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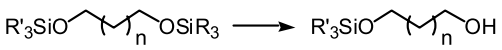
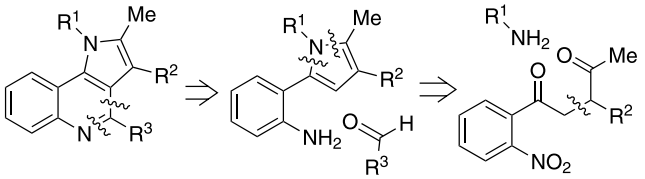
Contents

REPORT

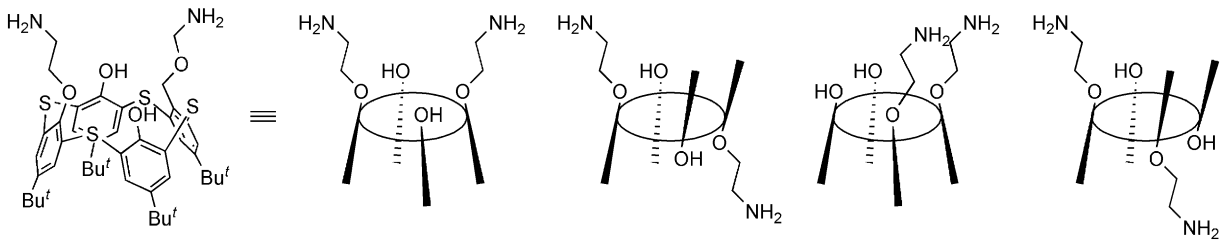


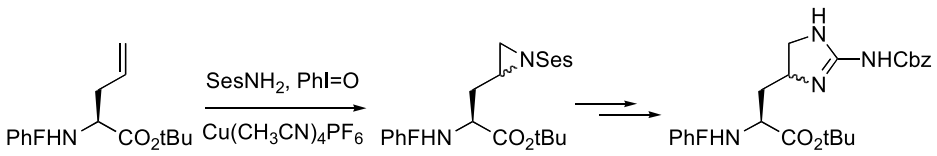
Reactions which allow for the selective deprotection of one silyl ether in the presence of another silyl ether are reviewed. This review covers examples reported in the literature since 1996. Examples are categorized by the type of silylated alcohol that is deprotected in the presence of the type of silyl ether that remains. A complete listing of examples in tabular form appears at the end of this manuscript.

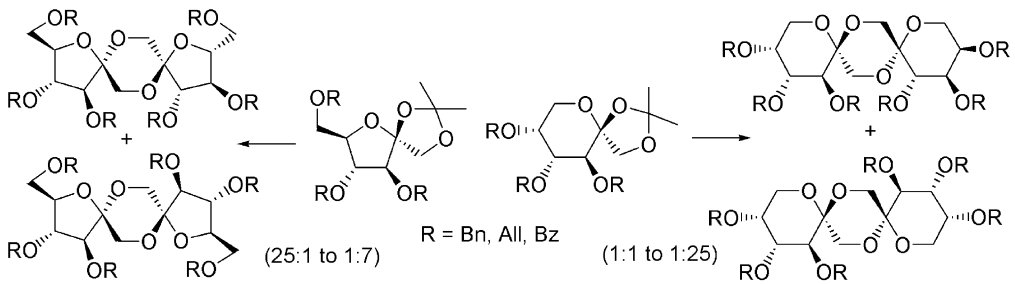
ARTICLES

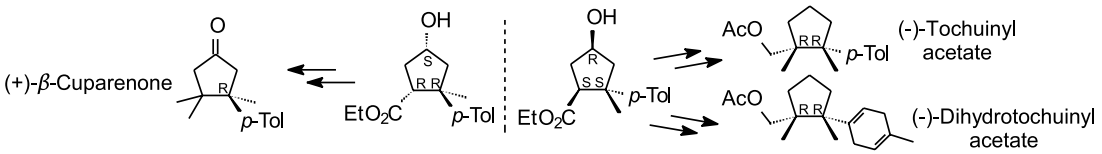


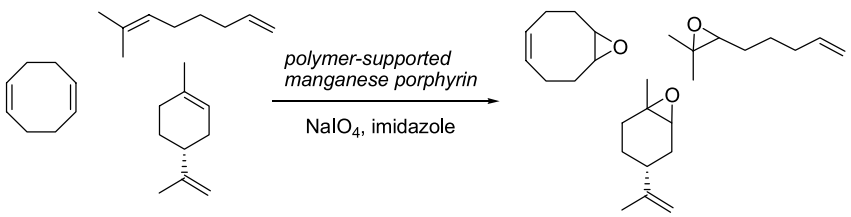
New 1,3,4-substituted pyrrolo[3,2-*c*]quinoline derivatives were synthesised in good yields by oxidative heteroannulation of internal imines starting from easily prepared substituted 5-(2-aminophenyl)pyrroles and commercially available aryl and heteroaryl aldehydes.

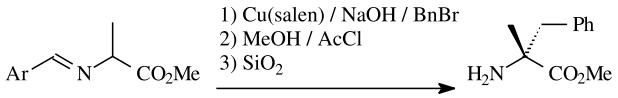




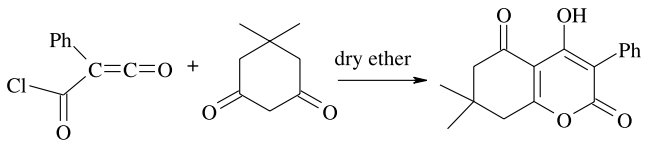


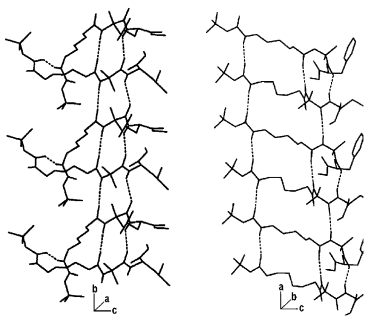


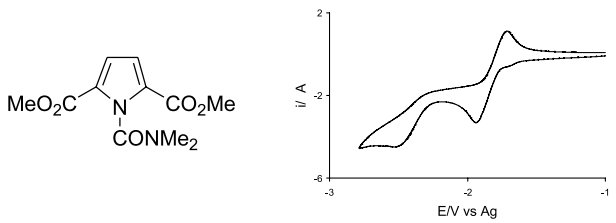


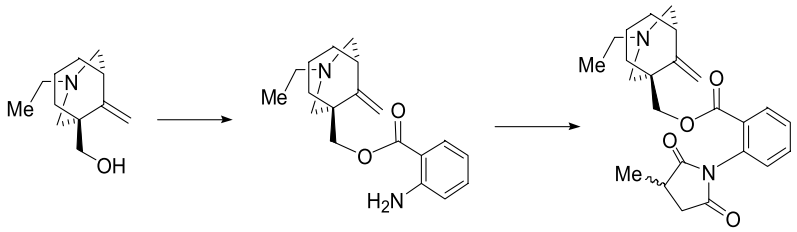


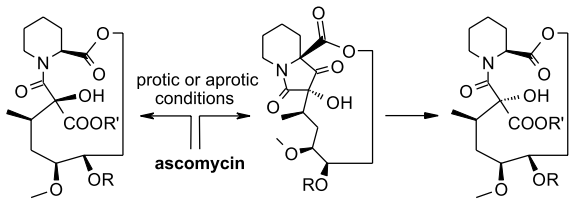
The effect of substituents on the aromatic rings of the salen ligand on the catalytic activity of the Cu(salen) catalyst was investigated. The influence of substituents on the aromatic ring of the alanine imine was also studied. The method used to prepare the catalyst was found to influence the enantioselectivity and a new procedure which avoids sephadex LH20 chromatography was developed.

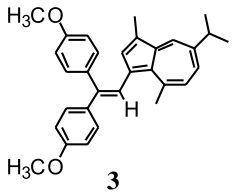












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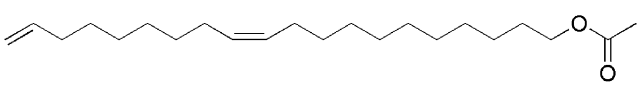

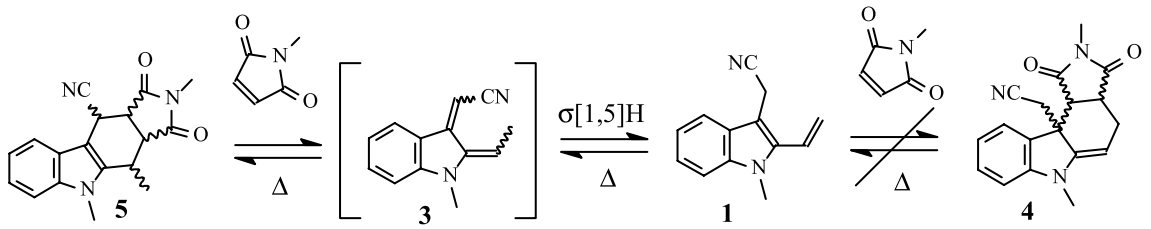

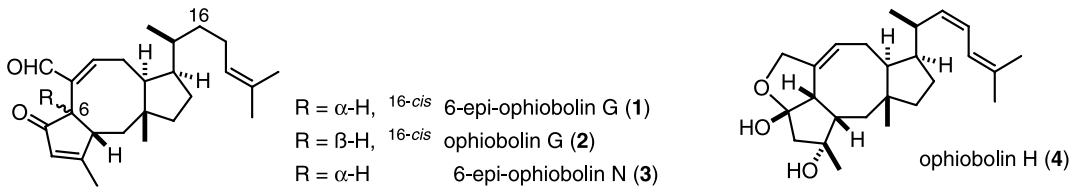

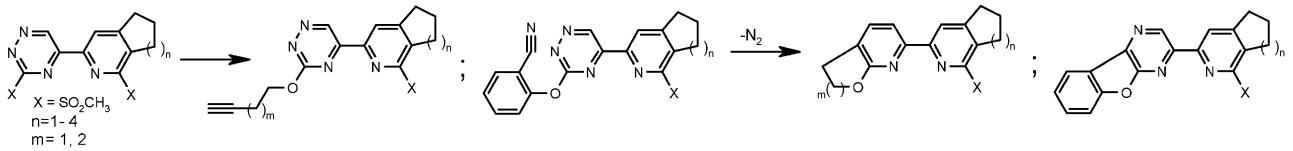

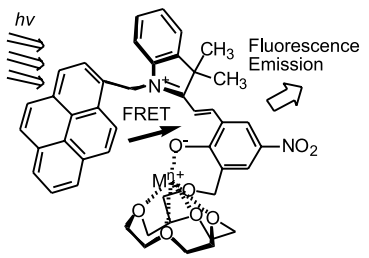

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Selective monodeprotection of bis-silyl ethers

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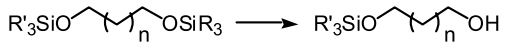
1. Introduction

As synthetic targets have grown more complex, protection/deprotection protocols have assumed prominent roles in synthetic organic chemistry.[1–3] The ability to efficiently protect and then deprotect hydroxyl groups has become increasingly important due to the abundance of these groups in natural products and the variety of transformations involving hydroxyl-containing intermediates. A number of di- and trialkylsilyl groups have emerged as means of temporarily rendering an alcohol inert; many of the more common silyl protecting groups are shown in Figure 1.

Complex products and the synthetic intermediates leading to their formation often contain multiple oxygen functionalities. The ability to selectively deprotect one silyl ether without affecting another silyl ether *in the same molecule* can be a crucial step in a synthetic scheme[4] (Scheme 1).



Scheme 1.

Such selective deprotection reactions have played important roles in, among others, the recently published syntheses of leucascandrolide A,[5,6] epothilone A[7] and B,[8,9] (+)-ambruticin S,[10] (+)-macbecin I,[11] (−)-laulimalide,[12,13] bafilomycin V_1,[14] gambierol[15] briarellin diterpenes,[16] (+)-spongistatin 1[17]and (+)-zampanolide.[18] In each of these examples, one silyl protecting group was removed to expose an alcohol while other silyl protected hydroxyl groups were left intact, allowing manipulation of only the newly available alcohol. The advantage of this approach is that different alcohols may be protected with the same functional group; but the reactivity of these functional groups can be controlled by careful selection of reagents and conditions.

Selective deprotection reactions of this nature were the subject of a review of the literature through the middle of 1996.[4] The present review serves as an update and cites examples published since the previous review through the end of 2003. It is important to note that, while many examples of selective desilylation reactions have been published as studies of a new method of deprotection, many more examples are found as one step in a multi-step synthesis of a natural or unnatural product, complicating systematic searches of the literature. Further, not all methods of selective desilylation can be extended to every

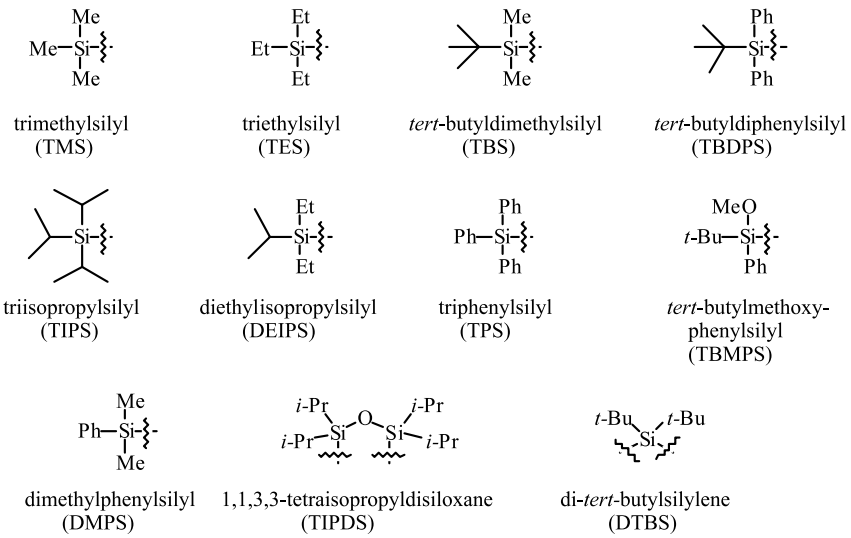
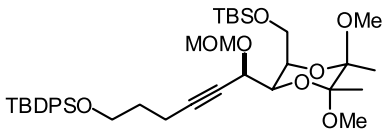


Figure 1.

system. For example, in studies toward the synthesis of the serinolipid, (+)-didemniserinolipid B, removal of a 1° TBS group in the presence of a 1° TBDPS group in a key intermediate **1** failed using HF–pyr despite precedent that such a selective desilylation was feasible,[19] while TBAF resulted in removal of both silyl protecting groups.



1

Similar problems associated with cleaving 1° and 2° TBS ethers in the presence of a 1° TBDPS ether have been reported,[20] when both the acidic reagent, PPTS, and the nucleophilic reagent, TBAF, resulted in multiple partial desilylation products with no evidence of the desired selective deprotection. These examples highlight one of the problems inherent in attempting selective deprotection of silyl ethers: as the differences between the protecting groups that are to be distinguished become smaller, the molecule's structural features and other functional groups may interfere with the desired outcome.

This review is intended as a resource for organic chemists interested in the use of selective desilylation reactions and is organized according to the type of silyl-protected alcohol (1°, 2°, phenolic, etc.) that is released in the presence of another silyl-protected alcohol (1°, 2°, phenolic, etc.) that remains intact. The text includes selected examples of selective deprotection reactions with the tables at the end providing a more complete overview of methods (Tables 4–14).

2. Mechanistic effects on selectivity

The mechanisms of desilylation have been reviewed elsewhere.[4] The steric and electronic environment of both the silicon and alcoholic carbon affect the rate of hydrolysis of the Si–O bond and these play critical roles in allowing the removal of one silyl group in the presence of another. In general, larger substituents around silicon or oxygen will slow the rate of reaction. Under acid conditions, substituent size on silicon is more important; under basic conditions, the effects of substituent size on silicon and oxygen are approximately the same.[21]

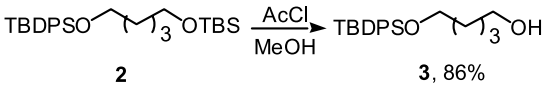
Electronic effects have been exploited in selective desilylation reactions. Electron-donating substituents on either the alcoholic carbon or the silicon accelerate the rate of acidic hydrolysis while electron-withdrawing groups accelerate base-mediated hydrolysis.[21] Although the effect is more significant when the electronic effect occurs through the oxygen,[21] manipulation of substituents on silicon has been used to overcome steric hindrance and allow the removal of bulkier silyl ethers in the presence of smaller and generally more labile silyl groups. Since electronic effects exert more influence on rates of acidic hydrolysis than steric effects,[22] discrimination between a TBS and TBDPS ether is relatively facile.[4] Under basic conditions, these groups react similarly.

3. Selective deprotection of silyl-protected 1° alcohols

3.1. In the presence of a 1° silyl ether

Selective removal of a silyl group from a 1° alcohol in the presence of another 1° silyl ether has been effected most commonly by taking advantage of differences in reactivity due to electronic and steric effects of substituents on the silicon.

3.1.1. Under acidic conditions. Acid hydrolysis of silyl ethers is accelerated by electron-donating substituents and slowed by electron-withdrawing groups. This allows a TBDPS group to remain unaffected while silyl groups such a TES and TBS undergo hydrolysis elsewhere in the same molecule. Thus, the well-established methods of removal of TES or TBS groups from protected 1° alcohols in the presence of 1° TBDPS ethers include HOAc/THF/H_2O,[23,24] mineral acids such as HCl[25] and H_2SO_4,[26] PPTS,[27] CSA,[28–30,31] TsOH,[32] and TFA.[33] Acetyl chloride in dry methanol has been reported to generate dry HCl in situ, allowing the selective deprotection of a 1°TBS ether in the presence of a 1° TBDPS ether (Scheme 2).[34]



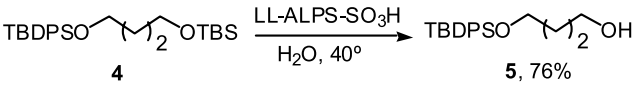
2 **3**, 86%

Scheme 2.

Preservation of a 1° silyl ether in the presence of another does not require the use of TBDPS groups. TIPS-protected 1° alcohols have been shown to survive acidic hydrolysis while a 1° TBS ether is cleaved with PPTS,[35] CSA,[36,37] and aqueous H_2SO_4.[38] TFA cleaves a 1° TES ether.[39]

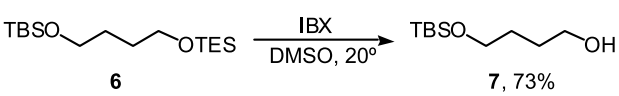
Polymer-supported acids have been shown to be useful in the deprotection of 1° TBS ethers without affecting 1° TBDPS ethers. A recent report describes the use of 'low-loading and alkylated polystyrene-supported sulfonic acid' (or LL–ALPS–SO_3H) to effect the selective deprotection of TBS-protected 1° alcohols in the presence of 1° TBDPS ethers (Scheme 3).[40] The temperature dependence of this reaction is noteworthy: at 40°, the reaction is selective but at 100°, the TBDPS group is also cleaved (Scheme 3).

In a similar fashion, montmorillonite K-10 has been shown to remove 1° TBS ethers without affecting 1° TBDPS ethers.[41]



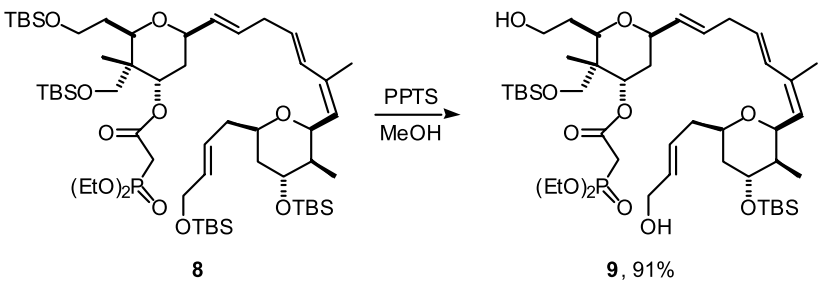
4 **5**, 76%

Scheme 3.



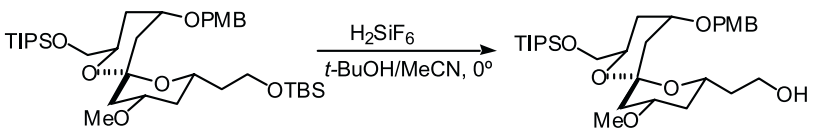
6 **7**, 73%

Scheme 4.



8 **9**, 91%

Scheme 5.



10 **11**, 86%

Scheme 6.

The use of *o*-iodoxybenzoic acid (IBX) as an acid in the selective cleavage of 1° TES ethers in the presence of TBS-protected 1° alcohols has been described (Scheme 4).[42] Although normally thought of as an oxidant, IBX in DMSO is sufficiently acidic to direct the hydrolysis of TES ethers without affecting benzyl ethers or ketals. The corresponding aldehyde was observed in small quantities, leading to the conclusion that deprotection of 1° TES ethers proceeds at a faster rate than oxidation.

Steric differences near the alcoholic carbons can also be exploited to allow selective desilylation of alcohols protected with the same silyl group. PPTS was used to deprotect two 1° TBS ethers in the presence of a more hindered 1° TBS ether in the total synthesis of (+)-lasonolide (Scheme 5).[43]

A similar scenario occurred in the HOAc-mediated selective removal of the less hindered TBS-protected 1° alcohol in the presence of another 1° TBS ether.[44]

Acidic fluoride-mediated selective deprotection of 1° silyl ethers is less common. Fluorosilicic acid (H_2SiF_6) was introduced as an agent in silyl deprotection in 1991[45,46] and its use in selective desilylations continues to grow. For example, a 1° TBS ether has been deprotected in the presence of a 1° TIPS ether using H_2SiF_6 in *t*-butanol and acetonitrile (Scheme 6).[47]

Similarly, HF–pyridine was used in the deprotection of a 1° TBS ether in the presence of a 1° TBDPS ether.[48]

An unusual example of the selective deprotection of TBDPS-protected 1° alcohol in the presence of a 1° TBS ether has been reported to occur upon stirring with a mixture of TBAF and HOAc in DMF or THF.[49] (Scheme 7) The effect of acid is important; without HOAc, TBS groups undergo more rapid hydrolysis.[49] No mechanism has been proposed to explain this selectivity. But, it seems likely that hypervalent silicon species are involved.[49]

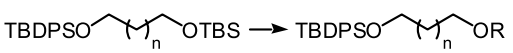
12 **13**, 82%

Scheme 7.

In recent years, Lewis acids have emerged as alternatives to the more traditional use of protic acids in selective deprotection reactions. For example, catalytic decaborane in MeOH/THF has been shown to cleave 1° TBS and TPS ethers without affecting TBDPS- or TIPS-protected 1° alcohols.[50] Trimethylsilyl triflate (TMS-OTf) has been used to deprotect a TBS-protected alcohol in the presence of a 1° TIPS ether.[51] TMS-OTf has also been used to catalyze the conversion of silyl ethers into diphenylmethyl ethers.[52] In this protocol, diphenylmethyl formate and catalytic TMS-OTf generate diphenylmethyl cations which rapidly effect deprotection of 1° TES, TBS and TIPS ethers. The rate of reaction is much slower for 1° TBDPS ethers, pointing to the possibility of selective deprotection.

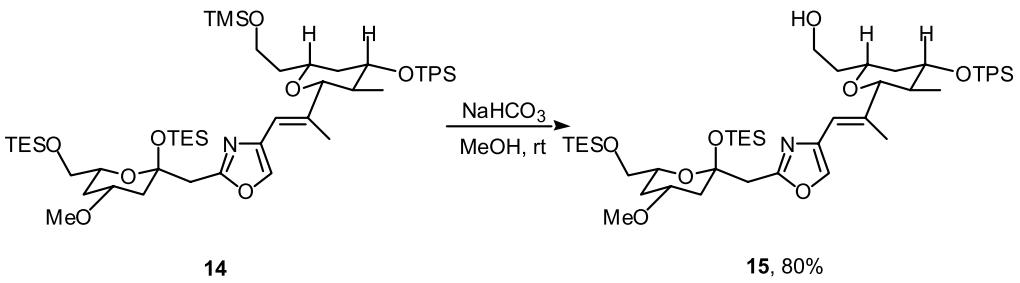
Transition metal salts have also proven useful in selectively removing silyl groups. Table 1 summarizes some of these results.

Table 1. Deprotection of 1°TBS ethers in the presence of 1°TBDPS ethers with Lewis Acids



Reagent	*n*	R	Yield (%)
$CeCl_3{\cdot}7H_2O$/NaI, CH_3CN	1	H	94[53,54]
$Ce(OTf)_4$, THF/MeOH	3	H	80[55]
$Cu(OTf)_2$, Ac_2O, CH_2Cl_2	6	Ac	90[56]
$InCl_3$, CH_3CN	3	H	89[57]
$ZrCl_4$, Ac_2O, CH_3CN	4	Ac	88[58]
$ZnBr_2$, H_2O, CH_2Cl_2	3	H	84[59]

Other Lewis acids that have been employed to cleave a 1° TBS ether in the presence of a 1° TBDPS ether include $Zn(BF_4)_2$[60] and $CeCl{\cdot}7H_2O$.[61] A series of bismuth(III) salts have been shown to effect deprotection of 1° TMS ethers in the presence of 1° TBS ethers.[62] TMS-and TBS-protected 1° benzylic alcohols undergo deprotection and oxidation with MnO_2 and $AlCl_3$ to the corresponding aldehyde without cleaving 1° TBS ethers.[63]



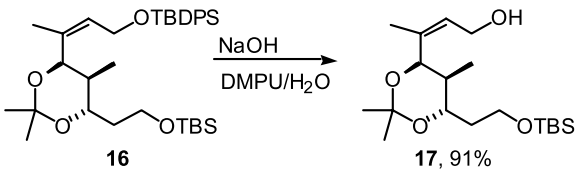
14 **15**, 80%

Scheme 8.

A heterogenous system of mesoporous MCM-41 in methanol has been shown to effect the removal of TMS and TES groups from 1° alcohols without affecting 1° TBS or TIPS ethers.[64]

3.1.2. Under basic/nucleophilic conditions. Base-mediated removal of silyl protecting groups is one of the oldest methods for the deprotection of silyl ethers.[65] As with acid-mediated deprotections, removal of the less-sterically hindered silyl group is a common strategy. For example, TMS-protected 1° alcohols have been desilylated in the presence of 1° TBS ethers using catalytic K_2CO_3 in MeOH.[66] A more challenging selective deprotection was reported in which a 1° TMS ether was hydrolyzed in the presence of a 1° TES ether as well as 2° TPS and a 3° TES ethers using $NaHCO_3$ in MeOH at room temperature (Scheme 8).[67]

Hydroxide bases mediate the deprotection of 1° TBDPS ethers in the presence of 1° trialkylsilyl ethers. For example, NaOH has also been used as a reagent to remove TBDPS-groups from protected 1° alcohols in the presence of 1° TBS ethers (Scheme 9).[68,69] Aqueous KOH in THF/MeOH was used to remove a TBDPS group from a protected 1° alcohol in the presence of a 1° TIPS ether.[16]



16 **17**, 91%

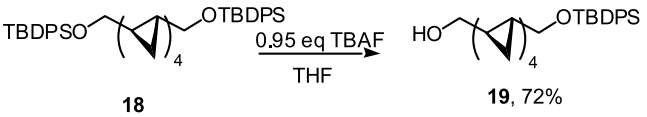
Scheme 9.

Similarly, a solution of 40% Bu_4NOH in H_2O and THF showed excellent selectivity (>99%) in removing TBDPS-protecting groups from 1° alcohols without affecting 1° TBS ethers.[49]

Verkade's non-ionic base, $P(MeNHCH_2CH_2)_3N$, has been shown to be more reactive in the desilylation of 1° TBS ethers than of 1° TBDPS ethers.[70]

Fluoride sources such as TBAF can deliver F^- to the silyl group, causing deprotection to occur. TBAF, used in slightly less than stoichiometric quantities, allowed the selective removal of a TBDPS group from a 1° alcohol in the presence of another 1° TBDPS ether.[71] (Scheme 10) When an excess of reagent was employed, both silyl groups were removed.

TBAF was also used to selectively remove one 1° TBS group in the presence of another on a self-assembled layer in which the monolayer is believed to have rendered two otherwise identical 1° TBS ethers non-equivalent.[72]



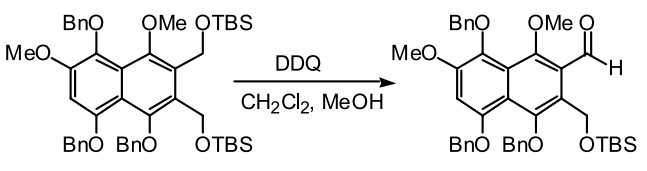
18 **19**, 72%

Scheme 10.

3.1.3. Miscellaneous conditions. Elemental Br_2[73] or I_2[74] in methanol have been used to selectively deprotect 1° TBS ethers in the presence of 1° TBDPS ethers. Interestingly, TBDPS ethers are cleaved using Br_2 in refluxing methanol but remain intact at room temperature.[75] When I_2 is used in refluxing methanol, TBDPS ethers are unaffected.[74] Alternatives to elemental halogens include IBr in CH_2Cl_2[76] and tetrabutylammonium bromide (TBAB) in methanol,[77] both of which effect the selective cleavage of 1° TBS ethers in the presence of 1° TBDPS ethers.

I_2 in methanolic KOH has been used at 0°C to achieve deprotection of a TBS-protected 1° alcohol in the presence of a 1° TBDPS ether with concomitant aldehyde oxidation and esterification.[78]

DDQ has been used to cleave TES- and TBS-protected 1° allylic alcohols in the presence of a 1° TIPS ether with oxidation of the resulting alcohol to an aldehyde under neutral conditions.[79] This reaction is specific for allylic and benzylic silyl ethers. But DDQ has been reported to allow two 1° benzylic TBS ethers to be distinguished from one another based on their different oxidation potentials resulting in selective deprotection and oxidation (Scheme 11).[80]



20 **21**, 80%

Scheme 11.

A polymer supported π-acid catalyst has been described as an alternative to DDQ that is less sensitive to degradation in aqueous media. Polymeric dicyano ketene acetal (DCKA) has been used in the selective deprotection of 1° TMS and TBS ethers in the presence of 1° TBDPS ethers.[81]

Catalytic hydrogenation on Pd/C in methanol has been shown to effect the deprotection of TES-protected 1° allylic alcohols without removing TBDPS or TIPS groups from other 1° allylic alcohols.[82] The solvent effect is significant; while the conversion of TES ether to alcohol was near 100% in MeOH, the same TES ether was virtually inert when the reaction was performed in acetonitrile. A more recent study shows TES, TPS and tributylsilyl groups can be removed from protected 1° alcohols in the presence of 1° TBS, TIPS or TBDPS ethers using catalytic hydrogenation over Pd/C.[83] And, 1° TBS ethers undergo selective hydrogenolysis in the presence of 1° TIPS and TBDPS ethers under these conditions. Interestingly, TES and TPS groups were also cleaved upon stirring with Pd/C in the absence of H_2 while TBS, TIPS and TBDPS groups were inert.[83]

Quinolinium fluorochromate (QFC) has been used to convert a 1° TBS ether into an aldehyde without affecting a 1° TBDPS ether.[84]

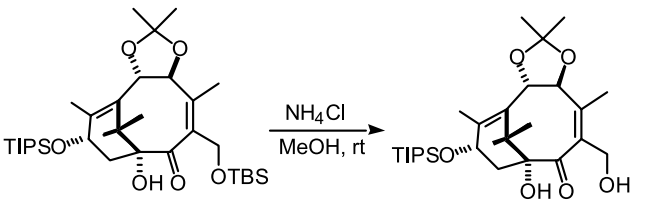
The phosphonium salt generated by the In situ reaction of triphenylphosphine and 2,4,4,6-tetrabromo-2,5-cyclohexadienone converts TMS-, TES- and TBS-protected 1° alcohols into alkyl bromides while leaving 1° TBDPS ethers unreacted.[85] A large excess of LiCl and water in DMF has been reported to effect the selective removal of TBS groups from protected 1° alcohols without affecting 1° TBDPS ethers.[86]

3.2. In the presence of a 2° silyl ether

Removal of a silyl protecting groups from a protected 1° alcohol in the presence of a 2° silyl ether is the oldest and perhaps most widely used form of selective silyl deprotection.[65] Typically, the ease of these reactions is largely due to the influence of steric bulk around the alcoholic carbon. In many synthetic schemes, though, a smaller, less sterically hindered silyl group is strategically placed on the 1° alcohol, enhancing the chances of selectivity in desilylation.

3.2.1. Under acidic conditions. The number of examples in which a 1° silyl ether is deprotected in the presence of a 2° silyl ether is enormous. So, in this section, only a few of those examples will be highlighted; a more complete listing is included in the tables at the end of this review.

Acidic conditions are often used to promote the selective removal of smaller silyl groups from protected 1° alcohols in the presence of 2° alcohols protected with bulkier silyl groups. Typically, TES or TBS groups protecting a 1° alcohol are cleaved without affecting a 2° TIPS or TBDPS ether. Acids used for such reactions include HOAc/THF/H_2O,[87–92] citric acid,[93] PPTS,[94–100] CSA,[5,36,37,101–103] TsOH,[99,104–107] TFA[108] and mineral acids such as H_2SO_4.[109] Ammonium chloride in MeOH at room temperature has been reported to remove a TBS group from a protected 1° alcohol in the presence of a 2° TIPS ether (Scheme 12).[110] The reaction conditions were sufficiently mild to allow survival of a ketal.



22 **23**, >91%

Scheme 12.

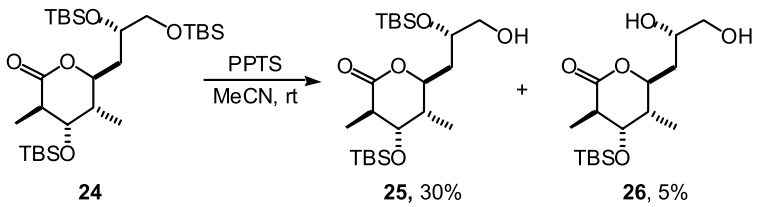
The less-common diphenyl-*tert*-butoxysilyl (DPTBS) group has been used to protect a 2° alcohol in the synthesis of the pectenotoxin skeleton and survives the use of CSA in MeOH/CH_2Cl_2 in the removal of a TBS group from a protected 1° alcohol.[111]

Another widely used means of deprotecting a 1° silyl ether in the presence of a 2° silyl ether involves the use of a TES group to protect the 1° alcohol and a TBS group to protect the 2° alcohol. Acidic reagents that have been shown to effect this transformation include CSA,[47,112] TFA,[10,113] triphenylphosphonium bromide ($Ph_3P{\cdot}HBr$)[114] and HCl.[115]

Selective deprotection of 1° silyl ethers in the presence of 2° silyl ethers in which the silyl groups are the same has also been described and illustrates the importance of steric demands around the alcohol's carbon. The selective deprotection of a 1° TES ether in the presence of a 2° TES ether has been effected using HOAc/THF/H_2O,[116] CSA,[47] PPTS,[117] and $Ph_3P{\cdot}HBr$.[114] However, a more frequently used strategy uses TBS groups to protect both the 1° and the 2° alcohol and reagents that have been shown to effect deprotection of 1° TBS ethers in the presence of 2° TBS ethers include HOAc/THF/H_2O,[118–120] CSA,[36,37,103,121–123] PPTS,[14,124–129] TsOH,[32,130] TFA,[131–136] and HCl.[137,138]

CSA has also been shown to be useful in the deprotection of a 1° TIPS ether in the presence of a 2° TIPS ether[139] and the deprotection of a 1° TBDPS ether in the presence of a 2° TBDPS ether.[140]

The choice of reagent in these reactions can be especially important.

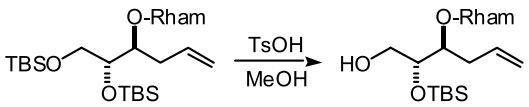


24 **25**, 30% **26**, 5%

Scheme 13.

For example, when PPTS in acetonitrile was used in the deprotection of a 1° TBS ether in the presence of a 2° TBS ether, the yield of monodeprotected product (**25**) was considerably diminished (Scheme 13).[123] However, attempts to use CSA or TsOH resulted in doubly deprotected product, **26**.

When two protecting groups are similar in size, care must also be taken to avoid over deprotection. The deprotection of a 1° TBS ether in the presence of a 2° TBS ether using TsOH has been reported (Scheme 14).[141] But to achieve high yields without desilylating the 2° position, the reaction was stopped and the recovered starting material was recycled.



27 **28**, 88%

Scheme 14.

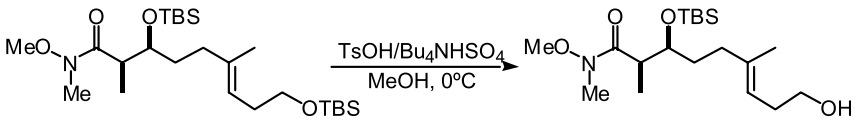
Phase-transfer catalysts have been useful in the deprotection of a 1° TBS ether in the presence of a 2° TBS ether.[142] Treatment of bis-silyl ether **29** with a 1:4 mixture of *p*TsOH and *n*-Bu_4NHSO_4 in MeOH led to the formation mono-protected product **30** in 89% yield (Scheme 15).[142]

Interestingly, despite its widespread use as an oxidant, Oxone® (2 $KHSO_5{\cdot}KHSO_4{\cdot}K_2SO_4$) has been reported to promote the deprotection of 1° TBS ethers in the presence of 2° TBS ethers but without oxidation of the newly released alcohol.[144]

Examples of HF-mediated desilylation reactions of 1° silyl ethers in the presence of 2° silyl ethers appear in the literature in abundance. For example, in the synthesis of (+)-mycotrienin I, a 1° TBS ether was deprotected in the presence of 2° TBS and TIPS ethers using HF·pyr in pyridine (Scheme 17).[145]

Typically, HF-mediated selective deprotection reactions rely upon the removal of a silyl group that is the same size as or smaller than the silyl group that is retained. Other examples of the use of HF·pyr in pyridine include deprotection of a 1° TES ether in the presence of a 2° TBS ether,[146,147] a 1° TBS ether in the presence of a 2° TBS ether,[18,148–150] a 1° TBS ether in the presence of a 2° TBDPS ether,[151] a 1° TBDPS ether in the presence of a 2° TBDPS ether,[152] and a 1° TBS ether in the presence of 2° TBS and TIPS ethers.[153]

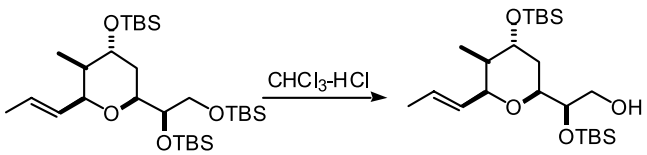
Other solvents may also be used with HF·pyr complex to effect selective desilylation. THF and pyridine/THF mixtures have been used in the HF·pyr-mediated deprotection of



29 **30**, 89%

Scheme 15.

'Acidic chloroform' was prepared by shaking $CHCl_3$ with concentrated HCl and separating the layers.[143] When tris-silylated substrate **31** was stirred at room temperature in this solvent, deprotection of the 1° TBS ether without hydrolysis of two 2° TBS ethers was observed (Scheme 16).[143]

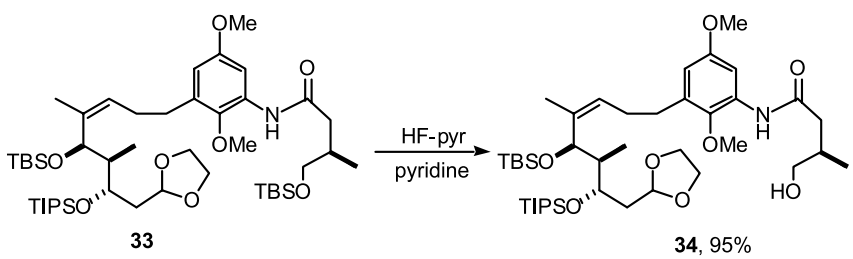


31 **32**, 97%

Scheme 16.

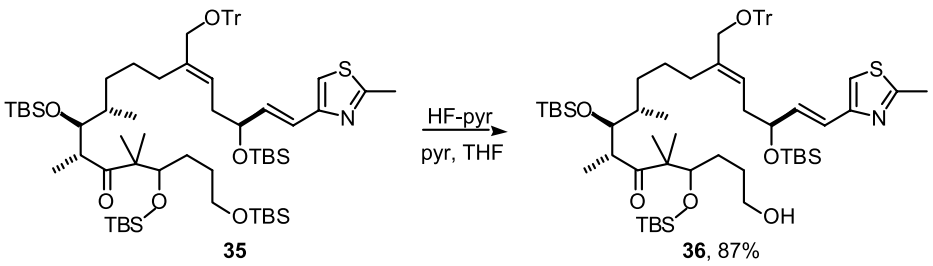
a 1° TBS ether in the presence of a 2° TBS ether,[154–161] a 1° TBDPS ether in the presence of a 2° TBDPS ether,[162] a 1° TBDPS ether in the presence of a 2° TIPS ether,[163] a 1° TBS ether in the presence of a 2° TBS and a 3° TES ether,[164] and a 1° TBS ether in the presence of 2° TBS and TIPS ethers.[165,166]

On occasion, it is advantageous to stop desilylation reactions prior to completion and resubmit the recovered starting material to deprotection conditions. For example, a 74% yield has been reported in the removal of a TBS group from a protected 1° alcohol in the presence of 2° TBS ethers using HF–pyr in THF–pyridine.[167] But when the recovered, fully protected compounds was re-treated with HF–pyr, the total yield increased to 87% (Scheme 18).[168]



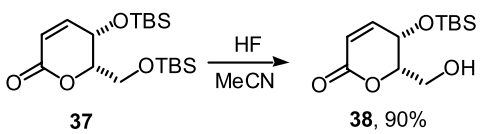
33 **34**, 95%

Scheme 17.



Scheme 18.

HF has also been used in the absence of pyridine to desilylate 1° silyl ethers in the presence of 2° silyl ethers. The solvent of choice for these reactions is acetonitrile although CH_3CN/THF mixtures have also been used.[169] Removal of a TBS group from a protected 1° alcohol in the presence of a 2° TBS ether was reported using 5% HF in CH_3CN (Scheme 19).[170]



Scheme 19.

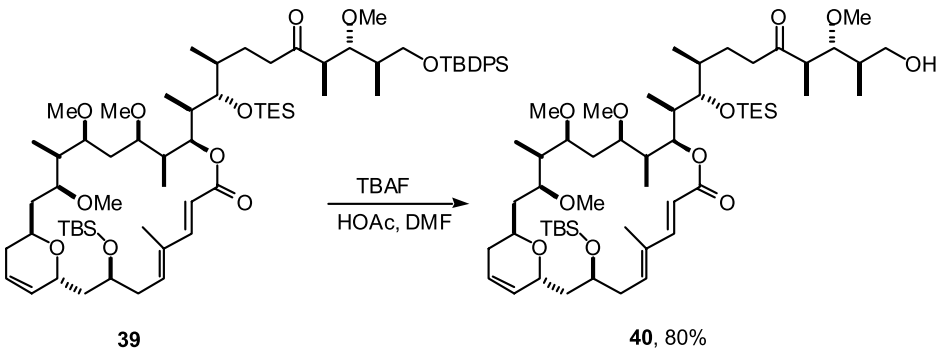
Other examples of HF-mediated selective deprotection include 1° TBS ethers in the presence of 2° TBDPS ethers[171,172] and a 1° TBS ether in the presence of a TPS-protected 2° alcohol.[173]

TBAF buffered with HOAc allows the selective deprotection of phenyl-substituted silyl groups and, thus, provides a means to reverse the usual trend of removing the less sterically demanding group from the protected 1° alcohol. For example, the selective cleavage of a 1° TBDPS ether in the presence of 2° TES and TBS ethers is typical of the use of HOAc-buffered TBAF in DMF (Scheme 20).[174]

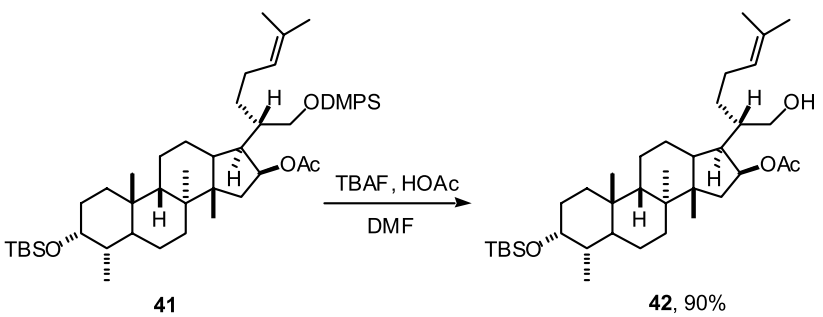
Other examples of this transformation using TBAF and HOAc in DMF have been reported.[142,175,176] THF has also been used as solvent in TBAF-HOAc mediated cleavage of 1° TBDPS ethers in the presence of 2° TBS ethers[7,150,177] and in the deprotection of a 1° TBDPS ether in the presence of 2° TES and TBS ethers.[178] Cleavage of two 1° TBDPS ethers and a 1° TIPS ether in the presence of a 2° TBS ether, a dithiane and a ketal was effected using a 20-fold excess of 1:1 TBAF:HOAc in THF.[179] And, a 1° DMPS ether has been cleaved in the presence of a 2° TBS ether using TBAF, HOAc and THF (Scheme 21).[180]

The combination of TBAF and HOAc also allows selective removal of like silyl group as evidenced by the selective deprotection of a 1° TBS ether in the presence of a 2° TBS ether[181,182] and a 1° TES ether in the presence of 2° TES and TBS ethers.[183]

Deprotection of 1° TBS ethers has been effected using H_2SiF_6 in *t*-amyl alcohol without cleaving 2° TIPS and TBS



Scheme 20.



Scheme 21.

ethers.[184] Similarly, a 1° TBS ether was deprotected in the presence of a 2° TIPS ether using H_2SiF_6 in a 4:1 mixture of CH_3CN and *t*-butyl alcohol.[184] Another example of the use of H_2SiF_6 to deprotect a 1° TBS ether in the presence of a 2° TBDPS ether employed CH_3CN and H_2O as solvent.[185]

Few examples of the use of Lewis acids for selective deprotection of 1° silyl ether in the presence of silyl-protected 2° alcohols have been reported. Removal of a TBS-group from a protected 1° alcohol in the presence of a 2° TBS ether occurs upon treatment with BCl_3 in THF.[186] $ZnBr_2$ and H_2O in refluxing CH_2Cl_2 has been shown to remove TES, TBS and TIPS groups from protected 1° alcohols but not TBDPS-protected 2° alcohols.[59] $Cu(OTf)_2$ in acetic anhydride converts 1° TBS ethers into acetates without affecting 2° TBDPS ethers.[56] TBS-protected 1° alcohols have been deprotected in the presence of 2° TBDPS ethers using catalytic $InCl_3$ in CH_3CN/H_2O.[57]

TMS-OTf has been reported to effect the deprotection of 1° TBS ethers in the presence of 2° TBDPS ethers.[51] TMS-OTf in the presence of diisopropylethylamine allowed the selective removal-at low temperature-of a TES group from a protected 1° alcohol followed by the rearrangement of an epoxide without affecting 2° TBS, TIPS or TBDPS ethers[187] (Scheme 22).

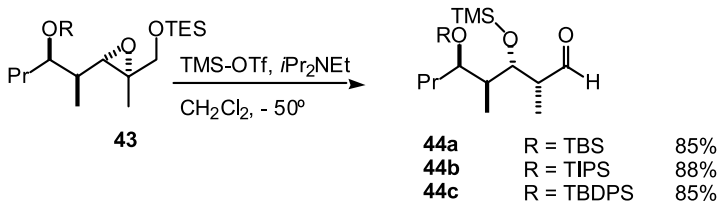
3.2.2. Under basic/nucleophilic conditions. In the absence of acid, fluoride-containing reagents can selectively remove a TBDPS group from a protected 1° alcohol in the presence of a 2° TBS ether. Under nucleophilic conditions, the stability of TBDPS and TBS ethers are approximately the same,[188] allowing the 1° TBDPS ether to undergo selective deprotection in the presence of a 2° TBS ether upon treatment with TBAF,[158] NH_4F in MeOH[189] or tris-(dimethylamino)sulfur (trimethylsilyl)difluoride (TAS-F).[190] For example, TBAF was used to effect the high-yield, selective desilylation of a 1° TBDPS ether in the presence of a 2° TBS ether as part of the total synthesis of oleandolide (Scheme 23).[158]

Fluoride-mediated deprotection reactions are not limited to the removal of TBDPS protecting groups. The deprotection of a 1° TBS ether in the presence of a 2° TIPS ether has been reported using TBAF·$3H_2O$ in THF.[47] A similar transformation was effected using anhydrous TBAF in THF.[191]

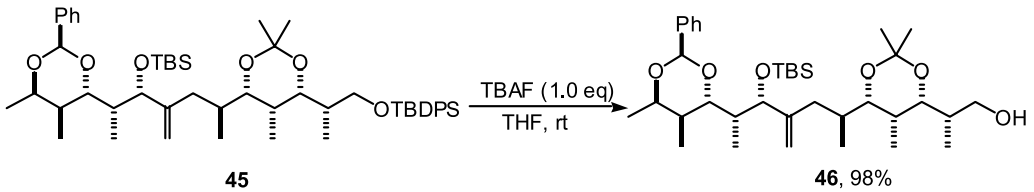
Alkoxysilyl groups are known to be especially sensitive to TBAF and this lability has been exploited in one step of the synthesis of phorboxazole in which a 1° TBMPS ether was cleaved in the presence of a 2° TBDPS ether (Scheme 24).[192]

More common, however, is the deprotection of 1° silyl ethers in the presence of 2° silyl ethers in which both alcohols are protected with the same silyl group. TBAF has been used to mediate the cleavage of 1° TES ethers in the presence of 2° TES ethers,[193–198] 1° TBS ethers in the presence of 2° TBS ethers[199–203] and 1° TBDPS ethers in the presence of 2° TBDPS ethers.[204,205]

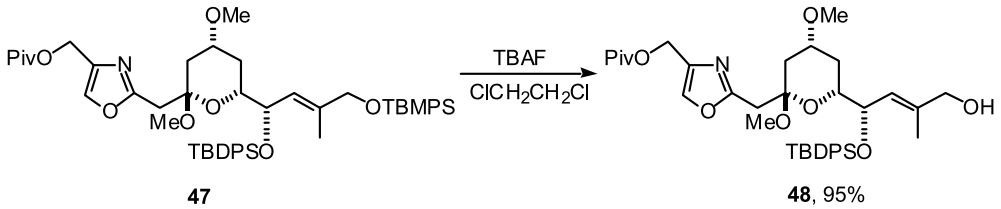
Other fluoride sources have proven useful in selective desilylation reactions. NH_4F in MeOH/H_2O has also been used in the selective desilylation of a 1° TBS ether in the presence of a 2° TBS ether.[206] KF in a mixed solvent of MeOH and THF was used to effect the deprotection of a 1° TES ether in the presence of 2° TES and TBS ethers.[17] This



Scheme 22.



Scheme 23.



Scheme 24.

reaction was not, however, entirely selective as a less sterically encumbered 2° TES ether and a very labile 2° TMS ether were also converted to alcohols.

Bronsted bases such as hydroxides, carbonates and hydrides have been used to effect selective deprotection reactions. A 1° TBDPS ether was deprotected in the presence of 2° TES and TBS ethers using NaOH, DMPU and H_2O (Scheme 25).[207] However, two less-sterically encumbered 2° TES ethers were hydrolyzed. The use of aqueous KOH in the presence of 18-crown-6 to remove a TBDPS group from a 1° alcohol without affecting a 2° TBS ether has been reported.[208] By contrast, NaOH left a 1° TBDPS ether intact when used to deprotect a 1° TBS ether in the presence of a 2° TBS ether (Scheme 26).[209]

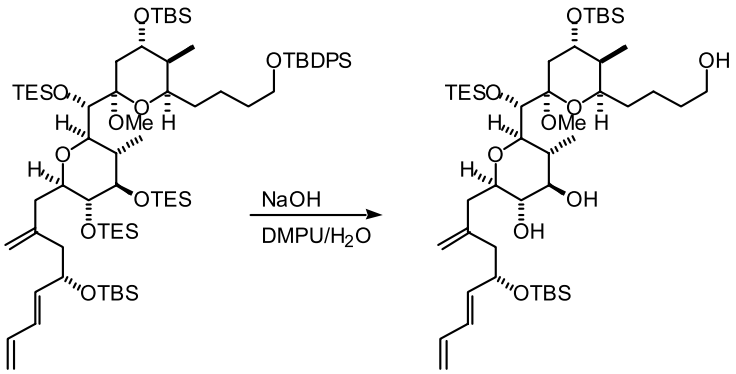
A mixture of NaH and propargyl alcohol in HMPA/THF at 0°C has been reported to effect the deprotection of a 1° TBDPS ether in the presence of a 2° TBS ether.[210] In the synthesis of (+)-milbemycin D, K_2CO_3 in MeOH was utilized to selectively remove a TBS group from a protected 1° alcohol in the presence of a 2° TBDPS ether. A 3° TMS ether was also cleaved under these conditions.[211]

3.2.3. Miscellaneous conditions. Carefully chosen oxidative conditions allow for the selective removal of a silyl group from a 1° alcohol in the presence of a 2° silyl ether with the resulting free carbinol undergoing oxidation. Swern conditions have been employed for the conversion of 1° TES ethers into aldehydes without desilylation of TES-protected 2° alcohols.[106,212,213] As part of the synthesis of antiglaucoma compounds, a 1° TES ether was deprotected and oxidized to the aldehyde without affecting a 2° TES ether or two THP-protected alcohols (Scheme 27).[213]

Swern conditions have also been used to deprotect and oxidize a 1° TMS ether in the presence of a 2° TMS ether[212] and a 1° TES ether in the presence of a 2° TBS ether.[214]

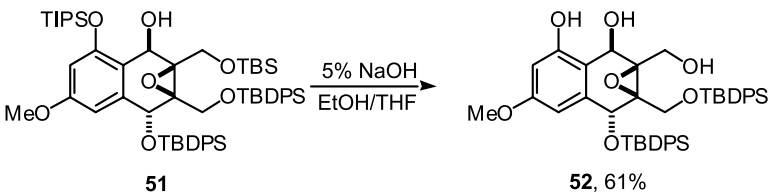
CrO_3·2 pyr in CH_2Cl_2 has been reported to selectively convert a 1° TES ether into an aldehyde without affecting a 2° TES ether.[215] Selective oxidative deprotection of 1° TBS ethers in the presence of 2° TBS ethers has been effected using quinolinium fluorochromate.[84]

Vilsmeier–Haack conditions have been reported to convert 1° TBS ethers to formate esters in the presence of 2° TBS ethers.[216,217] This methodology was extended to the preparation of formate esters from TIPS- and TBDPS-protected 1° alcohols in the presence of similarly protected 2° alcohols.[217] A more electrophilic reagent formed



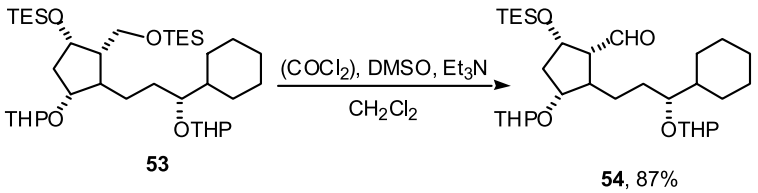
49 **50**, 60%

Scheme 25.



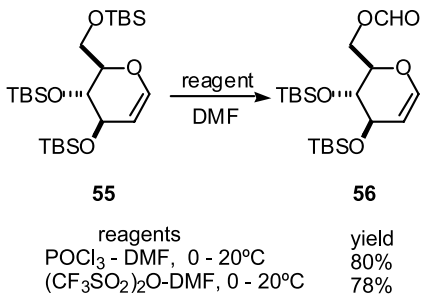
51 **52**, 61%

Scheme 26.



53 **54**, 87%

Scheme 27.



Scheme 28.

from $(CF_3SO_2)_2O$ and DMF proved useful as well[217] (Scheme 28).

Ceric ammonium nitrate (CAN) in isopropanol has been reported to deprotect 1° TBS ethers in the presence of 2° TBS ethers.[13,218] When CAN was adsorbed onto silica gel and used at 65°C in a 1:1 mixture of CCl_4 and isopropyl alcohol, TIPS-protected 1° alcohols also underwent desilylation in the presence of 2° TIPS ethers.[218] The mechanism for these reactions is believed to involve single electron transfers.[218]

DDQ and other π-acids have been used in silyl deprotections reactions and a single electron transfer mechanism has been proposed for these reactions.[219] A polymer-supported π-acid catalyst, polymeric dicyanoketene acetal (DCKA), has been shown to remove TBS groups from protected 1° alcohols in the presence of 2° TIPS and TBDPS ethers.[81]

CBr_4 in alcohol solvents has been used to effect the selective desilylation of 1° TBS ethers in the presence of 2° TBS ethers[220] and 1° TIPS ethers in the presence of 2° TIPS ethers.[221] Both reactions are catalytic in CBr_4 and proceed at reflux[221] or under photochemical conditions.[220]

A five-fold excess of LiBr with 18-crown-6 in acetone has been used to deprotect a 1° TBS ether in the presence of a 2° TBS ether.[222] Similarly, a 1° alcohol protected with a diphenyl-*t*-butoxysilyl group underwent deprotection in the presence of a 2° TBDPS ether using Na_2S in ethanol.[223] SiF_4 was used to deprotect a 1° TIPS ether in the presence of 2° TIPS and 3° TBS ethers in a total synthesis of hemibrevitoxin B.[224] And, a commercially available uronium salt, O-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyl-uronium tetrafluoroborate (or TBTU), mediated the selective removal of a TBS group from a protected 1° alcohol without affecting a 2° TBDPS ether.[225]

3.3. In the presence of a 3° silyl ether

Although not as common, deprotections of 1° silyl ethers in the presence of 3° silyl ethers occur in a similar manner as the more common removal of silyl groups from protected 1° alcohols in the presence of 2° silyl ethers.

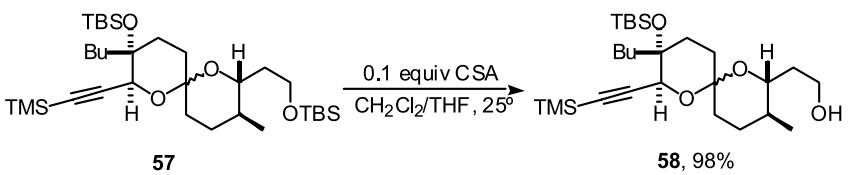
3.3.1. Under acidic conditions. CSA has proven useful for removing silyl protecting groups from protected 1° alcohols without affecting the more hindered 3° silyl ether. Catalytic CSA in CH_2Cl_2/MeOH at room temperature mediated the deprotection of 1° TBS ethers in the presence of 3° TBS ethers in high yield (Scheme 29).[226]

Similarly high yielding desilylations of 1° TES ethers in the presence of 3° TES ethers using CSA in MeOH/THF have been reported.[227,228] CSA-mediated removal of a TBS group from a protected 1° alcohol without affecting a 3° TES alcohol was slightly less successful.[228,229]

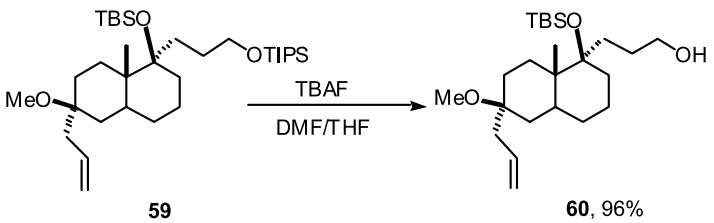
Amberlyst-15, an acidic resin, has been used in the synthesis of a portion of gambierol to remove a TES group from a protected 1° alcohol without deprotecting a 3° TBS ether or an acetal.[230]

Fluoride has also been used in acidic conditions to effect the deprotection of 1° silyl ethers in the presence of 3° silyl ethers. For example, HF in CH_3CN was used to remove TBS groups from protected 1° alcohols without affecting 3° TMS ethers.[231,232] HF–pyr in pyridine/THF effected the deprotection of a 1° TBS ether in the presence of a 3° TES ether.[94,233]

Likewise, TBAF buffered with HOAc has been used to selectively deprotect a 1° TBS ether in the presence of a 3° TBS ether in a step of Martin's asymmetric synthesis of erythromycin B[234] and to cleave a 1° TBDPS ether in the presence of 2° TBS and 3° TES ethers as a step in the synthesis of spongistatin 2.[178]



Scheme 29.



Scheme 30.

3.3.2. Under basic/nucleophilic conditions. TBAF has been used to deprotect a 1° TIPS ether in the presence of a 3° TBS ether in the preparation of precursors for the formation of macrocycles (Scheme 30).[235]

Tris(dimethylamino)sulfonium difluorotrimethylsilicate (or TAS-F) has been used in greater than stoichiometric quantities to deprotect a 1° TBDPS ether in the presence of a 3° TBS ether.[236]

3.3.3. Under miscellaneous conditions. SiF_4 in a 1:1 CH_2Cl_2/CH_3CN mixture at room temperature has been used to remove a TIPS group from a protected 1° alcohol in the presence of 2° TIPS and 3° TBS ethers.[224]

4. Selective deprotection of silyl-protected 2° alcohols

4.1. In the presence of 1° silyl ethers

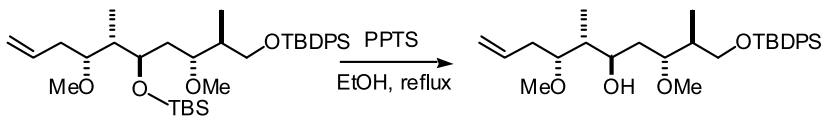
Selective removal of a silyl group from a protected 2° alcohol without affecting other protected alcohols requires consideration of the steric environments of all silyl ethers in the compound. The majority of selective deprotection reactions of silyl ethers rely upon steric factors, so desilylation of a silyl group from the more sterically encumbered alcohol requires a bulky silyl group on the less-hindered alcohol. Even when these conditions are applied, deprotection of 2° silyl ethers in the presence of 1° silyl ethers may prove impractical. Two such examples have recently appeared in which selective desilylation of a 2° TBS ether in the presence of a 1° TIPS ether could not be achieved[237] and global desilylation followed by monosilylation of the resulting 1° alcohol was more efficient than attempted selective deprotection reactions.[238]

4.1.1. Under acidic conditions. Deprotection of 2° silyl ethers in the presence of 1° silyl ethers require removal of a protecting group from a more sterically hindered alcohol and thus, strategies typically involve the attachment of smaller, more-labile silyl groups on the 2° alcohol and larger and more-robust silyl groups on the 1° alcohol. TMS and TES groups are most commonly used to protect the 2° alcohol with TIPS or TBDPS groups protecting the 1° alcohol. Acidic reagents can then effect deprotection of the 2° silyl ether in the presence of the 1° silyl ether and reagents that have been used for such selective deprotection reactions include TsOH,[37,239–246] PPTS,[247,248] CSA,[38,249] HOAc,[205,210] triphenylphosphonium bromide (Ph_3PHBr),[38] HCl[18,250–252] and H_2SO_4.[38,253]

The removal of a TBS group from a protected 2° alcohol in the presence of a 1° silyl ether is a less commonly used strategy but examples have been reported. Pattenden has described the deprotection of a 2° TBS ether in the presence of a 1° TBDPS ether using PPTS in refluxing ethanol (Scheme 31).[152]

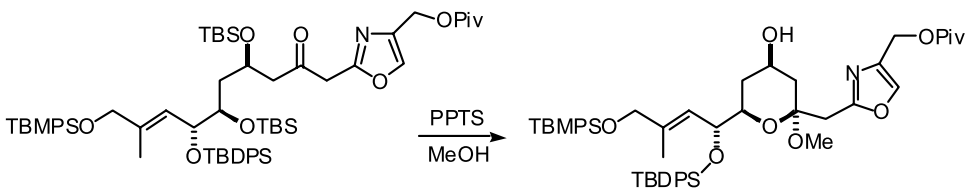
PPTS was also used to effect the deprotection of 2° TBS ethers in the presence of a 1° TBMPS and a 2° TBDPS ether (Scheme 32).[192] Although TBMPS groups are noted for the resistance to acid imparted by the electronic effects of an alkoxy ligand on silicon, a siginificant quantity of deprotected 1° alcohol was recovered.[192]

Acidic fluoride sources have also been used for deprotection of 2° silyl ethers in the presence of 1° silyl ethers. HF·pyridine has been used to deprotect 2° TES ethers in



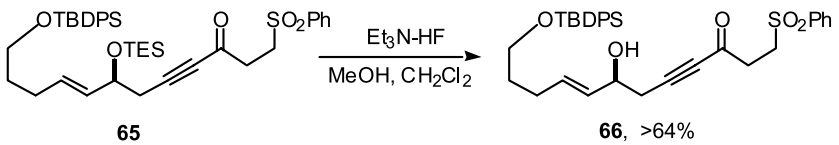
61 **62**, 90%

Scheme 31.



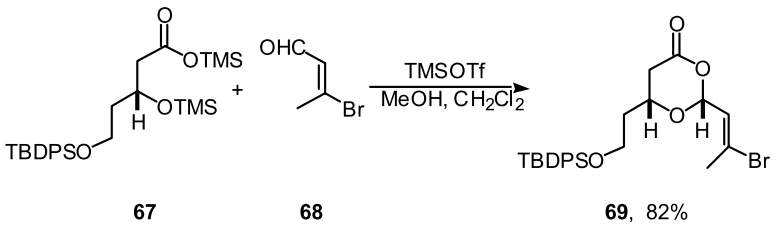
63 **64**, 58%

Scheme 32.



65 **66**, >64%

Scheme 33.



67 **68** **69**, 82%

Scheme 34.

the presence of 1° TBS,[66] TIPS[47,67,254] and TBDPS ethers.[255]

An excess of triethylamine trihydrofluoride (Et_3N-3 HF) has been shown to effect the cleavage of 2° and 3° TES ethers in the presence of a 1° TIPS ether.[256] In a similar example, a 2° TES ether underwent selective desilylation using Et_3N-HF without reaction with a 1° TBDPS ether (Scheme 33).[257]

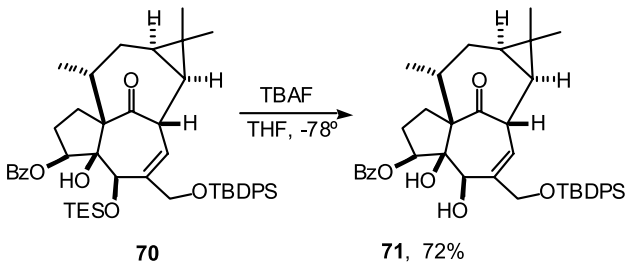
TBAF buffered with solid NH_4Cl has also been shown to effect the selective desilylation of a 2° TES ether in the presence of a 1° TBS ether.[258] And, H_2SiF_6 has been employed in the selective deprotection of a 2° TBS ether in the presence of a 1° TBDPS ether.[208]

Lewis acids have also been employed in selective desilylation reactions of 2° silyl ethers in the presence of 1° silyl ethers. TMS-OTf has been demonstrated to efficiently remove TBS groups from protected 2° alcohols in the presence of 1° TBDPS ethers at low temperature.[51,259] A 2° TMS ether was cleaved in the presence of a 1° TBDPS ether during the condensation of bis-silyl ether **67** with aldehyde **68** when TMS-OTf was used as a catalyst (Scheme 34).[160] On a larger scale, this reaction failed to proceed unless 5–10 mol% triflic acid was added, indicating that the true deprotection agent may have been triflic acid, produced from hydrolysis of TMS-OTf.

Catalytic $Cu(OTf)_2$ in acetic anhydride has been shown to convert 2° TBS ethers into acetates without reacting with 1° TBDPS ethers.[56] Similarly, catalytic $InCl_3$ in CH_3CN/H_2O cleaved a 2° TBS ether in the presence of a 1° TBDPS ether.[57] Excess $ZnBr_2$ in CH_2Cl_2 and H_2O mediated the deprotection of 2° TES and TBS ethers while leaving 1° TBDPS ethers unaffected.[59] Similar differences in reaction rates point to the use of $Zn(BF_4)_2$ in H_2O as an agent for the deprotection of 2° TBS ethers in the presence of 1° TBDPS ethers.[60] Selective deprotection of 2° TBS ethers has been observed upon heating with Montmorillonite K-10 in $MeOH/H_2O$. No reaction was observed when 1° TBDPS ethers were subjected to the same conditions.[41]

4.1.2. Under basic/nucleophilic conditions. TBAF mediates the removal of smaller, more labile TES group from protected 2° alcohols in the presence of a 1° TBDPS ether as illustrated in the synthesis of ingenol (Scheme 35).[260]

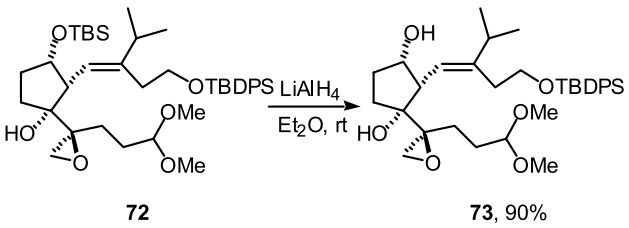
At room temperature, TBAF also mediates the selective cleavage of a 2° TMS ether in the presence of 1° and 2° TIPS ethers.[139]



70 **71**, 72%

Scheme 35.

$LiAlH_4$ in ether was used to effect the selective deprotection of a 2° TBS ether in the presence of a 1° TBDPS ether (Scheme 36).[261] These conditions permitted the survival of an acetal and an oxirane. But longer reaction time at higher temperature resulted in global desilylation and oxirane opening.



72 **73**, 90%

Scheme 36.

4.1.3. Under miscellaneous conditions. Selective desilylation of a 2° TES ether in the presence of a 1° TBS ether has been effected using Pd/C in MeOH.[262] A variation on this method that uses H_2 and Pd/C was applied to the deprotection of 2° TES ethers in the presence of a 1° TBDPS ether as part of the synthesis of phytoprostane F_1.[263] The need for H_2 in the latter instance may lie in the source of the Pd/C catalyst.[264] Significant differences in the ability of Pd/C from different suppliers to mediate deprotection of TES ethers in the absence of H_2 have been attributed to different acidities and the reaction in the absence of H_2 may be acid-catalyzed cleavage, not a Pd-mediated reaction.[264] A recent report compares the reactivities of several silyl protecting groups to hydrogenolysis over Pd/C and simply stirring with Pd/C: more acid-sensitive groups such as TES, TPS and *n*-Bu_3Si were removed under both sets of conditions while the larger, more robust groups, TBS, TIPS and TBDPS, were stable in the absence of H_2.[83]

Iodoetherification with I_2 and Ag_2CO_3 in ether or toluene has been shown to also effect the deprotection of 2° TES ethers in the presence of 1° TBS ethers[265,266] (Scheme 37).

IBr in CH_3CN effects a similar iodoetherification with deprotection of a 2° TBS ether in the presence of a 1° TBDPS ether.[267]

Excess LiCl in DMF/H_2O selectively deprotects a 2° TBS ether without affecting a 1° TBDPS ether. The phosphonium salt produced by the reaction of PPh_3 and 2,4,4,6-tetrabromo-2,5-cyclohexadienone mediates the selective conversion of a 2° TES ether into a 2° bromide in the presence of a 1° TBDPS ether.[85] The polymeric π-acid catalyst, dicyanoketene acetal (DCKA), has been shown to desilylate a 2° TBS ether in the presence of a 1° TBDPS ether.[81] A 2° TBS ether underwent deprotection in the presence of a 1° TBDPS and a 2° TBS ether upon treatment with dimethoxymethane and P_2O_5 (Scheme 38).[130]

4.2. In the presence of 2° silyl ethers

Removal of a silyl group from a protected 2° alcohol in the presence of another 2° silyl ether often depends on differences in the steric requirements of the silyl ligands. Occasionally, however, the steric and electronic environment of groups neighboring the alcoholic carbon play a role in diminishing the reactivity of one silyl ether more than another.

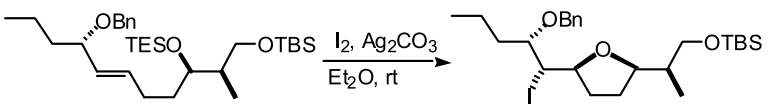
4.2.1. Under acidic conditions. Acid-mediated deprotection of one 2° silyl ether in the presence of another 2° silyl ether typically employs a strategy in which a smaller, more acid-sensitive silyl group is used to protect the alcohol that is to be released and a larger, more robust silyl ether is used protect the alcohol that is to remain protected. The most common approach is to protect one alcohol with a TMS or TES group and the other with a TBS, TIPS or TBDPS group. A number of acids have been employed in deprotections of this nature and include PPTS[248,268–277] TsOH,[147,242] TFA,[278–280] CSA,[155] HOAc,[208,281–288] HCl[289–291] and H_2SO_4.[38] TBS is sufficiently small to allow its removal from a protected 2° alcohol in the presence of 2° TIPS or TBDPS ethers using PPTS[192,193] CSA,[94,293] or HCl.[294–297] But, TBS can also be sufficiently large to allow it to remain in place protecting a 2° alcohol as a TES group was removed from another protected 2° alcohol using CSA.[155] PPTS has also been used to deprotect a 2° DEIPS ether in the presence of a 2° TIPS ether.[298]

A 2° TIPS ether was cleaved in the presence of a 2° TBS ether, highlighting the importance of the steric environment provided by the carbon framework of the molecule. Treatment of bis-silyl ether **78** with catalytic TsOH in MeOH at room temperature resulted in selective removal of the TIPS group from a protected 2° alcohol without affecting a 2° TBS ether that was more sterically encumbered (Scheme 39).[145]

The carbon skeleton of the silyl-protected diols and polyols can provide enough steric influence to allow like silyl ethers to be distinguished from one another. For example, TFA in wet THF allows the deprotection of a less-hindered 2° TES ether in the presence of another 2° TES ether (Scheme 40).[299]

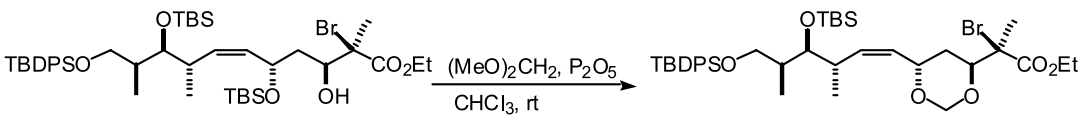
Similarly, CSA was used to remove a TBS group from a protected, less-hindered 2° alcohol without cleaving another 2° TBS ether.[300]

HF buffered with pyridine in THF has proven useful in the deprotection of a 2° TMS and a 2° TES ether in the presence of 2° TBS, 2° TES and 3° TES ethers as part of syntheses of



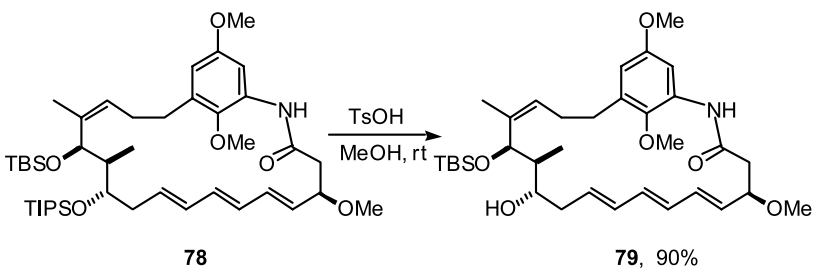
74 **75**, 92%

Scheme 37.



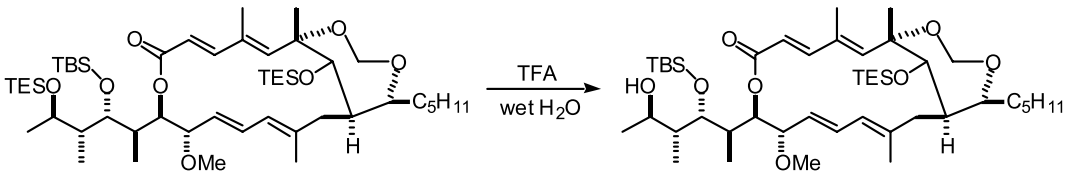
76 **77**, 80%

Scheme 38.



78 **79**, 90%

Scheme 39.



80 **81**, > 90%

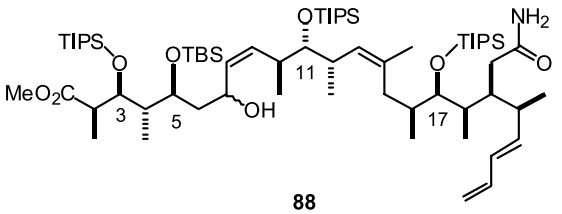
Scheme 40.

spongistatins.[164,301] A number of other examples of the deprotection of 2° TES ethers in the presence of 2° TBS ethers upon treatment with HF–pyr in THF have been reported.[115,116,255,302–304] Cleavage of a 2° TES ether in the presence of a 2° TIPS ether has been effected with HF–pyr in acetonitrile[305] or THF.[67,306] HF–pyr in THF also effects the selective deprotection of 2° TBS ethers in the presence of 2° TBDPS ethers.[6,307]

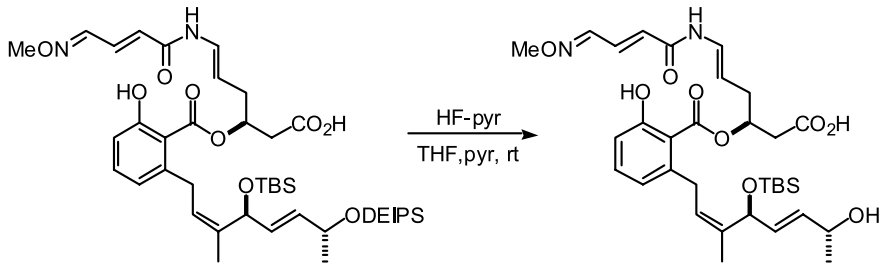
The challenge of selectively deprotecting silyl ethers with similar steric and electronic environments is illustrated by the relatively low yields achieved in the HF–pyr mediated cleavage of a 2° DEIPS ether in the presence of a 2° TBS ether (Scheme 41)[308,309] and a 2° TBS ether in the presence of another 2° TBS ether (Scheme 42).[310]

HF has also been buffered with Et_3N to effect the deprotection of a 2° TMS ether in the presence of a 2° TBS ether,[311] a 2° TES ether in the presence of a 2° TBS[117,312] and a 2° TBS ether in the presence of a 2° TIPS ether.[313] The role of the steric environment around the alcoholic carbons was again illustrated by the report of the selective desilylation of a 2° TIPS ether in the presence of a more sterically crowded 2° TBS ether using HF/Et_3N (Scheme 43).[306]

HF in CH_3CN has also proven useful in the selective desilylation of one 2° silyl ether in the presence of another 2° silyl ether. Aqueous HF in CH_3CN has been reported to effect the selective deprotection of a TES ether in the presence of 2° TBS[314] or TBDPS ether[315] and a 2° TBS ether in the presence of a more hindered 2° TBS ether.[316] As part of the total synthesis of (+)-discodermolide, 10% HF in CH_3CN was used to desilylate a 2° TBS and two of the three 2° TIPS ethers, leaving a third 2° TIPS ether.[317,318] Although which TIPS group remained was not included in the original publication, evidence indicates that the 2° TIPS ether on C-11 of **88** was the unreacted silyl ether.[319]

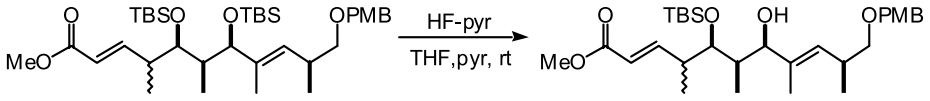


88



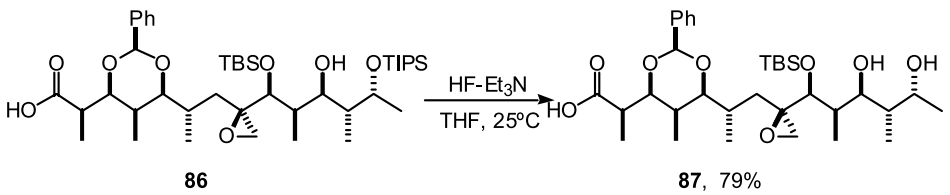
82 **83**, 43%

Scheme 41.



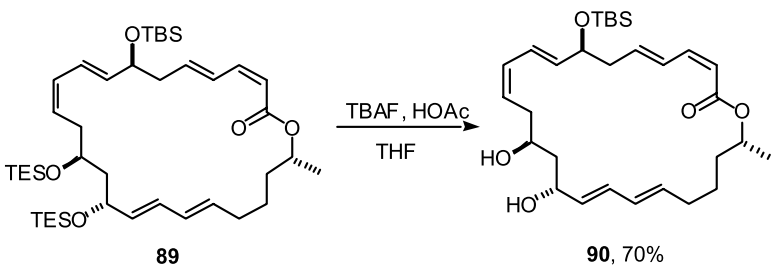
84 **85**, 56%

Scheme 42.



86 **87**, 79%

Scheme 43.



Scheme 44.

Other acidic fluoride-mediated methods of deprotection have been applied to this problem. Fluorosilicic acid in isopropanol at −40°C allowed the deprotection of a 2° TMS ether in the presence of a 2° TIPS and a 1° TBS ether.[184] Solvent and temperature were important. When *t*-BuOH was used as solvent at room temperature, the 1° TBS ether was lost and, when *t*-amyl alcohol was used at 0 °C, alkene isomerization was problematic.[184] Johnson also employed H_2SiF_6 with Et_3N in CH_3CN to effect the selective desilylation of a 2° TBS ether in the presence of a 2° TIPS ether.[320]

TBAF buffered with acetic acid was used to deprotect 2° TES ethers without affecting a 2° TBS ether. The addition of HOAc mediates the basicity of fluoride, preventing β-elimination of the TBS ether (Scheme 44).[321] Likewise, 2° TES ethers have undergone desilylation in the presence of 2° TES and TBS ethers using HOAc-buffered TBAF in THF.[183]

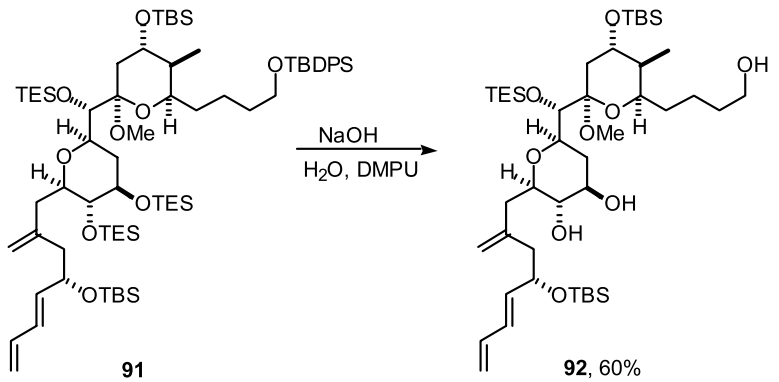
The use of $(NH_4)_2HF_2$ in NMP and DMF is yet another illustration of the importance of the steric environment around the alcoholic carbons. In this instance, a less hindered, equatorial 2° TES ether in a glycoside undergoes deprotection in the presence of an axial 2° TES ether.[322]

4.2.2. Under basic/nucleophilic conditions. K_2CO_3 in MeOH has proven useful in deprotecting 2° TMS ethers in the presence of 2° TBS[323] and TBDPS ethers.[324,325] Aqueous NaOH in DMPU was used to remove a TES group from a protected 2° alcohol in the presence of 2° TES and TBS ethers (Scheme 45).[207]

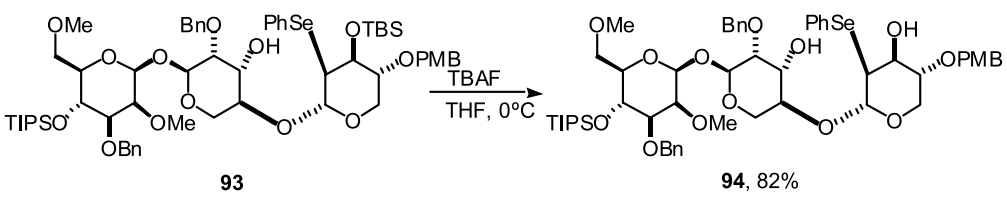
Although not typically used in silyl deprotection reactions, $LiAlH_4$ in THF was used to reductively deprotect a benzyl ether and, in the process, selectively desilylate a 2° TIPS ether in the presence of a 2° TBS ether.[326] In another unusual example, PhLi or MeLi were chosen to convert a tethered 2° silyl ether into an alcohol without reaction with a 2° TBS ether.[327]

TBAF has been widely used in the deprotection of 2° silyl ethers in the presence of other 2° silyl ethers. Often, 2° TES ethers are cleaved upon treatment with TBAF in THF and examples include the desilylation of 2° TES ethers in the presence of 2° TBS[210,328–330] and TBDPS[331,332] ethers. The selective deprotection of 2° TMS ethers in the presence of 2° TBS[333] and TIPS[139] ethers using TBAF/THF has also been reported. As part of the total synthesis of spongistatin 2, TBAF/THF was used to effect the desilylation of two 2° TES ethers in the presence of one 2° TES, two 2° TBS and one 3° TES ethers.[114]

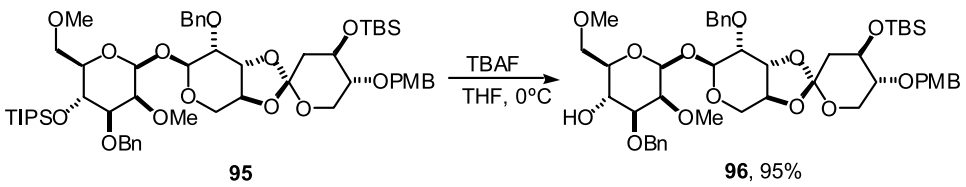
The selective desilylation of a 2° TIPS ether in the presence



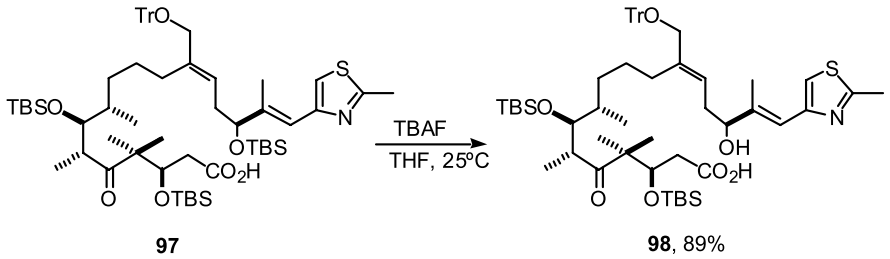
Scheme 45.



Scheme 46.



Scheme 47.



Scheme 48.

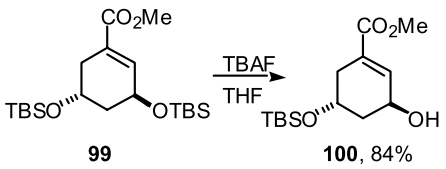
of a 2° TBS ether using TBAF at low temperature has been reported.[334] Although no explanation for the source of this selectivity was provided by the authors, the role of a nearby hydroxyl group was noted in determining which of two silyl groups was removed.[335] When bis-silyl ether **93** was treated with a slight excess of TBAF in THF, selective deprotection of the 2° TBS ether was observed (Scheme 46).[35] But when bis-silyl ether **95** in which the free hydroxyl was absent was exposed to the same conditions, the 2° TIPS ether was exclusively deprotected (Scheme 47).[335] It is unclear whether this difference in reactivity is due to the inherent conformational change required of the orthoester or the free hydroxyl group itself.[335]

Deprotection of 2° TBS ethers in the presence of other 2° TBS ethers using TBAF in THF has been an important step in several syntheses of epothilones and related compounds.[9,121,122,167,168,336–339] Treatment of tris-silyl ether **97** with excess TBAF in THF at 25 °C over 8 h afforded the monodeprotected product in high yield (Scheme 48).[167] Selectivity is due to less steric crowding around the silyl ether that undergoes cleavage.

In a variation, the carboxylic acid of **97** underwent deprotonation to form the carboxylate prior to deprotection.[338] It was suggested that selectivity was due to the trans-silylation from the silyl ether to the carboxylate, forming a silyl ester that was hydrolyzed upon workup. However, most applications of this protocol did not involve conversion of the carboxylic acid to the carboxylate salt prior to desilylation and reported selectivity was equally high.

Selective deprotection of a 2° TBS ether of a hemiacetal in the presence of a 2° TBS ether was effected upon treatment with TBAF in THF at 0°C.[340] A more sterically crowded 2° TBS ether was desilylated in the presence of another 2° TBS ether as part of the synthesis of callipeltoside A.[341] And, treatment of bis-silyl ether **99** with one equivalent of TBAF in THF resulted in selective deprotection of the 2° allylic TBS ether (Scheme 49).[342]

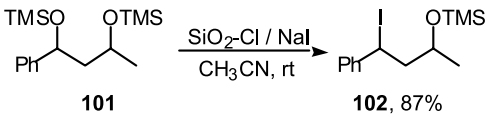
Other sources of fluoride have been used to deprotect one 2° silyl ether in the presence of another. KF in a 4:1 MeOH/THF mixture has been reported to effect the selective deprotection of 2° TMS and TES ethers in the presence of 2° TES and TBS ethers in the synthesis of (+)-spongistatin 1[17] and 2.[207] TAS-F in DMF was used to selectively deprotect 2° DEIPS ethers in the presence of 2° TES ethers as one of the final steps in the total synthesis of (+)-concanamycin F.[343]



Scheme 49.

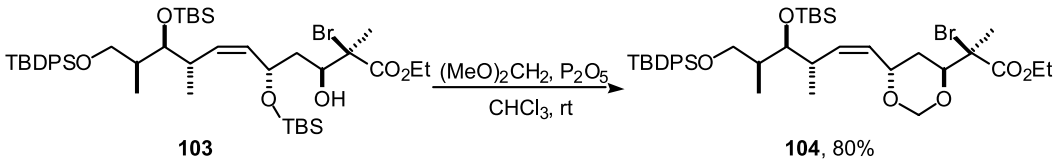
4.2.3. Under miscellaneous conditions. At room temperature, aqueous $NaIO_4$ in THF or ethanol has been shown to deprotect 2° TMS, TES, TBS, TIPS and TPS ethers—without evidence of oxidation—while leaving 2° TBDPS ethers largely intact.[344] At elevated temperatures, the reactivity of 2° TBDPS ethers becomes significant. Although the reaction is stoichiometric in $NaIO_4$, the mechanism of the reaction is unknown.

Silica chloride (SiO_2–Cl) and NaI in CH_3CN has been shown to convert silyl-protected allylic, benzylic and propargylic alcohols into the corresponding iodide.[345] Not surprisingly, this method has been used to selectively convert a 2° benzylic TMS ether into a 2° benzylic iodide in the presence of another 2° TMS ether (Scheme 50).[345]

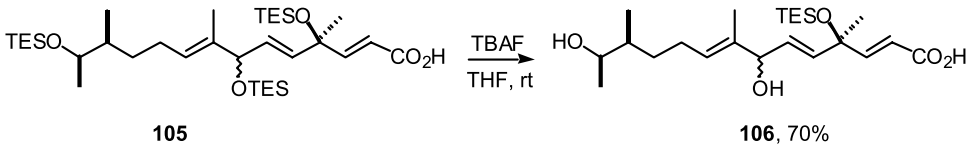


Scheme 50.

Tris-silyl ether **103** was treated with dimethoxymethane and P_2O_5 to form a cyclic acetal with concomitant desilylation of a 2° TBS ether without affecting another 2° TBS ether (Scheme 51).[130]



Scheme 51.



Scheme 52.

4.3. In the presence of a 3° silyl ether

Deprotection reactions involving 3° silyl ethers are rarely included in systematic studies of new methods for selective desilylation reactions. However, a number of examples of the selective deprotection of 2° silyl ethers in the presence of 3° silyl ethers have been reported as parts of natural and unnatural product syntheses. Typically, a small silyl group such as TMS or TES is removed from the protected 2° alcohol while another silyl group such as TES or TBS remains on the protected 3° alcohol.

4.3.1. Under acidic conditions. CSA has been reported to effect the selective deprotection of a 2° TMS ether in the presence of a 3° TES ether[346] and PPTS in CH_2Cl_2–MeOH was used to selectively deprotect a 2° TES ether in the presence of a 3° TES ether.[347] Similarly, aqueous CF_3SO_3H in THF was used to selectively deprotect a 2° TES ether in the presence of a 3° TBS ether and a 2° TBS ether in the presence of a 3° TBS ether.[348]

Aqueous HCl in a THF–CH_3CN mixture at −10 °C was used to effect the selective desilylation of a 2° TES ether in the presence of a 3° TES ether.[290,291] Under these conditions, a 2° TBS ether also survived. In a similar example, a 2° TES ether was deprotected in the presence of a 3° TBS ether using aqueous HCl in THF.[349]

More commonly, though, HF–pyridine has been the reagent of choice for selective desilylations of this type. In the total synthesis of spongistatin 2, HF–pyridine in THF/pyridine at 0 °C selectively cleaved a 2° TES ether in the presence of a 3° TES ether.[164] These conditions also led to the deprotection of a 1° TBS ether but left a 2° TBS ether intact. Later in the synthetic route, HF–pyr was used under similar conditions to effect the deprotection of 2° TMS and TES ethers in the presence of a 3° TES ether as well as 2° TES and TBS ethers.[164] Similarly, HF–pyr in pyridine has been shown to deprotect 2° TMS and TES ethers in the presence of a 3° TES ether and 2° TES and TBS ethers.[301] And, a 2° TES ether underwent selective desilylation in the presence of 3° TES, 2° TBS and 1° TIPS ethers upon treatment with HF–pyr in THF.[47]

Aqueous HF in CH_3CN was used to effect the deprotection of a 2° TBS ether in the presence of a 3° TBS ether.[350]

4.3.2. Under basic/nucleophilic conditions. Non-acidic sources of fluoride have also been employed to effect the desilylation of 2° silyl ethers in the presence of 3° silyl ethers. A two-fold excess of TBAF in THF allowed the selective cleavage of 2° TES ethers in the presence of a 3° TES ether (Scheme 52).[351]

Slow addition of an excess of TBAF in THF at 0 °C led to the selective deprotection of two less-hindered 2° TES ethers in the presence of a 3° TES ether as well as another 2° TES ether and three 2° TBS ethers (Scheme 53).[114] Attempts to use HF–pyr buffered with pyridine proved unsuccessful with large amounts of unreacted starting material and compromised selectivity.

KF in a 4:1 mixture of MeOH and THF was used to effect the selective cleavage of a 2° TMS ether in the presence of a 3° TES ether as well as 2° TES and TBS ethers in the synthesis of spongistatin 1[17] and 2.[207]

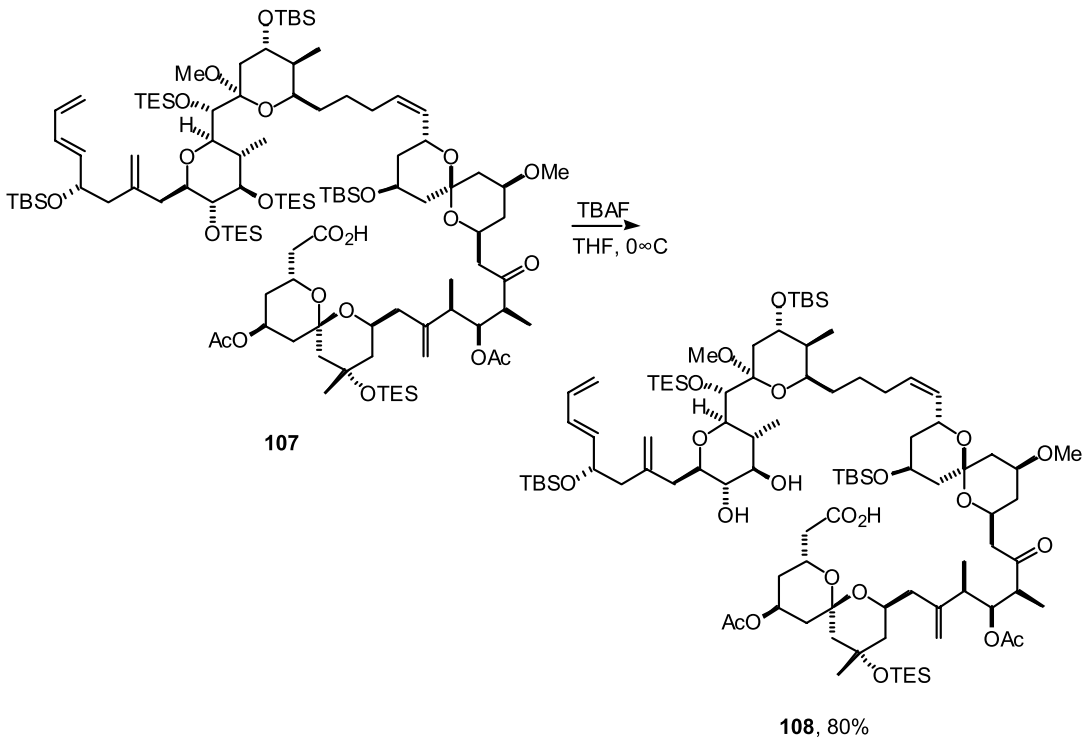
5. Selective deprotection of silyl-protected 3° alcohols

Reports of new methods for performing desilylation reactions rarely include examples of 3° silyl ethers. So, the examples presented here have been culled from multi-step syntheses of natural products. Typically, the steric crowding around 3° alcohols require that only the smallest silyl groups—TMS and TES groups—be used. To prevent desilylation of the *other* protected alcohol, larger silyl groups such as TIPS and TBDPS are usually employed.

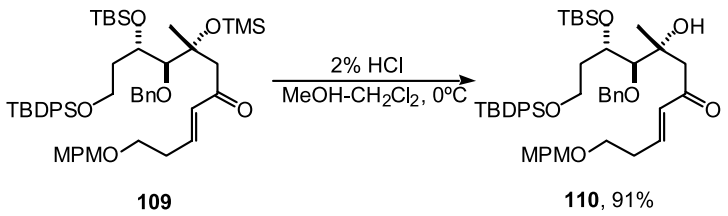
5.1. In the presence of 1° silyl ethers

5.1.1. Under acidic conditions. One equivalent of PPTS in MeOH allowed the deprotection of a 3° TMS ether in the presence of a 1° TIPS ether[352] and aqueous HOAc in THF was employed to selectively desilylate a 3° TMS ether in the presence of a TBDPS protected 1° alcohol.[353,354] The latter conditions also resulted in the hydrolysis of a THP-protected 1° alcohol and a 1° TES ether.

Aqueous HCl has also proven useful in the selective cleavage of 3° silyl ethers. A mixture of 1 N aqueous HCl in 5:1 THF–H_2O allowed the selective removal of a TMS group from a protected 3° alcohol in the presence of a 1°



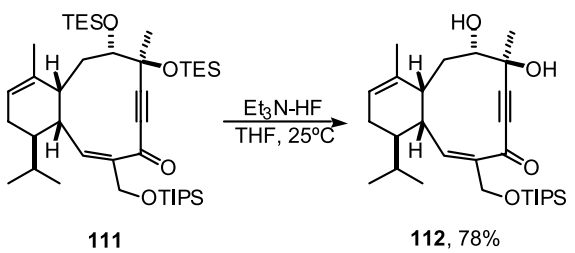
Scheme 53.



Scheme 54.

TIPS ether.[252] A 2° TES ether was also cleaved under these reaction conditions. A solution of 2% HCl in 3:1 CH_2Cl_2–MeOH was used to effect the selective desilylation of a 3° TMS ether in the presence of a 1° TBDPS and a 2° TBS ether (Scheme 54).[108] Similarly, a 3° TMS ether underwent deprotection in the presence of 1° and 2° TBS ethers when treated with aqueous HCl to a pH of 3.[355]

Et_3N–HF in THF was used to remove TES groups from protected 2° and 3° alcohols without affecting a 1° TIPS ether in the synthesis of sarcodictyins A and B (Scheme 55).[256]



Scheme 55.

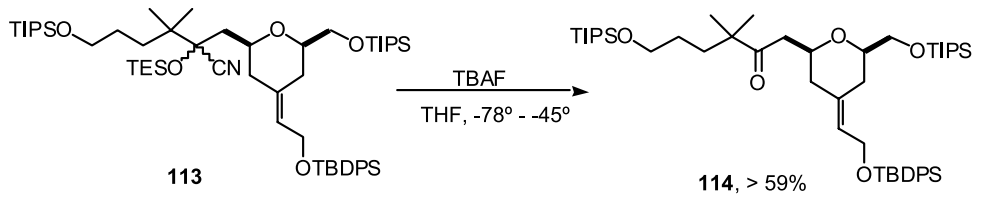
BF_3–OEt_2 in CH_2Cl_2 at ambient temperature has been shown to effect the selective deprotection of a 3° TMS ether in the presence of a 1° TBDPS ether.[245,246]

5.1.2. Under basic/nucleophilic conditions. One equivalent of TBAF in THF at low temperature was used to deprotect a 3° TES ether in the presence of 1° TIPS and TBDPS ethers with the resulting cyanohydrin reverting to a ketone (Scheme 56).[356]

K_2CO_3 in MeOH was used to selectively deprotect a 3° TMS ether in the presence of a 1° TBDPS ether and a 2° TBS ether.[108] The use of BH_3–SMe_2 in refluxing THF to effect the reduction of nitriles was also shown to deprotect 3° TMS ethers in the presence of 1° TBS ethers.[357]

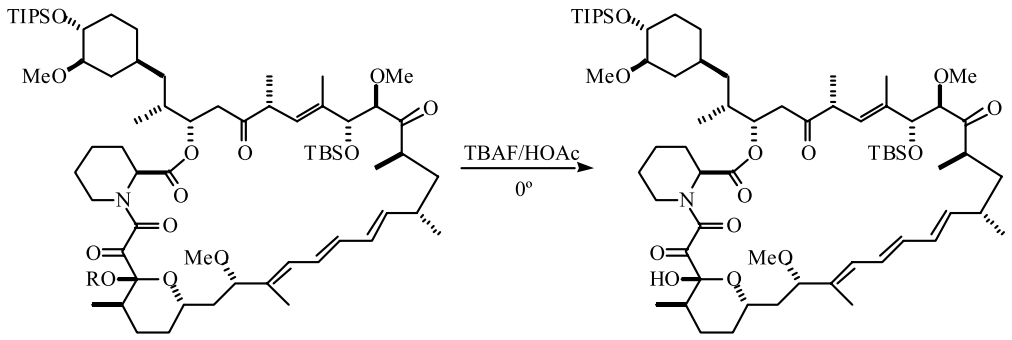
5.2. In the presence of 2° silyl ethers

5.2.1. Under acidic conditions. Examples of selective deprotection of 3° silyl ethers in the presence of 2° silyl ethers under acidic conditions often involve fluoride reagents. For example, in the total synthesis of (−)-rapamycin, TBAF buffered with HOAc was shown to deprotect 3° TMS and TES ethers in high yield while leaving 2° TBS



113 **114**, > 59%

Scheme 56.



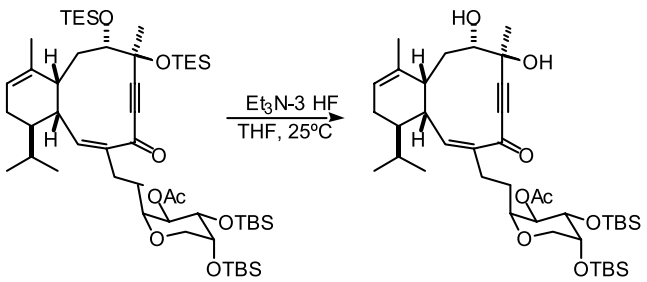
115 **a** R = TMS 30 min
b R = TES 3 h
c R = TBS 4 days

116 **a**, 90%
b, 90%
c, 20%

Scheme 57.

and TIPS ethers unaffected (Scheme 57).[358] When the 3° alcohol was protected with TBS, cleavage of the resulting 3° TBS ether was slow and low-yielding.

Et_3N–HF was used to desilylate 2° and 3° TES ethers in the presence of 2° TBS ethers as part of the total synthesis of eleutherobin (Scheme 58).[117,312]



117 **118**, 81%

Scheme 58.

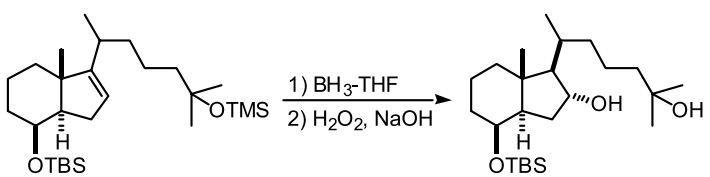
HF in CH_3CN was used to cleave a 3° TMS ether in the presence of a 2° TBS ether.[316] An allylic 2° TBS ether also underwent concomitant deprotection. H_2SiF_6 in H_2O/CH_3CN was chosen to effect the selective desilylation of a 3° TMS ether in the presence of a 2° TBDPS ether.[185,359] Under these conditions, a 1° TBS ether was also deprotected.

5.2.2. Under basic/nucleophilic conditions. As part of the total synthesis of (+)-milbemycin D, K_2CO_3 in MeOH was employed to deprotect a 3° TMS ether in the presence of a 2° TBDPS ether.[211] A 1° TBS ether was also cleaved under these reaction conditions.

A hydroboration–oxidation sequence intended to install an alcohol on what would become the D ring of a vitamin D analog also led to the deprotection of a 3° TMS ether in the presence of a 2° TBS ether (Scheme 59).[360]

6. Selective deprotection of aryl silyl and alkyl silyl ethers

As a general rule, when a substrate contains an alkyl silyl ether and an aryl silyl ether, acidic conditions will favor deprotection of the former and basic conditions will favor deprotection of the latter. When selectivity between two aryl



119 **120**, 68%

Scheme 59.

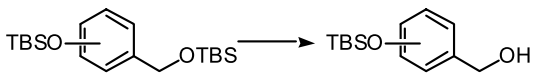
silyl ethers is desired, steric, electronic and neighboring group effects determine which silyl group is removed.

6.1. Selective deprotection of alkyl silyl ethers in the presence of an aryl silyl ether

6.1.1. Under acidic conditions. Many of the same acids used to selectively deprotect one alkyl silyl ether in the presence of another have been used to effect the deprotection of an alkyl silyl ether without cleaving an aryl silyl ether. The location of the silyl group on an alcoholic oxygen is a greater factor in its reactivity than the steric requirements of the group itself. Thus, acidic conditions can be used to deprotect TES and TBS protected 1° and 2° alcohols without affecting aryl silyl ethers. PPTS has been shown to cleave 1° TBS[361] or 2° TES ethers[362] in the presence of TBS-protected phenols. Similarly, CF_3CO_2H in CH_2Cl_2 was used to hydrolyze a 2° TBS ether in the presence of an aryl TBS ether during the total synthesis of (−)-doliculide.[363] And, catalytic quantities of a low-loading, alkylated polystyrene-supported sulfonic acid (LL–ALPS–SO_3H) have been shown to selectively desilylate a 1° TBS ether in the presence of a TBS-protected phenol.[40]

In situ generated HCl has also proven useful in cleaving 1° TBS ethers in the presence of aryl TBS ethers. Catalytic acetyl chloride in dry MeOH at 0 °C to room temperature allows rapid acid hydrolysis of a 1° TBS ether in the presence of a TBS-protected phenol.[34] TMSCl in wet CH_3CN also produces HCl in situ, leading to the selective deprotection of 1° TBS ethers in the presence of aryl TBS ethers.[364] A variation on this method includes NaI with TMSCl and H_2O to generate HI that effects similar results (Table 2).[364]

Table 2. Selective deprotection using in situ generated HCl

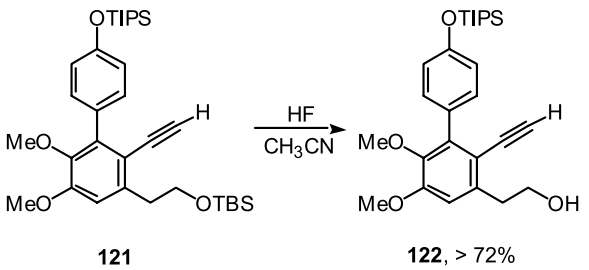


Isomer	Reagents	Yield (%)	Reference
para	AcCl, MeOH	80	34
meta	TMSCl, H_2O, CH_3CN	83	364
meta	TMSCl, H_2O, NaI, CH_3CN	86	364

A recent report describes the use of Nafion-H and NaI (or, bromodimethyl sulfonium bromide and NaI) to generate HI, allowing the selective cleavage of 1° and 2° TBS ethers in the presence of aryl TBS ethers.[365]

Oxone® in aqueous MeOH at room temperature has also been shown to allow the selective cleavage of 1° TBS ethers in the presence of aryl TBS ether.[144] Selectivity relies upon reaction kinetics, as longer reactions times result in high yields of deprotected phenol.

Fluoride reagents are typically not used in reactions in which retaining an aryl silyl ether is desired. However, HF in CH_3CN has been shown to selectively desilylate a 1° TBS ether in the presence of a TIPS-protected phenol (Scheme 60).[366]

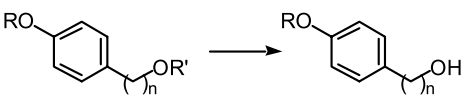


121 **122**, > 72%

Scheme 60.

Lewis acids have been introduced as reagents for the efficient deprotection of alkyl silyl ethers in the presence of aryl silyl ethers. Some of the Lewis acids shown to effect these conversions are $Sc(OTf)_3$,[367] $ZnBr_2$,[59] $BiCl_3$/NaI,[368] decaborane,[50] $InCl_3$,[57] $Ce(OTf)_4$,[55] $BiBr_3$,[369] TBS-OTf,[370] $ZrCl_4$,[58] and $Cu(OTf)_2$.[56] Examples of some of these selective desilylations are summarized in Table 3.

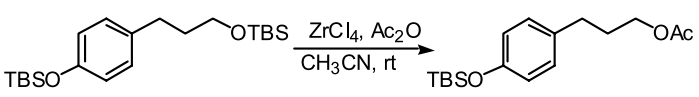
Table 3. Selective deprotection of alkyl vs aryl silyl ethers using Lewis acids



R	R′	*n*	Reagent	Yield (%)	Reference
TBS	TBS	3	$Sc(OTf)_3$, H_2O, CH_3CN	97	367
TBS	TES	3	$ZnBr_2$, H_2O, CH_2Cl_2	85	59
TBS	TBS	3	$ZnBr_2$, H_2O, CH_2Cl_2	87	59
TBS	TBS	1	$BiCl_3$, NaI, CH_3CN	81	368
TBS	TBS	1	$B_{10}H_{14}$, MeOH	94	50
TBS	TBS	1	$InCl_3$, H_2O, CH_3CN	82	57
TBDPS	TBS	3	$InCl_3$, H_2O, CH_3CN	90	57
TBDPS	TBS	3	$Ce(OTf)_4$, H_2O, THF	75	55
TBS	TBDPS	3	$Ce(OTf)_4$, H_2O, THF	70	55
TBS	TBS	2	$CeCl_3 \cdot 7\ H_2O$, CH_3CN	96	371

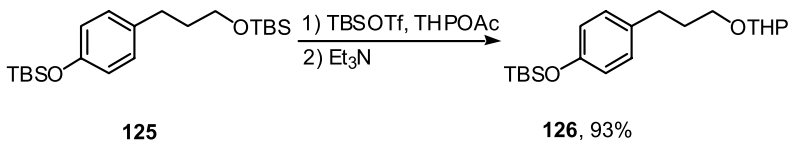
Also, $BiBr_3$ and H_2O in CH_3CN have been shown to effect the deprotection of 1° or 2° TBS ethers in the presence of aryl TBS ethers.[369]

When used in conjunction with acetic anhydride, $ZrCl_4$ has served as a catalyst for the conversion of 1° TBS ethers into the corresponding acetate without affecting aryl TBS or TIPS ethers (Scheme 61).[58] $Cu(OTf)_2$ allows the same selective conversion to occur.[56] And, TBSOTf and THP acetate effect the conversion of 1° TBS ethers into the corresponding THP ethers without affecting aryl TBS ethers (Scheme 62).[370]

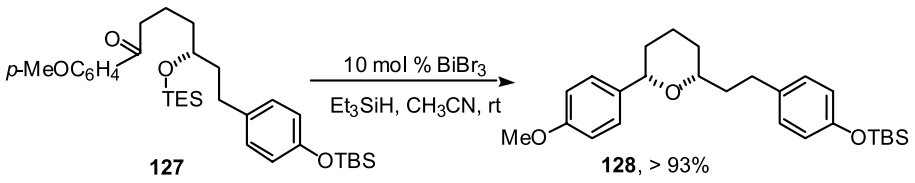


123 **124**, 88%

Scheme 61.



Scheme 62.

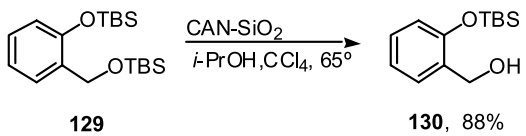


Scheme 63.

In a slightly different context, $BiBr_3/Et_3SiH$-mediated reductive etherification process forms cyclic ethers from δ-trialkylsiloxy-ketones.[372] Although cyclic ether formation is the desired outcome, the stability of an aryl TBS ether to the reaction conditions allows for selective reaction of alkyl silyl ether as illustrated in the synthesis of (−)-centrolobine (Scheme 63).[372] Experimental evidence suggests that the 2° TES, TBS and TIPS ethers react similarly and the reaction may be Bronsted acid-promoted, rather than Lewis acid-catalyzed.[372]

6.1.2. Under miscellaneous conditions. Iodine in methanol has been shown to effect the selective deprotection of 1° and 2° TBS ethers in the presence of aryl TBS ethers.[373] Similarly, CBr_4 in refluxing MeOH effects the selective desilylation of 1° TBS, TIPS and TBDPS ethers without cleaving TBS-, TIPS-, or TBDPS-protected phenols.[221] Although the exact mechanism is not clear, the possibility of In situ formation of catalytic amounts of acid under these conditions cannot be discounted.

Ceric ammonium nitrate on silica gel in a mixture of *i*-PrOH and CCl_4 effects the selective removal of a TBS group from protected 1° alcohol in the presence of an aryl TBS ether (Scheme 64).[218] This same transformation in comparable yield occurs using slightly more CAN in *i*-PrOH at room temperature.

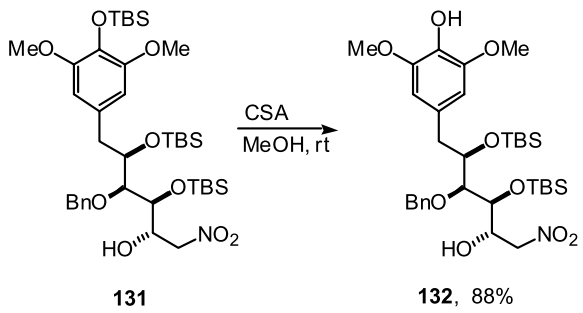


Scheme 64.

Catalytic hydrogenation has provided some interesting results of selective deprotection of alkyl silyl ethers in the presence of aryl silyl ethers. Alkyl TES ethers have been shown to be labile when subjected to catalytic Pd/C in MeOH while aryl TES ethers are stable.[262] However, hydrogenolysis of alkyl TBS ethers has been demonstrated using Pd/C as catalyst in a reaction in which aryl TBS ethers are inert.[374] An important supplier-dependent difference in the reactivity of Pd/C has been described which explains TES cleavage in the absence of H_2 as the result of residual acid in the preparation of the catalyst.[264]

6.2. Selective deprotection of aryl silyl ethers in the presence of alkyl silyl ethers

6.2.1. Under acidic conditions. Although uncommon, acid-mediated deprotections of aryl silyl ethers have been reported. Catalytic CSA in MeOH was used to effect the deprotection of an aryl TBS ether in the presence of 2° TBS ethers (Scheme 65).[375]



Scheme 65.

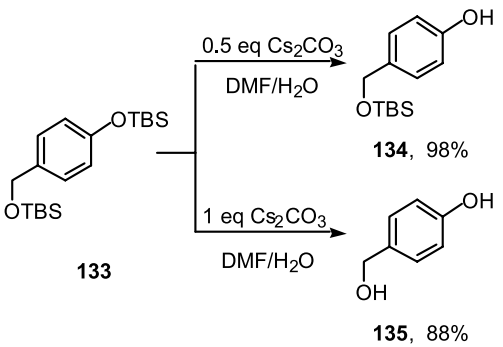
More typical, however, is the use of acidic resins and Lewis acids to promote selectivity of this nature. Montmorillonite K-10 in MeOH has been used to deprotect aryl TMS ethers at ambient temperature; 3° TMS ethers are unreactive under the same conditions.[376] Stirring with silica gel was used to deprotect an aryl TMS ether in the presence of 2° TBS ethers in the synthesis of cycloproparadicicol.[377]

The lability of the TMS group was exploited to allow the deprotection of a TMS-protected phenol in the presence of a 1° TBS ether upon treatment with $BiCl_3$, $Bi(O_2CCF_3)_3$ or $Bi(OTf)_3$ in MeOH.[62] Reaction times were crucial to the selectivity; selective cleavage of the aryl silyl ether was achieved within 2 min of exposure to the bismuth salt. But when reaction times were extended to 15 min and the reagent was $Bi(OTf)_3$, deprotection of both the alkyl and aryl silyl ethers was observed.[62]

$Zn(BF_4)_2$ in water has been shown to effect the deprotection of aryl TBS ethers without affecting 1° TBDPS ethers.[60]

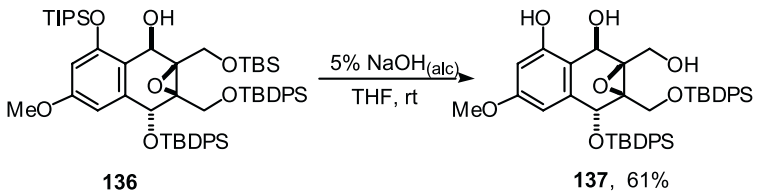
6.2.2. Under basic/nucleophilic conditions. Carbonate salts have been shown to effect the desilylation of silyl-protected phenols without affecting alkyl silyl ethers.

Excess K_2CO_3 and H_2O in refluxing EtOH was employed to effect the selective deprotection of an aryl TBS ether in the presence of a 2° TBS ether.[378] By contrast, Cs_2CO_3 in DMF–H_2O allows for the deprotection of TBS-protected phenols in the presence of 1° TBS ethers when 0.5 equivalents of base was used at room temperature (Scheme 66).[379] When a stoichiometric amount of Cs_2CO_3 was employed at 100 °C, both TBS groups were removed.



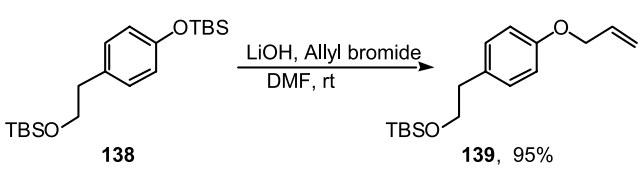
Scheme 66.

Hydroxide bases have been more widely used in desilylation reactions of aryl silyl ethers. At room temperature, excess LiOH in DMF[371] or KOH in EtOH[380] allowed the selective deprotection of TBS-protected phenols in the presence of alkyl TBS ethers. KOH in MeOH was used to remove the TBS group from the protected phenol in a steroid without affecting a 2° TBS ether.[381] But the selectivity of hydroxide bases is not universal for aryl silyl ethers; a 5% ethanolic NaOH solution in THF was used to effect the deprotection of a TIPS-protected phenol *and* a 1° TBS ether in the presence of 1° and 2° TBDPS ethers (Scheme 67).[209]



Scheme 67.

Aryl TBS ethers have been converted into the corresponding alkyl aryl ether by treatment with excess LiOH and alkyl bromide or iodide in DMF; no reaction at 1° or 2° TBS ethers was reported (Scheme 68).[382] When the alkyl halide was replaced with 2-fluoronitrobenzene, diaryl ethers could be formed without reaction of the alkyl silyl ether.[382]



Scheme 68.

An excess of solid NaOH and *n*-Bu_4NHSO_4, a phase transfer catalyst, in 1,4-dioxane has been used to effect the selective desilylation of aryl TBS ethers in the presence of 1° TES, TBS and TBDPS ethers as well as 2° TBS ethers.[383]

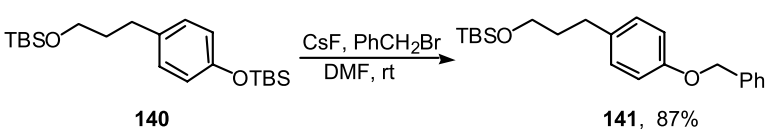
Triethylamine oxide in MeOH was used to effect the selective removal of TBS groups from protected phenols in the presence of 1° and 2° TBS ethers.[384] The mechanism is believed to involve nucleophilic attack of the amine oxide on the silicon of the protecting group. Similarly, 1,1,3,3-tetramethylguanidine (TMG) in CH_3CN has been shown to effect the deprotection of TBS and TBDPS protected phenols in the presence of 1° TBS and 1° and 2° TBDPS ethers.[385]

Fluoride sources, though, are more commonly used to effect the selective deprotection of aryl silyl ethers in the presence of alkyl silyl ethers. KF–Al_2O_3 has been shown to be a mild fluoride source that mediates the deprotection of TMS, TBS and TBDPS protected phenols, leaving similarly protected alcohols intact.[386] KF–Al_2O_3 in CH_3CN at 0 °C was used to deprotect an aryl TBS ether in the presence of a 2° TBS ether in the total synthesis of vancomycin.[387] However, three other phenolic TBS ethers survived these conditions due to steric and/or electronic factors.

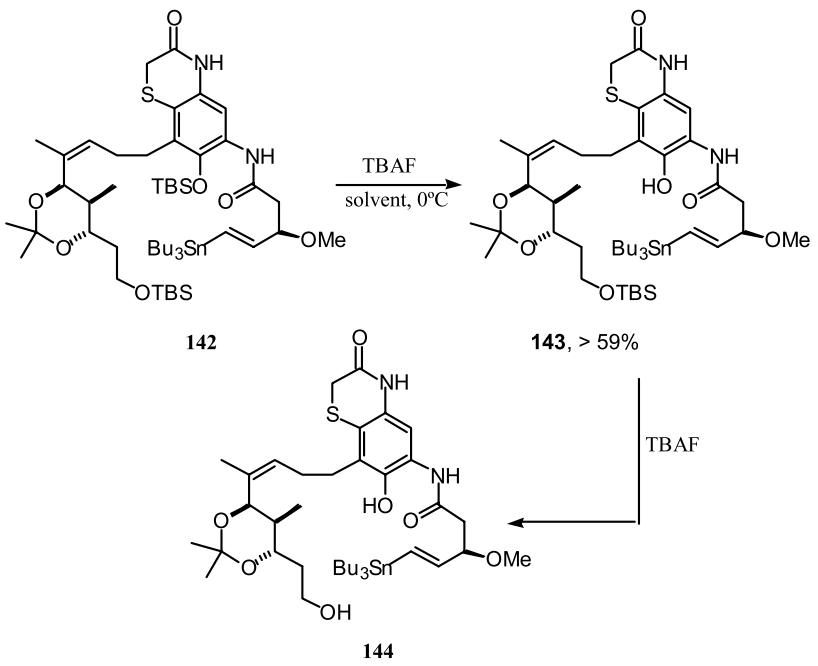
The effect of solvent on fluoride-induced desilylation reactions was highlighted in the solid-phase semisynthesis of vancomycin.[388] When CsF in DMF was applied to a substrate bearing TBS ethers of phenols and 2° alcohols, global desilylation was achieved. However, when CH_3CN was the solvent, deprotection of the aryl TBS ethers was achieved selectively and the 2° TBS ethers were unaffected.

By contrast, CsF and alkyl halides have been used to effect the selective conversion of a phenolic TBS ether to the corresponding alkyl aryl ether in the presence of a 1° TBS ether (Scheme 69).[389]

TBAF, however, is the most widely used reagent for the selective deprotection of phenolic silyl ethers in the presence of alkyl silyl ethers. TBAF in THF,[390] THF–H_2O[391] and toluene[392] have been shown to be effective in



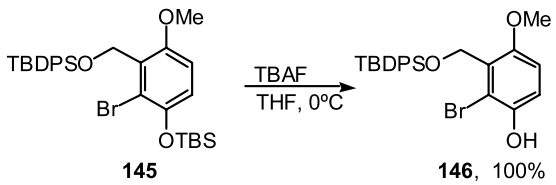
Scheme 69.



Scheme 70.

deprotecting TBS protected phenols in the presence of 1° TBS ethers. The importance of reaction conditions in determining selectivity was illustrated in the total synthesis of (+)-thiazinotrienomycin E when TBAF at 0 °C allowed the selective desilylation of a TBS-protected phenol in the presence of a 1° TBS ether.[69] But when the resultant monosilyl ether was treated with TBAF at room temperature, deprotection of the 1° TBS ether occurred (Scheme 70).[69]

Phenolic TBS ethers have also been cleaved in the presence of 2° TBS[393,394] or 1° TBDPS ethers using TBAF in THF (Scheme 71).[395,396]



Scheme 71.

Likewise, a phenolic TBS ether was cleaved in the presence of a 1° DEIPS and a 2° TBS ether using TBAF in THF at 0 °C.[308] Similar conditions were employed in the selective deprotection of a phenolic TIPS ether in the presence of a 2° TBDPS ether.[397]

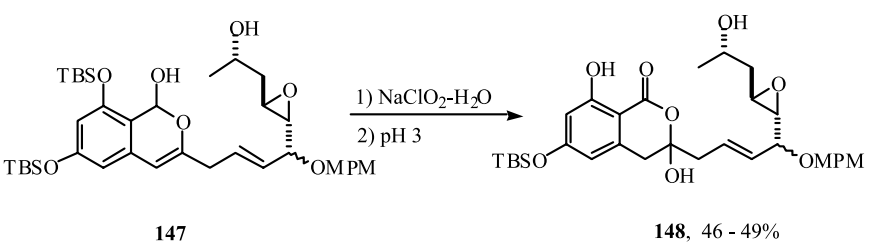
6.2.3. Under miscellaneous conditions. A 1:5 mixture of H_2O and DMSO at 90 °C was used to selectively deprotect an aryl TBS ether in the presence of a 1° TBS ether.[398] However, under similar conditions, 1° benzylic TBS ethers are actually more labile than the aryl silyl ether.[398]

Phenyliodine bis(trifluoroacetate), or PIFA, has been shown to effect the deprotection and oxidation of TMS, TBS and TIPS protected phenols in the presence of a 1° TBS ether.[392]

6.3. Selective deprotection of one aryl silyl ether in the presence of another

Deprotection of one phenolic silyl ether in the presence of another is typically a function of electronic differences between the protected hydroxyl groups due to ring substitution. On occasion, however, the differing rates of reaction among silyl groups of different size are exploited.

6.3.1. Under acidic conditions. Oxidation of hemiacetal **147** followed by work-up at pH 3 led to the selective deprotection of one aryl TBS ether in the presence of another (Scheme 72).[399] The proposed mechanism involves intramolecular migration of the TBS group closest to a newly formed carboxylate followed by hydrolysis of the resulting silyl ester.[399]

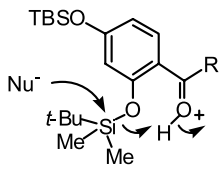


Scheme 72.

Differences in the rates of hydrolysis of TBS and TBDPS groups under acidic conditions were used to effect the selective desilylation of phenolic TBS ethers in the presence of phenolic TBDPS ethers in a copolymer of TBS-protected hydroxystyrene and TBDPS-protected hydroxystyrene.[400] Treatment of the copolymer with concentrated HCl in THF resulted in removal of the TBS group only.[400]

In situ generation of HCl by sonication of a 1:1 mixture of CCl_4 and MeOH allows the deprotection of aryl TBS ethers *that are ortho to a carbonyl* in the presence of other silyl ethers.[401] The proposed mechanism involves protonation of the neighboring carbonyl, forming a cyclic transition state which favors deprotection.[401]

$SbCl_5$ in CH_3CN has been shown to have no effect on TBS-protected nitrophenols while other TBS-protected phenols undergo rapid deprotection.[402]



149

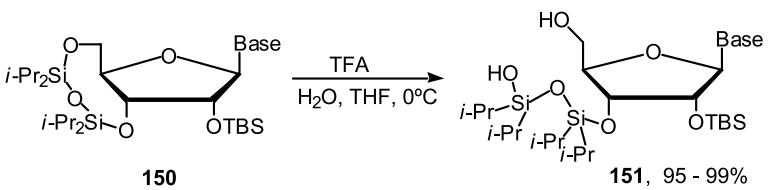
6.3.2. Under basic/nucleophilic conditions. Zirconium potassium phosphonate, $Zr(KPO_4)_2$, in acetone/water effects the desilylation of aryl silyl ethers at rates which vary according to the steric bulk around the silicon atom.[403] TES-protected phenols undergo deprotection at much faster rates than TBS- or TBDPS-protected phenols, implying that selective desilylation is possible.[403]

7. Selective deprotection reactions involving silylene- and disiloxane-protected diols

1,3-Diols can be protected by treatment with dialkyldichlorosilane or dialkylsilylditriflates to form silylenes. One silyl group protects two hydroxyl groups in much the same way that a ketal protects a diol. But the presence of a second oxygen atom bound to the silicon alters the chemistry of these protecting groups.

7.1. Deprotection of silylene- and disiloxane-protected diols in the presence of alkyl silyl ethers

A handful of examples of acid-mediated selective deprotection of silylenes in the presence of alkyl silyl ethers have been reported. Aqueous TFA in THF has been used to effect the cleavage of the 5′-end of a TIPDS protected ribonucleoside without affecting a 2° TBS ether (Scheme 73).[134,404,405]



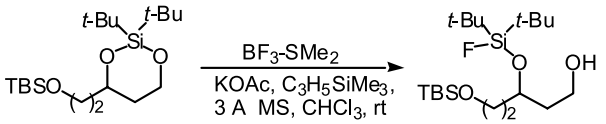
150 151, 95 - 99%

Scheme 73.

Similarly, a dimethylsilylene-protected 1,5-diol underwent selective deprotection in the presence of a 1° TPS ether using TFA–H_2O–THF.[406]

Lewis acids have been reported to effect the selective deprotection of silylenes in the presence of alkyl silyl ethers. A TIPDS-protected diol was shown to undergo selective desilylation when treated with Montmorillonite K-10 and MeOH and H_2O at 75°C but a 1° TBDPS ether was not cleaved.[41] However, 1° and 2° TBS ethers were susceptible to hydrolysis under these conditions.

More recently, the regioselective opening of a di-*t*-butylsilylene in the presence of 1° TIPS or TBS ethers using BF_3 has been reported (Scheme 74).[407]

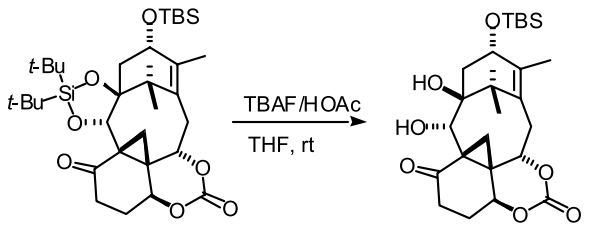


152 153, 95 - 99%

Scheme 74.

Fluoride sources are the most commonly used reagents for the desilylation of silylene-protected diols in the presence of alkyl silyl ethers. HF–pyridine in THF/pyridine has been used to selectively remove a DTBS group from a protected diol in the presence of 1° TIPS and 2° TBS ethers,[408] 1° and 2° TBS ethers,[409] a 1° TIPS ether[410] and 2° TBS,[157] TBDPS,[411] and DEIPS ethers.[412] Similarly, HF–pyridine was used to selectively deprotect a DTBS-protected diol in the presence of a 2° TBS ether.[413]

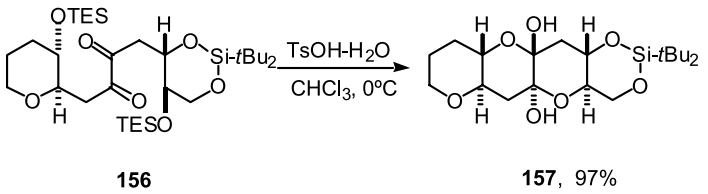
TBAF buffered with HOAc has also been used deprotect a DTBS-protected diol in the presence of a 2° TBS ether (Scheme 75).[302,414]



154 155, > 95%

Scheme 75.

One example of TBAF in THF mediating the selective desilylation of a TIPDS-protected diol in the presence of a 2° TBDPS ether has been reported.[415]



156 **157**, 97%

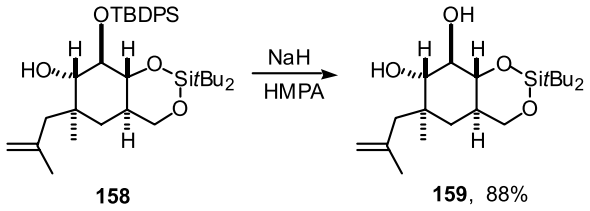
Scheme 76.

7.2. Deprotection of alkyl silyl ethers in the presence of silylene- and disiloxane-protected diols

Examples of selective deprotection reactions of alkyl silyl ethers in the presence of silylene protected diols are few in number but diverse in nature. TsOH–H_2O in CH_2Cl_2 at 0 °C effects the deprotection of a 2° TES ether in the presence of a DTBS protected diol (Scheme 76).[416]

TsOH–H_2O buffered with Et_3N in THF has been shown to deprotect a 2° TMS ether in the presence of a TIPDS-protected diol.[417] The same transformation occurred upon treatment with excess NH_3 in MeOH.[417]

A 2° TBDPS ether was selectively desilylated in the presence of a DTBS-protected diol upon treatment with NaH in HMPA (Scheme 77).[418]



158 **159**, 88%

Scheme 77.

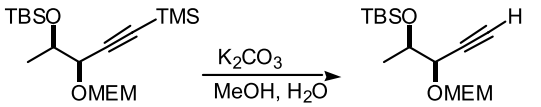
DDQ in CH_2Cl_2 mediates the deprotection of a 1° TBS ether in the presence of a DTBS-protected diol.[79] In this reaction, only allylic or benzylic silyl groups are removed as evidenced by the survival of a 1° TIPS ether in the same substrate.[79]

8. Protiodesilylation reactions of alkynyl silanes

Although somewhat different than the selective deprotection of bis-silyl ethers, the growing importance of selectivity between silyl protected terminal alkynes and silyl ethers warrants brief discussion. In general, protiodesilylation reactions of silyl-protected terminal alkynes are effected by treatment with mild base and, if the conditions are sufficiently mild, silyl-protected alcohols can be spared. When protected with a group larger than TMS, the alkynyl silane becomes more resistant to protiodesilylation.

8.1. Protiodesilylation of alkynyl silanes in the presence of silyl ethers

The most common method for deprotection of a TMS-protected terminal alkyne in the presence of a silyl ether involves treatment with K_2CO_3 in MeOH and H_2O.[419–422] For example, as part of the total synthesis of macrosphelide A, a TMS-protected alkyne underwent protiodesilylation in the presence of a 2° TBS ether upon treatment with K_2CO_3 in MeOH/H_2O (Scheme 78).[420]



160 **161**, > 87%

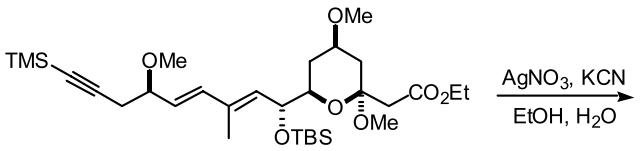
Scheme 78.

More vigorous conditions (aqueous KOH in MeOH/THF at reflux) were used to remove a TMS-group from an alkynyl silane *and a TBDPS group from a protected 1° alcohol* in the presence a 1° TIPS ether.[16]

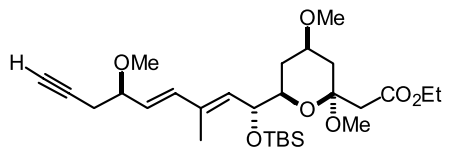
Table 4. Deprotection of 1° silyl ethers in the presence of another 1° silyl ether

Deprotection of:	In the presence of:				
	1° TMS	1° TES	1° TBS	1° TIPS	1° TBDPS
1° TMS		$NaHCO_3$[67]	$BiCl_3$[62] $Bi(O_2CCF_3)_3$[62] K_2CO_3[66] $NaHCO_3$[67] MCM-41[64]	MCM-41[64]	
1° TES			CSA[155] IBX/DMSO[42] MCM-41[64] H_2, Pd/C[83]	TFA[39] H_2, Pd/C[82] MCM-41[64]	CSA[31,228] H_2, Pd/C[82] TMS-OTf/HCO_2DPM/silica gel[52] $ZnBr_2$, H_2O[59] H_2, Pd/C[83]
1° TBS			HOAc[44] PPTS[43] TBAF[72] DDQ[80] MnO_2/$AlCl_3$[63] DMSO/H_2O[398] H_2, Pd/C[83]	H_2SO_4[38] CSA[36,37] PPTS[35] H_2SiF_6[47] TMS-OTf/Et_3N/MeOH[51] decaborane[50] $CeCl_3$·7 H_2O/NaI[53] H_2, Pd/C[82,83]	HCl[25,210] H_2SO_4[26] HOAc[23,24,92] TFA[33,108] PPTS[27,78,124,427,428] TsOH[32,130] CSA[28–30,429] LL-ALPS-SO_3H[40] Ac-Cl/MeOH[34] decaborane[50] $Cu(OTf)_2$/Ac_2O[56] $CeCl_3$·7 H_2O/NaI[53] $Ce(OTf)_4$, THF/H_2O[55] $PdCl_2(CH_3CN)_2$[430] $CeCl_3$·7 H_2O[61] $InCl_3$[57] $Zn(BF_4)_2$[60] $ZnBr_2$, H_2O[59] $ZrCl_4$/Ac_2O[58] TBAF[71] HF–pyr[48,431] H_2, Pd/C[82,83] I_2/KOH[78] I_2/MeOH[73] Br_2/MeOH[75] IBr[76] CCl_4/MeOH)))[432] Bu_4NBr_3/MeOH[77] LiCl/DMF[86] TMS-OTf/HCO_2DPM/silica gel[52]
1° TIPS					TMS-OTf/HCO_2DPM/silica gel[52]
1° TBDPS			TBAF/HOAc[49,69] NaOH[68,69,433] Bu_4NOH[49]	KOH[16]	HF–pyr[152] TBAF[71]

$AgNO_3$/KCN has been used to effect protiodesilylation of alkynyl silanes in the presence of 2° TBS ethers.[423] In the total synthesis of (+)-phorboxazole A, alkynylsilanne **162** was treated sequentially with $AgNO_3$ and KCN in an ethanol–water mixture to deprotect the terminal alkyne without affecting a 2° TBS ether (Scheme 79).[94]



162

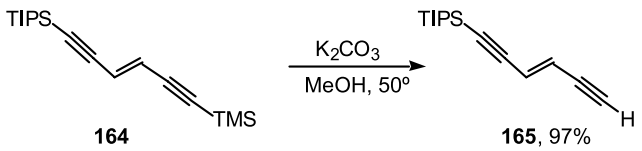


163, 75%

Scheme 79.

8.2. Selective protiodesilylation

When a compound contains two terminal alkynes, the attachment of different silyl groups allows for selective protiodesilylation. Not surprisingly, given the lability of alkynyl trimethylsilanes, the TMS group is the silyl group that is removed while the more robust TIPS group survives intact. Thus, treatment of bis-silaendiyne **164** with K_2CO_3 in MeOH at 50° affords the monodeprotected endiyne in good yield (Scheme 80).[424]



164 **165**, 97%

Scheme 80.

Similar results were achieved when NaOH in $EtOH/H_2O$ was used in the selective protiodesilylation of a TMS-protected alkyne in the presence of a TIPS-protected alkyne.[425]

Selective protiodesilylation of an alkynyl trimethylsilane in the presence of another was effected by treatment of $CaCO_3$ in MeOH.[426] The reaction required careful monitoring to avoid double deprotection and considerable amounts of unreacted starting material were recovered and reused.

9. Summary

The challenge of increasingly complex synthetic targets points to the continued use of protection/deprotection protocols in synthetic organic chemistry. Although the more traditional methods of deprotection of silyl ethers such as acid- and fluoride-mediated techniques are still widely used and often allow excellent chemoselectivity, the

Table 5. Deprotection of 1° silyl ethers in the presence of a 2° silyl ether

Deprotection of:	In the presence of:				
	2° TMS	2° TES	2° TBS	2° TIPS	2° TBDPS
1° TMS	HOAc[434] Swern Conditions[212] Swern Conditions[212]	$NaHCO_3$[67] Swern Conditions[212] CSA[47] PPTS[117] HBr/PPh_3[114] HOAc[116,435] HF–pyr[67] TBAF/HOAc[183,349] TBAF[193–198] KF[17] LiOH[67] Swern Conditions[106,212,213] CrO_3–2 pyr[215] HF–pyr[155]	HCl[115] HBr/PPh_3[114] CSA[47,112,155] PPTS[95] TFA[10,113] HF/pyr[146,147] HF[169] $TMSOTf/i\text{-}Pr_2NEt$[187] TBAF[47] TBAF/HOAc[183] KF[17] Swern Conditions[214]	TMS-OTf/$i\text{-}Pr_2NEt$[187] HF–pyr[67,254] LiOH[67]	Citric acid[93] TsOH[106] TMS-OTf/$i\text{-}Pr_2NEt$[187] TMS-OTf/Et_3N/MeOH[51]
1° TES					
1° TBS		HCl[137] HOAc[118–120] CSA[9,15,36,37,94,121–124,129,145,329,337,437–441] PPTS[14,43,123–129] TsOH[22,130,141] TsOH/Bu_4NHSO_4[142] TFA[108,131–133,135,136] Acidic $CHCl_3$[143] $Cu(OTf)_2$/Ac-O[56] BCl_3[186] HF–pyr[7,8,11,18,94,138,145,148–150,153,154,156,158–161,164–168,318,442–450] $HF^{170,451}$ H_2SiF_6[184] NH_4F[206] TBAF/HOAc[181,182] TBAF[199–203] TAS-F[452] CBr_4, hν[220,453] Jones reagent[138] LiBr/18-C-6[222] CAN/i-PrOH[13] $POCl_3$/DMF[216,217] $(CF_3SO_2)_2O$-DMF[217] H_2, Pd/C[83] QFC[84] Bu_4NBr_3/MeOH[77] $MnO_2/AlCl_3$[63] Oxone[144] CAN/SiO_2[218] TBAF/HOAc[179]	HCl[103, 454] H_2SO_4[109] HOAc[87–91] CSA[5,15,36,37,94,103,145,455] PPTS[100] TsOH[107] NH_4Cl/MeOH[110] HF–pyr[94,145,153,165,166] H_2SiF_6[184] TBAF[191] polymeric DCKA[81] TBAF·$3H_2O$[77]	HOAc[92] CSA[30,101,102,429] PPTS[96–99] TsOH[99,104,105] HF–pyr[6] HF[151,171,172,205] H_2SiF_6[185] TMS-OTf/Et_3N/MeOH[51] $Cu(OTf)_2$/Ac-$_2O$[56] $Zn(OTf)_2$[60] K_2CO_3[217] NaOH[209] TBTU[225] QFC[84] polymeric DCKA[81] $InCl_3$[57]
1° TIPS				CSA[139] TBAF[254] SiF_4[224] $POCl_3$-DMF[217] $(CF_3SO_2)_2O$-DMF[217] CBr_4/MeOH[221] CAN/SiO_2[218] HF–pyr[163] KOH/18-C-6[208]	CSA[205]
1° TBDPS		TBAF/HOAc[174,178] NaOH/DMPU[207]	NH_4F[189,456] NaOH/DMPU[207] KOH/18-C-6[208] TBAF/HOAc[7,12,49,142,150,174–178] TBAF[148,158,457] TAS-F[190] NaH, propargyl alcohol[210]		CSA[140] HF–pyr[152,162] NH_4F[189] TBAF[204,205] $POCl_3$-DMF[217] $(CF_3SO_2)_2O$-DMF[217]

development of new methods fill specialized roles and may allow for selective desilylation without unwanted side reactions.

The effect of changing the electronic environment on the silyl group can be profound in affecting the selectivity in deprotection reactions. But, with the exception of alkoxy-substituted silyl protecting groups, this remains an open area of research. Similarly, neighboring groups can mediate the delivery of reagents to specific silyl ethers and allow for selective deprotection. But, this has been largely unexploited with the exception of neighboring carbonyl groups directing the selective deprotection of aryl silyl ethers in the presence of other aryl silyl ethers.

Table 6. Deprotection of 1° silyl ethers in the presence of a 3° silyl ether

Deprotection of:	In the presence of:				
	3° TMS	3° TES	3° TBS	3° TIPS	3° TBDPS
1° TMS					
1° TES		CSA[227,228] PPTS[117] HF–pyr[254,301]	Amberlyst-15[230] TBAF/HOAc[349]		HOAc[353]
1° TBS	HF[231,232]	CSA[228,229] HF–pyr[94,149,164,233]	CSA[30,226,429] HF–pyr[449] TBAF/HOAc[234] TBAF[448] Oxone[144]		
1° TIPS			TBAF[235] SiF_4[224]		
1° TBDPS		TBAF/HOAc[178]	TAS-F[236]		

Table 7. Deprotection of 2° silyl ethers in the presence a 1° silyl ether

Deprotection of:	In the presence of:				
	1° TMS	1° TES	1° TBS	1° TIPS	1° TBDPS
2° TMS			TsOH[241] citric acid[184] H_2SiF_6[184] NaOH[454]	TBAF[139]	HOAc[458] CSA[249] TsOH[239,240,242] BF_3-OEt_2[458] TMS-OTf[160]
2° TES			HF–pyr[66] I_2/Ag_2CO_3[265,266] Pd/C, MeOH[262] TBAF/NH_4Cl[258] H_2, Pd/C[83]	HCl[252] HOAc[205] CSA[38,155] PPTS[248] H_2SO_4[38] Ph_3P-HBr[38] HF–pyr[47,67,254] HF-Et_3N[256]	HOAc[210] CSA[459] PPTS[460] TsOH[37,243,244,460] HCl[18,160,250,251] H_2SO_4[253] BF_3-OEt_2[37,460] Et_3N-HF[257] HF–pyr[255] TBAF[260,460] 2,4,4,6-tetrabromo*-*2,5-cyclohexadienone/PPh_3[85] H_2, Pd/C[263] $ZnBr_2$, H_2O[59]
2° TBS			$MnO_2/AlCl_3$[63]	CSA[248]	H_2SiF_6[208] PPTS[152,247] TMS-OTf/Et_3N[51] TMS-OTf[152,259] $Cu(OTf)_2/Ac_2O$[56] $InCl_3$[57] $LiAlH_4$[261,461] IBr[267] $P_2O_5/(MeO)_2CH_2$[130] LiCl/DMF[86] polymeric DCKA[81] $ZnBr_2$, H_2O[59] $Zn(BF_4)_2$[60]
2° TIPS					
2° TBDPS					

Table 8. Deprotection of 2° silyl ethers in the presence another 2° silyl ether

Deprotection of:	In the presence of:				
	2° TMS	2° TES	2° TBS	2° TIPS	2° TBDPS
2° TMS	SiO_2-Cl/NaI[345]	HF–pyr[164,301] TBAF[198] KF[207]	HOAc[281,282] PPTS[274] TsOH[242] CSA[273] HF–pyr[164,301] HF/Et_3N[311] BF_3-OEt_2[462,463] K_2CO_3[323–325] TBAF[333] KF[17,207]	TBAF[139] KF[17] NaOH[454] H_2SiF_6[184]	K_2CO_3[324,325] $NaIO_4$[344]
2° TES		TsOH[464] TFA[279,299] HF–pyr[164,301] TBAF/HOAc[183] $(NH_4)HF_2$[322] TBAF[114,329] KF[17] NaOH/DMPU[207]	HCl[290,291] HOAc[208,283,284] CSA[155,248] TsOH[147] PPTS[247,268–273,276,277] TFA[278,279,299] HF–pyr[47,115,116,164,255,301,303,304] HF-Et_3N[117,312] HF[314] $Zn(OTf)_2$/EtSH[465] $TiCl_3$(O-*i*Pr)[466] TBAF/HOAc[183,321] TBAF[114,210,328–330] KF[17] NaOH/DMPU[207] MCM-41[64] $PdCl_2/CuCl/H_2O$[467]	PPTS[94,275] H_2SO_4[38] TFA[280] HF–pyr[67,305,306] NH_4F[436] $Zn(OTf)_2$/EtSH[465] Amberlyst-15[468]	HCl[289] HOAc[205,285] HF[315] TBAF[331,332] K_2CO_3[469] $NaIO_4$[344]
2° TBS		TBAF[304]	H_2SO_4[470] CSA[183,300] HF–pyr[310,471] HF[316] BF_3-OEt_2[472] TBAF[9,121,122,167,168,276,277,336–342,473] $P_2O_5/(MeO)_2CH_2$[130] $MnO_2/AlCl_3$[63]	HCl[294,295] CSA[94,293] HF[317,318] Et_3N-3HF[313] H_2SiF_6/Et_3N[320] TBAF[35]	HCl[296,297] HOAc[286–288] PPTS[192,292] HF–pyr[6,307] TMS-OTf[152,259] BF_3-OEt_2[474] $Sc(OTf)_3$[367] $NaIO_4$[344]
2° TIPS			TsOH[145] HF/Et_3N[306] TBAF[334,335] $LiAlH_4$[326]	HF[317,318]	$NaIO_4$[344]
2° TBDPS					

Table 9. Deprotection of 2° silyl ethers in the presence of a 3° silyl ether

Deprotection of:	In the presence of:				
	3° TMS	3° TES	3° TBS	3° TIPS	3° TBDPS
2° TMS		CSA[346] HF–pyr[164,301] KF[17,207]			
2° TES		HCl[290,291] PPTS[347] HF–pyr[47,164,301] TBAF[114,351]	HCl[349] TfOH[348]		
2° TBS			TfOH[348] HF[350]		
2° TIPS					
2° TBDPS					

Table 10. Deprotection of 3° silyl ethers in the presence of a 1° silyl ether

Deprotection of:	In the presence of:				
	1° TMS	1° TES	1° TBS	1° TIPS	1° TBDPS
3° TMS			HCl[355] BH_3-SMe_2[357]	HCl[252] PPTS[352]	HCl[108] HOAc[353] BF_3-OEt_2[245,246] K_2CO_3[108]
3° TES			TBAF/NH_4Cl[258]	HF-Et_3N[256] TBAF[356]	TBAF[356]
3° TBS					
3° TIPS					
3° TBDPS					

Table 11. Deprotection of 3° silyl ethers in the presence of a 2° silyl ether

Deprotection of:	In the presence of:				
	2° TMS	2° TES	2° TBS	2° TIPS	2° TBDPS
3° TMS			HCl[108,355] HF[316] TBAF/HOAc[358] BH_3-THF[360] K_2CO_3[108]	TBAF/HOAc[358]	H_2SiF_6[185,359] K_2CO_3[211]
3° TES			HF-Et_3N[117,312] TBAF/HOAc[358]	TBAF/HOAc[358]	
3° TBS			TBAF/HOAc[358]	TBAF/HOAc[358]	
3° TIPS					
3° TBDPS					

Table 12. Deprotection of aryl silyl ethers in the presence of an alkyl silyl ether

Deprotection of:	In the presence of:				
	RO-TMS	1° TBS	2° TBS	1° TBDPS	2° TBDPS
ArOTMS		$BiCl_3$[62] $Bi(O_2CCF_3)_3$[62] $Bi(OTf)_3$[62] PIFA-MK10[392]	SiO_2[377]		
ArOTES					
ArOTBS		TBAF[69,308,390–392] KF-Al_2O_3[386] CsF, RX, DMF[389] K_2CO_3[378] $CsCO_3$, rt[379] Et_3NO[384] LiOH[371] NaOH/TBAH[383] KOH[380] LiOH/RX/DMF[382] TMG[385] PIFA-MK10[392] DMSO/H_2O[398]	CSA[375] TBAF[308,393,394] K_2CO_3[378] CsF/CH_3CN[388] Et_3NO[384] NaOH/TBAH[383] KOH[381] LiOH/RX/DMF[382] KF-Al_2O_3[387]	$Zn(BF_4)_2$[60] TBAF[395,396] NaOH/TBAH[383]	
ArOTIPS		PIFA-MK10[392]		NaOH[209]	TBAF[397] NaOH[209]
ArOTBDPS		PIFA-MK10[392]		TMG[385]	TMG[385]

Table 13. Deprotection of alkyl silyl ethers in the presence of an aryl silyl ether

Deprotection of:	In the presence of:				
	ArOTMS	ArOTES	ArOTBS	ArOTIPS	ArOTBDPS
1° TES		Pd/C, MeOH[262]	$ZnBr_2/H_2O$[59]	$ZnBr_2/H_2O$[59]	$ZnBr_2/H_2O$[59]
2° TES			PPTS[362] $BiBr_3/Et_3SiH$[372] $ZnBr_2/H_2O$[59]	$ZnBr_2/H_2O$[59]	$ZnBr_2/H_2O$[59]
1° TBS			PPTS[361] Nafion-H/NaI[365] LL-ALPS-SO_3H[40] AcCl/MeOH[34] TMS-Cl/H_2O[364] TMS-Cl/NaI/H_2O[364] Me_2SBr_2[365] TBSOTf, THPOAc[370] $BiBr_3/H_2O/CH_3CN$[369] $BiCl_3$/NaI[368] $CeCl_3{\cdot}7H_2O$[371] CuOTf, Ac_2O[56] $Sc(OTf)_3/H_2O$[367] $ZnBr_2/H_2O$[59] $InCl_3$[57] $ZrCl_4/Ac_2O$[58] decaborane[50] $Ce(OTf)_4$, THF/H_2O[55] CBr_4, MeOH[221] I_2/MeOH[373] CAN/SiO_2[218] Oxone/MeOH[144] H_2, Pd/C[374], CAN/SiO_2[218]	HF–pyr[366] BF_3-OEt_2[475] $ZnBr_2/H_2O$[59] $ZrCl_4/Ac_2O$[58]	HCl[476] $ZnBr_2/H_2O$[59] $InCl_3$[57] $BiOClO_4$[477]
2° TBS			Nafion-H/NaI[365] $BiBr_3/H_2O/CH_3CN$[369] $BiCl_3$/NaI[368] $CeCl_3{\cdot}7H_2O$[371] $ZnBr_2/H_2O$[59] TFA[363] $InCl_3$[57] Me_2SBr_2[365]	TFA[363] $ZnBr_2/H_2O$[59]	$ZnBr_2/H_2O$[59]
1° TIPS				CBr_4, MeOH[221] I_2/MeOH[373]	
1° TBDPS					$Sc(OTf)_3/H_2O$[367] CBr_4, MeOH[221] I_2/MeOH[373]

Table 14. Deprotection of aryl silyl ethers in the presence of another aryl silyl ether

Deprotection of:	In the presence of:				
	ArOTMS	ArOTES	ArOTBS	ArOTIPS	ArOTBDPS
ArOTMS					
ArOTES					
ArOTBS			$SbCl_5$[402] KF-Al_2O_3[387] CCl_4/MeOH[401] $NaClO_2$, NaH_2PO_4, pyridine[399]		HCl[400]
ArOTIPS					
ArOTBDPS					

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Biographical sketch

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A new entry to the substituted pyrrolo[3,2-*c*]quinoline derivatives of biological interest by intramolecular heteroannulation of internal imines

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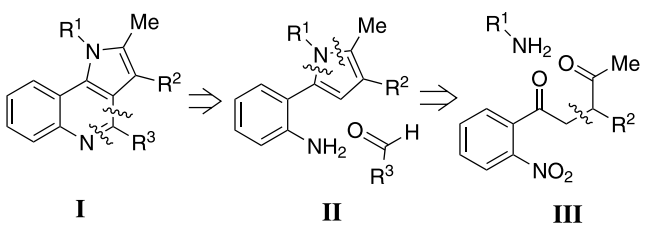
Abstract—New 1,3,4-substituted pyrrolo[3,2-*c*]quinoline derivatives were synthesised in good yields by oxidative heteroannulation of internal imines starting from easily prepared substituted 5-(2-aminophenyl)pyrroles and commercially available aryl and heteroaryl aldehydes. The reaction occurs as a one-pot process involving an intramolecular acid catalysed reaction.

1. Introduction

For many years, the pyrrolo[3,2-*c*]quinoline ring system (**I**, Scheme 1) has been known as a core structure unit of bioactive molecules of either synthetic[1] or natural source.[2] Several derivatives of such a tricyclic angular heterocycle possess a wide spectrum of biological activities,[3] including most notably antitumor properties,[4] gastric (H^+/K^+)-ATPase inhibitor,[5] hypotensive,[6] anti-inflammatory activities[7] and others. The relatively recent isolation of this framework from the organic extracts of *Martinella iquitosensis* roots, which evidenced antagonist properties against bradykinin receptors,[8] renewed interest has attracted several research groups to plan new synthetic approaches. The wide potential of such a skeleton along with our interest in targets featuring nitrogen containing aromatized polycyclic structures prompted us to develop an alternative synthetic route, so as to further investigate the interaction of such molecules with DNA.



Scheme 1. Disconnection approach of the pyrrolo[3,2-*c*]quinoline core.

A perusal of the literature highlights a large number of different synthetic pathways to such rings. However, only a few convenient cases concern entirely planar aromatic ring systems; such as those concerning the Fischer-indole synthesis,[9] metal mediated reactions,[1,10] and aryl radical cyclization onto pyrroles.[11]

As an extension of our ongoing work in the field directed to the development of new synthetic approaches to polycyclic nitrogen heterocycles,[12] as well as exploration of their biological and structural properties, we report here an alternative and convenient pathway leading, in good yields, to a series of new substituted pyrrolo[3,2-*c*]quinoline compounds.

The retrosynthetic approach proposed is illustrated in Scheme 1. It involves a double disconnection at the central pyridine ring to afford the 5-(*o*-aminophenyl) pyrroles **II**, which in turn would arise from the corresponding 1,4-diketone **III** and alkyl, aryl, heteroaryl amines simply formed by a Paal-Knorr reaction. Precursors **III** were easily synthesized according to the literature procedures[13,14] modified by us to achieve higher yields (see Section 4). Such a strategy, in combination with the possibility of using a wide range of commercially available reactants, allows functionalization of crucial positions of the pyrrole ring and can be suitable in combinatorial chemistry for the synthesis of a small library.

Reported synthetic pathways have mainly considered the

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construction of the pyrrolo[3,2-*c*]quinoline core starting from a preformed quinoline moiety,[15] fluorinated synthons,[16] substituted hydropyridine,[17] but limitations and/or low overall yields are often encountered. Often, the introduction of a specific substituent, especially in position 4 of the preformed skeleton, required laborious steps coupled with drastic experimental conditions. Moreover, it did not appear to offer much flexibility for preparing derivatives with groups other than simple alkyl ones in the 4 position.[18]

In this case, for our target molecules, we considered *o*-aminophenylpyrroles **II** as strategic precursors. Although, they have proved to be very versatile key intermediates, leading to a wide variety of pyrrolo-fused heterocycles,[19–22] to our knowledge, none of the previous synthetic pathways explored their reactivity via imine formation and intramolecular cyclization. The only reported example involving a carbonyl function concerned the use of formic acid, which in boiling benzene cyclized to the dihydro-pyrroloquinazoline ring.[14]

Here, we report our results on the development of a new and convenient one pot access to the title ring system starting from precursor **II**.

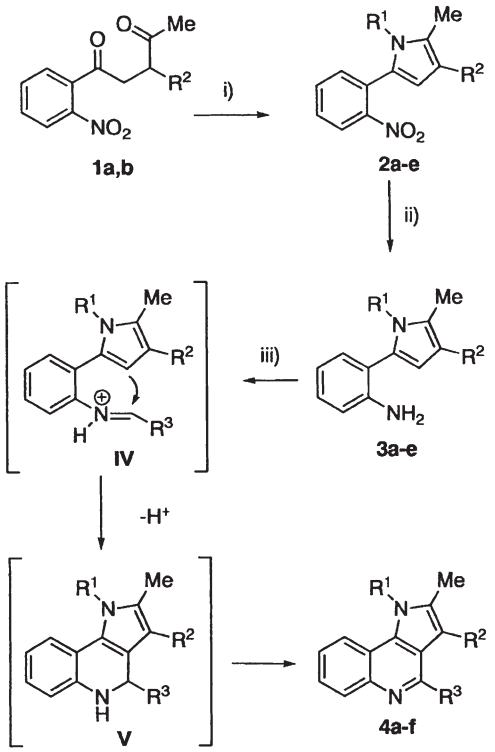
2. Results and discussion

The reaction sequence starts with the Paal-Knorr reaction between 1,4-diketones **1a**,**b**[13,14] and commercial alkyl, aryl, heteroaryl amines, which under reflux (3–8 h) in acetic acid, afforded the corresponding 5-(*o*-nitrophenyl)-1-substitued pyrroles **2a-e** in 60–87% isolated yield. Reduction with Pd/C in a Parr apparatus furnished the amino derivatives **3a-e**, in yields from good to excellent (Scheme 2).

Treatment of the latter amines with a slight excess of aldehydes in the presence of 15 mol% of *p*-toluenesulfonic acid (*p*-TsOH) in DMF at 100 °C provided compounds **4a-f** in good yields (72–94%) within 3 h.

Such a result may be reasonably accounted for on the basis of an intramolecular addition of pyrrole β-carbon on the transiently formed protonated imine (**IV**) to give the tricyclic intermediate (**V**), followed by spontaneous dehydrogenation (Scheme 2).

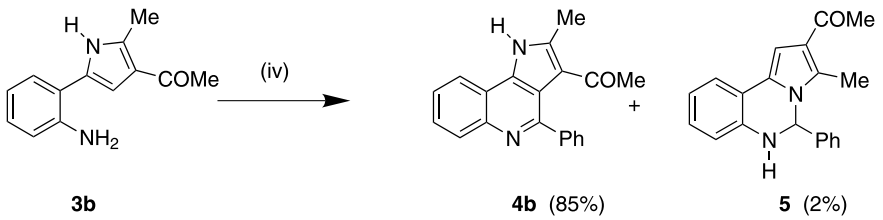
Attempted isolation of the imines **IV** or the cycloadduct **V** from the reaction as intermediates failed, even when operating under milder reaction condition.[23] Only during GC–MS monitorage of the reaction was a trace amount of a peak corresponding to the mass of the supposed imine **IV** or the dihydro cycloadduct **V** detected. Probably, this fact reflects the high reactivity of the internal nucleophile (pyrrole C-3) which immediately evolves to the fully aromatic system. It should be noted that isolation of the oxidized aromatic derivatives was also observed even in the presence of reductive condition,[11] confirming that the thermodynamic gain involved in the aromatization process is relevant.



	R^1	R^2	R^3	Yield%
1a		CO_2Et		78
1b		COMe		92
2a	Ph	CO_2Et		73
2b	H	COMe		80
2c	Me	COMe		87
2d	Et	COMe		60
2e	Ph	COMe		80
3a	Ph	CO_2Et		95
3b	H	COMe		97
3c	Me	COMe		91
3d	Et	COMe		82
3e	Ph	COMe		85
4a	Ph	CO_2Et	Ph	84
4b	H	COMe	Ph	85
4c	Me	COMe	Ph	77
4d	Et	COMe	Ph	84
4e	Ph	COMe	Ph	94
4f	Ph	COMe	5-Me-Furan-2*yl*	72

Reagents and conditions: i) R^1NH_2, AcOH, reflux, 3-8h; ii) H_2, 10% Pd/C, EtOH, rt, overnight; iii) R^3CHO, DMF, 100°C, 15 mol% *p*-TsOH within 3h.

Scheme 2. General procedure for the preparation of the novel pyrroloquinoline derivatives.



3b **4b** (85%) **5** (2%)

Reagents and conditions: (iv): PhCHO 1.1 eq, 15 mol% *p*-TsOH, DMF, 100°C, 2.5h.

Scheme 3. Formation of the competitive pyrrolo[1,2-*c*]quinazoline ring.

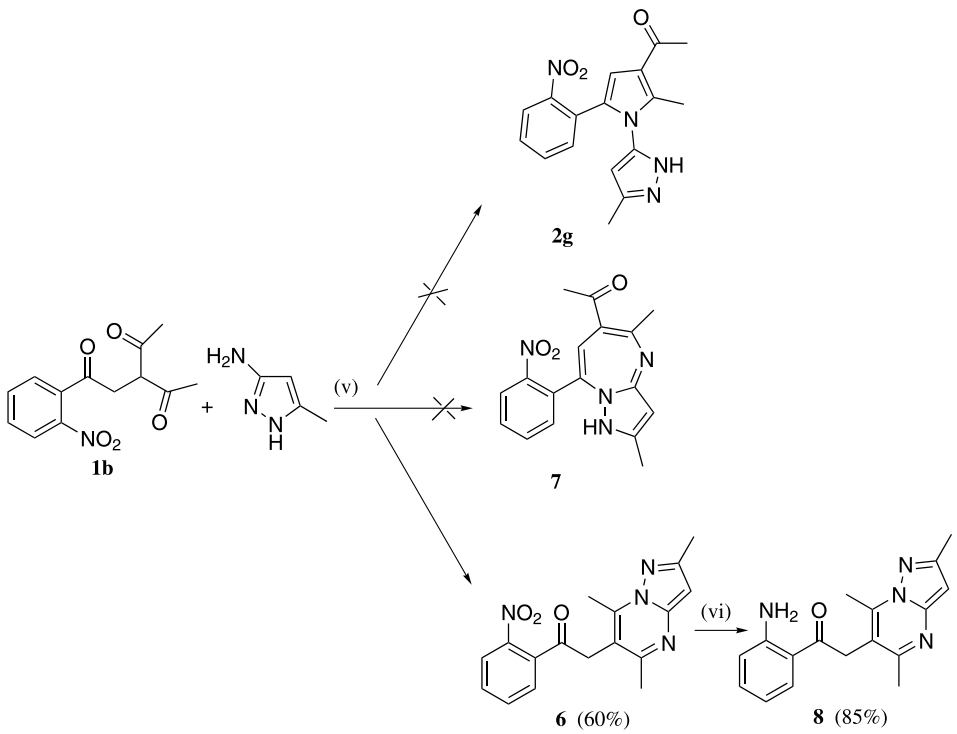
It is interesting to note the key step of this sequence is strongly related to a variant of the well-known Mannich reaction, and in particular the Pictet-Spengler condensation, which also features the first step on intramolecular addition of the position 3 of an indole derivative onto an in situ generated iminium ion.[24]

When the pyrroloaniline **3b** was treated with PhCHO under the same experimental conditions as before (Scheme 3), the expected compound **4b** was isolated as major product (85%) along with traces of the cycloadduct 5,6-dihydro-pyrrolo[1,2-*c*]quinazoline derivative **5** (2%) which appears to result from competitive NH pyrrole addition on the intermediate protonated imine.

In fact, besides the expected signals, the ^{1}H NMR spectrum of compound **5** exhibited a singlet at δ_H 6.62 ppm related to the dihydro pyrimidine CH together with another singlet at δ_H 7.06 ppm relative to the pyrrole CH. The signals for the corresponding carbon atoms in the ^{13}C NMR spectrum were found at δ_C 65.51 and 103.81 ppm, respectively.

In an attempt to introduce heteroaryl moieties on position 1, commercial 3-amino-5-methyl pyrazole was reacted with triketone **1b** (Scheme 4). Upon heating under reflux in acetic acid, a major compound (60% yields) with a peak *m*/*z* of 324 in the mass spectrum was isolated from the reaction mixture. NMR data of this product excluded the expected 1-pyrazol-2-yl pyrrole derivative **2g**. In fact, besides the signals for the (2-nitrophenyl) group, ^{1}H NMR spectrum showed a singlet at δ_H 6.36 ppm related to one proton, with a signal for the corresponding carbon atom at δ_C 94.30 ppm in the ^{13}C NMR spectrum, attributable to a pyrazole CH. Furthermore, the presence of a methylene group was also evidenced by a singlet at δ_H 4.57 ppm integrated for two protons in the ^{1}H NMR spectrum and by the corresponding carbon atom signal at δ_C 41.22 ppm in the ^{13}C NMR spectrum, confirmed by DEPT experiments.

Usually, 1,4-diketones and 2-amino-azoles give rise to 4+1 cyclo-condensation.[12b] In the case of triketone **1b**, other types of cyclo-condensation (3+3 or 4+3) could be envisaged. So, compounds **6** or **7** could be formed, respectively.



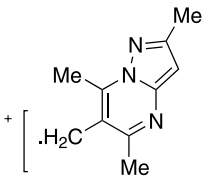
Reagents and conditions: (v) AcOH under reflux, 8h; (vi) H_2, PdC 10%, EtOH, rt, 12h.

Scheme 4. Competitive 3+3 cyclo-condensation in the attempted preparation of **2g**.

Analysis of the NMR data allowed us to assign the structure **6** to the isolated product. Unequivocal assignment of the signals was performed by 2D NMR experiments showing both one-bond and long-range heteronuclear C–H correlations. In addition to the one-bond correlation with the CH_2 carbon atom, the methylene protons exhibited ^2J connections with C-6 and carbonyl carbon atoms and ^3J connections with C-5 and C-7 carbon atoms. Analogously, besides the one-bond correlations, methyl protons exhibited ^2J correlations with the related C-*ipso* carbon atoms and ^3J correlations with the C-*ortho* carbon atoms. Therefore, C-6 carbon atom showed correlations with 5-Me and 7-Me protons, whereas C-3 carbon atom correlated with 2-Me protons. Correlations of pyrazole proton with C-2 and C-3a quaternary carbon atoms were also detected. Finally, the signals of the (2-nitrophenyl) group showed the appropriate correlations either interannular and to carbonyl carbon atom.

The same correlations were found in the 2D NMR spectra of the amino derivative **8** in turn obtained by hydrogenation of **6**. In this case, chemical shift variations $|\Delta\delta| \leq 0.68$ were detected for ^{13}C NMR signals of pyrazolo[1,5-*a*]pyrimidine ring carbon atoms, with the exception of C-6 ($\Delta\delta = +2.40$) and exocyclic methylene ($\Delta\delta = -3.41$) carbon atoms. As expected, the (2-aminophenyl) group showed in the ^1H and ^{13}C NMR spectra the appropriate signals of the reduction product of **6**.

Additional support was furnished by the fragmentation pattern as pointed out in the mass spectra of compound **6**, in which the base peak fragment at *m/z* 174, relative to the stable pyrazolopyrimidine scaffold, was detected (Fig. 1).



m/z 174

Figure 1. Base peak fragment observed at GC–MS (EI 70 eV) for compound **6**.

The above example indicates that the hetero-functionalization onto position 1 of the ring is strictly dependent on the nature and the reactivity of the heteroaryl amino species involved in the first reaction step. Thus, the simultaneous presence of supplementary nucleophilic site can divert from the expected 4+1 cyclo-condensation. Anyway, suitable hetero-aromatic amines could be employed at this purpose.[12a,b]

3. Conclusions

In summary, the above presented study allows easy access to fully aromatic pyrrolo[3,2-*c*]quinoline derivatives. This new approach is operationally simple and makes use of commercial available starting materials such as alkyl, aryl or heteroaryl amines concerned in step (i) and aryl or heteroaryl aldehydes[25] involved in step (iii). In contrast to previous methods, this new pathway allows convenient introduction onto position 1, 3 and 4 of the title ring system a variety of selected functional groups, which in turn are useful for structure–activity relationships studies or may be susceptible of further synthetic development. Although some limitation could be encountered, as observed for the cyclo-condensation of 3-aminopyrazole, the ready availability of the starting materials and ease of this procedure make this method ideal for an alternative new access to fully aromatic pyrrolo[3,2-*c*]quinoline derivatives.

4. Experimental

4.1. Materials and general methods

Unless otherwise specified, materials were purchased from commercial suppliers (Aldrich) and used without further purification. Acetic acid was distilled from acetic anhydride (3%, w/v) under argon Analytical thin layer chromatography was performed on Merck precoated silica gel (60 F_{254}) plates and column chromatography was accomplished on Merck silica gel 230–400 mesh (ASTM). Melting points were determined with a Buchi-Tottoli capillary apparatus and are uncorrected. IR spectra were determined in bromoform with a Jasco FT-IR 5300 spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC 250 spectrometer operating in FT mode in DMSO-d_6 solutions at 250.13 and 62.89 MHz, respectively. ^1H and ^{13}C chemical shift values are given in ppm relative to TMS (as internal standard) and DMSO-d_6 (centered at 39.50 ppm downfield from TMS), respectively. Coupling constants values are in Hz. ^{13}C chemical shift values were measured from proton fully decoupled spectra. Signals assignment was made on the basis both of known substituent effects and of one-bond multiplicities (indicated in parentheses) determined by DEPT-135 and confirmed by 2D C,H correlation experiments, using the standard Bruker pulse sequences XHDEPT.AUR and COLOC.AUR, for one-bond and long-range C,H interactions, respectively. Mass spectra (EI) were collected on a GC-MS-QP5050A Shimadzu mass spectrometer with ionization energy of 70 eV. Elemental analyses were performed on a Perkin–Elmer 240 °C elemental analyzer and the results were within ±0.3% of the theoretical values. Yields refer to purified products and are not optimized.

Analytical and spectroscopic data for compounds **1a**,**b** were consistent to those previously reported.[13,14] The yield of compound **1b** was optimized to 92% with respect to the reported 50%[13] by using the following modified procedure. To a solution of sodium ethoxide (80 mmol) in absolute ethanol (100 ml), acetylacetone (80 mmol) was added dropwise cooling with an ice-bath. After 1 h, the mixture was allowed to rt and the 2-nitrophenacyl bromide was added in small portions within 40 min. The reaction mixture was stirred at rt for 3 days and then quenched with 150 ml of water. A white solid was formed and was recrystallized from ethanol.

4.2. General method for the preparation of 5-(*o*-nitrophenyl)-1,3-substituted pyrroles (2a–e)

According to the procedure described[13] for **2a**–**e**, to a

solution of **1a**,**b** (10 mmol) in acetic acid (40 ml), the corresponding amine (10 mmol) was added. The mixture was heated under reflux for 3–8 h until disappearance of reactants (TLC monitorage). After cooling, the resultant solution was poured onto crushed ice. The so formed solid was filtered off, air-dried and recrystallized from ethanol.

Analytical and spectroscopic data for compounds **2a**–**c**,**e** were coincident to those previously reported.[13,14,20,21] Most detailed ^{1}H and ^{13}C NMR data, together with MS data, not previously reported, are now described.

4.2.1. 3-Acetyl-2-methyl-5-(2-nitrophenyl)-1*H*-pyrrole 2b. Yield 80%; white crystals, mp 206–207 °C [lit.[13] mp 208 °C]; δ_H 11.75 (1H, s, exchangeable with D_2O, N*H*), 7.92 (1H, d, *J* 7.5 Hz, H-3′), 7.74–7.64 (2H, m, H-5′ and -6′), 7.51 (1H, t, *J*=7.5 Hz, H-4′), 6.60 (1H, s, H-4), 2.49 (3H, s, 2-*Me*), 2.32 (3H, s, CO*Me*); δ_C 193.28 (s, CO), 147.52 (s, C-2′), 136.56 (s, C-2), 132.50 (d, C-5′), 130.32 (d, C-6′), 127.94 (d, C-4′), 125.62 (s, C-1′), 124.03 (d, C-3′), 123.80 (s, C-5), 121.76 (s, C-3), 110.33 (d, C-4), 28.30 (q, CO*Me*), 13.41 (q, 2-*Me*); *m*/*z* (EI) 244 (100, M^+), 229 (43), 183 (55), 77 (39), 43 (70%).

4.2.2. 3-Acetyl-1,2-dimethyl-5-(2-nitrophenyl)-1*H*-pyrrole 2c. Yield 87%; orange crystals, mp 115–116 °C [lit.[20] mp 116–117 °C]; δ_H 8.07 (1H, d, *J*=8.1 Hz, H-3′), 7.79 (1H, t, *J*=7.4 Hz, H-5′), 7.69 (1H, dd, *J*=8.1, 7.4 Hz, H-4′), 7.57 (1H, d, *J*=7.4 Hz, H-6′), 6.52 (1H, s, H-4), 3.27 (3H, s, N*Me*), 2.52 (3H, s, 2-*Me*), 2.28 (3H, s, CO*Me*); δ_C 193.36 (s, CO), 149.45 (s, C-2′), 135.97 (s, C-2), 133.46 (d, C-6′), 133.01 (d, C-5′), 129.85 (d, C-4′), 127.02 (s, C-5), 126.05 (s, C-1′), 124.08 (d, C-3′), 120.34 (s, C-3), 110.26 (d, C-4), 31.06 (q, N*Me*), 28.23 (q, CO*Me*), 11.41 (q, 2-*Me*); *m*/*z* (EI) 258 (96, M^+), 243 (80), 196 (81), 77 (64), 56 (100), 43 (86%).

4.2.3. 3-Acetyl-1-ethyl-2-methyl-5-(2-nitrophenyl)-1*H*-pyrrole 2d. Yield 60%; white crystals, mp 133–134 °C; [found: C, 66.29; H, 5.94; N, 10.27. $C_{15}H_{16}N_2O_3$ requires C, 66.16; H, 5.92; N, 10.29%]; ν_{max} 1643 (CO), 1527 and 1348 (NO_2) cm^{-1}; δ_H 8.05 (1H, d, *J*=7.8 Hz, H-3′), 7.78 (1H, t, *J*=7.2 Hz, H-5′), 7.69 (1H, dd, *J*=7.8, 7.2 Hz, H-4′), 7.60 (1H, d, *J*=7.2 Hz, H-6′), 6.46 (1H, s, H-4), 3.77 (2H, q, *J*=7.1 Hz, CH_2Me), 2.55 (3H, s, 2-*Me*), 2.26 (3H, s, CO*Me*), 1.04 (3H, t, *J*=7.1 Hz, CH$_2$*Me*); δ_C 193.37 (s, CO), 149.93 (s, C-2′), 135.02 (s, C-2), 133.48 (d, C-6′), 132.68 (d, C-5′), 129.99 (d, C-4′), 126.02 (s, C-1′), 125.92 (s, C-5), 123.91 (d, C-3′), 120.60 (s, C-3), 110.55 (d, C-4), 38.64 (t, *C*H$_2$Me), 28.21 (q, CO*Me*), 15.09 (q, CH$_2$*Me*), 11.28 (q, 2-*Me*); *m*/*z* (EI) 272 (100, M^+), 257 (33), 210 (25), 77 (21), 70 (71), 43 (70), 42 (91%).

4.2.4. 3-Acetyl-2-methyl-5-(2-nitrophenyl)-1-phenyl-1*H*-pyrrole 2e. Yield 80%; yellow crystals, mp 137–138 °C [lit.[20] mp 138 °C]; δ_H 7.82 (1H, d, *J*=8.0 Hz, H-3′), 7.61 (1H, t, *J*=7.3 Hz, H-5′), 7.49 (1H, dd, *J*=8.0, 7.3 Hz, H-4′), 7.45 (1H, d, *J*=7.3 Hz, H-6′), 7.39–7.33 (3H, m, N*Ph* H-3,5 and -4), 7.14 (2H, dd, *J*=7.9, 1.6 Hz, N*Ph* H-2,6), 6.79 (1H, s, H-4), 2.39 (3H, s, CO*Me*), 2.33 (3H, s, 2-*Me*); δ_C 193.81 (s, CO), 148.72 (s, C-2′), 136.35 (s, C-2),135.84 (s, N*Ph* C-1), 133.43 (d, C-6′), 132.71 (d, C-5′), 129.30 (d, C-4′), 129.16 (d, N*Ph* C-3,5), 128.45 (d, N*Ph*C-4), 128.02 (d, N*Ph* C-2,6), 128.02 (s, C-5), 126.23 (s, C-1′), 123.84 (d, C-3′), 121.29 (s, C-3), 111.07 (d, C-4), 28.53 (q, CO*Me*), 12.55 (q, 2-*Me*); *m*/*z* (EI) 320 (59, M^+), 305 (15), 261 (49), 228 (72), 186 (44), 118 (91), 77 (100), 51 (42), 43 (78%).

4.3. General method for the preparation of 5-(2-aminophenyl)-1-substituted-pyrroles (3a–e)

According to the procedure described[19] for **2a**–**c**,**e** compound **2d** was reduced overnight with hydrogen (50 psi) over 10% Pd–C in ethanol in a Parr apparatus at room temperature. The catalyst was filtered off and the solvent evaporated under reduced pressure. The obtained solid was recrystallized from ethanol.

Analytical and spectroscopic data for compounds **3a**–**c**,**e** were coincident to those previously reported.[13,14,20,21] Most detailed ^{1}H and ^{13}C NMR data, together with MS data, not previously reported, are now described.

4.3.1. 5-(2-Aminophenyl)-3-ethylester-2-methyl-1-phenyl-1*H*-pyrrole 3a. Yield 95%; orange crystals, mp 154–155 °C [lit.[21] mp 153 °C]; δ_H 7.35–7.30 (3H, m, N*Ph* H-3,5 and -4), 7.24 (2H, dd, *J*=7.8, 2.2 Hz, N*Ph* H-2,6), 6.86 (1H, ddd, *J*=8.0, 7.6, 1.3 Hz, H-4′), 6.69 (1H, dd, *J*=7.6, 1.3 Hz, H-6′), 6.54 (1H, dd, *J*=8.0, 0.8 Hz, H-3′), 6.49 (1H, s, H-4), 6.31 (1H, td, *J*=7.6, 0.8 Hz, H-5′), 4.81 (2H, s, exchangeable with D_2O, NH_2), 4.22 (2H, q, *J*=7.1 Hz, CH_2Me), 2.30 (3H, s, 2-*Me*), 1.28 (3H, t, *J*=7.1 Hz, CH$_2$*Me*); δ_C 164.56 (s, CO), 147.14 (s, C-2′), 137.26 (s, N*Ph* C-1), 136.36 (s, C-2), 131.50 (d, C-6′), 130.52 (s, C-5), 128.71 (d, N*Ph* C-3,5), 128.61 (d, C-4′), 128.05 (d, N*Ph* C-2,6 and -4), 116.04 (s, C-1′), 115.26 (d, C-5′), 114.29 (d, C-3′), 111.61 (s, C-3), 109.52 (d, C-4), 58.82 (t, *C*H$_2$Me), 14.45 (q, CH$_2$*Me*), 12.35 (q, 2-*Me*); *m*/*z* (EI) 320 (100, M^+), 275 (31), 274 (44), 130 (44), 118 (57), 77 (33), 51 (12%).

4.3.2. 3-Acetyl-5-(2-aminophenyl)-2-methyl-1*H*-pyrrole 3b. Yield 97%; white crystals, mp 130–131 °C [lit.[13] mp132 °C]; δ_H 11.39 (1H, s, exchangeable with D_2O, N*H*), 7.20 (1H, dd, *J*=7.5, 1.3 Hz, H-6′), 7.00 (1H, ddd, *J*=7.9, 7.5, 1.3 Hz, H-4′), 6.78 (1H, dd, *J*=7.9, 1.0 Hz, H-3′), 6.69 (1H, s, H-4), 6.63 (1H, td, *J*=7.5, 1.0 Hz, H-5′), 5.02 (2H, s, exchangeable with D_2O, NH_2), 2.49 (3H, s, 2-*Me*), 2.34 (3H, s, CO*Me*); δ_C 193.61 (s, CO), 144.90 (s, C-2′), 134.77 (s, C-2), 127.92 (d, C-6′), 127.49 (d, C-4′), 127.12 (s, C-5), 121.14 (s, C-3), 116.94 (s, C-1′), 116.50 (d, C-5′), 115.56 (d, C-3′), 108.52 (d, C-4), 28.40 (q, CO*Me*), 13.35 (q, 2-*Me*); *m*/*z* (EI) 214 (100, M^+), 199 (85), 172 (69), 171 (73), 100 (82), 77 (31), 43 (32%).

4.3.3. 3-Acetyl-5-(2-aminophenyl)-1,2-dimethyl-1*H*-pyrrole 3c. Yield 91%; white crystals, mp 134–135 °C [lit.[21] mp 135 °C]; δ_H 7.09 (1H, ddd, *J*=8.0, 7.3, 1.1 Hz, H-4′), 6.95 (1H, dd, *J*=7.3, 1.1 Hz, H-6′), 6.76 (1H, d, *J*=8.0 Hz, H-3′), 6.59 (1H, t, *J*=7.3 Hz, H-5′), 6.44 (1H, s, H-4), 4.81 (2H, exchangeable with D_2O, NH_2), 3.26 (3H, s, N*Me*), 2.51 (3H, s, 2-*Me*), 2.30 (3H, s, CO*Me*); δ_C 193.45 (s, CO), 147.19 (s, C-2′), 135.19 (s, C-2), 131.46 (d, C-6′), 130.00 (s, C-5), 129.15 (d, C-4′), 120.12 (s, C-3), 116.17 (s, C-1′), 115.83 (d, C-5′), 114.55 (d, C-3′), 109.49 (d, C-4), 30.63 (q, N*Me*), 28.28 (q, CO*Me*), 11.63 (q, 2-*Me*); *m*/*z* (EI) 228 (90,

M^+), 213 (100), 185 (52), 106 (64), 77 (23), 56 (51), 43 (26%).

4.3.4. 3-Acetyl-5-(2-aminophenyl)-1-ethyl-2-methyl-1*H*-pyrrole 3d. Yield 82%; yellow crystals, mp 88–89 °C; [found: C, 74.25; H, 7.51; N, 11.58. $C_{15}H_{18}N_2O$ requires C, 74.35; H, 7.49; N, 11.56%]; ν_{max} 3464 (NH_2), 3366 (NH_2), 1643 (CO) cm^{-1}; δ_H 7.10 (1H, ddd, *J*=7.9, 7.5, 1.3 Hz, H-4′), 6.97 (1H, dd, *J*=7.5, 1.3 Hz, H-6′), 6.75 (1H, d, *J*=7.9 Hz, H-3′), 6.60 (1H, t, *J*=7.5 Hz, H-5′), 6.42 (1H, s, H-4), 4.73 (2H, exchangeable with D_2O, N*H*$_2$), 3.70 (2H, q, *J*=7.1 Hz, C*H*$_2$Me), 2.53 (3H, s, CO*Me*), 2.30 (3H, s, 2-*Me*), 0.98 (3H, t, *J*=7.1 Hz, CH_2*Me*); δ_C 193.71 (s, CO), 147.26 (s, C-2′), 134.44 (s, C-2), 131.64 (d, C-6′), 129.39 (d, C-4′), 129.04 (s, C-5), 120.50 (s, C-3), 116.39 (s, C-1′), 115.99 (d, C-5′), 114.58 (d, C-3′), 110.16 (d, C-4), 38.29 (t, *C*H$_2$Me), 28.41 (q, CO*Me*), 15.57 (q, CH_2*Me*), 11.52 (q, 2-*Me*); *m*/*z* (EI) 242 (100, M^+), 227 (98), 199 (43), 99 (33), 77 (18), 43 (32%).

4.3.5. 3-Acetyl-5-(2-aminophenyl)-2-methyl-1-phenyl-1*H*-pyrrole 3e. Yield 85%; yellow crystals, mp 151–152 °C [lit.[21] mp 153 °C]; δ_H 7.40–7.32 (3H, m, N*Ph* H-3,5 and -4), 7.24 (2H, dd, *J*=7.5, 1.9 Hz, N*Ph* H-2,6), 6.88 (1H, ddd, *J*=8.0, 7.3, 1.3 Hz, H-4′), 6.71 (1H, dd, *J*=7.3, 1.3 Hz, H-6′), 6.70 (1H, s, H-4), 6.59 (1H, dd, *J*=8.0, 1.0 Hz, H-3′), 6.33 (1H, td, *J*=7.3, 1.0 Hz, H-5′), 4.93 (2H, s, exchangeable with D_2O, N*H*$_2$), 2.41 (3H, s, CO*Me*), 2.32 (3H, s, 2-*Me*); δ_C 193.97 (s, CO), 147.03 (s, C-2′), 137.02 (s, N*Ph* C-1), 135.36 (s, C-2), 131.53 (d, C-6′), 130.18 (s, C-5), 128.70 (d, N*Ph* C-3,5), 128.60 (d, C-4′), 128.02 (d, N*Ph* C-4), 127.98 (d, N*Ph* C-2,6), 120.77 (s, C-3), 116.13 (s, C-1′), 115.33 (d, C-5′), 114.36 (d, C-3′), 110.52 (d, C-4), 28.56 (q, CO*Me*), 12.74 (q, 2-*Me*); *m*/*z* (EI) 290 (100, M^+), 275 (98), 247 (40), 130 (61), 118 (42), 77 (83), 51 (49%).

4.4. General method for the preparation of 1,3,4-substituted-pyrrolo[3,2-*c*]quinoline derivatives (4a–f)

To a solution of aminopyrroles **3a**–**e** (0.57 mmol), in DMF (5 ml) commercial aldehydes (0.63 mmol) and catalytic amount of *p*-TsOH (15 mol%) were added. After stirring at 100 °C for 1–3 h, (TLC monitorage) the mixture was allowed to reach room temperature. Evaporation of the solvent under reduced pressure gave rise a dark residue which was dissolved in dichloromethane (30 ml) and washed with 3×10 ml of 5% aqueous $NaHCO_3$ solution. The organic extracts dried with $MgSO_4$ and evaporated in vacuo afforded a solid which was purified by column chromatography (eluant dichloromethane/ethyl acetate, 9:1, followed by recrystallization from ethanol).

In the case of aminopyrrole **3b**, along with the compound **4b** obtained in 85% of yield, compound **5** was isolated in 2% of yield.

4.4.1. 3-Ethylester-2-methyl-1,4-diphenyl-1*H*-pyrrolo[3,2-*c*]quinoline 4a. Yield 84%; white crystals, mp 273–274 °C; [found: C, 79.62; H, 5.48; N, 6.87. $C_{27}H_{22}N_2O_2$ requires C, 79.78; H, 5.46; N, 6.89%]; ν_{max} 1709 (CO) cm^{-1}; δ_H 8.08 (1H, dd, *J*=8.0, 1.3 Hz, H-6), 7.78–7.74 (3H, m, N*Ph* H-3,5 and -4), 7.71–7.61 (4H, m, N*Ph* H-2,6 and *Ph* H-2,6), 7.58–7.46 (4H, m, H-7 and *Ph* H-3,5 and -4), 7.22 (1H, ddd, *J*=8.1, 7.4, 1.3 Hz, H-8), 6.88 (1H, dd, *J*=8.1, 1.1 Hz, H-9), 3.58 (2H, q, *J*=7.2 Hz, C*H*$_2$Me), 2.31 (3H, s, 2-*Me*), 0.74 (3H, t, *J*=7.2 Hz, CH_2*Me*); δ_C 163.01 (s, CO), 151.86 (s, C-4), 144.07 (s, C-5a), 141.89 (s, *Ph* C-1), 137.88 (s, C-2 and N*Ph* C-1), 135.33 (s, C-9b), 130.66 (d, N*Ph* C-3,5), 130.45 (d, N*Ph* C-4), 129.97 (d, C-6), 128.75 (d, N*Ph* C-2,6), 128.29 (d, *Ph* C-4), 128.05 (d, *Ph* C-2,6 and -3,5), 126.95 (d, C-7), 125.58 (d, C-8), 119.91 (d, C-9), 116.70 (s, C-3a), 115.97 (s, C-9a), 115.62 (s, C-3), 60.00 (t, *C*H$_2$Me), 13.33 (q, CH_2*Me*), 11.65 (q, 2-*Me*); *m*/*z* (EI) 406 (92, M^+), 377 (100), 361 (61), 333 (46), 255 (13), 180 (19), 165 (34), 77 (20%).

4.4.2. 3-Acetyl-2-methyl-4-phenyl-1*H*-pyrrolo[3,2-*c*]quinoline 4b. Yield 85%; yellow crystals, mp 157–158 °C; [found: C, 80.15; H, 5.39; N, 9.30. $C_{20}H_{16}N_2O$ requires C, 79.98; H, 5.37; N, 9.33%]; ν_{max} 3225 (NH), 1647 (CO) cm^{-1}; δ_H 12.89 (1H, s, exchangeable with D_2O, N*H*), 8.39 (1H, dd, *J*=7.6, 1.5 Hz, H-9), 8.06 (1H, dd, *J*=8.0,1.3 Hz, H-6), 7.70–7.60 (4H, m, H-7, H-8, and *Ph* H-2,6), 7.51–7.48 (3H, m, *Ph* H-3,5 and -4), 2.55 (3H, s, 2-*Me*), 1.58 (3H, s, CO*Me*); δ_C 196.85 (s, CO), 153.66 (s, C-4), 143.25 (s, C-5a), 142.08 (s, *Ph* C-1), 138.98 (s, C-2), 135.26 (s, C-9b), 129.25 (d, C-6), 128.75 (d, *Ph* C-4), 128.59 (d, *Ph* C-3,5), 128.42 (d, *Ph* C-2,6), 127.22 (d, C-7), 125.99 (d, C-8), 120.84 (d, C-9), 117.74 (s, C-3), 116.14 (s, C-3a), 116.08 (s, C-9a), 31.24 (q, CO*Me*), 12.83 (q, 2-*Me*); *m*/*z* (EI) 300 (64, M^+), 285 (75), 255 (27), 128 (100), 114 (30%).

4.4.3. 3-Acetyl-1,2-dimethyl-4-phenyl-1*H*-pyrrolo[3,2-*c*]quinoline 4c. Yield 77%; yellow crystals, mp 184–185 °C; [found: C, 80.31; H, 5.75; N, 8.93. $C_{21}H_{18}N_2O$ requires C, 80.23; H, 5.77; N, 8.91%]; ν_{max} 1651 (CO) cm^{-1}; δ_H 8.56 (1H, dd, *J*=8.0, 1.1 Hz, H-9), 8.11 (1H, dd, *J*=7.8, 1.1 Hz, H-6), 7.70–7.55 (4H, m, *J* H-7, H-8 and *Ph* H-2,6), 7.51–7.47 (3H, m, *Ph* H-3,5 and -4), 4.13 (3H, s, N*Me*), 2.47 (3H, s, 2-*Me*), 1.51 (3H, s, CO*Me*); δ_C 197.76 (s, CO), 153.22 (s, C-4), 144.02 (s, C-5a), 141.73 (s, *Ph* C-1), 139.26 (s, C-2), 134.70 (s, C-9b), 129.66 (d, C-6), 128.76 (d, *Ph* C-4), 128.54 (d, *Ph* C-3,5), 128.40 (d, *Ph* C-2,6), 126.59 (d, C-7), 125.73 (d, C-8), 121.25 (d, C-9), 117.51 (s, C-3), 116.71 (s, C-9a), 115.70 (s, C-3a), 34.15 (q, N*Me*), 31.46 (q, CO*Me*), 10.94 (q, 2-*Me*); *m*/*z* (EI) 314 (89, M^+), 299 (100), 283 (47), 255 (53), 127 (65), 114 (31%).

4.4.4. 3-Acetyl-1-ethyl-2-methyl-4-phenyl-1*H*-pyrrolo[3,2-*c*]quinoline 4d. Yield 84%; yellow crystals, mp 197–198 °C; [found: C, 80.37; H, 6.16; N, 8.50. $C_{22}H_{20}N_2O$ requires C, 80.46; H, 6.14; N, 8.53%]; ν_{max} 1667 (CO) cm^{-1}; δ_H 8.50 (1H, dd, *J*=8.0, 1.2 Hz, H-9), 8.17 (1H, dd, *J*=8.1, 1.1 Hz, H-6), 7.75–7.65 (4H, m, H-7, H-8, and *Ph* H-2,6), 7.55–7.51 (3H, m, *Ph* H-3,5 and -4), 4.69 (2H, q, *J*=7.2 Hz, C*H*$_2$Me), 2.54 (3H, s, 2-*Me*), 1.52 (3H, s, CO*Me*), 1.50 (3H, t, *J*=7.2 Hz, CH_2*Me*); δ_C 197.83 (s, CO), 152.95 (s, C-4), 142.80 (s, C-5a), 140.47 (s, *Ph* C-1), 139.20 (s, C-2), 133.94 (s, C-9b), 129.20 (d, C-6), 128.88 (d, *Ph* C-4), 128.63 (d, *Ph* C-2,6), 128.58 (d, *Ph* C-3,5), 126.63 (d, C-7), 125.46 (d, C-8), 121.10 (d, C-9), 118.12 (s, C-3), 116.05 (s, C-3a and C-9a), 40.61 (t, *C*H$_2$Me), 31.46 (q, CO*Me*), 14.70 (q, CH_2*Me*), 10.52 (q, 2-*Me*); *m*/*z* (EI) 328 (88, M^+), 313 (100), 285 (56), 255 (41), 128 (19%).

4.4.5. 3-Acetyl-2-methyl-1,4-diphenyl-1*H*-pyrrolo[3,2-*c*]quinoline 4e. Yield 94%; yellow crystals, mp 259–260 °C; [found: C, 83.15; H, 5.37; N, 7.45. $C_{26}H_{20}N_2O$ requires C, 82.95; H, 5.35; N, 7.44%];. ν_{max} 1664 (CO) cm^{-1}; δ_H 8.10 (1H, dd, *J*=8.0, 1.1 Hz, H-6), 7.78–7.72 (5H, m, *Ph* H-2,6 and N*Ph* H-3,5 and -4), 7.63 (2H, dd, *J*=7.2, 1.9 Hz, N*Ph* H-2,6), 7.57–7.53 (4H, m, H-7 and *Ph* H-3,5 and -4), 7.24 (1H, ddd, *J*=8.0, 7.4, 1.1 Hz, H-8), 6.90 (1H, dd, *J*=8.0, 1.3 Hz, H-9), 2.20 (3H, s, 2-*Me*), 1.64 (3H, s, CO*Me*); δ_C 197.72 (s, CO), 153.43 (s, C-4), 143.81 (s, C-5a), 141.32 (s, *Ph*C-1), 139.64 (s, C-2), 137.76 (s, N*Ph* C-1), 135.17 (s, C-9b), 130.59 (d, N*Ph* C-3,5), 130.33 (d, N*Ph* C-4), 129.51 (d, C-6), 128.95 (d, *Ph*C-4), 128.65 (d, N*Ph* C-2,6 and *Ph*C-3,5), 128.47 (d, *Ph*C-2,6), 127.06 (d, C-7), 125.65 (d, C-8), 120.00 (d, C-9), 118.20 (s, C-3), 115.98 (s, C-9a), 115.90 (s, C-3a), 31.43 (q, CO*Me*), 11.55 (q, 2-*Me*); *m*/*z* (EI) 376 (49, M^+), 361 (100), 255 (8), 180 (16), 165 (17), 77 (11%).

4.4.6. 3-Acetyl-2-methyl-4-(5-methylfuran-2yl)-1-phenyl-1*H*-pyrrolo[3,2-*c*]quinoline 4f. Yield 72%; yellow crystals, mp 252–253 °C; [found: C, 78.69; H, 5.28; N, 7.38. $C_{25}H_{20}N_2O_2$ requires C, 78.93;H, 5.30; N, 7.36%]; ν_{max} 1672 (CO) cm^{-1}; δ_H 8.02 (1H, dd, *J*=8.1, 1.1 Hz, H-6), 7.76–7.73 (3H, m, N*Ph* H-3,5 and -4), 7.63 (2H, dd, *J*=7.1, 2.3 Hz, N*Ph* H-2,6), 7.53 (1H, ddd, *J*=8.1, 7.0, 1.3 Hz, H-7), 7.19 (1H, ddd, *J*=8.2, 7.0, 1.1 Hz, H-8), 7.13 (1H, d, *J*=3.5 Hz, *Furanyl* H-3), 6.84 (1H, dd, *J*=8.2, 1.3 Hz, H-9), 6.38 (1H, d, *J*=3.5 Hz, *Furanyl* H-4), 2.34 (3H, s, Furanyl-*Me*), 2.20 (3H, s, 2-*Me*), 2.01 (3H, s, CO*Me*); δ_C 197.60 (s, CO), 153.09 (s, *Furanyl* C-5), 152.16 (s, C-4), 143.92 (s, C-5a), 143.16 (s, *Furanyl* C-2), 138.73 (s, C-2), 137.81 (s, N*Ph* C-1), 135.13 (s, C-9b), 130.52 (d, N*Ph* C-3,5), 130.25 (d, N*Ph* C-4), 129.45 (d, C-6), 128.69 (d, N*Ph* C-2,6), 126.98 (d, C-7), 125.28 (d, C-8), 119.93 (d, C-9), 117.99 (s, C-3), 116.09 (s, C-9a), 114.20 (s, C-3a), 111.30 (d, *Furanyl* C-3), 108.71 (d, *Furanyl* C-4), 31.34 (q, CO*Me*), 13.26 (q, Furanyl-*Me*), 11.33 (q, 2-*Me*); *m*/*z* (EI) 380 (98, M^+), 365 (82), 337 (100), 293 (33), 77 (21), 43 (18%).

4.4.7. 2-Acetyl-3-methyl-5-phenyl-5,6-dihydro-pyrrolo[1,2-*c*]quinazoline 5. Yield 2%; yellow crystals, mp 195–196 °C; [found: C, 79.41; H, 5.97; N, 9.18. $C_{20}H_{18}N_2O$ requires C, 79.44; H, 6.00; N, 9.26%]; ν_{max} 3342 (NH), 1641 (CO) cm^{-1}; δ_H 7.52 (1H, d, *J*=7.4 Hz, H-10), 7.33 (1H, s, exchangeable with D_2O, N*H*), 7.27–7.23 (3H, m, H-3′,5′ and -4′), 7.06 (1H, s, H-1), 6.98 (1H, dd, *J*=7.8, 7.4 Hz, H-8), 6.92 (2H, dd, *J*=7.5, 1.1 Hz, H-2′,6′), 6.75 (1H, t, *J*=7.4 Hz, H-9), 6.72 (1H, d, *J*=7.8 Hz, H-7), 6.62 (1H, s, H-5), 2.43 (3H, s, 3-*Me*), 2.40 (3H, s, CO*Me*); δ_C 194.06 (s, CO), 141.29 (s, C-6a), 138.32 (s, C-1′), 132.28 (s, C-3), 128.54 (d, C-3′,5′), 128.01 (d, C-8), 127.23 (d, C-4′), 126.23 (s, C-10b), 125.21 (d, C-2′,6′), 121.88 (d, C-10), 121.59 (s, C-2), 118.55 (d, C-9), 115.95 (s, C-10a), 115.11 (d, C-7), 103.81 (d, C-1), 65.51 (d, C-5), 28.58 (q, CO*Me*), 10.85 (q, 3-*Me*); *m*/*z* (EI) 302 (94, M^+), 287 (17), 259 (20), 225 (100), 77 (10), 43 (15%).

4.4.8. Preparation of substituted pyrazolo[1,5-*a*]pyrimidine derivatives (6 and 8). When 3-amino-5-methyl-1*H*-pyrazole was employed as amine, under conditions specified in Section 4.2, 1-(2-nitro-phenyl)-2-(2,5,7-trimethyl-pyrazolo[1,5-*a*]pyrimidin-6-yl)-1-ethanone **6** was isolated (yield 60%) and crystallized from ethanol as white crystals, mp 149–150 °C; [found: C, 62.84; H, 4.99; N, 17.18. $C_{17}H_{16}N_4O_3$ requires C, 62.95; H, 4.97; N, 17.27%]; ν_{max} 1705 (CO), 1545 and 1350 (NO_2) cm^{-1}; δ_H 8.17 (1H, d, *J*=8.0 Hz, H-3′), 8.00 (1H, d, *J*=7.4 Hz, H-6′), 7.94 (1H, t, *J*=7.4 Hz, H-5′), 7.82 (1H, dd, *J*=8.0, 7.4 Hz, H-4′), 6.36 (1H, s, H-3), 4.57 (2H, s, CH_2), 2.67 (3H, s, 7-*Me*), 2.48 (3H, s, 5-*Me*), 2.41 (3H, s, 2-*Me*); δ_C 198.51 (s, CO), 157.94 (s, C-5), 153.13 (s, C-2), 147.30 (s, C-3a), 145.92 (s, C-2′), 143.94 (s, C-7), 135.41 (s, C-1′), 134.25 (d, C-5′), 131.91 (d, C-4′), 128.27 (d, C-6′), 124.47 (d, C-3′), 110.67 (s, C-6), 94.30 (d, C-3), 41.22 (t, *C*H_2), 23.23 (q, 5-*Me*), 14.30 (q, 2-*Me*), 13.41 (q, 7-*Me*); *m*/*z* (EI) 324 (30, M^+), 174 (100), 81 (53), 53 (34%).

Reduction of **6** under the conditions specified in Section 4.3 gave the 1-(2-amino-phenyl)-2-(2,5,7-trimethyl-pyrazolo[1,5-*a*]pyrimidin-6-yl)-1-ethanone **8**: yield 85%; white crystals, mp 177–178 °C; [found: C, 69.40; H, 6.18; N, 18.99. $C_{17}H_{18}N_4O$ requires C, 69.37; H, 6.16; N, 19.03%]; ν_{max} 3437 and 3350 (NH_2), 1616 (CO) cm^{-1}; δ_H 8.06 (1H, d, *J*=7.4 Hz, H-6′), 7.30 (1H, dd, *J*=8.2, 7.4 Hz, H-4′), 7.16 (2H, s, exchangeable with D_2O, NH_2), 6.80 (1H, d, *J*=8.2 Hz, H-3′), 6.63 (1H, t, *J*=7.4 Hz, H-5′), 6.33 (1H, s, H-3), 4.52 (2H, s, CH_2), 2.58 (3H, s, 7-*Me*), 2.41 (3H, s, 2-*Me*), 2.34 (3H, s, 5-*Me*); δ_C 197.91 (s, CO), 158.04 (s, C-5), 152.68 (s, C-2), 151.26 (s, C-2′), 147.24 (s, C-3a), 143.36 (s, C-7), 134.45 (d, C-4′), 131.33 (d, C-6′), 117.02 (d, C-3′), 116.15 (s, C-1′), 114.47 (d, C-5′), 113.07 (s, C-6), 94.06 (d, C-3), 37.81 (t, *C*H_2), 23.26 (q, 5-*Me*), 14.30 (q, 2-*Me*), 13.38 (q, 7-*Me*); *m*/*z* (EI) 294 (33, M^+), 201 (19), 174 (33), 120 (100), 92 (30), 65 (29).

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Stereoselective synthesis of all stereoisomers of vicinal and distal bis(*O*-2-aminoethyl)-*p*-*tert*-butylthiacalix[4]arene

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Abstract—*O*,*O*″- and *O*,*O*′-bis(2-aminoethyl)-*p*-*tert*-butylthiacalix[4]arenes of *anti* conformation have been prepared by the reduction of the corresponding *O*,*O*″- and *O*,*O*′-bis(cyanomethyl) ethers. Their *syn*-*O*,*O*″- and *O*,*O*′-counterparts have been prepared by alternative routes via the Mitsunobu reaction of thiacalix[4]arene with *N*-(2-hydroxyethyl)phthalimide and the reduction of a *O*,*O*′-disiloxanediyl-bridged *O*″,*O*‴-bis(cyanomethyl) ether of 1,2-alternate conformation, respectively. These products are expected to serve as useful precursors of highly elaborated synthetic receptors, including biscalixarenes.

1. Introduction

Calixarenes are one of the most extensively utilized scaffolds in the field of the host–guest chemistry.[1,2] A variety of sophisticated host molecules bearing a calixarene skeleton have been synthesized, which is partly due to the regio- and stereoselective functionalization methods developed for this class of compounds during the last decade.[1,2] Furthermore, calixarenes serve as potential building blocks for designing more elaborate structures like biscalixarenes, which are constructed through upper rim–upper rim,[3] lower rim–lower rim[4] or upper rim–lower rim covalent linkages or, alternatively generated through hydrogen bonding.[5] Recently, two of us reported a series of new biscalixarenes which consisted of two calix[4]arene (**1**) units linked through their lower rims with bridging moieties containing different aromatic or heteroaromatic units.[6] The complexation behavior of these biscalixarenes have been studied towards different metal ions (Na^+, K^+, Ca^{2+}, Pb^{2+} and Ag^+) and it has been found that these biscalixarenes bind silver ions selectively over other metal ions. In our continuing efforts to develop new receptors selective for soft metal ions, especially those of high-environmental loading such as cadmium, lead and mercury, we intended to replace the conventional calix[4]arene units of the biscalixarenes with thiacalix[4]arene **3**,[7] which has been shown to be an attractive host for soft metal ions.[8] However, during the course of the experiments, we have encountered a difficulty in preparing requisite *O*,*O*″-bis(2-aminoethyl)thiacalixarene **5**, as well as its *O*,*O*′-counterpart **6**, with *syn* arrangement of the two aminoethyl groups by applying the procedure used for the methylene-bridged analogs.[6] Thus, one precursor, *O*,*O*″-bis(cyanomethyl)thiacalixarene **7** was in an equilibrium state between the *syn* and *anti* conformational isomers in the solution under ambient conditions and the reduction of the equilibrium mixture with $LiAlH_4$ gave only the *anti* stereoisomer of the desired compound **5**.[9] It is well known that two propyl[1,2] and even two cyanomethyl[10] groups on the phenoxy oxygens of dialkylated calix[4]-arenes (e.g., **2**) are bulky enough to prevent the *syn*–*anti* isomerization via the oxygen-through-the-annulus rotation.[11] Although thiacalixarene **3** has approximately a 10% larger ring radius than the methylene-bridged analog **1**,[12] it has been reported that the dialkylation of **3** with iodopropane gave *syn*-*O*,*O*″-diether **4**,[13] indicating that two propyl groups are large enough to prevent the isomerization even in the case of thiacalix[4]arenes. Therefore, the behavior of bis(cyanomethyl) ether **7** is quite unique. In addition, the outcome of the reduction is of interest from the synthetic point of view, considering the fact that the dialkylation of calix[4]arenes with alkyl halides in the presence of a base preferentially affords *O*,*O*″-isomers of *syn* conformation,[14] by virtue of a circular intramolecular hydrogen bonding in the monoalkylated intermediate, and that the preparation of disubstituted calixarenes of *anti* conformation has, therefore, been the subject to be challenged.[15] *O*,*O*′-Bis(2-aminoethyl) counterpart **6** of *anti* conformation was also obtained as a single stereoisomer by the reduction of an equilibrium mixture of two conformers

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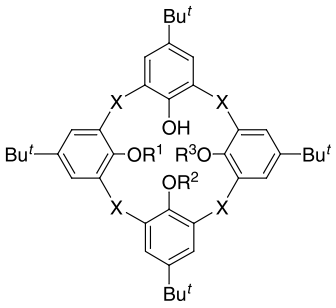
of *O*,*O*′-bis(cyanomethyl) ether **8**. We have succeeded in preparing each *syn* isomer of *O*,*O*″- and *O*,*O*′-bis(2-aminoethyl) ethers by an alternative route via the Mitsunobu reaction[16] or by using a *O*,*O*′-disiloxanediyl-bridged thiacalix[4]arene.[15] Herein, we report the synthesis of all four stereoisomers of bis(*O*-2-aminoethyl)-*p*-*tert*-butylthiacalix[4]arene and the conformational behavior of *O*,*O*″-bis(cyanomethyl) ether **7** in detail.



1: X=CH_2, R^1=R^2=R^3=H
2: X=CH_2, R^1=R^3=CH_2CN, R^2=H
3: X=S, R^1=R^2=R^3=H
4: X=S, R^1=R^3=Pr, R^2=H
5: X=S, R^1=R^3=$C_2H_4NH_2$, R^2=H
6: X=S, R^1=R^2=$C_2H_4NH_2$, R^3=H
7: X=S, R^1=R^3=CH_2CN, R^2=H
8: X=S, R^1=R^2=CH_2CN, R^3=H

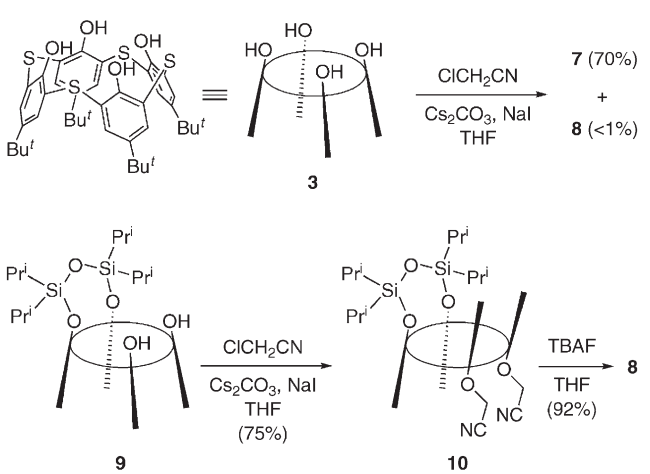
2. Results and discussion

2.1. Synthesis of all four stereoisomers of bis(*O*-2-aminoethyl)-*p*-*tert*-butylthiacalix[4]arene

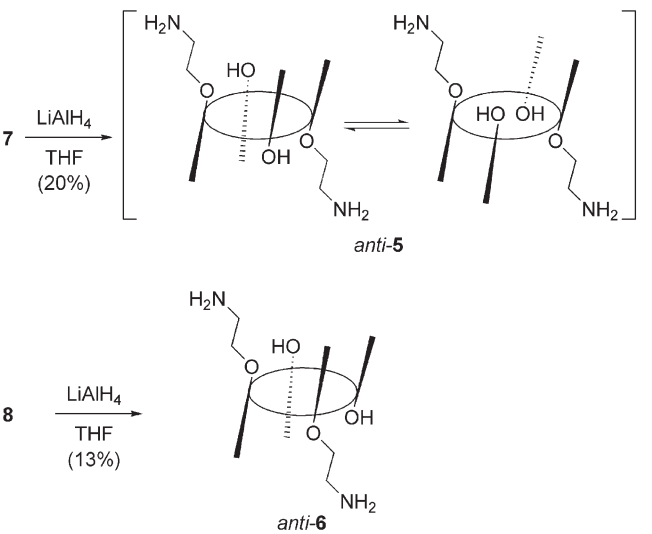
The alkylation of thiacalixarene **3** with chloroacetonitrile conducted under the same conditions as used for the conventional calixarene **1**[10] (**3**/$ClCH_2CN$/K_2CO_3/NaI=1:4:4:4) did not give the desired *O*,*O*″-bis(cyanomethyl) ether **7** but a complex, hardly isolable mixture. However, the reaction of **3** with 3.5 mol equiv. of chloroacetonitrile in the presence of 1 mol equiv. of Cs_2CO_3 and 3 mol equiv. of NaI in refluxing THF gave *O*,*O*″- and *O*,*O*′-bis(cyanomethyl) ethers **7** and **8** in 70 and <1% yields, respectively (Scheme 1). The yield of compound **7** decreased to 58 and 46%, and that of compound **8** increased to 5 and 3% when the reaction was performed using 1 mol equiv. of Na_2CO_3 and K_2CO_3, respectively. Thus, the choice of the base was important for the *O*,*O*″-distal dialkylation of thiacalixarene **3**. It was reported that the methylene-bridged analog **2** obtained by a similar etherification of conventional calixarene **1** adopted *syn* form in a cone conformation.[10] However, compound **7**, as well as compound **8**, was found to be in an equilibrium state between two conformers in the solution as discussed later.

Recently, we reported that 1,1,3,3-tetra(isopropyl)disiloxane-1,3-diyl (TIPDS) moiety was quite useful as a protective group for the proximal *O*,*O*′-dialkylation of calix[4]arenes.[15] It has been shown that the dialkylation of the TIPDS derivative of thiacalixarene **9** with alkyl halides in the presence of a base proceeds with high stereoselectivity to give, after removal of the TIPDS moiety, *syn*-*O*,*O*′-dialkylated products. The method was applied to prepare *O*,*O*′-bis(cyanomethyl) ether **8** (Scheme 1). Thus, treatment of **9** with chloroacetonitrile using Cs_2CO_3 as a base in THF gave *O*″,*O*‴-bis(cyanomethyl) ether **10** in excellent yield. The ^{1}H NMR spectrum of compound **10** showed two singlets for the *tert*-butyl protons (18H each) and four doublets for the aromatic protons (2H each), the magnetic equivalences suggesting C_s symmetric structure, that is, cone or 1,2-alternate conformation with the *syn* arrangement of the two cyanomethyl groups. As reported previously,[15] TIPDS derivatives of 1,2-alternate conformation can be distinguished from those of the other conformations by ^{1}H NMR spectrum, where some of the methyl protons of the TIPDS moiety are strongly shielded by the facing benzene rings, appearing around 0.4 ppm. The methyl signals of compound **10** appeared at 0.40, 0.80, 1.03 and 1.05 (6H each), which clearly assigned the conformation to be 1,2-alternate. Desilylation of **10** with tetrabutylammonium fluoride (TBAF) in THF liberated *O*,*O*′-bis(cyanomethyl) ether **8** in almost quantitative yield (Scheme 1).

Now that both the doubly cyanomethylated thiacalixarenes **7** and **8** were in hand in substantial quantities, their reduction to bis(2-aminoethyl) ethers **5** and **6** was examined (Scheme 2). Interestingly, treatment of the equilibrium mixture of two conformers **7** with $LiAlH_4$ in THF at 0 °C gave only one stereoisomer of *O*,*O*″-bis(2-aminoethyl) ether **5** though in 20% yield with concomitant formation of thiacalixarene **3** (40%) and its mono(2-aminoethyl) ether (25%). The stereochemistry of **5** was assigned to be *anti* by comparison of its ^{1}H NMR spectrum with that of *syn*-**5**



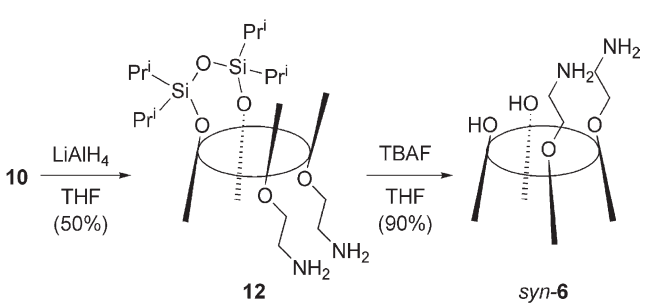
Scheme 1.



Scheme 2.

prepared by an alternative route via the Mitsunobu reaction (vide infra). The ^{1}H NMR spectrum of *anti*-**5** showed two singlets (18H each) for the *tert*-butyl protons and two singlets (4H each) for the aromatic protons, the magnetic equivalences suggesting that the compound was in an equilibrium state between two 1,2-alternate conformations. This was supported by the chemical shift values of the NCH_2 [one triplet (4H) at δ 2.40] and OCH_2 protons [one triplet (4H) at δ 3.82], which appeared considerably upfield similarly to those of compound **12** of 1,2-alternate conformation (δ 2.07–2.37, 3.66–3.77, respectively) (vide infra), indicating anisotropic shielding effects by the facing benzene rings. The reduction of the *O*,*O*′-bis(cyanomethyl) counterpart **8** also gave a single stereoisomer of *O*,*O*′-bis-(2-aminoethyl) ether **6**, the stereochemistry of which was assigned to be *anti* by comparison of its ^{1}H NMR spectrum with that of *syn*-**6** prepared by the reduction of the disiloxane-bridged bis(cyanomethyl) ether **10** (vide infra). The conformation of *anti*-**6** was determined to be 1,2-alternate based on the splitting patterns of the *tert*-butyl (two singlets, 18H each) and aromatic protons (four doublets, 2H each), combined with the upfield shifts of the NCH_2 (δ 2.26–2.32 and 2.44–2.48) and OCH_2 (δ 3.71–3.76 and 3.94–3.98) protons as observed for *anti*-**5** in the ^{1}H NMR spectrum.

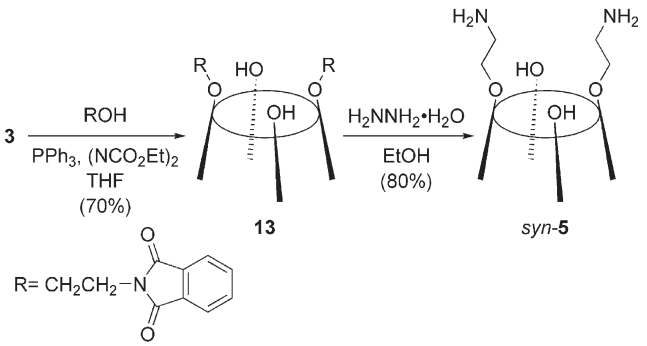
Both *O*,*O*″- and *O*,*O*′-bis(2-aminoethyl) ethers of *anti* conformation were thus obtained by the reduction of the corresponding conformationally mobile bis(cyanomethyl) ethers. Although the yields were only moderate, provision of the convenient route to the *anti*-di-*O*-substituted thiacalix[4]arenes should be noted. Although the precise reaction mechanisms for the obtainment of only *anti*-isomers is not clear at present, it may be said that the two cyanomethyl substituents flexibly arranged in *syn*-conformation may chelate tightly to the aluminum center to inhibit the expected reduction to aminoethyl moiety. On the other hand, bulky TIPDS group imposes substantial steric congestion on the calix framework,[15] which may allow the normal reduction of the *syn*-arranged cyanomethyl groups of compound **10**; we were pleased to know that the reduction actually proceeded smoothly to give bis-(2-aminoethyl) ether **12** in 50% yield after chromatographic purification (Scheme 3). The conformation of compound **12** was unambiguously determined to be 1,2-alternate with the *syn* arrangement of the two aminoethyl moieties, according to the same criteria as described for compound **10** (vide supra). Compound **12** on desilylation by treatment with TBAF liberated *syn*-**6** in 90% yield. The conformation of *syn*-**6** was determined to be cone based on the splitting



Scheme 3.

patterns of the *tert*-butyl (two singlets, 18H each) and aromatic protons (four doublets, 2H each), which suggested cone or 1,2-alternate conformation, combined with the chemical shift values of the NCH_2 (δ 3.59–3.63 and 3.93–3.95) and OCH_2 protons (δ 4.30–4.32 and 4.86–4.87); if the compound adopted 1,2-alternate conformation, these protons should appear at a higher field as those of *anti*-**5** (vide supra).

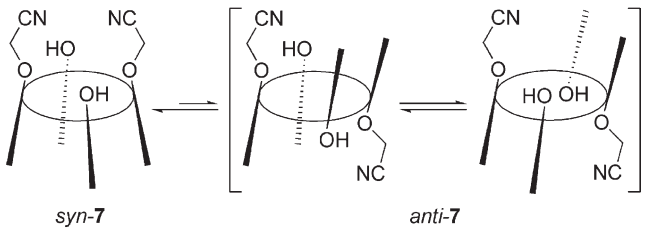
The *syn* isomer of *O*,*O*″-bis(2-aminoethyl) ether **5** could be prepared by an alternative route via the Mitsunobu reaction of thiacalixarene **3** with *N*-(2-hydroxyethyl)phthalimide, which gave *O*,*O*″-bis(2-phthalimidoethyl) ether **13** (Scheme 4). The hydrazinolysis of **13** in ethanol gave **5** in 80% yield. The *syn* conformation of compound **5**, as well as compound **13**, was deduced from an X-ray crystallographic analysis of a biscalix[4]arene prepared from compound **5**.[17] Thus, synthetic methods for all four stereoisomers of bis(*O*-2-aminoethyl)-*p*-*tert*-butylthiacalix[4]arene have now been provided. As thiacalix[4]arenes lack methylene bridges, which have been a probe for assigning the conformation of conventional calix[4]arenes in the NMR analysis,[1,18] it is sometimes difficult to elucidate their conformations from the NMR spectra. Compounds *syn*-**5** and **13** showed two singlets (18H each) for the *tert*-butyl protons and two singlets (4H each) for the aromatic protons in the ^{1}H NMR spectra, which suggested C_{2v}-symmetric structure, that is, cone or 1,3-alternate. Although these compounds are expected to adopt cone conformation by virtue of a circular intramolecular hydrogen bonding at the lower rim, the possibility of 1,3-alternate conformation cannot completely be ruled out.



Scheme 4.

2.2. Conformational analysis of *O*,*O*″-bis(cyanomethyl)-*p*-*tert*-butylthiacalix[4]arene (7)

The ^{1}H NMR spectrum of *O*,*O*″-bis(cyanomethyl) ether **7** revealed that it was a mixture of two isomers, the ratio being 68:32 in $CDCl_3$. Each isomer showed two singlets (18H each) for the *tert*-butyl protons and two singles (4H each) for the aromatic protons, the magnetic equivalences suggesting C_{2v}-symmetric structure. Thus, both may be assigned to *syn* isomers which adopt cone and 1,3-alternate conformations, respectively. Alternatively, one may be assigned to a *syn* and the other to an *anti* isomer, in the latter of which the phenol units rapidly interconvert via the oxygen-through-the-annulus rotation (Scheme 5). The OCH_2 protons of the minor isomer appeared considerably upfield (δ 4.64) similarly to those of compound **10** (δ 4.41),



Scheme 5.

which determined the conformation of the minor isomer to be 1,2-alternate with the *anti* arrangement of the two cyanomethyl groups. This conformation has also been found in the crystal structure of *O*,*O*″-dimethylthiacalix[4]arene.[19] The major isomer, which showed the OCH_2 signal at δ 5.44, was expected to adopt cone conformation by virtue of a circular intramolecular hydrogen bonding at the lower rim. The question now arises whether these isomers are stable enough to be isolated as in the case of conventional calix[4]arene,[10] or exist as an equilibrium mixture. It has been reported that tetrapropyl ether of thiacalixarene **3** gradually isomerizes in refluxing $CHCl_2CHCl_2$.[20] Actually, *syn*-*O*,*O*″-dipropyl ether **4** was found to isomerize under the same conditions to give, after 48 h, a 16:1 mixture of the *syn* and *anti* isomers. On the other hand, compound **7**, bearing smaller substituents than **4**, did not show any change after the same treatment. This indicates that the cyanomethyl group can pass through the thiacalix[4]arene annulus even at room temperature and that the two isomers of **7** are in an equilibrium state. Interestingly, the ratio of the isomers was found to change from 84:16 in $CDCl_2CDCl_2$, via 68:32 in $CDCl_3$ and 66:34 in THF-d_8, to 58:42 in DMSO-d_6 at room temperature. This means that the equilibrium shifts toward the *anti* isomer with increasing the solvent polarity, showing the importance of the circular intramolecular hydrogen bonding in the cone conformation.[21]

Compound **7**, which is conformationally mobile in solution, however, crystallized out in a pinched cone conformation with the *syn* arrangement of the two cyanomethyl groups, as is clear from the X-ray crystallographic analysis (Fig. 1): the two benzene rings (B and D) bearing a cyanomethyl moiety are almost parallel to each other and the two phenolic rings (A and C) are tilted so as to place the hydroxy groups inside the macrocycle in such a way that each hydroxy proton (H_A or H_C) forms hydrogen bondings with the same etheral oxygen (O_B) and with one bridging sulfur atom (S_1 or S_2), the bond lengths of H_A-O_B, H_C-O_B, H_A-S_1, and H_C-S_2 being 2.57, 2.28, 2.43, and 2.49 Å, respectively. The former

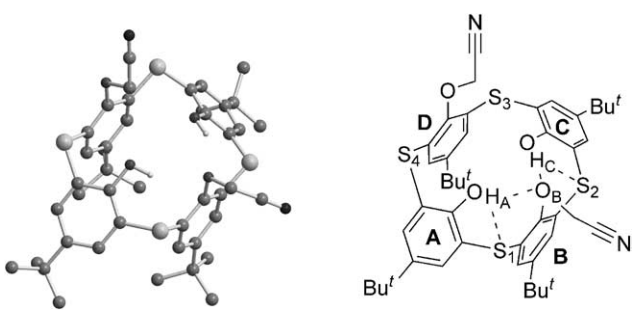


Figure 1. X-ray structure and its schematic view of compound **7**. H atoms except for OH groups are omitted for clarity.

type of asymmetric hydrogen boding between two hydroxy groups and only one ethereal oxygen is unique in calixarene chemistry. Interestingly, one of the two methylene moieties is oriented inside the macrocycle, while the other outside. The irregular inward orientation will be attributed to some packing forces.

The ^1H NMR spectrum of *O*,*O*′-bis(cyanomethyl) counterpart **8** also showed the presence of two conformers, the ratio being 67:33 in $CDCl_3$, 54:46 in $CDCl_2CDCl_2$, 70:30 in THF-d_8 and 43:57 in DMSO-d_6 at room temperature. Each conformer showed two singlets (18H each) for the *tert*-butyl protons and four doublets (2H each) for the aromatic protons, the OCH_2 signals appearing at δ 4.79 and 4.89 for one isomer and at δ 4.65 and 5.13 for the other, from which their conformations could not be deduced. However, it is apparent that the cyanomethyl group can pass through the thiacalix[4]arene annulus also in this proximally disubstituted case, considering the fact that *syn*-bis(cyanomethyl) ether **10** gave *anti*-bis(2-aminoethyl) ether **6** via compound **8**.

3. Conclusion

We have shown here the synthetic methods for all the stereoisomers of bis(*O*-2-aminoethyl)-*p*-*tert*-butylthiacalix[4]arenes. These compounds can be used as precursors for preparing a variety of new receptors, including biscalix[4]arenes, which may selectively recognize different types of cations, anions or salts. At present, work is in progress to prepare new biscalix[4]arenes in this laboratory.

4. Experimental

4.1. General

Melting points were taken using a Mitamura Riken MP-P apparatus. Microanalyses were carried out in the Microanalytical Laboratory of the Institute of Multidisciplinary Research for Advanced Materials, Tohoku University. IR spectra were recorded on a Shimadzu FTIR-8300 spectrometer. ^1H and ^{13}C NMR were recorded on a Bruker DPX-400 or DRX-500 spectrometer using tetramethylsilane (^1H NMR) or chloroform (^{13}C NMR) as an internal standard and $CDCl_3$ as a solvent. FAB mass spectra were recorded on a JEOL JMS-GCmate mass spectrometer using *m*-nitrobenzyl alcohol as a matrix. All reagents and solvents were obtained from commercial suppliers and used without further purification. Merck silica gel $60GF_{254}$ was used for TLC. Silica gel columns were prepared by use of Merck silica gel 60 (63–200 μm).

4.2. Synthesis of bis(*O*-2-aminoethyl)-*p*-*tert*-butylthiacalix[4]arenes

4.2.1. 5,11,17,23-Tetra-*tert*-butyl-25,27-bis(cyanomethoxy)-26,28-dihydroxythiacalix[4]arene (7). A mixture of *p*-*tert*-butylthiacalix[4]arene (**3**) (2.00 g, 2.77 mmol), chloroacetonitrile (745 mg, 9.87 mmol), NaI (1.25 g, 8.34 mmol) and Cs_2CO_3 (904 mg, 2.77 mmol) in dry THF (40 ml) was

stirred and heated under reflux for 7 days. After cooling, the mixture was quenched with 2 M HCl and extracted with chloroform. The chloroform layer was dried over anhydrous $MgSO_4$ and evaporated to dryness. The residue was purified by column chromatography with hexane–ethyl acetate (8:2) as the eluent to give bis(cyanomethyl) ether **7** (1.56 g, 70%), mp 305 °C; ^{1}H NMR (500 MHz) δ 0.82, 1.26 [18H: s, $C(CH_3)_3$×2 (major); s, $C(CH_3)_3$×2 (minor)], 1.34 [18H, s, $C(CH_3)_3$×2], 4.64, 5.44 [4H: s, OCH_2×2 (minor); s, OCH_2×2 (major)], 6.98, 7.14 [4H: s, ArH (major); s, ArH (minor)], 7.46, 7.69 [4H: s, ArH (minor); s, ArH (major)]; ^{13}C NMR (100 MHz) δ 30.5, 30.7, 30.9, 31.1, 31.2, 31.3, 31.4, 31.5, 31.7, 34.1, 34.3, 34.7, 56.6, 58.7, 114.6, 115.2, 119.2, 121.5, 128.6, 129.1, 131.7, 132.4, 132.9, 133.6, 134.2, 134.9, 143.6, 143.7, 149.7, 154.5, 155.2; FAB-MS *m/z* 798 (M^+). Anal. calcd for $C_{44}H_{50}N_2O_4S_4$: C, 66.13; H, 6.31. Found: C, 66.09; H, 6.12.

4.2.2. 5,11,17,23-Tetra-*tert*-butyl-25,26-bis(cyanomethoxy)-27,28-(2,2,4,4-tetraisopropyl-1,3,5-trioxa-2,4-disilapentane-1,5-diyl)thiacalix[4]arene (10). To a solution of disiloxane-bridged thiacalix[4]arene **9**[15] (5.00 g, 5.19 mmol) in dry THF (100 ml) were added Cs_2CO_3 (10.1 g, 31.0 mmol), chloroacetonitrile (2.35 g, 31.1 mmol) and NaI (4.67 g, 31.2 mmol). After refluxing for 65 h, the mixture was cooled to 0 °C, diluted with 2 M HCl and extracted with chloroform. The organic layer was washed with water and dried over anhydrous $MgSO_4$. After the solvent was evaporated, the residue was purified by column chromatography with hexane–ethyl acetate (9:1) as the eluent to give bis(cyanomethyl) ether **10** (4.06 g, 75%), mp 285–287 °C; ^{1}H NMR (400 MHz) δ 0.40 (6H, d, *J*=7.6 Hz, $CHCH_3$×2), 0.72–0.82 (2H, m, $CHCH_3$×2), 0.80 (6H, d, *J*=7.6 Hz, $CHCH_3$×2), 1.03 (6H, d, *J*=7.2 Hz, $CHCH_3$×2), 1.05 (6H, d, *J*=7.2 Hz, $CHCH_3$×2), 1.14–1.28 (2H, m, $CHCH_3$×2), 1.28 [18H, s, $C(CH_3)_3$×2], 1.35 [18H, s, $C(CH_3)_3$×2], 4.41 (4H, s, OCH_2×2), 7.36 (2H, d, *J*=2.4 Hz, ArH), 7.55 (2H, d, *J*=2.4 Hz, ArH), 7.60 (2H, d, *J*=2.4 Hz, ArH), 7.81 (2H, d, *J*=2.4 Hz, ArH). Anal. calcd for $C_{56}H_{76}N_2O_5S_4Si_2$: C, 64.57; H, 7.35; N, 2.69. Found: C, 64.80; H, 7.41; N, 3.00.

4.2.3. 5,11,17,23-Tetra-*tert*-butyl-25,26-bis(cyanomethoxy)-27,28-dihydroxythiacalix[4]arene (8). To a solution of disiloxane-bridged bis(cyanomethyl) ether **10** (312 mg, 0.300 mmol) in THF (15 ml) was added 1.0 M solution of TBAF in THF (0.30 ml, 0.30 mmol) at room temperature. After stirring for 1 h, the mixture was cooled to 0 °C, diluted with 2 M HCl and extracted with chloroform. The organic layer was washed with water and dried over anhydrous $MgSO_4$. After the solvent was evaporated, the residue was crystallized from chloroform–ethanol to give bis(cyanomethyl) ether **8** (221 mg, 92%), mp 195–200 °C; ^{1}H NMR (400 MHz) δ 1.10, 1.24 [18H: s, $C(CH_3)_3$×2 (minor); s, $C(CH_3)_3$×2 (major)], 1.21, 1.29 [18H: s, $C(CH_3)_3$×2 (minor); s, $C(CH_3)_3$×2 (major)], 4.65, 4.79 [2H: d, *J*=16 Hz, OCH×2 (minor); d, *J*=15 Hz, OCH×2 (major)], 4.89, 5.13 [2H: d, *J*=15 Hz, OCH×2 (major); d, *J*=16 Hz, OCH×2 (minor)], 7.27, 7.48 [2H: d, *J*=2.4 Hz, ArH (minor); d, *J*=2.4 Hz, ArH (major)], 7.42, 7.53 [2H: d, *J*=2.4 Hz, ArH (minor); d, *J*=2.4 Hz, ArH (major)], 7.50, 7.57 [2H: d, *J*=2.4 Hz, ArH (minor); d, *J*=2.5 Hz, ArH (major)], 7.55, 7.64 [2H: d, *J*=2.4 Hz, ArH (minor); d, *J*=2.5 Hz, ArH (major)]; FAB-MS *m/z* 798 (M^+). Anal. calcd for $C_{44}H_{50}N_2O_4S_4{\cdot}0.5H_2O$: C, 65.39; H, 6.36. Found: C, 65.65; H, 6.23.

4.2.4. *anti*-25,27-Bis(2-aminoethoxy)-5,11,17,23-tetra-*tert*-butyl-26,28-dihydroxythiacalix[4]arene (*anti*-5). $LiAlH_4$ (82 mg, 2.2 mmol) was added in small portions to a stirred solution of bis(cyanomethyl) ether **7** (300 mg, 0.375 mmol) in THF (20 ml) at 0 °C. The reaction was monitored by TLC and after the completion of the reaction, the excess of $LiAlH_4$ was carefully destroyed by adding 15% wet benzene. The mixture was filtered and the filtrate evaporated to leave a residue, which was purified by column chromatography with chloroform–ethanol (8:2) as the eluent to give bis(2-aminoethyl) ether *anti*-**5** (60.7 mg, 20%), mp 260 °C; ^{1}H NMR (400 MHz) δ 1.27 [18H, s, $C(CH_3)_3$×2], 1.32 [18H, s, $C(CH_3)_3$×2], 2.40 (4H, t, *J*=4.8 Hz, NCH_2×2), 3.82 (4H, t, *J*=4.8 Hz, OCH_2×2), 7.47 (4H, s, ArH), 7.51 (4H, s, ArH); FAB-MS *m/z* 806 (M^+). Anal. calcd for $C_{44}H_{58}N_2O_4S_4$: C, 65.47; H, 7.24. Found: C, 65.38; H, 7.17.

4.2.5. *anti*-25,26-Bis(2-aminoethoxy)-5,11,17,23-tetra-*tert*-butyl-27,28-dihydroxythiacalix[4]arene (*anti*-6). $LiAlH_4$ (82 mg, 2.2 mmol) was added in small portions to a stirred solution of bis(cyanomethyl) ether **8** (300 mg, 0.375 mmol) in THF (20 ml) at room temperature. The reaction was monitored by TLC and after the completion of the reaction, the excess of $LiAlH_4$ was carefully destroyed by adding 15% wet benzene. The mixture was filtered and the filtrate evaporated to give a residue, which was purified by column chromatography with chloroform–ethanol (9:1) as the eluent to give bis(2-aminoethyl) ether *anti*-**6** (39.5 mg, 13%), mp 240 °C; ^{1}H NMR (400 MHz) δ 1.24 [18H, s, $C(CH_3)_3$×2], 1.30 [18H, s, $C(CH_3)_3$×2], 2.26–2.32 (2H, m, NCH×2), 2.44–2.48 (2H, m, NCH×2), 3.71–3.76 (2H, m, OCH×2), 3.94–3.98 (2H, m, OCH×2), 7.41 (2H, d, *J*=2.5 Hz, ArH), 7.48 (2H, d, *J*=2.5 Hz, ArH), 7.53 (2H, d, *J*=2.5 Hz, ArH), 7.60 (2H, d, *J*=2.5 Hz, ArH); FAB-MS *m/z* 806 (M^+). Anal. calcd for $C_{44}H_{58}N_2O_4S_4$: C, 65.47; H, 7.24; N, 3.47. Found: C, 65.32; H, 7.03; N, 3.23.

4.2.6. 25,26-Bis(2-aminoethoxy)-5,11,17,23-tetra-*tert*-butyl-27,28-(2,2,4,4-tetraisopropyl-1,3,5-trioxa-2,4-disilapentane-1,5-diyl)thiacalix[4]arene (12). $LiAlH_4$ (112 mg, 2.95 mmol) was added in small portions to a stirred solution of bis(cyanomethyl) ether **10** (500 mg, 0.480 mmol) in a 1:1 mixture of dry THF and diethyl ether (20 ml) at room temperature. The reaction was monitored by TLC and after the completion of the reaction, the excess of $LiAlH_4$ was carefully destroyed by adding 15% wet benzene. The mixture was filtered and the filtrate evaporated to leave a residue, which was purified by column chromatography with chloroform–ethanol (9:1) as the eluent to give bis(2-aminoethyl) ether **12** (252 mg, 50%), mp 238–240 °C; ^{1}H NMR (400 MHz): δ 0.41 (6H, d, *J*=7.5 Hz, $CHCH_3$×2), 0.77 (6H, d, *J*=7.5 Hz, $CHCH_3$×2), 0.86 (2H, sept, $CHCH_3$×2), 1.01 (6H, d, *J*=7.5 Hz, $CHCH_3$×2), 1.08 (6H, d, *J*=7.5 Hz, $CHCH_3$×2), 1.18 (2H, sept, $CHCH_3$×2), 1.28 [18H, s, $C(CH_3)_3$×2], 1.34 [18H, s, $C(CH_3)_3$×2], 2.07–2.13 (2H, m, NCH×2), 2.31–2.37 (2H, m, NCH×2), 3.66–3.77 (4H, m, OCH_2×2), 7.34 (2H, d, *J*=2.5 Hz, ArH), 7.55 (2H, d, *J*=2.5 Hz, ArH), 7.58 (2H, d,

J=2.6 Hz, ArH), 7.75 (2H, d, J=2.6 Hz, ArH). Anal. calcd for $C_{56}H_{84}N_2O_5S_4Si_2$: C, 64.07; H, 8.07; N, 2.67. Found: C, 64.36; H, 8.29; N, 2.93.

4.2.7. *syn*-25,26-Bis(2-aminoethoxy)-5,11,17,23-tetra-*tert*-butyl-27,28-dihydroxythiacalix[4]arene (*syn*-6). To a solution of disiloxane-bridged bis(2-aminoethyl) ether **12** (200 mg, 0.191 mmol) in THF (20 ml) was added a 1.0 M solution of TBAF in THF (0.2 ml, 0.2 mmol) at room temperature. After stirring for 1 h, the mixture was cooled to 0 °C, diluted with 2 M HCl and extracted with chloroform. The organic layer was washed with water and dried over anhydrous $MgSO_4$. After the solvent was evaporated, the residue was crystallized from chloroform–ethanol to give bis(2-aminoethyl) ether *syn*-**6** (139 mg, 90%), mp 280 °C; ^{1}H NMR(400 MHz) δ 0.95 [18H, s, $C(CH_3)_3$×2], 1.13 [18H, s, $C(CH_3)_3$×2], 3.59–3.63 (2H, br m, NCH×2), 3.93–3.95 (2H, br m, NCH×2), 4.30–4.32 (2H, m, OCH×2), 4.86–4.87 (2H, br m, OCH×2), 7.10 (2H, d, J=2.4 Hz, ArH), 7.17 (2H, d, J=2.4 Hz, ArH), 7.39 (2H, d, J=2.4 Hz, ArH), 7.48 (2H, d, J=2.4 Hz, ArH); FAB-MS m/z 806 (M^+). Anal. calcd for $C_{44}H_{58}N_2O_4S_4$: C, 65.47; H, 7.24. Found: C, 65.21; H, 7.55.

4.2.8. 5,11,17,23-Tetra-*tert*-butyl-25,27-dihydroxy-26,28-bis(2-phthalimidoethoxy)thiacalix[4]arene (13). To an ice-cold mixture of compound **3** (721 mg, 1.00 mmol), *N*-(2-hydroxyethyl)phthalimide (1.75 g, 8.57 mmol) and triphenylphosphine (800 mg, 3.05 mmol) in THF (20 ml) was added dropwise diethyl azodicarboxylate (531 mg, 3.05 mmol) and the mixture was stirred at room temperature for 48 h. The solvent was evaporated and the residue was triturated with hot methanol to give a solid, which was purified by column chromatography with hexane–ethyl acetate (8:2) as the eluent to give bis(2-phthalimidoethyl) ether **13** (748 mg, 70%), mp 288–290 °C; ^{1}H NMR (400 MHz) δ 0.72 [18H, s, $C(CH_3)_3$×2], 1.27 [18H, s, $C(CH_3)_3$×2], 4.44 (4H, t, J=5.6 Hz, NCH_2×2), 4.87 (4H, t, J=5.6 Hz, OCH_2×2), 6.81 (4H, s, ArH), 7.24 (2H, s, OH), 7.47 (4H, s, ArH), 7.55–7.57 (4H, m, phthalimide ArH), 7.82–7.84 (4H, m, phthalimide ArH); FAB-MS m/z 1066 (M^+). Anal. calcd for $C_{60}H_{62}N_2O_8S_4{\cdot}0.5H_2O$: C, 66.95; H, 5.90; N, 2.60. Found: C, 67.08; H, 5.94; N, 2.58.

4.2.9. *syn*-25,27-Bis(2-aminoethoxy)-5,11,17,23-tetra-*tert*-butyl-26,28-dihydroxythiacalix[4]arene (*syn*-5). A solution of bis(2-phthalimidoethyl) ether **13** (1.15 g, 1.08 mmol) and hydrazine monohydrate (100 mg, 2.00 mmol) in ethanol (20 ml) was heated at 110 °C for 12 h. The ethanol was removed under reduced pressure and the residue was taken in chloroform, washed with 20% NH_4OH and dried over anhydrous $MgSO_4$. Removal of the solvent and crystallization from chloroform–ethanol furnished bis(2-aminoethyl) ether *syn*-**5** (696 mg, 80%), mp 298–300 °C; ^{1}H NMR (400 MHz) δ 0.88 [18H, s, $C(CH_3)_3$×2], 1.30 [18H, s, $C(CH_3)_3$×2], 3.32 (4H, t, J=5.6 Hz, NCH_2×2), 4.51 (4H, t, J=5.6 Hz, OCH_2×2), 7.11 (4H, s, ArH), 7.65 (4H, s, ArH); FAB-MS m/z 806 (M^+). Anal. calcd for $C_{44}H_{58}N_2O_4S_4$: C, 65.47; H, 7.24; N, 3.47. Found: C, 65.21; H, 7.02; N, 3.13.

4.3. X-ray analysis of compound 7

Data were collected on a Rigaku/MSC Mercury CCD diffractometer with monochromated Mo Kα radiation. The structure was solved by the direct methods and refined by the full-matrix least-squares method. Calculations were performed using the software package teXsan (v 1.10). Crystal data: $C_{44}H_{50}N_2O_4S_4$, M=799.13, monoclinic, a=12.976(3) Å, b=18.603(4) Å, c=18.335(4) Å, β=105.125(5)°, V=4272(1) Å^3, T=223 K, space group $P2_1/n$, Z=4, μ(Mo Kα)=2.65 cm^{-1}, 33,759 reflections measured, 11,723 unique (R_{int}=0.035). Final R_1=0.040, wR_2=0.043 for 5156 observed reflections data [$I>3\sigma(I)$]. GOF=0.71. The details of the crystal data have been deposited with Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-226829.

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Iminoiodane mediated aziridination of α-allylglycine: access to a novel rigid arginine derivative and to the natural amino acid enduracididine

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Abstract—The synthesis of fully protected aminodihydrohistidines in optically pure form is described starting from allylglycine derivatives. These compounds represent novel conformationally constrained analogues of arginine, one of them being, in addition, a protected form of the marine natural product, enduracididine. The key step of the strategy is a one-pot copper-catalyzed aziridination of *t*-butyl (*S*)-*N*-(9-phenyl-9*H*-fluoren-9-yl)allylglycinate ((*S*)-**16**) with 2-trimethylsilylethanesulfonamide in the presence of iodosylbenzene.

The conformational restriction of flexible bioactive molecules is a well known technique for increasing their intrinsic activity or their selectivity for a particular receptor subtype or enzyme isoform.[1,2] Such rigid ligands can also lead to greater lipophilicity and/or increased stability toward metabolic enzymes, both factors contributing to improved bioavailability of a given active substance. A case in point is arginine (**1**), the endogenous substrate of NO synthase, of which a number of conformationally restricted analogues have been prepared over the past years with these considerations in mind. Such rigid analogues have generally taken three forms. In the first, the essential guanidine function (or an isosteric equivalent) is incorporated in a chain-terminating heterocycle. These include, for instance, the N^{δ}–N^{ω} ethylene bridged analogue **2**[3] and the 2-aminopyrimidine derivative **3**[4] (Fig. 1).

In the second class of compounds, the 3-carbon tether is locked into a more rigid conformation either by introduction of a double bond (i.e. **4**)[5] or by incorporation as a ring (i.e., the guanidinophenylalanine derivative **5**).[5,6] Another approach to rigid arginine analogues consists in linking one of the nitrogen atoms of the guanidine functionality to one of the methylene groups. One such molecule is the piperidine derivative **6**, designed in this case to be a specific thrombin inhibitor.[7] Alternatively, a compound in which the terminal amino function is bonded to the methylene backbone would force arginine to adopt a highly folded conformation as opposed to the extended conformations exhibited by compounds **2**–**6**. Interestingly, such a compound, the aminodihydrohistidine **7** (enduracididine), has been isolated from natural sources. Thus, **7** was identified in 1968 as a component of a peptide antibiotic enduracidin,[8a] itself isolated from *Streptomyces fungicidicus*.[8b,c] Several years later, enduracididine was also shown to be a component of the antibiotic minosaminomycin, isolated from a plant source, *Lonchocarpus*

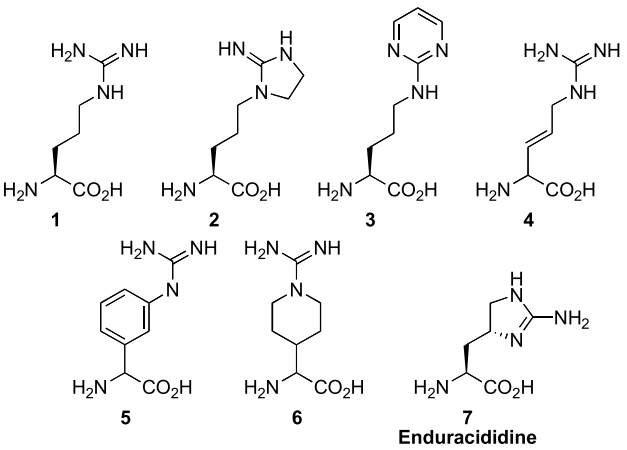

Figure 1. Arginine and rigid derivatives.

Keywords: Arginine mimetic; Allylglycine; Aziridine; Aziridination; Iminoiodane.
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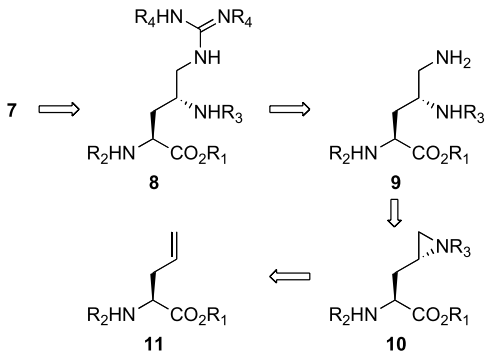
sericeus.[9] More recently, this amino acid has been identified in the cytotoxic fraction of extracts of a marine ascidian, *Leptoclinides dubius*.[10] While it has been reported that the structure of enduracididine was established by X-ray crystallography,[8a] the only published preparation of this compound was by way of a Bamberger cleavage of methyl histidinate which yielded a 1:1 mixture of enduracididine and its (4*S*) isomer.[11]

Thus, with the double incentive of preparing a new conformationally restricted arginine analogue and a natural product, we set about to develop a synthesis of enduracididine (in fact, a protected form of this molecule suitable for insertion in small biologically active peptide derivatives to furnish novel peptidomimetics). A retrosynthetic analysis of this compound (Scheme 1) suggested that it could be obtained from the guanidino derivative **8**, which in turn could be constructed from the diamine **9** for which purpose a variety of reagents are available.[12] The diamino compound **9** could then be prepared by amine (or equivalent) promoted opening of the aziridine **10** itself formed by diastereoselective aziridination of L-allylglycine **11**. The key step in this pathway then was the preparation of aziridine **10** from a suitably protected allylglycine derivative.

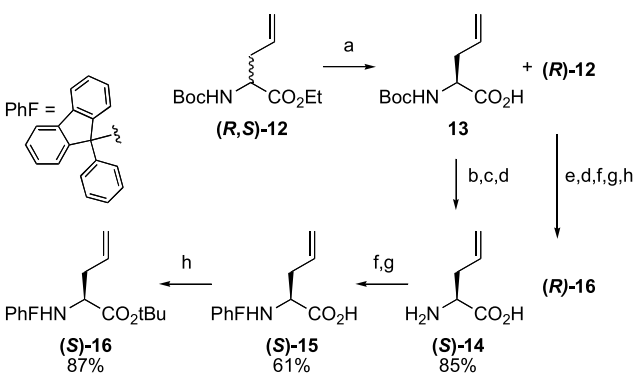


Scheme 1. Retrosynthetic analysis.

Interestingly, while both the epoxide[13] and cyclopropane[14] analogues of aziridine **10** have been described, derivatives of **10** itself have not.[15] A particularly attractive way of achieving this would be by application of the iminoiodane-mediated copper (I or II)-catalyzed aziridination of olefins.[16,17] In addition, the iminoiodane derived from trimethylsilylethanesulfonamide ($SesNH_2$) seemed particularly suited to this purpose as the Ses protecting group can be removed under mild conditions (F^-) avoiding possible racemization of the amino acid.[18] On the other hand, the application of this copper-catalyzed aziridination procedure to a nitrogen-containing olefinic substrate presented a certain challenge since it could be anticipated that the copper I or II salt would be sequestered by this nitrogen atom, thereby inhibiting the desired reaction. Indeed, while the copper-catalyzed iminoiodane-mediated aziridination procedure has been successfully used recently as a key step in the total synthesis of a number of natural products starting from non-nitrogenous substrates,[17] only very few examples[19,20] have been reported of this reaction being applied to nitrogen-containing starting materials. In this paper then, we report the results of our study in this regard and subsequent application to the synthesis of protected enduracididine **7**.

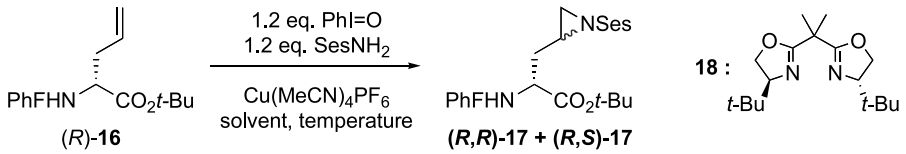
The optically pure starting allylglycine substrates were prepared by enzymatic resolution of racemic ethyl *N*-Boc-allylglycinate **12** using α-chymotrypsin (Scheme 2).[21] This procedure afforded (*S*)-*N*-Boc-allylglycine **13** with 98% optical purity and the unreacted isomer (*R*)-**12** (ee=94%). The choice of protecting groups for the carboxylic acid and amine functionalities of **13** was made based on the following considerations. Firstly, since in the retrosynthetic scheme we planned to generate a diamino intermediate **9**, protection of the carboxylic acid with a bulky *t*-butyl group was deemed necessary to prevent lactamization during this step. Secondly, while the *N*-Boc group may be considered both sufficiently bulky and electron-withdrawing to minimize the aforementioned possibility of copper sequestration during the aziridination step, preliminary experiments showed that the yield of aziridination products was very low.[22] The phenylfluorenyl group was considered a suitable choice in this case, combining both a large steric hindrance around the nitrogen atom with the possibility of selective removal. Thus, the Boc protecting group of compound **13** was removed by treatment with trifluoroacetic acid in dichloromethane (Scheme 2). The resulting free amino acid (*S*)-**14** was then transiently esterified by reaction with trimethylsilyl chloride. Treatment of the product in situ with 9-bromo-9-phenylfluorene in the presence of lead (II) nitrate[23] then provided after methanolic work-up the *N*-phenylfluorenyl (NPhF)-protected derivative (*S*)-**15**. Finally, reaction of the latter with *t*-butyl 2,2,2-trichloroacetimidate[24] yielded the desired fully protected (*S*)-allylglycine **16**. Similar treatment of ethyl (*R*)-*N*-Boc-allylglycine **12** then led to (*R*)-**16**. HPLC analysis of (*S*)-**16** and (*R*)-**16** using a chiral column (C-18 Waters Symmetry) showed that no epimerization had occurred during these deprotection/protection steps.

The (*R*) isomer of **16** was first used to optimize the copper-catalyzed aziridination of the double bond. We have previously demonstrated that the aziridination of a wide range of olefins can be achieved using a convenient one-pot procedure.[25] Thus, by simply mixing 1 equiv. of (*R*)-**16**, 1.2 equiv. of PhI=O and of $SesNH_2$ in the presence of 25 mol% of $Cu(CH_3CN)_4PF_6$ in acetonitrile at room



Scheme 2. Preparation of allylglycine derivatives. (a) α-chymotrypsin.[21] (b) TFA, CH_2Cl_2, rt, 2 h. (c) HCl, EtOH. (d) Propylene oxide, EtOH, reflux, 7 h. (e) 6 N HCl, reflux, 18 h. (f) TMSCl, CH_2Cl_2, reflux, 2 h. (g) PhFBr, $Pb(NO_3)_2$, CH_2Cl_2, rt, 84 h. (h) $Cl_3CC(=NH)O$*t*-Bu, cyclohexane, CH_2Cl_2, rt, 72 h.

Table 1. 'One-pot' copper-catalyzed aziridination of allylglycine derivative (*R*)-**16**



Entry	$Cu(CH_3CN)_4PF_6$	Solvent	Temperature	% Yield[a]	Diastereomeric ratio (*R*,*R*): (*R*,*S*)
1	25 mol%	CH_3CN	rt	32 (67)	65:35
2	25 mol%	C_6H_6	rt	26 (45)	67:33
3	25 mol%	CH_2Cl_2	rt	24 (44)	70:30
4	25 mol%	CH_3CN	5 °C	30 (74)	63:37
5	25 mol%	CH_3CN	45 °C	25 (53)	67:33
6	50 mol%	CH_3CN	rt	28 (60)	66:34
7	25 mol%+35 mol% **18**	CH_3CN	rt	23	70:30
8	25 mol%+35 mol% **18**	CH_3CN	−20 °C	16	75:25

[a] Yields in parentheses based on consumed substrate.

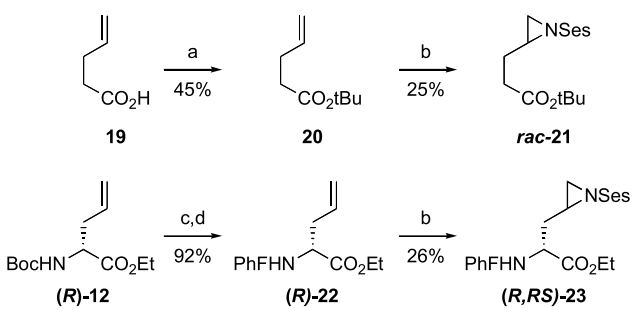
temperature, the desired *N*-Ses aziridine **17** was obtained in 32% overall yield (67% yield based on recovered starting material) (Table 1, entry 1). The aziridination was moderately diastereoselective providing a 65:35 ratio (as determined by ^1H NMR) of the (2*R*,4*R*) and (2*R*,4*S*) isomers, respectively, which could not be separated at this stage (see below for the determination of the absolute configurations).

Several attempts were made to improve the yield and/or the diastereoselectivity of the aziridination but all proved somewhat unsatisfactory. Thus (Table 1), while use of benzene or dichloromethane as the reaction solvent gave moderately higher diastereoselectivities (34 and 40%, respectively), this was at the detriment of yields (26 and 24%, respectively) (entries 2, 3). No substantial changes in yields and diastereoselectivities were observed when the reaction was run at colder (5 °C) or warmer (45 °C) temperatures (entries 4, 5). Unexpectedly, doubling the quantity of copper catalyst led to a slightly decreased aziridine yield (entry 6).

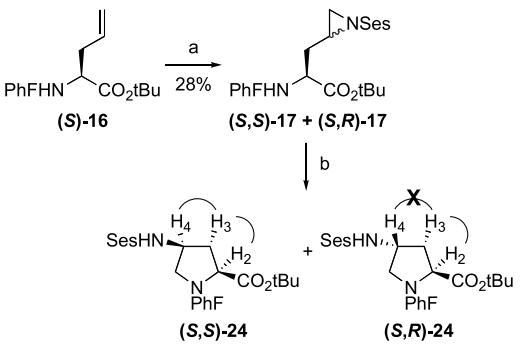
Evans has shown that high stereoselectivities can be obtained when the iminoiodane-mediated aziridination reaction of simple olefins (styrene, cinnamates) is conducted in the presence of chiral bis(oxazoline) catalysts.[26] When 35 mol% of such a ligand (the *t*-butyl derivative **18**) was added to the aziridination reaction medium of (*R*)-**16**, some improvement in diastereoselectivity was observed both at room temperature (40% de, entry 7) and at −20 °C (50% de, entry 8) but again, at the expense of overall product yield (23 and 16%, respectively).

In order to verify whether the presence of a nitrogen atom in the olefinic substrate was responsible for the relatively low aziridination yields, the same aziridination procedure was applied to *t*-butyl 4-pentenoate **20** (prepared by DCC/DMAP promoted esterification of carboxylic acid **19** by *t*-butanol) (Scheme 3). The yield of aziridine **21** (25%) was in fact no better than that starting from the analogous amine-containing substrate (*R*)-**16**, indicating that the nitrogen atom is most probably not interfering with the reaction. Moreover, when the bulky *t*-butyl ester of allylglycine **16** was replaced by a smaller ethyl ester as in (*R*)-**22** (prepared by selective removal of the *N*-Boc group of (*R*)-**12** followed by protection of the resulting amine with a phenylfluorenyl group),[23b] the yield of the corresponding aziridine (*R*)-**23** was again quite low (26%) and, in addition, no diastereoselectivity was observed. These results, combined with our previous observations,[18,27] strongly suggest that the bulky amine and carboxylic acid blocking groups of allylglycine **16** are not the source of the low yields of aziridinated product. The latter is more likely attributable to the previously described poor reactivity of terminal monosubstituted olefins under these conditions. The combined presence of both sterically demanding substituents does, however, appear to be necessary to ensure some diastereoselectivity in the aziridination step.

Since, despite many attempts, neither the yield nor the diastereoselectivity of the aziridination of (*R*)-allylglycine derivative **16** could be further improved, the best reaction conditions were now applied to (*S*)-**16** having the same C-2 configuration as arginine and enduracididine. As expected then, 'one-pot' aziridination of (*S*)-**16** with $SesNH_2$ provided an inseparable 7:3 mixture of aziridines **17** in 28% yield (65% based on consumed starting material) (Scheme 4). Attribution of the C-4 configuration of each isomer was made possible after intramolecular aziridine ring opening by the secondary amine of **17**, the resulting cyclized products being more amenable to NMR analysis. Thus, when the diastereomeric mixture of aziridines **17** was heated at 110 °C for 70 h in DMF, two major compounds were obtained. Separation of the compounds by column chromatography afforded the *cis* 4-aminoproline (*S*,*S*)-**24** in 46% yield[28] and the diastereomeric *trans*-4-aminoproline



Scheme 3. Aziridination of test substrates. (a) *t*-BuOH, DCC, DMAP, CH_2Cl_2, rt, 20 h. (b) $SesNH_2$ PhI=O, 25 mol% $Cu(CH_3CN)_4PF_6$, CH_3CN, rt, 16 h. (c) HCl_g, CH_2Cl_2, rt, 5 h. (d) PhFBr, K_3PO_4, CH_3NO_2, rt, 72 h.



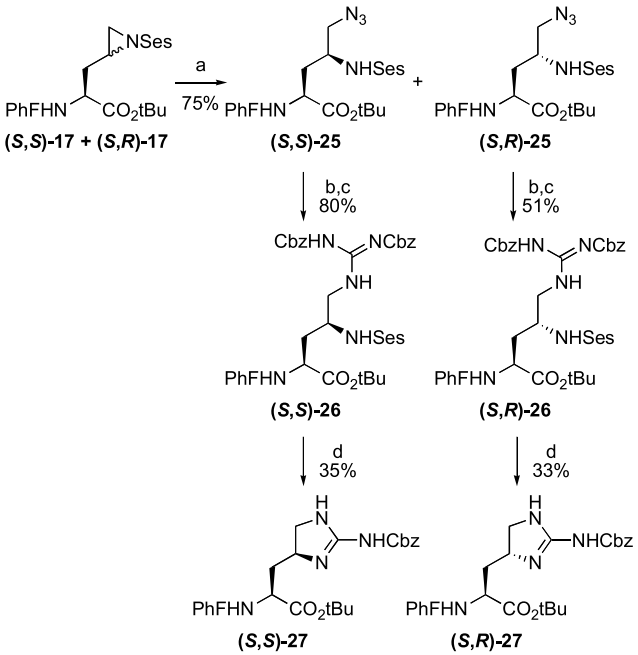
Scheme 4. Determination of the stereochemistry. (a) Conditions for step b in Scheme 3. (b) DMF, 110 °C, 70 h.

derivative (*S*,*R*)-**24** in 17% yield. The configuration of the Ses-amino group of the major compound (*S*,*S*)-**24** was clearly established by ^{1}H NMR NOESY and NOEDIFF experiments. Thus, while no direct correlation was observed between H-2 and H-4, strong NOE effects between H-2 and H-3 on one hand and H-3 and H-4 on the other hand were evident, indicating a *cis* relationship between H-2 and H-4. No such correlation could be observed between H-3 and H-4 in the minor product, though H-2 and H-3 were still strongly correlated, thereby corroborating the H-2/H-4 *trans* relationship in (*S*,*R*)-**24**. Based on these results, it may be deduced that the major diastereomer formed by aziridination of (*S*)-**16** is the (2*S*,4*S*) isomer and that of (*R*)-**16** the (2*R*,4*R*) isomer.

In order to prepare the required diamino intermediate of type **9**, opening of the aziridine ring of compound **17** (a mixture of the (*S*,*S*) and (*S*,*R*) isomers) by azide anion was then investigated (Scheme 5). Careful control of the reaction conditions was required in order to minimize the aforementioned intramolecular aziridine ring opening (heating with NaN_3 in DMF at 65 °C for 80 h in the presence of boron trifluoride etherate). The ^{1}H NMR spectrum of the crude reaction mixture showed that two major ring-opened products **25**, separated and purified by a combination of column chromatography on silica gel and HPLC, had been formed in a ratio identical to that of the diastereomeric components of starting material **17** (7:3). The major compound could thus be assigned the (2*S*,4*S*) configuration and the minor compound the (2*S*,4*R*) configuration.

The synthesis of the target rigid arginine derivatives was completed as shown in Scheme 5. Thus, reduction of the azide function of compound (*S*,*S*)-**25** with triphenylphosphine in the presence of water afforded the intermediate amine[29] which was reacted directly with *S*-methyl *N*,*N*′-bis(benzyloxycarbonyl)isothiourea[30] to give the protected guanidine derivative (*S*,*S*)-**26** in 80% yield. Treatment of this compound with cesium fluoride in DMF at 90 °C for 24 h then provided in one step the dihydroaminoimidazole derivative (*S*,*S*)-**27**, HPLC of which showed a diastereomeric purity of 99%. Identical treatment of (*S*,*R*)-**25** provided compound (*S*,*R*)-**27** (de of 98%). The latter is a protected form of enduracididine **7**.

In summary, we have described herein the first application of the one-pot copper-catalyzed iminoiodane-mediated



Scheme 5. End of the synthesis. (a) NaN_3, $BF_3{\cdot}OEt_2$, DMF, 65 °C, 80 h. (b) PPh_3, THF, H_2O, reflux, 20 h. (c) MeSC(=NCbz)NHCbz, $HgCl_2$, DMF, Et_3N, rt, 84 h. (d) CsF, DMF, 90 °C, 24 h.

aziridination procedure to an α-allylglycine derivative. This subsequently permitted the preparation of novel rigid analogues of arginine (i.e. **27**) starting from the stereoisomeric aziridinated products **17**. Interestingly, the (2*S*,4*R*)-isomer of **27** is a protected form of a marine natural product, enduracididine **7**. The present methodology therefore represents a versatile approach for the preparation of this compound, its isomers and its analogues.[31]

1. Experimental

1.1. General

Melting points were measured in capillary tubes on a Büchi B-540 apparatus and are uncorrected. IR spectra of samples were obtained either as KBr pellets or as films with a Nicolet 205 FT-IR or Fourier Perkin–Elmer 1600 FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR were determined on a Bruker AC 200 (200 MHz), AC 250 (250 MHz) or Aspect 3000 (300 MHz) instrument. ^{1}H and ^{13}C NMR chemical shifts are given as δ values with reference to Me_4Si as internal standard. Electron impact and chemical ionization mass spectra were recorded on an AEI MS-50 and AEI MS-9 spectrometer, respectively. High-resolution mass spectra were obtained using a Kratos MS-80 spectrometer. Optical rotations were determined with a JASCO P-1010 polarimeter. Thin-layer chromatography was performed on silica gel 60 plates with a fluorescent indicator. The plates were visualized with UV light (254 nm) and with a 3.5% solution of phosphomolybdic acid in ethanol. All column chromatography was conducted on silica gel 60 (230–240 mesh) at medium pressure (200 mbar). All solvents were distilled and stored over 4 Å molecular sieves before use. All reagents were purchased from the Aldrich Chemical Co.

and were used without further purification. Elemental analyses were performed at the ICSN, CNRS, Gif-sur-Yvette.

1.1.1. (*S*)-*N*-(*t*-Butyloxycarbonyl)allylglycine (13) and ethyl (*R*)-*N*-(*t*-butyloxycarbonyl)allylglycinate ((*R*)-12). These compounds were obtained by selective enzymatic saponification of the (*S*) enantiomer of racemic ethyl *N*-(*t*-butyloxycarbonyl)allylglycinate ((*R*,*S*)-**12**) with α-chymotrypsin following the procedure of Schricker et al.[21] The optical purity (ee) of each compound was determined by HPLC on a reverse phase C-18 Waters Symmetry column (4.6×250 mm) after derivatization using *o*-phthaldialdehyde and *N*-acetylcysteine as described.[32] Compound **13**: $[\alpha]_D^{22}$ +13 (c 1.15, MeOH), ee=98%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.46 (s, 9H), 2.50–2.70 (m, 2H), 4.40–4.52 (m, 1H), 4.95–5.05 (m, 1H, exchangeable with D_2O), 5.10–5.33 (m, 2H), 5.62–5.78 (m, 1H). Compound (*R*)-**12**: $[\alpha]_D^{22}$ −10 (c 0.92, MeOH), ee=94%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.29 (t, 3H, *J*=7.1 Hz), 1.48 (s, 9H), 2.50–2.60 (m, 2H), 4.27 (q, 2H, *J*=7.1 Hz), 4.42–4.52 (m, 1H), 5.10–5.28 (m, 3H, 1H, exchangeable with D_2O), 5.60–5.88 (m, 1H).

1.1.2. (*S*)-Allylglycine ((*S*)-14). To a solution of compound **13** (2.7 g, 12.7 mmol) in dichloromethane (12 mL) held at 0 °C was slowly added trifluoroacetic acid (9.7 mL, 127 mmol). After completion of the addition, the reaction mixture was stirred for 2 h at rt and then evaporated to dryness under vacuum. The residue was dissolved in ethanol (20 mL), 4 N HCl (3 mL) was added and the solution was once again evaporated to dryness, leaving (*S*)-**14** hydrochloride as a white powder (1.9 g, 98%): mp 206–208 °C.

A sample of the latter (500 mg, 3.3 mmol) in absolute ethanol (6.6 mL) was treated with propylene oxide (1.15 mL, 16.5 mmol), the mixture was refluxed for 7 h, cooled and the white precipitate of (*S*)-**14** was collected by filtration and washed with ether (324 mg, 85%). ESMS *m*/*z* 116 $(MH)^+$; ^{1}H NMR (300 MHz, D_2O) δ 2.53–2.72 (m, 2H), 3.80 (dd, 1H, *J*=5.1, 7.1 Hz), 5.23–5.32 (m, 2H), 5.69–5.85 (m, 1H); ^{13}C NMR (75 MHz, D_2O) δ 35.5, 54.6, 121.1, 131.9, 174.7.

1.1.3. (*S*)-*N*-(9-Phenyl-9*H*-fluoren-9-yl)allylglycine ((*S*)-15). To a suspension of (*S*)-allylglycine (*S*)-**14** (300 mg, 2.61 mmol) in anhydrous dichloromethane (5 mL) was added trimethylsilyl chloride (0.35 mL, 2.75 mmol). The reaction mixture was stirred at rt for 10 min and then refluxed for 2 h. The solution was cooled to rt, triethylamine (0.73 mL, 5.22 mmol) was added followed after 15 min by addition of lead (II) nitrate (0.59 g, 1.79 mmol) and of a solution of 9-bromo-9-phenylfluorene (1.14 g, 3.56 mmol) in dichloromethane (5 mL). The reaction mixture was stirred for 84 h at rt. Methanol (2.5 mL) was added, and after 2 h stirring, the mixture was filtered through Celite. The filtrate was evaporated in vacuo and the residue was chromatographed on silica gel (heptane–ethyl acetate 4:1 followed by 1:1) to afford compound (*S*)-**15** as a colorless solid (566 mg, 61%); mp 59–61 °C (lit.[23a] mp 63–64 °C); $[\alpha]_D^{23}$ − 152 (c 1.01, $CHCl_3$); IR (film) 2957, 2926, 1710, 1637 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.93–2.06 (m, 1H), 2.38–2.50 (m, 1H), 2.70 (t, 1H, *J*=5.4 Hz), 5.10–5.24 (m, 2H), 5.42–5.58 (m, 1H), 7.18–7.70 (m, 13H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 37.5, 54.9, 72.7, 120.5, 120.6, 124.9, 125.6, 125.9, 127.9, 128.2, 128.6, 128.9, 129.3, 129.4, 140.3, 141.3, 143.0, 146.6, 148.3, 174.7. HRESMS *m*/*z* 378.1466 $(M+Na)^+$ ($C_{24}H_{21}NO_2$+Na requires 378.1470).

1.1.4. *t*-Butyl (*S*)-*N*-(9-phenyl-9*H*-fluoren-9-yl)allylglycinate ((*S*)-16). To a solution of compound (*S*)-**15** (150 mg, 0.42 mmol) in dichloromethane (1 mL) was added a solution of *t*-butyl 2,2,2-trichloroacetimidate (184 mg, 0.84 mmol) in cyclohexane (0.85 mL). The reaction mixture was stirred for 72 h at rt, filtered through Celite and evaporated to dryness under vacuum. The residue was dissolved in dichloromethane (1 mL), treated again with the same quantity of reagent in cyclohexane and stirred for another 60 h. The residue obtained after filtration and evaporation was purified by chromatography on silica gel (heptane–ethyl acetate 19:1), affording compound (*S*)-**16** as a pale yellow solid (172 mg, 87%): mp 54–55 °C; $[\alpha]_D^{22}$ −173 (c 1.13, $CHCl_3$); IR (film) 3312, 2977, 1724, 1448 cm^{-1}; ESMS *m*/*z* 434 $(M+Na)^+$; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.22 (s, 9H), 2.21–2.32 (m, 2H), 2.71 (t, 1H, *J*=5.8 Hz), 3.12 (br s, 1H), 5.02–5.10 (m, 2H), 5.68–5.89 (m, 1H), 7.20–7.50 (m, 11H), 7.68–7.80 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.1, 40.4, 56.0, 73.2, 80.7, 117.3, 119.9, 125.5, 126.3, 127.2, 127.9, 128.3, 134.7, 140.3, 141.0, 145.1, 149.5, 149.6, 174.6. Anal. calcd for $C_{28}H_{29}NO_2$: C, 81.72; H, 7.10; N, 3.40. Found: C, 81.99; H, 7.17; N, 3.13.

1.1.5. *t*-Butyl (*R*)-*N*-(9-phenyl-9*H*-fluoren-9-yl)allylglycinate ((*R*)-16). A suspension of compound (*R*)-**12** (2.6 g, 10.7 mmol) in 6 N HCl was refluxed for 18 h. The reaction mixture was cooled and evaporated to dryness under vacuum by repeated co-evaporation with ethanol, affording (*R*)-allylglycine as the hydrochloride salt ((*R*)-**14** HCl) in quantitative yield. Treatment of this compound in the same manner as for (*S*)-**14** HCl provided compound (*R*)-**16** identical in all respects to (*S*)-**16** except for the optical rotation: $[\alpha]_D^{22}$ +152 (c 5.0, $CHCl_3$).

1.1.6. *t*-Butyl (2*R*,2′*RS*)-2-*N*-[(9-phenyl-9*H*-fluoren-9-yl)amino]-3-[*N*-(2-trimethylsilylethanesulfonyl)aziridin-2′-yl]propanoate ((*R*,*RS*)-17). To a suspension of activated 3 Å molecular sieves (250 mg) in acetonitrile (1.3 mL) were successively added compound (*R*)-**16** (310 mg, 0.75 mmol) and $Cu(CH_3CN)_4PF_6$ (70 mg, 0.19 mmol). A mixture of 2-trimethylsilylethanesulfonamide (178 mg, 0.98 mmol) and iodosylbenzene (217 mg, 0.98 mmol) was then introduced in five portions over a period of 1.5 h. The reaction mixture was stirred overnight at room temperature then filtered through a pad of Celite and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (heptane–ethyl acetate 19:1 then 9:1) to afford the *N*-(Ses)aziridines (*R*,*RS*)-**17** (142 mg, 32%) as an inseparable 7:3 mixture of diastereoisomers: IR (film) 1725, 1449, 1323 cm^{-1}; ESMS *m*/*z* 591 $(MH)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.05, 0.07 (2s, 9H), 1.05–1.15 (m, 2H), 1.20 (s, 9H), 1.25–1.45 (m, 1H), 1.8–2.05 (m, 2H), 2.4–2.7 (m, 2H), 2.75–3.1 (m, 3H), 7.2–7.45 (m, 11H), 7.65–7.75 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −1.9, −1.8, 9.4, 10.0, 28.0, 32.9, 33.6, 37.0, 37.5, 37.9, 48.7, 49.0,

54.6, 73.2, 81.5, 119.9, 120.1, 120.2, 125.6, 126.2, 126.4, 127.4, 128.0, 128.1, 128.3, 128.4, 128.5, 140.1, 141.2, 144.5, 144.7, 148.9, 149.2, 174.1, 174.2. Anal. calcd for $C_{33}H_{42}N_2O_4SSi$: C, 67.07; H, 7.16; N, 4.74; S, 5.43. Found: C, 67.27; H, 7.38; N, 4.56; S, 5.34.

1.1.7. *t*-Butyl (2*S*,2′*RS*)-2-*N*-(9-phenyl-9*H*-fluoren-9-yl)-amino-3-[*N*-(2-trimethylsilylethanesulfonyl)aziridin-2′-yl]propanoate ((*S*,*RS*)-17). Following the same procedure as for the preparation of (2*R*,4*RS*)-**17**, compound (*S*)-**16** (2.39 g, 5.8 mmol) in acetonitrile (25 mL) containing 3 Å molecular sieves (3.75 g) was treated with $Cu(CH_3CN)_4PF_6$ (0.63 g, 1.68 mmol) and a mixture of $SesNH_2$ (1.37 g, 7.6 mmol) and iodosylbenzene (1.7 g, 7.7 mmol) in five portions. After the usual work-up, the crude product was purified by chromatography on silica gel (ethyl acetate 1:19 then 1:9) affording compound (*S*,*RS*)-**17** as a 7:3 mixture of diastereomers (0.95 g, 28%) whose spectral characteristics were identical to those of compound (*R*,*RS*)-**17**.

1.1.8. *t*-Butyl 4-pentenoate (20). A solution of 4-pentenoic acid (1 g, 10 mmol), *t*-butanol (2.2 g, 40 mmol) and DMAP (20 mg, 0.16 mmol) in dichloromethane (5 mL) was treated at 0 °C with DCC (2.25 g, 11 mmol). The reaction mixture was stirred for 15 min at 0 °C and then for 20 h at rt. The precipitate was removed by filtration through Celite, the filtrate was evaporated under vacuum, the residue was taken up in dichloromethane (20 mL) and washed successively with 0.1 M HCl (2×20 mL), saturated aqueous $NaHCO_3$ (20 mL) and water (2×20 mL). The organic phase was dried over $MgSO_4$, the solvent was evaporated and the residue was chromatographed on silica gel affording compound **20**[33] as a colorless oil (710 mg, 45%): IR (film) 2979, 2932, 1732, 1153 cm^{-1}; ESMS *m*/*z* 179 $(M+Na)^+$; ^1H NMR (250 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.31–2.35 (m, 4H), 4.96–5.10 (m, 2H), 5.73–5.92 (m, 1H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 28.2, 29.2, 34.8, 80.3, 115.3, 137.1, 172.6.

1.1.9. *t*-Butyl (*R*,*S*)-3-[*N*-(2-trimethylsilylethanesulfonyl)-aziridin-2′yl]propanoate (*rac*-21). Following the same procedure as for the preparation of **17**, compound **20** (211 mg, 1.35 mmol) in acetonitrile (5.3 mL) containing 3 Å molecular sieves (0.7 g) was treated with $Cu(CH_3CN)_4PF_6$ (126 mg, 0.34 mmol) and a mixture of $SesNH_2$ (318 mg, 1.75 mmol) and iodosylbenzene (385 mg, 1.75 mmol) in five portions. After the usual work-up, the crude product was purified by chromatography on silica gel (ethyl acetate–heptane 1:4), affording compound *rac*-**21** as an orange oil (115 mg, 25%): IR (film) 3297, 2954, 1729 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 0.07 (s, 9H), 1.10–1.17 (m, 2H), 1.44 (s, 9H), 1.62–1.75 (m, 1H), 1.90–2.02 (m, 1H), 2.13 (d, 1H, *J*=4.6 Hz), 2.38 (t, 2H, *J*=7.0 Hz), 2.60 (d, 1H, *J*=7.0 Hz), 2.73–2.82 (m, 1H), 3.04–3.12 (m, 2H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ −1.9, 9.8, 26.9, 28.2, 32.6, 33.7, 38.0, 48.9, 80.9, 171.8. HRESMS *m*/*z* 358.1481 $(M+Na)^+$ ($C_{14}H_{29}NO_4SSi+Na^+$ requires 358.1484).

1.1.10. Ethyl (*R*)-*N*-(9-phenyl-9*H*-fluoren-9-yl)allylglycinate ((*R*)-22). HCl gas was bubbled through a solution of compound (*R*)-**12** (4.0 g, 16.4 mmol) in dichloromethane (50 mL) for 75 min. The reaction mixture was stirred at rt for 5 h and then evaporated to dryness under vacuum affording ethyl (*R*)-allylglycinate hydrochloride (3.0 g, 100%): mp 89–91 °C; $[\alpha]_D^{26}$ +1 (c 0.65, MeOH); IR (film) 3406, 2981, 1744, 1487 cm^{-1}; ESMS *m*/*z* 144 $(MH)^+$; ^1H NMR (250 MHz, $CDCl_3$) δ 1.30 (t, 3H, *J*=7.1 Hz), 2.75–2.95 (m, 2H), 4.05–4.35 (m, 3H), 5.26 (dd, 1H, *J*=1.5, 10.1 Hz), 5.33 (dd, 1H, *J*=1.5, 16.9 Hz), 5.75–5.95 (m, 1H), 8.70–8.90 (br s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 34.6, 52.9, 62.6, 121.5, 130.3, 168.8.

A solution of this compound (500 mg, 2.78 mmol) in nitromethane (5 mL) was then treated with potassium phosphate (1.18 g, 5.54 mmol) and 9-bromo-9-phenylfluorene (1.07 g, 3.3 mmol). The reaction mixture was stirred at rt for 72 h, ethanol (2 mL) was added and after 5 min of stirring, the mixture was filtered through Celite. The filter pad was washed with ethyl acetate, the filtrate and washings were combined, evaporated to dryness under vacuum and the residue was purified by column chromatography on silica gel (heptane–ethyl acetate 19:1), affording (*R*)-**22** as a pasty solid (985 mg, 92%): $[\alpha]_D^{22}$ +195 (c 2.01, $CHCl_3$); IR (film) 3314, 3062, 2979, 1729, 1447 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, 3H, *J*=7.1 Hz), 2.10–2.29 (m, 2H), 2.65–2.73 (m, 1H), 2.93 (br s, 1H), 3.58–3.70 (m, 1H), 3.70–3.82 (m, 1H), 4.97–5.01 (m, 1H), 5.01–5.05 (m, 1H), 5.61–5.76 (m, 1H), 7.13–7.72 (m, 13H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 39.9, 55.7, 60.5, 73.1, 117.7, 119.9, 120.0, 125.5, 126.3, 127.3, 127.5, 127.9, 128.4, 134.4, 140.3, 141.1, 144.9, 147.0, 149.3, 175.7. HRESMS *m*/*z* 406.1793 $(M+Na)^+$ ($C_{26}H_{25}NO_2+Na^+$ requires 406.1783).

1.1.11. Ethyl (2*R*,2′*RS*)-2-*N*-(9-phenyl-9*H*-fluoren-9-yl)-amino-3-[*N*-(2-trimethylsilylethanesulfonyl)aziridin-2′-yl]propanoate ((*R*,*RS*)-23). Following the same procedure as for the preparation of **17**, compound (*R*)-**22** (210 mg, 0.55 mmol) in acetonitrile (2.2 mL) containing 3 Å molecular sieves (0.3 g) was treated with $Cu(CH_3CN)_4PF_6$ (50 mg, 0.134 mmol) and a mixture of $SesNH_2$ (131 mg, 0.72 mmol) and iodosylbenzene (156 mg, 0.71 mmol) in five portions. After the usual work-up and chromatography of the residue on silica gel (ethyl acetate–heptane 1:9 then 1:5), compound (*R*,*RS*)-**23** was obtained as a 1:1 mixture of diastereomers (80 mg, 26%): IR (film) 3310, 2953, 1730, 1449 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.05 (s, 9H), 0.07 (s, 9H), 0.80–1.13 (m, 10H), 1.30–1.60 (m, 2H), 1.77–2.00 (m, 2H), 1.87 (d, 1H, *J*=4.4 Hz), 2.06 (d, 1H, *J*=4.4 Hz), 2.43 (d, 1H, *J*=7.0 Hz), 2.63–3.2 (m, 9H), 3.63–3.87 (m, 4H), 7.08–7.47 (m, 22H), 7.66–7.76 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −1.9, 9.5, 10.0, 30.5, 32.7, 33.3, 37.0, 37.1, 37.2, 37.3, 48.8, 49.0, 54.2, 54.4, 61.1, 73.1, 120.0, 120.2, 125.5, 126.2, 126.4, 127.5, 127.6, 128.2, 128.4–128.7, 140.1, 141.4, 144.3, 144.5, 148.5, 148.6, 148.9, 149.0, 175.2, 175.3. HRESMS *m*/*z* 585.2200 $(M+Na)^+$ ($C_{31}H_{38}N_2O_4SSi+Na^+$ requires 585.2219).

1.1.12. *t*-Butyl (2*S*,4*S*)- and (2*S*,4*R*)-1-*N*-(9-phenyl-9*H*-fluoren-9-yl)-4-*N*-(2-trimethylsilylethanesulfonyl)-aminoproline ((*S*,*S*)-24 and (*S*,*R*)-24, respectively). A solution of compound (*S*,*RS*)-**17** (147 mg, 0.25 mmol) in DMF (2 mL) was heated at 110 °C for 70 h. The solvent was removed under vacuum and the residue was purified by column chromatography on silica gel (heptane–ethyl acetate 7:1) affording by order of elution compounds

(*S*,*S*)-**24** and (*S*,*R*)-**24**. Compound (*S*,*S*)-**24**: (67 mg, 46%): $[\alpha]_D^{25}$ +102 (c 0.91, $CHCl_3$); IR (film) 3274, 2956, 1720, 1450 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.03 (s, 9H), 1.00–1.10 (m, 2H), 1.26 (s, 9H), 1.60–1.70 (m, 1H), 1.95–2.05 (m, 1H), 2.85–2.95 (m, 2H), 3.04 (dd, 1H, *J*=1.7, 10.7 Hz), 3.12 (dd, 1H, *J*=4.4, 9.7 Hz), 3.30 (d, 1H, *J*=9.7 Hz), 3.85–3.95 (m, 1H), 6.37 (d, 1H, *J*=10.3 Hz), 7.10–7.20 (m, 1H), 7.20–7.50 (m, 8H), 7.50–7.60 (m, 2H), 7.60–7.65 (m, 1H), 7.75–7.80 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −1.8, 10.7, 28.0, 38.5, 50.3, 53.5, 56.7, 59.4, 76.0, 81.4, 120.0, 120.4, 126.7, 127.0, 127.5, 127.6, 127.7, 128.2, 128.5, 128.7, 128.9, 139.4, 141.9, 142.4, 145.5, 148.1, 176.3. HRESMS *m*/*z* 591.2675 $(MH)^+$ ($C_{33}H_{43}N_2O_4SSi$ requires 591.2713).

Compound (*S*,*R*)-**24** (25 mg, 17%): $[\alpha]_D^{22}$ +18 (c 0.66, $CHCl_3$); IR (film) 3271, 2926, 2854, 1735, 1450 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 0.00 (s, 9H), 0.85–0.95 (m, 2H), 1.29 (s, 9H), 1.50–1.70 (m, 1H), 1.95–2.10 (m, 1H), 2.58 (t, 1H, *J*=9.0 Hz), 2.80–2.90 (m, 2H), 3.32 (dd, 1H, *J*=1.8, 9.7 Hz), 3.52 (dd, 1H, *J*=6.4, 9.0 Hz), 4.02 (d, 1H, *J*=8.9 Hz), 4.05–4.20 (m, 1H), 7.05–7.45 (m, 8H), 7.50–7.60 (m, 3H), 7.60–7.75 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −1.9, 10.8, 28.0, 40.0, 49.5, 52.1, 55.1, 60.3, 76.5, 80.5, 120.0, 120.3, 126.4, 126.8, 127.5, 127.6, 127.9, 128.1, 128.6, 128.7, 128.8, 140.3, 141.0, 142.6, 146.9, 147.6, 174.3. HRESMS *m*/*z* 591.2714 $(MH)^+$ ($C_{33}H_{43}N_2O_4SSi$ requires 591.2713).

1.1.13. *t*-Butyl (2*S*,4*S*)- and (2*S*,4*R*)-5-azido-2-*N*-(9-phenyl-9*H*-fluoren-9-yl)amino-4-*N*-(2-trimethylsilylethanesulfonyl)aminopentanoate ((*S*,*S*)-25 and (*S*,*R*)-25, respectively). To a solution of compound (*S*,*RS*)-**17** (870 mg, 1.47 mmol) in DMF (12 mL) were successively added under argon at rt solid sodium azide (400 mg, 6.15 mmol) and boron trifluoride etherate (0.75 mL, 6.1 mmol). The mixture was heated at 65 °C for 80 h, and after cooling to rt, water (120 mL) was added. The solution was extracted with ethyl acetate (3×200 mL), the organic extracts were combined, dried over $MgSO_4$ and evaporated. A first purification of the residue by column chromatography on silica gel (ethyl acetate–heptane 1:7) provided (*S*,*S*)-**25** and (*S*,*R*)-**25** as a mixture (760 mg, ~75%) contaminated with a small amount of the aminoproline derivative **24**. The isomeric azides were partially separated by careful chromatography on silica gel using ethyl acetate–toluene (1:18) as eluting solvent. Pure (*S*,*S*)-**25** (major diastereomer) was finally obtained by preparative HPLC of the enriched fraction on a PrepPak Deltapak C18 cartridge (15 μm, 100 Å, 47×250 mm) using 35:65 isocratic H_2O+0.1% CH_3CO_2H/CH_3CN+0.1% CH_3CO_2H: $[\alpha]_D^{25}$ −134 (c 1.0, $CHCl_3$); IR (film) 3286, 2926, 2103, 1728, 1449 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.08 (s, 9H), 1.05–1.15 (m, 2H), 1.25 (s, 9H), 1.50–1.60 (m, 1H), 1.70–1.80 (m, 1H), 2.65–2.75 (m, 1H), 2.85–3.05 (m, 2H), 3.08 (dd, 1H, *J*=6.1, 12.4 Hz), 3.18 (dd, 1H, *J*=5.8, 12.4 Hz), 3.45–3.60 (m, 1H), 7.20–7.50 (m, 11H), 7.70–7.80 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −1.8, 10.6, 28.0, 35.9, 50.0, 52.4, 54.2, 54.5, 73.3, 82.0, 120.1, 120.4, 126.0, 126.2, 126.4, 127.6, 128.2, 128.3, 128.8, 129.0, 140.3, 141.3, 143.6, 147.9, 148.5, 173.6. HRESMS *m*/*z* 656.2727 $(M+Na)^+$ ($C_{33}H_{43}N_5O_4SSi+Na^+$ requires 656.2703).

Minor diastereomer (*S*,*R*)-**25**: IR (film) 3283, 2954, 2929, 2103, 1724, 1449 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.09 (s, 9H), 1.00–1.10 (m, 2H), 1.25 (s, 9H), 1.50–1.64 (m, 2H), 2.47 (t, 1H, *J*=6.3 Hz), 2.79–3.03 (m, 3H), 3.28 (dd, 1H, *J*=4.4, 12.5 Hz), 3.35 (br s, 1H), 3.74–3.87 (m, 1H), 4.25 (d, 1H, *J*=9.1 Hz), 7.17–7.47 and 7.67–7.77 (m, 13H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −1.7, 10.4, 28.0, 38.5, 50.3, 50.8, 53.4, 55.1, 73.2, 81.9, 120.0, 120.3, 125.8, 126.1, 126.2, 127.5, 128.2, 128.6, 128.9, 140.2, 141.1, 144.2, 148.7, 149.2, 174.2. HRESMS *m*/*z* 656.2676 $(M+Na)^+$ ($C_{33}H_{43}N_5O_4SSi+Na^+$ requires 656.2703).

1.1.14. *t*-Butyl (2*S*,4*S*)-5-[*N*′,*N*″-bis(benzyloxycarbonyl)-guanidino]-2-*N*-(9-phenyl-9*H*-fluoren-9-yl)amino-4-*N*-(2-trimethylsilylethanesulfonyl)aminopentanoate ((*S*,*S*)-26). To a solution of compound (*S*,*S*)-**25** (100 mg, 0.16 mmol) in THF (9 mL) was added triphenylphosphine (55 mg, 0.21 mmol) and water (170 μL, 9.4 mmol). The reaction mixture was refluxed for 20 h, the solvent was evaporated and the residue was dried under vacuum. The latter was dissolved in DMF (1.8 mL) and *S*-methyl *N*,*N*′-bis(benzyloxycarbonyl)isothiourea (68 mg, 0.19 mmol), mercuric chloride (52 mg, 0.19 mmol) and triethylamine (66 μL, 0.47 mmol) were added. The reaction mixture was stirred for 84 h at rt, diluted with ethyl acetate (25 mL) and filtered. The filtrate was washed with a 10% aqueous citric acid solution (3×15 mL) and then with saturated aqueous NaCl (2×15 mL), dried over $MgSO_4$ and evaporated under vacuum. Chromatography of the residue on silica gel (ethyl acetate–heptane 1:3) afforded compound (*S*,*S*)-**26** as a pasty white solid (116 mg, 80%): $[\alpha]_D^{24}$ −55 (c 0.51, $CHCl_3$); IR (film) 3333, 2954, 1729, 1642 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 0.04 (s, 9H), 1.05–1.15 (m, 2H), 1.21 (s, 9H), 1.50–1.60 (m, 1H), 1.65–1.80 (m, 1H), 2.71 (dd, 1H, *J*=3.6, 8.0 Hz), 2.80–3.05 (m, 3H), 3.35–3.45 (m, 1H), 3.60–3.70 (m, 2H), 5.09 (s, 2H), 5.17 (d, 1H, *J*=12.0 Hz), 5.25 (d, 1H, *J*=12.0 Hz), 7.10–7.50 (m, 21H), 7.60–7.70 (m, 3H), 8.55 (t, 1H, *J*=5.3 Hz), 11.7 (br s, 1H). ^{13}C NMR (62.5 MHz, $CDCl_3$) δ −1.9, 10.3, 27.9, 35.6, 44.0, 49.7, 51.9, 54.2, 67.3, 68.4, 73.3, 82.1, 120.0, 120.4, 126.0, 126.1, 126.4, 127.5, 128.0, 128.1, 128.2, 128.3, 128.5, 128.6, 128.7, 128.8, 128.9, 129.9, 134.6, 136.7, 140.2, 141.3, 143.5, 147.8, 148.4, 153.7, 156.4, 163.6, 173.6. HRESMS *m*/*z* 940.3763 $(M+Na)^+$ ($C_{50}H_{59}N_5O_8SSi+Na^+$ requires 940.3751).

1.1.15. *t*-Butyl (2*S*,4*R*)-5-[*N*′,*N*″-bis(benzyloxycarbonyl)-guanidino]-2-*N*-(9-phenyl-9*H*-fluoren-9-yl)-4-*N*-(2-trimethylsilylethanesulfonyl)-2,4-diaminopentanoate ((*S*,*R*)-26). Following the same procedure as for the preparation of (*S*,*S*)-**27**, compound (*S*,*R*)-**25** (156 mg, 0.25 mmol) in THF (18 mL) was refluxed for 20 h in the presence of triphenylphosphine (112 mg, 0.43 mmol) and water (350 μL, 19.4 mmol). After evaporation, the residue, dissolved in DMF (3 mL), was treated with *S*-methyl *N*,*N*′-bis(benzyloxycarbonyl)isothiourea (108 mg, 0.3 mmol), mercuric chloride (82 mg, 0.3 mmol) and triethylamine (105 μL, 0.75 mmol) and the reaction mixture was stirred for 60 h at rt. Work up as before followed by chromatography of the residue on silica gel (ethyl acetate–heptane 1:5) provided compound (*S*,*R*)-**26** as a pasty white solid (125 mg, 51%): $[\alpha]_D^{23}$ −38 (c 1.04, $CHCl_3$); IR (film) 3331, 1729, 1641 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 0.04 (s,

9H), 1.00–1.10 (m, 2H), 1.19 (s, 9H), 1.40–1.60 (m, 1H), 1.60–1.80 (m, 1H), 2.30–2.45 (m, 1H), 2.75–3.00 (m, 3H), 3.25–3.30 (m, 1H), 3.80–3.95 (m, 1H), 5.10 (s, 2H), 5.18 (d, 1H, J=12.1 Hz), 5.24 (d, 1H, J=12.1 Hz), 5.43 (d, 1H, J=7.0 Hz), 7.20–7.50 (m, 21H), 7.60–7.70 (m, 2H), 8.40 (t, 1H, J=5.7 Hz), 11.7 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −1.8, 10.5, 28.0, 39.9, 44.1, 50.0, 51.9, 53.5, 67.3, 68.5, 73.2, 81.6, 120.0, 120.3, 125.7, 126.2, 126.6, 127.4, 128.1, 128.4–128.8, 134.6, 136.7, 140.3, 141.1, 144.2, 148.7, 149.4, 153.6, 157.3, 163.4, 174.4. HRESMS m/z 940.3741 $(M+Na)^+$ ($C_{50}H_{60}N_5O_8SSi+Na^+$ requires 940.3751).

1.1.16. *t*-Butyl (2*S*,4′*S*)-3-[2′-*N*-(benzyloxycarbonyl)-aminoimidazolidin-4′-yl]-2-*N*-(9-phenyl-9*H*-fluoren-9-yl)aminopropanoate ((*S*,*S*)-27). A mixture of compound (*S*,*S*)-**26** (281 mg, 0.31 mmol) and cesium fluoride (141 mg, 0.93 mmol) in DMF (3.5 mL) was heated at 90 °C for 24 h. Water (50 mL) was added and the solution was extracted with ethyl acetate (3×50 mL). The organic extracts were combined, dried over $MgSO_4$ and evaporated leaving a crude product which was purified by column chromatography on silica gel (heptane–ethyl acetate 1:1), affording compound (*S*,*S*)-**27** as a viscous oil which slowly solidified (65 mg, 35%). HPLC of an aliquot on a Waters C18 Symmetry column (4.6×250 mm) using water+0.1% CH_3CO_2H/CH_3CN+0.1% CH_3CO_2H as eluting solvents (85:15 to 12:88 gradient over 40 min; 1 mL/min flow rate) indicated that compound (*S*,*S*)-**27** was 99% pure: $[\alpha]_D^{23}$ −96 (c 0.84, $CHCl_3$); IR (film) 3389, 2929, 1724, 1657, 1622 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (s, 9H), 1.50–1.70 (m, 2H), 2.44–2.54 (m, 1H), 3.09 (dd, 1H, J=7.3, 9.3 Hz), 3.22 (br s, 1H), 3.69 (t, 1H, J=9.3 Hz), 4.01–4.14 (m, 1H), 5.05 (d, 1H, J=12.6 Hz), 5.13 (d, 1H, J=12.6 Hz), 6.56–6.84 (br s, 1H), 7.13–7.45 (m, 16H), 7.65–7.75 (m, 2H), 7.80–8.20 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.0, 41.1, 48.1, 51.7, 53.9, 66.5, 73.1, 81.6, 120.0, 120.4, 125.1, 126.2, 126.3, 127.4, 127.7, 127.9, 128.1, 128.4–128.5, 128.9, 137.6, 140.2, 141.1, 144.3, 148.8, 149.0, 163.2, 164.8, 174.4. HRESMS m/z 603.3009 $(MH)^+$ ($C_{37}H_{39}N_4O_4$ requires 603.2971).

1.1.17. *t*-Butyl (2*S*,4′*R*)-3-[2′-*N*-(benzyloxycarbonyl)-aminoimidazolidin-4′-yl]-2-*N*-(9-phenyl-9*H*-fluoren-9-yl)aminopropanoate ((*S*,*R*)-27). Following the same procedure as for the preparation of (*S*,*S*)-**27**, compound (*S*,*R*)-**26** (119 mg, 0.13 mmol) in DMF (1.5 mL) was treated with cesium fluoride (60 mg, 0.4 mmol) for 24 h at 90 °C. Work-up and purification as before afforded compound (*S*,*R*)-**27** as a pasty solid (26 mg, 33%). HPLC analysis of an aliquot under the same conditions as for (*S*,*S*)-**27** showed (*S*,*R*)-**27** to be 98% pure: $[\alpha]_D^{23}$ −100 (c 0.9, $CHCl_3$); IR (film) 2925, 1727, 1652, 1622 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.18 (s, 9H), 1.50–1.65 (m, 1H), 1.65–1.80 (m, 1H), 2.40–2.50 (m, 1H), 3.01 (dd, 1H, J=7.7, 9.7 Hz), 3.36 (t, 1H, J=9.7 Hz), 3.95–4.10 (m, 1H), 5.16 (d, 1H, J=12.5 Hz), 5.23 (d, 1H, J=12.5 Hz), 7.15–7.45 (m, 16H), 7.65–7.75 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.0, 40.4, 47.6, 53.4, 54.5, 67.8, 73.2, 81.8, 120.0, 120.3, 125.2, 126.2, 126.8, 127.5, 128.2, 128.3, 128.6, 128.7, 136.1, 140.4, 141.1, 143.8, 148.4, 149.2, 159.8, 161.1, 174.3. HRESMS m/z 603.3000 $(MH)^+$ ($C_{37}H_{39}N_4O_4$) requires 603.2971).

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28. A second minor (<10%) product was co-eluted with this compound whose NMR spectra indicated that it corresponded to the azetidine resulting from aziridine ring-opening at the C-4 position. ESMS *m/z* 591 $(MH)^+$; ^1H NMR (250 MHz, $CDCl_3$) δ 0.09 (s, 9H), 1.05–1.15 (m, 2H), 1.41 (s, 9H), 1.90–2.20 (m, 2H), 2.30–2.40 (m, 1H), 2.70–2.80 (m, 1H), 2.85–2.95 (m, 2H), 3.30–3.40 (m, 1H), 3.77 (dd, 1H, *J*=6.8, 9.4 Hz), 6.08 (d, 1H, *J*=7.2 Hz), 7.05–7.45 (m, 13H); ^{13}C NMR (75 MHz, $CDCl_3$) δ −1.8, 10.7, 22.2, 28.0, 46.1, 48.8, 56.2, 57.6, 76.3, 81.6, 120.2, 120.3, 126.0, 126.7, 127.8, 127.9, 128.0, 128.6, 129.0, 129.1, 140.1, 140.9, 141.5, 145.7, 146.9, 173.3.
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Carbohydrate-derived spiroketals: stereoselective synthesis of di-D-fructose dianhydrides

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Abstract—A one-pot synthesis of di-D-fructose dianhydrides (DFAs) having the 1,6,9,13-tetraoxadispiro[4.2.4.2]tetradecane and 1,7,10,15-tetraoxadispiro[5.2.5.2]hexadecane skeleton has been accomplished. The methodology relies on the ability of per-*O*-protected 1,2-*O*-isopropylidene β-D-fructofuranose and β-D-fructopyranose derivatives to undergo a tandem acetal cleavage-intermolecular glycosylation-intramolecular spiroketalization process by reaction with suitable acid promoters, such as boron trifluoride etherate or trifluoromethanesulfonic acid, in apolar organic solvents. Spirocyclization proceeds then under irreversible reaction conditions to give binary mixtures of di-D-fructofuranose (α,α and α,β diastereomers) or di-D-fructopyranose 1,2′:2,1′ dianhydrides (β,β and α,β), respectively, the stereochemical outcome being dependent on the non-participating or participating character of the protecting groups. Thus, benzylated and allylated derivatives afford, preferentially, the non-symmetric DFAs (α,β), with diastereomeric excess up to 92%. In contrast, the use of participating benzoyl groups favours the C_2-symmetric diastereomer in both series.

1. Introduction

The spiroketal unit represents a common feature in many biologically relevant natural products, including steroidal saponins, polyether ionophores, macrolide antibiotics, insect pheromones and toxic metabolites from algae and fungi,[1–4] being the target of much synthetic effort.[5–13] This structural element is also present in a unique class of cyclic disaccharides termed generically diketose dianhydrides, of which di-D-fructose dianhydrides (DAFs) are paradigmatic examples.[14] Some members of this class of compounds have been isolated from microorganisms[15] and higher plants.[16] Their potential use as sweeteners,[17,18] bifidogenic agents[19] or chiral templates[20,21] has triggered intense interest in the synthesis of these and related spiro-sugars.[22–27] The identification of DFAs as the major components of the thermolysis product of sucrose and D-fructose containing food materials, such as caramel and chicory,[28–30] and the need of pure standards for nutritional studies and analytical evaluation[31] has provided a further impetus.

Despite the variety of general methods existing for the construction of the spiroketal moiety, the control of the stereochemistry at the anomeric centres relies, almost exclusively, on the relative thermodynamic stability of the different isomers in the acid-catalyzed spiroketalization reaction. When all factors that control spiroketalization, that is, a maximum anomeric effect and minimum steric interactions, are reinforcing, a major isomer is produced. The stereoselectivity is lower when these factors are in conflict. In tricyclic systems,[32–35] however, such general statements must be applied carefully. A range of structures can usually accommodate the basic requirements, that is, oxygen substituents at anomeric centres in axial disposition and carbon substituents in equatorial disposition, with rather small differences in energy and low interconversion barriers.

In the case of DFAs, high yielding preparations have been previously achieved by protonic activation of D-fructose, sucrose or inulin with anhydrous hydrogen fluoride (HF) or its complex with pyridine.[36–38] Under such conditions, a fructosyl oxocarbenium cation is generated, which undergoes in situ glycosylation into the corresponding keto-disaccharide. Further spiroketalization is a reversible process that leads to a complex mixture of bis(spiro)disaccharides in which the two D-fructose constituents are joined through a central 1,4-dioxane ring. Up to five different

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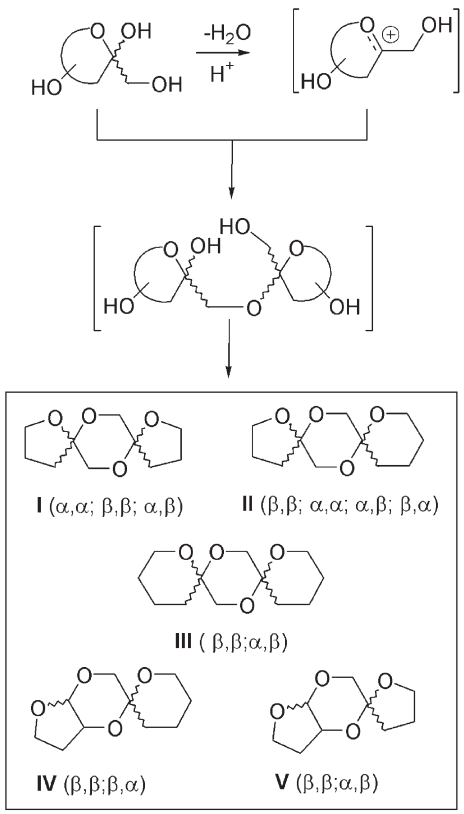
tricyclic cores (types I–V) and 13 DFA isomers that differ in the ring size, linking position and stereochemistry of the ketal stereocenters have been so far identified from reaction mixtures (Scheme 1).



Scheme 1. Acid-catalysed dimerization of ketoses.

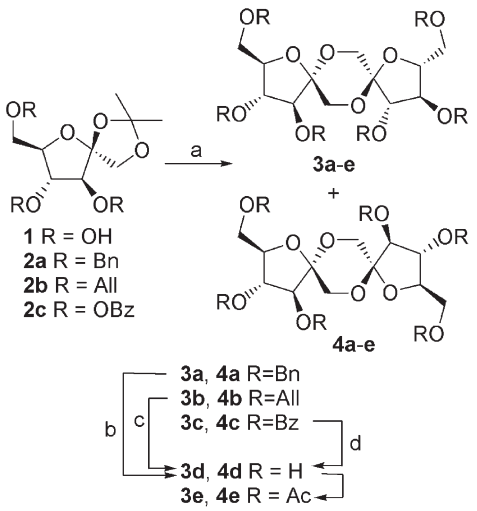
Difuranose DFA derivatives (types I and V) are formed at the early stages of the acid-promoted dimerization of D-fructose. However, they partially isomerize in the reaction medium to give mixed furanose–pyranose (types II and IV) and dipyranose species (type III). Although their relative proportions can be varied to some extent by modulation of the acid strength, isolation of pure samples from the isomeric mixtures remains a difficult task. We envisioned that isomerization reactions would be significantly slowed in anhydrous apolar solvents. Moreover, the use of protected D-fructose precursors should allow blocking the cyclic form of the monosaccharides and, eventually, controlling the stereochemical outcome of the dimerization process.[39] This concept has now been translated into stereoselective preparations of DFAs having the 1,6,9,13-tetraoxadispiro-[4.2.4.2]tetradecane and 1,7,10,15-tetraoxadispiro[5.2.5.2]-hexadecane core structure (types I and III, respectively). Our strategy relies on the ability of 1,2-*O*-isopropylidene D-fructose derivatives to undergo a tandem acetal cleavage—intermolecular glycosylation—intramolecular spiroketalization process by reaction with suitable acid promoters.[40,41] The scope and limitations of the methods as well as the factors influencing the relative proportions of the products are discussed.

2. Results and discussion

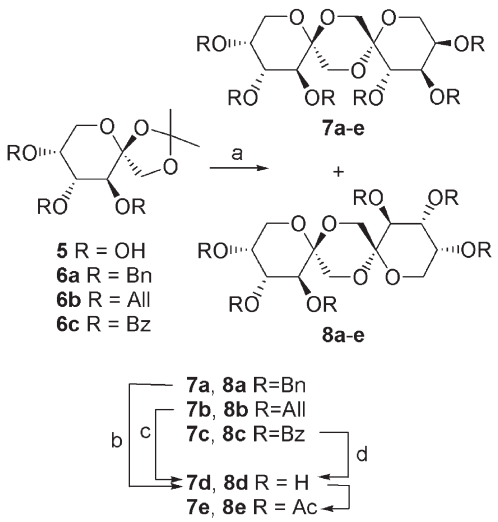
3,4,6-Tri-*O*-protected 1,2-*O*-isopropylidene-β-D-fructofuranoses **2a**-**c**, readily accessible from the known 1,2-*O*-isopropylidene-β-D-fructofuranose[42] **1**, were used as furanose-anchored precursors for the preparation of type I DFAs. First, a screening of their reactivity in the presence of a series of acid promoters was carried out (see Table 1 for selected results). Diethylaluminium chloride was found to be inefficient to provoke acetal cleavage in either toluene or dichloromethane, even using a large excess of reagent at 50 °C. Treatment with tin (IV) chloride or zinc chloride ($ZnCl_2{\cdot}Et_2O$) etherate resulted in removal of the isopropylidene group even at room temperature, but these reagents were unable to promote the subsequent glycosylation–spiroketalization reaction. A slight improvement was observed using $ZnCl_2{\cdot}Et_2O$ at 50 °C in toluene, although the final DFA products (**3a**–**c**, **4a**–**c**) were isolated in disappointingly low yields (4–15%) and poor stereoselectivities (Table 1, entries 1, 4 and 7). Interestingly, boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$) and trifluoromethanesulfonic acid (triflic acid, TfOH) succeeded in promoting the desired tandem transformations in toluene (see Table 1, entries 2, 5, and 8 for results using $BF_3{\cdot}Et_2O$), which is in agreement with their broad use for the cleavage of acetal protecting groups, as glycosylation promoters and as spiroketalization catalysts.[40,41,43,44] The use of dichloromethane as solvent was detrimental in the case of $BF_3{\cdot}Et_2O$; in contrast, it resulted in improved yields in the case of the protic acid promoter TfOH (Table 1, entries 3, 6 and 9). Employing these optimal reaction conditions, conversion

Table 1. Acid-promoted dimerization of 1,2-*O*-isopropyliden-D-fructose derivatives (**2a**–**c** and **6a**–**c**) to give DFAs (**3a**–**c**, **4a**–**c** and **7a**–**c**, **8a**–**c**, respectively)

Entry	Starting material	Acid promoter (equiv.)	Solvent	Temp. (°C)	Reaction time (h)	Yield (%)	Products (ratio)
1	**2a**	$ZnCl_2{\cdot}Et_2O$ (4.0)	Toluene	50	16	4	**3a**:**4a** (1:1)
2	**2a**	$BF_3{\cdot}Et_2O$ (1.0)	Toluene	−20	4.5	64	**3a**:**4a** (2:5)
3	**2a**	TfOH (1.5)	CH_2Cl_2	−78→20	1	75	**3a**:**4a** (1:2)
4	**2b**	$ZnCl_2{\cdot}Et_2O$ (4.0)	Toluene	50	16	12	**3b**:**4b** (2:3)
5	**2b**	$BF_3{\cdot}Et_2O$ (1.0)	Toluene	−20	3	75	**3b**:**4b** (1:5)
6	**2b**	TfOH (1.5)	CH_2Cl_2	−78→20	1	92	**3b**:**4b** (1:7)
7	**2c**	$ZnCl_2{\cdot}Et_2O$ (4.0)	Toluene	50	16	15	**3c**:**4c** (2:3)
8	**2c**	$BF_3{\cdot}Et_2O$ (2.0)	Toluene	4	16	40	**3c**:**4c** (24:1)
9	**2c**	TfOH (1.5)	CH_2Cl_2	−78→20	2	91	**3c**:**4c** (25:1)
10	**6a**	$BF_3{\cdot}Et_2O$ (1.5)	Toluene	−20	5	65	**7a**:**8a** (1:25)
11	**6a**	TfOH (1.5)	CH_2Cl_2	−78→20	1.5	76	**7a**:**8a** (1:20)
12	**6b**	$BF_3{\cdot}Et_2O$ (1.5)	Toluene	−20	5	68	**7b**:**8b** (1:7)
13	**6b**	TfOH (1.5)	CH_2Cl_2	−78→20	1.5	87	**7b**:**8b** (1:6)
14	**6c**	$BF_3{\cdot}Et_2O$ (2.0)	Toluene	20	72	80	**7c**:**8c** (1:1)
15	**6c**	TfOH (2.0)	CH_2Cl_2	−78→20	3	87	**7c**:**8c** (1:1)



Scheme 2. Synthesis of type I DFAs. Reagents: (a) $BF_3{\cdot}Et_2O$ in toluene or TfOH in CH_2Cl_2 (see Table 1); (b) H_2/Pd–C, AcOH–MeOH–HCOOH (90–97%); (c) $PdCl_2$, MeOH (70–80%); (d) NaOMe/MeOH (>95%); (e) Ac_2O-pyridine (>95%).



Scheme 3. Synthesis of type III DFAs. Reagents: (a) $BF_3{\cdot}Et_2O$ in toluene or TfOH in CH_2Cl_2 (see Table 1); (b) H_2/Pd–C, AcOH–MeOH–HCOOH (>95%); (c) $PdCl_2$, MeOH (70–75%); (d) NaOMe/MeOH (>95%); (e) Ac_2O-pyridine (>95%).

yields into the corresponding dispiro-disaccharides **3a–c**, **4a–c** in the range 75–92% were obtained (Scheme 2).

It is noteworthy that only two of the three possible type I DAF structures (the α,α, β,β and α,β diastereomers) were formed in all cases, namely the hexa-*O*-protected di-α-D-fructofuranose 1,2′:2,1′-dianhydride (**3a–c**) and α-D-fructofuranose β-D-fructofuranose 1,2′:2,1′-dianhydride derivatives (**4a–c**). Their relative proportions were strongly dependent on the nature of the hydroxyl protecting groups. Thus, in the case of ether-type groups (**2a** and **2b**) the non-symmetric diastereomer (**4a** and **4b**) was favored, with 43 and 75% diastereomeric excess (de) values over the C_2-symmetric dianhydride (**3a** and **3b**). The stereochemical outcome of the spiroketalization reaction was reversed for the benzoyl counterpart (**2c**), leading to a 92% de in favour of the α,α (**3c**) over the α,β isomer (**4c**).

To implement this approach for the stereoselective preparation of dispiro-difructopyranose dianhydrides (type III DFAs), 3,4,5-tri-*O*-benzyl- (**6a**),[45] 3,4,5-tri-*O*-allyl- (**6b**) and 3,4,5-tri-*O*-benzoyl-β-D-fructopyranose (**6c**),[46] available in three steps from D-fructose via the corresponding monoacetonide **5**,[46] were used as pyranose-anchored D-fructose templates. As a general rule, the acid-promoted dimerization process proceeded more slowly in these cases, in agreement with the lower stability of the six-membered cyclic oxocarbenium cation. Nevertheless, satisfactory conversion rates were obtained using either $BF_3{\cdot}Et_2O$ or TfOH by increasing the proportion of the catalyst, the temperature or using longer reaction times, TfOH in dichloromethane providing the higher yields on the corresponding DFA products. Binary mixtures of the corresponding hexa-*O*-protected di-β-D-fructopyranose 1,2′:2,1′-dianhydride (**7a–c**) and α-D-fructopyranose β-D-fructopyranose 1,2′:2,1′-dianhydride (**8a–c**) were obtained in all cases (Scheme 3). As previously observed in the furanose series, a strong stereodirecting effect of the hydroxyl protecting groups in the generation of the spiroketal stereocentres was observed. Thus, while the benzoyl derivatives (**7c** and **8c**) were obtained in identical relative proportion (Table 1, entries 14 and 15), the non-symmetric α,β-diastereomer (**8a** and **8b**) was favoured in the case of the benzylated or allylated pairs (Table 1, entries 10–13).

Pure samples of the hexa-*O*-protected individual DFA isomers could be obtained in all cases after column chromatography. Nevertheless, in the case of the benzoylated derivatives (**3c**, **4c** and **7c**, **8c**), replacing the benzoyl groups into acetyl (to give **3e**, **4e** and **7e**, **8e**, respectively) prior to column chromatography was advantageous for preparative purposes. The structure of all DFAs prepared in this study was confirmed by microanalytical, NMR and MS data. The chemical shifts of the anomeric C-2 (C-2′) carbon atoms are particularly useful for diagnostic purposes, behaving as a fingerprint for a given DFA core structure.[14] The structural assignment was further confirmed by transformation into the known fully unprotected DFAs (**3d**, **4d**, **7d** and **8d**) by removal of the *O*-protecting groups through standard methodologies. The relative proportions of stereoisomers in the mixtures was established by GC chromatography after derivatization of the unprotected DFAs as the corresponding hexa-*O*-trimethylsilyl derivatives, following the procedure previously reported for determination of DFAs in food products.[31]

The possibility to control not only the ring size but also the stereochemistry at the spiroketal centres in the synthesis of DFAs is noteworthy. We hypothesized that, in contrast to the mineral acid-catalyzed reaction, spiroketalization occurs in apolar organic solvents under virtually irreversible conditions, thus limiting isomerization processes. To confirm this point, the hexabenzylated C_2-symmetric dianhydride **7a** was subjected to the reaction conditions previously used to promote dimerization of **6a**. No isomerization into the favored non-symmetric diastereomer **8a** was detected after 24 h, supporting the above assumption. The preference for non-symmetric over C_2-symmetric DFA structures in the case of non-participating protecting groups (i.e., benzyl and allyl) can be rationalized in terms of

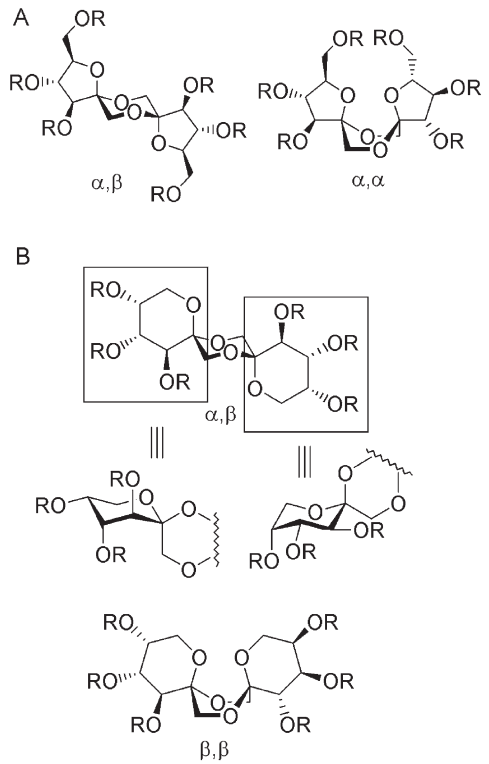


Figure 1. Conformations for non-symmetric and C_2-symemtric DFAs of types I (A) and III (B).

the relative stability of the incipient 1,4-dioxane ring leading to a given isomer. In the case of the α,β diastereomers, the central ring can accommodate the oxygen substituents in axial disposition and the carbon substituents in equatorial disposition in the chair conformation. Such situation does not prevail for the symmetric isomers, which must adopt a boat conformation at the central ring to accommodate the anomeric effect at both anomeric centres, a less favourable arrangement (Fig. 1(A) and (B)).[14]

The dimerization reaction of substrates bearing participating ester groups (**2c** and **6c**) probably proceeds through acyloxonium entities. The lower reactivity of these species as compared with the fructosyl cation is in agreement with the observed lower reactivity of benzoylated D-fructose derivatives towards dimerization (Table 1). In the furanose series, the formation of a *cis*-fused 2,3-acyloxonium cation intermediate (**9**) prevents O-1′ attack through the β-face in both the glycosylation and spirocyclization steps (Fig. 2(A)). Consequently, the thermodynamically less favoured di-α isomer **3c** is formed almost exclusively. In the pyranose series, however, glycosylation of the corresponding 2,3-acyloxonium cation (**10**) may compete with the attack by the benzoate group at C-5 to give a 2,5-acyloxonium intermediate (**11**), which blocks the nucleophilic attack by O-1′ through the α-face (Fig. 2(B)). At its turn, this cation will undergo selectively glycosylation-spiroketalization through the more accessible β-face to give **7c** (Fig. 2). It must be noticed that the α- and β-D-fructopyranose rings in α-fructopyranose β-fructopyranose 1,2′:2,1′-dianhydride derivatives adopts the $^4C_1$ and $^1C_4$ chair conformation, respectively, in order to fit the anomeric effect (Fig. 1(B)). The unfavourable steric interactions in the α-ring are

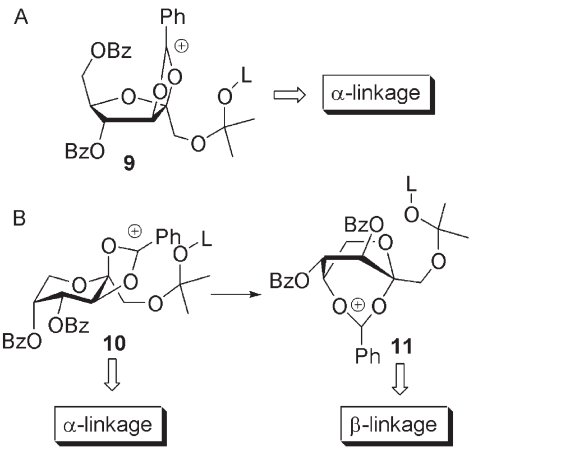


Figure 2. Probable structure of the acyloxonium cations involved in the dimerization reaction of benzoylated D-fructofuranose (A) and D-fructopyranose (B) precursors.

compensated by the gain in stability due to the chair arrangement of the central 1,4-dioxane ring in asymmetric dianhydrides, a situation that would not apply for a C_2-symmetric diastereomer. In fact, no dipyranose DFA derivatives having the α,α configuration have been reported up to date. Probably, the α-(2→1)-linked disaccharide derived from intermediate **10** would necessarily undergo spiroketalization through the β-face to give **8c**.

In conclusion, we have demonstrated that 1,2-*O*-isopropylidene-D-fructose derivatives are suitable precursors for the synthesis of difuranose and dipyranose DFAs. Boron trifluoride diethyl etherate complex and triflic acid are capable to promote acetal deprotection and dimerization to the corresponding spiro-cyclic disaccharides in organic solvents, under irreversible conditions. Both the ring size and the stereochemistry at the spiroketal centres can be controlled by judicious choice of the protecting groups in the monosaccharide template, non-participating groups favouring non-symmetric structures and participating groups the C_2-symmetric diastereomers.

3. Experimental

3.1. General methods

All solvents and reagents were purchased from commercial sources and used without further purification, except for toluene and dichloromethane, which were distilled under Ar stream over Na and CaH_2, respectively. 1,2-*O*-Isopropylidene-β-D-fructofuranose[42] (**1**), 1,2-*O*-isopropylidene-β-D-fructopyranose[46] (**5**) and 3,4,5-tri-*O*-benzoyl-1,2-*O*-isopropylidene-β-D-fructopyranose[46] (**6c**) were prepared according to described procedures. 3,4,5-Tri-*O*-benzyl-1,2-*O*-isopropylidene-β-D-fructopyranose (**6a**) has been previously obtained from **5** in 17% yield by treatment with benzyl bromide in tetrahydrofurane.[45] An improved preparation (70% yield), including full characterization data, is given hereinafter. Optical rotations were measured at room temperature in 1-cm or 1-dm tubes on a Perkin–Elmer 141 MC polarimeter. ^{1}H (and ^{13}C NMR) spectra were recorded at 300 (75.5) and 500 (125.7) MHz with Bruker 300 AMX and 500 DRX instruments, respectively. 2D

COSY, HMQC and HSQC experiments were used to assist on NMR assignments. Thin-layer chromatography (TLC) was carried out on aluminium sheets coated with Kieselgel 60 F254 (E. Merck), with visualisation by UV light and by charring with 10% H_2SO_4. Column chromatography was carried out on Silica Gel 60 (E. Merck, 230–400 mesh). FAB mass spectra were obtained with a Kratos MS-80 RFA instrument. The operating conditions were the following: the primary beam consisted of Xe atoms with a maximum energy of 8 keV; the samples were dissolved in thioglycerol, and the positive ions were separated and accelerated over a potential of 7 keV; NaI was added as cationizing agent. Elemental analyses were performed at the Instituto de Investigaciones Químicas (Sevilla, Spain).

Debenzylation of hexa-*O*-benzylated DAFs (**3a**, **4a**, **7a** and **8a**) or their mixtures was effected by catalytic hydrogenation with 10% Pd/C at 1 atm in 1:1 EtOAc–MeOH containing 10% formic acid. Deallylation reactions (**3b**, **4b**, **7b** and **8b**) were accomplished by treatment with $PdCl_2$ in MeOH.[47] Conventional debenzoylation (**3c**, **4c**, **7c** and **8c**) was carried out with methanolic NaOMe (1 M). Acetylation of fully unprotected DAF mixtures was performed with 1:1 Ac_2O–pyridine. In all cases, the physicochemical data for the individual fully unprotected DFAs (**3d**, **4d**, **7d** and **8d**) or the corresponding per-*O*-acetates (**3e**, **4e**, **7e** and **8e**) were identical to those previously reported.

3.1.1. 3,4,6-Tri-*O*-benzyl-1,2-*O*-isopropylidene-β-D-fructofuranose (2a). To a solution of **1** (1 g, 4.5 mmol) in DMF (15 mL), NaH (0.44 g, 18.2 mmol, 1.4 equiv.) and benzyl bromide (2.43 mL, 20 mmol, 1.5 equiv.) were added and the reaction mixture was stirred for 4 h at room temperature. Then, MeOH (5 mL) was added, the solvents were evaporated under reduced pressure and the residue was extracted with Et_2O (20 mL), washed with water (20 mL), dried ($MgSO_4$), concentrated and purified by column chromatography (1:8 EtOAc–petroleum ether) to furnish **2a** (1.78 g, 81%). R_f=0.33 (1:8 EtOAc–petroleum ether); $[\alpha]_D$=−27.3 (*c* 1.1, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.49, 1.53 (2s, each 3H, CMe_2), 3.46 (dd, 1H, $J_{5,6a}$=6.2 Hz, $J_{6a,6b}$=9.8 Hz, H-6a), 3.70 (dd, 1H, $J_{5,6b}$=6.2 Hz, H-6b), 3.99 (d, 1H, $J_{1a,1b}$=9.4 Hz, H-1a), 4.06 (d, 1H, $J_{3,4}$=5.0 Hz, H-3), 4.09 (d, 1H, H-1b), 4.17 (td, 1H, $J_{4,5}$=5.0 Hz, H-5), 4.19 (t, 1H, H-4), 4.60–4.77 (m, 6H, C*H*$_2$Ph), 7.33–7.39 (m, 15H, Ph); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 26.4, 26.5 (C*Me*$_2$), 71.3 (C-1), 72.2 (C-6), 73.5 (3*C*H$_2$Ph), 80.1 (C-5), 83.2 (C-3), 84.5 (C-4), 109.2 (C-2), 111.5 (*C*Me$_2$), 127.5–138.1 (Ph); FABMS: *m*/*z* 513 (100%, $[M+Na]^+$). Anal. Calcd for $C_{30}H_{34}O_6$: C, 73.45; H, 6.99; found: C, 73.52; H, 6.90.

3.1.2. 3,4,6-Tri-*O*-allyl-1,2-*O*-isopropylidene-β-D-fructofuranose (2b). To a solution of **1** (1.06 g, 4.8 mmol) in DMF (15 mL), NaH (0.87 g, 36 mmol) and allyl bromide (1.34 mL, 15.8 mmol) were added and the reaction mixture was stirred for 15 min at room temperature. Then water (5 mL) was added and the reaction mixture was extracted with Et_2O (5×40 mL). The organic layer was washed with H_2O (5×25 mL), dried ($MgSO_4$), and concentrated, and the residue purified by column chromatography (1:7 EtOAc–petroleum ether) to afford **2b** (1.31 g, 80%). R_f=0.50 (1:5 EtOAc–petroleum ether); $[\alpha]_D$=−32.1 (*c* 1.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38, 1.43 (2s, each 3H, CMe_2), 3.52 (dd, 1H, $J_{6a,6b}$=10.0 Hz, $J_{5,6a}$=6.2 Hz, H-6a), 3.56 (dd, 1H, $J_{5,6b}$=6.4 Hz, H-6b), 3.87 (d, 1H, $J_{3,4}$=6.5 Hz, H-3), 3.94, (dd, 1H, $J_{4,5}$=5.1 Hz, H-4), 3.98 (ddd, 1H, H-5), 3.99 (d, 1H, $J_{1a,1b}$=9.5 Hz, H-1a), 4.05 (d, 1H, H-1b), 3.97–4.17 (m, 6H, CH_2O), 5.13–5.30 (m, 6H, C*H*$_2$=CH), 5.83–5.95 (m, 3H, CH$_2$=C*H*); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 26.5, 26.6 (C*Me*$_2$), 71.2, 71.3 (3CH_2), 72.2 (C-6), 72.4 (C-1), 80.1 (C-5), 83.2 (C-3), 84.5 (C-4), 109.0 (C-2), 111.5 (*C*Me$_2$), 117.0, 117.4 (3CH=*C*H$_2$), 134.2, 134.5 (3*C*H=CH$_2$); FABMS: *m*/*z* 341 (30%, $[M+H]^+$). Anal. Calcd for $C_{18}H_{28}O_6$: C, 63.51; H, 8.29; found: C, 63.46; H, 8.58.

3.1.3. 3,4,6-Tri-*O*-benzoyl-1,2-*O*-isopropylidene-β-D-fructofuranose (2c). A solution of **1** (1 g, 4.5 mmol) and benzoyl chloride (2.65 mL, 23.3 mmol) in pyridine (8 mL) was stirred for 16 h at room temperature. Iced water (40 mL) was added to the reaction mixture and the suspension was extracted with CH_2Cl_2 (2×20 mL), the organic layer was dried ($MgSO_4$) and concentrated. The resulting residue was purified by column chromatography (1:5 EtOAc–petroleum ether) to yield **2c** (1.77 g, 73%). R_f=0.43 (1:4 EtOAc–petroleum ether); $[\alpha]_D$=−52.0 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.26, 1.44 (2s, each 3H, CMe_2), 4.21 (d, 1H, $J_{1a,1b}$=9.4 Hz, H-1a), 4.36 (d, 1H, H-1b), 4.51 (ddd, 1H, $J_{4,5}$=5.1 Hz, $J_{5,6a}$=4.6 Hz, $J_{5,6b}$=6.4 Hz, H-5), 4.67 (dd, 1H, $J_{6a,6b}$=11.6 Hz, H-6a), 4.82 (dd, 1H, H-6b), 5.87 (d, 1H, $J_{3,4}$=5.1 Hz, H-3), 5.95 (t, 1H, H-4), 7.39–8.12 (m, 15H, Ph); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 26.0 (C*Me*$_2$), 65.1 (C-6), 71.3 (C-1), 75.9 (C-3), 77.0 (C-4), 79.2 (C-5), 108.9 (C-2), 112.0 (*C*Me$_2$), 128.2–134.5 (Ph), 165.6, 165.7, 166.1 (3CO); FABMS: *m*/*z* 555 (100%, $[M+Na]^+$). Anal. Calcd for $C_{30}H_{28}O_9$: C, 67.66; H, 5.30; found: C, 67.68; H, 5.29.

3.1.4. 2,3,4-Tri-*O*-benzyl-1,2-*O*-isopropylidene-β-D-fructopyranose (6a). Compound **6a** was prepared from **5** (0.6 g, 2.72 mmol) as above described for **2a**, followed by column chromatography purification (1:9→1:7 EtOAc–petroleum ether). Yield: 0.90 g (70%); $[\alpha]_D$=−98.5 (*c* 1.0, CH_2Cl_2); Lit. $[\alpha]_D$=−81.2 (*c* 0.92, $CHCl_3$); R_f=0.30 (1:5 EtOAc–petroleum ether); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.56, 1.66 (2s, each 3H, CMe_2), 3.84 (m, 1H, H-5), 3.88 (m, 2H, H-6), 4.04 (dd, 1H, $J_{3,4}$=2.5 Hz, $J_{4,5}$=9.9 Hz, H-4), 4.08 (d, 1H, H-3), 4.09 (d, 1H, $J_{1a,1b}$=8.7 Hz, H-1a), 4.11 (d, 1H, H-1b), 4.58–5.16 (m, 6H, C*H*$_2$Ph), 7.31–7.49 (m, 15H, Ph); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 26.3, 27.2 (C*Me*$_2$), 62.4 (C-6), 67.5 (C-3), 70.0 (C-4), 70.2 (C-5), 71.8 (C-1), 71.6, 72.0, 75.4 (*C*H$_2$Ph), 104.2 (C-2), 111.8 (*C*Me$_2$), 127.4–138.5 (Ph); FABMS: *m*/*z* 513 (100%, $[M+Na]^+$). Anal. Calcd for $C_{30}H_{34}O_6$: C, 73.45; H, 6.99; found: C, 73.49; H, 6.87.

3.1.5. 2,3,4-Tri-*O*-allyl-1,2-*O*-isopropylidene-β-D-fructopyranose (6b). Compound **6b** was prepared from **5** (1.19 g, 5.4 mmol) as above described for **2b**, followed by column chromatography purification (1:7 EtOAc–petroleum ether). Yield: 1.37 g (75%); (1:7 EtOAc–petroleum ether). R_f=0.53 (1:5 EtOAc–petroleum ether); $[\alpha]_D$=−113.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.38, 1.43 (2s, each 3H, CMe_2), 3.66 (d, 1H, $J_{3,4}$=2.9 Hz, H-3), 3.71 (dd, 1H, $J_{4,5}$=9.7 Hz, H-4), 3.75 (m, 1H, H-5), 3.79 (m, 2H, H-6), 3.96 (d, 1H, $J_{1a,1b}$=8.4 Hz, H-1a), 4.03 (d, 1H, H-1b), 4.04–4.17 (m, 6H, CH_2O), 5.08–5.30 (m, 6H, C*H*$_2$=CH),

5.83–5.95 (m, 3H, CH_2=C*H*); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 26.1, 27.2 (CMe_2), 70.9, 71.1, 74.4 (3CH_2O), 111.8 (*C*Me$_2$), 116.4, 116.6, 117.4 (3CH=*C*H$_2$), 134.9, 135.2 (3*C*H=CH$_2$); FABMS: *m*/*z* 341 (100%, [M+H]$^+$). Anal. Calcd for $C_{18}H_{28}O_6$: C, 63.51; H, 8.29; found: C, 63.63; H, 8.00.

3.2. General procedure for the preparation of difructose dianhydrides (3a–c, 4a–c, 7a–c, 8a–c)

(a) By treatment with $BF_3{\cdot}Et_2O$. To a stirred 0.15 M solution of the corresponding 1,2-*O*-isopropylidene-D-fructose derivative **2a**–**c** or **6a**–**c** in dry toluene under Ar, the acid promoter $BF_3{\cdot}Et_2O$ was added. Reaction conditions (equivalents of acid, reaction temperature and reaction time) are collected in Table 1. The reaction mixture was quenched by addition of MeOH, washed with 5% aq. $NaHCO_3$, the organic layer was dried ($MgSO_4$), the solvents were evaporated under reduced pressure, and the products were separated by column chromatography with the eluent indicated in each case.

(b) By treatment with TfOH. To a stirred 50 mM solution of the corresponding 1,2-*O*-isopropylidene-D-fructose derivative **2a**–**c** or **6a**–**c** in freshly distilled CH_2Cl_2 under Ar at −78 °C, TfOH was added. The reaction mixture was allowed to warm up to room temperature and stirred for the indicated time (Table 1). Et_3N (0.5 mL) was added, the reaction mixture was stirred for 10 min, the solvents were evaporated under reduced pressure and the products were separated by column chromatography with the eluent indicated in each case.

Conversion yields are collected in Table 1. For benzylated (**3a**, **4a** and **7a**, **8a**) and allylated derivatives (**3b**, **4b** and **7b**, **8b**), efficient separations of the individual diastereomers were achieved after column chromatography. In the case of perbenzoylated derivatives (**3c**, **4c** and **7c**, **8c**), however, only small amounts of the pure DFAs could be obtained after a second column chromatography. Transformation into the corresponding per-*O*-acetates (**3e**, **4e** and **7e**, **8e**) allowed the efficient separation of individual isomers (column chromatography, eluent 1:3 EtOAc–petroleum ether). The relative proportions of C_2-symmetric versus non-symmetric diastereomers in the reaction mixtures were determined by GC after transformation into the corresponding mixtures of fully unprotected DFAs (**3d**, **4d** or **7d**, **8d**) and further derivatization as the corresponding hexa-*O*-trimethylsilyl derivatives, following the protocol previously reported.[31] The identity of the peaks was confirmed by comparison with authentic standards.

3.2.1. 3,4,6,3′,4′,6′-Hexa-*O*-benzyl-di-α-D-fructofuranose 1,2′:2,1′-dianhydride (3a). R_f=0.33 (1:4 EtOAc–petroleum ether); $[\alpha]_D$=+71.0 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.58 (dd, 2H, $J_{6a,6b}$=10.9 Hz, $J_{5,6a}$=4.9 Hz, H-6a), 3.61 (dd, 2H, $J_{5,6b}$=4.9 Hz, H-6b), 3.84 (d, 2H, $J_{1a,1b}$=12.7 Hz, H-1a), 3.87 (dd, 2H, $J_{3,4}$=3.8 Hz, $J_{4,5}$=6.8 Hz, H-4), 4.06 (d, 2H, H-3), 4.12 (d, 2H, H-1b), 4.20 (dt, 2H, H-5), 4.43–4.73 (m, 12H, CH_2Ph), 7.26–7.33 (m, 30H, Ph); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 59.9 (C-1), 68.0 (C-6), 72.2, 72.7, 73.5 (*C*H$_2$Ph), 79.3 (C-5), 83.2 (C-4), 88.0 (C-3), 105.4 (C-2), 127.5–138.1 (Ph); FABMS: *m*/*z* 887 (100%, [M+Na]$^+$). Anal. Calcd for $C_{54}H_{56}O_{10}$: C, 74.98; H, 6.52; found: C, 74.77; H, 6.29.

3.2.2. 3,4,6,3′,4′,6′-Hexa-*O*-allyl-di-α-D-fructofuranose 1,2′:2,1′-dianhydride (3b). R_f=0.60 (1:3 EtOAc–petroleum ether); $[\alpha]_D$=+92.1 (*c* 0.55, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.52 (dd, 2H, $J_{5,6a}$=5.4 Hz, $J_{6a,6b}$=11.0 Hz, H-6a), 3.56 (dd, 2H, $J_{5,6b}$=4.9 Hz, H-6b), 3.70 (dd, 2H, $J_{3,4}$=3.9 Hz, $J_{4,5}$=6.7 Hz, H-4), 3.71 (d, 2H, $J_{1a,1b}$=12.8 Hz, H-1a), 3.86 (d, 2H, H-3), 3.97 (d, 2H, H-1b), 4.01 (m, 2H, H-5), 4.00–4.18 (m, 12H, CH_2O), 5.14–5.29 (m, 12H, CH_2=CH), 5.83–5.93 (m, 6H, CH_2=C*H*); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 59.7 (C-1), 69.8 (C-6), 71.2, 71.4, 72.4 (6CH_2O), 79.3 (C-5), 83.2 (C-4), 87.9 (C-3), 105.4 (C-2), 117.0, 117.3, 117.6 (6CH=*C*H$_2$), 134.2, 134.5, 134.7 (6*C*H=CH$_2$). Anal. Calcd for $C_{30}H_{44}O_{10}$: C, 63.81; H, 7.85; found: C, 63.46; H, 8.05.

3.2.3. 3,4,6,3′,4′,6′-Hexa-*O*-benzoyl-di-α-D-fructofuranose 1,2′:2,1′-dianhydride (3c). R_f=0.21 (1:20 EtOAc–toluene); $[\alpha]_D$=−1.0 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.17 (d, 2H, $J_{1a,1b}$=12.2 Hz, H-1a), 4.21 (d, 2H, H-1b), 4.58 (ddd, 2H, $J_{4,5}$=5.4 Hz, $J_{5,6a}$=4.9 Hz, $J_{5,6b}$=3.1 Hz, H-5), 4.66 (dd, 2H, $J_{6a,6b}$=11.9 Hz, H-6a), 4.79 (dd, 2H, H-6b), 5.55 (dd, 2H, $J_{3,4}$=1.8 Hz, H-4), 5.77 (d, 2H, H-3), 7.39–8.07 (m, 30H, Ph); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 60.8 (C-1), 63.0 (C-6), 80.0 (C-3), 80.4 (C-5), 78.4 (C-4), 103.8 (C-2), 128.2–133.5 (Ph), 164.7, 165.6, 166.0 (CO); FABMS: *m*/*z* 971 (100%, [M+Na]$^+$). Anal. Calcd for $C_{54}H_{44}O_{16}$: C, 68.35; H, 4.67; found: C, 68.20; H, 4.51.

3.2.4. 3,4,6-Tri-*O*-benzyl-α-D-fructofuranose 3,4,6-tri-*O*-benzyl-β-D-fructofuranose 1,2′:2,1′-dianhydride (4a). R_f=0.32 (1:4 EtOAc–petroleum ether); $[\alpha]_D$=−5.2 (*c* 3.4, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.46 (d, 1H, $J_{1'a,1'b}$=10.5 Hz, H-1′a), 3.60 (dd, 1H, $J_{5',6'a}$=5.8 Hz, $J_{6'a,6'b}$=9.6 Hz, H-6′a), 3.67 (dd, 1H, $J_{5',6'b}$=6.8 Hz, H-6′b), 3.68 (dd, 1H, $J_{5,6a}$=5.1 Hz, $J_{6a,6b}$=10.8 Hz, H-6a), 3.75 (dd, 1H, $J_{5,6b}$=4.6 Hz, H-6b), 3.84 (1H, d, $J_{1a,1b}$=13.1 Hz, H-1a), 3.95 (1H, dd, $J_{3,4}$=2.0 Hz, $J_{4,5}$=5.4 Hz, H-4), 4.04 (d, 1H, H-3), 4.14 (m, 1H, H-5), 4.15 (d, 1H, H-1′b), 4.18 (d, 1H, H-1b), 4.20 (m, 1H, H-3′), 4.21 (m, 1H, H-4′), 4.27 (bdd, 1H, H-5′), 4.39–4.74 (m, 12H, CH_2Ph), 7.21–7.31 (m, 30H, Ph); ^{13}C NMR (125.5 MHz, $CDCl_3$) δ 62.5 (C-1), 63.3 (C-1′), 70.1 (C-6), 71.6 (C-6′), 71.9, 72.0, 72.2, 72.4, 73.2, 73.4 (*C*H$_2$Ph), 80.2 (C-5), 81.8 (C-4′), 83.6 (C-4), 84.7 (C-5′), 88.2 (C-3, C-3′), 99.6 (C-2′), 102.5 (C-2), 127.4–138.1 (Ph); FABMS: *m*/*z* 887 (100%, [M+Na]$^+$). Anal. Calcd for $C_{54}H_{56}O_{10}$: C, 74.98; H, 6.52. Found: C, 75.05; H, 6.50.

3.2.5. 3,4,6-Tri-*O*-allyl-α-D-fructofuranose 3,4,6-tri-*O*-allyl-β-D-fructofuranose 1,2′:2,1′-dianhydride (4b). R_f=0.40 (1:3 EtOAc–petroleum ether); $[\alpha]_D$=+4.3 (*c* 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.46 (d, 1H, $J_{1'a,1'b}$=11.5 Hz, H-1′a), 3.51 (dd, 1H, $J_{5',6'a}$=6.4 Hz, $J_{6'a,6'b}$=9.8 Hz, H-6′a), 3.58 (dd, 1H, $J_{5',6'b}$=5.8 Hz, H-6′b), 3.59 (dd, 1H, $J_{5,6a}$=3.1 Hz, $J_{6a,6b}$=11.5 Hz, H-6a), 3.61 (dd, 1H, $J_{5,6b}$=3.8 Hz, H-6b), 3.65 (d, 1H, $J_{3',4'}$=6.2 Hz, H-3′), 3.69, (d, 1H, $J_{1a,1b}$=12.0 Hz, H-1a), 3.75 (dd, 1H, $J_{3,4}$=2.6 Hz, $J_{4,5}$=5.8 Hz, H-4), 3.84 (d, 1H, H-3), 4.01 (m, 1H, H-4′), 4.03 (m, 1H, H-5), 4.06 (m, 1H, H-5′), 4.06 (d, 1H, H-1b),

3.95–4.12 (m, 12H, CH_2O), 4.14 (d, 1H, H-1′b), 5.10–5.30 (m, 12H, CH_2=CH), 5.78–5.92 (m, 6H, CH_2=C*H*); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 62.7 (C-1), 63.5 (C-1′), 70.3 (C-6), 71.9 (C-6′), 70.9, 71.1, 71.2, 71.9, 72.3, 72.4 (CH_2O), 80.1 (C-5), 81.4 (C-5′), 83.8 (C-4), 84.6 (C-3′), 84.7 (C-4′), 88.3 (C-3), 99.6 (C-2′), 102.4 (C-2), 116.8, 117.1, 117.2, 117.3, 117.4, 117.9 (CH=*C*H_2), 134.0, 134.3, 134.4, 134.5, 134.6, 134.8 (*C*H=CH_2). Anal. Calcd for $C_{30}H_{44}O_{10}$: C, 63.81; H, 7.85; found: C, 63.74; H, 7.79.

3.2.6. 3,4,6-Tri-*O*-benzoyl-α-D-fructofuranose 3,4,6-tri-*O*-benzoyl-β-D-fructofuranose 1,2′:2,1′-dianhydride (4c). R_f=0.58 (1:2 EtOAc–petroleum ether); $[\alpha]_D$=−46.0 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.90 (d, 1H, $J_{1'a,1'b}$=11.8 Hz, H-1′a), 3.98 (d, 1H, $J_{1a,1b}$=11.9 Hz, H-1a), 4.38 (d, 1H, H-1b), 4.42 (d, 1H, H-1′b), 4.50 (ddd, 1H, $J_{4',5'}$=4.9 Hz, $J_{5',6'a}$=3.5 Hz, $J_{5,6b}$=4.3 Hz, H-5′), 4.63 (m, 1H, H-5), 4.67 (dd, 1H, $J_{5,6a}$=4.5 Hz, $J_{6a,6b}$=12.1 Hz, H-6a), 4.74 (dd, 1H, $J_{5,6b}$=3.4 Hz, H-6b), 4.82 (dd, 1H, $J_{6'a,6'b}$=10.5 Hz, H-6′a), 4.84 (dd, 1H, H-6′b), 5.52 (dd, 1H, $J_{3,4}$=1.1 Hz, $J_{4,5}$=4.9 Hz, H-4), 5.66 (d, 1H, H-3), 5.69 (1H, d, $J_{3',4'}$=6.8 Hz, H-3′), 6.03 (1H, dd, $J_{4',5'}$=4.9 Hz, H-4′), 7.10–8.10 (m, 30H, Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 61.7 (C-1), 63.1 (C-1′), 63.6 (C-6), 70.1 (C-6′), 77.3 (C-4), 78.9 (C-5), 80.2 (C-5′), 81.9 (C-3), 83.6 (C-4′), 88.2 (C-3′), 99.9 (C-2′), 102.1 (C-2), 128.0–133.5 (Ph), 164.5–166.0 (CO); FABMS: *m*/*z* 971 (100%, $[M+Na]^+$). Anal. Calcd for $C_{54}H_{44}O_{16}$: C, 68.35; H, 4.67; found: C, 68.52; H, 4.86.

3.2.7. 3,4,5,3′,4′,5′-Hexa-*O*-benzyl-di-β-D-fructopyranose 1,2′:2,1′-dianhydride (7a). R_f=0.47 (1:2 EtOAc–petroleum ether); $[\alpha]_D$=−88.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.59 (d, 2H, $J_{1a,1b}$=12.1 Hz, H-1a), 3.67 (dd, 2H, $J_{5,6a}$=0.5 Hz, $J_{6a,6b}$=11.6 Hz, H-6a), 3.75 (dd, 2H, $J_{5,6b}$=1.8 Hz, H-6b), 3.77 (m, 2H, H-5), 3.85 (d, 2H, H-1b), 3.91 (d, 2H, $J_{3,4}$=9.8 Hz, H-3), 4.02 (d, 2H, $J_{4,5}$=3.0 Hz, H-4), 4.63–4.95 (m, 12H, C*H*$_2$Ph), 7.20–7.35 (m, 30H, Ph); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 62.8 (C-6), 63.3 (C-1), 68.7 (C-4), 70.3 (C-5), 71.6 (C-3), 71.7, 72.5, 74.6 (CH_2O), 97.3 (C-2), 127.2–138.9 (Ph). Anal. Calcd for $C_{30}H_{44}O_{10}$: C, 74.98; H, 6.53; found: C, 74.84; H, 6.36.

3.2.8. 3,4,5,3′,4′,5′-Hexa-*O*-allyl-di-β-D-fructopyranose 1,2′:2,1′-dianhydride (7b). R_f=0.50 (1:2 EtOAc–petroleum ether); $[\alpha]_D$=−138.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.58 (d, 2H, $J_{3,4}$=9.8 Hz, H-3), 3.60 (d, 2H, $J_{1a,1b}$=11.8 Hz, H-1a), 3.68 (dd, 2H, $J_{6a,6b}$=13.2 Hz, $J_{5,6a}$=1.9 Hz, H-6a), 3.72 (m, 2H, H-5), 3.73 (dd, H, $J_{5,6b}$=1.9 Hz, H-6b), 3.81 (d, 2H, $J_{4,5}$=3.1 Hz, H-4), 4.00 (d, 2H, H-1b), 4.09–4.18 (m, 11H, CH_2O), 4.35 (ddt, 1H, $^2J_{H,H}$=12.4 Hz, $^3J_{H,H}$=2.5 Hz, $^4J_{H,H}$=1.3 Hz, CH_2O), 5.08–5.31 (m, 12H, C*H*$_2$=CH), 5.85–5.97 (m, 6H, CH_2=C*H*); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 61.6 (C-6), 64.5 (C-1), 71.0, 71.2, 74.0 (CH_2O), 73.8 (C-5), 77.8 (C-4), 78.7 (C-3), 97.0 (C-2), 116.7, 116.8, 117.3 (CH=*C*H_2), 135.1, 135.2, 135.3 (*C*H=CH_2). Anal. Calcd for $C_{30}H_{44}O_{10}$: C, 63.81; H, 7.85; found: C, 63.81; H, 7.88.

3.2.9. 3,4,5,3′,4′,5′-Hexa-*O*-benzoyl-di-β-D-fructopyranose 1,2′:2,1′-dianhydride (7c). R_f=0.31 (1:2 EtOAc–petroleum ether); $[\alpha]_D$=−226.5 (*c* 0.38, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.84 (d, 2H, $J_{1a,1b}$=12.6 Hz, H-1a), 4.05 (dd, 2H, $J_{5,6a}$=1.3 Hz, $J_{6a,6b}$=13.2 Hz, H-6a), 4.15 (d, 2H, H-1b), 4.23 (dd, 2H, $J_{5,6b}$=1.0 Hz, H-6b), 5.70 (m, 2H, H-5), 5.83 (dd, 2H, $J_{3,4}$=10.7 Hz, $J_{4,5}$=3.5 Hz, H-4), 5.97 (d, 2H, H-3), 7.09–8.10 (m, 30H, Ph); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 62.8 (C-6), 63.3 (C-1), 68.7 (C-4), 70.3 (C-5), 71.6 (C-3), 97.3 (C-2), 128.1–133.6 (Ph), 164.4–171.7 (CO); FABMS: *m*/*z* 971 (100%, $[M+Na]^+$). Anal. Calcd for $C_{54}H_{44}O_{16}$: C, 68.35; H, 4.67; found: C, 68.22; H, 4.54.

3.2.10. 3,4,5-Tri-*O*-benzyl-α-D-fructopyranose 3,4,5-tri-*O*-benzyl-β-D-fructopyranose 1,2′:2,1′-dianhydride (8a). R_f=0.44 (1:2 EtOAc–petroleum ether) $[\alpha]_D$=−36.9 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.33 (d, 1H, $J_{1'a,1'b}$=11.6 Hz, H-1′a), 3.54 (d, 1H, $J_{5,6a}$=0 Hz, $J_{6a,6b}$=12.6 Hz, H-6a), 3.66 (dd, 1H, $J_{5',6'a}$=3.7 Hz, $J_{6'a,6'b}$=11.0 Hz, H-6′a), 3.71 (d, 1H, $J_{1a,1b}$=11.6 Hz, H-1a), 3.73 (m, 1H, H-5), 3.77 (d, 1H, $J_{3',4'}$=9.8 Hz, H-3′), 3.77 (d, 1H, H-1′a), 3.79 (m, 2H, H-3, H-4), 3.82 (dd, 1H, $J_{5,6b}$=1.8 Hz, H-6b), 3.87 (m, 1H, H-5′), 3.97 (dd, 1H, $J_{5',6'b}$=2.3 Hz, H-6′b), 4.02 (dd, 1H, $J_{4',5'}$=3.1 Hz, H-4′), 4.18 (1H, d, H-1′b), 4.44–5.02 (m, 12H, C*H*$_2$Ph), 7.24–7.37 (m, 30H, Ph); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 58.9 (C-6′), 60.6 (C-6), 61.2 (C-1), 61.3 (C-1′), 72.3 (C-5′), 71.3, 71.4, 72.1, 72.3, 73.5, 75.4 (*C*H_2Ph), 73.7 (C-4), 73.9 (C-3), 76.1 (C-3′), 77.6 (C-5), 78.3 (C-4′), 94.5 (C-2′), 95.8 (C-2), 127.5–138.5 (Ph); FABMS: *m*/*z* 887 (100%, $[M+Na]^+$). Anal. Calcd for $C_{54}H_{56}O_{10}$: C, 74.98; H, 6.52; found: C, 74.86; H, 6.54.

3.2.11. 3,4,5-Tri-*O*-allyl-α-D-fructopyranose 3,4,5-tri-*O*-allyl-β-D-fructopyranose 1,2′:2,1′-dianhydride (8b). R_f=0.40 (1:2 EtOAc–petroleum ether); $[\alpha]_D$=−34.6 (*c* 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.39 (d, 1H, $J_{1'a,1'b}$=11.4 Hz, H-1′a), 3.47 (d, 1H, $J_{3',4'}$=9.8 Hz, H-3′), 3.55 (d, 1H, $J_{3,4}$=3.0 Hz, H-3), 3.56 (d, 1H, $J_{1a,1b}$=12.1 Hz, H-1a), 3.59 (dd, 1H, $J_{5,6a}$=4.0 Hz, $J_{6a,6b}$=11.1 Hz, H-6a), 3.64 (dd, 1H, $J_{4,5}$=5.0 Hz, H-4), 3.73 (m, 4H, H-5, H-5′, H-6′a, H-6′b), 3.78 (d, 1H, H-1b), 3.79 (dd, 1H, $J_{4',5'}$=2.7 Hz, H-4′), 3.87 (dd, 1H, $J_{5,6b}$=4.0 Hz, H-6b), 3.99–4.15 (m, 11H, CH_2O), 4.21 (d, 1H, H-1′b), 4.35 (ddt, 1H, $^2J_{H,H}$=12.6 Hz, $^3J_{H,H}$=5.3 Hz, $^4J_{H,H}$=1.3 Hz, CH_2O), 5.09–5.17 (m, 12H, C*H*$_2$=CH), 5.80–5.95 (m, 6H, CH_2=C*H*); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 58.8 (C-6), 60.8 (C-1), 61.1 (C-6′), 61.4 (C-1′), 72.1 (C-5′), 73.7 (C-3′), 74.1 (C-5), 70.4, 70.9, 71.4, 71.6, 72.6, 74.6 (6CH_2O), 76.2 (C-4), 77.6 (C-3, C-4′), 94.3 (C-2′), 95.8 (C-2), 116.8, 116.9, 117.0, 117.2, 117.3, 117.4 (6CH=*C*H_2), 134.5, 134.9, 135.0, 135.1, 135.2, 135.3 (6*C*H=CH_2). Anal. Calcd for $C_{30}H_{44}O_{10}$: C, 63.81; H, 7.85; found: C, 63.98; H, 7.90.

3.2.12. 3,4,5-Tri-*O*-benzoyl-α-D-fructopyranose 3,4,5-tri-*O*-benzoyl-β-D-fructopyranose 1,2′:2,1′-dianhydride (8c). R_f=0.31 (1:2 EtOAc–petroleum ether); $[\alpha]_D$=−119.4 (*c* 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.83 (d, 1H, $J_{1'a,1'b}$=11.7 Hz, H-1′a), 3.89 (dd, 1H, $J_{5,6a}$=5.9 Hz, $J_{6a,6b}$=11.1 Hz, H-6a), 3.93 (d, 1H, $J_{1a,1b}$=11.5 Hz, H-1a), 3.95 (d, 1H, H-1b), 4.08 (d, 1H, H-1′b), 4.10 (m, 2H, H-6′a, H-6′b), 4.13 (dd, 1H, $J_{5,6b}$=10.6 Hz, H-6b), 5.56 (d, 1H, $J_{3,4}$=3.7 Hz, H-3), 5.61 (ddd, 1H, $J_{4,5}$=3.3 Hz, H-5), 5.73 (m, 1H, H-5′), 5.76 (d, 1H, $J_{3',4'}$=10.5 Hz, H-3′), 5.87 (dd, 1H, $J_{4,5}$=3.0 Hz, H-4), 7.22–8.15 (m, 30H, Ph); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 58.9 (C-6′), 60.6 (C-6), 61.2 (C-1), 61.3 (C-1′), 72.3 (C-5′), 73.7 (C-4), 73.9 (C-3), 76.1

(C-3′), 77.6 (C-5), 78.3 (C-4′), 94.5 (C-2′), 95.8 (C-2), 128.1–133.6 (Ph), 164.4–171.7 (CO); FABMS: *m/z* 971 (100%, $[M+Na]^+$).

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Enantioselective synthesis of natural (−)-tochuinyl acetate, (−)-dihydrotochuinyl acetate and (+)-β-cuparenone using both enantiomers of the same building block

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Abstract—The first enantioselective synthesis of tochuinyl acetate and dihydrotochuinyl acetate, two natural marine products isolated from *Tochuina tetraquetra* and *Gersemia rubiformis*, has been achieved starting from an enantiopure building block. The key feature of the present synthesis is complete control of two vicinal quaternary stereogenic centers present in the natural products and elucidation of their absolute stereochemistry, which was previously unknown. Furthermore, starting from the enantiomer of the same building block, the applied methodology provided a new approach towards natural (*R*)-(+)-β-cuparenone.

1. Introduction

The marine sesquiterpenes tochuinyl acetate (−)-**1** and dihydrotochuinyl acetate (−)-**2** were isolated[1] from the dendronotid nudibranch *Tochuina tetraquetra* and also from their feed, the soft coral *Gersemia rubiformis*. (+)-β-cuparenone, (+)-**3**, was isolated[2] from the essential oil of the 'Mayur pankhi' tree. These natural products, belonging to the aromatic sesquiterpene cuparene class, possess two vicinal quaternary centers in a cyclopentane ring, both stereogenic in (−)-**1** and (−)-**2**, and one in (+)-**3** (Fig. 1).

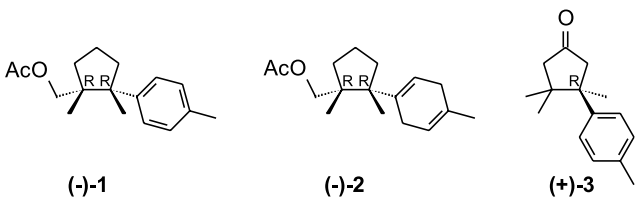

Figure 1. Natural tochuinyl acetate, (−)-**1**, and dihydrotochuinyl acetate, (−)-**2**, are represented with the absolute stereochemistry as determined in this work.

Owing to the difficulty associated with the construction of the adjacent quaternary centers on the sterically congested five-membered ring, their stereoselective synthesis has been a challenge for numerous organic synthetic chemists. However, there have only been a few reports on the enantioselective synthesis of natural (*R*)-(+)-β-cuparenone, (+)-**3**, or the enantiomer[3] and, to date, only racemic syntheses of tochuinyl acetate, **1**, and dihydrotochuinyl acetate, **2**, have been published[4] (the absolute configurations remain unknown). Starting from an enantiopure building block for the introduction and determination of the absolute stereochemistry, we have carried out the first enantioselective synthesis of (−)-**1** and (−)-**2** to determine the absolute stereochemistry of the two vicinal chiral centers present in the natural products. Our methodology is depicted in Scheme 1. Using the enantiomer of the same building block, the applied methodology allowed a new approach towards the known (*R*)-(+)-β-cuparenone, (+)-**3**. Our synthetic plan is outlined in Scheme 2.

2. Results and discussion

We recently reported the straightforward synthesis of the required enantiopure building blocks, (1*S*,2*S*,4*R*)-4-hydroxy-2-methyl-2-*p*-tolyl-cyclopentane carboxylic acid ethyl ester, (+)-**4**, and its enantiomer through enzymatic kinetic resolution of the corresponding racemic alcohol.[5]

The secondary alcohol of (+)-**4** was protected as its *tert*-butyldimethylsilyl ether (+)-**5**, using the standard method (Scheme 1).[6] Formation of the lithium enolate of the ester (+)-**5** with lithium diisopropylamide in THF and HMPA,

Keywords: Total synthesis; Configuration determination; Enantioselectivity; Cuparane family; Lipases.
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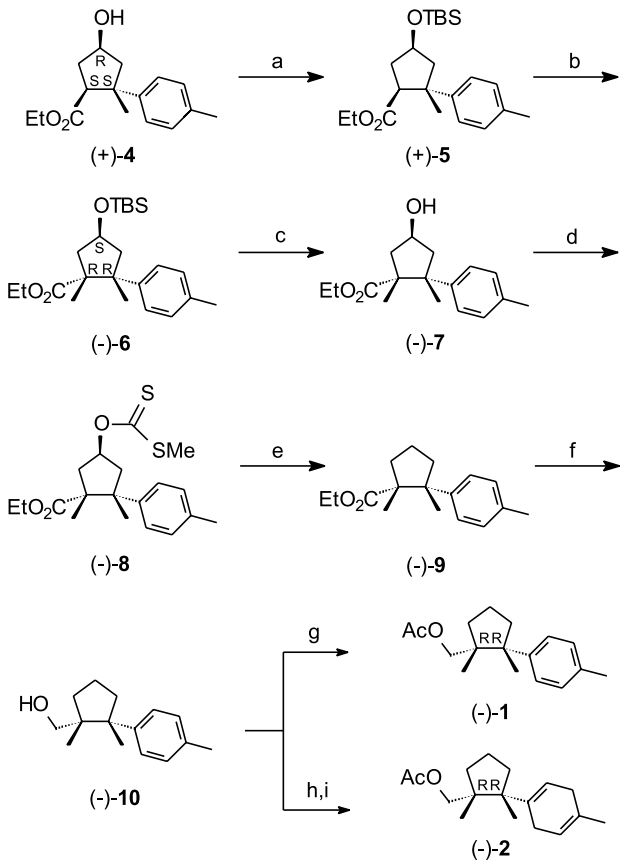
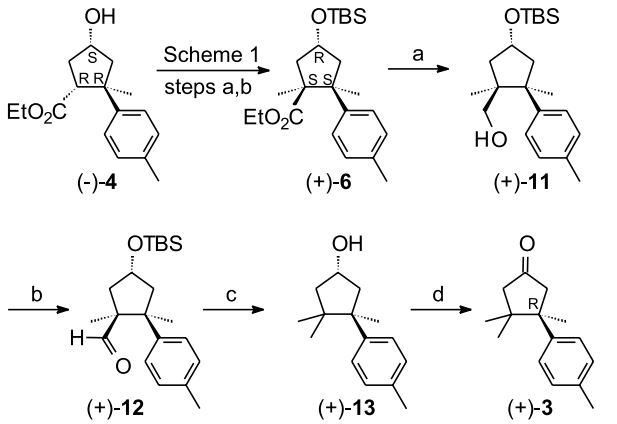


Scheme 1. Reagents and conditions: (a) TBSCl, Imidazole, DMF, rt, 100%; (b) LDA, HMPA, MeI, THF, −90 °C→rt, 93%; (c) TBAF, THF, rt, 98%; (d) NaH, CS_2, MeI, THF, 98%; (e) Bu_3SnH, AIBN, toluene, 96%; (f) $LiAlH_4$, Et_2O, 98%; (g) Ac_2O, pyridine, 95%; (h) Li, NH_3, *t*-BuOH, THF, −40 °C; (i) Ac_2O, pyridine, 80% for two steps.



Scheme 2. Reagents and conditions: (a) $LiAlH_4$, Et_2O, 95%; (b) NMO, TPAP, CH_2Cl_2, rt, 98%; (c) $NH_2NH_2{\cdot}H_2O$, NaOH, diethylene glycol, 160 °C, 95%; (d) NMO, TPAP, CH_2Cl_2, rt, 97%.

followed by addition of methyl iodide, furnished the alkylated ester (−)-**6** as the sole product.[7] In the ^1H NMR spectrum of this enantiomer, the upfield chemical shift of the ester methylene (δ 3.70 ppm), due to the shielding by the vicinal *cis* arene, established the stereochemistry at the newly created quaternary carbon atom in (−)-**6** like precedence.[4b,8] This stereochemistry will be confirmed by a NOESY experiment (not informative with (−)-**6**) on compound (+)-**11** (vide infra). Removal of the TBS protecting group of ester (−)-**6** (TBAF, THF) gave the corresponding alcohol (−)-**7** in 98% yield. Barton–McCombie deoxygenation of (−)-**7** proceeded by way of xanthate (−)-**8**, which was reduced smoothly with tri-*n*-butyltin hydride to provide the derivative (−)-**9** in an overall yield of 94% for the two steps.[9] Finally, reduction of ester (−)-**9** to give alcohol (−)-**10**, followed by acetylation, led to (−)-tochuinyl acetate, (−)-**1**, in 95% yield. The high enantiomeric purity of the compound was verified by chiral HPLC (>98% ee). On the other hand, Birch reduction of alcohol (−)-**10** furnished the crude dihydroalcohol, which on acetylation using acetic anhydride and pyridine gave (−)-dihydrotochuinyl acetate, (−)-**2**, in an overall yield of 80% for two steps.

The ^1H and ^{13}C NMR spectra of (−)-tochuinyl acetate, (−)-**1**, and (−)-dihydrotochuinyl acetate, (−)-**2**, were identical with those described for the natural isolated products[1] and in the racemic syntheses.[4] However, while the specific rotation of (−)-**1** [$[\alpha]_D^{25}=-38.0$ (*c* 1.0, CH_2Cl_2)] agrees with the value reported in the literature for the extracted product [lit.[1] $[\alpha]_D^{25}=-42.5$ (*c* 1.09, CH_2Cl_2)], the one of (−)-**2** [$[\alpha]_D^{25}=-45.5$ (*c* 1.0, CH_2Cl_2)] disagrees in the magnitude [lit.[1] $[\alpha]_D^{25}=-29.3$ (*c* 1.1, CH_2Cl_2)].

The enantioselective synthesis of (+)-β-cuparenone is depicted in Scheme 2. Starting from (−)-**4**, the alkylated ester (+)-**6** was obtained following the same first two steps as described in Scheme 1 for (−)-**6**, and reduction of (+)-**6** furnished alcohol (+)-**11** in 95% yield. The stereochemistry of the three stereocenters in (+)-**11** was confirmed using ^1H NMR NOESY experiments (Fig. 2). The four aromatic protons of the *p*-tolyl substituent resonated as an AB system at δ_H=7.22 and 7.10 ppm (J=8.2 Hz); a NOESY correlation between the Me–(C_6H_4), which resonated at δ_H=2.31 ppm, and the pair at δ_H=7.10 ppm showed the vicinal proximities of these protons. Furthermore, strong NOESY correlations among the other pair of aromatic protons at δ_H=7.22 ppm and H–C(4) and 2H–C(6) which resonated at δ_H=4.50 and 3.02 ppm and in addition, strong NOESY correlation among 2H–C(6) and H–C(4) established the *cis* spatial orientation among these protons and the *p*-tolyl group. Subsequent oxidation of (+)-**11** with catalytic tetrapropylammonium perruthenate (TPAP)[10] and NMO as the co-oxidant in dichloromethane provided the aldehyde (+)-**12** (98% yield). Huang–Minlon reduction[11] of the formyl group of (+)-**12** to methyl and, under the conditions of the reaction, removal of the protective TBS group, afforded directly the alcohol (+)-**13** in one step and 95% yield. Finally, conversion of alcohol (+)-**13** in (+)-β-cuparenone, (+)-**3**, was easily effected by TPAP oxidation as described

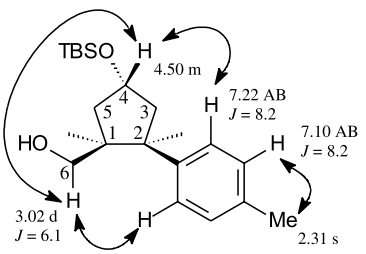


Figure 2. High-field ^1H NMR analysis and NOESY correlations of (+)-**11**.

above. The product showed the same $[\alpha]_D$ value, as well as the spectroscopic data (^1H and ^{13}C NMR) as those reported.[3]

3. Conclusion

In summary, an enantioselective synthesis of two marine aromatic sesquiterpenes, belonging to the cuparene class, has been achieved for the first time, and the absolute configurations of the two vicinal stereogenic quaternary centers present in the molecules have been fully determined; we trust that this will enrich the natural products databases. Furthermore, starting from the enantiomer of the previously utilized building block, the synthetic strategy was successfully applied to a new approach of the known natural (*R*)-(+)-β-cuparenone. The merits of this work are simple high-yielding reaction steps, secured absolute stereochemistry, and applicability of the methodology to the synthesis of the unnatural enantiomers of the present target molecules by reversing the enantiomers of the starting building block. Morever, our synthetic plan can also be adapted for the syntheses of other natural products with related structures in the field of cuparane and herbertane family.

4. Experimental

4.1. General experimental procedures

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution with Bruker AM-200 and Bruker AM-300 spectrometers. Infrared spectra were obtained as film using a Perkin–Elmer 1600 FT-IR spectrophotometer. Routine monitoring of reactions was performed using Merck Silica gel 60 F_{254}, aluminum supported TLC plates. Column chromatography was performed with Silica gel 60 (230–400 mesh) and gradients pentane/ether as eluent, unless otherwise stated. GC analyses were carried out on a Chrompack 9001 using a WCOT fused silica column (25 m×0.32 mm i.d.; CP-Wax-52 CB stationary phase; N_2 carrier gas: 50 kPa). Enantiomeric excess determinations were carried out using a commercial column from Daiser: CHIRALCEL OD-H® (250×4.6 mm; 10 μm) with hexane/*i*PrOH (98:2, v/v) and a flow rate of 1 mL/min. Specific rotations were recorded on a Perkin–Elmer 341 polarimeter. Microanalyses were performed on a ThermoFinnigan EA 1112 analyzer at our University. Unless otherwise stated, the solutions were dried over magnesium sulfate and evaporated in a rotary evaporator under reduced pressure.

4.1.1. (1*S*,2*S*,4*R*)-4-(*tert*-Butyl-dimethyl-silanyloxy)-2-methyl-2-*p*-tolyl-cyclopentanecarboxylic acid ethyl ester [(+)-5]. The alcohol (+)-**4** (675 mg, 2.58 mmol) was dissolved in DMF (10 mL), imidazole (530 mg, 7.78 mmol) and *tert*-butyldimethylsilyl chloride (590 mg, 3.91 mmol) were added, and the mixture was stirred for 1 h at rt. The solution was poured into water and extracted with ether. The combined organic extracts were washed with water, brine, dried, filtered, and concentrated. Column chromatography gave 970 mg (100%) of (+)-**5** as an oil; $[\alpha]_D^{25}$=+49.2 (*c*=1.0, $CHCl_3$); IR (film) ν 3041, 1749, 1256, 1031 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.29 and 7.11 (AB, 4H, *J*=8.3 Hz), 4.33 (quin., 1H, *J*=6.5 Hz), 4.10 (m, 2H), 3.04 (dd,1H, *J*=10.2, 8.3 Hz), 2.31 (s, 3H), 2.42–2.18 (m, 3H), 1.85 (dd, 1H, *J*=13.4, 5.9 Hz), 1.41 (s, 3H), 1.18 (t, 3H, *J*=7.1 Hz), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 173.6 (C), 146.5 (C), 135.2 (C), 128.8 (2×CH), 125.7 (2×CH), 71.4 (CH), 60.1 (CH_2), 53.2 (CH), 51.2 (CH_2), 47.4 (C), 38.5 (CH_2), 25.8 (3×CH_3), 25.5 (CH_3), 20.8 (CH_3), 18.0 (C), 14.2 (CH_3), −4.7 (2×CH_3–Si). Anal. Calcd for $C_{22}H_{36}O_3Si$: C, 70.16; H, 9.63. Found: C, 70.40; H, 9.61.

4.1.2. (1*R*,2*R*,4*S*)-4-(*tert*-Butyl-dimethyl-silanyloxy)-1,2-dimethyl-2-*p*-tolyl-cyclopentanecarboxylic acid ethyl ester [(−)-6]. To a cold (−90 °C) magnetically stirred solution of LDA [prepared from diisopropylamine (0.74 mL, 5.28 mmol) and *n*-BuLi (5.00 mmol, 3.13 mL of a 1.6 M solution in hexane)] in 10 mL of dry ether was added HMPA (1.29 mL, 7.44 mmol) followed by a solution of the ester (+)-**5** (500 mg, 1.33 mmol) in 3 mL of dry THF over a period of 10 min. The reaction mixture was stirred for 40 min at the temperature. Methyl iodide (1.34 mL, 21.52 mmol) was added to the reaction mixture, slowly warmed up to 0 °C and stirred for 5 h. The reaction mixture was partitioned between ether and, sequentially, 5% aqueous HCl, 10% aqueous $NaHCO_3$. The organic layers were dried ($MgSO_4$), concentrated, and chromatographed to give the alkylated ester (−)-**6** (486 mg, 93% yield) as an oil; $[\alpha]_D^{25}$=−17.6 (*c*=1.0, $CHCl_3$); IR (film) ν 3042, 1752, 1258, 1015 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.12 and 7.03 (AB, 4H, *J*=8.4 Hz), 4.68–4.55 (m, 1H), 3.65 (m, 2H), 2.91 (dd, 1H, *J*=14.0, 7.7 Hz), 2.62 (dd, 1H, *J*=14.1, 7.7 Hz), 2.27 (s, 3H), 1.81 (dd, 1H, *J*=13.8, 1.7 Hz), 1.65 (dd, 1H, *J*=13.9, 2.5 Hz), 1.55 (s, 3H), 1.41 (s, 3H), 0.85 (partially overlapped t, 3H, *J*=7.0 Hz), 0.85 (s, 9H), 0.06 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 176.4 (C), 143.2 (C), 135.4 (C), 128.3 (2×CH), 126.3 (2×CH), 71.4 (CH), 59.9 (CH_2), 57.2 (C), 51.4 (C), 49.1 (CH_2), 47.5 (CH_2), 25.8 (3×CH_3), 25.2 (CH_3), 20.7 (CH_3), 20.5 (CH_3), 17.9 (C), 13.5 (CH_3), −4.8 (2×CH_3–Si). Anal. Calcd for $C_{23}H_{38}O_3Si$: C, 70.72; H, 9.80. Found: C, 70.49; H, 9.78.

4.1.3. (1*R*,2*R*,4*S*)-4-Hydroxy-1,2-dimethyl-2-*p*-tolylcyclopentanecarboxylic acid ethyl ester [(−)-7]. To a solution of (−)-**6** (480 mg, 1.23 mmol) in dry THF (15 mL), tetra-*n*-butyl ammonium fluoride (1.0 M in THF, 2.5 mL, 2.50 mmol) was added dropwise. The reaction mixture was stirred at rt for 4 h. Cold water was added and the resultant mixture was extracted with ether. The combined organic extracts were washed with brine, dried, filtered, and concentrated. The residual oil was chromatographed to afford 332 mg (98%) of pure alcohol (−)-**7**; $[\alpha]_D^{25}$=−24.2 (*c*=1.0, $CHCl_3$); IR (film) ν 3405, 3034, 1741, 1191 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.13 and 7.04 (AB, 4H, *J*=8.3 Hz), 4.74 (m, 1H), 3.68 (m, 2H), 3.01 (dd, 1H, *J*=14.4, 7.9 Hz), 2.71 (dd, 1H, *J*=14.4, 7.9 Hz), 2.28 (s, 3H), 1.85 (dd, 1H, *J*=14.3, 2.3 Hz), 1.68 (dd, 1H, *J*=14.4, 3.0 Hz), 1.56 (s, 3H), 1.44 (s, 3H), 0.88 (t, 3H, *J*=7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.1 (C), 142.7 (C), 135.6 (C), 128.4 (2×CH), 126.2 (2×CH), 71.1 (CH), 60.0 (CH_2), 57.3 (C), 51.5 (C), 48.6 (CH_2), 46.7 (CH_2), 25.3 (CH_3), 20.7 (CH_3), 20.6 (CH_3), 13.6 (CH_3). Anal. Calcd for $C_{17}H_{24}O_3$: C, 73.88; H, 8.75. Found: C, 74.17; H, 8.72.

4.1.4. (1*R*,2*R*,4*S*)-1,2-Dimethyl-4-methylsulfanylthio carboxyoxy-2-*p*-tolyl-cyclopentanecarboxylic acid ethyl ester [(−)-8]. To a suspension of sodium hydride (63 mg, 1.30 mmol, 50% dispersion) in 5 mL of THF at 0 °C was added alcohol (−)-**7** (180 mg, 0.651 mmol). After the mixture was stirred for 30 min at 0 °C, carbon disulfide (303 mg, 241 μL, 2.61 mmol) and iodomethane (741 mg, 325 μL, 5.22 mmol) was added. The resulting mixture was stirred for another 1 h, carefully poured into ice, and extracted with ether. The organic layer was separated, dried ($MgSO_4$), filtered, and concentrated under reduced pressure to an oily residue, which was column chromatographed to provide 234 mg (98%) of xanthate (−)-**8**; $[\alpha]_D^{25}=-29.9$ (c=1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 and 7.07 (AB, 4H, J=8.5 Hz), 6.12 (tt, 1H, J=8.1, 2.7 Hz), 3.70 (m, 2H), 3.20 (dd, 1H, J=15.1, 8.1 Hz), 2.88 (dd, 1H, J=15.1, 8.3 Hz), 2.75 (s, 3H), 2.56 (s, 3H), 2.29 (s, 3H), 2.10 (dd, 1H, J=15.0, 1.7 Hz), 1.93 (dd, 1H, J=15.2, 2.7 Hz), 1.53 (s, 3H), 1.44 (s, 3H), 0.89 (t, 3H, J=7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 214.8 (C), 175.5 (C), 141.8 (C), 135.9 (C), 128.6 (2×CH), 126.1 (2×CH), 84.4 (CH), 60.3 (CH_2), 56.9 (C), 51.2 (C), 45.0 (CH_2), 43.3 (CH_2), 24.9 (CH_3), 20.8 (CH_3), 20.1 (CH_3), 19.0 (CH_3), 13.6 (CH_3). Anal. Calcd for $C_{19}H_{26}O_3S_2$: C, 62.26; H, 7.15; S, 17.50. Found: C, 61.99; H, 7.18; S, 17.69.

4.1.5. (1*R*,2*R*,4*S*)-1,2-Dimethyl-2-*p*-tolylcyclopentane carboxylic acid ethyl ester [(−)-9]. To a solution of xanthate (−)-**8** (188 mg, 0.513 mmol) and AIBN (15 mg) in toluene (5 mL) was added tri-*n*-butyltin hydride (252 mg, 230 μL, 0.866 mmol), and the reaction mixture heated under reflux for 40 min, cooled, and concentrated in vacuo. The resulting oily residue was chromatographed on column to provide 128 mg (96%) of (−)-**9**; $[\alpha]_D^{25}=-21.4$ (c=1.0, $CHCl_3$); IR (film) ν 3094, 3039, 1749, 1168 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 and 7.06 (AB, 4H, J=8.3 Hz), 3.70 (m, 2H), 2.66–2.54 (m, 1H), 2.40–2.31 (m, 1H), 2.29 (s, 3H), 2.03–1.75 (m, 3H), 1.66–1.56 (m, 1H), 1.40 (s, 3H), 1.34 (s, 3H), 0.90 (t, 3H, J=7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.6 (C), 143.6 (C), 135.3 (C), 128.3 (2×CH), 126.3 (2×CH), 59.8 (CH_2), 56.5 (C), 51.4 (C), 38.0 (CH_2), 36.0 (CH_2), 24.5 (CH_3), 21.1 (CH_2), 20.7 (CH_3), 20.5 (CH_3), 13.6 (CH_3). Anal. Calcd for $C_{17}H_{24}O_2$: C, 78.42; H, 9.29. Found: C, 78.71; H, 9.31.

4.1.6. (1*R*,2*R*)-(1,2-Dimethyl-2-*p*-tolyl-cyclopentyl)-methanol [(−)-10]. A solution of (−)-**9** (90 mg, 0.346 mmol) in dry ether (5 mL) was slowly added to a stirred slurry of $LiAlH_4$ (30 mg, 0.790 mmol) in dry ether (4 mL) at 0 °C. The solution was allowed to rise to rt. After 1 h, Celite (1 g) and $Na_2SO_4{\cdot}10H_2O$ (1 g) were added and the solution was stirred for a further 30 min. The mixture was filtered through a pad of $MgSO_4$ and concentrated. A column chromatography of the oil afforded 74 mg (98%) of pure (−)-**10**; $[\alpha]_D^{25}=-50.1$ (c=1.0, $CHCl_3$); IR (film) ν 3409, 3041, 1043 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 and 7.12 (AB, 4H, J=8.3 Hz), 3.12 and 3.05 (AB, 2H, J=11.3 Hz), 2.52–2.42 (m, 1H), 2.31 (s, 3H), 1.89–1.66 (m, 4H), 1.61–1.50 (m, 1H), 1.30 (s, 3H), 1.11 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.4 (C), 135.4 (C), 128.8 (2×CH), 126.6 (2×CH), 69.3 (CH_2), 49.4 (C), 49.2 (C), 37.4 (CH_2), 34.8 (CH_2), 25.0 (CH_3), 20.7 (CH_3), 20.2 (CH_2), 19.3 (CH_3). Anal. Calcd for $C_{15}H_{22}O$: C, 82.52; H, 10.16. Found: C, 82.86; H, 10.13.

4.1.7. (1*R*,2*R*)-Tochuinyl acetate [(−)-1]. To a magnetically stirred solution of the alcohol (−)-**10** (65 mg, 0.298 mmol) in pyridine (3 mL) was sequentially added acetic anhydride (185 mg, 200 μL, 1.81 mmol) and a catalytic amount of DMAP. The reaction mixture was stirred for 4 h at rt, then poured into 6 mL of 5% aqueous HCl and extracted with CH_2Cl_2 (3×10 mL). The combined organic phase was washed with 10% aqueous $NaHCO_3$ solution and brine, and dried ($MgSO_4$). Evaporation of the solvent and purification of the residue on a silica gel column furnished tochuinyl acetate (−)-**1** (74 mg, 95%) which exhibited ^{1}H and ^{13}C NMR spectra identical to those of the natural product; $[\alpha]_D^{25}=-38.0$ (c=1.0, CH_2Cl_2); IR (film) ν 3040, 1741, 1240, 1030 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 and 7.07 (AB, 4H, J=8.3 Hz), 3.59 and 3.35 (AB, 2H, J=10.9 Hz), 2.53–2.41 (m, 1H), 2.30 (s, 3H), 1.93 (s, 3H), 1.90–1.69 (m, 4H), 1.60–1.49 (m, 1H), 1.31 (s, 3H), 1.11 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0 (C), 142.9 (C), 135.3 (C), 128.5 (2×CH), 126.7 (2×CH), 70.5 (CH_2), 49.8 (C), 47.4 (C), 37.5 (CH_2), 34.8 (CH_2), 24.9 (CH_3), 20.8 (CH_3), 20.7 (CH_3), 20.2 (CH_2), 19.5 (CH_3). Anal. Calcd for $C_{17}H_{24}O_2$: C, 78.42; H, 9.29. Found: C, 78.72; H, 9.33.

4.1.8. (1*R*,2*R*)-Dihydrotochuinyl acetate [(−)-2]. To a solution of lithium (78 mg, 11.2 mmol) in 50 mL of freshly distilled ammonia was added dropwise, a solution of the alcohol (−)-**10** (60 mg, 0.275 mmol) and *tert*-butanol (0.5 mL) in 5 mL of dry THF over a period of 10 min. The reaction mixture was stirred overnight and then quenched with ammonium chloride. Ammonia was evaporated, the reaction mixture was diluted with water and extracted with ether (3×10 mL). The ether extract was washed with brine and dried ($MgSO_4$). Evaporation of the solvent furnished 55 mg of crude dihydro alcohol, which was immediately used for the acetylation step. To a magnetically stirred solution of the dihydro alcohol (55 mg, 0.251 mmol) in pyridine (3 mL) was sequentially added acetic anhydride (200 μL, 1.81 mmol) and a catalytic amount of DMAP, and stirred for 4 h at rt. The reaction mixture was then quenched with 10% aqueous HCl (6 mL) and extracted with CH_2Cl_2 (3×10 mL). The combined organic phase was washed with saturated aq. $NaHCO_3$ solution and brine, and dried ($MgSO_4$). Evaporation of the solvent and rapid purification of the residue on a silica gel column furnished the dihydrotochuinyl acetate (−)-**2** (58 mg, 80% from the alcohol (−)-**10**) which exhibited ^{1}H and ^{13}C NMR spectra identical to those of the natural product; $[\alpha]_D^{25}=-45.5$ (c=1.0, CH_2Cl_2); IR (film) ν 3051, 1744, 1238, 802 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.52 (br s, 1H), 5.37 (br s, 1H), 3.80 and 3.72 (AB, 2H, J=10.9 Hz), 2.75–2.65 (m, 2H), 2.62–2.53 (m, 2H), 2.18 (dt, 1H, J=12.8, 9.3 Hz), 2.00 (s, 3H), 1.79–1.56 (m, 3H), 1.63 (s, 3H), 1.48–1.39 (m, 2H), 1.05 (s, 3H), 1.02 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=171.4 (C), 138.5 (C), 130.5 (C), 119.1 (CH), 119.0 (CH), 70.0 (CH_2), 50.3 (C), 46.9 (C), 36.9 (CH_2), 35.3 (CH_2), 31.9 (CH_2), 28.3 (CH_2), 22.7 (CH_3), 22.6 (CH_3), 21.0 (CH_3), 20.1 (CH_3), 19.5 (CH_2). Anal. Calcd for $C_{17}H_{26}O_2$: C, 77.82; H, 9.99. Found: C, 77.59; H, 9.97.

4.1.9. (1*S*,2*S*,4*R*)-[4-(*tert*-Butyl-dimethyl-silanyloxy)-1,2-dimethyl-2-*p*-tolyl-cyclopentyl]-methanol [(+)-11]. A solution of (+)-**6** (260 mg, 0.666 mmol) in dry diethyl ether (50 mL) was slowly added at 0 °C to a stirred slurry of $LiAlH_4$ (51 mg, 1.34 mmol) in dry ether (5 mL). The solution was allowed to warm to rt. After 1 h, Celite (2 g) and $Na_2SO_4{\cdot}10H_2O$ (2 g) were added and the solution was stirred for a further 30 min. The mixture was filtered through a pad of $MgSO_4$ and concentrated. Column chromatography of the oil afforded 220 mg (95%) of pure (+)-**11**; $[\alpha]_D^{25}$=+30.2 (*c*=1.0, $CHCl_3$); IR (film) ν 3412, 3039, 1261, 1083 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.22 and 7.10 (AB, 4H, *J*=8.2 Hz), 4.50 (m, 1H), 3.02 (d, 2H, *J*=6.1 Hz), 2.84 (dd, 1H, *J*=14.1, 7.9 Hz), 2.31 (s, 3H), 2.15 (dd, 1H, *J*=14.0, 7.9 Hz), 1.77 (dd, 1H, *J*=14.1, 1.5 Hz), 1.62 (dd, 1H, *J*=14.1, 3.3 Hz), 1.46 (s, 3H), 1.19 (s, 3H), 0.88 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.2 (C), 135.4 (C), 128.8 (2×CH), 126.5 (2×CH), 70.6 (CH), 69.2 (CH_2), 49.7 (C), 49.5 (C), 48.6 (CH_2), 46.6 (CH_2), 26.2 (CH_3), 25.8 (3×CH_3), 20.8 (CH_3), 19.6 (CH_3), 17.9 (C), −4.7 (2×CH_3–Si). Anal. Calcd for $C_{21}H_{36}O_2Si$: C, 72.35; H, 10.41. Found: C, 72.09; H, 10.38.

4.1.10. (1*S*,2*S*,4*R*)-4-(*tert*-Butyl-dimethyl-silanyloxy)-1,2-dimethyl-2-*p*-tolyl-cyclopentanecarbaldehyde [(+)-12]. Tetrapropylammonium perruthenate (TPAP) (5 mol%, 10 mg, 0.028 mmol) was added in one portion to a stirred mixture of the alcohol (+)-**11** (190 mg, 0.545 mmol), *N*-methyl morpholine oxide (NMO) (130 mg, 1.11 mmol) and powdered 4 Å molecular sieves (370 mg) in CH_2Cl_2 (5 mL) at rt under argon atmosphere. On completion the reaction mixture was filtered through a short pad of celite, eluting with CH_2Cl_2. The filtrate was evaporated and the residue was purified by column chromatography on silica gel to afford 185 mg (98%) of aldehyde (+)-**12**; $[\alpha]_D^{25}$=+27.8 (*c*=1.0, $CHCl_3$); IR (film) ν 3043, 2719, 1250, 831 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.90 (s, 1H), 7.16 and 7.09 (AB, 4H, *J*=8.1 Hz), 4.52 (m, 1H), 2.63 (dd, 1H, *J*=13.9, 7.4 Hz), 2.51 (dd, 1H, *J*=14.0, 7.7 Hz), 2.30 (s, 3H), 1.82 (d, 1H, *J*=14.0 Hz), 1.62 (dd, 1H, *J*=13.9, 2.8 Hz), 1.51 (s, 3H), 1.31 (s, 3H), 0.89 (s, 9H), 0.06 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.4 (C), 140.9 (C), 135.9 (C), 129.0 (2×CH), 126.4 (2×CH), 70.5 (CH), 59.4 (C), 49.9 (C), 48.0 (CH_2), 44.5 (CH_2), 25.8 (3×CH_3), 25.4 (CH_3), 20.8 (CH_3), 17.9 (C), 16.6 (CH_3), −4.8 (2×CH_3–Si). Anal. Calcd for $C_{21}H_{34}O_2Si$: C, 72.78; H, 9.89. Found: C, 73.01; H, 9.86.

4.1.11. (1*S*,4*R*)-3,3,4-Trimethyl-4-*p*-tolyl-cyclopentanol [(+)-13]. A solution of the aldehyde (+)-**12** (137 mg, 0.395 mmol) and hydrazine hydrate (0.50 mL, 10.29 mmol) in diethylene glycol (3 mL) was heated to 160 °C for 4 h. The mixture was cooled to rt and treated with powdered sodium hydroxide (400 mg, 10.0 mmol). The reaction mixture was further heated to 180 °C for 7 h. It was then cooled to rt, poured into ice-cold water and extracted with ether. The extract was washed with brine and dried ($MgSO_4$). Evaporation of the solvent and purification of the residue by chromatography furnished the alcohol (+)-**13** (82 mg, 95%) as an oil; $[\alpha]_D^{25}$=+47.2 (*c*=1.0, $CHCl_3$); IR (film) ν 3424, 3039, 1059 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.21 and 7.11 (AB, 4H, *J*=8.2 Hz), 4.59 (m, 1H), 2.96 (dd, 1H, *J*=14.4, 8.3 Hz), 2.33 (s, 3H), 2.05 (dd, 1H, *J*=13.8, 8.0 Hz), 1.84–1.79 (m, 1H), 1.78–1.74 (m, 1H), 1.46 (s, 3H), 1.18 (s, 3H), 0.57 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.7 (C), 134.9 (C), 128.3 (2×CH), 126.7 (2×CH), 70.4 (CH), 50.8 (CH_2), 50.3 (C), 47.6 (CH_2), 44.9 (C), 26.5 (CH_3), 25.7 (CH_3), 24.5 (CH_3), 20.7 (CH_3). Anal. Calcd for $C_{15}H_{22}O$: C, 82.52; H, 10.16. Found: C, 82.41; H, 10.12.

4.1.12. (4*R*)-β-Cuparenone [(+)-3]. Tetrapropyl-ammonium perruthenate (TPAP) (5 mol%, 10 mg, 0.028 mmol) was added in one portion to a stirred mixture of the alcohol (+)-**13** (58 mg, 0.266 mmol), *N*-methyl morpholine oxide (NMO) (63 mg, 0.538 mmol) and powdered 4 Å molecular sieves (180 mg) in CH_2Cl_2 (5 mL) at rt under argon atmosphere. On completion the reaction mixture was filtered through a short pad of celite, eluting with CH_2Cl_2. The filtrate was evaporated and the residue was purified by column chromatography on silica gel to afford 56 mg (97%) of the β-cuparenone (+)-**3** which exhibited ^1H and ^{13}C NMR spectra identical to those of the natural product; $[\alpha]_D^{25}$=+45.3 (*c*=1.0, $CHCl_3$); IR (film) ν 3041, 1718, 1031 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.20 and 7.12 (AB, 4H, *J*=8.3 Hz), 3.12 (d, 1H, *J*=18.1 Hz), 2.41–2.24 (m, 3H), 2.31 (s, 3H), 1.40 (s, 3H), 1.22 (s, 3H), 0.72 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 218.0 (C), 141.2 (C), 135.8 (C), 128.7 (2×CH), 126.5 (2×CH), 52.4 (CH_2), 50.6 (CH_2), 47.7 (CH), 41.7 (CH), 26.2 (CH_3), 24.4 (CH_3), 24.1 (CH_3), 20.7 (CH_3). Anal. Calcd for $C_{15}H_{20}O$: C, 83.28; H, 9.32. Found: C, 82.98; H, 9.36.

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Chemoselective epoxidation of dienes using polymer-supported manganese porphyrin catalysts

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Abstract—Manganese porphyrin catalysts supported on different polymer resins were assessed in the selective epoxidation of three dienes. The recyclability of the catalysts was examined.

1. Introduction

Heterogeneously-supported manganese porphyrins have been widely investigated as catalysts in the epoxidation of alkenes. A survey of the literature revealed that these catalysts are most efficient in the epoxidation of conjugated alkene substrates (most commonly styrene and indene), followed by cyclic alkenes (cyclohexene, cyclooctene). In contrast, they are less successful in catalysing the epoxidation of less electron-rich terminal olefins such as dodec-1-ene and 1-hexene. Hence, in principle, supported manganese porphyrins may show good chemoselectivity in epoxidation of substrates containing more than one type of alkene. Although limonene has been widely chosen as a substrate to probe the steric hindrance of synthetic metalloporphyrins,[1–3] other dienes and polyolefins are rarely examined as substrates.

Previously, we immobilised the manganese complex of 5-(4-hydroxyphenyl)-10,15,20-triphenylporphyrin **1** on Merrifield and Argogel resins and examined their subsequent catalytic activity in the epoxidation of a wide range of mono-alkenes (styrene, stilbene, methylstyrene, cyclooctene, cyclohexene, norbornene, hex-1-ene and dodec-1-ene).[4] During the study, we discovered important compatibility issues between the nature of the linker group and catalyst activity and stability. The Merrifield resin-supported catalyst has been demonstrated to be robust, and may be subjected to several successive catalytic reactions without leaching. However, rates of turnover were slow, which we attributed to restricted mobility of the reactive metalloporphyrin moiety, imposed by the short spacer between the support and the catalyst. On the other hand, the flexible PEG spacer afforded by the Argogel-supported catalyst was found to be unstable, and extensive leaching was observed during catalytic recycling.

In this paper, we report the preparation of two new catalysts from commercially available polymer supports with more robust and longer linkers. These were compared with the Merrifield-supported catalyst in the chemoselective epoxidation of three types of dienes. Recyclability and selectivity of these catalysts were compared and contrasted.

2. Results and discussion

2.1. Catalyst preparation

Following previously reported procedures,[4] porphyrin **1** was tethered to commercially available Merrifield, bromo-Wang and carboxy bromo-Wang resins via an ether linkage, by treating the chlorinated/brominated resin beads with 5-(4-hydroxyphenyl)-10,15,20-triphenylporphyrin **1**, in the presence of K_2CO_3 at 80 °C for three days (Scheme 1)—Irori Kan™ reactors were used to protect the polymer beads from structural damage that may arise through prolonged mechanical stirring. Functionalized polymer supports **2**–**4** were thus obtained as dark purple beads. The reactions were monitored by following the displacement of the halide (%Cl or %Br analysis), whereas the final yields of the reactions were calculated from %N content. With the exception of **3**, yields were typically high. Subsequent metallation of the supported porphyrins **2**–**4** with $MnCl_2$ at high temperature

Keywords: Manganese porphyrins; Alkene epoxidation; Chemoselectivity; Polymer-supported catalysis.
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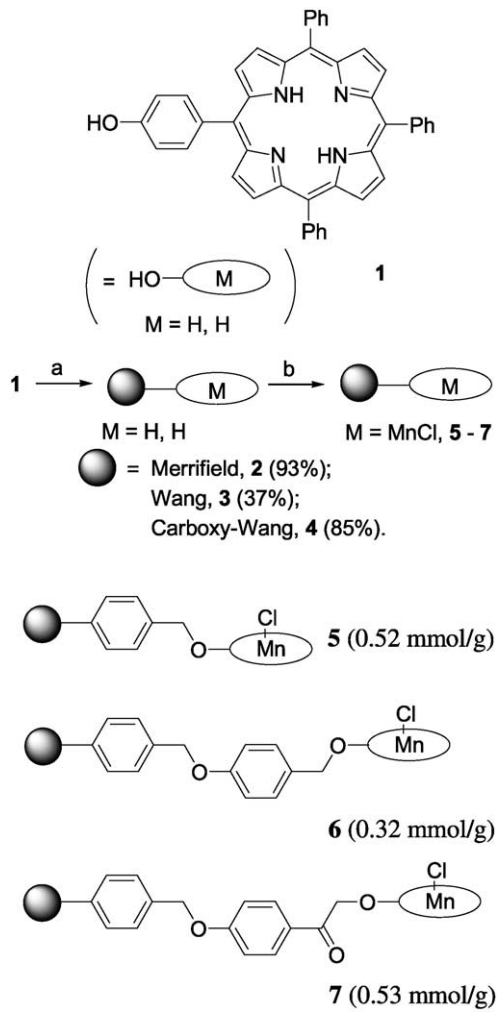


Scheme 1. Preparation of manganese porphyrins supported on commercially available resins: (a) Merrifield (**2**)/bromo-Wang (**3**)/carboxy bromo-Wang (**4**), K_2CO_3, DMF, 80 °C, 3 days; (b) $MnCl_2$ (50 equiv.), DMF, 165 °C, 2.5 h.

afforded the four supported manganese porphyrins **5**, **6** and **7**, respectively. Complexation was indicated by the disappearance of the ν(N–H) absorption band in the single-bead transmittance FTIR spectrum. The catalytic loading of the polymer beads, verified by %Mn analysis (ICP-AES), was between 0.26 and 0.53 mmol/g.

2.2. Catalytic epoxidation of dienes and recycling studies

Three diene substrates were chosen in the catalytic study (Fig. 1): 1,5-cyclooctadiene, 7-methyl-1,6-octadiene and limonene. Using previously optimised conditions (Scheme 2), the epoxidation reactions were performed employing 4 mol% of catalyst in the presence of imidazole, with sodium periodate as the oxidant.

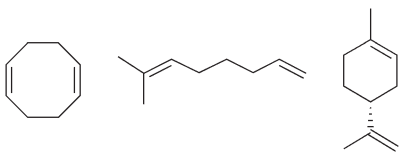
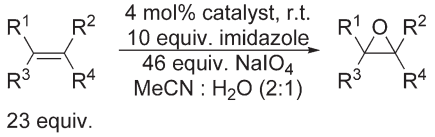


Figure 1. Chosen substrates for the chemoselective epoxidation.

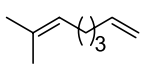
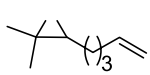
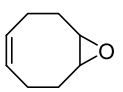


Scheme 2. General reaction conditions employed for catalytic epoxidation.

2.3. Epoxidation of 7-methyl-1,6-octadiene (Table 1, entries 1–3)

From previous studies, we expect the more electron-rich double bond to be epoxidised more favourably over the terminal alkene. Indeed, this proved to be the case (Table 1, entries 1–3). Tri-substituted epoxide was obtained exclusively in high yields (91–100%) in all cases.

Table 1. Selective epoxidation of 7-methyl-1,6-octadiene and cyclooctadiene

Entry	Substrate	Product	Catalyst	T/h	Yield/%[a]
1			**5**	33	94
2			**6**	33	91
3			**7**	24	100
4			**5**	36	76
5				42	68[b]
6			**6**	36	63
7			**7**	36	76[c]
8				36	51[b,d]

Reaction conditions outlined in Scheme 2.
[a] GC yields with respect to starting material.
[b] Carried out with recovered catalyst.
[c] Bis-epoxide (9%) was observed after 6 h.
[d] Formation of bis-epoxide was not observed in the second cycle.

Monitoring the progress of the reactions by GC (Fig. 2), the presence of an induction period of 4 h was detected before the Merrifield-supported catalyst **5** commenced its turnover. Beyond this, its activity was roughly comparable to that of Wang-supported **6**, which appeared to be the least robust, as its turnover ceased after 30 h. In contrast, the carboxy-Wang supported catalyst **7** gave the highest and quickest turnover, achieving 97% conversion within 12 h.

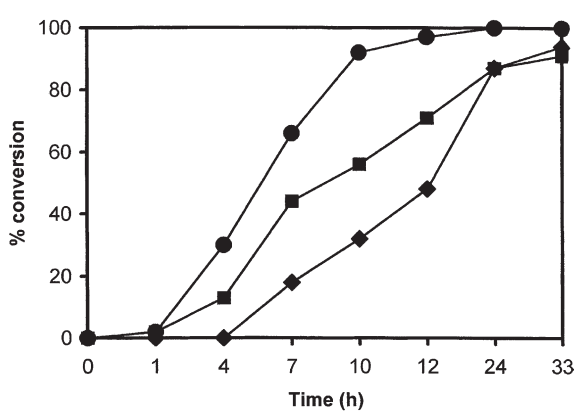


Figure 2. Rate of conversion of 7-methyl-1,6-octadiene catalysed by **5** (◆), **6** (■) and **7** (●).

2.4. Epoxidation of cyclooctadiene (Table 1, entries 4–8)

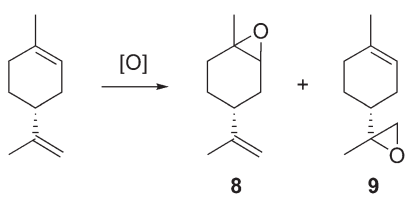
1,5-Cyclooctadiene contains two identical double bonds, from which it is possible to obtain mono- and bis-epoxide products. Most oxidants tend to reduce the diene to a mixture of products, including diols and ketones. Whilst the peroxide-based oxidants (such as that catalysed by MTO) tend to yield the bis-epoxide,[5,6] the synthetically more useful mono-epoxide may be obtained by the epoxidation of the diene by peracids, with yields of between 40 and 72%.[7,8]

A prior study by Jørgensen deployed an iron(II) phthalocyanine/iodosylbenzene system in the epoxidation of 1,5-cyclooctadiene, which led to the formation of a mixture of oxidised products in low yields.[9] In light of this, it was somewhat surprising to find that highly selective mono-epoxidation may be achieved using supported manganese porphyrins. Catalysts **5** and **6** gave exclusive formation of the mono-epoxide (Table 1, entries 4–6), whereas a small formation of bis-epoxide was observed with catalyst **7** (entry 7). In the latter case, the presence of the bis-epoxide was detected after 6 h.

Since catalysts **5** and **7** exhibited similar turnovers (entries 4 and 7), these were recovered by filtration. UV analysis of the reaction mixture (filtrate) did not exhibit any discernable absorption peaks that may be attributed to free or manganese porphyrin, thus indicating that no leaching of the catalyst occurred; the %Mn content of the recovered beads was also similar to the original value. The recovered catalysts were thus subjected to a second catalytic run. Both of the recycled catalysts showed much reduced catalytic activity (Table 1, entries 4 vs. 5, 7 vs. 8). In the reaction catalysed by recovered carboxy-Wang supported catalyst **7**, the slower turnover evidently suppressed the formation of the bis-epoxide.

2.5. Epoxidation of limonene

The monoterpene limonene contains an internal trisubstituted double bond and a disubstituted terminal double bond, and has been widely studied as a substrate in porphyrin epoxidation chemistry. Bis-epoxide may be obtained from simultaneous epoxidations, and the formation of 1, 2- and/or 8, 9-limonene oxides **8** and **9** (Scheme 3) was commonly used to denote the selectivity of the process, generally taken to reflect the steric environment of the metalloporphyrin.[2]



Scheme 3. Epoxidation of limonene.

Immobilisation of manganese porphyrin catalysts typically leads to a catastrophic effect on their selectivity towards the epoxidation of limonene, lowering the ratio from 9:1 in favour of the 1,2-epoxide **8**, to typically less than 1.7:1.[10] The most selective supported manganese catalyst reported to date is a Mn(III) porphyrin ionically bound to poly-(4-styrylmethyl)pyridinium chloride, which gave an overall yield of 91% in a 3:1 ratio.

Generally speaking, the covalently-bound manganese porphyrins **5**–**7** gave fairly comparable results, with ratios greater than 2:1 in most cases (Table 2). The selectivity of the process appeared to be dependent on the amount of oxidant employed. For catalysts **5** and **6**, a 2:1 oxidant-to-alkene ratio seemed to be optimal for both activity and selectivity (entries 1–3 and 4–6). In contrast, carboxy-Wang supported manganese porphyrin **7** appeared to be more robust, since the amount of oxidant did not affect its activity significantly. The highest chemoselectivity of 2.7:1 was obtained with catalysts **6** and **7** (Entries 5 and 7) with yields of between 66 and 68%. A small amount of the bis-epoxide was formed during the catalytic reaction employing catalyst **7**.

Table 2. Epoxidation of (*R*)-limonene

Entry	Catalyst	Oxidant:diene ratio	Time (h)	Yields 8:9[a]	Ratio 8:9
1	**5**	3:1	24	12:9	1.3:1
2	**5**	2:1	24	52:23	2.3:1
3	**5**	1:1	24	38:20	1.9:1
4	**6**	3:1	24	43:24	1.8:1
5	**6**	2:1	24	48:18	2.7:1
6	**6**	1:1	24	42:24	1.8:1
7	**7**	3:1	24	50:18	2.7:1
8	**7**	2:1	24	51:22[b]	2.3:1
9	**7**	1:1	24	30:16	1.9:1

Reaction conditions as outlined in Scheme 2, except for the amount of oxidant.
[a] GC yield with respect to starting material. No diastereomeric excess was observed in either product.
[b] 6% Bis-epoxide.

Once again, catalyst **7** was found to be the most active, compared to the other two catalysts. Under these conditions, the maximum yield of the 1,2-epoxide was reached within 12 h, whereupon the formation of the bis-epoxide became apparent, which coincides with the drop in yield (Fig. 3).

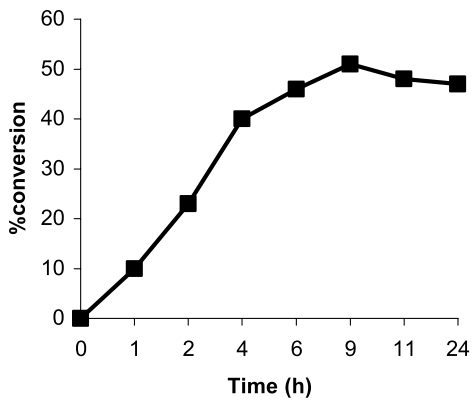


Figure 3. Rate of formation of 1,2-limonene epoxide **8** with catalyst **7**.

The diastereomeric ratio of products **8** and **9** was found to be 1:1 in each case, i.e. the inherent chirality of the starting material did not induce any facial selectivity in the epoxidation of either of the prochiral alkene moieties.

Finally, recyclability of the immobilised catalysts was tested by subjecting **5** and **7** to four successive catalytic

Table 3. Recycling catalysts **5** and **7** for the epoxidation of limonene

Entry	Catalyst (mol%)	Run	Time (h)	Yields 8:9[a]	Ratio 8:9
1	**5** (4)	1	24	52:23	2.3:1
2	**5** (4)	2	36	40:20	2.0:1
3	**5** (8)	2	24	54:19	2.8:1
4	**5** (8)	3	36	60:26	2.3:1
5	**5** (8)	4	36	48:17	2.8:1
6	**7** (4)	1	24	51:22 (6[b])	2.3:1
7	**7** (4)	2	24	48:23	2.1:1
8	**7** (8)	2	36	50:20	2.0:1
9	**7** (8)	3	36	57:26	2.2:1
10	**7** (8)	4	36	53:22	2.4:1

Oxidant/limonene (2:1).
[a] GC yields with respect to limonene. No diastereomeric excess was observed in either product.
[b] Bis-epoxide.

reactions (Table 3). For the Merrifield-supported porphyrin **5**, the recovered catalyst became increasingly less active—requiring either longer reaction times (entries 1 vs. 2 and 2 vs. 3) or higher catalytic loading (entries 2 vs. 3) to achieve comparable yields. In sharp contrast, catalyst **7** may be reused with no significant loss in activity in all four cycles (entries 8–10). Also, competitive formation of the bis-epoxide was not observed after the first catalytic run.

Remarkably, the selectivity was broadly maintained between the different cycles, and both catalysts may be subjected to four consecutive runs without significant changes in the ≥2:1 ratio.

3. Conclusion

To conclude, we have prepared and examined the comparative catalytic activity, selectivity and reusability of three polymer-supported manganese porphyrins **5**–**7**. Good chemoselectivity was observed in the epoxidation of three cyclic and acyclic dienes. Catalyst **7** consistently excelled in its catalytic activity and selectivity. As the length of the linkers was identical between catalysts **6** and **7**, we attribute its superiority to the higher reactive surface area of its beads (200–400 mesh), compared to the other two catalysts (100–200 mesh). All three resins may be recycled with varying successes, but encouragingly, no decline in selectivity was observed.

This work has led us to develop a new class of supported manganese porphyrin catalysts with improved selectivity and catalyst activity, which will be reported in due course.

4. Experimental

4.1. Materials

All resins were purchased from Novabiochem Wang bromo polystyrene and Merrifield resins are 1% cross-linked with bead sizes between 100 and 200 mesh. Brominated Wang resin was 1% cross-linked with bead sizes between 200 and 400 mesh. Anhydrous DMF was purchased from Aldrich and dichloromethane was freshly distilled from CaH_2 under nitrogen. Commercially available chemicals were purchased from Aldrich, Avocado, BDH, Fluka or Lancaster, and were used as received, unless otherwise stated. Manganese dichloride was purchased from Aldrich (99.999% purity).

The MacroKan™ used in the immobilisation of porphyrins is made of polypropylene with an internal volume of 2.4 mL, purchased from Irori Europe Limited (Tarporley Business Centre, Cheshire CW6 9UT, UK).

4.2. Instrumentation

Infrared spectra were recorded on a Perkin Elmer Spectrum One spectrometer. Spectra of porphyrin and metalloporphyrin samples were recorded as solutions in CCl_4 and $CHCl_3$, respectively, within a sealed cell with a path length of 0.1 mm with NaCl windows. Single bead FT-IR spectra (transmittance) were recorded with a beam-condensing accessory (BCA), using a diamond compressor to flatten the bead. UV spectra were recorded on a Perkin Elmer Lambda 18 spectrometer and performed in a thermostated (25 °C) cell of 10 mm path length. The samples were analysed in dichloromethane or in a solution of 20% piperidine in DMF.

^1H and ^{13}C NMR spectra were recorded using Bruker AM 360 and AVANCE 400 spectrometers NMR spectra of polymers were recorded using a Bruker AVANCE 400 spectrometer fitted with a special HR-MAS probe, with the resin placed in a 4 mm rotor. Chemical shifts were recorded in parts per million (δ: ppm) referenced to TMS (δ: 0) as an internal standard.

Mass spectra (MS) and high-resolution mass spectra (HRMS) were recorded using the EPSRC MS Services at University of Swansea, Wales or the Mass Spectrometry Service within the Department of Chemistry, King's College. FAB MS was run on a KRATOS 'MS89OMS' spectrometer. ES MS was run on a Micromass 'Q-TOF'. GC-MS spectra were recorded on a Varian Saturn 220 spectrometer equipped with an autosampler using a CP-Sil 8CB column.

4.3. Services

Elemental analyses were carried out by the Elemental Analysis Services at the University of North London (C, H, N) or University College London (halogens). %Mn was determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES) by Medac Ltd.

4.3.1. Synthesis of 4-(10,15,20-triphenyl-porphyrin-5-yl)-phenol 1.[11] To a refluxing solution of propionic acid (500 mL), were added pyrrole (13.9 mL, 0.2 mol), benzaldehyde (15.3 mL, 0.15 mol) and *p*-hydroxybenzaldehyde (6.1 g, 0.05 mol). The reaction mixture was stirred at reflux for 40 min. Propionic acid was then removed under vacuum. The black residue was first purified by column chromatography on silica, eluting with chloroform. The fraction containing TPPOH was suspended in CH_3OH, filtered and washed with CH_3OH to give 1.6 g (2.53 mmol, 5%) of the desired porphyrin as a purple solid, which was used in the subsequent steps without further purification. R_f 0.36 ($CHCl_3$); mp (220 °C. ^1H NMR (360 MHz, $CDCl_3$, 20 °C): δ 8.91–8.86 (m, 8H, H_7 and H_8-porphyrin), 8.23 (d, 6H,

^3J=7.6 Hz; H_2-Ph), 8.10 (d, 2H, ^3J=8.3 Hz; H_2-Ar), 7.80–7.75 (m, 9H; H_3-Ph, H_4-Ph), 7.22 (d, 2H, ^3J=8.3 Hz; H_3-Ar), 5.01 (s, 1H; OH), −2.76 (s, 2H; NH). ^{13}C NMR (90.5 MHz, $CDCl_3$, 20 °C): δ 155.8, 142.6, 136.1, 135.0, 132.0–131.0, 128.1, 127.1, 120.5, 114.1. UV–Vis (CH_2Cl_2): $\lambda_{max/nm}$ (ε): 415 (103 557), 515 (8461), 550 (3680), 589 (2152), 647 (2077) nm. HR-MS (FAB) (*m*/*z*): calcd for 630.2420, found: 630.2412 [M^+]. IR (CCl_4, cm^{-1}) ν_{NH} 3313, ν_{OH} 2962.

4.4. General procedure for the synthesis of Merrifield and Wang supported 4-(10,15,20-triphenyl-porphyrin-5-yl)-phenols

A Macro Irori Kan™ containing the appropriate resin (0.45 mmol) was placed in a N_2 purged three-necked round bottom flask with K_2CO_3 (0.34 g, 2.47 mmol), KI (0.164 g, 0.99 mmol) and *p*-4-(10,15,20-triphenyl-porphyrin-5-yl)-phenol **1** (0.47 g, 0.742 mmol). Anhydrous DMF (20 mL) was added via syringe and the mixture was magnetically stirred at 80 °C for 3 days. After cooling to room temperature, the beads were transferred to a sintered tube and washed successively with acetone/CH_3OH (1:1) (5 mL×5), acetone/CH_3OH/H_2O (1:1:1) (5 mL×5), acetone/CH_3OH (1:1) (5 mL×5), ethyl acetate (5 mL×5), CH_2Cl_2 (3 mL×5) and HPLC-grade pentane (5 mL×5). The resin was dried under vacuum at 50 °C for 2 h to give dark purple beads.

4.4.1. Merrifield 4-(10,15,20-triphenyl-porphyrin-5-yl)-phenol 2. Yield=93%, based on %N. After two treatments, elemental analysis found for a loading of 0.52 mmol g^{-1} Cl 0, N 2.90%; HR-MAS ^{1}H NMR (400 MHz, $CDCl_3$, 20 °C): δ 8.80 (br s, 8H, H_7 and H_8-porphyrin), 8.14 (br s, 6H, H_2-Ph), 7.59 (br s, 9H, H_3-Ph, H_4-Ph), 7.30–6.50 (br s, PS, H_3-Ar, H_2-Ar), 4.95 (br s, 2H, OCH_2), 1.8 (br s, PS), 1.39 (br s, PS), −2.75 (br s, 2H, NH); FT-IR (cm^{-1}): ν_{NH} 3317.

4.4.2. Wang 4-(10,15,20-triphenyl-porphyrin-5-yl)-phenol 3. Yield=37%, based on %N. After two treatments, elemental analysis found for a loading of 0.32 mmol g^{-1} Br 6, N 1.76%; FT-IR (cm^{-1}): ν_{NH} 3317 cm^{-1}.

4.4.3. Carboxy-Wang 4-(10,15,20-triphenyl-porphyrin-5-yl)-phenol 4. Yield=85%, based on %N. After one treatment, elemental analysis found for a loading of 0.53 mmol g^{-1} Br 0, N 2.93%. HR-MAS ^{1}H NMR (400 MHz, $CDCl_3$, 20 °C): δ 8.85 (br s, 8H, H_7 and H_8-porphyrin), 8.21 (br s, 6H, H_2-Ph), 7.71 (br s, 9H, H_3-Ph, H_4-Ph), 7.08–6.62 (br s, PS, H_3-Ar, H_2-Ar), 5.36 (br s, 2H, OCH_2), 1.59–1.33 (br s, PS), −2.78 (br s, 2H, NH); FT-IR (cm^{-1}): ν_{NH} 3319, ν_{CO} 1695.

4.5. General procedure for the synthesis of the supported manganese 4-(10,15,20-triphenyl-porphyrin-5-yl)-phenols

In an oven-dried three-neck flask equipped with an overhead stirrer and purged with N_2, the appropriate supported *p*-4-(10,15,20-triphenyl-porphyrin-5-yl)-phenol (0.5 mmol) was stirred in anhydrous DMF (30 mL). The flask was heated to 158 °C for 5 min before manganese chloride (3.13 g, 25 mmol) was added in one portion. The mixture was then stirred at 60 rpm at reflux for 2.5 h. After cooling to room temperature, the beads were transferred to a sintered tube and washed with CH_2Cl_2 (5 mL×10) and HPLC-grade pentane (5 mL×10). The resin was dried under vacuum at 50 °C for 2 h to give dark purple beads.

4.5.1. Merrifield 4-(10,15,20-triphenyl-porphyrin-5-yl)-phenol manganese chloride 5. 100% metallation, based on Mn%; elemental analysis found for a loading of 0.52 mmol g^{-1}, Mn 2.86%.

4.5.2. Wang 4-(10,15,20-triphenyl-porphyrin-5-yl)-phenol manganese chloride 6. 100% metallation, based on Mn%; elemental analysis found for a loading of 0.32 mmol g^{-1}, Mn 1.76%.

4.5.3. Carboxy Wang 4-(10,15,20-triphenyl-porphyrin-5-yl)-phenol manganese chloride 7. 100% metallation, based on Mn%; elemental analysis found for a loading of 0.53 mmol g^{-1}, Mn 2.92%. FT-IR (cm^{-1}): ν_{CO} 1695.

4.6. General epoxidation procedure

In a round-bottomed flask or in a Radley's carousel reaction tube, the appropriate catalyst (0.01 mmol), alkene (0.23 mmol) and axial ligand (0.1 mmol) were stirred in CH_3CN (3.7 mL) at room temperature. In a separate flask, $NaIO_4$ (0.46 mmol) was dissolved in H_2O (1.85 mL). This aqueous solution of $NaIO_4$ was transferred to the catalytic mixture. The progress of the reaction was monitored at regular intervals by analysing extracted aliquots by GC-MS. The yields of epoxides were based on the starting material consumed.

5. General procedure for the recovery and reuse of the catalyst

At the end of the epoxidation, the polymer-supported catalyst was filtered off via a filter syringe. The filtrate was extracted with CH_2Cl_2, washed with water, dried over Na_2SO_4 and evaporated. A small amount of the residue obtained was dissolved in dichloromethane and analysed by UV spectroscopy to determine if leaching of the porphyrin from the support had occurred. The catalyst beads in the syringe were washed with water, CH_2Cl_2 and HPLC-grade pentane and then dried under vacuum at 50 °C for 2 h. The recovered catalyst was then subjected to another catalytic cycle as described above.

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Influence of aromatic substituents on metal(II)salen catalysed, asymmetric synthesis of α-methyl α-amino acids

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Abstract—The influence of substituents on both the aromatic rings of the catalyst, and the benzylidene unit of the substrate are investigated in the (salen)copper(II) catalysed asymmetric benzylation of alanine derivatives. Catalysts with electron-donating, and electron-withdrawing substituents of various sizes and at various locations on the aromatic rings of the salen ligand were prepared, but all exhibited inferior enantioselectivity to the parent (salen)copper(II) complex. In contrast, the introduction of halogenated substituents onto the aromatic ring of the *N*-benzylidene alanine methyl ester substrate was found to enhance the enantioselectivity of the alkylation with a *para*-chloro substituent giving optimal results. A new procedure for the preparation of the catalysts which avoids the need for chromatography on sephadex LH20 is reported, and the optimal catalyst obtained in this way was found to be a cobalt(salen) complex.

1. Introduction

Interest in the asymmetric synthesis of α-amino acids and α,α-disubstituted amino acids by the alkylation of a prochiral enolate derived from glycine or an α-substituted amino acid has increased significantly in recent years. The most effective way of carrying out this process is to use a chiral catalyst under phase-transfer conditions, with the chiral catalyst also acting as a phase transfer catalyst.[1]

O'Donnell was the first to show that quaternary ammonium salts derived from cinchona alkaloids would catalyse the asymmetric alkylation of a glycine derived enolate, leading to non-racemic α-amino acids.[2] Recently, the groups of Lygo[3] and Corey,[4] have optimized this process and shown that the use of a 9-anthracenylmethyl group to quaternize the cinchona alkaloid results in a highly enantioselective catalyst which allows the synthesis of α-amino acids with >95% enantiomeric excess.[5] Attempts to extend this chemistry to enolates derived from other amino acids, thus allowing the synthesis of α,α-disubstituted amino acids were less successful.[6] Quaternized cinchona alkaloids can also be used to catalyse the alkylation of other enolates,[7] Michael additions,[8,9] aldol reactions,[10] and enone epoxidations.[11] They can also be used in conjunction with achiral palladium complexes to induce the asymmetric allylation of enolates.[12] Recently, polymer supported[9,13] and oligomeric[14] versions of cinchona derived phase transfer catalysts have been developed and used for asymmetric amino acid synthesis. The catalysts have also been used under micellar conditions.[15]

Synthetic quaternary ammonium salts derived from binaphthol have been developed by Maruoka.[16] These salts have been shown to act as asymmetric phase transfer catalysts for both the alkylation and dialkylation (with two different alkylating agents) of glycine derived imines, leading to both α-amino acids and α,α-disubstituted amino acids with excellent enantiomeric excesses. The asymmetric alkylation of β-keto-esters[17] and aldol reactions are also catalysed by these chiral ammonium salts.[18] Other groups have also investigated the use of synthetic phase transfer catalysts derived from ammonium[19] or guanidinium[20] salts and crown ethers.[21]

All the above work is based on the use of purely organic catalysts as asymmetric phase transfer catalysts. It was not until 1998 that Belokon' and Kagan reported that a metal complex could act as an asymmetric phase transfer catalyst. The sodium salt of TADDOL was found to catalyse the alkylation of alanine derivatives leading to α-methyl-α-amino acids with up to 82% enantiomeric excess.[22] Belokon' and Kagan have subsequently shown that the sodium salt of NOBIN[23] could act as an extremely rapid and enantioselective phase transfer catalyst for the same reaction. Nájera has recently reported that the sodium salt of BINOLAM is also an effective phase-transfer catalyst.[24]

Keywords: Catalyst; Phase-transfer; Asymmetric; Copper; Amino-acid.
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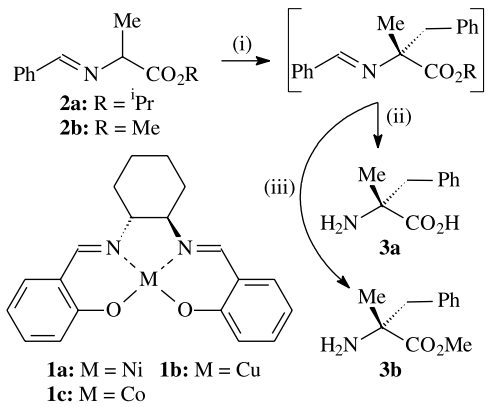
Chiral transition metal complexes have been used to catalyse a wide range of asymmetric transformations, and we have developed the asymmetric alkylation of amino acid enolates under phase transfer conditions catalysed by salen complexes of transition metals. In 1999, we reported that nickel(II)salen complex **1a** (10 mol%) would catalyse the asymmetric benzylation of alanine enolate **2a** leading to α-methyl phenylalanine **3a** (Scheme 1) with 30% enantiomeric excess.[25] The corresponding copper(II)salen complex **1b** was also studied and was found to be a far more effective catalyst. Just 2 mol% of complex **1b** was sufficient to catalyse the formation of compound **3a** with 88% enantiomeric excess. Recently, we have screened a wide range of other metal(salen) complexes for the alkylation of substrate **2b**, but whilst the Co(II)salen complex **1c** was found to be as active as complex **1b**, no superior catalyst was found.[26]



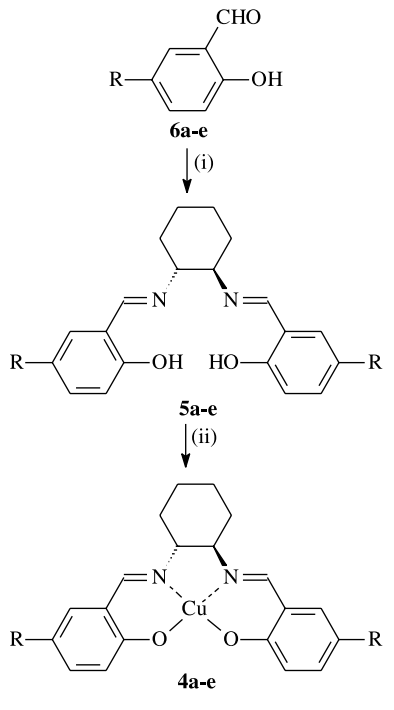
Scheme 1. Reagents: (i) **1a–c** (2–10 mol%)/NaOH (solid)/BnBr; (ii) H_3O^+/Δ; (iii) (R=Me), MeOH/AcCl then SiO_2.

Complex **1b** also catalysed the asymmetric alkylation of compound **2a** with other alkyl halides, giving α-methyl α-amino acids with 75–90% enantiomeric excess.[25] These reactions are carried out under solid–liquid phase transfer conditions with solid sodium hydroxide as the base, and both enantiomers of catalyst **1b** are equally readily available, thus allowing the synthesis of either enantiomer of an α-methyl α-amino acid. In subsequent work, we have also demonstrated that under appropriate reaction conditions, it is possible to use the readily available methyl ester substrate **2b**.[27] In addition, we have shown that the chemistry shown in Scheme 1 can be applied to amino acids other than alanine, thus allowing the synthesis of a range of non-racemic α,α-disubstituted α-amino acids.[28]

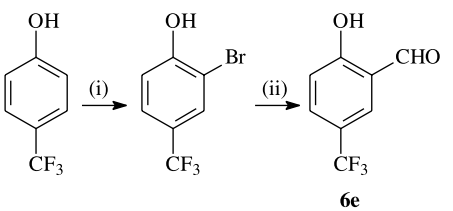
In this paper, we report the results of a study aimed at better understanding and optimizing the influence of various factors on the enantioselectivity of this reaction. In particular, the effect that substituents on the aromatic rings of catalyst **1b** have on the enantioselectivity of the catalyst are reported[25,29] In addition, the influence of the structure of the imine within substrate **2b** on the enantioselectivity of the reaction is studied.[29] Finally, a method for the preparation of the catalysts which avoids the use of size-exclusion chromatography on sephadex LH20 is described.

2. Results and discussion

Based on precedent from our work on asymmetric cyanohydrin synthesis using titanium(salen) complexes,[30] we expected that introduction of substituents at positions 3- and/or 5- of the aromatic rings of catalyst **1b** would enhance the enantioselectivity of the catalyst. These two positions (*ortho* and *para* to the phenol respectively) both allow the substituent to exert an electronic effect on the copper ion. However, we have previously shown that for the reaction shown in Scheme 1, introduction of a large *tert*-butyl group into the 3-position of the aromatic rings had a very negative impact on the enantioselectivity of the resulting catalyst.[25] Therefore, we initially investigated the synthesis and use of 5-substituted salen complexes **4a–e**. Compounds **4a–e** were prepared from the appropriate aldehyde via the corresponding salen ligands **5a–e** as shown in Scheme 2. Aldehydes **6b** and **6c** were commercially available, aldehydes **6a** and **6d** were prepared by literature routes,[31,32] and aldehyde **6e**[33] was prepared from 4-trifluoromethylphenol as shown in Scheme 3.[34]



Scheme 2. Reagents: (i) (*R*,*R*)-cyclohexane diamine/NaOMe; (ii) $CuBr_2$/NaOMe.



Scheme 3. Reagents: (i) Br_2; (ii) BuLi/DMF.

Catalysts **4a–e** were screened for catalytic activity in the alkylation of substrate **2b** with benzyl bromide to give α-methyl phenylalanine methyl ester **3b** as shown in Scheme 1. The enantiomeric excess of compound **3b** was readily determined by ^{1}H NMR analysis of the derived α-methylbenzyl ureas as previously reported.[25–29] The chemical yields and enantioselectivities observed using these catalysts are summarized in Table 1.

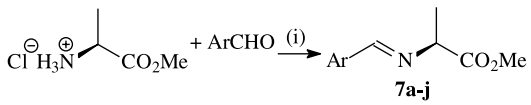
Table 1. Effect of 5-substituents on the enantioselectivity of catalysts **4a–e**

Entry	R	Yield (%)	ee (%)
1	^{t}Bu (**4a**)	39	80
2	OMe (**4b**)	78	45
3	NO_2 (**4c**)	60	0
4	F (**4d**)	54	42
5	CF_3 (**4e**)	68	25

The introduction of a large *tert*-butyl group into position 5 of the aromatic rings (**4a**) had no significant effect on the level of asymmetric induction observed using the catalyst (Table 1: entry 1). In contrast, complex **4b** containing a strongly electron donating group in the 5-position displayed significantly reduced asymmetric induction (Table 1: entry 2). Reasoning that if electron donating groups reduced the asymmetric induction, electron withdrawing groups might increase the asymmetric induction, the synthesis of complex **4c** containing nitro groups in the 5-position was undertaken. Unfortunately, whilst ligand **5c** could be prepared without difficulty, copper complex **4c** was totally insoluble and impossible to purify. When the catalytic activity of the crude complex was tested, no asymmetric induction was observed (Table 1: entry 3). The chemical yield obtained in this case is probably due to an uncatalysed background reaction.

To overcome the solubility problems observed with complex **4c**, the synthesis of fluorinated complexes **4d** and **4e** was undertaken and both complexes were obtained without difficulty. The 5-fluoro substituents in complex **4d** are inductively strongly electron withdrawing, but mesomerically strongly electron donating and the complex was found to exhibit very similar asymmetric induction to complex **4b** (Table 1: entry 4). In contrast, the trifluoromethyl groups in complex **4e** can only exhibit an inductively electron withdrawing effect, and this complex was found to be an even worse catalyst (Table 1: entry 5). Thus, the introduction of either electron donating or electron withdrawing substituents onto the 5-positions of complex **1b** was found to be detrimental for the asymmetric induction observed when the complexes were used as asymmetric phase transfer catalysts.

Having found no advantage in introducing substituents onto the aromatic rings of catalyst **1b**, the effect of substituents on the imine of substrate **2b** was investigated. Substrates **7a–j** were prepared from (*S*)-alanine methyl ester and the appropriate aldehyde as shown in Scheme 4. The alkylation of each of these substrates was carried out using benzyl bromide as the electrophile under the conditions shown in Scheme 1. The chemical yields and enantiomeric excesses of the α-methyl phenylalanine methyl ester **3b** obtained in each case are reported in Table 2.



7a-j

a: Ar = 4-$O_2NC_6H_4$; **b:** Ar = 4-$MeOC_6H_4$
c: Ar = 4-ClC_6H_4; **d:** Ar = 3-ClC_6H_4
e: Ar = 2-ClC_6H_4; **f:** Ar = 4-FC_6H_4
g: Ar = 4-BrC_6H_4; **h:** Ar = 4-IC_6H_4
i: Ar = 1-naphthyl; **j:** Ar = 2- naphthyl

Scheme 4. Reagents: (i) $Et_3N/MgSO_4$.

Table 2. Effect of imine structure on the enantioselectivity of the alkylation of substrates **7a–j**

Entry	Ar	Yield (%)	ee (%)
1	4-$O_2NC_6H_4$ (**7a**)	50	65
2	4-$MeOC_6H_4$ (**7b**)	79	71
3	4-ClC_6H_4 (**7c**)	71	92
4	3-ClC_6H_4 (**7d**)	53	81
5	2-ClC_6H_4 (**7e**)	43	70
6	4-FC_6H_4 (**7f**)	44	84
7	4-BrC_6H_4 (**7g**)	95	81
8	4-IC_6H_4 (**7h**)	67	86
8	1-Naphthyl (**7i**)	89	79
9	2-Naphthyl (**7j**)	62	77

Initially, the influence of electronic effects on the enantioselectivity was studied by the preparation of the 4-nitro **7a** and 4-methoxy **7b** substituted imines. It was anticipated that a strongly electron withdrawing group in the 4-position would acidify the α-proton and therefore possibly lower the enantioselectivity by increasing the rate of the uncatalysed background reaction. This was borne out by the observed 16% reduction in enantioselectivity (Table 2: entry 1) compared to the use of substrate **2b** under identical conditions (81% ee). In contrast however, an electron donating methoxy substituent was expected to increase the enantioselectivity of the reaction by reducing the acidity of the α-proton and hence reducing the rate of the background reaction. A possible reduction in chemical yield as a result of the lower acidity of the α-proton was also anticipated. In practice however (Table 2: entry 2), the chemical yield remained high and the enantioselectivity was reduced compared to substrate **2b**. This may indicate that the background reaction is not a significant factor when a catalyst is present and some other factor or factors are responsible for controlling the asymmetric induction.

Halo substituents offer the opportunity to introduce inductively electron withdrawing substituents onto the imine and to have this offset to some extent by a mesomerically electron donating effect. Therefore, a series of compounds **7c**, **f–h** were prepared in which a halogen was introduced at the 4-position of the imine. In each case, the enantioselectivity was at least as high as that observed using substrate **2b** (Table 2: entries 3, 6–8) and in the best case (the 4-chloro substituted derivative **7c**), a 10% increase in enantioselectivity to 91% was observed (Table 2: entry 3). The order of effectiveness of a 4-halo substituent was Cl>I>F>Br. The reason behind this ordering is not apparent, but the enantioselectivities observed with the bromo-, fluoro- and iodo- substituents are all within 5% of one another and this may be within the experimental error of

±3%. Thus, it appears that a 4-chloro substituent provides the optimal balance of electron withdrawing and electron donating effects to achieve the highest enantioselectivity and retain a good chemical yield. The importance of the chloro-substituent being in the 4-position was demonstrated by the preparation of the corresponding 3-chloro and 2-chloro derivatives **7d**,**e** respectively. The 3-chloro derivative which cannot exhibit any mesomeric effect gave an identical enantioselectivity to that observed using substrate **2b** (Table 2: entry 4). The 2-chloro derivative gave a lower enantioselectivity than that observed using substrate **2b** (Table 2: entry 5), and steric effects may be important in this case. To further probe the influence of steric effects, the synthesis of two naphthyl derivatives **7i**,**j** was undertaken. These two substrates both gave enantioselectivities that were comparable with, or just slightly lower than, substrate **2b** (Table 2: entries 8 and 9), suggesting that steric effects (at least in the plane of the aromatic ring) are not a significant factor in determining the enantioselectivity of the reaction.

In general, the effect of introducing substituents onto the imine on the enantioselectivity of the reaction was not as marked as the effect of introducing substituents onto the catalyst. However, in contrast to the results obtained with substituted catalysts, it was possible to both increase and decrease the enantioselectivity of the reaction compared to substrate **2b** (81% ee) by using an appropriately substituted imine.

Having successfully optimized the structure of the substrate, we returned to studies aimed at optimizing the structure of catalyst **1b**, this time using the benzylation of substrate **7c** as the test reaction. As a result of our related work in this area,[25–29] we have developed a working model to explain the mode of action of catalyst **1b**.[25,28] This model is shown in Figure 1. The key feature of the model is the formation of a hetero-polymetallic complex involving both copper(II) and sodium ions, with the latter coordinated by the salen oxygens, a process for which there is ample literature precedent.[35] In addition, our studies on changing the nature of the transition metal ion,[26] and in particular the fact that only the paramagnetic complexes derived from Cu(II) and Co(II) displayed high levels of asymmetric induction suggested that the reaction might proceed by a radical or radical anion mechanism.

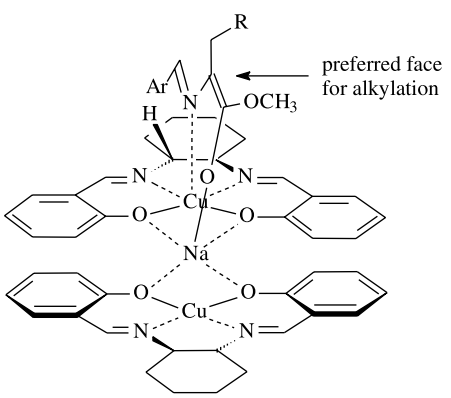
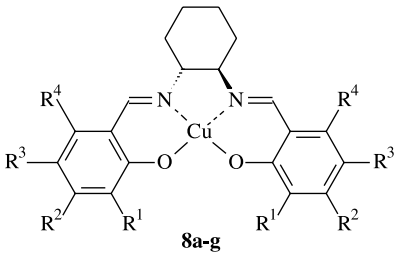


Figure 1. A model to explain the mode of action of catalyst **1b**.

Based on this model and mechanistic hypothesis, we decided to prepare a series of electron rich copper(II)salen complexes bearing hydroxy or methoxy substituents. It was hoped that if the substituents were located in the 3-position, then they would be able to assist with the coordination of the sodium ion and hence enhance the catalytic activity. In addition, there is literature precedent for hydroxyl substituents stabilizing radical anion formation in salen ligands.[36] Therefore, complexes **8a–f** were prepared and complex **4b** was also included in this study as was complex **8g**. The latter complex allowed us to investigate the effect of an alkyl group at the 4-position of the salen ring as this had not previously been studied. All of the aldehydes needed for the preparation of complexes **8a–g** are commercially available and were converted into the corresponding salen ligands by the route shown in Scheme 2. Copper complexes **8a–g** were then prepared by one of two methods. For complexes **8a**,**d**,**e**, the salen ligand was treated with copper(II) bromide and the resulting copper complex purified by gel permeation chromatography on sephadex LH-20 in the same way as for complexes **1b** and **4a**,**b**,**d**,**e**. However, during the course of this work the sephadex LH-20 required for the purification of complexes prepared in this way became commercially unavailable. Therefore, for complexes **8**,**b**,**c**,**f**,**g**, an alternative synthesis was developed which uses copper(II) acetate as the copper source. This method had the advantage that the copper complex could be purified simply by washing with suitable solvents (see Section 4 for details).



8a-g

a: R^1 = OH; R^2 = R^3 = R^4 = H
b: R^2 = OH; R^1 = R^3 = R^4 = H
c: R^3 = OH; R^1 = R^2 = R^4 = H
d: R^1 = OMe; R^2 = R^3 = R^4 = H
e: R^2 = OMe; R^1 = R^3 = R^4 = H
f: R^4 = OMe; R^1 = R^2 = R^3 = H
g: R^2= Me; R^1 = R^3 = R^4 = H
4b: R^3 = OMe; R^1 = R^2 = R^4 = H

The results of catalytic studies using copper complexes **8a–g** and **4b** in conjunction with substrate **7c** are summarized in Table 3. Previous work had suggested that

Table 3. Effect of oxygen or methyl substituents on the enantioselectivity of catalysts **4b** and **8a–g**

Entry	Complex	Yield (%)	ee (%)
1	**8a**	79	5
2	**8b**	46	4
3	**8c**	59	3
4	**8d**	39	14
5	**8e**	89	75
6	**4b**	61	52
7	**8f**	78	67
8	**8g**	56	74

a *tert*-butyl substituent in the 3-positions (R^1) would have a severely detrimental effect on the catalytic activity of the catalyst.[25] It was hoped that the much smaller size of a hydroxy or methoxy group combined with their sodium coordinating ability would overcome this steric effect. However, as entries 1 and 4 in Table 3 show, complexes **8a** and **8d** exhibited very poor levels of asymmetric induction. It is likely that the chemical yield observed in these cases is largely due to an uncatalysed alkylation as we have previously shown that significant formation of α-methyl phenylalanine occurs under the conditions of Scheme 1, even if the catalyst is omitted.[28]

The other two catalysts containing hydroxyl groups (**8b** and **8c**) also showed negligible levels of asymmetric induction (Table 3: entries 2 and 3). Catalysts **8a–c** will almost certainly form bis-sodium salts in situ, and this may prevent the formation of the bimetallic complex shown in Figure 1 and hence account for the lack of catalytic activity.

The methoxy containing catalysts **8d–f** and **4b** were all much more enantioselective than the corresponding hydroxy derivatives, even when the methoxy groups are in the 3-positions (R^1) (Table 3: compare entries 1 and 4). However, the highest chemical yield and asymmetric induction was observed with catalyst **8e** in which the methoxy groups are in the 4-positions (R^2) (Table 3: entry 5). In this position, the methoxy groups cannot coordinate the sodium ion in the model shown in Figure 1 and cannot exert any other obvious steric or electronic effect on the catalysis. It is notable that the second best results (Table 3: entry 7) were obtained with catalyst **8f** with the methoxy groups in the 6-position (R^4), the other position on the aromatic ring where the substituent cannot exert an apparent steric or electronic effect. Catalysts **8e** and **8f** were both significantly more active and enantioselective than catalyst **4b** (Table 3: entry 6) in which the methoxy group is *para* to the coordinating oxygen and so can exert a mesomeric electronic effect on the copper ion. Catalyst **8g** with methyl rather than methoxy groups in the R^4-positions showed essentially identical enantioselectivity to catalyst **8e**, suggesting that the enantioselectivity is not connected to the presence of an oxygen atom.

Since the catalysts used during this work were prepared by two different routes and since the method reported for the preparation of catalyst **1b** was no longer viable due to the commercial unavailability of sephadex LH20, it was decided to carry out a direct comparison of catalysts prepared by both synthetic routes. Two catalysts, **1b** and **8e**, were chosen for this study. Samples of both catalysts were prepared from the appropriate salen ligand using copper bromide/sodium methoxide followed by purification by chromatography on sephadex LH20 and by use of copper acetate followed by isolation of the catalyst by precipitation and purification by washing. The results obtained when the resulting catalysts were used to induce the asymmetric benzylation of substrate **7c** are compared in Table 4.

In the case of catalyst **1b**, the catalyst prepared using the copper acetate procedure was noticeably less enantioselective than the catalyst prepared by the copper bromide procedure (Table 4: entries 1 and 2). This may be partly due to the catalyst prepared from copper acetate being less pure due to the lack of a chromatographic purification, and use of an increased mol% of the catalyst did increase the enantioselectivity slightly (Table 4: entries 3 and 4). However, it was still not possible to match the enantioselectivity obtained using the catalyst prepared from copper bromide and increasing the mol% of catalyst above 4 mol% was severely detrimental to both the enantioselectivity and the chemical yield (Table 4: entry 5). The enantioselectivity of catalyst **1b** prepared using copper acetate could be further slightly increased by recrystallizing the catalyst from dichloromethane (Table 4: entry 6). This process was used in the literature to obtain crystals of complex **1b** suitable for X-ray analysis.[37] However, the recrystallization is very slow and low yielding and so not synthetically useful.

Table 4. Effect of method of preparation on the enantioselectivity of catalysts **1b** and **8e**

Entry	Catalyst (mol%)	Preparation	Yield (%)	ee (%)
1	**1b** (2)	$CuBr_2$	71	92
2	**1b** (2)	$Cu(OAc)_2$	94	78
3	**1b** (3)	$Cu(OAc)_2$	61	82
4	**1b** (4)	$Cu(OAc)_2$	78	81
5	**1b** (6)	$Cu(OAc)_2$	28	64
6	**1b**[a] (2)	$Cu(OAc)_2$	83	84
7	**8e** (2)	$CuBr_2$	89	75
8	**8e** (2)	$Cu(OAc)_2$	89	83
9	**8e** (3)	$Cu(OAc)_2$	59	80
10	**1c** (2)	$Co(OAc)_2$	89	85

[a] Catalyst was additionally recrystallized from dichloromethane.

In contrast, catalyst **8e** was found to be more enantioselective when prepared using copper acetate than when prepared using copper bromide (Table 4: entries 7 and 8). It may be that the purification by washing is more successful in the case of catalyst **8e** than catalyst **1b**. Increasing the amount of catalyst **8e** above the standard 2 mol% was found to have a detrimental effect on both the enantioselectivity and the chemical yield (Table 4: entry 9).

We have previously reported that Co(salen) complex **1c** (prepared using cobalt acetate) was as enantioselective as copper(salen) complex **1b** (prepared using copper bromide). This comparison was carried out using substrate **2b** and the enantioselectivities were 81 and 80% for catalysts **1b** and **1c** respectively.[26] Therefore, we decided to see if the enantioselectivity observed with catalyst **1c** could be further enhanced by the use of substrate **7c**. Treatment of substrate **7c** with benzyl bromide under the standard conditions (Scheme 1) using 2 mol% of complex **1c** as catalyst resulted in the formation of α-methyl phenylalanine methyl ester **3b** in 89% yield and 85% enantiomeric excess (Table 4: entry 10). This result is the best obtainable with any of the catalysts studied without the need for purification on sephadex LH20.

3. Conclusions

The enantioselectivity of (salen)Cu complexes as catalysts for the asymmetric benzylation of alanine derivatives was found to be strongly influenced by substituents on the aromatic rings of the salen ligand. All of the substituents

studied at any location on the aromatic rings had a negative effect on the enantioselectivity. In contrast, substituents on the aromatic ring of the *N*-arylidene alanine methyl ester substrate could have either a positive or negative effect. Optimal results were obtained using a *para*-chlorobenzylidene imine.

The mode of preparation of the catalyst was also found to influence its enantioselectivity. The best result (92% ee) was obtained when the catalyst was prepared using copper bromide and purified by chromatography on sephadex LH20. An alternative procedure using copper acetate and purification by washing with various solvents gave a less enantioselective (78% ee) catalyst derived from the unsubstituted salen ligand, though the enantioselectivity of the catalyst could be increased to 84% by further purification by recrystallization. In contrast however, for catalyst **8e** derived from the 4-methoxy-salen ligand, the copper acetate route was found to give a more enantioselective (83% ee) catalyst than the copper bromide procedure (75% ee). The most enantioselective catalyst (85% ee) which did not need purification by sephadex LH20 however was complex **1c** derived from cobalt acetate.

4. Experimental

^{1}H NMR, ^{13}C NMR and ^{19}F NMR spectra were recorded on a Bruker Avance 360 Spectrometer, (^{1}H 360 MHz, ^{13}C 90 MHz, and ^{19}F 338 MHz). The solvent for a particular spectrum is given in parentheses. ^{1}H and ^{13}C NMR Spectra were referenced to TMS and chemical-shift (δ) values, expressed in parts per million (ppm), are reported downfield of TMS. Chemical-shift values for ^{19}F spectra are relative to $CFCl_3$. The multiplicity of signals is reported as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br) or a combination of any of these. For ^{13}C NMR spectra, the peak assignments were made with the assistance of DEPT experiments.

Infrared spectra were recorded on a Perkin–Elmer FT-IR Paragon 1000 spectrometer, as a thin film between NaCl plates in the reported solvent, or as KBr disks. The characteristic absorption is reported as broad (br), strong (s), medium (m) or weak (w). Low and high resolution mass spectra were recorded at the EPSRC national service at the University of Wales, Swansea, or on a Bruker Apex III FTMS or Jeol AX505W spectrometer within the chemistry department at King's College. The sample was ionized by electron ionization (EI), chemical ionization with ammonia as the reagent gas (CI), fast atom bombardment (FAB) or electrospray ionization (ES). The major fragment ions are reported and only the molecular ions are assigned.

Optical rotations were recorded on a Perkin–Elmer 343 polarimeter in a thermostated cell of length 1 dm at 20 °C using the sodium D-line, and a suitable solvent that is reported along with the concentration (in g/100 mL). Melting points were determined with a Buchi Melting Point apparatus N° 520092 and are uncorrected. Elemental analyses were performed by the London School of Pharmacy.

Chromatographic separations were performed with silica gel 60 (230–400 mesh) and thin-layer chromatography was performed on polyester backed sheets coated with silica gel 60 F254, both supplied by Merck. Toluene was distilled from sodium prior to use.

4.1. 2-Hydroxy-5-trifluoromethylbenzaldehyde[33] 6e

In a three-neck flask under an argon atmosphere, 2-bromo-4-trifluoromethylphenol[34] (0.95 g, 4.2 mmol) was dissolved in THF (40 mL). The resulting solution was stirred and cooled to −60 °C and then *n*-BuLi (3.4 mL of a 2.5 M solution in hexane) was added dropwise. The reaction temperature was maintained at −60 °C for 1 h. Dimethyl formamide (1.6 mL, 21.1 mmol) was then added dropwise at the same temperature, stirred for another 5 min at −60 °C, and then the mixture was allowed to increase its temperature slowly until it reached room temperature and was stirred overnight. The reaction was hydrolysed with diluted hydrochloric acid and extracted 3 times with dichloromethane. The combined organic layers were dried and evaporated in vacuo to dryness to give a yellow oil which was chromatographed using dichloromethane/hexane (2:1) as eluent to give aldehyde **6e** (239 mg, 30%) as a white solid. Mp 59–60 °C; $\nu_{max}(CHCl_3)$ 3156 (s), 2960 (w), 2856 (w), 1665 (s), 1632 (m), 1596 (m), and 1496 cm^{-1} (m); $\delta_H(CDCl_3)$ 7.0–7.8 (3H, m, ArCH), 9.88 (1H, s, HCO), 11.23 (1H, s, OH); $\delta_C(CDCl_3)$ 119.1 (ArCH), 120.3 (ArC), 122.9 (q $^2J_{CF}$=34 Hz, Ar*C*CF$_3$), 124.0 (q $^1J_{CF}$=272 Hz, CF_3), 131.4 (q $^3J_{CF}$=4 Hz, ArCH), 133.8 (q $^3J_{CF}$=3 Hz, ArCH), 164.3 (ArC), 196.2 (HCO); $\delta_F(CDCl_3)$ −62.4 (CF_3); *m*/*z* (EI) 190 (M^+, 100), 189 (100), 172 (19), 161 (27), 144 (20). Found: C, 50.70%; H, 2.70%; $C_8H_5O_2F_3$ requires: C, 50.54%; H, 2.65%.

4.2. General procedure for the preparation of salen ligands

To a stirred mixture of aldehyde (20.0 mmol) and (1*R*,2*R*)-diaminocyclohexane dihydrochloride (1.87 g, 10.0 mmol) in methanol (37 mL) and ethanol (37 mL) was added a solution of NaOMe (5 mL of a 4.65 N solution) at room temperature. The resulting bright yellow solution was stirred under reflux overnight, then allowed to cool to room temperature, filtered and evaporated in vacuo. The yellow residue was taken up in dichloromethane (80 mL), filtered, then the organic layers were washed with water (2×30 mL) and brine (30 mL). The combined aqueous layers were back extracted with dichloromethane. The combined organic layers were dried and evaporated in vacuo to leave the desired ligand.

4.2.1. (1*R*,2*R*)-[*N*,*N*′-Bis-(2′-hydroxy-5′-*tert*-butylbenzylidene)]-1,2-diaminocyclohexane[38] 5a. Obtained as a pale yellow solid in 70% yield. Mp 115–116 °C; $[\alpha]_D^{20}$=−184 (*c* 0.9, $CHCl_3$); ν_{max}(KBr) 2959 (s), 2863 (m), 1632 (s), and 1492 cm^{-1} (s); $\delta_H(CDCl_3)$ 1.16 (9H, s, $C(CH_3)_3$), 1.3–1.8 (4H, m, CH_2CH_2), 3.2–3.3 (1H, m, CHN), 6.7–6.9 (1H, m, ArCH), 7.2–7.3 (3H, m, ArCH), 8.18 (1H, s, HC=N); $\delta_C(CDCl_3)$ 23.2 (CH_2), 30.4 (CH_3), 32.2 (CH_2), 32.9 (CMe_3), 71.8 (CHN), 115.2 (ArCH), 116.9 (ArC), 126.9 (ArCH), 128.4 (ArCH), 140.2 (ArC), 157.6 (ArC), 164.0 (HC=N).

4.2.2. (1*R*,2*R*)-[*N*,*N*′-Bis-(2′-hydroxy-5′-methoxy-benzylidene)]-1,2-diaminocyclohexane 5b. Obtained as a yellow solid in 88% yield. Mp 118–120 °C; $[\alpha]_D^{20}=-306$ (*c* 1.0, $CHCl_3$); ν_{max}($CHCl_3$) 2936 (m), 2860 (w), 1636 (s), and 1592 cm^{-1} (s); δ_H($CDCl_3$) 1.5–2.0 (4H, m, CH_2CH_2), 3.3–3.4 (1H, m, CHN), 3.72 (3H, s, OCH_3), 6.66 (1H, dd *J*=2.7 Hz, ArCH), 6.85 (1H, s, ArCH), 6.86 (1H, d *J*=2.7 Hz, ArCH), 8.21 (1H, s, HC═N), 12.83 (1H, s, OH); δ_C($CDCl_3$) 24.6 (CH_2), 33.5 (CH_2), 56.3 (OCH_3), 73.2 (CHN), 115.2 (ArCH), 117.9 (ArCH), 118.7 (ArC), 119.8 (ArCH), 152.4 (ArC), 155.5 (ArC), 164.9 (HC═N); *m*/*z* (CI) 383 (MH^+, 100), 249 (8). Found (ES) 383.1957, $C_{22}H_{27}N_2O_4$ (MH^+) requires 383.1971.

4.2.3. (1*R*,2*R*)-[*N*,*N*′-Bis-(2′-hydroxy-5′-nitro-benzylidene)]-1,2-diaminocyclohexane[38,39] 5c. Obtained as a yellow solid in 77% yield. Mp 198–200 °C; $[\alpha]_D^{20}=-27$ (*c* 1.0, $CHCl_3$); ν_{max}(KBr) 2926 (m), 1639 (s), 1538 (m), and 1484 cm^{-1} (m); δ_H($CDCl_3$) 1.4–1.5 (1H, m, CH_2), 1.6–1.8 (1H, m, CH_2), 1.9–2.0 (2H, m, CH_2), 3.3–3.5 (1H, m, CH–N), 6.8–6.9 (1H, m, ArCH), 8.0–8.10 (2H, m, ArCH), 8.28 (1H, s, HC═N); δ_C($CDCl_3$) 24.3 (CH_2), 33.1 (CH_2), 72.4 (CHN), 117.5 (ArC), 118.7 (ArCH), 128.3 (ArCH), 128.5 (ArCH), 139.9 (ArC), 164.1 (HC═N), 167.7 (ArC).

4.2.4. (1*R*,2*R*)-[*N*,*N*′-Bis-(2′-hydroxy-5′-fluoro-benzylidene)]-1,2-diaminocyclohexane 5d. Obtained as yellow crystals in 36% yield after recrystallization from hexane–isopropanol. Mp 121–123 °C; $[\alpha]_D^{20}=-494$ (*c* 0.4, $CHCl_3$); ν_{max}($CHCl_3$) 2938 (m), 2862 (m), 1636 (s) and 1589 cm^{-1} (m); δ_H($CDCl_3$) 1.4–1.5 (1H, m, CH_2), 1.6–1.7 (2H, m, CH_2), 1.8–1.85 (2H, m, CH_2), 3.2–3.3 (1H, m, CHN), 6.7–6.9 (3H, m, ArCH), 8.12 (1H, s, HC═N), 12.90 (1H, br s, OH); δ_C($CDCl_3$) 24.5 (CH_2), 33.3 (CH_2), 73.1 (CHN), 116.9 (d $^2J_{CF}$=23 Hz, ArCH), 118.2 (d $^3J_{CF}$=7 Hz, ArC), 118.7 (d $^3J_{CF}$=7 Hz, ArCH), 119.7 (d $^2J_{CF}$=23 Hz, ArCH), 155.7 (d $^1J_{CF}$=237 Hz, ArCF), 157.4 (d $^4J_{CF}$=1 Hz, ArCO), 164.1 (d $^4J_{CF}$=2 Hz, HC═N); δ_F($CDCl_3$) −126.3 (ArC–F); *m*/*z* (CI) 359 (MH^+, 100), 237 (7). Found (ES) 381.1372, $C_{20}H_{20}N_2O_2F_2Na$ ($M+Na^+$) requires 381.1385.

4.2.5. (1*R*,2*R*)-[*N*,*N*′-Bis-(2′-hydroxy-5′-trifluoromethyl-benzylidene)]-1,2-diaminocyclohexane 5e. Obtained as a yellow solid in a yield of 77%. Mp 120–122 °C; $[\alpha]_D^{20}=-250$ (*c* 0.4, $CHCl_3$); ν_{max}($CHCl_3$) 2936 (w), 1622 (s), and 1541 cm^{-1} (m); δ_H($CDCl_3$) 1.4–2.0 (4H, m, CH_2CH_2), 3.3–3.4 (1H, m, CHN), 6.9–7.4 (3H, m, ArCH), 8.22 (1H, s, HC═N), 13.64 (1H, s, OH); δ_C($CDCl_3$) 23.0 (CH_2), 31.9 (CH_2), 71.5 (CHN), 116.6 (ArCH), 116.9 (ArC), 120.0 (q $^2J_{CF}$=33 Hz, Ar*C*CF_3), 123.0 (q $^1J_{CF}$=271 Hz, CF_3), 127.7 (q $^3J_{CF}$=4 Hz, ArCH), 128.1 (q $^3J_{CF}$=3 Hz, ArCH), 162.7 (ArC), 162.9 (HC═N); δ_F($CDCl_3$) −62.0 (CF_3); *m*/*z* (CI) 459 (MH^+, 100), 190 (6), 52 (30). Found (ES) 481.1356, $C_{22}H_{20}N_2O_2F_6Na$ ($M+Na^+$) requires 481.1321.

4.2.6. (1*R*,2*R*)-[*N*,*N*-Bis-(2′-hydroxy-3′-methoxy-benzylidene)]-1,2-diaminocyclohexane.[40] Obtained as a yellow gel in 57% yield using the general procedure but with only a three hour reflux and ethyl acetate rather than dichloromethane used to extract the product. $[\alpha]_D^{20}=-490$ (*c* 0.05, $CHCl_3$); ν_{max}($CHCl_3$) 2937 (w), 2861 (w), 2254 (w), 1629 (s), and 1464 cm^{-1} (s); δ_H($CDCl_3$) 1.4–1.9 (4H, m, CH_2CH_2), 3.2–3.3 (1H, m, CHN), 3.80 (3H, s, OCH_3), 6.32 (1H, t *J*=7.75 Hz, ArCH), 6.71 (1H, dd *J*=7.75, 1.6 Hz, ArCH), 6.78 (1H, dd *J*=7.7, 1.7 Hz, ArCH), 8.17 (1H, s, HC═N); *m*/*z* (CI) 383 (M^+, 10), 242 (12), 210 (100), 193 (95), 170 (50). Found (ES) 405.1805, $C_{22}H_{26}N_2O_4Na$ ($M+Na^+$) requires 405.1785.

4.2.7. (1*R*,2*R*)-[*N*,*N*-Bis-(2′-hydroxy-4′-methoxy-benzylidene)]-1,2-diaminocyclohexane. Obtained as a yellow gel in 92% yield using the general procedure but with only a three hour reflux and ethyl acetate rather than dichloromethane used to extract the product. $[\alpha]_D^{20}=-1477$ (*c* 0.015, MeOH); ν_{max}($CHCl_3$) 3388 (br), 2935 (w), 1625 (s), 1580 (m), and 1514 cm^{-1} (m); δ_H($CDCl_3$) 1.3–2.1 (4H, m, CH_2CH_2), 3.2–3.3 (1H, m, CHN), 3.79 (3H, s, OCH_3), 6.33 (1H, dd *J*=8.6, 2.4 Hz, ArCH), 6.37 (1H, d *J*=2.4 Hz, ArCH), 7.01 (1H, d *J*=8.6 Hz, ArCH), 8.11 (1H, s, HC═N), 13.83 (1H, br s, OH); δ_C($CDCl_3$) 24.6 (CH_2), 33.5 (CH_2), 55.7 (OCH_3), 72.0 (CHN), 101.5 (ArCH), 106.6 (ArCH), 112.6 (ArC), 133.1 (ArCH), 163.8 (ArC), 164.1 (HC═N), 165.3 (ArC); *m*/*z* (CI) 383 (MH^+, 100), 249 (14), 152 (11). Found (ES) 383.1927, $C_{22}H_{27}N_2O_4$ (MH^+) requires 383.1965.

4.2.8. (1*R*,2*R*)-[*N*,*N*-Bis-(2′-hydroxy-6′-methoxy-benzylidene)]-1,2-diaminocyclohexane. Obtained as a yellow solid in 79% yield using the general procedure but with only a three hour reflux and ethyl acetate rather than dichloromethane used to extract the product. Mp 156–157 °C; $[\alpha]_D^{20}=-42.5$ (*c* 0.035, $CHCl_3$); ν_{max}($CHCl_3$) 2937 (w), 2360 (w), 2252 (w), 1624 (s), 1578 (m), and 1446 cm^{-1} (s); δ_H($CDCl_3$) 1.4–2.0 (4H, m, CH_2CH_2), 3.3–3.4 (1H, m, CHN), 3.73 (3H, s, OCH_3), 6.21 (1H, d *J*=8.2 Hz, ArCH), 6.48 (1H, d *J*=8.4 Hz, ArCH), 7.17 (1H, t *J*=7.7 Hz, ArCH), 8.70 (1H, s, HC═N), 14.37 (1H, s, OH); δ_C($CDCl_3$) 24.6 (CH_2), 33.5 (CH_2), 55.9 (OCH_3), 72.5 (CHN), 99.9 (ArCH), 108.3 (ArC), 110.5 (ArCH), 133.6 (ArCH), 160.0 (ArC), 161.3 (HC═N), 164.4 (ArC); *m*/*z* (CI) 384 (MH^+, 100), 249 (17), 152 (11). Found (ES) 383.1944, $C_{22}H_{27}N_2O_4$ (MH^+) requires 383.1965. Found (ES) 405.1764, $C_{22}H_{26}N_2O_4Na$ ($M+Na^+$) requires 405.1784.

4.2.9. (1*R*,2*R*)-[*N*,*N*-Bis-(2′,3′-dihydroxybenzylidene)]-1,2-diaminocyclohexane. To a stirred mixture of 2,3-dihydroxybenzaldehyde (2.0 g, 14.48 mmol) in methanol (100 mL) was added a solution of (1*R*,2*R*)-diaminocyclohexane dihydrochloride (1.35 g, 7.24 mmol) and sodium methoxide (0.78 g, 14.48 mmol) in methanol (100 mL) at room temperature. The resulting bright yellow solution was stirred under reflux for 2 h. Subsequently it was allowed to cool to room temperature and then evaporated in vacuo. The yellow residue was dissolved in dichloromethane (3×50 mL). The organic layers were washed with water (4×50 mL). The combined organic layers were dried ($MgSO_4$) and evaporated in vacuo to give a yellow gel. Crystallization from ethyl acetate and hexane at 4 °C overnight, gave an orange solid which was washed with hexane (2×10 mL) to obtain the desired compound (1.64 g 64%) as an orange solid. Mp 120–121 °C; $[\alpha]_D^{20}=-876$ (*c* 0.5, $CHCl_3$); ν_{max}($CHCl_3$) 3352 (br), 2937 (w), 2253 (w), and 1631 cm^{-1} (s); δ_H($CDCl_3$) 1.3–2.0 (4H, m, CH_2CH_2), 3.3–3.4 (1H, m, CHN), 6.56 (1H, t *J*=7.7 Hz, ArCH), 6.63 (1H, dd *J*=7.7, 1.6 Hz, ArCH), 6.85 (1H, dd *J*=7.7, 1.6 Hz, ArCH), 8.12 (1H, s, HC═N); δ_C($CDCl_3$) 24.5 (CH_2), 33.3

(CH_2), 71.3 (CHN), 117.2 (ArC), 117.0 (ArCH), 118.2 (ArCH), 122.7 (ArCH), 145.8 (ArC), 152.6 (ArC), 165.2 (HC═N); *m/z* (CI) 355 (MH^+, 3), 100 (15), 98 (100), 96 (20), 94 (12). Found (ES) 355.1653, $C_{20}H_{23}N_2O_4$ (MH^+) requires 355.1652.

4.2.10. (1*R*,2*R*)-[*N*,*N*-Bis-(2′,4′-dihydroxybenzylidene)]-1,2-diaminocyclohexane. To a stirred mixture of 2,4-dihydroxybenzaldehyde (2.0 g, 14.48 mmol) in methanol (100 mL) was added a solution of (1*R*,2*R*)-diaminocyclohexane dihydrochloride (1.35 g, 7.24 mmol) and sodium methoxide (0.78 g, 14.48 mmol) in methanol (100 mL) at room temperature. The resulting bright yellow solution was stirred under reflux for 2 h. The reaction mixture was cooled to room temperature and the yellow precipitate collected by filtration, washed with methanol (3×10 mL) and dried in vacuo to leave the desired compound (2.31 g, 90%) as a yellow solid. Mp >400 °C; $[\alpha]_D^{20}=-139$ (*c* 0.015, DMSO); ν_{max}(KBr) 2934 (w), 2524 (br), 1855 (br), and 1634 cm^{-1} (s); δ_H(DMSO-d_6) 1.4–1.9 (4H, m, CH_2CH_2), 3.28 (1H, m, CHN), 6.10 (1H, d *J*=2.1 Hz, ArCH), 6.21 (1H, dd *J*=8.5, 2.1 Hz, ArCH), 7.08 (1H, d *J*=8.5 Hz, ArCH), 8.27 (1H, s, HC═N), 9.99 (1H, br s, OH), 13.67 (1H, s, OH); δ_C(DMSO-d_6) 24.1 (CH_2), 33.1 (CH_2), 70.9 (CHN), 101.7 (ArCH), 107.2 (ArCH), 111.5 (ArC), 133.5 (ArCH), 161.9 (ArC), 164.1 (ArC), 164.4 (HC═N); *m/z* (CI) 355 (MH^+, 22), 235 (35), 189 (54), 136 (29), 115 (100). Found (ES) 355.1640, $C_{20}H_{23}N_2O_4$ (MH^+) requires 355.1652.

4.2.11. (1*R*,2*R*)-[*N*,*N*-Bis-(2′,5′-dihydroxybenzylidene)]-1,2-diaminocyclohexane.[41] To a stirred mixture of 2,5-dihydroxybenzaldehyde (2.0 g, 14.48 mmol) in methanol (100 mL) was added a solution of (1*R*,2*R*)-diaminocyclohexane dihydrochloride (1.35 g, 7.24 mmol) and sodium methoxide (0.78 g, 14.48 mmol) in methanol (100 mL) at room temperature. The resulting bright yellow solution was stirred under reflux for 2 h. Subsequently it was allowed to cool to room temperature and then evaporated in vacuo. The brown residue was dissolved in dichloromethane (3×50 mL) and filtered. The organic layers were washed with water (100 mL), dried ($MgSO_4$) and evaporated in vacuo to give a brown gel. The residue was crystallized from diethyl ether and hexane and the yellow precipitate was collected by filtration, washed with hexane (2×10 mL) and dried in vacuo to give the desired compound (795 mg, 31%) as a yellow solid. Mp 123–124 °C; $[\alpha]_D^{20}=-312$ (*c* 0.5, MeOH); ν_{max}(KBr) 3363 (br), 2935 (w), 1638 (s), and 1592 cm^{-1} (m); δ_H(DMSO-d_6) 1.4–1.9 (4H, m, CH_2CH_2), 6.6–6.7 (3H, m, ArCH), 8.94 (1H, s, HC═N), 9.20 (1H, s, OH), 12.46 (1H, s, OH); δ_C(CD_3OD) 25.7 (CH_2), 34.4 (CH_2), 74.1 (CHN), 120.2 (ArC), 118.1 (ArCH), 118.4 (ArCH), 121.5 (ArCH), 150.8 (ArC), 155.8 (ArC), 166.7 (HC═N); *m/z* (CI) 355 (MH^+, 81), 235 (100). Found (ES) 355.1645, $C_{20}H_{23}N_2O_4$ (MH^+) requires 355.1652.

4.3. General procedure for the preparation of copper(salen) complexes using copper bromide

To a solution of a chiral salen ligand (1.0 mmol) in methanol (5 mL) were added $CuBr_2$ (0.223 g, 1.0 mmol) and NaOMe (0.23 mL of a 4.6 N solution in MeOH). The resulting mixture was stirred for 3 h at room temperature and then the solvent was removed in vacuo. The crude residue was purified by gel permeation chromatography on LH-20 using EtOH/toluene (1:3) as eluent.

4.4. General procedure for the preparation of copper(salen) complexes using copper acetate

Solutions of the appropriate salen ligand (0.83 mmol) in ethanol (20 mL) and cuprous acetate monohydrate (0.17 g, 0.83 mmol) in water (2 mL) were mixed and refluxed under vigorous stirring for 2 h. After this time, the resulting solution was cooled to room temperature, filtered and the precipitate washed successively with water, methanol and diethyl ether (3×10 mL) to give the desired compound.

4.4.1. [(1*R*,2*R*)-[*N*,*N*-Bis-(2′-hydroxybenzylidene)]-1,2-diaminocyclohexanato]copper(II) 1b. Obtained as a purple solid in 82% yield using the copper acetate procedure. Mp >400 °C; $[\alpha]_D^{20}=-877$ (*c* 0.0219, $CHCl_3$); ν_{max}(KBr) 2931 (w), 1589 (s), and 1534 cm^{-1} (s); *m/z* (CI) 384 (29), 324 (34), 323 (100), 320 (32), 239 (28), 123 (28), 94 (47), 69 (27). Found (ES) 406.0729, $C_{20}H_{20}N_2O_2CuNa$ ($MH+Na^+$) requires 406.0713.

4.4.2. [(1*R*,2*R*)-[*N*,*N*′-Bis-(2′-hydroxy-5′-*tert*-butylbenzylidene)]-1,2-diaminocyclohexanato]copper(II) 4a. Obtained as a brown solid in a yield of 87% using the copper bromide procedure. Mp >270 °C; $[\alpha]_D^{20}=-604$ (*c* 0.013, $CHCl_3$); ν_{max}($CHCl_3$) 3018 (w), 2951 (s), 2861 (m), 1620 (s), 1525 (s), and 1470 cm^{-1} (s); *m/z* (ES) 496 (MH^+, 100), 331 (49). Found (ES) 496.2183, $C_{28}H_{37}N_2O_2Cu$ (MH^+) requires 496.2151.

4.4.3. [(1*R*,2*R*)-[*N*,*N*′-Bis-(2′-hydroxy-5′-methoxybenzylidene)]-1,2-diaminocyclohexanato]copper(II) 4b. Obtained as a brown solid in 79% yield using the copper bromide procedure. Mp >250 °C; $[\alpha]_D^{20}=-600$ (*c* 0.005, $CHCl_3$); ν_{max}($CHCl_3$) 2931 (m), 1634 (s), 1614 (m), 1538 (m), and 1468 cm^{-1} (s); *m/z* (CI) 444 (MH^+, 19), 383 (100), 232 (24), 151 (57). Found (ES) 444.1112, $C_{22}H_{25}N_2O_4Cu$ (MH^+) requires 444.1110.

4.4.4. [(1*R*,2*R*)-[*N*,*N*′-Bis-(2′-hydroxy-5′-nitro-benzylidene)]-1,2-diaminocyclohexanato]copper(II) 4c. Obtained using the copper bromide procedure without chromatography as an insoluble solid which was used without further purification. ν_{max}(KBr) 3062 (w), 2940 (w), 2862 (w), 1633 (m), 1601 (s), 1550 (m), and 1494 cm^{-1} (m).

4.4.5. [(1*R*,2*R*)-[*N*,*N*′-Bis-(2′-hydroxy-5′-fluoro-benzylidene)]-1,2-diaminocyclohexanato]copper(II) 4d. Obtained as a brown solid in a yield of 26% using the copper bromide procedure. Mp >270 °C; $[\alpha]_D^{20}=-640$ (*c* 0.025, $CHCl_3$); ν_{max}(KBr) 2942 (w), 1632 (s), 1538 (m), and 1462 cm^{-1} (s); *m/z* (ES) 420 (MH^+, 100), 140 (7). Found (ES) 420.0700, $C_{20}H_{19}N_2O_2F_2Cu$ (MH^+) requires 420.0711.

4.4.6. [(1*R*,2*R*)-[*N*,*N*′-Bis-(2′-hydroxy-5′-trifluoromethylbenzylidene)]-1,2-diaminocyclohexanato]copper(II) 4e. Obtained as a purple solid in a yield of 30% using the copper bromide procedure. Mp >270 °C. $[\alpha]_D^{20}=-352$ (*c* 0.013, $CHCl_3$); ν_{max}(KBr) 2936 (w), 2861 (w), 1633 (s), 1620 (s), and 1542 cm^{-1} (m); *m/z* (FAB) 520 (M^+, 48), 307 (19), 289 (12), 154 (100), 136 (69), 107 (27), 77 (33).

Found: C, 50.80%; H, 3.77%; N, 5.14%; $C_{22}H_{18}N_2O_2F_6Cu$ requires C, 50.82%; H, 3.49%; N, 5.39%.

4.4.7. [(1*R*,2*R*)-[*N*,*N*-Bis-(2′3′-dihydroxy-benzylidene)]-1,2-diaminocyclohexanato]copper(II) 8a. Obtained as a brown solid in 67% yield using the copper bromide procedure. Mp >400 °C; $[\alpha]_D^{20}=-1096$ (*c* 0.016, $CHCl_3$); ν_{max}(KBr) 3394 (br), 2933 (m), 2859 (w), 1626 (s), and 1551 cm^{-1} (m); *m*/*z* (ES) 438 (M+Na^+, 100), 281 (20), 227 (22), 179 (32). Found (ES) 416.0779, $C_{20}H_{21}N_2O_4Cu$ (MH^+) requires 416.0797. Found (ES) 438.0601, $C_{20}H_{20}N_2O_4CuNa$ (M+Na^+) requires 438.0611.

4.4.8. [(1*R*,2*R*)-[*N*,*N*-Bis-(2′4′-dihydroxy-benzylidene)]-1,2-diaminocyclohexanato]copper(II) 8b. Obtained as a brown solid in 83% yield using the copper acetate procedure. Mp >400 °C; $[\alpha]_D^{20}=-958$ (*c* 0.009, DMSO); ν_{max}(KBr) 3109 (br) 2934 (w), 1621 (s), and 1542 cm^{-1} (s); *m*/*z* (ES) 438 (M+Na^+, 100), 416 (MH^+, 63), 415 (86), 178 (17). Found (ES) 416.0783, $C_{20}H_{21}N_2O_4Cu$ (MH^+) requires 416.0791. Found (ES) 438.0601, $C_{20}H_{20}N_2O_4CuNa$ (M+Na^+) requires 438.0611.

4.4.9. [(1*R*,2*R*)-[*N*,*N*-Bis-(2′5′-dihydroxy-benzylidene)]-1,2-diaminocyclohexanato]copper(II) 8c. Obtained as a green/brown solid in 74% yield using the copper acetate procedure. Mp >400 °C; $[\alpha]_D^{20}=-957$ (*c* 0.011, $CHCl_3$); ν_{max}(KBr) 3375 (w), 2934 (w), 1621 (s), and 1542 cm^{-1} (s); *m*/*z* (ES) 416 (MH^+, 100). Found (ES) 416.0797, $C_{22}H_{21}N_2O_4Cu$ (MH^+) requires 416.0791.

4.4.10. [(1*R*,2*R*)-[*N*,*N*-Bis-(2′-hydroxy-3′-methoxy-benzylidene)]-1,2-diaminocyclohexanato]copper(II) 8d. Obtained as a red/brown solid in 94% yield using the copper bromide procedure. Mp 289–290 °C; $[\alpha]_D^{20}=-606$ (*c* 0.032, $CHCl_3$); ν_{max}(KBr) 3504 (br), 2932 (m), 1627 (s), and 1545 cm^{-1} (m); *m*/*z* (ES) 466 (M+Na^+, 100), 413 (10), 195 (30). Found (ES) 466.0990, $C_{22}H_{24}N_2O_4CuNa$ (M+Na^+) requires 466.0924.

4.4.11. [(1*R*,2*R*)-[*N*,*N*-Bis-(2′-hydroxy-4′-methoxy-benzylidene)]-1,2-diaminocyclohexanato]copper(II) 8e. Obtained as a brown solid in 75% yield using the copper bromide procedure and as a dark purple solid in 64% yield using the copper acetate procedure. Data for the product obtained using copper bromide. Mp >400 °C; $[\alpha]_D^{20}=-529$ (*c* 0.035, $CHCl_3$); ν_{max}(KBr) 3434 (br), 2933 (m), 1626 (s), and 1551 cm^{-1} (s); *m*/*z* (CI); 444 (MH^+, 52), 383 (98), 139 (100), 125 (41), 98 (96). Found (ES) 444. 1110, $C_{22}H_{25}N_2O_4Cu$ (MH^+) requires 444.1105. Data for the product obtained using copper acetate. Mp >400 °C; $[\alpha]_D^{20}=-968$ (*c* 0.022, $CHCl_3$); ν_{max}(KBr) 2933 (w), 1605 (s), and 1530 cm^{-1} (s); *m*/*z* (ES) 444 (MH^+, 100). Found (ES) 444. 1100, $C_{22}H_{25}N_2O_4Cu$ (MH^+) requires 444.1105.

4.4.12. [(1*R*,2*R*)-[*N*,*N*-Bis-(2′-hydroxy-6′-methoxy-benzylidene)]-1,2-diaminocyclohexanato]copper(II) 8f. Obtained as a brown solid in 79% yield using the copper acetate procedure. Mp 315–316 °C; $[\alpha]_D^{20}=-1074$ (*c* 0.005, $CHCl_3$); ν_{max}(KBr) 3424 (br), 3019 (m), 1637 (s), and 1544 cm^{-1} (m);. *m*/*z* (CI) 444 (MH^+, 20), 383 (67), 323 (17), 145 (18). Found (ES) 466. 0938, $C_{22}H_{24}N_2O_4CuNa$ (M+Na^+) requires 466.0924.

4.4.13. [(1*R*,2*R*)-[*N*,*N*-Bis-(2′-hydroxy-4′-methyl-benzylidene)]-1,2-diaminocyclohexanato]copper(II) 8g. Obtained as a brown solid in 64% yield using the copper acetate procedure. Mp 195–196 °C; $[\alpha]_D^{20}=-704$ (*c* 0.0135, $CHCl_3$); ν_{max}(KBr) 3406 (br), 2927 (m), 1609 (s), and 1527 cm^{-1} (m); *m*/*z* (ES) 412 (MH^+, 100). Found (ES) 412. 1200, $C_{22}H_{25}N_2O_4Cu$ (MH^+) requires 412.1207.

4.5. General procedure for the preparation of alanine Schiff bases

To a stirred suspension of alanine methyl ester hydrochloride (1.20 g, 7.20 mmol) in dichloromethane (10 mL), triethylamine (1.00 mL, 7.20 mmol), the appropriate aldehyde (7.20 mmol) and a small amount of $MgSO_4$ were added. The reaction mixture was stirred overnight at room temperature after which it was filtered and the solvent removed in vacuo. The residue was the taken up in diethyl ether (10 mL) and washed with Na_2CO_3(aq) (7×5 mL). The combined organic phases were then dried over $MgSO_4$ and evaporated to dryness to leave the desired product.

4.5.1. *para*-Nitrobenzylidene imine 7a. Obtained as an orange oil in 85% yield. $[\alpha]_D^{20}=+0.8$ (*c* 1.0, $CHCl_3$); ν_{max}(neat) 3107 (m), 2951 (m), 2854 (m), 1732 (s), 1601 (s), and 1518 cm^{-1} (s); δ_H($CDCl_3$) 1.58 (3H, d *J*=7 Hz, CH_3), 3.79 (3H, s, OCH_3), 4.25 (1H, q *J*=7 Hz, NCH), 7.97 (2H, d *J*=9 Hz, ArCH), 8.28 (2H, d *J*=9 Hz, ArCH), 8.44 (1H, s, HC=N); δ_C($CDCl_3$) 15.9 (CH_3), 52.6 (OCH_3), 68.5 (NCH), 124.4 (ArCH), 129.6 (ArCH), 141.8 (ArC), 149.8 (ArC), 161.4 (HC=N), 173.0 (CO_2); *m*/*z* (ES) 237 (MH^+, 50), 207 (100). Found (ES) 237.0873, $C_{11}H_{13}N_2O_4$ (MH^+) requires 237.0875.

4.5.2. *para*-Methoxybenzylidene imine[42] 7b. Obtained as a pale yellow oil in 78% yield. δ_H($CDCl_3$) 1.44 (3H, d *J*=7 Hz, CH_3), 3.67 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 4.02 (1H, q *J*=7 Hz, NCH), 6.84 (2H, d *J*=9 Hz ArCH), 7.64 (2H, d *J*=9 Hz, ArCH), 8.44 (1H, s, HC=N).

4.5.3. *para*-Chlorobenzylidene imine[43] 7c. Obtained as a yellow oil in 61% yield. δ_H($CDCl_3$) 1.47 (3H, d *J*=7 Hz, CH_3), 3.70 (3H, s, OCH_3), 4.11 (1H, q *J*=7 Hz, NCH), 7.45 (2H, d *J*=9 Hz, ArCH), 7.76 (2H, d *J*=9 Hz, ArCH), 8.22 (1H, s, HC=N).

4.5.4. *meta*-Chlorobenzylidene imine 7d. Obtained as a yellow oil in 68% yield. $[\alpha]_D^{20}=+0.6$ (*c* 1.0, $CHCl_3$); ν_{max} (neat) 3064 (w), 2984 (m), 2953 (m), 2871 (m), 1740 (s), 1645 (s), and 1570 cm^{-1} (s); δ_H($CDCl_3$) 1.42 (3H, d *J*=7 Hz, CH_3), 3.64 (3H, s, OCH_3), 4.04 (1H, q *J*=7 Hz, NCH), 7.21–7.31 (2H, m, ArCH), 7.49 (1H, m, ArCH), 7.71 (1H, s, ArCH), 8.16 (1H, s, HC=N); δ_C($CDCl_3$) 19.4 (CH_3), 52.0 (OCH_3), 67.7 (CH), 127.0 (ArCH), 128.0 (ArCH), 129.9 (ArCH), 131.1 (ArC), 134.8 (ArC), 137.5 (ArCH), 159.1 (HC=N), 172.7 (CO_2); *m*/*z* (CI) 228 (M(^{37}Cl)H^+, 30), 226 (M(^{35}Cl)H^+, 100), 166 (18). Found (EI) 225.0555, $C_{11}H_{12}NO_2^{35}Cl$ (M^+) requires 225.0557.

4.5.5. *ortho*-Chlorobenzylidene imine 7e. Obtained as a yellow oil in 67% yield. $[\alpha]_D^{20}=-0.1$ (*c* 1.0 $CHCl_3$); ν_{max}(neat) 3067 (w), 2986 (m), 2950 (m), 2890 (m), 1743 (s), 1636 (s), and 1592 cm^{-1} (m); δ_H($CDCl_3$) 1.45 (3H, d

J=7 Hz, CH_3), 3.67 (3H, s, OCH_3), 4.12 (1H, q J=7 Hz, NCH), 7.13–7.37 (3H, m, ArCH), 8.00 (1H, d J 7 Hz, ArCH), 8.66 (1H, s, HC=N); $\delta_C(CDCl_3)$ 18.4 (CH_3), 51.1 (OCH_3), 67.9 (NCH), 126.3 (ArCH), 127.4 (ArCH), 128.6 (ArCH), 130.6 (ArCH), 131.7 (ArC), 134.0 (ArC), 158.0 (HC=N), 171.6 (CO_2); *m/z* (CI) 226 (MH^+, 100). Found (ES) 226.0630, $C_{11}H_{13}NO_2^{35}Cl$ (MH^+) requires 226.0635.

4.5.6. *para*-Fluorobenzylidene imine 7f. Obtained as a yellow oil in 68% yield. $[\alpha]_D^{20}$=+26.1 (*c* 1.0, $CHCl_3$); ν_{max}(neat) 2986 (m), 2952 (m), 2872 (m), 1740 (s), 1644 (s), and 1501 cm^{-1} (s); $\delta_H(CDCl_3)$ 1.42 (3H, d J=7 Hz, CH_3), 3.64 (3H, s, OCH_3), 4.03 (1H, q J=7 Hz, NCH), 6.97 (2H, t J=9 Hz, ArCH), 7.7–7.8 (2H, m, ArCH), 8.18 (1H, s, HC=N); $\delta_C(CDCl_3)$ 20.0 (CH_3), 52.7 (OCH_3), 68.4 (CHN), 116.1 (ArCH), 116.3 (ArC), 131.0 (ArCH), 162.1 (HC=N), 166.3 (ArCF), 173.5 (CO_2); *m/z* (CI) 210 (MH^+, 100), 150 (20). Found (ES) 210.0927, $C_{11}H_{13}NO_2F$ (MH^+) requires 210.0930.

4.5.7. *para*-Bromobenzylidene imine[43,44] 7g. Obtained as a yellow oil in 74% yield. $\delta_H(CDCl_3)$ 1.42 (3H, d J=7 Hz, CH_3), 3.65 (3H, s, OCH_3), 4.04 (1H, q J=7 Hz, NCH), 7.43 (2H, d J=8.5 Hz, ArCH), 7.54 (2H, d J=8.5 Hz, ArCH), 8.16 (1H, s, HC=N).

4.5.8. *para*-Iodobenzylidene imine 7h. Obtained as a yellow oil in 72% yield from a reaction carried out following the general procedure with exclusion of light. $[\alpha]_D^{20}$=−0.1 (*c* 1.0, $CHCl_3$); ν_{max}(neat) 2983 (m), 2984 (m), 2870 (m), 1740 (s), 1642 (s), and 1585 cm^{-1} (s); $\delta_H(CDCl_3)$ 1.41 (3H, d J=7 Hz, CH_3), 3.64 (3H, s, OCH_3), 4.11 (1H, q J=7 Hz, NCH), 7.45 (2H, d J=9 Hz, ArCH), 7.76 (2H, d J=9 Hz, ArCH), 8.13 (1H, s, HC=N); $\delta_C(CDCl_3)$ 15.7 (CH_3), 52.7 (OCH_3), 68.3 (NCH), 98.3 (ArC), 130.4 (ArCH), 135.5 (ArCI), 137.9 (ArCH), 162.3 (HC=N), 173.1 (CO_2); *m/z* (CI) 318 (MH^+, 60), 192 (100). Found (EI) 316.9916 $C_{11}H_{12}NO_2I$ (M^+) requires 316.9913.

4.5.9. 1-Naphthylidene imine 7i. Obtained as a pale yellow oil in 90% yield. $[\alpha]_D^{20}$=+0.8 (*c* 1.0 $CHCl_3$); ν_{max}(neat) 3048 (m), 2984 (m), 2871 (m), 1740 (s), 1636 (s), 1590 (s), and 1509 cm^{-1} (s); $\delta_H(CDCl_3)$ 1.49 (3H, d J=7 Hz, CH_3), 3.64 (3H, s, OCH_3), 4.09 (1H, q J=7 Hz, NCH), 7.35–7.81 (7H, m, ArCH), 8.82 (1H, s, HC=N); $\delta_C(CDCl_3)$ 20.1 (CH_3), 52.7 (OCH_3), 69.3 (NCH), 124.7 (ArCH), 125.6 (ArCH), 126.2 (ArC), 127.4 (ArCH), 128.9 (ArC), 129.1 (ArCH), 129.7 (ArCH), 131.7 (ArCH), 135.7 (ArC), 161.4 (HC=N), 173.0 (CO_2); *m/z* (EI) 241 (M^+, 25), 182 (100), 166 (40), 154 (95), 139 (60), 127 (50). Found (ES) 242.1181, $C_{15}H_{16}NO_2$ (MH^+) requires 242.1181.

4.5.10. 2-Naphthylidene imine[45] 7j. Obtained as a yellow solid in 72% yield. $\delta_H(CDCl_3)$ 1.69 (3H, d J=7 Hz, CH_3), 3.89 (3H, s, OCH_3), 4.32 (1H, q J=7 Hz, NCH), 7.6–7.7 (2H, m, ArCH) 8.0–8.2 (5H, m, ArCH), 8.59 (1H, s, HC=N).

4.6. General procedure for the benzylation of *N*-arylidene (*S*)-alanine methyl esters

Imine **2b** or **7a–j** (0.88 mmol), powered sodium hydroxide (0.146 g, 3.66 mmol), catalyst **1b**,**c**, **4a–e**, or **8a–f** (2 mol%), dry toluene (2.5 mL) and benzyl bromide (0.126 mL, 1.06 mmol) were added to round bottomed flask under argon. The mixture was allowed to stir overnight at room temperature. MeOH (2 mL) and acetyl chloride (0.44 mL) were then added, and the reaction stirred for a further 4 h at room temperature under argon. The solvent was removed in vacuo and the residue was added to a silica gel column and eluted first with ethyl acetate (3×100 mL) and then with a mixture of ethyl acetate and ethanol (4:1) to give α-methyl phenylalanine methyl ester. If necessary, the amino ester was filtered through aluminium oxide to remove the last traces of copper salts.

4.7. Determination of the enantiomeric excess of α-methyl phenylalanine methyl ester

(*S*)-1-Phenylethyl isocyanate (1–2 drops, excess) was added to an NMR sample (in $CDCl_3$) of α-methyl phenylalanine methyl ester. The solution was left until the reaction was complete (usually overnight). The diastereomeric excess of the urea and hence the enantiomeric excess of the α-methyl phenylalanine methyl ester was determined by integration of the methylene proton region of the ^1H NMR spectrum of the urea.

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A convenient one-pot synthesis of substituted 2-pyrone derivatives

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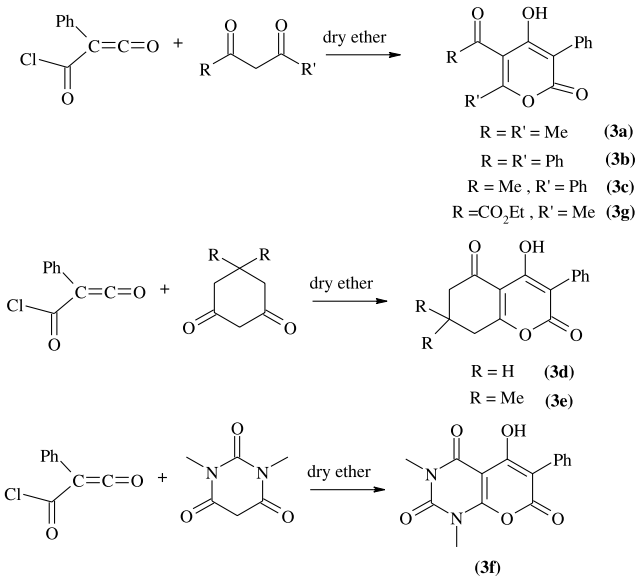
Abstract—2-Pyrone derivatives were prepared in a one step procedure from readily available (chlorocarbonyl)phenyl ketene and 1,3-diketones such as 2,4-pentanedione, 1,3-diphenyl-1,3-propanedione, 1-phenyl-1,3-butanedione, 1,3-cyclohexanedione, 5,5-dimethyl-1,3-cyclohexanedione, 1,3-dimethyl-pyrimidine-2,4,6-trione and ethyl 2,4-dioxopentanoate. A mechanism is presented to account for the formation of the products. This method provides an easy route to prepare 3,4,5,6-tetrasubstituted 2-pyrones in good to excellent yields and in a short experimental time.

1. Introduction

There are numerous reports in the literature on the synthesis of substituted 2-pyrone derivatives either by traditional approaches or by a process involving transition metal catalyzed reaction.[1–3] Furthermore, the synthesis of such compounds and their fused derivatives has been subject to several reviews which show high importance of this class of compounds.[4,5] Also interested are the 2-pyrone themselves, as some of the 2-pyrone with hydroxy, alkenyl, aryl and alkyl groups at 4 position were reported to be biologically active[6–8] and simple change in the substitution pattern on the 2-pyrone ring often leads to incredible diverse biological activity.[9] Although 2-pyrones have some aromatic character, they undergo Diels–Alder [4+2] cycloaddition reaction with alkynes and alkenes under suitable reaction conditions.[10–13] The synthesis of 2-pyrones substituted in the 4, 5 and 6 positions by the reaction of enamines with excess ketene has been reported in yields ranging from 25 to 50%.[14] 5-Ethoxycarbonyl-4-hydroxy-6-methyl-2-pyrones also were prepared in 64% yield by treatment of ethyl acetoacetate with malonyl chloride in toluene at reflux. The by-product of self-condensation of ethyl acetoacetate was separated by extraction, and attempts were made to optimize the pyrone preparation without any improvement.[15] This compound was also prepared in 66% yield upon treatment of the *t*-butyldiphenylsilyl enol ether of methyl acetoacetate and malonyl dichloride with $ZnBr_2$ followed by concentrated H_2SO_4.[16] Mild conversion of substituted 2-pyrones to 4-pyrones has been used in the synthesis of phenoxan, a naturally occurring compound with anti-HIV activity.[17,18]

2. Results and discussion

We have reported the reaction of (chlorocarbonyl)phenyl ketene **1** with carbonyl compounds to produce meldrumacid derivatives.[19,20] In this paper, we describe an investigation of the cycloaddition of **1** to 1,3-diketones to prepare 2-pyrone derivatives in a one step procedure in good to excellent yields (Scheme 1).



Compound	Yield (%)	Compound	Yield (%)
3a	82	**3e**	88
3b	92	**3f**	90
3c	85	**3g**	70
3d	90		

Scheme 1.

Keywords: 2-Pyrone; 1,3-Diketones; (Chlorocarbonyl)phenyl ketene.
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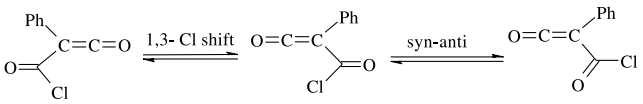
The cycloaddition of this isolable and stable ketene with 1,3-diketones proceeds by a simple and one step procedure. Compounds **3a**–**g** were prepared in 82, 92, 85, 90, 88, 90, and 70% yields, respectively.

Surprisingly, such an investigation has not been previously reported to prepare 2-pyrones from such a readily available and inexpensive starting material. It is pertinent to note that **1** undergoes a degenerate 1,3-shift of chlorine, as determined by ^{13}C NMR spectroscopy (Scheme 2).[21]

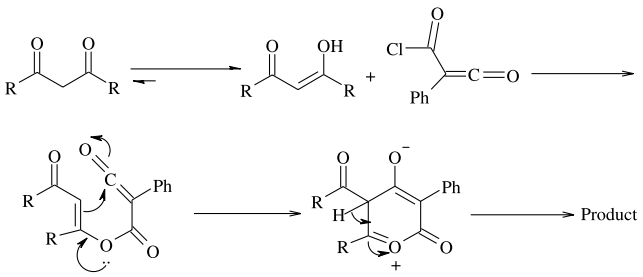


Scheme 2.

The chlorine atom can exchange between two carbonyl groups and also these ketenes can exist as a mixture of two conformers: *s-trans* and *s-cis*.[22] Thus the cycloaddition represented in scheme **1** accomplished by mixing the equimolar quantities of (chlorocarbonyl)phenyl ketene[23] and 1,3-diketones at ambient temperature in dry ether.

1,3-Diketones exist mainly in the enol forms, the influence of two carbonyl groups on the enol content is very striking, as we can see from the fact that 76% of 2,4-pentanedione is the enol form at equilibrium.[24] Therefore, the OH group of the enol form will attack the acyl chloride of the ketene followed by ring closure to produce the product.

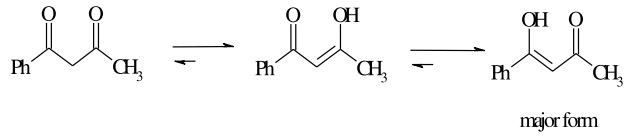
The reaction resulted in the formation of **3a** after 6 h. A plausible mechanism is shown below (Scheme 3).



Scheme 3.

Compound **3a** shows carbonyl absorption at 1741 and 1668 cm^{-1} in the IR spectrum. The ^1H NMR spectrum of **3a** indicated four kinds of proton signals with one signal quite downfield (δ 12.63 ppm) which is the proton of enol OH. This assignment is consistent with literature precedents for closely related products. The ^{13}C NMR and mass spectra are also in accordance with the proposed structure. In general all the spectral data support the structures for the compounds **3**(**a**–**g**). In the case of **3b**, the product formed instantly and it precipitates out within a second as a yellow solid.

It should be added that, β-ketoester such as ethyl acetoacetate have enol content in the range of 8% and diacetyl contain a little over 1% enol form. Attempts to bring β-ketoester in to reaction with **1** was mostly unsuccessful. Unsymmetrical 1,3-diketones such as 1-phenyl-1,3-butanedione and ethyl-2,4-dioxopentanoate have hydrogens between two different carbonyl groups (Scheme 4).



Scheme 4.

Based on the ^1H NMR and ^{13}C NMR only one product was obtained from the reaction of these unsymmetrical, 1,3-diketones and compound (**1**); this is indicative of formation of only one of these enol forms. The occurrence of a significant amount of one of the enol requires the presence of a group or groups capable of stabilizing the enol by delocalization of the π-electrons of the carbon–carbon double bond. In addition to the carbonyl group the double bond is conjugated with a phenyl ring.

1,2-Diketones do not contain more than 1% of the enol forms, therefore not enough to carry out the experiment of these forms with compound **1**.

3. Conclusions

In conclusion, we have shown that the condensation reaction of (chlorocarbonyl)phenyl ketene with 1,3-diketones, occurs efficiently in ether, providing a convenient and rapid synthesis of 2-pyrone derivatives in high yield, by a simple procedure and short experimental time. Furthermore, the products are solid and precipitate out from the reaction mixture and their purifications are simple.

4. Experimental

4.1. General

2,4-Pentanedione 1,3-diphenyl-1,3-propanedione, 1-phenyl-1,3-butanedione, ethyl 2,4-dioxopentanoate, 1,3-cyclohexanedione, 5,5-dimethyl-1,3-cyclohexanedione, and 1,3-dimethyl-pyrimidine-2,4,6-trione were obtained from Merck Chemical Co. and were used without further purification. Melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. IR spectra were measured on a Mattson 1000 FT-IR spectrometer. The proton and carbon-13 NMR spectra were recorded with a BRUKER DRX-500 AVANCE spectrometer at 500 and 125.77 MHz, respectively. Mass spectra were recorded on a MS-QP2000A Shimadzu mass spectrometer operating at an ionization potential of 70 eV. Elemental analyses were performed by National Iranian Oil Company lab (Tehran) using a Heracus CHN-O-Rapid analyzer.

4.1.1. 5-Acetyl-4-hydroxy-6-methyl-3-phenyl-2*H*-2-pyranone 3a. General procedure. (Chlorocarbonyl)phenyl ketene (0.36 g, 2 mmol) was added to 2,4-pentanedione (0.20 g, 2 mmol) in dry diethyl ether (20 ml) with stirring at ambient temperature for 6 h. The solid product was collected and recrystallized from benzene. 0.40 g. White crystals, 82% yield, mp 120–122 °C. MS, *m/z* (relative

intensity %): 244 (83 parent peak), 216 (100) base peak, 198 (44), 118 (59), 85 (52), 43 (98). IR (KBr): 1741, 1668 cm^{-1}. ^1H NMR ($CDCl_3$): δ 12.63 (1H, s, OH), 7.44–7.27 (5H, m, arom), 2.53 (3H, s, methyl protons), 2.46 (3H, s, acetyl protons). ^{13}C NMR ($CDCl_3$): δ 203.38 and 171.98 (2C═O), 165.49, 161.06 and 130.53 (3C), 130.43, 128.04 and 127.73 (3CH), 111.07 and 103.10 (2C), 32.77 and 23.09 ($2CH_3$). Anal. calcd for $C_{14}H_{12}O_4$: C, 68.83; H, 4.92%. Found: C, 68.80; H. 5.00%.

4.1.2. 5-Benzoyl-4-hydroxy-3,6-diphenyl-2*H*-2-pyranone 3b. 0.68 g. Yellow crystals, yield 92%, mp 173–175 °C. MS, *m/z* (%) 368 (38, parent peak), 340 (27), 105 (100, base peak), 77 (58). IR (KBr): 1741, 1666 cm^{-1}. ^1H NMR ($CDCl_3$): δ 10.16 (1H, s, OH), 7.62–7.17 (15H, m, arom). ^{13}C NMR: δ ($CDCl_3$) 197.39 and 166.67 (2C═O), 162.77, 161.11 and 136.77 (3C), 133.05 and 131.49 (2CH), 131.01 (C), 129.91 (CH), 129.71 (C), 129.58, 129.22 127.93, 127.92, 127.85 and 127.70 (6CH), 108.57and 104.01 (2C). Anal. calcd for $C_{24}H_{16}O_4$: C, 78.26; H, 4.34% Found; C, 78.00; H, 4.50%.

4.1.3. 5-Acetyl-4-hydroxy-3,6-diphenyl-2*H*-2-pyranone 3c. 0.62 g. Yellow crystals, yield 85%, mp 158–160 °C. MS, *m/z* (%): 306 (71, parent peak), 278 (54), 200 (31), 105 (100, parent peak), 77 (71), 43 (54), IR (KBr): 1732, 1658 cm^{-1}. ^1H NMR ($CDCl_3$): δ 11.90 (1H, s, OH), 7.66–7.27 (10H, m, arom), 2.01 (3H, s, acetyl proton). ^{13}CNMR ($CDCl_3$): δ 204.67 and 170.16 (2C═O), 163.98 and 160.94 (2C), 132.71 (CH), 132.36 and 130.35 (2C), 130.31, 129.58, 129.14, 128.02 and 127.83 (5CH), 110.58 and 103.94 (2C), 31.41 (CH_3). Anal. calcd for $C_{19}H_{14}O_4$: C, 74.5; H, 4.60%. Found; C, 74.4; H, 4.7%.

4.1.4. 4-Hydroxy-1-3-phenyl-7,8-dihydro-6*H*-chromene-2,5-dione 3d. 0.46 g. White crystals, yield 90%, mp 129–131 °C. MS, *m/z*: 256 (parent peak). IR (KBr): 1745, 1670 cm^{-1}. ^1H NMR ($CDCl_3$): δ 12.26 (1H, s, OH), 7.52–7.30 (5H, m, arom), 2.87 (2H, t, $^3J_{HH}$=5.75 Hz, CH_2), 2.61 (2H, t, $^3J_{HH}$=6.01 Hz, CH_2), 2.12 (2H, m, CH_2). ^{13}C NMR ($CDCl_3$): δ 202.12 and 176.07 (2C═O), 163.72 and 160.68 (2C), 129.80 (CH), 129.59 (C), 127.52 and 127.31 (2CH), 106.67 and 106.85 (C), 36.12, 27.85 and 19.12 ($3CH_2$). Anal. calcd for $C_{15}H_{12}O_4$: C, 70.30; H, 4.69%. Found; C, 69.80; H, 4.80%.

4.1.5. 4-Hydroxy-7,7-dimethyl-3-phenyl-7,8-dihydro-6*H*-chromene-2,5-dione 3e. 0.50 g. White crystals, yield 88%, mp 201–203 °C. MS, *m/z*: 284 (parent peak). IR (KBr): 1740, 1670 cm^{-1}. ^1H NMR: δ 12.13 (1H, s, OH), 7.55–7.30 (5H, m, arom), 2.78 (2H, s, CH_2), 2.52 (2H, s, CH_2) 1.20 (6H, s, $2CH_3$). ^{13}C NMR ($CDCl_3$): δ 2 01.72 and 174.61 (2C═O), 163.42, 160.94 (2C), 129.80 (CH), 129.53 (C), 127.53 and 127.33 (2CH), 105.75 and 102.80 (2C), 49.98 and 41.42 ($2CH_2$), 27.67 ($2CH_3$). Anal. calcd for $C_{17}H_{16}O_4$: C, 71.80; H, 5.63%. Found: C, 71.50; H, 5.70%.

4.1.6. 5-Hydroxy-1,3-dimethyl-6-phenyl-1*H*-pyrano[2,3-*d*]pyrimidine-2,4,7(3*H*)-trione 3f. 0.54 g. White crystals, yield 90%, mp 200–201 °C. MS, *m/z* (%): 300 (100, base peak and parent peak), 272 (88), 215 (47), 82 (45), 58 (5.2). IR (KBr), 1759, 1726, 1681 cm^{-1}. ^1H NMR ($CDCl_3$): δ 12.26 (1H, s, OH), 7.53–7.32 (5H, m, arom), 3.60 (3H, s, CH_3), 3.50 (3H, s, CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ 164.87, 163.68 and 158.16 (3C═O), 157.52 and 148.49 (2C), 130.19 (CH), 129.78 (C), 128.01 and 127.70 (2CH), 97.76 and 85.59 (2C), 29.54 and 28.08 ($2CH_3$). Anal. calcd for $C_{15}H_{12}N_2O_5$: C, 60.0; H, 4.0; N, 9.33%. Found: C, 60.0; H, 4.2; N, 9.3%.

4.1.7. Ethyl-2-(4-hydroxy-2-methyl-6-oxo-5-phenyl-6*H*-pyran-3-yl)-2-oxo-acetate 3g. 0.42 g. White crystals, yield 70%, mp 140.0 °C. MS, *m/z* (%): 302, (parent peak), IR (KBr), 1765, 1725, 1691 cm^{-1}. ^1H NMR: δ ($CDCl_3$) 9.67 (1H, s, OH), 7.46–7.27 (5H, m, arom), 4.42 (2H, q, $^3J_{HH}$=7.0 Hz, CH_2), 2.57 (3H, s, CH_3), 1.43 (3H, t, $^3J_{HH}$=7.0 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 187.75, 173.30 and 162.74 (3C═O), 162.60 and 160.36 (2C), 130.16 (CH), 128.87 (C), 128.70 and 128.55 (2CH), 107.77 and 104.09 (2C), 63.31 (OCH_2), 20.24 and 13.78 ($2CH_3$). Anal. calcd for $C_{16}H_{14}O_6$: C, 63.6; H, 4.6%. Found: C, 63.2; H, 4.8%.

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Hydrogen-bonded dimer can mediate supramolecular β-sheet formation and subsequent amyloid-like fibril formation: a model study

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Abstract—FT-IR data of six terminally blocked tripeptides containing Acp (ε-aminocaproic acid) reveals that all of them form supramolecular β-sheets in the solid state. Single crystal X-ray diffraction studies of two peptides not only support this data but also disclose the fact that the supramolecular β-sheet formation is initiated via dimer formation. The Scanning Electron Microscopic images of all peptides exhibit amyloid-like fibrils that show green birefringence after binding with Congo red, which is a characteristic feature of many neurodegenerative disease causing amyloid fibrils.

1. Introduction

The design of short model compounds that can form well-defined dimers and self-associate via non-covalent interactions to form supramolecular β-sheet structures is a highly emerging area of recent research.[1] Dimer systems, preferably those which give β-sheet structures, are important as a model for studying β-sheet interactions in proteins.[2] β-sheet formation between peptides is generally associated with the formation of insoluble aggregates of ill-defined structure. Dimer-mediated supramolecular β-sheet formation is particularly important as this mimics many neurotoxic amyloid sequences that self-assemble to form the β-sheet structure via dimerization.[3]

The molecular basis of fatal, neurodegenerative diseases like prion protein diseases,[4] Alzheimer's disease[2e,5] and other related diseases[6] involves an intermolecular β-sheet aggregation and subsequent formation of highly ordered fibrillar structures. Though it is well accepted that the amyloid fibrils consist of majorly β-sheet structure, the structure-function relationship of amyloidosis is still not clear. Moreover, some recent results demonstrate that not the matured fibril but the intermediates are potent neurotoxic agents in Alzheimer's disease.[7] So, deciphering the pathway(s) of fibril formation and neurotoxicity-associated with quaternary β-sheet assemblage is extremely significant for the developments of therapeutics for these diseases. All previous reports for establishing the pathway(s) of the quaternary structure formation of amyloid fibrils are based on methods, such as, CD spectropolarimetry, ANS binding fluorescence, different types of electron microscopic studies (SEM, TEM), Atomic Force Microscopy, and X-ray fiber-diffraction studies,[8] which are unable to elucidate the details of the quaternary structure and pathway(s) of fibril formation at the atomic level, unlike single crystal X-ray diffraction studies. It is, however, extremely difficult to get a crystal structure of a real amyloidogenic protein or a protein fragment due to its very low solubility and noncrystallinity. Although there are an increasing number of reports concerning the organization of β-sheets in Alzheimer's β-amyloid fibrils by solid-state NMR techniques,[9] we are still lacking the knowledge of the atomic details of the self-assembly mechanism and the intermediate(s) involved in the amyloid-fibrils formation. It is therefore, worthwhile to study a small model peptide containing non-protein amino acids[10] (stereochemically restricted/flexible) whose structure can be determined using single crystal X-ray diffraction studies as this may provide insights into how subunit (monomer or dimmer) self-associate to form β-sheet and amyloid-like fibril at the atomic resolution.

Our previous studies demonstrated that a tripeptide with an extended backbone conformation[11] and a turn forming

Keywords: Self-assembling peptides; Dimer; Aib; β-Sheet; Amyloid-like fibrils.
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tripeptide[12] self-assemble through various non-covalent interactions to form highly ordered β-sheet assemblage in crystals and an amyloid-like fibrillar structure in the solid state. However, we present here Acp (ε-aminocaproic acid) containing short synthetic tripeptides that form a hydrogen bonded dimer, which we believe can mediate the supramolecular β-sheet aggregation and subsequent amyloid-like fibril formation.

2. Result and discussion

The schematic presentations of all terminally protected tripeptides are shown in Figure 1. Here we have used an ε-aminocaproic acid residue (Acp) in which the two end moieties, NH and C═O can provide hydrogen bonding functionalities that serve to form intermolecular hydrogen bonds among the individual strands while the centrally located pentamethylene unit provides sufficient flexibility[13] to the peptide backbone to achieve an extended backbone conformation, a prerequisite for forming a β-sheet structure. Our first synthesized peptide **1**, having a centrally positioned flexible Gly residue fails to provide single crystals. The two residues Aib(2) and Leu(3) for peptide **2** and Aib(2) and Phe(3) for peptide **3**, containing hydrophobic groups in their side chains help to increase the crystallinity of these peptides and facilitate the association of each dimer, apart from their hydrogen bonding functionalities, through the interactions between the isobutyl side chain of Leu for peptide **2** and side chain Phe/Phe interaction for peptide **3**. In peptide **3**, a CH···π interaction[14] [the contact of a C(sp^2)–H group of the phenyl ring of molecule B of one asymmetric unit to the π cloud of the phenyl ring of molecule A of the neighboring asymmetric unit along crystallographic *a* axis is 2.74 Å] assists each dimer to bind together along the *a* direction. It is a well documented fact that π–π interactions have a significant role in amyloid aggregation.[15] For peptides **4**, **5** and **6** we have used Val, D-Val, and Ile respectively, replacing the terminal Leu residue from peptide **2**. All peptides were studied using FT-IR, NMR, scanning electron microscopy and optical microscopy.

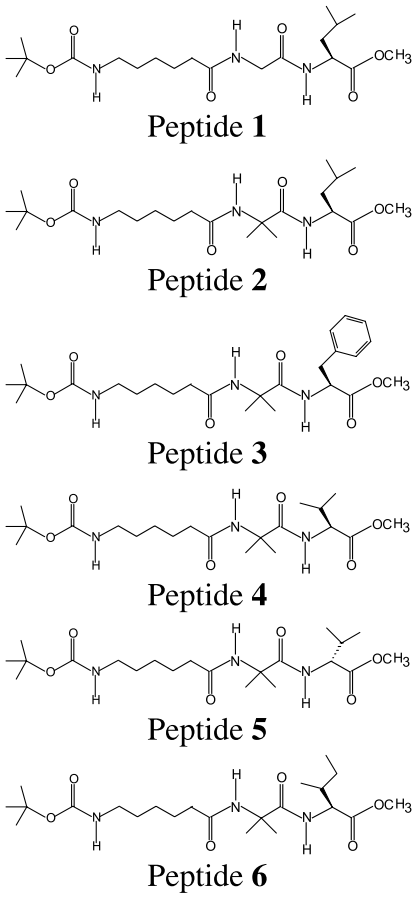

Figure 1. Schematic representation of peptides **1**, **2**, **3**, **4**, **5** and **6**.

2.1. Solid state FT-IR study

Preliminary information on the conformational preferences of all peptides were obtained from FT-IR studies.[16] The most informative frequency ranges are (i) 3500–3200 cm^{-1}, corresponding to the N–H stretching vibrations of the peptide and N-protecting urethane groups, and (ii) 1800–1600 cm^{-1}, corresponding to the C═O stretching vibrations of the peptide, urethane, and ester groups. Important IR data of all these reported compounds are listed in Table 1. In the 3500–3200 cm^{-1} region (Fig. 2), an intense band corresponding to strongly hydrogen bonded NH groups has been observed at 3375–3275 cm^{-1} for each of the reported peptides. In the 1800–1600 cm^{-1} region, several bands have been observed (Fig. 2). The bands at 1783–1740 and 1737–1610 cm^{-1} (Fig. 2) are assigned to the stretching of the C═O groups of free methyl ester moieties and hydrogen-bonded urethane groups respectively. The observed strong and medium bands at 1733–1690 cm^{-1} are attributed to the stretching of the C═O groups of hydrogen bonded esters and/or free urethanes.[16a,d–f] The strong bands at 1627–1650 cm^{-1} are assigned to the C═O groups of hydrogen bonded peptide moieties. The bands observed at 1627–1650 and 3227–3286 cm^{-1} are typical of a fully developed β-sheet conformation.[16] For peptides **2** and **3** strong bands are observed at nearly

Table 1. Infrared (IR) absorption frequencies (cm^{-1}) for all reported peptides in solid state (on KBr pellet)

Peptide	NH stretch	C═O stretch
Boc-Acp-Gly-Leu-OMe (**1**)	3307(st)	1745(m),1730(st),1681(m), 1651(st), 1645(sd)
Boc-Acp-Aib-Leu-OMe (**2**)	3315(st)	1707(w), 1685(st), 1660(st)
Boc-Acp-Aib-Phe-OMe (**3**)	3371(st), 3336(st), 3317(m), 3273(st)	1735(m), 1687(st), 1660(st), 1651(st)
Boc-Acp-Aib-Val-OMe (**4**)	3354(st), 3288(st)	1651–1633(st)
Boc-Acp-Aib-^{D}Val-OMe (**5**)	3354(st), 3290(st)	1712(m), 1701(st), 1681(v.w.), 1660(st)
Boc-Acp-Aib-Ile-OMe (**6**)	3373(st), 3340(st), 3269(st)	1735(st), 1687(vs), 1666(st), 1649(st)

st=strong, w=weak, v.w=very weak, m=medium, sd=shoulder, v.s=very strong.

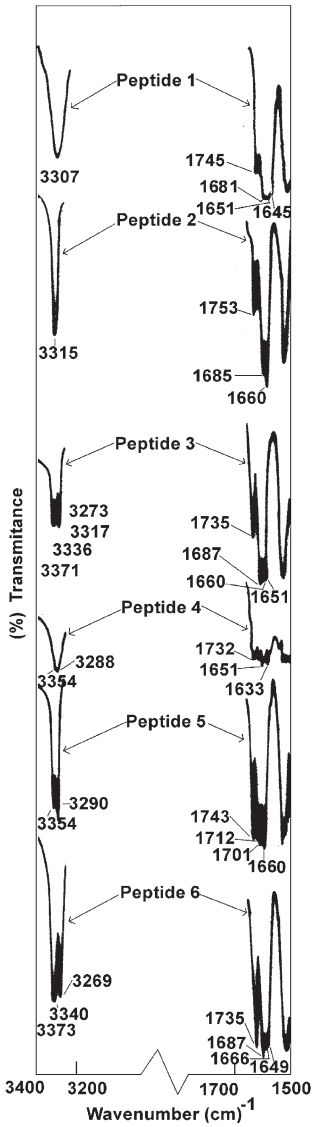

Figure 2. FT-IR spectra at the region 3200–3500 cm^{-1} (a) and 1500–1750 cm^{-1} (b) of peptides **1**, **2**, **3**, **4**, **5** and **6** in solid state.

1650 cm^{-1} region. For peptide **1**, a strong band at 1651 cm^{-1} with a shoulder at 1645 cm^{-1} has been observed in the solid state. All these data suggest an intermolecularly hydrogen bonded β-sheet structure for peptide **1** in the solid state. Similarly, FT-IR data corresponding to peptides **4**, **5** and **6** (Table 1) are indicative of the β-sheet-like structure in the solid state. It is suggested from the solid state FT-IR data that the solid state structure of all the reported peptides are similar and that they form intermolecularly hydrogen bonded β-sheet-like structures.

2.2. Single crystal X-ray diffraction study

Among all peptides, only peptides **2** and **3** give a single crystal suitable for X-ray diffraction analysis. ORTEP diagrams with the atom numbering schemes are provided in Figure 3(a) and in Figure 3(b) for peptides **2** and **3**, respectively. Interestingly, both Figures 3(a) and (b) show that there are two molecules (named A and B) in the asymmetric unit for both peptides and they are joined together by intermolecular hydrogen bonds to form a stable molecular dimer of two different conformers (Table 2 for peptide **2** and Table 3 for peptide **3**). There is no intramolecular hydrogen bond in molecules A and B for peptides **2** and **3**. In all cases, the Acp residue adopts an extended backbone conformation, while the centrally located Aib residue adopts a helical conformation, a very distinctive feature of the Aib residue.[17] However, the terminal residues, Leu for peptide **2** and Phe for peptide **3**, adopt extended backbone conformations. This provides an overall extended backbone structure with a small kink at the central place (due to the presence of a helix forming Aib residue) for each conformer of the molecular dimers of both peptides **2** and **3**. Each dimer then self-assembles via intermolecular hydrogen bonds forming an extended parallel β-sheet assemblage in both peptides **2** (Fig. 4(a)) and **3** (Fig. 4(b)). These β-sheets are themselves regularly stacked via van der Waals interactions to form a more complex quaternary structure (Fig. 5 for peptide **2** and Fig. 6 for peptide **3**). The hydrogen bonding parameters of peptides **2** and **3** are listed in Tables 2 and 3. There are three intermolecular hydrogen bonds N14A–H14A···O15B, N7A–H7A···O8B and N4A–H4A···O51B for peptide **2** and three (N1A–H1A···O2B, N4A–H4A···O5B, N11B–H11B···O12A) for peptide **3** that are responsible for connecting the individual molecules in the asymmetric unit to form and stabilize the dimer. There are three other intermolecular hydrogen bonds N4B–H4B···O51A, N7B–H7B···O8A and N14B–H14B···O15A for peptide **2** and two intermolecular hydrogen bonds N11A–H11A···O12B and N4B–H4B···O2A for peptide **3** which are involved in connecting the individual dimer to form the parallel β-sheet structure. Crystal data for these two peptides are detailed in Table 4. This data confirms our initial insight from the FT-IR data that these peptides have a sheet-like structure in their solid state. These crystal structures also support the fact that

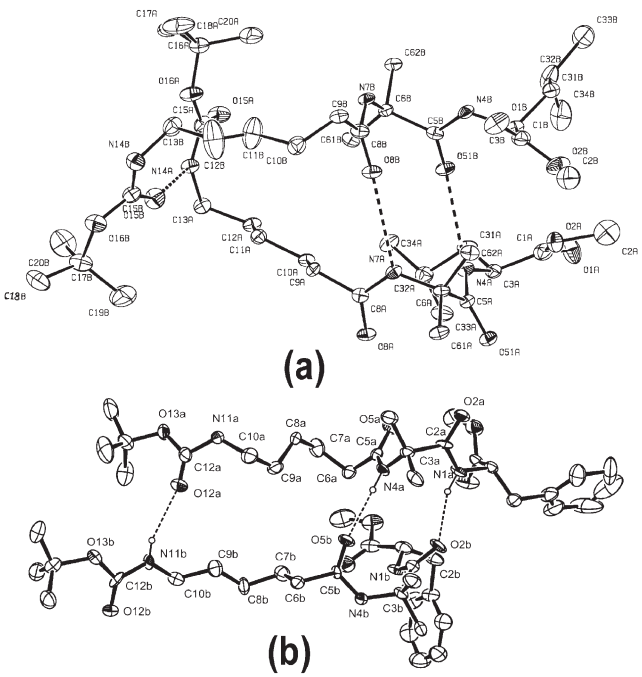

Figure 3. (a) The ORTEP diagram of peptide **2** and (b) the ORTEP diagram of peptide **3** with the atomic numbering scheme showing the dimer formation. Ellipsoids at 20% probability. The dimer formation via three intermolecular bonds is shown as dotted lines.

Table 2. Characteristics of peptide **2** (Boc-Acp-Aib-Leu-OMe) in molecules A and B

(a) Selected torsional angles[a] (°) of peptide **2**								
Residue	Molecule	ϕ	ψ	ω	θ_1	θ_2	θ_3	θ_4
Acp(1)	A	86.9	113.1	−173.6	176.9	176.6	173.5	56.7
	B	−102.7	127.9	178.4	−70.4	−158.0	179.5	145.4
Aib(2)	A	59.2	46.7	174.7	—	—	—	—
	B	−58.3	−50.2	−178.6	—	—	—	—
Leu(3)	A	−84.5	7.3	171.1	—	—	—	—
	B	−117.8	159.8	−177.1	—	—	—	—

(b) Intermolecular hydrogen bonding parameters of peptide **2**			
D–H···A	H···A (Å)	D···A (Å)	D–H···A (°)
N4A–H4A···O51B[b]	2.26	2.990	143
N4B–H4B···O51A	2.44	3.183	144
N7A–H7A···O8B[b]	2.20	3.041	166
N14A–H14A···O15B[b]	2.11	2.928	159
N14B–H14B···O15A[c]	2.12	2.956	136

[a] The torsion angles for rotation about the bonds of peptide backbone: (ϕ, ψ, ω); Torsions in the main chain in the N-terminal Acp residue about C^{α}–C^{β}, C^{β}–C^{γ}, C^{γ}–C^{δ} and C^{δ}–C^{ε}: θ_4 to θ_1, respectively.
[b] Symmetry equivalent x, $1+y$, z.
[c] Symmetry equivalent $1+x$, $-1+y$, z.

Table 3. Characteristics of peptide **3** (Boc-Acp-Aib-Phe-OMe) in molecules A and B

(a) Selected torsional angles[a] (°) of peptide **3**								
Residue	Molecule	ϕ	ψ	ω	θ_1	θ_2	θ_3	θ_4
Acp(1)	A	95.2	−117.8	−177.1	−146.1	−172.3	−66.0	−63.6
	B	−144.9	−106.4	−178.5	−179.4	−173.7	64.9	175.1
Aib(2)	A	−54.6	−45.7	−173.6	—	—	—	—
	B	−54.4	−41.5	−176.3	—	—	—	—
Phe(3)	A	−99.0	94.0	175.9	—	—	—	—
	B	−148.8	167.9	−180.0	—	—	—	—

(b) Intermolecular hydrogen bonding parameters of peptide **3**			
D–H···A	H···A (Å)	D···A (Å)	D–H···A (°)
N1A–H1A···O2B[b]	2.08	2.90	161
N4A–H4A···O5B[b]	2.12	2.97	171
N4B–H4B···O2A	2.12	2.87	144
N11A–H11A···O12B	2.16	3.01	169
N11B–H11B···O12A[c]	2.27	3.08	157

[a] The torsion angles for rotation about the bonds of peptide backbone: ϕ, ψ, ω; torsions in the main chain in the N-terminal Acp residue about C^{α}–C^{β}, C^{β}–C^{γ}, C^{γ}–C^{δ} and C^{δ}–C^{ε}: θ_4 to θ_1, respectively.
[b] Symmetry equivalents: $x-1$, y, z.
[c] Symmetry equivalents: $x+1$, y, z.

supramolecular β-sheet formation is mediated through dimerization for the representative peptides **2** and **3**.

2.3. Morphological study

The morphological similarity of the β-sheet assemblage of these reported peptides with amyloid fibrils has been studied using a scanning electron microscope (SEM). The SEM images of peptides **2**, **3** and **6** (Figs. 8, 9 and 12, respectively) of the dried fibrous material grown from ethyl acetate/methanol–water clearly demonstrate that the aggregate in the solid state is a bunch of long small

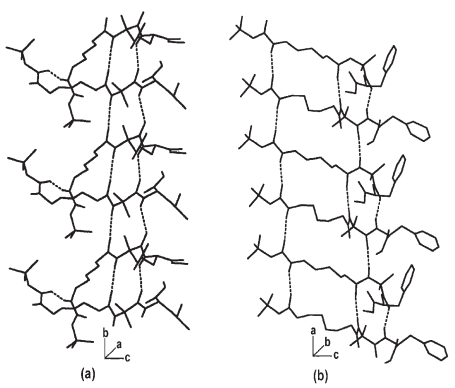

Figure 4. (a) Packing diagram of the peptide **2** in the *b* projection and (b) packing of peptide **3** along *a* projection illustrating intermolecular hydrogen bonding in solid state and the formation of continuous β-sheets columns. Hydrogen bonds are shown as dotted lines. Only hydrogen bonded H atoms are shown for clarity.

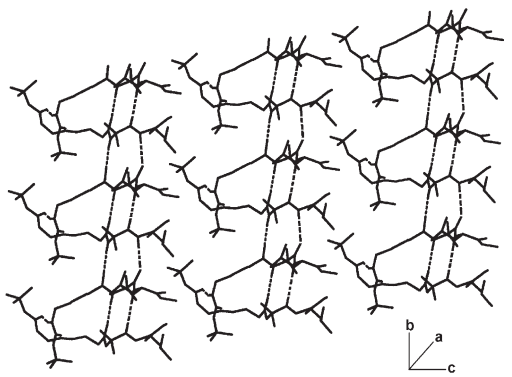

Figure 5. The packing of peptide **2** dictating the packing of individual columns via van der Waals' interactions along the crystallographic *c* axis into higher order β-sheet structure in crystal. Dotted lines are indicated for hydrogen bonds.

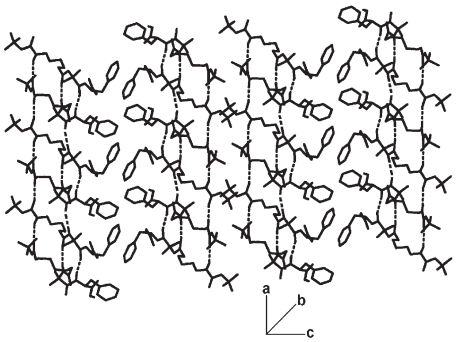

Figure 6. The crystal packing of peptide **3** showing the formation of a higher order β-sheet structure along the crystallographic *c* axis via van der Waals' interactions between the individual columns. Hydrogen bonds are shown as dotted lines.

Table 4. Crystallographic data for peptide **2** and peptide **3**

	Peptide **2**	Peptide **3**
Formula	$C_{22}H_{41}N_3O_6$	$C_{25}H_{39}N_3O_6$
Formule weight	443.68	477.59
Crystallizing solvent	Ethyl acetate	Methanol–water
Crystal system	Triclinic	Monoclinic
Temperature (K)	293	293
Space group	*P*1	P_{21}
a (Å)	9.354(14)	10.386(12)
b (Å)	9.844(14)	9.538(12)
c (Å)	15.72(2)	27.71(3)
α (°)	79.378(10)	90
β (°)	76.445(10)	93.985(10)
γ (°)	87.222(10)	90
U (Å^3)	1383(3)	2738.44
Z	2	4
D_{calcd} (g cm^{-3})	1.065	1.158
λ (Å)	0.71.73	0.71073
*R*1	0.0674	0.0866
*wR*2	0.2224	0.2070

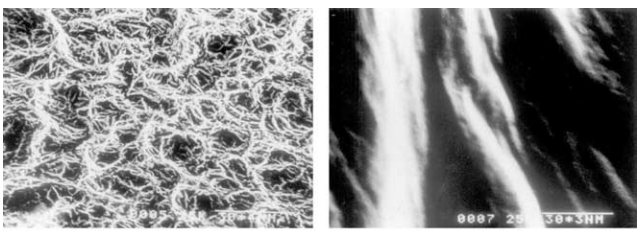

Figure 7. (a) Typical SEM image of peptide **1** taken from dried material growing from methanol–water, showing fibrillar network. (b) SEM image of peptide **1** in higher magnification showing intertwined helical filaments.

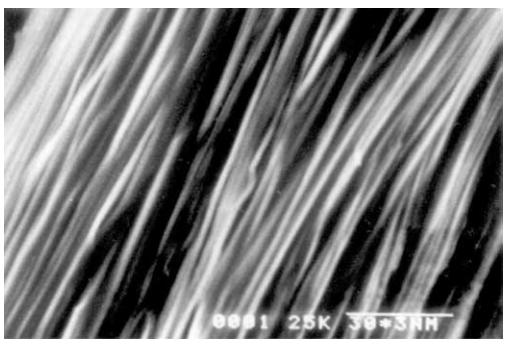

Figure 8. The SEM image of dried fibrous material of peptide **2** obtained from ethyl acetate solution.

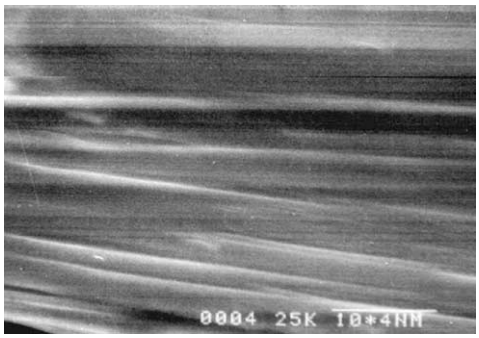

Figure 9. Typical SEM image of dried fibrous material of peptide **3** obtained from methanol–water solution.

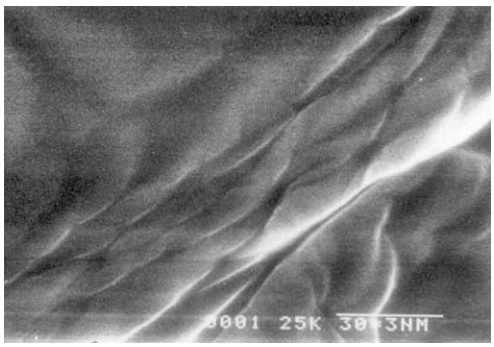

Figure 10. Typical SEM image of dried material of peptide **4** from methanol–water solution showing intertwined helical filaments.

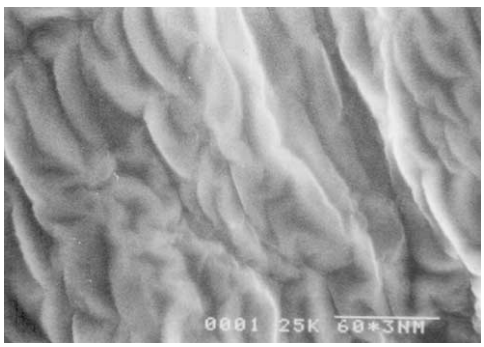

Figure 11. Typical SEM image of dried peptide **5** showing intertwined helical filaments obtained from methanol–water solution having reverse handedness to that of peptide **4**.

filaments, resembling amyloid fibrils. Morphologies of peptides **1**, **4** and **5** are the most remarkable. Figure 7(a) shows fibrillar network of peptide **1**, growing from methanol–water solvent system. Figure 7(b) shows higher magnification of the SEM picture of the peptide suggesting an intertwined helical nature of the fibrils. Figures 10 and 11 exhibit the SEM images of peptides **4** and **5** respectively from methanol–water. All these three (Figs. 7(b), 10 and 11) indicate that peptides **1**, **4** and **5** form inter-twined helical filaments, a special characteristic of many neurodegenerative disease causing amyloid fibrils.[18] Interestingly, the handedness of the helicity of these fibrils obtained from the two enantiomeric peptides (peptide **4** and peptide **5**) is reverse. This is presumably due to the presence of the amino acid residues with opposite chirality (L-Val and D-Val) in those peptides.

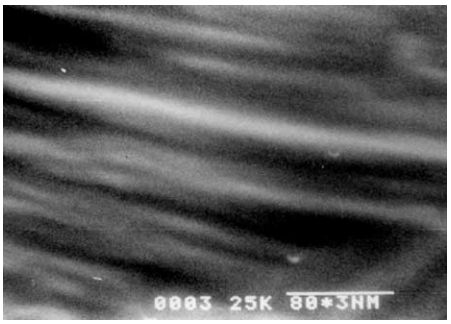

Figure 12. Typical SEM image of peptide **6**. Fibrils were grown from methanol–water solvent system.

2.4. Congo red binding studies

Air-dried drops of the solution of all these peptides were stained with a physiological dye Congo red. This led to the green birefringence with cross polarizers that is characteristic of amyloid fibrils when investigated microscopically.[19] Figure 13 is the representative picture of peptide **3** stained with Congo red, and exhibit distinct green birefringence under polarized light.

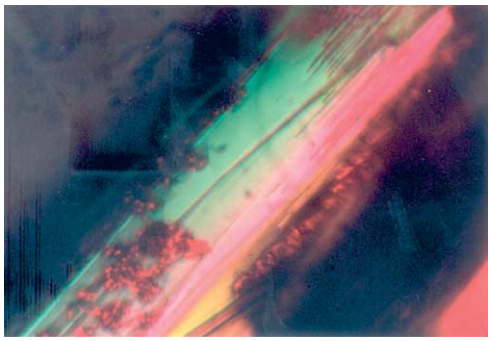

Figure 13. Congo red stained peptide **3** fibrils observed through crossed polarizers showing green-gold birefringence, a characteristic feature of amyloid fibrils.

3. Conclusion

FT-IR data of these peptides (peptides **1**, **2**, **3**, **4**, **5** and **6**) reveal that, all the reported peptides self-associate to form supramolecular β-sheet structure via intermolecular hydrogen bonds, in which intermolecular hydrogen bonds are present between the peptide linkages. Crystal structures of two peptides reveal that they form hydrogen bonded dimers that gives supramolecular β-sheets on self-assembly. Several previous reports suggest that many neurotoxic amyloidogenic peptide sequences can self-associate to form a quaternary β-sheet structure and ultimately form fibrils via dimerization.[3] Characterization of the peptide dimer at atomic resolution which self-assembles to form β-sheets is particularly important for better understanding of the pathway(s) and kinetics of amyloid fibril formation. We have established that quaternary β-sheet structure formation and amyloid-like fibril formation can be mediated by the dimerization of synthetic peptide molecules having extended backbone conformations using single crystal X-ray diffraction studies. The morphological resemblance of these peptides with Alzheimer's-associated Aβ peptides and other neurotoxic amyloid peptides indicates that the fibril model with parallel β-sheet assemblage[20] may be useful for exploring the structure-function relationship of amyloidogenic peptides and proteins.

4. Experimental

4.1. Synthesis of peptides

All peptides were synthesized by conventional solution phase methods by using racemization free fragment condensation strategy.[21] The Boc group was used for N-terminal protection and the C-terminus was protected as a methyl ester. Deprotections were performed using the saponification method. Couplings were mediated by dicyclohexylcarbodiimide-1-hydroxybenzotriazole (DCC/HOBt). All the intermediates were characterized by ^{1}H NMR (300 MHz) and thin layer chromatography (TLC) on silica gel and used without further purification. The final products were purified by column chromatography using silica (100–200 mesh size) gel as stationary phase and ethyl acetate/ethyl acetate–toluene mixture as eluent. The purified final compounds were fully characterized by 300 MHz ^{1}H NMR spectroscopy, mass spectrometry and elemental analysis. All chiral amino acids are of 'L' configuration unless otherwise it is mentioned.

4.1.1. Boc-Acp-OH (7). A solution of ε-aminocaproic acid (9.84 g, 75 mmol) in a mixture of dioxan (150 mL), water (75 mL) and 1 M NaOH (75 mL) was stirred and cooled in an ice-water bath. Di-*tert*-butylpyrocarbonate (18 g, 82.5 mmol) was added and stirring was continued at room temperature for 6 h. Then the solution was concentrated in vacuo to about 70–80 mL, cooled in an ice-water bath, covered with a layer of ethyl acetate (about 50 mL) and acidified with a dilute solution of $KHSO_4$ to pH 2–3 (Congo red). The aqueous phase was extracted with ethyl acetate and this operation was done repeatedly. The ethyl acetate extracts were pooled, washed with water and dried over anhydrous Na_2SO_4 and evaporated in vacuo. The pure material was obtained as waxy solid.

Yield=14.56 g (63 mmol, 84%). Elemental analysis calcd (%) for $C_{11}H_{21}N_1O_4$ (231): C, 57.14; H, 9.09; N, 6.06. Found: C, 57.37; H, 7.47; N, 6.24.

4.1.2. Boc-Acp(1)-Gly(2)-OCH_2Ph (8). Boc-Acp-OH (2.31 g, 10 mmol) was dissolved in a mixture of dichloromethane (DCM) (20 mL) in an ice-water bath. H-Gly-OCH_2Ph was isolated from the corresponding benzyl ester p-toluene sulfonate (6.74 g, 20 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration (10 mL) and this was added to the reaction mixture, followed immediately by di-cyclohexylcarbodiimide (DCC) (7.42 g, 36 mmol). The reaction mixture was allowed to come to room temperature and stirred for 48 h. DCM was evaporated, residue was taken in ethyl acetate (60 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine, then 1 M sodium carbonate (3×50 mL) and brine

(2×50 mL) and dried over anhydrous sodium sulfate, and evaporated in vacuo to yield **8** as a waxy solid.

Yield=2.8 g, (7.4 mmol, 74%). ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 7.29–7.30 (Ph, 5H, m); 6.11 (Gly(2) NH, 1H, t, J=6.0 Hz); 5.19 (–benzyl CH_2, 2H, s); 4.61 (Acp(1) NH, 1H, t, J=5.2 Hz); 4.07 ($C^{\alpha}H$ of Gly, 1H, t, J=8.2 Hz); 3.09–3.11 ($C^{\varepsilon}Hs$ of Acp(1), 2H, m); 2.22–2.34 ($C^{\alpha}Hs$ of Acp(1), 2H, m); 1.61–1.71 ($C^{\beta}Hs$ and $C^{\gamma}Hs$ of Acp(1), 4H, m); 1.30–1.35 ($C^{\delta}H_3$ of Acp(1), 2H, m); 1.44 (Boc-CH_3s, 9H, s). Elemental analysis calcd (%) for $C_{20}H_{30}N_2O_5$ (378): C, 63.49; H, 7.94; N, 7.41. Found: C, 63.65; H, 7.62; N, 7.25.

4.1.3. Boc-Acp(1)-Gly(2)-OH (9). To a sample of **8** (2.8 g, 7.4 mmol), MeOH (30 mL) and 2 M NaOH (15 mL) were added and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred. After 10 h methanol was removed in vacuo, the residue was taken in water (50 mL) and washed with diethyl ether (2×50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1 M HCl and it was extracted with ethyl acetate (3×50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated in vacuo to yield of **9** (1.58 g) as waxy solid.

Yield=1.58 g (5.5 mmol, 74%). ^{1}H NMR ($(CD_3)_2SO$, 300 MHz, δ in ppm): 12.2 (–COOH, 1H, b); 7.05 (Gly(2) NH, 1H, t, J=4.5 Hz); 3.56 ($C^{\alpha}H$ of Gly, 2H, t, J=7.5 Hz); 2.54–2.58 ($C^{\varepsilon}Hs$ of Acp(1), 2H, m); 1.78–1.81 ($C^{\alpha}Hs$ of Acp(1), 2H, m); 1.11–1.13 ($C^{\beta}Hs$+$C^{\gamma}Hs$ of Acp (1), 4H, m); 1.10 (Boc-CH_3s, 9H, s); 0.82–0.86 ($C^{\delta}Hs$ of Acp(1), 2H, m). Elemental analysis calcd (%) for $C_{13}H_{24}N_2O_5$ (288): C, 54.17; H, 8.33; N, 9.72. Found: C, 54.56; H, 8.21; N, 9.63.

4.1.4. Boc-Acp(1)-Gly(2)-Leu(3)-OMe (1). Boc-Acp-Gly-OH (1.44 g, 5 mmol) was dissolved in dichloromethane–DMF (10 mL) in an ice-water bath. H-Leu-OMe was isolated from methyl ester hydrochloride (1.82 g, 16 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 7 mL and it was added to the reaction mixture, followed immediately by dicyclohexylcarbodiimide (DCC) (1.03 g, 5 mmol) and HOBt (0.68 g, 5 mmol). The reaction mixture was allowed to come to room temperature and stirred for 72 h. The residue was taken in ethyl acetate (30 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCl (3×30 mL), brine, then 1 M sodium carbonate (3×30 mL) and brine (2×30 mL) and dried over anhydrous sodium sulfate and evaporated in vacuo to yield **1** as waxy solid. Purification was done by silica gel column (100–200 mesh) using 33% toluene/ethyl acetate as eluent.

Yield=1.45 g (3.5 mmol, 70%). R_f (33% toluene/ethyl acetate) 0.68; ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 6.62 (Leu(3) NH, 1H, d, J=9 Hz); 6.60 (Gly(2) NH, 1H, t, J=6 Hz); 4.69 (Acp(1) NH, 1H, t, J=4.8 Hz); 4.57–4.60 ($C^{\alpha}H$ of Leu, 1H, m); 3.96–4.01 ($C^{\alpha}Hs$ of Gly, 2H, m); 3.73 (–OCH_3, 3H, s); 3.07–3.13 ($C^{\varepsilon}H$ of Acp, 2H, m); 2.16–2.21 ($C^{\alpha}Hs$ of Acp(1), 2H, m); 1.56–1.71 ($C^{\beta}Hs$ and $C^{\gamma}Hs$ of Acp(1) and Leu(3), 4H, m); 1.50–1.52 ($C^{\delta}Hs$ of Acp(1), 2H, m); 1.44 (Boc-CH_3s, 9H, s); 0.93–0.94 ($C^{\delta}Hs$ of Leu(3), 6H, m). Mass spectral data M+Na^+=438, M_{calcd}=415. Elemental analysis calcd (%) for $C_{20}H_{37}N_3O_6$ (415): C, 57.83; H, 8.92; N, 10.12. Found: C, 57.94; H, 8.63; N, 10.32.

4.1.5. Boc-Acp(1)-Aib(2)-OMe (10). Boc-Acp-OH (9.25 g, 40 mmol) was dissolved in dichloromethane (DCM) (50 mL) in an ice-water bath. H-Aib-OMe was isolated from the corresponding methyl ester hydrochloride (12.29 g, 80 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 15 mL and this was added to the reaction mixture, followed immediately by dicyclohexylcarbodiimide (DCC) (8.24 g, 40 mmol). The reaction mixture was allowed to come to room temperature and stirred for 48 h. DCM was evaporated, residue was taken in ethyl acetate (60 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine, then 1 M sodium carbonate (3×50 mL) and brine (2×50 mL) and dried over anhydrous sodium sulfate, and evaporated in vacuo to yield Boc-Acp(1)-Aib(2)-OMe (10.04 g, 30.4 mmol, 76%) as a white solid.

Yield=10.04 g (30.4 mmol, 76%). Mp 85–86 °C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 6.09 (Aib(2) NH, 1H, s); 4.65 (Acp(1) NH, 1H, t, J=10.5 Hz); 3.73 (–OCH_3, 3H, s); 3.10–3.15 ($C^{\varepsilon}Hs$ of Acp(1), 2H, m); 2.14–2.19 ($C^{\alpha}Hs$ Acp(1), 2H, m); 1.56–1.68 ($C^{\beta}Hs$ and $C^{\gamma}Hs$ of Acp(1), 4H, m); 1.46–1.51 ($C^{\delta}H_3$ of Acp(1), 2H, m); 1.53 ($C^{\beta}H_3$ of Aib(2), 6H, s); 1.44 (Boc-CH_3s, 9H, s). Elemental analysis calcd (%) for $C_{16}H_{30}N_2O_5$ (330): C, 58.18; H, 9.09; N, 8.48. Found: C, 58.16; H, 9.06; N, 8.52.

4.1.6. Boc-Acp(1)-Aib(2)-OH (11). To a sample of **10** (10.04 g, 30.4 mmol), MeOH (75 mL) and 2 M NaOH (30 mL) were added and the progress of saponification was monitored by thin layer chromatography (TLC). The reaction mixture was stirred. After 10 h. methanol was removed in vacuo, the residue was taken into water (50 mL), washed with diethyl ether (2×50 mL). Then the pH of the aqueous layer was adjusted to 2 using 1 M HCl and it was extracted with ethyl acetate (3×50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated in vacuo to yield compound **11** (8.38 g) as a white solid.

Yield=8.38 g (26.5 mmol, 87.17%). Mp 106–107 °C.^{1}H NMR ($(CD_3)_2SO$, 300 MHz, δ in ppm): 11.78 (–COOH, 1H, br); 7.67 (Aib(2)–NH, 1H, s); 4.46 (Acp(1) NH,1H, t, J=10.5 Hz); 2.60–2.66 ($C^{\varepsilon}Hs$ of Acp(1), 2H, m); 1.76–1.81 ($C^{\alpha}Hs$ Acp(1), 2H, m); 1.15–1.23 ($C^{\beta}Hs$ and $C^{\gamma}Hs$ of Acp (1), 4H, m); 1.13 (Boc-CH_3s, 9H, s);1.07 ($C^{\beta}Hs$ of Aib,6H, s); 0.93–1.0 ($C^{\delta}Hs$ of Acp(1), 2H, m). Elemental analysis calcd (%) for $C_{15}H_{28}N_2O_5$ (316): C, 56.96; H, 8.86; N, 8.86. Found: C, 56.89; H, 8.78; N, 8.92.

4.1.7. Boc-Acp(1)-Aib(2)-Leu(3)-OMe (2). Boc-Acp(1)-Aib(2)-OH (1.58 g, 5 mmol) in DMF (10 mL) was cooled in an ice-water bath and H-Leu-OMe was isolated from the corresponding methyl ester hydrochloride (1.82 g, 10 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 7 mL and it was added to the reaction mixture, followed immediately by DCC (1.03 g, 5 mmol) and HOBt (0.68 g, 5 mmol). The reaction

mixture was stirred for 3 days. The residue was taken in ethyl acetate (60 mL) and the DCU was filtered off.The organic layer was washed with 2 M HCl (3×50 mL), brine, 1 M sodium carbonate (3×50 mL), brine (2×50 mL), dried over anhydrous sodium sulfate and evaporated in vacuo to yield white solid (1.64 g, 3.7 mmol). Purification was done by silica gel column (100–200 mesh) using ethyl acetate as eluent. Colourless single crystals were grown from ethyl acetate by slow evaporation.

Yield=1.64 g (3.7 mmol, 74%). R_f (ethyl acetate) 0.72. Mp 108–109 °C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 6.99 (Leu(3) NH, 1H, d, J=7.74 Hz); 6.05 (Aib(2) NH, 1H, s); 4.69–4.60 (Acp(1) NH, 1H, m); 4.53–4.58 (C^{α}H of Leu(3), 1H, m); 3.73 (OCH_3, 3H, s); 3.09–3.12 (C^{ε}Hs of Acp(1), 2H, m); 2.16–2.21 (C^{α}Hs of Acp(1), 2H, m); 1.62–1.67 (C^{β}Hs and C^{γ}Hs of Acp(1) and Leu(3), 4H, m); 1.57, 1.59 (C^{β}Hs of Aib(2), 6H, s); 1.47–1.53 (C^{δ}Hs of Acp(1), 2H, m); 1.44 (Boc-CH_3s, 9H, s); 0.87–0.94 (Leu(3) of C^{δ}Hs, 6H, m); MS data M+Na^+=466, M_{calcd}=443. Elemental analysis calcd (%) for $C_{22}H_{41}N_3O_6$ (443): C, 59.59; H, 9.25; N, 9.48. Found: C, 59.56; H, 9.11; N, 9.51.

4.1.8. Boc-Acp(1)-Aib(2)-Phe(3)-OMe (3). Boc-Acp(1)-Aib(2)-OH (1.58 g, 5 mmol) in DMF (10 mL) was cooled in an ice-water bath and H-Phe-OMe was isolated from the corresponding methyl ester hydrochloride (2.16 g, 10 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 7 mL and it was added to the reaction mixture, followed immediately by DCC (1.03 g, 5 mmol) and of HOBt (0.68 g, 5 mmol). The reaction mixture was stirred for 3 days. The residue was taken in ethyl acetate (60 mL) and the DCU was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine, 1 M sodium carbonate (3×50 mL), brine (2×50 mL), dried over anhydrous sodium sulfate and evaporated in vacuo to yield white solid (1.64 g, 3.7 mmol). Purification was done by silica gel column (100–200 mesh) using ethyl acetate as eluent. Colourless single crystals were grown from methanol–water mixture.

Yield=1.64 g (3.4 mmol, 68%). R_f (ethyl acetate) 0.65. Mp 92–93 °C. ^{1}H NMR ($CDCl_3$, 300 MHz, δ ppm): 7.11–7.35 (phenyl ring protons); 6.84 (Phe(3) NH, 1H, d, J=7.02 Hz); 5.98 (Aib NH(2), 1H, s); 4.8–4.86 (Acp(1)NH, 1H, m); 4.56–4.58 (C^{α}H of Phe(3), 1H, m); 3.73 (OCH_3, 3H, s); 3.21–3.14 (C^{β}Hs of Phe (3), 2H, m); 3.06–3.13 (C^{ε}Hs of Acp(1), 2H, m); 2.17–2.12 (C^{α}Hs of Acp(1), 2H, m); 1.53–1.67 (C^{β}Hs and C^{γ}Hs of Acp(1) 4H, m); 1.48, 1.50 (C^{β}Hs of Aib(2), 6H, s); 1.46–1.48 (C^{δ}Hs of Acp(1), 2H, m); 1.44 (Boc-CH_3s, 9H, s); MS data M+Na^++H^+=501, M_{calcd}=477. Elemental analysis calcd (%) for $C_{25}H_{39}N_3O_6$ (477): C, 62.89; H, 8.18; N, 8.81. Found: C, 62.87; H, 8.17; N, 8.83.

4.1.9. Boc Acp-Aib-Val-OMe (4). To a sample of **11** (1.58 g, 5 mmol) in DMF (10 mL) was cooled in an ice-water bath. H-Val-OMe was isolated from of the corresponding methyl ester hydrochloride (1.68 g, 10 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 7 mL and this was added to the reaction mixture, followed immediately by DCC (1.03 g, 5 mmol) and HOBt (0.68 g, 5 mmol). The reaction mixture was stirred for 3 days. The residue was taken in ethyl acetate (50 mL) and DCU was filtered off. The organic layer was washed with 2 M HCl (3×50 mL), brine, 1 M sodium carbonate (3×50 mL), brine (2×50 mL), dried over anhydrous sodium sulfate and evaporated in vacuo to yield of white solid (1.6 g). Purification was done by silica gel column using ethyl acetate as eluent.

Yield=1.6 g (3.73 mmol, 74%). R_f (ethyl acetate) 0.75. Mp 70–71 °C. ^{1}H NMR. ($CDCl_3$, 300 MHz, δ ppm): 7.12 (Val(3) NH, 1H, d, J=9 Hz); 6.03 (Aib(2) NH, 1H, s); 4.66 (Acp(1) NH, 1H, t, J=6 Hz); 4.48–4.52 (C^{α}H of Val(3), 1H, m); 3.74 (OCH_3, 3H, s); 3.07–3.14 (C^{ε}Hs of Acp(1), 2H, m); 2.17–2.21 (C^{α}Hs of Acp(1), 2H, m and C^{β}H of Val(3), 1H, m); 1.63–1.71 (C^{β}Hs and C^{γ}Hs of Acp(1), 4H, m); 1.57, 1.59 (C^{β}Hs of Aib(2), 6H, s); 1.47–1.53 (C^{δ}Hs of Acp(1), 2H, m); 1.44 (Boc-CH_3s, 9H, s); 0.90–0.96 (C^{γ}Hs of Val(3), 6H, m). Mass spectral data M+Na^+=452, M_{calcd}=429. Elemental analysis calcd for $C_{21}H_{39}N_3O_6$ (429): C, 58.74; H, 9.1; N, 9.79. Found: C, 58.60; H, 9.11; N, 9.71.

4.1.10. Boc Acp-Aib-D-Val-OMe (5). Boc-Acp-Aib-OH (1.58 g, 5 mmol) was dissolved in DMF (10 mL) in an ice-water bath. H-D-Val-OMe was isolated from methyl ester hydrochloride (1.68 g, 10 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 7 mL and it was added to the reaction mixture, followed immediately by dicyclohexylcarbodiimide (DCC) (1.03 g, 5 mmol) and HOBt (0.68 g, 5 mmol). The reaction mixture was allowed to come to room temperature and stirred for 72 h. The residue was taken in ethyl acetate (30 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCl (3×30 mL), brine, then 1 M sodium carbonate (3×30 mL) and brine (2×30 mL) and dried over anhydrous sodium sulfate and evaporated in vacuo to yield **5** as white solid. Purification was done by silica gel column (100–200 mesh) using ethyl acetate as eluent.

Yield=1.4 g (3.26 mmol, 65.2%). R_f (ethyl acetate) 0.74. Mp 86–87 °C. ^{1}H NMR. ($CDCl_3$, 300 MHz, δ ppm): 7.15 (D-Val(3) NH, 1H, d, J=7.8 Hz); 6.12 (Aib(2) NH, 1H, s); 4.63–4.68 (Acp(1)NH, 1H, t, J=7.5 Hz); 4.48–4.52 (D-Val(3) C^{α}H, 1H, m); 3.74 (OCH_3, 3H, s); 3.08–3.14 (C^{ε}Hs of Acp(1), 2H, m); 2.16–2.22 (C^{α}Hs of Acp(1), 2H, m and C^{β}H of D-Val(3), 1H, m); 1.63–1.7 (C^{β}Hs and C^{γ}Hs of Acp(1), 4H, m); 1.57, 1.59 (C^{β}Hs of Aib(2), 6H, s); 1.47–1.52 (C^{δ}Hs of Acp(1), 2H, m); 1.44 (Boc-CH_3s, 9H, s); 0.9–0.99 (C^{γ}Hs of D-Val(3), 6H, m). Mass spectral data M+Na^+=452, M_{calcd}=429. Elemental analysis calcd for $C_{21}H_{39}N_3O_6$ (429): C, 58.74; H, 9.1; N, 9.79. Found: C, 58.58; H, 9.17; N, 9.83.

4.1.11. Boc Acp-Aib-Ile-OMe (6). Boc-Acp-Aib-OH (1.58 g, 5 mmol) was dissolved in DMF (10 mL) in an ice-water bath. H-Ile-OMe was isolated from methyl ester hydrochloride (1.82 g, 10 mmol) by neutralization, subsequent extraction with ethyl acetate and concentration to 7 mL and it was added to the reaction mixture, followed immediately by dicyclohexylcarbodiimide (DCC) (1.03 g, 5 mmol) and HOBt (0.68 g, 5 mmol). The reaction mixture was allowed to come to room temperature and stirred for

72 h. The residue was taken in ethyl acetate (30 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 M HCl (3×30 mL), brine, then 1 M sodium carbonate (3×30 mL) and brine (2×30 mL) and dried over anhydrous sodium sulfate and evaporated in vacuo to yield **6** (1.6 g) in form of white solid. Purification was done by silica gel column (100–200 mesh) using ethyl acetate as eluent.

Yield=1.6 g (3.6 mmol, 72%). R_f (ethyl acetate) 0.73. Mp 75–76 °C. ^{1}H NMR ($CDCl_3$, 300 MHz δ ppm,): 7.14 (Ile(3) NH, 1H, d, J=8.3 Hz); 6.18 (Aib NH(2), 1H, s); 4.66 (Acp(1) NH, 1H, t, J=7.5 Hz); 4.49–4.53 (C^{α}H of Ile(3), 1H, m); 3.70 (OCH_3, 3H, s); 3.05–3.11 (C^{ε}Hs of Acp(1), 2H, m); 2.15–2.19 (C^{α}Hs of Acp(1), 2H, m); 1.87–1.95 (C^{β}Hs of Ile(3), 1H, m); 1.57–1.67 (Acp(1) C^{γ}Hs and C^{β}Hs, 4H, m); 1.53 (C^{β}Hs of Aib(2), 3H, s); 1.56 (C^{β}Hs of Aib(2), 3H, s); 1.471.41–1.49 (CδHs of Acp(1), 2H, m and C^{γ}Hs of Ile(3), 2H, m); 1.40 (Boc-CH_3s, 9H, s); 1.2–136 (C^{γ}Hs of Ile(3), 3H, m); 0.87–0.92 (C^{δ}Hs of Ile(3), 6H, m). Mass spectral data M+Na^+=466, M_{calcd}=443. Elemental analysis calcd for $C_{22}H_{41}N_3O_6$ (443): C, 59.59; H, 9.25; N, 9.48. Found: C, 59.56; H, 9.11; N, 9.51.

4.2. NMR experiments

All NMR studies were carried out on a Brüker DPX 300 MHz spectrometer at 300 K. Peptide concentrations were in the range 1–10 mM in $CDCl_3$.

4.3. FT-IR spectroscopy

The FT-IR spectra were taken using Shimadzu (Japan) model FT-IR spectrophotometer. The solid-state FT-IR measurements were performed using the KBr disk technique.

4.4. Morphological study

Morphologies of all reported tripeptides were investigated using optical microscopy and scanning electron microscopy (SEM). For the SEM study, fibrous materials (slowly grown from ethylacetate/methanol–water mixtures) were dried and gold coated. Then the micrographs were taken in a SEM apparatus (Hitachi S—415A).

4.5. Congo red binding study

An alkaline saturated Congo red solution was prepared. The peptide fibrils were stained by alkaline Congo red solution (80% methanol/20% glass distilled water containing 10 μL of 1% NaOH) for 2 min and then the excess stain (Congo red) was removed by rinsing the stained fibril with 80% methanol/20% glass distilled water solution for several times. The stained fibrils were dried in vacuum at room temperature for 24 h, then visualized at 100× or 500× magnification and birefringence was observed between crossed polarizers.

4.6. Single crystal X-ray diffraction studies

For peptide **2**, single crystals were obtained from ethyl acetate solution by slow evaporation. For peptide **3**, single crystals were obtained from methanol–water solution by slow evaporation. Crystal data for peptide **2** and peptide **3** were collected on a Marresearch Image Plate with Mo K_{α} radiation. The crystals were positioned at 70 mm from the image plate. 100 frames were measured at 2° intervals with a counting time of 2 min. Data analysis was carried out with the XDS program.[22] The structure was solved using direct methods with the Shelx86 program.[23] Non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms bonded to carbon were included in geometric positions and given thermal parameters equivalent to 1.2 times those of the atom to which they were attached. The structure was refined on F^2 using Shelxl.[24] Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre reference CCDC172055 for peptide **2** and CCDC 211390 for peptide **3**.

4.7. Mass spectrometry

Mass spectra were recorded on a Hewlett Packard Series 1100MSD mass spectrometer by positive mode electrospray ionization.

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Low temperature electrochemistry as a mechanistic probe for the partial reduction of heterocycles

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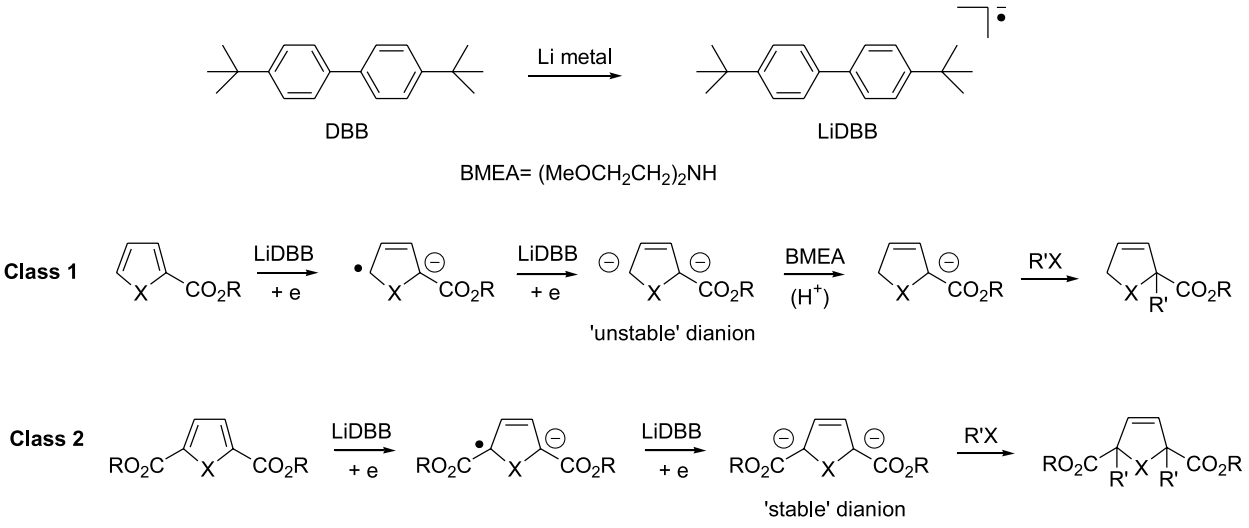
Abstract—The reduction of a series of electron deficient aromatic heterocycles has been examined using electrochemical techniques: the analysis was performed under anhydrous conditions at low temperature, so as to mimic typical synthetic reducing conditions.

1. Introduction

The use of lithium powder to effect the reduction of organic compounds is a well known technique in organic synthesis. Often, an electron transfer agent (such as naphthalene or a biphenyl) is added to the heterogeneous reaction mixture in order to facilitate reduction of the organic substrate.[1] Plausibly this additive is quickly reduced on or near the surface of the metal so forming a homogeneous electron transfer reagent in situ; generally the reduction of many organic compounds can be ineffective without such facilitators of electron transfer, especially at low temperatures.[2] Recently, we have reported on the reduction reactions of a range of aromatic heterocycles using lithium and di-*tert*-butylbiphenyl (DBB) in THF at low temperatures.[3] As expected, the presence of naphthalene or DBB was essential to allow reduction at a reasonable rate. While these reductive processes are related to the Birch reduction reaction they are accomplished without liquid ammonia solvent and hence we have called them 'ammonia free' reductions.[4]

Our studies have shown that the electron deficient heterocycles that can be reduced under ammonia free conditions fall into two distinct classes, Scheme 1. First, there are monosubstituted esters of pyrrole and furan that are



Scheme 1. X=O, NBoc.

Keywords: Reduction; Electrochemistry; Heterocycles.
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moderately electron deficient; we find that addition of a weak acid (bis-methoxyethylamine, BMEA; calculated pK_a 30) is required to allow reduction to occur.[4] We assume that this is present to protonate dianion intermediates (rather than radical anions which we consider too unreactive) formed in situ. Secondly, we have reduced several doubly activated heterocycles (furans, pyrroles and pyridines) which contain two ester electron withdrawing groups.[5,6] In this case, we know that a dianion is formed (because it can then be doubly trapped with a range of electrophiles) and addition of a weak acid is not necessary to allow reduction to take place.

In order to understand the electron transfer processes that are taking place under these reduction conditions, we were interested in developing an electrochemical analysis that would replicate the conditions of lithium in THF at −78 °C and would allow us to measure the reduction potentials of a variety of aromatic heterocycles. Therefore, we would be able to determine whether the formation of a dianion from class 1 and 2 aromatic heterocycles was mechanistically reasonable. We also sought kinetic data about the rate of electron transfer to these aromatic compounds so that we can understand the role of the electron transfer agent in the ammonia free reduction. In the long term, we aim to use electrochemistry as a technique to enable us to predict not only the products of a reduction but also the relative rate at which various aromatic compounds will be reduced under such lithium/THF conditions.

2. Results and discussion

2.1. Preparation of substrates

Our investigation into the electrochemical properties of heterocycles began with the preparation of a range of aromatic substrates using standard organic synthesis techniques as described elsewhere, Scheme 2.[6–11] In addition, the two electron carriers, DBB and naphthalene, which are essential for the reduction to occur rapidly, were also examined. Each of the aromatic heterocycles **3**–**10** has already been reduced under the ammonia free conditions using either **1** or **2** as an electron transfer agent.

2.2. Preliminary investigations into low temperature electrochemistry

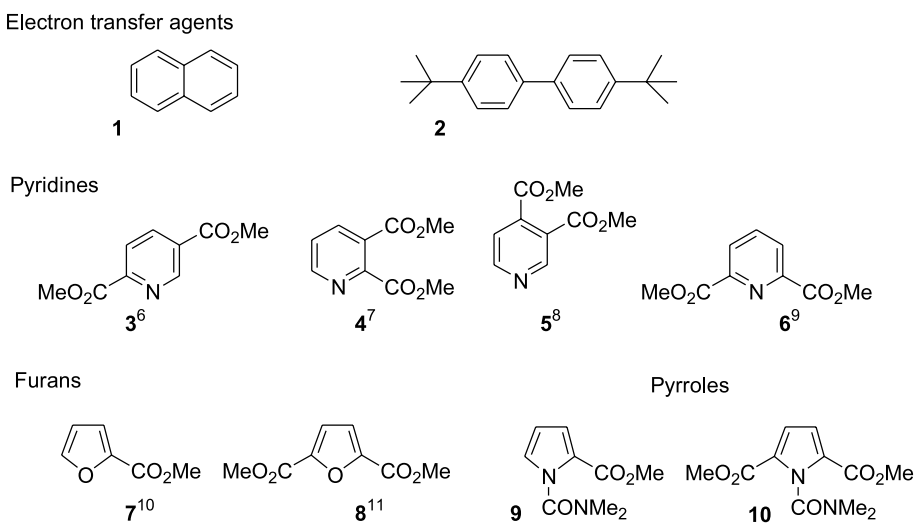
The focus of the initial tests centred on developing a system to allow the measurement of the reduction potentials of the heterocyclic substrates at low temperature, under conditions that mimicked those of the ammonia free reduction. A sealed cell was used, containing distilled THF and the supporting electrolyte, tetra-*n*-butylammonium perchlorate (0.5 M). Oxygen was removed by bubbling a stream of nitrogen through the solution. A length of silver wire was used as the quasi-reference electrode; a saturated calomel electrode could not be used due to the requirement for a saturated aqueous solution of KCl, which would allow water into the system. Note that the silver wire is a quasi-reference electrode, the potential of which may drift (±10 mV); however, in most cases the electrode was found to behave in a stable manner upon repetition of the electrochemistry experiments. A 1 mm platinum disc electrode was used for the working electrode.

For each substrate three parameters were investigated, the half wave potential, $E_{1/2}$, the diffusion coefficient, D, and the electron transfer rate constant, k_0. The half wave potential is defined by Eq. (1): where E_p^{Red} and E_p^{Ox} are the cathodic and anodic peak potentials in an electrochemically reversible (or quasi-reversible) cyclic voltammogram.

$$E_{1/2} = \frac{(E_p^{Red} + E_p^{Ox})}{2} \approx E^{0\prime} \quad (1)$$

Here, $E^{0\prime}$ is the formal potential of the redox couple, a measure of the thermodynamic 'ease' of reduction of the substrate. The diffusion coefficient, D, is a measure of the rate at which the molecule can diffuse through the solvent; it appears in Eq. (2) and can be inferred from the gradient of a plot of the peak current density, i_p, against the square root of the scan rate, ν, for electrochemically reversible processes.

$$i_p = 0.4463nFC_{bulk}\left(\frac{nF}{RT}\right)^{1/2}\nu^{1/2}D^{1/2} \quad (2)$$



Scheme 2.

The rate constant for transfer of the electron from the electrode to the substrate, k_0, is calculated using D and the Nicholson method;[12] the value reported is the average result over all scan rates.

The equations above were used to generate initial estimates for our parameters, which were then established by simulation of the cyclic voltammograms using the commercially available DIGISIM™ software package.[13]

Under electrochemical analysis all substrates gave a quasi-reversible one-electron reduction wave as shown in Figure 1. The results are collected in Table 1.

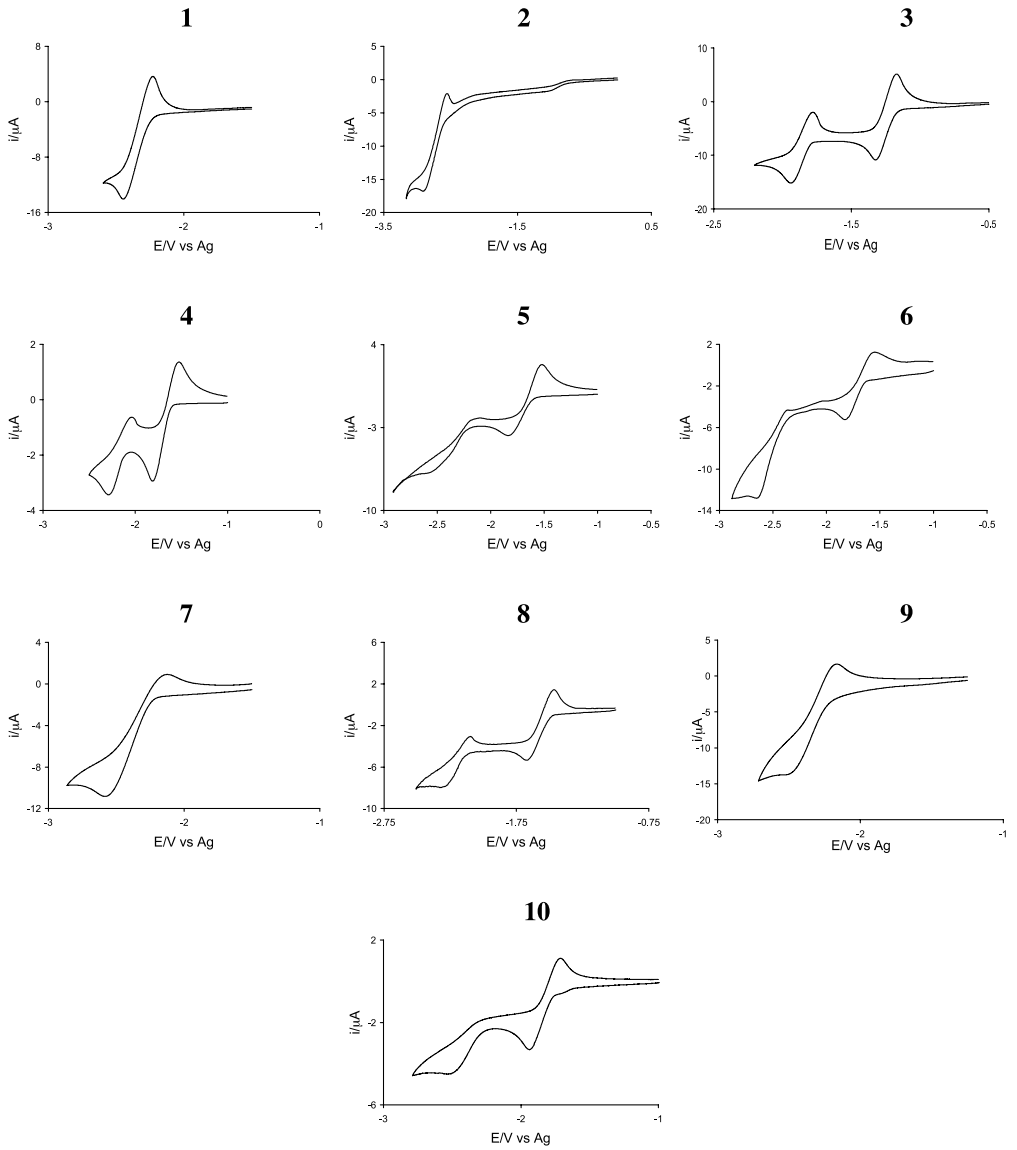


Figure 1. Voltammograms for compounds **1**–**10**, scan rate is 100 mV s^{-1}.

Table 1. First wave reduction data for compounds **1**–**10**, all at −74 °C

Compound	10^6 D/cm^2 s^{-1} (±0.02)	10^4 k_0/cm s^{-1} (±0.05)	$E_{1/2}$/V versus Ag (±0.05)	$E^{0\prime}$/V versus Ag (±0.1)
1	4.1	9.9	−2.3	−2.4
2	3.6	0.8[a]	−2.8	−2.9
3	0.3	2.1	−1.5	−1.5
4	0.7	1.6	−1.6	−1.6
5	0.7	1.9	−1.7	−1.8
6	0.4	2.6	−1.7	−1.8
7	1.2	0.1[a]	−2.4	−2.5
8	0.7	1.2	−1.6	−1.7
9	2.1	3.3	−2.4	−2.5
10	0.4	2.3	−1.8	−1.9

[a] k_0 and $E^{0\prime}$ obtained soley from simulation of voltammograms using DIGISIM™.

In addition, the four pyridine substrates (**3**–**6**)[14] and the di-esters of furan **8** and pyrrole **10** each showed a second quasi-reversible electron transfer, seen in the voltammogram by a second wave, indicating formation of the dianion, Figure 1. The results for the second wave potentials are given in Table 2.

Table 2. Data for dianion formation; all at −74 °C.

Compound	$E_{1/2}$/V versus Ag (±0.05)	$E^{0\prime}$/V versus Ag (±0.05)	10^4 k_0/cm s^{-1} (±0.5)
3	−2.2	−2.2	1.5
4	−2.1	−2.2	0.5
5	−2.4	−2.4	0.02
6	−2.5	−2.5	0.8
8	−2.2	−2.2	0.5
10	−2.5	−2.4	0.5

It should be noted that some distortion of the voltammograms due to ohmic effects is possible; however, since the formal potentials quoted, and which form a significant part of the discussion below, are deduced with reference to both peak potentials in the quasi-reversible voltammogram the values reported are thought to be reliable. The results presented above are important references to the proposed mechanism of reduction. The substrates which show a second electron reduction follow the 'stabilised dianion' mechanism described previously (see Scheme 1). The measured formal potential of DBB is more negative than the formal potential for the second electron transfer to these heterocycles (e.g. −2.9 V vs. −2.2 V for **8**), so in the presence of the DBB radical anion thermodynamics favour the formation of the dianion of the substrate, Scheme 3.

In the case of substrates **7** and **9**, which form an 'unstable' dianion, the potential for formation of the dianion lies outside the solvent decomposition limits of the system used. However, the formal potential for the radical anions formed from these heterocycles is less negative than that of the electron transfer agent DBB. Accordingly, it is thermodynamically possible that the radical anion of these heterocycles would be formed from that of DBB (and also from the radical anion of naphthalene). These electrochemistry experiments do not enable us to confirm unequivocally that dianions are present in the ammonia free reduction. However, the fact that reduction requires an amine (as a very weak acid) implies that a reactive species abstracts a proton from it. We do not consider the radical anion derived from electron deficient heterocycles to be sufficiently basic to do this, and this premise requires the formation of a small amount of dianion, which is quickly protonated to yield an enolate. The electron transfer agents (especially naphthalene) may also be capable of forming a small amount of dianion themselves and these can then be protonated and reduced (the dianions of naphthalene and biphenyl have both been reported in the literature).[2] We suspect that this process is competitive with reduction of the heterocycle.

2.2.1. Discussion of electrochemistry data. The experimentally determined values of k_0 all fall within the range of 1×10^{-4} to 3.5×10^{-4} cm s^{-1} with the exceptions of naphthalene, DBB and the furan **7**. The higher value of k_0 for naphthalene is indicative of relatively facile electron transfer which is due to its rigidity, so leading to a low inner sphere reorganisation energy upon electron transfer.[15] It is interesting to note that both the electron transfer agents (**1** and **2**) have comparatively large D values.

One possible explanation for the effectiveness of **1** and **2** at sequestering an electron from the surface of lithium metal, compared to the more electron deficient compounds **3**–**10**, is that ion-pairing effects are important in the transfer of an electron from the metal surface. During electron transfer on the lithium surface, an ion of Li^+ is transferred into solution for every electron removed. We speculate that the radical anions derived from **1** and **2** would be expected to have a lesser degree of ion-pairing than the corresponding radical anion derived from compounds **3**–**10**, all of which have an electronegative atom on which electron density will be concentrated. Therefore, there may be less of an energy barrier in transferring an electron from Li metal to the two all-carbon aromatic compounds. Once the electron is in solution, however, it can rapidly be passed from the electron transfer agent to the (more) electron deficient heterocycles and reduction ensues: electron transfer in solution may also involve the transfer of Li^+, but here from one arene to another which may not have the same barrier as that on the metal surface. This can be represented via a plot showing the change in free energy as the reaction progresses, Figure 2.

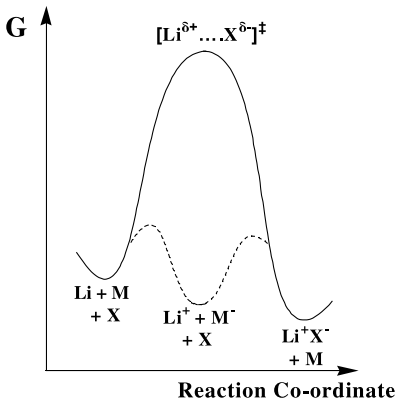
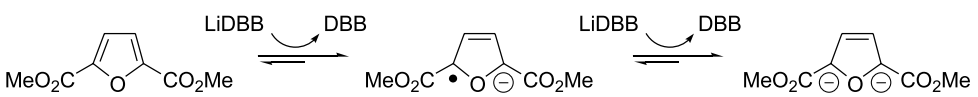


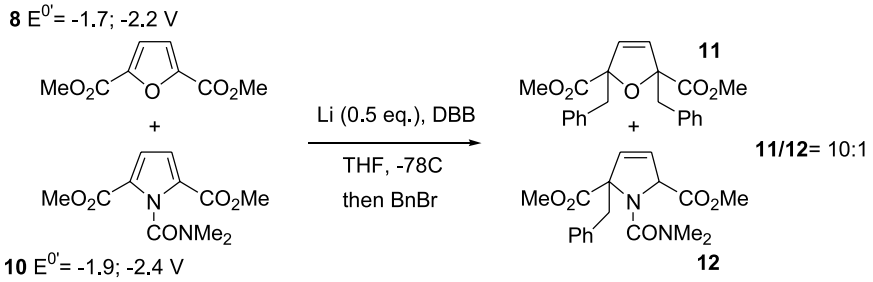
Figure 2. Schematic free energy diagram for the proposed reduction process: M=DBB, X=aromatic heterocycle.

2.3. Preliminary competition experiments

If the general mechanism that we propose in Scheme 1 is correct then we should be able to selectively reduce one type of heterocycle in the presence of another. To explore this possibility, a competition experiment was designed whereby equimolar amounts of pyrrole **10** and furan **8** were reduced with a sub-stoichiometric amount of lithium in



Scheme 3.



Scheme 4.

THF (with DBB present); the reaction was quenched with benzyl bromide. We deliberately sought a low conversion in order to avoid mass-action effects and we also made the assumption that the quenching of the reaction with an electrophile gives products which reflect the relative proportions of enolates present in solution. Analysis of the ^{1}H NMR spectrum from the crude reaction mixture revealed the presence of products **11**, from the furan **8**, as well as the mono-benzylated reduction product **12** from pyrrole **10** (these were compared against authentic standards prepared separately by the reduction of **8** and **10**, Scheme 4). Measurement of the integrals of the spectrum showed the amount of **12** to be $<10\%$ of the total reduced product. This was confirmed by integration of remaining starting material, which was strongly in favour of pyrrole **10**.

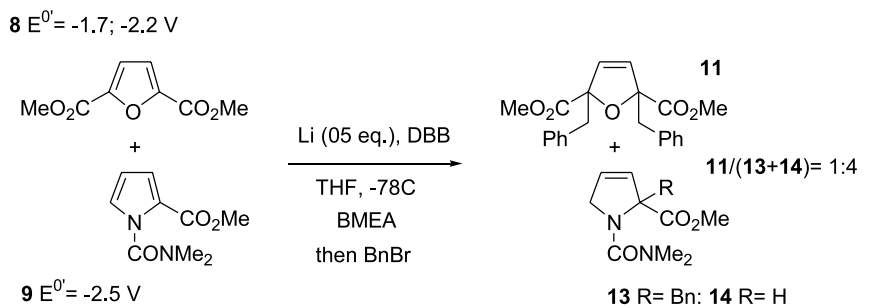
This result confirmed that the dominant product has arisen from the substrate with the least negative reduction potential. It is important to note that both **8** and **10** reduce under the same type of mechanism (formation of a stable dianion that is then quenched with an electrophile). Presumably, under these conditions the electron can add reversibly to the two heterocycles. Substrate **8** has a (second wave) formal reduction potential of -2.2 V versus Ag compared to compound **10** which has a (second wave) formal potential of -2.4 V versus Ag, giving a difference of 200 mV between the two. Using the relation between free energy and electrode potential the difference in the free energy of (double) reduction between **8** and **10** is 39 kJ mol^{-1}. This is a substantial difference, especially at -74 °C, and explains why **8** acts as an electron sink rather than **10**. The result suggests that when very electron deficient heterocycles (that form stable dianions) are used the ammonia free reduction will favour the formation of the thermodynamically most favourable dianion.

In addition, we also wanted to compare the reduction of two heterocycles, which reduce via different mechanisms (see Scheme 1). Under reducing conditions one of them (**9**) should be reduced by formation of an 'unstable' dianion species which will protonate irreversibly: the other (**8**) should form a stable dianion that will not be protonated at all. Therefore, in another competition experiment, equimolar amounts of compounds **8** and **9** were reduced with a sub-stoichiometric amount of lithium and DBB, Scheme 5. In this case, BMEA was added to protonate any reactive dianions present (we have precedent to show that BMEA does not react with the dianion derived from **8**[16]).

Analysis of the crude reaction mixture by ^{1}H NMR spectroscopy clearly showed that **9** was reduced in preference to **8** (proven by analysis of products **11**, **13**, **14** and remaining starting materials) despite **9** having a formal potential that was more negative than either the first or second wave potential of **8**.

One conceivable explanation for this difference in reactivity is that compound **9** is the only heterocycle capable of forming a reactive dianion, and each time a small amount is formed, it is removed (irreversibly) from the reaction by protonation by BMEA. Eventually, all of the available electrons in solution are partitioned through this reactive intermediate, which leads to the products derived from **9** after alkylation.

Whatever the explanation for this difference in reactivity it has been shown that the order of reduction of various aromatic compounds may (or may not) be predicted by their respective formal potentials. This depends upon whether they reduce by the same type of mechanism or not. We do know that selective reductions can be accomplished by



Scheme 5.

careful choice of the heterocycle and consideration of the likely mechanism of reduction.

2.4. Conclusions

This investigation into the electrochemical properties of heterocyclic compounds has produced data which have allowed us to test various aspects of the mechanism of the ammonia free Birch reduction. Development of analytical electrochemical techniques to encompass low temperature (anhydrous) conditions has been accomplished. An investigation into molecules which form stable dianions has shown that the thermodynamic reduction potential dictates product formation. This control has allowed chemoselective reduction to be achieved, as the equilibrium ratio of dianions is indicated by $E^{0\prime}$ and this type of reactivity could produce a powerful approach to predicting the outcome of synthetic reactions. Moreover, molecules that form unstable dianions can be reduced in the presence of more electron deficient counterparts that do not. Again, this change in relative reactivity could have synthetic applications.

Overall, this work has produced a set of electrochemical data on a series of heterocyclic compounds that has been enhanced by the use of competition experiments based on the relative rates of reduction of two substrates. This has given further insight into the interplay of factors controlling reduction and how these can be manipulated in a synthetically useful fashion. It has allowed us to develop a predictive tool that has direct relevance to preparative synthetic reactions and may now be applied to other functional groups and classes of compounds.

3. Experimental

3.1. General

For electrochemical experiments, a commercially available potentiostat (AUTOLAB PGSTAT30, Eco Chemie, The Netherlands) was employed. The airtight, small-volume electrochemical cell (ca. 25 mL) consisted of a three-electrode arrangement with a platinum wire counter electrode, and a silver wire quasi-reference electrode (Goodfellow Cambridge Ltd, Cambridge, UK). The working electrode employed was a 1 mm (diameter) platinum electrode housed in a Teflon™ insulating case; preliminary experiments employed working electrodes constructed from other material, such as lead, or a mercury-plated copper thin film electrode. The working electrodes were all carefully polished on a clean polishing pad (Kemet, UK) using a 1.0 μm aqueous alumina slurry (Beuhler, Lake Buff, Il., USA), and subsequently rinsed in de-ionised and doubly filtered water of resisitivity greater than 18 MΩ cm, taken from an Elgastat filter system (Vivendi, Bucks, UK). The electrode was carefully dried prior to immersing into the THF electrolyte. Tetra-*n*-butylammonium perchlorate (4.25 g, 12.5 mmol) was added to freshly distilled THF (25 mL) as the inert, supporting electrolyte (of concentration 0.5 M). All experiments were undertaken in an acetone/dry ice bath thermostatted at −74±2 °C; all electrolytic solutions were out-gassed for approximately 30 min using impurity-free nitrogen (BOC Gases, Guildford, Surrey, UK) to remove any trace oxygen dissolved in the electrolytes.

3.1.1. 1-Dimethylcarbamoyl-1*H*-pyrrole-2-carboxylic acid methyl ester 9. A solution of 1-*H*-pyrrole-2-carboxylic acid methyl ester (0.50 g, 4.0 mmol) in THF (10 mL) was added dropwise to a suspension of sodium hydride (0.19 g, 60% suspension in mineral oil, 4.8 mmol) in THF (10 mL) at 0 °C under an atmosphere of argon. The reaction was stirred for 30 min warmed to rt over 30 min. Carbamoyl chloride (0.73 mL, 8.0 mmol) was added and the reaction stirred for 14 h at rt. The reaction mixture was diluted with H_2O (10 mL) and extracted with EtOAc (3×30 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, petrol–EtOAc 65:35) to furnish the title compound **9** (0.51 g, 63%) as white needles. Mp 74–76 °C (from EtOAc); R_f (EtOAc) 0.61; ν_{max}(KBr)/cm^{-1} 3114, 2950, 1706, 1443, 1237; δ_H (400 MHz, $CDCl_3$) 7.01–6.97 (2H, m, ArH), 6.27 (1H, dd, J=2.8, 3.8 Hz, ArH), 3.83 (3H, s, O CH_3), 3.16 (3H, s, NCH_3), 2.70 (3H, s, NCH_3); δ_C (100 MHz, $CDCl_3$) 160.6, 153.5, 125.2, 122.7, 117.7, 110.4, 51.7, 37.9, 36.8; *m/z* (C.I.) 214 ($M+NH_4^+$, 6%), 197 ($M+H^+$, 100), 196 (M^+, 31), 181 (22), 165 (37), 72 (13); HRMS (C.I.) for $C_9H_{16}N_3O_3$ requires 214.1192, found ($M+NH_4^+$) 214.1200 (+4.0 ppm).

3.1.2. 1-Dimethylcarbamoyl-1*H*-pyrrole-2,5-dicarboxylic acid dimethyl ester, 10. *n*-Butyl lithium (10 mL 1.7 M solution in hexanes, 17 mmol) was added to a stirred solution of 2,2,6,6-tetramethylpiperidine (3.1 mL, 18 mmol) in THF (30 mL) at −78 °C under an atmosphere of argon. A solution of 1-dimethylcarbamoyl pyrrole (0.99 g, 7.2 mmol) in THF (10 mL) was added and the resulting solution stirred for 3 h. The reaction mixture was transferred via cannula into a solution of methyl chloroformate (1.7 mL, 22 mmol) in THF (10 mL) at −78 °C and stirred for a further 30 min. Saturated NH_4Cl solution (10 mL) was added and the reaction was extracted with Et_2O (2×30 mL). The combined organic extracts were washed with HCl (1 M, 30 mL) and brine (30 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, petrol–EtOAc 7:3) to furnish the title compound **10** (1.3 g, 70%) as orange plates. Mp 89–92 °C (from hexane); R_f (EtOAc) 0.65; ν_{max}(KBr)/cm^{-1} 3128, 2954, 1723, 1653, 1248; δ_H (400 MHz, $CDCl_3$) 6.93 (2H, s, ArH), 3.87 (6H, s, OCH_3), 3.24 (3H, s, NCH_3), 2.71 (3H, s, NCH_3); δ_C (100 MHz, $CDCl_3$) 160.0, 145.7, 126.6, 116.8, 52.2, 37.4, 36.7; *m/z* (C.I.) 272 ($M+NH_4^+$, 11%), 255 ($M+H^+$, 100) 195 (20); HRMS (C.I.) for $C_{11}H_{18}N_3O_5$ requires 272.1246, found ($M+NH_4^+$) 272.1236 (−4.0 ppm).

3.2. General procedure A: competition protocol

Sliced lithium wire (9.0 mg, 1.2 mmol) and DBB (530 mg, 2.0 mmol) were added to a Schlenk tube containing glass anti-bumping granules, under an atmosphere of argon and stirred for 2–3 h until the lithium was reduced to a fine powder. THF (20 mL) was added and the resultant turquoise solution was cooled to −78 °C and stirred for 30 min. A solution of substrate 1 (0.50 mmol), substrate 2

(0.50 mmol), BMEA (180 μL, 1.2 mmol) in THF (8 mL) was added dropwise to the reaction and the resulting mixture was stirred for 15 min. Benzyl bromide (200 μL, 2.0 mmol, filtered through K_2CO_3 and $MgSO_4$, was added and the mixture stirred for a further 10 min. The reaction was quenched with saturated NH_4Cl (10 mL), added to HCl (50 mL, 1 M) and extracted with EtOAc (3×30 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The residue was washed with petrol (200 mL) and the crude mixture analysed by ^1H NMR spectroscopy.

3.3. General procedure B: ammonia free Birch without BMEA protocol

Sliced lithium wire (28 mg, 4.0 mmol) and DBB (1.1 g, 4.0 mmol) were added to a Schlenk tube containing glass anti-bumping granules, under an atmosphere of argon and stirred for 2–3 h until the lithium was reduced to a fine powder. THF (20 mL) was added and the resultant turquoise solution was cooled to −78 °C and stirred for 30 min. A solution of substrate (1.0 mmol) in THF (8 mL) was added dropwise to the reaction and the resulting mixture was stirred for 15 min. Dibromoethane (filtered through K_2CO_3 and $MgSO_4$) was added to the reaction until the turquoise colour was quenched. Benzyl bromide (420 μL, 3.5 mmol, filtered through K_2CO_3 and $MgSO_4$) was added and the mixture stirred for a further 30 min. The reaction was quenched with saturated NH_4Cl (10 mL), added to HCl (50 mL, 1 M) and extracted with EtOAc (3×30 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash column chromatography.

3.4. General procedure C: ammonia free Birch with BMEA protocol

Sliced lithium wire (28 mg, 4.0 mmol) and DBB (1.1 g, 4.0 mmol) were added to a Schlenk tube containing glass anti-bumping granules, under an atmosphere of argon and stirred for 2–3 h until the lithium was reduced to a fine powder. THF (20 mL) was added and the resultant turquoise solution was cooled to −78 °C and stirred for 30 min. A solution of substrate (1.0 mmol), BMEA (180 μL, 1.2 mmol) and THF (8 mL) was added dropwise to the reaction and the resulting mixture was stirred for 15 min. Dibromoethane (filtered through K_2CO_3 and $MgSO_4$) was added to the reaction until the turquoise colour was quenched. Benzyl bromide (420 μL, 3.5 mmol, filtered through K_2CO_3 and $MgSO_4$) was added and the mixture stirred for a further 30 min. The reaction was quenched with saturated NH_4Cl (10 mL), added to HCl (50 mL, 1 M) and extracted with EtOAc (3×30 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash column chromatography.

3.4.1. 2,5-Dibenzyl-2,5-dihydro-furan-2,5-dicarboxylic acid dimethyl ester, 11. Furan-2,5-dicarboxylic acid dimethyl ester **8** (180 mg, 1.0 mmol) was subjected to reduction using general procedure B. The residue was purified by flash column chromatography (SiO_2, 500 mL petrol then petrol–EtOAc 4:1) to furnish the title compound **11** (150 mg, 40%) as a single (unassigned) diastereoisomer as white needles. Mp 94–96 °C (from Et_2O); R_f (petrol–EtOAc 4:1) 0.68; ν_{max}(KBr)/cm^{-1} 3030, 2952, 1752, 1496, 1454, 1435; δ_H (400 MHz, $CDCl_3$) 7.25–7.17 (10H, m, ArH), 5.95 (2H, s, C*H*═C*H*), 3.60 (6H, s, 2×OCH_3), 3.21 (2H, d, J=13.4 Hz, CH_AH_BPh), 3.15 (2H, d, J=13.4 Hz, CH_AH_BPh); δ_C (100 MHz, $CDCl_3$) 171.4, 135.1, 130.7, 130.4, 128.0, 126.7, 52.1, 44.4; *m/z* (C.I.) 384 ($M+NH_4^+$, 100%), 367 ($M+H^+$, 25); HRMS (C.I.) for $C_{22}H_{26}NO_5$ requires 384.1811, found ($M+NH_4^+$) 384.1797 (−3.6 ppm).

3.4.2. 2-Benzyl-1-dimethylcarbamoyl-2,5-dihydro-1*H*-pyrrole-2,5-dicarboxylic acid dimethyl ester, 12. 1-Dimethylcarbamoyl-1*H*-pyrrole-2,5-dicarboxylic acid dimethyl ester **10** (250 mg, 1.0 mmol) was subjected to reduction using general procedure B. The residue was purified by flash column chromatography (SiO_2, 500 mL petrol then petrol–EtOAc 3:2) to furnish the title compound **12** (67 mg, 19%) as a yellow oil (stereochemistry undefined). R_f (petrol–EtOAc) −0.12; ν_{max}(KBr)/cm^{-1} 2951, 1734, 1646, 1496, 1439, 1390; δ_H (400 MHz, $CDCl_3$) 7.23–7.15 (3H, m, ArH), 7.04–7.02 (2H, m, ArH), 5.82 (2H, t, J=1.8 Hz, C*H*═C*H*), 4.61 (1H, t, NCH), 3.76 (3H, s, CO_2CH_3), 3.69 (3H, s, CO_2CH_3), 3.57 (1H, d, J=14.4 Hz, CH_AH_BPh), 3.25 (1H, d, J=14.4 Hz, CH_AH_BPh), 2.63 (6H, s, N(CH_3)$_2$); δ_C (100 MHz, $CDCl_3$) 172.4, 168.8, 158.9, 136.8, 131.8, 130.5, 127.7, 127.3, 126.4, 77.5, 67.7, 52.4×2, 38.2, 37.8; *m/z* (C.I.) 347 ($M+H^+$, 100), 287 (28), 255 (42); HRMS (C.I.) for $C_{18}H_{23}N_2O_5$ requires 347.1607, found ($M+H^+$) 347.1622 (+4.2 ppm).

3.4.3. 2-Benzyl-1-dimethylcarbamoyl-2,5-dihydro-1*H*-pyrrole-2-carboxylic acid methyl ester 13 and (2-*RS*)-1-dimethylcarbamoyl-2,5-dihydro-1*H*-pyrrole-2-carboxylic acid methyl ester, 14. 1-Dimethylcarbamoyl-1*H*-pyrrole-2-carboxylic acid methyl ester **9** (200 mg, 1.0 mmol) was subjected to reduction using general procedure C. The residue was purified by flash column chromatography (SiO_2, 500 mL petrol then petrol–EtOAc 1:1) to furnish title compound **13** (92 mg, 31%) as a yellow oil and title compound **14** (75 mg, 37%) as a brown oil. Data for **13** R_f (EtOAc) 0.46; ν_{max}(film)/cm^{-1} 2949, 1736, 1642, 1625, 1496, 1454, 1388, 1244; δ_H (400 MHz, $CDCl_3$) 7.24–7.15 (3H, m, ArH), 7.08–7.05 (2H, m, ArH), 5.75 (1H, dt, J=1.8, 6.3 Hz, C*H*═CH), 5.63 (1H, dt, J=2.3, 6.3 Hz, CH═C*H*), 4.06 (1H, dt, J=2.0, 13.9 Hz, NCH_AH_B), 3.77 (0.5H, t, J=2.0 Hz, NCH_AH_B), 3.75–3.74 (3.5H, m, OCH_3 and NCH_AH_B), 3.58 (1H, d, J=14.1 Hz, CH_AH_BPh), 3.26 (1H, d, J=14.1 Hz, CH_AH_BPh), 2.74 (6H, s, N(CH_3)$_2$); δ_C (100 MHz, $CDCl_3$) 173.5, 160.8, 137.4, 133.6, 130.4, 129.6, 127.5, 126.1, 55.8, 53.6, 52.3, 38.6, 38.3; *m/z* (C.I.) 289 ($M+H^+$, 100), 229 (56), 197 (27), 72 (34); HRMS (C.I.) for $C_{16}H_{21}N_2O_3$ requires 289.1552, found ($M+H^+$) 289.1541 (−3.8 ppm).

Data for **14** R_f (EtOAc) 0.23; ν_{max}(film)/cm^{-1} 2952, 1753, 1642, 1622, 1389; δ_H (400 MHz, $CDCl_3$) 5.83 (2H, m, C*H*═C*H*), 5.48 (1H, m, *H*C(CO_2Me)), 4.36 (1H, m, NCH_AH_B), 4.07 (1H, m, NCH_AH_B), 3.68 (3H, s, OCH_3), 2.88 (6H, s, N(CH_3)$_2$); δ_C (100 MHz, $CDCl_3$) 171.5, 162.4, 127.7, 125.0, 67.3, 56.1, 52.2, 38.4; *m/z* (C.I.) 199 ($M+H^+$, 100%), 197 (54), 139 (44), 72 (31); HRMS (C.I.) for $C_9H_{-15}N_2O_3$ requires 199.1082, found ($M+H^+$) 199.1083 (+0.1 ppm).

Competition experiment 1: selective reduction of **8** and **10**. Furan-2,5-dicarboxylic acid dimethyl ester **8** (93 mg, 0.51 mmol) and 1-dimethylcarbamoyl-1*H*-pyrrole-2,5-dicarboxylic acid dimethyl ester **10** (130 mg, 0.51 mmol) were subjected to general procedure A, except that no BMEA was added to the reaction mixture.

Competition experiment 2: selective reduction of **8** and **9**. Furan-2,5-dicarboxylic acid dimethyl ester **8** (92 mg, 0.50 mmol) and 1-dimethylcarbamoyl-1 *H*-pyrrole-2-carboxylic acid methyl ester **9** (99 mg, 0.51 mmol) were subjected to general procedure A.

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Synthesis of simple analogues of methyllycaconitine—an efficient method for the preparation of the *N*-substituted anthranilate pharmacophore

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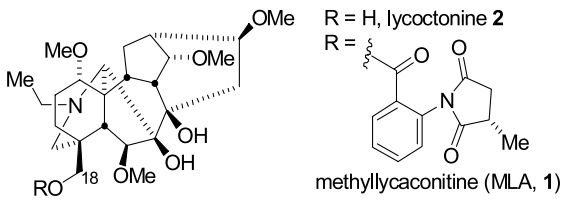
Abstract—The synthesis of several A and AE ring analogues of the alkaloid methyllycaconitine is reported. The key 2-(2″-methylsuccinimido)benzoate ester pharmacophore is introduced using an efficient two step procedure. Esterification of the alcohol precursors with *N*-(trifluoroacetyl)anthranilic acid under Steglich conditions followed by sodium borohydride mediated cleavage of the trifluoroacetyl group affords the anthranilate esters. Subsequent fusion with methylsuccinic anhydride affords the *N*-substituted anthranilate derivatives containing the key pharmacophore present in a range of commonly occurring *Delphinium* and *Aconitum* alkaloids.

Nicotinic acetylcholine receptors (nAchRs) are a family of ligand gated ion channels that are widely distributed in the human brain.[1,2] These receptors have numerous receptor subtypes composed of combinations of α2-7, α9-10 and β2-4 subunits.[3] nAchRs are involved in a number of physiological and behavioural conditions hence there is a pressing need for subtype selective agonists and antagonists to elucidate the biological roles of these receptors and to provide candidates for drug discovery. The α7 nAChR subtype is amongst the most prevalent in the brain and has been implicated as playing a key role in conditions such as schizophrenia, Alzheimer's disease and epilepsy.[4] Methyllycaconitine (MLA) **1**[5] is one of only a few compounds (including the peptide toxins α-bungarotoxin[6] and α-conotoxin ImI[7]) that binds with high affinity and selectivity to the α7 nAChR. MLA **1** is therefore a prime lead compound for development of new therapies targeting the α7 nAChR. We have therefore embarked on a programme to provide novel compounds that may help elucidate the key structural features of nAChR ligands that give rise to binding affinity, subtype selectivity and agonist/antagonist activity.



MLA **1** is the major toxic component of *Delphinium brownii*[8] and is a potent antagonist of the α7 nAChR in mammalian neuronal membranes. Furthermore, it exhibits very high selectivity for this subtype over other neuronal nAChRs rendering it a prime lead for the development of new therapeutic agents targeting the α7 nAChR. Structure activity studies on MLA have shown the *N*-substituted anthranilate ester moiety is an essential structural feature for pharmacological activity[9] and competitive ligand binding studies revealed that MLA **1** containing the 2-(2′-methylsuccinimido)benzoate ester sidechain displays ca. 10^3 times more potent inhibition that the parent alkaloid lycoctonine **2**.[10] It has also been proposed that the tertiary amine and ester sidechain of MLA form an acylated homocholine pharmacophore at physiological pH that gives rise to the high affinity nicotinic acetylcholine receptor binding.

A number of approaches to the synthesis of small molecule analogues of MLA incorporating the putative pharmacophore have been reported, including the synthesis of E,[11] AE[12] and AEF[13] ring systems, some of which display

Keywords: Methyllycaconitine; Anthranilate esters; Nicotinic acetylcholine receptors; Alkaloids.
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significant biological activity.[11,14] Given the demonstrated importance of the *N*-substituted anthranilate sidechain to the pharmacology of MLA analogues, we herein report[15] the full synthetic details for several analogues of MLA prepared using our recently developed efficient procedure for the introduction of this key structural unit.

Previous syntheses of *Delphinium* alkaloids and their analogues that contain a 2-(2′-methylsuccinimido)benzoate ester sidechain have made use of one of two methods (Scheme 1). The first method involves a two-step process using isatoic anhydride **3** to convert the alcohol into an anthranilate[16] then adding methylsuccinic anhydride **4** to form the desired cyclic imide. The second more convergent method involves direct addition of the entire 2-(3-methyl-2,5-dioxopyrrolidin-1-yl)benzoate group to the alcohol in a single step by esterification of the alcohol with acid **5**.

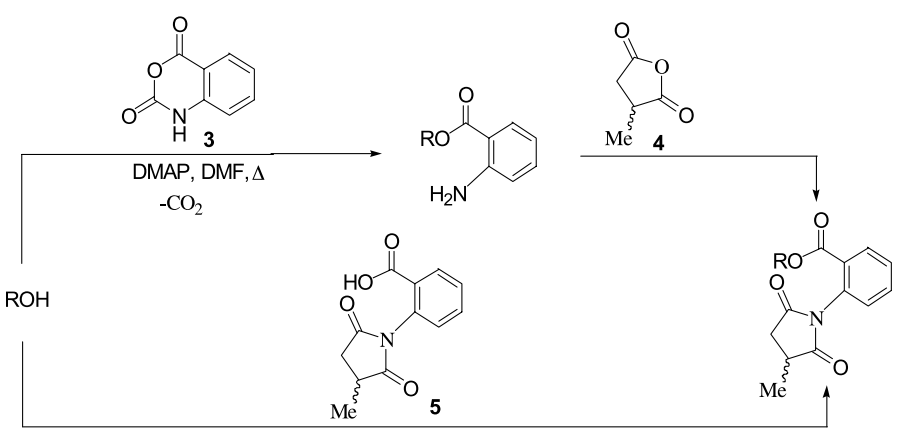
Kraus and Dneprovskaia[13b] reported the esterification of 2-(3-methyl-2,5-dioxopyrrolidin-1-yl)benzoic acid **5** by formation of the sodium salt of acid **5** followed by treatment with oxalyl chloride to generate the acid chloride, however our attempts to repeat this procedure met with little success. The same authors also reported that this high yielding esterification procedure failed to work using more hindered neopentyl-type alcohols giving mixtures of the desired ester and undesired isomeric carbamate by-product, and the procedure failed completely when using tertiary alcohols. They overcame this problem by effecting an S_N2 displacement of the neopentyl mesylate by the sodium salt of acid **5**. In our hands this procedure failed to give significant quantities of the anthranilate ester of the model compound, 1-methyl-3-piperidinemethanol **7** (Table 1). Bergmeier and co-workers[11a] reported that esterification of acid **5** using the coupling agent, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) was inconsistent with yields ranging from 6 to 60%.

Use of isatoic anhydride **3** as developed by Blagbrough[17] has been adopted by others to append the anthranilate ester group to MLA analogues.[13] In general, low yields (typically 40–65%) of the desired anthranilate esters are obtained which can be attributed to the hindered neopentyl environment of the C-18 hydroxyl group present in lycoctonine and many simpler analogues. The low yields of ester formation by these methods have prompted investigations into the introduction of this sidechain by alternative procedures,[13,18] however none of these methods has offered a general and high yielding solution to the problem of sidechain introduction.

In connection with our studies towards MLA analogues we turned our attention to the use of *N*-(trifluoroacetyl)anthranilic acid **6**[19] and the coupling procedure developed by Breslow[19] as an alternative to the isatoic anhydride mediated synthesis. Initial attempts to repeat this coupling procedure on stoichiometric quantities of the alkoxide salt derived from 1-methyl-3-piperidinemethanol (**7**, Table 1) did not result in practical yields of the coupled product. However, reaction of **7** with 3 equiv. of *N*-(trifluoroacetyl)-anthranilic acid **6** under Steglich conditions[11] (DCC/DMAP) followed directly by sodium borohydride mediated cleavage of the crude amide gave a gratifying 81% yield of the anthranilate ester. This compares favourably with the base catalysed reaction of isatoic anhydride, which in our hands proceeded in 72% yield (Table 1, entry 1).[16b] This high yielding and operationally simple anthranilate ester synthesis prompted us to explore the scope of this coupling reaction as an alternative approach.

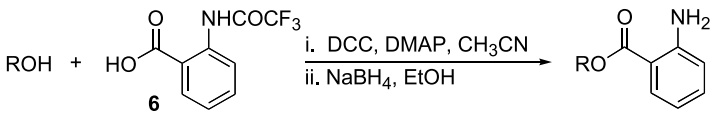
This two step coupling procedure was studied using a range of primary, neopentyl, secondary and tertiary alcohols together with the azabicyclic neopentyl alcohols **8**, **9** (Table 1) and diol **10** (Scheme 3). Heating ethyl 2-oxocyclohexane carboxylate with ethylamine and formaldehyde, according to the method of Iwai et al.[21] afforded the double Mannich adduct **11** (Scheme 2) that underwent Wittig reaction with the ylide derived from methyltriphenylphosphonium bromide to afford alkene **12** and thence alcohol **8** upon reduction of the ester with $LiAlH_4$. Reduction of keto ester **11** afforded a 1:1.25 mixture of the alcohols **13** and **14** and the major isomer **14** underwent smooth methylation to methyl ether **15**, followed by reduction of the ester to alcohol **9**. Direct reduction of **11** with $LiAlH_4$ afforded diol **10** that was used to probe the selective esterification of the neopentyl alcohol using this coupling procedure.

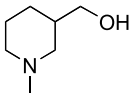
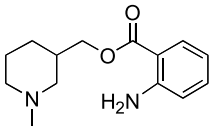
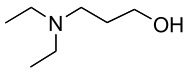
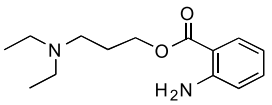
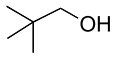
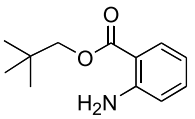
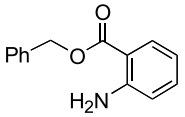
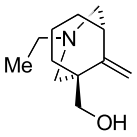
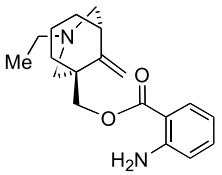
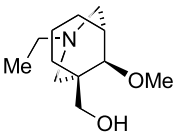
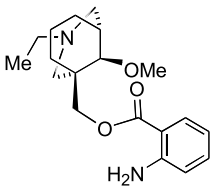
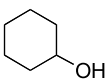
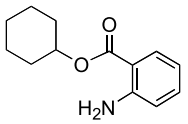
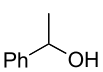
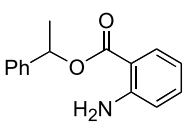
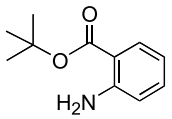
The reaction of a range of simple primary alcohols with *N*-(trifluoroacetyl)anthranilic acid **6** afforded good yields of the anthranilate esters in comparison with the isatoic anhydride mediated synthesis (Table 1, entries 2, 3 and 4).



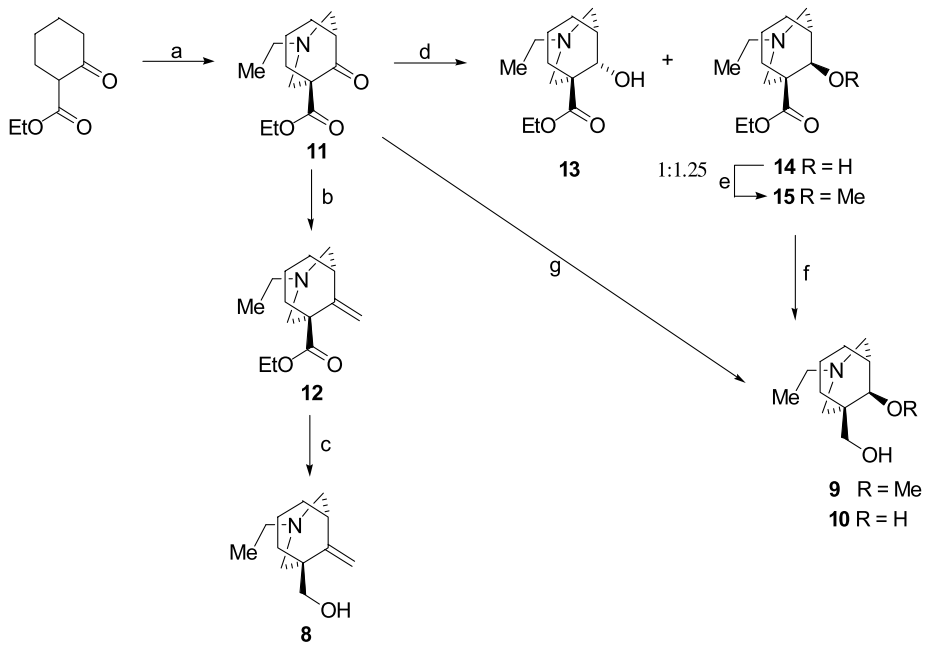
Scheme 1.

Table 1. Synthesis of anthranilate esters with *N*-(trifluoroacetyl)anthranilic acid **6**



Entry	Alcohol	Product	Yield of ester (%)[a]
1	 **7**	 **16**	81 (72)[b,c]
2		 **17**	85 (75)[b,c]
3		 **18**	94 (64)[b,d]
4		 **19**	85 (90)[b,e]
5	 **8**	 **20**	75 (40)[b,c]
6	 **9**	 **21**	78 (65)[b,d]
7		 **22**	97 (16)[b,c]
8		 **23**	91
9		 **24**	39 (77)[f]

[a] Yield of anthranilate ester prepared by alternative literature based methods in parentheses.
[b] Prepared by reaction with isatoic anhydride.
[c] Prepared according to Ref. 16b.
[d] See Ref. 17c.
[e] See Ref. 16b.
[f] See Ref. 19.



Scheme 2. Reagents and conditions: (a) $EtNH_2$, CH_2O, EtOH, reflux, 30 h, 27%; (b) *n*-BuLi, $MePPh_3Br$, THF, 92%; (c) $LiAlH_4$, THF, room temperature, 10 min, 94%; (d) $NaBH_4$, 1:1 THF/H_2O, 0 °C, 30 min, 81%; (e) NaH, MeI, THF, 0 °C, 70%; (f) $LiAlH_4$, THF, room temperature, 2 h, 91%; (g) $LiAlH_4$, THF, room temperature, 1 h, 54%.

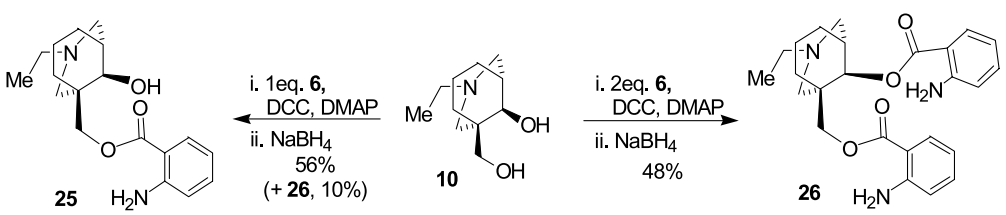
Extension of this procedure to AE bicyclic analogues of MLA, **8** and **9**[17d] (Table 1, entries 5 and 6) containing neopentyl substituted alcohols, also afforded coupled product in improved yield.

Secondary alcohols were observed to react readily with **6** to afford high yields of the anthranilate ester (Table 1, entries 7 and 8) providing the first direct, high yielding synthesis of anthranilate esters derived from secondary alcohols reported in the literature.[16] Attempts to promote the esterification of tertiary alcohols however, afforded lower yields of the coupled product, in line with the synthesis of *t*-butyl benzoate ester derivatives initially reported by Steglich.[20] The use of di-2-pyridyl thiocarbonate, recommended[22] for the synthesis of esters derived from tertiary alcohols, failed to give the desired product.

The extreme conditions associated with the isatoic anhydride mediated synthesis of anthranilate esters have been reported to lead to poor regioselectivity or possibly transesterification during reactions with diol substrates.[5a] We therefore investigated the new procedure to assess its potential for kinetic discrimination leading to the selective esterification of diol substrates (Scheme 3). To this end the reaction of bicyclic diol **10** with 1 equiv. of acid **6** favoured reaction at the primary hydroxyl to give mono-anthranilate ester **25** in 56%, together with a small quantity of the diester **26** (10%). Reaction of **10** with 2 equiv. of acid **6** gave the di-ester **26** in a moderate 48% yield.

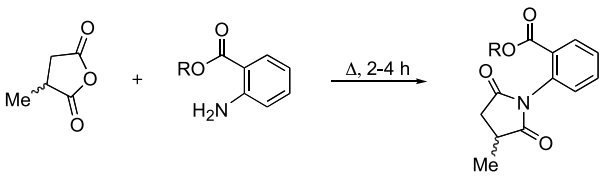
Finally, heating the anthranilate esters with 2 equiv. of 2-methylsuccinic acid at 125 °C according to the procedure of Blagbrough[17c] cleanly afforded the succinimide derivatives in good yield (Table 2). This procedure therefore offers a simple two step synthesis of the 2-(2′-methylsuccinimido)benzoate ester sidechain present in methyllycaconitine **1** and other *Delphinium* alkaloids.

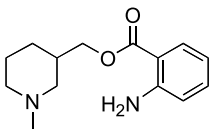
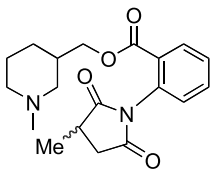
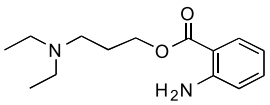
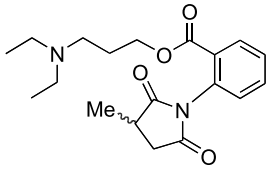
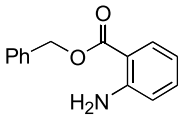
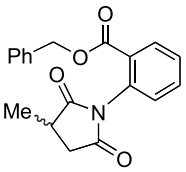
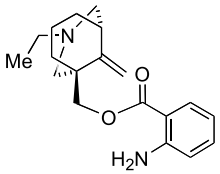
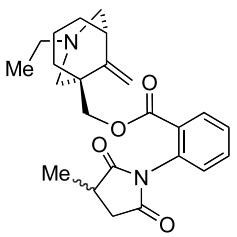
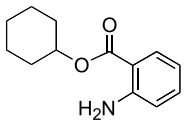
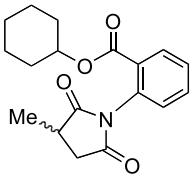
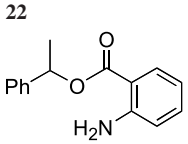
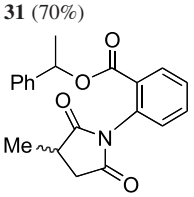
In conclusion, we have developed a practical, high yielding synthesis of anthranilate esters from primary and secondary alcohols using *N*-(trifluoroacetyl)anthranilic acid **6**. The reactions proceed under mild conditions and offer a practical alternative to existing procedures. The method has wide applicability for the synthesis of diterpenoid alkaloids such as methyllycaconitine **1** and their analogues and has been readily adopted by us for the synthesis of more complex tricyclic analogues of MLA.



Scheme 3.

Table 2. Reaction of anthranilate esters with methyl succinic anhydride



Entry	Anthranilate ester	Succinimide (yield %)
1	 **16**	 **27** (96%)
2	 **17**	 **28** (91%)
3	 **19**	 **29**[a] (73%)
4	 **20**	 **30** (84%)
5	 **22**	 **31** (70%)
6	 **23**	 **32** (70%)

[a] See Ref. 13a.

1. Experimental

1.1. General details

tert-Butyl 2-aminobenzoate **24** was synthesised using standard procedure given in Section 1.2 to give data which is in agreement literature values.[19] Benzyl 2-(3-methyl-2,5-dioxopyrrolidin-1-yl)benzoate **29** was synthesised using standard procedure given in Section 1.3 to give data which is in agreement literature values.[13a] All reactions were conducted in flame-dried or oven-dried glassware under a dry nitrogen atmosphere unless otherwise noted. Tetrahydrofuran was dried over sodium/benzophenone and distilled prior to use. Flash chromatography was performed using Merck Kieselgel 60 (230–400 mesh) with the indicated solvents. Thin layer chromatography (TLC) was carried out on precoated silica plates (Merck Kieselgel $60F_{254}$) and compounds were visualized by UV fluorescence or by staining with vanillin in methanolic sulfuric acid and heating. Infrared spectra were recorded with a Perkin Elmer 1600 series Fourier-transform infrared spectrometer as thin films between sodium chloride plates. Absorption maxima are expressed in wavenumbers (cm^{-1}) with the following abbreviations: s=strong, m=medium, w=weak and br=broad. ^1H and ^{13}C NMR spectra were obtained using a Bruker AC 200B or a Bruker AM 400 spectrometer. All chemical shifts are given in parts per million (ppm) downfield from tetramethylsilane as internal standard (^1H) or relative to $CDCl_3$ (^{13}C) and J values are given in Hz. ^1H NMR data are tabulated as s, singlet; d, doublet; t, triplet; q, quartet, m, multiplet, br, broad. High-resolution mass spectra were recorded using a VG70-SE spectrometer operating at nominal accelerating voltage of 70 eV. Chemical ionisation (CI) mass spectra were obtained with ammonia as the reagent gas.

1.1.1. Ethyl (1*R* *,5*R* *)-3-ethyl-9-oxo-3-azabicyclo-[3.3.1]nonane-1-carboxylate 11.[17d] A mixture of ethyl 2-oxo-cyclohexane-1-carboxylate (5.60 g, 32.9 mmol), ethylamine (1.48 g, 32.9 mmol, 40% aq. v/v) and formaldehyde (1.97 g, 65.8 mmol, 36% aq. v/v) in ethanol (500 ml) was heated under reflux for 30 h. After removal of the solvent at reduced pressure, the oily orange residue was dissolved in ether (200 ml) and extracted with 2 M hydrochloric acid (3×80 ml). The aqueous extract was made basic with 10% sodium hydroxide then extracted with ether (3×150 ml) and dried over anhydrous sodium sulfate. The solvent was removed at reduced pressure and the resultant dark orange oil was purified by flash chromatography (19:1 hexane–ethyl acetate) to afford the title compound **11** (2.10 g, 27%) as a bright yellow oil. ν_{max}(NaCl)/cm^{-1} 1738 (C=O, ester), 1718 (C=O, ketone); δ_H (400 MHz; $CDCl_3$) 1.07 (3H, t, J=7.2 Hz, NCH_2CH_3), 1.25 (3H, t, J=7.2 Hz, OCH_2CH_3), 1.45–1.53 (1H, m, 7B-H), 1.98–2.29 (3H, m, 6-CH_2, 8A-H), 2.35–2.54 (5H, m, 4B-H, 5-H, 8B-H, NCH_2CH_3), 2.76–2.87 (1H, m, 7A-H), 2.89 (1H, d, J_{gem}=11.4 Hz, 2B-H), 3.11 (1H, ddd, $J_{4A,5}$=2.2 Hz, $J_{4A,2A}$=2.2 Hz, J_{gem}=11.0 Hz, 4A-H), 3.18 (1H, dd, $J_{2A,4A}$=2.2 Hz, J_{gem}=11.4 Hz, 2A-H), 4.17 (2H, q, J=7.2 Hz, OCH_2CH_3); δ_C (100 MHz; $CDCl_3$) 12.6 (CH_3, NCH_2CH_3), 14.0 (CH_3, OCH_2CH_3), 20.4 (CH_2, C-7), 34.0 (CH_2, C-6), 36.7 (CH_2, C-8), 47.1 (CH, C-5), 51.0 (CH_2, NCH_2CH_3), 58.7 (quat., C-1), 59.8 (CH_2, C-4), 60.9 (CH_2, OCH_2CH_3), 61.5 (CH_2, C-2), 171.1 (quat., OC=O), 212.6 (quat., C-9); m/z (EI) 239 (M^+, 13), 224 ($M-CH_3$, 17), 222 (100), 210 ($M-C_2H_5$, 7), 196 (64), 194 ($M-OC_2H_5$, 32).

1.1.2. Ethyl (1*S* *,5*S* *)-3-ethyl-9-methylidene-3-aza-bicyclo[3.3.1]nonane-1-carboxylate 12. *n*-BuLi (5.3 ml,

8.48 mmol, 1.6 M solution in hexane) was added dropwise to a suspension of methyltriphenylphosphonium bromide (4.06 g, 11.37 mmol) in dry THF (40 ml) at −78 °C. The reaction mixture was stirred at 0 °C for 10 min then cooled to −78 °C and ethyl (1*R**,5*R**)-3-ethyl-9-oxo-3-azabicyclo[3.3.1]nonane-1-carboxylate **11** (680 mg, 2.84 mmol) in dry THF (10 ml) added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 1 h. The reaction was quenched with distilled water (10 ml) and the solvent removed at reduced pressure. The residue was dissolved in dry ether (40 ml) and extracted with 2 M hydrochloric acid (3×80 ml). The aqueous extract was made basic with 10% sodium hydroxide solution (250 ml) then extracted with ether (3×100 ml) then dried ($MgSO_4$) and concentrated in vacuo to leave the crude product which was purified by flash chromatography (19:1 hexane–ethyl acetate) to give the title compound **12** (624 mg, 92%) as a pale yellow oil. ν_{max}(NaCl)/cm^{-1} 2915 (CH), 1728 (C═O), 1651 (C═C), 1452, and 1252; δ_H (200 MHz; $CDCl_3$) 1.00 (3H, t, *J*=7.2 Hz, NCH_2CH_3), 1.19 (3H, t, *J*=7.1 Hz, OCH_2CH_3), 1.36–1.82 (1H, m, 7B-H), 1.66–1.89 (3H, m, 6-CH_2 and 8A-H), 2.03–2.26 (4H, m, 5-H, 8B-H and NCH_2), 2.31–2.34 (1H, m, 4B-H), 2.42–2.49 (1H, dd, $J_{2B,4B}$=1.7 Hz, J_{gem}=10.7 Hz, 2B-H), 2.59–2.74 (1H, m, 7A-H), 2.87–2.99 (2H, m, 2A-H and 4A-H), 4.09 (2H, q, *J*=7.1 Hz, OCH_2), 4.38 (1H, d, *J*=0.8 Hz, 10A-H) and 4.65 (1H, br s, 10B-H); δ_C (100 MHz; $CDCl_3$) 12.4 (CH_3, NCH_2CH_3), 14.0 (CH_3, OCH_2CH_3), 21.2 (CH_2, C-7), 33.3 (CH_2, C-6), 35.6 (CH_2, C-8), 40.8 (CH, C-5), 50.3 (quat., C-1), 51.8 (CH_2, NCH_2CH_3), 60.1 (CH_2, OCH_2CH_3), 60.4 (CH_2, C-4), 61.8 (CH_2, C-2), 103.2 (CH_2, C-10), 152.0 (quat., C-9) and 174.1 (quat., OC═O); *m*/*z* (EI) 237 (M^+, 50%), 222 (M−CH_3, 73), 208 (M−CH_3CH_2, 50), 164 (M−CH_3CH_2OCO, 69) and 58 (100). Found: M^+ 237.1743. $C_{14}H_{23}NO_2$ requires M^+ 237.1743.

1.1.3. (1*S,5*S**)-(3-Ethyl-9-methylidene-3-azabicyclo-[3.3.1]non-1-yl)methanol 8.** To a solution of ethyl (1*S**,5*S**)-3-ethyl-9-methyidene-3-azabicyclo[3.3.1]nonane-1-carboxylate **12** (250 mg, 1.05 mmol) in dry THF (25 ml) was added lithium aluminium hydride (80 mg, 2.11 mmol) and the mixture stirred, under an atmosphere of nitrogen, for 10 min. The reaction was then quenched by dropwise addition of water (10 ml), the volatiles removed in vacuo. The remaining aqueous mixture was extracted with ethyl acetate (2×30 ml) and the combined organic layers washed with brine (50 ml) then dried ($MgSO_4$) and concentrated in vacuo to give the crude product which was purified by flash chromatography (1:1 hexane–ethyl acetate) to give the title compound **8** (191 mg, 94%) as a clear oil. ν_{max}(NaCl)/cm^{-1} 3356 (OH), 2914 (CH), 1649 (C═C), 1471, 1451, and 1239; δ_H (200 MHz; $CDCl_3$) 1.05 (3H, t, *J*=7.2 Hz, NCH_2CH_3), 1.22–1.48 (2H, m, 6B-H and 7B-H), 1.59–1.74 (1H, m, 8A-H), 1.82–1.99 (3H, m, 5-H, 6A-H, 8B-H), 2.12–2.17 (1H, m, 4B-H), 2.26 (2H, q, *J*=7.2 Hz, NCH_2CH_3), 2.34–2.35 (1H, m, 2B-H), 2.56–2.78 (2H, m, 7A-H and 11-OH), 2.92–2.97 (2H, m, 2A-H and 4A-H), 3.44 (2H, m, OCH_2), 4.43 (1H, br s, 10A-H) and 4.68 (1H, br s, 10B-H); δ_C (50 MHz; $CDCl_3$) 12.5 (CH_3, NCH_2CH_3), 21.3 (CH_2, C-7), 34.1 (CH_2, C-6), 36.1 (CH_2, C-8), 41.8 (quat., C-1), 41.9 (CH, C-5), 52.1 (CH_2, NCH_2CH_3), 60.5 (CH_2, C-4), 62.3 (CH_2, C-2), 68.8 (CH_2, OCH_2), 101.0 (CH_2, C-10) and 155.0 (quat., C-9); *m*/*z* (EI) 195 (M^+, 13%), 180 (M−CH_3, 62), 178 (M−OH, 34), 164 (M−CH_2OH, 18) and 72 (100). Found: M^+ 195.1634. $C_{12}H_{21}NO$ requires M^+ 195.1623.

1.1.4. Ethyl (1*R,5*R**,9*R**)-3-ethyl-9-hydroxy-3-azabicyclo[3.3.1]nonane-1-carboxylate 14 and ethyl (1*R**,5*R**,9*S**)-3-ethyl-9-hydroxy-3-azabicyclo[3.3.1]-nonane-1-carboxylate 13.** A solution of ethyl (1*R**,5*R**) 3-ethyl-9-oxo-3-azabicyclo[3.3.1]nonane-1-carboxylate **11** (3.00 g, 12.54 mmol) in THF (15 ml) was added dropwise to a solution of sodium borohydride (0.24 g, 6.27 mmol) in THF (15 ml) and water (15 ml) at 0 °C and the mixture stirred for 30 min. The reaction mixture was then allowed to warm to room temperature and stirred for a further 2 h. After this time the reaction was quenched by the addition of 2.5 M NaOH (20 ml) and the volatile solvents removed in vacuo. The remaining aqueous mixture was extracted with diethyl ether (3×50 ml), the combined ether layers washed with brine (100 ml) then dried (Na_2SO_4) and concentrated in vacuo to give a residue which was purified by flash chromatography (4:1 hexane–ethyl acetate) to give: (i) ethyl (1*R**,5*R**,9*R**)-3-ethyl-9-hydroxy-3-azabicyclo-[3.3.1]nonane-1-carboxylate **14** (R_f 0.4) (1.36 g, 45%). ν_{max}(NaCl)/cm^{-1} 3527 (OH) and 1707 (C═O, ester); δ_H (400 MHz; $CDCl_3$) 1.03 (3H, t, *J*=6.9 Hz, NCH_2CH_3), 1.25 (3H, t, *J*=7.1 Hz, OCH_2CH_3), 1.45–1.46 (2H, m, 6B-H and 7B-H), 1.74–1.79 (1H, m, 8B-H), 1.94–2.09 (4H, m, 4B-H, 5-H, 6A-H and 8A-H), 2.20 (2H, q, *J*=7.1 Hz, NCH_2CH_3), 2.22–2.25 (1H, m, 2B-H), 2.57–2.61 (1H, m, 7A-H), 2.93 (1H, d, J_{gem}=11.0 Hz, 4A-H), 3.15 (1H, d, J_{gem}=11.0 Hz, 2A-H), 3.47 (1H, br s, OH), 3.87 (1H, s, 9-H) and 4.15 (2H, q, *J*=7.1 Hz, OCH_2CH_3); δ_C (100 MHz; $CDCl_3$) 12.7 (CH_3, NCH_2CH_3), 14.0 (CH_3, OCH_2CH_3), 20.8 (CH_2, C-7), 23.5 (CH_2, C-6), 27.5 (CH_2, C-8), 34.6 (CH, C-5), 46.7 (quat., C-1), 51.9 (CH_2, NCH_2CH_3), 58.2 (CH_2, C-4), 59.1 (CH_2, C-2), 60.7 (CH_2, OCH_2CH_3), 71.8 (CH, C-9) and 177.0 (quat., OC═O); *m*/*z* (EI) 241 (M^+, 40%), 224 (M−CH_3, 40), 224 (M−OH, 14), and 72 (100). Found: M^+ 241.16743. $C_{13}H_{23}NO_3$ requires M^+ 241.16780; (ii) ethyl (1*R**,5*R**, 9*S**)-3-ethyl-9-hydroxy-3-azabicyclo[3.3.1]nonane-1-carboxylate **13** (R_f 0.28) (1.09 g, 36%). ν_{max}(NaCl)/cm^{-1} 3508 (OH) and 1728 (C═O, ester); δ_H (400 MHz; $CDCl_3$) 1.04 (3H, t, *J*=7.1 Hz, NCH_2CH_3), 1.25 (3H, t, *J*=7.1 Hz, OCH_2CH_3), 1.40–1.46 (1H, m 7B-H), 1.74–1.79 (1H, m, 6B-H), 1.93–2.09 (3H, m, 6A-H and 8-CH_2), 2.17–2.24 (1H, m, 5-H), 2.25 (2H, q, *J*=7.1 Hz, NCH_2CH_3), 2.30–2.66 (3H, m, 2B-H, 4B-H and 7A-H), 2.94 (1H, d, J_{gem}=11.0 Hz, 4A-H), 3.15 (1H, d, J_{gem}=11.0 Hz, 2A-H), 3.47 (1H, br, OH), 3.87 (1H, br s, 9-H) and 4.13 (2H, q, *J*=7.1 Hz, OCH_2CH_3); δ_C (100 MHz; $CDCl_3$) 12.5 (CH_3, NCH_2CH_3), 14.1 (CH_3, OCH_2CH_3), 20.7 (CH_2, C-7), 31.0 (CH_2, C-6), 34.4 (CH_2, C-8), 35.2 (CH, C-5), 48.1 (quat., C-1), 51.6 (CH_2, NCH_2CH_3), 52.2 (CH_2, C-4), 53.4 (CH_2, C-2), 60.6 (CH_2, OCH_2CH_3), 71.6 (CH, C-9) and 176.4 (quat., OC═O); *m*/*z* (EI) 241 (M^+, 27%), 224 (M−CH_3, 100), 224 (M−OH, 8), 212 (M−C_2H_5, 38) and 196 (M−OC_2H_5, 60. Found: M^+ 241.1672. $C_{13}H_{23}NO_3$ requires M^+ 241.1678.

1.1.5. (1*S,5*R**,9*R**)-3-Ethyl-1-hydroxymethyl-3-azabicyclo[3.3.1]nonan-9-ol 10.** To a solution of (1*R**,5*R**)-ethyl 3-ethyl-9-oxo-3-azabicyclo[3.3.1]nonane-1-carboxylate **11** (200 mg, 0.836 mmol) in dry THF (20 ml) was added lithium aluminium hydride (63 mg, 1.67 mmol) and

the mixture stirred, under an atmosphere of nitrogen, for 1 h. The reaction was then quenched by the dropwise addition of water (10 ml) and the volatiles removed in vacuo. The remaining aqueous solution was extracted with ethyl acetate (2×30 ml) and the combined organic layers washed with brine (50 ml) then dried (Na_2SO_4) and concentrated in vacuo. The crude product was then purified by flash chromatography (9:1 dichloromethane–methanol) to give the title compound **10** (90 mg, 54%) as a clear oil. ν_{max}(NaCl)/cm^{-1} 3355 (OH), 2912 (CH), 1472, 1453, 1069 and 1037; δ_H (200 MHz; $CDCl_3$) 1.00 (3H, t, J=7.2 Hz, NCH_2CH_3), 1.19–1.28 (2H, m, 6-CH_2), 1.38–1.53 (2H, m, 7B-H and 8A-H), 1.75–2.05 (4H, m, 2B-H, 4B-H, 5-H and 8B-H), 2.16 (2H, q, J=7.2 Hz, NCH_2CH_3), 2.48–2.54 (1H, m, 7A-H), 2.61 (1H, d, J_{gem}=12.2 Hz, 4A-H), 2.96 (1H, d, J_{gem}=11.1 Hz, 2A-H), 3.29–3.45 (3H, m, OCH_2 and OH), 3.68 (1H, d, J=3.2 Hz, 9-H) and 3.71 (1H, br, 9-OH); δ_C (50 MHz; $CDCl_3$) 12.7 (CH_3, NCH_2CH_3), 20.6 (CH_2, C-7), 23.9 (CH_2, C-6), 26.6 (CH_2, C-8), 36.0 (CH, C-5), 37.9 (quat., C-1), 52.3 (CH_2, NCH_2CH_3), 58.4 (CH_2, C-4), 60.5 (CH_2, C-2), 70.6 (CH_2, OCH_2), and 74.9 (CH, C-9); m/z (EI) 199 (M^+, 28%), 184 (M−CH_3, 47), 182 (M−OH, 18) and 72 (100). Found: M^+ 199.1571. $C_{11}H_{21}NO_2$ requires M^+ 199.1572.

1.1.6. Ethyl (1*R,5*R**,9*R**)-3-ethyl-9-methoxy-3-azabicyclo[3.3.1]nonane-1-carboxylate 15.** To a suspension of sodium hydride (132 mg, 60% in oil, 3.32 mmol) in dry THF (10 ml) at 0 °C was added a solution of ethyl (1*R**,5*R**,9*R**)-3-ethyl-9-hydroxy-3-azabicyclo[3.3.1]-nonane-1-carboxylate **14** (200 mg, 0.83 mmol) in dry THF (10 ml). The mixture was then stirred for 1 h after which time iodomethane (0.70 g, 0.32 ml, 5.00 mmol) was added and the mixture was stirred at room temperature for 72 h. The reaction was then quenched by the careful addition of water (25 ml). The volatile solvents were removed in vacuo and the remaining aqueous solution extracted with ethyl acetate (3×20 ml). The combined organic layers were washed with brine (50 ml) then dried ($MgSO_4$) and concentrated in vacuo to give the crude product which was purified by flash chromatography (7:3 hexane–ethyl acetate) to give ethyl (1*R**,5*R**,9*R**)-3-ethyl-9-methoxy-3-azabicyclo[3.3.1]nonane-1-carboxylate **15** (R_f 0.4) (148 mg, 70%) as a clear oil. ν_{max}(NaCl)/cm^{-1} 2927 (C–H), 1731 (C=O, ester) and 1259 (C–O); δ_H (200 MHz; $CDCl_3$) 0.97 (3H, t, J=7.1 Hz, NCH_2CH_3), 1.19 (3H, t, J=7.1 Hz, OCH_2CH_3), 1.35–1.51 (2H, m, 6B-H and 7B-H), 1.70–1.80 (2H, m, 6A-H and 8B-H), 1.98–2.04 (2H, m, 5-H and 8A-H), 2.13–2.25 (4H, m, 2B-H, 4B-H and NCH_2CH_3), 2.44–2.54 (1H, m, 7A-H), 2.85 (1H, d, J_{gem}=10.8 Hz, 4A-H), 2.96 (1H, d, J_{gem}=11.1 Hz, 2A-H), 3.26 (3H, s, OCH_3), 3.47 (1H, d, J=3.6 Hz, 9-H) and 4.01–4.12 (2H, m, OCH_2CH_3); δ_C (50 MHz; $CDCl_3$) 12.6 (CH_3, NCH_2CH_3), 14.0 (CH_3, OCH_2CH_3), 20.5 (CH_2, C-7), 23.6 (CH_2, C-6), 26.5 (CH_2, C-8), 30.8 (CH, C-5), 46.9 (quat., C-1), 51.8 (CH_2, NCH_2CH_3), 55.9 (CH_3, OCH_3), 58.1 (CH_2, C-4), 60.1 (CH_2, C-2), 61.3 (CH_2, OCH_2CH_3), 81.4 (CH, C-9) and 175.2 (quat., OC=O); m/z (EI) 255 (M^+, 32%), 240 (M−CH_3, 44), 226 (M−C_2H_5, 30) and 224 (M−OCH_3, 100). Found: M^+ 255.1845. $C_{14}H_{25}NO_3$ requires M^+ 255.1834.

1.1.7. (1*R,5*S**,9*R**)-(3-Ethyl-9-methoxy-3-azabicyclo[3.3.1]non-1-yl)methanol 9.**[17d] A solution of ethyl (1*R**,5*R**,9*R**)-3-ethyl-9-methoxy-3-azabicyclo[3.3.1]-nonane-1-carboxylate **15** (546 mg, 2.14 mmol) in dry THF (10 ml) was added dropwise to a solution of lithium aluminium hydride (162 mg, 4.26 mmol) in THF (20 ml) and the mixture stirred, under an atmosphere of nitrogen, for 2 h. The reaction was then quenched by dropwise addition of water (20 ml), the volatiles removed in vacuo. The remaining aqueous mixture was extracted with ethyl acetate (2×20 ml). The combined organic layers were washed with brine (50 ml) then dried ($MgSO_4$) and concentrated in vacuo to give the crude product which was purified by flash chromatography (1:1 hexane–ethyl acetate) to give the title compound **9** (413 mg, 91%) as a clear oil. ν_{max}(NaCl)/cm^{-1} 3435 (OH) and 2947 (C–H); δ_H (200 MHz; $CDCl_3$) 0.99 (3H, t, J=7.1 Hz, NCH_2CH_3), 1.22–1.34 (2H, m, 6B-H and 7B-H), 1.37–1.45 (2H, m, 6A-H and 8B-H), 1.64–1.80 (2H, m, 5-H and 8A-H), 1.85–2.03 (2H, m, 2B-H and 4B-H) 2.13 (2H, q, J=7.1 Hz, NCH_2CH_3), 2.42–2.67 (1H, m, 7A-H), 2.61 (1H, d, J_{gem}=10.3 Hz, 4A-H), 2.97 (1H, d, J_{gem}=8.4 Hz, 2A-H), 3.14 (1H, d, J=3.1 Hz, 9-H), 3.29 (3H, s, OCH_3) and 3.24–3.28 (3H, m, OH and CH_2OH); δ_C (50 MHz; $CDCl_3$) 12.6 (CH_3, NCH_2CH_3), 20.4 (CH_2, C-7), 23.9 (CH_2, C-6), 27.1 (CH_2, C-8), 30.6 (CH, C-5), 38.1 (quat., C-1), 52.1 (CH_2, NCH_2CH_3), 55.0 (CH_3, OCH_3), 58.0 (CH_2, C-4), 60.9 (CH_2, C-2), 70.6 (CH_2, OCH_2OH) and 84.5 (CH, C-9); m/z (EI) 213 (M^+, 28%), 198 (M−CH_3, 59), 224 (M−OCH_3, 38) and 72 (100). Found: M^+ 213.1729. $C_{12}H_{23}NO_2$ requires M^+ 213.1729.

1.1.8. *N*-(Trifluoroacetyl)anthranilic acid 6. Anthranilic acid (15.13 g, 0.11 mol) was carefully added in portions over 15 min to a 250 ml round bottom flask containing vigourously stirred trifluoroacetic anhydride (30.7 ml, 0.22 mol). After 1 h the mixture was cooled to 0 °C and carefully quenched by the addition of water (100 ml). The mixture was then filtered and the crude product recystallised from ethanol/water to give the title compound **6** (18.77 g, 73%) as colourless crystals, mp 178–180 °C (lit.[19] mp 179–182 °C).

1.2. Standard procedure for the formation of 2-aminobenzoate esters using *N*-(trifluoroacetyl)anthranilic acid 6

To a solution of alcohol (1 mmol), *N*-(trifluoroacetyl)-anthranilic acid **179** (2 mmol) and 4-(dimethylamino)-pyridine (0.1 mmol) in acetonitrile (5 ml) was added 1,3-dicyclohexylcarbodiimide (2 mmol) and the mixture stirred, under an atmosphere of nitrogen, at 40 °C for 24 h. After this time the mixture was cooled, filtered and the filtrate evaporated to dryness. The crude mixture was then dissolved in dichloromethane (20 ml), washed with aq. sodium bicarbonate (20 ml) and brine (20 ml) then dried ($MgSO_4$) and concentrated in vacuo to leave the crude *N*-(trifluoroacetyl)anthranilate ester. This residue was suspended in absolute ethanol (10 ml), sodium borohydride (2 mmol) added, and the mixture stirred for 2 h. The reaction was quenched by the addition of water and the volatile solvent removed in vacuo. The remaining aqueous solution was extracted with ethyl acetate (2×30 ml) and the combined organic layers washed with brine (50 ml) then dried ($MgSO_4$) and concentrated in vacuo to leave the crude product, which was purified by flash chromatography to afford the anthranilate ester.

1.2.1. 1-Methyl-piperidin-3-ylmethyl 2-aminobenzoate 16. This reaction was carried out according to the standard procedure using 1-methyl-3-piperidinemethanol **7** (50 mg, 0.39 mmol), *N*-(trifluoroacetyl)anthranilic acid **6** (180 mg, 0.77 mmol), 4-(dimethylamino)pyridine (5 mg, 0.04 mmol), 1,3-dicyclohexylcarbodiimide (160 mg, 0.77 mmol) and sodium borohydride (29 mg, 0.77 mmol) using 5:1 dichloromethane–methanol as solvent for flash chromatography to afford the title compound **16** (78 mg, 81%) as a cream solid, mp 51–52 °C. ν_{max}(NaCl)/cm^{-1} 3480 and 3369 (NH_2), 1686 (C═O), 1618, 1589, 1560, 1456, 1296 and 1245; δ_H (400 MHz; $CDCl_3$) 1.07 (1H, ddd, $J_{4'A,5'A\text{-}H}$=4.5 Hz, $J_{4'A,5'B}$=4.5 Hz, J_{gem}=11.4 Hz, 4′A-H), 1.55–1.82 (4H, m, 2′A-H, 4′B-H and 5′-CH_2), 1.91 (1H, td, J_{gem}=11.2 Hz, $J_{6'A,5'A}$=11.2 Hz, $J_{6'A,5'B}$=3.0 Hz, 6′A-H), 2.03–2.17 (1H, br m, 3′-CH), 2.27 (3H, s, N-CH_3), 2.78 (1H, br d, J_{gem}=11.2 Hz, 6′B-H), 2.93 (1H, dt, J_{gem}=10.8 Hz, $J_{2'B,3'A}$=1.6 Hz, $J_{2'B,6'B}$=1.6 Hz, 2′B-H), 4.06–4.17 (2H, m, OCH_2), 5.72 (2H, br, NH_2), 6.60–6.64 (2H, m, 3-H and 5-H), 7.24 (1H, t, *J*=7.7 Hz, 4-H) and 7.82 (1H, d, *J*=7.5 Hz, 6-H); δ_C (100 MHz; $CDCl_3$) 24.7 (CH_2, C-5′), 26.7 (CH_2, C-4′), 35.9 (CH, C-3′), 46.5 (CH_3, N-*C*H_3), 55.9 (CH_2, C-6′), 59.1 (CH_2, C-2′), 66.9 (CH_2, OCH_2), 110.7 (quat., C-1), 116.2 (CH, C-3), 116.6 (CH, C-5), 131.0 (CH, C-6), 133.9 (CH, C-4), 150.5 (quat., C-2) and 167.9 (quat., C═O); *m*/*z* (EI) 248 (M^+, 82%), 233 (M−CH_3, 2), 128 (M−C_7H_6NO, 65), 120 (C_7H_6NO, 35), 112 (M−$C_7H_6NO_2$, 59) and 111 ($C_7H_{13}N$, 100). Found: M^+ 248.1536. $C_{14}H_{20}N_2O_2$ requires M^+ 248.1536.

1.2.2. 3-(Diethylamino)propyl 2-aminobenzoate 17. This reaction was carried out according to the standard procedure using 3-(diethylamino)-1-propanol (28 mg, 0.21 mmol), *N*-(trifluoroacetyl)anthranilic acid **6** (100 mg, 0.43 mmol), 4-(dimethylamino)pyridine (3 mg, 0.02 mmol), 1,3-dicyclohexylcarbodiimide (89 mg, 0.43 mmol) and sodium borohydride (16 mg, 0.43 mmol) using 5:1 dichloromethane–methanol as solvent for flash chromatography to afford the title compound **17** (45 mg, 85%) as a pale yellow oil. ν_{max}(NaCl)/cm^{-1} 3477 and 3370 (NH_2), 1689 (C═O), 1617, 1588, 1562, 1467 and 1245; δ_H (400 MHz; $CDCl_3$) 1.07 (6H, t, *J*=7.2 Hz, 2×NCH_2CH_3), 2.00 (2H, quin, *J*=6.3 Hz, 2′-CH_2), 2.65–2.73 (6H, m, 2×NCH_2CH_3 and 3′-CH_2), 4.33 (2H, t, *J*=6.3 Hz, 1′-CH_2), 5.73 (2H, br, NH_2), 6.61–6.67 (2H, m, 3-H and 5-H), 7.26 (1H, td, *J*=7.1 Hz, 1.6, 4-H) and 7.83 (1H, dd, *J*=8.0 Hz, 1.6, 6-H); δ_C (50 MHz; $CDCl_3$) 11.2 (CH_3, NCH_2CH_3), 22.9 (CH_2, C-2′), 46.7 (CH_2, NCH_2CH_3), 49.1 (CH_2, C-3′), 62.6 (CH_2, C-1′), 110.7 (quat., C-1), 116.1 (CH, C-3), 116.5 (CH, C-5), 131.0 (CH, C-6), 134.0 (CH, C-4), 150.4 (quat., C-2) and 167.9 (quat., C═O); *m*/*z* (EI) 250 (M^+, 46%), 235 (M−CH_3, 31), 221 (M−CH_2CH_3, 6), 178 (M−$N(CH_2CH_3)_2$, 11), 120 (M−$C_7H_{16}NO$, 62) and 86 ($(CH_3CH_2)_2NCH_2$, 100). Found: M^+ 250.1699. $C_{14}H_{22}N_2O_2$ requires M^+ 250.1681.

1.2.3. 2,2-Dimethylpropyl 2-aminobenzoate 18. The reaction was carried out according to the standard procedure using neopentyl alcohol (90 mg, 1.02 mmol), *N*-(trifluoroacetyl)anthranilic acid **6** (529 mg, 2.27 mmol), 4-(dimethylamino)pyridine (14 mg, 0.113 mmol), 1,3-dicyclohexylcarbodiimide (468 mg, 2.27 mmol) and sodium borohydride (85 mg, 2.27 mmol) using 6:4 hexane–ethyl acetate as the solvent for flash chromatography to afford the title compound **18**[17d] (198 mg, 94%) as a pale yellow oil. ν_{max}(NaCl)/cm^{-1} 3483 and 3372 (NH_2), 2957 (C–H), 1690 (C═O), 1617, 1589, 1560, 1371, 1293, 1245, 1161 and 1105; δ_H (200 MHz; $CDCl_3$) 1.03 (9H, br s, 3×2′-CH_3), 3.96 (2H, s, 1′-CH_2), 5.55 (2H, br, NH_2), 6.62–6.70 (2H, m, 3-H and 5-H), 7.26 (1H, t, *J*=7.7 Hz, 4-H) and 7.89 (1H, d, *J*=8.3 Hz, 6-H); δ_C (50 MHz; $CDCl_3$) 26.6 (CH_3, 2′-CH_3), 31.5 (quat., C-2′), 73.6 (CH_2, C-1′), 111.4 (quat., C-1), 116.6 (CH, C-3), 116.9 (CH, C-5), 131.0 (CH, C-6), 133.9 (CH, C-4), 149.9 (quat., C-2) and 168.0 (quat., OC═O); *m*/*z* (EI) 207 (M^+, 71%), 120 (M−$C_5H_{11}O$, 98), 119 (M−$C_5H_{12}O$, 98), 92 (M−$C_6H_{11}O_2$, 100). Found: M^+ 207.1261. $C_{12}H_{17}NO_2$ requires M^+ 207.1259.

1.2.4. Benzyl 2-aminobenzoate 19.[16b] The reaction was carried out according to the standard procedure using benzyl alcohol (500 mg, 4.62 mmol), *N*-(trifluoroacetyl)anthranilic acid **6** (2.15 g, 9.23 mmol), 4-(dimethylamino)pyridine (56 mg, 0.46 mmol), 1,3-dicyclohexylcarbodiimide (1.9 g, 9.25 mmol) and sodium borohydride (350 mg, 9.25 mmol) using 5:1 hexane–ethyl acetate as the solvent for flash chromatography to afford the title compound **19** (893 mg, 85%) as a pale oil. ν_{max}(NaCl)/cm^{-1} 3482 and 3373 (NH_2), 3031, 1690 (C═O), 1615, 1587, 1560, 1292 and 1242; δ_H (200 MHz; $CDCl_3$) 5.32 (2H, s, 1′-CH_2), 5.72 (2H, br, NH_2), 6.60–6.67 (2H, m, 3-H and 5-H), 7.21 (1H, td, *J*=7.7 Hz, 1.4, 4-H), 7.32–7.47 (5H, m, 5×Ar-H) and 7.94 (1H, dd, *J*=8.2 Hz, 1.4, 6-H); δ_C (50 MHz; $CDCl_3$) 65.8 (CH_2, 1′-CH_2), 110.3 (quat., C-1), 116.0 (CH, C-3), 116.5 (CH, C-5), 127.8 (CH, Ar), 127.9 (CH, Ar), 128.4 (CH, Ar), 131.1 (CH, C-6), 134.0 (CH, C-4), 136.1 (quat., Ar), 150.4 (quat., C-2) and 167.6 (quat., C═O); *m*/*z* (EI) 227 (M^+, 65%), 150 (M−C_6H_5, 5), 120 (M−C_7H_7O, 28) and 91 (C_7H_7, 100). Found: M^+ 227.0943. $C_{14}H_{13}NO_2$ requires M^+ 227.0946.

1.2.5. (1′*S,5′*S**)-(3-Ethyl-9-methyidene-3-azabicyclo-[3.3.1]non-1-yl)methyl 2-aminobenzoate 20.** The reaction was carried out according to the standard procedure using (1*S**,5*S**)-(3-ethyl-9-methyidene-3-azabicyclo[3.3.1]non-1-yl)methanol **8** (50 mg, 0.257 mmol), *N*-(trifluoroacetyl)-anthranilic acid **6** (120 mg, 0.515 mmol), 4-(dimethylamino)pyridine (3 mg, 0.026 mmol), 1,3-dicyclohexylcarbodiimide (107 mg, 0.518 mmol) and sodium borohydride (49 mg, 1.29 mmol) using 1:1 hexane–ethyl acetate as solvent for flash chromatography to afford the title compound **20** (60 mg, 75%) as a pale yellow oil. ν_{max}(NaCl)/cm^{-1} 3482 and 3373 (NH_2), 2919 (CH), 1688 (C═O), 1652 (C═C), 1616, 1589, 1456, 1293 and 1244; δ_H (200 MHz; $CDCl_3$) 1.06 (3H, t, *J*=7.1 Hz, NCH_2CH_3), 1.46–2.09 (6H, m, 5′-H, 6′-CH_2, 7′B-H and 8′-CH_2), 2.19–2.33 (3H, m, 4′B-H and NCH_2CH_3), 2.43–2.44 (1H, m, 2′B-H), 2.74–2.83 (1H, m, 7′A-H), 3.00–3.11 (2H, m, 2′A-H and 4′A-H), 4.21 (2H, s, OCH_2), 4.56 (1H, br s, 10′A-H) and 4.78 (1H, br s, 10′B-H), 5.72 (2H, br, NH_2), 6.61–6.69 (2H, m, 3-H and 5-H), 7.22–7.30 (1H, m, 4-H) and 7.85 (1H, dd, *J*=1.6 Hz, 7.5, 6-H); δ_C (50 MHz; $CDCl_3$) 12.5 (CH_3, NCH_2CH_3), 21.4 (CH_2, C-7′), 33.9 (CH_2, C-6′), 36.4 (CH_2, C-8′), 40.9 (quat., C-1′), 41.7 (CH, C-5′), 52.1 (CH_2, NCH_2CH_3), 60.4 (CH_2, C-4′), 62.8 (CH_2, C-2′), 69.9 (CH_2, OCH_2), 101.7 (CH_2, C-10′), 110.9 (quat., C-1), 116.3 (CH, C-3), 116.7 (CH, C-5), 131.1 (CH, C-6), 134.0 (CH, C-4), 150.4 (quat., C-2), 155.9 (quat., C-9′) and 168.0 (quat., C═O); *m*/*z* (EI) 314 (M^+, 31%), 299 (M−CH_3, 19), 178

(M−$NH_2C_6H_4CO_2$, 100) and 120 ($NH_2C_6H_4CO$, 60). Found: M^+ 314.1979. $C_{19}H_{26}N_2O_2$ requires M^+ 314.1994.

1.2.6. (1′*S* *,5′*R* *,9′*R* *)-(3-Ethyl-9-methoxy-3-azabicyclo[3.3.1]non-1-yl)methyl 2-aminobenzoate 21.[17d] The reaction was carried out according to the standard procedure using (1*R* *,5*S* *,9*R* *)-(3-ethyl-9-methoxy-1-3-azabicyclo[3.3.1]non-1-yl)methanol **9** (258 mg, 1.19 mmol), *N*-(trifluoroacetyl)anthranilic acid **6** (556 mg, 2.39 mmol), 4-(dimethylamino)pyridine (72 mg, 0.60 mmol), 1,3-dicyclohexylcarbodiimide (492 mg, 2.39 mmol) and sodium borohydride (135 mg, 3.58 mmol) using 1:1 hexane–ethyl acetate as solvent for flash chromatography to afford the title compound **21** (312 mg, 78%) as a clear oil. ν_{max} (NaCl)/cm^{-1} 3481 and 3371 (NH_2), 2969 and 2971 (C–H), 1689 (C═O), 1617, 1588, 1561, 1487, 1455, 1379, 1294 and 1244; δ_H (200 MHz; $CDCl_3$) 1.04 (3H, t, *J*=7.1 Hz, NCH_2CH_3), 1.21–1.57 (3H, m, 7′B-H and 6′-CH_2), 1.62–1.95 (2H, m, 8′-CH_2), 2.05–2.30 (5H, m, 2′B-H, 4′B-H, 5′-H and NCH_2CH_3), 2.54–2.67 (1H, m, 7′A-H), 2.94 (1H, d, J_{gem}=11.0 Hz, 4′A-H), 3.06 (1H, d, J_{gem}=10.2 Hz, 2′A-H), 3.16 (1H, br s, 9-H), 3.31 (3H, s, OCH_3), 4.05 (2H, m, OCH_2), 5.75 (2H, br, NH_2), 6.62–6.69 (2H, m, 3-H and 5-H), 7.26 (1H, td, *J*=7.2 Hz, 0.8. 4-H) and 7.85 (1H, dd, *J*=1.4 Hz, 8.3, 6-H); δ_C (50 MHz; $CDCl_3$) 12.7 (CH_3, NCH_2CH_3), 20.4 (CH_2, C-7′), 24.3 (CH_2, C-6′), 28.0 (CH_2, C-8′), 30.7 (CH, C-5′), 38.2 (quat., C-1′), 52.3 (CH_2, NCH_2CH_3), 56.0 (CH_3, OCH_3), 58.3 (CH_2, C-4′), 61.4 (CH_2, C-2′), 69.3 (CH_2, OCH_2), 81.1 (CH, C-9′), 110.9 (quat., C-1), 116.2 (CH, C-3), 116.7 (CH, C-5), 130.9 (CH, C-6), 133.9 (CH, C-4), 150.5 (quat., C-2) and 168.0 (quat., OC═O); *m*/*z* (EI) 332 (M^+, 50%), 317 (M−CH_3, 35), 301 (M−OCH_3, 30), 196 (M−$NH_2C_6H_4CO_2$, 25), 165 (M−$C_8H_9NO_2$, 64), 120 ($NH_2C_6H_4CO$, 59) and 72 (100). Found: M^+ 332.2094. $C_{19}H_{28}N_2O_3$ requires M^+ 332.2099.

1.2.7. Cyclohexyl 2-aminobenzoate 22.[23] The reaction was carried out according to the standard procedure, with the esterification step being left for 48 h, using cyclohexanol (200 mg, 2.0 mmol), *N*-(trifluoroacetyl)anthranilic acid **6** (920 mg, 3.94 mmol), 4-(dimethylamino)pyridine (24.0 mg, 0.196 mmol), 1,3-dicyclohexylcarbodiimide (812 mg, 3.94 mmol) and sodium borohydride (75 mg, 1.98 mmol) using 1:1 hexane–ethyl acetate as solvent for flash chromatography to afford the title compound **22** (426 mg, 97%) as a clear oil. ν_{max} (NaCl)/cm^{-1} 3479 and 3369 (NH_2), 2935 and 2858 (C–H), 1686 (C═O), 1615; δ_H (200 MHz; $CDCl_3$) 1.28–1.93 (10H, m, 5×CH_2), 4.98–5.06 (1H, m, 1′-H), 5.61 (2H, br, NH_2), 6.62–6.70 (2H, m, 3-H and 5-H), 7.26 (1H, t, *J*=7.7 Hz, 4-H), 7.91 (1H, d, *J*=8.2 Hz, 6-H); δ_C (50 MHz; $CDCl_3$) 23.5 (CH_2, C-3′), 25.3 (CH_2, C-4′), 31.5 (CH_2, C-2′), 72.1 (CH, C-1′), 111.3 (quat., C-1), 116.0 (CH, C-3), 116.5 (CH, C-5), 131.0 (CH, C-6), 133.7 (CH, C-4), 150.2 (CH, C-2), 167.4 (quat., OC═O); *m*/*z* (EI) 219 (M^+, 43%), 137 (M−C_6H_{10}, 94) and 119 (M−$C_6H_{12}O$, 100). Found: M^+ 219.1259. $C_{13}H_{17}NO_2$ requires M^+ 219.1259.

1.2.8. 1-Phenylethyl 2-aminobenzoate 23. The reaction was carried out according to the standard procedure, with the esterification step being left for 48 h, using 1-phenylethanol (200 mg, 1.63 mmol), *N*-(trifluoroacetyl)anthranilic acid **6** (763 mg, 3.27 mmol), 4-(dimethylamino)pyridine (20 mg, 0.163 mmol), 1,3-dicyclohexylcarbodiimide (675 mg, 3.27 mmol) and sodium borohydride (123 mg, 3.23 mmol) using 7:3 hexane–ethyl acetate as solvent for flash chromatography to afford the title compound **23** (335 mg, 85%) as a pale yellow oil. ν_{max}(NaCl)/cm^{-1} 3485 and 3373 (NH_2), 3031, 2980, 1687 (C═O), 1616, 1560, 1487, 1292 and 1240; δ_H (200 MHz; $CDCl_3$) 1.65 (3H, d, *J*=6.7 Hz, 2′-CH_3), 5.44 (2H, br, NH_2), 6.07 (1H, q, *J*=6.7 Hz, 1′-CH), 6.62–6.70 (2H, m, 3-H and 5-H), 7.22–7.46 (6H, m, 5×Ar-H and 4-H) and 7.98 (1H, dd, *J*=8.4 Hz, 1.8, 6-H); δ_C (50 MHz; $CDCl_3$) 22.6 (CH_3, C-2′), 72.1 (CH, C-1′), 110.7 (quat., C-1), 116.2 (CH, C-3), 116.6 (CH, C-5), 125.9 (CH, Ar), 127.7 (CH, Ar), 128.5 (CH, Ar), 131.2 (CH, C-6), 134.1 (CH, C-4), 142.0 (quat., Ar), 150.4 (quat., C-2) and 167.2 (quat., C═O); *m*/*z* (EI) 241 (M^+, 50%), 150 (M−C_7H_7, 73), 120 (C_7H_6NO, 41) and 105 (C_6H_5CO, 33). Found: M^+ 241.1102. $C_{15}H_{15}NO_2$ requires M^+ 241.1103.

1.2.9. (1′*S* *,5′*S* *,9′*R* *)-3-Ethyl-9-hydroxy-3-azabicyclo[3.3.1]non-1-ylmethyl 2-aminobenzoate 25. The reaction was carried out according to the standard pro cedure using (1*S* *,5*R* *,9*R* *)-3-ethyl-1-hydroxymethyl-3-azabicyclo[3.3.1]nonan-9-ol **10** (200 mg, 1.00 mmol), *N*-(trifluoroacetyl)anthranilic acid **6** (234 mg, 1.00 mmol), 4-(dimethylamino)pyridine (61 mg, 0.50 mmol), 1,3-dicyclohexylcarbodiimide (207 mg, 1.00 mmol) and sodium borohydride (76 mg, 2.01 mmol) using 1:1 hexane–ethyl acetate as solvent for flash chromatography to afford the title compound **25** (178 mg, 56%) as a pale yellow oil. ν_{max} (NaCl)/cm^{-1} 3481 (NH) and 3371 (NH and OH), 2969 and 2971 (C–H), 1689 (C═O), 1617, 1588, 1561, 1487, 1455, 1379, 1294 and 1244; δ_H (200 MHz; $CDCl_3$) 1.09 (3H, t, *J*=6.9 Hz, NCH_2CH_3), 1.34–1.59 (4H, m, 6′-CH_2, 7′B-H and 8′A-H), 1.71–2.31 (6H, m, 2′B-H, 4′B-H, 5′-H, 8′B-H and NCH_2CH_3), 2.57–2.66 (1H, m, 7′A-H), 2.92–3.05 (3H, m, 2′A-H, 4′A-H and 9′-OH), 3.51 (1H, br s, 9′-H), 3.66 (1H, d, J_{gem}=11.5 Hz, OCH_AH_B), 4.47 (1H, d, J_{gem}=11.5 Hz, OCH_AH_B), 5.72 (2H, br, NH_2), 6.62–6.69 (2H, m, 3-H and 5-H), 7.28 (1H, t, *J*=7.3 Hz, 4-H) and 7.85 (1H, d, *J*=8.3 Hz, 6-H); δ_C (50 MHz; $CDCl_3$) 12.8 (CH_3, NCH_2CH_3), 20.6 (CH_2, C-7′), 24.1 (CH_2, C-6′), 27.2 (CH_2, C-8′), 35.3 (CH, C-5′), 38.8 (quat., C-1′), 52.2 (CH_2, NCH_2CH_3), 58.7 (CH_2, C-4′), 60.7 (CH_2, C-2′), 68.9 (CH_2, OCH_2), 71.0 (CH, C-9′), 110.2 (quat., C-1), 116.2 (CH, C-3), 116.7 (CH, C-5), 131.1 (CH, C-6), 134.3 (CH, C-4), 150.7 (quat., C-2) and 168.6 (quat., C═O); *m*/*z* (EI) 318 (M^+, 35%), 303 (M−CH_3, 9), 198 (M−$NH_2C_6H_4CO$, 22), 182 (M−$NH_2C_6H_4CO_2$, 55), 120 ($NH_2C_6H_4CO$, 35) and 72 (100). Found: M^+ 318.1940. $C_{18}H_{26}N_2O_3$ requires M^+ 318.1943. A second fraction afforded (1*S* *,5*R* *,9*R* *)-9-(2-aminobenzoyl)-3-ethyl-3-azabicyclo[3.3.1]non-1-ylmethyl 2-aminobenzoate **26** (44 mg, 10%) as a yellow oil, for which the spectroscopic data was in agreement with that reported in the procedure described below.

1.2.10. (1′*S* *,5′*R* *,9*R* *)-9-(2-Aminobenzoyl)-3-ethyl-3-azabicyclo[3.3.1]non-1-lymethyl 2-aminobenzoate 26. The reaction was carried out according to the standard procedure using (1*S* *,5*R* *,9*R* *) 3-ethyl-1-hydroxymethyl-3-azabicyclo[3.3.1]nonan-9-ol **10** (50 mg, 0.250 mmol), *N*-(trifluoroacetyl)anthranilic acid **6** (120 mg, 0.515 mmol), 4-(dimethylamino)pyridine (3 mg, 0.026 mmol), 1,3-dicyclohexylcarbodiimide (107 mg, 0.519 mmol) and sodium

borohydride (49 mg, 1.29 mmol) using 1:1 hexane–ethyl acetate as solvent for flash chromatography to afford the title compound **26** (52 mg, 48%) as a yellow oil. ν_{max} (NaCl)/cm^{-1} 3483 and 3373 (NH_2), 2924 and 2779 (C–H), 1687 (C=O), 1616, 1588, 1561, 1487, 1454, 1383, 1294 and 1242; δ_H (200 MHz; $CDCl_3$) 1.07 (3H, t, J=7.1 Hz, NCH_2CH_3), 1.57–2.05 (5H, m, 6′-CH_2, 7′B-H and 8′-CH_2), 2.15–2.42 (5H, m, 2′B-H, 4′B-H, 5′-H and NCH_2CH_3), 2.65–2.86 (1H, m, 7′A-H), 3.05 (2H, m, 2′A-H and 4′A-H), 3.99–4.12 (2H, m, OCH_2), 5.11 (1H, d, J=3.5 Hz, 9′-H), 5.60–5.80 (4H, br, NH_2), 6.61–6.70 (4H, m, 2×3-H and 2×5-H), 7.21–7.31 (2H, m, 2×4-H) and 7.85–7.96 (2H, m, 2×6-H); δ_C (50 MHz; $CDCl_3$) 12.7 (CH_3, NCH_2CH_3), 20.5 (CH_2, C-7′), 25.0 (CH_2, C-6′), 28.5 (CH_2, C-8′), 33.2 (CH, C-5′), 37.7 (quat., C-1′), 52.0 (CH_2, NCH_2CH_3), 57.9 (CH_2, C-4′), 61.1 (CH_2, C-2′), 68.7 (CH_2, OCH_2), 74.3 (CH, C-9′), 110.6 and 110.8 (quat., 2×C-1), 116.2 (CH, 2×C-3), 116.5 and 116.6 (CH, 2×C-5), 130.9 and 131.0 (CH, 2×C-6), 133.9 and 134.0 (CH, 2×C-4), 150.4 and 150.6 (quat., 2×C-2) and 167.1 and 167.7 (quat., 2×OC=O); m/z (EI) 437 (M^+, 11%), 317 (M−$NH_2C_6H_4CO$, 5), 300 (M−$NH_2C_6H_4CO_2$, 58), 164 [M−2×($NH_2C_6H_4CO_2$), 100] and 120 ($NH_2C_6H_4CO_2$, 92). Found: M^+ 437.2315. $C_{25}H_{31}N_3O_4$ requires M^+ 437.2315.

1.3. Standard procedure for the formation of 2-(3-methyl-2,5-dioxopyrrolidin-1-yl)benzoate esters using methylsuccinic anhydride

2-Aminobenzoate ester (1 mmol) and methylsuccinic anhydride (3 mmol) were heated together at 125 °C for 3 h. After this time the crude mixture was dissolved in warm ethyl acetate (10 ml), washed with sat. sodium bicarbonate solution (30 ml) and brine (30 ml) then dried ($MgSO_4$) and concentrated in vacuo. The crude product was purified by flash chromatography to afford the 2-(3-methyl-2,5-dioxopyrrolidin-1-yl)benzoate ester.

1.3.1. 1-Methyl-piperidin-3-ylmethyl 2-(3-methyl-2,5-dioxopyrrolidin-1-yl)benzoate 27. The reaction was carried out according to the standard procedure using 1-methyl-piperidin-3-ylmethyl 2-aminobenzoate **16** (200 mg, 0.81 mmol) and methylsuccinic anhydride **28** (276 mg, 2.41 mmol) using 4:1 dichloromethane–methanol as solvent for flash chromatography to afford the title compound **27** (266 mg, 96%) as a clear oil. ν_{max} (NaCl)/cm^{-1} 2938 (C–H), 1776 (O=C–N–C=O), 1712 (C=O), 1578, 1492, 1393 and 1263; δ_H (400 MHz; $CDCl_3$) 1.01–1.04 (1H, m, 4″A-H), 1.44 (3H, br d, 3′-CH_3), 1.58–1.80 (4H, m, 2″ A-H, 4″ B-H and 5″ –CH_2), 1.93 (1H, d, J_{gem}=10.3 Hz, 6″ A-H), 2.07–2.13 (1H, br m, 3″-H), 2.28 (3H, s, N-CH_3), 2.43–2.56 (1H, br m, 3′-H), 2.77 (1H, d, J_{gem}=10.9 Hz, 6″ B-H), 2.87 (1H, d, J_{gem}=10.7 Hz, 2″ B-H), 3.02–3.08 (2H, br m, 4′-CH_2), 4.01–4.12 (2H, m, OCH_2), 7.22 (1H, d, J=7.8 Hz, 3-H), 7.49 (1H, td, J=7.6 Hz, 1.4, 5-H), 7.63 (1H, td, J=7.2 Hz, 1.8, 4-H) and 8.08 (1H, dd, J=1.2 Hz, 6.9, 6-H); δ_C (100 MHz; $CDCl_3$) 17.1 (CH_3, 3′-CH_3), 25.2 (CH_2, C-5″), 27.2 (CH_2, C-4″), 35.8 (CH, C-3″), 36.4 (CH, C-3′), 37.6 (CH_2, C-4′), 47.1 (CH_3, NCH_3), 56.6 (CH_2, C-6″), 59.5 (CH_2, C-2″), 68.4 (CH_2, OCH_2), 127.9 (quat., C-1), 129.9 (CH, C-5), 130.4 (CH, C-3), 131.1 (CH, C-6), 133.4 (quat., C-2), 133.9 (CH, C-4), 164.9 (quat., OC=O), 176.5 (quat., C-5′) and 180.5 (quat., C-2′); m/z (EI) 344 (M^+, 45%), 329 (M−CH_3, 5), 216 (M−$C_7H_{14}NO$, 36), 188 (M−$C_8H_{14}NO_2$, 40), 128 ($C_8H_{14}NO_2$, 81) and 41 (100). Found: M^+ 344.1734. $C_{19}H_{24}N_2O_4$ requires M^+ 344.1736.

1.3.2. 3-(Diethylamino)propyl 2-(3-methyl-2,5,dioxopyrrolidin-1-yl)benzoate 28. The reaction was carried out according to the standard procedure using 3-(diethylamino)propyl 2-aminobenzoate **17** (300 mg, 1.198 mmol) and methylsuccinic anhydride (410 mg, 3.595 mmol) using 4:1 dichloromethane–methanol as solvent for flash chromatography to afford the title compound **28** (377 mg, 91%) as a yellow oil. ν_{max} (NaCl)/cm^{-1} 1777 (N–C=O), 1713 (C=O) and 1573; δ_H (200 MHz; $CDCl_3$) 1.11 (6H, br t, 2×NCH_2CH_3), 1.30 (3H, br d, 3′-CH_3), 1.94 (2H, m, 2″-CH_2), 2.31–2.46 (2H, m, 4′-CH_2), 2.55–2.64 (1H, m, 3′-H), 2.90–3.01 (6H, m, 2×NCH_2CH_3 and 3″-CH_2), 4.19 (2H, br t, 1″-CH_2), 7.14 (1H, dd, J=1.0 Hz, 7.8, 3-H), 7.40 (1H, td, J=7.6 Hz, 1.3, 5-H), 7.54 (1H, td, J=7.6 Hz, 1.6, 4-H) and 7.92 (1H, dd, J=1.4 Hz, 7.7, 6-H); δ_C (50 MHz; $CDCl_3$) 8.1 (CH_3, 2×NCH_2CH_3), 16.9 (CH_3, 3′-CH_3), 23.0 (CH_2, C-2″), 36.4 (CH_2, C-4′), 36.7 (CH, C-3′), 45.9 (CH_2, 2×NCH_2CH_3), 48.2 (CH_2, C-3″), 61.8 (CH_2, C-1″), 126.6 (quat., C-1), 129.0 (CH, C-5), 129.2 (CH, C-3), 130.7 (CH, C-6), 132.1 (quat., C-2), 133.2 (CH, C-4), 164.0 (quat., OC=O), 177.3 (quat., C-5″) and 179.6 (quat., C-2′); m/z (EI) 346 (M^+, 5%), 331 (M−CH_3, 30), 274 (M−$(C_2H_5)_2N$, 4), 188 (M−$C_8H_{16}NO_2$, 27) and 86 ($C_5H_{12}N$, 100). Found: M^+ 346.1882. $C_{19}H_{26}N_2O_4$ requires M^+ 346.1893.

1.3.3. (1″*S,5″*S**,3′*R**)- and (1″ *S**,5″ *S**,3″*S**)-(3-Ethyl-9-methyidene-3-azabicyclo[3.3.1]non-1-yl)methyl 2-(3-methyl-2,5-dioxopyrrolidin-1-yl)benzoate 30.** The reaction was carried out according to the standard procedure using (1′*S**,5′*S**)-(3-ethyl-9-methyidene-3-azabicyclo-[3.3.1]non-1-yl)methyl 2-aminobenzoate **20** (45 mg, 0.143 mmol) and methylsuccinic anhydride (48 mg, 0.429 mmol) using 7:3 hexane–ethyl acetate as solvent for flash chromatography to afford the title compound 30 (49 mg, 84%) as a clear oil. ν_{max}(NaCl)/cm^{-1} 2920 (C–H), 1779 (N–C=O), 1715 (C=O), 1602, 1492, 1262 and 1186; δ_H (200 MHz; $CDCl_3$) 1.21 (3H, t, J=7.2 Hz, NCH_2CH_3), 1.38–1.62 (4H, m, 3′-CH_3 and 6″ B-H), 1.68–2.10 (4H, m, 6″ A-H, 7″ B-H and 8″-CH_2), 2.15–2.16 (1H, m, 5″-H), 2.20–2.30 (4H, m, 2″ B-H, 4″ B-H and NCH_2CH_3), 2.42–2.79 (3H, br m, 3′-H, 4″ A-H and 7″ A-H), 3.00–3.09 (3H, br m, 2″ A-H and 4′-CH_2), 4.15 (2H, s, OCH_2), 4.49 (1H, br s, 10″ A-H), 4.75 (1H, br s, 10″ B-H), 7.24 (1H, dd, J=0.9 Hz, 7.7, 3-H) 7.51 (1H, td, J=7.7 Hz, 1.3, 5-H), 7.64 (1H, td, J=7.7 Hz, 1.5, 4-H) and 8.10 (1H, dd, J=1.0 Hz, 7.7, 6-H); δ_C (50 MHz; $CDCl_3$) 12.5 (CH_3, NCH_2CH_3), 16.3 (CH_3, 3′-CH_3), 21.4 (CH_2, C-7″), 33.9 (CH_2, C-6″), 35.2 (CH_2, C-4′), 36.8 (CH_2, C-8″), 36.9 (CH, C-3′), 40.9 (quat., C-1″), 41.7 (CH, C-5″), 52.0 (CH_2, NCH_2CH_3), 60.4 (CH_2, C-4″), 62.8 (CH_2, C-2″), 70.7 (CH_2, OCH_2), 101.6 (CH_2, C-10″), 126.3 (quat., C-1), 129.2 (CH, C-5), 129.3 (CH, C-3), 129.8 (CH, C-6), 131.4 (quat., C-2), 133.4 (CH, C-4), 156.9 (quat., C-9″), 164.5 (quat., OC=O), 176.0 (quat., C-5′) and 179.4 (quat., C-2′); m/z (EI) 410 (M^+, 4%), 439 (M−CH_3, 14), 194 (M−$C_{12}H_{10}O_3N$, 27) and 178 (M−$C_{12}H_{10}O_4N$, 100). Found: M^+ 410.2229. $C_{24}H_{30}N_2O_4$ requires M^+ 410.2206.

1.3.4. Cyclohexyl 2-(3-methyl-2,5-dioxopyrrolidin-1-yl)benzoate 31. The reaction was carried out according to

the standard procedure using cyclohexyl 2-aminobenzoate **22** (100 mg, 0.456 mmol) and methylsuccinic anhydride (156 mg, 1.37 mmol) using 1:1 hexane–ethyl acetate as solvent for flash chromatography to afford the title compound **31** (101 mg, 70%) as a yellow oil. ν_{max} (NaCl)/cm^{-1} 2937 and 2859 (C–H), 1781 (O═C–N–C═O), 1715 (C═O), 1602, 1492, 1453, 1390 and 1259; δ_H (200 MHz; $CDCl_3$) 1.25–1.94 (13H, m, 5×CH_2 and 3′-CH_3), 2.46–2.61 (1H, m, 3′-H), 3.05–3.15 (2H, m, 4′-CH_2), 4.85–4.94 (1H, m, 1″-H), 7.23 (1H, dd, *J*=1.4 Hz, 7.8, 3-H), 7.50 (1H, td, *J*=7.5 Hz, 1.1, 5-H), 7.63 (1H, td, *J*=7.6 Hz, 1.5, 4-H) and 8.10 (1H, d, *J*=6.9 Hz, 6-H); δ_C (50 MHz; $CDCl_3$) 16.3 (CH_3, 3′-CH_3), 23.6 (CH_2, C-3″), 25.2 (CH_2, C-4″), 31.4 (CH_2, C-2″), 35.1 (CH_2, C-4′), 36.8 (CH, C-3′), 73.4 (CH, C-1″), 126.2 (quat., C-1), 129.1 (CH, C-5), 129.5 (CH, C-3), 131.2 (CH, C-6), 131.3 (quat., C-2), 132.9 (CH, C-4), 164.3 (quat., OC═O), 175.8 (quat., C-5′) and 179.7 (quat., C-2′); *m/z* (EI) 315 (M^+, 2%), 216 (M−$C_6H_{11}O$, 100) and 188 (M−$C_7H_{11}O_2$, 40). Found: M^+ 315.1466. $C_{18}H_{21}NO_4$ requires M^+ 315.1470.

1.3.5. 1.1.1-Phenylethyl 2-(3-methyl-2,5-dioxopyrrolidin-1-yl)benzoate 32. The reaction was carried out according to the standard procedure using 1-phenylethyl 2-aminobenzoate **23** (70 mg, 0.29 mmol) and methylsuccinic anhydride (132 mg, 1.16 mmol) using 1:1 hexane–ethyl acetate as solvent for flash chromatography to afford the title compound **32** (49 mg, 50%) as an orange oil. ν_{max} (NaCl)/cm^{-1} 2979, 1775 (O═C–N–C═O), 1713 (C═O), 1602, 1493, 1454, 1390 and 1259; δ_H (400 MHz; $CDCl_3$) 1.24–1.39 (3H, m, 3′-CH_3), 1.62 (3H, d, *J*=6.5 Hz, 2″-CH_3), 2.37–2.43 (1H, m, 3′-H), 2.86–3.04 (2H, m, 4′-H), 6.01 (1H, q, *J*=6.5 Hz, 1″-CH), 2.23–7.40 (6H, m, 3-H and 5×Ar-H), 7.50 (1H, td, *J*=7.7 Hz, 1.1, 5-H), 7.64 (1H, td, *J*=7.5 Hz, 1.5, 4-H) and 8.14 (1H, d, *J*=7.5 Hz, 6-H); δ_C (100 MHz; $CDCl_3$) 16.7 (CH_3, 3′-CH_3), 22.5 (CH_3, C-2″), 35.8 (CH_2, C-4′), 37.4 (CH, C-3′), 74.1 (CH, C-1″), 126.9 (quat., C-1), 128.7 (CH, Ar), 128.7 (CH, Ar), 128.9 (CH, Ar), 129.2 (CH, C-5), 129.9 (CH, C-3), 130.2 (CH, Ar), 132.1 (CH, C-6), 132.5 (CH, Ar), 132.9 (quat., C-2), 133.8 (CH, C-4), 141.7 (quat., Ar), 164.5 (quat., OC═O), 176.3 (quat., C-5′) and 180.5 (quat., C-2′); *m/z* (EI) 337 (M^+, 6%), 322 (M−CH_3, 8) and 188 (M−C_8H_9O, 100). Found: M^+ 337.1313. $C_{20}H_{19}NO_4$ requires M^+ 337.1314.

Acknowledgements

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On the reactivity of ascomycin at the binding domain. Part 2: Hydroxide mediated rearrangement reactions

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Abstract—The natural product ascomycin represents a highly functionalised 23-membered macrocycle with a polyketide backbone. Within the binding domain, ascomycin features the unusual pattern of a masked tricarbonyl moiety, which potentially allows for high structural diversity via simple isomerisation events. Herein, highly stereoselective, hydroxide mediated rearrangement reactions at the binding domain are reported.

1. Introduction

FK 506 **1** is a 23-membered macrolactam isolated from the fermentation broth of *Streptomyces tsukubaensis 9993*.[7–9] Interestingly, ascomycin **2**, a compound which had been isolated earlier as a consequence of its antifungal activities, and whose structure had originally not been elucidated, was later shown to be a close structural analogue of FK 506 (ethyl in position 21 instead of allyl).[10–13] Pimecrolimus (Elidel®, SDZ ASM 981, **3**), derived from ascomycin and featuring a more lipophilic cyclohexyl-part, has been shown to possess a high therapeutic potential for the treatment of

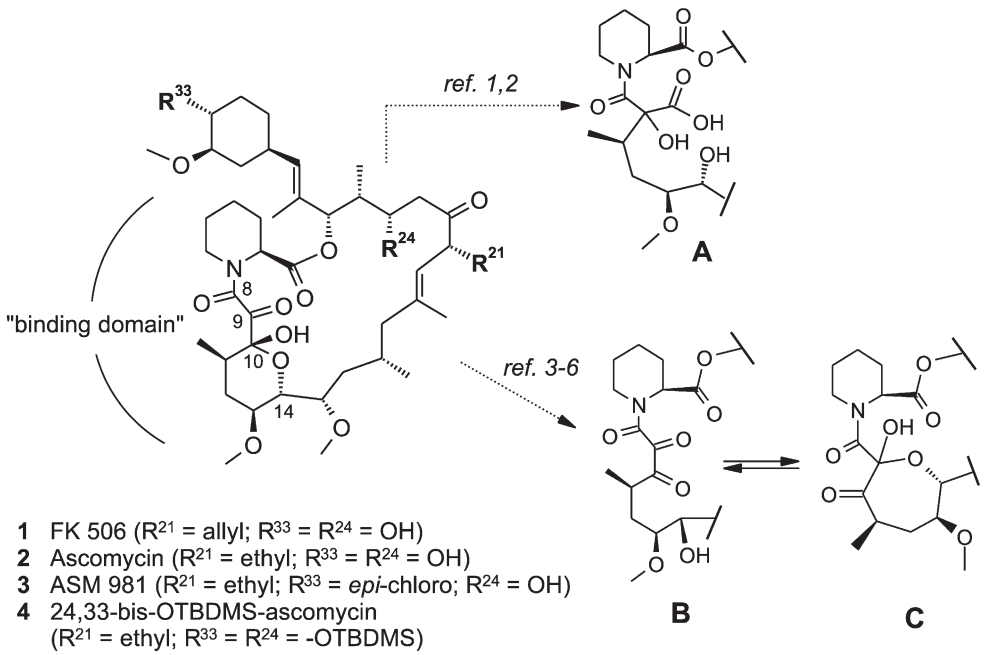

Figure 1.

Keywords: Ascomycin; Ring contraction; Rearrangement; Binding domain; Tricarbonyl.
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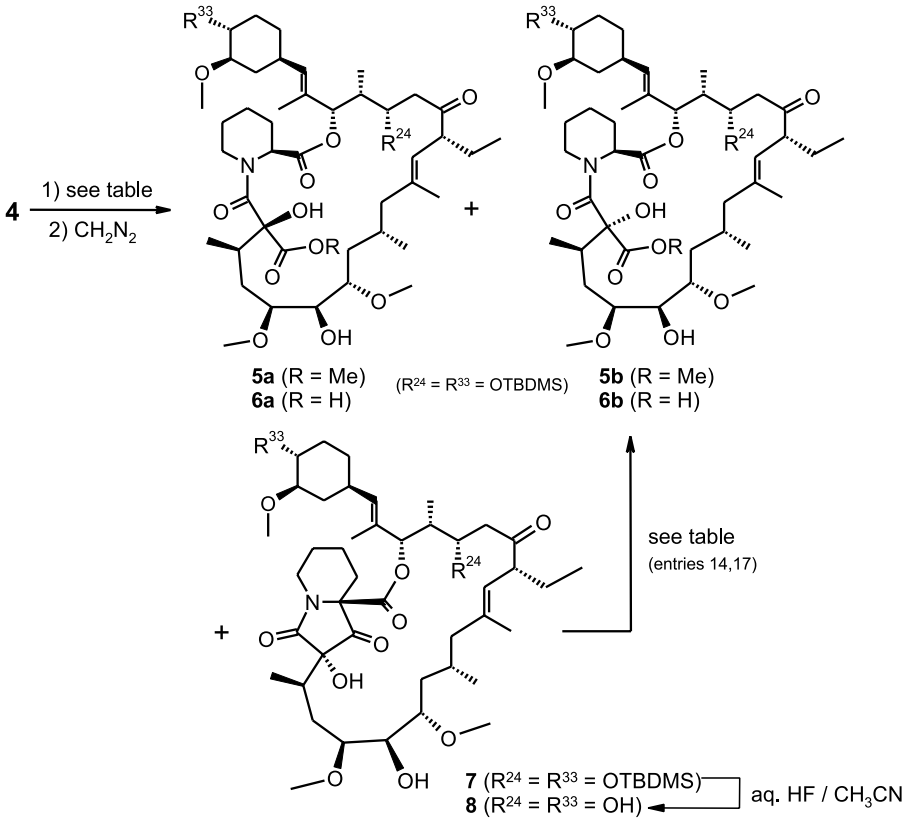
inflammatory skin diseases. Pimecrolimus cream 1%, which combines a skin selective, anti-inflammatory activity with a low risk for systemic side effects, has successfully been introduced into the market for the treatment of atopic dermatitis and contact dermatitis.[14–16] In the left hand part ('binding domain', Fig. 1),[17–21] ascomycin features the unusual pattern of three adjacent carbonyl groups (C8–C10), whereby one carbonyl group (C10) is involved in hemiketal formation. The inherently labile hemiketal structure at C10 potentially allows the formation of numerous alternative isomers via liberation of the tricarbonyl portion, tautomerisation, enolisation and non-specific



Scheme 1.

Table 1.

Entry[a]	Educt	Base	Solvent	Time	**5a/5b**[b]	Products (isol. %)[c]
1	**4**	5 equiv. LiOH	THF/H_2O=3:1	12 min	82:18	**5a** (72), **5b** (11)
2	**4**	5 equiv. KOH	THF/H_2O=3:1	10 min	83:17	**5a** (70), **5b** (12)
3	**4**	5 equiv. NaOH	THF/H_2O=3:1	10 min	86:14	**5a** (69), **5b** (9)
4	**4**	10 equiv. $Ca(OH)_2$	THF/H_2O=3:1	30 min	>98:2	**5a** (91) or **6a** (95%)[d]
5	**4**	10 equiv. $Ca(OH)_2$	THF/H_2O=7:1	60 min	>98:2	**5a** (91)
6	**4**	10 equiv. $Ca(OH)_2$	THF/H_2O=20:1	3 h	>98:2	**5a** (86)
7	**4**	10 equiv. $Ca(OH)_2$	THF/H_2O=80:1	30 h	>98:2	**5a** (74)
8	**4**	1.2 equiv. LiOH	DMSO	20 min	<2:98	**5b** (75) or **6b** (78)[d]
9	**4**	1.2 equiv. KOH	DMSO	30 min	<2:98	**5b** (78)
10	**4**	1.2 equiv. KOH	THF/18-crown-6	40 min	<2:98	**5b** (82), **7**(trace)
11	**4**	1.2 equiv. KOH	THF/18-crown-6	30 min	n.d.	**5b** (75), **7** (6)
12	**4**	1.2 equiv. KOH	THF/18-crown-6	10 min	n.d.	**5b** (52), **7** (24)
13	**4**	1.2 equiv. KOH	THF/18-crown-6	1.5 min	n.d.	**7** (74),[d] **4** (11), **6b** (trace)
14	**7**	1.2 equiv. KOH	THF/18-crown-6	40 min	0:100	**5b** (85) or **6b** (78)[d]
15	**4**	5 equiv. NaOD	THF/D_2O=3:1	12 min	81:19	**5a** (71),[e] **5b** (9)[f]
16	**4**	10 equiv. $Ca(OH)_2$	THF/D_2O=3:1	30 min	>98:2	**5a** (93)[e]
17	**7**	5 equiv. NaOD	THF/D_2O=3:1	40 min	0:100	**5b** (71%)[f]
18	**6a**	5 equiv. NaOD	THF/D_2O=3:1	15 min	100:0	**5a** (91)[e]
19	**6b**	5 equiv. NaOD	THF/D_2O=3:1	15 min	0:100	**5b** (89)[e]

[a] All reactions were carried out at room temperature.
[b] Determined by ^1H NMR of the crude reaction mixture after esterification with diazomethane.
[c] Isolated yields (not optimised) after esterification with diazomethane.
[d] Acidic work up without esterification.
[e] No deuterium incorporations detected.
[f] >95% deuterium incorporation at C2.

re-hemiketalsation events (**B**, **C** in Fig. 1).[3–6] The resulting structural diversity at the binding domain translates into a tremendous reactivity towards manifold reaction conditions.[22–27] As a consequence, selective reactions directed to other parts of the macrocycle are not easily achieved without provoking concomitant transformations within this peculiar unit. Thus, several attempts to cleave the endocyclic ester linkage led to a facile ring contraction via rearrangement of the C9/C10 (**A** in Fig. 1) region instead of yielding the desired 1,26-*seco*-derivative. For this reaction, a benzilic acid type rearrangement process has been proposed.[1,2] As part of our research program on the ascomycins, aiming at the generation of more stable and structurally less flexible binding domain mimics, we decided to investigate this reaction in more detail, in order to assess its scope and limitations and to get more insight into the reaction pathway(s) involved.

2. Results and discussion

2.1. Hydroxide mediated rearrangement reactions

In order to prevent cross-reactivity at the potentially base sensitive β-hydroxyketone portion of ascomycin (C22–C24),[28] 24,33-bis-OTBDMS-protected ascomycin **4** was chosen as the starting material for our investigations (Scheme 1, Table 1). First, we treated **4** with lithium hydroxide in THF–water solution (Table 1, entry 1). After an acidic work up, followed by esterification of the crude reaction mixture with diazomethane, two diastereoisomers, the ring contracted 10(*S*)-α-hydroxy acid methyl ester **5a** together with minor amounts of the not yet known 10(*R*)-epimer **5b** could be isolated (for stereo chemical determinations see Section 2.3). Replacement of lithium hydroxide by potassium or sodium hydroxide led to similar results (entries 2 and 3). Notably, replacement of lithium hydroxide by calcium hydroxide resulted in an almost complete diastereoselectivity of the rearrangement reaction. Thus, the reaction of **4** with 10 equiv. calcium hydroxide in THF/water (3:1) solution, afforded after esterification almost exclusively the 10(*S*)-isomer **5a** in high yield (entry 4). Changing the amount of water in the calcium hydroxide mediated rearrangement reaction had a strong influence on the reaction times required but no effect on stereo selectivity (entries 5–7). Remarkably, a complete change in stereo selectivity is observed under aprotic conditions. Thus, the treatment of **4** with powdered lithium or potassium hydroxide in dimethylsulfoxide (DMSO) solution or with potassium hydroxide in anhydrous tetrahydrofuran in the presence of 18-crown-6, yielded, dependent on the work up procedure applied, the diastereoisomerically pure 10(*R*)-hydroxy acid **6b** or the corresponding methyl ester **5b** in reasonable yields (entries 8–10). Careful monitoring of the latter reaction revealed that the 10(*R*)-hydroxy acid **6b** is formed via the unexpected novel intermediate **7**. Thus, short treatment of **4** with KOH/18-crown-6 led preferentially to **7** together with minor amounts of **5b**, whereas prolonged reaction times resulted in an increase in **6b** at the expense of **7** (entries 10–13). As could be anticipated from these results, treatment of pure **7** under identical reaction conditions, gave diastereoisomerically pure **6b** or (after esterification) **5b** (entry 14). In order to answer the question whether or not a similar intermediate might also be involved in the formation of the 10(*S*)-hydroxy-acid **6a**, 24,33-bis-OTBDMS-ascomycin **4** was treated with calcium deuteroxide or sodium deuteroxide in THF–D_2O solution. Analysis of the NMR spectra of the products **5a**,**b** showed no deuterium–hydrogen exchange in **5a** but a quantitative deuterium incorporation at C2 of **5b** (entries 15 and 16). Complete deuteration at C2 of **5b** could be confirmed by the absence of the H2-signal, a highfield shift and signal broadening of $H6_{eq}$ and $H3_{eq}$ in the ^1H NMR and a downfield shift of the C2-resonance by 0.2 ppm. As expected, analogous treatment of **7** gave **5b** deuterated almost quantitatively at C2 (entry 17). Treatment of isolated **6a** and **6b** with NaOD in THF–D_2O resulted in no deuteration at any position, thus giving evidence that the deuterium incorporation at C2 of **6b** when starting from **4**, occurs not after the formation of **6b**. Furthermore, as no

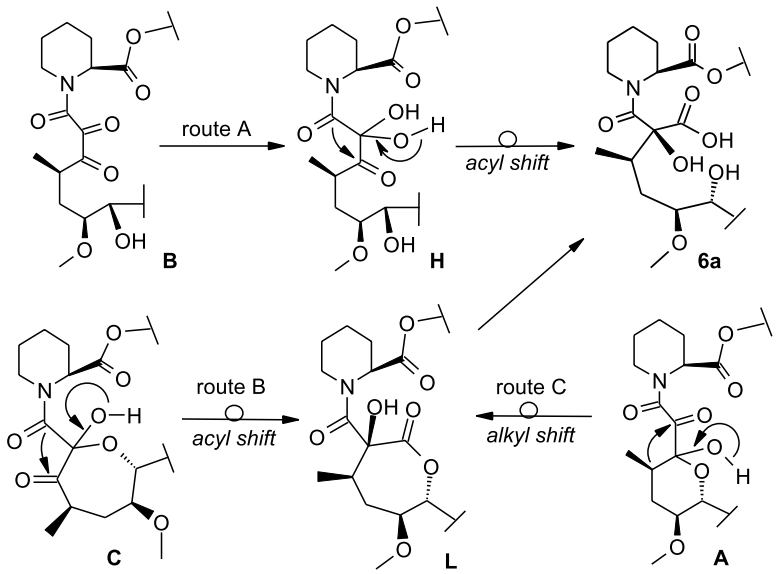
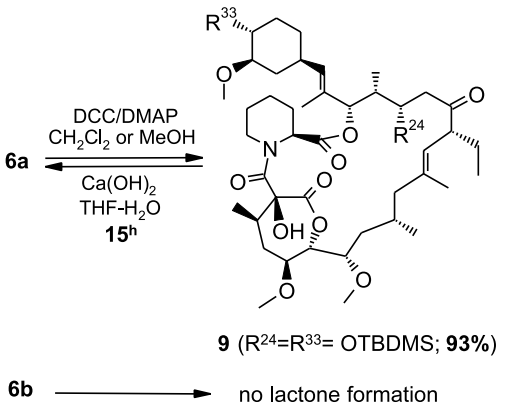


Figure 2.

hydrogen–deuterium exchange is observed when **4** is converted to **6a**, using calcium hydroxide in THF–D_2O solution (entry 16), this confirms that the configurations at all other potentially base labile positions (i.e., C2, C21 and C11) of **5a** and **6a** are not affected and thus are identical with the corresponding configurations in the starting material **4**. In summary, these experiments clearly demonstrate that involvement and deprotonation at C2 occurs in the formation of the 10(*R*)-hydroxy-acid **6b**, but can be excluded for the 10(*S*)-hydroxy-acid **6a**. The diastereoisomers **6a** and **6b** are thus formed via distinct, highly diastereoselective reaction pathways.

2.2. Discussion of reaction mechanisms

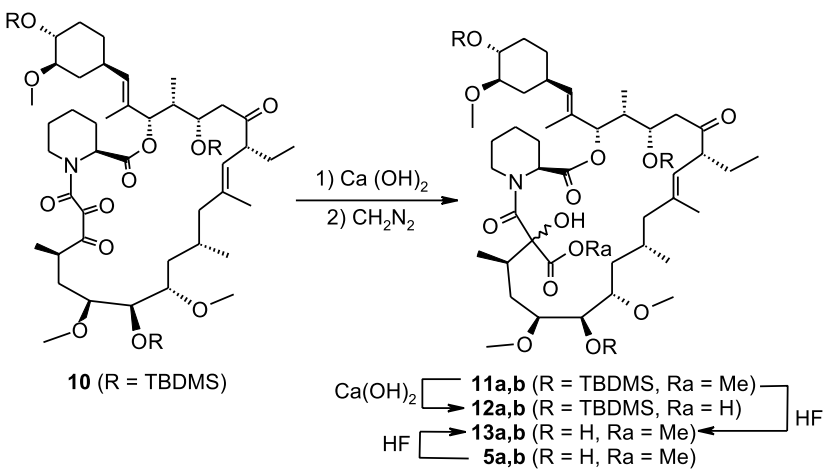
2.2.1. Formation of 6a. Taking into consideration the ketone/hemiketal equilibrium at the binding domain (compare Fig. 1), at least three different pathways for the formation **6a** (Fig. 2) can be formulated. Route A suggests an acyl migration as the key step of the rearrangement process, initiated by a nucleophilic attack of a hydroxide ion at the most reactive central carbonyl of the tricarbonyl form (**B→H→6a**, intermolecular nucleophile induced acyl shift). This benzilic acid type rearrangement reaction has already been proposed from the results of a study with 9-^{13}C-labelled material: the ^{13}C-label was mostly found in the carboxyl group which is in agreement with an acyl(C8)-migration.[1,2] However, the labelling study fits also to route B, which supposes essentially the same mechanism, but with participation of the 14-hydroxy group as internal nucleophile (**C→L→6a**, intramolecular nucleophile induced acyl shift), thus leading to a seven-membered lactone intermediate which might yield the final product on lactone hydrolysis. Such a lactone intermediate could also be formed if the hydroxyl ion acts rather as a base than as a nucleophile (route C; **A→L→6a**, base induced alkyl shift). Although a seven-membered lactone derivative (**L**) was not found in the rearrangement reactions, it cannot be excluded as intermediate because its formation may be much slower than its hydrolysis to the final product. In order to shed some light on this, we attempted to prepare such lactone derivatives, starting from the corresponding acids **6a** and **6b** (Scheme 2).



Scheme 2.

Lactonisation of **6a** was performed with *N*,*N*′-dicyclohexylcarbodiimide (DCC) in the presence of catalytic amounts of 4-dimethylaminopyridine (DMAP) in dichloromethane to provide **9** in excellent yield. Apparently, the carboxyl function and the 14-hydroxy group in **6a** are in a perfect orientation for lactonisation, since even in methanol only **9** instead of the expected methyl ester **5a** was obtained. In contrast, all attempts to convert the 10(*R*)-hydroxy acid **6b** to the corresponding lactone failed. Hydrolysis of **9** with calcium hydroxide (10 equiv.) in THF/water (3:1) resulted in the acid **6a** as expected, but the reaction time was markedly longer (15 h) than the conversion of **4** to **6a** under comparable reaction conditions (30 min; compare Table 1, entry 4). This clearly rules out **9** as an intermediate during the rearrangement process and supports the proposed intermolecular benzilic acid type rearrangement event (compare Fig. 2). Additional evidence for this suggestion was gained starting from the easily available 14,24,33-tris-OTBDMS-protected ascomycin derivative **10**,[29] in which the binding domain is fixed in the tricarbonyl form by blocking the 14-hydroxy group (Scheme 3).

Thus, treatment of the characteristically yellow coloured **10** with calcium hydroxide (10 equiv.) in THF/water (3:1) led almost instantaneously to the disappearance of the yellow colour and afforded in a fast reaction (<5 min) and with

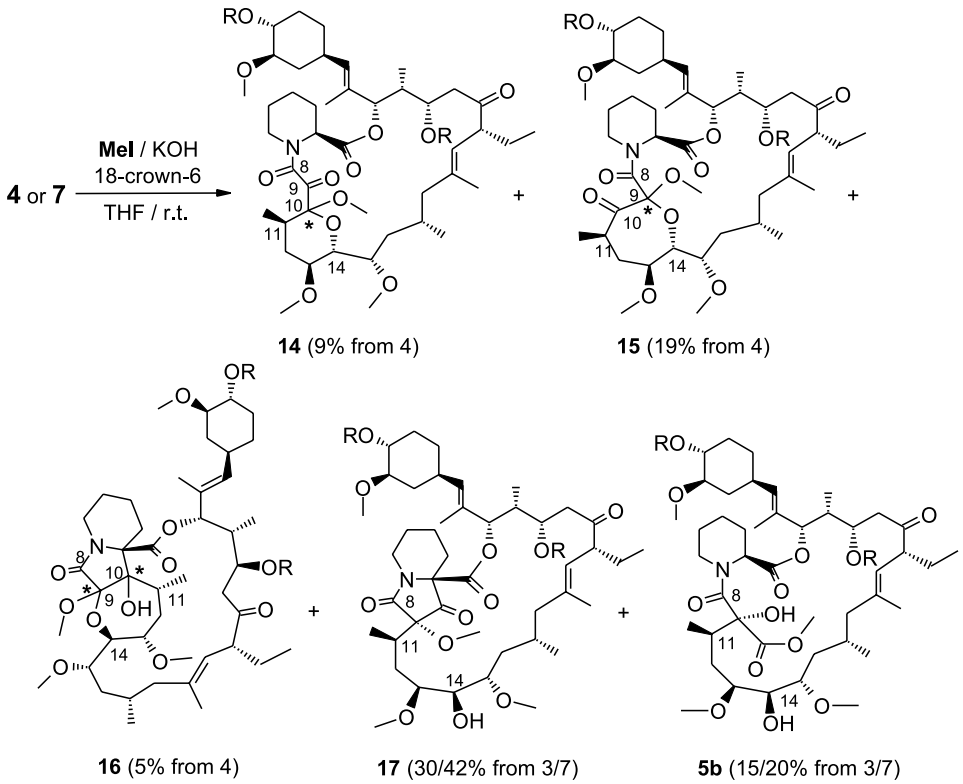


Scheme 3.

excellent diastereoselectivity after esterification with diazomethane (10(*S*)/10(*R*)=96:4) the 14,24,33-tris-OTBDMS-protected hydroxy acid methyl esters **11a** and **11b**. Saponification of the separated esters with excess calcium hydroxide in tetrahydrofuran–water solution allowed the isolation of the corresponding acids **12a** and **12b** as well. Desilylation of **11a** and **5a**, using aqueous hydrogen fluoride in acetonitrile solution furnished the same hydroxy acid methyl ester **13a**, thus corroborating that **5a**, **11a**, **12a** and **13a** differ only with respect to their protection pattern but exhibit the same stereochemistry at C10. The same relationship could be shown for the 10(*S*)-epimers **5b**, **11b**, **12b** and **13b**, respectively.

2.2.2. Formation of 6b and 7. Inspection of the structure of **7** clearly reveals that a rearrangement process and a cyclisation event are required for its formation. Therefore, two general pathways, a cyclisation prior to a rearrangement or vice versa should be considered. In order to gain some insight into the reaction pathway involved, we repeated the reaction leading to **7** and **6b** in the presence of excess methyl iodide as trapping reagent (Scheme 4).

In the event, 24,33-bis-OTBDMS-ascomycin **4** was added in one portion at room temperature to a well stirred suspension of powdered potassium hydroxide (1.5 equiv.), 18-crown-6 (0.5 equiv.) and methyl iodide (10 equiv.) in tetrahydrofuran. After an acidic work up a complex mixture was obtained which could be separated by column chromatography on silica gel to afford the O-methylated derivatives **14**-**17** and the 10(*R*)-hydroxy ester **5b**. Using **7** as a starting material and applying the same reaction conditions led in an overall yield of 62% to the compounds **17** and **5b** as well, thus emphasising once again the key role of **7** as intermediate and further confirming that **17** has the same configurations as **7** at all chiral positions. The compounds **14** and **15** represent trapped versions of the potential equilibrium products of **4** and thus their formation is explicable. Inspective is the formation of the O-methyl-derivative **16** since in this compound a cyclisation between C2 and C10 together with an intact (not rearranged) carbon chain (C8–C11) can easily be recognised. Interestingly, compound **15** is completely stable under the conditions of its formation. Thus, no further cyclisation to **16** is observed. Taking this into consideration, a reaction mechanism involving a cyclisation prior to a rearrangement can be proposed (Fig. 3). Thus, cyclisation between C2 and C10 may start from the O-deprotonated seven-membered hemiketal form **C** via a, most probably, intramolecular assisted deprotonation at C2 (no intramolecular assistance is possible in the O-methylated derivative **15**) followed by ring closure to give the intermediate **D**, which has the possibility to be in a hemiketal/ketone equilibrium with the unmasked α-ketoamide form **F**. Taking into consideration intermediate **F**, an α-ketol-type rearrangement easily explains the formation of **7** which in turn, upon a hydroxide mediated *retro*-ester condensation event, provides the hydroxy acid **6b**. The rearrangement process **F** to **7** is not unlikely, since thereby the destabilising electronic repulsion of two adjacent carbonyls (C8, C9) is removed. However, although the suggested reaction pathway is supported by the quenching experiment, other reaction



Scheme 4.

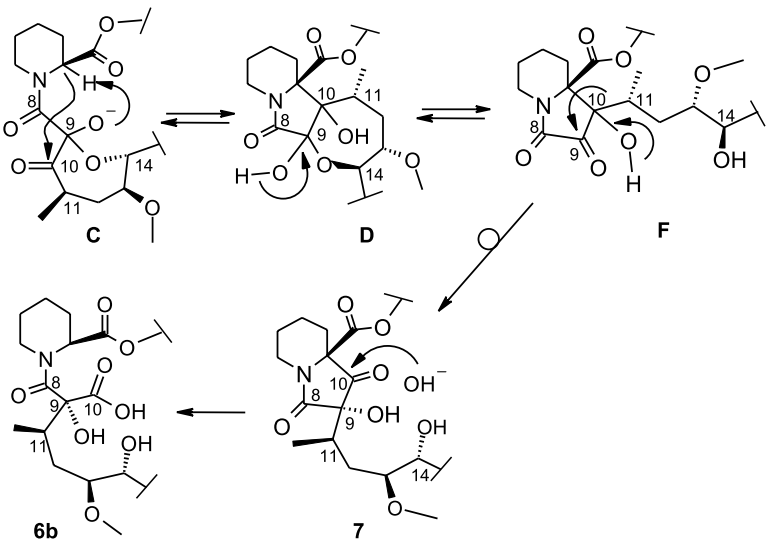
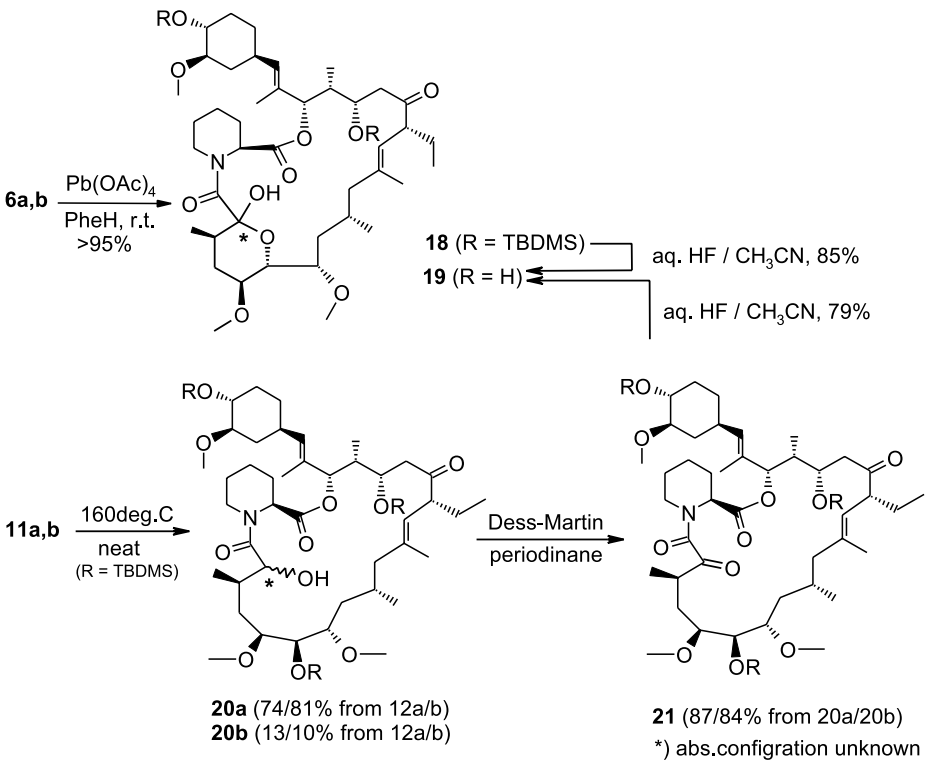


Figure 3.

pathways can be proposed as well. Further attempts to identify decisive experiments which allow the mechanism to be unambiguously defined are ongoing.

2.3. Stereochemical assignments

2.3.1. Stereochemical correlations via regioselective degradation. As already shown above, the ring contracted hydroxy acid derivatives **5a**, **6a**, **11a**, **12a** and **13a** differ only with respect to their substitution pattern and not in their stereochemical arrangement. The same applies to the series **5b**, **6b**, **11b**, **12b** and **13b**. From the mechanistic investigations and the trapping experiments it is also clear that the rearrangement product **7** and its O-methylated congener **17** relate to the 10(*R*) hydroxy acid **6b**. However, since the formation of the **6b** evidently involves position C2, **6a** and **6b** may not only differ at the newly created chiral center (C10), but also in the configuration at C2. In order to compare those isomers with respect to their relative configurations, it was necessary to remove or modify the chirality at C10 without affecting the remaining chiral positions. This could be achieved by an oxidative decarboxylation of the diastereoisomeric acids **6a** and **6b** or by a thermal decarboxylation of the 14,24,33-tri-s-OTBDMS-protected hydroxy acids **12a** and **12b** (Scheme 5).



Scheme 5.

Thus, reacting **6a** or **6b** with excess lead tetra acetate in benzene solution afforded, independent of the starting material applied, the common degradation product **18**, which upon desilylation provided the nor-9-carbonyl-ascomycin derivative **19** in high overall yield. It is interesting to note, that **18** exists in deuterochloroform solution exclusively in the 9(*R*)-hemiketal form (NOE from 9-OH to H14) and as a single conformer with *E*-configuration at the amide bond. In contrast, the deprotected congener **19** is in equilibrium with the free 9-keto form (hemiketal form/keto-form=5:1) whereby the hemiketal form adopts most probably exclusively the *E*-amide and the keto form the *Z*-amide orientation.[30] Alternatively, heating up **12a** or **12b** in the absence of solvent to 160 °C for 10 min accomplished a clean decarboxylation to give mixtures of the α-hydroxy amides **20a** and **20b**, which differ only in their configuration at C10. As expected, oxidative conversion of both, applying the Dess–Martin protocol,[31] gave the 14,24,33-tris-OTBDMS-protected α-keto amide **21**, which could be deprotected to **18** as well, thus once again corroborating that **6a** and **6b** (or **12a** and **12b**) differ only in their configuration at C10. Together with the deuteration experiments, listed in Table 1, this also substantiate, that all other chiral positions of **6a**,**b** and its differently protected congeners are identical as compared to those in natural ascomycin.

2.3.2. X-ray analysis of the compounds 8 and 13a. After having carefully established the relative stereochemical relationships, crystalline material for X-ray analysis was required in order to determine absolute configurations. Gratifyingly, suitable crystals could be obtained from the compounds **8** (**8**·3H_2O from methanol–water) and **13a** (**13a**·THF from tetrahydrofuran). ORTEP-plots of the structures (atomic displacement ellipsoids drawn at the 50% probability level, hydrogen atoms drawn as spheres of arbitrary radius) are depicted in Figures 4 and 5.[32]

The rearrangement product **13a** (and consequently all of its

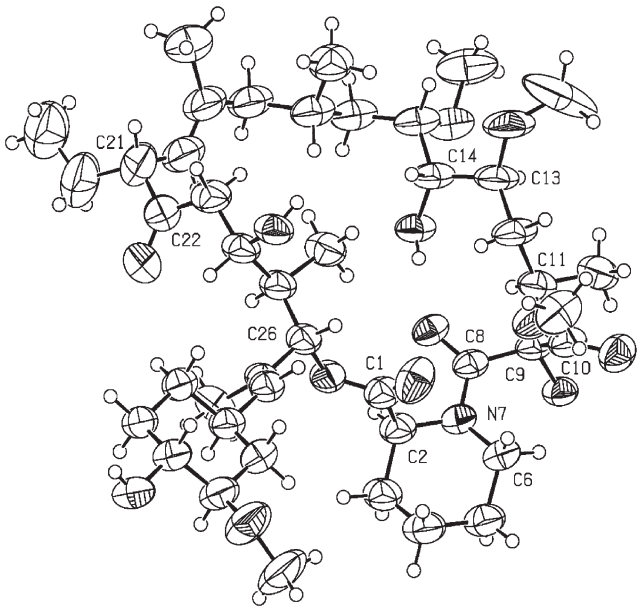


Figure 4.

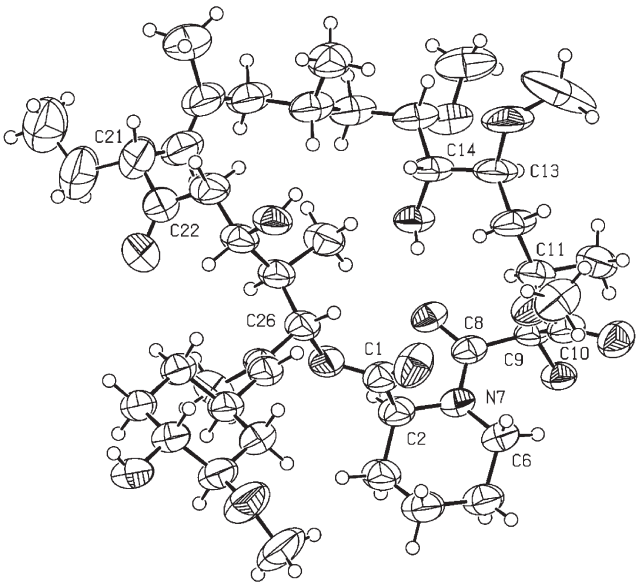


Figure 5.

only differently protected congeners: i.e., **5a**, **6a**, **11a** and **12a**) exhibits the *S*-configuration at the quaternary carbon C10 and the natural *S*-stereochemistry at C2. Consequently, and as deduced from the selective degradations, the series **5b**, **6b**, **11b** and **12b** should exhibit the same stereochemistry at C2 (*S*) but the opposite configuration (*R*) at C10. The latter is in agreement with the X-ray structure of **8** (Fig. 5), which exhibits the 2(*R*), 10(*S*)-configuration. With the aid of the X-ray-structure of **8**, the absolute configurations of congeners **7** and **17** at C2 and C10, together with the 2-(*R*)-configuration at C2 of the cyclised but not yet rearranged compound **16** could be assigned unambiguously as well.

3. Summary

Starting from 24,33-bis-OTBDMS-ascomycin **4** carefully chosen reaction conditions allow either the diastereoselective preparation of the 10(*S*)-α-hydroxy acid **6a** or its 10(*R*)-congener **6b** in reasonable yields. Mechanistic investigations confirmed, that **6a** is formed via an benzilic acid type rearrangement process as proposed earlier,[1,2] whereas, trapping experiments and the isolation of the novel rearrangement product **7** clearly suggest, that the 10(*R*)-isomer **6b**, which has been isolated for the first time, is formed through a cascade of reaction steps including tautomerisation, cyclisation and a acyloin-rearrangement followed by a *retro*-ester condensation. The relative and absolute stereochemistry at newly formed chiral centers of most of the compounds disclosed herein could be determined unambiguously via regioselective degradation reactions and X-ray analysis. The structures of all compounds are fully supported by one- and two-dimensional NMR data. Since the tricarbonyl portion of ascomycin is a source of lability, the findings described herein may be of use for researchers in the field, who are attempting to replace the tricarbonyl by more stable binding domain mimics.

4. Experimental

4.1. General

All NMR spectra were recorded on a BRUKER AVANCE 500 MHz spectrometer (resonance frequencies 500.13 MHz for ^{1}H, 125.76 MHz for ^{13}C), equipped with a broadband inverse probe head with *z*-gradients, in 0.6 ml $CDCl_3$ (Merck Uvasol®, 99.8% D) at 301 K. Chemical shifts are given in values of ppm, referenced to residual $CHCl_3$ signals (7.26 for ^{1}H, 77.0 for ^{13}C). Proton and carbon-13 signal assignments were deduced from ^{1}H, ^{13}C, gradient-selected $^{1}H,^{1}H$-COSY (correlated spectroscopy), gradient-selected inverse $^{1}H,^{13}C$-HSQC (heteronuclear single-quantum correlation), and gradient-selected inverse $^{1}H,^{13}C$-HMBC (heteronuclear multiple-bond correlation) experiments. Stereochemical information was extracted from two-dimensional T-ROESY (transverse rotating-frame Overhauser effect spectroscopy) or selective one-dimensional ROESY[20] experiments. Routine mass spectroscopy (ESI, electrospray ionisation) was performed on a Finnigan Navigator AQA mass spectrometer with HP 1100 LC system, using methanol (Merck LiChrosolv®, gradient grade) as solvent. Solutions of approx. 50–100 μg/ml of the test compound in acetonitrile (Merck LiChrosolv®) were used for injection. Two scans in each experiment were applied, with 25 and 50 V cone voltages, respectively. The probe temperature was 523 K. High-resolution mass spectra (HRMS) were measured on a Finnigan MAT900 S mass spectrometer or on a 9.4T Bruker APEX III Fourier Transform mass spectrometer in positive-ESI mode. Unit cell determination and intensity data collection for compound **8** were performed on an Enraf Nonius CAD4 with graphite monochromatised Cu(K_α) radiation. Non-hydrogen atoms were refined with anisotropic displacement parameters, hydrogen atoms were calculated in idealised positions and refined using a riding model. Unit cell determination and intensity data collection for **13b** were performed on a Bruker AXS SMART 6000 CCD, with Cu(K_α) radiation from rotating anode generator with Osmic multilayer mirrors. A semi-empirical absorption correction was applied, based on the intensities of symmetry-related reflections measured at different angular settings. The structures were solved by direct methods and refined by full-matrix least-squares on F^2. All reactions were monitored by HPTLC (Merck HPTLC-plates, silica gel 60, F_{254}). Visualisation of the reaction components was obtained by spraying with a solution of molybdatophosphoric acid (20% in EtOH/H_2O, 3:1). Flash column chromatography was performed on silica gel (Merck, silica gel 60, 0.04–0.063 mm, 230–400 mesh ASTM) at approx. 3–5 bar. Solvents and reagents (reagent grade) were used as purchased. Samples for micro-elementary analysis were subjected to size exclusion chromatography (Sephadex® LH20, in order to get rid of minor low molecular weight impurities which might originate from the solvents used for chromatography) and lyophilised from dioxane or benzene at high vacuum.

4.2. Preparation of 5a, 5b, 6a, 6b and 7 and 2-deuterio-5b according to Table 1

Compounds **5a**,**b** (*entry* 1). To a solution of 5.1 g 24,33-bis-OTBDMS-ascomycin **4** (5 mmol) in 300 ml tetrahydrofuran and 35 ml water were added 25 ml (25 mmol, 5 equiv.) of an aq. 1 N-lithium hydroxide solution and the resultant slightly turbid mixture was magnetically stirred for 12 min at room temperature. For work up the mixture was partitioned between ethyl acetate (600 ml) and 1 N hydrochloric acid (150 ml). The aqueous layer was separated and washed twice with ethyl acetate (100 ml). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and concentrated at reduced pressure to a volume of about 100 ml. The resultant solution was titrated with an approx. 1 M ethereal diazomethane solution until the yellow colour did not disappear. Excess diazomethane was removed in a slight stream of nitrogen and the remaining solution was evaporated to dryness at the rotary evaporator. Flash column chromatography (silica gel, toluene/acetonitrile=5:1) provided **5b** as the first and **5a** as the second fraction. The product fractions were evaporated under reduced pressure and dried for 15 h under high vacuum to give 3.71 g (72%) **5a** and 0.57 g (11%) **5b** as amorphous powders.

Compounds **5a**,**b** (*entries* 2 and 3). Starting from 5.1 g 24,33-bis-OTBDMS-ascomycin **4**, but using 1 N aqueous potassium hydroxide (or sodium hydroxide) solution, the reaction, work up, esterification and purification was performed as described above to give the title compounds as colourless foams in yields as indicated in Table 1.

Compound **5a** *or* **6a** (*entry* 4). To a solution of 10.2 g 24,33-bis-OTBDMS-ascomycin **4** (10 mmol) in 300 ml tetrahydrofuran and 50 ml water were added 7.41 g (100 mmol, 10 equiv.) calcium hydroxide in one portion and the resultant suspension was stirred at room temperature until TLC indicated the complete consumption of the starting material (30 min). The mixture was partitioned between ethyl acetate (1200 ml) and 1 N hydrochloric acid (400 ml). The aqueous layer was separated and extracted twice with ethyl acetate (200 ml). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate and evaporated to dryness at reduced pressure to give the crude acid **6a**.

Isolation as hydroxy acid **6a**. Half of the above crude product was subjected to a short flash column chromatography (silica gel, dichloromethane/methanol=10:1). The product containing fraction was evaporated at the rotary evaporator, re-dissolved in 200 ml ethyl acetate, washed with 20 ml 1 N-hydrochloric acid (to remove salt forming impurities originating from the silica gel) and brine, dried over anhydrous sodium sulfate and filtered through a Whatman® (A) glass microfibre filter. The filtrate was evaporated at reduced pressure and dried on high vacuum to give 4.93 g (95%) **6a** as an amorphous powder.

Isolation as hydroxy acid, *methyl ester* **5a**. The remaining crude acid **6a** was dissolved in 100 ml ethyl acetate, titrated with an approx. 1 M ethereal diazomethane solution until the yellow colour remained. Excess diazomethane was removed in a slight stream of nitrogen and the remaining solution was evaporated to dryness at the rotary evaporator. Flash column chromatography (silica gel, toluene/ethyl

acetate=2:1) provided 4.79 g (91%) **5b** as amorphous powder.

Compound **5a** (*entries* 5–7). To solutions of in each case 2.0 g (1.96 mmol) **4** in mixtures of 70 ml tetrahydrofuran/water=7:1, 20:1 and 80:1 were added 1.45 g (10 equiv., 19.6 mmol) calcium hydroxide and the reactions were stirred at room temperature until TLC indicated complete consumption of the starting material (1, 3 and 30 h). Work up, esterification and purification as described above provided 1.88 (91%), 1.77 (86%) and 1.53 g (74%) **5a** as colorless foams.

Compound **5b** *and* **6b** (*entry* 8). To a solution of 3.0 g (2.94 mmol) 24,33-bis-OTBDMS-ascomycin **4** in 30 ml dimethyl sulfoxide (DMSO) were added 85 mg (1.2 equiv.; 3.53 mmol) powdered lithium hydroxide, monohydrate in one portion and the resultant suspension was vigorously stirred at room temperature (30 min). The reaction mixture was partitioned between ethyl acetate (200 ml) and 1 N-hydrochloric acid (50 ml). The organic layer was five times washed with water, dried over anhydrous sodium sulfate and evaporated at reduced pressure. The residual oil was subjected to size exclusion chromatography (Sephadex® LH20, ethyl acetate) in order to remove DMSO completely and the relevant fraction was evaporated to dryness. The crude product was divided into two equal parts and further manipulated as described above (entry 4) to give 1.19 g (78%) **6b** and 1.16 g (75%) **5b** as amorphous powders.

Compound **5b** (*entry* 9). Starting from 1.0 g (0.98 mmol) 24,33-bis-OTBDMS-ascomycin **4** in 10 ml DMSO and 66 mg (1.2 equiv.; 1.18 mmol) powdered potassium hydroxide, the reaction was performed and worked up as described above (30 min reaction time) to give after esterification and purification 0.81 g (78%) **5b** as amorphous powder.

Compounds **5b** *and* **7** (*entries* 11–14). To solutions of in each case 2.0 g (1.96 mmol) 24,33-bis-OTBDMS-ascomycin **4** and 100 mg (0.38 mmol, 20 mol%) 18-crown-6 in 100 ml tetrahydrofuran were added 132 mg (1.2 equiv.; 2.36 mmol) powdered potassium hydroxide and the suspensions were allowed to stir at room temperature for 1.5/10/30 and 40 min, respectively. For work up, the mixtures were partitioned between ethyl acetate (300 ml) and 1 N-hydrochloric acid (60 ml). The organic layers were washed with brine, dried over anhydrous sodium sulfate and evaporated at reduced pressure to give the crude products as slightly brownish foams.

The crude products of the 40/30 and 10 min runs were redissolved in 40 ml dichloromethane and titrated with an approx. 1 M ethereal solution of diazomethane until the characteristic yellow colour remained. The resultant solutions were evaporated and subjected to flash column chromatography (silica gel, toluene/ethyl acetate=2:1 to 1:1) to give the title compounds **5b** and **7** as amorphous powders. 40 min run (entry 10): 1.69 g (82%) **5b**; 30 min run (entry 11): 1.55 g (75%) **5b** and 0.12 g (6%) **7**; 10 min run (entry 12): 1.07 g (52%) **5b** and 0.48 g (24%) **7**. The crude product of the 1.5 min run (entry 13) was directly subjected to flash column chromatography (silica gel, toluene/ethyl acetate=1:1) to give 0.22 g (11%) of recovered starting material **4** and 1.48 g (74%) **7** as amorphous powders.

Compound **5b** *from* **7** (*entry* 14). To a solution of 0.5 g (0.49 mmol) **7** and 25 mg (0.098 mmol, 20 mol%) 18-crown-6 in 25 ml tetrahydrofuran were added 33 mg (1.2 equiv.; 0.59 mmol) powdered potassium hydroxide and the suspensions were allowed to stir at room temperature for 40 min. Work up, esterification and purification as described above provided 0.44 g (85%) **5b** as amorphous powder.

Compound **5a** and 2-deuterio-**5b** (*entry* 15). To a solution of 0.51 g 24,33-bis-OTBDMS-ascomycin **4** (0.5 mmol) in 30 ml tetrahydrofuran and 3.5 ml deuterium oxide were added 2.5 ml (2.5 mmol, 5 equiv.) of an aq. 1 N-sodium deuterium oxide solution in deuterium oxide and the resultant mixture was stirred for 12 min at room temperature. Work up, esterification and purification as described for entry 1 provided 0.38 g (71%) **5a** and 0.047 g (9%) 2-deuterio-**5b**.

Compound **5a** (*entry* 16). 1.02 g 24,33-bis-OTBDMS-ascomycin **4** (1 mmol) were added in one portion to a pre-stirred (10 min) suspension of 0.74 g (10 mmol, 10 equiv.) calcium hydroxide in 30 ml tetrahydrofuran and 5 ml deuterium oxide. After 30 min, an acidic work up, followed by esterification and purification as described for entry 4 provided 0.98 g (93%) **5a**. No deuteration could be seen by MS and ^{1}H NMR.

Compound 2-deuterio-**5b** *from* **7** (*entry* 17). To a solution of 0.51 g **7** (0.5 mmol) in 30 ml tetrahydrofuran and 3.5 ml deuterium oxide were added 2.5 ml (2.5 mmol, 5 equiv.) of a 1 N-sodium deuteride solution in deuterium oxide and the resultant mixture was magnetically stirred for 40 min at room temperature. Work up, esterification and purification as described for entry 1 provided 0.37 g (71%) 2-deuterio-**5b** as amorphous powder.

Compound **5a** *and* **5b** *from* **6a** *and* **6b** (*entries* 18 *and* 19). To a solution of 0.52 g (0.5 mmol) **6a** or **6b** in 30 ml tetrahydrofuran and 3.5 ml deuterium oxide were added 2.5 ml (2.5 mmol, 5 equiv.) of a 1 N-sodium deuteride solution in deuterium oxide and the resultant mixtures was stirred for 15 min at room temperature. Work up, esterification and purification as described for above provided 0.48 g (91%) **5a** and 0.47 g (89%) **5b** as amorphous powders.

4.2.1. Compound 5a. CHN ($C_{56}H_{101}NO_{13}Si_2$) calcd: 63.90/9.67/1.33, found: 63.64/9.51/1.24. HRMS (M+Na; calcd/found): 1074.6709/1074.6713. ^{13}C NMR ($CDCl_3$, *Z*/*E*=3:2), δ (*Z*/*E*-isomer, ppm): 168.38/168.93 (C1); 53.99/56.63 (C2); 26.32/28.20 (C3); 20.95/20.60 (C4); 24.93/25.01 (C5); 43.58/42.00 (C6); 169.47/168.93 (C8); 81.61/81.43 (C9); 172.16/172.16 (C10); 35.08/36.02 (C11); 36.10/36.08 (C12); 79.23/82.19 (C13); 74.39/73.48 (C14); 77.69/77.51 (C15); 41.0br/n.d. (C16); 28.80/26.55 (C17); 47.22/47.43 (C18); 139.17/139.72 (C19); 122.92/123.89 (C20); 55.03/56.25 (C21); 209.29/209.24 (C22); 45.40/48.40 (C23); 67.70/68.28 (C24); 38.98/39.47 (C25); 83.95/

81.25 (C26); 131.19/131.57 (C27); 12.25/11.30 (C28); 136.46/135.72 (C29); 35.08/35.11 (C30); 36.37/36.16 (C31); 84.19/84.10 (C32); 75.18/75.11 (C33); 33.93/33.87 (C34); 30.81/30.67 (C35); 23.25/23.53 (C36); 11.63/11.57 (C37); 52.86/52.75 (10-OMe); 56.76/56.63 (13-OMe); 57.42/59.07 (15-OMe); 57.89/57.83 (32-OMe); 14.33/13.43 (11-Me); 20.05/20.61 (17-Me); 18.50/16.60 (19-Me); 9.00/9.35 (25-Me); 25.91, 25.86, 25.78, 18.13, 18.04, −4.03, −4.36, −4.52, −4.75 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (*Z*-isomer, ppm): 5.22 (d, *J*=5.5 Hz, H-2); 4.03 (d, *J*=12.5 Hz, H-6a); 3.09 (H-6b); 2.66 (H-11); 3.29 (H-13); 3.52 (H-14); 3.51 (H-15); 4.92 (d, *J*=10.2 Hz, H-20); 3.13 (H-21); 2.75 (dd, *J*=17.5+7.9 Hz, H-23a); 2.36 (dd, *J*=17.5+5.4 Hz, H-23b); 4.17 (dd, *J*=7.9+5.4 Hz, H-24); 5.13 (d, *J*=9.9 Hz, H-26); 5.25 (d, *J*=9.7 Hz, H-29); 2.94 (H-32); 3.40 (H-33); 1.01 (d, 3H, *J*=6.7 Hz, 11-Me); 1.74 (s, 3H, 19-Me); 1.62 (s, 3H, 27-Me); 0.07, −0.03, (s, each 3H, Si−Me), 0.06 (s, 6H, Si−Me); 0.89 (s, 18H, $(CH_3)_3$); 3.82 (s, 3H, 10-OMe); 5.48 (s, 9-OH); δ (*E*-isomer, ppm): 5.00 (br s, H-2); 4.56 (d, *J*=13.5 Hz, H-6a); 2.98 (H-6b); 2.35 (H-11); 3.20 (H-13); 3.48 (H-14); 3.44 (H-15); 4.69 (d, *J*=10.0 Hz, H-20); 3.31 (H-21); 2.85 (dd, *J*=14.7+10.0 Hz, H-23a); 2.28 (dd, *J*=14.7+4.4 Hz, H-23b); 4.08 (dd, *J*=10.0+4.4 Hz, H-24); 5.19 (d, *J*=8.0 Hz, H-26); 5.31 (d, *J*=8.6 Hz, H-29); 2.94 (H-32); 3.40 (H-33); 0.93 (d, 3H, *J*=6.6 Hz, 11-Me); 1.83 (s, 3H, 19-Me); 1.55 (s, 3H, 27-Me); 0.07, 0.02, (s, each 3H, Si−Me), 0.01 (s, 6H, Si−Me); 0.87, 0.85 (s, each 9H, $(CH_3)_3$); 3.62 (s, 3H, 10-OMe); 4.20 (br s, 9-OH).

4.2.2. Compound 5b. CHN ($C_{56}H_{101}NO_{13}Si_2$) calcd: 63.90/9.67/1.33, found: 63.72/9.61/1.28. HRMS (M+Na; calcd/found): 1074.6709/1074.6716. ^{13}C NMR ($CDCl_3$, single rotamer), δ (ppm): 169.68 (C1); 53.32 (C2); 25.97 (C3); 20.59 (C4); 25.13 (C5); 44.28 (C6); 168.25 (C8); 82.17 (C9); 172.92 (C10); 36.66 (C11); 34.53 (C12); 79.64 (C13); 75.31 (C14); 77.59 (C15); 35.35 (C16); 28.06 (C17); 48.07 (C18); 139.09 (C19); 123.60 (C20); 55.15 (C21); 209.60 (C22); 45.83 (C23); 67.85 (C24); 39.93 (C25); 80.56 (C26); 131.74 (C27); 12.49 (C28); 134.92 (C29); 35.07 (C30); 36.41 (C31); 84.19 (C32); 75.17 (C33); 33.93 (C34); 30.82 (C35); 23.54 (C36); 11.62 (C37); 52.99 (10-OMe); 56.37 (13-OMe); 57.50 (15-OMe); 57.88 (32-OMe); 15.43 (11-Me); 20.32 (17-Me); 17.82 (19-Me); 9.37 (25-Me); 25.93, 25.87, 18.15, 18.06, −4.33, −4.52, −4.74 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (ppm): 5.27 (d, *J*=4.8 Hz, H-2); 3.97 (d, *J*=13.5 Hz, H-6a); 3.24 (H-6b); 2.69 (H-11); 3.26 (H-13); 3.40 (H-14); 3.46 (ddd, *J*=9.4+4.6+1.7 Hz, H-15); 4.88 (d, *J*=9.9 Hz, H-20); 3.15 (H-21); 2.60 (dd, *J*=17.5+5.5 Hz, H-23a); 2.46 (dd, *J*=17.5+6.5 Hz, H-23b); 4.17 (ddd, *J*=6.5+5.5+2.1 Hz, H-24); 5.13 (d, *J*=7.5 Hz, H-26); 5.24 (d, *J*=8.9 Hz, H-29); 2.93 (ddd, *J*=11.4+8.5+4.6 Hz, H-32); 3.40 (H-33); 0.93 (d, 3H, *J*=6.7 Hz, 11-Me); 1.71 (d, 3H, *J*=1.2 Hz, 19-Me); 1.60 (d, 3H, *J*=1.2 Hz, 27-Me); 0.08, 0.06, 0.03, −0.06 (s, each 3H, Si−Me); 0.89, 0.86 (s, each 9H, $(CH_3)_3$); 4.06 (br s, 9-OH); 3.80 (s, 3H, 10-OMe).

4.2.3. Compound 2-deuterio-5b. ^{13}C NMR ($CDCl_3$, single rotamer), δ (ppm): 169.67 (C1); 53.10 (C2); 25.9 (br) (C3); 20.53 (C4); 25.13 (C5); 44.24 (C6); 168.23 (C8); 82.15 (C9); 172.92 (C10); 36.64 (C11); 34.56 (C12); 79.59 (C13); 75.32 (C14); 77.59 (C15); 35.33 (C16); 28.11 (C17); 48.07 (C18); 139.10 (C19); 123.59 (C20); 55.13 (C21); 209.58 (C22); 45.91 (br) (C23); 67.81 (C24); 39.88 (C25); 80.63 (br) (C26); 131.73 (C27); 12.46 (C28); 133.5 br (C29); 35.06 (C30); 36.40 (C31); 84.18 (C32); 75.16 (C33); 33.92 (C34); 30.81 (C35); 23.52 (C36); 11.61 (C37); 53.00 (10-OMe); 56.35 (13-OMe); 57.49 (15-OMe); 57.88 (32-OMe); 15.41 (11-Me); 20.31 (17-Me); 17.83 (19-Me); 9.34 (25-Me); 25.93, 25.86, 18.14, 18.06, −4.24, −4.34, −4.52, −4.75 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (ppm): H2 absent; 3.94 (d, *J*=13.9 Hz, H-6_{eq}); 3.23 (m, overlapped, H-6_{ax}); 2.68 (m, H-11); 3.24 (m, H-13); 3.37 (m, H-14); 3.46 (ddd, *J*=9.4+4.6+1.3 Hz, H-15); 4.87 (d, *J*=9.7 Hz, H-20); 3.14 (m, H-21); 2.60 (dd, *J*=17.8+5.3 Hz, H-23a); 2.44 (dd, *J*=17.8+6.6 Hz, H-23b); 4.16 (ψtd, *J*=6.1+1.5 Hz, H-24); 5.13 (d, *J*=7.6 Hz, H-26); 5.24 (d, *J*=8.7 Hz, H-29); 2.25 (m, H-30); 2.92 (m, H-32); 3.38 (m, H-33); 0.82 (t, *J*=7.3 Hz, CH_3-37); 0.07, 0.06, 0.02, −0.07 (4s, 24H, 24-Si(t.Bu)*Me*$_2$+32-Si(t.Bu)*Me*$_2$); 0.88, 0.85 (2s, 18H, 24-Si(*t*Bu)Me$_2$+32-Si(*t*Bu)Me$_2$); ~4.0 (s, very broad, 9-O*H*); 3.80 (s, 10-OMe); 0.92 (d, 3H, *J*=6.9 Hz, 11-Me); 3.31 (s, 3H, 13-OMe); 3.36 (s, 3H, 15-OMe); 1.70 (s, 3H, 19-Me); 1.59 (s, 3H, 27-Me); 3.38 (s, 3H, 32-OMe).

4.2.4. Compound 6a. CHN ($C_{55}H_{99}NO_{13}Si_2$) calcd: 63.61/9.61/1.35, found: 63.39/9.58/1.21. HRMS (M+Na; calcd/found): 1060.6553/1060.6549. ^{13}C NMR (CD_3OD, single rotamer), δ (ppm): 169.55 (C1); 53.39 (C2); 26.18 (C3); 20.50 (C4); 24.53 (C5); 43.41 (C6); 170.14 (C8); 81.54 (C9); 172.05 (C10); 35.75 (C11); 32.99 (C12); 79.71 (C13); 74.26 (C14); 77.90 (C15); 37.36 (C16); 27.63 (C17); 46.74 (C18); 139.82 (C19); 122.36 (C20); 55.44 (C21); 210.37 (C22); 44.3 br (C23); 69.03 (C24); 39.96 (C25); 78.8 (C26); 132.07 (C27); 11.95 (C28); 132.7br (C29); 34.84 (C30); 36.03 (C31); 84.13 (C32); 75.06 (C33); 33.57 (C34); 30.62 (C35); 23.29 (C36); 10.63 (C37); 55.44 (13-OMe); 57.14 (15-OMe); 56.76 (32-OMe); 13.55 (11-Me); 19.25 (17-Me); 16.92 (19-Me); 8.57 (25-Me); 25.09, 24.99, 17.57, 17.48, −5.46, −5.56, −5.65, −5.89 (2×TBDMS). ^{1}H NMR (CD_3OD, selected data), δ (ppm): 5.15 (br d, *J*=4.5 Hz, H-2); 4.31 (d, *J*=12.5 Hz, H-6a); 3.10 (ddd, *J*=12.5+12.5+2.9 Hz, H-6b); 2.53 (H-11); 3.30 (H-13); 3.56 (dd, *J*=5.7+3.9 Hz, H-14); 3.51 (H-15); 4.94 (d, *J*=9.9 Hz, H-20); 3.26 (H-21); 2.71 (br dd, *J*=17.0+5.2 Hz, H-23a); 2.48 (dd, *J*=17.0+6.0 Hz, H-23b); 4.21 (ddd, *J*=6.0+5.2+2.6 Hz, H-24); 5.23 (H-26); 5.23 (H-29); 3.00 (ddd, *J*=11.2+8.5+4.5 Hz, H-32); 3.43 (H-33); 1.07 (d, 3H, *J*=6.6 Hz, 11-Me); 1.75 (s, 3H, 19-Me); 1.65 (d, 3H, *J*=1.2 Hz, 27-Me); 0.09, 0.08, 0.07, −0.01 (s, each 3H, Si−Me); 0.90, 0.89 (s, each 9H, $(CH_3)_3$).

4.2.5. Compound 6b. CHN ($C_{55}H_{99}NO_{13}Si_2$) calcd: 63.61/9.61/1.35, found: 63.40/9.54/1.39. HRMS (M+Na; calcd/found): 1060.6553/1060.6551. ^{13}C NMR ($CDCl_3$, mixture of rotamers >9:1), δ (major rotamer, ppm): 170.19 (C1); 55.38 (C2); 26.23 (C3); 21.57 (C4); 25.30 (C5); 45.87 (C6); 173.83 (C8); 83.48 (C9); 173.35 (C10); 40.57 (C11); 33.92 (C12); 78.03 (C13); 74.10 (C14); 77.27 (C15); 34.40 (C16); 25.86 (C17); 47.20 (C18); 137.33 (C19); 122.14 (C20); 54.53 (C21); 208.88 (C22); 49.41 (C23); 68.22 (C24); 42.65 (C25); 79.22 (C26); 130.54 (C27); 13.77 (C28); 130.54 (C29); 34.94 (C30); 36.49 (C31); 84.13 (C32); 75.10 (C33);

33.73 (C34); 30.95 (C35); 22.74 (C36); 11.76 (C37); 56.22 (13-OMe); 57.09 (15-OMe); 57.99 (32-OMe); 13.77 (11-Me); 20.62 (17-Me); 18.92 (19-Me); 10.61 (25-Me); 2×25.86, 18.13, 17.95, −4.52, −4.57, −4.68, −4.73 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (major rotamer, ppm): 5.65 (m, H-2); 5.20 (d, J=12.4 Hz, H-6$_{eq}$); 2.75 (ψt, J=12.6 Hz, H-6$_{ax}$); 2.60 (m, H-11); 3.28 (m, H-13); 3.54 (m, H-14); 3.51 (m, H-15); 4.95 (d, J=10.9 Hz, H-20); 3.22 (m, H-21); 2.89 (dd, J=18.8+7.9 Hz, H-23a); 2.24 (dd, J=18.8+2.2 Hz, H-23b); 4.20 (ψtd, J=8.4+2.5 Hz, H-24); 4.54 (s, broad, H-26); 5.02 (d, J=8.8 Hz, H-29); 2.22 (m, H-30); 2.95 (m, H-32); 3.40 (m, H-33); 0.79 (t, J=7.4 Hz, CH_3-37); 0.08, 0.07, 0.06, 0.01, 0.89, 0.87 (6s, 42H, 2×TBDMS); 4.53 (s, 9-O*H*); 13.5 (broad, 10-COO*H*); 0.96 (d, 3H, J=6.9 Hz, 11-Me); 3.30 (s, 3H, 13-OMe); 3.02 (s, very broad, 14-O*H*); 3.32 (s, 3H, 15-OMe); 1.78 (s, 3H, 19-Me); 1.49 (s, 3H, 27-Me); 3.40 (s, 3H, 32-OMe).

4.2.6. Compound 7. HRMS (M+Na; calcd/found): 1042.6447/1042.644. ^{13}C NMR ($CDCl_3$), δ (ppm): 163.89 (C1); 72.86 (C2); 33.19 (C3); 20.67 (C4); 23.72 (C5); 37.28 (C6); 170.54 (C8); 203.27 (C9); 76.70 (C10); 33.92 (C11); 30.00 (C12); 77.94 (C13); 71.81 (C14); 79.48 (C15); 39.42 (C16); 26.35 (C17); 47.79 (C18); 140.21 (C19); 123.88 (C20); 56.42 (C21); 208.50 (C22); 47.35 (C23); 68.10 (C24); 39.31 (C25); 86.06 (C26); 130.93 (C27); 11.03 (C28); 137.59 (C29); 35.17 (C30); 36.13 (C31); 84.08 (C32); 75.12 (C33); 33.85 (C34); 30.64 (C35); 22.21 (C36); 11.48 (C37); 55.12 (13-OMe); 59.74 (15-OMe); 57.91 (32-OMe); 16.94 (11-Me); 20.50 (17-Me); 16.16 (19-Me); 9.59 (25-Me); 25.87, 25.83, 18.16, 18.04, −3.78, −4.27, −4.51, −4.73 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (ppm): 2.47 (br d, J=13.6 Hz, H-3a); 1.48 (H-3b); 4.36 (dd, J=13.4+4.8 Hz, H-6a); 3.20 (H-6b); 2.52 (H-11); 3.87 (H-13); 3.88 (H-14); 3.22 (H-15); 4.72 (d, J=10.8 Hz, H-20); 3.24 (H-21); 2.82 (dd, J=15.0+11.1 Hz, H-23a); 2.17 (dd, J=15.0+4.1 Hz, H-23b); 4.11 (dd, J=11.1+4.1 Hz, H-24); 5.17 (d, J=10.6 Hz, H-26); 5.36 (d, J=8.9 Hz, H-29); 2.95 (ddd, J=11.2+8.5+4.5 Hz, H-32); 3.40 (H-33); 0.76 (d, 3H, J=7.3 Hz, 11-Me); 1.82 (s, 3H, 19-Me); 1.62 (s, 3H, 27-Me); 0.08, 0.07 (s, each 3H, Si–Me); 0.01 (s, 6H, Si–Me); 0.89, 0.85 (s, each 9H, $(CH_3)_3$); 4.25 (s, 10-OH); 2.42 (s, 14-OH).

4.2.7. Compound 8. To a solution of 3.0 g (2.94 mmol) **7** in 120 ml acetonitrile were added 10 ml aqueous hydrogen fluoride (40 w/w%). The mixture was stirred for 6 h at room temperature and then partitioned between ethyl acetate and a saturated solution of aqueous sodium hydrogen carbonate. The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate and evaporated at reduced pressure. Flash column chromatography (silica gel, ethyl acetate) provided 2.07 g (89%) **8** as amorphous powder. 1.0 g of the amorphous material was dissolved in 5 ml methanol and water (~10 ml) was added until the solution got slightly turbid. The solution was filtered through a Whatman® glass filter (type GF) and the clear solution was allowed to stand at room temperature for 2 weeks. The crystals thereby formed were used for X-ray analysis. CHN ($C_{43}H_{69}NO_{12}$, from the amorphous powder) calcd: 65.21/8.78/1.77, found: 64.92/8.43/1.59. HRMS (M+Na; calcd/found): 814.4714/814.4719. ^{13}C NMR ($CDCl_3$/d_6-DMSO=6:1), δ (ppm): 164.50 (C1); 72.05 (C2); 30.27 (C3); 20.72 (C4); 23.55 (C5); 37.59 (C6); 170.46 (C8); 76.25 (C9); 204.24 (C10); 34.18 (C11); 31.79 (C12); 78.64 (C13); 73.66 (C14); 78.67 (C15); 37.07 (C16); 27.13 (C17); 48.18 (C18); 139.42 (C19); 124.08 (C20); 55.46 (C21); 209.97 (C22); 46.25 (C23); 66.82 (C24); 38.75 (C25); 84.90 (C26); 130.76 (C27); 11.9 (C28); 134.45 (C29); 34.84 (C30); 34.68 (C31); 83.89 (C32); 73.19 (C33); 31.71 (C34); 30.24 (C35); 22.56 (C36); 11.38 (C37); 56.11 (13-OMe); 58.55 (15-OMe); 56.43 (32-OMe); 14.89 (11-Me); 20.69 (17-Me); 16.47 (19-Me); 9.03 (25-Me). ^{1}H NMR ($CDCl_3$/d_6-DMSO=6:1, selected data), δ (ppm): 2.50 (br d, J=13.5 Hz, H-3a); 2.00 (H-3b); 4.35 (br dd, J=13.7+3.0 Hz, H-6a); 3.07 (H-6b); 2.44 (H-11); 3.59 (H-13); 3.54 (H-14); 3.29 (H-15); 4.78 (d, J=10.4 Hz, H-20); 3.21 (ddd, J=10.4+8.4+5.5 Hz, H-21); 2.79 (dd, J=16.1+8.6 Hz, H-23a); 2.45 (H-23b); 3.99 (ddd, J=7.9+5.5+1.7 Hz, H-24); 5.17 (d, J=9.0 Hz, H-26); 5.36 (d, J=9.2 Hz, H-29); 3.02 (ddd, J=11.5+8.8+4.3 Hz, H-32); 3.39 (H-33); 0.87 (d, 3H, J=7.1 Hz, 11-Me); 1.77 (s, 3H, 19-Me); 1.62 (s, 3H, 27-Me).

4.2.8. Compound 9. A solution of 1.5 g (1.44 mmol) **6a** and 0.70 g (3 equiv., 4.33 mmol) 1,1′-carbonyldiimidazole (CDI) and 5 mg (2.8 mol%) 4-dimethylaminopyridine (DMAP) in 25 ml dichloromethane was stirred for 2 h at room temperature. For work up the mixture was partitioned between ethyl acetate and 1 N-hydrochloric acid. The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate and evaporated to dryness at reduced pressure. The residual foam was subjected to flash column chromatography (silica gel, toluene/ethyl acetate=5:1) to give 1.37 g (93%) **9** as amorphous powder. CHN ($C_{55}H_{97}NO_{12}Si_2$) calcd: 64.73/9.58/1.37, found: 64.59/9.40/1.21. HRMS (M+Na; calcd/found): 1042.6447/1042.6441. ^{13}C NMR ($CDCl_3$, mixture of rotamers <1:9), δ (major rotamer, ppm): 168.50 (C1); 56.94 (C2); 27.78 (C3); 20.15 (C4); 24.97 (C5); 41.54 (C6); 169.89 (C8); 80.79 (C9); 168.22 (C10); 33.36 (C11); 36.42 (C12); 76.55 (C13); 80.51 (C14); 76.5 br (C15); 31.66 (C16); 25.98 (C17); 49.20 (C18); 136.34 (C19); 125.89 (C20); 53.23 (C21); 212.98 (C22); 46.46 (C23); 70.45 (C24); 42.04 (C25); 76.5 br (C26); 133.88 (C27); 12.47 (C28); 132.62 (C29); 34.90 (C30); 36.41 (C31); 84.18 (C32); 75.13 (C33); 33.91 (C34); 30.60 (C35); 25.44 (C36); 11.45 (C37); 57.31 (13-OMe); 56.32 (15-OMe); 57.83 (32-OMe); 17.93 (11-Me); 21.30 (17-Me); 15.96 (19-Me); 11.81 (25-Me); 26.00, 25.86, 18.14, 17.96, −4.39, −4.52, −4.75 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (major rotamer, ppm): 4.14 (br s); 4.48 (d, J=13.3 Hz, H-6a); 3.85 (br dd, J=13.3+13.3 Hz, H-6b); 2.06 (H-11); 3.49 (H-13); 4.63 (d, J=8.7 Hz, H-14); 3.59 (d, J=11.4 Hz, H-15); 4.94 (d, J=9.7 Hz, H-20); 3.33 (H-21); 3.02 (d, J=19.1 Hz, H-23a); 2.63 (dd, J=19.1+8.7 Hz, H-23b); 4.13 (H-24); 5.57 (s, H-26); 5.24 (d, J=9.2 Hz, H-29); 2.93 (ddd, J=11.4+8.6+4.6 Hz, H-32); 3.38 (H-33); 0.92 (d, 3H, J=6.3 Hz, 11-Me); 1.36 (s, 3H, 19-Me); 1.63 (s, 3H, 27-Me); 0.07, 0.06 (s, each 3H, Si–Me); 0.03 (s, 6H, Si–Me); 0.89, 0.82 (s, each 9H, $(CH_3)_3$); 5.49 (br s, 9-OH).

4.2.9. Compounds 11a and 11b. To a solution of 20 g (17.62 mmol) 14,24,33-tris-OTBDMS-ascomycin **10** in 250 ml tetrahydrofuran were added 15 ml water and 20 g

(15.3 equiv., 270 mmol) calcium hydroxide and the resultant suspension was stirred for 30 min at room temperature (TLC-monitoring revealed completion of the reaction in <5 min). For work up the mixture was partitioned between ethyl acetate (800 ml) and 1 N-hydrochloric acid (200 ml). The organic layer was separated, washed twice with brine, dried over anhydrous sodium sulfate and evaporated to dryness at reduced pressure. The residue was dissolved in 200 ml dichloromethane and titrated with an approx. 1 M ethereal solution of diazomethane until the characteristic yellow colour remained. After evaporation at reduced pressure the mixture of **11a**,**b** was subjected to flash column chromatography (silica gel, cyclohexane/ethyl acetate=10:1) to give 17.69 g (86%) **11a** and 514 mg (2.5%) **11b** as amorphous powders.

4.2.10. Compound 11a. CHN ($C_{62}H_{115}NO_{13}Si_3$) calcd: 63.82/9.93/1.20, found: 63.73/9.82/1.00. HRMS (M+Na; calcd/found): 1188.7574/1188.7578. ^{13}C NMR ($CDCl_3$, *Z*/*E*~3:7), δ (*Z*(only selected data due to extreme signal broadening)/*E*-isomer, ppm): n.d./168.32 (C1); 53.53/56.35 (C2); n.d./28.50 (C3); n.d./20.95 (C4); n.d./25.07 (C5); 43.14/41.81 (C6); n.d./168,45 (C8); 82.44/81.88 (C9); n.d./171.84 (C10); n.d./39.59 (C11); n.d./31.77 (C12); 81.33/85.89 (C13); 76.98/76.14 (C14); 79.46/78.16 (C15); n.d./42.29 (C16); n.d./26.54 (C17); 46.50/46.70 (C18); n.d./140.39 (C19); n.d./123.38 (C20); n.d./56.59 (C21); n.d./209.07 (C22); n.d./48.29 (C23); n.d./68.24 (C24); n.d./39.59 (C25); n.d./84.16 (C26); n.d./131.21 (C27); 12.64/11.10 (C28); n.d./136.61 (C29); 35.03/35.13 (C30); 36.48/36.15 (C31); n.d./84.10 (C32); n.d./75.12 (C33); n.d./33.87 (C34); 30.84/30.65 (C35); 23.37/23.10 (C36); 11.54/11.54 (C37); 53.11/52.70 (10-OMe); n.d./55.85 (13-OMe); 60.20/60.38 (15-OMe); 57.84/57.84 (32-OMe); 14.74/13.82 (11-Me); 20.42/20.14 (17-Me); 16.61/16.14 (19-Me); n.d./9.19 (25-Me); 26.03, 25.86, 25.78, 18.42, 18.14, 18.03, −3.67, −4.35, −4.52, −4.75 (3×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (*Z*-isomer, ppm): 5.16 (H-2); 4.08 (H-6a); 3.35 (H-6b); 2.50 (H-11); 3.72 (d, *J*=8.4 Hz, H-14); 4.87 (d, *J*=10.2 Hz, H-20); 3.19 (H21); 2.47 (H-23a); 2.42 (H-23b); 4.08 (H-24); 2.95 (ddd, *J*=11.3+8.5+4.4 Hz, H-32); 3.40 (H-33); 1.06 (d, 3H, *J*=6.6 Hz, 11-Me); 1.72 (s, 3H, 19-Me); 1.62 (s, 3H, 27-Me); 0.90, 0.89,0.85 (s, each 9H, $(CH_3)_3$); 0.07, 0.06, 0.06 (s, each 6H, Si–Me); 3.84 (s, 3H, 10-OCH_3); δ (*E*-isomer, ppm): 4.99 (br s, H-2); 4.58 (H-6a); 3.04 (H-6b); 2.20 (H-11); 2.85 (H-13); 3.67 (d, *J*=8.4 Hz, H-14); 3.05 (H-15); 4.60 (d, *J*=9.8 Hz, H-20); 3.30 (H-21); 2.84 (dd, *J*=14.8+10.8 Hz, H-23a); 2.24 (dd, *J*=14.8+4.1 Hz, H-23b); 4.09 (dd, *J*=10.8+4.1 Hz, H-24); 5.14 (d, *J*=10.3 Hz, H-26); 5.31 (d, *J*=8.9 Hz, H-29); 2.95 (ddd, *J*=11.3+8.5+4.4 Hz, H-32); 3.40 (H-33); 0.95 (d, 3H, *J*=6.6 Hz, 11-Me); 1.83 (s, 3H, 19-Me); 1.55 (s, 3H, 27-Me); 0.90, 0.89,0.85 (s, each 9H, $(CH_3)_3$); 0.08, 0.07, 0.02 (s, each 6H, Si–Me); 3.62 (s, 3H, 10-OCH_3); 5.43 (s, 9-OH).

4.2.11. Compound 11b. CHN ($C_{62}H_{115}NO_{13}Si_3$) calcd: 63.82/9.93/1.20, found: 63.63/9.89/1.01. HRMS (M+Na; calcd/found): 1188.7574/1188.7580. ^{13}C NMR ($CDCl_3$, *Z*/*E*=4:1), δ (*Z*/*E*-isomer, ppm): 169.02/169.78 (C1); 53.84/55.89 (C2); 26.23/n.d. (C3); 20.97/n.d. (C4); 25.33/25.05 (C5); 43.72/39.96 (C6); 167.39/166.49 (C8); 83.13/81.74 (C9); 172.99/173.35 (C10); 38.60/n.d. (C11); 31.57/30.68 (C12); 84.75/85.5 br (C13); 76.34/n.d. (C14); 79.24/78.72 (C15); 41.49/n.d. (C16); 27.00/26.41 (C17); 46.56/46.90 (C18); 140.49/139.96 (C19); 123.75/123.75 (C20); 56.24/57.77 (C21); 211.78/208.73 (C22); 42.74/47.92 (C23); 72.29/67.87 (C24); 41.90/39.25 (C25); 77.08/82.6 br (C26); 132.70/131.20 (C27); 14.38/11.05 (C28); 130.37/136.58 (C29); 34.94/35.17 (C30); 36.65/36.13 (C31); 84.12/84.12 (C32); 75.10/75.08 (C33); 33.77/33.92 (C34); 30.93/n.d. (C35); 22.83/22.68 (C36); 11.47/11.47 (C37); 52.95/52.94 (10-OMe); 56.95/55.89 (13-OMe); 60.88/60.88 (15-OMe); 57.98/n.d. (32-OMe); 15.977n.d. (11-Me); 20.56/20.2 br (17-Me); 16.60/16.10 (19-Me); 10.06/n.d. (25-Me); 26.09, 26.00, 25.92, 25.86, 18.41, 18.13, 17.88, −4.47, −4.52, −4.72, −4.93 (3×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (*Z*-isomer, ppm): 5.31 (br s, H-2); 4.22 (d, *J*=14.5 Hz, H-6a); 2.90 (H-6b); 2.51 (H-11); 3.30 (H-13); 3.67 (d, *J*=8.4 Hz, H-14); 3.05 (H-15); 4.83 (d, *J*=10.2 Hz, H-20); 3.14 (ddd, *J*=10.2+9.7+4.2 Hz, H-21); 2.57 (dd, *J*=14.1+9.5 Hz, H-23a); 2.16 (H-23b); 4.03 (br d, *J*=9.5 Hz, H-24); 5.15 (H-26); 5.14 (d, *J*=9.8 Hz, H-29); 2.94 (H-32); 3.40 (H-33); 0.94 (d, 3H, *J*=6.9 Hz, 11-Me); 1.71 (s, 3H, 19-Me); 1.65 (s, 3H, 27-Me); 0.08, 0.07 (s, each 6H, Si–Me), 0.03, −0.05 (s, each 3H, Si–Me); 0.90, 0.89, 0.88 (s, each 9H, $(CH_3)_3$); 3.81 (s, 3H, 10-OCH_3); δ (*E*-isomer, ppm): 5.14 (H-2); 4.43 (d, *J*=13.2 Hz, H-6a); 3.12 (H-6b); 3.65 (H-14); 2.92 (H-15); 4.57 (d, *J*=10.2 Hz, H-20); 3.25 (H-21); 2.84 (H-23a); 2.19 (H-23b); 4.10 (H-24); 5.20 (d, *J*=10.0 Hz, H-26); 5.35 (d, *J*=9.0 Hz, H-29); 2.94 (H-32); 3.40 (H-33); 1.80 (s, 3H, 19-Me); 1.55 (s, 3H, 27-Me); 0.08 (s, 9H, Si–Me), 0.07 (s, 6H, Si–Me), 0.02 (s, 3H, Si–Me); 0.90, 0.89, 0.88 (s, each 9H, $(CH_3)_3$); 3.84 (s, 3H, 10-OCH_3).

4.2.12. Compound 12a. A suspension of 2.0 g (1.71 mmol) **11a** and 1.9 g (15 equiv., 25.7 mmol) calcium hydroxide in 50 ml tetrahydrofuran and 10 ml water was stirred for 40 h at room temperature. For work up the mixture was partitioned between ethyl acetate and 1 N-hydrochloric acid. The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate and evaporated to dryness at reduced pressure to give 1.9 g (96%) **12a** as an amorphous powder which required no further purification. HRMS (M+Na; calcd/found): 1174.7418/1174.7413. ^{13}C NMR ($CDCl_3$, mixture of rotamers <1:9), δ (major rotamer, selected data, ppm): n.d. (C1); 57.18 (C2); 28.63 (C3); 20.79 (C4); 25.24 (C5); 42.62 (C6); n.d. (C8); 83.53 (C9); n.d. (C10); 40.37 (C11); 31.72 (C12); n.d. (C13); 75.16 (C14); 79.58 (C15); 41.19 (C16); 27.18 (C17); 47.25 (C18); 140.19 (C19); 123.42 (C20); 56.42 (C21); 209.20 (C22); 48.58 (C23); 68.45 (C24); 39.89 (C25); 84.14 (C26); 131.37 (C27); n.d. (C28); n.d. (C29); 35.14 (C30); 36.20 (C31); 84.14 (C32); 75.16 (C33); 33.91 (C34); 30.67 (C35); 23.09 (C36); 11.53 (C37); 55.79 (13-OMe); 60.13 (15-OMe); 57.84 (32-OMe); 14.05 (11-Me); 20.04 (17-Me); 16.11 (19-Me); 8.8 br (25-Me); 26.05, 25.94, 25.87, 25.82, 18.30, 18.15, 18.03, −3.77, −4.30, −4.52, −4.74, −4.79 (3×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (major rotamer, ppm): 5.73 (br s, H-2); 4.53 (H-6a); 3.26 (H-6b); 2.26 (H-11); 3.00 (H-13); 3.71 (d, *J*=7.9 Hz, H-14); 2.90 (H-15); 4.57 (H-20); 3.19 (H-21); 2.87 (dd, *J*=14.7+10.8 Hz, H-23a); 2.20 (H-23b); 4.09 (dd, *J*=11.0+3.3 Hz, H-24); 5.15 (H-26); 5.32 (H-29); 2.95 (ddd, *J*=11.4+8.7+4.6 Hz, H-32); 3.39 (H-33); 0.94 (d, 3H,

J=6.8 Hz, 11-Me); 1.80 (s, 3H, 19-Me); 1.54 (s, 3H, 27-Me); 0.08, 0.01 (s, each 6H, Si−Me), 0.07, 0.06 (s, each 3H, Si−Me); 0.90, 0.86 (s, each 9H, $(CH_3)_3$), 0.89 (s, 18H, $(CH_3)_3$); 6.66 (br s, COOH).

4.2.13. Compound 12b. Starting from 0.35 g (0.3 mmol) **11b**, the reaction was performed as described above to give 0.33 g (96%) **12b**. CHN ($C_{61}H_{113}NO_{13}Si_3$) calcd: 63.55/9.88/1.21, found: 63.55/9.70/1.21. HRMS (M+Na; calcd/found): 1174.7418/1174.7420. ^{13}C NMR ($CDCl_3$, *Z*/*E*=3:1), δ (*Z*/*E*-isomer, ppm): 168.16/169.18 (C1); 55.31/57.05 (C2); 27.09/28.63 (C3); 21.17/20.38 (C4); 25.01/24.94 (C5); 45.25/40.96 (C6); 171.86/172.84 (C8); 83.97/81.26 (C9); n.d./n.d. (C10); 41.50/42.97 (C11); 30.44/30.00 (C12); 80.55/79.54 (C13); 75.68/74.85 (C14); 82.05/84.53 (C15); 40.67/41.11 (C16); 29.21/27.28 (C17); 47.26/47.26 (C18); 139.41/139.88 (C19); 124.00/123.87 (C20); 55.86/56.21 (C21); 209.75/208.95 (C22); 43.15/48.20 (C23); 70.36/67.90 (C24); 34.98/39.30 (C25); 78.10/83.33 (C26); 132.33/130.98 (C27); 13.68/11.04 (C28); 131.10/136.95 (C29); 34.93/35.18 (C30); 36.45/36.19 (C31); 84.09/84.15 (C32); 75.09/75.17 (C33); 33.83/33.92 (C34); 30.84/30.65 (C35); 24.44/22.88 (C36); 11.65/11.50 (C37); 60.48/60.18 (13-OMe); 56.10/55.86 (15-OMe); 57.87/57.87 (32-OMe); 14.06/15.98 (11-Me); 21.35/20.01 (17-Me); 17.05/16.41 (19-Me); 10.89/8.64 (25-Me); 25.95, 25.86, 25.81, 18.30, 18.15, 18.03, −3.68, −4.36, −4.47, −4.52, −4.62, −4.76 (3×TBDMS). ^1H NMR ($CDCl_3$, selected data), δ (*Z*-isomer, ppm): 5.30 (br s, H-2); 4.56 (H-6a); 3.25 (H-6b); 1.81 (H-11); 3.00 (H-13); 3.74 (d, *J*=7.9 Hz, H-14); 2.95 (H-15); 4.60 (H-20); 3.27 (H-21); 2.85 (dd, *J*=15.0+11.1 Hz, H-23a); 2.20 (H-23b); 4.09 (dd, *J*=11.1+3.8 Hz, H-24); 5.19 (d, *J*=10.9 Hz, H-26); 5.35 (d, *J*=9.0 Hz, H-29); 2.93 (H-32); 3.39 (H-33); 1.12 (d, 3H, *J*=7.0 Hz, 11-Me); 1.81 (s, 3H, 19-Me); 1.55 (s, 3H, 27-Me); 0.90 (s, 18H, Si−Me); 0.0–0.1 (overlapped, $(CH_3)_3$); δ (*E*-isomer, ppm): 5.11 (H-2); 4.96 (br d, *J*=13.0 Hz, H-6a); 3.13 (H-6b); 1.85 (H-11); 3.18 (H-13); 3.72 (d, *J*=7.9 Hz, H-14); 3.02 (H-15); 5.05 (d, *J*=10.3 Hz, H-20); 3.13 (H-21); 2.51 (dd, *J*=17.0+7.4 Hz, H-23a); 2.44 (dd, *J*=17.0+4.0 Hz, H-23b); 4.16 (H-24); 5.14 (H-26); 5.11 (H-29); 2.93 (H-32); 3.39 (H-33); 0.97 (d, 3H, *J*=7.0 Hz, 11-Me); 1.71 (s, 3H, 19-Me); 1.59 (s, 3H, 27-Me); 0.90 (s, 18H, Si−Me); 0.0–0.1 (overlapped, $(CH_3)_3$).

4.2.14. Compound 13a. (1) from **5a**. To a solution of 2.1 g (2 mmol) **5a** in 100 ml acetonitrile were added 9 ml aqueous hydrogen fluoride (40 w/w%). The mixture was stirred for 5 h at room temperature and then partitioned between ethyl acetate and a saturated solution of aqueous sodium hydrogen carbonate. The organic layer was separated, washed with brine, dried over anhydrous sodium sulfate and evaporated at reduced pressure. Flash column chromatography (silica gel, ethyl acetate) provided 1.50 g (93%) of analytically pure **13a** as amorphous powder. 1.0 g of the amorphous powder was dissolved in 30 ml tetrahydrofuran and concentrated to a volume of about 15 ml and the resulting clear solution was stored for 1 week at 4 °C. The crystals formed thereof (0.4 g) were used for X-ray analysis. (2) from **11a**. Starting from 0.4 g (0.34 mmol) **11a**, the reaction, work up and purification was performed as described above (reaction time 7 h) to provide 0.22 g (79%) **12a** as amorphous powder. HRMS (M+Na; calcd/found): 846.4980/846.4975. ^{13}C NMR ($CDCl_3$, *Z*/*E*=1:1), δ (*Z*/*E*-isomer, ppm): 169.51/n.d. (C1); 54.33/56.8 br (C2); 26.16/n.d. (C3); 20.74/n.d. (C4); 25.02/24.9 br (C5); 44.12/41.61 (C6); 168.90/n.d. (C8); 81.85/n.d. (C9); 171.96/n.d. (C10); 33.56/n.d. (C11); 30.42/n.d. (C12); 79.42/77.67 (C13); 72.86/n.d. (C14); 77.67/76.83 (C15); 36.53/35.17 (C16); 29.92/27.86 (C17); 47.65/48.78 (C18); 139.48/139.3 br (C19); 123.92/124.47 (C20); 54.48/n.d. (C21); 211.71/n.d. (C22); 45.41/45.65 (C23); 66.67n.d. (C24); 38.73/39.42 (C25); 82.54/n.d. (C26); 130.94/131.41 (C27); 12.34/13.3 br (C28); 133.86/130.49 (C29); 34.96*/34.90* (C30); 34.51/34.74 (C31); 84.17/84.17 (C32); 73.49/73.49 (C33); 31.22/31.22 (C34); 30.62/30.53 (C35); 23.70/n.d. (C36); 11.69*/11.57* (C37); 52.84/53.4 br (10-OMe); 57.65/56.14 (13-OMe); 57.97/n.d. (15-OMe); 56.48/56.48 (32-OMe); 15.26/19.76 (11-Me); 19.74/21.3 br (17-Me); 18.28/16.3 br (19-Me); 9.20/8.7 br (25-Me). ^1H NMR ($CDCl_3$, selected data), δ (*Z*-isomer, ppm): 5.31 (d, *J*=5 Hz, H-2); 4.08 (d, *J*=13.3 Hz, H-6a); 3.00 (H-6b); 2.66 (H-11); 3.30 (H-13); 3.38 (H-14); 3.49 (H-15); 4.99 (d, *J*=9.8 Hz, H-20); 3.09 (H-21); 2.64 (dd, *J*=18.3+4.7 Hz, H-23a); 2.48 (dd, *J*=18.3+8.4 Hz, H-23b); 3.98 (H-24); 5.10 (d, *J*=8.5 Hz, H-26); 5.29 (d, *J*=10.0 Hz, H-29); 2.98 (H-32); 3.40 (H-33); 1.01 (d, 3H, *J*=6.5 Hz, 11-Me); 1.72 (s, 3H, 19-Me); 1.58 (s, 3H, 27-Me); 3.81 (s, 3H, 10-OMe); 5.53 (br s, OH); 4.62 (br s, OH); δ (*E*-isomer, ppm): 4.88 (br s, H-2); 2.66 (H-11); 3.35 (H-13)*; 3.38 (H-14); 3.30 (H-15)*; 4.94 (br s, H-20); 2.75 (dd, *J*=16.8+5.8 Hz, H-23a); 2.60 (H-23b); 5.07 (d, *J*=9.5 Hz, H-29); 2.98 (H-32); 3.40 (H-33); 0.97 (d, *J*=6.5 Hz, 11-Me); 1.64 (s, 3H, 19-Me); 1.58 (s, 3H, 27-Me); 3.71 (br s, 3H, 10-OMe); 5.72 (br s, OH); 4.58 (br s, OH); (*) opposite assignment possible.

4.2.15. Compound 13b. Starting from 0.9 g (0.86 mmol) **5b** or 0.1 g (0.086 mmol) **11b** the deprotections were carried as described above to give 0.64 g (93%) or 81 mg (89%) **13b** as amorphous powders. HRMS (M+Na; calcd/found): 846.4980/846.4972. ^{13}C NMR ($CDCl_3$, single rotamer), δ (ppm): 169.92 (C1); 53.26 (C2); 25.25 (C3); 20.89 (C4); 25.15 (C5); 45.06 (C6); 168.28 (C8); 81.55 (C9); 172.46 (C10); 37.48 (C11); 33.99 or 33.93 (C12); 81.07 (C13); 75.68 (C14); 76.91 (C15); 33.99 or 33.93 (C16); 27.34 (C17); 49.19 (C18); 138.31 (C19); 125.09 (C20); 54.53 (C21); 211.92 (C22); 46.43 (C23); 66.04 (C24); 38.73 (C25); 83.78 (C26); 130.56 (C27); 12.03 (C28); 134.44 (C29); 34.99 (C30); 34.44 (C31); 84.25 (C32); 73.49 (C33); 31.27 (C34); 30.35 (C35); 23.18 (C36); 11.55 (C37); 52.68 (10-OMe); 57.26 (13-OMe); 56.93 (15-OMe); 56.38 (32-OMe); 17.17 (11-Me); 20.95 (17-Me); 16.86 (19-Me); 9.65 (25-Me). ^1H NMR ($CDCl_3$, selected data), δ (ppm): 5.32 (d, *J*=4.9 Hz, H-2); 3.88 (br d, *J*=14.2 Hz, H-6a); 3.02 (br dd, *J*=14.2+13.5 Hz, H-6b); 2.65 (H-11); 3.17 (H-13); 3.17 (H-14); 3.37 (H-15); 4.80 (d, *J*=9.9 Hz, H-20); 3.17 (H-21); 2.62 (H-23a); 2.55 (dd, *J*=18.6+9.1 Hz, H-23b); 3.96 (br d, *J*∼9 Hz, H-24); 4.93 (d, *J*=9.4 Hz, H-26); 5.36 (d, *J*=9.0 Hz, H-29); 2.98 (H-32); 3.41 (H-33); 1.02 (d, 3H, *J*=6.9 Hz, 11-Me); 1.70 (s, 3H, 19-Me); 1.49 (s, 3H, 27-Me); 4.75 (br s, 9-OH); 3.83 (s, 3H, 10-OCH_3).

4.2.16. Compounds 14-17 and 5b. (a) from **4**. To a magnetically stirred solution of 5.0 g (4.9 mmol) 24,33-bis-OTBDMS-ascomycin **4**, 500 mg (0.39 equiv., 1.9 mmol)

18-crown-6 and 3.48 g (5 equiv., 24.5 mmol, 1.53 ml) iodomethane in 150 ml tetrahydrofuran were added 0.41 g (1.5 equiv.; 7.35 mmol) powdered potassium hydroxide. After stirring for 40 min at room temperature, the mixture was partitioned between ethyl acetate (500 ml) and 1 N-hydrochloric acid (100 ml). The organic layer was washed with brine, dried over anhydrous sodium sulfate and evaporated at reduced pressure. Separation by flash column chromatography (silica gel, gradient/toluene/ethyl acetate=7:1 to 2:1) afforded 0.96 g (19%) **15**, 0.26 g (5%) **16**, 0.46 g (9%) **14**, 1.52 g (30%) **17** and 0.77 g (15%) **5b** (in the given order) as amorphous powders. (b) from **7**. Starting from 0.5 g **7** (0.49 mmol) the reaction and work up was performed as described above to give a mixture of **17** and **5b**. Flash column chromatography (toluene/ethyl acetate=3:1) afforded 213 mg (42%) **17** and 103 mg (20%) **5b**, respectively (Scheme 4).

4.2.17. Compound 14. CHN ($C_{56}H_{99}NO_{12}Si_2$) calcd: 65.01/9.65/1.35, found: 65.33/9.45/1.21. HRMS (M+Na; calcd/found): 1056.6604/1056.6605. ^{13}C NMR ($CDCl_3$, *Z*/*E*=3:1), δ (*Z*/*E*-isomer, ppm): 168.93/168.93 (C1); 50.81/56.17 (C2); 26.70/27.74 (C3); 21.21/20.72 (C4); 25.034/25.55 (C5); 43.1 br/38.35 (C6); 164.99/165.91 (C8); 196.54/196.54 (C9); 100.31/100.95 (C10); 32.35/31.29 (C11); 32.81/30.65 (C12); 74.02/74.21 (C13); 76.16/76.72 (C14); 76.98/79.48 (C15); 35.31/36.78 (C16); 26.55/28.55 (C17); 47.46/42.5 br (C18); 141.13/134.86 (C19); 121.45/126.61 (C20); 56.39/55.61 (C21); 211.15/210.66 (C22); 43.49/50.2 (C23); 72.54/67.87 (C24); 41.55/39.30 (C25); 76.16/82.8 br (C26); 133.28/131.08 (C27); 13.72/11.6 br (C28); 130.8 br/136.4 br (C29); 34.97/35.16 (C30); 36.54/36.14 (C31); 84.17/84.17 (C32); 75.15/75.15 (C33); 33.92/33.92 (C34); 35.31/35.31 (C35); 22.61/24.50 (C36); 11.31/11.65 (C37); 49.17/48.95 (10-OMe); 56.49/55.16 (13-OMe); 57.05/60.05 (15-OMe); 57.86/57.77 (32-OMe); 15.01/15.88 (11-Me); 18.55/21.46 (17-Me); 15.96/19.4 br (19-Me); 10.12/9.78 (25-Me); 25.86, 25.83, 18.13, 18.01, 17.87, −4.09, −4.51, −4.75, −4.99 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (*Z*-isomer, ppm): 5.14 (d, *J*=6.0 Hz, H-2); 3.40 (H-6a); 3.32 (H-6b); 2.10 (H-11); 3.45 (H-13); 3.62 (dd, *J*=9.5+1.6 Hz, H-14); 3.43 (H-15); 4.74 (d, *J*=10.2 Hz, H-20); 3.19 (ddd, *J*=10.0+10.0+4.2 Hz, H-21); 2.39 (H-23a); 2.24 (H-23b); 4.08 (ddd, *J*=9.0+3.4+3.4 Hz, H-24); 5.27 (br s, H-26); 5.12 (d, *J*=9.4 Hz, H-29); 2.93 (ddd, *J*=11.2+8.5+4.4 Hz, H-32); 3.37 (H-33); 1.05 (d, 3H, *J*=6.9 Hz, 11-Me); 1.62 (s, 3H, 19-Me); 1.61 (s, 3H, 27-Me); 0.07, 0.06, 0.01, −0.06 (s, each 3H, Si−Me); 0.88, 0.87 (s, each 9H, $(CH_3)_3$); 3.38 (s, 3H, 10-OMe); δ (*E*-isomer, ppm): 4.26 (d, *J*=5.4 Hz, H-2); 4.38 (br dd, *J*=13.0+3.4 Hz, H-6a); 3.20 (H-6b); 2.10 (H-11); 3.37 (H-13); 3.73 (H-14); 3.76 (H-15); 4.94 (d, *J*=10.1 Hz, H-20); 3.34 (H-21); 2.93 (H-23a); 2.35 (H-23b); 4.12 (dd, *J*=10.2+4.3 Hz, H-24); 5.22 (d, *J*=10.2 Hz, H-26); 5.33 (d, *J*=9.0 Hz, H-29); 2.93 (ddd, *J*=11.2+8.5+4.4 Hz, H-32); 3.37 (H-33); 1.09 (d, 3H, *J*=6.8 Hz, 11-Me); 1.74 (d, 3H, *J*=1.2 Hz, 19-Me); 1.53 (d, 3H, *J*=1.2 Hz, 27-Me); 0.07, 0.06, 0.02, 0.01 (s, each 3H, Si−Me); 0.89, 0.87 (s, each 9H, $(CH_3)_3$); 3.34 (s, 3H, 10-OMe).

4.2.18. Compound 15. CHN ($C_{56}H_{99}NO_{12}Si_2$) calcd: 65.01/9.65/1.35, found: 64.78/9.59/1.30. HRMS (M+Na; calcd/found): 1056.6604/1056.6603. ^{13}C NMR ($CDCl_3$, mixture of rotamers=1:4), δ (major rotamer, ppm): 169.92 (C1); 56.03 (C2); 28.02 (C3); 21.00 (C4); 25.17 (C5); 39.77 (C6); 165.39 (C8); 103.13 (C9); 209.31 (C10); 39.17 (C11); 39.59 (C12); 77.68 (C13); 78.67 (C14); 77.2 br (C15); 35.65 (C16); 25.7 br (C17); 40.2 br (C18); 139.17 (C19); 123.70 (C20); 56.03 (C21); 212.5 br (C22); n.d. (C23); 70.8 br (C24); 41.43 (C25); 78.67 (C26); 132.18 (C27); 12.6 br (C28); 134.5 br (C29); 35.04 (C30); 36.29 (C31); 84.09 (C32); 75.09 (C33); 33.85 (C34); 30.67 (C35); 23.66 (C36); 11.26 (C37); 55.25 (9-OMe); 57.13 (13-OMe); 56.15 (15-OMe); 57.84 (32-OMe); 16.92 (11-Me); 19.87 (17-Me); 15.88 (19-Me); 10.65 (25-Me); 25.86, 18.14, 17.94, −4.10, −4.46, −4.52, −4.74 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (major rotamer, ppm): 5.59 (br d, *J*=4.5 Hz, H-2); 4.37 (d, *J*=13.5 Hz, H-6a); 2.84 (br s, H-6b); 2.94 (H-11); 3.42 (H-13); 3.49 (H-14); 3.62 (H-15); 4.68 (d, *J*=10.3 Hz, H-20); 3.30 (H-21); 2.57 (br s, H-23a); 2.27 (H-23b); 4.05 (ddd, *J*=6.6+6.6+2.3 Hz, H-24); 5.22 (H-26); 5.32 (d, *J*=9.1 Hz, H-29); 2.94 (H-32); 3.38 (H-33); 1.19 (d, 3H, *J*=6.6 Hz, 11-Me); 1.74 (s, 3H, 19-Me); 1.59 (s, 3H, 27-Me); 0.07, 0.06, 0.02, 0.01 (s, each 3H, Si−Me); 0.89, 0.87 (s, each 9H, $(CH_3)_3$); 3.62 (s, 3H, 9-OMe); δ (minor rotamer, ppm): 5.10 (H-2); 2.94 (H-11); 4.91 (d, *J*=10.0 Hz, H-20); 3.20 (H-21); 2.75 (dd, *J*=17.5+5.5 Hz, H-23a); 2.36 (dd, *J*=17.5+5.9 Hz, H-23b); 4.28 (H-24); 5.22 (H-29); 2.94 (H-32); 3.38 (H-33); 1.15 (d, 3H, *J*=6.6 Hz, 11-Me); 0.06, 0.05, 0.05, 0.01 (s, each 3H, Si−Me); 0.88, 0.85 (s, each 9H, $(CH_3)_3$); 3.32 (s, 3H, 9-OMe).

4.2.19. Compound 16. CHN ($C_{56}H_{99}NO_{12}Si_2$) calcd: 65.01/9.65/1.35, found: 65.06/9.57/1.17. HRMS (M+Na; calcd/found): 1056.6604/1056.6603. ^{13}C NMR ($CDCl_3$), δ (ppm): 170.58 (C1); 73.17 (C2); 28.11 (C3); 21.58 (C4); 23.42 (C5); 39.71 (C6); 166.4 (C8); 99.04 (C9); 80.28 (C10); 33.36 (C11); 34.15 (C12); n.d. (C13); 75.06 (C14); 78.46 (C15); n.d. (C16); 27.97 (C17); 38.7 br (C18); 136.3 br (C19); 124.71 (C20); 54.04 (C21); ~214 br (C22); n.d. (C23); n.d. (C24); n.d. (C25); n.d. (C26); 131.2 br (C27); 13.8 br (C28); 127.9 br (C29); 35.35 (C30); 36.38 (C31); 84.22 (C32); 75.23 (C33); 33.99 (C34); 30.85 (C35); 25.14 (C36); 11.88 (C37); 54.41 (9-OMe); ~58 br (13-OMe); 56.04 (15-OMe); 57.63 (32-OMe); 17.9 br (11-Me); 21.46 (17-Me); 17.58 (19-Me); 10.04 (25-Me); 25.85, 25.84, 18.17, 18.00, −4.16, −4.53, −4.82 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (ppm): 2.19 (d, *J*=13.7 Hz, H-3a); 2.02 (H-3b); 4.01 (d, *J*=12.5 Hz, H-6a); 2.57 (br s, H-6b); 1.71 (H-11); 3.02 (br s, H-13); 3.68 (br d, *J*~9.0 Hz, H-14); 3.50 (br s, H-15); 4.97 (d, *J*=8.5 Hz, H-20); 3.40 (H-21); 4.22 (br s, H-24); 4.83 (br s, H-26); 5.41 (br d, *J*=6.5 Hz, H-29); 2.96 (ddd, *J*=11.4+8.5+4.6 Hz, H-32); 3.38 (H-33); 0.97 (br s, 3H, 11-Me); 1.74 (s, 3H, 19-Me); 1.61 (s, 3H, 27-Me); 0.06, 0.06, 0.04, 0.01 (s, each 3H, Si−Me); 0.88, 0.86 (s, each 9H, $(CH_3)_3$); 3.77 (s, 3H, 9-OMe); 3.69 (s, 10-OH).

4.2.20. Compound 17. CHN ($C_{56}H_{99}NO_{12}Si_2$) calcd: 65.01/9.65/1.35, found: 64.70/9.54/1.23. HRMS (M+Na; calcd/found): 1056.6604/1056.6599. ^{13}C NMR ($CDCl_3$), δ (ppm): 163.87 (C1); 72.44 (C2); 31.82 (C3); 20.40 (C4); 24.33 (C5); 37.24 (C6); 168.06 (C8); 204.20 (C9); 84.34 (C10); 34.21 (C11); 30.33 (C12); 79.46 (C13); 74.07 (C14); 78.83 (C15); 39.06 (C16); 26.69 (C17); 47.73 (C18); 140.31 (C19); 123.72 (C20); 56.20 (C21); 208.67 (C22); 47.32

(C23); 67.98 (C24); 39.30 (C25); 86.26 (C26); 130.76 (C27); 11.11 (C28); 137.65 (C29); 35.17 (C30); 36.09 (C31); 84.07 (C32); 75.09 (C33); 33.84 (C34); 30.60 (C35); 22.31 (C36); 11.47 (C37); 54.26 (10-OMe); 57.10 (13-OMe); 59.60 (15-OMe); 57.88 (32-OMe); 16.45* (11-Me); 20.50 (17-Me); 16.41* (19-Me); 9.89 (25-Me); 25.86, 25.83, 18.15, 18.04, −3.83, −4.28, −4.52, −4.74 (2×TBDMS). ^{1}H NMR ($CDCl_3$), δ (ppm): 2.44 (br d, J=12.5 Hz, H-3a); 1.42 (H-3b); 4.44 (br dd, J=13.0+3.0 Hz, H-6a); 3.23 (H-6b); 2.57 (H-11); 3.57 (H-13); 3.68 (dd, J=7.0+3.6 Hz, H-14); 3.26 (H-15); 4.73 (d, J=10.7 Hz, H-20); 3.23 (H-21); 2.80 (dd, J=15.0+10.5 Hz, H-23a); 2.21 (dd, J=15.0+4.5 Hz, H-23b); 4.10 (dd, J=10.5+4.5 Hz, H-24); 5.10 (d, J=10.4 Hz, H-26); 5.36 (d, J=9.0 Hz, H-29); 2.95 (ddd, J=11.3+8.6+4.5 Hz, H-32); 3.40 (H-33); 0.80 (d, 3H, J=7.5 Hz, 11-Me); 1.82 (s, 3H, 19-Me); 1.60 (s, 3H, 27-Me); 0.07, 0.06, 0.01, 0.00 (s, each 3H, Si−Me); 0.89, 0.85 (s, each 9H, $(CH_3)_3$); 3.16 (s, 3H, 10-OMe).

4.2.21. Compound 18. (a) from **6a**. To a solution of 0.5 g (0.48 mmol) hydroxy acid **6a** in 30 ml benzene were added 1.0 g (4.7 equiv., 2.26 mmol) lead tetra acetate in one portion. The resultant suspension was stirred for 30 min at room temperature and then partitioned between ethyl acetate and a saturated aqueous sodium hydrogen carbonate solution. The aqueous layer was removed and the organic layer was washed twice with brine, dried over sodium sulfate and evaporated to dryness at reduced pressure. The residue was subjected to a short flash column chromatography to give 0.46 g (96%) **18** as an amorphous powder. (b) from **6b**. Starting from 0.5 g **6b** the reaction, work up and purification was performed as described above to give 0.47 g (98%) **18**, CHN ($C_{54}H_{97}NO_{11}Si_2$) calcd: 65.35/9.85/1.41, found: 65.30/9.83/1.22. HRMS (M+Na; calcd/found): 1014.6498/1014.6508. ^{13}C NMR ($CDCl_3$, single rotamer), δ (ppm): 169.74 (C1); 57.00 (C2); 27.53 (C3); 20.65 (C4); 25.85 (C5); 42.8 br (C6); 169.39 (C8); 97.43 (C10); 36.69 (C11); 32.71 (C12); 73.72 (C13); 72.87 (C14); 75.59 (C15); 31.84 (C16); 25.56 (C17); 49.65 (C18); 137.2 br (C19); 123.37 (C20); 54.40 (C21); 210.86 (C22); 46.4 br (C23); 70.40 (C24); 39.4 br (C25); n.d. (C26); n.d. (C27); 10.9 br (C28); 135.35 (C29); 35.06 (C30); 36.25 (C31); 84.27 (C32); 75.11 (C33); 34.02 (C34); 30.65 (C35); 25.79 (C36); 11.44 (C37); 56.21 (13-OMe); 57.23 (15-OMe); 57.75 (32-OMe); 15.77 (11-Me); 20.08 (17-Me); 14.43 (19-Me); 10.9 br (25-Me); 25.93, 25.79, 18.13, 18.04, −4.11, −4.52, −4.75 (2×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (ppm): 5.37 (H-2); 4.47 (br d, J=13.0 Hz, H-6a); 2.76 (H-6b); 1.82 (H-11); 2.07 (ddd, J=12.3+4.6+4.6 Hz, H-12a); 3.39 (H-13); 3.81 (dd, J=9.7+1.8 Hz, H-14); 3.54 (ddd, J=11.5+4.7+1.4 Hz, H-15); 5.05 (br s, H-20); 3.31 (H-21); 2.72 (br s, H-23a); 2.54 (br s, H-23b); 4.00 (br s, H-24); 5.05 (br s, H-26); 5.35 (d, J=8.7 Hz, H-29); 2.92 (ddd, J=11.2+8.5+4.5 Hz, H-32); 3.40 (H-33); 0.81 (d, 3H, J=6.6 Hz, 11-Me); 1.52 (s, 3H, 19-Me); 1.46 (s, 3H, 27-Me); 0.07, 0.06, 0.05, 0.03 (s, each 3H, Si−Me); 0.88, 0.85 (s, each 9H, $(CH_3)_3$); 5.64 (10-OH).

4.2.22. Compound 19. Starting from 0.3 g (0.27 mmol) **21** or 0.4 g (0.4 mmol) **18**, the deprotection and work up was performed as described above for **13a** to give after a flash column chromatography (silica gel, ethyl acetate) 0.17 g (82%) or 0.24 g (78%) **19** as amorphous powders. HRMS (M+Na; calcd/found): 786.4768/786.4757. ^{13}C NMR ($CDCl_3$, hemiketal form/ketone form=5:1), δ (hemiketal/ketone, ppm): 169.81 or 169.73/169.43 (C1); 56.92/52.63 (C2); 28.39/26.11 (C3); 21.06/21.29 (C4); 25.57/25.20 (C5); 42.05/44.38 (C6); 169.81 or 169.73/166.55 (C8); 97.64/202.68 (C10); 37.10/40.08 (C11); 32.31/32.21 (C12); 73.48/80.79 (C13); 73.11/73.19 (C14); 75.94/78.09 (C15); 32.86/35.58 (C16); 25.11/27.55 (C17); 49.34/48.51 (C18); 139.01/138.56 (C19); 123.84/124.76 (C20); 53.76/55.11 (C21); 213.94/211.87 (C22); 47.51/45.35 (C23); 67.01/69.94 (C24); 41.90/38.50 (C25); 78.31/82.79 (C26); 132.51/130.92 (C27); 13.53/12.46 (C28); 130.61/133.47 (C29); 34.92/34.99 (C30); 34.73/34.49 (C31); 84.17/84.21 (C32); 73.52/73.52 (C33); 31.22/31.21 (C34); 30.54/30.41 (C35); 24.43/23.29 (C36); 11.55/11.61 (C37); 55.89/57.35 (13-OMe); 57.84/57.05 (15-OMe); 56.50/56.43 (32-OMe); 15.75/14.70 (11-Me); 20.09/21.04 (17-Me); 14.70/14.78 (19-Me); 9.77/9.84 (25-Me). ^{1}H NMR ($CDCl_3$, selected data), δ (hemiketal form, ppm): 5.53 (s, H-2); 4.40 (d, J=13.0 Hz, H-6a); 3.05 (br dd, J=13.0+13.0 Hz, H-6b); 1.79 (H-11); 3.49 (H-13); 3.83 (H-14); 3.54 (ddd, J=11.2+5.0+2.1 Hz, H-15); 4.88 (d, J=10.1 Hz); 3.41 (H-21); 2.98 (dd, J=14.8+4.0 Hz, H-23a); 2.53 (dd, J=14.8+9.0 Hz, H-23b); 3.80 (H-24); 5.25 (s, H-26); 5.27 (d, J=9.2 Hz, H-29); 2.99 (H-32); 3.40 (H-33); 0.80 (d, 3H, J=6.6 Hz, 11-Me); 1.54 (s, 3H, 19-Me); 1.62 (s, 3H, 27-Me); 5.62 (s, 10-OH); δ (ketone form, ppm): 5.21 (d, J=5.5 Hz, H-2); 3.80 (H-6a); 3.00 (H-6b); 3.40 (H-11); 3.80 (H-14); 3.26 (H-15); 4.85 (d, J=10.2 Hz, H-20); 3.39 (H-21); 2.71 (dd, J=18.0+3.3 Hz, H-23a); 2.53 (H-23b); 3.98 (H-24); 5.08 (d, J=8.0 Hz, H-26); 5.32 (d, J=9.3 Hz, H-29); 2.99 (H-32); 3.40 (H-33); 1.21 (d, 3H, J=7.0 Hz, 11-Me); 1.59 (s, 3H, 19-Me); 1.57 (s, 3H, 27-Me).

4.2.23. Compounds 20a and 20b. (a) from **12a**. A small glass tube was charged with 0.5 g (0.43 mmol) **12a** and a magnetic stirring bar and immersed in a preheated (160 °C) oil bath. After approx. 2 min, carbon dioxide formation occurred in the clear liquid which ceased after two additional minutes. After 8 min, the mixture was cooled down to room temperature, diluted in 3 ml dichloromethane and subjected to flash column chromatography (silica gel, dichloromethane/acetone=20:1) to give after evaporation and drying of the relevant fractions at high vacuum 0.36 g (74%) **20a** and 63 mg (13%) **20b** as amorphous powders. (b) from **12b**. Starting from 100 mg (0.087 mmol) **12b**, the decarboxylation was performed as described above to give 78 mg (81%) **20a** and 10 mg (10%) **20b**.

4.2.24. Compound 20a. CHN ($C_{60}H_{113}NO_{11}Si_3$) calcd: 64.99/10.27/1.26, found: 65.20/10.05/1.22. HRMS (M+Na; calcd/found): 1130.7519/1130.7525. ^{13}C NMR ($CDCl_3$, Z/E=1:2), δ (Z/E-isomer, ppm): 169.73/169.34 (C1); 52.88/54.66 (C2); 26.60/27.50 (C3); 21.01/20.32 (C4); 26.38/24.68 (C5); 43.60/40.01 (C6); 172.99/174.20 (C8); 71.50/69.05 (C10); 34.40/33.04 (C11); 33.41/32.23 (C12); 79.64/79.72 (C13); 78.11/72.67 (C14); 80.36/81.53 (C15); 40.69/37.28 (C16); 31.20/32.33 (C17); 47.08/45.88 (C18); 139.16/140.91 (C19); 124.10/123.43 (C20); 54.88/55.20 (C21); 208.80/209.65 (C22); 45.88/47.92 (C23); 68.37/67.52 (C24); 39.21/38.58 (C25); 81.74/83.13 (C26); 131.65/130.81 (C27); 12.04/11.58 (C28); 135.30/136.76 (C29); 35.01/35.15 (C30); 36.23/36.15 (C31); 84.09/84.09 (C32);

75.11/75.11 (C33); 33.88/33.88 (C34); 30.73/30.66 (C35); 23.34/23.36 (C36); 11.49/11.49 (C37); 59.92/55.83 (13-OMe); 57.11/58.26 (15-OMe); 57.84/57.90 (32-OMe); 12.98/13.82 (11-Me); 20.23/21.33 (17-Me); 16.54/18.54 (19-Me); 11.49/9.70 (25-Me); 26.14, 26.00, 25.91, 25.86, 25.85, 18.15, 18.06, 18.02, −3.62, −4.16, −4.34, −4.52, −4.75, −4.90 (3×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (*Z*-isomer, ppm): 5.39 (d, *J*=4.8 Hz, H-2); 3.73 (br d, *J*=13.3 Hz, H-6a); 3.20 (H-6b); 4.40 (d, *J*=2.1 Hz, H-10); 1.85 (H-11); 3.27 (H-13); 3.71 (dd, *J*=8.0+1.4 Hz, H-14); 3.29 (H-15); 4.78 (d, *J*=10.5 Hz, H-20); 3.19 (H-21); 2.78 (dd, *J*=17.4+8.0 Hz, H-23a); 2.36 (dd, *J*=17.4+4.6 Hz, H-23b); 4.14 (ddd, *J*=8.0+4.6+2.3 Hz, H-24); 5.19 (d, *J*=8.7 Hz, H-26); 5.27 (d, *J*=8.9 Hz, H-29); 2.95 (H-32); 3.40 (H-33); 0.79 (d, 3H, *J*=6.6 Hz, 11-Me); 1.78 (s, 3H, 19-Me); 1.57 (s, 3H, 27-Me); 0.10, 0.09, 0.08, 0.06, 0.05, 0.01 (s, each 3H, Si−Me); 0.92, 0.89, 0.88 (s, each 9H, $(CH_3)_3$); δ (*E*-isomer, ppm): 4.46 (d, *J*=5.1 Hz, H-2); 4.39 (d, *J*=12.0 Hz, H-6a); 3.16 (H-6b); 4.22 (s, H-10); 1.85 (H-11); 3.27 (H-13); 3.95 (dd, *J*=8.0+1.6 Hz, H-14); 3.08 (H-15); 4.66 (d, *J*=10.3 Hz, H-20); 3.23 (H-21); 2.85 (dd, *J*=16.7+10.3 Hz, H-23a); 2.31 (dd, *J*=16.7+4.0 Hz, H-23b); 4.09 (ddd, *J*=10.3+4.0+1.4 Hz, H-24); 5.16 (d, *J*=9.6 Hz, H-26); 5.34 (d, *J*=8.7 Hz, H-29); 2.95 (H-32); 3.40 (H-33); 0.80 (d, 3H, *J*=6.6 Hz, 11-Me); 1.80 (d, 3H, *J*=1.0 Hz, 19-Me); 1.64 (d, 3H, *J*=1.1 Hz, 27-Me); 0.09, 0.08, 0.08, 0.07, 0.02, 0.01 (s, each 3H, Si−Me); 0.91, 0.89, 0.87 (s, each 9H, $(CH_3)_3$).

4.2.25. Compound 20b. CHN ($C_{60}H_{113}NO_{11}Si_3$) calcd: 64.99/10.27/1.26, found: 65.22/10.11/1.25. HRMS (M+Na; calcd/found): 1130.7519/1130.7520. ^{13}C NMR ($CDCl_3$, mixture of rotamers >9:1), δ (major rotamer, ppm): 168.69 (C1); 53.13 (C2); 26.40 (C3); 21.24 (C4); 25.06 (C5); 43.16 (C6); 174.94 (C8); 72.56 (C10); 34.96 (C11); 29.96 (C12); 83.35 (C13); 75.01 (C14); 79.53 (C15); 42.61 (C16); 27.48 (C17); 48.01 (C18); 139.66 (C19); 123.57 (C20); 55.16 (C21); 210.21 (C22); 45.83 (C23); 68.99 (C24); 40.53 (C25); 78.70 (C26); 132.34 (C27); 13.70 (C28); 131.17 (C29); 34.96 (C30); 36.61 (C31); 84.14 (C32); 75.17 (C33); 33.87 (C34); 30.95 (C35); 23.55 (C36); 11.50 (C37); 56.68 (13-OMe); 60.64 (15-OMe); 57.99 (32-OMe); 21.31 (11-Me); 20.27 (17-Me); 15.97 (19-Me); 9.71 (25-Me); 25.99, 25.86, 25.80, 18.39, 18.16, 17.97 (3×TBDMS). ^{1}H NMR ($CDCl_3$, selected data, δ (major rotamer, ppm): 5.17 (d, *J*=5.3 Hz, H-2); 3.66 (br d, *J*=13.0 Hz, H-6a); 3.11 (H-6b); 4.22 (s, H-10); 1.85 (H-11); 2.87 (d, *J*=11.0 Hz, H-13); 3.66 (d, *J*=8.5 Hz, H-14); 2.97 (H-15); 4.87 (d, *J*=10.3 Hz, H-20); 3.11 (H-21); 2.67 (dd, *J*=17.2+5.0 Hz, H-23a); 2.35 (dd, *J*=17.2+6.6 Hz, H-23b); 4.27 (H-14); 5.11 (d, *J*=4.4 Hz, H-26); 5.10 (d, *J*=9.4 Hz, H-29); 2.95 (H-32); 3.39 (H-33); 1.16 (d, 3H, *J*=7.1 Hz, 11-Me); 1.76 (d, 3H, *J*=0.7 Hz, 19-Me); 1.59 (d, 3H, *J*=1.0 Hz, 27-Me); 0.08, 0.08, 0.07, 0.06, 0.05, −0.01 (s, each 3H, Si−Me); 0.91, 0.89, 0.85 (s, each 9H, $(CH_3)_3$); 3.48 (br s, 10-OH).

4.2.26. Compound 21. (a) from **20a**. To a solution of 0.3 g (0.27 mmol) **20a** in 10 ml dichloromethane were added 0.34 g (8.12 mmol, 3 equiv.). Dess−Martin periodinane and the suspension was stirred for 5 h at room temperature. The resultant mixture was directly subjected to a short flash column chromatography (silica gel, dichloromethane/acetone=50:1) to afford 0.26 g (87%) **21**. (b) from **20b**. Starting from 45 mg (0.041 mmol) **20b** and applying the same reaction conditions and work up as described above provided 38 mg (84%) **21** as amorphous powder: CHN ($C_{60}H_{111}NO_{11}Si_3$) calcd: 65.11/10.11/1.27, found: 65.00/9.92/1.15. HRMS (M+Na; calcd/found): 1128.7363/1128.7363. ^{13}C NMR ($CDCl_3$, mixture of rotamers >9:1), δ (major rotamer, ppm): 168.84 (C1); 51.36 (C2); 25.53 (C3); 20.65 (C4); 25.73 (C5); 44.58 (C6); 167.08 (C8); 203.70 (C10); 40.46 (C11); 31.78 (C12); 82.37 (C13); 75.75 (C14); 78.24 (C15); 42.93 (C16); 26.56 (C17); 46.40 (C18); 139.92 (C19); 123.48 (C20); 55.79 (C21); 208.97 (C22); 47.73 (C23); 67.70 (C24); 38.75 (C25); 82.95 (C26); 130.87 (C27); 11.25 (C28); 136.17 (C29); 35.08 (C30); 36.27 (C31); 84.19 (C32); 75.18 (C33); 34.03 (C34); 30.72 (C35); 23.61 (C36); 11.69 (C37); 56.85 (13-OMe); 61.09 (15-OMe); 57.78 (32-OMe); 16.48 (11-Me); 20.54 (17-Me); 16.02 (19-Me); 8.87 (25-Me); 26.05, 25.87, 25.73, 18.41, 18.18, 18.05, −3.95, −4.17, −4.40, −4.49, −4.74, −4.85 (3×TBDMS). ^{1}H NMR ($CDCl_3$, selected data), δ (major rotamer, ppm): 5.24 (br d, *J*=4.0 Hz, H-2); 3.43 (H-6a); 3.05 (ddd, *J*=13.8+13.8+2.8 Hz, H-6b); 3.28 (H-11); 2.13 (dd, *J*=14.7+10.5 Hz, H-12a); 2.79 (d, *J*=9.6 Hz, H-13); 3.63 (d, *J*=9.2 Hz, H-14); 3.20 (H-15); 4.61 (d, *J*=10.3 Hz, H-20); 3.22 (H-21); 2.87 (dd, *J*=16.0+9.9 Hz, H-23a); 2.22 (dd, *J*=16.0+4.0 Hz, H-23b); 4.17 (dd, *J*=9.9+4.0 Hz, H-24); 4.92 (d, *J*=9.9 Hz, H-26); 5.35 (d, *J*=8.7 Hz, H-29); 2.93 (ddd, *J*=11.2+8.5+4.4 Hz, H-32); 3.40 (H-33); 1.19 (d, 3H, *J*=7.6 Hz, 11-Me); 1.78 (s, 3H, 19-Me); 1.44 (s, 3H, 27-Me); 0.10, 0.08, 0.06, 0.03 (s, each 3H, Si−Me), 0.07 (s, 6H, Si−Me); 0.93, 0.89, 0.88 (s, each 9H, $(CH_3)_3$).

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Reactions of azulenes with 1,2-diaryl-1,2-ethanediols in methanol in the presence of hydrochloric acid: comparative studies on products, crystal structures, and spectroscopic and electrochemical properties

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Abstract—Although reaction of guaiazulene (**1a**) with 1,2-diphenyl-1,2-ethanediol (**2a**) in methanol in the presence of hydrochloric acid at 60 °C for 3 h under aerobic conditions gives no product, reaction of **1a** with 1,2-bis(4-methoxyphenyl)-1,2-ethanediol (**2b**) under the same reaction conditions as **2a** gives a new ethylene derivative, 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene (**3**), in 97% yield. Similarly, reaction of methyl azulene-1-carboxylate (**1b**) with **2b** under the same reaction conditions as **1a** gives no product; however, reactions of 1-chloroazulene (**1c**) and the parent azulene (**1d**) with **2b** under the same reaction conditions as **1a** give 2-[3-(1-chloroazulenyl)]-1,1-bis(4-methoxyphenyl)ethylene (**4**) (81% yield) and 2-azulenyl-1,1-bis(4-methoxyphenyl)ethylene (**5**) (15% yield), respectively. Along with the above reactions, reactions of **1a** with 1,2-bis(4-hydroxyphenyl)-1,2-ethanediol (**2c**) and 1-[4-(dimethylamino)phenyl]-2-phenyl-1,2-ethanediol (**2d**) under the same reaction conditions as **2b** give 2-(3-guaiazulenyl)-1,1-bis(4-hydroxyphenyl)ethylene (**6**) (73% yield) and (*Z*)-2-[4-(dimethylamino)phenyl]-1-(3-guaiazulenyl)-1-phenylethylene (**7**) (17% yield), respectively. Comparative studies of the above reaction products and their yields, crystal structures, spectroscopic and electrochemical properties are reported and, further, a plausible reaction pathway for the formation of the products **3**–**7** is described.

Azulenes have become readily available by synthesis over the past 40 years and aroused considerable interest as a representative example of non-benzenoid aromatic hydrocarbons, because of their facile electrophilic substitution reactions and insusceptibility to Diels–Alder-type addition reactions.

As a series of basic studies on the creation of novel functional materials with a delocalized π-electron system and their potential utility, we have been working on a facile preparation, the molecular and crystal structures, spectroscopic and characteristic chemical properties and, further, electrochemical behavior of mono- and dicarbocations stabilized by a 3-guaiazulenyl group for the past several years. These products can be readily obtained by the condensation reactions of naturally occurring guaiazulene (**1a**) with the corresponding aldehyde compounds in acetic acid (and methanol) in the presence of hexafluorophosphoric acid (and tetrafluoroboric acid), respectively.[1–7] During the course of our investigations, we have quite recently found that the reaction of **1a** with 1,2-bis(4-methoxyphenyl)-1,2-ethanediol (**2b**) in methanol in the presence of hydrochloric acid at 60 °C for 3 h gave a new ethylene derivative, 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene (**3**) (97% yield) and, further, have found that the reduction of α,α′-bis(3-guaiazulenylmethylium) bis(tetrafluoroborate) (**8**), whose carbonium ion is known,[8,9] with zinc powder in trifluoroacetic acid at 0 °C for 5 min afforded (*E*)-1,2-di(3-guaiazulenyl)ethylene (**9**), efficiently (94% yield), which enabled us to compare the spectroscopic properties, crystal structures and electrochemical behavior of **3** under the same analytical conditions as **9**. Although **9** is also a known compound,[10–12] which was prepared, in 72% yield, by the McMurry reaction of guaiazulene-3-carbaldehyde, and the spectroscopic (UV–vis and ^{1}H NMR) and electrochemical properties of **9** were reported, nothing has really been documented regarding other spectroscopic

Keywords: Azulenes; Carbonium ions; Electrochemistry; Electron donors; Reduction; X-ray crystal structures.
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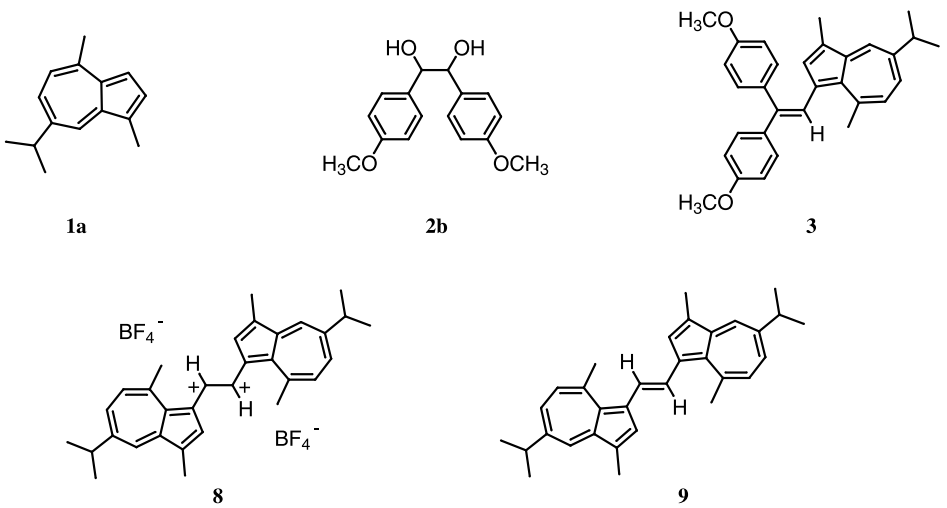


1a **2b** **3**

8 **9**

properties (i.e. IR, MS and ^{13}C NMR) and the X-ray crystal structure of **9**. Now, our interest has been focused on a comparative study of the reactions of guaiazulene (**1a**), methyl azulene-1-carboxylate (**1b**), 1-chloroazulene (**1c**) and the parent azulene (**1d**) with 1,2-bis(4-methoxyphenyl)-1,2-ethanediol (**2b**) in methanol in the presence of hydrochloric acid at 60 °C for 3 h along with the reactions of **1a** with 1,2-diphenyl-1,2-ethanediol (**2a**), 1,2-bis(4-hydroxyphenyl)-1,2-ethanediol (**2c**) and 1-[4-(dimethylamino)phenyl]-2-phenyl-1,2-ethanediol (**2d**) under the same reaction conditions as **2b**. We now wish to report our detailed studies on the molecular structures of the above reaction products and their yields and, further, their spectroscopic properties, crystal structures and electrochemical behavior compared with those of **9**.

1. Results and discussion

1.1. Reaction of guaiazulene (1a) with 1,2-bis(4-methoxyphenyl)-1,2-ethanediol (2b): an efficient preparation and spectroscopic properties of 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene (3)

Compound **3** was prepared using a methanol as a solvent as shown in Section 3.1.2, whose molecular structure was established on the basis of elemental analysis and spectroscopic data [UV–vis, IR, MALDI-TOF- and EI-MS, ^{1}H and ^{13}C NMR including 2D NMR (i.e. H–H COSY, NOESY, HMQC=^{1}H detected heteronuclear multiple quantum coherence and HMBC=^{1}H detected heteronuclear multiple bond connectivity)].

Compound **3** (97% yield) was dark-green needles [mp 150 °C, determined by thermal analysis (TGA and DTA)]. The UV–vis [λ_{max} (CH_3CN) nm] spectrum is shown in Figure 1(a). A comparative study of the UV–vis spectrum of **3** with those of guaiazulene (**1a**)[13] and (*E*)-1,2-di(3-guaiazulenyl)ethylene (**9**) (see Section 3.1.17) showed that: (i) similarly, as in the case of **9** [see Fig. 1(b)], no characteristic UV–vis absorption bands for guaiazulene were observed, indicating the formation of the molecule **3** with a delocalized π-electron system; and (ii) although the spectral pattern of the characteristic UV–vis absorption bands of **3** resembled that of **9**, the longest absorption wavelength of **3** (λ_{max} 635 nm, log ε=2.54) showed a hypsochromic shift (Δ 26 nm) and a hypochromic effect in comparison with that of **9** (λ_{max} 661 nm, log ε=3.13). The IR (KBr) spectrum showed four specific bands based on the C–O at ν_{max} 1242 and 1034 cm^{-1}, and the aromatic C=C at ν_{max} 1605 and 1508 cm^{-1}. The MALDI-TOF-MS (without any matrix reagent) spectrum showed only a molecular ion peak at *m*/*z* 436 (M^+, 100%). The molecular formula $C_{31}H_{32}O_2$ was determined by the exact EI-MS (70 eV) spectrum. The elemental analysis confirmed the molecular formula $C_{31}H_{32}O_2$. The ^{1}H NMR (C_6D_6) spectrum showed signals based on the 3-guaiazulenyl group, signals based on the two 4-methoxyphenyl groups which were not equivalent, and a signal based on the >C=C*H*– unit, whose signals were carefully assigned

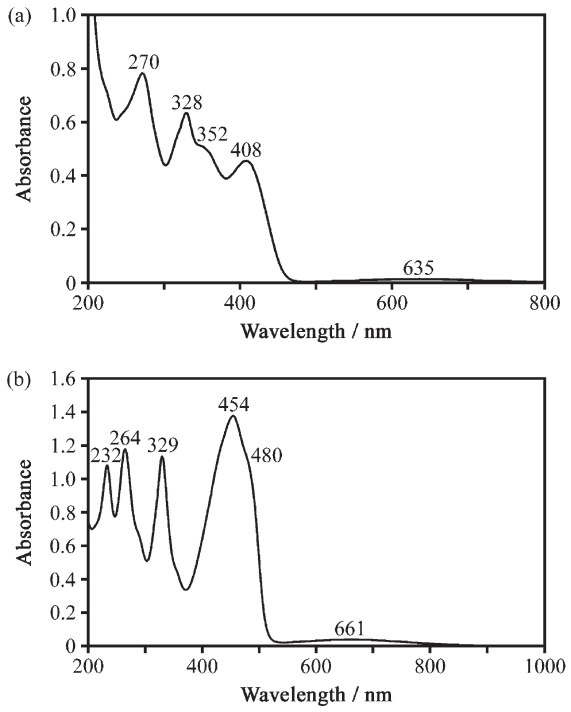


Figure 1. The UV–vis spectra of **3** (a) and **9** (b) in CH_3CN. Concentrations, **3**: 0.010 g/L (22.9 μmol/L), **9**: 0.012 g/L (28.5 μmol/L). Length of the cell, 1 cm each.

using the computer-assisted simulation analysis, H–H COSY and NOESY techniques. The proton signals of the Me-1‴ (δ 2.31) and H-2‴ (7.44) of the 3-guaiazulenyl group and the >C═C*H*– (7.77) unit for **3** showed apparent up-field shifts in comparison with those [i.e. the Me-1′,1″ (2.57) and H-2′,2″ (8.14) of the two 3-guaiazulenyl groups and the –*H*C═C*H*– (8.12 equiv.) unit] of **9**. The ^{13}C NMR (C_6D_6) spectrum exhibited twenty-six carbon signals assigned by HMQC and HMBC techniques. Although the carbon signal of the >C═*C*H–(δ125.6) unit of **3** coincided with those of the –H*C*═*C*H– (125.2 equiv.) unit of **9**, the carbon signals of the C-2‴ (140.4), C-3a‴ (135.5) and C-3‴ (127.2) of the 3-guaiazulenyl group for **3** showed apparent down- and up-field shifts in comparison with those [i.e. the C-2′,2″ (136.1), C-3a′,3a″ (132.5) and C-3′,3″ (128.8) of the two 3-guaiazulenyl groups] of **9**. From a comparative study of the chemical shifts (δ, ppm) for the proton and carbon signals of **3** with those of **1**[13] and **9**, it can be inferred that: (i) although the planes of the two 3-guaiazulenyl groups for **9** are co-planar with that of the –HC═CH– unit, forming the molecule **9** with a delocalized π-electron system, the plane of the 3-guaiazulenyl group for **3** twists from that of the >C═CH– unit owing to the influence of steric hindrance and repulsion between the 3-guaiazulenyl group and the (*Z*)-4-methoxyphenyl group; however, (ii) the molecule **3** with a delocalized π-electron system is apparently formed in an organic solvent (e.g. acetonitrile or benzene). The elemental analysis and these spectroscopic data for **3** led to the molecular structure, 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene.

1.2. X-ray crystal structure of 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene (3) compared with those of (*E*)-1,2-di(3-guaiazulenyl)ethylene (9), *trans*-stilbene (10) and (*Z*)-1-chloro-2-(4-methylphenyl)-1,2-diphenylethylene (11)

The crystal structure of compound **3** was then determined by means of X-ray diffraction, producing accurate structural parameters. The ORTEP drawing of **3**, indicating the

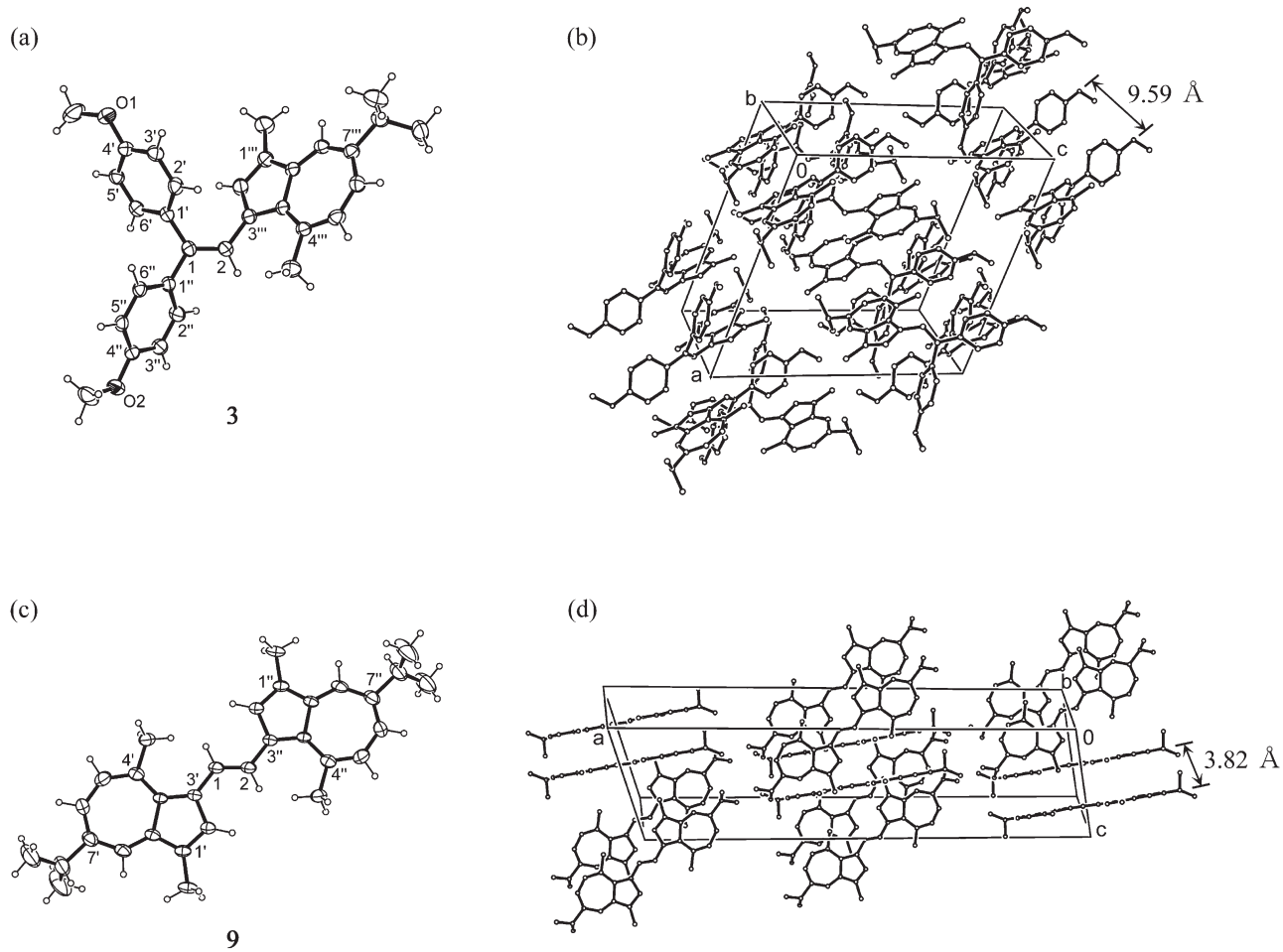
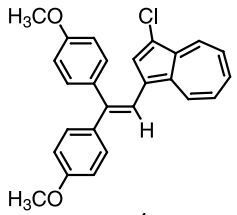


Figure 2. The ORTEP drawings with the numbering scheme (30% probability thermal ellipsoids) of **3** (a) and **9** (c) and the packing structures of **3** (b) and **9** (d); hydrogen atoms are omitted for reasons of clarity, respectively. The bond distances (Å) of **3** are as follows: C1–C2; 1.343(4), C1–C1′; 1.487(4), C1′–C2′; 1.390(4), C2′–C3′; 1.377(5), C3′–C4′; 1.377(5), C4′–C5′; 1.377(5), C5′–C6′; 1.382(5), C6′–C1′; 1.390(5), C4′–O1; 1.373(4), O1–CH_3; 1.397(6), C1–C1″; 1.486(4), C1″–C2″; 1.394(4), C2″–C3″; 1.373(5), C3″–C4″; 1.374(4), C4″–C5″; 1.376(4), C5″–C6″; 1.380(4), C6″–C1″; 1.382(4), C4″–O2; 1.375(4), O2–CH_3; 1.415(5), C1‴–C2‴; 1.382(5), C2‴–C3‴; 1.407(5), C3‴–C3a‴; 1.416(5), C3a‴–C4‴; 1.393(4), C4‴–C5‴; 1.390(5), C5‴–C6‴; 1.388(5), C6‴–C7‴; 1.383(4), C7‴–C8‴; 1.384(5), C8‴–C8a‴; 1.382(4), C8a‴–C1‴; 1.413(5), C3a‴–C8a‴; 1.515(4), C1‴–C9‴; 1.507(5), C4‴–C10‴; 1.508(5), C7‴–C11‴; 1.528(5), C11‴–C12‴; 1.511(6), C11‴–C13‴; 1.516(6) and C2–C3‴; 1.464(4). The bond distances (Å) of **9** are as follows: C1–C2; 1.32(1), C1′–C2′; 1.378(8), C2′–C3′; 1.452(8), C3′–C3a′; 1.391(7), C3a′–C4′; 1.404(8), C4′–C5′; 1.369(9), C5′–C6′; 1.40(1), C6′–C7′; 1.362(10), C7′–C8′; 1.397(9), C8′–C8a′; 1.351(8), C8a′–C1′; 1.388(8), C3a′–C8a′; 1.533(8), C1′–C9′; 1.509(9), C4′–C10′; 1.512(9), C7′–C11′; 1.561(10), C11′–C12′ 1.43(1), C11′–C13′; 1.52(1) and C1–C3′; 1.444(8).

molecular structure, 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene, compared with that of (*E*)-1,2-di(3-guaiazulenyl)ethylene (**9**) is shown in Figure 2(a) together with the selected bond distances. As the result, the structural parameters of **3** revealed that: (i) the C–C bond distance between the >C═CH– unit [1.343(4) Å] was characteristically longer than those of the –HC═CH– units of **9** [1.32(1) Å] and *trans*-stilbene (**10**) [1.326(2) Å];[14] however, the C–C bond distance between the >C═CH– unit coincided with that of the >C═CCl– unit of (*Z*)-1-chloro-2-(4-methylphenyl)-1,2-diphenylethylene (**11**) [1.340(3) Å];[15] (ii) although the crystal structure of **9** was planar from the dihedral angles between the least-squares planes, the planes of the two 4′- and 4″-methoxyphenyl and 3‴-guaiazulenyl groups of **3** twisted by 51.5°, 136.9° and 47.4° from that of the >C═CH– unit, respectively, owing to the influence of large steric hindrance and repulsion between those three groups; (iii) the average C–C bond distances for the seven- and five-membered rings of the 3‴-guaiazulenyl group (1.405 and 1.427 Å) coincided with the bond distances observed for those of the 3′- and 3″-guaiazulenyl groups of **9** (1.403 and 1.429 Å each); and (iv) the average C–C bond distances for the benzene rings of the two 4′- and 4″-methoxyphenyl groups (1.382 and 1.380 Å) coincided with the bond distances observed for those of the two benzene rings of **10** (1.386 Å each).[14] Along with the crystal structures of **3** and **9** [see Fig. 2(a) and (c)], the packing structures of **3** and **9** revealed that: although the molecule **3** did not form a π-stacking structure in the single crystal as shown in Figure 2(b), the molecule **9** formed a π-stacking structure in the single crystal as shown in Figure 2(d), whose average *inter*-plane distance between the over-lapping molecules was 3.82 Å. Thus, the reason why the yield of **9** as single crystals was high (94% yield) can be inferred to be that **9** readily forms an accumulation (i.e. an *inter*-molecular π-stacking structure) in the recrystallization solvent, providing the single crystals of **9** efficiently.

1.3. A comparative study of the reactions of methyl azulene-1-carboxylate (1b), 1-chloroazulene (1c) and the parent azulene (1d) with 1,2-bis(4-methoxyphenyl)-1,2-ethanediol (2b) under the same conditions as the reaction of guaiazulene (1a) with 2b

The reactions of methyl azulene-1-carboxylate (**1b**), 1-chloroazulene (**1c**) and the parent azulene (**1d**) with **2b** under the same reaction conditions as **1a** (see Section 3.1.2) were investigated. As the result, it was found that: although the reaction of **1b** with **2b** gave no product (see Section 3.1.4), the reactions of **1c** and **1d** with **2b** afforded 2-[3-(1-chloroazulenyl)]-1,1-bis(4-methoxyphenyl)ethylene (**4**) (81% yield) and 2-azulenyl-1,1-bis(4-methoxyphenyl)-ethylene (**5**) (15% yield), respectively, whose molecular structures were established on the basis of elemental analysis and spectroscopic data [UV–vis, IR, EI-MS, ^{1}H and ^{13}C NMR including 2D NMR (i.e. H–H COSY, NOESY, HMQC and HMBC)] (see Sections 3.1.6 for **4**; 3.1.8 for **5**). Similarly, as in the case of 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene (**3**), the ^{1}H NMR signals of **4** and **5** were carefully assigned using the computer-assisted simulation analysis. Furthermore, the reaction of **1d** with **2b** gave, besides **5**, a chromatographically inseparable mixture of several bis[bis(4-methoxyphenyl)vinyl]azulenes (**5′**) (41% yield), showing a very complicated ^{1}H NMR spectrum. The structures of the products **5′** were presumed on the basis of elemental analysis and spectroscopic data (IR and EI-MS) (see Section 3.1.8).



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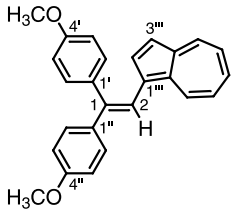


5

1.4. X-ray crystal structure of 2-[3-(1-chloroazulenyl)]-1,1-bis(4-methoxyphenyl)ethylene (4) compared with that of 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)-ethylene (3)

Although an X-ray crystallographic analysis of compound **5** has not yet been achieved because of difficulty in obtaining a single crystal suitable for this purpose, the crystal structure of compound **4** has been determined. The ORTEP drawing of **4**, indicating the molecular structure, 2-[3-(1-chloroazulenyl)]-1,1-bis(4-methoxyphenyl)ethylene, is shown in Figure 3(a) together with the selected bond distances. As the result, the structural parameters of **4** revealed that: (i) the C–C bond distance between the >C═CH– unit [1.347(3) Å] coincided with that of the >C═CH– unit of **3** [1.343(4) Å]; (ii) similarly, as in the case of **3** [see Fig. 2(a)], the planes of the two 4′- and 4″-methoxyphenyl and 3‴-(1-chloroazulenyl) groups of **4** twisted by 70.9°, 30.8° and 16.4° from that of the >C═CH– unit, respectively, owing to the influence of large steric hindrance and repulsion between those three groups; (iii) the average C–C bond distances for the seven- and five-membered rings of the 3‴-(1-chloroazulenyl) group (1.400 and 1.417 Å) coincided with the bond distances observed for those of the 3‴-guaiazulenyl group of **3** (1.405 and 1.427 Å); and (iv) the average C–C bond distances for the benzene rings of the two 4′- and 4″-methoxyphenyl groups (1.382 and 1.386 Å) coincided with the bond distances observed for those of the two 4′- and 4″-methoxyphenyl groups of **3** (1.382 and 1.380 Å). Along with the crystal structure of **4**, the packing structure of **4** revealed that: although the molecule **3** did not form a π-stacking structure in the single crystal as shown in Figure 2(b), the molecule **4** formed a π-stacking structure in the single crystal as shown in Figure 3(b), whose average *inter*-plane distance between the over-lapping molecules was 4.04 Å.

1.5. A comparative study of the reactions of guaiazulene (1a) with 1,2-diphenyl-1,2-ethanediol (2a), 1,2-bis(4-hydroxyphenyl)-1,2-ethanediol (2c) and 1-[4-(dimethylamino)phenyl]-2-phenyl-1,2-ethanediol (2d) under the same conditions as the reaction of 1a with 1,2-bis(4-methoxyphenyl)-1,2-ethanediol (2b)

The reactions of **1a** with 1,2-diphenyl-1,2-ethanediol (**2a**), 1,2-bis(4-hydroxyphenyl)-1,2-ethanediol (**2c**) and 1-[4-(dimethylamino)phenyl]-2-phenyl-1,2-ethanediol (**2d**) under the same conditions as the reaction of **1a** with **2b** (see Section 3.1.2) were investigated. As the result, it was

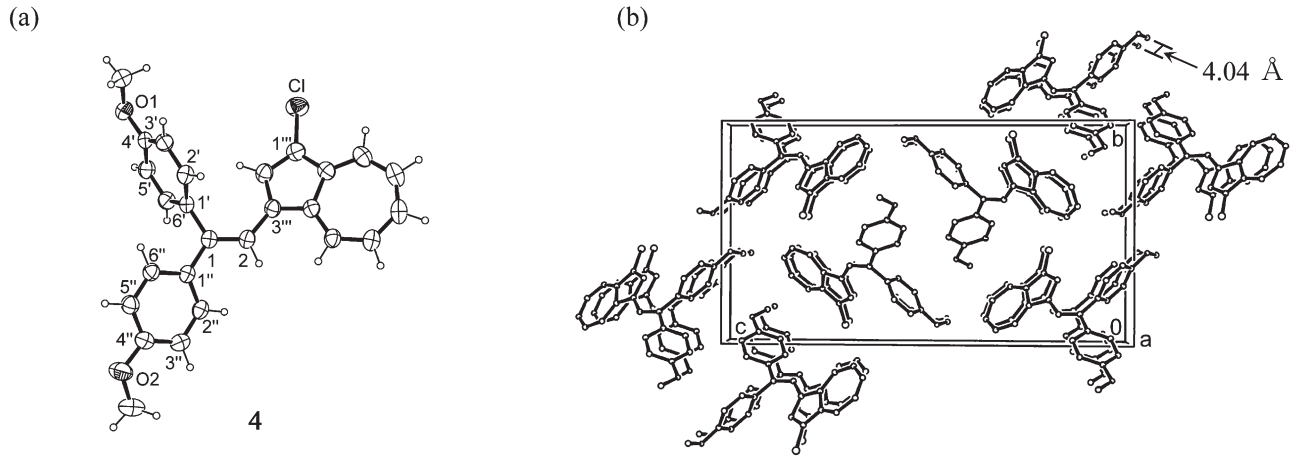
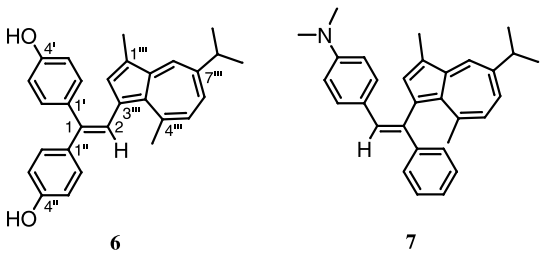


Figure 3. The ORTEP drawing (a) with the numbering scheme (30% probability thermal ellipsoids) of **4** and the packing structure (b) of **4**; hydrogen atoms are omitted for reasons of clarity. The bond distances (Å) of **4** are as follows: C1–C2; 1.347(3), C1–C1′; 1.498(3), C1′–C2′; 1.376(3), C2′–C3′; 1.382(3), C3′–C4′; 1.381(3), C4′–C5′; 1.383(3), C5′–C6′; 1.375(3), C6′–C1′ 1.395(3), C4′–O1; 1.370(3), O1–CH_3; 1.423(3), C1–C1″; 1.486(3), C1″–C2″; 1.385(3), C2″–C3″; 1.384(4), C3″–C4″; 1.390(4), C4″–C5″; 1.379(3), C5″–C6″; 1.375(3), C6″–C1″; 1.398(3), C4″–O2; 1.366(3), O2–CH_3; 1.393(3), C1‴–C2‴; 1.377(4), C2‴–C3‴; 1.412(3), C3‴–C3a‴; 1.413(3), C3a‴–C4‴; 1.387(3), C4‴–C5‴; 1.388(4), C5‴–C6‴; 1.392(4), C6‴–C7‴; 1.373(4), C7‴–C8‴; 1.390(4), C8‴–C8a‴; 1.378(4), C8a‴–C1‴; 1.393(4), C3a‴–C8a‴; 1.487(3), C1‴–Cl; 1.732(3) and C2–C3‴; 1.448(3).

found that: although the reaction of **1a** with **2a** gave no product (see Section 3.1.10), the reactions of **1a** with **2c** and **2d** afforded 2-(3-guaiazulenyl)-1,1-bis(4-hydroxyphenyl)-ethylene (**6**) (73% yield) and (*Z*)-2-[4-(dimethylamino)-phenyl]-1-(3-guaiazulenyl)-1-phenylethylene (**7**) (17% yield), respectively, whose molecular structures were established on the basis of elemental analysis and spectroscopic data [UV–vis, IR, EI- and FAB-MS, ^{1}H and ^{13}C NMR including 2D NMR (i.e. H–H COSY, NOESY, HMQC and HMBC)] (see Sections 3.1.12 for **6**; 3.1.14 for **7**). Similarly, as in the case of 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene (**3**), the ^{1}H NMR signals of **6** and **7** were carefully assigned using the computer-assisted simulation analysis.



1.6. X-ray crystal structure of (*Z*)-2-[4-(dimethylamino)phenyl]-1-(3-guaiazulenyl)-1-phenylethylene (7) compared with those of 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene (3), *trans*-stilbene (10) and [4-(dimethylamino)phenyl]-3-guaiazulenylmethylium tetrafluoroborate (12)

The crystal structure of compound **7**, indicating the molecular structure, (*Z*)-2-[4-(dimethylamino)phenyl]-1-(3-guaiazulenyl)-1-phenylethylene, is shown in Figure 4(a) together with the selected bond distances. As the result, the structural parameters of **7** revealed that: (i) the C–C bond distance between the –HC═C< unit [1.347(4) Å] coincided with that of the >C═CH– unit of **3** [1.343(4) Å]; (ii) similarly, as in the case of **3** [see Fig. 2(a)], the planes of the 4‴-(dimethylamino)phenyl, 3′-guaiazulenyl and phenyl groups of **7** twisted by 153.4°, 82.6° and 22.1° from that of the –HC═C< unit, respectively, owing to the influence of large steric hindrance and repulsion between those three groups; (iii) the average C–C bond distances for the seven- and five-membered rings of the 3′-guaiazulenyl group (1.409 and 1.420 Å) coincided with the bond distances observed for those of the 3‴-guaiazulenyl group of **3** (1.405 and 1.427 Å); (iv) the average C–C bond distance for the benzene ring of the C-1 position (1.382 Å) coincided with the bond distances observed for those of the two benzene rings of *trans*-stilbene (**10**) (1.386 Å each);[14] and (v) the plane of the dimethylamino group was co-planar with that of the benzene ring of the C-2 position and, further, although the C4‴–N and C1‴–C2 bond distances [1.382(3) and 1.463(4) Å] were longer than the C4–N and C1–Cα bond distances [1.359(7) and 1.414(7) Å] of [4-(dimethylamino)phenyl]-3-guaiazulenylmethylium tetrafluoroborate (**12**)[5] with the resonance forms of the 3-guaiazulenylium **12**′ and quinonoid **12**″ structures in the single crystal, the dimethylaminobenzene ring clearly indicated the bond alternation between the single and double bonds, which coincided with the bond alternation pattern observed for the dimethylaminobenzene ring of **12**, in comparison with the benzene ring of the C-1 position. Along with the crystal structure of **7**, the packing structure of **7** revealed that: similarly, as in the case of **3**, the molecule **7** did not form a π-stacking structure in the single crystal as shown in Figure 4(b). A comparative study of the C–C bond distances for the partial structures (i.e. azulenylethylene units) of **3**, **4**, **7** and **9** is shown in Table 1.

1.7. A plausible reaction pathway for the formation of 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene (3), 2-[3-(1-chloroazulenyl)]-1,1-bis(4-methoxyphenyl)-ethylene (4), 2-azulenyl-1,1-bis(4-methoxyphenyl)-ethylene (5) and 2-(3-guaiazulenyl)-1,1-bis(4-hydroxyphenyl)ethylene (6)

In 1932 Bachmann and Moser reported the pinacol–

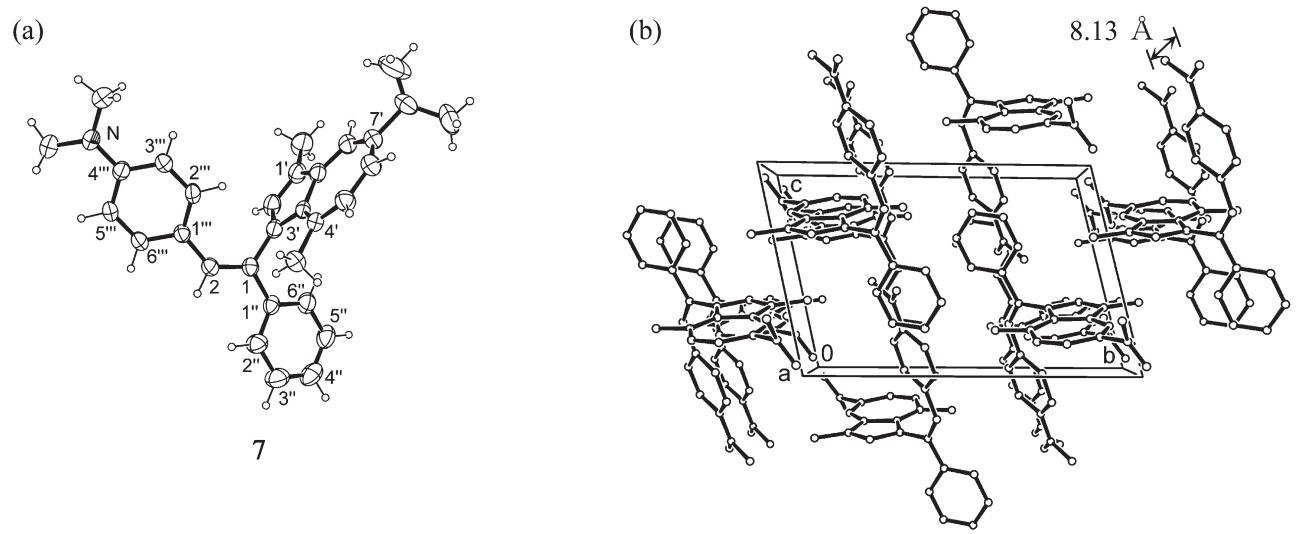
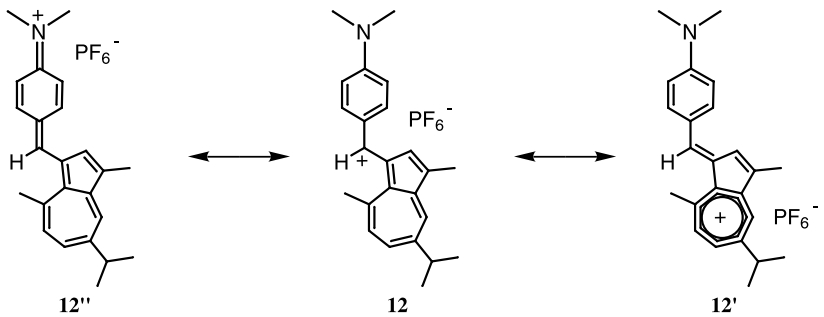
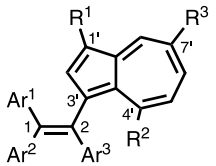


Figure 4. The ORTEP drawing (a) with the numbering scheme (30% probability thermal ellipsoids) of **7** and the packing structure (b) of **7**; hydrogen atoms are omitted for reasons of clarity. The bond distances (Å) of **7** are as follows: C1–C2; 1.347(4), C2–C1‴; 1.463(4), C1‴–C2‴; 1.395(4), C2‴–C3‴; 1.372(4), C3‴–C4‴; 1.389(4), C4‴–C5‴; 1.394(4), C5‴–C6‴; 1.364(4), C6‴–C1‴; 1.392(4), C4‴–N; 1.382(3), N–C7‴; 1.427(4), N–C8‴; 1.438(4), C1′–C2′; 1.373(4), C2′–C3′; 1.420(4), C3′–C3a′; 1.404(4), C3a′–C4′; 1.410(4), C4′–C5′; 1.382(4), C5′–C6′; 1.399(4), C6′–C7′; 1.386(5), C7′–C8′; 1.384(4), C8′–C8a′; 1.394(4), C8a′–C1′; 1.397(4), C3a′–C8a′; 1.502(4), C1–C3′; 1.489(4), C1–C1″; 1.487(4), C1″–C2″; 1.387(4), C2″–C3″; 1.382(4), C3″–C4″; 1.367(5), C4″–C5″; 1.381(5), C5″–C6″; 1.379(4) and C6″–C1″; 1.395(4).



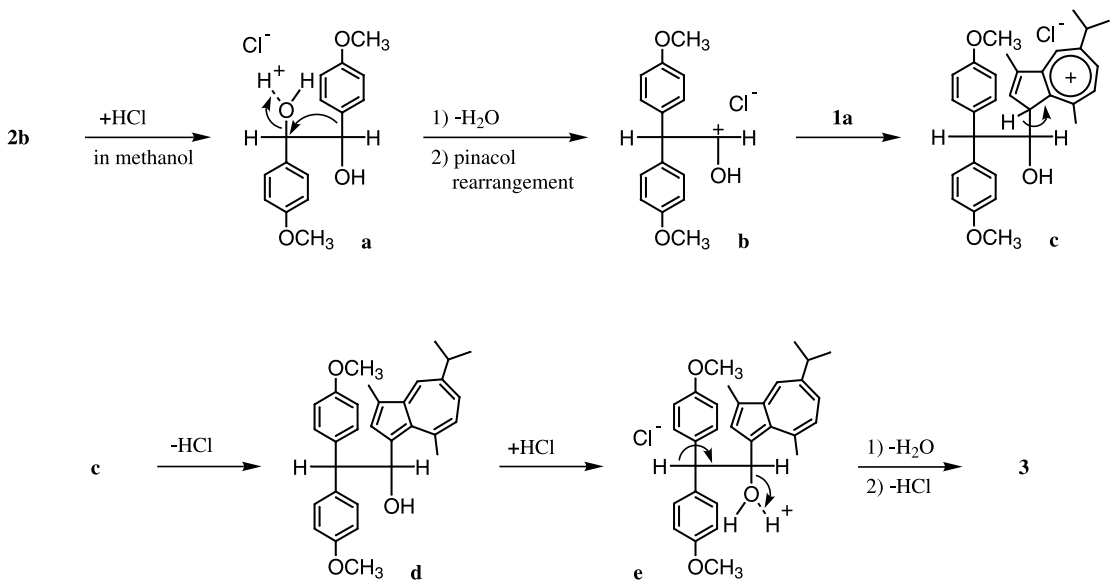
pinacolone rearrangement; namely, on the relative migratory aptitudes of aryl groups, and concluded that the migration (%) of the *p*-anisyl (*p*-$CH_3OC_6H_4$–) group was extremely high.[16] From this result, a plausible reaction pathway for the formation of compound **3** can be inferred as shown in Scheme 1: (i) upon heating 1,2-bis(4-methoxyphenyl)-1,2-ethanediol (**2b**) in methanol in the presence of hydrochloric acid at 60 °C under aerobic conditions, it is

Table 1. The C–C bond distances (Å) for the azulenylethylene units of **3**, **4**, **7** and **9**



3: $Ar^1 = Ar^2 = p\text{-}CH_3OC_6H_5$, Ar^3 = H, $R^1 = R^2 = CH_3$, $R^3 = i$-Pr
4: $Ar^1 = Ar^2 = p\text{-}CH_3OC_6H_5$, Ar^3 = H, R^1 = Cl, $R^2 = R^3$ = H
7: $Ar^1 = p\text{-}(CH_3)_2NC_6H_5$, Ar^2 = H, $Ar^3 = C_6H_5$, $R^1 = R^2 = CH_3$, $R^3 = i$-Pr
9: $Ar^1 = Ar^3$ = H, Ar^2 = 3-guaiazulenyl group, $R^1 = R^2 = CH_3$, $R^3 = i$-Pr

Atom	Compound			
	3	**4**	**7**	**9**
C1–C2	1.343(4)	1.347(3)	1.347(4)	1.32(1)
C2–C3′	1.464(4)	1.448(3)	1.487(4)	1.444(8)
C1′–C2′	1.382(5)	1.377(4)	1.373(4)	1.378(8)
C2′–C3′	1.407(5)	1.412(3)	1.420(4)	1.452(8)
C3′–C3a′	1.416(5)	1.413(3)	1.404(4)	1.391(7)
C3a′–C4′	1.393(4)	1.387(3)	1.410(4)	1.404(8)
C4′–C5′	1.390(5)	1.388(4)	1.382(4)	1.369(9)
C5′–C6′	1.388(5)	1.392(4)	1.399(4)	1.40(1)
C6′–C7′	1.383(4)	1.373(4)	1.386(5)	1.362(10)
C7′–C8′	1.384(5)	1.390(4)	1.384(4)	1.397(9)
C8′–C8a′	1.382(4)	1.378(4)	1.394(4)	1.351(8)
C8a′–C1′	1.413(5)	1.393(4)	1.397(4)	1.388(8)
C8a′–C3a′	1.515(4)	1.487(3)	1.502(4)	1.533(8)

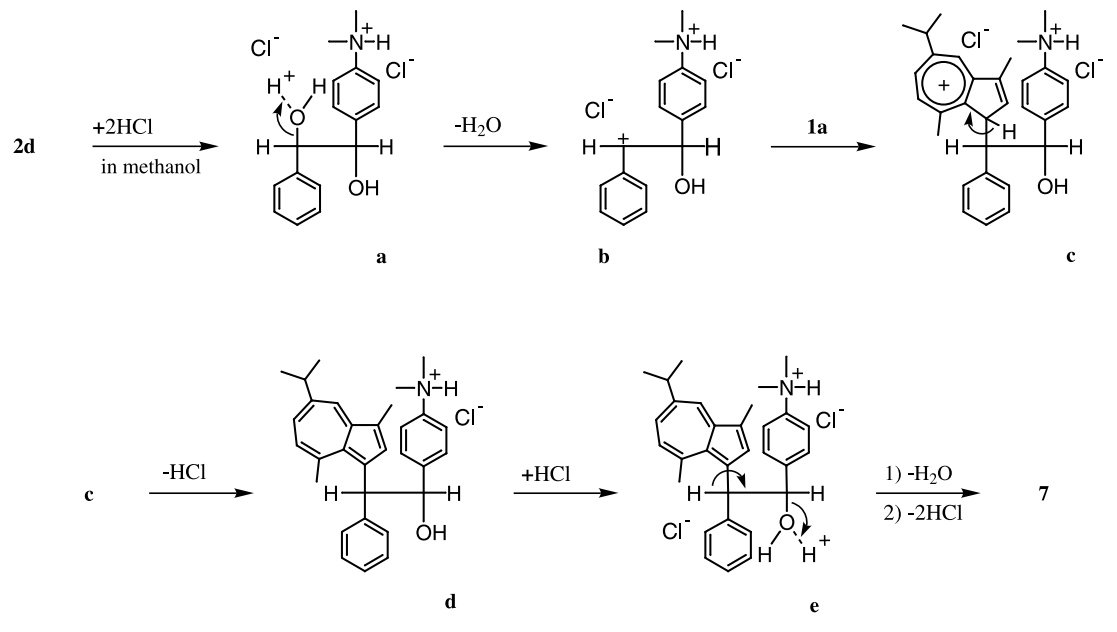


Scheme 1. A plausible reaction pathway for the formation of **3** from the reaction of guaiazulene (**1a**) with 1,2-bis(4-methoxyphenyl)-1,2-ethanediol (**2b**) in methanol in the presence of hydrochloric acid at 60 °C for 3 h under aerobic conditions.

gradually converted into the pinacol rearrangement product **b** via **a**; and, further, (ii) the reaction of guaiazulene (**1a**) with the carbocation **b** generated under the reaction conditions rapidly affords **3** presumably via **c**, **d** and **e**. A plausible reaction pathway for the formation of the products **4**–**6** can be inferred to be the same as that for **3** (see Scheme 1).

1.8. A plausible reaction pathway for the formation of (*Z*)-2-[4-(dimethylamino)phenyl]-1-(3-guaiazulenyl)-1-phenylethylene (7)

From the molecular structure of the resulting product, (*Z*)-2-[4-(dimethylamino)phenyl]-1-(3-guaiazulenyl)-1-phenylethylene (**7**), obtained by the reaction of guaiazulene (**1a**) with 1-[4-(dimethylamino)phenyl]-2-phenyl-1,2-ethanediol (**2d**), a plausible reaction pathway for the formation of compound **7** can be inferred as shown in Scheme 2: (i) upon heating **2d** in methanol in the presence of hydrochloric acid at 60 °C under aerobic conditions, it is gradually converted into the dehydration product **b**, simultaneously possessing a protonated amino group at the C-4′ position, via **a**; and, further, (ii) the reaction of **1a** with the carbocation **b** generated under the reaction conditions rapidly affords **7** presumably via **c**, **d** and **e**.



Scheme 2. A plausible reaction pathway for the formation of **7** from the reaction of guaiazulene (**1a**) with 1-[4-(dimethylamino)phenyl]-2-phenyl-1,2-ethanediol (**2d**) in methanol in the presence of hydrochloric acid at 60 °C for 3 h under aerobic conditions.

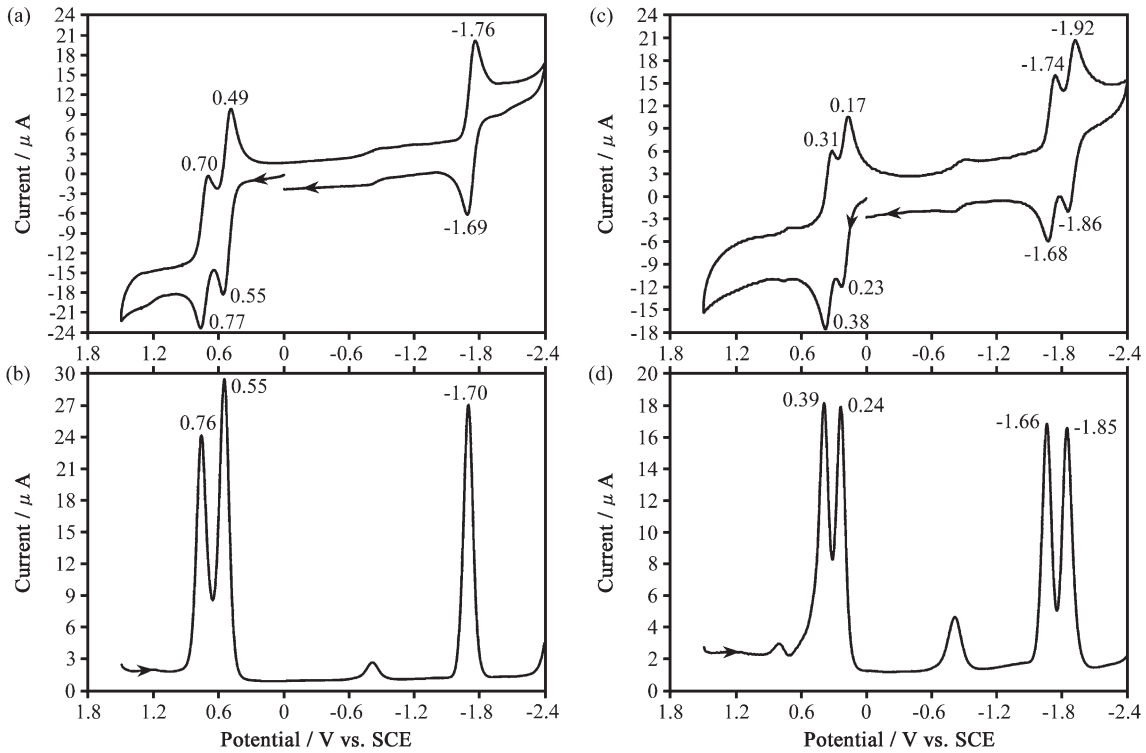
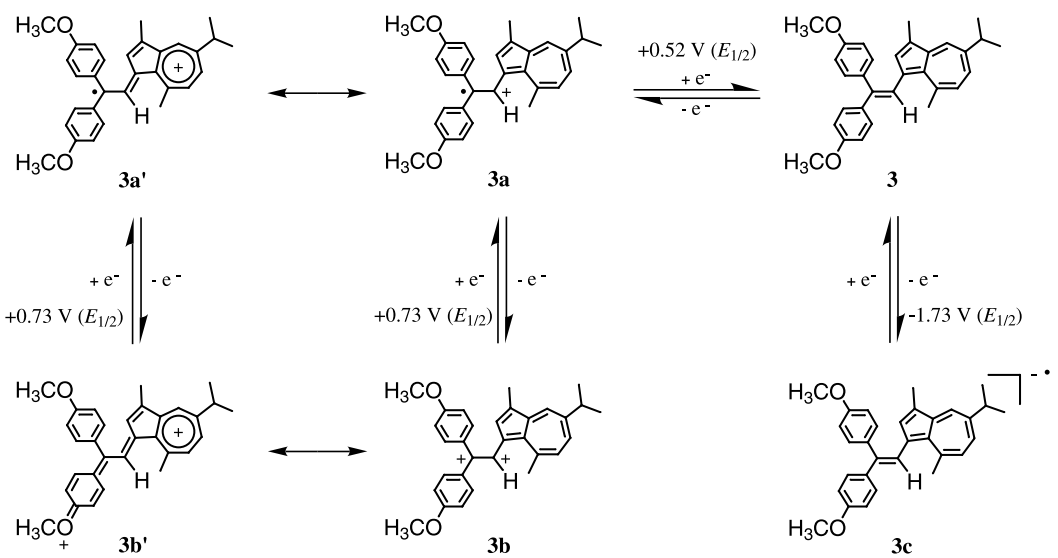


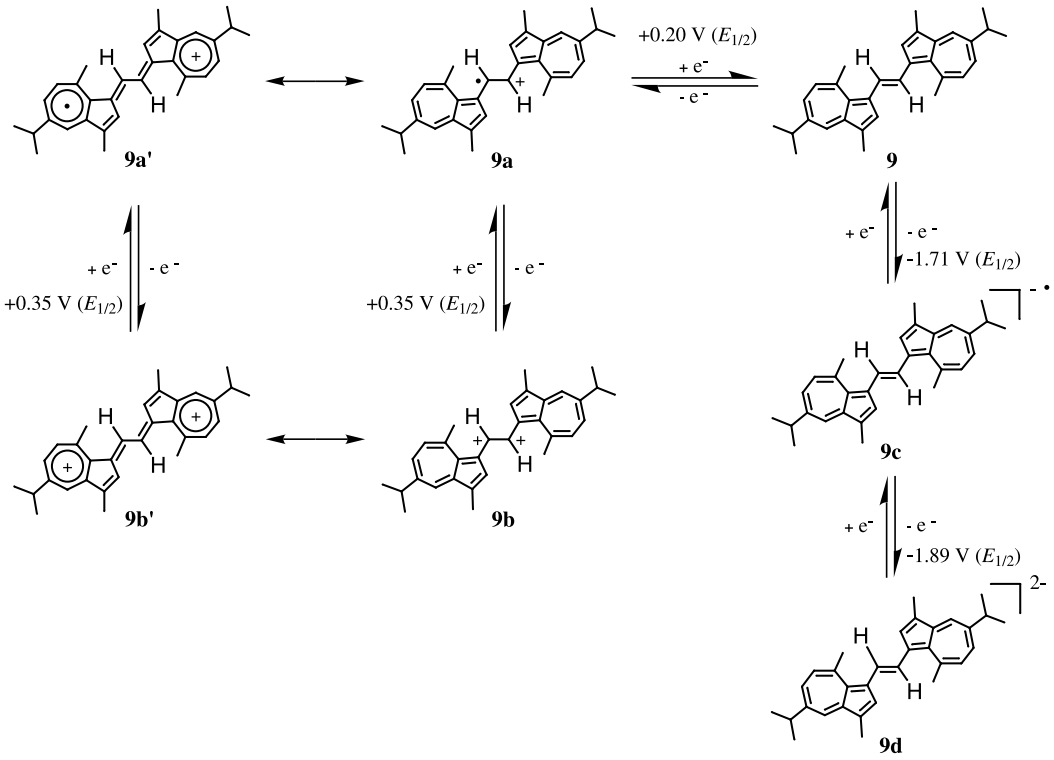
Figure 5. Cyclic and differential pulse voltammograms of **3** (3.0 mg, 6.9 μmol) (a, b) and **9** (2.0 mg, 4.8 μmol) (c, d) in 0.1 M [n-Bu_4N]BF_4, CH_3CN (10 mL) at a glassy carbon (ID: 3 mm) and platinum wire served as the working and auxiliary electrodes; scan rates 100 mV s^{-1} at 25 °C under argon, respectively. For comparative purposes, the oxidation potential using ferrocene as a standard material showed +0.45 (E_p) V by DPV and +0.42 ($E_{1/2}$) V by CV under the same electrochemical conditions as **3** and **9**.

1.9. Electrochemical behavior of 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene (3) compared with that of (*E*)-1,2-di(3-guaiazulenyl)ethylene (9)

We have been interested further in a comparative study of the electrochemical properties of compound **3** and (*E*)-1,2-di(3-guaiazulenyl)ethylene (**9**). The electrochemical behavior of **3** was, therefore, measured by means of CV and DPV (Potential/V vs. SCE) in 0.1 M [n-Bu_4N]BF_4, CH_3CN. Three redox potentials observed by DPV were positioned at the E_p values of +0.76, +0.55 and −1.70 V, while the corresponding three reversible redox potentials determined by CV were located at the values of +0.73 ($E_{1/2}$), +0.52 ($E_{1/2}$) and −1.73 ($E_{1/2}$) V as shown in Figure 5(a) and (b). From a comparative study of the redox potentials of **3** with those of **9** [see Fig. 5(c) and (d)] under the same electrochemical conditions as **3**, a plausible electron transfer mechanism of **3** and **9** based on their CV and DPV data can be inferred as shown in Schemes 3 (for



Scheme 3. A plausible electron transfer mechanism based on the CV and DPV data of **3**.



Scheme 4. A plausible electron transfer mechanism based on the CV and DPV data of **9**.

3) and 4 (for **9**); namely, (i) **3** stepwise undergoes two-electron oxidation at the potentials of +0.52 ($E_{1/2}$) and +0.73 ($E_{1/2}$) V by CV (corresponding to +0.55 and +0.76 V by DPV), generating an electrochemically stable dication **3b′** via the cation-radical **3a** and its resonance form **3a′** (and/or via **3a** and the resonance form **3b** of the dication **3b′**). Similarly, as in the case of **3**, **9** stepwise undergoes two-electron oxidation at the potentials of +0.20 ($E_{1/2}$) and +0.35 ($E_{1/2}$) V by CV (corresponding to +0.24 and +0.39 V by DPV), generating an electrochemically stable dication **9b′** via the cation-radical **9a** and its resonance form **9a′** (and/or via **9a** and the resonance form **9b** of the dication **9b′**). Thus, **3** is less susceptible to oxidation than **9**; (ii) **3** is reduced to the anion-radical **3c** at the potential of −1.73 ($E_{1/2}$) V by CV (corresponding to −1.70 V by DPV). Thus, the one-electron reduction potential of **3** coincides with that of **9** [−1.71 ($E_{1/2}$) V by CV (corresponding to −1.66 V by DPV)]; and, further, (iii) **9** is stepwise reduced to the dianion **9d** at the potential of −1.89 ($E_{1/2}$) V by CV (corresponding to −1.85 V by DPV) via the anion-radical **9c** at the potential of −1.71 ($E_{1/2}$) V by CV (corresponding to −1.66 V by DPV). As the result, the CV and DPV data indicated **3** and **9** serve as an electron donor, respectively. Along with the cation-radicals and the dications generated from **3** and **9**, the anion-radicals **3c**, **9c** and the dianion **9d** are also electrochemically stable.

1.10. A comparative study of the electrochemical behavior of 2-[3-(1-chloroazulenyl)]-1,1-bis(4-methoxyphenyl)ethylene (4), 2-azulenyl-1,1-bis(4-methoxyphenyl)ethylene (5) and 2-(3-guaiazulenyl)-1,1-bis(4-hydroxyphenyl)ethylene (6) with that of 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene (3)

The electrochemical behavior of compounds **4**, **5** and **6** was measured under the same electrochemical conditions as **3** [see Fig. 5(a) and (b)]. As the result, it was found that (i) **4** stepwise undergoes two-electron oxidation at the potentials of +0.77 ($E_{1/2}$) and +0.88 ($E_{1/2}$) V by CV (corresponding to +0.78 and +0.91 V by DPV), generating an electrochemically stable dication via an electrochemically stable cation-radical and, further, **4** is reduced to the anion-radical

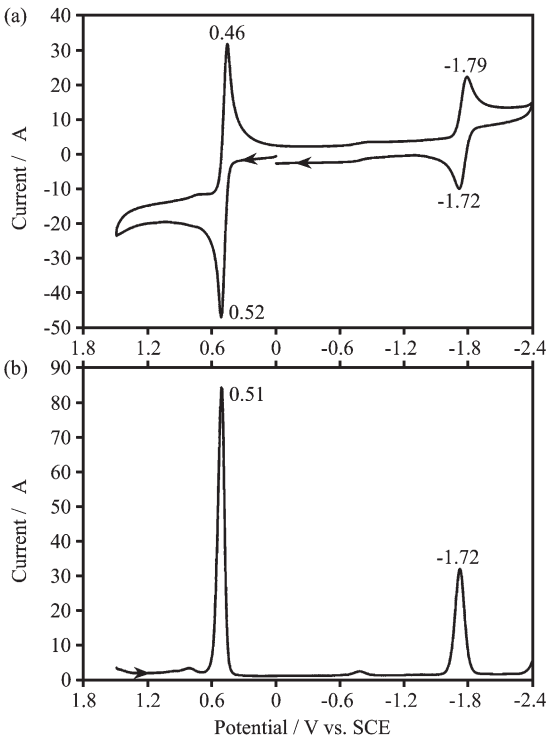
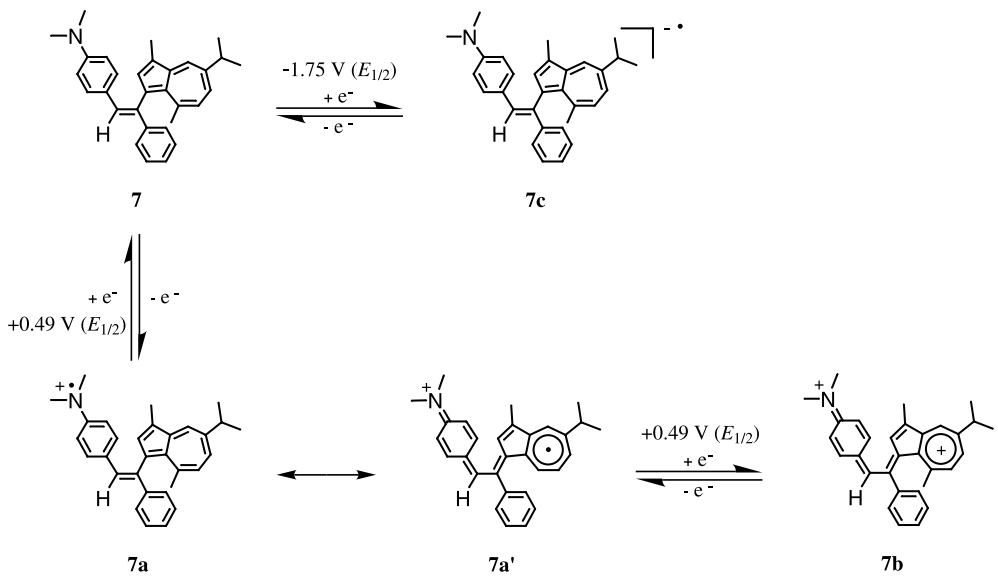


Figure 6. Cyclic (a) and differential pulse (b) voltammograms of **7** (3.0 mg, 7.1 μmol) under the same electrochemical conditions as **3** and **9**.



Scheme 5. A plausible electron transfer mechanism based on the CV and DPV data of **7**.

at the potential of −1.32 ($E_{1/2}$) V by CV (corresponding to −1.31 V by DPV), generating an electrochemically stable anion-radical. Thus, although **4** is less susceptible to oxidation than **3**, **4** is more susceptible to reduction than **3**; (ii) **5** stepwise undergoes three-electron oxidation at the potentials of +0.69 (E_{pa}, irreversible; E_{pc}: +0.47 V), +0.77 ($E_{1/2}$) and +0.98 ($E_{1/2}$) V by CV (corresponding to +0.55, +0.69, +0.80 and +1.00 V by DPV), generating an electrochemically stable trication-radical via an electrochemically unstable cation-radical and an electrochemically stable dication and, further, **5** is reduced to the anion-radical at the potential of −1.53 ($E_{1/2}$) V by CV (corresponding to −1.51 V by DPV), generating an electrochemically stable anion-radical. Thus, although **5** is less susceptible to oxidation than **3**, **5** stepwise undergoes three-electron oxidation and, further, **5** is more susceptible to reduction than **3**; and (iii) **6** stepwise undergoes two-electron oxidation at the potentials of +0.50 ($E_{1/2}$) and +0.70 ($E_{1/2}$) V by CV (corresponding to +0.53 and +0.72 V by DPV), generating an electrochemically stable dication via an electrochemically stable cation-radical and, further, **6** is reduced to the anion-radical at the potential of −1.78 (E_{pc}, irreversible) V by CV (corresponding to −1.77 V by DPV), generating an electrochemically unstable anion-radical. Thus, the two-electron oxidation potentials of **6** coincide with those of **3**. Although **6** and **3** undergo one-electron reduction, respectively, generating an electrochemically unstable anion-radical from **6** and an electrochemically stable anion-radical from **3**, the one-electron reduction potential [(E_{pc}) V by CV] of **6** coincides with that of **3** [−1.76 (E_{pc}) V by CV, see Fig. 5(a)].

1.11. A comparative study of the electrochemical behavior of (*Z*)-2-[4-(dimethylamino)phenyl]-1-(3-guaiazulenyl)-1-phenylethylene (7) with that of 2-(3-guaiazulenyl)-1,1-bis-(4-methoxyphenyl)ethylene (3)

The electrochemical behavior of **7** was measured under the same electrochemical conditions as **3**. Two redox potentials observed by DPV were positioned at the E_p values of +0.51 and −1.72 V, while the corresponding two reversible redox potentials determined by CV were located at the values of +0.49 ($E_{1/2}$) and −1.75 ($E_{1/2}$) V as shown in Figure 6(a) and (b). From a comparative study of the redox potentials of **7** with those of **3** [see Fig. 5(a) and (b)], a plausible electron transfer mechanism of **7** based on its CV and DPV data can be inferred as shown in Scheme 5; namely, (i) **7** undergoes two-electron oxidation at a potential of +0.49 ($E_{1/2}$) V by CV (corresponding to +0.51 V by DPV), generating an electrochemically stable dication **7b** via the cation-radical **7a** and its resonance form **7a′**. Thus, **7** is susceptible to two-electron oxidation than **3**; and (ii) **7** is reduced to the anion-radical **7c** at the potential of −1.75 ($E_{1/2}$) V by CV (corresponding to −1.72 V by DPV). Thus, the one-electron reduction potential of **7** coincides with that of **3**. As the result, the CV and DPV data indicated **7** serves as an electron donor. Along with the dication **7b** generated from **7**, the anion-radical **7c** is also electrochemically stable.

1.12. Reaction of 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene (3) with *N*-chlorosuccinimide (NCS)

The chemistry on stilbenes has been studied to a considerable extent, and the physical and chemical properties, the biological activities, and the functions for those numerous molecules have been well documented. For example, it is well known that diethylstilbestrol (DES)[17,18] and chlorotrianisene (**13**)[17] exhibit significant estrogenic activity. On the other hand, naturally occurring guaiazulene (**1a**) has been widely used clinically as anti-inflammatory and anti-ulcer agents. Furthermore, 3-chloroguaiazulene (**14**) was isolated from a deep sea coral, gorgonian.[19] Along with the title investigations, 'reactions of azulenes with 1,2-diaryl-1,2-ethanediols in methanol in the presence of hydrochloric acid', our interest has been focused on preparation and estrogenic activity of 1-chloro-1-(3-guaiazulenyl)-2,2-bis(4-methoxyphenyl)ethylene (**15**), possessing a similar-type structure as **13**, with a view to a comparative study with the estrogenic activity of **13**.

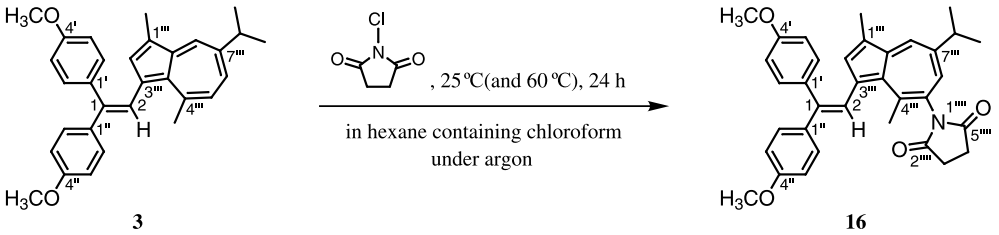
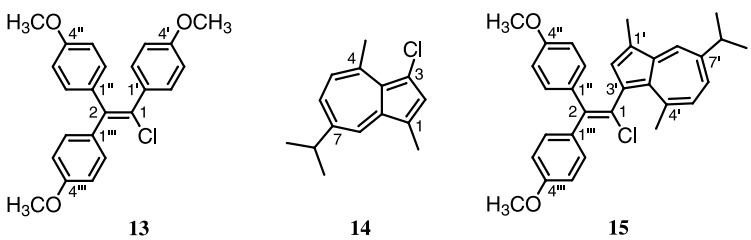


Figure 7. The reaction of 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene (**3**) with *N*-chlorosuccinimide (NCS) in hexane containing chloroform at 25 °C (and 60 °C) for 24 h under argon.

Similarly, as in the case of the preparation of 1-chloroazulene (**1c**) (see Section 3.1.5), the reactions of 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene (**3**) with *N*-chlorosuccinimide (NCS) at 25 °C (and 60 °C) were carried out, giving the same result, as shown in Figure 7 and Section 3.1.19. As the result, although this reaction did not give the target compound **15**, 1,1-bis(4-methoxyphenyl)-2-{3-[5-(succinimidyl)guaiazulenyl]}ethylene (**16**) was obtained in 10% yield, besides the recovered starting material **3** (81%). The molecular structure of the product **16** was established on the basis of elemental analysis and spectroscopic data [UV–vis, IR, EI-MS, ^{1}H and ^{13}C NMR including 2D NMR (i.e. H–H COSY, NOESY, HMQC and HMBC)] (see Section 3.1.19). Similarly, as in the case of 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene (**3**), the ^{1}H NMR signals of **16** was carefully assigned using the computer-assisted simulation analysis. In 1991 Nozoe and his co-workers reported that the reaction of guaiazulene (**1a**) with *N*-bromosuccinimide (NBS) in benzene at room temperature for 30 min gave 1-(5-guaiazulenyl)succinimide, possessing a similar partial structure as **16**, in 1% yield along with eight other products, the recovered starting material **1a** (15%) and a polar resinous substance (18% yield).[20] Moreover, the electrochemical behavior of **16** was measured under the same electrochemical conditions as **3** [see Fig. 5(a) and (b)]. As the result, it was found that **16** stepwise undergoes two-electron oxidation at the potentials of +0.64 ($E_{1/2}$) and +0.78 ($E_{1/2}$) V by CV (corresponding to +0.66 and +0.80 V by DPV), generating an electrochemically stable dication via an electrochemically stable cation-radical and, further, **16** is reduced to the anion-radical at the potential of −1.59 ($E_{1/2}$) V by CV (corresponding to −1.56 V by DPV), generating an electrochemically stable anion-radical. Thus, although **16** is less susceptible to oxidation than **3**, **16** is more susceptible to reduction than **3**, owing to the influence of the succinimidyl group substituted at the C-5 position of the 3-guaiazulenyl group. Studies on the preparation of **15** and, further, the estrogenic activity of **3**–**7**, **9** and **16** compared with that of **13** (and DES) are noteworthy, and are currently under intensive investigation.

2. Conclusion

We have reported the following five points in this paper: (i) although reaction of guaiazulene (**1a**) with 1,2-diphenyl-1,2-ethanediol (**2a**) in methanol in the presence of hydrochloric acid at 60 °C for 3 h under aerobic conditions gave no product, reaction of **1a** with 1,2-bis(4-methoxyphenyl)-1,2-ethanediol (**2b**) under the same reaction conditions as **2a** afforded a new ethylene derivative, 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene (**3**), in 97% yield; (ii) similarly, reaction of methyl azulene-1-carboxylate (**1b**) with **2b** under the same reaction conditions as **1a** gave no product; however, reactions of 1-chloroazulene (**1c**) and the parent azulene (**1d**) with **2b** under the same reaction conditions as **1a** afforded 2-[3-(1-chloroazulenyl)]-1,1-bis(4-methoxyphenyl)ethylene (**4**) (81% yield), 2-azulenyl-1,1-bis(4-methoxyphenyl)ethylene (**5**) (15% yield) and a chromatographically inseparable mixture of several bis[bis(4-methoxyphenyl)vinyl]azulenes (**5′**) (41% yield), respectively; (iii) along with the above reactions, reactions of **1a** with 1,2-bis(4-hydroxyphenyl)-1,2-ethanediol (**2c**) and 1-[4-(dimethylamino)phenyl]-2-phenyl-1,2-ethanediol (**2d**) under the same reaction conditions as **2b** gave 2-(3-guaiazulenyl)-1,1-bis(4-hydroxyphenyl)ethylene (**6**) (73% yield) and (*Z*)-2-[4-(dimethylamino)phenyl]-1-(3-guaiazulenyl)-1-phenylethylene (**7**) (17% yield), respectively; (iv) comparative studies on the above reaction products and their yields, crystal structures, spectroscopic and electrochemical properties have been reported and, further, a plausible reaction pathway for the formation of the products **3**–**7** has been described; and (v) reactions of **3** with *N*-chlorosuccinimide (NCS) in a mixed solvent of hexane and chloroform (4:1, vol/vol) at 25 °C (and 60 °C) for 24 h under argon respectively gave 1,1-bis(4-methoxyphenyl)-2-{3-[5-(succinimidyl)guaiazul-



enyl]}ethylene (**16**) in 10% yield, besides the recovered starting material **3** (81%).

3. Experimental

3.1. General

Thermal (TGA/DTA) and elemental analyses were taken on a Shimadzu DTG-50H thermal analyzer and a Yanaco MT-3 CHN corder, respectively. MALDI-TOF- and EI- (and FAB-) MS spectra were taken on a Shimadzu/Kratos Kompact-MALDI 4 and a JEOL The Tandem Mstation JMS-700 TKM data system, respectively. UV–visible and IR spectra were taken on a Beckman DU640 spectrophotometer and a Shimadzu FTIR-4200 Grating spectrometer, respectively. NMR spectra were recorded with a JEOL GX-500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) cryospectrometer at 25 °C. The ^{1}H NMR spectra were assigned using the computer-assisted simulation analysis (the software: gNMR developed by Adept Scientific plc) on a DELL Dimension XPS T500 personal-computer with a Pentium III processor. Cyclic and differential pulse voltammograms were measured by an ALS Model 600 electrochemical analyzer.

3.1.1. Preparation of 1,2-bis(4-methoxyphenyl)-1,2-ethanediol (2b). To a powder of $NaBH_4$ (100 mg, 2.64 mmol) was added a solution of commercially available 1,2-bis(4-methoxyphenyl)-1,2-ethanedione (500 mg, 1.85 mmol) in ethanol (5 mL). The mixture was stirred at 25 °C for 30 min. The crude product, 1,2-bis(4-methoxyphenyl)-1,2-ethanediol (**2b**), thus obtained was recrystallized from ethanol–water (1:3, vol/vol) to provide a ca. 3:1, chromatographically inseparable mixture of *meso* (1*R*,2*S*)-1,2-bis(4-methoxyphenyl)-1,2-ethanediol (**2b′**) and two enantiomeric, (1*R*,2*R*)- and (1*S*,2*S*)-1,2-bis(4-methoxyphenyl)-1,2-ethanediol (**2b″**), forms as stable crystals (380 mg, 1.39 mmol, 75% yield).

Compound **2b**. White plates, mp 167 °C [determined by thermal analysis (TGA and DTA)]. Found: C, 70.26; H, 6.52%. Calcd for $C_{16}H_{18}O_4$: C, 70.06; H, 6.61%; exact EI-MS (70 eV), found: *m/z* 274.1187 (M^+, 54%) and 256.1085 ($[M-H_2O]^+$, 100%); calcd for $C_{16}H_{18}O_4$: M^+, *m/z* 274.1205 and $[M-H_2O]^+$, *m/z* 256.1099; IR ν_{max} (KBr) cm^{-1}, 3344 (O–H), 2901, 2835 (C–H), 1612, 1516 (aromatic C=C) and 1254, 1030 (C–O). The relative intensity of the ^{1}H NMR signals for the *meso* **2b′** and the enantiomers **2b″** showed a ratio of ca. 3:1.

Compound **2b′**. ^{1}H NMR (CD_3CN), δ 3.21, 3.22 (1H each, dd, *J*=4.6, 2.6 Hz, OH-1,2), 3.77 (6H, s, MeO-4′,4″), 4.645, 4.653 (1H each, brd dd, *J*=4.6, 2.6 Hz, H-1,2), 6.83 (4H, brd ddd, *J*=8.5, 2.5, 1.0 Hz, H-3′,5′,3″,5″) and 7.15 (4H, brd ddd, *J*=8.5, 2.5, 1.0 Hz, H-2′,6′,2″,6″); ^{13}C NMR (CD_3CN), δ 159.9 (C-4′,4″), 134.9 (C-1′,1″), 129.4 (C-2′,6′,2″,6″), 113.9 (C-3′,5′,3″,5″), 78.0 (C-1,2) and 55.8 (MeO-4′, 4″).

Compound **2b″**. ^{1}H NMR (CD_3CN), δ 3.68, 3.69 (1H each, dd, *J*=6.0, 2.3 Hz, OH-1,2), 3.73 (6H, s, MeO-4′,4″), 4.55, 4.54 (1H each, brd dd, *J*=6.0, 2.3 Hz, H-1,2), 6.76 (4H, brd ddd, *J*=8.5, 2.5, 1.0 Hz, H-3′,5′,3″,5″) and 7.05 (4H, brd ddd, *J*=8.5, 2.5, 1.0 Hz, H-2′,6′,2″,6″); ^{13}C NMR (CD_3CN), δ 159.9 (C-4′,4″), 134.4 (C-1′,1″), 129.4 (C-2′,6′,2″,6″), 114.0 (C-3′,5′,3″,5″), 79.0 (C-1,2) and 55.8 (MeO-4′,4″).

3.1.2. Reaction of guaiazulene (1a) with 1,2-bis(4-methoxyphenyl)-1,2-ethanediol (2b) in methanol in the presence of hydrochloric acid. To a solution of commercially available guaiazulene (**1a**) (50 mg, 252 μmol) in methanol (1.0 mL) was added a solution of 1,2-bis(4-methoxyphenyl)-1,2-ethanediol (**2b**) (60 mg, 219 μmol) in methanol (1.5 mL) containing 36% hydrochloric acid (0.2 mL) at 60 °C. The mixture was stirred at 60 °C for 3 h under aerobic conditions and then evaporated in vacuo. The residue thus obtained was carefully separated by silica-gel column chromatography (several times) with hexane–ethyl acetate–benzene (90:5:5, vol/vol/vol) as an eluant. The crude product, 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene (**3**), thus obtained was recrystallized from methanol to provide pure **3** as stable crystals (93 mg, 213 μmol, 97% yield).

Compound **3**. Dark-green needles, mp 150 °C [determined by thermal analysis (TGA and DTA)]. Found: C, 85.29; H, 7.44%. Calcd for $C_{31}H_{32}O_2$: C, 85.28; H, 7.39%; R_f=0.35 on silica-gel TLC (hexane–AcOEt–benzene=90:5:5, vol/vol/vol); UV–vis λ_{max} (CH_3CN) nm (log ε), 270 (4.33), 328 (4.24), 352sh (4.14), 408 (4.10) and 635 (2.54); IR ν_{max} (KBr) cm^{-1}, 2905, 2866 (C–H), 1605, 1508 (aromatic C=C) and 1242, 1034 (C–O); MALDI-TOF-MS (without any matrix reagent), *m/z* 436 (M^+, 100%); exact EI-MS (70 eV), found: *m/z* 436.2398 (M^+, 100%); calcd for $C_{31}H_{32}O_2$: M^+, *m/z* 436.2402. ^{1}H NMR (C_6D_6), signals based on the 3-guaiazulenyl group at δ 1.16 (6H, d, *J*=6.9 Hz, $(CH_3)_2$CH-7‴), 2.31 (3H, brd s, Me-1‴), 2.70 (1H, sept, *J*=6.9 Hz, Me_2CH-7‴), 2.94 (3H, s, Me-4‴), 6.63 (1H, d, *J*=10.6 Hz, H-5‴), 7.02 (1H, dd, *J*=10.6, 2.0 Hz, H-6‴), 7.44 (1H, brd s, H-2‴), 7.91 (1H, d, *J*=2.0 Hz, H-8‴) and signals based on the 1,1-bis(4-methoxyphenyl) groups at δ 3.24 (3H, s, MeO-4′), 3.33 (3H, s, MeO-4″), 6.70 (2H, ddd, *J*=8.5, 2.5, 1.0 Hz, H-3′,5′), 6.84 (2H, ddd, *J*=8.5, 2.5, 1.0 Hz, H-3″,5″), 7.38 (2H, ddd, *J*=8.5, 2.5, 1.0 Hz, H-2′,6′), 7.49(2H, ddd, *J*=8.5, 2.5, 1.0 Hz, H-2″,6″) and a signal based on the >C=C*H*– unit at δ 7.77 (1H, brd s, H-2); ^{13}C NMR (C_6D_6), δ 159.6 (C-4″), 159.1 (C-4′), 146.2 (C-4‴), 140.4 (C-2‴), 140.2 (C-7‴), 139.7 (C-8a‴), 138.7 (C-1), 137.7 (C-1″), 135.5 (C-3a‴), 134.6 (C-6‴), 134.0 (C-1′), 133.2 (C-2′,6′), 133.2 (C-8‴), 129.6 (C-2″,6″), 127.2 (C-3‴), 126.5 (C-5‴), 125.6 (C-2), 125.1 (C-1‴), 114.1 (C-3′,5′), 114.1 (C-3″,5″), 54.8 (MeO-4″), 54.6 (MeO-4′), 37.9 (Me_2CH-7‴), 27.8 (Me-4‴), 24.5 ($(CH_3)_2$CH-7‴) and 12.9 (Me-1‴).

3.1.3. X-ray crystal structure of 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene (3). A total 6319 reflections with $2\theta_{max}$=55.0° were collected on a Rigaku AFC-5R automated four-circle diffractometer with graphite monochromated Mo-*K*α radiation (λ=0.71069 Å, rotating anode: 50 kV, 180 mA) at 296 K. The structure was solved by direct methods (SIR97) and expanded using Fourier techniques (DIRDIF94). The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares

refinement was based on F^2. All calculations were performed using the teXsan crystallographic software package. Crystallographic data have been deposited at the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK and copies can be obtained on request, free of charge, by quoting the publication citation and the deposition number CCDC 200992.

Crystallographic data for **3**: $C_{31}H_{32}O_2$ (FW=436.59), dark-green needle [from ethyl acetate–methanol=1:5 (vol/vol), the crystal size, 0.40×0.20×0.50 mm^3], monoclinic, $P2_1/n$ (#14), a=15.427(2) Å, b=10.103(3) Å, c=16.706(2) Å, β=105.371(9)°, V=2510.5(8) Å^3, Z=4, D_{calcd}=1.155 g/cm^3, μ(Mo-$K\alpha$)=0.70 cm^{-1}, scan width=(1.52+0.30 tan θ)°, scan mode=$\omega-2\theta$, scan rate=8.0°/min, measured reflections=6319, observed reflections=3574, no. of parameters=298, $R1$=0.052, $wR2$=0.160 and goodness of fit indicator=1.22.

3.1.4. Reaction of methyl azulene-1-carboxylate (1b) with 1,2-bis(4-methoxyphenyl)-1,2-ethanediol (2b) in methanol in the presence of hydrochloric acid. To a solution of methyl azulene-1-carboxylate[21] (**1b**) (41 mg, 220 μmol), whose compound was prepared according to a method based on the references,[22,23] in methanol (1.0 mL) was added a solution of 1,2-bis(4-methoxyphenyl)-1,2-ethanediol (**2b**) (60 mg, 219 μmol) in methanol (1.5 mL) containing 36% hydrochloric acid (0.2 mL) at 60 °C. The mixture was stirred at 60 °C for 3 h under aerobic conditions and then evaporated in vacuo. No product was observed by silica-gel TLC [solv. hexane–ethyl acetate (8:2, vol/vol)] of the residue thus obtained. Furthermore, the thus-obtained residue was carefully separated by silica-gel column chromatography with hexane–ethyl acetate (8:2, vol/vol) as an eluant, giving only the starting material **1b** (40 mg). No product was obtained.

3.1.5. Preparation of 1-chloroazulene (1c). Compound **1c** was prepared according to a method based on the reference.[23] To a solution of *N*-chlorosuccinimide (NCS) (150 mg, 1.12 mmol) in hexane (15 mL) was added a solution of commercially available azulene (**1d**) (100 mg, 0.78 mmol) in hexane (5.0 mL). The mixture was stirred at 25 °C for 18 h under argon and then evaporated in vacuo. The residue thus obtained was carefully separated by silica-gel column chromatography with hexane as an eluant, giving pure 1-chloroazulene[23] (**1c**) as a blue paste (89 mg, 0.55 mmol, 71% yield).

3.1.6. Reaction of 1-chloroazulene (1c) with 1,2-bis(4-methoxyphenyl)-1,2-ethanediol (2b) in methanol in the presence of hydrochloric acid. To a solution of 1-chloroazulene (**1c**) (27 mg, 166 μmol) in methanol (1.0 mL) was added a solution of 1,2-bis(4-methoxyphenyl)-1,2-ethanediol (**2b**) (75 mg, 275 μmol) in methanol (1.5 mL) containing 36% hydrochloric acid (0.18 mL) at 60 °C. The mixture was stirred at 60 °C for 3 h under aerobic conditions and then evaporated in vacuo. The residue thus obtained was carefully separated by silica-gel column chromatography with hexane–ethyl acetate–benzene (90:5:5, vol/vol/vol) as an eluant. The starting material **1c** (2 mg, 12 μmol, 7%) was recovered. The crude product, 2-[3-(1-chloroazulenyl)]-1,1-bis(4-methoxyphenyl)ethylene (**4**), thus obtained was recrystallized from methanol to provide pure **4** as stable crystals (54 mg, 135 μmol, 81% yield).

Compound **4**. Dark-green prisms, mp 137 °C [determined by thermal analysis (TGA and DTA)]. Found: C, 77.88; H, 5.42%. Calcd for $C_{26}H_{21}ClO_2$: C, 77.90; H, 5.28%; R_f=0.14 on silica-gel TLC (hexane–AcOEt–benzene=90:5:5, vol/vol/vol); UV–vis λ_{max} (CH_3CN) nm (log ε), 268 (4.48), 328 (4.44), 358 (4.24), 407 (4.22) 657 (2.58) and 665 (2.58); IR ν_{max} (KBr) cm^{-1}, 2927, 2831 (C–H), 1609, 1508 (aromatic C=C), 1242, 1034 (C–O) and 740 (C–Cl); exact EI-MS (70 eV), found: m/z 400.1219 (M^+, 100%); calcd for $C_{26}H_{21}ClO_2$: M^+, m/z 400.1230. ^{1}H NMR (C_6D_6), signals based on the 3-(1-chloroazulenyl) group at δ 6.53 (1H, dd, J=9.6, 9.6 Hz, H-7‴), 6.56 (1H, dd, J=9.6, 9.6 Hz, H-5‴), 7.00 (1H, dd, J=9.6, 9.6 Hz, H-6‴), 7.40 (1H, s, H-2‴), 8.06 (1H, d, J=9.6 Hz, H-8‴), 8.07 (1H, d, J=9.6 Hz, H-4‴) and signals based on the 1,1-bis(4-methoxyphenyl) groups at δ 3.26 (3H, s, MeO-4′), 3.33 (3H, s, MeO-4″), 6.75 (2H, ddd, J=8.5, 2.3, 1.4 Hz, H-3′,5′), 6.84 (2H, ddd, J=8.7, 2.5, 1.5 Hz, H-3″,5″), 7.23 (2H, ddd, J=8.5, 2.3, 1.4 Hz, H-2′,6′), 7.43 (2H, ddd, J=8.7, 2.5, 1.5 Hz, H-2″,6″) and a signal based on the >C=C*H*– unit at δ 7.38 (1H, s, H-2); ^{13}C NMR (C_6D_6), δ 159.9 (C-4″), 159.8 (C-4′), 140.0 (C-1), 139.3 (C-6‴), 137.1 (C-1″), 136.8 (C-3a‴), 135.4 (C-8a‴), 134.8 (C-2‴), 134.6 (C-8‴), 134.5 (C-4‴), 133.7 (C-1′), 132.0 (C-2′,6′), 129.2 (C-2″,6″), 126.0 (C-3‴), 123.5 (C-7‴), 122.8 (C-5‴), 118.1 (C-1‴), 117.8 (C-2), 114.8 (C-3′,5′), 114.1 (C-3″,5″), 54.9 (MeO-4″) and 54.7 (MeO-4′).

3.1.7. X-ray crystal structure of 2-[3-(1-chloroazulenyl)]-1,1-bis(4-methoxyphenyl)ethylene (4). A total 5477 reflections with $2\theta_{max}$=55.0° were collected on a Rigaku AFC-5R automated four-circle diffractometer with graphite monochromated Mo-$K\alpha$ radiation (λ=0.71069 Å, rotating anode: 50 kV, 180 mA) at 296 K. The structure was solved by direct methods (SIR97) and expanded using Fourier techniques (DIRDIF94). The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on F^2. All calculations were performed using the teXsan crystallographic software package. CCDC No.: 225123.

Crystallographic data for **4**: $C_{26}H_{21}ClO_2$ (FW=400.90), dark-green prism [from methanol, the crystal size, 0.40×0.30×0.50 mm^3], monoclinic, $P2_1/n$ (#14), a=6.280(2) Å, b=13.481(4) Å, c=24.902(1) Å, β=94.78(1)°, V=2101.0(7) Å^3, Z=4, D_{calcd}=1.267 g/cm^3, μ(Mo-$K\alpha$)=2.01 cm^{-1}, scan width=(0.73+0.30 tan θ)°, scan mode=ω, scan rate=8.0°/min, measured reflections=5477, observed reflections=2704, no. of parameters=262, $R1$=0.039, $wR2$=0.116 and goodness of fit indicator=1.25.

3.1.8. Reaction of azulene (1d) with 1,2-bis(4-methoxyphenyl)-1,2-ethanediol (2b) in methanol in the presence of hydrochloric acid. To a solution of commercially available azulene (**1d**) (35 mg, 273 μmol) in methanol (1.0 mL) was added a solution of 1,2-bis(4-methoxyphenyl)-1,2-ethanediol (**2b**) (30 mg, 109 μmol) in methanol (1.5 mL) containing 36% hydrochloric acid (0.2 mL) at 60 °C. The mixture was stirred at 60 °C for 3 h under aerobic conditions and then evaporated in vacuo. The residue thus

obtained was carefully separated by silica-gel column chromatography (several times) with hexane–ethyl acetate–benzene (90:5:5, vol/vol/vol) as an eluant, giving the recovered starting material **1d** (8 mg, 62.4 μmol, 23%), 2-azulenyl-1,1-bis(4-methoxyphenyl)ethylene (**5**) as a dark-green paste (6 mg, 16.4 μmol, 15% yield) and a chromatographically inseparable mixture of bis[bis(4-methoxyphenyl)vinyl]azulenes (**5′**). The product **5** thus obtained was recrystallized from hexane–ethyl acetate–benzene (90:5:5, vol/vol/vol) to provide pure **5** as stable crystals. A mixture of **5′** thus obtained was recrystallized from ethyl acetate–methanol (1:5, vol/vol) to provide **5′** as stable crystals (27 mg, 45 μmol, 41% yield).

Compound **5**. Dark-green blocks, mp 145 °C [determined by thermal analysis (TGA and DTA)]. Found: C, 85.19; H, 6.15%. Calcd for $C_{26}H_{22}O_2$: C, 85.22; H, 6.05%; R_f=0.25 on silica-gel TLC (hexane–AcOEt–benzene=90:5:5, vol/vol/vol); UV–vis λ_{max} (CH_3CN) nm (log ε), 225sh (4.38), 265 (4.49), 308sh (4.37), 320 (4.43), 347 (4.33), 403 (4.25) and 639 (2.46); IR ν_{max} (KBr) cm^{-1}, 3017, 2835 (C–H) and 1605, 1508 (aromatic C=C) and 1246, 1030 (C–O); exact EI-MS (70 eV), found: *m/z* 366.1621 (M^+, 100%); calcd for $C_{26}H_{22}O_2$: M^+, *m/z* 366.1620. ^1H NMR (C_6D_6), signals based on the azulenyl group at δ 6.63 (1H, dd, *J*=9.8, 9.8 Hz, H-5‴), 6.72 (1H, dd, *J*=9.8, 9.8 Hz, H-7‴), 7.07 (1H, d, *J*=4.0 Hz, H-3‴), 7.11 (1H, dd, *J*=9.8, 9.8 Hz, H-6‴), 7.58 (1H, d, *J*=4.0 Hz, H-2‴), 7.72 (1H, d, *J*=9.8 Hz, H-4‴), 8.26 (1H, d, *J*=9.8 Hz, H-8‴) and signals based on the 1,1-bis(4-methoxyphenyl) groups at δ 7.49 (2H, ddd, *J*=8.6, 2.5, 1.0 Hz, H-2″,6″), 7.33 (2H, ddd, *J*=8.6, 2.5, 1.0 Hz, H-2′,6′), 6.87 (2H, ddd, *J*=8.6, 2.5, 1.0 Hz, H-3″,5″), 6.82 (2H, ddd, *J*=8.6, 2.5, 1.0 Hz, H-3′,5′), 3.34 (3H, s, MeO-4″), 3.31 (3H, s, MeO-4′) and a signal based on the >C=C*H*– unit at δ 7.58 (1H, s, H-2); ^{13}C NMR (C_6D_6), δ 159.7 (C-4″), 159.6 (C-4′), 142.4 (C-3a‴), 139.8 (C-1), 137.7 (C-6‴), 137.61 (C-8a‴), 137.58 (C-2‴), 137.5 (C-1″), 136.3 (C-4‴), 134.5 (C-1′), 133.9 (C-8‴), 132.3 (C-2′,6′), 129.2 (C-2″,6″), 128.1 (C-1‴), 123.7 (C-5‴), 122.3 (C-7‴), 119.3 (C-2), 119.0 (C-3‴), 114.6 (C-3′,5′), 113.7 (C-3″,5″), 54.8 (MeO-4″) and 54.7 (MeO-4′).

Compound **5′**. Dark-green prisms, mp >220 °C [decomp., determined by thermal analysis (TGA and DTA)]. Found: C, 82.82; H, 6.12%. Calcd for $C_{42}H_{36}O_4$: C, 83.42; H, 6.00%; R_f=0.03 on silica-gel TLC (hexane–AcOEt–benzene=90:5:5, vol/vol/vol); IR ν_{max} (KBr) cm^{-1}, 2997, 2831 (C–H) and 1605, 1508 (aromatic C=C) and 1246, 1034 (C–O); exact EI-MS (70 eV), found: *m/z* 604.2614 (M^+, 100%); calcd for $C_{42}H_{36}O_4$: M^+, *m/z* 604.2613.

3.1.9. Preparation of 1,2-diphenyl-1,2-ethanediol (2a). To a powder of $NaBH_4$ (100 mg, 2.64 mmol) was added a solution of commercially available 1,2-diphenyl-1,2-ethanedione (500 mg, 2.38 mmol) in ethanol (5 mL). The mixture was stirred at 25 °C for 30 min. The crude product, 1,2-diphenyl-1,2-ethanediol (**2a**), thus obtained was recrystallized from ethanol–water (1:3, vol/vol) to provide a ca. 25:1, chromatographically inseparable mixture of *meso* (1*R*,2*S*)-1,2-diphenyl-1,2-ethanediol (**2a′**), and two enantiomeric, (1*R*,2*R*)- and (1*S*,2*S*)-1,2-diphenyl-1,2-ethanediol (**2a″**), forms as stable crystals (350 mg, 1.63 mmol, 68% yield).

Compound **2a**. White plates, mp 132 °C [determined by thermal analysis (TGA and DTA)]. Found: C, 78.82; H, 6.58%. Calcd for $C_{14}H_{14}O_2$: C, 78.48; H, 6.59%; exact EI-MS (70 eV), found: *m/z* 214.1004 (M^+, 46%) and 196.0918 ($[M-H_2O]^+$, 100%); calcd for $C_{14}H_{14}O_2$: M^+, *m/z* 214.0994 and $[M-H_2O]^+$, *m/z* 196.0888; IR ν_{max} (KBr) cm^{-1}, 3371, 3310 (O–H), 2901 (C–H), 1601, 1497 (aromatic C=C) and 1281, 1034 (C–O). The relative intensity of the ^1H NMR signals for the *meso* **2a′** and the enantiomers **2a″** showed a ratio of ca. 25:1.

Compound **2a′**. ^1H NMR (CD_3CN), δ 3.36, 3.37 (1H each, dd, *J*=4.6, 2.9 Hz, OH-1,2), 4.75, 4.76 (1H each, brd dd, *J*=4.6, 2.9 Hz, H-1,2) and 7.20–7.30 (10H, m, protons for two phenyl groups); ^{13}C NMR (CD_3CN), δ 142.7 (C-1′,1″), 128.6 (C-2′,6′,2″,6″), 128.24 (C-3′,5′,3″,5″), 128.15 (C-4′,4″) and 78.4 (C-1,2).

Compound **2a″**. ^1H NMR (CD_3CN), δ 3.75, 3.76 (1H, dd, *J*=6.0, 2.3 Hz, OH-1,2), 4.64, 4.65 (1H each, brd dd, *J*=6.0, 2.3 Hz, H-1,2) and 7.20–7.30 (10H, m, protons for two phenyl groups).

3.1.10. Reaction of guaiazulene (1a) with 1,2-diphenyl-1,2-ethanediol (2a) in methanol in the presence of hydrochloric acid. To a solution of commercially available guaiazulene (**1a**) (50 mg, 252 μmol) in methanol (1.0 mL) was added a solution of 1,2-diphenyl-1,2-ethanediol (**2a**) (60 mg, 219 μmol) in methanol (1.5 mL) containing 36% hydrochloric acid (0.2 mL) at 60 °C. The mixture was stirred at 60 °C for 3 h under aerobic conditions and then evaporated in vacuo. No product was observed by silica-gel TLC [solv. hexane–ethyl acetate–benzene (90:5:5, vol/vol/vol)] of the residue thus obtained. Furthermore, the thus-obtained residue was carefully separated by silica-gel column chromatography with hexane–ethyl acetate–benzene (90:5:5, vol/vol/vol) as an eluant, giving only the starting material **1a** (49 mg). No product was obtained.

3.1.11. Preparation of 1,2-bis(4-hydroxyphenyl)-1,2-ethanediol (2c). To a powder of $NaBH_4$ (50 mg, 1.32 mmol) was added a solution of commercially available 1,2-bis(4-hydroxyphenyl)-1,2-ethanedione (100 mg, 0.41 mmol) in methanol (3.0 mL). The mixture was stirred at 0 °C for 1 h. After the reaction, distilled-water (10 mL) was added to the mixture and then the resulting product was extracted with diethyl ether (10 mL×3). The extract was evaporated in vacuo. The crude product, 1,2-bis(4-hydroxyphenyl)-1,2-ethanediol (**2c**), thus obtained was recrystallized from ethanol to provide a ca. 12:1, chromatographically inseparable mixture of *meso* (1*R*,2*S*)-1,2-bis(4-hydroxyphenyl)-1,2-ethanediol (**2c′**), and two enantiomeric, (1*R*,2*R*)- and (1*S*,2*S*)-1,2-bis(4-hydroxyphenyl)-1,2-ethanediol (**2c″**), forms as stable crystals (51 mg, 0.21 mmol, 51% yield).

Compound **2c**. White plates, mp >177 °C [decomp. determined by thermal analysis (TGA and DTA)]. Found: C, 68.81; H, 5.71%. Calcd for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73%; exact FAB-MS (3-nitrobenzyl alcohol matrix), found: *m/z* 246.0906; calcd for $C_{14}H_{14}O_4$: M^+, *m/z* 246.0892. The relative intensity of the ^1H NMR signals

for the *meso* **2c**′ and the enantiomers **2c**″ showed a ratio of ca. 12:1.

Compound **2c**′. ^{1}H NMR (CD_3OD), δ 4.63 (2H, s, H-1,2), 6.68 (4H, brd ddd, J=8.6, 2.5, 1.0 Hz, H-3′,5′,3″,5″) and 7.04 (4H, brd ddd, J=8.6, 2.5, 1.0 Hz, H-2′,6′,2″,6″); ^{13}C NMR (CD_3OD), δ 157.7 (C-4′,4″), 133.7 (C-1′,1″), 129.8 (C-2′,6′,2″,6″), 115.5 (C-3′,5′,3″,5″) and 78.8 (C-1,2).

Compound **2c**″. ^{1}H NMR (CD_3OD), δ 4.50 (2H, s, H-1,2), 6.58 (4H, brd ddd, J=8.6, 2.5, 1.0 Hz, H-3′,5′,3″,5″) and 6.89 (4H, brd ddd, J=8.6, 2.5, 1.0 Hz, H-2′,6′,2″,6″); ^{13}C NMR (CD_3OD), δ 157.7 (C-4′,4″), 133.5 (C-1′,1″), 129.6 (C-2′,6′,2″,6″), 115.5 (C-3′,5′,3″,5″) and 80.1 (C-1,2).

3.1.12. Reaction of guaiazulene (1a) with 1,2-bis(4-hydroxyphenyl)-1,2-ethanediol (2c) in methanol in the presence of hydrochloric acid. To a solution of commercially available guaiazulene (**1a**) (18 mg, 91 μmol) in methanol (1.0 mL) was added a solution of 1,2-bis(4-hydroxyphenyl)-1,2-ethanediol (**2c**) (20 mg, 81 μmol) in methanol (1.5 mL) containing 36% hydrochloric acid (0.2 mL) at 60 °C. The mixture was stirred at 60 °C for 3 h under aerobic conditions. After the reaction, distilled-water (10 mL) was added to the mixture and then the mixture was extracted with diethyl ether (10 mL×2). The extract was washed with water, dried ($MgSO_4$) and evaporated in vacuo. The residue thus obtained was carefully separated by silica-gel column chromatography with hexane–ethyl acetate–benzene (7:2:1, vol/vol/vol) as an eluant. The starting material **1a** (5 mg, 25 μmol, 27%) was recovered. The crude product, 2-(3-guaiazulenyl)-1,1-bis(4-hydroxyphenyl)ethylene (**6**), thus obtained was recrystallized from benzene to provide pure **6** as stable crystals (24 mg, 59 μmol, 73% yield).

Compound **6**. Dark-green prisms, mp 123 °C [determined by thermal analysis (TGA and DTA)]. Found: C, 85.44; H, 6.73%. Calcd for $C_{29}H_{28}O_2$: C, 85.26; H, 6.91%; R_f=0.15 on silica-gel TLC (hexane–AcOEt–benzene=7:2:1, vol/vol/vol); UV–vis λ_{max} (CH_3CN) nm (log ε), 270 (4.42), 327 (4.34), 348 (4.25), 406 (4.18) and 636 (2.74); IR ν_{max} (KBr) cm^{-1}, 3395 (O–H), 2959, 2866 (C–H) and 1609, 1509 (aromatic C=C); exact EI-MS (70 eV), found: m/z 408.2078 (M^+, 100%); calcd for $C_{29}H_{28}O_2$: M^+, m/z 408.2089. ^{1}H NMR (C_6D_6), signals based on the 3-guaiazulenyl group at δ 1.16 (6H, d, J=6.9 Hz, (CH_3)$_2$CH-7‴), 2.29 (3H, brd s, Me-1‴), 2.69 (1H, sept, J=6.9 Hz, Me$_2$CH-7‴), 2.89 (3H, s, Me-4‴), 6.60 (1H, d, J=10.6 Hz, H-5‴), 7.00 (1H, dd, J=10.6, 2.0 Hz, H-6‴), 7.37 (1H, brd s, H-2‴), 7.89 (1H, d, J=2.0 Hz, H-8‴) and signals based on the 1,1-bis(4-hydroxyphenyl) groups at δ 3.95 (1H, s, OH-4′), 4.05 (1H, s, OH-4″), 6.41 (2H, ddd, J=8.6, 2.5, 1.0 Hz, H-3′,5′), 6.53 (2H, ddd, J=8.5, 2.5, 1.0 Hz, H-3″,5″), 7.24 (2H, ddd, J=8.5, 2.5, 1.0 Hz, H-2′,6′), 7.34 (2H, ddd, J=8.5, 2.5, 1.0 Hz, H-2″,6″) and a signal based on the >C=CH– unit at δ 7.69 (1H, s, H-2); ^{13}C NMR (C_6D_6), δ 155.7 (C-4″), 155.3 (C-4′), 146.2 (C-4‴), 140.3 (C-2‴), 140.2 (C-7‴), 139.7 (C-8a‴), 138.6 (C-1), 137.5 (C-1″), 135.4 (C-3a‴), 134.5 (C-6‴), 133.9 (C-1′), 133.2 (C-2′,6′), 133.1 (C-8‴), 129.6 (C-2″,6″), 127.1 (C-3‴), 126.5 (C-5‴), 125.4 (C-2), 125.1 (C-1‴), 115.4 (C-3′,5′), 115.3 (C-3″,5″), 37.9 (Me$_2$$C$H-7‴), 27.8 (Me-4‴), 24.5 ((CH_3)$_2$CH-7‴), 12.8 (Me-1‴).

3.1.13. Preparation of 1-[4-(dimethylamino)phenyl]-2-phenyl-1,2-ethanediol (2d). To a powder $NaBH_4$ (100 mg, 2.64 mmol) was added a solution of commercially available 4-(dimethylamino)benzoin (400 mg, 1.57 mmol) in ethanol (5 mL). The mixture was stirred at 25 °C for 1 h. After the reaction, distilled-water (15 mL) was added to the mixture and then the resulting product was extracted with diethyl ether (15 mL×2). The extract was dried ($MgSO_4$) and evaporated in vacuo. The pure product, 1-[4-(dimethylamino)phenyl]-2-phenyl-1,2-ethanediol (**2d**), was obtained as stable crystals (365 mg, 1.41 mmol, 90% yield).

Compound **2d**. White plates, mp 109 °C [determined by thermal analysis (TGA and DTA)]. Found: C, 74.74; H, 7.42; N, 5.43%. Calcd for $C_{16}H_{19}NO_2$: C, 74.68; H, 7.44; N, 5.44%; exact FAB-MS (3-nitrobenzyl alcohol matrix), found: m/z 257.1426; calcd for $C_{16}H_{19}NO_2$: M^+, m/z 257.1416. ^{1}H NMR (CD_3CN), δ 2.90 (6H, s, (CH_3)$_2$N-4′), 3.12 (1H, d, J=4.0 Hz, OH-2), 3.21 (1H, d, J=4.0 Hz, OH-1), 4.62 (1H, dd, J=6.0, 4.0 Hz, H-2), 4.70 (1H, dd, J=6.0, 4.0 Hz, H-1), 6.67 (2H, brd ddd, J=8.9, 2.5, 1.0 Hz, H-3′,5′), 7.09 (2H, brd ddd, J=8.9, 2.5, 1.0 Hz, H-2′,6′) and 7.22–7.31 (5H, m, protons for a phenyl group); ^{13}C NMR (CD_3CN), δ 151.3 (C-4′), 143.2 (C-1″), 130.3 (C-1′), 129.1 (C-2′,6′), 128.6 (C-2″,6″), 128.3 (C-3″,5″), 128.0 (C-4″), 112.8 (C-3′,5′), 78.5 (C-1), 78.3 (C-2) and 40.8 ((CH_3)$_2$N-4′).

3.1.14. Reaction of guaiazulene (1a) with 1-[4-(dimethylamino)phenyl]-2-phenyl-1,2-ethanediol (2d) in methanol in the presence of hydrochloric acid. To a solution of commercially available guaiazulene (**1a**) (46 mg, 232 μmol) in methanol (1.0 mL) was added a solution of 1-[4-(dimethylamino)phenyl]-2-phenyl-1,2-ethanediol (**2d**) (60 mg, 233 μmol) in methanol (2.0 mL) containing 36% hydrochloric acid (0.2 mL) at 60 °C. The mixture was stirred at 60 °C for 3 h under aerobic conditions. After the reaction, the reaction solution was carefully neutralized with aq. $NaHCO_3$ and then the mixture was extracted with diethyl ether (10 mL×2). The extract was washed with water, dried ($MgSO_4$) and evaporated in vacuo. The residue thus obtained was carefully separated by silica-gel column chromatography (several times) with hexane–benzene (6:4, vol/vol) as an eluant. The starting material **1a** (3 mg, 15 μmol, 7%) was recovered. The crude product, (*Z*)-2-[4-(dimethylamino)phenyl]-1-(3-guaiazulenyl)-1-phenylethylene (**7**), thus obtained was recrystallized from methanol–AcOEt (5:1, vol/vol) to provide pure **7** as stable crystals (17 mg, 40.5 μmol, 17% yield).

Compound **7**. Dark-green prisms, mp 166 °C [determined by thermal amalysis (TGA and DTA)]. Found: C, 88.62; H, 7.93; N, 3.35%. Calcd for $C_{31}H_{33}N$: C, 88.74; H, 7.93; N, 3.34%; R_f=0.15 on silica-gel TLC (hexane–benzene=6:4, vol/vol); UV–vis λ_{max} (CH_3CN) nm (log ε), 244 (4.30), 288 (4.45), 305sh (4.28), 349 (4.30), 372sh (4.13), 619 (2.61), 672sh (2.52) and 744sh (2.07); exact FAB-MS (3-nitrobenzyl alchol matrix), found: m/z 419.2620; calcd for $C_{31}H_{33}N$: M^+, m/z 419.2613. ^{1}H NMR (C_6D_6), signals based on the 3-guaiazulenyl group at δ 1.21 (6H, d, J=6.9 Hz,

($C\mathit{H}_3$)$_2$CH-7′), 2.59 (3H, brd s, Me-1′), 2.79 (1H, sept, J=6.9 Hz, (CH_3)$_2$CH-7′), 2.84 (3H, brd s, Me-4′), 6.67 (1H, brd d, J=10.9 Hz, H-5′), 7.10 (1H, brd dd, J=10.9, 2.0 Hz, H-6′), 7.60 (1H, brd s, H-2′), 8.25 (1H, d, J=2.0 Hz, H-8′) and signals based on the 4-(dimethylamino)phenyl group at δ 2.29 (6H, s, (CH_3)$_2$N-4‴), 6.20 (2H, ddd, J=9.0, 2.5, 1.0 Hz, H-3‴,5‴), 7.039 (2H, ddd, J=9.0, 2.5, 1.0 Hz, H-2‴,6‴) and signals based on the phenyl group at δ 7.040 (1H, brd dddd, J=7.7, 7.7, 1.5, 1.5 Hz, H-4″), 7.116, 7.119 (1H each, brd ddd, J=7.7, 7.7, 1.5 Hz, H-3″,5″), 7.400, 7.413 (1H each, brd ddd, J=7.7, 1.5, 1.5 Hz, H-2″,6″), and a signal based on the –HC═C< unit at δ 7.40 (1H, s, H-2); ^{13}C NMR (C_6D_6), δ 149.4 (C-4‴), 146.9 (C-4′), 145.8 (C-1″), 141.0 (C-2′), 139.4 (C-7′), 138.6 (C-8a′), 136.8 (C-1), 135.0 (C-6′), 134.4 (C-3a′), 133.4 (C-8′), 130.8 (C-2‴,6‴), 129.4 (C-2), 128.5 (C-3″,5″), 127.5 (C-3′), 127.2 (C-1‴), 126.9 (C-2″,6″), 126.8 (C-5′), 126.5 (C-4″), 125.9 (C-1′), 112.4 (C-3‴,5‴), 39.7 ((CH_3)$_2$N-4‴), 38.1 ((CH_3)$_2$CH-7′), 25.6 (Me-4′), 24.7 (($\mathit{C}H_3$)$_2$CH-7′) and 13.1 (Me-1′).

3.1.15. X-ray crystal structure of (*Z*)-2-[4-(dimethylamino)phenyl]-1-(3-guaiazulenyl)-1-phenylethylene (7). A total 5981 reflections with $2\theta_{max}$=55.0° were collected on a Rigaku AFC-5R automated four-circle diffractometer with graphite monochromated Mo-$K\alpha$ radiation (λ=0.71069 Å, rotating anode: 50 kV, 180 mA) at 296 K. The structure was solved by direct methods (SIR97) and expanded using Fourier techniques (DIRDIF94). The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on F^2. All calculations were performed using the teXsan crystallographic software package. CCDC No.: 228482.

Crystallographic data for **7**: $C_{31}H_{33}N$ (FW=419.61), dark-green prism [from ethyl acetate–methanol=1:5 (vol/vol), the crystal size, 0.40×0.40×0.60 mm^3], triclinic, $P-1$ (#2), a=10.368(4) Å, b=13.765(4) Å, c=9.083(4) Å, α=101.98(3)°, β=101.38(3)°, γ=95.14(3)°, V=1231.5(8) Å^3, Z=2, D_{calcd}=1.132 g/cm^3, μ(Mo-$K\alpha$)=0.64 cm^{-1}, scan width=(1.47+0.30 tan θ)°, scan mode=$\omega-2\theta$, scan rate=8.0°/min, measured reflections=5981, observed reflections=5670, no. of parameters=289, $R1$=0.064, $wR2$=0.202 and goodness of fit indicator=1.58.

3.1.16. Preparation of α,α′-bis(3-guaiazulenylmethylium) bis(tetrafluoroborate) (8). To a solution of commercially available guaiazulene (**1a**) (357 mg, 1.8 mmol) in acetic acid (3 mL) was added a solution of commercially available glyoxal (40% aqueous solution, 70 μL, ca. 0.6 mmol) in acetic acid (4 mL) containing tetrafluoroboric acid (42% aqueous solution, 0.3 mL). The mixture was stirred at 25 °C for 1 h under aerobic conditions, giving a precipitation of a dark-purple solid of **8**, and then was centrifuged at 2.5 krpm for 1 min. The crude product thus obtained was carefully washed with diethyl ether, and was recrystallized from acetonitrile–diethyl ether (1:5, vol/vol) (several times) to provide pure **8** as stable crystals (350 mg, 0.59 mmol, 98% yield).

Compound **8**. Dark-purple plates, mp >160 °C [decomp., determined by thermal analysis (TGA and DTA)]. Found: C, 64.87; H, 5.99%. Calcd for $C_{32}H_{36}B_2F_8$: C, 64.68; H, 6.11%; UV–vis λ_{max} (CF_3COOH) nm (log ε), 255 (4.63), 318 (4.29), 407 (4.39), 432sh (4.41), 466 (4.51) and 526 (4.64); IR ν_{max} (KBr) cm^{-1}, 1056 and 520 (BF_4^-); exact FAB-MS (3-nitrobenzyl alcohol matrix), found: m/z 420.2814; calcd for $C_{32}H_{36}$: [M−2BF_4]$^+$, m/z 420.2817. ^{1}H NMR (CF_3COOD), signals based on the α,α′-bis(3-guaiazulenylmethylium) moiety with a delocalized π-electron system at δ 1.38 (12H, d, J=7.0 Hz, ($C\mathit{H}_3$)$_2$CH-7,7′), 2.43 (6H, s, Me-1,1′), 3.34 (6H, s, Me-4,4′), 3.35 (2H, sept, J=7.0 Hz, Me$_2$CH-7,7′), 7.88 (2H, brd s, H-2,2′), 8.30 (2H, dd, J=11.0, 2.0 Hz, H-6,6′), 8.47 (2H, d, J=2.0 Hz, H-8,8′), 8.48 (2H, d, J=11.0 Hz, H-5,5′) and 8.73 (2H, brd s, HC$^+$-α,α′); ^{13}C NMR (CF_3COOD), δ 177.6 (C-7,7′), 164.5 (C-8a,8a′), 159.6 (C-4,4′), 153.8 (C-3a,3a′), 153.2 (C-5,5′), 150.7 (C-1,1′), 150.6 (C-3,3′), 146.2 (C-6,6′), 139.6 (C-8,8′), 138.4 (HC$^+$-α,α′), 138.0 (C-2,2′), 41.7 (Me$_2\mathit{C}$H-7,7′), 28.9 (Me-4,4′), 23.4 (($\mathit{C}H_3$)$_2$CH-7,7′) and 13.6 (Me-1,1′).

3.1.17. Preparation of (*E*)-1,2-di(3-guaiazulenyl)ethylene (9). To a solution of **8** (80 mg, 134.6 μmol) in trifluoroacetic acid (2 mL) was added a zinc powder (440 mg, 6.73 mmol) under argon. The mixture was stirred at 0 °C for 5 min under argon. After the reaction, the zinc powder was removed by using a centrifugal separator. The reaction solution was carefully neutralized with aq. $NaHCO_3$ and then the resulting product was extracted with hexane (20 mL×2). The extract was washed with water, dried ($MgSO_4$) and evaporated in vacuo, giving a dark-green solid. The crude product thus obtained was recrystallized from CH_2Cl_2–hexane (1:5, vol/vol) (several times) to provide pure **9** as stable single crystals (53 mg, 126.0 μmol, 94% yield).

Compound **9**. Dark-green plates, mp 226 °C [determined by thermal analysis (TGA and DTA)] (lit.[10] 219–220 °C). Found: C, 91.74; H, 8.97%. Calcd for $C_{32}H_{36}$: C, 91.37; H, 8.63%; R_f=0.39 on silica-gel TLC (hexane–AcOEt–benzene=90:5:5, vol/vol/vol); UV–vis λ_{max} (CH_3CN) nm (log ε), 232 (4.58), 264 (4.62), 329 (4.60), 454 (4.68), 480sh (4.59) and 661 (3.13); IR ν_{max} (KBr) cm^{-1}, 2954 and 948 (*trans*-CH═CH–); MALDI-TOF-MS (without any matrix reagent), m/z 420 (M$^+$, 100%); exact EI-MS (70 eV), found: m/z 420.2832 (100%); calcd for $C_{32}H_{36}$: M$^+$, m/z 420.2817. ^{1}H NMR (C_6D_6), signals based on the two 3-guaiazulenyl groups at δ 1.20 (12H, d, J=7.0 Hz, ($C\mathit{H}_3$)$_2$CH-7′,7″), 2.57 (6H, brd s, Me-1′,1″), 2.73 (2H, sept, J=7.0 Hz, Me$_2$CH-7′, 7″), 2.93 (6H, s, Me-4′,4″), 6.56 (2H, d, J=11.0 Hz, H-5′,5″), 6.99 (2H, dd, J=11.0, 2.0 Hz, H-6′,6″), 7.97 (2H, d, J=2.0 Hz, H-8′,8″), 8.14 (2H, brd s, H-2′,2″) and a signal based on the –CH═CH– unit at δ 8.12 (2H, s, H-1,2); ^{13}C NMR (C_6D_6), δ 146.3 (C-4′,4″), 141.2 (C-8a′,8a″), 140.0 (C-7′,7″), 136.1 (C-2′,2″), 134.6 (C-6′,6″), 133.2 (C-8′,8″), 132.5 (C-3a′,3a″), 128.8 (C-3′,3″), 126.8 (C-5′,5″), 126.1 (C-1′,1″), 125.2 (C-1,2), 37.7 (Me$_2\mathit{C}$H-7′,7″), 28.3 (Me-4′,4″), 24.2 (($\mathit{C}H_3$)$_2$CH-7′,7″) and 12.9 (Me-1′,1″).

3.1.18. X-ray crystal structure of (*E*)-1,2-di(3-guaiazulenyl)ethylene (9). A total 3234 reflections with $2\theta_{max}$=55.0° were collected on a Rigaku AFC-5R automated four-circle diffractometer with graphite monochromated Mo-$K\alpha$ radiation (λ=0.71069 Å, rotating

anode: 50 kV, 180 mA) at 296 K. The structure was solved by direct methods (SIR97) and expanded using Fourier techniques (DIRDIF94). The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on F^2. All calculations were performed using the teXsan crystallographic software package. CCDC No.: 203374.

Crystallographic data for **9**: $C_{32}H_{36}$ (FW=420.64), dark-green plate (the crystal size, 0.30×0.10×0.60 mm^3), monoclinic, *C*2/*c* (#15), *a*=38.874(10) Å, *b*=5.33(1) Å, *c*=12.677(7) Å, β=107.29(4)°, *V*=2509(5) $Å^3$, *Z*=4, D_{calcd}=1.113 g/cm^3, μ(Mo-*K*α)=0.62 cm^{-1}, scan width=(1.42+0.30 tan θ)°, scan mode=ω−2θ, scan rate=8.0°/min, measured reflections=3234, observed reflections=1564, no. of parameters=145, *R*1=0.089, *wR*2=0.242 and goodness of fit indicator=1.88.

3.1.19. Reaction of 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene (3) with *N*-chlorosuccinimide (NCS). To a solution of commercially available *N*-chlorosuccinimide (NCS) (16 mg, 120 μmol) in hexane (1.0 mL) was added a solution of 2-(3-guaiazulenyl)-1,1-bis(4-methoxyphenyl)ethylene (**3**) (52 mg, 119 μmol) in hexane (3.0 mL) containing chloroform (1.0 mL). The mixture was stirred at 25 °C for 24 h under argon and then evaporated in vacuo. The residue thus obtained was carefully separated by silica-gel column chromatography with hexane–ethyl acetate–benzene (5:4:1, vol/vol/vol) as an eluant. The starting material **3** (42 mg, 96 μmol, 81%) was recovered. The crude product, 1,1-bis(4-methoxyphenyl)-2-{3-[5-(succinimidyl)guaiazulenyl]}ethylene (**16**), thus obtained was recrystallized from methanol to provide pure **16** as stable crystals (6 mg, 12 μmol, 10% yield). Similarly, the reaction of **3** with NCS at 60 °C for 24 h under argon gave the same result as the above reaction at 25 °C.

Compound **16**. Dark-green prisms, mp 157 °C [determined by thrmal analysis (TGA and DTA)]. Found: C, 78.21; H, 6.82; N, 2.57%. Calcd for $C_{35}H_{35}NO_4$: C, 78.77; H, 6.61; N, 2.62%; R_f=0.20 on silica-gel TLC (hexane–AcOEt–benzene=5:4:1, vol/vol/vol); UV–vis λ_{max} (CH_3CN) nm (log ε), 273 (4.52), 332 (4.41), 357 (4.33), 410 (4.24) and 642 (2.54); IR ν_{max} (KBr) cm^{-1}; 2959, 2835 (C–H), 1713 (C=O), 1605, 1508 (aromatic C=C) and 1246, 1030 (C–O); exact EI-MS (70 eV), found: *m*/*z* 533.2571 (M^+, 100%); calcd for $C_{35}H_{35}NO_4$: M^+, *m*/*z* 533.2566. ^{1}H NMR (C_6D_6), signals based on the 3-[5-(succinimidyl)guaiazulenyl] group at δ 1.12 (6H, d, *J*=6.9 Hz, $(CH_3)_2$CH-7‴), 1.90–2.05 (4H, m, H-3a,b⁗,4a,b⁗), 2.26 (3H, s, Me-1‴), 2.67 (1H, sept, *J*=6.9 Hz, Me_2CH-7‴), 2.77 (3H, s, Me-4‴), 7.09 (1H, d, *J*=1.7 Hz, H-6‴), 7.37 (1H, brd s, H-2‴), 7.81 (1H, d, *J*=1.7 Hz, H-8‴) and signals based on the 1,1-bis(4-methoxyphenyl) groups at δ 3.25 (3H, s, MeO-4′), 3.32 (3H, s, MeO-4″), 6.74 (2H, ddd, *J*=8.6, 2.5, 1.0 Hz, H-3′,5′), 6.80 (2H, ddd, *J*=8.6, 2.5, 1.0 Hz, H-3″,5″), 7.374 (2H, ddd, *J*=8.6, 2.5, 1.0 Hz, H-2′,6′), 7.374 (2H, ddd, *J*=8.6, 2.5, 1.0 Hz, H-2″,6″) and a signal based on the >C=C*H*– unit at δ 7.53 (1H, s, H-2); ^{13}C NMR (C_6D_6), δ 175.7 (C-2⁗,5⁗), 159.7 (C-4″), 159.3 (C-4′), 144.4 (C-4‴), 141.1 (C-8a‴), 141.0 (C-2‴), 139.6 (C-7‴), 138.8 (C-1), 137.2 (C-1″), 135.4 (C-6‴), 133.72 (C-3a‴), 133.66 (C-1′), 133.2 (C-2′,6′), 133.0 (C-8‴), 130.7 (C-3‴), 129.7 (C-2″,6″), 126.4 (C-1‴), 125.77 (C-5‴), 125.71 (C-2), 114.11 (C-3′,5′), 114.05 (C-3″,5″), 54.8 (MeO-4″), 54.6 (MeO-4′), 38.1 (Me_2CH-7‴), 28.4 (C-3⁗,4⁗), 24.2 (($CH_3)_2$CH-7‴), 21.9 (Me-4‴) and 12.8 (Me-1‴).

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13. **1a**: UV–vis λ_{max} (CH_3CN) nm (log ε), 213 (4.10), 244 (4.39), 284 (4.61), 301sh (4.03), 348 (3.65), 365 (3.46), 600 (2.68), 648sh (2.61) and 721sh (2.20). ^{1}H NMR (C_6D_6), (1.22 (6H, d, *J*=7.0 Hz, $(CH_3)_2$CH-7), 2.615 (3H, s, Me-4), 2.624 (3H, brd s, Me-1), 2.83 (1H, sept, *J*=7.0 Hz, Me_2CH-7), 6.79 (1H, d, *J*=11.0 Hz, H-5), 7.22 (1H, dd, *J*=11.0, 2.0 Hz, H-6), 7.31 (1H, d, *J*=4.0 Hz, H-3), 7.69 (1H, d, *J*=4.0 Hz, H-2) and 8.22 (1H, d, *J*=2.0 Hz, H-8); ^{13}C NMR (C_6D_6), (144.1 (C-4), 139.7 (C-7), 138.1 (C-8a), 137.2 (C-3a), 136.7 (C-2), 134.6 (C-6), 133.2 (C-8), 125.4 (C-1), 125.2 (C-5), 113.5 (C-3), 38.5 (Me_2CH-7), 24.8 (($CH_3)_2$CH-7), 24.0 (Me-4) and 13.0 (Me-1); DPV (E_p), +0.65 and −1.77 V; CV, +0.69 (E_{pa}) and −1.79 ($E_{1/2}$) V under the same electrochemical conditions as **3**.
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Synthesis of a natural insect repellent isolated from thrips

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Abstract—A convenient, high yield procedure for the synthesis of (11*Z*)-11,19-eicosadienyl acetate (**1**) has been developed. This compound shows strong repellent activity against ants.

1. Introduction

The principal approach to prevention of vector-borne diseases is avoidance. Although vaccines or chemoprophylactic drugs are available against important vector-borne diseases such as yellow fever and malaria, the use of repellents against biting arthropods is highly recommended. In addition, fire-ants, leaf-cutter-ants, termites and other insects are very serious pests[1] in the tropics causing severe damage both to crops and reforestation.

For personal protection most authorities recommend repellents containing N,N-diethyl-*m*-toluamide (DEET)[2,3] whereas the use of permethrin-containing repellents is limited to clothing, shoes and gear. Due to several limitations due to toxic side effects,[4] so-called 'natural repellents' have been in the focus of interest[5–7] for quite a long time and they can be regarded as an alternative to several repellents used up to now.

2. Results and discussion

During our own efforts in the development of insect repellent compounds[8] we became interested in the composition of the low volatile defensive secretion excreted by *Suocerathrips linguis* (Thysanoptera, Phlaeothripidae)[9]—small insect living on leaves of *Sansevieria* plants.

GC-MS analysis of this defensive secretion against ants revealed the presence of a multi-component mixture containing several well-known acetates of long chain (mono-un)-saturated (C16···C20) alcohols. Additionally an hitherto unknown compound of m/z=336 was detected. From the presence of a m/z=61 and a very small m/z=276 as well as from the results of an in situ dimethyl disulfide derivatisation[8] a (11*Z*)-11,19-eicosadienyl acetate structure (**1**) for this compound seemed most likely. Although a direct determination of the absolute configuration of the internal double bond from the GC-MS data was not possible, we assigned a (11*Z*) configuration to **1** since (*E*)-isomers are only scarcely found in insect allomones and pheromones. In order to prove these assumptions as well as to investigate its repellent activity against ants a straightforward synthesis of **1** was called for.

Retrosynthetic analysis revealed a suitably protected 11-dodecynol as an ideal starting material. Thus, 10-bromo-decanol (**2**) was protected as its tetrahydropyranyl acetal by treatment of **2** with 3,4-dihydro-2*H*-pyran (DHP) in the presence of catalytic amounts of pyridinium *p*-toluene sulfonate (PPTS)[10,11] to afford **3** in 80% isolated yield. Reaction of **3** with lithium acetylide ethylenediamine complex[12] gave 79% of **4** that was allowed to react with 8-chloro-1-octene (**5**) in the presence of *n*-BuLi to yield the 19-icosen-11-ynyl-tetrahydropyranyl acetal **6**. Compound **5** was easily accessed from commercially available 1,5-dichloro-pentane (**7**) and allylmagnesium chloride[13,14] in the presence of LiCl and $CuCl_2$.

The deprotection of **6** with an ion exchange resin in methanol proceeded very smoothly and gave 19-icosen-11-yn-1-ol (**8**) in almost quantitative yield. Acetylation of **8** gave 98% of the corresponding acetate **9** that was subjected to a partial hydrogenolysis using the *Lindlar* catalyst in hexane containing quinoline. Chromatographic work-up finally gave 92% of the target compound **1**.

The GC-MS spectra of synthesised **1** were identical to the compound obtained from *S. linguis* in every aspect. In order

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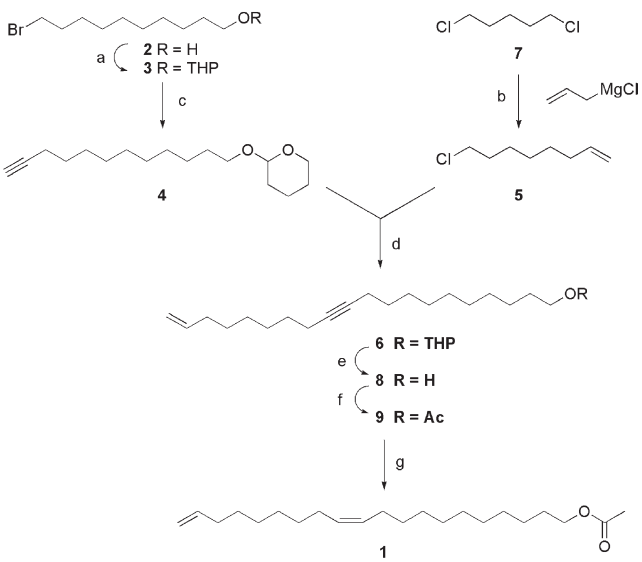
to prove the anti-repellent activity of **1** a bio-assay was performed using colonies of the ant *Myrmica rubra*. Evaluation of these data reveals, that a 10% solution of (11*Z*)-**1** retreats >85–90% of the ants looking for prey. Thus this assay showed **1** as an excellent repellent since the food protected by **1** was left nearly untouched by the predators.

It seems reasonable to assume that **1** spreads fast on surfaces and in predators like ants and mites—using mainly olfactory sense—a coating of sensory organs will make these insects 'blind'. In consequence, the ants are not longer able to find their prey; additionally, it can be expected that they get serious problems to evaluate their own trail pheromones.

The synthesis of analogues and their biological screening against a variety of insects is presently under investigation in our laboratories (Scheme 1).



Scheme 1. (a) DHP, PPTA, CH_2Cl_2; (b) LiCl, $CuCl_2$, THF; (c) lithium-acetylide, DMSO; (d) *n*-BuLi, THF/HMPT; (e) Amberlyst15 (H^+-form), methanol; (f) Ac_2O/pyridine; (g) *Lindlar*-catalyst, H_2, hexane.

3. Experimental

3.1. General

Melting points are uncorrected (*Leica* hot stage microscope), optical rotations were obtained using a Perkin–Elmer 341 polarimeter (1 cm micro cell), NMR spectra were recorded using the Varian spectrometers Gemini 200, Gemini 2000 or Unity 500 (δ given in ppm, *J* in Hz, internal Me_4Si or internal CCl_3F), IR spectra (film or KBr pellet) on a Perkin–Elmer FT-IR spectrometer Spectrum 1000, MS spectra were taken on a Intectra GmbH AMD 402 (electron impact, 70 eV) or on a Finnigan MAT TSQ 7000 (electrospray, voltage 4.5 kV, sheath gas nitrogen) instrument; for elemental analysis a Foss-Heraeus Vario EL instrument was used; TLC was performed on silica gel (Merck 5554, detection by UV absorption or by treatment with a solution of 10% sulfuric acid, ammonium molybdate and cerium$^{(IV)}$) sulfate followed by gentle heating. The solvents were dried according to usual procedures.

3.1.1. 10-Bromodecyltetrahydro-2*H*-2-pyranyl ether (3). A solution of 10-bromo-1-decanol (**2**) (3.0 g, 11.39 mmol) containing DHP (1.43 g, 17.0 mmol) and PPTA (28 mg, 0.11 mmol) in dry dichloromethane (50 ml) was stirred for 2 days at room temperature, then an aq. solution of Na_2CO_3 (2 M, 11 ml) was added, the layers were separated and the organic phase was dried (K_2CO_3). The solvents were removed and the residue purified by chromatography (silica gel, hexane/ethyl acetate 9:1) to afford **3** (2.92 g, 80%) as a colourless liquid. R_f (hexane/ethyl acetate 9:1)=0.5; IR (film): ν=2927s, 2854s, 1465m, 1455m, 1440m, 1383m, 1365m, 1352m, 1322m, 1260m, 1200s, 1184m, 1163m, 1136s, 1120s, 1078s, 1034s, 988m, 905m, 869m, 815m, 722m, 646m, 564w cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ=1.25–1.35 (m, 10H, C*H*$_2$), 1.40 (m, 2H, C*H*$_2$), 1.45–1.60 (m, 6H, C*H*$_2$), 1.70 (m, 1H, C*H*$_2$), 1.80 (m, 3H, C*H*$_2$–CH$_2$Br, C*H*$_2$, THP), 3.35 (m, 3H, C*H*$_2$Br, C*H*$_2$–O), 3.45 (m, 1H, C*H*$_2$, THP), 3.70 (ddd, $^2J_{H,H}$=9.5 Hz, $^3J_{H,H}$=6.84 Hz, $^2J_{H,H}$=6.84 Hz, 1H, C*H*$_2$), 3.85 (m, 1H, C*H*$_2$, THP), 4.55 (dd, $^3J_{H,H}$=4.35 Hz, $^3J_{H,H}$=2.48 Hz, 1H, C*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ=19.8 (*C*H$_2$, THP), 25.6 (*C*H$_2$, THP), 26.2 (*C*H$_2$), 28.2 (*C*H$_2$), 28.8 (*C*H$_2$), 29.4 (*C*H$_2$), 29.5 (*C*H$_2$), 29.5 (*C*H$_2$), 29.8 (*C*H$_2$), 30.9 (*C*H$_2$, THP), 32.9 (*C*H$_2$–CH$_2$Br), 34.0 (*C*H$_2$–Br), 62.3 (*C*H$_2$–O, THP), 67.7 (*C*H$_2$–O), 98.8 (*C*H); MS (GC-MS, e.i., 70 eV): *m*/*z* (%)=85 (100), 101 (8), 115 (2), 137 (2), 163 (1), 190 (1), 219 (1), 241 (1), 247 (2), 267 (1), 292 (1), 319 (3); HRMS for $C_{15}H_{29}BrO_2$: calcd 320.13509; found: 320.13511. Anal. calcd for $C_{15}H_{29}BrO_2$ (321.29): C, 56.07; H, 9.10; found: C, 55.87; H, 9.21.

3.1.2. 11-Dodecynyltetrahydro-2*H*-2-pyranyl ether (4). To a suspension of the lithium acetylide ethylendiamine complex (2.9 g, 32 mmol) in dry DMSO (17 ml) at 15–20 °C within 2 h a solution of **3** (4.9 g, 15.27 mmol) in dry DMSO) (17 ml) was slowly added and stirring was continued for 12 h. The reaction was quenched by the addition of water (15 ml), hexane (15 ml) and again water (15 ml). The aq. layer was extracted with hexane (3×100 ml), the combined organic phases washed with brine (3×25 ml), dried (Na_2SO_4), the solvents were removed under diminished pressure and the residue purified by chromatography (silica gel, hexane/ethyl acetate 98:2) to yield **4** (3.19 g, 79%) as a colourless liquid. R_f (hexane/ethyl acetate 9:1)=0.5; IR (film): ν=3312m, 2929s, 2855s, 2118w, 1465s, 1455s, 1441m, 1384m, 1136s, 1121s, 1079s, 1034s, 990s, 906m, 869m, 844w, 815m, 722m, 628m cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ=1.20–1.40 (m, 13H, C*H*$_2$), 1.55 (m, 8H, C*H*$_2$), 1.75 (m, 1H, C*H*$_2$), 1.90 (dd, $^4J_{H,H}$=2.69 Hz, $^4J_{H,H}$=2.49 Hz, 1H, C*H*), 2.15 (m, 2H, C*H*$_2$–C≡C), 3.35 (m, 1H, C*H*$_2$–O), 3.45 (m, 1H, C*H*$_2$–O, THP), 3.70 (ddd, $^2J_{H,H}$=9.53 Hz, $^3J_{H,H}$=6.84 Hz, $^3J_{H,H}$=6.84 Hz, 1H, C*H*$_2$–O), 3.85 (m, 1H, C*H*$_2$, THP), 4.55 (dd, $^3J_{H,H}$=4.57 Hz, $^3J_{H,H}$=2.49 Hz, 1H, O–C*H*–O, THP); ^{13}C NMR (100 MHz, $CDCl_3$): δ=18.5 (*C*H$_2$–C≡C), 19.8 (*C*H$_2$, THP), 25.6 (*C*H$_2$, THP), 26.3 (*C*H$_2$), 28.6 (*C*H$_2$), 28.8 (*C*H$_2$), 29.1 (*C*H$_2$), 29.5 (*C*H$_2$), 29.5 (*C*H$_2$), 29.6 (*C*H$_2$), 29.8 (*C*H$_2$), 30.9 (*C*H$_2$, THP), 62.3 (*C*H$_2$–O, THP), 67.7 (*C*H$_2$–O), 68.0 (*C*H≡C), 84.7 (*C*≡C), 98.8 (O–*C*H–O, THP); MS (GC-MS, e.i., 70 eV): *m*/*z* (%)=85 (100), 101 (28), 115 (4), 135 (1), 165 (1), 195 (1), 225 (1), 265 (1), 266 (1); HRMS for $C_{17}H_{30}O_2$: calcd 266.22458; found: 266.22459. Anal. calcd for $C_{17}H_{30}O_2$ (266.42): C, 76.64; H, 11.35; found: C, 76.52; H, 11.51.

3.1.3. 8-Chloro-1-octene (5). A mixture of $CuCl_2$ (1.9 g, 14.06 mmol) and LiCl (1.2 g, 28.04 mmol) was stirred in abs. THF (100 ml) at room temperature overnight. 1,5-dichloropentane (10.0 g, 70.98 mmol) in dry THF (30 ml) was added to this deep red solution at −15 °C and stirred at this temperature for another 3 h. Then a solution of allylmagnesium chloride (106 mmol, 2 M in THF) was added, the mixture was allowed to warm to room temperature and stirred overnight. Then aq. hydrochloric acid (1 M, 100 ml) was added, the aq. phase extracted with ether (3×100 ml) and the combined organic phases were dried (Na_2SO_4), die solvents removed and the residue was subjected to chromatography (silica gel, hexane) to afford **5** (3.0 g, 29%) as a colourless liquid. R_f (hexane)=0.69; IR (film): ν=3077m, 2931s, 2857s, 1641m, 1445m, 1308m, 994m, 911m, 728m cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ=1.30–1.50 (m, 6H, CH_2), 1.75 (m, 2H, CH_2), 2.05 (m, 2H, CH_2), 3.50 (t, $^3J_{H,H}$=6.7 Hz, 2H, CH_2–Cl), 4.90 (m, 1H, CH_2=C), 4.95 (m, 1H, CH_2=C), 5.80 (m, 1H, C*H*=*C*); ^{13}C NMR (100 MHz, $CDCl_3$): δ=26.8 (*C*H_2), 28.4 (*C*H_2), 28.8 (*C*H_2), 32.6 (*C*H_2), 33.7 (*C*H_2), 45.1 (*C*H_2–Cl), 114.3 (*C*H_2=C), 138.8 (*C*H=C); MS (GC-MS, e.i., 70 eV): *m*/*z* (%)=41 (100), 54 (16), 55 (56), 68 (39), 82 (9), 83 (5), 95 (2), 104 (20), 118 (3), 146 (1); HRMS for $C_9H_{15}Cl$: calcd 146.08623; found: 146.08625. Anal. calcd for $C_9H_{15}Cl$ (146.66): C, 65.52; H, 10.31; found: C, 65.47; H, 10.55.

3.1.4. 19-Icosen-11-ynyltetrahydro-2*H*-2-pyranyl ether (6). To a −10 °C cold solution of **4** (0.8 g, 3.0 mmol) in abs. THF (15 ml) a solution of butyllithium (3.15 mmol, 1.6 M in hexane) was slowly added, stirring at that temperature was continued for 1 h and a solution of **5** (3.5 g, 3.15 mmol) in HMPT (8 ml) was slowly added at −18 °C. The mixture was allowed to warm to room temperature and stirring was continued for another 12 h, then the reaction was stopped by the addition of water (11 ml). The phases were separated, the aq. phase was extracted with hexane (3×100 ml), the combined organic layers were washed with water (2×20 ml) and brine (15 ml) and dried (Na_2SO_4). After evaporation of the solvents the residue was purified by chromatography (silica gel, hexane/ethyl acetate 98:2) to yield **6** (0.64 g, 57%) as a colourless liquid. R_f (hexane/ethyl acetate 9:1)=0.6; IR (film): ν=3333w, 3076w, 2926s, 2855s, 2360w, 1737w, 1676w, 1640m, 1465m, 1440m, 1352m, 1323m, 1284w, 1260m, 1200s, 1184m, 1137s, 1079s, 1034s, 992m, 908s, 869m, 815m cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ=1.20–1.60 (m, 28H, CH_2), 1.70 (m, 1H, CH_2), 1.80 (m, 1H, CH_2), 2.0 (dd, $^3J_{H,H}$=6.84 Hz, $^3J_{H,H}$=6.84 Hz, 2H, CH_2–C=C), 2.15 (m, 4H, CH_2–C≡C), 3.35 (ddd, $^2J_{H,H}$=9.53 Hz, $^3J_{H,H}$=6.63 Hz, $^3J_{H,H}$=6.63 Hz, 1H, CH_2–O), 3.45 (m, 1H, CH_2–O, THP), 3.70 (ddd, $^2J_{H,H}$=9.51 Hz, $^3J_{H,H}$=6.83 Hz, $^3J_{H,H}$=6.84 Hz, 1H, CH_2–O), 3.85 (m, 1H, CH_2–O, THP), 4.55 (dd, THP, $^3J_{H,H}$=4.35 Hz, $^3J_{H,H}$=2.69 Hz, 1H, O–C*H*–O), 4.90 (m, 1H, CH_2=C), 4.95 (m, 1H, CH_2=C) 5.80 (m, 1H, C*H*=C); ^{13}C NMR (100 MHz, $CDCl_3$): δ=18.8 (*C*H_2), 18.9 (*C*H_2), 19.8 (*C*H_2), 25.6 (*C*H_2), 26.3 (*C*H_2), 28.7 (*C*H_2), 28.7 (*C*H_2), 28.8 (*C*H_2), 28.9 (*C*H_2), 29.1 (*C*H_2), 29.2 (*C*H_2), 29.3 (*C*H_2), 29.4 (*C*H_2), 29.5 (*C*H_2), 29.6 (*C*H_2), 29.8 (*C*H_2), 30.8 (*C*H_2), 33.8 (*C*H_2), 62.3 (*C*H_2–O, THP), 67.7 (*C*H_2–O), 80.1 (*C*≡C), 80.3 (*C*≡C), 98.8 (*C*H, THP), 114.1 (*C*H_2=C), 139.0 (*C*H=C); MS (GC-MS, e.i., 70 eV): *m*/*z* (%)=85 (100), 101 (25), 109 (7), 135 (7), 149 (2), 163 (1), 177 (1), 189 (1), 219 (1), 221 (1%), 247 (1), 265 (2), 279 (1), 303 (2), 305 (1); HRMS for $C_{25}H_{44}O_2$: calcd 376.3341; found: 376.3342. Anal. calcd for $C_{25}H_{44}O_2$ (376.62): C, 79.73; H, 11.78; found: C, 79.56; H, 11.85.

3.1.5. 19-Icosen-11-yn-1-ol (8). A solution of **6** (0.55 g, 1.46 mmol) in methanol (15 ml) was stirred with ion exchange resin (Amberlyst 15, H^+-form, 0.5 g) for 2 days. The resin was filtered off and the filtrate was evaporated followed by a chromatographic purification (silica gel, hexane/ethyl acetate 9:1) to afford **8** (0.39 g, 91%) as a greasy solid. R_f (hexane/ethyl acetate 9:1)=0.1; IR (film): ν=3331s, 3078m, 2927s, 2854s, 1829w, 1642m, 1461m, 1436m, 1335m, 1288m, 1262m, 1224w, 1190w, 1133m, 1060m, 1042m, 1024m, 994m, 969m, 910s cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ=1.20–1.40 (m, 19H, CH_2), 1.45 (m, 4H, CH_2), 1.55 (m, 2H, CH_2), 2.0 (dd, $^3J_{H,H}$=6.84 Hz, $^3J_{H,H}$=6.84 Hz, 2H, CH_2–C=C), 2.15 (m, 4H, CH_2–C≡C), 3.60 (t, $^3J_{H,H}$=6.63 Hz, 2H, CH_2–OH), 4.90 (m, 1H, CH_2=C), 4.95 (m, 1H, CH_2=C), 5.8 (m, 1H, C*H*=C); ^{13}C NMR (100 MHz, $CDCl_3$): δ=18.8 (*C*H_2, 2C), 25.8 (*C*H_2), 28.6 (*C*H_2), 28.7 (*C*H_2), 28.8 (*C*H_2), 28.9 (*C*H_2), 29.2 (3×*C*H_2), 29.5 (2×*C*H_2), 29.6 (*C*H_2), 32.9 (*C*H_2), 33.8 (*C*H_2), 63.1 (*C*H_2–OH), 80.2 (*C*≡C), 80.3 (*C*≡C), 114.1 (*C*H_2=C), 139.0 (*C*H=C); MS (GC-MS, e.i., 70 eV): *m*/*z* (%)=67 (100), 95 (63), 121 (39), 135 (60), 150 (15), 163 (4), 191 (1), 210 (1), 235 (1), 249 (1), 263 (1), 292 (1); HRMS for $C_{20}H_{36}O$: calcd 292.2766; found: 292.2766. Anal. calcd for $C_{20}H_{36}O$ (292.50): C, 82.12; H, 12.41; C, 81.93; H, 12.55.

3.1.6. 19-Icosen-11-ynyl acetate (9). To a solution of **8** (1.3 g, 4.45 mmol) in dry pyridine (8.2 ml) acetic anhydride (4.3 ml) was added and the mixture was stirred for 3.5 h, then the volatiles were removed under diminished pressure. The residue was suspended in hexane (100 ml) and washed with water (3×100 ml) and the solvents were evaporated to afford **9** (1.45 g, 98%) as a colourless liquid. R_f (hexane/ethyl acetate 9:1)=0.65; IR (film): ν=3076w, 2929s, 2856s, 1743s, 1641w, 1465m, 1437m, 1387m, 1365m, 1332w, 1238s, 1039m, 995w, 910m cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ=1.20–1.40 (m, 19H, CH_2), 1.45 (m, 4H, CH_2), 1.60 (m, 2H, CH_2), 2.0 (m, 5H, CH_2–C=C, CH_3), 2.15 (m, 4H, CH_2–C≡C), 4.05 (t, $^3J_{H,H}$=6.84 Hz, 2H, CH_2–O), 4.90 (m, 1H, CH_2=C), 4.95 (m, 1H, CH_2=C), 5.80 (m, 1H, C*H*=C); ^{13}C NMR (100 MHz, $CDCl_3$): δ=18.8 (2×*C*H_2), 21.0 (*C*H_2), 26.0 (*C*H_2), 28.7 (2×*C*H_2), 28.9 (2×*C*H_2), 29.1 (*C*H_2), 29.2 (2×*C*H_2), 29.3 (*C*H_2), 29.5 (*C*H_2), 29.6 (*C*H_2), 33.8 (*C*H_2), 64.6 (*C*H_2–O), 80.2 (2×*C*≡C), 114.2 (*C*H_2=C), 139.0 (*C*H_2=C), 171.1 (*C*=O); MS (GC-MS, e.i., 70 eV): *m*/*z* (%)=63 (100), 178 (65), 192 (76), 203 (7), 217 (7), 231 (6), 238 (7), 252 (18), 263 (7), 277 (9), 291 (19), 305 (28), 319 (9), 320 (3), 334 (3); HRMS for $C_{22}H_{38}O_2$: calcd 334.2872; found: 334.2872. Anal. calcd for $C_{22}H_{38}O_2$ (334.54): C, 78.99; H, 11.45; found: C, 78.77; H, 11.54.

3.1.7. (11Z)-11,19-Icosadienyl acetate (1). A solution of **9** (0.27 g, 0.81 mmol) in hexane (10 ml) containing quinoline (0.125 ml) and *Lindlar*-catalyst (42 mg) was stirred under hydrogen (1 atm) for 1 h, then the catalyst was removed and the solvents were evaporated. Chromatographic purification

(silica gel, hexane/ethyl acetate 95:5) gave **1** (0.25 g, 92%) as a colourless liquid. R_f (hexane/ethyl acetate 9:1)=0.57; IR (film): ν=3467w, 3077m, 3004s, 2924s, 2853s, 1744s, 1641s, 1464s, 1387s, 1365s, 1237s, 1039s, 994s, 909s, 810w, 723s, 634m, 606m cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ=1.20–1.40 (m, 22H, CH_2), 1.60 (m, 2H, CH_2), 2.0 (m, 9H, CH_2–C=C, CH_3), 4.05 (t, $^3J_{H,H}$=6.7 Hz, 2H, CH_2–O), 4.90 (m, 1H, CH_2=C), 4.95 (m, 1H, CH_2=C), 5.35 (ddd, $^3J_{H,H}$=9.6 Hz, $^3J_{H,H}$=6.02 Hz, $^3J_{H,H}$=5.81 Hz, 2H, C–CH=CH–C), 5.80 (m, 1H, CH=C); ^{13}C NMR (100 MHz, $CDCl_3$): δ=21.0 (*C*H_3), 26.0 (*C*H_2, 2C), 27.2 (*C*H_2), 27.3 (*C*H_2), 28.7 (*C*H_2), 29.0 (*C*H_2), 29.1 (*C*H_2), 29.2 (*C*H_2), 29.3 (2×*C*H_2), 29.6 (2×*C*H_2), 29.7 (*C*H_2), 29.8 (*C*H_2), 33.8 (*C*H_2), 64.7 (*C*H_2–O), 114.1 (*C*H_2=C), 129.8 (–*C*H=CH–), 129.9 (–*C*H=CH–), 139.1 (*C*H=C), 194.4 (*C*=O); MS (GC-MS, e.i., 70 eV): *m*/*z* (%)=43 (100), 55 (83), 81 (53), 95 (40), 121 (12), 149 (4), 164 (2), 191 (1), 219 (1), 247 (1), 276 (1), 293 (1), 308 (1), 336 (2); HRMS for $C_{22}H_{40}O_6$: calcd 336.3028; found: 336.3028. Anal. calcd for $C_{22}H_{40}O_6$ (336.55): C, 78.51; H, 11.98; found: C, 78.43; H, 12.07.

3.2. Bio-assay

Two colonies of *M. rubra* (about 25 individuals each, Antstore, Berlin) were reared separately in glass tanks. During the assay the ants were allowed to choose between two pieces of prey (ca 75 mg turkey meat) that were placed on a sheet of paper at a distance of ca. 50 mm. Each piece was surrounded by a circle (20 mm radius) soaked (50 μl) either with pure methanol (for reference) or a solution of **1** (0.5 μl in 50 μl methanol). Statistical evaluation of the test results was made using the χ^2 test for pairwise comparison of the number of ants ($p<0.05$).

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Computational insight into the thermal reactivity of *N*-methyl-3-cyanomethyl-2-vinylindole. Competition between two pericyclic reactions☆,☆☆

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Abstract—The direct cycloaddition of a *N*-methyl-3-cyanomethyl-2-vinylindole (**1**) with a *N*-methylmaleimide (**2**) is not observed. The in situ formation of an indolo-2,3-quinodimethane intermediate (**3**) leads instead of the normal cycloadduct to an 1,2,3,4-tetrahydrocarbazole (**5**). To help our understanding of this reaction, we performed a DFT study. The formation of both, the direct cycloadduct (**4**) and the intermediate (**3**) are found to be not thermodynamically favorable. However, the small amount of **3** formed in the medium reacts with the dienophile in this way explaining the regio-selectivity of the reaction. The diastereo-selectivity is finally explained by a Curtin–Hammett-type energy profile.

1. Introduction

The carbazole ring is an important building block for the construction of polymers and it is also the core of a wide range of alkaloids. Indeed, on the one hand its electronic properties induced by the juxtaposition of several aromatic rings play an important part in the construction of polymers with thermal,[1] electrical,[2] photo-electrical,[3] or photo-luminescent[4] properties. On the other hand, the planar structure of this pharmacophore is a key factor in the biological activity of natural carbazole alkaloids such as staurosporine (inhibitor of protein kinase C), carbazomycin (antifungal agent) or ellipticine (anticancer agent). Thus, based on the biological activity of those naturally occurring alkaloids, a large number of non-natural analogues has been synthesized with different therapeutic applications.[5] These promising therapeutic and material applications of carbazole containing compounds explain the considerable interest in the chemistry of this system.

For several years, our laboratory has been interested in the synthesis of anticancer agents and natural products, both containing the carbazole ring. As in other groups,[6] our synthetic scheme of this ring is based on a thermal Diels–Alder cycloaddition of a dienophile with a diene containing an indole ring (a 2-vinylindole[7] or a gramine[8]). Both of these reactants are chosen to afford the desired substitution on the tetrahydrocarbazole obtained, which lead to the corresponding substituted carbazole ring after oxidation.

We recently reported a synthetic route, starting from an *N*-methyl-3-cyanomethyl-2-vinylindole **1** and *N*-methyl-maleimide **2**.[7] Surprisingly, the expected cycloadduct **4** (Scheme 1) has not been recovered and this route appeared to be very efficient for the synthesis of 1,2,3,4-substituted-tetrahydrocarbazoles **5**(**a**-**d**). However, the mechanism of this reaction is not trivial and is supposed to proceed in two steps via the in-situ formation of an indolo-2,3-quino-dimethane intermediate (Scheme 2). These steps are then assumed to be both a competition between two reactions:

(i) a [1,5] H sigmatropic shift converting the 2-vinylindole **1** into an indolo-2,3-quinodimethane intermediate **3** versus a Diels–Alder reaction between **1** and **2**. This step is introduced to explain the regio-selectivity,
(ii) an inversion of configuration in the previously formed

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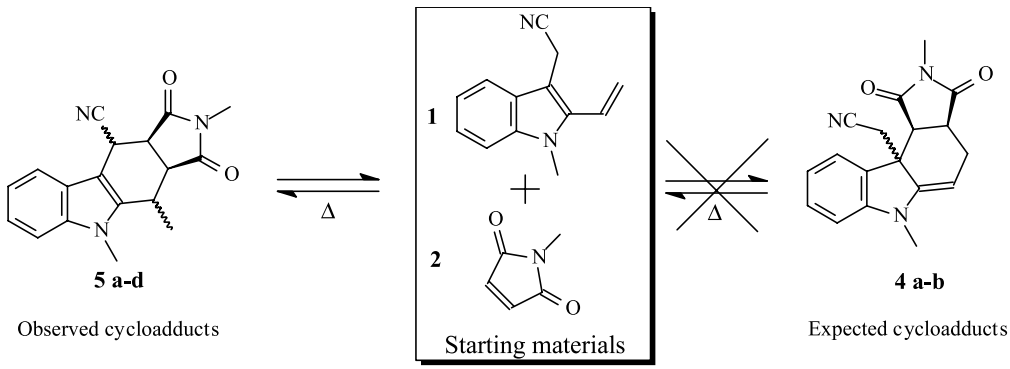
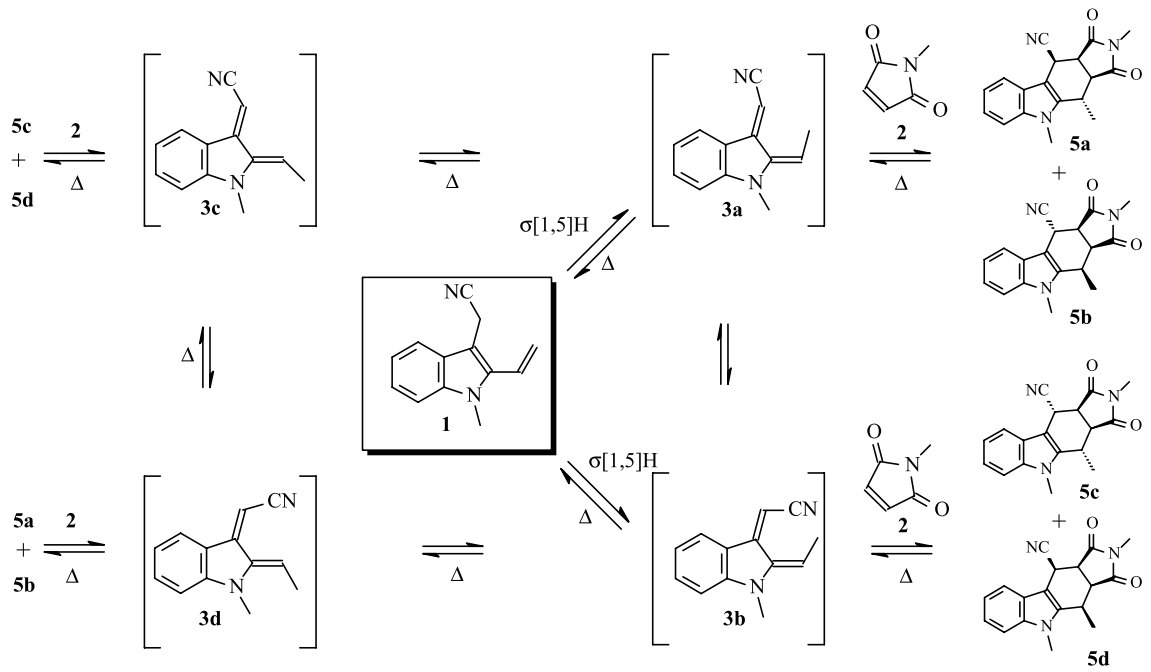


Scheme 1. [4+2] Cycloaddition between *N*-methyl-3-cyanomethyl-2-vinylindole (**1**) and *N*-methylmaleimide (**2**): expected **4**(**a**,**b**) and observed **5**(**a**-**d**) cycloadducts.[7]



Scheme 2. Presentation of the proposed mechanism for the reaction between an *N*-methyl-3-cyanomethyl-2-vinylindole (**1**) with an *N*-methylmaleimide (**2**). (Only one of the two possible enantiomer is represented).

indolo-2,3-quinodimethane intermediate versus a Diels–Alder cycloaddition of the dienophile **2** on this same intermediate. This step is introduced to explain the diastereo-selectivity.

However, the reason for the regio-selectivity of the reaction is still not clear nor is the reason for the stereo-selectivity. Therefore, with the view to help our understanding of this reaction and in fact to confirm or invalidate the postulated mechanism, we performed a theoretical study. Thus, the different possible reaction pathways have been investigated (Scheme 2) and the results obtained are presented.

2. Methodology

According to the extended computational works of several groups in the field of pericyclic reactions,[9] and in particular that of Houk et al.,[10] it appears that the density functional theory (DFT) using the B3LYP[11] functional (or UB3LYP for diradical species) and the 6-31G* basis[12] set is the most suitable method to perform our study, which involves Diels–Alder cycloaddition, [1,5] H sigmatropic shift and diradical species. However, the computational cost of this method is great for systems containing a large number of heavy atoms, as in our case (23).

For the study of pericyclic reactions occurring in large systems, Jursic et al.[13] overcome the problem of the 'cpu-time' consuming geometry optimization and frequency calculations by using the dual level method B3LYP/6-31G*//AM1.[14] We thus initiated our study with this method, supported in our choice by the fact that semi-empirical calculations on indolo-2,3-quinodimethanes have already been reported in the literature.[15] However, geometries obtained with the AM1 hamiltonian (as well as with the PM3[16] one) were not acceptable, because of the non-planar geometry of the indole nitrogen in the indolo-2,3-quinodimethane intermediate. We consequently

abandoned this path and increased the level of theory for the optimization part, leading to the dual level B3LYP/6-31G*//B3LYP/3-21G approach,[17] which appears to give an adequate balance between the quality of the result and the cost of the calculations. Thus, all geometries reported here are obtained with the B3LYP functional and the 3-21G basis set, and all energies are computed by a single point calculation on the previously optimized structure, using the same DFT functional and the 6-31G* basis set. Restricted (RB3LYP) and unrestricted (UB3LYP) wave-function were used for closed and open-shell species, respectively.

In order to produce theoretical energy barriers, vibrational frequencies were calculated at the B3LYP/3-21G, and used un-scaled to compute the zero point energies, thermal corrections, vibrational entropies and their contribution to activation enthalpies, entropies and free energies. These frequency calculations also allowed us to determine the nature of each stationary point where all frequencies were positive for reactants and products, and only one imaginary frequency was found for transition states, corresponding to the vibration of the reaction coordinate.

The contribution of the solvent effect on geometries and to activation Gibbs Free Energies was calculated via the self-consistent reaction field (SCRF[18]) method using the B3LYP/6-31G*//B3LYP/3-21G level of theory and the Onsager model[19] of solvation (a dielectric constant of 2.1 was used to simulate the toluene continuum).

All calculations were performed with Gaussian98 series of programs[20] and run locally on a SGI Octane or on a IBM SP at the Centre Informatique National de l'Enseignement Supérieur (CINES). Structures were drawn with Gopenmol software.[21]

3. Computational results

The insight into the mechanism proposed in Scheme 2 is divided into two parts. The first part deals with the reactivity of the *N*-methyl-3-cyanomethyl-2-vinylindole (**1**) while the second one focuses on the reactivity of the intermediates **3**.

All of the coordinates and energies of the DFT optimized species within this context are provided in the Supporting Information, as well as a study to validate the calculation method employed.

3.1. The thermal reactivity of 1

In this section are reported the results obtained for the study of the thermal reactivity between *N*-methyl-3-cyanomethyl-2-vinylindole (**1**) and *N*-methylmaleimide (**2**). It is divided into four parts, the first one focuses on the conformational equilibrium of the reactant **1** with the view to point out its different conformations and especially those involved in the sigmatropic shift and in the cycloaddition. These two pericyclic reactions are then studied in the second and in the third part, respectively. Finally a comparison between these two reactions is made.

3.1.1. Conformational analysis of 1. The compound **1** only possesses three bonds which can freely rotate: the one of the *N*-methyl substituent, the one in position 2 of the indole ring (included in the vinyl group) and the one in position 3 of the indole ring (bearing the cyanomethyl group). Only eight conformers have been found, but in fact only four of them are energetically and chemically different due to the existence of a symmetric relationship in relation to the indole ring-containing plane. Hereafter, these conformers will be referred as **1a**, **1b**, **1c**, **1d** and their corresponding image are **1a′**, **1b′**, **1c′**, **1d′**.

From the analysis of the results, it appears that the relative free energy differences between each conformer are not significant at the experimental temperature of 438 K, and because of the very low rotational barriers which separate each conformer, we can assume that there is a fast equilibrium between them, leading to a constant statistical relative proportion of each of these conformers during the reaction. The application of the Boltzmann law leads to the estimation of the relative abundance of each conformer of **1** of 20:42:8:29 per cent for, respectively, **1a**(**1a′**):**1b**(**1b′**):**1c**(**1c′**):**1d**(**1d′**) in the experimental conditions as reported in Figure 1.

3.1.2. The sigmatropic [1,5] H shift occurring in 1. Since the pioneering works of Hoffmann and Woodward on pericyclic reactions,[22] it is known that a sigmatropic rearrangement occurs via a concerted pathway, controlled by the frontier molecular orbital (FMO). In the present study, the FMO control explains the orientation of the cyano group in the intermediate **3** formed.

Indeed, in our proposed mechanism, only **1a**, **1b** (represented in Fig. 1) and their corresponding image through the plane containing the indole ring (**1a′** and **1b′**, not represented) possess an adapted geometry for a thermal suprafacial H-shift. Consequently, only four transition states are located on the PES of the system ($\mathbf{TS}_5$, $\mathbf{TS}_6$, $\mathbf{TS}'_5$, and $\mathbf{TS}'_6$, respectively). Because of the FMO control of this reaction, the new formed π bond have to rotate: (i) clockwise if the shift occurs in **1a′** and **1b′** ($\mathbf{TS}'_5$ and $\mathbf{TS}'_6$), (ii) anti-clockwise if the shift occurs in **1a** and **1b** ($\mathbf{TS}_5$ and $\mathbf{TS}_6$). Therefore, **1a** (**1a′**) leads to the compounds **3a** (**3a′**) via $\mathbf{TS}_5$ ($\mathbf{TS}'_5$), and **1b** (**1b′**) leads to the compound **3b** (**3b′**) via $\mathbf{TS}_6$ ($\mathbf{TS}'_6$). Thus, the FMO control of the sigmatropic shift implies the formation of two isomers of **3** (**3a** and **3b**) as previously proposed in Scheme 2.

The activation barriers of these sigmatropic rearrangements have been reported in Table 1 for the unprimed family. The structure of two reactants **1a** and **1b** and their sigmatropic products (**3a** and **3b**) are shown in Figure 2, with the energetic profile of the reaction along with the pathway calculated to reproduce experimental conditions, by taking into account the thermal, the entropic and the solvent effects. It can be seen in this figure that the relative free energy of the two new products **3a** and **3b** is higher than those of the reactants. These two reactions are thus thermodynamically unfavored and this explains why these corresponding products cannot be isolated experimentally. Concerning the activation barriers (Table 1), it can be noticed that they are smaller than the

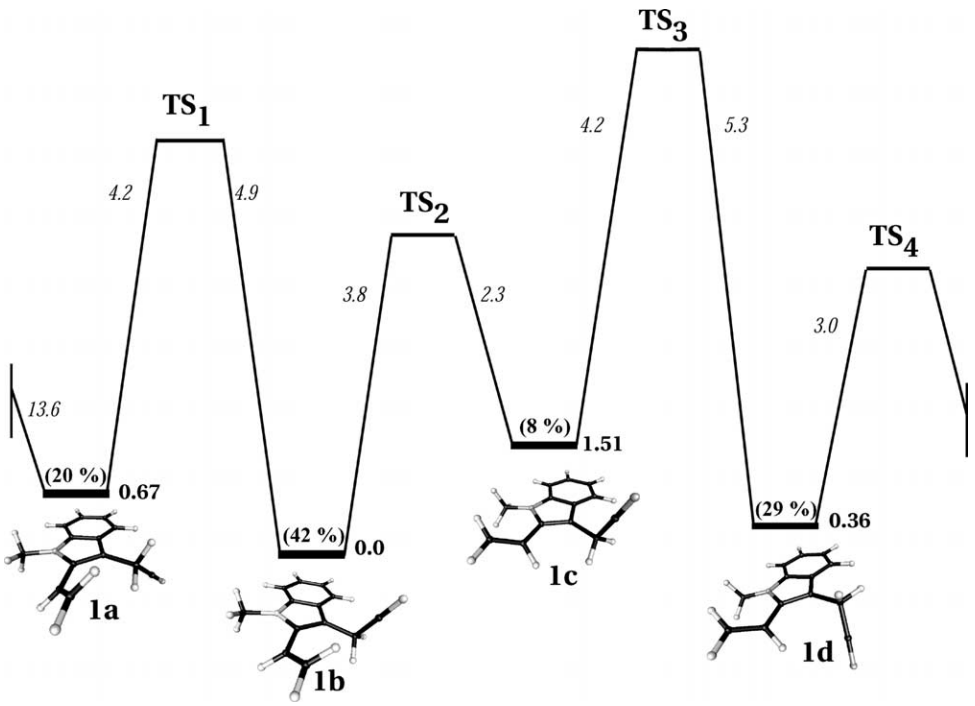


Figure 1. Conformational interchange pathway for the compound **1**. The free energies reported are given in kcal mol^{-1} and have been calculated at 438 K using the B3LYP/6-31G*//B3LYP/3-21G level and the Onsager model of solvation. Free energies of activation are reported in italic. The relative free energy of each conformer is reported in bold (relative to **1b**). The relative abundance of each conformer is reported in parenthesis.

one found for the pentadiene: 30.5 kcal mol^{-1} from **1a** and 32.9 kcal mol^{-1} from **1b** compared to 33.0 kcal mol^{-1} from the pentadiene in its *cis* conformation (result reported in the Supporting Information). This reaction is then more favorable than the one that occurs with pentadiene, even if it implies the breakdown of the aromatic delocalization of the indole ring. In fact, this can be explained by the rise of the lability of the migrant proton, due to the proximity of the cyano group and by the fact that the loss of the aromaticity of the indole ring is more or less compensated for the creation of electronic delocalization between the new π bonds formed and the cyano group. Concerning the solvent effect, it is negligible as expected for this kind of intermolecular reaction where there is no significant variation of the dipolar moment along the reaction coordinate.

Table 1. Electronic energies ($\Delta E^{\neq a}$), enthalpies ($\Delta H^{\neq b}$), entropies ($\Delta S^{\neq b}$) and free energies [$\Delta G^{\neq b}$ (gas phase) and $\Delta G_{tol}^{\neq b,c}$ (toluene solution)] at 438 K, for all reactions presented. Reverse reaction energies are given in parenthesis

	$\Delta E^{\neq a}$	$\Delta H^{\neq b}$	$\Delta S^{\neq b}$	$\Delta G^{\neq b}$	$\Delta G_{tol}^{\neq b,c}$
1a→TS$_1$→1b	2.84 (3.80)	2.07 (3.15)	−5.99 (−4.13)	4.69 (4.96)	4.23 (4.90)
1b→TS$_2$→1c	3.19 (1.62)	2.61 (1.12)	−2.10 (−2.23)	3.53 (2.10)	3.82 (2.31)
1c→TS$_3$→1d	6.77 (7.13)	5.97 (6.27)	−7.15 (−7.37)	9.10 (9.50)	4.18 (5.33)
1d→TS$_4$→1a	1.81 (2.07)	1.26 (1.37)	−2.42 (−3.93)	2.32 (3.09)	3.01 (2.70)
1a→TS$_5$→3a	30.46 (28.67)	29.46 (27.78)	−8.56 (−6.05)	33.21 (30.43)	32.62 (30.39)
1b→TS$_6$→3b	32.86 (26.57)	31.93 (25.68)	−7.10 (−4.93)	35.04 (27.84)	34.96 (27.60)
1a→TS$_7$→4a (*endo*)	25.40 (42.30)	24.87 (43.03)	−50.39 (5.57)	46.94 (40.59)	47.56 (40.88)
1b→TS$_7$→4a (*endo*)	26.36	25.96	−48.52	47.21	48.23
1a→TS$_8$→4b (*exo*)	26.19 (43.00)	25.82 (44.01)	−45.88 (−9.25)	47.23 (39.96)	47.34 (40.00)
1b→TS$_8$→4b (*exo*)	27.14	26.90	−47.30	47.50	48.01
3a→TS$_9$→3b	33.19 (29.62)	33.47 (29.99)	2.94 (4.50)	32.18 (28.02)	33.19 (28.72)
3b→TS$_{10}$→3d	28.52 (29.97)	28.54 (29.87)	1.59 (3.12)	27.17 (29.32)	27.63 (29.83)
3d→TS$_{11}$→3c	33.06 (33.31)	33.12 (33.35)	0.71 (0.75)	32.81 (33.22)	32.96 (33.82)
3c→TS$_{12}$→3a	30.14 (32.00)	30.31 (32.24)	1.41 (2.19)	29.69 (31.28)	29.05 (30.47)
3a→TS$_{13}$→5a (*endo*)	17.41 (49.42)	17.10 (50.12)	−50.32 (1.14)	39.14 (49.62)	39.25 (49.27)
3b→TS$_{14}$→5c (*endo*)	16 97 (57.31)	16.74 (58.04)	−49.43 (0.41)	38.39 (57.86)	38.63 (57.28)
3c→TS$_{15}$→5d (*endo*)	14.52 (48.23)	13.81 (48.53)	−47.54 (−4.5)	37.70 (50.50)	37.92 (49.81)
3d→TS$_{16}$→5b (*endo*)	14.24 (48.44)	13.63 (48.81)	−53.49 (−3.60)	37.06 (50.39)	37.36 (50.51)
3a→TS$_{17}$→5b (*exo*)	19.45 (51.54)	19.45 (52.48)	−43.20 (6.34)	38.37 (49.70)	38.26 (49.24)
3b→TS$_{18}$→5d (*exo*)	21.39 (56.80)	21.18 (57.45)	−46.76 (−0.02)	42.10 (57.46)	41.70 (56.65)
3c→TS$_{19}$→5c (*exo*)	14.34 (52.98)	13.88 (53.63)	−49.75 (2.39)	35.67 (52.58)	35.83 (51.42)
3d→TS$_{20}$→5a (*exo*)	15.13 (49.25)	14.61 (49.79)	−51.66 (0.20)	37.24 (49.71)	36.98 (49.28)

[a] Electronic energies from B3LYP/6-31G*//B3LYP/3-21G calculations, including the ZPE correction (in kcal mol^{-1}).
[b] For evaluation of the thermodynamic properties, the B3LYP/3-21G computed kinetic contributions are used with the following conditions: frequencies are not scaled, P=1 atm, T=438 K (energies in kcal mol^{-1}, entropies in cal mol^{-1} K^{-1}).
[c] Solvent (toluene) effect evaluated with the Onsager model at the B3LYP/6-31G*//B3LYP/3-21G level.

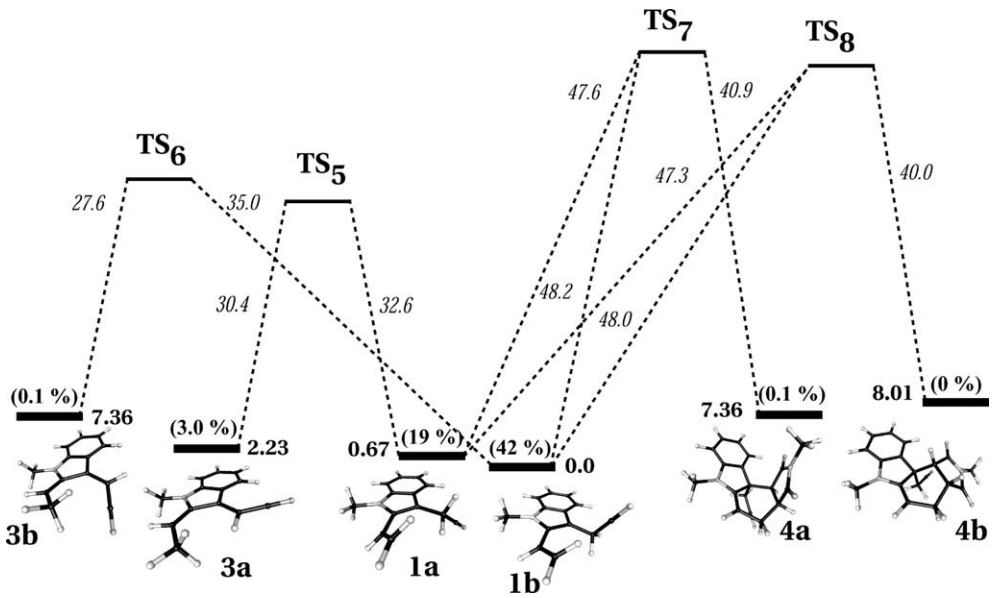


Figure 2. Representation of the two possible pericyclic reactions for **1** when **2** is present in the medium. The [1,5] H shift which leads to **3a** or **3b** and the Diels–Alder cycloaddition which leads to **4a** or **4b**. The free energies reported are given in kcal mol^{-1} and have been calculated at 438 K using the B3LYP/6-31G*//B3LYP/3-21G level, and the Onsager model of solvent. Free energies of activation are reported in italic. The relative free energy of each specie is reported in bold (relative to **1b**). The relative abundance of each specie is reported in parenthesis.

3.1.3. The cycloaddition between 2 and 1. Even if the product resulting from this cycloaddition was not experimentally observed, it is not precluded its feasibility, that is, the occurrence of a transition state joining the reactants and the desired product. Indeed, our search for transition states of this reaction succeeded in and we have found two concerted asynchronous transition states, corresponding to the *endo* and to the *exo* approach of the dienophile on compounds **1a** and **1b**. Two different approaches of the dienophile on two different conformers of **1** (**1a** and **1b**) lead only to two transition structures ($\mathbf{TS}_7$ and $\mathbf{TS}_8$). This is because, both positions of the cyanomethyl group in the two reacting conformers collapsed in the transition state to only one position that is available for this substituent. Thus the *endo* approach of the dienophile on **1a** and **1b** leads to **4a** via the $\mathbf{TS}_7$, and the *exo* approach leads to **4b** via the $\mathbf{TS}_8$. The relative free energy of the two possible cycloadducts **4a** and **4b**, and their geometries have been reported in Figure 2 at the experimental temperature of 438 K, and has been found to be higher than the ones of the reactants. This reaction is therefore thermodynamically unfavored. Moreover, the energy barriers of the reaction have been reported in Table 1. These barriers are higher than the one found for the cycloaddition between butadiene and ethylene (see reaction R_2 in Supporting Information), which is known to be a poorly effective reaction (25.40 kcal mol^{-1} for the smallest barrier against 18.0 kcal mol^{-1} for the reaction R_2). Consequently, we can conclude that the cycloaddition of **2** with **1a** and **1b** is not only thermodynamically unfavored, but also kinetically unfavored.

Concerning the solvent effect, its contribution does not dramatically change the fate of the reaction. However, we can notice that it reverses the order of the *endo*:*exo* selectivity by destabilising the *endo* approach. The larger variation of the dipolar moment along the pathway of this approach as compared to the one of the *exo* approach, could account for this observation.

3.1.4. Comparison of these two reactions. From the results reported in Figure 2, we can see that:

(i) none of these two reactions leads to a product more stable than the reactants,
(ii) considering only the four possible products, **3a** is the more stable,
(iii) the energy barriers of the sigmatropic shift are smaller than for cycloadditions,
(iv) the energy barriers of these two pericyclic reactions are higher than those reported for the conformational equilibrium of **1**.

This last point means that the conformational equilibrium of the reactant **1** cannot influence the course of the reaction due to the very low energy barriers found. The reaction could therefore, proceed by any of the possible conformations of **1**. The first point is in fact the most important result because it indicates that all reverse reactions are kinetically favored. As a consequence, in the experimental thermal condition used, we can assume that the control of this first step of the reaction is thermodynamic. This control imposes a constant thermodynamic equilibrium between each specie reported in Figures 1 and 2, it is then possible to estimate the relative abundance of each of these species during the reaction using the Boltzmann law (Fig. 2).

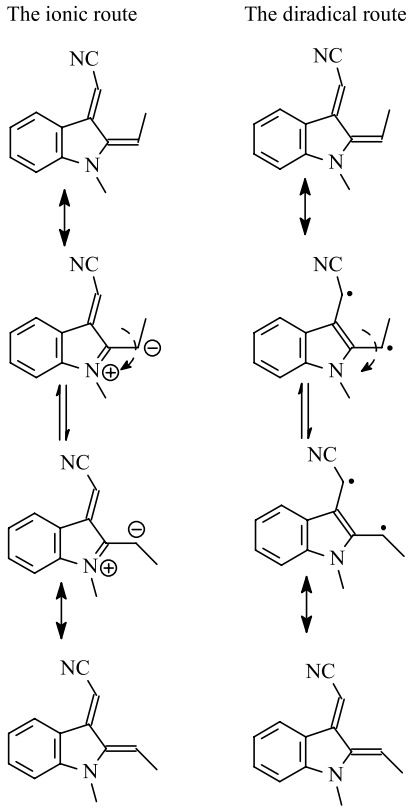
This calculation highlights that the major component (96%) when the thermodynamic equilibrium state is obtained, is the starting vinylindole; with a small amount of **3** (3%) present, allowing the reaction to proceed by this way.

3.2. The thermal reactivity of 3

Here we report the results obtained for the study of the thermally-activated-reaction between indolo-2,3-quinodimethane (**3**) and *N*-methylmaleimide (**2**). This section is

divided into three parts, the first one focuses on the exchange occurring between each of the possible isomers of **3**, and the second one deals with their cycloaddition with **2**. Finally, a comparison between these two reactions is presented.

3.2.1. Inversion of configuration of the enamine double bond. As stated above, the sigmatropic shift that **1a** and **1b** (or on **1a**′ and **1b**′) undergone can only lead to the two conformers **3a** and **3b** (or **3a**′ and **3b**′) respectively. However, after their formation they can invert their configuration either by ionic or diradical routes, as shown in Scheme 3. Because we cannot know a priori if the cycloaddition between **3** and **2** can be controlled either by a thermodynamic or a kinetic process, it is important to investigate the reality of this inversion of configuration. This study could be useful to predict the diastereoselectivity of this step of the reaction.



Scheme 3. The two possible (ionic or diradical) routes for the inversion of configuration of compound **3**.

No acceptable ionic pathway was found with Gaussian98. Indeed, in all of our attempts to find these paths, we observed that the reverse sigmatropic shift occurred before a stable ionic transition structure was found. This result therefore tends to indicate that the ionic path allowing the inversion of configuration of the enamine double bond:

(i) either presents a very high energy barrier which crosses the surface of the reverse sigmatropic shift avoiding the possibility of an inversion of configuration by this way,
(ii) or simply does not exist.

On the contrary, we succeeded in locating the diradical one, both for the singlet and the triplet states. The energy difference between these two pathways is not significant (less than 0.5 kcal mol^{-1}) and we have decided only to report here the results concerning the singlet spin state.

The structure of each transition states can be found in the supporting information. The four different isomers and their transition structures for interconversion are shown in Figure 4 where energetic profiles take into account for thermal, entropic and solvent effects to reproduce experimental conditions. All the energy barriers for the singlet spin state pathway are reported in Table 1.

From these calculations, it is important to notice that the energy barriers of these inversions are of the same range as the ones obtained for the sigmatropic shift and are in reality certainly lower considering the fact that the spin contamination of our DFT wavefunction has not been corrected for, which is known to increase the energy of diradical species.[10d] This reaction is then kinetically allowed and leads to two new conformers of the two indolo-2,3-quinodimethanes primarily formed by the sigmatropic shift (**3c** and **3d**). So if the indolo-2,3-quinodimethane intermediates **3a** and **3b** are not immediately trapped in the mixture during another reaction, an equilibrium between all these four isomers (and their corresponding projection through the indole-containing plan) can be formed.

3.2.2. The cycloaddition between 2 and 3. Indolo-2,3-quinodimethanes are known to be highly reactive in the Diels–Alder cycloaddition reaction.[15] In the present case, four different isomers of the indolo-2,3-quinodimethane (**3**) could be generated in-situ and therefore should react with the dienophile **2**, still present due to its lack of reactivity towards **1**.

The search for these transition states led to the location on the PES of two saddle points (of order 1), joining the reactants and the desired product for each isomer of **3**, corresponding to the *endo* and the *exo* approach of the dienophile **2**. The energy barriers of these eight asynchronous concerted transition structures are reported in Table 1 and their geometries can be found in the supporting information.

From these transition structures, only four diastereoisomers of the adduct **5** can be obtained (**5a**, **5b**, **5c** and **5d**). A conformational analysis of these four diastereoisomers reveals that they can only adopt two different conformations (bent or stretched) as shown in Figure 3 with **5a** as an example.

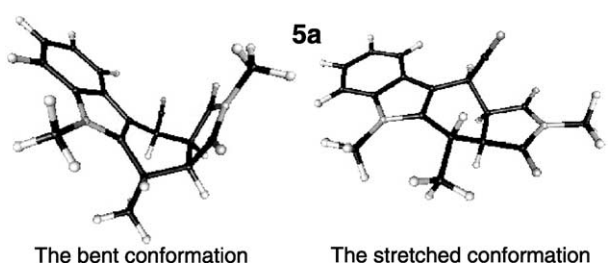


Figure 3. The two possible conformations of the cycloadduct corresponding to the cycloaddition of **3** with **2**, we report here as an example the diastereoisomer **5a**.

The following discussion will only deal with the more stable conformation of each of the diastereoisomers, which are bent for **5a** and **5c** and stretched for **5b** and **5d**, corresponding to an axial position of the methyl substituent in each case. These four cycloadducts were placed along the pathway drawn in Figure 4 for the *exo* approach of the dienophile (for a better legibility, the *endo* approach was omitted in this figure).

The analysis of these results shows that all energy barriers involved in this reaction are smaller than those found for the cycloaddition of **1** with **2**, indicating that they are kinetically more favorable. However, they are not as low as for the reactivity of other quinodimethane-like compounds, whose energy barrier ($\Delta E^{\neq}$) is generally found to be in the range of 5–17 kcal mol^{-1} for a cycloaddition.[9a,d] This increase in the energy barrier can be attributed to the cyano group. Indeed, as reported by Manoharan et al.,[9d] the partial delocalization (π) of the peripheral ring of the diene during the formation of the **TS** is an important parameter to decrease the activation energy of the cycloaddition between a quinodimethane-like compound and a dienophile. Because the cyano group is conjugated with the the diene, the formation of the **TS** is less favorable than with ordinary quinodimethanes and the activation energy increase.

Solvation proved to have little influence on this reaction as the dielectric constant used to simulate the solvent is not large enough to affect the fate of the reaction.

3.2.3. Comparison between these two reactions. From the results reported in Figure 4, we can see that:

(i) the energy barriers of the inversion of configuration are smaller than those of the cycloaddition,
(ii) the cycloadducts are more stable than, not only the different isomers of **3**, but also the different conformers of **1**. (i.e., the reaction free energies are negative).

The profile of this reaction is then totally different than the one obtained previously. Indeed, the second point indicates that the reverse reaction of each cycloaddition is not favorable and we can now assume that these cycloadditions are controlled by kinetic factors. Furthermore, the first point opens the possibility of an equilibrium between each of the isomers of **3** and, according to the Curtin–Hammett principle, the abundance of the different products at the end of the cycloaddition therefore depends on the relative energies of the eight transition structures with respect to each other. According to this principle, the abundance of each diastereoisomer of **5** can be calculated and in our case leads to the prediction that compound **5c** should be the major adduct (Fig. 4).

4. Discussion

4.1. The role of the temperature

The temperature is linked to the kinetics of a reaction by the relation:

$$\ln K_i = -\frac{\Delta G_i^{\neq}}{RT} \quad (1)$$

where $\Delta G_i^{\neq}$ is the activation free energy and K_i the kinetic

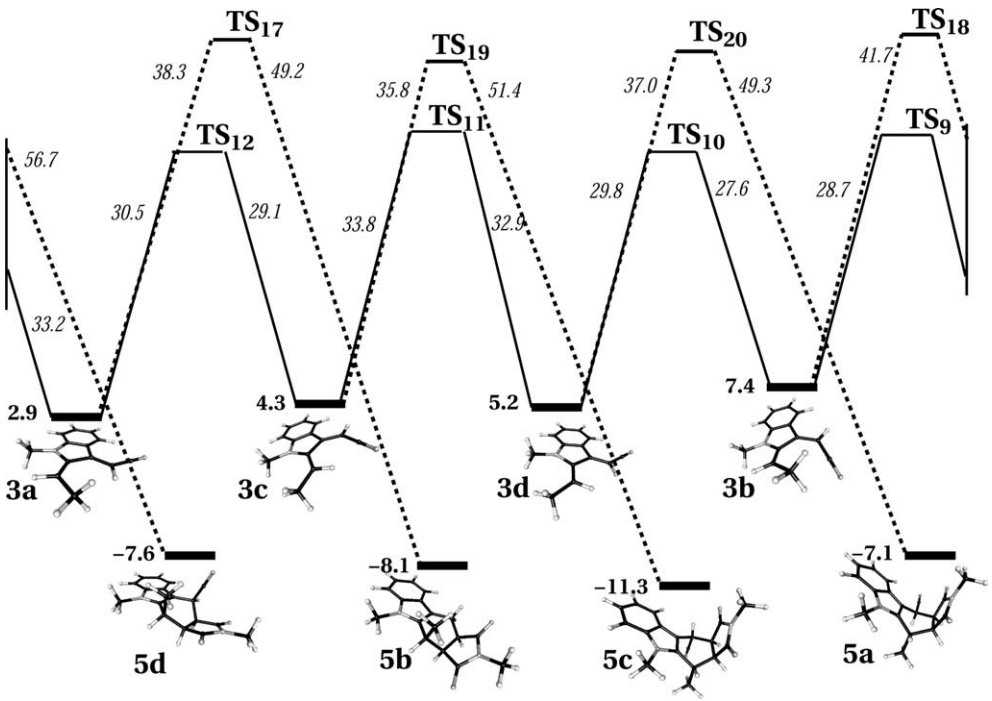
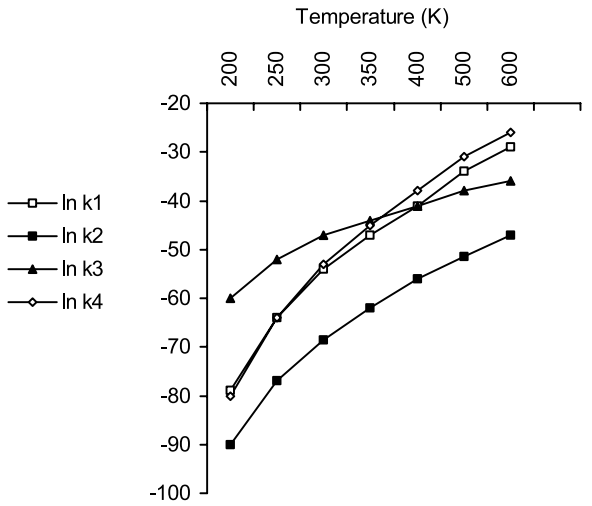


Figure 4. Representation of the pathway corresponding to the interconversion of **3** and of the *exo* cycloaddition between **2** and **3**. The free energies reported are given in kcal mol^{-1} and have been calculated at 438 K using the B3LYP/6-31G*//B3LYP/3-21G level of theory and the Onsager model of solvation. Free energies of activation are reported in italic. The relative free energy of each specie is reported in bold (relative to **1b**).

constant of the reaction *i*. The curve ln $K_i=f(T)$ is shown in Graph 1, for a representative sigmatropic shift (**1a**→**TS**$_5$→**3a**), an inversion of configuration (**3a**→**TS**$_{12}$→**3c**) and two cycloadditions (**1b**→**TS**$_8$→**4b**, **3c**→**TS**$_{19}$→**5c**), in order to assess the influence of the temperature on the kinetics of all these reactions. As expected, two families appear: one containing the two intramolecular reactions for which the weak entropic dependence induces a fast acceleration of the kinetic when the temperature increases, and another one containing the intermolecular cycloadditions for which the larger entropic dependence induces a slower acceleration of their kinetics.



Graph 1. Temperature dependence on the kinetics of each studied reaction: (1) the sigmatropic shift of **1a**, (2) the cycloaddition between **1b** and **2** (*exo* approach of the dienophile), (3) the cycloaddition between **3c** and **2** (*exo* approach of the dienophile) and (4) the inversion of configuration of **3a** to give **3c**.

When comparing two reactions, two cases can arise:

(i) no crossing is observed between their representative kinetic curves: this is the case for the sigmatropic shift and the cycloaddition between **1** and **2**. Therefore, the comparison of their $\Delta E^{\neq}$ and $\Delta G^{\neq}$ leads to the same conclusion concerning the kinetic difference between the two reactions. It is, not necessary to take into account the temperature in this case.

(ii) a crossing is observed between their representative kinetic curves: this is the case between the inversion of configuration and the cycloaddition of **3** with **2**. Therefore, the comparison of $\Delta E^{\neq}$ and $\Delta G^{\neq}$ between these two reactions leads to two opposite conclusions according to the temperature. Indeed, in this case, for $T<350$ K, the cycloaddition is faster than the inversion of configuration but for $T>350$ K it is the contrary.

In this work, because of the observed crossing lines in Graph 1, all energy barriers are compared at the experimental temperature of 438 K.

4.2. The regio-selectivity

From the results obtained in this study, we can see that the direct cycloaddition **1** and **2** is found not to be energetically favored, leading to a less stable adduct than the starting reactants (Fig. 2). This reaction can, however, occur by another route, since the small amount of indolo-2,3-quinodimethane **3** (provided by the sigmatropic shift) can react with the dienophile, leading this time to the stable adduct **5** (Fig. 5). Therefore, based on the mechanism proposed in Scheme 1, our calculations lead to a set of results which agreed with the experimental data and explain without ambiguity the regio-selectivity of the reaction. Thus, the consistencies of these results with experiments allow us to validate the mechanism proposed.

4.3. The diastereo-selectivity

Concerning the reaction involving **1** and **2**, our calculations show that the key stage, to assess the relative abundance of each diastereo-isomer of the compound **5** at the end of the reaction (i.e., its diastereo-selectivity), is the cycloaddition between **3** and **2**, which is found to follow the Curtin–Hammett principle. This kinetic control of the reaction is in agreement with the experimental data in so far as the majority product isolated is not the thermodynamic one (indeed, 81% of isolated cycloadduct correspond to the diastereo-isomer **5a**,[7] which is according to our calculations not the thermodynamically preferred compound). However, when we apply this principle to calculate the relative abundance of each of the four possible diastereo-isomer, we find that **5c** should be in the majority, which is not in agreement with the experimental data. This disappointing result is in fact not surprising, indeed, for a reaction, which follows the Curtin–Hammett principle, the abundance of each product only depends on the fine difference between the free energy of each transition structure leading to these products. The ability to accurately predict the diastereo-selectivity then depends only on the accuracy of the computational method used to calculate the free energies of these transition structures and we suppose that the dual method B3LYP/6-31G*//B3LYP/3-21G we used is not accurate enough to assess with exactness these energies. Despite this uncertainty the overall mechanism cannot be questioned.

4.4. The solvent effect

The solvent effects are seen to cause lowering of the barriers and reaction free energies almost uniformly, so that there is no important change in the relative order of $\Delta G^{\neq}$ when comparing two different reactions and the toluene consequently has no influence on the regio-selectivity of the reaction. However, if one wants to deal with the diastereo-selectivity, this effect must be taken into account because it acts on the fine difference in free energies of the transition structures.

4.5. Influence of the cyanomethyl group

The cyano group of **1** seems to be the key factor of the reaction between **1** and **2**. Indeed, it acts on the regio-selectivity by making the sigmatropic shift possible (by increasing the lability of the migrant hydrogen and contributing to the stability of the intermediate by creating a new electronic delocalization to compensate the loss of the

aromaticity of the indole ring) and also act on the diastereoselectivity by increasing the activation barrier of the cycloaddition between **3** and **2** and by the fact of allowing the inversion of configuration of **3**.

5. Conclusion

A theoretical study based on the B3LYP/6-31G*//B3LYP/3-21G calculations has been used to investigate the thermal reactivity of *N*-methyl-3-cyanomethyl-2-vinylindole towards the Diels–Alder cycloaddition with *N*-methylmaleimide, in both the gas phase and in toluene solution. Through the analysis of the different results obtained, three separate conclusions arise:

(i) the determination of the reaction pathway corresponding to the mechanism proposed, allowed us to reproduce and explain the regio-selectivity of this unusual reaction giving credence to the mechanism proposed,

(ii) the determination of the diastereo-selectivity for the cycloaddition between **3** and **2** failed. But we have demonstrated that it is controlled by the kinetics of this particular reaction. The relative abundance of each possible diastereoisomer of **5** is then shown to depend only on the free energy of each transition structure leading to the respective compound, in accordance with the Curtin–Hammett principle. We believe that choosing a more accurate calculation method would give a better accordance with experimental results,

(iii) the cyano group borne by **1** is the key factor of the reaction. Indeed, it controls the regio-selectivity by decreasing the activation barrier of the sigmatropic shift, allowing the formation of **3** when the direct cycloaddition with the dienophile is not possible. This group also controls the diastereoselectivity by increasing the activation barrier of the cycloaddition between the indol-2,3-quinodimethane intermediate **3** and the dienophile **2**, which is found to be too large for this kind of reaction, allowing the formation of the four different isomers of **3**.

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Cytotoxic sesterterpenes, 6-epi-ophiobolin G and 6-epi-ophiobolin N, from marine derived fungus *Emericella variecolor* GF10

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Abstract—Two new sesterterpenes, 6-epi-ophiobolin G (**1**) and 6-epi-ophiobolin N (**3**), and six known ophiobolins were isolated from the extracts of the fungus, *Emericella variecolor* GF10, which was separated from marine sediment. The planar structures of the new compounds were deduced from analysis of the 2D NMR spectra, and the stereochemistry was determined by extensive examination of the NOESY spectrum. Additionally, the configuration of the C-6 proton in ophiobolin G (**2**) was revised from α to β, and the unsolved stereochemistry of ophiobolin H (**4**) was determined by its physicochemical evidence and the chemical correlation with ophiobolin K (**8**). Ophiobolin K (**8**) showed cytotoxic activity against various tumor cell lines, including adriamycin-resistant mouse leukemia cells (P388), with IC_{50} of 0.27–0.65 μM.

1. Introduction

Ophiobolins are a group of naturally occurring sesterterpenes with an unusual tricyclic or tetracyclic structure showing a broad spectrum of inhibitory activity against nematodes, fungi, and bacteria, and cytotoxic activity against cancer cells.[1] Ophiobolin A (**9**) is the first known member of this family, and its absolute structure was determined by X-ray crystallography of the bromo-methoxy derivative.[2] This compound was reported to inhibit calmodulin-activated cyclic nucleotide phosphodiesterase[3] and induce apoptotic cell death in the L1210 cell line.[4] Although the mechanisms of these activities remain unclear, these findings imply ophiobolin's potential for biological and pharmaceutical uses.

During our search for bioactive substances from marine microorganisms, we previously reported a novel anthracycline, komodoquinone A, from the solid-state fermentation of a marine *Streptomyces* sp. KS3.[5,6] Further study led us to the isolation of two new ophiobolins, 6-epi-ophiobolin G (**1**) and 6-epi-ophiobolin N (**3**), and six known ophiobolins, ophiobolin G (**2**),[7] ophiobolin H (**4**),[7] 6-epi-ophiobolin C (**5**),[8] ophiobolin C (**6**),[8,9] 6-epi-ophiobolin K (**7**),[10] and ophiobolin K (**8**)[10] from the culture broth of the marine derived fungus, *Emericella variecolor* GF10. This paper presents the isolation of these compounds and the structural elucidation of 6-epi-ophiobolin G (**1**), ophiobolin G (**2**), 6-epi-ophiobolin N (**3**), and ophiobolin H (**4**).

2. Results and discussion

The fungus strain of *E. variecolor* GF10 was separated from marine sediment collected at 70 m depth in the Gokasyo Gulf, Mie Prefecture, Japan. The GF10 strain was cultured at 30 °C for 2 weeks in MG medium (malt extract 20 g, glucose 20 g, bact peptone 1 g, artificial seawater 1000 mL) or barley solid medium (barley 15 g, artificial seawater 25 mL). The EtOAc soluble portion of the 2-butanone extracts of these cultures were fractionated by silica gel column chromatography and purified by reversed-phase HPLC to obtain two new sesterterpenes named 6-epi-ophiobolin G (**1**) and 6-epi-ophiobolin N (**3**) together with six known sesterterpenes. The structures of the six known sesterterpenes were identified as ophiobolin G (**2**),[7] ophiobolin H (**4**),[7] 6-epi-ophiobolin C (**5**),[8] ophiobolin C (**6**),[8,9] 6-epi-ophiobolin K (**7**),[10] and ophiobolin K (**8**)[10] by comparison of the MS and NMR data with those of the authentic compounds.

The molecular formula of compound **1** was determined as $C_{25}H_{34}O_2$ by HRFABMS in conjunction with NMR analysis. The ^1H NMR spectrum of **1** showed the signals

Keywords: Ophiobolin; Marine fungus; *Emericella variecolor*; Cytotoxic; Sesterterpene.
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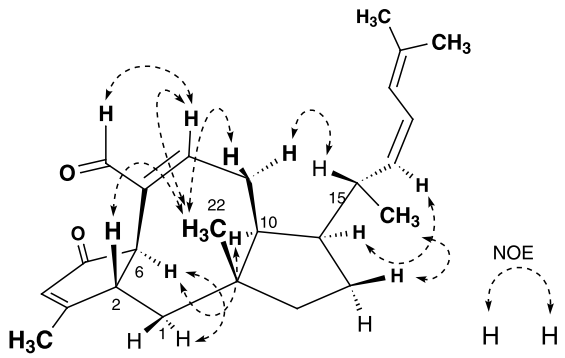


Figure 1. Key NOE correlations of 6-epi-ophiobolin G (**1**).

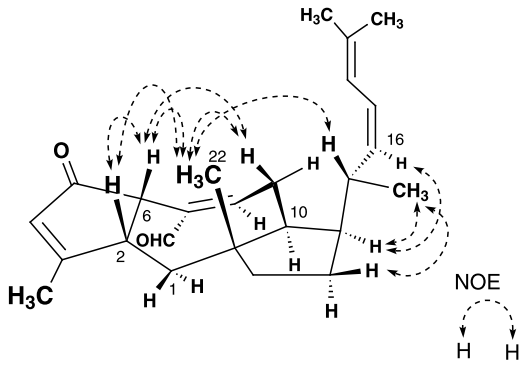


Figure 2. Key NOE correlations of ophiobolin G (**2**).

due to one aldehyde proton, five olefinic protons, four singlet methyl protons (three for vinylic methyls and one for angular methyl), and one doublet methyl protons. Detailed interpretation of a combination of $^1H-^1H$ COSY, HMQC, and HMBC spectral data of **1** revealed that **1** is a sesterterpene having a tricyclic ophiobolane skeleton,[1] which consists of one each of ketone and aldehyde, eight olefinic carbons (five of them are carbons bearing a proton, and other three are quaternary carbons), one quaternary carbon, five methine carbons, four methylene carbons, and five methyl carbons. The ^1H and ^{13}C NMR data of compound **1** closely resembled those of ophiobolin G (**2**). These evidences indicated that compound **1** is a stereoisomer of ophiobolin G (**2**). The stereostructure of **1** was elucidated by detailed analysis of the NOESY spectra of **1** and **2**. Thus, the NOESY spectrum of **1** showed the NOE correlations from H-6 (δ_H 3.38, brs) to H-10 (δ_H 2.61, m) and H-1α (δ_H 1.15, t); H_3-22 (δ_H 0.85, s) to H-2 (δ_H 2.65, m), H-8 (δ_H 6.79, d), and H-9β (δ_H 2.20, m), and the lack of NOE from H-6 to H-2 gave a clear indication that the A/B ring is *trans*-fused and the H-2 proton is on the same side with the C-22 methyl group (Fig. 1). On the other hand, the A/B-*cis* ring structure in ophiobolin G (**2**) was deduced from the strong NOESY correlation between H-2 (δ_H 3.11, brs) and H-6 (δ_H 4.16, brs) (Fig. 2). These evidences indicated that compound **1** is the 6-epi isomer of ophiobolin G (**2**). It has been reported that the C-1 carbon of ophiobolin having an A/B-*cis* ring structure resonates at higher field in comparison with the A/B-*trans* ophiobolin[11] and the proton signal at C-2 of the 6-epi isomer having H-6α is shielded by ca. 0.2–0.3 ppm in comparison with the A/B-*cis* isomer having H-6β.[12] These phenomena were also observed in this study (Tables 1 and 2); thus, the C-1 carbon and the H-2 proton of compound **1** and ophiobolin G (**2**) were observed at δ_C 46.1 and δ_C 35.7; δ_H 2.65 (m) and δ_H 3.11 (brs), respectively. The orientation of H-6 in ophiobolin G (**2**) has been previously reported to be α. Based on the above findings, the orientation of the H-6 proton in ophiobolin G

Table 1. ^1H NMR data for 6-epi-ophiobolin G (**1**), ophiobolin G (**2**), 6-epi-ophiobolin N (**3**), ophiobolin H (**4**), 6-epi-ophiobolin C (**5**), ophiobolin C (**6**), 6-epi-ophiobolin K (**7**), and ophiobolin K (**8**). (600 MHz in $CDCl_3$, δ_H (mult., *J* (Hz)))

	1	2	3	4	5	6	7	8
1α	1.15 (t, 13.2)	1.34 m	1.16 (t, 13.0)	1.42 m	1.56 m	1.23 m	1.51 m	1.21 m
1β	2.03 m	1.89 m	2.04 m	1.52 m	1.76 m	1.78 m	1.83 m	1.77 m
2	2.65 m	3.11 brs	2.68 m	2.24 m	2.15 m	2.36 m	2.13 m	2.36 m
4	6.02 s	6.05 s	6.04 s	2.06 (d, 13.5)	2.42 (d, 16.5)	2.47 (d, 20.1)	2.44 (d, 16.4)	2.51 (d, 18.9)
				2.15 (d, 13.5)	3.08 (d, 16.5)	2.77 (d, 20.1)	3.04 (d, 16.4)	2.78 (d, 18.9)
6	3.38 brs	4.16 brs	3.45 (d, 4.3)	3.15 m	3.35 (d, 10.3)	3.24 (d, 9.6)	3.24 (d, 11.0)	3.26 (d, 10.3)
8	6.79 (d, 6.1)	7.06 m	6.84 (d, 4.4)	5.62 m	6.89 (d, 5.5)	7.18 (t, 8.5)	6.80 (d, 4.9)	7.11 (t, 8.5)
9α	2.92 (d, 20.6)	2.88 (d, 19.1)	2.71 m	2.46 m	2.61 m	2.28 m	2.79 m	2.11 m
9β	2.20 m	2.30 m	2.23 m	1.69 m	2.21 m	2.42 m	2.36 (d, 17.1)	2.95 m
10	2.61 m	1.90 m	2.71 m	1.59 m	2.61 m	2.63 m	2.49 m	1.59 m
12	1.44 m	1.34 m	1.43 m	1.36 m	1.47 m	1.39 m	1.40 m	1.40 m
	1.52 m	1.40 m	1.51 m	1.42 m	1.47 m	1.43 m	1.45 m	1.40 m
13α	1.28 m	1.27 m	1.25 m	1.27 m	1.16 m	1.43 m	1.18 m	1.25 m
13β	1.67 m	1.66 m	1.61 m	1.73 m	1.56 m	1.52 m	1.61 m	1.61 m
14	1.88 m	1.83 m	1.74 m	2.06 m	1.76 m	2.36 m	1.82 m	2.07 m
15	2.58 m	2.51 m	1.43 m	2.67 m	1.47 m	1.62 m	2.47 m	2.71 m
16	5.10 (t, 10.5)	5.11 m	0.99 m	5.19 m	0.98 m	1.15 m	5.06 (t, 11.2)	5.18 (t, 9.7)
			1.43 m		1.47 m	1.23 m		
17	6.09 (t, 10.5)	5.98 m	1.93 m	5.98 m	1.91 m	1.95 m	6.02 (t, 11.2)	6.01 m
			1.93 m		2.07 m	2.07 m		
18	6.00 (d, 10.5)	5.98 m	5.12 (t, 6.9)	5.98 m	5.12 (t, 6.9)	5.07 (t, 6.9)	5.95 (d, 11.2)	5.97 m
20	2.06 s	2.21 s	2.07 s	1.22 s	1.44 s	1.33 s	1.37 s	1.34 s
21	9.27 s	9.38 s	9.31 s	4.59 (d, 12.1)	9.20 s	9.20 s	9.09 s	9.21 s
				4.78 (d, 12.1)				
22	0.85 s	0.78 s	0.86 s	0.89 s	0.84 s	0.88 s	0.76 s	0.95 s
23	0.96 (d, 6.6)	0.86 (d, 6.6)	0.90 (d, 6.6)	0.87 (d, 6.6)	0.89 (d, 6.6)	0.76 (d, 6.6)	0.91 (d, 6.6)	0.92 (d, 6.6)
24	1.76 s	1.74 s	1.61 s	1.72 s	1.60 s	1.58 s	1.69 s	1.74 s
25	1.82 s	1.82 s	1.69 s	1.80 s	1.69 s	1.66 s	1.76 s	1.81 s

Table 2. ^{13}C NMR data for 6-epi-ophiobolin G (**1**), ophiobolin G (**2**), 6-epi-ophiobolin N (**3**), ophiobolin H (**4**), 6-epi-ophiobolin C (**5**), ophiobolin C (**6**), 6-epi-ophiobolin K (**7**), and ophiobolin K (**8**) (150 MHz in $CDCl_3$, δ_C)

	1	2	3	4	5	6	7	8
1	46.1	35.7	45.8	35.9	41.6	36.1	41.4	35.1
2	49.3	48.8	49.0	50.9	49.7	50.9	49.6	50.3
3	177.6	177.4	177.5	80.0	76.7	76.8	76.7	76.9
4	130.5	130.7	130.2	50.8	55.2	54.8	55.0	54.9
5	207.7	207.1	207.4	116.5	217.1	217.4	216.9	217.2
6	50.2	48.3	50.0	52.8	49.1	48.5	48.9	48.6
7	140.2	137.7	140.4	138.8	142.0	141.5	141.4	141.4
8	158.1	160.3	156.9	123.3	159.8	164	160.5	163.9
9	31.1	29.6	31.0	24.9	31.2	24.8	30.8	25.5
10	44.0	46.4	43.1	55.0	43.3	53.5	43.8	53.6
11	45.6	46.0	45.0	43.5	44.6	43.9	44.7	43.9
12	44.5	40.3	44.6	42.9	45.7	42.6	45.7	42.6
13	27.9	27.8	27.1	26.6	27.4	22.9	27.7	26.5
14	52.3	46.4	51.5	47.1	51.8	45.3	52.1	47.2
15	32.9	35.3	31.8	35.5	32.2	32.8	32.6	35.3
16	135.9	137.4	37.2	137.9	37.2	37	135.6	137.1
17	124.2	122.3	25.7	121.7	26.0	26.0	124.0	122.5
18	120.1	120.2	124.4	120.3	124.7	124.5	120.0	120.0
19	136.7	135.7	131.6	135.0	131.7	131.4	136.4	136.1
20	17.4	18.7	17.2	25.6	26.1	25.5	25.9	25.7
21	193.2	194.9	193.0	71.5	194.5	196.2	194.7	196.2
22	23.1	24.5	23.1	18.7	23.7	19.0	23.3	18.7
23	21.4	20.6	18.6	20.2	18.9	16.5	21.3	20.4
24	18.2	18.1	17.7	18.1	18.0	17.6	18.2	18.1
25	26.7	26.4	25.7	26.4	25.9	25.7	26.5	26.6

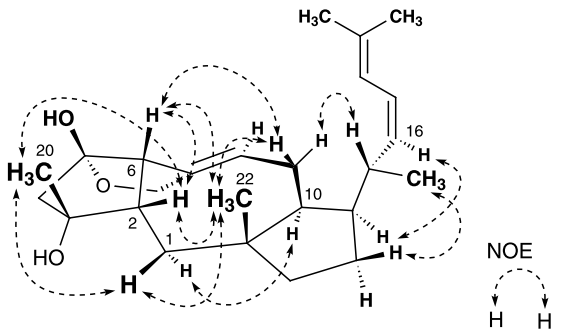
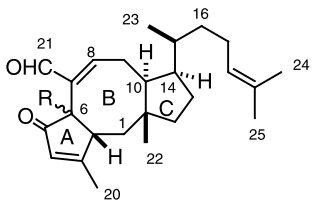


Figure 3. Key NOE correlations of ophiobolin H (**4**).

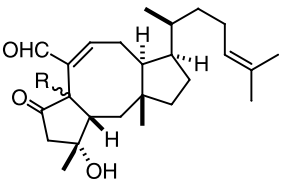
(**2**) should be revised to be β and compound **1** should be 6-epi-ophiobolin G having H-6α.

Compound **3** named 6-epi-ophiobolin N has a molecular formula of $C_{25}H_{36}O_2$ as determined by HRFABMS. The proton and carbon signals ascribable to the A and B rings in **3** were closely similar to those of **1**, while other signals were almost identical to those of 6-epi-ophiobolin C (**5**) (Tables 1 and 2). This suggested that **3** has a hybrid structure of **1** and **5**. NOE correlations from H-6 (δ_H 3.45, d) to H-10 (δ_H 2.71, m) and H-1α (δ_H 1.16, t); H_3-22 (δ_H 0.86, s) to H-1β (δ_H 2.04, m), H-2 (δ_H 2.68, m), H-8 (δ_H 6.84, d), and H-9β (δ_H 2.23, m) in the NOESY spectrum of **3** indicated that **1** and **3** share the same stereostructure. Consequently, the structure of compound **3**, 6-epi-ophiobolin N, was clarified to be a 16,17-dihydro analogue of 6-epi-ophiobolin G (**1**), and also the 6-epi isomer of the previously reported congener named anhydrozizanin A.[8]

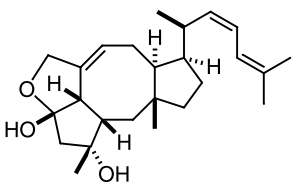
The physical data of compound **4** were identical with those of ophiobolin H.[7] The unsolved stereochemistry of ophiobolin H (**4**) led us to do further structural examination of this compound. The NOESY spectrum of **4** exhibited NOE correlations from H-6 (δ_H 3.15, m) to H-2 (δ_H 2.24, m), H-9β (δ_H 1.69, m), and H_3-22 (δ_H 0.89, s); H-10 (δ_H 1.59, m) to H-1α (δ_H 1.42, m) and H-14 (δ_H 2.06, m) indicating a *cis* fusion of the ring A/B. Further observation of the NOE correlations from H_3-20 (δ_H 1.22, s) to H-2 (δ_H 2.24, m) and H-1β (δ_H 1.52, m) suggested that the methyl group at C-3 is β-orientation (Fig. 3). This was corroborated by the fact that the reduction of ophiobolin K (**8**)[10] with $CeCl_3/6H_2O$ and $NaBH_4$ afforded a single product, which was identical with ophiobolin H (**4**) on the basis of HPLC, TLC, ^1H NMR, and HRFABMS comparison. Furthermore, the orientation of the hydroxyl group at C-5 could be deduced as β, since the stereostructure having 5β-hydroxyl group, which was shown in Figure 3, is only reasonable one to be able to explain the presence of the above NOE correlations. Based on these findings, the unsolved



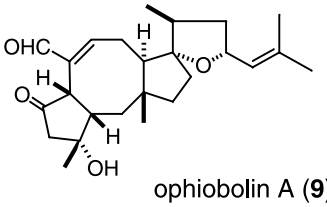
R = α-H, 16-*cis* 6-epi-ophiobolin G (**1**)
R = ß-H, 16-*cis* ophiobolin G (**2**)
R = α-H 6-epi-ophiobolin N (**3**)



R = α-H 6-epi-ophiobolin C (**5**)
R = ß-H ophiobolin C (**6**)
R = α-H, 16-*cis* 6-epi-ophiobolin K (**7**)
R = ß-H, 16-*cis* ophiobolin K (**8**)



ophiobolin H (**4**)



ophiobolin A (**9**)

Chart 1.

Table 3. Cytotoxic activity of ophiobolin K (**8**) against various cultured tumor cells

Compound	IC_{50} value (μM)							
	T-47D	MDA-MB-231	HOP18	NCI-H460	HCT116	ACHN	P388	P388/ADR[a]
Adriamycin	0.048	0.095	0.11	0.0061	0.055	0.048	0.012	2.56
Ophiobolin K (**8**)	0.35	0.57	0.65	0.57	0.33	0.27	0.51	0.36

[a] Adriamycin-resistant cells.

stereochemistry at C-3, C-5, and C-6 of ophiobolin H (**4**) was determined to be as depicted in Chart 1.

The compounds obtained here are well correlated, and all can be considered as congeners of ophiobolin A (**9**). The stereochemistry at C-14 and C-15 and the absolute stereostructure were deduced from those of ophiobolin A,[2,11] whose absolute stereostructure was determined by X-ray crystallography of its bromo-methoxy derivative, and ophiobolin C,[9] of which asymmetric total synthesis has been accomplished.

These compounds showed cytotoxicity against the neuroblastoma cell line, Neuro 2A. The treatment of 1–3 μM of these compounds induced cell death accompanied by shrinkage in cell soma and chromatin condensation at 12 or 24 h after drug application. Ophiobolin K (**8**) was further tested with various cultured cell lines. As showed in Table 3, **8** showed seven times stronger cytotoxic activities against P388/ADR tumor cells than adriamycin.

The liquid culture of the GF10 strain in the MG medium produced ophiobolins in poor yield (0.1–0.6 mg/L for compounds **1**–**4**, 2–3 mg/L for compounds **5**–**8**). On the other hand, the culture in the solid-state medium based on cereals produced ophioblins in higher yield. In the case of the rice medium or soybean medium, 0.5–1.5 mg of **1**–**4** and 5–10 mg of **5**–**8** were produced in both 100 g medium, while in the cases of the barley medium, corn medium, or potato medium, 1–5 mg of **1**–**4** and 10–30 mg of **5**–**8** were produced in each 100 g medium, respectively.

3. Experimental

3.1. General experimental procedures

NMR spectra were recorded on a Varian Unity Inova 600 (600 MHz) spectrometer using the solvent peak as the internal standard. Spots on TLC were detected by spraying 1% $Ce(SO_4)_2$/10% H_2SO_4 [1 g $Ce(SO_4)_2$, 100 mL 10% aq. H_2SO_4] with subsequent heating. Artificial seawater was prepared by Aquamarine (Yashima Pure Chemical Co. LTD, Japan). Other instruments used to obtain physical data and the experimental conditions for chromatography were the same as in our previous paper.[5]

3.2. Fungus material, culture conditions, and extraction

The *E. variecolor* GF10 strain was separated from the marine sediment collected from a depth at 70 m in Gokasyo Gulf, Mie Prefecture, Japan, in 2002 and deposited in our laboratory. The GF10 strain was classified as *E. variecolor* from its cultural characteristics and 16S rDNA sequence. MG medium (malt extract: 20 g, glucose: 20 g, bact peptone: 1 g, artificial seawater: 1000 mL) was used as seed medium and liquid medium. Rice solid medium (rice: 25 g, artificial seawater: 50 mL, in a 500 mL flask), barley solid medium (barley: 15 g, artificial seawater: 25 mL, in a 500 mL flask), soybean solid medium (soybean: 50 g, artificial seawater: 75 mL, in a 500 mL flask), corn solid medium (canned corn: 100 g, solid Aquamarine: 1.4 g, liquid Aquamarine: 1 mL, in a 500 mL flask), and potato solid medium (sliced fresh potato: 100 g, solid Aquamarine: 1.4 g, liquid Aquamarine: 1 mL, in a 500 mL flask) were used as solid medium. They were all autoclaved before use. The GF10 strain was cultured in the seed medium at 30 °C for 5 days. Then, the broth of the strain was inoculated into the production medium and cultured under static conditions at 30 °C for 2 weeks. The culture of the MG medium was filtered, and then the filtrate was partitioned with 2-butanone, and the residue was extracted with acetone. The organic extracts were combined and evaporated under reduced pressure to give an extract, which was further partitioned into an EtOAc–H_2O mixture. The EtOAc layer was evaporated under reduced pressure to give an EtOAc extract. For solid-state fermentation, the culture was extracted with acetone and a mixed solvent (EtOAc–MeOH–acetone, 1:2:4), and then the organic solvent was combined and evaporated under reduced pressure to give an extract. The extract was partitioned into an EtOAc–H_2O mixture, and the EtOAc layer was evaporated under reduced pressure to afford an EtOAc extract.

3.3. Isolation of ophiobolins (1–8)

The EtOAc extract (4.5 g) of the MG medium culture (1 L×10) was fractionated by SiO_2 column chromatography (*n*-hexane–EtOAc) to give five fractions (A–E). The active fraction C (70 mg) was further separated by reversed-phase HPLC (Cosmosil 5C18-AR, 10×250 mm, MeOH–H_2O=85:15) to furnish 6-epi-ophiobolin K (**7**, 12 mg), ophiobolin K (**8**, 11 mg), 6-epi-ophiobolin C (**5**, 9 mg), 6-epi-ophiobolin G (**1**, 2 mg), 6-epi-ophiobolin C (**6**, 10 mg), and 6-epi-ophiobolin N (**3**, 3 mg). The purification of the EtOAc extract (3.4 g) of the barley solid medium culture (barley 40 g×20) by the same procedure gave ophiobolin G (**2**, 15 mg), 6-epi-ophiobolin G (**1**, 9 mg), ophiobolin H (**4**, 8 mg), and 6-epi-ophiobolin N (**3**, 9 mg). For quantitative analyses, the EtOAc extracts were fractionated by SiO_2 column chromatography (*n*-hexane–EtOAc) and the fractions containing ophiobolins were analyzed by HPLC (Cosmosil 5C18-AR, 10×250 mm, MeOH–H_2O=80:20, UV 230 nm).

3.3.1. 6-epi-Ophiobolin G (1). Amorphous powder; $[\alpha]_D^{23}$=+117° (*c* 1.05, MeOH); IR (KBr) ν_{max} cm^{-1}: 2965, 1705, 1680; UV ($CHCl_3$) λ_{max} (ε): 228 nm (27000); ^1H and

^{13}C NMR data: shown in Tables 1 and 2; FABMS: *m/z* 389 [(M+Na)$^+$]; HRFABMS: found *m/z* 389.2466. Calcd for $C_{25}H_{34}O_2Na$: 389.2456.

3.3.2. Ophiobolin G (2). Amorphous powder; $[\alpha]_D^{23}$=+26° (*c* 0.88, MeOH); UV ($CHCl_3$) λ_{max} (ε): 227 nm (29700); ^{1}H and ^{13}C NMR: data shown in Tables 1 and 2; FABMS: *m/z* 367 [(M+H)$^+$]; HRFABMS: found *m/z* 367.2642. Calcd for $C_{25}H_{35}O_2$: 367.2637.

3.3.3. 6-epi-Ophiobolin N (3). Amorphous powder; $[\alpha]_D^{23}$=+88° (*c* 0.34, MeOH); IR (KBr) ν_{max} cm^{-1}: 2970, 1702, 1670; UV ($CHCl_3$) λ_{max} (ε): 226 nm (19000); ^{1}H and ^{13}C NMR: data shown in Tables 1 and 2; FABMS: *m/z* 391 [(M+Na)$^+$]; HRFABMS: found *m/z* 391.2634. Calcd for $C_{25}H_{36}O_2Na$: 391.2613.

3.3.4. Ophiobolin H (4). Amorphous powder; $[\alpha]_D^{23}$=+44° (*c* 0.12, MeOH); UV ($CHCl_3$) λ_{max} (ε): 240 nm (16800); ^{1}H and ^{13}C NMR: data shown in Tables 1 and 2; FABMS: *m/z* 409 [(M+Na)$^+$]; HRFABMS: found *m/z* 409.2717. Calcd for $C_{25}H_{38}O_3Na$: 409.2719.

3.4. Reduction of ophiobolin K (8)

A solution of **8** (1 mg) in EtOH (0.2 mL) was treated with $CeCl_3/6H_2O$ (2 mg) and $NaBH_4$ (2 mg), and the mixture was stirred at 0 °C for 0.5 h. The reaction mixture was diluted with 5 mL of mixed solvent (*n*-hexane–EtOAc=2:1) and then filtered with a silica gel pad. The filtrate was evaporated under reduced pressure, and the resulting residue was purified by HPLC (Cosmosil 5SL, 10×250 mm, *n*-hexane–EtOAc, 4:1) to obtain a product (0.8 mg), which was identified with ophiobolin H (**4**) by HPLC, TLC, ^{1}H NMR, and HRFABMS.

3.5. Assay for activity in Neuro 2A cells

Neuro 2A cells were grown in Dulbecco's modified essential medium (DMEM) with 10% fetal bovine serum (FBS). The cells were kept in incubator at 37 °C with 5% CO_2. The cells were plated on 24-well plates at a density of 2×10^4 per well with 1 mL of culture medium. After 24 h cultivation, the medium was exchanged for fresh medium, and the testing sample in 10 μL of EtOH was added to each well. After 12 or 24 h incubation, morphological changes in the cells were observed under microscope.

3.6. Assay for cytotoxic activity

NCI-H460, HOP18 (human lung carcinoma), MDA-MB-231, T-47D (human breast carcinoma), ACHN (human 'renal carcinoma), HCT116 (human colon carcinoma), P388 (mouse leukemia cells) and P388/ADR (adriamycin resistant cells) were cultured in RPMI-1640 medium supplemented with 10% FBS. All cells were maintained at 37 °C with 5% CO_2. Cells were seeded into 96-well plates (1×10^4 cells/well) and incubated for 24 h. The test sample, dissolved in DMSO, was added in serial dilutions and the cells were further incubated for 72 h. In vitro cytotoxic activity was evaluated by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay or WST-1 [5-(2,4-disulfophenyl)-2-(4-iodophenyl)-2H-tetrazolium, inner salt, sodium salt] assay.

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Application of intramolecular cycloaddition/retro cycloaddition reactions for the synthesis of unsymmetrical 2,2′-bipyridine and 2-benzofuropyrazin-2-ylpyridine analogues☆

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Abstract—1,2,4-Triazines bearing cycloalkeno[*c*]pyridine substituents at the 5-position, **2a**–**d**, prepared by an intermolecular Diels–Alder reaction of bi-5,5-triazines with cyclic enamines, were provided with an alkynyloxy or a 2-cyanophenoxy group at the 3-position of the triazinyl unit. A subsequent intramolecular Diels–Alder reaction of the former, followed by loss of N_2 leads to two new classes of 2,2′-bipyridine analogues containing different heterocyclic units, namely cycloalkeno[*c*]pyridine and 2,3-dihydrofuro- or 2,3-dihydropyrano[2,3-*b*]pyridine **8a**–**h**; the intramolecular reaction of the 2-cyanophenoxy compound gives benzo[4,5]furo[2,3-*b*]pyrazine **10a**–**c**.

1. Introduction

Aromatic biheterocycles and their complexes with metal ions have found extensive use in coordination and supramolecular chemistry.[2] Particularly interesting and useful are functionalized 2,2′-bipyridines which have been shown to exhibit important applications in the area of catalysis,[3] metal containing polymers,[4] molecular electronics[5] and optoelectronic devices[6] and as photoactivated species.[7] Also monofunctionalized or unsymmetrical bisfunctionalized 2,2′-bipyridines have received considerable attention. These compounds are frequently used as reactive intermediates[8] or fine products[9] and are attractive building blocks for supramolecular chemistry.[10]

Various methods exist for the synthesis of unsymmetrical bipyridines, of which the transition-metal catalyzed heteroaryl cross-coupling reactions of specially prepared pyridines[11] or Krönke–Potts[12,13] and Friedlander strategies[14] are the most often employed. However, recent efforts have centered around the synthesis of unsymmetrical 2,2′-bipyridines by inverse electron demand [4+2] cycloaddition/retro-cycloaddition Diels–Alder (DA-rDA) reaction of suitably substituted 1,2,4-triazines.[15] In previous reports we demonstrated the use of dimeric 1,2,4-triazines **1** as precursors for the preparation of a range of symmetrical **3** and unsymmetrical, annulated 2,2′-bipyridines (**4**, **5**)[16–19] (Scheme 1). This approach is based on the regioselective, intermolecular DA-rDA reaction of **1** with cyclic enamines to give a common intermediate **2**. Subsequent treatment of **2** with an appropriate enamine leads to **3**, **4** or **5** depending on the dienophile used. The presence of alkylsulfanyl substituent in **2** makes these compounds attractive starting materials for the synthesis of more elaborated biheterocycles, because this group can be easily converted into an alkylsulfonyl group, which is more reactive toward nucleophilic reagents.

We now show that intramolecular DA-rDA reactions of **2**, substituted with a dienophilic side chain on the 1,2,4-triazine ring, provide ready access to unsymmetrical 2,2′-bipyridines **8a**–**h** consisting of two different heterocyclic units: cycloalkeno[*c*]pyridines and dihydrofuro[2,3-*b*]- or dihydropyrano[2,3-*b*]pyridines. The furo- and pyranopyridine functionalities have emerged as useful pharmacophores in several therapeutic areas.[20] This approach evolved from the developments in 1,2,4-triazine annulation chemistry[21] and from the high reactivity of this heterocycle toward nucleophilic displacements.[22] The essential features of the strategy are summarized in the sequence depicted in Scheme 2, wherein methylsulfonyl derivatives **6a**–**d** were envisaged as a key intermediates and the primary subgoals of the project.

Nucleophilic replacement of methylsufinate from the latter

☆ See Ref. 1.

Keywords: Dimeric triazines; Intramolecular Diels–Alder reaction; 2,2′-Bipyridine analogues.
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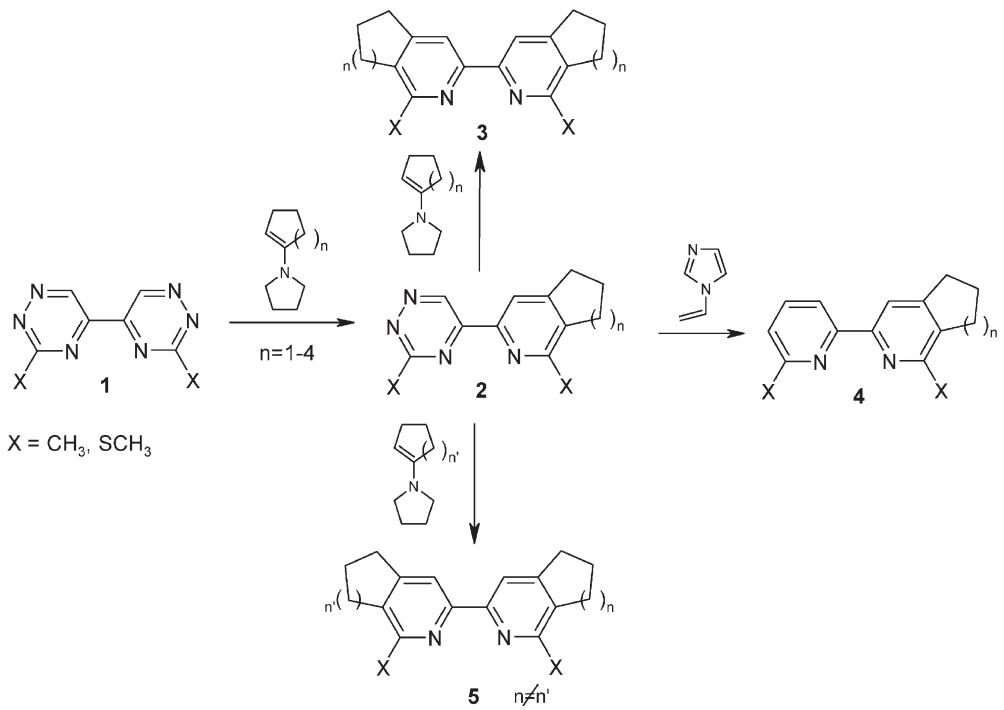
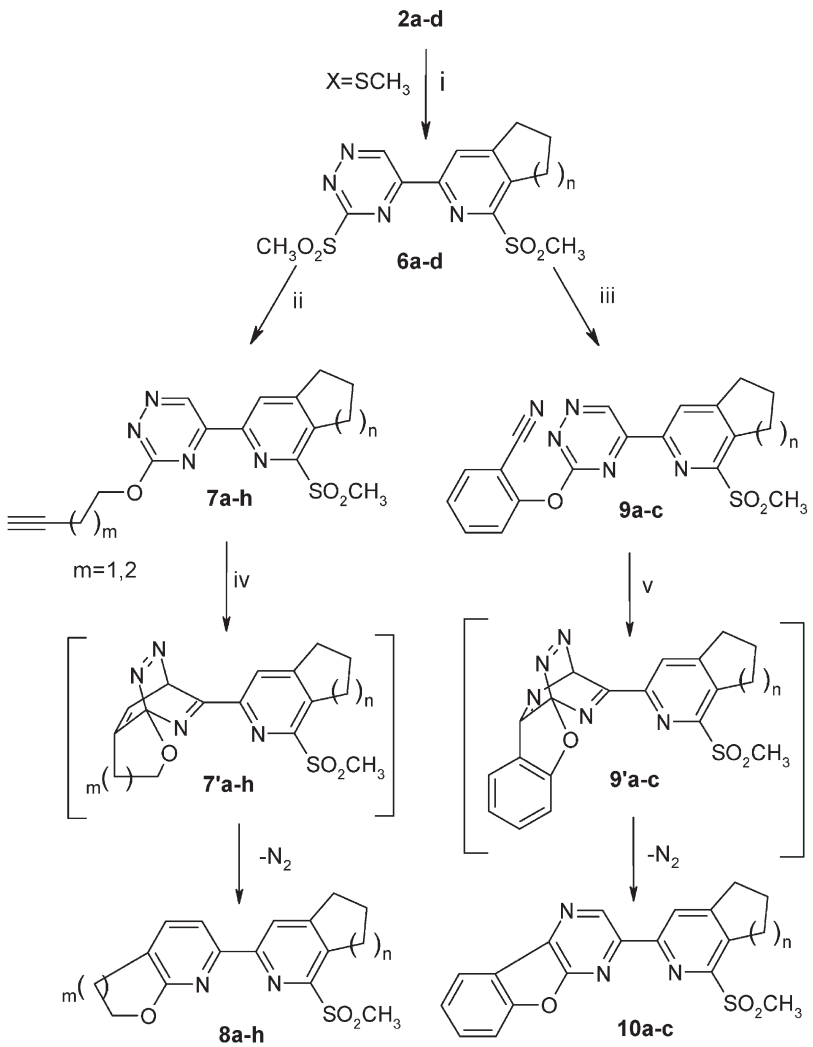


Scheme 1.



Scheme 2. (i) $KMnO_4/H_2O/C_6H_6/t\text{-}Bu_4N^+Br^-$; (ii) 4-pentyn-1-ol or 3-butyn-1-ol, NaH, DMA; (iii) 2-cyanophenol, THF, 0 °C; (iv) bromobenzene, reflux; (v) nitrobenzene, reflux.

Table 1. Yields, melting points of compounds **6a–d**, **7a–h** and **8a–h**

Compound	*n*	*m*	Time [h]	Yield [%]	Mp [°C]	Compound	*n*	*m*	Time [h]	Yield [%]	Mp [°C]
6a	1	—	3	64	242–243	**7g**	3	2	1.5	76	101–102
6b	2	—	3	79	306–307	**7h**	4	2	2.5	81	141–142
6c	3	—	3	93	243–244	**8a**	1	1	21	37	236–237
6d	4	—	3	98	258–259	**8b**	2	1	12	85	216–217
7a	1	1	0.6	38	169–170	**8c**	3	1	2	80	215–216
7b	2	1	0.3	75	179–180	**8d**	4	1	3	80	236–237
7c	3	1	0.5	95	161–162	**8e**	1	2	41	33	263–264
7d	4	1	0.5	80	178–179	**8f**	2	2	46	55	254–255
7e	1	2	1	29	156–157	**8g**	3	2	103	83	242–243
7f	2	2	1.5	30	153–154	**8h**	4	2	76	84	253–254

with alkoxides bearing an acetylene unit at the terminus should provide the desired 1,2,4-triazine derivatives **7a–h** with the appropriate dienophilic side chain, which may be converted into the target molecules **8a–h** via an intramolecular DA-rDA reaction (Scheme 2).

2. Results and discussion

The required **2a–d** (X=SCH_3) were obtained via a regioselective intermolecular DA-rDA reaction of easily available 3,3′-dimethylsulfanyl-5,5′-bi-1,2,4-triazine **1** with cyclic enamines according to our published method.[18] The sulfides **2a–d** were efficiently oxidized to the methylsulfones **6a–d**. The highest yields of the latter, isolated as precipitated solids from the reaction mixtures, were obtained when the oxidation reaction was carried out with potassium permanganate under phase transfer catalytic conditions. When the reaction of **2a–d** with the oxidizing agent was followed by TLC, it became evident that the intermediates were quickly converted into the desired sulfones **6a–d**. Condensation of these compounds in crude form with the sodium salts of 4-hydroxy-1-butyne and 5-hydroxy-1-pentyne in DMF at 0 °C led smoothly, in a chemoselective manner to 3-(3-butynyloxy)- **7a–d** and 3-(4-pentynyloxy)-1,2,4-triazin-3-yl-cycloalkeno[*c*]pyridines **7e–h**, respectively. These low temperature conditions were necessary in order to avoid the nucleophilic replacement of methylsulfinate from the pyridine part of **6a–h**, since electron deficient azines bearing an alkylsulfonyl substituent easily undergo nucleophilic substitution with a wide variety of nucleophiles.[22] Compounds **7a–d** underwent an intramolecular DA-rDA reaction in refluxing bromobenzene within 0.5 h to give the desired dihydrofuro[2,3-*b*]pyridines **8a–d** in moderate to good yield. It should be noted that methylsulfonyl function in **8a–d** may potentially be utilized as a handle for the introduction of further functionality through ipso nucleophilic substitution reactions. Finally, thermolysis of **7e–h** in refluxing bromobenzene afforded the corresponding dihydropyrano[2,3-*b*]pyridines **8e–h** in good yield. The formation of a 6-membered ring in **8e–h** is less favorable, and more time for completion is required than the reaction which yields a 5-membered ring. The structures of all products were confirmed by spectroscopic methods and microanalysis. Table 1 shows the reaction conditions, yields and melting points of compounds **6**, **7** and **8**.

The intermolecular/intramolecular retro Diels–Alder reaction method described above can easily be extended to the synthesis of more elaborate heteroaryl-condensed pyridines **10a–c**. In this case 2-cyanophenol was selected as potential dienophile. Nucleophilic displacement of methylsulfinate from **2a–d** with sodium 2-cyanophenoxide (generated in situ by treatment of 2-cyanophenol with sodium hydride in anhydrous DMF) afforded the corresponding cyanophenoxy derivatives **9a–c** in good yield. Heating of **9a–c** in nitrobenzene at reflux for 16 h gave the desired pyrazinylpyridine analogues **10a–c** (Table 2).

Table 2. Yields, melting points of compounds **9a–c** and **10a–c**

Compound	*n*	Time [h]	Yield [%]	Mp [°C]
9a	1	3	53	220–222
9b	2	3	80	222–223
9c	3	3	70	216–217
10a	1	4	52	250–251
10b	2	16	75	297–298
10c	3	4	54	260–261

In conclusion, we have developed the above-mentioned synthetic strategy for the preparation of unsymmetrical 2,2′-bipyridine analogues consisting of different cycloalkenopyridines and dihydrofuro[2,3-*b*]- or dihydropyrano[2,3-*b*]pyridines and heteroaryl-condensed pyridines. The synthesized compounds contain a methylsulfonyl group on the cycloalkeno[*c*]pyridine ring which opens access to further substituted molecules.

3. Experimental

Melting points are uncorrected. IR spectra were measured with a Magna IR-760 spectrophotometer. The ^{1}H NMR spectra were recorded in deuteriochloroform on a Varian-Gemini 200 MHz spectrometer. Mass spectra were measured with an AMD 604 (AMD Intectra GmbH, Germany) spectrometer [electron impact and liquid secondary ion mass spectrometry (LSIMS) methods]. Elemental analyses were recorded on Perkin–Elmer 2400-CHN analyzer. Column chromatography was performed on silica gel (230–400 mesh, 60 Merck). All solvents used were dried and distilled before use according to standard procedures.[23] Merck 60F_{254} plates were used for analytical (TLC) chromatography.

3.1. General procedure for the oxidation of methylsulfanyl derivatives 2a–d to methylsulfonyl derivatives 6a–d

A solution of $KMnO_4$ (12 mmol) in water (32 ml) was added to a solution of **2a–d** (1 mmol) and catalytic amounts of *t*-$Bu_4N^+Br^-$ (0.005 g) in a mixture of AcOH (3 ml) and benzene (37 ml). The reaction mixture was stirred at room temperature for 3 h. A saturated solution of $Na_2S_2O_5$ in water was added to the mixture until the purple color disappeared. The organic layer was separated and water phase was extracted (3×50 ml) with benzene. The organic layers were combined and dried over $MgSO_4$. After removal of the solvent the crude compound was used to the next step without purification.

3.1.1. 1-Methylsulfonyl-3-(3-methylsulfonyl-1,2,4-triazin-5-yl)-6,7-dihydro-5*H*-cyclopenta[*c*]pyridine 6a. Yellow crystals, mp 242–243 °C; IR (KBr) ν_{max}/cm^{-1} 2980, 1339 (SO_2), 1150 (SO_2); ^1H NMR ($CDCl_3$): δ=2.09–2.35 (m, 2H, CH_2), 3.1 (t, 2H, *J*=6.4 Hz, CH_2), 3.45 (t, 2H, *J*=6.2 Hz, CH_2), 3.46 (s, 3H, SO_2Me), 3.50 (s, 3H, SO_2Me), 8.51 (s, 1H, pyridine-H), 10.21 (s, 1H, triazine-H); HRMS (EI): *m*/*z* calcd for $C_{13}H_{14}O_4N_4S_2$ (M^+), 354.0456; found, 354.0446.

3.1.2. 1-Methylsulfonyl-3-(3-methylsulfonyl-1,2,4-triazin-5-yl)-5,6,7,8-tetrahydroisoquinoline 6b. Yellow crystals, mp 306–307 °C; IR (KBr) ν_{max}/cm^{-1} 2970, 1340 (SO_2), 1150 (SO_2); ^1H NMR ($CDCl_3$) δ=1.87–1.97 (m, 4H, 2×CH_2), 3.2 (t, 2H, *J*=6.4 Hz, CH_2), 3.38 (t, 2H, *J*=6.19 Hz, CH_2), 3.52 (s, 3H, SO_2Me), 3.62 (s, 3H, SO_2Me), 8.60 (s, 1H, pyridine-H), 10.25 (s, 1H, triazine-H); HRMS (EI): *m*/*z* calcd for $C_{14}H_{16}O_4N_4S_2$ (M^+), 368.0613; found, 368.0600.

3.1.3. 1-Methylsulfonyl-3-(3-methylsulfonyl-1,2,4-triazin-5-yl)-6,7,8,9-tetrahydro-5*H*-cyclohepta[*c*]pyridine 6c. Yellow crystals, mp 243–244 °C; IR (KBr) ν_{max}/cm^{-1} 2923, 1329 (SO_2), 1140 (SO_2); ^1H NMR ($CDCl_3$): δ=1.65–2.05 (m, 6H, 3×CH_2), 3.04 (t, 2H, *J*=6.3 Hz, CH_2), 3.48 (t, 2H, *J*=6.1 Hz, CH_2), 3.52 (s, 3H, SO_2Me), 3.60 (s, 3H, SO_2Me), 8.70 (s, 1H, pyridine-H), 10.31 (s, 1H, triazine-H); HRMS (EI): *m*/*z* calcd for $C_{15}H_{18}O_4N_4S_2$ (M^+), 382.0769; found, 382.0757.

3.1.4. 1-Methylsulfonyl-3-(3-methylsulfonyl-1,2,4-triazin-5-yl)-5,6,7,8,9,10-hexahydrocycloocta[*c*]pyridine 6d. Yellow crystals, mp 258–259 °C; IR (KBr) ν_{max}/cm^{-1} 2940, 1340 (SO_2), 1140 (SO_2); ^1H NMR ($CDCl_3$): δ=1.58–1.78–2.15 (m, 8H, 4×CH_2), 3.01 (t, 2H, *J*=6.3 Hz, CH_2), 3.39 (t, 2H, *J*=6.1 Hz, CH_2), 3.55 (s, 3H, SO_2Me), 3.61 (s, 3H, SO_2Me), 8.64 (s, 1H, pyridine-H), 10.25 (s,1H, triazine-H); HRMS (EI): *m*/*z* calcd for $C_{16}H_{20}O_4N_4S_2$ (M^+), 396.0926; found, 396.0932.

3.2. General procedure for the nucleophilic substitution of 6a–d with 3-butyn-1-ol and 4-pentyn-1-ol

To a mixture of 3-butyn-1-ol (1 mmol) or 4-pentyn-1-ol (1 mmol) and 60% NaH in mineral oil (1.1 mmol) in dry DMF (5 ml), the substrate **6a–d** (1 mmol), was added. The mixture was stirred at 0 °C for 15 min, then at room temperature (see Table 1). The reaction mixture was poured into ice/H_2O and acidified with AcOH. The precipitate was filtered off and compounds **7a–h** were used to the next step without purification.

3.2.1. 3-(3-But-3-ynyloxy-1,2,4-triazin-5-yl)-1-methysulfonyl-6,7-dihydro-5*H*-cyclopenta[*c*]pyridine 7a. Yellow crystals, mp 169–170 °C; IR (KBr) ν_{max}/cm^{-1} 3293 (–C≡CH), 2965, 1349 (SO_2), 1140 (SO_2); ^1H NMR ($CDCl_3$): δ=2.08 (t, 1H, *J*=2.6 Hz, ≡CH), 2.20–2.36 (qui, 2H, *J*=7.7 Hz, CH_2), 2.86 (dt, 2H, J_1=2.6 Hz, J_2=6.9 Hz, CH_2), 3.10 (t, 2H, *J*=7.6 Hz, CH_2), 3.44 (s, 3H, SO_2Me), 3.45 (t, 2H, *J*=7.6 Hz, CH_2), 4.78 (t, 2H, *J*=6.9 Hz, OCH_2), 8.60 (s, 1H, pyridine-H), 9.90 (s, 1H, triazine-H); HRMS (EI): *m*/*z* calcd for $C_{16}H_{17}O_3N_4S$ $(MH)^+$, 345.1021; found, 345.0992.

3.2.2. 3-(3-But-3-ynyloxy-1,2,4-triazin-5-yl)-1-methysulfonyl-5,6,7,8-tetrahydroisoquinoline 7b. Yellow crystals, mp 179–180 °C; IR (KBr) ν_{max}/cm^{-1} 3290 (–C≡CH), 2965, 1350 (SO_2), 1145 (SO_2); ^1H NMR ($CDCl_3$): δ=1.70–1.85 (m, 4H, 2×CH_2), 2.05 (t, 1H, *J*=2.6 Hz, ≡CH), 2.45 (dt, 2H, J_1=2.6 Hz, J_2=6.9 Hz, CH_2), 2.80 (t, 2H, *J*=7.2 Hz, CH_2), 3.30 (t, 2H, *J*=6.0 Hz, CH_2), 3.50 (s, 3H, SO_2Me), 4.75 (t, 2H, *J*=6.9 Hz, OCH_2), 8.42 (s, 1H, pyridine-H), 9.85 (s, 1H, triazine-H); HRMS (LIMS): *m*/*z* calcd for $C_{17}H_{19}O_3N_4S$ $(MH)^+$, 359.1178; found, 359.1199.

3.2.3. 3-(3-But-3-ynyloxy-1,2,4-triazin-5-yl)-1-methysulfonyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[*c*]pyridine 7c. Yellow crystals, mp 161–162 °C; IR (KBr) ν_{max}/cm^{-1} 3290 (–C≡CH), 2960, 1359 (SO_2), 1135 (SO_2); ^1H NMR ($CDCl_3$): δ=1.62–1.98 (m, 6H, 3×CH_2), 2.07 (t, 1H, *J*=2.6 Hz, ≡CH), 2.45 (dt, 2H, J_1=2.8 Hz, J_2=7.0 Hz, CH_2), 3.02 (t, 2H, *J*=5.4 Hz, CH_2), 3.38 (t, 2H, *J*=5.0 Hz, CH_2), 3.53 (s, 3H, SO_2Me), 4.77 (t, 2H, *J*=6.8 Hz, OCH_2), 8.45 (s, 1H, pyridine-H), 9.80 (s, 1H, triazine-H); HRMS (EI): *m*/*z* calcd for $C_{18}H_{21}O_3N_4S$ (M^+), 373.1344; found, 373.1329.

3.2.4. 3-(3-But-3-ynyloxy-1,2,4-triazin-5-yl)-1-methysulfonyl-5,6,7,8,9,10-hexahydrocycloocta[*c*]pyridine 7d. Yellow crystals, mp 178–179 °C; IR (KBr) ν_{max}/cm^{-1} 3285 (–C≡CH), 2945, 1350 (SO_2), 1135 (SO_2); ^1H NMR ($CDCl_3$): δ=1.38–1.45 (m, 4H, 2×CH_2), 1.70–2.05 (m, 4H, 2×CH_2), 2.08 (t, 1H, *J*=2.6 Hz, ≡CH), 2.87 (dt, 2H, J_1=2.7 Hz, J_2=6.9 Hz, CH_2), 2.96 (t, 2H, *J*=6.1 Hz, CH_2), 3.36 (t, 2H, *J*=6.2 Hz, CH_2), 3.55 (s, 3H, SO_2Me), 4.79 (t, 2H, *J*=6.8 Hz, OCH_2), 8.50 (s, 1H, pyridine-H), 9.82 (s, 1H, triazine-H); HRMS (EI): *m*/*z* calcd for $C_{19}H_{22}O_3N_4S$ (M^+), 386.1413; found, 386.1414.

3.2.5. 3-(4-Pent-4-ynyloxy-1,2,4-triazin-5-yl)-1-methysulfonyl-6,7-dihydro-5*H*-cyclopenta[*c*]pyridine 7e. Yellow crystals, mp 156–157 °C; IR (KBr) ν_{max}/cm^{-1} 3265 (–C≡CH), 2965, 1370 (SO_2), 1145 (SO_2); ^1H NMR ($CDCl_3$): δ=2.00 (t, 1H, *J*=2.6 Hz, ≡CH), 2.09–2.35 (m, 4H, 2×CH_2), 2.49 (dt, 2H, J_1=2.7 Hz, J_2=7.0 Hz, CH_2), 3.10 (t, 2H, *J*=7.7 Hz, CH_2), 3.44 (s, 3H, SO_2CH_3), 3.46 (t, 2H, *J*=7.6 Hz, CH_2), 4.78 (t, 2H, *J*=6.8 Hz, OCH_2), 8.62 (s, 1H, pyridine-H), 9.89 (s, 1H, triazine-H); HRMS (LIMS): *m*/*z* calcd for $C_{17}H_{19}O_3N_4S$ $(MH)^+$, 359.1178; found, 359.1177.

3.2.6. 3-(3-Pent-4-ynyloxy-1,2,4-triazin-5-yl)-1-methylsulfonyl-5,6,7,8-tetrahydroquinoline 7f. Yellow crystals, mp 153–154 °C; IR (KBr) ν_{max}/cm^{-1} 3295 (–C≡CH), 2960, 1345 (SO_2), 1145 (SO_2); ^1H NMR ($CDCl_3$): δ=1.87–1.84 (m, 4H, 2×CH_2), 1.99 (t, 1H, J=2.6 Hz, ≡CH), 2.10–2.25 (m, 2H, CH_2), 2.49 (dt, 2H, J_1=2.6 Hz, J_2=7.0 Hz, CH_2), 2.96 (t, 2H, J=6.3 Hz, CH_2), 3.34 (t, 2H, J=6.4 Hz, CH_2), 3.52 (s, 3H, SO_2Me), 4.78 (t, 2H, J=6.2 Hz, OCH_2), 8.23 (s, 1H, pyridine-H), 9.45 (s, 1H, triazine-H); HRMS (LIMS): m/z calcd for $C_{18}H_{21}O_3N_4S$ $(MH)^+$, 373.1334; found, 373.1344.

3.2.7. 3-(4-Pent-4-ynyloxy-1,2,4-triazin-5-yl)-1-methylsulfonyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[*c*]pyridine 7g. Yellow crystals, mp 101–102 °C; IR (KBr) ν_{max}/cm^{-1} 3280 (–C≡CH), 2955, 1349 (SO_2), 1135 (SO_2); ^1H NMR ($CDCl_3$): δ=1.68–1.84 (m, 6H, 3×CH_2), 1.99 (t, 1H, J=2.6 Hz, ≡CH), 2.17 (qui, 2H, J=6.3 Hz, CH_2), 2.49 (dt, 2H, J_1=2.5 Hz, J_2=6.8 Hz, CH_2), 3.00 (t, 2H, J=6.4 Hz, CH_2), 3.40 (t, 2H, J=6.4 Hz, CH_2), 3.42 (s, 3H, SO_2Me), 4.78 (t, 2H, J=6.2 Hz, OCH_2), 8.45 (s, 1H, pyridine-H), 9.80 (s, 1H, triazine-H); HRMS (EI): m/z calcd for $C_{19}H_{23}O_3N_4S$ (M^+), 387.1485; found, 387.1487.

3.2.8. 3-(4-Pent-4-ynyloxy-1,2,4-triazin-5-yl)-1-methylsulfonyl-5,6,7,8,9,10-hexahydrocycloocta[*c*]pyridine 7h. Yellow crystals, mp 141–142 °C; IR (KBr) ν_{max}/cm^{-1} 3290 (–C≡CH), 2965, 1339 (SO_2), 1145 (SO_2); ^1H NMR ($CDCl_3$): δ=1.35–1.45 (m, 4H, 2×CH_2), 1.87–1.94 (m, 4H, 2×CH_2), 2.00 (t, 1H, J=2.6 Hz, ≡CH), 2.20 (qui, 2H, J=6.3 Hz, CH_2), 2.50 (dt, 2H, J_1=2.5 Hz, J_2=6.8 Hz, CH_2), 2.96 (t, 2H, J=6.4 Hz, CH_2), 3.40 (t, 2H, J=6.4 Hz, CH_2), 3.55 (s, 3H, SO_2Me), 4.78 (t, 2H, J=6.2 Hz, OCH_2), 8.48 (s, 1H, pyridine-H), 9.81 (s, 1H, triazine-H); HRMS (EI): m/z calcd for $C_{20}H_{24}N_4SO_3$ (M^+), 400.1569; found, 400.1559.

3.3. General procedure for the intramolecular Diels–Alder reaction of 7a–h. Synthesis of furo- and pyrano[2,3-*b*]pyridine derivatives 8a–h

A stirred solution of the **7a–h** in bromobenzene (approx. 0.4 g in 10 ml of solvent) was heated at reflux under nitrogen (see Table 1). After this time, the reaction mixture was cooled to room temperature and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography using chloroform as eluent to give a white solid.

3.3.1. 3-(2,3-Dihydrofuro[2,3-*b*]pyridin-6-yl)-1-methylsulfonyl-6,7-dihydro-5*H*-cyclopenta[*c*]pyridine 8a. White crystals, mp 236–237 °C; IR (KBr) ν_{max}/cm^{-1} 2919, 1351 (SO_2), 1140 (SO_2), 1078 (C–O–C); ^1H NMR ($CDCl_3$): δ=2.10–2.30 (qui, 2H, J=7.6 Hz, CH_2), 3.01 (t, 2H, J=7.7 Hz, CH_2), 3.33 (t, 2H, J=6.4 Hz, CH_2), 3.38 (t, 2H, J=6.0 Hz, CH_2), 3.39 (s, 3H, SO_2Me), 4.70 (t, 2H, J=8.5 Hz, OCH_2), 7.55–7.63 (d, 1H, J=7.6 Hz, pyridine-H), 7.93 (d, 1H, J=7.6 Hz, pyridine-H), 8.48 (s, 1H, pyridine-H); HRMS (EI): m/z calcd for $C_{16}H_{16}O_3N_2S$ (M^+), 316.0881; found, 316.0881. The hygroscopic nature of the compound led to variability in the microanalytical data.

3.3.2. 3-(2,3-Dihydrofuro[2,3-*b*]pyridin-6-yl)-1-methylsulfonyl-5,6,7,8-tetrahydroisoquinoline 8b. White crystals, mp 216–217 °C; IR (KBr) ν_{max}/cm^{-1} 2919, 1302 (SO_2), 1127 (SO_2), 1028 (C–O–C); ^1H NMR ($CDCl_3$): δ=1.65–1.90 (m, 4H, 2×CH_2), 2.95 (t, 2H, J=6.8 Hz, CH_2), 3.30–3.40 (m, 4H, 2×CH_2), 3.50 (s, 3H, SO_2Me), 4.70 (t, 2H, J=7.2 Hz, OCH_2), 7.50 (d, 1H, J=7.6 Hz, pyridine-H), 7.80 (d, 1H, J=7.6 Hz, pyridine-H), 8.30 (s, 1H, pyridine-H); HRMS (EI): m/z calcd for $C_{17}H_{18}O_3N_2S$ (M^+), 330.1038; found, 330.1038. Anal. calcd for $C_{17}H_{18}O_3N_2S{\cdot}0.25H_2O$: C, 60.98; H, 5.38; N, 8.37. Found: C, 60.86; H, 5.51; N, 8.01.

3.3.3. 3-(2,3-Dihydrofuro[2,3-*b*]pyridin-6-yl)-1-methylsulfonyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[*c*]pyridine 8c. White crystals, mp 215–216 °C; IR (KBr) ν_{max}/cm^{-1} 2929, 1305 (SO_2), 1130 (SO_2), 1020 (C–O–C); ^1H NMR ($CDCl_3$): δ=1.60–1.95 (m, 6H, 3×CH_2), 2.95 (t, 2H, J=6.8 Hz, CH_2), 3.28–3.40 (m, 4H, 2×CH_2), 3.50 (s, 3H, SO_2Me), 4.71 (t, 2H, J=7.2 Hz, OCH_2), 7.80 (d, 1H, J=7.6 Hz, pyridine-H), 7.83 (d, 1H, J=7.6 Hz, pyridine-H), 8.32 (s, 1H, pyridine-H). Anal. calcd for $C_{18}H_{20}O_3N_2S{\cdot}0.25H_2O$: C, 61.98; H, 5.88; N, 8.03. Found: C, 61.94; H, 5.83; N, 8.06.

3.3.4. 3-(2,3-Dihydrofuro[2,3-*b*]pyridin-6-yl)-1-methylsulfonyl-5,6,7,8,9,10-hexahydrocycloocta[*c*]pyridine 8d. White crystals, mp 236–237 °C; IR (KBr) ν_{max}/cm^{-1} 2945, 1315 (SO_2), 1135 (SO_2), 1015 (C–O–C); ^1H NMR ($CDCl_3$): δ=1.30–1.45 (m, 4H, 2×CH_2), 1.69–1.95 (m, 4H, 2×CH_2), 2.93 (t, 2H, J=6.8 Hz, CH_2), 3.21–3.40 (m, 4H, 2×CH_2), 3.51 (s, 3H, SO_2Me), 4.71 (t, 2H, J=7.2 Hz, OCH_2), 7.81 (d, 1H, J=7.6 Hz, pyridine-H), 7.84 (d, 1H, J=7.6 Hz, pyridine-H), 8.35 (s, 1H, pyridine-H). Anal. calcd for $C_{19}H_{22}O_3N_2S$: C, 63.66; H, 6.19; N, 7.82. Found: C, 63.65; H, 6.21; N, 7.89.

3.3.5. 7-(1-Methylsulfonyl-6,7-dihydro-5*H*-cyclopenta[*c*]-pyridin-3-yl)-3,4-dihydro-2*H*-pyrano[2,3-*b*]pyridine 8e. White crystals, mp 263–264 °C; IR (KBr) ν_{max}/cm^{-1} 2924, 1306 (SO_2), 1127 (SO_2), 1055 (C–O–C); ^1H NMR ($CDCl_3$): δ=2.00–2.15 (m, 2H, CH_2), 2.20 (qui, 2H, J=7.6 Hz, CH_2), 2.88 (t, 2H, J=7.7 Hz, CH_2), 3.01 (t, 2H, J=7.7 Hz, CH_2), 3.38 (t, 2H, J=7.6 Hz, CH_2), 3.39 (s, 3H, SO_2Me), 4.20 (t, 2H, J=5.2 Hz, CH_2), 7.61 (d, 1H, J=7.7 Hz, pyridine-H), 7.94 (d, 1H, J=7.7 Hz, pyridine-H), 8.49 (s, 1H, pyridine-H); HRMS (EI): m/z calcd for $C_{17}H_{18}N_2SO_3$ (M^+), 330.1038; found, 330.1033. Anal. calcd for $C_{17}H_{18}O_3N_2S{\cdot}0.25H_2O$: C, 60.98; H, 5.38; N, 8.37. Found: C, 60.70; H, 5.35; N, 7.98.

3.3.6. 7-(1-Methylsulfonyl-5,6,7,8-tetrahydroisoquinolin-3-yl)-3,4-dihydro-2*H*-pyrano[2,3-*b*]pyridine 8f. White crystals, mp 254–255 °C; IR (KBr) ν_{max}/cm^{-1} 2929, 1299 (SO_2), 1130 (SO_2), 1060 (C–O–C); ^1H NMR ($CDCl_3$): δ=1.75–1.92 (m, 4H, 2×CH_2), 2.04–2.10 (m, 2H, CH_2), 2.80–2.95 (m, 4H, 2×CH_2), 3.27 (t, 2H, J=6.1 Hz, CH_2), 3.48 (s, 3H, SO_2Me), 4.42 (t, 2H, J=5.3 Hz, OCH_2), 7.50 (d, 1H, J=7.6 Hz, pyridine-H), 7.82 (d, 1H, J=7.6 Hz, pyridine-H), 8.29 (s, 1H, pyridine-H); HRMS (EI): m/z calcd for $C_{18}H_{20}O_3N_2S$ (M^+), 344.1205; found, 344.1194. Anal. calcd for $C_{18}H_{20}O_3N_2S$: C, 62.79; H, 5.81; N, 8.14. Found: C, 62.65; H, 5.93; N, 7.92.

3.3.7. 7-(1-Methylsulfonyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[*c*]pyridin-3-yl)-3,4-dihydro-2*H*-pyrano[2,3-*b*]-pyridine 8g. White crystals, mp 243–244 °C; IR (KBr)

ν_{max}/cm^{-1} 2949, 1305 (SO_2), 1145 (SO_2), 1035 (C–O–C); ^1H NMR ($CDCl_3$): δ=1.60–1.92 (m, 6H, 3×CH_2), 2.05 (qui, 2H, J=6.4 Hz, CH_2), 2.85–3.05 (m, 4H, 2×CH_2), 3.35 (t, 2H, J=6.1 Hz, CH_2), 3.50 (s, 3H, SO_2Me), 4.42 (t, 2H, J=5.3 Hz, OCH_2), 7.50 (d, 1H, J=7.6 Hz, pyridine-H), 7.84 (d, 1H, J=7.6 Hz, pyridine-H), 8.33 (s, 1H, pyridine-H). Anal. calcd for $C_{19}H_{22}O_3N_2S$: C, 63.69; H, 6.15; N, 7.82. Found: C, 63.67; H, 6.15; N, 7.75.

3.3.8. 7-(1-Methylsulfonyl-5,6,7,8,9,10-hexahydrocycloocta[*c*]pyridin-3-yl)-3,4-dihydro-2*H*-pyrano[2,3-*b*]pyridine 8h. White crystals, mp 253–254 °C; IR (KBr) ν_{max}/cm^{-1} 2930, 1305 (SO_2), 1135 (SO_2), 1035 (C–O–C); ^1H NMR ($CDCl_3$): δ=1.35–1.41 (m, 4H, 2×CH_2), 1.60–1.92 (m, 4H, 2×CH_2), 2.04 (qui, 2H, J=6.4 Hz, CH_2), 2.85–3.05 (m, 4H, 2×CH_2), 3.35 (t, 2H, J=6.1 Hz, CH_2), 3.50 (s, 3H, SO_2Me), 4.42 (t, 2H, J=5.3 Hz, OCH_2), 7.53 (d, 1H, J=7.6 Hz, pyridine-H), 7.86 (d, 1H, J=7.6 Hz, pyridine-H), 8.36 (s, 1H, pyridine-H). Anal. calcd for $C_{20}H_{24}O_3N_2S$ 0.25H_2O: C, 63.75; H, 6.50; N, 7.44. Found: C, 63.85; H, 6.40; N, 7.56.

3.4. General procedure for the nucleophilic substitution reaction of 6a–c with 2-cyanophenol

60% NaH in mineral oil (1.1 mmol) was added to a solution of 2-cyanophenol (1.0 mmol) in dry DMF (5 ml), and the mixture was stirred at 0 °C for 15 min. The substrate **6a–c** (1 mmol) was added, and the mixture was stirred for 3 h at room temperature. The reaction mixture was then poured into ice/H_2O and acidified with AcOH. The precipitate was filtered off and purified by column chromatography using chloroform as eluent to give light yellow compounds **9a–c**.

3.4.1. 2-[5-(1-Methylsulfonyl-6,7-dihydro-5*H*-cyclopenta[*c*]pyridin-3-yl)-1,2,4-triazin-3-yloxybenzonitrile 9a. Yellow crystals, mp 220–222 °C; IR (KBr) ν_{max}/cm^{-1} 2929, 2234 (–C≡N) 1334 (SO_2), 1120 (SO_2); ^1H NMR ($CDCl_3$): δ=2.24–2.32 (m, 2H, CH_2), 3.10 (t, 2H, J=7.8 Hz, CH_2), 3.42–3.47 (m, 2H, CH_2), 3.44 (s, 3H, SO_2Me), 7.41–7.50 (m, 2H, Ar), 7.70–7.82 (m, 2H, Ar), 8.57 (s, 1H, pyridine-H), 9.84 (s, 1H, triazine-H); HRMS (EI): m/z calcd for $C_{19}H_{15}O_3N_5S$ (M^+), 393.0896; found, 393.0889.

3.4.2. 2-[5-(1-Methylsulfonyl-5,6,7,8-tetrahydroisoquinolin-3-yl)-1,2,4-triazin-3-yloxybenzonitrile 9b. Yellow crystals, mp 222–223 °C; IR (KBr) ν_{max}/cm^{-1} 2939, 2238 (–C≡N) 1348 (SO_2), 1127 (SO_2); ^1H NMR ($CDCl_3$): δ=1.84–1.97 (m, 4H, 2×CH_2), 2.97 (t, 2H, J=6.0 Hz, CH_2), 3.35 (t, 2H, J=6.1 Hz, CH_2), 3.52 (s, 3H, SO_2Me), 7.41–7.50 (m, 2H, Ar), 7.70–7.82 (m, 2H, Ar), 8.40 (s, 1H, pyridine-H), 9.94 (s, 1H, triazine-H); HRMS (EI): m/z calcd for $C_{20}H_{17}N_5SO_3$ (M^+), 407.1044; found, 407.1052.

3.4.3. 2-[5-(1-Methylsulfonyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[*c*]pyridin-3-yl)-1,2,4-triazin-3-yloxybenzonitrile 9c. Yellow crystals, mp 216–217 °C; IR (KBr) ν_{max}/cm^{-1} 2929, 2232 (–C≡N) 1304 (SO_2), 1127 (SO_2); ^1H NMR ($CDCl_3$): δ=1.57–1.79 (m, 4H, 2×CH_2), 1.90–1.93 (m, 2H, CH_2), 3.00–3.05 (m, 2H, CH_2), 3.38–3.43 (m, 2H, CH_2), 3.53 (s, 3H, SO_2Me), 7.41–7.49 (m, 2H, Ar), 7.70–7.76 (m, 2H, Ar), 8.44 (s, 1H, pyridine-H), 9.93 (s, 1H, triazine-H); HRMS (EI): m/z calcd for $C_{21}H_{19}O_3N_5S$ (M^+), 421.1209; found, 421.1204.

3.5. Intramolecular Diels–Alder reaction of 9a–c. General procedure for the synthesis of benzofuro[2,3-*b*]pyrazines derivatives 10a–c

A stirred solution of **9a–c** in nitrobenzene (approx. 0.4 g in 10 ml of solvent) was heated at reflux under nitrogen for 16 h. After this time, the reaction mixture was cooled to room temperature, the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography using chloroform as eluent to give white solid.

3.5.1. 3-(1-Methylsulfonyl-6,7-dihydro-5*H*-cyclopenta[*c*]pyridin-3-yl)benzo[4,5]furo[2,3-*b*]pyrazine 10a. White crystals, mp 250–251 °C; IR (KBr) ν_{max}/cm^{-1} 2927, 1299 (SO_2), 1126 (SO_2); ^1H NMR ($CDCl_3$): δ=2.00–2.28 (m, 2H, CH_2), 3.10 (t, 2H, J=7.7 Hz, CH_2), 3.39–3.45 (m, 2H, CH_2), 3.47 (s, 3H, SO_2Me), 7.48–7.58 (m, 1H, Ar), 7.68–7.74 (m, 2H, Ar), 8.25–8.29 (m, 1H, Ar), 8.57 (s, 1H, pyridine-H), 9.69 (s, 1H, pyrazine-H); HRMS (EI): m/z calcd for $C_{19}H_{15}N_3SO_3$ (M^+), 365.0834; found, 365.0802.

3.5.2. 3-(1-Methylsulfonyl-5,6,7,8-tetrahydroisoquinolin-3-yl)benzo[4,5]furo[2,3-*b*]pyrazine 10b. White crystals, mp 297–298 °C; IR (KBr) ν_{max}/cm^{-1} 2956, 1300 (SO_2), 1133 (SO_2); ^1H NMR ($CDCl_3$): δ=1.84–1.96 (m, 4H, 2×CH_2), 2.98 (t, 2H, J=6.3 Hz, CH_2), 3.32 (t, 2H, J=6.1 Hz, CH_2), 3.55 (s, 3H, SO_2Me), 7.49–7.56 (m, 1H, Ar), 7.68–7.72 (m, 2H, Ar), 8.21–8.26 (m, 1H, Ar), 8.36 (s, 1H, pyridine-H), 9.58 (s, 1H, pyrazine-H); HRMS (EI): m/z calcd for $C_{20}H_{17}N_5SO_3$ (M^+), 379.0978; found, 379.0992.

3.5.3. 3-(1-Methylsulfonyl-6,7,8,9-tetrahydro-5*H*-cyclohepta[*c*]pyridin-3-yl)benzo[4,5]furo[2,3-*b*]pyrazine 10c. White crystals, mp 260–261 °C; IR (KBr) ν_{max}/cm^{-1} 2935, 1296 (SO_2), 1120 (SO_2); ^1H NMR ($CDCl_3$): δ=1.67–1.99 (m, 6H, 3×CH_2), 3.00–3.09 (m, 2H, CH_2), 3.35–3.41 (m, 2H, CH_2), 3.57 (s, 3H, SO_2Me), 7.48–7.57 (m, 1H, Ar), 7.68–7.73 (m, 2H, Ar), 8.24–8.28 (m, 1H, Ar), 8.41 (s, 1H, pyridine-H), 9.58 (s, 1H, pyrazine-H); HRMS (EI): m/z calcd for $C_{21}H_{19}N_3SO_3$ (M^+), 393.1147; found, 393.1166.

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Fluorescence emission control and switching of oxymethylcrowned spirobenzopyrans by metal ion

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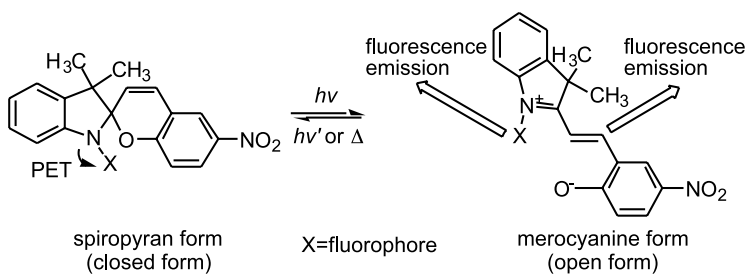
Abstract—Oxymethylcrowned spirobenzopyran **1** and pyrenylspirobenzopyran **2** were synthesized, and fluorescence emission of their corresponding merocyanine form was examined in the presence of metal ions. For **2**, fluorescence emission derived from the pyrene moiety was completely quenched by photoinduced electron transfer (PET) of the nitrogen atom when the merocyanine form was not produced, namely, without metal ions. However, when **2** was converted to the merocyanine form by the complexation of its crown ether with a metal ion, fluorescence resonance energy transfer (FRET) from the pyrene to the merocyanine moieties took place to produce fluorescence emission. This result demonstrates that the spirobenzopyran isomerization can function as a fluorescence emission switch. Fluorescence quantum yield measurement for **1** and **2** showed that fluorescence emission depends on the binding metal ion in which the fluorescence quantum yield generally increased with the increase of metal ion radius.

1. Introduction

Photochromic compounds are defined as compounds showing a reversible photoinduced phenomenon in which a photosensitive compound is converted to another isomer exhibiting a different absorption spectrum in the visible region. For recent several decades, various photochromic compounds have been designed, and their properties have been examined at the viewpoint of practical application, physical properties such as colorability, decoloration rate, photofatigue resistance and so on.[1] On the other hand, fluorescence emission is a versatile property to be applied not only to analytical chemistry but also to biological chemistry.[2] For instance, a combination of azacrown ether with fluorophore affords a fluorescence ion-indicator, where metal ion binding to the azacrown ether results in enhancement of fluorescence emission by suppression of photoinduced electron transfer (PET) of the nitrogen atom in the azacrown ether.

Recently, some photochromic compounds such as spirobenzopyran[3] and spironaphthooxazine[4] are reported to produce fluorescence emission when they adopt the merocyanine (open) form. It is, however, clear that there is experimental difficulty to study fluorescence emission of spiropyrans as the merocyanine (open) form isomerizes back to the spiropyran (closed) form when stopping UV irradiation (Scheme 1). Furthermore, the merocyanine form



Scheme 1. Isomerization of spirobenzopyran.

Keywords: Spirobenzopyran; Crown ether; Fluorescence emission; Metal ion.
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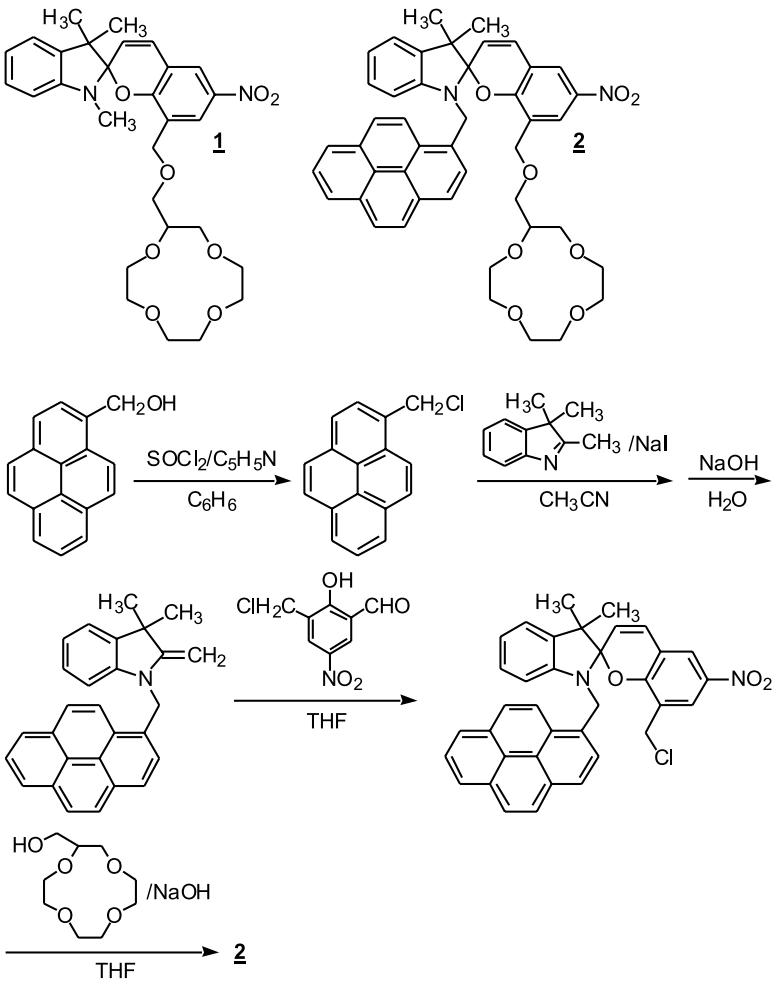
restores to the spiropyran by probe light to observe fluorescence spectra.

The combination of photochromic compounds with ion-responsive molecules such as crown ether[5] and calixarene[6] has been reported to afford ion-responsive photochromic compounds. In the case of spirobenzopyrans,[7] a fascinating property is that spirobenzopyran bearing the crown ether moiety, crowned spirobenzopyran, tends to isomerize to the merocyanine form in the presence of a metal ion without UV irradiation.[8] Furthermore, the merocyanine form of spirobenzopyran is considered as a zwitter-ion in which the nitrogen atom has a positive charge.[1] The nitrogen atom is known to quench fluorescence emission through PET, but the positive charge on the nitrogen atom suppresses the quenching. Therefore, when a fluorophore is introduced at the nitrogen atom of spirobenzopyran, switching of fluorescence emission derived from the fluorophore is expected by spirobenzopyran isomerization accompanying PET switching (Scheme 1). Those properties of spirobenzopyran prompted us to investigate fluorescence emission of crowned spirobenzopyran. In this paper, we report spirobenzopyran derivatives bearing both oxymethylcrown ether and pyrene moieties, which show ion-responsible fluorescence emission.

2. Results and discussion

2.1. Synthesis of oxymethylcrowned spirobenzopyrans

Oxymethycrowned spirobenzopyran **1** was synthesized according to our previous work,[9] while oxymethylcrowned spirobenzopyran **2** was synthesized with the outline as shown in Scheme 2. As a fluorophore to be introduced at the nitrogen atom of spirobenzopyran, we chose a pyrene moiety, as its fluorescence property is well known. Commercially available 1-pyrenemethanol was converted to 1-chloromethylpyrene by the reaction with thionyl-chloride in the presence of pyridine. The reaction of 1-chloromethylpyrene with 2,3,3-trimethylindolenine in the presence of sodium iodide afforded 1-(1-pyrenyl)-methyl-3,3-dimethyl-2-methyleneindoline after treatment with aqueous sodium hydroxide, but purification by gel permeation chromatography resulted in some decomposition. Condensation of 1-(1-pyrenyl)methyl-3,3-dimethyl-2-methyleneindoline with 3-chloromethyl-5-nitrosalicyl-aldehyde produced chloromethyl(pyrenylmethyl)spiro-benzopyran. Finally, the reaction of chloromethyl(pyrenyl-methyl)spirobenzopyran with hydroxymethyl-12-crown-4 in the presence of powdered sodium hydroxide afforded desired product **2** in 12% yield after purification by gel permeation chromatography.



Scheme 2. Synthesis outline.

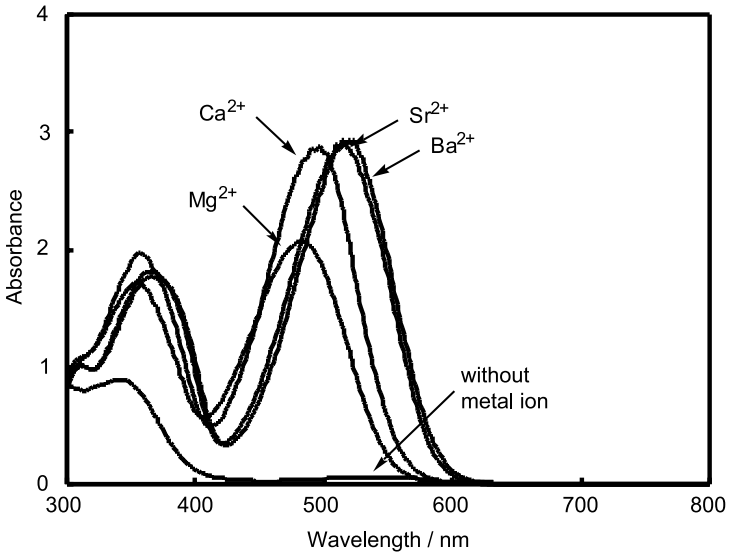


Figure 1. UV–Vis absorption spectra of **1** in the presence of an alkaline-earth metal ion.

2.2. Fluorescence emission and metal ion binding

UV–Vis absorption and fluorescence emission spectra of **1** were measured using alkali and alkaline-earth metal perchlorates in acetonitrile at room temperature. In the presence of an equal amount of an alkali metal ion (1×10^{-4} mol dm^{-3}), only Li^+ could induce discernible isomerization to the merocyanine form, however, the fluorescence emission was too weak to obtain reliable data (the data not shown). On the other hand, UV–Vis absorption spectra indicated that significant isomerization to the merocyanine form was induced by alkaline-earth metal ions (Fig. 1). As discussed later, more than 95% of **1** was converted to the merocyanine form with alkaline-earth metal ions except for Mg^{2+}. With decreasing the metal ion radius from Ba^{2+} to Mg^{2+}, a significant blue-shift in UV–Vis absorption spectra was observed reflecting the polar atmosphere induced by a metal ion.[9,10] Fluorescence emission and excitation spectra of **1** in the presence of alkaline-earth metal ions are summarized in Figures 2 and 3, respectively. A similar blue-shift depending on the metal ion was observed in both of the spectra as was the case in UV–Vis absorption spectra. Without metal ions, any fluorescence emission was not detected. This means that the fluorescence emission is derived only from the merocyanine form.

In the case of **2**, UV–Vis absorption spectra (Fig. 4) showed a strong absorption peak at 343 nm assigned to the absorption of the pyrene moiety. Without metal ions, namely, when **2** adopted the spiropyran form, any meaningful fluorescence emission was not detected even with excitation at 343 nm. This result indicates that fluorescence emission derived from the pyrene moiety is completely quenched by PET of the nitrogen atom, namely, that the fluorescence emission switch is off-state as expected. Addition of an equal amount of an alkaline-earth metal ion (5×10^{-5} mol dm^{-3}) to the solution of **2** induced significant isomerization to the merocyanine form in a similar way to **1** as shown in UV–Vis absorption spectra in

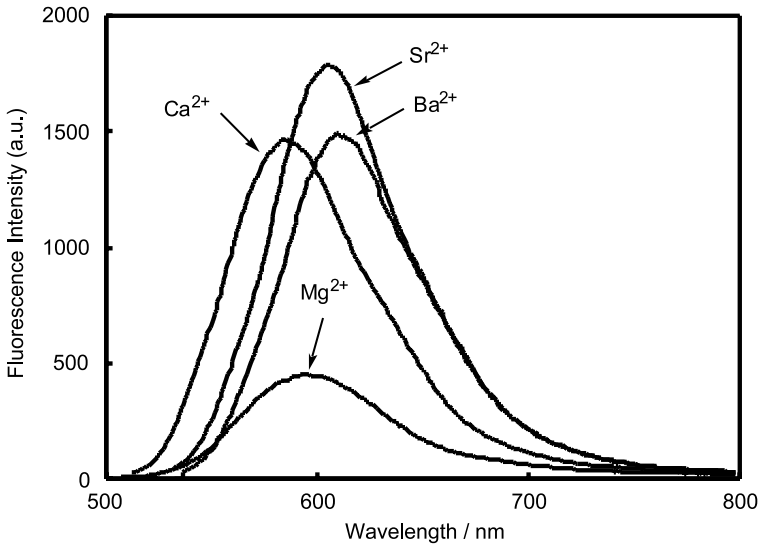


Figure 2. Fluorescence emission spectra of **1** in the presence of an alkaline-earth metal ion. The wavelengths of excitation light were 495, 500, 520 and 525 nm for the Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} solutions, respectively.

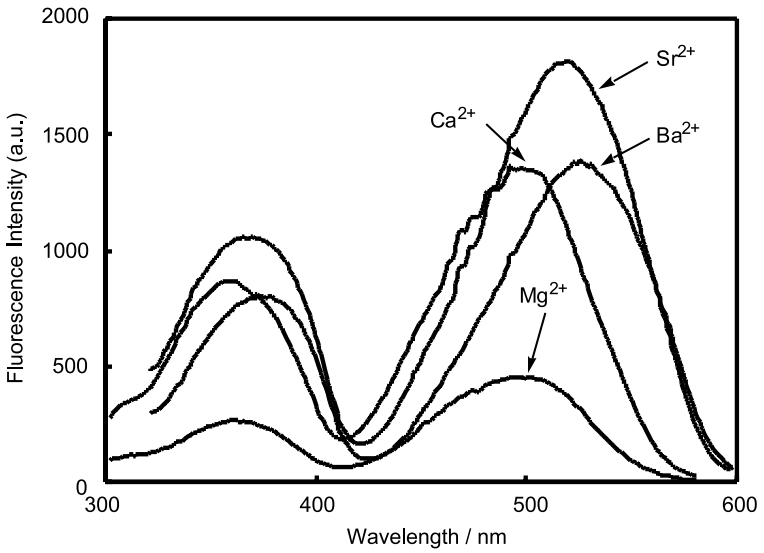


Figure 3. Excitation spectra of **1** in the presence of an alkaline-earth metal ion. The wavelengths detecting fluorescence emission were 590, 590, 605 and 615 nm for the Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} solutions, respectively.

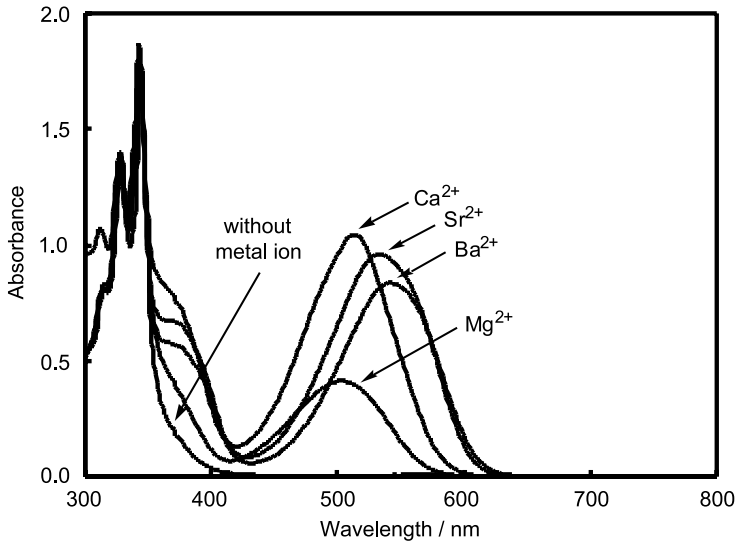


Figure 4. UV–Vis absorption spectra of **2** in the presence of an alkaline-earth metal ion.

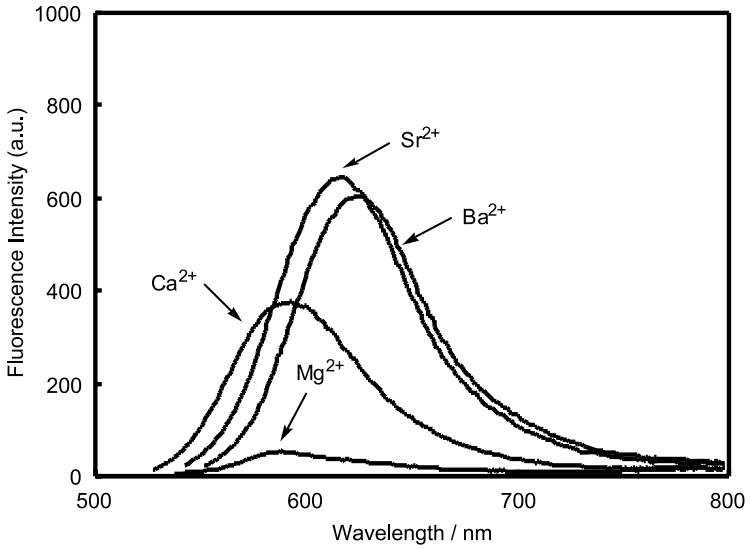


Figure 5. Fluorescence emission spectra of **2** in the presence of an alkaline-earth metal ion. The wavelengths of excitation light were 525, 515, 530 and 540 nm for the Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} solutions, respectively.

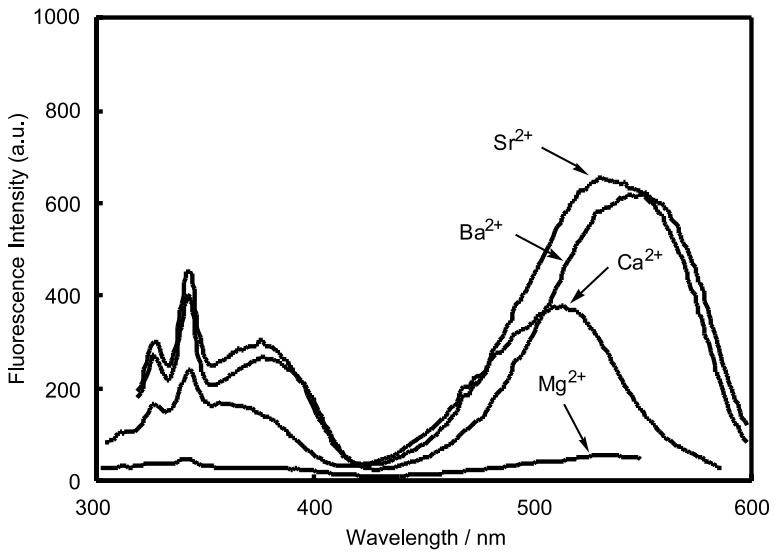
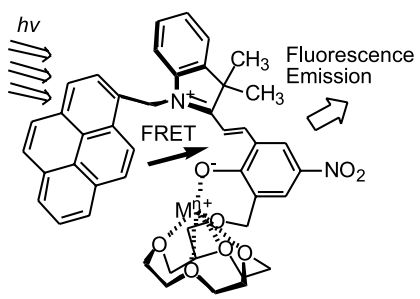


Figure 6. Excitation spectra of **2** in the presence of an alkaline-earth metal ion. The wavelengths detecting fluorescence emission were 590, 595, 615 and 625 nm for the Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} solutions, respectively.

Figure 4, while alkali metal ions hardly showed discernible spectral change (the data not shown). The fluorescence emission and excitation spectra in the presence of alkaline-earth metal ions are summarized in Figures 5 and 6. In Figure 5, fluorescence emission spectra for **2** showed a similar tendency to those for **1**. Fluorescence emission derived from the pyrene moiety was hardly detected again even with excitation at 343 nm, although **2** adopted the merocyanine form. However, the fluorescence excitation spectra in Figure 6 showed strong peaks at 343 nm, which are consistent with the strong peaks in UV–Vis absorption spectra in Figure 4. As those strong peaks at 343 nm are derived from the pyrene moiety, the pyrene moiety obviously contributes the fluorescence emission of the merocyanine moiety. Therefore, it is suggested that fluorescence resonance energy transfer (FRET) took place from the pyrene to the merocyanine moieties, resulting in fluorescence emission of the merocyanine moiety (Scheme 3).[11] The FRET efficiency, where the pyrene and the merocyanine moieties were donor and acceptor, respectively, was evaluated as 12% in the presence of Sr^{2+}. This means that the isomerization between the spiropyran and the merocyanine forms functions as a fluorescence emission switch through the PET of the nitrogen atom. The fluorescence emission switch of the fluorophore introduced at the nitrogen atom of the spirobenzopyran is on- and off-states in the merocyanine and the spiropyran forms, respectively.



Scheme 3. Fluorescence emission and FRET of **2**.

2.3. Fluorescence quantum yield

In order to determine the fluorescence quantum yields, Φ (%), the conversion ratio (%) of oxymethylcrowned spirobenzopyrans to the merocyanine form from the spiropyran form was evaluated, where only their merocyanine form produced fluorescence emission. Therefore, extinction coefficient, ε for the merocyanine form was determined to evaluate the conversion ratio. When the interaction of a metal ion with the oxymethylcrowned spirobenzopyrans is strong enough, the conversion is regarded as 100% in the presence of excess amount of the metal ion. In cases of Ca^{2+}, Sr^{2+}, and Ba^{2+}, the UV–Vis spectra of **1** and **2** did not show any change when the concentration of metal ions was more than two-fold concentration of oxymethylcrowned spirobenzopyrans. In the case of Mg^{2+}, five- and ten-fold excess amounts of Mg^{2+} were necessary until there was no change in spectra. The obtained values for extinction coefficient (ε) are summarized in Table 1. For **1**, ε decreased with a decrease of the metal ion radius, namely, an increase of the charge density of the metal ion, accompanying successive blue-shifts. Similar tendency was reported with the azacrowned spirobenzopyran.[12] To the contrary, ε of **2** increased with increasing the charge density of the metal ion with blue-shift, and Ca^{2+} afforded the maximum ε.

Table 1. Extinction coefficients $\varepsilon/10^4$ mol^{-1} dm^{3a}

	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}
1	2.66 (484)	2.92 (491)	2.96 (517)	2.98 (524)
2	1.85(503)	2.12 (513)	1.96 (532)	1.71 (541)

[a] Wavelength (nm) at the maximum absorption was shown in parenthesis.

Fluorescence quantum yields Φ of the merocyanine form were determined using 9,10-diphenylanthracene as the standard, in which Φ of 9,10-diphenylanthracene in cyclohexane is known as ca. 98%. The equation as shown in Scheme 4 was applied to determine Φ.

In the equation, I, A, and n represent integrated area of the

$$\Phi_{sa} = \Phi_{st} \frac{I_{sa} * A_{st} * (n_{sa})^2}{I_{st} * A_{sa} * (n_{st})^2}$$

Scheme 4. Equation for Φ.

fluorescence emission peak, absorbance at excitation wavelength, and refractive index of solvent, while subscripts of st and sa mean standard and sample, respectively. According to the equation, Φ for 9,10-diphenylanthracene in acetonitrile was calculated as 100%. Influence of the metal ion concentration on Φ was examined in the presence of various concentrations of Mg^{2+} and Sr^{2+}. The range of Mg^{2+} concentration was between 5×10^{-6} and 100×10^{-6} mol dm^{-3} and that of Sr^{2+} concentration was between 4×10^{-6} and 10×10^{-6} mol dm^{-3}. On the other hand, the concentration of **1** and **2** was constant in 5×10^{-6} mol dm^{-3}. Intensity of fluorescence emission increased with the increase of metal ion concentration, and finally, it became constant. Φ of **1** with Mg^{2+} and Sr^{2+} were 1.6±0.2 and 3.5±0.1%, respectively, and Φ of **2** with Sr^{2+} was 2.1±0.2% regardless of the metal ion concentration. Those results obviously indicate that intensity of the fluorescence emission is depending on concentration of the merocyanine form without influence of metal ion concentration.

Φ in the presence of various metal ions are shown in Table 2. In the case of **1**, Sr^{2+} showed the most effective fluorescence emission, and the combination of **2** with Sr^{2+} and Ba^{2+} produced fluorescence emission effectively. On the other hand, smaller ions such as Mg^{2+} and Ca^{2+} afforded smaller Φ. Although a heavy atom is known to suppress fluorescence emission, rather larger ions, in other words, heavier ions such as Sr^{2+} and Ba^{2+} afforded larger Φ. The fact that smaller metal ions to produce polar atmosphere such as Mg^{2+} and Ca^{2+} strongly interacted with the merocyanine form as reported in our previous work[9,10] suggests that the polar atmosphere induced by metal ions decreased Φ. However, Φ for the merocyanine form of spirobenzopyran without metal ions has been reported to be 1.2% in ethanol[13] being comparable to Φ for **2** with Ca^{2+}, 0.99%. This comparison suggests that the structure arrangement of the merocyanine rather than the polar atmosphere induced by metal ions influenced Φ. Furthermore, the notable maximum point in Φ for **1** with Sr^{2+} also supports the conclusion that Φ is depending on the radius of metal ions to arrange the merocycnine structure but not on the polar atmosphere induced by metal ions. Therefore, fluorescence emission control of the merocyanine seems to be possible through structure arrangement of the merocyanine induced by molecular recognition. On the other hand, as the fluorescence emission intensity is depending on metal ion concentration, the oxymethylcrowned spirobenzopyran can be one of fluorescence ion-indicators.

Table 2. Fluorescence quantum yield Φ/%[a]

	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}
1	1.8 (495)	2.9 (500)	3.4 (520)	2.8 (525)
2	—[b]	0.99 (515)	1.9 (530)	2.0 (540)

[a] Wavelength (nm) of excitation light was shown in parenthesis.
[b] Fluorescence emission was too weak to determine Φ.

3. Conclusions

In summary, spirobenzopyran bearing both oxymethylcrown and pyrene moieties demonstrated that spirobenzopyran isomerization functioned as a fluorescence emission switch through PET switching induced by change in charge on the nitrogen atom. Fluorescence quantum yield measurement showed dependence of the fluorescence quantum yield on the binding metal ion.

4. Experimental

4.1. General

All chemicals for synthesis were of available purity and used without further purification. For spectral measurements, spectroscopic grade acetonitrile was used as a solvent, while all metal perchlorates were of the commercially highest purity. Oxymethyl-12-crown-4-spirobenzopyran **1** was synthesized according to the procedure in Ref. 9.

4.2. Synthesis of oxymethyl-12-crown-4-pyrenylspirobenzopyran 2

A benzene solution (100 mL) of 1-pyrenemethanol (1.16 g, 5 mmol) with pyridine (790 mg, 10 mmol) was placed to a three-necked flask at room temperature. A benzene solution (20 mL) of $SOCl_2$ (1.19 g 10 mmol) was added to the mixture dropwise at ambient temperature, and then, the reaction mixture was refluxed for 6 h. After cooling, the reaction mixture was poured into aq. HCl (5 wt%), and the organic layer was separated. The obtained crude product (1-chloromethylpyrene, pale yellow solid, 53%) by solvent evaporation was dried under vacuum and used for the following synthesis.

Under nitrogen atmosphere, 1-chloromethylpyrene (1.25 g, 5 mmol), 2,3,3-trimethylindolenine (954 mg, 6 mmol), NaI (900 mg, 6 mmol), and acetonitrile (150 mL) were placed into a three-necked flask, and the reaction mixture was refluxed for 12 h. After evaporation of acetonitrile, the obtained residue was treated with aq. NaOH (0.3 mol dm^{-3}, 100 mL) for 10 min. The reaction mixture was poured into water, and the product was extracted with chloroform. The obtained crude product (1-(1-pyrenyl)methyl-3,3-dimethyl-2-methyleneindoline, purple liquid, quantitative) by solvent evaporation was dried under vacuum and used for the subsequent synthesis without further purification.

3-Chloromethyl-5-nitrosalicylaldehyde (1.08 g, 5 mmol) and dry THF (90 mL) were put to a three-necked flask under nitrogen atmosphere. A dry THF solution (10 mL) of 1-(1-pyrenyl)methyl-3,3-dimethyl-2-methyleneindoline (1.87 g, 5 mmol) was added to the mixture, and the reaction mixture was refluxed for 4 h. After cooling, the solvent was evaporated, and purification by gel permeation chromatography afforded the pure product, chloromethyl(pyrenylmethyl)spirobenzopyran in 51% yield as purple-red solid.

Under nitrogen atmosphere, chloromethyl(pyrenylmethyl)-spirobenzopyran (571 mg, 1 mmol), hydroxymethyl-12-crown-4 (618 mg, 3 mmol), and THF (20 mL) were placed

to a three-necked flask. Powdered NaOH (360 mg, 9 mmol) was added to the mixture, and the reaction mixture was stirred for 1 h at room temperature. Acetic acid (540 mg, 9 mmol) was added to the reaction mixture. The reaction mixture was poured into water, and the product was extracted with chloroform. Purification of the product obtained after solvent evaporation was conducted with gel permeation chromatography to afford the compound in 12% yield as purple-red viscous oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.34 (3H, s, CH_3), 1.35 (3H, s, CH_3), 3.4–3.9 (17H, m, OCH_2), 4.3–4.4 (2H, m, $PyCH_2$), 5.09 (2H, s, $PhCH_2$), 5.76 (1H, d, J=10.4 Hz, CH═), 6.41 (1H, d, J=8.0 Hz, ArH), 6.62 (1H, d, J=10.0 Hz, CH═), 6.90 (1H, t, J=7.4 Hz, ArH), 7.06 (1H, t, J=7.6 Hz, ArH), 7.16 (1H, d, J=7.2 Hz, ArH), 7.82 (1H, s, ArH), 7.94 (1H, d, J=7.6 Hz, ArH), 7.9–8.3 (9H, m, ArH); IR (neat, cm^{-1}): 3019 (CH_2), 1219 (OCH_2), 743 (C═C); m/z 740 (M^+). Anal. Calcd for $C_{45}H_{44}N_2O_8$: C 72.96, H 5.99, N 3.78, Found: C 72.89, H 5.79, N 3.81.

4.3. Spectral measurement

Spectral measurement was carried out using acetonitrile as the solvent at room temperature. The UV–Vis spectra were taken after allowing a measuring solution to stand overnight under dark condition. For UV–Vis spectra measurement, both concentrations for **1** and alkaline-earth metal perchlorates were 1×10^{-4} mol dm^{-3} in Figure 1, and those in Figure 4 were 5×10^{-5} mol dm^{-3}. In the case of fluorescence emission and excitation spectra measurement, concentrations for **1**, **2**, and alkaline-earth metal perchlorates were 5×10^{-6} mol dm^{-3}, and all measurement was carried out under argon atmosphere at room temperature.

For FRET efficiency evaluation,[14] both concentrations for **2** and Sr^{2+} were 5×10^{-5} mol dm^{-3}. Merocyanine was excited at 530 and 345 nm individually, and the former corresponds to the absorption band of merocyanine, λ_M, and the latter is the absorption band of pyrene overlapping with that of merocyanine, λ_P. Fluorescence intensity of merocyanine was evaluated at 615 nm. In general, the relationship among absorbance ($A(\lambda)$), transmitted light intensity ($I_L(\lambda)$), original incident light intensity ($I_{LO}(\lambda)$), the fraction of light intensity absorbed by sample to $I_{LO}(\lambda)$ ($I_{LR}(\lambda)$) is presented as follows:

$$-A(\lambda) = \log\frac{I_L(\lambda)}{I_{LO}(\lambda)}$$

$$I_{LR}(\lambda) = 1 - 10^{-A(\lambda)} = \frac{I_{LO}(\lambda) - I_L(\lambda)}{I_{LO}(\lambda)}$$

When only merocyanine moiety is excited at λ_M, fluorescence intensity of merocyanine ($I_{F-M}(\lambda_{ex}=\lambda_M)$) is explained with instrumental function coefficient (k), incident light intensity ($I_{LO}(\lambda_M)$), absorbance ($A(\lambda_M)$), and fluorescence quantum yield (Φ).

$$I_{F-M}(\lambda_{ex} = \lambda_M) = kI_{LO}(\lambda_M)(1 - 10^{-A(\lambda^M)})\Phi$$

On the other hand, when both pyrene and merocyanine moieties are excited at λ_P, and FRET from the pyrene moiety to the merocyanine moiety takes place, fluorescence intensity of merocyanine ($I_{F-M}(\lambda_{ex}=\lambda_P)$) is explained with instrumental function coefficient (k), incident light intensity ($I_{LO}(\lambda_P)$), absorbance ($A(\lambda_P)$), fluorescence quantum yield (Φ), FRET efficiency (E), and the fraction of light intensity absorbed by the pyrene moiety to the total light intensity absorbed at λ_P (P).

$$I_{F-M}(\lambda_{ex} = \lambda_P) = kI_{LO}(\lambda_P)(1 - 10^{-A(\lambda_P)})(PE + 1 - P)\Phi$$

P is defined with light intensity absorbed by the pyrene moiety ($I_{LR-P}(\lambda_P)$) and by the merocyanine moiety ($I_{LR-M}(\lambda_P)$), which are explained with absorbances for the pyrene moiety ($A_P(\lambda_P)$) and for the merocyanine moiety ($A_M(\lambda_P)$),

$$P = \frac{I_{LR-P}(\lambda_P)}{I_{LR-P}(\lambda_P) + I_{LR-M}(\lambda_P)}$$

$$= \frac{1 - 10^{-A_P(\lambda_P)}}{1 - 10^{-A_P(\lambda_P)} + 1 - 10^{-A_M(\lambda_P)}}$$

As the light intensity is corrected to be constant regardless of wavelength in the instrument for fluorescence spectra measurement, the FRET efficiency E is explained with P, $I_{F-M}(\lambda_{ex}=\lambda_M)$, $I_{F-M}(\lambda_{ex}=\lambda_P)$, $A(\lambda_M)$, and $A(\lambda_P)$.

$$E = \frac{1}{P}\left(\frac{I_{F-M}(\lambda_{ex} = \lambda_P)(1 - 10^{-A(\lambda_M)})}{I_{F-M}(\lambda_{ex} = \lambda_M)(1 - 10^{A(\lambda_P)})} - 1 + P\right)$$

The absorbances for the pyrene and merocyanine moieties at 345 nm, $A_P(\lambda_P)$ and $A_M(\lambda_P)$, were estimated as 0.1075 and 0.06, respectively, while the absorbance at 530 nm was 0.0959. Therefore, the E was calculated at 0.12.

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