.63; N, 4.40.

Compound **11e** (24%): 1,3-bis[4-(9-carbazolyl)phenyl]azulene, green needles, mp 261–263 °C; ^{1}H NMR ($CDCl_3$) δ=8.73 (d, J=9.8 Hz, 2H), 8.32 (s, 1H), 8.19 (d, J=7.7 Hz), 7.92 (d, J=8.1 Hz, 4H), 7.75 (d, J=8.1 Hz, 4H), 7.70 (t, J=9.8 Hz, 1H), 7.58 (d, J=7.7 Hz, 4H), 7.47

(tm, J=7.7 Hz, 4H), 7.33 (tm, J=7.7 Hz, 4H), 7.25 (t, J=9.8 Hz, 2H) ppm; ^{13}C NMR ($CDCl_3$) δ=141.0, 139.5, 137.2, 137.0, 136.4, 136.2, 136.1, 131.0, 129.7, 127.3, 126.0, 124.1, 123.4, 120.3, 120.0, 109.9 ppm; IR (KBr) ν_{max}=3036m, 1687m, 1673m, 1655m, 1638m, 1625m, 1596m, 1578m, 1561m, 1543m, 1524s, 1508s, 1499s, 1477s, 1449s, 1422s, 1377m, 1361s, 1313s, 1227s, 1169m, 1146m, 1106m, 1031m, 936m, 914m, 835s, 771w, 744s, 719s, 649m, 632m, 566m, 530m, 518m, 437m, 421m cm^{-1}; UV–vis (CH_2Cl_2) λ_{max}=242 (log ε=4.92), 288sh (4.68), 294 (4.78), 316 (4.74), 337sh (4.49), 377 (4.05), 390sh (4.01), 407 (3.25), 462 (2.79), 584sh (2.15), 617 (2.27), 653sh (2.10) nm; MS (70 eV) m/z (rel int): 610 (M^+, 100), 305 (13), 276 (12), 241 (10), 166 (12). Anal. Calcd for $C_{46}H_{30}N_2 \cdot 1.4H_2O$: C, 86.86; H, 5.20; N, 4.40. Found: C, 86.99; H, 5.08; N, 4.37.

Compound **11f** (53%): 1,3-bis(4-bromophenyl)azulene,[44] green needles, mp 192–194 °C. ^{1}H NMR ($CDCl_3$) δ=8.48 (d, J=9.8 Hz, 2H), 8.04 (s, 1H), 7.63 (d, J=8.4 Hz, 4H), 7.62 (t, J=9.8 Hz, 1H), 7.49 (d, J=8.4 Hz, 2H), 7.17 (t, J=9.8 Hz, 2H) ppm; ^{13}C NMR ($CDCl_3$) δ=139.4, 136.8, 136.7, 136.2, 135.9, 131.8, 131.3, 129.3, 124.1, 120.7 ppm; IR (KBr) ν_{max}=3040w, 2927m, 2846m, 1573s, 1561s, 1543s, 1525s, 1509m, 1498s, 1479s, 1459m, 1449m 1438m 1429m, 1409m, 1378m, 1362m, 1322m, 1293m, 1264m, 1219s, 1175m, 1138w, 1100m, 1074s, 1007s, 936m, 877w, 831s, 810s, 736s, 719m, 693w, 671w, 656w, 639w, 619w, 601w, 574m, 537m, 507m, 492w cm^{-1}; UV–vis (CH_2Cl_2) λ_{max}=228sh (log ε=4.24), 252 (4.33), 286sh (4.47), 304 (4.61), 324sh (4.37), 384 (3.92), 560sh (2.29), 614 (2.44), 637 (2.32), 7.49 (1.77) nm; MS (70 eV) m/z (rel int): 440 (M^+, 40), 438 (M^+, 100), 436 (M^+, 53), 278 (14), 276 (53), 274 (12), 202 (12), 138 (11). Anal. Calcd for $C_{22}H_{14}Br_2$: C, 60.31; H, 3.22. Found: C, 60.28; H, 3.34.

4.5. Alternative synthesis of 1,3-bis[4-(*N*,*N*-dimethylamino)phenyl]azulene (11c)

In a 20 ml pressure-resistant glass bottle was charged 100 mg (0.228 mmol) of **11f**, 8.0 mg (36 mmol) of palladium acetate, 20 mg (69 mmol) of (t-Bu)$_3$PHBF$_4$, 65.0 mg (0.676 mmol) of NaOBu-t, and 5 ml of toluene. The bottle was evacuated at −100 °C and 0.255 g (5.00 mmol) of dimethylamine, obtained from the aqueous solution by mixing with a NaOH solution, was introduced into the bottle at the same temperature. The resulting bottle was sealed and heated at 110 °C in an oil bath with occasional swirling for 7 days. The resulting reaction mixture was poured into water and extracted with ether (20 ml×3). The combined organic layer was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue was chromatographed on neutral alumina with a 1:1 mixture of hexane and chloroform as eluent to give 61.0 mg (73%) of **11c** as green microcrystals.

4.6. Alternative synthesis of 1,3-bis[4-(*N*,*N*-diphenylamino)phenyl]- and 1,3-bis[4-(9-carbazoylyl)phenyl]-azulenes (11d and e)

To a solution of 110 mg (0.250 mmol) of **11f** in 7 ml of toluene were added 1.7 mg (7.5 mmol) of palladium acetate, 4.4 mg (15 mmol) of (t-Bu)$_3$PHBF$_4$, 72.1 mg (0.750 mmol) of NaOBu-t, and 0.75 mmol of the amine. This flask was evacuated and refilled with argon five times and then refluxed at 110 °C for 7 h. Then the resulting reaction mixture was poured into water and extracted with ether (20 ml×3). The combined organic layer was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel to give the product.

4.7. Synthesis of *N*,*N*,*N*′,*N*′-tetrasubstituted 1,3-bis(4-aminophenylethynyl)azulenes (14a–c)

To a solution of 152 mg (0.400 mmol) of 1,3-diiodoazulene and 1.20 mmol of *N*,*N*-disubstituted 4-ethynylaniline in 15 ml of triethylamine were added 6.7 mg (0.010 mmol) of bis(triphenylphosphine)palladium dichloride, 11 mg (0.040 mmol) of triphenylphosphine, and 9.1 mg (0.048 mmol) of CuI. This flask was evacuated and refilled with argon five times and then refluxed at 50 °C for 5 h. Then the resulting reaction mixture was poured into water and extracted with ether (20 ml×3). The combined organic layer was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel to give the product.

Compound **14a** (48%): 1,3-bis{4-(dimethylamino)phenylethynyl}azulene, mp 204–206 °C. ^{1}H NMR ($CDCl_3$) δ=8.52 (d, J=9.7 Hz, 2H), 8.06 (s, 1H), 7.59 (t, J=9.7 Hz, 1H), 7.49 (d, J=9.0 Hz, 4H), 7.19 (t, J=9.7 Hz, 2H), 6.69 (d, J=9.0 Hz, 4H), 2.98 (s, 12H) ppm; ^{13}C NMR ($CDCl_3$) δ=149.9, 141.3, 140.8, 139.7, 136.9, 132.5, 124.7, 111.9, 111.4, 110.8, 95.0, 82.7, 40.3 ppm; IR (KBr) ν_{max}=3448w, 2882w, 2793w, 2182w, 1892w, 1870w, 1846w, 1831w, 1812w, 1802w, 1794w, 1774w, 1751w, 1719m, 1702m, 1686m, 1674w, 1655w, 1638m, 1607s, 1571m, 1561w, 1532s, 1499m, 1476w, 1459w, 1438s, 1408w, 1389w, 1368s, 1286m, 1231m, 1187s, 1064m, 1003w, 948m, 867w, 805s, 754w, 726s, 670w, 644w, 575w, 549m, 517m cm^{-1}; UV–vis (CH_2Cl_2) λ_{max}=250 (log ε=4.38), 309sh (4.65), 341 (4.77), 412 (4.22), 665 (2.49) nm; MS (70 eV) m/z (rel int): 414 (M^+, 100), 262 (18). Anal. Calcd for $C_{30}H_{26}N_2 \cdot 1/3H_2O$: C, 85.68; H, 6.39; N, 6.66. Found: C, 85.80; H, 6.34; N, 6.60.

Compound **14b** (50%): 1,3-bis{4-(diphenylamino)phenylethynyl}azulene, mp 152–153 °C. ^{1}H NMR ($CDCl_3$) δ=8.55 (d, J=9.7 Hz, 2H), 8.08 (s, 1H), 7.65 (t, J=9.7 Hz, 1H), 7.46 (d, J=8.8 Hz, 4H), 7.28 (t, J=8.0 Hz, 8H), 7.26 (t, J=9.7 Hz, 2H), 7.13 (d, J=8.0 Hz, 8H), 7.06 (t, J=8.0 Hz, 4H), 7.04 (d, J=8.8 Hz, 4H) ppm; ^{13}C NMR ($CDCl_3$) δ=147.6, 147.3, 141.6, 141.1, 139.9, 137.0, 132.3, 129.4, 125.3, 124.8, 123.4, 122.6, 116.9, 110.9, 94.2, 84.1 ppm; IR (KBr) ν_{max}=3448w, 3033m, 2189w, 1945w, 1924w, 1896w, 1870m, 1794w, 1774m, 1761w, 1719m, 1655m, 1638m, 1618w, 1587s, 1562w, 1543m, 1523w, 1509w, 1491s, 1459w, 1439w, 1389w, 1362w, 1315m, 1275s, 1175m, 1075w, 1028w, 893w, 861w, 834m, 752s, 728w, 695s, 646w, 618m, 575w, 511m, 475w cm^{-1}; UV–vis (CH_2Cl_2) λ_{max}=254 (log ε=4.48), 310 (4.68), 370 (4.84), 412sh (4.39), 650 (2.33) nm; MS (70 eV): m/z (rel int) 662 (M^+, 100), 331 (20). Anal. Calcd for $C_{50}H_{34}N_2$: C, 90.60; H, 5.17; N, 4.23. Found: C, 90.32; H, 5.34; N, 4.04.

Compound **14c** (72%): 1,3-bis(4-carbazolylphenylethynyl)-azulene, mp 214–216 °C. ^{1}H NMR ($CDCl_3$) δ=8.69 (d, J=9.8 Hz, 2H), 8.69 (d, J=9.8 Hz, 2H), 8.24 (s, 1H), 8.16 (dm, J=8.3 Hz, 4H), 7.87 (dm, J=8.3 Hz, 4H), 7.78 (t, J=9.8 Hz, 1H), 7.62 (dm, J=8.3 Hz, 4H), 7.58 (d, J=8.3 Hz, 4H), 7.44 (tm, J=8.3 Hz, 4H), 7.41 (t, J=9.8 Hz, 2H), 7.32 (t-like, J=8.3 Hz, 4H) ppm; ^{13}C NMR ($CDCl_3$) δ=142.2, 141.7, 140.2, 137.2, 137.2, 132.9, 126.9, 126.1, 126.0, 125.0, 123.6, 122.9, 120.4, 120.2, 110.5, 109.8, 93.6, 85.9 ppm; IR (KBr) ν_{max}=3048m, 3023m, 2927m, 2866m, 2193m, 1647w, 1637w, 1625m, 1598m, 1561m, 1524m, 1499s, 1477m, 1408m, 1390m, 1359s, 1334m, 1314m, 1293m, 1227s, 1182m, 1169m, 1148m, 1119m, 1104m, 1054w, 1028w, 1015m, 1003m, 929w, 914m, 883w, 825m, 772w, 746s, 722s, 669m, 658m, 624m, 574m, 565m, 537m, 491w, 459w, 445w, 435m, 421m cm^{-1}; UV–vis (CH_2Cl_2) λ_{max}=239 (log ε=5.03), 259sh (4.81), 284sh (4.61), 294 (4.72), 331sh (4.83), 345 (4.91), 406 (4.21), 431sh (4.14), 574sh (2.51), 629 (2.27), 695sh (2.10), 781sh (1.78) nm; MS (70 eV) m/z (rel int): 658 (M^+, 100), 329 (28). Anal. Calcd for $C_{50}H_{30}N_2 \cdot 0.8H_2O$: C, 89.21; H, 4.73; N, 4.16. Found: C, 89.17; H, 4.80; N, 3.87.

4.8. Cyclic voltammetry

A standard three-electrode cell configuration was employed using a glassy carbon disk working electrode, a Pt wire auxiliary electrode, and an Ag wire as an Ag/Ag$^+$ quasi-reference electrode. The reference electrode was calibrated at the completion of each measurement on a saturated calomel electrode (SCE). Cyclic voltammetry was measured in a dichloromethane solution for **11** and **14** with tetrabutylammonium perchlorate as a supporting electrolyte and a scan rate of 0.1 Vs^{-1} at 25 °C.

4.9. OLED fabrication

The OLED structures **A** and **B** employed in this study are shown in the inset of Figure 2.

Structure **A**: organic layers were fabricated by high-vacuum (10^{-7}–10^{-6} Torr) thermal evaporation onto a glass substrate precoated with an ITO layer with a sheet resistance of 10 Ω/square. Prior to use, the ITO was degreased with solvents and cleaned in an UV–ozone chamber before loading into the evaporation system. A 10 nm-thick CuPc, **11c**, **d**, or **e** as the HIL, a 50 nm-thick TPTE as the HTL, a 60 nm-thick Alq_3 as the light-emitting layer, a 0.5 nm-thick LiF as the electron-injecting layer, and a 150 nm-thick aluminum metal as a cathode were deposited on the substrate. Devices were encapsulated under nitrogen in a glass-to-glass epoxy sealed package.

Structure **B**: organic layers were fabricated by high-vacuum (10^{-7}–10^{-6} Torr) thermal evaporation onto a glass substrate precoated with an ITO layer with a sheet resistance of 10 Ω/square. Prior to use, the ITO was degreased with solvents and cleaned in an UV–ozone chamber before loading into the evaporation system. A 10 nm-thick CuPc, **11c**, **d**, or **e** as the HIL, a 30 nm-thick TPTE as the HTL, a 40 nm-thick Alq_3 with 1.2–1.5 wt% of DCJTB as the light-emitting layer, a 40 nm-thick Alq_3 and a 0.5 nm-thick LiF as the electron-injecting layer, and a 100 nm-thick aluminum metal as a cathode were deposited on the substrate. Devices were encapsulated under nitrogen in a glass-to-glass epoxy sealed package.

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43. It was found that this azulene derivative **11b** showed inclusion of various solvents in a solid state. Structural analysis of the solvent-included solids is now under investigation.
44. Recently, we reported the X-ray crystallographic analysis of **11f** and clarified the unusual out-of-plane deformation of the azulene ring, see: Ohta, A.; Thanh, N. C.; Terasawa, K.; Fujimori, K.; Kuroda, S.; Oda, M. *Tetrahedron Lett.* **2006**, *47*, 2815–2819.

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Asymmetric synthesis of (+)-cardiobutanolide

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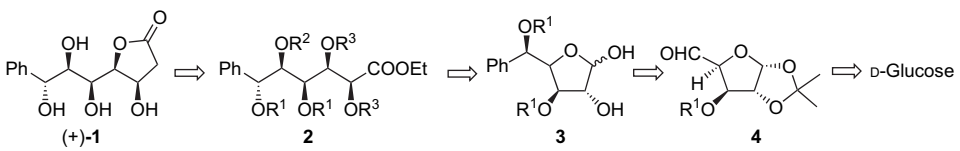
Abstract—A formal total synthesis of (+)-cardiobutanolide has been accomplished from D-glucose, a readily available precursor.

1. Introduction

(+)-Cardiobutanolide **1**, a γ-lactone was isolated in 2003 from *Goniothalamus cardiopetalus* of the family Annonaceae.[1] The related natural products isolated from the same tree have been used as a traditional medicine in Asia to treat rheumatism, edema, and as a mosquito repellent. Because of the structural complexity of having five contiguous chiral centers in the molecule and potential pharmacological activity, (+)-cardiobutanolide **1** has recently attracted the attention of synthetic organic chemists. Two syntheses of the molecule involving chiral starting materials have already appeared in the literature.[2] While this manuscript was in preparation, a third synthesis appeared employing a chiron strategy.[3] For the last several years, we were involved in the synthesis of bioactive natural products having lactone as a sub-unit using sugars as cheap starting materials.[4] In this paper, we report our approach for asymmetric synthesis of (+)-cardiobutanolide **1**.

The retrosynthetic analysis of (+)-**1** is shown in Scheme 1. It was conceived that the five-membered lactone ring can be constructed after Arndt–Eistert homologation of an ester **2**, which can be synthesized from a hemiacetal **3**. The requisite chirality at the 7-carbon of (+)-**1** in the side chain of **3** would be created by a diastereoselective addition of PhMgX to the corresponding aldehyde **4**, which in turn, could be derived from D-glucose. The chirality at the 5- and 6-carbon atoms in the natural product would be directly translated from D-glucose. The other two chiral centers at 3- and 4-carbon atoms would be created by Sharpless asymmetric dihydroxylation reaction.

The synthesis of (+)-cardiobutanolide **1** involved 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylopentodialdo-1,4-furanose **5**, which was readily synthesized from D-glucose using a literature procedure.[5] The diastereoselective addition of PhMgBr to the aldehyde **5** resulted in the unwanted chelation controlled product **6a** and the desired C-5 epimer **6b** in a ratio of 12:1 (Scheme 2). The diastereomeric ratio could be improved by subjecting the mixture to an oxidation (PDC)–reduction ($NaBH_4$) sequence to obtain the desired isomer **6b** in a ratio of 10:1, which could be separated by column chromatography resulting in the pure **6b**. After protecting the benzylic hydroxyl group as a benzyl ether, the acetonide of **7** was hydrolyzed with dilute sulfuric acid in dioxane to provide a hemiacetal whose diol was oxidatively cleaved with $NaIO_4$ to give an aldehyde **8** that was directly subjected to Wittig olefination reaction with a stabilized ylide ($Ph_3P{=}CHCO_2Et$). This gave an inseparable mixture of α,β-unsaturated ester **9** in favor of *E*-isomer (*E*/*Z* ratio=3:1). Our strategy required that the olefination product **9** should have trans geometry for the subsequent incorporation of chiral diol via Sharpless asymmetric dihydroxylation.



Scheme 1. Retrosynthetic analysis.

Keywords: Cardiobutanolide; Asymmetric dihydroxylation; Lactone; D-Glucose.
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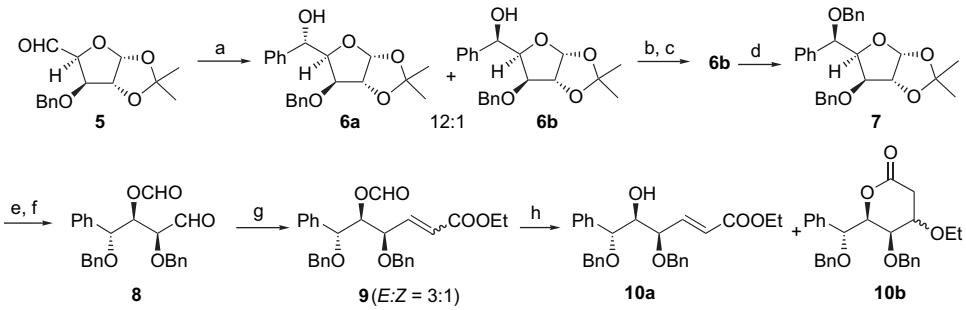


Scheme 2. Reagents and conditions: (a) PhMgBr, THF, 0 °C–rt, 30 min (90%); (b) PDC, CH_2Cl_2, 4 Å MS, catalytic amount of CH_3CO_2H, 6 h (82%); (c) $NaBH_4$, MeOH, 0 °C–rt, 4 h (98%), dr=10:1; (d) NaH, BnBr, TBAI, THF, 0 °C–rt, 2 h (98%); (e) 0.4% H_2SO_4, dioxane, 100 °C, 6 h (80%); (f) $NaIO_4$, H_2O/MeOH, 98%; (g) $Ph_3P{=}CHCO_2Et$, CH_2Cl_2, rt, 2 h, 98%; (h) K_2CO_3, EtOH, 0 °C–rt (96%).

Keeping this in mind, we envisaged that the cis-isomer of the α,β-unsaturated ester, on exposure to K_2CO_3/EtOH, would provide **10b** after deprotection of the formate ester and lactonization followed by 1,4-addition of an ethoxide group. Under the above basic conditions, the trans-isomer of **9**, after deprotection of the formyl group, would remain as hydroxyl ester **10a** as it would not lactonize because of the geometrical constraint.[6] This was indeed the case. The required *trans*-ester **10a** could be obtained in pure form using the above protocol (Scheme 2). The δ-hydroxyl group of **10a** was protected as the corresponding TIPS ether that was subjected to Sharpless asymmetric dihydroxylation using AD-mix β (Scheme 3). The desired diol **11** was obtained in 94% yield with a ratio of 4:1. The minor isomer could be separated after the formation of acetonide ester **12**. Conversion of **12** to **14** was carried out using the Arndt–Eistert homologation protocol.[7] Thus, the ester **12** was hydrolyzed with LiOH in aqueous methanol to obtain carboxylic acid that was converted into α-diazoketone **13** via reaction of diazomethane with the mixed anhydride. Wolff rearrangement of the **13** using silver benzoate afforded the homologated ester **14** in 68% yield. Exposure of the **14** to TFA/H_2O (10:1) resulted in deprotection of the TIPS ether as well as acetonide with in situ lactonization to give **15** as a clean product (Scheme 3). Since the conversion of **15** into the natural product (+)-**1** has recently been carried out,[3] we stopped our synthesis at this stage.

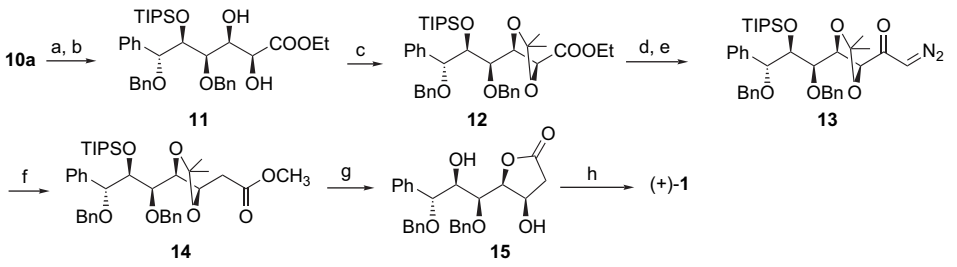
In conclusion, we have achieved a formal total synthesis of (+)-cardiobutanolide **1** from D-glucose, which is a readily available starting material. The key steps in the synthesis are Sharpless asymmetric dihydroxylation, Arndt–Eistert homologation, and lactonization reactions.

2. Experimental

2.1. General methods

Chemicals were purchased and used without further purification. ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-LA400 spectrometer. All chemical shifts are quoted on the δ scale, TMS as internal standard, and coupling constants are reported in hertz. Routine monitoring of reactions were performed by TLC, and using 0.2 mm Kieselgel 60 F_{254} precoated aluminum sheets, commercially available from Merck. Visualization was done by fluorescence quenching at 254 nm, by exposure to iodine vapor. All the column chromatographic separations were done by using silica gel (Acme's, 60–120 mesh). Petroleum ether used was of boiling range 60–80 °C. Reactions that needed anhydrous conditions were run under an atmosphere of nitrogen or argon using flame-dried glassware. The organic extracts were dried over *anhydrous* sodium sulfate. Evaporations of solvents were performed at reduced pressure. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under nitrogen. Dichloromethane was distilled from CaH_2. AD-mix β and $(DHQD)_2PHAL$ were purchased from Aldrich. $MeSO_2NH_2$, CSA, and 2,2-dimethoxypropane were obtained from Lancaster.

2.1.1. 3-*O*-Benzyl-1,2-*O*-isopropylidene-5-*C*-phenyl-α-L-idopentofuranose (6a and 6b). To a solution of α-D-pentodialdose **5** (8.89 g, 32.0 mmol) in *anhydrous* THF (160.0 mL), PhMgBr (48.0 mL, 1 M soln in THF, 48.0 mmol) was added under an N_2 atmosphere at 0 °C and stirred at room temperature for 12 h. The reaction mixture was quenched with saturated NH_4Cl and extracted with



Scheme 3. Reagents and conditions: (a) TIPSOTf, 2,6-lutidine, DCM, 0 °C–rt (98%); (b) AD-mix β, $(DHQD)_2PHAL$, $MeSO_2NH_2$, *t*-BuOH/H_2O, 0 °C, 24 h (94%), dr 4:1; (c) 2,2-dimethoxy propane, CSA, DCM, 0 °C–rt, 2 h (91%); (d) LiOH·H_2O, MeOH/H_2O (4:1) (91%); (e) Et_3N, ClCOOEt, CH_2N_2, THF, 0 °C–rt (60%); (f) PhCOOAg, Et_3N, MeOH, 2 h (68%); (g) TFA/H_2O (10:1), DCM, 24 h (98%); (h) Ref. 3.

ethyl acetate. The combined organic layers were washed with brine, dried over *anhydrous* Na_2SO_4, and concentrated. The residue was purified over silica gel to give a separable diastereomeric mixture (dr 12:1) of benzylic alcohol **6a** (9.45 g, 83%) and **6b** (0.80 g, 7%). Compound **6a**: IR (thin film, cm^{-1}) 3548, 3020; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.31 (s, 3H), 1.49 (s, 3H), 3.64 (d, J=2.9 Hz, 1H), 4.31 (dd, J=7.3, 4.1 Hz, 1H), 4.43 (ABq, J=11.5 Hz, $\Delta\nu$=98.1 Hz, 2H), 4.61 (d, J=3.7 Hz, 1H), 5.06 (d, J=7.3 Hz, 1H), 6.02 (d, 3.4 Hz, 1H), 7.26–7.41 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.2, 26.8, 71.8, 72.4, 82.1, 82.2, 84.5, 105.2, 111.9, 127.1, 127.7, 128.0, 128.1, 128.4, 128.6, 136.9, 139.6. MS (FAB) 357 (M^++1). Anal. Calcd for $C_{21}H_{24}O_5$: C, 70.77; H, 6.79; found: C, 70.71; H, 6.83.

2.1.2. 3-*O*-Benzyl-1,2-*O*-isopropylidene-5-*C*-phenyl-α-D-glucopentofuranose (6b). To a solution of alcohol **6a** and **6b** (5.36 g, 15.05 mmol) in dry DCM (90.0 mL), PDC (8.49 g, 22.58 mmol), powdered 4 Å MS (12.04 g), and glacial acetic acid (600 μL) were added. The reaction was exothermic, refluxing gently and the orange color of the solution turned to dark brown within 5 min. The reaction mixture was stirred for 6 h; Celite was added and again stirred for 10 min. The reaction mixture was filtered over silica gel under vacuo and washed with DCM. The organic layer was concentrated to yield the ketone (4.37 g, 82%). The ketone (4.37 g, 12.34 mmol) was dissolved in MeOH (40.0 mL) and cooled to 0 °C. To this cooled solution $NaBH_4$ (1.87 g, 49.39 mmol) was added portion wise and stirred for 4 h. The reaction mixture was quenched with saturated NH_4Cl and MeOH was removed on a rotavapor. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with water and brine and placed over *anhydrous* Na_2SO_4. The organic layer was concentrated in vacuo and chromatographed over silica gel to give a separable diastereomeric mixture (10:1) of alcohol **6b** (3.91 g, 89%) and **6a** (0.38 g, 9%) as a syrup. Compound **6b**: $[\alpha]_D^{25}$ −85.18 (c 1.35, $CHCl_3$); IR (thin film, cm^{-1}) 3550, 3045; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.31 (s, 3H), 1.46 (s, 3H), 4.00 (d, J=2.4 Hz, 1H), 4.32 (dd, J=5.9, 3.2 Hz, 1H), 4.57 (ABq, J=11.5 Hz, $\Delta\nu$=90.6 Hz, 2H), 4.62 (d, J=3.9 Hz, 1H), 5.10 (d, J=6.1 Hz, 1H), 6.03 (d, J=3.9 Hz, 1H), 7.26–7.39 (m, 10H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.1, 26.7, 71.9, 72.2, 81.6, 82.4, 82.7, 105.1, 111.6, 126.0, 127.6, 128.0, 128.4, 128.7, 136.6, 141.2. MS (FAB) 357 (M^++1). Anal. Calcd for $C_{21}H_{24}O_5$: C, 70.77; H, 6.79; found: C, 70.84; H, 6.71.

2.1.3. 3,5-Di-*O*-benzyl-1,2-*O*-isopropylidene-5-*C*-phenyl-α-D-glucopentofuranose (7). To a suspension of NaH (0.67 g, 16.8 mmol) in *anhydrous* THF (50.0 mL), alcohol **6b** (3.0 g, 8.4 mmol) in *anhydrous* THF (10.0 mL) was slowly added at 0 °C, stirred for 30 min, followed by addition of benzyl bromide (1.10 mL, 9.26 mmol) and a catalytic amount of TBAI. The resulting mixture was stirred at room temperature for 2 h, quenched with NH_4Cl, and extracted with ether. The organic layer was washed with brine, dried over *anhydrous* Na_2SO_4, and concentrated in vacuo. The residue was purified over silica gel to give the benzyl ether **7** (3.68 g, 98%) as a white solid. Mp 68–69 °C; $[\alpha]_D^{25}$ −60.00 (c 0.80, $CHCl_3$); IR (thin film, cm^{-1}) 3025, 1240; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.26 (s, 3H), 1.40 (s, 3H), 4.23 (m, 2H), 4.36 (m, 2H), 4.59 (m, 2H), 4.72 (m, 2H), 5.85 (d, J=3.7 Hz, 1H), 7.20–7.46 (m, 15H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.3, 26.7, 53.4, 70.3, 72.4, 78.2, 81.8, 82.1, 82.9, 105.0, 111.5, 127.5, 127.6, 127.8, 128.1, 128.3, 128.4, 137.7, 138.2, 139.3. MS (FAB) 447 (M^++1). Anal. Calcd for $C_{28}H_{30}O_5$: C, 75.31; H, 6.77; found: C, 75.23; H, 6.85.

2.1.4. (2*S*,3*R*,4*R*)-2,4-Bis-(benzyloxy)-3-formyloxy-4-phenyl-1-butanal (8). A solution of the acetonide **7** (9.0 g, 20.17 mmol) in dioxane (60.0 mL) was treated with 0.4% H_2SO_4 (21.0 mL) at 100 °C for 20 h. The reaction mixture was quenched with solid Na_2CO_3 and concentrated. The residue was dissolved in ethyl acetate, washed with water and brine, and dried over Na_2SO_4. The organic layer was concentrated and chromatographed over silica gel to give (6.55 g, 80%) hemiacetal as a white solid. The hemiacetal (4.3 g, 10.6 mmol) was dissolved in MeOH (305.0 mL) and treated with 0.6 N $NaIO_4$ aqueous solution (308.0 mL). The reaction mixture was stirred at room temperature for 1 h and then concentrated on rotavapor. The residue was diluted with water and extracted with DCM. The combined organic layers were washed with brine and dried over *anhydrous* Na_2SO_4. The solvent was removed in vacuo and the residue was chromatographed over silica gel to give formido aldehyde **8** (4.2 g, 98%) as an oily liquid; $[\alpha]_D^{25}$ −8.52 (c 0.68, $CHCl_3$); IR (thin film, cm^{-1}) 3040, 1720; ^1H NMR ($CDCl_3$, 400 MHz): δ 4.11 (d, J=10.9 Hz, 1H), 4.38 (d, J=11.2 Hz, 1H), 4.47 (d, J=2.7 Hz, 1H), 4.62 (ABq, J=11.7 Hz, $\Delta\nu$=84.6 Hz, 2H), 4.65 (d, J=9.0 Hz, 1H), 5.55 (dd, J=9.0, 2.5 Hz, 1H), 7.17–7.40 (m, 15H), 7.62 (s, 1H), 9.58 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 53.4, 70.5, 73.2, 73.7, 77.9, 82.1, 127.9, 128.0, 128.1, 128.33, 128.39, 128.4, 128.5, 128.6, 128.8, 137.2, 158.9, 199.8. MS (FAB) 405 (M^++1). Anal. Calcd for $C_{25}H_{24}O_5$: C, 74.24; H, 5.98; found: C, 74.31; H, 5.92.

2.1.5. (4*R*,5*R*,6*R*)-Ethyl-4,6-bis-(benzyloxy)-5-(formidohydroxy)-5-phenyl-(2*E*/*Z*)-hexenoate (9). To a solution of formido aldehyde **8** (203.0 mg, 0.5 mmol) in dry DCM (3.0 mL), ethoxycarbonylmethylenetriphenylphosphorane (348 g, 1.0 mmol) was added and stirred at room temperature for 2 h. The reaction mixture was quenched with water and extracted with DCM. The organic layer was washed with water and brine and dried over *anhydrous* Na_2SO_4. The solvent was removed under vacuo and purified over silica gel to give an inseparable mixture (*E*/*Z* 3:1) of *trans*- and *cis*-enoate **9** (238 mg, 98%). IR (thin film, cm^{-1}) 3048, 1645; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.24 (t, J=7.3 Hz, 0.75H), 1.27 (t, J=7.3 Hz, 2.75H), 4.09 (d, J=11.2 Hz, 1H), 4.14–4.21 (m, 2H), 4.35 (dd, J=11.5, 2.7 Hz, 2H), 4.55–4.73 (m, 3H), 5.30 (dd, J=8.8, 1.5 Hz, 0.75H), 5.44 (dd, J=9.0, 1.7 Hz, 0.25H), 5.68 (d, J=8.6 Hz, 0.25H), 5.91 (dd, J=11.7, 1.2 Hz, 0.25H), 6.11 (dd, J=15.8, 1.4 Hz, 0.75H), 6.79 (dd, J=15.8, 5.8 Hz, 0.75H), 7.15–7.14 (m, 15H), 7.65 (d, J=2.7 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1, 53.4, 60.6, 70.6, 72.1, 75.2, 75.9, 78.4, 123.9, 127.7, 127.8, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 137.2, 137.5, 143.7, 159.0, 165.6. MS (FAB) 475 (M^++1). Anal. Calcd for $C_{29}H_{30}O_6$: C, 73.40; H, 6.37; found: C, 73.35; H, 6.47.

2.1.6. (4*R*,5*R*,6*R*)-Ethyl-4,6-bis-(benzyloxy)-5-(hydroxy)-5-phenyl-(2*E*)-hexenoate (10a) and (5*R*,6*R*,7*R*)-4-ethoxy-5,7-bis-(benzyloxy)-6-(1-phenylmethyl)-3,4,5,6-tetrahydro-4*H*-pyran-2-one (10b). The mixture of α,β-unsaturated

ester **9** (6.8 g, 14.3 mmol) was dissolved in EtOH (45.0 mL) and cooled to 0 °C. To this cooled solution, K_2CO_3 (2.96 g, 21.42 mmol) was added and stirred at room temperature for 1 h. The reaction mixture was quenched with 2 N HCl at 0 °C and stirred for 2 h. The aqueous layer was extracted with ethyl acetate, washed with brine, and placed over *anhydrous* Na_2SO_4. The solvent was removed under vacuo and chromatographed to give the *trans*-ester **10a** (4.78 g, 72%) and the corresponding lactone **10b** (1.51 g, 23%). Compound **10a**: $[\alpha]_D^{25}$ +56.18 (*c* 2.62, $CHCl_3$); IR (thin film, cm^{-1}) 3452, 1740; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.28 (t, *J*=7.1 Hz, 3H), 3.71 (dd, *J*=8.3, 2.2 Hz, 1H), 4.08 (d, *J*=11.2 Hz, 1H), 4.19 (q, *J*=7.1 Hz, 2H), 4.33 (dd, *J*=15.1, 11.2 Hz, 2H), 4.41 (d, *J*=8.3 Hz, 1H), 4.46 (dd, *J*=6.4, 1.2 Hz, 1H), 4.63 (d, *J*=11.5 Hz, 1H), 6.09 (dd, *J*=15.9, 1.2 Hz, 1H), 6.97 (dd, *J*=15.8, 6.6 Hz, 1H), 7.17 (d, *J*=7.3 Hz, 1H), 7.23–7.39 (m, 15H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.2, 60.5, 70.4, 71.7, 76.2, 76.8, 80.9, 123.5, 127.7, 127.91, 127.96, 128.0, 128.2, 128.3, 128.4, 128.5, 137.5, 137.8, 138.8, 145.4, 165.9. MS (FAB) 447 (M^++1). Anal. Calcd for $C_{28}H_{30}O_5$: C, 75.31; H, 6.77; found: C, 75.25; H, 6.84. Compound **10b**: $[\alpha]_D^{25}$ −8.00 (*c* 0.60, $CHCl_3$); IR (thin film, cm^{-1}) 3455, 1758; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.14 (t, *J*=6.8 Hz, 3H), 2.56 (dd, *J*=17.8, 2.4 Hz, 1H), 2.84 (dd, *J*=17.8, 4.9 Hz, 1H), 3.40–3.50 (m, 2H), 3.77 (ABq, *J*=4.9 Hz, $\Delta\nu$=6.6 Hz, 1H), 4.19 (d, *J*=4.4 Hz, 1H), 4.71 (d, *J*=8.6 Hz, 1H), 4.42 (d, *J*=11.2 Hz, 1H), 4.60–4.73 (m, 4H), 7.24–7.48 (m, 15H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 15.2, 32.6, 60.2, 64.5, 70.2, 71.3, 71.7, 72.6, 78.0, 80.2, 126.8, 127.5, 127.6, 127.7, 127.8, 128.1, 128.2, 128.3, 128.4, 137.6, 137.7, 138.2, 168.8. MS (FAB) 447 (M^++1). Anal. Calcd for $C_{28}H_{30}O_5$: C, 75.31; H, 6.77; found: C, 75.42; H, 6.65.

2.1.7. (4*R*,5*R*,6*R*)-Ethyl-4,6-bis(benzyloxy)-2,3-dihydroxy-6-phenyl-5-(triisopropylsilyloxy)-hexanoate (11). To a solution of alcohol **10a** (564 mg, 1.14 mmol) in dry DCM (5.0 mL), 2,6-lutidine (400 μL, 3.43 mmol) was added at 0 °C. The solution was stirred for 30 min, and then TIPSOTf was added and was further stirred for 12 h at room temperature. The reaction mixture was quenched with water and diluted with DCM. The organic layer was washed with water and brine and dried over *anhydrous* Na_2SO_4. The organic layer was concentrated in vacuo. The residue was chromatographed on silica gel to give the TIPS ether (695 mg, 98%). After that to a 1:1 solution of *t*-BuOH and H_2O (10.0 mL), AD-mix β (4.12 g) was added and stirred for 5 min. To this stirred solution $(DHQD)_2PHAL$ (103.0 mg) and $MeSO_2NH_2$ (172.0 mg) was added at room temperature. The reaction mixture was cooled to 0 °C, TIPS ether (1.03 g, 1.93 mmol) was added, and was further stirred at same temperature for 24 h. The reaction mixture was quenched with Na_2SO_3. After 30 min EtOAc was added and organic phase was separated. The combined organic layer was washed twice with 1 M $KHSO_4$ solution, 5% $NaHCO_3$, brine and dried over *anhydrous* Na_2SO_4. The organic layer was evaporated in vacuo and residue was purified on silica gel to give a inseparable mixture of diol **11** (1.12 g, 92%). ^1H NMR ($CDCl_3$, 400 MHz): δ 0.91 (m, 3H), 1.01 (m, 18H), 1.22 (t, *J*=17.3 Hz, 3H), 2.96 (d, *J*=8.5 Hz, 1H), 3.32 (d, *J*=2.9 Hz, 1H), 3.87 (dd, *J*=9.8, 3.4 Hz, 1H), 4.17–4.34 (m, 4H), 4.51 (m, 3H), 4.73 (d, *J*=4.6 Hz, 1H), 7.09–7.47 (m, 15H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 12.6, 12.8, 14.1, 18.0, 18.1, 61.5, 70.1, 70.8, 73.0, 73.1, 76.0, 76.7, 82.7, 127.5, 127.6, 127.8, 127.9, 128.0, 128.1, 128.2, 128.7, 138.1, 138.2, 138.5, 173.8. MS (FAB) 638 (M^++1). Anal. Calcd for $C_{37}H_{52}O_7Si$: C, 69.78; H, 8.23; found: C, 69.69; H, 8.33.

2.1.8. (4*S*,5*R*)-Ethyl-5-((1*R*,2*R*,3*R*)-1,3-bis(benzyloxy)-3-phenyl-2-(triisopropylsilyloxy)propyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylate (12). To the solution of diol **11** (2.34 g, 3.58 mmol) in DCM (12.0 mL), 2,2-dimethoxy propane (4.4 mL, 35.78 mmol) and catalytic amount of CSA were added at 0 °C. The reaction mixture was stirred at room temperature for 2 h under nitrogen atmosphere, quenched with water, and extracted with DCM. The combined organic layer was washed with brine, dried over *anhydrous* Na_2SO_4, and concentrated in vacuo. The residue was purified on column chromatography to give ester **12** (2.26 g, 91%) as a separable mixture in a ratio of 4:1. $[\alpha]_D^{25}$ −25.61 (*c* 2.15, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 0.77 (m, 3H), 0.97 (d, *J*=7.3 Hz, 18H), 1.08 (m, 3H), 1.47 (d, *J*=9.1 Hz, 6H), 3.96 (m, 2H), 4.14 (m, 2H), 4.34 (m, 2H), 4.50 (m, 3H), 4.68 (s, 2H), 7.27–7.44 (m, 15H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.4, 13.8, 18.1, 18.3, 25.9, 27.1, 61.2, 70.3, 73.4, 75.8, 76.7, 78.1, 78.2, 79.8, 87.8, 111.4, 126.7, 126.9, 127.4, 127.8, 127.9, 128.1, 128.7, 138.2, 139.1, 139.3, 171.2. MS (FAB) 678 (M^++1). Anal. Calcd for $C_{40}H_{56}O_7Si$: C, 70.97; H, 8.34; found: C, 70.87; H, 8.43.

2.1.9. 1-[5-(1,3-Bis-benzyloxy-3-phenyl-2-triisopropylsilanyloxy-propyl)-2,2-dimethyl-[1,3]dioxolan-4-yl]-2-diazo-ethanone (13). To a ice cooled solution of **12** (300 mg, 0.43 mmol) in methanol/water (5.0 mL, 4:1) was added lithium hydroxide monohydrate (108.7 mg, 2.59 mmol) at 0 °C. The mixture was brought to 25 °C and was further stirred for 2 h. The pH of the solution was adjusted to 7.0 by addition of aqueous NH_4Cl, the solvent was evaporated and the residue so obtained was extracted with chloroform to give an acid. To this acid (730.0 mg, 1.09 mmol) in THF (8.0 mL) at 0 °C, triethylamine (451.0 μL, 3.29 mmol) and ethylchloroformate (204.3 μL, 2.18 mmol) were added one after the other. After 15 min, the reaction mixture was brought to room temperature for 30 min and was filtered over Celite. To this filtrate a freshly prepared solution of diazomethane in diethyl ether [(prepared from *N*-nitrosomethyl urea (1.10 g) and KOH (2.0 g)] was added dropwise over a period of 30 min. The mixture was stirred for 1.5 h at room temperature. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography to give **13** (453.7 mg, 60%). $[\alpha]_D^{25}$ −21.96 (*c* 2.85, $CHCl_3$); IR (thin film, cm^{-1}) 3371, 3031, 2107, 1637, 1071; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.77 (m, 3H), 0.93 (d, *J*=7.3 Hz, 18H), 1.26 (d, *J*=10.9 Hz, 6H), 3.83 (m, 1H), 4.19 (m, 1H), 4.35 (m, 3H), 4.51 (m, 2H), 4.67 (ABq, *J*=11.7 Hz, $\Delta\nu$=41.0 Hz, 2H), 7.18–7.41 (m, 15H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.3, 18.1, 18.3, 26.0, 26.9, 53.1, 70.5, 73.7, 75.4, 78.0, 79.9, 81.3, 83.1, 110.7, 127.1, 127.2, 127.3, 127.6, 127.8, 128.0, 128.09, 128.13, 128.6, 138.3, 138.9, 139.2, 193.8. MS (FAB) 674 (M^++1). Anal. Calcd for $C_{39}H_{52}N_2O_6Si$: C, 69.61; H, 7.79; found: C, 69.72; H, 7.65.

2.1.10. Methyl 2-((4*R*,5*S*)-5-((1*R*,2*R*,3*R*)-1,3-bis(benzyloxy)-3-phenyl-2-(triisopropylsilyloxy)propyl)-2,2-dimethyl-1,3-dioxolan-4-yl)acetate (14). To a solution of α-diazoketone **13** (316.0 mg, 0.45 mmol) in anhydrous MeOH (6.0 mL) was added, dropwise, a solution of silver benzoate (38.0 mg, 0.14 mmol) in triethylamine (731 μL) under dry N_2 at −10 °C. The reaction mixture was further stirred for 1.5 h. The solvent was evaporated and the residue was purified by column chromatography to give **14** (217 mg, 68%) as a syrupy liquid. $[\alpha]_D^{25}$ −2.79 (*c* 3.25, $CHCl_3$); IR (thin film, cm^{-1}) 3030, 2928, 1743, 1071; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.72 (m, 3H), 0.96 (d, *J*=7.3 Hz, 18H), 1.29 (m, 6H), 2.41 (dd, *J*=15.8, 9.5 Hz, 1H), 2.72 (dd, *J*=15.8, 2.2 Hz, 1H), 3.62 (s, 3H), 3.80 (t, *J*=9.0 Hz, 1H), 3.95 (dd, *J*=9.3, 1.5 Hz, 1H), 4.15 (d, *J*=11.5 Hz, 1H), 4.31 (m, 3H), 4.45 (d, *J*=7.3 Hz, 1H), 4.60 (ABq, *J*=11.9 Hz, Δν=20.6 Hz, 2H), 7.24–7.43 (m, 15H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.4, 13.8, 18.1, 18.3, 25.9, 27.1, 61.2, 70.3, 73.4, 75.8, 76.7, 78.1, 78.2, 79.8, 87.8, 111.4, 126.7, 126.9, 127.4, 127.8, 127.9, 128.1, 128.7, 138.2, 139.1, 139.3, 171.2. MS (FAB) 678 (M^++1). Anal. Calcd for $C_{40}H_{56}O_7Si$: C, 70.97; H, 8.34; found: C, 70.92; H, 8.41.

2.1.11. 5-(1,3-Bis-benzyloxy-2-hydroxy-3-phenyl-propyl)-4-hydroxy-dihydro-furan-2-one (15). To a stirred solution of **14** (103 mg, 0.15 mmol) in CH_2Cl_2 (2.0 mL) was added a mixture of water (1.0 mL) and TFA (0.1 mL). The reaction mixture was stirred for 24 h. The reaction mixture was quenched with solid $NaHCO_3$, extracted with DCM, washed with brine, and placed over *anhydrous* $NaSO_4$, evaporated under reduced pressure to provide the lactonized product **15** (69 mg, 98%) as a solid. Mp 136–138 °C; $[\alpha]_D^{25}$ −49.5 (*c* 1.2, $CHCl_3$); IR (thin film, cm^{-1}) 3397, 3030, 2924, 1767, 1060; ^1H NMR ($CDCl_3$, 400 MHz): δ 2.50 (d, *J*=17.3 Hz, 1H), 2.64 (dd, *J*=17.6, 4.9 Hz, 1H), 3.88 (dd, *J*=8.6, 1.5 Hz, 1H), 4.13 (d, *J*=8.5 Hz, 1H), 4.40–4.45 (m, 3H), 4.55–4.60 (m, 3H), 4.81 (d, *J*=11.2 Hz, 1H), 7.24–7.44 (m, 15H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 39.4, 69.7, 70.4, 74.3, 75.1, 75.5, 80.9, 83.1, 127.9, 128.1, 128.2, 128.4, 128.6, 128.8, 137.4, 137.5, 138.3, 175.2. MS (FAB) 450 (M^++1). Anal. Calcd for $C_{27}H_{28}O_6$: C, 72.30; H, 6.29; found: C, 72.38; H, 6.22.

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Effects of steric bulk and stereochemistry on the rates of diketopiperazine formation from *N*-aminoacyl-2,2-dimethylthiazolidine-4-carboxamides (Dmt dipeptide amides)—a model for a new prodrug linker system

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Abstract—A peptide-like self-immolative molecular clip is required for release of active drugs from prodrugs by endopeptidases. Upon cleavage from the carrier, this clip must collapse and release the drug rapidly. A series of aminoacyl-5,5-dimethylthiaproline (Aaa-Dmt) *N*-(2-(4-nitrophenyl)ethyl)amides were designed. Boc-L-aminoacyl fluorides were coupled with *R*-DmtOH to give Boc-L-Aaa-*R*-DmtOH, which were converted to the Boc-L-Aaa-*R*-Dmt *N*-(2-(4-nitrophenyl)ethyl)amides. The L,*S* diastereomeric series was prepared by the reaction of Boc-Aaa PFP esters with *S*-DmtOH. The L-Aaa-Dmt *N*-(2-(4-nitrophenyl)ethyl)amides were allowed to cyclise to diketopiperazines (DKPs) in aqueous buffers, expelling 2-(4-nitrophenyl)ethylamine as a model for amine-containing drugs. Reaction rates were dependant on pH. In the L,*R* diastereomeric series, increasing steric bulk of the Aaa side-chain (Gly, Ala, Phe, Val) led to decrease in the reaction rate. However, in the L,*S* series, the greatest rate of reaction was observed for the most bulky amino-acid (Val), with $t_{½}$=15 min at pH 8.0. The effects of steric bulk and stereochemistry are rationalised through conformational analysis (NMR and X-ray crystallography) of the starting dipeptide amides, the product diketopiperazines and key analogues. Since the dipeptides are (almost) exclusively in the *cis*-amide conformation, trans–cis interconversion is not relevant. The data suggest that steric interactions in the reacting conformations of the dipeptide amides, as they form the tetrahedral intermediates, are the controlling factors. Thus, L-Aaa-*S*-Dmt amides are shown to be excellent candidates for incorporation into the design of novel prodrugs.

1. Introduction

Prodrugs have an increasingly important role in the selective delivery of potent drugs to their intended site of action in the body. In a prodrug, a part or the whole of the pharmacophore is masked by a group, which is removed, usually by the catalytic activity of an enzyme, in the target site. The utility of the prodrug approach can be to increase the concentration of the active drug in the target tissue or to diminish the concentration in an organ to which the drug is toxic. The masking group can also carry functionality to increase aqueous solubility or to modify the biodistribution of the prodrug. For example, macromolecular prodrugs, in which many molecules of the drug are attached to a soluble polymer, are selectively retained in solid tumours owing to leaky vasculature and poor lymphatic drainage.[1] Prodrugs can be bipartite or tripartite in concept (Scheme 1). In bipartite prodrugs, the trigger (which is the masking group)[2] is joined directly to the effector (the drug moiety to be released); the activating enzyme modifies the trigger chemically, causing the effector to be released. Of course, the design of bipartite prodrugs relies on the possibility of appropriate modes of attachment of the trigger to the effector. Several reductively activated prodrug systems work in this way.[3,4] Where this is not possible, a linker is interposed between the trigger unit and the effector, making a tripartite prodrug (Scheme 1). In this system, the activating enzyme triggers release of the linker–effector unit; the linker then decomposes spontaneously to expel the effector (drug). Such linkers can be as simple as a carbamate ester,[5] providing a carbamate anion as a good leaving group from the trigger; carbon dioxide is then lost rapidly to give the amine drug. 4-Aminobenzyl carbamate esters are also useful linkers.[6]

Peptidases present particular problems as activating enzymes, in terms of prodrug design in general and linker design in particular. Exopeptidases, particularly carboxypeptidases,

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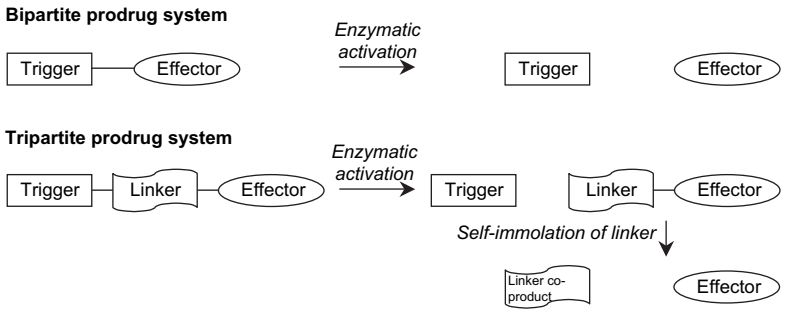
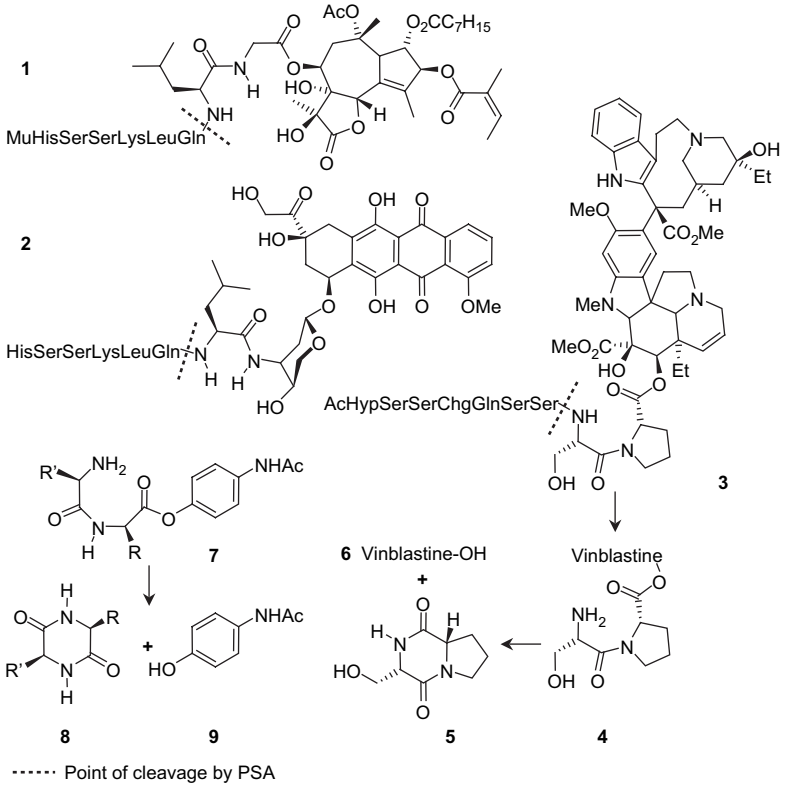


Scheme 1. Schematic representation of the release of drugs from bipartite and tripartite prodrugs systems. The trigger or the trigger–linker unit, respectively, comprises the group which masks the pharmacophore of the drug (effector).

can release amine-containing drugs directly from amides at the C-terminal of short peptides; for example, doxorubicin is released from the polymeric prodrug PK1 (in which it is linked to the polymeric backbone through the sequence GlyPheLeuGly) by cathepsin B.[7] However, some activating enzymes are endopeptidases, cleaving only amide bonds between amino acids. In several cases, this leads to the release of a drug molecule still carrying one or more aminoacyl unit; this may or may not be deleterious to the desired pharmacological activity. For example, prodrug **1** is cleaved by prostate-specific antigen (PSA) in malignant prostate tissue to release a thapsigargin analogue still carrying a Leu residue,[8] as shown in Scheme 2. Similarly, PSA-mediated cleavage of prodrug **2** releases leucyl-doxorubicin in prostate tumours.[9,10] Interestingly, PSA does not release doxorubicin from HisSerSerLysLeuGln-doxorubicin. In both cases, the released Leu-drug construct does have cytotoxic activity. To address this problem for a drug in which a pendant Leu would be deleterious to activity, prodrug **3** (Scheme 2) has been developed.[11] PSA causes cleavage of the Ser–Ser bond, releasing SerPro-vinblastine **4**. The N-terminal primary amine of this dipeptide ester then attacks the ester carbonyl, forming a diketopiperazine **5** and expelling the cytotoxic drug **6**. Similarly, dipeptide esters **7** of paracetamol have been proposed as prodrugs to release the analgesic **9** slowly by forming diketopiperazines **8**.[12]

In both of these systems where a dipeptide is the linker, the drug is a good leaving group, forming either an alkyl ester or a phenyl ester. Only a limited number of drugs carry an alcohol in the pharmacophore, whereas many carry a primary or secondary amine. Design of a linker where the drug is



Scheme 2. Examples of prodrugs triggered by an endopeptidase. The thapsigargin analogue prodrug **1** and the leucyl-doxorubicin prodrug **2** both release drug carrying one aminoacyl unit upon cleavage by prostate-specific antigen (PSA). PSA-mediated cleavage of the vinblastine prodrug **3** releases the Ser-Pro vinblastine ester **4**, which cyclises to form the diketopiperazine **5**, expelling the vinblastine alcohol **6**. The prodrug system **7** cyclises spontaneously, forming the diketopiperazines **8** and expelling paracetamol **9**.

attached to the C-terminus of a dipeptide through an amide is more challenging, owing to the much lower electrophilic reactivity of amides. In this paper, we report our development of a dipeptide linker suitable for amine-containing drugs.

2. Design of the dipeptide amide linker

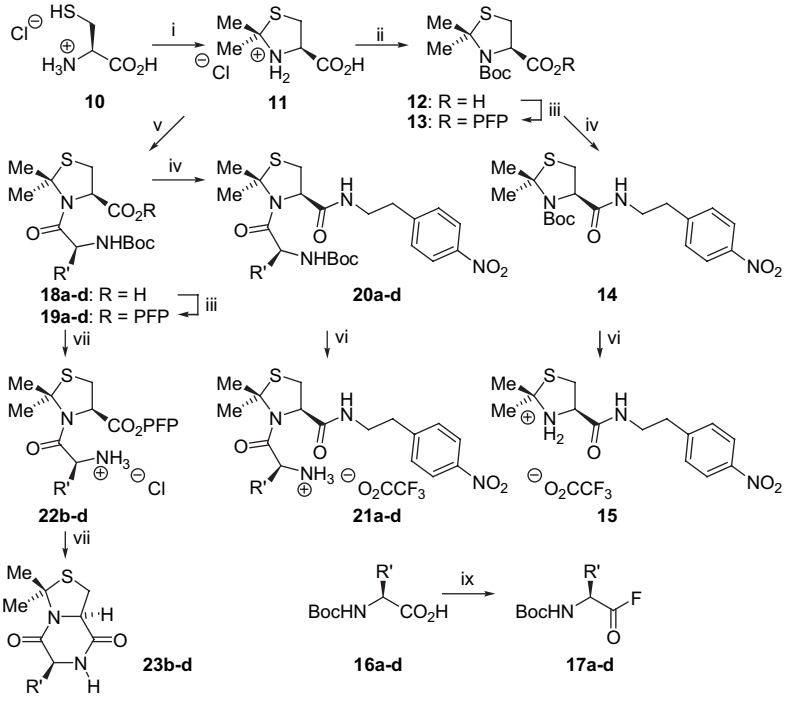
In peptides with free N-terminal amines, the N-terminal amino-acid pair can cyclise slowly to give a diketopiperazine (DKP); the C-terminal remainder of the peptide (an amine leaving group) is expelled. The rate of DKP formation depends on the proportion of the dipeptide in the reacting cis conformation.[13] However, most peptide sequences adopt only the trans conformation, because it is energetically favourable. The presence of proline at the penultimate position greatly enhances the rate of DKP formation and expulsion of the amine leaving group, owing to the greater propensity to adopt a *cis*-amide conformation (AlaPro is 13% cis[13,14]). This phenomenon was exploited in the SerPro linker in prodrug **3**; replacement of Pro with acyclic amino acids suppressed release of vinblastine.[11]

Interestingly, replacement of Pro with 2,2-dimethylthiazolidine-4-carboxylic acid (Dmt) has been reported to force peptides into 100% cis conformation,[14,15] putatively owing to the major steric clash between the side-chain of the N-terminal amino-acid and the geminal dimethyl unit. Since the conformation, and thus biological activity of peptides, is critically dependant on whether amino-acyl prolines are in the *cis* or *trans* amide rotamer, there exist several enzymes, which catalyse the interconversion, including cyclophilins and Pin1.[16–18] Control of conformation by 5,5-dimethylproline and by Dmt has been exploited in studying the biologically active conformations of peptides.[19–26]

Since peptides containing Dmt exist mainly in the *cis* (*Z*) tertiary amide rotamer, it is likely that aminoacyl-Dmt amides also adopt predominantly this conformation. We, therefore, postulated that, despite the lower electrophilicity of amides, these aminoacyl-Dmt amides should cyclise, expelling the amine (drug). To test this hypothesis and to study the effects of relative stereochemistry and steric bulk of the amino-acid side-chain on the rate of cyclisation and release, a series of N-terminal-protected aminoacyl-Dmt dipeptide amides were designed. In these dipeptide amides, 2-(4-nitrophenyl)-ethylamine was used as a simple model for drugs containing primary aliphatic amines. The protecting group was chosen to be Boc, which could be removed under acidic conditions, which prevent cyclisation. The dipeptide amide salts could then be placed in aqueous buffers of various pH values and the rates of cyclisation and release of the model drug from these candidate prodrug linkers could be measured.

3. Chemical synthesis

The synthetic approach to the first diastereomeric series of target dipeptide amide salts **21a–d** is shown in Scheme 3. In this series, L-amino acids carrying side chains with diverse steric bulk (Gly, L-Ala, L-Val, L-Phe) were coupled to *R*-2,2-dimethylthiazolidine-4-carboxylic acid derivatives. Firstly, 2,2-dimethylthiazolidine-4-carboxylic acid **11** (*R*-Dmt) was prepared in good yield from condensation of L-Cys hydrochloride **10** with 2,2-dimethoxypropane by the method of Kemp and Carey.[27] Initially, it was planned to carry out the peptide synthesis in the conventional manner for dipeptide



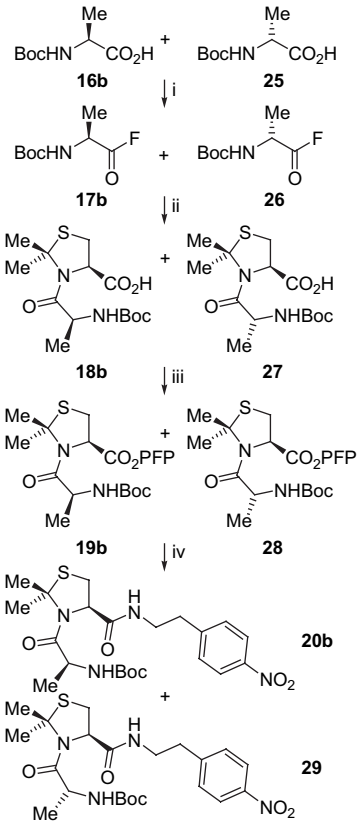
Scheme 3. Synthesis of dipeptide *N*-(2-(4-nitrophenyl)ethyl)amides **21a–d** and diketopiperazines **23b–d**. PFP=pentafluorophenyl. Reagents and conditions: (i) $(MeO)_2CMe_2$, acetone, N_2, Δ; (ii) Boc_2O, Pr^i_2NEt, MeCN; (iii) PFPOH, DCC, EtOAc, N_2; (iv) 2-(4-nitrophenyl)ethylamine hydrochloride (**24**), Et_3N, CH_2Cl_2; (v) **17a–d**, Pr^i_2NEt, DMF; (vi) CF_3CO_2H, CH_2Cl_2; (vii) HCl, CH_2Cl_2; (viii) Et_3N, CH_2Cl_2; (ix) 2,4,6-trifluoro-1,3,5-triazine, pyridine, CH_2Cl_2.

amides, by forming the C-terminal amide at the beginning of the synthesis. Protection of the secondary amine of **11** with Boc proceeded in poor yield, owing to the presence of three bulky substituents on the carbons adjacent to this nitrogen. Boc-*R*-DmtOH **12** was then activated as its pentafluorophenyl ester **13** for coupling with 2-(4-nitrophenyl)ethylamine **24** to form the required BocDmt *N*-(2-(4-nitrophenyl)ethyl)amide **14** in excellent yield. Removal of the Boc protection afforded the Dmt *N*-(2-(4-nitrophenyl)ethyl)amide salt **15** but all attempts to couple this with activated amino-acid derivatives led to opening of the thiazolidine ring, shown by observation of cysteinyl peptides in the crude multi-component reaction mixtures.

In view of the difficulty of achieving couplings to Dmt with the C-terminal amide already in place, an alternative strategy was investigated, coupling of *N*-protected amino acids with **11**, followed by installation of the *N*-(2-(4-nitrophenyl)ethyl)-amide. As noted above, the secondary amine of **11** is severely sterically hindered and is also deactivated as a nucleophile by the carboxylate and by the sulfur. Thus, a reactive and sterically small acylating agent is required for coupling. Wöhr et al.[28] have successfully coupled Fmoc-protected amino-acyl fluorides with **11** in moderate-to-good yields. Adapting this procedure to the required Boc-protected analogues, BocGlyOH **16a**, Boc-L-AlaOH **16b**, Boc-L-ValOH **16c** and Boc-L-PheOH **16d** were converted to the corresponding acyl fluorides **17a–d**, respectively (Scheme 3) by treatment with cyanuric fluoride in the presence of pyridine. These acyl fluorides proved to be unstable and were used immediately in crude form for reaction with **11** in dry DMF. The Boc-protected dipeptides **18a–d** were obtained in 27–35% yields after careful chromatography. Activation as the pentafluorophenyl esters **19a–d** and coupling with 2-(4-nitrophenyl)ethylamine then efficiently afforded the required Boc-protected amino-acyl Dmt *N*-(2-(4-nitrophenyl)ethyl)-amides **20a–d**. Brief treatment with trifluoroacetic acid in dichloromethane then afforded the amino-acyl Dmt *N*-(2-(4-nitrophenyl)ethyl)amide salts **21a–d**, which were sufficiently stable to be stored prior to the cyclisation rate studies.

The diketopiperazines **23b–d** were also required both as HPLC analytical standards for the kinetics studies of cyclisation and as compounds in their own right for study of their conformations, to aid in understanding the relative cyclisation reaction rates. Treatment of the Boc-dipeptide pentafluorophenyl esters **19b–d** with hydrogen chloride removed the Boc protection, giving the dipeptide PFP ester salts **22b–d**. Since all these carry the excellent pentafluorophenoxy leaving group, the corresponding free bases cyclised very rapidly to afford the required diketopiperazines **23b–d** in good yields.

To confirm that the two sets of signals seen in many of the NMR spectra did indeed correspond to rotamers about the tertiary amide bond and did not indicate racemisation of the N-terminal acyl fluoride prior to coupling, one synthetic sequence was conducted with a mixture of enantiomers of BocAla of known composition. This series of experiments would lead to mixtures of diastereoisomers; firstly, the NMR spectra of the individual diastereoisomers could be observed and compared with the spectra from the series starting with homochiral Boc-L-Ala and secondly, examination of the molar ratio of diastereoisomers at each step in the synthetic sequence would reveal and quantify any significant loss of stereochemical integrity. As shown in Scheme 4, the acid fluorides of a mixture comprising 40% Boc-L-AlaOH **16b** and 60% Boc-D-Ala **25** were prepared as above. This mixture of enantiomeric acyl fluorides **17b** and **26** was coupled with homochiral *R*-Dmt **11** to give a mixture of the L,*R* product **18b** and the D,*R* product **27** in 36% yield. As expected, the ^{1}H NMR spectrum of the mixture showed distinct sets of signals for the two diastereoisomers, in the molar ratio 2:3, respectively, *without prior chromatographic purification*. The ^{1}H–^{1}H COSY spectrum of this mixture highlighted these separate sets of signals, confirming that the diastereoisomers could be distinguished by this technique. Notably, only one set of signals corresponded to the product **18b** derived from homochiral Boc-L-AlaOH **16b** alone, confirming that the sets of signals in the spectra of the latter did correspond to rotamers and not to diastereoisomers. Furthermore, the similarity of the ratio of products to the ratio of starting Boc-Ala enantiomers confirms that there is little or no diastereoselectivity in the coupling reaction, despite the modest yield. Moving forward in the sequence, the mixture of **18b** and **27** was converted to a mixture of the corresponding pentafluorophenyl esters **19b** and **28** in high yield. The



Scheme 4. Experiments to demonstrate that no racemisation and no diastereomeric induction took place during the acyl fluoride coupling and subsequent steps towards the dipeptide *N*-(2-(4-nitrophenyl)ethyl)amides. PFP=pentafluorophenyl. The molar ratio of enantiomeric starting materials BocAlaOH **16b/25** was 2:3 in the mixture; molar ratios of diastereomeric pairs of intermediates and products **18b/27**, **19b/28** and **20b/29** were 2:3 in the mixtures, as determined by ^{1}H NMR. Reagents and conditions: (i) 2,4,6-trifluoro-1,3,5-triazine, pyridine, CH_2Cl_2; (ii) **11**, Pr^i_2NEt, DMF; (iii) PFPOH, DCC, EtOAc, N_2; (iv) 2-(4-nitrophenyl)ethylamine hydrochloride, Et_3N, CH_2Cl_2.

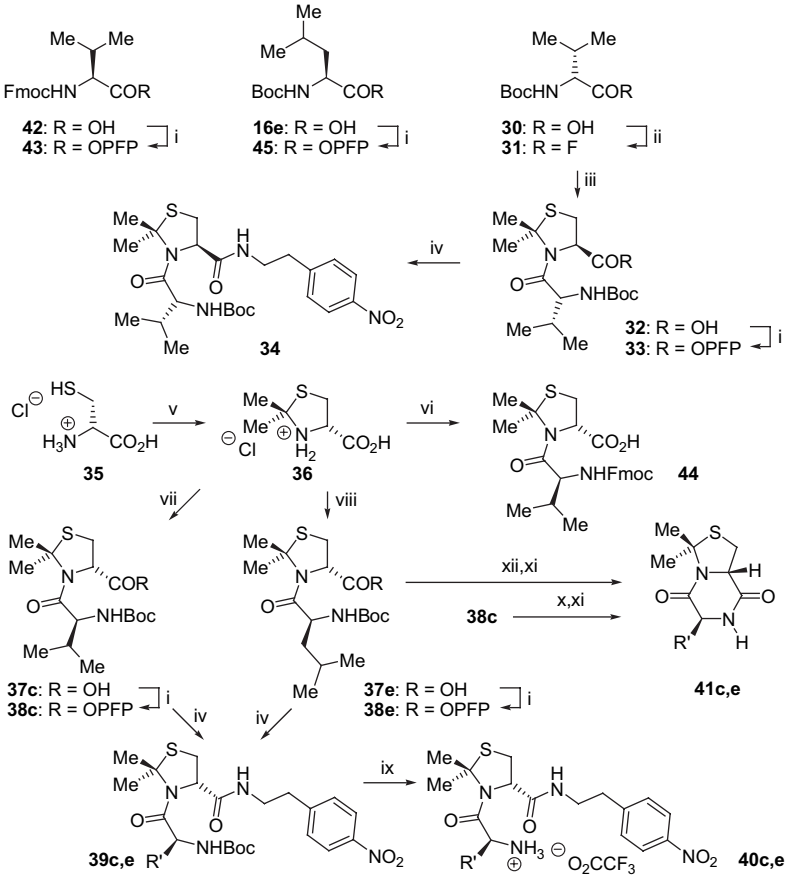
^{19}F NMR spectrum of this mixture was complex and indicated the presence of two conformers for each of the two diastereoisomers, with a diastereomeric ratio of 2:3 again. A similar ratio was seen in the ^{1}H NMR spectrum. These active esters then reacted with 2-(4-nitrophenyl)ethylamine **24** in the usual way to give a 2:3 mixture of **20b** and **29**. This series of experiments did show that the diastereoisomers were distinguishable by NMR at each stage and that little or no racemisation had taken place.

Scheme 5 shows the synthetic approaches to the two target dipeptide amides **40c** and **40e** in the L,*S* diastereomeric series. As a model experiment, D-ValOH **16c** was converted to its acyl fluoride **17c**, which was coupled with **11** in the usual way to give Boc-D-Val-*R*-DmtOH **32** in 50% yield. This dipeptide was then taken through to the pentafluorophenyl ester **33** and the Boc-protected dipeptide amide **34** in the usual way. In the target L,*S* series, condensation of D-Cys **35** with 2,2-dimethoxypropane gave *S*-DmtOH **36** and coupling with Boc-L-Val acyl fluoride **17c** gave the L,*S*-Boc-dipeptide **37c** in 35% yield. The corresponding pentafluorophenyl ester **38c** was then converted to the DKP **41c**, by deprotection and treatment with base, and to the protected dipeptide amide **39c** and through to the target L-Val-*S*-Dmt dipeptide amide salt **40c**, as for the L,*R* diastereoisomer **21c**, above. As expected, the ^{1}H NMR spectra of the enantiomers **34** and **39c** were identical.

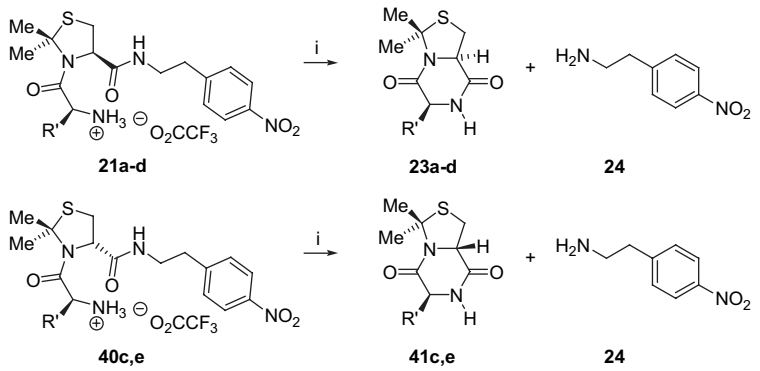
In the light of the relatively higher yields obtained for couplings of BocVal to the Dmt unit in this diastereomeric series, couplings with more sterically demanding pentafluorophenyl esters were explored. As a model, Fmoc-L-ValOH **42** was converted to its pentafluorophenyl ester **43**, which was coupled effectively with **36** to give Fmoc-L-Val-*S*-DmtOH **44**. Similarly, Boc-L-LeuOPFP **45** reacted smoothly with **36** to afford **37e**, from which the dipeptide pentafluorophenyl ester **38e** was readily obtained. As with the lower homologue **38c**, this material could be converted to the corresponding DKP **41e** and, through the Boc-dipeptide amide **39e**, to the target L-Leu-*S*-Dmt amide salt **40e**.

4. DKP formation studies

The rates of cyclisation of the dipeptide amides **21a–d**, **40c** and **40e** to form the DKPs **23a–d**, **41c** and **41e**, respectively, and to expel the model drug **24** (Scheme 6) were studied in aqueous buffer at pH 8.0 and 7.0, to reflect the physiological pH range. The reaction of the L-Val-*S*-Dmt dipeptide amide **40c** was also studied at pH 6.0. Experiments were conducted in duplicate or triplicate at 37 °C. HPLC analysis of the reaction mixtures was performed at appropriate time points to enable the determination of the rates of the reactions. UV detection at 275 nm reported on the loss of the starting dipeptide amides and production of **24** (through the



Scheme 5. Synthesis of the *S*-thiazolidine series of dipeptide amides **40c** and **40e** and diketopiperazines **41c** and **41e**. PFP=pentafluorophenyl. Reagents and conditions: (i) PFPOH, DCC, EtOAc, N_2; (ii) 2,4,6-trifluoro-1,3,5-triazine, pyridine, CH_2Cl_2; (iii) **11**, Pr^i_2NEt, DMF; (iv) 2-(4-nitrophenyl)ethylamine hydrochloride, Et_3N, CH_2Cl_2; (v) $(MeO)_2CMe_2$, acetone, N_2, Δ; (vi) **43**, Pr^i_2NEt, THF, DMF; (vii) **17c**, Pr^i_2NEt, DMF; (viii) **45**, Pr^i_2NEt, DMF; (ix) CF_3CO_2H, CH_2Cl_2; (x) HCl, CH_2Cl_2; (xi) Et_3N, CH_2Cl_2; (xii) CF_3CO_2H.



Scheme 6. Studies on rates of ring-closure of dipeptide amides **21a–d**, **40c** and **40e** to diketopiperazines **23a–d**, **41c** and **41e**, expelling the model drug 2-(4-nitrophenyl)ethylamine **24**. Reagent and conditions: (i) aqueous buffer pH 6.0, 7.0 or 8.0.

nitrophenyl chromophore), whereas simultaneous detection at 225 nm allowed quantification of the product DKPs. Examples of graphs showing consumption of the starting materials and formation of the products are shown in Figure 1. Each reaction followed first-order kinetics closely, as expected, and the calculated half lives are shown in Table 1. A wide range of half lives was seen at pH 8.0 (15 min to 28 h) and at pH 7.0 (1 h to >45 h).

Comparison of the cyclisation half lives at pH 8.0 with the corresponding values at pH 7.0 indicates that each dipeptide amide reacts some 2.5–4 times faster at higher pH. Since, it is only the unprotonated free-base dipeptide that carries the nucleophilic primary amine that attacks the secondary amide carbonyl, this consistently observed effect of pH on the overall reaction rate reflects the amount of this prototropic form that is present in solution. Since the pK_a values of the N-terminal amines are likely to be ca. 8–9, changing the pH by one pH unit will have a marked effect on the concentration of the free-base form in solution and, thus, on the reaction rates. In the case of **40c**, further lowering the pH to 6.0 slows the reaction a further 10-fold, reflecting the very low concentration of the reactive prototropic form in this acidic milieu.

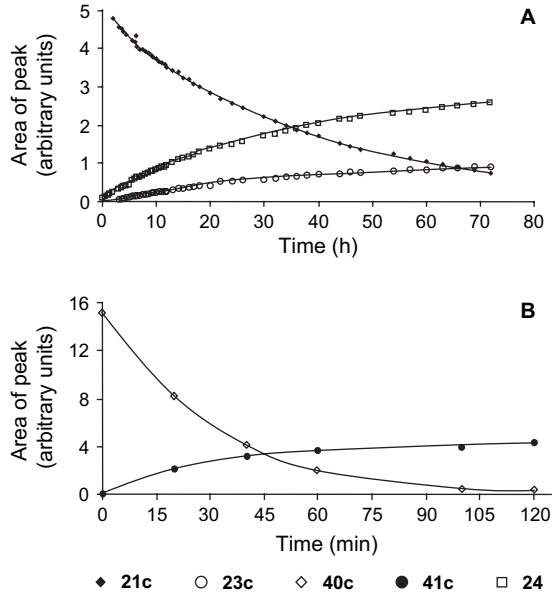


Figure 1. Examples of graphs obtained for typical experiments measuring consumption of dipeptide amides **21c** (A) and **40c** (B), formation of DKPs **23c** and **41a** and expulsion of 2-(4-nitrophenyl)ethylamine **24** in aqueous solution at pH 8.0.

Table 1. Half lives of cyclisation of dipeptide *N*-(2-(4-nitrophenyl)ethyl)-amides **21a–d**, **40c** and **40e** in aqueous buffer at pH 6.0, 7.0 and 8.0, forming the corresponding diketopiperazines **23a–d**, **41c** and **41e** and expelling 2-(4-nitrophenyl)ethylamine **24**

Compound	R	Stereoisomer[a]	$t_{½}$ (37 °C, h)		
			pH 8.0	pH 7.0	pH 6.0
21a	H	*R*	10–11[b]	42	ND[c]
21b	Me	L,*R*	3–4[b]	8–9[b]	ND[c]
21c	$CHMe_2$	L,*R*	26–27[b]	>45	ND[c]
21d	CH_2Ph	L,*R*	28–29[b]	ND[c]	ND[c]
40c	$CHMe_2$	L,*S*	0.25–0.3[b]	0.75–1.25[b]	10
40e	CH_2CHMe_2	L,*S*	1.0–1.5[b]	2.5	ND[c]

[a] L/D refers to the configuration of the N-terminal amino-acid; *R*/*S* refers to the configuration of the thiazolidine.
[b] Range of values from >1 experiment.
[c] Not determined.

In the L,*R* diastereomeric series, the effect of increasing the steric bulk of the amino-acid side-chain was not straightforward. It had been predicted that increasing the bulk would increase the rate of reaction by increasing the proportion of the reactive *Z* tertiary amide rotamer in solution. Comparison of the half-life of cyclisation of the Gly-*R*-Dmt amide **21a** with that of the L-Ala-*R*-Dmt analogue **21b** would suggest that this prediction was true. However, the L-Val-*R*-Dmt and L-Phe-*R*-Dmt dipeptide amides **21c** and **21d**, containing the much more bulky isopropyl and benzyl side chains, respectively, cyclise around nine times slower at pH 8.0 than does the L-Ala-*R*-Dmt analogue **21b**. Thus, with the exception of the Gly analogue, increase of steric bulk of the side-chain *decreases* the reaction rate in this series.

The converse is seen in other diastereomeric series. In general, the L,*S* dipeptide amides cyclise much faster than do the L,*R* analogues. Within the series, the L-Val-*S*-Dmt dipeptide amide **40c** reacts some 3–4 times faster than does the L-Leu-*S*-Dmt dipeptide amide **40e**. The isobutyl group of the latter, although formally larger than the isopropyl group of the former, is less sterically demanding at the critical β-carbon atom. The Gly-*R*-Dmt dipeptide amide **21a** is also formally a member of this diastereomeric series and its reaction rates are ca. 40 times slower than those of **40c** and 10–15 times slower than those of **40e**. Thus, in this

series, increasing the steric bulk of the side-chain *increases* the reaction rate.

Clearly, simple consideration of the *Z*/*E* tertiary amide rotamer equilibrium cannot provide the complete rationalisation of these effects. A detailed study of conformations of the dipeptide amides and related precursors was therefore undertaken, along with examination of conformations of likely intermediates in the courses of the cyclisations.

5. Conformational studies—dipeptides

5.1. NMR studies—L,*R* diastereomeric series

As expected, the ^{1}H NMR spectrum of **12** shows the presence of two rotamers about the Boc–N carbamate bond, in approximately equal amounts. Figure 2 shows the signals corresponding to the 4-H of the thiazolidine in $(CD_3)_2SO$ at 20 and 80 °C. At lower temperature, the signals for 4-H are distinct and, at higher temperature, the signals are fully coalesced.

The dipeptides were designed such that the geminal dimethyl unit should provide sufficient steric bulk to perturb the slow equilibrium between the rotamers about the tertiary amide peptide bond in favour of the cis (*Z*) conformer, which is the one required for the formation of the DKPs and expulsion of the 2-(4-nitrophenyl)ethylamine leaving group. As an indicator of the populations of the rotamers of the N-terminal free-base dipeptides (some of which were expected to cyclise too quickly for satisfactory measurement of rotamer populations), the ratios of the rotamers of the Boc-aminoacyl-DMT amides **20a–d**, **39c**,**e** were measured by ^{1}H NMR spectroscopy. Firstly, the spectrum (in $(CD_3)_2SO$ at 20 °C) of the simplest Boc-dipeptide amide (**20a**), which contains glycine and is thus a member of both diastereomeric series, showed two sharp singlets at δ 1.32 and 1.38 in the approximate ratio of 1:11, corresponding to the protons of the Boc group. The remainder of the spectrum also showed evidence for major and minor populations but the peaks were overlapping, precluding other measurements of the ratio of rotamers. To confirm that these populations did indeed correspond to amide rotamers, spectra were run at temperatures ranging from 20 to 100 °C. The energy barrier to rotation proved to be low for this type of restricted amide bond rotation, with significant broadening of the peaks at 30 °C and full coalescence to a sharp spectrum at 40 °C.

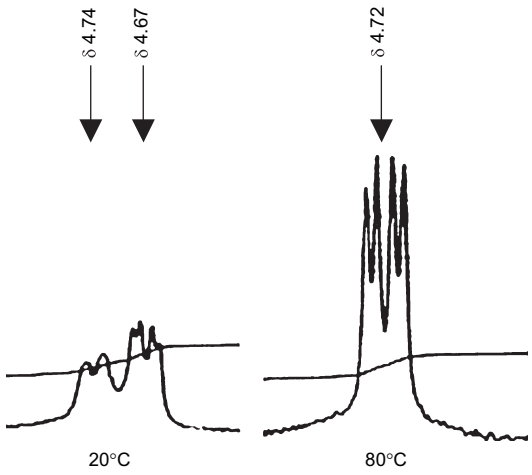


Figure 2. Parts of ^{1}H NMR spectra of BocDmtOH **12** in $(CD_3)_2SO$ at 20 and 80 °C.

Although this study did indicate that there was a strong preference for one of the rotamers, it did not show whether the more abundant rotamer was *Z* or *E* and identification of the conformation of the major rotamer required a NOESY spectrum at 20 °C. Firstly, the peaks of the major rotamer were fully assigned using a COSY spectrum and some conformational information was derived from the coupling constants. Interestingly, the signals for the Gly methylene protons were widely separated by some 0.47 ppm, which suggested a conformation in which they were located relatively close in space to the chiral 4-C of the thiazolidine. In the 2-(4-nitrophenyl)ethyl unit, the protons of the methylene adjacent to the amide nitrogen were magnetically inequivalent (δ 3.39 and 3.47), as expected, but the protons of the more remote methylene resonated as a simple triplet, reflecting its greater distance from the chiral centre. In the thiazolidine, a COSY cross-peak was seen between 4-H and 5_β-H (δ 3.31) but not between 4-H and 5_α-H (δ 3.10); this lack of a cross-peak is consistent with the multiplicity of the 5_α-H signal as a simple doublet, coupling only to its geminal partner. The lack of observed coupling between 4-H and 5_α-H points to a dihedral angle close to 90° between these protons. In the NOESY spectrum, a cross-peak was seen between 4-H and 5_α-H but no peak was seen between 4-H and 5_β-H, confirming the above assignment. A strong cross-peak diagnostic for the identity of the major rotamer was present between 4-H and the downfield Gly methylene proton, whereas a weak peak was seen between 4-H and the other Gly methylene proton. Cross peaks between the geminal dimethyl unit and the Gly methylene protons were absent, confirming that the glycine was close in space to the 4-C of the thiazolidine.

Similar conformational studies were carried out on the other three Boc-protected dipeptide amides in the *R*-Dmt series. The 1-D ^{1}H NMR spectra of **20b–d** in $(CD_3)_2SO$ at 20 °C each showed only one set of signals, indicating the presence of only one rotamer about the tertiary amide bond in each case. The identity of the single rotamer of the Ala analogue **20b** was established by a NOESY experiment, which showed a connectivity between Ala α-H and the thiazolidine 4-H. Correlations between the geminal methyls and all the protons of the BocAla moiety were correspondingly absent, confirming the *Z* tertiary amide conformation for the sole rotamer of **20b**. The spectra of **20c** and **20d** were similarly indicative of the *Z* rotamers. Thus, all four members of this L,*R* diastereomeric series exist either predominantly or exclusively in this conformation in DMSO solution, which is the one required for cyclisation.

5.2. Crystal structure of Boc-L-Ala-*R*-Dmt *N*-(2-(4-nitrophenyl)ethyl)amide 20b

Boc-L-Ala-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **20b** formed crystals, from ethyl acetate/hexane, of a suitable quality for X-ray crystallography. The crystal structure is shown in Figure 3. This molecule, which exists in DMSO solution as the *Z*

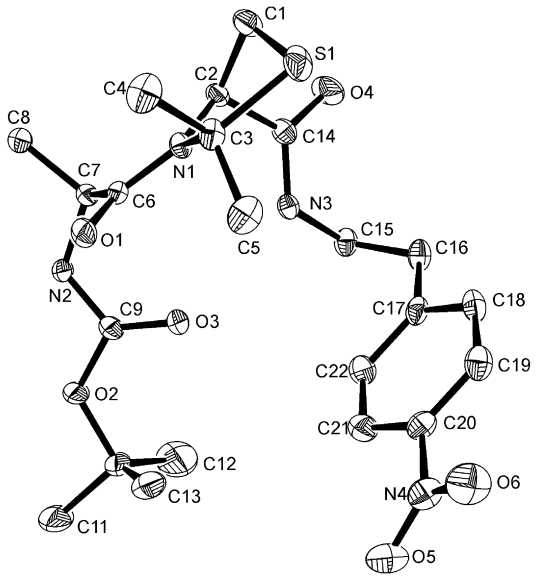


Figure 3. X-ray crystal structure plot of one molecule within the asymmetric unit of Boc-L-Ala-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **20b**, showing crystallographic numbering. Ellipsoids are represented at 30% probability and hydrogens are omitted for clarity.

rotamer, also adopts this conformation in the solid state. In addition to any conformational preference, which may be driven by steric factors, there is an intramolecular hydrogen bond tying the Boc carbonyl oxygen at the N-terminal of the dipeptide to the secondary amide N–H at the C-terminal. The intermolecular hydrogen bonds from the central tertiary amide carbonyl oxygen to the N-terminal Boc-N–H form a chain through the crystal.

5.3. NMR studies—L,*R* diastereomeric series

In the L,*S* diastereomeric series, the conformations of the tertiary amides of two examples were examined, in addition to that of BocGly-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **20a** which, as noted above, can be considered as a member of both series. ^{1}H NMR spectra of the enantiomers Boc-D-Val-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **34** and Boc-L-Val-*S*-DmtNH$(CH_2)_2C_6H_4NO_2$ **39c** showed the presence of two rotamers in the abundance ratio of 4:1; the major rotamer was again the *cis*-amide *Z* conformer, although a variable-temperature study showed that coalescence of the sets of signals was not yet complete at the highest temperature studied (80 °C). The signals for the rotamers of most tertiary amides coalesce below this temperature and that the current observation indicates a relatively high energy barrier to rotation in this molecule. The same ratio of tertiary amide rotamers was present in the solution of the homologue Boc-L-Leu-D-DmtNH$(CH_2)_2C_6H_4NO_2$ **39e**.

Thus, the *Z*/*E* ratio of rotamers is strongly in favour of the isomer in each case and the geminal dimethyl unit at the 4-position of the thiazolidine has been effective in achieving this desired outcome. However, direct comparison of the rotamer ratios for the diastereomers Boc-L-Val-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **20c** and Boc-L-Val-*S*-DmtNH$(CH_2)_2C_6H_4NO_2$ **39c** suggests that this sterically driven bias towards the *Z* rotamer is not as effective in the latter, owing to the greater distance between the isopropyl side-chain of the Val and the *pseudo*-axial 2-methyl group on the thiazolidine. Also noteworthy is the effect of steric bulk of the side-chain of the amino-acid on the chemical shifts of the diastereotopic thiazolidine 5-protons (Table 2). In the L,*R* series (**20b–d**), the simple doublet for 5-H cis to 4-H resonated downfield to the signal for 5-H trans to 4-H but in the L,*S* (and D,*R*) diastereomeric series (**34**, **39c**, **39e**), the chemical shifts are reversed; this may reflect a subtle conformational difference in the thiazolidine ring between the two series. The chemical shifts of the corresponding protons in the Gly analogue **20a** resembled those of the L,*S*/D,*R* series.

Table 2. ^{1}H NMR chemical shifts of the diastereotopic thiazolidine 5-protons in the Boc-Aaa-DmtNH$(CH_2)_2C_6H_4NO_2$ analogues **20a–d**, **34**, **39c** and **39e**

Compound	Stereoisomer[a]	δ (5-H_{trans})[b]	δ (5-H_{cis})[c]	$\Delta\delta$ (5_{trans}-H−5_{cis}-H)
20a	*R*	3.31	3.10	+0.21
20b	L,*R*	3.24	3.32	−0.08
20c	L,*R*	3.12	3.63	−0.41
20d	L,*R*	2.89	3.20	−0.31
34	D,*R*	3.36	3.04	+0.32
39c	L,*S*	3.36	3.04	+0.32
39e	L,*S*	3.45	3.05	+0.30

[a] L/D refers to the configuration of the N-terminal amino-acid; *R*/*S* refers to the configuration of the thiazolidine.
[b] 5-H trans to 4-H.
[c] 5-H cis to 4-H.

Examination of the ^{1}H NMR spectra of the dipeptide amide salts **21a–d** showed the presence of only one conformer in solution; this is presumably the *Z* tertiary amide rotamer, in the light of the similarity of the spectra to those of the *N*-Boc-protected precursors **20a–d**.

NOESY spectroscopy of the dipeptide pentafluorophenyl ester hydrochloride salts **22c** and **22d** gave some contrasting conformational information. The ^{1}H NMR spectrum of **22c** showed the presence of only one conformer in DMSO solution. Whereas the corresponding NOESY spectrum did allow assignment of the signals for the individual 5-H protons, no cross peaks were seen between signals for protons on the Val moiety and those on the thiazolidine; thus the sole rotamer could not be identified. However, in the case of the Phe analogue **22d**, the NOESY spectrum of a DMSO solution revealed through-space interaction between the upfield Me (δ 1.87) and the *ortho*-protons on the phenyl group. There is also connectivity between the downfield Me (δ 1.92) and the Phe α-H (δ 4.24). These data suggest that this dipeptide ester salt may be exclusively in the *E* amide conformation. This conformation may be favoured by the considerable steric bulk of the pentafluorophenyl ester driving the Phe residue towards the geminal dimethyl unit.

6. Conformational studies—diketopiperazines

As a contribution to understand the marked different rates of ring-closure to form the DKPs and to expel the 2-(4-nitrophenyl)ethylamine, NMR and crystallographic studies were carried out on the various DKPs. The *cyclo*-L-Ala-*R*-Dmt and *cyclo*-L-Val-*R*-Dmt DKPs **23b** and **23c** were crystalline

compounds and thus amenable for the determination of conformation in the solid state by X-ray crystallography. Although there have been several studies reported in the literature on the conformations of prolyl DKPs,[29–32] there are no reports to date for similar studies with their thiaproline analogues.

6.1. NMR studies—L,*R* diastereomeric series

DKP **23d**, derived from L-Phe and *R*-2,2-dimethylthiazolidine-4-carboxylic acid, formed a non-crystallisable gum and an attempt was made to determine its conformation in solution by NOESY spectroscopy and by examination of ^{1}H–^{1}H NMR coupling constants. Although the former was relatively uninformative about conformation, it did serve to assign the two singlet signals due to the methyl groups and to distinguish the signals for the two 1-H protons. This NOESY spectrum showed NOE connectivity between one 1-H double doublet (δ 3.26) and the 8a-H (δ 4.53); thus the signal at δ 3.26 is due to the 1-H on the lower (α) face of the molecule. NOE connectivity was also seen between the upfield methyl singlet at δ 1.87 and the β-face 1-H at δ 3.19; thus this upfield singlet signal (δ 1.87) is due to the methyl on the β-face. Another NOE connection was seen between the *ortho*-protons of the phenyl group and 6-H, suggesting that the phenyl group was pointing away from the heterocycle.

The boat conformation of the DKP ring of **23d** was confirmed by the presence of the five-bond coupling ^{5}J= 0.8 Hz between the axial 8a-H and the axial 6-H.[33] Therefore, the $PhCH_2$ side-chain is in an equatorial position and is far from the geminal dimethyl unit. The thiazolidine is probably in the half-chair conformation, with a *trans*-diaxial coupling ^{3}J=11.3 Hz between 8a-H and 1_{β}-H. The corresponding axial–equatorial coupling to 1_{α}-H is ^{3}J=5.9 Hz. A four-bond coupling ^{4}J=1.6 Hz is observed between 8a-H and NH, as seen for **23b**. The coupling constants from the Ph-CH_2 protons to 6-H are ^{3}J=10.2 and 3.9 Hz; thus the Ph cannot be antiperiplanar to the 6-H, as a coupling constant of 10.2 Hz is only consistent with antiperiplanar H–C–C–H; the phenyl is either orientated towards the nitrogen or towards the carbonyl oxygen in a staggered conformation. These data contrast with those reported[33] for the analogous ditryptophenaline, in which the two corresponding coupling constants between Ph-CH_2 and 6-H are ^{3}J=4.4 and 3.1 Hz; these values are used to support a conformation with Ph located over the DKP ring. We have also noted a similar conformation for *cyclo*-L-Phe-D-Pro.[34] However, the data for **23d** are consistent with the X-ray structure-derived conformation reported[35] for *cyclo*-L-Phe-L-Pro, in which the DKP is boat conformation, CH_2Ph is equatorial and the phenyl is antiperiplanar to the carbonyl. Further evidence for the boat conformation of the DKP ring is provided by the five-bond coupling ^{5}J=0.8 Hz between 8a-H and 6-H. Maes et al.[33] observed a similar coupling in ditryptophenaline, a natural product from *Aspergillus flavus* var. *columnaris*, which contains a *cyclo*-L-Phe-L-Pro unit fused to a heterocycle through the Pro side-chain loop, and used it to confirm that the DKP is in a boat conformation in solution with the corresponding protons in axial and cis. Thus 8a-H and 6-H in **23d** are also shown to be 1,4-diaxial and cis, an arrangement only available in a boat conformation.

6.2. Crystal structure of *cyclo*-L-Ala-*R*-Dmt 23b

A crystallographic quality crystal of *cyclo*-L-Ala-*R*-Dmt **23b** was grown from ethyl acetate/hexane and the X-ray crystal structure was determined. Figure 4 shows the intermolecular hydrogen-bonding pattern evident in the crystal and the structure of a single molecule, together with the crystallographic numbering scheme. The intermolecular hydrogen-bonding forms a ribbon through the crystal, with the N–H being hydrogen-bonded to the corresponding secondary amide carbonyl of the adjacent molecule. The tertiary amide carbonyl (from the dimethylthiaproline unit) does not participate in hydrogen-bonding. Within an individual molecule of **23b**, the DKP ring is in a flattened boat conformation, with the thiazolidine in a half-chair. This ring conformation places the geminal methyl groups in equatorial and axial positions; the 6-methyl occupies a *pseudo*-equatorial position. Notably, this 6-methyl group is remote in space from the geminal dimethyl unit and, indeed, from other sterically bulky groups.

NMR observations show that a similar conformation is also likely to be adopted in solution in chloroform. The coupling constants between the axial 8a-H and the 1_{β}-H and 1_{α}-H protons are ^{3}J=10.2 and 6.6 Hz, respectively. These values are consistent with the crystallographic dihedral angles (8a-H)-(8a-C)-(1-C)-(1_{β}-H) of 166° and (8a-H)-(8a-C)-(1-C)-(1_{α}-H) of 44°, according to the Karplus relationship. The axial 6-H couples with the adjacent N–H by ^{3}J=1.0 Hz, which corresponds to the crystallographic dihedral angle of 97°. Longer-range couplings are also observed. A four-bond coupling ^{4}J=1.6 Hz is seen between 8a-H and the NH; the coupling path between these atoms contains four atoms in one plane and one terminal atom out of that plane, thus the W-arrangement is almost adopted. A five-bond coupling

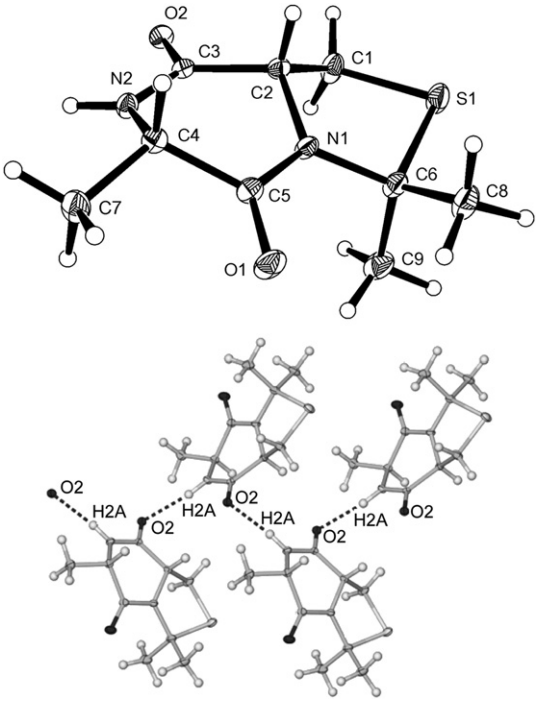


Figure 4. (Upper) Plot of the asymmetric unit in the X-ray crystal structure of *cyclo*-L-Ala-*S*-Dmt **23b**. Ellipsoids are represented at 30% probability. (Lower) Intermolecular hydrogen-bonding pattern for **23b** in the crystal.

^5J=0.8 Hz was observed between the axial 8a-H and the axial 6-H, confirming the boat conformation.[33]

6.3. Crystal structure of *cyclo*-L-Val-*R*-Dmt 23c

As for *cyclo*-L-Ala-*R*-Dmt **23b**, a crystal of *cyclo*-L-Val-*R*-Dmt **23c** was grown from ethyl acetate/hexane and the X-ray crystal structure was determined. Figure 5 shows the intermolecular hydrogen-bonding pattern evident in the crystal and the structure of a single molecule, together with the crystallographic numbering scheme. The intermolecular hydrogen-bonding motif in this crystal is different from that in the crystal of *cyclo*-L-Ala-*R*-Dmt **23b**, above, in that the hydrogen-bonded pairs are formed between the N–H hydrogens of each of a pair of molecules and the secondary amide carbonyls. Again, the tertiary amide carbonyls do not take part in hydrogen-bonding.

The X-ray crystallographic study of *cyclo*-L-Val-*R*-Dmt **23c** shows that this molecule adopts a conformation similar to that of *cyclo*-L-Ala-*R*-Dmt **23b**. Again, the DKP ring is in a boat conformation, with the thiazolidine in a half-chair. The geminal methyl groups at position-3 are in equatorial and axial positions; the 6-isopropyl occupies a *pseudo*-equatorial position. This 6-isopropyl group is again remote in space from the bulky geminal dimethyl unit. The solution conformation of *cyclo*-L-Val-*R*-Dmt **23c** in $(CD_3)_2SO$ is similar, as shown by the ^{1}H NMR spectrum. The ^3J coupling constants between the axial 8a-H and the 1_β-H and 1_α-H are 11.7 and 5.9 Hz, respectively, and are again consistent with the dihedral angles (8a-H)-(8a-C)-(1-C)-(1_β-H) of 169° and (8a-H)-(8a-C)-(1-C)-(1_α-H) of 41°. In this case, the coupling between the axial 6-H and the adjacent N–H is too small to be resolved; the dihedral angle in the crystal structure is 98°. The diagnostic five-bond coupling ^5J= 1.2 Hz was again observed between the axial 8a-H and the axial 6-H, confirming the boat conformation in solution.[33] Turning to the conformation of the side-chain, a ^3J=2.3 Hz coupling is seen between 6-H and the adjacent Me_2CH; in the solid state, the corresponding dihedral angle is 70°. Interestingly, Young et al.[32] reported an analogous coupling constant ^3J=2.7 Hz for coupling between the Val α-H and the Val β-H in *cyclo*-L-Val-L-Pro, which, together with lanthanide shift-reagent studies, is interpreted as showing that this DKP adopts the same conformation with respect to the isopropyl side-chain.

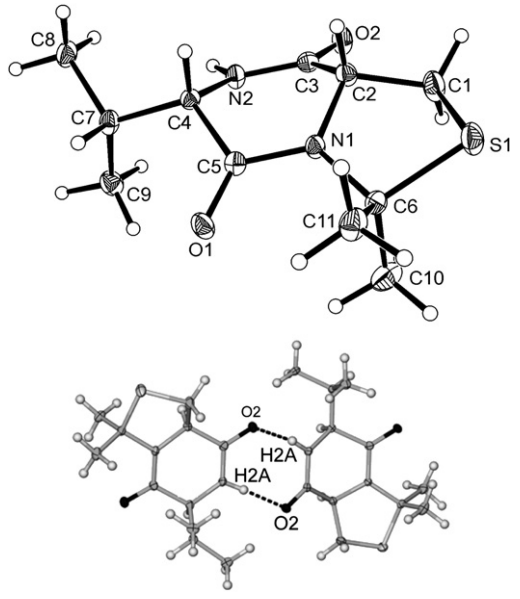


Figure 5. (Upper) Plot of one molecule in the asymmetric unit of *cyclo*-L-Val-*R*-Dmt **23c**. Ellipsoids are represented at 30% probability. (Lower) Intermolecular hydrogen-bonding pattern for **23b** in the crystal.

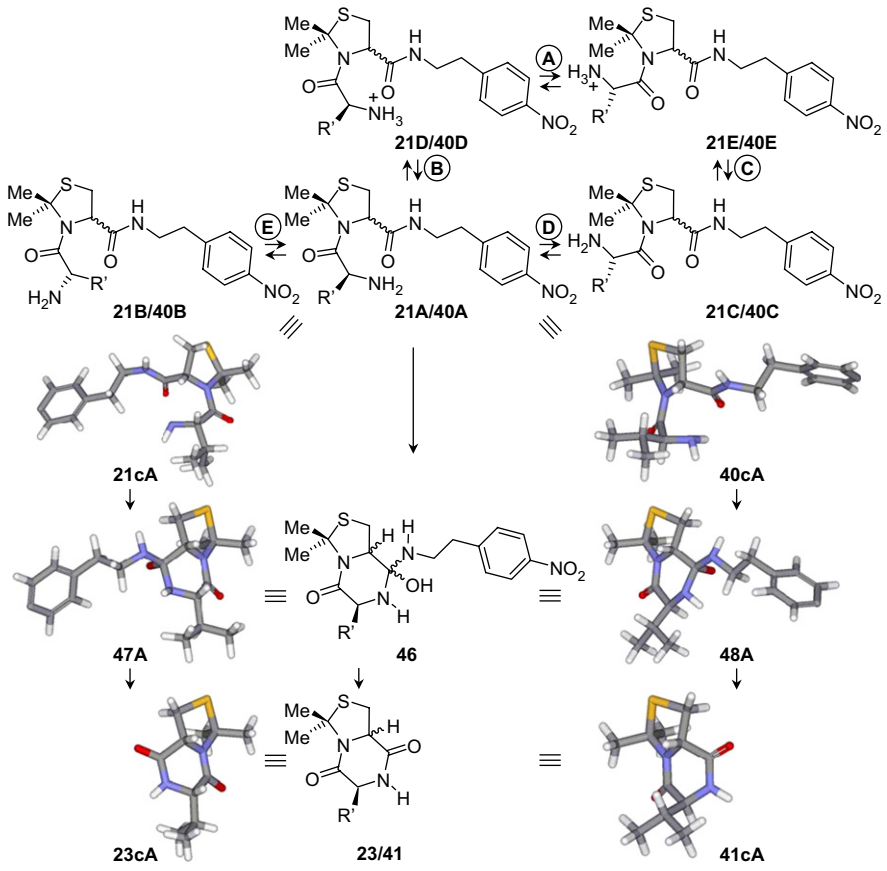
6.4. NMR studies—L,*S* diastereomeric series

In the L,*S* diastereomeric series, neither *cyclo*-L-Val-*S*-Dmt **41c** nor *cyclo*-L-Leu-*S*-Dmt **41e** formed crystals of a suitable quality for crystallography. However, ^{1}H NMR spectroscopy allowed some inferences to be made about their conformations in solution.

In contrast to the L,*R* series, where the coupling constant between 6-H and the adjacent N–H was very small, this coupling was much larger in the L,*S* series, with ^3J=3.5 Hz for **41c** and with ^3J=5.0 Hz for **41e**. These values are inconsistent with the boat conformations of the DKP rings but indicate that the DKPs are likely to be flattened from the boat form. This flattening would relieve steric compression between the 6-side-chain substituents (Me_2HC– or Me_2CHCH_2–) and 8a-H. Indeed the coupling constants show that the flattening is greater in **41c** than in **41e**, reflecting the greater steric demand of the isopropyl group compared to that of the 2-methylpropyl group. With the DKP tending towards planarity, the side chains are remote in space from the geminal dimethyl unit. As expected, 8a-H did not couple with 6-H in either compound in this series, since these protons are now trans and no longer 1,4-diaxial. The conformations of the five-membered rings are likely to be half chairs, as indicated by the coupling constants between 8a-H (now on the β-face) and 1_α-H (10.9 Hz for **41c** and 10.5 for **41e**) and between 8a-H and 1_β-H (^3J=5.5 Hz for **41c** and ^3J=5.9 Hz for **41e**).

7. Conclusions

At pH 8.0 and 7.0, there are trends relating the bulk of the side-chain of the N-terminal amino-acid and the rate of cyclisation. There are also marked differences in the rate of cyclisation between the diastereoisomers. As shown in Table 1, in the L,*R* series, the fastest rates are observed for L-Ala-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **21b** at both pH values. Increasing the steric bulk of the side-chain from Me to isopropyl or benzyl (in L-Val-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **21c** or L-Phe-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **21d**, respectively) slows the cyclisation by ca. seven-fold. Interestingly, the smallest analogue, Gly-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **21a**, also cyclised some three-fold slower than did **21b**. Scheme 7 shows some of the relevant conformational and prototropic equilibria involved in the cyclisation, which aid in the rationalisation of these observations. Firstly, cyclisation can only occur from the free-base forms **21A/40A**, **21B/40B** and **21C/40C**, in which the amine is nucleophilic, rather than from the cationic protonated forms **21D/40D** and **21E/40E**. However, the prototropic equilibria B and C would be rapid and the pK_a of **21D/40D** and **21E/40E** would be expected to be largely independent of the steric bulk of the



Scheme 7. Cyclisation of dipeptide *N*-(2-(4-nitrophenyl)ethyl)amides to form diketopiperazines, with expulsion of **24**, showing selected conformations and prototropic equilibria of starting dipeptide amides and reacting conformations of intermediates. The sequence **21A/40A**→**46**→**23/41** shows the general course of the reaction; sequences **21cA**→**47A**→**23cA** and **40cA**→**48A**→**41cA** show the specific structures for the diastereoisomers of the L-Val-derived analogues. Structures **21cA**, **47A**, **40cA**, **48A** and **41cA** are derived from energy-minimising MM2 calculations; structure **23cA** is an X-ray crystal structure. In structures **21cA**, **47A**, **40cA** and **48A**, the nitro group has been omitted for clarity.

side-chain R′. Thus, equilibria B and C can be discounted as sources of the differences in rates of ring-closure. As the core peptide bond in all the dipeptides is a tertiary amide, the populations of the *Z* and *E* amide rotamers in the reaction mixtures could be predicted to be an important determinant of the rate of cyclisation, since only the Z conformer places the primary amine sufficiently close to the secondary amide carbonyl carbon, the electrophile in the key step. Since the rate of interconversion of the tertiary amide rotamers is relatively slow at ambient temperature, the 2,2-dimethylthiazolidine unit was designed to bias the equilibrium (equilibrium D, Scheme 7) towards the required *Z* conformer **21A/40A**. As noted above, it was not possible to measure the populations of the tertiary amide bond rotamers directly in the free-base dipeptide amides **21A/40A**, owing to the possible perturbation of the equilibrium by the cyclisation reaction consuming one of the components. However, ^{1}H NMR spectra of the protonated (and thus unreactive) forms of salts **21a–d** showed that each existed solely in the *Z* tertiary amide conformation, irrespective of the bulk of the side-chain R′. Moreover, the state of protonation of L-Ala-L-ProNH_2 has been reported to have little effect on the cis/trans conformational equilibrium.[14] Thus, equilibrium A is not relevant to the effects of side-chain bulk on the rate of cyclisation. Similarly, as discussed above, the electrically neutral *N*-Boc precursors **20a–d** were shown to exist solely or >90% as the *Z* tertiary amide rotamers. Taken together, these conformational data suggest that the free-base dipeptide amides are likely to be all (almost) exclusively in the reactive *Z* tertiary amide conformation.

If the bulk of the side-chain R′ does not influence the rate of cyclisation by perturbing the *Z*/*E* tertiary amide rotamer population in the starting materials, then the question remains at which point in the cyclisation process does the nature of R′ have its effect? Since the primary amine has to be located close to the secondary amide carbonyl as the molecules move towards the first transition state, the conformation about the carbonyl–C_α bond in the N-terminal amino-acid is also important, i.e., the dipeptides must adopt conformer **21A** (Scheme 7), rather than conformers such as **21B**, in which the amine is pointing away from the target secondary amide. No data are available on the populations of these two types of conformers but one may speculate, on steric grounds, that the unreactive conformer **21** was more likely when R′=H in Gly-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **21a** and the reactive conformer **21** is more favoured when R′ is large, in L-Ala-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **21a**, L-Val-*R*-DmtNH$(CH_2)_2$-$C_6H_4NO_2$ **21c** and L-Phe-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **21d**. This would rationalise the slow cyclisation of the Gly analogue.

Now, comparing the rates of formation of DKPs from the remaining members of the L,*R* diastereomeric series, is the steric bulk of R′ exerting its effect on the first step of

the reaction, as the primary amine of the reactive conformers **21** attack the secondary amide carbonyl to form the tetrahedral intermediates **46** or is this effect evident in the second step of the reaction, the collapse of the intermediate to form the DKP **23/41** with expulsion of the leaving group 2-(4-nitrophenyl)ethylamine **24**? To answer this question, the structures of the key intermediates were compared. Structure **21cA** (Scheme 7) is a 3-D representation of the reacting conformation of **21c** (R′=isopropyl, derived from L-Val), generated using a MM2-minimised structure. Similarly, structure **47A** is a 3-D representation of the minimised structure of intermediate **46** (R′=isopropyl, derived from L-Val) and structure **23cA** is a 3-D representation of the crystal structure of DKP **23c** (R′=isopropyl, derived from L-Val). Examination of the DKP structure **23cA**, as discussed in detail above, shows that the isopropyl group is *pseudo*-equatorial in the boat conformation of the DKP ring and is thus remote from the geminal dimethyl unit and, indeed, from all other sterically demanding moieties. The conformations of the other DKPs in the L,*R* series are similar. Thus, increasing the steric bulk of R′ in this series would not cause increase in the steric crowding in the DKPs **23** and it is therefore highly unlikely that the effect of bulky R′ on slowing the overall reaction rate derives influences from the second step, the collapse of the tetrahedral intermediate. Similarly, the tetrahedral intermediates **46** are shown to have *pseudo*-chair conformations for the six-membered ring, as shown in the L-Val-derived analogue structure **47A**. In this conformation, again, the R′ group occupies a sterically open region of space and thus variations in steric bulk of R′ in this intermediate are similarly unlikely to influence the overall reaction rate significantly.

However, the detail of the conformation required for the starting dipeptide amide **21** as it enters the initial nucleophilic reaction reveals the origin of the rate-slowing effect of bulky R′. As can be seen in structure **21cA** (Scheme 7, derived from L-Val), the conformation in which the primary amine can approach the target electrophile requires a particular local conformation about the carbonyl–C_α bond in which R′ is fully eclipsing the carbonyl oxygen. Thus increasing the steric bulk of R′ will make this conformation increasingly unfavourable and thus slows the initial step of the cyclisation.

In the L,*S* diastereomeric series, which also includes Gly-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **21a**, the opposite trend in overall cyclisation reaction rates is evident, with increasing *local* steric bulk of the side-chain R′ tending to speed the reaction (Table 1). The Gly analogue **21a** may be a special case, as noted above, involving unfavourable populations of the conformers in equilibrium E (Scheme 7). Additionally, the rates of cyclisation of the major members of this series, L-Val-*S*-DmtNH$(CH_2)_2C_6H_4NO_2$ **40c** and L-Leu-*S*-DmtNH$(CH_2)_2C_6H_4NO_2$ **40e**, are also much faster than those of the L,*R* series, with the L,*S* Val analogue **40c** reacting ca. 100 times faster than its diastereoisomer **21c**. As with the L,*R* series, examination of the structures of the reacting conformations enabled rationalisation of the relative rates. Structure **41cA** shows the conformation of the L-Val derived DKP, as discussed above. In this structure, the isopropyl group is not close in space to either of the geminal dimethyl groups, so variation of this group would not be expected to influence the energy of the DKP greatly. This group is also remote from the methyl groups in intermediate **48A**; thus steric crowding is also unimportant here. However, in the L,*S* series, as with the L,*R* series, it is the steric crowding in the reacting conformation of the initial dipeptide amide substrate that is critical. As shown in example structure **40cA** (Scheme 7), the reactive conformation of the carbonyl–C_α bond has the α-hydrogen almost eclipsing the tertiary amide carbonyl oxygen. This arrangement is clearly much more favourable on steric grounds than that in the L,*R* diastereoisomer **21cA**, with R′ eclipsing this oxygen. Thus the reacting conformation is more sterically accessible in the L,*S* series, facilitating the rapid formation of the tetrahedral cyclic intermediates. Comparing the rates of cyclisation of the L-Val analogue **40c** and the L-Leu analogue **40e**, one may then postulate that the greater steric demand of the tertiary isopropyl centre may drive the molecule more effectively into this reacting conformation than does the lesser demand of the secondary centre of the isobutyl group.

The cyclisation of peptides containing Dmt to give DKPs has not previously been studied. A related system, the cyclisation of L-alanyl-L-prolinamide in water to give *cyclo*-L-Ala-L-Pro and ammonia, was the subject of a detailed kinetic analysis by Capasso et al.[13] This is a relatively slower process than the cyclisations of aminoacyl-Dmt amides reported in this paper. In the $AlaProNH_2$ study, it was noted that the trans→cis conformational interchange of the tertiary amide was fast[36,37] and became rate-limiting only under conditions were the cyclisation was fast.[13] Interestingly, Sager et al.[38] note that the cis conformers of pentapeptides containing L-Pro or L-Dmt can be readily cyclised to give cyclic pentapeptides but the corresponding trans conformers tend to produce cyclic dimers (cyclic decapeptides) under the same conditions. Limitation of rate in this conformational interchange step may be important, in principle, in the cyclisations here, particularly those in the L-Aaa-*S*-DmtNH$(CH_2)_2C_6H_4NO_2$ diastereomeric series, but it is unlikely to affect the global rate of reaction since the vast majority of these reactant molecules are already in the cis conformation. As expected, pH had a marked effect on the rate of cyclisation of L-Ala-L-$ProNH_2$, where the trend was for the reaction to be faster at higher pH.[13] However, replotting the data to account for the fraction of the reactive free-base form in solution indicated that the cyclisation of this form was faster at high and low pH values and had a minimum between pH 7 and 10. These observations were used to support parallel mechanisms involving general-base catalysis and general-acid catalysis.[13] In the present case, there was a trend towards faster cyclisation at higher pH within the range pH 6.0–8.0, which correlated with increased fractions of the unprotonated reactive forms, but reactions at more extreme pH values (outside the physiological range) were not studied.

In this paper, we have reported the cyclisations of two diastereomeric series of aminoacyl-Dmt *N*-(2-(4-nitrophenyl)-ethyl)amides, giving the cyclic dipeptides (DKPs) and releasing 2-(4-nitrophenyl)ethylamine as a model for drugs carrying primary amines. The rate of ring-closure and release depends on the relative configurations of the two chiral centres, the L,*S* dipeptide amides react much more rapidly. Each of the Aaa-Dmt dipeptides adopts 80–100% the *cis* tertiary amide conformation; thus the rate of ring-closure is

effectively independent of cis–trans interconversion. Thus the L-Val-L-Dmt and L-Leu-L-Dmt units are shown to be highly effective self-immolative molecular clips for incorporation into prodrug linkers designed for cleavage/triggering by endopeptidases. These linkers release amine drugs rapidly, whereas the existing dipeptide cyclisation technology[11,12] is effective only in releasing OH-containing drugs where the ester is a better electrophile and the alcohol is a better leaving group. Aminoacyl-Dmt units, therefore, have great potential utility in prodrug design and development.

8. Experimental

8.1. General

^{1}H NMR spectra were recorded on Varian GX270 or EX400 spectrometers of samples in $CDCl_3$, unless otherwise stated. IR spectra were recorded on a Perkin–Elmer 782 spectrometer as KBr discs, unless otherwise stated. Mass spectra were obtained using fast atom bombardment (FAB) ionisation in the positive ion mode, unless otherwise stated. The chromatographic stationary phase was silica gel. DCC refers to *N*,*N*′-dicyclohexylcarbodiimide, THF refers to tetrahydrofuran, DMF refers to dimethylformamide, HOBt refers to 1-hydroxybenzotriazole, DMAP refers to 4-dimethylaminopyridine and citric acid refers to a 5% aqueous solution of 3-carboxy-3-hydroxypentanedioic acid. THF was dried with Na. Solutions in organic solvents were dried with $MgSO_4$. Solvents were evaporated under reduced pressure. The aqueous $NaHCO_3$ and brine were saturated. Experiments were conducted at ambient temperature, unless otherwise stated. Melting points were measured either with a Thermo Galen Kofler block (uncorrected) or with a differential scanning calorimeter.

8.2. *R*-2,2-Dimethyltetrahydrothiazole-4-carboxylic acid hydrochloride (11)

L-Cys·HCl·H_2O **10** (3.5 g, 20 mmol) was boiled under reflux in acetone (250 mL) and 2,2-dimethoxypropane (50 mL) for 6 h under N_2. The solid was collected by filtration to afford **11** (3.45 g, 87%) as a white solid: mp 125–130 °C (lit.[27] mp 165–168 °C); IR ν_{max} 3412, 2600, 1744 cm^{-1}; NMR (D_2O) δ_H 2.06 (6H, s, 2×Me), 2.92 (1H, dd, *J*=15.2, 3.9 Hz, 5-H), 3.00 (1H, dd, *J*=15.2, 5.9 Hz, 5-H), 4.06 (1H, dd, *J*=5.9, 3.9 Hz, 4-H).

8.3. *R*-3-(1,1-Dimethylethoxycarbonyl)-2,2-dimethyltetrahydrothiazole-4-carboxylic acid (12)

Compound **11** (1.0 g, 5.1 mmol) was stirred with Pr^i_2NEt (722 mg, 5.6 mmol) and di-*tert*-butyl dicarbonate (1.45 g, 6.6 mmol) in dry MeCN (10 mL) for 2 days. The evaporated residue, in Et_2O, was filtered (Celite®). The evaporated residue, in CH_2Cl_2, was washed with aq H_2SO_4 (100 mM, cold), H_2O and brine. Drying, evaporation and recrystallisation (hexane) afforded **12** (150 mg, 11%) as a white solid: mp 112–114 °C (lit.[27] mp 114–114.5 °C); NMR ($CDCl_3$, 20 °C) δ_H 1.43 (4.5H, br s) and 1.53 (4.5H, br s, Bu^t), 1.80 (4.5H, m) and 1.87 (1.5H, br s, 2×Me), 3.1–3.28 (2H, m, 5-H_2), 4.83 (1H, m) and 4.98 (1H, m, 4-H); NMR ($(CD_3)_2SO$, 20 °C) δ_H 1.35 (5.4H, br s) and 1.43 (3.6H, br s) (Bu^t), 1.70 (br s), 1.73 (br s) and 1.75 (br s, 2×Me), 3.03 (d, *J*=12.1 Hz) and 3.34 (m, 5-H_2), 4.67 (0.6H, dd, *J*=4, 2 Hz) and 4.74 (0.4H, br d, *J*=5 Hz, 4-H); NMR ($(CD_3)_2SO$, 80 °C) δ_H 1.41 (9H, s, Bu^t), 1.73 (3H, s, 2-Me), 1.77 (3H, s, 2-Me), 3.05 (1H, dd, *J*=12.1, 3.0 Hz, 5-H), 3.35 (1H, dd, *J*=12.1, 6.9 Hz, 5-H), 4.72 (1H, dd, *J*=6.9, 3.0 Hz, 4-H).

8.4. Pentafluorophenyl *R*-3-(1,1-dimethylethoxycarbonyl)-2,2-dimethyltetrahydrothiazole-4-carboxylate (13)

Compound **12** (1.10 g, 4.2 mmol) was stirred with pentafluorophenol (850 mg, 4.6 mmol) and DCC (850 mg, 4.6 mmol) in EtOAc (10 mL) at 0 °C under N_2 for 3 h. Filtration and evaporation gave **13** (1.62 g, 90%) as white needles: mp 107–109 °C (lit.[27] mp 104–105 °C); NMR δ_H 1.46 (6.3H, s, Bu^t), 1.52 (2.7H, s, Bu^t), 1.80 (0.9H, s, 2-Me), 1.83 (3H, s, 2-Me), 1.90 (2.1H, s, 2-Me), 3.28 (0.7H, br d, *J*=12.5 Hz, 5-H), 3.41 (0.3H, m, 5-H), 3.47 (1H, dd, *J*=12.5, 7.0 Hz, 5-H), 4.05 (0.3H, m, 5-H), 5.18 (0.7H, dd, *J*=6.6, 1.6 Hz, 4-H), 5.26 (0.3H, dd, *J*=6.6, 2.3 Hz, 4-H); NMR δ_F −162.2 (0.3F, m, 4′-F), −161.8 (0.7F, m, 4′-F), −157.8 (0.6F, t, *J*=21.1 Hz, 3′,5′-F_2), −157.3 (1.4F, t, *J*=21.1 Hz, 3′,5′-F_2), −152.6 (1.4F, d, *J*=17.2 Hz, 2′,6′-F_2), −151.90 (0.6F, d, *J*=17.2 Hz, 2′,6′-F_2).

8.5. *R*-2,2-Dimethyl-3-(1,1-dimethylethoxycarbonyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (14)

2-(4-Nitrophenyl)ethylamine hydrochloride **24**·HCl (85 mg, 0.42 mmol) was stirred with Et_3N (85 mg, 0.84 mmol) and **13** (180 mg, 0.42 mmol) in CH_2Cl_2 (2.0 mL) for 2 h. Evaporation and chromatography ($CHCl_3$/MeOH, 9:1) afforded **14** (160 mg, 93%) as a viscous yellow oil: IR (film) ν_{max} 3325, 1681, 1519, 1346 cm^{-1}; NMR δ_H 1.43 (9H, s, Bu^t), 1.70 (3H, s, 2-Me), 1.75 (3H, s, 2-Me), 2.95 (2H, m, ArCH_2), 3.23 (2H, m, 5-H_2), 3.53 (1H, m) and 3.68 (1H, m, NHCH_2), 4.74 (1H, m, 4-H), 7.42 (2H, d, *J*=8.6 Hz, Ar 2,6-H_2), 8.18 (2H, d, *J*=8.6 Hz, Ar 3,5-H_2); NMR δ_C 19.6, 28.7, 36.0, 40.7, 67.5, 82.0, 93.7, 110.0, 124.0, 129.9, 146.9, 171.3; MS *m*/*z* 410.1764 (M+H) ($C_{19}H_{28}N_3O_5S$ requires 410.1750), 354 (M−Me_2C=CH_2), 336 (M−Bu^{t}O), 310 (M−Boc). Found: H, 6.59; N, 9.94. $C_{19}H_{27}N_3O_5S$ requires H, 6.65; N, 10.27%.

8.6. *R*-2,2-Dimethyl-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide trifluoroacetate salt (15)

Compound **14** (1.30 g, 3.2 mmol) was stirred with CF_3CO_2H (20 mL) and CH_2Cl_2 (10 mL) for 2 h. Evaporation afforded **15** (1.35 g, quant.) as a highly hygroscopic gum: NMR δ_H 1.82 (3H, s, 2-Me), 1.93 (3H, s, 2-Me), 2.97 (2H, m, ArCH_2), 3.20 (1H, dd, *J*=12.5, 6.2 Hz, 5-H), 3.59 (1H, dd, *J*=12.5, 8.2 Hz, 5-H), 3.64 (2H, m, NHCH_2), 5.22 (1H, dd, *J*=8.2, 6.2 Hz, 4-H), 7.35 (2H, d, *J*=8.6 Hz, Ar 2,6-H_2), 7.56 (1H, br t, *J*=5.9 Hz, NH), 8.15 (2H, d, *J*=8.6 Hz, Ar 3,5-H_2), 9.76 (2H, br, N^+H_2); MS *m*/*z* 310.1238 (M+H) ($C_{14}H_{20}N_3O_3S$ requires 310.1225).

8.7. 1,1-Dimethylethyl *N*-(fluorocarbonylmethyl)carbamate (17a)

BocGlyOH **16a** (1.0 g, 5.7 mmol) was stirred with pyridine (450 mg, 5.7 mmol) in dry CH_2Cl_2 (30 mL) and added

slowly to 2,4,6-trifluoro-1,3,5-triazine (1.54 g, 11.4 mmol) in dry CH_2Cl_2 (10 mL), then stirred for 3 h. The mixture was washed with ice-water (3×). Drying and evaporation afforded crude **17a** (800 mg) as a white gum: NMR δ_H 1.47 (9H, s, Bu^t), 4.11 (2H, t, J=5.5 Hz, CH_2), 5.05 (1H, br, NH); NMR δ_F 30.21 (1F, s, CO_2F).

8.8. 1,1-Dimethylethyl *S*-*N*-(1-fluorocarbonyl-2-methylpropyl)carbamate (17c)

BocValOH **16c** (2.5 g, 11 mmol) was stirred with pyridine (910 mg, 11.5 mmol) in dry CH_2Cl_2 (30 mL) and added slowly to 2,4,6-trifluoro-1,3,5-triazine (3.1 g, 23 mmol) in dry CH_2Cl_2 (30 mL). Stirring was continued under N_2 at −10 °C for 2 h. The mixture was washed with ice-water (3×100 mL). Drying and evaporation afforded crude **17c** (2.22 g) as a white solid: mp 38–42 °C (lit.[39] mp 36–38 °C); NMR δ_H 0.99 (3H, d, J=7.0 Hz, Val-Me), 1.04 (3H, d, J=6.6 Hz, Val-Me), 1.46 (9H, s, Bu^t), 2.25 (1H, m, Val β-H), 4.40 (1H, dd, J=8.6, 4.7 Hz, Val α-H), 4.96 (1H, br d, NH); NMR δ_F 32.29 (1F, s, CO_2F).

8.9. 1,1-Dimethylethyl *S*-*N*-(1-fluorocarbonyl-2-phenylethyl)carbamate (17d)

BocPheOH **16d** was treated with pyridine and 2,4,6-trifluoro-1,3,5-triazine, as for the synthesis of **17c**, to give crude **17d** (57%) as a colourless oil: NMR δ_H 1.42 (9H, s, Bu^t), 3.15 (1H, dd, J=14.4, 6.2 Hz, Phe β-H), 3.17 (1H, dd, J=14.4, 5.9 Hz, Phe β-H), 4.74 (1H, br d, J=5.9 Hz, Phe α-H), 4.86 (1H, d, J=6.6 Hz, NH), 7.10–7.30 (5H, m, Ph-H_5); NMR δ_F 29.21 (1F, s, CO_2F).

8.10. *R*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)glycyl)-2,2-dimethyltetrahydrothiazole-4-carboxylic acid (18a)

Compound **17a** (810 mg, 4.6 mmol) was stirred with **11** (1.00 g, 5.1 mmol) and Pr^i_2NEt (1.24 g, 9.6 mmol) in dry DMF (100 mL) for 3 h. The evaporated residue, in EtOAc, was washed with cold citric acid and brine. Drying, evaporation and chromatography (EtOAc/Et_2O/AcOH, 13:6:1) afforded **18a** (560 mg, 35%) as a pale buff solid: mp 152–155 °C: IR ν_{max} 3385, 1748, 1672, 1625, 1539 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 1.38 (9H, s, Bu^t), 1.74 (3H, s, 2-Me), 1.76 (3H, s, 2-Me), 3.28 (2H, m, 5-H_2), 3.50 (1H, dd, J=17.2, 6.2 Hz) and 3.87 (1H, dd, J=16.8, 5.5 Hz, Gly-H_2), 5.10 (1H, m, 4-H), 6.78 (1H, t, J=6 Hz, NH); MS m/z 319.1339 (M+H) ($C_{13}H_{23}N_2O_5S$ requires 319.1328), 263 (M−Me_2C=CH_2). Found: C, 47.80; H, 6.72; N, 8.85. $C_{13}H_{22}N_2O_5S\cdot0.5H_2O$ requires C, 47.99; H, 6.51; N, 8.61%.

8.11. 1,1-Dimethylethyl *S*-*N*-(1-(fluorocarbonyl)ethyl)-carbamate (17b) and *R*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-alanyl)tetrahydrothiazole-4-carboxylic acid (18b)

BocAlaOH **16b** was treated with pyridine and 2,4,6-trifluoro-1,3,5-triazine, as for the synthesis of **17c**, to give crude **17b** as a white solid. This material was treated with **11**, as for the synthesis of **18a** except that the chromatographic eluant was EtOAc/Et_2O/AcOH (49:49:2), to give **18b** (30% from **11**) as a pale yellow solid: mp 93–94 °C: IR ν_{max} 3338, 1705, 1655, 1534 cm^{-1}; NMR δ_H 1.31 (3H, d, J=6.6 Hz, Ala-Me), 1.40 (9H, s, Bu^t), 1.81 (3H, s, 2-Me), 1.92 (3H, s, 2-Me), 3.24 (1H, dd, J=12.1, 5.5 Hz, 5-H), 3.45 (1H, d, J=12.1 Hz, 5-H), 4.53 (1H, qn, J=7 Hz, Ala α-H), 4.85 (1H, d, J=5.1 Hz, 4-H), 5.68 (1H, d, J=7.8 Hz, NH); MS m/z 333.1489 (M+H) ($C_{14}H_{25}N_2O_5S$ requires 333.1484), 277 (M−Me_2C=CH_2), 259 (M−Bu^{t}O).

8.12. *R*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-valinyl)tetrahydrothiazole-4-carboxylic acid (18c)

Compound **17c** was treated with **11**, as for the synthesis of **18a** except that the chromatographic eluant was hexane/EtOAc/AcOH (70:29:1), to give **18c** (27%) as a colourless gummy solid: IR ν_{max} 3338, 1705, 1655, 1534 cm^{-1}; NMR δ_H 0.90 (3H, d, J=7.0 Hz, Val-Me), 1.0 (3H, d, J=6.6 Hz, Val-Me), 1.39 (9H, s, Bu^t), 1.82 (3H, s, 2-Me), 1.88 (1H, m, Val β-H), 1.92 (3H, s, 2-Me), 3.21 (1H, dd, J=12.1, 5.5 Hz, 5-H), 3.44 (1H, d, J=12.1 Hz, 5-H), 4.50 (1H, dd, J=9.4, 5.5 Hz, Val α-H), 5.02 (1H, d, J=5.1 Hz, 4-H), 5.60 (1H, d, J=9.4 Hz, NH), 8.52 (1H, br, OH); MS m/z 361.1822 (M+H) ($C_{16}H_{29}N_2O_5S$ requires 361.1797), 305 (M−Me_2C=CH_2), 261 (M−Boc).

8.13. *R*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-phenylalanyl)tetrahydrothiazole-4-carboxylic acid (18d)

Compound **17d** was treated with **11**, as for the synthesis of **18a** except that the chromatographic eluant was EtOAc/AcOH (99:1), to give **18d** (30%) as a pale yellow gum: NMR δ_H 1.29 (9H, s, Bu^t), 1.68 (3H, s, 2-Me), 1.77 (3H, s, 2-Me), 2.80 (1H, dd, J=13.3, 7.1 Hz, Phe β-H), 2.88 (1H, dd, J=13.3, 6.1 Hz, Phe β-H), 2.97 (1H, dd, J=11.7, 5.9 Hz, 5-H), 3.12 (1H, d, J=11.7 Hz, 5-H), 4.34 (1H, ddd, J=9.0, 7.1, 6.1 Hz, Phe α-H), 4.68 (1H, d, J=5.9 Hz, 4-H), 6.50 (1H, d, J=9.0 Hz, NH), 7.26 (5H, m, Ph-H_5); MS m/z 409.1812 (M+H) ($C_{20}H_{29}N_2O_5S$ requires 409.1797), 353 (M−Me_2C=CH_2), 335 (M−Bu^{t}O), 309 (M−Boc).

8.14. Pentafluorophenyl *R*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)glycyl)tetrahydrothiazole-4-carboxylate (19a)

Compound **18a** (450 mg, 1.4 mmol) was stirred with pentafluorophenol (290 mg, 1.6 mmol) and DCC (330 mg, 1.6 mmol) in EtOAc (5.0 mL) at 0 °C under N_2 for 3 h. The mixture was filtered (Celite®) and the solid was washed with cold EtOAc. The evaporated residue, in hexane, was kept at 4 °C for 16 h and filtered. Evaporation gave **19a** (700 mg, 92%) as a colourless oil: NMR δ_H 1.45 (9H, s, Bu^t), 1.88 (3H, s, 2-Me), 1.92 (3H, s, 2-Me), 3.45 (1H, dd, J=12.5, 5.5 Hz, 5-H), 3.51 (1H, d, J=12.1 Hz, 5-H), 3.67 (1H, dd, J=17.2, 3 Hz) and 4.13 (1H, dd, J=17.2, 6.6 Hz) (Gly-H_2), 5.20 (1H, d, J=5.3 Hz, 4-H), 5.37 (1H, br, NH); NMR δ_F −161.3 (2F, m, 3′,5′-F_2), −156.4 (1F, t, J=21.4 Hz, 4′-F), −152.0 (2F, d, J=16.8 Hz, 2′,6′-F_2); MS m/z 485.1185 (M+H) ($C_{19}H_{22}F_5N_2O_5S$ requires 485.1170), 429 (M−Me_2C=CH_2), 411 (M−Bu^{t}O), 385 (M−Boc).

8.15. Pentafluorophenyl *R*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-alanyl)tetrahydrothiazole-4-carboxylate (19b)

Compound **18b** was treated with pentafluorophenol and DCC, as for the synthesis of **19a**, to give **19b** (89%) as a colourless oil: NMR δ_H 1.36 (2.1H, d, J=6.6 Hz, Ala-Me), 1.37 (0.9H, d, J=6.6 Hz, Ala β-H_3), 1.39 (6.3H, s, Bu^t), 1.43 (2.7H, s, Bu^t), 1.85 (2.1H, s, 2-Me), 1.94 (2.1H, s, 2-Me), 2.05 (0.9H, s, 2-Me), 2.09 (0.9H, s, 2-Me), 3.33 (0.3H, dd, J=12.7, 2.9 Hz, 5-H), 3.40 (0.7H, dd, J=12.5, 5.5 Hz, 5-H), 3.46 (0.3H, m, 4-H), 3.49 (0.7H, d, J=12.1 Hz, 4-H), 4.39 (0.7H, qn, J=7 Hz, Ala α-H), 4.79 (0.3H, qn, J=7 Hz, Ala α-H), 5.19 (0.7H, d, J=5.5 Hz, 4-H), 5.24 (0.3H, br, NH), 5.40 (0.7H, d, J=7.8 Hz, NH), 5.57 (0.3H, dd, J=6.6, 3.1 Hz, 4-H); NMR δ_F −161.7 (0.6F, m, 3′,5′-F_2), −161.6 (1.4F, dt, J=21.0, 18.4 Hz, 3′,5′-F_2), −157.1 (0.3F, t, J=21.0 Hz, 4′-F), −156.7 (0.7F, t, J=21.0 Hz, 4′-F), −151.9 (0.6F, d, J=17.1 Hz, 2′,6′-F_2), −150.8 (1.4F, d, J=17.1 Hz, 2′,6′-F_2); MS *m/z* 499.1335 (M+H) ($C_{20}H_{24}F_5N_2O_5S$ requires 499.1326), 443 (M−Me_2C=CH_2), 425 (M−Bu^{t}O).

8.16. Pentafluorophenyl 2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-valinyl)tetrahydrothiazole-4-carboxylate (19c)

Compound **18c** was treated with pentafluorophenol and DCC, as for the synthesis of **19a**, to give **19c** (93%) as a colourless oil: IR (film) ν_{max} 3440, 1718, 1669, 1521 cm^{-1}; NMR δ_H 0.94 (2.1H, d, J=6.6 Hz, Val-Me), 0.99 (2.1H, d, J=6.6 Hz, Val-Me), 1.03 (0.9H, d, J=7.0 Hz, Val-Me), 1.10 (0.9H, d, J=7.0 Hz, Val-Me), 1.33 (6.3H, s, Bu^t), 1.43 (2.7H, s, Bu^t), 1.86 (2.1H, s, 2-Me), 1.91 (0.7H, m, Val β-H), 1.95 (2.1H, s, 2-Me), 1.99 (1.8H, s, 2×2-Me), 3.32 (0.3H, dd, J=12.5, 3.5 Hz, 5-H), 3.40 (0.7H, dd, J=12.5, 5.5 Hz, 5-H), 3.44 (0.3H, d, J=6.2 Hz, 5-H), 3.50 (0.7H, d, J=12.1 Hz, 5-H), 4.28 (0.7H, dd, J=9.0, 6.2 Hz, Val α-H), 4.54 (0.3H, dd, J=9.8, 6.2 Hz, Val α-H), 5.12 (0.3H, d, J=10.2 Hz, NH), 5.24 (0.7H, d, J=9.0 Hz, NH), 5.38 (0.7H, d, J=4.7 Hz, 4-H), 5.62 (0.3H, dd, J=6.6, 3.5 Hz, 4-H); NMR δ_F −161.9 (1.4F, dd, J=22.4, 18.4 Hz, 3′,5′-F_2), −161.6 (0.6F, dd, J=21.0, 17.1 Hz, 3′,5′-F_2), −157.1 (0.7F, dt, J=22.4 Hz, 4′-F), −152.6 (0.3F, dt, J=17.1 Hz, 4′-F), −151.8 (0.6F, d, J=17.1 Hz, 2′,6′-F_2), −150.3 (1.4F, d, J=18.4 Hz, 2′,6′-F_2); MS *m/z* 527.1660 (M+H) ($C_{22}H_{28}F_5N_2O_5S$ requires 527.1639), 471 (M−Bu^{t}O), 427 (M−Boc), 328 (M−BocVal).

8.17. Pentafluorophenyl *R*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-phenylalanyl)tetrahydrothiazole-4-carboxylate (19d)

Compound **18d** was treated with pentafluorophenol and DCC, as for the synthesis of **19a**, to give **19d** (92%) as a white solid: mp 35-36 °C; IR ν_{max} 3442, 1792, 1716, 1659, 1523 cm^{-1}; NMR δ_H 1.39 (9H, s, Bu^t), 1.77 (3H, s, 2-Me), 1.86 (3H, s, 2-Me), 2.40 (1H, dd, J=12.5, 5.5 Hz, 5-H), 2.89 (1H, dd, J=12.5, 10.9 Hz, Phe β-H), 3.00 (1H, d, J=12.5 Hz, 5-H), 3.25 (1H, dd, 12.5, 3.9 Hz, Phe β-H), 4.37 (1H, d, J=5.5 Hz, 4-H), 4.51 (1H, m, Phe α-H), 5.42 (1H, d, J=7.8 Hz, NH), 7.33 (5H, m, Ph-H_5); NMR δ_F −161.8 (2F, dd, J=21.0, 17.1 Hz, 3′,5′-F_2), −156.9 (1F, t, J=21.0 Hz, 4′-F), −150.7 (1F, d, J=18.4 Hz, 2′,6′-F_2); MS *m/z* 575.1635 (M+H) ($C_{26}H_{28}F_5N_2O_5S$ requires 575.1639), 519 (M−Me_2C=CH_2), 501 (M−Bu^{t}O), 475 (M−Boc).

8.18. *R*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)glycyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (20a)

Compound **19a** was treated with **24**·HCl and Et_3N, as for the synthesis of **14** except that the chromatographic eluant was EtOAc, to give **20a** (76%) as a white solid: mp 65–66 °C; NMR ($(CD_3)_2SO$, 80 °C) δ_H 1.38 (9H, s, Bu^t), 1.72 (3H, s, 2-Me), 1.74 (3H, s, 2-Me), 2.91 (2H, t, J=7.4 Hz, $ArCH_2$), 3.10 (1H, d, J=12.9 Hz, 5$_α$-H), 3.20 (1H, dd, J=16.4, 5.5 Hz, Gly-H), 3.31 (1H, m, 5$_β$-H), 3.39 (1H, m) and 3.47 (1H, m) ($ArCH_2CH_2$), 3.67 (1H, dd, J=16.4, 5.9 Hz, Gly-H), 4.79 (1H, d, J=5.5 Hz, 4-H), 6.67 (1H, br t, NH), 7.50 (2H, d, J=8.6 Hz, Ar 2,6-H_2), 8.13 (2H, d, J=8.6 Hz, Ar 3,5-H_2); MS *m/z* 467.1943 (M+H) ($C_{21}H_{31}N_4O_6S$ requires 467.1964), 411 (M−Me_2C=CH_2), 393 (M−Bu^{t}O), 367 (M−Boc).

8.19. *R*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-alanyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (20b)

Compound **19b** was treated with **24**·HCl and Et_3N, as for the synthesis of **14** except that the chromatographic eluant was EtOAc/hexane (4:1), to give **20b** (75%) as a white solid: mp 156–158 °C: IR ν_{max} 3319, 1696, 1657, 1601, 1520 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 1.12 (3H, d, J=7.0 Hz, Ala-Me), 1.37 (9H, s, Bu^t), 1.61 (3H, s, 2-Me), 1.71 (3H, s, 2-Me), 2.95 (2H, m, $ArCH_2$), 3.24 (1H, dd, J=12.1, 5.5 Hz, 5$_β$-H), 3.32 (2H, m, 5$_α$-H+$ArCH_2CH$), 3.53 (1H, m, $ArCH_2CH$), 3.84 (1H, m, Ala α-H), 4.73 (1H, d, J=5.5 Hz, 4-H), 7.06 (1H, d, J=5.9 Hz, NH), 7.49 (2H, d, J=9.0 Hz, Ar 2,6-H_2), 8.13 (2H, d, J=8.6 Hz, Ar 3,5-H_2) 8.13 (1H, br, NH); MS *m/z* 481.2142 (M+H) ($C_{22}H_{33}N_4O_6S$ requires 481.2121), 425 (M−Me_2C=CH_2), 381 (M−Boc).

8.20. *R*-(2,2-Dimethyl-3-(1,1-dimethylethoxycarbonyl)-L-valinyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (20c)

Compound **19c** was treated with **24**·HCl and Et_3N, as for the synthesis of **14** except that the chromatographic eluant was EtOAc/hexane (1:1), to give **20c** (73%) as a white solid: mp 233.8 °C (DSC); IR ν_{max} 3331, 1700, 1653, 1519 cm^{-1}; NMR δ_H 0.95 (3H, d, J=6.6 Hz, Val-Me), 1.01 (3H, d, J=6.6 Hz, Val-Me), 1.44 (9H, s, Bu^t), 1.65 (3H, s, 2-Me), 1.81 (3H, s, 2-Me), 1.87 (1H, m, Val β-H), 3.02 (2H, m, $ArCH_2$), 3.12 (1H, dd, J=12.1, 5.5 Hz, 5$_β$-H), 3.50 (1H, m, $ArCH_2CH$), 3.63 (1H, d, J=12.1 Hz, 5$_α$-H), 3.75 (1H, m, Val α-H), 3.79 (1H, m, $ArCH_2CH$), 4.67 (1H, d, J=5.5 Hz, 4-H), 4.90 (1H, d, J=7.8 Hz, NHBoc), 7.38 (2H, d, J=9.0 Hz, Ar 2,6-H_2), 7.98 (1H, br, N$H$$CH_2$), 8.14 (2H, d, J=8.6 Hz, Ar 3,5-H_2); MS *m/z* 509.2434 (M+H) ($C_{24}H_{37}N_4O_6S$ requires 509.2434), 453 (M−Bu^{t}O), 409 (M−Boc), 310 (M−BocVal).

8.21. *R*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-phenylalanyl)-*N*-(2-(4-nitrophenyl)ethyl)-tetrahydrothiazole-4-carboxamide (20d)

Compound **19d** was treated with **24**·HCl and Et_3N, as for the synthesis of **20c**, to give **20d** (95%) as a white solid: mp 45–46 °C: IR ν_{max} 3442, 1699, 1652, 1518 cm^{-1}; NMR δ_H 1.43 (9H, s, Bu^t), 1.58 (3H, s, 2-Me), 1.60 (3H, s, 2-Me), 2.30 (1H, dd, J=11.7, 5.9 Hz, Phe β-H), 2.89 (2H, m, Phe β-H+5$_\beta$-H), 2.97 (2H, t, J=7.0 Hz, $ArCH_2$), 3.20 (1H, d, J=11.7 Hz, 5$_\alpha$-H), 3.41 (1H, m, $ArCH_2CH$), 3.69 (1H, m, $ArCH_2CH$), 3.74 (1H, d, J=5.5 Hz, 4-H), 4.13 (1H, m, Phe α-H), 4.98 (1H, d, J=5.9 Hz, Phe NH), 7.18 (2H, m, Phe 2′,6′-H_2), 7.33 (3H, m, Phe 3′,4′,5′-H_3), 7.35 (2H, d, J=8.6 Hz, Ar 2,6-H_2), 8.10 (2H, d, J=9 Hz, Ar 3,5-H_2), 8.10 (1H, m, NH); MS m/z 557.2433 (M+H) ($C_{28}H_{37}N_4O_6S$ requires 557.2434), 501 (M−Me_2C=CH_2), 457 (M−Boc).

8.22. *R*-2,2-Dimethyl-3-glycyl-*N*-(2-(4-nitrophenyl)-ethyl)tetrahydrothiazole-4-carboxamide trifluoroacetate salt (21a)

Compound **20a** (50 mg, 0.11 mmol) was stirred in CF_3CO_2H (0.4 mL) and CH_2Cl_2 (1.6 mL) for 45 min. Evaporation afforded **21a** (53 mg, quant.) as a highly hygroscopic colourless gum: NMR ($(CD_3)_2SO$) δ_H 1.76 (3H, s, 2-Me), 1.78 (3H, s, 2-Me), 2.90 (2H, t, J=6.6 Hz, $ArCH_2$), 3.18 (1H, m, Gly-H), 3.20 (d, J=12.1 Hz, 5-H), 3.34 (1H, dd, J=12.5, 5.9 Hz, 5-H), 3.42 (2H, br q, $ArCH_2CH_2$), 3.89 (1H, m, Gly-H), 4.85 (1H, d, J=6.2 Hz, 4-H), 7.50 (2H, d, J=9.0 Hz, Ar 2,6-H_2), 8.02 (3H, br, N^+H_3), 8.15 (2H, d, J=8.6 Hz, Ar 3,5-H_2), 8.26 (1H, t, J=6.2 Hz, NH); MS m/z 367.1448 (M+H) ($C_{16}H_{24}N_4O_4S$ requires 367.1400), 310 (M−Gly), 225 (M−$CH_2CH_2C_6H_4NO_2$).

8.23. 3-(L-Alanyl)-*R*-2,2-dimethyl-*N*-(2-(4-nitrophenyl)-ethyl)tetrahydrothiazole-4-carboxamide trifluoroacetate salt (21b)

Compound **20b** was treated with CF_3CO_2H, as for the synthesis of **21a** except that the reaction time was 20 min, to give **21b** (quant.) as a pale buff solid: mp 119–121 °C: IR ν_{max} 3410, 1655, 1600, 1517 cm^{-1}; NMR (CD_3OD) δ_H 1.34 (3H, d, J=6.6 Hz, Ala-Me), 1.69 (3H, s, 2-Me), 1.72 (3H, s, 2-Me), 2.85 (1H, dt, J=12.9, 6.6 Hz) and 2.89 (1H, dt, J=12.9, 6.6 Hz, $ArCH_2$), 3.10 (1H, dd, J=12.9, 1.6 Hz, 5-H), 3.33 (2H, m, 5-H, NC*H*H), 3.54–3.61 (1H, dt, J=13.7, 6.6 Hz, NC*H*H), 3.68 (1H, q, J=7.0 Hz, Ala α-H), 4.74 (1H, dd, J=5.9, 1.6 Hz, 4-H), 7.39 (2H, d, J=9.0 Hz, Ar 2,6-H_2), 8.07 (2H, d, J=9.0 Hz, Ar 3,5-H_2); MS m/z 381.1603 (M+H) ($C_{17}H_{25}N_4O_4S$ requires 381.1597).

8.24. *R*-2,2-Dimethyl-*N*-(2-(4-nitrophenyl)ethyl)-3-(L-valinyl)tetrahydrothiazole-4-carboxamide trifluoroacetate salt (21c)

Compound **20c** was treated with CF_3CO_2H, as for the synthesis of **21a** except that the reaction time was 15 min, to give **21c** (quant.) as a colourless highly hygroscopic gum: NMR ($(CD_3)_2SO$) δ_H 0.95 (3H, d, J=6.6 Hz, Val-Me), 1.00 (3H, d, J=6.6 Hz, Val-Me), 1.80 (3H, s, 2-Me), 1.82 (3H, s, 2-Me), 2.10 (1H, m, Val β-H), 2.98 (2H, t, J=7.0 Hz, $ArCH_2$), 3.32 (1H, dd, J=12.5, 2.3 Hz, 5-H), 3.45 (1H, dd, J=12.1, 5.9 Hz, 5-H), 3.53 (3H, m, NCH_2 and Val α-H), 4.95 (1H, dd, J=5.9, 2.3 Hz, 4-H), 7.57 (2H, d, J=8.6 Hz, Ar 2,6-H_2), 8.16 (3H, br, N^+H_3), 8.23 (2H, d, J=8.6 Hz, Ar 3,5-H_2), 8.33 (1H, t, J=5.9 Hz, NH); MS m/z 409.1911 (M+H) ($C_{19}H_{29}N_4O_4S$ requires 409.1910).

8.25. *R*-2,2-Dimethyl-*N*-(2-(4-nitrophenyl)ethyl)-3-(L-phenylalanyl)tetrahydrothiazole-4-carboxamide trifluoroacetate salt (21d)

Compound **20d** was treated with CF_3CO_2H, as for the synthesis of **21c**, to give **21d** (quant.) as a colourless highly hygroscopic gum: NMR (CD_3OD) δ_H 1.64 (3H, s, 2-Me), 1.70 (3H, s, 2-Me), 2.55 (1H, dd, J=12.5, 5.9 Hz, 5-H), 2.90 (2H, t, J=7.0 Hz, $ArCH_2$), 2.95 (1H, dd, J=13.3, 8.6 Hz, Phe β-H), 3.08 (1H, d, J=12.5 Hz, 5-H), 3.10 (1H, dd, J=13.3, 5.6 Hz, Phe β-H), 3.42 (2H, m, $NHCH_2$), 4.08 (1H, m, Phe α-H), 4.20 (1H, d, J=4.6 Hz, 4-H), 4.98 (1H, d, J=5.9 Hz, Phe NH), 7.27 (2H, m, Phe 2′,6′-H_2), 7.36 (3H, m, Phe 3′,4′,5′-H_3), 7.50 (2H, d, J=8.6 Hz, Ar 2,6-H_2), 8.18 (2H, d, J=8.6 Hz, Ar 3,5-H_2), 8.23 (1H, t, J=6.6 Hz, NH), 8.38 (3H, br, N^+H_3); MS m/z 457.1907 (M+H) ($C_{23}H_{29}N_4O_4S$ requires 457.1910).

8.26. Pentafluorophenyl 3-(L-alanyl)-*R*-2,2-dimethyl-tetrahydrothiazole-4-carboxylate hydrochloride (22b)

HCl was bubbled through **19b** (90 mg, 0.18 mmol) in CH_2Cl_2 (5.0 mL) for 30 min. Evaporation afforded **22b** (quant.) as a highly hygroscopic yellow solid: IR ν_{max} 3423, 1793, 1670, 1521 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 1.20 (3H, d, J=7.0 Hz, Ala-Me), 1.74 (3H, s, 2-Me), 1.77 (3H, s, 2-Me), 3.17 (1H, dd, J=12.1, 10.2 Hz, 5-H), 3.25 (1H, dd, J=12.1, 6.6 Hz, 5-H), 4.10 (1H, q, J=7.0 Hz, Ala α-H), 4.68 (1H, dd, J=10.4, 6.6 Hz, 4-H), 6.30 (3H, br, N^+H_3); MS m/z 215 (M−C_6F_5O).

8.27. Pentafluorophenyl *R*-2,2-dimethyl-3-(L-valinyl)-tetrahydrothiazole-2-carboxylate hydrochloride (22c)

Compound **19c** was treated with HCl, as for the synthesis of **22b** except that the reaction time was 1 h, to give **22c** (120 mg, 90%) as a white wax: IR ν_{max} 3433, 1790, 1667, 1520 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 0.82 (3H, d, J=6.6 Hz, Val-Me), 0.99 (3H, d, J=7.4 Hz, Val-Me), 1.75 (6H, s, 2×Me), 2.29–2.37 (1H, d septet, J=2.3, 7.0 Hz, Val β-H), 3.13 (1H, dd, J=11.3, 10.2 Hz, 5$_\beta$-H), 3.22 (1H, dd, J=11.7, 6.2 Hz, 5$_\alpha$-H), 3.89 (1H, m, Val α-H), 4.60–4.64 (1H, dd, J=6.2, 1.2 Hz, 4-H), 5.94 (3H, br, N^+H_3); NMR δ_F −171.5 (1F, tt, J=23.7, 6.8 Hz, 4′-F_2), −165.1 (2F, dd, J=23.7, 19.6 Hz, 3′,5′-F_2), −161.5 (2F, dd, J=19.6, 6.8 Hz, 2′,6′-F_2); MS m/z 427 (M+H), 243 (M−C_6F_5O).

8.28. Pentafluorophenyl *R*-2,2-dimethyl-3-(L-phenylalanyl)tetrahydrothiazole-2-carboxylate hydrochloride (22d)

Compound **19d** was treated with HCl, as for the synthesis of **22b** except that the reaction time was 1.5 h, to give **22d** (quant.) as a colourless highly hygroscopic viscous oil: IR ν_{max} 3434, 1791, 1669, 1520 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 1.66 (3H, s, 2-Me), 1.74 (3H, s, 2-Me), 2.75 (1H, t, J=10.9 Hz, 5$_\beta$-H), 3.04 (1H, dd, J=14.1, 4.7 Hz, Phe β-H),

3.06 (1H, dd, J=14.1, 4.3 Hz, Phe β-H), 3.13 (1H, dd, J=11.7, 5.9 Hz, 5_α-H), 4.34 (1H, t, J=4.3 Hz, Phe α-H), 4.46 (3H, br, N^+H_3), 4.60 (1H, dd, J=10.5, 5.5 Hz, 4-H), 7.25 (5H, m, Ph-H_5), 8.31 (1H, s, NH); NMR δ_F −171.5 (2F, tt, J=22.4, 6.6 Hz, 4′-F_2), −165.1 (1F, dd, J=22.4, 19.7 Hz, 3′,5′-F_2), −161.8 (2F, dd, J=19.7, 6.6 Hz, 2′,6′-F_2), MS m/z 475.1131 (M+H) ($C_{21}H_{20}F_5N_2O_3S$ requires 475.1115), 291 (M−C_6F_5O).

8.29. 6*S*,8a*R*-3,3,6-Trimethyltetrahydrothiazolo[3,4-*a*]-pyrazine-5,8-dione (23b)

Compound **22b** (190 mg, 480 μmol) was stirred with Et_3N (97 mg, 1.0 mmol) in CH_2Cl_2 (5.0 mL) for 5 min. Evaporation and chromatography (EtOAc) afforded **23b** (15 mg, 15%) as a white solid: mp 155.8 °C (DSC); IR ν_{max} 3436, 1693, 1652 cm^{-1}; NMR δ_H 1.46 (3H, d, J=7.0 Hz, 6-Me), 1.85 (3H, s, 3-Me), 1.89 (3H, s, 3-Me), 3.25 (1H, dd, J=12.5, 6.6 Hz, 1_α-H), 3.30 (1H, dd, J=12.5, 10.2 Hz, 1_β-H), 4.07 (1H, ddq, J=7.0, 1.0, 0.8 Hz, 6-H), 4.57 (1H, dddd, J=10.5, 6.6, 1.6, 0.8 Hz, 8a-H), 7.28 (1H, br, NH); MS m/z 368 (M+mNBA), 215.0862 (M+H) ($C_9H_{15}N_2O_2S$ requires 215.0854). Found C, 50.43; H, 6.55; N, 12.9. $C_9H_{14}N_2O_2S$ requires C, 50.45; H, 6.58; N, 13.07%.

8.30. 6*S*,8a*R*-3,3-Dimethyl-6-(1-methylethyl)tetrahydrothiazolo[3,4-*a*]pyrazine-5,8-dione (23c)

Compound **22c** was treated with Et_3N, as for the synthesis of **23b** except that the reaction time was 10 min, to give **23c** (87%) as a white solid: mp 186.4 °C (DSC); IR ν_{max} 3213, 1692 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 0.83 (3H, d, J=7.0 Hz, CHCH_3), 1.0 (3H, d, J=7.0 Hz, CHCH_3), 1.76 (3H, s, 3-Me), 1.77 (3H, s, 3-Me), 2.34 (1H, d septet, J=7.0, 2.3 Hz, CHMe$_2$), 3.14 (1H, dd, J=11.7, 10.2 Hz, 1_β-H), 3.23 (1H, dd, J=11.7, 5.9 Hz, 1_α-H), 3.34 (1H, br, NH), 3.89 (1H, dd, J=2.3, 1.2 Hz, 6-H), 4.64 (1H, ddd, J=11.7, 5.9, 1.2 Hz, 8a-H); MS m/z 396 (M+mNBA), 243.1168 (M+H) ($C_{11}H_{19}N_2O_2S$ requires 243.1167).

8.31. 6*S*,8a*R*-3,3-Dimethyl-6-phenylmethyltetrahydrothiazolo[3,4-*a*]pyrazine-5,8-dione (23d)

Compound **22d** was treated with Et_3N, as for the synthesis of **23b** except that the reaction time was 1.5 h, to give **23d** (81%) as a colourless gum: IR (film) ν_{max} 3377, 1794, 1688 cm^{-1}; NMR δ_H 1.87 (3H, s, 3-Me), 1.92 (3H, s, 3-Me), 2.82 (1H, dd, J=14.4, 10.2 Hz, PhCH), 3.19 (1H, dd, J=12.1, 10.5 Hz, 1_β-H), 3.26 (1H, dd, J=12.1, 5.9 Hz, 1_α-H), 3.56 (1H, dd, J=14.4, 3.9 Hz, PhCH), 4.24 (1H, ddd, J=10.2, 3.9, 0.8 Hz, 6-H), 4.53 (1H, dddd, J=11.3, 5.9, 1.6, 0.8 Hz, 8a-H), 5.82 (1H, s, NH), 7.21–7.37 (5H, m, Ph-H_5); MS m/z 291.1169 (M+H) ($C_{15}H_{18}N_2O_2S$ requires 291.1167).

8.32. *R*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-D-alanyl)tetrahydrothiazole-4-carboxylic acid (27) and *R*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-alanyl)tetrahydrothiazole-4-carboxylic acid (18b)

A mixture of Boc(D-Ala)F **26** (308 mg, 1.6 mmol) and **17b** (154 mg, 800 μmol) was stirred with **11** (500 mg, 2.5 mmol) and Et_3N (480 mg, 4.8 mmol) in dry DMF (50 mL) for 16 h. The evaporated residue, in EtOAc, was washed with cold citric acid and brine. Drying and evaporation afforded a mixture of **27** and **18b** (300 mg, 36%) as a white solid: mp 153–155 and 160–162 °C; IR ν_{max} 3316, 1748, 1718, 1643, 1660, 1540, 1512 cm^{-1}; NMR δ_H 1.30 (1.2H, d, J=2.7 Hz, Ala-Me (**18b**)), 1.32 (1.8H, d, J=3.1 Hz, Ala-Me (**27**)), 1.40 (3.6H, s, Bu^t (**18b**)), 1.42 (5.4H, s, Bu^t (**27**)), 1.81 (1.2H, s, 2-Me (**18b**)), 1.84 (1.8H, s, 2-Me (**27**)), 1.87 (1.8H, s, 2-Me (**27**)), 1.91 (1.2H, s, 2-Me (**18b**)), 3.23 (0.4H, dd, J=11.7, 5.5 Hz, 5-H (**18b**)), 3.34 (0.6H, dd, J=12.1, 5.5 Hz, 5-H (**27**)), 3.40 (0.6H, d, J=12.1 Hz, 5-H (**27**)), 3.45 (0.4H, d, J=11.7 Hz, 5-H (**18b**)), 4.25 (0.6H, qn, J=6.6 Hz, Ala α-H (**27**)), 4.53 (0.4H, qn, J=7.0 Hz, Ala α-H (**18b**)), 4.87 (0.4H, d, J=5.1 Hz, 4-H (**18b**)), 5.36 (0.6H, d, J=8.6 Hz, NH (**27**)), 5.59 (0.6H, d, J=5.1 Hz, 4-H (**27**)), 5.79 (0.4H, d, J=8.2 Hz, NH (**18b**)); MS m/z 333.1494 (M+H) ($C_{14}H_{25}N_2O_5S$ requires 333.1484), 277 (M−Me_2C=CH_2).

8.33. Pentafluorophenyl *R*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-D-alanyl)tetrahydrothiazole-4-carboxylate (28) and pentafluorophenyl *R*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-alanyl)tetrahydrothiazole-4-carboxylate (19b)

The above mixture of **27** and **18b** was treated with pentafluorophenol and DCC, as for the synthesis of **19a**, to give a mixture of **28** and **19b** (80%) as a colourless gum: NMR δ_H 1.30 (2.1H, d, J=6.6 Hz, Ala-Me (**28**)), 1.37 (0.9H, d, J=6.6 Hz, Ala-Me (**19b**)), 1.39 (2.7H, s, Bu^t (**19b**)), 1.43 (6.3H, s, Bu^t (**28**)), 1.85 (0.9H, s, 2-Me (**19b**)), 1.87 (2.1H, s, 2-Me (**28**)), 1.89 (2.1H, s, 2-Me (**28**)), 1.94 (0.9H, s, 2-Me (**19b**)), 3.37 (0.3H, dd, J=12.5, 3.5 Hz, 5-H (**28**)), 3.40 (0.7H, dd, J=12.5, 5.9 Hz, 5-H (**28**)), 3.49 (1H, d, J=12.5 Hz, 5-H), 4.22 (0.7H, qn, J=7.0 Hz, Ala α-H (**28**)), 4.38 (0.3H, m, Ala α-H (**19b**)), 5.01 (0.7H, d, J=9.0 Hz, NH (**28**)), 5.19 (0.7H, d, J=5.5 Hz, 4-H (**28**)), 5.58 (0.3H, d, J=3.5 Hz, 4-H (**19b**)), 6.13 (0.3H, t, J=3.9 Hz, NH (**19b**)); NMR δ_F −163.8 (2F, t, J=21.0 Hz, 3′,5′-F_2), −162.1 (2F, t, J=21.0 Hz, 3′,5′-F_2), −161.5 (2F, m, 3′,5′-F_2), −161.1 (2F, t, J=22.4 Hz, 3′,5′-F_2), 156.4 (1F, t, J=21.0 Hz, 4′-F), −156.6 (1F, t, J=21.0 Hz, 4′-F), 157.1 (1F, t, J=21.0 Hz, 4′-F), −157.8 (1F, t, J=21.0 Hz, 4′-F), −150.9 (2F, d, J=17.1 Hz, 2′,6′-F_2), −151.9 (2F, d, J=17.1 Hz, 2′,6′-F_2), −152.1 (2F, d, J=18.4 Hz, 2′,6′-F_2), −152.6 (2F, d, J=17.1 Hz, 2′,6′-F_2); MS m/z 499.1340 (M+H) ($C_{20}H_{24}F_5N_2O_5S$ requires 499.1326), 443 (M−Me_2C=CH_2), 425 (M−Bu^{t}O).

8.34. *R*-(2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-D-alanyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (29) and *R*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-alanyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (20b)

The above mixture of **28** and **19b** was treated with **24**·HCl and Et_3N, as for the synthesis of **20b**, to give a mixture of **29** and **20b** (220 mg, 76%) as a white solid: mp 69–80 °C; NMR ($(CD_3)_2SO$) δ_H 0.93 (1.8H, d, J=6.6 Hz, Ala-Me (**29**)), 1.11 (1.2H, d, J=6.6 Hz, Ala-Me (**20b**)), 1.36 (3.6H, s, Bu^t (**20b**)), 1.37 (5.4H, s, Bu^t (**20b**)), 1.59 (1.2H, s, 2-Me (**20b**)), 1.68 (1.8H, s, 2-Me (**29**)), 1.70 (1.8H, s, 2-Me

(**29**)), 1.71 (1.2H, s, 2-Me (**20b**)), 2.88 (0.8H, m, $ArCH_2$ (**20b**)), 2.95 (1.2H, m, $ArCH_2$ (**29**)), 3.00–4.00 (5H, m, 5-H_2, $NHCH_2$, Ala α-H), 4.72 (0.4H, d, *J*=4.1 Hz, 4-H (**20b**)), 5.25 (0.6H, d, *J*=4.3 Hz, 4-H (**29**)), 7.05 (0.4H, d, *J*=5.9 Hz, NH (**20b**)), 7.13 (0.6H, d, *J*=7.4 Hz, NH (**29**)), 7.49 (0.8H, d, *J*=8.2 Hz, Ar 2,6-H_2 (**20b**)), 7.51 (1.2H, d, *J*=8.6 Hz, Ar 2,6-H_2 (**29**)), 8.13 (2H, d, *J*=8.6 Hz, Ar 3,5-H_2), 8.17 (1H, d, *J*=5.9 Hz, Ala NH); MS *m*/*z* 481.2128 (M+H) ($C_{22}H_{33}N_4O_6S$ requires 481.2121), 425 (M−Me_2C=CH_2), 381 (M−Boc).

8.35. *S*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-D-valinyl)tetrahydrothiazole-4-carboxylic acid (32)

Compound **31** (prepared as **17c**) (270 mg, 1.1 mmol) was stirred with **11** (250 mg, 1.1 mmol) and Pr^i_2NEt (310 mg, 2.4 mmol) in dry DMF (10 mL) for 16 h. The evaporated residue, in EtOAc, was washed with cold citric acid, cold H_2O and brine. Drying, evaporation and chromatography (hexane/EtOAc/AcOH, 49:49:2) afforded **32** (200 mg, 50%) as a colourless gum: IR (film) ν_{max} 3329, 1714, 1657, 1462 cm^{-1}; NMR δ_H 0.92 (3H, d, *J*=6.2 Hz, Val-Me), 0.94 (3H, d, *J*=6.6 Hz, Val-Me), 1.43 (9H, s, Bu^t), 1.85 (3H, s, 2-Me), 1.89 (3H, s, 2-Me), 2.05 (1H, m, Val β-H), 3.27 (1H, dd, *J*=12.1, 5.7 Hz, 5-H), 3.40 (1H, d, *J*=12.1 Hz, 5-H), 3.86 (1H, t, *J*=9.6 Hz, Val α-H), 5.42 (1H, d, *J*=10.1 Hz, NH), 5.66 (1H, d, *J*=5.7 Hz, 4-H); MS *m*/*z* 361.1810 (M+H) ($C_{16}H_{29}N_2O_5S$ requires 361.1797), 305 (M−Me_2C=CH_2).

8.36. Pentafluorophenyl *R*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-D-valinyl)tetrahydrothiazole-4-carboxylate (33)

Compound **32** was treated with pentafluorophenol and DCC, as for the synthesis of **19a**, to give **33** (41%) as a colourless oil: NMR δ_H 0.89 (2.1H, d, *J*=7.0 Hz, Val-Me), 0.98 (2.1H, d, *J*=6.6 Hz, Val-Me), 1.04 (0.9H, d, *J*=7.0 Hz, Val-Me), 1.10 (0.9H, d, *J*=7.0 Hz, Val-Me), 1.45 (6.3H, s, Bu^t), 1.48 (2.7H, s, Bu^t), 1.89 (2.1H, s, 2-Me), 1.93 (2.1H, s, 2-Me), 2.00 (0.9H, s, 2-Me), 2.02 (1H, m, Val β-H), 2.06 (0.9H, s, 2-Me), 3.30 (0.3H, dd, *J*=12.9, 3.1 Hz, 5-H), 3.43 (0.3H, d, *J*=4.7 Hz, 5-H), 3.54 (0.7H, dd, *J*=12.5, 6.2 Hz, 5-H), 3.83 (0.7H, t, *J*=9.4, 5-H), 5.03 (0.7H, d, *J*=9.8 Hz, 4-H), 5.08 (0.3H, d, *J*=9.0 Hz, 4-H), 5.62 (0.3H, dd, *J*=6.6, 2.3 Hz, Val α-H), 5.67 (0.7H, dd, *J*=3.9, 2.0 Hz, Val α-H), 6.2 (1H, m, NH).

8.37. *R*-(2,2-Dimethyl-3-(1,1-dimethylethoxycarbonyl)-D-valinyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (34)

Compound **33** was treated with **24**·HCl and Et_3N, as for the synthesis of **20c**, to give **34** (17%) as a colourless gum: IR (film) ν_{max} 3320, 1702, 1655, 1514 cm^{-1}; NMR δ_H 0.83 (3H, d, *J*=6.6 Hz, Val-Me), 0.92 (3H, d, *J*=6.6 Hz, Val-Me), 1.42 (7.2H, s, Bu^t), 1.55 (1.8H, s, Bu^t), 1.79 (2.4H, s, 2-Me), 1.81 (2.4H, s, 2-Me), 1.88 (0.6H, s, 2-Me), 1.91 (0.6H, s, 2-Me), 1.92 (1H, m, Val β-H), 2.97 (2H, t, *J*=7.0 Hz, $ArCH_2$), 3.04 (1H, d, *J*=12.5 Hz, 5_α-H), 3.36 (1H, dd, *J*=12.5, 7.0 Hz, 5_β-H), 3.44 (1H, m, $ArCH_2CH$), 3.80 (1H, m, $ArCH_2CH$), 3.86 (1H, t, *J*=9.0 Hz, Val α-H), 4.85 (1H, d, *J*=9.4 H, ValNH), 5.07 (0.2, d, *J*=6.2 Hz, 4-H), 5.36 (0.8H, d, *J*=6.6 Hz, 4-H), 6.03 (0.2H, br, $NHCH_2$), 6.41 (0.8H, br, $NHCH_2$), 7.35 (0.4H, d, *J*=8.6 Hz, Ar 2,6-H_2), 7.40 (1.6H, d, *J*=8.6 Hz, Ar 2,6-H_2), 8.15 (0.4H, d, *J*=9.0 Hz, Ar 3,5-H_2), 8.17 (1.6H, d, *J*=8.6 Hz, Ar 3,5-H_2); MS *m*/*z* 509.2437 (M+H) ($C_{24}H_{37}N_4O_6S$ requires 509.2434).

8.38. *S*-2,2-Dimethyltetrahydrothiazole-4-carboxylic acid hydrochloride (36)

D-Cys·HCl·H_2O **35** was treated with acetone and 2,2-dimethoxypropane, as for the synthesis of **11**, to give **36** (78%) as a white solid: mp 166–170 °C (lit.[39] mp 165–168 °C for *R* enantiomer); IR ν_{max} 3412, 2600, 1743 cm^{-1}; NMR (D_2O) δ_H 2.06 (6H, s, 2×Me), 2.92 (1H, dd, *J*=15.2, 3.9 Hz, 5-H), 3.00 (1H, dd, *J*=15.2, 5.9 Hz, 5-H), 4.06 (1H, dd, *J*=5.9, 3.9 Hz, 4-H).

8.39. *S*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-valinyl)tetrahydrothiazole-4-carboxylic acid (37c)

Compound **17c** (1.08 g, 4.6 mmol) was stirred with **36** (1.00 g, 5.1 mmol) and Pr^i_2NEt (1.24 g, 9.6 mmol) in dry DMF (100 mL) for 16 h. The evaporated residue, in EtOAc, was washed with cold citric acid, cold H_2O and brine. Drying, evaporation and chromatography (hexane/EtOAc/AcOH, 70:28:2) afforded **37c** (630 mg, 35%) as a colourless gummy solid: IR ν_{max} 3434, 2970, 1723, 1605, 1513 cm^{-1}; NMR δ_H 0.87–0.95 (6H, m, 2×Val-Me), 1.43 (9H, s, Bu^t), 1.85 (3H, s, 2-Me), 1.88 (3H, s, 2-Me), 2.00 (1H, m, Val β-H), 3.20–3.50 (2H, m, 5-H+Val α-H), 3.85 (1H, t, *J*=9.8 Hz, 5-H), 5.36 (1H, d, *J*=10.2 Hz, 4-H), 5.68 (0.7H, d, *J*=5.1 Hz, NH), 8.63 (1H, br, OH); MS *m*/*z* 361.1810 (M+H) ($C_{16}H_{29}N_2O_5S$ requires 361.1797), 305 (M−Me_2C=CH_2).

8.40. *S*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-leucyl)tetrahydrothiazole-4-carboxylic acid (37e)

Compound **45** (1.5 g, 3.8 mmol) was stirred with **36** (833 mg, 4.2 mmol) and Pr^i_2NEt (1.63 g, 12.6 mmol) in dry DMF (40 mL) for 16 h. The evaporated residue, in EtOAc, was washed with cold 5% aq citric acid, cold H_2O and brine. Drying and evaporation afforded crude **37e** (2.4 g) as a gummy oil: NMR δ_H 0.81–0.90 (6H, m, 2×Leu-Me), 1.40 (9H, s, Bu^t), 1.50–1.60 (2H, m, Leu β-H_2+Leu γ-H), 1.83 (3H, s, 2-Me), 1.86 (3H, s, 2-Me), 3.28 (2H, m, 5-H_2), 4.17 (1H, m, Leu α-H), 5.30 (1H, d, *J*=5.92 Hz, NH), 5.70 (1H, m, 4-H); MS *m*/*z* 375.1948 (M+H) ($C_{17}H_{31}N_2O_5S$ requires 375.1954), 319 (M−Me_2C=CH_2).

8.41. Pentafluorophenyl *S*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-valinyl)tetrahydrothiazole-4-carboxylate (38c)

Compound **37c** was treated with pentafluorophenol and DCC, as for the synthesis of **19a**, to give gave **38c** (68%) as a colourless oil: NMR δ_H 0.88 (3H, d, *J*=6.6 Hz, Val-Me), 0.97 (3H, d, *J*=6.6 Hz, Val-Me), 1.45 (9H, s, Bu^t), 1.88 (3H, s, 2-Me), 1.92 (3H, s, 2-Me), 2.04 (1H, m, Val

β-H), 3.55 (1H, dd, *J*=12.5, 6.6 Hz, 5-H), 3.82 (1H, t, *J*=9.4 Hz, 5-H), 5.08 (1H, br d, *J*=9.8 Hz, 4-H), 5.61 and 5.66 (1H, 2×d, *J*=4.7 Hz, Val α-H), 6.22 (1H, br d, *J*=3.9 Hz, NH); MS *m/z* 527.1656 (M+H) ($C_{22}H_{28}F_5N_2O_5S$ requires 527.1639), 471 (M−Bu^{t}O).

8.42. Pentafluorophenyl *S*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-leucyl)tetrahydrothiazole-4-carboxylate (38e)

Compound **37e** was treated with pentafluorophenol and DCC, as for the synthesis of **19a**, to give crude **38e** as a pale yellow oil: NMR δ_H 0.88 (3H, d, *J*=6.4 Hz, Leu-Me), 0.90 (3H, d, *J*=6.4 Hz, Leu-Me), 1.44 (9H, s, Bu^t), 1.55–1.65 (2H, m, Leu β,γ-H_3), 1.86 (3H, s, 2-Me), 1.91 (3H, s, 2-Me), 3.47 (1H, dd, *J*=12.7, 9.8 Hz, $5_β$-H), 3.54 (1H, dd, *J*=12.7, 4.8 Hz, $5_α$-H), 4.17 (1H, dt, *J*=2.7, 8.7 Hz, Leu α-H), 4.96 (1H, d, *J*=9.0 Hz, 4-H), 6.17 (1H, d, *J*=9.0 Hz, NH); NMR δ_F −161.5 (2F, t, *J*=17.1 Hz, 3′,5′-F_2), −156.6 (1F, t, *J*=22.4 Hz, 4′-F), −152.1 (2F, d, *J*=18.4 Hz, 2′,6′-F_2). MS *m/z* 541.1797 (M+H) ($C_{23}H_{30}N_2O_5F_5S$ requires 541.1796), 485 (M−Me_2C=CH_2), 441 (M−Boc).

8.43. *S*-(2,2-Dimethyl-3-(1,1-dimethylethoxycarbonyl)-L-valinyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (39c)

Compound **38c** was treated with **24**·HCl and Et_3N, as for the synthesis of **20c**, to give **39c** (49%) as a colourless oil: IR ν_{max} 3323, 1698, 1605, 1520 cm^{-1}; NMR δ_H 0.83 (3H, d, *J*=6.6 Hz, Val-Me), 0.92 (3H, d, *J*=6.6 Hz, Val-Me), 1.42 (7.2H, s, Bu^t), 1.55 (1.8H, s, Bu^t), 1.80 (2.4H, s), 1.82 (2.4H, s), 1.89 (0.6H, s) and 1.91 (0.6H, s, 2-Me_2), 1.95 (1H, m, Val β-H), 2.81 (1.6H, dt, *J*=14.1, 7.0 Hz, ArCH_2), 3.04 (0.8H, d, *J*=12.5 Hz, $5_β$-H), 3.36 (1H, dd, *J*=12.5, 6.6 Hz, $5_α$-H), 3.44 (1H, m, ArCH_2*CH*), 3.77 (1H, m, ArCH_2*CH*), 3.86 (1H, t, *J*=9.0 Hz, Val α-H), 4.92 (1H, d, *J*=9.8 Hz, Val NH), 5.07 (0.2H, d, *J*=7.0 Hz, 4-H), 5.36 (0.8H, d, *J*=6.2 Hz, 4-H), 6.09 (0.2H, br, N*H*CH_2), 6.47 (0.8H, t, *J*=5.5 Hz, N*H*CH_2), 7.35 (0.4H, d, *J*=8.6 Hz, Ar 2,6-H_2), 7.40 (1.6H, d, *J*=8.6 Hz, Ar 2,6-H_2), 8.13 (0.4H, d, *J*=8.2 Hz, Ar 3,5-H_2), 8.16 (1.6H, d, *J*=8.6 Hz, Ar 3,5-H_2); MS *m/z* 509.2432 (M+H) ($C_{24}H_{37}N_4O_6S$ requires 509.2434), 453 (M−Bu^{t}O). Some ^{1}H NMR peaks from the minor isomer overlapped with other peaks from the major isomer and thus were not identifiable.

8.44. *S*-(2,2-Dimethyl-3-(1,1-dimethylethoxycarbonyl)-L-leucyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (39e)

Compound **38e** was treated with **24**·HCl and Et_3N, as for the synthesis of **20c**, to give **39e** (58%) as a white solid: mp 108–109 °C; NMR δ_H 0.84 (2.4H, d, *J*=6.4 Hz, Leu-Me), 0.90 (2.4H, d, *J*=6.4 Hz, Leu-Me), 0.98 (1.2H, d, *J*=6.4 Hz, 2×Leu-Me), 1.41 (7.2H, s, Bu^t), 1.43 (1.8H, s, Bu^t), 1.60–1.70 (3H, m, Leu β,γ-H_3), 1.78 (2.4H, s, 2-Me), 1.80 (2.4H, s, 2-Me), 1.84 (0.6H, s, 2-Me), 1.85 (0.6H, s, 2-Me), 2.97 (2H, t, *J*=7.0 Hz, ArCH_2), 3.05 (1H, d, *J*=12.3 Hz, $5_β$-H), 3.45 (2H, m, ArCH_2*CH*, $5_α$-H), 3.85 (1H, m, ArCH_2*CH*), 4.11 (0.8H, m, Leu α-H), 4.30 (0.2H, m, Leu α-H), 4.81 (0.8H, d, *J*=8.6 Hz, LeuNH), 4.92 (0.2H, d, *J*=8.6 Hz, LeuNH), 5.30 (0.2H, d, *J*=7.0 Hz, 4-H), 5.42 (0.8H, d, *J*=7.0 Hz, 4-H), 6.15 (1H, t, *J*=5.1 Hz, N*H*CH_2), 7.41 (2H, d, *J*=8.6 Hz, Ar 2,6-H_2), 8.15 (0.4H, d, *J*=8.3 Hz, Ar 3,5-H_2), 8.19 (1.6H, d, *J*=8.6 Hz, Ar 3,5-H_2); MS *m/z* 523.2596 (M+H) ($C_{25}H_{39}N_4O_6S$ requires 523.2590), 467 (M−Me_2C=CH_2), 423 (M−Boc).

8.45. *S*-2,2-Dimethyl-*N*-(2-(4-nitrophenyl)ethyl)-3-(L-valinyl)tetrahydrothiazole-4-carboxamide trifluoroacetate salt (40c)

Compound **39c** was treated with CF_3CO_2H, as for the synthesis of **21c**, to give **40c** (quant.) as a highly hygroscopic viscous oil: NMR ($(CD_3)_2SO$) δ_H 0.82 (3H, d, *J*=7.4 Hz, Val-Me), 0.84 (3H, d, *J*=7.0 Hz, Val-Me), 1.80 (6H, s, 2×2-Me), 2.02 (1H, m, Val β-H), 2.88 (2H, t, *J*=7.0 Hz, ArCH_2), 3.08 (1H, d, *J*=12.1 Hz, 5-H), 3.38 (2H, m, NHCH_2), 3.52 (1H, dd, *J*=12.1, 4.7 Hz, 5-H), 4.60 (1H, m, Val α-H), 5.05 (1H, d, *J*=5.9 Hz, 4-H), 7.49 (2H, d, *J*=8.6 Hz, Ar 2,6-H_2), 8.15 (2H, d, *J*=8.6 Hz, Ar 3,5-H_2), 8.23 (1H, t, *J*=5.9 Hz, NH); MS *m/z* 409.1911 (M+H) ($C_{19}H_{29}N_4O_4S$ requires 409.1910).

8.46. *S*-2,2-Dimethyl-*N*-(2-(4-nitrophenyl)ethyl)-3-(L-leucyl)tetrahydrothiazole-4-carboxamide trifluoroacetate salt (40e)

Compound **39e** was treated with CF_3CO_2H, as for the synthesis of **21c**, to give **40e** (quant.) as colourless highly hygroscopic gum: NMR (CD_3OD) δ_H 0.90 (3H, d, *J*=4.7 Hz, Leu-Me), 0.98 (3H, d, *J*=4.3 Hz, Leu-Me), 1.60–1.70 (3H, m, Leu β,γ-H_3), 1.89 (3H, s, 2-Me), 1.92 (3H, s, 2-Me), 2.95–3.07 (3H, m, 5-H, ArCH_2), 3.38–3.52 (2H, m, ArCH_2*CH*, 5-H), 3.70 (1H, m, ArCH_2*CH*), 3.95 (1H, m, Leu α-H), 5.08 (1H, d, *J*=6.2 Hz, 4-H), 7.52 (2H, d, *J*=7.0 Hz, Ar 2,6-H_2), 8.20 (2H, d, *J*=7.4 Hz, Ar 3,5-H_2); MS *m/z* 423.2077 (M+H) ($C_{20}H_{31}N_4O_4S$ requires 423.2066).

8.47. 6*S*,8a*S*-3,3-Dimethyl-6-(1-methylethyl)tetrahydrothiazolo[3,4-*a*]pyrazine-5,8-dione (41c)

Compound **38c** was treated with HCl, as for the synthesis of **22b**, to give crude pentafluorophenyl *S*-2,2-dimethyl-3-(*N*-(L-valinyl)tetrahydrothiazole-2-carboxylate hydrochloride (quant.) as a pale yellow gummy solid: IR ν_{max} 3412 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 0.88 (3H, d, *J*=6.6 Hz, Val-Me), 0.92 (3H, d, *J*=7.0 Hz, Val-Me), 1.78 (3H, s, 2-Me), 1.82 (3H, s, 2-Me), 2.06 (1H, m, Val β-H), 3.11 (1H, dd, *J*=11.7, 10.9 Hz, 5-H), 3.22 (1H, dd, *J*=11.7, 5.5 Hz, 5-H), 3.32 (3H, br, N^+H_3), 4.64 (1H, dd, *J*=10.9, 5.5 Hz, 4-H), 5.19 (1H, m, Val α-H); NMR δ_F −171.46 (2F, m, 3′,5′-F_2), −165.12 (1F, dt, *J*=23.7 Hz, 4′-F), −161.53 (2F, dd, *J*=19.7, 6.6 Hz, 2′,6′-F_2); MS *m/z* 427 (M+H), 243 (M−C_6F_5O). This material was treated with Et_3N, as for the synthesis of **23b**, to give **41c** (61%) as a white solid: mp 180–182 °C; NMR ($(CD_3)_2SO$) δ_H 0.88 (3H, d, *J*=6.6 Hz, 6-CMe), 0.91 (3H, d, *J*=6.6 Hz, 6-CMe), 1.78 (3H, s, 3-Me), 1.82 (3H, s, 3-Me), 2.07 (1H, m, 6-CH), 3.11 (1H, dd, *J*=11.7, 10.5 Hz, $1_α$-H), 3.23 (1H, dd, *J*=11.7, 5.5 Hz, $1_β$-H), 3.40 (1H, dd, *J*=5.9, 3.5 Hz, 6-H), 4.64 (1H, dd, *J*=10.9, 5.5 Hz, 8a-H), 8.90 (1H, d, *J*=3.5 Hz, NH); MS *m/z* 396 (M+*m*NBA), 243.1176 (M+H) ($C_{11}H_{19}N_2O_2S$

requires 243.1167). Found C, 54.60; H, 7.39; N, 11.4. $C_{11}H_{18}N_2O_2S$ requires C, 54.52; H, 7.49; N, 11.56%.

8.48. 6*S*,8a*S*-3,3-dimethyl-6-(2-methylpropyl)tetrahydrothiazolo[3,4-*a*]pyrazine-5,8-dione (41e)

Compound **38e** (700 mg, 1.30 mmol) was stirred with CF_3CO_2H (1 mL) and CH_2Cl_2 (4 mL) for 20 min. Evaporation afforded crude pentafluorophenyl *S*-2,2-dimethyl-3-(*N*-(L-leucyl)tetrahydrothiazole-2-carboxylate trifluoroacetate salt (quant.) as a gummy solid. This material (554 mg, 1 mmol) was stirred with Et_3N (202 mg, 2 mmol) in CH_2Cl_2 (3.0 mL) for 30 min. Evaporation and chromatography (EtOAc) afforded **41e** (150 mg, 59%) as a white solid: mp 153–155 °C; NMR δ_H 0.95 (3H, d, *J*=6.6 Hz, CHC*H*$_3$), 1.00 (3H, d, *J*=6.6 Hz, CHC*H*$_3$), 1.60–1.78 (3H, m, 6-CH_2CH), 1.88 (3H, s, 3-Me), 1.89 (3H, s, 3-Me), 3.23 (1H, dd, *J*=12.1, 10.9 Hz, 1$_\alpha$-H), 3.32 (1H, dd, *J*=12.1, 5.9 Hz, 1$_\beta$-H), 3.87 (1H, dt, *J*=10.0, 5.0 Hz, 6-H), 4.55 (1H, dd, *J*=10.5, 5.5 Hz, 8a-H), 6.29 (1H, br, NH); MS *m*/*z* 257.1316 ($C_{12}H_{21}N_2O_2S$ requires 257.1324).

8.49. *N*-(Fluoren-9-ylmethoxycarbonyl)-L-valine pentafluorophenyl ester (43)

FmocValOH **42** was treated with pentafluorophenol and DCC, as for the synthesis of **19a**, to give **43** (70%) as a white solid: mp 114–117 °C (lit.[40] mp 122–123 °C); NMR δ_H 1.03 (3H, d, *J*=6.6 Hz, Val-Me), 1.10 (3H, d, *J*=6.6 Hz, Val-Me), 2.40 (1H, m, Val β-H), 4.24 (1H, t, *J*=6.6 Hz, C*H*CH$_2$O), 4.46 (2H, d, *J*=6.6 Hz, CH$_2$O), 4.67 (1H, dd, *J*=9.4, 5.1 Hz, Val α-H), 5.27 (1H, d, *J*=9.4 Hz, NH), 7.30 (2H, t, *J*=7.4 Hz, Ar-H$_2$), 7.38 (2H, t, *J*=7.4 Hz, Ar-H$_2$), 7.58 (2H, d, *J*=7.4 Hz, Ar-H$_2$), 7.75 (2H, d, *J*=7.8 Hz, Ar-H$_2$); NMR δ_F −161.2 (2F, t, *J*=20.9 Hz, 3′,5′-F$_2$), −156.6 (1F, t, *J*=20.9 Hz, 4′-F), −151.5 (2F, d, *J*=18.0 Hz, 2′,6′-F$_2$).

8.50. *S*-2,2-Dimethyl-3-(*N*-(fluoren-9-ylmethoxycarbonyl)-L-valinyl)tetrahydrothiazole-4-carboxylic acid (44)

Compound **43** (1.16 g, 2.3 mmol), in dry THF (10 mL), was added slowly to **36** (495 mg, 2.5 mmol) and Pr^i_2NEt (970 mg, 7.5 mmol) in dry DMF (30 mL) at 0 °C under N_2 for 3 h. The mixture was then slowly warmed to 20 °C and stirred for 16 h. The evaporated residue, in EtOAc, was washed with cold 5% aq citric acid and brine. Drying, evaporation and chromatography (hexane/EtOAc/AcOH, 25:25:1) afforded **44** (600 mg, 50%) as a white solid: mp 95–97 °C; NMR δ_H 0.92 (3H, d, *J*=7.0 Hz, Val-Me), 0.94 (3H, d, *J*=6.6 Hz, Val-Me), 1.84 (3H, s, 2-Me), 1.89 (3H, s, 2-Me), 2.10 (1H, m, Val β-H), 3.25 (1H, dd, *J*=12.1, 5.5 Hz, 5-H), 3.35 (1H, d, *J*=12.5 Hz, 5-H), 3.90 (1H, t, *J*=9.8 Hz, Val α-H), 4.20 (1H, t, *J*=7.0 Hz, C*H*CH$_2$O), 4.37 (1H, dd, *J*=10.9, 7.0 Hz, CHO), 4.45 (1H, dd, *J*=10.5, 7.0 Hz, CHO), 5.43 (1H, d, *J*=10.1 Hz, NH), 5.52 (1H, d, *J*=5.1 Hz, 4-H), 7.28 (2H, t, *J*=7.4 Hz, Ar-H$_2$), 7.38 (2H, t, *J*=7.4 Hz, Ar-H$_2$), 7.54 (2H, d, *J*=6.6 Hz, Ar-H$_2$), 7.75 (2H, d, *J*=7.4 Hz, Ar-H$_2$).

8.51. *N*-(1,1-Dimethylethoxycarbonyl)-L-leucine pentafluorophenyl ester (45)

Boc-L-LeuOH **16e** was treated with pentafluorophenol and DCC, as for the synthesis of **19a**, to give **45** (79%) as a colourless oil (lit.[41] oil): NMR δ_H 1.02 (6H, d, *J*=6.3 Hz, 2×Leu-Me), 1.47 (9H, s, Bu^t), 1.67 (2H, dd, *J*=9.7, 8.2 Hz, Leu β-H), 1.8 (2H, m, Leu β-H+Leu γ-H), 4.41 (0.1H, m, Leu α-H), 4.62 (0.9H, m, Leu α-H), 5.75 (0.1H, br, NH), 4.92 (0.9H, d, *J*=8.2 Hz, NH); NMR δ_F −162.1 (1.8F, t, *J*=21 Hz, 3′,5′-F$_2$), −161.9 (0.2F, t, *J*=21.0 Hz, 3′,5′-F$_2$), −157.6 (0.9F, t, *J*=22.4 Hz, 4′-F), −157.4 (0.1F, t, *J*=22.4 Hz, 4′-F), −152.7 (0.2F, d, *J*=19.7 Hz, 2′,6′-F$_2$),

Table 3. Crystal data and structure refinement of **20b**, **23b** and **23c**

Compound	**20b**	**23b**	**23c**
Empirical formula	$C_{22}H_{32}N_4O_6S$	$C_9H_{14}N_2O_2S$	$C_{11}H_{18}N_2O_2S$
Formula weight	480.58	214.28	242.33
Crystal system	Monoclinic	Orthorhombic	Triclinic
Space group	$P2_1$	$P2_12_12_1$	$P1$
a/Å	7.2780(1)	6.1600(1)	5.7440(2)
b/Å	21.0700(2)	6.5280(1)	9.7410(3)
c/Å	16.5780(2)	25.9050(5)	11.0190(4)
α/°	—	—	91.399(1)
β/°	94.700(1)	—	94.980(1)
γ/°	—	—	98.341(2)
U/Å^3	2533.65(5)	1041.70(3)	607.29(4)
Z	4	4	2
D_c/g cm^{-3}	1.260	1.366	1.325
M/mm^{-1}	0.170	0.287	0.255
F(000)	1024	456	260
Crystal size/mm	0.22×0.08×0.08	0.40×0.20×0.10	0.15×0.10×0.10
Theta min, max/°	3.81, 27.47	4.06, 27.86	3.60, 27.45
Index ranges	$-9\le h\le 9$; $-27\le k\le 27$; $-21\le l\le 21$	$-8\le h\le 8$; $-8\le k\le 8$; $-34\le l\le 34$	$-7\le h\le 7$; $-12\le k\le 12$; $-14\le l\le 14$
Reflections collected	48362	15756	9382
Independent reflections, *R*(int)	11591, 0.0738	2475, 0.0470	5031, 0.0391
Reflections observed (>2)	7928	2180	4185
Data/restraints/parameters	11591/5/625	2475/1/136	5031/5/307
Goodness-of-fit on F^2	1.005	1.037	1.040
Final *R*1, *wR*2 indices [*I*>2(*I*)]	0.0451, 0.0789	0.0298, 0.0697	0.0364, 0.0760
Final *R*1, *wR*2 indices (all data)	0.0886, 0.0904	0.0386, 0.0730	0.0513, 0.0818
Flack parameter	0.01(5)	0.02(8)	0.04(5)
Largest diff. peak and hole/eÅ^{-3}	0.226, −0.284	0.192, −0.219	0.253, −0.242

−152.2 (1.8F, d, J=18.4 Hz, 2′,6′-F_2); MS m/z 795 (2 M), 398.1394 (M+H) ($C_{17}H_{21}N_1O_4S$ requires 398.1391).

8.52. X-ray crystallography

Single crystals of compounds **20b**, **23b** and **23c** were analysed at 150(2) K using graphite-monochromated Mo Kα radiation and a Nonius Kappa CCD diffractometer. Details of the data collections, solutions and refinements are given in Table 3. The structures were uniformly solved using SHELXS-97[42] and refined using full-matrix least squares in SHELXL-97.[43]

Convergence was uneventful, other than for the following noteworthy points:

The asymmetric unit in **20b** and **23c** consists of two molecules. In **23b** and **23c**, the NH hydrogens were located and refined at 0.89 Å from the relevant parent nitrogens.

In all three structures, the NH and carbonyl groups are implicated in defining the supramolecular topology. In particular, the lattices in **20b** and **23b** are dominated by the hydrogen-bonded chains of molecules, whereas in **23c**, discrete intermolecular interactions occur between pairs of adjacent molecules in the gross array. Intermolecular hydrogen-bonding is also evident in **20b.**

Crystallographic data for all three compounds have been deposited with the Cambridge Crystallographic Data Centre as supplementary publications CCDC 603701–603703. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223 336033, e-mail: deposit@ccdc.cam.ac.uk].

8.53. Cyclisation studies

The HPLC equipment comprised a Dionex autosampler (Model ASl—100/ASl—100T), Dionex pump (Model P 580), Dionex column thermostat for HPLC and GPC (Model 585) and a Dionex UV–vis detector (Model UVD 170S/340S). A C_{18} Hypersil (Thermoquest BDS) column (250×4.6 mm) was used. The mobile phase was composed of a mixture (65/35, v/v) of acetonitrile and aqueous phosphate buffer (0.066 M), adjusted to pH 6.0, 7.0 and 8.0 with aqueous potassium hydroxide (19 M) using a Hanaa pH meter (Model 210). The phosphate buffer was filtered in a Vacuubrand GmbH vacuum system (Model ME 4) with a Schleicher and Schuell filter membrane (pore size and diameter of 0.45 and 47 mm). The flow rate during the assays was 0.8 mL min^{-1} and detection was accomplished at λ=275 and 225 nm. For each kinetic run, the dipeptide amide salt **21a**, **21b**, **21c**, **21d**, **40c** or **40e** (1.0 mg) was added to the buffer (1.0 mL) at the appropriate pH and the mixture was stirred at 37 °C. Samples were withdrawn at appropriate time points for direct HPLC analysis. The HPLC runs were also conducted at 37 °C.

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Tandem [2+2] cycloaddition and Cope rearrangement in reactions of cross-conjugated azatrienes with conjugated ketenes: a facile single step synthesis of novel azocinone derivatives

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Abstract—A facile single step synthesis of novel azocinone derivatives involving tandem [2+2] cycloaddition and Cope rearrangement in the reactions of cross-conjugated azatrienes with vinyl/isopropenyl ketenes supported by theoretical calculations is reported.

1. Introduction

Nitrogen heterocycles are among the most useful and their utility has been widely demonstrated in the chemistry of natural products, in material sciences, and in pharmaceutical chemistry.[1] Their synthesis has attracted considerable attention due to their large importance as building blocks for many therapeutically useful materials, as well as for the wide range of potential biological activity of both synthetic and naturally occurring derivatives. The hetero Diels–Alder reactions of azadienes are one of the most versatile routes for the synthesis of such nitrogen heterocycles. Extensive efforts have been carried out toward the Diels–Alder cycloadditions involving azadienes containing one or more nitrogen atoms and the rapid developments in this area have been reviewed.[1] A large variety of nitrogen heterocycles have been synthesized in our laboratory by the cycloaddition reactions of azadienes with a number of dienophiles.[2]

Recently, Saito and co-workers[3] have reported the diene transmissive hetero Diels–Alder (DTHDA) reactions of some in situ generated cross-conjugated azatrienes, providing a synthetic access to ring fused heterocyclic frameworks.

However, despite being excellent synthons, their initial [4+2] cycloaddition reaction has been restricted to a few reactive heterocummulenes. The lack of extensive efforts in this area may be due to their reported sensitivity to varying reaction conditions and the non-availability of pure starting materials. Our continued interest in the utilization of azadienes in cycloaddition reactions,[2] coupled with the synthetic potential of cross-conjugated azatrienes prompted us to explore the isolation of azatrienes as stable synthons prior to their utilization in hetero DA reactions.

The procedure for the synthesis of stable cross-conjugated azatrienes involves the treatment of the corresponding ketone **1** (10 mmol) with aromatic amine **2** (15 mmol) in the presence of titanium tetrachloride (10 mmol) and triethylamine (22 mmol) in dry toluene at 0 °C (Scheme 1).

After completion, the reaction mixture was passed through a silica column and distillation of the solvent under reduced pressure yielded solid compounds in very good yields (71–88%).

Saito et al. examined the reactions of azatriene **3c** with disubstituted ketenes and obtained [2+2] cycloadducts, which upon heating in toluene underwent [1,3] sigmatropic rearrangement to yield pyridone derivatives.[3b] Recently, Alcaide and co-workers have reported that the reactions of 1-azabuta-1,3-dienes with vinyl/isopropenyl ketenes leading to the initial formation of *cis*-3,4-divinyl-β-lactams as [2+2] adducts, which upon thermolysis in refluxing toluene underwent [3,3] sigmatropic shift to yield azocinone derivatives.[5] However, the methodology used suffers several disadvantages, for example, 3,4-divinyl-β-lactams, used for these transformations, were prepared following a multiplet strategy with natural loss of yields. Also, despite being a novel synthetic route for few azocinones, the scope of this reaction

Keywords: Azatrienes; Ketenes; Cycloadditions; Cope rearrangement.
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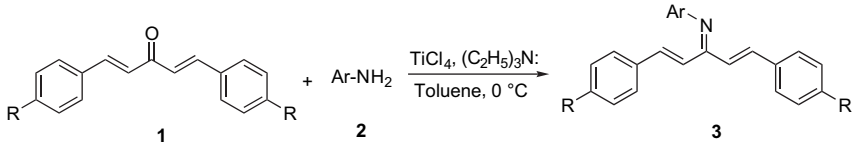


R = OCH_3, H, Cl
Ar = *p*-methoxyphenyl, *p*-tolyl, phenyl

3a. R = OCH_3, Ar = *p*-methoxyphenyl (79%)
3b. R = OCH_3, Ar = *p*-tolyl (82%)
3c. R = OCH_3, Ar = phenyl (88%)
3d. R = H, Ar = *p*-methoxyphenyl (84%)
3e. R = H, Ar = *p*-tolyl (87%)
3f. R = H, Ar = phenyl (83%)
3g. R = Cl, Ar = *p*-methoxyphenyl (76%)
3h. R = Cl, Ar = phenyl (81%)
3i. R = Cl, Ar = *p*-tolyl (71%)

Scheme 1.

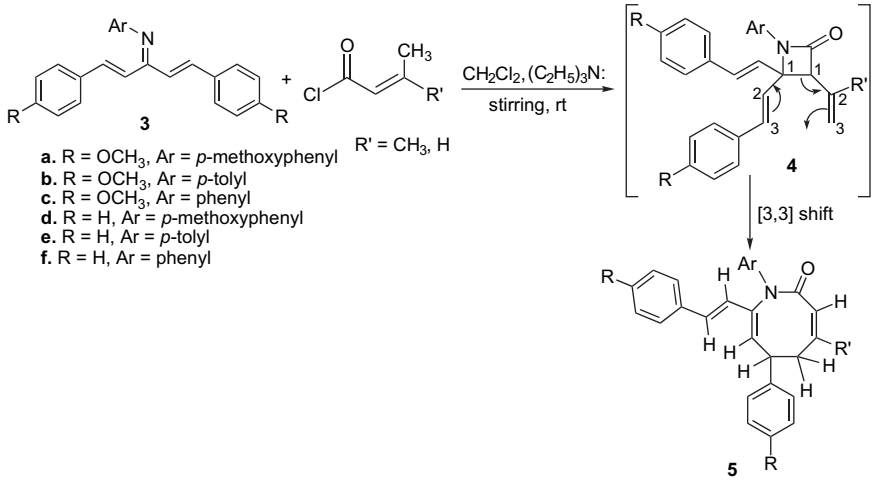
was limited to only few possible structural variants at N of 1-azabuta-1,3-diene.

In order to explore their scope and synthetic potential, we have examined the reactions of the isolated azatrienes **3** with conjugated ketenes viz. vinyl/isopropenyl ketenes, which themselves are known to participate either as 2π or 4π component in [2+2] and [4+2] cycloaddition reactions,[4] generated in situ from the corresponding acid chlorides in the presence of triethylamine in dry dichloromethane at room temperature. Interestingly, these reactions resulted in a facile single step synthesis of novel azocinone derivatives in excellent yields (72–89%), instead of the expected trialkyl 3,4,4-azitidinin-2-ones.

The formation of azocinones **5** in these reactions is probably the result of initial [2+2] cycloadditions and highly efficient [3,3] sigmatropic rearrangements of the so formed [2+2] cycloadducts, 3,4,4-trisubstituted-2-azetidinones **4**, as transient intermediates. The comparison of tlc of the crude reaction mixture with that of the pure product clearly ruled out any possibility of the observed Cope rearrangement taking place during processes such as recrystallization, work up, etc.

The mechanism proposed above was further corroborated by the energy minimization calculations performed at AM1 level using MOPAC program.[6] The calculations reveal that the product **5** is more stable than the initial [2+2] cycloadduct **4** by 28.9 kcal mol^{-1} (Scheme 2).

The energy minimization calculations for 3,4,4-trisubstituted-2-azetidinones **4** indicate an energy of 130.8 kcal mol^{-1} with apical distance (C_{12}–C_{17}) of 3.54 Å and dihedral angles −89° (C_{14}–C_2–C_6–C_{12}) and −15° (C_2–C_{14}–C_{15}–C_{17}) (Fig. 1). On the other hand, the energy minimization



a. R = OCH_3, Ar = *p*-methoxyphenyl
b. R = OCH_3, Ar = *p*-tolyl
c. R = OCH_3, Ar = phenyl
d. R = H, Ar = *p*-methoxyphenyl
e. R = H, Ar = *p*-tolyl
f. R = H, Ar = phenyl

R' = CH_3, H

No.	R	Ar	R'	Yield(%)
5a.	OCH_3	*p*-methoxyphenyl	CH_3	85
5b.	OCH_3	*p*-methoxyphenyl	H	82
5c.	OCH_3	*p*-tolyl	CH_3	81
5d.	OCH_3	*p*-tolyl	H	88
5e.	OCH_3	phenyl	CH_3	89
5f.	OCH_3	phenyl	H	83
5g.	H	*p*-methoxyphenyl	CH_3	72
5h.	H	*p*-methoxyphenyl	H	78
5i.	H	*p*-tolyl	CH_3	86
5j.	H	*p*-tolyl	H	80
5k.	H	phenyl	CH_3	77
5l.	H	phenyl	H	76

Scheme 2.

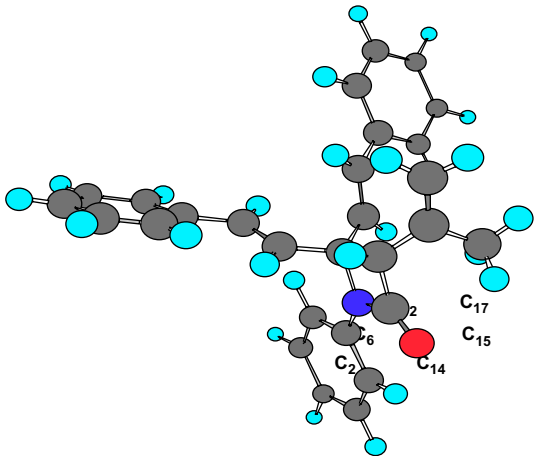


Figure 1. Energy minimized structure of intermediate **4**.

calculations on 3,4-divinyl azetidinone earlier utilized in thermolytic Cope rearrangement,[5] have shown its ground state energy as 80.6 kcal mol^{-1} with apical distance of 3.87 Å (Figs. 2 and 3).[6]

In line with the arguments advanced above, the reduction in dihedral angle increases the energy of the system to 102.2 kcal mol^{-1}, a difference of 21.6 kcal. The resultant energy barrier of 21.6 kcal mol^{-1} is naturally more difficult to attain than 4.1 kcal mol^{-1} required for conversion of **4** to **5**. This is also in agreement with the harsh conditions required for the earlier reported Cope rearrangement.[5]

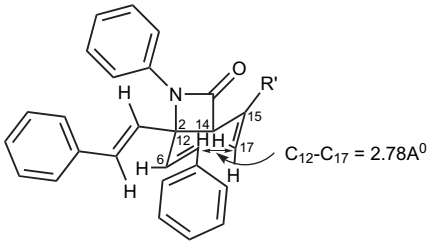


Figure 2. Most probable conformation of **4** for Cope rearrangement.

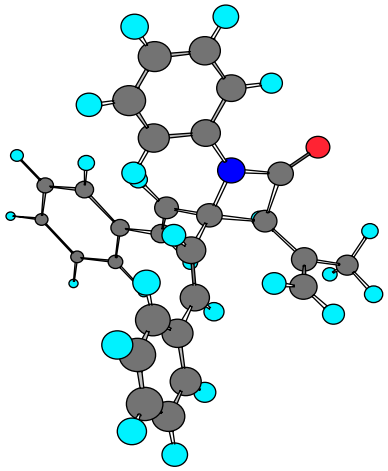


Figure 3. Energy minimized most probable conformer of [2+2] adduct **4** required for Cope rearrangement.

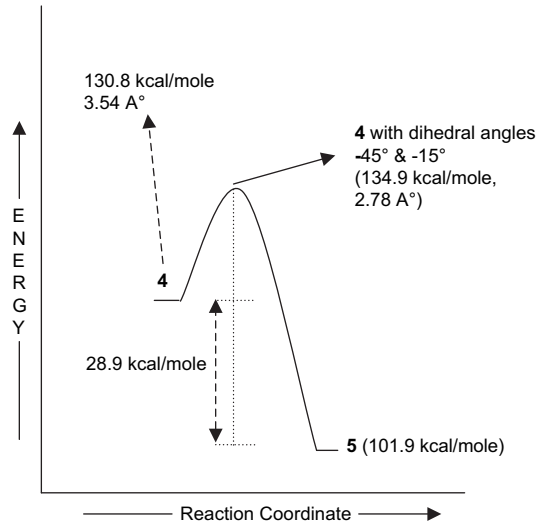


Figure 4. A probable graphical representation of reaction pathway.

The methodology has been generalized for the synthesis of other azocinone derivatives **5** (**c–l**) in the reactions of cross-conjugated azatrienes (**3b–3f**) with conjugated ketenes without even traces of corresponding [2+2] cycloadducts. Since, the synthesis of eight- and nine-membered rings is comparatively difficult due to their unfavorable enthalpic and entropic factors,[7,8] an easy and convenient route for the synthesis of such eight membered lactams has been developed (Fig. 4).

In conclusion, an unprecedented single pot synthesis of stable cross-conjugated azatrienes **3** (**a–f**) along with the tandem [2+2] cycloaddition and highly facile [3,3] sigmatropic rearrangement in their reactions with conjugated ketenes, leading to facile synthesis of various functionalised azocinone derivatives, has been reported. Alcaide and co-workers in their communication exploited just the azadienic system having limited variants thus imposing structural limitations on the reaction site and restraining the diversity of the formed azocinone derivatives. Whereas the systems considered in our manuscript widens the scope of this methodology, as many structural variants present in the cross-conjugated system acting as 1-azadiene opens up a channel to obtain novel functionalized azocinones. The facile nature of the observed [3,3] sigmatropic shift has been supported by the theoretical studies.

2. Experimental

2.1. General

Melting points were determined by open capillary method using Veego Precision Digital Melting Point apparatus (MP-D) and are uncorrected. IR spectra were recorded on a Shimadzu D-8001 FT-spectrophotometer. ^{1}H NMR spectra were recorded in deuterochloroform with Bruker AC-E 200 (200 MHz) spectrometer using TMS as an internal standard. Chemical shift values are expressed as parts per million downfield from TMS and *J* values are in hertz. Splitting patterns are indicated as s: singlet, d: doublet and br s: broad singlet. ^{13}C NMR spectra were also recorded on a Bruker

AC-E 200 (50.4 MHz) spectrometer in a deuterochloroform using TMS as an internal standard. Mass spectra were recorded on Shimadzu GCMS-QP-2000 mass spectrometer. Elemental analyses were performed on Heraus CHN-O-Rapid Elemental Analyzer. Column chromatography was performed on a silica gel (60–120) mesh or Harrison Research Chromatotron using 2 mm plates (Silica gel PF_{254}).

2.2. Starting materials

Cross-conjugated ketones **1** were prepared according to the reported procedure.[9] Crotyl and 3,3-dimethylacryl chlorides were prepared from their corresponding acids and thionyl chloride. Thionyl chloride was distilled before use. Aromatic amines used were commercially available. Dichloromethane was dried over di-phosphorous pentoxide and stored over molecular sieves (4 Å).

2.3. General procedure for the preparation of cross-conjugated azatrienes (3)

To the solution of **1** (10 mmol) and aryl amine **2** (15 mmol) in toluene (30 mL) was added triethylamine (22 mmol) and reaction mixture was stirred. After stirring for 5 min $TiCl_4$ (10 mmol) was added to the reaction mixture dropwise by keeping the reaction temperature at 0 °C. After completion (tlc), the reaction mixture was passed through a silica column and distillation of the solvent under reduced pressure yielded solid compounds, which were recrystallized using ethyl acetate–hexane mixture (1:5, v/v).

2.4. General procedure for the reaction of azatrienes with isopropenyl/vinyl ketenes

To a well-stirred solution of azatrienes **3** (10 mmol) and triethylamine (15 mmol) in dry methylene chloride (30 mL) was added dropwise a solution of 3,3-dimethylacryl chloride/crotyl chloride in dry methylene chloride (30 mL) over a period of 0.5 h at room temperature. After completion of the reaction (tlc), the reaction mixture was first washed with saturated sodium bicarbonate solution (2×25 mL) and water (2×50 mL) and the organic layer was dried over anhydrous sodium sulfate. Removal of solvent under reduced pressure yielded the crude product, which was purified by silica gel column chromatography using a mixture of ethyl acetate and hexane (1:10, v/v).

2.4.1. (4-Methoxy-phenyl)-{3-(4-methoxy-phenyl)-1-[2(4-methoxy-phenyl)-vinyl]-allylidene}-amine (3a). Reddish yellow prismatic crystalline solid, Yield: 79%; mp 161–163 °C; Anal. Calcd for $C_{26}H_{25}NO_3$: C, 78.17; H, 6.31; N, 3.51. Found: C, 78.34; H, 6.42; N, 3.42%. IR (KBr): ν_{max}=1602, 1510, 1463, 1033 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=3.82 (s, 6H, 2×–OCH_3), 3.84 (s, 3H, –OCH_3), 6.68 (d, J=16.5 Hz, 1H, olefinic), 6.84–6.93 (m, 7H, 6ArH and 1H, olefinic), 7.10 (d, J=16.5 Hz, 1H, olefinic), 7.13 (d, J=16.5 Hz, 1H, olefinic), 7.25–7.55 (m, 6H, ArH) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=55.2 (–OCH_3), 55.3 (–OCH_3), 114.0, 114.1, 114.2, 120.7, 122.7, 124.2, 128.3, 128.6, 128.9, 129.1, 136.9, 137.3, 144.2, 156.3, 160.2, 160.4, and 163.0. *m/z*: 399 (M^+).

2.4.2. {3-(4-Methoxy-phenyl)-1-[2-(4-methoxy-phenyl)-vinyl]-allylidene}-*p*-tolyl-amine (3b). Reddish yellow solid, Yield: 82%; mp 148–149 °C; Anal. Calcd for $C_{26}H_{25}NO_2$: C, 81.43; H, 6.57; N, 3.65. Found: C, 81.37; H, 6.51; N, 3.59%. IR (KBr) ν_{max}=1602, 1510, 1463, 1033 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=2.35 (s, 3H, –CH_3), 3.81 (s, 3H, –OCH_3), 3.84 (s, 3H, –OCH_3), 6.66 (d, J=16.5 Hz, 1H, olefinic), 6.80–6.94 (m, 6H, ArH), 7.12 (d, J=15.9 Hz, 1H, olefinic), 7.15 (d, J=15.9 Hz, 1H, olefinic), 7.25–7.36 (m, 4H, ArH), 7.47 (d, J=16.5 Hz, 1H, olefinic), 7.53 (d, J=8.9 Hz, 2H, ArH) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=20.9, 55.3, 55.4, 114.2, 114.4, 120.7, 121.2, 123.4, 124.0, 128.3, 129.1, 129.3, 129.7, 130.3, 133.1, 137.5, 148.3, 160.3, 160.5, and 163.2. *m/z*: 383 (M^+).

2.4.3. {3-(4-Methoxy-phenyl)-1-[2-(4-methoxy-phenyl)-vinyl]-allylidene}-phenyl-amine (3c). Yellow solid, Yield: 88%; mp 155–156 °C; Anal. Calcd for $C_{25}H_{23}NO_2$: C, 81.27; H, 6.27; N, 3.79. Found: C, 81.35; H, 6.34; N, 3.73%. IR (KBr): ν_{max}=1603, 1510, 1462, 1033 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=3.83 (s, 6H, 2×–OCH_3), 6.66 (d, J=16.5 Hz, 1H, olefinic), 6.78–7.05 (m, 7H, ArH), 7.11 (d, J=15.9 Hz, 1H, olefinic), 7.13 (d, J=15.9 Hz, 1H, olefinic), 7.21–7.33 (m, 4H, ArH), 7.49 (d, J=16.5 Hz, 1H, olefinic), 7.55 (d, J=8.9 Hz, 2H, ArH) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=55.4, 114.1, 114.5, 120.2, 121.7, 122.6, 123.7, 125.6, 127.8, 128.5, 129.6, 130.2, 132.9, 136.8, 149.5, 159.9, 160.2, and 163.1. *m/z*: 369 (M^+).

2.4.4. (4-Methoxy-phenyl)-(3-phenyl-1-styryl-allylidene)-amine (3d). Yellow solid, Yield: 84%; mp 119–120 °C; Anal. Calcd for $C_{24}H_{21}NO$: C, 84.92; H, 6.24; N, 4.13. Found: C, 85.02; H, 6.29; N, 4.18%. IR (KBr): ν_{max}=1602, 1510, 1460, 1033 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=3.83 (s, 3H, –OCH_3), 6.70 (d, J=16.5 Hz, 1H, olefinic), 6.86–6.97 (m, 7H, ArH), 7.13 (d, J=15.8 Hz, 1H, olefinic), 7.15 (d, J=16.5 Hz, 1H, olefinic), 7.29–7.38 (m, 5H, ArH), 7.41 (d, J=15.8 Hz, 1H, olefinic), 7.56 (d, J=8.7 Hz, 2H, ArH) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=55.3, 113.9, 114.6, 121.6, 122.6, 124.7, 127.3, 128.4, 128.6, 128.9, 129.4, 137.2, 138.5, 145.9, 156.8, 160.1, 160.7, and 162.9. *m/z*: 339 (M^+).

2.4.5. (3-Phenyl-1-styryl-allylidene)-*p*-tolyl-amine (3e). Yellow solid, Yield: 87%; mp 107–108 °C; Anal. Calcd for $C_{24}H_{21}N$: C, 89.12; H, 6.54; N, 4.33. Found: C, 89.22; H, 6.47; N, 4.38%. IR (KBr): ν_{max}=1603, 1512, 1465, 1033 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=2.37 (s, 3H, –CH_3), 6.81 (d, J=16.5 Hz, 1H, olefinic), 6.85 (d, J=8.4 Hz, 2H, ArH), 7.17 (d, J=15.9 Hz, 1H, olefinic), 7.19–7.64 (m, 14H, 12ArH and 2 olefinic) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=20.9, 121.1, 122.5, 126.3, 127.3, 127.5, 128.7, 128.8, 128.9, 129.2, 129.4, 133.5, 135.8, 136.2, 137.8, 138.1, 148.0, and 162.8. *m/z*: 323 (M^+).

2.4.6. Phenyl-(3-phenyl-1-styryl-allylidene)-amine (3f). Reddish yellow solid, Yield: 83%; mp 79–80 °C; Anal. Calcd for $C_{23}H_{19}N$: C, 89.28; H, 6.19; N, 4.53. Found: C, 89.21; H, 6.25; N, 4.48%. IR (KBr): ν_{max}=1602, 1510, 1463, 1033 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=6.76 (d, J=16.5 Hz, 1H, olefinic), 6.90–7.13 (m, 3H,

2ArH and 1 olefinic), 7.18 (d, J=16.5 Hz, 1H, olefinic), 7.22–7.63 (m, 13H, ArH), 7.75 (d, J=16.5 Hz, 1H, olefinic) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=120.9, 122.3, 123.8, 125.9, 127.5, 128.3, 128.7, 128.9, 129.0, 130.4, 134.7, 135.7, 136.1, 138.0, 143.3, 150.7, and 162.9. m/z: 309 (M^+).

2.4.7. {3-(4-Chloro-phenyl)-1-[2-(4-chloro-phenyl)-vinyl]-allylidene}-(4-methoxy-phenyl)-amine (3g). Yellow crystalline solid, Yield: 76%; mp 110–111 °C; Anal. Calcd for $C_{24}H_{19}Cl_2NO$: C, 70.60; H, 4.69; N, 3.43. Found: C, 70.66; H, 4.62; N, 3.52%. IR (KBr): ν_{max}=1603, 1510, 1462, 1033 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=3.81 (s, 3H, –OCH_3), 6.76 (d, J=16.5 Hz, 1H, olefinic), 7.01 (d, J=15.9 Hz, 1H, olefinic), 7.08 (d, J=16.5 Hz, 1H, olefinic), 7.19–7.31 (m, 4H, ArH), 7.36 (d, J=8.9 Hz, 2H, ArH), 7.37 (d, J=8.9 Hz, 2H, ArH), 7.49 (d, J=8.4 Hz, 2H, ArH), 7.52 (d, J=8.4 Hz, 2H, ArH), 7.66 (d, J=15.9 Hz, 1H, olefinic) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=55.4, 114.1, 120.8, 122.9, 124.7, 126.5, 127.6, 128.2, 128.7, 129.5, 130.1, 134.8, 135.6, 137.6, 137.8, 141.5, 152.6, and 161.1. m/z: 408 (M^+).

2.4.8. {3-(4-Chloro-phenyl)-1-[2-(4-chloro-phenyl)-vinyl]-allylidene}-*p*-tolyl-amine (3h). Yellow solid, Yield: 81%; mp 124–125 °C; Anal. Calcd for $C_{24}H_{19}Cl_2N$: C, 73.47; H, 4.88; N, 3.57. Found: C, 73.51; H, 4.83; N, 3.62%. IR (KBr): ν_{max}=1602, 1512, 1462, 1033 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=2.39 (s, 3H, –CH_3), 6.78 (d, J=16.5 Hz, 1H, olefinic), 6.99 (d, J=8.6 Hz, 2H, ArH), 7.05 (d, J=15.9 Hz, 1H, olefinic), 7.18 (d, J=16.5 Hz, 1H, olefinic), 7.21 (d, J=8.7 Hz, 2H, ArH), 7.19–7.31 (m, 4H, ArH), 7.35–7.52 (m, 4H, ArH), 7.66 (d, J=15.9 Hz, 1H, olefinic) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=20.8, 114.2, 121.6, 122.5, 124.9, 126.5, 127.8, 128.4, 128.9, 129.1, 129.5, 134.8, 135.6, 137.6, 137.8, 141.5, 152.6, and 161.1. m/z: 391 (M^+).

2.4.9. {3-(4-Chloro-phenyl)-1-[2-(4-chloro-phenyl)-vinyl]-allylidene}-phenyl-amine (3i). Yellow solid, Yield: 71%; mp 131–132 °C; Anal. Calcd for $C_{23}H_{17}Cl_2N$: C, 73.02; H, 4.53; N, 3.70. Found: C, 72.94; H, 4.59; N, 3.61%. IR (KBr): ν_{max}=1603, 1510, 1464, 1033 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=6.74 (d, J=16.5 Hz, 1H, olefinic), 7.01 (d, J=8.7 Hz, 2H, ArH), 7.03 (d, J=15.9 Hz, 1H, olefinic), 7.16 (d, J=16.5 Hz, 1H, olefinic), 7.23 (d, J=8.7 Hz, 2H, ArH), 7.14–7.35 (m, 4H, ArH), 7.36–7.51 (m, 5H, ArH), 7.67 (d, J=15.9 Hz, 1H, olefinic) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=114.1, 121.8, 122.6, 125.1, 126.3, 127.9, 128.2, 129.2, 129.9, 131.5, 134.5, 135.9, 137.2, 137.8, 141.8, 152.7, and 161.5. m/z: 377 (M^+).

2.4.10. 1,6-Bis-(4-methoxy-phenyl)-8-[2-(4-methoxy-phenyl)-vinyl]-4-methyl-5,6-dihydro-1*H*-azocinone (5a). Colorless crystalline solid, Yield: 85%; mp 202–203 °C; Anal. Calcd for $C_{31}H_{31}NO_4$: C, 77.31; H, 6.49; N, 2.91. Found: C, 77.39; H, 6.53; N, 2.99. IR (KBr): ν_{max}=1662, 1508, 1338, 1299, 1031 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=1.84 (s, 3H, –CH_3), 2.28 (unresolved dd, J=13.1 Hz, 1H, –CH_2), 2.72 (unresolved dd, J=13.1 Hz, 1H, –CH_2), 3.77 (s, 6H, 2×–OCH_3), 3.83 (s, 3H, –OCH_3), 4.08–4.12 (m, 1H, –CH), 5.93 (d, J=10.0 Hz, 1H, olefinic), 5.99 (br s, 1H, olefinic), 6.34 (d, J=16.2 Hz, 1H, olefinic), 6.42 (d, J=16.2 Hz, 1H, olefinic), 6.74–6.94 (m, 6H, ArH), 7.15–7.35 (m, 6H, ArH) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=26.1 (–CH_3), 39.9 (–CH_2), 42.6 (–CH), 55.2 (–OCH_3), 55.3(–OCH_3), 96.1, 113.9, 114.3, 120.1, 122.3, 126.4, 127.7, 127.9, 129.1, 130.7, 132.1, 132.3, 134.5, 138.7, 140.2, 157.6, 158.6, 159.4, and 168.5. m/z: 481 (M^+).

2.4.11. 1,6-Bis-(4-methoxy-phenyl)-8-[2-(4-methoxy-phenyl)-vinyl]-5,6-dihydro-1*H*-azocin-2-one (5b). Colorless solid, Yield: 82%; mp 191–192 °C; Anal. Calcd for $C_{30}H_{29}NO_4$: C, 77.06; H, 6.21; N, 3.00. Found: C, 77.13; H, 6.13; N, 2.93%. IR (KBr): ν_{max}=1660, 1512, 1338, 1299, 1030 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=2.41–2.47 (m, 1H, –CH_2), 2.76–2.82 (m, 1H, –CH_2), 3.81 (s, 6H, 2×–OCH_3), 3.83 (s, 3H, –OCH_3), 4.13–4.18 (m, 1H, –CH), 5.93 (br s, 1H, olefinic), 5.99 (d, J=10.1 Hz, 1H, olefinic), 6.02 (br s, 1H, olefinic), 6.43 (br s, 2H, olefinic), 6.71–6.89 (m, 6H, ArH), 7.13–7.38 (m, 6H, ArH) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=37.5, 40.8, 55.2, 55.5, 96.2, 114.1, 114.5, 120.5, 122.3, 123.7, 126.4, 127.7, 129.8, 131.4, 132.2, 133.8, 135.4, 137.6, 138.9, 141.2, 142.5, 157.4, and 168.5. m/z: 467 (M^+).

2.4.12. 6-(4-Methoxy-phenyl)-8-[2-(4-methoxy-phenyl)-vinyl]-4-methyl-1-*p*-tolyl-5,6-dihydro-1*H*-azocin-2-one (5c). Pale white solid, Yield: 81%; mp 212–213 °C; Anal. Calcd for $C_{31}H_{31}NO_3$: C, 79.97; H, 6.71; N, 3.01. Found: C, 80.05; H, 6.78; N, 2.96%. IR (KBr): ν_{max}=1663, 1510, 1330, 1290, 1030 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=1.83 (s, 3H, –CH_3), 2.34 (s, 3H, –CH_3), 2.39–2.43 (m, 1H, –CH_2), 2.64–2.74 (m, 1H, –CH_2), 3.83 (s, 6H, 2×–OCH_3), 4.09–4.13 (m, 1H, –CH), 5.88 (br s, 1H, olefinic), 6.01 (br s, 1H, olefinic), 6.45 (br s, 2H, olefinic), 6.77 (d, J=8.7 Hz, 2H, ArH), 6.88 (d, J=8.8 Hz, 2H, ArH), 6.99–7.41 (m, 8H, ArH) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=20.8 (CH_3), 25.9 (–CH_3), 40.3 (–CH_2), 42.9 (–CH), 55.3 (–OCH_3), 96.2, 113.8, 114.4, 120.5, 121.8, 123.5, 125.6, 126.7, 129.4, 130.7, 131.8, 134.2, 135.2, 136.1, 138.4, 142.4, 156.5, 157.2, and 168.2. m/z: 465 (M^+).

2.4.13. 6-(4-Methoxy-phenyl)-8-[2-(4-methoxy-phenyl)-vinyl]-1-*p*-tolyl-5,6-dihydro-1*H*-azocin-2-one (5d). Colorless solid, Yield: 88%; mp 183–184 °C; Anal. Calcd for $C_{30}H_{29}NO_3$: C, 79.80; H, 6.47; N, 3.10. Found: C, 79.85; H, 6.51; N, 3.01%. IR (KBr): ν_{max}=1661, 1514, 1330, 1293, 1030 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=2.34 (s, 3H, –CH_3), 2.43–2.48 (m, 1H, –CH_2), 2.74–2.80 (m, 1H, –CH_2), 3.82 (s, 6H, 2×–OCH_3), 4.17–4.20 (m, 1H, –CH), 5.87–5.91 (m, 1H, olefinic), 5.97 (d, J=10.2 Hz, 1H, olefinic), 6.02 (br s, 1H, olefinic), 6.45 (br s, 2H, olefinic), 6.77 (d, J=8.7 Hz, 2H, ArH), 6.89 (d, J=8.7 Hz, 2H, ArH), 6.99–7.42 (m, 8H, ArH) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=21.0, 37.7, 40.6, 55.4, 96.1, 113.9, 114.4, 120.3, 121.6, 122.8, 125.9, 127.3, 129.4, 132.5, 133.4, 135.6, 136.7, 137.4, 138.8, 140.9, 141.8, 156.9, and 168.3. m/z: 451 (M^+).

2.4.14. 6-(4-Methoxy-phenyl)-8-[2-(4-methoxy-phenyl)-vinyl]-4-methyl-1-phenyl-5,6-dihydro-1*H*-azocin-2-one (5e). Colorless prismatic crystalline solid, Yield: 89%; mp 196–197 °C; Anal. Calcd for $C_{30}H_{29}NO_3$: C, 79.80; H,

6.47; N, 3.10. Found: C, 79.89; H, 6.51; N, 3.14%. IR (KBr): ν_{max}=1660, 1510, 1335, 1295, 1030 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=1.82 (s, 3H, $-CH_3$), 2.29 (unresolved dd, J=13.1 Hz, 1H, $-CH_2$), 2.74 (unresolved dd, J=13.1 Hz, 1H, $-CH_2$), 3.79 (s, 6H, 2×$-OCH_3$), 4.09–4.14 (m, 1H, –CH), 5.94 (d, J=10.1 Hz, 1H), 6.02 (br s, 1H, olefinic), 6.33 (d, J=16.2 Hz, 1H), 6.44 (d, J=16.2 Hz, 1H), 6.79–7.39 (m, 13H, ArH) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=26.2 ($-CH_3$), 39.8 ($-CH_2$), 42.9 (–CH), 55.2 ($-OCH_3$), 96.2, 114.1, 114.5, 120.7, 121.8, 124.4, 125.8, 126.2, 128.5, 129.6, 132.3, 133.7, 135.5, 138.9, 141.8, 156.5, 158.4, 159.2, and 168.2. m/z: 451 (M^+).

2.4.15. 6-(4-Methoxy-phenyl)-8-[2-(4-methoxy-phenyl)-vinyl]-1-phenyl-5,6-dihydro-1*H*-azocin-2-one (5f). White solid, Yield: 83%; mp 178–179 °C; Anal. Calcd for $C_{29}H_{27}NO_3$: C, 79.61; H, 6.22; N, 3.20. Found: C, 79.68; H, 6.29; N, 3.13%. IR (KBr): ν_{max}=1663, 1510, 1330, 1295, 1030 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=2.37–2.42 (m, 1H, $-CH_2$), 2.73–2.79 (m, 1H, $-CH_2$), 3.82 (s, 6H, 2×$-OCH_3$), 4.16–4.21 (m, 1H, –CH), 5.93 (br s, 1H, olefinic), 6.05 (br s, 1H, olefinic), 6.43 (br s, 2H, olefinic), 6.79 (d, J=8.8 Hz, 2H, ArH), 6.87–7.39 (m, 11H, ArH) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=39.1, 41.1, 55.2, 96.1, 113.8, 114.2, 120.4, 121.8, 122.6, 124.9, 126.7, 128.3, 130.7, 133.2, 133.7, 135.1, 136.3, 137.2, 141.5, 143.5, 156.8, and 168.2. m/z: 437 (M^+).

2.4.16. 1-(4-Methoxy-phenyl)-4-methyl-6-phenyl-8-styryl-5,6-dihydro-1*H*-azocin-2-one (5g). Pale white solid, Yield: 72%; mp 217–218 °C; Anal. Calcd for $C_{29}H_{27}NO_2$: C, 82.63; H, 6.46; N, 3.32. Found: C, 82.52; H, 6.41; N, 3.24%. IR (KBr): ν_{max}=1661, 1517, 1333, 1295, 1030 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=1.92 (s, 3H, $-CH_3$), 2.32–2.38 (m, 1H, $-CH_2$), 2.76–2.79 (m, 1H, $-CH_2$), 3.78 (s, 3H, $-OCH_3$), 4.14–4.20 (m, 1H, –CH), 6.02 (br s, 1H, olefinic), 6.03 (br s, 1H, olefinic), 6.50 (br s, 2H, olefinic), 6.87 (d, J=8.8 Hz, 2H, ArH), 7.16–7.43 (m, 12H, ArH) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=26.1 ($-CH_3$), 37.9 ($-CH_2$), 41.1(–CH), 55.3 ($-OCH_3$), 96.2, 114.0, 120.2, 124.8, 126.5, 127.2, 127.9, 128.2, 130.3, 132.4, 132.9, 133.1, 136.5, 138.8, 140.2, 142.4, 145.8, 157.3, and 168.6. m/z: 421 (M^+).

2.4.17. 1-(4-Methoxy-phenyl)-6-phenyl-8-styryl-5,6-dihydro-1*H*-azocin-2-one (5h). Colorless solid, Yield: 78%; mp 189–190 °C; Anal. Calcd for $C_{28}H_{25}NO_2$: C, 82.53; H, 6.18; N, 3.44. Found: C, 82.57; H, 6.12; N, 3.36%. IR (KBr): ν_{max}=1660, 1515, 1330, 1295, 1030 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=2.42–2.48 (m, 1H, $-CH_2$), 2.80–2.83 (m, 1H, $-CH_2$), 3.79 (s, 3H, $-OCH_3$), 4.16–4.19 (m, 1H, –CH), 5.90 (br s, 1H, olefinic), 6.03 (d, J=10.0 Hz, 1H, olefinic), 6.17 (d, J=10.0 Hz, 1H, olefinic), 6.52 (br s, 2H, olefinic), 6.89 (d, J=8.8 Hz, 2H, ArH), 7.17–7.46 (m, 12H, ArH) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=37.4 ($-CH_2$), 40.7 (–CH), 55.2 ($-OCH_3$), 96.1, 114.1, 124.5, 126.4, 126.5, 127.0, 127.2, 127.9, 128.5, 129.1, 131.3, 131.5, 131.9, 133.1, 136.2, 139.1, 142.2, 157.8, and 168.1. m/z: 407 (M^+).

2.4.18. 4-Methyl-6-phenyl-8-styryl-1-*p*-tolyl-5,6-dihydro-1*H*-azocin-2-one (5i). Colorless solid, Yield: 86%; mp 167–168 °C; Anal. Calcd for $C_{29}H_{27}NO$: C, 85.89; H, 6.71; N, 3.45. Found: C, 85.83; H, 6.62; N, 3.39%. IR (KBr): ν_{max}=1664, 1513, 1331, 1292, 1030 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=1.83 (s, 3H, $-CH_3$), 2.35 (s, 3H, $-CH_3$), 2.25–2.33 (m, 1H, $-CH_2$), 2.73–2.82 (m, 1H, $-CH_2$), 4.15–4.19 (m, 1H, –CH), 5.99 (br s, 1H, olefinic), 6.05 (br s, 1H, olefinic), 6.53 (br s, 2H, olefinic), 6.78 (d, J=8.8 Hz, 2H, ArH), 6.89 (d, J=8.8 Hz, 2H, ArH), 7.10–7.45 (m, 10H, ArH) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=20.9, 25.8 ($-CH_3$), 37.6 ($-CH_2$), 40.7 (–CH), 96.1, 114.2, 121.5, 122.6, 123.3, 124.9, 126.7, 127.9, 129.8, 130.8, 131.9, 132.2, 132.8, 133.4, 134.5, 135.4, 142.9, 157.8, and 168.2. m/z: 405 (M^+).

2.4.19. 6-Phenyl-8-styryl-1-*p*-tolyl-5,6-dihydro-1*H*-azocin-2-one (5j). Colorless solid, Yield: 80%; mp 159–160 °C; Anal. Calcd for $C_{28}H_{25}NO$: C, 85.90; H, 6.44; N, 3.58. Found: C, 86.01; H, 6.52; N, 3.49%. IR (KBr): ν_{max}= 1662, 1511, 1330, 1299, 1030 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=2.37 (s, 3H, $-CH_3$), 2.35–2.43 (m, 1H, $-CH_2$), 2.72–2.80 (m, 1H, $-CH_2$), 4.13–4.18 (m, 1H, –CH), 5.92 (br s, 1H, olefinic), 6.07 (d, J=8.9 Hz, 1H, olefinic), 6.17 (br s, 1H, olefinic), 6.53 (br s, 2H, olefinic), 6.79 (d, J=8.8 Hz, 2H, ArH), 6.93 (d, J=8.8 Hz, 2H, ArH), 7.05–7.41 (m, 10H, ArH) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=20.9, 37.5 ($-CH_2$), 40.4 (–CH), 96.2, 114.2, 120.8, 122.5, 123.1, 123.7, 125.5, 126.8, 127.3, 127.9, 129.1, 130.3, 132.7, 134.5, 136.3, 136.8, 142.5, 158.1, and 168.3. m/z: 391 (M^+).

2.4.20. 4-Methyl-1,6-diphenyl-8-styryl-5,6-dihydro-1*H*-azocin-2-one (5k). Colorless solid, Yield: 77%; mp 172–173 °C; Anal. Calcd for $C_{28}H_{25}NO$: C, 85.90; H, 6.44; N, 3.58. Found: C, 85.97; H, 6.49; N, 3.63%. IR (KBr): ν_{max}= 1661, 1510, 1326, 1290, 1030 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=1.86 (s, 3H, $-CH_3$), 2.30–2.36 (m, 1H, $-CH_2$), 2.74–2.81 (m, 1H, $-CH_2$), 4.21–4.26 (m, 1H, –CH), 5.91 (br s, 1H, olefinic), 6.02 (br s, 1H, olefinic), 6.56 (br s, 2H, olefinic), 6.79–7.15 (m, 6H, ArH), 7.20–7.41 (m, 9H, ArH) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=26.1 ($-CH_3$), 37.6 ($-CH_2$), 40.5 (–CH), 96.2, 114.3, 121.3, 122.6, 123.7, 125.5, 126.4, 127.2, 129.5, 130.6, 132.2, 132.8, 133.4, 134.5, 135.4, 136.1, 142.7, 156.7, and 168.4. m/z: 391 (M^+).

2.4.21. 1,6-Diphenyl-8-styryl-5,6-dihydro-1*H*-azocin-2-one (5l). Colorless solid, Yield: 76%; mp 208–209 °C; Anal. Calcd for $C_{27}H_{23}NO$: C, 85.91; H, 6.14; N, 3.71. Found: C, 85.82; H, 6.19; N, 3.65%. IR (KBr): ν_{max}=1660, 1515, 1327, 1290, 1030 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$, Me_4Si): δ=2.35–2.41 (m, 1H, $-CH_2$), 2.70–2.78 (m, 1H, $-CH_2$), 4.15–4.22 (m, 1H, –CH), 5.98 (br s, 1H, olefinic), 6.06 (d, J=10.1 Hz, 1H, olefinic), 6.13 (br s, 1H, olefinic), 6.57 (br s, 2H, olefinic), 6.73–7.39 (m, 15H, ArH) ppm; ^{13}C NMR (50.4 MHz, $CDCl_3$, Me_4Si): δ=37.9 ($-CH_2$), 40.6 (–CH), 96.1, 113.9, 120.5, 121.9, 122.6, 123.2, 125.1, 125.4, 127.6, 129.4, 130.3, 131.6, 131.8, 133.6, 135.9, 136.8, 141.9, 157.8, and 168.5. m/z: 377(M^+).

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Synthesis of dispirooxindolecycloalka[*d*]pyrimidino[2,3-*b*]-thiazole pyrrolidine/thiapyrrolizidine ring systems

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Abstract—The synthesis of a new class of spirooxindolo pyrrolidines and spirooxindolo thiapyrrolizidines has been accomplished by a three component, one-pot 1,3-dipolar cycloaddition reaction. The cycloaddition was found to be highly regioselective.

1. Introduction

The azomethine ylide represents one of the most reactive and versatile classes of 1,3-dipoles and is readily trapped by a range of dipolarophiles forming substituted pyrrolidines.[1] The reactions of azomethine ylides have been studied more than any other dipoles due to their remarkable synthetic potentials.[2] Spiropyrrolidines and spiropyrrolizidines have gained much attention in recent years due to their interesting biological activities.[3,4] The synthesis of spirooxindole ring systems has also attracted considerable attention since they are the basic building units in many natural products like gelsemine, pseudotabersonine, morroniside, etc.[5] Molecules with the thiazolidine nucleus have shown a wide spectrum of bioactivities like anti-inflammatory and anti-hypertensive activities.[6,7] Apart from the well established pharmacological activities of the thiazolone ring fused to a cycloheptene system,[8] the pyrimidine ring fused with substituted seven- and eight-membered carbocycles has shown anticancer and herbicidal activities.[9–11]

Since we have been involved in [3+2] cycloaddition chemistry for some years[12,13] and some of our synthesized molecules have shown good biological activities,[14] we decided to synthesize some complex spiroheterocyclic ring systems incorporating the foresaid bioactive moieties anticipating an overall bioactivity. These complex spiroheterocycles are synthesized by reacting suitable non-stabilized azomethine ylides with various substituted (*E*)-arylmethylene derivatives of octahydro/decahydro cycloalka[*d*]thiazolo[3,2-*a*]-pyrimidine-3-ones.

Keywords: Azomethine ylide; Regioselective; Spiropyrrolidines; Spirothiapyrrolizidines.
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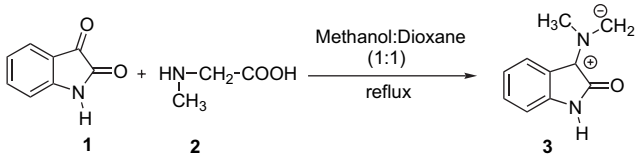
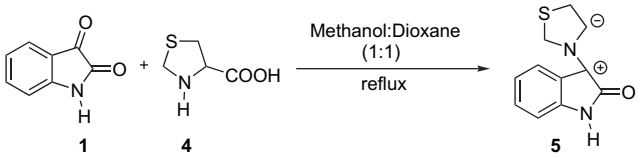
2. Results and discussion

Azomethine ylides can be prepared by several methods from easily available starting materials. Among the methods, the 'decarboxylation route' offers a general method in which an aldehyde or a ketone is reacted with α-amino acids.[15]

The in situ generated azomethine ylide is trapped by dipolarophiles to give cycloadducts. In our synthetic study, we have generated two types of azomethine ylides, namely cyclic and acyclic, by reacting *N*-methyl glycine **2** and L-thiazolidine-4-carboxylic acid **4** with isatin **1** (Schemes 1 and 2). The ylides so generated were reacted with arylidene octahydro/decahydro cycloalka[*d*]thiazolo[3,2-*a*]pyrimidine-3-ones as dipolarophiles to yield novel dispiropolycyclic complex heterocycles.

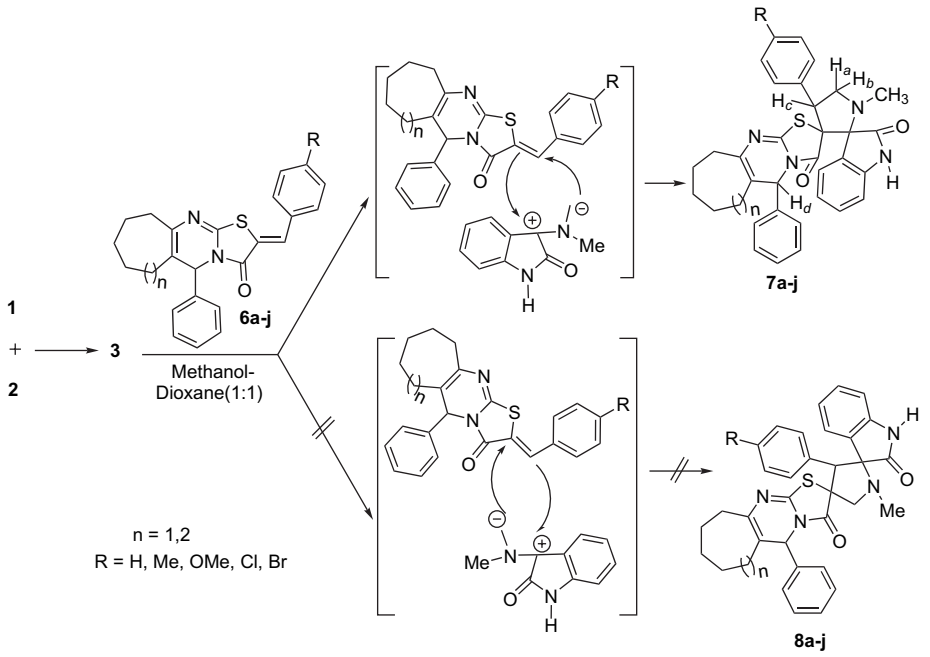


Scheme 1.



Scheme 2.

The required dipolarophiles **6a–j** were prepared in three steps by heating mono arylmethylene cycloalkanones with



Scheme 3.

thiourea in ethanolic potassium hydroxide giving cycloalka[*d*]pyrimidine-2-thiones, which were cyclized with monochloroacetic acid followed by condensation with various substituted benzaldehydes.[16,17]

When an equimolar mixture of isatin **1**, sarcosine **2** and dipolarophiles **6a–j** in methanol–dioxane (1:1) was heated to reflux, a series of novel spirooxindolo-spirothiazolo pyrimidino pyrrolidines **7a–j** were obtained in good yields (Scheme 3). The in situ generated *anti* 1,3-dipole[18,19] was trapped by conformationally restricted *S-cis* enone dipolarophiles to afford a series of cycloadducts.

A series of solvent systems such as methanol, acetonitrile and methanol–dioxane (1:1) were investigated to find the best reaction conditions. Among the solvents used, methanol–dioxane mixture (1:1) was found to be the best in terms of higher yields and shortest reaction times (Table 1). A single regioisomer was isolated in all the cases studied. No trace of the other regioisomers **8a–j** were found even after prolonged reaction times.

Table 1. Cycloaddition of ylides generated from isatin and sarcosine with the dipolarophiles

Entry	Product	*n*	R	Yield (%) (A/B/C)[a]	Reaction time (h) (A/B/C)[a]
1	**7a**	1	H	68/76/84	12/9/5
2	**7b**	1	*p*-Me	64/70/79	11/10/5
3	**7c**	1	*p*-OMe	69/72/79	14/11/5.5
4	**7d**	1	*p*-Cl	69/72/88	12/9/4.5
5	**7e**	1	*p*-Br	68/72/87	11.5/9/4
6	**7f**	2	H	63/74/87	10/9/4
7	**7g**	2	*p*-Me	70/73/83	13/10/4
8	**7h**	2	*p*-OMe	69/74/81	13/10/4.5
9	**7i**	2	*p*-Cl	69/74/81	11/9/3
10	**7j**	2	*p*-Br	69/74/88	12/10/3.5

[a] Solvent system: A=methanol, B=acetonitrile, C=methanol–dioxane (1:1).

The structure and regiochemistry of the products **7a–j** were established by IR, $^{1}H/^{13}C$ NMR spectroscopic and mass spectrometric studies. For instance, the IR spectrum of cycloadduct **7a** showed three characteristic peaks at 1721, 1712 and 3250 cm^{-1} corresponding to the oxindole ring carbonyl, the thiazolidinone ring carbonyl and the secondary amide NH groups, respectively.

The ^{1}H NMR spectrum of **7a** displayed multiplets in the region δ 0.96–2.25 due to the cycloheptyl ring protons. A sharp singlet at δ 2.14 was accounted for the *N*-methyl protons. Three doublet of doublets appeared at δ 3.35, 4.06 and 3.96 for H_a and H_b of N–CH_2 protons and benzylic proton (H_c) of pyrrolidine ring, which explained the observed regiochemistry.

If the other regioisomer **8a** had formed then a singlet for the benzylic proton (H_c) and two doublets for the *N*-methylene (H_a and H_b) protons would be observed. Also, the regiochemistries of the cycloadducts **7a**[20] and **7f**[21] were unambiguously corroborated by their X-ray crystallographic data (Figs. 1 and 2).

The signals appearing at δ 174.2 and 177.1 in the ^{13}C NMR spectrum reveal the presence of the thiazolone and the oxindole ring carbonyl groups, respectively. The two spiro quaternary carbons at C2 and C4 positions appeared at δ 79.7 and 70.7.

We performed a similar three component reaction in which the azomethine ylide **5** generated from L-thiazolidine 4-carboxylic acid **4** and isatin **1** was reacted with dipolarophiles **6a–j** to yield a series of novel dispiro-thio-pyrrolizidines **9a–j** in good yields. The products were formed by the regioselective cycloaddition of the ylide **5** across the exocyclic double bond of the dipolarophiles **6a–j** (Scheme 4). As in the case of the cycloaddition reactions described in

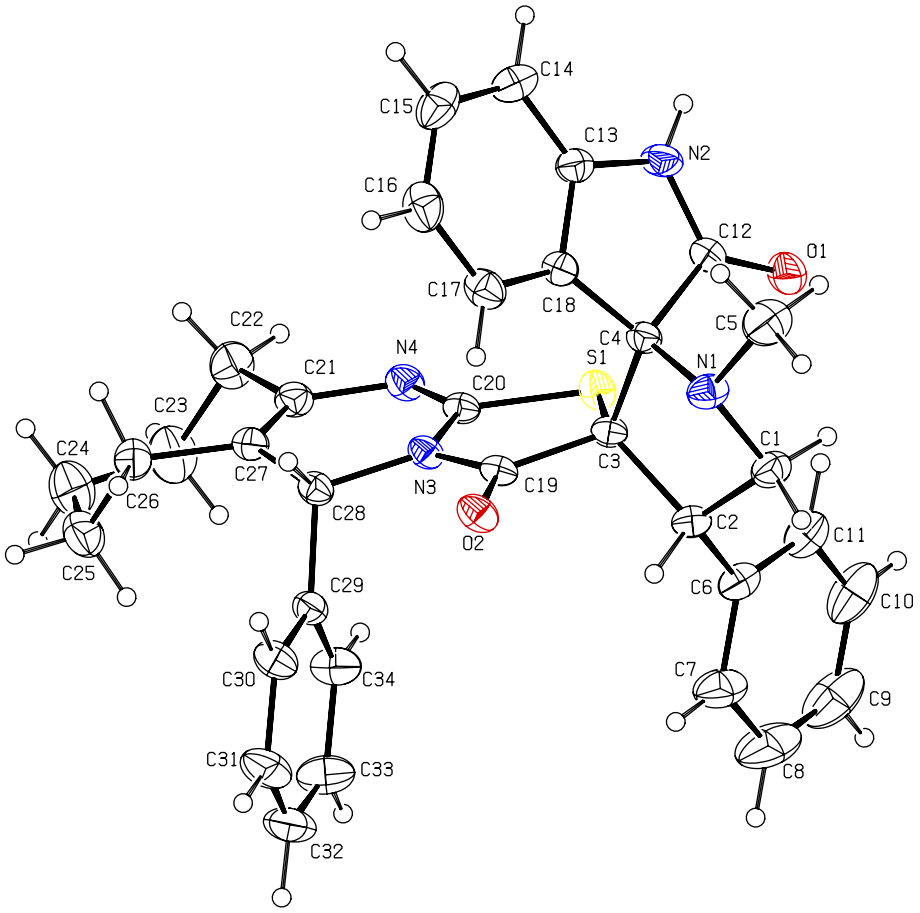


Figure 1. ORTEP diagram of **7a**.

Scheme 3, we noticed that the reaction proceeded to give the best yield of the products in methanol–dioxane (1:1) (Table 2).

The structure and stereochemistry of the cycloadducts **9a–j** were confirmed by their spectral data. Thus, the thiazolone keto carbonyl of **9a** exhibited a peak at 1710 cm^{-1} in the IR spectrum showing an increase of 10 cm^{-1} from the normal value observed for benzylidene pyrimidine thiazolo-3-one, indicating the loss of conjugation. Also, the IR spectrum showed characteristic absorption due to a secondary amide at 3250 cm^{-1} and the oxindole carbonyl stretching band at 1720 cm^{-1}. In the ^1H NMR spectrum, apart from the multiplets observed for the cycloheptyl protons in the region δ 0.95 and 2.34, the benzylic proton on pyrrolidine ring H_b appeared at δ 3.82 as a doublet and the H_e and H_f protons were seen as two doublets at δ 3.58 and 4.00, respectively. The H_a proton was found as a multiplet in the region δ 4.95–4.99. The benzylic proton H_g on the pyrimidine ring appeared at δ 5.04 as a singlet. In contrast, if **10a** was formed, the H_b proton would appear as a singlet and the *N*–CH proton of the thiapyrrolizidine ring would manifest itself as a doublet of doublets. The ^{13}C NMR spectrum of **9a** showed peaks for two spiro quaternary carbons at δ 69.5 and 74.5. The carbonyl carbons resonated at δ 173.7 and 176.6. And the remaining signals for aromatic and aliphatic carbons confirmed the proposed structure. No trace of the other regioisomer **10a** was detected.

3. Conclusion

In conclusion, the synthesis of a series of novel dispirooxindolo pyrrolidines and dispirooxindolo thiapyrrolizidines has been achieved in a one-pot, three component cycloaddition reaction. It was observed that the non-stabilized azomethine ylide generated added regioselectively across the exocyclic double bonds of the dipolarophiles to give novel spiroheterocycles. The evaluation of the biological activities of the synthesized compounds is in progress. All of the cycloadducts have shown moderate to good activities against human pathogenic bacterias like *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi* 'A', *Salmonella paratyphi* 'H', *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Escherichia coli* and fungi like *Candida albicans* and *Candida tropicalis*.

4. Experimental

4.1. General considerations

Infra red spectra were recorded on a Shimadzu IR-8300 series FT-IR spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded on a JEOL 300 and JEOL 400 MHz instrument in $CDCl_3$ solvent with TMS as a standard. Mass spectra were recorded by JEOL-DX303 HF

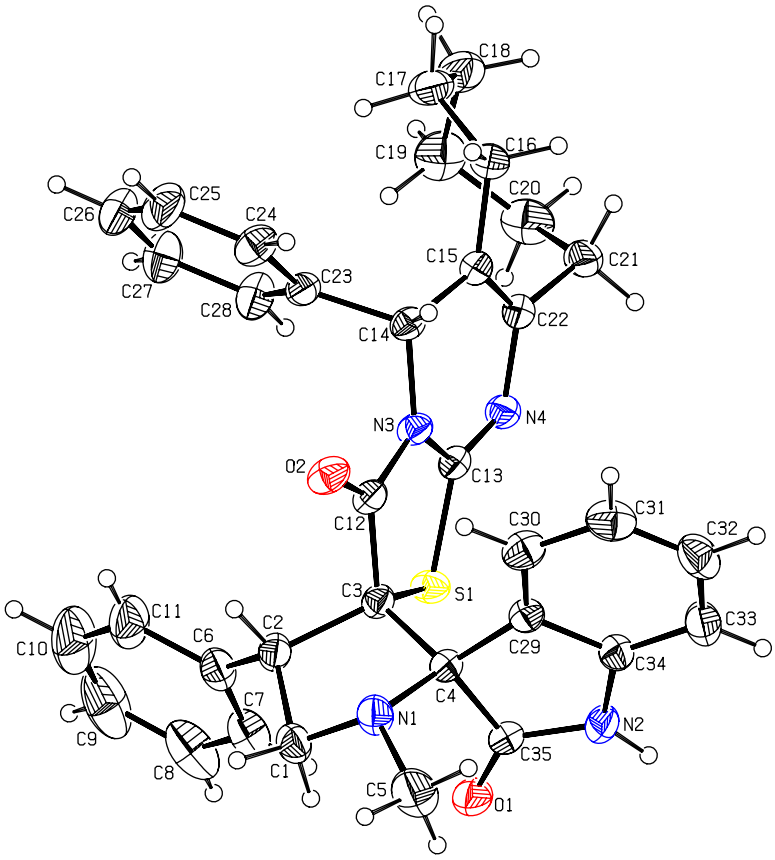
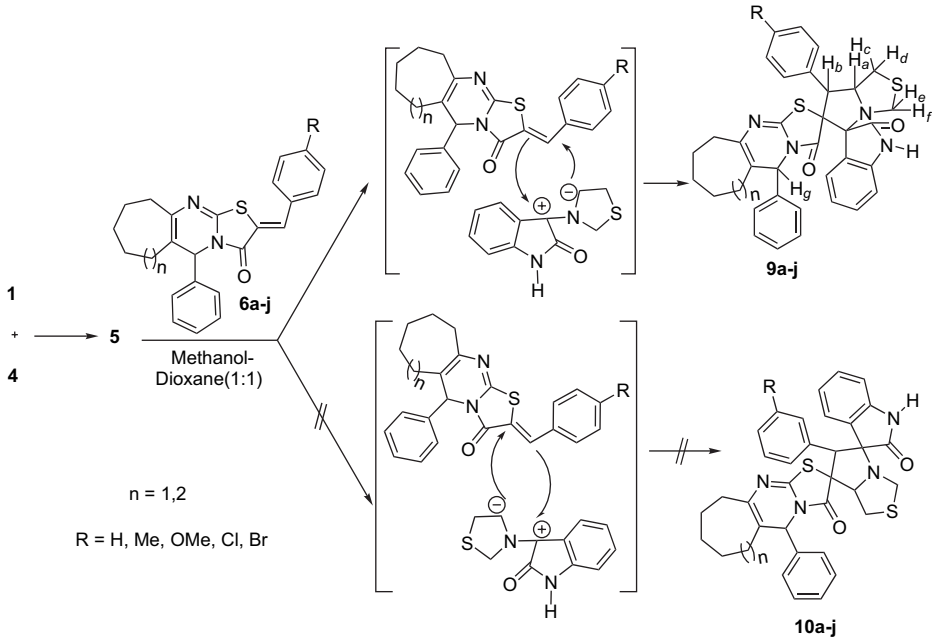


Figure 2. ORTEP diagram of **7f**.

mass Spectrometer. Elemental analyses were carried out by Perkin–Elmer CHNS 2400 instrument. X-ray diffraction data collection was performed by using Endraf-Nonius CHD4 diffractometer and Smart CCD Area diffractometer.

Column chromatography was performed on silica gel (ACME, 100–200 mesh). Routine monitoring of the reaction was made using thin layer chromatography developed on glass plates coated with silica gel-G (ACME) of 25 mm thickness and visualized with iodine.



Scheme 4.

Table 2. Cycloaddition of ylides generated from isatin and L-thiaproline with the dipolarophiles

Entry	Product	*n*	R	Yield (%) (A/B/C)[a]	Reaction time (h) (A/B/C)[a]
11	**9a**	1	H	66/70/80	13/10.5/4
12	**9b**	1	*p*-Me	67/77/80	14/11/4.5
13	**9c**	1	*p*-OMe	66/77/81	13/12/5
14	**9d**	1	*p*-Cl	68/72/83	12/10/3.5
15	**9e**	1	*p*-Br	69/73/85	12/9/3.5
16	**9f**	2	H	63/73/84	14/11/3.5
17	**9g**	2	*p*-Me	61/69/79	16/13/5
18	**9h**	2	*p*-OMe	64/70/79	15/13/5
19	**9i**	2	*p*-Cl	65/77/86	15/12/3.5
20	**9j**	2	*p*-Br	70/77/89	13/9/3.5

[a] Solvent system: A=methanol, B=acetonitrile, C=methanol–dioxane (1:1).

4.2. General procedure for the synthesis of cycloadducts 7a–j

A mixture of isatin **1** (0.176 g, 1.2 mmol), sarcosine **2** (0.106 g, 1.2 mmol) and 5-phenyl-2-(arylmethylene)-5,6,7,8,9,10-hexahydrocyclohepta/5,6,7,8,9,10,11-heptahydrocycloocta[*d*]thiazolo[3,2-*a*]pyrimidin-3(2*H*)-ones **6a–j** (1 mmol) in methanol–dioxane (1:1, 20 mL) was refluxed until the disappearance of the starting materials as shown by the TLC analysis (R_f=0.35–0.40). The reaction mixture was then concentrated in vacuo and extracted with water (50 mL) and dichloromethane (50 mL). The organic layer was washed with brine solution, dried with anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography with hexane–ethyl acetate (8:2) mixture to get compounds **7a–j** in good yields. The cycloadducts **7a** and **7f** were recrystallized from methanol by slow evaporation method for X-ray crystallographic analysis.

4.2.1. 1-*N*-Methyl-spiro[2.3′]oxindole-spiro[3.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″-hexahydrocyclohepteno[1,2-*d*]-thiazolo[3,2-*a*]pyrimidin-3″-one-4-phenyl-pyrrolidine (7a). Colourless solid (0.470 g, 84%). Mp 235–236 °C. IR (KBr): 3250, 2921, 1721, 1712, 1649, 1583 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.96–2.25 (m, 10H, cycloheptyl), 2.14 (s, 3H, N-Me), 3.35 (dd, 1H, H_a, J_1=8.0 Hz, J_2=8.8 Hz), 3.96 (dd, 1H, H_c, J_1=8.8 Hz, J_2=10.0 Hz), 4.06 (dd, 1H, H_b, J_1=10.0 Hz, J_2=8.0 Hz), 5.02 (s, 1H, H_d), 6.72–7.40 (m, 14H, Ar-H), 8.46 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 25.4, 26.1, 30.8, 31.7, 35.3, 53.9, 57.7, 60.3, 70.7, 79.7, 110.0, 118.4, 123.0, 124.3, 127.6, 127.7, 128.3, 128.4, 128.5, 130.2, 130.2, 137.3, 139.1, 141.0, 142.7, 150.2, 174.2, 177.1. MS *m/z*: 560.7 (M^+). Anal. Calcd for $C_{34}H_{34}N_4O_2S$: C, 72.82; H, 5.75; N, 9.99. Found: C, 72.71; H, 5.68; N, 9.88.

4.2.2. 1-*N*-Methyl-spiro[2.3′]oxindole-spiro[3.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″-hexahydrocyclohepteno[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3″-one-4-(*p*-methyl)-phenyl-pyrrolidine (7b). Fluffy white solid (0.454 g, 79%). Mp 230–231 °C. IR (KBr): 3245, 2921, 1719, 1710, 1652, 1583 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.77–2.28 (m, 10H, cycloheptyl), 2.16 (s, 3H, N-Me), 2.33 (s, 3H, CH_3), 3.38 (dd, 1H, H_a, J_1=8.0 Hz, J_2=8.8 Hz), 4.00 (dd, 1H, H_c, J_1=8.8 Hz, J_2=10.0 Hz), 4.06 (dd, 1H, H_b, J_1=10.0 Hz, J_2=8.0 Hz), 5.04 (s, 1H, H_d), 6.74–7.40 (m, 13H, Ar-H), 8.75 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 21.1, 25.4, 26.1, 30.7, 30.9, 31.7, 34.2, 35.2, 35.5, 53.6, 57.7, 60.3, 70.8, 79.7, 110.0, 118.4, 122.9, 124.4, 127.6, 128.0, 128.3, 128.4, 129.2, 130.1, 134.2, 137.2, 139.2, 141.0, 142.7, 150.4, 174.3, 177.1. MS *m/z*: 574.6 (M^+). Anal. Calcd for $C_{35}H_{34}N_4O_2S$: C, 73.14; H, 5.96; N, 9.74. Found: C, 73.02; H, 5.89; N, 9.70.

4.2.3. 1-*N*-Methyl-spiro[2.3′]oxindole-spiro[3.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″-hexahydrocyclohepteno[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3″-one-4-(*p*-methoxy)phenyl-pyrrolidine (7c). Colourless solid (0.466 g, 79%). Mp 202–204 °C. IR (KBr): 3240, 2931, 1720, 1710, 1649, 1581 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.78–2.20 (m, 10H, cycloheptyl), 2.16 (s, 3H, N-Me), 3.38 (dd, 1H, H_a, J_1=8.0 Hz, J_2=8.8 Hz), 3.96 (dd, 1H, H_c, J_1=8.8 Hz, J_2=10.0 Hz), 4.04 (dd, 1H, H_b, J_1=10.0 Hz, J_2=8.0 Hz), 3.79 (s, 3H, OMe), 5.05 (s, 1H, H_d), 6.70–7.41 (m, 13H, Ar-H), 8.50 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 25.3, 26.1, 30.7, 31.6, 35.2, 35.5, 53.4, 55.1, 57.8, 60.2, 71.0, 79.5, 110.0, 113.7, 118.3, 122.9, 124.3, 127.6, 128.3, 128.4, 129.3, 130.1, 131.3, 139.1, 140.9, 142.7, 150.4, 158.8, 174.2, 177.2. MS *m/z*: 590.7 (M^+). Anal. Calcd for $C_{35}H_{36}N_4O_3S$: C, 71.16; H, 5.80; N, 9.48. Found: C, 71.22; H, 5.75; N, 9.40.

4.2.4. 1-*N*-Methyl-spiro[2.3′]oxindole-spiro[3.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″-hexahydrocyclohepteno[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3″-one-4-(*p*-chloro)-phenyl-pyrrolidine (7d). Colourless solid (0.523 g, 88%). Mp 177–179 °C. IR (KBr): 3240, 2921, 1719, 1710, 1643, 1590 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.78–2.26 (m, 10H, cycloheptyl), 2.12 (s, 3H, N-Me), 3.31 (dd, 1H, H_a, J_1=8.0 Hz, J_2=8.8 Hz), 3.93 (dd, 1H, H_c, J_1=8.8 Hz, J_2=10.0 Hz), 4.00 (dd, 1H, H_b, J_1=10.0 Hz, J_2=8.0 Hz), 5.00 (s, 1H, H_d), 6.70–7.33 (m, 13H, Ar-H), 8.50 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 25.4, 26.1, 30.8, 31.5, 31.6, 35.2, 35.5, 53.6, 57.6, 60.3, 70.3, 79.5, 110.2, 118.5, 123.1, 124.1, 127.7, 128.4, 128.5, 128.6, 128.6, 130.3, 131.6, 133.4, 135.8, 139.0, 141.0, 142.8, 150.1, 174.0, 177.2. MS *m/z*: 595.1 (M^+). Anal. Calcd for $C_{34}H_{31}N_4O_2SCl$: C, 68.69; H, 5.24; N, 9.41. Found: C, 68.60; H, 5.18; N, 9.34.

4.2.5. 1-*N*-Methyl-spiro[2.3′]oxindole-spiro[3.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″-hexahydrocyclohepteno[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3″-one-4-(*p*-bromo)-phenyl-pyrrolidine (7e). Pale yellow powder (0.556 g, 87%). Mp 216–218 °C. IR (KBr): 3245, 2931, 1720, 1712, 1640, 1589 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.88–2.33 (m, 10H, cycloheptyl), 2.19 (s, 3H, N-Me), 3.40 (dd, 1H, H_a, J_1=8.0 Hz, J_2=8.8 Hz), 3.94 (dd, 1H, H_c, J_1=8.8 Hz, J_2=10.0 Hz), 4.06 (dd, 1H, H_b, J_1=10.0 Hz, J_2=8.0 Hz), 5.11 (s, 1H, H_d), 6.77–7.47 (m, 13H, Ar-H), 8.73 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 21.0, 25.3, 26.1, 30.8, 31.6, 35.2, 35.5, 53.6, 57.5, 60.3, 70.2, 79.4, 110.1, 118.5, 121.7, 123.0, 124.1, 127.7, 128.4, 128.5, 128.6, 130.3, 131.5, 132.0, 136.3, 139.0, 141.0, 142.7, 149.9, 174.0, 177.1. MS *m/z*: 639.6 (M^+). Anal. Calcd for $C_{34}H_{31}N_4O_2SBr$: C, 63.84; H, 4.88; N, 8.76. Found: C, 63.90; H, 4.93; N, 8.83.

4.2.6. 1-*N*-Methyl-spiro[2.3′]oxindole-spiro[3.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″,11″-heptahydrocyclo-octeno[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3″-one-4-phenyl-pyrrolidine (7f). Colourless solid (0.499 g, 87%). Mp 199–201 °C. IR (KBr): 3240, 2931, 1724, 1712, 1642,

1589 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.85–2.28 (m, 12H, cyclooctyl), 2.24 (s, 3H, N-Me), 3.47 (dd, 1H, H_a, J_1=8.0 Hz, J_2=8.8 Hz), 4.06 (dd, 1H, H_c, J_1=8.8 Hz, J_2=10.0 Hz), 4.26 (dd, 1H, H_b, J_1=10.0 Hz, J_2=8.0 Hz), 5.04 (s, 1H, H_d), 6.74–7.40 (m, 14H, Ar-H), 8.20 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 25.8, 26.3, 28.4, 28.7, 29.0, 31.3, 35.4, 52.4, 58.0, 58.9, 70.8, 80.2, 109.9, 116.0, 122.8, 124.2, 125.4, 126.9, 127.7, 128.4, 128.5, 128.5, 128.7, 130.0, 130.2, 130.4, 137.8, 138.0, 140.1, 142.5, 150.8, 174.3, 177.0. MS *m/z*: 574.4 (M^+). Anal. Calcd for $C_{35}H_{34}N_4O_2S$: C, 73.14; H, 5.96; N, 9.74. Found: C, 73.06; H, 5.91; N, 9.68.

4.2.7. 1-*N*-Methyl-spiro[2.3′]oxindole-spiro[3.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″,11″-heptahydrocyclooctено-[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3″-one-4-(*p*-methyl)-phenyl-pyrrolidine (7g). Fluffy white solid (0.488 g, 83%). Mp 221–223 °C. IR (KBr): 3245, 2921, 1720, 1711, 1643, 1596 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.88–2.27 (m, 12H, cyclooctyl), 2.23 (s, 3H, N-Me), 2.32 (s, 3H, Ar-CH_3), 3.45 (dd, 1H, H_a, J_1=8.0 Hz, J_2=8.8 Hz), 4.03 (dd, 1H, H_c, J_1=8.8 Hz, J_2=10.0 Hz), 4.22 (dd, 1H, H_b, J_1=10.0 Hz, J_2=8.0 Hz), 5.03 (s, 1H, H_d), 6.73–7.82 (m, 13H, Ar-H), 8.84 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 21.1, 25.8, 26.3, 28.4, 28.7, 28.9, 31.3, 35.3, 52.0, 58.1, 58.8, 70.9, 80.1, 109.8, 115.9, 122.8, 124.3, 126.9, 127.6, 128.3, 128.4, 129.2, 130.1, 130.3, 134.7, 137.2, 138.0, 140.1, 142.4, 174.4, 176.9. MS *m/z*: 588.7 (M^+). Anal. Calcd for $C_{36}H_{36}N_4O_2S$: C, 73.43; H, 6.16; N, 9.50. Found: C, 73.38; H, 6.10; N, 9.40.

4.2.8. 1-*N*-Methyl-spiro[2.3′]oxindole-spiro[3.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″,11″-heptahydrocyclooctено-[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3″-one-4-(*p*-methoxy)-phenyl-pyrrolidine (7h). Pale yellow powder (0.489 g, 81%). Mp 215–217 °C. IR (KBr): 3250, 2921, 1718, 1710, 1645, 1585 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.88–2.27 (m, 12H, cyclooctyl), 2.17 (s, 3H, N-Me), 3.80 (s, 3H, OMe), 3.45 (dd, 1H, H_a, J_1=8.0 Hz, J_2=8.8 Hz), 3.97 (dd, 1H, H_c, J_1=8.8 Hz, J_2=10.0 Hz), 4.20 (dd, 1H, H_b, J_1=10.0 Hz, J_2=8.0 Hz), 5.04 (s, 1H, H_d), 6.79–7.42 (m, 13H, Ar-H), 8.33 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 25.8, 26.3, 28.4, 28.7, 29.0, 31.3, 35.3, 51.8, 55.2, 58.2, 58.8, 71.1, 80.0, 109.7, 113.9, 115.9, 122.8, 124.3, 127.0, 127.7, 128.3, 128.4, 129.8, 130.3, 131.3, 138.1, 140.1, 142.3, 158.9, 174.4, 176.8. MS *m/z*: 604.7 (M^+). Anal. Calcd for $C_{36}H_{36}N_4O_3S$: C, 71.49; H, 5.99; N, 9.26. Found: C, 71.58; H, 5.72; N, 9.11.

4.2.9. 1-*N*-Methyl-spiro[2.3′]oxindole-spiro[3.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″,11″-heptahydrocyclooctено-[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3″-one-4-(*p*-chloro)-phenyl-pyrrolidine (7i). Fluffy white solid (0.493 g, 81%). Mp 209−211 °C. IR (KBr): 3240, 2920, 1724, 1712, 1640, 1590 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.85−2.26 (m, 12H, cyclooctyl), 2.22 (s, 3H, N-Me), 3.45 (dd, 1H, H_a, J_1=8.0 Hz, J_2=8.8 Hz), 3.96 (dd, 1H, H_c, J_1=8.8 Hz, J_2=10.0 Hz), 4.20 (dd, 1H, H_b, J_1=10.0 Hz, J_2=8.0 Hz), 5.05 (s, 1H, H_d), 6.78–7.40 (m, 13H, Ar-H), 8.40 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 25.8, 26.3, 28.4, 28.9, 29.0, 31.2, 35.3, 52.0, 58.0, 59.0, 70.4, 80.0, 110.0, 116.1, 122.9, 124.0, 127.0, 127.7, 128.5, 128.7, 130.5, 131.6, 133.4, 136.2, 138.0, 140.0, 142.5, 150.6, 174.2, 177.1. MS *m/z*: 608.5 (M^+). Anal. Calcd for $C_{35}H_{33}N_4O_2SCl$: C, 69.00; H, 5.45; N, 9.19. Found: C, 69.11; H, 5.55; N, 9.12.

4.2.10. 1-*N*-Methyl-spiro[2.3′]oxindole-spiro[3.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″,11″-heptahydrocyclooctено-[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3″-one-4-(*p*-bromo)-phenyl-pyrrolidine (7j). White solid (0.575 g, 88%). Mp 207–209 °C. IR (KBr): 3270, 2921, 1720, 1710, 1649, 1596 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.85–2.26 (m, 12H, cyclooctyl), 2.26 (s, 3H, N-Me), 3.45 (dd, 1H, H_a, J_1=8.0 Hz, J_2=8.8 Hz), 3.98 (dd, 1H, H_c, J_1=8.8 Hz, J_2=10.0 Hz), 4.18 (dd, 1H, H_b, J_1=10.0 Hz, J_2=8.0 Hz), 5.06 (s, 1H, H_d), 6.74–7.45 (m, 13H, Ar-H), 8.75 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 24.9, 25.8, 26.3, 27.4, 28.3, 28.9, 29.0, 31.2, 35.5, 52.1, 57.9, 59.1, 70.3, 80.0, 110.1, 116.1, 121.7, 122.9, 123.9, 125.4, 126.9, 127.7, 128.7, 130.5, 131.6, 132.0, 136.8, 138.0, 140.0, 142.7, 150.6, 174.2, 177.2. MS *m/z*: 653.6 (M^+). Anal. Calcd for $C_{35}H_{33}N_4O_2SBr$: C, 64.31; H, 5.08; N, 8.57. Found: C, 64.23; H, 4.97; N, 8.49.

4.3. General procedure for the synthesis of cycloadducts 9a–j

A mixture of isatin **1** (0.176 g, 1.2 mmol), L-thiazolidine-4-carboxylic acid **4** (0.106 g, 1.2 mmol) and 5-phenyl-2-(arylmethylene)-5,6,7,8,9,10-hexahydrocyclohepta/5,6,7,8,9,10,11-heptahydrocycloocta[*d*]thiazolo[3,2-*a*]pyrimidin-3(2*H*)-ones **6a–j** (1 mmol) in methanol–dioxane (1:1, 20 mL) was refluxed until the disappearance of starting materials as shown by the TLC analysis (R_f=0.35–0.40). The reaction mixture was then concentrated in vacuo and extracted with water (50 mL) and dichloromethane (50 mL). The organic layer was washed with brine solution, dried with anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by column chromatography with hexane–ethyl acetate (8:2) mixture to get compounds **9a–j** in good yields.

4.3.1. Spiro[4.3′]oxindole-spiro[5.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″-hexahydrocyclohepteno[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3″-one-6-phenyl-thiapyrrolizidine (9a). Colourless solid (0.483 g, 80%). Mp 219–221 °C. IR (KBr): 3250, 2912, 1720, 1710, 1639, 1589 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.95–2.34 (m, 10H, cycloheptyl), 2.77 (dd, 1H, H_c, J_1=6.8 Hz, J_2=9.7 Hz), 2.92 (dd, 1H, H_d, J_1=5.8 Hz, J_2=9.7 Hz), 3.58 (d, 1H, H_e, J=5.8 Hz), 3.82 (d, 1H, H_b, J=9.7 Hz), 4.00 (d, 1H, H_f, J=5.8 Hz), 4.95–4.99 (m, 1H, H_a), 5.04 (s, 1H, H_g), 6.85–7.64 (m, 14H, Ar-H), 8.63 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 25.3, 26.1, 30.6, 31.6, 32.4, 35.5, 47.4, 58.6, 60.7, 69.5, 74.5, 110.4, 118.7, 122.8, 123.4, 127.9, 128.1, 128.5, 128.5, 128.6, 130.0, 130.6, 135.7, 139.3, 140.6, 142.4, 150.0, 173.7, 176.6. MS *m/z*: 604.0 (M^+). Anal. Calcd for $C_{35}H_{32}N_4O_2S_2$: C, 69.50; H, 5.33; N, 9.26. Found: C, 69.40; H, 5.47; N, 9.20.

4.3.2. Spiro[4.3′]oxindole-spiro[5.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″-hexahydrocyclohepteno[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3″-one-6-(*p*-methyl)-phenyl-thiapyrrolizidine (9b). White solid (0.495 g, 80%). Mp 212–214 °C. IR (KBr): 3286, 2921, 1720, 1712, 1644, 1587 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.80–2.31 (m, 10H, cycloheptyl), 2.33 (s, 3H, Ar-CH_3), 2.70 (dd, 1H, H_c, J_1=6.9 Hz,

J_2=9.6 Hz), 2.92 (dd, 1H, H_d, J_1=5.8 Hz, J_2=9.6 Hz), 3.58 (d, 1H, H_e, J=5.7 Hz), 3.80 (d, 1H, H_b, J=9.6 Hz), 4.01 (d, 1H, H_f, J=5.7 Hz), 4.92–4.99 (m, 1H, H_a), 5.03 (s, 1H, H_g), 6.76–7.57 (m, 13H, Ar-H), 8.65 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 22.1, 25.3, 26.1, 30.6, 31.6, 31.6, 32.4, 35.5, 47.4, 58.2, 60.6, 69.5, 74.6, 110.4, 118.6, 122.8, 123.5, 128.0, 128.1, 128.4, 128.5, 129.3, 129.8, 130.5, 132.6, 137.5, 139.3, 140.6, 142.3, 150.1, 173.7, 176.6. MS *m/z*: 618.5 (M^+). Anal. Calcd for $C_{36}H_{34}N_4O_2S_2$: C, 69.87; H, 5.53; N, 9.05. Found: C, 69.94; H, 5.59; N, 9.11.

4.3.3. Spiro[4.3′]oxindole-spiro[5.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″-hexahydrocyclohepteno[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3″-one-6-(*p*-methoxy)-phenyl-thiapyrrolizidine (9c). Pale yellow solid (0.488 g, 81%). Mp 191–193 °C. IR (KBr): 3286, 2922, 1720, 1713, 1640, 1595 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.80–2.33 (m, 10H, cycloheptyl), 2.70 (dd, 1H, H_c, J_1=6.9 Hz, J_2=9.6 Hz), 2.92 (dd, 1H, H_d, J_1=5.8 Hz, J_2=9.6 Hz), 3.58 (d, 1H, H_e, J=5.7 Hz), 3.77 (d, 1H, H_b, J=9.6 Hz), 3.80 (s, 3H, Ar-OCH_3), 4.01 (d, 1H, H_f, J=5.7 Hz), 4.87–4.95 (m, 1H, H_a), 5.03 (s, 1H, H_g), 6.73–7.57 (m, 13H, Ar-H), 8.42 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 25.3, 26.1, 30.6, 31.7, 32.5, 35.6, 47.4, 55.1, 58.1, 60.0, 69.0, 74.1, 110.3, 114.0, 118.6, 122.8, 123.5, 127.7, 128.1, 128.5, 130.5, 131.1, 139.3, 140.6, 142.2, 159.5, 173.1, 176.9. MS *m/z*: 602.7 (M^+). Anal. Calcd for $C_{36}H_{34}N_4O_3S_2$: C, 71.73; H, 5.68; N, 9.29. Found: C, 71.64; H, 5.59; N, 9.21.

4.3.4. Spiro[4.3′]oxindole-spiro[5.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″-hexahydrocyclohepteno[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3″-one-6-(*p*-chloro)-phenyl-thiapyrrolizidine (9d). Pale yellow solid (0.530 g, 83%). Mp 217–219 °C. IR (KBr): 3286, 2922, 1720, 1713, 1640, 1595 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.80–2.32 (m, 10H, cycloheptyl), 2.68 (dd, 1H, H_c, J_1=6.9 Hz, J_2=9.6 Hz), 2.91 (dd, 1H, H_d, J_1=5.9 Hz, J_2=9.6 Hz), 3.58 (d, 1H, H_e, J=5.7 Hz), 3.77 (d, 1H, H_b, J=9.6 Hz), 4.02 (d, 1H, H_f, J=5.7 Hz), 4.86–4.94 (m, 1H, H_a), 5.06 (s, 1H, H_g), 6.77–7.57 (m, 13H, Ar-H), 8.74 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 25.3, 26.1, 30.6, 31.5, 31.6, 32.4, 35.4, 47.4, 58.2, 60.7, 69.5, 74.1, 110.5, 118.8, 122.9, 123.2, 128.1, 128.2, 128.6, 130.6, 131.3, 133.8, 134.2, 139.1, 140.6, 142.3, 149.7, 173.4, 176.5. MS *m/z*: 639.8 (M^+). Anal. Calcd for $C_{35}H_{31}N_4O_2S_2Cl$: C, 65.76; H, 4.88; N, 8.76. Found: C, 65.79; H, 4.80; N, 8.82.

4.3.5. Spiro[4.3′]oxindole-spiro[5.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″-hexahydrocyclohepteno[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3″-one-6-(*p*-bromo)-phenyl-thiapyrrolizidine (9e). Pale yellow solid (0.581 g, 85%). Mp 198–200 °C. IR (KBr): 3286, 2922, 1720, 1713, 1640, 1595 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.88–2.25 (m, 10H, cycloheptyl), 2.61 (dd, 1H, H_c, J_1=6.8 Hz, J_2=9.7 Hz), 2.83 (dd, 1H, H_d, J_1=5.8 Hz, J_2=9.7 Hz), 3.51 (d, 1H, H_e, J=5.8 Hz), 3.68 (d, 1H, H_b, J=9.7 Hz), 4.95 (d, 1H, H_f, J=5.8 Hz), 4.81–4.84 (m, 1H, H_a), 4.99 (s, 1H, H_g), 6.64–7.49 (m, 13H, Ar-H), 8.95 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 26.1, 30.6, 31.5, 32.4, 35.4, 47.5, 58.3, 60.7, 69.4, 74.0, 110.5, 118.8, 122.0, 122.8, 123.1, 128.1, 128.2, 128.6, 130.6, 131.7, 134.7, 139.1, 140.6, 142.4, 149.7, 173.4, 176.6. MS *m/z*: 683.6 (M^+). Anal. Calcd for $C_{35}H_{31}N_4O_2S_2Br$: C, 61.48; H, 4.56; N, 8.19. Found: C, 61.31; H, 4.49; N, 8.26.

4.3.6. Spiro[4.3′]oxindole-spiro[5.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″,11″-heptahydrocycloocteno[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3″-one-6-phenyl-thiapyrrolizidine (9f). Yellow solid (0.519 g, 84%). Mp 198–200 °C. IR (KBr): 3250, 2930, 1720, 1710, 1640, 1595 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.85–2.18 (m, 12H, cyclooctyl), 2.75 (dd, 1H, H_c, J_1=6.8 Hz, J_2=9.7 Hz), 2.94 (dd, 1H, H_d, J_1=5.8 Hz, J_2=9.7 Hz), 3.60 (d, 1H, H_e, J=5.8 Hz), 3.90 (d, 1H, H_b, J=9.7 Hz), 3.97 (d, 1H, H_f, J=5.8 Hz), 4.76–4.85 (m, 1H, H_a), 5.00 (s, 1H, H_g), 6.82–7.49 (m, 14H, Ar-H), 8.63 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 25.7, 26.3, 28.4, 29.0, 31.2, 32.5, 32.6, 35.7, 46.7, 47.1, 57.0, 58.8, 59.3, 70.0, 74.7, 110.3, 116.1, 122.7, 123.2, 125.3, 127.2, 127.4, 127.9, 128.4, 128.7, 128.9, 129.8, 130.0, 130.8, 136.2, 140.1, 142.2, 150.6, 173.9, 176.5. MS *m/z*: 618.8 (M^+). Anal. Calcd for $C_{36}H_{34}N_4O_2S_2$: C, 69.87; H, 5.53; N, 9.05. Found: C, 69.80; H, 5.47; N, 8.98.

4.3.7. Spiro[4.3′]oxindole-spiro[5.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″,11″-heptahydrocycloocteno[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3″-one-6-(*p*-methyl)-phenyl-thiapyrrolizidine (9g). Fluffy white solid (0.499 g, 79%). Mp 203–205 °C. IR (KBr): 3260, 2912, 1720, 1712, 1643, 1593 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.97–2.18 (m, 12H, cyclooctyl), 2.33 (s, 3H, Ar-CH_3), 2.74 (dd, 1H, H_c, J_1=6.8 Hz, J_2=9.6 Hz), 2.94 (dd, 1H, H_d, J_1=5.8 Hz, J_2=9.6 Hz), 3.61 (d, 1H, H_e, J=5.8 Hz), 3.91 (d, 1H, H_b, J=9.6 Hz), 3.99 (d, 1H, H_f, J=5.8 Hz), 4.95–4.99 (m, 1H, H_a), 5.04 (s, 1H, H_g), 6.85–7.64 (m, 13H, Ar-H), 8.63 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 21.1, 25.0, 25.7, 26.3, 27.0, 28.4, 28.8, 29.0, 31.3, 32.5, 35.8, 46.8, 47.1, 56.8, 58.8, 59.4, 61.7, 70.1, 74.8, 110.3, 116.1, 122.7, 123.3, 125.8, 127.4, 128.6, 129.4, 130.7, 133.2, 137.6, 140.2, 142.2, 150.7, 173.9, 176.5. MS *m/z*: 632.8 (M^+). Anal. Calcd for $C_{37}H_{33}N_4O_2S_2$: C, 69.74; H, 5.73; N, 8.85. Found: C, 69.66; H, 5.67; N, 8.77.

4.3.8. Spiro[4.3′]oxindole-spiro[5.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″,11″-heptahydrocycloocteno[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3″-one-6-(*p*-methoxy)-phenyl-thiapyrrolizidine (9h). Pale yellow fluffy solid (0.512 g, 79%). Mp 192–194 °C. IR (KBr): 3250, 2930, 1723, 1711, 1643, 1591 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.83–2.22 (m, 12H, cyclooctyl), 2.74 (dd, 1H, H_c, J_1=6.8 Hz, J_2=9.6 Hz), 2.95 (dd, 1H, H_d, J_1=5.8 Hz, J_2=9.6 Hz), 3.61 (d, 1H, H_e, J=5.8 Hz), 3.82 (s, 3H, OMe), 3.88 (d, 1H, H_b, J=9.6 Hz), 4.02 (d, 1H, H_f, J=5.8 Hz), 4.80–4.89 (m, 1H, H_a), 5.02 (s, 1H, H_g), 6.80–7.52 (m, 13H, Ar-H), 8.70 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 25.7, 26.3, 28.3, 28.8, 29.0, 31.2, 32.5, 47.2, 55.1, 56.7, 59.4, 70.2, 74.9, 110.3, 114.0, 116.0, 122.6, 123.3, 127.4, 127.4, 128.3, 128.3, 128.5, 128.7, 130.6, 131.1, 137.8, 140.2, 142.3, 150.7, 159.1, 173.9, 176.7. MS *m/z*: 648.8 (M^+). Anal. Calcd for $C_{37}H_{36}N_4O_3S_2$: C, 68.49; H, 5.59; N, 8.63. Found: C, 68.40; H, 5.51; N, 8.67.

4.3.9. Spiro[4.3′]oxindole-spiro[5.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″,11″-heptahydrocycloocteno[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3″-one-6-(*p*-chloro)-phenyl-thiapyrrolizidine (9i). Fluffy white solid (0.531 g, 86%). Mp 220–221 °C. IR (KBr): 3150, 2920, 1724, 1710, 1643, 1590 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.83–2.24 (m, 12H, cyclooctyl), 2.72 (dd, 1H, H_c, J_1=6.8 Hz, J_2=9.6 Hz), 2.93 (dd,

1H, H_d, J_1=5.8 Hz, J_2=9.6 Hz), 3.61 (d, 1H, H_e, J=5.8 Hz), 3.89 (d, 1H, H_b, J=9.6 Hz), 4.02 (d, 1H, H_f, J=5.8 Hz), 4.90–4.96 (m, 1H, H_a), 5.03 (s, 1H, H_g), 6.79–7.50 (m, 13H, Ar-H), 8.40 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 26.0, 26.5, 28.6, 29.2, 29.3, 30.3, 31.5, 32.7, 47.4, 57.1, 59.8, 70.3, 74.5, 110.7, 116.5, 123.0, 123.3, 127.8, 128.8, 128.9, 129.1, 131.0, 131.7, 134.1, 135.0, 138.1, 140.4, 142.7, 150.6, 174.0, 176.7. MS *m/z*: 653.3 (M^+). Anal. Calcd for $C_{36}H_{33}N_4O_2S_2Cl$: C, 66.19; H, 5.09; N, 8.57. Found: C, 66.11; H, 5.01; N, 8.48.

4.3.10. Spiro[4.3′]oxindole-spiro[5.2″]-5″-phenyl-5″,6″,7″,8″,9″,10″,11″-heptahydrocycloocteno[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3″-one-6-(*p*-bromo)-phenyl-thiapyrrolizidine (9j). Fluffy white solid (0.620 g, 89%). Mp 204–206 °C. IR (KBr): 3286, 2921, 1719, 1710, 1649, 1595 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.91–2.20 (m, 12H, cyclooctyl), 2.72 (dd, 1H, H_c, J_1=6.8 Hz, J_2=9.6 Hz), 2.93 (dd, 1H, H_d, J_1=5.8 Hz, J_2=9.6 Hz), 3.62 (d, 1H, H_e, J=5.8 Hz), 3.87 (d, 1H, H_b, J=9.6 Hz), 4.03 (d, 1H, H_f, J=5.8 Hz), 4.92–4.95 (m, 1H, H_a), 5.03 (s, 1H, H_g), 6.84–7.50 (m, 13H, Ar-H), 8.91 (s, 1H, NH). ^{13}C NMR ($CDCl_3$): δ 25.7, 26.3, 28.3, 29.0, 29.1, 31.1, 32.5, 47.3, 57.0, 58.9, 59.6, 70.0, 74.1, 110.5, 116.3, 122.1, 122.8, 130.5, 130.8, 131.5, 131.8, 131.8, 132.0, 135.2, 137.8, 140.1, 142.4, 150.4, 173.7, 176.7. MS *m/z*: 697.7 (M^+). Anal. Calcd for $C_{36}H_{33}N_4O_2S_2Br$: C, 61.97; H, 4.76; N, 8.03. Found: C, 61.91; H, 4.68; N, 7.94.

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